Full Paper

Synthesis and Antimycobacterial Activity of Azetidine-, Quinazoline-, and Triazolo-thiadiazole-containing Pyrazines

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The re-emergence of tuberculosis (TB) as a global health problem over the past few decades, accompanied by the rise of drug-resistant strains of *Mycobacterium tuberculosis*, emphasizes the need for the discovery of new therapeutic drugs against this disease. The emerging serious problem both in terms of TB control and clinical management prompted us to synthesize a novel series of *N*-[2-(substituted aryl)-3-chloro-4-oxoazetidin-1-yl]-2-(pyrazin-2-yloxy)acetamide, 6-(substituted aryl)-3-[(pyrazin-2-yloxy)methyl][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole, and *N*-[6-({2-[(pyrazin-2-yloxy)acetyl] hydrazino}sulfonyl)-2-methyl-4-oxo-1,4-dihydroquinazolin-3(2*H*)yl]-substituted aryl sulfonamides. The compounds were synthesized using the appropriate synthetic route. All synthesized compounds were assayed *in vitro* for antimycobacterial activity against the H37 Rv strain of *Mycobacterium tuberculosis*. The minimum inhibitory concentration (MIC) was determined for the test compounds as well as for the reference standards. The compound which exhibited good antimycobacterial activity contains the substituents fluorine and methoxy. These electron-withdrawing or -donating substituents amend the lipophilicity of the test compounds which, in turn, alter the permeability across the bacterial cell membrane. Compounds **28**, **37**, and **43** showed good antimycobacterial activity while compound **51** showed a promising antimycobacterial activity.

Keywords: Antimycobacterial activity / Azetidine / Pyrazine / Quinazoline / Triazothiadiazole

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Introduction

Tuberculosis (TB) is the disease caused by *Mycobacterium tuberculosis*. Approximately two billion people are infected world-wide. The World Health Organization estimates that about two million people die every year from TB due to the lack of an inability to afford proper health care [1]. Overcrowding and ill-nourishment of poor people living in large cities leads to a high incidence of the disease due to the ease at which the infection can be transferred [2]. This contributes to the accelerated speed at which TB spreads in underdeveloped countries. There is also an alarming increase in cases of TB caused by multidrug-resistant strains of *Mycobacterium tuberculosis*, due, in part, to inadequate drug therapy as a result of incorrectly selected medications or suboptimal drug dosing [3].

Despite the undoubted success of widespread implementation of the DOTS (directly observed therapy, short-course) strategy, only 70% of the world population is getting the benefit of the same. Tuberculosis is a key driver of the increase in synergy with the HIV epidemic, which is having a devastating impact in some parts of the world such as the WHO African Region, where 31% of new TB cases are attributable to HIV co-infection [4]. Furthermore, the emergence of strains of *Mycobacterium tuberculosis* resistant to all the first-line drugs is causing serious concern in some countries [5]. No new classes of drugs for

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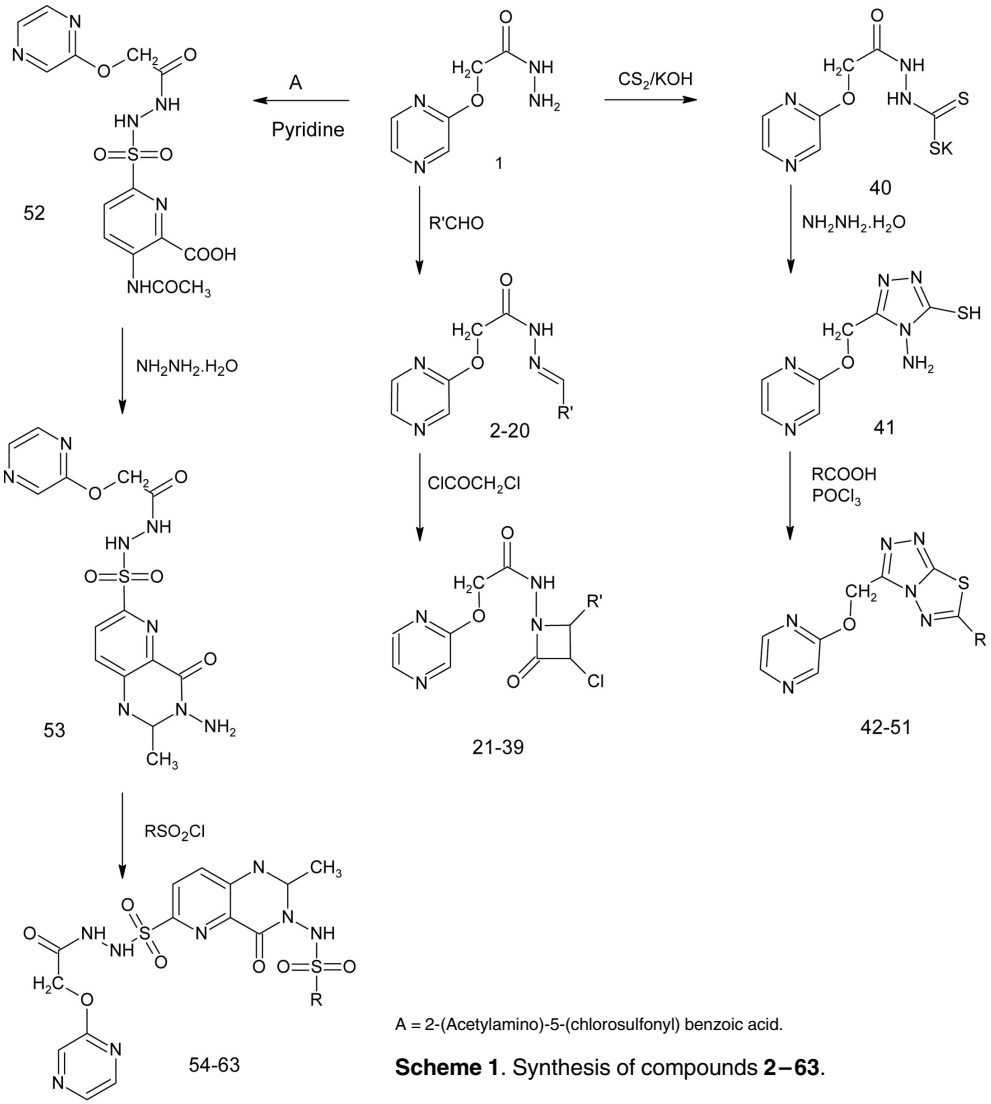


A = 2-(Acetylamino)-5-(chlorosulfonyl) benzoic acid.

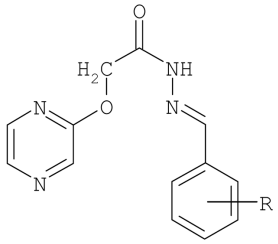
Scheme 1. Synthesis of compounds **2 – 63**.

TB have been developed in the past 30 years, reflecting the inherent difficulties in discovery and clinical testing of new agents and the lack of pharmaceutical industry research in the area [6].

