Synthesis, antibacterial and antifungal activity of 5-aryl amido-6-isopropyl-2-(mercapto/ hydroxy)-4-[(4'-difluoro methoxy) (3'-hydroxy) phenyl]-1,4-dihydro pyrimidine

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ABSTRACT. Various isopropylacetoacetanilides were treated with 4-(difluoromethoxy)-3-hydroxy benzaldehyde and thiourea/urea in the presence of acid catalyst to produce the 1,4-dihydropyrimidine derivatives (**4a-5i** and **5a-5i**) with good yields. All the synthesized compounds were characterized by mass, ¹H-NMR and IR spectrum, also evaluated for antibacterial and antifungal activity against two Gram +ve and two Gram -ve bacterial and fungi strains. The compounds **4e**, **4g** and **5d** were found comparatively promising active against all the bacterial and fungal strains.

1. INTRODUCTION

Multi-component coupling reactions (MCR's), involving the inherent formation of several bonds in one step, have proven to be efficient and powerful tool for the rapid formation of complex heterocyclic compounds in recent years [1]. The unique features of these reactions are their operational simplicity, structural diversity, versatility, high synthetic efficiency, atom-economy, and single step synthesis without isolating the intermediates. The synthesis of 1,3-dihydropyrimidines via one-pot condensation of aldehyde, β-dicarbonyl compound, and thiourea/urea known as Biginelli reaction [2] is one of the most recognized and often used MCR's for the synthesis of these valuable heterocyclic compounds.

Microbial infections are a growing problem in contemporary medicine. According to statistical evidence provided by WHO, many of the drugs treatment breakthroughs of the last century could be lost through the spread of antimicrobial resistance [3]. For instance, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are important pathogens causing invasive diseases such as sepsis, meningitis, necrotizing fasciitis, pneumonia [4], nosocomial pneumonia [5], cystic fibrosis, acute leukemia, organ transplants, and intravenous-drug addiction [6]. Some of these pathogens have been reported to develop resistance [7] to the well-known commercially available drugs. As a result, many infectious diseases may one day become uncontrollable and could rapidly spread throughout the world. Consequently, the discovery of potent antibiotic drugs is considered to be one of the greatest scientific and medical goals.

There is a growing interest pertaining to the synthesis of bioactive heterocyclic compounds in pharmacy. Among various heterocyclic compounds, pyrimidine derivatives have apparently gained considerable importance owing to their varied biological activities such as adenosine receptor antagonist [8], anti-inflammatory [9], CDK inhibitor [10], calcium channel antagonist [11] and anti-tumor [12] activities. On the other hand, a promising diverse pharmacological activity is shown by the pyrimidine nucleus such as anti-biotic, anti-fungal [13], anti-cancer [14], HIV protease inhibitor [15] and chemotherapeutic activities [16].

In the present work, we synthesized new several compounds via a one-pot, multi-component reaction of 4-(difluoromethoxy)-3-hydroxy benzaldehyde with various 1-Aryl amino-4-methylpentane-1:3-dione and thiourea/urea (Scheme 2). The biological activities (MIC) of the synthesized compounds were compared with known standard drugs.

2. EXPERIMENTAL

Thin layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365 nm). IR spectra were recorded on a FT-IR-8400 spectrophotometer using DRS prob. $^1\text{H-NMR}$ (400 MHz) spectra were recorded on a Bruker AVANCE II spectrometer in DMSO-d_6 . Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu). Melting points were measured in open glass capillaries and are uncorrected. Elemental analysis of the all the synthesized compounds were carried out on Elementar Vario EL III Carlo Erba 1108 model, and the results are in agreements with the structures assigned.

Reaction Scheme

Reagents & Conditions: (a) Dioxane, KOH, 14-20 hrs, 120°C , reflux

Scheme 1: Synthesis of 1-Arylamino-4-methylpentane-1:3-dione

Reagents & Conditions: (a) Methanol, conc. HCl, 14hrs, 65°C , reflux

Scheme 2: Synthesis of 1,4-dihydropyrimidine derivatives

Procedure for synthesis of substituted 1-Aryl amino-4-methylpentane-1:3-dione (2a-2i)

A mixture of Aniline (0.5gm, 5.37 mol) and 4-methyl-3-oxopentanoate (0.83, 6.4 mol) in 1,4-dioxane was refluxed in the presence of KOH for 14 hrs. The reaction mixture is monitored by TLC. After the completion of reaction, the product was poured into crushed ice, neutralize with dil. HCl make pH 4-5 and the separated solid was filtered out and crystallized from ethanol m.p 127°C.

Similarly, other 1-Arylamino-4-methylpentane-1;3-dione (2a-2i) have been synthesized.

