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Design, synthesis and evaluation of thiazole based amides for their antitubercular and PknG inhibitory activity

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A series of thiazole based amides have been designed and synthesized by solution-phase amide coupling of 2-aminothiazole and its derivatives with naturally occurring aromatic and heteroaromatic acids in excellent yield *via* DIC/HOBt protocol All the compounds have been evaluated for their antitubercular activity against *M. tuberculosis* virulent strain *M. tuberculosis H37Rv* and PknG inhibitory activity in the presence and absence of the inhibitors The compounds display moderate to significant PknG inhibitory activity (9.1-15.6% inhibition at 100 μM) as compared to the standard inhibitors and very moderate *in vitro* antitubercular activities against *M. tuberculosis* virulent strain *M. tuberculosis H37Rv*.

Keywords: Peptides, heterocycles, benzothiazole, *M. tuberculosis*, STPK inhibitor, HOBt, DIC

Tuberculosis is an infectious disease, which is caused by the bacteria *Mycobacterium tuberculosis* (*M. tuberculosis*). It is one of the most important global health problems. According to WHO, 1.5 million people died from tuberculosis out of 10 million new reported cases. The virulence of pathogenic mycobacteria is due to its capacity to survive for a prolonged period within macrophages[1-3]. It is revealed by genomic study that the genome of *Mycobacterium tuberculosis* constitutes of 11 serine/threonine protein kinases, out of which protein kinase G (PknG) plays a pivotal role in regulatory process within the mycobacterial cell and signaling cascade of the infected host cell[4-6]. Although PknG is not required for mycobacterial growth, it is crucial for the survival within host macrophage by blocking phagosome-lysosome fusion within infected macrophages[4,7]. Furthermore, PknG has also been reported to be necessary for acquiring intrinsic antibiotic resistance in bacteria[8]. Therefore, blocking PknG with the use of the specific PknG inhibitor like AX20017, will result in the inhibition of internalized mycobacteria. These findings make PknG an attractive and promising drug target for the treatment of *M. tuberculosis*. There are reports which have identified several small molecules (Figure 1), which act as PknG inhibitors, but their numbers are very limited[9-11]. Therefore, it is of utmost importance to search for new small molecules that act as PknG inhibitors.

Thiazole and its natural or synthetic derivatives displayed a variety of biological as well as pharmacological activities like antitubercular, analgesic, anti-inflammatory, antibacterial and antifungal, central nervous system stimulation, algicidal, anti-HIV, antiallergic, antischizophrenic, antihypertension, antihypnotics, anti-tumor and for the treatment of pain[12-24]. Furthermore, thiazole derivatives were also reported to display anti degenerative activity and coupled with other aromatic ring systems, can form new biologically active compounds[25]. Nitazoxanide (**NTZ**) and its hydrolyzed active metabolite Tizoxanide (**TIZ**), which have been approved by FDA for the treatment of protozoal infection in 2002 have effectively killed both the replicating and nonreplicating *Mtb* with MIC value 16 μg/mL, revealed the presence of thiazole unit in their chemical structure (Figure 2)[26,27]. Recently Sacchettini and their co-workers also reported **I** and **II** as inhibitors for *Mycobacterium tuberculosis* ATP Phosphoribosyl Transferase (HisG)[28]. Several Distamycin analogs (**III**) were also reported to have antimicrobial activities (Figure 2)[29].

A closer look at the chemical structure of compounds shown in Figure 2, showed the presence of thiazole unit having an amide group at its 2-position. Keeping in view of the above discussion, we were interested in synthesizing a series of small molecules having the 2-amidothiazole unit in their

chemical structure and to evaluate them for their antitubercular and pknG inhibitory activity.

Results and Discussion

Chemistry

Standard DIC/HOBt protocol for acid and amine coupling has been adopted for the synthesis of targeted compounds. The synthetic strategy started from the reaction between 2-aminothiazole and phenylacetic acid by using 1-hydroxy benzotriazole (HOBt), diisopropyl carbodiimide (DIC) and 4-dimethylamino pyridine (DMAP) as coupling reagents in anhydrous CH_2Cl_2 under N_2 atmosphere for 5 h at 0-5°C to get the desired 2-phenyl-N-(1,3-thiazol-2-yl)acetamide **(1)** in 86% yield (Scheme I).

The structure of compound **1** has been established based on their spectroscopic data and microanalysis. The IR spectrum of the compound **1** exhibited absorption bands at 3309 cm^{-1} for the NH group and at 1675 cm^{-1} for CO which proves the presence of both the groups in compound **1**. The ESI-MS spectrum (Mass Spectrum) of the compound **1** showed a peak at *m/z* 219 $[M+H]^+$ corresponding to its molecular formula $C_{11}H_{10}N_2OS$. In ^1H NMR

