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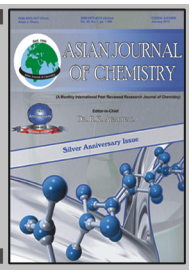

Synthesis and Biological Evaluation of Novel Thio-1,4-dihydropyrimidine-5-carboxylate Derivatives

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We have synthesized ten novel isopropyl 2-(4-substituted benzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate derivatives (**5a-j**). All the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, MS and elemental analysis data. Newly synthesized compounds were screened for antiinflammatory activity on acetic acid induced writhing in mice and carrageenan induced paw oedema in rats. Compounds (**5g**) and (**5b**) showed a potent antiinflammatory activity (100 % at 100 mg/kg b.w) compared to reference standard drug, nimesulide (100% at 50 mg/kg b.w). The other compounds showed good antiinflammatory activity. All the synthesized compounds were also screened for antioxidant activity, among those three compounds were shown good antioxidant activity by using the *in vitro* method.

Key Words: Thio-1,4-dihydropyrimidine-5-carboxylate, Antiinflammatory, Antioxidant activity.

INTRODUCTION

Heterocycles are ubiquitous to among pharmaceutical compounds[1]. Pyrimidine moiety is an important class of N-containing heterocycles widely used as key building blocks for pharmaceutical agents. These compounds exhibit a wide spectrum of pharmacophore, as they act as bactericidal, fungicidal[2-5], analgesic[6], antioxidant[7], antihypertensive[8], antifilarial[9] and antitumor agents[10]. Preclinical data from literature indicates the continuing research in polysubstituted pyrimidine as potential antitumor agents[11-13]. Among these, thiouracils in particular are used as antiinflammatory and virucidal agents[14]. The biological and synthetic significance places this scaffold at a prestigious position in medicinal chemistry research.

The key role pyrimidines play in cellular processes has made them valuable leads for drug discovery. One important class of pyrimidines is 2-thiopyrimidine (2-TP) and its derivatives, which are also recognized as 2-mercaptopyrimidine compounds[15]. In 2-thiopyrimidine ring, sulfur atom serves as an interesting replacement for the existing oxygen atom bonded to C-2 in uridine base[16,17]. Based on this approach, 2-thiopyrimidines have attracted significant attention of synthetic chemists and biochemists[18-20]. Pathak *et al.*[21] have reported potential activity of 2-thiopyrimidine derivatives against *Mycobacterium tuberculosis* (Mtb).

One-step synthesis of 3,4-dihydropyrimidin-2(1*H*)-one by three-component condensation of aldehydes, ethyl acetoacetate and urea in alcohol using strong mineral acid was first reported by Biginelli[22]. These products, popularly known as Biginelli compounds possess several pharmaceutical properties like antibacterial, antiviral, antiinflammatory, antihypertensive and antitumor agents[23]. Based on the literature reports and recognizing the prospective of substituted thiopyrimidenes as antiinflammatory and antimicrobial agents, in a continued quest for new potential molecules, we designed and synthesized novel isopropyl 2-(4-substituted benzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate derivatives (**5a-j**) having substituted benzylthio groups. Structures of the products were characterized by IR, ^{1}H NMR, ^{13}C NMR, LC-MS and elemental analysis.

