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ARTICLE

Synthesis and evaluation of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones as potential antitubercular agents

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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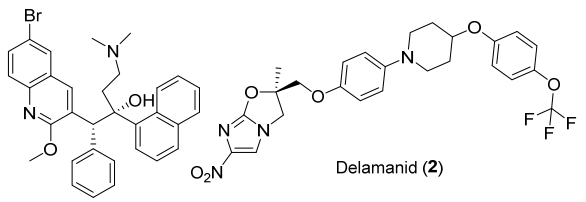
A number of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones were designed, synthesized and screened against *Mycobacteria* as a part of our program to develop new antitubercular agents. It was observed that some of the compounds have significant antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) and *Mycobacterium bovis* BCG (ATCC 35743). The active compounds were studied for cytotoxicity against four cell lines and were found to be non-cytotoxic. The results showed that the compounds **13b** and **29e** were found to exhibit very good antimycobacterial activity (MIC in the range of 6-8 μM) and the thienopyrimidinones as a class have potential to be developed as antitubercular agents.

1. Introduction

Tuberculosis (TB) is a major global health problem and as per the global tuberculosis report 2014[1] which includes data compiled from 202 countries and territories, in 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease. Occurrence of multidrug resistant tuberculosis (MDR-TB) is complicating the situation further as globally, 3.5% of new and



Bedaquiline (**1**) Delamanid (**2**)

Figure 1. Structures of bedaquiline and delamanid

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Electronic Supplementary Information (ESI) available: Details of synthesis, spectral data, activity data. See DOI: 10.1039/x0xx00000x

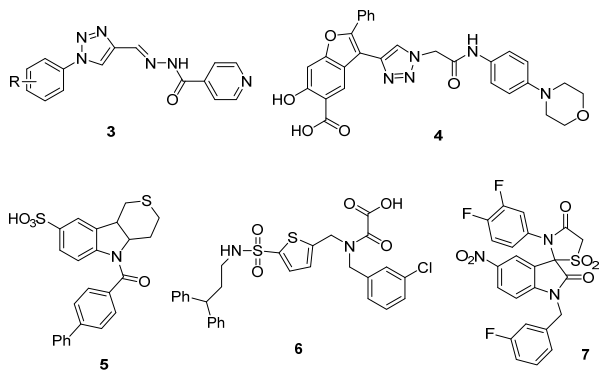


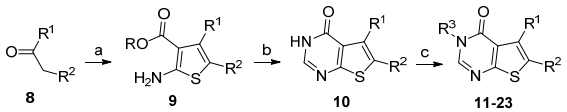
Figure 2. Structures of compounds **3-7** exhibiting antimycobacterial activity

20.5% of previously treated TB cases were estimated to have had MDR-TB in 2013. These facts indicate that development of new TB drugs is of great importance. In addition, the emergence of extensively drug-resistant TB (XDR-TB) also makes the efforts in this direction necessary as on average, an estimated 9.0% of patients with MDR-TB had extensively drug resistant TB (XDR-TB). However, the number of new drugs approved for TB treatment is negligible and on December 28, 2012 the U.S. Food and Drug Administration (FDA) approved bedaquiline (**1**) (Figure 1) as part of combination therapy in adults to treat pulmonary multi-drug resistant tuberculosis, the first new treatment in 40 years. Subsequently, delamanid (**2**) received conditional approval by European Medicines Agency (EMA) for the treatment of MDR-TB in November 2013. The search for new scaffolds is necessary to overcome the problem of limited choice of current TB drugs. The research in this direction has resulted in a few hits e.g. **3**[2], **4**[3], **5**[4,5], **6**[6] and **7**[7] (Figure 2).

We wished to explore the potential of thienopyrimidinones as antitubercular agents. Substituted thienopyrimidinones exhibit various biological activities as well as constitute the part of the molecular skeleton of a number of biologically active compounds but their potential as antitubercular agents is rarely explored[8]. The substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **10** can be easily prepared from substituted 2-aminothiophene-3-carboxylates **9** which in turn are prepared in one step by Gewald synthesis[9,10] from easily available aldehydes or ketones **8** as starting materials (Scheme 1). The thieno[2,3-*d*]pyrimidin-4(3*H*)-ones of type **10** can be functionalized at various positions and thus provide an opportunity to have a number of compounds available for biological activity screening. A focused library of thienopyrimidinones was designed, synthesized and screened against mycobacteria[11]. Encouragingly, some of the molecules exhibited significant antimycobacterial activity therefore the work was continued further and the results are reported herein.

