Synthesis and biological screening of novel pyrazole and isoxazole derivatives

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Novel 1,5-disubstituted pyrazole and isoxazole derivatives like, aryl 5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid 8a-e, [(aryl)-piperazin-1-yl]-[5-(3-fluoro-4-methoxyphenyl)-isoxazol-3-yl]-methanone 9a-e, aryl 5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid 10a-e have been synthesized and characterized using IR, 1H NMR, 13C NMR and mass spectral analysis. All the synthesized compounds have been screened for their antibacterial activity against S. aureus, E. coli, B. subtilis, P. aeruginosa, S. pyogenes, K. terrigena and K. pneumoniae and antifungal activity against T. viride, A. flavus, A. brasillansis, and C. albicans. Interestingly all the synthesized compounds exhibit good antibacterial and antifungal activity.

Keywords: Pyrazole, isoxazole, antibacterial, antifungal

Azole containing compounds exhibit both antibacterial and antifungal activities and some of them are in clinical practice as antimicrobial agents. Because of the extensive use of theseazole drugs, some microbial strains have developed resistance to these drugs. The increasing number of azole drug resistant strains has initiated research to develop new antimicrobial compounds. Some pyrazole derivatives are extensively studied and used as antimicrobial agents1-20. Pyrazoles are an important class of heterocyclic compound and many pyrazole derivatives are reported to have broad spectrum of biological activities like anti-inflammatory21-23, antifungal24, herbicidal25,26, antitumor, cytotoxic27, and antiviral28,29 activities. Pyrazole derivatives also act as antiangiogenic agents30, A3 adenosine receptor antagonists31, neuropeptide YY5 receptor antagonists32, kinase inhibitor for treatment of type 2 diabetes, hyperlipidemia, obesity33, and thrombopiotininmetics34. Recently urea derivatives of pyrazoles have been reported as potent inhibitors of p38 kinase35. As the high electronegativity of halogens (particularly chlorine and fluorine) in the aromatic part of the drug molecules plays an important role in enhancing their biological activity, we became interested to incorporate 4-fluoro or 4-chloro substitution in the aryl rings of 1, 5-diaryl pyrazoles. As a part of our ongoing research, aiming the synthesis of new antimicrobial compounds36, here in we report the synthesis of novel pyrazole derivatives and their antimicrobial activities.

Results and Discussion

The target compounds 8a-e, 9a-e and 10a-e were synthesized according to Scheme I. Initially, acetophenone 1 was treated with diethyl oxalate in presence of sodium hydride to obtain diketo ester 2. The formation of ester 2 was confirmed by 1H NMR which showed the absence of carbonyl peak at 1690 cm⁻¹. The 1H NMR spectrum showed disappearance of acid proton at δ14.10 and change in IR frequency of acid to amide from 1661 to 1639 cm⁻¹.

The 1H NMR spectrum showed disappearance of acid proton and appearance of piperazine protons between δ2.25-3.40 and 3.60-3.90. Similarly, compound 2 was treated with hydrazine hydrate to obtain pyrazole ester 4. The structure of compound 4 was confirmed by 1H NMR which showed NH proton of pyrazole at δ13.72-13.85 and...
absence of carbonyl peak at 1690 cm\(^{-1}\) in IR. Further compound 4 was alkylated using different alkyl or aryl halide to obtain corresponding N-substituted pyrazole esters 7a-e. Structures of 7a-e were established by \(^1\)H NMR which displayed the proton peaks due to alkyl or aryl substitution and the disappearance of NH proton peak. Compounds 7a-e were further subjected to alkaline hydrolysis to afford acid derivatives 10a-e. Structures of compounds 10a-e were confirmed by \(^1\)H NMR wherein absence of ester peaks at \(\delta\) 13.40 confirmed their formation. Similarly, the structures of all the other derivatives were confirmed similarly and the results are presented in the experimental part. Physical data of the final compounds are presented in Table I.

All the synthesized pyrazole and isoxazole derivatives (8a-e, 9a-e and 10a-e) were tested for their \textit{in vitro} antibacterial activity against clinically isolated bacterial strains such as \textit{S.aureus}, \textit{E.coli}, \textit{B.subtilis}, \textit{P.aeruginosa}, \textit{S.pyogens}, \textit{K.terrigena} and \textit{K.pneumonia} by using disc method and minimum inhibitory concentration (MIC). Antibacterial results are summarized in Table II, which indicated that, most of the synthesized compounds registered the MIC at 100\,\mu g/mL or less, that
is, these compounds exhibited comparable or at most two fold less activity against all bacterial strains as compared with standard drug Chloramphenicol.