EXPERIMENTAL

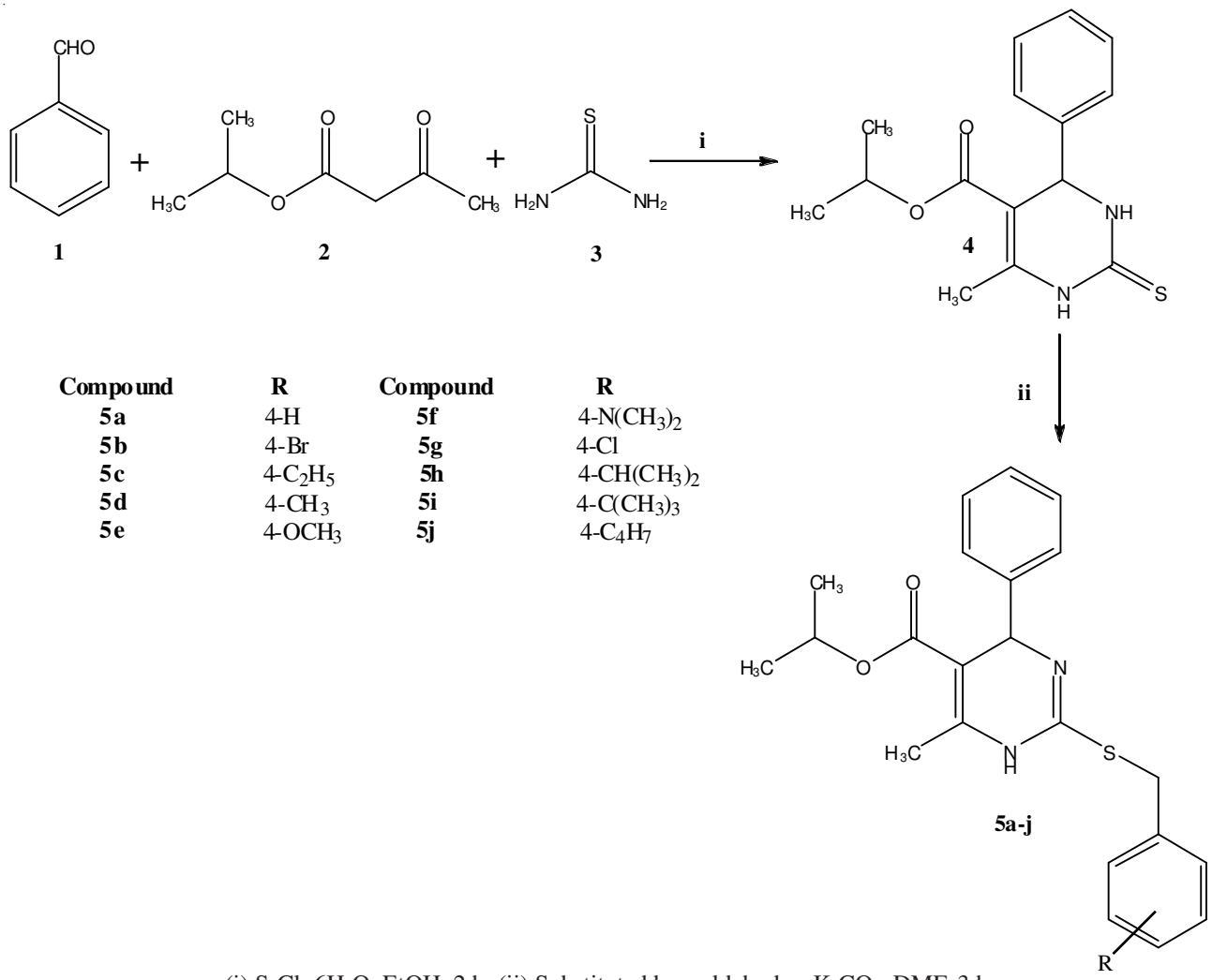
All reagents and solvents (Aldrich or Merck) were purchased and used without further purification. Melting points were determined on a Fisher-Johns melting point apparatus were uncorrected. Crude products were purified by column chromatography on silica gel of 60-120 mesh. IR spectra were obtained on a Perkin Elmer BX serried FT-IR 5000 spectrometer using KBr pellet. NMR spectra were recorded on a Bruker 400 MHz spectrometer for ^{1}H NMR. The ^{13}C NMR spectra were recorded on JEOL. The chemical shifts were reported as ppm down field using TMS as an internal standard. LC-MS spectra were recorded on a MASPEC low resolution mass spectrometer operating at 70 eV.

General procedure for the synthesis of 5-isopropoxycarbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-thione (4)[24]: To a solution of isopropyl acetoacetate (1 mmol), aldehyde (1.1 mmol), thiourea (1.5 mmol), $SrCl_2 \cdot 6H_2O$ (1 mmol, 10 mol %) and EtOH (20 mL). The mixture was heated at 40 ºC and the progress of the reaction was monitored by TLC. After completion of the reaction (*ca.* 3-5 h) the solution was cooled to room temperature and poured into crushed ice. The resultant solid product was collected and purified by column chromatography (**Scheme-I**).

