Evaluation and Docking Study of Pyrazine Containing 1, 3, 4-Oxadiazoles Clubbed with Substituted Azetidin-2-one: A New Class of Potential Antimicrobial and Antitubercular

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ABSTRACT

Background Tuberculosis (TB) caused by Mycobacterium tuberculosis is one of the main killers of people all over the world. The major hurdles with existing therapy are the lengthy regimen and appearance of multi drug resistant (MDR) and extensively drug resistant (XDR) strains of M.tuberculosis.

Aims The present work was aimed to synthesize and determine antitubercular and antimicrobial potential of some novel 3-chloro-4-aryl-1-[4-(5-pyrazin-2-yl[1,3,4]oxadiazole-2-ylmethoxy)-phenyl]-azetidin-2-one derivatives **7**(**a-h**) from pyrazinoic acid as precursor, which is a well-established antitubercular agent. Here we report the synthesis of a new class of heterocyclic molecules in which pyrazine, 1, 3, 4-oxadiazole and azetidinone moieties were present in one frame work.

Methods Pyrazinoic acid (**1**) was esterified first (**2**) followed by amination to produce hydrazide (**3**) which was refluxed with POCl3 to obtain 2-chloromethyl-5pyrazino-1, 3, 4-oxadiazole (**4**). This was then further reacted with 4-amino phenol to obtain 4-[5-pyrazino-1, 3, 4-oxadiazol-2-yl-methoxy]-phenyl amine (**5**) which on condensation with various aromatic aldehydes afforded a series Schiff's bases **6(a-h)**. Dehydrative annulations of **6(a-h)** in the presence of chloroacetyl chloride and triethylamine yielded 3-chloro-4-aryl-1-[4-(5-pyrazin-2-yl-[1, 3, 4]oxadiazole-2-ylmethoxy)-phenyl]-azetidin-2-one derivatives **7(a-h)**. Antibacterial, antifungal and antitubercular potential of all the synthesized compounds were assessed. Docking study was performed using the software VLife Engine tools of Vlifemds 4.6 on the protein lumazine synthase of M. tuberculosis (PDB entry code 2C92).

Results The present studies demonstrated that synthesized oxadiazole derivatives have good antimicrobial activity against the various microorganisms. Among the synthesized derivative, **7b** and **7g** were found to be prominent compounds which have potential antibacterial, antifungal and antitubercular activity (with MIC 3.12 µg/ml and high dock score ranging from −59.0 to −54.0) against Mycobacterium tuberculosis.

Conclusions Derivatives **7b** and **7g** would be effective lead candidates for tuberculosis therapy.

Introduction

Microbial infections remain the chief cause of mortality worldwide. Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is one of the main killers of people all over the world. The major hurdles with existing therapy are the lengthy regimen and appearance of multi drug resistant (MDR) and extensively drug resistant (XDR) strains of *M.tuberculosis* and this made the condition most frightening [1, 2]. Therefore, there is an urgent demand for a new class of an-

timicrobial agent with a different mode of action and it led medicinal chemists to explore a wide variety of chemical structures. In pursuit of this goal, our research efforts herein have been directed towards the discovery of new chemical entities that are effective antimicrobial and antitubercular agents.

Nitrogen containing heterocyclic compounds are well studied for their broad spectrum of activities [3–7]. Among them 1, 3, 4-oxadiazole and their derivatives constitute a crucial class of organic compounds which have been reported to possess versatile activities. Oxadiazole is a cyclic compound containing one oxygen and two nitrogen atoms in a five-member ring [8]. Oxadiazoles have occupied a unique place in the field of medicinal chemistry due to its wide range of activities [9]. From the literature survey, oxadiazole nucleus has been found to possess anti-inflammatory [10–12], anticonvulsant [13], antifungal and antibacterial [14, 15], antiviral [16], antioxidant [17], analgesic [12], and antitubercular activity [18].

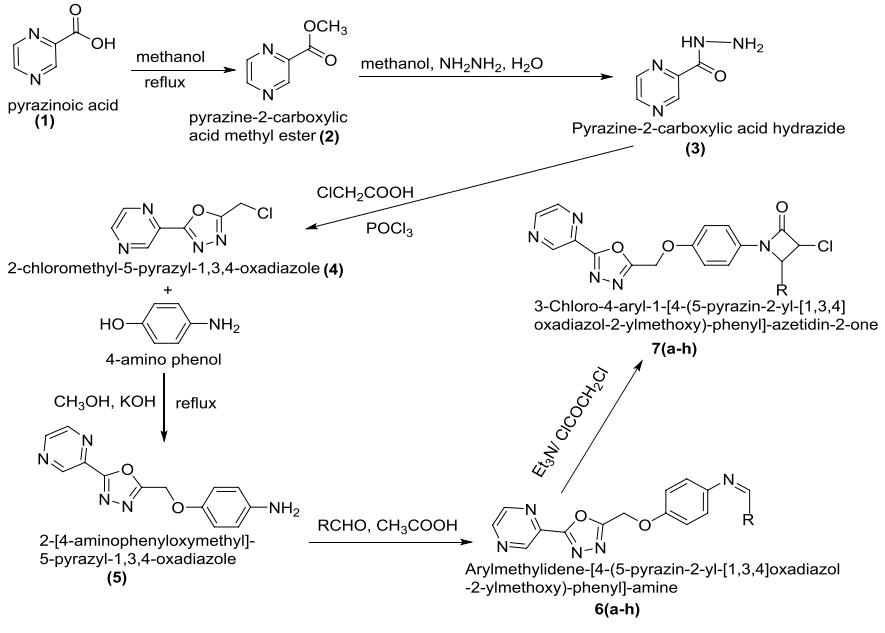
β-Lactams are the most successful antimicrobials [19–22] till recent days, unless microorganisms producing β- lactamase. The 2-azetidinone (β-lactam) ring system is the common structural feature of a number of broad spectrum β-lactam antibiotics, including penicillins, cephalosporins, carbapenems, nocardicins, monobactams, clavulanic acid, sulbactams and tazobactams, which have been widely used as chemotherapeutic agents to treat bacterial infections and microbial diseases [23–30]. A large number of 3-chloro- 2-azetidinones possesses potent antimicrobial [31], antitubercular [32, 33], antioxidant [34], inhibitors of cholesterol absorption [35], vasopressin V1a antagonists [36] activity.

Pyrazine derivatives are well-known and an important aromatic heterocyclic compound having two-nitrogen-containing six-membered ring. They can carry substituents at one or more of the four ring carbon atoms. Pyrazinamide is one of the most effective antitubercular drugs. Various pyrazine derivatives and pyrazinamide analogs also exhibit high antibacterial activity, e. g. pyrazinoic acid esters [37], pyrazine thiocarboxamide and *N*-hydroxymethyl pyrazine thiocarboxamide [38], and ring substituted pyrazinylchalcones [39]. It is also reported 4-mono- and 4-disubstituted 1-pyrazinoyl thiosemicarbazides exhibit high tuberculostatic activity [40, 41].

After extensive literature search, it was observed that, till date enough efforts have not been made to combine these moieties as a single molecular scaffold and to study its biological activity [42–44]. This initiated us to synthesizing compounds containing the oxadiazole, azetidinone and pyrazine ring systems in the same matrix to serve as a new scaffold. In this paper, we have reported the synthesis of a new class of heterocyclic molecules in which pyrazine, 1, 3, 4-oxadiazole and azetidinone moieties were present in one frame work. Further this new class of heterocyclic molecules was explored for their antitubercular, antibacterial and antifungal potential.

Results and Discussion

Pyrazine-2-carboxylic acid methyl ester **(2)** was prepared in quantitative yield according to a known method [45]. This compound on amination with hydrazine hydrate afforded pyrazine-2-carboxylic acid hydrazide (**3**). Compound **3** refluxed with $POCl_3$ to obtain 2-chloro methyl-5-pyrazyl-1, 3, 4-oxadiazole (**4**). This was then further reacted with 4-amino phenol to obtain 2-[4aminophenyloxymethyl]-5-pyrazyl-1, 3, 4-oxadiazole **(5)**.The condensation of compound (**5**) with various aromatic aldehydes yielded aryl methylene-[4-(5-pyrazin-2-yl-[1,3,4] oxadiazole-2-ylmethoxy)-phenyl]-amine derivatives **6(a-h)**. Compounds **6(a-h)**, on reaction with chloroacetyl chloride in the presence of triethylamine underwent dehydrative annulation



▶ **Fig. 1** Synthesis of 3-chloro-4-aryl-1-[4-(5-pyrazin-2-yl [1,3,4]oxadiazole-2-ylmethoxy)-phenyl]-azetidin-2-one derivatives **7**(**a–h**).

