

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF 2-(2-ARYLIDENEHAZINYL)-4-(3-CHLOROPHENYL)-6-HYDROXYPYRIMIDINE-5-CARBONITRILE

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ABSTRACT

A series of novel 2-(2-arylidenehydrazinyl)-4-(3-chlorophenyl)-6-hydroxypyrimidine-5-carbonitrile (4a-f) were synthesized from the reactions of 4-(3-chlorophenyl)-6-hydroxy-2-mercaptopyrimidine-5-carbonitrile. The newly synthesized compounds were characterized using by IR, ¹H-NMR, Mass spectroscopy and elemental analysis. In addition, the synthesized compounds were screened for their in vitro antibacterial and antifungal activities.

Keywords: Pyrimidine, antibacterial, antifungal activity.

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INTRODUCTION

Pyrimidine is a six-member organic heterocyclic compound consisting of two nitrogen atoms in the ring at 1 and 3 positions. Many pyrimidine derivatives exhibit a broad spectrum of biological activities such as antimicrobial,¹ anti-inflammatory,^{2, 3} antitumor,⁴⁻⁵ CXCR2 receptor antagonists,⁶ analgesic activities,⁷⁻⁸ antineoplastic agents,⁹ renin inhibitors,¹⁰ anticancer.¹¹

In view of applications of pyrimidine derivatives in the clinical field, we report the synthesis of a series of 2-(2-arylidenehydrazinyl)-4-(3-chlorophenyl)-6-hydroxypyrimidine - 5-carbonitrile (**4a-f**). Synthesis of target compounds has been done by condensation of various aromatic aldehydes with 4-(3-chlorophenyl)-2-hydrazinyl-6-hydroxypyrimidine-5-carbonitrile (**3**). The structures of the synthesized products were confirmed by the spectral (IR, ¹H NMR and Mass-4b and 4c) and elemental analysis.

EXPERIMENTAL

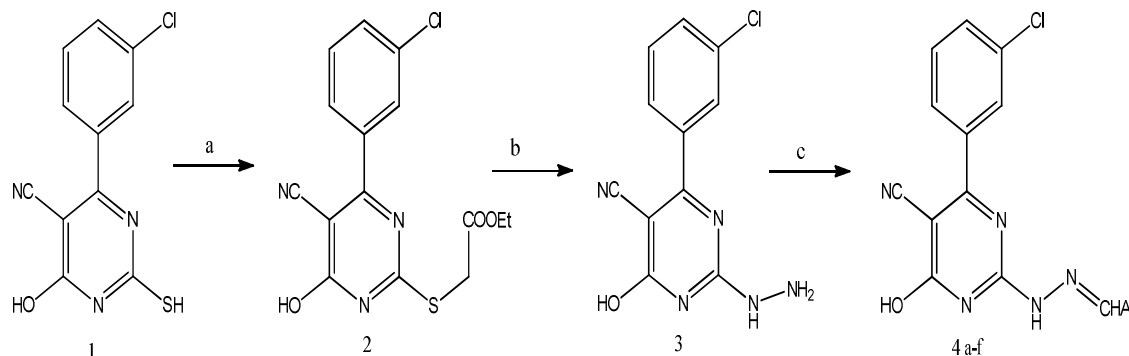
Melting points were measured in open capillary tubes and are uncorrected. The progress of the reaction was monitored by thin layer chromatography (TLC) on Merck silica gel 60 F₂₅₄ aluminum sheets and visualized by UV light. IR spectra were recorded on Shimadzu FTIR (KBr) – 408 spectrophotometers. The ¹H NMR spectra were recorded at 400 MHz in DMSO-d₆ using TMS as internal standard and are given in δ units. The LC-MS spectra were recorded on WATER, Q-TOF Micro mass. The synthesized compounds were named using ChemDraw Ultra software (v.12.0). Compound **1** was prepared from the reaction of 3-chlorobenzaldehyde, ethyl cyanoacetate, and thiourea as per reported method.^{4, 12} Further compounds **1** was converted into **3** as described in literature.^{13, 14}

Synthesis of ethyl 2-(4-(3-chlorophenyl)-5-cyano-6-hydroxypyrimidin-2-ylthio) acetate (**2**)

Compound **1** (1mmol) was dissolved in 15 ml DMF. To this solution ethyl chloroacetate (1mmol) was added dropwise and a catalytic amount of triethylamine. The reaction mixture was stirred at for 7h until the reaction was completed monitored by TLC. The reaction mixture was poured into ice-cold water.

Separated solid was filtered, washed with water, dried. The residue obtained was purified by recrystallization from ethanol. The structure of compound **2** was confirmed by IR and ¹H NMR.

