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ScienceDirect
European Journal of Medicinal Chemistry 41 (2006) 1293–1300
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://france.elsevier.com/direct/ejmech

Original article

Synthesis, analgesic, anti-inflammatory, ulcerogenic index and antibacterial activities of novel 2-methylthio-3-substituted-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*]pyrimidin-4(3*H*)-ones

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Received 13 December 2005; received in revised form 12 June 2006; accepted 14 June 2006
Available online 22 August 2006

Abstract

A variety of novel 2-methylthio-3-substituted-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*]pyrimidin-4(3*H*)-ones have been synthesized by reacting (2-methylthio-4-oxo-3*H*-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*]pyrimidin-3-yl)dithiocarbamic acid methyl ester (**5**) with a variety of amines. The starting material dithiocarbamate (**5**) was synthesized from 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo (*b*) thiophene (**1**) by a novel innovative route. The title compounds were investigated for analgesic, anti-inflammatory, ulcerogenicity index and antibacterial activities. While the test compounds exhibited significant activity, the compounds 1-methyl-3-(2-methylthio-4-oxo-3*H*-5,6,7,8-tetrahydrobenzo (*b*) thieno [2,3-*d*]pyrimidin-3-yl)thiourea (**A1**), 1-dimethyl-3-(2-methylthio-4-oxo-3*H*-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*]pyrimidin-3-yl)thiourea (**A2**), 1-diethyl-3-(2-methylthio-4-oxo-3*H*-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*]pyrimidin-3-yl)thiourea (**A3**) and 1-pyrrolidinyl-3-(2-methylthio-4-oxo-3*H*-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*]pyrimidin-3-yl)thiourea (**A4**) showed more potent analgesic activity and the compounds 1-dimethyl-3-(2-methylthio-4-oxo-3*H*-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*]pyrimidin-3-yl)thiourea (**A2**), 1-diethyl-3-(2-methylthio-4-oxo-3*H*-5,6,7,8-tetrahydrobenzo (*b*) thieno-[2,3-*d*]pyrimidin-3-yl)thiourea (**A3**) and 1-pyrrolidinyl-3-(2-methylthio-4-oxo-3*H*-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*] pyrimidin-3-yl)thiourea (**A4**) showed more potent anti-inflammatory activity than the reference standard diclofenac sodium.

Keywords: Thienopyrimidine; Thioureas; Pyrimidine; Analgesic; Anti-inflammatory

1. Introduction

Bacterial infections often produce pain and inflammation. In normal practice, two groups of agents (chemotherapeutic, analgesic and anti-inflammatory) are prescribed simultaneously. Unfortunately, none of drug possesses these three activities in a single component. On our going medicinal chemistry research programme we have find that quinazolines and condensed quinazolines exhibit potent antimicrobial [1] and central nervous system (CNS) activities like analgesic and anti-inflammatory [2] activities. Recently reports have shown that thienopyrimidines (bioisotere of quinazoline) possess CNS and antibacterial activities [3–5]. Exploiting the bioisosterism concept, we have documented 2-phenyl-3-substituted quinazolines [6], 2,3-disubstituted quinazolines [7], 2-methyl-3-substituted quinazolin-4-(*3H*)-ones [8] and 2-methylthio-3-substituted quinazolin-4-(*3H*)-ones [9] that exhibited good analgesic and anti-inflammatory activities. The present work is an extension of our ongoing efforts towards the development and identification of new molecules by bioisotere concept, we have aimed to synthesize some novel 2-methylthio-3-substituted-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*] pyrimidin-4(3*H*)-ones and screened for analgesic, anti-inflammatory, ulcerogenicity index and antibacterial activities.

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doi:10.1016/j.ejmech.2006.06.005

2. Chemistry

The title compounds were synthesized by nucleophilic substitution of (2-methylthio-4-oxo-3*H*-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*]pyrimidin-3-yl) dithiocarbamic acid methyl ester (**5**) with different amines. The starting material (2-methylthio-4-oxo-3*H*-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*] pyrimidin-3-yl)dithiocarbamic acid methyl ester (**5**) was synthesized by reacting the amino group of 3-amino-2-methylthio-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*] pyrimidin-4(3*H*)-one (**4**) with carbon disulfide, sodium hydroxide and dimethyl sulfate. Compound **4** inturn was synthesized from 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo (*b*) thiophene (**1**) by a novel innovative route (Fig. 1). Thus 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo(*b*) thiophene (**1**), was treated with carbon disulfide and sodium hydroxide to afford sodium salt of dithiocarbamate, which upon methylation with dimethyl sulfate afforded methyl ester of dithiocarbamic acid **2**. Compound **2** on treatment with hydrazine hydrate yielded the thiosemicarbazide **3a** which undergoes internal cyclization to afford 3-amino-2-mercapto-5,6,7,8-tetrahydrobenzo (*b*) thieno [2,3-*d*]pyrimidin-4(3*H*)-one (**3**). Compound **3** upon methylation with dimethyl sulfate yielded the desired 3-amino-2-methylthio-5,6,7,8-tetrahydrobenzo (*b*) thieno-[2,3-*d*]pyrimidin-4(3*H*)-one (**4**). Spectral data (IR, NMR, and mass spectra) confirmed the structures of the synthesized compounds, the purity of these compounds was ascertained by microanalysis.

