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ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · JANUARY 2013

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2012.12.055 · Source: PubMed

European Journal of Medicinal Chemistry 62 (2013) 677–687

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

New 2-benzylsulfanyl-nicotinic acid based 1,3,4-oxadiazoles: Their synthesis and biological evaluation

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ARTICLE INFO

Article history:
Received 20 August 2012
Received in revised form
13 December 2012
Accepted 23 December 2012
Available online 23 January 2013

Keywords:
2-Benzylsulfanyl-nicotinic acid
1,3,4-Oxadiazoles
Antimicrobial
Antitubercular activity

ABSTRACT

A novel series of 5-(2-benzylsulfanyl-pyridin-3-yl)-2-(substituted)-sulfanyl-1,3,4-oxadiazoles **6a**–**j** were synthesized from key intermediate 5-(2-benzylsulfanyl-pyridin-3-yl)-3*H*-[1,3,4]oxadiazole-2-thione **5**. Nucleophilic substitution reactions with different electrophiles (E+), such as haloacetate and haloalkyl groups, were performed to get target compounds **6a**–**j**. Compounds were characterized by NMR, mass, IR spectra and C, H, N analyses. All compounds were evaluated for their antimicrobial and antimycobacterial activities; selected analogs were screened for their anticancer activity on 60 tumor cell lines at single dose 1.00^{-5} M. Unfortunately, none of the compounds showed a significant antitumor activity on 60 human tumor cell lines. However, compounds **6g** and **6f** with benzothiazole moiety (12.5 and 25 μg/ml) showed promising activity against *Escherichia coli* compared to ampicillin; compounds **6d**, **6j** bearing triazole and morpholine, respectively, showed promising antitubercular activity (25 μg/ml) compared to rifampicin.

1. Introduction

The emergence of "Antimicrobial resistance" (AMR) is complex and serious health problem. The increasing incidence of infection caused by rapid development of AMR has reached an alarming level and became a complex and challenging health problem. Resistant microorganisms (they include bacteria, viruses and some parasites) are able to withstand attack by antimicrobial medicines such as antibiotics, antiviral and antitumor drugs. Mortality has been increasing in the developing countries highly affected by infectious diseases especially tuberculosis (TB) due to the emergence of AMR [1]. TB results in an estimated 1.7 million deaths each year and the worldwide number of new cases (more than 9 million) is higher than at any other time in history [2]. Further, TB in association with human immunodeficiency virus (HIV-1) infection has been viewed as an emerging global epidemic.

On the other hand, the development of resistance to chemotherapeutic agents is a common obstacle in the treatment of different types of cancers [3]. TB and cancer are called the 'big killer and intractable diseases'. AMR is one of the most important factors contributing to the failure of current therapies for TB and cancer. Hence, there is an urgent need to develop potent, fast-acting, new classes of agents likely to be unaffected by existing resistance mechanisms with low toxicity. Heterocyclic compounds play an important role in an untiring effort aimed at developing new antimicrobial and antitumor agents with new mechanism of action.

Pyridine is one of the most ubiquitous heterocyclic compounds in nature (e.g., in the coenzyme vitamin B_6 family and in numerous alkaloids) and its central role as versatile building block in the synthesis of natural products as well as biologically active compounds has led to a continued interest in the practical synthesis of pyridine derivatives [4]. Pyridine bases are widely used in pharmaceuticals as nicotinamides and nicotinic acid derivatives, especially; pyridine-3-carboxylic acid derivatives are very useful as antimicrobial, fungicidal, agricultural and industrial chemicals and have extensively been documented for their wide variety of pharmacological activities such as antimicrobial, analgesic, anti-inflammatory, anti-HIV and antitubercular [5–9].

On the other hand, 1,3,4-oxadiazoles are thermally stable and neutral heteroaromatic molecules and associated with potent pharmacological activity due to the presence of toxophoric –N=C–O– linkage [10]. 1,3,4-Oxadiazoles display quite a broad spectrum of biological activities such as antimicrobial [11], antimycobacterial [12], antiviral [13] and anticancer [14]. Further, they are very good

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http://dx.doi.org/10.1016/j.ejmech.2012.12.055

bioisosteres of amides and esters, which can contribute substantially in increasing pharmacological activity by participating in hydrogen bonding interactions with the receptors [15]. In addition, the free SH has a strong nucleophilic character and can easily be derivatized by many electrophiles (E+) showing different physicochemical properties, such as haloacetate and haloalkyl groups. Besides, the study of literature reveals that number of pyridine-oxadiazole hybrids bearing different substituents via linker like, zibotentan, especially, that is in late clinical trial as an anticancer agent, showed important biological activities (Fig. 1) [16].

On the basis of literature studies, it was supposed that presence of carboxamide, acyl or alkyl linkage on oxadiazole moiety leads to an increase in biological activity, especially in case of *Mycobacterium tuberculosis*; linker plays an important role on their penetration into the microbial cell and also the synthesis of heterocyclic compounds containing multi-structure in a molecule has received much attention in recent years [17]. Moreover, the diverse biological activity data of pyridines and 1,3,4-oxadiazoles encouraged us to envisage the combination of both pharmacophores in a compact system and to synthesize a new series of pyridine-oxadiazoles substituted with haloacetate and different heterocyclic moieties at C-2 position of oxadiazole nucleus. For preliminary research we selected thiazole, benzothiazole, pyrimidine, triazole, piperazine, morpholine and epichlorohydrin heterocyclic groups as substituents to incorporate with 1,3,4-oxadiazole-pyridine framework due to their diverse biological properties [13,18]. Therefore, it was planned to synthesize hybrid compounds that resembles zibotentan structure and comprise both pyridine and oxadiazole substituted with the aforementioned heterocyclic ring systems via two carbon acyl or aliphatic linker. Such hybridization was designed in order to investigate the effect of such structural variation on biological activities [19].

In view of above mentioned findings and as a part of our general search for biologically active newer pyridine-oxadiazole hybrid motif, we report herein the synthesis, antimicrobial, antitubercular and antitumor activity of new series of 5-(2-benzylsulfanyl-pyridin-3-yl)-3*H*-[1,3,4]oxadiazole-2-thione (5) and its *S*-alkyl derivative **6a**–**j** (Fig. 2).

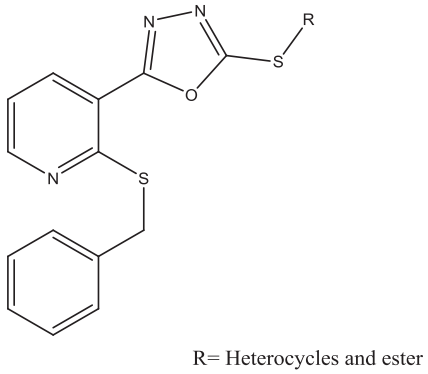


Fig. 2. Skeleton of target compounds.

2. Chemistry

Esterification of 2-benzylsulfanyl-nicotinic acid with methanol and few drops of concentrated sulfuric acid afforded 2-benzylsulfanyl-nicotinic acid methyl ester **3**. The key intermediate in the synthesis of new oxadiazoles bearing different heterocycles **6a**–**j** is 2-benzylsulfanyl-nicotinic acid hydrazide **4**, which was prepared by hydrazinolysis of **3** with hydrazine hydrate. 5-(2-Benzylsulfanyl-pyridin-3-yl)-3*H*-[1,3,4]oxadiazole-2-thione **5** was obtained by intramolecular cyclization of **4** with carbon disulfide in the presence of potassium hydroxide and 95% ethanol under reflux followed by acidification with dilute hydrochloric acid as outlined in Scheme 1.

The synthesis of 2-chloro-*N*-4-(substituted)-acetamides **1a**–**g** was accomplished by reacting chloroacetyl chloride with corresponding hetero amines **a**–**g** in corresponding solvent and reaction condition as outlined Scheme 2.

Final compounds 5-(2-benzylsulfanyl-pyridin-3-yl)-2-(substituted)-sulfanyl-1,3,4-oxadiazoles **6a**–**j** were synthesized by refluxing equimolar mixture of **5**, with different synthesized hetero acetamides **1a**–**g** and other substituents in appropriate solvent and reaction condition as shown in Scheme 3.

