UNIVERSIDADE FEDERAL DE GOIÁS
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA TROPICAL E SAÚDE PÚBLICA

JULIANA RODRIGUES

Identificação de novos fármacos antimaláricos através de estratégia de quimiogenômica por reposicionamento e validação experimental

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UNIVERSIDADE FEDERAL DE GOIÁS
INSTITUTO DE PATOLOGIA TROPICAL E SAÚDE PÚBLICA

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JULIANA RODRIGUES

Identificação de novos fármacos antimaláricos através de estratégia de quimiogenômica por reposicionamento e validação experimental

Tese apresentada ao Programa de Pós-Graduação em Medicina Tropical e Saúde Pública da Universidade Federal de Goiás para obtenção do Título de Doutor.

Orientadora: Profa. Dra Carolina Horta Andrade
Co-orientador: Prof. Dr. Pedro Vitor Lemos Cravo

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ATA DA REUNIÃO DA BANCA EXAMINADORA DA DEFESA DE JULIANA RODRIGUES - Ao primeiro dias do mês de março do ano de 2018 (1°/03/2018), às 13:30 horas, reuniram-se os componentes da Banca Examinadora: Profs. Drs. CAROLINA HORTA ANDRADE, ÉVERTON KORT KAMP FERNANDES, ANA MARIA DE CASTRO, BRUNO JUNIOR NEVES e FÁBIO TRINDADE MARANHÃO COSTA, para, sob a presidência da primeira, e em sessão pública realizada no CENTRO DE EVENTOS DA UFG/CAMPUS SAMAMBAIA, procederem à avaliação da defesa de tese intitulada: **"IDENTIFICAÇÃO DE NOVOS FÁRMACOS ANTIMALÁRICOS ATRAVÉS DE ESTRATÉGIA DE QUIMIOGENÔMICA POR REPOSICIONAMENTO E VALIDAÇÃO EXPERIMENTAL"** em nível de **DOUTORADO**, área de concentração em **PARASITOLOGIA**, de autoria de **JULIANA RODRIGUES** discente do PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA TROPICAL E SAÚDE PÚBLICA, da Universidade Federal de Goiás. A sessão foi aberta pela Orientadora Profa. Dra. CAROLINA HORTA ANDRADE, que fez a apresentação formal dos membros da Banca e orientou a Candidata sobre como utilizar o tempo durante a apresentação de seu trabalho. A palavra a seguir, foi concedida ao autor da tese que, em 30 minutos, procedeu à apresentação de seu trabalho. Terminada a apresentação, cada membro da Banca argüiu a Candidata, tendo-se adotado o sistema de diálogo seqüencial. Terminada a fase de argüição, procedeu-se à avaliação da defesa. Tendo-se em vista o que consta na Resolução n°. 1034/2014 do Conselho de Ensino, Pesquisa, Extensão e Cultura (CEPEC), que regulamenta o Programa de Pós-Graduação em Medicina Tropical e Saúde Pública a Banca, em sessão secreta, expressou seu Julgamento, considerando a candidata **Aprovada** ou **Reprovada**:

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Em face do resultado obtido, a Banca Examinadora considerou a candidata Habilitada, (**Habilitada ou não Habilitada**), cumprindo todos os requisitos para fins de obtenção do título de **DOUTOR EM MEDICINA TROPICAL E SAÚDE PÚBLICA**, na área de concentração em **PARASITOLOGIA**, pela Universidade Federal de Goiás. Cumpridas as formalidades de pauta, às 17h15min, a presidência da mesa encerrou esta sessão de defesa de tese e para constar eu, JOSÉ CLEMENTINO DE OLIVEIRA NETO, secretário do Programa de Pós-Graduação em Medicina Tropical e Saúde Pública lavrei a presente Ata que depois de lida e aprovada, será assinada pelos membros da Banca Examinadora e por mim em duas vias de igual teor.
A Banca Examinadora aprovou a seguinte alteração no título da Tese:

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Dedico minha tese aos meus Pais que me incentivaram sem medidas para que eu pudesse chegar até aqui. Este foi sempre o maior sonho deles, concretizá-lo se tornou o meu maior desafio!

SUMÁRIO

TABELAS

FIGURAS

SIMBOLOS, SIGLAS E ABREVIATURAS

BLAST	*Basic local Alignment*
CS	Circunsporozoíto
DHF	Dihidrofolato
DHFR	Enzima dihidrofolato redutase
DHPS	Enzima dihidrofolato sintetase
GTS	*Global Technical Strategy*
IMC	Complexo de membranas internas
MLC	Miosina de cadeia leve
MyoA	Miosina A
P. cynomolgy	*Plasmodium cynomolgy*
P. falciparum	*Plasmodium falciparum*
P. knowlesi	*Plasmodium knowlesi*
P. malariae	*Plasmodium malariae*
P. ovale	*Plasmodium ovale*
P. vivax	*Plasmodium vivax*
PABA	Ácido paraaminobenzóico
Pf3D7	Cepa de *P. falciparum* sensível à cloroquina
Pfcrt	*Plasmodium falciparum chloroquine-resistance transporter*
Pfmdr1	*Plasmodium falciparum multidrug-resistance 1*
PfW2	Cepa-resistente de *Plasmodium falciparum*
QSAR	Quantitative structure–activity relationship
SP	Sulfadoxina-pirimetamina
STICH	Search Tool for Interacting Chemicals

TDR	Terapeutic Drug Research
THF	Tetrahidrofolato
TTD	Terapeutic Target Database
WHO	World Health Organization

RESUMO

Identificação de novos fármacos antimaláricos através de estratégia de quimiogenômica por reposicionamento e validação experimental

A malária é uma doença infecciosa grave que acomete bilhões de pessoas em todo o mundo. *Plasmodium falciparum* é o agente causador de malária grave em humanos, enquanto que *P. vivax* é o mais amplamente distribuído, sendo a principal espécie causadora de malária no Brasil. Atualmente, seu controle é baseado principalmente em equemas terapêuticos. No entanto, a eficácia do tratamento é comprometida pela resistência do parasito a todos os fármacos disponíveis, inclusive à artemisinina. Existe, portanto, uma necessidade urgente da descoberta de novos fármacos antimaláricos. O reposicionamento de fármacos é uma importante estratégia para acelerar a descoberta de fármacos, pois visa a identificação de novos usos para fármacos já aprovados. Assim, o objetivo deste trabalho foi utilizar uma estratégia de reposicionamento de fármacos por quimiogenômica, com foco em *P. falciparum* e *P. vivax*, a fim de identificar fármacos já aprovados que sejam eficazes contra parasitos de malária. Inicialmente, realizou-se uma aproximação de genômica comparativa na base de dados da *TDR Targets Database* para selecionar alvos presentes exclusivamente em *P. falciparum* e *P. vivax*, mas sem homólogos em humanos. Cada um dos alvos selecionados foi então usado para interrogar as bases de dados de fármacos, DrugBank, *Therapeutic Target Database* e STITCH. Os alvos de *P. falciparum* e *P. vivax* foram alinhados com os seus alvos homólogos utilizando o BLAST. Foram considerados para estudos subsequentes apenas os que apresentaram ≥ 80% de sobreposição entre as duas sequências para o alvo do fármaco correspondente. Assim, os fármacos identificados foram submetidos a uma busca na literatura visando selecionar fármacos que nunca foram testados experimentalmente contra parasitos de malária. Em seguida, foi realizada uma predição da atividade dos fármacos selecionados, utilizando modelos de QSAR binários desenvolvidos em nosso laboratório. Ao final, a estratégia de quimiogenômica permitiu selecionar seis fármacos com potencial atividade antimalárica. Estes fármacos foram submetidos a ensaios *in vitro* em estágios assexuados de *P. falciparum* (cepas 3D7, sensível a cloroquina, e W2, multi-resistente). Dentre eles, a epirrubicina se destacou por exibir uma potente atividade *in vitro* contra a cepa 3D7 (IC_{50} = 140 nM). Além disso, este fármaco demonstrou ser cerca de duas vezes mais ativa contra cepas resistentes W2 (IC_{50} = 69 nM), exibindo, inclusive, maior atividade do que a cloroquina. No momento, experimentos *in vitro* em estágios sexuados (conversão de oocineto) e ensaios *in vivo* com *P. berghei* e *P. chabaudi* estão sendo

realizados. A epirrubicina se mostrou, portanto, um candidato interessante a fármaco antimalárico. Futuros estudos são necessários para investigar o seu mecanismo de ação e potencial toxicidade e, eventualmente, avançar no processo de desenvolvimento do fármaco.

Palavras - Chave: Malária; Tratamento; Resposicionamento de fármacos; Quimiogenômica; Epirrubicina

ABSTRACT

Identification of new antimalarial drugs through drug repositioning chemogenomics approach and experimental evaluation

Malaria is an infectious disease of possible chronic evolution that affects billions of people in the tropics and subtropics. *P. falciparum* is the most lethal malaria parasite of humans, while *P. vivax* is the most widely distributed. The effectiveness of the antimalarial treatment is compromised by the ability of the parasite to evolve resistance to the compounds and by the lack of new effective antimalarials, underscoring the urgent need for the discovery of new drugs. One of the strategies that has been gradually explored in the search for new therapies is the so-called "drug repurposing" approach. In this context, the goal of the present study was to use a drug repurposing-chemogenomics strategy to identify effective drugs against malaria parasites. A comparative genomics tool available from the TDR Targets Database was used through to select targets expected to be present exclusively in *P. falciparum* and *P. vivax* parasites, but absent in humans. Each of the selected targets was then used as a *query* in the following databases: Drugbank, Therapeutic Target Database and STITCH. The *P. falciparum* and *P. vivax* targets were aligned with their predicted homologue targets, using pairwise BLAST, to compare functionally relevant regions. Only those where ≥ 80% overlap was observed between the two sequences for the corresponding drug target were considered for subsequent studies. Thereafter, the drugs identified were submitted to a bibliographic search to find drugs that were never evaluated against malaria parasites in the past. A prediction of active compounds was performed through binary QSAR models. The selected drugs were submitted to *in vitro* assays using asexual stages of *P. falciparum* (strains 3D7, chloroquine-sensitive, and W2, multidrug-resistant). Epirubicin displayed potent *in vitro* activity against the 3D7 strain (IC_{50} = 140 nM). In addition, the drug was shown to be about twice as active against the W2 resistant strain (IC_{50} = 69 nM), exhibiting even greater activity than chloroquine. At present, *in vitro* experiments in sexual stages (ookinete conversion) and *in vivo* assays with *P. berghei* and *P. chabaudi* are being carried out. In conclusion, epirubicin is a good antimalarial drug candidate, although future studies are required to investigate its mechanism of action, potential toxicity, and eventually, to advance in the drug development process.

Keywords: Malaria; Treatment; Drug repositioning; Chemogenomics; Epirubicin

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1 INTRODUÇÃO / REVISÃO DA LITERATURA

1.1 Malária

A malária é uma das principais doenças em países tropicais e subtropicais do mundo, causada por parasitos protozoários do gênero *Plasmodium*. Das 150 espécies de *Plasmodium* descritas, cinco são as que prioritariamente parasitam o homem: *P. malariae, P. vivax, P. falciparum, P. ovale, P. knowlesi.* Além disso, a espécie *P. cynomolgy* foi recentemente descrita como também causador de infecção humana (TA et al., 2014). No entanto, as maiores taxas de mortalidade e morbidade da malária são causadas por *P. falciparum* e *P. vivax*, respectivamente. A espécie mais amplamente disseminada em todo o mundo é o *P. vivax*, sendo encontrada em quase todas as regiões onde a malária é endêmica. O *P. falciparum* causa a forma mais grave da doença, sendo o principal responsável pelas causas de morte por malária (CAI H, ZHOU Z, GU J, 2013). A malaria é transmitida aos humanos através da picada da fêmea de mosquitos do gênero *Anopheles,* principalmente *A. darlingi,* (HOFFMAN et al., 2002) ou ainda por transfusão sanguínea ou transmissão congênita (BRASIL, 2013).

As primeiras manifestações clinicas da malária geralmente ocorrem entre 9 e 14 dias após o mosquito inocular o parasito no hospedeiro. A malária caracteriza-se incialmente por sintomas inespecíficos, tais como dores de cabeça, fadiga, febre, emagrecimento e náuseas, sendo os sintomas descritos comumente confundidos com gripe (WHO, 2010, WHO, 2012). A condição *sine qua non* para o diagnóstico de malária é de ocorrência de arrepios e tremores, seguidos de febre, os quais duram entre 2 e 3 dias (WHO, 2012, 2015c). Os sintomas subsequentes e os padrões das doenças variam para cada tipo de malária, consistindo em febre superior a 40°C, cefaleia intensa, vertigens, delírio e convulsão. Se um indivíduo está infectado com malária e a doença não for tratada prontamente com medicamentos antimaláricos eficazes, a doença pode progredir rapidamente e levar o indivíduo a óbito (WHO, 2015c).

1.1.1 Dados Epidemiológicos

A Organização Mundial de Saúde (OMS) estima que existem 3,2 bilhões de pessoas no Mundo com risco de infecção por malária, sendo que em 2016 foram estimados 212 milhões de casos e 429.000 mortes no mundo (WHO, 2016) (Figura 1). Aproximadamente 45% dos casos de malária ocorrem na África e 20% nas Américas. O Brasil, juntamente com a Venezuela, é líder de casos nas Américas, sendo 99,5% destes reportados na região denominada Amazônia Legal com mais de 85% dos casos no Brasil sendo causados por *P. vivax* (LIMA et al., 2015; WHO, 2016).

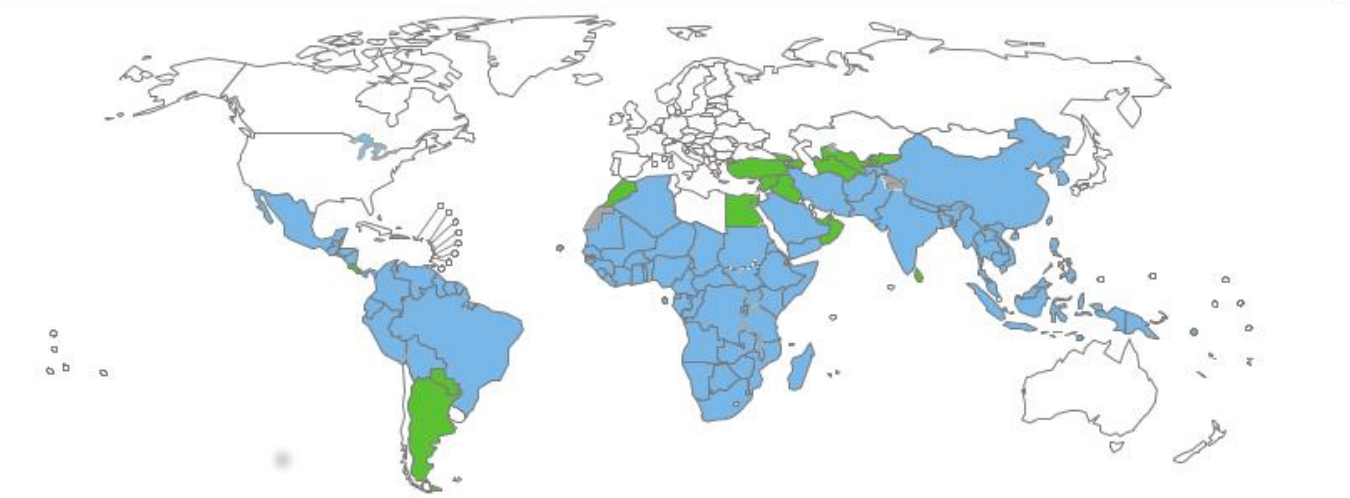
No Brasil, houve um decréscimo de casos de malária entre os anos de 2000 a 2015, um levantamento aponta que o país registrou o menor número casos nesses 15 anos. Em 2015 foram notificados cerca de 143 mil casos de malária, havendo uma redução de 85%, se comparados ao ano de 2000 (ALEXANDRE PENIDO, 2016). Os incentivos financeiros e a ampliação do quantitativos dos agentes de saúde, possibilitaram que esses índices fossem alcançados.

Segundo a OMS, em áreas com intensa transmissão de malária, o grupo mais suscetível a desenvolver malária são crianças menores de 5 anos e gestantes (WHO, 2015b), dado apresentarem pouca reduzida ou imunidade à doença. Desta forma o grupo supracitado que vive na África Subsariana, está quantificado com o maior índice de mortalidade no Mundo.

Já os indivíduos adultos que estão em áreas de intensa transmissão são menos suscetíveis de ficarem gravemente doentes ou morrerem, uma vez que a imunidade contra a malária se acumula gradualmente entre aqueles que são infectados continuamente e sobrevivem (WHO, 2015a), através da imunidade adquirida.

Vale ressaltar que em detrimento do aquecimento Global e o aumento significativo das migrações humanas, a malária é hoje considerada uma doença com possibilidade de reemergir em áreas onde já foi erradica ou controlada (BARRY; ARNOTT, 2014). Pois, viajantes, imigrantes ou militares infectados retornam de áreas endêmicas da malária, tornando possível os casos de reemersão da doença.

Entretanto, podem ocorrer infecções assintomáticas subjacentes, e desta forma, o indivíduo passar ser um reservatório para a transmissão da malária (GABRIELI; SMIDLER; CATTERUCCIA, 2014).



Países em que a malária ocorreu em 2016

Países em que a malária não ocorreu em 2000 e 2016

Países em que a malária ocorreu em 2000

Figura 1- Distribuição geográfica da malária no Mundo, entre 2000 e 2016 (adaptado de WORLD HEALTH ORGANIZATION, 2016).

1.1.2. Ciclo biológico

O ciclo do *Plasmodium*, parasito Apicomplexa, é complexo, envolvendo reprodução assexuada e sexuada, que se alternam entre o hospedeiro humano e o mosquito vetor do gênero *Anopheles* (MIAO, J ; CHEN, Z; WANG, Z; SHRESTHA, S; LI, X; LI, R; CUI, 2017) (Figura 2).

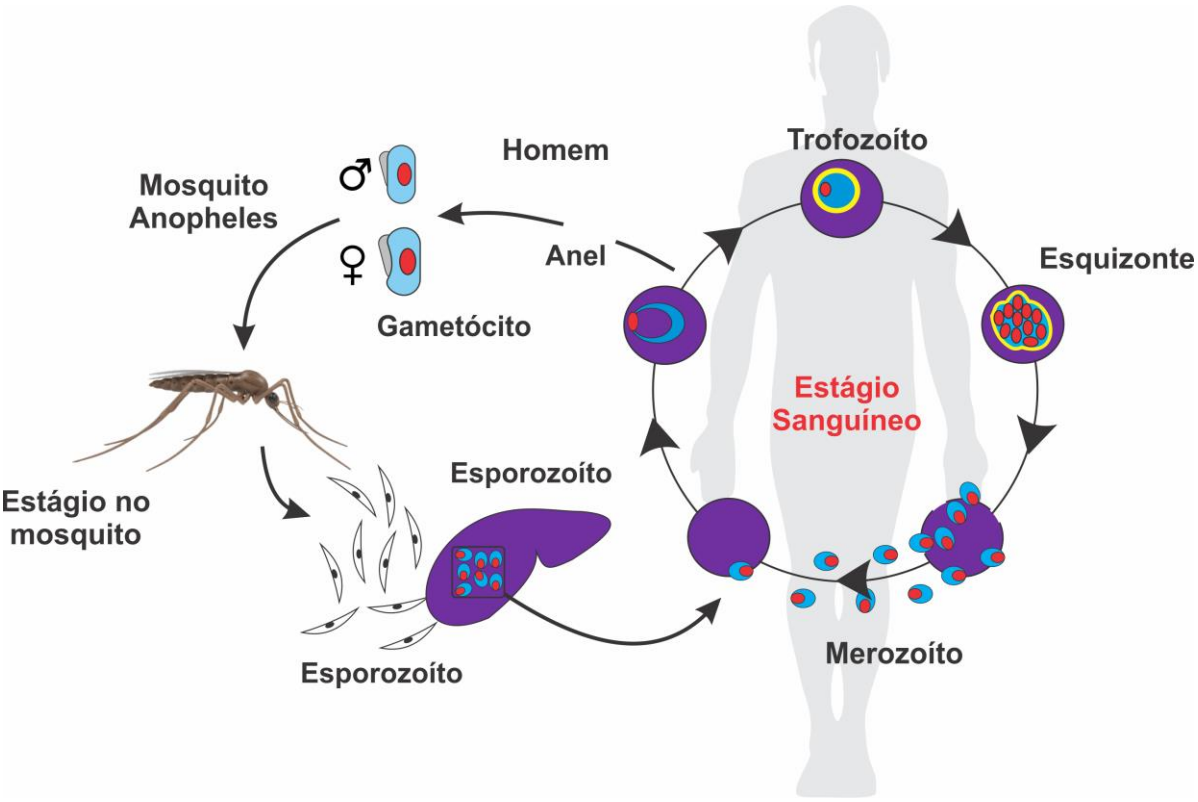


Figura 2 - Ilustração esquemática do ciclo de vida do *Plasmodium*, evidenciando as fases sexuada no mosquito *Anopheles* e assexuada nos seres humanos (adaptado de ENOMOTO et al., 2012).

O ciclo assexuado ou esquizogônico inicia-se quando os esporozoítos, formas infectantes contidas na saliva do mosquito, são inoculados no homem durante o repasto sanguíneo. Uma vez injetados na pele, os esporozoítos usam sua mobilidade ativa para alcançar os capilares sanguíneos e termina sua jornada no fígado, período que pode levar cerca de 30 a 60 min (LUÍS REY, 2008; SILVA *et al*., 2012). Nem todos os esporozoítos inoculados deixam a pele, isto depende da espécie de parasito e do grau de vascularização no sítio de inoculação. Outros eventos que ocorrem durante a jornada do esporozoíto, tais como a passagem através das barreiras endoteliais da pele e do fígado precisam ser esclarecidas, assim como é controversa a interação entre estas formas do parasito com as células de Kupffer (fagócitos do fígado) (MÉNARD et al., 2008).

Após penetrar nas células do fígado, os esporozoítos se transformam e se multiplicam assexuadamente para produzir milhares de merozoitos livres (estágio do fígado). Cada um desses merozoitos assexuados invade um eritrócito e entra em outra fase de reprodução assexuada, e depois fazem a clivagem da célula do fígado, liberando de 8 a 32 merozoítos que invadem os eritrócitos (estágio sanguíneo). Nos eritrócitos infectados, o desenvolvimento dos parasitos é acompanhado de mudanças morfológicas (anel, trofozoítos e esquizontes), que irão repetir o ciclo eritrocítico (ENOMOTO et al., 2012).

Após algum tempo de evolução da infecção malárica, algumas formas já não se dividem mais e são denominados gametócitos masculinos e femininos. Os anofelinos fazem o repasto sanguíneo, incolulam os gametócitos que por sua vez formarão microgametas masculinos (exflagelação) e femininos através do ciclo sexuado. Esses gametas se unem formando os zigotos ou oocinetos, que se dirigem para o resvestimento epitelial da parede intestinal do inseto (LUÍS REY, 2008; CDC, 2012;), que se dividem por equizogonia, formando os esporozoítos (LUÍS REY, 2008; LIMA et al., 2015;).

1.2. Medidas de Controle

As principais estratégias de controle da malária concentram-se na redução das taxas de infecção e de mortalidade. Na ausência de uma vacina eficaz, as medidas utilizadas são baseadas em prevenção, através da minimização à exposição ao vetor, e do tratamento dos sintomas e infecção da doença.

Em 2015, a OMS realizou uma conferência de Estratégia Técnica Global (*Global Technical Strategy)* com o objetivo de estabelecer estratégias para reduzir as taxas de incidência e mortalidade de malária em pelo menos 90% até 2030 (WHO, 2015b). No entanto, em 1953, uma proposta semelhante foi também feita pela OMS, mas os objetivos não foram alcançados. Assim, uma avaliação dos acertos e erros do programa de 1953 foi realizada, com intuito de elaborar novas estratégias para erradicação da malária. Desta fora, a conferência de 2015 ressaltou a importância de se utilizar dados de vigilância de alta qualidade para a tomada de decisões (MNZAVA et al., 2015a). Também destacou que medidas inovadoras serão essenciais para atingir os objetivos, como por exemplo o desenvolvimento de novos fármacos para o controle do parasito bem como o controle vetorial baseado em novos inseticidas (ALONSO; ENGELS, 2017).

1.2.1. Controle vetorial

Historicamente, o controle vetorial tem sido o pilar para prevenir a transmissão da malária (ALONSO; ENGELS, 2017). Essa estratégia resulta de um conjunto de ações integradas que tem por base as características dos locais de intervenção, como a biologia do vetor, a taxa de transmissão e morbilidade da doença nessa área, infraestruturas de saúde existentes e seus recursos, condições ambientais e a situação econômica (CDC, 2012; MULAMBA et al., 2014). Algumas variáveis devem ser consideradas para que a campanha de controle do vetor seja efetiva, tais como colaboração entre os serviços de saúde e entidades públicas e privadas, sensibilização da comunidade através da educação,

exigência de uma legislação e regulamentos efetivos que deem suporte, assim como o uso racional de inseticidas e boas práticas de manuseio dos mesmos (WHO MALARIA POLICY ADVISORY COMMITTEE AND SECRETARIAT, 2015; WHO, 2015c; WHO, 2016).

Entretanto, nos últimos anos, é observado o desenvolvimento de resitência pelos mosquitos aos inseticidas, particularmente à classe dos piretróides, que são amplamente usados em mosquiteiros impregnados com inseticidas (MNZAVA et al., 2015b; WHO MALARIA POLICY ADVISORY COMMITTEE AND SECRETARIAT, 2015).

Desta forma, é necessário o monitoramento e gestão eficazes da resistência aos inseticidas e ainda apoiar a disponibilidade e acessibilidade de novos produtos de controle de vetores (MULAMBA et al., 2014; WHO, 2015b).

1.2.2. Controle parasitário

Um diagnóstico em tempo hábil e tratamentos adequados são estratégias fundamentais para o controle da malária. O uso adequado de fármacos antimaláricos ameniza os sintomas e complicações que levam ao risco de morte (WHO, 2015c).

1.2.3. Vacinas

Registaram-se progressos no controle da malária, com reduções substanciais na mortalidade e morbidade resultados estes, que puderam ser obtidos através da administração de fármacos em massa e medidas de controle vetorial, incluindo a distribuição de mosquiteiros impregnados com inseticida de longa duração e pulverização residual interna (HEALER et al., 2017; WHO, 2015a).

No entanto, a resistência emergente dos parasitos aos últimos antimaláricos existentes, como artemisinina (*P. falciparum*) e cloroquina (*P. vivax*), representa uma ameaça significativa para a sustentabilidade dessas intervenções (HYDE, 2007, HENRIQUES *et al.,* 2013b, HEALER et al., 2017). Desta forma, desenvolver uma vacina eficaz contra a malária é uma estratégia importante na saúde pública, combinada à quimioterapia, controle vetorial e ações sanitárias e ambientais.

Atualmente, existem 30 formulações vacinais para *P. falciparum* em desenvolvimento, sendo que 18 destas já estão em fase clínica de testes. Em contrapartida, há apenas 15 candidatos vacinais contra *P. vivax,* e apenas 2 destes estão em ensaios clínicos (BARRY e ARNOTT 2014).

Em julho de 2015, órgãos regulatórios europeus licenciaram a vacina antimalárica RTS`S. Esta vacina é baseada em proteínas recombinantes (proteínas virais de hepatite e

proteínas de *P. falciparum*) (EUROPEAN MEDICINES AGENCY, 2015). Esta vacina já está sendo testada em crianças e jovens na África. Entretanto, resultados da fase clínica III demonstram que a vacina apresenta baixa eficácia e provavelmente não atingirá o objetivo de erradicar a malária (MAHMOUDI; KESHAVARZ, 2017).

Desta forma, as vacinas em estágios mais avançados oferecem proteção parcial, não bloqueando a transmissão da doença e as que protegem totalmente, se deparam com desafios tecnológicos em termos de produção, formulação e entrega que ainda precisam ser superados (THE MALERA CONSULTATIVE GROUP ON VACCINES, 2011).

Apesar da vacinação ser a melhor forma de erradicar e/ou prevenir doenças, ainda as principais formas de combate a transmissão da malária são baseadas no uso de medicamentos, controle vetorial e saúde pública (BISPO et al., 2013). Portanto, o planejamento e descoberta de novos fármacos antimaláricos é de suma importância para o controle da malária.

1.3. Tratamento

Os fármacos antimaláricos são baseados em produtos naturais ou compostos sintéticos produzidos a partir da década de 40. Esses fármacos são específicos para cada etapa do ciclo de vida do *Plasmodium* (MAKINDE; OKOSUN, 2011; WORLD HEALTH ORGANIZATION, 2016).

Dois critérios gerais são utilizados para a classificação dos fármacos antimaláricos, sendo eles: (*i*) baseado na estrutura química e/ou principal mecanismo de ação; e (*ii*) baseado no local/fase de ação. Neste último caso, os fármacos podem ser classificados como: esquizonticidas sanguíneos, quando atuam sobre a fase intra-eritrocitária; esquizonticidas teciduais, quando previnem o desenvolvimento de esquizontes hepáticos; hipnozoiticidas, que atuam sobre os hipnozoítos hepáticos; e gametocidas, quando atuam nas formas sexuais intra-eritrocitárias (Tabela 1) (FRANÇA; DOS SANTOS; FIGUEROA-VILLAR, 2008, HENRIQUES et al., 2013).

Tabela 1 – Fármacos antimaláricos, divididos por grupos químicos e classificados de acordo com o principal mecanismo de ação e fase do ciclo de atuação.

Fármacos antimaláricos		
Quinina e Derivados	**Mecanismo de Ação**	**Fases do ciclo de atuação**
Quinina Amodiaquina Cloroquina Halofantrina	Interferência na destoxificação do grupo heme	Esquizoticida sanguíneos Esquizoticida tecidual Hiptonozoiticida Gametocida
Primaquina		Gametocida
Antanogistas de Folatos	**Mecanismo de Ação**	**Fases do ciclo de atuação**
Pirimetamina	Inibição da síntese de ácidos nucleicos	Esquizonticida sanguíneos
Proguanila		Esquizonticida teciduais
Sulfonamidas	**Mecanismo de Ação**	**Fases do ciclo de atuação**
Sulfadoxina	Inibição da síntese de ácidos nucleícos	Esquizonticida sanguíneos
Atovaquona	Interferência na cadeia de transferência de elétrons mitocondriais	Esquizonticidas sanguíneos
Artemisinina e derivados	**Mecanismo de Ação**	**Fases do ciclo de atuação**
Artemisinina Dihidroartemisinina Artesunato Artemeter	Geração de stress Oxidativo	Esquizonticidas sanguíneos Gametocida (*P. falciparum)*
Antibióticos	**Mecanismo de Ação**	**Fases do ciclo de atuação**
Tetraciclina Doxicilina Clindamicina	Inibidores da Síntese Proteica	Esquizonticidas sanguíneos

1.3.1. Fármacos quinolínicos

As quinolinas incluem alguns dos fármacos antimaláricos mais utilizados e podem ser divididas em dois grupos: fármacos tipo 1 que incluem as 4-aminoquinolonas (cloroquina, piperaquina) e derivados de base de Mannich (amodiaquina) (Figura 3); e fármacos tipo 2, que são os aril amino-álcoois, como a quinina e mefloquina (Figura 4). Os fármacos tipo 1 são bases fracas diprotonadas e hidrofílicas em pH neutro, enquanto os fármacos tipo 2 são bases fracas e solúveis em lipídeos em pH neutro (OLLIARO; TAYLOR; RIGAL, 2001).

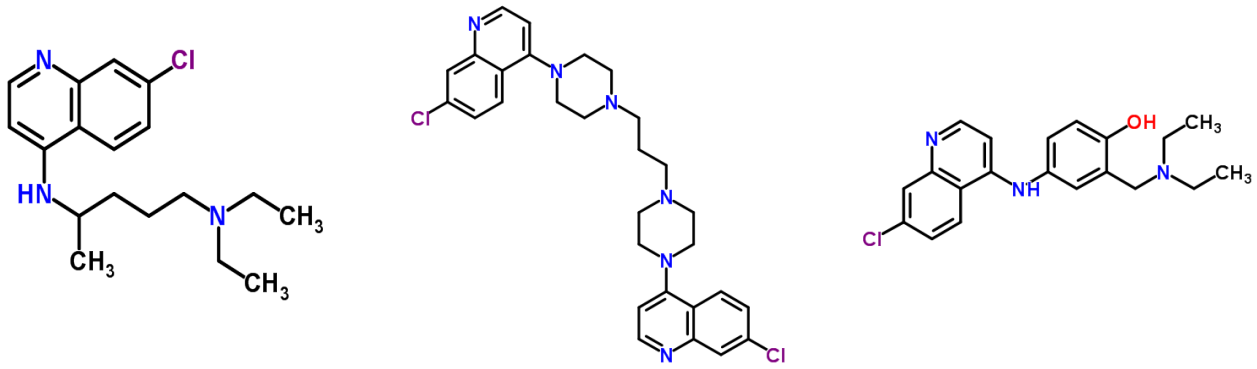


Figura 3 - Estruturas químicas da cloroquina, piperaquina e amodiaquina

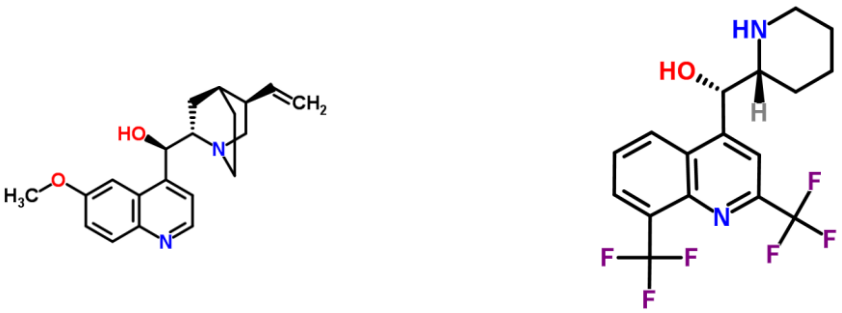


Figura 4- Estruturas químicas da quinina e mefloquina.

A cloroquina (Figura 3) foi a primeira 4-aminoquinolina utilizada no tratamento da malária. Introduzida na terapêutica no final dos anos 40 para a prevenção de malária em larga escala, este fármaco já foi considerado o tratamento padrão devido a sua eficácia, acessibilidade e segurança, mesmo durante a gravidez.

O mecanismo de ação da cloroquina tem sido alvo de intensas pesquisas durante décadas. Estudos sugerem que o mecanismo de ação da cloroquina envolve o sistema processo de polimerização do heme no vacúolo digestivo. Acredita-se que cloroquina possa se ligar a hematina, um dímero de heme e impedir o processo de desintoxicação de heme

livre, levando o parasito a morte pelo acumulo de heme tóxico (CHOMBRINCK *et al*., 2013; EGAN, 2008; FITCH, 2004; PETERSEN, EASTMAN, LANZER, 2011).

Entretanto, o uso inapropriado levou ao surgimento de resistência do parasito, em países endêmicos, exceto nos países da América central. Mutações nos genes *pfcrt* (*Plasmodium falciparum chloroquine-resistance transporter*) e *pfmdr1* (*Plasmodium falciparum multidrug-resistance 1*) estão relacionados com o principal mecanismo de resistência à cloroquina (WALLER et al., 2003; ARAV-BOGER; HAPIRO, 2005).

O gene *pfcrt* é um determinante principal da resistência à cloroquina, enquanto que *pfmdr* foi identificado como um fator modulador (ECKER et al., 2012). Este fato pode ser justificado pois a presença de *pfmdr1* cloroquina resistente está relacionada com a adaptação em reposta às mudanças fisiológicas que são resultados de outras mutações genéticas associada com *pfcrt* (IBRAHEEM et al., 2014).

1.3.2. Antagonistas de folato

Antagonistas de folato podem atuar como excelentes esquizonticidas sanguíneos. Entretanto, os parasitos da malária têm desenvolvido resistência a esses fármacos. Muitos dos antifolatos são tóxicos ao ser humano e apresentam baixa biodisponibilidade (FRANÇA; DOS SANTOS; FIGUEROA-VILLAR, 2008).

Estes fármacos são divididos em dois grupos: antifolatos do tipo I: são incluídos os competidores do ácido paraminobenzóico (PABA), que interrompem a formação do ácido di-hidroxifólico, necessário para a síntese de ácidos nucléicos, através da inibição da di-hidropteroato sintase, neste grupo incluem as sulfonamidas e sulfonas (Figura 5).

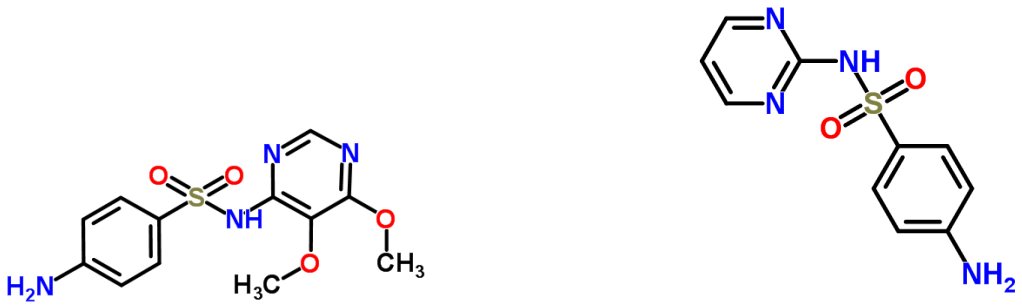


Figura 5 - Estruturas químicas das sulfas: sulfadoxina e sulfadiazina.

Já os antifolatos do grupo 2 inibem a enzima dihidrofolato redutase (DHFR), prevenindo a redução de dihidrofolato (DHF) a tetrahidrofolato (THF), sendo o THF cofator

necessário para biossíntese de timidilato, nucleotídeos purínicos e alguns aminoácidos. O mecanismo de resistência a esses fármacos é conferido por mutações dos genes que codificam as respectivas enzimas, *dhps* e *dhfr* (ABDUL-GHANI; FARAG; ALLAM, 2013).

A pirimetamina (Figura 6) é contraindicada para o tratamento da malária na fase aguda, pois atua contra o *P. falciparum* de forma lenta. Desta forma, tem sido administrada combinada com outros fármacos, tais como sulfadoxina (Figura 5), dapsona (Figura 7) e cloroquina (Figura 3).

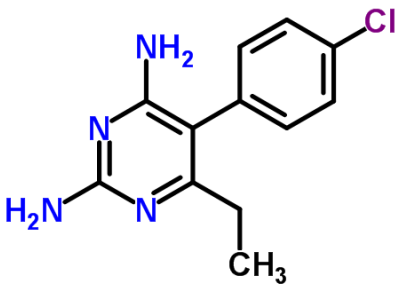


Figura 6 - Estrutura química da pirimetamina

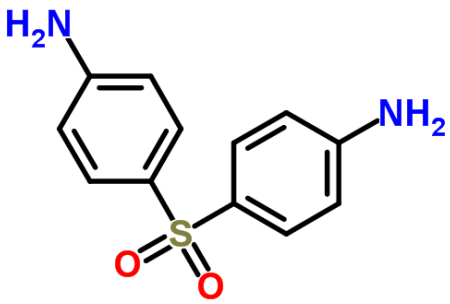
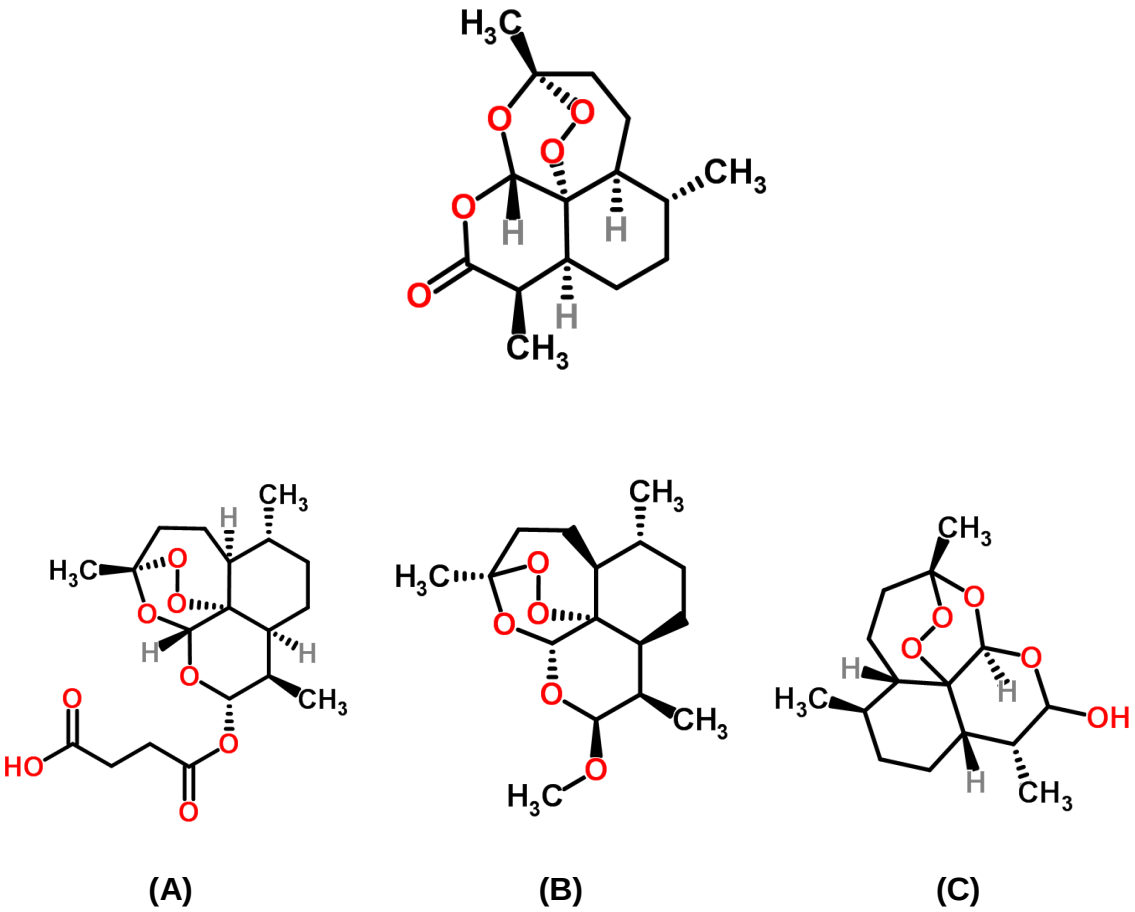


Figura 7 - Estrutura química da dapsona

1.3.3. Artemisinina e derivados

A artemisinina, uma substância obtida da planta *Artemisia annua*, (Figura 8) foi descoberta na China em 1967, com o objetivo de ajudar os militares no norte do Vietnã na guerra contra os Estados Unidos (MILLER; SU, 2011). Na medicina tradicional chinesa, essa planta já era utilizada há anos. A partir daí muitos derivados da artemisinina vem sendo sintetizados por companhias farmacêuticas. Os principais derivados são artesunato (Figura

8), artemeter, e dihidroatemisinina. Estes derivados sintéticos apresentam uma melhor biodisponibilidade oral quando comparados à artemisinina (MESHNICK, 2002; MUTI et al., 2010).



Figuras 8- Estruturas químicas da artemisina e seus principais derivados: artesunato (A), artemeter (B) e dihidroartemisina (C).

As artemisininas são lactonas sesquiterpênicas, apresentando um grupo endoperóxido essencial para a atividade antimalárica (EASTMAN; FIDOCK, 2009;ISMAIL et al., 2016). Elas são esquizonticidas sanguíneos de ação rápida, pouco solúvel em água, eficaz no tratamento de ataque agudo de malária, incluindo a malária cerebral e a resistente a cloroquina (MESHNICK, 2002).

O mecanismo de ação envolve a formação de complexo de transição, por intermédio dos átomos de ferro do Heme e de endoperóxido. A posição relativa da artemisinina com respeito ao grupo heme é determinada por interações esteroesletrônicas entre ambos, os quais afetam o rearranjo do complexo até o rompimento da ligação Fe-0 (O'NEILL; BARTON; WARD, 2010).

É importante ressaltar que Youyoy Tu, pesquisadora chinesa responsável por descobrir e extrair a artemisinina da planta *Artemisia annua*, recebeu o Prêmio Nobel de Medicina em 2015. A sua descoberta propiciou uma redução de cerca de 100 mil casos de mortes por ano, e trouxe mais expectativa de vida para diversas pessoas de áreas pobres do mundo (AMARAL, 2015).

Todavia, a resistência do *P. falciparum* a artemisinina foi já reportada em cinco países da grande Sub Região Mekong, no sudeste Asiático: Camboja, República Democrática Popular do Lao, Mianmar, Tailândia e Vietnam (WHO, 2015c). Um polimorfismo do gene *k-13-helice* parece ser um marcador molecular capaz de rastrear o surgimento e propagação de *P. falciparum* resistente a artemisinina (ARIEY et al., 2014).

1.3.4. Resistência aos fármacos antimaláricos

A eficácia da quimioterapia é comprometida pela capacidade de o parasito da malária desenvolver resistência ao fármaco e pela escassez de novos antimaláricos. A resistência aos antimaláricos vem sendo observada e registrada em *P. falciparum*, *P. malarie* e *P. vivax* (RODRIGUES et al., 2010). Em *P. falciparum*, a resistência aos fármacos já surgiu para todos os antimaláricos usados atualmente (amodiaquina, cloroquina, mefloquina, quinina e sufadoxina pirimetamina, e mais recentemente em derivados de artemisinina)(HOTT et al., 2015; VERLINDEN; LOUW; BIRKHOLTZ, 2016).

Os principais antimaláricos aprovados na atualidade para uso clínico agem principalmente em duas vias metabólicas do parasito: degradação de hemoglobina e síntese de ácidos nucleicos (KRISHNA, 2006, ALONSO; ENGELS, 2017).

Exceto para os derivados de artemisinina, a resistência do parasito tem evoluído e se tornado comum para os antimaláricos usados atualmente. Um dos fenômenos que contribuem para o surgimento e aumento de resistência aos fármacos é que a resistência a diferentes fármacos é muitas vezes controlada por mecanismos moleculares semelhantes e, consequentemente, a evolução de resistência a um fármaco específico pode ter impacto sobre a eficácia de outros (PARHIZGAR, 2017). Por exemplo, a resistência a fármacos derivados de quinina, tais como a mefloquina e lumefantrina, bem como para os derivados de artemisinina estruturalmente não relacionados, tem sido associada a mutações e / ou amplificação da proteína homólogo -1 de multirresistência, *pfmdr1* (WOODROW; KRISHNA, 2006).

Da mesma forma, a resistência aos fármacos que bloqueiam a síntese de ácido nucleico, tais como sulfadoxina, pirimetamina e proguanila, é em grande parte atribuída por mutações pontuais em genes que codificam duas enzimas, di-hidrofolato redutase (*dhfr*) e a

dihidropteroate sintase (*dhps*) (LEBRAS; DURAND, 2003). Ao considerar a descoberta de novos fármacos antimaláricos, é essencial estudar novas alternativas como alvos moleculares. Um exemplo é o apicoplasto, um plastídio não-fotossintetizante que foi descrito pela primeira vez em a década de 1990, que, recentemente, confirmou ter sido adquirido por endossimbiose secundária de uma alga contendo um plastídio (MCFADDEN; YEH, 2017).

1.4. Planejamento e descoberta de fármacos

O processo de descoberta e desenvolvimento de novos fármacos é bastante complexo, podendo ser dividido didaticamente em duas grandes fases: descoberta e desenvolvimento de fármacos (Figura 9 A).

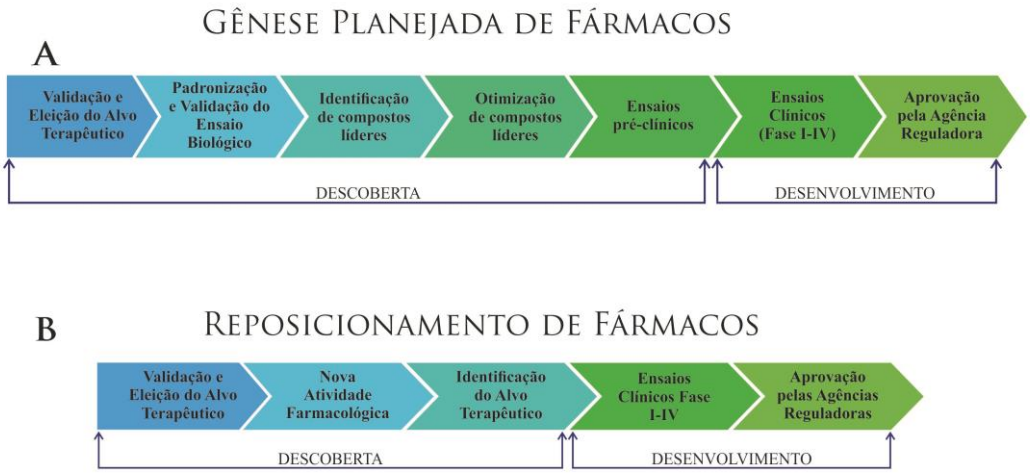


Figura 9- Etapas envolvidas na gênese planejada de novos fármacos (**A**) e reposicionamento de fármacos (**B**). Adaptada de NEVES et al., 2015)

Nos estágios iniciais da fase de descoberta, as pesquisas se concentram no estudo e caracterização de alvos macromoleculares com elevado potencial e identificação de moléculas pequenas capazes de modular o alvo selecionado. Uma vez identificado um alvo molecular promissor, é fundamental a sua validação, ou seja, é preciso verificar qual é a relevância deste alvo no processo fisiopatológico em questão (WERMUTH, 2008).

Nesta fase, buscam-se identificar moléculas que apresentem propriedades desejadas para um fármaco, principalmente potência e seletividade, mas que ainda precisam ser otimizadas em relação a outras propriedades, como farmacocinéticas e toxicidade

(KOUTSOUKAS et al., 2011). Os compostos ativos que se destacam nesta fase são selecionados compostos-líderes ou protótipos (*lead compounds*). A fase seguinte tem por objetivo otimização dos compostos líderes em relação às suas propriedades farmacodinâmicas e farmacocinéticas (OPREA; OVERINGTON, 2015).

Na etapa de desenvolvimento clínico, os ensaios clínicos são realizados em humanos e cada fase (fase I a IV) é executada com determinado número de indivíduos saudáveis ou pacientes, de acordo com a fase em questão (FISHER; COTTINGHAM; KALBAUGH, 2015; KINCH, 2016).

