Full Paper

Synthesis and Pharmacological Investigation of 3-Subsituted-amino-2-methylsulfanyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-ones as Analgesic and Anti-Inflammatory Agents

V. Alagarsamy[1], V. Raja Solomon[2], G. Deepa[3], P. Parthiban[3], and G. V. Anjana[3]

[1] Medicinal Chemistry Research Laboratory, Dayananda Sagar College of Pharmacy, Kumaraswamy Layout, Bangalore, India
[2] Medicinal & Process Chemistry Division, Central Drug Research Institute, Lucknow, India
[3] Medicinal Chemistry Research Laboratory, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankovil, India

In the present work, design, synthesis, and pharmacological evaluation of the analgesic, anti-inflammatory, and ulcerogenic-index activities of new 3-subsituted-amino-2-methylsulfanyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-ones, structurally planed by exploiting a clear concept of bio-isosterism, are reported. All compounds exhibited significant analgesic and anti-inflammatory activity. Compounds **A1, A3** showed higher analgesic activity and more potent anti-inflammatory activity than that of the reference compound diclofenac sodium. Interestingly, the test compounds showed only mild ulcerogenic potential when compared to that of acetylsalicylic acid.

Keywords: Analgesic / Anti-Inflammatory activity / Thienopyrimidin-4-one

Received: February 4, 2007; accepted: May 16, 2007

DOI 10.1002/ardp.200700017

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) represent the standard therapy for the management of inflammation and pain. However, long-term clinical use of NSAIDs is associated with significant side effects of gastrointestinal lesions, bleeding, and nephrotoxicity. Therefore, the discovery of new, safer anti-inflammatory drugs represents a challenging goal for such a research area [1 – 4]. In our ongoing medicinal chemistry research program we have documented that quinazolines and condensed quinazolines exhibit potent central nervous system (CNS) activities: analgesic, anti-inflammatory [5], and anticonvulsant [6]. In continuation, we have reported 2-phenyl-3-substituted quinazolines (Fig. 1, **I**) [7], 2-methyl-3-substituted quinazolines (Fig. 1, **II**) [8], 2-methylthio-3-substituted quinazolines (Fig. 1, **III**) [9]; 2,3-disubstituted quinazolines [10] exhibited good analgesic and anti-inflammatory activities. Literature evidences have shown that thienopyrimidines (bioisostere of quinazoline) possess CNS and antibacterial activities [11 – 13]. Recently, Barreiro *et al*. reviewed that bioisosterism is an useful strategy for the lead optimization process and molecular modification for rational drug design [14].