Literature survey revealed that the pyrazine ring is important for antimycobacterial activity [7]. In addition, many azetidine, quinazoline, and triazolo-thiadiazole derivatives exhibit a wide variety of biological activities such as antimicrobial [8], anti-inflammatory [9], antihistaminic [10], antihypertensive [11], hypnotic [12], anticonvulsant [13], etc. In view of the fact that the pyrazine ring possesses antimycobacterial activity, and as a part of our ongoing studies in the area of antibacterial and antimycobacterial agents [14, 15], we have synthesized some novel *N*-[2-(substituted aryl)-3-chloro-4-oxoazetidin-1-yl]-2-(pyrazin-2-yloxy) acetamides **21 – 39**, 6-(substituted aryl)-3-[(pyrazin-2-yloxy)methyl][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole **42 – 51**, and *N*-[6-({2-[(pyrazin-2-yloxy)acetyl]hydrazino}sulfonyl)-2-methyl-4-oxo-1,4-dihydroquinazolin-3(2*H*)-yl]-substituted aryl sulfonamide **54 – 63**, with the aim of obtaining new broad-spectrum antimycobacterial agents with less toxicity.

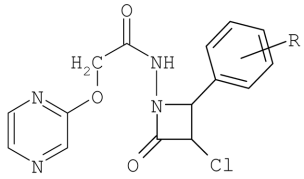
In the present investigation, different derivatives of *N*-[2-(substituted aryl)-3-chloro-4-oxoazetidin-1-yl]-2-(pyrazin-2-yloxy) acetamide **21 – 39**, 6-(substituted aryl)-3-[(pyrazin-2-yloxy)methyl][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole **42 – 51**, and *N*-[6-({2-[(pyrazin-2-yloxy)acetyl]hydrazino}-sulfonyl)-2-methyl-4-oxo-1,4-dihydroquinazolin-3(2*H*)-yl]-substituted aryl sulfonamide **54 – 63** were synthesized (Scheme 1) and evaluated for their antimycobacterial activity.

Table 1. Physical and analytical data of compounds **2–20**.



Compound	R	Molecular formula	M.p. (°C)	Yield (%)
2	2,4-Dichloro	$C_{13}H_{10}Cl_2N_4O_2$	224–225	71
3	2,6-Dichloro	$C_{13}H_{10}Cl_2N_4O_2$	178–179	68
4	2-Chloro	$C_{13}H_{11}ClN_4O_2$	209–210	65
5	2-Fluoro	$C_{13}H_{11}FN_4O_2$	196–197	72
6	4-Bromo-2-nitro	$C_{13}H_{10}BrN_5O_4$	173–174	64
7	2-Nitro	$C_{13}H_{11}N_5O_4$	217–218	52
8	2-Methoxy	$C_{14}H_{14}N_4O_3$	165–166	59
9	3,4,5-Trimethoxy	$C_{16}H_{18}N_4O_5$	143–144	58
10	3,5-Dimethoxy	$C_{15}H_{16}N_4O_4$	152–153	46
11	3-Chloro	$C_{13}H_{11}ClN_4O_2$	182–183	65
12	3-Fluoro	$C_{13}H_{11}FN_4O_2$	137–138	63
13	3-Nitro	$C_{13}H_{11}N_5O_4$	153–154	64
14	3-Methoxy	$C_{14}H_{14}N_4O_3$	162–163	78
15	4-Bromo	$C_{13}H_{11}BrN_4O_2$	143–144	53
16	4-Methyl	$C_{14}H_{14}N_4O_2$	184–185	64
17	4-Chloro	$C_{13}H_{11}ClN_4O_2$	202–203	58
18	4-Fluoro	$C_{13}H_{11}FN_4O_2$	168–169	62
19	4-Nitro	$C_{13}H_{11}N_5O_4$	190–191	55
20	4-Methoxy	$C_{14}H_{14}N_4O_3$	171–172	63

Table 2. Physical and analytical data of compounds **21–39**



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Compound	R	Molecular formula	M.p. (°C)	Yield (%)
21	2,4-Dichloro	$C_{15}H_{11}Cl_3N_4O_3$	169–170	56
22	2,6-Dichloro	$C_{15}H_{11}Cl_3N_4O_3$	172–173	54
23	2-Chloro	$C_{15}H_{12}Cl_2N_4O_3$	185–186	46
24	2-Fluoro	$C_{15}H_{12}ClFN_4O_3$	212–213	59
25	4-Bromo-2-nitro	$C_{15}H_{11}BrClN_5O_5$	218–219	61
26	2-Nitro	$C_{15}H_{12}ClN_5O_5$	156–157	52
27	2-Methoxy	$C_{16}H_{15}ClN_4O_4$	195–196	49
28	3,4,5-Trimethoxy	$C_{18}H_{19}ClN_4O_6$	201–202	45
29	3,5-Dimethoxy	$C_{17}H_{17}ClN_4O_5$	221–222	62
30	3-Chloro	$C_{15}H_{12}Cl_2N_4O_3$	182–183	53
31	3-Fluoro	$C_{15}H_{12}ClFN_4O_3$	245–246	57
32	3-Nitro	$C_{15}H_{12}ClN_5O_5$	275–276	52
33	3-Methoxy	$C_{16}H_{15}ClN_4O_4$	164–165	59
34	4-Bromo	$C_{15}H_{12}BrClN_4O_3$	235–236	46
35	4-Methyl	$C_{16}H_{15}ClN_4O_3$	191–192	48
36	4-Chloro	$C_{15}H_{12}Cl_2N_4O_3$	227–228	59
37	4-Fluoro	$C_{15}H_{12}ClFN_4O_3$	235–236	42
38	4-Nitro	$C_{15}H_{12}ClN_5O_5$	212–213	45
39	4-Methoxy	$C_{16}H_{15}ClN_4O_4$	238–239	41

Results and discussion

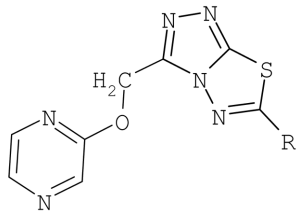
In the present investigation, different derivatives of *N*-[2-(substituted aryl)-3-chloro-4-oxoazetidin-1-yl]-2-(pyrazin-2-yloxy) acetamide **21–39**, 6-(substituted aryl)-3-[(pyrazin-2-yloxy)methyl][1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazole **42–51**, and *N*-[6-({2-[(pyrazin-2-yloxy)acetyl]hydrazino}sulfonyl)-2-methyl-4-oxo-1,4-dihydroquinazolin-3(2H)-yl]-substituted aryl sulfonamide **54–63** were synthesized and evaluated for their physical, analytica,l and spectral data.