General procedure for the synthesis of isopropyl 2-(4-substitutedbenzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (5a-j): An ice cold solution of 5-isopropoxycarbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-thione (1 mmol) in DMF (4 vol), potassium carbonate (1.5 mmol) and substituted benzyl bromides (1.3 mmol) was taken in a 1 L round bottomed flask equipped with magnetic stirrer and stirred for 3 h. The residual portion was poured on to crushed ice, neutralized with dilute acid and the product obtained isopropyl 2-(4-substituted benzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (**5a-j**). It was filtered, washed with water, dried and recrystallized from ethanol (**Scheme-I**).

Isopropyl 2-(benzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (5a): Pale yellow solid; Yield 69 %; m.p. 196-198 ºC; IR (KBr, ν_{max}, cm^{-1}): 3313 (NH), 1709 (CO), 1625 (C-N), 1559 (C-C), 641 (C-S); ^{1}H NMR (δ, DMSO-d_6): 1.24 (d, 6H, OCH-$(CH_3)_2$), 2.10 (s, 1H, NH), 2.35 (s, 3H, Ar-CH_3), 4.21 (s, 1H, -CH), 4.91 (m, 1H, OCH-$(CH_3)_2$), 5.10 (s, 2H, -SCH_2), 7.20-7.52 (m, 10H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 170.10, 162.63, 152.96, 142.95, 136.89, 129.63, 129.34, 128.42, 128.14, 127.37, 127.15, 120.22, 66.23, 48.19, 38.25, 23.53, 21.44; LCMS: (m/z) 381 [M+H]. Anal. calcd. for $C_{22}H_{24}N_2O_2S$: C, 69.42; H, 6.35; N, 7.33. Found: C, 69.58; H, 6.47; N, 7.29.

Isopropyl 2-(4-bromobenzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (5b): Yellow solid; Yield 63 %; m.p. 215-216 ºC; IR (KBr, ν_{max}, cm^{-1}): 3315 (NH), 1716 (CO), 1613 (C-N), 1528 (C-C), 638 (C-S); ^{1}H NMR (δ, DMSO-d_6): 1.30 (d, 6H, OCH-$(CH_3)_2$), 2.15 (s, 1H, NH), 2.39 (s, 3H, Ar-CH_3), 4.27 (s, 1H, -CH), 4.93 (m, 1H, OCH-$(CH_3)_2$), 5.24 (s, 2H, -SCH_2), 7.08-7.91 (m, 9H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 170.59, 163.89, 152.71, 143.48, 136.76, 132.66, 132.18, 129.70, 127.63, 127.41, 120.88, 119.93, 67.66, 48.39, 39.42, 23.78, 21.53; LCMS: (m/z) 460 [M + 2H]. Anal. calcd. for $C_{22}H_{23}N_2O_2SBr$: C, 57.50; H, 5.03; N, 6.13. Found: C, 57.71; H, 4.88; N, 6.21.



Compound	R	Compound	R
5a	4-H	**5f**	4-$N(CH_3)_2$
5b	4-Br	**5g**	4-Cl
5c	4-C_2H_5	**5h**	4-$CH(CH_3)_2$
5d	4-CH_3	**5i**	4-$C(CH_3)_3$
5e	4-OCH_3	**5j**	4-C_4H_7

(i) $SrCl_2 \cdot 6H_2O$, EtOH, 2 h; (ii) Substituted benzaldehydes, K_2CO_3, DMF, 3 h

