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Preliminary communication

Synthesis and preliminary evaluation of some *N*-[5-(2-furanyl)-2-methyl-4-oxo-4*H*-thieno[2,3-*d*]pyrimidin-3-yl]-carboxamide and 3-substituted-5-(2-furanyl)-2-methyl-3*H*-thieno[2,3-*d*]pyrimidin-4-ones as antimicrobial agents

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Abstract

Two series of *N*-[5-(2-furanyl)-2-methyl-4-oxo-4*H*-thieno[2,3-*d*]pyrimidin-3-yl]-carboxamide (**4a**–**m**) and 3-substituted-5-(2-furanyl)-2-methyl-3*H*-thieno[2,3-*d*]pyrimidin-4-ones (**5a**–**m**) were synthesised using appropriate synthetic route. All the test compounds **4a**–**m** and **5a**–**m** were assayed in vitro for antibacterial activity against two different strains of Gram-negative (*Escherichia coli* and *S. typhi*) and Gram-positive (*S. aureus*, *B. subtilis*) bacteria and the antimycobacterial activity was evaluated against *M. tuberculosis* and *M. avium* strains. The minimum inhibitory concentration (MIC) was determined for test compounds as well as for reference standards. The test compounds have shown significant antibacterial and antimycobacterial activity against all the microbial strains used, when tested in vitro. In general, along with the thienopyrimidinone ring, substituted amido or imino side chain at position 3 is essential for antimicrobial activity. Among the compounds tested, compounds **4c**, **4e** and **4g** in *N*-[5-(2-furanyl)-2-methyl-4-oxo-4*H*-thieno[2,3-*d*]pyrimidin-3-yl]-carboxamide series and compounds **5c**, **5e** and **5g** in 3-substituted-5-(2-furanyl)-2-methyl-3*H*-thieno[2,3-*d*]pyrimidin-4-ones series were found to be the most potent. Further the toxicity of most potent compounds **4c**, **4e** and **4g** and **5c**, **5e** and **5g** were assessed using hemolytic assay and minimal hemolytic concentration (MHCs) were determined. In general, test compounds were found to be non-toxic up to a dose level of 200 μmol L^{-1} (MHC).

Keywords: MIC (minimum inhibitory concentration); Thieno[2,3-*d*]pyrimidin-4-one; Synthesis; Hemolytic assay; Antibacterial and Antimycobacterial activity

1. Introduction

The deterioration of the human population due to the enhance prevalence of infectious diseases is becoming a world wide problem. Over the last few years, tuberculosis is retrieving its place among these infectious diseases and today, nearly one-third of the world's population is infected with *Mycobacterium tuberculosis*, with approximately three million patients deceasing every year [1]. The contemporary treatment of these infectious diseases involves administration of a multi-drug regimen over a long period of time, and consequently a high level of patient non-compliance is allied with the existing therapy [2,3]. Along with this lack of patient compliance, a multi-drug treatment has also led to the rapid emergence of multidrug-resistant strains [4–7]. The resistance problem demands that a renewed effort should be made to seek antimicrobial agents effective against pathogenic microorganisms resistant to current treatment [8]. Further the treatment of infectious diseases is even more difficult in immuno-

Abbreviations: MIC; MHC; LD_{50}.

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deficient patients such as those infected with the human immunodeficiency virus (HIV) [9]. Recent studies show that the application of an appropriate dosage regimen with highly potent antimicrobial agents not only eradicates bacterial growth but also minimizes the probability of resistance formation [10–12]. The biochemical basis of both intrinsic and acquired resistance are now known [13–15] and has contributed significantly towards the designing of new entities by rational strategies that can be used to counteract the resistance. The development of new potential drugs which will be devote of the side-effect profile of currently available drugs will be one of the possible solution to treat various infectious disease with multidrug treatment over a long period of time. Moreover, in immunocompromised patients, tubercular pathology is often accompanied by other bacterial infections; it is therefore essential to develop the new entities, which will act both as antibacterial and antimycobacterials.

Earlier, we reported triazolobenzothiazole derivatives as one of the pharmacophores for antimycobacterial activity [16]. Literature survey reveals that various substituted thieno-pyrimidines/pyrimidinones are known to possess a wide range of pharmacological activities like anti-inflammatory [17,25], antimalarial [18], antibacterial [19,20], spasmolytic [21], etc. In view of the fact that some thieno[2,3-*d*]pyrimidine possess antibacterial properties and as part of our ongoing studies in the area of, antibacterial and antimycobacterial agents [22,23], we have synthesized two series of some novel *N*-[5-(2-furanyl)-2-methyl-4-oxo-4*H*-thieno[2,3-*d*]pyrimidin-3-yl]-carboxamide (**4a**–**m**) and 3-substituted-5-(2-furanyl)-2-methyl-3*H*-thieno[2,3-*d*]pyrimidin-4-ones (**5a**–**m**) with the aim of obtaining the new broad spectrum antimicrobial agents which will be devoid of side effects associated with current therapy.

Thus, in the present investigation, thirteen different derivatives of **4a**–**m** and **5a**–**m** were synthesised and evaluated for their antibacterial and antimycobacterial activity. The most potent compound of each series was further evaluated for their toxicity using hemolytic assay.

2. Chemistry

The synthesis of title compounds **4a**–**m** and **5a**–**m** has been carried out as depicted in Fig. 1. The starting material 2-amino-4-(2-furanyl)-thiophene-3-carboxylic acid ethyl ester (**1**) was prepared by condensing 2-cyano-3-(2-furanyl)-but-2-enoic acid ethyl ester with sulphur, according to the Gewald method [24]. The

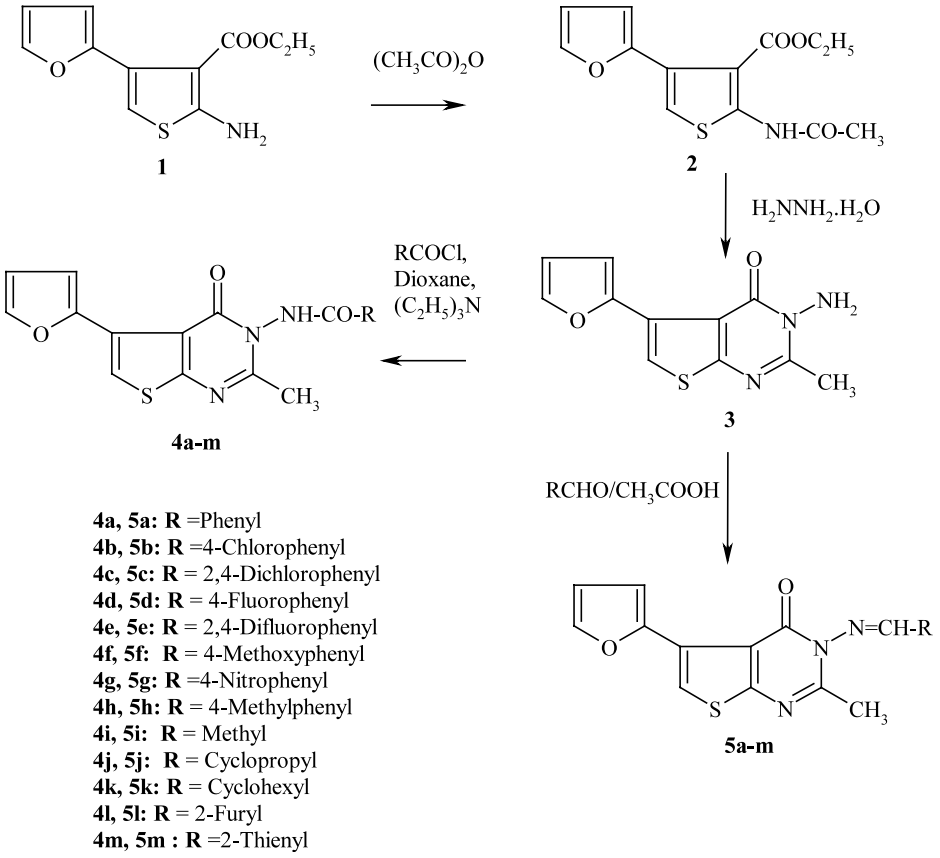

Fig. 1. General scheme for the synthesis of compounds **4a**–**m** and **5a**–**m**.

