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Synthesis & Antimicrobial Activity of Some Novel Pyrimidine-2,4(*1H,3H*)-diones

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Abstract: The aim of current research work was to synthesized some novel pyrimidine-2,4-diones by condensing various substituted amines with 3-substituted -6-chlorouracil. The structure of the synthesized compounds were characterized using physical & spectral data. Novel pyrimidine-2,4-(1H,3H)-diones were then screened for their antimicrobial profile using kirby bauer disc diffusion method. The anti-bacterial data reveals that compounds OBP01 and OBP02 had better activity against tested *B. subtilis* (Gram-positive) whereas OBP03 against *E. coli* (Gram-negative). OBP03 also found to have better anti-fungal activity than rest of the compounds when tested against *Aspergillus niger* & *Penicillium marneffei*. This study lead us to conclude that pyrimidine-2,4(1H,3H)-diones are potential agents to treat infection.

Keywords: Pyrimidine-2,4-diones; Antimicrobial Activity; Synthesis; Biological Activity.

INTRODUCTION

In spite of remarkable growth in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. There impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance.[1] During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics[2] has lead to the search of new antimicrobial agents with the goal to discover new chemical structures, which overcome the above disadvantage.[3]

Pyrimidine-2,4-dione is a 6-membered heterocyclic ring system having two nitrogen atoms at 1 & 3 position of the ring. In the 1967, a number of substituted pyrimidine-2,4-diones were synthesized and intensively studied as good reversible inhibitors of thymidine phosphorylase.[4] The small and simple pyrimidine nucleus is present in compounds involved in research aimed at evaluating new products that possess biological activities, such as anti-viral, anti-malarial agents, adenosine receptor ligands, anti-cancer agents, compounds targeting delayed-type hypersensivity and anti-convulsant agents. After that pyrimidine-2,4-dione derivatives have been studied extensively and found to have diverse chemical reactivity and broad spectrum of biological activity.[5-9]

EXPERIMENTAL SECTION

Melting points were measured in open capillary tubes on a Buchi 530 melting point apparatus and were uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on Shimadzu Affinity-1 (KBr) and Brucker Advance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. All exchangeable protons were confirmed by addition of D_2O.

