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Synthesis and Screening of 3-Formyl-2-thio-1,2,3,4-tetrahydro pyrimidine Analogues as Antibacterial Agents

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5-Acyl-6-methyl-4-substituted-2-thio-1,2,3,4-tetrahydropyrimidines were prepared by cyclocondensation between appropriate aldehyde, acetoacetate and thiourea using aluminium chloride and conc. hydrochloric acid. These compounds upon treatment with dimethyl formamide and phosphorous oxychloride furnish the title compounds. The structures of these compounds have been confirmed on the basis of their analytical, IR and NMR spectral data. The title compounds have been tested for antibacterial activity against *Eshcereshia coli*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. They have also been tested for antifungal activity against *Aspergillus niger* and *Penicillium chrysogenum*.

Key Words: 3-Formyl-2-thio-1,2,3,4-tetrahydro pyrimidine, Antibacterial.

INTRODUCTION

The resistance of common pathogens to standard antibiotic therapy is rapidly becoming a major problem throughout the world. The resistance of multi drug-resistant gram positive bacteria is increasing and infections caused by *Eshcereshia coli*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enter'ococci* and *Pneumococci* are particularly problematic. There is a real perceived need for the discovery of new compounds endowed with antibacterial property.

As a result, there is an urge for a new antimicrobial for this critical situation, such as increased prevalence of multidrug-resistant bacteria and the emergence of deadly infectious diseases.

In recent years, substituted 2-oxo-1,2,3,4-tetrahydropyrimidines received significant attention owing to their diverse range of biological properties such as calcium channel modulation, 1-adrenoreceptor selective antagonism, HIV gp120-CD_4 inhibition, antiviral, anticancer with mitotic kinesin inhibition, inhibition of Walker carcinosarcoma, as a oral antihypertensive, blood platelet aggregation inhibition, useful for the treatment of benign prostatic hyperplasia, antiinflammatory, antifungal and antibacterial. The presence of several interacting functional groups in these compounds also determines their great synthetic potential[1].

Pyrimidine is the most important member of all the diazines as this ring system occurs widely in living organisms. Purines, uric acid, alkoxan, barbituric acid and a majority of antimalarials and antibacterials also contain the pyrimidine ring.

Pyrimidine was first isolated by Gabriel and colman in 1899. Since pyrimidine is symmetrical about the line passing C-2 and C-5 the positions C-4 and C-6 are equivalent and so are N-1 and N-3. When a hydroxyl or amino group is present at the 2,4,6-position then they are tautomeric with oxo and imino, respectively[2,3].

In the present paper, the synthesis and antibacterial screening to investigate the relationship between the various physicochemical parameters and antibacterial activity of synthesized 3-formyl derivatives of 5-acyl-6-methyl-4-substituted-2-thio-1,2,3,4-tetrahydropyrimidines have been described.

EXPERIMENTAL

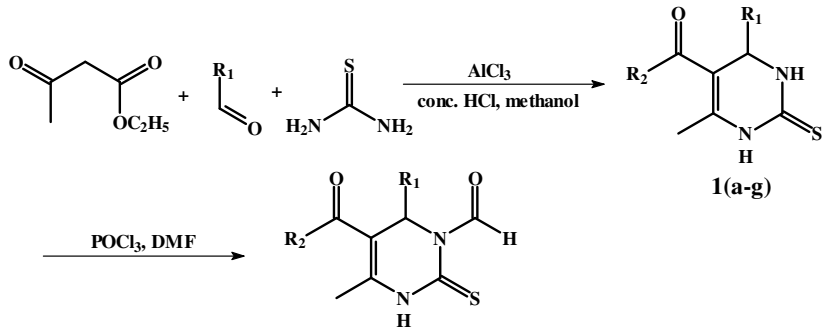
Melting points of the synthesized compounds were determined in open capillary tubes and are therefore uncorrected. The structures of the title compounds were established on the basis of elemental analysis and spectral data. The IR spectra were recorded on FTIR 4100 spectrophotometer. ^{1}H NMR spectra were recorded on Varian-NMR 400 MHz spectrometer using $CDCl_3$/DMSO-d_6 as solvent with TMS as an internal standard. Purity of the synthesized compounds was checked by silica gel-G plate using hexane and ethyl acetate as solvent system.

