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Review

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Computer-Aided Drug Discovery Approaches against the Tropical Infectious Diseases Malaria, Tuberculosis, Trypanosomiasis, and Leishmaniasis

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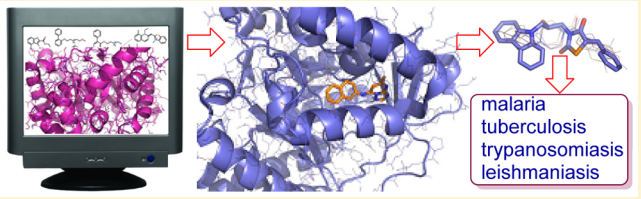
ABSTRACT: Despite the tremendous improvement in overall global health heralded by the adoption of the Millennium Declaration in the year 2000, tropical infections remain a major health problem in the developing world. Recent estimates indicate that the major tropical infectious diseases, namely, malaria, tuberculosis, trypanosomiasis, and leishmaniasis, account for more than 2.2 million deaths and a loss of approximately 85 million disability-adjusted life years annually.



The crucial role of chemotherapy in curtailing the deleterious health and economic impacts of these infections has invigorated the search for new drugs against tropical infectious diseases. The research efforts have involved increased application of computational technologies in mainstream drug discovery programs at the hit identification, hit-to-lead, and lead optimization stages. This review highlights various computer-aided drug discovery approaches that have been utilized in efforts to identify novel antimalarial, antitubercular, antitrypanosomal, and antileishmanial agents. The focus is largely on developments over the past 5 years (2010−2014).

KEYWORDS: *tropical infections, computer-aided drug discovery, malaria, tuberculosis, trypanosomiasis, leishmaniasis*

1.0. INTRODUCTION

On September 8, 2000, world leaders adopted the Millennium Declaration during the 55th session (Millennium Summit) of the United Nations (UN) General Assembly at UN headquarters in New York.[1] The Millennium Declaration is the blueprint that set forth and articulated measures toward achievement of the eight ambitious Millennium Development Goals (MDGs) to reduce extreme poverty, hunger, and disease in the world by the year 2015.[2] The sixth MDG, "to combat HIV-AIDS [human immunodeficiency virus-acquired immune deficiency syndrome], malaria, and other diseases", specifically addresses the deleterious health and economic impact of infectious diseases.[3] Concerted global efforts initiated immediately prior to and after the Millennium Summit toward attainment of this goal have inexorably spearheaded an epidemiological transition in the pattern of morbidity and mortality characterized by a gradual decline in the causes due to communicable diseases and a concomitant increase in the causes by noncommunicable diseases.[4,5] Nevertheless, infectious diseases that have a predominant prevalence in the tropical and subtropical regions of the world, namely, malaria, tuberculosis (TB), human African trypanosomiasis (HAT), American trypanosomiasis (Chagas' disease), and leishmaniasis, still comprise the single most significant proportion of disease burden in developing countries.[6,7]

As itemized in Table 1, tropical infectious diseases are estimated to account for more than 2.2 million deaths and an approximate loss of 85 million disability-adjusted life years (DALYs) annually in the developing world.[3,8] Interventions to curtail the health and economic impact of these infectious ailments have involved preventative strategies such as vaccination, chemoprophylaxis, public health awareness campaigns, and vector control measures. However, the bedrock of successful control of tropical infections has so far been effective case management through chemotherapy. Unfortunately, clinical use of the available drugs for tropical infections is severely restricted by several liabilities including limited efficacy, exorbitant treatment costs, intolerable host toxicity, and inevitable emergence of drug-resistant microbes. There is thus an ever-increasing need for continued search for safe, effective, and affordable medicines for the management of these diseases. The global drug discovery enterprise has endeavored to meet this need, and there currently are numerous ongoing efforts to discover and develop new drugs for tropical infectious diseases.

The discovery of drugs for tropical infectious diseases has traditionally relied on phenotypic whole-cell screening approaches.[11,12] However, mechanistic target-based approaches have gained traction and are currently used complementarily

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Table 1. Global Burden of Disease Due to Major Tropical Infectious Diseases[a]

infection	global prevalence (millions)	population at risk (millions)	annual mortality (thousands)	DALYs,[b] 2004 (millions)	regions of highest prevalence
malaria[9]	198	3200	584	46.5	sub-Saharan Africa, Asia, South and Latin America, Middle East, and Pacific Islands
tuberculosis[10]	11	2000	1100	34.7[8]	sub-Saharan Africa and Southeast Asia
leishmaniasis[3,8]	12	350	51	2.1	India, South Asia, sub-Saharan Africa, Latin America, Caribbean, and Mediterranean region
HAT[c,3,8]	0.3	60	48	1.5	sub-Saharan Africa
Chagas' disease[3,8]	10	120	14	0.7	Latin America and Caribbean

[a]Quoted estimates were obtained from the cited references. [b]DALYs, disability-adjusted life years. [c]HAT, human African trypanosomiasis.

with the empirical cell-based assays.[13] This complementarity is best illustrated by the observation that whereas phenotypic cell-based screening has been relatively more successful than target-based approaches in delivering first-in-class small-molecule drugs, target-based drug discovery has been much more successful for follower drugs.[14,15] Target-based drug discovery approaches have lately been emphasized as a way of harvesting the significant investment made in the genomic and proteomic initiatives as well as to allow for the exploitation of large chemical libraries of small-molecule compounds readily available through combinatorial chemistry.[16] In any case, because most anti-infective agents exert therapeutic effects by binding to and regulating the biological activity of a particular mechanistic target in the pathogenic microbe, target-driven approaches are best suited for the discovery of rational therapies with a well-founded molecular basis.

In addition to the experimental in vitro target-based screening of compound libraries, a variety of computational methods are gaining greater utility in the discovery of anti-infective agents for tropical infections. As the terminology suggests, computer-aided drug discovery (CADD) refers to the utilization of computer technology and software to underpin drug discovery efforts. Also referred to as in silico drug discovery, it is the application of computational methods to support, guide, and streamline the processes of drug discovery, design, development, and optimization. These methods have proven useful right from the initial stages of hit identification and hit-to-lead development to the more involved lead optimization stages of drug discovery.

The two most commonly reported computational approaches to drug discovery are structure-based methods (primarily molecular docking studies) and ligand-based approaches such as ligand-based pharmacophore methods and three-dimensional quantitative structure−activity relationship (3D QSAR) models.[17,18] Additionally, a range of filtering methods are used to eliminate unattractive compounds on the basis of unfavorable physicochemical, pharmacokinetic, and toxicity properties as well as to enrich data sets with drug-like compounds.[19,20]

1.1. Structure-Based Computational Approaches. Structure-based drug discovery (SBDD) relies on information from the 3D structure of the drug target in the search for new compounds active against the particular target. The 3D structural information is almost always derived from the crystal structure of the drug target, preferably cocrystallized with a known ligand, and is used to guide the discovery of new ligands either by informing the design of novel compounds or through the identification of new ligands from the screening of virtual compound libraries. The main advantages of target-based approaches, particularly against validated targets of known structure, is that inhibitor progression can be guided more rationally through the pursuit of desirable steric and electronic complementarity between the target and designed ligands. Potency and selectivity can be more readily optimized secure in the knowledge of the intended target.[21]

The most commonly used SBDD approach is molecular docking,[17] whereby computer software simulates ligand−target binding and predicts binding conformations and molecular interactions between ligands and target macromolecules. Aside from its application in the screening and prioritization of compounds with desirable predicted binding energies, molecular docking is also important in elucidating possible binding modes and attendant ligand−target interactions. Another popular SBDD approach involves the use of structure-based pharmacophore models for virtual screening of compound libraries or to guide the design of novel ligands with potential affinity for the target.

In cases when the 3D crystal structure of a target of interest is not available, a theoretical 3D structure of the protein may be built to support SBDD techniques. Such models, referred to as homology models, can be used in a manner similar to crystal structures, enabling docking studies, investigation of ligand−target interactions, and mechanisms of action studies. A significant number of structure-based CADD studies are based on homology models. The use of homology models facilitates the utilization of structure-based CADD even in the absence of experimental 3D protein structures. The homology model of a target protein is based on another protein (i.e., the template) for which the experimental 3D structure is available, and it therefore comes as no surprise that the major limitation to the use of homology models is that the accuracy of the model is highly dependent on the sequence identity between target protein and the template. A sequence identity >40% is usually considered adequate for correct alignment.[22] It is self-evident that adequate sequence identity in and around the active site is important in homology models meant to be used for molecular docking studies. This sequence identity requirement also applies for those regions away from the binding interface where conformational changes could affect ligand binding. Poor sequence identity in these regions, including gaps in template or query sequences, could significantly compromise the utility of a homology model to support SBDD even in cases when the rest of the protein exhibits satisfactory sequence identity.

1.2. Ligand-Based Computational Approaches. Ligand-based drug design (LBDD) approaches are useful in the absence of the 3D crystal structure (or valid homology model) of the intended drug target, which renders SBDD impracticable. The generation and use of ligand-based methods offer a viable CADD alternative in cases when a set of active inhibitors/ligands is known. Ligand-based pharmacophores are built based on molecular descriptors derived from the known active ligands, such as H-bond donors, H-bond acceptors, hydrophobic groups, and ionizable groups. These models attempt to identify the ligand features necessary for ligand−target complementarity and

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Table 2. Antimalarial Drug Targets That Have Recently Been Used in CADD Approaches

target[a]	function
P. falciparum macrophage migratory inhibitory factor (*Pf* MIF)[28]	*Pf* MIF is a homologue of the human immunoregulatory cytokine MIF; it is released by *P. falciparum* upon malaria infection and mediates the host's immune response; inhibition of tautomerase activity in the *Pf* MIF may affect the cytokine activity
P. falciparum cytochrome bc1 complex[27,62]	this is a key element in the mitochondrial respiratory chain and is essential for survival of *P. falciparum*; blocking of the oxidation site (Qo) of cytochrome inhibits pyrimidine biosynthesis, leading to parasite death
P. falciparum FKBP35[28]	FKBP35 belongs to the immunophilin family, also known as FK506 binding proteins (FKBPs); it is involved in the calcineurin pathway essential for the growth of plasmodia
P. falciparum plasmepsins (*Pf* PM)[29,30,58]	plasmepsins are protease enzymes involved in hemoglobin degradation and are necessary for growth and maturation of plasmodia
P. falciparum enoyl-acyl carrier protein (ACP) reductase[31,50]	enoyl ACP reductase catalyzes the final step of the type II fatty acid biosynthesis; being an oxidoreductase, the enzyme requires the reduced form of nicotinamide adenine dinucleotide (NAD^+) cofactor, NADH
P. falciparum dihydroorotate dehydrogenase (DHODH)[32]	DHODH is a flavin mononucleotide (FMN)-dependent mitochondrial enzyme involved in de novo pyrimidine biosynthesis; it catalyzes oxidation of dihydroorotate to orotate
P. falciparum falcipain-2[33,61]	an essential cysteine protease for hemoglobin degradation in the food vacuole at the trophozoite stage
P. falciparum heat shock protein 90 (Hsp90)[34]	Hsp90 is a molecular hub responsible for maintaining the survival and life cycle of plasmodia
P. vivax SUB1 protease[35]	SUB1 protease is a subtilisin-like serine protease that plays a role in both merozoite egress and invasion of erythrocytes
P. falciparum falcipain-3[36,61]	falcipain-3 is a cysteine protease belonging to the papain family; it is localized in the food vacuole and is involved in hemoglobin degradation
P. falciparum sulfur mobilization S (SufS) and *P. falciparum* sulfur mobilization E (SufE)[37]	SufS and SufE are localized in the apicoplast and are involved in the sulfur mobilization (SUF) pathway of Fe-S cluster biogenesis; the SUF pathway is necessary for apicoplast maintenance and parasite survival
P. falciparum phosphatase (PF2D7_1305500)[39]	an atypical mitogen-activated protein kinase phosphatase that is an essential element of intraerythrocytic development expressed throughout the life cycle
P. vivax vivapain-2[40]	a cysteine protease that plays an essential role in hemoglobin hydrolysis and is thus important for the survival and growth of the parasite
P. falciparum ribosomal phosphoprotein P1 (RPP1)[44]	an acidic phosphoprotein that forms ribosomal P protein complexes in association with the neutral phosphoprotein (P0) and acidic phosphoprotein (P2); the P protein is immunogenic and is involved in protein translation
P. falciparum GTP cyclohydrolase I[47]	a rate-limiting enzyme in the folate pathway; mutations of its encoding gene are responsible for the antifolate resistance and have influenced evolution of antifolate resistance
P. falciparum calcium adenosine triphosphate synthase (ATPase)[49]	a calcium ATPase involved in calcium transport in plasmodia; it is a target of artemisinin and related endoperoxides antimalarial agents
P. falciparum NADH:dehydrogenase-2 (*Pf* NDH2)[59]	*Pf* NDH2 is a mitochondrial enzyme serving as a metabolic choke point in the respiratory chain of the parasite's mitochondria

[a]References given are citations in which the target has been used as the basis for antimalarial CADD.

thus biological activity, thereby making it possible to perform molecular similarity searches of large compound databases.[17]

Shape-based similarity searching, also referred to as topomeric searching, provides an alternative ligand-based approach for the identification of potentially bioactive compounds. In this approach, collections of molecules are screened for compounds that exhibit 3D shape similarity to known active ligands, under the premise that similarly shaped molecules are likely to exhibit similar biological properties. In addition to allowing for fast and efficient screening of large compound databases, this approach also enables the retrieval of compounds with relatively diverse topology while also avoiding unwanted chemical functionalities. Topomeric searching has successfully supported lead-hopping efforts and the identification of novel bioactive compounds.[23,24]

Furthermore, if the biological activity of a range of known inhibitors has been reliably determined, then 3D QSAR models can be built to establish a mathematical correlation between various properties of the compounds (steric, electrostatic, functional, etc.) and their corresponding biological activities. Validated 3D QSAR models can be very useful in guiding the design and optimization of potential bioactive compounds by facilitating the prediction of the biological activity of newly designed compounds and guiding the selection of compounds for synthesis and testing.[19]

1.3. Integration of Structure- and Ligand-Based Approaches. Combining SBDD and LBDD approaches represents an emerging popular approach to CADD due to its association with the efficient provision of optimum results. Typically, this approach entails initial virtual high-throughput screening (vHTS) of prefiltered compound databases. This is followed by more detailed docking studies on a more refined, smaller subset of compounds to identify the final cluster of high-priority compounds for synthesis and biological evaluation.[17]

