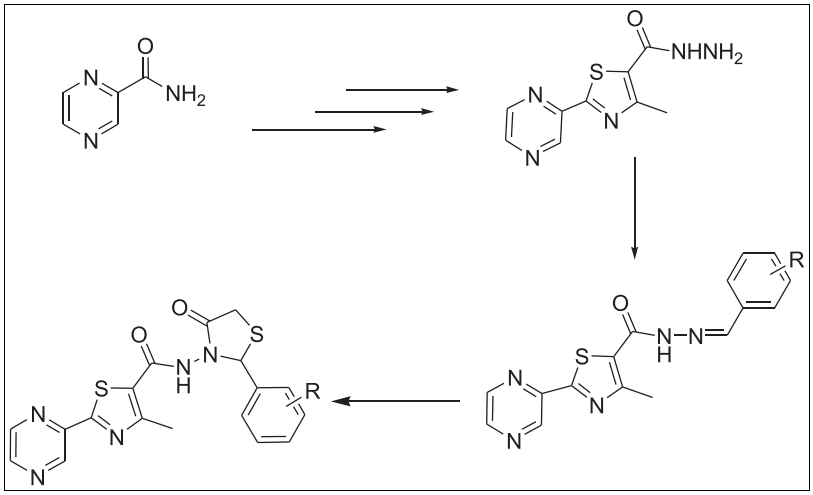
Synthesis and Antitubercular activity of New Thiazolidinones with Pyrazinyl and Thiazolyl scaffolds

Sambhaji T. Dhumal,[a] Amarsinh R. Deshmukh,[a] Lalit D. Khillare,[a] Manisha Arkile,[b] Dhiman Sarkar,[b] and Ramrao A. Mane[a]*

[a]Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, 431004, India
[b]Combi Chem-Bio Resource Centre, CSIR-National Chemical Laboratory, Pune, 411008, India
*E-mail: manera2011@gmail.com

Additional Supporting Information may be found in the online version of this article.
Received June 17, 2015
DOI 10.1002/jhet.2552
Published online in 00 Month 2015 Wiley Online Library (wileyonlinelibrary.com).



Emergence of multidrug resistant and extensively drug resistant tuberculosis has prompted to develop new molecular entities to treat the disease. A series of new 4-thiazolidinones with pyrazinyl and thiazolyl scaffolds has been synthesized, and their antitubercular activity is reported. The title 4-thiazolidinones, *N*-(pyrazinyl substituted thiazoloylamino)-2-aryl-4-thiazolidinones (**6a–j**) have been first time prepared using pyrazinamide as a starting material via five successive steps. The purity and the structures of the intermediates (carboethoxythiazole, acid hydrazide, and azomethines) and title thiazolidinones (**6a–j**) have been confirmed by TLC and spectral analyses, respectively. An antitubercular screening of the new 4-thiazolidinones has been performed on bacterial strains, *Mycobacterium tuberculosis H37Ra* and *Mycobacterium BCG* using the solutions of different concentrations of the compounds (**6a–j**) and the screening results are presented. Compound **6a** has displayed notable antitubercular activity.

J. Heterocyclic Chem., **00,** 00 (2015).

INTRODUCTION

Tuberculosis (TB) is a major global health problem and highly contagious infectious disease, caused by *Mycobacterium tuberculosis* (*Mtb*). One third of the world's population is currently infected with *Mtb* [1]. In 2012, the World Health Organization reported 8.6 million new cases and 1.3 million TB deaths [2]. Increasing incidence of multidrug resistant TB (resistant to at least isoniazid and rifampicin), extensively drug resistant TB (XDR-TB; multidrug resistant plus resistant to fluroquinoline and aminoglycoside), totally drug resistant TB, and coinfection with HIV remain a serious problem [3]. Currently available TB treatment is an administration of combination of first-line antituberculars; rifampicin, isoniazid, ethambutol, and pyrazinamide (PZA) for 6 months and has side effects and noncompliance. Because of the serious threats of TB, the search and development of new bioactive therapeutic lead as effective anti-TB drugs against the resistant strains having fewer to no side effects is currently gaining more importance. As such, the development of novel molecular scaffolds targeting Mtb is an area of increasing active investigation globally [4].

First-line antitubercular agent, PZA is a nicotinamide analog. This in combination with rifampicin displays sterilizing activity, the ability to kill the dormant non-growing tubercle bacilli of low metabolism activity [5]. This synergetic effect of PZA and rifampicin against dormant subpopulation of Mtb is responsible to decrease the time of treatment from 12 to 6 months. PZA is a prodrug, and after getting converted into pyrazinoic acid [6], acts as inhibitor of mycobacterial fatty acid synthase-I [7]. Pyrazines possess wide range of pharmacological activities, viz antitubercular [8],

antifungal [9], antitumor [10], and antibacterial [11]. The previously mentioned clinical properties of PZA/its derivatives have increased the interest to synthesize its more new derivatives/analogues with the hope to obtain potentially active antitubercular compounds [4].