Chemistry

To a solution of 2-(pyrazin-2-yloxy)acetohydrazide **1** in ethanol, sodium acetate and various aromatic aldehydes were added. This mixture was refluxed for 12 h. Excess solvent was removed under vacuum to get compounds **2–20**. The structures of compounds **2–20** were confirmed on the basis of elemental analyses and spectral data (Table 1). The IR spectra showed NH-stretching bands at 3215–3230 cm^{-1} and the disappearance of NH_2-stretching bands at 3200–3400 cm^{-1}. In the ^{1}H-NMR spectrum, the azomethane proton appeared as a multiplet at δ = 2.1–2.3 ppm integrating for one proton. Cyclization of compounds **2–20** with chloroacetyl chloride in the presence of TEA results in the formation of *N*-[2-(substituted aryl)-3-chloro-4-oxoazetidin-1-yl]-2-(pyrazin-2-yloxy) acetamides **21–39**. The structure was confirmed by elemental analyses and spectral data (Table 2). Due to the formation of the azetidine ring, the characteristic peaks in the IR spectra were observed at 1720–1740 cm^{-1} attributed to the monocyclic carbonyl group and the absence of a peak at 1660–1680 cm^{-1}. In the ^{1}H-NMR, the cyclized compounds showed the absence of a signal at δ = 2.1–2.3 ppm for azomethane, which appeared as β-lactum proton as a duplet at δ = 2.2–2.4 ppm integrating for one proton. In the FAB-MS spectra, the molecular ion peak [M^+], which appeared with different intensities, confirmed the molecular weight of the compounds. An appearance of the isotopic peak [M^+ + 2] along with the molecular ion peak confirmed the presence of a halogen atom in the compounds.

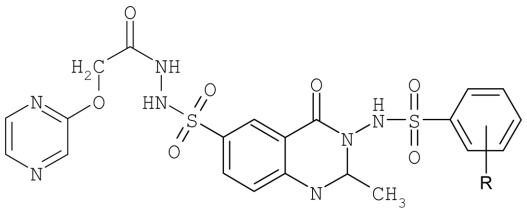
2-(Pyrazin-2-yloxy)acetohydrazide **1** on treatment with carbon disulphide and potassium hydroxide gives the potassium salt of 2-[(pyrazin-2-yloxy)acetyl] hydrazine carbodithioic acid. Compound **40** was cyclized with hydrazine hydrate to get 4-amino-5-[(pyrazin-2-yloxy)-methyl]-4*H*-1,2,4-triazole-3-thiol **41**, which was treated with various aromatic carboxylic acids in the presence of phosphorous oxychloride to yield 6-(substituted aryl)-3-[(pyrazin-2-yloxy)methyl][1,2,4]triazolo[3,4-*b*][1,3,4]thiadi-

Table 3. Physical and analytical data of compounds **42 – 51**.



Compound	R	Molecular formula	M.p. (°C)	Yield (%)
42	4-Chlorophenyl	$C_{14}H_9ClN_6OS$	189 – 190	56
43	4-Fluorophenyl	$C_{14}H_9FN_6OS$	165 – 167	57
44	4-Bromophenyl	$C_{14}H_9BrN_6OS$	209 – 210	65
45	3,5-Dinitrophenyl	$C_{14}H_8N_8O_5S$	156 – 157	53
46	4-Methyl phenyl	$C_{15}H_{12}N_6OS$	196 – 197	64
47	Phenyl	$C_{14}H_{10}N_6OS$	215 – 216	51
48	Benzyl	$C_{15}H_{12}N_6OS$	276 – 277	68
49	2-Furanyl	$C_{12}H_8N_6O_2S$	168 – 169	41
50	3,5-Dimethoxy phenyl	$C_{16}H_{14}N_6O_3S$	145 – 146	56
51	3-Pyridinyl	$C_{13}H_9N_7OS$	191 – 192	49

Table 4. Physical and analytical data of compounds **54 – 63**.



Compound	R	Molecular formula	M.p. (°C)	Yield (%)
54	–	$C_{20}H_{20}N_8O_7S_2$	214 – 215	52
55	4-Chloro	$C_{20}H_{19}ClN_8O_7S_2$	192 – 192	59
56	3-Nitro	$C_{20}H_{19}N_9O_9S_2$	245 – 246	61
57	4-Hydroxy	$C_{20}H_{20}N_8O_8S_2$	236 – 237	53
58	4-Methoxy	$C_{21}H_{22}N_8O_8S_2$	199 – 200	57
59	3,5-Dimethoxy	$C_{22}H_{24}N_8O_9S_2$	260 – 261	41
60	2,4-Dichloro	$C_{20}H_{18}Cl_2N_8O_7S_2$	278 – 279	46
61	4-Fluoro	$C_{20}H_{19}FN_8O_7S_2$	245 – 246	42
62	4-Methyl	$C_{21}H_{22}N_8O_7S_2$	231 – 232	65
63	2-Nitro-4-chloro	$C_{20}H_{18}ClN_9O_9S_2$	224 – 225	63

azoles **42 – 51**. Their structures were confirmed using elemental and spectral analyses (Table 3). The IR absorption peaks of **41** at 3167, 3270, and 2578 cm^{-1} were assigned to NH_2- and SH-groups. When **41** was converted to **42 – 51**, the SH-peak disappeared and the new characteristic peak of C-S-C appeared at 690 cm^{-1}. The evident change in the ^{1}H-NMR spectrum of **40** after cyclization is that the signals of the amino and mercapto proton are at δ = 5.81 and 13.39 ppm, respectively. The chemical shifts of the triazole methyl group are seen in the range δ = 2.30 to 2.51 ppm.

2-(Acetylamino)-5-(chlorosulfonyl) benzoic acid was condensed with 2-(pyrazin-2-yloxy)acetohydrazide **1** to yield 2-(acetylamino)-5-({2-[(pyrazin-2-yloxy)acetyl] hydrazino}sulfonyl)benzoic acid **52**, which was then reacted with hydrazine hydrate to afford *N*′-[(3-amino-2-methyl-4-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)sulfonyl]-2-(pyrazin-2-yloxy)acetohydrazide **53**. Compound **53** was then reacted with various substituted aryl sulphonyl chlorides to give the titled compounds **54 – 63**. The structures of the compounds were confirmed on the basis of elemental and spectral data (Table 4). In the IR spectra of **51**, the NH-stretching band was clearly observed at 3200 – 3500 cm^{-1} and not the bands for NH_2 of the reactant. The –OH stretching band was observed at 3390 cm^{-1}. This, again, was absent in compound **53** because of the cyclization of **52**. The peaks of NH_2 and CH_3 were observed at 3226 and 3235 cm^{-1}, respectively, in compound **53**, and in compounds **54 – 63** the NH_2 stretching band was absent due to substitution of the substituted aryl sulphonyl group. In the NMR spectra of **55**, δ = 2.0 ppm and be attributed to NH of SO_2NH, which is absent in compound **53**. In compounds **54 – 63**, the NH_2 proton of compound **53** at δ = 3.13 ppm was absent. These observations confirmed that the reaction is complete. In the FABMS spectra, the molecular ion peak [M^+], which appeared at different intensities, confirmed the molecular weight of the compounds. An appearance of the isotopic peak [$M^+ + 2$] along with the molecular ion peak confirmed the presence of a halogen atom in the compounds.

