Synthesis of some novel 2-mercapto-3-(substituted amino)-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-ones as analgesic and anti-inflammatory agents

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Abstract

A variety of novel 2-mercapto-3-(substituted amino)-5,6,7,8-tetrahydro-3*H*-benzo[4,5] thieno[2,3-*d*]pyrimidin-4-ones were synthesized by reacting 3-Amino-2-mercapto-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one with different aldehydes and ketones; the starting material 3-amino-2-mercapto-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 thiophene by a novel innovative route. The title compounds were investigated for analgesic, anti-inflammatory and ulcerogenic index activities. While the test compounds exhibited significant activity, compounds **AS1, AS2** and **AS3** showed more potent analgesic activity. The compound **AS3** showed more potent anti-inflammatory activity when compared to the reference standard diclofenac sodium. Interestingly the test compounds showed only mild ulcerogenic potential when compared to aspirin.

Keywords: Thienopyrimidine, thioureas, pyrimidine, analgesic, anti-inflammatory

Introduction

Quinazolines and condensed quinazolines are found to possess potent analgesic and anti-inflammatory activities. On our going medicinal chemistry research programme we have found that quinazolines and condensed quinazolines exhibit potent central nervous system (CNS) activities like analgesic, anti-inflammatory[1] and anticonvulsant.[2] 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 (Fig 1, **I**),[6] 2-methyl-3-substituted quinazolines (Fig 1, **II**),[7] 2-methylthio-3-substituted quinazolines (Fig 1, **III**),[8] 2,3-disubstituted quinazolines[9] they 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 designed some 2-mercapto-3-(substituted amino)-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-ones. The title compounds were synthesized by reacting 3-amino-2-mercapto-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-mercapto-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-tetrahydrobenzothiophene **1**, by a novel innovative route (Scheme 1). Spectral data (IR, NMR and mass spectra) confirmed the structures of the synthesized compounds; the purity of these compounds was ascertained by microanalysis (Table 1). The synthesized compounds were tested for their analgesic, anti-inflammatory and ulcerogenic index activities.