All the final compounds **6a**–**j** was reported for the first time and gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

3. Biology

The MICs of synthesized compounds were carried out by broth microdilution method as described by Rattan [20]. Antibacterial activity was screened against 2 g positive (*Staphylococcus aureus* MTCC 96 and *Streptococcus pyogenes* MTCC 442) and 2 g negative (*Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 2488) bacteria by using ampicillin as a standard antibacterial agent. Antifungal activity was screened against three fungal species *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus*

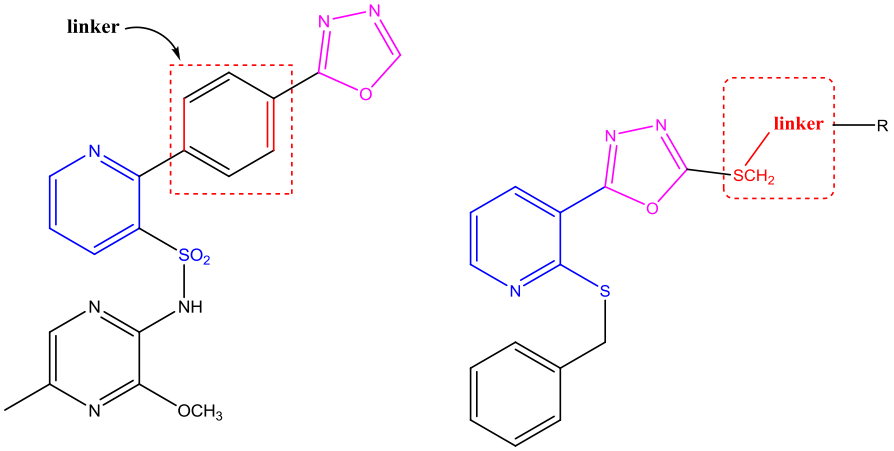
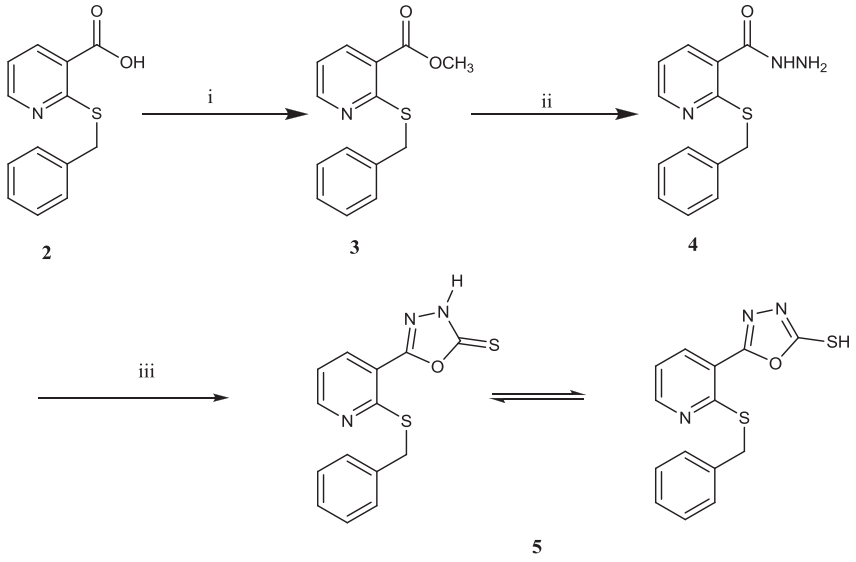


Fig. 1. Resemblances between zibotentan (anticancer agent) and target compounds **6a**–**g**.



Scheme 1. Synthetic protocol for intermediate 5-(2-benzylsulfanyl-pyridin-3-yl)-3*H*-[1,3,4]oxadiazole-2-thione **5**. (i) Methanol, conc. H_2SO_4, refluxed 10 h; (ii) $N_2H_4 \cdot 2H_2O$, methanol, refluxed 5 h; (iii) CS_2/KOH, methanol, refluxed 5 h.

clavatus MTCC 1323, and griseofulvin was used as a standard antifungal agent. The antimicrobial screening data are shown in Table 1.

All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and tested against above mentioned known drugs. Mueller Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test. Inoculum size for test strain was adjusted to 10^8 Colony Forming Unit (CFU) per milliliter by comparing the turbidity. DMSO was used as diluents to get desired concentration of drugs to test upon standard bacterial strains. Minimum inhibitory concentration (MIC) of compounds was determined against *M. tuberculosis* $H_{37}Rv$ strain by using Lowenstein-Jensen medium (conventional method) as described by Rattan and is shown in Table 2 [20].

National Cancer Institute (NCI) of United States selected six compounds to be evaluated for their *in vitro* antitumor activity. The data of screening at a single high dose (10^{-5} M) on 60 human tumor cell lines is reported as mean graph of the percent growth of treated cells in Table 2 using procedure reported by NCI [21].

4. Result and discussion

4.1. Analytical results

The IR spectra of the compound **4** exhibited broad stretching bands at around 3284, 3184 cm^{-1} due to NH/NH_2 and strong stretching band at 1636 cm^{-1} due to amide −C═O group. The ^1H NMR spectra showed sharp singlet at δ 9.60 due to NH and broad singlet at δ 4.19 due to NH_2 which vanished on D_2O exchange. Two doublets at δ 8.46 and δ 7.69 integrating for each proton were attributable to the C_6 and C_4 protons of pyridine moiety, respectively. The rest of the aromatic protons of pyridine and benzene moieties appeared as multiplets between δ 7.33 and δ 7.02. The SCH_2 protons resonated as a singlet at δ 4.33.

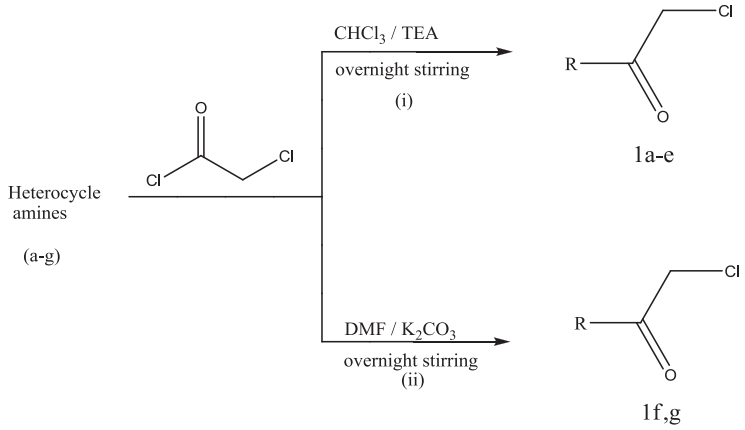
When compound **4** was converted to the corresponding oxadiazole **5** the absorption bands due to NH/NH_2 and amide carbonyl group disappeared in IR spectrum of **5**, instead, new absorption bands due to NH, C═N, C═S, C−O−C were observed at 3266, 1605, 1234, 1326 and 1164 cm^{-1}, respectively. Furthermore, the ^1H NMR spectrum of compound **5** showed no signal belonging to amine and amide; while new signal belonging to thiol−thione tautomerism was recorded at δ 14.67 and also in the ^{13}C NMR spectrum the C-2 and C-5 carbon atom of 1,3,4-oxadiazole ring at δ 157.50 and δ 136.37, respectively.

In general, for compounds **6a**–**j** the sharp stretching band at 3266, 1234 cm^{-1} due to NH and C═S of oxadiazole ring, respectively disappeared indicating *S*-alkylation and also the ^1H NMR spectrum of compounds **6a**–**j** showed the presence of new peak between δ 3.72–4.67 for the SCH_2 group indicating the formation of *S*-alkyl derivative. Similarly, the ^{13}C NMR spectrum of **6a**–**j** showed the absence of the C═S signal at δ 181.22. However in compound **4** this was present which is strong evidence for *S*-alkylation. In particular, the ^1H NMR spectrum of compound **6h** showed new additional signals derived from ester group which were observed at δ 1.16–1.19 (−$OCH_2\underline{CH_3}$) and δ 4.11–4.17 (−$O\underline{CH_2}CH_3$) integrating for three protons and two protons respectively, and also in the ^{13}C NMR spectrum this group resonated at δ 13.83 and δ 61.53, respectively.

