Structure-based Design of Inhibitors Against Maltosyltransferase GlgE

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ABSTRACT

The emergence of *Mycobacterium tuberculosis* (Mtb) drug resistant strains calls for research of novel anti-TB drugs. In this study, structure-based pharmacophore generation, virtual screening, molecular docking and *de novo* lead optimization were employed in the search for possible inhibitors of Maltosyltransferase GlgE enzyme, a recently validated anti-TB drug target. Chemical libraries containing only natural products were screened. The top hits were docked and the resulting leads were subsequently modified using *De Novo Evolution*. Three modified compounds were found to have greater binding energy than the substrate. All were derived from the lead compound ZINC39010596 (5,7-dihydroxy-2-propan-2-yl-8-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl]oxychromen-4-one). Toxicity predictions of these compounds revealed that they are non-carcinogenic, non-mutagenic, and biodegradable.

Key words: Tuberculosis, Maltosyltransferase GlgE, Structure-based Pharmacophore Generation, Virtual Screening, Molecular Docking.

INTRODUCTION

Tuberculosis (TB) is a highly contagious airborne disease brought by the pathogen *Mycobacterium tuberculosis* (*Mtb*) that causes infection in the lungs[1]. Despite the fact that the causative organism was discovered 100 years ago and drugs against this agent are already available, TB remains to be a worldwide health problem[2]. It is considered by the WHO as one of the leading causes of death, affecting approximately 8.7 million people in 2011 alone, 1.4 million of which have died[3]. Management of TB is usually done using antibacterial drugs like isoniazid and rifampicin[4]. However, the emergence of multi-drug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) strains have now made these drugs less effective in killing the mycobacterium[5]. Thus, there is great need to develop novel anti-TB drugs.

One recently validated drug target for *Mtb* is the enzyme Maltosyltransferase GlgE (Rv1327c)[6].

It is part of a previously unrecognized four step α-glucan pathway that converts the disaccharide trehalose into α-glucan polysaccharide which is a component of the extracellular α-glucan capsule of *Mtb,* important for its virulence and persistence.By employing traditional and chemical reverse genetics, GlgE inactivation caused rapid death of *Mtb in vitro* in mice by two mechanisms. The first is called "self-poisoning"; in which accumulation of the substrate ofMaltosyltransferaseGlgE, particularly maltose-1-phosphate (M1P), proved fatal to the bacterium because it elicits pleiotropic stresses which includes inhibition of respiration, induction of stringent response and DNA damage. Interestingly, *Mtb* responds to the stress by generating more trehalose, the M1P precursor, in a misguided stress protection response that further fuels the accumulation of M1P, worsening the case scenario.The second but independent death mechanism is based on the fact that the GlgE pathway works side-by-side with the Rv3032 pathway. As long as the redundant Rv3032 is functioning, the GlgE pathway could be responsible as a whole for the viability and virulence of *Mtb*. However, simultaneousinhibition of both pathways is lethal. This implies thatwhilemonotherapy with GlgE inhibitors is sufficient to kill *Mtb* as effective as first-line drugs like isoniazid, it could be further improved by combination therapy with Rv3032 inhibitors and could also avoid the possibility of development of resistance.This makes MaltosyltransferaseGlgE a promising anti-TB drug target. Furthermore, it is absent in humans, but is present in almost all mycobacteria and other opportunistic pathogens like *Pseudomonas* and *Burkholderia* species. The structure of its homologue from *Streptomyces coelicolor* was released only in 2011 and was reported to have similar catalytic properties with *Mtb*GlgEtogether with a conserved active site[7]. With an available structure of the drug target, structure-based drug design may now be employed.

In this work, structure-based screening and *in silico* optimization[8] was carried out to search for potential inhibitors of *Mtb*GlgE.Specifically, we sought natural products that possess significant structural and chemical complementarities to the target receptor[9]. Accordingly, a pharmacophore[10] based on the binding site in GlgE wasgenerated and used to screen several compounds databases of natural products. The top hits were docked to the drug target and were rank-ordered based on their

binding energy. The high-binding compounds were further optimized structurally. Lastly, the variants of the leads were evaluated for their potential toxicity properties.

MATERIALS AND METHODS

All computational procedures were done on Accelrys Discovery Studio (DS) Client v2.5.0.9164 installed on a computer running on Microsoft® Windows 7 Home Premium 64-bit Operating System using a processor of Intel® Core™ i3 CPU @ 2.27 GHz and installed memory (RAM) of 4.00 GB.

