PYRIMIDINES AS POTENT CYTOTOXIC AND ANTI-INFLAMMATORY AGENTS

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INTRODUCTION

Pyrimidines are the most important six-membered heterocyclic compounds containing two nitrogen atoms. Pyrimidine occurs in living system in the form of nucleic acid and vitamins. As pyrimidine is a basic nucleus in DNA and RNA, it has been found to be associated with diverse biological activities. The molecule containing pyrimidine nucleus possess wide range of biological activities such as antimalarial [1], antibacterial [2], antifungal [3], anti-inflammatory [4], cytotoxic [5], and antitubercular [6] activity. Furthermore, the intermediates used chalcones are known for their anti-inflammatory [7], antifungal [8], antimalarial [9], and anti-inflammatory [10] activities. By considering the above facts, it was contemplated to synthesize a new series of pyrimidines (PM-PM). The final synthesized compounds have been screened for their in vitro anti-inflammatory and in vitro cytotoxic activity studies.

METHODS

Melting points were determined by capillary method and were uncorrected. The infrared (IR) spectra were recorded using Shimadzu Perkin Elmer-8201 PIR spectrometer using a thin film of potassium bromide pellet technique and frequencies are expressed in cm⁻¹. ¹H Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 11400 NMR spectrometer. All spectra were taken in CDCl₃ and dimethyl sulfoxide (DMSO). Chemical shift values are reported in ppm relative to tetramethylsilane (δ=0) as an internal standard. Mass spectra were recorded on JeolSX-102/DA-6000 mass spectrometer (6 kV, 10 Ma) as FAB gas. The purity of the synthesized compounds was checked on silica gel coated plates by using ethyl acetate:Chloroform (9:1) as a solvent and observed in ultraviolet light.

General procedure

Synthesis of 1-(4-nitrophenyl)-3-substituted-phenylprop-2-en-1-one [11]

A mixture of 4-nitrocetophenone (0.01 mol) and substituted aromatic aldehydes (0.01 mol) in ethanol (20 ml) were stirred for 24 hrs in the

MATTER

Chalcones [1-(4-nitrophenyl)-3-substituted-phenylprop-2-en-1-one] were synthesized from various substituted aldehydes with 4-nitrocetophenone and cyclized with urea and glacial acetic acid to give pyrimidine derivatives [4-(4-nitrophenyl)-6-substituted-phenylpyrimidin-2-ol].

Methods:

Methods: Synthesis of 4-(4-nitrophenyl)-6-substituted-phenylpyrimidin-2-ol (PM-PM) [12]

A mixture of substituted chalcones (0.01 mol) in 20 ml of ethanol/glacial acetic acid and urea (0.01 mol) in 20% NaOH was refluxed for 20 hrs. After completion of the reaction, the mixture was poured into crushed ice cold water, and recrystallized from ethanol. The purity of the compound was checked by TLC using chloroform:Ethyl acetate (1:9) as solvent.

Spectral data

4-(4-chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-ol (PM-PM)

IR (KBr)cm⁻¹: 1501(C=C str), 816(Ar, C-H bend), 3015(Ar, C-H str), 1669(C=N str), 3432(O-H str), 1335(C-N str), 732(C-Cl str); ¹H NMR (400 MHz, DMSO-d₆): 7.1-7.7 (m, 9H, Ar-H), 8.3 (s, 1H, OH); MS: m/z 328(M⁺).

4-(4-fluorophenyl)-6-(4-nitrophenyl)pyrimidin-2-ol (PM-PM)

IR (KBr)cm⁻¹: 1501(C=C str), 1351(C-N str), 1266(C-F str); ¹H NMR (400 MHz, DMSO-d₆): 7.1-7.7 (m, 9H, Ar-H), 8.3 (s, 1H, OH); MS: m/z 309(M⁺).