Antimycobacterial activity

The MIC values of the test compounds are summarized in Table 5. For the comparison of the MIC values, the intermediate compounds are also included in Table 5. The results revealed that the MIC values of compounds **2 – 20** are lower than the MIC values of compounds **21 – 39**. This proves the importance of the azetidine ring for imparting antimycobacterial activity to a compound. **28** and **37** show the best antimycobacterial activity of this class of compounds. The activity may be due to the presence of more electronegative groups like fluorine and methoxy. These groups also impart lipophilicity to a compound. The MIC values of **42 – 51** show promising antibacterial activity in some of the compounds. **43** and **51** showed good antimycobacterial activity. Again, the activity may be due to the presence of a fluorine on the aromatic ring. The literature survey revealed the importance of the pyridine ring in imparting biological activity. The compounds containing a pyridine ring show maximum antimycobacterial activity. The fact that compounds **54 – 63**

Table 5. *In-vitro* antimycobacterial activity of the test compounds.

Compound	Antimycobacterial Activity[a] (MIC)	Compound	Antimycobacterial Activity[a]
2	176	**31**	19
3	150	**32**	35
4	>250	**33**	17
5	165	**34**	26
6	124	**35**	89
7	174	**36**	47
8	140	**37**	7
9	115	**38**	25
10	NA	**39**	23
11	>250	**40**	>250
12	138	**41**	>250
13	192	**42**	100
14	177	**43**	1
15	123	**44**	28
16	155	**45**	79
17	127	**46**	NA
18	>250	**47**	NA
19	NA	**48**	NA
20	163	**49**	3
21	41	**50**	12
22	78	**51**	0.4
23	49	**52**	>250
24	23	**53**	>250
25	19	**54**	>250
26	25	**55**	>250
27	12	**56**	156
28	3	**57**	189
29	8	**58**	NA
30	49	**59**	23
Std. 1[b]	0.2	**60**	25
Std. 2[c]	0.005	**61**	32
		62	>250
		63	NA

[a] MIC: Minimum inhibitory concentration.

[b] Std.1: Rifampin.

[c] Std. 2: Isoniazid (INH); NA: not active at 500 μg/mL concentration; DMF has no antimycobacterial activity at the concentrations used to dissolve the test compounds.?

did not show promising results may be due to their high molecular weight.

Experimental

General

Melting points were determined in open capillaries using the microprocessor-based melting point apparatus, Model PMP-DM (Veego Instruments, Corp., Mumbai, India) and are uncorrected. Purity of the compounds was checked by precoated TLC plates (E. Merck, Kieselgel 60 F_{25}, Mumbai, India). IR spectra were recorded using KBr pellets on Perkin-Elmer 337 spectrophotometer from Perkin-Elmer International Incorporation, Rorkreuz, Switzerland (ν_{max} in cm^{-1}), ^{1}H-NMR spectra were taken on a Varian 300 MHz instrument at RSIC (Regional Sophisticated Instrumentation Centre), IIT (Indian Institute of Technology), Powai, Mumbai, India. 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).

*Synthesis of compounds **2–20***

To a solution of **1** (0.01 mol) in ethanol (50 mL), sodium acetate (0.01 mol) and various aromatic aldehydes (0.02 mol) were added and the reaction mixture was heated under reflux for 6 h. Excess of solvent was removed under vacuum. The residue so obtained was washed with diethyl ether and recrystallized from methanol. Using the same procedure, 19 different compounds were synthesized.

*Compound **2***

R_f: 0.61 (acetonitrile/methanol, 1:1); IR: 3222 (NH), 1720 (C=O stretching), 1660 (N=C stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.10–2.20 (m, 1H, -N=CH-), 4.83 (s, 2H, OCH_2), 7.15–7.62 (m, 3H, ArH), 7.60 (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%): 327 [M^+ + 2], (100). Anal. calcd. for $C_{13}H_{10}Cl_2N_4O_2$ (325.16): C, 48.02; H, 3.10; N, 17.23. Found: C, 48.10; H, 3.15; N, 17.26.

*Compound **3***

R_f: 0.58 (acetonitrile/methanol, 1:1); IR: 3221 (NH), 1721 (C=O stretching), 1655 (N=C stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.10–2.21 (m, 1H, -N=CH-), 4.83 (s, 2H, OCH_2), 7.10–7.66 (m, 3H, ArH), 7.60 (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%): 327 [M^+ + 2] (100). Anal. calcd. for $C_{13}H_{10}Cl_2N_4O_2$ (325.16): C, 48.02; H, 3.10; N, 17.23. Found: C, 48.10; H, 3.15; N, 17.26.

*Compound **5***

R_f: 0.48 (acetonitrile/methanol, 1:1); IR: 3219 (NH), 1722 (C=O stretching), 1657 (N=C stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.11–2.21 (m, 1H, -N=CH-), 4.83 (s, 2H, OCH_2), 7.35–7.70 (m, 4H, ArH), 7.60 (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%): 276 [M^+ + 2] (100). Anal. calcd. for $C_{13}H_{11}FN_4O_2$ (274): C, 56.93; H, 4.04; N, 20.43. Found: C, 56.95; H, 4.06; N, 20.47.

*Compound **6***

R_f: 0.52 (acetonitrile/methanol, 1:1); IR: 3219 (NH), 1722 (C=O stretching), 1657 (N=C stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.11–2.21 (m, 1H, -N=CH-), 4.83 (s, 2H, OCH_2), 7.19–7.77 (m, 3H, ArH), 7.60 (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%): 382 [M^+ + 2] (100). Anal. calcd. for $C_{13}H_{10}BrN_5O_4$ (380): C, 41.07; H, 2.65; N, 18.42. Found: C, 41.00; H, 2.69; N, 18.44.

*Compound **8***

R_f: 0.41 (acetonitrile/methanol, 1:1); IR: 3222 (NH), 1720 (C=O stretching), 1659 (N=C stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.10–2.20 (m, 1H, -N=CH-), 3.73 (s, 3H, OCH_3), 4.83 (s, 2H, OCH_2), 7.15–7.71 (m, 4H, ArH), 7.60 (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%): 288 [M^+ + 2] (100). Anal. calcd. for $C_{14}H_{14}N_4O_3$ (286): C, 58.74; H, 4.93; N, 19.57. Found: C, 58.78; H, 4.97; N, 19.59.

Compound ***10***

R_f: 0.59 (acetonitrile/methanol, 1:1); IR: 3223 (NH), 1722 (C=O stretching), 1653 (N=C stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.12 – 2.21 (m, 1H, -N=CH-), 3.73 (s, 6H, OCH_3), 4.84 (s, 2H, OCH_2), 7.22 – 7.74 (m, 3H, ArH), 7.61 (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%): 316 [M^+] (100). Anal. calcd. for $C_{15}H_{16}N_4O_4$ (316.32): C, 56.96; H, 5.10; N, 17.71. Found: C, 56.99; H, 5.06; N, 17.74.