reaction afforded the starting material in 70% yield. Compound **1** was converted into 2-acetylamino-4-(2-furanyl)-thiophene-3-carboxylic acid ethyl ester (**2**), by nucleophilic acylation of amino group with Ac_2O, according to modified literature method [25]. Zinc dust was added in catalytic amount to improve the overall yield of compound **2** (percentage yield 80%). The compound **2** was cyclised into 3-amino-5-(2-furanyl)-2-methyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (**3**) by treatment with hydrazine hydrate [25]. Further this key intermediate **3** was converted into various derivatives of **4a**–**m** by treatment with substituted acid chlorides in presence of triethylamine. Using the same key intermediate various derivatives of second series of title compounds **5a**–**m** was prepared by treatment with substituted aldehydes in glacial acetic acid. The reaction proceeds at this step in two steps via nucleophilic addition and elimination reaction. The loss of water molecule is the rate determining step and the reaction is catalysed by glacial acetic acid.

All the synthesised compounds were characterised by their physical, analytical and spectral data (Table 1). The IR spectra of compound **1** showed the presence of characteristic absorption peak at 3308 and 3200 (N–H stretching) and at 1730 (C=O stretching). The compound **2** showed the absorption band at 3255 (N–H stretching) due to conversation of NH_2 into amide, while Compound **3** showed the presence of characteristic absorption peaks at 3308 and 3200 (N–H stretching) due to free amino group and at 1005 (cyclic pyrimidine). The IR spectra of title compound **4a**–**m** and **5a**–**m** shows presence of band around 3255 (N–H stretching in **4a**–**m**) and 1620–1628 (C=N stretching in **5a**–**m**) and absence of peak at 3308 and 3200 due to conversion of free amino group into amide and imide side chain in **4a**–**m** and **5a**–**m**, respectively. All the haologanated title compounds (**4b**–**e** and **5b**–**e**) showed the absorption bands at 590–760 (C–X stretching). Nitro substituted compound **4g** and **5g** showed broadband at 1490–1510 (O=N=O stretching). Further the EIMS, ^{1}H- and ^{13}C-NMR spectral data of all the synthesised compounds are in conformity with the structure assigned.

In the EIMS spectra, molecular ion peaks [M^+], which appeared at different intensities, confirm the molecular weight of the examined compounds. Molecular ion peaks were the base peak for the compounds **4a**, **4f**–**m**, **5a** and **5f**–**m**, whereas appearance of an isotopic peaks [$M^+ + 1$, $M^+ + 2$] along with the molecular ion peak confirms the presence of halogen atom in compound **4b**–**e** and **5b**–**e**. The elemental analyses of all the synthesised compounds were found within the limit of $\pm 0.4\%$ of theoretical values. The corresponding ^{1}H-,

Table 1
Physical and analytical data of compounds **4a**–**m** and **5a**–**m**

Compound	R	Molecular formula [a] (Mol. wt.)	M.p (°C)	Yield [b] (%)	Mass [M^+]
4a	phenyl	$C_{18}H_{13}N_3O_3S$ (351)	264–266	83	351
4b	4-chlorophenyl	$C_{18}H_{12}ClN_3O_3S$ (385)	190–192	62	387 [c]
4c	2,4-dichlorophenyl	$C_{18}H_{11}Cl_2N_3O_3S$ (419)	230–232	72	421 [c]
4d	4-fluorophenyl	$C_{18}H_{12}FN_3O_3S$ (369)	227–229	66	371 [c]
4e	2,4-difluorophenyl	$C_{18}H_{11}F_2N_3O_3S$ (387)	235–236	65	389 [c]
4f	4-methoxyphenyl	$C_{19}H_{15}N_3O_4S$ (381)	223–224	47	381
4g	4-nitrophenyl	$C_{18}H_{12}N_4O_5S$ (396)	167–169	70	396
4h	4-methylphenyl	$C_{19}H_{15}N_3O_3S$ (365)	269–270	65	365
4i	methyl	$C_{13}H_{11}N_3O_3S$ (289)	238–240	40	289
4j	cyclopropyl	$C_{15}H_{13}N_3O_3S$ (315)	210–211	63	315
4k	cyclohexyl	$C_{18}H_{19}N_3O_3S$ (357)	269–270	58	357
4l	2-furyl	$C_{16}H_{11}N_3O_4S$ (341)	240–241	60	341
4m	2-thienyl	$C_{16}H_{11}N_3O_3S_2$ (357)	249–250	57	357
5a	phenyl	$C_{18}H_{13}N_3O_2S$ (335)	234–236	63	335
5b	4-chlorophenyl	$C_{18}H_{12}ClN_3O_2S$ (369)	218–219	75	371 [c]
5c	2,4-dichlorophenyl	$C_{18}H_{11}Cl_2N_3O_2S$ (403)	225–226	65	405 [c]
5d	4-fluorophenyl	$C_{18}H_{12}FN_3O_2S$ (353)	151–153	50	355 [c]
5e	2,4-difluorophenyl	$C_{18}H_{11}F_2N_3O_2S$ (371)	113–115	58	373 [c]
5f	4-methoxyphenyl	$C_{19}H_{15}N_3O_3S$ (365)	155–156	58	365
5g	4-nitrophenyl	$C_{18}H_{12}N_4O_4S$ (380)	248–250	42	380
5h	4-methylphenyl	$C_{19}H_{15}N_3O_2S$ (349)	199–200	41	349
5i	methyl	$C_{13}H_{11}N_3O_2S$ (273)	209–210	73	273
5j	cyclopropyl	$C_{15}H_{13}N_3O_2S$ (299)	148–150	57	299
5k	cyclohexyl	$C_{18}H_{19}N_3O_2S$ (341)	250–251	70	341
5l	2-furyl	$C_{16}H_{11}N_3O_3S$ (325)	210–211	60	325
5m	2-thienyl	$C_{16}H_{11}N_3O_2S_2$ (341)	250–251	63	341

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

^{13}C-NMR and mass spectral data are presented in Section 5.

3. Pharmacology

All the test compounds was assayed in vitro for antibacterial activity against two different strains of Gram-negative (*E. coli* and *S. typhi*) and Gram-positive (*S. aureus*, *B. subtilis*) bacteria and the antimycobacterial activity was evaluated against *M. tuberculosis* and *M avium* strains using standard protocol [26]. The minimum inhibitory concentration (MIC) was determined by the test tube dilution technique using Mueller–Hinton nutrient broth (for antibacterial) and modified Kirchner's culture medium containing 0.5% sterilised horse serum for antimycobacterial activity. The MIC values were also tested for three well-known antibiotics (ampicillin, penicillin-G, and chloramphenicol) to compare the antibacterial activity of our test compounds with the antibiotics, which are currently in therapy. Rifampin and isoniazid (INH) were used as reference standard for antimycobacterial activity (see Section 5). Further the toxicity of most potent compounds **4c**, **4e** and **4g** and **5c**, **5e** and **5g** from each series were assessed using hemolytic assay and minimal hemolytic concentration (MHCs) and LD_{50} values were determined [27].

