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SYNTHESIS AND ANTI INFLAMMATORY ACTIVITY OF 3-(2-4-METHYL-2-(2-NITROPHENYLAMINO)-6-(PIPERAZIN-1-YL METHYL) PYRIMIDIN-5-YL)-2H-CHROMEN-2-ONE

P. R. Logesh Kumar[1]* and Dr. Girendra Gautam[2]

[1]Research Scholar, Department of Pharmacy, Bhagwant University, Sikar Road, Ajmer, Rajasthan - 305004, India.

[2]Research Guide, Professor & Head, Faculty of Pharmacy, Bhagwant University, Sikar Road, Ajmer, Rajasthan - 305004, India.

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***Corresponding Author**
P. R. Logesh Kumar
Research Scholar,
Department of Pharmacy,
Bhagwant University, Sikar Road, Ajmer, Rajasthan - 305004, India.

ABSTRACT

The pyrimidine ring system has wide occurrence in nature as substituted and ring fused compounds and derivatives, including the nucleotides, thiamine (vitaminB1) and alloxan. It is also found in many synthetic compounds such as barbiturates and the HIV drug, zidovudine. Although pyrimidine derivatives such as uric acid and alloxan were known in the early 19th century, a laboratory synthesis of a pyrimidine was not carried out until 1879, when Grimaux reported the preparation of barbituric acid from urea and malonic acid in the presence of phosphorus oxychloride.

KEYWORDS: Pyrimidine, 2-nitrophenylamine, spectral analysis, anti inflammatory.

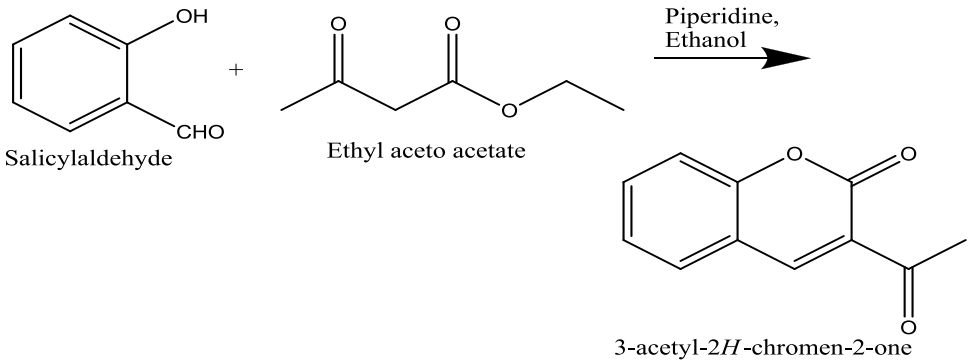
INTRODUCTION

The pyrimidine ring system has wide occurrence in nature as substituted and ring fused compounds and derivatives, including the nucleotides, thiamine (vitaminB1) and alloxan. It is also found in many synthetic compounds such as barbiturates and the HIV drug, zidovudine. Although pyrimidine derivatives such as uric acid and alloxan were known in the early 19th century, a laboratory synthesis of a pyrimidine was not carried out until 1879, when Grimaux reported the preparation of barbituric acid from urea and malonic acid in the presence of phosphorus oxychloride. The systematic study of pyrimidines began in 1884 with

Pinner, who synthesized derivatives by condensing ethyl acetoacetate with amidines. Pinner first proposed the name "pyrimidine" in 1885. The parent compound was first prepared by Gabriel & Colman in 1900, by conversion of barbituric acid to 2,4,6-trichloropyrimidine followed by reduction using zinc dust in hot water.

