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COMPUTER-AIDED DESIGN OF DIPHENYLETHERS AS EFFECTIVE ANTI – *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Tuberculosis (TB) is the second cause of death from an infectious disease in adults worldwide. However many antimycobacterials have been discovered up to date, but their success is hampered by *Mycobacterium tuberculosis* resistance. Computer-aided drug design is considered as one of the most efficient approaches that accelerate the process of drug discovery. Therefore, the aim of this *in silico* study is to evaluate antimycobacterial activity of diphenylether derivatives using computer aided drug design tools (Tripos Sybyl-X, and MOE packages). Enoyl Acyl carrier protein reductase enzyme (InhA) was selected as drug target, because of its vital role in type II fatty acid biosynthesis. 3D crystal structure of InhA (PDB 2X23) was obtained from protein data bank and prepared for docking. Twenty three diphenylether derivatives and known InhA inhibitor, PT70, were docked into the active site of the InhA enzyme. The obtained data (scores) were converted to free binding energy using special equation. The visualization within the active site showed the hydrogen bonding, π–π and van der Waals' interaction interactions between the enzyme and the tested ligands. The results of this study revealed that, among the docked compounds, compounds a1 and a19 were exhibited the lowest binding energies (high affinities) with values of -17.85 kcal/mol and-17.00 kcal/mole respectively, whereas the known inhibitor, PT70, showed-16.33 kcal/mol. It is observed that the length of the side chain R1 attached to ring A and type of side chain R2 and R3 attached to ring B played a vital role on the activity of the compounds.

Further *in vitro* studies await to be conducted in order to corroborate antimycobacterials activity of diphenylether derivatives.

KEYWORDS: Computer-Aided Design, *in silico*, Diphenylethers, InhA, Tuberculosis, *Mycobacterium Tuberculosis*.

INTRODUCTION

Tuberculosis (TB) is a disease caused by the bacteria *Mycobacterium tuberculosis*. It most commonly affects the lungs, as well as it can be disseminated all over the body.[1]

The transmission of TB is airborne by the carrier person. The bacteria are contained in small, airborne droplets created by coughing or sneezing.[2]

Tuberculosis (TB) is second only to the human immunodeficiency virus (HIV) as the greatest killer in the world. The Global Tuberculosis Report 2014 estimates that there were 9.0 million new TB cases in 2013 and 1.5 million TB deaths.[3]

In Sudan, the estimated incidence was 50,000 cases in 2009, where the estimated prevalence was 209 cases per 100,000 of the population.[4]

Isoniazid INH is a pro-drug, requiring oxidative activation by the *M. tuberculosis* catalase-peroxidase enzyme KatG.[5] After enzymatic oxidation, the drug binds tightly to the enzyme enoyl acyl carrier protein (ACP) reductase, InhA.[6] The occurrence of resistance to INH is more frequently than resistance to any other drugs due to the wide use of this drug.[7] In a study, among the INH-resistant clinical isolates, 50–80% of cases were due to mutations in the katGgene, leading to decrease of activation of INH.[8]

Target discovery is an important early step in the drug discovery process. Three-dimensional structural information derived from x-ray crystallogarphy of enzyme-inhibitor complexes is applied for the design of new types of inhibitors.[9]

Therefore, this study was conducted to address the following objective:

To use computer aided drug design tools to construct a slow onset and direct inhibitors of *Mycobacterium tuberculosis* enoyl-ACP reductase (InhA), which is an enzyme involved in the type II fatty acid biosynthesis process.

MATERIALS AND METHODS

Computational Details

The computational studies were carried out on a personal computer with 2.2 GHz Intel Quad Core processor and 4GB (RAM).Video projector, operating under the Windows 7(64 bit). Structure construction, optimization and visualization were carried out using the molecular modeling packages SYBYL-X suite of programs (v. 1.1, Tripos Inc., USA) and Molecular Operating Environment (MOE) (v.2008.10, Chemical computing group).

Preparation of Receptor Enzyme

The selected enzyme is Mycobacterium enoyl-ACP reductase whose crystal structures are available online and one of them has PDB Code 2X23.[10] The structure was downloaded from protein data bank (http://www.rcsb.org/). The enzyme plays important role in Mycolic acid biosynthesis. The selected 3D structure of enzyme was having slow and tight inhibitor 2-(o-Tolyloxy)-5-hexylphenol (PT70). The enzyme have four identical subunits, therefore, chain A was used.