(Staudinger's ketene–imine reaction) to afford 3-chloro-4-aryl-1-[4-(5-pyrazin-2-yl-[1,3,4]oxadiazole-2-ylmethoxy)-phenyl]-azetidin-2-one derivatives **7(a-h)**.These reactions are summarized in ▶**Fig. 1**. The substituents of the compounds are given in ▶**Table 1**. Yields of the synthesized compounds were moderate to fair (54–70 %). The physical and molecular data are listed in ▶**Tables 2** and ▶**3**. The purity of the compounds was monitored by TLC and the structure of the compounds was deduced on the basis of their elemental analysis and spectral data. Both analytical and spectral data of all the synthesized derivatives were in full agreement with the proposed structures. The structures assigned to **7(a-h)** were supported by IR, NMR and MASS spectra. The IR spectrum of titled compounds **7(a-h)** revealed C = C aromatic characteristic stretching absorption between 1492–1591 cm^{-1} and aromatic C-H stretching absorption manifested between 2993–3065 cm^{-1}. The aromatic C-H stretching for methyl moiety manifested between 2807–2927 cm^{-1}. The ring stretching mode (C = O) of azetidinone for titled compounds **7(a-h)**, manifested frequency between 1730–1789 cm^{-1}, that indicates formation of β-lactam ring of azetidinone, while C-Cl bending for azetidinone is revealed by characteristic bands between 2933–3065 cm^{-1}. The oxadiazole ring manifested C-O-C stretching absorption between 1053–1089 cm^{-1}. The asymmetric bending absorptions of aromatic NO_2 function was manifested at 1531 cm^{-1} and the symmetric bending absorption was manifested at 1394 cm^{-1} for **7e** and **7h**. The aromatic C-O bending for ether moiety of **7g** was manifested at 1162 cm^{-1} and aromatic O-H stretching of hydroxyl function of **7d** was manifested at 3208 cm^{-1}. The C-Cl bending frequency for aromatic chloro function in **7a** and **7b** manifested at 642 cm^{-1}. In ^1H NMR all the aromatic rings were observed as multiplets at δ scale between 6.96–811 ppm in the titled compounds **7(a-h)** and three protons of pyrazine moiety manifested as multiplets at δ scale between 8.22–837 ppm in the titled compounds **7(a-h)**. The one proton of CH-Cl of azetidinone having one proton in its proximity, thus were observed as doublet at δ scale between 5.75–5.77 ppm. The two protons of a CH_2 function having no proton in its proximity thus, shows singlet peak between 5.27–5.29 in the titled compounds **7(a-h)**, a singlet peak is seen at 3.77 ppm in **7g** for methoxy function and presence of one proton for aromatic C-OH manifested singlet at 5.15 ppm in **7d.** In the MASS spectrum, the m/z 68 and m/z 79 due to formation of $[C_2N_2O]^+$ and $[C_3H_2NOCl]^+$ depicted the presence of oxadiazole and azetidinone ring in the titled compounds.

The antimicrobial activity of the synthesized derivatives was assessed by agar cup plate method [46] and the average diameter of zone of inhibition (mm) and MIC (μg/ml) [47] were determined in comparison with standard drugs.

All the synthesized compounds were screened for their *in vitro* antibacterial activity against two gram positive (*S. aureus, B.subtilis*) and two gram negative (*P.aeruginosa, E. coli*) bacteria taking Amoxicillin as a standard drug and *in vitro* antifungal activity against two fungus strains *C.albicans* and *A. niger* taking Miconazole as a standard drug (▶**Table 4**).

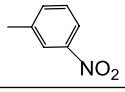
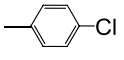
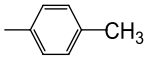
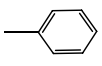
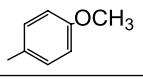
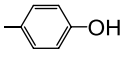
The results of zone of inhibition studies as shown in ▶**Table 4** showed that the compounds **7b**, **7e** and **7g** have greater antibacterial potential than other derivatives against the gram positive (*B. subtilis* and *S.aureus*) and gram negative (*E.coli* and *P.aeruginosa)* bacterias. Similarly antifungal activity of **7b**, **7e** and **7g** derivatives displayed higher zone of inhibition as compared to other derivatives against *C.albicans* and *A.niger.* The antimicrobial (antibacterial and antifungal) potential of the synthesized compounds was further confirmed by determination of MIC of all the derivatives. The MIC is defined as the minimum concentration of compounds required to completely inhibit the bacterial growth. It was clearly observed from the results as shown in ▶**Table 4** that the derivatives **7b**, **7e** and **7g** showed greater antibacterial potential against gram positive and gram negative bacterias as compared to other derivatives.

The series synthesized was found to be more active against *S.aureus and E.coli* compared to other bacterias used. In comparison to standard drug compound **7b** and **7g** exhibited two fold increase in antibacterial activity against *S.aureus* and compound **7b** against *E.coli.* Compound **7g** also exhibited two fold increase in activity against *P.aeruginosa* compared to standard drug.

The oxadiazole derivatives are found to be more potent against *C.albicans* compared to *A. niger*. The derivative **7g** with electron donating group in substitution and **7b** and **7e** with electron withdrawing substitutions displayed potent activity against *C.albicans.* In perticular the compounds **7g** with MIC 12.5 μg/ml is endowed with maximum potency against *C. albicans*, being two times more active than the standard drug Miconazole.

Compound **7b** and **7g** exhibited potential activity against *A. niger* but not as potent as compared to the standard drug. Other oxadiazole derivatives have shown fair to medium antibacterial and antifungal activities. No inhibitory effect was observed for DMSO.

▶**Table 1** Various substitutions (R) used.

Compound	R	Compound	R
7a		**7e**	
7b		**7f**	
7c		**7g**	
7d		**7h**	

▶ **Table 2** Physical and molecular properties of synthesized aryl methylene-[4-(5-pyrazin-2-yl-[1,3,4]oxadiazole-2-ylmethoxy)-phenyl]-amine derivatives.

Compound	Physical state	Mol. Formula	Mol. Weight	Percentage Yield (in %)	M.P (°C)	Solubility	R_f value
6a	Brownish crystals	$C_{20}N_5O_2ClH_{14}$	392	55	222 – 224	DMSO, MeOH, $CHCl_3$	0.55
6b	Pale Brownish crystals	$C_{20}N_5O_2ClH_{14}$	392	61	188 – 190	DMSO, MeOH, $CHCl_3$	0.48
6c	Brownish crystals	$C_{20}N_5O_2H_{15}$	358	66	217 – 218	DMSO, MeOH, $CHCl_3$	0.41
6d	Yellowish Brown crystals	$C_{20}N_5O_3H_{15}$	374	52	202 – 204	DMSO, MeOH, $CHCl_3$	0.39
6e	Reddish Brown crystals	$C_{20}N_6O_4H_{14}$	403	72	179 – 180	DMSO, MeOH, $CHCl_3$	0.57
6f	Dark Yellowish crystals	$C_{21}N_5O_2H_{17}$	372	56	182 – 184	DMSO, MeOH, $CHCl_3$	0.33
6g	Pale Brownish crystals	$C_{21}N_5O_3H_{17}$	388	77	197 – 198	DMSO, MeOH, $CHCl_3$	0.52
6h	Brown crystals	$C_{20}N_6O_4H_{14}$	403	67	208 – 210	DMSO, MeOH, $CHCl_3$	0.59

▶ **Table 3** Physical and molecular properties of synthesized 3-chloro-4-aryl-1-[4-(5-pyrazin-2-yl [1,3,4] oxadiazole-2-ylmethoxy)-phenyl]-azetidin-2-one derivatives.