No final da fase clínica III, caso aprovado pelas agências regulatórias, o fármaco é lançado no mercado e prossegue para os estudos de fase IV. Assim, o fármaco será comercializado e monitorado para que possíveis efeitos adversos a longo prazo sejam detectados (NG, 2008;MULLARD, 2016). É necessário ressaltar que apesar de todo o esforço empenhando na descoberta de novos fármacos, a taxa de insucesso desse processo é demasiadamente alta. Estima-se que apenas 10% dos candidatos a fármacos em fases clinicas chegam ao mercado (HAY et al., 2014).

Atualmente, os fármacos que estão disponíveis na terapêutica requereram entre 12 a 24 anos de pesquisa, desenvolvimento e inovação (PD&I), desde a concepção do projeto ao lançamento do medicamento no mercado. Além disso, estima-se que o custo varia entre 500 milhões a 2 bilhões de dólares, dependendo da terapia ou empresa (MORGAN et al., 2011;BARAS; BARAS; SCHULMAN, 2012;DIMASI, JOSEPH A. , GRABOWSKI, HENRY G., HANSEN, 2015). Portanto, novas estratégias estão sendo utilizadas para descoberta de novos fármacos.

1.4.1. Reposicionamento de fármacos

Uma estratégia que vem sendo bastante explorada na descoberta de fármacos é o reposicionamento de fármacos (DUBUS et al., 2009; LIU et al., 2013). Este processo consiste na identificação de um novo uso/nova indicação para um fármaco já aprovado para uso clínico em humanos (Figura 9B). Pelo fato de tais fármacos já serem aprovados para tratamentos em seres humanos para outros fins, podem mais facilmente entrar em ensaios clínicos, acelerando acentuadamente o processo de descoberta e diminuindo significativamente os seus custos e riscos (NEVES et al., 2015; SBARAGLINI et al., 2016).

Estratégias de reposicionamento de fármacos dependem da integração de uma série de conceitos biológicos e metodologias de análise bioinformática que permitem identificar alvos terapêuticos (proteínas do patógeno) e fármacos já disponíveis com potencialidade de

inibir esses alvos, resultando na eliminação ou inibição do crescimento do patógeno alvo (EKINS et al., 2011; NOVAC, 2013). Diferentes bases de dados existentes na *web* disponibilizam informações sobre milhares de compostos terapêuticos que atuam sobre alvos proteicos específicos, a via metabólica envolvida e que doenças já foram tratadas ou estão fase de testes (LIU et al., 2013).

Fármacos identificados por esta via podem então ser sujeitos a ensaios experimentais de inibição do parasito *in vitro*, denominados de ensaios primários. Subsequentemente, aqueles compostos que apresentem níveis satisfatórios de inibição *in vitro*, são usados em ensaios em modelos experimentais, onde se avalia o seu nível de inibição do crescimento *in vivo*, denominados de ensaios secundários. Em última análise, este paradigma poderá levar a propor novas terapias (LI et al., 2016; SARDANA et al., 2011; YU; RAMSEY, 2016).

Exemplos de fármacos reposicionados incluem a sildenafila, originalmente desenvolvida para tratamento da hipertensão arterial e angina e através de serendipidade foi reposicionada para ser o primeiro tratamento da disfunção eréctil no homem; e miltefosina, originalmente utilizada no tratamento do câncer e reposicionada para tratamento da leishmaniose visceral, a talidomida indicada orignalmente para o tratamento de náuseas e reposicionada para o tratamento de eritema nodoso em leprosos (ANDERSEN et al., 1998; ASHBURN; THOR, 2004; KITCHEN; VAUGHN; SKILLMAN, 2006; BERMAN, 2015).

A busca por novos fármacos demanda um longo tempo para as pesquisas, recursos financeiros onerosos e ainda um elevado risco de perda dos investimentos. Dentre as abordagens mais utilizadas, o reposicionamento de fármacos (drug repositioning, therapeutic switching) e a triagem (screening) de bibliotecas químicas de compostos sintetizados, ganharam destaque e se tornaram prioridade nos programas de Drug Discovery, tanto na indústria como nos setores não industriais (acadêmicos e governamental) (BAJORATH, 2012; OPREA; OVERINGTON, 2015).

1.4.1.1. Reposicionamento de fármacos para malária

O reposicionamento de fármacos já demonstrou sua importância para a descoberta de novos antimaláricos, uma vez que antibióticos de amplo espectro já demonstraram ter atividade antimalárica. Por exemplo, as tetraciclinas como a doxiciclina, foi utilizada com sucesso na quimioprofilaxia e no tratamento da malária (RIECKMANN et al, 1971, CLYDE; GILMAN; MCCARTHY, 1975;). A doxiciclina foi aprovada pela primeira vez para uso clínico em 1967 como um antibiótico de amplo espectro. Este fármaco é parcialmente eficaz contra as fases hepáticas de malária (profilaxia causal) e tem demonstrado ação esquizonticida de

ação lenta (RIECKMANN et al,1971; RIECKMANN, 1983). O antibiótico doxiciclina é usado com sucesso como fármaco preventivo para os viajantes que migram para zonas endêmicas da malária e também, é usado de maneira profilática em terapia combinada com agentes de ação rápida como a quinina e quinidina (LALLOO et al., 2007; TAN et al., 2011).

Da mesma forma, a clindamicina, um antibiótico lincosamida que foi originalmente utilizado nas décadas de 1940-1950 para o tratamento da acne (Christian e Krueger, 1975), com inúmeras aplicações, é usado clinicamente para a malária (e a toxoplasmose) e com atividade antimalárica descrita pela primeira vez na década de 1970 (MILLER et al., 1974).

O grupo sulfonamida é uma estrutura presente em muitos fármacos utilizados para tratar uma ampla variedade de doenças humanas, incluindo doença renal, glaucoma, epilepsia e obesidade e, mais recentemente, como candidatos à terapia de câncer (TEMPERINI et al., 2008(SUPURAN, 2011). Embora existam mais de 100 medicamentos contendo sulfonamida aprovados pelo FDA (SMITH; JONES, 2008), apenas alguns foram explorados em terapias antiparasitárias. As sulfonamidas antibacterianas, sulfadiazina e sulfadoxina, que inibem a síntese de folato, foram testadas em terapias combinadas para tratar infecções por *T. gondii* e *P. falciparum* (DOBERSTYN et al., 1976).

O fármaco sulfametoxazol-trimetoprim é usado para tratar a toxoplasmose e malária ocasionada por *P. falciparum* (MANYANDO et al., 2013). Dapsona, um antibiótico com grupo sulfona que foi usado pela primeira vez para tratar a hanseníase e outras doenças como dermatite herpetiforme e pneumonia ocasionada por *P*. *carinii* em pacientes com AIDS (WOLF et al., 2002), foi introduzida no tratamento da malária no final da década de 1990. Entretanto, foi descontinuada em 2008 por ocasionar anemia hemolítica em 20% dos pacientes (MERCKX et al., 2003).

Desta forma existe uma necessidade continua de descobrir e desenvolver novos antimaláricos que sejam eficazes contra os parasitos resistentes. Assim a descoberta e desenvolvimento de novos fármacos através das estratégias computacionais para complementar as abordagens tradicionais reduz consideravelmente o custo, o tempo e os riscos na pesquisa de quimioterapia (WADOOD et al., 2013).

As ferramentas *in silico* usadas na descoberta e desenvolvimento de fármacos podem ser amplamente classificadas em bancos de dados bioquímicos, quimioinformática e ferramentas usadas em "*Drug Design"* baseadas em estruturas e em ligantes (TALELE; KHEDKAR; RIGBY, 2010).

1.5. Quimiogenômica

O sequenciamento do genoma de referência de *P. falciparum* foi concluído em 2002 por Gardner e colaboradores (GARDNER et al., 2002), combinado com o sequenciamento contínuo do genoma de outras espécies de *Plasmodium* (AURRECOECHEA et al., 2017), que tem auxiliado a diminuir as barreiras para a identificação de novos alvos de fármacos, bem como o estudo de mecanismo de ação de fármacos já conhecidos (GARDNER et al., 2002;CARLTON et al., 2008; BERENSTEIN et al., 2016c).

O *P. falciparum* apresenta um genoma nuclear constuído de 23 milhões de pares de bases que codificam cerca de 5.300 genes, distribuídos em 14 cromossomos (GARDNER et al., 2002). Os genes envolvidos na variação antigênica estão concentrados nas regiões sub-teloméricas dos cromossomos. Em comparação com os genomas de outros microorganismos eucarióticos de vida livre, o genoma deste parasito intracelular codifica menos enzimas e proteínas transposrtadoras, entretanto, uma grande proporção de genes é dedicada as interações parasito-hospedeiro. Muitas proteínas com codificação nuclear são direcionadas ao apicoplasto, uma organela envolvida no metabolismo de ácidos graxos e isoprenóides (AURRECOECHEA et al., 2009; GARDNER et al., 2002).

O crescimento de dados genômicos e fenotípicos em grande escala, bem como dados de compostos químicos e fármacos, tem permitido um avanço na área reposicionamento de fármacos. Atualmente, encontram-se disponíveis vários bancos de dados, que armazenam dados de acesso público e gratuito sobre compostos químicos, fármacos e genes, tais com EuPathDB (AURRECOECHEA et al., 2017),TDR *Targets Database* (MAGARIÑOS et al., 2012), *DrugBank* (WISHART et al., 2006a), *Therapeutic Target Database* (TTD) (CHEN; JI; CHEN, 2002a), PubChem (WANG et al., 2015b), entre outros.

Uma estratégia que pode ser utilizada para a busca e priorização de alvos é a quimiogenômica (NEVES et al., 2015). A genômica comparativa se baseia principalmente no estudo da homologia e dinâmica evolutiva dos organismos, seus respectivos genes e proteínas, podendo apresentar grande utilidade no entendimento da evolução das espécies pela comparação de seus genomas completos ou de genes-específicos de cada espécie (HARDISON, 2003).

Os avanços da área da genômica permitiram que novas ferramentas computacionais de bioinformática e quimiogenômica pudessem ser aplicadas aos estudos de reposiciomento de fármacos (NEVES et al., 2015). A quimiogenômica é uma estratégia interdisciplinar que envolve a investigação das interações de todo o espaço biológico disponível (genes, proteínas) com todo o espaço químico disponível (BREDEL; JACOBY, 2004).

Desta forma, o reposicionamento de fármacos utilizando estratégias computacionais, bioinformática e quimiogenômica tem se mostrado promissor contra diversos parasitos, tais como: *Schistossoma mansoni* (NEVES et al., 2015)*, P. falciparum* (BISPO et al., 2013)*, Tripanossoma cruzi* (RODRIGUES et al., 2015) *e Toxoplasma gondii* (ANDREWS; FISHER; SKINNER-ADAMS, 2014)*.*

2 JUSTIFICATIVA

A malária causa cerca de meio milhão de mortes anualmente no mundo, sendo o *P. falciparum* responsável por 90% de todos os casos. Na ausência de uma vacina eficaz, a principal forma de controle da doença é baseada no tratamento farmacológico. Relatórios recentes sobre a resistência à artemisinina no Sudeste Asiático justificam a descoberta urgente de novos fármacos para o tratamento da malária. No entanto, a maioria dos compostos bioativos não conseguem avançar a cascata do desenvolvimento pois não oferecem a segurança necessária. Assim, se torna evidente a necessidade de se identificarem e disponibilizarem novos antimaláricos para uso clínico em humanos, seguros e eficazes.

Além disso, o sequenciamento completo do genoma de *P. falciparum* combinado com o sequenciamento contínuo do genoma das outras espécies de *Plasmodium*, proporcionaram um recurso inestimável para a para a priorização, identificação e estudo da funcionalidade de novos candidatos a alvos terapêuticos de fármacos e ao mesmo tempo permitiram o desenvolvimento e aplicação de ferramentas computacionais de quimiogenômica aplicadas a estudos de reposicionamento de fármacos. Levando em consideração o fato de que proteínas que compartilham algum tipo de semelhança estrutural também podem compartilhar os mesmos ligantes, o estudo de reposicionamento *in silico* de novos fármacos para malária justifica-se pelas seguintes razões:

1- O genoma do *P. falciparum* e *P. vivax*, bem como a lista de todas proteínas expressas nos principais estágios do ciclo evolutivo do parasito encontram-se disponíveis na literatura;
2- Diversas bases de dados armazenam informações de alvos biológicos, além de informações químicas e farmacológicas de fármacos e candidatos a fármacos;
3- O reposicionamento *in silico* de fármacos para malária pode ser conduzido através da busca por proteínas do parasito que tenham homologia com alvos terapêuticos de fármacos disponíveis nas bases de dados DrugBank, TTD e STITCH;
4- Estudos computacionais podem ser úteis para reduzir o custo operacional) e tempo necessários para realização dos ensaios biológicos *ex vivo* em *P. vivax*;
5- Os fármacos identificados como potenciais contra o *P. falciparum* e *P vivax*, também poderão servir como ponto de partida (*scaffolds*) para o planejamento e síntese de análogos estruturais com maior potência, seletividade e permeabilidade.

3 OBJETIVOS

3.1- Objetivo Geral

O objetivo geral deste trabalho foi utilizar uma estratégia de reposicionamento de fármacos por quimiogenômica, com foco em *P. falciparum* e *P. vivax*, a fim de identificar fármacos já aprovados que sejam eficazes contra parasitos de malária.

3.2- Objetivos Específicos

- Compilar uma biblioteca de potenciais alvos terapêuticos (proteínas) presentes em *P. falciparum* e *P. vivax* e ausentes em humanos, através da estratégia de genômica comparativa;
- Realizar uma busca baseada em homologia em várias bases de dados, com o objetivo de identificar fármacos aprovados para uso em humanos com potencial atividade antimalárica;
- Determinar a conservação estrutural das regiões funcionais para as proteínas de *P. falciparum* e *P. vivax* que apresentam considerável homologia para com alvos terapêuticos de fármacos;
- Priorizar fármacos para a avaliação biológica utilizando as predições de modelos de QSAR desenvolvidos com dados fenotípicos de atividade antimalárica (*P. falciparum*) frente a cepas resistentes à cloroquina (W2) e sensíveis à cloroquina (3D7);
- Determinar a atividade antimalárica *in vitro* dos fármacos selecionados em *P. falciparum*, utilizando cepas sensíveis à cloroquina (3D7) e resistentes (W2).

4 MÉTODOS

4.1. Estratégias computacionais

4.1.1. Compilação de uma lista de potenciais alvos terapêuticos através de genômica comparativa

Com o intuito de identificar e listar alvos terapêuticos em *P. falciparum* e *P. vivax,* foi utilizada a estratégia de genômica comparativa, através da base de dados *TDR Targets Database* (TDR, http://tdrtargets.org). A TDR é uma plataforma de bioinformática que procura explorar a disponibilidade de diversos conjuntos de dados genômicos e químicos para facilitar a identificação e priorização de fármacos e alvos de fármacos em agentes patogênicos causadores de doenças negligenciadas (MAGARIÑOS et al., 2012).

Na base de dados TDR, foi selecionanada a opção *targets,* na qual permite selecionar genes/alvos de acordo com critérios estabelecidos em filtros subsequentes, assim foram escolhidos os patógenos de interesse (*P. falciparum* e *P. vivax*). O próximo critério (filtro) de seleção foi baseado na distribuição filogenética (Figura 8). Essa abordagem de genômica comparativa permitiu selecionar alvos presentes exclusivamente nos parasitos *P. falciparum* e *P. vivax*, mas ausentes em humanos. A intenção foi compilar uma lista de alvos contra os quais fármacos potencialmente identificados possuíssem seletividade antimalárica, ou seja, uma probabilidade reduzida de apresentarem toxicidade para células de humanos.

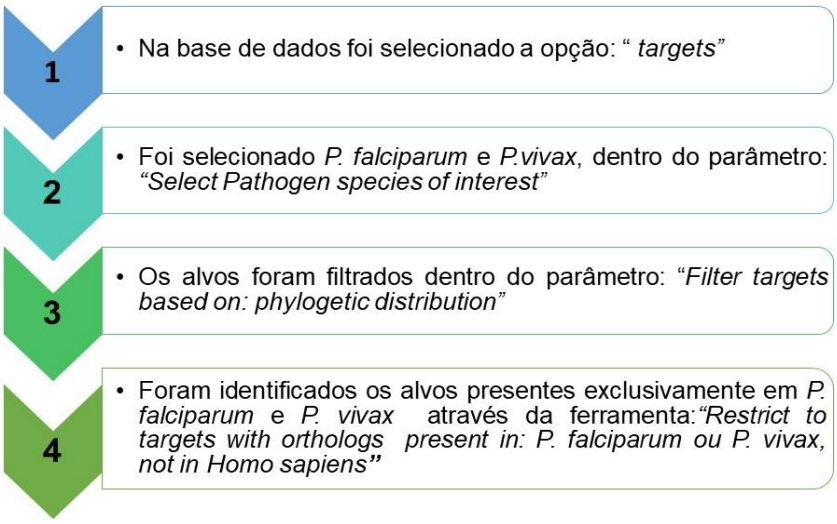


Figura 10 - Fluxograma da metodologia para compilação de uma lista de potenciais alvos terapêuticos através de genômica comparativa, utilizando a plataforma TDR *Targets*.

Cada um dos alvos potenciais correspondentes aos parâmetros selecionados foi computado numa planilha de Excel, em que se registraram individualmente cada um dos seguintes parâmetros: “Gene ID”, “Gene Ontology” (componente celular, função molecular e processo biológico) e “Sequência FASTA do alvo”. Deste modo foi criada uma biblioteca virtual de potenciais alvos terapêuticos (proteínas) presentes em *P. falciparum* e *P. vivax* mas ausentes em humanos, ou com grau de similaridade insuficiente para representar homologia evolutiva.

4.1.2. Identificação de fármacos já aprovados para uso clínico em humanos, com potencial atividade contra *P. falciparum* e *P. vivax*

Cada uma das proteínas da biblioteca gerada em 4.1.1 foi considerada como alvo potencial e, consequentemente, usada para interrogar três bases de dados disponíveis publicamente que fornecem informações detalhadas sobre fármacos e seus alvos: *Therapeutic Targets Database* (TTD) (ZHU et al., 2012), *DrugBank* (WISHART et al., 2006a) e STITCH (KUHN et al., 2014). A estratégia de pesquisa nos bancos de dados DrugBank e TTD foi baseada no princípio de homologia, em que cada consulta (*query*- proteína de *P. falciparum* ou *P. vivax*) resultou em uma comparação por similaridade com todos os alvos de fármacos conhecidos e armazenados em cada uma das bases de dados. Nos casos em que foram identificados alvos de fármacos homólogos, todas as proteínas com um valor estatístico de similaridade correspondente a um *E-value* ≤ 10^{-20} para DrugBank e TTD foram listados como alvos em potencial. Por outro lado, o banco de dados STITCH integra dados dispersos na literatura e de várias bases de dados de vias biológicas, relações fármaco-alvo e afinidades de ligação entre moléculas. A rede resultante pode ser explorada de forma interativa, ou utilizando como base uma pontuação de confiança (*score*), que varia de 0 a 1. Usando a pontuação, um conjunto de interações de confiança estatística variável entre fármacos e alvos é definido (ou seja, proteínas com anotações sobre Gene Ontology). Esse *score* pode ser então utilizado para filtragem das afinidades fármaco-alvo mais apropriadas/desejáveis. Assim, no caso da base de dados STITCH, foram considerados para estudos subsequentes apenas aqueles alvos com uma pontuação ≥ 0,8 (KUHN et al., 2014).

Os alvos previstos pelas três bases de dados descritas foram ainda filtrados, considerando apenas as proteínas que interagiram com fármacos já aprovados para uso em humanos. Desta forma, a classe de nutracêuticos foi excluída, uma vez que é improvável

que estes compostos apresentem atividade antimalárica.

4.1.3. Alinhamento

Estruturas superimpostas permitem comparar características funcionalmente relevantes, resíduos conservados necessários para catálise e resíduos críticos para a ligação do ligante. Assim, os alvos de *P. falciparum* ou *P. vivax* selecionados foram alinhados com os seus alvos homólogos preditos através da ferramenta *pairwise* BLAST (AGARWAL; STATES, 1998a). Deste modo, foram considerados para estudos subsequentes mais aprofundados apenas os casos em que ocorreram ≥ 80% de sobreposição entre as duas sequências para o alvo do fármaco correspondente.

4.1.4. Determinação da conservação estrutural das regiões funcionais para as proteínas de *P. falciparum* e *P. vivax* com os alvos homólogos de fármacos

O servidor ConSurf (ASHKENAZY et al., 2010a) é uma ferramenta de bioinformática que estima a conservação evolutiva de posições de aminoácidos numa proteína com base nas relações filogenéticas entre sequências homólogas. O ConSurf foi utilizado para a caracterização adicional das regiões funcionais nos alvos homólogos de fármacos, em comparação com os alvos de *P. falciparum* e *P. vivax*. Desta forma, o grau de conservação dos aminoácidos dentro dos sítitos ativos foi estimado utilizando 150 proteínas de homólogos com sequências semelhantes obtidas a partir do banco de dados UniProt (APWEILER et al., 2010) e identificadas pelo método de CSI-BLAST (*E-value* de corte ≤ 1^{-10}) (BIEGERT; SÖDING, 2009). As sequências foram agrupadas e as que apresentaram elevada similaridade (> 95%) foram excluídas usando o algoritmo de CD-HIT(LI; GODZIK, 2006), com o objetivo de filtrar as sequências redundantes. Do mesmo modo, as sequências que partilharam menos do que o corte de identidade estabelecido (<35%) também foram ignoradas (APWEILER et al., 2010) (Apweiler et al. 2004). Um alinhamento de múltiplas sequências (MSA) das sequências homólogas foram construídos através do MAFFT-L-INS-i (KATOH; STANDLEY, 2013) e foi utilizado para construir uma árvore filogenética através do algoritmo de Neighbor-Joining (KOYUTÜRK, 2010). Pontuações de conservação posição-específica foram calculadas utilizando um modelo bayesiano (KRUSCHKE, 2010). Em seguida, a conservação das regiões funcionais dos alvos homólogos de fármacos foi visualmente comparada com os correspondentes alvos de *Plasmodium* e os resultados satisfatórios foram classificados como: de alta conservação (≥ 80%) ou conservação moderada (79-60%).

4.1.5. Busca na literatura

Uma pesquisa bibliográfica foi realizada através de buscas no PubMed (HAROON, 1998), PubChem Bioassay(PUBCHEM, 2016) e SciFinder (WARR et al., 2011), com a finalidade de identificar dentre os fármacos selecionados, quais ainda não tinham sido avaliados experimentalmente contra parasitos de malária. Foram considerados como "avaliados" apenas os fármacos que tinham sido submetidos a ensaios fenotípicos *in vitro* ou ensaios *in vivo*, para qualquer espécie *Plasmodium*. Portanto, se um determinado fármaco foi verificado como "não" testado, significa que não foram identificados registros de publicação, ou que o estudo ou ensaio resultante da pesquisa foi insuficientemente informativo para inferir a potencial utilidade do composto como um fármaco antimalárico (NEVES et al., 2015). Para a realização da pesquisa bibliográfica, foram utilizadas as seguintes buscas: 1. ("drug name"[MeSH Terms] OR "drug name"[All Fields]) AND ("Plasmodium"[MeSH Terms] OR "Plasmodium"[All Fields]) and 2. ("drug name"[MeSH Terms] OR "drug name"[All Fields]) AND ("malaria"[MeSH Terms] OR "malaria"[All Fields]),

4.2. Estratégias Experimentais

4.2.1. Cultivo das cepas

As cepas de *P. falciparum* 3D7 (sensível a cloroquina) e *P. falciparum* W2 (resistente) foram cultivadas em meio RPMI completo (RPMI 1640, Sigma EUA), suplementado com 25 mM de bicarbonato de sódio, 25 mM de hepes, 2 mM de L – glutamina, 40 mg de gentamicina, 0,36 mM de hipoxantina, 10 mM de glicose, 10% de plasma humano dos grupos sanguíneos A + e 2% de hematócrito (0+) sob atmosfera de 5% de CO_2.

4.2.2. Sincronização da cultura

Para se obter um predomínio de formas jovens dos parasitos (anel), seguiu-se o protocolo recomendado de sincronização (LAMBROS; VANDERBERG, 1979) utilizando D-sorbitol (Sigma). Inicialmente, foram confeccionados esfregaços sanguíneos das placas de cultura para se determinar o estágio de amadurecimento dos parasitos. A cultura selecionada (contendo 1% de formas jovens) foi centrifugada (400 g/10 min/T.A.), sendo o sobrenadante desprezado e o sedimento ressuspendido em solução de D-sorbitol a 5% por 10 minutos. Passado esse tempo, a suspensão de hemácias com o D-sorbitol foi centrifugada (400 g/10 min/T.A.), o sobrenadante desprezado e as hemácias ressuspendidas com o meio de cultura completo. As hemácias em meio completo foram novamente plaqueadas contendo agora, apenas formas jovens dos parasitos (anéis).

4.2.3. Avaliação da atividade antimalárica dos fármacos por fluorescência de SYBR® Green

Com intuito de avaliar a atividade antimalárica dos fármacos *in vitro,* as culturas sincronizadas foram diluídas com sangue não infectado para que a parasitemia fosse ajustada a 0,5%. Para os ensaios foram utilizadas placas de 96 poços de poliestireno, onde 150 µl da suspensão de hemácias infectadas foram distribuídas por poço. Em uma segunda placa, os candidatos fármacos foram diluídos de maneira seriada, em meio RPMI completo, e 50 µl de cada concentração foi adicionado em triplicata nos poços contendo a suspensão de hemácias infectadas. As placas foram mantidas incubadas por 72 horas sob atmosfera de 5% de CO_2, e ao final desse período, foram seladas com papel alumínio e congeladas a -20°C por até três dias. Após o descongelamento das placas, 100 µl da solução de hemácias infectadas incubadas com as drogas foram adicionados em poços de uma nova placa de 96, de cor preta, aos quais foram adicionados 100 µl de solução de lise (20 mM de Tris, 5 mM de EDTA, 0,008% de saponina e 0,08% de Triton X-100) e 0,4 µl de SYBR® Green (10,000x). As placas foram incubadas no escuro por uma hora e após este tempo, a fluorescência associada ao SYBR® Green intercalado ao DNA foi mensurada em um leitor de microplaca CLARIOstar, Labtech BMG) a 490 nm de excitação e 540 nm de emissão. A eficácia dos fármacos foi determinada comparando a porcentagem de inibição em relação aos poços controle (crescimento dos parasitos na ausência de drogas), e o IC_{50} foi determinado utilizando o software Prism v5.0 (GraphPad Prism, San Diego).

5 ARTIGOS

Artigo 1 – “Computational chemogenomics drug repositioning strategy enables the discovery of Epirubicin as a new repurposed hit for *P. falciparum* and *P. vivax*. ”

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Artigo 2- “In Silico Chemogenomics Drug Repositioning Strategies for Neglected Tropical Diseases”

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Artigo 1

Title: Computational chemogenomics drug repositioning strategy enables the discovery of Epirubicin as a new repurposed hit for *P. falciparum* and *P. vivax*

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Abstract

Widespread resistance against all antimalarial drugs thwarts current efforts to control the disease and urges the discovery of new effective treatments. Drug repositioning to treat parasitic diseases is increasingly becoming an attractive proposition because it has lower overall development costs and shorter development timelines. Here, we performed a comparative chemogenomics approach to select *Plasmodium falciparum* and *P. vivax* proteins as potential drug targets and analyzed these using a computer-assisted drug repositioning workflow to identify approved drugs with antimalarial activity potential. Among seven drugs identified as potent antimalarial candidates, the anthracycline epirubicin was selected for further experimental validation for its antimalarial activity and mode of action. Epirubicin was shown to be efficacious against an *in vivo* murine model and potent against multidrug-resistant *P. falciparum* strains and *P. vivax* field isolates in nanomolar range. Transmission blocking activity was observed for epirubicin *in vitro* and *in vivo*. Finally, using yeast-based haploinsufficiency chemical genomic profiling, we aimed to shed some light into the compound's mechanism of action. Beyond the target predicted by computational approach, a DNA gyrase in the apicoplast, functional assays suggested a GlcNac-1-P-transferase (GPT) enzyme as a second target in the parasite. Docking

calculations predicted the binding mode of epirubicin with DNA gyrase and GPT protein. As epirubicin is originally an antitumoral agent and presents associated toxicity, its potent antiplasmodial activity not only against *P. falciparum* but also *P. vivax* in different stages of parasite lifecycle supports the use of this drug's scaffold for lead optimization in malaria drug discovery.

Keywords: Malaria; drug repositioning; chemogenomics; epirubicin; DNA gyrase

1. Introduction

Malaria is an infectious disease caused by protozoan parasites from the *Plasmodium* genus that are transmitted by the bite of *Anopheles* mosquitoes. Among the five malaria parasite species capable of infecting humans, *P. falciparum* and *P. vivax* are in the spotlight due to their wide geographic distribution and increasing morbidity and mortality rates. With nearly 200 million cases reported in 2017 [1], malaria still imposes a heavy burden upon tropical and subtropical areas of the globe, accounting for more than 400,000 deaths annually. Moreover, although *P. vivax* causes less fatalities, its particular biology confers it a wider geographic distribution with a larger number of people at risk of infection.

In the absence of an effective vaccine against malaria, chemotherapy constitutes the main approach towards disease control. The introduction of Artemisinin Combined Therapies (ACTs) has widely contributed to reducing malaria-associated incidence and mortality [2]. However, malaria parasites have long evolved resistance to antimalarial drugs available, including to the ones used in ACTs [3], menacing the control efforts. One of the underlying phenomena contributing to the sudden decline of antimalarial efficacy is cross-resistance, i.e. resistance to an antimalarial drug may help evolving resistance against compounds with similar molecular mechanisms [4]. Hence, new antimalarial drugs with novel molecular targets and distinct modes of action are urgently needed.

Malaria drug discovery efforts traditionally focus on *P. falciparum* due both to its high mortality rates and the availability of continuous *in vitro* culture. However, severe *P. vivax* cases occur[5] , and could be

linked to chloroquine resistance[6], which has been reported worldwide; therefore highlighting the need for identifying novel anti-*P. vivax* drugs. The lack of a continuous *in vitro* culture system and the rapid onset of circulating gametocytes in the peripheral blood upon infection [7] represent major bottlenecks in fighting vivax malaria, reinforcing the need to look beyond the blood stage for drug development and consequently, disease control and/or elimination.

The drug development process is long, costly and very prone to failure. Thus, it is crucial to advance strategies to improve the success rate in malaria drugs R&D. Drug repositioning is one such strategy [8] that resorts to approved and marketed drugs as starting points for the development of new therapies. As all approved drugs have detailed information on their pharmacokinetic and safety profiles, when a new application for a drug is identified, the molecule can be rapidly advanced into clinical trials. Although few drugs have been repurposed to treat malaria [9,10], most have resulted from either serendipity or phenotypical screening of approved drugs libraries [11,12], with little prior evidence-based data to support their efficacy. Consequently, such approaches can be time-consuming and expensive. In a bid to boost drug repositioning efforts, rational-based approaches based on *in silico* evaluations allow predicting molecule efficacy, narrowing down the panel of drugs for experimental evaluation [13].

We have previously developed an *in silico* target-based chemogenomics workflow [14], which led to identifying approved drugs with predicted and/or confirmed activity against *P. falciparum* apicoplast [15], energy metabolism in *Leishmania* [16] and multiple life-cycle stages of *Schistosoma mansoni* [14,17]. In the present work, we carried out a comparative phylogenomics approach in order to identify predicted targets of *P. falciparum* and *P. vivax*, that are present in the parasite's proteome but expected to be absent in humans. Among a list of approved drugs against these targets, we identify an approved drug from the class of anthracyclines, epirubicin, which is used for chemotherapy to treat breast cancer in combination with other drugs [18]. Driven by the remarkable results obtained from preliminary *in vitro* phenotypic screens, we sought to explore epirubicin's antiplasmodial potential throughout the parasite's life cycle and carried out functional investigations to explore its mechanism of action.

2. Material and Methods

2.1. Repurposing of drugs from public databases

Initially, we compiled a list of *P. falciparum* and *P. vivax* proteins with orthologues predicted to be absent in humans and rodents using TDR Targets Database [19]. Then, a sequence-based similarity search (e-value $\leq 10^{-20}$) was performed between *Plasmodium* proteins and all drug targets available in DrugBank [20] and TTD [21] according to methodology developed by Neves and co-workers [14]. Conserved amino acid residues are believed to perform important structural and/or functional roles in the protein. Herein, all the predicted *Plasmodium* protein targets were analyzed to determine if they share functional amino acid residues with their homologous human drug targets. Initially, predicted *Plasmodium* targets were aligned with their homologous drug targets using pairwise BLAST [22]. Subsequently, functional regions among the approved drug targets and *Plasmodium* targets were compared using the ConSurf server [23,24] using default parameters.

2.2. Machine Learning models

Two datasets with phenotypic bioassay data for 3D7 (susceptible to chloroquine) [25–30] and W2 (resistant to chloroquine) [26,28,31–35] strains of *P. falciparum* were compiled from the PubChem BioAssay database (https://pubchem.ncbi.nlm.nih.gov/bioassay) [36,37]. The compounds with reproducible potency ($IC_{50} \leq 1$ μM) were considered active, whereas the remaining compounds (IC_{50} >1 μM) were considered inactive, following the antiplasmodial cutoff of < 1 μM for sensitive and resistant strains of *Plasmodium* spp, suggested by the GHIT-coordinated committee [38]. All chemical structures and correspondent biological information were carefully curated according to the protocols proposed by Fourches and colleagues [39–41]. Finally, a curated dataset containing 2,853 active and 2,853 inactive compounds was used for modeling activity against the 3D7 sensitive strain, while a dataset containing 3,086 active and 3,086 inactive compounds was used for modeling the W2 resistant strain. The chemical structures of selected compounds were translated into Morgan fingerprints using the open-source cheminformatics toolkit RDKit v.2.4.0. (http://www.rdkit.org). Then, machine learning

(ML) models were built separately for 3D7 and W2 datasets by combining these fingerprints with the GBM method [42]. All ML classifiers were implemented using the R v.3.0.3.[43]. The models were developed using the 5-fold external cross-validation procedure. Models were built using the modeling set while the compounds in momentary external set (fold) were employed for evaluation of predictive performance. Finally, the statistical performance of ML models was estimated using sensitivity (SE), specificity (SP), correct classification rate (CCR), and Cohen's kappa coefficient (κ).

After modeling, the most predictive models developed for 3D7 and W2 were employed for prediction of antiplasmodial activity of prioritized drugs. The reliability of predictions was estimated based on applicability domain, i.e., the Euclidean distances among each drug and the training set compounds used to develop ML models. If an investigated drug exceeded a distance threshold defined using default parameter Z at 0.5, the prediction was considered to be less trustworthy [44].

2.3. *In vitro* assay against *P. falciparum* asexual stages

P. falciparum strains 3D7 (drug-sensitive), Dd2 and W2 (multi drug-resistant) were cultured in RPMI 1640 medium (Sigma) supplemented with 10% human plasma [45]. Synchronous cultures were obtained from treatment with a 5% D-sorbitol (Sigma-Aldrich) solution. Drug inhibition assays were performed by distributing ring-synchronized parasites (0.5% parasitemia and 2% hematocrit) in 96-well microplates in the presence of different concentrations of epirubicin (Sigma, E9406) in triplicate. Chloroquine was used as antimalarial control. After 72 h of incubation, parasitemia was determined by fluorometry using SybrGreen fluorescent dye, according to Hartwig et al. [46]. EC_{50} values were calculated by plotting Log dosing *vs* growth inhibition (expressed as percentage relative to drug-free control). Drugs tested were purchased from Sigma-Aldrich.

2.4. Cytotoxicity assay in mammalian cell lines

Cytotoxicity of epirubicin was assessed using MTT assay to quantify cell viability of two cell lines: fibroblast-like cell line from monkey kidney tissue (COS-7) and human hepatoma (HepG2) via

mitochondrial activity as previously described [47]. Briefly, cells were cultivated at 5% CO_2 and 37ºC using Dulbecco's Modified Eagle Medium supplemented with 10% heat-inactivated fetal bovine serum. Cells were seeded at a density of 10^5 cells per well and when the confluence was about 70%, cells were incubated for 72 h with a serial dilution of epirubicin. Absorbance reading at OD_{570} nm was done in a plate spectrophotometer and the percent viability of cells was expressed as a percentage relative to untreated control.

2.5. *In vivo* activity on murine *Plasmodium* asexual stages

In vivo activity of epirubicin in a murine model of infection was evaluated in groups of 5 to 6 female C57BL/6JUnib mice (6-7 weeks old) obtained from the Center for Bioterism - UNICAMP (nº4574-1/2017 of the Ethics and Animal Utilization Committee at UNICAMP). The animals were housed in polypropylene cages in a pathogen free animal facility at a temperature of 20 ± 3°C and relative humidity of 60 ± 5%, in a cycle of 12h light/12h darkness. At 3h after intraperitoneal inoculation of 10^6 GFP-expressing *P. yoelii* 17XNL-infected erythrocytes, animals were randomly divided into experimental groups and treatment was initiated (day 0). Epirubicin was administered intraperitoneally at 2, 4 and 6 mg/kg. Untreated control groups received only the drug vehicle (10% DMSO). Treatment was administered daily until day 3 post-infection, following a modified Peter's 4-day suppressive test [48]. Parasitemia was measured by diluting 1 μL of whole blood obtained from mice tail in 100 μL of PBS followed by flow cytometry analysis using 488 nm laser with 530/40 emission filters (FACSCalibur) [49]. Antimalarial activity was expressed as a percentage of inhibition relative to the control group.

2.6. Sample collection of *P. vivax* field isolates

Due to the lack of a continuous *in vitro* culture, antimalarial assays in *P. vivax* require fresh blood samples to be performed. For this purpose, vivax malaria patients were recruited at Fundação de Medicina Tropical Doutor Heitor Viera Dourado (FMT-HVD), Manaus, Amazonas, Brazil. Ten mL of

blood samples were collected from exclusively non-severe vivax malaria adult patients diagnosed by microscopy. Only patients with parasitemia higher than 1,000 parasites/µL and without any antimalarial treatment history in the last 60 days were included, after agreement to participate in the study by signing the Informed Consent Form. The project was approved by the Research Ethics Committee (nº 2.584969 of April 6, 2018).

2.7. *Ex vivo* susceptibility against *P. vivax* clinical isolates

Ex vivo susceptibility of *P. vivax* isolates was evaluated by schizont maturation assay. For this assay, only blood samples containing more than 40% of ring parasites were used. Blood samples were centrifuged at 400 g, the pellet was washed twice in McCoy 5A medium, passed through a cellulose column for leukocyte removal and washed twice in medium. Parasitized erythrocyte suspension at 2% hematocrit was added to 96-well plates containing epirubicin (0.0095 to 5 µM) or chloroquine (0.00195 to 1 µM) in duplicate for each of the ten serial dilutions, as well as untreated control wells. Parasites were cultured in hypoxic atmosphere (5% CO_2, 5% O_2, 90% N_2) at 37°C for 35 to 50 h. The number of schizonts per 200 asexual parasites was determined for each drug concentration and control [50] and EC_{50} values were calculated using the online software ICestimator (http://www.antimalarial-icestimator.net/).

2.8. *Anopheles aquasalis* infection by standard membrane-feeding assay (SMFA)

Anopheles aquasalis mosquitoes used for this purpose were bred in the insectary of the Laboratory of Entomology at FMT-HVD, Manaus, Amazonas, Brazil, according to an established methodology [51]. For *An. aquasalis* infection, blood from *P. vivax*-infected patients was centrifuged at 400 g for 5 minutes and resuspended in non-immune AB serum to a 40% hematocrit in the presence of epirubicin (5 or 10 µM) or the vehicle (DMSO 0.1%) as a control, and given immediately to feed 100 adult females of *An. aquasalis* (3 to 5 days) per group. After 2 h of feeding, the fully engorged mosquitoes were transferred to a new cage with available 10% sugar solution in a room with controlled

temperature (24 to 26°C) and humidity (70 to 80%) until dissection. At seven days post-infection, the midguts from each experimental group were dissected in PBS, placed in a cover glass, stained with 0.1% Merbromin® and analyzed by microscopy to determine the infection status. Infection rate was expressed as the percentage of mosquitoes with at least one oocyst and the infection intensity was determined as the arithmetic mean of the oocysts counted per dissected midgut.

2.9. *In vitro* inhibition of *P. berghei* ookinete formation by conversion assay

The conversion assay evaluates a compound's potential of inhibiting ookinete formation *in vitro* [52]. Briefly, Balb/c mice were infected intraperitoneally with a mutant *P. berghei* line expressing luciferase (nLuc) under ookinete-specific promoter. After 3-4 days, blood with circulating gametocytes was collected from mice and infected blood was incubated in 96-well plates with culture medium (RPMI 1640, 25 mM HEPES, 50 mg/L hypoxanthine and 1% PSN-penicillin/streptomycin/neomycin, pH 8,3) containing a serial dilution of epirubicin. After 24h of incubation at 21ºC, substrate for nLuc (Nano-Glo® Luciferase Assay System - PROMEGA) was added and plates were read in a luminometer. EC_{50} values were calculated by plotting Log dosing *vs* growth inhibition (expressed as percentage relative to drug-free control).

2.10. Chemical genomic profiling

Chemical genomic profiling was performed with a *S. cerevisiae* heterozygous yeast library for the ~ 6000 genes (Invitrogen cat. No. 95401.H4Pool), in which single deletions are identified by molecular barcodes flanked by universal sequences [53]. IC_{20} for epirubicin was determined as described previously [54]. Briefly, *S. cerevisiae* BY4743 wildtype strain (initial OD_{600} of 0.1) was incubated in 384-well plates in the presence of serial dilution of epirubicin in YDP liquid medium (1% w/v yeast extract; 2% w/v peptone; 2% w/v glucose) at 30 °C with double orbital agitation of 200 rpm for 24h. For competition assays, the library pool was inoculated (initial OD_{600} of 0.1) in YPD liquid medium in 48 well plates at 30 °C with 200 rpm agitation in the presence of epirubicin IC_{20} concentration for 12h (approximately 5

generations). The culture was diluted 1:20 for further 12 hours of growth (~ 10 generations). Every 5 generations, cells were harvested and pellets were collected for gDNA extraction with the Wizard® Genomic DNA Purification Kit (Promega). The upstream molecular barcodes were PCR amplified with U1 and U2 primers containing Illumina pre-adapters for multiplexing sequencing using the Illumina HiSeq 2500 platform from the University of Sao Paulo Genome Sequencing Center. Based on the barcode sequences, sequencing analysis was carried out by creating a "virtual genome" to which reads were aligned and quantified per barcode. Heterozygous strains were ranked based on their epirubicin sensitivity after 10 generations. Strains most depleted within treated populations compared to untreated control (top hits, p-value < 0.001) were followed by gene ontology (GO) analysis.

2.11. Homology Modelling studies

As there were no 3D crystallographic structures available of *Plasmodium* DNA gyrase or GlcNac-1-P-transferase (GPT) proteins at the time we conducted this research, they were constructed by homology modeling using the SwissModel server [55,56]. The primary sequences of each protein were obtained at the Uniprot server [57] and their FASTA sequences were submitted to SwissModel to obtain the homology models. The models that presented higher sequence identity and coverage with the templates were selected to the refinement step. After modeling, we refined the structures using the GalaxyRefine server [58] and MolProbity server [59–61] to add hydrogen atoms and to analyze the quality statistics of the modeled proteins.

2.12. Molecular docking calculations

Docking calculations of epirubicin were performed using the Glide software [62] in extra precision (XP) mode. For DNA gyrase, we performed docking calculations of the protein with DNA strands (called gyrase-DNA) and without DNA strands (gyrase). We prepared the protein structures through Protein Preparation Wizard tool [63,64], following the standard protocol [65]. The ligand structure was retrieved from PubChem [66] and prepared through LigPrep tool [67], according to tutorial. The protein grid coordinates were built based on ligands co-crystallized in the structures of homologue proteins:

levofloxacin (from PDB ID 5EIX) for the DNA gyrase grid and tunicamycin (from PDB ID 6BW5) for the GPT grid. The Visual Molecular Dynamics program (VMD) [68] was used for the visual inspection of 3D docking poses and to render the 3D molecular images.

2.13. Statistical analysis

Statistical analysis was performed using Graphpad Prism 6v software. To calculate the EC_{50} in asexual and sexual blood stages of parasites and cell lines, a non-linear regression curve was made with the data of the concentrations expressed in logarithmic scale. The inhibition potential of chloroquine and epirubicin in *P. vivax* isolates was compared by the Mann-Whitney test. In the membrane feeding assay, infection rates between groups were compared using one-way ANOVA and Dunnet's post-test. For *in vivo* data, comparison of group parasitemia was compared using ANOVA and Dunnet's post-test. Data was considered statistically significant when $p < 0.05$.

3. RESULTS

3.1. Computational chemogenomics screening

We developed a computational chemogenomics framework (Figure 1A) and used it in the repurposing of new drugs to treat malaria. Initially, we carried out a genomewide phylogenomics analysis in the TDR Targets Database to prioritize *P. falciparum* and *P. vivax* proteins without orthologues in humans and rodents. Consequently, we obtained a list of 2,830 *P. falciparum* proteins and 2,914 *P. vivax* proteins. We further filtered these proteins by removing from the list all peptides that were annotated as hypothetical, resulting in a panel of 1,095 and 636 proteins for *P. falciparum* and *P. vivax*, respectively.

Subsequently, putative antiplasmodial drugs were screened using the underlying assumption that homologous proteins have enhanced probability of sharing the same ligands [14,69]. A sequence-based similarity search was performed between *Plasmodium* proteins and all drug targets available in

DrugBank [20] and the Therapeutic Targets Database (TTD) [16,21], which provide detailed information about drugs and their targets. This strategy resulted in a list of 115 potential *Plasmodium* targets (i.e., 57 and 58 confirmed targets for *P. falciparum* and *P. vivax*, respectively) that could interact with 36 approved drugs (18 for *P. falciparum* and 18 *P. vivax*).

In order to increase the confidence in drug-target predictions, we investigated the conservation of functional regions of prioritized *Plasmodium* proteins and their drug targets homologues. This strategy allowed us to prioritize *Plasmodium* proteins with conserved binding sites, thereby increasing the probability that each of the drugs bears biological activity. Consequently, we selected 28 approved drugs (15 for *P. falciparum* and 13 for *P. vivax*) that could potentially interact with 12 potential druggable *Plasmodium* targets for further analysis. A bibliographic research allowed to identify which among the selected drugs had not been tested or proposed as antimalarial before, leading to a final list of 12 approved drugs. Detailed information about the list of predicted drugs and their associated *Plasmodium* targets are provided in Supplementary Table S1.

3.2. Machine Learning (ML) models

ML models were built to prioritize which drugs would be experimentaly evaluated, by distinguishing between active vs. inactive drugs against blood stages of *P. falciparum* 3D7 (susceptible to chloroquine) and W2 (multi-drug resistant) strains. Initially, two datasets with phenotypic bioassay data for 3D7 (susceptible to chloroquine) [25–30] and W2 (resistant to chloroquine) [26,28,31–35] strains of *P. falciparum* were compiled from the PubChem BioAssay database [36,37]. Subsequently, all chemical structures and correspondent biological information were carefully curated according to the protocols proposed by Fourches and colleagues [40,41,70]. Further, ML models were trained by combining Avalon fingerprints (generated from chemical structures) and Gradient Boosting Machine (GBM) algorithm [42]. According to the statistical results of a 5-fold external cross-validation procedure (see Materials and Methods), the model developed for prediction of antiplasmodial activity against the 3D7 strain showed correct classification rate (CCR) = 74%, sensitivity (SE) = 73%, specificity (SP) = 74%, and coverage

= 70%. The model developed for the W2 strain showed CCR = 71%, SE = 71%, SP = 72%, and coverage = 70%. The twelve drugs prioritized from computational chemogenomics analysis were submitted to ML models for prediction of the antiplasmodial activity. The predictions were considered reliable when they were within the chemical space (i.e., applicability domain) of the training set compounds used to build ML models. At the end of this process, seven drugs (epirubicin, phenoxybenzamine, besifloxacin, nedocromil, isoprenaline, mesalazine, and captopril) with potential antiplasmodial activity were selected for biological evaluation.

3.3. *In vitro* screening of selected drugs

We carried out a primary *in vitro* screen to determine each drug's ability to inhibit growth of the chloroquine-sensitive *P. falciparum* 3D7 strain at 5 μM (Figure 1B). The drugs used from the list of potential candidates from *in silico* approach were tested in parallel with chloroquine and pyrimethamine as controls. These results showed that epirubicin (Figure 1C), which we predicted to inhibit *P. falciparum* DNA gyrase subunit A, was capable of inhibiting *P. falciparum* 3D7 growth at nearly 100%, and, therefore, we decided to investigate its antimalarial activity agaisnt other parasite species and determine its possible mode-of-action.

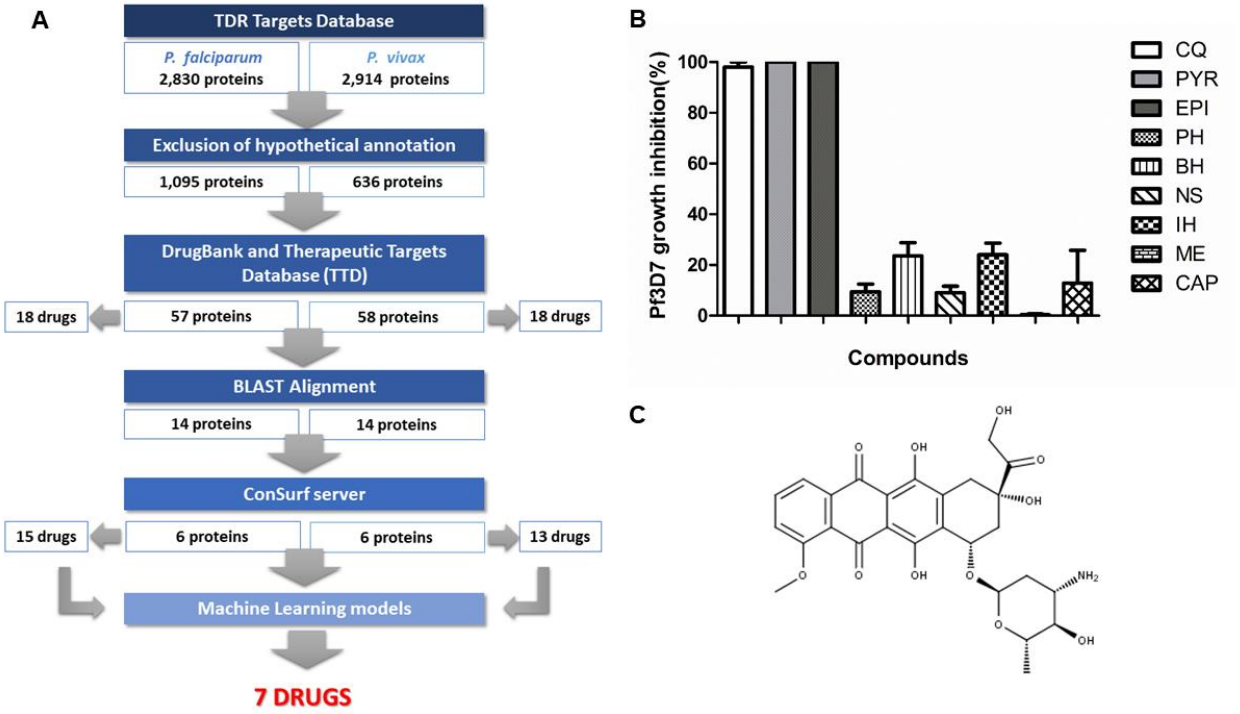

Figure 1. Chemogenomics strategy for drug repurposing. (A) Flowchart summarizing the *in silico* repositioning chemogenomics strategy and corresponding results. (B) Phenotypic screening for inhibition of *P. falciparum* 3D7 growth *in vitro* using compounds candidates for repositioning at 5 µM. (C) Chemical structure of epirubicin. CQ: chloroquine; PYR: pyrimethamine; EPI: epirubicin; PH: phenoxybenzamine hydrochloride; BH: besifloxacin hydrochloride; NS: nedocromil sodium; IH: isoproterenol hydrochloride; ME: mesalazine; CAP: captopril.

