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Research Article

DESIGN AND SYNTHESIS OF NOVEL 4-(4-FLUORO-3-METHYLPHENYL)-6-(SUBSTITUTED ARYL)-1,6-DIHYDROPYRIMIDIN-2-OL DERIVATIVES AS POTENT ANTI-INFLAMMATORY AND ANALGESIC AGENTS

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ABSTRACT

Objective: Pyrimidine heterocycles possessing hydroxy group has a unique place in medicinal chemistry and also plays a key role in biological processes. In the biological functions at cellular level pyrimidine plays imperative roles which lead the researchers to design a variety of its derivatives. The aim of the present study was to synthesize the novel set of 4-(4-fluoro-3-methylphenyl)-6-(substituted aryl)-1,6-dihydropyrimidin-2-ol derivatives. These compounds were screened for their analgesic and anti-inflammatory activities.

Methods: A novel series of 4-(4-fluoro-3-methylphenyl)-6-(substituted aryl)-1,6-dihydro pyrimidin-2-ol derivatives were furnished in two steps starting from 4-fluoro-3-methyl acetophenone through chalcone formation. Human red blood cell membrane stabilization method and carrageenan-induced rat paw edema test were performed for screening *in vitro* and *in vivo* anti-inflammatory activity, respectively. Tail-flick technique was performed for screening analgesic activity.

Results: All the synthesized 4-(4-fluoro-3-methylphenyl)-6-(substituted aryl)-1,6-dihydro pyrimidin-2-ol derivatives were characterized by Fourier-transform infrared spectroscopy, ^{1}H nuclear magnetic resonance, mass spectroscopy, and bases of elemental analysis. The result of biological screening revealed that many of the new derivatives were endowed with improved anti-inflammatory and analgesic activities.

Conclusion: Nature of the substituent played a major role in anti-inflammatory and analgesic activities. The pyrimidine derivative with chlorophenyl substitution exhibited potent anti-inflammatory and analgesic activities. From the results, it was concluded that 6-(4-chlorophenyl)-4-(4-fluoro-3-methyl phenyl)-1,6-dihydropyrimidin-2-ol was the most active compound.

Keywords: Pyrimidine, Chalcone, Anti-inflammatory, Analgesic, Carrageenan, Acetic acid.

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INTRODUCTION

Pyrimidine is ubiquitous in nature and found in a large group of biologically active compounds such as nucleic acids, vitamins, and coenzymes. They play a key role in the human physiological process. Pyrimidine [1] is a six-membered heterocycle with two nitrogen atoms situated in a 1,3-arrangement. The other name of pyrimidine is *m*-diazine or 1,3-diazine. Both nitrogen atoms in pyrimidines resemble pyridine nitrogen. The aromatic ring consists of a lone pair of electrons in the sp^2 hybrid orbital which belongs to the nitrogen atoms in its plane. These lone pairs are not needed for aromatic sextet; hence, they are basic in nature similar to pyridine. In the biological functions at cellular level pyrimidine plays a key role, which leads the researchers to design a variety of its derivatives. Pyrimidine heterocycles possessing hydroxyl group have a unique place in medicinal chemistry [2] and also plays a key role in biological processes [3]. The pharmacologically active drugs, namely barbituric acid and its several derivatives (e.g., Veranal) (Fig. 1) possess pyrimidine moiety in its main nucleus [4]. Pyrimidine derivatives provide a variety of biological activities such as cytotoxic [5], antimalarial [6], antioxidant [7], tyrosinase inhibitory [8], anti-inflammatory [9], cyclin-dependant kinase inhibitors [10], alopecia agent [11], and antibacterial [12].

The inflammation process involves a cascade that can be elicited by numerous stimuli (e.g. infectious agents, ischemia, and antigen-antibody interaction). Nonsteroidal anti-inflammatory drugs (NSAIDs) represent a heterogeneous family of pharmacologically potent compounds used to alleviate acute and chronic inflammation, pain, and fever. Almost two decades ago, steroidal drugs, namely prednisolone, dexamethasone, and betamthansone were considered to be the choicest and effective anti-inflammatory drugs. The serious and enormous adverse effects caused by either short-term or long-term usage of steroid therapy necessitated accelerated research toward the development of NSAIDs since the past two decades [13,14]. In the past decade, copious enhances have taken place in the understanding of pathogenesis, and as a result, momentous progress has been made and is still being made in the development of flawless NSAIDs [15]. Motivated by the aforesaid findings, and pursuing our studies on pyrimidine moiety, a new series of 4-(4-fluoro-3-methylphenyl)-6-(substituted aryl)-1,6-dihydropyrimidin-2-ol derivatives were synthesized and tested for their anti-inflammatory and analgesic activities.

METHODS

Chemicals and reagents

The chemicals and reagents used in this work were obtained from various chemical units Avra, Sigma-Aldrich, SRL and SD Fine Chem. The solvents used were of LR grade and purified before their use. The silica gel G used for analytical chromatography (thin-layer chromatography [TLC]) was obtained from E. Merck India Ltd. Solvent systems used were n-hexane:acetone (7:3).

