Pharmacophoric screening of newly synthesized isoniazid derivatives and their antimycobacterial activity

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Abstract: *Mycobacterium tuberculosis* is clinically recognized as a causative agent of Tuberculosis. Keeping in view, this study was endeavored to screen our previously synthesized seventeen INH analogues for their antimycobacterial potential using proportion method. During this process, INH and all the seventeen compounds were examined at different concentrations of 0.05, 0.1 and 0.2µg/mL which were prepared using Lowenstein-Jensen (LJ) base. For drug susceptibility test, three Mycobacterial strains ATCC H37Rv, known INH-sensitive and INH-resistant strains were selected, sub-cultured on LJ Medium and serial diluted to achieve 1:10, 1:100, 1:1000 and 1:10000 from calibrated bacterial suspension Mcfarland No. 1. Dilutions of 1:100 and 1:10000 were added to drug free medium and 1:100 bacterial suspension was added to each of the test concentrations and finally incubated for 4-6 weeks at 37°C. It was observed that only compounds II and XI were active against MTb. Compounds III, IX and X also showed activity but were less potent. Ligand Scout 3.02[il_10] was used to perform pharmacophore-based screening where important pharmacophoric features were identified in the structures of these compounds which could be related to their observed antimycobacterial activity.

Keywords: Pyridine-4-carbohydrazide, antitubercular activity, dilution method, pharmacophore mapping.

INTRODUCTION

Tuberculosis TB is one of the most ancient diseases and is still commonly found around the globe. It is caused by obligate aerobic acid fast bacilli called *Mycobacterium tuberculosis* Mtb, which grow very slowly and mostly cause pulmonary infections (Smith, 2003). In 1993, World Health Organization WHO declared TB as a global crisis. It is considered as a foremost reason of mortality after HIV & malaria, and an economic burden for developing countries. Re-emergence of this life-threatening disorder associated with the interface of AIDS/HIV also present an alarming situation. Another dilemma is the emergence of multi drug-resistant strains of TB. (Baker, 1994; Kremer and Besra, 2002). Despite these, no fresh antitubercular treatment came into the clinical site for the past decades. The researchers have been trying to develop new vaccines and drugs for complete eradication of this deadliest disease.

For rational drug designing the study of pharmacophoric features in bioactive molecules might help and guide us in highlighting their important binding regions (Mason *et al.*, 2001; Cavalli *et al.*, 2002; Khedkar *et al.*, 2007). Pharmacophore modeling is commonly employed where active ligand is known but target's (receptor/protein) 3D structure is unclear. It is believed that agents with same structural domains bind to the target in the same way as the compounds for known biological activity. Determination of pharmacophoric features, their active conformations which are required to interact with the target and establishment of a 3D relationship are the components of pharmacophore mapping (Hou and Xu, 2004; Vyas *et al.*, 2008)

Various analogues of isoniazid were synthesized in the past with the aim to develop lead molecules for antitubercular drug discovery (Ferreira *et al.*, 2008; Hearn *et al.*, 2009; Sankar and Pandiarajan, 2010; Rodrigues *et al.*, 2013; Rychtarčíková *et al.*, 2014). Considering this, we have reported the synthesis of novel seventeen sulphonyl, benzoyl and phenacyl INH derivatives I-XVII (table 1) with antimicrobial activity (Naeem *et al.*, 2015; Naeem *et al.*, 2016). Here we have attempted to test the potential of these analogues against sensitive and resistant strains of mycobacterium.

MATERIALS AND METHOD

Dimethyl sulfoxide (DMSO), glycerol and LJ base were purchased from Musaji Adam & Sons, Karachi, Pakistan. Sterile Distilled Water was used to perform Serial dilution of the test compounds under Telsar AH-100 Laminar Flow. Inspissation was carried out in INSPI Max

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inspissator. Memmert incubator was used for incubation of inoculated LJ medium.