4. Results and discussion

In the present investigation, 13 different derivatives of *N*-[5-(2-furanyl)-2-methyl-4-oxo-4*H*-thieno[2,3-*d*]pyrimidin-3-yl]-carboxamide (**4a**–**m**) and 3-substituted-5-(2-furanyl)-2-methyl-3*H*-thieno[2,3-*d*]pyrimidin-4-ones (**5a**–**m**) were synthesised and evaluated for their antibacterial and antimycobacterial activity. Synthesis of title compounds **4a**–**m** and **5a**–**m** have been carried out as depicted in Fig. 1. All the synthesised compounds were purified and were characterised by their physical, analytical and spectral data (Table 1).

The MIC values of test compounds are summarised in Table 2. For the sake of comparison, the MICs of compound **2** and **3** are also included in the Table 2. The results revealed that all the test compounds **4a**–**m** and **5a**–**m** exhibited remarkable in vitro activity against all tested microbial (bacterial and mycobacterial) strains. The MICs values are in the range of 4–218 μmol L^{-1}.

The structure activity relationship reveals that thienopyrimidinone ring is crucial for both the antibacterial and antimycobacterial activity as compound **2** was found to be inactive compare to compound **3** against all test organisms at concentration >1000 μmol L^{-1}. The substituted-amido or imino side chain at position 3 on thienopyrimidinone ring contribute significantly towards antimicrobial activity along with thienopyrimidinone pharmacophore as compound **3** was found to be less active compare to compound **4a**–**m** and **5a**–**m**. The different substituents in compounds **4a**–**m** and **5a**–**m** over the side chain at position 3 of thienopyrimidinone ring exert significant influence on the biological activity. In general, aromatic substituted compounds **4a**–**h** and **5a**–**h** at position 3 were found to be more active than aliphatic (**4i**–**k** and **5i**–**k**) and heterocyclic (**4l**, **4m**, **5l** and **5m**) substituents. Further the presence of electron withdrawing groups (halogen, alkoxy and nitro substituents in **4b**–**4g** and **5b**–**5g**) on the aromatic ring, in general increases the potency of test compounds compare to compounds having electron donating groups (alkyl substituents in **4h** and **5h**) and unsubstituted one (**4a** and **5a**). Literature survey reveals that the lipophilicity of test compound has significant influence on the antimicrobial activity [28,29]. In general, electron withdrawing or donating groups amends the lipophilicity of test compounds, which in turn alters permeability across the bacterial cell membranes. In general, compounds **4a**–**m** and **5a**–**m** were found to be more active against Gram-positive bacteria compare to Gram negative. This may be due to absence of unique outer membrane of peptidoglycon in Gram-positive bacteria and thereby the cell wall of Gram-positive bacteria is more permeable to the test compounds. Further the MIC values of compounds **4a**–**m** were found to be comparatively higher (less potent) when measured after 21 days than 14 days measurement against mycobacterial strain and 24 h measurements against bacterial strains. These values were also found to be higher than the MIC values of **5a**–**m** (both in bacterial and mycobacterial strains), which indicates that the test compounds were metabolising with time in the biological environment. In order to confirm this fact in vitro, the most potent compound **4e** was incubated in phosphate buffer solution (pH 7.4) at 37 °C and after every 12 h 20 μL of aliquot was analysed by Analytical RP-HPLC (reverse phase high performance column chromatography) over a period of 15 days. Further the individual peaks were collected from HPLC, freeze-dried and characterised by LCMS (Shimadzu high-sensitivity liquid chromatograph mass spectrometer-2010). Result of this study shows that (Fig. 5), after 14 days the compound **4e** (parent peak, retention time: 36. min) get hydrolysed (appearance of second peak at 30 min). This hydrolysed product (metabolite of compound **4e)** was found to be less lipophilic compare to parent compound as it elute before the parent peak from C_{18} column in RP HPLC. As shown in Fig. 2, at physiological pH, the test compound **4e** undergo hydrolysis across amide side chain (as the observed mass, Fig. 4 was found to be in agreement with the hydrolysis product of compound **4e**). Alike **4e** it may be possible that all the test compounds (**4a**–**m**) undergoes hydrolysis and therefore the MIC values increases with time, particularly the electron

Table 2
In vitro antibacterial and antimycobacterial activity of test compounds [a]

Compound	Antibacterial activity (MIC) [b]				Antimycobacterial activity (MIC) [b]			
	Gram positive		Gram negative		*M. tuberculosis*		*M avium*	
	S. aureus	*B. subtilis*	*E. coli*	*S. typhi*	14 [c]	21 [d]	14 [c]	21 [d]
2	[e]	[e]	[e]	[e]	[e]	[e]	[e]	[e]
3	500	550	580	560	482	500	510	515
4a	50	55	54	42	50	99	48	110
4b	10	9	10	12	9	16	8	20
4c	5	4	6	6	5	16	4	22
4d	9	9	9	11	9	15	9	25
4e	4	5	4	5	4	15	4	20
4f	10	9	10	12	9	16	8	20
4g	6	5	7	6	6	16	6	22
4h	30	46	55	48	41	96	40	101
4i	55	65	70	98	78	200	86	211
4j	56	57	60	108	88	209	89	218
4k	59	60	66	97	105	203	88	215
4l	50	56	65	78	41	96	40	101
4m	51	55	60	74	46	106	45	121
5a	55	59	64	66	50	52	48	50
5b	10	9	9	8	9	10	10	12
5c	4	4	6	5	4	5	4	5
5d	10	9	9	8	9	10	10	12
5e	5	4	5	6	4	5	4	5
5f	11	11	12	12	14	15	14	15
5g	4	5	7	7	6	7	6	7
5h	58	56	59	65	52	58	56	57
5i	65	69	74	76	60	62	58	62
5j	64	66	71	70	66	67	59	61
5k	65	72	77	76	64	62	57	62
5l	57	59	62	64	52	56	62	54
5m	58	56	69	65	50	54	52	65
Std1 [f]	50	4	4	49	NA	NA	NA	NA
Std2 [g]	6	22	5	5	NA	NA	NA	NA
Std3 [h]	4	6	5	6	NA	NA	NA	NA
Std4 [i]	8	7	189	199	4	5	5	5
Std5 [j]	in active	in active	in active	in active	4	4	4	4

[a] DMF has no antimicrobial activity at the concentration used to dissolve the test compounds.
[b] MIC: minimum inhibitory concentration.
[c] Antimycobacterial activity was measured after 14 days of incubation.
[d] Antimycobacterial activity was measured after 21 days of incubation.
[e] Compound **2** was found to be inactive at >1000 mmol L^{-1}. NA: indicates activity not measured.
[f] **Std1**: Ampicillin.
[g] **Std2**: Penicillin-G.
[h] **Std3**: Chloramphenicol.
[i] **Std4**: Rifampin.
[j] **Std5**: Isoniazid (INH).