Yield: 87%, mp 216-218 °C. IR (KBr) cm⁻¹: 3453, 3301 (enolic OH), 2942 (CH aliphatic), 2222 (–CN), 1734 (C=O ester), 1665 (C=O pyrimidine), 1251 (C-O-C), 884 (C-S-C), 780 (Ar-Cl); ¹H-NMR (400 MHz, DMSO-d₆, ppm): 1.13 (t, J=7.12, 3H, –CH₃), 4.11 (t, J=7.12, 2H, –OCH₂), 4.07 (s, 2H, –SCH₂), 7.58 (t, 1H, Ar-H), 7.66 (d, 1H, Ar-H), 7.88 (d, 1H, Ar-H), 7.89 (s, 1H, Ar-H).



(a) ClCH₂COOEt / K₂CO₃ / DMF rt (b) Hydrazine hydrate / Methanol reflux
(c) aromatic aldehyde / AcOH / EtOH reflux.

Scheme-1

Synthesis of 4-(3-chlorophenyl)-2-hydrazinyl-6-hydroxypyrimidine-5-carbonitrile (**3**)

Compound **2** (1 mmol) was dissolved in 20 ml ethanol. To this solution hydrazine hydrate (1.1 mmol) was added. The reaction mixture was refluxed for 4 h until the reaction was completed, monitored by TLC. After completion of the reaction, the contents were cooled to room temperature. The separated solid was filtered, washed with ethanol, dried and used for the next reaction. Yield: 83%. M.p. 260-262 °C. IR (KBr) cm⁻¹: 3501-3301 (enolic OH, NH & NH₂), 2206 (–CN), 780 (Ar-Cl).

Synthesis of 2-(2-arylidenehydrazinyl)-4-(3-chlorophenyl)-6-hydroxypyrimidine-5-carbonitrile (**4a-f**)

A mixture of Compound **3** (1 mmol), aromatic aldehyde (1 mmol), and the glacial acetic acid catalytic amount in absolute ethanol (15 ml) was refluxed for 4 h. A formed precipitate was filtered off, washed with water and dried. The residue obtained was purified by silica gel column chromatography.

2-((E)-2-(4-hydroxybenzylidene)hydrazinyl)-4-(3-chlorophenyl)-6-hydroxypyrimidine-5-carbonitrile (**4a**)

Yield 88%. m.p. 250 °C. IR (KBr) cm⁻¹: 3514 (enolic OH), 3399 (OH), 3241 (NH), 3072 (Ar-H), 2215 (CN), 1680 (–HC=N–), 777 (Ar-Cl). ¹H-NMR: 6.88 (d, J=8.6, 2H, Ar-H), 7.63 (t, J=7.84, 1H, Ar-H), 7.71 (d, 1H, Ar), 7.88 (d, 2H, Ar-H), 7.92 (d, 2H, Ar-H), 8.13 (s, 1H, Ar N=CH), 10.07 (s, 1H, Ar-OH), 12.36 (s, 1H, NH), 12.52 (s, 1H, Py-OH).

2-((E)-2-(4-hydroxy-3-methoxybenzylidene)hydrazinyl)-4-(3-chlorophenyl)-6-hydroxypyrimidine-5-carbonitrile (**4b**)

Yield: 90%, m.p. 320 °C. IR (KBr) cm⁻¹: 3530 (OH), 3252 (enolic OH), 3216 (NH), 3070 (Ar-H), 2208 (CN), 1670 (–HC=N–), 780 (Ar-Cl). ¹H-NMR: 3.88 (s, 3H, OCH₃), 6.83 (d, J=8.12, 2H, Ar-H), 7.27 (dq, J=6.84, 1H, Ar-H), 7.58 (t, J=7.8, 1H, Ar-H), 7.66 (d, 1H, J=7.8, Ar-H), 7.70 (s, 1H, Ar-H), 8.08 (s, 1H, Ar N=CH), 9.64 (s, 1H, Ar-OH), 12.19 (s, 1H, NH), 12.49 (s, 1H, Py-OH). LC-MS, m/z: 396.29 (M+1).

2-((E)-2-(2-fluorobenzylidene) hydrazinyl)-4-(3-chlorophenyl)-6-hydroxypyrimidine-5-carbonitrile (4c)

Yield 88%. m.p. 326 °C. IR (KBr) cm^{-1} : 3466 (enolic OH), 3204 (NH), 3022 (Ar-H), 2211 (CN), 1656 (-HC=N-), 776 (Ar-Cl). $^1\text{H-NMR}$: 3.88 (s, 3H, OCH_3), 6.83 (d, $J=8.12$, 2H, Ar-H), 7.27 (dq, $J=6.84$, 1H, Ar-H), 7.58 (t, $J=7.8$, 1H, Ar-H), 7.66 (d, 1H, $J=7.8$, Ar-H), 7.70 (s, 1H, Ar-H), 8.08 (s, 1H, Ar N=CH), 12.48 (br s, 2H, NH, Py-OH). LC-MS, m/z : 368.31 (M+1).

