Journal of the Korean Chemical Society
2012, *Vol. 56, No. 2*
Printed in the Republic of Korea
http://dx.doi.org/10.5012/jkcs.2012.56.2.236

Design and Synthesis of *p*-hydroxybenzohydrazide Derivatives for their Antimycobacterial Activity

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(Received September 28, 2011; Accepted February 14, 2012)

ABSTRACT. The main mycobacterial infection in human is tuberculosis caused by Mycobacterium tuberculosis. Tuberculosis is the leading infectious cause of death in the world. Therefore there is continuing and compelling need for new and improved treatment for tuberculosis. The entire logic towards design of new compounds containing 4-hydroxy-N'-(1,3-thiazoldin-2-yldene)benzohydrazide moiety is basically for superior antimycobacterial activity. The recent advances in QSAR and computer science have provided a systematic approach to design a structure of any compound and further, the biological activity of the compound can be predicted before synthesis. The 3D-QSAR studies for the set of 4-hydroxy-N'-(1,3-thiazoldin-2-yldene)benzohydrazide and their derivatives were carried out by using V-life MDS (3.50). The various statistical methods such as Multiple Linear Regression (MLR), Partial Least Square Regression (PLSR), Principle Component Regression(PCR) and K nearest neighbour (kNN) were used. The kNN showed good results having cross validated r^2 0.9319, r^2 for external test set 0.8561 and standard error of estimate 0.2195. The docking studies were carried out by using Schrodinger GLIDE module which resulted in good docking score in comparison with the standard isoniazid. The designed compounds were further subjected for synthesis and biological evaluation. Antitubercular evaluation of these compounds showed that (4.a), (4.d) and (4.g) found as potent inhibitor of H37RV.

Key words: 3D QSAR, GLIDE, Benzohydrazide

INTRODUCTION

Tuberculosis has become a primary health threat to the mankind. *Mycobacterium tuberculosis* is a very successful pathogen, which causes tuberculosis and is the greatest single infectious cause of mortality worldwide, killing approximately two million people annually.

Tuberculosis (TB) is a worldwide pandemic caused by different species of mycobacteria. The latest statistics reveals that there are around 8 million new cases each year, out of which developing countries show major share. Among HIV-infected people with weakened immune system, TB is a leading killer epidemic. Every year about 2 million people living with HIV/AIDS die from TB. Furthermore, in recent times the appearance of multidrug- resistant TB (MDR-TB), a form of TB that does not respond to the standard treatments, is more common.[1-10]

An additional concern is the rise in multi drug resistance (MDR). Increasing incidence of MTB strains resistant to one or more first line TB drugs such as isoniazid,[11] pyrazinamide[12] and rifampicin[13] has recently intensified the need to develop new and more efficient drugs for the treatment of mycobacterial infections.

The rapid development of drug resistance in microbes, the toxicity and sideeffects of existing antituberculous drug. The lack of bactericidal preparations effective against stable Mycobacterium and wide spread of the HIV virus are factors stimulating the efforts directed toward the creation of a qualitatively new generation of antitubercular drugs.

Discovering three-dimensional pharmacophores which can explain the activity of a series of ligands is one of the most significant contributions of computational chemistry to drug discovery.[11] A QSAR is a mathematical relationship between a biological activity of a molecular system and its geometric and chemical characteristics. QSAR attempts to find consistent relationships between biological activity and molecular properties, so that these rules can be used to evaluate the activity of new compounds 3D models are more easily interpretable than 2D descriptor or fingerprint-based QSAR models, making it easier to suggest new compounds for synthesis.[12,13] Docking is the simulation of stable structures between receptor and known drugs, natural ligands or imaginary compounds and is very useful for interpreting activities of known ligands. It an

operation in which one molecule is brought into the vicinity of another while calculating the interaction energies of the many mutual orientations of the two interacting species. Docking procedure is used as a guide to identify the preferred orientation of one molecule relative to the other.

Literature survey reveals that 4-hydroxybenzohydrazide ring is important for antitmycobacterial activity.[14] In addition, many thiazoline derivatives exhibit a wide varietyof biological activities such as antimicrobial,[15] anti-inflammatory,[16] antihistaminic,[17] antihypertensive,[18] hypnotic,[19] and anticonvulsant,[20] etc. In view of the fact that 4-hydroxy benzohydrazide ring possess antimycobacterial activity and as a part of our ongoing studies in the area of antibacterial and antitubercular agents[21] we have synthesized N'-[-(3,4 disubstituted)-1,3-thiazol-2ylidene)]4-hydroxybenzohydrazide compounds from (4.a-4.h) with the aim of obtaining the new broad spectrum antimicrobial agents, which will devoid of side effects associated with current therapy.

EXPERIMENTAL

3D QSAR

Chemical data: A series of 54 molecules belonging to *p*-hydroxybenzohydrazide derivatives *Mycobaterium tuberculosis* inhibitors were taken and used to generate 3D-QSAR.

Hardware and software: All the 3D QSAR work was performed using the Molecular Design Suite (VLife MDS software package, version 3; from VLife Sciences, Pune, India), on Dell Desktop Computers with a Dual core processor of Intel and Windows operating system.

