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Research Article

Synthesis, Characterization and Pharmacological Evaluation of Novel Mannich bases of Thienopyrimidine Derivatives

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Abstract: Thienopyrimidines has received considerable attention as they are endowed with variety of biological activities and have wide range of therapeutic properties. Thienopyrimidines, the structural analogues of biogenic purine class, has high significant in field of pharmaceutical and biotechnological sciences, with wide spectrum of biological activities. A series thienopyrimidines can be easily synthesized by Gewald reaction. The first step of this multicomponent reaction is the Knoevenagel-Cope condensation of carbonyl compound (ketone or aldehyde) with an activated nitrile (α-cyanoester), yielding an α,β– unsaturated nitrile. This intermediate is then thiolated at the methylene group by elemental sulfur, followed by an intramolecular cyclization yielding a polysubstituted-2-aminothiophene. The structure of compounds were characterized by ^{1}H-NMR, IR and mass spectral analysis, and evaluated for their anti-inflammatory activity by carrageen induced paw oedema method and standard drug used for anti-inflammatory activity is Diclofenac Sodium.
Keywords: Gewald reaction, 2-aminothiophene, thieno [2, 3-d] pyrimidine derivatives, anti inflammatory activity.

INTRODUCTION

Medicinal chemistry [1] or Pharmaceutical chemistry are disciplines at the intersection of chemistry, especially organic chemistry and pharmacology and various other biological activities. It is concerned with design, chemical synthesis and development for pharmaceutical drugs.

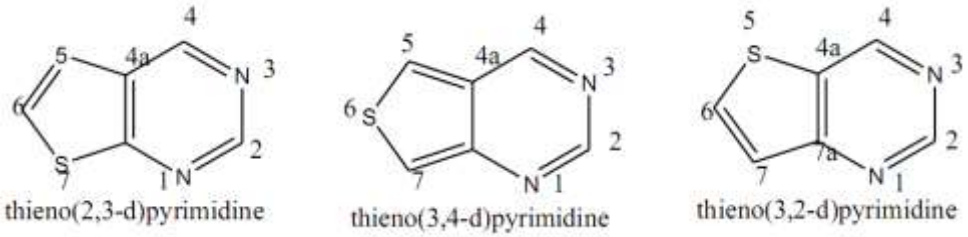
It is the application of chemical research techniques to the synthesis of pharmaceuticals thereby, medicinal chemistry almost always geared towards drug discovery and development.

Heterocyclic containing the thienopyrimidine moiety are of interest because of their interesting pharmacological and biological activities. Thus, over the last two decades many thienopyrimidines have been found to exhibit a variety of pronounced activities such as antimicrobial [2], anti-inflammatory [3], antibacterial [4], antiviral [5], anti-hypertensive [6] and anti-tumour [7] activities. Some of the compounds showed potent and specific cytotoxicity against several leukemia cell lines. Representative compounds among the synthesized thienopyrimidines were tested and evaluated as anti-tumour agents and for cytotoxicity against some cancer cell lines. A pyrimidine nucleus fused with another heterocyclic has found wide applications in the design and discovery of novel molecules and drugs. Thieno [2, 3-D] Pyridine [8] occupies a special position among fused pyrimidines because they are structural analogues of biogenic purines and can be considered as potential nucleic acid anti-metabolites.

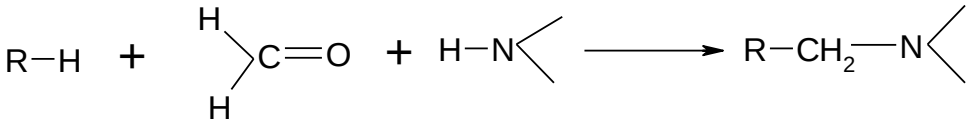
Thienopyrimidines are a class of fused heterocycles which are common sources for the development of new potential therapeutic agents [9]. There are three isomeric thienopyrimidines corresponding to the three possible types of annulation of thiophene to the pyrimidine ring vizthieno [2, 3-d] pyrimidine, thieno [3, 4-d] pyrimidine, and thieno [3, 2-d] pyrimidines. The three isomeric thienopyrimidines [10]

Many thienopyrimidines were reported for their antimicrobial and antifungal activities. Many compounds were screened for their anticancer activity too. Literature survey showed that the 2,4-diaminothieno[2,3-d]pyrimidines have the property of inhibition of dihydrofolatereductase. Thieno [2,3-d]pyrimidin-4(3H)-one compounds have been reported to be effective inhibitors of 17β-HSD1, which results in inhibition of the E_2 dependent tumor growth and hence

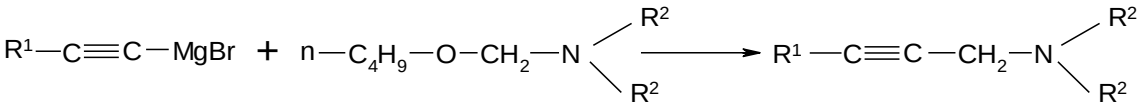
these compounds are useful for the treatment and prevention of breast cancer and other hormone dependent disorders.



