Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2017, Volume 5, Issue 5
ISSN: 2321–3647(online)

Synthesis and Evaluation of 3-Formyl -2-Thio - 1, 2, 3, 4 - Tetrahydropyrimidine Analogues for their Antimicrobial Activity

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ABSTRACT

Pyrimidine is a synthetic heterocyclic aromatic organic compound similar to pyridine and benzene, with two nitrogen atoms at positions 1 and 3 of the six membered ring. It shows isomerisation with two other forms of diazine. Aldehydes undergo cyclocondensation reaction with thiourea and ethyl acetoacetate in presence of Aluminium chloride and Conc. HCl in methanol. On cooling the solid separated was filtered and was washed with cold methanol, dried and recrystallized from methanol. Various substituted pyrimidines were synthesized using different aldehydes. Purity and the homogeneity of all the title compounds were confirmed by their sharp melting points and TLC. The newly synthesized compounds were subjected to physico-chemical and elemental analysis. The structure of all the newly synthesized compounds was confirmed by IR, ^{1}H NMR and Mass Spectral data. The data of antibacterial activity against *S. aureus, E. coli, B. subtilis* and *S. epidermidis* was tested and Antifungal activity against *A. niger, P. chrysogenum and A. flavus* and zone of inhibition was recorded. Aldehydes with electron releasing groups (IId, IIg) have shown much better antibacterial activity and compounds IIc and IIf have shown better anti fungal activity than the other derivatives.

Keywords: Pyrimidine, Thiourea, Ethyl acetoacetate and Tetrahydropyrimidines.

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Received 19 April 2017, Accepted 25 April 2017

Please cite this article as: Lingala S *et al.*, Synthesis and Evaluation of 3-Formyl -2-Thio - 1, 2, 3, 4 - Tetrahydropyrimidine Analogues for their Antimicrobial Activity. American Journal of Pharmacy & Health Research 2017.

INTRODUCTION

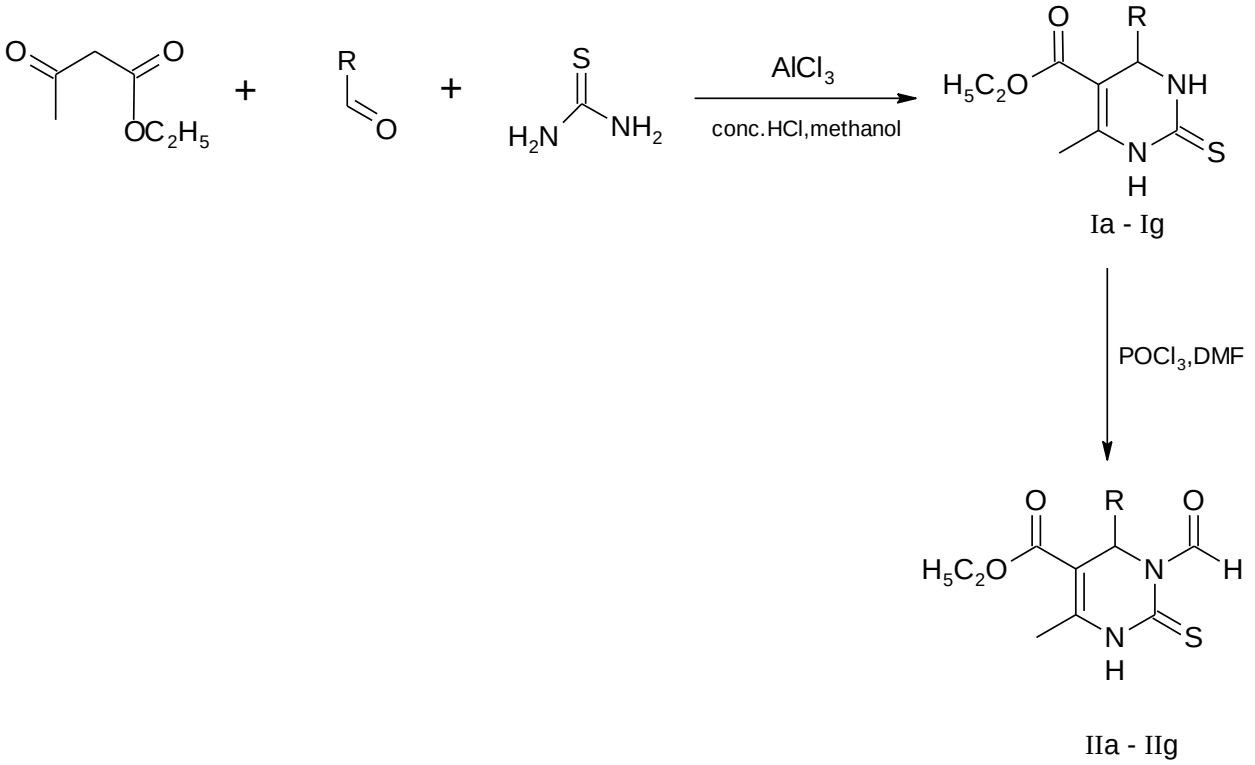
Pyrimidine is a synthetic heterocyclic aromatic organic compound similar to pyridine and benzene, with two nitrogen atoms at positions 1 and 3 of the six membered ring. It shows isomerisation with two other forms of diazine[1]. Three nucleobases found in nucleic acids (cytosine, thymine and uracil) are pyrimidine derivatives. Pyrimidine shows many common properties with pyridine as the number of the nitrogen atoms increases in the ring the pi electrons become less energetic and the electrophilic aromatic substitution gets very difficult while the nucleophilic aromatic substitution becomes easier[2,3]. The SAR studies give insights into the molecular properties causing receptor affinity and selectivity. The promising nature of the compounds may be attributed to the substitutions at the hydrophobic domain. These compounds had electron withdrawing and donating groups at the ortho, meta & para positions of the hydrophobic aryl ring[4,5]. A literature survey on pyrimidine derivatives reveals that these compounds possess varied biological actions like antimicrobial, antibacterial, anthelmintic, antiviral and anti-cancer activities. We have made an attempt in the present study towards the synthesis of different aldehyde derivatives of pyrimidine and evaluate how the molecule will influence the antimicrobial activity[6,7,8].