Structure conformation generation: Structures of compounds were sketched using the 2D structure draw application and converted to 3D structures. All the structures were minimized and optimized with the Merck Molecular Force Field (MMFF) method taking the root mean square gradient (RMS) of 0.01 kcal/mol Å and the iteration limit to 10,000. All the structures were ionized at neutral pH 7. Conformers for each structure were generated using ConfGen by applying OPLS-2005 force field method and least energy conformer was selected for further study and all the compounds were aligned by template based method.

Biological Activities

The negative logarithm of the measured IC_{50} (μM) against *Mycobaterium tuberculosis* as pIC_{50} [pIC_{50}=-log ($IC_{50}\times10^{-6}$)] was used as dependent variable, thus correlating the data linear to the free energy change. Since some compound exhibited insignificant/no inhibition, such compounds were excluded from the present study.[21-29]

Protocol: In the present study, (7.48650 to 31.8668)× (-16.7361 to 0.3877 / -8.4230 to 7.30490) Å grid at the interval of 2.00 was generated around the aligned compounds. The steric, electrostatic and hydrophobic interaction energies are computed at the lattice points of the grid using a methyl probe of charge +1 of gasteiger- marsili type. The QSAR models were developed using Stepwise (SW) Forward - Backward, Simulated (SA) Annealing and Genetic Algorithm (GA) variable selection method with pIC_{50} activity field as dependent variable and physico-chemical descriptors as independent variable having cross-correlation limit of 0.9, 0.7 and 1.0 for model 1, model 2 and model 3 respectively. Selection of test and training set was done by sphere exclusion method having dissimilarity value of 4.2, 5.3 and 4.9 for model 1, model 2 and model 3 respectively. Variance cut off point was 0.0.numbers of maximum and minimum neighbors were 5 and 2 respectively.

Docking

Protocol: All the docking calculations were performed using "Standard precision (SP) and Extra Precision (XP)" mode of Glide 8.5 program; Schrodinger LLC and the 2005 implementation of OPLS_2005 force field. The best docked structure was chosen using a Glide score (Gscore) function. The Gscore was modified and extended version of the empirically based Chemscore function. Another scoring function used by Glide was Emodel, which itself derived from a combination of Gscore, coloumbic, van der Waals and the strain energy of the ligand. Beside this the energy, contacts which included good, bad and ugly were also used for the evaluation of the docked complexes.

The molecules chosen from QSAR prediction were used for docking purpose. Among them, the first twenty nine compounds showed very good glide score. These molecules were again selected for docking via standard precision method. To obtain the precise results, these molecules were then subjected for extra precision method. Both the results were noted and compared. Extra precision method was showing good results in the form of Glide score, Emodel score, Energy. H-bond contacts, good and bad vander Waals forces, and Glide pose number.

The following steps were undertaken for Molecular

docking studies.[30-38]

Ligand preparation[31]: The selected co-crystallized ligand i.e. ligand which was already binded to protein, consequently by opening ligprep window ligand structures were taken. The force field parameter was selected as Molecular Mechanics Force Field (MMFF). The possible states of ligand generated were 32. By keeping remaining data default, ligand preparation was done.

Protein preparation: Protein preparation was done by clicking on window protein preparation wizard. All hydrogens were added. The preprocessing was done. Then, unwanted chain 'B' from the protein was removed as the protein selected was the homodimer. Water molecules were removed from the protein. Heterostates were generarted. The state having lowest penalty and highest probability was selected. After going to window, Impref minimization, all hydrogens and force field OPLS_2005 was selected.

Grid preparation: Receptor was defined and the co-crystallized ligand was differentiated from the active site of receptor 'A' chain. The atoms were scaled by vander Waals radii of 1.0 with the partial atomic charge less than 0.25 defaults. Grid generation was done with selection of rigid docking. The amino acids were not movable so scaling factor was applied upto not less than 0.7. By keeping remaining data unchanged, grid was prepared (*Fig*. 3).

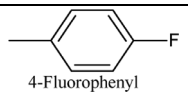
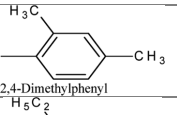
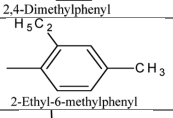
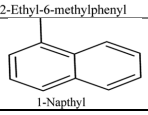
Standard precision (SP) and extra precision (XP) mode: Standard precision docking was having precision between extra precision (XP) and high throughput screening (HTVS). XP docking was used for refining molecules which were giving good results in SP docking.

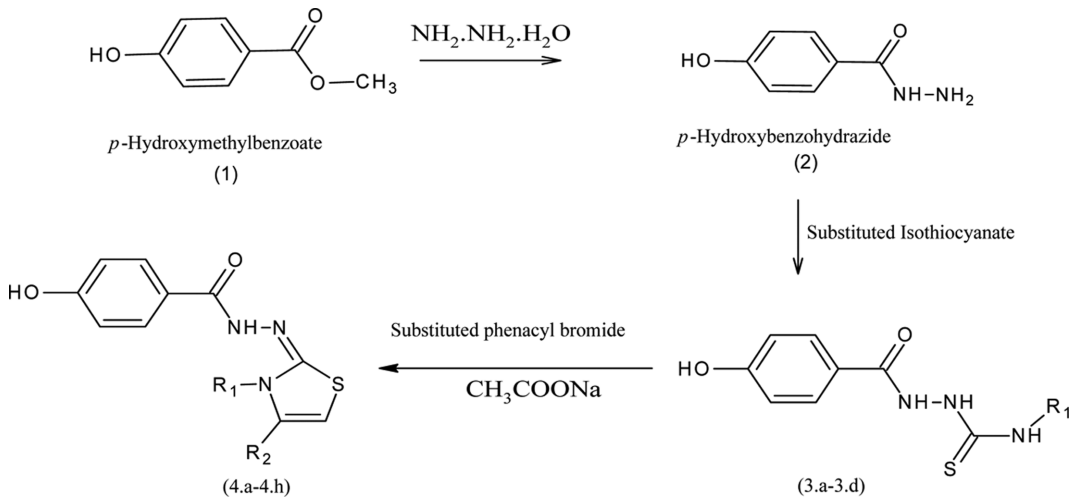
The extra precision docking was performed by using prepared ligands and preprocessed protein. The module Glide was selected from the maestro and XP docking was performed which was indicated good results in the form of docking score, emodel score, glide energy, H- bond contacts and vander Waals forces. The comparative analysis of the docking parameters was carried out with isoniazid (Standard).