The 3-D structure of enzyme is generally not complete. It needs extensive checking for the missing bonds and atoms. Using the computer based SYBYL protein preparation tools; all water molecules were removed, both polar and nonpolar hydrogen atoms were added, side chains were repaired, chain termini were fixed, protonation types were set for the reorientation of the hydrogen atoms which would be more favorable to hydrogen bonding. Atomic charges were assigned to the receptor using MMFF99 force field.[11] and to the ligand. The protein complex was minimized using AMBER7 FF99 force field. Finally the 3D structure of the prepared protein was saved as PDB file.

Selection of Binding Site

The protomol is a representation of the receptor's binding cavity in which putative ligands are aligned.[12] Protomols can be produced by one of four routes: automatic: Surflex-Dock finds the largest cavity in the receptor protein; ligand-based: by a ligand in the same coordinate space as the receptor; residue-based: by specified residues in the receptor; multi-channel surface: finds multiple cavities and select one use.

The complexed ligand from the crystal structure was used to construct the protomol, which was then stored as MOL2 file. Ligands were docked directly from MOL2 files.

Small molecules preparation

Library of compounds belongs to Diphenylether class which were designed using builder module of SYBYL. The ligand geometries were optimized with the Powel method.[13] using the MMFF94 and MMFF94 charges for all atoms, until a gradient 0.05 kcal/mol/A was reached.

The derivatives of diphenyl ethers were docked with InhA, (Figure 1 and table 1) showing the main framework of these compounds.

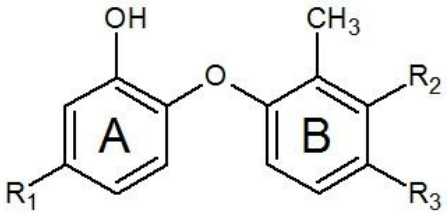

FIGURE 1: The main framework of the compounds used in the docking study.

TABLE1: Structure of diphenyl ether derivatives.

Compound	R_1	R_2	R_3
a1	Octyl	H	H
a2	Octyl	H	Cl
a3	Octyl	H	NH_2
a4	Heptyl	H	Cl
a5	Heptyl	H	NH_2
a6	Hexyl	H	Cl
a7	Hexyl	H	NH_2
a8	Hexyl	Cl	H
a9	Hexyl	NH_2	H
a10	Hexyl	Cl	Cl
a11	Octyl	Cl	Cl
a12	Heptyl	Cl	Cl
a13	Hexyl	H	Br
a14	Hexyl	Br	H
a15	Hexyl	H	OH
a16	Hexyl	OH	H
a17	Hexyl	CH_3	H
a18	Hexyl	H	CH_3
a19	Hexyl	OH	OH
a20	Hexyl	Br	Br
a21	Hexyl	H	NO_2
a22	Hexyl	NO_2	NO_2
a23	Hexyl	NO_2	H
PT70	Hexyl	H	H

MOLECULAR DOCKING PROCEDURE

Ligand docking was performed with the Surflex-Dock software of SYBYL package using the procedure reported by A. Jain.[14] CScore (Consensus Score).[15] module was implemented to calculate and rank the docking scores for the resulting docking conformations.

CScore integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of the receptor. The strengths of individual scoring functions combine to produce a consensus that is more accurate than any single function for evaluating ligand-receptor. CScore was automatically computed from the six scores (0, 1, 2, 3, 4, and 5); the best CScore is five. Structures with scores of 3 or 4 merit further consideration. Structures with a CScore of 0 are consistently considered bad by all scoring functions and should be dropped.

Surflex-Dock's initial implementation used the empirical scoring function Hammerhead procedure.[16] to screen for the binding of flexible molecules to a protein binding site. Input to Surflex-Dock consists of the 3D structure of a receptor protein with hydrogens and binding site empty of co-crystallized ligand. The protomol, a set of probes (CH4, N-H, C=O) complementary to the active site, with 3D ligands, with proper atom types, and hydrogens, beside, in any arbitrary optimized conformation.

