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Research Article

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Synthesis and antifungal activities of diaryl pyrazoles carboxamide derivatives

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ABSTRACT

*Series of seven 5-phenyl-1H-pyrazole-3-carboxylic acid amide derivatives were prepared by varying the active part (amide group) of pyrazole. All the synthesized compounds were characterized by, ^{1}H NMR, ^{13}C NMR and mass spectrometry and were screened, antifungal activity, against four fungal pathogens such as Candida albicans, Aspergillus flavus, Aspergillus fumigatus and Aspergillus niger using broth microdilution method. Active compounds were also screened for MIC (minimum inhibitory concentration) and MFC (minimum fungicidal concentrations). Two compounds **5b** and **5f** were found to be more potent than standard actidione drugs, against Candida albicans strains.*

Key words: Pyrazole carboxamide, antifungal activity, MIC and MFC.

INTRODUCTION

Frequencies of pathogenic fungal infections are life threatening and caused by opportunistic fungal pathogens has increased significantly in immunocompromised patients [1, 2] over the past two decades. Since Candida albicans (C. albicans) and Aspergillus fumigatus (A. fumigatus) are the main causative fungi in which Candida albicans has been identified as the major opportunistic pathogen in the etiology of fungal infections; however, the frequency of other Candida species is increasing dramatically [3]. Current antifungal therapy suffers from drug-resistant strains of pathogenic bacteria towards available antibiotics and many drugs have become resistant to routine antifungal drugs. Therefore, there is urgent need for development of new drugs alternative as well as effective drugs with higher efficiency, broader spectrum, and lower toxicity.

A number of antifungal azoles were discovered in the last three decades and are introduced in clinical practice up till now [4, 10]. Despite some significant advances in this field, there is a continuing increase in the incidence of fungal infections, together with a gradual rise in azole resistance [11]. However clinical use of azoles is limited because of an increase of resistant strains, particularly during the long-term treatment [14-19]. Therefore, identification of new azole antifungal agents which are not associated with the emergence of resistance is needed. This goal could be achieved by developing novel and potent antifungal azoles to overcome resistance and develop effective therapies.
Pyrazoles are one of the oldest classes of bioactive compounds which are widely used agents. The clinical significance of this class of compounds has stimulated the synthesis of new lead compounds retaining the ‘core’ pyrazole chromophore. Pyrazoles are known to possess numerous chemical and biological activities such as

antimicrobial [20], antifungal [21], antitumor [22], antileukemia [23], antitubercular [24], antidepressant [25], and anticonvulsant [26]. Literature also revealed that trifluoromethyl group is responsible for the biological activity and therefore is the subject of considerable developing interest [27]. The increased activity is attributed to the presence of fluorine atoms (highly electronegative) in the molecules which increases the lipophilicity and affects the partitioning of molecules into membranes and facilitates hydrophobic interactions of the molecules with specific binding sites on either receptor or enzymes [28].

Encouraged by these observations and continous effort on the studies of azole optimization based biheterocyclic rings [29-33], we report the synthesis of new class of azoles, where in potent amide group is linked to pyrazole moiety at C-5 position and observe the additive effect of substituent on the pyrazole nuclei towards the antifungal activity.

EXPERIMENTAL SECTION

All solvents were dried and purified by standard techniques just before use. The progress of the reaction was monitored by thin layer chromatography (TLC) using pre-coated Merck silica gel 60 F_{254} TLC plate. The spots were visualized by UV or by iodine vapour. Melting points (m.p. values) were determined on melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker at 400 MHz spectrometer (Germany) using tetramethylsilane (TMS) as an internal standard. The chemical shift values are recorded on δ scale and the coupling constants (*J*) are in Hertz. Mass spectrometery was recorded on waters, Q-TOF MICROMASS (LC-MS).

General Synthesis:

Synthesis of ethyl 4-(3,5-bistrifluoromethyl) phenyl)-2,4-dixobutanoate (2): 3,5 –bis-trifluoro acetophenone (1 mol) was treated with diethyl oxalate (1.5 mol) and sodium hydride (2 mol) in toluene at rt. The reaction mixture was stirred overnight and progress of the reaction was monitored on TLC. After completion of the reaction, the solvent was evaporated and the mixture was then poured on to crushed ice and acidified with dil. HCl to obtain white solid which was recrystallized from ethanol.