Synthesis of Designed Compounds

Scheme: The synthesis of the intermediate and target compounds were performed by the reaction illustrated in *Scheme* 1. Compound 2 namely, 4-hydroxybenzohydrazide was synthesized in excellent yield by amination of compound 1. Reaction of 2 with alkyl/aryl isothiocynate in ethanol gives compounds 3.a-3.d Condensation of product 3.a-i with substituted phenacyl bromides affords

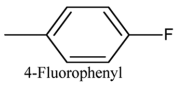
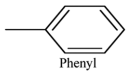
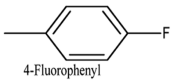
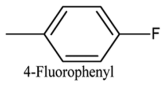
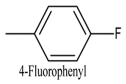
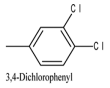
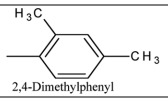
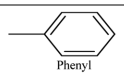
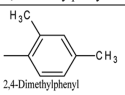
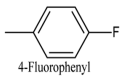
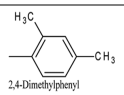
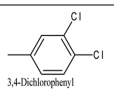
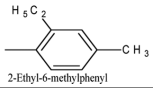
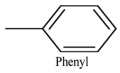
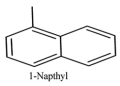
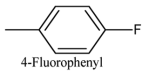
***Table* 1.** Predicted compds (3.a-3.d)

Compd	R_1
3.a	 4-Fluorophenyl
3.b	 2,4-Dimethylphenyl
3.c	 2-Ethyl-6-methylphenyl
3.d	 1-Napthyl



***Scheme* 1.**

***Table* 2.** Predicted compds (4.a-4.h)

Compd	R_1	R_2
4.a	 4-Fluorophenyl	 Phenyl
4.b	 4-Fluorophenyl	 4-Fluorophenyl
4.c	 4-Fluorophenyl	 3,4-Dichlorophenyl
4.d	 2,4-Dimethylphenyl	 Phenyl
4.e	 2,4-Dimethylphenyl	 4-Fluorophenyl
4.f	 2,4-Dimethylphenyl	 3,4-Dichlorophenyl
4.g	 2-Ethyl-6-methylphenyl	 Phenyl
4.h	 1-Napthyl	 4-Fluorophenyl

compounds from 4.a-4.h (*Table* 1 and 2).

1) Chemistry

· General procedures

Chemicals were obtained from Alfa Aesar (UK), Loba Chemie/S.D. Fine-Chem./E. Merck. Melting points (m.p.) were detected with open capillaries using Thermonik Precision Melting point cum Boiling point apparatus (C-PMB-2, Mumbai, India) and are uncorrected. IR spectra (KBr) were recorded on FTIR-8400s spectrophotometer (Shimadzu, Japan). HNMR was obtained using a BRUKER AVANCE II 400 Spectrophotometer using $CDCl_3$. All chemical shift values were recorded as d (ppm). The purity of compounds was checked by thin layer chromatography (Merck, silica gel, HF, type 60, 0.25 mm). The elemental analysis was performed at RTM Nagpur University, India. Elemental analyses for C. H, N were within_0.4% of theoretical values.

2) Synthesis procedure

· Synthesis of Compound 2

The mixture of compd (1) (3.04 g, 0.02 mol) and hydrazine hydrate (99%), (30 mL, 0.6 mol) was refluxed for 12 h. The excess solvent was removed under vacuum upto 10 mL. The reaction mixture was cooled at 4-5^0. The separated solid crystals (2) were filtered and washed with cold water. The crystals were dried and recrystallized from ethanol (99.9%).

Yield: 1.20 g (60.91%). mp 190-192^o (ethanol), R_f : 0.46 (ethanol: water). IR (KBr): cm^{-1} 3191 (alcohol O-H & C-O stretching), 1506 (C=C vibrations), 1609 (C-O stretching), 1328 (alcohol O-H stretching), 884 (benzene 1,4-disubstituted).

· General Procedure for Synthesis of 3.a-3.d

To a solution of compd (2) (0.01 mol) in dry ethanol (190 mL), various aliphatic/aromatic isothiocynates (0.01 mol) were added and the reaction mixture was refluxed for 20 h. The excess solvent was removed under vacuum upto 10 mL. The residue was washed with diethyl ether and recrystallized using methanol.