MATERIALS AND METHOD

The solvents and chemicals used for the experimental work have been procured from S.D. Fine Chemicals, E. Merck, India and Qualigens, India. Silica gel G used for the chromatography (TLC) was procured from S.D. Fine Chemicals, India. Melting points of the newly synthesized compounds were determined in an open glass capillary using a Kjeldahl flask containing liquid paraffin and are uncorrected. The proton magnetic resonance spectra (1H NMR) were recorded on Bruker 300 MHz instrument (Bruker, Germany) in DMSO/CDCl3 using TMS as internal standard. The infrared spectra of compounds were recorded in KBr on a FTIR- -8400S, Fourier Transform (Shimadzu), Japan infrared spectrophotometer. Mass spectra were recorded on LC-MS/MS (API-4000 TM), Applied BioSystems, MDS SCIEX (Canada).

Scheme of the experiment:

Aldehydes undergo condensation reactions with thiourea and ethyl acetoacetate in presence of Aluminium chloride and Conc. HCl in methanol. Various substituted pyrimidines were synthsized using different aldehydes. These Substituted pyrimidines were formylated using dimethylformamide and phosphorous oxychloride.



SCHEME - I

General procedure for the synthesis of 5-acyl-6-methyl-4-substituted-2- thio-1,2,3,4-tetrahydropyrimidines (Ia-Ig)

The new compounds are synthesized by an already reported cyclocondensation reaction between aldehyde, thiourea and acetoacetate. A mixture of appropriate aldehyde (benzaldehyde) (0.02 mol), ethyl acetoacetate (0.02mol), thiourea (0.03mol), aluminium chloride (0.01mol), Con. Hydrochloric acid (2 drops) in methanol were refluxed for 4 hrs [9]. The solid thus separated on cooling was filtered, washed with cold methanol, dried and recrystallized from methanol. M.P : 210 - 212°C, Yield : 76 %.