1.4. Prefiltering of Compound Databases. It is evident from the preceding sections that CADD relies heavily on the computational screening of large databases of structurally diverse molecules to identify compounds of interest. Although the sheer size of many of the available compound databases means that a vast and diverse chemical space is available for interrogation, it also poses the unavoidable challenge that many of the compounds contained in these databases may not be biologically relevant. By implication, a lot of time and computational resources may be spent screening compounds that have no chance of serving as viable hit and lead compounds for drug discovery. Prefiltering of compound databases has therefore become a vital initial step in many CADD efforts, whereby a wide range of filters are applied to eliminate compounds on the basis of unfavorable physicochemical, pharmacokinetic, and toxicity properties. The screening of the prefiltered databases can be rendered more efficient through their enrichment with drug-like compounds and, when applicable, the prioritization of privileged structures. The rationale and methods of prefiltering compound databases have been adequately reviewed elsewhere.[19,20]

1.5. Preamble. The overall goals of this review are four-fold. The first goal is to highlight the increasing and demonstrable capability of CADD tools to rapidly and cost effectively identify medicinal chemistry starting points from both target- and phenotypic whole cell-based screening utilized in drug discovery

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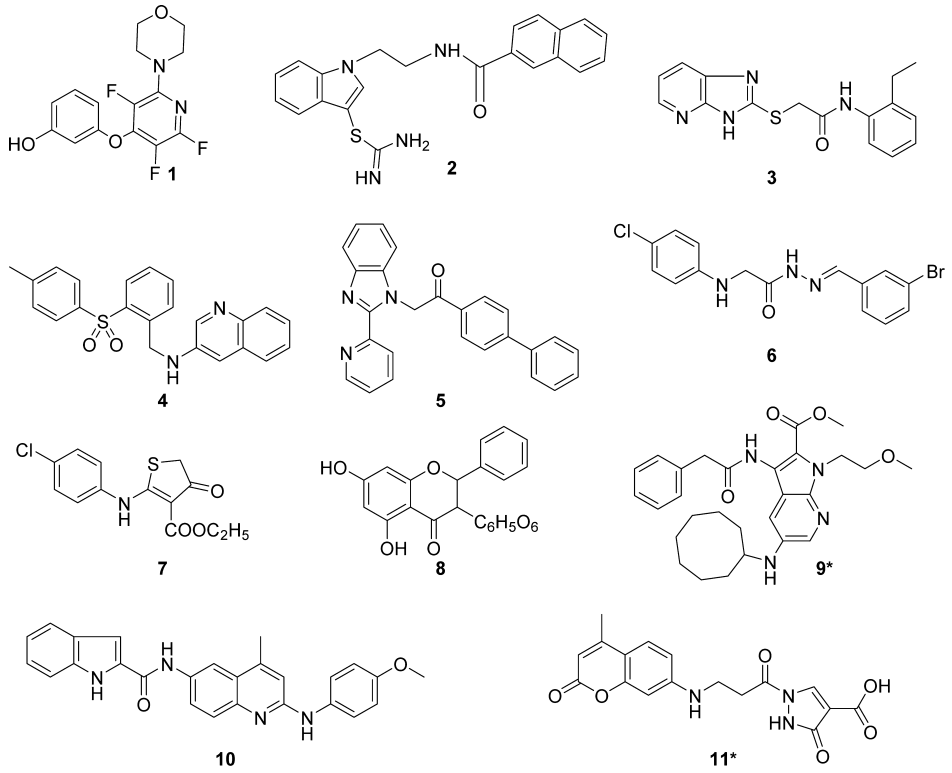

Figure 1. Some antiplasmodial hits identified through structure-based virtual screening. (∗) Experimental biological data not reported.

for the causative agents of the major tropical infectious diseases, namely, *Plasmodium falciparum* (malaria), *Mycobacterium tuberculosis* (TB), *Trypanosoma brucei* (HAT), *Trypanosoma cruzi* (Chagas' disease), and *Leishmania* spp. (leishmaniasis). Second, the review aims to highlight the major gaps and bottlenecks that currently exist in progressing chemical matter identified from CADD approaches to preclinical and clinical candidate status. Third, the review draws attention to the need for computational chemists to work closely alongside medicinal chemists to collectively weed out compounds identified from CADD approaches that have structural motifs associated with nonspecific and misleading activity across a wide range of assays.

Fourth, the review points out the need for confirmation of CADD predictions with experimentally derived biological activity. One of the unfortunate characteristics of CADD is that, in some cases, compounds predicted to be biologically active by computational means turn out to be inactive when evaluated experimentally. Consequently, it is acknowledged that blindly following up hits or leads identified by computational means but reported without confirmation of biochemical or whole-cell biological activity may be dangerous and misleading and should be treated with caution as they can easily lead researchers in the wrong direction. Nevertheless, many published CADD studies still include computationally derived hits or leads without attendant biochemical or whole-cell biological data. For the purposes of this review, hit compounds having no attendant biological data have been clearly designated as such in their accompanying descriptions and, when applicable, their corresponding compound numbers are followed by an asterisk (*). Any researcher would be well advised to begin any follow up of such compounds with an initial experimental confirmation of biological activity.

2.0. MALARIA

Malaria, the most deadly protozoan infection of humans, is caused by apicomplexan hemoprotozoa parasites of the genus *Plasmodium* transmitted through bites of infected female anopheline mosquitoes. Four plasmodial species, namely, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*, cause human malaria. The majority of fatalities arise from *P. falciparum* infection. According to the World Health Organization (WHO), an estimated 3.2 billion people in 97 countries and territories are at risk of malaria infection. In 2013, 198 million malaria cases occurred globally, leading to 584,000 deaths.[9] Although a global health problem, malaria is predominantly a tropical disease. Africa accounts for approximately 90% of global malaria mortality, bearing heavily on children aged under 5 years, who account for 78% of all fatalities.[9]

Although concerted efforts focused at vector control, proper case management, and combination chemotherapies have led to a notable reduction in global malaria mortality and morbidity, the inexorable emergence of drug-resistant malarial parasites creates a compelling need for continued search for newer antimalarials. In this section, we focus on some recently reported computer-aided antimalarial drug discovery efforts and their potential impact in the future treatment of malaria.

2.1. Drug Targets Utilized in Antimalarial CADD Approaches. Several essential malarial proteins have provided a wide range of established and potential drug targets that have been utilized in antimalarial CADD approaches. Table 2 provides a summary of these targets and their functions in plasmodial metabolism.

2.2. Structure-Based Approaches. An impressive range of therapeutically relevant malarial proteins have been identified and characterized, from enzymes involved in biosynthesis and hemoglobin digestion to receptors and transporters involved in

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membrane transport and signaling. This diversity of characterized protein targets has gone on to underpin numerous structure-based antimalarial drug discovery studies, as elaborately reviewed by Drinkwater and McGowan.[25] In some cases for which the 3D structures of potential antimalarial drug targets are yet to be elucidated, many groups have opted to apply homology modeling to generate 3D structures of the target proteins. Although there are numerous antimalarial CADD studies, only the more recent studies with accompanying biological data are included in this review.

2.2.1. Structure-Based Virtual Screening. Structure-based virtual screening approaches have by far been the most utilized methods in drug discovery efforts for antiplasmodial compounds. For example, Dahlgren et al.[26] discovered four small-molecule inhibitors of *P. falciparum* macrophage migratory inhibitory factor (*Pf*MIF) by structure-based virtual screening of Maybridge and ZINC drug-like commercial databases. Glide SP and Glide XP protocols were successively used to dock compounds on the crystal structure of *Pf*MIF (PDB: 2WKF). Seventeen compounds were selected for biological evaluation on the basis of the docking score, favorable protein−ligand interactions, physicochemical properties, and structural diversity. Four compounds had very attractive inhibition constant (K_i) values ranging between 8 and 99 μM with high selectivity for *Pf*MIF over the human MIF. The most active compound, **1** (Figure 1), had a K_i of 8.6 μM against *Pf*MIF and was used for further substructure searches and synthetic derivation.

Similarly, Carrasco et al.[27] identified four active compounds against the chloroquine-resistant W2 strain of *P. falciparum* by structure-based virtual screening of a drug-like database included in the MOE package. A crystal structure of *Saccharomyces cerevisiae* bc1 complex (PDB ID: 3CX5) was validated by docking the experimentally known inhibitors of *P. falciparum* bc1 complex because both species have high sequence identity in the oxidation pocket (Qo). The validated Qo model was employed to screen the database using the Autodock program, and 23 compounds were selected and in vitro assayed for antiplasmodial activity. Four compounds showed antiplasmodial activity below 10 μM, with the most potent compound, **2**, exhibiting an IC_{50} of 1.56 μM.

In yet another study, Harikishore et al.[28] identified a potential antiplasmodial hit against the *P. falciparum* FKBD35 protein from the ChemDiv database using structure-based pharmacophore modeling and docking methods. A structure-based pharmacophore model was first developed based on the *P. falciparum* FKBD35-FK506 X-ray crystal structure. Consequently, pharmacophore-based screening of the ChemDiv library resulted in a focused library of 13000 molecules. Library refinement with absorption, distribution, metabolism, and excretion (ADME) filters resulted in 2600 molecules. The docking program GOLD was then employed in docking the focused library into the active site of *P. falciparum* FKBP35. The top-scored compounds were then prioritized on the basis of contact of key catalytic residues such as Asp56, Tyr101, and Ile75. Of 46 compounds tested experimentally, compound **3**, containing a purine-like ring system linked to a phenyl ring via a thioacetamide linkage, selectively inhibited plasmodial FKBD35 with IC_{50} values of 132 nM and 125 nM for *P. falciparum* and *P. vivax*, respectively.

Song et al.[29] identified five moderate nonpeptide inhibitors of *P. falciparum* plasmepsin II by structure-based screening with Specs and MayBridge databases. Two different Glide docking protocols, Standard-precision (SP) and Extra-precision (XP), were performed on the plasmepsin II structure (PDB code: 1LEE). The top-ranked compounds were further visually inspected for key interactions with residues Asp34 and Asp214, structural diversity, binding modes, and conformation similarities to the cocrystal ligand. Of the commercially obtained 89 compounds, 5 compounds revealed moderate inhibitory potencies with IC_{50} values ranging from 4.62 to 9.47 μM. The most active compound, **4**, showed an IC_{50} of 7 μM against falcipain-2. In a closely related study, Saify et al.[30] identified a benzimidazole class of compounds as *P. falciparum* plasmepsin II inhibitors by structure-based virtual screening using an in-house database containing more than 1000 compounds. Thirty compounds were synthesized and evaluated for inhibition of the growth of cultured intraerythrocytic *P. falciparum*. Four benzimidazole compounds showed inhibition of parasite growth at $\leq$3 μM, with compound **5** exhibiting good antiplasmodial activity (IC_{50} = 0.16 μM) and inhibition of plasmepsin-II (IC_{50} = 14.7 μM).

Samal et al.[31] performed structure-based virtual screening using the iResearch database to identify inhibitors of *P. falciparum* enoyl-ACP reductase. One of the identified inhibitors was selected for synthetic derivation on the bases of the docking score, binding site analysis, and synthetic accessibility. Eight synthetic derivatives showed moderate activity against *P. falciparum* enoyl-ACP reductase with IC_{50} values below 100 μM. Compound **6** was the most potent, with an IC_{50} value of 7 μM. In another study aimed at the discovery of novel *P. falciparum* dihydroorotate dehydrogenase (*Pf*DHODH) inhibitors, Xu et al.[32] adopted a virtual screening strategy integrating molecular docking and MM-GBSA rescoring based on the cocrystal structure of *Pf*DHODH complexed with DSM1 (PDB: 3I65). Dihydrothiophenone hit compound **7** was identified from the SPECS database (ID: AG-690/40639878) and showed good inhibitory activity against *Pf*DHODH with an IC_{50} value of 1.11 μM. A series of derivatives obtained through synthetic optimization were tested against *Pf*DHODH and showed good selectivity for the plasmodial enzyme over the human DHODH.

Wang et al.[33] identified 10 natural products as *P. falciparum* falcipain-2 inhibitors from an in-house natural products database using structure-based virtual screening. The identified compounds showed moderate inhibitory activities against falcipain-2 with IC_{50} values in the range of 3.18−68.19 μM. Compound **8** exhibited in vitro antiplasmodial activity against a chloroquine-sensitive strain (*P. falciparum* 3D7; IC_{50} = 5.54 μM) and a chloroquine-resistant strain (*P. falciparum* Dd2; IC_{50} = 4.05 μM). In addition, Wang et al.[34] studied the static and dynamic differences between the *P. falciparum* heat shock protein 90 (Hsp90) and the human orthologue. The study revealed that the ATP-binding pocket has a *P. falciparum* specific extension but differs in the tertiary structure and dynamics. Subsequent structure-based screening identified 7-azaindole derivative **9*** as an inhibitor of *P. falciparum* Hsp90.

Bouillon et al.[35] performed structure-based virtual screening using a homology model of the *P. vivax* SUB1 protease to identify potential inhibitors. To build homology models for *P. vivax* SUB1 protease, seven template structures with high resolution and low BLAST expectation value were selected. In addition, 50 additional homologous sequences were included for optimal multiple sequence alignment. Fifty models were built from their respective sequence alignments with the seven template structures. The validated model with the lowest rmsd between template and model binding pockets was selected for structure-based virtual screening of the ChemDiv database. The database

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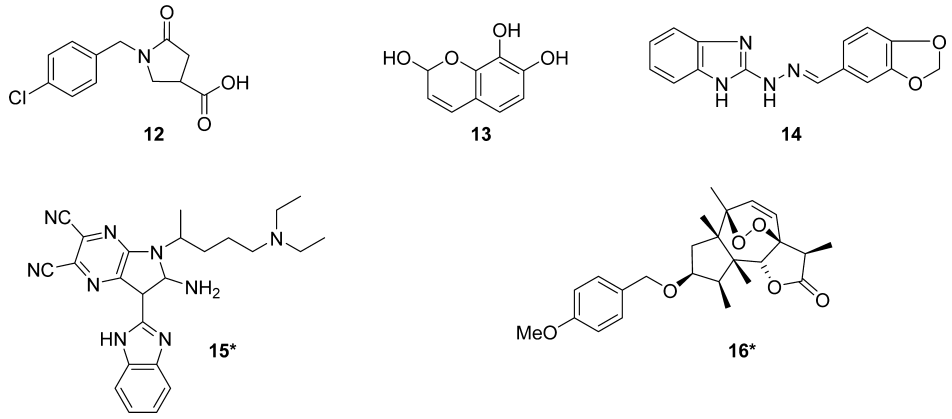

Figure 2. Antiplasmodial hit compounds identified through structural modeling campaigns. (∗) Experimental biological data not reported.

was first filtered with the OpenEye filter program, resulting in 149,992 hits. These hits were then docked in parallel into the active site of the homology model of *P. vivax* SUB1 using FlexX, FlexX-Pharm, and ICM software programs. The best scored 306 compounds were tested for inhibition of *Pv*SUB1r enzymatic activity at a concentration of 50 μM. Of the 37 compounds that displayed >30% inhibition, compound **10** showed an apparent K_i value of 6 μM against *P. vivax* SUB1.