4-(4-hydroxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-ol (PM-PM)

IR (KBr)cm⁻¹: 1501(C=C str), 865(Ar, C-H bend), 3010(Ar, C-H str), 1676(C=N str), 3400(O-H str), 1320(C-N str); ¹H NMR (400 MHz, DMSO-d₆): 7.1-7.6 (m, 9H, Ar-H), 8.1 (s, 1H, OH); MS: m/z 309(M⁺).

Anti-inflammatory activity

The synthesized compounds were screened for their anti-inflammatory activity using carrageenan-induced rat hind paw edema method [13]. All the experiments were carried out as per the rules and regulations...
of institutional animal ethics committee (Animal Ethics Committee, K.S. Hegde Medical Academy, Deralakatte, Mangalore - 575 018 Ref. No. KSHEMA/AEC/31/2010). Results obtained were expressed as mean ± SEM, and the student's t-test was used to determine the significance difference between the control group and rats treated with the test compounds. Anti-inflammatory activity of synthesized compounds was compared with standard drug diclofenac sodium 10 mg/kg body weight showing 64.5% inhibition of rat paw edema whereas tested compounds showed inhibition ranging from 31.52% to 60.39% after 120 min. The compounds PM<sub>1</sub>, PM<sub>2</sub>, PM<sub>5</sub>, and PM<sub>6</sub> showed moderate anti-inflammatory activity compared to the standard drug diclofenac. The results are tabulated in Table 2.

**Cytotoxic activity**

All the test compounds were screened for cytotoxic activity against Ehrlich Ascites Carcinoma (EAC) cells. The tumor cells aspirated from the peritoneal cavity of tumor-bearing mice was washed thrice with normal saline and checked for viability using trypan blue exclusion method [14]. The cell suspension (1 million cells in 0.1 ml) was added to tubes containing various concentrations of the test compounds and volume was made up to 1 ml using phosphate buffered saline. Control tubes contained only cell suspension. The assay mixtures were incubated for 3 hrs, at 37°C, and then, percent of dead cells were evaluated by trypan blue exclusion method. Compounds PM<sub>1</sub>, PM<sub>2</sub>, PM<sub>5</sub>, and PM<sub>6</sub> induced the greatest effect on EAC cells with an activity more than 60% at a concentration of 200 µg/ml. The results are summarized in Table 3.

**RESULTS AND DISCUSSION**

**Anti-inflammatory activity**

The in vivo anti-inflammatory activity of the synthesized compounds by carrageenan-induced rat hind paw edema method showed that compounds PM<sub>1</sub>, PM<sub>2</sub>, PM<sub>5</sub>, and PM<sub>6</sub> exhibited significant activity. The presence of pyrimidine moiety with electron withdrawing groups

| Compound code | R            | Molecule weight | M.P°C   | Physical state | % yield |
|---------------|--------------|-----------------|---------|----------------|---------|
| PM<sub>1</sub>| 4-Cl         | 327             | 222-224 | White crystals | 67      |
| PM<sub>2</sub>| 4-F          | 295             | 210-212 | Brown crystals | 70      |
| PM<sub>3</sub>| 3-OH         | 309             | 231-233 | White crystals | 64      |
| PM<sub>4</sub>| 4-CH<sub>3</sub> | 324             | 250-252 | Brown crystals | 78      |
| PM<sub>5</sub>| 3-NO<sub>2</sub> | 304             | 240-242 | White crystals | 80      |
| PM<sub>6</sub>| 4-CN         | 304             | 240-242 | White crystals | 80      |
such as chloro, fluoro, nitro, and cyano accounted for significant anti-inflammatory activity.

Cytotoxic activity

The test compounds were screened for their cytotoxic activity against EAC cells using trypan blue exclusion method. Compounds PM1, PM2, PM3, PM4, PM5, and PM6 induced significant effect on EAC cells with an activity more than 60% at a concentration of 200 µg/ml. The presence of pyrimidine moiety with substitution and group such as chloro, fluoro, nitro, and cyano has accounted for their remarkable cytotoxic activity.

CONCLUSION

Most of the synthesized compounds resulted in good yield, and most of them showed potent anti-inflammatory and cytotoxic activities.

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