Synthesis of ethyl3-(3, 5-bistrifluoromethyl) phenyl)-1-(4-fluorophenyl)-1*H*-pyrazole-5-carboxylate (3): The mixture of ethyl ester **2**(1.0 mol) and 4-fluorophenyl hydrazine hydrochloride (1.1 mol) in ethanol: acetic acid (2:1) was stirred at 100^{o}C for 6h. After completion of the reaction, the solvent was evaporated and the mixture was then poured on to crushed ice to obtain the white solid which was recrystallized from ethanol.

Synthesis of (3, 5-bis (trifluoromethyl) phenyl)-1- (4-fluorophenyl) - N-phenyl-1*H*-pyrazole-5-carboxylic acid (4): Ethyl ester **3** (1.0 mol) was added to the ethanolic NaOH (1.2 mol) solution and the mixture was stirred at rt for 4h. The progress of the reaction was monitored on TLC. After complete hydrolysis, the excess solvent was removed. The residue was dissolved in water and acidified using dil HCl to obtain the white solid which was recrystallized from ethanol.

General synthesis of (3, 5-bis (trifluoromethyl) phenyl)-1- (4-fluorophenyl)-1*H*-pyrazol-5-yl)(4-(4-trifluoromethyl) substituted amine/piperazin-1-yl)methanone (5a-g): To a cooled solution of pyrazole carboxylic acid **4** (1.0 mol) in Dimethyl formamide, HOBt (1.1 mol) was added followed by subsititued piperazine/ aryl amine (1.0 mol). TEA (2.0 mol) and EDC.HCl (1.1 mol) were added thereafter and the reaction mixture was stirred overnight. The mixture was then poured on to crushed ice; the product was filtered and washed with water. The final products were purified by column chromatography using hexane: ethyl acetate as eluant.

(3, 5-bis (trifluoromethyl) phenyl)-1- (4-fluorophenyl)-1*H*-pyrazol-5-yl)(4-(4-trifluoromethyl)piperazin-1-yl)methanone (5a): Yield 80%, yellow solid, m. p: 130-132^{0}C. ^{1}H NMR (400MHz, $CDCl_3$) : δ 3.32-3.35(m, 4H, piperazine –CH_2-), 4.0(m,2H, piperazine –CH_2-), 4.34(m, 2H, piperazine–CH_2-), 7.08-7.16(m, 6H, Ar-H), 7.26-7.29(m, 2H, Ar-H), 7.36-7.40(m, 1H, Ar-H), 7.66(S, 2H, Ar-H), 7.85(s,1H, Ar-H).

(3, 5-bis (trifluoromethyl) phenyl)-1- (4-fluorophenyl)-1H-pyrazol-5-yl)(4-(2-methoxyphenyl)piperazin-1-yl)methanone(5b): Yield 82%, White solid, m.p: 152-154^{0}C. ^{1}H NMR (400MHz, $CDCl_3$): δ 3.19(m, 4H, piperazine–CH_2-), 3.90(s, 3H, Ar-H), 4.05(m, 2H, piperazine–CH_2-), 4.34(m, 2H, piperazine –CH_2-), 6.90-6.96(m, 2H, Ar-H), 7.07-7.14(m, 4H, Ar-H), 7.26-7.29(m, 3H, Ar-H), 7.66(S, 2H, Ar-H). CMR (100MHz, $CDCl_3$): 42.93, 47.39, 50.69, 51.41, 55.44, 111.36, 116.39, 116.62, 118.98, 121.41, 122.28, 123.51, 124.12, 126.84, 127.38, 27.47,

128.58, 131.58, 131.76, 132.10, 132.43, 132.77, 134.89, 137.92, 140.76, 152.30, 161.16, 161.70, 163.65. Lc-MS (M+1): 593.163

(3, 5-bis (trifluoromethyl) phenyl)-1- (4-fluorophenyl)-1H-pyrazol-5-yl)(4-(3,4-dichlorophenyl)piperazin-1-yl)methanone(5c): Yield 76%, White solid, m.p: 140-142^{0}C. ^{1}H NMR (400MHz, $CDCl_3$): 3.25-3.28(m, 4H, piperazine–CH_2-), 3.98(m, 2H, piperazine–CH_2-), 4.31(m, 2H, piperazine –CH_2-), 6.75-6.78(m, 1H, Ar-H), 6.98(m, 1H, Ar-H), 7.11-7.16(m, 3H, Ar-H), 7.25-7.31(m, 3H, Ar-H), 7.65(s, 2H, Ar-H), 7.80(s, 2H, Ar-H).