Retrieval of MaltosyltransferaseGlgE structure and compound libraries

Because the structure of Maltosyltransferase Glge from *Mtb*is not yet available, the crystal data of its closest homologue from another actinomycete, *Streptomyces coelicolor*, was used in this study. The 2.09 angstroms resolution 3D structure of MaltosyltransferaseGlgE Isoform I in complex with the ligand maltose (PDB code: 3ZT5) was retrieved from the RCSB Protein Data Bank.Only chain A among the four identical subunits (A, B, C and D)of the protein was retained. The protein was cleaned, prepared and minimized using the *Clean Protein* tool,*Prepare Protein*and*Minimization* protocol. To determine the extent of modification that occurred on the protein due to the application of the said protocols, the RMSD value was computed using the*Align and Superimpose Proteins* protocol.

Natural products catalogs were obtained from ZINC database as SDF files (http://zinc.docking.org/browse/catalogs/natural-products), namely; Ambinter Natural Products, AnalytiCon Discovery NP, IBScreen NP,Indofine Natural Products, Molecular Diversity Preservation International, Nubbe Natural Products, Princeton NP, SelleckBiochemicals NP, Specs Natural Products, TCM Database @ Taiwan and UEFS Natural Products. In addition to these, other compound libraries, particularly "MEGx: Pure Natural Products" and "NATx: Semi-Synthetic Screening Compounds based on Natural Product Scaffolds" were obtained from AnalytiCon Discovery (http://www.ac-discovery.com/content/Products&Technologies) while Otava Drug-like Green Collection was retrieved from Otava Chemicals (http://www.otavachemicals.com/download-compound-libraries/doc_details/5-

drug-like-green-collection-sdf). Lastly, the ligand maltose was obtained as SDF from the PDB. The compounds were then preparedusing the *Prepare Ligands* protocol and built into separate databases using the *Build 3D Database* protocol.

Structure-based Pharmacophore Generation

The bound maltose was used to locate the active site of the enzyme. A site sphere was generated on the active site using the *Define Sphere from Selection* tool. Site sphere optimization was done by adjusting the size of the sphere until the docked pose is as close as possible to the pose of the original crystallized structure. Afterwards, maltose was deleted and the protein was defined as the receptor using the *Define Selected Molecule as Receptor* tool.The active site was then analyzed for donor, acceptor, and hydrophobic features by generating a pharmacophore using the *Interaction Generation* protocol. The initial pharmacophore features were further trimmed using the *Cluster Current Feature* and *Keep Only Cluster Centers* tools for all acceptor, donor, and hydrophobe features.

Virtual screening and molecular docking

The abovementioned databases of molecules were then screened using the pharmacophore generated earlier by running the *Screen Library* protocol using rigid fitting method. Compounds with fit value greater than or equal to 3.0 were subjected to second screening, this time using the flexible fitting method. From here, compounds with fit value greater than or equal to 4.0 were subjected to molecular docking. Maltose was rescreened regardless of the fit value.

Molecular docking was done using the*Dock Ligands (CDOCKER)* protocol.The poses generated were evaluated by calculating their binding energies using the *Calculate Binding Energies* protocolwith *In Situ Ligand Minimization* and then ranked accordingly. Moreover, visual inspection of the docked poses was also conducted by generating ligand interaction diagrams. From this, the lead compounds were identified.

Chemical Modification (De Novo) of the Lead Compounds

The structures of the chosen lead compounds were modified using the *De Novo Evolution* protocol, *Full Evolution mode*. For this, the highest ranking pose of the lead compound was used as the ligand scaffold. The fragment library used was the built-in small fragment library, organic.str.The binding energies of the generated modified compounds werecalculated and ranked accordingly, while ligand interaction diagrams were also generated and further analyzed for molecular interactions.
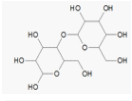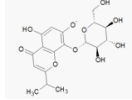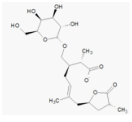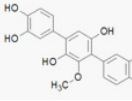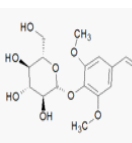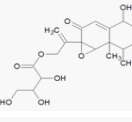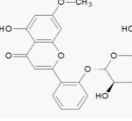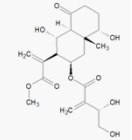
Toxicity Prediction of Modified Lead Compounds
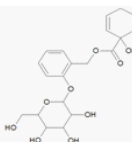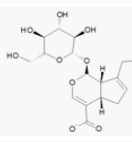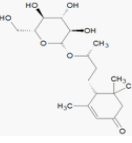
The modified lead compounds were further tested for properties like carcinogenicity, mutagenicity and biodegradability using the *Toxicity Prediction (Extensible)* protocol.