Literature survey reveals that 4-thiazolidinone is a versatile scaffold observed in various pharmaceutical agents and displays broad spectrum of biological activities such as antitubercular [12], anti-inflammatory [13], anticancer [14], antihyperglycemic [15], antifungal [16], anticonvulsant [17], antihistaminic [18], and anti-HIV [19]. Some of the thiazoles are the key scaffolds/clinical drugs and have displayed numerous biological and pharmaceutical activities such as antitubercular [20], anti-inflammatory [21], anticancer [22], antibacterial [23], antifungal [24], and antiviral [25].

Considering the therapeutic significance of pyrazines, thiazoles, and thiazolidinones here it was thought to synthesize some new 4-thiazolidinones having these biodynamic rings, i.e., thiazole and pyrazine within one molecular framework with expectation to obtain the new leads with enhanced antitubercular activity, because of the combined synergetic effect of the rings.

RESULTS AND DISCUSSION

Chemistry. The target compounds have been synthesized by following multistep route, designed on the basis of retrosynthetic analysis, starting from PZA.

In the first step, PZA (**1**) was refluxed with phosphorus pentasulphide in pyridine and converted into thio PZA (**2**). The freshly prepared thio PZA was then condensed with 2-chloroethylacetoacetate in refluxed ethanol and obtained 5-carboethoxy-4-methyl (pyrazinyl-2-yl) thiazole (**3**) following Hantzsch Synthesis. A mixture of hydrazine hydrate and carboethoxythiazole (**3**) was dissolved in ethanol, and the solution was refluxed for getting the respective acid hydrazide (**4**). Condensation of aryl aldehydes and the acid hydrazide (**4**) has been carried in PEG-400 at 110°C and obtained the corresponding Schiff bases/azomethines (**5a–j**). Finally, the cyclocondensation of mercaptoacetic acid and azomethines /Schiff bases has been carried in glycerol at 100°C and obtained the title new compounds *N*-(pyrazinyl substituted thiazoloylamino)-2-aryl-4-thiazolidinones, i.e., 4-methyl-*N*-(4-oxo-2-phenylthiazolidin-3-yl)-2-(pyrazin-2-yl)thiazole-5-carboxamides (**6a–j**, Scheme 1).

All the newly synthesized compounds have been characterized using their IR, ^{1}H NMR, ^{13}C NMR, and HRMS spectral data. The IR spectrum of compound (**6a**) indicates that formation of product as it shows a characteristics absorption peak at 1706 cm^{-1}, which corresponds to the carbonyl group of cyclic amide. The ^{1}H NMR spectrum of compound (**6a**) displays a pair of doublet at δ 3.81 and 3.98 ppm, because of the geminal coupling between two hydrogens of S–CH_2–C=O and a sharp singlet at δ 5.88 and 10.77 ppm, corresponds to the signals because of protons of N–CH–S and NH–C=O, The presence of three characteristics carbon signals is observed at δ 29.31, 61.6, and 169.01 ppm in ^{13}C NMR spectrum of (**6a**) owing to the signals of carbons of S-CH_2-CO, N-CH-S and C=O groups, respectively, confirming the presence of a 4-thiazolidinone ring in (**6a**). The HRMS spectrum further strengthen the structure assigned as 4-methyl-*N*-(4-oxo-2-phenylthiazolidin-3-yl)-2-(pyrazin-2-yl)thiazole-5-carboxamide as it displays $[M+H]^+$ ion peak at *m/z* 398.0735 for the molecular formula $C_{18}H_{15}N_5O_2S_2$ (Scheme 1).

Biological evaluation. ***Antitubercular activity.*** All the synthesized compounds were evaluated for there *in vitro* activity against all *M. Tuberculosis H37Ra*, *M. Bovis BCG*. All compounds showed moderate activity against Mycobacterium species (Table 1). All compounds did not show any activity against other bacteria, which means it does not affect human's normal flora. Compound (**6a**) showed 70% inhibition at 30 μg/mL concentration, which is highest among the series. The title compound (**6a**) is a parent compound not having any substituent in the benzonoid ring. The other compounds (**6b-j**), most of them are having electron donating substituents at four position, responsible for enhancing electron availability and activation of benzonoid ring. This is the only difference with respect to the electron availability, causing more favor to (**6a**) to display better antitubercular activity than the others.