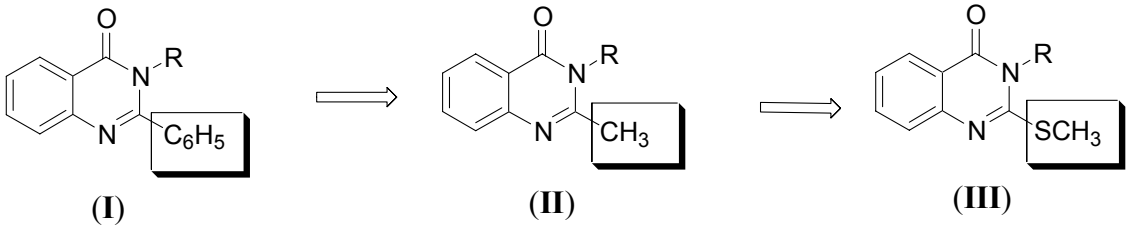
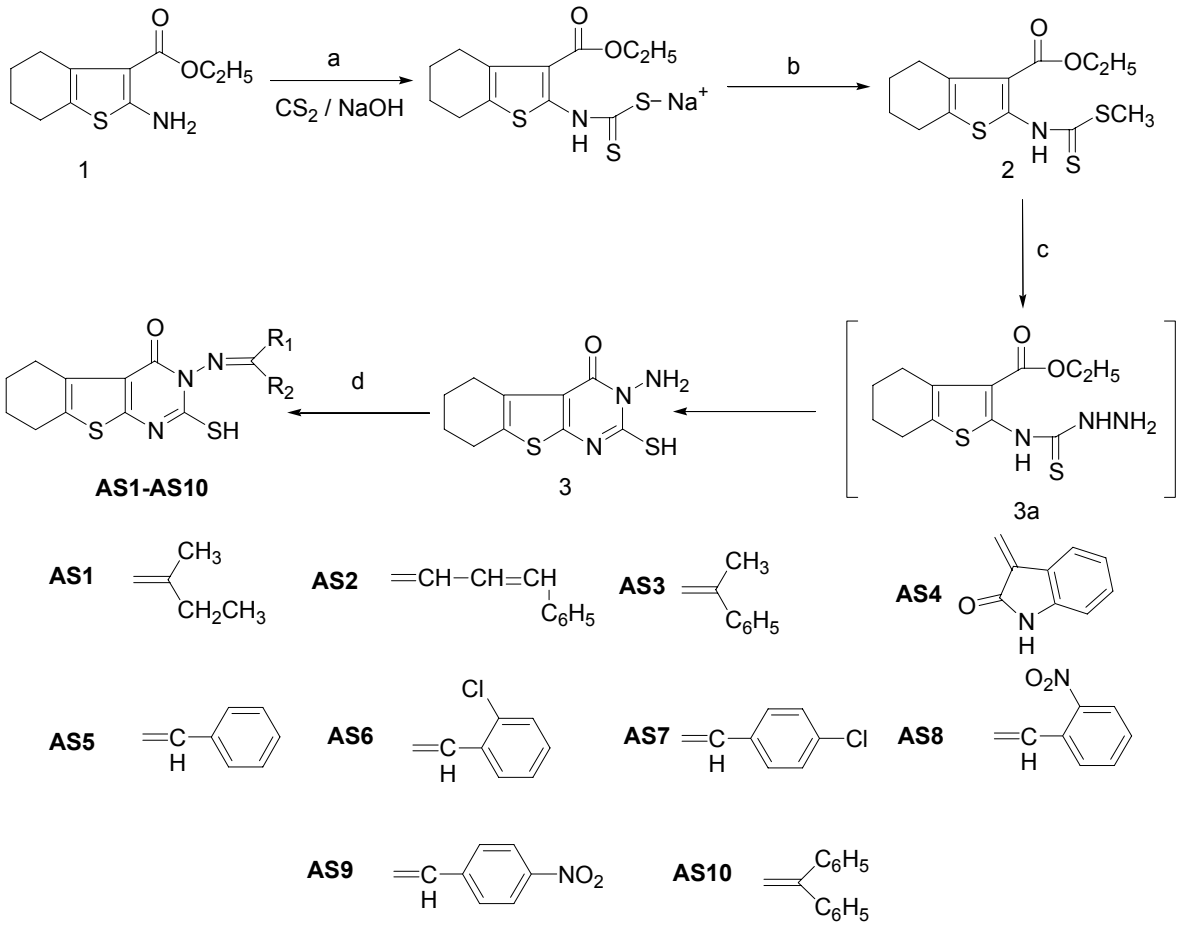


Figure 1. Some lead molecules of quinazolines.

Results and Discussion

Synthetic pathway depicted in Scheme 1 outlines the chemistry of present work. The key intermediate 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-tetrahydrobenzothiophene **1** in dimethyl sulphoxide, the sodium salt of dithiocarbamic acid obtained was methylated with dimethyl sulphate to get methyl 2-methylsulfanylthiocarbonylamino-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester **2**. The compound **2** and hydrazine hydrate when refluxed in ethanol yielded the desired 3-amino-2-mercapto-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one **3.** The product obtained was cyclic and not an open chain thiosemicarbazide **3a**. The IR spectrum of **3** 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. NMR spectrum of **3** showed multiplet at δ 1.52-1.91 for cyclohexyl and singlet at 3.24 and 5.42 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 spectrum is also in agreement with the molecular weight of the compound.



Scheme 1. Synthesis of 2-mercapto-3-(substituted amino)-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-ones.

The title compounds 2-mercapto-3-(substituted amino)-5,6,7,8-tetrahydro-3*H*-benzo[4,5] thieno[2,3-*d*]pyrimidin-4-ones **AS1-AS10** were obtained by the condensation of amino group of 3-amino-2-mercapto-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one **(3)** with different aldehydes and ketones. The formation of title product is indicated by the disappearance of peak due to 3-NH_2 of the starting material in IR and NMR spectra of all the compounds **AS1-AS10**. The IR and NMR spectra of 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 spectra is in agreement with the molecular weight of the compounds. Elemental (C,H,N) analysis indicated that the calculated and observed values were within the acceptable limits (± 0.4%). Characterization data of the title compounds is represented in Table 1.

