Synthesis and antifungal activity of 1, 5-diaryl pyrazole substituted thiazole derivatives

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Received 7 August 2014; accepted (revised) 13 April 2015

A series of eight derivatives of 5-bistrifluoromethyl phenyl pyrazole substituted thiazole have been prepared and characterized by \textsuperscript{1}H and \textsuperscript{13}C NMR, and mass spectrometry. All the synthesized derivatives have been screened for antifungal activity against four fungal pathogens such as \textit{Candida albicans}, \textit{Aspergillus flavus}, \textit{Aspergillus fumigatus} and \textit{Aspergillus niger} using broth microdilution method. Active compounds have also been screened for MIC (minimum inhibitory concentration) and MFC (minimum fungicidal concentration). Three compounds 8b, 8f and 8h are found to be equi-potent with standard drug actidione, against \textit{Candida albicans} and \textit{Aspergillus flavus} strains.

\textbf{Keywords}: Pyrazole-thiazole, antifungal activity, MIC, MFC, biological assay

Over the past three decades, the occurrence of fungal infections has consistently increased and commonly seen in patients undergoing organ transplants or anti-cancer chemotherapy and patients with AIDS\textsuperscript{1,2}. Since \textit{Candida albicans}, \textit{Aspergillus flavus} and \textit{Aspergillus fumigatus} are the main causative fungi in which \textit{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\textsuperscript{3}. Despite a large number of antibiotics and chemotherapeutics available for medical use, there is still a need for better drugs, as current antifungal therapy suffers from drug related toxicity and many of these fungi have become resistant to routine antifungal drugs\textsuperscript{4}.

Azoile based antifungal drugs have been developed in the past decades and are in clinical practice\textsuperscript{5-7}. Therefore, identification of new azoile based antifungal agents is warranted. Literature survey revealed that composition of different pharmacophores that linked biheterocyclic rings (azoles) is a growing field to discover new drugs, due to the possible collective effect\textsuperscript{8}. Biheterocyclic pyrazole incorporated thiazole\textsuperscript{9}, thiadiazole\textsuperscript{10}, 1,2,4-oxadiazole\textsuperscript{11}, 1,2,4-triazoles and benzoxazoles\textsuperscript{12} exhibited enhanced pharmacological activity. Similarly, pyrazole and thiazole are familiar class of heterocyclic (azoles) moieties and together possess a wide variety of biological activities including antitumour\textsuperscript{13}, antimicrobial\textsuperscript{14}, anticancer\textsuperscript{15}, EP1 receptor antagonists\textsuperscript{16} and antiviral\textsuperscript{17} and their utility as medicine have drawn attention in the recent years in combating various diseases. Literature also revealed that trifluoromethyl group is responsible for the enhanced biological activity and therefore, is the subject of considerable growing interest\textsuperscript{18}. Encouraged by these observations and in continuation of our research work on the studies of azoile based compounds containing biheterocyclic rings\textsuperscript{19}, we report the synthesis of new class of azoles, wherein potent thiazole moiety is linked to pyrazole moiety at C-3 position and the effect of substituted phenyl-thiazole ring on the antifungal activity.