Procedure for synthesis of 5-phenyl amido-6-isopropyl-2-mercapto-4-[(4'-difluoro methoxy) (3'-hydroxy) phenyl]-1,4-dihydropyrimidine (4a)

A mixture of 4-(difluoromethoxy)-3-hydroxybenzaldehyde (0.5gm, 2.65 mol), 4-methyl-3-oxo-N-phenylpentanamide (0.5gm, 5.45 mol) and thiourea (0.20 gm, 2.04 mol) with 2-3 drops of Conc. HCl in methanol (15 ml) was refluxed at 65°C for 14 hrs. The reaction mixture is monitored by TLC. After the completion of reaction, cool the reaction mixture at room temperature. The excess of solvent was distilled out and solid was filtered out and crystallized from ethanol. Yield 61 %, m.p. 160°C. (C₂₁H₂₁F₂N₃O₃S; Required: C, 58.19; H, 4.88; N, 9.69; found: C, 58.12; H, 4.50; N, 9.63 %).

5-(4-chloro phenyl amido)-6-isopropyl-2-mercapto-4-[(4'-difluoro methoxy) (3'-hydroxy) phenyl]-1,4-dihydro pyrimidine(4f); The physical and spectral data of compound is as following. Obtained as light yellow colour in 79% Yield; m.p 182 °C; IR (KBr, cm⁻¹): 3294 (C-N-H str.), 3331 (-OH), 2931(C-H str.)(asym), 2620 (C-S-H str.), 1621 (C=N str.), 1323 (C-N str.), 1107 (C-F) ; ¹H-NMR (DMSO-d₆, δ ppm): 1.50 (s, 3H, -CH₃), 1.67 (s, 3H, -CH₃), 3.84 (s, 1H, -CH, isopropyl), 4.72 (s, 1H, -CH, chiral), 6.73-6.75 (dd, 1H, -CH, aromatic), 6.80-6.83 (d, 1H, -CH, aromatic), 7.02 (s, 1H, -CH -CHF₂), 7.07-7.09 (d, 1H, -CH, aromatic), 7.20 (s, 1H, -NH, pyrimidine ring), 7.31-7.40 (dd, 2H, -CH, aromatic), 7.46-7.64 (dd, 2H, -CH, aromatic), 9.07 (s, 1H, -SH), 10.01 (s, 1H, -NH, amide), 10.03 (s, 1H, -OH). Maas : (m/z) 467 ; Anal. Calcd. for C₂₁H₂₀ClF₂N₃O₃S: C: 53.90%, H: 4.31%, N: 8.98%; Found: C: 53.79%, H: 4.12%, N: 8.86%.

5-Arylamido-6-isopropyl-2-mercapto-4-[(4'-difluoro methoxy) (3'-hydroxy) phenyl]-1,4-dihydro pyrimidine (4a-4i) were synthesized. The physical data are recorded in Table no.I.

Procedure for synthesis of 5-phenyl amido-6-isopropyl-2-hydroxy-4-[(4'-difluoro methoxy) (3'-hydroxy) phenyl]-1,4-dihydro pyrimidine (5a)

A mixture of 4-(difluoromethoxy)-3-hydroxybenzaldehyde (0.5gm, 2.65 mol), 4-methyl-3-oxo-N-phenylpentanamide (0.5gm, 5.45 mol) and urea (0.17 gm, 1.75 mol) with 2-3 drops of Conc. HCl in methanol (15 ml) was refluxed for 12-14 hrs. The reaction mixture is monitored by TLC. After the completion of reaction, cool the reaction mixture at room temperature. The excess of solvent was distilled out and solid was filtered out and crystallized from ethanol. Yield 61 %, m.p. 158°C. (C₂₁H₂₁F₂N₃O₄; Required: C, 60.43; H, 5.07; N, 10.07; found: C, 60.12; H, 5.10; N, 10.02%).