Compound 9e exhibited excellent comparable activity against S. aureus while compounds 8d, 9c and 10a showed excellent activity against E. coli. Compounds 8c, 9c and 10e exhibited good activity against B. subtilis, compounds 10a and 10c showed promising activity against P. aeruginosa while compounds 8c and 8d were active against S. pyogenes. Compounds 8b, 8c and 10e exhibited good activity against K. terrigena. All these compounds registered MIC at 60µg/mL comparable to the standard drug. Remaining compounds 8a, 9a, 9b, 9d, 9e, 10b and 10d exhibited moderate antibacterial activity with MIC ranging from 70-100 µg/mL as compared to the standard drug.

All the synthesized pyrazole and isoxazole derivatives (8a-e, 9a-e and 10a-e) were screened for their in vitro antifungal activity against clinically isolated bacterial strains such as T. viride, A. flavus, A. brasillansis and C. albicans by using disc method and minimum inhibitory concentration (MIC) at very low concentration 100 µg/ml. The results obtained are summarized in Table III

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<th>Entry</th>
<th>S.aureus</th>
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<th>B.subtilis</th>
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Experimental Section

Melting points of compounds were determined in open capillary tubes in silicon oil bath using Veego melting point apparatus and are uncorrected. Purity of
compounds was monitored by TLC on silica F254 coated aluminum plates (Merck) as adsorbent and U.V. light and iodine chamber as a visualizing agent. IR spectra (KBr in cm\(^{-1}\)) were recorded on a Shimadzu Model FTIR-435. NMR spectra were recorded on a Varian Mercury TH-300 and TH-400 operating at 300 and 400 MHz (\(^{1}H\) NMR) and 100 MHz (\(^{13}C\) NMR) using CDCl\(_3\) as a solvent and TMS as an internal standard (Chemical shift in ppm). All chemicals and solvents used are of analytical grade.

### Table III — Minimum fungal inhibitory concentrations (MIC µg/mL) of the compounds 8a-e, 9a-e, 10a-e

<table>
<thead>
<tr>
<th>Entry</th>
<th>(T.) viride</th>
<th>A. flavus</th>
<th>A. brazillians</th>
<th>C. albicans</th>
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<tbody>
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Synthesis of ethyl 5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylate, 3. Ethyl 4-(3-fluoro-4-methoxyphenyl)-4-hydroxy-2-oxobut-3-enoate 2 (1 mol) and hydroxyl amine hydrochloride (3 mole equiv) in ethanol (20 mL) was refluxed for 8 hr. After removal of ethanol in vacuum, the oil obtained was poured into ice cold water which solidified. The solid was filtered and purified by silica-flash chromatography (hexane/ethyl acetate = 8:2). Yield: 85%, m.p. 135–40°C; FT-IR: 1732 (C=O, ester), 1519 (N-O), 1458 (C=C), 1253 cm\(^{-1}\); \(^{1}H\) NMR (400 MHz, DMSO): \(\delta\) 1.42-1.46 (t, 3H, \(-CH\_\text{F}\)); 3.85 (s, 3H, \(-O-CH\_\text{F}\)); 7.51-7.57 (m, 2H, \(\text{ArH}\)).

Synthesis of ethyl 5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylate, 4. Compound 2 (25.0 g, 0.095 mol) and hydrazine hydrate (0.28 mol) in ethanol (25 mL) was refluxed for 8 hr. After evaporation of ethanol in vacuum, the oil obtained poured into ice cold water. The solid obtained was filtered and purified by silica gel flash chromatography (hexane/ethyl acetate = 8:2). (21.42 g) Yield: 87%, m.p. 158–162°C; FT-IR: 1716 (C=O, ester), 1690 (N-O), 1494 (C=C), 1260 (C-F), 1138 cm\(^{-1}\); \(^{1}H\) NMR (400 MHz, DMSO): \(\delta\) 1.35-1.39 (t, 3H, \(-CH\_\text{F}\)); 3.89 (s, 3H, \(-O-CH\_\text{F}\)); 4.31-4.36 (q, 2H, \(J = 7.2\) Hz, \(-O-\text{CH}_{2}-CH\_\text{F}\)); 7.11-7.30 (m, 2H, \(\text{ArH}\)), 7.55-7.62 (m, 2H, \(\text{ArH}\)), 13.72-13.85 (bs, 1H, \(-NH\)), \(m/z\) 265 (M+H).

