Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 2151–2161
Bioorganic & Medicinal Chemistry

Synthesis and preliminary evaluation of some pyrazine containing thiazolines and thiazolidinones as antimicrobial agents

Chandrakant G. Bonde* and Naresh J. Gaikwad

Department of Pharmaceutical Sciences, Nagpur University Campus, Nagpur University, Amravati Road, Nagpur 440033, India

Received 6 November 2003; revised 17 February 2004; accepted 23 February 2004

Abstract—A series of *N*′-[3,4-disubstituted-1,3-thiazol-2(3H)-ylidene]-2-(pyrazin-2-yloxy)acetohydrazide **11**–**66** and *N*′-[(2Z)-3-(4-bromophenyl)-4-oxo-1,3-thiazolidin-2-ylidene]-2-(pyrazin-2-yloxy)acetohydrazide **68**–**74** were synthesized using appropriate synthetic route. The entire test compounds **11**–**66** and **68**–**74** were assayed in vitro for antibacterial activity against two different strains of Gram-negative (*E. coli* and *S. typhi*), Gram-positive (*S. aureus* and *B. subtilis*) bacteria and the antimycobacterial activity was evaluated against H_{37}Rv strain of *Mycobacterium tuberculosis*. The minimum inhibitory concentration (MIC) was determined for test compounds and for reference standards. The test compounds showed significant antibacterial and antimycobacterial activity against the microbial strains used, when tested in vitro. In general, pyrazine ring and substituted thiazoline ring are essential for antimicrobial activity. Among the compounds tested, compounds **11**, **12** and **40** were found to be most potent. The toxicity of most potent compounds **11**, **12** and **40** were determined using hemolytic assay and minimal hemolytic concentration (MHCs) were determined. The test compounds were found to be nontoxic up to a dose level of 250 μg/mL.
© 2004 Elsevier Ltd. All rights reserved.

1. Introduction

The deterioration of human population due to the enhance prevalence of infectious diseases is becoming a worldwide problem. Over the last few years, tuberculosis is retrieving its place among theses infectious diseases and today, nearly one-third of the world's population is infected with *Mycobacterium tuberculosis* with approximately three million patients deceasing every year.[1] The contemporary treatment of these infectious diseases involves administration of multi-drug regimen over a long period of time,[2,3] which lead to patient noncompliance and rapid emergence of multi-drug resistance strain.[4–7] The resistance problem demands to seek antimicrobial agents effective against pathogenic microorganisms resistant to current treatment.[8] Furthermore, treatment of infectious diseases is more difficult in immunodeficient patients such as those infected with human immunodeficiency virus (HIV).[9] Recent studies showed that the application of appropriate dosage regimen with highly potent antimicrobial agents not only eradicates bacterial growth but also minimizes the probability of resistance formation.[10–12] The biochemical basis of both intrinsic and acquired resistance are now known[13–15] and has contributed significantly towards the design of new entities by rational strategies that can be used to counteract the resistance. The development of new potential drugs, which will be devoid of side-effect profile of currently available drugs, will be one of the possible solutions to treat various infectious diseases with multi-drug treatment over a long period of time. Moreover, in immunocompromized patients, tubular pathology is often accompanied by bacterial infections; therefore there is a need to develop the new entities, which will act both as antibacterial and antimycobacterials.

Literature survey reveals that pyrazine ring is important for antimycobacterial activity.[16] In addition, many thiazoline derivatives exhibit a wide variety of biological activities such as antimicrobial,[17] antiinflammatory,[18] antihistaminic,[19] antihypertensive,[20] hypnotic[21] and anticonvulsant,[22] etc. In view of the fact that pyrazine ring possess antimycobacterial activity and as a part of our ongoing studies in the area of antibacterial and antimycobacterial agents,[16] we have synthesized some novel *N*′-[3,4-disubstituted-1,3-thiazol-2(3H)-ylidene]-2-(pyrazin-2-yloxy)acetohydrazide and *N*′-[(2Z)-3-(4-bromophenyl)-4-oxo-1,3-thiazolidin-2-ylidene]-2-(pyrazin-2-yloxy)acetohydrazide with the aim of obtaining

Keywords: Antimicrobial; Thiazoline; Thiazolidinones; Hemolytic assay.
* Corresponding author. Tel.: +91-712-2500324; fax: +91-712-25003-55; e-mail: chandubonde@yahoo.co.in

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.02.024

the new broad spectrum antimicrobial agents, which will devoid of side effects associated with current therapy.

Thus in the present investigation, 56 different derivatives of *N*′-[3,4-disubstituted-1,3-thiazol-2(3H)-ylidene]-2-(pyrazin-2-yloxy)acetohydrazide and 8 different derivatives of *N*′-[(2Z)-3-(4-bromophenyl)-4-oxo-1,3-thiazolidin-2-ylidene]-2-(pyrazin-2-yloxy)acetohydrazide were synthesized and evaluated for their antibacterial and antimycobacterial activity. The most potent compounds of each series were further evaluated for their toxicity using hemolytic assay.

2. Chemistry

The synthesis of the intermediate and target compounds were performed by the reaction illustrated in Figure 1.

Compound **2** namely ethyl(pyrazin-2-yloxy)acetate was synthesized in an excellent yield by electrophilic substitution on 2-hydroxy pyrazine by ethyl chloroacetate under reflux condition. Compound **2** on amination with hydrazine hydrate afforded 2-(pyrazin-2-yloxy)acetohydrazide **3**. Reaction of **3** with alkyl/aryl isothiocynate in ethanol gives compounds **4**–**10**. The structures of the compounds **4**–**10** were confirmed on the basis of elemental analysis and spectral data. The IR spectra showed NH and CS stretching bands at 3215–3230 and 1309–1348 cm^{-1}, respectively. The ^1H NMR showed downfield signal at δ 11.6–14.23 attributed to 3-substituted NH and NH of 2-methoxy amido appeared as a singlet signal at δ 10.25–10.8. These two signals disappeared with D_2O. The other protons appeared at the expected chemical shifts. Condensation of **4**–**10** with appropriate phenacyl bromide in boiling ethanol containing anhydrous sodium acetate may lead to formation of *N*′-[3,4-disubstituted-1,3-thiazol-2(3H)-ylidene]-2-(pyrazin-2-yloxy)acetohydrazide **11**–**66** and/or *N*-[(2Z)-2-[(aryl/alkyl substituted)imino]-4-(aryl/alkyl substituted)-1,3-thiazol-3(2H)-yl]-2-(pyrazin-2-yloxy)acetamide **67**. In fact, only one product was obtained as confirmed by TLC. The structure of the products **11**–**66** and **67** was based on previous discussion of the structures of similar compounds.[23] The structures of the reaction products were confirmed by elemental analysis, IR, ^1H NMR and FABMS analyses. IR spectra revealed that the disappearance of NH band at 3215–3230 cm^{-1}. The ^1H NMR spectra also lacked the NH signals and showed new singlet signal at δ 5.8–6.1 attributed to C_5–H of thiazoline ring. The reaction of **4**–**10** with chloroacetic acid in boiling ethanol containing fused sodium acetate afforded the corresponding *N*′-[(2Z)-3-(4-alkyl/aryl substituted)-4-oxo-1,3-thiazolidin-2-ylidene]-2-(pyrazin-2-yloxy)acetohydrazide. Compounds **68**–**74** and not *N*-{(2Z)-2-[(4-alkyl/aryl substituted)imino]-4-oxo-1,3-thiazolidin-3-yl}-2-(pyrazin-2-yloxy)acetamide as expected from our previous discussion.[23]

3. Result and discussion

In the present investigation, different derivatives of *N*′-[3,4-disubstituted-1,3-thiazol-2(3H)-ylidene]-2-(pyrazin-2-yloxy)acetohydrazide **11**–**66** and *N*′-[(2Z)-3-(4-alkyl/aryl substituted)-4-oxo-1,3-thiazolidin-2-ylidene]-2-(pyrazin-2-yloxy)acetohydrazide. Compounds **68**–**74** were synthesized and evaluated for their physical, analytical and spectral data (Tables 1 and 2).