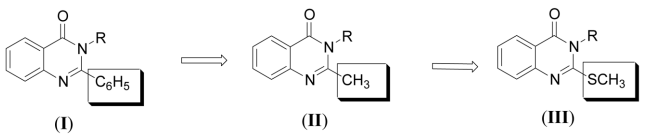
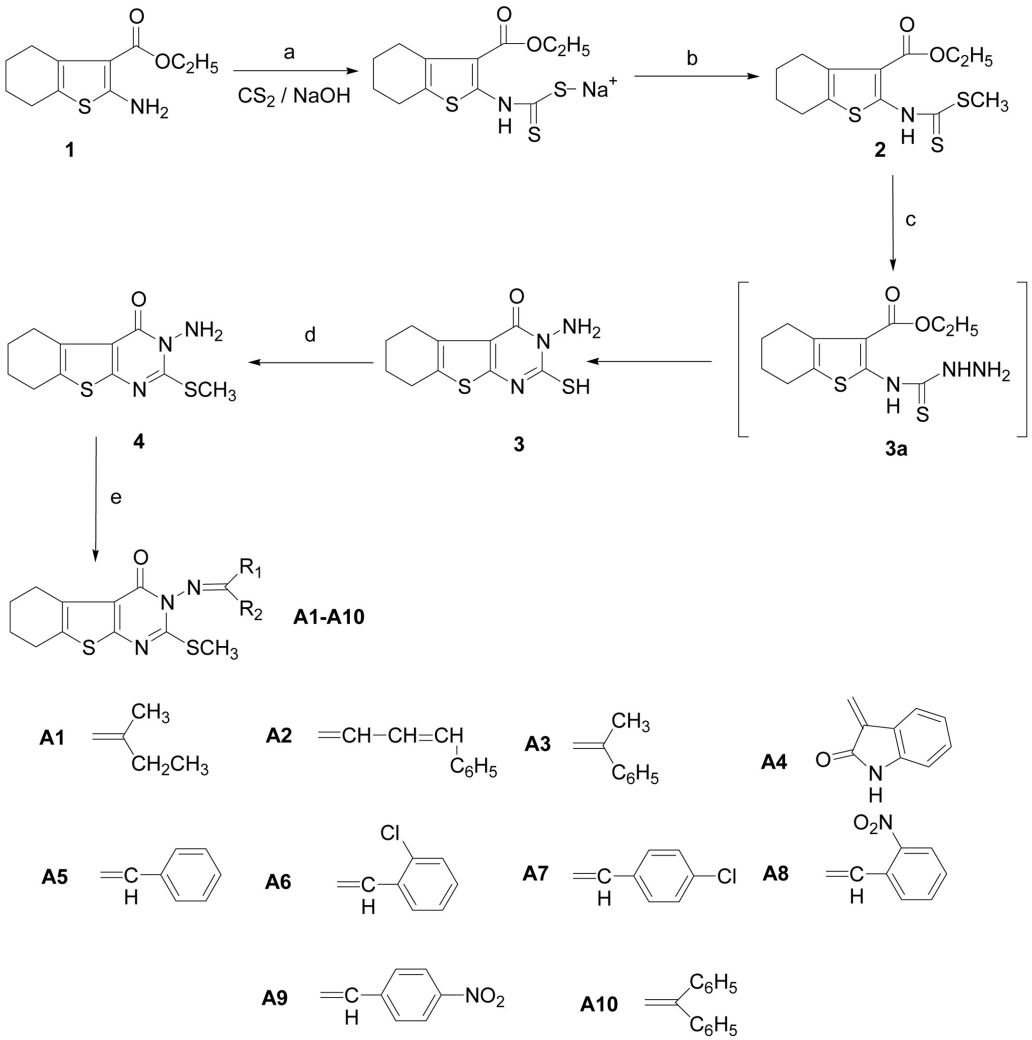


Figure 1. Some lead molecules of quinazolines.

The bioisostere concept is an oversimplification of the role of scaffolds for activity, unless it plays a pivotal role for function or interaction such as for β-lactams in peni-

Correspondence: V. Alagarsamy, Medicinal Chemistry Research Laboratory, Dayananda Sagar College of Pharmacy, Kumaraswamy Layout, Bangalore-560 078, India.
E-mail: samy_veera@yahoo.com
Fax: +91 80 2666-0789



Reagents and Conditions: (a) DMSO, rt, 30 min; (b) $(CH_3)_2SO_4$, 5-10°C, 2 h; (c) NH_2NH_2, ethanol reflux for 8 h; (d) DMF/NaOH, $(CH_3)_2SO_4$, 5 – 10°C, 3 h; (e) $(R_2R_1)CO$; glacial CH_3COOH reflux, 30 h.

Scheme 1. Synthesis route of presented compounds.

cillins. On the basis of these results, we surmised that replacement of the condensed quinazoline ring system with a thienopyrimidines bioisostere group could give compounds with improved biological activities by illustrating the old and well-known isosterism thiophene / phenyl having similar geometry and electronic features (Fig. 2). The present work is an extension of our ongoing efforts towards the development and identification of new molecules; by the bioisotere concept, we have designed some 3-subsituted-amino-2-methylsulfanyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-ones (Scheme 1). The title compounds were synthesized by reacting 3-amino-2-methylsulfanyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*] pyrimidin-4-one with a variety of aldehydes and ketones. The starting material, 3-amino-2-methylsulfanyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]-thieno[2,3-*d*]pyrimidin-4-one, was synthesized from 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo[*b*]thiophene (Scheme 1). The synthesized compounds were tested for their analgesic, anti-inflammatory, and ulcerogenic index activities.

Figure 2. Bioisostere concept for design.

