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 sp² 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. 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 chalone 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, ¹H 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, Chalone, Anti-inflammatory, Analgesic, Carrageenan, Acetic acid.
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 procedure [17]. The starting material 4-fluoro-3-methyl acetophenone 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 before use. The synthesized compounds were evaluated for their anti-inflammatory and analgesic activities.

Production of 4-(4-fluoro-3-methylphenyl)-3-(4-methylphenyl)prop-2-en-1-one (1a)

A solution of 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.

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.
2c 4-(4-fluoro-3-methylphenyl)-6-(2-nitrophenyl)-1,6-dihydropyrimidine-2-o1

Yield 79%, m.p. 162°C, FT-IR (KBr) cm⁻¹: 3095, 2930, 1717, 1402, 1396, 1365, 1275, 1078, 997, 753. 3075 (Ar-C-H); 1657.22 (C=O); 1583 (Ar=C=O); 3611 (OH); 1256 and 1142 (NH); 1509 (400 MHz, CDCl₃, 5 ppm) 2.387 (s, 3, H, CH₃); 1.641 (s, 1, H, O-H); 2.192 (1H, 1, C-H); 3.521 (d, J=6.8Hz, 1H, C=H); 3.981 (d=J=8.8Hz, 1H, C=H); 7.184 (m, 1H, C=H); 7.154 (m, 1H, C=H); 7.394 (m, 1H, C=H); 7.761-7.924 (m, 3H, C=H, C=H, and C-H); MS (EI) m/z: 342 (M⁺).

2f 6-(4-dimethylamino)phenyl)-4-(4-fluoro-3-methylphenyl)-1,6-dihydropyrimidine-2-o1

Yield 82%, m.p. 158°C, FT-IR (KBr) cm⁻¹: 1053.59 (C=O); 2919.47 (C=O); 3434.74 (NH); 1648.76 (C=O); 1594 (C=O); 1299.42 (C=O); 1249 (400 MHz, CDCl₃, 5 ppm) 2.378 (s, 3, H, CH₃); 1.597 (s, 1, H, O-H); 2.194 (m, 1H, N-H); 3.075 (s, 6H, N-(CH₃)₃); 3.521 (d, J=6.8Hz, 1H, C=H); 3.981 (d=J=8.8Hz, 1H, C=H); 7.184 (m, 1H, C=H); 7.154 (m, 1H, C=H); 7.394 (m, 1H, C=H); 7.761-7.924 (m, 3H, C=H, C=H, and C-H); MS (EI) m/z: 325 (M⁺).

2g 6-(4-chlorophenyl)-4-(4-fluoro-3-methylphenyl)-1,6-dihydropyrimidine-2-o1

Yield 84%, m.p. 166°C, FT-IR (KBr) cm⁻¹: 983.19 (C=O); 2942.18 (C=O); 3819.23 (C=O); 3053.32 (Ar=O); 1662.57 (C=O); 1592 (C=O); 3246.75 (NH); 3433 (400 MHz, CDCl₃, 5 ppm) 2.377 (s, 3, H, CH₃); 1.771 (s, 1, H, O-H); 2.188 (d, J=12.8Hz, 1H, C=H); 7.150 (m, J=8.4Hz, 1H, C=H); 3.703 (m, 2H, C=H and C-H); 7.603 (m, 2H, C=H and C-H); 7.508-7.922 (m, 4H, C=H, C=H and C=H); MS (EI) m/z: 316 (M⁺).

2h 6-(anthracen-9-yl)-4-(4-fluoro-3-methylphenyl)-1,6-dihydropyrimidine-2-o1

Yield 70%, m.p. 171°C, FT-IR (KBr) cm⁻¹: 1307.27 (C=O); 2922.80 (C=O); 3005.10 (C=O); 3430.91 (C=O); 1565.05 (C=O); 1463.52 (C=O); 1365 (C=O); 1269 (C=O); 1188 (C=O); 3.518 (d, J=6.8Hz, 1H, C=H). 1389 (C=O); 1269 (C=O); 1188 (C=O); 3.518 (d, J=6.8Hz, 1H, C=H). 1269 (C=O); 1188 (C=O); 3.518 (d, J=6.8Hz, 1H, C=H); 7.173 (m=8.4Hz, 1H, C=H); 7.527-8.883 (m, 9H, C=H); 7.997-7.932 (m, 3H, Ar-H); MS (EI) m/z: 382 (M⁺).

2i 6-(5-bromo-2-hydroxy-3-methylphenyl)-4-(4-fluoro-3-methylphenyl)-1,6-dihydropyrimidine-2-o1

Yield 73%, m.p. 160°C, FT-IR (KBr) cm⁻¹: 1072 (C=O); 851 (C=Br); 2928 (C=Br); 2926 (C=H); 1607 (C=O); 3431 (C=O); 3421 (CH₃); 1623 (C=O); 1565 (C=O); 1463 (C=O); 1365 (C=O); 1269 (C=O); 1188 (C=O); 3.518 (d, J=6.8Hz, 1H, C=H); 7.896 (m, 1H, C=H); 7.527-8.883 (m, 9H, C=H); 7.997-7.932 (m, 3H, Ar-H); MS (EI) m/z: 382 (M⁺).

In vivo anti-inflammatory activity

The human red blood cell (HRBC) membrane stabilization method

The blood was collected from a healthy human volunteer and mixed with equal volume of saline solution (2% dextrose, 0.08% 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:

\[
\% \text{Membrane stabilization} = 100 - \frac{OD \text{ of Test}}{OD \text{ of Control}} \times 100
\]
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-CH3). 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 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 CH3. 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)**

The 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 2h exhibited good activity when compared to that of standard drug. It may be due to the presence of halogen group on the phenyl ring 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 chloride 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-methoxyphenyl)-6-(2-bromophenyl)-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, 1H-NMR, mass spectroscopy, and elemental analysis. These derivatives were evaluated for their analgesic activity and anti-inflammatory activity. The results showed that all the compounds exhibited good activity when compared to that of standard drug. Compounds with halogen substituents 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 dimethoxyphenyl 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.
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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