In Mannichreaction **[**11]; formaldehyde and an amine (usually as its hydrochloride) are condensed with an organic compound containing active hydrogen. The essential feature of the reaction is replacement of the active hydrogen atom by an amino methyl or a substituted amino methyl group. Hence, this reaction is also known as amino methylation.

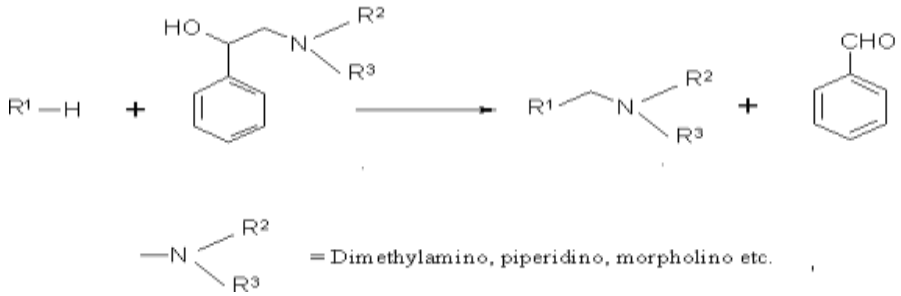


The reaction conditions of Mannich reactions vary with the nature of the substrates. Alkyl ketones are refluxed in alcoholic solutions for several hours with an amine hydrochloride and para formaldehyde or formalin. Phenols are treated with amines, formalin or para formaldehyde in hot alcoholic solution for few hours, or the mixture is allowed to stand at room temperature for few days. Heterocyclic compounds, carboxylic acids and their derivatives are reacted in aqueous or alcoholic medium with formalin in water at room temperature. In the case of alkynes, the reaction is carried out in the presence of copper salts. Several acetylenic magnesium halides have been amino methylated using amino methyl butyl ethers.



Mannich reaction **[**12]is generally carried out by mixing the substrate, aldehyde, and the amine in equimolar amounts. However, in some cases the amine and aldehyde are condensed first and then allowed to react with the substrate. In the latter method the initial condensation products are sometimes isolable. In other cases, condensation between aldehyde and substrate is allowed to take place before addition of the amine.

A great variety of substrates has been successfully amino methylated by the reagent 2-amino-1-phenylethanol in the presence of lead acetate.



Mannich bases often crystallize from the reaction mixture or the bases can be separated by extraction with aqueous hydrochloric acid. In some cases concentration of the reaction mixture or addition of a neutral solvent is necessary.

EXPERIMENTAL WORKS

All the chemicals were obtained from S.D. Fine chem. Limited (Mumbai). All the glassware is of borosilicate grade. Melting points were determined in open capillaries and are uncorrected. The melting point of organic compound was determined by thiel's melting point apparatus. The purity of the compounds was ascertained by TLC on silica gel-G plate. TLC is an important method for synthetic chemistry to infer the formation of the compound based on the R_f value since different compound will have different R_f values. It also help in the confirming the reaction. The solvent used

was Chloroform: Ethyl acetate: Formic acid (5:4:1), Iodine chamber was used for visualization of the spots.

Characterizations of synthesized compounds were done by spectral studies.

Fourier transform infrared (FT-IR) spectra were taken in KBr on a Thermo Nicolet Nexus 670 spectrophotometer. ^{1}HNMR spectra were recorded on BRUKER AVANCE 300MHz spectrophotometer in $CDCl_3$ with TMS as internal standard. The chemical shift values are in delta (ppm).

Mass spectra were recorded on Polaris Q apparatus (Thermo Electron) and the fragmentations were obtained by electronic impact (EI). The data is given as mass to charge ratio (m/z) and nominal masses were used for the calculation of molecular weights of the synthesized products.