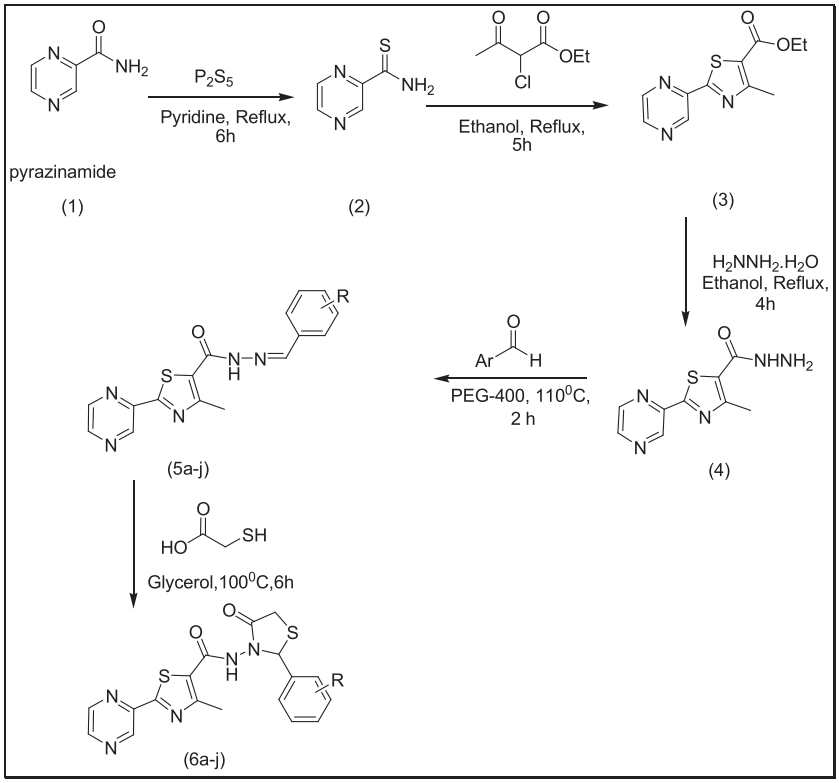
CONCLUSION

New *N*-(pyrazinylsubstituted thiazoloylamino)-2-aryl-4-thiazolidinones (**6a–j**) have been synthesized by developing convenient multistep synthetic route starting from readily available PZA, an existing antitubercular drug. The antitubercular evaluation has also been carried out of the new thiazolidinones using their lower concentrations. All of them have shown moderate activity against *M. tuberculosis H37Ra* and *M. Bovis BCG*. The most active compound among them is **6a**.

EXPERIMENTAL

Methods and Materials. The chemicals used were of laboratory grade. Melting points of all the synthesized compounds were determined in open capillary tubes and are uncorrected. The IR spectra (In KBr pellets) were recorded on Brukar FTIR spectrometer.^{1}H NMR spectra were recorded on a Brukar DRX-300 and 300 MHz NMR spectrometer, and ^{13}C NMR spectra were recorded on a Brukar DRX-75 and 75 MHz NMR in $CDCl_3$/DMSO-d_6 using tetramethylsilane as an internal standard and chemical shifts are in δ (ppm). High-resolution mass spectra (HRMS) were recorded on Agilent 6520 (Q-TOF)

Scheme 1. Synthesis of 4-methyl-*N*-(4-oxo-2-phenyl-thiazolidin-3-yl)-2-(pyrazin-2-yl)thiazole-5-carboxamides (**6a–j**).



ESI-HRMS instrument. The purity of each of the compound was checked by thin-layer chromatography (TLC) using silica-gel, $60\,F_{254}$ aluminum sheets as an adsorbent, and visualization was accomplished by iodine/ultraviolet light.

Synthesis of pyrazine-2-carbothiamide (2), (prepared by following reported method [26]). A mixture of pyrazine-2-carboxamide (**1**) (0.01 mol) and phosphorous pentasulphide (0.004 mole) was dissolved in pyridine (8 mL) and then the solution was refluxed for 4 h. The progress of the reaction was monitored by TLC using ethyl acetate: hexane (6:4) as solvents. After 4 h, reaction mass was poured in ice water and kept overnight, the solid obtained was filtered and was washed with water and the obtained crude solid was crystallized from ethanol-DMF. (yield 59%, mp 191−193°C). The melting point is an agreement with the reported.

Synthesis of ethyl-4-methyl-2-(pyrazin-2-yl)thiazole-5-carboxylate (3). Pyrazine-2-carbothiamide (**2**) (0.01 mol) and 2-chloroethylacetoacetate (0.02 mol) were dissolved in ethanol (10 mL), and the solution was refluxed. The progress of the reaction were monitored by TLC using ethyl acetate: hexane (3:7) as solvents. After refluxing for 5 h, ethanol was removed from the reaction mass under reduced pressure. The residue obtained was added in ice cold water, neutralized by $NaHCO_3$.The solid obtained was filtered, washed with water and crystallized from ethanol.