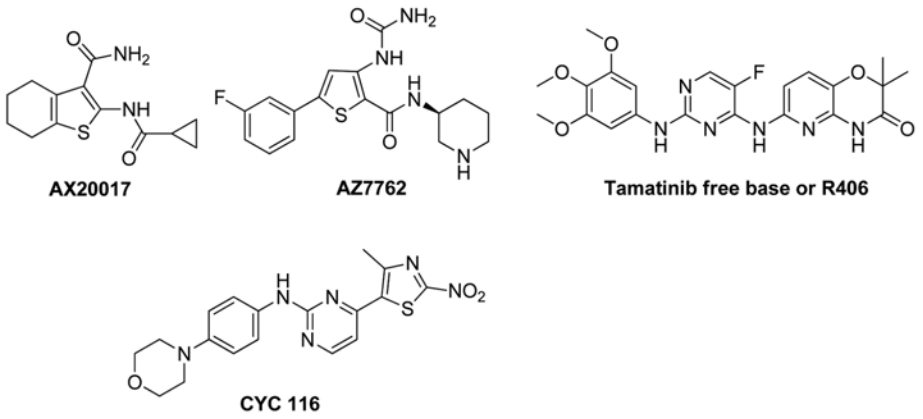


Figure 1 — Bacterial Protein Kinase Inhibitors

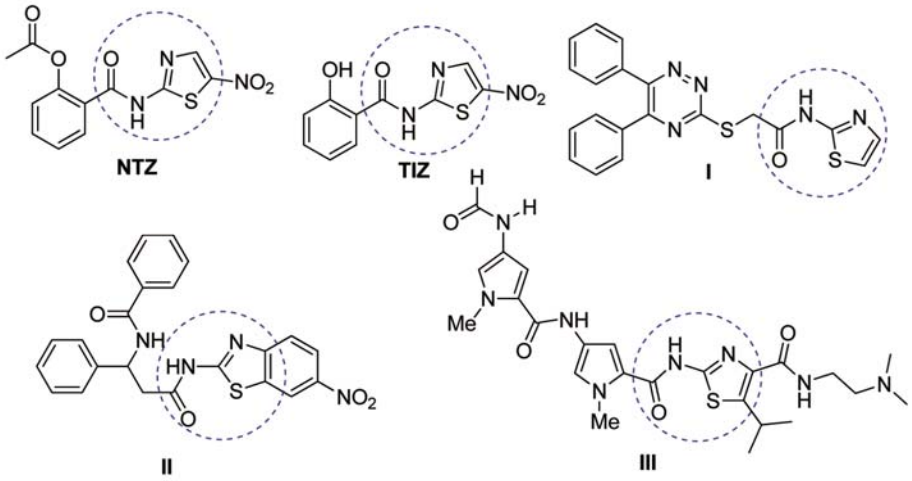
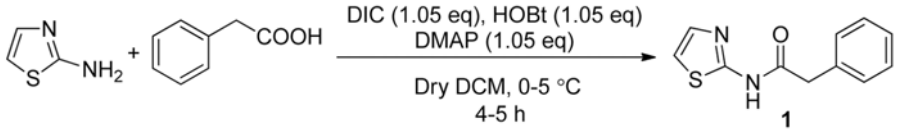


Figure 2 — Antibacterial amides derived from thiazoles



Scheme I — Synthesis of prototype compound **1**

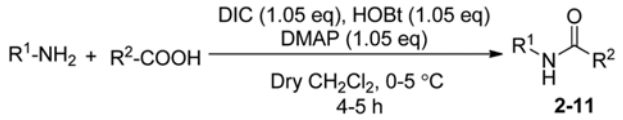
spectrum, H-4 and H-5 proton of the thiazole ring was observed as two distinct doublets at δ 7.47 and 7.02 respectively with coupling constant J = 2.3 Hz. Whereas all the other five aromatic protons of the phenyl ring appeared as a multiplet at δ 7.40-7.28. The methylene proton appeared as a singlet at δ 3.89. In the ^{13}C NMR spectrum, the carbonyl group appeared at δ 169.0. The quaternary carbon of the thiazole ring was observed at δ 159.0 while the two CH carbon of thiazole ring were observed at δ 136.9 and 113.0 respectively. Quaternary carbon of the phenyl ring was observed at δ 134.2 whereas the other five aromatic CH's of the phenyl ring were observed at δ 129.1, 128.4, and 126.9 respectively. The methylene carbon signal was observed at δ 42.4.

After the successful synthesis and spectroscopic characterization of the compound **1**, we have further synthesized a series of compounds **2-11**, obtained by the coupling with different naturally occurring aromatic acids with 2-amino thiazole and its various derivatives by adopting the above DIC/HOBt coupling protocol (Scheme II, Table I).

The structures of all the above compounds **2-11** were also confirmed on the basis of their spectroscopic data and microanalyses. In the case of ^{1}H NMR spectra of compound **9**, **10** and **11**, the two CH protons were observed at around δ 7.76 and 6.94 with a coupling constant around 10.0 Hz, which confirms the *trans* geometry. In the case of compound **8**, the CH_2 group was observed at δ 4.23 with coupling constant 3.8 Hz. Furthermore, in ^{13}C NMR, compounds **2-11** showed the usual peak as expected. The IR spectrum of the compounds **2-11** also exhibited absorption bands around 3300 and 1670 cm^{-1} which proved the presence of NH and CO group respectively. The ESI-MS spectra of the compounds **2-11** showed their characteristic $[M+H]^+$ peaks.

Biology

The above-synthesized compounds (**1-11**) were evaluated against *M. tuberculosis* virulent strain *M. tuberculosis H37Rv*. The MIC (Minimum inhibitory concentration) values were determined using the agar microdilution method[30-32]. The results are given in Table II.



Scheme II — General scheme for the synthesis of compounds **2-11**

As evident from Table II, among all the tested compounds only three compounds **5**, **8**, and **11** were found active at the minimum inhibitory concentration (MIC) 12.5 μg/mL against *M. tuberculosis*, while the rest of the compounds were inactive at MIC 12.5 μg/mL. The most plausible explanation may be that these compounds are unable to penetrate the cell wall of *M. tuberculosis*.

All the above compounds **1-11**were also screened for their mycobacterial serine-threonine protein kinase G (PknG) inhibitory activity in the presence and the absence of the inhibitors. Among all the above-screened, compounds **1-11** showed moderate inhibition ranging from 9.1-15.6%. AX20017 was used as a standard PknG inhibitor which showed 41% inhibition of enzyme activity at 100 μm[33]. The results are tabulated below (Table III).