Compound	Mol. Formula	Mol. Weight	Physical state	Percentage Yield (in %)	M.P	Solubility	R_f value
7a	$C_{22}H_{15}Cl_2N_5O_3$	468.29	Brownish crystals	64	176 – 177	DMSO, MeOH, $CHCl_3$	0.81
7b	$C_{22}H_{15}Cl_2N_5O_3$	468.29	Pale Brownish crystals	70	162 – 164	DMSO, MeOH, $CHCl_3$	0.79
7c	$C_{22}H_{16}ClN_5O_3$	433.85	Brownish crystals	58	209 – 210	DMSO, MeOH, $CHCl_3$	0.68
7d	$C_{22}H_{16}ClN_5O_4$	449.85	Light Brown crystals	55	175 – 176	DMSO, MeOH, $CHCl_3$	0.73
7e	$C_{22}H_{15}ClN_6O_5$	478.84	Dark Brown crystals	61	140 – 142	DMSO, MeOH, $CHCl_3$	0.86
7f	$C_{25}H_{26}ClN_5O_3$	479.96	Yellowish crystals	54	152 – 154	DMSO, MeOH, $CHCl_3$	0.65
7g	$C_{23}H_{18}ClN_5O_4$	463.87	Pale Brownish crystals	62	134 – 135	DMSO, MeOH, $CHCl_3$	0.77
7h	$C_{23}H_{19}ClN_6O_5$	494.89	Brown crystals	67	171 – 172	DMSO, MeOH, $CHCl_3$	0.83

The encouraging results from the antibacterial and antifungal studies impelled us to go for preliminary screening of synthesized compounds against *M. tuberculosis*. Therefore, all the synthesized compounds were screened for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv (MTCC200) by Lowenstein-Jensen (L.J.) Agar (MIC) method [48] using isoniazid and rifampicin as standard drugs. Results as shown in ▶ **Table 5** depicted that out of eight screened compounds **7b** and **7g** having para substituted aromatic ring displayed potential antitubercular activity with MIC 3.12 µg/ml against the strain *Mycobacterium tuberculosis* H37Rv. Substitution at ortho position on the aromatic ring in compound **7a** have MIC value 6.25 µg/ml shows considerable antitubercular activity.

Compounds **7e, 7d, 7h** showed good activity (MIC 25–50 mg/ml) which is attributed to 3-nitro, 4-hydroxy and 2-nitro substituents whereas the MIC values of compounds **7c** and **7f** which having phenyl alkyl substitution were found to be more than 100 µg/ml and exhibited lesser antitubercular activity.

Although the synthesized derivatives specifically **7b**, **7g** and **7a** displayed potent antitubercular activity but they are still lesser active as compared to standard drugs (isoniazid and rifampicin). The results obtained reveals that the nature of substituents on the aromatic ring have a great impact on the antitubercular activities of the test compounds.

In silico Molecular Docking studies were performed with the synthesized compounds using VLife MDS 4.6 [49].

Protein Preparation

After conducting ample literature study lumazine synthase of *M. tuberculosis* (PDB entry code 2C92), was selected as the biological target for carrying out the docking study of our synthesized compounds. The enzyme lumazine synthase is not present in the human or animal host [50, 51]. Hence, they are promising candidates for the inhibition of *M. tuberculosis* growth. Lumazine synthase inhibitors can be considered as potential lead compounds for the design of therapeutically useful antitubercular agents.

- The crystal structures of the target protein were obtained from Protein Data Bank, downloaded from www.rcsb.org and saved in standard 3D coordinate format. The protein Data Bank (PDB) provides the 3-D structure of all the proteins by NMR or by X-ray crystallography.
- The selected lumazine synthase was prepared using biopredicta module of the VLife MDS 4.6.
- The enhancement of the crude PDB structure of receptor was done by completing the incomplete residues. The water molecules were removed and hydrogens were added in to the protein structure to retain the geometry, the co-crystallized ligand lying within the receptor was modified by assigning missing bond order and hybridization states.
- The structure was then optimized by the optimization module of the Vlife MDS 4.6 using Merck molecular force field. The optimized receptor was then saved as mol file and used for docking study.
- The assorted 3D view of the protein PDB 2C92 is shown in ▶ **Fig. 2**

▶ **Table 4** Antibacterial and Antifungal evaluation of synthesized 3-chloro-4-aryl-1-[4-(5-pyrazin-2-yl-[1,3,4] oxadiazole-2-ylmethoxy)-phenyl]-azetidin-2-one derivatives.

Compound	Zone of inhibition (mm) against microorganisms					
	G+ve bacteria		G-ve bacteria		Fungal species	
	B. subtilis	*S. aureus*	*E. coli*	*P.aeruginosa*	*C.albicans*	*A. niger*
7a	23 ± 0.86	25 ± 0.66	25.3 ± 0.47	21 ± 0.86	21.3 ± 0.24	22 ± 0.86
7b	25 ± 0.66	28.3 ± 0.47	29 ± 0.63	22.3 ± 0.24	22 ± 0.66	24 ± 0.66
7c	19.3 ± 0.47	20 ± 0.63	20.3 ± 0.24	16.6 ± 0.26	19.3 ± 0.47	21.3 ± 0.47
7d	18.6 ± 0.26	19.6 ± 0.26	19.6 ± 0.26	17 ± 0.63	20.3 ± 0.24	20 ± 0.63
7e	24.3 ± 0.24	26.3 ± 0.47	26 ± 0.66	22 ± 0.86	22.3 ± 0.47	23 ± 0.66
7f	20 ± 0.86	22 ± 0.86	23.3 ± 0.24	20 ± 0.63	20.3 ± 0.47	22.3 ± 0.47
7g	26.3 ± 0.47	27.3 ± 0.24	28 ± 0.66	24 ± 0.86	23 ± 0.66	25 ± 0.66
7h	23 ± 0.06	25 ± 0.66	25 ± 0.66	21.3 ± 0.47	21 ± 0.86	22 ± 0.86
Control	NZ	NZ	NZ	NZ	NZ	NZ
Amoxicillin	29.3 ± 0.47	31.3 ± 0.24	30 ± 0.86	28.3 ± 0.24	ND	ND
Miconazole	ND	ND	ND	ND	26.3 ± 0.47	28 ± 0.86

ND: Not Determined. NZ: No Zone of Inhibition.

▶ **Table 5** Antimicrobial activity of the synthesized 3-chloro-4-aryl-1-[4-(5-pyrazin-2-yl [1,3,4]oxadiazole-2-ylmethoxy)-phenyl]-azetidin-2-one derivatives (MIC's in µg/ml*).

Compound	MIC (µg/ml)						
	Bacterial strains				Fungal strains		*M. tuberculosis*
	B.subtilis	*S.aureus*	E.coli	*P.aeruginosa*	*C.albicans*	*A.niger*	
7a	100	100	25	200	50	50	6.25
7b	50	50	6.25	200	25	25	3.12
7c	200	200	100	400	100	100	>100
7d	200	200	100	400	50	100	50
7e	100	100	12.5	200	25	50	25
7f	200	200	100	200	50	50	>100
7g	50	50	12.5	100	12.5	25	3.12
7h	100	100	25	200	50	50	50
Control	–	–	–	–	–	–	–
Amoxicillin	50	100	12.5	200	ND	ND	ND
Miconazole	ND	ND	ND	ND	25	12.5	ND
Rifampicin	ND	ND	ND	ND	ND	ND	0.25
Isoniazid	ND	ND	ND	ND	ND	ND	0.20

* MIC- Minimum inhibitory concentration. ND: Not Determined.

Ligand Preparation [49]

Ligand preparation was done by

- Drawing the structures using ChemSketch 12 and Chem Draw Ultra 7 in 2D and saved as MDL Molfile format.
- Converting the ligands to 3D format using VLife Engine tools of Vlifemds 4.6.
- Then energetically minimizing the 3D structures up to the rms gradient of 0.01 using Merck Molecular Force Field (MMFF) by using Vlifemds 4.6 software.
- Then generating the conformers of all the synthesized ligands by Monte Carlo method. For doing this, all rotatable bonds of the ligands were selected and number of seeds used for searching the conformational space was set as 5.
- Energetically minimizing all the conformers up to the rms gradient of 0.01 and then saved in separate folder.
- Molecular alignment: Using the template-based technique the molecules of the dataset were aligned, for alignment of the molecules the most active molecule was used as a template. The alignment of all the molecules on the template shown in ▶ **Fig. 3**.

Docking Studies [49]

Docking simulations were performed using Biopredicta tool in the grip docking mode.

- The active site selection was done by choosing the cavity having maximum hydrophobic surface area.