Following are some specific examples

3.a: Yield: 1.9 g (94.73%). mp 100-102^o (methanol), R_f: 0.54 (ethylacetate : cyclohexane : methanol, 7: 2.5: 0.5). IR (KBr): cm^{-1} 3133 (N-H stretching), 1631(C-N stretching), 1501 (C=C stretching), 1342 (C-O stretching), 1208 (C-S stretching), 1019 (C-F stretching), 833 (benzene 1, 4-disubstituted).

3.b: Yield: 1.53 g (78.06%). mp 152-154^o (methanol), R_f: 0.61 (ethylacetate : cyclohexane : methanol, 7.5 : 2.5 : 0.5). IR (KBr): cm^{-1} 3219 (N-H stretching), 1609 (C-N stretching), 1532 (C=C stretching), 1351 (C-O stretching), 1238 (C-S stretching), 846 (benzene 1,4-disubstituted).

3.c: Yield: 2.05 g (69.26%). mp 81-83^o (methanol), R_f: 0.72 (ethylacetate : cyclohexane : methanol, 7: 2.5: 0.5). IR (KBr): cm^{-1} 3246 (N-H stretching), 1632 (C-N stretching), 1508 (C=C stretching), 1346 (C-O stretching), 1234 (C-S stretching), 845 (benzene 1,4-disubstituted).

3.d: Yield: 1.85 g (88.09%). mp 100-102^o (methanol), R_f: 0.63 (ethylacetate : cyclohexane : methanol, 7: 2.5: 0.5). IR (KBr): cm^{-1} 3184 (N-H stretching), 1640 (C-N stretching), 1507 (C=C stretching), 1332 (C-O stretching), 1207 (C-S stretching), 814 (benzene 1,4-disubstituted).

· General Procedure for Synthesis of Compound 4.a-4.h

The mixture of the thiosemicarbazide (0.01 mol) (3.a-3.d) appropriate phenacyl bromide (0.01 mol) and sodium acetate (0.2 mol) in ethanol (50 mL) was refluxed for 7 h. The mixture was cooled, diluted with enough water to develop turbidity and left overnight to obtain the product.

The product was filtered, dried and recrystallized using aqueous ethanol.

4.a: Yield: 1.20 g (86.95%). mp 128-130^o (methanol), R_f: 0.64 (ethylacetate : cyclohexane : methanol, 7: 2.5 :

0.5). IR (KBr): cm^{-1} 3178 (N-H stretching), 1607 (C=N stretching), 1507 (C=C vibrations), 1347 (C-O stretching), 1051 (C-F stretching), 814 (benzene 1,4-disubstituted).

4.b: Yield: 1.29 g (88.35%). mp 196-198° (methanol), R_f: 0.59 (ethylacetate : cyclohexane : methanol, 7: 2.5 : 0.5). IR (KBr): cm^{-1} 3178 (N-H stretching), 1640 (C=N stretching), 1533 (C=C vibrations), 1339 (C-O stretching), 1017 (C-F stretching), 807 (benzene 1,4-disubstituted).

4.c: Yield: 1.32 g (80.98%). mp 133-135° (methanol), R_f: 0.53 (ethylacetate : cyclohexane : methanol, 7: 2.5 : 0.5). IR (KBr): cm^{-1} 3184 (N-H stretching), 1640 (C=N stretching), 1533 (C=C vibrations), 1339 (C-O stretching), 1019 (C-F stretching), 805 (benzene 1,4-disubstituted), 644 (C-Cl stretching).

4.d: Yield: 0.96 g (76.19%). mp 121-123° (methanol), R_f: 0.62 (ethylacetate : cyclohexane : methanol, 7: 2.5 : 0.5). IR (KBr): cm^{-1} 3183 (N-H stretching), 1640 (C=N stretching), 1533 (C=C vibrations), 1338 (C-O stretching), 811 (benzene 1,4-disubstituted).

4.e: Yield: 1.12 g (78.32%). mp 130-132° (methanol), R_f: 0.44 (ethylacetate : cyclohexane : methanol, 7: 2.5 : 0.5). IR (KBr): cm^{-1} 3185 (N-H stretching), 1640 (C=N stretching), 1532 (C=C vibrations), 1339 (C-O stretching), 1020 (C-F stretching), 811 (benzene 1,4-disubstituted).

4.f: Yield: 1.20 g (74.53%). mp 108-110° (methanol), R_f: 0.71 (ethylacetate : cyclohexane : methanol, 7:2.5:0.5). IR (KBr): cm^{-1} 3013 (N-H stretching), 1641 (C=N stretching), 1535 (C=C vibrations), 1338 (C-O stretching), 808 (benzene 1,4-disubstituted), 643 (C-Cl stretching).

4.g: Yield: 0.98 g (72.59%). mp 196-198° (methanol), R_f: 0.73 (ethylacetate : cyclohexane : methanol, 7:2.5:0.5). IR (KBr): cm^{-1} 2351 (N-H stretching), 1633 (C=N stretching), 1539 (C=C vibrations), 1340 (C-O stretching), 842 (benzene 1,4-disubstituted).

4.h: Yield: 1.10 g (78.57%). mp 93-95° (methanol), R_f: 0.65 (ethylacetate : cyclohexane : methanol, 7:2.5:0.5). IR (KBr): cm^{-1} 2581(N-H stretching), 1640 (C=N stretching), 1533 (C=C vibrations), 1340 (C-O stretching), 1019 (C-F stretching), 804 (benzene 1,4-disubstituted).