3.4. Epirubicin is effective *in vitro* against *Plasmodium* asexual stages in the nanomolar range

Considering that epirubicin was shown to be potent at 5 µM, we sought to determine its EC_{50}. The drug was tested in serially diluted concentrations against drug-sensitive *P. falciparum* 3D7 and multi drug-resistant W2 and Dd2 strains, with chloroquine as control. As shown in Table 1, we found that epirubicin has a modest effect against the drug-sensitive strain *P. falciparum* 3D7 when compared to chloroquine (around 10-fold less active), but the EC_{50} just over 100 nM (111 ± 22.4 nM), places it in the medium nanomolar range (Figure 2A). Importantly, the drug showed similar or even greater

potency against drug-resistant strains Dd2 and W2 (EC_{50} = 99 ± 25.1 and 69 ± 5.1 nM, respectively) (Figure 2C).

Seeking to further understand epirubicin's antimalarial activity, we followed epirubicin-treated and untreated *P. falciparum* cultures for 60 h in order to evaluate morphological changes in the parasites (Figure 2D). Epirubicin showed activity towards the early trophozoite stage, since morphological deformities could be observed only 24 h after incubation. The treated parasites were not viable since no ring- or trophozoite stage parasites were observed when the culture was continued to the 48 h or 60 h, respectively.

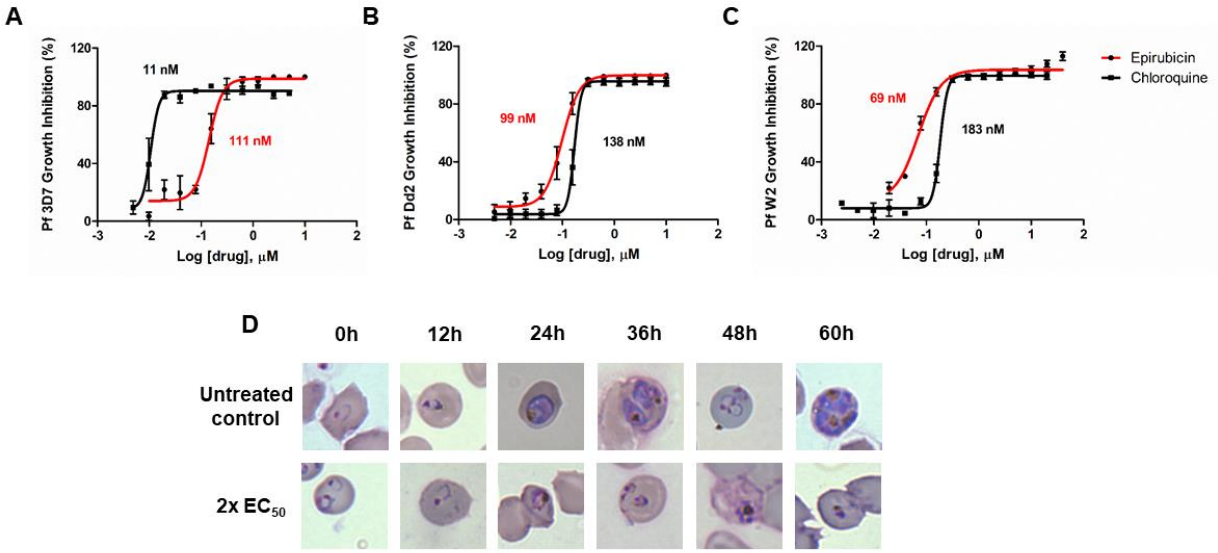

Figure 2. *In vitro* antimalarial activity of epirubicin against *P. falciparum* strains. Inhibition curves for epirubicin *in vitro* against (A) chloroquine-sensitive (3D7) and (B, C) multidrug-resistant (Dd2 and W2) *P. falciparum* strains. Data derive from three independent experiments. (D) Microscopy of Giemsa-stained thin smears of highly synchronized parasite cultures starting at the ring stage (1% parasitemia). First line shows parasites treated with DMSO (control) while second line show culture continuously treated with epirubicin at concentrations 2-fold the EC_{50} value and DMSO (control). Images were collected using an Olympus microscope equipped with 100× objective and a camera.

We also evaluated epirubicin's cytotoxicity *in vitro* using two mammalian cell lines. Non-tumoral mammalian cell line (COS7) was shown to be moderately sensitive to epirubicin, presenting half maximal cytotoxic concentration (CC_{50}) around 5 μM (5.48 ± 0.72 μM). Predictably, we found epirubicin to be significantly toxic to tumoral HepG2 cells (0.08 ± 0.03 μM) with 70-fold more sensitivity when compared to COS7 cells. Taking into account epirubicin's CC_{50} in COS7 cells, we calculated its selectivity index around 49 (Table 1).

3.5. Epirubicin mitigates mice parasitemia *in vivo* but does not resolve the infection

After demonstrating epirubicin's activity against *P. falciparum in vitro*, we investigated whether this activity is sustained in an *in vivo* model of infection. *P. yoelii 17XNL*-infected mice were treated with epirubicin for four days (days 0-3 p.i.). As shown in Table 2, treating animals with 2 mg/kg showed no potency on inhibiting parasitemia. The intermediate dose of 4 mg/kg showed 86,8% of inhibition on day 3 p.i. but on day 5 p.i., this inhibition dropped below 50%. However, 6 mg/kg of epirubicin significantly reduced parasitemia compared to untreated control by 96% and 86% on day 3 p.i and at day 5 p.i (48 h after the end of treatment). On day 7 p.i., all three doses tested reached less than 14% of parasite growth inhibition.

3.6. Epirubicin inhibits parasite maturation of *P. vivax* clinical isolates

Ex vivo inhibition potential of epirubicin was evaluated in *P. vivax* isolates by the schizont maturation assay using 9 clinical isolates from *P. vivax*-infected patients. Blood samples used for this purpose showed an initial parasitemia of 5,407.33 ± 2,372.21 parasites/μL with 65.67% (ranging from 40 to 88%) of the observed parasites in the ring stage. *Ex vivo* activity of epirubicin was tested in all 9 isolates, whereas chloroquine activity was evaluated in parallel in 4 of those (Figure 3A). Epirubicin and chloroquine EC_{50} was 20.42 nM (range, 8.76 to 60.05 nM) and 17.13 nM (range, 2.16 to 48.76 nM), respectively. No significant difference was observed between the compounds' inhibition potencies ($p = 0.4140$; Mann-Whitney).

3.7. Epirubicin blocks transmission by inhibiting *P. berghei* ookinete development *in vitro*

A conversion assay using sexual stages of *P. berghei* with ability to fertilize *in vitro* was conducted to examine epirubicin's transmission blocking potential. Epirubicin showed activity in a dose-response manner, inhibiting approximately 80% of ookinete development at 1 μM with an EC_{50} of 0.39 μM (Figure 3B). The result suggests that epirubicin interferes in fertilization, sexual recombination and consequent ookinete development in *P. berghei*.

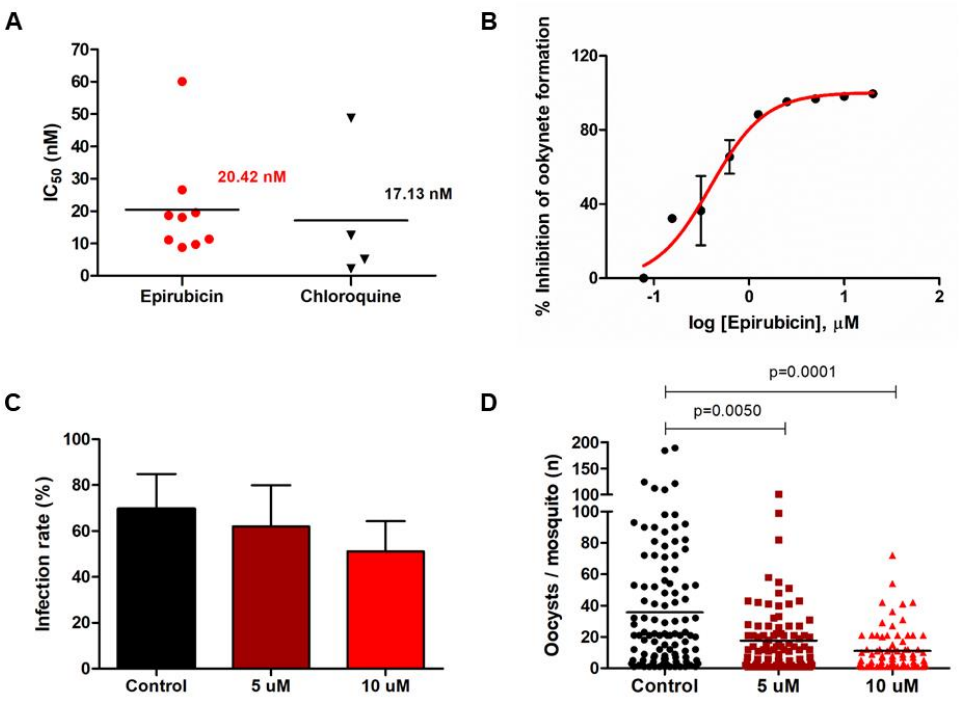

Figure 3. Blood stage and transmission blocking activity of Epirubicin on *P. vivax*. (A) *Ex vivo* drug susceptibility of epirubicin in clinical isolates of *P. vivax* from Manaus, Brazil. (B) Infection rates and (C) oocyst intensity in membrane feeding assay (MFA) using *An. aquasalis* mosquitoes exposed to *P. vivax*-infected blood. For membrane feeding assays, seven biological replicates were performed. (D) *In vitro* inhibition of *P. berghei* gamete fertilization and ookinete conversion by epirubicin on conversion assay.

3.8. Epirubicin blocks *in vivo* parasite transmission in *P. vivax*-infected *Anopheles* mosquitoes

Once epirubicin's transmission blocking activity was verified *in vitro*, we aimed to evaluate its activity *in vivo* for inhibition of mosquito infection using membrane feeding assays. For this purpose, seven clinical samples of *P. vivax* were used. The prevalence of oocysts in the mosquitoes was not significantly reduced in the presence of epirubicin ($p = 0.1636$; ANOVA-Dunnet) (Figure 3C). Among infected mosquitoes, epirubicin led to a dose-dependent decrease in the absolute number of oocysts found in mosquitoes midguts. While the control group presented a mean of 23.83 oocysts per mosquito, the groups treated with 5 and 10 μM of epirubicin presented, respectively, 11.22 and 5.54 – i.e., a decrease of 53% and 76% in oocyst intensity (Figure 3D).

3.9. Chemical genomic profiling identifies protein glycosylation affected by epirubicin

First steps in investigating the mechanism of action of epirubicin were taken with the chemical genomic profiling assay, based on the premise that diploid yeast hemizygous for a gene is hypersensitive to an inhibitor drug directed to the product of that gene. Epirubicin inhibition assays performed in wild type yeast revealed an EC_{20} of 2.9 μM (data not shown), which was the drug concentration used to perform the chemical genomic profiling competition assays. Pools of approximately 6,000 heterozygous yeast strains were grown for 10 generations under treatment. Analysis of the haploinsufficiency profile of heterozygous yeast strains allowed the identification of epirubicin-sensitive strains (Table S2), four of which presented statistical significance in their depletion compared to the control (Figure 4B). A mutant strain that has reduced dose of an essential gene involved in protein N-glycosylation, *Δalg7*, which codes for the dolichol phosphate *N*-acetylglucosamine-1-phosphotransferase (GPT) [71] was found among the hits, which indicates that protein glycosylation can be potentially affected by epirubicin treatment.

Considering that *P. falciparum* has a GPT orthologue present in its genome [72], we suggest that epirubicin could interfer with isoprenoid metabolism in the malaria parasite, either via apicoplast disruption or GPT enzyme inhibition (Figure 4C).

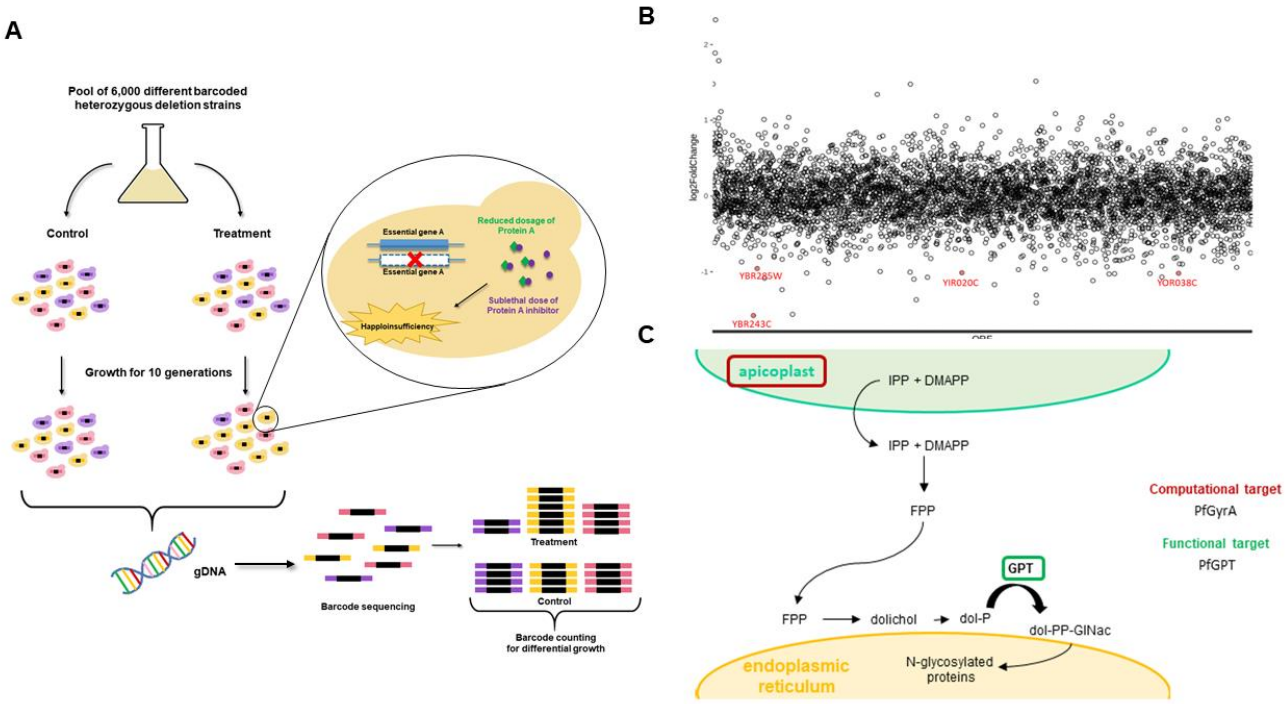

Figure 4. Investigation of epirubicin's mechanism of action. (A) Chemical genomic profilin assay. A pool of ~6,000 barcoded heterygous strains deleted for each gene of S. cerevisiae's genome is cultivated in the presence and absence of a sublethal dose of Epirubicin. Yeast culture is diluted 1:20 at 5 generations and growth is allowed until 10 generations. Genomic DNA is collected for sequencing. Barcode counting allows identification of strains depleted under treatment, suggesting hypersensitivity to Epirubicin. (B) Drug-induced haploinsufficiency profiling of mutant haploid heterozygous S. cerevisiae upon treatment with epirubicin IC_{20}. Log fold change is plotted on y-axis as a function of depleted yeast strains alphabetically ordered by their respective ORF. The lower the log fold change value, the more sensitive the strain is. Red dots highlighted in the chart represent the ORFs that meet the conditions p-value < 0.001 and log2 fold change < 0. (C) Scheme summarizing the proposed mechanism for epirubicin's mode of action in the malaria parasite. Abbreviations: gDNA, genomic DNA; IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate; FPP, farnesyl

pyrophosphate; dol-P, dolichol phosphate; GPT, dolichol phosphate N-acetylglucosamine-1-phosphotransferase; dol-PP-GlcNAc, dolichol pyrophosphate N-acetylglucosamine

3.10. Molecular modeling and docking studies of epirubicin with its two predicted targets

Considering the predicted and functional molecular targets identified for epirubicin, we sought to explore its potential to bind to each of the proposed targets. We built the *P. falciparum* DNA gyrase (*Pf*GyrA) and dolichol phosphate N-acetylglucosamine-1-phosphotransferase (*Pf*GPT) structures through a homology modeling approach. The DNA gyrase model was based on PDB ID 2XKJ from *Acinetobacter baumannii* [73], which had 30.04% sequence identity with *Pf*GyrA. This model was analyzed at MolProbity server and showed a MolProbity score of 1.83, reflecting in a single score the crystallographic resolution at which those values would be expected [61] (Table S3). Also, the Ramachandran plot of the DNA gyrase model showed that 96.96% of the residues lie in the most favorable regions (Table S3 and Fig. S1). The *Pf*GPT model was based on PDB ID 6BW5 from *Homo sapiens* [74] which had 45.27% sequence identity with *P. falciparum* GPT. The *Pf*GPT model showed 97.84% of their residues in the most favorable regions of the Ramachandran plot and MolProbity score of 1.93 (Table S4 and Fig. S2).

We then performed molecular docking calculations in order to investigate the binding mode and shed some light into the affinity of epirubicin against the two targets. Epirubicin docked in *Pf*GyrA (Figure 5A) and *Pf*GPT (Figure 5B) presented good binding affinities, with docking scores of -7.96 and -7.92 kcal·mol^{-1}, respectively. Moreover, docking calculations of epirubicin with *Pf*GyrA structure with DNA strand and without DNA strand (data not shown), reinforces the fact that epirubicin could act as a DNA intercalating agent, since it has better docking scores in gyrase-DNA (-7.96 kcal·mol^{-1}) compared to gyrase without DNA strand (-6.38 kcal·mol^{-1}).

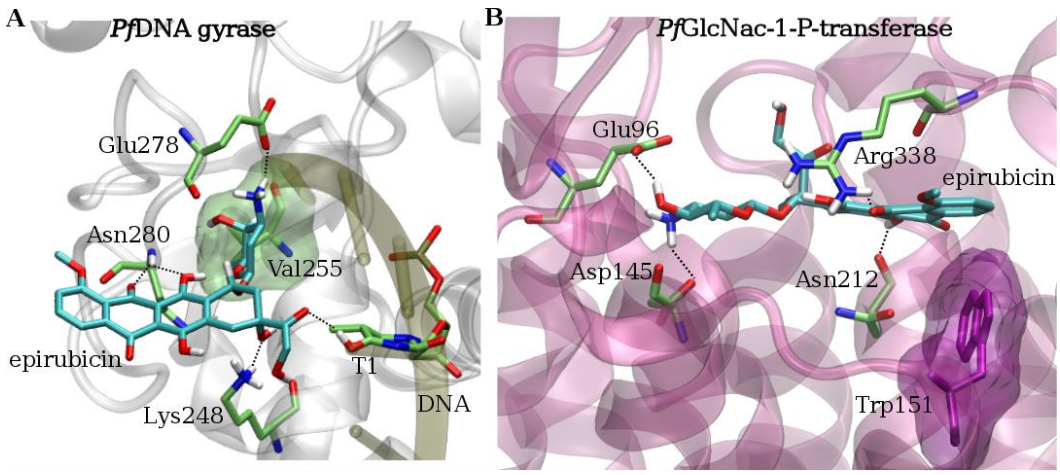

Figure 5. 3D intermolecular interactions between epirubicin and its two potential molecular targets. (A) Epirubicin (carbon atoms in cyan) docked within DNA binding site of gyrase-DNA (docking score -7.96 kcal·mol-1), highlighting the main hydrogen bonds (dotted black lines) within epirubicin and the amino acid residues Lys248, Glu278 and Asn280, as well as the interaction with DNA base pair thymine (T1). Epirubicin also made hydrophobic interaction with Val255 residue (transparent green surface). (B) Docked pose of epirubicin with GPT protein (docking score -7.92 kcal·mol-1), highlighting the main hydrogen bonds (dotted black lines) with the amino acid residues Glu96, Asp145, Asn212 and Arg338. Epirubicin was also able to make *T-stacking* interaction with Trp151 residue (transparent purple surface). Amino acids are colored as: carbon atoms in green, oxygen in red, hydrogen in white, nitrogen in blue.

4. DISCUSSION

We have used a combined comparative chemogenomics and bioinformatics approach to identify new drugs against *P. falciparum* and *P. vivax*, the two most important of the human malaria parasites. In doing so, we employed the strategy of drug repurposing, which allows identifying new applications for drugs that have already been approved for use in humans, hence providing the quickest possible transition from bench to bedside. Thus, we generated a final list of seven drugs that were expected to be active, but have never been tested as antimalarials previously, especially against *P. vivax*. These drugs are anticipated to inhibit different parasitic targets and span several different original indications,

including antivirals, anticancer, antibiotics, etc. (Table S1). Then, we proceeded to test the *in vitro* ability to inhibit growth of the drug-sensitive strain of *P. falciparum* 3D7 of this set of drugs. Out of these, epirubicin was shown to be potent, with an IC_{50} at the nanomolar level. Mogire and colleagues also identified epirubicin *in silico* as a potential candidate for drug repurposing against *P. falciparum*, but they did not tested the drug in experimental assays[75]. However, it is worth noting that we investigated *P. vivax* proteins as potential drugs targets in our repurposing pipeline and also experimentally validated epirubicin against malarial parasites.

Epirubicin is an anthracycline which is the 4'-epi-isomer of doxorubicin. The compound exerts its effects by interfering with DNA synthesis and function, having reported activity over both mammalian and bacterial cells[76]. Epirubicin inhibits nucleic acid and protein biosynthesis by intercalation between base pairs, forming an epirubicin-DNA complex that inhibits topoisomerase II activity. Its interference with both DNA replication and transcription is also given by inhibition of DNA helicase activity [76].

Subsequent fine *in vitro* tests to calculate the drug's EC_{50} confirmed that it was potent against both sensitive and multidrug resistant *P. falciparum* strains. This is an important observation in light of the fact that the great majority of the parasite population in natural settings is nowadays highly resistant to most drugs worldwide, including chloroquine. For instance, in Brazil, an area of chloroquine resistance, the mean parasite EC_{50} among *P. falciparum* Amazonian isolates has been shown to be nearly 300 nM[77], which is nearly four-fold higher than the activity we reported here for epirubicin in resistant *P. falciparum* strain W2. This is highly relevant because it is possible that epirubicin is effective against both chloroquine-sensitive and chloroquine-resistant parasites. Interestingly, analysis of epirubicin's pharmacokinetic profile allowed us to observe that its EC_{50} for *P. falciparum* strain 3D7 can be achieved in plasma concentration at about 10 hours after intravenous infusion in human patients (approximately 64 μg/L)[78], which represents an enormous advantage in clinical settings.

Lack of cross resistance of epirubicin with clinically used antimalarial compounds is likely due to its predicted target, the DNA gyrase subunit A, involved in the process of DNA synthesis in the malarial apicoplast, whilst the targets of chloroquine are related to inhibition of haem polymerization in the

parasite's food vacuole[79]. Consequently, because the targets and their respective localization within the cell are very distinct, the phenomenon of cross-resistance between the two drugs is highly unlikely. This phenotype of epirubicin's activity in chloroquine-resistant strain was corroborated *in vivo*. The compound's efficacy was shown in nonlethal *P. yoelli* 17X, a chloroquine-resistant strain[80], by inhibition of mice parasitemia until day 7 post-infection.

We also found that epirubicin is capable of inhibiting maturation of blood-stage parasites in samples from *P. vivax* clinical isolates from Manaus, Brazil. Evaluating the drug's potency against *P. vivax* comes in an extremely relevant context of selection of parasite resistance to chloroquine [81], the golden standard antimalarial drug for malaria vivax treatment in endemic regions for over 60 years [82]. Nonetheless, given that in the last years *P. vivax* resistance has been linked to increasing numbers of reports of infection severity [83], validating the activity of a repurposed drug against *P. vivax* is timely.

In addition to activity against asexual blood stage parasites, epirubicin also was shown to be able in blocking parasite transmission, probably inhibiting gamete fertilization and consequent ookinete formation. When assessing *in vivo* transmission blocking activity through membrane feeding assay, epirubicin was able to reduce the number of *P. vivax* oocysts found in the midguts of infected mosquitoes. Many features of *P. vivax* biology have direct impact on its pathogenesis[7,84] and turn their infected patients in efficient infection reservoirs, i.e. its ability to generate asymptomatic infections, to produce early circulating gametocytes soon after establishment of infection and to develop dormant liver stages make this species very skilled when it comes to transmission ability. For this reason, the data on transmission blocking holds promise on epirubicin's potential to reduce host-to-vector transmission and curb new infections by blocking the transmission capacity of the vectors.

The first steps in elucidating the possible mechanism of action of epirubicin on the parasite were taken using the haploinsufficiency chemical genomic profiling assay, an approach based on the premise that yeast containing a gene expressed in heterozygosity will become hypersensitive to an inhibitor compound directed to the product of that gene. By exploiting a collection of mutant yeast strains, this methodology allows to explore biochemical pathways and molecular targets affected by a compound at

the genomic level. Since the therapeutic target predicted by computational screening for epirubicin in *Plasmodium* is the subunit A of the apicoplast DNA gyrase (derived from prokaryotic plasmid)[85], we expected to find the depletion of the strain mutant for *TOP2* gene, which encodes DNA topoisomerase II, an yeast enzyme structural and phylogenetically related to apicoplast gyrase [86]. Unfortunately, strains heterozygous for *TOP2* were underrepresented in the initial strain pool, not allowing statistically evaluating the sensitivity of this strain under epirubicin treatment and functionally validating *P. falciparum* DNA gyrase (*Pf*GyrA) as an epirubicin target.

The molecular target identified, Alg7, is essential in yeast and conserved among eukaryotes [87]. Alg7 is the enzyme Dol-P-dependent GlcNAc-1-P transferase (GPT) [71], which participates in the initial stages of N-glycoprotein synthesis [88]. *P. falciparum* has an Alg7 ortholog [89,90] whose product is responsible for the synthesis of glycans bound to dolichol [87], a class of isoprenoids that participate in the biosynthesis of N-linked oligosaccharides [91]. As it has been shown that the essential role of the *Plasmodium* apicoplast in the blood stage is the production of isoprenoid precursors [92], disruption of apicoplast function by epirubicin leads to inhibition of dolichol biosynthesis and thereby protein *N*-glycosylation. Additionally, resistance to epirubicin in cancer cells can occur mediated by WNT glycoproteins, which are glycosylated by GPT [93]. In this way, chemical genomic profiling results support our *in silico* prediction of epirubicin action upon the parasite apicoplast. Moreover, our docking calculations showed that epirubicin binds with similar affinity to both *Pf*GyrA and GPT, which indicates that its action upon protein glycosylation might not only be a consequence of apicoplast disruption, but also a direct target.

We have applied a computer-based drug repositioning workflow to propose new drug candidates for treatment malaria, focusing in the neglected parasite *P. vivax*. In doing so, we have demonstrated that epirubicin has a potent effect blocking parasite development of sexual and asexual life stages in both *P. falciparum* and *P. vivax* despite having been shown to present significant toxicity when used as an anticancer agent. Epirubicin is predicted to target the parasite's GyrA, however, functional validation identified a second putative target for epirubicin, GPT enzyme involved in protein *N*-glycosylation.

Both molecular targets identified, either through computational or functional assays, are involved, in a broader perspective, in the metabolism of isoprenoids, a biological process essential to eukaryotic cells. Hence, epirubicin acts upon vital parasite functions, corroborating its antimalarial activity throughout the *Plasmodium* life cycle. Collectively, our findings encourage further investigation on other possible inhibitors of isoprenoid metabolism in the parasite as well as using epirubicin's chemical structure as a scaffold for the development of novel and potent antimalarial agents.

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interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflicts of interest

None to declare.

Author Contributions

* L.T.F. and J.R. have equally contributed to this work. #C.H.A., P.V.L.C. and F.T.M.C. have equally directed this work. All authors approved the final version of the manuscript.

References

1 WHO. *World Malaria Report 2018*. 2018http://www.who.int/malaria/world_malaria_report_2010/en/index.html.

2 World Health Organization. *World Malaria Report 2016*. 2016.

3 World Health Organization. World Malaria Report 2015. 2015.

4 Paloque L, Witkowski B, Lelièvre J, Ouji M, Ben Haddou T, Ariey F *et al.* Endoperoxide-based compounds: cross-resistance with artemisinins and selection of a Plasmodium falciparum lineage with a K13 non-synonymous polymorphism. *J Antimicrob Chemother* 2018; **73**: 395–403.

5 Alexandre MA, Ferreira CO, Siqueira AM, Magalhães BL, Mourão MPG, Lacerda M V. *et al.* Severe Plasmodium vivax malaria, Brazilian Amazon. *Emerg Infect Dis* 2010; **16**: 1611–1614.

6 Marques MM, Costa MRF, Santana Filho FS, Vieira JLF, Nascimento MTS, Brasil LW *et al.* Plasmodium vivax chloroquine resistance and anemia in the western brazilian amazon. *Antimicrob Agents Chemother* 2014; **58**: 342–347.

7 Boyd M, Kitchen S. On the infectiousness of patients infected with Plasmodium vivax and Plasmodium falciparum. *Am J Trop Med Hyg* 1937; **17**: 253–62.

8 Nicola Nosengo. Can you teach old drugs new tricks? *Nature* 2016; **534**: 314–316.

9 Tan KR, Magill AJ, Parise ME, Arguin PM. Doxycycline for malaria chemoprophylaxis and

treatment: Report from the CDC expert meeting on malaria chemoprophylaxis. *Am J Trop Med Hyg* 2011; **84**: 517–531.

10 Manyando C, Njunju EM, D'Alessandro U, Van geertruyden JP. Safety and Efficacy of Co-Trimoxazole for Treatment and Prevention of Plasmodium falciparum Malaria: A Systematic Review. *PLoS One* 2013; **8**. doi:10.1371/journal.pone.0056916.

11 Andrews KT, Fisher G, Skinner-Adams TS. Drug repurposing and human parasitic protozoan diseases. *Int J Parasitol Drugs Drug Resist* 2014; **4**: 95–111.

12 Kundu CN, Das S, Nayak A, Satapathy SR, Das D, Siddharth S. Anti-malarials are anti-cancers and vice versa - One arrow two sparrows. *Acta Trop* 2015; **149**: 113–127.

13 Liu Z, Fang H, Reagan K, Xu X, Mendrick DL, Slikker W *et al.* In silico drug repositioning: what we need to know. *Drug Discov Today* 2013; **18**: 110–5.

14 Neves BJ, Braga RC, Bezerra JCB, Cravo PVL, Andrade CH. In Silico Repositioning-Chemogenomics Strategy Identifies New Drugs with Potential Activity against Multiple Life Stages of Schistosoma mansoni. *PLoS Negl Trop Dis* 2015; **9**. doi:10.1371/journal.pntd.0003435.

15 Bispo NA, Culleton R, Silva LA, Cravo P. A Systematic In Silico Search for Target Similarity Identifies Several Approved Drugs with Potential Activity against the Plasmodium falciparum Apicoplast. *PLoS One* 2013; **8**. doi:10.1371/journal.pone.0059288.

16 Silva LA, Vinaud MC, Castro AM, Cravo PVL, Bezerra JCB. In silico search of energy metabolism inhibitors for alternative leishmaniasis treatments. *Biomed Res Int* 2015; **2015**. doi:10.1155/2015/965725.

17 Lima MNN, Melo-Filho CC, Cassiano GC, Neves BJ, Alves VM, Braga RC *et al.* QSAR-Driven Design and Discovery of Novel Compounds With Antiplasmodial and Transmission Blocking Activities. *Front Pharmacol* 2018; **9**: 146.

18 Earl H, Iddawela M. Epirubicin as adjuvant therapy in breast cancer. *Expert Rev Anticancer Ther* 2004; **4**: 189–195.

19 Magariños MP, Carmona SJ, Crowther GJ, Ralph SA, Roos DS, Shanmugam D *et al.* TDR targets: A chemogenomics resource for neglected diseases. *Nucleic Acids Res* 2012; **40**: 1118–1127.

20 Law V, Knox C, Djoumbou Y, Jewison T, Guo AC, Liu Y *et al.* DrugBank 4.0: Shedding new light on drug metabolism. *Nucleic Acids Res* 2014; **42**: 1091–1097.

21 Li YH, Yu CY, Li XX, Zhang P, Tang J, Yang Q *et al.* Therapeutic target database update 2018: enriched resource for facilitating bench-to-clinic research of targeted therapeutics. *Nucleic Acids Res* 2018; **46**: D1121–D1127.

22 Agarwal P, States DJ. Comparative accuracy of methods for protein sequence similarity search. *Bioinformatics* 1998; **14**: 40–7.

23 Glaser F, Pupko T, Paz I, Bell RE, Bechor-Shental D, Martz E *et al.* ConSurf: identification of functional regions in proteins by surface-mapping of phylogenetic information. *Bioinformatics* 2003; **19**: 163–4.

24 Ashkenazy H, Erez E, Martz E, Pupko T, Ben-Tal N. ConSurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. *Nucleic Acids Res* 2010; **38**: W529-33.

25 National Center for Biotechnology Information. PubChem BioAssay Database; AID=449703, Source=Genomics Institute of the Novartis Research Foundation, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=449703 (accessed Jan. 22, 2017). .

26 Plouffe D, Brinker A, McNamara C, Henson K, Kato N, Kuhen K *et al.* In silico activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput screen. *Proc Natl Acad Sci* 2008; **105**: 9059–9064.

27 National Center for Biotechnology Information. PubChem BioAssay Database; AID=524790, Source=National Institute of Allergy and Infectious Diseases, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=524790 (accessed Jan. 22, 2017). .

28 Yuan J, Johnson RL, Huang R, Wichterman J, Jiang H, Hayton K *et al.* Genetic mapping of targets mediating differential chemical phenotypes in Plasmodium falciparum. *Nat Chem Biol* 2009; **5**: 765–771.

29 National Center for Biotechnology Information. PubChem BioAssay Database; AID=1828, Source=NIH Chemical Genomics Center, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=1828 (accessed Jan. 22, 2017). .

30 National Center for Biotechnology Information. PubChem BioAssay Database; AID=660866, Source=Medicines for Malaria Venture, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=660866 (accessed Jan. 22, 2017). .

31 National Center for Biotechnology Information. PubChem BioAssay Database; AID=449704, Source= Genomics Institute of the Novartis Research Foundation, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=449704 (accessed Jan. 22, 2017). .

32 National Center for Biotechnology Information. PubChem BioAssay Database; AID= 524796, Source=National Institute of Allergy and Infectious Diseases, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=524796 (accessed Jan. 22, 2017). .

33 National Center for Biotechnology Information. PubChem BioAssay Database; AID= 1883, Source=NIH Chemical Genomics Center, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=1883 (accessed Jan. 22, 2017). .

34 Cross RM, Maignan JR, Mutka TS, Luong L, Sargent J, Kyle DE *et al.* Optimization of 1,2,3,4-Tetrahydroacridin-9(10 H)-ones as Antimalarials Utilizing Structure–Activity and Structure–Property Relationships. *J Med Chem* 2011; **54**: 4399–4426.

35 National Center for Biotechnology Information. PubChem BioAssay Database; AID= 606570, Source=NIH University of South Florida, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=606570 (accessed Jan. 22, 2017). .

36 Wang Y, Xiao J, Suzek TO, Zhang J, Wang J, Zhou Z *et al.* PubChem's BioAssay Database. *Nucleic Acids Res* 2012; **40**: D400–D412.

37 Wang Y, Bolton E, Dracheva S, Karapetyan K, Shoemaker BA, Suzek TO *et al.* An overview of the PubChem BioAssay resource. *Nucleic Acids Res* 2010; **38**: D255-66.

38 Katsuno K, Burrows JN, Duncan K, Huijsduijnen RH Van, Kaneko T, Kita K *et al.* Hit and lead criteria in drug discovery for infectious diseases of the developing world. *Nat Publ Gr* 2015; : 1–8.

39 Fourches D, Muratov E, Tropsha A. Trust, but verify: on the importance of chemical structure curation in cheminformatics and QSAR modeling research. *J Chem Inf Model* 2010; **50**: 1189–204.

40 Fourches D, Muratov E, Tropsha A. Curation of chemogenomics data. *Nat Chem Biol* 2015; **11**: 535–535.

41 Fourches D, Muratov E, Tropsha A. Trust, but Verify II: A Practical Guide to Chemogenomics Data Curation. *J Chem Inf Model* 2016; **56**: 1243–1252.

42 Friedman JH. Greedy Function Approximation: A Gradient Boosting Machine. *Ann Stat* 2001; **29**: 1189–1232.

43 R Development Core Team. *R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing*. R Foundation for Statistical Computing: Vienna, Austria, 2008.

44 Tropsha A, Golbraikh A. Predictive QSAR modeling workflow, model applicability domains, and virtual screening. *Curr Pharm Des* 2007; **13**: 3494–504.

45 Trager W, Jensen JB. Human malaria parasites in continuous culture. *Science* 1976; **193**: 673–5.

46 Russell B, Malleret B, Suwanarusk R, Anthony C, Kanlaya S, Lau YL *et al.* Field-based flow cytometry for ex vivo characterization of plasmodium vivax and P. falciparum antimalarial sensitivity. *Antimicrob Agents Chemother* 2013; **57**: 5170–5174.

47 Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the MTT assay. *Cold Spring Harb Protoc* 2018; : 469–471.

48 Peters W. The chemotherapy of rodent malaria, XXII. The value of drug-resistant strains of P. berghei in screening for blood schizontocidal activity. *Ann Trop Med Parasitol* 1975; **69**: 155–171.

49 Ono T, Tadakuma T, Rodriguez A. Plasmodium yoelii yoelii 17XNL constitutively expressing GFP throughout the life cycle. *Exp Parasitol* 2007; **115**: 310–313.

50 Russell BM, Udomsangpetch R, Rieckmann KH, Kotecka BM, Coleman RE, Sattabongkot J. Simple In Vitro Assay for Determining the Sensitivity of Plasmodium vivax Isolates from Fresh Human Blood to Antimalarials in Areas where P . vivax Is Endemic. *Antimicrob Agents Chemother* 2003; **47**: 170–173.

51 Pimenta PFP, Orfano AS, Bahia AC, Duarte APM, Ríos-velásquez CM, Melo FF *et al.* An overview of malaria transmission from the perspective of Amazon Anopheles vectors. *Mem Inst Oswaldo Cruz* 2015; **110**: 23–47.

52 Calit J, Drobescu I, Gaitán XA, Borges MH, Ramos MS, Eastman RT *et al.* Screening the Pathogen Box against Plasmodium sexual stages using a new nanoluciferase based transgenic line of P. berghei identifies transmission-blocking compounds. *Antimicrob Agents Chemother* 2018. doi:10.1128/AAC.01053-18.

53 Giaever G, Shoemaker DD, Jones TW, Liang H, Winzeler EA, Astromoff A *et al.* Genomic profiling of drug sensitivities via induced haploinsufficiency. *Nat Genet* 1999; **21**: 278–283.

54 Hoepfner D, Helliwell SB, Sadlish H, Schuierer S, Filipuzzi I, Brachat S *et al.* High-resolution chemical dissection of a model eukaryote reveals targets, pathways and gene functions. *Microbiol Res* 2014; **169**: 107–120.

55 Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, Schmidt T *et al.* SWISS-MODEL: Modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids Res* 2014; **42**: 1–7.

56 Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R *et al.* SWISS-MODEL: Homology modelling of protein structures and complexes. *Nucleic Acids Res* 2018; **46**: W296–W303.

57 Bateman A. UniProt: A worldwide hub of protein knowledge. *Nucleic Acids Res* 2019; **47**: D506–D515.

58 Ko J, Park H, Heo L, Seok C. GalaxyWEB server for protein structure prediction and refinement. *Nucleic Acids Res* 2012; **40**: 294–297.

59 Hintze BJ, Lewis SM, Richardson JS, Richardson DC. Molprobity's ultimate rotamer-library distributions for model validation. *Proteins Struct Funct Bioinforma* 2016; **84**: 1177–1189.

60 Davis IW, Leaver-Fay A, Chen VB, Block JN, Kapral GJ, Wang X *et al.* MolProbity: All-atom contacts and structure validation for proteins and nucleic acids. *Nucleic Acids Res* 2007; **35**: 375–383.

61 Chen VB, Arendall WB, Headd JJ, Keedy DA, Immormino RM, Kapral GJ *et al.* MolProbity: All-atom structure validation for macromolecular crystallography. *Acta Crystallogr Sect D Biol Crystallogr* 2010; **66**: 12–21.

62 Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA *et al.* Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *J Med Chem* 2006; **49**: 6177–6196.

63 Madhavi Sastry G, Adzhigirey M, Day T, Annabhimoju R, Sherman W. Protein and ligand preparation: Parameters, protocols, and influence on virtual screening enrichments. *J Comput Aided Mol Des* 2013; **27**: 221–234.

64 Schrödinger Release 2015-2: Protein Preparation Wizard, Schrödinger, LLC, New York, NY, 2015. .

65 Banks JL, Beard HS, Cao Y, Cho AE, Damm W, Farid R *et al.* Integrated Modeling Program, Applied Chemical Theory (IMPACT). *J Comput Chem* 2005; **26**: 1752–1780.

66 Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A *et al.* PubChem substance and compound databases. *Nucleic Acids Res* 2016; **44**: D1202–D1213.

67 Schrödinger Release 2015-2: LigPrep, Schrödinger, LLC, New York, NY, 2015. .

68 Humphrey W, Dalke A, Schulten K. VDM: Visual Molecular Dynamics. *J Mol Graph* 1996; **7855**: 33–38.

69 Andrade CH, Neves BJ, Melo-Filho CC, Rodrigues J, Silva DC, Braga RC *et al.* In Silico

Chemogenomics Drug Repositioning Strategies for Neglected Tropical Diseases. *Curr Med Chem* 2018; **26**: 1–22.

70 Fourches D, Muratov E, Tropsha A. Trust, But Verify: On the Importance of Chemical Structure Curation in Cheminformatics and QSAR Modeling Research. *J Chem Inf Model* 2010; **50**: 1189–1204.

71 Zhu X, Lehrman MA. Cloning, sequence and expression of cDNA encoding Hamster UDP-GlcNAc:dolichol phosphate N-acetylglucosamine-1-phosphate transferase. *J Biol Chem* 1990; **265**: 14250–14255.

72 Imlay L, Odom AR. Isoprenoid Metabolism in Apicomplexan Parasites. *Curr Clin Microbiol Reports* 2014; **1**: 37–50.

73 Veselkov DA, Laponogov I, Pan X-S, Selvarajah J, Skamrova GB, Branstrom A *et al.* Structure of a quinolone-stabilized cleavage complex of topoisomerase IV from Klebsiella pneumoniae and comparison with a related Streptococcus pneumoniae complex . *Acta Crystallogr Sect D Struct Biol* 2016; **72**: 488–496.

74 Yoo J, Mashalidis EH, Kuk ACY, Yamamoto K, Kaeser B, Ichikawa S *et al.* GlcNAc-1-P-transferase-tunicamycin complex structure reveals basis for inhibition of N-glycosylation. *Nat Struct Mol Biol* 2018; **25**: 217–224.

75 R.M. M, H.M. A, R.W. M, D.W. J, A.C. C, B. A *et al.* Target-similarity search using Plasmodium falciparum proteome identifies approved drugs with anti-malarial activity and their possible targets. *PLoS One* 2017; **12**: 1–24.

76 Knox C, Law V, Jewison T, Liu P, Ly S, Frolkis A *et al.* DrugBank 3.0: A comprehensive resource for 'Omics' research on drugs. *Nucleic Acids Res* 2011; **39**. doi:10.1093/nar/gkq1126.

77 e Silva LFR, de Magalhães PM, Costa MRF, Alecrim M das GC, Chaves FCM, Hidalgo A de F *et al.* In vitro susceptibility of Plasmodium falciparum Welch field isolates to infusions prepared from Artemisia annua L. cultivated in the Brazilian Amazon. *Mem Inst Oswaldo Cruz* 2012; **107**: 859–866.

78 Robert J. Clinical Pharmacokinetics of Epirubicin. *Clin Pharmacokinet* 1994; **26**: 428–438.

79 Cravo P, Culleton R, Afonso A, Ferreira I, do Rosario V. Mechanisms of Drug Resistance in Malaria: Current and New Challenges. *Antiinfect Agents Med Chem* 2006; **5**: 63–73.

80 Warhurst DC, Killick-Kendrick R. Spontaneous Resistance to Chloroquine in a Strain of Rodent Malaria (Plasmodium berghei yoelii). *Nature* 1967; : 44–47.

81 Filho FS de S, Arcanjo AR de L, Chehuan YM, Costa MR, Martinez-Espinosa FE, Vieira JL *et al.* Chloroquine- Resistant Plasmodium vivax, Brazilian Amazon. *Emerg Infect Dis* 2007; **13**: 1125–1126.

82 Coatney GR. Pitfalls in a Discovery: The Chronicle of Chloroquine. *Am J Med Hyigiene* 1963; **12**: 121–128.

83 Price RN, Douglas NM, Anstey NM. New developments in Plasmodium vivax malaria: Severe disease and the rise of chloroquine resistance. *Curr Opin Infect Dis* 2009; **22**: 430–435.

84 Mueller I, Galinski MR, Baird JK, Carlton JM, Kochar DK, Alonso PL *et al.* Key gaps in the knowledge of Plasmodium vivax, a neglected human malaria parasite. *Lancet Infect Dis* 2009; **9**: 555–566.

85 Arisue N, Hashimoto T. Phylogeny and evolution of apicoplasts and apicomplexan parasites. *Parasitol Int* 2015; **64**: 254–259.

86 Lynn R, Giaever G, Swanberg SL, Wang JC. Tandem regions of yeast DNA Topoisomerase II share homology with different subunits of bacterial gyrase. *Science (80-)* 1986; **233**: 647–649.

87 Samuelson J, Banerjee S, Magnelli P, Cui J, Kelleher DJ, Gilmore R *et al.* The diversity of dolichol-linked precursors to Asn-linked glycans likely results from secondary loss of sets of glycosyltransferases. *Proc Natl Acad Sci* 2005; **102**: 1548–1553.

88 Barnes G, Hansen WJ, Holcomb CL, Rine J. Asparagine-linked glycosylation in Saccharomyces cerevisiae: genetic analysis of an early step. *Mol Cell Biol* 1984; **4**: 2381–2388.

89 Robert V, Bourgouin C, Depoix D, Thouvenot C, Lombard M-N, Grellier P. Malaria and obesity: obese mice are resistant to cerebral malaria. *Malar J* 2008; **7**: 81.

90 Lombard J. The multiple evolutionary origins of the eukaryotic N-glycosylation pathway. *Biol Direct* 2016; **11**. doi:10.1186/s13062-016-0137-2.

91 Naik RS, Venkatesan M, Gowda DC. Plasmodium falciparum: The Lethal Effects of Tunicamycin and Mevastatin on the Parasite Are Not Mediated by the Inhibition of N-Linked Oligosaccharide Biosynthesis. *Exp Parasitol* 2001; **98**: 110–114.

92 Yeh E, DeRisi JL. Chemical rescue of malaria parasites lacking an apicoplast defines organelle function in blood-stage plasmodium falciparum. *PLoS Biol* 2011; **9**. doi:10.1371/journal.pbio.1001138.

93 Yuan G, Regel I, Lian F, Friedrich T, Hitkova I, Hofheinz RD *et al.* WNT6 is a novel target gene of caveolin-1 promoting chemoresistance to epirubicin in human gastric cancer cells.

Oncogene 2013; **32**: 375–387.

Table 1. Antiplasmodial and cytotoxic in vitro activity of epirubicin

	EC_{50} (nM)			COS-7		HepG2	
Drug	**Pf 3D7**	**Pf Dd2**	**Pf W2**	**CC_{50} (nM)**	**SI**	**CC_{50} (nM)**	**SI**
Epirubicin	110.7 ± 22.4	99.5 ± 25.1	68.9 ± 5.1	5480 ± 720	49.4	80 ± 30	0.7
Chloroquine	11.3 ± 3.6	137.8 ± 34.1	182.8 ± 11.5	ND		ND	

EC_{50}/CC_{50}: half of the maximal inhibitory concentration

SI: Selectivity index calculated from CC_{50} / EC_{50} (Pf 3D7)

ND: not determined

Data derived from three independent experiments

Table 2. *In vivo* parasitemia inhibition of *P. yoelii* 17XNL-infected mice treated with epirubicin for 4 days.

Dose (mg/kg)	**Mean percentage parasitemia (inhibition[a]) ± SD**		
	Day 3	**Day 5**	**Day 7**
Nontreated	1.3 ± 0.8 (0)	1.7 ± 0.4 (0)	3.6 ± 1.5 (0)
2	0.6 ± 0.4 (56.9 ± 14.5)	2.2 ± 1.5 (6.8 ± 9.8)	6.9 ± 5.3 (7.4 ± 12.9)
4	0.2 ± 0.2 (86.8 ± 9.7)	1.4 ± 1.2 (46.1 ± 26.0)	7.5 ± 6.1 (13.0 ± 22.6)
6	0.1 ± 0.1 (96.4 ± 3.2)	0.4 ± 0.3 (86.1 ± 11.3)	1.8 ± 1.3 (13.9 ± 67.1)

[a] Values are expressed as mean percentage parasitemia inhibition relative to non-treated control.

Data derive from three independent experiments.