Table 1. Characterization data of 2-mercapto-3-(substituted amino)-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-ones

Comp. Code	Mol. formula	Mol. Wt[a].	Mp, ^{o}C	Yield %	Calculated/Found, %		
					C	H	N
AS1	$C_{14}H_{17}N_3OS_2$	307	235-237	81	54.69	5.57	13.67
					54.65	5.59	13.61
AS2	$C_{19}H_{17}N_3OS_2$	367	213-214	73	62.10	4.66	11.43
					62.17	4.68	11.47
AS3	$C_{18}H_{17}N_3OS_2$	355	259-261	77	60.82	4.82	11.82
					60.83	4.79	11.87
AS4	$C_{18}H_{14}N_4O_2S_2$	382	261-263	72	56.52	3.68	14.64
					56.57	3.63	14.71
AS5	$C_{17}H_{15}N_3OS_2$	341	197-199	81	59.79	4.42	12.31
					59.76	4.43	12.34
AS6	$C_{17}H_{14}N_3OS_2Cl$	375	268-270	76	54.31	3.75	11.17
					54.30	3.79	11.19
AS7	$C_{17}H_{14}N_3OS_2Cl$	375	214-216	74	54.31	3.75	11.17
					54.36	3.72	11.23
AS8	$C_{17}H_{14}N_4O_3S_2$	386	266-268	79	52.83	3.65	14.49
					52.86	3.62	14.56
AS9	$C_{17}H_{14}N_4O_3S_2$	386	277-279	72	52.83	3.65	14.49
					52.81	3.63	14.45
AS10	$C_{23}H_{19}N_3OS_2$	417	283-285	79	66.16	4.59	10.06
					66.19	4.63	10.11

[a]Molecular weight determination by mass spectral analysis

The biological screening results revealed that all the test compounds **(AS1-AS10**) showed significant analgesic activity (Table 2). Compound **AS1** with N-3-aliphatic substituent showed good activity, when it was replaced by araliphatic group (compound **AS2** and **AS3**) leads to retaining in activity. Placement of aryl group at N-3 (compounds **AS4, AS5** and **AS10**) results in decreasing activity. Placement of electron withdrawing group at N-3 aryl ring (compounds **AS6-AS9**) results in further decrease in activity. Compound 3-*sec*-butylideneamino-2-mercapto-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (**AS1**) emerged as the most active analgesic agent and it is more potent when compared to the reference standard diclofenac sodium.

Table 2. Analgesic activity (tail-flick technique)