4.2. Biological results

4.2.1. Antimicrobial activity

The investigation of antibacterial screening data is summarized in Table 1. Results that showed MIC value less than that of standard drug were considered promising, results reveal that 2-chloro-*N*-4-(substituted)-acetamides showed moderate activity against all the bacterial strain except compound **1f**, **1g** having benzothiazole moiety showed good activity against *S. aureus* , *E. coli* and *P. aeruginosa*. 2-Benzylsulfanyl-nicotinic acid hydrazide **4** exhibited good activity against *S. aureus* (125 μg/ml), while 1,3,4-oxadiazole **5** was found to be more active than hydrazide **4** at an MIC value of 100 μg/ml against *S. aureus*, *S. pyogenes* and *E. coli*. The antibacterial screening results reveal that most of the final compounds **6b**, **6c**, **6d**, **6e** and **6g** showed good bacterial inhibition (250 μg/ml) while compounds **6a** and **6f** having thiazole and benzothiazole, respectively, possessed pronounced activity (100–125 μg/ml) against *S. aureus*. Compound **6i** bearing epichlorohydrin exhibited highest activity (62.5 μg/ml) against *S. aureus* and *S. pyogenes* whereas compounds **6b**, **6d**, **6e** and **6f** displayed good activity (100 μg/ml) against *S. pyogenes*. Compound **6g** clubbed with benzothiazole



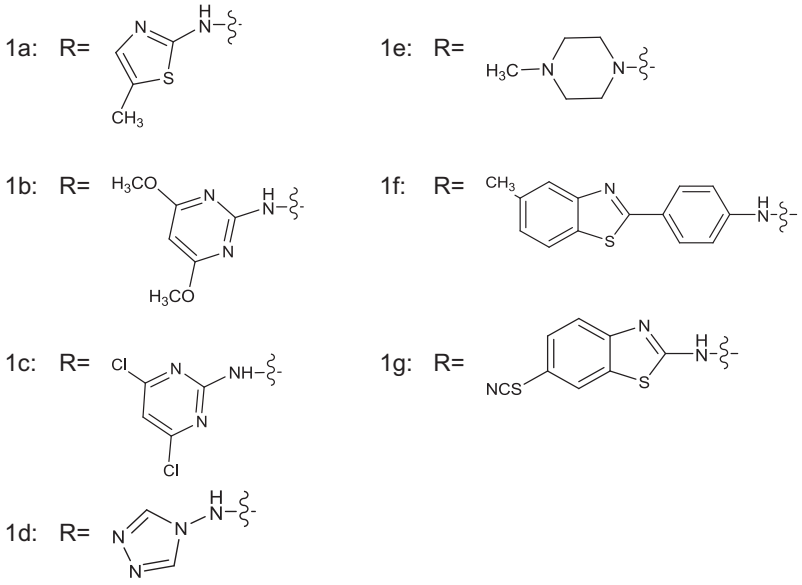
Hetero amines:

a = 5-Methyl-thiazol-2-ylamine, e= 1-Methyl-piperazine

b= 4,6-Dimethoxy-pyrimidin-2-ylamine, f= 4-(5-Methyl-benzothiazol-2-yl)-phenylamine

c= 4,6-Dichloro-pyrimidin-2-ylamine, g= 6-Thiocyanato-benzothiazol-2-ylamine

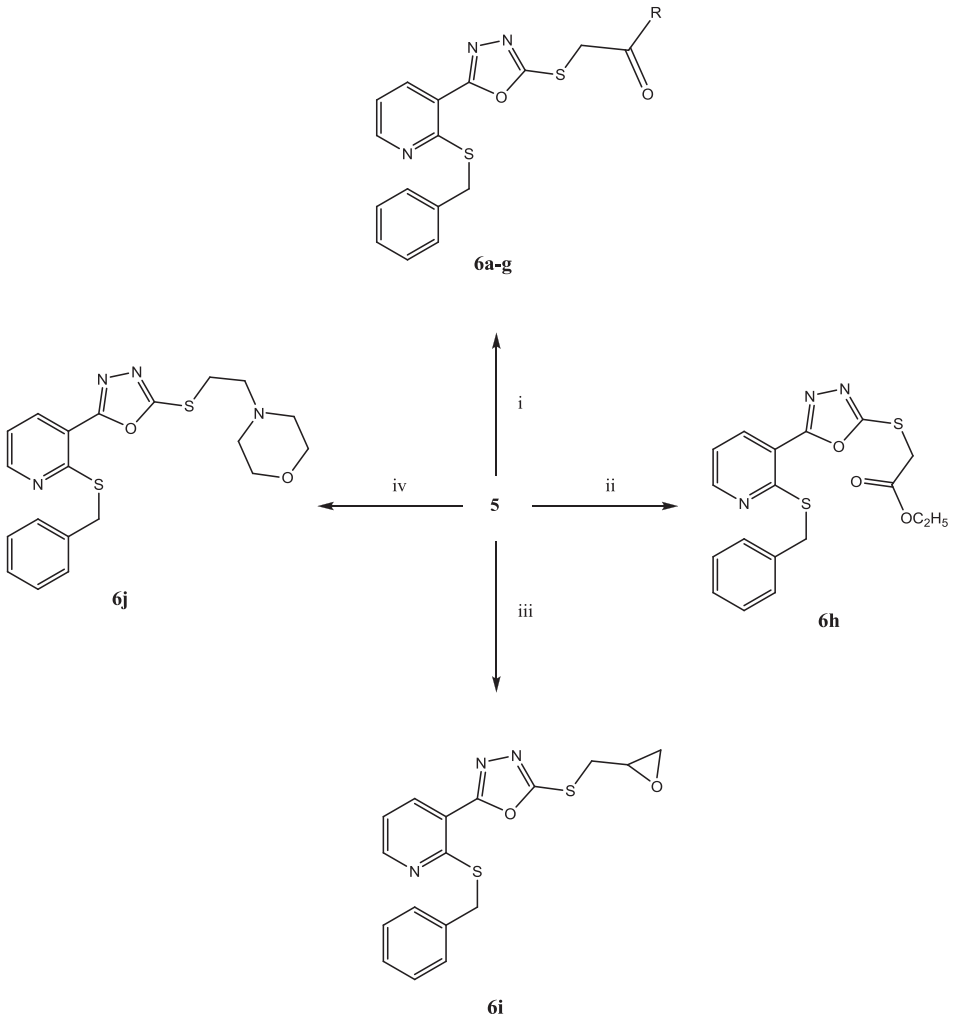
d= [1,2,4]Triazol-4-ylamine



Scheme 2. Synthetic protocol for intermediates 2-chloro-*N*-4-(substituted)-acetamides **1a**–**g** (i) chloro-acetyl chloride, chloroform, TEA, overnight stirring; (ii) chloro-acetyl chloride, DMF, K_2CO_3, overnight stirring.

having thiocyanato group was found to be the most active compound at an MIC value of 12.5 μg/ml as well as compounds **6f** and **6a** also showed higher activity at MIC value of 25 μg/ml and 62.5 μg/ml, respectively, against *E. coli*. Compounds **6a**, **6f** and **6g** having thiazole, benzothiazole and thiocyanato benzothiazole, respectively, showed higher activity (62.5 μg/ml) whereas others displayed moderate activity against *P. aeruginosa*. From the results, it can be concluded that compound **6i** showed significant activity toward gram positive bacteria whereas **6g** exhibited significant activity against gram negative bacteria. Compounds **6a** and **6f** were found to exhibit promising activity higher than that of ampicillin against both gram positive and gram negative bacteria.

The investigation of antifungal screening data is summarized in Table 1. Results reveal that, the synthesized compounds showed variable degree of inhibition against the tested fungal species, 2-chloro-*N*-4-(substituted)-acetamides **1a**–**c**,**1g** possessed good activity (200–500 μg/ml) against *C. albicans* except compounds **1d** and **1f**. None of the substituted acetamides **1a**–**g** showed any significant antifungal activity against *A. niger* and *A. clavatus* while hydrazide **4** exhibited good activity (500 μg/ml) against *C. albicans* and oxadiazole **5** showed moderate activity (200 μg/ml) against *A. clavatus*. Compound **6d** having triazole substituent exhibited better activity (100 μg/ml) whereas other compounds possessed good activity (200–500 μg/ml) except compounds **6c**, **6h** and **6j** against *C. albicans*. All the compounds showed weak activity against *A. niger* and *A. clavatus* except **6g** having thiocyanato benzothiazole that was found to be good active compound at an MIC value of 100 μg/ml against *A. clavatus*.



Scheme 3. Synthetic protocol for 5-(2-benzylsulfanyl-pyridin-3-yl)-2-(substituted)-sulfanyl-1,3,4-oxadiazoles **6a**–**j** (i) **1a**–**g**, DMF, K_2CO_3; (ii) ethyl bromoacetate, acetone, K_2CO_3, refluxed 4 h; (iii) epichlorohydrin, methanol, KOH, refluxed 5 h; (iv) 4-(2-chloro-ethyl)-morpholine, ethanol, KOH, refluxed 8 h.

4.2.2. Antitubercular activity

The investigation of antitubercular activity screening data is summarized in Table 1. The encouraging results from the antibacterial studies impelled us to go for preliminary screening of synthesized compounds against *M. tuberculosis* $H_{37}Rv$. Rifampicin (40 μg/ml) was used as a reference drug because rifampicin (RIF) is first-line antibiotic in the treatment of tuberculosis (TB), leprosy and a growing number of Gram-positive bacteria such as multidrug resistant *S. aureus* and used as dual antitubercular–antibacterial agents. The data show compounds **6d**, **6j** bearing triazole and morpholine, respectively, as the most active amongst the tested compounds and it was also found to exhibit higher antitubercular activity (25 μg/ml) in comparison to rifampicin (40 μg/ml) against *M. tuberculosis* $H_{37}Rv$. Compounds **6a**, **6f** and **6g** showed comparable activity (50–62.5 μg/ml) which is attributed due to thiazole, and benzothiazole substituents.