Scheme-I: Synthetic pathway for the compounds **5a-j**

Isopropyl 2-(4-ethylbenzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (5c): Yellow solid; Yield 75 %; m.p. 174-175 ºC; IR (KBr, ν_{max}, cm^{-1}): 3310 (NH), 1711 (CO), 1609 (C-N), 1546 (C-C), 654 (C-S); ^{1}H NMR (δ, DMSO-d_6): 1.18 (t, 3H, -ArCH$_2$-CH$_3$), 1.26 (d, 6H, OCH-(CH$_3$)$_2$), 2.10 (s, 1H, NH), 2.34 (s, 3H, Ar-CH$_3$), 2.47 (q, 2H, -ArCH$_2$-CH$_3$), 4.23 (s, 1H, -CH), 4.90 (m, 1H, OCH-(CH$_3$)$_2$), 5.15 (s, 2H, -SCH$_2$), 6.91-7.40 (m, 9H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 169.92, 162.53, 152.98, 142.74, 142.13, 134.12, 129.44, 129.19, 128.48, 127.50, 127.21, 120.38, 66.35, 48.22, 38.44, 27.19, 23.51, 21.47, 17.62; LCMS: (m/z) 409 [M + H]. Anal. calcd. for $C_{24}H_{28}N_2O_2S$: C, 70.57; H, 6.91; N, 6.86. Found: C, 70.78; H, 7.01; N, 6.77.

Isopropyl 6-methyl-2-(4-methylbenzylthio)-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (5d): Pale yellow solid; Yield 65 %; m.p. 223-224 ºC; IR (KBr, ν_{max}, cm^{-1}): 3312 (NH), 1710 (CO), 1617 (C-N), 1544 (C-C), 648 (C-S); ^{1}H NMR (δ, DMSO-d_6): 1.24 (d, 6H, OCH-(CH$_3$)$_2$), 2.10 (s, 1H, NH), 2.28-2.33 (s, 6H, -CH$_3$), 4.21 (s, 1H, -CH), 4.89 (m, 1H, OCH-(CH$_3$)$_2$), 5.10 (s, 2H, -SCH$_2$), 7.08-7.35 (m, 9H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 169.73, 162.51, 152.88, 142.77, 135.67, 132.84, 129.95, 129.31, 129.23, 127.35, 127.09, 120.43, 66.43, 48.10, 38.57, 23.49, 21.85, 21.40; LCMS: (m/z) 395 [M + H]. Anal. calcd. for $C_{23}H_{26}N_2O_2S$: C, 70.05; H, 6.69; N, 7.16. Found: C, 70.17; H, 6.53; N, 7.23.

Isopropyl 2-(4-methoxylbenzylthio)-6-methyl-4-phenyl-1,4-dihydro-pyrimidine-5-carboxylate (5e): Yellow solid; Yield 76 %; m.p. 170-171 ºC; IR (KBr, ν_{max}, cm^{-1}): 3310 (NH), 1706 (CO), 1606 (C-N), 1568 (C-C), 645 (C-S); ^{1}H NMR (δ, DMSO-d_6): 1.30 (d, 6H, OCH-(CH$_3$)$_2$), 2.15 (s, 1H, NH), 2.38 (s, 3H, Ar-CH$_3$), 3.76 (s, 3H, -ArOCH$_3$), 4.26 (s, 1H, -CH), 4.92 (m, 1H, OCH-(CH$_3$)$_2$), 5.20 (s, 2H, -SCH$_2$), 6.84-7.36 (m, 9H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 170.51, 163.19, 153.97, 153.24, 143.58, 129.80, 129.23, 128.10, 127.91, 127.44, 120.94, 67.51, 54.11, 48.43, 39.34, 23.80, 21.69; LCMS: (m/z) 411 [M + H]. Anal. calcd. for $C_{23}H_{26}N_2O_3S$: C, 67.29; H, 6.38; N, 6.82. Found: C, 67.18; H, 6.29; N, 6.93.

Isopropyl 2-(4-(dimethylamino)benzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (5f): Yellow solid; Yield 82 %; m.p. 210-212 ºC; IR (KBr, ν_{max}, cm^{-1}): 3314 (NH), 1718 (CO), 1610 (C-N), 1538 (C-C), 642 (C-S); ^{1}H NMR (δ, DMSO-d_6): 1.30 (d, 6H, OCH-(CH$_3$)$_2$), 2.18 (s, 1H, NH), 2.39 (s, 3H, Ar-CH$_3$), 2.94 (s, 6H, -ArN(CH$_3$)$_2$), 4.28 (s, 1H, -CH), 4.92 (m, 1H, OCH-(CH$_3$)$_2$), 5.25 (s, 2H, -SCH$_2$), 6.65-7.38 (m, 9H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 170.48, 163.62, 153.97, 143.63, 137.18, 129.97, 129.63, 127.80, 127.64, 125.60, 120.96, 114.43, 67.50, 48.49, 40.78, 39.51, 23.87, 21.49; LCMS: (m/z) 424 [M + H]. Anal. calcd. for $C_{24}H_{29}N_3O_2S$: C, 68.05; H, 6.90; N, 9.92. Found: C, 67.94; H, 6.97; N, 9.79.