In another elegant antimalarial CADD effort, Chintakrindi et al.[36] designed inhibitors of *P. falciparum* falcipain-3 based on the 7-amino-4-methylcoumarin moiety of its well-known substrate benzyloxycarbonyl-Phe-Arg-7-amino-4-methylcoumarin. A set of novel nonpeptidic inhibitors were designed using the Ludi de novo technique incorporating a receptor-based alignment protocol. On the basis of the docking score obtained using the GOLD software, one compound was prioritized for synthetic derivation. The activities of designed inhibitors were predicted and validated using various 3D QSAR techniques. Among others, compound **11*** (Figure 1) was predicted as a potential falcipain-3 inhibitor, although no biological data were experimentally obtained.

2.2.2. Homology Modeling and Binding Site Recognition. Several studies report the generation and application of homology models of a diverse range of antimalarial drug targets for molecular docking studies as well as for investigating ligand−protein interactions. For example, Charan et al.[37] employed homology modeling and protein−protein docking methods to explore the structure and interaction of *P. falciparum* sulfur mobilization S (SufS) and *P. falciparum* sulfur mobilization E (SufE) enzymes. Crystal structures of the *Escherichia coli* CsdBNifS homologue (PDB: 1C0N) and *E. coli* SufE (PDB: 1MZG) were used as templates for constructing models of *P. falciparum* SufS and *P. falciparum* SufE, respectively. The validated models of *P. falciparum* SufS and *P. falciparum* SufE were then docked with the GRAMMX server to predict protein−protein interactions.[38] The modeling study revealed that the proximal position of conserved cysteine residues in the *P. falciparum* SufS−*P. falciparum* SufE complex may allow sulfide transfer from the pyridoxal phosphate cofactor-bound active site of *P. falciparum* SufS.

Homology models have also found extensive use in target-based virtual screening, an integral aspect of SBDD. Campbell et al.[39] developed a homology model of a putative *P. falciparum* phosphatase from *P. falciparum* 3D7 (PF3D7_1305500) that is considered important for the parasite's intraerythrocytic development and expressed throughout the life cycle. The validated model was then used for structure-based virtual screening of the ChEMBL-NTD database, yielding three compounds, **12**, **13**, and **14** (Figure 2), with moderate in vitro activity against *P. falciparum* 3D7 and C9 parasites.

Yadav et al.[40] modeled the 3D structure of *P. vivax* vivapain-2, which was used to search three publicly available databases (STITCH 3.1,[41] DrugBank,[42] and Therapeutic Target Database[43]) for similar validated targets with their associated drugs. The natural product E-64 was docked to the model and showed that Asn281, Cys283, Val396, and Asp398 were the key amino acid residues involved in the protein−ligand interactions. A pharmacophore model was then developed on the basis of this complex. Pharmacophore-based virtual screening of the DrugBank database proposed three hypothetical hit compounds (structures not published). Similar homology modeling was undertaken by Kumari et al.[44] on the basis of the 3D structure of ribosomal phosphoprotein P1 (RPP1) using the iterative threading assembly refinement (I-TASSER) ab initio method.[45] The validated model was then used to perform structure-based virtual screening with DockBlaster,[46] which identified a number of compounds as having good interactions with RPP1, although no biological data were provided.

Kümpornsin et al.[47] have constructed a decameric model for *P. falciparum* GTP cyclohydrolase I based on *Thermus thermophilus*, and the model revealed conservation of the key residues for substrate binding. On the basis of the model, an H279S mutant was constructed, which explained the loss of enzymatic activity. Reker et al.[48] applied advanced ligand, protein sequence, and pocket-based computational approaches to suggest plasmodial kinases as targets for pyrrolopyrazine class of compounds (such as compound **15***, Figure 2) initially identified as inhibitors for herbicidal target 4-diphosphocytidyl-2C-methyl-D-erythritol synthase through HTS.

2.2.3. Molecular Dynamics (MD) Simulations. MD simulations refer to the use of computational methods to characterize and/or predict the time-dependent motions and interactions of atoms and molecules in macromolecular systems, including biomolecules such as proteins, nucleic acids, and carbohydrates. MD can be considered a branch of applied physics that is based on the application of Newton's equations of motion and the principles of statistical molecular mechanics. In drug discovery, MD simulations have been widely employed to gain insight into receptor−drug interactions at an atomic level.[49−51] An important application of MD simulations in drug discovery is in the calculation of the binding energies corresponding to ligand−receptor interactions. These free energy calculations are carried

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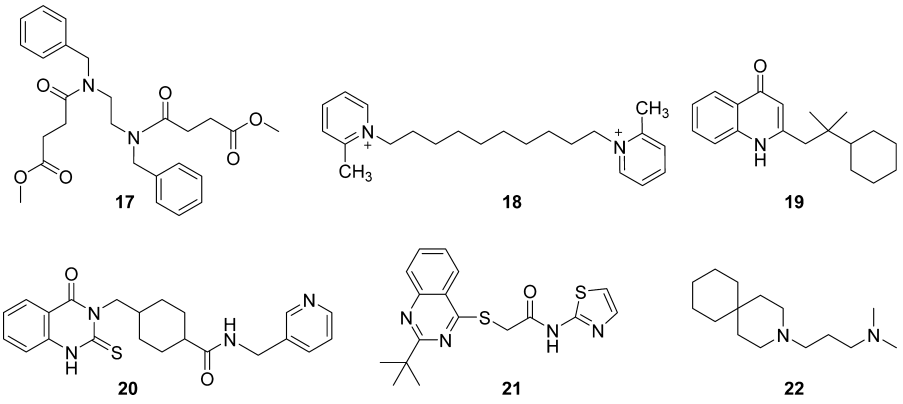

Figure 3. Antiplasmodial hits identified through pharmacophore modeling, molecular similarity searches, and integrated structure- and ligand-based approaches.

out using the molecular mechanics Poisson−Boltzmann surface area (MM-PBSA) approach.[50] In addition, MD simulations help in understanding and predicting the flexibility and folding of protein targets and homology models, the effects of mutations in the binding sites of proteins, and the transport of ions and small drug molecules across membrane proteins.[49−51] Although MD simulations are time-consuming tasks owing to the computationally intensive calculations required, their use in conjunction with experimental methods has enhanced the understanding of protein structure−function relationships and receptor−drug interactions in drug discovery. Examples of the application of MD simulations in antimalarial CADD are presented in this subsection.

By enabling the identification of key amino acid residues within the binding site, site-directed mutagenesis is an important technique for detailed investigation of ligand−protein interactions. The capacity now exists to undertake such studies computationally (i.e., in silico mutagenesis), enabling application of MD simulations in antimalarial drug discovery. For example, the binding mode of compound **16*** to *P. falciparum* calcium-ATPase (ATP6) has been elucidated using a combination of 3D pharmacophore modeling, molecular docking studies, and in silico mutagenesis to explain the binding mode of thaperoxides to *P. falciparum* ATP6. In addition, in silico mutagenesis and MD studies showed the importance of Phe264 and electrostatic interactions between Lys260 in helix H3 and Lys1036 and Asp1038 in L6/7 loop for the binding of thaperoxides.[52]

Similarly, Lindert and McCammon[53] performed extensive MD simulations on the tetrameric *P. falciparum* enoyl-acyl carrier protein (ACP) reductase in different states of cofactor and ligand binding to understand the dynamics of protein−ligand complexes. Analyses of different MD simulations of the complexes revealed that the active-site binding pocket is dynamic and wide, and the dynamics of the ligand within the binding site showed many stabilizing interactions besides known interactions of crystal structures. In addition, fragment-based docking revealed that the druggable enoyl-ACP reductase hotspots are located in the central cavity at the interface of the subunit C-termini.

2.3. Ligand-Based Approaches. *2.3.1. Two-Dimensional (2D) and Three-Dimensional (3D) QSAR Modeling.* 2D and 3D QSAR are commonly used methods for modeling the physical and chemical properties of compounds. 2D QSAR refers to topological representation, which defines the connectivity of atoms in the molecule in terms of the presence and nature of chemical bonds.[54] 3D QSAR techniques, such as comparative molecular field analysis (CoMFA) and molecular similarity indices in a comparative analysis (CoMSIA), explain the 3D structures of the target forming highly specific interactions with a ligand. This approach has been pursued by several research groups. One such study was reported by Ghasemi and Shiri,[55] who performed CoMFA, CoMSIA, and docking studies of 2-pyrimidinecarbonitrile-based analogues on the *P. falciparum* falcipain. Their work showed that bulky and hydrophobic substituents may increase potency. Similar studies have been reported by Li et al.,[56] Villalobos et al.,[57] Sharma et al.,[58] and Raj et al.[59] working on a wide range of privileged antimalarial scaffolds. Although none of these studies reported experimentally derived biological data, these QSAR approaches are useful in deriving correlations between compounds and their biological activities and are thus facilitating lead optimization and the design of compounds with desirable biological activities.

2.3.2. Pharmacophore Modeling. Pharmacophore modeling has also gained greater traction in antiplasmodial drug discovery. This technique is best exemplified by the work of McKay et al.,[60] who developed a predictive pharmacophore model for selective inhibitors of *P. falciparum* plasmepsins (*Pf*PM) using HypoGen algorithm. The model was validated using a training set composed of 40 known actives and 960 decoy molecules and employed a 3D search query to screen the Specs and Asinex Platinum commercial databases. The virtual hit compounds were ranked according to their fit values and 15 compounds selected by visual inspection and subsequently tested against *P. falciparum* 3D7, *Pf*PM II, *Pf*PM IV, and human cathepsin D. The most active compound, **17** (Figure 3), showed an IC_{50} value of 5.24 μM in the whole-cell parasite assay and a K_i of 7 μM in the *Pf*PM II and *Pf*PM IV enzyme inhibition assay and was prioritized for lead optimization.

Another successful antimalarial CADD campaign was reported by Sullivan et al.,[61] who identified 12 antiplasmodial compounds using a novel quantum modeling approach. Unlike other models, descriptors of quantum models are derived from a special representation of quantum fields. Three separate quantum models were developed on the basis of a training set containing 26 known antimalarial compounds and the validated model used to filter potentially active compounds from a database compiled from multiple sources including Enamine and ChemBridge. Twelve compounds were selected on the basis of commercial availability and validated in vitro in both anti-*P. falciparum* and mammalian cytotoxicity assays. Six compounds showed good antiplasmodial activity in the nanomolar range, with the most active compound, **18**, showing an IC_{50} value of 27 nM in vitro.

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2.3.3. Molecular Similarity Searches. Sharma et al.[62] employed similarity-searching and chemoinformatics approaches to select compounds for HTS experiments in the identification of selective inhibitors against *P. falciparum* NADH:dehydrogenase-2 (*Pf*NDH2). Substructure search using the only known *Pf*NDH2 inhibitor, 1-hydroxy-2-dodecyl-4-(1*H*)quinolone, was first performed using a BioFocus database. A spectrophotometric assay of the resultant 1175 compounds identified 54 active compounds with IC_{50} < 20 μM. Further studies using seven different similarity search methods (MDL MACCS keys, FCFP2, ECFP2, turbo similarity algorithm, PCA, Bayesian modeling, and bioisostere similarity) were performed using the 54 active compounds in parallel with the BioFocus database, resulting in 34356 hits. On the basis of the scoring, ADME, and structural diversity, the best 16050 compounds were selected for HTS against *Pf*NDH2. This work identified 48 compounds with IC_{50} values ranging from 100 nM to 10 μM. The most active compound, **19** (Figure 3), had an IC_{50} of 78 nM.

2.4. Integrated Structure- and Ligand-Based Approaches. Integration of structure- and ligand-based methods is theoretically associated with greater efficiency in hit identification. In our literature review, three recent applications of this approach were encountered. The first account is described by Rodrigues et al.,[63] who employed ligand- and structure-based virtual screening approaches to identify potential inhibitors of *P. falciparum* bc1 complex from the ZINC and MOE databases. Twenty-three compounds were prioritized for screening and evaluated for antiplasmodial activity against the *P. falciparum* chloroquine-resistant W2 strain. Six compounds showed IC_{50} values <30 μM, with the most active compound, **20** (Figure 3), exhibiting an IC_{50} of 1.97 μM in vitro.

Shah et al.[64] performed combined ligand- and structure-based virtual screening with Chembridge and Asinex commercial databases to identify *P. falciparum* falcipain-2 and falcipain-3 inhibitors. In the ligand-based screening, three previously identified inhibitors were used as queries for substructure searches. A total of 1500 hits were obtained and subsequently docked with the falcipain-2 crystal structure using a previously validated Glide XP protocol.[65] The top 300 molecules were selected on the basis of the E model score. Biological evaluation was carried out on 69 compounds selected on the basis of such criteria as formation of hydrogen bonds by ligand atoms with key residues, reasonable ligand geometry, and commercial availability. Up to 28 compounds showed inhibition of falcipain-2 in the low micromolar range with IC_{50} values of 5−48 μM. Furthermore, some of these compounds were active against cultured malaria parasites with IC_{50} < 10 μM. Compound **21** was the most active, with an IC_{50} of 4.64 μM. The role of water molecules in the ligand-binding domain of falcipain-2 and falcipain-3 with these ligands was further studied by statistical thermodynamics. The study found that hydrogen bond networks with key residues, displacement of unfavorable hydration sites by complementary groups of the ligands, and chemical reactivity of electrophiles to the catalytic cysteine are some of the important factors that contribute to potent inhibition of falcipain-2 and falcipain-3.

Mugumbate et al.[66] screened the ZINC database using two different protocols involving structure- and ligand-based methods. In one protocol, the Lipinski's "rule of five" was first used to screen the ZINC database for drug-like properties. The resulting 1.1 million compounds were further clustered to avoid very similar compounds, and the structurally diverse compounds were subjected to structure-based screening with the falcipain-2 crystal structure using the GOLD program. In the second protocol, 25000 drug-like compounds were extracted from a drug-like subset using the ZINC web browser tool. The data set was further filtered on the basis of the properties of nine nonpeptide inhibitors of falcipain-2 using the OpenEye drug-like filter program. The top 100 compounds were selected on the basis of similarity scores and subjected to structure-based screening using Autodock. The best ligands were selected on the basis of the docking score and visual inspection using such criteria as ligand adopted position and orientation in the binding site, formation of hydrogen bonds, and chemical diversity. Of the 60 compounds commercially obtained, 19 compounds showed low micromolar inhibitory activity against cultured chloroquine-resistant *P. falciparum* W2 strain. Compound **22** was one of the most active compounds, with an IC_{50} value of 1.32 μM against cultured parasites.