Comp. code	Dose (mg/kg)	Percent Analgesic activity			
		0.5 h	1 h	2 h	3 h
AS1	10	46 ± 1.05**	50 ± 1.39**	59 ± 2.36**	42 ± 1.32*
	20	63 ± 1.21***	66 ± 1.52***	72 ± 1.42***	50 ± 1.39**
AS2	10	38 ± 1.39*	39 ± 1.62**	45 ± 1.43**	35 ± 1.62*
	20	49 ± 1.42**	54 ± 1.71**	59 ± 1.62***	41 ± 1.31*
AS3	10	43 ± 1.46*	46 ± 1.37**	48 ± 1.51**	38 ± 1.67*
	20	56 ± 1.17**	57 ±1.82**	66 ± 1.67***	46 ± 1.56**
AS4	10	37 ± 1.46*	39 ± 1.47*	45 ± 1.47*	30 ± 1.52*
	20	49 ± 1.53**	49 ± 1.36**	57 ± 1.28**	38 ± 1.38*
AS5	10	33 ± 1.41*	36 ± 1.48*	44± 1.23*	29 ± 1.45*
	20	48 ± 1.29**	50 ± 1.62**	55± 1.38**	40± 1.25*
AS6	10	31 ± 1.73*	37 ± 1.66*	38± 1.38*	33 ± 1.62*
	20	43 ±1.37*	46 ± 1.42**	46 ± 1.72**	40 ± 1.38*
AS7	10	35 ± 1.46*	38± 1.27*	39 ± 1.82*	29 ±1.27*
	20	42 ± 1.28*	45 ± 1.18**	45 ± 1.26**	39 ± 1.13*
AS8	10	31 ± 1.35*	35 ± 1.27*	39± 1.31*	27 ± 1.16*
	20	42 ± 1.32*	49 ± 1.23**	50± 1.56**	35 ± 1.42*
AS9	10	33 ± 1.51*	36 ± 1.19*	42 ± 1.52*	34 ± 1.19*
	20	40 ± 1.37*	45 ± 1.42**	46 ± 1.81**	39 ± 1.67*
AS10	10	32 ± 1.48*	39 ± 1.29*	44 ± 1.57*	36 ± 1.64*
	20	41 ± 1.23*	49 ± 1.82**	53 ± 1.31**	42 ± 156*
Control		2 ± 0.35	6 ± 0.49	4 ± 0.59	4± 0.91
Diclofenac	10	37 ± 1.69*	43 ± 1.42***	45 ± 092**	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<0.01and ***p<0.001 as compared with the respective control

The anti-inflammatory activity data (Table 3) indicated that all the test compounds protected rats from carrageenan-induced inflammation. The compound 3-*sec*-butylideneamino-2-mercapto-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (**AS1)** showed more potent anti-inflammatory activity and compounds **AS2** and **AS3** showed equipotent anti-inflammatory activity when compared to the reference standard diclofenac sodium.

Table 3. Anti-inflammatory activity (carrageenan induced rat paw oedema method)

Comp. code	Dose (mg/kg)	Percent Protection			
		0.5 h	1 h	2 h	3 h
AS1	10	39 ± 1.65*	41 ± 1.52**	45 ± 1.15**	35 ± 1.32*
	20	47 ± 1.42**	56 ± 1.36***	61 ±1.27***	46 ± 1.38**
AS2	10	33 ± 1.63*	37 ± 1.38*	40 ± 1.46*	34 ± 1.26*
	20	46 ± 1.41**	53 ± 1.83**	58 ± 1.29***	38 ± 1.51*
AS3	10	37 ± 1.83*	37 ± 1.46*	42 ± 1.57*	33 ± 1.57*
	20	47 ± 1.35**	55 ± 1.29***	59 ±1.05***	34 ± 1.16*
AS4	10	32 ± 1.36*	38 ± 1.30*	40 ± 1.26*	28 ± 1.93*
	20	41 ± 1.62**	46 ± 1.29**	55± 1.52***	37 ± 1.26*
AS5	10	30 ± 1.73*	34 ± 1.15*	37 ± 1.93*	31 ± 1.07*
	20	38 ± 1.17*	45 ± 1.09**	48 ± 1.07**	37 ± 1.27*
AS6	10	29 ± 1.29*	34 ± 1.18*	37 ± 1.38*	34 ± 1.29*
	20	37 ± 1.16*	43 ± 1.93**	49 ± 1.17**	37 ± 1.06*
AS7	10	33 ± 1.45*	33 ± 1.29*	34 ± 1.52*	30 ± 1.32*
	20	37 ± 1.32*	38 ± 1.26*	45 ± 1.07**	38 ± 1.26*
AS8	10	31 ± 1.67*	36 ± 1.31*	36 ± 1.32*	33 ± 1.61*
	20	36 ± 1.36*	43 ± 1.42*	52 ± 1.39**	39 ± 1.53*
AS9	10	30 ± 1.25*	35 ± 1.81*	38 ± 1.27*	26 ± 1.15*
	20	39 ± 1.37*	43 ± 1.41**	48 ± 1.91**	27 ± 1.37*
AS10	10	33 ± 1.72*	37 ± 1.63*	39 ± 1.62*	35 ± 1.48*
	20	39 ± 1.39*	48 ± 1.37**	51 ± 1.31**	38 ± 1.60*
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<0.01and ***p<0.001 as compared with the respective control

The ulcer index of the test compounds (Table 4) reveals that the compounds **AS6-AS9** 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.