General procedure for the synthesis of ethyl 1-(substitutedphenyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylate, 5a-e

Ethyl 4-(3-fluoro-4-methoxyphenyl)-4-hydroxy-2-oxobut-3-enoate 2 (4.85 mol) and hydrazine hydrate (0.28 mol) in ethanol were refluxed for 8 hours. After evaporation of ethanol in vacuum, the oil obtained was filtered and washed with water. The solid compound was dried under vacuum at 50°C. The crude product was purified by crystallization in methanol to yield 2 as a yellow solid (25.46 g, 95%), m.p. 90-95°C; FT-IR: 1738 (C=O, ester), 1690 (C=O, ketone) 1442 (C=C), 1265 cm\(^{-1}\) (C-F); \(^{1}H\) NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.25-1.44 (t, 3H, \(J = 7.2\) Hz, \(-CH\_\text{F}\)); 3.98 (s, 3H, \(-O-\text{CH}_{2}-CH\_\text{F}\)); 4.36-4.43 (q, 2H, \(J = 7.2\) Hz, \(-O-\text{CH}_{2}-CH\_\text{F}\)); 6.99-7.02 (m, 2H, \(\text{ArH}\)), 7.73-7.81 (m, 2H, \(\text{ArH}\)).

Synthesis of ethyl 5-(3-fluoro-4-methoxyphenyl)-isoxa-zole-3-carboxylate, 3. Ethyl 4-(3-fluoro-4-methoxyphenyl)-4-hydroxy-2-oxobut-3-enoate 2 (1 mol) and hydrazine hydrate (3 mole equiv) in ethanol (20 mL) was refluxed for 8 hr. After removal of ethanol in vacuum, the oil obtained was poured into ice cold water which solidified. The solid was filtered and purified by silica-flash chromatography (hexane/ethyl acetate = 8:2). Yield: 85%, m.p. 135–40°C; FT-IR: 1732 (C=O, ester), 1519 (N-O), 1458 (C=C), 1253 cm\(^{-1}\) (C-F); \(^{1}H\) NMR (400 MHz, DMSO): \(\delta\) 1.35-1.39 (t, 3H, \(-CH\_\text{F}\)); 3.89 (s, 3H, \(-O-\text{CH}\_\text{F}\)); 4.31-4.36 (q, 2H, \(J = 7.2\) Hz, \(-O-\text{CH}_{2}-CH\_\text{F}\)); 7.11-7.30 (m, 2H, \(\text{ArH}\)), 7.55-7.62 (m, 2H, \(\text{ArH}\)), 13.72-13.85 (bs, 1H, \(-NH\)), \(m/z\) 265 (M+H).

Ethyl 1-(4-chlorophenyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylate, 5b. Yield 86%; m.p. 94-96°C; IR (KBr): 2933 (C=H), 1738 (C=O, ester), 1690 (C=O, ketone) 1442 (C=C), 1265 cm\(^{-1}\) (C-F); \(^{1}H\) NMR (400 MHz, DMSO): \(\delta\) 1.25-1.44 (t, 3H, \(J = 7.2\) Hz, \(-CH\_\text{F}\)); 3.98 (s, 3H, \(-O-\text{CH}_{2}-CH\_\text{F}\)); 4.36-4.43 (q, 2H, \(J = 7.2\) Hz, \(-O-\text{CH}_{2}-CH\_\text{F}\)); 6.99-7.02 (m, 2H, \(\text{ArH}\)), 7.73-7.81 (m, 2H, \(\text{ArH}\)).

Ethyl 5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylate, 5b. Yield 86%; m.p. 94-96°C; IR (KBr): 2933 (C=H), 1738 (C=O, ester),
1693 (C=O), 1489 (C=C), 1278 (C-F), 1129 cm$^{-1}$ (C-N);
$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.33-1.36 (t, 3H, CH$_2$-$CH_3$), 3.84 (s, 3H, O-CH$_2$-$CH_3$), 4.33 (q, 2H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 6.93-6.97 (m, 2H, ArH), 7.15-7.18 (m, 2H, ArH), 7.32-7.44 (m, 4H, ArH).