Isopropyl 2-(4-chlorobenzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (5g): Yellow solid; Yield 80 %; m.p. 252-253 ºC; IR (KBr, ν_{max}, cm^{-1}): 3312 (NH), 1721 (CO), 1628 (C-N), 1552 (C-C), 648 (C-S); ^{1}H NMR (δ, DMSO-d_6): 1.30 (d, 6H, OCH-(CH$_3$)$_2$), 2.16 (s, 1H, NH), 2.38 (s, 3H, Ar-CH$_3$), 4.27 (s, 1H, -CH), 4.91 (m, 1H, OCH-(CH$_3$)$_2$), 5.22 (s, 2H, -SCH$_2$), 7.18-7.42 (m, 9H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 170.65, 163.58, 153.60, 143.51, 135.48, 132.13, 130.77, 129.95, 129.69, 127.71, 127.41, 120.67, 67.58, 48.40, 39.25, 23.83, 21.54; LCMS: (m/z) 415 [M + H]. Anal. calcd. for $C_{22}H_{23}N_2O_2SCl$: C, 63.67; H, 5.61; N, 6.77. Found: C, 63.78; H, 5.69; N, 6.93.

Isopropyl 2-(4-isopropylbenzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (5h): Yellow solid; Yield 78 %; m.p. 185-187 ºC; IR (KBr, ν_{max}, cm^{-1}): 3316 (NH), 1714 (CO), 1618 (C-N), 1528 (C-C), 652 (C-S); ^{1}H NMR (δ, DMSO-d_6): 0.98 (d, 6H, -ArCH(CH$_3$)$_2$), 1.26 (d, 6H, OCH-(CH$_3$)$_2$), 2.12 (s, 1H, NH), 2.33 (s, 3H, Ar-CH$_3$), 2.64 (m, 1H, -ArCH(CH$_3$)$_2$), 4.20 (s, 1H, -CH), 4.89 (m, 1H, OCH-(CH$_3$)$_2$), 5.10 (s, 2H, -SCH$_2$), 7.12-7.38 (m, 9H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 169.37, 162.45, 153.08, 146.81, 142.98, 133.77, 129.12, 128.83, 127.47, 127.35, 127.14, 120.42, 66.52, 48.19, 38.83, 34.67, 24.18, 23.53, 21.42; LCMS: (m/z) 423 [M + H]. Anal. calcd. for $C_{25}H_{30}N_2O_2S$: C, 71.04; H, 7.18; N, 6.65. Found: C, 70.89; H, 7.23; N, 6.74.

Isopropyl 2-(4-*tert*-butylbenzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (5i): Pale yellow solid; Yield 71 %; m.p. 201-202 ºC; IR (KBr, ν_{max}, cm^{-1}): 3318 (NH), 1713 (CO), 1616 (C-N), 1538 (C-C), 647 (C-S); ^{1}H NMR (δ, DMSO-d_6): 1.28 (d, 6H, OCH-(CH$_3$)$_2$), 1.36 (s, 9H, -ArC(CH$_3$)$_3$), 2.12 (s, 1H, NH), 2.35 (s, 3H, Ar-CH$_3$), 4.22 (s, 1H, -CH), 4.89 (m, 1H, OCH-(CH$_3$)$_2$), 5.10 (s, 2H, -SCH$_2$), 7.12-7.36 (m, 9H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 169.94, 162.47, 152.54, 149.12, 143.28, 133.17, 129.63, 129.47, 127.55, 127.13, 123.18, 119.34, 66.79, 48.24, 38.63, 33.72, 30.65, 23.40, 21.39; LCMS: (m/z) 437 [M + H]. Anal. calcd. for $C_{26}H_{32}N_2O_2S$: C, 71.51; H, 7.40; N, 6.43. Found: C, 71.57; H, 7.49; N, 6.36.