Antimycobacterial activity

Antitubercular activity of INH and its seventeen synthetic analogues was performed using proportion method (Canetti *et al.*, 1963; Canetti *et al.*, 1969; Anochie *et al.*, 2011). For the growth of mycobacterium, LJ medium is primarily intended to be used with fresh eggs (Egg-based LJ media). Stock solution (1000µg/mL) of INH and each test compound was prepared using DMSO as solvent which then serial diluted to 100µg/mL and 10µg/mL and further diluted using egg-based LJ to have final test concentrations of 0.05, 0.1 and 0.2µg/mL. Afterwards the contents were poured to pre-sterilized universal bottles which were then inspissated in slanted manner for 45 minutes at 85°C to solidify drug-containing slopes. Same conditions were given to bottles containing egg-based LJ medium to produce drug-free slopes (control).

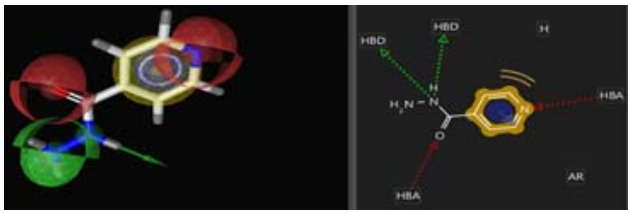


Fig. 1: Pharmacophoric regions of INH

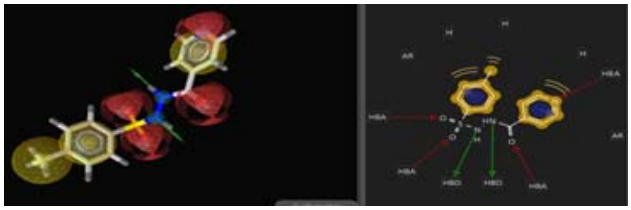


Fig. 2: Pharmacophoric regions of Compound I

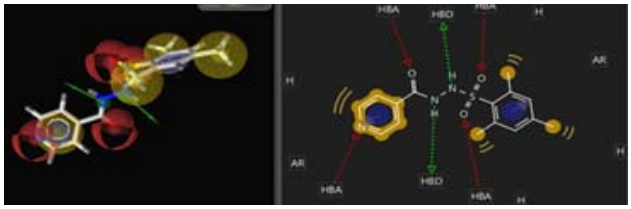


Fig. 3: Pharmacophoric regions of Compound II

Three strains $H_{37}Rv$, INH-sensitive INH-S and INH-resistant INH-R were chosen for drug susceptibility test DST. Serial dilution of each strain was performed from calibrated bacterial suspension equivalent to McFarland No. 1 and serial diluted tenfold to a dilution of 100 and 10000 to adjust turbidity of bacterial suspensions. These were then added in drug-free as well as drug-containing slopes of each test concentration. Finally, slopes were incubated for 4-6 weeks at 37°C. The inoculated media are examined for contamination after 1 week of incubation. Slopes were checked visually for the presence of mycobacterium and followed the critical proportion of 1%.

If the growth on the media containing drug is ≥ to the growth on 10^{-4} control, the strain is considered resistant to that particular drug concentration. On the other hand, if the growth on the media containing one drug is < to the growth on 10^{-4} control, the strain is considered susceptible to that particular drug concentration.

Pharmacophore-based screening

Pharmacophoric regions were examined with the help of software Chem Draw Ultra 8.0 and Ligand Scout3.02[il_10] (Wolber and Langer, 2005; Khan *et al.*, 2010) and classified as Hydrogen bond donor(s) HBD, Hydrogen bond acceptor(s) HBA, Ionic interaction (positive ionizable) PI, Hydrophobic and Aromatic regions. There is a possibility that all or some of these regions might be involved in binding to the target site.

Ligand Scout is software which allows rapid and transparent derivation of 3D feature-based pharmacophores from ligand structural information in a totally automated and suitable way.