5-(4''-chlorophenylamido)-6-isopropyl -2-hydroxy-4-[(4'-difluoromethoxy) (3'-hydroxy) phenyl]-1, 4-dihydro pyrimidine (5f); the physical and spectral data of compound is as following. Obtained as pale yellow colour in 77% Yield; m.p 182 °C; IR (KBr, cm⁻¹): 3298 (N-H str.), 3293 (-OH), 3026 (C-H str.)(sym), 1583 (C=N str.), 1309 (C-N str.), 1143 (C-F) ; ¹H-NMR (DMSO-d₆, δ ppm): 1.48 (s, 3H, -CH₃), 1.58 (s, 3H, -CH₃), 3.82 (s, 1H, -CH, isopropyl), 4.64-4.65 (d, 1H, -CH, chiral), 6.81-6.83 (d, 1H, -CH, aromatic), 6.91 (s, 1H, - CHF₂), 7.02-7.09 (t, 1H, -CH, aromatic), 7.16-7.17 (d, 1H, -CH, aromatic), 7.20 (s, 1H, -NH, pyrimidine ring), 7.37-7.40 (d, 2H, -CH, aromatic), 7.63-7.66 (d, 2H, -CH, aromatic), 7.90 (s, 1H, -OH), 9.96 (s, 1H, -NH, amide), 10.05 (s, 1H, -OH, aromatic). Maas: (m/z) 451; Anal. Calcd. for C₂₁H₂₀ClF₂N₃O₄: C: 55.82%, H: 4.46%, N: 9.30%; Found: C: 53.79%, H: 4.12%, N: 9.26%.

5-Arylamido-6-isopropyl-2-hydroxy-4-[(4'-difluoromethoxy)(3'-hydroxy) phenyl]-1, 4-dihydro pyrimidine (5a-5i) were synthesized. The physical data are recorded in Table no.I.

Table no. I

Sr. No.	Ar	Molecular Formula	M.W.	M.P. °C	Yield %	% Nitrogen	
						Calculated	Found
4a	C ₆ H ₅ -	C ₂₁ H ₂₁ F ₂ N ₃ O ₃ S	434	160	61	9.69	9.63
4b	4-CH ₃ - C ₆ H ₅ -	C ₂₂ H ₂₃ F ₂ N ₃ O ₃ S	448	151	88	9.39	9.25
4c	4-OCH ₃ -C ₆ H ₄ -	C ₂₂ H ₂₃ F ₂ N ₃ O ₄ S	464	163	86	9.07	9.01
4d	2- OCH ₃ -C ₆ H ₄ -	C ₂₂ H ₂₃ BrF ₂ N ₃ O ₄ S	513	159	81	9.07	9.00
4e	4-F- C ₆ H ₄ -	C ₂₁ H ₂₀ F ₃ N ₃ O ₃ S	452	137	75	9.31	9.27
4f	4-Cl- C ₆ H ₄ -	C ₂₁ H ₂₀ ClF ₂ N ₃ O ₃ S	467	182	79	8.98	8.86
4g	2- Cl- C ₆ H ₄ -	C ₂₁ H ₂₀ ClF ₂ N ₃ O ₃ S	452	174	65	8.98	8.26
4h	2,4-(Cl) ₂ - C ₆ H ₃ -	C ₂₁ H ₁₉ Cl ₂ F ₂ N ₃ O ₃ S	503	169	74	8.34	8.38
4i	4-Br- C ₆ H ₄ -	C ₂₁ H ₂₀ BrF ₂ N ₄ O ₅ S	479	134	77	8.20	8.18
5a	C ₆ H ₅ -	C ₂₁ H ₂₁ F ₂ N ₃ O ₄	418	158	86	10.07	10.02
5b	4-CH ₃ - C ₆ H ₅ -	C ₂₂ H ₂₃ F ₂ N ₃ O ₄	432	151	88	9.74	9.69
5c	4-OCH ₃ -C ₆ H ₄ -	C ₂₂ H ₂₃ F ₂ N ₃ O ₅	447	163	83	9.39	9.25
5d	2- OCH ₃ -C ₆ H ₄ -	C ₂₁ H ₂₀ BrF ₂ N ₃ O ₄	447	159	79	9.39	9.35
5e	4-F- C ₆ H ₄ -	C ₂₁ H ₂₀ F ₃ N ₃ O ₄	436	137	74	9.65	9.61
5f	4-Cl- C ₆ H ₄ -	C ₂₁ H ₂₀ ClF ₂ N ₃ O ₄	451	182	77	9.30	9.26
5g	2- Cl- C ₆ H ₄ -	C ₂₁ H ₂₀ ClF ₂ N ₃ O ₄	451	174	65	9.30	9.23
5h	2,4-(Cl) ₂ - C ₆ H ₄ -	C ₂₁ H ₁₉ Cl ₂ F ₂ N ₃ O ₄	503	169	74	8.64	8.58
5i	4-Br- C ₆ H ₄ -	C ₂₁ H ₂₀ BrF ₂ N ₃ O ₄	496	134	76	8.47	8.37