- The docking simulation was done using GRIP batch docking. In this, all generated conformers of one ligand were put as one batch in GRIP docking wizard. Likewise, the batches for all other ligands were put.
- All the conformers were virtually docked at the defined cavity of the receptor.
- The parameters considered for docking study were – rotation angle: 30 ° (the ligand gets rotated for different poses by rotation angle), number of placements: 30 (by placements, the method will check all the 30 possible placements into the active site pocket and will result out few best placements out of 30), scoring function: dock score, exhaustive method.
- For each ligand, all the conformers with their best placements and their dock score will be saved in output folder. The method also highlights the best placement of best conformer of one particular ligand which is having best (minimum) dock score.
- Different types of interactions were studied between docked 3D ligands and 3D macromolecule target as shown in ▶ **Fig. 4**.

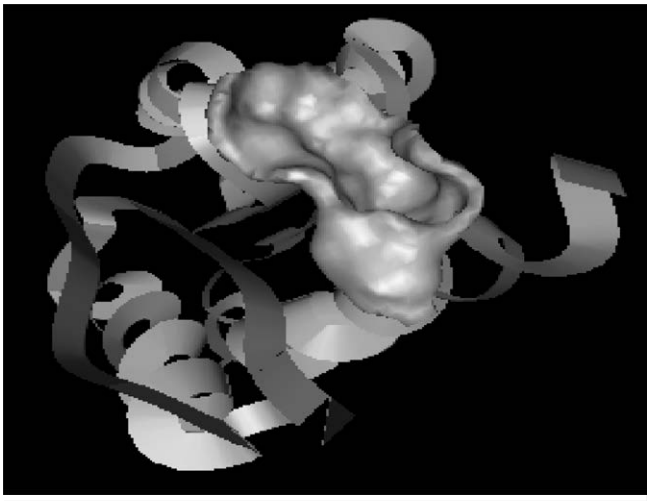
Here we have listed only best ligands and their dock score in ▶ **Table 6**. The ligand forming most stable drug-receptor complex is the one which is having minimum dock score. After docking simulation, the best docked conformer of each ligand and receptor were merged and were checked for various interactions of ligand with receptor like hydrogen bonding, hydrophobic bonding and van der waal's interaction. Compounds were found to exhibit hydrogen bonding, hydrophobic bonding and van der Waal's interaction with the receptor. ▶ **Fig. 4** shows the docking position and interactions of the active compounds in the receptor cavity. All the compounds were found to exhibit large number of van der Waal's bonding with wide range of residues. Some of the common residues involved in this type of interaction are ASP74A, ARG154A, SER109A, ARG157A, ASN72A and THR110A. However ARG154A and ARG157A were found to be the common residues involved in docking of all the ligands.

From the dock score, compounds **7b**, **7a** and **7g** were found to have highest negative dock score ranging from − 59.0 to − 54.0 (▶ **Table 6**). It means that these formed most stable drug-receptor complex amongst other compounds.

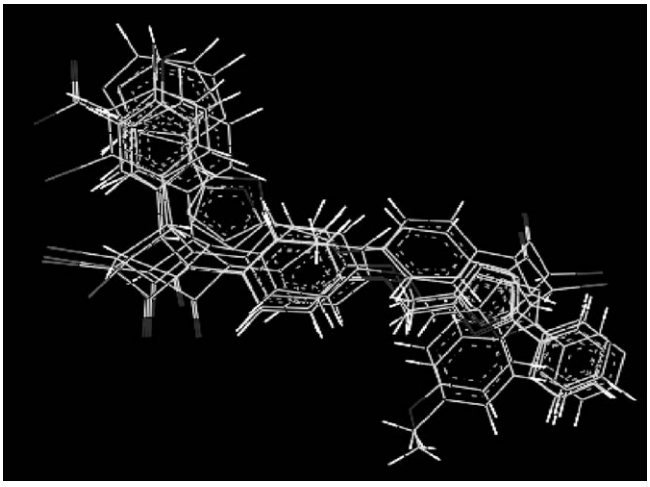
Pharmacophore Modeling [49]

Pharmacophore modeling was carried out in the molsign module of Vlife MDS 4.6 software. A series of derivatives was first aligned on the active molecule. Alignment of small organic molecules is one of the important tasks in drug design and bioactivity prediction. A pharmacophore model is a set of three dimensional features that are necessary for bioactive ligands. Six different pharmacophore types are currently supported include H bond acceptor (HBA) and donor (HBD), hydrophobic feature, aromatic ring and positive and negative ionizable centers (Ku). Three fields are required to set the parameters for pharmacophore identification. In the first field Primary Pharmacophore Feature Count, value 3 indicates the minimum number of pharmacophore features generated for an alignment. The next is a number for Tolerance which indicates the flexibility in percentage allowed while comparing two feature combinations across two molecules. Here the value entered is 10 Å. It is also required to set the Max Distance Allowed between two features, the value is set to 10. Pharmacophore feature and Distance between features are shown in ▶ **Fig. 5** and ▶ **Fig. 6**. ▶ **Fig 5** shows pharmacophore for synthesized molecules indicating the pharmacophoric features like hydrogen bond donor (green) and hydrophobic (orange), Hydrogen bond acceptor (blue), Aliphatic (buff) which are required for activity. The larger tessellated spheres are indicative of the common pharmacophores identified in the molecules; the smaller solid features are of the individual molecules.

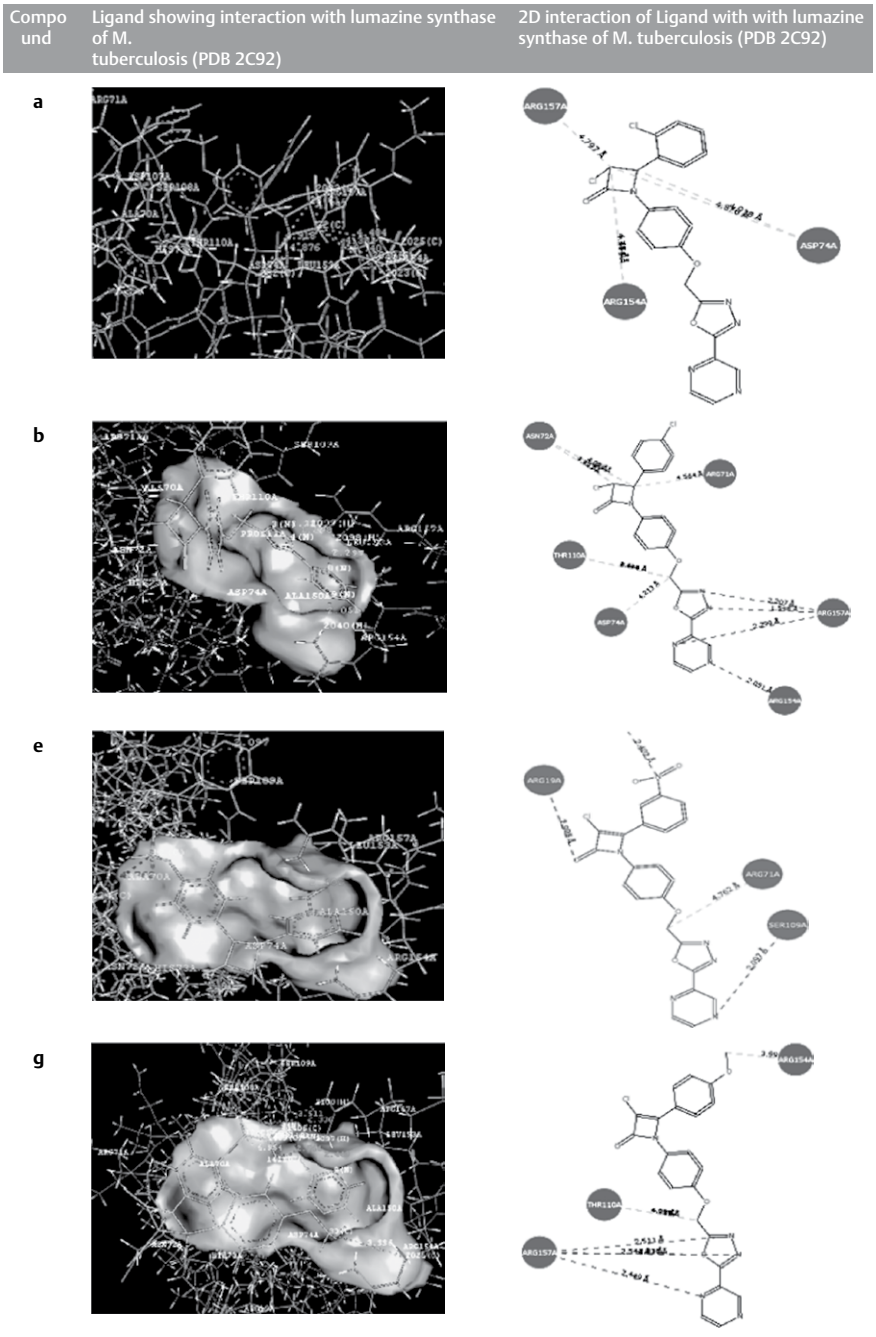
Literature survey indicates that electron-withdrawing groups or electron releasing groups increases lipophilicity of the test compounds, which in turn enhances permeability across the mycobacterial cell membrane. The presence of electronegative atom such as chloro group in **7b** has shown better activity than **7c** and **7f** which have alkyl substitutions on the phenyl ring. This clearly indicates the importance of electronegative atom at para position of aromatic ring for better activity. Also **7g** bearing a methoxy group at para position have shown good antitubercular activity. The antitu-



▶ **Fig. 2** Structure of PDB (2C9B) with cavity assorted view.