Artigo 2

In Silico Chemogenomics Drug Repositioning Strategies for Neglected Tropical Diseases

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Abstract: Only ~1% of all drug candidates against Neglected Tropical Diseases (NTDs) have reached clinical trials in the last decades, underscoring the need for new, safe and effective treatments. In such context, drug repositioning, which allows finding novel indications for approved drugs whose pharmacokinetic and safety profiles are already known, is emerging as a promising strategy for tackling NTDs. Chemogenomics is a direct descendent of the typical drug discovery process that involves the systematic screening of chemical compounds against drug targets in high-throughput screening (HTS) efforts, for the identification of lead compounds. However, different to the one-drug-one-target paradigm, chemogenomics attempts to identify all potential ligands for all possible targets and diseases. In this review, we summarize current methodological development efforts in drug repositioning that use state-of-the-art computational ligand- and structure-based chemogenomics approaches. Furthermore, we highlighted the recent

progress in computational drug repositioning for some NTDs, based on curation and modeling of genomic, biological, and chemical data. Additionally, we also present in-house and other successful examples and suggest possible solutions to existing pitfalls.

Keywords: Neglected Tropical Diseases, drug repositioning, chemogenomics, similarity search, machine learning, pharmacophores, protein alignment, docking.

1. INTRODUCTION

Neglected tropical diseases (NTDs) are a heterogeneous group of infections unified not by evolution, geography or pathophysiology but by their endemicity, especially in low-income populations in developing regions of Africa, Asia, and the Americas, thus perpetuating the poverty of the poorest (BUSCAGLIA; KISSINGER; AGÜERO, 2015). NTDs represent a broad range of parasitic diseases, which include either protozoa: Chagas disease, human African trypanosomiasis and Leishmaniasis; viruses: Dengue/Chikungunya and rabies; bacteria: Buruli ulcer, leprosy, trachoma and endemic treponematoses; or helminths: schistosomiasis, dracunculiasis, echinococcosis, foodborne trematodiases, lymphatic filariasis, onchocerciasis, soil-transmitted helminthiasis and taeniasis/cysticercosis ("Centers for Disease Control and Prevention. Neglected tropical diseases. http://www.cdc.gov/globalhealth/ntd/diseases/index.html (Accessed July 18, 2016).", [s.d.], "World Health Organization. Neglected tropical diseases. http://www.who.int/neglected_diseases/diseases/en (Accessed July 20, 2016).", [s.d.]); mycetoma, a group of infections caused by certain fungi or bacteria (ZIJLSTRA et al., 2016); and *vivax* malaria (BASSAT et al., 2016; WAHEED et al., 2015).

Recent estimates of Word Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) showed that 149 developing countries distributed in Africa, Asia, and the Americas are affected by at least one NTD. Furthermore, it is believed that these diseases kill an estimated 534,000 people every year and affects more than one billion people worldwide ("Centers for Disease Control and Prevention. Neglected tropical diseases. http://www.cdc.gov/globalhealth/ntd/diseases/index.html (Accessed July 18, 2016).", [s.d.], "World Health Organization. Neglected tropical diseases. http://www.who.int/neglected_diseases/diseases/en (Accessed July 20, 2016).", [s.d.]).

Consequently, the impact of NTDs on these impoverished and marginalized populations is associated with malnutrition, chronic disability and premature death, stigma and social exclusion, and loss of educational and employment opportunities (HOFSTRAAT; VAN BRAKEL, 2016; LITT; BAKER; MOLYNEUX, 2012; WEISS, 2008). These characteristics highlight the enormous economic and healthcare challenges for the countries affected.

Notwithstanding, these economic and healthcare challenges, current chemotherapy for NTDs is restricted to few options of drugs and present several limitations such as high toxicity, lack of efficacy, difficulties in administration, high costs, and increasing drug resistance, all of which constitute serious hurdles regarding access to patients (NWAKA; HUDSON, 2006; RENSLO; MCKERROW, 2006; WYATT et al., 2011). Moreover, one of the biggest problems to fully understanding NTD pathogens is related to the occurrence of multiple parasite strains and/or species, complex life-cycles, and a lack of appropriate *in vitro*/*in vivo* culture systems (TIBAYRENC; AYALA, 2012).

All these facts fully justify the establishment of public and private initiatives to invest in research and development (R&D) for new drugs against NTDs. Nevertheless, the pharmaceutical industry still underinvests in this area, mostly because of the costly and risky nature of the R&D process and the corresponding little financial return for these diseases (DIMASI et al., 2010; DIMASI; HANSEN; GRABOWSKI, 2003; GOUPIL; MCKERROW, 2014; KOLA; LANDIS, 2004). As a consequence, only ~1% of all drug candidates that reached the clinical trials in last decades were for NTDs (PEDRIQUE et al., 2013).

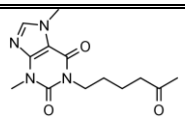
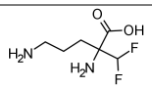
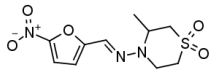
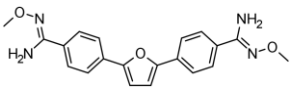
2. Drug repositioning for NTDs

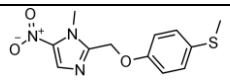
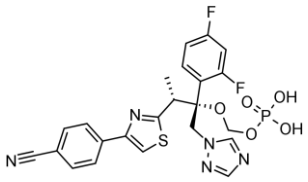
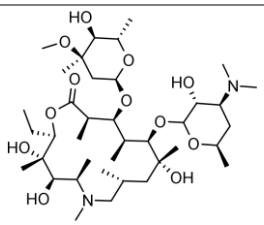
Drug repositioning has emerged as a promising strategy for NTDs. Also referred to as drug repurposing, drug reprofiling or drug rescue, this strategy consists of finding novel indications for approved drugs, or drugs that have failed for one indication, either pre- or post-approval, or have been abandoned in development (ASHBURN; THOR, 2004; AUBÉ, 2012; CHONG; SULLIVAN, 2007). The appeal of this strategy is readily recognized because of the investigated drug is not again subjected to the early stages of R&D process, and owing to its safety and pharmacokinetic profiles are already known. Shorter routes to the clinic are possible because hit identification, hit-to-lead and lead optimization, pre-clinical studies, bulk manufacturing, formulation development and even phase I clinical trials have, in many cases, already been completed and can, therefore, be bypassed (ASHBURN; THOR, 2004; CHONG; SULLIVAN, 2007; NOVAC, 2013; SBARAGLINI et al., 2016). Hence, compared with *de novo*

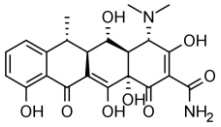
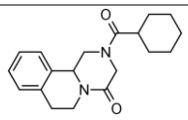
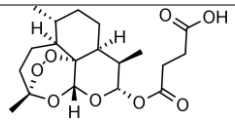
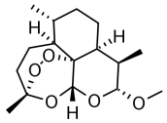
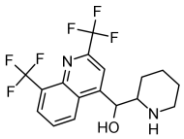
drug discovery, drug repositioning mostly bypasses the initial six to nine years typically required for the conception of new chemical entities, entering to preclinical testing or clinical trials directly. Accordingly, drug repositioning can reduce R&D risks, costs and timelines to the market, and consequently provide strategic advantages for drug discovery for NTDs (JIN; WONG, 2014; NOVAC, 2013).

In the same manner with the prominent example of sildenafil (“Sildenafil: an oral drug for impotence.”, 1998), many known repositioned drugs for treating NTDs or its complications were discovered from serendipitous findings or resulted from a better understanding of the drugs mechanism of action (BOLGÁR et al., 2013). Table 1 summarizes successfully repositioned drugs and repositioning candidates in clinical trials for the treatment of NTDs. Among the highlighted examples, some repositioned drugs resulted in innovative therapies. For instance, miltefosine was initially developed as an anticancer drug and is now used as the first oral treatment for visceral leishmaniasis (BERMAN, 2015; SINDERMANN et al., 2004). Praziquantel, the first well tolerable, effective, and broad-spectrum agent for treating schistosomiasis, was initially developed for treating helminthiasis on veterinary practice (MCMAHON, 1981; MCMAHON; KOLSTRUP, 1979; SANTOS et al., 1979). These examples show that repositioned drugs play a central role in the treatment of these diseases.

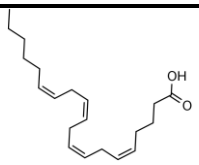
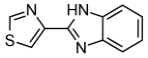
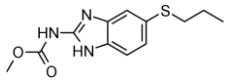
Table 1. Successfully repositioned drugs and repositioning candidates in clinical trials for treatment of NTDs or its complications.
(Table 1) continued

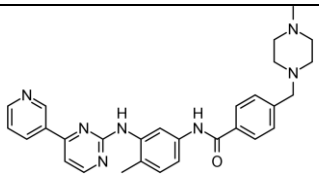
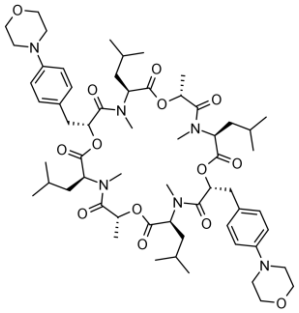
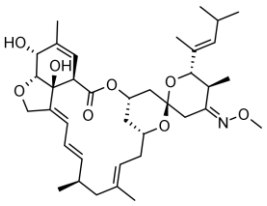
Drug	Structure	Original indication	New indication	Status	References
Pentoxifylline		Muscle pain and peripheral artery disease	Tegumentary leishmaniasis	Phase II/III	("ClinicalTrials.gov. Antimony Plus Pentoxifylline in Cutaneous Leishmaniasis. https://clinicaltrials.gov/ct2/show/NCT01381055 (Accessed July 21, 2016)", [s.d.]; DE SÁ OLIVEIRA et al., 2000; LESSA et al., 2001)
Allopurinol		Chronic gout	Tegumentary leishmaniasis	Phase II	(BARZILAI; FRIEDMAN; TRAU, 1995; MARTINEZ; GONZALEZ; VERNAZA, 1997; MARTINEZ; MARR, 1992)
Eflornithine		Facial hirsutism	African trypanosomiasis	Approved	(OPIGO; WOODROW, 2009; PRIOTTO et al., 2009; SCHMID et al., 2012)
Nifurtimox		Chagas	African trypanosomiasis	Approved	(ALIROL et al., 2013; OPIGO; WOODROW, 2009; PRIOTTO et al., 2009)
Pafuramidine		Pneumocystis pneumonia	African trypanosomiasis	Phase III	("ClinicalTrials.gov. Trial of DB289 for the Treatment of Stage I African Trypanosomiasis. https://clinicaltrials.gov/ct2/show/NCT00803933 (Accessed July 21, 2016)", [s.d.]; MDACHI et al., 2009; POHLIG et al., 2016)

Fexinidazole		Fungal infections	Chagas disease and African trypanosomiasis	Phase II/III	(“ClinicalTrials.gov. Pivotal Study of Fexinidazole for Human African Trypanosomiasis in Stage 2. https://clinicaltrials.gov/ct2/show/NCT01685827 (Accessed July 21, 2016)”, [s.d.], “ClinicalTrials.gov. Study to Evaluate Fexinidazole Dosing Regimens for the Treatment of Adult Patients With Chagas Disease. https://clinicaltrials.gov/ct2/show/NCT02498782 (Accessed July 23, 2016).”, [s.d.], “ClinicalTrials.gov. Trial to Determine Efficacy of Fexinidazole in Visceral Leihmaniasis Patients in Sudan. https://clinicaltrials.gov/ct2/show/NCT01980199 (Accessed July 23, 2016).”, [s.d.]; TARRAL et al., 2014)
Fosravuconazole		Fungal infections	Chagas disease	Phase I	(“Drugs for Neglected Diseases initiative. New benznidazole regimens/combos. http://www.dndi.org/diseases-projects/portfolio/new-benz-regimens (Accessed July 23, 2016).”, [s.d.])
Azithromycin		Bacterial infections	Vivax malaria and trachoma	Approved	(ANDERSEN et al., 1998; BURR et al., 2014; HART et al., 2014; KITCHEN; VAUGHN; SKILLMAN, 2006)

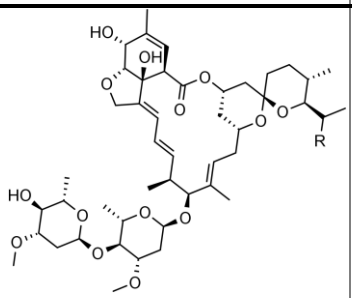
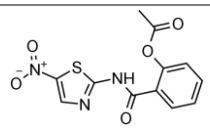
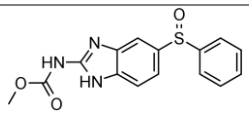
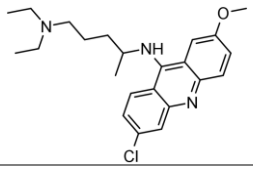
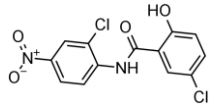
Doxycycline		Bacterial infections	Vivax malaria, lymphatic filariasis, and onchocerciasis	Approved	(ANDERSEN et al., 1998; HOERAUF et al., 2008; KITCHEN; VAUGHN; SKILLMAN, 2006; PANG; LIMSOMWONG; SINGHARAJ, 1988; SUPALI et al., 2008; TAMAROZZI et al., 2012; TAYLOR et al., 2005; TAYLOR; HOERAUF; BOCKARIE, 2010)
Praziquantel		Veterinary helminths	Schistosomiasis and taeniasis	Approved	(MCMAHON, 1981; MCMAHON; KOLSTRUP, 1979; RIM et al., 1978, 1979; SANTOS et al., 1979; ZWIERZ; MACHNICKA, 1985)
Artesunate		Malaria	Schistosomiasis	Phase III	(LIU et al., 2014; PÉREZ DEL VILLAR et al., 2012; SAEED et al., 2016; SHU-HUA, 2005)
Artemether		Malaria	Schistosomiasis	Phase III	(LIU et al., 2014; PÉREZ DEL VILLAR et al., 2012; SAEED et al., 2016; SHU-HUA, 2005)
Mefloquine		Malaria	Urinary schistosomiasis	Phase II	(BASRA et al., 2013; "ClinicalTrials.gov. Activity of Mefloquine Against Urinary Schistosomiasis. https://clinicaltrials.gov/ct2/show/NCT01132248 (Accessed July 21, 2016)", [s.d.])

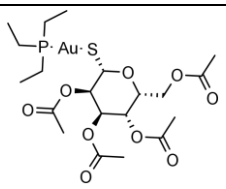
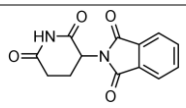
(Table 1) continued

Drug	Structure	Original indication	New indication	Status	References
Arachidonic acid		Dietary supplementation	Schistosomiasis	Phase II	(BARAKAT et al., 2015; "ClinicalTrials.gov. Arachidonic Acid Treatment Against Schistosomiasis Infection in Children. https://clinicaltrials.gov/ct2/show/NCT02144389 (Accessed July 23, 2016)", [s.d.])
Thiabendazole		Veterinary helminths	Soil-transmitted helminthiasis	Approved	(BARANSKI; CARNEIRO FILHO, 1966; SWINTON; REAVES, 1965; "Thiabendazole (Mintezol)-a new anthelmintic.", 1967; VAN DE ERVE, 1966)
Albendazole		Veterinary helminths	Lymphatic filariasis and soil-transmitted helminthiasis	Approved	(BETHONY et al., 2006; OTTESEN; ISMAIL; HORTON, 1999)

Imatinib		Cancer	Lymphatic filariasis	Phase II	("ClinicalTrials.gov. Efficacy and Microfilaricidal Kinetics of Imatinib for the Treatment of Loa Loa. https://clinicaltrials.gov/ct2/show/NCT02644525 (Accessed July 21, 2016)", [s.d.]; O'CONNELL et al., 2015)
Emodepside		Veterinary helminths	Onchocerciasis and lymphatic filariasis	Phase I	("ClinicalTrials.gov. First in Man Clinical Trial of Emodepside (BAY 44-4400). https://clinicaltrials.gov/ct2/show/NCT02661178 (Accessed July 25, 2016)", [s.d.]; CRISFORD et al., 2015)
Moxidectin		Veterinary helminths	Onchocerciasis	Phase II	(AWADZI et al., 2014; "ClinicalTrials.gov. Study Evaluating Orally Administered Moxidectin In Subjects With Onchocerca Volvulus Infection. https://clinicaltrials.gov/ct2/show/NCT00300768 (Accessed July 25, 2016)", [s.d.]; TAGBOTO; TOWNSON, 1996)

(Table 1) continued

Drug	Structure	Original indication	New indication	Status	References
Ivermectin	 B_{1a}: R = CH_2CH_3; B_{1b}: R = CH_3	Veterinary helminths	Onchocerciasis and strongyloidiasis	Approved	(LINDLEY, 1987; MARTI et al., 1996; NIGHTINGALE, 1997; WHITE et al., 1987)
Nitazoxanide		Intestinal protozoal diseases	Human fascioliasis and tapeworm infections	Approved	(GALVAN-RAMIREZ et al., 2007; LATEEF et al., 2008; PEREZ-MOLINA et al., 2011; ROSSIGNOL; ABAZA; FRIEDMAN, 1998; ROSSIGNOL; MAISONNEUVE, 1984; ZUMAQUERO-RÍOS et al., 2013)
Oxfendazole		Veterinary helminths	Cysticercosis	Phase I	("ClinicalTrials.gov. Phase I Trial Evaluating the Safety and Pharmacokinetics of Oxfendazole. https://clinicaltrials.gov/ct2/show/NCT02234570 (Accessed July 23, 2016).", [s.d.])
++Quinacrine		Malaria	Taeniasis	Phase II	(KOUL et al., 2000)
Niclosamide		Molluscicide	Taeniasis	Approved	(BRKIĆ; GLISIĆ; SIMIĆ, 1968; DITZEL; SCHWARTZ, 1967)

Auranofin		Rheumatoid arthritis	Amoebiasis	Phase I	(“ClinicalTrials.gov. Auranofin PK Following Oral Dose Administration. https://clinicaltrials.gov/ct2/show/NCT02089048 (Accessed July 25, 2016)”, [s.d.])
Thalidomide		Nausea	Erythema nodosum in leprosy	Approved	(NIGHTINGALE, 1998)

All listed drugs in Table 1 represent successful examples of drug repositioning for NTDs, but current chemotherapy to most of those diseases remains restricted to a few options, and the R&D pipeline has not been satisfactorily filled. Therefore, repositioning of effective and safe drugs still represents an urgent demand for these global health problems (ANDREWS; FISHER; SKINNER-ADAMS, 2014; GOUPIL; MCKERROW, 2014). In this review, we summarize the recent progress in computational drug repositioning based on curation and modeling of genomic, biological, and chemical data. We also present in-house and literature examples and suggest possible solutions to existing pitfalls.

3. Chemogenomics: Integrating chemical and biological spaces

Chemogenomics is a direct descendent of the typical drug discovery process that involves the systematic screening of chemical compounds against biological targets in high-throughput screening (HTS) efforts, for the identification of lead compounds (BREDEL; JACOBY, 2004). However, contrary to one-drug-one-target paradigm, chemogenomics attempts to identify all potential ligands for all possible targets/diseases (CARON et al., 2001; MESTRES, 2004). Therefore, analyzing chemogenomics data consists of a never-ending learning process aimed at completing a matrix (Fig. **1**), where compounds are reported as rows, biological targets or pathogens are usually reported as columns, and reported values are typically potencies or phenotypic effects (ROGNAN, 2007)

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Several technological advances have contributed to a better understanding of molecular mechanisms of diseases and resulted in opportunities to screen compounds against a large panel of protein targets and pathogens. Huge progress has been made in genomic consortiums targeting biological pathways of pathogens, development of appropriate culture systems, availability of large commercial compound libraries, as well as capabilities for collecting, and analyzing biological, genetic, and chemical data (HURLE et al., 2013; LI et al., 2016). Consequently, large freely available databases such as PubChem BioAssay (WANG et al., 2012), ChEMBL (GAULTON et al., 2012), BindingDB (CHEN; LIU; GILSON, 2001), DrugBank (KNOX et al., 2011; WISHART et al., 2006b), and Therapeutic Targets Database (TTD) (CHEN; JI; CHEN, 2002b; ZHU et al., 2009), containing millions of chemicals tested in various biological Fig. **1**) using basic assumptions: (i) compounds sharing particular chemical scaffold/similarity should also share targets and (ii) targets sharing similar ligands should share similar binding sites. (KLABUNDE, 2007; ROGNAN, 2007). In the subsequent sections, we review recent representative methodological development efforts in drug repositioning that use state-of-the-art computational ligand- and target-based chemogenomic approaches.

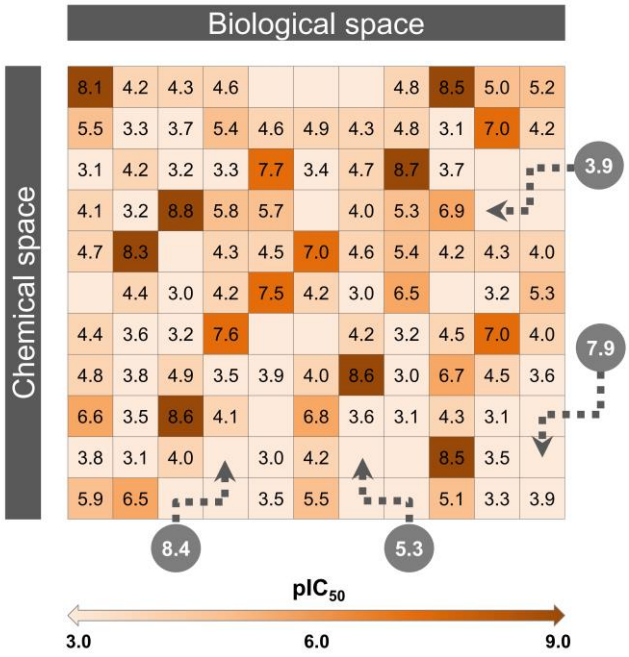

Fig. (1). Chemical−biological interaction space in a chemogenomic study. The interaction between chemical compounds and biological targets or pathogens can be visualized as a correlation matrix, where compounds are indexed by rows and targets/pathogens by columns. Each matrix cell contains potency value (pIC_{50}) or phenotypic effect of a given compound on a given target/pathogen. Colored cells in brown highlights the most promisor biological activities while the beige scale highlights

the less promisor activities. Grey arrows represent the computational methods applied to fill activity gaps in matrix by predicting specific pIC_{50} values.

Assays are continuously growing. Fully exploitation of this rich source of big data became highly attractive to medicinal chemists, having a profound positive influence in the drug discovery process (ALVES et al., 2015a, 2015b; BRAGA et al., 2014b; BRUNO JUNIOR NEVES, RAFAEL FERREIRA DANTAS, MARIO ROBERTO SENGER, WALTER CÉSAR GÓES VALENTE, JOÃO DE MELLO REZENDE-NETO, WILLIAN TÁVORA CHAVES, LEE KAMENTSKY, ANNE CARPENTER, 2016; LUO et al., 2014; MELO-FILHO et al., 2016; NEVES et al., 2016c; ZHANG et al., 2013), including in computational drug repositioning (ACHENBACH et al., 2011; BERENSTEIN et al., 2016a; DUBUS et al., 2009; EKINS et al., 2011; MARTORANA; PERRICONE; LAURIA, 2016).

We believe that the fully knowledge of the integration of chemical and biological data in NTDs is a goal far from being completed. Nevertheless, the computational curation and modeling of available data represent a significant step in this direction (FOURCHES; MURATOV; TROPSHA, 2010, 2015, 2016). Computational chemogenomics studies are important to NTDs because they can be applied to fill existing gaps in that matrix by predicting new biological indications (see grey arrows in

3.1. Ligand-based chemogenomic approaches

The basic concept of ligand-based chemogenomic approaches is that compounds sharing enough similarity to existing biologically annotated ligands have enhanced probability to share the same biological profile (KLABUNDE, 2007; ROGNAN, 2007). The basic principle, with the advantages and disadvantages of these methods, are listed below.

3.1.1. Ligand similarity search

Ligand similarity search is used to predict new biological activities for a wide database of drugs by measuring their level of structural similarity with a set of ligands with known activity (DUBUS et al., 2009). Several works have been published regarding the application of this approach for drug repositioning (KEISER et al., 2007, 2009). For instance, using a panel of approved and investigational drugs annotated with their protein targets, Keiser and colleagues (KEISER et al., 2009) performed a chemical similarity search for each pair of ligands and built a network map of pharmacological space based on the similarity between drugs. Chemical similarities between drugs predicted thousands of unanticipated drug−target associations. Then, 23 drug–target associations were subsequently experimentally confirmed, five of which were

potent at low nanomolar concentrations (KEISER et al., 2009). Fig. **2A** shows the schematic representation of calculation of Tanimoto similarity using bit strings, a wide-used ligand-based similarity method. As we can see, both query ligands and the database of drugs are represented as 2D molecular fingerprints, a string made up of binary digits that account for the absence (0) or presence (1) of representative fragments in chemical structure. Thus, fingerprints allow the comparison of chemical structures based on a simplified representation of bit sequences (MUEGGE; MUKHERJEE, 2016). This comparison must then be expressed in a way that can be quantified. Many similarity criteria can be used to afford this, among them, the Tanimoto coefficient, which is equal to the number of common bits set to 1 in both fingerprints divided by the total number of bits set to 1 between both fingerprints. It also assumes that two structures can be considered similar if this coefficient is higher than 0.85 (CERETO-MASSAGUÉ et al., 2015; NEVES et al., 2016a). When a high similarity between a drug and a known ligand is achieved, the first can be related to the biological activity of the ligand.

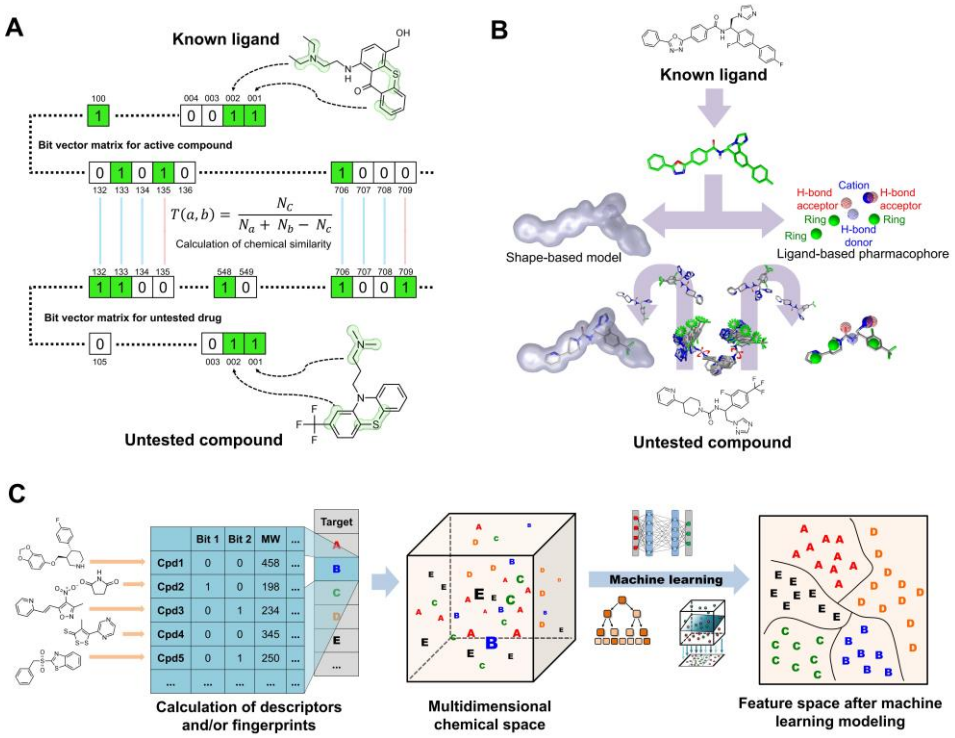

Fig. (2). Schematic representation of ligand-based chemogenomic methods. (**A**) calculation of Tanimoto similarity using bit strings (encoded by the presence and absence of structural fragments) generated for two compounds. N_a represents the number of features present in known ligand and not in untested compound, N_b represents the number of features present in untested compound and not in known ligand, and N_c the number of features present in both chemical structures; (**B**) fitting of a compound into pharmacophore and shape-based models generated from a known active ligand. Hydrogen bond donors and acceptors are represented as red and blue checkered spheres, respectively; aromatic rings by green spheres; lipophilic areas are represented as yellow spheres; and cations and

anions are represented by red and blue solid spheres; and (**C**) steps of multi-target QSAR modeling using machine learning approaches.

3.1.2. Ligand-based pharmacophores and shape-based models

Ligand-based pharmacophores represent the 3D-arrangement of the key pharmacophore features (i.e., hydrogen-bond donors or acceptors, aromatic rings, hydrophobic areas, positively and negatively ionizable groups, and metal coordinating areas) (CAPORUSCIO; TAFI, 2011; VUORINEN; SCHUSTER, 2015). Another ligand-based method is the 3D shape-based strategy that relies on the 3D surface shape, volume and pharmacophore features described previously (KOES; CAMACHO, 2014; KORTAGERE; KRASOWSKI; EKINS, 2009). In this manner, both strategies could be employed to find new, untested compounds with a high chance to interact with a known target (Fig. **2B**). The generation of both models basically involves four steps: (i) compilation and curation of a dataset of active and inactive compounds, collected either from the literature and, if available, from in-house *in vitro* studies; (ii) alignment of the bioactive conformations of the molecules to determine the best overlay of corresponding features; (iii) statistical validation and selection of best pharmacophoric/shape hypothesis using appropriate metrics (BRAGA; ANDRADE, 2013); and (iv) screening of untested compounds based on principle that chemical structures sharing similar pharmacophoric features or surface shapes are likely to share similar biological activities. These methods deliver good predictivity if the active ligands are known to bind to a target at a particular location, but flexibility represents one of the main difficulties in model generation/validation because the bioactive conformations of the ligands are usually not known (BOSTRÖM; GREENWOOD; GOTTFRIES, 2003; VUORINEN; SCHUSTER, 2015).

3.1.3. Multi-target QSAR models

The last decade has witnessed tremendous progress in cheminformatics, due in large part to the use of machine learning methods (DOBCHEV; PILLAI; KARELSON, 2014; LAVECCHIA, 2014; MELVILLE; BURKE; HIRST, 2009) for the development of multi-target quantitative structure–activity relationship (mt-QSAR) techniques (LIU et al., 2011; SPECK-PLANCHE; CORDEIRO, 2015). Unlike traditional one-target QSAR techniques, mt-QSAR can simultaneously predict the probability of activity against different biological targets (proteins, pathogens, cell lines, etc.) by using large and heterogeneous datasets of chemicals. In simple terms, a unique QSAR model for multiple outputs (LIMA et al., 2016; SPECK-PLANCHE; CORDEIRO, 2015).

The mt-QSAR models range from straightforward and parametric equations derived from linear machine learning methods as well as from nonlinear models derived from nonlinear machine learning techniques. Initially, molecular fingerprints and/or descriptors are calculated for each chemical structure in different sets of compounds with experimental activity against different targets (Fig. **2C**). Then, machine learning methods are used to establish quantitative relationships between descriptors and biological activities. This step involves empirically discovering a function that maps between the feature vectors and activities (CHERKASOV et al., 2014). Lastly, the quality of resulting mt-QSAR model is measured by using appropriate metrics regarding its ability to correctly predict the activities of compounds experimentally evaluated (TROPSHA, 2010). Once validated, the generated model can be used to fill existing holes in the matrix of chemogenomics data by predicting the activity of each compound against different biological targets.

Some machine learning methods perform better than others in the presence of irrelevant or mutually correlated descriptors. It is fairly common, as part of the training phase of the algorithm, to choose a subset of the original features that are helpful for building a predictive model and not strongly correlated with other features (CHERKASOV et al., 2014; MITCHELL, 2014). However, the influence of various factors on the prediction performance of models is decreasing in the following row: data quality > molecular descriptors > machine learning method (MURATOV et al., 2010). Therefore, data curation procedures such as structure standardization, analysis of duplicates, concordance analysis between original and compiled bioactivities (FOURCHES; MURATOV; TROPSHA, 2010, 2015, 2016), as well as dataset balancing (CHANG et al., 2013; ZAKHAROV et al., 2014) should be performed to ensure consistency and quality of models.

3.2. Target-based chemogenomic approaches

Recent advances in genome sequencing of several NTD pathogens provide an invaluable resource to improve our understanding of their fundamental biology. Overall, genomic studies combined with functional genomics profiles of gene expression at both the transcriptome and proteome levels, as well as metabolomics data are revealing molecular make-up of NTD pathogens (BUSCAGLIA; KISSINGER; AGÜERO, 2015). Besides, thousands of 3D structures of different pathogen targets have been solved by X-ray crystallography and nuclear magnetic resonance (NMR) and stored in public databases such as the RCSB Protein Data Bank (PDB) (ROSE et al., 2015)

Consequently, the availability of these resources enabled the development and application of target-based chemogenomic approaches to screening existing drugs against NTD pathogens. Target-based chemogenomic approaches are divided into two groups, depending on whether the amino acid sequence or the 3D structure of proteins is compared (ROGNAN, 2007). The basic principles, with the advantages and disadvantages of these methods, are discussed below.

3.2.1. Proteome-wide alignment

Proteome-wide alignment tools can be applied to drug repositioning using the underlying assumption that proteins sharing enough similarity (orthology) have enhanced the probability of sharing the same ligands (KLABUNDE, 2007; POLLASTRI; CAMPBELL, 2011; ROGNAN, 2007). For performing such analysis, one could use the Pfam database (http://pfam.xfam.org), which is a collection of multiple sequence alignments and profile hidden Markov models (HMMs). Each Pfam HMM represents a protein family or domain. By searching a protein sequence against the Pfam library of HMMs, you can determine which domains it carries i.e. its domain architecture (FINN et al., 2016; SONNHAMMER; EDDY; DURBIN, 1997) (Fig. **3A**).

However, despite the simplicity of the idea that proteins sharing enough similarity have enhanced the probability of sharing the same ligands, the identification of orthologous proteins for distantly related organisms is a daunting task, because of the complexity of the routes of gene evolution that often involves horizontal gene fusion and fission, lineage-specific gene loss, and other events that complicate the evolutionary scenarios (KRISTENSEN et al., 2011b). Therefore, the assumption that drug activity obtained for a protein of a different organism can be transferred toward the protein of interest need to be taken prudently, and with the aid of different approaches such as sequence alignments (ALTSCHUL et al., 1997; LI, 2003; WANG et al., 2015b), phylogenetic trees, graph representation (KRISTENSEN et al., 2011b), characterization of functional residues and domains (ASHKENAZY et al., 2010a; GLASER et al., 2003), and chokepoint analysis of metabolic pathways (SINGH; MALIK; SHARMA, 2007; TAYLOR et al., 2013), many of them available in the Pfam database (FINN et al., 2016).

For example, trying to explore potential drug targets in 1-deoxy-*D*-xylulose 5-phosphate (DOXP) pathway of *Plasmodium falciparum*, Jomaa and colleagues (JOMAA et al., 1999) discovered that a functional DOXP reductoisomerase had a high degree of sequence similarity with the bacterial and blue algal protein sequences of the enzyme DOXP reductoisomerase. Based on this, two know inhibitors of bacterial DOX reductoisomerase, fosmidomycin, and its

analog FR-900089, were investigated in experimental malaria models. Both compounds showed *in vitro* activity at nanomolar concentrations across three *P. falciparum* strains and cured mice infected with the rodent malaria parasite *P. vincke* (JOMAA et al., 1999).

Some concerns are raised about the exploration of pathogen targets with orthologues in humans aiming to avoid selectivity issues and possibly adverse effects. However, the situation is radically different when the orthologous protein is the target of an approved drug. In this case, orthologous proteins may provide evidence of druggability and offer potential scaffolds, and the selectivity issue can be anticipated and solved, and eventually the initial difficulty can be turned into an opportunity (BEGHYN et al., 2011; NJOROGE et al., 2014). On the other hand, the exploration of orthologous proteins depend on the availability of genomic data for the targeted pathogen, and is not able to identify drugs that interact with non-protein targets (SATERIALE et al., 2014).

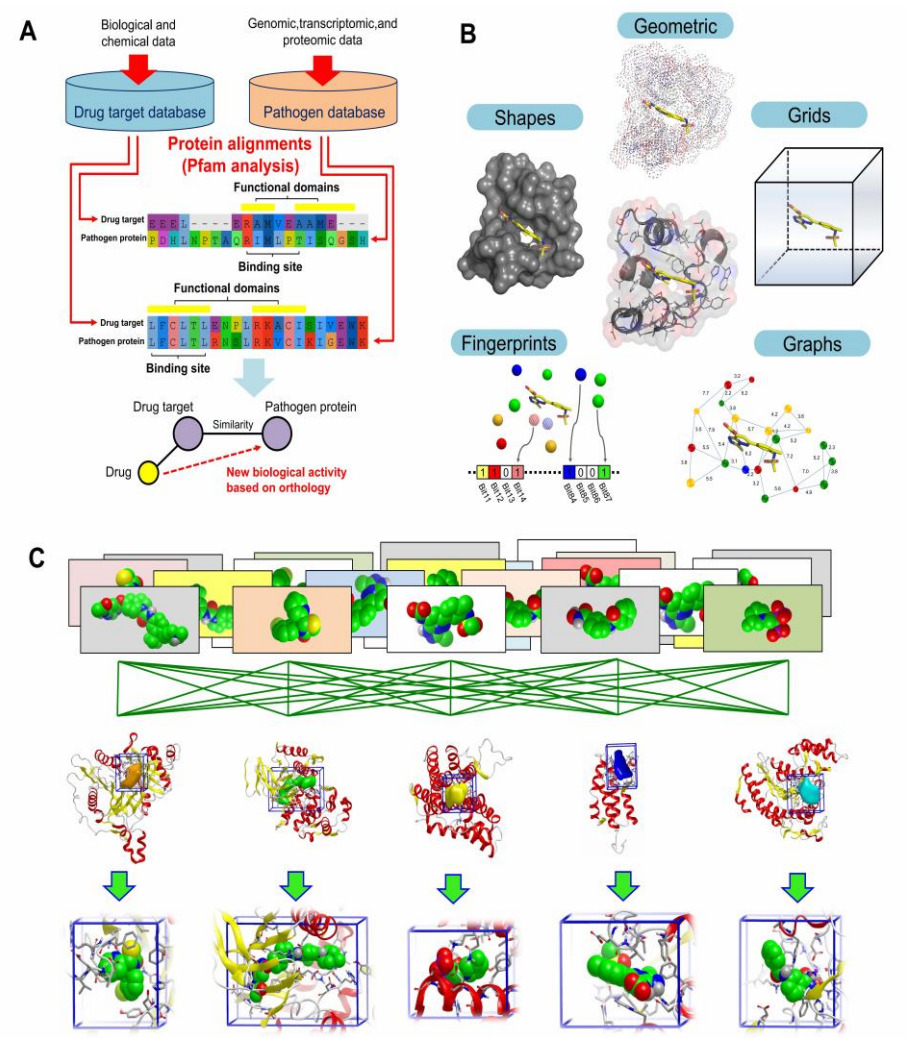

Fig. (3). Schematic representation of the target-based chemogenomic methods. (**A**) Comparison of pathogen proteins with known drug targets using sequence alignment, and analysis of Pfam database for identification of domain organization and conserved binding site amino acid residues; (**B**) Different levels of binding site representation for subsequent comparisons; and (**C**) fitting of multiple ligands into multiple targets in panel docking strategy.

3.2.2. Binding site similarity

The comparison of proteins on the binding site level sing 3D structural data is a promising chemogenomic approach motivated by the assumption that binding sites sharing similar characteristics should share similar ligands (ROGNAN, 2007). Methodologically, the comparison of binding sites can reveal similarities by 3D structural fold similarities or sequence motifs common to proteins with similar functions. However, their

u applicability can also be extended to the identification of patterns in proteins which do not share similar functions, or even do not seem to have any evolutionary relationship (EHRT; BRINKJOST; KOCH, 2016; MILLETTI; VULPETTI, 2010). For example, in a systematic pairwise comparison of the proto-oncogene *Pim-1* kinase binding site with 6,412 druggable protein-ligand binding sites, De Franchi and colleagues (DE FRANCHI et al., 2010) found that the adenosine triphosphate (ATP) binding site of synapsin I (a target involved in the regulation of neurotransmitter release) could recognize kinase inhibitors. Additionally, biochemical validation of this hypothesis showed that staurosporine, as well as three other kinase inhibitors effectively bound to synapsin I with nanomolar affinities (DE FRANCHI et al., 2010). This kind of similarity can exclusively be identified by of efficient binding site comparison methods showing their impact on drug repositioning.

The tools for binding site comparison could be classified according to the type of chemical features and algorithms used to represent the ligand binding pocket (EHRT; BRINKJOST; KOCH, 2016) (Fig. **3B**). For example, shape-based distribution algorithms evaluate the similarities between binding sites based on their surfaces (BATISTA et al., 2014; BINKOWSKI; JOACHIMIAK, 2008; DAS; KOKARDEKAR; BRENEMAN, 2009). The fingerprint-based algorithms generate a group of pharmacophoric features that are subsequently converted to a matrix of fingerprints, thus enabling extremely rapid comparisons (WOOD et al., 2012). Graph-based algorithms encode objects such as atoms, functional groups, or pharmacophoric features as nodes connected by edges that can be labeled by distances between two nodes (NAJMANOVICH; KURBATOVA; THORNTON, 2008; SCHMITT; KUHN; KLEBE, 2002). Geometry-based algorithms compare binding sites by purely geometric configuration of atoms or

pharmacophoric features in the 3D space (TU; SHI, 2013; YETURU; CHANDRA, 2008). Grid-based algorithms compare biding sites using molecular interaction/property fields, which are used to detect locations where interactions of amino acid residues with particular pharmacophoric features would be most favorable (BARONI et al., 2007; GOODFORD, 1985; TOTROV, 2011).

3.2.3. Panel docking

Panel docking strategy represents a robust approach in drug repositioning. It involves fitting the drugs into the binding sites of a panel of 3D protein targets to predict their binding affinities (Fig. **3C**). Detailed analyses of these binding characteristics potentially identify novel molecular targets for the drug which may be relevant for its experimental validation on biochemical assays or targeted pathogen (KHARKAR; WARRIER; GAUD, 2014). Mechanistically, panel docking calculations can be accomplished using a search algorithm and the scoring function. The search algorithm generates various possible poses to fit the ligands into the binding sites of the proteins while the scoring function orders them by a score (CERQUEIRA et al., 2015; FERREIRA et al., 2015; LIONTA et al., 2014).

In theory, panel docking is computationally more expensive when compared to conventional docking methods because it must deal with a broad range of protein targets. However, both docking strategies share the same problems. The critical issues in docking include the limited ability to exploit the protein flexibility and low accuracy of the score functions (BRAGA et al., 2014a; NEVES et al., 2016a). Consequently, some streamlined strategies have been developed to solve these methodological limitations. To incorporate protein flexibility in docking, ensemble methods make use of multiple input conformations of a target using a set of different 3D structures available on PDB or extracted from multiple conformations produced by molecular dynamics simulations (HOU et al., 2015; LIAO et al., 2011). On the other hand, consensus strategy could be applied by averaging the values of multiple scoring functions, providing better accuracy as compared to the individual scores (BRAGA et al., 2014a; ZHONG; ZHANG; XIU, 2010).

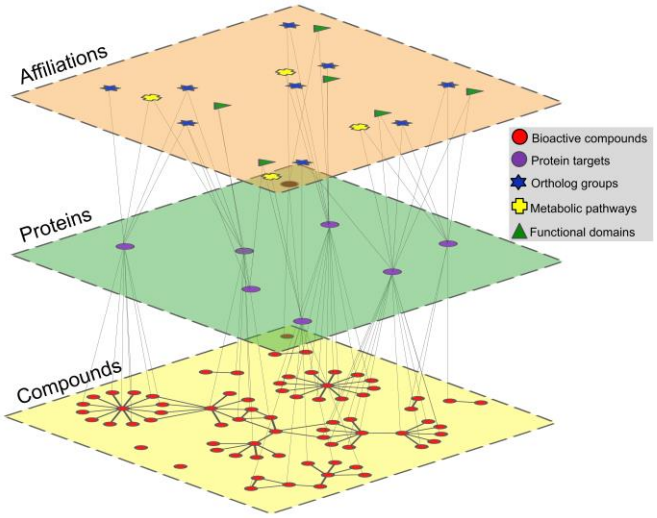

Fig. (4). Multilayer network representation of chemical and biological data. First layer contains drugs as nodes (red circles) and chemical similarity relations as edges. Second layer contains proteins as nodes (purple circles). Links between these two layers represent known and significant bioactivity data of a compound against a defined protein target. The top layer contains functional domains (green triangles), ortholog groups (blue stars), and metabolic pathways (yellow crosses). Links between the second and third layers represent affiliations of proteins to each of these functional annotations (BERENSTEIN et al., 2016a).

In addition to consensus scoring, post-processing techniques such as molecular mechanics combined with Poisson–Boltzmann surface area (MM-PBSA) (KOLLMAN et al., 2000; WANG et al., 2001) or with generalized Born and surface area (MM-GBSA) (LYNE; LAMB; SAEH, 2006) methods, can be used to better estimate the free energy of binding considering the binding contributions (i.e., ligand desolvation, intramolecular, and conformational entropy penalties upon binding), and, therefore, can be employed to rescore the docking results (BROWN; MUCHMORE, [s.d.]; GUIMARÃES; CARDOZO, 2008).

3.3. Modeling complex multilayer networks

Multilayer networks have been used to represent or predict regulatory and functional interactions among genes, proteins, biochemical pathways, and drugs of different organisms, leading to the identification of thousands of interactions in a relatively short time, even in the presence of scarce target inhibition data for the NTD pathogen of interest (BERENSTEIN et al., 2016a). Various computational resources can be explored to build multilayer network inferences. For instance, TDR Targets database (AGÜERO et al., 2008; MAGARIÑOS et al., 2012) encompasses

extensive genomic, biochemical and pharmacological data related to NTD pathogens and model organisms, as well as computationally predicted druggability for potential targets and compound desirability information (AGÜERO et al., 2008; MAGARIÑOS et al., 2012). Other information such as analysis of domain annotations, orthology relationships, as well as metabolic pathways and enzyme commission (EC) number can be obtained from Pfam database (FINN et al., 2016), InterPro database (JONES et al., 2014), OrthoMCL (LI, 2003), and Kyoto Encyclopedia of Genes and Genomes (KEGG) (OGATA et al., 2000), respectively.

Multilayer networks can be represented as a set of objects (nodes) and relations (edges) distributed in three different layers, each including a different type of information (Fig. **4**). The relationships of representing edges stem from the existence of well-established theorems and algorithms to identify network level properties, which are not apparent when looking at single interactions. For example, in the first layer of Fig. **4**, chemical compounds are represented as nodes and edges connecting compounds with the same substructure or chemically similar. Nodes in the second layer represents proteins from hundreds of pathogen and non-pathogen organisms, but no connection is initially established. In the third layer, nodes represent proteins with shared functional relations such as orthology, functional domains, and participate in the same biochemical pathways. Lastly, bioactivity data information obtained from different sources (e.g., ChEMBL (GAULTON et al., 2012), PubChem BioAssay (WANG et al., 2012), and TDR Targets (AGÜERO et al., 2008; MAGARIÑOS et al., 2012)) can be used to establish links between pairs of compounds, between compounds and known protein targets, and between proteins and functional affiliation classes (BERENSTEIN et al., 2016a).

3.4. Practical applications of *in silico* chemogenomics drug repositioning for NTDs

In the current scenario, computational chemogenomics tools still remain poorly explored in drug repositioning for NTDs. Despite the small number of examples available in the literature, most of them led the discovery of new indications with potency or affinity values in the low micromolar range. Below, we discuss some *in-house* examples and other successful applications for the

identification of potent drugs for NTDs using ligand- and/or structure-based chemogenomic approaches.

3.4.1. Schistosomiasis

Schistosomiasis is caused by flatworms of the genus *Schistosoma* that affects millions of people worldwide, ranking second only after malaria regarding its public health importance. The current reliance on a single drug, i.e., praziquantel, for treatment and control of this disease calls for the urgent discovery of novel anti-schistosomal drugs (COLLEY et al., 2014). Aiming at discovering new drugs, our group has implemented a proteome-wide alignment screen of a dataset of 2,114 proteins to identify drugs that are approved for clinical use in humans that could be active against multiple life stages of *S. mansoni* (NEVES et al., 2015). Initially, protein sequences of *S. mansoni* genes with differential expression in the intra-mammalian stages (PROTASIO et al., 2012) were curated and aligned against the sequences of drug targets in the DrugBank (KNOX et al., 2011; WISHART et al., 2006b), TTD (CHEN; JI; CHEN, 2002b; ZHU et al., 2009), and STITCH (KUHN et al., 2008, 2012) databases. Inclusion and exclusion criteria such as alignment coverage and conservation state of functional amino acid residues were also incorporated in the drug screening. Then, a chemical space analysis of predicted drugs was generated using *k*-means clustering integrated to principal component analysis aiming at removing drugs clustered in the chemical space of known inactive anti-schistosomal compounds. As a result, 27 genes encoding potential drug targets for a total of 115 approved drugs were identified. Among the combined list of potential drugs targeting schistosome proteins, 47 were reported in the literature to exhibit anti-schistosomal activity against at least one species or parasite stage (NEVES et al., 2015). In a following study, our group predicted paroxetine, an antidepressant drug, as *S. mansoni* serotonin transporter (*Sm*SERT) inhibitor. Studies dating back to the 1970s and 1980s have shown that among parasitic flatworms, in particularly *S. mansoni*, serotonin is an important modulator of neuromuscular function and increases metabolic activity by stimulating glucose uptake, glycogen breakdown, and lactate excretion (BOYLE; YOSHINO, 2005; BOYLE; ZAIDE; YOSHINO, 2000; FONTANA et al., 2009; MANSOUR, 1985; PATOCKA; RIBEIRO, 2007; RAHMAN; METTRICK; PODESTA, 1985). Then, *in vitro* assays performed by our group confirmed that paroxetine had a pronounced

effect on schistosomula viability (EC_{50} = 2.5 μM after 72h of exposure) and against adult male and female *S. mansoni* worm motility (EC_{50} = 5.1 μM and 9.9 μM after 72h of exposure, respectively) (BRUNO JUNIOR NEVES, RAFAEL FERREIRA DANTAS, MARIO ROBERTO SENGER, WALTER CÉSAR GÓES VALENTE, JOÃO DE MELLO REZENDE-NETO, WILLIAN TÁVORA CHAVES, LEE KAMENTSKY, ANNE CARPENTER, 2016). Lastly, homology modeling and docking studies with *Sm*SERT and human SERT revealed insights into the chemical basis of paroxetine anti-schistosomal activity and selectivity.