Overall, the ability to identify antiplasmodial hit compounds through computational means has been consistently proven in recent years. In any case, there is no shortage of characterized antimalarial targets and known active ligands to support both structure- and ligand-based antimalarial drug discovery efforts. Nevertheless, it is quite apparent that SBDD methods using both protein crystal structures and homology models have been quite widely applied for malaria. This comes as no surprise considering the diversity and range of characterized malaria protein targets,[47] and there still exists considerable opportunity for further application of structure-based CADD against malaria targets. Several interesting hits have been identified using this approach, some of which exhibit in vitro antiplasmodial activity in the lower micromolar and even nanomolar range. Such confirmed hits could form the basis for onward progression by way of dedicated structure−activity relationship (SAR) studies through the design, synthesis, and biological evaluation of analogues.

The ligand-based approaches have also contributed several confirmed hit compounds, including some very promising ones exhibiting in vitro antiplasmodial activity in the nanomolar range.[46] Also noteworthy is the diverse range of ligand-based techniques that have been successfully applied, ranging from QSAR studies to pharmacophore modeling to substructure molecular similarity searches. This, coupled with the sheer number and diversity of known antimalarial compounds that have been reported over the years, means that LBDD will continually be a very productive source of antimalarial hit compounds.

3.0. TUBERCULOSIS

Tuberculosis is an infectious disease that typically affects the lungs (i.e., pulmonary TB) and is caused by the bacterium *Mycobacterium tuberculosis*. Tuberculosis remains a major global health concern and is currently ranked as the second leading cause of death from infectious diseases worldwide after HIV. The WHO estimated 8.6 million new TB cases in 2012, with >80% of these cases being reported in Asia and Africa. In the same year, there were an estimated 1.3 million TB deaths, including an estimated 74000 deaths among children. Approximately 75% of total TB deaths occurred in African and Southeast Asian regions.[67]

Current TB therapy faces major challenges such as extended treatment regimens, poor toxicity profiles, and, more importantly, the development of clinically significant resistance by the causative microorganism to available therapies. There were an estimated 450,000 new cases of multidrug-resistant TB (MDR-TB) worldwide in 2012 and approximately 170,000 deaths from

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Table 3. Protein Targets That Have Been Utilized in Antimycobacterial CADD

protein target[a]	function
β-ketoacyl−acyl carrier protein synthase III (FabH)[68,69]	FabH is an attractive potential drug target involved in the mycobacterial type II fatty acid synthase (FAS II) biosynthetic pathway; this pathway yields mycolic acid and other fatty acids essential for mycobacterial survival
nucleoid-associated protein HU[70]	nucleoid-associated protein HU is a newly identified potential drug target vital for maintaining chromosomal integrity and regulating DNA function
acyl-CoA carboxylases (ACCases)[71]	ACCases catalyze biosynthesis of mycolic acids and multimethyl-branched fatty acids, essential components of the mycobacterial cell wall
M. tuberculosis NAD^+-dependent ligase (LigA)[72−74]	DNA ligases catalyze the formation of a phosphodiester linkage between adjacent termini in double-stranded DNA and are vital enzymes in DNA replication and repair; LigA is essential for *M. tuberculosis* survival and multiplication
6-hydroxymethyl-7,8-dihydropteroate synthase (DHPS)[75]	DHPS is the first enzyme in the folate pathway, involved in the biosynthesis of 6-hydroxymethyl-7,8-dihydropteroate, a precursor for folate synthesis; this enzyme has also been successfully exploited in antibacterial and antimalarial drug discovery
ATP synthase (ATPase)[76,86]	a central enzyme involved in the energy metabolism of *M. tuberculosis* and other bacteria through the synthesis of ATP via oxidative phosphorylation; it is essential for survival
type II dehydroquinase[77,97]	type II dehydroquinase is the third enzyme of the shikimic acid pathway, the biosynthetic route by which *M. tuberculosis* synthesizes aromatic amino acids and other important aromatic metabolites; the enzyme has been validated as an antimycobacterial target
pantothenate synthetase (PS)[78]	pantothenate synthetase catalyzes the condensation of pantoate and β-alanine to furnish pantothenate, an essential precursor of coenzyme A
dihydrofolate reductase (DHFR)[79,91,101]	an integral enzyme in the folate pathway involved in the reduction of dihydrofolate to THF, an essential cofactor for purine and thymidine biosynthesis; DHFR is a validated drug target that has been successfully exploited in antibacterial and antimalarial drug discovery
2-*trans*-enoyl-ACP (CoA) reductase (InhA)[80,99,103]	InhA is the fourth enzyme of the FAS II fatty acid biosynthetic pathway and is the target for the established antimycobacterial drug isoniazid
alanine racemase[81]	alanine racemase catalyzes the racemization of L-alanine to D-alanine, an essential precursor for mycobacterial cell wall synthesis
DNA gyrase (DNAg)[82,83]	DNA gyrase is involved in integral cellular processes such as DNA replication, transcription, and recombination; it is the target for the quinolone antibacterials such as ciprofloxacin
β-Subunit of RNA polymerase (rpoB)[85]	RNA polymerase is involved in transcription and elongation of RNA and is the target of the established anti-TB drug rifampicin
Mycobacterium tuberculosis aryl acid adenylating enzyme (MbtA)[87,88,93,94,98]	MbtA is involved in biosynthesis of mycobactins, small-molecule iron chelators (siderophores) involved in iron acquisition; mycobactins are critical for virulence and growth in iron-deficient conditions
dihydrodipicolinate synthase (DHDPS)[96]	DHDPS is a key enzyme of the lysine/diaminopimelate biosynthetic pathway, important for both cell wall and amino acid biosynthesis
chorismate mutase[100]	an essential enzyme in the shikimic acid pathway
lipoate protein ligase B (LipB)[102]	LipB is involved in the biosynthesis of the lipoic acid cofactor; it is a very promising drug target as it is essential for mycobacterial survival

[a]References given are citations within the main text in which the given protein target has been used as the basis for antimycobacterial CADD.

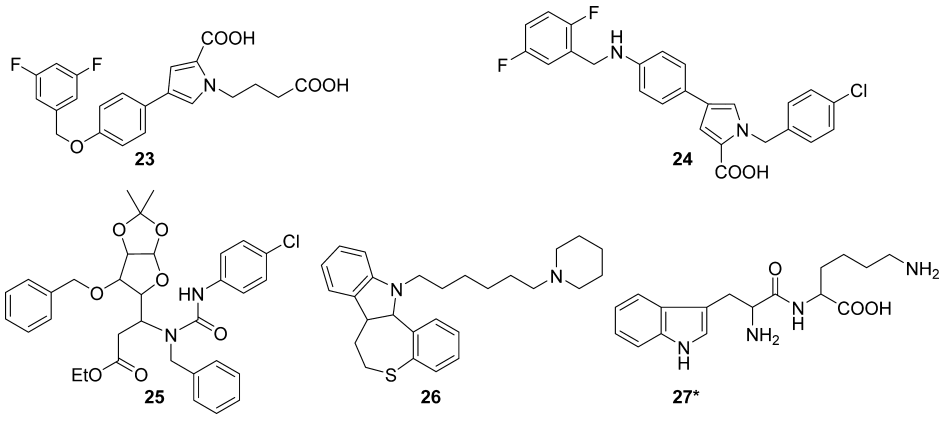

Figure 4. Examples of compounds with antimycobacterial activity identified through molecular docking and target-based virtual screening. (∗) Experimental biological data not reported.

MDR-TB.[67] Clinical cases of extensively drug resistant TB (XDR-TB) are also on the rise. In 2013, 54 countries and territories reported treating XDR-TB cases. Globally, 3232 XDR-TB cases were enrolled in treatment, up from 1852 cases in 2012 and reflecting increases in enrollments in 17 high MDR-TB burden countries.[10]

In this section, we focus on the application of CADD in the search for anti-TB chemotherapeutics, citing pertinent examples and highlighting instances of successful identification of highly potent compounds.

3.1. Range of Targets Used for Antimycobacterial CADD. As will be seen from later discussions, an impressively wide range of protein targets involved in diverse cellular processes and biochemical pathways has formed the basis of antimycobacterial CADD efforts. These targets are summarized in Table 3.

3.2. Structure-Based Drug Discovery Methods for Tuberculosis. *3.2.1. Molecular Docking Studies and Target-Based Virtual Screening.* Several studies have reported the use of molecular docking approaches in the identification of

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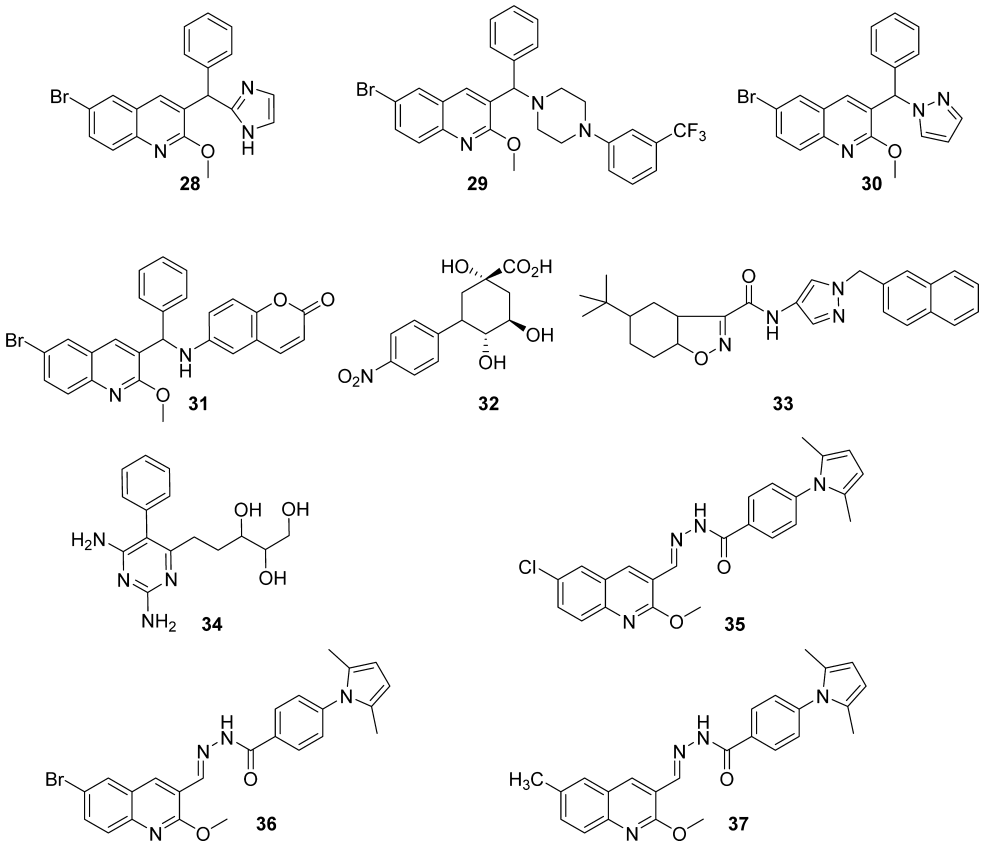

Figure 5. Examples of compounds with antimycobacterial activity identified through docking studies to investigate ligand−protein interactions.

inhibitors against various *M. tuberculosis* drug targets. Several of these studies have employed these methods to screen in-house databases or virtually generated combinatorial libraries. In one such study, a structurally diverse virtual library of compounds was docked into the active site of *M. tuberculosis* β-ketoacyl−acyl carrier protein synthase III (FabH). The FabH is an attractive potential drug target involved in the mycobacterial type II fatty acid synthase (FAS II) biosynthetic pathway that generates mycolic acid and other fatty acids essential for the mycobacterial cell wall. The compound library was designed on the basis of the structures of known FabH inhibitors. Eight compounds from the molecular docking studies for synthesis were selected on the basis of predicted binding energies, drug-like properties, and synthetic accessibility. These compounds were synthesized and tested against a reference strain of *M. tuberculosis* H37Rv, leading to the identification of compound **23** (Figure 4) with a MIC value of 12.5 μg/mL.[68] A similar study on the same target led to the identification of 12 structurally related novel FabH inhibitors such as compound **24**, all of which showed strong inhibition on the growth of *M. tuberculosis* H37Rv.[69]

In silico docking methods have been instrumental in the identification of several different classes of compounds with antimycobacterial activity through molecular docking of publicly available[70,71] as well as in-house[72−74] compound databases. For example, Srivastava and co-workers separately reported the identification of glycosyl ureide,[72] glycofuranosylated diamine,[73] and *N*-substituted tetracyclic indole[71] derivatives that inhibited *M. tuberculosis* NAD^+-dependent DNA ligase (LigA). These compounds were identified through molecular docking of an in-house database of ≈15000 compounds using the binding site derived from the crystal structure of the enzyme. Promising compounds such as the glycosylureide compound **25** and the tetracyclic indole compound **26** showed significant selectivity for *M. tuberculosis* LigA over human DNA ligase I. Docking and kinetic studies further suggested that the binding sites of these compounds and NAD^+ overlap with each other.

Notably, molecular docking studies have also proven to be useful in the identification of small peptide inhibitors of antimycobacterial drug targets. Docking studies of a series of dipeptide ligands were carried out to identify small peptide inhibitors of 6-hydroxymethyl-7,8-dihydropteroate synthase (DHPS) that had superior potency, ligand−protein interactions, and selectivity for mycobacterial proteins. The docking studies identified Trp-Lys (compound **27***) as a potent small peptide inhibitor of *M. tuberculosis* DHPS that appeared to bind to the highly conserved pterin-binding pocket. Docking calculations also indicated that this dipeptide had 70- and 90-fold higher selectivities for *M. tuberculosis* DHPS as compared to human dihydrofolate reductase (DHFR) and human thymidylate synthase, respectively.[75] However, no experimental inhibitory data for compound **27*** were reported.

