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Synthesis and Biological evaluation of some novel 2-Hydroxy Pyrimidines

M. Mumtaz Mohammed Hussain[a*],

K. Ishwar Bhat[b]

B. C. Revanasiddappa[b],

D. R. Bharathi[c]

[a] Department of Pharmaceutical Chemistry, SJM College of Pharmacy Chitradurga, 577502, Karnataka, India.
[b] Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Nitte University, Mangalore - 575018, Karnataka, India
[c] Department of Pharmacology, SJM College of Pharmacy Chitradurga – 577502, Karnataka, India.

Corresponding Authors:
M. Mumtaz Mohammed Hussain
Lecturer, Dept. of Pharmaceutical Chemistry, SJM College of Pharmacy, JMIT Campus, Chitradurga – 577502, Karnataka, India
Email: mumtazmhussain@gmail.com

Abstract: A novel series of 2-hydroxy pyrimidines **(4a-l)** were synthesized via (E)-thienyl chalcones **(3a-l)**. (E)- thienyl chalcones were prepared by the reaction of thiophene-2-aldehyde **(1)** with various substituted acetophenones **(2)** in presence of NaOH in alcohol medium. Treatment of these chalcones **(3a-l)** with urea gave the title compounds 2-hydroxy Pyrimidines **(4a-l)**. The structures of the newly synthesized compounds were assigned on the basis of IR, ^{1}H-NMR, Mass spectral studies and elemental analysis. All the final synthesized compounds were evaluated for their *In-Vitro* antibacterial, antifungal and antitubercular activities. Some of the compounds have exhibited promising antibacterial, antifungal and antitubercular activities.

Keywords: Chalcones, 2-hydroxy pyrimidines, antibacterial activity, antifungal activity.

Introduction

Chalcones show impressive physiological properties and some of them posses wide range of activities such as antibacterial[1], antiviral[2], antitubercular[3] antifungal[4], anti-inflammatory[4] etc. chalcones act as one of the key intermediate for the synthesis of various heterocyclic compounds.

Pyrimidine derivatives are reported to possess antiviral[5], antileshmanial[6], antimicrobial[7], anti-inflammatory[7], anthelmentic[8] etc., Several important sulfa drugs like Sulfadiazine, Sulfamerazine and Sulfadimidine contains Pyrimidine moiety. A variety of natural products such as alkaloids also contain the pyrimidine ring system, these include hypoxanthine and xanthine, which occur in tea, and caffeine and theophylline (the constituents of tea leaves).

The key intermediate Chalcones **(3a-l)** have been synthesized by Claisen-Schmidt

condensation reaction by reacting thiophene-2-carbaldehyde and various substituted aromatic ketones in alcohol medium as per the reported procedure[9]. The resulted Chalcones **(3a-l)** undergoes selective cyclization with urea in presence of NaOH in alcohol medium to yield the title compounds 2-hydroxy Pyrimidine derivatives.

By considering the above facts and in search of small molecules as potential drug candidates, in the present study we have planned to synthesize a novel series of 2-hydroxy pyrimidines followed by their *In-vitro* antibacterial, antifungal and antitubercular evaluation. The reaction sequence leading to the formation of title compounds is depicted in **Scheme-01**

Experimental

Melting pints were recorded in open capillaries with electrical melting point apparatus and were uncorrected. IR spectra (KBr disks) were recorded using Bruker-α IR spectrophotometer. ^{1}H NMR is recorded in Bruker Avance (400 MHz) Spectrometer in $CDCl_3$ solution, with TMS as an internal standard reference. Mass spectra were recorded on a Micromass Q-TOF spectrometer. Elemental analysis was carried out using Vario Elementary Model CHN analyzer instrument. TLC was performed on silica gel coated plates for monitoring the reactions.

