Research Article **CODEN: AJPAD7** **ISSN: 2321-0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF DIHYDRO-5-SUBSTITUTED-2-THIOXOPYRIMIDINES

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ABSTRACT

In this study, synthesis of dihydro-5-substituted-2-thioxopyrimidine derivatives has been described. The route of preparation involved the use of thiobarbituric acid as starting material and treated with substituted aldehyde compounds (aromatic and aliphatic) to give the required derivatives. A total of six derivatives were synthesized and the compounds were purified by chromatographic methods and identified by spectroscopic methods; FTIR, H^1 NMR and also by measuring its melting point. The synthesized compounds were tested for antibacterial activity against two bacterial strains, of them one is gram positive strain *Staphylococcus aureus* and one gram negative strain *Bacillus subtilis*. The compounds were also evaluated for antifungal activity against two fungal strains *Rhizopus* and *Asperigillus*. The phenolic compounds viz., benzyl **(3)**, 2-hydroxy **(4)**, 4-dimethylamino **(5)**, 4-dimethylaminocinnamyl **(6)** derivatives were found to be active against both the fungal and bacterial strains. The non-phenolic compounds viz., formyl **(1)**, glutryl **(2)** derivatives exhibited significant activity against the gram negative bacteria *Bacillus subtilis* revealing that substitution by aromatic nuclei than aliphatic nuclei enhances the antimicrobial activity.

KEYWORDS

Antimicrobial activity, Thiobarbituric acid and Thioxopyrimidine.

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INTRODUCTION

The pyrimidinoyl and thioxopyrimidinoyl groups are of great importance in treating biological system. Analgesic, anti-arrhythmic, anticancer, antipyretic and anti-inflammatory activities are observed in some pyrimidinoyl and thioxopyrimidinoyl derivatives[1]. Among the derivatives of pyrimidines, 2-thioxopyrimidine substances have been observed

that have antiviral, anticancer[2], antimalarial, anaesthetic, fungicidal, insecticidal activity, and antimicrobial action [3].

Condensation of active hydrogen compounds with aldehydes/ketones is known as Knoevenagel condensation. Cross aldol-type condensation of thiobarbituric acid with aromatic aldehydes using acetic acid as a catalyst is available for the preparation [4]. The parent nucleus 4, 6-dioxo-2-thioxopyrimidine has a reactive methylene group (with active hydrogens) flanked between two carbonyl groups, which makes it a versatile molecule for the synthesis of various substituted (e.g., amino carbonyl derivatives, arylidiene derivatives, coupled products with aryldiazonium salts, 5-arylazo compounds etc) and fused heterocyclic systems (e.g., pyrano and benzopyran [2, 3-d] pyrimidines, benzofuro and furo [2, 3-d] pyrimidines etc) in Figure No.1.

The biological profiles of compounds presenting this subunit are related to its relative acidity and its capacity to stabilize free radicals, mimicking the fragment of thiobarbituric acid.

In view of proven potentiality of thiobarbituric acid analogues which contain 4, 6-dioxo-2-thioxopyrimidine pharmacophore, it has been planned to synthesize various analogues of 4, 6-dioxo-2-thioxopyrimidines containing other interesting structural features such as substituted aromatic aldehydes responsible for antimicrobial activity. In our present work, we described a rapid and convenient method for the synthesis of 5-arylidene thiobarbituric acids and new substituted thioxopyrimidine derivatives under uncatalyzed conditions using water as the solvent which exhibit both antibacterial and antifungal activities.

MATERIALS AND METHODS

All the melting points reported in this thesis were determined in open capillaries using "Stuart Melting Point Apparatus", expressed in (^{o}C) and are uncorrected. Purity of the compounds was checked by using the glass plates coated with Silica gel G and spots were detected by iodine vapor. UV spectra were recorded on Systronics UV-visible spectrometer. The IR spectra were recorded using KBr Pellets on a Perkin-Elmer Spectrum BX-I Infrared spectrophotometer (cm^{-1}). ^{1}H NMR spectra were recorded on GE Omega 300 and 400 MHz spectrometer or Bruker AC (400MHz)andGemini-2000(Varian 200 MHz) spectrometer using TMS as internal standard (chemical shifts in δ ppm)Aldehydes were procured from Merck, SD fine chemicals, Aldrich and Sigma. All other chemicals are of AR grade or LR grade.