RESULTS

Antimycobacterial activity

The antimycobacterial study of INH and compounds I - XVII against $H_{37}RV$, INH-S and INH-R were presented in table 2. According to the findings, INH was found active against $H_{37}Rv$ and INH-S at 0.1 and 0.2µg/mL. It was clear that only the sulphonyl products II and III and phenacyl derivatives IX, X and X1 were active against Mtb $H_{37}Rv$ and INH-S strains in which compound II and XI were equipotent to that of INH.

Structurally, compound II had three methyl groups at *ortho* and *para* positions, which made the compound more lipophilic that might support INH to retain its activity. Whereas derivatives III, IX and X possessed one bromo, chloro and fluoro groups at *para* position respectively. Compound XI possessed one nitro group at *meta* position. From these findings, it can easily be concluded that the attachment of electron withdrawing groups (such as -Br, -Cl, -F and $-NO_2$) cause INH to retain the antimycobacterial activity.

Pharmacophore-based screening

Pharmacophoric regions of INH and compounds I-XVII were given in table 3 and presented in figs. 1-18. According to Ligand Scout, INH possessed six while compounds I-XVII had ten to fourteen pharmacophoric binding regions. INH showed one aromatic and one hydrophobic ring with two HBA and HBD.

All compounds displayed two aromatic rings except VII which had one more aromatic ring. Compounds IV, VI and XII showed one, VIII, XI, XV, XVI and XVII displayed two, I, III, V, VII, IX, X and XIV expressed three, II and XIII pictured four hydrophobic rings respectively.

Table 1: Synthetic INH analogues I – XVII

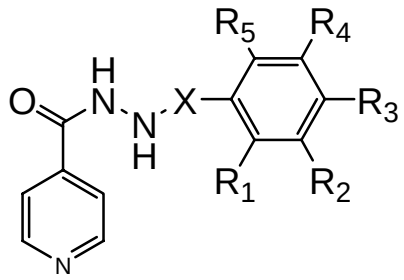
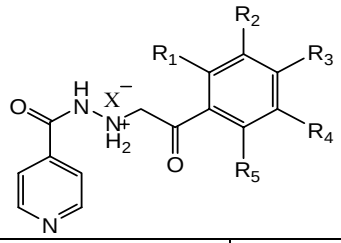
S.No.	Compound Name	
Sulphonyl and benzoyl analogues 		
I	N□-[(4-methylbenzene)sulfonyl]pyridine-4-carbohydrazide	X= SO_2,R_1, R_2, R_4, R_5 = H, R_3 = CH_3
II	N□-[(2,4,6-trimethylbenzene)sulfonyl]pyridine-4-carbohydrazide	X= SO_2,R_2, R_4= H, R_1, R_3, R_5 = CH_3
III	N□-[(4-bromobenzene)sulfonyl]pyridine-4-carbohydrazide	X= SO_2,R_1, R_2, R_4, R_5 = H, R_3 = Br
IV	N□-[(4-nitrobenzene)sulfonyl]pyridine-4-carbohydrazide	X= SO_2,R_1, R_2, R_4, R_5 = H, R_3 = NO_2
V	N□-[(4-methylbenzoyl)]pyridine-4-carbohydrazide	X= CO,R_1, R_2, R_4, R_5 = H, R_3 = CH_3
VI	N□-[(3,5-dinitrolbenzoyl)]pyridine-4-carbohydrazide	X= CO,R_1, R_3, R_5 = H, R_2, R_4= NO_2
Phenacyl analogues 		
VII	[2-oxo-2-(4-phenylphenyl)ethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_4, R_5 = H, R_3 = C_6H_5
VIII	[2-(3,4-dihydroxyphenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium chloride	X = Cl, R_1, R_4, R_5= H, R_2, R_3 = OH
IX	[2-(4-chlorophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_4, R_5 = H, R_3 = Cl
X	[2-(4-fluorophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_4, R_5 = H, R_3 = F
XI	[2-(3-nitrophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_3, R_4, R_5 = H, R_2 = NO_2
XII	[2-(4-nitrophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_4, R_5 = H, R_3 = NO_2
XIII	[2-(2,4-difluorophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium chloride	X = Cl, R_2, R_4, R_5 = H, R_1, R_3 = F
XIV	[2-(4-bromophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_4, R_5 = H, R_3 = Br
XV	[2-(2,5-dimethoxyphenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_2,R_3, R_5= H, R_1,R_4 = OCH_3
XVI	[2-(4-methoxyphenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_4, R_5 = H, R_3 = OCH_3
XVII	(2-oxo-2-phenylethyl) (pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_3, R_4, R_5 = H