**Ethyl 5-(3-fluoro-4-methoxyphenyl)-1-(4-methoxyphenyl)-1H-pyrazole-3-carboxylate, 5c:** Yield 81%; m.p. 94-99°C; IR (KBr): 1739 cm$^{-1}$ (C=O, ester), 1484°C (C-F), 1278 cm$^{-1}$ (C-N); $^1$H NMR (400 MHz, CDCl$_3$): δ 1.36-1.40 (t, 3H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 3.85 (s, 3H, O-CH$_2$-$CH_3$), 4.30 (q, 2H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 6.03-6.07 (m, 2H, ArH), 7.15-7.20 (m, 3H, ArH), 7.32-7.40 (m, 2H, ArH).

**Ethyl 1-(3-chlorophenyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylate, 5e:** Yield 85%; m.p. 110-120°C; IR (KBr): 1739 cm$^{-1}$ (C=O, ester), 1484°C (C-F), 1275 cm$^{-1}$ (C-N); $^1$H NMR (400 MHz, CDCl$_3$): δ 1.30-1.38 (t, 3H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 3.85 (s, 3H, O-CH$_2$-$CH_3$), 4.32 (q, 2H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 6.03-6.07 (m, 2H, ArH), 7.15-7.22 (m, 3H, ArH), 7.32-7.40 (m, 2H, ArH).

**Ethyl 1-(3-fluoro-4-methoxyphenyl)isoxazole-3-carboxylic acid, 6:** Compound 3 (15.0 g, 1.0 mol) and NaOH (5.0 g) were taken in methanol (50 mL) and the mixture was refluxed for 8-10 hr. After completion of the reaction as indicated on TLC, the reaction mixture was poured into ice cold water. The resultant mixture was acidified with dil. HCl to obtain the product. Yield 89%; $^1$H NMR (400 MHz, CDCl$_3$): δ 3.81 (s, 3H, O-CH$_2$-$CH_3$), 6.92 (s, 1H, ArH), 7.08-7.13 (m, 1H, ArH), 7.48-7.54 (m, 2H, ArH).

**General procedure for the synthesis of ethyl 5-(3-fluoro-4-methoxyphenyl)-1-(alkyl/aryl)-1H-pyrazole-3-carboxylate, 7a-e**

To a solution of compound 4 (1.0 mol) in ethanol (10 mL), alkyl or aryl halide (1.2 mol) was added and refluxed for 6-8 hr. The reaction was monitored on TLC, after the completion of reaction 10 gm crushed ice was added to the reaction mixture. The crude product obtained was filtered and purified by column chromatography using hexane: ethyl acetate (8:2) as eluent to obtain the pure product in 73-81% yield.

**Ethyl 5-(3-fluoro-4-methoxyphenyl)-1-methyl-1H-pyrazole-3-carboxylate, 7a:** Yield 88%; m.p. 122-24°C; IR (KBr): 1739 cm$^{-1}$ (C=O, ester), 1484°C (C-F), 1278 cm$^{-1}$ (C-N); $^1$H NMR (400 MHz, CDCl$_3$): δ 1.34-1.35 (t, 3H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 3.84 (s, 3H, O-CH$_2$-$CH_3$), 3.98 (s, 3H, CH$_3$), 4.32-4.35 (q, 2H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 7.08-7.20 (m, 2H, ArH), 7.56-7.67 (m, 2H, ArH).

**Ethyl 1-(cyanomethyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylate, 7b:** Yield 83%; m.p. 119-22°C; IR (KBr): 1739 cm$^{-1}$ (C=O, ester), 1484°C (C-F), 1278 cm$^{-1}$ (C-N); $^1$H NMR (400 MHz, CDCl$_3$): δ 1.35-1.38 (t, 3H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 3.90 (s, 3H, O-CH$_2$-$CH_3$), 4.34-4.44 (q, 2H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 4.87 (s, 2H, CH$_2$-CN), 6.96-7.01 (m, 2H, ArH), 7.46-7.55 (m, 2H, ArH).

