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

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ABSTRACT: In the present work, we had synthesized some novel pyrimidine-2,4-diones by condensing various substituted amines with 3-substituted -6-chlorouracil. The structure of the synthesized compounds was characterized using physical & spectral data. Novel pyrimidine-2,4-(1H,3H)-diones were then screened for their antimicrobial profile using Kirby Bauer Disc Diffusion(KBDD) method. The anti-bacterial data reveals that compounds OBP-08 and OBP-10 had better activity against tested gram-positive organism whereas OBP-06 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 may be the desired scaffold to generate lead anti-infective agents.

KEYWORDS: Pyrimidine-2,4-dione; Antimicrobial Activity; Synthesis; Bioactive Agents.

INTRODUCTION

Heterocyclic compounds containing five or six membered ring with one or more nitrogen atoms are always of great importance in the pharmaceutical sector as having the bio-isosteric factor.

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.[1,2] 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.[1,3-7]

Pyrimidine-2, 4-diones (uracils) are the inhibitors of the Gram-positive DNA polymerase IIIC. DNA polymerase IIIC is the polymerase required for the replication of chromosomal DNA in Gram-positive bacteria with low G + C content and thus represents a highly interesting target for therapy of some of the microorganisms.[8-10]

In continuation of our previous research work[1], we have synthesized some more novel analogs of pyrimidine-2,4-dione scaffold and evaluated their antimicrobial activity.

MATERIALS & METHODS

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.

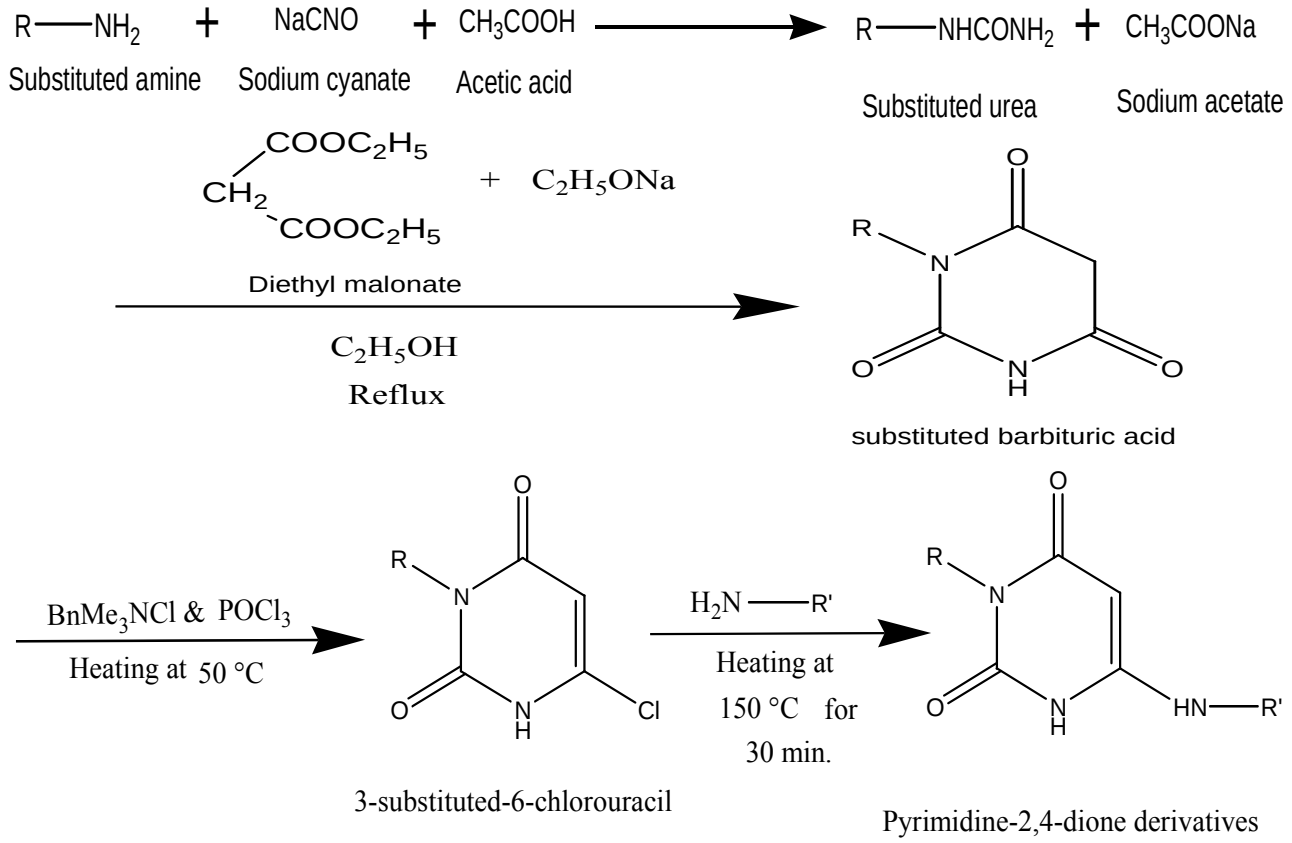

Figure 1 Synthetic route for derivatization of Pyrimidine-2,4-dione[Ref. 1]

The procedure adopted for the synthesis of novel pyrimidine-2,4-dione analogs were referred from references [1, 4, 11-13].