Antimycobacterial Activity

The stock solution (2-4 mg/mL) of test compounds was prepared in a mixture of sterile water and dimethylformamide (8:2) solvent. The stock solution was sterilized by passing through a 0.2 mm polycarbonate sterile membrane (Nuclepore) filters. Further, the serial dilution of test compounds was carried out and the following concentration was used: 1000, 500, 250, 125, 62, 32, 16, 8, 4 and 1 mg/mL.Test compounds at various concentrations were added using the BACTEC 460 radiometric system. Compounds effecting <90% inhibition in the primary screen (MIC>12.5 mg/mL) were not evaluated further. Compounds demonstrating at least 90% inhibition in the primary screen were re-tested at lower concentration (MIC) in a broth microdilution assay with alamar blue. The MIC was defined as the lowest concentration inhibiting 90% of the inoculum. After 72 h exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 Nonradioactive Cell Proliferation Assay.[25-27]

BACTEC radiometric method of susceptibility testing: Inocula for susceptibility testing were either from a positive BACTEC isolation vial with a growth index (GI) of 500 or more, or a suspension of organisms isolated earlier on a conventional medium. The culture was well mixed with a syringe and 0.1 mL of a positive BACTEC culture was added to each of the vials containing the test drugs.The drug vials contained rifampicin (0.25 mg/mL). A control vial was inoculated with a 1 : 100 dilution of the culture.A suspension equivalent to a McFarland No. 1 standard was prepared in the same manner as a BACTEC positive vial, when growth from a solid medium was used. Each vial was tested immediately on a BACTEC instrument to provide CO in the headspace. The vials were incubated at 378C and tested daily with a BACTEC instrument. When the GI in the control reads at least 30, the increase in GI (θGI) from the previous day in the control was compared with that in the drug vial. The following formula was used to interpret results:

ΔGI control $>\Delta GI$ drug = susceptible
ΔGI control $<\Delta GI$ drug = resistant

If a clear susceptibility pattern (the difference of ΔGI of control and the drug bottle) was not seen at the time the control GI is 30 the vials were read for 1 or 2 additional days to establish a definite pattern of ΔGI differences.

RESULTS AND DISCUSSION

3D-QSAR

Among these methods (*Table* 3), the kNN showed good results having cross validated r^2 0.9319, r^2 for external test set 0.8561 and standard error of estimate 0.2195. This statistical data along with distribution points (*Fig.* 1) and fitness plot (*Fig.* 2) was used for design of new compounds. Using the result of kNN (*Table* 3), 140 new compounds having different aliphatic and aromatic substitution at position R_1, and aromatic bulky substitution at position R_2,

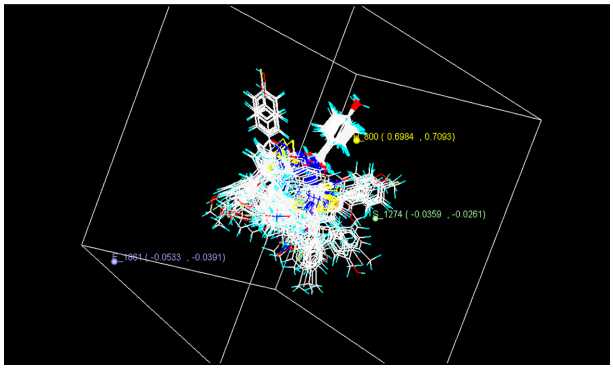


***Fig.* 1.** Distribution points of benzohydrazide derivatives (XVI.a-XXI.i).

were designed and were subjected for the generic prediction. Out of them, first eight compounds (*Table* 4) which were showed better predicted activity (PIC_{50}) were chosen for synthesis and those showed predicted activity (PIC_{50}) below 3.29 were omitted.

Docking

Results were obtained in the form of docking score, emodel score, glide energy, H- bond contacts and vander Waals forces (*Table* 5). The comparative analysis of the docking parameters was carried out with isoniazid (Standard) (*Table* 6). The H-bonds and vander Waals contacts