Scheme-01

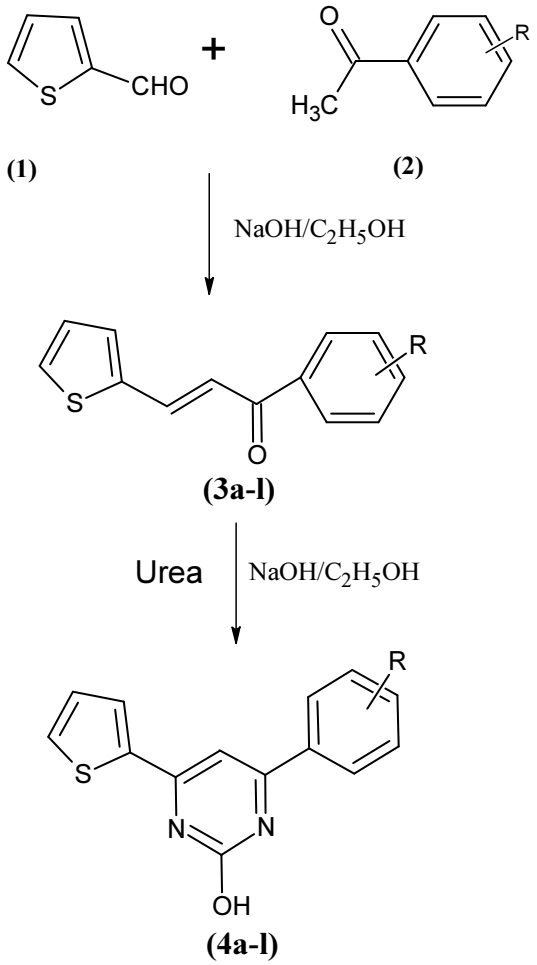

Fig. 1: Reaction scheme of synthesis of 2-hydroxy pyrimdines (4a-l)

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Synthesis of 2-hydroxy pyrimidines (4a-l)

A mixture of Chalcones **(3a-l)** (0.01 mol) and urea (0.01 mol) was dissolved in ethanol (30 ml) containing few drops of NaOH. The reaction mixture was refluxed on water bath at 70-80^{0}C gently for about 8-10 hrs, the progress of the reaction was monitored by TLC (benzene: chloroform 8:2). The hot reaction mixture was then filtered and allowed to cool. The resulting solid so obtained was filtered, washed several times with distilled water, dried and recrystallized from ethanol.The physical data of 2-hydroxy pyrimidines **(4a-l)** is given in table-1.

Table-1: Physical data of 2-Hydroxy Pyrimidines **(4a-l)**

Comp	R-$COCH_3$	M.W.	M.P (^{0}C)	Elemental analysis Calculated (Found)			Yield (%)
				C (%)	H (%)	N (%)	
4a	4-NO_2	299	65-67	56.18 (56.15)	3.03 (3.01)	14.04 (14.00)	55
4b	4-Cl	288	75-77	58.23 (58.18))	3.14 (3.09)	12.28 (12.23)	50
4c	4-CH_3	268	60-62	67.14 (67.05)	4.51 (4.46)	10.44 (10.39)	60
4d	4-NH_2	269	90-92	62.43 (62.40)	4.12 (4.07)	15.60 (15.20)	70
4e	H	254	55-57	66.12 (66.04)	3.96 (3.91)	11.02 (10.99)	50
4f	4-F	272	120-122	61.75 (61.66)	3.33 (3.29)	10.29 (10.27)	52
4g	3-NO_2	299	215-217	56.18 (56.12)	3.03 (3.00)	14.04(14.01)	55
4h	4-Br	333	190-192	50.46 (50.39)	2.72 (2.69)	8.41 (8.38)	70
4i	4-OCH_3	284	199-201	63.26 (63.21)	4.25 (4.21)	9.85 (9.81)	67
4j	4-OH	270	150-152	62.21 (62.17)	3.73 (3.68)	10.36 (10.33)	72
4k	1-Naphthyl	304	221-223	71.03 (70.98)	3.97 (3.93)	9.20 (9.17)	65
4l	2-Thiophene	260	183-185	55.36 (55.29)	3.10 (3.04)	10.76 (10.70)	60

M.W: Molecular Weight; M.P: Melting point in ^{0}C

4-(4-nitrophenyl)-6-(thiophen-2-yl) pyrimidin-2-ol (4a)

IR (cm^{-1}): 3451 (OH), 3100 (C-H), 1654 (C=N), 1581 (C=C), 1522 & 1343 (NO_2), 698 (C-S).**^{1}H-NMR (δ PPM):** 6.64-7.98 (m, 8H, Ar-H) 10.46 (s, 1H, OH).**Mass:** 300 (M+1)

4-(4-chlorophenyl)-6-(thiophen-2-yl) pyrimidin-2-ol (4b)

IR (cm^{-1}): 3442 (OH), 3105 (C-H), 1671 (C=N), 1588 (C=C), 825 (C-Cl), 700 (C-S).**^{1}H NMR (δ PPM):** 7.08-7.97 (m, 9H, 8H, Ar-H), 11.68 (s, 1H, OH).**Mass:** 289 (M+1)

4-(thiophen-2-yl)-6-(p-tolyl) pyrimidin-2-ol (4c)

IR (cm^{-1}): 3443 (OH), 3097 (C-H), 1658 (C=N), 1593 (C=C), 719 (C-S).**^{1}H NMR (δ PPM):** 2.43 (s, 3H, CH_3), 7.08-7.95 (m, 8H, Ar-H), 9.52 (s, 1H, OH).**Mass:** 269 (M+1)