Antibacterial and antifungal activity

The newly synthesized compounds were screened for their antibacterial activity against Gram-positive (*S. aureus* ATCC 6538, *M. luteus* ATCC 9345), Gram negative (*E. coli* ATCC 4230, *S. typhi* ATCC 14028) bacteria, as described by the guidelines in NCCLS-approved standard document M7-A4, using the micro dilution broth procedure [17]. Ampicillin trihydrate was used as the reference antibacterial agent. The antifungal activities of the newly synthesized chemical compounds were tested against yeast strain (*C. albicans* ATCC 14053) according to the guidelines in NCCLS-approved standard document M27-A2, using the micro dilution broth procedure [18]. Fluconazole was used as the reference antifungal agent. The solutions of test compounds and reference drug were prepared by dissolving in DMSO at a concentration of 2560 µg/mL. The 2-fold dilutions of the compounds and the reference drug were prepared (1280, 640, 320, 160, 80, 40, 20,

10 µg/mL). Antibacterial activities of the newly synthesized chemical compounds were performed in Mueller-Hinton broth medium at a pH of 7.2 with an inoculum of $(1-2) \times 10^3$ cells/mL by the spectrophotometric method, and an aliquot of 100µL solution was added to each tube of serial dilution. The chemical compounds-broth medium serial tube dilutions inoculated with each bacterium were incubated on a rotary shaker at 37°C for 18 hrs at 150 rpm. The minimum inhibitory concentration (MIC) of each chemical compound was recorded as the lowest concentration of each chemical compound in the tubes with no growth (i.e., no turbidity) of inoculated bacteria. Minimum inhibitory concentration (MIC, µg/mL) was measured and compared with control; the MIC values of the compounds (**4a-4i** and **5a-5i**) are represented in table no. II.

Antibacterial and antifungal activity of compounds **4a-4i** and **5a-5i** in MIC (µg/mL).

Table no. II

Comp. Id	Ar	Antibacterial Activity				Antifungal Activity
		Gram-positive bacteria		Gram-negative bacteria		
		<i>S. aureus</i>	<i>M. luteus</i>	<i>E. coli</i>	<i>S. thphi</i>	
4a	C ₆ H ₅ -	80	40	160	80	160
4b	4-CH ₃ - C ₆ H ₅ -	80	160	160	80	160
4c	4-OCH ₃ - C ₆ H ₄ -	80	160	160	80	80
4d	2- OCH ₃ - C ₆ H ₄ -	160	80	160	80	80
4e	4-F- C ₆ H ₄ -	40	40	40	80	80
4f	4-Cl- C ₆ H ₄ -	160	80	80	160	160
4g	2- Cl- C ₆ H ₄ -	40	40	80	40	40
4h	2,4-(Cl) ₂ - C ₆ H ₄ -	160	160	80	80	80
4i	4-Br- C ₆ H ₄ -	160	80	80	40	40
5a	C ₆ H ₅ -	40	40	80	80	80
5b	4-CH ₃ - C ₆ H ₅ -	80	80	160	160	160
5c	4-OCH ₃ - C ₆ H ₄ -	160	80	80	40	40
5d	2- OCH ₃ - C ₆ H ₄ -	80	40	40	40	40
5e	4-F- C ₆ H ₄ -	80	80	80	40	40
5f	4-Cl- C ₆ H ₄ -	160	80	80	160	160
5g	2- Cl- C ₆ H ₄ -	80	160	80	80	160
5h	2,4-(Cl) ₂ - C ₆ H ₄ -	80	160	80	80	160
5i	4-Br- C ₆ H ₄ -	80	80	80	40	40
Ampicillin	-	20	20	40	20	-
Fluconazole	-	-	-	-	-	10

From the result of biological evaluation, it has been observed that the compounds exhibited interesting biological activity, however with a degree of variation. Most of the compounds tested were found to have comparable antibacterial and exhibit low antifungal activity. From the Table-II, it can be observed that compounds **4a**, **4i** and **5c** were moderate active against *S. aureus*, *M. luteus*, *Escherichia coli*, *S. thyphi*., *C. albicans*. Compounds **4e**, **4g**, **5a**, **5d**, **5e** and **5i** were give promising activity against *S. aureus*, *M. luteus*, *Escherichia coli*, *S. thyphi*., *C. albicans*.

3. CONCLUSION

In summary, we have synthesized a series of new 1,4-dihydropyrimidine derivatives. All the newly synthesized compounds were confirmed with spectroscopic data like $^1\text{H-NMR}$, Mass, IR Spectra, elemental analysis and evaluated antibacterial and antifungal activity. The antibacterial and antifungal study shows that the compounds **4e**, **4g**, **4e**, **5a**, **5d**, **5e** and **5i** have promising activity with MICs between 40 and 80 $\mu\text{g/mL}$. The importance of such work lies in the possibility that the new compounds might be more efficacious drugs against bacteria, which could be helpful in designing more potent antibacterial agent for therapeutic use.

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