Yield 72%, mp 91–92°C; IR (KBr) ν cm^{-1}: 3260, 3180, 2979, 1704, 1523, 1665, and 1254; ^1H NMR (300 MHz, $CHCl_3$-*d*) δ ppm = 1.40 (t, 3H, CH_3), 2.82 (s, 3H, CH_3), 4.38 (q, 2H, CH_2), 8.62 (d, J = 12 Hz, 2H), and 9.43 (s, 1H); HRMS *m/z*: 250.0643 $[M+H]^+$ for $C_{11}H_{11}N_3O_2S$.

Synthesis of 4-methyl-2-(pyrazin-2-yl)thiazole-5-carbohydrazide (4). A mixture of ethyl-4-methyl-2-(pyrazin-2-yl) thiazole-5-carboxylate (**3**) (0.01 mol) and excess of hydrazine hydrate (0.03 mol) was refluxed in ethanol. The progress of the reaction was monitored by TLC using ethyl acetate: hexane (3:7) as solvents. Then after 4 h, ethanol was removed under reduced pressure. The obtained residue was then poured in ice cold water. The solid that was obtained was filtered, washed with water and crystallized from ethanol.

Yield 74 %, mp 208–211°C; IR (KBr)) ν cm^{-1}: 3307, 3060, 1674, 1602 and 1585; ^1H NMR (300 MHz, $CHCl_3$-*d*) δ ppm = 2.80 (s, 3H), 4.16 (br. s., 2H), 7.20 (br. s., 1 H), 8.50–8.77 (m, 2H), and 9.44 (s, 1H), LCMS *m/z*: 235.58 $[M]^+$ for $C_9H_9N_5OS$.

General Procedure for the synthesis of N-arylidene-4-methyl-2-(pyrazin-2-yl)thiazole-5-carbohydrazide (5a–j). 4-Methyl-2-(pyrazin-2-yl)thiazole-5-carbohydrazide (**4**) (0.01 mol) and benzaldehydes (0.01 mol) were dissolved in PEG-400 (8 mL), and the solution was stirred at 110°C. The progress of the reaction was monitored by TLC using ethyl acetate: hexane (3:7) as solvents. After 2 h of stirring, the reaction

Table 1

Antimycobacterial activity of compounds (**6a–j**).

Compound code	Antimycobacterial activity (% Inhibition) at 30 μg/mL concentration the of compound	
	M. tuberculosis H37Ra	*M. bovis BCG*
6a	71.16	62.67
6b	39.24	28.65
6c	48.94	46.72
6d	27.64	37.83
6e	17.96	31.72
6f	37.14	33.54
6g	42.12	21.48
6h	14.82	24.16
6i	29.65	45.21
6j	28.49	19.46
Rifampicin	90 (0.03 ug/mL Conc.)	91 (0.04 ug/mL Conc.)

Table 2

Physical data of *N*-benzylidene-4-methyl-2-(pyrazin-2-yl)thiazole-5-carbohydrazides (**5a–j**).

Entry	Compounds	R	Yield[a] (%)	Melting point (°C)
1	5a	4-H	85	207–209
2	5b	4-CH_3	77	218–220
3	5c	4-Cl	82	252–255
4	5d	4-OCH_3	73	210–211
5	5e	4-F	80	172–174
6	5f	2-F	72	182–184
7	5 g	2-Cl	74	195–198
8	5 h	3,4-OCH_3	68	222–224
9	5i	4-$N(CH_3)_2$	74	232–234
10	5j	4-OH	78	168–171

[a]Isolated yields.

Table 3

Physical data of 4-methyl–N-(4-oxo-2-phenylthiazolidin-3-yl)-2-(pyrazine-2- yl)thiazol-5-carboxamides (**6a–j**).

Entry	Compounds	R	Yields[a] (%)	Melting points (°C)
1	6a	4-H	78	205–207
2	6b	4-CH_3	72	170–172
3	6c	4-Cl	82	106–107
4	6d	4-OCH_3	76	168–170
5	6e	4-F	84	174–177
6	6f	2-F	62	205–208
7	6g	2-Cl	66	146–148
8	6h	3,4-OCH_3	68	172–174
9	6i	4-$N(CH_3)_2$	72	156–158
10	6j	4-OH	72	171–172

[a]Isolated yields.

mass was poured in cold water and the obtained crude product was crystallized from ethanol-DMF. The physical data of the compounds (**5a–j**) of the series are given in the Table 2.

Synthesis of N-benzylidene-4-methyl-2-(pyrazin-2-yl)-thiazole-5-carbohydrazide (5a). IR (KBr) $\nu\,cm^{-1}$: 3391, 3157, 3048, 1668, 1574 and 1385; ^1H NMR (300 MHz, DMSO-d_6) δ ppm = 2.93 (s, 3 H), 7.45–7.79 (m, 5H), 7.91 (s, 1H), 8.62–8.65 (d, J = 12 Hz, 2H), and 9.44 (s, 2H); LCMS m/z: 324.01 $[M+H]^+$ for $C_{16}H_{13}N_5OS$.