4-(4-aminophenyl)-6-(thiophen-2-yl) pyrimidin-2-ol (4d)

IR (cm^{-1}): 3317 (OH), 3217 (NH), 3099 (C-H), 1634 (C=N), 1599 (C=C), 681 (C-S).**^{1}H NMR (δ PPM) :** 4.9 (s, 2H, NH_2). 6.64-7.87 (m, 8H, Ar-H), 9.88 (s, 1H, OH).**Mass:** 270 (M+1)

Biological evaluation

Antimicrobial Activity

The *In-Vitro* anti-microbial screening of the newly synthesized 2-hydroxy pyrimidines was carried out against Gram-positive organisms (*E. Fecalis and S. Aureus),*Gram-negative organisms *(K.pneumoneae and E. Coli)* and *fungi (C. albicans and A. niger*) by conventional tube dilution method[10] and compared with that of the standard drug Ciprofloxacin and Flucanazole respectively.

Dilutions of each drug were prepared with brain heart infusion broth (BHI) for MIC. A stock solution of drug with concentration 1000 µg/10µl

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was prepared in DMF. In the initial tube 20 µl of drug was added into the 380 µl of brain heart infusion broth. For dilutions 200 µl of brain heart infusion broth was added into the next 10 tubes separately. Then from the initial tube 200 µl was transferred to the first tube containing 200 µl of brain heart infusion broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200 µl was transferred to second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-10} dilution for each drug. From the last tube 200 µl of solution was discarded. From the maintained stock cultures of required organisms, 5 µl was taken and added into 2ml of brain heart infusion broth (Himedia). In each serially diluted tube 200 µl of above culture suspension was added. The tubes were incubated for 24 hrs and observed for turbidity. MIC of each drug was defined as the lowest concentration that produces no visible turbidity after incubation time.

In the antibacterial activity, compounds **4c, 4e, 4g** and **4h** have showed significant activity against gram positive organisms, and most of the synthesized compounds displayed moderate activity aginst *K.pneumoneae* and compounds **4a** and **4g** showed good activity aginst *E.Coli.* In the antifungal activity compounds **4c, 4d, 4k** and **4l** showed good activity against *C.albicans* and compounds **4a, 4c, 4d** and **4k** showed good activity against *A.fumigatus.* The antimicrobial data of the compounds **(4a-l)** is given in table-2.

Table-2: Antibacterial and Antifungal activity of 2-hydroxy Pyrimidines **(4a-l)**

Comp	Minimum Inhibitory Concentration in (µg)					
	E.Fecalis	*S. Aureus*	*K. Pneumoniae*	*E. coli*	*C. albicans*	*A. fumigatus*
4a	16.6	16.6	31.25	8.3	8.3	8.3
4b	8.3	31.25	31.25	16.6	16.6	125
4c	2	31.25	31.25	62.5	2.0	8.3
4d	8.3	16.6	31.25	16.6	4.15	8.3
4e	2	250	125	16.6	62.5	62.5
4f	16.6	16.6	62.5	16.6	62.5	31.25
4g	4.15	4.15	16.6	8.3	8.3	31.25
4h	31.25	4.15	62.5	62.5	R	8.3
4i	62.5	250	125	250	8.3	125
4j	62.5	250	250	250	16.6	125
4k	62.5	500	R	R	4.15	125
4l	62.5	250	250	125	4.15	250
Ciprofloxacin[a]	**1µg**	**2µg**	**1µg**	**2µg**	**-**	**-**
Fluconazole [a]	**-**	**-**	**-**	**-**	**16.6µg**	**8.3 µg**

* Resistant, [a]Standard drugs

Antitubercular activity

The antitubercular activity of the synthesized Pyrimidines **(4a-l)** were carried out by using Middle brook 7H9 agar medium against *M. tuberculosis* $H_{37}Rv$ strain by Microplate Alamar Blue Assay (MABA)[11] method. The Middle brook 7H9 agar medium containing different derivatives, standard drug as well as control was inoculated with *M. tuberculosis* $H_{37}Rv$ strain. The inoculated plates were incubated at 37° C for four weeks. At the end of four weeks they were checked for growth.

The new compounds **4a, 4d, 4e, 4f and 4g** have showed significant antitubercular activity with MIC ranging at 12.5 µg. INH was used as standard

drug for comparison purpose. The antitubercular data of the compounds **(4a-l)** is given in table-3.

Table-3: Antitubercular activity of 2-hydroxy Pyrimidines **(4a-l)**

Comp	MIC in µg
4a	12.5
4b	25
4c	25
4d	12.5
4e	12.5
4f	12.5
4g	12.5
4h	25
4i	50
4j	50
4k	50
4l	50
INH	**0.2**

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