Results and discussion

The synthetic pathway depicted in Scheme 1 outlines the chemistry of the present work. The intermediate compound 3-amino-2-mercapto-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one **3** was prepared by treating carbon disulphide and sodium hydroxide solution simultaneously to a vigorously stirred solution of 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo[*b*]thiophene **1** in dimethyl sulphoxide. The sodium salt of dithiocarbamic acid obtained was methylated with dimethyl sulphate to get the 2-methylsulfanylthiocarbonylamino-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester **2**. Compound **2** and hydrazine hydrate, when refluxed in ethanol, yield the desired key intermediate compound **3.** The product obtained was cyclic and not an open chain thiosemicarbazide **3a**. It was confirmed by IR spectra of **3**. It showed intense peaks at 3300, 3200 cm^{-1} for amino (NH_2), 2550 cm^{-1} for mercapto (SH), and 1680 cm^{-1} for carbonyl (C=O) stretching. The NMR spectra of compound **3** showed a multiplet at δ 1.5–1.9 for cyclohexyl and singlets at 3.2 and 5.4 for SH and NH_2, respectively. Data from the elemental analyses have been found to be in conformity with the assigned structure. Further, the molecular ion recorded in the mass spectra is also in agreement with the molecular weight of the compound.

The 3-amino-2-methylsulfanyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one **4** was obtained by dissolving **3** in 2% alcoholic sodium hydroxide solution and methylating it with dimethyl sulphate by stirring at room temperature. The IR spectra of **4** showed the disappearance of the NH- and C=S-stretching signals of cyclic thiourea. It showed a peak for carbonyl (C=O) stretching at 1690 cm^{-1}. The ^{1}H-NMR spectra of compound **4** showed singlets at δ 3.9 and d 6.5 for SCH_3 and NH_2, respectively; a multiplet at δ 1.3–1.7 was observed for the cyclohexyl (8H) protons. Data from the elemental analyses and molecular ion recorded in the mass spectra further confirmed the assigned structure.

The title compounds 3-subsituted-amino-2-methylsulfanyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one **A1–A10** were obtained by condensation of the amino group of 3-amino-2-methylsulfanyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (**4**) with different aldehydes and ketones. The formation of the title compound is indicated by the disappearance of a peak due to NH_2-group of the starting material in IR and NMR spectra of all the compounds **A1–A10**. The IR and NMR spectra these compounds showed the presence of peaks due to ($N=CR^1R^2$), carbonyl (C=O), and cyclohexyl ring. The molecular ion recorded in the mass spectrum is in agreement with the molecular weight of the compounds. Table 1 gives the physicochemical data of 3-subsituted-amino-2-methylsulfanyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-d]pyrimidin-4-ones **A1–A10** and Table 2 shows the IR and NMR spectral data of the newly synthesized compounds **A1–A10**.

Table 1. Physicochemical characterization data of 3-subsitute-damino-2-methylsulfanyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]-thieno[2,3-d]pyrimidin-4-ones **A1–A10**.

Compd.	Mol. Formula	Mol. Wt[a)]	Mp (°C)	Yield (%)
A1	$C_{15}H_{19}N_3OS_2$	321	221–223	85
A2	$C_{20}H_{19}N_3OS_2$	381	241–245	70
A3	$C_{19}H_{19}N_3OS_2$	369	217–219	79
A4	$C_{19}H_{16}N_4O_2S_2$	396	226–228	76
A5	$C_{18}H_{17}N_3OS_2$	355	221–223	83
A6	$C_{18}H_{16}N_3OS_2Cl$	389	231–233	79
A7	$C_{18}H_{16}N_3OS_2Cl$	389	247–249	71
A8	$C_{18}H_{16}N_4O_3S_2$	400	219–220	81
A9	$C_{18}H_{16}N_4O_3S_2$	400	253–255	76
A10	$C_{24}H_{21}N_3OS_2$	431	266–268	71

[a)] Molecular weight determination by mass spectral analysis (M+H).

The pharmacological screening results revealed that all the test compounds **A1–A10** showed significant analgesic activity (Table 3). Compound **A1** with a N-3 aliphatic substituent showed good activity, replacement by an araliphatic group (compound **A2** and **A3**) leads to retaining the activity. Placement of an aryl group at N-3 (compounds **A4**, **A5**, and **A10**) results in decreasing activity. Placement of electron withdrawing groups at the N-3 aryl ring (compounds **A6–A9**) results in further decrease of activity. Compound 3-*sec*-butylideneamino-2-methylsulfanyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one **A1** emerged as the most active analgesic agent. It is also more potent than to the reference standard diclofenac sodium and 2,3-disubstituted quinazolin-4(3*H*)-ones which we reported earlier [8, 9].