(3, 5-bis (trifluoromethyl) phenyl)-1- (4-fluorophenyl)-1H-pyrazol-5-yl)(4-(N-tertbutoxy carbonyl piperazin-1-yl)methanone (5d): Yield 83%, White solid, m.p : 116-118^{0}C. ^{1}H NMR (400MHz, $CDCl_3$): 1.48(s, 9H, CH_3), 3.54 (m, 4H, piperazine –CH_2-), 3.80(m, 2H, piperazine –CH_2), 4.10 (m, 4H, piperazine –CH_2-), 7.10-7.15(m, 3H, Ar-H), 7.25-7.28(3.54, m, 2H, Ar-H), 7.65(s, 2H, Ar-H), 7.84(s, 3H, Ar-H).

(3,5-bis (trifluoromethyl) phenyl)-1- (4-fluorophenyl)-1H-pyrazol-5-yl)(4-*p*- tolylpiperazin-1-yl) methanone(5e): Yield 78%, White solid, m.p: 120-122^{0}C. ^{1}H NMR (400MHz, $CDCl_3$): 2.27(s, 3H, Ar-H) 3.20-3.23 (m, 4H, piperazine –CH_2-), 3.97-3.98(m, 2H, piperazine –CH_2-), 4.28(m, 2H, piperazine–CH_2-), 6.85-6.88(m, 2H, Ar-H), 7.08-7.15(m, 4H, Ar-H), 7.25-7.29(m, 2H, Ar-H), 7.65(s,2h, Ar-H), 7.84(s, 1H, Ar-H).

3-(3,5-bis(trifluoromethyl)phenyl)-1-(4-fluorophenyl)-n-phenyl-1H-pyrazole-5-carboxamide (5f): Yield 72%, White solid, m.p: 158-156^{0}C. ^{1}H NMR (400MHz, $CDCl_3$): 7.12-7.19 (m, 3h, Ar-H), 7.29 (s, 1H, -CH- pyrazole), 7.30-7.39 (m, 2H, Ar-H), 7.65 (s, 2H, Ar-H), 7.71-7.72 (d, 2H, Ar-H), 7.85 (s, 1H, Ar-H).

3-(3,5-bis(trifluoromethyl)phenyl)-N,1-bis(4-fluorophenyl)-1H- pyrazole -5-carboxamide (5g): Yield 72%, White solid, m.p: 166-168^{0}C. ^{1}H NMR (400MHz, $CDCl_3$): 7.03-7.05(t, 2H, Ar-H), 7.14-7.15(m, 2H, Ar-H), 7.27(s, 1H, -CH-pyrazole), 7.32-7.34(m, 2H, Ar-H), 7.65(s, 2H, Ar-H), 7.67-7.69(m, 3H, Ar-H), 7.86(s, 1H, Ar-H), n8.72(s, 1H, broad singlet amide). CMR (100MHz, $CDCl_3$) 109.18, 115.63, 115.85, 116.58, 116.80, 118.65, 121.36, 121.50, 121.58, 122.48, 122.52, 122.55, 124.08, 126.79, 127.52, 127.61, 128.60, 131.25, 131.85, 132.19, 132.52, 132.86, 133.60, 133.63, 134.66, 134.69, 142.36, 147.62, 158.21, 158.83, 160.63, 161.39,163.89. Lc-MS (M+1): 512.1105

Biology

In Vitro Antifungal activity:

The synthesized compounds were screened for their antifungal activity against *Aspergillus flavus* (NCIM 544), *Aspergillus fumigatus* (NCIM 902), *Aspergillus niger* (NCIM 584) and *Candida albicans* (NCIM 3471). The test cultures were grown separately in Sabouards Dextrose broth (SDB) (Hi Media, India) at room temperature for 48 h. After checking the purity 100 µl of test cultures were spread on Sabouards Dextrose Agar plates (SDA), using cork bore 6mm diameter wells were made on plates. The test compounds were dissolved in DMSO. Each well was filled with 50 µl volume of test compound. The DMSO used as negative control and Actidione of 1000 µg/ml concentration used as positive control. Inoculated plates were kept at room temperature for 48 h. Each plate was then observed for inhibition zones [34].