Instruments

All the melting points were taken in open glass capillary and are uncorrected. ^{1}H nuclear magnetic resonance (NMR) spectra were taken on a Bruker ultra shield (400 MHz) NMR spectrometer in $CDCl_3$ using tetramethylsilane [(CH3)4Si] as the internal standard. Chemical shift (δ) are expressed in ppm. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. All the IR spectra were recorded in KBr pellets on a Jasco Fourier-transform infrared (FT-IR)

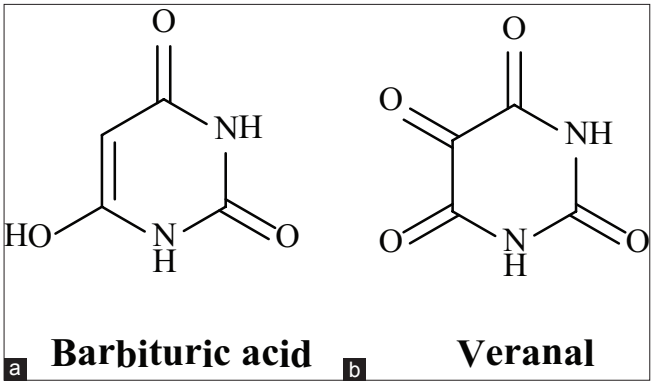


Fig. 1: (a and b) Hypnotic drugs with pyrimidine nucleus

410 spectrometer. Elemental analyses were performed on a Perkin Elmer model 240c analyzer and were within ±0.4% of the theoretical values.

Animals

The animals were maintained in colony cages at 25±2°C, relative humidity of 45–55%, under a 12 h light and dark cycle and were fed standard animal feed [16]. All the animals were acclimatized for a week before use. The synthesized compounds were evaluated for their anti-inflammatory and analgesic activities.

Experimental work

Preparation of (E)-1-(4-fluoro-3-methylphenyl)-3-(substituted aryl)prop-2-en-1-one (1a-1i)

The key intermediates (E)-1-(4-fluoro-3-methylphenyl)-3-(substituted aryl) prop-2-en-1-one (1a-1i) were prepared according to the reported literature [17]. The starting material 4-fluoro-3-methyl acetophenone (0.02 mol) was treated with aromatic aldehydes (0.02 mol) in the presence of catalytic amount of lithium hydroxide. Ethanol (20 ml) was used as a solvent. The reaction mixture was kept for constant stirring using a multistage magnetic stirrer at room temperature until the solution turns turbid. The reaction was monitored by TLC (n-hexane:acetone – 7:3). Then, the reaction mixture was poured into crushed ice and neutralized with the help of dil.HCl. The precipitate was filtered under vacuum, washed with cold ethanol and distilled water. The obtained chalcones were purified by recrystallization and column chromatography.

1a (E)-1-(4-fluoro-3-methylphenyl)-3-(thiophen-2-yl) prop-2-en-1-one
Greenish yellow crystals (EtOH), yield = 81%; m.p. 101–103°C. FT-IR (KBr) cm^{-1}: 1657 (C=O, chalcone), 1585 (C=C), 1149 (C-F), 2949 (C-CH_3), ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm): 2.358(s, 3H, CH_3), 7.430 (d, 1H, α-H), 7.921 (d, 1H, β-H), 7.42 (s, 1H, Ar-H), 7.32 (d, 1H, Ar-H), 7.848 (d, 1H, Ar-H), 7.86 (d, 1H, Ar-H), 7.433 (d, 1H, Ar-H), 7.972 (s, 1H, Ar-H). MS (EI) m/z: 247 (M^+). Anal. calcd for $C_{14}H_{11}FOS$: C, 68.27; H, 4.50; F, 7.71; O, 6.50; S, 13.02.

1b (E)-3-(2-bromophenyl)-1-(4-fluoro-3-methylphenyl) prop-2-en-1-one
Yellow crystals (EtOH), yield = 76%; m.p. 100–102°C. FT-IR (KBr) cm^{-1}: 1658 (C=O, chalcone), 1588 (C=C), 2924 (C-CH_3), 1153(C-F), 752.9 (C-Br). ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm): 2.384 (s, 3H, -CH_3), 7.374 (d, 1H, α-H), 8.155 (d, 1H, β-H), 7.119-7.93 (d, 7H, Ar-H). MS (EI) m/z: 319 (M^+). Anal. calcd for $C_{16}H_{12}BrFO$: C, 60.21; H, 3.79; F, 5.95; O, 5.01; Br, 25.04.

1c (E)-1-(4-fluoro-3-methylphenyl)-3-(2-nitrophenyl) prop-2-en-1-one
Orange crystals (EtOH), yield = 77%; m.p. 94–96°C. FT-IR (KBr) cm^{-1}: 1674 (C=O, chalcone), 1513(C=C), 2878 (C-CH_3), 1292 (C-F), 1341 (C-NO_2).^{1}HNMR(400 MHz, $CDCl_3$, δ ppm): 2.39 (s, 3H, CH_3), 7.86 (d, 1H, α-H), 8.09 (d, 1H, β-H), 7.585 (s, 1H, Ar-H), 7.173 (d, 1H, Ar-H), 7.71 (d, 1H, Ar-H) 8.15 (s, 1H, Ar-H), 8.112 (d, 1H, Ar-H), 7.582 (d, 1H, Ar-H), 7.928 (d, Ar-H). MS (EI) m/z: 284 (M^+). Anal. calcd for $C_{16}H_{12}FNO_3$: C, 67.36; H, 4.24; F, 6.66; N, 4.91;O,16.83.