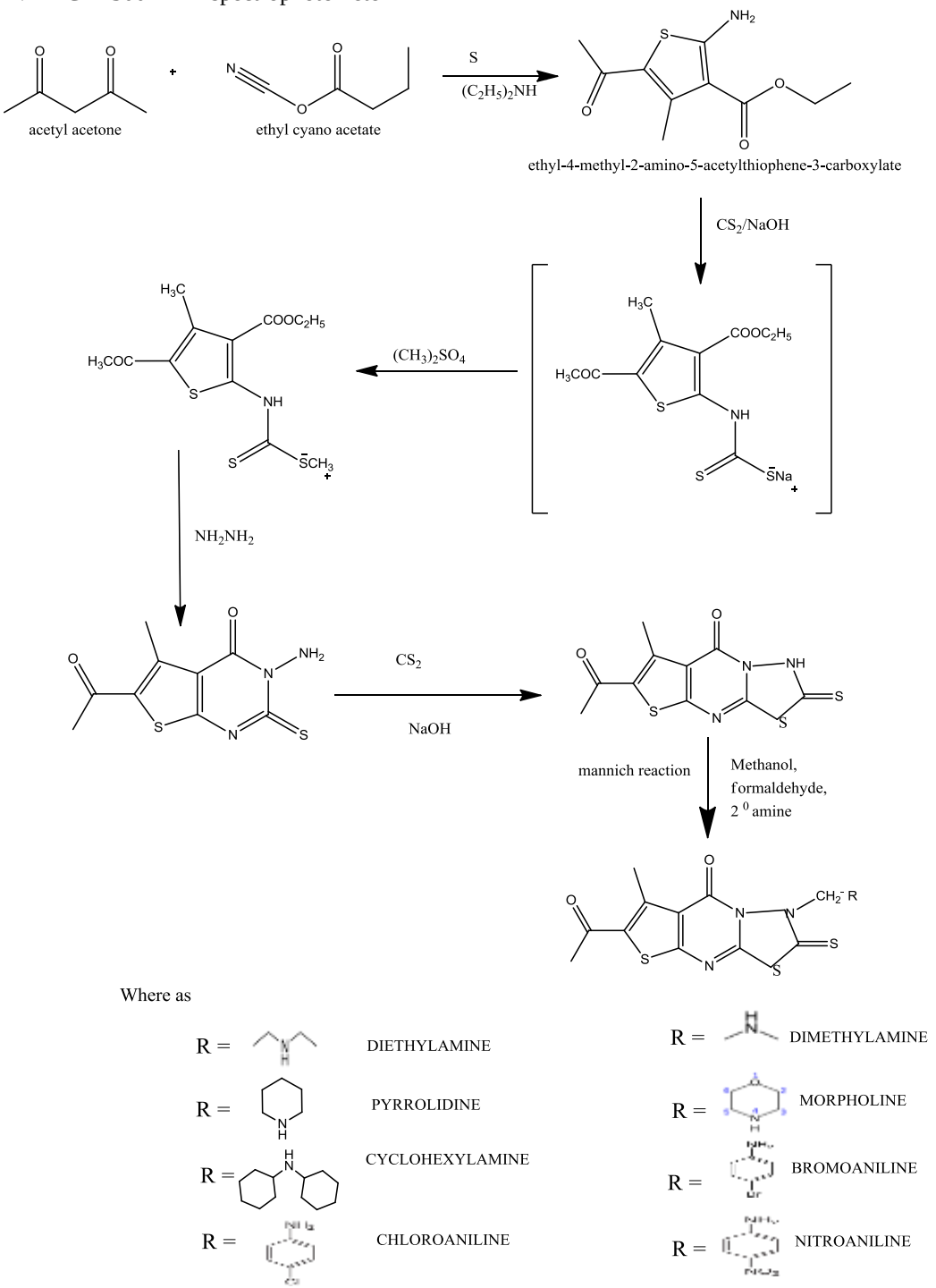


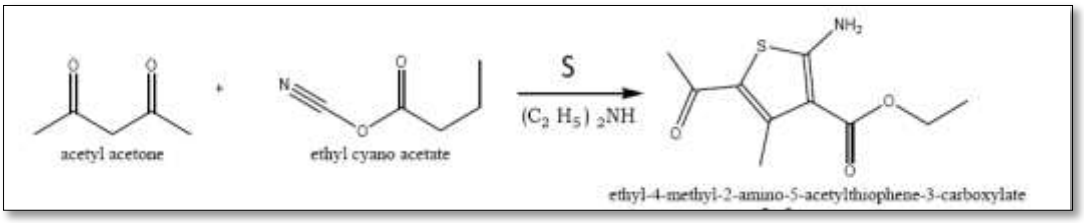
Fig-1: Scheme of Work

Scheme of the Work

Experimental Procedure [13]

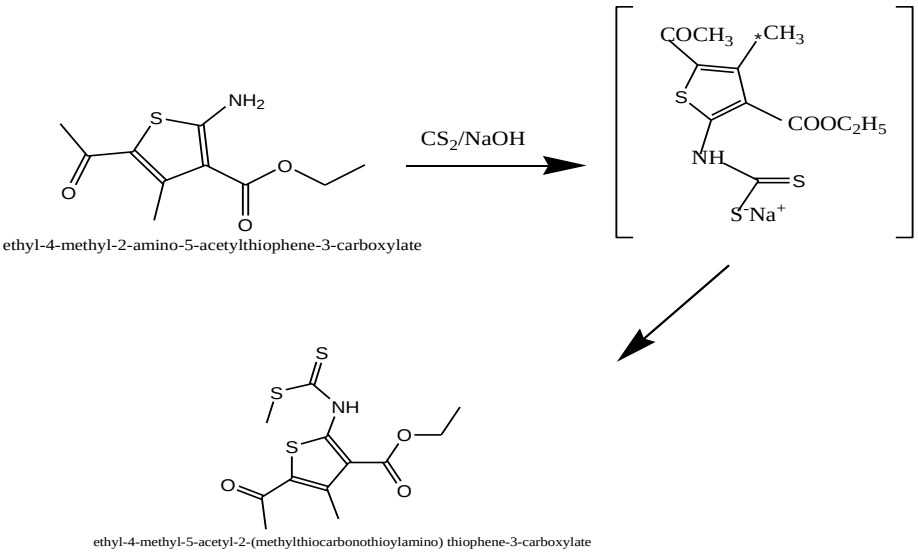
Step-1: Synthesis of Ethyl-4-methyl-2-amino-5-acetylthiophene-3-carboxylate (1)

A mixture of acetyl acetone (0.01 mol), ethyl cyano acetate(0.01 mol), sulfur (0.01 mol) and diethyl amine (0.01mol) was heated at 70`C under stirring in absolute ethanol (20 mL) for 4h, then the mixture was left for 24h at 0`C. The solid formed was collected by filtration, washed with ethanol (20mL), dried and crystallized from absolute ethanol.