Synthesis of N′-(4-chlorobenzylidene)-4-methyl-2-(pyrazin-2-yl)thiazole-5-carbohydrazide (5c). IR (KBr) $\nu\,cm^{-1}$: 3360, 3112, 2958, 1664 and 857; ^1H NMR (300 MHz, DMSO-d_6) δ = 2.51 (s, 3H, CH_3), 7.25 (s, 1H, CH), 7.37–7.38 (m, 4H, Ar-H), 8.49–9.29 (m, 3H, pyrazine-H), and 11.64 (s, 1H, Amido N-H); HRMS m/z: 358.0521 $[M+H]^+$ for $C_{16}H_{12}ClN_5OS$.

Synthesis of N-(4-methoxybenzylidene)-4-methyl-2-(pyrazin-2-yl)thiazole-5-carbohydrazide (5d). IR (KBr) $\nu\,cm^{-1}$: 3312, 3112, 3048, 1662, 1648 and 1320; ^1H NMR (300 MHz, DMSO-d_6) δ = 2.13 (s, 3H, CH_3), 3.10 (s, 3H, OCH_3), 6.79 (s, 1H, CH), 6.20–6.22 (dd, J = 8 Hz, 2H, Ar-H), 6.93–6.95 (dd, J = 8 Hz, 2H, Ar-H), 7.59 (s, 1H, NH), 7.90–8.69 (m, 3H, pyrazine-H); HRMS m/z: 354.1016 $[M+H]^+$ for $C_{17}H_{15}N_5O_2S$.

General procedure for the synthesis of 4-methyl–N-(4-oxo-2-arylthiazolidin-3-yl)-2-(pyrazine-2-yl)thiazol-5-carboxamide (6a–j). A mixture of N-arylidene-4-methyl-2-(pyrazin-2-yl)thiazole-5-carbohydrazide (**5a–j**) (0.01 mol) and mercaptoacetic acid (0.04 mol) in glycerol (8 mL) was stirred at 100°C. The progress of the reaction was monitored by TLC using ethyl acetate: hexane (4:7) as solvents. After 2 h of stirring, the reaction mass poured in cold water, washed with $NaHCO_3$, and the obtained crude product was crystallized from ethanol. The physical data of the compounds (**6a–j**) of the series are given in the Table 3.

Synthesis of 4-Methyl–N-(4-oxo-2-pheylthiazolidin-3-yl)-2-(pyrazine-2-yl)thiazol-5-carboxamide (6a). IR (KBr) $\nu\,cm^{-1}$: 3376, 2957, 2929, 2836, 1706, 1664, and 1511; ^1H NMR (300 MHz, DMSO-d_6) δ ppm = 2.50 (s, 3H, CH_3), 3.81–3.98 (dd, 2H, CH_2), 5.93 (s, 1H, CH), 7.35–7.53.(m, 5H, Ar-H), 8.69–9.26 (m, 3H, pyrazine-H), and 10.77 (s, 1H, Amido N-H);^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm = 16.92, 29.31, 61.68, 124.94, 127.87, 128.56, 129.10, 137.83, 140.65, 144.59, 144.70, 146.59, 157.87, 159.86, 165.79, and 169.01; HRMS m/z: 398.0735 $[M+H]^+$ for $C_{18}H_{15}N_5O_2S_2$.

Synthesis of 4-Methyl-N-(4-oxo-2-(p-tolyl)thiazolidin-3-yl)-2-(pyrazin-2-yl)thiazole-5-carboxamide (6b). IR (KBr) $\nu\,cm^{-1}$: 3389, 2968, 2923, 1707, 1665, and 1648; ^1H NMR (300 MHz, DMSO-d_6) δ ppm = 2.50 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 3.78–3.95 (dd, 2H, CH_2), 5.88 (s, 1H, CH), 7.19–7.21 (dd, J = 8 Hz, 2H, Ar-H), 7.37–7.40 (dd, J = 8 Hz, 2H, Ar-H), 8.70–9.27 (m, 3H, pyrazine-H), and 10.74 (s, 1H, Amido N-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm = 16.93, 20.75, 29.31, 61.55, 124.94,

127.84, 129.08, 134.73, 138.56, 140.65, 144.71, 144.99, 146.60, 157.91, 159.84, 165.76, and 168.98; HRMS *m/z*: 412.0879 $[M+H]^+$ for $C_{19}H_{17}O_2N_5S_2$.