General method for the synthesis of substituted 2-thioxopyrimidines (1-6)

A mixture of substituted aldehyde (10 mmol) and thiobarbituric acid (10 mmol) in water (40 mL) was stirred at 95-100 ºC for 2 h. Then the solid was filtered and washed subsequently with boiling water and finally with ether and drying in vacuum. The residue was dissolved in warm ethanol and recrystallized [5] in Figure No.2.

The Physicochemical data of the compounds is given in the Table No. 1.

Anti microbial activity

It is evident that 2-thioxopyrimidines exhibit a pronounced antimicrobial activity. Therefore, it has been worthwhile to screen the synthesized substituted 2-thioxopyrimidines for anti bacterial and antifungal activity.

Materials

Two bacterial test organisms, one gram-positive bacteria: *Staphylococcus aureus* and one gram-negative bacteria: *Bacillus subtilis* and two fungal test organisms: *Rhizopus* and *Aspergillus* were selected and obtained from the institute of Microbial technology. Cultures of test organisms were maintained on nutrient agar slants and were sub-cultured in Petri dishes prior to testing. The media used was nutrient agar and nutrient broth.

Various substituted thioxopyrimidines were synthesized as described earlier. Stock solutions of the synthesized compounds were prepared in concentrations of 1mg/ml using ethanol as solvent for anti-bacterial and anti-fungal activity.

Method

The anti microbial activity of title compounds has been assayed against four different strains by agar

diffusion method. One gram-positive bacteria, *Staphylococcus aureus*, one gram-negative bacteria, *Bacillus subtilis* and two fungal organisms are *Rhizopus* and *Aspergillus.*

Generally, the antimicrobial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria and fungi in nutrient broth or agar. The inhibition can be measured by two methods: one is serial dilution method and the other is diffusion method. The serial dilution method is very much useful for the determination of the anti bacterial and anti fungal activity. The agar diffusion method is of three types. They are:

- Cup-plate method,
- Filter-paper method,
- Gradient plate method

The method adopted in this investigation was cup-plate method.

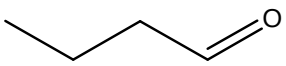
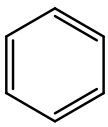
Anti-bacterial and antifungal activity was performed by cup-plate method by measuring zone of inhibition. All the test compounds were screened for antibacterial and antifungal activity against bacterial strains *Staphylococcus aureus*, *Bacillus subtilis* and fungal strains *Rhizopus* and *Aspergillus* at a concentration of 100µg/ml. Streptomycin was used as standard drug at a concentration of 100µg/ml.

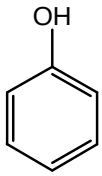
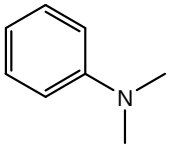
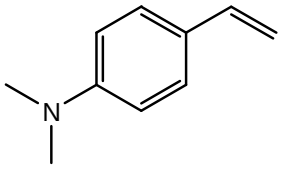
RESULTS AND DISCUSSION

A total of six compounds were synthesized in this series. The compounds were obtained in good yield ranging from 70-80%. The physical data such as melting point, recrystallization solvent and yield are given in Table No.1. ^{1}HNMR spectrum was taken for compound **5** which also supported the structures assigned. The compound **5** showed a singlet at δ 7.1-7.3 due to C_6H_5CH=, multiplets at δ 7-8 due to aryl and olefinic protons, a peak at δ 2.9 due to Ar-N$(CH_3)_2$ was observed in case of compound **5**. The mass spectrum of the compound **5** showed a characteristic molecular ion peaks (M^++1) at m/z 258 and the fragmentation pattern was found to be characteristic to its structure. The spectral data of the compounds was given in Table No. 2.

Among all the series of compounds, some of them gave promising results. The aromatic compounds viz., benzyl **(3)**, 2-hydroxy **(4)**, 4-dimethylamino **(5)**, 4-dimethylaminocinnamyl **(6)** derivatives were found to be active against both the fungal and bacterial strains. The aliphatic compounds viz., formyl **(1)**, glutryl **(2)** derivatives exhibited significant activity against the gram negative bacteria *Bacillus subtilis*. Among the aromatic compounds 4-dimethylamino **(5)**, 4-dimethyl aminocinnamyl **(6)** derivatives showed more appreciable activity than benzyl derivative **(3)** against all the four strains. But the benzyl derivative **(3)** exhibited more potent activity against the fungal strain *Rhizopus* than the 4-dimethylaminocinnamyl **(6)** derivative. The data was given in the Table No. 3.