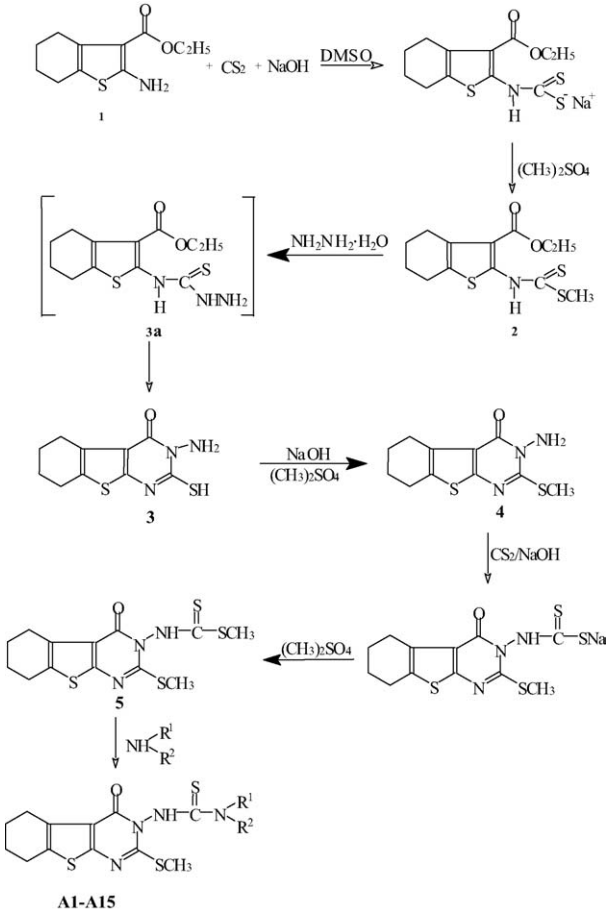

Fig. 1. Synthetic protocol of 2-methylthio-3-substituted-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*]pyrimidin-4-(*3H*)-ones.

3. Results and discussion

3.1. Analgesic activity

Test for analgesic activity was performed by tail-flick technique [10] using Wistar albino mice. The results of analgesic activity indicate that all the test compounds exhibited significant activity (Table 1). Compound **A1** with methyl substitution showed good activity; with increased lipophilicity (dimethyl group), compound **A2** showed an increase in activity. Further increase in lipophilicity (diethyl group) compound **A3** led to even greater activity. Substitution with alicyclic amine (pyrrolidine) compound **A4** showed moderate increase in activity. Placement of alicyclic amines with additional heteroatoms (compounds **A5** and **A6**) led to a decrease in activity. Aromatic substitution **A7**–**A15** showed still lower activity. The compounds with aliphatic substitution (compounds **A1**–**A4**) showed better activity. Compound 1-pyrrolidinyl-3-(2-methylthio-4-oxo-3*H*-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*]pyrimidin-3-yl) thiourea (**A4**) was found to be the most active analgesic agent and was more potent than diclofenac sodium.

3.2. Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan-induced paw edema test in rats [11]. The anti-inflammatory activity data (Table 2) indicated that all the test compounds protected rats from carrageenan-induced inflammation and are more potent than our earlier reported 2,3-disubtituted quinazolin-4(3*H*)-ones. Compounds **A2**–**A4** showed more anti-inflammatory activity than diclofenac sodium.

3.3. Evaluation of ulcerogenicity index [12–14]

The ulcer index of the test compounds (Table 3) reveals that the compounds **11**–**14** possessing electron withdrawing groups exhibited higher ulcer index than the other test compounds. The high ulcer index score for these compounds may be due to the suppression of the prostaglandin synthesis.

3.4. Antibacterial activity

Evaluation of antibacterial activity was done by agar dilution method [15]. The results of antibacterial activity (Table 4) indicate that all the test compounds exhibited moderate activity against the tested bacteria. Compound **A6** showed good activity against *Pseudomonas aeruginosa*, and *Proteus vulgaris*; compound **A8** exhibited good activity against *P. aeruginosa*, *Salmonella typhimurium* and *P. vulgaris*; compound **A13** exhibited good activity against *Bacillus subtilis*, *Edwersiella tarda* and *S. typhimurium*. While the compound **A14**

Table 1
PAA of test compounds (Tail-flick technique)