Anti-inflammatory activity was evaluated by a carrageenan-induced paw oedema protocol in rats [16]. The anti-inflammatory activity data (Table 4) indicated that all the test compounds protected rats moderately from carrageenan-induced inflammation at 30 min of reaction time; the activity increased at 1 h and it reached to peak level at 2 h. Declining in activity was observed at 3 h. Compounds **A1** and **A3** showed more potent anti-inflammatory activity and the compound **A2** showed equipotent anti-inflammatory activity when compared to the reference standard diclofenac sodium.

The ulcer index of the test compounds (Table 4) revealed that open chain aliphatic-substituted com-

Table 2. IR and NMR spectral data of the newly synthesized compounds **A1 – A10**.

Compd.	IR (cm^{-1}) (KBr)		^{1}H-NMR ($CDCl_3$) δ
	C=O	C=N	
A1	1690	1620	1.1 – 1.6 (m, 8H, $(CH_2)_4$), 1.8 – 1.9 (q, 2H, CH_2CH_3), 2.1 – 2.2 (t, 3H, CH_2CH_3), 2.7 – 2.8 (s, 3H, CH_3), 3.0 – 3.1 (s, 3H, SCH_3)
A2	1686	1624	0.9 – 1.4 (m, 8H, $(CH_2)_4$), 2.9 – 3.0 (s, 3H, SCH_3), 5.9 (d, 1H, C*H*), 6.3 – 6.6 (m, 1H, C*H*), 6.8 (d, 1H, C*H*), 6.9 – 7.4 (m, 5H, Ar-*H*)
A3	1685	1625	0.9 – 1.4 (m, 8H, $(CH_2)_4$), 1.7-1.8 (s, 3H, CH_3), 2.8-2.9 (s, 3H, SCH_3) and 7.1-7.5 (m, 5H, Ar-*H*)
A4	1675	1618	1.0 – 1.6 (m, 8H, $(CH_2)_4$), 3.0 – 3.1 (s, 3H, SCH_3), 7.1 – 7.8 (m, 4H, Ar-*H*), 8.4 (br s, 1H, N*H*)
A5	1681	1626	1.2 – 1.7 (m, 8H, $(CH_2)_4$), 3.3 – 3.4 (s, 3H, SCH_3), 6.8 (s, 1H, C*H*), 7.1 – 7.6 (m, 5H, Ar-*H*)
A6	1679	1625	0.9 – 1.2 (m, 8H, $(CH_2)_4$), 3.1 – 3.2 (s, 3H, SCH_3), 6.4 (s, 1H, C*H*), 7.9 – 8.3 (m, 4H, Ar-*H*)
A7	1683	1619	0.8 – 1.4 (m, 8H, $(CH_2)_4$), 3.2 – 3.3 (s, 3H, SCH_3), 6.4 (s, 1H, C*H*), 8.1 – 8.4 (m, 4H, Ar-*H*)
A8	1687	1620	1.0 – 1.6 (m, 8H, $(CH_2)_4$), 3.1 – 3.2 (s, 3H, SCH_3), 6.4 (s, 1H, C*H*), 7.5 – 8.0 (m, 4H, Ar-*H*)
A9	1690	1626	1.2 – 1.7 (m, 8H, $(CH_2)_4$), 3.1 – 3.2 (s, 3H, SCH_3), 6.5 (s, 1H, C*H*), 7.7 – 8.2 (m, 4H, Ar-*H*)
A10	1686	1632	1.0 – 1.5 (m, 8H, $(CH_2)_4$), 3.1 – 3.2 (s, 3H, SCH_3), 7.3 – 8.0 (m, 10H, Ar-*H*)

Table 3. Analgesic activity of the synthesized compounds **A1 – A10** by Tail-Flick Technique.