1d (E)-1-(4-fluoro-3-methylphenyl)-3-(4-hydroxy-3-methoxy-5-nitro phenyl)prop-2-en-1-one
Pale yellow crystals (EtOH), yield = 78%; m.p. 91–93°C. FT-IR (KBr) cm^{-1}: 1684 (C=O, chalcone), 1546 (C=C), 2944 (C-CH_3), 1103 (C-F), 1230 (C-OCH_3), 1366 (NO_2), 3200 (OH).^{1}HNMR (400 MHz, $CDCl_3$ δ ppm) CH_3, 2.186 (s, 3H, CH_3), 1.727 (s, 1H, OH) 4.043 (s, 3H, -OCH_3), 7.664 (d, 1H, α-H), 8.245 (d, 1H, β-H), 7.285-8.245(m, 5H, Ar-H). MS (EI) m/z: 331.09 (M^+). Anal. calcd for $C_{17}H_{14}NO_5$: C, 61.63; H, 4.26; F, 5.73; N, 4.23; O, 24.15.

1e (E)-3-(3,4-dimethoxyphenyl)-1-(4-fluoro-3-methylphenyl)prop-2-en-1-one
Lemon yellow crystals (EtOH), yield = 81%; m.p. 85–87°C. FT-IR (KBr) cm^{-1}: 1656 (C=O, chalcone), 1583(C=C), 2930 (C-CH_3), 1254(C-F), 1142 (C-OCH_3). ^{1}HNMR(400 MHz, $CDCl_3$ δ ppm): 2.35 (s, 3H, CH_3), 3.936 (d, 6H, -OCH_3), 7.739 (d, 1H, α-H), 7.905 (d, 1H, β-H), 7.38 (s, 1H, Ar-H), 7.25 (d, 1H, Ar-H), 7.864 (d, 1H, Ar-H), 7.23 (s, 1H, Ar-H), 7.080 (d, 1H, Ar-H), 6.913 (d, 1H, Ar-H). MS (EI) m/z: 301 (M^+). Anal. calcd for $C_{18}H_{17}FO_3$: C, 71.99; H, 5.71; F, 6.33; O, 15.98.

1f (E)-3-(4-(dimethylamino)phenyl)-1-(4-fluoro-3-methylphenyl) prop -2-en-1-one
Bright red crystals (EtOH), yield = 83%; m.p. 93–95°C. FT-IR (KBr) cm^{-1}: 1651 (C=O, chalcone), 1593(C=C), 2923 (C-CH_3), 1243(C-F). ^{1}HNMR(400 MHz, $CDCl_3$ δ ppm): 2.37 (s, 3H, CH_3), 3.06 (s, 6H, N(CH_3), 7.913 (d, 1H, α-H), 7.577 (d, 1H, β-H), 7.34 (s, 1H, Ar-H), 7.03 (d, 1H, Ar-H), 7.849 (d, 1H, Ar-H), 7.78 (s, 2H, Ar-H), 6.734 (d, 2H, Ar-H). MS (EI) m/z: 284 (M^+). Anal. calcd for $C_{18}H_{18}FNO$: C, 76.30; H, 6.40; F, 6.71; O, 5.65; N, 4.94.

1g (E)-3-(4-chlorophenyl)-1-(4-fluoro-3-methylphenyl) prop-2-en-1-one
Lemon yellow crystals (EtOH), yield = 77%; m.p. 101–103°C. FT-IR (KBr) cm^{-1}: 1663 (C=O, chalcone), 1591(C=C), 2960 (C-CH_3), 1243(C-F), 819 (C-Cl). ^{1}HNMR(400 MHz, $CDCl_3$ δ ppm): 2,38 (s, 3H, CH_3), 7,591 (d, 1H, α-H), 7,93 (d, 1H, β-H), 7,58 (s, 1H, Ar-H), 7,433 (d, 1H, Ar-H), 7,61 (d, 1H, Ar-H), 7,60 (s, 1H, Ar-H), 7,75 (d, 1H, Ar-H), 7,47 (d, 1H, Ar-H). MS (EI) m/z: 275 (M^+). Anal. calcd for $C_{16}H_{12}ClFO$: C, 69.95; H, 4.40; Cl, 12.91; F, 6.92; O, 5.82.

1h (E)-3-(anthracen-9-yl)-1-(4-fluoro-3-methylphenyl) prop-2-en-1-one
Orange crystals (EtOH), yield = 79%; m.p. 91–93°C. FT-IR (KBr) cm^{-1}: 1658 (C=O, chalcone), 1591(C=C), 2924 (C-CH_3), 1261 (C-F). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm): 2.376 (s, 3H, CH_3), 7.558 (d, 1H, α-H), 7.964 (d, 1H, β-H), 7.602 (s, 1H, Ar-H), 7.964 (d, 1H, Ar-H), 7.37 (d, 1H, Ar-H), 7.43-9.03 (m, 9H, Anthracene-H). MS (EI) m/z: 340 (M^+). Anal. calcd for $C_{24}H_{17}FO$: C, 84.68; H, 5.03; F, 5.58; O, 4.70.