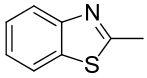
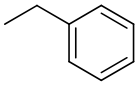
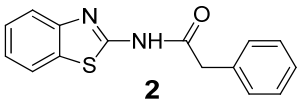
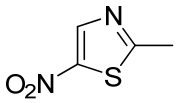
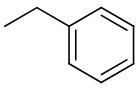
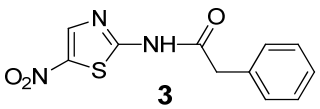
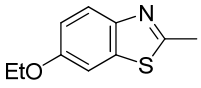
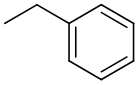
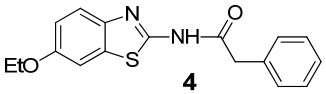
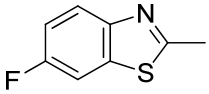
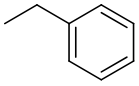
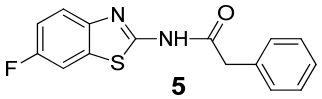
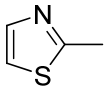
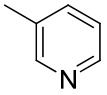
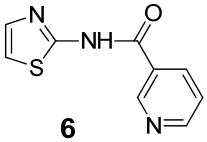
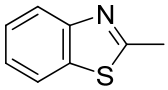
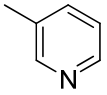
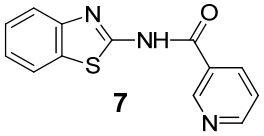
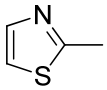
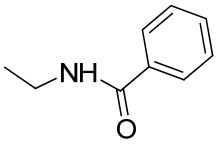
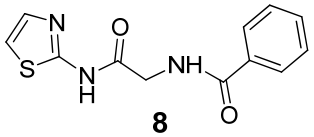
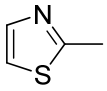
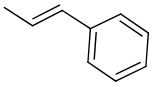
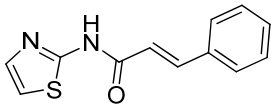
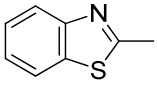
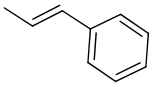
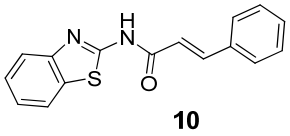
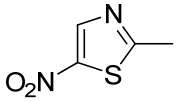
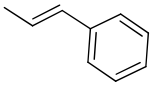
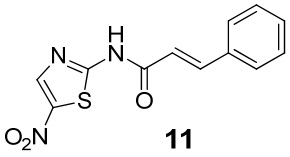
From Table III, it is clear that compounds **1**, **2**, **3**, and **4** inhibit 31, 38, 34, and, 31% relative to standard compound AX20017 respectively. It is clear from the structure that the compound having the benzothiazolyl group at one end and the benzyl group at the other end are the most active compounds. Furthermore, the compounds obtained from phenylacetic acid are found to be more active than the compounds obtained from nicotinic acid, cinnamic acid, and hippuric acid. On the other hand, it is found that compounds obtained from the amines having electron-donating groups are more active than those having the electron-withdrawing groups.

Experimental Section

Materials and Methods

All chemicals were purchased from commercial sources (Sigma Aldrich and Spectrochem) and used as such without purification. Progress of the reactions was monitored by TLC on E. Merck Kieselgel 60 F_{254}, with detection by spraying a 20% aq $KMnO_4$ solution and UV light. Column chromatography was performed over silica gel (60-120 mesh E. Merck). IR spectra were recorded with KBr (Pressed Pellet Technique) with a Perkin−Elmer Spectrum RX-1 (4000-450 cm^{-1}) spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-200 instrument in $CDCl_3$+CCl_4 or $CDCl_3$+DMSO-d_6 solvent. Chemical shift values (δ) are reported in ppm relative to TMS (tetramethylsilane) as internal standard and coupling constants (J) in Hertz. Splitting pattern has been described as follows: s

Table I — The reaction of different naturally occurring aromatic acids and heterocyclic amines

Entry	R^1	R^2	Product	Isolated Yield (%)
1			 **2**	85
2			 **3**	84
3			 **4**	88
4			 **5**	84
5			 **6**	78
6			 **7**	83
7			 **8**	80
8			 **9**	84
9			 **10**	85
10			 **11**	81

(singlet), d (doublet), t (triplet), q (quartet), m (multiplet). ESI mass spectra were recorded using Quattro II (Micromass). Elemental analyses were determined by using a Perkin–Elmer 2400 II elemental analyzer.

Chemistry

General procedure for the synthesis of compounds 1-11

To a solution of aromatic acid (1.05 eq) in dry dichloromethane (25 mL) at 0-5°C, anhydrous 1-hydroxybenzotriazole (1.05 eq.) and

Table II — *In vitro* antitubercular activities of compounds **1-11** against *M. tuberculosis* virulent strain *M. tuberculosis H37Rv.*

S. No.	Compd	MIC (μg/mL) *M. tuberculosis* H37Rv
1	**1**	> 12.5
2	**2**	> 12.5
3	**3**	> 12.5
4	**4**	> 12.5
5	**5**	12.5
6	**6**	> 12.5
7	**7**	> 12.5
8	**8**	12.5
9	**9**	> 12.5
10	**10**	> 12.5
11	**11**	12.5
12	Isoniazid (INH)	0.02
13	Rifampicin	0.20
14	Ethambutol	2.0

MIC = Minimum inhibitory concentration, the lowest concentration of the compound which inhibits the growth of mycobacterium >90%. Isoniazid, rifampicin, and ethambutol used as the control, against *M. tuberculosis H37 Rv.*