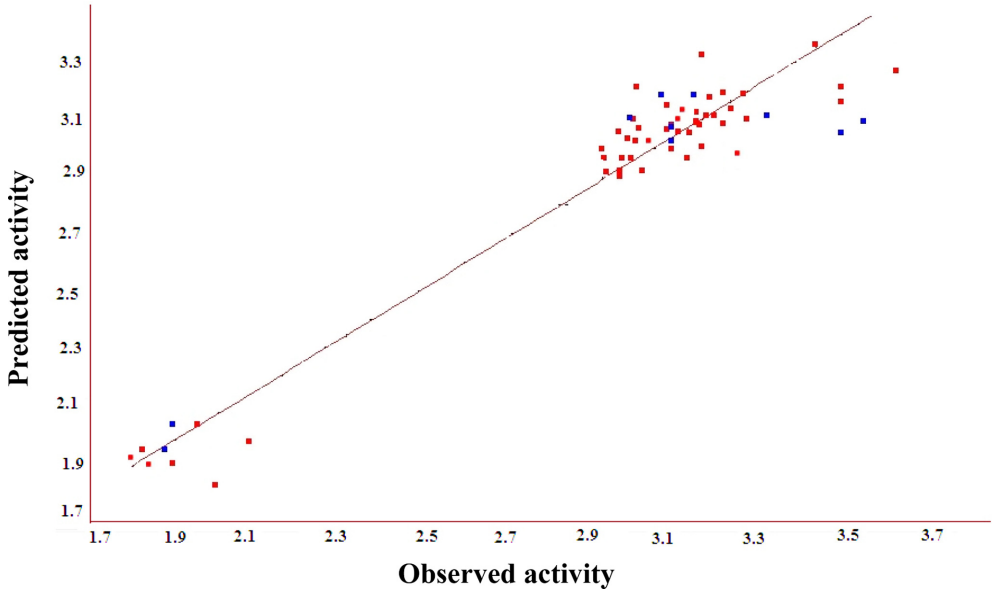


***Fig.* 2.** Actual vs. predicted activities of benzohydrazide derivatives (XVI.a-XXI.i).

***Table* 3.** Comparative result of QSAR methods of benzohydrazide derivatives (XVI.a-XXI.i)

Sr. No	Method	r^2	q^2	"F" test	Pred_r^2	Pred_r^2se
1	MR	0.5934	0.5124	22.3774	0.6019	0.2922
2	PLS	0.5285	0.4427	26.3368	0.4712	0.3367
3	PCR	0.5113	0.4369	24.5912	0.7076	0.2507
4	**kNN**	**-**	**0.9319**	**-**	**0.8561**	**0.2195**

***Table* 4.** Predicted activity of compds. (4.a-4.h)

Sr. No	Compd.	R_1	R_2	Predicted activity (PIC_{50})
1	4.a	4-Fluorophenyl	Phenyl	3.2963
2	4.b	4-Fluorophenyl	4-Fluorophenyl	3.2954
3	4.d	4-Fluorophenyl	3,4-Dichlorophenyl	3.2934
4	4.c	2,4-Dimethylphenyl	Phenyl	3.2139
5	4.g	2,4-Dimethylphenyl	3,4-Dichlorophenyl	3.2165
6	4.e	2,4-Dimethylphenyl	4-Fluorophenyl	3.2960
7	4.f	2-Ethyl-6-methylphenyl	Phenyl	3.2955
8	4.h	1-Napthyl	4-Fluorophenyl	3.2954

***Table* 5.** XP docking of compds (4.a-4.h)

Sr. No.	Compd.	Glide score	Emodel score	Glide energy	Pose number	H-bond	Good vdw	Bad vdw	Ugly vdw
1	4.a	-6.65	-78.9	-51.4	1	1	307	3	0
2	4.b	-5.34	-76.6	-48.1	2	1	308	5	0
3	4.c	-10.45	-60.3	-48.2	8	2	385	16	0
4	4.d	-7.20	-87.2	-53.1	2	2	249	3	0
5	4.e	-8.00	-53.8	-40.5	12	2	413	20	2
6	4.f	-4.68	-52.2	-42.3	14	0	393	29	0
7	4.g	-6.47	-75.2	-51.5	2	0	261	15	0
8	4.h	-3.35	-68.8	-47.3	60	1	265	9	0

***Table* 6.** XP docking of isoniazid (as standard)

Sr. No.	Compd.	G-score	Emodel score	Glide energy	Pose number	H-bond	Good vdw	Bad vdw	Ugly vdw
1	Isoniazid	-4.09	-27.5	-22.6	3	0	99	1	0
2	Isoniazid	-3.96	-30.6	-23.5	8	1	158	2	0

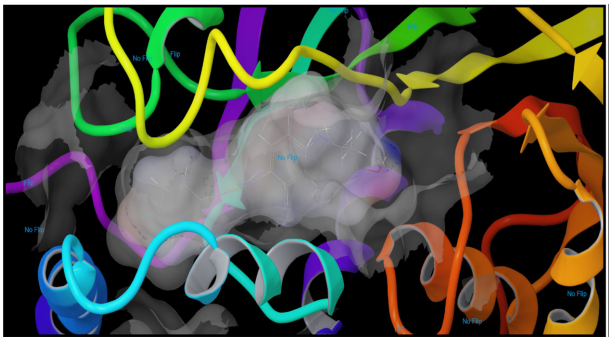


***Fig.* 3.** Grid selected for docking of compds. (4.a-4.h) with 2NSD receptor.

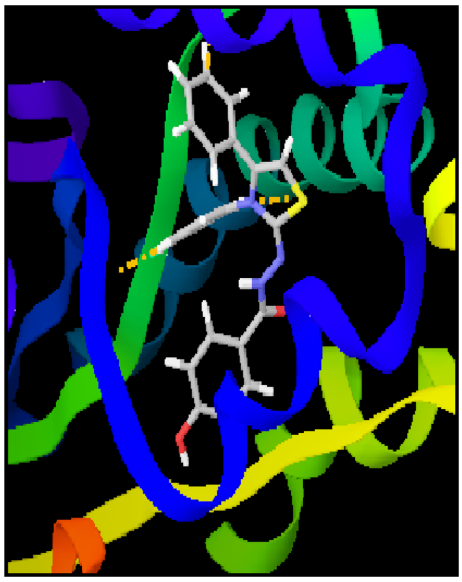


***Fig.* 4.** Compd. (4.a) docked in active site of 2NSD.

(good, bad and ugly) to the receptor were visualized using default settings to analyze the binding modes of the ligands

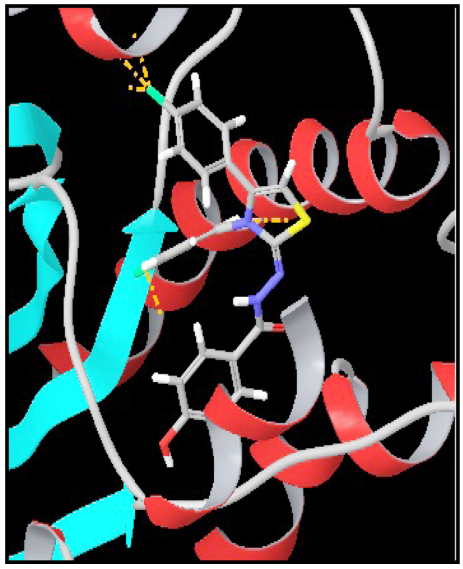


***Fig.* 5.** Compd. (4.b) docked in active site of 2NSD.

to receptor (*Fig.* 3 to *Fig.* 8).