2. Results and discussion

2.1 Chemistry



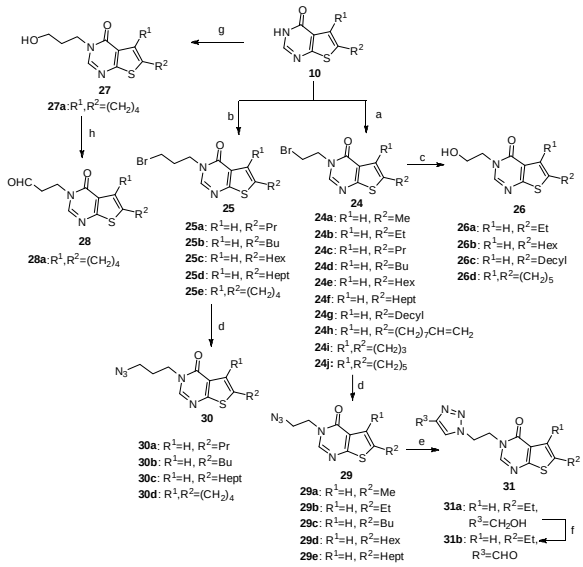
11a: R^1=H, R^2=Me, R^3=Pr
11b: R^1=H, R^2=Me, R^3=i-Pr
11c: R^1=H, R^2=Me, R^3=i-Bu
11d: R^1=H, R^2=Et, R^3=Et
11e: R^1=H, R^2=Et, R^3=Pr
11f: R^1=H, R^2=Et, R^3=i-Pr
11g: R^1=H, R^2=Et, R^3=Pent
11h: R^1=H, R^2=Et, R^3=Oct
11i: R^1=H, R^2=Pr, R^3=Pr
11j: R^1=H, R^2=Pr, R^3=i-Pr
11k: R^1=H, R^2=Pr, R^3=Oct
11l: R^1=H, R^2=Pent, R^3=Me
11m: R^1=H, R^2=Pent, R^3=Pr
11n: R^1, R^2=-$(CH_2)_4$, R^3=Me
12a: R^1=H, R^2=Et, R^3=CH_2Ph
12b: R^1=H, R^2=Pr, R^3=CH_2Ph
12c: R^1=H, R^2=Bu, R^3=CH_2Ph
12d: R^1=H, R^2=Pent, R^3=CH_2Ph
12e: R^1=H, R^2=Hex, R^3=CH_2Ph
13a: R^1=H, R^2=Pent, R^3=CH_2CN
13b: R^1=H, R^2=Hept, R^3=CH_2CN

14a: R^1=H, R^2=Pent, R^3=CH_2-cyclopropyl
14b: R^1=H, R^2=Hex, R^3=CH_2-cyclopropyl
14c: R^1=H, R^2=Hept, R^3=CH_2-cyclopropyl
15a: R^1=H, R^2=Pr, R^3=CH_2-(4-bromomethyl)phenyl
16a: R^1=H, R^2=Pent, R^3=2-(1,3-dioxolan-2-yl)ethyl
16b: R^1,R^2=-$(CH_2)_4$, R^3=2-(1,3-dioxolan-2-yl)ethyl
16c: R^1,R^2=-$(CH_2)_4$, R^3=2-(1,3-dioxan-2-yl)ethyl
17a: R^1=H, R^2=Me, R^3=CH_2-CO-2,4-difluorophenyl
17b: R^1=H, R^2=Pr, R^3=CH_2-CO-2,4-difluorophenyl
17c: R^1=H, R^2=Pent, R^3=CH_2-CO-2,4-difluorophenyl
18a: R^1=H, R^2=Bu, R^3=allyl
19a: R^1=H, R^2=Pent, R^3=CH_2COOEt
19b: R^1=H, R^2=Et, R^3=CH_2COOEt d
20b: R^1=H, R^2=Et, R^3=CH_2COOH
19c: R^1=H, R^2=Me, R^3=CH_2COOEt d
20c: R^1=H, R^2=Me, R^3=CH_2COOH
21a: R^1=H, R^2=Bu, R^3=CH_2-$CH(OEt)_2$ e
22a: R^1=H, R^2=Bu, R^3=CH_2-CHO
21b: R^1=H, R^2=Me, R^3=CH_2-$CH(OEt)_2$
23a: R^1=H, R^2=Pr, R^3=CH_2-CO-thiophen-2-yl
23b: R^1=H, R^2=Hex, R^3=CH_2-CO-thiophen-2-yl
23c: R^1,R^2=-$(CH_2)_4$, R^3=CH_2-CO-thiophen-2-yl