1i (E)-3-(5-bromo-2-hydroxy-3-methoxyphenyl)-1-(4-fluoro-3-methyl phenyl)prop-2-en-1- one
Orange crystals (EtOH), yield = 69%; m.p. 93–95°C, FT-IR (KBr) cm^{-1}: 1656 (C=O, chalcone), 1581(C=C), 2923 (C-CH_3), 1195 (C-F), C-OH (3243), C-OCH_3 (1255), C-Br (705): ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm): 1,617 (s, 3H, CH_3), 7,34 (d, 1H, α-H), 9,879 (s, 1H, β-H), 7,203 (d, 5H, Ar-H), 7,285(s, 1H, OH), 3,943(s, 3H, C-OCH_3): MS (EI) m/z: 365 (M^+): Anal. calcd for $C_{17}H_{14}FBrO_3$: C, 55.91; H, 3.83; Br, 21.88; F, 5.20; O, 13.14.

Synthesis of 4-(4-fluoro-3-methylphenyl)-6-(substituted aryl)-1,6-dihydropyrimidin-2-ol. (2a–2i)

The mixture of chalcones (0.002 mol), urea (0.002 mol), and 5ml of HCl in absolute ethanol (20 ml) was refluxed on a water bath for 6–8 h. The reaction was monitored by TLC. After completion of the reaction, 40% ammonia was added to neutralize the reaction mixture. The reaction mixture was kept in the refrigerator for 2 h. The precipitate obtained was filtered under vacuum and recrystallized using ethanol to obtain 4-(4-fluoro-3-methylphenyl)-6-(substituted aryl)-1,6-dihydropyrimidin-2-ol derivatives.

2a 4-(4-fluoro-3-methylphenyl)-6-(thiophen-2-yl)-1,6-dihydropyrimidin-2-ol.
Yield 75%, m.p. 168°C, FT-IR (KBr) cm^{-1}: 1041.13 (C-F); 2918.24 (C-CH_3 Str); 3073.70 (Ar C-H str); 1667.52 (C=N str); 1585.11 (Ar C=C);

3449.10 (NH str); ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.388 (s, 3H, CH_3); 1.593 (m, 1H, N-H); 2.194 (d, 1H, C_6'-H pyrimidine); 7.112 (d, 1H, C5'-H pyrimidine); 7.134 (m, 2H, C_5 and C_2-H); 7.125 (d, J=5.2 Hz, 1H, C_6-H); 7.304 (d, J=7.6Hz, 1H, C_3'-H); 7.397 (d, J= 3.7 1H, C_4'¬-H): MS (EI) m/z: 288 (M^+). Anal. calcd for $C_{15}H_{13}FN_2OS$; C, 62.48; H, 4.54; F, 6.59; N, 9.72; O, 5.55; S, 11.12.

2b 4-(4-fluoro-3-methylphenyl)-6-(2-bromophenyl)-1,6-dihydropyrim idin-2-ol
Yield 69%, m.p. 154°C, FT-IR (KBr) cm^{-1}:934.23 (C-F); 2924 (C-CH_3 Str); 753 (C-Br); 3050 (Ar C-H str); 1662 (C=N str); 1588 (Ar C=C); 3624 (OH): ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.388 (s, 3H, CH_3); 1.640 (s, 1H, N-H); 2.192 (d, J=2Hz, 1H, $C_{6'}$-H pyrimidine); 7.119 (d, 1H, $C_{5'}$-H pyrimidine); 7.163 (m, 2H, C_5 and C_2-H); 7.425 (m, 1H, C_6-H); 7.141–8.153 (m, 4H, Ar-H): MS (EI) m/z: 360 (M^+). Anal. calcd for $C_{17}H_{14}BrFN_2O$; C, 56.53; H, 3.91; Br, 22.12; F,5.26; N, 7.76; O, 4.43.

2c 4-(4-fluoro-3-methylphenyl)-6-(2-nitrophenyl)-1,6-dihydropyrimi dine-2-ol
Yield 79%, m.p. 162°C, FT-IR (KBr) cm^{-1}: 1029.45 (C-F); 2927.46 (C-CH_3 Str); 3073.32 (Ar C-H str); 1669.54 (C=N str); 1511 (Ar C=C); 1340 (NO_2): ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.394 (s, 3H, CH_3); 2.193 (m, 1H, N-H); 3.519 (d, J=1.6Hz, 1H, $C_{6'}$-H pyrimidine); 7.173 (d, 1H, $C_{5'}$-H pyrimidine); 7.269 (m, 1H, C_5-H); 7.308 (m, 1H, C_5-H); 7.544 (m, 1H, C_6-H); 7.51 (m, 1H, $C_{4''}$-H); 7.94 (m, 1H, $C_{3''}$-H); 7.867 (m, 1H, $C_{6''}$-H); 8.152 (m, 1H, C_3"-H): MS (EI) m/z: 327 (M^+). Anal. calcd for $C_{17}H_{14}FN_3O_3$; C, 62.38; H, 4.13; F, 5.08; N, 12.84; O, 14.66.