Table 4. Ulcerogenicity index

Cmp.Code	Ulcer index	Comp.Code	Ulcer index
AS1	0.61±0.26*	**AS7**	0.83±0.31*
AS2	0.65±0.23*	**AS8**	0.86±0.27*
AS3	0.73±0.29*	**AS9**	0.81±0.23*
AS4	0.64±0.23*	**AS10**	0.74±0.26*
AS5	0.69±0.26*	Control	0.15±0.32
AS6	0.91±0.23*	Aspirin	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

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 the C-2 position also we 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**)[6], whereas the C-2 methyl series lead molecule showed 50% analgesic and 44% anti-inflammatory activity (Fig 1, **II**)[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 (Fig 1, **III**)[9]. The results of the analgesic and anti-inflammatory activities of the present series showed that enhancement of activity (56% analgesic and 46% anti-inflammatory activity). Interestingly these compounds showed negligible ulcer index unlike other Non Steroidal Anti-inflammatory Drugs (NSAID's), Hence this series could be developed as a novel class of analgesic and anti-inflammatory agents. However further structural modification is planned to increase the analgesic and anti-inflammatory activities with the decreased ulcerogenic index.

Experimental Section

General Procedures. 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 ^1H NMR spectra on Varian 300 MHz spectrometer, using tetramethylsilane as internal standard. Elemental analyses were performed on Carlo Erba 1108. The 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzothiophene **1**, was prepared as per the procedure described by Gewald and Schinke[10].

Synthesis of 2-methylsulfanylthiocarbonylamino-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester (2) To a vigorously stirred solution of 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzothiophene **(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 stirr 30 min more. Dimethyl sulphate (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 (br, NH), 1690 (vs, C=O,), 1060 (s, C=S,) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.41-1.83 (m, 8H, (CH_2)$_4$), 2.01 (t, 3H, $COOCH_2CH_3$), 2.43 (s, 3H, SCH_3), 4.11 (q, 2H, 2-$COOCH_2CH_3$), and 7.30 (s, 1H, $NHCSSCH_3$, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$): δ 15.6, 18.5, 21.9, 28.1, 37.3 (2C), 61.3, 129.2, 134.8, 136.5, 144.4, 164.3, 197.5; 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-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-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 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 (br, NH_2,), 2550 (m, SH), 1680 (vs, C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.52-1.91 (m, 8H, (CH_2)$_4$), 3.24 (s, 1H, S*H*, D_2O exchangeable), 5.42 (br s, 2H, NH_2, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$): δ 22.8, 28.5, 37.1 (2C), 134.7, 138.2, 145.1, 146.3, 160.5, 169.7; 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-*sec*-Butylideneamino-2-mercapto-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*] pyrimidin-4-one (AS1). A mixture of 3-amino-2-mercapto-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-d]pyrimidin-4-one 1.0 gm (0.004 mol) and ethyl methyl ketone 0.3 ml (0.004 mol) in glacial acetic acid was refluxed for 25 h. The reaction mixture was poured into ice water. The solid obtained was recrystallized from ethanol, yield =81%, mp 235-237° C; IR (KBr) (cm^{-1}): 2543 (m, SH), 1685 (vs, C=O), 1616 (vs, C=N); ^{1}H-NMR($CDCl_3$) : δ 0.92-1.51 (m, 8H, (CH_2)$_4$), 1.71 (q, 2H, CH_2CH_3), 2.14 (t, 3H, CH_2CH_3), 2.30 (s, 3H, CH_3), 3.51 (s, 1H, S*H*, D_2O exchangeable); ^{13}C-NMR ($CDCl_3$): δ 8.9, 17.5, 21.3, 22.4, 28.4, 37.2 (2C), 133.2, 136.8, 143.8, 144.8, 157.7, 165.1, 172.3. Similarly the compounds **AS2-AS10** were synthesized.