Isopropyl 6-methyl-4-phenyl-2-((1,2,3,4-tetrahydro-naphthalen-1-yl)methylthio)-1,4-dihydropyrimidine-5-carboxylate (5j): Pale yellow solid; Yield 69 %; m.p. 221-223 ºC; IR (KBr, ν_{max}, cm^{-1}): 3315 (NH), 1720 (CO), 1632 (C-N), 1555 (C-C), 650 (C-S); ^{1}H NMR (δ, DMSO-d_6): 1.28 (d, 6H, OCH-(CH$_3$)$_2$), 1.48-1.52 (m, 4H, -2CH$_2$), 2.12 (s, 1H, NH), 2.36 (s, 3H, Ar-CH$_3$), 2.78 (t, 2H, -CH$_2$), 3.20 (m, 1H, -CH), 3.48 (d, 2H, -SCH$_2$), 4.24 (s, 1H, -CH), 4.90 (m, 1H, OCH-(CH$_3$)$_2$), 6.84-7.38 (m, 9H, Ar-H); ^{13}C NMR (δ, DMSO-d_6): 169.78, 163.10, 153.19, 143.06, 135.83, 135.17, 129.39, 128.64, 127.49, 127.21, 126.30, 124.27, 120.48, 66.74, 48.28, 39.31, 32.46, 30.29, 28.62, 23.58, 21.44, 18.73; LCMS: (m/z) 435 [M + H]. Anal. calcd. for $C_{26}H_{30}N_2O_2S$: C, 71.84; H, 6.73; N, 6.58. Found: C, 71.97; H, 6.73; N, 6.58.

Pharmacological screening

Antiinflammatory activity: All the synthesized compounds were tested for their antiinflammatory activity using Carrageenan induced rat hind paw oedema method of Winter *et al.*[8]. The oedema hind paw was induced by injection of 0.1 mL of 1 % Carrageenan solution into subplanter region of right hind paw. The volume of the paw was measured plethysmographically immediately and after 120 min the injection of the irritant. The difference in volume gave the amount of oedema developed. Percentage inhibition of the oedema between control group and the compound treated group was calculated and compared with the group receiving standard drug at 50 mg/kg b.w. The results are tabulated in Table-1.

TABLE-1
ANTIINFLAMMATORY ACTIVITY OF (**5a-j**) (100 mg/kg b.w) AND NIMESULIDE (50 mg/kg b.w).

Compound	Paw oedema thickness (mm)					
	30 m (X ± SE)	Oedema inhibition (%)	60 m (X ± SE)	Oedema inhibition (%)	120 m (X ± SE)	Oedema inhibition (%)
Control	1.3 ± 0.05	–	1.5 ± 0.03	–	1.7 ± 0.03	–
5a	1.3 ± 0.05	7.6	1.2 ± 0.05**	20.0	1.3 ± 0.08**	23.5
5b	1.1 ± 0.03	**15.3**	1.1 ± 0.00**	**26.6**	1.1 ± 0.03**	**41.1**
5c	1.2 ± 0.05	7.6	1.3 ± 0.03**	13.3	1.3 ± 0.03**	23.5
5d	1.2 ± 0.06	7.6	1.1 ± 0.05**	26.6	1.2 ± 0.03**	29.4
5e	1.2 ± 0.03	7.6	1.1 ± 0.03**	26.6	1.2 ± 0.05**	29.4
5f	1.2 ± 0.03	7.6	1.1 ± 0.06**	26.6	1.4 ± 0.03**	17.6
5g	1.2 ± 0.03	**15.3**	1.1 ± 0.00**	**26.6**	1.1 ± 0.03**	**41.1**
5h	1.4 ± 0.00	7.6	1.2 ± 0.03**	20.0	1.3 ± 0.10**	23.5
5i	1.2 ± 0.05	7.6	1.2 ± 0.05**	20.0	1.3 ± 0.06**	23.5
5j	1.1 ± 0.03	15.3	1.2 ± 0.03**	20.0	1.3 ± 0.05**	23.5
Nimesulide	1.1 ± 0.05	15.3	1.1 ± 0.00**	26.6	1.0 ± 0.00**	41.1

Data represent mean values ± SE of six mice per group and the percent changes versus 30, 60 and 120 m post-carrageenan injection.
Data were analyzed using one-way ANOVA followed by Turkey–Krammer Multiple comparison test $^{**}p < 0.01$.
Per cent oedema inhibition was calculated as regards saline control group.
**Significant difference from the control value at $p < 0.01$.
SE = Standard error. The active compounds are marked in bold letters.