2d 4-(4-fluoro-3-methylphenyl)-6-(4-hydroxy-3-methoxy-5-nitrophen yl)-1,6-dihydro pyrimidine-2-ol
Yield 71%, m.p. 172°C, FT-IR (KBr) cm^{-1}: 1044.45 (C-F); 2921.34 (C-CH_3 Str); 3076.17 (Ar C-H str); 1610.70 (C=N str); 1546 (Ar C=C); 3403.75 (NH str); 3735 (OH); 1463 (NO_2); 1269 (OCH_3): ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.193 (s, 3H, CH_3); 1.606 (s, 1H, O-H); 11.273 (m, 1H, N-H); 3.518 (d, J=4.8Hz, 1H, C_6'-H pyrimidine); 4.051 (s, 3H, OCH_3); 7.128 (m, 3H, C_2,$C_{5'}$,$C_{5''}$-H); 7.673 (m, 1H, C_6-H); 8.253 (m, 2H, $C_{2''}$-H and C_6"-H): MS (EI) m/z: 373 (M^+). Anal. calcd for $C_{17}H_{14}FN_3O_3$; C, 62.38; H, 4.13; F,5.08; N, 12.84; O, 14.66

2e 6-(3,4-dimethoxyphenyl)-4-(4-fluoro-3-methylphenyl)-1,6-dihydro pyrimidine-2-ol
Yield 66%, m.p. 144°C, FT-IR (KBr) cm^{-1}: 1023.84 (C-F); 2932.06 (C-CH_3 Str); 3075.07 (Ar C-H str); 1657.22 (C=N str); 1583 (Ar C=C); 3611 (OH); 1256 and 1142 (OCH_3): ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm) 2.387 (s, 3H, CH_3); 1.641 (s, 1H, O-H); 2.192 (m, 1H, N-H); 3.515 (d, J=4.8Hz, 1H, $C_{6'}$-H pyrimidine); 3.981 (d, J=8Hz, 6H, OCH_3); 6.941 (d, J=8.4Hz, 1H, $C_{5'}$-H pyrimidine); 7.184 (m, 1H, C_2-H); 7.154 (m, 1H, C_5-H); 7.394 (m, 1H, C_6-H); 7.761-7.924 (m, 3H, $C_{2''}$-H, $C_{5''}$-H and $C_{6''}$-H): MS (EI) m/z: 342 (M^+). Anal. calcd for $C_{19}H_{19}FN_2O_3$; C, 66.66; H, 5.59; F, 5.55; N, 8.18; O, 14.02

2f 6-(4-(dimethylamino)phenyl)-4-(4-fluoro-3-methylphenyl)-1,6-dihy dropyrimidine-2-ol
Yield 82%, m.p. 158°C, FT-IR (KBr) cm^{-1}: 1053.59 (C-F); 2919.47 (C-CH_3 Str); 3434.74 (Ar NH str); 1648.76 (C=N str); 1594 (Ar C=C); 1299.42 (C-N str) ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.378 (s, 3H, CH_3); 1.597 (s, 1H, O-H); 2.194 (m, 1H, N-H); 3.075 (s, 6H, N-$(CH_3)_2$); 3.521 (d, J=5.6Hz, 1H, $C_{6'}$-H pyrimidine); 6.734 (d, J=5.2Hz, 1H, $C_{5'}$-H pyrimidine); 7.133 (m, 1H, C_2-H); 7.342 (m, 1H, C_5-H); 7.586 (m, 1H, C_6-H); 7.544-7.913 (m, 4H, $C_{2''}$-H, $C_{5''}$-H and $C_{6''}$-H): MS (EI) m/z: 325 (M^+). Anal. calcd for $C_{19}H_{20}FN_3O_4$; C, 70.13; H, 6.20; F, 5.84; N, 12.19; O, 4.92.

2g 6-(4-chlorophenyl)-4-(4-fluoro-3-methylphenyl)-1,6-dihydropyrimi dine-2-ol
Yield 84%, m.p. 166°C, FT-IR (KBr) cm^{-1}:983.19 (C-F); 2924.18 (C-CH_3 Str); 819.23 (C-Cl); 3053.32 (Ar C-H str); 1662.57 (C=N str); 1592 (Ar C=C); 3246.75 (NH str); 3433 (OH): ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.377 (s, 3H, CH_3); 1.771 (s, 1H, O-H); 1.740 (m, 1H, N-H); 2.188 (d, J=1.2Hz, 1H, $C_{6'}$-H pyrimidine); 7.150 (m, J=8.4Hz, 1H, $C_{5'}$-H pyrimidine); 7.403 (m, 2H, $C_{2''}$-H and $C_{6''}$-H); 7.603 (m, 2H, $C_{3''}$-H and $C_{5''}$-H); 7.508-7.922 (m, 4H, C_2-H, C_5-H and C_6-H): MS (EI) m/z: 316 (M^+). Anal. calcd for $C_{17}H_{14}ClFN_2O$; C, 64.46; H, 4.45; Cl, 11.19; F, 6.00; N, 8.84; O, 5.05.