Compound code	Dose (mg kg^{-1})	PAA			
		30 min	1 h	2 h	3 h
A1	10	46 ± 1.15*	46 ± 1.42*	53 ± 1.31**	36 ± 1.47*
	20	61 ± 1.92***	65 ± 1.31***	69 ± 1.03***	50 ± 1.73**
A2	10	52 ± 1.22**	55 ± 1.07**	55 ± 1.36**	43 ± 1.47*
	20	60 ± 1.51***	69 ± 1.56***	74 ± 1.84***	47 ± 1.72*
A3	10	55 ± 1.09**	57 ± 1.32**	65 ± 1.36***	46 ± 1.32*
	20	69 ± 1.05***	74 ±1.93***	75 ± 1.51***	58 ± 1.17**
A4	10	56 ± 1.21**	62 ± 1.15***	66 ± 1.42***	47 ± 1.26*
	20	73 ± 1.05***	76 ± 1.39***	77 ± 1.31***	60 ± 1.39***
A5	10	35 ± 1.16*	42 ± 0.95*	43 ± 1.05**	32 ± 1.32*
	20	46 ± 1.51*	53 ± 1.49**	59 ± 1.67**	36 ± 1.09*
A6	10	37 ± 1.09*	40 ± 1.18*	39 ± 1.16*	35 ± 1.32*
	20	50 ± 1.34**	52 ± 1.02**	55 ± 1.37**	37 ± 1.49*
A7	10	39 ± 1.12*	39 ± 1.12*	47 ± 1.12*	30 ± 1.08*
	20	46 ± 1.93*	54 ± 1.41**	56 ± 1.39**	37 ± 1.23*
A8	10	35 ± 1.16*	38 ± 1.04**	45 ± 1.34*	31 ± 1.63*
	20	43 ± 1.92*	49 ± 1.37*	53 ± 1.16**	38 ± 1.81*
A9	10	33 ± 1.12*	35 ± 1.26*	40 ± 1.41*	31 ± 1.61*
	20	41 ± 1.16*	47 ± 1.32*	48 ± 1.39*	36 ± 1.74*
A10	10	34 ± 1.21*	38 ± 1.90*	43 ± 1.23*	32 ± 1.71*
	20	43 ± 1.21*	48 ± 1.42*	51 ± 1.60**	39 ± 1.83*
A11	10	29 ± 1.17*	30 ± 1.19*	31 ± 1.17*	26 ± 1.06*
	20	43 ± 1.32*	45 ± 1.03*	46 ± 1.39*	34 ± 1.15*
A12	10	26 ± 1.37*	32 ± 1.41*	34 ± 1.39*	27 ± 1.31*
	20	41 ± 1.43*	52 ± 1.26**	49 ± 1.92**	37 ± 1.39*
A13	10	39 ± 1.93*	41 ± 1.71*	46 ± 1.63*	27 ± 1.32*
	20	49 ± 1.05**	50 ± 1.63**	49 ± 1.37**	30 ± 1.61*
A14	10	36 ± 1.31*	40 ± 1.46*	42 ± 1.21*	23 ± 1.72*
	20	45 ± 1.46*	47 ± 1.52*	45 ± 1.47*	27 ± 1.43*
A15	10	27 ± 1.39*	38 ± 1.69*	42 ± 1.31*	25 ± 1.51*
	20	41 ± 1.63*	45 ± 1.32*	49 ± 1.92**	33 ± 1.32*
Control		2 ± 0.35	6 ± 0.49	4 ± 0.59	4 ± 0.91
Diclofenac	10	37 ± 1.69*	43 ± 1.42*	45 ± 0.92*	33 ± 0.96*
	20	46 ± 0.95*	55 ± 1.16**	62 ± 1.49***	39 ± 1.13*

Each value represents the mean ± S.D. ($n = 6$). Significance levels $^{*}P < 0.5$, $^{**}P < 0.01$and $^{***}P < 0.001$ as compared with the respective control.

exhibited good activity against *Salmonella paratyphi*, *E. tarda* and *Klebsiella pneumoniae*.

4. Conclusions

In our earlier studies [6–9] we observed that the presence of alkyl groups exhibited more analgesic and anti-inflammatory activities over aryl groups at the N-3 position. Hence in the C-2 position also we made a substitution in such a way as to increase lipophilicity of the molecule. The placement of such a group enhanced the analgesic and anti-inflammatory activities. To compare the increase in activity we have taken the average of all the readings of reaction time noted for each compound for each pharmacological activity. The most active compound of the C-2 phenyl series showed 43% analgesic and 36% anti-inflammatory activity [6], whereas the C-2 methyl series lead molecule showed 50% analgesic and 44% anti-inflammatory activity [7]. Introduction of sulfur atom at C-2 position in the above series i.e. by placing methyl thio group at C-2 position showed 54% analgesic and 43% anti-inflammatory activity [9].

Replacement of benzene ring of the benzpyrimidine (quinazoline) series by its bioisostere along with the cyclohexyl ring afforded the present series with the increased lipophilicity. The results of the analgesic and anti-inflammatory activities of the present series showed that enhancement of activity (65% analgesic and 45% anti-inflammatory activity) but there is no increase in antibacterial activity. Interestingly these compounds showed mild ulcer index unlike other Non Steroidal Anti Inflammatory Drugs (NSAIDs). Hence this series could be developed as a novel class of analgesic and anti-inflammatory agents. However further structural modification is planned to increase not only the analgesic and anti-inflammatory activities also the antibacterial activity.

5. Experimental protocols

5.1. Chemistry

Melting points were determined in open capillary tubes on a Thomas Hoover apparatus and are uncorrected. IR spectra were recorded in KBr on a Shimadzu FT-IR, 8300 spectrometer (cm^{-1}), mass spectra on a MASPEC msw 9629 mass spectrometer at 70 eV and NMR spectra on varian 300 MHz spectrometer, using tetramethylsilane as internal standard. Elemental analyses were performed on Carlo erba 1108.

Table 2
Percent anti-inflammatory activity of test compounds (carrageenan-induced paw edema test in rats)