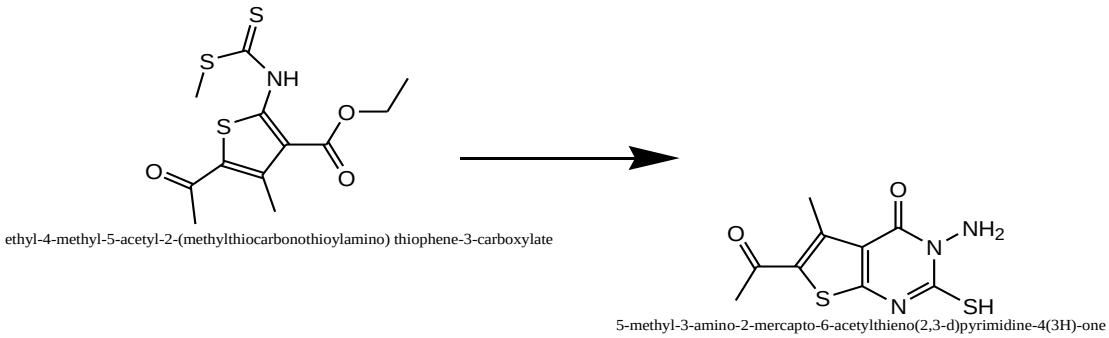
Step-2: Synthesis of Ethyl-4-methyl-5-acetyl-2 (methyl thio carbon thioyl amino)thiophene-3-carboxylate (2)

To a vigorously stirred solution of 1 (0.02 mol) in dimethylsulfoxide DMSO (10mL) at room temperature, carbon disulfide(0.02 mol) and aqueous sodium hydroxide were added simultaneously over 30min; stirring was continued for further 30min. Dimethylsulfate (0.02 mol) was added drop wise to the reaction mixture under stirring at 5-10`C. It was stirred for another 2 h and poured into ice-water. The solid obtained was filtered off, dried and recrystallized from ethanol.



Step-3: Synthesis of 5-methyl-3-amiono-2-mercapto-6-acetylthieno [2, 3-d] pyrimidine-4(3H)-one (3):

A solution of 9 (0.01mol) in ethanol (30mL) and hydrazine hydrate (0.01 mol) was added and refluxed on a water bath until the methyl mercaptan evolution ceased after 8h. After cooling the solid obtained was filtered off, dried and recrystallized from ethanol or acetone-mixture.



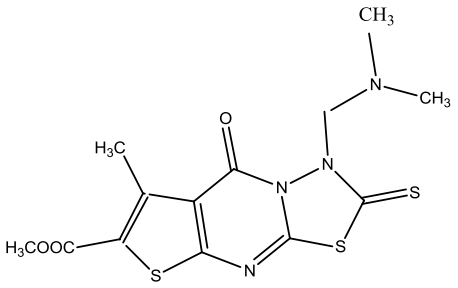
General experimental procedure for synthesis of derivatives:

To the synthesized thienopyrimidine basic moiety compound add methanol, formaldehyde and mannich base all are taken in equimolar concentration. And are subject for reflux for 2 hrs at 45-50^{0}C . After that filter the formed product and recrystallized by using ethanol and dried.

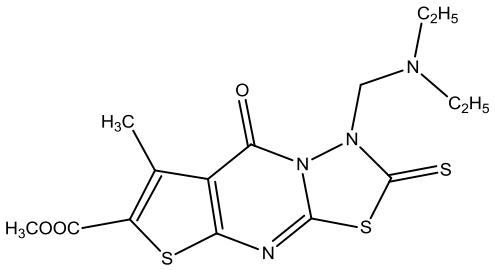
The reaction was evaluated by using TLC plate technique and the used solvents are Acetone & Hexane in different ratios. The formed product was tested for melting point, and compare with that of the standard value.

Synthesized Compounds

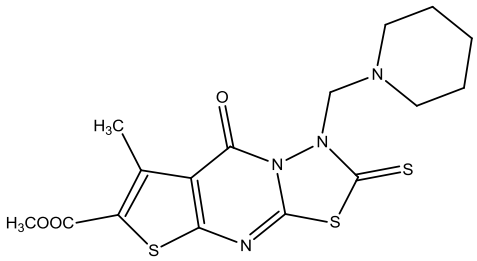
COMPOUND-1:



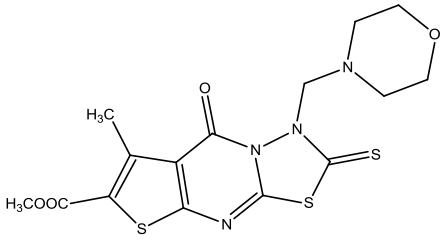
COMPOUND-2:



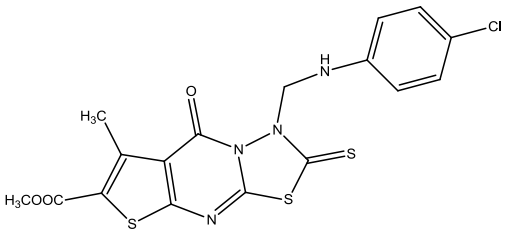
COMPOUND-3:



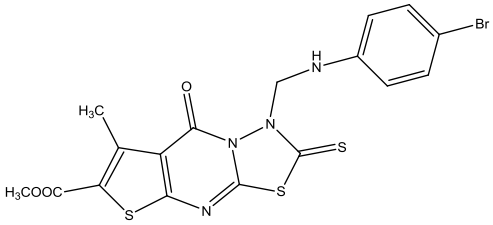
COMPOUND-4:



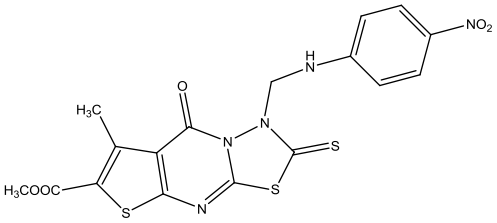
COMPOUND-5:



COMPOUND-6:



COMPOUND-7:



COMPOUND-8:

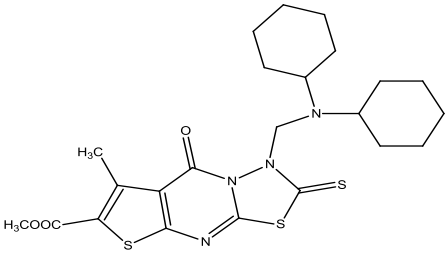
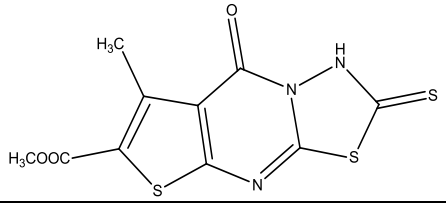
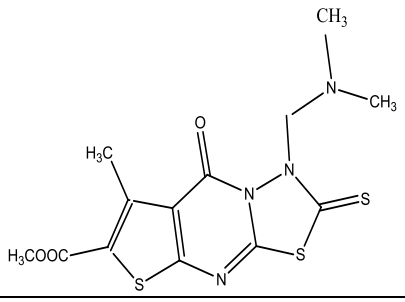
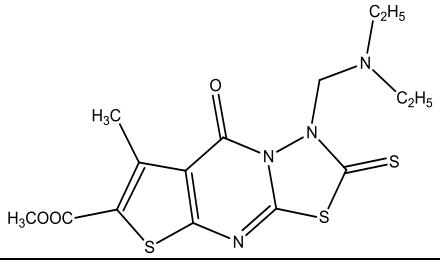
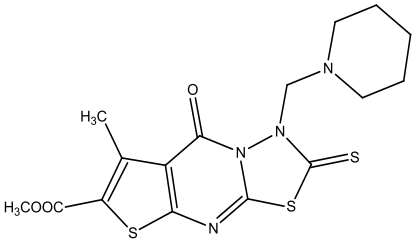
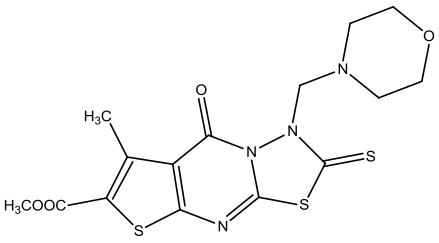
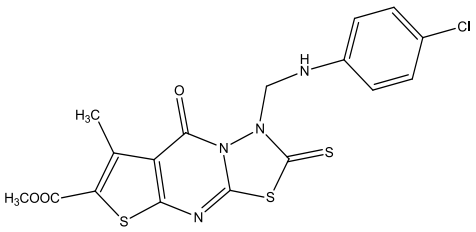
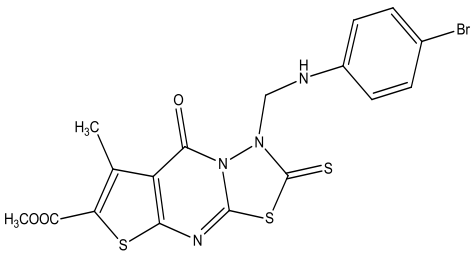
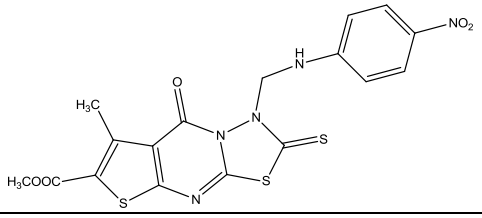
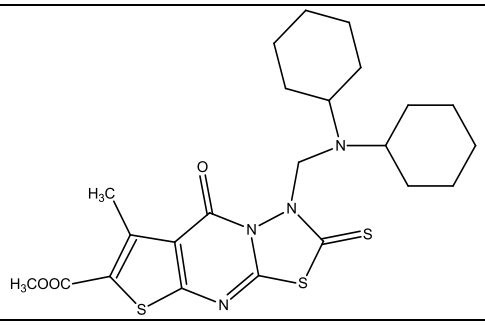