Surflex-Dock was allowed to produce up to 20 different docking conformations for each ligand. All docking conformations in the receptor were then relaxed and ranked with the CScore, and the conformations with best scores were checked visually. Docking results were analyzed in details by visual inspection and by measuring geometrical parameters defining the particular ligand-receptor.

RESULTS AND DISCUSSION

The target of this study was to design potent antitubercular agents which can interact efficiently with the structurally well-defined target, the *Mycobacteium tuberculosis* enoyl-acyl carrier protein reductase. During the study; the enzyme active site was investigated to understand its steric and electrostatic features. Docking study has been conducted for different derivatives of diphenylether skeleton to have an insight into the possible binding interactions between the compounds and the residues constructing the binding site.

Triclosan inhibits InhA directly. Although it is a relatively weak inhibitor of InhA, many attempts has been done to improve its affinity toward InhA.[17] In addition, the INH-NAD adduct was shown to be a slow onset inhibitor of InhA. It was reported that slow onset inhibition is linked to ordering of an active site loop (residues 195–210 in InhA), which leads to a closure of the substrate-binding pocket.[18]

To design a slow onset diphenyl ether, it hypothesized that there must be an entropic penalty for loop ordering. Introduction of a methyl group ortho to the diphenyl ether linkage resulted in a compound, PT70 that is a slow onset inhibitor of InhA. The crystal structure of PT70 bound to InhA showed that slow onset inhibition is linked to ordering of the substrate-binding loop.[10]

InhA is a NADH dependent enzyme; therefore much care should be taken during enzyme preparation regarding the co-factor geometry and atoms types. In some rare reports it has been mentioned that docking could be performed in presence or absence of the co-factor.however in the present study, the co-factor was left in the active site during docking process.[19]

Docking study which was performed for a database of 24 compounds resulted in an interesting group of compounds with considerable interactions.

Validating the Docking Methodology

The Surflex-Dock methodology was first tested on known inhibitor PT70 (2-(o-Tolyloxy)-5-hexylphenol), which was docked with the the active site of InhA. Results revealed great consistency with the reported interactions, regarding hydrogen bonding and hydrophobic interactions.[10]

The 3D and 2D visualization of the results (figure 2 A and 2B) were carried out using MOE and the Lig-X module of the MOE package. Docking results of PT70 have revealed two hydrogen bonds; both are extended from the phenolic oxygen of the compound, which played the role of acceptor atom.

The two donor atoms are the hydrogen of the OH of TYR158 at a distance of 2.45Å, and the hydrogen of the 2′-hydroxyl moiety of the nicotinamide ribose of the NAD1270 which is 2.55Å apart. The phenolic benzene ring is forming π-π overlapping with the nicotinamide ring of NAD1270.

Molecular Modeling of Diphenylethers

In the current study 24 compounds were carefully designed. In attempt to model new diphenylethers, compounds were manually built and different substituents were attached to the different positions of the two benzene rings. The possible substitution patterns over the diphenylethers, substituents were represented by R1, R2 and R3 which could cover a range of different include: -CH3, Hexayl, Heptyl, Octayl-OH, -NO2, NH2,-Cl,-Br (Figure 1).

All the studied inhibitors were docked into the binding site of InhA and the energy scores of the inhibitors are also shown in Table 2.