RESULTS AND DISCUSSION

The structure of Maltosyltransferase GlgE(PDB code: 3ZT5) was first subjected to the *Prepare Protein* protocol to address certain issues[11] like missing atoms, missing loop regions, etc. in order to standardize the protein for subsequent computational processes. *Minimization* was done to minimize the energy of the protein through geometry optimization. These protocols may bring about conformational changes; thus it is made sure that the extent of modification that occurred is well within the allowable range. In docking simulations, the calculated RMSD between the main chain atoms must be below 1.5 Å[12]. In this study, the computed RMSD was 0.721 Å (Fig. 1) which is well within the acceptable range, thus validating the prepared protein.On the other hand, the compound databases were subjected to the *Prepare Ligands* protocol to generate reasonable starting ligand structures and in order to apply the Lipinski filter. The Lipinski filter is a collection of criteria (H-bond donors < 5, H-bond acceptor < 10, Molecular Mass < 500, log P < 5) that are commonly observed in drug-like candidates[13]. Subsequently, the prepared ligands were built into separate 3D databases.

To create a pharmacophore, the active site of the protein was first identified using the bound maltose. A site spherewhose radius is 8.0 Åand has x, y and z coordinates of 2.855, -31.798, 2.869, respectively, was generated. It was made sure that it encompasses all the amino acids in the active site (Fig. 2). The RMSD between the original and docked poses of maltose (Fig. 3) was 0.7755 Å,
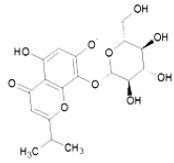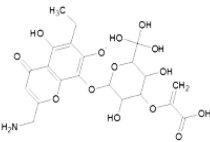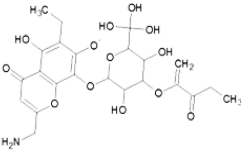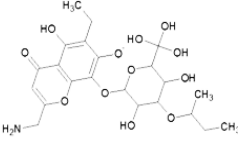
Table 1: Summary of the Properties of Maltose and the Top 10 Hits

Compound ID	Structure	Binding Energy (kcal/mol)	Fit Value (Rigid)	Fit Value	Database Source
Maltose		-414.22924	3.05	4.15	RCSB Protein Data Bank
ZINC39010596		-309.58203	3.14	4.60	TCM Database @ Taiwan
ZINC31163371		-308.17681	3.23	4.43	AnalytiCon Discovery NP
ZINC31166471		-300.72453	3.64	4.18	Ambinter Natural Products
Compound623		-296.90711	3.44	4.12	MEGx: Pure Natural Products
ZINC04102166		-275.95849	3.22	4.15	Nubbe Natural Products
ZINC31155668		-273.75658	3.34	4.10	AnalytiCon Discovery NP
ZINC13515708		-268.60091	3.28	4.00	Nubbe Natural Products
Compound125		-265.03806	4.07	4.00	MEGx: Pure Natural Products
ZINC08382359		-263.19436	3.69	4.21	Specs Natural Products
ZINC31170351		-261.73699	3.99	4.64	AnalytiCon Discovery NP

Table 2: Actual number of polar, van der Waals, H-bonds, charged and pi-pi or pi-cation interactions of each of the top ten hits with only the amino acid residues in the *active site* of protein which comprises the following: Lys264, Asn268, Val279, Trp281, Ala282, Gln324, Tyr357, Asp359, Arg392, Asp394, Asn395, Glu423, Asp480, Lys534 and Tyr535

ID	Polar	van der Waals	H-bonds	Charged	Pi
Maltose (native)	14	1	11	0	0
Maltose (docked)	13	2	12	0	0
ZINC39010596	10	3	7	0	3
ZINC31163371	12	1	4	1	0
ZINC31166471	9	5	3	1	3
Compound623	8	6	7	0	1
ZINC04102166	10	2	6	1	0
ZINC31155668	8	4	3	0	0
ZINC13515708	10	5	3	0	1
Compound125	10	3	3	0	0
ZINC08382359	10	5	5	0	3
ZINC31170351	11	3	3	0	0

Table 3: Structures of ZINC39010596 and the top 3 modified ligands

Compound ID	Structure	Binding Energy (kcal/mol)
ZINC39010596		-309.58
Ligand 1		-426.00
Ligand 2		-424.65
Ligand 3		-414.68

which is still within the acceptable range[14]. Initially, thepharmacophore has a total of 273 features but was further trimmed down to 19 features by clustering the features and keeping cluster centers. It has 6 acceptor (green), 7 donor (violet) and 6 hydrophobe features (light blue).