Scheme 1. Reagents and conditions: a. Gewald synthesis[9,10]; b. Ref[12,13]; c. R^3X, K_2CO_3, DMF or acetonitrile, RT; d.NaOH, EtOH, H_2O, RT; e. Conc HCl, CH_3CN, RT

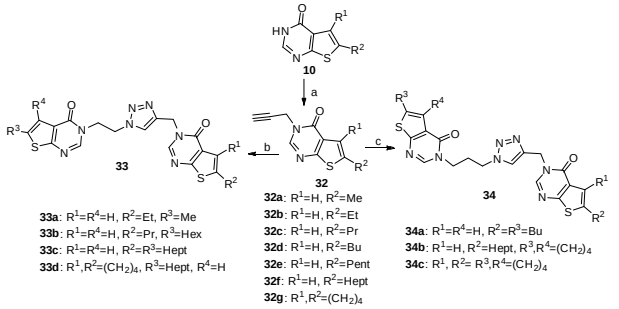
The substituted 2-aminothiophene-3-carboxylates and thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **10** were prepared as reported in our earlier work[12,13,14]. A number of variously substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones with general structures **11** to **23** were obtained from thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **10** by reaction with the corresponding halides in DMF or acetonitrile in the presence of potassium carbonate, and further functional group transformations, as shown in Scheme 1. The structures of these compounds were confirmed by spectral methods[14]. Further functional group conversions provided the compounds with various other substituents.

The utility of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **10** was further explored to obtain various bromides, azides, triazoles, alcohols and aldehydes as exemplified in the Scheme 2. The scheme also shows the flexibility and potential to get a large number of compounds for the biological activity study.

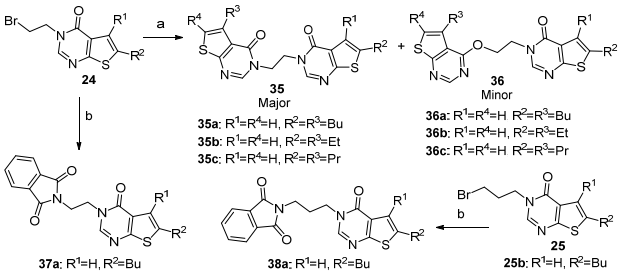


Scheme 2. Reagents and conditions: a. $BrCH_2CH_2Br$, K_2CO_3, dry DMF, RT; b. $BrCH_2CH_2CH_2Br$, K_2CO_3, dry DMF, RT; c. K_2CO_3, DMF, RT; d. NaN_3, DMF, 80^{0}C; e. Propargyl alcohol, t-BuOH, water, $CuSO_4.5H_2O$, sodium ascorbate, RT; f. IBX, DMSO, RT, 3 h; g.$BrCH_2CH_2OH$, K_2CO_3, dry DMF, RT, 8 h; h. IBX, EtOAc, reflux, 6h.

The efforts were continued further (Scheme 3) wherein thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **10** were reacted with propargyl bromide to obtain the acetylenic compounds **32** which were subjected to Click reaction with azides **29** or **30** to get the triazoles **33** or **34**.



Scheme 3. Reagents and conditions: a. Propargyl bromide, K_2CO_3, DMF, RT; b. Thienopyrimidinone **29**, t-BuOH, water, $CuSO_4.5H_2O$, sodium ascorbate, RT; b. Thienopyrimidinone **30**, t-BuOH, water, $CuSO_4.5H_2O$, sodium ascorbate, RT.



Scheme 4. Reagents and conditions: a. Required thienopyrimidinone **10**, K_2CO_3, DMF, RT; b. Potassium phthalimide, DMF, KI, 130 ^{0}C, 10 h.

Efforts to prepare the dimeric compounds **35** by reaction of the bromides **24** with thienopyrimidinones **10** or dibromoethane with excess thienopyrimidinones **10** afforded the desired compounds **35** as major products and the compounds **36** as minor products (Scheme 4). The reactions of bromides **24** or **25** with potassium phthalimide afforded corresponding compounds **37** or **38**.