Due to the better activity against tested microorganisms, compounds **6a**, **6d**, **6g**, **6i** and **6j** have been selected for further development and biological studies like antiprotozoal activity, cytotoxicity, antimicrobial activity against resistant gram-positive organisms such as vancomycin-resistant *Escherichia faecium* (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP), methicillin-resistant *S. aureus* (MRSA) to acquire more information about structure–activity relationships in our laboratories.

4.2.3. Antitumor activity

NCI of United States selected compounds **6a**, **6b**, **6c**, **6e**, **6f** and **6j** to be evaluated for their *in vitro* anticancer activity Table 2. Anticancer activity results show that only compounds **6a** and **6e** have a growth inhibitory (GI) > 50%. Interestingly, compound **6a** showed selectivity on line leukemia CCRF-CEM, HL-60(TB) and MOLT-4 with GI values of 62.16, 50.23 and 58.22%, respectively; on non-small cell lung cancer NCI-H522 show values of 81.51%; on colon cancer SW-620 with 55.08%; renal cancer 786-0 with 77.81%; and breast cancer MDA-MB231/ATCC 88.98 and T-47D with 62.55%. Compound **6e** showed selectivity on line leukemia MOLT-4 with GI values of 51.6%; non-small cell lung cancer with 56.74 and renal cancer UO-31 with 69.13%. Unfortunately, none of the compounds showed a significant growth inhibition for evaluation against the 60 cell panel at five concentration levels by NCI.

5. Conclusion

We herein report the successful synthesis of (**6a**–**j**), new pyridine-oxadiazoles substituted with heterocyclic and aliphatic moieties, starting from commercially available 2-mercaptonicotinic acid. They have been characterized by spectral studies. All the synthesized compounds have been investigated for their antimicrobial and antimycobacterial activities; selected compounds

Table 1
Minimum inhibitory concentrations (MICs, μg/ml).

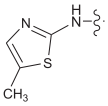
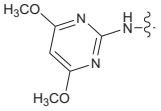
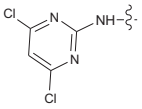
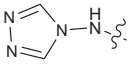
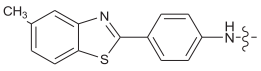
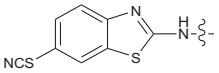
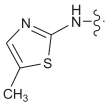
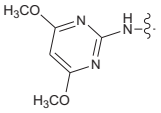
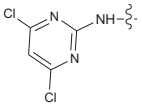
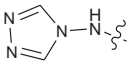
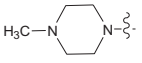
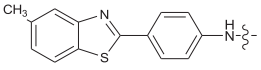
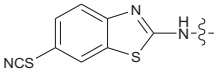
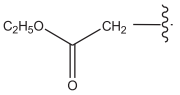
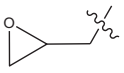
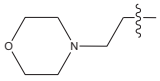
Compound	R	Gram positive bacteria		Gram negative bacteria		Fungal species			*M. tuberculosis* $H_{37}Rv$ (% inhibition)
		S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*	*A. clavatus*	
		MTCC-96	MTCC-443	MTCC-442	MTCC-2488	MTCC-227	MTCC-282	MTCC-323	
1a		500	250	250	250	200	500	500	250 (97%)
1b		500	100	125	125	500	1000	>1000	250 (98%)
1c		500	200	200	500	250	>1000	>1000	500 (88%)
1d		500	250	250	250	1000	>1000	>1000	1000 (90%)
1f		250	125	100	100	1000	>1000	500	250 (99%)
1g		250	125	100	100	500	1000	500	200 (99%)
4	–	125	125	125	200	500	500	250	250 (99%)
5	H	100	100	100	125	1000	1000	200	100 (99%)
6a		125	200	62.5	62.5	250	1000	1000	50 (99%)
6b		250	100	125	250	500	500	500	250 (99%)
6c		250	200	200	200	1000	500	500	100 (98%)
6d		250	100	125	125	100	1000	500	25 (99%)
6e		250	100	125	200	500	500	1000	250 (97%)
6f		100	100	25	62.5	250	1000	500	62.5 (99%)
6g		250	250	12.5	62.5	500	100	250	50 (99%)
6h		500	500	125	200	1000	1000	1000	100 (98%)
6i		62.5	62.5	250	125	500	1000	1000	500 (97%)

Table 1 (*continued*)

Compound	R	Gram positive bacteria		Gram negative bacteria		Fungal species			*M. tuberculosis* $H_{37}Rv$ (% inhibition)
		S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*	*A. clavatus*	
		MTCC-96	MTCC-443	MTCC-442	MTCC-2488	MTCC-227	MTCC-282	MTCC-323	
6j		500	200	200	200	1000	1000	1000	25 (99%)
Ampicillin		250	100	100	100	–	–	–	–
Griseofulvin		–	–	–	–	500	100	100	–
Rifampicin									40 (99%)

were evaluated for antitumor activity. The antimicrobial results indicate that the 2-thiobenzyl nicotinic-oxadiazoles substituted with thiazole, benzothiazole and epichlorohydrin emerged as promising antimicrobials showing better activity while analogs bearing triazole substituent showed better antifungal activity. The antitubercular results reveal that compounds **6d** and **6j** bearing triazole and morpholine, respectively, showed better antitubercular activity (25 μg/ml) compared to rifampicin. Meanwhile, compounds **6a**, **6f**, **6g** and **6i** are considered to be the most active broad spectrum antimicrobial members in this study. Compound **6g** could be identified as the most biologically active member within this study with an interesting dual antitubercular and antibacterial profile. Consequently, such type of compounds would represent a fruitful matrix for the future development of a new class of dual antitubercular–antibacterial agents that deserves further investigation and derivatization.

6. Experimental

6.1. Chemical protocol

All the reagents and solvents were purchased from commercial suppliers Fisher Scientific Ltd. and Rankem India Ltd. 2-Mercaptonicotinic acid was obtained as gift sample from Sajjan Ind. Ltd. All the solvents were dried and distilled before use. Melting points were determined in open capillaries on PMP-DM scientific melting point apparatus and are uncorrected. The progress of each reaction and the purity of the compounds were monitored by ascending thin layer chromatography (TLC) on silica gel G (Merck), visualized by iodine vapor or UV light. The IR spectra (in potassium bromide pellets) were recorded on a Thermo Scientific Nicolet iS10 FT-IR spectrometer and the wave numbers were given in cm^{-1}. The ^1H NMR spectra were recorded ($CDCl_3$/DMSO-d_6 mixture) on a Bruker HSP 400 NMR spectrometer, Bruker Avance II 400 NMR spectrometer and Varian Gemini 200, 400 MHz spectrometer. The ^{13}C NMR spectra were recorded ($CDCl_3$/DMSO-d_6 mixture) on a Bruker Avance II 400 NMR spectrometer operating at 100 MHz. Chemical shifts (*d*) are reported in parts per million (ppm) using TMS as an internal standard. The splitting pattern abbreviations are designed as s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; br, broad. The mass spectra were recorded on micromass Q-T of micro (TOF MS ES^+). Microanalysis of the compounds was done on a Heraeus Carlo Erba 1180 CHN analyzer and the values were recorded within ±0.5% of the theoretical values. All spectral data were consistent with the proposed structure.

2-Benzylsulfanyl-nicotinic acid (**2**) was prepared by the literature procedure [22].

6.1.1. General procedure for the preparation of 2-chloro-N-4-(substituted)-acetamides (**1a**–**e**)

To a stirred solution of corresponding hetero amines **a**–**e** (0.04 mol) and triethyl amine (0.04 mol) in chloroform (200 ml) was added drop wise chloroacetyl chloride (0.04 mol) at 0–5 °C. After addition, the resulting mixture was stirred overnight at room temperature. Reaction completion was monitored through thin layer chromatography using hexane:ethyl acetate (8:2) as mobile phase. The reaction mixture was concentrated to get solid and as such taken in the methanol solution. The precipitates thus separated out were allowed to stand 2 h. The resultant solid **1a**–**e** was filtered, washed, dried and as such taken for the next step.