2-((E)-2-(3-hydroxybenzylidene) hydrazinyl)-4-(3-chlorophenyl)-6-hydroxypyrimidine-5-carbonitrile (4d)

Yield 92%. m.p. 310 °C. IR (KBr) cm^{-1} : 3467 (enolic OH), 3210 (NH), 3068 (Ar-H), 2208 (CN), 1656 (-HC=N-), 779 (Ar-Cl). $^1\text{H-NMR}$: 6.87 (dd, $J=6.16$ and 1.76 , 1H, Ar-H), 7.22 (t, $J=7.88$, 1H, Ar-H), 7.35 (d, $J=7.64$, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 7.51-7.59 (m, 2H, Ar-H), 7.84-7.87 (m, 2H, Ar-H), 9.43 (s, 1H, Ar-OH), 12.40 (s, 2H, Py-OH, NH).

2-((E)-2-((furan-2-yl)methylene)hydrazinyl)-4-(3-chlorophenyl)-6-hydroxypyrimidine-5-carbonitrile (4e)

Yield: 90%. m.p. 300 °C. IR (KBr) cm^{-1} : 3462 (enolic OH), 3221 (NH), 3042 (Ar-H), 2208 (CN), 1667 (-HC=N-), 791 (Ar-Cl). $^1\text{H-NMR}$: 6.60 (t, $J=1.72$, 1H, Ar-H), 7.20 (d, $J=3.36$, 1H, Ar-H), 7.58 (m, 2H, Ar-H), 7.75 (d, 1H, $J=1.32$, Ar-H), 7.86 (m, 2H, Ar-H), 8.16 (s, 1H, Ar N=CH), 11.83 (s, 1H, NH), 12.65 (s, 1H, Py-OH).

2-((E)-2-(3,4,5-trimethoxybenzylidene) hydrazinyl)-4-(3-chlorophenyl)-6-hydroxypyrimidine-5-carbonitrile (4f)

Yield 90%. m.p. 320 °C. IR (KBr) cm^{-1} : 3467 (enolic OH), 3220 (NH), 3043 (Ar-H), 2208 (CN), 1656 (-HC=N-), 779 (Ar-Cl). $^1\text{H-NMR}$: 3.74 (s, 3H, OCH_3), 3.88 (s, 6H, OCH_3), 7.26 (s, 2H, Ar-H), 7.61 (m, 2H, Ar-H), 7.8 (m, 2H, Ar-H), 8.11 (s, 1H, Ar N=CH), 12.65 (s, 2H, Py-OH, NH).

Table-1: Synthesis of 2-(2-arylidenehydrazinyl)-4-(3-chlorophenyl)-6-hydroxypyrimidine-5-carbonitrile (4a- f)

Entry	R-CHO	Yield ^a (%)	M.P.(°C)	Comp. no.
1	4-OH C_6H_4	88	250	4a
2	4-OH,3- OCH_3 C_6H_4	90	320	4b
3	4-F C_6H_4	88	326	4c
4	3-OH C_6H_4	92	310	4d
5	Furyl	90	300	4e
6	3,4,5-(OCH_3) $_3\text{C}_6\text{H}_2$	90	320	4f

Antimicrobial Activity

Synthesized compounds (4a-f) were evaluated for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* (two-gram negative) and *Staphylococcus aureus*, *Streptococcus pyogenes* (two gram-positive) using Broth dilution method.¹⁵ Ampicillin used as a standard drug, the MIC results are represented in Table-2. Synthesized compounds (4a-f) also tested for their antifungal activity against three fungi *Candida albicans*, *Aspergillus niger*, *Aspergillus clavatus*, griseofulvin used as standard drug. The MIC results are represented in Table-3.