3.4.2. Human African trypanosomiasis

Human African trypanosomiasis, or sleeping sickness, is a parasitic disease caused by *Trypanosoma brucei* that affects tens of thousands of people annually (HOTEZ et al., 2007). The disease is fatal unless treated and current treatments are limited in safety, efficacy, and resistant parasites (CROFT; BARRETT; URBINA, 2005). Aiming at identifying new drugs, Bland and colleagues (BLAND et al., 2011) performed a search of potential drug targets examining the *T. brucei* genome for the presence of orthologous of known, chemically validated drug targets that have compounds that have successfully passed phase II clinical trials. Because the *T. brucei* infiltrates the central nervous system, they restricted their focus to orthologous targets related to compounds capable of crossing the blood-brain barrier, e.g., cyclic adenosine monophosphate (AMP) phosphodiesterases (*Tbr*PDE_{B1} and *Tbr*PDE_{B2}). Consequently, the authors hypothesized that exploration of human PDE inhibitors chemotypes could identify good starting points for the discovery of new trypanocidal agents. Subsequent *in vitro* studies were carried using a portfolio of PDE inhibitors, in which it was observed that the human PDE_4 inhibitor piclamilast inhibited *Tbr*PDE_{B1} and *Tbr*PDE_{B2} (IC_{50} = 4.7 μM and 11.4 μM, respectively) quickly killed the bloodstream forms of *T. brucei* (EC_{50} = 9.5 μM). Finally, homology modeling and docking studies with *Tbr*PDE_{B1} and human PDE_4 revealed insights into the chemical basis of piclamilast trypanocidal activity (BLAND et al., 2011). A similar approach was recently reported, describing the profiling of human lysine deacetylase inhibitors as a starting point for antiparasitic agents (WANG et al., 2015a).

3.4.3. Leishmaniasis

Leishmaniasis is a group of diseases caused by obligate intracellular protozoa of the genus *Leishmania*, that produce a wide range of clinical manifestations, from localized (cutaneous and mucocutaneous) to systemic (visceral) lethal forms (KOBETS; GREKOV; LIPOLDOVA, 2012). It is estimated that 350 million people live in areas of transmission of this parasite (WHO, 2010). Its current treatment has several limitations such as the development of parasitic resistance, toxicity of some drugs, high cost and only one oral drug available, miltefosine (FREITAS-JUNIOR et al., 2012). To find new drugs against this disease, Chavali and colleagues (CHAVALI et al., 2008) developed a metabolic network method that incorporates single gene essentiality and synthetic lethality to prioritize a set of 560 potential *Leishmania major* targets. Then, the prioritized protein sequences of *L. major* were aligned with the sequence of drug targets in the DrugBank (KNOX et al., 2011; WISHART et al., 2006b) and STITCH (KUHN et al., 2008, 2012) databases to screen for approved drugs that can potentially be used as antileishmanials (CHAVALI et al., 2012). Subsequently, inclusion and exclusion criteria such as druggability, FDA approved status, and drug toxicity was imposed in the drug screening pipeline. In parallel, computational predictions of abnormal growth phenotype (lethal or growth-reducing *in silico* gene deletions) from metabolic reconstruction were also applied for filtering *L. major* proteins. As a result, 15 proteins representing potential drug targets for a total of 240 approved drugs were identified. From the combined list of potential drugs targeting essential genes, nine of them have been reported in the literature to exhibit activity against *L. major*. It is important to note that halofantrine, an antimalarial drug prioritized using this strategy, showed significant antileishmanial activity (EC_{50} = 9.5 µM) when experimentally evaluated *in vitro* against *L. major* promastigotes. Furthermore, synthetic lethality predictions also aided in the prediction of superadditive drug combinations. For proof-of-concept, double-drug combinations containing disulfiram, a drug used to treat chronic alcoholism, and antibiotics/antipsychotics produced a more pronounced antileishmanial activity *in vitro* when compared with the sum of the inhibitory effects of the individual drugs (CHAVALI et al., 2012).

In another work, aiming at finding approved drugs with potential antileishmanial activity, our group carried out a study integrating bio- and cheminformatics approaches. Initially, using the TDR Targets database

(AGÜERO et al., 2008; MAGARIÑOS et al., 2012), we compiled a list of 229 *Leishmania* genes, specific for the genus, and common to five species of the parasite (*L. major*, *L. braziliensis*, *L. amazonensis*, *L. infantum* and *L. mexicana*). Moreover, we explored potential targets that could be essential to the process of development and differentiation for these five species of *Leishmania*. Then, the predicted primary amino acid sequences in FASTA format were obtained from the GeneDB database (LOGAN-KLUMPLER et al., 2012). Each protein sequence was used to search for homologous validated targets for other diseases in three databases of approved drugs (DrugBank (KNOX et al., 2011; WISHART et al., 2006b), STITCH (KUHN et al., 2008, 2012), and TTD (CHEN; JI; CHEN, 2002a; ZHU et al., 2009)). Consequently, 68 unique homologous proteins, interacting with 124 drugs, were found. They were homologous proteins of 37 potential targets from *Leishmania*. We found that from these 124 drugs, 28 were previously reported as being active against *Leishmania*. Additionally, an *in-house* QSAR model developed based on phenotypic screening against *L. infantum* was applied for the prediction of antileishmanial activity. Finally, five drugs not yet tested against *Leishmania* spp. were selected for *in vitro* biological evaluation.

3.4.4. Malaria

Although significant progress towards malaria prevention and control have been achieved over the last 15 years, human malaria parasites still claim nearly half a million lives worldwide every year (CIBULSKIS et al., 2016). The absence of an effective vaccine, shortage of new effective treatments and parasite resistance to currently available compounds, underscore the urgent need for identifying new antimalarial therapies. As such, drug repositioning has been increasingly regarded as an attractive strategy for fast-track identification of new effective and safe antimalarials.

The main antimalarial drugs approved for clinical use act mainly on two parasite metabolic pathways: hemoglobin digestion and nucleic acid synthesis. However, parasite resistance has evolved and became common for the currently used antimalarial drugs, including artemisinin and its derivatives (TILLEY et al., 2016). One of the underlying phenomena contributing to the emergence of drug resistance, is that resistance to different drugs is often controlled by similar molecular mechanisms and consequently the evolution of resistance to one

particular compound may impact on the efficacy of others. For instance, resistance to quinine-derived drugs, such as mefloquine and lumefantrine, as well as to the structurally unrelated artemisinin derivatives, has been shown to be modulated by mutations and/or amplification of the multidrug resistance protein homologue-1 (*mdr-1*) (WOODROW; KRISHNA, 2006). Similarly, resistance to drugs that block parasite nucleic acid synthesis, such as sulfadoxine, pyrimethamine and proguanil, is largely conferred by point mutations in genes encoding two enzymes, dihydrofolate reductase (*dhfr*) and the dihydropteroate synthase (*dhps*) (LE BRAS; DURAND, 2003).

Considering the design of new antimalarial drugs, is imperative to investigate alternative antimalarial molecular targets and biochemical pathways. One such strategy has focused on the apicoplast, a non-photosynthetic malarial plastid firstly described in the 1990's (MCFADDEN et al., 1996; WILSON; WILLIAMSON; PREISER, 1994) and recently confirmed to be acquired by secondary endosymbiosis of a plastid-containing red algae (MOORE et al., 2008). The apicoplast's genome is small (≈ 35kb), and the organelle harbors several unique metabolic functions, mostly accomplished by proteins that are nuclear-encoded and later imported into its lumen (RALPH et al., 2004). These unique metabolic features represent an attractive starting point for therapeutic intervention, since they are mostly of prokaryotic origin, a fact that may heighten the target selectivity of antimalarial drugs and/or reduce the probabilities of toxicity to humans. The antibiotic doxycycline targets the 16S rRNA of the apicoplast and has been repositioned, being successfully used as a preventive medicine for travelers travelling to malaria endemic zones and also in a combination therapy for malaria treatment (BUDIMULJA et al., 1997; LALLOO et al., 2007; YEH; DERISI, 2011). Several other approved drugs, mainly antibiotics, have also shown to inhibit several different targets in the malaria apicoplast and are currently under scrutiny towards repositioning (CHAKRABORTY, 2016). Our group has developed a computational-driven approach, based on protein orthology search, to identify approved drugs with the potential of interfering with the biology of the apicoplast (BISPO et al., 2013). In doing so, we suggested an array of drugs that are expected to inhibit several metabolic processes within the apicoplast, such as DNA repair, replication, nucleic acid metabolism, fatty acid synthesis, proteolysis and phosphorylation (BISPO et al., 2013). The drugs

identified were distributed within a wide range of classes, including antibiotics (e.g., isoxyl), antifungal agents (e.g., nitroxoline), anti-ulcer agents (e.g., sucralfate), antihypertensive agents (e.g., aliskiren) and anticancer agents (e.g., bleomycin) (BISPO et al., 2013).

Anticancer drugs also represent interesting starting points for identifying new antimalarial treatments and many of these agents have been proposed as antimalarials (KUNDU et al., 2015). However, since the primary molecular targets of anticancer agents are mammalian cells, the issue of toxicity to humans is often a main concern about their safety. One interesting way to maximize the chance of identifying safe antimalarials is to use computer-based comparative phylogenomics approaches that allow identifying the genes and corresponding encoded proteins that are present in malaria parasites, but absent in humans. To this purpose, there are web-based databases, such as TDR Targets (AGÜERO et al., 2008; MAGARIÑOS et al., 2012) and PlasmoDB (BAHL, 2003) that allow to carry out a genomewide comparison between the human and parasite genomes to identify selective drug targets. We carried out such an approach and identified 2,830 and 2,914 targets of *Plasmodium falciparum* and *Plasmodium vivax*, respectively, with no orthologues in humans. Each of the target's primary protein sequence was then used to search for homologous validated targets for other diseases in three databases of approved drugs (DrugBank (KNOX et al., 2011; WISHART et al., 2006b), STITCH (KUHN et al., 2008, 2012), and TTD (CHEN; JI; CHEN, 2002a; ZHU et al., 2009)). Predicted drug-target interactions were further refined using pairwise alignment and conservation state of functional regions by BLAST (ALTSCHUL et al., 1997) and ConSurf (ASHKENAZY et al., 2010a; GLASER et al., 2003), respectively. This strategy allowed us to identify 10 targets that are expected to be amenable to inhibition by 11 approved drugs with good safety profile, that have never been proposed as antimalarials before. Future *in vitro* and *in vivo* tests will allow determining the efficacy of each of these drugs against malaria parasites.

CONCLUSION

Academia plays a significant role in the early stages of drug development for NTDs. The R&D gap left behind by big pharma companies needs to be filled, to seek out hope for 534,000 people from the world's poorest areas, dying every year. In this context, chemogenomics and drug repositioning strategies stand out

as potential tools to investigate approved drugs with already known safety and pharmacokinetic profiles that could interact with all possible pathogen targets. Is notorious to mention that there is no single best solution for reaching this goal. To overcome different obstacles, we highlighted the importance of combined methodological efforts in drug repositioning using state-of-the-art computational ligand- and target-based chemogenomic approaches for NTDs.

Chemogenomics potential pitfalls emerge from a high dependence of search results on the query employed, the variety of descriptors used, activity landscapes and activity cliffs, conclusions derived from incomplete data and databases compiled with a small list of active and inactive compounds. In addition, 3D structure-based methods to compare binding sites or to estimate the protein-ligand interactions are still not sensitive to the scoring binding site similarities and estimation of scoring energy. Given the complexity of biological data, a critical analysis is necessary to interpret the findings appropriately. Despite the challenges and limitations, we showed successful examples of *in silico* chemogenomics drug repositioning approaches for the identification of new hits for schistosomiasis, Human African trypanosomiasis, leishmaniasis, and malaria. These developments are impressive, especially given the short period, but there is still a long way to go.

LIST OF ABBREVIATIONS

NTDs, neglected tropical diseases; WHO, Word Health Organization; CDC, Centers for Disease Control and Prevention; R&D, research and development; HTS, high-throughput screening; TTD, Therapeutic Target Database; mt-QSAR, multi-target quantitative structure–activity relationships; RMN, nuclear magnetic resonance; PDB, Protein Data Bank; DOXP, 1-deoxy-D-xylulose 5-phosphate; ATP, adenosine triphosphate; EC, enzyme commission; KEGG, Kyoto Encyclopedia of Genes and Genomes; SERTs, serotonin transporters; AMP, adenosine monophosphate; PDE, cyclic AMP phosphodiesterase; MDR1, multidrug resistance protein homologue-1; DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthase.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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[1] Buscaglia, C.A.; Kissinger, J.C.; Agüero, F. Neglected Tropical Diseases in the Post-Genomic Era. *Trends Genet.*, **2015**, *31*, 539–555.

[2] Centers for Disease Control and Prevention. Neglected tropical diseases. http://www.cdc.gov/globalhealth/ntd/diseases/index.html (Accessed July 18, 2016).

[3] World Health Organization. Neglected tropical diseases. http://www.who.int/neglected_diseases/diseases/en (Accessed July 20, 2016).

[4] Zijlstra, E.E.; van de Sande, W.W.J.; Welsh, O.; Mahgoub, E.S.; Goodfellow, M.; Fahal, A.H. Mycetoma: A Unique Neglected Tropical Disease. *Lancet Infect. Dis.*, **2016**, *16*, 100–112.

[5] Waheed, A.A.; Ghanchi, N.K.; Rehman, K.A.; Raza, A.; Mahmood, S.F.; Beg, M.A. Vivax Malaria and Chloroquine Resistance: A Neglected Disease as an Emerging Threat. *Malar. J.*, **2015**, *14*, 146.

[6] Bassat, Q.; Velarde, M.; Mueller, I.; Lin, J.; Leslie, T.; Wongsrichanalai, C.; Baird, J.K. Key Knowledge Gaps for Plasmodium Vivax Control and Elimination. *Am. J. Trop. Med. Hyg.*, **2016**, *107*, 475–479.

[7] Litt, E.; Baker, M.C.; Molyneux, D. Neglected Tropical Diseases and Mental Health: A Perspective on Comorbidity. *Trends Parasitol.*, **2012**, *28*, 195–201.

[8] Weiss, M.G. Stigma and the Social Burden of Neglected Tropical Diseases. *PLoS Negl. Trop. Dis.*, **2008**, *2*, e237.

[9] Hofstraat, K.; van Brakel, W.H. Social Stigma towards Neglected Tropical Diseases: A Systematic Review. *Int. Health*, **2016**, *8*, i53–i70.

[10] Nwaka, S.; Hudson, A. Innovative Lead Discovery Strategies for Tropical Diseases. *Nat. Rev. Drug Discov.*, **2006**, *5*, 941–955.

[11] Wyatt, P.G.; Gilbert, I.H.; Read, K.D.; Fairlamb, A.H. Target Validation: Linking Target and Chemical Properties to Desired Product Profile. *Curr. Top. Med. Chem.*, **2011**, *11*, 1275–1283.

[12] Renslo, A.R.; McKerrow, J.H. Drug Discovery and Development for Neglected Parasitic Diseases. *Nat. Chem. Biol.*, **2006**, *2*, 701–710.

[13] Tibayrenc, M.; Ayala, F.J. Reproductive Clonality of Pathogens: A Perspective on Pathogenic Viruses, Bacteria, Fungi, and Parasitic Protozoa. *Proc. Natl. Acad. Sci.*, **2012**, *109*, E3305–E3313.

[14] DiMasi, J.A.; Hansen, R.W.; Grabowski, H.G. The Price of Innovation: New Estimates of Drug Development Costs. *J. Health Econ.*, **2003**, *22*, 151–185.

[15] Kola, I.; Landis, J. Can the Pharmaceutical Industry Reduce Attrition Rates? *Nat. Rev. Drug Discov.*, **2004**, *3*, 711–715.

[16] Goupil, L.S.; McKerrow, J.H. Introduction: Drug Discovery and Development for Neglected Diseases. *Chem. Rev.*, **2014**, *114*, 11131–11137.

[17] DiMasi, J.A.; Feldman, L.; Seckler, A.; Wilson, A. Trends in Risks Associated With New Drug Development: Success Rates for Investigational Drugs. *Clin. Pharmacol. Ther.*, **2010**, *87*, 272–277.

[18] Pedrique, B.; Strub-Wourgaft, N.; Some, C.; Olliaro, P.; Trouiller, P.; Ford, N.; Pécoul, B.; Bradol, J.-H. The Drug and Vaccine Landscape for Neglected Diseases (2000–11): A Systematic Assessment. *Lancet Glob. Heal.*, **2013**, *1*, e371–e379.

[19] Aubé, J. Drug Repurposing and the Medicinal Chemist. *ACS Med. Chem. Lett.*, **2012**, *3*, 442–444.

[20] Ashburn, T.T.; Thor, K.B. Drug Repositioning: Identifying and Developing New Uses for Existing Drugs. *Nat. Rev. Drug Discov.*, **2004**, *3*, 673–683.

[21] Chong, C.R.; Sullivan, D.J. New Uses for Old Drugs. *Nature*, **2007**, *448*, 645–646.

[22] Sbaraglini, M.L.; Vanrell, M.C.; Bellera, C.L.; Benaim, G.; Carrillo, C.; Talevi, A.; Romano, P.S. Neglected Tropical Protozoan Diseases: Drug Repositioning as a Rational Option. *Curr. Top. Med. Chem.*, **2016**, *16*, 2201–2222.

[23] Novac, N. Challenges and Opportunities of Drug Repositioning. *Trends Pharmacol. Sci.*, **2013**, *34*, 267–272.

[24] Jin, G.; Wong, S.T.C. Toward Better Drug Repositioning: Prioritizing and Integrating Existing Methods into Efficient Pipelines. *Drug Discov. Today*, **2014**, *19*, 637–644.

[25] Sildenafil: An Oral Drug for Impotence. *Med. Lett. Drugs Ther.*, **1998**, *40*, 51–52.

[26] Bolgár, B.; Arany, Á.; Temesi, G.; Balogh, B.; Antal, P.; Mátyus, P. Drug Repositioning for Treatment of Movement Disorders: From Serendipity to Rational Discovery Strategies. *Curr. Top. Med. Chem.*, **2013**, *13*, 2337–2363.

[27] Sindermann, H.; Croft, S.L.; Engel, K.R.; Bommer, W.; Eibl, H.J.; Unger, C.; Engel, J. Miltefosine (Impavido): The First Oral Treatment against Leishmaniasis. *Med. Microbiol. Immunol.*, **2004**, *193*, 173–180.

[28] Berman, J. Miltefosine, an FDA-Approved Drug for the "orphan Disease", Leishmaniasis. *Expert Opin. Orphan Drugs*, **2015**, *3*, 727–735.

[29] Santos, A.T.; Blas, B.L.; Noseñas, J.S.; Portillo, G.P.; Ortega, O.M.; Hayashi, M.; Boehme, K. Preliminary Clinical Trials with Praziquantel in Schistosoma Japonicum Infections in the Philippines. *Bull. World Health Organ.*, **1979**, *57*, 793–799.

[30] McMahon, J.E. Praziquantel: A New Schistosomicide against Schistosoma Mansoni. *Arzneimittelforschung.*, **1981**, *31*, 592–594.

[31] McMahon, J.E.; Kolstrup, N. Praziquantel: A New Schistosomicide against Schistosoma Haematobium. *Br. Med. J.*, **1979**, *2*, 1396–1369.

[32] Sundar, S.; Jha, T.K.; Thakur, C.P.; Engel, J.; Sindermann, H.; Fischer, C.; Junge, K.; Bryceson, A.; Berman, J. Oral Miltefosine for Indian Visceral Leishmaniasis. *N. Engl. J. Med.*, **2002**, *347*, 1739–1746.

[33] Ben Salah, A.; Ben Messaoud, N.; Guedri, E.; Zaatour, A.; Ben Alaya, N.; Bettaieb, J.; Gharbi, A.; Belhadj Hamida, N.; Boukthir, A.; Chlif, S.; Abdelhamid, K.; El Ahmadi, Z.; Louzir, H.; Mokni, M.; Morizot, G.; Buffet, P.; Smith, P.L.; Kopydlowski, K.M.; Kreishman-Deitrick, M.; Smith, K.S.; Nielsen, C.J.; Ullman, D.R.; Norwood, J.A.; Thorne, G.D.; McCarthy, W.F.; Adams, R.C.; Rice, R.M.; Tang, D.; Berman, J.; Ransom, J.; Magill, A.J.; Grogl, M. Topical Paromomycin with or without Gentamicin for Cutaneous Leishmaniasis. *N. Engl. J. Med.*, **2013**, *368*, 524–532.

[34] Sundar, S.; Jha, T.K.; Thakur, C.P.; Sinha, P.K.; Bhattacharya, S.K. Injectable Paromomycin for Visceral Leishmaniasis in India. *N. Engl. J. Med.*, **2007**, *356*, 2571–2581.

[35] Neal, R.A.; Murphy, A.G.; Olliaro, P.; Croft, S.L. Aminosidine Ointments for the Treatment of Experimental Cutaneous Leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.*, **1994**, *88*, 223–225.

[36] Berman, J.D. U.S. Food and Drug Administration Approval of AmBisome (Liposomal Amphotericin B) for Treatment of Visceral Leishmaniasis. *Clin. Infect. Dis.*, **1999**, *28*, 49–51.

[37] Bern, C.; Adler-Moore, J.; Berenguer, J.; Boelaert, M.; Boer, M. den; Davidson, R.N.; Figueras, C.; Gradoni, L.; Kafetzis, D.A.; Ritmeijer, K.; Rosenthal, E.; Royce, C.; Russo, R.; Sundar, S.; Alvar, J. Reviews Of Anti-infective Agents: Liposomal Amphotericin B for the Treatment of Visceral Leishmaniasis. *Clin. Infect. Dis.*, **2006**, *43*, 917–924.

[38] Sundar, S.; Chakravarty, J.; Agarwal, D.; Rai, M.; Murray, H.W. Single-Dose Liposomal Amphotericin B for Visceral Leishmaniasis in India. *N. Engl. J. Med.*, **2010**, *362*, 504–512.

[39] Prado Sampaio, S.A.; Castro, R.M.; Dillon, N.L.; Costa Martins, J.E. Treatment of Mucocutaneous (American) Leishmaniasis with Amphotericin B: Report of 70 Cases. *Int. J. Dermatol.*, **1971**, *10*, 179–181.

[40] Crofts, M.A. Use of Amphotericin B in Mucocutaneous Leishmaniasis. *J. Trop. Med. Hyg.*, **1976**, *79*, 111–113.

[41] ClinicalTrials.gov. High Dose Fluconazole in Cutaneous Leishmaniasis in Bahia and Manaus. https://clinicaltrials.gov/ct2/show/NCT01953744 (Accessed July 21, 2016).

[42] Emad, M.; Hayati, F.; Fallahzadeh, M.K.; Namazi, M.R. Superior Efficacy of Oral Fluconazole 400 Mg Daily versus Oral Fluconazole 200 Mg Daily in the Treatment of Cutaneous Leishmania Major Infection: A Randomized Clinical Trial. *J. Am. Acad. Dermatol.*, **2011**, *64*, 606–608.

[43] Sousa, A.Q.; Frutuoso, M.S.; Moraes, E.A.; Pearson, R.D.; Pompeu, M.M.L. High-Dose Oral Fluconazole Therapy Effective for Cutaneous Leishmaniasis Due to Leishmania (Vianna) Braziliensis. *Clin. Infect. Dis.*, **2011**, *53*, 693–695.

[44] Sousa, A.Q.; Lima Pompeu, M.M.; Pearson, R.D. Disseminated Cutaneous Leishmaniasis, a Patient with 178 Lesions Cured with Fluconazole. *Am. J. Trop. Med. Hyg.*, **2016**, *94*, 1–2.

[45] de Sá Oliveira, T.; Capp Neto, M.; Martins, B.J.; Rodrigues, H.A.; Antonino, R.M.; Magalhães, A. V. Action of Pentoxifylline on Experimental Cutaneous Leishmaniasis due to Leishmania (Leishmania) Amazonensis. *Memórias do Inst. Oswaldo Cruz*, **2000**, *95*, 477–282.

[46] Lessa, H.A.; Machado, P.; Lima, F.; Cruz, A.A.; Bacellar, O.; Guerreiro, J.; Carvalho, E.M. Successful Treatment of Refractory Mucosal Leishmaniasis with Pentoxifylline plus Antimony. *Am. J. Trop. Med. Hyg.*, **2001**, *65*, 87–89.

[47] ClinicalTrials.gov. Antimony Plus Pentoxifylline in Cutaneous Leishmaniasis. https://clinicaltrials.gov/ct2/show/NCT01381055 (Accessed July 21, 2016).

[48] Martinez, S.; Marr, J.J. Allopurinol in the Treatment of American Cutaneous Leishmaniasis. *N. Engl. J. Med.*, **1992**, *326*, 741–744.

[49] Barzilai, A.; Friedman, J.; Trau, H. Treatment of Cutaneous Leishmaniasis with Allopurinol. *J. Am. Acad. Dermatol.*, **1995**, *32*, 518.

[50] Martinez, S.; Gonzalez, M.; Vernaza, M.E. Treatment of Cutaneous Leishmaniasis with Allopurinol and Stibogluconate. *Clin. Infect. Dis.*, **1997**, *24*, 165–169.

[51] Schmid, C.; Kuemmerle, A.; Blum, J.; Ghabri, S.; Kande, V.; Mutombo, W.; Ilunga, M.; Lumpungu, I.; Mutanda, S.; Nganzobo, P.; Tete, D.; Mubwa, N.; Kisala, M.; Blesson, S.; Mordt, O.V. In-Hospital Safety in Field Conditions of Nifurtimox Eflornithine Combination Therapy (NECT) for T. B. Gambiense Sleeping Sickness. *PLoS Negl. Trop. Dis.*,

2012, *6*, e1920.

[52] Opigo, J.; Woodrow, C. NECT Trial: More than a Small Victory over Sleeping Sickness. *Lancet*, **2009**, *374*, 7–9.

[53] Priotto, G.; Kasparian, S.; Mutombo, W.; Ngouama, D.; Ghorashian, S.; Arnold, U.; Ghabri, S.; Baudin, E.; Buard, V.; Kazadi-Kyanza, S.; Ilunga, M.; Mutangala, W.; Pohlig, G.; Schmid, C.; Karunakara, U.; Torreele, E.; Kande, V. Nifurtimox-Eflornithine Combination Therapy for Second-Stage African Trypanosoma Brucei Gambiense Trypanosomiasis: A Multicentre, Randomised, Phase III, Non-Inferiority Trial. *Lancet*, **2009**, *374*, 56–64.

[54] Alirol, E.; Schrumpf, D.; Amici Heradi, J.; Riedel, A.; de Patoul, C.; Quere, M.; Chappuis, F. Nifurtimox-Eflornithine Combination Therapy for Second-Stage Gambiense Human African Trypanosomiasis: Medecins Sans Frontieres Experience in the Democratic Republic of the Congo. *Clin. Infect. Dis.*, **2013**, *56*, 195–203.

[55] Pohlig, G.; Bernhard, S.C.; Blum, J.; Burri, C.; Mpanya, A.; Lubaki, J.-P.F.; Mpoto, A.M.; Mununga, B.F.; N'tombe, P.M.; Deo, G.K.M.; Mutantu, P.N.; Kuikumbi, F.M.; Mintwo, A.F.; Munungi, A.K.; Dala, A.; Macharia, S.; Bilenge, C.M.M.; Mesu, V.K.B.K.; Franco, J.R.; Dituvanga, N.D.; Tidwell, R.R.; Olson, C.A. Efficacy and Safety of Pafuramidine versus Pentamidine Maleate for Treatment of First Stage Sleeping Sickness in a Randomized, Comparator-Controlled, International Phase 3 Clinical Trial. *PLoS Negl. Trop. Dis.*, **2016**, *10*, e0004363.

[56] ClinicalTrials.gov. Trial of DB289 for the Treatment of Stage I African Trypanosomiasis. https://clinicaltrials.gov/ct2/show/NCT00803933 (Accessed July 21, 2016).

[57] Mdachi, R.E.; Thuita, J.K.; Kagira, J.M.; Ngotho, J.M.; Murilla, G.A.; Ndung'u, J.M.; Tidwell, R.R.; Hall, J.E.; Brun, R. Efficacy of the Novel Diamidine Compound 2,5-Bis(4-Amidinophenyl)- Furan-Bis-O-Methlylamidoxime (Pafuramidine, DB289) against Trypanosoma Brucei Rhodesiense Infection in Vervet Monkeys after Oral Administration. *Antimicrob. Agents Chemother.*, **2009**, *53*, 953–957.

[58] Tarral, A.; Blesson, S.; Mordt, O.V.; Torreele, E.; Sassella, D.; Bray, M.A.; Hovsepian, L.; Evène, E.; Gualano, V.; Felices, M.; Strub-Wourgaft, N. Determination of an Optimal Dosing Regimen for Fexinidazole, a Novel Oral Drug for the Treatment of Human African Trypanosomiasis: First-in-Human Studies. *Clin. Pharmacokinet.*, **2014**, *53*, 565–580.

[59] ClinicalTrials.gov. Pivotal Study of Fexinidazole for Human African Trypanosomiasis in Stage 2. https://clinicaltrials.gov/ct2/show/NCT01685827 (Accessed July 21, 2016).

[60] ClinicalTrials.gov. Study to Evaluate Fexinidazole Dosing Regimens for the Treatment of Adult Patients With Chagas Disease. https://clinicaltrials.gov/ct2/show/NCT02498782 (Accessed July 23, 2016).

[61] ClinicalTrials.gov. Trial to Determine Efficacy of Fexinidazole in Visceral Leihmaniasis Patients in Sudan. https://clinicaltrials.gov/ct2/show/NCT01980199 (Accessed July 23, 2016).

[62] Drugs for Neglected Diseases initiative. New benznidazole regimens/combos. http://www.dndi.org/diseases-projects/portfolio/new-benz-regimens (Accessed July 23, 2016).

[63] Kitchen, L.W.; Vaughn, D.W.; Skillman, D.R. Reviews Of Anti-Infective Agents: Role of US Military Research Programs in the Development of US Food and Drug Administration–Approved Antimalarial Drugs. *Clin. Infect. Dis.*, **2006**, *43*, 67–71.

[64] Andersen, S.L.; Oloo, A.J.; Gordon, D.M.; Ragama, O.B.; Aleman, G.M.; Berman, J.D.; Tang, D.B.; Dunne, M.W.; Shanks, G.D. Successful Double-Blinded, Randomized, Placebo-Controlled Field Trial of Azithromycin and Doxycycline as Prophylaxis for Malaria in Western Kenya. *Clin. Infect. Dis.*, **1998**, *26*, 146–150.

[65] Hart, J.D.; Edwards, T.; Burr, S.E.; Harding-Esch, E.M.; Takaoka, K.; Holland, M.J.; Sillah, A.; Mabey, D.C.W.; Bailey, R.L. Effect of Azithromycin Mass Drug Administration for Trachoma on Spleen Rates in Gambian Children. *Trop. Med. Int. Heal.*, **2014**, *19*, 207–211.

[66] Burr, S.E.; Hart, J.; Edwards, T.; Harding-Esch, E.M.; Holland, M.J.; Mabey, D.C.W.; Sillah, A.; Bailey, R.L. Anthropometric Indices of Gambian Children after One or Three Annual Rounds of Mass Drug Administration with Azithromycin for Trachoma Control. *BMC Public Health*, **2014**, *14*, 1176.

[67] Pang, L.; Limsomwong, N.; Singharaj, P. Prophylactic Treatment of Vivax and Falciparum Malaria with Low-Dose Doxycycline. *J. Infect. Dis.*, **1988**, *158*, 1124–1127.

[68] Supali, T.; Djuardi, Y.; Pfarr, K.M.; Wibowo, H.; Taylor, M.J.; Hoerauf, A.; Houwing-Duistermaat, J.J.; Yazdanbakhsh, M.; Sartono, E. Doxycycline Treatment of Brugia Malayi –Infected Persons Reduces Microfilaremia and Adverse Reactions after Diethylcarbamazine and Albendazole Treatment. *Clin. Infect. Dis.*, **2008**, *46*, 1385–1393.

[69] Taylor, M.J.; Makunde, W.H.; McGarry, H.F.; Turner, J.D.; Mand, S.; Hoerauf, A. Macrofilaricidal Activity after Doxycycline Treatment of Wuchereria Bancrofti: A Double-Blind, Randomised Placebo-Controlled Trial. *Lancet*, **2005**, *365*, 2116–2121.

[70] Taylor, M.J.; Hoerauf, A.; Bockarie, M. Lymphatic Filariasis and Onchocerciasis. *Lancet*, **2010**, *376*, 1175–1185.

[71] Tamarozzi, F.; Tendongfor, N.; Enyong, P.A.; Esum, M.; Faragher, B.; Wanji, S.; Taylor, M.J. Long Term Impact of Large Scale Community-Directed Delivery of Doxycycline for the Treatment of Onchocerciasis. *Parasit. Vectors*, **2012**, *5*, 53.

[72] Hoerauf, A.; Specht, S.; Büttner, M.; Pfarr, K.; Mand, S.; Fimmers, R.; Marfo-Debrekyei, Y.; Konadu, P.; Debrah, A.Y.; Bandi, C.; Brattig, N.; Albers, A.; Larbi, J.; Batsa, L.; Adjei, O.; Büttner, D.W. Wolbachia Endobacteria Depletion by Doxycycline as Antifilarial Therapy Has Macrofilaricidal Activity in Onchocerciasis: A Randomized Placebo-Controlled Study. *Med. Microbiol. Immunol.*, **2008**, *197*, 295–311.

[73] Rim, H.J.; Park, S.B.; Lee, J.S.; Joo, K.H. Therapeutic Effects of Praziquantel (Embay 8440) against Taenia Solium Infection. *Korean J. Parasitol.*, **1979**, *17*, 67–72.

[74] Zwierz, C.; Machnicka, B. Treatment with Praziquantel the Taenia Saginata Infection in Men. *Bull. Inst. Marit. Trop. Med. Gdynia*, **1985**, *36*, 77–80.

[75] Rim, H.J.; Park, C.Y.; Lee, J.S.; Joo, K.H.; Lyu, K.S. Therapeutic Effects of Praziquantel (Embay 8440) against Hymenolepis Nana Infection. *Korean J. Parasitol.*, **1978**, *16*, 82–87.

[76] Shu-Hua, X. Development of Antischistosomal Drugs in China, with Particular Consideration to Praziquantel and the Artemisinins. *Acta Trop.*, **2005**, *96*, 153–167.

[77] Liu, Y.-X.; Wu, W.; Liang, Y.-J.; Jie, Z.-L.; Wang, H.; Wang, W.; Huang, Y.-X. New Uses for Old Drugs: The Tale of Artemisinin Derivatives in the Elimination of Schistosomiasis Japonica in China. *Molecules*, **2014**, *19*, 15058–15074.

[78] Saeed, M.E.M.; Krishna, S.; Greten, H.J.; Kremsner, P.G.; Efferth, T. Antischistosomal Activity of Artemisinin Derivatives in Vivo and in Patients. *Pharmacol. Res.*, **2016**, *110*, 216–226.

[79] Pérez del Villar, L.; Burguillo, F.J.; López-Abán, J.; Muro, A. Systematic Review and Meta-Analysis of Artemisinin Based Therapies for the Treatment and Prevention of Schistosomiasis. *PLoS One*, **2012**, *7*, e45867.

[80] Basra, A.; Mombo-Ngoma, G.; Capan Melser, M.; Akerey Diop, D.; Wurbel, H.; Mackanga, J.-R.; Furstenau, M.; Manego Zoleko, R.; Adegnika, A.A.; Gonzalez, R.; Menendez, C.; Kremsner, P.G.; Ramharter, M. Efficacy of Mefloquine Intermittent Preventive Treatment in Pregnancy Against Schistosoma Haematobium Infection in Gabon: A Nested Randomized Controlled Assessor-Blinded Clinical Trial. *Clin. Infect. Dis.*, **2013**, *56*, e68–e75.

[81] ClinicalTrials.gov. Activity of Mefloquine Against Urinary Schistosomiasis. https://clinicaltrials.gov/ct2/show/NCT01132248 (Accessed July 21, 2016).

[82] Barakat, R.; Abou El-Ela, N.E.; Sharaf, S.; El Sagheer, O.; Selim, S.; Tallima, H.; Bruins, M.J.; Hadley, K.B.; El Ridi, R. Efficacy and Safety of Arachidonic Acid for Treatment of School-Age Children in Schistosoma Mansoni High-Endemicity Regions. *Am. J. Trop. Med. Hyg.*, **2015**, *92*, 797–804.

[83] ClinicalTrials.gov. Arachidonic Acid Treatment Against Schistosomiasis Infection in Children. https://clinicaltrials.gov/ct2/show/NCT02144389 (Accessed July 23, 2016).

[84] Swinton, N.W.; Reaves, L.E. Successful Therapy of Strongyloidiasis with Thiabendazole. *Lahey Clin. Found. Bull.*, **1965**, *14*, 117–120.

[85] Baranski, M.C.; Carneiro Filho, M. Massive Infestation with Larva Migrans. Treatment with Thiabendazole. *Rev. Bras. Med.*, **1966**, *23*, 233–235.

[86] Thiabendazole (Mintezol)-a New Anthelmintic. *Med. Lett. Drugs Ther.*, **1967**, *9*, 99–101.

[87] Van de Erve, J. Systemic Thiabendazole Treatment of Creeping Eruption. *J. S. C. Med. Assoc.*, **1966**, *62*, 263–264.

[88] Ottesen, E.A.; Ismail, M.M.; Horton, J. The Role of Albendazole in Programmes to Eliminate Lymphatic Filariasis. *Parasitol. Today*, **1999**, *15*, 382–386.

[89] Bethony, J.; Brooker, S.; Albonico, M.; Geiger, S.M.; Loukas, A.; Diemert, D.; Hotez, P.J. Soil-Transmitted Helminth Infections: Ascariasis, Trichuriasis, and Hookworm. *Lancet*, **2006**, *367*, 1521–1532.

[90] ClinicalTrials.gov. Efficacy and Microfilaricidal Kinetics of Imatinib for the Treatment of Loa Loa. https://clinicaltrials.gov/ct2/show/NCT02644525 (Accessed July 21, 2016).

[91] O'Connell, E.M.; Bennuru, S.; Steel, C.; Dolan, M.A.; Nutman, T.B. Targeting Filarial Abl-like Kinases: Orally Available, Food and Drug Administration–Approved Tyrosine Kinase Inhibitors Are Microfilaricidal and Macrofilaricidal. *J. Infect. Dis.*, **2015**, *212*, 684–693.

[92] Crisford, A.; Ebbinghaus-Kintscher, U.; Schoenhense, E.; Harder, A.; Raming, K.; O'Kelly, I.; Ndukwe, K.; O'Connor, V.; Walker, R.J.; Holden-Dye, L. The Cyclooctadepsipeptide Anthelmintic Emodepside Differentially Modulates Nematode, Insect and Human Calcium-Activated Potassium (SLO) Channel Alpha Subunits. *PLoS Negl. Trop. Dis.*, **2015**, *9*, e0004062.

[93] ClinicalTrials.gov. First in Man Clinical Trial of Emodepside (BAY 44-4400). https://clinicaltrials.gov/ct2/show/NCT02661178 (Accessed July 25, 2016).

[94] Awadzi, K.; Opoku, N.O.; Attah, S.K.; Lazdins-Helds, J.; Kuesel, A.C. A Randomized, Single-Ascending-Dose, Ivermectin-Controlled, Double-Blind Study of Moxidectin in Onchocerca Volvulus Infection. *PLoS Negl. Trop. Dis.*, **2014**, *8*, e2953.

[95] ClinicalTrials.gov. Study Evaluating Orally Administered Moxidectin In Subjects With Onchocerca Volvulus Infection. https://clinicaltrials.gov/ct2/show/NCT00300768 (Accessed July 25, 2016).

[96] Tagboto, S.K.; Townson, S. Onchocerca Volvulus and O. Lienalis: The Microfilaricidal Activity of Moxidectin Compared with that of Ivermectin in Vitro and in Vivo. *Ann. Trop. Med. Parasitol.*, **1996**, *90*, 497–505.

[97] Lindley, D. Merck's New Drug Free to WHO for River Blindness Programme. *Nature*, **1987**, *329*, 752–752.

[98] White, A.T.; Newland, H.S.; Taylor, H.R.; Erttmann, K.D.; Larijani, E.K.; Nara, A.; Aziz, M.A.; D'Anna, S.A.; Williams, P.N.; Greene, B.M. Controlled Trial and Dose-Finding Study of Ivermectin for Treatment of Onchocerciasis. *J. Infect. Dis.*, **1987**, *156*, 463–470.

[99] Nightingale, S.L. From the Food and Drug Administration. *JAMA*, **1997**, *277*, 703.

[100] Marti, H.; Haji, H.J.; Savioli, L.; Chwaya, H.M.; Mgeni, A.F.; Ameir, J.S.; Hatz, C. A Comparative Trial of a Single-Dose

Ivermectin versus Three Days of Albendazole for Treatment of Strongyloides Stercoralis and Other Soil-Transmitted Helminth Infections in Children. *Am. J. Trop. Med. Hyg.*, **1996**, *55*, 477–481.

[101] Rossignol, J.F.; Abaza, H.; Friedman, H. Successful Treatment of Human Fascioliasis with Nitazoxanide. *Trans. R. Soc. Trop. Med. Hyg.*, **1998**, *92*, 103–104.

[102] Zumaquero-Ríos, J.L.; Sarracent-Pérez, J.; Rojas-García, R.; Rojas-Rivero, L.; Martínez-Tovilla, Y.; Valero, M.A.; Mas-Coma, S. Fascioliasis and Intestinal Parasitoses Affecting Schoolchildren in Atlixco, Puebla State, Mexico: Epidemiology and Treatment with Nitazoxanide. *PLoS Negl. Trop. Dis.*, **2013**, *7*, e2553.

[103] Galvan-Ramirez, M.L.; Rivera, N.; Loeza, M.E.; Avila, X.; Acero, J.; Troyo, R.; Bernal, R. Nitazoxanide in the Treatment of Ascaris Lumbricoides in a Rural Zone of Colima, Mexico. *J. Helminthol.*, **2007**, *81*, 255–259.

[104] Rossignol, J.F.; Maisonneuve, H. Nitazoxanide in the Treatment of Taenia Saginata and Hymenolepis Nana Infections. *Am. J. Trop. Med. Hyg.*, **1984**, *33*, 511–512.

[105] Lateef, M.; Zargar, S.A.; Khan, A.R.; Nazir, M.; Shoukat, A. Successful Treatment of Niclosamide- and Praziquantel-Resistant Beef Tapeworm Infection with Nitazoxanide. *Int. J. Infect. Dis.*, **2008**, *12*, 80–82.

[106] Perez-Molina, J.A.; Diaz-Menendez, M.; Gallego, J.I.; Norman, F.; Monge-Maillo, B.; Ayala, A.P.; Lopez-Velez, R. Evaluation of Nitazoxanide for the Treatment of Disseminated Cystic Echinococcosis: Report of Five Cases and Literature Review. *Am. J. Trop. Med. Hyg.*, **2011**, *84*, 351–356.

[107] ClinicalTrials.gov. Phase I Trial Evaluating the Safety and Pharmacokinetics of Oxfendazole. https://clinicaltrials.gov/ct2/show/NCT02234570 (Accessed July 23, 2016).

[108] Koul, P.A.; Wahid, A.; Bhat, M.H.; Wani, J.I.; Sofi, B.A. Mepacrine Therapy in Niclosamide Resistant Taeniasis. *J. Assoc. Physicians India*, **2000**, *48*, 402–403.

[109] Ditzel, J.; Schwartz, M. Worm Cure without Tears. The Effect of Niclosamide on Taeniasis Saginata in Man. *Acta Med. Scand.*, **1967**, *182*, 663–664.

[110] Brkić, D.; Glisić, L.; Simić, P. Yomesan in Therapy of Taeniasis and Hymenolepis Nanae. *Med. Glas.*, **1968**, *22*, 107–108.

[111] ClinicalTrials.gov. Auranofin PK Following Oral Dose Administration. https://clinicaltrials.gov/ct2/show/NCT02089048 (Accessed July 25, 2016).

[112] Nightingale, S.L. From the Food and Drug Administration. *JAMA*, **1998**, *280*, 872.

[113] Andrews, K.T.; Fisher, G.; Skinner-Adams, T.S. Drug Repurposing and Human Parasitic Protozoan Diseases. *Int. J. Parasitol. Drugs Drug Resist.*, **2014**, *4*, 95–111.

[114] Bredel, M.; Jacoby, E. Chemogenomics: An Emerging Strategy for Rapid Target and Drug Discovery. *Nat. Rev. Genet.*, **2004**, *5*, 262–275.

[115] Caron, P.R.; Mullican, M.D.; Mashal, R.D.; Wilson, K.P.; Su, M.S.; Murcko, M.A. Chemogenomic Approaches to Drug Discovery. *Curr. Opin. Chem. Biol.*, **2001**, *5*, 464–470.

[116] Mestres, J. Computational Chemogenomics Approaches to Systematic Knowledge-Based Drug Discovery. *Curr. Opin. Drug Discov. Devel.*, **2004**, *7*, 304–313.

[117] Rognan, D. Chemogenomic Approaches to Rational Drug Design. *Br. J. Pharmacol.*, **2007**, *152*, 38–52.

[118] Li, J.; Zheng, S.; Chen, B.; Butte, A.J.; Swamidass, S.J.; Lu, Z. A Survey of Current Trends in Computational Drug Repositioning. *Brief. Bioinform.*, **2016**, *17*, 2–12.

[119] Hurle, M.R.; Yang, L.; Xie, Q.; Rajpal, D.K.; Sanseau, P.; Agarwal, P. Computational Drug Repositioning: From Data to Therapeutics. *Clin. Pharmacol. Ther.*, **2013**, *93*, 335–341.

[120] Wang, Y.; Xiao, J.; Suzek, T.O.; Zhang, J.; Wang, J.; Zhou, Z.; Han, L.; Karapetyan, K.; Dracheva, S.; Shoemaker, B.A.; Bolton, E.; Gindulyte, A.; Bryant, S.H. PubChem's BioAssay Database. *Nucleic Acids Res.*, **2012**, *40*, D400-12.

[121] Gaulton, A.; Bellis, L.J.; Bento, A.P.; Chambers, J.; Davies, M.; Hersey, A.; Light, Y.; McGlinchey, S.; Michalovich, D.; Al-Lazikani, B.; Overington, J.P. ChEMBL: A Large-Scale Bioactivity Database for Drug Discovery. *Nucleic Acids Res.*, **2012**, *40*, D1100-7.

[122] Chen, X.; Liu, M.; Gilson, M.K. BindingDB: A Web-Accessible Molecular Recognition Database. *Comb. Chem. High Throughput Screen.*, **2001**, *4*, 719–725.

[123] Knox, C.; Law, V.; Jewison, T.; Liu, P.; Ly, S.; Frolkis, A.; Pon, A.; Banco, K.; Mak, C.; Neveu, V.; Djoumbou, Y.; Eisner, R.; Guo, A.C.; Wishart, D.S. DrugBank 3.0: A Comprehensive Resource for "Omics" Research on Drugs. *Nucleic Acids Res.*, **2011**, *39*.

[124] Wishart, D.S.; Knox, C.; Guo, A.C.; Shrivastava, S.; Hassanali, M.; Stothard, P.; Chang, Z.; Woolsey, J. DrugBank: A Comprehensive Resource for in Silico Drug Discovery and Exploration. *Nucleic Acids Res.*, **2006**, *34*, D668-72.

[125] Chen, X.; Ji, Z.L.; Chen, Y.Z. TTD: Therapeutic Target Database. *Nucleic Acids Res.*, **2002**, *30*, 412–415.

[126] Zhu, F.; Han, B.; Kumar, P.; Liu, X.; Ma, X.; Wei, X.; Huang, L.; Guo, Y.; Han, L.; Zheng, C.; Chen, Y. Update of TTD: Therapeutic Target Database. *Nucleic Acids Res.*, **2010**, *38*, D787-91.

[127] Neves, B.J.; Dantas, R.F.; Senger, M.R.; Valente, W.C.G.; Rezende-Neto, J. de M.; Chaves, W.T.; Kamentsky, L.; Carpenter, A.; Silva-Junior, F.P.; Andrade, C.H. The Antidepressant Drug Paroxetine as a New Lead Candidate in Schistosome Drug Discovery. *Med. Chem. Commun.*, **2016**, *7*, 1176–1182.

[128] Neves, B.J.; Dantas, R.F.; Senger, M.R.; Melo-Filho, C.C.; Valente, W.C.G.; de Almeida, A.C.M.; Rezende-Neto, J.M.;

Lima, E.F.C.; Paveley, R.; Furnham, N.; Muratov, E.; Kamentsky, L.; Carpenter, A.E.; Braga, R.C.; Silva-Junior, F.P.; Andrade, C.H. Discovery of New Anti-Schistosomal Hits by Integration of QSAR-Based Virtual Screening and High Content Screening. *J. Med. Chem.*, **2016**, *59*, 7075–7088.

[129] Zhang, L.; Fourches, D.; Sedykh, A.; Zhu, H.; Golbraikh, A.; Ekins, S.; Clark, J.; Connelly, M.C.; Sigal, M.; Hodges, D.; Guiguemde, A.; Guy, R.K.; Tropsha, A. Discovery of Novel Antimalarial Compounds Enabled by QSAR-Based Virtual Screening. *J. Chem. Inf. Model.*, **2013**, *53*, 475–492.

[130] Alves, V.M.; Muratov, E.; Fourches, D.; Strickland, J.; Kleinstreuer, N.; Andrade, C.H.; Tropsha, A. Predicting Chemically-Induced Skin Reactions. Part II: QSAR Models of Skin Permeability and the Relationships between Skin Permeability and Skin Sensitization. *Toxicol. Appl. Pharmacol.*, **2015**, *284*, 273–280.

[131] Alves, V.M.; Muratov, E.; Fourches, D.; Strickland, J.; Kleinstreuer, N.; Andrade, C.H.; Tropsha, A. Predicting Chemically-Induced Skin Reactions. Part I: QSAR Models of Skin Sensitization and Their Application to Identify Potentially Hazardous Compounds. *Toxicol. Appl. Pharmacol.*, **2015**, *284*, 262–272.

[132] Braga, R.C.; Alves, V.M.; Silva, M.F.B.; Muratov, E.; Fourches, D.; Tropsha, A.; Andrade, C.H. Tuning hERG out: Antitarget QSAR Models for Drug Development. *Curr. Top. Med. Chem.*, **2014**, *14*, 1399–1415.