G-score: The docking studies were performed using standard precision mode of Glide. The results of the docking studies were generated in the form of G-score. The more negative value of G-score indicated that the compound may be more potent and indicated the good binding potential of the compound. The G-score of the standard ligand i.e. isoniazid, in case of docking with 2NSD, was found as -4.09. The G-score of the compounds (4.a), (4.b), (4.c), (4.d), (4.e), (4.f), (4.g) and (4.h) were also found as -6.65, -5.34, -10.45, -7.20, -8.00, -4.08, -6.47 and -3.35 respectively. Close analysis of these results suggested that designed compounds were comparable with standard antitubercular agent, isoniazid. Besides the G-score, other

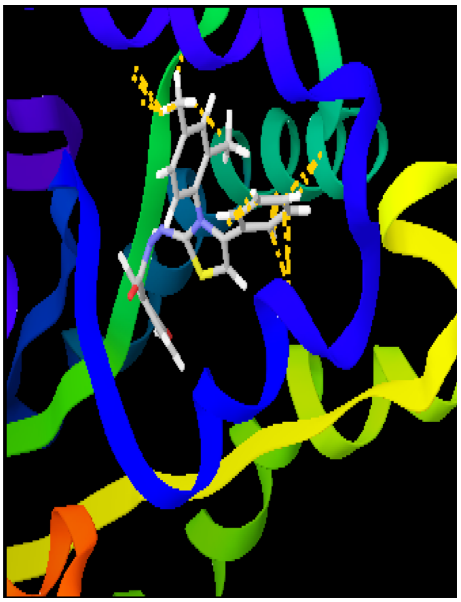


***Fig.* 6.** Compd. (4.c) docked in active site of 2NSD.

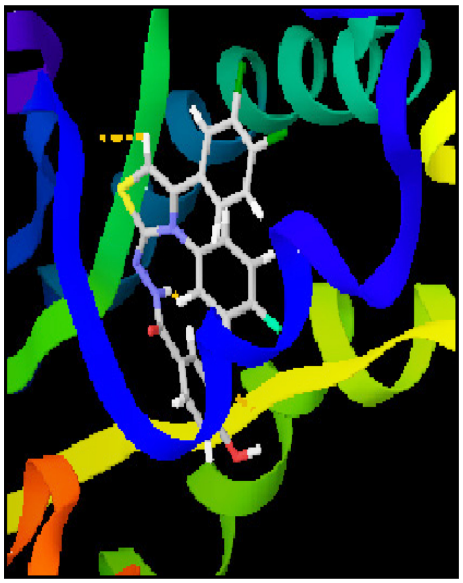


***Fig.* 7.** Compd. (4.d) docked in active site of 2NSD.

parameters like, energy and the E-model were also taken into consideration for the evaluation of the docking results. The values of the energy and E-model were found significantly closer to the values of the standard isoniazid.

H-Bond interaction: The number of H-bond interactions in the standard compound, isoniazid, was compared with those of the designed compounds. In case of docking with 2NSD, the numbers of H-bond interactions of the standard compound, isoniazid, was found as 4, while those of compounds (4.a), (4.b), (4.c), (4.d), (4.e), (4.f), (4.g) and (4.h) were found to be 1, 1, 2, 2, 2, 0, 0 and 1, respectively. This has indicated the requirement of additional functional groups which can form possible H-bond interaction with the 2NSD. When docked with 2NSD, the compd (4.f) and (4.g) has not shown H-bonding.

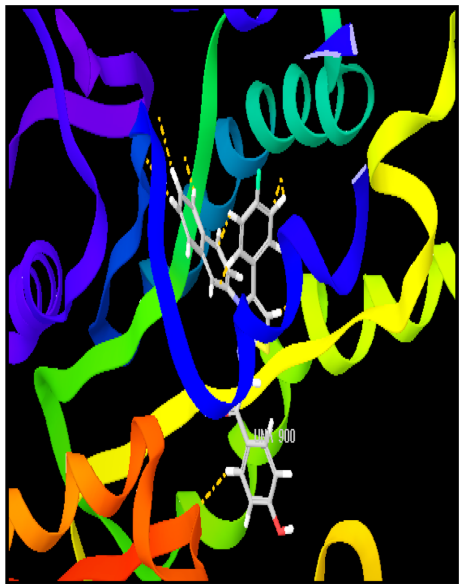


***Fig.* 8.** Compd. (4.h) docked in active site of 2NSD.

Contacts: It was well established and accepted fact that number of good vander Waals interactions decides the binding affinity for any ligand with receptor enzyme protein. Therefore, the analysis was done in the form of the binding modes and abilities, considering the number of good, bad and ugly van der Waals (vdW) interactions of the standard, designed compounds with 2NSD active binding site. In all, the docking scores were the net results of the number of H-bonds and number of good van der Waals contacts and penalties due to number of bad Van der Waals contacts.