2-Mercapto-3-(3-phenyl-allylideneamino)-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*] pyrimidin-4-one (AS2) IR (KBr) (cm^{-1}): 2537 (m, SH), 1689 (vs, C=O), 1614 (s, C=N); ^{1}H-NMR ($CDCl_3$): δ 1.02-1.60 (m, 8H, (CH_2)$_4$), 3.41 (s, 1H, S*H*, D_2O exchangeable), 6.03 (s, 1H, C*H*), 6.51 (s, 1H, C*H*), 6.94 (s, 1H, C*H*), 6.91-7.23 (m, 5H, Ar-*H*); ^{13}C-NMR ($CDCl_3$): δ 22.4, 28.6, 37.2 (2C), 120.7, 128.2 (2C), 128.9 (2C), 130.1, 134.2, 135.7, 136.9, 138.6, 144.8, 146.9, 155.8, 164.2, 172.9.

2-Mercapto-3-(1-phenyl-ethylideneamino)-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*] pyrimidin-4-one (AS3) IR (KBr) (cm^{-1}): 2541 (m, SH), 1675 (vs, C=O), 1615 (s, C=N); ^{1}H-NMR ($CDCl_3$): δ 1.10-1.62 (m, 8H, (CH_2)$_4$), 2.02 (s, 3H, CH_3), 3.41 (s, 1H, S*H*, D_2O

exchangeable), 7.32-7.71 (m, 5H, Ar-*H*); ^{13}C-NMR ($CDCl_3$): δ 19.6, 22.4, 28.7, 37.2 (2C), 129.4 (2C), 131.2 (2C), 132.1, 132.9, 133.3, 135.4, 144.7, 145.6, 157.2, 164.7, 172.3.

2-Mercapto-3-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (AS4) IR (KBr) (cm^{-1}): 2535 (m, SH), 1675 (vs, C=O), 1611 (s, C=N); ^{1}H-NMR ($CDCl_3$) : δ 1.02-1.81 (m, 8H, (C*H*$_2$)$_4$), 3.31 (s, 1H, S*H*, D_2O exchangeable), 7.82-8.24 (m, 4H, Ar-*H*), 8.51 (s, 1H, N*H*, D_2O exchangeable); ^{13}C NMR ($CDCl_3$): δ 22.1, 28.4, 37.3 (2C), 122.4, 124.1, 125.6, 131.4, 133.2, 133.9, 135.7, 139.1, 144.8, 145.9, 157.4, 162.3, 165.8, 172.5.

3-(Benzylidene-amino)-2-mercapto-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*] pyrimidin-4-one (AS5) IR (KBr) (cm^{-1}): 2549 (m, SH), 1683 (vs, C=O), 1617 (s, C=N); ^{1}H-NMR ($CDCl_3$) : δ 0.80-1.32 (m, 8H, (C*H*$_2$)$_4$), 3.63 (s, 1H, S*H*, D_2O exchangeable), 6.51 (s, 1H, C*H*), 7.31-7.82 (m, 5H, Ar-*H*); ^{13}C-NMR ($CDCl_3$): δ 22.7, 28.3, 37.3 (2C), 129.6 (2C), 130.1 (2C), 131.9, 132.2, 133.1, 136.4, 144.7, 145.3, 156.4, 164.2, 172.1.

3-[(2-Chloro-benzylidene)-amino]-2-mercapto-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (AS6) IR (KBr) (cm^{-1}): 2545 (m, SH), 1680 (vs, C=O), 1620 (s, C=N); ^{1}H-NMR ($CDCl_3$) : δ 0.91-1.23 (m, 8H, (C*H*$_2$)$_4$), 3.62 (s, 1H, S*H*, D_2O exchangeable), 6.63 (s, 1H, C*H*), 7.32-7.81 (m, 4H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 22.3, 28.2, 37.5 (2C), 127.7, 130.4, 131.9, 132.7, 133.9 (2C), 135.6, 137.3, 144.6, 145.9, 156.9, 165.2, 172.5.