3.2.2. Docking Studies To Investigate Ligand−Protein Interactions and Mechanism of Action. Aside from its application in the screening and prioritization of compounds with desirable predicted binding energies, another important application of molecular docking studies is elucidating possible binding modes and attendant ligand−target interactions. This information is derived from the inspection of the predicted binding poses provided by the majority of docking software currently in use. This information is important in explaining structure−activity relationships and thereby guiding the design of analogues with potentially improved fit and optimized interactions with the particular target. In other cases, molecular docking studies have been used to investigate potential

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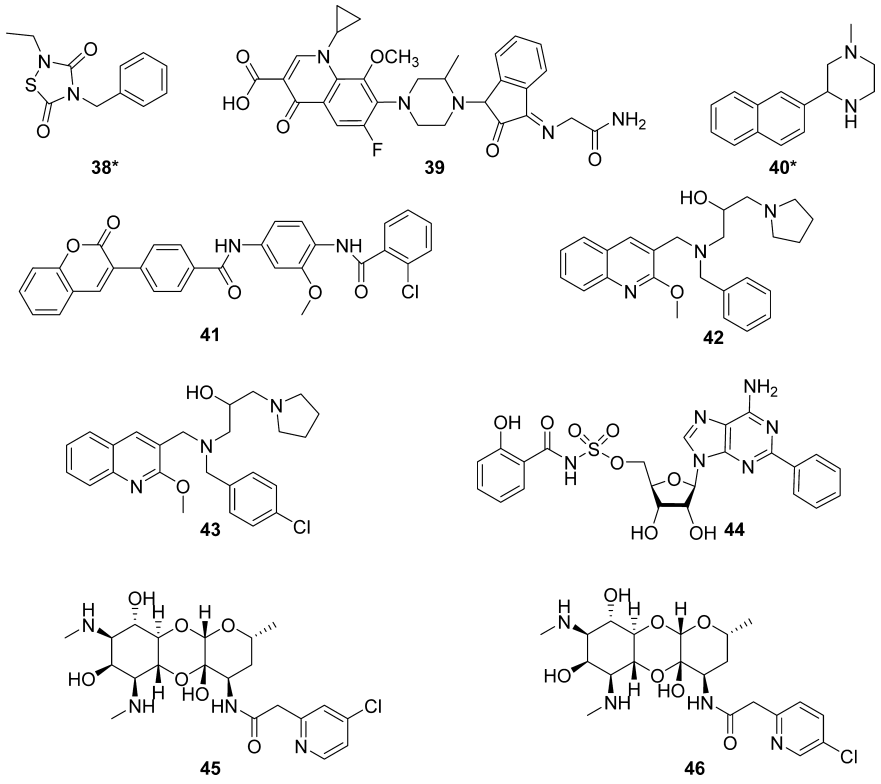

Figure 6. Examples of compounds with antimycobacterial activity identified through homology modeling. (∗) Experimental biological data not reported.

mechanisms of action in instances when the mechanism of action of known active compounds is still unknown. Several examples of this application of molecular docking can be found in anti-TB drug discovery, as highlighted below.

One such study involved the use of molecular docking studies to reveal important ligand−target interactions of a series of novel inhibitors of the proton pump of *M. tuberculosis* ATPase. In this study, two new series of compounds based on the structure of diarylquinoline R207910 (DARQ) were designed, synthesized, and screened against *M. tuberculosis* H37Rv. DARQ, a quinoline compound that targets the proton pump of bacterial ATPase, has been shown to effectively inhibit mycobacterial growth. Compounds **28**−**31** (Figure 5) showed excellent bactericidal activity comparable to that of the standard drug isoniazid; all had minimum inhibitory concentrations (MIC) values of 6.25 μg/mL. Docking studies carried out to elucidate possible ligand−target interactions indicated possible crucial interactions with residues Arg186 of the ATPase subunit a, as well as Glu61, Leu68, and Phe65 of the ATPase subunit c. These interactions were comparable to those of the known inhibitor DARQ.[76] Ligand docking studies have also been used to reveal the key ligand−target interactions of compound **32**, a potent *M. tuberculosis* type II dehydroquinase (DHQase) inhibitor (K_i = 54 nM). This compound was identified through the design and screening of 5-aryl analogues of a known type II DHQase inhibitor. Docking studies to investigate potential ligand−protein interactions identified a potential key electrostatic binding interaction between the aromatic rings of the compounds and Arg19, a residue that is essential for the activity of *M. tuberculosis* type II DHQase.[77]

Molecular docking studies were used to gain additional insight into ligand−protein interactions of *M. tuberculosis* pantothenate synthetase (PS) inhibitors. Information from these studies was used to guide the design of new potential inhibitors and led to the identification of potent isoxazole carboxamide-based inhibitor derivatives, such as compound **33**, a potent inhibitor of PS (IC_{50} = 90 nM).[78]

Following the observation that *M. tuberculosis* DHFR contained a tightly bound glycerol close to the binding site, a feature that is absent in human DHFR, a series of potential *M. tuberculosis* DHFR inhibitors incorporating a glycerol-mimicking triol were designed on the basis of the structures of pyrimethamine and other known pyrimidine-2,4-diamine inhibitors of various DHFRs. Compound **34** showed selective in vitro inhibition of *M. tuberculosis* DHFR, with only very modest inhibition of the growth of the yeast containing the human or the *S. cerevisiae* enzyme. The other inhibitors exhibited less or no selectivity, and docking studies were carried out to investigate the reason for this and rationalize the structure−activity observations. These docking studies suggested that, whereas the glycerol-mimicking triol was expectedly involved in a hydrogen-bonding network, substitution of the 5-phenyl group was associated with loss of selectivity due to disruption of ideal binding contacts between DHFR and the inhibitors. This was not the case with the unsubstituted **34**.[79]

Twenty-three quinoline analogues were tested for inhibitory activity against *M. tuberculosis* H37Rv. Ciprofloxacin and norfloxacin were assayed for comparison. To investigate the potential ligand−protein interactions with their putative target *M. tuberculosis* 2-*trans*-enoyl-ACP (CoA) reductase (InhA), the same compounds were also docked into the InhA active site. InhA is the fourth enzyme of the FAS II fatty acid biosynthetic pathway and is the target for the established antimycobacterial drug isoniazid. Compounds **35**−**37** exhibited the highest activity against the tested mycobacteria with MIC values of 0.2 μg/mL.

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Docking studies revealed possible hydrogen bonding and other interactions between active compounds and the enzyme target.[80]

3.2.3. Role of Homology Modeling in Antimycobacterial Drug Discovery. As mentioned previously, an experimental 3D structure (homology model) of a target protein may be built to support SBDD in cases when the 3D crystal structure of the particular target is not available. Homology models of several antimycobacterial drug targets have been built and successfully used to support the structure-based discovery of compounds with antitubercular activity. Examples are highlighted in this section.

M. tuberculosis alanine racemase (Alr) catalyzes the racemization of L-alanine to D-alanine, an essential precursor for bacterial cell wall synthesis. A homology model of the enzyme was built and used to screen 25 structural derivatives of D-cycloserine previously designed and prioritized on the basis of their drug-likeness. The screening identified compound **38*** (Figure 6) as a promising virtual hit that merited bioassay and further preclinical evaluation, although no further experimental evaluation was reported.[81]

A homology model for *M. tuberculosis* DNA gyrase (DNAg) was built using the crystal structural coordinates of A and B subunits of the *E. coli* DNAg as template. This model was then used to investigate the ligand−protein interactions of a series of gatifloxacin analogues that had been previously synthesized and tested for activity against *M. tuberculosis* DNAg. The results of the docking of the compounds with GyrA were very well correlated with the previously determined experimental results (r^2 = 0.87; n = 7), but GyrB docking results were poorly correlated (r^2 = 0.47; n = 8). This suggested that these compounds inhibited the GyrA subunit of *M. tuberculosis* DNAg. Furthermore, the docking studies revealed three possible key hydrogen-bonding interactions between the most active compound, **39** (IC_{50} = 3.0 μg/mL against *M. tuberculosis* DNA gyrase), and the amino acid residues Lys49 and Asn172 of GyrA.[82]

Another unrelated study utilized homology models of mutant and wild type *M. tuberculosis* DNAg A subunit (GyrA) to investigate the ligand−protein interactions of a series of GyrA inhibitors. These inhibitors were designed by rational modification of the fluoroquinolone structure at positions known to contribute to the DNAg binding and broad-spectrum antibacterial activity properties. This approach led to the identification of several ligands that had excellent predicted binding affinities against GyrA. For example, compound **40*** showed a high binding affinity of −9.2 kcal/mol, superior to the binding affinity derived for the established fluoroquinolone antibacterials moxifloxacin and gatifloxacin. The docking studies also highlighted important hydrophobic ligand−protein interactions and suggested that increasingly lipophilic substituents on either of the positions of the fluoroquinolone structure considerably increased the binding affinities. The experimental assay results of the designed ligands were not reported.[83]

In an attempt to identify compounds with activity against nonreplicating persistent forms of *M. tuberculosis*, a homology model of *M. tuberculosis* DevR was developed. This model was used in the generation of a pharmacophore that was applied in the virtual screening of compounds from the ZINC database. Docking studies and MD simulations on prioritized compounds led to the identification of 11 compounds for in vitro biological assays. This work identified compound **41** that specifically inhibited DevR activity (IC_{50} < 26.2 μg/mL) and drastically reduced the hypoxic survival of *M. tuberculosis*.[84] A similar study reported the first homology model for the β-subunit of the *M. tuberculosis* RNA polymerase (rpoB), an enzyme involved in transcription and elongation of RNA and the target of the established anti-TB drug rifampicin. The homology model was then applied for the molecular docking analysis of 60 structural analogues of rifampicin, 43 of which had lower binding energy values than rifampicin.[85]

Molecular docking studies have also been carried out on bedaquiline, a drug recently approved for use against MDR-TB by the U.S. Food and Drug Administration. Bedaquiline was docked into a homology model of the *M. tuberculosis* ATPase binding site to identify the key ligand−protein interactions and shed light on the structural features essential for interaction with the target. This information was then used to design a novel series of 12 arylquinoline derivatives that also showed reasonable 3D similarity to bedaquiline as well as acceptable predicted ADME profiles. These compounds were synthesized and tested in vitro for activity against *M. tuberculosis* H37Rv and cytotoxicity against the VERO C1008 cell line. Several compounds exhibited good antimycobacterial activity and low cytotoxicity. The most promising compounds were **42** (MIC = 5.18 μM and MIC/CC_{50} = 152.86) and **43** (MIC = 5.59 μM and MIC/CC_{50} = 160.57). Docking studies with compound **42** revealed that its mode of binding is similar to that of bedaquiline. Notably, a correlation was found between log MIC values versus docking scores for this series of compounds (R^2 = 0.705), suggesting inhibition of *M. tuberculosis* ATPase as a possible mechanism of action.[86]

M. tuberculosis aryl acid adenylating enzyme (MbtA) is involved in the biosynthesis of mycobactins, small-molecule iron chelators (siderophores) involved in iron acquisition and critical for virulence and growth of *M. tuberculosis* in iron-deficient conditions. The identification of the bisubstrate MbtA inhibitor 5′-*O*-[*N*-(salicyl)sulfamoyl]adenosine (Sal-AMS) afforded the opportunity for the development of Sal-AMS analogues as potential MbtA inhibitors. A detailed study has reported the construction of a complete homology model of MbtA and, through the application of MD simulations, the design of a series of Sal-AMS analogues that were docked and analyzed within the MbtA homology model. In addition, these analogues were synthesized and subjected to MbtA inhibition assays and cytotoxicity testing, as well as antitubercular activity screening against whole-cell *M. tuberculosis* H37Rv under iron-deficient and iron-replete conditions. The study reported the identification of analogues with remarkably potent and selective antitubercular activity that compared favorably against isoniazid, a first-line antitubercular drug. Overall, the whole-cell antitubercular activity of the compounds correlated well with the in vitro enzyme inhibition results. Among the tested analogues, compound **44** exhibited the most potent in vitro MbtA inhibition ($K_{i,app}$ = 0.27 nM).[87] Clear trends in the SAR data emerged for this series of inhibitors, and the same group of researchers went further to develop and report a quantitative linear interaction energy model that achieved good correlation (R^2 = 0.7) in the prediction of the binding affinities of aryl acid-AMP bisubstrate inhibitors of MbtA.[88]

Another notable success arising from structure-based computational methods was the recent report of the identification of a novel series of semisynthetic spectinomycin analogues with excellent pharmacological profiles and exceptional antimycobacterial activity, including activity against both MDR-TB and XDR-TB. Molecular docking studies of a series of spectinamide analogues into the active site of a homology model of the *M. tuberculosis* ribosomal 16S helix were used to guide the rational structural derivation of more active compounds. This led

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to the identification of optimized leads **45** and **46**, which exhibited improved antimycobacterial activity against a panel of drug-susceptible and -resistant strains of *M. tuberculosis* strains. These compounds also reduced lung mycobacterial burden and increased survival in in vivo murine infection models and also showed favorable pharmacokinetic and safety profiles.[89]

3.3. Ligand-Based Antimycobacterial Drug Discovery Efforts. *3.3.1. Ligand-Based Pharmacophore and 3D QSAR Approaches.* Ligand-based pharmacophore approaches have been used in attempts to identify new antimycobacterial agents and explore structure−activity relationships. For example, pharmacophores derived from ethambutol and the antitubercular agent 2-phenyl-3-nitrozoimidazo[1,2-*a*]pyrimidine (IMP) were used in the design of a novel series of 17 spiro-isoxazolidine compounds. Several of these compounds were found to significantly inhibit the growth of *M. tuberculosis* H37Rv in vitro. The most effective inhibitor was compound **47** (Figure 7),

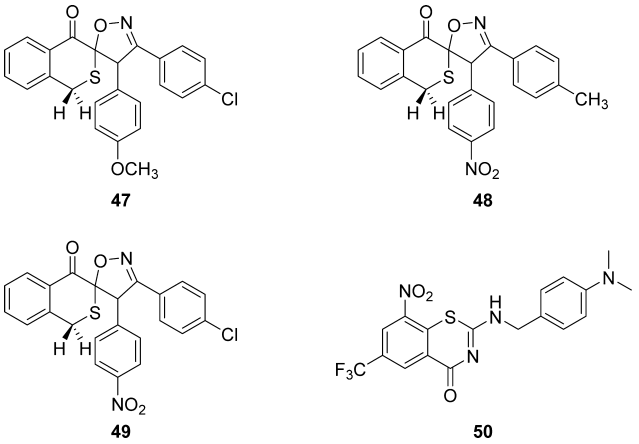

Figure 7. Examples of compounds with antimycobacterial activity identified through ligand-based pharmacophore approaches.

which produced 93% inhibition of *M. tuberculosis* at a concentration of 6.25 μg/mL. Interestingly, compounds **48** and **49** that also showed activity against HIV-1 (IC_{50} = 11 μM) exhibited good antimycobacterial activity.[90]

Priyadarsini and co-workers[91] separately developed a ligand-based pharmacophore model and a 3D QSAR model for *M. tuberculosis* DHFR inhibitors. The pharmacophore model was developed on the basis of 24 known and structurally diverse DHFR inhibitors, and was found to have excellent predictive capability (R = 0.93) when validated against a test set of 204 known DHFR inhibitors. Furthermore, the group also developed a highly predictive 3D QSAR model based on a set of newly synthesized substituted benzothiazole/benzimidazole compounds with antimycobacterial activity. Cross-validation of the two independently developed models suggested that both models yielded similar results.