Table 1: Physical Properties of Synthesized Compounds

Compound	Structure	Molecular Formula	M. Wt	M.P^{0}C	%Yield
CP		$C_{10}H_{10}N_3O_3S_2$	221	232	62.33
CP-1		$C_{13}H_{17}N_4O_3S_2$	281	157	61.04

CP-2		$C_{15}H_{21}N_4O_3S_2$	349	159	77.41
CP-3		$C_{16}H_{21}N_4O_3S_2$	381	213	70
CP-4		$C_{15}H_{19}N_4O_4S_2$	383	242	63.02
CP-5		$C_{17}H_{22}N_4O_3S_2Cl$	395.5	238	65.36
CP-6		$C_{17}H_{22}N_4O_3S_2Br$	438	262	54.27
CP-7		$C_{17}H_{22}N_5O_5S_2$	440	224	64.72

CP-8		$C_{23}H_{33}N_4O_3S_2$	477	248	71.8

PHARMACOLOGICAL EVALUATION

Acute Oral Toxicity [14]

Introduction

Acute oral toxicity defines to those adverse effects occurring following oral administration of a single dose of substances or multiple doses given within 24 hrs. The various methods used to evaluate the acute oral toxicity are as follows.

1. Fixed dose procedure (OECD guideline-420)
2. Acute toxic class methods (OECD guideline-423)
3. Ups and down procedure (OECD guideline-425)

OECD guideline-423

Experimental Protocol (Acute toxic class method in mice) [15]

In the present study, the acute oral toxicity of synthesized novel mannich bases containing thienopyrimidine moiety derivatives were performed by acute toxic class method. In this method, the toxicity of synthesized compounds was tested using a step wise procedure, each step using three mice of a single sex. The mice were fasted prior to dosing (food but not water should be with held) for three to four hours. Following the period of pasting, the animal should be weighed and synthesized drug was administered orally at a dose of 2000 mg/kg body weight. Animals were observed individually after dosing at least once during the first 30 min; periodically during the first 24hrs with special attention given during the first 4hrs and daily thereafter, for a total 14 days. The test procedure commences with a starting dose of 2000mg/kg body weight as per OECD-423 Guidelines.

Introduction to Inflammation

Inflammation [16]

Inflammation (Latin, īnflammo, "Iignite, set a light") is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function.

Types of Inflammation

Inflammation can be classified into two types.

- Acute inflammation
- Chronic inflammation

Evaluation methods [17]

The three important aspects of inflammation that render themselves readily to measurement are erythema, edema and formation of granulation tissue. Compounds claimed to posses anti-inflammatory activity can be evaluated either by their ability to reduce one or more of these phenomena in experimentally induced inflammation or by testing their anti-inflammatory activity in experimental apparatus produced in animals. The commonly employed methods are

a). Erythema assays [18]

In this methods irradiation of shaven back skin of guinea pig with UV light cause erythema which can be reduced by anti-inflammatory agents.

b).Edema assays [19]

The edema can be produced in experimental animals by the local injection of substance like Formaldehyde, Carrageenan, Histamine, Dextron and Egg-albumin.

c).Granuloma assays [20]

There are two types of granuloma assays such as cotton wool pellet and granuloma pouch method.

d). Experimental arthritis assays [21]

Poly arthritis induced in rats by injection of dead tubercle bacilli suspended in liquid paraffin is frequently used method. Kaolin, talc and even mercury have also been injected directly into joints of and pigeons to induced arthritis.

e). Miscellaneous

Localized inflammatory reaction can be produced in rats by-

1. Intrapleural injection of turpentine.
2. Intraperitonial injection of formaldehyde.

Experimental procedure for fresh egg white induced paw edema method

Male or female wistar-albino rats with a body weight between 100 and 150g are used. They were acclimated to laboratory conditions for seven days before commencement of experiments, and were allowed free access to standard drug pellet diet and water ad libitum. The animals are starved overnight. To insure uniform hydration, the rats receive the test drug at dose level of 100mg/kg body weight suspended in

5% acacia solution. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.05 ml of 1% solution of egg albumin into the plantar side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured with Plethysmometer immediately after injection, again after 1h, 2h, and 3h and 5hrs % inhibition was calculated by following formula.

%Inhibition = [(Control-Test)/Control] X 100

Fig. 2: Paw Oedema

The values are calculated by Dunnett method by comparing all the compounds with control in One-way Analysis of Variance (ANOVA) and are expressed in Mean ± SEM.

Table 2: Group of animals, drugs and their doses:

Groups	Sample	Dose
Group-1	Control (5% Gum acacia Suspension)	10ml/kg
Group-2	Standard (Diclofenac sodium)	5mg/kg
Group-3	Compound-1	100 mg/kg
Group-4	Compound-2	100 mg/kg
Group-5	Compound-3	100 mg/kg
Group-6	Compound-4	100 mg/kg
Group-7	Compound-5	100 mg/kg
Group-8	Compound-6	100 mg/kg
Group-9	Compound-7	100 mg/kg
Group-10	Compound-8	100 mg/kg

RESULTS & DISCUSSION

IR Spectral Data of Synthesized Compounds [22]

The synthesized compounds of the present study were characterized by Fourier transform infrared (FT-IR) spectra were taken in KBr pellets on a Thermo Nicolet Nexus 670 spectrophotometer. All the synthesized compounds of the present study showed expected characteristic absorption bands for C-H (Ar), CH_2, N (CH_3), C=N, C=S, C-Cl, C-NO_2, C-OH, groups.