\textbf{Results and Discussion}

Considering the importance of biheterocyclic compounds, it was planned to synthesize thiazole substituted pyrazole derivatives as possible antifungal agents. The target compounds 8a-h were synthesized as per Scheme I. Accordingly, ethyl 4-(3,5-bis(trifluoromethyl)phenyl)-2,4-dioxobutanoate 2 was obtained by Claisen condensation of 3,5-(bis-trifluoromethyl)acetophenone 1 with diethyl oxalate in presence of NaH. The structure of compound 2 was confirmed by \textsuperscript{1}H NMR which showed presence of ethyl ester protons at \(\delta\) 1.44 (\(-\text{CH}_3\)) and 4.36 (\(-\text{CH}_2\)-). Two singlets at \(\delta\) 8.5, integrating for two protons and \(\delta\) 8.2 integrating for
one proton were attributed to bis-trifluoromethyl phenyl ring. Ester 2 on reaction with 4-fluorophenyl hydrazine hydrochloride afforded pyrazole ester 3. The ester 3 was purified by silica gel column chromatography using hexane and ethyl acetate (9:1) as eluent. Structure of pyrazole ester 3 was ascertained by IR, ¹H NMR and NOE. The ester carbonyl stretching frequencies for compound 3 appeared at 1748 cm⁻¹. The ¹H NMR spectrum of 3 showed proton signals of bis-trifluoromethyl phenyl ring at δ 7.89 and 7.70, integrating for one and two protons, respectively. The pyrazole proton appeared as singlet at δ 7.21. The protons of 4-fluorophenyl ring appeared as two multiplets at δ 7.37 and 7.17. The ethyl ester protons appeared at δ 4.47 (-CH₂-) and 1.44 (-CH₃). The shielding of two protons of bis-trifluoromethyl phenyl ring in 3 could be attributed to the fact that these protons appear in the shielding zone of 4-fluorophenyl ring. This fact was confirmed by observing the NOE effect. In the NOESY spectrum of compound 3, appearance of weak cross peak between δ 7.70 and 7.37 and cross peak between δ 7.70 and 7.17 clearly confirmed the close proximity of the two aromatic rings. Pyrazole ester 3, on alkaline hydrolysis afforded the 5-(3,5-bis(trifluoromethyl)phenyl)-1-(4-fluorophenyl)-1H-pyrazole-3-carboxylic acid 4. The formation of the acid 4 was confirmed by disappearance of ethyl ester proton peaks at δ 4.47 (-CH₂-) and 1.44 (-CH₃) in its ¹H NMR and disappearance of C=O stretching band at 1748 cm⁻¹ in its IR spectrum. Carboxylic acid 4 was converted into amide in situ using thionyl chloride and ammonium hydroxide to obtain 5. The corresponding amide 5 was converted into nitrile 6 by stirring in POCl₃. Formation of nitrile was confirmed by IR which displayed a CN stretching at 2246 cm⁻¹. The nitrile derivative 6 was further converted to the

Reagents and reaction conditions: (a) diethyl oxalate, NaH, toluene, RT; (b) 4F-C₆H₄-NH-NH₂, ethanol, reflux 100°C; (c) NaOH, ethanol; (d) SOCl₂, CHCl₃, reflux, NH₄OH; (e) POCl₃, RT; (f) H₂S; (g) sub. phenacyl bromide, ethanol, reflux

Scheme I — Synthethic pathway for the formation of 8a-h
corresponding thioamide 7 by passing H₂S gas. The IR spectrum of 7 showed the disappearance of CN stretching band. The formation of thiourea was also confirmed by its ¹H NMR spectrum which revealed the amide NH₂ protons at δ 9.46 and 9.78 while other aromatic protons appeared in the aromatic region. Thiourea 7 was then refluxed in ethanol with different substituted phenacyl bromides to afford the target compounds 8a-h. The structure of all the derivatives was confirmed by spectral analysis and the results are presented in the experimental section.

**Antifungal activity**

All the synthesized compounds were tested for antifungal activity against fungi *C. albicans*, *A. flavus*, *A. fumigatus* and *A. niger*. The MIC, MFC were determined in the range of concentration 1000 to 250 µg/mL against the microorganisms tested and the results are presented in Table 1. Analysis of MICs and MFCs revealed some lead molecule with appreciable antifungal activity. Out of the tested compounds (7, 8a-h), parent compound 7 did not show any appreciable activity against tested species. The thiazole substituted derivatives 8a-h showed moderate to good activity against some fungal species compared to the standard drug. However, it was noteworthy that the substitution on phenyl ring exhibited increase in activity irrespective of its nature (electron donating or electron withdrawing substituent). The phenyl substituted derivative 8a was inactive against all tested microorganisms. Compound 8b (fluoro) exhibited two fold higher (MIC at 250 µg/mL) activity than the standard actidione against *A. flavus*, while 8c (chloro), 8d (bromo), 8e (methyl) and 8g (nitro) was inactive against all tested microorganisms. Compound 8f (methoxy) exhibited four fold higher activity (MIC at 250 µg/mL) than the standard actidione against *C. albicans*, while it registered (MIC at 500 µg/mL) comparable activity with standard actidione against *A. flavus*. Compound 8h (3,5-bistrifluoromethyl) registered MIC at 1000 µg/mL against *C. albicans* and MIC at 500 µg/mL against *A. flavus*, comparable with standard actidione. The MFC of the compounds 8b, 8f and 8h was same or four fold higher than the corresponding MIC results. Finally it can be concluded that this class of molecules certainly holds great promise towards the pursuit to discover novel class of antifungal agents.