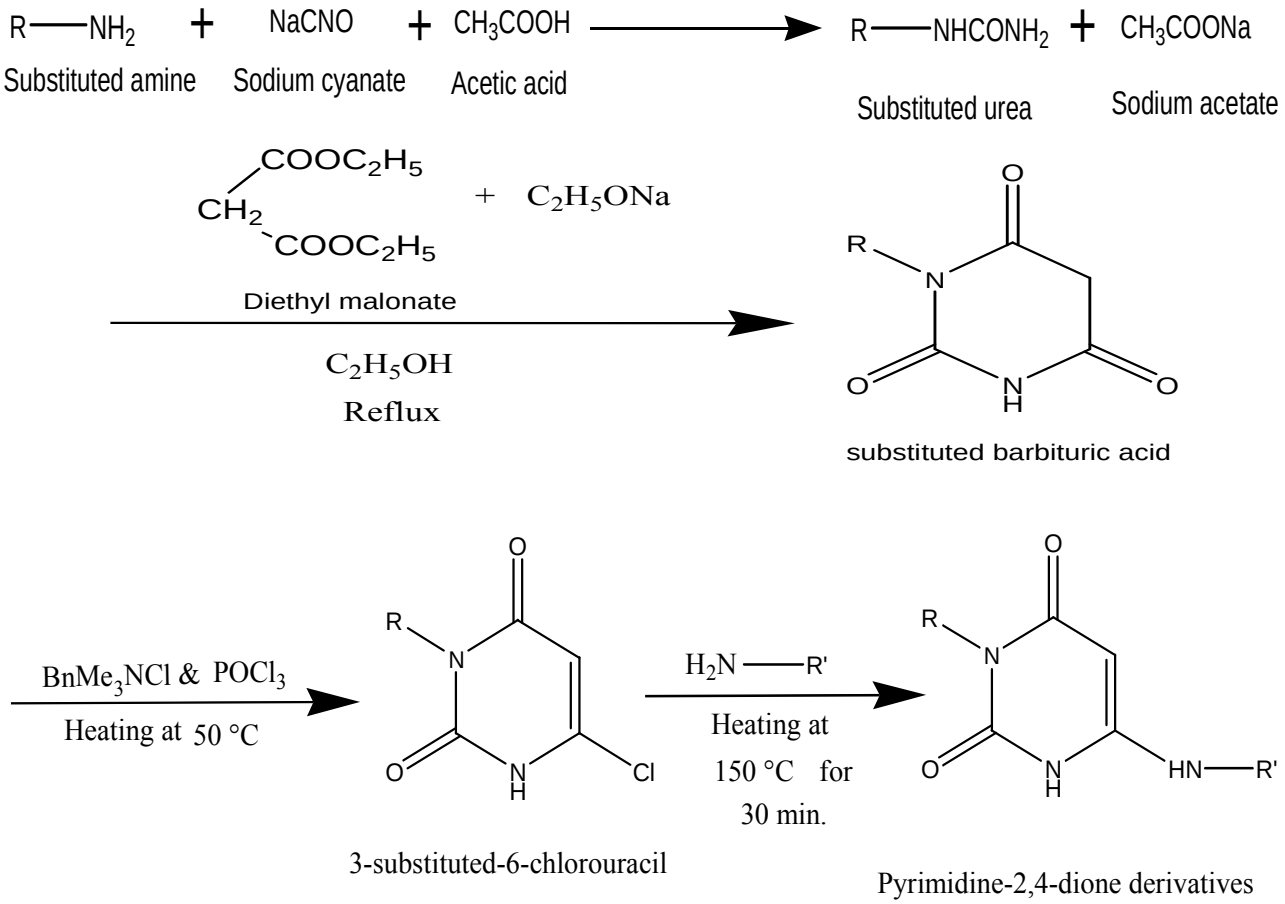

Figure 1: Synthetic Scheme for Synthesizing the Pyrimidine-2,4-dione Derivatives

General procedure for preparation of substituted urea[6, 11]

0.1mol p-Substituted aniline and 30ml conc. HCl were taken in a 500ml two neck RBF. To this mixture, a solution of 0.1mol sodium cyanate in 15ml water was added and refluxed for 6 hours. The water was evaporated. Then 30ml ethanol was added and the suspension was heated. The warm mixture was filtered and the filtered solid was washed with hot ethanol. Concentration and cooling of filtrate gave the product. Reaction was monitored by TLC and melting point was determined.

General procedure for preparation of substituted barbituric acid[6, 11, 12, 13]

0.05mol clean sodium metal was taken in a 500ml RBF and then 25ml absolute ethanol was added. When all the sodium had dissolved, 0.05mol substituted urea and 0.05mol diethyl malonate were added, the reaction mixture was refluxed for 7 hours at 110ºC. The mixture was allowed to cool and conc. aqueous HCl was added until the solution was acidic. The solvent was evaporated under reduced pressure using vacuum evaporator and 100ml ethanol was added to the residue with heating. The hot mixture was filtered and

the filtered solid was washed with ethanol. Concentration of filtrate gave the substituted barbituric acid. Reaction was monitored by TLC and melting point was determined.

General procedure for preparation of N_3-substituted-6-chlorouracil[6, 12, 13]

0.03mol Substituted barbituric acid was dissolved in 25ml acetone in a 250ml RBF, then 0.06mol benzyltrimethylammonium chloride and 7ml phosphoryl chloride were added and stirred for 10 minutes. Reaction mixture was heated at 50ºC on oil bath for 6 hours. The reaction mixture was cooled to room temperature and evaporated to dryness. The residue was quenched with ice chips and maintained at 0ºC for 10 minutes. The solution was extracted with ethyl acetate (4×15ml) and the combined organic extract was dried over sodium sulphate and solvent was evaporated. It was crystallized by ethanol. Reaction was monitored by TLC and melting point was determined.

General procedure for preparation of Pyrimidine-2,4-dione derivatives[6, 12, 13]

0.01mol N_3-Substituted-6-chlorouracil and 0.02mol p-substituted aniline were taken and stirred for 10 min. in a 250ml RBF. The reaction mixture was heated at 150°C on oil bath for 30 min. After cooling, the product was crystallized with ethanol. Reaction was monitored by TLC and melting point of product was determined.