Table III — Bio-evaluation of compounds against Mycobacterial PknG

S. No.	Compd	Inhibition at 100μM (%)
1	**1**	12.8
2	**2**	15.6
3	**3**	14.0
4	**4**	12.9
5	**5**	10.7
6	**6**	10.2
7	**7**	10.2
8	**8**	10.7
9	**9**	10.9
10	**10**	9.1
11	**11**	NA
12	AX20017	41%

NA = not active

4-dimethylaminopyridine (1.05 eq.) were added under nitrogen atmosphere. The reaction mixture was allowed to stir for 10 min. A solution of N, N'-diisopropylcarbodiimide (1.05 eq.) diluted with dry dichloromethane (10 mL) was added slowly to the reaction mixture. The reaction mixture was allowed to stir for 10 min at 0-5°C. A solution of aromatic amine in dry dichloromethane (15 mL) was added slowly to the above reaction mixture while maintaining the temperature of the reaction between 0-5°C under nitrogen atmosphere. After the completion of the reaction, the reaction mixture was concentrated under reduced pressure while maintaining the temperature of the water bath not exceeding 40°C. The residue, so obtained, was purified by column chromatography (SiO_2, 60-120 mesh) using appropriate eluent to give the desired compounds **1-11** in good yields.

2-Phenyl-N-(1,3-thiazol-2-yl)acetamide, 1

It was obtained by using 2-aminothiazole (0.25 g, 2.50 mmol), phenylacetic acid (0.36 g, 2.64 mmol), DMAP (0.32 g, 2.62 mmol), HOBt (0.35 g, 2.59 mmol), DIC (0.4 mL, 2.55 mmol) and dichloromethane (50 mL) as described above to give compound **1** as a white solid powder (0.47 g. Yield 86%). R_f 0.5 (50% hexane:ethylacetate). m.p.153-154°C. IR (KBr): 3414, 3309, 2937, 2370, 1675, 1580, 1344, 1275, 1063, 716 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$+CCl_4): δ 7.47 (d, J = 2.34 Hz, 1H, CH, ArH), 7.40-7.30 (m, 5H, CH, ArH), 7.02 (d, J = 2.36 Hz, 1H, CH, ArH), 3.89 (s, 2H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$+CCl_4): δ 169.0 (CO), 159.0 (ArC), 136.9 (ArCH), 134.2 (ArC), 129.1 (2ArCH), 128.4(2ArCH), 126.9 (ArCH), 113.0 (ArCH), 42.4 (CH_2); MS (ESI^+): *m/z* 219 $[M+H]^+$. Anal. Calcd for $C_{11}H_{10}N_2OS$: C, 60.53; H, 4.62; N, 12.83. Found: C, 65.49; H, 4.64; N, 12.81%.

N-(1,3-Benzothiazol-2-yl)-2-phenylacetamide, 2

It was obtained by using 2-aminobenzothiazole (0.25 g, 1.66 mmol), phenylacetic acid (0.24 g, 1.76 mmol), DMAP (0.21 g, 1.71 mmol), HOBt (0.24 g, 1.78 mmol), DIC (0.3 mL, 1.91 mmol) and dichloromethane (50 mL) as described above to give compound **2** as a white solid powder (0.38 g. Yield 85%). R_f 0.5 (50% hexane:ethylacetate). m.p.159-160°C. IR (KBr): 3485, 3201, 2973, 2366, 1695, 1579, 1272, 1054, 716 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 7.86 (d, J = 4.96 Hz, 1H, ArH), 7.77 (d, J = 5.30 Hz, 1H, ArH), 7.47-7.42 (m, 1H, ArH), 7.42-7.28 (m, 4H, ArH), 7.22-7.20 (m, 2H, ArH), 3.85 (s, 2H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$+DMSO-d_6.): δ 168.5 (CO), 156.6 (ArC), 147.3 (ArC), 133.2 (ArC), 130.4 (ArC), 127.9 (2ArCH), 126.9 (2ArCH), 125.4 (ArCH), 124.5 (ArCH), 121.9 (ArCH), 119.8 (ArCH), 119.1 (ArCH), 40.8 (CH_2); MS (ESI^+): *m/z* 269 $(M+H)^+$. Anal. Calcd for $C_{15}H_{12}N_2OS$: C, 67.14; H, 4.51; N, 10.44. Found: C, 67.11; H, 4.54; N, 10.41%.

N-(5-Nitro-1,3-thiazol-2-yl)-2-phenylacetamide, 3

It was obtained by using 2-amino-5-nitrothiazole (0.25 g, 1.72 mmol), phenylacetic acid (0.25 g, 1.83 mmol), DMAP (0.22 g, 1.80 mmol), HOBt

(0.24 g, 1.78 mmol), DIC (0.3 mL, 1.91 mmol) and dichloromethane (50 mL) as described above to give compound **3** as a white solid powder (0.38 g. Yield 84%). R_f 0.5 (40% hexane:ethylacetate). m.p. 195-196°C. IR (KBr): 3429, 3278, 3150, 2925, 2365, 1705, 1654, 1561, 1345, 1145, 770, 722 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 8.31 (s, 1H, ArH), 7.35-7.27 (m, 5H, ArH), 3.85 (s, 2H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$+ DMSO-d_6.): δ 169.7 (CO), 164.2 (ArC), 141.6 (ArC), 140.3 (ArCH), 132.7 (ArC), 128.3 (2ArCH), 127.5 (2ArCH), 126.2 (ArCH), 41.1 (CH_2); MS (ESI^+): *m/z* 264 $(M+H)^+$. Anal. Calcd for $C_{11}H_9N_3O_3S$: C, 50.18; H, 3.45; N, 15.96. Found: C, 50.15; H, 3.48; N, 15.94%.