*6.1.1.1. 2-Chloro-N-(5-methyl-thiazol-2-yl)-acetamide (**1a**).* Creamy solid (88%); m.p. 182–184 °C; IR (KBr) cm^{-1}: 3194 (–NH str.), 3063 (CH str. in thiazole ring), 2955 (–CH_2 str.), 1704 (C=O str.); ^1H NMR (DMSO, 400 MHz) δ: 2.49 (s, 3H, CH_3), 4.45 (s, 2H, CH_2), 7.40 (s, 1H, CH–thiazole), 11.60 (s, 1H, CONH disappeared on D_2O exchange).

*6.1.1.2. 2-Chloro-N-(4,6-dimethoxy-pyrimidin-2-yl)-acetamide (**1b**).* Light brown solid (86%); m.p. 168–170 °C; IR (KBr) cm^{-1}: 3265 (–NH str.), 3058 (Ar–H str.), 2924 (–CH_2 str.), 1682 (C=O str.); ^1H NMR (DMSO, 400 MHz) δ: 3.81 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.34 (s, 2H, CH_2), 7.55 (s, 1H, CH–pyrimidine), 11.10 (s, 1H, CONH disappeared on D_2O exchange).

*6.1.1.3. 2-Chloro-N-(4,6-dichloro-pyrimidin-2-yl)-acetamide (**1c**).* Off white solid (82%); m.p. 163–165 °C; IR (KBr) cm^{-1}: 3195 (–NH str.), 3032 (Ar–H str.), 2938 (–CH_2 str.), 1698 (C=O str.); ^1H NMR (DMSO, 400 MHz) δ: 4.29 (s, 2H, CH_2), 7.29 (s, 1H, CH–pyrimidine), 10.82 (s, 1H, CONH disappeared on D_2O exchange).

*6.1.1.4. 2-Chloro-N-[1,2,4]triazol-4-yl-acetamide (**1d**).* Off white solid (60%); m.p. 138–140 °C; IR (KBr) cm^{-1}: 3345 (–NH str.), 2960 (–CH_2 str.), 1652 (C=O str.); ^1H NMR (DMSO, 400 MHz) δ: 4.38 (s, 2H, CH_2), 7.98 (s, 2H, CH–triazole), 8.98 (s, 1H, CONH disappeared on D_2O exchange).

*6.1.1.5. 2-Chloro-1-(4-methyl-piperazin-1-yl)-ethanone (**1e**).* Brown solid (75%); m.p. 53–55 °C; IR (KBr) cm^{-1}: 3238 (–NH str.), 2977 (–CH_2 str.), 1669 (C=O str.); ^1H NMR (DMSO, 400 MHz) δ: 2.19 (s, 3H, CH_3), 2.47 (bs, 4H, $N(CH_2)_2$), 3.25 (bs, 4H, $N(CH_2)_2$), 4.21 (s, 2H, CH_2).

*6.1.1.6. Preparation of 2-chloro-N-[4-(5-methyl-benzothiazol-2-yl)-phenyl]-acetamide (**1f**).* To a stirred solution of 4-(5-methyl-benzothiazol-2-yl)-phenylamine **f** (5 g, 0.02 mol) and potassium carbonate (5.25 g, 0.04 mol) in dimethylformamide (200 ml) was added drop wise chloroacetyl chloride (4.7 g, 0.04 mol). After addition, the resulting mixture was stirred overnight at room temperature. Reaction completion was monitored through thin layer chromatography using to toluene:ethyl acetate (8:2) as mobile phase. After completion of the reaction, the reaction mixture was quenched with ice water and stirred for 1 h. The solid product was filtered, dried and as such taken for the next step.

Table 2
In vitro screening of compounds (growth percentage) on 60 tumor cell lines at single dose 1.00^{-5} M.

Panel/cell line	Compound/growth %					
	6a	**6b**	**6c**	**6e**	**6f**	**6j**
Leukemia						
CCRF-CEM	37.84	108.04	100.80	–	93.13	103.30
HL-60(TB)	49.77	97.33	98.32	70.34	97.30	100.58
K-562	77.25	98.19	92.83	83.02	101.28	117.02
MOLT-4	41.78	93.24	95.73	48.24	92.15	103.46
RPMI-8226	63.94	101.28	100.29	61.50	92.21	103.94
SR	67.93	96.23	101.44	79.80	82.63	104.12
Non-Small cell lung cancer						
A549/ATCC	51.02	96.48	104.04	68.50	103.74	112.13
EKVX	72.12	105.48	102.82	70.73	89.86	108.24
HOP-62	81.10	99.65	90.57	97.00	96.81	115.59
HOP-92	–	–	–	43.26	93.36	–
NCI-H226	70.17	101.18	83.68	–	80.80	113.84
NCI-H23	80.60	103.48	102.32	87.63	96.60	104.18
NCI-H460	81.10	111.56	105.09	90.14	100.66	122.10
NCI-H522	18.49	88.19	83.12	71.29	87.86	105.23
Colon cancer						
HCC-2998	89.46	109.08	102.74	90.48	106.15	116.34
HCT-116	82.36	100.80	97.25	94.74	95.38	107.92
HCT-15	77.28	105.37	106.24	86.53	110.57	105.27
HT29	81.64	100.10	109.71	93.73	100.11	111.38
KM12	44.92	–	–	72.79	–	107.38
SW-620	89.97	106.58	102.90	97.25	103.98	106.89
COLO 205	–	–	–	109.81	–	–
CNS cancer						
SF-268	59.14	103.74	99.75	67.47	91.56	106.81
SF-295	90.08	97.13	96.77	89.57	107.09	105.81
SF-539	85.53	94.54	101.08	98.59	92.89	131.71
SNB-19	65.26	107.49	105.78	82.05	104.16	114.74
SNB-75	70.63	92.54	85.69	82.98	95.59	79.64
U251	72.82	105.69	105.76	83.94	105.95	117.21
Melanoma						
LOX IMVI	73.07	97.61	94.90	90.29	93.56	108.01
MALME-3M	100.37	98.19	94.05	97.12	99.33	111.40
M14	84.14	96.65	91.95	99.56	97.41	107.96
MDA-MB-435	96.46	104.91	104.95	93.79	107.49	114.42
SK-MEL-2	94.03	102.24	101.58	100.95	89.79	–
SK-MEL-28	105.57	113.51	112.51	106.70	114.02	107.28
SK-MEL-5	78.02	104.56	100.58	84.77	89.89	102.24
UACC-257	94.94	105.14	113.12	96.81	112.79	146.67
UACC-62	71.67	107.43	101.18	76.81	104.15	112.93
Ovarian cancer						
IGROV1	58.54	113.55	105.14	75.27	99.69	98.28
OVCAR-3	82.23	102.30	105.60	92.03	100.46	119.85
OVCAR-4	66.97	100.34	106.38	57.36	98.84	106.42
OVCAR-5	87.50	100.60	106.21	100.88	107.45	119.15
OVCAR-8	70.31	104.79	101.66	97.71	94.29	109.20
NCI/ADR-RES	74.05	102.15	100.71	89.18	99.51	106.67
SK-OV-3	96.19	103.35	110.28	100.62	105.30	108.55
Renal cancer						
A498	73.66	125.16	111.69	78.49	104.57	107.13
ACHN	66.47	106.83	102.53	80.83	90.76	124.25
CAKI-1	75.26	99.45	95.76	93.36	87.76	100.33
RXF 393	73.04	107.20	104.62	89.09	114.34	122.58
SN12C	46.34	106.28	102.74	64.24	101.65	114.35
TK-10	82.27	98.56	109.49	115.08	117.87	117.73
UO-31	22.19	80.64	67.66	30.87	59.34	89.38
786-0	–	–	–	109.37	–	–
Prostate cancer						
PC-3	46.43	103.98	94.53	57.42	91.78	110.91
DU-145	57.96	108.02	107.15	81.74	87.54	111.92
Breast cancer						
MCF-7	62.78	95.52	92.01	76.68	81.24	94.38
MDA-MB231/ATCC	11.02	108.07	92.02	56.48	71.41	113.93
HS 578T	86.18	107.23	97.37	93.24	92.32	133.25
BT-549	75.70	99.60	101.77	95.44	91.60	112.50
T-47D	37.45	89.91	98.48	55.34	87.79	101.38
MDA-MB-468	57.53	98.19	98.85	74.76	92.20	98.30

2-Chloro-*N*-(6-thiocyanato-benzothiazol-2-yl)-acetamide **5g** was prepared by the similar procedure.

*6.1.1.7. 2-Chloro-N-[4-(5-methyl-benzothiazol-2-yl)-phenyl]-acetamide (**1f**).* (76%); m.p. 195–197 °C; IR (KBr) cm^{-1}: 3366 (–NH str.), 3040 (Ar–H str.), 2920 ($-CH_2$ str.), 1663 (C=O str.); ^{1}H NMR (DMSO, 400 MHz) δ: 2.45 (s, 3H, CH_3), 4.32 (s, 2H, CH_2), 7.39 (s, 1H, Ar–H), 7.79 (s, 2H, Ar–H), 7.90 (d, 2H, Ar–H), 8.10 (s, 2H, Ar–H), 10.70 (s, 1H, CONH disappeared on D_2O exchange).