RESULTS & DISCUSSION

Pyrimidine-2, 4(*1H, 3H*)-diones were synthesized according to synthetic scheme as shown in Figure 1.

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

S. No.	Compd. Code	Mol. Formula	Mol. Wt.	Melting Point ºC	% Yield	R_f Value
1.	OBP-06	$C_{16}H_{14}N_4O_3$	310.31	208 -210	56.73	0.60[a]
2.	OBP-07	$C_{17}H_{12}N_4O_4S$	368.37	200 -202	52.94	0.90[a]
3.	OBP-08	$C_{18}H_{14}N_4O_4S$	382.39	170 -172	42.85	0.95[a]
4.	OBP-09	$C_{18}H_{14}N_4O_4S$	382.39	180 -182	45.71	0.85[a]
5.	OBP-10	$C_{19}H_{16}N_4O_4S$	396.42	220 -222	50.00	0.80[a]
a = Dichloromethane : Methanol (9 : 1) and b = Dichloromethane : Methanol (8 : 2)						

3-(4-methoxyphenyl)-6-(pyridine-2-ylamino)pyrimidine-2,4(1*H*, 3*H*)-dione (OBP-06)

^{1}H-NMR (δ in ppm, $CDCl_3$), 3.8 (s, 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, 8H, 4×-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), 779 (mono substituted benzene).

3-(4-hydroxyphenyl)-6-(6-hydroxybenzothiazol-2-ylamino)pyrimidine-2,4(1*H*, 3*H*)-dione (OBP-07)

^{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, 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), 779 (mono substituted benzene), 609 (C-S).

3-(4-methoxyphenyl)-6-(6-hydroxybenzothiazol-2-ylamino)pyrimidine-2,4(1*H*, 3*H*)-dione (OBP-08)

^{1}H-NMR (δ in ppm, $CDCl_3$), 3.8 (s, 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, 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), 856 (para substituted benzene), 779 (mono substituted benzene), 709 (C-S).

3-(4-hydroxyphenyl)-6-(6-methoxybenzothiazol-2-ylamino)pyrimidine-2,4(1*H*, 3*H*)-dione (OBP-09)

^{1}H-NMR (δ in ppm, $CDCl_3$), 3.8 (s, 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, 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), 856 (para substituted benzene), 785 (mono substituted benzene), 648 (C-S).

3-(4-methoxyphenyl)-6-(6-methoxybenzothiazol-2-ylamino)pyrimidine-2,4(1*H*, 3*H*)-dione (OBP-10)

^{1}H-NMR (δ in ppm, $CDCl_3$), 3.8 (s, 6H, 2×-OCH_3), 4.0 (s, 1H, C-NH), 4.8 (s, 1H, -CH=C(N‹)$_2$), 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), 1627 (C=N), 1612 (aromatic), 856 (para substituted benzene), 771 (mono substituted benzene), 694 (C-S).

Antibacterial 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.	OBP-06	08	06	15	14
2.	OBP-07	07	06	17	16
3.	OBP-08	08	06	19	14
4.	OBP-09	05	07	14	14
5.	OBP-10	09	08	18	15
6.	Ampicillin	12	13	28	26

Antifungal 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.	OBP06	08	05	18	14
2.	OBP07	08	07	17	16
3.	OBP08	06	07	14	15
4.	OBP09	07	05	16	13
5.	OBP10	07	06	17	17
6.	Griseofulvin	09	08	21	19

A series of pyrimidine-2,4-dione derivatives have been schematically designed and synthesized using traditional wt lab techniques. All the compounds were characterized using physical and spectral data. Pyrimidine-2,4-diones were then evaluated for their antimicrobial activity using bacterial(B. subtilis & E. coli) as well as fungal strains(A. niger & P. marneffei) and 50 µg/ml & 100 µg/ml concentration of test compounds. All the test compounds have good antibacterial activity out of which OBP-08 had shown maximum activity against B. subtilis whereas OBP-07 have significant activity against both the bacterial strains. All the test compounds have good antifungal activity out of which OBP-06 found to be having maximum activity against A. niger whereas OBP-10 found to have good activity against both the fungal strains.

CONCLUSION

In the present study, we designed and successfully synthesized five novel pyrimidine-2,4-dione analogs which have a significant antimicrobial activity against gram positive & gram negative bacteria as well fungal strains. This study could help us to tailor the structure to get the lead molecule as anti-infective agent.

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