2.2 Biological evaluation

2.2.1 Antimycobacterial activity

All the synthesized compounds were screened for their *in vitro* activity against *M. tuberculosis* H37Ra (MTB) (ATCC 25177) and *M. bovis* BCG (BCG) (ATCC 35743) using two fold dilution technique, in order to determine the actual minimum inhibitory concentration (MIC). Activity against MTB was determined through the XTT reduction menadione assay (XRMA) reading absorbance at 470 nm as per the protocol described by Singh *et al.*[15] Briefly, compound solution (2.5 µl) was added in a total volume of 250 µl of M. pheli medium consisting of the MTB, BCG; sealed with plate sealers and allowed to incubate for 8 days (active stage) and 12 days (dormant stage) at 37 ^{0}C. The XRMA was then carried out to estimate viable cells present in different wells of the assay plate. To all wells, 200 µM XTT was added and incubated at 37 ^{0}C for another 20 min. It was followed by addition of 60 µM of Menadione and incubated at 37 ^{0}C for 40 min. The optical density was measured using a microplate reader (Spectramaxplus 384 plate reader, Molecular Devices Inc.) at 470 nm filter against a blank prepared from well free of cells. Absorbance obtained from cells treated with 1% DMSO alone was considered as 100% cell growth. The nitrate reductase (NR) assay was performed to estimate inhibition of *M. bovis* BCG by compounds[16]. Briefly, in NR assay, 80 µl of culture from incubated 96 wells plate was taken into another 96 wells plate, then 80 µl of 1% sulfanilic acid in 20% of conc. HCl was added, incubated for 10 min at room temperature and then 80 µl of 0.1 % N-(1-Naphthyl)ethylenediamine dihydrochloride solution in distilled water was added. Finally, absorbance for the NR assay was measured at 540 nm.

In vitro activity against MTB and *M. bovis* BCG at active (8 days) and dormant (12 days) stages was performed using the XRMA and NR assay, respectively, as described above. Percentage inhibition was calculated using the following formula:

% inhibition = [(control–CMP) / (control–blank)] x 100

where 'control' is the activity of mycobacteria without compounds, 'CMP' is the activity of mycobacteria in the presence of compounds and 'blank' is the activity of the culture medium without mycobacteria.

2.2.2 Cytotoxicity:

To check the selectivity, selected thienopyrimidinones were assayed for their cytotoxic effects in four different cell lines THP-1, MCF-7, A549 and HCT 116 using MTT assay[17,18,19] (Table 2). The cell lines were maintained under standard cell culture conditions under 5% CO_2 at 37°C in 95% air humidified environment. Each concentration was tested in duplicates in a single experiment. GI_{50} values were calculated using OriginPro Software[17,18,20].

2.2.3 Selectivity Index:

The selectivity index (SI) was calculated by dividing the 50% growth inhibition concentration (GI50) for cell lines (THP-1, A549, MCF-7 and HCT 116) by the MIC for *in vitro* activity against active/dormant MTB and BCG[21].

2.2.4 Results of antimycobacterial activity

All the newly synthesized compounds were screened for their *in vitro* activity against Mycobateria by using *M. tuberculosis* H37Ra (MTB) and *M. bovis* BCG (BCG) species and the detailed results are given in supplementary data. In the primary screening, 12 compounds (**11g**, **11m**, **13b**, **14a**, **14b**, **14c**, **24g**, **24h**, **25d**, **26c**, **29d** and **29e**) were found to be active against both the species (Table 1) which were selected for further dose response screening[14].

Compounds **13b** and **29e** showed very promising activity against active MTB, with MIC_{90} 2.51 µg/mL (8.68 µM) and 2.07 µg/mL (6.5 µM) respectively. Compounds **24h**, **26c** and **29d** showed MIC_{90} values in the range of 3 to 8 µg/mL while remaining compounds showed MIC_{90} values in the range of 10-50 µg/mL.

Similarly, activity was observed against *M. bovis* BCG also. Compounds **24g**, **29e**, **13b**, **14b**, **14c** and **24h** showed MIC < 3 µg/mL while compounds **11m**, **11g**, **29d** and **25d** showed MIC < 10 µg/mL.

Table no 1. Antimycobacterial activity data for various thienopyrimidinones

Comp no	*M. tuberculosis* H37 Ra (Active Stage)	*M. tuberculosis* H37 Ra (Dormant Stage)	*M. bovis* BCG (Active Stage)	*M. bovis* BCG (Dormant Stage)
	MIC_{90} (µg/mL)	MIC_{90} (µg/mL)	MIC_{90} (µg/mL)	MIC_{90} (µg/mL)
11g	44.13	43.76	10.09	20.76
11m	42.89	36.32	8.51	9.82
13b	**2.51**	**5.20**	**1.89**	**3.02**
14a	38.32	>50	4.95	8.07
14b	45.86	42.70	1.37	2.34
14c	49.31	49.13	1.52	2.01
24g	24.58	35.21	2.29	3.35
24h	3.50	6.31	2.42	1.40
25d	17.51	18.49	6.30	9.37
26c	6.70	15.02	21.43	23.42
29d	8.42	11.54	4.08	4.51
29e	**2.07**	**8.33**	**1.30**	**2.26**
RIF	0.51	0.75	0.45	0.81

RIF indicates Rifampicin, MIC_{90} indicates minimum inhibitory concentration for 90% (or greater) inhibition (µg/mL).