withdrawing groups favours this hydrolysis compare to electron donating group and therefore significant increase in the MIC values of compounds **4b**–**g** were found at $t = 21$ days. These results also reflect that substituted amido side chain in compound **4a**–**m** is necessary for antimicrobial activity; in turns it may be essential to maintain the lipophilicity of test compounds. Among the compound tested, compound **4c**, **4e** and **4g** in series of *N*-[5-(2-furanyl)-2-methyl-4-oxo-4*H*-thieno[2,3-*d*]pyrimidin-3-yl]-carboxamide (**4a**–**m**) and **5c**, **5e** and **5g** in series the of 3-substituted-5-(2-furanyl)-2-methyl-3*H*-thieno[2,3-*d*]pyrimidin-4-ones (**5a**–**m**) was found to be equipotent as reference standards. Further the toxicity of most potent compounds (**4c**, **4e**, **4g**, **5c**, **5e**, and **5g**) of each series were assessed using hemolytic assay and minimal hemolytic concentration (MHC) and LD_{50} values (Log concentration of test compound required for 50% hemolysis) was determined from the graph of % lyses against Log concentration (Fig. 3). The most potent compounds (**4c**, **4e**, **4g**, **5c**, **5e**, and **5g**) were found to be non-toxic up to a dose level of 200 μmol L^{-1} (MHC) and their corresponding LD_{50}

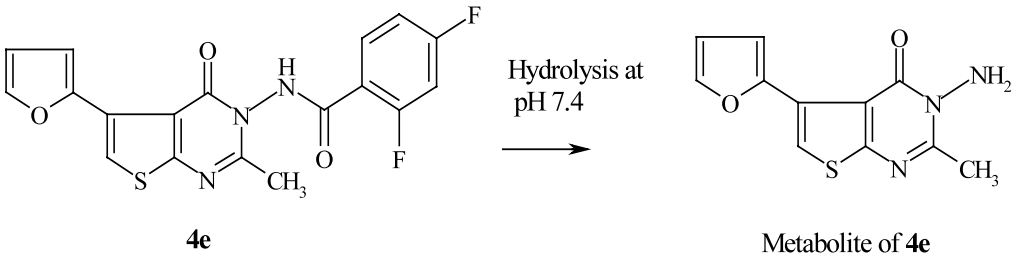

Fig. 2. Hydrolysis of compound **4e** at pH 7.4.

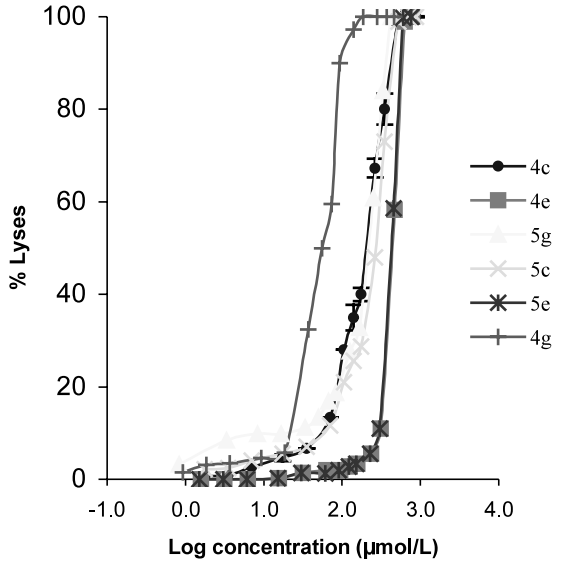

Fig. 3. Graph of % lyses against Log concentration for compound **4c**, **4e**, **4g**, **5c**, **5e** and **5g**.

values were found to be within a range of 550–850 μmol L^{-1} (**4c**, 850; **4e**, 880; **4g**, 550; **5c**, 820; **5e**, 835; and **5g**, 750 μmol L^{-1}). Thus, in comparison with antibiotics commonly used in therapy, our most potent compounds show similar or slightly less in vitro antimicrobial activity against various bacterial (Gram-positive and

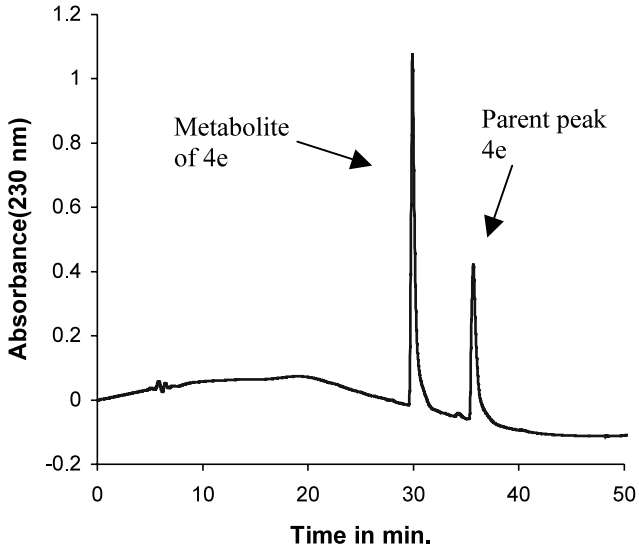

Fig. 5. Analytical reverse phase high performance liquid chromatogram of compound **4e** and its metabolite. RP-HPLC conditions: Beckman Gold Nouveau System equipped with a 168 photodiode-array detector. Column; Vydac 218TP53 (C_{18}, 300 Å, 5 mm 3.2 mm i.d. × 250 mm). 0.5 mL min^{-1}, Buffer A = 0.1% TFA in water. Buffer B = 90% ACN (acetonitrile) containing 10% buffer A. Linear gradient from 0% B to 90% B over 30 min, total run time 50 min. Retention time (Rt) for parent peak 36 min, metabolite Rt = 30 min.

Gram-negative) and mycobacterial strains with marked reduced toxicity (hemolytic activity), suggested that this

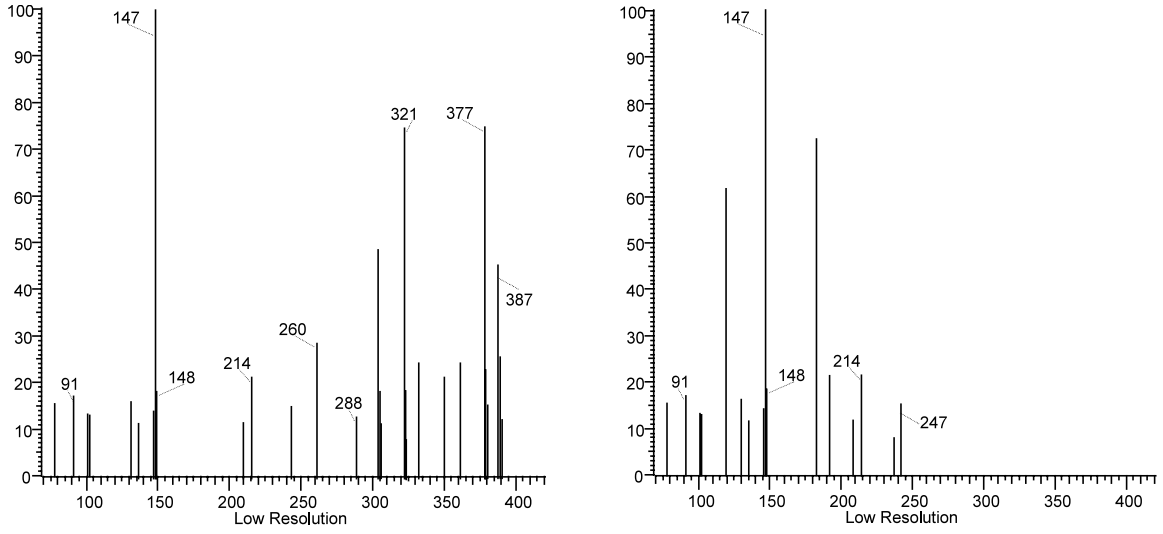

Fig. 4. LCMS of compound **4e** and its metabolite. Expected molecular weight of compound **4e** [389, $M^+ + 2$], metabolite [247, M^+].

class of compounds could be use as potent broad-spectrum antimicrobial agent to treat various clinical conditions associated with multiple infectious diseases. Further studies are in progress to optimise these lead compounds and fully characterised the mode of action.