N-(6-Ethoxy-1,3-benzothiazol-2-yl)-2-phenylacetamide, 4

It was obtained by using 2-amino-6-ethoxybenzothiazole (0.25 g, 1.29 mmol), phenylacetic acid (0.19 g, 1.39 mmol), DMAP (0.17 g, 1.39 mmol), HOBt (0.18 g, 1.33 mmol), DIC (0.3 mL, 1.91 mmol) and dichloromethane (50 mL) as described above to give compound **4** as a white solid powder (0.35 g. Yield 88%). R_f 0.5 (40% hexane:ethylacetate). m.p.130-132°C. IR (KBr): 3421, 3238, 3182, 2935, 2355, 1690, 1602, 1260, 1057, 725 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+CCl_4): δ 7.40 (dd, J_1 = 0.9 Hz, J_2 = 5.8 Hz, 1H, ArH), 7.35-7.28 (m, 6H, ArH), 7.05 (dd, J_1 = 1.7 Hz, J_2 = 5.8 Hz, 1H, ArH) 4.12 (q, J = 4.6 Hz, 2H, OCH_2), 3.85 (s, 2H, CH_2), 1.46 (t, J = 4.6 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$+ DMSO-d_6): δ 169.3 (CO), 155.6 (ArC), 155.2 (ArC), 142.5(ArC), 134.4 (ArC), 132.7 (ArC) , 128.9 (2ArCH), 127.9 (2ArCH), 126.5 (ArCH), 120.7 (ArCH), 114.8 (ArCH), 104.5 (ArCH), 63.4 (OCH_2), 41.9 (CH_2), 14.5 (CH_3); MS (ESI^+): *m/z* 313 $(M+H)^+$. Anal. Calcd for $C_{17}H_{16}N_2O_2S$: C, 65.36; H, 5.16; N, 8.97. Found: C, 65.33; H, 5.19; N, 8.94%.

N-(6-Fluoro-1,3-benzothiazol-2-yl)-2-phenylacetamide, 5

It was obtained by using 2-amino-6-fluorobenzothiazole (0.25 g, 1.49 mmol), phenylacetic acid (0.21 g, 1.54 mmol), DMAP (0.19 g, 1.55 mmol), HOBt (0.21 g, 1.55 mmol), DIC (0.3 mL, 1.91 mmol) and dichloromethane (50 mL) as described above to give compound **5** as a white solid powder (0.36 g. Yield 84%). R_f 0.5 (40% hexane:ethylacetate). m.p.179-180°C. IR (KBr): 3430, 2858, 1615, 1551, 1215, 765 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 7.50-7.46 (m, 1H, ArH), 7.38-7.34 (m, 1H, ArH), 7.32-7.25 (m, 5H, ArH), 7.14-7.08 (m, 1H, ArH), 3.83 (s, 2H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$+ DMSO-d_6.): δ 169.7 (CO), 157.6 (ArC), 145.0 (ArC), 134.3 (ArC), 132.7 (ArC), 132.5 (ArC), 129.0 (2ArCH), 128.1 (2ArCH), 126.6 (ArCH), 121.3(ArCH), 121.1(ArCH), 113.9 (ArCH), 107.7 (ArCH), 41.8 (CH_2); MS (ESI^+): *m/z* 287 $(M+H)^+$. Anal. Calcd for $C_{15}H_{11}FN_2OS$: C, 62.92; H, 3.87; N, 9.78. Found: C, 62.89; H, 3.90; N, 9.74%.

N-(1,3-Thiazol-2-yl)pyridine-3-carboxamide, 6

It was obtained by using 2-aminothiazole (0.25 g, 2.50 mmol), nicotinic acid (0.32 g, 2.60 mmol), DMAP (0.32 g, 2.62 mmol), HOBt (0.35 g, 2.59 mmol), DIC (0.4 mL, 2.55 mmol) and dichloromethane (50 mL) as described above to give compound **6** as a white solid powder (0.40 g. Yield 78%). R_f 0.5 (2:98 MeOH:$CHCl_3$). m.p.> 200°C. IR (KBr): 3025, 2364, 1650, 1561, 1215, 765 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 9.24 (s, 1H, ArH), 8.67 (d, J = 4.7 Hz, 1H, ArH), 8.35 (d, J = 8.0 Hz, 1H, ArH), 7.49-7.34 (m, 2H, ArH), 6.91 (d, J = 3.5 Hz, 1H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$+DMSO-d_6.): δ 163.5 (CO), 158.6 (ArC), 152.4 (ArCH), 149.1 (ArCH), 136.9 (ArCH), 135.5 (ArCH), 127.9 (ArC), 123.1 (ArCH), 113.4 (ArCH); MS (ESI^+): *m/z* 206 $(M+H)^+$. Anal. Calcd for $C_9H_7N_3OS$: C, 52.67; H, 3.44; N, 20.47. Found: C, 52.63; H, 3.48; N, 20.43%.