▶ **Fig. 3** Alignment of all the molecules.

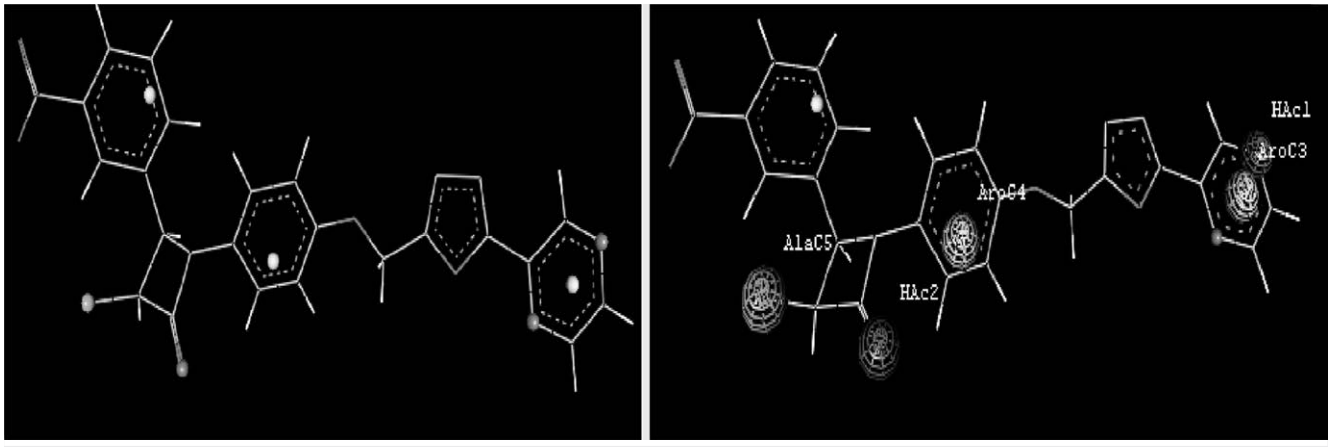


▶ **Fig. 4** Binding modes (docking view) and 2D interaction of **7a,7b,7e** and **7g** with lumazine synthase of M. tuberculosis (PDB 2C92), where dark blue and light blue lines represent various hydrogen bonding and hydrophobic interactions with amino acid residues.

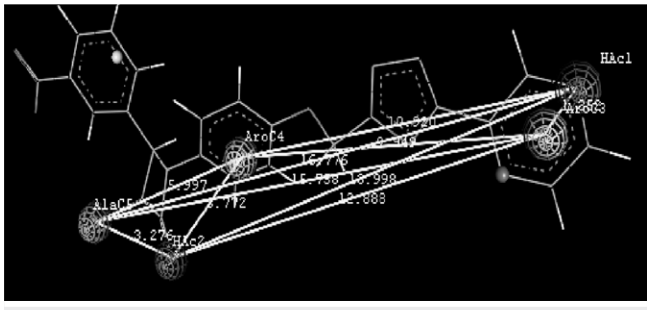
bercular activities of the synthesized derivatives were found in the following order: para chloro or para methoxy substitution on aromatic ring > ortho chloro substitution on aromatic ring > 3-nitro substitution on aromatic ring > para hydroxy or ortho nitro substitution on aromatic ring > compounds with alkyl substitution or unsubstituted aromatic ring. These results make the synthesized novel oxadiazole-substituted azitidinone derivatives interesting lead molecule for more synthetic and biological evaluation. It can be concluded that this class of compounds certainly hold great promise towards pursuit to discover novel class of antimicrobial and antimycobacterial agents. The compounds **7b** and **7g** with potential activity against the selected microorganisms may be regarded as precursor compounds for searching for new derivatives showing potent antimicrobial and antimycobacterial activity.

▶ **Table 6** Ligand-receptor interaction of target compounds.

Compound	Dock score	Distance (in ˚A)	Amino acid	Binding group
7a	−62.923	4.876	ASP74A	-C–Cl of azatidinone ring
		4.9181	ASP74A	-C–N of azatidinone ring
		4.3814	ARG154A	-C–Cl of azatidinone ring
		4.7966	ARG157A	-C–Cl of azatidinone ring
7b	−69.449	4.1296	ASN72A	-C–N of azatidinone ring
		3.8212	ASN72A	-C–Cl of azatidinone ring
		4.564	ARG71A	-C–N of azatidinone ring
		3.645	THR110A	-C- of $–CH_2O$-
		4.213	ASP74A	-C- of $–CH_2O$-
		2.207	ARG157A	–N– of Oxadiazole ring
		1.598	ARG157A	–N– of Oxadiazole ring
		2.299	ARG157A	–N– of Pyrazine
		2.051	ARG154A	–N– of Pyrazine
7e	−56.415	1.906	ARG19A	O of C = O of azatidinone ring
		2.277	ARG19A	O of C = O of azatidinone ring
		2.097	SER109A	–N– of Pyrazine
		2.402	ARG157A	-N- of $-NO_2$ group
		4.762	ARG71A	-C- of $–CH_2O$-
7g	−64.705	3.99	ARG154A	–C– of OCH_3
		4.99	THR110A	-C- of $–CH_2O$-
		2.511	ARG157A	–N– of Oxadiazole ring
		2.54	ARG157A	–N– of Oxadiazole ring
		2.449	ARG157A	–N– of Pyrazine



▶ **Fig. 5** Pharmacophore for synthesized molecules indicating the pharmacophoric features.



▶ **Fig. 6** Distance between pharmacophoric features.

Conclusion

The present research work reports the successful synthesis of various *3-chloro-4-aryl-1-[4-(5-pyrazin-2-yl-*[1,3,4] *oxadiazole-2-ylmethoxy)-phenyl]-azetidin-2-one derivatives* **7(a-h)** and assessment of their antimicrobial and anti-tuberculosis activity. On the basis of results of biological studies, better antimicrobial activity was observed with derivatives having electron withdrawing or electron releasing groups on the aromatic ring. It was found that the para position of such substituents is responsible for better activity. Also, some of the derivatives were found to be potential antimycobacterial agent. The docking score

of the synthesized compounds correlated with the antitubercular activity study. Another important feature of the study was pharmacophore modeling which provided different features of the compounds helpful in binding. So, compound **7b** and **7g** due to their better activity against *M.tuberculosis* H37Rv strain are the best choice for the preparation of new derivatives in order to further improve antimicrobial and antitubercular activity in future.

Acknowledgement

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Conflict of Interest

The authors confirm that this article content has no conflicts of interest.

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Supporting Information

Evaluation and Docking Study of Pyrazine Containing 1, 3, 4-Oxadiazoles Clubbed with Substituted Azetidin-2-one: A New Class of Potential Antimicrobial and Antitubercular

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Material and Methods

Experimental

Reagents: Starting materials, reagents and solvents were purchased from Sigma-Aldrich, USA and Merck, Germany and were used further without any purification.

Equipments: The progress of reaction was confirmed by using thin layer chromatography (TLC) method on silica gel plates (Merck, Germany) of 3x15 cm coated with silica gel G. The spots on TLC plates were visualized by using UV lamp (254nm) and R_f values were calculated. Melting points were determined on digital melting point apparatus (Flora; Perfit, India) and were uncorrected. Elemental analysis (C, H, N) was conducted using Carlo Erba analyzer model 1108. The IR spectra (in NaCl prism) were recorded on Schimadzu FT-IR spectrophotometer using Nujol method. The ^{1}H NMR spectra were recorded (in DMSO) on a BRUKER AVANCE-400 MHz spectrometer using TMS as an internal standard, values of chemical shift were expressed in ppm. The spin multiplicities are indicated by the symbols, s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). Mass spectra (ESI, 70ev) were recorded on Water, Q-TOF Micromass (LC-MS).