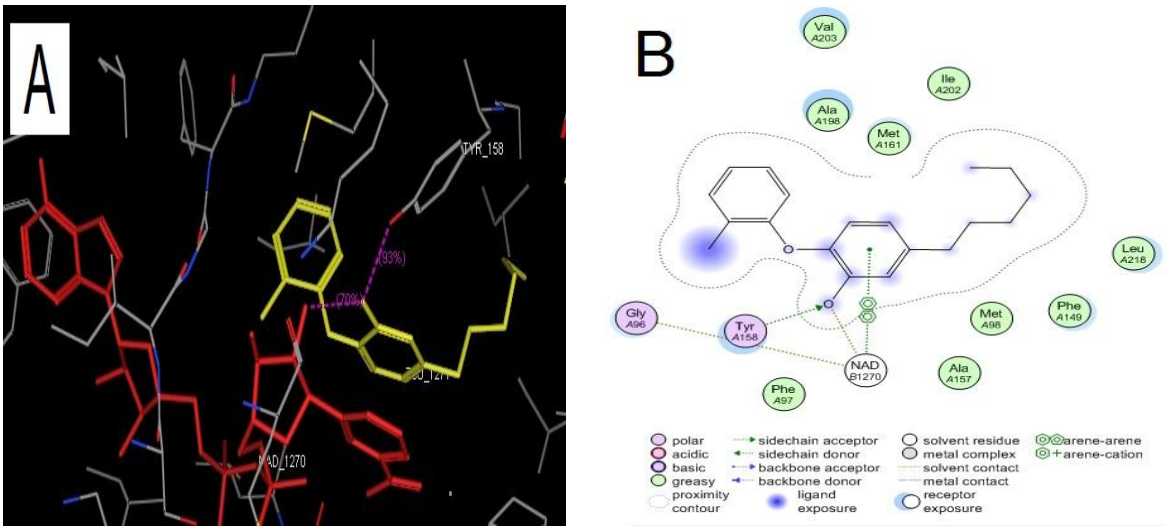

FIGURE 2: Visualization of PT7 within InhA active site. (A) 3D picture of binding interactions of PT70 in the active site (PT70 is yellow and NAD is red). (B) 2D view of the active site with explanatory key. All are generated by MOE.

Surflex-dock predicts the binding affinities of the ligand-protein complex in the form of $-\log(K_d)$ referred as total score.Therefore, scoring values were converted into free energy of binding values using the following equation: $kcal/mol=0.59\ln(10^{-pkd})$ (Holt et al., 2010), the resulted Surflex docking scores have shown a range of values between 13.14-6.69 corresponding to binding energy range of -17.85-(-9.09) kcal/mol (table 2). The small range between the maximum and the minimum scores, indicates that the binding interactions of these compounds is somewhat similar(figure 3).

The total score of compounds **a1 and a19** were higher than that of the PT70 and were exhibited the lowest binding energies (high affinities) with values of -17.85 kcal/mol and-17.00 kcal/mole respectively, whereas the known inhibitor, PT70, showed-16.33 kcal/mol (table 2). Crash score revealed that inappropriate penetration into the binding site was in

favor of compounds PT70 and **a1** in the series, followed by a15, a23, a9, a7, a8, a3, a14, a5, a16, a2, a4, a6, a18, a17, a12, a19 and other compounds. Charge and van der Waals interactions between the protein and the ligand suggested that a19, a22, a21, a17, a23, a20, a18, a13, a14, a11 and a12 were the superior ligands than PT70 to bind with InhA followed by a15, a16, a2, a3, a4, a6, a5, a1, a10, a8, a7 and a9. Helmholtz free energies of interactions for protein–ligand atom pairs prefer all the compounds over those of PT70. All the compounds showed better hydrogen bonding, complex (ligand–protein), and internal (ligand–ligand) energies than PT70. Scoring of compounds with respect to the reward for hydrogen bonding, lipophilic contact, and rotational entropy, along with intercept terms revealed that all the compounds showed increased interactions with the protein than PT70. The C-score (Consensus score) indicated the summary of all the forces interacted between the ligands and InhA. Compounds a12, a13, a14, a15, a1, a6, a5, a19, a21, a11, a10 a8, a3 and PT70 showed best CScore of five.

TABLE 2: Surflex-Dock scores (kcal/mol) and the corresponding binding energies of diphenylethers derivatives.