Virtual screening allows the rapid selection of a subset of compounds predicted to have significant interactions with the given target out of a large database of molecules. In this study, the use of a pharmacophore was incorporated into virtual screening protocol via pre-screening – a method proven to have the advantage of reducing the size of the database to be docked because docking is the more time-consuming process compared to virtual screening[15]. Pharmacophore-based virtual screening in DS has been documented to give successful hits

in past studies[16,17]. Virtual screening in DS is done by the *Screen Library* protocol which has two options for fitting the ligands in the receptor, rigid fitting and flexible fitting, both of which were done in this study with the best hits of the rigid fitting being subjected to the flexible fitting method. The minimum fit value for the compounds to pass the rigid screening test was arbitrarily set to 3.0 while the minimum fit value for the flexible screening was set to 4.0 in order to trim down the number of compounds to be docked to the receptor molecule (Fig.4).

The top hits from virtual screening were then subjected to molecular docking using the *CDOCKER* protocol which is a CHARMm based docking tool that generates random ligand poses and places them in a rigid receptor. *In situ ligand minimization* was done during the calculation of the
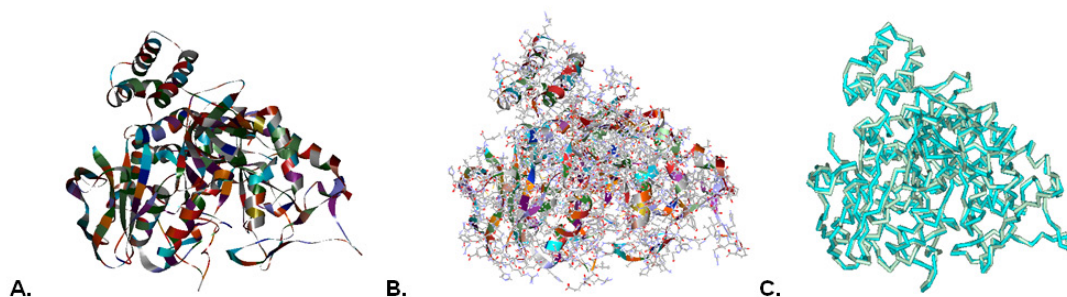
Fig. 1: (A) Original structure of MaltosyltransferaseGlgE, (B) Cleaned, prepared and minimized structure, (C) Superimposition of the original and prepared protein structures (RMSD = 0.721 Å)
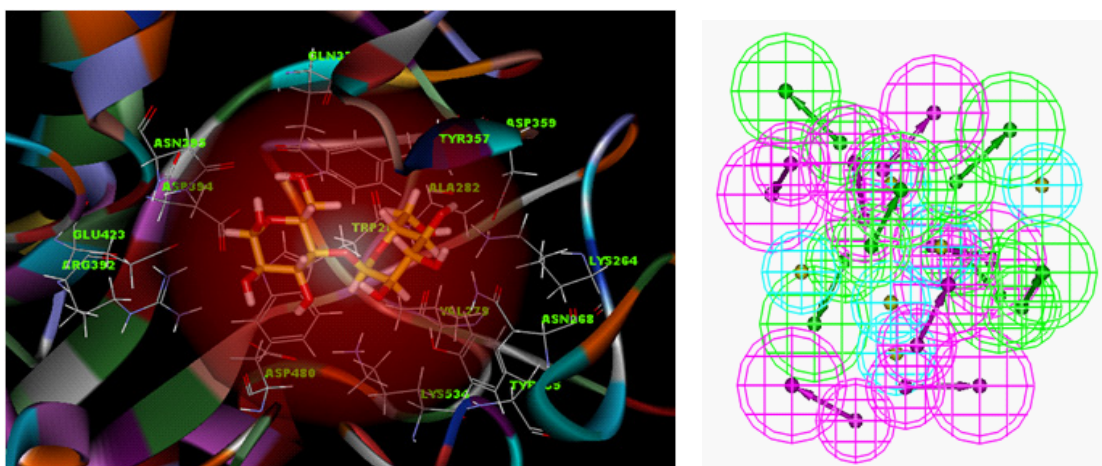