3-[(4-Chloro-benzylidene)-amino]-2-mercapto-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (AS7) IR (KBr) (cm^{-1}): 2532 (m, SH), 1685 (vs, C=O), 1622 (s, C=N); ^{1}H-NMR ($CDCl_3$) : δ 1.21-1.70 (m, 8H, (C*H*$_2$)$_4$), 3.41 (s, 1H, S*H*, D_2O exchangeable), 6.60 (s, 1H, C*H*), 7.91-8.23 (m, 4H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 21.9, 28.0, 37.3 (2C), 127.5, 130.0, 131.8, 132.4, 133.5 (2C), 135.6, 137.9, 145.1, 146.2, 156.6, 165.2, 172.2.

2-Mercapto-3-[(2-nitro-benzylidene)-amino]-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (AS8). IR (KBr) (cm^{-1}): 2530 (m, SH), 1676 (vs, C=O), 1618 (s, C=N); ^{1}H-NMR ($CDCl_3$): δ 1.03-1.81 (m, 8H, (C*H*$_2$)$_4$), 3.51 (s, 1H, S*H*, D_2O exchangeable), 6.71 (s, 1H, C*H*), 7.52-7.81 (m, 4H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 21.8, 28.9, 36.9 (2C), 125.5, 128.4, 131.5, 133.4, 134.2, 136.3, 137.5, 145.8, 146.7, 149.9, 156.2, 165.7, 172.2.

2-Mercapto-3-[(4-nitro-benzylidene)-amino]-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*] pyrimidin-4-one (AS9). IR (KBr) (cm^{-1}): 2535 (m, SH), 1683 (vs, C=O), 1633 (s, C=N); ^{1}H-NMR ($CDCl_3$): δ 1.31-1.84 (m, 8H, (C*H*$_2$)$_4$), 3.60 (s, 1H, S*H*, D_2O exchangeable), 6.73 (s, 1H, C*H*), 7.53-7.81 (m, 4H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 22.1, 28.5, 37.8 (2C), 124.9, 128.7, 131.3, 133.8, 134.7, 137.9, 145.9, 146.7, 146.9, 149.9, 156.9, 165.8, 172.9.

3-(Benzhydrylidene-amino)-2-mercapto-5,6,7,8-tetrahydro-3H-benzo[4,5]thieno[2,3-*d*] pyrimidin-4-one (AS10). IR (KBr) (cm^{-1}): 2545 (m, SH), 1674 (vs, C=O), 1619 (s, C=N); ^{1}H-NMR ($CDCl_3$) : δ 0.92 -1.53 (m, 8H, (C*H*$_2$)$_4$), 3.51 (s, 1H, S*H*, D_2O exchangeable), 7.24-8.12 (m, 10H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 22.6, 28.5, 37.3 (2C), 130.4 (2C), 130.9, 132.7, 133.0, 133.5, 133.9, 157.6, 133.4, 136.4, 139.5, 143.8, 144.9, 145.9, 146.3, 152.6, 156.9, 165.2, 172.9.

Pharmacology

The synthesized compounds were evaluated for analgesic, anti-inflammatory and ulcerogenic activities. Student 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% carboxyl methyl cellulose as vehicle) by the same route of administration. Each group consisted of six animals. The Institutional Animal Ethics Committee approved the protocol of the animal studies.

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 12h light and dark cycle; they were fed standard animal feed. All the animals were acclimatized for a week before use.

Analgesic activity. Test for analgesic activity was performed by tail-flick technique using Wistar albino mice (25 - 35 gm) of either sex selected by random sampling technique. [11,12] 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 30min, 1, 2 and 3h after the treatment, and cut-off time was 10 sec. The percent analgesic activity (PAA) was calculated by the following formula,

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

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.[13] Diclofenac sodium 10, 20 mg/kg was administered as a standard drug for comparison. The test compounds were administered at two dose levels (10 mg, 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 3h 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.*[14] 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 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 fourth day, pylorus was ligated, 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.[15]

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