2h 6-(anthracen-9-yl)-4-(4-fluoro-3-methylphenyl)-1,6-dihydropyrimi dine-2-ol
Yield 70%, m.p. 171°C, FT-IR (KBr) cm^{-1}: 1037.27 (C-F); 2922.80 (C-CH_3 Str); 2854.51 (C-H str) 1658.91 (C=N str); 1455 (Ar C=C); 3439.50 (NH str); 3739 (OH); ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.382 (s, 3H, CH_3); 1.622 (s, 1H, O-H); 2.193 (m, 1H, N-H); 3.518 (d, J=5.6Hz, 1H, $C_{6'}$-H pyrimidine); 7.173 (m, J=8.4Hz, 1H, $C_{5'}$-H pyrimidine); 7.527-8.883 (m, 9H, Anthracene-H); 7.999-7.932 (m, 3H, Ar-H): MS (EI) m/z: 382 (M^+). Anal. calcd for $C_{17}H_{14}ClFN_2O$; C, 64.46; H, 4.45; Cl, 11.19; F, 6.00; N, 8.84; O, 5.05.

2i 6-(5-bromo-2-hydroxy-3-methoxyphenyl)-4-(4-fluoro-3-methylphe nyl)-1,6-dihydro pyrimidine-2-ol
Yield 73%, m.p. 160°C, FT-IR (KBr) cm^{-1}: 1072 (C-F): 851 (C-Br): 2928 (C-CH_3 Str): 2926 (C-H str) 1607 (C=N str): 1399 (Ar C=C): 3421 (NH str): 3562 (OH): 1223 (O-CH_3); ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.49 (s, 3H, CH_3); 1.798 (s, 1H, N-H); 2.052 (m, 1H, O-H); 3.89 (3H, OCH_3); 4.519 (d, J=8.2Hz, 1H, $C_{6'}$-H pyrimidine); 7.69 (1H, OH); 7.06 (m, J=7.6Hz, 1H, $C_{5'}$-H pyrimidine); 6.91 (1H, $C_{4''}$-H); 7.55 (1H, $C_{6''}$-H); 7.22 (1H, C_2-H); 7.29 (1H, C_5-H); 7.91 (1H, C_6-H): MS (EI) m/z: 407 (M^+). Anal. calcd for $C_{17}H_{14}ClFN_2O$; C, 53.09; H, 3.96; Br, 19.63; F, 4.67; N, 6.88; O, 11.79.

Anti-inflammatory activity

In vitro anti-inflammatory screening

The human red blood cell (HRBC) membrane stabilization method
The blood was collected from a healthy human volunteer and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl) and centrifuged at 3000 rpm for 10 min. The packed cells were washed with iso-saline (0.36%) and a 10% suspension was made. Various concentrations of 4-(4-fluoro-3-methylphenyl)-6-(substituted aryl)-1,6-dihydropyrimidin-2-ol (2a–2j) were prepared (75, 150, and 200 µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline, and 0.5 ml of HRBC suspension were added. It was incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min, and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (75, 150, and 200 µg/ml) was used as the reference standard, and the control was prepared by omitting the compounds under examination.

The percentage of HRBC membrane stabilization or protection was calculated using the following formula:

$$\%\text{Membranestabilization} = 100 - \frac{\text{O.D Of Test}}{\text{O.D Of Control}} \times 100$$

Ethical approval

All experiments have been examined and approved by the Institutional Animal Ethics Committee at the GITAM University, Visakhapatnam, India (Approved proposal No:- IAEC/GIP-1287/CAD-UGC/ approved/3/2015). Animal experiments were also performed in accordance with the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals [18].

***In vivo* anti-inflammatory activity**

Wistar albino rats of either sex (200 – 250 gms) were procured from Ghosh Enterprises kolkatta, West Bengal. A total of 72 rats were divided into 12 groups of 6 rats each. They were allowed for fasting overnight and given water *ad libitum*. Group I was given only 1% sodium carboxymethyl cellulose suspension (1 ml/kg) and was used as carrageenan-treated control. Group II was treated with the standard drug diclofenac (100mg/kg). Similarly, the rest of the groups were administered with test drugs (2a–2i), respectively. The test drugs (100 mg/kg body weight) and the standard drugs (100 mg/kg body

weight) were administered orally with the help of the oral catheter. After 30 min, 0.05 ml of 1% carrageenan suspension was slowly injected subcutaneously into the subplantar region of the left hind paw to all the groups to produce inflammation. After the administration of carrageenan, the volume of its displacement was measured volumetrically by comparing with 0 min reading and again after every 1, 2, 3, and 4 h of induction with plethysmometer apparatus and compared. The percentage increase of paw thickness was determined at 0, 1, 2, 3, and 4 h after induction of inflammation.

The anti-inflammatory activity was expressed as percentage inhibition.

$$\%\text{Inhibition} = \frac{\text{Control} - \text{test}}{\text{Control}} \times 100$$

Analgesic activity

The acetic acid writhing test was performed on Wistar albino rats by following the method of Berkowitz *et al.* [19]. Test compounds were given to the animals at the dose of 50 mg/kg, 30 min later the animals have injected intraperitoneally with 0.25 ml/rat of 0.5% acetic acid. The mean number of writhes for each experimental groups and the percentage decrease compared with the control group was calculated after 60 min.