Fig. 2: (A) The generated site sphere based on the binding site of maltose in the protein showing all the amino acids in the active site, (B) The clustered pharmacophore composed of 19 features: 6 acceptor (green), 7 donor (violet) and 6 hydrophobe (light blue) features

binding energy (BE) to remove any ligand van der Waals clashes prior to BE calculations. The docking results showed that the magnitude of binding energy of the top 10 hits were still lower compared to that of the original bound ligand maltose whose BE is -414.23 kJ/mol (Table 1). Further analysis of the molecular interactions involved in the binding of the compounds with the receptor showed that maltose has the highest number of H-bonds involved (Table 2). However, it is observed that it has a much lower number of van der Waals interaction compared to the other compounds. The top hit ZINC39010596 also has a high number of H-bonds with an additional pi-pi and pi-cation interactions with Tyr357 and Arg392, respectively. Yet, these interactions proved to be insufficient to surpass the high binding energy of maltose.

According to Syson et al.[7], the amino acid residues in the active site of the protein include the following: Lys264, Asn268, Val279, Trp281, Ala282, Gln324, Tyr357, Asp359, Arg392, Asp394, Asn395, Glu423, Asp480, Lys534 and Tyr535. Thus, it is essential for the lead compound/s to have significant interactions with these residues. However, only two of the top hits have interactions with all the residues, namely ZINC13515708 and ZINC08382359, which are 7th and 9th in the rank respectively. The top hit ZINC39010596 lacked interaction with Ala282. Maltose, on the other hand,
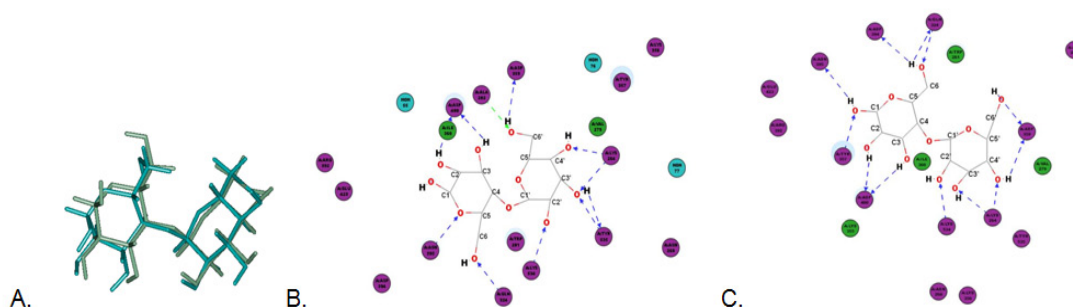
Fig. 3: (A) Superimposition of maltose in the original protein and the docked maltose in the prepared protein (RMSD = 0.7755 Å), (B) Ligand interaction diagram of maltose in the original protein; polar (magenta circles), van der Waals (green circles), H-bond with a. acid side chain (blue arrow), H-bond with a. acid main chain (green arrow), water molecules (aqua blue circles), (C) Ligand Interaction Diagram of the docked maltose in the prepared and minimized protein
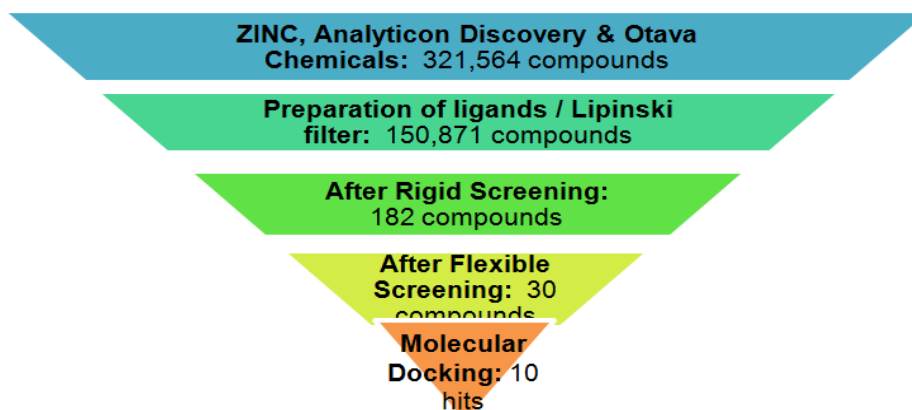

Fig. 4: Summary of the virtual screening process used in this study. The number of compounds was trimmed dramatically from the original database through the application of different filters and screening processes

demonstrated interactions with all the residues. It is alsointeresting to note that the following compounds ZINC39010596, ZINC31163371, Compound623, ZINC04102166, ZINC31155668, ZINC13515708 and ZINC08382359 have a common structural feature which is a glucopyranose unit similar to that of maltose. Besides, most of the top hitshave a high number of hydroxyl units, just like maltose, which may be the reason why these compounds have comparable binding energies.The lead compound ZINC39010596 or 5,7-dihydroxy-2-propan-2-yl-8-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl]oxychromen-4-one is a glycosylated flavone derivative wherein the phenyl ring was substituted by an isopropyl group (Fig. 5).