MIC and MFC: The Minimum Inhibitory concentrations (MICs) were determined by broth dilution technique. The SDB tubes containing test compounds were serially diluted. The 48 h grown cultures of fungi was inoculated in each tube. The tubes were incubated at room temperature for 24 h along with control. The lowest concentration required to arrest the growth of fungi was regarded as MIC. To get the minimum fungicidal concentration (MFC), a loopful was taken from the MIC tubes and streaked on SDA plates. The growth was observed after incubation at 37^{0}C at 24h. The lowest concentration which showed no growth was recorded as MFC [35].

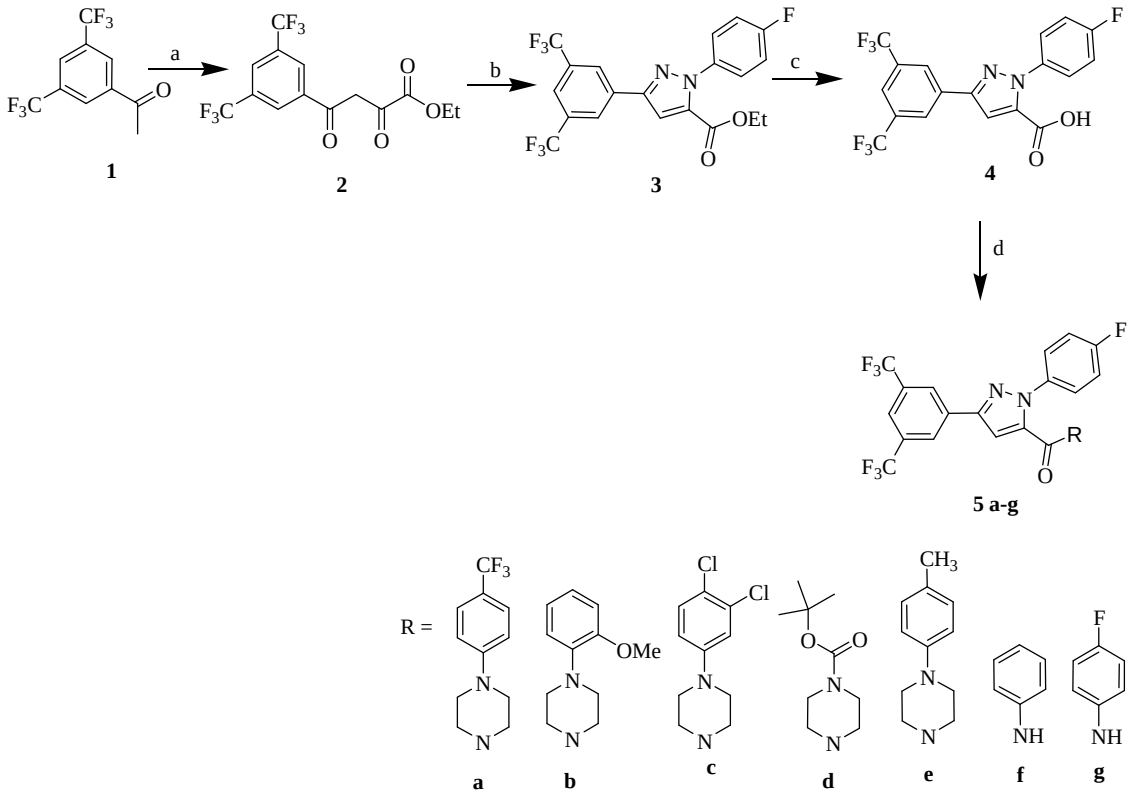
RESULTS AND DICUSSION

Chemistry

Considering the importance of pyrazole derivatives, it was planned to synthesize pyrazole substituted amide as possible antifungal agents. The target compounds **5a-g** was synthesized as per **Scheme 1.** Accordingly ester **2** was obtained from reaction of 3, 5-bistrifluoromethyl acetophenone **1** with diethyl oxalate in presence of NaH using clasien condensation reaction. Ester **2** on reaction with 4-fluoro phenyl hydrazine hydrochloride afforded pyrazole ester **3**. The structure of compound **3** was confirmed by ^{1}H NMR which displayed a triplet at δ 1.63 and quartet at δ 4.66. The pyrazole ester derivative **3** on alkaline hydrolysis afforded the 3-(3, 5-bis (trifluromethyl) phenyl)-1-(4-