**Ethyl 1-(cyclopropylmethyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylate, 7e:** Yield 83%; m.p. 128-30°C; IR (KBr): 1739 cm$^{-1}$ (C=O, ester), 1484°C (C-F), 1286 (C-C), 1131 cm$^{-1}$ (C-N); $^1$H NMR (400 MHz, CDCl$_3$): δ 0.45-0.78 (m, 5H, cyclopropyl), 1.34-1.37 (t, 3H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 3.82 (s, 3H, CH$_2$-Ph), 3.91 (s, 3H, O-CH$_2$-$CH_3$), 4.31-4.41 (q, 2H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 7.06-7.20 (m, 1H, ArH), 7.57-7.62 (m, 3H, ArH).

**Ethyl 1-allyl-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylate, 7d:** Yield 73%; m.p. 124-27°C; IR (KBr): 1739 cm$^{-1}$ (C=O, ester), 1484°C (C-F), 1286 cm$^{-1}$ (C-N); $^1$H NMR (400 MHz, CDCl$_3$): δ 1.35-1.38 (t, 3H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 3.96 (s, 3H, O-CH$_2$-$CH_3$), 4.29-4.36 (q, 2H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 4.45 (m, 1H, CH$_2$-CH$_2$-CH$_3$), 5.16-5.19 (m, 2H, CH$_2$-CH$_2$-CH$_2$), 5.76-5.87 (m, 1H, CH$_2$-CH$_2$-CH$_3$), 7.06-7.19 (m, 1H, ArH), 7.55-7.65 (m, 3H, ArH).

**Ethyl 1-benzyl-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylate, 7e:** Yield 81%; m.p. 132-35°C; IR (KBr): 1739 cm$^{-1}$ (C=O, ester), 1488°C (C-C), 1289 (C-F), 1132 cm$^{-1}$ (C-N); $^1$H NMR (400 MHz, CDCl$_3$): δ 1.30-1.38 (t, 3H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 3.88 (s, 3H, O-CH$_2$-$CH_3$), 4.27-4.36 (q, 2H, J = 7.1 Hz, -O-CH$_2$-$CH_3$), 5.02 (s, 2H, CH$_2$-Ph), 7.07-7.32 (m, 1H, ArH), 7.56-7.74 (m, 8H, ArH).
General procedure for the synthesis of 1-(substituted-phenyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid, 8a-e

Compound 5a-e (1.0 g, 1.0 mol) and NaOH (0.5 g) were taken in methanol (10 mL) and the mixture was refluxed for 8-10 hr. After completion of the reaction as indicated on TLC, the reaction mixture was poured into ice cold water. The resultant mixture was acidified with dil. HCl to obtain the product which was purified by silica-flash chromatography (chloroform/methanol = 8:2).

1-(4-Chlorophenyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid, 8a: Yield 89%; m.p. 160-65°C; IR (KBr): 2932 (-C-H), 1700 (C=O), 1494 (C=C), 1260 (C-F), 1138 cm⁻¹ (C-N); ¹H NMR (400 MHz, CDCl₃): δ 3.83 (s, 3H, -OCH₃), 6.97-7.00 (m, 2H, ArH), 7.18-7.22 (m, 2H, ArH), 7.38-7.40 (m, 2H, ArH), 7.56-7.60 (m, 2H, ArH) 13.10 (bs, 1H, -COOH); MS: m/z 247 (M+H).

5-(Fluoro-4-methoxyphenyl)-1-(4-fluorophenyl)-1H-pyrazole-3-carboxylic acid, 8b: Yield 76%; m.p. 200-205°C; IR (KBr): 2932 (-C-H), 1689 (C=O), 1484 (C=C), 1279 (C-F), 1123 cm⁻¹ (C-N); ¹H NMR (400 MHz, CDCl₃): δ 3.80 (s, 3H, -OCH₃), 6.95-6.99 (m, 2H, ArH), 7.17-7.20 (m, 2H, ArH), 7.30-7.41 (m, 4H, ArH), 13.00 (bs, 1H, -COOH); MS: m/z 331 (M+H).

5-(Fluoro-4-methoxyphenyl)-1-(3-fluorophenyl)-1H-pyrazole-3-carboxylic acid, 8c: Yield 84%; m.p. 144-48°C; IR (KBr): 2936 (-C-H), 1692 (C=O), 1492 (C=C), 1254 (C-F), 1132 cm⁻¹ (C-N); ¹H NMR (400 MHz, CDCl₃): δ 3.80 (s, 3H, -OCH₃), 3.83 (s, 3H, -OCH₃), 6.98-7.02 (m, 3H, ArH), 7.15-7.18 (m, 2H, ArH), 7.22-7.28 (m, 2H, ArH), 12.92 (bs, 1H, -COOH); MS: m/z 243 (M+H).