All synthesized compounds showed -CH (aromatic) absorption bands in the region of 3160cm^{-1} , 3163cm^{-1} , 3059cm^{-1} , 3049 cm^{-1}, 3059cm^{-1}. All the synthesized compounds showed C=N absorption bands in the region of 1509cm^{-1} , 1593cm^{-1} , 1561cm^{-1} , 1568cm^{-1} , 1568cm^{-1}.

All the synthesized compounds showed C=S absorption bands in the region of 1444cm^{-1} , 1444cm^{-1} , 1469cm^{-1} , 1465cm^{-1} , 1468cm^{-1} respectively. Compound-2 showed C-Cl absorption bands in the region of 837cm^{-1}. Compound-3 showed –NO_2 absorption bands in the region of 1469cm^{-}Compound-6 showed –N $(CH_3)_2$ absorption bands in the region of 1164cm^{-1} respectively. The strong bands at 1600-1430 cm^{-1} corresponding to initial NH_2 were absent, which was the most characteristic evidence of the Cyclization.

H ^{1}NMR Spectral Data of Synthesized Compounds [23]

The synthesized compounds of the present study were characterized by H^{1}NMR spectra.
All the synthesized compounds of the present study showed expected characteristic peaks for C-H (Ar), CH_2, CH_3,NH groups[24].

All synthesized compounds showed -CH (aromatic) peaks in the region of 6.5-8.5.
All the synthesized compounds showed CH_3 peaks in the region of 2-4.2.

All the synthesized compounds showed CH_2 peaks in the region 1.6-3.2. Basic moiety showed NH peak in the region of 1.4-1.7.

Table 3: Mass Spectral Data of The Synthesized Compounds [25]

Compounds	Molecular Mass	M^+ or M^- ion peak
CP	221	222
CP-1	281	282
CP-2	349	348
CP-3	381	380
CP-4	383	383
CP-5	394	393
CP-6	438	436
CP-7	440	440
CP-8	477	480

The values are calculated by Dunnett method by comparing all the compounds with control in One-way Analysis of Variance (ANOVA) and are expressed in Mean ± SEM.