IR (KBr) (cm^{-1}) : 1748 (C=O),1230 (C-O),1026 (C=S), 3280 (-NH-), 1470 (Aromatic ring)

^{1}H NMR (DMSOd_6): δ ppm : 1.18(3H,t,ethyl CH3 at C-5), 2.1-2.4(3H, s,C6-CH3), 4.3(2H,q,-OCH2), 5.4(1H,s,-CH=), 7.4-7.8(5H,m,Ar-CH=), 10.1(1H,s,OCH-), 9.5(1H,s,-NH-), 9.3(1H,s,-NH-). **EI-MS: m/z = 276 (M^+).**

General procedure for the preparation of 3-formyl derivatives of 5-acyl-6-methyl-4-substituted-2-thio-1,2,3,4- tetrahydropyrimidines (IIa-IIg)

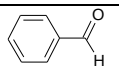
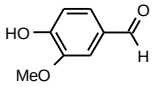
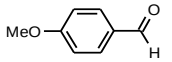
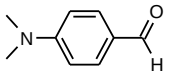
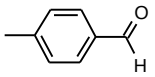
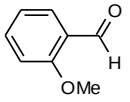
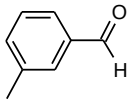
To the suspension of the above synthesized compound 5-acyl-6-methyl-4-substituted-2-thio-1,2,3,4-tetrahydropyrimidines (0.02 mol) in 20 ml of dry dimethylformamide, phosphorous oxychloride (0.02 mol) was added in a ice bath. The resulting mixture was heated at 70^{o}C and

kept there for 40 minutes and then was poured into 150 ml of ice-water to yield the solid product. The solid product thus separated was filtered and washed with cold water. It was then dried and recrystallized from ethanol[10]. The physical data of all the newly prepared compounds is given in Table-1. M.P : 230 – 232°C, Yield : 73%.

IR (KBr)(cm^{-1}) : 1735 (C=O),1210 (C-O),1050 (C=S), 3300 (-NH-), 1495 (Aromatic ring)

^{1}H NMR (DMSOd_6): δ ppm : 1.11(3H,t,ethyl CH3 at C-5), 2.3-2.5(3H, s,C6-CH3), 4.0(2H,q,-OCH2), 5.1(1H,s,-CH=), 7.2-7.3(5H,m,Ar-CH=), 10.3(1H,s,OCH-), 9.7(1H,s,-NH-), 8.2(1H,s,-CHO). **EI-MS: m/z = 304 (M^+).**

Table 1: Physical data of the title compounds (IIa - IIg)

Sl.No.	Compound	Substituents (R)	Molecular Formula	Molecular Weight	Melting Point °C	Yield %
1	IIa		$C_{15}H_{16}N_2O_3S$	281	232-234	65
2	IIb		$C_{16}H_{18}N_2O_5S$	313	236-238	70
3	IIc		$C_{16}H_{18}N_2O_4S$	311	220-222	65
4	IId		$C_{17}H_{21}N_3O_3S$	325	210-212	70
5	IIe		$C_{17}H_{18}N_6O$	322	224-226	70
6	IIf		$C_{15}H_{13}N_5O_2$	295	218-220	60
7	IIg		$C_{15}H_{12}N_5OCl$	313	230-232	75

Anti Bacterial Activity

The antibacterial activity of all the newly prepared compounds was tested *in vitro* against *S. aureus* (NCIM-5021), *E. coli* (NCIM-2545), *S. epidermidis* (NCIM-2493) and *B. subtilis* (NCIM-2655) by the cup-plate agar diffusion method, using Muller-Hinton agar medium[11]. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone were done as per the standard procedure[12]. Each test compound was dissolved in dimethyl sulfoxide. Benzyl penicillin was employed as reference standard (1000µg/ml) to compare the results. All the compounds were tested at a concentration of 0.05ml (50µg) and 0.1ml (100µg) level and DMSO as a control which did not show any inhibition. The cups each of 8mm 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 ml and 0.1ml) were added separately in the cups and petridishes were subsequently incubated at 37ºC±1ºC for 24 hours for antibacterial activity[13]. Zone of inhibition produced by each compound was measured in mm and the results are presented in the Table -2. All the newly synthesized compounds have shown antibacterial activity.