RESULTS AND DISCUSSION

The title compounds 2a-2i were synthesized as per the protocol shown in Scheme 1. In the present work, by substituting different aryl moiety at the C-6 position of 4-(4-fluoro-3-methylphenyl)-1,6-dihydropyrimidin-2-ol, a sequence of novel pyrimidine derivatives 2a-2i was synthesized. The presence of particular groups was identified from IR spectra by means of some characteristic absorption bands. The IR spectrum of chalcones showed characteristic intense absorption bands at 1657 (C=O, chalcone), 1585 (C=C), 1149 (C-Fl), and 2949 (C-CH_3). The formation of pyrimidine was confirmed from the absorption bands of IR spectra. The absorption band at 3449.10 indicates NH Stretch of the pyrimidine ring. Further, it can also be confirmed from the ^{1}H NMR spectral data. A strong peak at δ 1.593 ppm integrating for N-H proton of pyrimidines. The spectrum also revealed a doublet at δ 2.194 ppm for the proton of C-6-H of the pyrimidine ring. A singlet peak at δ 2.34 ppm for three protons which might be assigned to CH_3. The structure of title compounds 2a–2i was further confirmed by the appearance of various other peaks in NMR spectroscopy corresponding to the assigned structure. In addition, the data of the mass spectrum further confirmed their molecular weight and purity.

Biological activities

In vitro anti-inflammatory activity (HRBC membrane stabilization method)

In vitro anti-inflammatory activity of test compounds was evaluated using the HRBC membrane stabilization method. The anti-inflammatory activity results (Table 1) revealed that all the test compounds showed better activity when compared to that of standard drug. From the results, it was observed that the compounds 2g and 2b exhibited good activity when compared to that of standard drug. It may be due to the presence of halogen group on the phenyl ring attached at position-6 of pyrimidine. The compound 2i also showed good activity. The presence of bulk anthracene group attached to the pyrimidine ring may contribute to its activity. The compound 2a with thiophene moiety attached to the pyrimidine ring also showed good activity. Rest of the compounds showed moderate anti-inflammatory activity.

In vivo anti-inflammatory activity (carrageenan-induced rat paw edema method)

In vivo anti-inflammatory activity of test compounds was evaluated using carrageenan-induced rat paw edema method. The anti-inflammatory activity results (Table 2) revealed that all the test compounds showed better activity when compared to that of standard drug. The phenyl ring substituted with hydroxyl, methoxy, and nitro substituents attached at position-6 of pyrimidine causes a decrease in the activity of the compound 2d. The compound 2e possessing dimethoxyphenyl ring at position-6 of pyrimidine ring exhibited moderate anti-inflammatory activity when compared to the reference standard diclofenac sodium. Replacement of dimethoxy phenyl ring with 5-bromo-2-hydroxy-3-methoxy phenyl ring 2i leads to an increase in the activity. With increased lipophilicity, the compound with dimethylamino substituent 2f and nitrophenyl substituents 2c showed the least activity. Among all tested compounds para chloro analog 2g exhibited a better activity which was more potent than diclofenac. Compounds with anthracene moiety 2h, 2-bromo phenyl 2b and thiophene ring 2a also showed better activity.

Analgesic activity

Entire test compounds 2a–2i were tested for their analgesic activity by the tail-flick technique using Wistar albino mice. The results of the analgesic study were summarized in Table 2. Compounds 2g with 4-chlorophenyl derivative and 2b with 2-bromophenyl derivative showed similar analgesic activity compared to standard drug diclofenac sodium. Replacement of chlorine group with nitro or dimethylamino or methoxy or hydroxyl groups results in a sharp fall in the activity. It may be due to a decrease in the lipophilicity. From the results, it was found that the pyrimidine derivatives with halogen substituents showed better activity when compared to other derivatives. Compounds 6-(4-chlorophenyl)-4-(4-fluoro-3-methylphenyl)-1,6-dihydropyrimidin-2-ol 2g and 4-(4-fluoro -3-methylphenyl)-6-(2-bromo phenyl)-1,6-dihydropyrimidin-2-ol 2b were found to be the most active analgesic agent and it showed similar potency when compared to the reference standard diclofenac sodium.