*6.1.1.8. 2-Chloro-N-(6-thiocyanato-benzothiazol-2-yl)-acetamide (**1g**).* (74%); m.p. 180–182 °C; IR (KBr) cm^{-1}: 3382 (–NH str.), 3080 (Ar–H str.), 2880 ($-CH_2$ str.), 2155 (–SCN), 1682 (C=O str.); ^{1}H NMR (DMSO, 400 MHz) δ: 4.52 (s, 2H, CH_2), 7.52 (s, 1H, Ar–H), 8.09 (s, 1H, Ar–H), 8.12 (s, 1H, Ar–H), 9.92 (s, 1H, CONH disappeared on D_2O exchange).

*6.1.2. 2-Benzylsulfanyl-nicotinic acid hydrazide (**4**)*

2-Benzylsulfanyl-nicotinic acid **2** (10 g, 0.04 mol) and few drops of concentrated sulfuric acid as a catalyst in methanol (500 ml) was heated under reflux for 10 h. The reaction progress was monitored by thin layer chromatography using toluene:ethyl acetate (7:3) as the mobile phase. After the completion of the reaction, reaction mixture was poured into ice cold water and allowed to stand overnight in the freezer. Afterward the respective ester **3** (chewing gum type white semisolid) obtained was filtered, washed with 10% sodium bicarbonate solution and as such taken for the next step. To a solution of **3** in methanol (250 ml) was added hydrazine hydrate (4.0 g, 0.085 mol) with constant stirring for 10 min. The resulting mixture was heated under reflux for 5 h. Reaction completion was monitored by thin layer chromatography using toluene:ethyl acetate: methanol (7:2:1) as the mobile phase and allowed to stand overnight. The white crystals **4** formed were filtered, washed and after drying recrystallized from ethanol. White crystalline solid (65%); m.p. 195–197 °C; IR (KBr) cm^{-1}: 3284, 3184 (NH/NH_2), 1636 (C=O); ^{1}H NMR (DMSO, 400 MHz) δ: 4.19 (bs, 2H, NH_2 disappeared on D_2O exchange), 4.33 (s, 2H, CH_2), 7.05–7.02 (m, 1H, 4′-Hbn), 7.22–7.12 (m, 3H, 3′/5′/1-Hbn/py), 7.33–7.31 (m, 2H, 2′/6′-Hbn), 7.69 (dd, 1H, 6-Hpyr), 8.46 (dd, 1H, 2-Hpyr), 9.60 (s, 1H, CONH disappeared on D_2O exchange).

*6.1.3. 5-(2-Benzylsulfanyl-pyridin-3-yl)-3H-[1,3,4]oxadiazole-2-thione (**5**)*

A mixture of 2-benzylsulfanyl-nicotinic acid hydrazide **4** (5 g, 0.018 mol), carbon disulfide (2 g, 0.03 ml), potassium hydroxide (2 g, 0.04 mol) and methanol (250 ml) was heated under reflux for 5 h. Afterward the reaction mixture was cooled to room temperature, poured into cold water and acidified with diluted hydrochloric acid solution to bring the pH between 3 and 4. The precipitates thus separated out were allowed to stand overnight. The resultant off white colored solid **5** was filtered, washed and after drying recrystallized from acetone. Off white solid (78%); m.p. 187–189 °C. IR (KBr) cm^{-1}: 3266 (NH), 1326, 1164 (C–O–C), 1234 (C=S); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.35 (s, 2H, CH_2), 7.25–7.15 (m, 4H, 3′/4′/5′/5-Hbn/py), 7.36–7.34 (m, 2H, 2′/6′-Hbn), 8.08–8.06 (dd, 1H, 6-Hpy), 8.57–8.56 (dd, 1H, 2-Hpy), 14.67 (s, 1H,thiol–thione tautomerism).

*6.1.4. General procedure for the preparation of 5-(2-benzylsulfanyl-pyridin-3-yl)-2-(substituted)-sulfanyl-1,3,4-oxadiazole (**6a–g**)*

A mixture of **5** (0.006 mol), dimethylformamide (25 ml), potassium carbonate (0.012 mol) and corresponding hetero acetamides **1a–g** (0.006 mol) was heated to 90–120 °C for 8–15 h. Completion of reaction was periodically observed by thin layer chromatography using appropriate mobile phase system. After completion of reaction, filtered the inorganics, the filtrate obtained was quenched with ice water and stirred for 1 h and the solid separated was filtered and dried through pump to afford title compounds **6a–g**.

*6.1.4.1. 2-[5-(2-Benzylsulfanyl-pyridin-3-yl)-[1,3,4]oxadiazol-2-ylsulfanyl]-N-(5-methyl-thiazol-2-yl)-acetamide (**6a**).* A mixture of **5** (2 g, 0.006 mol), dimethylformamide (25 ml), potassium carbonate (1.6 g, 0.012 mol) and 2-chloro-*N*-(5-methyl-thiazol-2-yl)-acetamide **1a** (1.5 g, 0.006 mol) was heated to 90 °C for 8 h. Completion of reaction was periodically observed by thin layer chromatography using mobile phase toluene:methanol (8:2). After completion of reaction, filtered the inorganics, the filtrate obtained was quenched with ice water and stirred for 1 h and the solid separated was filtered and dried through pump to afford title compound **6a** as off white solid (72%); m.p. 200–202 °C; IR (KBr) cm^{-1}: 3363 (–NH str.), 1679 (C=O str.); ^1H NMR (DMSO, 400 MHz) δ: 2.33 (s, 3H, CH_3), 4.40 (s, 2H, SCH_2), 4.47 (s, 2H, SCH_2), 7.162 (s, 1H, thiazole–H), 7.165–7.426 (m, 6H, Ar–Hbn/py), 8.19–8.21 (dd, 1H, Ar–Hpy), 8.68–8.69 (dd, 1H, Ar–Hpy), 12.34 (s, 1H, CONH disappeared on D_2O exchange); ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 13.07, 33.78, 34.16, 117.50, 118.79, 122.70, 126.19, 128.32, 128.89, 136.62, 137.67, 138.14, 150.56, 157.42 162.68, 163.80, 169.27, 172.20; Anal. found (calc.) for $C_{20}H_{17}N_5O_2S_3$ (%): C, 52.61 (52.73); H, 3.55 (3.76); N, 15.26 (15.37); mass *m*/*z* (M^+) 455.

*6.1.4.2. 2-[5-(2-Benzylsulfanyl-pyridin-3-yl)-[1,3,4]oxadiazol-2-ylsulfanyl]-N-(4,6-dimethoxy-pyrimidin-2-yl)-acetamide (**6b**).* A mixture of **5** (2 g, 0.006 mol), dimethylformamide (25 ml), potassium carbonate (1.6 g, 0.012 mol) and 2-chloro-*N*-(4,6-dimethoxy-pyrimidin-2-yl)-acetamide **1b** (1.39 g, 0.006 mol) was heated to 95–100 °C for 14 h. Completion of reaction was periodically observed by thin layer chromatography using mobile phase toluene:methanol (7:3). After completion of reaction, filtered the inorganics, the filtrate obtained was quenched with ice water and stirred for 1 h and the solid separated was filtered and dried through pump to afford title compound **6b** as off white solid (65%); m.p. 232–234 °C; IR (KBr) cm^{-1}: 3309 (–NH str.), 1655 (C=O str.), 1267, 1082 (C–O–C str.); ^1H NMR (DMSO, 400 MHz) δ: 3.73 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 4.39 (s, 2H, SCH_2), 4.52 (s, 2H, SCH_2), 7.11 (s, 1H, pyrimidine–H), 7.12–7.40 (m, 6H, Ar–Hbn/py), 8.09–8.11 (dd, 1H, Ar–Hpy), 8.52–8.53 (dd, 1H, Ar–Hpy), 11.12 (s, 1H, CONH disappeared on D_2O exchange); ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 37.29, 39.32, 55.78 116.29, 119.54, 126.27, 128.13, 128.79, 136.68, 137.88, 150.25, 157.12, 157.84, 162.32, 163.79, 170.57, 171.35; Anal. found (calc.) for $C_{22}H_{20}N_6O_4S_2$ (%): C, 53.06 (53.21); H, 4.15 (4.06); N, 16.86 (16.92); mass *m*/*z* (M^+) 496.