[133] Luo, M.; Wang, X.S.; Roth, B.L.; Golbraikh, A.; Tropsha, A. Application of Quantitative Structure–Activity Relationship Models of 5-HT 1A Receptor Binding to Virtual Screening Identifies Novel and Potent 5-HT 1A Ligands. *J. Chem. Inf. Model.*, **2014**, *54*, 634–647.

[134] Melo-Filho, C.C.; Dantas, R.F.; Braga, R.C.; Neves, B.J.; Senger, M.R.; Valente, W.C.G.; Rezende-Neto, J.M.; Chaves, W.T.; Muratov, E.N.; Paveley, R.A.; Furnham, N.; Kamentsky, L.; Carpenter, A.E.; Silva-Junior, F.P.; Andrade, C.H. QSAR-Driven Discovery of Novel Chemical Scaffolds Active against Schistosoma Mansoni. *J. Chem. Inf. Model.*, **2016**, *56*, 1357–1372.

[135] Dubus, E.; Ijjaali, I.; Barberan, O.; Petitet, F. Drug Repositioning Using in Silico Compound Profiling. *Future Med. Chem.*, **2009**, *1*, 1723–1736.

[136] Martorana, A.; Perricone, U.; Lauria, A. The Repurposing of Old Drugs or Unsuccessful Lead Compounds by in Silico Approaches: New Advances and Perspectives. *Curr. Top. Med. Chem.*, **2016**, *16*, 2088–2106.

[137] Ekins, S.; Williams, A.J.; Krasowski, M.D.; Freundlich, J.S. In Silico Repositioning of Approved Drugs for Rare and Neglected Diseases. *Drug Discov. Today*, **2011**, *16*, 298–310.

[138] Achenbach, J.; Tiikkainen, P.; Franke, L.; Proschak, E. Computational Tools for Polypharmacology and Repurposing. *Future Med. Chem.*, **2011**, *3*, 961–968.

[139] Berenstein, A.J.; Magariños, M.P.; Chernomoretz, A.; Agüero, F. A Multilayer Network Approach for Guiding Drug Repositioning in Neglected Diseases. *PLoS Negl. Trop. Dis.*, **2016**, *10*, e0004300.

[140] Fourches, D.; Muratov, E.; Tropsha, A. Curation of Chemogenomics Data. *Nat. Chem. Biol.*, **2015**, *11*, 535.

[141] Fourches, D.; Muratov, E.; Tropsha, A. Trust, but Verify: On the Importance of Chemical Structure Curation in Cheminformatics and QSAR Modeling Research. *J. Chem. Inf. Model.*, **2010**, *50*, 1189–1204.

[142] Fourches, D.; Muratov, E.N.; Tropsha, A. Trust, But Verify II: A Practical Guide to Chemogenomics Data Curation. *J. Chem. Inf. Model.*, **2016**, DOI:10.1021/acs.jcim.6b00129.

[143] Klabunde, T. Chemogenomic Approaches to Drug Discovery: Similar Receptors Bind Similar Ligands. *Br. J. Pharmacol.*, **2007**, *152*, 5–7.

[144] Keiser, M.J.; Setola, V.; Irwin, J.J.; Laggner, C.; Abbas, A.I.; Hufeisen, S.J.; Jensen, N.H.; Kuijer, M.B.; Matos, R.C.; Tran, T.B.; Whaley, R.; Glennon, R. a; Hert, J.; Thomas, K.L.H.; Edwards, D.D.; Shoichet, B.K.; Roth, B.L. Predicting New Molecular Targets for Known Drugs. *Nature*, **2009**, *462*, 175–181.

[145] Keiser, M.J.; Roth, B.L.; Armbruster, B.N.; Ernsberger, P.; Irwin, J.J.; Shoichet, B.K. Relating Protein Pharmacology by Ligand Chemistry. *Nat. Biotechnol.*, **2007**, *25*, 197–206.

[146] Muegge, I.; Mukherjee, P. An Overview of Molecular Fingerprint Similarity Search in Virtual Screening. *Expert Opin. Drug Discov.*, **2016**, *11*, 137–148.

[147] Cereto-Massagué, A.; Ojeda, M.J.; Valls, C.; Mulero, M.; Garcia-Vallvé, S.; Pujadas, G. Molecular Fingerprint Similarity Search in Virtual Screening. *Methods*, **2015**, *71*, 58–63.

[148] Neves, B.J.; Muratov, E.; Machado, R.B.; Andrade, C.H.; Cravo, P.V.L. Modern Approaches to Accelerate Discovery of New Antischistosomal Drugs. *Expert Opin. Drug Discov.*, **2016**, *11*, 557–567.

[149] Vuorinen, A.; Schuster, D. Methods for Generating and Applying Pharmacophore Models as Virtual Screening Filters and for Bioactivity Profiling. *Methods*, **2015**, *71*, 113–134.

[150] Caporuscio, F.; Tafi, A. Pharmacophore Modelling: A Forty Year Old Approach and Its Modern Synergies. *Curr. Med. Chem.*, **2011**, *18*, 2543–2553.

[151] Koes, D.R.; Camacho, C.J. Shape-Based Virtual Screening with Volumetric Aligned Molecular Shapes. *J. Comput. Chem.*, **2014**, *35*, 1824–1834.

[152] Kortagere, S.; Krasowski, M.D.; Ekins, S. The Importance of Discerning Shape in Molecular Pharmacology. *Trends Pharmacol. Sci.*, **2009**, *30*, 138–147.

[153] Braga, R.C.; Andrade, C.H. Assessing the Performance of 3D Pharmacophore Models in Virtual Screening: How Good Are

They? *Curr. Top. Med. Chem.*, **2013**, *13*, 1127–1138.

[154] Boström, J.; Greenwood, J.R.; Gottfries, J. Assessing the Performance of OMEGA with Respect to Retrieving Bioactive Conformations. *J. Mol. Graph. Model.*, **2003**, *21*, 449–462.

[155] Lavecchia, A. Machine-Learning Approaches in Drug Discovery: Methods and Applications. *Drug Discov. Today*, **2014**, *20*, 318–331.

[156] Melville, J.L.; Burke, E.K.; Hirst, J.D. Machine Learning in Virtual Screening. *Comb. Chem. High Throughput Screen.*, **2009**, *12*, 332–343.

[157] Dobchev, D.; Pillai, G.; Karelson, M. In Silico Machine Learning Methods in Drug Development. *Curr. Top. Med. Chem.*, **2014**, *14*, 1913–1922.

[158] Liu, Q.; Zhou, H.; Liu, L.; Chen, X.; Zhu, R.; Cao, Z. Multi-Target QSAR Modelling in the Analysis and Design of HIV-HCV Co-Inhibitors: An in-Silico Study. *BMC Bioinformatics*, **2011**, *12*, 294.

[159] Speck-Planche, A.; Cordeiro, M.N.D.S. Multi-Target QSAR Approaches for Modeling Protein Inhibitors. Simultaneous Prediction of Activities Against Biomacromolecules Present in Gram-Negative Bacteria. *Curr. Top. Med. Chem.*, **2015**, *15*, 1801–1813.

[160] Lima, A.N.; Philot, E.A.; Trossini, G.H.G.; Scott, L.P.B.; Maltarollo, V.G.; Honorio, K.M. Use of Machine Learning Approaches for Novel Drug Discovery. *Expert Opin. Drug Discov.*, **2016**, *11*, 225–239.

[161] Cherkasov, A.; Muratov, E.N.; Fourches, D.; Varnek, A.; Baskin, I.I.; Cronin, M.; Dearden, J.; Gramatica, P.; Martin, Y.C.; Todeschini, R.; Consonni, V.; Kuz'Min, V.E.; Cramer, R.; Benigni, R.; Yang, C.; Rathman, J.; Terfloth, L.; Gasteiger, J.; Richard, A.; Tropsha, A. QSAR Modeling: Where Have You Been? Where Are You Going To? *Journal of Medicinal Chemistry*, **2014**, *57*, 4977–5010.

[162] Tropsha, A. Best Practices for QSAR Model Development, Validation, and Exploitation. *Mol. Inform.*, **2010**, *29*, 476–488.

[163] Mitchell, J.B.O. Machine Learning Methods in Chemoinformatics. *Wiley Interdiscip. Rev. Comput. Mol. Sci.*, **2014**, *4*, 468–481.

[164] Muratov, E.N.; Artemenko, A.G.; Varlamova, E. V; Polischuk, P.G.; Lozitsky, V.P.; Fedchuk, A.S.; Lozitska, R.L.; Gridina, T.L.; Koroleva, L.S.; Sil'nikov, V.N.; Galabov, A.S.; Makarov, V. a; Riabova, O.B.; Wutzler, P.; Schmidtke, M.; Kuz'min, V.E. Per Aspera Ad Astra: Application of Simplex QSAR Approach in Antiviral Research. *Future Med. Chem.*, **2010**, *2*, 1205–1226.

[165] Chang, C.Y.; Hsu, M.T.; Esposito, E.X.; Tseng, Y.J. Oversampling to Overcome Overfitting: Exploring the Relationship between Data Set Composition, Molecular Descriptors, and Predictive Modeling Methods. *J. Chem. Inf. Model.*, **2013**, *53*, 958–971.

[166] Zakharov, A. V; Peach, M.L.; Sitzmann, M.; Nicklaus, M.C. QSAR Modeling of Imbalanced High-Throughput Screening Data in PubChem. *J. Chem. Inf. Model.*, **2014**, *54*, 705–712.

[167] Rose, P.W.; Prlić, A.; Bi, C.; Bluhm, W.F.; Christie, C.H.; Dutta, S.; Green, R.K.; Goodsell, D.S.; Westbrook, J.D.; Woo, J.; Young, J.; Zardecki, C.; Berman, H.M.; Bourne, P.E.; Burley, S.K. The RCSB Protein Data Bank: Views of Structural Biology for Basic and Applied Research and Education. *Nucleic Acids Res.*, **2015**, *43*, D345-56.

[168] Pollastri, M.P.; Campbell, R.K. Target Repurposing for Neglected Diseases. *Future Med. Chem.*, **2011**, *3*, 1307–1315.

[169] Finn, R.D.; Coggill, P.; Eberhardt, R.Y.; Eddy, S.R.; Mistry, J.; Mitchell, A.L.; Potter, S.C.; Punta, M.; Qureshi, M.; Sangrador-Vegas, A.; Salazar, G.A.; Tate, J.; Bateman, A. The Pfam Protein Families Database: Towards a More Sustainable Future. *Nucleic Acids Res.*, **2016**, *44*, D279–D285.

[170] Sonnhammer, E.L.; Eddy, S.R.; Durbin, R. Pfam: A Comprehensive Database of Protein Domain Families Based on Seed Alignments. *Proteins*, **1997**, *28*, 405–420.

[171] Kristensen, D.M.; Wolf, Y.I.; Mushegian, A.R.; Koonin, E. V. Computational Methods for Gene Orthology Inference. *Brief. Bioinform.*, **2011**, *12*, 379–391.

[172] Wang, Y.; Coleman-Derr, D.; Chen, G.; Gu, Y.Q. OrthoVenn: A Web Server for Genome Wide Comparison and Annotation of Orthologous Clusters across Multiple Species. *Nucleic Acids Res.*, **2015**, *43*, W78–W84.

[173] Li, L. OrthoMCL: Identification of Ortholog Groups for Eukaryotic Genomes. *Genome Res.*, **2003**, *13*, 2178–2189.

[174] Altschul, S.F.; Madden, T.L.; Schäffer, A. a.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D.J. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs. *Nucleic Acids Res.*, **1997**, *25*, 3389–3402.

[175] Glaser, F.; Pupko, T.; Paz, I.; Bell, R.E.; Bechor-Shental, D.; Martz, E.; Ben-Tal, N. ConSurf: Identification of Functional Regions in Proteins by Surface-Mapping of Phylogenetic Information. *Bioinformatics*, **2003**, *19*, 163–164.

[176] Ashkenazy, H.; Erez, E.; Martz, E.; Pupko, T.; Ben-Tal, N. ConSurf 2010: Calculating Evolutionary Conservation in Sequence and Structure of Proteins and Nucleic Acids. *Nucleic Acids Res.*, **2010**, *38*, W529-33.

[177] Singh, S.; Malik, B.K.; Sharma, D.K. Choke Point Analysis of Metabolic Pathways in E.histolytica: A Computational Approach for Drug Target Identification. *Bioinformation*, **2007**, *2*, 68–72.

[178] Taylor, C.M.; Wang, Q.; Rosa, B.A.; Huang, S.C.-C.; Powell, K.; Schedl, T.; Pearce, E.J.; Abubucker, S.; Mitreva, M. Discovery of Anthelmintic Drug Targets and Drugs Using Chokepoints in Nematode Metabolic Pathways. *PLoS Pathog.*, **2013**, *9*, e1003505.

[179] Jomaa, H.; Wiesner, J.; Sanderbrand, S.; Altincicek, B.; Weidemeyer, C.; Hintz, M.; Türbachova, I.; Eberl, M.; Zeidler, J.;

Lichtenthaler, H.K.; Soldati, D.; Beck, E. Inhibitors of the Nonmevalonate Pathway of Isoprenoid Biosynthesis as Antimalarial Drugs. *Science*, **1999**, *285*, 1573–1576.

[180] Njoroge, M.; Njuguna, N.M.; Mutai, P.; Ongarora, D.S.B.; Smith, P.W.; Chibale, K. Recent Approaches to Chemical Discovery and Development Against Malaria and the Neglected Tropical Diseases Human African Trypanosomiasis and Schistosomiasis. *Chem. Rev.*, **2014**, *114*, 11138–11163.

[181] Beghyn, T.B.; Charton, J.; Leroux, F.; Laconde, G.; Bourin, A.; Cos, P.; Maes, L.; Deprez, B. Drug to Genome to Drug: Discovery of New Antiplasmodial Compounds. *J. Med. Chem.*, **2011**, *54*, 3222–3240.

[182] Sateriale, A.; Bessoff, K.; Sarkar, I.N.; Huston, C.D. Drug Repurposing: Mining Protozoan Proteomes for Targets of Known Bioactive Compounds. *J. Am. Med. Informatics Assoc.*, **2014**, *21*, 238–244.

[183] Ehrt, C.; Brinkjost, T.; Koch, O. Impact of Binding Site Comparisons on Medicinal Chemistry and Rational Molecular Design. *J. Med. Chem.*, **2016**, *59*, 4121–4151.

[184] Milletti, F.; Vulpetti, A. Predicting Polypharmacology by Binding Site Similarity: From Kinases to the Protein Universe. *J. Chem. Inf. Model.*, **2010**, *50*, 1418–1431.

[185] De Franchi, E.; Schalon, C.; Messa, M.; Onofri, F.; Benfenati, F.; Rognan, D. Binding of Protein Kinase Inhibitors to Synapsin I Inferred from Pair-Wise Binding Site Similarity Measurements. *PLoS One*, **2010**, *5*, e12214.

[186] Batista, J.; Hawkins, P.C.; Tolbert, R.; Geballe, M.T. SiteHopper - a Unique Tool for Binding Site Comparison. *J. Cheminform.*, **2014**, *6*, P57.

[187] Binkowski, T.A.; Joachimiak, A. Protein Functional Surfaces: Global Shape Matching and Local Spatial Alignments of Ligand Binding Sites. *BMC Struct. Biol.*, **2008**, *8*, 45.

[188] Das, S.; Kokardekar, A.; Breneman, C.M. Rapid Comparison of Protein Binding Site Surfaces with Property Encoded Shape Distributions. *J. Chem. Inf. Model.*, **2009**, *49*, 2863–2872.

[189] Wood, D.J.; de Vlieg, J.; Wagener, M.; Ritschel, T. Pharmacophore Fingerprint-Based Approach to Binding Site Subpocket Similarity and Its Application to Bioisostere Replacement. *J. Chem. Inf. Model.*, **2012**, *52*, 2031–2043.

[190] Najmanovich, R.; Kurbatova, N.; Thornton, J. Detection of 3D Atomic Similarities and Their Use in the Discrimination of Small Molecule Protein-Binding Sites. *Bioinformatics*, **2008**, *24*, i105–i111.

[191] Schmitt, S.; Kuhn, D.; Klebe, G. A New Method to Detect Related Function Among Proteins Independent of Sequence and Fold Homology. *J. Mol. Biol.*, **2002**, *323*, 387–406.

[192] Tu, H.; Shi, T. Ligand Binding Site Similarity Identification Based on Chemical and Geometric Similarity. *Protein J.*, **2013**, *32*, 373–385.

[193] Yeturu, K.; Chandra, N. PocketMatch: A New Algorithm to Compare Binding Sites in Protein Structures. *BMC Bioinformatics*, **2008**, *9*, 543.

[194] Totrov, M. Ligand Binding Site Superposition and Comparison Based on Atomic Property Fields: Identification of Distant Homologues, Convergent Evolution and PDB-Wide Clustering of Binding Sites. *BMC Bioinformatics*, **2011**, *12*, S35.

[195] Baroni, M.; Cruciani, G.; Sciabola, S.; Perruccio, F.; Mason, J.S. A Common Reference Framework for Analyzing/Comparing Proteins and Ligands. Fingerprints for Ligands And Proteins (FLAP): Theory and Application. *J. Chem. Inf. Model.*, **2007**, *47*, 279–294.

[196] Goodford, P.J. A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules. *J. Med. Chem.*, **1985**, *28*, 849–857.

[197] Cerqueira, N.M.F.S.A.; Gesto, D.; Oliveira, E.F.; Santos-Martins, D.; Brás, N.F.; Sousa, S.F.; Fernandes, P.A.; Ramos, M.J. Receptor-Based Virtual Screening Protocol for Drug Discovery. *Arch. Biochem. Biophys.*, **2015**, *582*, 56–67.

[198] Ferreira, L.; dos Santos, R.; Oliva, G.; Andricopulo, A. Molecular Docking and Structure-Based Drug Design Strategies. *Molecules*, **2015**, *20*, 13384–13421.

[199] Lionta, E.; Spyrou, G.; Vassilatis, D.K.; Cournia, Z. Structure-Based Virtual Screening for Drug Discovery: Principles, Applications and Recent Advances. *Curr. Top. Med. Chem.*, **2014**, *14*, 1923–1938.

[200] Kharkar, P.S.; Warrier, S.; Gaud, R.S. Reverse Docking: A Powerful Tool for Drug Repositioning and Drug Rescue. *Future Med. Chem.*, **2014**, *6*, 333–342.

[201] Braga, R.C.; Alves, V.M.; Silva, A.C.; Liao, L.M.; Andrade, C.H. Virtual Screening Strategies in Medicinal Chemistry: The State of the Art and Current Challenges. *Curr. Top. Med. Chem.*, **2014**, *14*, 1899–1912.

[202] Hou, X.; Li, K.; Yu, X.; Sun, J.; Fang, H. Protein Flexibility in Docking-Based Virtual Screening: Discovery of Novel Lymphoid-Specific Tyrosine Phosphatase Inhibitors Using Multiple Crystal Structures. *J. Chem. Inf. Model.*, **2015**, *55*, 1973–1983.

[203] Liao, C.; Sitzmann, M.; Pugliese, A.; Nicklaus, M.C. Software and Resources for Computational Medicinal Chemistry. *Future Med. Chem.*, **2011**, *3*, 1057–1085.

[204] Zhong, S.; Zhang, Y.; Xiu, Z. Rescoring Ligand Docking Poses. *Curr. Opin. Drug Discov. Devel.*, **2010**, *13*, 326–334.

[205] Kollman, P.A.; Massova, I.; Reyes, C.; Kuhn, B.; Huo, S.; Chong, L.; Lee, M.; Lee, T.; Duan, Y.; Wang, W.; Donini, O.; Cieplak, P.; Srinivasan, J.; Case, D.A.; Cheatham, T.E. Calculating Structures and Free Energies of Complex Molecules: Combining Molecular Mechanics and Continuum Models. *Acc. Chem. Res.*, **2000**, *33*, 889–897.

[206] Wang, J.; Morin, P.; Wang, W.; Kollman, P.A. Use of MM-PBSA in Reproducing the Binding Free Energies to HIV-1 RT

of TIBO Derivatives and Predicting the Binding Mode to HIV-1 RT of Efavirenz by Docking and MM-PBSA. *J. Am. Chem. Soc.*, **2001**, *123*, 5221–5230.

[207] Lyne, P.D.; Lamb, M.L.; Saeh, J.C. Accurate Prediction of the Relative Potencies of Members of a Series of Kinase Inhibitors Using Molecular Docking and MM-GBSA Scoring. *J. Med. Chem.*, **2006**, *49*, 4805–4808.

[208] Brown, S.P.; Muchmore, S.W. High-Throughput Calculation of Protein-Ligand Binding Affinities: Modification and Adaptation of the MM-PBSA Protocol to Enterprise Grid Computing. *J. Chem. Inf. Model.*, *46*, 999–1005.

[209] Guimarães, C.R.W.; Cardozo, M. MM-GB/SA Rescoring of Docking Poses in Structure-Based Lead Optimization. *J. Chem. Inf. Model.*, **2008**, *48*, 958–970.

[210] Magariños, M.P.; Carmona, S.J.; Crowther, G.J.; Ralph, S. a.; Roos, D.S.; Shanmugam, D.; Van Voorhis, W.C.; Agüero, F. TDR Targets: A Chemogenomics Resource for Neglected Diseases. *Nucleic Acids Res.*, **2012**, *40*, 1118–1127.

[211] Agüero, F.; Al-Lazikani, B.; Aslett, M.; Berriman, M.; Buckner, F.S.; Campbell, R.K.; Carmona, S.; Carruthers, I.M.; Chan, a W.E.; Chen, F.; Crowther, G.J.; Doyle, M. a; Hertz-Fowler, C.; Hopkins, A.L.; McAllister, G.; Nwaka, S.; Overington, J.P.; Pain, A.; Paolini, G. V; Pieper, U.; Ralph, S. a; Riechers, A.; Roos, D.S.; Sali, A.; Shanmugam, D.; Suzuki, T.; Van Voorhis, W.C.; Verlinde, C.L.M.J. Genomic-Scale Prioritization of Drug Targets: The TDR Targets Database. *Nat. Rev. Drug Discov.*, **2008**, *7*, 900–907.

[212] Jones, P.; Binns, D.; Chang, H.-Y.; Fraser, M.; Li, W.; McAnulla, C.; McWilliam, H.; Maslen, J.; Mitchell, A.; Nuka, G.; Pesseat, S.; Quinn, A.F.; Sangrador-Vegas, A.; Scheremetjew, M.; Yong, S.-Y.; Lopez, R.; Hunter, S. InterProScan 5: Genome-Scale Protein Function Classification. *Bioinformatics*, **2014**, *30*, 1236–1240.

[213] Ogata, H.; Goto, S.; Sato, K.; Fujibuchi, W.; Bono, H.; Kanehisa, M. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.*, **2000**, *27*, 29–34.

[214] Colley, D.G.; Bustinduy, A.L.; Secor, W.E.; King, C.H. Human Schistosomiasis. *Lancet*, **2014**, *383*, 2253–2264.

[215] Neves, B.J.; Braga, R.C.; Bezerra, J.C.B.; Cravo, P.V.L.; Andrade, C.H. In Silico Repositioning-Chemogenomics Strategy Identifies New Drugs with Potential Activity against Multiple Life Stages of Schistosoma Mansoni. *PLoS Negl. Trop. Dis.*, **2015**, *9*, e3435.

[216] Protasio, A. V; Tsai, I.J.; Babbage, A.; Nichol, S.; Hunt, M.; Aslett, M. a; De Silva, N.; Velarde, G.S.; Anderson, T.J.C.; Clark, R.C.; Davidson, C.; Dillon, G.P.; Holroyd, N.E.; LoVerde, P.T.; Lloyd, C.; McQuillan, J.; Oliveira, G.; Otto, T.D.; Parker-Manuel, S.J.; Quail, M. a; Wilson, R.A.; Zerlotini, A.; Dunne, D.W.; Berriman, M. A Systematically Improved High Quality Genome and Transcriptome of the Human Blood Fluke Schistosoma Mansoni. *PLoS Negl. Trop. Dis.*, **2012**, *6*, e1455.

[217] Kuhn, M.; Szklarczyk, D.; Franceschini, A.; von Mering, C.; Jensen, L.J.; Bork, P. STITCH 3: Zooming in on Protein-Chemical Interactions. *Nucleic Acids Res.*, **2012**, *40*, D876-80.

[218] Kuhn, M.; von Mering, C.; Campillos, M.; Jensen, L.J.; Bork, P. STITCH: Interaction Networks of Chemicals and Proteins. *Nucleic Acids Res.*, **2008**, *36*, D684-8.

[219] Patocka, N.; Ribeiro, P. Characterization of a Serotonin Transporter in the Parasitic Flatworm, Schistosoma Mansoni: Cloning, Expression and Functional Analysis. *Mol. Biochem. Parasitol.*, **2007**, *154*, 125–133.

[220] Fontana, A.C.K.; Sonders, M.S.; Pereira-Junior, O.S.; Knight, M.; Javitch, J.A.; Rodrigues, V.; Amara, S.G.; Mortensen, O. V. Two Allelic Isoforms of the Serotonin Transporter from Schistosoma Mansoni Display Electrogenic Transport and High Selectivity for Serotonin. *Eur. J. Pharmacol.*, **2009**, *616*, 48–57.

[221] Mansour, T.E. Serotonin Receptors in Parasitic Worms. *Adv. Parasitol.*, **1985**, *23*, 1–36.

[222] Rahman, M.S.; Mettrick, D.F.; Podesta, R.B. Schistosoma Mansoni: Effects of in Vitro Serotonin (5-HT) on Aerobic and Anaerobic Carbohydrate Metabolism. *Exp. Parasitol.*, **1985**, *60*, 10–17.

[223] Boyle, J.P.; Zaide, J. V; Yoshino, T.P. Schistosoma Mansoni: Effects of Serotonin and Serotonin Receptor Antagonists on Motility and Length of Primary Sporocysts in Vitro. *Exp. Parasitol.*, **2000**, *94*, 217–226.

[224] Boyle, J.P.; Yoshino, T.P. Serotonin-Induced Muscular Activity in Schistosoma Mansoni Larval Stages: Importance of 5-HT Transport and Role in Daughter Sporocyst Production. *J. Parasitol.*, **2005**, *91*, 542–550.

[225] Hotez, P.J.; Molyneux, D.H.; Fenwick, A.; Kumaresan, J.; Sachs, S.E.; Sachs, J.D.; Savioli, L. Control of Neglected Tropical Diseases. *N. Engl. J. Med.*, **2007**, *357*, 1018–1027.

[226] Croft, S.L.; Barrett, M.P.; Urbina, J.A. Chemotherapy of Trypanosomiases and Leishmaniasis. *Trends in Parasitology*, **2005**, *21*, 508–512.

[227] Bland, N.D.; Wang, C.; Tallman, C.; Gustafson, A.E.; Wang, Z.; Ashton, T.D.; Ochiana, S.O.; McAllister, G.; Cotter, K.; Fang, A.P.; Gechijian, L.; Garceau, N.; Gangurde, R.; Ortenberg, R.; Ondrechen, M.J.; Campbell, R.K.; Pollastri, M.P. Pharmacological Validation of Trypanosoma Brucei Phosphodiesterases B1 and B2 as Druggable Targets for African Sleeping Sickness. *J. Med. Chem.*, **2011**, *54*, 8188–8194.

[228] Wang, Q.; Rosa, B.A.; Nare, B.; Powell, K.; Valente, S.; Rotili, D.; Mai, A.; Marshall, G.R.; Mitreva, M. Targeting Lysine Deacetylases (KDACs) in Parasites. *PLoS Negl. Trop. Dis.*, **2015**, *9*, e0004026.

[229] Kobets, T.; Grekov, I.; Lipoldova, M. Leishmaniasis: Prevention, Parasite Detection and Treatment. *Curr. Med. Chem.*, **2012**, *19*, 1443–1474.

[230] WHO. Control of the Leishmaniases. In: *WHO Expert Committee on the Control of Leishmaniases*; Geneva, **2010**; pp. 1–

186.

[231] Freitas-Junior, L.H.; Chatelain, E.; Kim, H.A.; Siqueira-Neto, J.L. Visceral Leishmaniasis Treatment: What Do We Have, What Do We Need and How to Deliver It? *Int. J. Parasitol. Drugs Drug Resist.*, **2012**, *2*, 11–19.

[232] Chavali, A.K.; Whittemore, J.D.; Eddy, J.A.; Williams, K.T.; Papin, J.A. Systems Analysis of Metabolism in the Pathogenic Trypanosomatid Leishmania Major. *Mol. Syst. Biol.*, **2008**, *4*, 177.

[233] Chavali, A.K.; Blazier, A.S.; Tlaxca, J.L.; Jensen, P.A.; Pearson, R.D.; Papin, J.A. Metabolic Network Analysis Predicts Efficacy of FDA-Approved Drugs Targeting the Causative Agent of a Neglected Tropical Disease. *BMC Syst. Biol.*, **2012**, *6*, 27.

[234] Logan-Klumpler, F.J.; De Silva, N.; Boehme, U.; Rogers, M.B.; Velarde, G.; McQuillan, J.A.; Carver, T.; Aslett, M.; Olsen, C.; Subramanian, S.; Phan, I.; Farris, C.; Mitra, S.; Ramasamy, G.; Wang, H.; Tivey, A.; Jackson, A.; Houston, R.; Parkhill, J.; Holden, M.; Harb, O.S.; Brunk, B.P.; Myler, P.J.; Roos, D.; Carrington, M.; Smith, D.F.; Hertz-Fowler, C.; Berriman, M. GeneDB--an Annotation Database for Pathogens. *Nucleic Acids Res.*, **2012**, *40*, 98–108.

[235] Chen, X.; Ji, Z.L.; Chen, Y.Z. TTD: Therapeutic Target Database. *Nucleic Acids Res.*, **2002**, *30*, 412–415.

[236] Cibulskis, R.E.; Alonso, P.; Aponte, J.; Aregawi, M.; Barrette, A.; Bergeron, L.; Fergus, C.A.; Knox, T.; Lynch, M.; Patouillard, E.; Schwarte, S.; Stewart, S.; Williams, R. Malaria: Global Progress 2000 – 2015 and Future Challenges. *Infect. Dis. Poverty*, **2016**, *5*, 61.

[237] Tilley, L.; Straimer, J.; Gnädig, N.F.; Ralph, S.A.; Fidock, D.A. Artemisinin Action and Resistance in Plasmodium Falciparum. *Trends Parasitol.*, **2016**, *xx*, 1–15.

[238] Woodrow, C.J.; Krishna, S. Antimalarial Drugs: Recent Advances in Molecular Determinants of Resistance and Their Clinical Significance. *Cell. Mol. Life Sci.*, **2006**, *63*, 1586–1596.

[239] Le Bras, J.; Durand, R. The Mechanisms of Resistance to Antimalarial Drugs in Plasmodium Falciparum. *Fundam. Clin. Pharmacol.*, **2003**, *17*, 147–153.

[240] Wilson, R.J.; Williamson, D.H.; Preiser, P. Malaria and Other Apicomplexans: The "plant" connection. *Infect. Agents Dis.*, **1994**, *3*, 29–37.

[241] McFadden, G.I.; Reith, M.E.; Munholland, J.; Lang-Unnasch, N. Plastid in Human Parasites. *Nature*, **1996**, *381*, 482–482.

[242] Moore, R.B.; Oborník, M.; Janouškovec, J.; Chrudimský, T.; Vancová, M.; Green, D.H.; Wright, S.W.; Davies, N.W.; Bolch, C.J.S.; Heimann, K.; Šlapeta, J.; Hoegh-Guldberg, O.; Logsdon, J.M.; Carter, D.A. A Photosynthetic Alveolate Closely Related to Apicomplexan Parasites. *Nature*, **2008**, *451*, 959–963.

[243] Ralph, S.A.; van Dooren, G.G.; Waller, R.F.; Crawford, M.J.; Fraunholz, M.J.; Foth, B.J.; Tonkin, C.J.; Roos, D.S.; McFadden, G.I. Metabolic Maps and Functions of the Plasmodium Falciparum Apicoplast. *Nat. Rev. Microbiol.*, **2004**, *2*, 203–216.

[244] Budimulja, A.S.; Syafruddin; Tapchaisri, P.; Wilairat, P.; Marzuki, S. The Sensitivity of Plasmodium Protein Synthesis to Prokaryotic Ribosomal Inhibitors. *Mol. Biochem. Parasitol.*, **1997**, *84*, 137–141.

[245] Yeh, E.; DeRisi, J.L. Chemical Rescue of Malaria Parasites Lacking an Apicoplast Defines Organelle Function in Blood-Stage Plasmodium Falciparum. *PLoS Biol.*, **2011**, *9*, e1001138.

[246] Lalloo, D.G.; Shingadia, D.; Pasvol, G.; Chiodini, P.L.; Whitty, C.J.; Beeching, N.J.; Hill, D.R.; Warrell, D.A.; Bannister, B.A. UK Malaria Treatment Guidelines. *J. Infect.*, **2007**, *54*, 111–121.

[247] Chakraborty, A. Understanding the Biology of the Plasmodium Falciparum Apicoplast; an Excellent Target for Antimalarial Drug Development. *Life Sci.*, **2016**, *158*, 104–110.

[248] Bispo, N.A.; Culleton, R.; Silva, L.A.; Cravo, P. A Systematic in Silico Search for Target Similarity Identifies Several Approved Drugs with Potential Activity against the Plasmodium Falciparum Apicoplast. *PLoS One*, **2013**, *8*, e59288.

[249] Kundu, C.N.; Das, S.; Nayak, A.; Satapathy, S.R.; Das, D.; Siddharth, S. Anti-Malarials Are Anti-Cancers and Vice Versa - One Arrow Two Sparrows. *Acta Trop.*, **2015**, *149*, 113–127.

[250] Bahl, A. PlasmoDB: The Plasmodium Genome Resource. A Database Integrating Experimental and Computational Data. *Nucleic Acids Res.*, **2003**, *31*, 212–215.

6 DISCUSSÃO

No presente trabalho foi possível identificar novos candidatos a fármacos antimaláricos através da estratégia de quimiogenômica por reposicionamento e validação experimental dos fármacos selecionados em ensaios *in vitro* em *P. falciparum* (artigos 1 e 2).

6.1- Descoberta de novos fármacos para doenças negligenciadas, através da estratégia de quimiogenômica por reposicionamento

No artigo 2, foi elaborada uma revisão sobre a descoberta de novos fármacos para doenças negligenciadas utilizando a estratégia de quimiogenômica por reposicionamento. Sabe-se o quanto é onerosa e dispendiosa a P&D de novos fármacos, até o que o mesmo esteja disponível para o uso clinico. Infelizmente os investimentos para o planejamento e desenvolvimento de novos fármacos contra doenças negligenciadas são limitados, e as estratégias computacionais tem sido aliada para acelerar as novas descobertas.

A quimiogenômica representa um campo promissor para a descoberta de novos fármacos e envolve investigação de bibliotecas moleculares capazes de interagir com alvos biológicos de interesse utilizando bioensaios automatizados de alta vazão (BREDEL; JACOBY, 2004; NEVES, 2015). Tem por objetivo identificar todos os possíveis ligantes para todos os alvos biológicos possíveis (CARON et al., 2001; ROGNAN, 2007).

A busca por similaridade de ligantes é usada para prever novas atividades biológicas para um amplo banco de dados de fármacos (*DrugBank*, por exemplo), medindo seu nível de similaridade estrutural como um conhecido conjunto de ligantes (DUBUS et al., 2009). Muitas predições já foram realizadas através desta abordagem. Keiser e colaboradores, por exemplo, usando fármacos aprovados com seus respectivos alvos (anotados), conseguiram prever milhares de associações de fármacos e alvos, os quais ainda não eram conhecidos (KEISER et al., 2007, 2009). Posteriormente, as 23 associações preditas foram confirmadas experimentalmente, sendo que cinco eram potentes a baixas concentrações namomolares (KEISER et al., 2009).

O Projeto Genoma Humano (HUMAN; PROJECT, 2001) veio contribuir para o avanço da ciência nestas últimas décadas e com o desenvolvimento de novos fármacos. Muitos outros organismos foram sequenciados e seu genoma depositado em bancos de dados, disponíveis de forma gratuita, para consulta e uso das informações neles contidas (AURRECOECHEA et al., 2017). Assim, avanços recentes no sequenciamento dos patógenos causadores das DTN, fornece recursos inestimáveis para compreender a biologia, metabolismo e interações dos organismos com seus hospedeiros.

As ferramentas de alinhamento de proteínas podem ser aplicadas ao reposicionamento de fármacos, considerando o pressuposto de que as proteínas que compartilham algum tipo de semelhança estrutural, podem compartilhar o mesmo ligante (ROGNAN, 2007, KLABUNDE, 2007, POLLASTRI; CAMPBELL, 2011, KLUG; GELB; POLLASTRI, 2016). Entretanto, vale ressaltar que apesar desta abordagem parecer simplista, a identificação de proteínas ortólogas é bem complexa e não podemos nos esquecer da história evolutiva dos organismos, nas quais podem passar por mutações, recombinações, fluxo gênico, etc (KRISTENSEN et al., 2011a). Portanto, sugere-se que outras abordagens sejam aliadas para que haja um confiabilidade tais como: alinhamentos de proteínas (ALTSCHUL et al., 1997; WANG et al., 2015b), árvores filogenéticas e análise de grafos(KRISTENSEN et al., 2011a), análise de domínios e resíduos funcionais (ASHKENAZY et al., 2010a; GLASER et al., 2003) e identificação de *chokepoints* em redes metabólicas (SINGH; MALIK; SHARMA, 2007, TAYLOR et al., 2013).

A abordagem de quimiogenômica na busca de novos alvos já foi aplicada em outros trabalhos no nosso grupo de pesquisa, como na identificação de candidatos a fármacos esquistossomicidas (NEVES et al., 2015), e anti-chagásicos (RODRIGUES et al., 2015). Também foi possível a identificação de fármacos aprovados com atividade no metabolismo energético em *Leishmania* (SILVA et al., 2015).

Em um dos trabalhos supracitados possível a identificação de fármacos aprovados com atividade predita e/ou confirmada contra o apicoplasto de *P. falciparum* (BISPO et al., 2013). Foram compiladas proteínas específicas de aplicoplasto de *P. falciparum* e interrogadas em 3 bases de dados (DrugBank, Stich e TTD), assim foi possível, identificar que esses interagiam com 47

proteinas envolvidas na biologia do apicoplasto de *P. falciparum*, sendo que 15 desses alvos putativos tiveram atividade com medicamentos já aprovados para o uso clinico, entretanto, atividade antimalárica nunca fora avaliada.

Ressaltamos que os fármacos ácido azelaico (Figura 11) e a tiocarlida (Figura 12), foram preditos em nosso trabalho (Artigo 1) como candidatos a antimaláricos, e que existem concomitância nos alvos propostos para os trabalhos de Bispo (BISPO et al., 2013) e Rodrigues (Artigo 1), corroborando com a idéia de que aliar diversas abordagens, nos confere maior acurácia nos resultados obtidos (WANG et al., 2015b).

Figura 11- Estrutura química do ácido azelaico

Outros estudos *in silico* (RANAKRISHNAN et al., 2017) identificaram recentemente o acido azelaico como um candidato antimalárico, usaram análises comparativas de sequências e estruturas de fármacos aprovados pelo FDA (em inglês *Food and Drug Administration*), originalmente usados outros agentes patogênicos. Foi proposta uma avaliação comparativa dos sítios de ligação entre os alvos conhecidos e seus respectivos homólogos (*P. falciparum*), baseando na disponilidade de detalhes moleculares das proteínas alvo: complementos alvos-inibidores, complementos de alvo-substrato e locais de ligação de ligantes desconhecidos (RANAKRISHNAN et al., 2015)

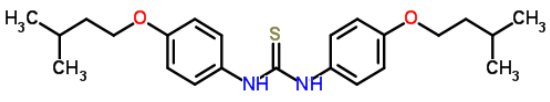


Figura 12- Estrutura química da tiocarlida.

O ácido azelaico (Figura 11) demonstra uma boa capacidade de se difundir através de biomembranas (BOTT et al., 2011), embora, esta propriedade não seja um pré-requisito para medicamentos cujo alvo seja o apicoplasto. Mas é uma vantagem, tendo em vista que o fármaco terá que atravessar 6 membranas. Entretanto, o ácido azelaico é comercialmente disponibilizado, em creme de uso tópico, portanto, testá-lo como antimalárico apresenta grandes desafios.

A tiocarlida (Figura 12), um derivado de tiouréia que foi utilizado com sucesso para o tratamento clínico da tuberculose durante a década de 1960 (PHETSUKSIRI et al., 1999), mostrou exibir atividade antimicrobacteriana significativa *in vitro* e é eficaz contra cepas resistentes a múltiplos fármacos de *Mycobacterium tuberculosis* (PHETSUKSIRI et al., 2003). Bispo e colaboradores (BISPO, et al., 2015) sugeriram que o isoxyl pode ser capaz de inibir o alvo homólogo de *P. falciparum stearoyl-CoA delta 9 desaturase*, esta enzima está correlacionada a síntese de ácidos graxos no apicoplasto. Este fármaco apresenta baixa toxicidade, com efeitos colaterais não descritos, tornando um excelente candidato a ser reposicionado como antimalárico.

Assim, sugere-se que, outras abordagens de quimioinformática sejam exploradas, com o intuito de investigar se o presente fármaco demonstra ou não atividade antimalárica. Como por exemplo, comparar os sitios de ligações das proteínas (alvos), através de dados estruturais em 3D (Artigo 2), a partir do

pressuposto que sítios com características semelhantes compartilham ligantes semelhantes (ROGNAN, 2007). Esta abordagem tem sido considerada promissora considerando o exposto acima.

Podemos citar um outro trabalho do nosso grupo, em que Neves e colaboradores (NEVES et al., 2016b), através da abordagem de quimiogenômica por reposicionamento *in silico*, identificaram a paroxetina com atividade anti-esquistomicida *in vitro*. Os ensaios demostraram que houveram redução da motilidade dos vermes adultos (fêmeas e machos), a medida que as concentrações de paroxetina eram aumentadas (NEVES et al., 2016b). Deve-se considerar que a motilidade/atividade motora está correlacionada com aspectos biológicos importantes do *Schistosoma mansoni,* que vão desde a prevalência em seu hospedeiro até a perpetuação da espécie, nos processos de cópula (NOËL, 2008).

Uma outra abordagem a ser utilizada para a descoberta de novos fármacos é o uso das “redes em multicamadas”, elas são utilizadas para representar ou prever interações regulatórias e funcionais entre genes, proteínas, caminhos bioquímicos e fármacos de diferentes organismos, levando à identificação de milhares de interações em um tempo relativamente curto, mesmo que os dados dos patógenos/alvos das DNT sejam escassos e que sejam escassos (BERENSTEIN et al., 2016b). Vários recursos computacionais podem ser explorados para criar inferências de rede multicamada. Por exemplo, a base de dados TDR *Targets* (AGÜERO et al., 2008; MAGARIÑOS et al., 2012) engloba dados genômicos, bioquímicos e farmacológicos relacionados a patógenos DNT e organismos modelo, bem como a capacidade de verificação computacional para potenciais alvos e informações de desejos compostos (AGÜERO et al., 2008; MAGARIÑOS et al., 2012).

A abordagem de redes foi aplicada por Berenstein (BERENSTEIN et al., 2016a) para sugerir alvos para compostos órfãos que são ativos contra o *P. falciparum* em telas de alto rendimento. Estre trabalho forneceu uma lista prioritária de proteínas para moléculas de consulta e demonstrou a capacidade de propor novas hipóteses testadas para cada composto.

Esta estratégia torna-se muito interessante pois permite modelar grandes conjuntos de dados genômicos e informações químicas extensivas (KEISER et al., 2009; CHENG et al., 2012; EMIG et al., 2013). Vale ressaltar que o banco

genômico do *Plasmodium* o PlasmoDB (AURRECOECHEA et al., 2009), abrange informações significativas, portanto, realizar a mineração destes dados torna-se complexo. Assim, as análises multivariadas são técnicas particularmente favoráveis, no intuito de priorizar e identificar medicamentos órfãos biotivos.

O que temos observado é que no cenário atual, as ferramentas computacionais de quimiogenômica continuam sendo pouco exploradas no reposicionamento de fármacos, principalmente para as Doenças Tropicais Negligenciadas. Apesar da pequena quantidade de exemplos disponíveis na literatura, a maioria conduziu a identificação/descoberta de novos candidatos a fármacos contra as DTN, algumas delas usando abordagens quimogenômicas baseadas em ligantes e/ou estrutura.

6.2- Abordagem de quimiogenômica por reposicionamento identifica epirrubicina como um potente fármaco antimalárico

No artigo 1, objetivamos identificar novos fármacos antimaláricos, através da estratégia de quimiogenômica por reposicionamento e validação experimental. Para isso, inicialmente selecionamos os alvos de *P. falciparum* e *P. vivax*, baseando-se em seletividade, ou seja, todas as proteínas presentes nos parasitos da malária, mas que não tinham homólogos em seres humanos. Por conseguinte, realizamos uma abordagem filogenômica, através de um filtro de seleção disponível no *TDR Targets Database,* que permitiu agrupar as proteínas de acordo com sua distribuição filogenética.

A filogenômica estuda as relações evolutivas baseadas em análises genômicas comparativas dos dados no genoma, e é indispensável na avaliação de diversas hipóteses biológicas, por exemplo na distribuição e disseminação da patogenicidade bacteriana, convergência ou divergência adaptativa, origem das organelas, ou resolução de árvores filogenéticas (PHILIPPE et al., 2005, CHAN; RAGAN, 2013). As relações taxonômicas são inferidas com base na homologia de um antepassado comum, observando sequências genômicas ou a abordagem de todo o genoma (CHAN et al., 2009; PUIGBÒ; WOLF; KOONIN, 2010).

Ao usar a abordagem filogenômica, foi possível identificar um total de 5.744 possíveis alvos de malária (proteínas), e ausentes em seres humanos,

dos quais 2.830 e 2.914 estão presentes nos proteomas previstos para *P. falciparum e P. vivax*, respectivamente. Em seguida, as proteínas hipotéticas foram removidas, visto que é pouco provável que tenham outros homólogos conhecidos além dos parasitos da malária, resultando em total de 1.095 e 636 alvos para *P. falciparum* e *P. vivax*, respectivamente. As proteínas hipotéticas apresentam funções desconhecidas e representam um desafio não só para a genômica funcional, mas também à biologia geral (GALPERIN, 2001).

Os experimentos bioquímicos/moleculares tradicionais podem atribuir funções precisas para genes nesses genomas. No entanto, o processo é demorado e dispendioso, observa-se que mesmo nos genomas mais completamente sequenciados, pouco mais de 50% dos genes, foram anotados. Assim, as ferramentas de bioinformática têm sido grandes aliadas, para realizar predições das funções das proteínas. Em 2011, Oladele e colaboradores(OLADELE; SADIKU; BEWAJI, 2011) caracterizaram *in silico* 11 proteínas hipotéticas do *P. falciparum*, demonstrando que estudos sobre as proteínas hipotéticas ainda são incipientes.

Após a compilação dos alvos antimaláricos baseados em seletividade, foram identificados alvos homólogos e seus respectivos fármacos aprovados para uso em humanos. Dessa forma, foram identificados 57 e 58 alvos confirmados para *P. falciparum* e *P. vivax*, respectivamente. A busca por similaridade é uma ferramenta de bioinformática que permite identificar proteínas homólogas, ou seja, as proteínas que originaram de um ancestral comum e que provavelmente possuem a mesma função (VERLI, 2014). Muitos alvos de *P. falciparum* e *P. vivax* encontrados possuíam homologia com alvos de fármacos humanos. Isso significa que, além do fármaco se ligar a proteínas de *P. falciparum* e *P. vivax,* se ligam também às proteínas humanas. Para o reposicionamento de fármacos, isso não é necessariamente um problema, uma vez que a dose para o efeito terapêutico de um fármaco pode variar no tratamento de doenças distintas.

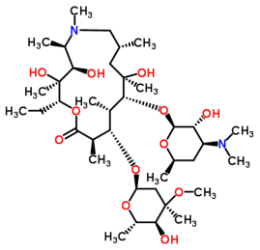
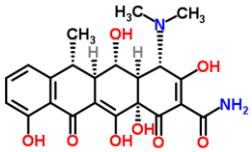
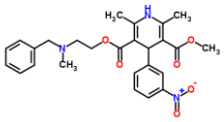
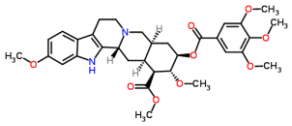
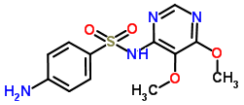
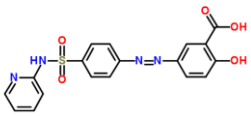
Para aumentar a probabilidade de que cada um dos alvos identificados correspondia a homólogos verdadeiros encontrados nos bancos de dados (DrubBank, TTD e Stich), foram considerados apenas proteínas cuja cobertura (ou seja, o comprimento da sequência que apresenta semelhança entre duas proteínas) fosse ≥ a 80%, verificada utilizando o BLASTp. O BLAST não avalia a

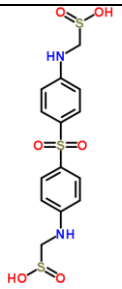
homologia entre as sequências. No entanto, é possível inferir que duas sequências são homólogas quando apresentarem grande identidade/similaridade, mesmo que apresentem um *e-value* baixo, com cobertura alta (AGARWAL; STATES, 1998a). Ao final deste filtro, foi possível obter uma lista com 14 e 21 alvos preditos, para *P. falciparum* e *P.vivax*, respectivamente.

Posteriormente, realizou-se uma comparação das regiões funcionais entre os alvos de fármacos aprovados e os seus homólogos em *Plasmodium*, para estimar a conservação do sítio ativo e a preservação da afinidade para os fármacos preditos. Para isso, utilizou-se o servidor ConSurf, que avalia quais aminoácidos são estruturais e funcionais para uma proteína, podendo calcular a porcentagem de conservação do sítio ativo entre os alvos homólogos (GLASER et al., 2003, ASHKENAZY et al., 2010b).Esta abordagem permitiu gerar uma lista final de proteínas com características funcionalmente relevantes e resíduos conservados, aumentando assim, a probabilidade de que cada um dos fármacos tenha atividade biológica. Foi então realizada uma busca na literatura com intuito de verificar quais dos fármacos selecionados já tinham sido testados em parasitos de malária. Quando o fármaco já tivesse sido testado como antimalárico, este não foi considerado nos estudos subsequentes.

Os resultados obtidos nesta abordagem nos permitiram selecionar 7 fármacos (Tabela 2) que já foram testados *in vitro* ou *in vivo* contra o parasito da malaria ou são/foram usados como antimaláricos. Desta forma, este achado valida parcialmente a nossa abordagem de quimiogenômica por reposicionamento para identificar fármacos aprovados com atividade contra *P. falciparum* e *P. vivax*. Assim a estratégia poderá também ser utilizada para outros agentes patogênicos.