Using the structures and activity data of 160 benzothiazinone compounds, a 2D ligand-based pharmacophore model was developed that had good predictive power for the activity of this class of compounds against seven strains of *M. tuberculosis*. The model was then applied in the design and filtering of a virtual combinatorial library of benzothiazinone compounds. This led to the identification of several compounds with excellent in vitro activity against *Mycobacterium smegmatis* and MDR *M. tuberculosis* 2745/09. Compound **50** was the most active, exhibiting MICs of 0.001 and 0.03 μg/mL against *M. smegmatis* and MDR *M. tuberculosis* 2745/09, respectively.[92]

Tawari and Degani[93] reported the development of a ligand-based pharmacophore for predicting the binding modes of potential nucleoside bisubstrate inhibitors of MbtA. The pharmacophore model was developed using a set of 63 nucleoside bisubstrate analogues with known activity against the enzyme and was then used to generate a 3D QSAR model with good predictive power (Pearson R = 0.85).[93] A similar study also generated a ligand-based 3D QSAR model using a previously reported set of quinaxaline-1,4-di-*N*-oxide derivatives. The model was highly predictive (R^2 = 0.881) and may find future use in the rational development of anti-TB drugs.[94]

A novel method of identifying and discriminating between phase-specific inhibitors of drug-tolerant *M. tuberculosis* H37Rv has been recently reported. The method, based on a systematic algorithm, demonstrated that molecules targeting the replicative and nonreplicative phases of *M. tuberculosis* have different chemical and molecular properties, and on this basis a descriptor-based computational model was developed that could differentiate between active and inactive compounds in both phases. The study also showed that certain structural groups (such as nitro, alkyne, enamine) were dominant in inhibitors of both phases.[95]

3.3.2. Integrated Structure- and Ligand-Based Approaches. Combined structure- and ligand-based approaches have increasingly been applied in the discovery of novel compounds with antimycobacterial activity. The utility of applying different computational strategies is highlighted in the study by Garg and co-workers.[96] This study reported the use of different computational approaches to identify potential *M. tuberculosis* dihydrodipicolinate synthase (DHDPS) inhibitors, a key enzyme of the lysine/diaminopimelate (DAP) biosynthetic pathway that is important for both cell wall and amino acid biosynthesis. The first approach involved the computational generation of a combinatorial library of 4088 drug-like pyruvate analogues. The second approach involved the ligand-based virtual screening of the prefiltered NCI and PubChem databases of compounds using a 3D superimposition approach with pyruvate as the template. This approach retrieved a total of 2640 pyruvate-like compounds. The third approach involved the screening of 3847 anti-infective compounds retrieved from PubChem database using Lipinski's "rule of five", which identified 1750 compounds that had no 2D/3D similarity with pyruvate. The three sets of compounds were then docked into the binding site of *M. tuberculosis* DHDPS. The molecular docking studies identified 374 pyruvate analogues, 300 pyruvate-like compounds, and 25 anti-infectives that had binding energies superior to the pyruvate substrate. There was considerable structural diversity among the identified compounds, and the docking studies also provided insights into possible binding modes and important ligand−protein interactions. However, no experimental enzyme inhibition data or antimycobacterial activity was reported.[96]

A combination of receptor-based and ligand-based approaches was also used in the identification of inhibitors of *M. tuberculosis* type II DHQase, the third enzyme of the shikimic acid pathway and a validated antimycobacterial drug target. To begin with, a 2D fingerprint search was carried out on ≈4 million compounds from the ZINC database to identify compounds with structural similarity to known inhibitors of type II DHQase. The similarity search yielded a set of 268,186 unique compounds that were subsequently screened using a 3D pharmacophore generated from the putative bound conformation of the 3-nitrophenyl

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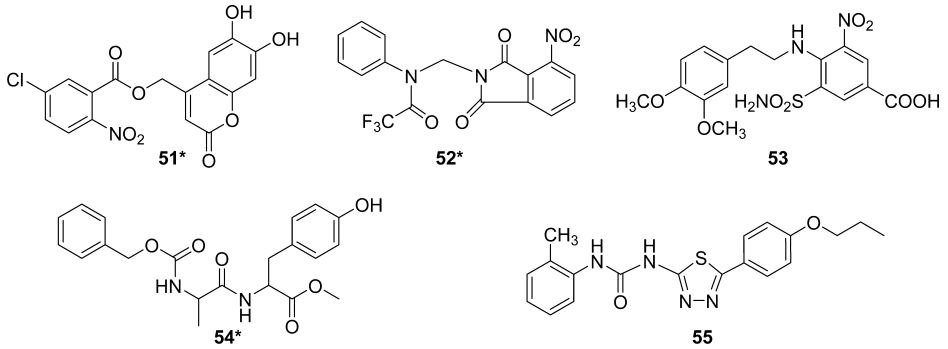

Figure 8. Examples of compounds with antimycobacterial activity identified through integrated ligand- and structure-based methods. (∗) Experimental biological data not reported.

derivative of quinate, the most active of the known inhibitors. The 3D pharmacophore search yielded 4821 compounds, of which 4808 were successfully docked into the *M. tuberculosis* type II DHQase binding site. In addition, a 3D QSAR model was developed using the structures and activities of 45 known *M. tuberculosis* type II DHQase inhibitors reported in the literature. The model was used to predict the biological activity of 59 high-scoring compounds identified from the docking studies, which led to the final selection of 42 novel potential inhibitors of *M. tuberculosis* type II DHQase. Some of these compounds, such as compounds **51*** and **52*** (Figure 8), had excellent docking scores. The selected compounds bore a diverse range of molecular scaffolds, thereby offering new medicinal chemistry starting points for the design of novel *M. tuberculosis* type II DHQase inhibitors.[97] However, no experimentally derived biological data for these compounds were reported.

In another antimycobacterial CADD approach involving MbtA, two different 3D QSAR models were developed from the structures and activities of 80 known MbtA inhibitors selected from literature sources. These validated and highly predictive models (R^2 values >0.8) were separately developed using different partial atomic charges along with different combinations of descriptor fields. The 3D QSAR models were then used to predict the biological activities of 196 compounds identified from the ligand-based virtual screening of the ZINC database. At least 60 compounds had a higher predicted activity than that of the most active known inhibitor. Further analysis including molecular docking and MD studies identified five high-priority virtual hits. The docking studies also provided insights into the probable ligand−protein interactions that are important for optimal binding. Experimental enzyme inhibition and/or antimycobacterial activity was not reported.[98]

In a study on *M. tuberculosis* InhA inhibitors, both molecular docking studies and a 3D QSAR model predicted the same molecule to be the most active inhibitor from a combinatorial library of 300 compounds. However, no experimental biological data for the ligand are reported.[99] In another study, inhibitors of *M. tuberculosis* chorismate mutase (CM), an essential enzyme in the shikimate pathway, have also been identified by applying a combination of ligand- and structure-based methods. Initially, an in-house library of ≈15000 compounds was screened using a ligand-based pharmacophore approach. Ninety-five compounds were identified and subjected to molecular docking studies using the crystal structure of the enzyme, and this enabled the selection of 15 compounds for bioassay. Enzyme inhibition assays identified four inhibitors of *M. tuberculosis* CM, with compound **53** having the lowest K_i of 5.7 μM. Furthermore, analysis of docking modes revealed important H-bonding and van der Waals interactions with the binding site residues.[100]

Kumar and Siddiqi[101] incorporated the use of structural interaction fingerprint (SIFt) screening in their integrated computational approach. They reported the integrated use of 3D pharmacophore-based virtual screening, molecular docking studies, and SIFt screening for the identification of inhibitors of *M. tuberculosis* DHFR. The 3D pharmacophore models were generated using *M. tuberculosis* DHFR−inhibitor crystal structure complexes. Virtual screening of 59275 compounds from the Maybridge database using these models identified 632 compounds of interest, which were further narrowed to 183 promising hit molecules by docking studies into the DHFR binding site. Molecular docking poses of the identified compounds were examined using SIFts generated from 78 known DHFR inhibitors that led to the selection of 20 promising and structurally diverse virtual hits. However, experimental bioassay results are not reported.[101]

Billones[102] also reported the use of a combination of computational methods in the identification of inhibitors of *M. tuberculosis* lipoate protein ligase B (LipB). Lipoate protein ligase B is involved in the biosynthesis of the lipoic acid cofactor and is a very promising drug target as it is essential for mycobacterial survival. Specifically, a combination of virtual screening, molecular docking, and in silico ADME profiling was undertaken. A total of 153,000 compounds from the NCI database were screened on the basis of a pharmacophore derived from LipB. A validated docking methodology was then used to dock the identified virtual hits into the LipB binding site. Nine compounds had superior binding energies to the known inhibitor decanoic acid and also featured important polar and H-bonding interactions with the target protein. These compounds were further analyzed using in silico ADME and toxicity filters, which included predicted solubility, absorption, plasma protein binding, CYP2D6 inhibition, and hepatotoxicity. These studies identified compound **54*** that had satisfactory predicted ADME and toxicity properties and favorable ligand−protein interactions.[102] No accompanying experimental inhibition data on *M. tuberculosis* LipB were reported.

In an effort to improve their chances of finding viable hit compounds as well as allow for a comparison of different methods, some research groups opt to apply different computational methods in parallel. For example, Pauli and co-workers[103] reported the use of two independent virtual screening approaches for the identification of InhA inhibitors. A total of ≈1 million preselected compounds from the ZINC database were screened. The first approach involved an initial screening of the compounds based on a four-point 3D pharmacophore

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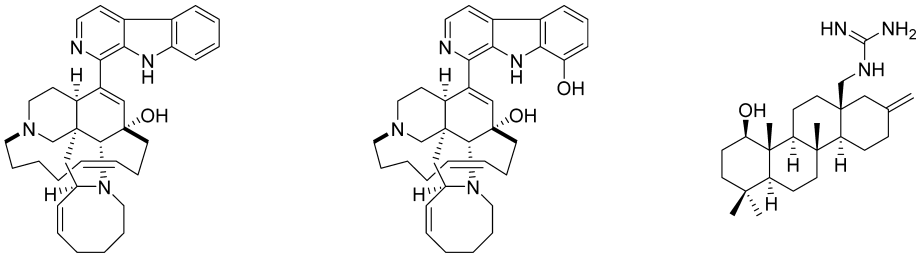

Figure 9. Natural products with potential antimycobacterial activity.[106]

derived from both structure-based and ligand-based information. Selected compounds were then docked into the *M. tuberculosis* InhA binding site and subjected to in silico toxicity prediction. This approach yielded a final subset of 34 compounds. The second approach involved dedicated molecular docking studies that utilized four well-established docking programs. Compounds that achieved very similar docking conformations and predicted ligand−target interactions in at least three of the docking programs were selected for in silico toxicity prediction. This approach yielded a final subset of 21 compounds. Interestingly, there was no superposition of compounds identified by the two approaches. After analysis of predicted toxicity and ADME properties of the 55 compounds identified by both methods, 6 compounds were selected for in vitro testing for inhibitory activity against InhA. Compound **55** was identified as the most promising inhibitor with a K_i value of 20 (±2) μM against the 2-*trans*-dodecenoyl-CoA substrate.[103]

3.4. Natural Products. The importance of incorporating modern drug discovery techniques into natural product drug discovery is self-evident. These modern approaches include, among others, the adoption of computational drug discovery approaches.[104,105] Whereas virtual screening, molecular docking, and other computational techniques have predominantly been applied to collections of synthetic compounds, efforts have been made to leverage them to complement and enrich natural product drug discovery.

In what is an innovative application of established computational approaches to natural products, in silico tools were used to characterize the chemical space of a diverse group of natural products with previously reported in vitro activity against different *M. tuberculosis* strains, which was compared with the chemical space of established conventional anti-TB drugs as well as those drugs currently in development. The study found that the natural products had comparable compliance to the "rule of five" when compared to conventional drugs, as well as solubility and permeability profiles similar to the drugs currently in development. Furthermore, molecular docking studies revealed potential targets for some of the natural products. The findings suggested that manzamine A (**56**) and 8-hydroxymanzamine A (**57**) could be inhibitors of InhA, whereas scytoscalarol (**58**) has binding affinity for arabinosyltransferase *M. tuberculosis* EmbC (Figure 9).[106] Experimental confirmation of the activity of these compounds against arabinosyltransferase *M. tuberculosis* EmbC would be the next logical step of this study.

From the preceding discussions, it can be seen that SBDD methods using both protein crystal structures and homology models have been quite widely applied in TB drug discovery, with remarkable success in some instances.[85] These molecular docking studies have led to the identification of bioactive hit compounds and have also been used quite extensively in the investigation of unknown ligand−protein interactions. There exists untapped potential for structure-based CADD, which can be largely supported by the widening of the currently limited pool of crystallized TB protein drug targets.

A significant number of the structure-based CADD studies for TB are based on homology models. As we have noted previously, the major limitation to the use of homology models is that the accuracy of any homology model is highly dependent on the sequence identity between target and template. Whereas some of the homology models for the TB drug targets had moderate sequence identity with their templates (*M. tuberculosis* DevR at 32%,[84] *M. tuberculosis* DNAg at 35%,[79] and *M. tuberculosis* rpoB at 47.4%[85]), others had remarkably high sequence identity (MbtA at 76%[84] and *M. tuberculosis* Alr at 94.1%[81]). The moderate sequence identities were explicitly acknowledged as limitations in the respective studies. The sequence identities for some of the homology models were not reported, such as for the *M. tuberculosis* ATPase binding site[86] and *M. tuberculosis* ribosomal 16S helix.[83] Despite this limitation, several structure-based CADD initiatives did manage to identify hit compounds with confirmed bioactivity, such as against *M. tuberculosis* ribosomal 16S helix, *M. tuberculosis* DNAg, and *M. tuberculosis* DevR.

On their own, LBDD methods appear to have been applied only to a limited extent in TB drug discovery. The application of ligand-based methods integrated with structure-based approaches appears to be the favored tactic. Regrettably, the majority of the TB studies reporting integrated ligand- and structure-based CADD do not report experimental biological data for the hit compounds identified. It is our opinion that, for this reason, the true impact of this integrated approach in contributing viable antimycobacterial hit compounds cannot therefore be adequately assessed.