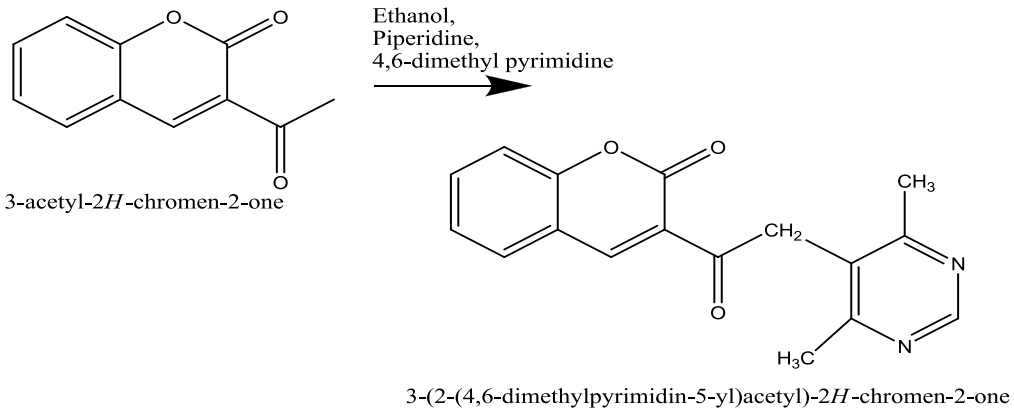
MATERIALS AND METHODS

Scheme of the work



STEP: 1 SYNTHESIS OF 3-ACETYL-2H-CHROMEN-2-ONE.

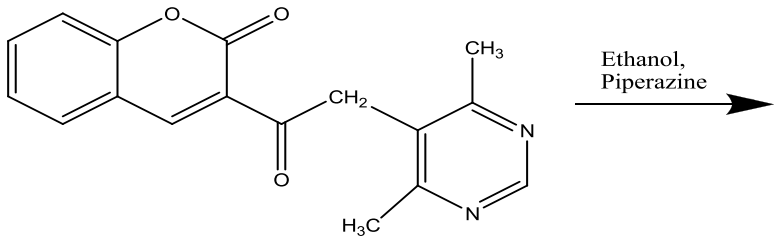
A mixture of salicylaldehyde (0.1 mole) and ethyl aceto acetate (0.2 mole) in ethanol were taken in round bottom flask. To this mixture few drops of piperidine were added and refluxed for 2-3 hours. After completion of reaction the content is poured onto crushed ice. The solid is filtered, dried and recrystalized from ethanol.



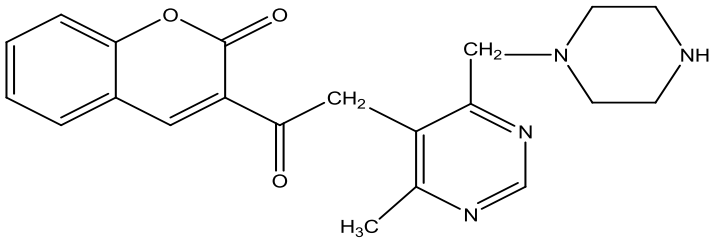
STEP: 2 SYNTHESIS OF 3-(2-(4,6-DIMETHYL PYRIMIDIN-5YL)ACETYL)2H-CHROMEN-2-ONE.

Equimolar quantities of 3-acetyl-2H-chromen-2-one and 4, 6-dimethylpyrimidine were refluxed in absolute ethanol using piperidine as a catalyst for 8-10 hours. The solution

mixture is concentrated and poured onto crushed ice. The compound thus obtained is filtered at pump dried and recrystalized from ethanol to get pure crystalline solid.



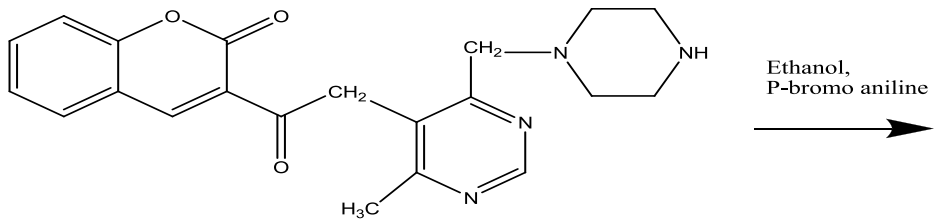
3-(2-(4,6-dimethylpyrimidin-5-yl)acetyl)-2*H*-chromen-2-one



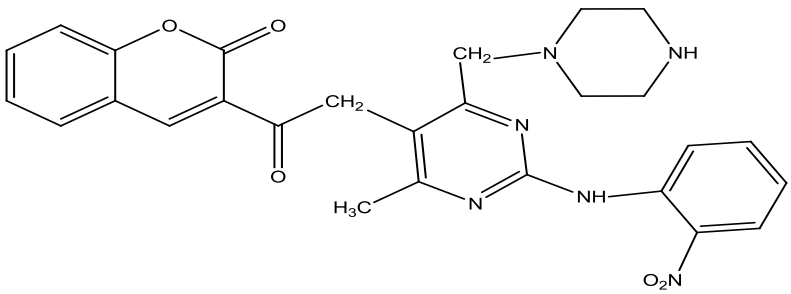
3-(2-(4-methyl-6-(piperazin-1-ylmethyl)pyrimidin-5-yl)acetyl)-2*H*-chromen-2-one

STEP: 3 SYNTHESIS OF 3-(2-(4-METHYL-6-PIPERAZINE-1-YLMETHYL) PYRIMIDINE-5YL) ACETYL-2H-CHROMEN-2ONE.