5. Experimental

5.1. Chemistry

Melting points were determined in open capillaries using Thermonik Precision Melting point cum Boiling point apparatus, Model C-PMB-2 and are uncorrected. Purity of synthesised compounds was checked by pre-coated TLC plates (E. Merck Kieselgel 60 F_{254}). IR spectra were recorded using KBr pellets on a Perkin–Elmer 337 Spectrophotometer (ν_{max} in cm^{-1}). ^{1}H- and ^{13}C-NMR spectra on a Bruker W.M. 400 Spectrometer at 360 MHz using TMS (tetramethylsilane) as internal standard. The chemical shifts (δ) are reported in part per million (ppm) relative to TMS in $CDCI_3$ solution. Signal multiplicities are presented by s (singlet), d (doublet), t (triplet), q (quartet), br-s (broad singlet) and m (multiplet). Mass spectra (EI-MS) were recorded at 70 eV on a JEOL D-300 spectrometer. Elemental analyses were carried out using Heraus Carlo Erba 1180 CHN analyser and values within limit of $\pm$0.4% of the theoretical values were taken into consideration. All the chemicals used for the synthesis were purchased from Aldrich Company Ltd, Dorset (UK).

5.1.1. Synthesis of 2-amino-4-(2-furanyl)-thiophene-3-carboxylic acid ethyl ester (**1**)

Starting material 2-amino-4-(2-furanyl)-thiophene-3-carboxylic acid ethyl ester (**1**) was prepared from 2-cyano-3-(2-furanyl)-but-2-enoic acid ethyl ester according to the literature method [24].

White crystals; R_f: 0.56 ($CHCl_3$); yield 70%; m.p.: 86–87 °C; IR (KBr): 3308 and 3200 (N–H stretching), 2962 and 2872 (C–H stretching), 1730 (C=O stretching), 1250 (C–O stretching), 1056 (O–C_2H_5 stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.13 (s, 3H, –CO–O–CH_2–CH_3), 4.43–4.50 (q, 2H, –CO–O–CH_2–CH_3), 5.86 (s, 2H, NH_2), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.57 (s, 1H, thiophene) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.2 (–O–CH_2–CH_3), 39.5 (–O–CH_2–CH_3), 109.1–110.7 (furyl), 125.4–127.23 (thiophene), 142.6–142.9 (furyl), 170.2 (–$COOC_2H_5$) ppm; EIMS (m/z, %): 237 [M^+, 100%].

5.1.2. Synthesis of 2-acetylamino-4-(2-furanyl)-thiophene-3-carboxylic acid ethyl ester (**2**)

A mixture of **1** (0.004 mol), acetic anhydride (0.012 mol) and zinc dust (0.28g) was warmed on a water bath for 10 min with constant stirring. The resulting clear solution was cooled at room temperature and solid so obtained was filtered. The residue so obtained was recrystallised from MeOH [25].

White crystals; R_f: 0.66 ($CHCl_3$); yield 80%; m.p.: 103–104 °C; IR (KBr): 3255 (N–H stretching), 2962 and 2872 (C–H stretching), 1730 (C=O stretching), 1250 (C–O stretching), 1056 (O–C_2H_5 stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.13 (s, 3H, –CO–O–CH_2–CH_3), 1.17 (s, 3H, –NH–CO–CH_3), 4.43–4.50 (q, 2H, –CO–O–CH_2–CH_3), 5.86–5.89 (m, 1H, NH), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.57 (s, 1H, thiophene) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.1 (–O–CH_2–CH_3), 26.2 (–NH–CO–CH_3), 39.4 (–O–CH_2–CH_3), 109.1–110.2 (furyl), 125.4–127.3 (thiophene), 142.6–142.9 (furyl), 170.0 and 172.6 (–$COOC_2H_5$ and –NH–CO–CH_3), ppm; EIMS (m/z, %): 279 [M^+, 100%]. Anal. ($C_{13}H_{13}NSO_4$) C, H, N.

5.1.3. Synthesis of 3-amino-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**3**)

To a solution of **2** (0.014 mol) in dry EtOH (10 mL), hydrazine hydrate (80%, 0.014 mol) was added and the reaction mixture was heated under reflux for 3 h on water bath. Excess of the solvent was distilled-off in vacuo. The residue so obtained was recrystallised from C_3H_6O [25].

White crystals; R_f: 0.61 ($CHCl_3$); yield 57%; mp: 174–175 °C; IR (KBr): 3308 and 3200 (N–H stretching), 2962 and 2872 (C–H stretching), 1730 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.30 (s, 3H, CH_3), 4.86–4.89 (m, 2H, NH_2), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.57 (s, 1H, thiophene) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.0 (–CH_3), 109.0–111.1 (furyl), 125.4–127.23 (thiophene), 142.6 (furyl), 150.4 (pyrimidine), 170.2 (–CO of pyrimidine) ppm; EIMS (m/z, %): 247 [M^+, 100%]. Anal. ($C_{11}H_9N_3SO_2$) C, H, N.

5.1.4. Synthesis of N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-carboxamide (**4a–m**)

A mixture of **3** (0.002 mol) and Et_3N (0.002 mol) in dry dioxane (10 mL) was heated at 70–80 °C for 30 min. To this reaction mixture, various substituted acid chloride (0.002 mol) was added dropwise with constant stirring. The reaction mixture was heated at 100–110 °C for 4 h. Excess of the solvent was distilled-off in vacuo and 50 mL of ice-cold water was added. The residue so obtained was filtered, washed with $NaHCO_3$ solution (5%) and recrystallised from EtOH. Using the above procedure 13 such compounds **4a–m** were synthesised and characterised and their spectral data are as follows.

5.1.4.1. N-[5-(2-Furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-benzamide (**4a**).

White crystals; R_f: 0.51 ($CHCl_3$); IR (KBr): 3202 (N–H stretching), 2960 and 2874 (C–H stretching), 1731 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.31 (s, 3H,

CH_3), 5.85–5.89 (m, 1H, –N*H*–CO–R), 6.43–6.46 (q, 2H, furyl), 7.41–7.42 (d, 1H, furyl), 7.11–7.57 (m, 1H, thiophene and 5H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.2 (–*C*H_3), 108.0–109.6 (furyl), 125.4–127.2 (thiophene and aromatic), 140.1 (–NH–*CO*–R), 142.6 (furyl), 150.0 (pyrimidine), 170.8 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 351 [M^+, 100%].

*5.1.4.2. 4-Chloro-N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-benzamide (**4b**).* White crystals; R_f: 0.52 ($CHCl_3$); IR (KBr): 3210 (N–H stretching), 2965 and 2870 (C–H stretching), 1729 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.30 (s, 3H, C*H*$_3$), 5.86–5.89 (m, 1H, –N*H*–CO–R), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.10–7.57 (m, 1H, thiophene and 4H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.3 (–*C*H_3), 108.5–109.2 (furyl), 124.4–127.2 (thiophene and aromatic), 139.2 (–NH–*CO*–R), 141.6 (furyl), 151.0 (pyrimidine), 170.4 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 387 [M^+ +2, 89%].

*5.1.4.3. 2,4-Dichloro-N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-benzamide (**4c**).* White crystals; R_f: 0.55 ($CHCl_3$); IR (KBr): 3205 (N–H stretching), 2964 and 2876 (C–H stretching), 1728 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.29 (s, 3H, C*H*$_3$), 5.80–5.88 (m, 1H, –N*H*–CO–R), 6.43–6.46 (q, 2H, furyl), 7.43–7.44 (d, 1H, furyl), 7.11–7.66 (m, 1H, thiophene and 3H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.9 (–*C*H_3), 109.6–110.0 (furyl), 125.4–127.2 (thiophene and aromatic), 141.8 (–NH–*CO*–R), 142.9 (furyl), 152.0 (pyrimidine), 173.1 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 421 [M^+ +2, 76%].