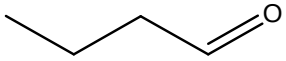
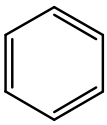
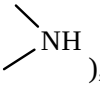
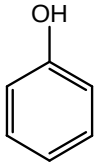
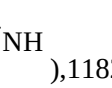
Table No.1: Physicochemical data of Dihydro-5-substituted-2- thioxopyrimidines (1-6)

Compound	R	M.P ^{0}C	Yield in %
1	H	145-148	74
2		186-189	75
3		225-230	64

4		228-230	62
5		242-245	78
6		204-207	74

Recrystallization Solvent: Ethanol

Table No.2: Physicochemical data of Dihydro-5-substituted-2- thioxopyrimidines (1-6)

Compound	R	IR, ^{1}HNMR and MASS data
1	H	2843 cm^{-1}(=CH-), 1634 cm^{-1}(C=O), 1229 cm^{-1}(-C-S-NH), 1440 cm^{-1} ().
2		1522 cm^{-1}(), 1645 cm^{-1} (C=O), 1150cm^{-1}(-C-S-NH).
3		3052cm^{-1}(Ar C-H), 1510 cm^{-1}(), 1669cm^{-1} (C=O), 1251cm^{-1}(-C-S-NH).
4		3350 cm^{-1}(O-H), 3465 cm^{-1}(N-H), 2893 cm^{-1}(Ar C-H), 1647 cm^{-1} (C=O), 1450 cm^{-1} (),1182 cm^{-1}(-C-S-NH).

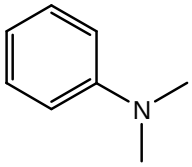
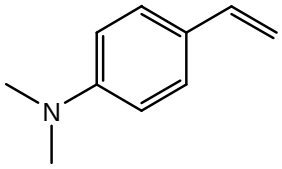
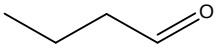
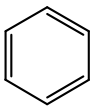
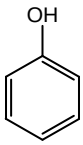
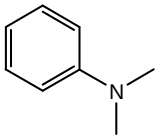
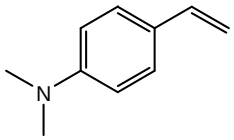
5		3455cm^{-1}(N-H), 1490 cm^{-1}(), 3118 cm^{-1} (ArC-H), 1669 cm^{-1} (C=O), 1234cm^{-1} (-C-S-NH). δ 2.9 (s, 6H, $(CH_3)_2$,N), δ 4.14 (s, 1H, -CO-CH_2-CO-), δ 7.3-7.7(m, 4H, Ar-H), δ 8.06 (s, 1H, PhCH=C). (M^++1) peak at m/z 258
6		3475 cm^{-1}(N-H), 2902 cm^{-1}(Ar C-H), 1648 cm^{-1} (C=O), 1572 cm^{-1}(C=C), 1489 cm^{-1} (), 1236cm^{-1}(-C-S-NH).

Table No.3: Antibacterial activity of Dihydro-5-substituted-2- thioxopyrimidines (1-6)

Compound	**R**	**Zone of inhibition in mm**			
		Rhizo-pus	**Asperi-gillus**	**S.aureus**	**Bacillus**
1	H	12	11	14	12
2		11	11	13	12
3		17	13	12	8
4		13	14	14	13
5		14	15	15	15