RESULTS AND DISCUSSION

Initially 6-(3-chlorophenyl) -4-oxo- 2-thioxo - 1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1) was synthesized as per reported method. Compound 1 on reaction with ethyl chloroacetate was converted to ethyl 2-((4-(3-chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)acetate (2). Further compound 2 was treated with hydrazine hydrate under the refluxed condition and converted to 2-

hydrazinyl-4-(3-chlorophenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (3). Finally, a series of 2-(2-arylidenehydrazinyl)-4-(3-chlorophenyl)-6-oxo-1,6-dihydro pyrimidine-5-carbonitrile (4a-f) was synthesized by condensation of compound 3 with various aromatic aldehydes. The formation of target compounds (4a-f) was supported by spectral analysis. The FT-IR showed absorption bands in the range of 3530-3463 cm^{-1} for enolic -OH, 3204-3316 cm^{-1} for -NH-, 2950-3070 cm^{-1} for aromatic C-H, 2208-2220 cm^{-1} for -CN, 1600-1670 cm^{-1} for azomethine group (-CH=N-) and 770-780 cm^{-1} for Ar-Cl. The ^1H NMR spectra of title compounds (4a-h) showed peaks of enolic -OH, hydrazide (NH), aromatic, imine (-CN=N-) proton. The ^1H NMR spectra of compounds (4a-f) showed broad singlet at δ 11.83-12.65 indicating the presence of hydrazide -NH- and enolic -OH. The sharp singlet around δ 8.10 indicating the presence of azomethine (-CH=N-) proton. The appearance of signals in the range of 6.8-7.8 was due to aromatic protons. In the mass spectrum compound, 4b showed a peak at m/z 396.29 (M+1) and compound 4c showed a peak at m/z 368.31 (M+1). All the newly synthesized compounds 4a-f were screened in vitro for their antimicrobial activities. The MIC values ($\mu\text{g/ml}$) of 4a-f along with standard drugs against selected microbes are shown in Table-2 and 3. All compounds 4a-f shows mild to moderate antibacterial activity against selected microbes as compared to standard drug chloramphenicol.

Table 2: In vitro antibacterial activities of compounds (4a- f)(Minimum inhibition concentration, $\mu\text{g/ml}$)

S.No.	E. coli	P. aeruginosa	S. aureus	S. pyogenus
4a	500	200	125	200
4b	500	500	200	62.5
4c	100	250	250	100
4d	200	125	125	100
4e	100	125	200	200
4f	62.5	100	125	250
AMPICILLIN	100	--	250	100
CHLORAMPHENICOL	50	50	50	50

Table 3: In vitro antifungal activities of compounds (4a- f)
(Minimum inhibition concentration, $\mu\text{g/ml}$)

Compounds	C. albicans	A. niger	A. clavatus
4a	250	500	500
4b	1000	1000	1000
4c	1000	250	250
4d	1000	1000	1000
4e	500	>1000	>1000
4f	250	500	500
NYSTATIN	100	100	100
GRESEOFULVIN	500	100	100

But as compared to another standard drug ampicillin all synthesized compounds show good for mild activity. Compounds 4c, 4e, 4f shows promising activity against E. coli, compounds 4d, 4e, 4f shows good activity against P. aeruginosa, compounds 4a, 4d, 4f shows good activity against S. Aureus and compounds 4b, 4c, 4d shows good activity against S. pyogenus. Out of which 4f exhibited most promising activity against E. coli that is 62.5 $\mu\text{g/ml}$ and 4b exhibited most promising activity against S. pyogenus that is 62.5 $\mu\text{g/ml}$. Microbial activity data showed that compound 4a, 4b showed mild antibacterial activity (>200 $\mu\text{g/ml}$) against E. coli, compounds 4a, 4b, and 4c exhibited mild antibacterial (>200 $\mu\text{g/ml}$) against P. aeruginosa, compounds 4b, 4c and 4e exhibited mild antibacterial (>200 $\mu\text{g/ml}$)

against *S. Aureus* and compounds 4a, 4e and 4f exhibited mild antibacterial ($>200\mu\text{g/ml}$) against *S. pyogenus* as compared to standard drug Ampicilin ($100\mu\text{g/ml}$). All compounds 4a-f shows mild to moderate antifungal activity against selected microbes as compared to standard drug griseofulvin. Compounds 4a, 4e, 4f exhibit good antifungal activity against *C. albicans* and other compounds shows mild antifungal activity.

CONCLUSION

In present study, design, and synthesis of a new pyrimidine, 2-(2-arylidenehydrazinyl)-4-(3-chlorophenyl)-6-oxo-1,6-dihydro pyrim-idine-5-carbonitrile (4a-h) derivatives are described. All the derivatives were characterized by physical and spectral analysis. All the synthesized compounds were evaluated for their antibacterial and antifungal activities. It was noted that the most of the derivatives were show mild to moderate and good antibacterial and antifungal activities. As per result of antibacterial activity compounds 4c, 4e and 4f show most promising activity while 4b shows excellent activity against *S. pyogenus*. Compound s 4a, 4c and 4f shows have remarkable antifungal activity.

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