Compound	Dose (mg/kg)	Percent Analgesic activity			
		30 min	**1 h**	**2 h**	**3 h**
A1	10	49 ± 1.23**	51 ± 1.21**	57 ± 2.31***	41 ± 1.26*
	20	63 ± 1.41***	65 ± 1.37***	72 ± 1.36***	50 ± 1.26**
A2	10	42 ± 1.19**	46 ± 1.34**	48 ± 1.41**	39 ± 1.28*
	20	47 ± 1.37**	51 ± 1.32***	54 ± 1.41***	40 ± 1.32*
A3	10	48 ± 1.18*	49 ± 1.25**	53 ± 1.61**	42 ± 1.13*
	20	56 ± 1.31***	58 ±1.53***	65 ± 1.29***	43 ± 1.51**
A4	10	35 ± 1.29*	40 ± 1.51*	41 ± 1.37**	25 ± 1.41*
	20	50 ± 1.41**	54 ± 1.51***	50 ± 1.72***	34 ± 1.35*
A5	10	35 ± 1.34*	38 ± 1.27*	47 ± 1.42**	23 ± 1.17*
	20	44 ± 1.27**	50 ± 1.39**	55 ± 1.72***	35 ± 1.42*
A6	10	35 ± 1.51*	41 ± 1.63*	44 ± 1.72**	24 ± 1.39*
	20	47 ±1.36**	49 ± 1.51**	54 ± 1.31**	35 ± 1.82**
A7	10	37 ± 1.37*	39 ± 1.21*	43 ± 1.52*	28 ± 1.62*
	20	45 ± 1.62**	49 ± 1.81**	49 ± 1.73**	42 ± 1.29*
A8	10	34 ± 1.28*	37 ± 1.56*	44 ± 1.52*	26 ± 1.29*
	20	46 ± 1.51*	51 ± 1.62**	54 ± 1.53**	31 ± 1.72*
A9	10	36 ± 1.63*	39 ± 1.82*	46 ± 1.35*	27 ± 1.61*
	20	45 ± 1.31**	48 ± 1.37**	54 ± 1.29**	33 ± 1.82*
A10	10	36 ± 1.37*	40 ± 1.18*	46 ± 1.42*	30 ± 1.58*
	20	43 ± 1.29**	51 ± 1.31**	52 ± 1.81**	37 ± 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 ± SD (n = 6).
Significance levels * $p < 0.5$, ** $p < 0.01$ and *** $p < 0.001$ as compared with the respective control.

pound **A1** showed negligible ulcer index, whereas the araliphatic-substituted compounds **A2** and **A3** exhibited a small increase in ulcer index, and the aryl-substituted compounds **A4**, **A5**, and **A10** showed a further increase in ulcer index. Compounds **A6 – A9** containing aryl-substituted and electron-withdrawing groups exhibited a higher ulcer index than the other test compounds. When compared to the reference standard acetylsalicylic acid (Aspirin[1], ulcer index 1.73 ± 0.41) the test compounds exhibited about 35% to 50% of the ulcer index of reference standards. Compound **A1** exhibited the least ulcer index (0.56 ± 0.31) among the test compounds, which is about one third of the ulcer index of reference standards acetylsalicylic acid. Compound **A6** showed the highest ulcer index (0.85 ± 0.21) among the test compounds, which is about 50% of the ulcer index of reference standards acetylsalicylic acid and diclofenac.

Table 4. Anti-inflammatory activity of synthesized compounds **A1–A10** by carrageenan-induced rat paw oedema method.