The MIC values of the test compounds are summarized in Table 3. For comparison, the MICs of compounds **2**, **3** and **4**–**10** are included in Table 3. The results revealed that the test compounds **11**–**66** exhibit remarkable antimycobacterial activity against H37 Rv strain of *M. tuberculosis*. The MIC values are in the

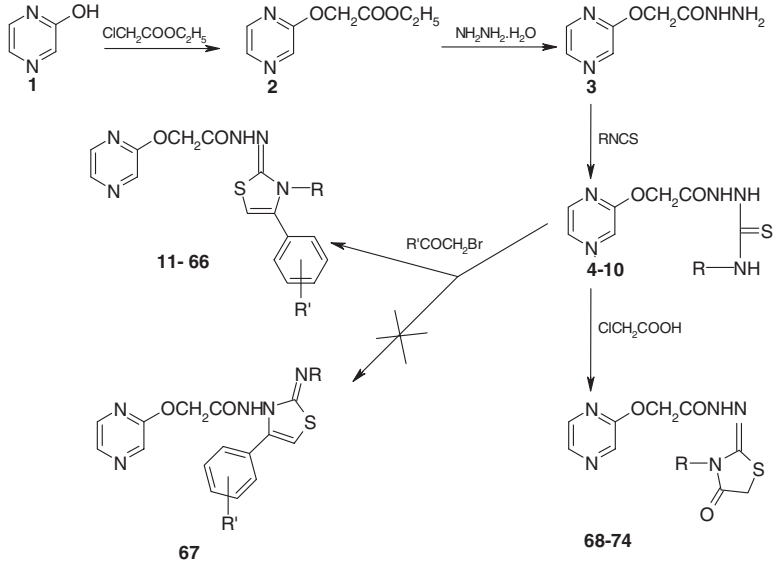

Figure 1. General scheme for synthesis of compounds **11**–**74** (R = 4-chloro-2-nitrophenyl, 4-chlorophenyl, 2,4-dichlorophenyl, 4-flurophenyl, *n*-butyl, *t*-butyl, R′ = 4-bromo, 4-chloro, 4-fluro, 4-methoxy, 4-hydroxy, 2,4-dichloro methyl).

Table 1. Physical and analytical data of compounds

Compound	R	R	Molecular formula[a]	MP	Yield[b]	Mass [M+]
11	4-Chloro-2-nitrophenyl	4-Bromo	$C_{21}H_{14}N_6O_4SClBr$	169–170	62	561[c]
12	4-Chloro-2-nitrophenyl	4-Chloro	$C_{21}H_{14}N_6O_4SCl_2$	172–175	65	517[c]
13	4-Chloro-2-nitrophenyl	4-Fluro	$C_{21}H_{14}N_6O_4SClF$	185–186	72	504[c]
14	4-Chloro-2-nitrophenyl	4-Methoxy	$C_{22}H_{17}N_6O_5SCl$	212–213	59	512[c]
15	4-Chloro-2-nitrophenyl	4-Hydroxy	$C_{21}H_{15}N_6O_5SCl$	218–219	58	498[c]
16	4-Chloro-2-nitrophenyl	2,4-Dichloro	$C_{21}H_{13}N_6O_4SCl_3$	217–218	56	551[c]
17	4-Chloro-2-nitrophenyl	—	$C_{21}H_{15}N_6O_4SCl$	156–157	67	482[c]
18	4-Chloro-2-nitrophenyl	4-Methyl	$C_{22}H_{17}N_6O_4SCl$	169–170	63	497[c]
19	4-Chlorophenyl	4-Bromo	$C_{21}H_{15}N_5O_2SClBr$	159–160	49	516[c]
20	4-Chlorophenyl	4-Chloro	$C_{21}H_{15}N_5O_2SCl_2$	165–166	59	472[c]
21	4-Chlorophenyl	4-Fluro	$C_{21}H_{15}N_5O_2SClF$	178–179	63	459[c]
22	4-Chlorophenyl	4-Methoxy	$C_{22}H_{18}N_5O_3SCl$	170–172	47	467[c]
23	4-Chlorophenyl	4-Hydroxy	$C_{21}H_{16}N_5O_3SCl$	195–196	58	453[c]
24	4-Chlorophenyl	2,4-Dichloro	$C_{21}H_{14}N_5O_2SCl_3$	201–202	59	506[c]
25	4-Chlorophenyl	—	$C_{21}H_{16}N_5O_2SCl$	221–222	46	437[c]
26	4-Chlorophenyl	4-Methyl	$C_{22}H_{18}N_5O_2SCl$	226–227	65	451[c]
27	2,4-Dichlorophenyl	4-Bromo	$C_{21}H_{14}N_5O_2SCl_2Br$	235–236	68	551[c]
28	2,4-Dichlorophenyl	4-Chloro	$C_{21}H_{14}N_5O_2SCl_3$	198–199	73	506[c]
29	2,4-Dichlorophenyl	4-Fluro	$C_{21}H_{14}N_5O_2SCl_2F$	201–202	75	494[c]
30	2,4-Dichlorophenyl	4-Methoxy	$C_{22}H_{17}N_5O_3SCl_2$	209–210	65	502[c]
31	2,4-Dichlorophenyl	4-Hydroxy	$C_{21}H_{15}N_5O_3SCl_2$	199–200	58	488[c]
32	2,4-Dichlorophenyl	2,4-Dichloro	$C_{21}H_{13}N_5O_2SCl_4$	182–183	52	541[c]
33	2,4-Dichlorophenyl	—	$C_{21}H_{15}N_5O_2SCl_2$	191–191	63	472
34	2,4-Dichlorophenyl	4-Methyl	$C_{22}H_{17}N_5O_2SCl_2$	256–257	69	486[c]
35	4-Ethoxyphenyl	4-Bromo	$C_{23}H_{20}N_5O_3SBr$	248–249	71	526[c]
36	4-Ethoxyphenyl	4-Chloro	$C_{23}H_{20}N_5O_3SCl$	243–244	46	481[c]
37	4-Ethoxyphenyl	4-Fluro	$C_{23}H_{20}N_5O_3SF$	256–257	59	469[c]
38	4-Ethoxyphenyl	4-Methoxy	$C_{24}H_{23}N_5O_3S$	196–197	58	477
39	4-Ethoxyphenyl	4-Hydroxy	$C_{23}H_{21}N_5O_4S$	209–210	64	463
40	4-Ethoxyphenyl	2,4-Dichloro	$C_{23}H_{19}N_5O_3SCl$	258–259	52	516[c]
41	4-Ethoxyphenyl	—	$C_{23}H_{21}N_5O_3S$	186–187	63	447
42	4-Ethoxyphenyl	4-Methyl	$C_{24}H_{23}N_5O_3S$	249–250	68	461
43	*n*-Butyl	4-Bromo	$C_{19}H_{20}N_5O_2SBr$	267–268	59	462[c]
44	*n*-Butyl	4-Chloro	$C_{19}H_{20}N_5O_2SCl$	135–136	76	417[c]
45	*n*-Butyl	4-Fluro	$C_{19}H_{20}N_5O_2SF$	168–169	78	405[c]
46	*n*-Butyl	4-Methoxy	$C_{20}H_{23}N_5O_3S$	233–234	54	413
47	*n*-Butyl	4-Hydroxy	$C_{19}H_{21}N_5O_3S$	265–266	63	399
48	*n*-Butyl	2,4-Dichloro	$C_{19}H_{19}N_5O_2SCl_2$	241–242	75	452[c]
49	*n*-Butyl	—	$C_{19}H_{21}N_5O_3S$	215–216	68	383
50	*n*-Butyl	4-Methyl	$C_{20}H_{23}N_5O_2S$	222–223	59	397
51	Isopropyl	4-Bromo	$C_{18}H_{18}N_5O_2SBr$	189–190	81	448[c]
52	Isopropyl	4-Chloro	$C_{18}H_{18}N_5O_2SCl$	169–170	55	403[c]
53	Isopropyl	4-Fluro	$C_{18}H_{18}N_5O_2SF$	158–159	56	391[c]
54	Isopropyl	4-Methoxy	$C_{19}H_{21}N_5O_3S$	178–179	67	399
55	Isopropyl	4-Hydroxy	$C_{18}H_{19}N_5O_3S$	171–172	53	385
56	Isopropyl	2,4-Dichloro	$C_{18}H_{17}N_5O_2SCl_2$	163–164	68	438[c]
57	Isopropyl	—	$C_{18}H_{19}N_5O_2S$	204–205	56	369
58	Isopropyl	4-Methyl	$C_{19}H_{21}N_5O_2S$	256–257	75	383
59	*t*-Butyl	4-Bromo	$C_{19}H_{21}N_5O_2SBr$	198–199	68	462[c]
60	*t*-Butyl	4-Chloro	$C_{19}H_{21}N_5O_2SCl$	166–167	79	417[c]
61	*t*-Butyl	4-Fluro	$C_{19}H_{21}N_5O_2SF$	185–186	54	405[c]
62	*t*-Butyl	4-Methoxy	$C_{20}H_{23}N_5O_3S$	143–144	68	413
63	*t*-Butyl	4-Hydroxy	$C_{19}H_{21}N_5O_3S$	168–169	56	399
64	*t*-Butyl	2,4-Dichloro	$C_{19}H_{19}N_5O_2SCl_2$	184–185	54	452[c]
65	*t*-Butyl	—	$C_{19}H_{21}N_5O_2S$	196–197	81	383
66	*t*-Butyl	4-Methyl	$C_{20}H_{23}N_5O_2S$	212–213	76	397

[a] CHN analyses were found to be within the limit of ±0.4%.
[b] All the compounds were recrystallized from ethanol.
[c] Values represent [M+2] due to appearance of an isotopic peak.

range of 1.5–12.5 while the antibacterial activity against the Gram-positive and Gram-negative stains of bacteria is moderate, also compound no **11**, **12** and **40** showed good antibacterial activity against both the Gram-positive and Gram-negative stains of bacteria.