Methodology

The scheme for the synthesis of 5-pyrazyl-2-substitutedsulfanyl-1, 3, 4-oxadiazole derivatives from pyrazinoic acid is shown in Figure **1** and corresponding R for **5a–h** is given in Table **1**

Synthesis and spectral data of compounds ***2-7***

Pyrazine-2-carboxylic acid methyl ester **(2)**: Pyrazine-2-carboxylic acid methyl ester was synthesized from pyrazinoic acid following the procedure as reported earlier with slight modifications.[42] Pyrazinoic acid (0.1mol), 60ml methanol and 1.4ml conc. H_2SO_4 were refluxed for 7hrs. The progress of reaction was monitored via TLC till single spot. The mixture was then cooled and poured in crushed ice, was made alkaline, which was followed by extraction with ether. Ether layers were evaporated and kept aside to obtain shiny pale brownish crystals of compound **2** in good yield. Recrystallisation was done using methanol.

2-pyrazylhydrazide **(3)**: Mixture of ester **2** and hydrazine hydrate in 1:1 portion and 30 ml of ethanol were taken in a round bottom flask and refluxed for 4-6 hrs.[42] The progress of reaction was monitored via TLC till single spot. Excess of ethanol was removed by distillation. Product **3** separates out upon cooling in good yield which was filtered and was recrystallised using methanol.

2-chloromethyl-5pyrazino-1, 3, 4-oxadiazole **(4)**: The compound **4** was synthesized by refluxing a mixture of compound **3** (0.001mole), $POCl_3$ (15ml) and chloroacetic acid (0.002mole) on oil bath at 110°C for 7hrs. Progress of the reaction was checked by TLC using toluene: ethyl acetate: methanol (70:20:10) as mobile phase. The excess of $POCl_3$ was distilled off and cooled residue was poured into ice cold water. The content was neutralized with ammonia to obtain crude product **4** which was filtered, dried and recrystallized by using ethanol.

4-[5-pyrazino-1,3,4-oxadiazol-2-yl-methoxy]-phenyl amine **(5)**: Solution of compound **4** (0.01mole) in 15ml methanol part wise was added in 15ml methanolic solution of 4-amino phenol (0.012mole) and KOH (0.15mole). After addition, reaction mixture was

refluxed on water bath for 12hrs. Progress of the reaction was checked by TLC using toluene: ethyl acetate (75:25) as mobile phase. Then the reaction mixture was cooled and poured on crushed ice with stirring. The product compound **5** was filtered, washed well with cold water, then dried and recrystallized with ethanol.

***Arylmethylene-[4-(5-pyrazin-2-yl-[1,3,4]oxadiazole-2-ylmethoxy)-phenyl]-amine derivatives* 6(a-h)**: The compound **6** was synthesized by adding solution of compound **5** (0.1mole), methanol, substituted aldehyde(0.1mole) and few drops of glacial acetic acid followed by reflux on water bath for 4hrs. Progress of the reaction was checked by TLC. The reaction mixture was cooled, poured on ice, recrystallized with methanol to obtain compound **6(a-h)**.

a]*2-[4-[2-chlorobenzylidene]aminophenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole(6a)*: Obtained in 55% as Brownish crystals; mp (°C): 222-224; IR (Nujol, cm^{-1}): 3062 (Ar C-H str), 2900 (C-H str), 1462,1541(Ar C=C str), 1259 (C-O bend),1059 (C-O-C bend in oxadiazole),1672 (C=N), 642(C-Cl mono substituted in benzene), 1574 (CH=N str, azomet); ^{1}H NMR (DMSO, 400MHz, ppm): 8.24-8.39 (m,3H,CH of pyrazine), 5.27(s, 2H, CH_2), 7.01- 7.12 (m,4H, Ar), 7.34-7.71(m,4H, Ar-Cl), 8.54(s1H,N=CH, azomet).

b]*2-[4-[4-chlorobenzylidene]aminophenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole(6b)*: Obtained in 61% as Pale Brownish crystals; mp (°C): 188-190; IR (Nujol, cm^{-1}): 3026 (Ar C-H str), 2928 (C-H str), 1490,1518(Ar C=C str), 1265(C-O bend),1055 (C-O-C bend. in oxadiazole),1677 (C=N), 715(C-Cl monosubstituted in benzene), 1571(CH=N str, azomet); ^{1}H NMR (DMSO, 400MHz, ppm): 8.24-8.38 (m,3H,CH of pyrazine), 5.27(s, 2H, CH_2), 7.01 -7.12 (m,4H, Ar), 7.34-7.71 (m, 4H, Ar-Cl) , 8.54(s1H,N=CH,azomet).

c]*2-[4-[benzylidene]aminophenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole(6c)*: Obtained in 66% as Brownish crystals; mp (°C): 217-218; IR (Nujol, cm^{-1}): 3018 (Ar C-H str), 2920 (C-H str), 1492, 1565(Ar C=C str), 1235 (C-O bend.),1084 (C-O-C bend. in oxadiazole), 1640 (C=N), 1574 (CH=N str, azomet); ^{1}H NMR (DMSO, 400MHz, ppm): 8.20-8.35 (m,3H,CH of pyrazine), 5.27(s, 2H, CH_2),), 7.01-7.78 (m,9H, ArH), 7.34, 8.52(s1H,N=CH, azomet).

d]*2-[4-[4-hydroxybenzylidene]aminophenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole(6d)*: Obtained in 52% as Yellowish Brown crystal; mp (°C): 202-204; IR (Nujol, cm^{-1}): 3030 (Ar C-H str), 2906 (C-H str), 1541, 1579(Ar C=C str), 1263 (C-O bend.), 1070 (C-O-C bend. in oxadiazole),1635 (C=N), 3205 (Ar-OH str), 1569(CH=N str, azomet); ^{1}H NMR (DMSO, 400MHz, ppm): 8.21-8.36 (m,3H,CH of pyrazine), 5.27(s, 2H, CH_2),), 7.01-7.12 (m,4H, ArH), 7.34-7.69 (m, 4H, Ar-OH), 8.52 (s1H,N=CH,azomet), 5.12

(s,1H,C-OH).

e]*2-[4-[3-nitrobenzylidene]aminophenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole(6e)*: Obtained in 72% as Reddish Brown crystals; mp (°C): 179-180; IR (Nujol, cm^{-1}): 3021 (Ar C-H str), 2910 (C-H str),1525(Ar C=C str), 1202 (C-O bend.),1073 (C-O-C str in oxadiazole),1672 (C=N), 1530 (asymAr N-O str.), 1289 (sym Ar N-O bend..), 1571 (CH=N str, azomet); ^{1}H NMR (DMSO, 400MHz, ppm): 8.23-8.39 (m,3H,CH of pyrazine), 5.27 (s, 2H, CH_2), 7.01 -7.12 (m,4H, ArH), 7.34-7.88, (m, 4H, Ar-NO_2), 8.52 (s1H,N=CH,azomet).

f]*2-[4-[4-methylbenzylidene]aminophenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole(6f)*: Obtained in 56% as Dark Yellowish crystals; mp (°C): 182-184; IR (Nujol, cm^{-1}): 3069 (Ar C-H str), 2928 (C-H str), 1360(C-H bend.) 1501, 1581(Ar C=C str), 1263(C-O), 1055 (C-O-C str in oxadiazole), 1672 (C=N), 1573 (CH=N str, azomet); ^{1}H NMR (DMSO, 400MHz, ppm): 8.24-8.39 (m,3H,CH of pyrazine), 5.26(s, 2H, CH_2), 7.01 -7.12 (m,4H, Ar), 7.19-7.85 (m, 4H, Ar- CH_3), 8.55 (s,1H, N= CH azomet),2.38 (s,3H, CH_3).

g]*2-[4-[4-methoxybenzylidene]aminophenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole(6g)*: Obtained in 77% as Pale Brownish crystals; mp (°C): 197-198; IR (Nujol, cm^{-1}): 3190 (Ar C-H str), 2922 (C-H str.), 1455, 1490 (Ar C=C str), 1221(C-O bend.), 1063 (C-O-C str in oxadiazole), 1640 (C=N), 1155(C-O bend. in ether),1571(CH=N str, azomet); ^{1}H NMR (DMSO, 400MHz, ppm): 8.21-8.36 (m,3H,CH of pyrazine), 5.27 (s, 2H, CH_2),), 7.01-7.12 (m,4H, ArH), 6.90-7.86 (m, 4H, Ar- OCH_3), 8.52 (s1H,N=CH,azomet.),3.77 (s,3H, CH_3).

h]*2-[4-[2-nitrobenzylidene]aminophenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole(6h)*: Obtained in 67% Brown crystals; mp (°C): 208-210; IR (Nujol, cm^{-1}): 3010 (Ar C-H str), 2904 (C-H str), 1527(Ar C=C str), 1070 (C-O-C bend. in oxadiazole),1652 (C=N), 1510 (asym Ar N-O str.), 1369 (sym Ar N-O bend.), 1574(CH=N str, azomet); ^{1}H NMR (DMSO, 400MHz, ppm): 8.21-8.35 (m,3H,CH of pyrazine), 5.27(s, 2H, CH_2),), 7.01 & 7.12 (m,4H, ArH), 7.34-8.52 (m, 4H, Ar-NO_2), 8.52(s1H,N=CH, azomet).