flurophenyl)-1-H-pyrazole-5-carboxylic acid **4** was confirmed by ^{1}H NMR wherein the ester peaks dissappered**.** Coupling of the acid **4** with different substituted piperazines and different substituted anilines afforded the final target carboxamide derivatives **5a-g,** respectively. The structure of all the derivatives was confirmed by spectral analysis and the results are presented in the experimental section.

Scheme 1. Synthethic pathway for the compounds 6a-j



Reagent and reaction conditions: (a) diethyl oxalate, NaH, toluene, rt; (b) 4F-C_6H_4-NH-NH_2.HCl, EtOH, 100^0C
(c) NaOH, ethanol, rt; (d) HOBt, sub. piperazine/ aryl amine, TEA, EDC.HCl, dmf, rt.

Biology:

All the synthesized compounds were tested for antifungal activity against fungi *C. albicans*, *A. flavus*, *A. fumigatus* and *A. niger*. To evaluate the activity of the synthesized compounds, the zone of inhibition, minimum inhibitory concentrations (MIC) and minium fungical concentration (MFC) were carried out using dimethyl sulfoxide (DMSO) and determined by using agar diffussion method. Known antibotic Actidione (reference drug) was used for comparison studies. The MIC, MFC were determined in the range of concentration 1000 to 250 µg/mL against the microorganisms tested are reported in Table 1. Analysis of MICs and MFCs revealed few lead molecules with appreciable antifungal activity. Out of the tested compounds (**4, 5a-g**), parent compound **4** did not show any appreciable activity against tested species. The amide substituted derivatives **5a-g** showed moderate to good activity against some fungal species compared to the standard drug. However, it is noteworthy that the amide linkage is responsible for enchancement of activity. Compound **5a**, **5c**, **5d**, **5e** and **5g** was inactive against all tested microorganisms. Compound **5b** and **5f** exhibited four fold (MIC at 250 µg/mL) and two fold (MIC at 500 µg/mL) excellent activity than the standard actidione respectively, against *C. albicans*. The MFC of the compounds **5b** and **5f** was four fold or two fold higher than the corresponding MIC results. Based on the observation, it can be concluded that this class of molecules **5b** and **5f** certainly holds great promise and further studies are necessary to lead them as drug molecule.

Table 1: Minimum Inhibitory concentrations (MIC) and Minimum fungicidal concentration (MFC) of the compounds (4, 5a-g)

Compds	MIC and MFC µg/mL							
	C. albicans		*A.flavus*		*A.fumigatus*		*A.niger*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Actidione	**1000**	**1000**	**500**	**2000**	**1000**	**2000**	**500**	**500**
4	NA	NA	NA	NA	NA	NA	NA	NA
5a	NA	NA	NA	NA	NA	NA	NA	NA
5b	**250**	**500**	NA	NA	NA	NA	NA	NA
5c	NA	NA	NA	NA	NA	NA	NA	NA
5d	NA	NA	NA	NA	NA	NA	NA	NA
5e	NA	NA	NA	NA	NA	NA	NA	NA
5f	**500**	**500**	NA	NA	NA	NA	NA	NA
5g	NA	NA	NA	NA	NA	NA	NA	NA

CONCLUSION

A group of novel 1, 5 -diaryl pyrazoles were synthesized and tested for their antifungal activities. Compounds having the piperazine and aryl amine linkage showed pronounced activity, particularly compounds with 2OMe-piperidine and aryl amine. It can be concluded that the amide linkage is responsible for the activity against tested fungi as the parent carboxylic acid derivative exhibited no activity. In particular, **5b** showed excellent activity against *C. albicans***.** However, it is immature to arrive at any conclusion on structure aspect of these molecules and further evaluation is needed.

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