The structure–activity relationship revealed that thiazoline ring is essential for antibacterial activity as compounds **2**, **3** and **4**–**10** showed comparatively less activity than **11**–**66**. Amide linkage plays an important role in imparting the antimycobacterial activity, as compound **2**

Table 2. Physical and analytical data of compounds

Compound	R	Molecular formula[a]	MP	Yield[b]	Mass [M+]
4	4-Chloro-2-nitrophenyl	$C_{13}H_{11}N_6O_4SCl$	245–246	86	382.78[c]
5	4-Chlorophenyl	$C_{13}H_{12}N_5O_2SCl$	220–221	84	337.78[c]
6	2,4-Dichlorophenyl	$C_{13}H_{11}N_5O_2SCl_2$	215–216	84	372.22[c]
7	4-Ethoxyphenyl	$C_{15}H_{17}N_5O_3S$	201–202	89	347.39
8	*n*-Butyl	$C_{11}H_{17}N_5O_2S$	152–154	91	269.32
9	Isopropyl	$C_{10}H_{15}N_5O_2S$	233–234	75	283.34
10	*t*-Butyl	$C_{11}H_{17}N_5O_2S$	174–175	81	269.32
68	4-Chloro-2-nitrophenyl	$C_{15}H_{11}N_6O_5SCl$	256–257	76	422.80[c]
69	4-Chlorophenyl	$C_{15}H_{12}N_5O_3SCl$	247–248	66	377.80[c]
70	2,4-Dichlorophenyl	$C_{15}H_{11}N_5O_3SCl_2$	218–219	71	412.25[c]
71	4-Ethoxyphenyl	$C_{17}H_{17}N_5O_4S$	253–254	84	387.41
72	*n*-Butyl	$C_{13}H_{17}N_5O_3S$	206–207	76	323.37
73	Isopropyl	$C_{12}H_{15}N_5O_3S$	225–226	64	309.34
74	*t*-Butyl	$C_{13}H_{17}N_5O_3S$	241–242	69	323.37

[a] CHN analyses were found to be within the limit of ±0.4%.
[b] All the compounds were recrystallized from ethanol.
[c] Values represent [M+2] due to appearance of an isotopic peak.

is less active than compound **3**. Amide linkage and thiazoline ring contributes significantly towards antimicrobial activity. The different substituent in compounds **11**–**66** over the side chain at 3 and 4 position of thiazoline ring exerts significant influence on biological activity. In general, aromatic substituted compounds at 3-position were found to be more active than aliphatic substituents. Further, the presence of electron-withdrawing groups (both halogen and nitro substituents) in **11**–**17** showed maximum antimycobacterial activity. Literature survey reveals that electrons-withdrawing or donating groups amend the lipophilicity of the test compounds, which in turn alters permeability across the bacterial cell membrane. Further the MIC values of **11**–**66** were found to be comparatively less after 21 days than 14 days against mycobacterium strain and 24 h against bacterial strains. These values were also found to be higher than MIC values of **11**–**66**, which indicated that the test compounds were metabolizing with time in the biological environment. In order to confirm this fact in vitro, the most potent compound **12** was incubated in phosphate buffer solution (pH 7.4) at 37 °C and after every 12 h, 20 μL of aliquot was analyzed in Analytical RP-HPLC (reverse phase high performance column chromatography) over a period of 15 days. Result of the study showed that after 14 days, the compound **12** (parent peak, retention time: 14.63 min) get metabolized (appearance of second peak at 9.05 min, Fig. 3). Among the compounds tested, **11**, **12** and **40** were found to be most potent. Again in comparison to thiazoline ring, thiazolidine ring showed less activity **68**–**74**. Thus thiazolidine ring is not essential for imparting the antibacterial activity to the compounds containing pyrazine ring. Further, the toxicity of most potent compounds (**11**, **12** and **40**) were assessed using hemolytic assay and minimal hemolytic concentration (MHC) and LD_{50} values (Log concentration of test compound required for 50% hemolysis) were determined from the graph of % lyses against Log concentration (Fig. 2). The most potent compounds (**11**, **12** and **40**) were found to be nontoxic up to a dose level of 250 μg/mL (MHC) and their corresponding LD_{50} values were found to be within the range of 560–770 μg/mL (11, 770; 12, 650; 40, 560; μg/mL). Thus, in comparison with antibiotics commonly used in therapy, our most potent compounds showed similar or slightly less in vitro antimicrobial activity against various bacterial (Gram-positive and Gram-negative) and mycobacterial strains with marked reduction in toxicity (hemolytic activity), suggested that this class of compounds could be used as potent broad-spectrum antimicrobial agent to treat various clinical conditions associated with multiple infectious diseases. Further studies are in progress to optimize these lead compounds and to characterize the mode of action.

3.1. Antimicrobial activity

All the test compounds were assayed in vitro for antibacterial activity against two different strains of Gram-negative (*E. coli* and *S. typhi*) and Gram-positive (*S. aureus* and *B. subtilis*) bacteria and the antimycobacterial activity was evaluated against H37 Rv strain of *M. tuberculosis* strain using standard protocol.[24] The minimum inhibitory concentration (MIC) was determined by the test tube dilution technique using Mueller–Hinton nutrient broth (for antibacterial) and modified Kirchner's culture medium containing 0.5% sterilized horse serum for antimycobacterial activity. The MIC values were also tested for standard antibiotics (Ampicillin, Penicillin-G and Chloramphenicol) to compare the antibacterial activity of our test compounds. Rifampin and Isoniazid (INH) were used as standard for antimycobacterial activity. Further, the toxicity of most potent compounds **11**, **12** and **40** were assessed using hemolytic assay and minimal hemolytic concentration (MHCs) and LD_{50} values were determined.[25]

4. Experimental

Melting points were determined in open capillaries using microprocessor-based melting point apparatus, Model

Table 3. In vitro antibacterial and antimycobacterial activity of test compounds

Compound	Antibacterial activity[a]				Antimycobacterial activity[b]	
	Gram positive		Gram negative		*M. tuberculosis*	
	S. aureus	*B. subtilis*	*E. coli*	*S. typhi*	14[c]	21[d]
2	e	e	e	e	e	e
3	e	e	e	e	e	e
4	157	185	175	>200	52	61
5	168	195	125	139	62	74
6	187	169	174	125	57	65
7	158	142	156	121	85	92
8	123	158	147	159	45	56
9	165	158	141	123	56	63
10	109	136	125	158	51	49
11	3	5	8	4	1.0	6
12	6	1	2	9	0.3	4
13	19	15	17	15	6.1	9
14	17	14	17	13	2.7	8
15	15	12	32	19	4.0	7
16	19	11	23	15	3.6	5
17	12	25	63	16	5.2	9
18	12	15	25	23	4.5	12
19	19	13	25	14	10.7	16
20	37	36	42	25	11.5	23
21	14	19	25	36	12.7	17
22	12	16	21	39	11.6	16
23	16	21	29	24	12.4	24
24	21	29	32	12	10.8	13
25	32	18	39	25	12.5	26
26	23	48	56	89	12.1	52
27	41	26	48	39	10.5	22
28	9	11	15	12	11.9	16
29	12	9	14	18	12.2	11
30	7	9	13	19	9.6	15
31	27	35	36	57	10.8	21
32	23	45	59	58	10.6	15
33	42	39	54	62	12.4	23
34	17	16	28	69	11.5	21
35	25	36	84	56	8.9	17
36	26	52	54	63	8.7	19
37	59	63	68	74	10.2	22
38	26	39	85	56	8.7	14
39	15	25	23	69	10.4	15
40	1	7	2	3	1.5	8
41	25	69	58	49	3.5	15
42	26	28	39	52	3.1	12
43	26	95	56	26	7.4	19
44	69	58	45	16	7.6	13
45	26	25	27	16	7.6	15
46	59	48	63	47	6.6	18
47	56	57	49	26	7.9	16
48	28	63	67	69	7.2	13
49	49	56	57	26	9.2	21
50	68	59	57	58	8.7	20
51	28	68	54	17	8.1	15
52	14	28	64	37	8.7	16
53	57	58	29	27	8.5	21
54	24	26	54	95	7.5	22
55	27	65	45	47	8.4	11
56	56	85	54	25	8.2	14
57	56	26	128	64	10	18
58	63	58	25	69	9.5	13
59	85	76	35	25	6.3	18
60	46	28	95	23	7.2	19
61	95	25	26	36	6.9	13
62	56	38	39	25	5.8	17
63	69	37	35	26	7.1	12