Compound code	Dose (mg kg^{-1})	Percent protection			
		30 min	1 h	2 h	3 h
A1	10	33 ± 1.29*	37 ± 1.39*	40 ± 1.16**	29 ± 1.09*
	20	43 ± 1.15**	49 ± 1.27**	53 ± 1.32***	41 ± 1.31**
A2	10	36 ± 1.07*	38 ± 1.19*	43 ± 1.32**	29 ± 1.51*
	20	48 ± 1.47**	56 ± 1.37***	62 ± 1.76***	40 ± 1.05**
A3	10	39 ± 1.32**	42 ± 1.51**	46 ± 2.32**	35 ± 1.32*
	20	47 ± 1.16**	59 ± 1.06***	62 ± 2.03***	46 ± 1.26**
A4	10	35 ± 1.19*	41 ± 1.04**	45 ± 1.47**	34 ± 1.03*
	20	46 ± 1.32**	59 ± 1.41***	59 ± 1.32**	42 ± 1.26**
A5	10	29 ± 1.15*	30 ± 1.42*	36 ± 1.31*	25 ± 1.25*
	20	39 ± 1.26**	49 ± 2.41**	57 ± 1.63***	34 ± 1.33*
A6	10	29 ± 1.61*	35 ± 1.56*	32 ± 1.31*	29 ± 1.51*
	20	40 ± 1.34**	50 ± 1.51***	56 ± 1.64***	32 ± 1.62*
A7	10	27 ± 1.26*	39 ± 1.42**	40 ± 1.32**	32 ± 1.21*
	20	42 ± 1.47**	46 ± 1.53**	48±1.39**	38 ± 1.39*
A8	10	25 ± 1.51*	35 ± 1.43*	39 ± 1.18**	30 ± 1.62*
	20	39 ± 1.54**	46 ± 2.38**	44 ± 1.83**	39 ± 1.04**
A9	10	22 ± 1.01*	32 ± 1.26**	35 ± 1.24*	27 ± 1.23*
	20	36 ± 1.23*	41 ± 1.38**	42 ± 1.46**	31 ± 1.33*
A10	10	25 ± 1.16*	34 ± 1.37*	37 ± 1.31*	29 ± 1.23*
	20	38 ± 1.32*	42 ± 1.63**	45 ± 1.47**	32 ± 1.26*
A11	10	26 ± 1.16*	29 ± 1.51*	33 ± 1.18*	26 ± 1.14*
	20	39 ± 1.28**	44 ± 1.83**	49 ± 1.14**	30 ± 1.83*
A12	10	23 ± 1.02*	27 ± 1.32*	30 ± 1.36*	24 ± 1.21*
	20	35 ± 1.19*	39 ± 1.42*	42 ± 1.42**	25 ± 1.32*
A13	10	31 ± 1.43*	35 ± 1.33**	33 ± 1.17*	24 ± 1.74*
	20	42 ± 1.22**	43 ± 1.62**	52 ± 1.95***	42 ± 1.92**
A14	10	27 ± 1.41*	30 ± 1.32*	31 ± 1.26*	21 ± 1.04*
	20	36 ± 1.56*	39 ± 1.39**	47 ± 1.45**	37 ± 1.21*
A15	10	29 ± 1.18*	29 ± 1.12*	31 ± 1.39*	23 ± 1.86*36
	20	33 ± 1.53*	34 ± 1.36**	39 ± 1.46**	36 ± 1.54*
Control		5.1 ± 0.29	6.1 ± 0.27	5.7 ± 0.32	3.2 ± 0.93
Diclofenac	10	32 ± 0.63*	38 ± 1.58*	39 ± 1.97*	33 ± 0.93*
	20	45 ± 1.61**	52 ± 0.92**	60 ± 1.52***	42 ± 1.36*

Each value represents the mean ± S.D. ($n = 6$). Significance levels *$P < 0.5$, **$P < 0.01$and ***$P < 0.001$ as compared with the respective control.

Table 3
Evaluation of ulcerogenicity index

Drug	Ulcer index
A1	0.91 ± 0.21*
A2	0.89 ± 0.27*
A3	0.83 ± 0.24*
A4	0.87 ± 0.27*
A5	0.93 ± 0.24*
A6	0.90 ± 0.29*
A7	0.73 ± 0.16*
A8	0.89 ± 0.36*
A9	0.94 ± 0.13
A10	0.79 ± 0.21*
A11	1.09 ± 0.23**
A12	1.13 ± 0.16**
A13	1.06 ± 0.31**
A14	1.17 ± 0.26**
A15	0.97 ± 0.38*
Control	1.05 ± 0.32
Aspirin	1.73 ± 0.41**

Dose 20 mg for test compounds and 200 mg kg^{-1} for aspirin. Each value represents the mean ± S.E.M. ($n = 6$). Significance levels *$P < 0.05$ and **$P < 0.01$ as compared with the respective control.

The 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo (*b*) thiophene (**1**), was prepared as per the procedure described by Gewald and Schinke [16].

*5.1.1. Synthesis of methyl N-(3-carbethoxy-4,5,6,7-tetrahydrobenzo (b) thienyl) dithiocarbamate (**2**)*

To a vigorously stirred solution of 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo (*b*) thiophene (**1**) (4.5 g, 0.02 mol) in dimethyl sulfoxide (10 ml) at room temperature, carbon disulfide (1.98 g, 0.026 mol) and aqueous sodium hydroxide (1.2 ml, 20 mol solution) were added simultaneously over 30 min, the stirring was continued for further 30 min. Dimethyl sulfate (2.5 g, 0.02 mol) was added drop wise to the reaction mixture with stirring at 5–10 °C, it was further stirred for 2 h and poured into ice-water, the solid obtained was filtered, dried and recrystallized from ethanol. Yield = 85%, m.p. 135–137 °C. IR: 3210 (NH), 1690 (C=O), 1060 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.4–1.8 (m, 8H, $(CH_2)_4$), 2.0 (t, 3H, -COOCH$_2$C$\underline{H}_3$), 4.1 (q, 2H, -COOC$\underline{H}_2$CH$_3$), 4.4 (s, 3H, -SCH$_3$) and 7.3 (s, 1H, -N$\underline{H}$CSSCH$_3$, D_2O exchangeable).MS (*m*/*z*): 315 (M^+). Anal. Calcd. for $C_{13}H_{17}NO_2S_3$: C, 49.51; H, 5.44; N, 4.47. Found: C, 49.49; H, 5.42; N, 4.51.