	Total Score[a]	Free Binding Energy	Crash[b]	Polar[c]	D-SCORE[d]	PMF_SCORE[e]	G_CORE[f]	CHEM-SCORE[g]	C-SCORE[h]
a1	13.14	-17.85	-0.73	1.03	-180.2362	-63.5433	-337.3717	-47.5337	5
a19	12.51	-17.00	-3.71	1.05	-1321.4130	-44.1820	-365.1193	-47.5199	5
PT 70	12.02	-16.33	-0.50	0.00	-380.2705	0.0000	-173.5397	-5.4798	5
a3	11.59	-15.75	-2.12	1.15	-185.9497	-59.7770	-369.0004	-45.2118	5
a5	11.24	-15.27	-2.34	1.05	-180.7259	-53.4001	-352.8474	-44.5918	5
a9	11.08	-15.05	-1.54	1.02	-160.8591	-53.7011	-319.9391	-41.0915	2
a15	11.03	-14.98	-1.26	1.11	-349.2238	-54.7807	-314.5496	-41.4249	5
a7	10.89	-14.79	-1.72	1.11	-170.3677	-55.2966	-312.8334	-41.5096	4
a18	10.51	-14.28	-3.31	1.06	-521.4943	-27.4057	-347.1778	-46.1603	3
a2	10.43	-14.17	-2.66	1.13	-194.5092	-58.7810	-363.8615	-49.1404	4
a4	10.28	-13.97	-2.71	1.13	-185.8408	-53.8663	-333.3535	-47.1570	2
a23	10.21	-13.87	-1.47	0.62	-708.4664	-28.5515	-318.7924	-36.9922	2
a16	10.07	-13.68	-2.45	1.02	-333.9415	-60.9966	-303.5453	-42.3846	4
a14	9.68	-13.15	-2.18	0.89	-386.7067	-44.8289	-307.5982	-43.6599	5
a17	9.61	-13.06	-3.44	0.00	-712.4984	-28.9308	-342.3371	-43.7400	2
a6	9.54	-12.96	-2.84	1.13	-180.8160	-52.0621	-319.9754	-45.6930	5
a8	9.41	-12.78	-1.89	0.98	-171.2764	-53.1005	-300.5019	-44.3313	5
a12	8.09	-10.99	-3.68	0.01	-381.4765	-56.4628	-356.4370	-46.3973	5
a13	8.08	-10.98	-4.56	1.15	-391.4439	-62.1988	-320.3024	-47.2957	5
a11	7.77	-10.56	-4.08	0.00	-383.1625	-45.2524	-347.6905	-46.9279	5
a22	7.76	-10.54	-5.03	1.04	-1142.2776	-75.6326	-328.2200	-50.7967	3
a20	7.72	-10.49	-4.34	0.92	-588.9266	-21.4593	-334.2676	-40.7454	3
a10	7.20	-9.78	-4.56	1.14	-178.0889	-51.7161	-304.6161	-46.4564	5

TABLE 2: Surflex-Dock scores (kcal/mol) and the corresponding binding energies of diphenylethers derivatives (continue).

	Total Score[a]	Free Binding Energy	Crash[b]	Polar[c]	D-SCORE[d]	PMF_SCORE[e]	G_SCORE[f]	CHEM-SCORE[g]	C-SCORE[h]
a21	6.69	-9.09	-4.89	1.35	-1086.0759	-28.1039	-294.3200	-39.5360	5

[a]Total Score expressed as -log(Kd) to represent binding affinities. The total score includes the Crash score.

[b]Crash score revealing the inappropriate penetration into the binding site. Crash scores close to 0 are favorable. Negative numbers indicate penetration.

[c]Polar indicating the contribution of the polar non-hydrogen bonding interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds.

[d]D score for charge and van der Waals interactions between the protein and the ligand.[20]

[e]PMF score indicating the Helmholtz free energies of interactions for protein–ligand atom pairs (Potential of Mean Force, PMF).[21]

[f]G score showing hydrogen bonding, complex (ligand–protein), and internal (ligand–ligand) energies.[22]

[g]Chem score points for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept term.[23]

[h]C Score (Consensus Score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor. CSCORE computed from Total Score, ChemScore, G-Score, D-Score, and PMF-Score.

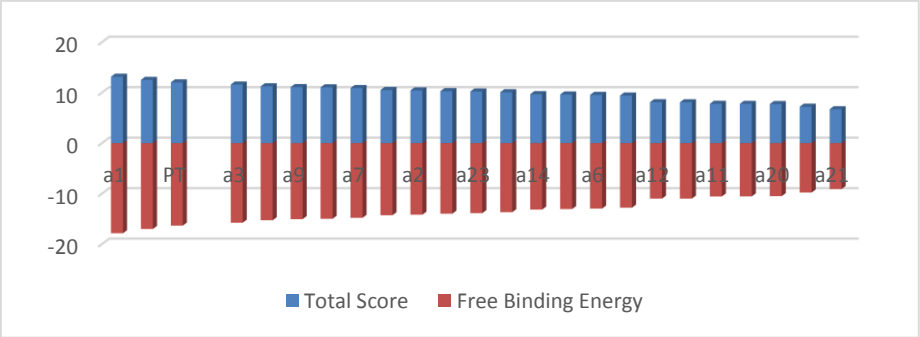

FIGURE 3: Surflex-Dock total scores and the corresponding binding energies (kcal/mol) of diphenylethers derivatives.