Compound	Dose (mg/kg)	Percent Protection			
		30 min	1 h	2 h	3 h
A1	10	42 ± 1.59*	45 ± 1.57**	47 ± 1.41**	39 ± 1.62*
	20	49 ± 1.23**	60 ± 1.38***	65 ±1.18***	48 ± 1.29**
A2	10	36 ± 1.41*	37 ± 1.36*	44 ± 1.61**	30 ± 1.25*
	20	50 ± 1.52**	55 ± 1.17***	60 ± 1.51***	36 ± 1.68*
A3	10	39 ± 1.71*	43 ± 1.58*	46 ± 1.90**	39 ± 1.51*
	20	48 ± 1.62**	59 ± 1.83***	63 ±1.25***	37 ± 1.61*
A4	10	34 ± 1.80*	37 ± 1.34*	42 ± 1.27*	28 ± 1.06*
	20	45 ± 1.34**	49 ± 1.72**	58 ± 1.31***	30 ± 1.32*
A5	10	30 ± 1.18*	36 ± 1.84*	36 ± 1.25*	26 ± 1.96*
	20	36 ± 1.68*	49 ± 1.27**	51 ± 1.42**	38 ± 1.73*
A6	10	29 ± 1.67*	38 ± 1.42*	40 ± 1.61*	28 ± 1.83*
	20	36 ± 1.29*	49 ± 1.42**	52 ± 1.36**	43 ± 1.42*
A7	10	30 ± 1.63*	37 ± 1.39*	40 ± 1.52*	23 ± 1.37*
	20	39 ± 1.42*	47 ± 1.56**	56 ± 1.51**	40 ± 1.34*
A8	10	35 ± 1.62*	39 ± 1.94*	41 ± 1.31*	24 ± 1.73*
	20	39 ± 1.31*	48 ± 1.73**	55 ± 1.52**	32 ± 1.69*
A9	10	32 ± 1.34*	39 ± 1.08*	41 ± 1.73*	25 ± 135*
	20	43 ± 1.63*	46 ± 1.26**	50 ± 1.94**	27 ± 1.28*
A10	10	36 ± 1.34*	40 ± 1.82*	41 ± 1.79*	29 ± 1.37*
	20	42 ± 1.51**	50 ± 1.45**	53 ± 1.83***	37 ± 1.29*
Control		5.1 ± 0.29	6.1 ± 0.27	5.7 ± 0.32	3.2 ± 0.51
Diclofenac	10	32 ± 0.63*	38 ± 1.58*	39 ±197**	33 ± 0.93*
	20	45 ± 1.61**	52 ± 0.92***	60 ± 1.52***	42 ± 1.36*

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

Table 5. Evaluation of ulcerogenicity index of synthesized compounds **A1–A10**.

Compd.	Ulcer index	Compd.	Ulcer index
A1	0.56 ± 0.31*	**A7**	0.83 ± 0.36*
A2	0.63 ± 0.20*	**A8**	0.80 ± 0.32*
A3	0.59 ± 0.19	**A9**	0.82 ± 0.39*
A4	0.68 ± 0.27*	**A10**	0.71 ± 0.18*
A5	0.67 ± 0.36*	**Control**	0.15 ± 0.32
A6	0.85 ± 0.21*	**Acetylsalicylic acid**	1.73 ± 0.41**

Each value represents the mean ± SD (n = 6).
Significance levels * $p < 0.05$ and ** $p < 0.01$ as compared with the respective control.

Conclusions

In our earlier studies [7–10], we observed that the presence of alkyl groups at the N-3 position exhibited stronger analgesic and anti-inflammatory activities than having aryl groups in this position. Hence, in the C-2 position, we also made a substitution in such a way 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 (Fig. 1, **I**, [7]), whereas the C-2 methyl series lead molecule showed 50% analgesic and 44% anti-inflammatory activity (Fig. 1, **II**, [8]). Introduction of a sulfur atom at the C-2 position in the above series, *i.e.* by placing a methylthio group at C-2 position, showed 54% analgesic and 43% anti-inflammatory activity (Fig. 1, **III**). The present study indicates that the replacement of 2-methylthio-3-substituted benzopyrimidines by its bioisostere 2-methylthio-3-substituted thienopyrimidines (Fig. 2) leads to enhancement in the analgesic and anti-inflammatory activity (60% analgesic and 50% anti-inflammatory activity). Interestingly, these compounds also showed 35% of ulcer index of the reference NSAID's acetylsalicylic acid. Hence, this series could be developed as a novel class of analgesic and anti-inflammatory agents.

Experimental

Chemistry

Melting points (mp) were taken in open capillaries on Thomas Hoover melting point apparatus (Thomas Hoover Capillary

Apparatus, Philadelphia, PA, USA) and are uncorrected. The IR spectra were recorded in potassium bromide disks on a Perkin-Elmer Model-398 spectrometer (Perkin-Elmer, Norwalk, CT, USA). The ^{1}H spectra were recorded on a DPX-300 MHz Bruker FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA). The chemical shifts were reported as parts per million (δ ppm) tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument (JEOL, Tokyo, Japan) using fast atom bombardment (FAB positive). Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer (Perkin-Elmer) and values were within the acceptable limits of the calculated values. The progress of the reaction was monitored on readymade silica gel plates (Merck, Darmstadt, Germany) using chloroform-methanol (9 : 1) as a solvent system. Iodine was used as a developing agent. Elemental (C, H, N) analysis indicated that the calculated and observed values were within the acceptable limits (± 0.4%). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (UK), or Spectrochem Pvt. Ltd (India) and were used without further purification.