Antioxidant screening (*in vitro*)

Hydrogen peroxide scavenging activity: A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4). Various concentrations (12.5, 25, 50, 100 mg/mL) of 1 mL of the test samples or standard, ascorbic acid[25] in methanol were added to 2 mL of hydrogen peroxide solution in phosphate buffer saline. The absorbance was measured at 230 nm after 10 min[26].

Nitric oxide scavenging activity: The reaction mixture (6 mL) containing sodium nitroprusside (10 mM, 4 mL), phosphate buffer saline (pH 7.4, 1 mL) and test samples or standard, ascorbic acid solution in dimethyl sulphoxide (1 mL) at various concentrations (12.5, 25, 50, 100 mg/mL) was incubated at 25 ºC for 150 min. After incubation, 0.5 mL of reaction mixture containing nitrite ion was removed, 1 mL of sulphanilic acid reagent was added to this, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 1 mL of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 0.5 h in diffused light. A pink coloured chromophore was formed. The absorbance was measured at 640 nm[27].

Lipid peroxidation inhibitory activity: Egg lecithin (3 mg/mL phosphate buffer, pH 7.4) was sonicated in an ultrasonic sonicator for 10 min to ensure proper liposome formation. Test samples or standard, ascorbic acid (100 mL) of different concentrations (12.5, 25, 50, 100 mg/mL) were added to liposome mixture (1 mL); the control was without test sample. Lipid peroxidation was induced by adding ferric chloride (10 mL, 400 mM) and L-ascorbic acid (10 mL, 200 mM). After incubation for 1 h at 37 ºC the reaction was stopped by adding hydrochloric acid (2 mL, 0.25 N) containing trichloroacetic acid (150 mg/mL), thiobarbituric acid (3.75 mg/mL) and butylated hydroxy anisole (0.50 mg/mL). The reaction mixture was subsequently boiled for 15 min, cooled, centrifuged at 1000 rpm for 15 min and the absorbance of the supernatant was measured at 532 nm[28]. For all the above antioxidant methods, experiments were done in triplicate and average is taken, the % inhibition at different concentration was calculated by the following formula:

$$\% \text{ Inhibition} - [1-(V_t/V_c)] \times 100$$

where V_t - mean absorption of test compound and V_c - mean absorption of control.

The IC_{50} value was derived from the % inhibition at different concentration.

RESULTS AND DISCUSSION

The synthesis of thio-1,4-dihydropyrimidine-5-carboxylate derivatives (**5a-j**) was carried out according to **Scheme-I**. Benzaldehyde (**1**) and isopropyl acetoacetate (**2**) in ethanol was refluxed with thiourea (**3**) using ethanol as solvent in basic conditions to yield 5-isopropoxy-carbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-thione. It was synthesized by the multicomponent Biginelli reaction. The Biginilli compound **4** was treated with substituted benzaldehydes in presence of potassium carbonate and to afford the titled compounds (**5a-j**). The reaction sequences are outlined in **Scheme-I**.

All the synthesized compounds were obtained in good to high yields. Products were purified and characterized by various spectroscopic techniques. The IR spectra of compounds (**5a-j**) showed characteristic absorption bands at 3318-3310, 1721-1706, 1632-1606, 1568-1525 and 652-647 cm^{-1} corresponding to the N-H str, ester group of C-O str, C-N str, C-C str and C-S str functions in the structures. Similarly the ^1H NMR spectra showed peaks due to in the range of δ 1.24-1.30 for OCH-$(CH_3)_2$, δ 2.10-2.18 for NH, δ 2.28-2.39 for Ar-CH_3, δ 4.20-4.28 for -CH, δ 4.89-4.92 for OCH-$(CH_3)_2$, δ 3.48-5.25 for -SCH_2 and δ 6.65-7.91 for Ar-H. The mass spectrum of all the compounds showed molecular ion peak at M + H, at M + 2H corresponding to its molecular formula, which confirmed its chemical structure. The IR, ^1H NMR, ^{13}C NMR, mass spectra and elemental analysis showed the structure of various thio-1,4-dihydropyrimidine-5-carboxylate derivatives (**5a-j**).