Compound-2 (0.1 mole) is to be dissolve in piperazine (0.1 mole) is refluxed in ethanol for 8-10 hours. The content is evaporated to dryness and the product so obtain is wash with water repeatedly and recrystalized from ethanol.



3-(2-(4-methyl-6-(piperazin-1-ylmethyl)pyrimidin-5-yl)acetyl)-2*H*-chromen-2-one



3-(2-(4-methyl-2-(2-nitrophenylamino)-6-(piperazin-1-ylmethyl)pyrimidin-5-yl)acetyl)-2*H*-chromen-2-one

STEP: 4 SYNTHESIS OF 3-(2-4-METHYL-2-(2-NITROPHENYLAMINO)-6-(PIPERAZIN-1-YL METHYL) PYRIMIDIN-5-YL)-2H-CHROMEN-2-ONE.

Compound-3 (0.1 mole) is to be dissolve in acetone (50 ml) which is to be added to 2-nirto phenylamine (0.1 mole) in acetone (50 ml) and contents are to be refluxed in ethanol for 6-8 hours. The reaction mixture is reduced to half of its volume and poured onto crushed ice. The product so obtain is wash with water repeatedly, dried and recystalized from ethanol.

Physical characterization

- ✓ Molecular formula : $C_{27}H_{26}N_6O_3$
- ✓ Molecular weight (gm) : 514 g/mol
- ✓ Soluble in Methanol, Ethanol and DMSO.
- ✓ Melting point : 105ºC
- ✓ Melting points were determined using Veego Digital melting point apparatus.
- ✓ The purity of synthesis compound was monitored on TLC.
- ✓ Absorbent used : Precoated Silica gel- G plate
- ✓ Mobile Phase : Chloroform : Ethanol (2:8)
- ✓ R_f value: 0.89.

Biological screening

***In-Vitro* Anti-Inflammatory Activity**

Inflammation is normal protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents and local response of living mammalian tissue to injurious agents, which may be due to physical agents like heat, cold, radiation, trauma; Chemical agents like organic and inorganic; Infective agents like bacteria, virus, parasites; Immunological agents like antigen-antibody reactions, cell mediated reaction. In the present study invitro anti-inflammatory activity was checked for the synthesized compounds.

HRBC Membrane Stabilisation method

The method involves the stabilization of human red blood cell membrane by hypotonicity induced membrane lysis.

Principle

The lysosomal enzymes released during inflammatory condition produce a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. The anti-inflammatory agents act by either inhibiting the lysosomal enzymes or by stabilizing the lysosomal membrane since the human red blood cell membrane are

similar to lysosomal membrane components. The prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of the drug.

Reagents

- HRBC suspension : 10%
- Alsiever solution
- Isotonic saline : 0.85%
- Phosphate buffer : 0.15M, pH-7.2
- Hypotonic saline : 0.36%

Preparation of Alsievier's solution

2g dextrose + 0.8g sodium citrate + 0.05g citric acid + 0.42g sodium chloride was made up with distilled water to 100ml.

Preparation of 0.5 ml of 10% HRBC Suspension

To 3 ml of blood, add 3 ml of Alsievier's solution and centrifuge at 3000 rpm for 20 minutes then packed cells were washed with isotonic saline and later 10% v/v suspension of the packed cells was made with isotonic saline.