Tabela 2. Fármacos preditos na abordagem de quimiogenômica por reposicionamento, e que foram relatados na literatura como potencial atividade antimalárica.

Fármaco	Estrutura Química	Categoria original do Fármaco
Azithromycina		Antibiótico
Doxiciclina		Antibiótico
Nicardipina		Agente anti-hipertensivo
Reserpina		Agente anti-hipertensivo
Sulfadoxina		Antimalárico
Sulfasalazina		Agente antirreumático

Sulfoxona		Antibiótico

Azitromicina é um antibiótico macrolídeo semi-sintético da classe dos “azalídeos”. Tal como outros antibióticos macrolídeos, a azitromicina inibe a síntese de proteínas bacterianas pela ligação à subunidade ribossomal 50S do ribossoma bacteriano 70S (ANDERSEN et al., 1995).

Este fármaco apresentou excelente perfil de segurança em crianças e gestantes em avaliações de profilaxia antimalárica no Quênia, na Indonésia e na Tailândia por investigadores militares em meados da década de 1990. A eficácia da profilaxia foi excelente (99%) para a malária de *P. vivax*, mas para malária ocasionada de *P. falciparum*, variou de 70% a 83%, mesmo com administração diária (ANDERSEN et al., 1998; KITCHEN; VAUGHN; SKILLMAN, 2006).

Estudos randomizados em Bangladesh demonstraram que a azitromicina combinada ao artesunato, pode ser uma alternativa promissora para o tratamento de malária não complicada ocasionada por *Plasmodium falciparum*. Pacientes entre 8-65 anos receberam doses de azitromicina/artesunato durante 3 dias (30mg/Kg de azitromicina e 4mg/Kg por dia de artesunato). A taxa de cura em 42 dias foi de 97%, e não foram registrados efeitos adversos graves (THRIEMER et al., 2010).

Além disso, a atividade da azitrocimina foi obsevada no apicoplasto de *Plasmodium falciparum*, estudos demonstraram que o antibiótico bloqueou o desenvolvimento do aplicoplasto levando a fenótipos incomuns que afetam a biologia do parasito (UDDIN; MCFADDEN; GOODMAN, 2017). A organela demonstra ser essencial para a sobrevivência de *Plasmodium* e a perda do apicoplasto se manifesta como uma resposta de "morte retardada" no parasito. O apicoplasto evoluiu a partir de cianobactérias em um evento endosimbiótico complexo, resultando uma arquitetura e maquinaria de expressão de genes bastante bacteriana (CHAKRABORTY, 2016). Estudos

demonstram que o aplicoplasto é suscetível a uma ampla gama de antibióticos (UDDIN; MCFADDEN; GOODMAN, 2017).

O antibiótico doxiciclina é um derivado sintético de tetraciclina com atividade antimicrobiana similar a vibramicina, fabricado pela Pfizer, possui propriedades antimaláricas de ação lenta ligadas aos ribossomos, inibindo assim a síntese protéica (TAN et al., 2011). A doxiciclina se liga de forma reversível às subunidades ribossômicas de 30 S e, possivelmente, à subunidade ribossomal 50S, bloqueando a ligação do RNAt ao RNAm inibindo a síntese de proteínas bacterianas.

Embora se saiba que a doxiciclina seja um esquizonticida sanguíneo, o mecanismo de ação exato não está bem definido (MAZIER et al., 1993). Em *P. falciparum*, observou-se que a doxiciclina bloqueia a expressão de genes dos aplicoplastos tornando -os não funcionais e desta forma impedem o desenvolvimento de parasitos viáveis (DAHL et al., 2006).

Recomenda-se o uso da doxiclina para indivíduos que irão viajar para locais onde a malária é endêmica, alguns trabalhos demonstram a eficácia deste fármaco como medida profilática (TAN et al., 2011; EYAL; RAHAV; ELI, 2017). 55 Individuos do sexo masculino em regime militar foram submetidos a quimioprofilaxia de doxiclina, a administração do medicmanto foi feita até 3 dias após o termino da viagem, sendo que 13 desenvolveram malária de *P. vivax* e nenhum apresentou malária de *P. falciparum* (RIECKMANN et al., 1993).

Embora a doxiciclina seja um valioso antimalárico em terapia combinada requer administração diária e pode causar fototoxicidade, desconforto gastrointestinal e diarréia. Além disso, a doxiciclina não pode ser administrada a mulheres grávidas ou crianças devido à descoloração e desenvolvimento dos dentes (TAN et al., 2011).

A nicardipina é um poderoso bloqueador de canais de cálcio com ação vasodilatadora marcada. Tem propriedades anti-hipertensivas e é eficaz no tratamento de angina e espasmos coronários sem apresentar efeitos de cardiodepressão. Também foi reposicionado no tratamento da asma e melhora a ação de agentes antineoplásicos específicos (BOLTON et al., 2008).

Sabe-se que a resistência de *P. falciparum* à cloroquina é revertida *in vitro* por alguns compostos, incluindo antagonistas do cálcio, o que poderia aumentar o acúmulo do fármaco no vacúolo alimentar do parasito (AZEVEDO et al.,

2013). No entanto, esse mecanismo de resistência pode ser insuficiente quando o nível de cálcio aumenta (MILLER et al., 2013).

O cálcio é considerado um dos mais importantes sinalizadores celulares e desta forma sua concentração celular é livre estritamento reguladas. Em mamíferos por exemplo se complxam como cálcio diferentes afincidades e especicificidades (SILVA et al., 2009). As quinases dependentes de cálcio são consideradas um dos grupos mais importantes de enzimas para o parasito da malária. Dentre essas a PfCDK1, é indispensável para a invasão dos eritrócitos e motilidade dos parasitos, é expressa durante a o ciclo intraeritrocítico e esporogonia no mosquito (AZEVEDO et al., 2013; GAJI et al., 2014). A PfCDK3, por exemplo é uma proteína essencial para ciclo sexudado do parasito, pois o nocaute do gene que codifica essa proteína reduz em duas vezes a habilidade do oocineto em infectar o intestino do mosquito (LI; BAKER; COX, 2000; MILLER et al., 2013; SIMON; HUART; WILMANNS, 2015).

A sulfadoxina é utilizada em combinação com pirimetamina para o tratamento ou a prevenção da malária. Também pode ser usado para tratar várias infecções em outros mamíferos (BOLTON et al., 2008).

A sulfadoxina e a pirimetamina são indicadas para o tratamento da malária de *Plasmodium falciparum* em pacientes com suspeita de resistência à cloroquina (DOBERSTYN et al., 1976; MARKS et al., 2005). Sulfadoxina ajuda a inibir a enzima dihidropteroate sintetase, que é uma enzima necessária na conversão de PABA em ácido fólico. Como o ácido fólico é vital para a síntese, reparação e metilação do DNA, que é vital para o crescimento celular em *Plasmodium falciparum*. Com deficiência deste nutriente vital, o parasita tem dificuldade em reproduzir (AHMED et al., 2006; DAS et al., 2010; LUMB; SHARMA, 2011).

Este fármaco é recomendado para o tratamento preventivo intermitente da malária durante a gravidez na maior parte da África subsaariana (WHITE et al., 2014). Estudos tem evidenciado que a resistência ao SP (sulfadoxina-pirimetamina) está relacionada a mutações no gene dhfr e dhps de *Plasmodium falciparum* (ABDUL-GHANI; FARAG; ALLAM, 2013; MARKS et al., 2005).

Um trabalho realizado no sudeste da Nigéria demonstrou a prevalência de mutações triplas no Pfdhfr, tais mutações podem comprometer a eficácia da quimioprevenção através da SP. Serão necessários monitoramentos contínuos

através de marcadores moleculares, avaliar estratégias alternativas de tratamento preventivo e buscar novos fármacos para prevenir a malária na gravidez (ESU et al., 2018).

Conforme exposto acima, a quimioterapia atualmente disponivel pode apresentar inúmeros desafios, tais como: efeitos adversos (vômitos ou mau estado geral), que podem exigir via parenteral de administração; nem todas as espécies e cepas de plasmódios são igualmente sensíveis aos medicamentos antimaláricos; os medicamentos não agem todos sobre as mesmas fases evolutivas do Plasmodium e disseminação de cepas resistentes. A partir do observado é emergente a necessidade da descoberta de novos antimaláricos.

Desta forma, seguindo o fluxo de trabalho proposto na metodologia, após a busca na literatura, foram preditos 11 novos candidatos antimaláricos, dentre eles, Quatro foram avaliados experimentalmente (ácido azelaico, captopril, mesalazina e epirrubicina). Estes fármacos foram testados juntamente com os compostos controle (cloroquina e pirimmetamina).

A epirrubicina (Figura 13) é uma antraciclina, isômero 4'-epi da doxorrubicina. Este fármaco exerce seus efeitos antitumorais por interferência na síntese e função do DNA. As propriedades gerais dos fármacos desta classe incluem: interação com o DNA de diversas formas (intercalação entre os pares de bases, quebras de cadeia de DNA e inibição com a enzima topoisomerase II) (KNOX et al., 2011).

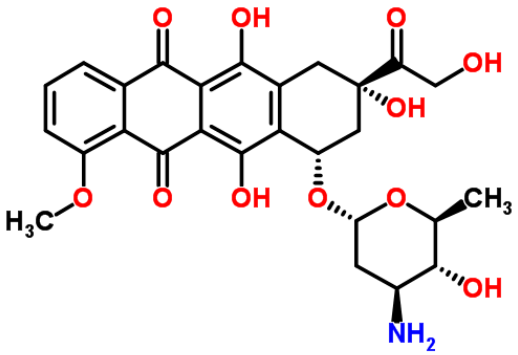


Figura 13- Estrutura química da epirrubicina

A maioria desses compostos foi isolada de fontes naturais. No entanto, eles não possuem a especificidade dos antibióticos antimicrobianos e, portanto,

produzem toxicidade significativa. As antraciclinas estão entre os medicamentos antitumorais mais importantes disponíveis. A epirrubicina também pode inibir a atividade da polimerase, afetar a regulação da expressão gênica e produzir danos nos radicais livres ao DNA (ALI et al., 2013; HALE; DEUTSCH; LAHIRI, 2017).

O alvo homólogo de DNA-topoisomerase II em *Plasmodium* foi previsto ser a subunidade A de DNA gyrase tanto em *P. falciparum* quanto em *P. vivax* . Devido ao seu perfil potencial de inibição, a epirrubicina foi então continuada para a determinação da sua concentração inibitória a 50% (IC_{50}). Para isso, utilizou-se um método à base de fluorescência que faz uso do SYBR Green, e as concentrações diluídas foram testadas contra o *P. falciparum* 3D7 e W2, em triplicatas, juntamente com a cloroquina (controle). Apesar do fato de que a epirrubicina tem um efeito modesto quando comparada à cloroquina (cerca de 7 vezes), ela ainda exibe o IC_{50} um pouco mais de 100 nM (140 nM), que está posicionada na faixa nanomolar média. Ressaltamos que a epirrubicina mostrou ser cerca de duas vezes mais ativa contra a cepa multi - resistente W2 (69 nM) exibindo maior atividade do que a cloroquina em si.

Assim, os testes *in vitro* confirmaram que o fármaco era potente contra o parasito sensível (3D7) e mais ainda contra a cepa multi - resistente ao fármaco (W2). Esta é uma observação relevante, pois à grande maioria da população de parasitos em ambientes naturais atualmente, é resistente à maioria dos antimaláricos disponíveis, incluindo a cloroquina (WELLEMS et al., 1990,SILVA et al., 2012).

No Brasil, por exemplo, em uma área de resistência à cloroquina, o IC_{50} médio entre os isolados amazônicos de *P. falciparum* mostrou ser de quase 300 nM (SILVA et al., 2012), que é três vezes maior do que a atividade encontrada para a epirrubicina. Isto é altamente relevante porque é possível que a epirrubicina seja eficaz contra os parasitos sensíveis à cloroquina e resistentes à cloroquina, o que representa uma enorme vantagem, uma vez que o medicamento pode ser usado para tratar pacientes infectados com parasitas resistentes aos medicamentos

Nossos dados sugerem que a epirrubicina é ativa contra parasitos resistentes à cloroquina, uma vez que seu alvo previsto é a subunidade A de DNA-girase, envolvida no processo de síntese de DNA no apicoplasto, dos

Plasmodium, enquanto, que os alvos da cloroquina estão relacionados com a inibição da atividade da heme-polimerase presente no vacúolo digestivo do parasito (WELLEMS et al.,1990; FIDOCK et al., 2000; CRAVO et al., 2006).

Uma outra interpretação subjacente importante de nossos dados é a proposta da subunidade A de DNA-girase como alvo para a terapia de malária. A subunidade A da DNA-girase é um alvo atraente, principalmente porque o apicoplasto da malária é uma organela que resulta da endossimbiose secundária, algumas de suas enzimas (como a subunidade A da DNA-girase) são de origem procariótica e, portanto, podem ser bloqueadas usando fármacos menos tóxicos, como antibióticos (GARCÍA-ESTRADA et al., 2010). Embora a enzima tenha sido sugerida como um alvo putativo de malária antes (GARCÍA-ESTRADA et al., 2010; RINIKER et al., 2013; TANG GIRDWOOD; NENORTAS; SHAPIRO, 2015), não existem estudos que demonstrem que o alvo seja inibido pela epirrubicina e com tal eficácia.

A epirrubicina é um fármaco antineoplásico da classe de antraciclina, que é conhecida por não possuir especificidade e, portanto, produz toxicidade significativa, como aplasia de medula óssea, mucosite grau 4 e sangramento gastrointestinal(WISHART et al., 2006, ALI et al., 2013; HALE; DEUTSCH; LAHIRI, 2017). A razão para isso é provavelmente pelo que de que os fármacos dentro desta classe possuem um ou mais destinos fora do alvo (como a DNA topoisomerase-alfa II humana) que inibe a atividade da polimerase humana, afetando a regulação da expressão gênica e produzindo danos nos radicais livres para DNA.

Assim, é possível que a potência da epirrubicina em relação aos parasitos da malária supere em grande parte a toxicidade sobre as células de mamíferos, permitindo que ele seja usado em concentrações muito menores do que as necessárias para o tratamento do câncer e consequentemente evitando sua toxicidade. Por este motivo, estudos futuros envolverão a avaliação do índice de seletividade do medicamento, a fim de verificar se ele pode ser facilmente reposicionado para o tratamento da malária.

7 CONCLUSÕES

Na presente tese de doutorado foram realizados estudos envolvendo estratégias modernas para o reposicionamento de novos fármacos para o tratamento da malária, através de uma abordagem de genômica comparativa e estratégias *in silico.*

Através da abordagem de genômica comparativa, foi possível obter uma biblioteca com 5.827 potenciais alvos terapêuticos presentes em *P. falciparum* e *P.vivax* e ausentes em seres humanos.

Após uma busca baseada em homologia em 3 bases de dados de fármacos aprovados (DrugBank, Stitch e TTD), foi possível identificar 115 alvos homólogos que interagem com 36 fármacos aprovados para usos em humanos, que dessa forma, teriam potencial atividade antimalárica. Assim, foram selecionados os fármacos que possuíram 60% ou mais de conservação em seu sitio ativo, totalizando uma lista com 29 fármacos potencialmente antimaláricos.

Para a realização dos ensaios biológicos, foram priorizados os fármacos que foram preditos através de modelos de QSAR binários desenvolvidos com dados fenotípicos de atividade antimalárica (*P. falciparum*) frente a cepas resistentes à cloroquina (W2) e sensíveis à cloroquina (3D7).

Ao final das análises *in silico*, os fármacos selecionados foram avaliados *in vitro* em *P. falciparum*, e destes e a epirrubicina se destacou por exibir uma potente atividade *in vitro* contra a cepa 3D7 (IC_{50} = 140 nM). Além disso, este fármaco demonstrou ser cerca de duas vezes mais ativa contra cepas multi-resistentes W2 (IC_{50} = 69 nM), exibindo, inclusive, maior atividade do que a cloroquina A epirrubicina é um fármaco aprovado como antineoplásico e antibacteriano. Neste trabalho, propusemos que o fármaco interage com a subunidade A da DNA-girase de *P. falciparum*. Este alvo é relevante, pois está envolvido nos processos de síntese do DNA do apicoplasto de *Plasmodium*, o que o torna um alvo promissor para o tratamento da malária.

Desta forma, sugere-se que uma abordagem *in silico* seja realizada para buscar todos os possíveis inibidores de DNA-girase, bem como testar a eficácia dos fármacos remanescentes que foram identificados neste trabalho. Além

disso, a epirrubicina será submetida outros ensaios *in vitro* envolvendo cepas resistentes, e ainda, a ensaios *in vivo* com *P. berghei* e *P. chabaudi*.

REFERÊNCIAS

ABDUL-GHANI, R.; FARAG, H. F.; ALLAM, A. F. **Sulfadoxine-pyrimethamine resistance in Plasmodium falciparum:** A zoomed image at the molecular level within a geographic context. Acta Tropica, 2013.

ACHENBACH, J. et al. **Computational tools for polypharmacology and repurposing**. Future Medicinal Chemistry, v. 3, n. 8, p. 961–968, jun. 2011.

AGARWAL, P.; STATES, D. J. **Comparative accuracy of methods for protein sequence similarity search.** Bioinformatics (Oxford), v. 14, n. 1, p. 40–47., 1998a.

AGARWAL, P.; STATES, D. J. **Comparative accuracy of methods for protein sequence similarity search**. Bioinformatics (Oxford, England), v. 14, n. 1, p. 40–7, 1998b.

AGÜERO, F. et al. **Genomic-scale prioritization of drug targets: the TDR Targets database.** Nature reviews. Drug discovery, v. 7, n. 11, p. 900–7, 2008.

AHMED, A. et al. **Prevalence of mutations associated with higher levels of sulfadoxine-pyrimethamine resistance in Plasmodium falciparum isolates from Car Nicobar Island and Assam, India.** Antimicrobial Agents and Chemotherapy, v. 50, n. 11, p. 3934–3938, 2006.

ALEXANDRE PENIDO. **Portal da Saúde**. Disponível em: <http://portalsaude.saude.gov.br/index.php/cidadao/principal/agencia-saude/23368-brasil-registra-menor-numero-de-casos-de-malaria-nos-ultimos-35-anos/>. Acesso em: 15 ago. 2017.

ALI, N. et al. **Successful engraftment and survival following allogeneic hematopoietic stem cell transplant in a child with familial hemophagocytic lymphohistiocytosis.** Indian Journal of Pediatrics, v. 80, n. 1, p. 65–66, 2013.

ALIROL, E. et al. **Nifurtimox-Eflornithine Combination Therapy for Second-Stage Gambiense Human African Trypanosomiasis**: Medecins Sans Frontieres Experience in the Democratic Republic of the Congo. Clinical Infectious Diseases, v. 56, n. 2, p. 195–203, jan. 2013.

ALONSO, P.; ENGELS, D. **Drug-based vector control: a potential new paradigm**. Malaria Journal, v. 16, n. 1, p. 200, 2017.

ALTSCHUL, S. F. et al. **Gapped BLAST and PSI-BLAST**: A new generation of protein

database search programs Nucleic Acids Research, 1997.

ALVES, V. M. et al. **Predicting chemically-induced skin reactions. Part I:** QSAR models of skin sensitization and their application to identify potentially hazardous compounds. Toxicology and applied pharmacology, v. 284, n. 2, p. 262–272, 2015a.

ALVES, V. M. et al. **Predicting chemically-induced skin reactions. Part II:** QSAR models of skin permeability and the relationships between skin permeability and skin sensitization. Toxicology and Applied Pharmacology, v. 284, n. 2, p. 273–280, abr. 2015b.

AMARAL, E. F. **Malária** : Aspectos históricos e utilização da Artemisinina em seu tratamento. p. 34, 2015.

ANDERSEN, S. L. et al. **Activity of azithromycin as a blood schizonticide against rodent and human plasmodia in vivo.** American Journal of Tropical Medicine and Hygiene, v. 52, n. 2, p. 159–161, 1995.

ANDERSEN, S. L. et al. **Successful double-blinded, randomized, placebo-controlled field trial of azithromycin and doxycycline as prophylaxis for malaria in western Kenya. Clinical infectious diseases** : an official publication of the Infectious Diseases Society of America, v. 26, n. 1, p. 146–150, jan. 1998.

ANDERSON, S. **Graphical representation of molecules and substructure-search queries in MACCStm.** Journal of Molecular Graphics, v. 2, n. 3, p. 83–90, 1984.

ANDREWS, K. T.; FISHER, G.; SKINNER-ADAMS, T. S. Drug repurposing and human parasitic protozoan diseases. **International Journal for Parasitology: Drugs and Drug Resistance**, v. 4, n. 2, p. 95–111, 2014.

APWEILER, R. et al. **The Universal Protein Resource (UniProt) in 2010.** Nucleic Acids Res, v. 38, p. D142-8, 2010.

ARIEY, F. et al. **A molecular marker of artemisinin-resistant Plasmodium falciparum malaria**. Nature, v. 505, n. 7481, p. 50–5, 2014.

ASHBURN, T. T.; THOR, K. B. **Drug repositioning:** identifying and developing new uses for existing drugs. Nature reviews. Drug discovery, v. 3, n. 8, p. 673–83, ago. 2004.

ASHKENAZY, H. et al. **ConSurf 2010**: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. Nucleic Acids Res, v. 38, n. Web Server issue, p. W529-33, 2010a.

ASHKENAZY, H. et al. **ConSurf 2010**: calculating evolutionary conservation in

sequence and structure of proteins and nucleic acids. Nucleic Acids Res**,** v. 38, n. Web Server issue, p. W529-33, 2010b.

AUBÉ, J. **Drug Repurposing and the Medicinal Chemist**. ACS Medicinal Chemistry Letters, v. 3, n. 6, p. 442–444, jun. 2012.

AURRECOECHEA, C. et al. **PlasmoDB:** A functional genomic database for malaria parasites. Nucleic Acids Research, v. 37, n. SUPPL. 1, 2009.

AURRECOECHEA, C. et al. **EuPathDB:** The eukaryotic pathogen genomics database resource. Nucleic Acids Research, v. 45, n. D1, p. D581–D591, 2017.

AWADZI, K. et al. **A Randomized, Single-Ascending-Dose, Ivermectin-Controlled, Double-Blind Study of Moxidectin in Onchocerca volvulus Infection**. PLoS Neglected Tropical Diseases, v. 8, n. 6, p. e2953, jun. 2014.

AZEVEDO, M. F. et al. **Inhibition of Plasmodium falciparum CDPK1 by conditional expression of its J-domain demonstrates a key role in schizont development. Biochemical** Journal, v. 452, n. 3, p. 433–441, 2013.

BAHL, A. **PlasmoDB**: the Plasmodium genome resource. A database integrating experimental and computational data. Nucleic Acids Research, v. 31, n. 1, p. 212–215, jan. 2003.

BAJORATH, J. **Progress in computational medicinal chemistry.** Journal of Medicinal Chemistry, v. 55, n. 8, p. 3593–3594, 2012.

BARAKAT, R. et al. **Efficacy and Safety of Arachidonic Acid for Treatment of School-Age Children in Schistosoma mansoni High-Endemicity Regions**. American Journal of Tropical Medicine and Hygiene, v. 92, n. 4, p. 797–804, abr. 2015.

BARANSKI, M. C.; CARNEIRO FILHO, M. **Massive infestation with larva migrans. Treatment with thiabendazole**. Revista brasileira de medicina, v. 23, n. 4, p. 233–235, abr. 1966.

BARAS, A. I.; BARAS, A. S.; SCHULMAN, K. A. **Drug development risk and the cost of capital.** Nature Reviews Drug Discovery, v. 11, n. April, p. 347–348, 2012.

BARONI, M. et al. **A Common Reference Framework for Analyzing/Comparing Proteins and Ligands. Fingerprints for Ligands And Proteins (FLAP)**: Theory and Application. Journal of Chemical Information and Modeling, v. 47, n. 2, p. 279–294, mar. 2007.

BARRY, A. E.; ARNOTT, A. **Strategies for designing and monitoring malaria vaccines targeting diverse antigens**. Frontiers in Immunology, 2014.

BARZILAI, A.; FRIEDMAN, J.; TRAU, H. **Treatment of cutaneous leishmaniasis with allopurinol**. Journal of the American Academy of Dermatology, v. 32, n. 3, p. 518, mar. 1995.

BASRA, A. et al. **Efficacy of Mefloquine Intermittent Preventive Treatment in Pregnancy Against Schistosoma haematobium Infection in Gabon**: A Nested Randomized Controlled Assessor-Blinded Clinical Trial. Clinical Infectious Diseases, v. 56, n. 6, p. e68–e75, mar. 2013.

BASSAT, Q. et al. **Key Knowledge Gaps for Plasmodium vivax Control and Elimination.** American Journal of Tropical Medicine and Hygiene, v. 107, n. 8, p. 475–479, jul. 2016.

BASTOS, M. F. et al. **Fucosylated chondroitin sulfate inhibits Plasmodium falciparum cytoadhesion and merozoite invasion**. Antimicrobial Agents and Chemotherapy, v. 58, n. 4, p. 1862–1871, 2014.

BATISTA, J. et al. **SiteHopper - a unique tool for binding site comparison.** Journal of Cheminformatics, v. 6, n. Suppl 1, p. P57, 2014.

BEGHYN, T. B. et al. **Drug to genome to drug: discovery of new antiplasmodial compounds.** Journal of medicinal chemistry, v. 54, n. 9, p. 3222–40, maio 2011.

BERENSTEIN, A. J. et al. **A Multilayer Network Approach for Guiding Drug Repositioning in Neglected Diseases.** PLoS Neglected Tropical Diseases, v. 10, n. 1, 2016a.

BERENSTEIN, A. J. et al. **A Multilayer Network Approach for Guiding Drug Repositioning in Neglected Diseases**. PLoS Neglected Tropical Diseases, v. 10, n. 1, 2016b.

BERMAN, J. **Miltefosine, an FDA-approved drug for the "orphan disease", leishmaniasis**. Expert Opinion on Orphan Drugs, v. 3, n. 6, p. 727–735, jun. 2015.

BETHONY, J. et al. **Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm**. The Lancet, v. 367, n. 9521, p. 1521–1532, maio 2006.

BIEGERT, A; SÖDING, J. **Sequence context-specific profiles for homology searching.** Proceedings of the National Academy of Sciences of the United States of America, v. 106, n. 10, p. 3770–3775, 2009.

BINKOWSKI, T. A.; JOACHIMIAK, A. **Protein Functional Surfaces:** Global Shape Matching and Local Spatial Alignments of Ligand Binding Sites. BMC Structural Biology, v. 8, n. 1, p. 45, 2008.

BISPO, N. A. et al. **A Systematic In Silico Search for Target Similarity Identifies Several Approved Drugs with Potential Activity against the Plasmodium falciparum Apicoplas**t. PLoS ONE, v. 8, n. 3, 2013.

BLAND, N. D. et al. **Pharmacological Validation of Trypanosoma brucei Phosphodiesterases B1 and B2 as Druggable Targets for African Sleeping Sickness.** Journal of Medicinal Chemistry, v. 54, n. 23, p. 8188–8194, dez. 2011.

BOLGÁR, B. et al. **Drug repositioning for treatment of movement disorders**: from serendipity to rational discovery strategies. Current topics in medicinal chemistry, v. 13, n. 18, p. 2337–2363, 2013.

BOLTON, E. E. et al. PubChem: **Integrated Platform of Small Molecules and Biological Activities**. Annual Reports in Computational Chemistry, v. 4, p. 217–241, 2008.

BOSTRÖM, J.; GREENWOOD, J. R.; GOTTFRIES, J. **Assessing the performance of OMEGA with respect to retrieving bioactive conformations**. Journal of molecular graphics & modelling, v. 21, n. 5, p. 449–62, mar. 2003.

BOTT, C. Y. et al. **Plasmodium falciparum Apicoplast Drugs:** Targets or Off-Targets ? 2011.

BOYLE, J. P.; YOSHINO, T. P. S**erotonin-induced muscular activity in Schistosoma mansoni larval stages: importance of 5-HT transport and role in daughter sporocyst production**. The Journal of parasitology, v. 91, n. 3, p. 542–550, 2005.

BOYLE, J. P.; ZAIDE, J. V; YOSHINO, T. P. S**chistosoma mansoni: effects of serotonin and serotonin receptor antagonists on motility and length of primary sporocysts in vitro**. Experimental parasitology, v. 94, n. 4, p. 217–226, 2000.

BRAGA, R. C. et al. **Virtual Screening Strategies in Medicinal Chemistry:** The state of the art and current challenges. Current topics in medicinal chemistry, v. 14, n. 16, p. 1899–1912, 2014a.

BRAGA, R. C. et al. **Tuning hERG out:** Antitarget QSAR Models for Drug Development. Current topics in medicinal chemistry, v. 14, n. 11, p. 1399–1415, maio 2014b.

BRAGA, R. C.; ANDRADE, C. H. **Assessing the performance of 3D pharmacophore models in virtual screening:** how good are they? Current topics in medicinal chemistry, v. 13, n. 9, p. 1127–1138, 2013.

BRASIL. **Situação epidemiológica da malária no Brasil**, 2000 a 2011. Boletim Epidemiológico, v. 44, n. 1, p. 16, 2013.

BREDEL, M.; JACOBY, E. **Chemogenomics: an emerging strategy for rapid target and drug discovery**. Nature Reviews Genetics, v. 5, n. 4, p. 262–275, 2004.

BREIMAN, L. **Random forests**. Machine Learning, v. 45, n. 1, p. 5–32, 2001.

BRKIĆ, D.; GLISIĆ, L.; SIMIĆ, P. **Yomesan in therapy of taeniasis and Hymenolepis nanae.** Medicinski glasnik, v. 22, n. 4, p. 107–108, abr. 1968.

BROWN, S. P.; MUCHMORE, S. W. H**igh-throughput calculation of protein-ligand binding affinities**: modification and adaptation of the MM-PBSA protocol to enterprise grid computing. Journal of chemical information and modeling, v. 46, n. 3, p. 999–1005, [s.d.].

BRUNO JUNIOR NEVES, RAFAEL FERREIRA DANTAS, MARIO ROBERTO SENGER, WALTER CÉSAR GÓES VALENTE, JOÃO DE MELLO REZENDE-NETO, WILLIAN TÁVORA CHAVES, LEE KAMENTSKY, ANNE CARPENTER, F. P. S.-J. AND C. H. A. **The antidepressant drug paroxetine as a new lead candidate in schistosome drug discovery.** Med. Chem. Commun., v. 7, n. 6, p. 1176–1182, 2016.

BUDIMULJA, A. S. et al. **The sensitivity of Plasmodium protein synthesis to prokaryotic ribosomal inhibitors**. Molecular and Biochemical Parasitology, v. 84, n. 1, p. 137–141, jan. 1997.

BURR, S. E. et al. **Anthropometric indices of Gambian children after one or three annual rounds of mass drug administration with azithromycin for trachoma control.** BMC Public Health, v. 14, n. 1, p. 1176, dez. 2014.

BURROWS, J. N. et al. **New developments in anti-malarial target candidate and product profiles.** Malaria Journal, v. 16, n. 1, p. 26, 2017.

BUSCAGLIA, C. A.; KISSINGER, J. C.; AGÜERO, F. **Neglected Tropical Diseases in the Post-Genomic Era**. Trends in Genetics, v. 31, n. 10, p. 539–555, out. 2015.

CAI H, ZHOU Z, GU J, W. Y. **Comparative Genomics and Systems Biology of Malaria Parasites Plasmodium.** Curr Bioinform, v. 7, n. 4, p. 1–22, 2013.

CAPORUSCIO, F.; TAFI, A. **Pharmacophore modelling: a forty year old approach and its modern synergies.** Current medicinal chemistry, v. 18, n. 17, p. 2543–53, jan. 2011.

CARLTON, J. M. et al. **Comparative genomics of the neglected human malaria parasite Plasmodium vivax**. Nature, v. 455, n. 7214, p. 757–63, 2008.

CARNEIRO, P. F. et al. **Synthesis and antimalarial activity of quinones and structurally-related oxirane derivatives**. European Journal of Medicinal Chemistry, v. 108, p. 134–140, 2016.

CARON, P. R. et al. **Chemogenomic approaches to drug discovery. Current opinion in chemical biology**, v. 5, n. 4, p. 464–470, 2001.

CDC. CDC - **Malaria - About Malaria - Biology - Malaria Parasites**. USA Government, 2012. Disponível em: <http://www.cdc.gov/malaria/about/biology/parasites.html>

Centers for Disease Control and Prevention. **Neglected tropical diseases**. http://www.cdc.gov/globalhealth/ntd/diseases/index.html (Accessed July 18, 2016).

CERETO-MASSAGUÉ, A. et al. **Molecular fingerprint similarity search in virtual screening**. Methods, v. 71, p. 58–63, jan. 2015.

CERQUEIRA, N. M. F. S. A. et al. **Receptor-based virtual screening protocol for drug discovery.** Archives of Biochemistry and Biophysics, v. 582, p. 56–67, set. 2015.

CHAKRABORTY, A. U**nderstanding the biology of the Plasmodium falciparum apicoplast;** an excellent target for antimalarial drug development. Life Sciences, v. 158, p. 104–110, ago. 2016.

CHAN, C. X. et al. **Lateral transfer of genes and gene fragments in prokaryotes**. Genome biology and evolution, v. 1, p. 429–438, 2009.

CHAN, C. X.; RAGAN, M. A. **Next-generation phylogenomics.** Biology direct, v. 8, n. 1, p. 3, 2013.

CHANG, C. Y. et al.. Journal of **Oversampling to overcome overfitting: Exploring the relationship between data set composition, molecular descriptors, and predictive modeling methods** Chemical Information and Modeling, v. 53, n. 4, p. 958–971, 2013.

CHAVALI, A. K. et al. **Systems analysis of metabolism in the pathogenic trypanosomatid Leishmania major**. Molecular systems biology, v. 4, p. 177, mar. 2008.

CHAVALI, A. K. et al. **Metabolic network analysis predicts efficacy of FDA-approved drugs targeting the causative agent of a neglected tropical disease**. BMC Systems Biology, v. 6, n. 1, p. 27, 2012.

CHEN, X.; JI, Z. L.; CHEN, Y. Z. **TTD:** Therapeutic Target Database. Nucleic Acids

Research, v. 30, n. 1, p. 412–415, 2002a.

CHEN, X.; JI, Z. L.; CHEN, Y. Z. **TTD:** Therapeutic Target Database. Nucleic acids research, v. 30, n. 1, p. 412–415, jan. 2002b.

CHEN, X.; LIU, M.; GILSON, M. K. **BindingDB:** a web-accessible molecular recognition database. Combinatorial Chemistry & High Throughput Screening, v. 4, n. 8, p. 719–725, dez. 2001.

CHENG, F. et al. **Prediction of drug-target interactions and drug repositioning via network-based inference**. PLoS Computational Biology, v. 8, n. 5, 2012.

CHERKASOV, A. et al. **QSAR modeling:** Where have you been? Where are you going to?Journal of Medicinal Chemistry, jan. 2014.

CHONG, C. R.; SULLIVAN, D. J. **New uses for old drugs**. Nature, v. 448, n. 7154, p. 645–646, ago. 2007.

CIBULSKIS, R. E. et al. **Malaria:** Global progress 2000 – 2015 and future challenges. Infectious Diseases of Poverty, v. 5, n. 1, p. 61, dez. 2016.

ClinicalTrials.gov. **Activity of Mefloquine Against Urinary Schistosomiasis.** https://clinicaltrials.gov/ct2/show/NCT01132248 (Accessed July 21, 2016).

ClinicalTrials.gov. **Antimony Plus Pentoxifylline in Cutaneous Leishmaniasis**. https://clinicaltrials.gov/ct2/show/NCT01381055 (Accessed July 21, 2016).

ClinicalTrials.gov. **Arachidonic Acid Treatment Against Schistosomiasis Infection in Children**. https://clinicaltrials.gov/ct2/show/NCT02144389 (Accessed July 23, 2016).

ClinicalTrials.gov. **Auranofin PK Following Oral Dose Administration.** https://clinicaltrials.gov/ct2/show/NCT02089048 (Accessed July 25, 2016). [s.d.].

ClinicalTrials.gov. **Efficacy and Microfilaricidal Kinetics of Imatinib for the Treatment of Loa Loa**. https://clinicaltrials.gov/ct2/show/NCT02644525 (Accessed July 21, 2016).

ClinicalTrials.gov. **First in Man Clinical Trial of Emodepside (BAY 44-4400).** https://clinicaltrials.gov/ct2/show/NCT02661178 (Accessed July 25, 2016). [s.d.].

ClinicalTrials.gov. **Phase I Trial Evaluating the Safety and Pharmacokinetics of Oxfendazole.** https://clinicaltrials.gov/ct2/show/NCT02234570 (Accessed July 23, 2016).

ClinicalTrials.gov. **Pivotal Study of Fexinidazole for Human African Trypanosomiasis in Stage 2.** https://clinicaltrials.gov/ct2/show/NCT01685827 (Accessed July 21, 2016).

ClinicalTrials.gov. **Study Evaluating Orally Administered Moxidectin In Subjects With Onchocerca Volvulus Infection.** https://clinicaltrials.gov/ct2/show/NCT00300768 (Accessed July 25, 2016).

ClinicalTrials.gov. **Study to Evaluate Fexinidazole Dosing Regimens for the Treatment of Adult Patients With Chagas Disease.** https://clinicaltrials.gov/ct2/show/NCT02498782 (Accessed July 23, 2016). [s.d.].

ClinicalTrials.gov. **Trial of DB289 for the Treatment of Stage I African Trypanosomiasis.** https://clinicaltrials.gov/ct2/show/NCT00803933 (Accessed July 21, 2016).

ClinicalTrials.gov. **Trial to Determine Efficacy of Fexinidazole in Visceral Leihmaniasis Patients in Sudan**. https://clinicaltrials.gov/ct2/show/NCT01980199 (Accessed July 23, 2016).

CLYDE, D. F.; GILMAN, R. H.; MCCARTHY, V. C. **Antimalarial effects of clindamycin in man.** American Journal of Tropical Medicine and Hygiene, v. 24, n. 2, p. 369–370, 1975.

COHEN, J. **A coefficient of agreement of nominal scales**. Educational and Psychological Measurement, v. 20, p. 37–46, 1960.

COLLEY, D. G. et al. **Human schistosomiasis.** Lancet, v. 383, n. 9936, p. 2253–64, jun. 2014.

CRAVO, P. et al. **Mechanisms of Drug Resistance in Malaria:** Current and New Challenges. Anti-Infective Agents in Medicinal Chemistry, v. 5, n. 1, p. 63–73, 2006.

CRAVO, P.; NAPOLITANO, H.; CULLETON, R. **How genomics is contributing to the fight against artemisinin-resistant malaria parasites**. Acta Tropica, v. 148, p. 1–7, 2015.

CRISFORD, A. et al. **The Cyclooctadepsipeptide Anthelmintic Emodepside Differentially Modulates Nematode, Insect and Human Calcium-Activated Potassium (SLO) Channel Alpha Subunits**. PLOS Neglected Tropical Diseases, v. 9, n. 10, p. e0004062, out. 2015.

CROFT, S. L.; BARRETT, M. P.; URBINA, J. A. C**hemotherapy of trypanosomiases and leishmaniasis**. Trends in Parasitology, 2005.

CROSS, R. M. et al. **Optimization of 1,2,3,4-Tetrahydroacridin-9(10 H)-ones as Antimalarials Utilizing Structure–Activity and Structure–Property Relationships.** Journal of Medicinal Chemistry, v. 54, n. 13, p. 4399–4426, jul.

2011.

DAHL, E. L. et al. **Tetracyclines specifically target the apicoplast of the malaria parasite Plasmodium falciparum**. Antimicrobial Agents and Chemotherapy, v. 50, n. 9, p. 3124–3131, 2006.

DAS, M. K. et al. **High chloroquine treatment failure rates and predominance of mutant genotypes associated with chloroquine and antifolate resistance among falciparum malaria patients from the island of Car Nicobar, India.** Journal of Antimicrobial Chemotherapy, v. 65, n. 6, p. 1258–1261, 2010.

DAS, S.; KOKARDEKAR, A.; BRENEMAN, C. M. **Rapid Comparison of Protein Binding Site Surfaces with Property Encoded Shape Distributions.** Journal of Chemical Information and Modeling, v. 49, n. 12, p. 2863–2872, dez. 2009.

DE FRANCHI, E. et al. **Binding of Protein Kinase Inhibitors to Synapsin I Inferred from Pair-Wise Binding Site Similarity Measurements**. PLoS ONE, v. 5, n. 8, p. e12214, ago. 2010.

DE SÁ OLIVEIRA, T. et al. **Action of pentoxifylline on experimental cutaneous leishmaniasis due to Leishmania (Leishmania) amazonensis.** Memórias do Instituto Oswaldo Cruz, v. 95, n. 4, p. 477–282, 2000.

DILL, J. D. . et al. **Search and Retrieval Using an Automated Molecular Access System. 182nd National Meeting of the American Chemical Society**. Anais...New York: 1981

DIMASI, JOSEPH A. , GRABOWSKI, HENRY G., HANSEN, R. W. T**he cost of drug counterfeiting.** European Pharmaceutical Review, v. 20, n. 1, p. 5, 2015.

DIMASI, J. A. et al. **Trends in Risks Associated With New Drug Development:** Success Rates for Investigational Drugs. Clinical Pharmacology & Therapeutics, v. 87, n. 3, p. 272–277, mar. 2010.

DIMASI, J. A.; HANSEN, R. W.; GRABOWSKI, H. G. **The price of innovation:** new estimates of drug development costs. Journal of health economics, v. 22, n. 2, p. 151–85, mar. 2003.

DITZEL, J.; SCHWARTZ, M. Worm cure without tears. **The effect of niclosamide on taeniasis saginata in man**. Acta medica Scandinavica, v. 182, n. 5, p. 663–664, nov. 1967.

DOBCHEV, D.; PILLAI, G.; KARELSON, M. I**n silico machine learning methods in drug development.** Current Topics in Medicinal Chemistry, v. 14, n. 16, p. 1913–1922, out. 2014.

DOBERSTYN, E. B. et al. **Single dose therapy of falciparum malaria using pyrimethamine in combination with diformyldapsone or sulfadoxine**. American Journal of Tropical Medicine and Hygiene, v. 25, n. 1, p. 14–19, 1976.

Drugs for Neglected Diseases initiative. **New benznidazole regimens/combos.** http://www.dndi.org/diseases-projects/portfolio/new-benz-regimens (Accessed July 23, 2016).

DUBUS, E. et al. **Drug repositioning using in silico compound profiling.** Future medicinal chemistry, v. 1, n. 9, p. 1723–36, 2009.

DURANT, J. L. et al. **Reoptimization of MDL keys for use in drug discovery.** Journal of Chemical Information and Computer Sciences, v. 42, n. 6, p. 1273–1280, 2002.

EASTMAN, R. T.; FIDOCK, D. A. **Artemisinin-based combination therapies:** a vital tool in efforts to eliminate malaria. Nature reviews. Microbiology, v. 7, n. 12, p. 864–874, 2009.

EHRT, C.; BRINKJOST, T.; KOCH, O. **Impact of Binding Site Comparisons on Medicinal Chemistry and Rational Molecular Design.** Journal of Medicinal Chemistry, v. 59, n. 9, p. 4121–4151, maio 2016.

EKINS, S. et al. **In silico repositioning of approved drugs for rare and neglected diseases.** Drug Discovery Today, v. 16, n. 7–8, p. 298–310, 2011.

EMIG, D. et al. **Drug Target Prediction and Repositioning Using an Integrated Network-Based Approach**. PLoS ONE, v. 8, n. 4, 2013.

ENOMOTO, M. et al. **Blockage of spontaneous ca2+ oscillation causes cell death in intraerythrocitic plasmodium falciparum**. PLoS ONE, v. 7, n. 7, 2012.

ESU, E. et al. **Prevalence of the Pfdhfr and Pfdhps mutations among asymptomatic pregnant women in Southeast Nigeria**. 2018.

EUROPEAN MEDICINES AGENCY. **First malaria vaccine receives positive scientific opinion from EMA**. Press release, v. 44, n. July, p. 1–3, 2015.

EYAL, M.; RAHAV, G.; ELI, S. **Vivax malaria chemoprophylaxis: the role of Atovaquone-proguanil, compared to other options.** Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, n. January 2018, 2017.

FERREIRA, L. et al. **Molecular Docking and Structure-Based Drug Design** Strategies. Molecules, v. 20, n. 7, p. 13384–13421, jul. 2015.

FIDOCK, D. A. et al. **Mutations in the P. falciparum digestive vacuole**

transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Molecular cell, v. 6, n. 4, p. 861–71, 2000.

FINN, R. D. et al. **The Pfam protein families database**: towards a more sustainable future. Nucleic Acids Research, v. 44, n. D1, p. D279–D285, jan. 2016.

FISHER, J. A.; COTTINGHAM, M. D.; KALBAUGH, C. A. **Peering into the pharmaceutical "pipeline**": Investigational drugs, clinical trials, and industry priorities. Social Science and Medicine, v. 131, p. 322–330, 2015.

FONTANA, A. C. K. et al. **Two allelic isoforms of the serotonin transporter from Schistosoma mansoni display electrogenic transport and high selectivity for serotonin.** European Journal of Pharmacology, v. 616, n. 1–3, p. 48–57, 2009.

FOURCHES, D.; MURATOV, E.; TROPSHA, A. **Trust, but verify: On the importance of chemical structure curation in cheminformatics and QSAR modeling research.** Journal of Chemical Information and Modeling, 2010.

FOURCHES, D.; MURATOV, E.; TROPSHA, **A. Curation of chemogenomics data**. Nature Chemical Biology, v. 11, n. 8, p. 535–535, 2015.

FOURCHES, D.; MURATOV, E.; TROPSHA, A. **Trust, but Verify II:** A Practical Guide to Chemogenomics Data Curation. Journal of Chemical Information and Modeling, v. 56, n. 7, p. 1243–1252, 2016.

FRANÇA, T. C. C.; DOS SANTOS, M. G.; FIGUEROA-VILLAR, J. D. **Malária: Aspectos históricos e quimioterapia**. Quimica Nova, v. 31, n. 5, p. 1271–1278, 2008.

FREITAS-JUNIOR, L. H. et al. **Visceral leishmaniasis treatment:** What do we have, what do we need and how to deliver it? International Journal for Parasitology: Drugs and Drug Resistance, v. 2, p. 11–19, 2012.

FRIEDMAN, J. H. **Greedy Function Approximation:** A Gradient Boosting Machine. The Annals of Statistics, v. 29, n. 5, p. 1189–1232, 2001.

GABRIELI, P.; SMIDLER, A.; CATTERUCCIA, F. **Engineering the control of mosquito-borne infectious diseases.** Genome biology, v. 15, n. 11, p. 535, 2014.

GAJI, R. Y. et al. **Expression of the essential kinase PFCDPK1 from plasmodium falciparum in toxoplasma gondii facilitates the discovery of novel antimalarial drugs**. Antimicrobial Agents and Chemotherapy, v. 58, n. 5, p. 2598–2607, 2014.

GALPERIN, M. Y. **Conserved “hypothetical” proteins:** New hints and new puzzles. Comparative and Functional Genomics. Anais...2001

GALVAN-RAMIREZ, M. L. et al. **Nitazoxanide in the treatment of Ascaris lumbricoides in a rural zone of Colima,** Mexico. Journal of Helminthology, v. 81, n. 3, p. 255–259, set. 2007.

GARCÍA-ESTRADA, C. et al. **DNA topoisomerases in apicomplexan parasites: promising targets for drug discovery**. Proceedings. Biological sciences / The Royal Society, v. 277, n. 1689, p. 1777–1787, 2010.

GARDNER, M. J. et al. **Genome sequence of the human malaria parasite Plasmodium falciparum**. Nature, v. 419, n. 6906, p. 498–511, 2002.

GAULTON, A. et al. **ChEMBL:** a large-scale bioactivity database for drug discovery. Nucleic acids research, v. 40, n. Database issue, p. D1100-7, jan. 2012.

GEDECK, P.; ROHDE, B.; BARTELS, C. Article QSAR − **How Good Is It in Practice ?** Comparison of Descriptor Sets on an Unbiased Cross Section of Corporate Data Sets QSAR - How Good Is It in Practice ? Comparison of Descriptor Sets on an Unbiased Cross Section of Corporate Data Sets. p. 1924–1936, 2006.

GLASER, F. et al. **ConSurf:** Identification of functional regions in proteins by surface-mapping of phylogenetic information. Bioinformatics, v. 19, n. 1, p. 163–164, 2003.

GOMES, M. N. et al. **QSAR-driven design, synthesis and discovery of potent chalcone derivatives with antitubercular activity**. European Journal of Medicinal Chemistry, v. 137, p. 126–138, set. 2017.

GOODFORD, P. J. A **Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules.** Journal of medicinal chemistry, v. 28, n. 7, p. 849–857, 1985.

GOUPIL, L. S.; MCKERROW, J. H. **Introduction: Drug Discovery and Development for Neglected Diseases.** Chemical Reviews, v. 114, n. 22, p. 11131–11137, nov. 2014.

GUIMARÃES, C. R. W.; CARDOZO, M. MM-GB/SA **Rescoring of Docking Poses in Structure-Based Lead Optimization**. Journal of Chemical Information and Modeling, v. 48, n. 5, p. 958–970, maio 2008.

HALE, O.; DEUTSCH, P. G. P.; LAHIRI, A. **Epirubicin extravasation:** consequences of delayed management. BMJ Case Reports, v. 2017, p. bcr2016218012, 2017.

HARDISON, R. C. **Comparative genomics**. PLoS Biology, 2003.

HAROON, A. **PubMed** (http://www.ncbi.nlm.nih.gov/PubMed).

HART, J. D. et al. **Effect of azithromycin mass drug administration for trachoma on spleen rates in Gambian children.** Tropical Medicine & International Health, v. 19, n. 2, p. 207–211, fev. 2014.

HAY, M. et al. Clinical development success rates for investigational drugs. Nature Biotechnology, v. 32, n. 1, p. 40–51, 2014.

HEALER, J. et al. **Vaccines to Accelerate Malaria Elimination and Eventual Eradication**. Cold Spring Harbor perspectives in medicine, p. 279–295, 2017.

HENRIQUES, G. et al. A**rtemisinin resistance in rodent malaria - mutation in the AP2 adaptor mu-chain suggests involvement of endocytosis and membrane protein trafficking**. Malar J, v. 12, p. 118, 2013.

HOERAUF, A. et al. **Wolbachia endobacteria depletion by doxycycline as antifilarial therapy has macrofilaricidal activity in onchocerciasis: a randomized placebo-controlled study**. Medical Microbiology and Immunology, v. 197, n. 3, p. 295–311, set. 2008.