Table 2: Antimycobacterial activity of INH and Compounds I – XVII

Mtb strain / Compound	Mtb H37Rv			INH-S			INH-R		
	Test concentration (µg/mL)			Test concentration (µg/mL)			Test concentration (µg/mL)		
	0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2
INH	R	S	S	R	S	S	R	R	R
I	R	R	R	R	R	R	R	R	R
II	R	S	S	R	S	S	R	R	R
III	R	R	S	R	R	S	R	R	R
IV	R	R	R	R	R	R	R	R	R
V	R	R	R	R	R	R	R	R	R
VI	R	R	R	R	R	R	R	R	R
VII	R	R	R	R	R	R	R	R	R
VIII	R	R	R	R	R	R	R	R	R
IX	R	R	S	R	R	S	R	R	R
X	R	R	S	R	R	S	R	R	R
XI	R	S	S	R	S	S	R	R	R
XII	R	R	R	R	R	R	R	R	R
XIII	R	R	R	R	R	R	R	R	R
XIV	R	R	R	R	R	R	R	R	R
XV	R	R	R	R	R	R	R	R	R
XVI	R	R	R	R	R	R	R	R	R
XVII	R	R	R	R	R	R	R	R	R

R = Resistant, S = Sensitive

Table 3: Pharmacophoric Regions of INH and Compounds I – XVII

Compound	Aromatic Ring	Hydrophobic Ring	Hydrogen Bond Acceptor	Hydrogen Bond Donor	Positive Ionizable region	Total
INH	01	01	02	02	-	06
I	02	03	04	02	-	11
II	02	04	04	02	-	12
III	02	03	04	02	-	11
IV	02	01	06	02	-	11
V	02	03	03	02	-	10
VI	02	01	07	02	-	12
VII	03	03	03	03	01	13
VIII	02	02	05	04	01	14
IX	02	03	03	02	01	11
X	02	03	04	02	01	12
XI	02	02	05	02	01	12
XII	02	01	05	02	01	11
XIII	02	04	05	02	01	14
XIV	02	03	03	02	01	11
XV	02	02	05	02	01	12
XVI	02	02	04	02	01	11
XVII	02	02	03	02	01	10

(-) = no region

Compounds V, VII, IX, XIV and XVII presented three HBA regions. Compounds I, II, III, X and XVI had four, VIII, XI, XII, XIII and XV bore five HBA features in their structures while IV and VI demonstrated six and seven HBA regions respectively.