The 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo[*b*]thiophene **1**, was prepared by the procedure described by Gewald *et al.* [15].

2-Methylsulfanylthiocarbonylamino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic acid ethyl ester ***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 sulphoxide (10 mL) at room temperature, carbon disulphide (1.98 g, 0.026 mol) and aqueous sodium hydroxide (1.2 mL, 20 mol solution) were added simultaneously over 30 min. Then the mixture was allowed to stir for 30 min more. Dimethyl sulphate (2.5 g, 0.02 mol) was added dropwise to the reaction mixture with stirring at 5 – 10°C, it was further stirred for 2 h and then poured into ice water; the solid obtained was filtered, dried, and recrystallized from ethanol. Yield = 85%, mp. 135 – 137°C. IR: 3210 (NH), 1690 (C=O), 1060 cm^{-1} (C=S); ^{1}H-NMR ($CDCl_3$): δ 1.4 – 1.8 (m, 8H, $(CH_2)_4$), 2.0 (t, 3H, 2-$COOCH_2CH_3$), 4.1 (q, 2H, 2-$COOCH_2CH_3$), 4.4 (s, 3H, SCH_3), 7.3 (s, 1H, $NHCSSCH_3$, 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

3-Amino-2-mercapto-5,6,7,8-tetrahydro-3H-benzo[4,5]thieno[2,3-d]pyrimidin-4-one ***3***

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 refluxed on a water bath until the methylmercaptan evolution ceased (8 h). After cooling, the solid obtained was filtered, dried, and recrystallized from ethanol-acetone mixture. Yield = 75%, mp. 251 – 252°C. IR: 3300, 3200 (NH_2), 2550 (SH), 1680 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$): δ 1.5 – 1.9 (m, 8H, $(CH_2)_4$), 3.2 (s, 1H, S*H*), 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.

3-Amino-2-methylsulfanyl-5,6,7,8-tetrahydro-3H-benzo[4,5]thieno[2,3-d]pyrimidin-4-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 sulphate (1.25 g, 0.01 mol) was added dropwise 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%, mp. 216 – 219°C; IR: 3350, 3330 (NH_2), 1690 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 1.3 – 1.7 (m, 8H, $(CH_2)_4$), 3.9 (s, 3H, SCH_3), 6.5 (s, 2H, 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.

General synthetic procedure for 3-subsituted-amino-2-methylsulfanyl-5,6,7,8-tetrahydro-3H-benzo[4,5]thieno-[2,3-d]pyrimidin-4-ones ***A1 – A10***

A mixture of 3-amino-2-methylsulfanyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*] pyrimidin-4-one **4** (1.0 g, 0.004 mol) and appropriate ketones (0.3 mL, 0.004 mol) in glacial acetic acid was refluxed for 30 h. The reaction mixture was poured into ice water. The solid obtained was recrystallized from ethanol. The physical and spectral data of the synthesized compounds are presented in Table 1 and Table 2.

Pharmacological screening

The synthesized compounds were evaluated for analgesic anti-inflammatory activities and the ulcerogenic index. 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 the analgesic and anti-inflammatory testing, but for ulcerogenicity studies intraperitoneally as suspension in 10% v/v Tween-20. Each test group consisted of six animals. The animals were procured from the Tetrex 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 with animals.

Analgesic activity

Test for analgesic activity was performed by tail-flick technique [16, 17] using Wistar albino mice (25 – 35 g) of either sex selected by random sampling technique. Diclofenac sodium at a dose level of 10 mg/kg and 20 mg/kg was administered orally as reference drug for comparison. The test compounds at two dose levels (10, 20 mg/kg) were administered orally. The reaction time was recorded at 30 min, 1, 2, and 3 h after the treatment, and cut-off time was 10 sec. 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.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan-induced paw oedema test in rats [18]. Diclofenac sodium 10, 20 mg/kg was administered as a standard drug for comparison. The test compounds were administered at two dose levels (10 and 20 mg/kg). The paw volumes were measured using the mercury displacement technique with the help of a plethysmograph

immediately before and 30 min, 1, 2, and 3 h after carrageenan injection. The percent inhibition of paw oedema was calculated using the following formula:

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.