***3-chloro-4-aryl-1-[4-(5-pyrazin-2-yl[1,3,4]oxadiazole-2-ylmethoxy)-phenyl]-azetidin-2-one derivatives* 7(a-h)**: Triethylamine (0.005 mol, 0.795 ml) was added to a solution of Schiff's base **6(a-h)** (0.01 mol) in acetone followed by drop wise addition of a solution of chloroacetyl chloride (0.01 mol, 1.13 ml) with stirring. The mixture was refluxed upto 6 hrs and the reaction progress was monitored *via* TLC till single spot. The contents were then poured onto the crushed ice. The solid material was filtered, washed with petroleum ether and recrystallized by ethanol to obtain compounds **7(a-h)**.

a]*2-[4-[4-[2-chlorophenyl]-3-chloroazetidinonyl]phenyloxymethyl]-5-pyrazyl-1,3,4 oxadiazole* (**7a)**: Obtained in 64% as Brownish crystals; mp (°C): 176-177; IR (Nujol,

cm^{-1}): 3026 (Ar C-H str), 2928 (C-H str), 1517(Ar C=C str), 1275(C-O),1055 (C-O-C str in oxadiazole), 1677 (C=N), 642(C-Cl mono substituted in benzene), 1751(C=O str in azetidinone), 734 (C-Cl); ^{1}H NMR (DMSO, 400MHz, ppm): 8.23-8.36 (m,3H,CH of pyrazine), 5.27 (s, 2H, CH_2), 7.05-7.14 (m,4H, phenyl), 7.19-7.42 (m,4H,CH in phenyl), 5.14 (s,1H,CH-Ar), 5.75-5.76 (d,1H, CH-Cl of azetidinone). Ms (m/z): 468(M^+), 470 (M+2) other fragments peak are 320, 290, 214, 196, 179, 163, 147, 103, 79, 69. Anal. Calcd. for $C_{22}H_{15}Cl_2N_5O_3C$, 56.43; H, 3.23; N, 14.96 Found: C,56.47; H, 3.24; N, 14.99.

b]*2-[4-[4-[4-chlorophenyl]-3-chloroazetidinonyl]phenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole* (**7b**): Obtained in 70% as Pale Brownish crystals; mp (°C): 162-164; IR (Nujol, cm^{-1}): 3015 (Ar C-H str), 2900 (C- H str), 1492,1573 (Ar C=C str), 1235 (C-O),1084 (C-O-C str in oxadiazole), 1645 (C=N), 710 (C-Cl monosubstituted in benzene), 1738 (C=O str in azetidinone), 1107.19, 782 (C-Cl); ^{1}H NMR (DMSO, 400MHz, ppm): 8.23-8.37 (m,3H,CH of pyrazine), 5.29 (s, 2H, CH_2), 6.98 -7.14 (m,4H, phenyl), 7.16-7.39 (m,4H,CH in phenyl), 5.18 (s,1H,CH-Ar), 5.76-7.77 (d,1H, CH-Cl of azetidinone). Ms (m/z): 468(M^+), 470(M+2) other fragments peak are 320, 290, 214, 196, 179, 163, 147, 103, 79, 69. Anal. Calcd. for $C_{22}H_{15}Cl_2N_5O_3C$, 56.43; H, 3.23; N,14.96 Found: C,56.47; H, 3.27; N, 15.00.

c] *2-[4-[4-phenyl-3-chloroazetidinonyl]phenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole* (**7c**): Obtained in 58% as Brownish crystals; mp (°C): 209-210; IR (Nujol, cm^{-1}): 3050 (Ar C-H str), 2926 (C- H str), 1511, 1571(Ar C=C str), 1273(C-O), 1086 (C-O-C str in oxadiazole), 1637 (C=N), 1739 (C=O str in azetidinone), 678 (C-Cl); ^{1}H NMR (DMSO, 400MHz, ppm): 8.23-8.37 (m,3H,CH of pyrazine), 5.29 (s, 2H, CH_2), 6.99 -7.14 (m,4H, phenyl), 7.16-7.37 (m,5H,CH in phenyl), 5.19 (s,1H,CH-Ar), 5.75-5.76 (d,1H, CH-Cl of azetidinone). Ms (m/z): 433(M^+), 435 (M+2) other fragments peak are 253, 180, 177, 161, 152,147, 103, 79, 68. Anal. Calcd. for $C_{22}H_{16}ClN_5O_3$ C, 60.91; H, 3.72; N,16.14 Found: C,60.89; H, 3.75;N,16.18.

d]*2-[4-[4-[4-hydroxyphenyl]-3-chloroazetidinonyl]phenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole* (**7d**): Obtained in 55% as Light Brown crystals; mp (°C): 175-176; IR (Nujol, cm^{-1}): 3026 (Ar C-H str), 2919 (C- H str), 1498 (Ar C=C str), 1192(C-O), 1093 (C-O-C str in oxadiazole), 1670 (C=N), 1756 (C=O str in azetidinone), 732(C-Cl), 3208 (Ar-OH str). ; ^{1}H NMR (DMSO, 400MHz, ppm): 8.23-8.36 (m,3H,CH of pyrazine), 5.29(s, 2H, CH_2), 6.89-7.15 (m,4H, phenyl), 7.17-7.38 (m,4H,CH in phenyl), 5.18 (s,1H,CH-Ar), 5.75-5.76 (d,1H, CH-Cl of azetidinone), 5.15 (s,1H,aromatic C-OH). Ms (m/z): 449(M^+), 451 (M+2) other fragments peak are 302, 272, 253,196, 168, 177, 161, 147, 103, 79, 68. Anal. Calcd. for $C_{22}H_{16}ClN_5O_4C$, 58.74; H, 3.59; N, 15.57 Found: C,58.77; H, 3.63; N,15.55.

e]*2-[4-[4-[3-nitrophenyl]-3-chloroazetidinonyl]phenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole* (**7e**): Obtained in 61% as Dark Brown crystals; mp (°C): 140-142; IR (Nujol, cm^{-1}): 3065 (Ar C- H str), 2922 (C-Haliph), 1531, 1591(Ar C=C str), 1273 (C-O), 1053 (C-O-C str in oxadiazole), 1754 (C=O str in azetidinone), 710 (C-Cl), 1531 (asymAr N-Ostr.), 1394 (sym Ar N-Ostr.) ; ^{1}H NMR (DMSO, 400MHz, ppm): 8.23-8.36 (m,3H,CH of pyrazine), 5.29 (s, 2H, CH_2), 6.88 -7.14 (m,4H, phenyl), 7.57-8.11 (m,4H,CH in phenyl), 5.18 (s,1H,CH-Ar), 5.76-7.77 (d,1H, CH-Cl of azetidinone). Ms (m/z): 478 (M^+), 480 (M+2) other fragments peak are 331, 301, 253, 225, 177, 161, 147, 122, 103, 79, 68. Anal. Calcd. for $C_{22}H_{15}ClN_6O_5C$, 55.18; H, 3.16; N, 17.55 Found: C, 55.22; H, 3.19; N, 17.59.