*5.1.4.4. 4-Fluoro-N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-benzamide (**4d**).* White crystals; R_f: 0.49 ($CHCl_3$); IR (KBr): 3200 (N–H stretching), 2965 and 2872 (C–H stretching), 1732 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.32 (s, 3H, C*H*$_3$), 5.76–5.84 (m, 1H, –N*H*–CO–R), 6.41–6.47 (q, 2H, furyl), 7.36–7.43 (d, 1H, furyl), 7.14–7.57 (m, 1H, thiophene and 4H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.5 (–*CH*$_3$), 110.0–110.9 (furyl), 125.4–127.3 (thiophene and aromatic), 139.5 (–NH–*CO*–R), 142.6 (furyl), 150.3 (pyrimidine), 170.6 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 371 [M^+ +2, 56%].

*5.1.4.5. 2,4-Difluoro-N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-benzamide (**4e**).* White crystals; R_f: 0.59 ($CHCl_3$); IR (KBr): 3209 (N–H stretching), 2962 and 2876 (C–H stretching), 1733 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.35 (s, 3H, C*H*$_3$), 5.76–5.89 (m, 1H, –N*H*–CO–R), 6.43–6.49 (q, 2H, furyl), 7.43–7.46 (d, 1H, furyl), 7.11–7.57 (m, 1H, thiophene and 3H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 22.0 (–*C*H_3), 42.6 (–O*C*H_3), 108.0 (furyl), 125.4–127.2 (thiophene and aromatic), 140.6 (–NH–*CO*–R), 142.6 (furyl), 150.4 (pyrimidine), 170.2 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 389 [M^+, 67%].

*5.1.4.6. 4-Methoxy-N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-benzamide (**4f**).* White crystals; R_f: 0.53 ($CHCl_3$); IR (KBr): 3199 (N–H stretching), 2959 and 2875 (C–H stretching), 1730 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.28 (s, 3H, CH_3), 2.51 (s, 3H, –Ar–O–C*H*$_3$), 5.66–5.69 (m, 1H, –N*H*–CO–R), 6.41–6.43 (q, 2H, furyl), 7.41–7.43 (d, 1H, furyl), 7.11–7.98 (m, 1H, thiophene and 4H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 19.2 (–*C*H_3), 108–109.7 (furyl), 125.4–127.2 (thiophene and aromatic), 140.5 (–NH–*CO*–R), 142.1 (furyl), 150.6 (pyrimidine), 170.0 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 381 [M^+,100%].

*5.1.4.7. 4-Nitro-N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-benzamide (**4g**).* White crystals; R_f: 0.56 ($CHCl_3$); IR (KBr): 3200 (N–H stretching), 2962 and 2872 (C–H stretching), 1730 (C=O stretching), 1490–1500 (O=N=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.30 (s, 3H, C*H*$_3$), 5.86–5.89 (m, 1H, –N*H*–CO–R), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.10–7.87 (m, 1H, thiophene and 4H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.0 (–*C*H_3), 109–110.1 (furyl), 125.4–127.2 (thiophene and aromatic), 140.1 (–NH–*CO*–R), 142.6 (furyl), 150.4 (pyrimidine), 170.3 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 396 [M^+,100%].

*5.1.4.8. 4-Methyl-N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-benzamide (**4h**).* White crystals; R_f: 0.41 ($CHCl_3$); IR (KBr): 3200 (N–H stretching), 2965 and 2878 (C–H stretching), 1728 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.34 (s, 6H, –C*H*$_3$, –NH–CO–Ar–C*H*$_3$), 5.84–5.89 (m, 1H, –N*H*–CO–R), 6.43–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.10–7.81 (m, 1H, thiophene and 4H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.6 (–*C*H_3), 109.0–110.4 (furyl), 125.4–127.2 (thiophene and aromatic), 140.3 (–NH–*CO*–R), 142.6 (furyl), 150.0 (pyrimidine), 170.6 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 365 [M^+, 100%].

*5.1.4.9. Methyl-N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-acetamide (**4i**).* White crystals; R_f: 0.42 ($CHCl_3$); IR (KBr): 3201 (N–H stretching), 2960 and 2873 (C–H stretching), 1730 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.32 (s, 6H, –C*H*$_3$, –NH–CO–C*H*$_3$), 5.86–5.89 (m, 1H, –N*H*–CO–R), 6.44–6.46 (q, 2H, furyl), 7.10–7.20 (m, 1H, thiophene), 7.40–7.42 (d, 1H, furyl) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.6 (–*C*H_3), 109.0–111.0 (furyl), 125.4–127.3 (thiophene), 140.1 (–NH–*CO*–R), 142.6

(furyl), 150.3 (pyrimidine), 170.7 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 289 [M^+, 100%].

*5.1.4.10. Cyclopropyl-N-[5-(2-furan-yl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-acetamide (**4j**).* White crystals; R_f: 0.45 ($CHCl_3$); IR (KBr): 3205 (N–H stretching), 2962 and 2872 (C–H stretching), 1733 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.56–0.91 (m, 4H, cyclopropyl), 1.44–1.90 (m, 1H, cyclopropyl), 2.34 (s, 3H, –C*H*$_3$), 5.86–5.89 (m, 1H, –N*H*–CO–R), 6.44–6.46 (q, 4H, furyl), 7.10–7.20 (m, 1H, thiophene), 7.40–7.42 (d, 2H, furyl) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 14.2 (*C*H of cyclopropyl), 20.4 (–*C*H$_3$), 109.1–110.1 (furyl), 25.2–27.4 (*C*H$_2$ of cyclopropyl), 125.4–127.3 (thiophene), 140.0 (–NH–*CO*–R), 142.6 (furyl), 150.2 (pyrimidine), 170.5 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 315 [M^+,100%].

*5.1.4.11. Cyclohexyl-N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-acetamide (**4k**).* White crystals; R_f: 0.46 ($CHCl_3$); IR (KBr): 3200 (N–H stretching), 2963 and 2877 (C–H stretching), 1730 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.44–1.90 (m, 11H, cyclohexyl), 2.30 (s, 3H, –C*H*$_3$), 5.86–5.89 (m, 1H, –N*H*–CO–R), 6.44–6.46 (q, 2H, furyl), 7.10–7.20 (m, 1H, thiophene), 7.40–7.42 (d, 1H, furyl) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 14.2 (cyclohexyl), 20.1 (–*C*H$_3$), 25.0–27.6 (cyclohexyl), 108.0–109.1 (furyl), 125.4–127.2 (thiophene), 140.1 (–NH–*CO*–R), 142.6 (furyl), 150.2 (pyrimidine), 170.4 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 357 [M^+,100%].

*5.1.4.12. 2-Furyl-N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-acetamide (**4l**).* White crystals; R_f: 0.47 ($CHCl_3$); IR (KBr): 3209 (N–H stretching), 2962 and 2871 (C–H stretching), 1729 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.34 (s, 3H, C*H*$_3$), 5.86–5.89 (m, 1H, –N*H*–CO–R), 6.44–6.76 (q, 4H, furyl), 7.10–7.20 (m, 1H, thiophene), 7.40–7.42 (d, 2H, furyl) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.6 (–*C*H$_3$), 109.0–110.5 (furyl), 125.4–127.3 (thiophene), 140.4 (–NH–*CO*–R), 142.6 (furyl), 150.2 (pyrimidine), 170.0 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 341 [M^+, 100%].