General procedure for the synthesis of 5-acyl-6-methyl-4-substituted-2-thio-1,2,3,4-tetrahydro pyrimidines (1a-g): These compounds were synthesized by the cyclocondensation reaction between corresponding aldehyde, ethylacetoacetate and thiourea. The mixture of appropriate aldehyde (0.02 mol), ethyl acetoacetate (0.02 mol), thiourea (0.03 mol), aluminium chloride (0.01 mol), conc. hydrochloric acid (2 drops) in methanol were refluxed for 4 h. The solid thus separated on cooling was filtered, washed with cold methanol, dried and recrystallized from methanol.

General procedure for the synthesis of 3-formyl derivatives of 5-acyl-6-methyl-4-substituted-2-thio-1,2,3,4-tetrahydropyrimidines (2a-g): To a suspension of respective 5-acyl-6-methyl-4-substituted-2-thio-1,2,3,4-tetrahydro pyrimidines (0.02 mol) in 20 mL of dry dimethyl formamide, phosphorous oxychloride (0.02 mol) was added in ice-bath. The resulting solution was heated at 70 ºC and kept for 40 min and then was poured into 150 mL of ice-water to yield the solid product. The solid product thus separated was filtered, washed with cold water, dried and recrystallized from ethanol (**Scheme-I**).

Ethyl 3-formyl-6-methyl-2-thio-4-phenyl-1, 2, 3, 4-tetrahydro pyrimidine 5-carboxylate (2a): Yield 65 % m.p.: 232 ºC. IR (KBr, ν_{max}, cm^{-1}): 1735 (C=O), 1275 (C-O), 1125 (C=S), 2850 (CH_3), 3350 (NH), 1130 (C-N), 1495 (aromatic ring) ^{1}H NMR 1.11 (t, 3H, ethyl CH_3), 2.3-2.5 (s, 3H, C6-CH_3), 4.0 (q, 2H, OCH_2), 5.1 (s, 1H, methine), 7.2-7.3 (m, 5Hph), 10.3 (s, 1H, formyl CH) 9.7 (s, 1H, NH).

Ethyl-3-formyl 4-(3-methoxy-4-hydroxyphenyl)-6-methyl-2-thio1,2,3,4-tetrahydro pyrimidine-5-carboxylate (2b): Yield 71 % m.p.: 236 ºC. IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O), 1267 (C-O), 1115 (C=S), 2832 (CH_3), 3310 (NH), 1130 (C-N), 1485 (aromatic ring). ^{1}H NMR 0.11 (t, 3H, ethyl CH_3, 2.5 (s, 3H, C_6-$CH_3)_4$, (q, 2H, OCH_2), 5.1 (s, 1H, methine), 6.7 (m, 3Hph), 9.54 (s, 1H, formyl CH), 8.9 (s, 1H, NH). 3.8 (s, 3H, CH_3O), 10.2 (s, 1H, OH).



Scheme-I

Ethyl 3-formyl-4-(4-methoxy phenyl)-6-methyl-2-thio1,2,3,4-tetrahydro pyrimidine 5-carboxylate (2c): Yield 67 %, m.p.: 146 ºC. IR (KBr, ν_{max}, cm^{-1}): 1737 (C=O), 1315 (C-O), 1057 (C=S), 2875 (CH_3), 3330 (NH), 1250 (C-N), 1475 (aromatic ring). ^{1}H NMR 1.11 (t, 3H, ethyl CH_3), 2.3-2.5 (s, 3H, C_6-CH_3), 4 (q, 2H, OCH_2), 5.1 (s, 1H, methine), (6.8, 6.9, 7.1, 7.1) (m, 4Hph), 10.2 (s, 1H, formyl CH), 9.5 (s, 1H, NH), 3.7, 3.8, 4.0 (s, 3H, CH_3O).