2.2.5 Results of Cytotoxicity and Selectivity Index:

All the synthesized compounds were further evaluated against four human cancer cell lines (MCF-7, A549, THP-1 and HCT116) to check the toxicity of these compounds (Table no 2)[14]. The GI_{50} (> 100 µg/mL) values of the compounds **13b** and **29e** indicate that the compounds are potent and specific inhibitors against MTB. The compounds **11m**, **24g**, **24h**, **25d** and **29d** were found to be most active antiproliferative compounds with IC_{50} in the range of 14.70 -

45.44 µg/mL against MCF7, A549 and HCT 116 cell lines. The compound **29d** showed highest cytotoxicity (GI_{50} 14.70 µg/mL) against A549. GI_{90} studies also indicated that the compound **29d** had highest cytotoxicity (GI_{90} 74.66 µg/mL) against A549[14].

The selectivity of selected thienopyrimidinones towards human cell lines against MTB is described in terms of the selectivity index (Table 3). The selectivity index reflects the concentration of the compound at which it is active against *mycobacteria* but is not toxic towards host cells. A higher selectivity index indicates that the compound can be used as a therapeutic agent. The compounds **13b (SI: >40)** and **29e (SI: >45)** showed very high SI index, which are actually good inhibitors of *M. tuberculosis* and *M. bovis* BCG. Although the selectivity index of rifampicin is very high, it is important to consider the significance of this study with respect to the developing resistance among microorganisms against available antibiotics. MDR and XDR mycobacterial strains have been reported to exhibit resistance against known anti-TB drugs such as isoniazid, rifampicin, ethambutol and pyrazinamide[1]. Hence, there is a great need to screen new compounds having therapeutic potential and our efforts in the present study were directed towards this. According to a study of Hartkoorn *et al.*[22] on the drug susceptibility of TB, antimycobacterial activity was considered to be specific when the selectivity index was >10. In the current study, both **13b** and **29e** exhibited selectivity index of >40 indicating their potential as antitubercular agents.

Table No 2. Cytotoxicity profile of selected compounds against four human cancer cell lines

	***In vitro* cytotoxicity of selected thienopyrimidinone compounds**			
Comp no	**MCF-7 (Breast)**	**A549 (Lung)**	**HCT 116 (Colon)**	**THP-1 (Leukemia)**
	GI_{50} (µg/mL)	**GI_{50} (µg/mL)**	**GI_{50} (µg/mL)**	**GI_{50} (µg/mL)**
11g	>100	>100	>100	>100
11m	33.80	27.36	>100	>100
13b	>100	>100	>100	>100
14a	>100	>100	>100	>100
14b	24.83	>100	17.64	>100
14c	>100	>100	>100	>100
24g	20.52	35.08	>100	>100
24h	26.12	45.44	42.41	>100
25d	19.68	34.15	>100	>100
26c	>100	>100	>100	>100
29d	>100	16.17	14.7	>100
29e	>100	>100	>100	>100
RIF	>100	>100	>100	>100
Paclitaxel	0.0048	0.0035	0.0260	0.1374

GI_{50} indicates concentration to inhibit 50% growth of cells.

2.2.6. Structure-activity relationship

The preliminary studies reported herein indicate that the compounds with thienopyrimidinone structural unit have potential to be studied further and to be developed as antitubercular agents. Some of the conclusions drawn from the structures of the compounds studied in the present work and antitubercular activity exhibited[14] are as follows:

The compounds with longer alkyl chain at 6 position of thieno[2,3-*d*]pyrimidin-4(3*H*)-one were observed to exhibit better antitubercular activity than the corresponding compounds with shorter alkyl chain at 6 position and same functional groups at 3 position e.g. the antitubercular activity was observed in the order of **29c** < **29d** < **29e**. Similarly **13a** was observed to be less active than **13b**. The compounds with general structures **24** and **25** with 2-bromoethyl and 3-bromopropyl side chains respectively at 3 position and alkyl chains at 6 position exhibited similar trend in activity with **24a** < **24e** < **24f** < **24g** and **25a** < **25c** < **25d**.