*5.1.2. Synthesis of 3-Amino-2-mercapto-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d] pyrimidin-4(3H)-one (**3**)*

To a solution of **2** (3.15 g, 0.01 mol) in ethanol 30 ml was treated with hydrazine hydrate (4.3 g, 0.01 mol, 99%) and

Table 4
Antibacterial activity[a] of test compounds by agar-dilution method

Compound Code	*B. subtilis*	*S. paratyphi*	*E. Tarda*	*P. aeruginosa*	*S .typhimurium*	*P. vulgaris*	*K. pneumoniae*
A1	39.06	39.06	39.06	156.25	78.12	78.12	78.12
A2	78.12	156.25	156.25	39.06	39.06	39.06	78.12
A3	78.12	78.12	78.12	156.25	78.12	39.06	78.12
A4	156.25	39.06	78.12	39.06	156.25	78.12	156.25
A5	78.12	39.06	78.12	39.06	39.06	78.12	78.12
A6	39.06	39.06	39.06	9.76	78.12	9.76	156.25
A7	78.12	156.25	78.12	78.12	156.25	78.12	39.06
A8	78.12	78.12	39.06	9.76	9.76	9.76	39.06
A9	156.25	78.12	39.06	78.12	78.12	78.12	156.25
A10	78.12	39.06	78.12	78.12	156.25	156.25	156.25
A11	78.12	39.06	78.12	39.06	78.12	39.06	39.06
A12	39.06	78.12	39.06	39.06	78.12	78.12	78.12
A13	9.76	156.25	9.76	78.12	9.76	39.06	78.12
A14	39.06	9.76	9.76	39.06	78.12	78.12	9.76
A15	39.06	78.12	78.12	39.06	39.06	39.06	39.06
Norflo-xacin	2.44	0.60	0.60	0.018	4.88	1.22	9.76

[a] MIC values (μg ml^{-1}).

refluxed on a water bath until the methylmercaptan evolution ceases (8 h). After cooling, the solid obtained was filtered, dried and recrystallized from ethanol/acetone mixture. Yield = 75%, m.p. 251–252 °C. IR: 3300, 3200 (NH_2), 1680 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.5–1.9 (m, 8H, $(CH_2)_4$), 3.2 (s, 1H, -SH) and 5.4 (s, 2H, -NH_2, D_2O exchangeable). MS (*m*/*z*): 253 (M^+). Anal. Calcd. for $C_{10}H_{11}N_3OS_2$: C, 47.47; H, 4.38; N, 16.68. Found: C, 47.40; H, 4.33; N, 16.66.

5.1.3. Synthesis of 3-Amino-2-methylthio-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d] pyrimidin-4(3H)-one (4)

To an ice cold solution of **3** (2.53 g, 0.01 mol) in dimethyl formamide (50 ml), sodium hydroxide (0.4 g, 0.01 mol) was added and the mixture was stirred for 1 h. To this dimethyl sulfate (1.25 g, 0.01 mol) was added drop wise with constant stirring. After the addition was completed, the reaction mixture was further stirred for 3 h at room temperature. It was then poured into ice-water and the solid obtained was filtered, washed with water, dried and recrystallized from ethanol/acetone mixture. Yield = 79%, m.p. 216–219 °C. IR: 3350, 3330 (NH_2), 1690 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.3–1.7 (m, 8H, $(CH_2)_4$), 3.9 (s, 3H, SCH_3) and 6.5 (s, 2H, 3-NH_2, D_2O exchangeable); MS (*m*/*z*) 267 (M^+). Anal. Calcd. for $C_{11}H_{13}N_3OS_2$: C, 49.42; H, 4.90; N, 15.71. Found: C, 49.49; H, 4.87; N, 15.73.

5.1.4. Synthesis of (2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d pyrimidin-3-yl) dithiocarbamic acid methyl ester (5)

To a vigorously stirred solution of **4** (5.34 g, 0.02 mol) in dimethyl sulfoxide (10 ml) at room temperature, carbon disulfide (1.6 ml, 0.026 mol) and sodium hydroxide (1.2 ml, 20 mol solution) were added drop wise over 30 min, then the mixture was allowed to stir for 30 min more. Dimethyl sulfate (2.5 g, 0.02 mol) was added at 5–10 °C, stirring was continued for 3 h and the reaction mixture was poured into ice-water, the solid obtained was filtered, washed with water, dried and recrystallized from ethanol. Yield = 71%, m.p. 119–121 °C. IR: 3290 (NH), 1680 (C=O), 1160 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.5–1.9 (m, 8H, $(CH_2)_4$), 3.4 (s, 3H, 2-SCH_3), 3.6 (s, 3H, 3-SCH_3) and 4.9 (s, 1H, NH, D_2O exchangeable). MS (*m*/*z*): 357 (M^+). Anal. Calcd. for $C_{13}H_{15}N_3OS_4$: C, 43.67; H, 4.22; N, 11.75. Found: C, 43.68; H, 4.19; N, 11.79.

5.1.5. Synthesis of 1-methyl-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl)thiourea (A1)

A mixture of **5** (3.57 g, 0.01 mol) and methylamine (0.62 g, 0.02 mol) in *N*,*N*-dimethyl formamide (20 ml) was refluxed for 29 h, cooled and poured into ice-water, the solid obtained was filtered, dried and recrystallized from ethanol. Yield = 70%, m.p. 193–195 °C. IR: 3300 (NH), 1680 (C=O), 1150 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.2–1.7 (m, 8H, $(CH_2)_4$), 2.3 (s, 3H, NCH_3), 2.7 (s, 3H, SCH_3), 4.6 (s, 1H, NH, D_2O exchangeable) and 4.9 (s, 1H, NH, D_2O exchangeable). MS (*m*/*z*): 340 (M^+). Anal. Calcd. for $C_{13}H_{16}N_4OS_3$: C, 45.92; H, 4.74; N, 16.47. Found: C, 45.89; H, 4.76; N, 16.42.