Ethyl-4-[4-(dimethylamino)phenyl]-3-formyl-6-methyl-2-thio-1,2,3,4-tetrahydro pyrimidine 5-carboxylate (2d): Yield 28, m.p.: 230 ºC. IR ((KBr, ν_{max}, cm^{-1}): 1700 (C=O), 1300 (C-O), 1050 (C=S), 2845 (CH_3), 3342 (NH), 1200 (C-N) 1490 (aromatic ring). ^{1}H NMR 1.11 (t, 3H, ethyl CH_3), 2.3-2.5 (s, 3H, C_6-CH_3), 4 (q, 2H, OCH_2), 5.2 (s, 1H, methine), 7.3, 7.6 (m, 4Hph), 10.3 (s, 1H, formyl CH), 9.6 (s, 1H, NH), 3.0 (s, 6H, H_3C-N-CH_3).

Ethyl-4-[4-methylphenyl]-3-formyl-6-methyl-2-thio-1,2,3,4-tetrahydro pyrimidine 5-carboxylate (2e): Yield 86 %, m.p.: 164 ºC. IR (KBr, ν_{max}, cm^{-1}): 1810 (C=O), 1285 (C-O), 1112 (C=S), 2832 (CH_3), 3335 (NH), 1315 (C-N), 1482 (aromatic ring). ^{1}H NMR 1.1 (t, 3H, ethyl CH_3), 2.3 (S, 6H, $C_6H_5CH_3$, CH_3-C=C), 4.0 (q, 2H, OCH_2), 5.3 (S, 1H, methine), 7.1 (m, 4H, phenyl), 8.2 (s, 1H, formyl CH), 7.5 (S, 1H, NH).

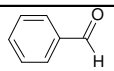
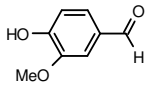
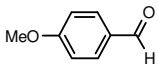
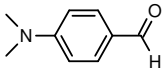
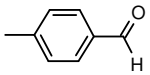
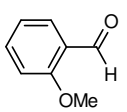
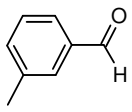
Ethyl-4-[2-methoxyphenyl]-3-formyl-6-methyl-2-thio-1,2,3,4-tetrahydro pyrimidine 5-carboxylate (2f): Yield 67 %, m.p.: 186 ºC. IR (KBr, ν_{max}, cm^{-1}): 1800 (C=O), 1257 (C-O), 1035 (C=S), 2775 (CH_3), 3400 (NH), 1190 (C-N), 1475 (aromatic ring). ^{1}H NMR 1.0 (t, 3H, ethyl CH_3), 2.4 (s, 3H, C_6-CH_3), 4.0 (q, 2H, OCH_2), 5.7 (s, 1H, methine), 3.8 (s, 3H, CH_3O), 7.9 (s, 1H, formyl CH), 7.3 (s, 1H, NH), 6.8, 7.0, 7.2 (m, 4H, phenyl).

Ethyl-4-[3-methylphenyl]-3-formyl-6-methyl-2-thio-1,2,3,4-tetrahydro pyrimidine 5-carboxylate (2g): Yield 62 %, m.p.: 194 ºC. IR (KBr, ν_{max}, cm^{-1}): 1690 (C=O), 1225 (C-O), 1046 (C=S), 2835 (CH_3), 3349 (NH), 1160 (C-N) 1469 (aromatic ring). ^{1}H NMR 1.1 (t, 3H, ethyl CH_3), 2.3 (s, 6H, m-tolyl, allyl), 4.1 (q, 2H, OCH_2), 5.3 (s, 1H, methine), 7.0, 7.12, 7.2, 7.2 (m, 4H, phenyl), 8.2 (s, 1H, formyl CH), 7.5 (s, 1H, NH).

Antibacterial activity: Antibacterial activity of these seven compounds was tested *in vitro* against *Staphylococcus aureus* (NCIM-5021), *Escherichia coli* (NCIM-2545), *Staphylococcus epidermidis* (NCIM-2493) and *Bacillus subtilis* (NCIM-2545) by the cup-plate agar diffusion method, using Mueller-Hinton agar medium. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone were done as per the standard procedure. Each test compound was dissolved in dimethyl sulfoxide. Benzyl penicillin was employed as reference standards (1000 µg/mL) to compare results. All the compounds were tested at a concentration of 0.05 mL (50 µg) and 0.1 mL (100 µg) level and DMSO as a control did not show any inhibition. The cups each of 8 mm diameter were made by scooping out medium with a sterilized cork borer from a petridish which was inoculated with the organisms. The solutions of each test compound, control and reference standard (0.05 and 0.10 mL) were added separately in the cups and petridishes were subsequently incubated at 37 ± 1 ºC for 24 h for antibacterial activity. Zone of inhibition produced by each compound was measured in mm.