Compound ***12***

R_f: 0.74 (acetonitrile/methanol, 1:1); IR: 3219 (NH), 1722 (C=O stretching), 1657 (N=C stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.11 – 2.21 (m, 1H, -N=CH-), 4.83 (s, 2H, OCH_2), 7.29 – 7.81 (m, 4H, ArH), 7.60 (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%): 276 [M^+ + 2] (100). Anal. calcd. for $C_{13}H_{11}FN_4O_2$ (274): C, 56.93; H, 4.04; N, 20.43. Found: C, 56.97; H, 4.09; N, 20.45.

Compound ***14***

R_f: 0.32 (acetonitrile/methanol, 1:1); IR: 3222 (NH), 1720 (C=O stretching), 1659 (N=C stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.10 – 2.20 (m, 1H, -N=CH-), 3.75 (s, 3H, OCH_3), 4.83 (s, 2H, OCH_2), 7.14 – 7.75 (m, 4H, ArH), 7.60 (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%): 288 [M^+ + 2] (100). Anal. calcd. for $C_{14}H_{14}N_4O_3$ (286): C, 58.74; H, 4.93; N, 19.57. Found: C, 58.78; H, 4.95; N, 19.59.

Compound ***15***

R_f: 0.46 (acetonitrile/methanol, 1:1); IR: 3229 (NH), 1720 (C=O stretching), 1663 (N=C stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.11 – 2.19 (m, 1H, -N=CH-), 4.82 (s, 2H, OCH_2), 7.11 – 7.84 (m, 4H, ArH), 7.25 (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%): 337 [M^+ + 2] (100%). Anal. calcd. for $C_{13}H_{11}BrN_4O_2$ (335.16): C, 46.59; H, 3.31; N, 16.72. Found: C, 46.62; H, 3.33; N, 16.75.

Compound ***16***

R_f: 0.52 (acetonitrile/methanol, 1:1); IR: 3229 (NH), 1720 (C=O stretching), 1663 (N=C stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.47 (d, 3H, CH_3), 2.12 – 2.20 (m, 1H, -N=CH-), 4.82 (s, 2H, OCH_2), 7.20 – 7.62 (m, 4H, ArH), 7.25 (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%): 272 [M^+ + 2] (100). Anal. calcd. for $C_{14}H_{14}N_4O_2$ (270): C, 62.21; H, 5.22; N, 20.73. Found: C, 62.19; H, 5.25; N, 20.76.

Compound ***18***

R_f: 0.39 (acetonitrile/methanol, 1:1); IR: 3219 (NH), 1721 (C=O stretching), 1656 (N=C stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.11 – 2.21 (m, 1H, -N=CH-), 4.83 (s, 2H, OCH_2), 7.20 – 7.68 (m, 4H, ArH), 7.60 (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%): 276 [M^+ + 2] (100). Anal. calcd. for $C_{13}H_{14}FN_4O_2$ (274): C, 56.93; H, 4.04; N, 20.43. Found: C, 56.90; H, 4.06; N, 20.40.

Compound ***20***

R_f: 0.91 (acetonitrile/methanol, 1:1); IR: 3222 (NH), 1720 (C=O stretching), 1659 (N=C stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.10 – 2.20 (m, 1H, -N=CH-), 3.71 (s, 3H, OCH_3), 4.83 (s, 2H, OCH_2), 7.21 – 7.63 (m, 4H, ArH), 7.60 (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%): 288 [M^+ + 2] (100). Anal. calcd. for $C_{14}H_{14}N_4O_3$ (286): C, 58.74; H, 4.93; N, 19.57. Found: C, 58.77; H, 4.91; N, 19.55.

Synthesis of compounds ***21 – 39***

To a solution of compound **21 – 39** (0.01 mol) and TEA (12 mL) in absolute alcohol (100 mL), chloroethyl acetate (0.02 mol) was added dropwise with constant stirring over the period of 1 h. After stirring for 1 h, the reaction mixture was refluxed for 2 h. The reaction mixture was cooled and poured in ice-cold water. The separated solid was filtered off, dried, and recrystallized from petroleum ether (60 – 80). Using the same procedure, 19 such compounds were synthesized.

Compound ***21***

R_f: 0.35 (acetonitrile/methanol, 1:1); IR: 3229 (NH), 1720 (β-lactum C=O), 1698 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.20 – 2.33 (d, 1H, -CH-β-lactum), 2.36 – 2.40 (d, 1H, -β-lactum) , 4.82 (s, 2H, OCH_2), 7.20 – 7.70 (m, 3H, ArH), 7.60 (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%): 403.6 [M^+ + 2] (100). Anal. calcd. for $C_{15}H_{11}Cl_3N_4O_3$ (401.6): C, 44.86; H, 2.76; N, 13.95. Found: C, 44.89; H, 2.78; N, 13.99.

Compound ***22***

R_f: 0.46 (acetonitrile/methanol, 1:1); IR: 3228 (NH), 1721 (β-lactum C=O), 1698 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.10 – 2.20 (d, 1H, -CH-β-lactum), 2.37 – 2.40 (d, 1H, -β-lactum), 4.82 (s, 2H, OCH_2), 7.16 – 7.65 (m, 3H, ArH), 7.60 (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%): 403.6 [M^+ + 2] (100). Anal. calcd. for $C_{15}H_{11}Cl_3N_4O_3$ (401.6): C, 44.86; H, 2.76; N, 13.95. Found: C, 44.85; H, 2.75; N, 13.96.

Compound ***24***

R_f: 0.72 (acetonitrile/methanol, 1:1); IR: 3227 (NH), 1722 (β-lactum C=O), 1699 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.20 – 2.30 (d, 1H, -CH-β-lactum), 2.37 – 2.40 (d, 1H, -β-lactum), 4.82 (s, 2H, OCH_2), 7.35 – 7.70 (m, 4H, ArH), 7.60 (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%): 352.74 [M^+ + 2] (100). Anal. calcd. for $C_{15}H_{12}ClFN_4O_3$ (350.74): C, 51.37; H, 3.45; N, 15.97. Found: C, 51.39; H, 3.43; N, 16.01.

Compound ***25***

R_f: 0.59 (acetonitrile/methanol, 1:1); IR: 3229 (NH), 1725 (β-lactum C=O), 1697 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.21 – 2.29 (d, 1H, -CH-β-lactum), 2.37 – 2.41 (d, 1H, -β-lactum), 4.82 (s, 2H, OCH_2), 7.19 – 7.77 (m, 3H, ArH), 7.60 (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%): 458.64 [M^+ + 2] (100). Anal. calcd. for $C_{15}H_{11}BrClN_5O_5$ (456.64): C, 39.45; H, 2.43; N, 16.34. Found: C, 39.48; H, 2.49; N, 15.36.