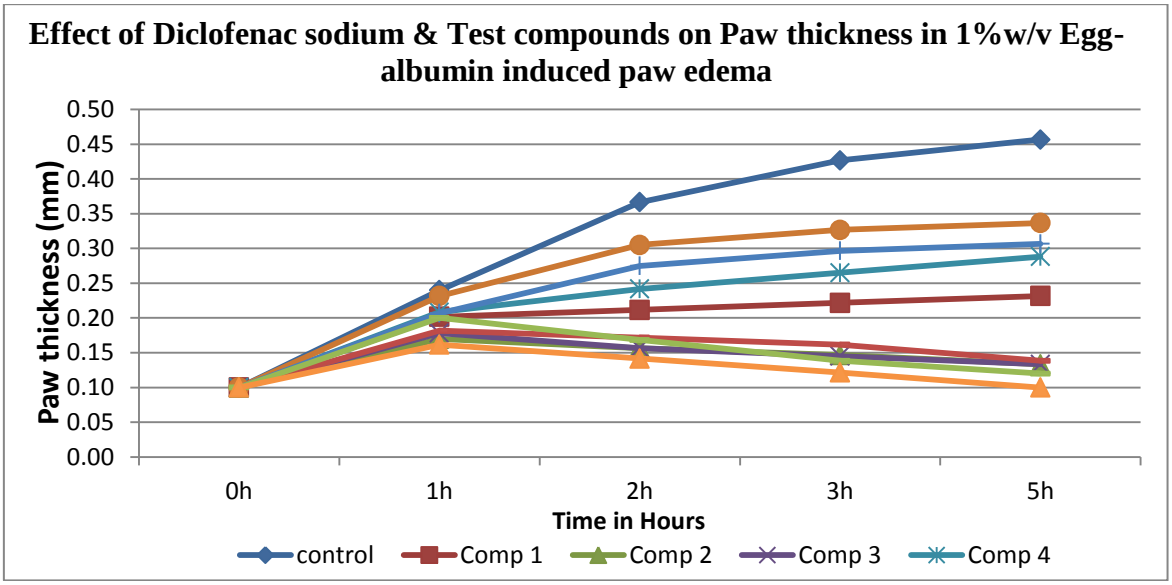


Fig 3: Effect of Diclofenac sodium & Test compounds on Paw thickness

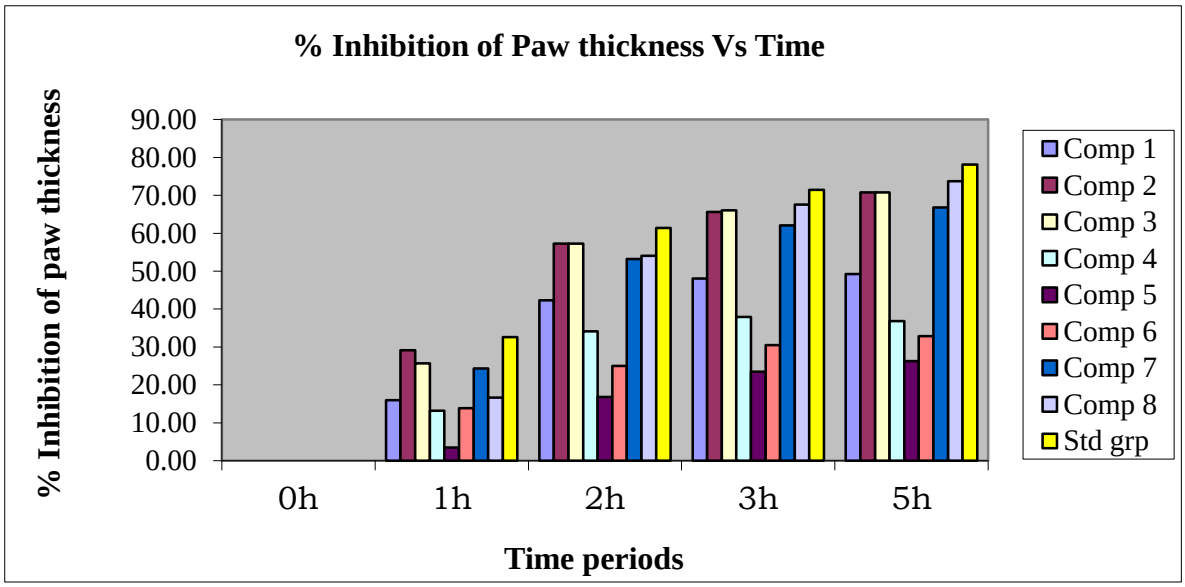


Fig 4: % Inhibition of Paw thickness

Table 4 : Comparison of significance of synthesized compounds with control

Groups	1 hr	2 hr	3 hr	5 hr
Control	0.24±0.00*	0.3667±0.006*	0.4267±0.008	0.4567±0.009
CP-1	0.2017±0.001*	0.2117±0.001	0.2217±0.001*	0.2317±0.001*
CP-2	0.17±0.00**	0.1567±0.002**	0.1467±0.002**	0.1333±0.004**
CP-3	0.1783±0.001**	0.1567±0.002**	0.1450±0.002**	0.1333±0.002**
CP-4	0.2083±0.001*	0.2417±0.001	0.2650±0.002*	0.2883±0.003
CP-5	0.2317±0.001	0.3050±0.002	0.3267±0.002	0.3367±0.002
CP-6	0.2067±0.002	0.2750±0.002	0.2967±0.002	0.3667±0.002
CP-7	0.1817±0.001	0.1717±0.002	0.1617±0.001	0.1383±0.011
CP-8	0.2±0.00**	0.1683±0.001**	0.1383±0.001**	0.1200±0.002**
Standard	0.1617±0.001**	0.1417±0.001**	0.1217±0.001**	0.100±0.002**

Value are mean ± SEM (n=6), *=significant at P<0.05, **= significant P<0.01 significantly different compare to control.

SUMMARY AND CONCLUSION

In the present study novel mannich base of thienopyrimidine derivatives were synthesized. The synthesized compounds were characterized by IR, ^{1}HNMR & Mass Spectrum. All the synthesized compounds showed characteristic absorption peaks in IR, ^{1}HNMR and Mass Spectrum. The first chapter deals with the introduction of therapeutic agents based on thienopyrimidine moiety, Literature survey on the investigation carried out by the earlier workers in the synthesis and evaluation of hetero cyclic system containing thienopyrimidine moiety, aim and scope of work, experimental work including detailed procedure for synthesis of title compounds and physical data of synthesized compounds containing molecular formula, molecular weights, percentage yield and spectral data and finally describes results of experimental investigations and discussion of results.

Acute oral toxicity studies were performed according to the OECD guideline-423 method. From the toxicity studies the data revealed that all the synthesized compounds proved to be non toxic at tested dose levels and well tolerated by the experimental animals as their LD_{50} cut of values > 2000 mg/kg body weight. The synthesized novel mannich bases of thienopyrimidine derivatives were subjected to Invivo anti-inflammatory evaluation.

Anti-inflammatory activity of the synthesized compounds was evaluated by carragean induced rat paw edema method. The activity was studied at the dose levels of 100 mg/kg body weight and their effects were measured at 1, 2, 3 and 5 hrs. The paw volume of the rat in inhibiting inflammation by the synthesized compounds at different time intervals is measured by mercury displacement method.

The anti-inflammatory studies revealed that all the synthesized novel mannich bases of thienopyrimidine derivatives showed significant anti-inflammatory activity when compared with that of standard drug Diclofenac sodium. CP-5, CP-6 and CP-8 showed greater Pharmacological activity due to the presence of more electron withdrawing groups (Br, Cl & NO_2), whereas CP-1, CP-2, CP-3, CP-4 and CP-7 showed mild to moderate activity. Therefore further studies required to develop pharmacologically more promieting compounds in these series.

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