Diphenylether a1

This compound has been selected from the docked diphenylethers, on basis of scoring and binding interaction. It is observed that the octayl side chain attached to ring (A) increase its binding affinity compare to PT70 with hexayl side chain. The docking score of this

compound was found to be 13.14 which corresponding to -17.85kcal/mol whereas the PT70 showed 12.02 which corresponding to -16.33 kcal/mol with similar Cscore value of 5. The docking interactions of this compound were similar to those of PT70 (figure 4). As the hydroxyl group of the compound is interacting with the enzyme via two hydrogen bonds, one with the OH of Tyr158 (2.74Å) and the other with 2′-hydroxyl of the nicotinamide ribose of the NAD1270 (2.70Å). Benzene ring (A) has shown π-π (face-face) stacking with the nicotinamide ring of NAD1270. Ring (B) of the compound is in van der Waal's contact with the residues constructing the hydrophobic pocket of the enzyme (Ala198, Met161, Ile202, and Val203).

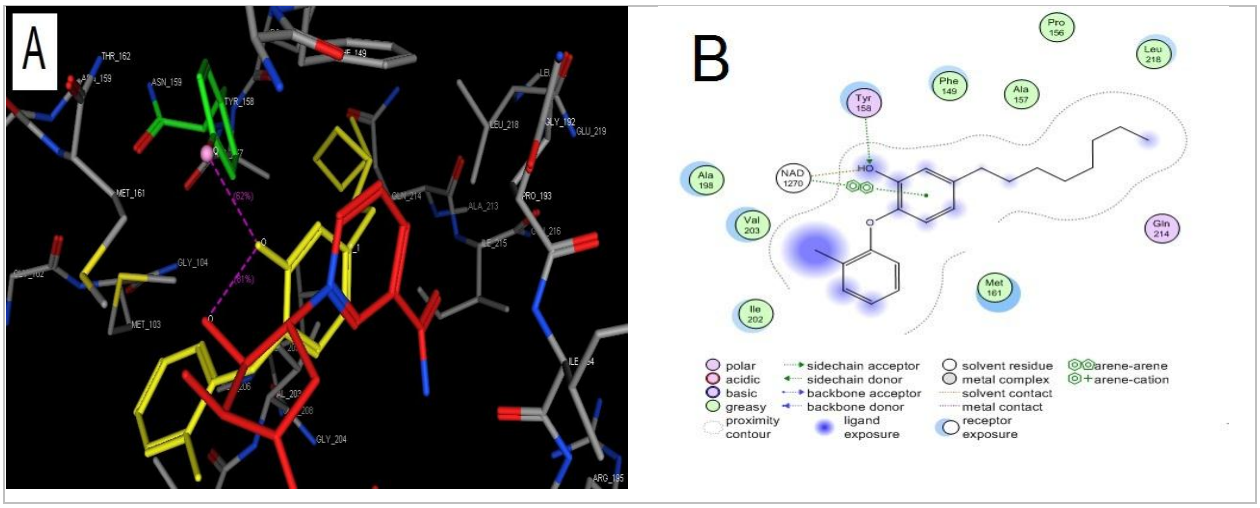

FIGURE 4: Visualization of Diphenylether a1 within InhA active site. (A) 3D picture of binding interactions of a1 in the active site (a1 is yellow and NAD is red). (B) 2D view of the active site with explanatory key. All are generated by MOE.