5.1.6. Synthesis of 1-dimethyl-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl) thiourea (A2)

Compound **A2** was prepared by adopting the same procedure as for **A1** and obtained in 72% yield, m.p. 161–162 °C. IR: 3350 (NH), 1690 (C=O), 1130 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.4–1.9 (m, 8H, $(CH_2)_4$), 2.3–2.5 (s, 6H, $N(CH_3)_2$), 2.6–2.7 (s, 3H, SCH_3), 4.9 (s, 1H, NH, D_2O exchangeable). MS (*m*/*z*): 354 (M^+). Anal. Calcd. for $C_{14}H_{18}N_4OS_3$: C, 47.50; H, 5.12; N, 15.82. Found: C, 47.49; H, 5.09; N, 15.83.

5.1.7. Synthesis of 1-diethyl-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl)thiourea (A3)

Compound **A3** was prepared by adopting the same procedure as for **A1** and obtained in 73% yield, m.p. 176–177 °C. IR: 3310 (NH), 1685 (C=O), 1120 (C=S) cm^{-1}. ^{1}H-NMR

($CDCl_3$) δ 1.5–2.0 (m, 8H, $(CH_2)_4$), 2.3–2.5 (m, 4H, $N(CH_2CH_3)_2$), 2.8–3.0 (m, 6H, $N(CH_2CH_3)_2$), 3.1–3.3 (s, 3H, SCH_3), 4.8–4.9 (s,1H, NH, D_2O exchangeable). MS (*m*/*z*): 382 (M^+). Anal. Calcd. for $C_{16}H_{22}N_4OS_3$: C, 50.30; H, 5.80; N, 14.66. Found: C, 50.29; H, 5.76; N, 14.65.

*5.1.8. Synthesis of 1-pyrrolidinyl-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl) thiourea (**A4**)*

Compound **A4** was prepared by adopting the same procedure as for **A1** and obtained in 69% yield, m.p. 186–187 °C. IR: 3360 (NH), 1670 (C=O), 1130 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.1–1.6 (m, 8H, $(CH_2)_4$), 1.8–2.0 (m, 8H, $N(CH_2–CH_2)_2$), 2.9–3.0 (s, 3H, SCH_3), 4.6–4.7 (s, 1H, NH, D_2O exchangeable. MS (*m*/*z*): 380 (M^+). Anal. Calcd. for $C_{16}H_{20}N_4OS_3$: C, 50.57; H, 5.30; N, 14.74. Found: C, 50.53; H, 5.32; N, 14.74.

*5.1.9. Synthesis of 1-morpholinyl-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl) thiourea (**A5**)*

Compound **A5** was prepared by adopting the same procedure as for **A1** and obtained in 73% yield, m.p. 236–237 °C. IR: 3350 (NH), 1680 (C=O), 1140 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.3–1.9 (m, 8H, $(CH_2)_4$), 2.3–2.5 (m, 4H, $N(CH_2–CH_2)_2O$), 2.8–3.0 (m, 4H, $N(CH_2–CH_2)_2O$), 3.1–3.2 (s, 3H, SCH_3), 4.7–4.8 (s, 1H, NH, D_2O exchangeable). MS (*m*/*z*): 396 (M^+). Anal. Calcd. for $C_{16}H_{20}N_4O_2S_3$: C, 48.52; H, 5.08; N, 14.12. Found: C, 48.49; H, 5.09; N, 14.14.

*5.1.10. Synthesis of 1-piperazinyl-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d] pyrimidin-3-yl) thiourea (**A6**)*

Compound **A6** was prepared by adopting the same procedure as for **A1** and obtained in 72% yield, m.p. 209–211 °C. IR: 3310 (NH), 1690 (C=O), 1130 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.1–1.6 (m, 8H, $(CH_2)_4$), 2.5–2.7 (m, 4H, $N(CH_2–CH_2)_2N$), 3.0–3.2 (m, 4H, $N(CH_2–CH_2)_2N$), 3.3–3.4 (s, 3H, SCH_3), 4.3–4.4 (s, 1H, NH, D_2O exchangeable), 4.8–4.9 (s, 1H, NH, D_2O exchangeable). MS (*m*/*z*): 395 (M^+). Anal. Calcd. for $C_{16}H_{21}N_5OS_3$: C, 48.65; H, 5.35; N, 17.73. Found: C, 48.63; H, 5.37; N, 17.69.

*5.1.11. Synthesis of 1-phenyl-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl) thiourea (**A7**)*

Compound **A1** was prepared by adopting the same procedure as for **A1** and obtained in 76% yield, m.p. 239–241 °C. IR: 3310 (NH), 1670 (C=O), 1140 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.3–1.9 (m, 8H, $(CH_2)_4$), 3.1–3.2 (s, 3H, SCH_3), 4.5–4.6 (s, 1H, NH, D_2O exchangeable), 4.8–4.9(s, 1H, NH, D_2O exchangeable), 6.9–7.3 (m, 5H, ArH). MS (*m*/*z*): 402 (M^+). Anal. Calcd. for $C_{18}H_{18}N_4OS_3$: C, 53.78; H, 4.51; N, 13.93. Found: C, 53.81; H, 4.50; N, 13.91.