**Experimental Section**

All anhydrous solvent 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 F₂₅₄ 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 IR spectra were recorded on Shimadzu IR affinity. The ¹H and ¹³C NMR spectra were recorded on a Bruker instrument at 200 and 400 MHz spectrometer (Germany) using tetramethylsilane (TMS) as an internal standard. The chemical shift values are recorded on scale and the coupling constants (J) are in Hertz. Mass spectrometry was recorded on Waters, Q-TOF MICROMASS (LC-MS).

**Synthesis of ethyl 4-(3, 5-bistrifluoromethyl)phenyl)-2, 4-dioxobutanoate (2):** To a solution of sodium hydride (2.0 mol) in toluene, diethyl oxalate (1.5 mol) was added at RT. To this a solution of 3,5-bistrifluoromethyl acetonphenone (1.0 mol) in toluene (1.5 mol) was added at RT. 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 the white solid, m.p. 60-62°C.

**Synthesis of ethyl 3-(3, 5-bistrifluoromethyl)phenyl)-1-(4-fluorophenyl)-1H-pyrazole-5-carboxylate 3:** The solution 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°C for 6 hr. After completion of the reaction, the solvent was evaporated and the mixture was then poured on to crushed ice to obtain the crude product as white solid. The pyrazole ester 3 was purified by silica gel column chromatography using hexane: ethyl acetate (9:1) as eluent.

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**Table 1 — Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) for the compounds 8a-h**

<table>
<thead>
<tr>
<th>Compd*</th>
<th><em>C. albicans</em></th>
<th><em>A. flavus</em></th>
<th><em>A. fumigatus</em></th>
<th><em>A. niger</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
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<tr>
<td>8b</td>
<td>NA</td>
<td>NA</td>
<td>250</td>
<td>250</td>
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<tr>
<td>8f</td>
<td>250</td>
<td>250</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>8h</td>
<td>1000</td>
<td>NA</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>Actidione</td>
<td>1000</td>
<td>1000</td>
<td>500</td>
<td>1000</td>
</tr>
</tbody>
</table>

*compounds which showed MIC and MFC < 1000 µg/mL are only reported. NA-compounds showed MIC and MFC > 1000 µg/mL.
m.p. 84-86°C. 1H NMR (500 MHz, CDCl3): δ 1.44 (m, 3H, -CH3), 4.47 (m, 2H, -CH2-), 7.11-7.13 (m, 2H, Ar-H), 7.21 (s, 1H, pyrazole-CH3), 7.30-7.34 (m, 2H, Ar-H), 7.66 (s, 2H, Ar-H), 7.85 (s, 1H, Ar-H).

Synthesis of 5-(3,5-bis(trifluoromethyl)phenyl)-1-(4-fluorophenyl)-1H-pyrazole-3-carboxylic acid 4: A solution of ethyl 5-(3,5-bis(trifluoromethyl)phenyl)-1-(4-fluorophenyl)-1H-pyrazole-3-carboxylate 3 (1.0 mol) in ethanolic NaOH (1.2 mol) was stirred for 4 hr at RT. After completion of the reaction, the mixture was poured on to crushed ice to obtain white solid. The progress of the reaction was monitored on TLC. The reaction mixture was cooled and filtered. The solid obtained was washed with cold ethanol. The final products were purified by column chromatography using hexane:ethyl acetate as eluent.

5-(3,5-bis(trifluoromethyl)phenyl)-3-(5-(4-chlorophenyl)thiazol-2-yl)-1H-pyrazole 5a: Yield 70%, White solid, m.p. 184-86°C.

5-(3,5-bis(trifluoromethyl)phenyl)-3-(5-(4-bromo-phenyl)thiazol-2-yl)-1H-pyrazole 5b: Yield 80%, White solid, m.p. 218-20°C.

5-(3,5-bis(trifluoromethyl)phenyl)-3-(5-(4-chlorophenyl)thiazol-2-yl)-1H-pyrazole 5c: Yield 84%, White solid, m.p. 178-80°C.