Table 2: Antibacterial activity of synthesized derivatives (IIa – IIg)

Compound	Zone of Inhibition (mm)							
	E. coli		***B. subtilis***		***S. epidermidis***		***S. aureus***	
	0.05ml	**0.1ml**	**0.05ml**	**0.1ml**	**0.05ml**	**0.1ml**	**0.05ml**	**0.1ml**
IIa	11	13	14	16	16	18	13	15
IIb	16	19	15	18	18	21	14	18
IIc	15	17	14	16	12	14	10	13
IId	17	20	15	18	12	16	20	21
IIe	16	18	17	19	12	15	17	18
IIf	15	18	17	18	12	16	12	16
IIg	17	18	15	17	23	26	13	15
Negative Ctrl.	--	--	--	--	--	--	--	--
Std	31	32	28	33	25	27	28	31

--No activity, Negative Control - DMSO

Anti Fungal Activity

Cup plate method using PDA (potato dextrose agar) medium was employed to study the antifungal activity of substituted pyrimidines IIa - IIg against *A. nige*, *P. chrysogenum* and *A. flavus*. Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5ml). Fluconazole was employed as reference standard (1000µg/ml) to compare the results. All the compounds were tested at a concentration of 0.05ml (50µg) and 0.1ml (100µg) level and DMSO as a control which did not show any inhibition. The cups each of 8mm diameter were made by scooping out medium with a sterilized cork borer from a petridish which was inoculated with the organisms[14,15]. The solutions of each test compound, control and reference standard (0.05 ml and 0.1ml) were added separately into the cups. The petridishes were subsequently incubated at 37ºC±1ºC for 24 hrs for antifungal activity. The Zone of inhibition produced by each compound was measured in mm and the results are presented in the Table -3

Table 3: Antifungal activity of synthesized derivatives (IIa – IIg)

Compound	Zone of Inhibition (mm)					
	A. niger		*P. chrysogenum*		*A. flavus*	
	0.05ml	0.1ml	0.05ml	0.1ml	0.05ml	0.1ml
IIa	12	17	11	21	10	12
IIb	11	14	14	26	8	10
IIc	16	23	18	28	9	12
IId	10	14	13	22	7	10
IIe	13	21	12	14	9	12
IIf	12	18	13	16	10	13
IIg	11	14	11	10	7	9
Negative Ctrl.	--	--	--	--	--	--
Std	24	28	22	27	22	26

--No activity, Negative Control - DMSO

RESULTS AND DISCUSSION

The purity and homogeneity of all the newly synthesized compounds were confirmed by their sharp melting points and TLC. In all the cases, the compounds were obtained in solid state and the yields varied from minimum 40% to a maximum of 84%.

The structures of these compounds were confirmed by C, H, and N analytical data, IR and ^{1}H NMR and Mass spectral data. From the antibacterial screening it was observed that all the newly synthesized compounds exhibited activity against all the organisms employed as indicated in Table -2. The compound **IId** showed maximum zone of inhibition (20 & 21 mm) against *S. aureus* and zone of inhibition (17 & 20 mm) against *E. coli.* Compounds **IIg** and **IIb** have shown good antibacterial activity against *S. epidermidis* with zone of inhibition of 23 & 26 and 18 & 21mm followed by compound **IIe** with zone of inhibition of 17 & 19 mm on *B. subtilis.*The compound **IIc** has shown least activity on *S. aureus* with zone of inhibition of 10 & 13 mm.

The antifungal activity of the compounds studied against *A. niger* and *P.chrysogenum* and *A. flavus* is shown in Table -3**.** Fluconazole was used as reference standard for inhibitory activity against fungi. The compound **IIc** showed maximum zone of inhibition (16 & 23 mm) against *A. niger* and zone of inhibition (18 & 28 mm) against *P. chrysogenum* followed by **IIe** with zone of inhibition of 13 & 21 mm on *A. niger*. Compound **IIb** has shown good activity on *P. chrysogenum* with zone of inhibition of 14 & 26 mm. all the synthesized compounds have shown least activity on *A. flavus.*

CONCLUSION

The study suggested that 3-formyl-2-thio-1,2,3,4-tetrahydropyrimidine analogues have antibacterial activity against *S. aureus, E. coli, B. subtilis and S. epidermidis.* Aldehydes with

electron releasing groups (IId, IIg) have much better antibacterial activity than the other derivatives. Aldehydes with electron withdrawing groups (IIc) had shown much better anti fungal activity.

ACKNOWLEDGEMENTS

The authors are thankful to the Management of SVS Group of Institutions, Warangal, for providing laboratory facilities and financial support.

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