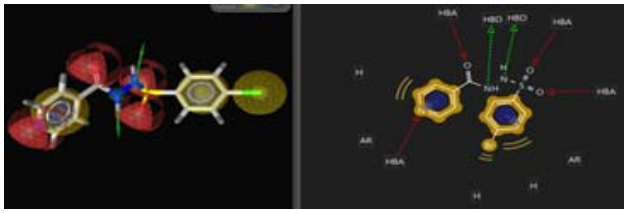


Fig. 4: Pharmacophoric regions of Compound III

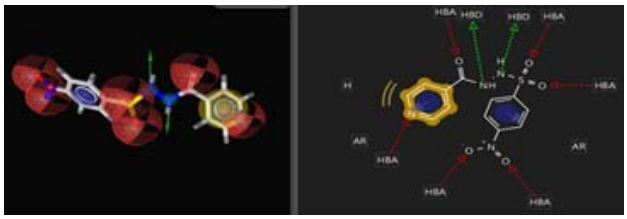


Fig. 5: Pharmacophoric regions of Compound IV

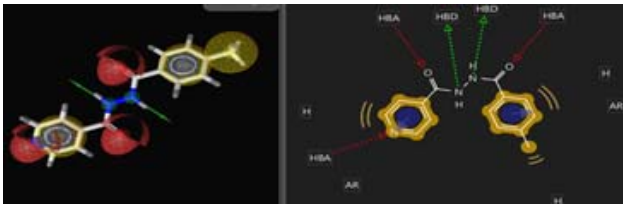


Fig. 6: Pharmacophoric regions of Compound V

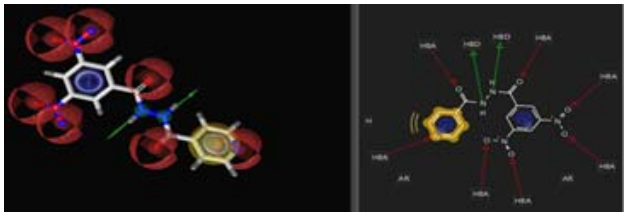


Fig. 7: Pharmacophoric regions of Compound VI

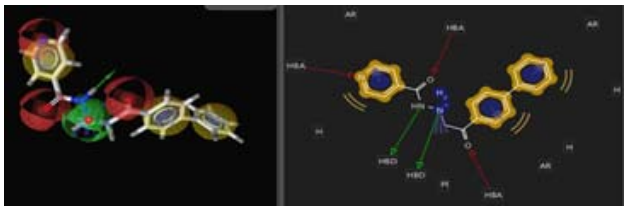


Fig. 8: Pharmacophoric regions of Compound VII

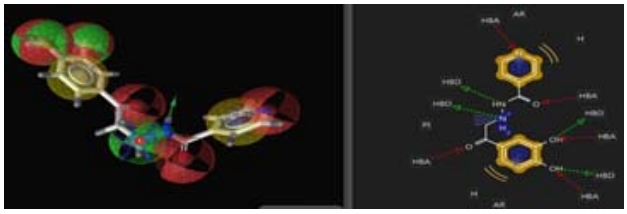


Fig. 9: Pharmacophoric regions of Compound VIII

All compounds depicted two HBD regions except VII and VIII each with three and four donor regions respectively. Derivatives VII-XVII showed the presence of one positive ionizable fraction.