6		15	17	17	16
Standard	Streptomycin	18	23	30	30

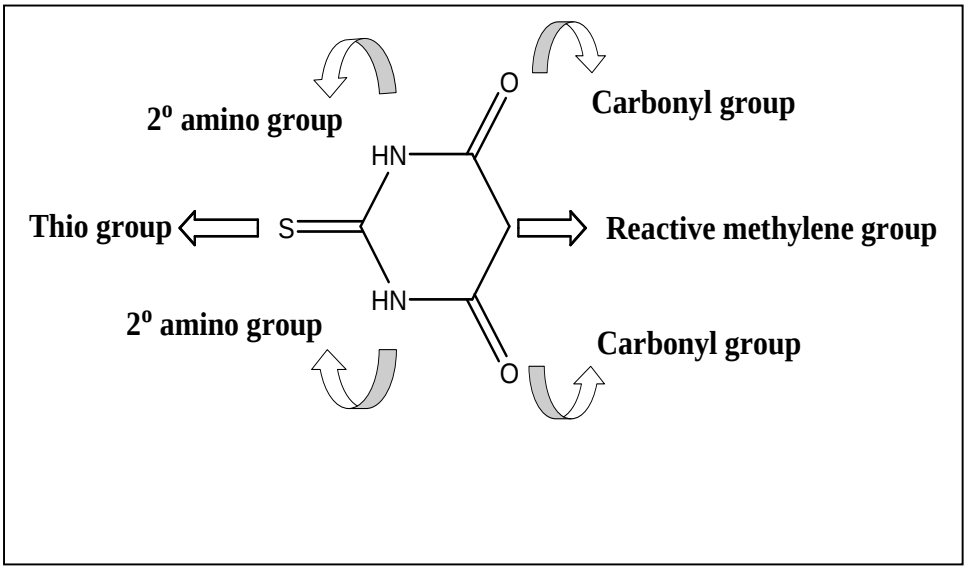
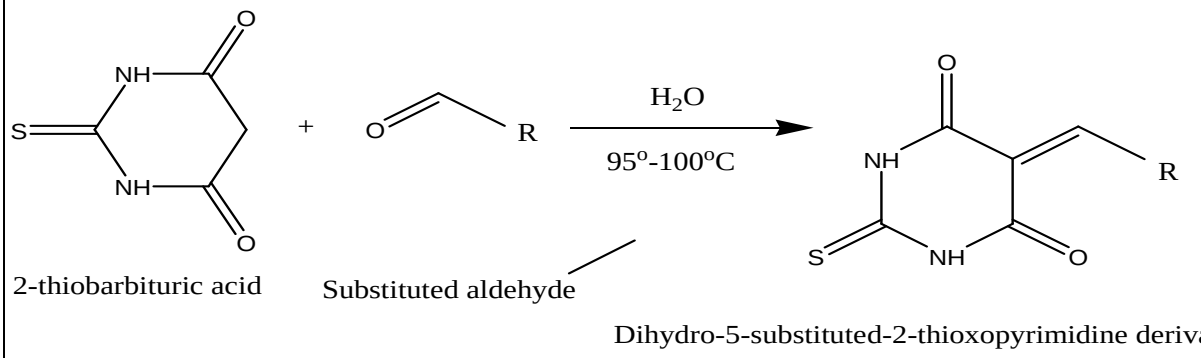


Figure No.1: Structural features of the parent nucleus

SCHEME



2-thiobarbituric acid

Substituted aldehyde

Dihydro-5-substituted-2-thioxopyrimidine derivatives

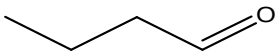
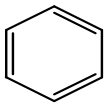
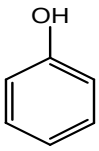
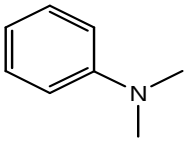
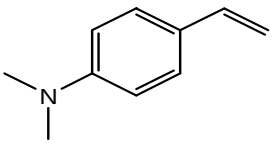
S.No.	R	Name of the Compound
1.	H	Dihydro-5-methylene-2-thioxopyrimidine -4,6(1*H*,5*H*)-dione
2.		5-(tetrahydro-4,6-dioxo-2-thioxopyrimidin -5(6H)-ylidene)pentanal
3.		Dihydro-5-benzylidene-2-thioxopyrimidine -4,6(1H,5H)-dione
4.		Dihydro-5-(2-hydroxybenzylidene)-2-thioxo pyrimidine-4,6(1H,5H)-dione
5.		Dihydro-5-(4-dimethylaminobenzylidene)-2-thioxopyrimidine-4,6(1H,5H)-dione
6.		Dihydro-5-(4-dimethylamino-3-phenylallylidene)-2-thioxopyrimidine-4,6(1H,5H)-dione

Figure No.2: Schematic Synthesis of Thioxopyrimidine

CONCLUSION

Compound 4-dimethylaminocinnamyl **(6)** derivative was equipotent to the standard drug Streptomycin. Among the series of compounds, aromatic derivatives namely 4-dimethylamino **(5)** and 4-dimethylaminocinnamyl **(6)** compounds showed more appreciable activity than benzyl derivative **(3)** against all the four strains when compared to that of aliphatic derivatives formyl **(1)** and glutryl **(2)** compounds revealing that substitution by aromatic nuclei than aliphatic nuclei enhances the antimicrobial activity.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to Sri Venkateswara University, Tirupati for providing the spectral data of the compounds.

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