*6.1.4.3. 2-[5-(2-Benzylsulfanyl-pyridin-3-yl)-[1,3,4]oxadiazol-2-ylsulfanyl]-N-(4,6-dichloro-pyrimidin-2-yl)-acetamide (**6c**).* A mixture of **5** (2 g, 0.006 mol), dimethylformamide (25 ml), potassium carbonate (1.6 g, 0.012 mol) and 2-chloro-*N*-(4,6-dichloro-pyrimidin-2-yl)-acetamide **1c** (1.45 g, 0.006 mol) was heated to 95–100 °C for 14 h. Completion of reaction was periodically observed by thin layer chromatography using mobile phase toluene:methanol (7:3). After completion of reaction, filtered the inorganics, the filtrate obtained was quenched with ice water and stirred for 1 h and the solid separated was filtered and dried through pump to afford title compound **6c** as grayish brown solid (61%); m.p. 228–230 °C; IR (KBr) cm^{-1}: 3289 (–NH str.), 1662 (C=O str.); ^1H NMR (DMSO, 400 MHz) δ: 4.22 (s, 2H, SCH_2), 4.43 (s, 2H, SCH_2), 7.21 (s, 1H, pyrimidine–H), 7.22–7.49 (m, 6H, Ar–Hbn/py), 8.18–8.19 (dd, 1H, Ar–Hpy), 8.46–8.47 (dd, 1H, Ar–Hpy), 11.98 (s, 1H, CONH disappeared on D_2O exchange); ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 33.13, 36.55, 117.39, 118.94, 119.46, 127.14, 128.19, 128.82, 135.21, 136.22, 151.36, 158.04 161.98, 162.9, 163.84, 169.23 Anal. found (calc.) for $C_{20}H_{14}Cl_2N_6O_2S_2$ (%): C, 47.39 (47.53); H, 2.65 (2.79); N, 16.56 (16.63); mass *m*/*z* (M^+) 505.

*6.1.4.4. 2-[5-(2-Benzylsulfanyl-pyridin-3-yl)-[1,3,4]oxadiazol-2-ylsulfanyl]-N-[1,2,4]triazol-4-yl-acetamide (**6d**).* A mixture of **5** (2 g, 0.006 mol), dimethylformamide (25 ml), potassium carbonate (1.6 g, 0.012 mol) and 2-chloro-*N*-[1,2,4]triazol-4-yl-acetamide **1d** (0.96 g, 0.006 mol) was heated to 90 °C for 10 h. Completion of reaction was periodically observed by thin layer chromatography using mobile phase toluene:methanol (8:2). After completion of reaction, filtered the inorganics, the filtrate obtained was quenched with ice water and stirred for 1 h and the solid separated was filtered and dried through pump to afford title compound **6d** as golden brown solid (69%); m.p. 210–212 °C; IR (KBr) cm^{-1}: 3264 (–NH str.), 1682 (C=O str.); ^1H NMR (DMSO, 400 MHz) δ: 4.26 (s, 2H, SCH_2), 4.39 (s, 2H, SCH_2), 7.20–7.48 (m, 6H, Ar–Hbn/py), 8.08 (s, 2H, triazole–H), 8.16–8.18 (dd, 1H, Ar–Hpy), 8.27–8.28 (dd, 1H, Ar–Hpy), 10.88 (s, 1H, CONH disappeared on D_2O exchange); ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 33.49, 39.23, 117.23, 118.34, 127.10, 128.11, 129.07, 136.29, 137.43, 146.35, 151.16, 157.93 161.62, 163.20, 174.35 Anal. found (calc.) for $C_{18}H_{15}N_7O_2S_2$ (%): C, 50.69 (50.81); H, 3.42 (3.55); N, 22.96 (23.04); mass *m*/*z* (M^+) 425.

*6.1.4.5. 2-[5-(2-Benzylsulfanyl-pyridin-3-yl)-[1,3,4]oxadiazol-2-ylsulfanyl]-1-(4-methyl-piperazin-1-yl)-ethanone (**6e**).* A mixture of **5** (2 g, 0.006 mol), dimethylformamide (25 ml), potassium carbonate (1.6 g, 0.012 mol) and 2-chloro-1-(4-methyl-piperazin-1-yl)-ethanone **1e** (1.05 g, 0.006 mol) was heated to 90 °C for 8 h. Completion of reaction was periodically observed by thin layer chromatography using mobile phase toluene:methanol (8:2). After completion of reaction, filtered the inorganics, the filtrate obtained was quenched with ice water and stirred for 1 h and the solid separated was filtered and dried through pump to afford title compound **6e** as light brown solid (41%); m.p. 158–160 °C; IR (KBr) cm^{-1}: 1664 (C=O str.), 1337 (C–N str.); ^1H NMR (DMSO, 400 MHz) δ: 2.25 (s, 3H, CH_3), 2.44 (t, 4H, piperazine–N–CH_2), 3.32 (t, 4H, piperazine–N–CH_2), 4.37 (s, 2H, SCH_2), 4.50 (s, 2H, SCH_2), 7.20–7.45 (m, 6H, Ar–Hbn/py), 8.01–8.03 (dd, 1H, Ar–Hpy), 8.48–8.49 (dd, 1H, Ar–Hpy); ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 28.13, 33.19, 37.34, 44.35, 54.67, 61.53, 116.63, 118.29, 126.17, 128.02, 128.66, 136.89, 137.53, 150.76, 158.19 161.85, 163.20, 171.27; Anal. found (calc.) for $C_{21}H_{23}N_5O_2S_2$ (%): C, 57.01 (57.12); H, 5.45 (5.25); N, 15.71 (15.86); mass *m*/*z* (M^+) 441.

*6.1.4.6. Preparation of 2-[5-(2-benzylsulfanyl-pyridin-3-yl)-[1,3,4]oxadiazol-2-ylsulfanyl]-N-[4-(5-methyl-benzothiazol-2-yl)-phenyl]-acetamide (**6f**).* A mixture of **5** (2 g, 0.006 mol), dimethylformamide (25 ml), potassium carbonate (1.6 g, 0.012 mol) and 2-chloro-*N*-[4-(5-methyl-benzothiazol-2-yl)-phenyl]-acetamide **1f** (1.86 g, 0.006 mol) was heated to 115–120 °C for 15 h. Completion of reaction was periodically observed by thin layer chromatography using mobile phase toluene:methanol:ethyl acetate (7:2:1). After completion of reaction, filtered the inorganics, the filtrate obtained was quenched with ice water and stirred for 1 h and the solid separated was filtered and dried through pump to afford title compound **6f** as buff yellowish solid (52%); m.p. 204–206 °C; IR (KBr) cm^{-1}: 3263 (–NH str.), 1661 (C=O str.); ^1H NMR (DMSO, 400 MHz) δ: 2.45 (s, 3H, CH_3), 4.06 (s, 2H, SCH_2), 4.50 (s, 2H, SCH_2), 6.79–8.05 (m, 13H, Ar–H), 8.58–8.59 (dd, 1H, Ar–Hpy), 8.69–8.70 (dd, 1H, Ar–Hpy), 10.35 (s, 1H, CONH disappeared on D_2O exchange); ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 19.27, 33.92, 40.26, 117.88, 119.31, 121.86, 123.45, 126.65, 126.98, 127.13, 128.49, 129.11, 132.54, 135.87, 136.14, 137.56, 141.14, 151.19, 155.87, 158.21 163.02, 163.20, 170.42, 172.69; Anal. found (calc.) for $C_{30}H_{23}N_5O_2S_3$ (%): C, 61.81 (61.94); H, 3.45 (3.99); N, 12.01 (12.04); mass *m*/*z* (M^+) 581.

*6.1.4.7. Preparation of 2-[5-(2-benzylsulfanyl-pyridin-3-yl)-[1,3,4]oxadiazol-2-ylsulfanyl]-N-(6-thiocyanato-benzothiazol-2-yl)-acetamide (**6g**).* A mixture of **5** (2 g, 0.006 mol), dimethylformamide (25 ml), potassium carbonate (1.6 g, 0.012 mol) and 2-chloro-*N*-(6-

thiocyanato-benzothiazol-2-yl)-acetamide **1g** (1.69 g, 0.006 mol) was heated to 100–105 °C for 12 h. Completion of reaction was periodically observed by thin layer chromatography using mobile phase toluene:methanol:ethyl acetate (7:2:1). After completion of reaction, filtered the inorganics, the filtrate obtained was quenched with ice water and stirred for 1 h and the solid separated was filtered and dried through pump to afford title compound **6g** as buff yellowish solid (59%); m.p. 200–202 °C; IR (KBr) cm^{-1}: 33,014 (–NH str.), 1652 (C=O str.), 2158 (SCN str.); ^1H NMR (DMSO, 400 MHz) δ: 3.98 (s, 2H, SCH_2), 4.32 (s, 2H, SCH_2), 6.95–8.08 (m, 9H, Ar–H), 7.92–7.93 (dd, 1H, Ar–Hpy), 8.27–8.28 (dd, 1H, Ar–Hpy), 9.87 (s, 1H, CONH disappeared on D_2O exchange); ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 33.64, 40.24, 110.39 116.59, 119.38, 121.45, 123.56, 124.57, 126.59, 128.37, 128.87, 129.49, 135.67, 137.73, 150.11, 152.49, 157.39 162.24, 162.10, 171.37, 172.35; Anal. found (calc.) for $C_{24}H_{16}N_6O_2S_4$ (%): C, 52.31 (52.54); H, 2.85 (2.94); N, 15.16 (15.32); mass m/z (M^+) 548.