Synthesis of N-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)-4-methyl-2-(pyrazin-2-yl)thiazole-5-carboxamide (6c). IR (KBr) $\nu\,cm^{-1}$: 3360, 2961, 1700, 1664, 1525 and 753; ^1H NMR (300 MHz, DMSO-d_6) δ ppm = 2.72 (s, 3H, CH_3), 3.80–4.00 (dd, 2H, CH_2), 5.93 (1H, s, CH), 7.45–7.48. (dd, J = 8Hz, 2H, Ar-H), 7.74–7.95 (dd, J = 8Hz, 2H, Ar-H), 8.71–9.28 (m, 3H, pyrazinyl), and 10.76 (s, 1H, Amido N-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm = 16.98, 20.75, 60.92, 124.77, 128.56, 129.84, 133.61, 137.16, 140.67, 144.73, 144.99, 146.63, 158.03, 159.88, 165.83, and 168.84; HRMS *m/z*: 432.0332 $[M+H]^+$ for $C_{18}H_{14}ClN_5O_2S_2$.

Synthesis of N-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)-4-methyl-2-(pyrazin-2-yl)thiazole-5-carboxamide (6d). IR (KBr) $\nu\,cm^{-1}$: 3345, 2963, 1706, 1657, 1652, and 1527; ^1H NMR (300 MHz, DMSO-d_6) δ ppm = 2.64 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 3.80–4.00 (dd, 2H, CH_2), 5.97 (s, 1H, CH), 6.88–6.92 (dd, J = 8 Hz, 2H, Ar-H) 7.35–7.39 (dd, J = 8 Hz, 2H, Ar-H), 8.52–8.64 (m, 3H, pyrazine-H), and 9.36 (s, 1H, Amido N-H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ ppm = 17.50, 30.14, 60.11, 62.42, 116.11, 123.92, 128.01, 129.68, 130.07, 132.50, 134.06, 147.74, 151.61, 157.91, 159.82, 160.29, 162.42, 164.40, 165.05, and 170.13; HRMS *m/z*: 428.0843 $[M+H]^+$ for $C_{19}H_{17}N_5O_3S_2$.

Synthesis of N-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)-4-methyl-2-(pyrazin-2-yl)thiazole-5-carboxamide (6e). ^1H NMR (300 MHz, $CDCl_3$) δ = 2.50 (s, 3H, CH_3), 3.59–3.85 (dd, 2H, CH_2), 5.89 (s, 1H, CH), 6.93–7.02 (dd, J = 8 Hz, 2H, Ar-H), 7.30–7.34 (dd, J = 8Hz, 2H, Ar-H), 8.12–8.21 (m, 3H, pyrazine-H), and 9.22 (s, 1H, Amido N-H), ^{13}C NMR (125 MHz, $CHCl_3$-*d*) δ ppm = 17.45, 30.10, 63.01, 122.18, 123.89, 128.02, 128.68, 129.11, 134.03, 136.81, 147.77, 151.61, 157.91, 159.52, 160.26, 165.08, and 170.12; HRMS *m/z*: 416.0643 $[M+H]^+$ for $C_{18}H_{14}FN_5O_2S_2$.

Synthesis of N-(2-(2-fluorophenyl)-4-oxothiazolidin-3-yl)-4-methyl-2-(pyrazin-2-yl)thiazole-5-carboxamide (6f). IR (KBr) $\nu\,cm^{-1}$: 3354, 2967, 1706, 1655, and 1641; ^1H NMR (300 MHz, DMSO-d_6) δ ppm = 2.61 (s, 3H, CH_3), 3.70–3.84 (dd, 2H, CH_2), 6.28 (s, 1H, CH), 7.04–7.52 (m, 4H, Ar-H), 8.44–8.62 (m, 3H, pyrazine-H), and 9.33 (s, 1H, Amido N-H); ^{13}C NMR ($CHCl_3$-d, 125 MHz) δ ppm = 17.60, 29.82, 59.76, 123.85, 127.65, 128.60, 130.38, 130.49, 133.83, 134.81, 141.75, 144.05, 145.78, 146.00, 160.45, 166.50, and 170.41; HRMS *m/z*: 416.0643 $[M+H]^+$ for $C_{18}H_{14}FN_5O_2S_2$.

Synthesis of N-(2-(2-chlorophenyl)-4-oxothiazolidin-3-yl)-4-methyl-2-(pyrazin-2-yl)thiazole-5-carboxamide (6g). IR (KBr) $\nu\,cm^{-1}$: 3360, 2952, 2923, 1704, 1667, 1515, and 748 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ ppm = 2.72 (s, 3H, CH_3), 3.78–3.92 (dd, 2H, CH_2), 6.50 (s, 1H, CH), 7.36–7.56.(m, 4H, Ar-H), 7.89–8.66 (m, 3H, pyrazinyl), and 9.40 (s, 1H, Amido N-H); ^{13}C NMR ($CHCl_3$-d, 125 MHz) δ ppm = 17.60, 29.82, 59.76, 123.85, 127.65, 128.60, 130.38, 130.49, 133.83, 134.81, 141.75, 144.05, 145.78, 146.00, 160.45, 166.50, and 170.41; HRMS *m/z*: 432.0332 $[M+H]^+$ for $C_{18}H_{14}ClN_5O_2S_2$.