Synthesis

Synthetic studies started with reaction of *p*-hydroxymethylbenzoate (1) which on amination with hydrazine hydrate leads to form common intermediate *p*-hydroxybenzohydrazide (2). This intermediate (2) when undergoes nucleophilic addition reaction with aryl isothiocyanates were resulted in the formation of thiosemicarbazides (3.a-3.d) (*Table* 7, p. 21). The cyclization reaction (Step-III, p. 23) was carried out by using different phenacyl bromides, actual cyclization takes place by using sodium acetate. Step-III was resulted in the formation of compounds (4.a-4.h) (*Table* 2, p.23).

***Table* 7.** *In Vitro* antimycobacterial activity of compds (4.a-4.h)

Compd	MIC (μg/mL)									
	100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
4.a	S	R	R	R	R	R	R	R	R	R
4.b	R	R	R	R	R	R	R	R	R	R
4.c	R	R	R	R	R	R	R	R	R	R
4.d	S	R	R	R	R	R	R	R	R	R
4.e	R	R	R	R	R	R	R	R	R	R
4.f	R	R	R	R	R	R	R	R	R	R
4.g	S	R	R	R	R	R	R	R	R	R
4.h	R	R	R	R	R	R	R	R	R	R

All synthesized compounds were further characterized by melting point. The compounds showed single spot in TLC which assured the completion of reactions and the purity of the compounds.

IR spectra of synthesized compd (2) of first step showed the presence of characteristic absorption peaks at 3191(N-H Stretching), 1506 (C=C vibrations), 1609 (C-O stretching), 1328 (alcohol O-H Stretching), 884 (benzene 1,4 -disubstituted).

2-[(*p*-hydroxyphenyl)-carbonyl]-*N*-(substituted alkyl/aryl)-hydrazinecarbothioamide: Addition of substituted isothiocyantes to solution of compd (2) in ethanol afforded corresponding 2-[(4-hydroxyphenyl)carbonyl]-N-substitustedhydrazine-carbothioamide (3.a-3.d). In the reaction, the compd (2) act as a nucleophile and having lone pair of electron on nitrogen.

The IR spectra of compounds (3.a-3.d) showed NH and CS stretching bands at 3133-3246 and 1207-1238 cm^{-1} respectively. The C=C and C-O stretching was shown at 1501-1532 cm^{-1} and 1332-1351 cm^{-1} respectively. The compd (3.a) showed characteristic C-F stretching band at 1019 cm^{-1}.

N'-[(-3-substituted alkyl/aryl)-(4-substituted aryl)-1,3-thiazolidin)-2-ylidene]-*p*-hydroxybenzohydrazide: The reaction of (3.a-3.d) with phenacyl bromide in boiling ethanol containing sodium acetate undergo nucleophilic substitution-elimination reaction afforded the corresponding N'-[(3-substitutedalkyl/aryl-4-oxo-1,3-thiazolidin)-2-ylidene]-*p*-hydroxy benzohydrazide (4.a-4.h).

The IR spectra of compounds (4.a-4.h) showed NH and CN stretching bands at 2351-3184 cm^{-1} and 1607-1641 cm^{-1} respectively. The C=C and C-O stretching was obtained at 1507-1539 cm^{-1} and 1338-1347 cm^{-1} respectively. The compd (4.a), (4.b), (4.c), (4.e) and (4.h) showed characteristic C-F stretching band at 1017-1051 cm^{-1}. The compd (4.f) showed C-Cl stretching at 643 cm^{-1}.

In ^{1}H NMR spectrum of the compounds (4.a-4.h), the signal due to aromatic proton appeared as multiplet in the range of δ value 7.2-8.1 integrating for four protons. The cyclized compounds (4.a-4.h) showed absence of a signal at δ 2.1-2.3 for imine. The compounds (4.a-4.h) showed characteristic peak at δ 6.58-6.98 (s, 1H, Ar-OH). The compd (4.d), (4.e) and (4.f) showed characteristic peak at δ 2.30-2.49 (s, 6H, CH_3), while compd (4.g) showed peak at δ 2.58 (s, 6H, CH_3), 1.65 (s, 3H, ethyl CH_3), 1.90 (s, 2H, ethyl CH_2).

In the EIMS spectra, molecular ion peaks [M^+], which appeared at different intensities, confirmed the molecular weight of the compounds (4.a-4.h). Molecular ion peaks were the base peaks for the compounds.

The elemental analysis of the compounds (4.a-4.h) were found within the limits of the theoretical values.

Antimycobacterial Activity

The anti mycobacterial activity of compounds were assessed against *M. tuberculosis* using microplate alamar blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method.

All the newly synthesized compounds (4.a-4.h) were assayed in vitro for their antimycobacterial activity against *M. tuberculosis* H_{37}Rv. Antimycobacterial activity was carried out at 100, 50, 25, 12.5, 6.25, 3.125, 1.6, 0.8, 0.4 and 0.2 μg/mL. The solvent DMSO was used for these dilutions. For comparison, Isoniazid was employed as the reference antimycobacterial agent as the same drug having structural similarity with the tested drug. Their results are shown in *Table* 7.

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