*5.1.12. Synthesis of 1-(4-methoxyphenyl)-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl)thiourea (**A8**)*

Compound **A8** was prepared by adopting the same procedure as for **A1** and obtained in 69% yield, m.p. 239–242 °C. IR: 3360 (NH), 1680 (C=O), 1150 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.1–1.6 (m, 8H, $(CH_2)_4$), 3.3–3.4 (s, 3H, SCH_3), 3.8–3.9 (s, 3H, OCH_3), 4.4–4.5 (s, 1H, NH, D_2O exchangeable), 4.6–4.7 (s, 1H, NH, D_2O exchangeable), 6.5–6.9 (m, 4H, ArH) MS (*m*/*z*): 432 (M^+). Anal. Calcd. for $C_{19}H_{20}N_4O_2S_3$: C, 52.82; H, 4.66; N, 12.96. Found: C, 52.79; H, 4.69; N, 12.94.

*5.1.13. Synthesis of 1-(3-methylphenyl)-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl) thiourea (**A9**)*

Compound **A9** was prepared by adopting the same procedure as for **A1** and obtained in 76% yield, m.p. 193–194 °C. IR: 3290 (NH), 1685 (C=O), 1150 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.1–1.7 (m, 8H, $(CH_2)_4$), 1.8–1.9 (s, 3H, CH_3), 3.5–3.6 (s, 3H, SCH_3), 4.5–4.6 (s, 1H, NH, D_2O exchangeable), 4.9–5.0 (s, 1H, NH, D_2O exchangeable), 6.6–7.1 (m, 4H, ArH). MS (*m*/*z*): 416 (M^+). Anal. Calcd. for $C_{19}H_{20}N_4OS_3$: C, 54.85; H, 4.84; N, 13.46. Found: C, 54.81; H,4.88; N, 13.42.

*5.1.14. Synthesis of 1-(4-methylphenyl)-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl) thiourea (**A10**)*

Compound **A10** was prepared by adopting the same procedure as for **A1** and obtained in 71% yield, m.p. 211–213 °C. IR: 3310 (NH), 1680 (C=O), 1145 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.3–1.8 (m, 8H, $(CH_2)_4$), 1.9–2.0 (s, 3H, CH_3), 3.5–3.6 (s, 3H, SCH_3), 4.3–4.4 (s, 1H, NH, D_2O exchangeable), 4.8–4.9 (s, 1H, NH, D_2O exchangeable), 6.7–7.2 (m, 4H, ArH). MS (*m*/*z*): 416 (M^+). Anal. Calcd. for $C_{19}H_{20}N_4OS_3$: C, 54.85; H, 4.84; N, 13.46. Found: C, 54.90; H, 4.85; N, 13.52.

*5.1.15. Synthesis of 1-(4-nitrophenyl)-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl) thiourea (**A11**)*

Compound **A11** was prepared by adopting the same procedure as for **A1** and obtained in 76% yield, m.p. 153–155 °C. IR: 3350 (NH), 1685 (C=O), 1140 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.2–1.7 (m, 8H, $(CH_2)_4$), 3.5–3.6 (s, 3H, SCH_3), 4.5–4.6 (s, 1H, NH, D_2O exchangeable), 4.9–5.0 (s, 1H, NH, D_2O exchangeable), 6.7–7.1 (m, 4H, ArH). MS (*m*/*z*): 447 (M^+). Anal. Calcd. for $C_{18}H_{17}N_5O_3S_3$: C, 48.36; H, 3.83; N, 15.66. Found: C, 48.32; H, 3.81; N, 15.63.

*5.1.16. Synthesis of 1-(3-nitrolphenyl)-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl) thiourea (**A12**)*

Compound **A12** was prepared by adopting the same procedure as for **A1** and obtained in 73% yield, m.p. 169–171 °C.

IR: 3310 (NH), 1680 (C=O), 1130 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.2–1.8 (m, 8H, $(CH_2)_4$), 3.1–3.2 (s, 3H, SCH_3), 4.2–4.3 (s, 1H, NH, D_2O exchangeable), 4.6–4.7 (s, 1H, NH, D_2O exchangeable), 6.9–7.3 (m, 4H, ArH). MS (*m/z*): 447 (M^+). Anal. Calcd. for $C_{18}H_{17}N_5O_3S_3$: C, 48.36; H, 3.83; N, 15.66. Found: C, 48.29; H, 3.76; N, 15.67.

5.1.17. Synthesis of 1-(4-chlorophenyl)-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl) thiourea (*A13*)

Compound **A13** was prepared by adopting the same procedure as for **A1** and obtained in 72% yield, m.p. 222–225 °C. IR: 3310 (NH), 1690 (C=O), 1150 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.2–1.8 (m, 8H, $(CH_2)_4$), 3.2–3.3 (s, 3H, SCH_3), 4.3–4.4 (s, 1H, NH, D_2O exchangeable), 4.7–4.8 (s, 1H, NH, D_2O exchangeable), 6.5–7.0 (m, 4H, ArH). MS (*m/z*): 436 (M^+). Anal. Calcd. for $C_{18}H_{17}N_4OS_3Cl$: C, 49.70; H, 3.93; N, 12.88. Found: C, 49.53; H, 3.95; N, 13.79.