HOFFMAN, S. L. et al. **Plasmodium, human and Anopheles genomics and malaria**. Nature, v. 415, n. 6872, p. 702–709, 2002.

HOFSTRAAT, K.; VAN BRAKEL, W. H. **Social stigma towards neglected tropical diseases:** a systematic review. International Health, v. 8, n. suppl 1, p. i53–i70, mar. 2016.

HOTEZ, P. J. et al. **Control of Neglected Tropical Diseases**. New England Journal of Medicine, v. 357, n. 10, p. 1018–1027, set. 2007.

HOTT, A. et al. **Artemisinin-resistant Plasmodium falciparum parasites exhibit altered patterns of development in infected erythrocyte**s. Antimicrobial Agents and Chemotherapy, v. 59, n. 6, p. 3156–3167, 2015.

HOU, X. et al. **Protein Flexibility in Docking-Based Virtual Screening:** Discovery of Novel Lymphoid-Specific Tyrosine Phosphatase Inhibitors Using Multiple Crystal Structures. Journal of Chemical Information and Modeling, v. 55, n. 9, p. 1973–1983, 2015.

HUMAN, T.; PROJECT, G. **Human Genome Project. Genome,** v. 26, n. October 1990, p. 8–10, 2001.

HURLE, M. R. et al. **Computational Drug Repositioning:** From Data to Therapeutics. Clinical Pharmacology & Therapeutics, v. 93, n. 4, p. 335–341, 2013.

HYDE, J. E. **Drug-resistant malaria** - An insightFEBS Journal, 2007.

ISMAIL, H. M. et al. **Artemisinin activity-based probes identify multiple molecular targets within the asexual stage of the malaria parasites Plasmodium falciparum 3D7.** Proceedings of the National Academy of Sciences of the United States of America, v. 113, n. 8, p. 2080–5, 2016.

JIN, G.; WONG, S. T. C. **Toward better drug repositioning: prioritizing and integrating existing methods into efficient pipeline**s. Drug discovery today, v. 19, n. 5, p. 637–644, maio 2014.

JOMAA, H. et al. **Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs**. Science (New York, N.Y.), v. 285, n. 5433, p. 1573–6, set. 1999.

JONES, P. et al. **InterProScan 5:** genome-scale protein function classification. Bioinformatics, v. 30, n. 9, p. 1236–1240, maio 2014.

KATOH, K. et al. **Improvement in the accuracy of multiple sequence alignment program** MAFFT. Genome informatics, v. 16, n. 1, p. 22–33, jan. 2005.

KATOH, K.; STANDLEY, D. M. **MAFFT multiple sequence alignment software version 7: Improvements in performance and usability**. Molecular Biology and Evolution, v. 30, n. 4, p. 772–780, 2013.

KEISER, M. J. et al. **Relating protein pharmacology by ligand chemistry**. Nature biotechnology, v. 25, n. 2, p. 197–206, 2007.

KEISER, M. J. et al. **Predicting new molecular targets for known drugs.** Nature, v. 462, n. 7270, p. 175–181, 2009.

KHARKAR, P. S.; WARRIER, S.; GAUD, R. S. **Reverse docking:** a powerful tool for drug repositioning and drug rescue. Future medicinal chemistry, v. 6, n. 3, 2014.

KINCH, M. S. **2015 in review:** FDA approval of new drugsDrug Discovery Today, 2016.

KITCHEN, L. W.; VAUGHN, D. W.; SKILLMAN, D. R. **Reviews Of Anti-infective Agents:** Role of US Military Research Programs in the Development of US Food and Drug Administration–Approved Antimalarial Drugs. Clinical Infectious Diseases, v. 43, n. 1, p. 67–71, jul. 2006.

KLABUNDE, T. **Chemogenomic approaches to drug discovery: similar receptors bind similar ligands.** British journal of pharmacology, v. 152, n. 1, p. 5–7, 2007.

KLUG, D. M.; GELB, M. H.; POLLASTRI, M. P. **Repurposing strategies for tropical disease drug discovery.** Bioorganic & medicinal chemistry letters, v. 26, n. 11, p. 2569–2576, 2016.

KNOX, C. et al. **DrugBank 3.0:** A comprehensive resource for “Omics” research on

drugs. Nucleic Acids Research, v. 39, n. SUPPL. 1, 2011.

KOBETS, T.; GREKOV, I.; LIPOLDOVA, M. **Leishmaniasis:** prevention, parasite detection and treatment. Current medicinal chemistry, v. 19, n. 10, p. 1443–74, jan. 2012.

KOES, D. R.; CAMACHO, C. J. **Shape-based virtual screening with volumetric aligned molecular shapes.** Journal of Computational Chemistry, v. 35, n. 25, p. 1824–1834, set. 2014.

KOLA, I.; LANDIS, J. **Can the pharmaceutical industry reduce attrition rates?** Nature reviews. Drug discovery, v. 3, n. 8, p. 711–5, ago. 2004.

KOLLMAN, P. A. et al. **Calculating Structures and Free Energies of Complex Molecules:** Combining Molecular Mechanics and Continuum Models. Accounts of Chemical Research, v. 33, n. 12, p. 889–897, dez. 2000.

KORTAGERE, S.; KRASOWSKI, M. D.; EKINS, S. **The importance of discerning shape in molecular pharmacology.** Trends in Pharmacological Sciences, v. 30, n. 3, p. 138–147, 2009.

KOUL, P. A. et al. **Mepacrine therapy in niclosamide resistant taeniasis**. The Journal of the Association of Physicians of India, v. 48, n. 4, p. 402–403, abr. 2000.

KOUTSOUKAS, A. et al. **From in silico target prediction to multi-target drug design:** Current databases, methods and applicationsJournal of Proteomics, 2011.

KOYUTÜRK, M. **Algorithmic and analytical methods in network biology**. Wiley Interdisciplinary Reviews: Systems Biology and Medicine, v. 2, n. 3, p. 277–292, 2010.

KRISTENSEN, D. M. et al. **Computational methods for Gene Orthology inference.** Briefings in Bioinformatics, v. 12, n. 5, p. 379–391, 2011a.

KRISTENSEN, D. M. et al. **Computational methods for Gene Orthology inference**. Briefings in Bioinformatics, v. 12, n. 5, p. 379–391, set. 2011b.

KRUSCHKE, J. K. **What to believe:** Bayesian methods for data analysisTrends in Cognitive Sciences, 2010.

KUHN, M. et al. **STITCH:** interaction networks of chemicals and proteins. Nucleic acids research, v. 36, n. Database issue, p. D684-8, jan. 2008.

KUHN, M. et al. **STITCH 3:** Zooming in on protein-chemical interactions. Nucleic Acids Research, v. 40, n. D1, 2012.

KUHN, M. et al. **STITCH 4**: Integration of protein-chemical interactions with user data. Nucleic Acids Research, v. 42, n. D1, 2014.

KUNDU, C. N. et al. **Anti-malarials are anti-cancers and vice versa - One arrow two sparrows.** Acta Tropica, v. 149, p. 113–127, 2015.

LALLOO, D. G. et al. **UK malaria treatment guidelines.** Journal of Infection, v. 54, n. 2, p. 111–121, fev. 2007.

LAMBROS, C.; VANDERBERG, J. P. **Synchronization of Plasmodium falciparum erythrocytic stages in culture**. The Journal of parasitology, v. 65, n. 3, p. 418–20, 1979.

LATEEF, M. et al. **Successful treatment of niclosamide- and praziquantel-resistant beef tapeworm infection with nitazoxanide.** International Journal of Infectious Diseases, v. 12, n. 1, p. 80–82, jan. 2008.

LAVECCHIA, A. **Machine-learning approaches in drug discovery:** methods and applications. Drug Discovery Today, v. 20, n. 3, p. 318–331, 2014.

LE BRAS, J.; DURAND, R. **The mechanisms of resistance to antimalarial drugs in Plasmodium falciparum**. Fundamental & clinical pharmacology, v. 17, n. 2, p. 147–53, 2003.

LESSA, H. A. et al. **Successful treatment of refractory mucosal leishmaniasis with pentoxifylline plus antimony.** The American journal of tropical medicine and hygiene, v. 65, n. 2, p. 87–89, ago. 2001.

LI, J. et al. **A survey of current trends in computational drug repositioning**. Briefings in Bioinformatics, v. 17, n. 1, p. 2–12, 2016.

LI, J. L.; BAKER, D. A.; COX, L. S. **Sexual stage-specific expression of a third calcium-dependent protein kinase from Plasmodium falciparum**. Biochimica et Biophysica Acta - Gene Structure and Expression, v. 1491, n. 1–3, p. 341–349, 2000.

LI, L. OrthoMCL: **Identification of Ortholog Groups for Eukaryotic Genomes**. Genome Research, v. 13, n. 9, p. 2178–2189, set. 2003.

LI, W.; GODZIK, A. Cd-hit: **A fast program for clustering and comparing large sets of protein or nucleotide sequences.** Bioinformatics, v. 22, n. 13, p. 1658–1659, 2006.

LIAO, C. et al. **Software and resources for computational medicinal chemistry**. Future Medicinal Chemistry, v. 3, n. 8, p. 1057–1085, jun. 2011.

LIMA, A. N. et al. Use of machine learning approaches for novel drug discovery. Expert

Opinion on Drug Discovery, v. 11, n. 3, p. 225–239, mar. 2016.

LIMA, R. B. S. et al. I**n vitro and in vivo anti-malarial activity of plants from the Brazilian Amazon**. Malaria journal, v. 14, n. 1, p. 508, 2015.

LINDLEY, D. **Merck's new drug free to WHO for river blindness programme.** Nature, v. 329, n. 6142, p. 752–752, out. 1987.

LIONTA, E. et al. **Structure-based virtual screening for drug discovery: principles, applications and recent advances**. Current Topics in Medicinal Chemistry, v. 14, n. 16, p. 1923–1938, jan. 2014.

LITT, E.; BAKER, M. C.; MOLYNEUX, D. **Neglected tropical diseases and mental health: a perspective on comorbidity.** Trends in Parasitology, v. 28, n. 5, p. 195–201, maio 2012.

LIU, Q. et al. **Multi-target QSAR modelling in the analysis and design of HIV-HCV co-inhibitors**: an in-silico study. BMC Bioinformatics, v. 12, n. 1, p. 294, 2011.

LIU, Y.-X. et al. **New Uses for Old Drugs:** The Tale of Artemisinin Derivatives in the Elimination of Schistosomiasis Japonica in China. Molecules, v. 19, n. 9, p. 15058–15074, set. 2014.

LIU, Z. et al. **In silico drug repositioning:** what we need to know. Drug discovery today, v. 18, n. 3–4, p. 110–5, 2013.

LOGAN-KLUMPLER, F. J. et al. **GeneDB--an annotation database for pathogens.** Nucleic acids research, v. 40, p. 98–108, jan. 2012.

LUÍS REY. **Bases da Parasitologia Médica**. 2. ed. Rio de Janeiro: [s.n.].

LUMB, V.; SHARMA, Y. D. **Novel K540N mutation in Plasmodium falciparum dihydropteroate synthetase confers a lower level of sulfa drug resistance than does a K540E mutation.** Antimicrobial Agents and Chemotherapy, v. 55, n. 5, p. 2481–2482, 2011.

LUO, M. et al. **Application of Quantitative Structure–Activity Relationship Models of 5-HT 1A Receptor Binding to Virtual Screening Identifies Novel and Potent 5-HT 1A Ligands**. Journal of Chemical Information and Modeling, v. 54, n. 2, p. 634–647, fev. 2014.

LYNE, P. D.; LAMB, M. L.; SAEH, J. C. **Accurate Prediction of the Relative Potencies of Members of a Series of Kinase Inhibitors Using Molecular Docking and MM-GBSA Scoring**. Journal of Medicinal Chemistry, v. 49, n. 16, p. 4805–4808, ago. 2006.

MAGARIÑOS, M. P. et al. **TDR targets**: A chemogenomics resource for neglected

diseases. Nucleic Acids Research, v. 40, n. D1, 2012.

MAHMOUDI, S.; KESHAVARZ, H. **Efficacy of phase 3 trial of RTS, S/AS01 malaria vaccine:** The need for an alternative development plan. Human Vaccines and Immunotherapeutics, v. 5515, n. March, p. 1–4, 2017.

MAKINDE, O. D.; OKOSUN, K. O. **Impact of Chemo-therapy on Optimal Control of Malaria Disease with Infected Immigrants**. BioSystems, v. 104, n. 1, p. 32–41, 2011.

MANSOUR, T. E. **Serotonin Receptors in Parasitic Worms.** Advances in Parasitology, v. 23, p. 1–36, 1985.

MANYANDO, C. et al. **Safety and Efficacy of Co-Trimoxazole for Treatment and Prevention of Plasmodium falciparum Malaria:** A Systematic Review. PLoS ONE, v. 8, n. 2, p. e56916, 2013.

MARKS, F. et al. **Parasitological rebound effect and emergence of pyrimethamine resistance in Plasmodium falciparum after single-dose sulfadoxine-pyrimethamine.** The Journal of infectious diseases, v. 192, n. 11, p. 1962–5, 2005.

MARTI, H. et al. **A comparative trial of a single-dose ivermectin versus three days of albendazole for treatment of Strongyloides stercoralis and other soil-transmitted helminth infections in children.** The American journal of tropical medicine and hygiene, v. 55, n. 5, p. 477–481, nov. 1996.

MARTINEZ, S.; GONZALEZ, M.; VERNAZA, M. E. **Treatment of cutaneous leishmaniasis with allopurinol and stibogluconate**. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, v. 24, n. 2, p. 165–169, fev. 1997.

MARTINEZ, S.; MARR, J. J. **Allopurinol in the Treatment of American Cutaneous Leishmaniasis.** New England Journal of Medicine, v. 326, n. 11, p. 741–744, mar. 1992.

MARTORANA, A.; PERRICONE, U.; LAURIA, A. **The Repurposing of Old Drugs or Unsuccessful Lead Compounds by in Silico Approaches**: New Advances and Perspectives. Current topics in medicinal chemistry, v. 16, n. 19, p. 2088–2106, 2016.

MAYROSE, I. et al. **Comparison of site-specific rate-inference methods for protein sequences**: empirical Bayesian methods are superior. Molecular biology and evolution, v. 21, n. 9, p. 1781–91, set. 2004.

MAZIER, D. et al. **Activity of Doxycyclineagainst Preerythrocytic Malaria.** Journal of Infectious Diseases, v. 168, n. 6, p. 1603–1604, 1993.

MCFADDEN, G. I. et al. **Plastid in human parasites.** Nature, v. 381, n. 6582, p. 482–482, jun. 1996.

MCFADDEN, G. I.; YEH, E. **The apicoplast:** now you see it, now you don't. International Journal for Parasitology, v. 47, n. 2–3, p. 137–144, 2017.

MCMAHON, J. E. **Praziquantel:** a new schistosomicide against Schistosoma mansoni. Arzneimittel-Forschung, v. 31, n. 3a, p. 592–594, 1981.

MCMAHON, J. E.; KOLSTRUP, N. **Praziquantel:** a new schistosomicide against Schistosoma haematobium. British medical journal, v. 2, n. 6202, p. 1396–1369, dez. 1979.

MDACHI, R. E. et al. **Efficacy of the Novel Diamidine Compound 2,5-Bis(4-Amidinophenyl)- Furan-Bis-O-Methlylamidoxime (Pafuramidine, DB289) against Trypanosoma brucei rhodesiense Infection in Vervet Monkeys after Oral Administration.** Antimicrobial Agents and Chemotherapy, v. 53, n. 3, p. 953–957, mar. 2009.

MELO-FILHO, C. C. et al. **QSAR-Driven Discovery of Novel Chemical Scaffolds Active against Schistosoma mansoni**. Journal of chemical information and modeling, v. 56, n. 7, p. 1357–72, jul. 2016.

MELVILLE, J. L.; BURKE, E. K.; HIRST, J. D. **Machine learning in virtual screening. Combinatorial chemistry & high throughput screening**, v. 12, n. 4, p. 332–43, maio 2009.

MÉNARD, R. et al. **Plasmodium pre-erythrocytic stages: what's new? Trends in Parasitology,** v. 24, n. 12, p. 564–569, 2008.

MERCKX, A. et al. **Identification and initial characterization of three novel cyclin-related proteins of the human malaria parasite Plasmodium falciparum.** The Journal of biological chemistry, v. 278, n. 41, p. 39839–39850, 2003.

MESHNICK, S. R. **Artemisinin:** Mechanisms of action, resistance and toxicity. International Journal for Parasitology. Anais...2002

MESTRES, J. **Computational chemogenomics approaches to systematic knowledge-based drug discovery**. Current opinion in drug discovery & development, v. 7, n. 3, p. 304–313, maio 2004.

MIAO, J ; CHEN, Z; WANG, Z; SHRESTHA, S; LI, X; LI, R; CUI¶, L. **Sex-Specific Biology of the Human Malaria Parasite Revealed from the Proteomes of**

Mature Male and Female Gametocytes. Biochemistry, 2017.

MILLER, J. B. et al. **Calcium/Calmodulin-dependent protein kinase is negatively and positively regulated by calcium, providing a mechanism for decoding calcium responses during symbiosis signaling**. The Plant cell, v. 25, n. 12, p. 5053–66, 2013.

MILLER, L. H. et al. **Evaluation of clindamycin in combination with quinine against multidrug resistant strains of Plasmodium falciparum.** American Journal of Tropical Medicine and Hygiene, v. 23, n. 4, 1974.

MILLER, L. H.; SU, X. **Artemisinin:** Discovery from the Chinese herbal garden. Cell, v. 146, n. 6, p. 855–858, 2011.

MILLETTI, F.; VULPETTI, A. **Predicting Polypharmacology by Binding Site Similarity:** From Kinases to the Protein Universe. Journal of Chemical Information and Modeling, v. 50, n. 8, p. 1418–1431, ago. 2010.

MITCHELL, J. B. O. **Machine learning methods in chemoinformatics.** Wiley Interdisciplinary Reviews: Computational Molecular Science, v. 4, n. October, p. n/a-n/a, 2014.

MNZAVA, A. P. et al. **Implementation of the global plan for insecticide resistance management in malaria vectors:** progress, challenges and the way forward. Malaria journal, v. 14, p. 173, 2015a.

MNZAVA, A. P. et al. **Implementation of the global plan for insecticide resistance management in malaria vectors:** progress, challenges and the way forward. Malaria Journal, v. 14, n. 1, p. 173, 2015b.

MOORE, R. B. et al. A **photosynthetic alveolate closely related to apicomplexan parasites**. Nature, v. 451, n. 7181, p. 959–963, fev. 2008.

MORGAN, S. et al. **The cost of drug development:** A systematic reviewHealth Policy, 2011.

MUEGGE, I.; MUKHERJEE, P. **An overview of molecular fingerprint similarity search in virtual screening**. Expert Opinion on Drug Discovery, v. 11, n. 2, p. 137–148, fev. 2016.

MULAMBA, C. et al. **Widespread pyrethroid and DDT resistance in the major malaria vector anopheles funestus in East Africa is driven by metabolic resistance mechanisms.** PLoS ONE, v. 9, n. 10, 2014.

MULLARD, A. **2015 FDA drug approvals**. Nature Reviews Drug Discovery, v. 15, n. 2, p. 73–76, 2016.

MURATOV, E. N. et al. **Per aspera ad astra: application of Simplex QSAR approach in antiviral research.** Future medicinal chemistry, v. 2, n. 7, p. 1205–26, jul. 2010.

MUTI, R. et al. Kombinasi Ekstrak Batang Talikuning dan Artemisin sebagai Obat **Antimalaria terhadap Plasmodium berghei Combination of Anamirta cocculus Stem Extract and Artemisin as an Antimalaria Agent against Plasmodium berghei**. Kedokteran Brawijaya, v. 26, n. 1, p. 8–13, 2010.

NAJMANOVICH, R.; KURBATOVA, N.; THORNTON, J. Detection of 3D atomic similarities and their use in the discrimination of small molecule protein-binding sites. Bioinformatics, v. 24, n. 16, p. i105–i111, ago. 2008.

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION**. PubChem BioAssay Database;** AID=1828, Source=NIH Chemical Genomics Center, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=1828 (accessed Jan. 22, 2017). [s.d.].

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. **PubChem BioAssay Database**; AID=449703, Source=Genomics Institute of the Novartis Research Foundation, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=449703 (accessed Jan. 22, 2017). [s.d.].

National Center for Biotechnology Information. **PubChem BioAssay Database;** AID=449704, Source= Genomics Institute of the Novartis Research Foundation, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=449704 (accessed Jan. 22, 2017). [s.d.].

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. **PubChem BioAssay Database;** AID=524790, Source=National Institute of Allergy and Infectious Diseases, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=524790 (accessed Jan. 22, 2017). [s.d.].

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. **PubChem BioAssay Database;** AID=660866, Source=Medicines for Malaria Venture, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=660866 (accessed Jan. 22, 2017). [s.d.].

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. **Pubchem Bioassay database;** AID= 1883, Source=NIH Chemical Genomics Center, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=1883 (accessed Jan. 22, 2017). [s.d.].

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. **PubChem BioAssay Database**; AID= 524796, Source=National Institute of Allergy and Infectious Diseases, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=524796 (accessed Jan. 22, 2017). [s.d.].

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. **PubChem BioAssay Database;** AID= 606570, Source=NIH University of South Florida, https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=606570 (accessed Jan. 22, 2017). [s.d.].

NEVES, B. J. et al. **In Silico Repositioning-Chemogenomics Strategy Identifies New Drugs with Potential Activity against Multiple Life Stages of Schistosoma mansoni**. PLoS Neglected Tropical Diseases, v. 9, n. 1, 2015.

NEVES, B. J. BRUNO JUNIOR NEVES **Reposicionamento de fármacos e planejamento de novos compostos ativos contra Schistosoma mansoni** Goiânia BRUNO JUNIOR NEVES Reposicionamento de fármacos e planejamento de novos compostos ativos contra Schistosoma mansoni Goiânia. [s.l: s.n.].

NEVES, B. J. et al. **Modern approaches to accelerate discovery of new antischistosomal drugs**. Expert opinion on drug discovery, v. 11, n. 6, p. 557–67, 2016a.

NEVES, B. J. et al. **Discovery of New Anti-Schistosomal Hits by Integration of QSAR-**Based Virtual Screening and High Content Screening. 2016b.

NEVES, B. J. et al. **Discovery of New Anti-Schistosomal Hits by Integration of QSAR-**Based Virtual Screening and High Content Screening. Journal of medicinal chemistry, v. 59, n. 15, p. 7075–88, ago. 2016c.

NG, R. **Drugs:** From Discovery to Approval, Second Edition. [s.l: s.n.].

NICOLA NOSENGO. **Can you teach old drugs new tricks?** Nature, v. 534, n. 7607, p. 314–316, 2016.

NIGHTINGALE, S. L. **From the Food and Drug Administration.** JAMA, v. 277, n. 9, p. 703, mar. 1997.

NIGHTINGALE, S. L. **From the Food and Drug Administration.** JAMA, v. 280, n. 10, p. 872, set. 1998.

NJOROGE, M. et al. **Recent Approaches to Chemical Discovery and Development Against Malaria and the Neglected Tropical Diseases Human African Trypanosomiasis and Schistosomiasis**. Chemical reviews, v. 114, n. 22, p.

11138–11163, jul. 2014.

NOËL, F. **Sistema neuromuscular e controle da motilidade no verme adulto. In**: CARVALHO, O.; COELHO, PMZ., AND LENZI, H. (Eds.). . Schitosoma mansoni e esquistossomose: uma visão multidisciplinar [online]. Rio de Janeiro: FIOCRUZ, 2008. p. 207–244.

NOVAC, N. **Challenges and opportunities of drug repositioningTrends in** Pharmacological Sciences, 2013.

NWAKA, S.; HUDSON, A. I**nnovative lead discovery strategies for tropical diseases. Nature reviews**. Drug discovery, v. 5, n. 11, p. 941–55, nov. 2006.

O'CONNELL, E. M. et al. **Targeting Filarial Abl-like Kinases: Orally Available, Food and Drug Administration–Approved Tyrosine Kinase Inhibitors Are Microfilaricidal and Macrofilaricidal.** Journal of Infectious Diseases, v. 212, n. 5, p. 684–693, set. 2015.

O'NEILL, P. M.; BARTON, V. E.; WARD, S. A. **The molecular mechanism of action of artemisinin** - The debate continues. Molecules, v. 15, p. 1705–1721, 2010.

OGATA, H. et al. **KEGG:** Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Research, v. 27, n. 1, p. 29–34, jan. 2000.

OLADELE, T. O.; SADIKU, J. S.; BEWAJI, C. O. **In silico characterization of some hypothetical proteins in the proteome of Plasmodium falciparum**. CENTREPOINT JOURNAL (Science Edition), v. 17, n. 2, p. 129–139, 2011.

OLLIARO, P.; TAYLOR, W. R. J.; RIGAL, J. **Controlling malaria:** Challenges and solutions. Tropical Medicine and International Health, v. 6, n. 11, p. 922–927, 2001.

OPIGO, J.; WOODROW, C. **NECT trial:** more than a small victory over sleeping sickness. The Lancet, v. 374, n. 9683, p. 7–9, jul. 2009.

OPREA, T. I.; OVERINGTON, J. P. **Computational and Practical Aspects of Drug Repositioning.** Drug repurposing, rescue, and repositioning, v. 0, n. 0, p. 1–8, 2015.

OTTESEN, E. A.; ISMAIL, M. M.; HORTON, J. **The Role of Albendazole in Programmes to Eliminate Lymphatic Filariasis.** Parasitology Today, v. 15, n. 9, p. 382–386, set. 1999.

PANG, L.; LIMSOMWONG, N.; SINGHARAJ, P. **Prophylactic Treatment of Vivax and Falciparum Malaria with Low-Dose Doxycycline.** Journal of Infectious Diseases, v. 158, n. 5, p. 1124–1127, nov. 1988.

PARHIZGAR, A. R. **Introducing new antimalarial analogues of chloroquine and amodiaquine:** A narrative review. Iranian Journal of Medical Sciences, v. 42, n. 2, p. 115–128, 2017.

PATOCKA, N.; RIBEIRO, P. **Characterization of a serotonin transporter in the parasitic flatworm, Schistosoma mansoni:** cloning, expression and functional analysis. Molecular and biochemical parasitology, v. 154, n. 2, p. 125–133, 2007.

PEDRIQUE, B. et al. **The drug and vaccine landscape for neglected diseases (2000–11):** a systematic assessment. The Lancet Global Health, v. 1, n. 6, p. e371–e379, dez. 2013.

PEREZ-MOLINA, J. A. et al. **Evaluation of Nitazoxanide for the Treatment of Disseminated Cystic Echinococcosis**: Report of Five Cases and Literature Review. American Journal of Tropical Medicine and Hygiene, v. 84, n. 2, p. 351–356, fev. 2011.

PÉREZ DEL VILLAR, L. et al. **Systematic review and meta-analysis of artemisinin based therapies for the treatment and prevention of schistosomiasis**. PloS one, v. 7, n. 9, p. e45867, jan. 2012.

PHETSUKSIRI, B. et al. **Antimycobacterial activities of isoxyl and new derivatives through the inhibition of mycolic acid synthesis**. Antimicrobial Agents and Chemotherapy, v. 43, n. 5, p. 1042–1051, 1999.

PHETSUKSIRI, B. et al. **Unique Mechanism of Action of the Thiourea Drug Isoxyl on Mycobacterium tuberculosis**. Journal of Biological Chemistry, v. 278, n. 52, p. 53123–53130, 2003.

PHILIPPE, H. et al. **Phylogenomics.** Annual Review of Ecology, Evolution, and Systematics, v. 36, n. 1, p. 541–562, 2005.

PLOUFFE, D. et al. **In silico activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput scree**n. Proceedings of the National Academy of Sciences, v. 105, n. 26, p. 9059–9064, jul. 2008.

POHLIG, G. et al. **Efficacy and Safety of Pafuramidine versus Pentamidine Maleate for Treatment of First Stage Sleeping Sickness in a Randomized, Comparator-Controlled, International Phase 3 Clinical Trial**. PLOS Neglected Tropical Diseases, v. 10, n. 2, p. e0004363, fev. 2016.

POLLASTRI, M. P.; CAMPBELL, R. K. **Target repurposing for neglected diseases.** Future medicinal chemistry, v. 3, n. 10, p. 1307–15, 2011.

PRIOTTO, G. et al. **Nifurtimox-eflornithine combination therapy for second-stage**

African Trypanosoma brucei gambiense trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial. The Lancet, v. 374, n. 9683, p. 56–64, jul. 2009.

PROTASIO, A. V et al. **A systematically improved high quality genome and transcriptome of the human blood fluke Schistosoma mansoni**. PLoS neglected tropical diseases, v. 6, n. 1, p. e1455, jan. 2012.

PUBCHEM. **PubChem Compound**. Disponível em: <https://www.ncbi.nlm.nih.gov/pccompound>.

PUIGBÒ, P.; WOLF, Y. I.; KOONIN, E. V. **The tree and net components of prokaryote evolution**. Genome Biology and Evolution, v. 2, p. 745–756, 2010.

R DEVELOPMENT CORE TEAM. R: A **Language and Environment for Statistical Computing.** R Foundation for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing, 2008.

RAHMAN, M. S.; METTRICK, D. F.; PODESTA, R. B. **Schistosoma mansoni:** effects of in vitro serotonin (5-HT) on aerobic and anaerobic carbohydrate metabolism. Experimental parasitology, v. 60, n. 1, p. 10–17, 1985.

RALPH, S. A. et al. **Metabolic maps and functions of the Plasmodium falciparum apicoplast.** Nature Reviews Microbiology, v. 2, n. 3, p. 203–216, mar. 2004.

RENSLO, A. R.; MCKERROW, J. H. **Drug discovery and development for neglected parasitic diseases**. Nature chemical biology, v. 2, n. 12, p. 701–710, dez. 2006.

RIECKMANN, K. H**. Falciparum Malaria**: The Urgent Need for Safe and Effective Drugs. Annual Review of Medicine, v. 34, n. 1, p. 321–335, 1983.

RIECKMANN, K. H. et al. **Recent military experience with malaria chemoprophylaxis.** Medical Journal of Australia, v. 158, n. 7, p. 446–449, 1993.

RIECKMANN KH, POWELL RD, MCNAMARA JV, WILLERSON D JR, LASS L, FRISCHER H, C. P. **Effects of tetracycline against chloroquine-resistant and chloroquine-sensitive Plasmodium falciparum**. The American Journal of Tropical Medicine and Hygiene, v. 20, n. 6, p. 811–815, 1971.

RIM, H. J. et al. **Therapeutic effects of praziquantel (Embay 8440) against Hymenolepis nana infection.** The Korean Journal of Parasitology, v. 16, n. 2, p. 82–87, 1978.

RIM, H. J. et al. **Therapeutic effects of praziquantel (Embay 8440) against Taenia solium infection**. The Korean Journal of Parasitology, v. 17, n. 1, p. 67–72, 1979.

RINIKER, S. et al. **Open-source platform to benchmark fingerprints for ligand-based virtual screening**. Journal of Cheminformatics, v. 5, n. 1, p. 26, 2013.

RODRIGUES, J. et al. **Identificação de novos fármacos contra a doença de Chagas através de estratégia de genômica e bioinformática.** p. 77–84, 2015.

RODRIGUES, L. A. et al. **Experimental evolution of resistance to artemisinin combination therapy results in amplification of the mdr1 gene in a rodent malaria parasite.** PLoS One, v. 5, n. 7, p. e11593, 2010.

ROGNAN, D. Chemogenomic approaches to rational drug design 1. Br.J.Pharmacol., v. 152, n. 1, p. 38–52, 2007.

ROSE, P. W. et al. **The RCSB Protein Data Bank:** views of structural biology for basic and applied research and education. Nucleic acids research, v. 43, n. Database issue, p. D345-56, jan. 2015.

ROSSIGNOL, J. F.; ABAZA, H.; FRIEDMAN, H. **Successful treatment of human fascioliasis with nitazoxanide.** Transactions of the Royal Society of Tropical Medicine and Hygiene, v. 92, n. 1, p. 103–104, 1998.

ROSSIGNOL, J. F.; MAISONNEUVE, H. **Nitazoxanide in the treatment of Taenia saginata and Hymenolepis nana infections**. The American journal of tropical medicine and hygiene, v. 33, n. 3, p. 511–512, maio 1984.

RUSSELL, B. et al. **Field-based flow cytometry for ex vivo characterization of plasmodium vivax and P. falciparum antimalarial sensitivity**. Antimicrobial Agents and Chemotherapy, v. 57, n. 10, p. 5170–5174, 2013.

SAEED, M. E. M. et al. A**ntischistosomal activity of artemisinin derivatives in vivo and in patients**. Pharmacological Research, v. 110, p. 216–226, ago. 2016.

SANTOS, A. T. et al. **Preliminary clinical trials with praziquantel in Schistosoma japonicum infections in the Philippines**. Bulletin of the World Health Organization, v. 57, n. 5, p. 793–799, 1979.

SARDANA, D. et al. **Drug repositioning for orphan diseases**. Briefings in Bioinformatics, v. 12, n. 4, p. 346–356, 2011.

SATERIALE, A. et al. **Drug repurposing:** mining protozoan proteomes for targets of known bioactive compounds. Journal of the American Medical Informatics Association, v. 21, n. 2, p. 238–244, mar. 2014.

SBARAGLINI, M. L. et al. **Neglected Tropical Protozoan Diseases:** Drug Repositioning as a Rational Option. Current topics in medicinal chemistry, v. 16, n. 19, p. 2201–2222, 2016.

SCHMID, C. et al. **In-Hospital Safety in Field Conditions of Nifurtimox Eflornithine Combination Therapy (NECT) for T. b. gambiense Sleeping Sickness.** PLoS Neglected Tropical Diseases, v. 6, n. 11, p. e1920, nov. 2012.

SCHMITT, S.; KUHN, D.; KLEBE, G. **A New Method to Detect Related Function Among Proteins Independent of Sequence and Fold Homology.** Journal of Molecular Biology, v. 323, n. 2, p. 387–406, out. 2002.

SHU-HUA, X. **Development of antischistosomal drugs in China, with particular consideration to praziquantel and the artemisinins.** Acta Tropica, v. 96, n. 2–3, p. 153–167, nov. 2005.

Sildenafil: an oral drug for impotence. **The Medical letter on drugs and therapeutics**, v. 40, n. 1026, p. 51–52, maio 1998.

SILVA, L. A. et al. **In silico search of energy metabolism inhibitors for alternative leishmaniasis treatments**. BioMed Research International, v. 2015, 2015.

SILVA, L. F. R. E et al. **In vitro susceptibility of Plasmodium falciparum Welch field isolates to infusions prepared from Artemisia annua L. cultivated in the Brazilian Amazon.** Memorias do Instituto Oswaldo Cruz, v. 107, n. 7, p. 859–866, 2012.

SILVA, B. V. et al. **Proteíns quinases:** Características estruturais e inibidores químicos. Quimica Nova, v. 32, n. 2, p. 453–462, 2009.

SIMON, B.; HUART, A.-S.; WILMANNS, M. **Molecular mechanisms of protein kinase regulation by calcium/calmodulin**. Bioorganic & Medicinal Chemistry, v. 23, n. 12, p. 2749–2760, 2015.

SINDERMANN, H. et al. **Miltefosine (Impavido):** the first oral treatment against leishmaniasis. Medical microbiology and immunology, v. 193, n. 4, p. 173–80, nov. 2004.

SINGH, S.; MALIK, B. K.; SHARMA, D. K. **Choke point analysis of metabolic pathways in E.histolytica:** a computational approach for drug target identification. Bioinformation, v. 2, n. 2, p. 68–72, 2007.

SMITH, D. A.; JONES, R. M. **The sulfonamide group as a structural alert:** A distorted story? Current opinion in drug discovery & development, v. 11, n. 1, p. 72–9, 2008.

SONNHAMMER, E. L.; EDDY, S. R.; DURBIN, R. **Pfam: a comprehensive database of protein domain families based on seed alignments.** Proteins, v. 28, n. 3, p. 405–20, jul. 1997.

SPANGENBERG, T. et al. **The Open Access Malaria Box:** A Drug Discovery Catalyst for Neglected Diseases. PLoS ONE, v. 8, n. 6, 2013.

SPECK-PLANCHE, A.; CORDEIRO, M. N. D. S. **Multi-Target QSAR Approaches for Modeling Protein Inhibitors. Simultaneous Prediction of Activities Against Biomacromolecules Present in Gram-Negative Bacteria.** Current topics in medicinal chemistry, v. 15, n. 18, p. 1801–1813, 2015.

SUPALI, T. et al. **Doxycycline Treatment of Brugia malayi –Infected Persons Reduces Microfilaremia and Adverse Reactions after Diethylcarbamazine and Albendazole Treatment.** Clinical Infectious Diseases, v. 46, n. 9, p. 1385–1393, maio 2008.

SUPURAN, C. T. **Carbonic anhydrase inhibitors and activators for novel therapeutic applications.** Future Med Chem, v. 3, n. 9, p. 1165–1180, 2011.

SWINTON, N. W.; REAVES, L. E. **Successful therapy of Strongyloidiasis with thiabendazole.** Lahey Clinic Foundation bulletin, v. 14, n. 3, p. 117–120, 1965.

TA, T. H. et al. **First case of a naturally acquired human infection with Plasmodium cynomolgi.** Malaria journal, v. 13, p. 68, 2014.

TAGBOTO, S. K.; TOWNSON, S. **Onchocerca volvulus and O. lienalis:** the microfilaricidal activity of moxidectin compared with that of ivermectin in vitro and in vivo. Annals of tropical medicine and parasitology, v. 90, n. 5, p. 497–505, out. 1996.

TALELE, T.; KHEDKAR, S.; RIGBY, A. **Successful Applications of Computer Aided Drug Discovery:** Moving Drugs from Concept to the Clinic. Current Topics in Medicinal Chemistry, v. 10, n. 1, p. 127–141, 2010.

TAMAROZZI, F. et al. **Long term impact of large scale community-directed delivery of doxycycline for the treatment of onchocerciasis**. Parasites & Vectors, v. 5, n. 1, p. 53, 2012.

TAN, K. R. et al. **Doxycycline for malaria chemoprophylaxis and treatment: Report from the CDC expert meeting on malaria chemoprophylaxis.** American Journal of Tropical Medicine and Hygiene. Anais...2011

TANG GIRDWOOD, S. C.; NENORTAS, E.; SHAPIRO, T. A. T**argeting the gyrase of Plasmodium falciparum with topoisomerase poisons.** Biochemical Pharmacology, v. 95, n. 4, p. 227–237, 2015.

TARRAL, A. et al. **Determination of an Optimal Dosing Regimen for Fexinidazole, a Novel Oral Drug for the Treatment of Human African Trypanosomiasis:**

First-in-Human Studies. Clinical Pharmacokinetics, v. 53, n. 6, p. 565–580, jun. 2014.

TAYLOR, C. M. et al. **Discovery of Anthelmintic Drug Targets and Drugs Using Chokepoints in Nematode Metabolic Pathways.** PLoS Pathogens, v. 9, n. 8, 2013.

TAYLOR, M. J. et al. **Macrofilaricidal activity after doxycycline treatment of Wuchereria bancrofti: a double-blind, randomised placebo-controlled trial.** The Lancet, v. 365, n. 9477, p. 2116–2121, jun. 2005.

TAYLOR, M. J.; HOERAUF, A.; BOCKARIE, M. **Lymphatic filariasis and onchocerciasis.** The Lancet, v. 376, n. 9747, p. 1175–1185, out. 2010.

TEMPERINI, C. et al. Carbonic anhydrase inhibitors. **Sulfonamide diuretics revisited--old leads for new applications?** Organic & biomolecular chemistry, v. 6, n. 14, p. 2499–506, 2008.

THE MALERA CONSULTATIVE GROUP ON VACCINES. **A research agenda for malaria eradication**: Vaccines. PLoS Medicine, v. 8, n. 1, 2011.

Thiabendazole (Mintezol)-a new anthelmintic. **The Medical letter on drugs and therapeutics,** v. 9, n. 25, p. 99–101, dez. 1967.

THRIEMER, K. et al. **Azithromycin combination therapy for the treatment of uncomplicated falciparum malaria in Bangladesh:** an open-label randomized, controlled clinical trial. The Journal of infectious diseases, v. 202, n. 3, p. 392–8, 2010.

TIBAYRENC, M.; AYALA, F. J. **Reproductive clonality of pathogens:** A perspective on pathogenic viruses, bacteria, fungi, and parasitic protozoa. Proceedings of the National Academy of Sciences, v. 109, n. 48, p. E3305–E3313, nov. 2012.

TILLEY, L. et al. **Artemisinin Action and Resistance in Plasmodium falciparum.** Trends in Parasitology, v. xx, n. 9, p. 1–15, 2016.

TOTROV, M. **Ligand binding site superposition and comparison based on Atomic Property Fields:** identification of distant homologues, convergent evolution and PDB-wide clustering of binding sites. BMC Bioinformatics, v. 12, n. Suppl 1, p. S35, 2011.

TRAGER, W.; JENSEN, J. B. **Human malaria parasites in continuous culture.** Science (New York, N.Y.), v. 193, n. 4254, p. 673–5, 1976.

TROPSHA, A. **Best Practices for QSAR Model Development, Validation, and Exploitation**. Molecular Informatics, v. 29, n. 6–7, p. 476–488, jul. 2010.

TU, H.; SHI, T. Ligand Binding Site Similarity Identification Based on Chemical and Geometric Similarity. The Protein Journal, v. 32, n. 5, p. 373–385, jun. 2013.

UDDIN, T.; MCFADDEN, G. I.; GOODMAN, C. D. **Validation of putative apicoplast targeting drugs using a chemical supplementation assay in cultured human malaria parasites**. Antimicrobial Agents and Chemotherapy, p. AAC.01161-17, 2017.

VAN DE ERVE, J. **Systemic thiabendazole treatment of creeping eruption**. Journal of the South Carolina Medical Association (1975), v. 62, n. 7, p. 263–264, jul. 1966.

VAPNIK, V. **The Nature of Statistical Learning Theory**. 2nd. ed. New York: Springer, 2000.

VERLI, H. **Bioinformática da Biologia à Flexibilidade Molecular.** [s.l: s.n.]. v. 53

VERLINDEN, B. K.; LOUW, A.; BIRKHOLTZ, L.-M. **Resisting resistance:** is there a solution for malaria? Expert Opinion on Drug Discovery, v. 11, n. 4, p. 395–406, 2016.

VIERA, A. J.; GARRETT, J. M. **Understanding interobserver agreement:** the kappa statistic. Family medicine, v. 37, n. 5, p. 360–363, maio 2005.

VUORINEN, A.; SCHUSTER, D. **Methods for generating and applying pharmacophore models as virtual screening filters and for bioactivity profiling**. Methods, v. 71, p. 113–134, jan. 2015.

WADOOD, A. et al. **In-silico drug design:** An approach which revolutionarised the drug discovery process. open Access Drug Design and Delivery, v. 1, n. 3, p. 1–4, 2013.

WAHEED, A. A. et al**. Vivax malaria and chloroquine resistance:** a neglected disease as an emerging threat. Malaria Journal, v. 14, n. 1, p. 146, dez. 2015.

WANG, J. et al. **Use of MM-PBSA in Reproducing the Binding Free Energies to HIV-1 RT of TIBO Derivatives and Predicting the Binding Mode to HIV-1 RT of Efavirenz by Docking and MM-PBSA**. Journal of the American Chemical Society, v. 123, n. 22, p. 5221–5230, jun. 2001.

WANG, Q. et al. **Targeting Lysine Deacetylases (KDACs) in Parasites.** PLOS Neglected Tropical Diseases, v. 9, n. 9, p. e0004026, set. 2015a.

WANG, Y. et al. **An overview of the PubChem BioAssay resource.** Nucleic Acids Research, v. 38, n. Database issue, p. D255-66, jan. 2010.

WANG, Y. et al. **PubChem's BioAssay Database**. Nucleic acids research, v. 40, n.

Database issue, p. D400-12, jan. 2012.

WANG, Y. et al. **OrthoVenn:** A web server for genome wide comparison and annotation of orthologous clusters across multiple species. Nucleic Acids Research, v. 43, n. W1, p. W78–W84, 2015b.

WARR, W. A. et al. **SciFinder ® Overview.** Science & Technology Libraries, v. 29, n. 4, p. P4, 2011.

WEISS, M. G. **Stigma and the Social Burden of Neglected Tropical Disease**s. PLoS Neglected Tropical Diseases, v. 2, n. 5, p. e237, maio 2008.

WELLEMS, T. E. et al. **Chloroquine resistance not linked to mdr-like genes in a Plasmodium falciparum cross**. Nature, v. 345, n. 6272, p. 253–255, 1990.

WERMUTH, C. G. **The Practice of Medicinal Chemistry.** [s.l: s.n.].

WHITE, A. T. et al. **Controlled Trial and Dose-Finding Study of Ivermectin for Treatment of Onchocerciasi**s. Journal of Infectious Diseases, v. 156, n. 3, p. 463–470, set. 1987.

WHITE, N. J. et al. **Malaria.** The Lancet, v. 383, n. 9918, p. 723–735, 2014.

WHO. **Control of the leishmaniases.** WHO Expert Committee on the Control of Leishmaniases. Geneva: [s.n.].

WHO. **Reversing the Incidence of Malaria 2000–2015**. 2015a.

WHO. **Global technical strategy for malaria 2016-2030**. World Health Organization. [s.l: s.n.]. Disponível em: <http://apps.who.int/iris/bitstream/10665/176712/1/9789241564991_eng.pdf?ua=1>.

WHO, W. H. O. **World Malaria** Report. [s.l: s.n.].

WHO, W. H. O. **WORLD MALARIA REPORT 2015**. [s.l: s.n.].

WILSON, R. J.; WILLIAMSON, D. H.; PREISER, P. **Malaria and other Apicomplexans: the “plant” connection**. Infectious agents and disease, v. 3, n. 1, p. 29–37, 1994.

WISHART, D. S. et al. **DrugBank: a comprehensive resource for in silico drug discovery and exploration.** Nucleic Acids Research, v. 34, n. 90001, p. D668–D672, 2006a.

WISHART, D. S. et al. **DrugBank: a comprehensive resource for in silico drug discovery and exploration.** Nucleic acids research, v. 34, n. Database issue, p. D668-72, jan. 2006b.

WOLF, R. et al. **Dapsone.** Dermatol Online J, v. 8, n. 1, p. 2, 2002.

WOOD, D. J. et al. **Pharmacophore fingerprint-based approach to binding site subpocket similarity and its application to bioisostere replacement.** Journal of chemical information and modeling, v. 52, n. 8, p. 2031–43, ago. 2012.

WOODROW, C. J.; KRISHNA, S. **Antimalarial drugs: recent advances in molecular determinants of resistance and their clinical significance**. Cellular and molecular life sciences : CMLS, v. 63, n. 14, p. 1586–96, 2006.

WORLD HEALTH ORGANIZATION. **World Malaria Report 2016**. World Health Organization. [s.l: s.n.].

World Health Organization. **Neglected tropical diseases.** http://www.who.int/neglected_diseases/diseases/en (Accessed July 20, 2016).

WORLD HEALTH ORGANIZATION MALARIA POLICY ADVISORY COMMITTEE AND SECRETARIAT. **Malaria Policy Advisory Committee to the WHO:** conclusions and recommendations of sixth biannual meeting (September 2014). Malaria journal, v. 14, p. 107, 2015.

WYATT, P. G. et al. **Target validation:** linking target and chemical properties to desired product profile. Current topics in medicinal chemistry, v. 11, n. 10, p. 1275–83, jan. 2011.

YANG, H. et al. Therapeutic target database update 2016: Enriched resource for bench to clinical drug target and targeted pathway information. Nucleic Acids Research, v. 44, n. D1, p. D1069–D1074, 2016.

YEH, E.; DERISI, J. L. **Chemical Rescue of Malaria Parasites Lacking an Apicoplast Defines Organelle Function in Blood-Stage Plasmodium falciparum**. PLoS Biology, v. 9, n. 8, p. e1001138, ago. 2011.

YETURU, K.; CHANDRA, N. **PocketMatch:** A new algorithm to compare binding sites in protein structures. BMC Bioinformatics, v. 9, n. 1, p. 543, 2008.

YU, A. Z.; RAMSEY, S. A. **A Computational Systems Biology Approach for Identifying Candidate Drugs for Repositioning for Cardiovascular Disease**. Interdisciplinary Sciences: Computational Life Sciences, 2016.

YUAN, J. et al. **Genetic mapping of targets mediating differential chemical phenotypes in Plasmodium falciparum.** Nature Chemical Biology, v. 5, n. 10, p. 765–771, out. 2009.

ZAKHAROV, A. V et al. **QSAR modeling of imbalanced high-throughput screening data in PubChem.** Journal of chemical information and modeling, v. 54, n. 3, p. 705–12, mar. 2014.

ZHANG, L. et al. **Discovery of novel antimalarial compounds enabled by QSAR-based virtual screening.** Journal of Chemical Information and Modeling, v. 53, n. 2, p. 475–492, 2013.

ZHANG, S. et al. **A novel Automated Lazy Learning QSAR (ALL-QSAR) approach:** Method development, applications, and virtual screening of chemical databases using validated ALL-QSAR models. Journal of Chemical Information and Modeling, v. 46, n. 5, p. 1984–1995, 2006.

ZHONG, S.; ZHANG, Y.; XIU, Z. **Rescoring ligand docking poses.** Current opinion in drug discovery & development, v. 13, n. 3, p. 326–334, maio 2010.

ZHU, F. et al. **Update of TTD:** Therapeutic Target Database. Nucleic Acids Research, v. 38, n. SUPPL.1, 2009.

ZHU, F. et al. **Therapeutic target database update 2012:** A resource for facilitating target-oriented drug discovery. Nucleic Acids Research, v. 40, n. D1, 2012.

ZIJLSTRA, E. E. et al. **Mycetoma:** a unique neglected tropical disease. The Lancet Infectious Diseases, v. 16, n. 1, p. 100–112, jan. 2016.

ZUMAQUERO-RÍOS, J. L. et al**. Fascioliasis and Intestinal Parasitoses Affecting Schoolchildren in Atlixco, Puebla State, Mexico**: Epidemiology and Treatment with Nitazoxanide. PLoS Neglected Tropical Diseases, v. 7, n. 11, p. e2553, nov. 2013.

ZWIERZ, C.; MACHNICKA, B. **Treatment with praziquantel the Taenia saginata infection in men. Bulletin of the Institute of Maritime and Tropical Medicine in Gdynia,** v. 36, n. 1–4, p. 77–80, 1985.