Evaluation of ulcerogenicity index

Ulceration in rats was induced as described by Goyal *et al*. [19]. 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 intraperitoneally. One group was administered with acetylsalicylic acid intraperitoneally at a dose of 20 mg/kg once daily for three days. The remaining group of animals was administered with test compounds intraperitoneally at a dose of 20 mg/kg. On day four, pylorus was ligated. Animals were fasted for 36 h before the pylorus ligation procedure [20]. Four hours after the ligation, animals were sacrificed. The stomach was removed and opened along the greater curvature. Ulcer index was determined by the method of Ganguly and Bhatnagar [21] and recorded in Table 5.

Statistical analysis

Statistical analysis of the biological activity of the synthesized compounds on animals was evaluated using a one-way analysis of variance (ANOVA). In all cases, post-hoc comparisons of the means of individual groups were performed using Tukey's test. A significance level of $P < 0.05$ denoted significance in all cases. All values are expressed as mean ± SD (standard deviations). For statistical analysis, we have used GraphPad Prism 3.0 version. (GraphPad Prism 3.0 version; GraphPad Software, Inc., San Diego, CA, USA).

References

[1] J. van Ryn, R. M. Botting, *Inflamm. Res.* **1995**, *44*, 1–10.

[2] J. van Ryn, G. Trummlitz, M. Pairet, *Curr. Med. Chem.* **2000**, *7*, 1145–1161.

[3] J. van Ryn, *Nature New Biol* **1971**, *231*, 232–235.

[4] M. Beuck, *Angew. Chem. Int. Ed.* **1999**, *38*, 631–633.

[5] V. Alagarsamy, R. Revathi, S. Vijayakumar, K. V. Ramseshu, *Pharmazie* **2003**, *58*, 233–236.

[6] V. Alagarsamy, A. Thangathirupathy, S. C. Mandal, S. Rajasekaran, *et al*., *Indian J. Pharm. Sci*. **2006**, *68*, 108–111.

[7] V. Alagarsamy, V. R. Solomon, G. Vanikavitha, V. Paluchamy, *et al*., *Biol. Pharm. Bull.* **2002**, *25*, 1432–1435.

[8] V. Alagarsamy, G. Murugananthan, R. Venkateshperumal, *Biol. Pharm. Bull*. **2003**, *26*, 1711–1714.

[9] V. Alagarsamy, R. Rajesh, R. Meena, S. Vijaykumar, *et al*., *Biol. Pharm. Bull*. **2004**, *27*, 652–656.

[10] V. Alagarsamy, V. Muthukumar, N. Pavalarani, P. Vasanthanathan, R. Revathi, *Biol. Pharm. Bull.* **2003**, *26*, 557–559.

[11] A. E. Abdel–Rahman, E. A. Bakhite, E. A. Al–Taifi, *Pharmazie* **2003**, *58*, 372–377.

[12] R. V. Chambhare, B. G. Khadse, A. S. Bobde, R. H. Bahekar, *Eur. J. Med. Chem*. **2003**, *38*, 89–100.

[13] N. A. Santagati, A. Caruso, V. M. C. Cutuli, F. Caccamo, *Farmaco* **1995**, *50*, 689–695.

[14] L. M. Lima, E. J. Barreiro, *Curr. Med. Chem*. **2005**, *12*, 23–57.

[15] K. Gewald, E. Schinke, H. Bottcher, *Chem. Ber*. **1966**, *99*, 94–100.

[16] S. K. Kulkarni, *Life Sci.* **1980**, *27*, 185–188.

[17] R. E. Amour, D. L. Smith, *J. Pharm. Exp. Therap.* **1941**, *72*, 74–78.

[18] C. A. Winter, E. A. Risely, G. W. Nuss, *Exp. Biol. Med.* **1962**, *111*, 544–547.

[19] R. K. Goyal, A. Chakrabarti, A. K. Sanyal, *Planta med.* **1985**, *29*, 85–88.

[20] M. Shay, S. A. Komarov, D. Fels, D. Meranze, *et al*., *Gastroenterology* **1945**, *5*, 43–61.

[21] A. K. Ganguly, O. P. Bhatnagar, *Can. J. Physilo. Pharmacol*. **1973**, *51*, 748–750.