The compounds **11a-m** in the present work, having alkyl chains both at 3 and 6 positions of thienopyrimidinone unit, did not exhibit antitubercular activity. Also, the compounds having benzyl group at 3 position of thienopyrimidinone and 2-6 carbon alkyl chain at position 6 (compounds **12a-e**) did not exhibit antitubercular activity. Compounds **14a-c** with cyclopropylmethyl group at position 3 and pentyl, hexyl or heptyl chain at position 6 of thienopyrimidinone exhibited moderate antitubercular activity. Compounds having propargylic side chain at position 3 of thienopyrimidinone moiety and alkyl chain ranging from methyl to heptyl at position 6, with general structure **32**, did not exhibit antitubercular activity. A detailed study with more number of compounds would be necessary to refine the structure-activity relationship conclusions drawn from the present preliminary results.

Table No 3. Selectivity index (SI) of selected thienopyrimidinones on human cell lines against *Mycobacterium tuberculosis* H37Ra and *M. bovis* BCG

Comp no	SI on MCF-7		SI on A549		SI on HCT 116		SI on THP-1	
	Against H37Ra	Against BCG	Against H37Ra	Against BCG	Against H37Ra	Against BCG	Against H37Ra	Against BCG
	Active stage of Mycobacterium tuberculosis H37Ra and *M. bovis* BCG							
11g	2	10	2	10	2	10	2	10
11m	1	4	1	3	2	12	2	12
13b	**40**	**53**	**40**	**53**	**40**	**53**	**40**	**53**
14a	3	20	3	20	3	20	3	20
14b	1	18	2	73	0	13	2	73
14c	2	66	2	66	2	66	2	66
24g	1	9	1	15	4	44	4	44
24h	7	11	13	19	12	18	29	41
25d	1	3	2	5	6	16	6	16
26c	15	5	15	5	15	5	15	5
29d	12	25	2	4	2	4	12	25
29e	**48**	**77**	**48**	**77**	**48**	**77**	**48**	**77**
RIF	196	222	196	222	196	222	196	222

3. Experimental

General experimental procedures and spectral data for all compounds prepared for antimycobacterial activity testing described in this article and detailed screening results are given in the supplementary data. The spectral data for active compounds are given below.

3.1. Spectral data for active compounds

3.1.1. 6-Ethyl-3-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11g)

^{1}H NMR (200MHz, $CDCl_3$): δ 0.91 (t, J = 7Hz, 3H), 1.28-1.44 (m, 7H), 1.68-1.82 (m, 2H), 2.88 (q, J = 7 Hz, 2H), 3.99 (t, J = 7 Hz, 2 H), 7.17 (s, 1 H), 7.92 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.80, 15.21, 22.17, 23.91, 28.61, 29.17, 46.80, 117.44, 124.77, 145.62, 145.87, 157.22, 162.24. IR ($CHCl_3$): 1672 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{13}H_{18}ON_2S$ + H]: 251.1213, found: 251.1210; [$C_{13}H_{18}ON_2S$ + Na]: 273.1032 found: 273.1027. Melting Point: 74^{0}C.

3.1.2. 6-Pentyl-3-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (11m)

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, J = 7Hz , 3H), 1.00 (t, J = 7Hz, 3H), 1.27-1.47 (m, 4H), 1.62-1.94 (m, 4H), 2.84 (t, J =8Hz, 2H), 3.98 (t, J =7Hz, 2H), 7.16 (s, 1H), 8.01 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.93, 13.83, 22.20, 22.67, 30.44, 30.60, 30.94, 48.28, 118.08, 124.67, 144.44, 145.57, 157.13, 162.11. IR ($CHCl_3$): 1672 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{14}H_{20}ON_2S$ + H]: 265.1369, found: 265.1367; [$C_{14}H_{20}ON_2S$ + Na]: 287.1189 found: 287.1185. Melting Point: 141^{0}C.

3.1.3. 2-(6-Heptyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)acetonitrile (13b)

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, J =7Hz, 3H), 1.23-1.49 (m, 8H), 1.63-1.78 (m, 2H), 2.86 (t, J=8Hz, 2H), 4.91 (s, 2H), 7.19 (s, 1H), 8.04 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.95, 22.48, 28.79 (2C), 30.51, 30.91, 31.57, 33.47, 113.82, 118.03, 124.09, 143.64, 146.23, 155.93, 162.25. IR ($CHCl_3$): 1686, 2358 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{15}H_{19}ON_3S$ + H]: 290.1322,

found: 290.1318; [$C_{15}H_{19}ON_3S$ + Na]: 312.1141, found: 312.1136. Melting Point: 86^{0}C.