*5.1.4.13. 2-Thienyl-N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-acetamide (**4m**).* White crystals; R_f: 0.49 ($CHCl_3$); IR (KBr): 3201 (N–H stretching), 2960 and 2872 (C–H stretching), 1730 (C=O stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.30 (s, 3H, C*H*$_3$), 5.86–5.89 (m, 1H, –N*H*–CO–R), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.10–7.50 (m, 4H, thiophene) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.0 (–*C*H$_3$), 109.0–109.6 (furyl), 125.4–127.3 (thiophene), 140.0 (–NH–*CO*–R), 142.6 (furyl), 150.1 (pyrimidine), 170.7 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 357 [M^+, 100%].

*5.1.5. Synthesis of 3-substituted-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5a**–**m**)*

A mixture of **3** (0.002 mol) and various substituted aldehyde (0.002 mol) in 10 mL of acetic acid was heated under reflux for 4 h. Excess of the solvent was distilled-off in vacuo and 50 mL of ice-cold water was added. The residue so obtained was filtered, and recrystallised from EtOH. Using the above procedure eleven such compounds **5a**–**m** were synthesised and characterised and their spectral data are as follows.

*5.1.5.1. 3-(Benzylidene-amino)-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5a**).* White crystals; R_f: 0.36 ($CHCl_3$); IR (KBr): 2960 and 2871 (C–H stretching), 1731 (C=O stretching), 1620 (C=N stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.10 (s, 3H, C*H*$_3$), 2.20–2.41 (m, 1H, –N=*CH*–R), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.10–7.77 (m, 1H, thiophene and 5H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.2 (–*C*H$_3$), 36.7 (–N=*CH*–R), 109.0–109.2 (furyl), 125.4–127.3 (thiophene and aromatic), 142.6 (furyl), 150.0 (pyrimidine), 170.0 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 335 [M^+, 100%].

*5.1.5.2. 3-[(4-Chloro-benzylidene)-amino]-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5b**).* White crystals; R_f: 0.34 ($CHCl_3$); IR (KBr): 2965 and 2873 (C–H stretching), 1733 (C=O stretching), 1620 (C=N stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.11 (s, 3H, C*H*$_3$), 2.20–2.42 (m, 1H, –N=*CH*–R), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.10–7.87 (m, 1H, thiophene and 4H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.2 (–*C*H$_3$), 36.5 (–N=*CH*–R), 109.0–110.1 (furyl), 125.4–127.3 (thiophene and aromatic), 142.6 (furyl), 150.0 (pyrimidine), 170.0 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 371 [M^+ +2, 90%].

*5.1.5.3. 3-[(2,4-Chloro-benzylidene)-amino]-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5c**).* White crystals; R_f: 0.38 ($CHCl_3$); IR (KBr): 2960 and 2875 (C–H stretching), 1730 (C=O stretching), 1622 (C=N stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.09 (s, 3H, CH_3), 2.20–2.46 (m, 1H, –N=*CH*–R), 6.44–6.47 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.11–7.57 (m, 1H, thiophene and 3H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.9 (–*C*H$_3$), 36.8 (–N=*CH*–R), 109.0–109.9 (furyl), 125.4–127.2 (thiophene and aromatic), 142.6 (furyl), 150.8 (pyrimidine), 170.6 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 405 [M^+ +2, 60%].

*5.1.5.4. 3-[(4-Fluoro-benzylidene)-amino]-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5d**).* White crystals; R_f: 0.39 ($CHCl_3$); IR (KBr): 2962

and 2870 (C–H stretching), 1731 (C=O stretching), 1620 (C=N stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.17 (s, 3H, CH_3), 2.20–2.41 (m, 1H, –N=C*H*–R), 6.44–6.46 (q, 2H, furyl), 7.39–7.40 (d, 1H, furyl), 7.12–7.97 (m, 1H, thiophene and 4H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.8 (–*C*H_3), 36.5 (–N=*C*H–R), 108.2–109.4 (furyl), 125.4–127.2 (thiophene and aromatic), 142.6 (furyl), 150.0 (pyrimidine), 170.0 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 355 [M^+ +2, 70%].

*5.1.5.5. 3-[(2,4-Fluoro-benzylidene)-amino]-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5e**).* White crystals; R_f: 0.40 ($CHCl_3$); IR (KBr): 2962 and 2871 (C–H stretching), 1730 (C=O stretching), 1620 (C=N stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.09 (s, 3H, CH_3), 2.21–2.40 (m, 1H, –N=C*H*–R), 6.44–6.46 (q, 2H, furyl), 7.02–7.04 (d, 1H, furyl), 7.10–7.97 (m, 1H, thiophene and 3H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.5 (–*C*H_3), 36.4 (–N=*C*H–R), 109.2–110.1 (furyl), 125.4–127.2 (thiophene and aromatic), 142.6 (furyl), 150.8 (pyrimidine), 170.9 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 373 [M^+ +2, 90%].

*5.1.5.6. 3-[(4-Methoxy-benzylidene)-amino]-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5f**).* White crystals; R_f: 0.31 ($CHCl_3$); IR (KBr): 2965 and 2874 (C–H stretching), 1733 (C=O stretching), 1620 (C=N stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.01 (s, 3H, CH_3), 2.11 (s, 3H, –N=CH–Ar–O–CH_3), 2.41–2.47 (m, 1H, –N=C*H*–R), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.12–7.87 (m, 1H, thiophene and 4H, Ar–H) ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.5 (–*C*H_3), 36.2 (–N=*C*H–R), 42.1 (*C*H_3–O–C_6H_4–), 109.1–109.9 (furyl), 125.4–127.2 (thiophene and aromatic), 142.6 (furyl), 150.0 (pyrimidine), 170.4 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 365 [M^+, 100%].

*5.1.5.7. 3-[(4-Nitro-benzylidene)-amino]-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5g**).* White crystals; R_f: 0.36 ($CHCl_3$); IR (KBr): 2962 and 2872 (C–H stretching), 1730 (C=O stretching), 1620 (C=N stretching), 1490–1510 (O=N=O stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.09 (s, 3H, CH_3), 2.40–2.41 (m, 1H, –N=C*H*–R), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.10–7.57 (m, 1H, thiophene and 4H, Ar–H), ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.8 (–*C*H_3), 36.7 (–N=*C*H–R), 109.0–110.7 (furyl), 125.4–127.3 (thiophene and aromatic), 142.6 (furyl), 150.2 (pyrimidine), 170.0 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 380 [M^+, 100%].

*5.1.5.8. 3-[(4-Methyl-benzylidene)-amino]-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5h**).* White crystals; R_f: 0.37 ($CHCl_3$); IR (KBr): 2959 and 2868 (C–H stretching), 1728 (C=O stretching), 1620 (C=N stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.11 (s, 6H, –CH_3, –N=CH–Ar–CH_3), 2.20–2.41 (m, 1H, –N=C*H*–R), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.10–7.77 (m, 1H, thiophene and 4H, aromatic), ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.0 (–*C*H_3 and –N=CH–Ar–*C*H_3), 36.1 (–N=*C*H–R), 109.0–110.1 (furyl), 125.4–127.2 (thiophene and aromatic), 142.6 (furyl), 150.1 (pyrimidine), 170.1 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 349 [M^+, 100%].