Diphenylether a19:

The substituents R_2 and R_3 with OH atoms cause favorable charge and van der Waal's interactions with hydrophobic pocket of the enzyme compared to PT70 with H atoms and increase its binding affinity slightly. The docking score of this compound was found to be 12.51 which corresponding to -17.00 kcal/mol whereas the PT70 showed 12.02 which corresponding to -16.33 kcal/mol with similar Cscore value of 5. Compared with PT70, compound a19 also reveals similar interactions (figure 5). However only one hydrogen bond interaction between the hydroxyl group of the compound and 2′-hydroxyl of the nicotinamide ribose of the NAD1270 is formed. Benzene ring (A) has shown π-π (face- face) stacking with the nicotinamide ring of NAD1270. Ring (B) of the compound is in van der Waal's contact with the residues constructing the hydrophobic pocket of the enzyme (Leu218, Pro193, Ala157, 149Phe, Ile202, and Val203).

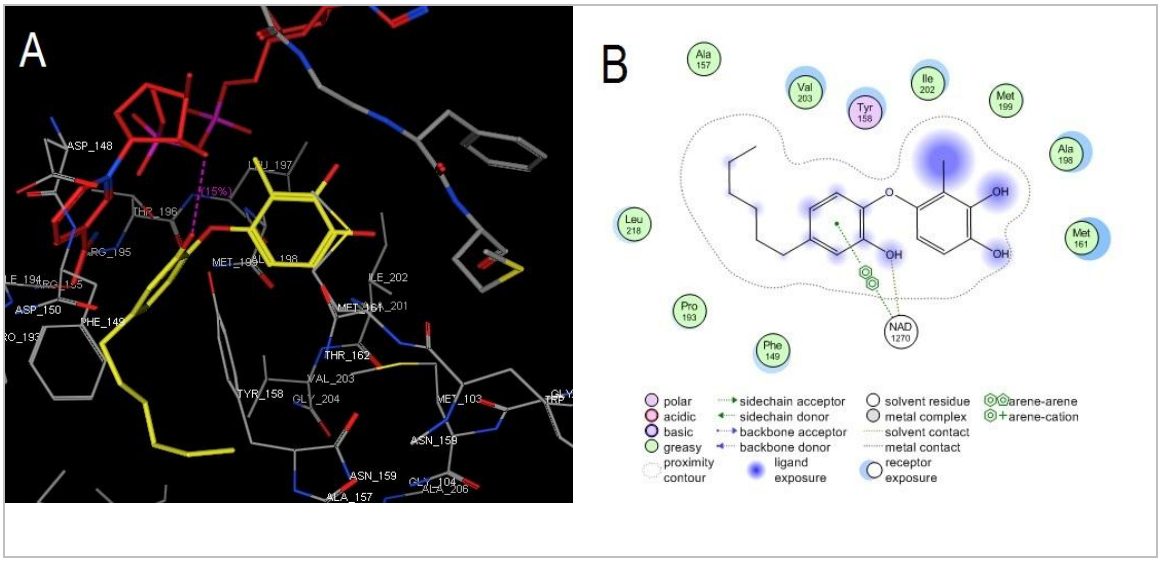

FIGURE 5: Visualization of Diphenylether a19 within InhA active site. (A) 3D picture of binding interactions of a19 in the active site (a19 is yellow and NAD is red). (B) 2D view of the active site with explanatory key. All are generated by MOE.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This study was conducted because of the urgent need for the development of drugs for tuberculosis which is so far threatening lives of millions of people all over the world. Those drugs should be inexpensive, can be easily synthesized, and capable of controlling the disease without being harmful to human.

During this study about 24 compounds were modeled and designed using molecular docking technique. Molecular modeling and docking studies suggested that compounds a1 and a19 interacted with InhA enzyme more efficiently than PT70 and hence, these can be further developed to improve their anti-tubercular activity. It is observed that the length of the side chain R1 attached to ring A and type of side chain R2 and R3 attached to ring B played a pivotal role on the activity of the compounds.

The diphenylether derivatives obtained from modifications in positions R_1 of ring (A) and R_2 and R_3 of ring (B) offers significant information about the kind of modification that can be tolerated for this region in the binding site of InhA, opining up the possibility of generating new compounds by combination of effective substituents at these positions in the diphenylether framework.

Recommendations

Further studies are recommended to be conducted, such as determination of the IC_{50} values of diphenylether derivatives, to test the cytotoxicity of those compounds, and to assess the ADMET properties of the compounds.

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