3.1.4. 3-(Cyclopropylmethyl)-6-pentylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (14a)

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.32-0.48 (m, 2H), 0.55-0.70 (m, 2H), 0.88 (t, *J* =7Hz, 3H), 1.17-1.44 (m, 5H), 1.63-1.75 (m, 2H), 2.82 (t, *J* =7Hz, 2H), 3.86 (d, *J* =7Hz , 2H), 7.14 (s, 1H), 7.99 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 4.03 (2C), 10.88, 13.83, 22.22, 30.45, 30.60, 30.95, 50.80, 118.14, 124.66, 144.39, 145.26, 157.31, 162.28. IR ($CHCl_3$): 1671 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{15}H_{20}ON_2S$ + H]: 277.1369, found: 277.1369; [$C_{15}H_{20}ON_2S$ + Na]: 299.1189, found: 299.1186. Melting Point: 73^{0}C.

3.1.5. 3-(Cyclopropylmethyl)-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (14b)

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.36-0.48 (m, 2H), 0.60-0.72 (m, 2H), 0.89 (t, *J* =7Hz, 3H), 1.17-1.45 (m, 7H), 1.62-1.77 (m, 2H), 2.84 (t, *J* =7Hz, 2H), 3.88 (d, *J* =7Hz , 2H), 7.16 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 4.14 (2C), 10.98, 14.03, 22.51, 28.59, 30.62, 31.01, 31.46, 50.93, 118.26, 124.80, 144.56, 145.31, 157.45, 162.39. IR ($CHCl_3$): 1683 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{16}H_{22}ON_2S$ + H]: 291.1526, found: 291.1519; [$C_{16}H_{22}ON_2S$ + Na]: 313.1345 found: 313.1336. Melting Point: 49^{0}C.

3.1.6. 3-(Cyclopropylmethyl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (14c)

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.35-0.48 (m, 2H), 0.58-0.69 (m, 2H), 0.87 (t, *J* =7Hz, 3H), 1.14-1.47 (m, 9H), 1.68-1.76 (m, 2H), 2.83 (t, *J* =7Hz, 2H), 3.86 (d, *J* =7Hz , 2H), 7.15 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 4.07 (2C), 10.91, 13.98, 22.52, 28.80, 28.86, 30.53, 30.97, 31.62, 50.84, 118.18, 124.71, 144.45, 145.27, 157.35, 162.32. IR ($CHCl_3$): 1678 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{17}H_{24}ON_2S$ + H]: 305.1682, found: 305.1683; [$C_{17}H_{24}ON_2S$ + Na]: 327.1502 found: 327.1500. Melting Point: 57^{0}C.

3.1.7. 3-(2-Bromoethyl)-6-decylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (24g)

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, *J* =7Hz, 3H), 1.19-1.45 (m, 14H), 1.72 (t, *J* =7Hz, 2H), 2.85 (t, *J* = 8Hz, 2H), 3.77 (t, *J* =6Hz, 2H), 4.38 (t, *J* = 6Hz, 2H), 7.16 (s, 1H), 7.99 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.07, 22.63, 28.91, 29.25(2C), 29.46, 29.52, 29.76, 30.60, 31.02, 31.84, 48.73, 118.03, 124.53, 145.08, 145.66, 157.02, 162.75. IR ($CHCl_3$): 1676 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{18}H_{27}ON_2S^{79}Br$ + H]: 399.1100, found: 399.1102; [$C_{18}H_{27}ON_2S^{79}Br$ + Na]: 421.0920, found: 421.0920. Melting Point: 73^{0}C.

3.1.8. 3-(2-Bromoethyl)-6-(non-8-enyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (24h)

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23-1.49 (m, 8H), 1.65-1.83 (m, 2H), 1.97-2.09 (m, 2H), 2.84 (t, *J* =8Hz, 2H), 3.76 (t, *J* =6Hz, 2H), 4.38 (t, *J* =6Hz, 2H), 4.90-5.07 (m, 2H), 5.73-5.90 (m, 1H), 7.15 (s, 1H), 7.99 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.80, 28.83, 28.90, 29.07, 29.76, 30.58, 30.98, 33.70, 48.74, 114.19, 118.05, 124.53, 139.05, 145.03, 145.67, 157.02, 162.75. IR ($CHCl_3$): 1674 cm^{-1}. HRMS (ESI) m/z calculated for $C_{17}H_{23}ON_2{}^{79}BrS$ + H]: 383.0787, found: 383.0786; [$C_{17}H_{23}ON_2{}^{79}BrS$ + Na]: 405.0607, found: 405.0605. Melting Point: 150^{0}C.