Antfungal activity: Same cup plate method using PDA (potato dextrose agar) medium was employed to study the antifungal activity of substituted pyrimidines **2a-g** against *Aspergillus niger* and *Penicillium chrysogenum*. Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 mL). Fluconazole was employed as reference standard (1000 µg/mL) to compare the results.

TABLE-1
CHARACTERISATION DATA OF THE TITLE COMPOUNDS (**2a-g**)

Comp.	Aldehyde (R_1)	m.f.	Yield (%)	m.p. (ºC)	Analysis found (calcd) (%)		
					C	H	N
2a		$C_{15}H_{16}N_2O_3S$	65	232	59.21	5.20	9.20
2b		$C_{16}H_{18}N_2O_5S$	71	236	54.80	5.10	8.00
2c		$C_{16}H_{18}N_2O_4S$	67	146	57.40	5.30	8.30
2d		$C_{17}H_{21}N_3O_3S$	28	230	58.78	6.00	12.10
2e		$C_{16}H_{18}N_2O_3S$	86	164	60.37	5.66	8.80
2f		$C_{16}H_{18}N_2O_4S$	67	186	57.48	5.30	8.30
2g		$C_{16}H_{18}N_2O_3S$	62	194	60.37	5.66	8.80

RESULTS AND DISCUSSION

The purity and homogeneity of all the title compounds were confirmed by their sharp melting points and TLC. In all cases, these compounds were obtained in solid state and the yields varied from 40-80 %. The synthesized compounds were subjected to physico-chemical and elemental analysis. The structures of these compounds were confirmed by analytical data (Table-1), IR and ^{1}H NMR spectral data. Anti-microbial activity data suggested that 3-formyl-2-thio-1,2,3,4-tetrahydropyrimidine analogues have strong antibacterial activity against *Staphylococcus aureus*, *Eshcereshia coli*, *Bacillus subtilis* and *Staphylococcus epidermidis* (Table-2).

TABLE-2
ANTIBACTERIAL ACTIVITY DATA OF 3-FORMYL-2-THIO-1,2,3,4-TETRAHYDROPYRIMIDINE ANALOGUES (**2a-g**)

Organism	Compounds															
	2a		**2b**		**2c**		**2d**		**2e**		**2f**		**2g**		Standard	
	0.05 mL	0.10 mL	0.05 mL	0.10 mL	0.05 mL	0.10 mL	0.05 mL	0.10 mL	0.05 mL	0.10 mL	0.05 mL	0.10 mL	0.05 mL	0.10 mL	0.05 mL	0.10 mL
E. coli	11	13	18	19	15	17	17	19	16	18	15	18	17	18	31	32
B. subtilis	14	16	15	18	14	16	15	17	17	19	17	18	15	17	28	33
S. epidermidis	16	18	18	21	12	14	12	16	12	15	12	16	25	28	25	27
S. aureus	13	15	14	18	10	13	20	21	17	18	19	12	13	15	28	31

Aldehydes with electron withdrawing groups (**2b**) and electron releasing groups (**2d, 2g**) have much better antibacterial activity than the other derivatives. Aldehydes with electron withdrawing groups (**2c**, **2f**) and electron releasing groups (**2e**) have much better antifungal activity (Table-3).

TABLE-3
ANTIFUNGAL ACTIVITY OF SUBSTITUTED 3-FORMYL-2-THIO-1,2,3,4-TETRAHYDROPYRIMIDINE (**2a-g**)

Organism	Compounds															
	2a		**2b**		**2c**		**2d**		**2e**		**2f**		**2g**		Standard	
	0.05 mL	0.10 mL	0.05 mL	0.10 mL	0.05 mL	0.10 mL	0.05 mL	0.10 mL	0.05 mL	0.10 mL	0.05 mL	0.10 mL	0.05 mL	0.10 mL	0.05 mL	0.10 mL
A. niger	12	17	11	14	16	23	10	14	11	28	12	18	12	14	24	28
P. chrysogenum	13	23	12	28	18	28	13	22	12	14	13	16	11	10	22	27

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