(*continued on next page*)

Table 3 (*continued*)

Compound	Antibacterial activity[a]				Antimycobacterial activity[b]	
	Gram positive		Gram negative		*M. tuberculosis*	
	S. aureus	*B. subtilis*	*E. coli*	*S. typhi*	14[c]	21[d]
64	36	98	56	63	6.6	15
65	56	85	65	25	8.7	18
66	45	95	46	23	8.2	13
68	104	85	73	41	85	89
69	125	189	NA	79	102	105
70	158	175	86	96	148	154
71	109	135	168	125	169	175
72	186	163	NA	NA	156	159
73	174	163	156	185	191	196
74	124	189	NA	175	128	135
Std1[f]	12.5	2.6	25	12.5	NA	NA
Std2[g]	0.01	0.09	0.015	0.016	NA	NA
Std3[h]	5	7	6	7	NA	NA
Std4[i]	1	0.9	51	57	0.2	0.22
Std5[j]	Inactive	Inactive	Inactive	Inactive	0.005	0.008

[a] DMF has no antimicrobial activity at the concentration used to dissolve the test compounds.
[b] MIC: minimum inhibitory concentration.
[c] Antimycobacterial activity was measured after 14 days of incubation.
[d] Antimycobacterial activity was measured after 21 days of incubation.
[e] Compound **2** was found to be inactive at 200 μg/mL. NA: indicates activity not measured.
[f] Std1: Ampicillin.
[g] Std2: Penicillin-G.
[h] Std3: Chloramphenicol.
[i] Std4: Rifampin.
[j] Std5: Isoniazid (INH).

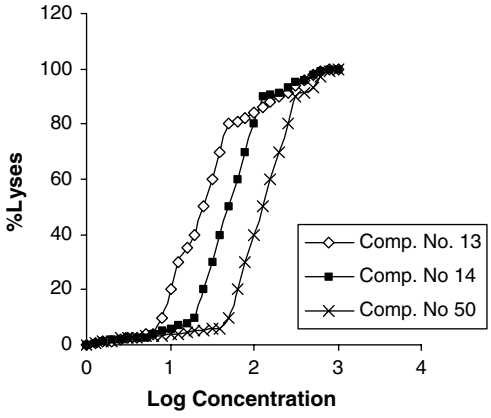

Figure 2. Graph of % lyses against Log concentration for compounds **11**, **12** and **40**.

PMP-DM (Mumbai, India) and are uncorrected. Purity of the compounds was checked by precoated TLC plates (E. Merck Kieselgel 60 F25, Mumbai, India). IR spectra were recorded using KBr pellets on Perkin–Elmer 337 spectrophotometer from Perkin–Elmer International Incorporation, Rorkreuz, Switzerland (V_{max} in cm^{-1} ^1H NMR spectra on a Varian 300 MHz instrument at RSIC, IIT, Powai. Elemental analyses were carried out using FLASH EA 1112 CHN analyzer from Thermo Finnigen, Italy. Mass spectra (FAB-MS) were recorded on 70 eV on Jeol D-300 spectrometer (Jeol Ltd., Tokyo, Japan).

4.1. Syntheses

Specific examples presented below illustrate general synthetic procedures.

Synthesis of **2**: To a mixture of TEA (6.58 g, 0.065 mol), 2-hydroxy pyrazine (5 g, 0.0575 mol), was added dropwise a solution of ethyl chloroacetate (10.87 mL, 0.065 mol) in 1:4 dioxane (50 mL). The temperature was maintained at 90 °C for 1 h and then the reaction mixture was stirred for 7–8 h. The excess solvent was removed under reduced pressure to obtain sticky mass, which was poured into ice-cold water and extracted from chloroform. The chloroform was removed under vacuum and solid product obtained was recrystallized from chloroform. Yield 72%, Rf: 0.56 (acetonitrile:methanol, 1:1); mp 68–69 °C, IR: 1730 (C=O stretching), 2985, 2989 (CH_2CH_3 stretching) cm^{-1}; ^1H NMR ($CDCl_3$): 1.37 (t, 3H, CH_3), 4.34–4.61 (q, 2H, CH_2), 4.70 (s, 2H, OCH_2), 8.76 (t, 1H, pyrazine C5-H), 8.89 (d, 1H, pyrazine C6-H), 9.254 (d, 1H, pyrazine C2-H), FABMS (*m/z*, 100%): 182.15 [M^+, 100%); Anal. found: C, 52.74; H, 5.53; N, 15.37. For $C_8H_{10}N_2O_3$ (182.17): C, 52.73; H, 5.59; N, 15.32.

Synthesis of **3**: A mixture of **2** (3.75 g, 0.02 mol), 85% hydrazine hydrate (4.12 mL, 0.08 mol) in ethanol (40 mL) was refluxed for 12 h. The excess solvent was removed under reduced pressure and the reaction mixture was cooled at 4–5 °C. The solid crystals separated were filtered, washed with cold water, dried and recrystalized from ethanol. Yield 62%, Rf: 0.62 (sceto-

nitrile:methanol, 1:1); mp 170–171 °C, IR: 3382, 3355 ($NHNH_2$), 1733 (C=O stretching), cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.40 (s, 2H, NH_2), 4.70 (s, 2H, OCH_2), 7.78 (s, 1H, CONH) 8.74 (t, 1H, pyrazine C5-H), 8.85 (d, 1H, pyrazine C6-H), 9.24 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 168.13 [M^+, 100%); Anal. found: C, 42.86; H, 4.80; N, 33.32. For $C_6H_8N_4O_2$ (168.15): C, 42.83; H, 4.80; N, 33.31.

Synthesis of **4**: To a solution of **3** (0.01 mol) in ethanol (50 mL), various aliphatic/aromatic isothiocynates (0.01 mol) were added and the reaction mixture was refluxed for 12 h. Excess solvent was removed under vacuum. The residue was washed with diethyl ether and recrystallized using methanol.

Yield 85%, Rf: 0.68 (acetonitrile:methanol, 1:1); mp 245–246 °C, IR: 3215, 3230 (NH), 1731 (C=O stretching), 1315 (C=S stretching) cm^{-1}; ^1H NMR ($CDCl_3$): 4.70 (s, 2H, OCH_2), 7.24–7.52 (m, 3H, ArH), 7.78 (s, 1H, CONH), 8.74 (t, 1H, pyrazine C5-H), 8.85 (d, 1H, pyrazine C6-H), 9.24 (d, 1H, pyrazine C2-H), 10.40 (s, 1H, NH), 11.81 (s, 1H, NHAr), FABMS (m/z, 100%): 384.75 ([M^++2], 100%); Anal. found: C, 40.78; H, 2.92; N, 21.98. For $C_{13}H_{11}N_6O_4SCl$ (382.78): C, 40.79; H, 2.90; N, 21.96.

5. Yield 81%, Rf: 0.65 (acetonitrile:methanol, 1:1); mp 220–221 °C, IR: 3217, 3232 (NH), 1738 (C=O stretching), 1313 (C=S stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.71 (s, 2H, OCH_2), 7.39–7.62 (m, 4H, ArH), 7.75 (s, 1H, CONH), 8.73 (t, 1H, pyrazine C5-H), 8.82 (d, 1H, pyrazine C6-H), 9.21 (d, 1H, pyrazine C2-H), 10.44 (s, 1H, NH), 11.83 (s, 1H, NHAr), FABMS (m/z, 100%): 339.76 ([M^++2], 100%); Anal. found: C, 46.20; H, 3.56; N, 20.76. For $C_{13}H_{12}N_5O_2SCl$ (337.78): C, 46.22; H, 3.58; N, 20.73.