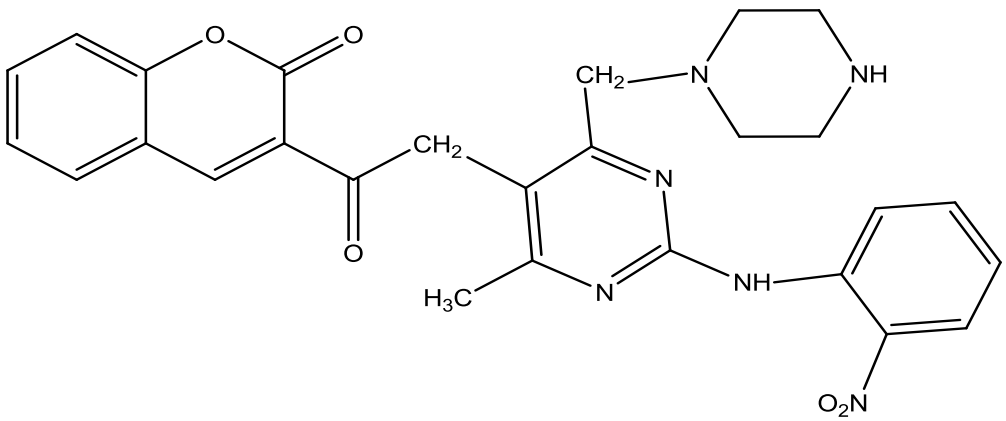
Preparation of Hypotonic Saline

0.36g of sodium chloride in 100 ml of distilled water.

Preparation of Isotonic Saline

0.85g of sodium chloride in 100 ml of distilled water.

Spectral analysis



IUPAC Name

3-(2-(4-methyl-2-(2-nitrophenylamino)-6-(piperazin-1-ylmethyl)pyrimidin-5-yl)acetyl)-2*H*-chromen-2-one

^{1}HNMR Interpretation

^{1}HNMR Spectral data Absorption position (in PPM)	
7.27 - 7.53	m, 6H, CH
6.77 – 6.89	d, 6H, CH
5.47	s, 3H, CH_2
4.8	d, 3H, NH

RESULTS AND DISCUSSION

Synthesis

The present study report the synthesis of pyrimidine derivatives nucleophillic substitution of salicylaldehyde with ethyl aceto acetate was carried out stepwise at different temperature by various amines. The first step involves substitution of ethyl aceto acetate and the next by 4,6-dimethyl pyrimidine. The final pyrimidine derivative in the synthesized compound 3 was replaced by 2-nitrophenylamine. Since the report regarding this compound suggest a pyrimidine posses a good bioactive moiety.

Physical Characterization

Melting points of the synthesized compound was taken in open capillary tubes and was uncorrected and were found to be in the range 115-130˚C.

TLC was performed using precoated silica gel plates of 0.25mm thickness. Eluents used were chloroform, ethanol (2:8) spots were visualized in U.V. light.

At room temperature solubility of newly synthesized compounds were determined by various organic solvents and it was found that all compounds were freely soluble in Methanol, Ethanol, DMSO and DMF.

In-Vitro Anti-Inflammatory Activity

The synthesized compounds are to be used for this study. They are to be made into doses of 1000 µg/ml with DMSO (5.0%) solution. Diclofenac sodium is taken as standard. The reaction mixture (4.5 ml) consist of 2 ml of hypotonic saline (0.36% sodium chloride), 1 ml of 0.15 M phosphate buffer (Ph 7.4), 1 ml of the test solution (1000 µg/ml) in normal saline and 0.5 ml of HRBC suspension in normal saline. For control test, 1 ml isotonic saline is to be used instead of test solution while product control lacked RBC. The mixture is then

incubated at 56˚C for 30 minutes, then to be cooled under running tap water and centrifuged at 3000 rpm for 20 minutes. The absorbances of the supernatants are read at 560 nm. Percent membrane stabilization activity is calculated as follows:

$$\% \text{ stabilization} = \frac{\text{OD of test control-OD of test sample}}{\text{OD of test control}} \times 100$$

S.No	Compound code	Percentage Stabilization
1	2-nitrophenylamine	80
2	STD (Diclofenac)	87.4

CONCLUSION

In the present study certain pyrimidine derivatives were synthesized and characterized by ^{1}HNMR. The synthesized compound show characteristic absorption peaks –in ^{1}HNMR spectra. Expected molecules in (m+) fragments were observed for the entire compounds in mass spectra.

The synthesized compound was subjected to biological evaluation. The compound were evaluated for anti-inflammatory studies revealed that the substitution of different aromatic amines to parent pyrimidine nucleus show the moderate activity.

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