*5.1.5.9. 3-Ethylideneamino-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5i**).* White crystals; R_f: 0.38 ($CHCl_3$); IR (KBr): 2962 and 2872 (C–H stretching), 1735 (C=O stretching), 1625 (C=N stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.12 (s, 6H, CH_3 and –N=CH–CH_3), 2.20–2.40 (m, 1H, –N=C*H*–R), 6.44–6.46 (q, 2H, furyl), 7.41–7.42 (d, 1H, furyl), 7.12–7.57 (m, 1H, thiophene), ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.5 (–*C*H_3 and –N=CH–*C*H_3), 36.2 (–N=*C*H–R), 108.8–109.6 (furyl), 125.4–127.3 (thiophene), 142.6 (furyl), 150.1 (pyrimidine), 170.1 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 273 [M^+, 100%].

*5.1.5.10. 3-(Cyclopropylmethylene-amino)-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5j**).* White crystals; R_f: 0.33 ($CHCl_3$); IR (KBr): 2962 and 2872 (C–H stretching), 1730 (C=O stretching), 1626 (C=N stretching), cm^{-1}; ^{1}H–NMR ($CDCl_3$): δ 0.86–0.88 (d, 4H, CH_2 of cycolpropyl), 1.22–1.23 (m, 1H, C*H* of cyclopropyl), 2.09 (s, 3H, –CH_3), 2.20–2.40 (m, 1H, –N=C*H*–R), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.10–7.57 (m, 1H, thiophene), ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.0 (–*C*H_3), 24.4 (–*C*H_2–*C*H_2– of cyclopropyl), 29.6 (–*C*H– of cyclopropyl), 36.1 (–N=*C*H–R), 109.2–110.1 (furyl), 125.4–127.3 (thiophene), 142.6 (furyl), 150.1 (pyrimidine), 170.6 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 299 [M^+, 100%].

*5.1.5.11. 3-(Cyclohexylmethylene-amino)-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5k**).* White crystals; R_f: 0.39 ($CHCl_3$); IR (KBr): 2960 and 2876 (C–H stretching), 1730 (C=O stretching), 1628 (C=N stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.43–1.92 (m, 11H, cyclohexyl), 2.11 (s, 3H, CH_3), 2.20–2.40 (m, 1H, –N=C*H*–R), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.1–7.57 (m, 1H, thiophene), ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 14.2 (cyclohexyl), 20.4 (–*C*H_3), 25.0–27.6 (cyclohexyl), 36.0 (–N=*CH*–R), 109.1–109.8 (furyl), 125.4–127.2 (thiophene), 142.6 (furyl), 150.7 (pyrimidine), 170.1 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 341 [M^+, 100%].

*5.1.5.12. 3[(Furan-2-ylmethylene)amino]-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5l**).* White crystals; R_f: 0.46 ($CHCl_3$); IR (KBr): 2962

and 2872 (C–H stretching), 1730 (C=O stretching), 1620 (C=N stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.08 (s, 3H, CH_3), 2.20–2.42 (m, 1H, –N=*CH*–R), 6.44–6.46 (q, 4H, furyl), 7.40–7.42 (d, 2H, furyl), 7.11–7.57 (m, 1H, thiophene), ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.5 (–CH_3), 36.5 (–N=*CH*–R), 109.0–110.5 (furyl), 125.4–127.2 (thiophene), 142.6 (furyl), 150.0 (pyrimidine), 170.6 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 325 [M^+, 100%].

*5.1.5.13. 3[(Thiophen-2-ylmethylene)amino]-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-one (**5m**).* White crystals; R_f: 0.36 ($CHCl_3$); IR (KBr): 2960 and 2875 (C–H stretching), 1733 (C=O stretching), 1620 (C=N stretching), cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.12 (s, 6H, CH_3), 2.20–2.42 (m, 1H, –N=*CH*–R), 6.44–6.46 (q, 2H, furyl), 7.40–7.42 (d, 1H, furyl), 7.10–7.57 (m, 4H, thiophene), ppm; ^{13}C-NMR (360 MHz, $CDCl_3$): δ 20.9 (–CH_3), 36.1 (–N=*CH*–R), 109.1–109.8 (furyl), 125.4–127.2 (thiophene), 142.6 (furyl), 150.8 (pyrimidine), 170.8 (–*C*O of pyrimidine) ppm; EIMS (*m*/*z*, %): 341 [M^+, 100%].

5.2. Pharmacology

5.2.1. Antibacterial and antimycobacterial activity

All the test compounds was assayed in vitro for antibacterial activity against two different strains of Gram-negative (*E. coli* ATCC 25922 and *S. typhi* CNCTC 786) and Gram-positive (*S. aureus* ATCC 3750, *B. subtilis* 6633) bacteria and the antimycobacterial activity was evaluated against *M. tuberculosis* CNCTC My 331/88 and *M. avium* CNCTC My 330/88 strains. The MIC was determined by the test tube dilution technique using Mueller–Hinton nutrient broth (for antibacterial) and modified Kirchner's culture medium containing 0.5% sterilised horse serum for antimycobacterial activity [26]. The MIC values were also tested for three well-known antibiotics (penicillin-G, ampicillin, and chloramphenicol) to compare the antibacterial activity of our test compounds with the antibiotics, which are currently in therapy. Rifampcin and isoniazid (INH) were used as reference standard for antimycobacterial activity. The stock solution (2–4 mg mL^{-1}) of test compounds was prepared in a mixture of sterile water and dimethylformamide (8:2) solvent. The stalk solution was sterilised by passing through a 0.2 μm polycarbonate sterile membrane (Nuclepore) filters. Further the serial dilution of test compounds was carried out and the following concentration was used: 1000, 500, 250, 125, 62, 32, 16, 8, 4 and 1 μmol L^{-1}. Test compounds at various concentrations were added to culture medium in a sterilised borosilicate test tube and different bacterial strains were inoculated at 10^6 bacilli mL^{-1} concentration. The tubes were incubated at 37 °C for 24 h for antibacterial activity and 14 and 21 days for antimycobacterial activity and then examined for the presence or absence of growth of the test organisms. All experiments were performed in triplicate. The MIC values were obtained from the lowest concentration of the test compound where the tubes remained clear, indicated that the bacterial growth was completely inhibited at this concentration. The MIC values were expressed in μmol L^{-1} and the results are shown in Table 2.

5.2.2. Hemolytic assay

Fresh human blood (5 mL) was centrifuged at 700 × *g* (3500 rpm) for 10 min and the erythrocytes (RBCs) were collected. The erythrocytes were washed and centrifuged three times with phosphate buffered saline (PBS, pH 7.4, 50 mL) and the supernatant was decanted carefully. The erythrocyte suspension was diluted two times with saline solution and total erythrocytes were counted using haemocytometer. Finally necessary dilution was carried out with saline solution to get total count to 6.4×10^8 cells mL^{-1}. The stock solution of the test compounds was made at an initial concentration of 4 mg mL^{-1} using the same saline solution. Assay was carried out in 1 mL eppendorf tubes. In an eppendorf, varying volumes (900–650 μL) of the saline solution was added. To this varying volume of the stock solution of test compounds (1–250 μL) was added followed by 100 μL of RBC suspension. For the positive control i.e. total cell lyses, 10 μL of 20% w/v Triton was added instead of the test compounds and the plain saline solution was use as control. The eppendorf tubes were then incubated at 37 °C for 30 min, tubes were centrifuge at 4000 rpm for 10 min and carefully 200 μL of the supernatant was transferred to a 96-well microtritre plates with U-shaped wells. The absorbance of the samples was read at 404 nm with Spectra Max-190 microplate spectrophotometer. The percentage lyses was determined as $(A_{\text{test compounds}} - A_{\text{blank}})/(A_{\text{positive control}} - A_{\text{blank}})*100$. Further the % lyses was plotted against Log concentration of test compounds used (Fig. 3) and from the graph MHC and LD_{50} (concentration required for 50% lyses) was determined [27].

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