6. Yield 78%, Rf: 0.62 (acetonitrile:methanol, 1:1); mp 215–216 °C, IR: 3212, 3235 (NH), 1734 (C=O stretching), 1317 (C=S stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.72 (s, 2H, OCH_2), 7.21–7.54 (m, 3H, ArH), 7.74 (s, 1H, CONH), 8.73 (t, 1H, pyrazine C5-H), 8.82 (d, 1H, pyrazine C6-H), 9.21 (d, 1H, pyrazine C2-H), 10.43 (s, 1H, NH), 11.82 (s, 1H, NHAr), FABMS (m/z, 100%): 374.21 ([M^++2], 100%); Anal. found: C, 41.92; H, 2.91; N, 18.85. For $C_{13}H_{11}N_5O_2SCl_2$ (372.23): C, 41.95; H, 2.98; N, 18.81.

7. Yield 86%, Rf: 0.59 (acetonitrile:methanol, 1:1); mp 201–202 °C, IR: 3214, 3236 (NH), 1733 (C=O stretching), 1318 (C=S stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.22, (t, 3H, CH_2 CH_3), 3.61(q, 2H, CH_2 CH_3), 4.72 (s, 2H, OCH_2), 7.01–7.49 (m, 4H, ArH), 7.74 (s, 1H, CONH), 8.73 (t, 1H, pyrazine C5-H), 8.82 (d, 1H, pyrazine C6-H), 9.21 (d, 1H, pyrazine C2-H), 10.43 (s, 1H, NH), 11.82 (s, 1H, NHAr), FABMS (m/z, 100%): 347.39 ([M^+], 100%); Anal. found: C, 51.84; H, 4.94; N, 20.19. For $C_{15}H_{17}N_5O_3S$ (372.23): C, 51.86; H, 4.93; N, 20.16.

8. Yield 78%, Rf: 0.62 (acetonitrile:methanol, 1:1); mp 152–153 °C, IR: 3209, 3232 (NH stretching), 2984, 2989, 2991 ($CH_2CH_2CH_2CH_3$ stretching) 1733 (C=O stretching), 1315 (C=S stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.94 (t, 3H, butyl CH_3) 1.39 (m, 2H, butyl CH_2), 1.53 (m, 2H, butyl CH_2), 3.62 (m, 2H, butyl CH_2), 4.71 (s, 2H, OCH_2), 7.02–7.61 (m, 4H, ArH), 7.73 (s, 1H, CONH), 8.74 (t, 1H, pyrazine C5-H), 8.81 (d, 1H, pyrazine C6-H), 9.22 (d, 1H, pyrazine C2-H), 10.44 (s, 1H, NH), 11.81 (s, 1H, NHAr), FABMS (m/z, 100%): 283.33 ([M^+], 100%); Anal. found: C, 46.61; H, 6.06; N, 24.74. For $C_{11}H_{17}N_5O_2S$ (283.35): C, 46.63; H, 6.05; N, 24.72.

9. Yield 83%, Rf: 0.56 (acetonitrile:methanol, 1:1); mp 233–234 °C, IR: 3213, 3229 (NH stretching), 2986, 2990 ($CH(CH_3)_2$ stretching), 1732 (C=O stretching), 1315 (C=S stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.2 (dd, 6H, isopropyl CH_3), 4.00 (m, 1H, isopropyl CH), 4.71 (s, 2H, OCH_2), 7.74 (s, 1H, CONH) 8.73 (t, 1H, pyrazine C5-H), 8.82 (d, 1H, pyrazine C6-H), 9.21 (d, 1H, pyrazine C2-H), 10.44 (s, 1H, NH), 11.82 (s, 1H, NHAr), FABMS (m/z, 100%): 269.35 ([M^+], 100%); Anal. found: C, 44.64; H, 5.62; N, 26.05. For $C_{10}H_{15}N_5O_2S$ (269.32): C, 44.60; H, 5.61; N, 26.00.

10. Yield 86%, Rf: 0.60 (acetonitrile:methanol, 1:1); mp 174–175 °C, IR: 3213, 3224 (NH stretching), 2981, 2986, 2990, ($C(CH_3)_3$ stretching) 1731 (C=O stretching), 1316 (C=S stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.2 (dd, 9H, *t*-butyl CH_3), 4.72 (s, 2H, OCH_2), 7.73 (s, 1H, CONH), 8.71 (t, 1H, pyrazine C5-H), 8.84 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), 10.45 (s, 1H, NH), 11.83 (s, 1H, NHAr), FABMS (m/z, 100%): 283.32 ([M^+], 100%); Anal. found: C, 46.62; H, 6.05; N, 24.73. For $C_{11}H_{17}N_5O_2S$ (283.35): C, 46.63; H, 6.05; N, 24.72.

Synthesis of **11**: The mixture of the thiosemicarbazide (0.01 mol), appropriate phenacyl bromide (0.01 mol) and sodium acetate (0.2 mol) in ethanol (50 mL) was refluxed for 7 h. The mixture was cooled, diluted with enough water to develop turbidity and left overnight to obtain the product. The product was filtered, dried and recrystallized using aqueous ethanol. The yields and physical constants are summarized in Table 1.

Rf: 0.68 (acetonitrile:methanol, 1:1), IR: 3225 (NH), 1731 (C=O stretching), 1576, 1480, 1051 (thiazoline), 3001 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.71 (s, 2H, OCH_2), 6.02 (s, 1H, thiazoline), 7.14–7.62 (m, 7H, ArH), 7.77 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 563.81 ([M^++2], 100%); Anal. found: C, 44.94; H, 2.52; N, 14.98. For $C_{21}H_{14}N_6O_4SClBr$ (561.80): C, 44.90; H, 2.51; N, 14.96.

12. Rf: 0.61 (acetonitrile:methanol, 1:1), IR: 3226 (NH), 1732 (C=O stretching), 1577, 1481, 1050 (thiazoline), 3002 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.73 (s, 2H, OCH_2), 6.04 (s, 1H, thiazoline), 7.09–7.72 (m, 7H, ArH), 7.78 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 517.37 ([M^++2], 100%); Anal. found: C, 48.72; H, 2.74; N, 16.29. For $C_{21}H_{14}N_6O_4SCl_2$ (517.35): C, 48.76; H, 2.73; N, 16.25.

13. Rf: 0.72 (acetonitrile:methanol, 1:1), IR: 3225 (NH), 1733 (C=O stretching), 1576, 1481, 1051 (thiazoline), 3003 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.72 (s, 2H, OCH_2), 5.99 (s, 1H, thiazoline), 7.19–7.63 (m, 7H, ArH), 7.79 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 500.87 ([M^++2], 100%); Anal. found: C, 50.3 8; H, 2.84; N, 16.76. For $C_{21}H_{14}N_6O_4SCl_2$ (500.89): C, 50.36; H, 2.82; N, 16.78.

14. Rf: 0.58 (acetonitrile:methanol, 1:1), IR: 3225 (NH), 1738 (C=O stretching), 1572, 1486, 1057 (thiazoline), 3005 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.2 (t, 3H, OCH_3–C_6H_4), 4.72 (s, 2H, OCH_2), 5.97 (s, 1H, thiazoline), 7.18–7.65 (m, 7H, ArH), 7.76 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 514.95 ([M^++2], 100%); Anal. found: C, 51.56; H, 3.33; N, 16.36. For $C_{22}H_{17}N_6O_5SCl$ (512.93): C, 51.52; H, 3.34; N, 16.38.

15. Rf: 0.62 (acetonitrile:methanol, 1:1), IR: 3228 (NH), 1734 (C=O stretching), 1573, 1485, 1056 (thiazoline), 3004 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.72 (s, 2H, OCH_2), 5.96 (s, 1H, thiazoline), 7.12–7.69 (m, 7H, ArH), 7.78 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.19 (s, 1H, C_6H_4OH), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 500.8 ([M^++2], 100%); Anal. found: C, 51.56; H, 3.33; N, 16.36. For $C_{21}H_{15}N_6O_5SCl$ (498.9): C, 50.56; H, 3.03; N, 16.85.

16. Rf: 0.63 (acetonitrile:methanol, 1:1), IR: 3225 (NH), 1733 (C=O stretching), 1578, 1482, 1052 (thiazoline), 3005 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.73 (s, 2H, OCH_2), 6.07 (s, 1H, thiazoline), 7.09–7.72 (m, 6H, ArH), 7.78 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 553.76 ([M^++2], 100%); Anal. found: C, 45.73; H, 2.39; N, 15.25. For $C_{21}H_{13}N_6O_4SCl_3$ (551.79): C, 45.71; H, 2.38; N, 15.23.