N-(1,3-Benzothiazol-2-yl)pyridine-3-carboxamide, 7

It was obtained by using 2-aminobenzothiazole (0.25 g, 1.66 mmol), nicotinic acid (0.21 g, 1.71 mmol), DMAP (0.21 g, 1.71 mmol), HOBt (0.24 g, 1.78 mmol), DIC (0.3 mL, 1.91 mmol) and dichloromethane (50 mL) as described above to give compound **7** as a white solid powder (0.35 g. Yield 83%). R_f 0.5 (50:50 Ethylacetate:Hexane). m.p.> 200°C. IR (KBr): IR (KBr): 3415, 3364, 3321, 2666, 2360, 1675, 1600, 1280, 741, 640 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 9.34 (d, J = 0.9 Hz, 1H, ArH), 8.77 (d, J = 2.2 Hz, 1H, ArH), 8.52-8.49 (m, 1H, ArH), 7.87 (d, J = 5.0 Hz, 1H, ArH), 7.77 (d, J = 5.7 Hz, 1H, ArH), 7.50-7.39 (m, 2H, ArH), 7.33-7.28 (m, 1H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$+ DMSO-d_6.): δ 163.3 (CO), 157.4 (ArC), 151.2 (ArCH), 147.7 (ArCH), 146.1 (ArC), 134.2 (ArCH), 129.6 (ArC), 126.3 (ArC), 124.4 (ArCH), 121.9 (ArCH), 121.7 (ArCH), 119.9 (ArCH), 118.4 (ArCH); MS (ESI^+): 256 $(M+H)^+$. Anal. Calcd for $C_{13}H_9N_3OS$: C, 61.16; H, 3.55; N, 16.46. Found: C, 61.12; H, 3.58; N, 16.42%.

N-{2-Oxo-2-[(1,3-thiazol-2-yl)amino] ethyl}be nzamide, 8

It was obtained by using 2-aminothiazole (0.25 g, 2.50 mmol), hippuric acid (0.46 g, 2.56 mmol), DMAP (0.32 g, 2.62 mmol), HOBt (0.35 g, 2.59 mmol), DIC (0.4 mL, 2.55 mmol) and dichloromethane (50 mL) as described above to give compound **8** as a white solid powder (0.52 g. Yield 80%). R_f 0.5 (40% hexane:ethylacetate). m.p.> 200°C. IR (KBr): 3560, 3345, 2925, 2365, 1780, 1650, 1530, 1290, 1167, 955, 705 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 8.65 (bs, 1H, NH), 7.92 (d, J = 4.6 Hz, 2H, ArH), 7.50-7.38 (m, 4H, ArH), 6.98-6.97 (m, 1H, ArH), 4.23 (d, J = 3.8 Hz, 2H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$+ DMSO-d_6.): δ 173.0 (CO), 172.1 (CO), 163.1 (ArC), 142.5 (ArC), 138.9 (ArCH), 136.4 (ArCH), 133.2 (2ArCH), 132.5 (2ArCH), 118.0 (ArCH), 47.8 (ArCH); MS (ESI^+): 262 $(M+H)^+$. Anal. Calcd for $C_{12}H_{11}N_3O_2S$: C, 55.16; H, 4.24; N, 16.08. Found: C, 55.14; H, 4.28; N, 16.02%.

(2*E*)-3-Phenyl-N-(1,3-thiazol-2-yl)prop-2-enamide, 9

It was obtained by using 2-aminothiazole (0.25 g, 2.50 mmol), cinnamic acid (0.39 g, 2.63 mmol), DMAP (0.32 g, 2.62 mmol), HOBt (0.35 g, 2.59 mmol), DIC (0.4 mL, 2.55 mmol) and dichloromethane (50 mL) as described above to give compound **9** as a white solid powder (0.48 g. Yield 84%). R_f 0.5 (40% hexane:ethylacetate). m.p.> 200°C. IR (KBr): 3531, 2860, 1625, 1541, 1325, 1215, 760, 670 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 7.76 (d, J = 10.0 Hz, 1H, CH), 7.59-7.57 (m, 2H, ArH), 7.47-7.40 (m, 4H, ArH), 7.04 (d, J = 2.0 Hz, 1H, ArH), 6.94 (d, J = 10.0 Hz, 1H, CH); MS (ESI^+): 231 $(M+H)^+$. Anal. Calcd for $C_{12}H_{10}N_2OS$: C, 62.59; H, 4.38; N, 12.16. Found: C, 62.55; H, 4.41; N, 12.13%.

(2*E*)-N-(1,3-Benzothiazol-2-yl)-3-phenylprop-2-enamide, 10

It was obtained by using 2-aminobenzothiazole (0.25 g, 1.66 mmol), cinnamic acid (0.26 g, 1.75 mmol), DMAP (0.21 g, 1.71 mmol), HOBt (0.24 g, 1.78 mmol), DIC (0.3 mL, 1.91 mmol) and dichloromethane (50 mL) as described above to give compound **10** as a white solid powder (0.40 g. Yield 85%). R_f 0.5 (50% hexane:ethylacetate). m.p.> 200°C. IR (KBr): 3426, 3171, 2950, 2365, 1687, 1595, 1550, 1155, 745 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 7.73 (d, J = 10.0 Hz, 1H, CH), 7.70-7.57 (m, 1H, ArH), 7.47-7.43 (m, 2H, ArH), 7.32-7.24 (m, 4H, ArH), 7.17-6.73 (m, 2H, ArH), 6.78 (d, J = 10.0 Hz, 1H, CH); MS (ESI): 281 $(M+H)^+$. Anal. Calcd for $C_{16}H_{12}N_2OS$: C, 68.55; H, 4.31; N, 9.99. Found: C, 68.52; H, 4.35; N, 9.95%.

(2*E*)-N-(5-Nitro-1,3-thiazol-2-yl)-3-phenylprop-2-enamide, 11

It was obtained by using 2-amino-5-nitrothiazole (0.25 g, 1.72 mmol), cinnamic acid (0.27 g, 1.82 mmol), DMAP (0.22 g, 1.80 mmol), HOBt (0.24 g, 1.78 mmol), DIC (0.3 mL, 1.91 mmol) and dichloromethane (50 mL) as described above to give compound **11** as a white solid powder (0.38 g. Yield 81%). R_f 0.5 (50% hexane:ethylacetate). m.p.> 200°C. IR (KBr): 3447, 3145, 2927, 2705, 1685, 1625, 1555, 1337, 1155, 760, 480 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 8.35 (d, J = 1.1 Hz, 1H, ArH), 7.87 (d, J = 10.2 Hz, 1H, CH), 7.60-7.42 (m, 5H, ArH), 6.90 (d, J = 10.5 Hz, 1H, CH); MS (ESI^+): 276 $(M+H)^+$. Anal. Calcd for $C_{12}H_9N_3O_3S$: C, 52.36; H, 3.30; N, 15.26. Found: C, 52.33; H, 3.23; N, 15.22%.