5.1.18. Synthesis of 1-(4-bromophenyl)-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl) thiourea (*A14*)

Compound **A14** was prepared by adopting the same procedure as for **A1** and obtained in 76% yield, m.p. 242–243 °C. IR: 3300 (NH), 1690 (C=O), 1150 (C=S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.2–1.8 (m, 8H, $(CH_2)_4$), 3.0–3.1 (s, 3H, SCH_3), 4.2–4.3 (s, 1H, NH, D_2O exchangeable), 4.8–5.0 (s, 1H, NH, D_2O exchangeable), 7.1–7.6 (m, 4H, ArH). MS (*m/z*): 481 (M^+). Anal. Calcd. for $C_{18}H_{17}N_4OS_3Br$: C, 44.95; H, 3.56; N, 11.64. Found: C, 44.93; H, 3.52; N, 11.63.

5.1.19. Synthesis of 1-(diphenyl)-3-(2-methylthio-4-oxo-3H-5,6,7,8-tetrahydrobenzo (b) thieno[2,3-d]pyrimidin-3-yl) thiourea (*A15*)

Compound **A15** was prepared by adopting the same procedure as for **A1** and obtained in 70% yield. m.p 264-265 °C. IR: 3360 (NH), 1685 (C = O), 1130 (C = S) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.4–1.9 (m, 8H, $(CH_2)_4$), 3.0–3.1 (s, 3H, SCH_3), 4.8–4.9 (s, 1H, NH, D_2O exchangeable), 6.6–7.3 (m, 10H, ArH). MS (*m/z*): 478 (M^+). Anal. Calcd. for $C_{24}H_{22}N_4OS_3$: C, 60.30; H, 4.63; N, 11.72. Found: C, 60.29; H, 4.65; N, 12.69.

5.2. Pharmacology

The synthesized compounds were evaluated for analgesic, anti-inflammatory, ulcerogenic index and antimicrobial activities. Student's *t*-test was performed to ascertain the significance of all the exhibited activities. The test compounds and the standard drugs were administered in the form of a suspension (1% carboxy methyl cellulose as a vehicle) by oral route of administration for analgesic and anti-inflammatory but for ulcerogenicity studies by intra peritoneally as suspension in 10% v/v Tween. Each group consisted of six animals. The animals were procured from the National Biological Center, Madurai, India, and were maintained in colony cages at 25 ± 2 °C, relative humidity of 45–55%, under a 12 h light and dark cycle; they were fed standard animal feed. All the animals were acclimatized for a week before use. The Institutional Animal Ethics committee approved the protocol adopted for the experimentation of animals.

5.2.1. Analgesic activity

Test for analgesic activity was performed by tail-flick technique [10] using Wistar albino mice (25–35 g) of either sex selected by random sampling technique. Diclofenac sodium at a dose level of 10 and 20 mg kg^{-1} was administered orally as reference drug for comparison. The test compounds at two dose levels (10, 20 mg kg^{-1}) were administered orally. The reaction time was recorded at 30 min, 1–3 h after the treatment, and cut-off time was 10 s. The percent analgesic activity (PAA) was calculated by the following formula,

$$\mathrm{PAA} = \left[\frac{T_2 - T_1}{10 - T_1}\right] \times 100$$

where T_1 is the reaction time (s) before treatment, and T_2 is the reaction time (s) after treatment.

5.2.2. Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan-induced paw edema test in rats [11]. Diclofenac sodium 10, 20 mg kg^{-1} was administered as a standard drug for comparison. The test compounds were administered at two dose levels (10 mg, 20 mg kg^{-1}). The paw volumes were measured using the mercury displacement technique with the help of a plethysmograph immediately before and 30 min, 1–3 h after carrageenan injection. The percent inhibition of paw edema was calculated using the following formula

$$\text{Percent inhibition } I = 100[1 - (a - x)/(b - y)]$$

Where *x* is the mean paw volume of rats before the administration of carrageenan and test compounds or reference compound (test group), *a* is the mean paw volume of rats after the administration of carrageenan in the test group (drug treated), *b* is the mean paw volume of rats after the administration of carrageenan in the control group, *y* is the mean paw volume of rats before the administration of carrageenan in the control group.

5.2.3. Evaluation of ulcerogenicity index

Ulceration in rats was induced as described by Goel et al. [12]. Albino rats of wistar strain weighing 150–200 g of either sex were divided into various groups each of six animals. Control group of animals were administered only with 10% v/v Tween 80 suspension intraperitonially. One group was administered with Aspirin (German Remedies) intraperitoneally in a dose of 200 mg kg^{-1} once daily for 3 days. The remaining group of animals was administered with test compounds intraperitoneally in a dose of 20 mg kg^{-1}. On fourth day. Pylorus was ligated as per the method of Shay et al. [13]. Animals were fasted for 36 h before the pylorus ligation procedure. Four hours after the ligation, animals were sacrificed. The stomach

was removed and opened along with the greater curvature. Ulcer index was determined by the method of Ganguly and Bhatnagar [14] and recorded in Table 3.

5.2.4. Antibacterial activity

Evaluation of antibacterial activity by agar dilution method [15]. The standard strains were procured from the American Type Culture Collection (ATCC), Rockville, USA, and the pathological strains were procured from the Department of Microbiology, Madurai Medical College and Research Institute, Madurai, India. The antibacterial activity of the synthesized compounds was screened against the following bacterial strains: *P. vulgaris* ATCC 9484, *S. typhimurium* ATCC 33068, *K. pneumoniae* ATCC 13883, *E. tarda*, *P. aeruginosa* ATCC 2853, *B. subtilis* ATCC 6051, and *S. paratyphi*. All bacteria were grown on Muller–Hinton Agar (Hi-media) plates (37 °C, 24 h) and the minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited the growth on agar plates, disregarding a single colony or faint haze caused by the inoculums. The MIC of the test compounds was compared with the reference drug norfloxacin.

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