17. Rf: 0.56 (acetonitrile:methanol, 1:1), IR: 3226 (NH), 1732 (C=O stretching), 1579, 1484, 1052 (thiazoline), 3005 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.73 (s, 2H, OCH_2), 6.05 (s, 1H, thiazoline), 7.02–7.73 (m, 9H, ArH), 7.77 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 553.76 ([M^++2], 100%); Anal. C, 52.25; H, 3.12; N, 17.44. For $C_{21}H_{15}N_6O_4SCl$ (482.90): C, 52.23; H, 3.13; N, 17.40.

18. Rf: 0.65 (acetonitrile:methanol, 1:1), IR: 3226 (NH), 1737 (C=O stretching), 1573, 1484, 1055 (thiazoline), 3003 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.2 (t, 3H, CH_3–C_6H_4), 4.72 (s, 2H, OCH_2), 5.97 (s, 1H, thiazoline), 7.18–7.65 (m, 7H, ArH), 7.76 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 498.94 ([M^++2], 100%); Anal. found: C, 53.15; H, 3.43; N, 16.95. For $C_{22}H_{17}N_6O_4SCl$ (496.93): C, 53.18; H, 3.45; N, 16.91.

19. Rf: 0.58 (acetonitrile:methanol, 1:1), IR: 3223 (NH), 1732 (C=O stretching), 1574, 1482, 1052 (thiazoline), 3004 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.71 (s, 2H, OCH_2), 6.05 (s, 1H, thiazoline), 7.14–7.52 (m, 8H, ArH), 7.77 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 518.82 ([M^++2], 100%); Anal. found: C, 48.83; H, 2.92; N, 16.94. For $C_{21}H_{15}N_5O_2SClBr$ (516.80): C, 48.81; H, 2.93; N, 16.91.

23. Rf: 0.59 (acetonitrile:methanol, 1:1), IR: 3226 (NH), 1733 (C=O stretching), 1572, 1484, 1057 (thiazoline), 3003 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.72 (s, 2H, OCH_2), 5.95 (s, 1H, thiazoline), 7.16–7.64 (m, 8H, ArH), 7.75 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.20 (s, 1H, C_6H_4OH) 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 500.8 ([M^++2], 100%); Anal. found: C, 55.54; H, 3.55; N, 15.46. For $C_{21}H_{16}N_5O_3SCl$ (453.90): C, 55.57; H, 3.56; N, 15.43.

26. Rf: 0.65 (acetonitrile:methanol, 1:1), IR: 3226 (NH), 1734 (C=O stretching), 1575, 1484, 1056 (thiazoline), 3004 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.3 (t, 3H, CH_3–C_6H_4), 4.72 (s, 2H, OCH_2), 5.98 (s, 1H, thiazoline), 7.18–7.65 (m, 8H, ArH), 7.75 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 453.95 ([M^++2], 100%); Anal. C, 58.49; H, 4.05; N, 15.53. For $C_{22}H_{18}N_5O_2SCl$ (451.93): C, 58.47; H, 4.04; N, 15.50.

30. Rf: 0.77 (acetonitrile:methanol, 1:1), IR: 3224 (NH), 1739 (C=O stretching), 1573, 1485, 1056 (thiazoline), 3003 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.26 (t, 3H, OCH_3–C_6H_4), 4.72 (s, 2H, OCH_2), 5.97 (s, 1H, thiazoline), 7.09–7.71 (m, 7H, ArH), 7.76 (s, 1H, CONH) 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 504.35 ([M^++2], 100%); Anal. found: C, 52.63; H, 3.42; N, 13.97. For $C_{22}H_{17}N_5O_3SCl_2$ (502.37): C, 52.60; H, 3.41; N, 13.94.

32. Rf: 0.67 (acetonitrile:methanol, 1:1), IR: 3224 (NH), 1735 (C=O stretching), 1579, 1483, 1053 (thiazoline), 3004 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.73 (s, 2H, OCH_2), 6.07 (s, 1H, thiazoline), 7.18–7.70 (m, 6H, ArH), 7.78 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 543.25 ([M^++2], 100%); Anal. found: C, 46.62; H, 2.44; N, 12.97. For $C_{21}H_{13}N_5O_2SCl_4$ (541.24): C, 46.60; H, 2.42; N, 12.94.

33. Rf: 0.56 (acetonitrile:methanol, 1:1), IR: 3225 (NH), 1731 (C=O stretching), 1575, 1481, 1055 (thiazoline), 3002 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.72 (s, 2H, OCH_2), 6.04 (s, 1H, thiazoline), 7.09–7.71 (m, 9H, ArH), 7.77 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 474.36 ([M^++2], 100%); Anal. C, 53.42; H, 3.22; N, 14.84. For $C_{21}H_{15}N_6O_4SCl$ (472.35): C, 53.40; H, 3.20; N, 14.83.

37. Rf: 0.71 (acetonitrile:methanol, 1:1), IR: 3223 (NH), 1734 (C=O stretching), 1575, 1482, 1053 (thiazoline), 3002 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.72 (s, 2H, OCH_2), 6.01 (s, 1H, thiazoline), 7.08–7.61 (m, 8H, ArH), 7.79 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 467.48 ([M^++2], 100%); Anal. found: C, 59.31; H, 4.35; N, 15.09. For $C_{23}H_{20}N_5O_3FS$ (465.50): C, 59.35; H, 4.33; N, 15.05.

39. Rf: 0.67 (acetonitrile:methanol, 1:1), IR: 3224 (NH), 1733 (C=O stretching), 1571, 1484, 1055 (thiazoline), 3003 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.22, (t, 3H, CH_2CH_3), 3.61 (q, 2H, CH_2CH_3), 4.72 (s, 2H, OCH_2), 6.07 (s, 1H, thiazoline), 7.03–7.67 (m, 8H, ArH), 7.78 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.19 (s, 1H, C_6H_4OH) 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 463.51 ([M^+], 100%); Anal. found: C, 59.65; H, 4.55; N, 15.13. For $C_{21}H_{15}N_5O_4S$ (498.9): C, 59.60; H, 4.57; N, 15.11.

41. Rf: 0.72 (acetonitrile:methanol, 1:1), IR: 3224 (NH), 1735 (C=O stretching), 1575, 1482, 1052 (thiazoline), 3004 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.72 (s, 2H, OCH_2), 5.99 (s, 1H, thiazoline), 7.05–7.49 (m, 9H, ArH), 7.78 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 447.53 ([M^+], 100%); Anal. found: C, 61.73; H, 4.73; N, 15.65. For $C_{23}H_{21}N_5O_3S$ (447.51): C, 61.73; H, 4.73; N, 15.65.

43. Rf: 0.53 (acetonitrile:methanol, 1:1), IR: 3226 (NH), 1732 (C=O stretching), 1575, 1481, 1053 (thiazoline), 3002 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.94 (t, 3H, butyl CH_3), 1.39 (m, 2H, butyl CH_2), 1.53 (m, 2H, butyl CH_2), 3.62 (m, 2H, butyl CH_2), 4.71 (s, 2H, OCH_2), 5.99 (s, 1H, thiazoline), 7.16–7.68 (m, 4H, ArH), 7.77 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 464.38 ([M^++2], 100%); Anal. found: C, 49.33; H, 4.32; N, 15.17. For $C_{19}H_{20}N_5O_2SBr$ (462.36): C, 49.36; H, 4.30; N, 15.15.

47. Rf: 0.62 (acetonitrile:methanol, 1:1), IR: 3225 (NH), 1734 (C=O stretching), 1571, 1487, 1056 (thiazoline), 3001 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.95 (t, 3H, butyl CH_3), 1.38 (m, 2H, butyl CH_2), 1.54 (m, 2H, butyl CH_2), 3.63 (m, 2H, butyl CH_2) δ 4.72 (s, 2H, OCH_2), 5.94 (s, 1H, thiazoline), 7.19–7.56 (m, 4H, ArH), 7.75 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.20 (s, 1H, C_6H_4OH) 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 399.49 ([M^++2], 100%); Anal. found: C, 57.19; H, 5.31; N, 17.33. For $C_{19}H_{21}N_5O_3S$ (399.47): C, 57.13; H, 5.30; N, 17.32.