3.1.9. 3-(3-Bromopropyl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (25d)

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, *J* =7Hz, 3H), 1.18-1.40 (m, 8H), 1.58-1.80 (m, 2H), 2.25-2.49 (m, 2H), 2.82 (t, *J* =7Hz, 2H), 3.41 (t, *J* =6Hz, 2H), 4.17 (t, *J* =6Hz, 2H), 7.12 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.93, 22.46, 28.74, 28.79, 29.78, 30.47, 30.90, 31.06, 31.55, 45.19, 117.92, 124.59, 144.82, 145.53, 157.13, 162.36. IR ($CHCl_3$): 1670 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{16}H_{23}ON_2{}^{79}BrS$ + H]: 371.0787, found: 371.0785; [$C_{16}H_{23}ON_2{}^{79}BrS$ + Na]: 393.0607, found: 393.0605. Melting Point: 75^{0}C.

3.1.10. 6-Decyl-3-(2-hydroxyethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (26c)

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, *J* =7Hz, 3H), 1.18-1.43 (m, 14H), 1.62-1.78 (m, 2H), 2.82 (t, *J* = 8Hz, 2H), 2.97(t, *J* =5Hz, 1H), 3.97 (q, *J* = 5Hz, 2H), 4.16 (t, *J* =5Hz, 2H), 7.08 (s, 1H), 7.99 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.08, 22.65, 28.96, 29.27(2C), 29.49, 29.54, 30.61, 31.03, 31.85, 49.42, 60.70, 117.91, 124.48, 144.92, 146.31, 157.78, 162.56. IR ($CHCl_3$): 1675, 3408(bs) cm^{-1}. HRMS (ESI) m/z calculated for [$C_{18}H_{28}O_2N_2S$ + H]: 337.1944, found: 337.1937; [$C_{18}H_{28}O_2N_2S$ + Na]: 359.1764 found: 359.1756. Melting Point: 62^{0}C.

3.1.11. 3-(2-Azidoethyl)-6-hexylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (29d)

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, J =7Hz, 3H), 1.22-1.45 (m, 6H), 1.65-1.76 (m, 2H), 2.84 (t, J =7Hz, 2H), 3.76 (t, J =6Hz, 2H), 4.12 (t, J =6Hz, 2H), 7.14 (s, 1H), 7.94 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.99, 22.47, 28.55, 30.58, 30.96, 31.42, 46.15, 49.46, 118.01, 124.50, 145.06, 145.71, 157.11, 162.67. IR ($CHCl_3$): 1674, 2106 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{14}H_{19}ON_5S$ + H]: 306.1383, found: 306.1378; [$C_{14}H_{19}ON_5S$ + Na]: 328.1203 found: 328.1197. Melting Point: 50^{0}C.

3.1.12. 3-(2-Azidoethyl)-6-heptylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (29e)

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, J=7Hz, 3H), 1.19-1.45 (m, 8H), 1.62-1.82 (m, 2H), 2.84 (t, J=7Hz, 2H), 3.76 (t, J =5Hz, 2H), 4.12 (t, J=6Hz, 2H), 7.15 (s,1H), 7.94 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.94, 22.49, 28.79, 28.83, 30.52, 30.94, 31.58, 46.09, 49.43, 117.95, 124.47, 144.99, 145.70, 157.02, 162.55. IR ($CHCl_3$): 1676, 2122 cm^{-1}. HRMS (ESI) m/z calculated for [$C_{15}H_{21}ON_5S$ + H]: 320.1540, found: 320.1537; [$C_{15}H_{21}ON_5S$ + Na]: 342.1359 found: 342.1354. Melting Point: 50^{0}C.

4. Conclusions

In conclusion, thienopyrimidinones with varying structural features were synthesized for evaluation of antimycobacterial activity. From our initial screening studies, it was found that the thienopyrimidinones **11g**, **11m**, **13b**, **14a**, **14b**, **14c**, **24g**, **24h**, **25d**, **26c**, **29d** and **29e** exhibited significant activity against *Mycobacterium tuberculosis* H37Ra and *Mycobacterium bovis* BCG. The compounds **13b** and **29e** exhibited greater efficiency in terms of mycobacterial inhibition, specificity and selectivity. Moreover, both the compounds showed satisfactory biocompatibility against human cancer cell lines. These compounds are good candidates for further activity-guided fractionation in the search for new active therapeutic compounds. Our studies point to the possibility of accessing more active compounds based on present encouraging findings as the compounds described in the present work have various functional groups for further structural modifications.

Acknowledgements

We thank CSIR, New Delhi for financial support (OSDD program, Project no HCP0001) and Dr. C. V. Ramana for fruitful discussions.

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