Synthesis of N-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidin-3-yl)-4-methyl-2-(pyrazin-2-yl)thiazole-5-carboxamide (6h). IR (KBr) $\nu\,cm^{-1}$: 3362, 2964, 1715, 1668, 1654, and 1254; ^1H NMR (300 MHz, DMSO-d_6) δ ppm = 2.65 (s, 3H, CH_3), 3.67–3.77 (dd, 2H, CH_2), 3.90 (s, 3H, OCH_3), 4.10 (s, 3H, OCH_3), 5.97 (s, 1H, CH), 6.80–7.02.(m, 3H, Ar-H), 7.91–8.63 (m, 3H, pyrazine-H), and 9.36 (s, 1H, Amido N-H); HRMS *m/z*: 458.0952 $[M+H]^+$ for $C_{20}H_{19}N_5O_4S_2$.

Antitubercular screening. All the chemicals such as XTT (Sodium Salt Powder), sulfanilic acid, sodium nitrate, HCl, NEED rifampicin, and DMSO were purchased from Sigma-Aldrich, USA. Dubos medium was purchased from DIFCO, USA.

Microbial strains such as *Mycobacterium tuberculosis H37Ra* (ATCC 25177) and *M. bovis* BCG (*ATCC* 35734) were obtained from AstraZeneca, India. The stock culture was maintained at −80°C and subcultured once in a liquid medium before inoculation into an experimental culture. Cultures were grown in Dubos media (enrichment media).

Antimycobacterium testing. For antimycobacterial assay, M. pheli medium (minimal essential medium) was used for both *M.tuberculosis H37Ra* and *M.bovis BCG*. It contains 0.5 g KH_2PO_4, 0.25 g trisodium citrate, 60 mg $MgSO_4$, 0.5 g aspargine, and 2 mL glycerol in distilled water (100 mL) followed by pH adjustment to 6.6. Stock solutions of the compounds were prepared in DMSO and were used for further antimycobacterial testing.

M. tuberculosis H37Ra (ATCC 25177) was grown to logarithmic phase ($O.D._{595} \sim 1.0$) in a defined medium (M. pheli_medium). The stock culture was maintained at −70°C and subcultured once in M. pheli_medium before inoculation into experimental culture. Drugs were solubilized in dimethyl sulfoxide (DMSO) and stored in aliquots at −20°C. XTT sodium salt powder (Sigma) was prepared as a 1.25 mM stock solution in sterile 1× PBS and used immediately. Menadione (Sigma) was always freshly prepared as a 6 mM solution in DMSO before use. Compounds were screened for their inhibitory effect on MTB by following XTT Reduction Menadione Assay protocol published earlier [27]. Briefly, in all wells of assay plate 200 μM XTT was added as a final concentration and incubated at 37°C for 20 min. Then 60 μM Menadione was added as a final concentration and incubated at 37°C for 40 min. The optical density was read on a micro plate reader (Spectramax plus384 plate reader, Molecular Devices Inc) at 470 nm filter against a blank prepared from cell-free wells. Absorbance given by cells treated with the vehicle alone was taken as

100% cell growth. All experiments were performed in triplicates along with rifampicin as standard (MIC 0.03 (μg/mL), and the quantitative value was expressed as the average ± standard deviation.

For *M. bovis BCG* nitrate reduction (NR) assay was used [28]. An additional 0.1% inoculated culture of 1 OD was mixed into drug solution, and NR was carried out after 12 days. Briefly 1:1:1 ration of culture:sulfanulic acid in HCl:NEED in D/W was added. Optical density was measured at 540 nm filter against a blank prepared from cell-free wells.

Acknowledgments. Authors are thankful to Professor D.B. Ingle for his invaluable discussions and guidance. The authors are very much thankful to CDRI, Lucknow, for providing spectral facilities. Authors are also thankful to National Chemical Laboratory, Pune, for screening antitubercular/antibacterial activity. One of the authors S.T.D. is grateful to U.G.C., New Delhi, India, for financial assistance in the form of NET Senior Research Fellowship.

REFERENCES AND NOTES

[1] http://www.who.int/tb/challenges/xdr/xdr_map_june08.pdf.

[2] Zitko, J.; Servusova B., Janoutova, A.; Paterova, P.; Mandikova, J.; Garaj, V.; Vejsova, M.; Marek, J.; Dolezal, M. Bioorg Med Chem 2015, 23, 174.

[3] Boogaard, J. D.; Kibiki, G. S.; Kisanga, E. R.; Boeree, M. J.; Aarnoutse, R. E. Antimicrob Agents Chemother 2009, 53, 849.