50. Rf: 0.52 (acetonitrile:methanol, 1:1), IR: 3225 (NH), 1736 (C=O stretching), 1573, 1482, 1059 (thiazoline), 3004 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): 0.96 (t, 3H, butyl CH_3), 1.34 (t, 3H, CH_3–C_6H_4), 1.39 (m, 2H, butyl CH_2), 1.56 (m, 2H, butyl CH_2), 3.65 (m, 2H, butyl CH_2), 4.72 (s, 2H, OCH_2), 5.98 (s, 1H, thiazoline), 7.15–7.61 (m, 4H, ArH), 7.76 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 397.53 ([M^+], 100%); Anal. found: C, 60.45; H, 5.85; N, 17.64.For $C_{20}H_{23}N_5O_2S$ (397.50): C, 60.43; H, 5.83; N, 17.62.

52. Rf: 0.51 (acetonitrile:methanol, 1:1), IR: 3224 (NH), 1732 (C=O stretching), 1573, 1488, 1056 (thiazoline), 3002 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.23 (dd, 6H, isopropyl CH_3), 4.03 (m, 1H, isopropyl CH), 4.72 (s, 2H, OCH_2), 6.06 (s, 1H, thiazoline), 7.08–7.76 (m, 4H, ArH), 7.79 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 405.87 ([M^++2], 100%); Anal. found: C, 48.72; H, 2.74; N, 16.29. For $C_{18}H_{18}N_5O_2SCl$ (403.89): C, 48.76; H, 2.73; N, 16.25.

55. Rf: 0.49 (acetonitrile:methanol, 1:1), IR: 3223 (NH), 1733 (C=O stretching), 1575, 1490, 1060 (thiazoline), 3005 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.22 (dd, 6H, isopropyl CH_3), 4.05 (m, 1H, isopropyl CH), 4.73 (s, 2H, OCH_2), 6.05 (s, 1H, thiazoline), 7.05–7.66 (m, 4H, ArH), 7.79 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.18 (s, 1H, C_6H_4OH), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 385.46 ([M^+], 100%); Anal. found: C, 56.05; H, 4.94; N, 18.12. For $C_{18}H_{19}N_5O_3S$ (385.44): C, 56.09; H, 4.96; N, 18.17.

57. Rf: 0.73 (acetonitrile:methanol, 1:1), IR: 3225 (NH), 1734 (C=O stretching), 1578, 1485, 1054 (thiazoline), 3003 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.73 (s, 2H, OCH_2), 5.58 (s, 1H, thiazoline), 7.03–7.69 (m, 9H, ArH), 7.78 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 369.46 ([M^+], 100%); Anal. C, 58.54; H, 5.19; N, 18.99. For $C_{18}H_{19}N_5O_2S$ (369.44): C, 58.52; H, 5.18; N, 18.96.

59. Rf: 0.59 (acetonitrile:methanol, 1:1), IR: 3226 (NH), 1732 (C=O stretching), 1575, 1481, 1053 (thiazoline), 3002 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.39 (s, 9H, *t*-butyl), 4.72 (s, 2H, OCH_2), 6.01 (s, 1H, thiazoline), 7.06–7.69 (m, 4H, ArH), 7.76 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 464.35 ([M^++2], 100%); Anal. found: C, 49.34; H, 4.31; N, 15.18. For $C_{19}H_{20}N_5O_2SBr$ (462.36): C, 49.36; H, 4.30; N, 15.15.

62. Rf: 0.64 (acetonitrile:methanol, 1:1), IR: 3225 (NH), 1733 (C=O stretching), 1576, 1484, 1057 (thiazoline), 3003 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.21 (t, 3H, OCH_3–C_6H_4), 1.40 (s, 9H, *t*-butyl), 4.73 (s, 2H, OCH_2), 6.03 (s, 1H, thiazoline), 7.06–7.69 (m, 4H, ArH), 7.76 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (m/z, 100%): 413.48 ([M^+], 100%); Anal. found: C, 57.16; H, 5.32; N, 17.59. For $C_{20}H_{23}N_5O_3S$ (413.49): C, 57.12; H, 5.30; N, 17.54.

66. Rf: 0.65 (acetonitrile:methanol, 1:1), IR: 3225 (NH), 1734 (C=O stretching), 1573, 1486, 1054 (thiazoline), 3005 (ArH stretching) cm^{-1}; ^1H NMR ($CDCl_3$): 1.34 (t, 3H, CH_3–C_6H_4), 1.40 (s, 9H, *t*-butyl), 4.72 (s, 2H, OCH_2), 5.97 (s, 1H, thiazoline), 7.15–7.61 (m, 4H, ArH), 7.76 (s, 1H, CONH), 8.75 (t, 1H, pyrazine C5-H), 8.86 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (*m*/*z*, 100%): 397.51 ([M^+], 100%); Anal. found: C, 60.46; H, 5.84; N, 17.61. For $C_{20}H_{23}N_5O_2S$ (397.50): C, 60.43; H, 5.83; N, 17.62.

Synthesis of **68**: A mixture of the thiosemicarbazide (0.01 mol), chloroacetic acid (0.01 mol) and sodium acetate (0.2 mol) in ethanol (60 mL) was refluxed for 10 h. The mixture was cooled, diluted with enough water to develop turbidity and left overnight to obtain the product. The product was filtered, dried and recrystallized using aqueous ethanol. The yields and physical constants are summarized in Table 2. Yield 71%, Rf: 0.56 (acetonitrile:methanol, 1:1); mp 256–257 °C, IR: 3224 (NH), 1732 (C=O stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.76 (s, 2H, thiazolidine CH_2), 4.70 (s, 2H, OCH_2), 7.15–7.61 (m, 3H, ArH), 7.75 (s, 1H, CONH), 8.73 (t, 1H, pyrazine C5-H), 8.84 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (*m*/*z*, 100%): 424.82 ([M^++2], 100%); Anal. found: C, 42.68; H, 2.65; N, 19.91. For $C_{15}H_{11}N_6O_5SCl$ (382.78): C, 42.61; H, 2.63; N, 19.88.

70. Yield 71%, Rf: 0.61 (acetonitrile:methanol, 1:1); mp 218–219 °C, IR: 3214 (NH), 1731 (C=O stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.98 (s, 2H, thiazolidine CH2), 4.72 (s, 2H, OCH_2), 7.15–7.62 (m, 3H, ArH), 7.75 (s, 1H, CONH), 8.73 (t, 1H, pyrazine C5-H), 8.82 (d, 1H, pyrazine C6-H), 9.21 (d, 1H, pyrazine C2-H), FABMS (*m*/*z*, 100%): 414.26 ([M^++2], 100%); Anal. found: C, 43.71; H, 2.71; N, 17.01. For $C_{15}H_{11}N_5O_3SCl_2$ (372.23): C, 43.70; H, 2.69; N, 16.99.

74. Yield 69%, Rf: 0.65 (acetonitrile:methanol, 1:1); mp 241–242 °C, IR: 3212 (NH stretching), 2982, 2987, 2989, ($C(CH_3)_3$ stretching) 1732 (C=O stretching), cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.2 (dd, 9H, *t*-butyl CH_3), 3.01 (s, 2H, thiazolidine CH2), 4.72 (s, 2H, OCH_2), 7.74 (s, 1H, CONH), 8.71 (t, 1H, pyrazine C5-H), 8.84 (d, 1H, pyrazine C6-H), 9.23 (d, 1H, pyrazine C2-H), FABMS (*m*/*z*, 100%): 323.37 ([M^+], 100%); Anal. found: C, 48.30; H, 5.32; N, 21.71. For $C_{13}H_{17}N_5O_2S$ (283.35): C, 48.29; H, 5.30; N, 21.66.

4.2. Microbiology

All the test compounds were assayed in vitro for antibacterial activity against two different strains of Gram-negative (*E. coli* ATCC3750 and *S. typhi* NCTC 786) and Gram-positive (*S. aureus* ATCC 3750, *B. subtilis* 6633) bacteria and the antimycobacterial activity was evaluated against $H_{37}Rv$ strain of *M. tuberculosis*. The MIC was determined by the test tube dilution technique using Mueller–Hinton nutrient broth (for antibacterial) and modified Kirchner's culture medium containing 0.5% sterilized horse serum for antimycobacterial activity.[24] The MIC values were also tested for three well-known antibiotics (penicillin-G, ampicillin and chloramphenicol) to compare the antibacterial activity of our test compounds with the antibiotics, which are currently in therapy. Rifampcin and isoniazid (INH) were used as reference standard for antimycobacterial activity. The stock solution (2–4 μg/mL) of test compounds was prepared in a mixture of sterile water and dimethylformamide (8:2) solvent. The stock solution was sterilized by passing through a 0.2 mm polycarbonate sterile membrane (Nuclepore) filters. Further, the serial dilution of test compounds was carried out and the following concentration was used: 1000, 500, 250, 125, 62, 32, 16, 8, 4 and 1 μg/mL. Test compounds at various concentrations were added to culture medium in a sterilized borosilicate test tube and different bacterial strains were inoculated at 10^6 bacilli/mL concentration. The tubes were incubated at 37 °C for 24 h for antibacterial activity and 14 and 21 days for antimycobacterial activity and then examined for the presence or absence of growth of the test organisms. All experiments were performed in triplicate. The MIC values were obtained from the lowest concentration of the test compound where the tubes remained clear, indicated that the bacterial growth was completely inhibited at this concentration. The MIC values were expressed in μg/mL and the results are shown in Table 3.