Compound ***27***

R_f: 0.49 (acetonitrile/methanol, 1:1); IR: 3227 (NH), 1721 (β-lactum C=O), 1697 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.20 – 2.33 (d, 1H, -CH-β-lactum), 2.36 – 2.40 (d, 1H, -β-lactum), 3.73 (s, 3H, OCH_3), 4.82 (s, 2H, OCH_2), 7.15 – 7.71 (m, 4H, ArH), 7.60 (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%): 365 [M^+ + 2] (100). Anal. calcd. for $C_{16}H_{15}ClN_4O_4$ (362.78): C, 52.97; H, 4.17; N, 15.44. Found: C, 52.90; H, 4.20; N, 15.48.

Compound ***28***

R_f: 0.64 (acetonitrile/methanol, 1:1); IR: 3227 (NH), 1721 (β-lactum C=O), 1697 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.20–2.33 (d, 1H, -CH-β-lactum), 2.36–2.40 (d, 1H, -β-lactum), 3.71 (s, 9H, OCH_3), 4.82 (s, 2H, OCH_2), 7.44–7.71 (m, 2H, ArH), 7.60 (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%): 425 [M^+ + 2] (100). Anal. calcd. for $C_{16}H_{19}ClN_4O_6$ (422.83): C, 51.30; H, 4.53; N, 13.25. Found: C, 51.15; H, 4.55; N, 13.28.

Compound ***29***

R_f: 0.26 (acetonitrile/methanol, 1:1); IR: 3225 (NH), 1725 (β-lactum C=O), 1693 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.10–2.21 (d, 1H, -CH-β-lactum), 2.32–2.36 (d, 1H, -β-lactum), 4.83 (s, 2H, OCH_2), 7.22–7.74 (m, 3H, ArH), 7.65 (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%): 395 [M^+] (100). Anal. calcd. for $C_{17}H_{17}ClN_4O_6$ (392.80): C, 51.98; H, 4.36; N, 14.26. Found: C, 52.00; H, 4.31; N, 14.30.

Compound ***30***

R_f: 0.26 (acetonitrile/methanol, 1:1); IR: 3230 (NH), 1729 (β-lactum C=O), 1699 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.11–2.23 (d, 1H, -CH-β-lactum), 2.33–2.38 (d, 1H, -β-lactum), 4.83 (s, 2H, OCH_2), 7.20–7.70 (m, 4H, ArH), 7.71 (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%): 369 [M^+ + 2] (100). Anal. calcd. for $C_{15}H_{12}Cl_2N_4O_3$ (367.19): C, 49.07; H, 3.29; N, 15.26. Found: C, 49.10; H, 3.21; N, 15.29.

Compound ***31***

R_f: 0.55 (acetonitrile/methanol, 1:1); IR: 3229 (NH), 1721 (β-lactum C=O), 1699 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.21–2.29 (d, 1H, -CH-β-lactum), 2.37–2.40 (d, 1H, -β-lactum), 4.82 (s, 2H, OCH_2), 7.29–7.81 (m, 4H, ArH), 7.60 (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%): 353 [M^+ + 2] (100). Anal. calcd. for $C_{15}H_{12}ClFN_4O_3$ (350.74): C, 51.37; H, 3.45; N, 15.97. Found: C, 51.40; H, 3.49; N, 15.99.

Compound ***33***

R_f: 0.57 (acetonitrile/methanol, 1:1); IR: 3227 (NH), 1721 (β-lactum C=O), 1697 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.20–2.33 (d, 1H, -CH-β-lactum), 2.36–2.40 (d, 1H, -β-lactum), 3.73 (s, 3H, OCH_3), 4.82 (s, 2H, OCH_2), 7.14–7.75 (m, 4H, ArH), 7.60 (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%): 365 [M^+ + 2] (100). Anal. calcd. for $C_{16}H_{15}ClN_4O_4$ (362.78): C, 52.97; H, 4.17; N, 15.44. Found: C, 53.05; H, 4.19; N, 15.46.

Compound ***34***

R_f: 0.78 (acetonitrile/methanol, 1:1); IR: 3230 (NH), 1729 (3.1.2.11 β-lactum C=O), 1699 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.11–2.23 (d, 1H, -CH-β-lactum), 2.33–2.38 (d, 1H, -β-lactum), 4.83 (s, 2H, OCH_2), 7.11–7.84 (m, 4H, ArH), 7.71 (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%): 413.5 [M^+ + 2] (100). Anal. calcd.for $C_{15}H_{12}BrClN_4O_3$ (411.64): C, 43.80; H, 2.95; N, 13.63. Found: C, 43.77; H, 2.94; N, 13.61.

Compound ***36***

R_f: 0.65 (acetonitrile/methanol, 1:1); IR: 3230 (NH), 1729 (β-lactum C=O), 1699 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.11–2.23 (d, 1H, -CH-β-lactum), 2.33–2.38 (d, 1H, -β-lactum), 4.83 (s, 2H, OCH_2), 7.20–7.70 (m, 4H, ArH), 7.71 (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%): 369 [M^+ + 2] (100). Anal. calcd. for $C_{15}H_{12}Cl_2N_4O_3$ (367.19): C, 49.07; H, 3.29; N, 15.26. Found: C, 49.15; H, 3.37; N, 15.29.

Compound ***37***

R_f: 0.43 (acetonitrile/methanol, 1:1); IR: 3229 (NH), 1721 (β-lactum C=O), 1699 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.21–2.29 (d, 1H, -CH-β-lactum), 2.37–2.40 (d, 1H, -β-lactum), 4.82 (s, 2H, OCH_2), 7.20–7.68 (m, 4H, ArH), 7.60 (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%): 353 [M^+ + 2] (100). Anal. calcd. for $C_{15}H_{12}ClFN_4O_3$ (350.74): C, 51.37; H, 3.45; N, 15.97. Found: C, 51.41; H, 3.46; N, 15.95.

Compound ***39***

R_f: 0.55 (acetonitrile/methanol, 1:1); IR: 3227 (NH), 1721 (β-lactum C=O), 1697 (C=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.20–2.33 (d, 1H, -CH-β-lactum), 2.36–2.40 (d, 1H, -β-lactum), 3.73 (s, 3H, OCH_3), 4.82 (s, 2H, OCH_2), 7.15–7.71 (m, 4H, ArH), 7.60 (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%): 365 [M^+ + 2] (100). Anal. calcd. for $C_{16}H_{15}ClN_4O_4$ (362.78): C, 52.97; H, 4.17; N, 15.44. Found: C, 52.91; H, 4.19; N, 15.48.

Synthesis of compound ***40***

A mixture of the thiosemicarbazide (0.01 mol), carbon disulphide (0.01 mol), and potassium hydroxide (0.1 mol) in ethanol (50 mL) was heated under reflux for 5 h. The mixture was cooled to separate the products. The product was recrystallized from hydro-alcohol.

Synthesis of compound ***41***

A mixture of **40** (3.75 g, 0.02 mol) and 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. The separated solid crystals were filtered, washed with cold water, dried, and recrystallized from absolute alcohol.