[4] (a) Chauhan, K.; Sharma, M.; Trivedi, P.; Chaturvedi, V.; Chauhan, P. M. S. Bioorg Med Chem Lett 2014, 24, 4166; (b) Altamari, J. M.; Hockey, S. C.; Boshoff, H. I.; Sajid, A.; Henderson, L. C. Chem Med Chem 2015, 10, 787.

[5] Zhang, Y. Front Biosci 2004, 9, 1136.

[6] Judge, V.; Narasimhan, B.; Ahuja, M. Hygeia J D Med 2012, 4, 1.

[7] Sayahi, H.; Zimhony, O.; Jacobs, W. R.; Shekhtman, A.; Welch, J. T. Bioorg Med Chem Lett 2011, 21, 4804.

[8] Sriram, D.; Yogeeswari, P.; Reddy, S. P. Bioorg Med Chem Lett 2006, 16, 2113.

[9] Dolezal, M.; Cmedlova, P.; Palek, L.; Vinsova, J.; Kunes, J.; Buchta, V.; Jampilek, J.; Kralova, K. Eur J Med Chem 2008, 43, 1105.

[10] Zhang, Y.; Wang, X-L.; Liu, W.; Yang, Y-S.; Tang, J-F.; Zhu, H-L. Bioorg Med Chem 2012, 20, 6356.

[11] Zitko, J.; Dolezal, M.; Svobodova, M.; Vejsova, M.; Kunes, J.; Kucera, R.; Jilek, P. Bioorg Med Chem 2011, 19, 1471.

[12] (a) Sharma, S.; Sharma, P. K.; Kumar, N.; Dudhe, R. Biomed Pharmacother 2011, 65, 244; (b) Pathak, R.B; Chovatia, P.T; Parekh, H. H. Bioorg Med Chem Lett 2012, 22, 5129–5133.

[13] Murphy, G. J.; Holder, J. C. Trends Pharmacol Sci 2000, 2, 469.

[14] (a) Havrylyuk, D.; Zimenkovsky, B.; Vasylenko, O.; Gzella, A.; Lesyk, R. J Med Chem 2012, 55, 8630; (b) Havrylyuk D.; Zimenkovsky B.; Vasylenko O.; Day C.W.; Smee D.F.; Grellier P.; Lesyk R. Eur J Med Chem 2013, 66, 228–237.

[15] (a) Bhosle, M. R.; Mali, J. R.; Pal, S.; Srivastava, A. K.; Mane, R. A. Bioorg Med Chem Lett 2014, 24, 2651.: (b) Raza, S.; Srivastava, S. P.; Srivastava, D. S.; Srivastava, A.K.; Haq, W.; Katti S.B.; Eur J Med Chem 2013, 63, 611–620.

[16] Jain, A. K.; Vaidya, A.; Ravichandran, V.; Kashaw, S. K.; Agrawal, R. K. Bioorg Med Chem 2012, 03, 69.

[17] Archana, Srivastava, V. K.; Kumar, A. Eur J Med Chem 2002, 37, 873.

[18] (a) Diurno, M. V.; Mazzoni, O.; Piscopo, E.; Calignano, A.; Giordano, F.; Bolognesel, A. J Med Chem 1992, 35, 2910.: (b) Arya, K.; Rawat, D.S.; Dandia, A.; Sasai, H. J Flu Chem 2012,137, 117–122.

[19] Rawal, R. K.; Tripathi, R.; Katti, S. B.; Pannecouque, C.; Clercq, E. D. Bioorg Med Chem 2007, 15, 1725–1731.

[20] Makam, P.; Kankanala, R.; Prakash, A.; Kannan, T. Eur J Med Chem 2013, 69, 564.

[21] Khillare, L. D.; Bhosle, M.; Deshmukh, A.; Mane, R. A. Med Chem Res 2015, 24, 1380.

[22] Romagnoli, R.; Baraldi, P. G.; Salvador, M. K.; Camacho, M. E.; Preti, D.; Tabrizi, M. E.; Basatto, M.; Brancale, A.; Hamel, E.; Bortolozzi, R.; Basso, G.; Viola, g. Bioorg Med Chem 2012, 20, 7083.

[23] Kalhor, M.; Salehifar, M.; Nikokar, I. Med Chem Res 2014, 23, 2947.

[24] Sarojini, B. K.; Krishna, B. G.; Darshanraj, C. G.; Bharath, B. R.; Manjunatha, H. Eur J Med Chem 2010, 45, 3490.

[25] Stankova, I.; Chuchkov, K.; Shishkov, S.; Kostova, K.; Mukova, L.; Galabov, A. S. Amino Acids 2009, 37, 383.

[26] Radwan S.M.; Bakhite, E.A. Monatshefte fur Chemie 1999, 130, 1117.

[27] Singh, U.; Akhtar, S.; Mishra, A.; Sarkar D. J Micro Methods 2011, 84, 202.

[28] Khan, A.; Sarkar, D. J Micro Methods 2008, 73, 6.