4.3. Hemolytic assay

Fresh human blood (5 mL) was centrifuged at 700*g* (3500 rpm) for 10 min and the erythrocytes (RBCs) were collected. The erythrocytes were washed and centrifuged three times with phosphate buffered saline (PBS, pH 7.4, 50 mL) and the supernatant was decanted carefully. The erythrocyte suspension was diluted two times with saline solution and total erythrocytes were counted using haemocytometer. Finally necessary dilution was carried out with saline solution to get total count to 6.4×10^8 cells/mL. The stock solution of the test compounds was made at an initial concentration of 4 mg/mL using the same saline solution. Assay was carried out in 1 mL eppendorf tubes. In an eppendorf, varying volumes (900–650 μL) of the saline solution was added. To this varying volume of the stock solution of test compounds (1–250 μL) was added followed by 100 μL of RBC suspension. For the positive control that is, total cell lyses, 10 μL of 20% w/v Triton was added instead of the test compounds and the plain saline solution was used as control. The eppendorf tubes were then incubated at 37 °C for 30 min, tubes were centrifuged at 4000 rpm for 10 min and carefully 200 μL of the supernatant was transferred to 96-well microtritre plates with U-shaped wells. The absorbance of the samples was read at 404 nm with Spectra Max-190 microplate spectrophotometer. The percentage lyses was determined as $(A_{\text{test compounds}} - A_{\text{blank}})/(A_{\text{positive control}} - A_{\text{blank}}) \times 100$. Further the % lyses was plotted against Log concentration of test compounds used (Fig. 3) and from the graph MHC and LD_{50} (concentration required for 50% lyses) was determined.[25]

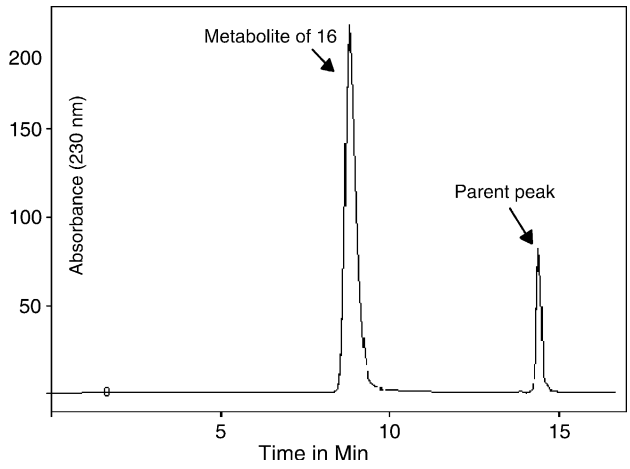

Figure 3. Analytical reverse phase high performance liquid chromatogram of compound **12** and its metabolite. RP-HPLC conditions: Beckman Gold Nouveau System equipped with a 168 photodiode-array detector. Column; Vydac 218TP53 (C18, 300 Å, 5 mm 3.2 mm i.d. × 250 mm). 0.5 mL/min, Buffer A=0.1% TFA in water. Buffer B=90% ACN (acetonitrile) containing 10% buffer A. Linear gradient from 0% B to 90% B over 10 min, total run time 20 min. Retention time (Rt) for parent peak 9.05 min, metabolite Rt=14.63 min.

5. Conclusion

We have described the synthesis, antimicrobial activity and Hemolytic studies of pyrazine containing thiazoline and thiazolidine derivatives. The number of compounds showed good potency in in vitro assays. Out of which compounds **11**, **12** and **40** showed good potency against Gram-positive, Gram-negative bacteria and *M. tuberculosis*. Again compounds **28** and **30** showed good potency against Gram-positive organisms. The antimycobacterial activity of all the compounds are less in 21 days than in 14 days, means the test drug is metabolizing with time in biological environment. As some compounds show good antibacterial and antimycobacterial activity, such compounds would represent a fruitful matrix for the development of a new class of dual antibacterial–antimycobacterial agents that would deserve further investigation and derivatization.

Acknowledgements

The authors are thankful to Dr. A. S. Bobade, Department of Chemotherapy, Haffkine Institute for Training Research and Testing, Parel, Mumbai 12 for his assistance during the antimicrobial testing and enzymatic assay. Thanks to Dr. Ravi Chambare, Novartis Enterprises Limited, Navi Mumbai for technical support.

References and notes

1. Rouhi, A. M. *Chem. Eng. News* **1999**, *17*, 52.
2. Clarck, C.; Jacobs, M.; Appelbaum, P. *J. Clin. Microbiol.* **1998**, *36*, 3579.
3. Davidson, P. T.; Le, H. Q. *Drugs* **1992**, *43*, 651.
4. Vincent, T. A. *Int. J. Antimicrob. Agents* **2000**, *16*, 317.
5. Tan, Y. T.; Tillett, D. J.; Mckay, I. A. *Mol. Med. Today* **2000**, *6*, 309.
6. MacManus, M. C. *Am. J. Health-Syst. Pharm.* **1997**, *54*, 1420.
7. Heinemann, J. A. *Drug Discov. Today* **1999**, *4*, 72.
8. Chopra, I.; Hodgson, J.; Metcalf, B.; Poste, G. *Antimicrob. Agents Chemother.* **1997**, *41*, 497.
9. Sander, P.; Bottger, E. C. *Chemotherapy* **1999**, *45*, 95.
10. Chan, W. C.; Li, R. C.; Ling, J. M. *J. Antimicrob. Chemother.* **1999**, *43*, 55.
11. Bush, K. *Curr. Opin. Chem. Biol.* **1997**, *1*, 169.
12. Stapleton, P.; Shannon, K.; Phillips, I. *J. Antimicrob. Chemother.* **1995**, *36*, 483.
13. Chopra, I. J. *Antimicrob. Chemother.* **1992**, *30*, 737.
14. Coleman, K.; Athalye, M.; Clancey, A.; Davison, M.; Payne, D. J.; Perry, C. R.; Chopra, I. J. *Antimicrob. Chemother.* **1994**, *33*, 1091.
15. Sykes, R. B.; Bonner, D. P. *Br. Med. Bull.* **1984**, *40*, 96.
16. Bonde, C. G.; Nadkarni, B. A.; Khadse, B. G. *Indian J. Heter. Chem.* **2001**, *10*, 271.
17. Sihgh, S. P.; Parmar, S. S.; Raman, K.; Stenherb, V. I. *Chem. Pharm. Bull.* **1981**, *81*, 197.
18. Patel, P. B.; Trivedi, J. T. *Ind. Chem. Soc.* **1987**, *54*, 765.
19. Vittoria, D. M.; Mazzoni, O.; Piscopo, E.; Caligmano, A.; Bolognese, A. *J. Med. Chem.* **1992**, *35*, 2910.
20. Omar, A. M.; Eshba, N. H. *J. Pharm. Sci.* **1984**, *73*, 1166.
21. Chaudhary, M.; Parmar, S. S.; Chaudhari, S. K.; Ramasastry, B. V. *J. Pharm. Sci.* **1976**, *65*, 443.
22. Ragab, F. A.; Hussain, m. M.; Hanna, M. m.; Hassan, G. S. *J. Pharm. Sci.* **1993**, *34*, 387.
23. Hassan, I. Y.; Koussi, A. A.; Farghaly, Z. S. *Chem. Pharm. Bull.* **1998**, *46*(5), 863.
24. *A Manual of Clinical Microbiology*; Sahm, D. F., Washington, J. A., Ballows, A., Housler, W. J., Herrmann, K. L., Isenberg, H. D., Shadomy, H. J., Eds.; fifth ed.; ASM: Washington, DC, 1991; p 1105.
25. Kikuchi, K. E.; Bernard, M.; Sadownik, A.; Regen, S. L.; Armstrong, D. *Antimicrob. Agents Chemother.* **1997**, *41*, 1433.