4.0. TRYPANOSOMIASIS AND LEISHMANIASIS

The etiological agents for Chagas' disease, HAT, and leishmaniasis belong to the Trypanosomatidae family of the order Kinetoplastida.[107] *Trypanosoma cruzi* is responsible for Chagas' disease in South America, whereas *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* cause HAT in West and Central Africa and East Africa, respectively. Several species of the genus *Leishmania* cause cutaneous, mucocutaneous, and visceral leishmaniases endemic in 88 countries in the horn of Africa, South Asia, and Latin America. Because of their occurrence among marginalized populations, the current therapeutic landscape in the management of trypanosomatid diseases is severely restricted by antiquity, low efficacy, intolerable toxicity, and complicated treatment regimens of available antitrypanosomatidal agents.[108]

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Table 4. Antitrypanosomatid Drug Targets Utilized in CADD Approaches

target[a]	function
glyceraldehyde-3-phosphate dehydrogenase[113,114]	key enzyme in carbohydrate metabolism in trypanosomatids catalyzing conversion of glyceraldehyde-3-phosphate to 1,3-biphosphoglycerate, with concomitant reduction of NAD^+ to NADH
trypanothione reductase[117−119]	essential enzyme catalyzing regeneration of oxidized trypanothione, a critical component of the antioxidant defense systems in trypanosomatids
trypanosomal cysteine proteases[120]	rhodesain and cruzain cysteine proteases are essential enzymes for the survival, infectivity, and pathogenicity of trypanosomes;they are involved in a range of functions such as degradation of host immunoglobulins, formation of variant coat glycoproteins, and entry into the brain
tubulin[128]	tubulin is a cytoskeletal protein that polymerizes to from microtubules essential for cell division and maintenance of cellular cytoskeleton

[a]References given are citations in which the target has been used as the basis for antitrypanosomatid CADD.

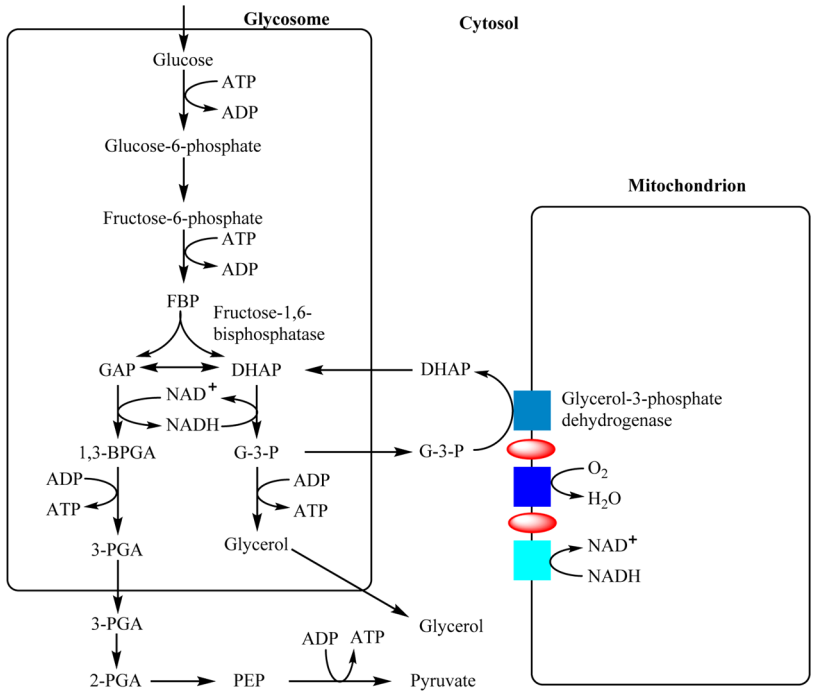

Figure 10. Glycolytic pathway in *Trypanosoma brucei* bloodstream trypanosomes.[109] 1,3-BPGA, 1,3-bisphosphoglycerate; 2-PGA, 2-phosphoglycerate; 3-PGA, 3-phosphoglycerate; DHAP, dihydroxyacetone; FBP, fructose-1,6-bisphosphate; GAP, glyceraldehyde-3-phosphate; PEP, phosphoenolpyruvate.

This section highlights some recent efforts at antitrypanosomatid drug discovery spearheaded using CADD techniques. Although there are fewer success stories than in other tropical diseases discussed in this review, the efforts are encouraging, and there is potential for new lessons that may inform breakthrough discoveries in the near future.

4.1. Antitrypanosomatid Drug Targets. Phylogenetic studies suggest that members of the Trypanosomatidae family share several biochemical features distinct from those in higher eukaryotes. A broad-spectrum drug clinically useful against all of the parasitic trypanosomatids is therefore a theoretical prospect.[109] It is envisaged that the evolving CADD approaches may hasten realization of this prospect by facilitating more elaborate comparative biochemistry and orthologue searching than are routinely applied in antitrypanosomatid drug discovery efforts. Some of the protein targets that have been utilized in antitrypanosomatid CADD efforts are shown in Table 4. They include glyceraldehyde-3-phosphate dehydrogenase (GAPDH), trypanothione reductase (TryR), cysteine proteases, and tubulin.

4.2. Structure-Based Drug Discovery for Trypanosomatids. *4.2.1. Target-Based Virtual Screening.* The application of target-based virtual screening to antitrypanosomatid drug discovery is best exemplified by studies targeting GAPDH and TryR. The use of GAPDH is based on the fact that differences in energy metabolism between vertebrate host and infectious microbes are eagerly sought after for anti-infective drug discovery due to their potential for selective targeting. Such is the case for the carbohydrate metabolism in the trypanosomatids. Whereas most other eukaryotic cells, including the mammalian host, have all of their glycolytic enzymes within the cytosolic compartment, the trypanosomatids have the first seven enzymes of glycolysis (Figure 10), two enzymes of the glycerol metabolism, and several enzymes of the pentose phosphate pathway ensconced inside glycosomes, microbody organelles that are phylogenetically related to peroxisomes of higher eukaryotes.[110,111] This compartmentation has fuelled the search for antitrypanosomatid drugs for decades on the basis of the assumption that glycosomal enzymes in kinetoplastids could be sufficiently different from those in the cytosol of the mammalian host.[110]

The glycolytic pathway is rendered even more attractive for antitrypanosomal drug discovery by the observation that bloodstream trypanosomes lack a functional citric acid (Kreb's) cycle and mitochondrial oxidative phosphorylation and are thus solely dependent on glycolysis for the generation of

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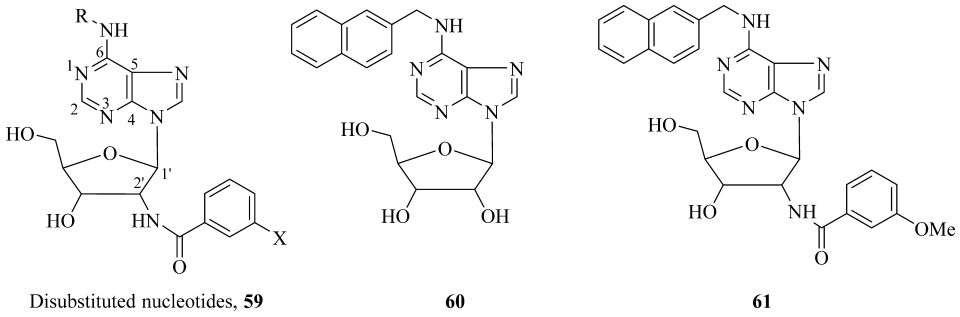

Figure 11. Designed adenosine analogue inhibitors of trypanosomatid glyceraldehyde-3-phosphate dehydrogenase.[113,114] R = diphenylmethyl, phenethyl, (*S*)-α-methylbenzyl, 1-naphthalenemethyl, 4-aminomethyl-1-naphthalenemethyl, 7-methyl-1-naphthalenemethyl; X = methoxy, chloro.

ATP for their energy requirements.[112] Consequently, a number of enzymes with significant influence on the carbohydrate metabolism have been exploited in antitrypanosomatid drug discovery campaigns. The GAPDH is one of the most notable enzymes exploited in this endeavor through structure-based CADD approaches.

Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) is a key glycolytic homotetrameric enzyme that catalyzes the conversion of glyceraldehyde-3-phosphate (G3P) to 1,3-biphosphoglycerate (1,3-BPG), with concomitant reduction of NAD^+ to NADH. Computational studies of the crystal structures of the trypanosomatid GAPDH in *T. brucei*, *T. cruzi*, and *Leishmania mexicana* to the human GAPDH indicate significant differences around the binding pocket for the adenosyl moiety of the bound NAD^+ cosubstrate. Thus, structural modification of the adenosyl group could be used to design compounds that selectively and competitively block the binding of NAD^+ to trypanosomatid GAPDHs.[113] On the basis of this proposition, and using adenosine as the template, Bressi et al.[113] designed, synthesized, and evaluated a series of adenosine analogues for their enzyme binding affinities as well as in vitro antitrypanosomatid activity. As shown in Figure 11, the design strategy involved modification of the adenosine N^6 position and the ribose 2′-position to give disubstituted nucleotides **59**.

Preliminary studies by Gelb et al. had showed that the N^6-(1-naphthalenemethyl)-adenosine **60** was the most potent inhibitor of *L. mexicana* GAPDH with an IC_{50} of 150 μM, 330-fold more potent than adenosine. Guided by molecular modeling, enzyme binding optimization studies led to the synthesis of N^6-(1-naphthalenemethyl)-2′-(3-methoxybenzamido)adenosine, **61**, that inhibited *L. mexicana* GAPDH with an IC_{50} of 280 nM, which is >5 orders of magnitude lower than the IC_{50} of adenosine.[114] In addition, this compound did not detectably inhibit human GAPDH when tested up to its solubility limit of approximately 40 μM, implying a good selectivity index toward trypanosomatid GAPDH. A few compounds prepared in this study were tested for their ability to block the growth of bloodstream *T. brucei*, with moderate to good activity when compared to the original adenosine lead compound.[115]

Trypanosomatids depend on spermidine for growth and survival. Consequently, enzymes involved in spermidine synthesis and utilization are promising targets for drug development. One such enzyme is TryR, a typical flavin-containing NADPH-dependent cysteine oxidoreductase homologous to glutathione reductase and thioredoxin reductase that are present in higher eukaryotes. Trypanothione reductase is involved in the regeneration of oxidized trypanothione, a critical component of the antioxidant defense systems in trypanosomatids and a well-validated target for antitrypanosomal drug development.[116]

In an effort to discover new chemotypes for antitrypanosomal drug discovery, Maccari et al.[117] applied a fast virtual screening protocol that combined information about the crystallographic protein structure of *T. cruzi* TryR and the structure of active ligands. The choice of the screening protocol was informed by two factors. First, the presence of many charged centers and lack of well-defined hydrophobic moieties in trypanothione make pharmacophore modeling from the protein−substrate complex difficult. Second, in addition to their structural diversity, known inhibitors of TryR have varied binding modes and biological data, thus making a common ligand-based approach complicated. The applied protocol utilized 19 known competitive inhibitors of TryR sharing different scaffolds and physicochemical properties to model a commercially available Asinex database using a sequence of computer softwares. A total of 5640 unique structures were preselected and the best 100 molecules chosen on the basis of their structural diversity. Selected compounds were commercially sourced and subjected to in vitro biological evaluation. This work led to the identification of nine compounds with micromolar activity against *T. cruzi* TryR and holding promise for the future treatment of Chagas' disease.[114] Structures of three representative hit compounds, **62** (K_i = 5.7 ± 0.1 μM), **63** (K_i = 9.3 ± 0.3 μM), and **64** (K_i = 11.8 ± 0.3 μM), are shown in Figure 12.

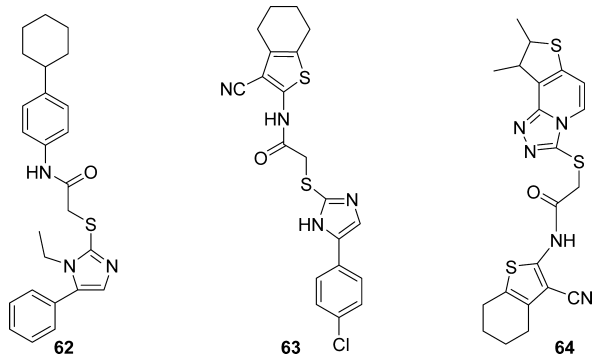

Figure 12. Novel compounds with good in vitro activity against *Trypanosoma cruzi* trypanothione reductase.[117]

4.2.2. Docking Studies To Investigate Ligand−Protein Interactions and Mechanism of Action. As discussed under Tuberculosis: Docking Studies To Investigate Ligand−Protein Interactions and Mechanism of Action, molecular docking studies have been applied to good effect in the investigation of ligand−protein interactions as well as elucidation of the mechanism of action. One such work in antitrypanosomatid drug discovery was reported by Venkatesan and Dubey, who

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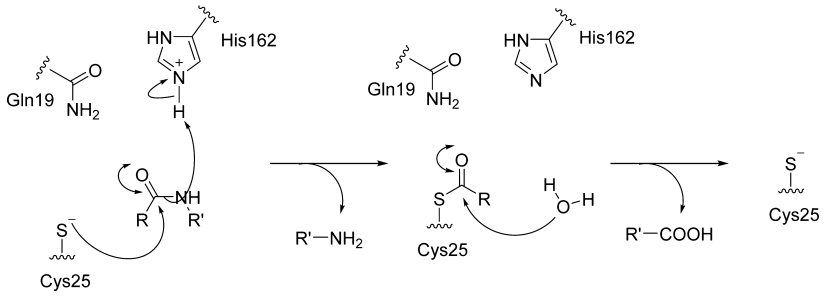

Figure 13. Proposed catalytic mechanism for cysteine proteases.[121]

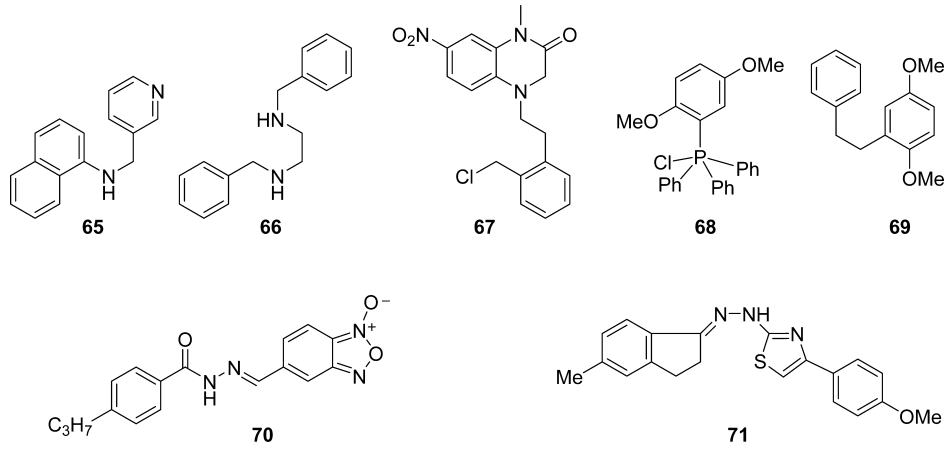

Figure 14. Examples of compounds with antitrypanosomal activity identified through ligand-based drug discovery approaches.[123−127]

utilized automatic analysis of poses (AuPosSOM) computer software to cluster and classify protein−ligand interactions between *Leishmania infantum* TryR and the NCI Diversity set II compounds.[118] The majority of the top 50 ranked hits were tricyclic compounds. In silico molecular docking of 9-aminoacridine, phenothiazine, and quinone derivatives on *L. infantum* TryR using AutoDock4 confirmed earlier observation that the various structural classes have varying and unique binding modes at the enzyme active site.[119] The results indicated that quinone derivatives bind at the flavin adenine dinucleotide binding domain, whereas 9-aminoacridines and phenothiazines produce their inhibitory effect by binding at the Z site of TryR. Although no experimentally derived enzyme inhibitory data were reported, the modeled binding modes provide valuable insight into the interactions of these compounds at the *L. infantum* TryR active sites, which could be exploited for rational design and synthesis of specific inhibitors.