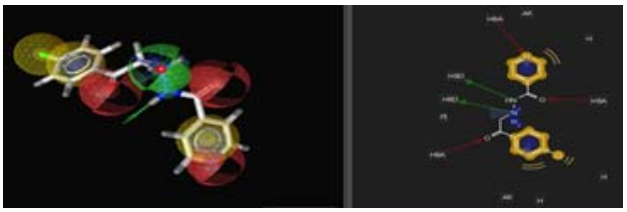


Fig. 10: Pharmacophoric regions of Compound IX

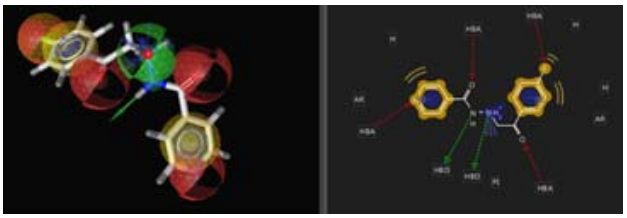


Fig. 11: Pharmacophoric regions of Compound X

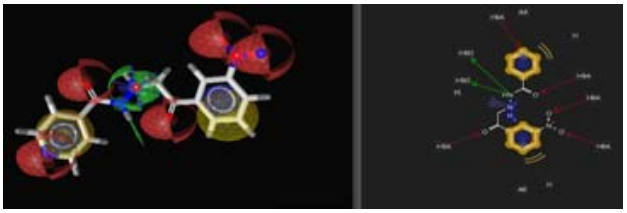


Fig. 12: Pharmacophoric regions of Compound XI

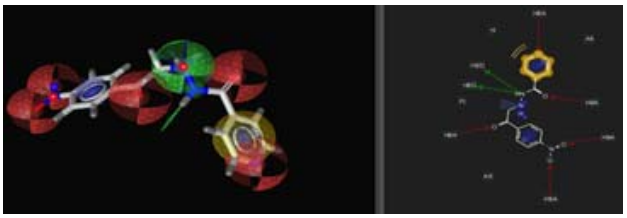


Fig. 13: Pharmacophoric regions Compound XII

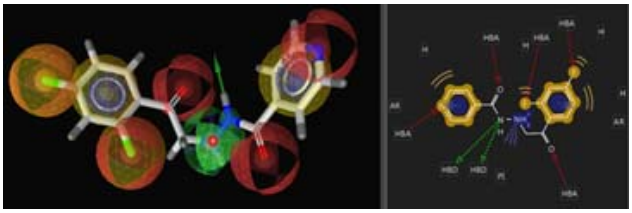


Fig. 14: Pharmacophoric regions of Compound XIII

DISCUSSION

If we seek to ascertain a link between the results of antimycobacterial activity and *in-silico* studies, a single theory cannot be possible. All of the synthesized compounds possessed three parts; substituted aromatic ring, spacer (sulphonyl, benzoyl and phenacyl group) and the parent group (INH). Among sulphonyl derivatives, compound II sustaining the activity of INH had three hydrophobic regions on the benzene ring indicating the

presence of three *ortho* and *para* methyl groups which could be accountable for more lipophilicity and hence retention of antimycobacterial activity.

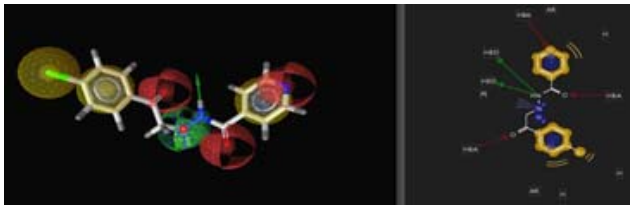


Fig. 15: Pharmacophoric regions of Compound XIV

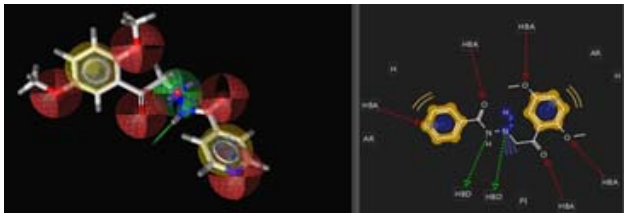


Fig. 16: Pharmacophoric regions of Compound XV

Fig. 17: Pharmacophoric regions of Compound XVI

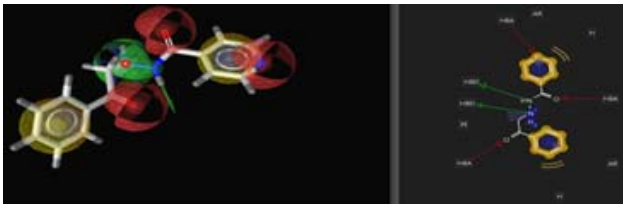


Fig. 18: Pharmacophoric regions of Compound XVII

In phenacyl derivatives of INH, the active compounds showed variable binding areas. However product XI with *meta* nitro group having one aromatic, one hydrophobic and two HBA regions found active. The hydrophobic ring in compound IX and X substituted with *para*-chlorine and *para* fluorine were also found active.

CONCLUSIONS

As a result of this study, new anti-T.B. drugs would be available for the patients.

Tuberculosis remains a big challenge because of resistance to already known drugs. It is the job of researchers to design and develop such a drug molecule which is active against the resistant strains of mycobacterium. In this concern, we offered these derivatives for further exploration as antimycobacterial agents.

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