R_f: 0.44 (acetonitrile/methanol, 1:1); IR: 3335, 3269 (NH-stretching), 1494, 1467, 1420 (triazole) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.11–2.19 (m, 1H, -N=CH-), 4.80 (s, 2H, OCH_2), 5.81 (s, 2H, NH_2), 8.73 (t, 1H, pyrazine C5-H), 8.82 (d, 1H, pyrazine C6-H), 9.21 (d, 1H, pyrazine C2-H), 13.39 (s, 1H, SH); FABMS (*m*/*z*, 100%): 224 [M^+] (100). Anal. calcd. for $C_7H_8N_6OS$ (224.25): C, 37.49; H, 3.60; N, 37.48. Found: C, 37.52; H, 3.56; N, 37.52.

Synthesis of compounds ***42–51***

A mixture of **41** (0.02 mol) and an aromatic acid (0.02 mol) in $POCl_3$ (40 mL) was refluxed for 7 h. The reaction mixture was gradually poured onto crushed ice by stirring and potassium carbonate was added to the mixture by stirring. An appropriate amount of potassium hydroxide was added to reach pH 8 and the mixture was left to stand overnight. The solid which then separated was filtered, washed with cold water, dried, and recrystallized from absolute alcohol to get the final products.

*Compound **42***

R_f: 0.66 (acetonitrile/methanol, 1:1); IR: 1626 (C=N stretching), 1266 (N-N=C), 694 (C-S-C) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 7.39 – 7.81 (m, 4H, ArH),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%): 346 [M^+ + 1] (100). Anal. calcd. for $C_{14}H_9ClN_6OS$ (344.78): C, 48.77; H, 2.63; N, 24.37. Found: C, 48.81; H, 2.68; N, 24.46.

*Compound **43***

R_f: 0.62 (acetonitrile/methanol, 1:1); IR: 1627 (C=N stretching), 1268 (N-N=C), 695 (C-S-C) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 7.21 – 7.72 (m, 4H, ArH), 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%): 330 [M^+ + 2] (100). Anal. calcd. for $C_{14}H_9FN_6OS$ (328.33): C, 51.22; H, 2.76; N, 25.60. Found: C, 51.27; H, 2.78; N, 25.57.

*Compound **45***

R_f: 0.80 (acetonitrile/methanol, 1:1); IR: 1628 (C=N stretching), 1263 (N-N=C), 692 (C-S-C) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 7.29 – 7.79 (m, 3H, ArH), 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%): 400 [M^+] (100). Anal. calcd. for $C_{14}H_9ClN_6OS$ (400.33): C, 42.00; H, 2.01; N, 27.99. Found: C, 42.04; H, 2.06; N, 28.05.

*Compound **46***

R_f: 0.63 (acetonitrile/methanol, 1:1); IR: 1627 (C=N stretching), 1261 (N-N=C), 691 (C-S-C) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 7.21 – 7.71 (m, 3H, ArH), 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%): 325 [M^+ + 1] (100). Anal. calcd. for $C_{14}H_8N_8O_5S$ (324.37): C, 55.54; H, 3.73; N, 25.91. Found: C, 55.52; H, 3.75; N, 25.86.

*Compound **47***

R_f: 0.57 (acetonitrile/methanol, 1:1); IR: 1615 (C=N stretching), 1263 (N-N=C), 691 (C-S-C) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 7.25 – 7.78 (m, 5H, ArH), 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%): 311 [M^+ + 1] (100). Anal. calcd. for $C_{14}H_{10}N_6OS$ (310.34): C, 54.18; H, 3.25; N, 27.08. Found: C, 54.24; H, 3.27; N, 27.19.

*Compound **48***

R_f: 0.75 (acetonitrile/methanol, 1:1); IR: 1629 (C=N stretching), 1261 (N-N=C), 689 (C-S-C) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.71 (s, 2H, CH_2), 7.10 – 7.82 (m, 5H, ArH), 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%): 325 [M^+ + 1] (100). Anal. calcd. for $C_{15}H_{12}N_6OS$ (324.37): C, 55.54; H, 3.73; N, 25.91. Found: C, 55.59; H, 3.75; N, 25.82.

*Compound **49***

R_f: 0.46 (acetonitrile/methanol, 1:1); IR: 1629 (C=N stretching), 1226 (furan C-O-C), 694 (C-S-C) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 7.45 – 8.13 (m, 3H, furan), 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%): 300 [M^+] (100). Anal. calcd. for $C_{12}H_8N_6O_2S$ (300.30): C, 48.00; H, 2.69; N, 27.99. Found: C, 48.05; H, 2.66; N, 27.95.

*Synthesis of compound **52***

A mixture of **3** and 2-(acetylamino)-5-(chlorosulfonyl)benzoic acid (0.01 mol), in ethanol (25 mL) and pyridine (1 mL) was refluxed in an oil bath at 120°C for 4 h. The reaction mixture was cooled and poured into crushed ice, filtered, and washed with water. The product was recrystallized from ethanol.

R_f: 0.58 (acetonitrile/methanol, 1:1); IR: 3390 (OH-stretching), 1680, 1733 (C=O stretching), 3226, 3235 (NH-stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.00 (s, 2H, 2 NH), 4.83 (s, 2H, OCH_2), 7.80 – 8.70 (m, 3H, ArH), 8.00 (s, 1H, NH), 8.73 (t, 1H, pyrazine C5-H), 8.82 (d, 1H, pyrazine C6-H), 9.21 (d, 1H, pyrazine C2-H), 11.00 (s, 1H, OH); FABMS (*m*/*z*, 100%): 367 [M^+] (100). Anal. calcd. for $C_{13}H_{13}N_5O_6S$ (367.34): C, 42.51; H, 3.57; N, 19.06. Found: C, 42.55; H, 3.61; N, 19.10.

*Synthesis of compound **53***

A mixture of **52** and hydrazine hydrate in ethanol was refluxed on a water bath for 3 h. The content was cooled and poured onto crushed ice, filtered, and washed with water. The isolated product was recrystallized from ethanol.

R_f: 0.63 (acetonitrile/methanol, 1:1); IR: 3390 (OH-stretching), 3226, 3235 (NH-stretching), 1680, 1675 (C=O stretching), 1160 (S=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.47 (d, 3H, CH_3), 2.00 (s, 1H, NH), 3.13 (s, 2H, NH_2), 4.00 (s, 1H, NH of quinazolinone), 4.83 (s, 2H, OCH_2), 4.90 (q, 1H, CH), 6.90 – 8.10 (m, 3H, ArH), 8.00 (s, 1H, NH), 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%): 367 [M^+] (100). Anal. calcd. for $C_{14}H_{16}N_8O_5S$ (408.40): C, 41.17; H, 3.95; N, 27.44. Found: C, 41.21; H, 3.97; N, 27.48.