The use of molecular docking studies in the elucidation of the mechanism of action of antitrypanosomatids is best exemplified by the work of Mendez-Lucio et al., who studied the mechanism of inhibition of cysteine proteases by the nitriles.[120] Cysteine proteases are involved in numerous biochemical processes and have been targeted in drug discovery for a variety of diseases. In antiparasitic drug discovery, falcipain, cruzain, and rhodesain have long provided drug targets for *P. falciparum* malaria, *T. cruzi* Chagas' disease, and *T. brucei* sleeping sickness, respectively. The catalytic mechanism of proteolytic action of cysteine proteases is shown in Figure 13.[121] For cruzain, the enzyme active site is composed of the sulfhydryl group of Cys25 that exists as a thiolate anion, the imidazole ring of His162 that exists as an imidazolium cation, and the amide group of Gln19 that provides a stabilizing effect on tetrahedral enzyme−substrate intermediates. Nucleophilic attack of the thiolate on the protein substrate is followed by proton transfer from the imidazolium cation forming an amine product and an acyl−enzyme complex. Subsequent deacylation facilitated by a water molecule releases a carboxylic acid product and regenerates the thiolate moiety.[122]

Although nitrile-containing molecules are well-known inhibitors of cruzain that act via covalent reaction at Cys25, the energetics involved in the binding are not yet clear. Toward unravelling the mechanism of this binding, Mendez-Lucio et al. studied the molecular recognition of cysteine proteases by nitriles using density functional theory and quantum semi-empirical computational calculations. A number of propositions were made from the results of this study: (1) the molecular interaction starts with a nucleophilic attack from Cys25 to the nitrile inhibitor followed by a proton transfer from His162; (2) the interaction could be managed by modulating the proton transfer from His162 to the inhibitor; and (3) an increased electrophilicity in the carbon on the nitrile moiety might facilitate the nucleophilic attack from Cys25, thus decreasing activation energy while increasing inhibitory activity.[120] The structural features suggested by the study data could be used to improve inhibitory activity of nitriles against cysteine proteases.

4.3. Ligand-Based Antitrypanosomatid Drug Discovery Efforts. *4.3.1. Ligand-Based Pharmacophore Modeling and QSAR Studies.* For a period spanning over a decade, Castillo-Garit et al. have pursued ligand-based discovery for anti-*T. cruzi* agents using two QSAR models developed and validated using a data set of 440 compounds of various structural classes. The data set is composed of 143 antitrypanosomal compounds and 297 compounds having other clinical uses such as antivirals, sedative/hypnotics, diuretics, anticonvulsants, hemostatics, oral hypoglycemics, antihypertensives, antihelminthics, and anti-

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cancer compounds.[123−125] The QSAR modeling involved the use of both stochastic and nonstochastic bond-based quadratic indices and linear discriminant analysis (LDA). The models showed reliable predictive capability with high accuracies (87−95%), good specificity (66−85%), and high sensitivity (88−100%) for both training and test sets, hence capable of accurately discriminating between antitrypanosomally active and inactive compounds.[123] The two models have proven useful and have consequently been applied in the rational discovery of new trypanosomicidal compounds.

In one of the reported studies, Castillo-Garit et al. applied the developed QSAR models in the in silico predictions of the antiepimastigote (AE) activity of nine synthesized compounds and their epimastigote inhibitory activity experimentally determined.[123] This work led to identification of four compounds with >70% inhibitory activity against epimastigotes at 100 μg/mL. When evaluated for epimastigote inhibition and cytotoxicity against mammalian cells at 10 μg/mL in vitro, compounds **65** and **66** (Figure 14) showed attractive antitrypanosomal activity (AE = 81.31 and 78.22%, respectively) and high selectivity indices (cytotoxicity = 1.37 and 15.44%, respectively) comparable to that of the clinically used drug nifurtimox (AE = 85.45%, cytotoxicity = 0.6%).[123] Not surprisingly, the two compounds have very close structural resemblance, which further gives credibility to the predictive capability of the applied mathematical QSAR models.

In a related study by the same research group, another set of nine synthesized nitro-based compounds were subjected to in silico evaluation for antitrypanosomal activity using two model equations and the resultant theoretical predictions correlated with experimental data.[124] Compound **67** gave the best predicted score for activity (>80% in both equations), which correlated to high in vitro potency characterized by epimastigote inhibitory activity of 100%, amastigote susceptibility activity of 56% (nifurtimox = 94%), and cytotoxicity of 0%, hence less toxic than nifurtimox in vitro.[124] In yet another study, the two LDA-based QSAR algorithms gave high predictive scores for antitrypanosomal activity of 11 synthetic compounds.[125] Compound **68** scored the highest predicted activity in both models showing high correlation with its good experimental epimastigotes inhibitory effect (IC_{50} = 3.24 μM), being orders of magnitude better than the reference drug benznidazole (IC_{50} = 54.7 μM). On the basis of its good cytotoxicity/activity index of 5.5, synthetic tractability, and visual comparison with other compounds in the test set, compound **69** (IC_{50} = 46.52 μM) appears attractive for further synthetic optimization.

In another inspiring LBDD work for antichagasic compounds, Jorge et al.[126] developed and utilized two predictive QSAR models to design six benzofuroxan derivatives. The QSAR models were developed on the basis of the Hansch and VolSurf analyses of a set of substituted-[*N*-(benzofuroxan-5-yl)-methylene]benzohydrazides for which in vitro anti-*T. cruzi* activity had previously been determined. The 2D Hansch analysis was composed of nine descriptors related to steric, electronic, hydrophobic, and topological properties, whereas the 3D VolSurf analysis was composed of 110 descriptors related to molecular size and shape, hydrophile/lipophile balance, molecular diffusion, calculated *n*-octanol/water partition coefficient, calculated distribution coefficient, charge state, 3D pharmacophore, and some ADME properties of the training set. The QSAR models suggested that hydrophobicity, steric, and electronic properties are the most crucial features for the anti-*T. cruzi* activity of the benzofuroxan derivatives and largely influenced the design strategy. The six designed compounds were synthesized and in vitro assayed for antitrypanosomal activity. In what may be regarded as an endorsement of the powerful role of predictive QSAR models in drug discovery, not only were the predicted inhibitory values in good agreement with the experimentally derived values but also five of the six designed compounds had better activity than the training set, further confirming the predictability of the developed models. The most active compound, **70**, had excellent inhibitory activity against *T. cruzi* epimastigotes in vitro (IC_{50} = 3.04 μM), approximately 7-fold more potent than benznidazole (IC_{50} = 20.84 μM) and equipotent to nifurtimox (IC_{50} = 3.78 μM).

In a similar but unrelated research study, Noguera et al.[127] developed two QSAR algorithms based on a set of 17 previously synthesized and in vitro assayed 4-arylthiazolylhydrazones and applied the models to predict antichagasic activity of three newly designed and synthesized analogues as well as postulate conformational parameters for their biological activity. The modeling rightly predicted growth inhibitory activity of the designed compounds, with the most active compound, **71**, exhibiting an IC_{50} value of 4.16 ± 0.12 μM against *T. cruzi* epimastigotes in vitro, which was slightly better than that of the reference drug benznidazole (IC_{50} = 5.39 ± 0.25 μM) but comparable to those of previously synthesized 4-arylthiazolylhydrazones. Conformational analysis revealed that the antichagasic efficacy of 4-arylthiazolylhydrazones was highly correlated to their spatial orientation. The modeling postulated that among the four possible minimum energy orientations around the C═N—NH—C═N portion of the thiazolylhydrazone moiety, the pharmacologically active conformer adopted a *cis* conformation with a dihedral angle of approximately −85°.[127]

4.3.2. Integrated Structure- and Ligand-Based Approaches. A recent literature account utilizing integrated structure- and ligand-based techniques was reported by Goodarzi et al.[128] in an effort to discover antileishmanial agents using tubulin as the target protein. Tubulin is a heterodimeric protein consisting of α and β subunits, which polymerize to form microtubules that have a number of functions within eukaryotic organisms, including chromosome segregation, motility, and maintenance of cellular morphology. The assembly−disassembly process is critical for the proper functioning of microtubules within the cell.[129] Due to its essential functions in cellular homeostasis, tubulin is an established drug target in the chemotherapy of a number of diseases, most notably cancer and helminth infections. Previous studies have demonstrated potential targeting of tubulin in kinetoplastid drug discovery.[130]

The study by Goodarzi et al. utilized QSAR and docking studies to elucidate novel antileishmanial diarylsulfides and sulfonamides active against *Leishmania donovani* α,β-tubulin. The two compound classes were modeled using Dragon descriptors and multiple linear and support vector algorithms. The compounds were proposed using the QSAR models built by combining substructures of the active compounds of both classes. Docking studies using *L. donovani* α,β-tubulin revealed several compounds with high affinity for the protein active site comparable to those of existing antileishmanial compounds.[128] However, no experimental data were provided.

By far, ligand-based approaches have been applied in CADD for antitrypanosomatids to a greater extent than structure-based methods. This can be rationalized by the fact that there is limited activity in target-based drug discovery for neglected diseases compared to phenotypic whole-cell screening as alluded to in the Introduction. In the absence of any available crystal structure of

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the target protein, LBDD is invariably the only applicable CADD approach. As illustrated in the reviewed studies in this section, the usefulness of theoretical predictions is enhanced by the accompanying biological data experimentally derived through phenotypic whole-cell assays. When in vitro activity and acceptable selectivity indices are consistently demonstrated through replicate assays, reverse pharmacology to determine the mechanistic target and molecular basis for bioactivity would be the next logical step.[13] This would facilitate the application of the more rational SBDD.

Nevertheless, the reported literature accounts of the use of SBDD for kinetoplastids indicate a slow but steady change of prospects in this therapeutic area. There is a likelihood that future CADD approaches will see a rise in the structure-based techniques as molecular and genetic tools find greater utility in the elucidation of the crystal structures of relevant kinetoplastid drug targets. The critical factor that will ultimately enhance applicability of SBDD for antitrypanosomatids and their progression into the clinic will be scrupulous target identification and validation. As a minimum, the selected targets should satisfy the criteria for essentiality, assayability, resistance potential, toxicity potential, and structural information.[131]

5.0. CONCLUSIONS AND FUTURE OUTLOOK

There is an urgent need for the development of novel drugs targeting tropical infectious diseases to supplement currently available medicines. Such efforts hinge on the identification of novel classes of bioactive molecules that target both established and nontraditional but exploitable drug targets. Computational approaches have demonstrable capability to play an important role in this endeavor. Whereas the various computational techniques have already contributed notably to drug discovery efforts in the subject therapeutic areas, there has been very limited success so far in delivering drugs discovered by this approach to clinical use. However, the consistent identification of a diverse range of promising compounds as reported herein is very encouraging, and the breakthrough into clinical drug development and mainstream use may yet be forthcoming. It is the contention of the authors that CADD approaches represent the best opportunity so far for enhancing productivity pipelines in tropical infectious diseases. The easy availability of many diverse chemical libraries in pharmaceutical companies, virtual discovery organizations, and academic institutions and the increased genomic information facilitative of computational predictions make CADD a very attractive venture for improved and efficient drug discovery for tropical infections.[132]

Going forward, some key areas of CADD need to be addressed. The gap between computational predictions and experimental findings needs to be narrowed to avoid blindly following up on hits or leads identified by computational means but reported without confirmation of biochemical or whole-cell biological activity. In any case, it is advisible to begin any follow up of such compounds with an initial experimental confirmation of biological activity. The continued refinement of predictive models and algorithms should improve the accuracy of computational prediction of biological activity. Furthermore, the potential for computational techniques to aid in the elucidation of SAR and mechanisms of action is yet to be fully utilized. Far too many studies report only the computational identification of compounds of interest and their biological activity. More emphasis needs to be placed on progressing such studies by mounting dedicated SAR and mechanistic investigations that can further harness the power of CADD and improve the chances of identifying truly valuable bioactive compounds with clinical utility.

A word of caution: as is the case with HTS and other approaches utilized in hit identification, there needs to be an element of vigilance when data generated from CADD studies are interpreted. This is particularly important with regard to the designation of active compounds and their subsequent prioritization for further progression. Caution must be taken to weed out compounds that possess structural motifs associated with nonspecific and misleading "activity" across a wide range of bioassays. Several classes of compounds exhibiting such confounding false signals across a variety of assays have been identified and are collectively referred to as pan-assay interference compounds (PAINS).[133] Further attempts to develop and optimize such compounds is a futile exercise that results only in unnecessary wastage of resources in the form of time, effort, and money. This is a strong argument for the need for computational chemists to work closely alongside medicinal chemists so as to promptly identify and exclude PAINS from genuine hit compounds and hence ensure realization of the full potential of CADD approaches.

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Notes

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ABBREVIATIONS

ADME, absorption, distribution, metabolism, and excretion; CADD, computer-aided drug discovery and development; DALYs, disability-adjusted life years; HAT, human African trypanosomiasis; HTS, high-throughput screening (either in vitro assays or in silico computation); IC_{50}, half-maximal inhibitory concentration, i.e., the concentration of a compound that causes 50% inhibition of a biological process in vitro; LBDD, ligand-based drug design; LDA, linear discriminant analysis; MD, molecular dynamics; MDR-TB, multidrug-resistant tuberculosis; MIC, minimum inhibitory concentration; PAINS, pan assay interference compounds; QSAR, quantitative structure−activity relationship; SBDD, structure-based drug discovery; XDR-TB, extensively drug resistant tuberculosis

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