Bio-evaluation methods

Determination of antitubercular activity against *M. tuberculosis* H37Rv strain (Agar microdilution method)[30-32]

Determination of MIC and drug susceptibility of the test compounds/drugs against *M. tuberculosis* H37Rv were determined by agar microdilution methodology. In this method, two-fold dilutions of every test compound were added into 7H10 agar and *M. tuberculosis* H37Rv was used as the test organism. The MIC of each tested compound was determined by incorporating lower concentrations of the tested compound in the middle brook 7H10 agar medium supplemented with OADC. A culture of *M. tuberculosis* H37Rv, which was growing on L-J medium was harvested in 0.85% saline with 0.05% Tween-80. A suspension of 1 µg/mL concentration of extracts/compounds was prepared in dimethyl sulphoxide. This suspension was added to (in tubes) 7H10 middle Brook's medium (containing 1.7 mL medium and 0.2 mL OADC supplement) at different concentrations of the test compounds keeping the volume constant (0.1 mL). The medium was allowed to cool gradually keeping the tubes in slanting position. After this, these tubes were incubated at 37°C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5×10^4 bacilli per test tube). These tubes were then allowed to incubate at 37°C.

The growth of bacilli was observed after 30 days of incubation. Tubes having the test compounds were compared with control tubes where medium alone was incubated with H37Rv. The concentration at which complete inhibition of colonies took place was taken as an active concentration of test compounds.

Purification of *Mycobacterium tuberculosis* PKnG[33]

The synthesized compounds were tested or screened against the mycobacterial serine-threonine protein kinase G (PknG). The recombinantly purified enzyme was used for the present study. In short, the *Mycobacterium tuberculosis* (MTB) genomic DNA was used as a template for the amplification of the *pknG* gene by PCR. The gene was cloned in pTriEx4 vector using the primers containing the desired restriction enzyme sites. For expression in *pknG*, *E coli* with *Hind*III flanking sites was subcloned in pTriEx4 vector *E. coli* BL21 (DE3). Cells were transformed with pTriEX4-*pknG* and transformants were allowed to grow in LB medium containing ampicillin (100 µg/mL) at temperature 37°C, till OD at 600 nm reached 0.6. IPTG was then added to a final concentration of 0.8 mM and cultures were further expedited for an additional 4 h at 37°C with shaking. Cells were harvested by centrifugation at 5000 × g for 15 min and resuspended in binding buffer [sodium phosphate 20.0 mM (*p*H 7.4), NaCl 50.0 mM, imidazole 5.0 mM, PMSF 1.0 mM] and sonicated on ice for 2 min. After sonication, TritonX-100 was added in cell lysate at an eventual concentration of 1% before centrifugation at 30000 × g for 30 min at temperature 4°C. The supernatant was loaded onto Ni_2+-NTA column, washed with 60 mM imidazole and 6-His-PknG was eluted with 200 mM imidazole. Affinity purified 6-His-PknG was further purified by size exclusion chromatography using a sephacryl 200 column and AKTA Prime protein purification system (GE healthcare).

Screening of compounds against Kinase activity

All the synthesized compounds were dissolved completely in DMSO. Primary efficacy was determined by using 100 µM concentration of each compound, which was screened using purified PknG as an enzyme and myelin basic protein as a substrate. The activity and the inhibition were determined with the help of luciferase activity mediated by ATP, by ADP–Glo (Promega, USA). Briefly, ADP-Glo™ Kinase Assay is a luminescent kinase assay, which measures ADP formed from a kinase reaction. ADP is converted into ATP, which is then converted into light by Ultra-Glo™ Luciferase.

Conclusions

In the present study, we have synthesized and characterized a series of thiazole unit based compounds having an amide group at its 2-position by using different aromatic acids. A few of these compounds have good PknG inhibitory activity. Nearly all the compounds showed very moderate antitubercular activity against the *H37Rv* virulent strain of *M. tuberculosis*. These findings led us to the identification of a new prototype of compounds for further optimization and development to get novel compounds having promising PknG inhibitory activity.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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References

1 Nguyen L & Pieters J, *Trends Cell Biol*, 15 (2005) 269. https://doi.org/10.1016/j.tcb.2005.03.009

2 Russell D G, *Nat Rev Mol Cell Biol*, 2 (2001) 569. https://doi.org/10.1038/35085034

3 Walburger A, Koul A, Ferrari G, Nguyen L, Prescianotto-Baschong C, Huygen K, Klebl B, Thompson C, Bacher G & Pieters J, *Science*, 304 (2004) 1800. https://doi.org/10.1126/science.1099384

4 Nguyen L, Walburger A, Houben E, Koul A, Muller S, Morbitzer M, Klebl B, Ferrari G & Pieters J, *J Bacteriol*, 187 (2005) 5852. https://doi.org/10.1128/JB.187.16.5852-5856.2005

5 O'Hare H M, Duran R, Cervenansky C, Bellinzoni M, Wehenkel A M, Pritsch O, Obal G, Baumgartner J, Vialaret J, Johnsson K & Alzari P M, *Mol Biol*, 70 (2008) 1408. https://doi.org/10.1111/j.1365-2958.2008.06489.x

6 Cole S T, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon S V, Eiglmeier K, Gas S, Barry C.E.III, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail M A, Rajandream M A, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston J E, Taylor K, Whitehead S & Barrell B G, *Nature*, 393 (1998) 537. https://doi.org/10.1038/31159