RESULTS

Pyrimidine-2, 4(*1H, 3H*)-diones were synthesized according to synthetic scheme as shown in figure 1. First, the appropriate urea was reacted with diethyl malonate in the refluxing sodium ethoxide/ethanol to form the substituted barbituric acid as described in the literature. The *N*-alkylbarbituric acid was specifically chlorinated at C_6 by refluxing in $POCl_3$ in the presence of water. The final step employed the resulting substituted 6-chlorouracil and the appropriate aniline heated for 30 min. at 150°C on oil bath.[10] The structure of new compounds prepared during present investigation has been authentically established by their melting points, IR and ^{1}H-NMR studies.

S. No.	Compd. Code	Mol. Formula	Mol. Wt.	Melting Point ºC	% Yield	R_f Value
1.	OBP01	$C_{17}H_{15}N_3O_4$	325.32	245 - 247	64.51	0.75[a]
2.	OBP02	$C_{16}H_{13}N_3O_4$	311.29	238 -242	59.23	0.80[b]
3.	OBP03	$C_{19}H_{19}N_3O_3$	337.37	198 -200	58.06	0.78[b]
4.	OBP04	$C_{15}H_{12}N_4O_3$	296.28	244 -246	55.55	0.85[a]
5.	OBP05	$C_{20}H_{21}N_3O_3$	351.4	186 -188	53.1	0.65[a]
a = Dichloromethane : Methanol (9 : 1) and b = Dichloromethane : Methanol (8 : 2)						

Table 1: Physicochemical Data of Pyrimidine-2,4-dione Derivatives

3-(4-hydroxyphenyl)-6-(4-methoxyphenylamino)pyrimidine-2,4(1*H*, 3*H*)-dione (OBP01)

^{1}H-NMR (δ in ppm, $CDCl_3$), 3.2 (d, 3H, -OCH_3), 4.0 (s, 1H, C-NH), 4.8 (s, 1H, -CH=C(NH-)$_2$), 5.0 (s, 1H, -OH), 6.0 (s, 1H, -CONH-), 6.2-7.0 (s, 8H, 4×-CH=CH-), 8.0 (s, 1H, Ar-NH-). IR (cm^{-1}, KBr), 3263 (NH), 2924 (CH), 2529 (OH), 1720 (CO), 1612 (aromatic), 833 (para substituted benzene), 725 (mono substituted benzene).

3-(4-hydroxyphenyl)-6-(4-hydroxyphenylamino)pyrimidine-2,4(1*H*, 3*H*)-dione (OBP02)

^{1}H-NMR (δ in ppm, $CDCl_3$), 4.0 (s, 1H, C-NH), 4.8 (s, 1H, -CH=C(NH-)$_2$), 5.0 (s, 2H, 2×-OH), 6.0 (s, 1H, -CONH-), 6.2-7.0 (s, 8H, 4×-CH=CH-), 8.0 (s, 1H, Ar-NH-). IR (cm^{-1}, KBr), 3263 (NH), 2924 (CH), 2529 (OH), 1720 (CO), 1612 (aromatic), 825 (para substituted benzene), 725 (mono substituted benzene).

6-(3-ethyl-4-methylphenylamino)-3-(4-hydroxyphenyl)pyrimidine-2,4(1*H*, 3*H*)-dione (OBP03)

^{1}H-NMR (δ in ppm, $CDCl_3$), 1.2 (s, 3H, Ph-CH_2CH_3), 2.2 (s, 3H, Ph-CH_3), 2.6 (s, 2H, CH_2-Ph), 4.0 (s, 1H, C-NH), 4.8 (s, 1H, -CH=C(NH-)$_2$), 5.0 (s, 1H, -OH), 6.0 (s, 1H, -CONH-), 6.2-7.0 (s, 6H, 3×-CH=CH-), 8.0 (s, 1H, Ar-NH-). IR (cm^{-1}, KBr), 3263 (NH), 2924 (CH), 2529 (OH), 1720 (CO), 1612 (aromatic), 825 (para disubstituted benzene), 702 (mono substituted benzene).