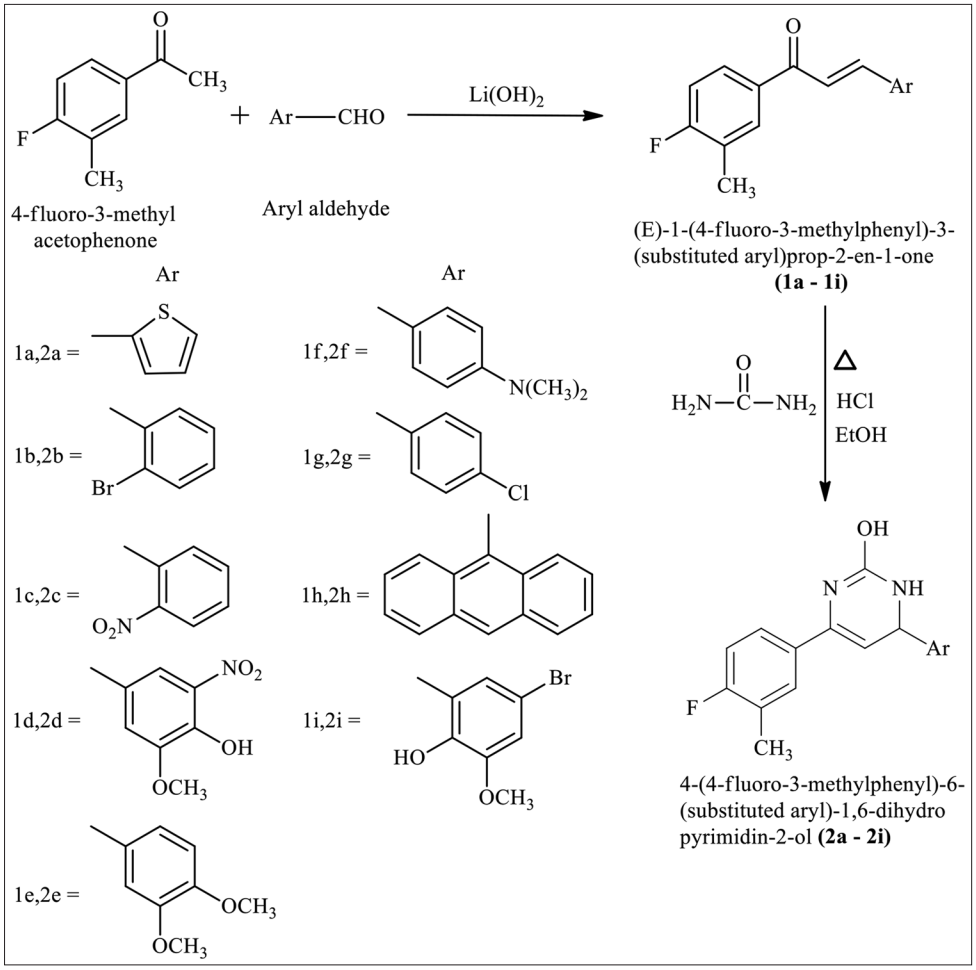
CONCLUSION

In summary, a series of novel pyrimidine derivatives 2a–2i were synthesized and characterized by FT-IR, ^{1}H-NMR, mass spectroscopy, and elemental analysis. These derivatives were evaluated for their analgesic

Table 1. Anti-inflammatory activity of pyrimidine (2a–2i) HRBC method

S.No	Compound	Concentration (µg/ml)	%Stabilization
1.	2a	75	50.21
		150	69.12
		200	80.16
2.	2b	75	49.84
		150	58.12
		200	82.56
3.	2c	75	39.06
		150	55.15
		200	71.46
4.	2d	75	41.65
		150	55.51
		200	65.23
5.	2e	75	44.61
		150	56.18
		200	76.35
6.	2f	75	46.54
		150	54.21
		200	64.11
7.	2g	75	51.17
		150	64.25
		200	91.12
8.	2h	75	46.93
		150	61.62
		200	74.45
9.	2i	75	49.44
		150	66.16
		200	88.61
10.	Diclofenac	75	64.26
		150	78.74
		200	94.67

HRBC: Human red blood cell



Scheme 1: Synthesis of 4-(4-fluoro-3-methylphenyl)-6-(substituted aryl)-1,6-dihydropyrimidin-2-ol (2a -2i)

Table 2: Effect of pyrimidine derivatives in carrageenan-induced paw edema in rats and analgesic activity

Compound	1st h	2nd h	3rd h	4th h	% inhibition of edema after 4 h	Analgesic activity % decrease of writhes in 60 min after treatment relative to control
2a	0.621±0.121	0.761±0.018	0.564±0.118	0.479±0.024	66.2	53.2
2b	0.512±0.027	0.696±0.015	0.551±0.042	0.463±0.036	67.4	59.2
2c	0.812±0.015	1.006±0.027	0.862±0.016	0.624±0.021	56.0	41.3
2d	0.853±0.121	0.961±0.042	0.832±0.052	0.716±0.041	49.6	36.2
2e	0.712±0.013	0.836±0.043	0.629±0.021	0.561±0.071	60.5	48.2
2f	0.831±0.016	0.932±0.053	0.861±0.042	0.706±0.061	50.3	40.6
2g	0.457±0.026	0.532±0.112	0.416±0.061	0.392±0.051	72.4	60.2
2h	0.517±0.017	0.589±0.056	0.501±0.066	0.456±0.011	67.9	50.4
2i	0.463±0.051	0.561±0.012	0.426±0.006	0.439±0.013	69.1	55.3
Diclofenac	0.436±0.011	0.529±0.020	0.431±0.019	0.407±0.025	71.3	62.3
Control	0.8412±0.046	1.119±0.061	1.261±0.037	1.421±0.053	-	-

and anti-inflammatory activities. In general, chlorine substituted compounds exhibited potent analgesic and anti-inflammatory activities. From the study, it was concluded that in this series nature of the substituent played a major role in analgesic and anti-inflammatory activity than its position. Among several tested compounds, 6-(4-chlorophenyl)-4-(4-fluoro-3-methylphenyl)-1,6-dihydro pyrimidin-2-ol 2g showed better analgesic and anti-inflammatory activities which were more potent than reference standard diclofenac. Hence, this analog could be developed as a new class of analgesic and anti-inflammatory agent.

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AUTHOR'S CONTRIBUTIONS

Muralidharan V: Performed the experiments. Dr. C. Asha Deepti: Conceived the idea, study design and finalized the manuscript. Dr. S. Raja: Assisted in experimental work and helped in the preparation of the manuscript.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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