Antiinflammatory screening: The results of tested compounds as well as reference standard were measured before administration of Carrageenan inflammation. After the Carrageenan inflammation was administered on rats, the effect was measured in the intervals of 30, 60 and 120 min. The

per cent oedema inhibition was calculated reference to saline control group, as depicted in Table-1. All the newly obtained compounds (**5a-j**) were tested for antiinflammatory activity. Compared to the standard, Nimesulide, bulk of the compounds exhibited moderate to good anti-inflammatory activity. The results revealed that, while **5b** and **5g** have shown potent anti-inflammatory activity, compounds **5a, 5c, 5d, 5e, 5f, 5h, 5i** and **5j** exhibited good antiinflammatory activities.

Antioxidant activity: All the synthesized compounds **5a-j** were screened for their *in vitro* antioxidant activity by various methods such as scavenging of hydrogen peroxide, scavenging of nitric oxide radical and lipid peroxidation inhibitory activity. *In vitro* antioxidant activity of synthesized compound is summarized in Table-2. The investigation of antioxidant screening revealed that some of the tested compounds showed moderate to good antioxidant activity. Particularly, benzylthiol derivatives (**5a-j**) showed more promising antioxidant activity as compared to that of standard, ascorbic acid. This could be due the availability of free thiol group. In scavenging of nitric oxide radical techniques compounds **5j** and **5i** shown low IC_{50} value than the standard. While, **5j**, **5i** and **5a** has shown more potent activity by scavenging of hydrogen peroxide. All the compounds showed higher IC_{50} value than the standard by lipid peroxidation inhibitory activity. Derivatives with aliphatic group on benzene ring having good antioxidant activity compared with the other compounds in their series. **5h**, **5a**, **5f**, **5b**, **5g** and **5d** having moderate to good antioxidant activity.

TABLE-2
ANTIOXIDANT ACTIVITY (IC_{50} VALUES) OF COMPOUNDS (**5a–j**)

Compounds	IC_{50} (Mean ± SD) µg/mL		
	Scavenging of NO radical	Scavenging of H_2O_2	Lipid peroxidation inhibitory activity
5a	56 ± 0.052	32 ± 0.318	39 ± 0.066
5b	60 ± 0.066	49 ± 0.121	43 ± 0.318
5c	65 ± 0.453	58 ± 0.318	78 ± 0.045
5d	63 ± 0.183	53 ± 0.066	68 ± 0.087
5e	69 ± 0.318	66 ± 0.162	63 ± 0.162
5f	59 ± 0.453	41 ± 0.087	46 ± 0.453
5g	62 ± 0.045	46 ± 0.024	57 ± 0.279
5h	54 ± 0.024	36 ± 0.121	37 ± 0.318
5i	46 ± 0.333	30 ± 0.183	34 ± 0.087
5j	44 ± 0.279	29 ± 0.087	31 ± 0.121
Standard	47 ± 0.087	33 ± 0.121	26 ± 0.333

S.D. = Standard deviation (average of three determination); Standard = Ascorbic acid.

Conclusion

In conclusion, we have described simple and efficient protocol for the synthesis of novel thio-1,4-dihydropyrimidine-5-carboxylate derivatives (**5a-j**) with good yields. All the synthesized compounds have been investigated for their anti-inflammatory, antibacterial and antifungal activities. With our newly synthesized compounds, it is evident that **5b** and **5g** have highest antiinflammatory activity; **5a**, **5i** and **5j** have anti-oxidant activity. Accordingly, these novel class of thio-1,4-dihydropyrimidine-5-carboxylate derivatives reported from our laboratory emerge as a valuable lead series with great potential to be used as antiinflammatory and antioxidant activity agents and as promising candidates for further efficacy evaluation.

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