5-(3,5-bis(trifluoromethyl)phenyl)-3-(5-(4-methylphenyl)thiazol-2-yl)-1H-pyrazole 5d: Yield 80%, White solid, m.p. 176-78°C.

5-(3,5-bis(trifluoromethyl)phenyl)-3-(5-(4-ethylphenyl)thiazol-2-yl)-1H-pyrazole 5e: Yield 76%, White solid, m.p. 188-90°C.

5-(3,5-bis(trifluoromethyl)phenyl)-3-(5-(4-iso-propylphenyl)thiazol-2-yl)-1H-pyrazole 5f: Yield 76%, White solid, m.p. 196-98°C.

5-(3,5-bis(trifluoromethyl)phenyl)-3-(5-(4-tert-butylphenyl)thiazol-2-yl)-1H-pyrazole 5g: Yield 70%, White solid, m.p. 204-4°C.

5-(3,5-bis(trifluoromethyl)phenyl)-3-(5-(4-chloro-2-naphthyl)thiazol-2-yl)-1H-pyrazole 5h: Yield 78%, White solid, m.p. 194-96°C.

5-(3,5-bis(trifluoromethyl)phenyl)-3-(5-(4-fluorophenyl)thiazol-2-yl)-1H-pyrazole 5i: Yield 76%, White solid, m.p. 202-24°C.
7.32-7.36 (m, 3H, Ar-H, pyrazole-CH-), 7.49-7.53 (m, 3H, Ar-H, thiazole-CH-), 7.82-7.87 (m, 2H, Ar-H), 8.01 (s, 2H, Ar-H), 8.12 (s, 1H, Ar-H); HRMS (M+1)^+: 548.0986.

5-(3, 5-Bis(trifluoromethyl)phenyl)-1-(4-fluorophenyl)-3-(5-(4-nitrophenyl) thiazol-2-yl)-1H-pyrazole 8f: Yield 68%, White solid, m.p. 212-14°C, ^1H NMR (200 MHz, CDCl_3): δ 3.8 (s, 3H, OCH_3), 7.02-7.04 (m, 2H, Ar-H), 7.32-7.38 (m, 2H, Ar-H), 7.49-7.53 (m, 2H, Ar-H), 7.63 (s, 1H, pyrazole-CH-), 7.86-8.01 (m, 5H, Ar-H), 8.12 (s, 1H, Ar-H); HRMS (M+1)^+: 564.0987.

5-(3, 5-Bis(trifluoromethyl)phenyl)-1-(4-fluorophenyl)-3-(5-(4-methoxyphenyl) thiazol-2-yl)-1H-pyrazole 8g: Yield 76%, White solid, m.p. 182-84°C, ^1H NMR (400 MHz, CDCl_3): δ 7.11-7.16 (m, 2H, Ar-H), 7.31-7.36 (m, 3H, Ar-H, pyrazole-CH-), 7.50 (s, 1H, thiazole-CH-), 7.60-7.65 (m, 2H, Ar-H), 7.70 (s, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 7.94-7.97 (m, 2H, Ar-H). HRMS (M+1)^+: 670.0704.

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 Agar plates (SDA), using Dextrose broth (SDB) (Hi Media, India) at RT for 48 hr. The fungi was inoculated in each tube. The tubes were incubated at RT for 24 hr 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°C at 24 hr. The lowest concentration which showed no growth was recorded as MFC.

Conclusion

In conclusion, eight 1,5-diaryl pyrazole thiazole derivatives 8a-h were synthesized and investigated for their antifungal activities. The synthesized compounds 7, 8a, 8c, 8d, 8e and 8g were inactive against the fungal species while compounds 8b, 8f and 8h exhibited higher antifungal activities against two fungi in vitro. In particular, 8f showed excellent activity against C. albicans. It was noteworthy that derivatives possessing both electron donating (methoxy) as well as electron withdrawing substituent (flouro) on phenyl ring significantly enhance the antifungal activity compared to the other substitutions on phenyl ring. It is immature to arrive at any conclusion on structure activity aspect of these molecules and further studies are warranted.

Acknowledgments

The authors thank CSIR, New Delhi for financial support. SAIF, Punjab University and CIF, S.P. Pune University is also acknowledged for providing spectral analysis.

References