*Synthesis of compounds **54 – 63***

A mixture of **53** and substituted aryl sulphonyl chloride (0.01 mol), in ethanol (25 mL) and pyridine (1 mL) was refluxed in an oil bath at 120°C for 4 h. The reaction mixture was cooled and poured in crushed ice, filtered and washed with water. The product was recrystallized from ethanol.

*Compound **55***

R_f: 0.47 (acetonitrile/methanol, 1:1); IR: 3390 (OH-stretching), 3224, 3218 (NH-stretching), 1720, 1675 (C=O stretching), 1166 (S=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.47 (d, 3H, CH_3), 2.00 (s, 2H, NH_2), 4.00 (s, 1H, NH of quinazolinone), 4.83 (s, 2H, OCH_2), 4.90 (q, 1H, CH), 6.90 – 8.40, (m, 7H, ArH), 8.00 (s, 1H, NH), 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%): 585 [M^+ + 2] (100). Anal. calcd. for $C_{20}H_{19}ClN_8O_7S_2$ (583.00): C, 41.20; H, 3.28; N, 19.22. Found: C, 41.24; H, 3.30; N, 19.26.

*Compound **56***

R_f: 0.52 (acetonitrile/methanol, 1:1); IR: 3391 (OH-stretching), 3225, 3219 (NH-stretching), 1721, 1676 (C=O stretching), 1165 (S=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.47 (d, 3H, CH_3), 2.00 (s, 2H, NH_2), 4.00 (s, 1H, NH of quinazolinone), 4.83 (s, 2H, OCH_2), 4.90 (q, 1H, CH), 6.96 – 8.20 (m, 7H, ArH), 8.00 (s, 1H, NH), 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%): 594 [M^+] (100). Anal. calcd. for $C_{20}H_{19}N_9O_9S_2$ (593.56): C, 40.47; H, 3.23; N, 21.24. Found: C, 40.54; H, 3.26; N, 21.19.

*Compound **57***

R_f: 0.71 (acetonitrile/methanol, 1:1); IR: 3389 (OH-stretching), 3225, 3219 (NH-stretching), 1722, 1676 (C=O stretching), 1168 (S=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.47 (d, 3H, CH_3), 2.00 (s, 2H, NH_2), 4.00 (s, 1H, NH of quinazolinone), 4.83 (s, 2H, OCH_2), 4.90 (q, 1H, CH), 6.95 – 8.25, (m, 7H, ArH), 8.00 (s, 1H, NH), 8.73 (t, 1H, pyrazine C5-H), 8.82 (d, 1H, pyrazine C6-H), 9.20 (s, 1H,

C_6H_4OH), 9.21 (d, 1H, pyrazine C2-H), FABMS (*m*/*z*, 100%): 565 [M^+] (100). Anal. calcd. for $C_{20}H_{20}N_8O_8S_2$ (564.56): C, 42.55; H, 3.57; N, 19.85. Found: C, 42.41; H, 3.59; N, 19.79.

*Compound **59***

R_f: 0.77 (acetonitrile/methanol, 1:1); IR: 3391 (OH-stretching), 3225, 3215 (NH-stretching), 1722, 1676 (C=O stretching), 1164 (S=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (t, 6H, OCH_3-C_6H_4), 1.47 (d, 3H, CH_3), 2.00 (s, 2H, NH_2), 4.00 (s, 1H, NH of quinazolinone), 4.83 (s, 2H, OCH_2), 4.90 (q, 1H, CH), 6.90–8.40, (m, 6H, ArH), 8.00 (s, 1H, NH), 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%): 609 [M^+] (100). Anal. calcd. for $C_{22}H_{24}N_8O_9S_2$ (608.61): C, 43.42; H, 3.97; N, 18.41. Found: C, 43.46; H, 3.96; N, 18.37.

*Compound **61***

R_f: 0.45 (acetonitrile/methanol, 1:1); IR: 3392 (OH-stretching), 3225, 3214 (NH-stretching), 1721, 1674 (C=O stretching), 1165 (S=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.47 (d, 3H, CH_3), 2.00 (s, 2H, NH_2), 4.00 (s, 1H, NH of quinazolinone), 4.83 (s, 2H, OCH_2), 4.90 (q, 1H, CH), 7.10–8.40 (m, 7H, ArH), 8.00 (s, 1H, NH), 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%): 566 [M^+] (100%). Anal. calcd. for $C_{20}H_{19}FN_8O_7S_2$ (566.55): C, 42.40; H, 3.38; N, 19.78. Found: C, 42.51; H, 3.41; N, 19.81.

*Compound **63***

R_f: 0.59 (acetonitrile/methanol, 1:1); IR: 3386 (OH-stretching), 3231, 3211 (NH-stretching), 1714, 1672 (C=O stretching), 1162 (S=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.46 (d, 3H, CH_3), 2.10 (s, 2H, NH_2), 3.90 (s, 1H, NH of quinazolinone), 4.76 (s, 2H, OCH_2), 5.01 (q, 1H, CH), 7.20–8.20 (m, 6H, ArH), 8.10 (s, 1H, NH), 8.75 (t, 1H, pyrazine C5-H), 8.82 (d, 1H, pyrazine C6-H), 9.21 (d, 1H, pyrazine C2-H); FABMS (*m*/*z*, 100%): 630 [M^+ + 2] (100). Anal. calcd. for $C_{20}H_{18}ClN_9O_9S_2$ (628.00): C, 38.25; H, 2.89; N, 20.07. Found: C, 38.28; H, 2.92; N, 20.11.

Antimycobacterial activity

All the tested compounds were assayed *in vitro* for antimycobacterial activity against the H37 Rv strain of *M. tuberculosis* strain using a standard protocol [16]. 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 rifampin and isoniazid (INH). As this class of compounds has a similarity with pyrazinamide, yet pyrazinamide is a prodrug, it does not give the antimycobacterial activity *in vitro*, so INH is used. Although rifampin does not have a structural similarity with the synthesized analogues, it contains the piperidine ring and it inhibits DNA-dependent RNA polymerase in bacterial cells by binding its beta-subunit and showed the very good antimycoacterial activity *in vitro*. Therefore, for comparison with the most potent existing drug, rifampin is used as one of the standards.

Microbiology

All the test compounds were assayed *in vitro* for antimycobacterial activity and evaluated against the H37Rv strain of *M. tuberculosis*. The MIC was determined by the test tube dilution technique using modified Kirchner's culture medium containing 0.5% sterilized horse serum for antimycobacterial activity. 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 passage through 0.2 mm polycarbonate sterile membrane filters (Nuclepore). Further, the serial dilution of the test compounds was carried out and the following concentration was used: 1000, 500, 250, 125, 62, 32, 16, 8, 4, and 1 g/mL. Test compounds at various concentrations were added to the culture medium in a sterilized borosilicate test tube and the H37 Rv strain of mycobacterium tuberculosis was inoculated at 10^6 bacilli/mL concentration. The tubes were incubated at 37°C for 14 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, indicating 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 5.

The authors have declared no conflict of interest.

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