3-(4-hydroxyphenyl)-6-(pyridine-2-ylamino)pyrimidine-2,4(1*H*, 3*H*)-dione (OBP04)

^{1}H-NMR (δ in ppm, $CDCl_3$), 4.0 (s, 1H, C-NH), 4.8 (s, 1H, -CH=C(NH-)$_2$), 5.0 (s, 1H, -OH), 6.0 (s, 1H, -CONH-), 6.2-7.0 (s, 6H, 3×-CH=CH-), 8.0 (s, 1H, Ar-NH-). IR (cm^{-1}, KBr), 3263 (NH), 2924 (CH), 2529 (OH), 1720 (CO), 1627 (C=N), 1612 (aromatic), 825 (para substituted benzene), 763 (mono substituted benzene).

6-(3-ethyl-4-methylphenylamino)-3-(4-methoxyphenyl)pyrimidine-2,4(1*H*, 3*H*)-dione (OBP05)

^{1}H-NMR (δ in ppm, $CDCl_3$), 1.2 (s, 3H, Ph-CH_2CH_3), 2.2 (s, 3H, Ph-CH_3), 2.6 (s, 2H, CH_2-Ph), 3.2 (d, 3H, -OCH_3), 4.0 (s, 1H, C-NH), 4.8 (s, 1H, -CH=C(NH-)$_2$), 6.0 (s, 1H, -CONH-), 6.2-7.0 (s, 6H, 3×-CH=CH-), 8.0 (s, 1H, Ar-NH-). IR (cm^{-1}, KBr), 3263 (NH), 2924 (CH), 2529 (OH), 1720 (CO), 1612 (aromatic), 833 (para disubstituted benzene), 725 (mono substituted benzene).

Anti-bacterial Activity

The antibacterial activity of newly synthesized pyrimidine-2,4-dione derivatives evaluated against gram positive bacteria *viz. Bacillus subtillis* and gram negative bacteria *viz. Escherichia coli*. The standard drug used is Ampicillin. In this method, Petri-plates were filled with liquefied agar medium to uniform thickness. After solidified of medium, plates were inoculated with test micro-organisms and then filter paper discs dipped in the test compounds solution in DMSO and standard drug solution in DMSO were placed in each quadrant of plate. These plates were incubated at 37±1ºC for 24 hrs. The drug will diffuse into the agar medium are prevent the growth of microbes and produce a clear zone of inhibition.

Table 2: Results of Antibacterial activity of Novel Pyrimidine-2,4-diones

S. No.	Compounds Code	Mean Zone of Inhibition in mm (50µg/ml)		Mean Zone of Inhibition in mm (100µg/ml)	
		B.subtillis	***E.coli***	***B.subtillis***	***E.coli***
1.	OBP01	09	08	20	17
2.	OBP02	10	06	23	16
3.	OBP03	05	10	12	18
4.	OBP04	07	06	17	15
5.	OBP05	07	08	16	17
11.	Ampicillin	12	13	28	26

Anti-fungal Activity

The antifungal activity of newly synthesized pyrimidine-2,4-dione derivatives evaluated against two organisms *aspergillus niger* and

penicillium marneffei. The standard drug used is Griseofulvin.

The stock culture of micro-organisms were aseptically inoculated in 50 ml of nutrient broth and incubated at 37±1ºC for 48 hrs.

Table 3: Results of Antifungal activity of Novel Pyrimidine-2,4-diones

S. No.	Compounds Code	Mean Zone of Inhibition in mm (50µg/ml)		Mean Zone of Inhibition in mm (100µg/ml)	
		A.niger	***P. marneffei***	***A.niger***	***P. marneffei***
1.	OBP01	06	05	15	13
2.	OBP02	07	06	14	16
3.	OBP03	07	08	16	18
4.	OBP04	05	06	13	17
5.	OBP05	07	06	15	15
6.	Griseofulvin	09	08	21	19

CONCLUSION

We have successfully synthesized a series of pyrimidine-2,4-dione derivatives and antibacterial and antifungal activity data of the prepared compounds showed that the pyrimidine-2,4-diones shown very good anti-bacterial activity against Gram-positive organism compared to those of the compounds shown against Gram-negative organism.

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