f]2-[4-[4-[4-methylphenyl]-3-chloroazetidinonyl]phenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole (**7f**): Obtained in 54% Yellowish crystals; mp (°C): 152-154; IR (Nujol, cm^{-1}): 2993 (Ar C-H str), 2807 (C- H str), 1367(C-Hstr.) 1455, 1498 (Ar C=C str), 1201(C-O), 1089 (C-O-C str in oxadiazole), 1646 (C=N), 1737 (C=O str in azetidinone), 720 (C-Cl).; ^{1}H NMR (DMSO, 400MHz, ppm): 8.22-8.37 (m,3H,CH of pyrazine) , 5.29(s, 2H, CH_2), 6.96- 7.18 (m,4H, phenyl), 7.19-7.21 (m,4H, CH in phenyl), 5.16 (s,1H,CH-Ar) 5.76-7.77 (d,1H, CH-Cl of azetidinone), 2.39 (s,3H, CH_3). Ms (m/z): 447(M^+), 449(M+2) other fragments peak are 300, 270, 253, 194, 177, 166, 161, 147, 103, 91, 79, 68. Anal. Calcd. for $C_{25}H_{26}ClN_5O_3$ C, 62.56; H, 5.46; N, 14.59 Found: C, 62.60; H, 5.49; N, 14.63.

g]*2-[4-[4-[4-methoxyphenyl]-3-chloroazetidinonyl]phenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole* (**7g**): Obtained in 62% as Pale Brownish crystals; mp (°C): 134-135; IR (Nujol, cm^{-1}): 3010 (Ar C-H str), 2944 (C- H str), 1494, 1527 (Ar C=C str), 1081 (C-O-C str in oxadiazole), 1651 (C=N), 1789(C=O str in azetidinone), 724 (C-Cl), 1162 (C-O str. in ether); ^{1}H NMR (DMSO, 400MHz, ppm): 8.23-8.36 (m,3H,CH of pyrazine), 5.29 (s, 2H, CH_2), 6.99 -7.14 (m,4H, phenyl), 7.16-7.36 (m,4H,CH in phenyl), 5.20 (s,1H,CH-Ar), 5.74-5.75 (d,1H, CH-Cl of azetidinone), 3.77 (s,3H, CH_3). Ms (m/z): 463(M^+), 465(M+2) other fragments peak are 320, 290, 253, 214, 196, 177, 161, 147, 103, 79, 68. Anal. Calcd. for $C_{23}H_{18}ClN_5O_4$ C, 59.55; H, 3.91;N, 15.10 Found: C, 59.59; H, 3.90;N, 15.13.

h]*2-[4-[4-[2-nitrophenyl]-3-chloroazetidinonyl]phenyloxymethyl]-5-pyrazyl-1,3,4-oxadiazole* (**7h**): Obtained in 67% as Brown crystals; mp (°C): 171-172; IR (Nujol, cm^{-1}): 3065 (Ar C-H str), 2922 (C- H str), 1531, 1581(Ar C=C str), 1273(C-O),1053 (C-O-C str in oxadiazole), 1655 (C=N), 1757 (C=O str in azetidinone), 701(C-Cl), 1531(asym Ar N-Ostr.), 1384(sym Ar N-Ostr.).; ^{1}H NMR (DMSO, 400MHz, ppm): 8.23-8.37 (m ,3H,CH of pyrazine), 5.27(s, 2H, CH_2), 6.91-7.18 (m, 4H, phenyl), 7.51-8.21 (m,4H,CH in phenyl), 5.18 (s,1H,CH-Ar), 5.76-7.77 (d,1H, CH-Cl of azetidinone). Ms (m/z): 478(M^+), 480(M+2) other fragments peak are 316, 286, 253, 210, 182, 177, 161, 147, 103, 79, 68. Anal. Calcd. for $C_{23}H_{19}ClN_6O_5$C, 55.82; H, 3.87; N, 16.98 Found: C, 55.86; H, 3.91; N, 17.02.

Antimicrobial Evaluation

In vitro anti-bacterial activity

Test strain

The various test microorganisms *Bacillus subtilis* (MTCC121), *Staphylococcus aureus* (MTCC737), *Escherichia coli* (MTCC1687) and *Pseudomonas aeruginosa* (MTCC1688) were obtained from the Department of Microbiology, MM Institute of Medical Sciences and Research, MMU, Ambala, India, and used to evaluate the antibacterial potential of the synthesized compounds. Cultures of the test bacteria were grown on Nutrient agar

(NA) slant and incubated at 37°C for 1-3 days, depending upon the growth period of bacteria.

Evaluation technique

The antibacterial evaluation was carried out by using agar cup-plate method.[43,44] Sample solution used was prepared by dissolving each test compound (50 mg) in DMSO (50 ml, 1000 µg /ml). For all compounds the sample size was fixed at 0.1 ml. Cups of 6 mm diameter were scooped out using a sterilized cork borer in agar medium contained in a petri dish which was previously inoculated with the microorganisms. The test compound solution (0.1 ml) was added in the cups and petri dishes were incubated at 37°C for 48 hours. Ampicillin was used as reference drugs and dimethylformamide as a negative control. Zone of inhibition produced by each compound was measured in mm and the averages based on triplicate measurements were recorded.

In vitro anti-fungal activity

Test strain

The clinical isolates of *Aspergillus niger* (MTCC281) and *Candida albicans* (MTCC183) were obtained from the Department of Microbiology, MM Institute of Medical Sciences and Research, MMU, Ambala, India. The isolate was subcultured on Sabouraud Dextrose Agar (Hi-Media) at 37°C for 48-72 hours.

Evaluation technique

All the synthesized compounds were assessed for their *in vitro* anti-fungal activity against pathogenic fungi using cup plate method with Sabouraud's dextrose agar

media. [43, 44] Different concentrations of compounds in DMSO were added into each labeled well (DMSO, solvent as a control) and incubated at 37°C for 72 hours. Experiment was carried in triplicate. The zone of inhibition was calculated by measuring the diameter of the zone using Miconazole as standard drug.

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) was calculated using a method as reported earlier [43, 44] Nutrient agar was prepared first, sterilized, and then cooled to 45°C with gentle shaking. It was inoculated with microorganism culture before pouring into the sterilized petri dishes. The culture media were allowed to set and thereafter the cups were made by punching into the agar surface with sterile cork borer and scooping out the punched part of the agar. Each test compounds (0.1ml) was added into the cups. Two fold diluted solutions of the synthesized compounds (6.5, 12.5, 25, 50, 100, 200, 400, 800, and 1600 µg/ml) and reference drugs were used. The drug solutions were allowed to diffuse into the medium for some time. The plates were incubated at 30-35°C for 24-48 hours. MIC values were determined at the end of the incubation period.

***Anti-tubercular activity*:**

Drug susceptibility of MIC of the synthesized compounds against *M. tuberculosis* $H_{37}RV$ (MTCC200) were performed by Lowenstein-Jensen (L.J) Agar (MIC) method,[45] where primary 1000, 500 and 250 mg/ml and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25 and 3.25 mg/ml dilutions of each test compound were added to liquid L. J. Medium and then media were sterilized by inspissations method. A culture of M. tuberculosis H37Rv

growing on L. J. medium was harvested in 0.85% saline in bijou bottles. First stock solution of 2000 mg/ml concentration of all test compounds were prepared in DMSO.These tubes were then incubated at 37°C for 24 h followed by streaking of M. tuberculosis H37Rv (5×104 bacilli per tube). Incubation of these tubes was then done at 37°C. After 12 days, 22 days and finally 28 days of incubation growth of bacilli was seen. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M.tuberculosis* H_{37}RV (MTCC200) bacillus. The concentration at which no development of colonies occurred or <26 colonies was taken as MIC concentration of test compound. Also the strain *M.tuberculosis* H_{37}RV (MTCC200) was tested with drug isoniazide and rifampicin as standard drugs.

List of Abbreviations

Aliph- Aliphatic
Anal.- Analysis
Bend- Bending
Calcd- Calculated
$CDCl_3$- Deuterated chloroform
DMSO- Dimethyl sulfoxide
FT-IR- Fourier transform infra red
INH- Isoniazide
L.J.- Lowenstein-Jensen
LC-MS- Liquid chromatography–mass spectrometry
m/z- Mass / charge
MDR- Multi drug resistant
MeOH- Methanol
MHz- Megahertz
MIC- Minimum inhibitory concentration
MP- Melting point
Ms- Mass spectrum
NA- Nutrient agar
NMR- Nuclear magnetic resonance
PAS- p-aminosalicylic acid
ppm- Parts per million
PZA- Pyrazinamide
Str- Stretching
TB- Tuberculosis
TLC- Thin layer chromatography
TMS- Tetramethylsilane
UV- Ultra violet
XDR- Extensively drug resistant