In our desire to search for compounds with higher binding energies than that of maltose, modification of the lead compounds was further employed. The top three lead compounds, ZINC39010596, ZINC31163371 and ZINC31166471 were the ones modified using the *De Novo Evolution* protocol. In the *De Novo Evolution* protocol of DS2.5, modifications (i.e. addition of small fragments) are made to an existing ligand scaffold to maximize binding. Ludi scoring was used by the protocol to rank the compounds. Approximately sixty modified molecules were generated by the *De Novo Evolution* protocol for all three. To trim this down, it was intuitively decided that only the compounds with binding energies higher than -400 kcal/mol were considered. We noticed that some of the modified compounds have high molecular weight, even greater than 500 g/mol, thus violating the Lipinski rule of five. However, recent studies showed that there are actually some good drug candidates that violate this rule[18].
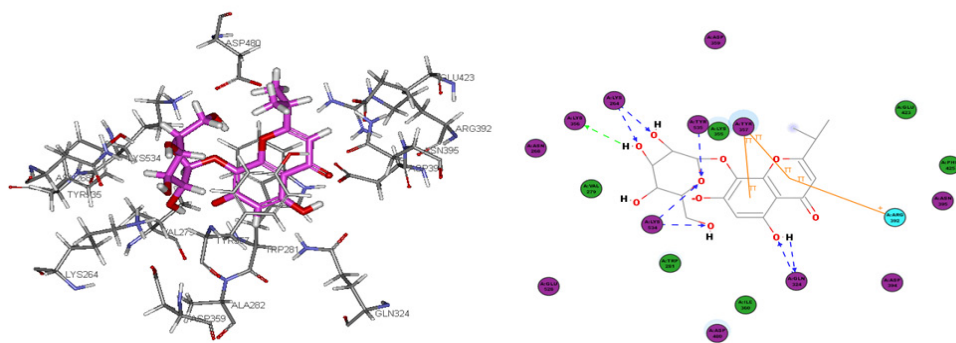
Fig. 5: 3D representation and 2D ligand interaction diagram of ZINC39010596; polar (magenta circles), van der Waals (green circles), H-bond with a. acid side chain (blue arrow), H-bond with a. acid main chain (green arrow), pi-pi interactions (orange lines)

We were delighted to find three compounds that have more negative binding energies than the intermediate maltose (BE = -414.23 kcal/mol), all of which originated from ZINC39010596. It is observed that there are similar interactions that occur for the top three modified ligands 1, 2 and 3. All have pi-pi interactions with Trp281, H-bonds with Lys264, Gln324, Arg392, Asp394, Glu423, Asp480, Tyr535 and charged interaction with Tyr535. However, there are still some noticeable differences like for instance; Ligand 1 has an additional H-bond with Lys534 but lacked visible interaction with Ala282. Nonetheless, the similarity in interactions was expected because there are actually only minor differences in the structures of these compounds (Table 3).

Furthermore, it is also important to evaluate *a priori* the pharmacodynamics (e.g. toxicity, degradability, etc.) properties of the molecule. Thus, properties like carcinogenicity and biodegradability were predicted using the *Toxicity Prediction (Extensible)* protocol of DS2.5. Generally, it is important for a drug to be noncarcinogenic and nonmutagenic. Overall, the calculations showed that the top three ligands are noncarcinogenici and nonmutagenic and are also biodegradable, albeitthey may be irritant to skin and the eyes.

CONCLUSION

Pharmacophore generation, virtual screening, molecular docking (CDOCKER) and *de novo* lead modification were employed in this study for the search of possible inhibitors of MaltosyltransferaseGlgE, a recently validated anti-TB target. Three compounds that have relatively higher binding energies than the intermediate maltose were identified, all of which were derived from ZINC39010596. Toxicity predictions of these compounds also revealed that they are non-carcinogenic, non-mutagenic and biodegradable,

although irritant to the skin and eyes. Experimental bioactivity measurements of potential anti-TB compoundssuch asGlgE inhibitors are underway in our group.

ACKNOWLEDGMENTS

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