6.1.5. [5-(2-Benzylsulfanyl-pyridin-3-yl)-[1,3,4]oxadiazol-2-ylsulfanyl]-acetic acid ethyl ester (**6h**)

A mixture of **5** (2 g, 0.006 mol), potassium carbonate (1.68 g, 0.012 mol) and ethyl bromoacetate (1.1 g, 0.006 mol) in acetone (50 ml) was heated under reflux for 4 h. Reaction completion was monitored through thin layer chromatography using *n*-hexane:ethyl acetate (8:2) as mobile phase. After completion of reaction, filtered the inorganics, the filtrate obtained was concentrated to remove acetone. The white crystalline solid was washed with water, dried and recrystallized using ethanol to yield **6h** as off white crystals (61%); m.p. 168–170 °C; IR (KBr) cm^{-1}: 1738 (C=O str.), 1315, 1139 (C–O–C str.); ^1H NMR (DMSO, 400 MHz) δ: 1.16–1.19 (t, 3H, CH_3 of ester), 4.11–4.17 (q, 2H, CH_2 of ester), 4.29 (s, 2H, SCH_2), 4.49 (s, 2H, SCH_2), 7.22–7.44 (m, 6H, Ar–Hbn/py), 8.21–8.24 (dd, 1H, Ar–Hpy), 8.69–8.71 (dd, 1H, Ar–Hpy); ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 13.83, 33.78, 34.16, 61.53, 116.20, 119.04, 126.80, 128.12, 128.97, 136.37, 137.34, 150.76, 157.12 162.32, 163.20, 167.17; Anal. found (calc.) for $C_{18}H_{17}N_3O_3S_2$ (%): C, 55.61 (55.80); H, 4.45 (4.42); N, 10.96 (10.84); mass m/z (M^+) 387.

6.1.6. 2-Benzylsulfanyl-3-(5-oxiranylmethylsulfanyl-[1,3,4]oxadiazol-2-yl)-pyridine (**6i**)

A mixture of **5** (2 g, 0.006 mol), potassium hydroxide (0.7 g, 0.012 mol) and epichlorohydrin (0.5 g, 0.006 mol) in methanol (50 ml) was heated under reflux for 5 h. Reaction completion was monitored through thin layer chromatography using toluene:ethyl acetate (8:2) as mobile phase. After completion of reaction, the reaction mixture was concentrated, quenched with ice water and stirred for 1 h. The precipitated product was filtered and dried through pump to afford title compound **6i** as golden brown solid (45%); m.p. 198–200 °C; IR (KBr) cm^{-1}: 2958, 2839 (CH str.); ^1H NMR (DMSO, 400 MHz) δ: 2.59 (dd, 2H, CH_2 of oxiranyl ring), 2.76 (m, H, CH of oxiranyl ring), 3.72 (d, 2H, SCH_2), 4.36 (s, 2H, SCH_2), 7.12–7.36 (m, 6H, Ar–Hbn/py), 8.06–8.08 (dd, 1H, Ar–Hpy), 8.33–8.35 (dd, 1H, Ar–Hpy); ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 34.02, 42.16, 47.93, 54.59, 115.32, 118.78, 127.12, 128.39, 128.92, 136.63, 137.78, 151.72, 156.89, 161.74, 163.91; Anal. found (calc.) for $C_{17}H_{15}N_3O_2S_2$ (%): C, 57.03 (57.12); H, 4.45 (4.23); N, 11.66 (11.76); mass m/z (M^+) 357.

6.1.7. 4-{2-[5-(2-Benzylsulfanyl-pyridin-3-yl)-[1,3,4]oxadiazol-2-ylsulfanyl]-ethyl}-morpholine (**6j**)

A mixture of **5** (2 g, 0.006 mol), ethanol (50 ml), potassium hydroxide (1.68 g, 0.012 mol) and 4-(2-chloro-ethyl)-morpholine (1.2 g, 0.006 mol) was heated under reflux for 8 h. Reaction completion was monitored through thin layer chromatography using toluene:ethyl acetate (8:2) as mobile phase. After completion of reaction, the reaction mixture was cooled down, quenched with ice water and stirred for 1 h. The precipitated product was filtered and dried through pump to afford title compound **6i** as creamy white solid (42%); m.p. 193–195 °C; IR (KBr) cm^{-1}: 2949, 2829 (CH str.), 1329 (C=N str.), 1272, 1093 (C–O–C str.); ^1H NMR (DMSO, 400 MHz) δ: 2.55–2.58 (m, 4H, NCH_2 of morpholine ring), 2.65–2.68 (t, 2H, aliphatic–CH_2), 3.20–3.23 (m, 4H, OCH_2 of morpholine ring), 4.31–4.34 (t, 2H, SCH_2), 4.67 (s, 2H, SCH_2), 7.25–7.58 (m, 6H, Ar–Hbn/py), 8.06–8.09 (dd, 1H, Ar–Hpy), 8.67–8.69 (dd, 1H, Ar–Hpy); ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 28.10, 33.96, 39.66, 49.39, 60.13, 117.04, 119.89, 126.12, 128.39, 128.63, 135.21, 136.64, 151.12, 156.72 161.52, 162.98, 167.95; Anal. found (calc.) for $C_{20}H_{22}N_4O_2S_2$ (%): C, 57.82 (57.95); H, 5.45 (5.35); N, 13.43 (13.52); mass m/z (M^+) 414.

6.2. Biological protocol

6.2.1. In vitro evaluation of antimicrobial activity

The antimicrobial susceptibility testing was performed *in vitro* by broth microdilution method using Mueller-Hinton broth. In brief, the MIC determination of the synthesized compounds was carried out in side-by-side comparison with ampicillin against Gram-positive bacteria (*S. aureus* and *S. pyogenes*) and Gram-negative (*E. coli* and *P. aeruginosa*). The antifungal activity was assayed against three fungal species (*C. albicans*, *A. niger* and *A. clavatus*) and griseofulvin was used as a standard antifungal agent. The minimal inhibitory concentrations (MIC, μg/ml) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain. Serial twofold dilutions of all samples were prepared in triplicate in microtiter plates and inoculated with a suitably prepared cell suspension to achieve the required start concentration. Serial dilutions were prepared in primary and secondary screening. DMSO was used as diluents to get desired concentration of drugs to test upon standard bacterial strains. The control tube containing no antibiotic was immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. Each synthesized drug was diluted obtaining 2000 μg/ml concentration, as a stock solution. In primary screening 500, 250 and 125 μg/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5, 6.250, 3.125 and 1.5625 μg/ml concentrations.

The highest dilution showing at least 99% inhibition is taken as MIC.

6.2.2. In vitro evaluation of antitubercular activity

The preliminary antitubercular screening for test compounds was obtained for *M. tuberculosis* $H_{37}Rv$, the MIC of each drug was determined by broth dilution assay by L. J. agar (MIC) method [23] where primary 1000, 500 and 250 μg/ml and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25 and 3.25 μg/ml dilutions of each test compound were added liquid L. J. Medium and then media were sterilized by inspissation method. A culture of *M. tuberculosis* $H_{37}Rv$ growing on L. J. medium was harvested in 0.85% saline in bijou bottles. All test compound makes first stock solution of 2000 μg/ml concentration of compounds was prepared in DMSO. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* $H_{37}Rv$ (5 × 104 bacilli per tube). These tubes were

then incubated at 37 °C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* $H_{37}Rv$. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The standard strain *M. tuberculosis* $H_{37}Rv$ was tested with known drug rifampicin.

Acknowledgments

The authors are thankful to Department of Chemistry, VNSGU, Surat for providing necessary research facilities, Sajjan India Ltd for providing some free chemical samples and NCI, Bethesda, MD, USA for performing the antitumor screening. One of the authors Amit C. Purohit is also thankful to Maa Foundation for providing research scholarship. We also thank CDRI Lucknow for elemental analysis and SAIF Chandigarh for spectral analysis of the compounds.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.12.055.

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