7 Scherr N, Honnappa S, Kunz G, Mueller P, Jayachandran R & Winkler F, *Proc Natl Acad Sci* (*USA*) 104 (2007) 12151. https://doi.org/10.1073/pnas.0702842104

8 Wolff K A, Nguyen H T, Cartabuke R H, Singh A, Ogwang S & Nguyen L, *Antimicrobial Agents Chemother*, 53 (2009) 3515. https://doi.org/10.1128/AAC.00012-09

9 Kurosu M & Begari E, *Drug Dev Res* 71 (2010) 168. https://doi.org/10.1002/ddr.20362

10 Anand N, Singh P, Sharma A, Tiwari S, Singh V, Singh D K, Srivastava K K, Singh B N & Tripathi R P, *Bioorg Med Chem*, 20 (2012) 5150. https://doi.org/ 10.1016/j.bmc. 2012.07.009

11 Kanehiro Y, Tomioka H, Pieters J, Tatano Y, Kin H, Lizasa H & Yoshiyama H, *Front Microbiol*, 9 (2018) 1517. https://doi.org/10.3389/fmicb.2018.01517

12 Pattan S R, Dighe N S, Nirmal S A, Merekar A N, Laware R B, Shinde H V & Musmade D S, *Asian J Research Chem*, 2 (2009) 196.

13 Sharma R N, Xavier F P, Vasu K K, Chaturvedi S C & Pancholi S S, *J Ezym Inhib Med Chem*, 24 (2009) 890. https://doi.org/10.1080/14756360802519558

14 Argyropoulou I, Geronikaki A, Vicini P & Zani F, *Arkivoc*, VI (2009) 89. https://doi.org/10.3998/ark.5550190.0010.611

15 Trautman H D & Longe L M, *J Am Chem Soc*, 70 (1948) 3436. https://doi.org/10.1021/ja01190a064

16 Surrey A R, *J Am Chem Soc*, 71 (1949) 3354. https://doi.org/10.1021/ja01178a023

17 Bhattacharya P, Leonard J T & Roy K, *Bioorg Med Chem*, 13 (2005) 1159. https://doi.org/10.1016/j.bmc.2004.11.022

18 Alemagna A, Bacchetti T & Beltrame P, *Tetrahedron*, 24 (1968) 3209. https://doi.org/10.1016/S0040-4020(01)92619-5

19 Hargrave K D, Hess F K & Oliver J T, *J Med Chem*, 26 (1983) 1158. https://doi.org/10.1021/jm00362a014

20 Patt W C, Hamilton H W, Taylor M D, Ryan M J, Taylor D G, Connolly C J C, Doharty A M, Klutchko S R, Sircar I, Steinbaugh B A, Bately B L, Painchand C A, Rapundalo S T, Michniewicz B M & Olzon S C J, *J Med Chem*, 35 (1992) 2562. https://doi.org/10.1021/jm00092a006

21 Jaen J C, Wise L D, Caprathe B W, Tecle H, Bergmeier S, Humblet C C, Heffner T G, Meltzner L T & Pugsley T A, *J Med Chem*, 33 (1990) 311. https://doi.org/10.1021/jm00163a051

22 Ergenc N, Capan G, Gunay N S, Oskirimli S, Gungor M, Ozbey S & Kendi E, *Arch Pharm*, 332 (1999) 343. https://doi.org/10.1002/(SICI)15214184(199910)332:10<343 ::AID-ARDP343>3.0.CO;2-0

23 Taori K, Paul V J & Luesch H, *J Am Chem Soc*, 130 (2008) 1806. https://doi.org/10.1021/ja7110064

24 Carter J S, Kramer S, Talley J J, Penning T, Collins P, Graneto M J, Seibert K, Koboldt C, Masferrer J & Zweifel B, *Bioorg Med Chem Lett*, 9 (1999) 1171. https://doi.org/10.1016/S0960-894X(99)00157-2

25 Panico A M, Geronikaki A, Mgonzo R, Cardile V, Gentile B & Doytchinova I, *Bioorg Med Chem*, 11 (2003) 2983. https://doi.org/10.1016/S0968-0896(03)00149-4

26 Megraud F, Occhialini A & Rossignol J F, *Antimicrob Agents Chemother*, 42 (1998) 2836. https://doi.org/10.1128/AAC.42.11.2836

27 Adagu S, Nolder D, Warhurst D C & Rossignol J F, *J Antimicrob Chemother*, 49 (2002) 103. https://doi.org/10.1093/jac/49.1.103

28 Cho Y, Ioerger T R & Sacchettini J C, *J Med Chem*, 51 (2008) 5984. https://doi.org/10.1021/jm800328v

29 Khalaf A I, Waigh R D, Drummond A J, Pringle B, McGroarty I, Skellem G G & Suckling C J, *J Med Chem*, 47 (2004) 2133. https://doi.org/10.1021/jm031089x

30 Saito H, Tomioka H, Sato K, Emori M, Yamane T & Yamashita K, *Antimicrob Agents Chemother*, 35 (1991) 542. https://doi.org/10.1128/AAC.35.3.542

31 Mitchison D A, Allen B W, Carol L, Dickinson J M & Aber V R, *J Med Microbiol*, 5 (1972) 165. https://doi.org/10.1099/00222615-5-2-165

32 Jenson K A, *Bull Int Union Tuberc*, 25 (1955) 89. https://doi.org/10.1111/j.1746-1561.1955.tb06806.x

33 ChaurasiyaS K & Srivastava K K, *Bioorg Med Chem Microbiol*, 9 (2009) 271. https://doi.org/10.1186/1471-2180-9-271