5-(Fluoro-4-methoxyphenyl)-1-(3-fluorophenyl)-1H-pyrazole-3-carboxylic acid, 8d: Yield 79%; m.p. 170-174°C; IR (KBr): 2967 (-C-H), 1692 (C=O), 1489 (C=C), 1262 (C-F), 1137 cm⁻¹ (C-N); ¹H NMR (400 MHz, CDCl₃): δ 3.83 (s, 3H, -OCH₃), 7.01-7.08 (m, 2H, ArH), 7.18-7.23 (m, 3H, ArH), 7.32-7.40 (m, 2H, ArH), 7.50-7.58 (m, 1H, ArH), 13.1 (bs, 1H, -COOH); MS: m/z 331 (M+H).

1-(3-Chlorophenyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid, 8e: Yield 89%; m.p. 155-60°C; IR (KBr): 2936 (-C-H), 1693 (C=O), 1482 (C=C), 1275 (C-F), 1128 cm⁻¹ (C-N); ¹H NMR (400 MHz, CDCl₃): δ 3.81 (s, 3H, -OCH₃), 7.00-7.08 (m, 2H, ArH), 7.12-7.22 (m, 3H, ArH), 7.46-7.60 (m, 2H, ArH), 13.08 (bs, 1H, -COOH); MS: m/z 247 (M+H).

General procedure for the synthesis of [4-(aryl-piperciniz-1-yl) [5-(3-fluoro-4-methoxyphenyl)isoaxazol-3-yl)methanone, 9a-e

A mixture of carboxylic acid 6 (3.5 mol), EDC.HCl (5.4 mol), HOBr (5.4 mol) in anhydrous DMF (20 mL) was stirred at RT. Substituted pipercazine (3.5 mol) was then added and the mixture was further stirred at RT for 10-12 hr. After completion of the reaction as indicated by TLC, the reaction mixture was quenched in crushed ice. The precipitated solid was washed with NaHCO₃ and dil. HCl. The product thus obtained was purified by silica gel column chromatography using hexane: ethyl acetate as eluent.

[4-(3,4-Dichlorophenyl) piperciniz-1-yl][5-(3-fluoro-4-methoxyphenyl)isoaxazol-3-yl)methanone, 9a: Yield 88%; m.p. 185-88°C; IR (KBr): 1658 (C=O), 1519 (N-O), 1489 (C=C), 1289 (C-F), 1130 cm⁻¹ (C-N); ¹H NMR (400 MHz, CDCl₃): δ 3.20-3.39 (m, 4H, CH₂-pipercazine), 3.80-3.88 (m, 4H, CH₂-pipercazine), 3.95 (s, 3H, -OCH₃), 7.11-7.15 (m, 1H, ArH), 7.20-7.25 (m, 3H, ArH), 7.30-7.38 (m, 2H, ArH), 7.75-7.90 (m, 2H, ArH); MS: m/z 451 (M+H).

[5-(Fluoro-4-methoxyphenyl) isoaxazol-3-yl][4-(3-trifluoromethyl)pheny]piperciniz-1-yl)methanone, 9b: Yield 83%; m.p. 110-116°C; IR (KBr): 1655 (C=O), 1519 (N-O), 1489 (C=C), 1289 (C-F), 1130 cm⁻¹ (C-N); ¹H NMR (400 MHz, CDCl₃): δ 3.20-3.39 (m, 4H, CH₂-pipercazine), 3.80-3.88 (m, 4H, CH₂-pipercazine), 3.95 (s, 3H, -OCH₃), 7.11-7.15 (m, 1H, ArH), 7.20-7.25 (m, 3H, ArH), 7.30-7.38 (m, 2H, ArH), 7.75-7.90 (m, 2H, ArH); MS: m/z 450 (M+H).

[5-(Fluoro-4-methoxyphenyl) isoaxazol-3-yl][4-(2-methoxyphenyl)piperciniz-1-yl)methanone, 9c: Yield 78%; m.p. 134-38°C; IR (KBr): 1660 (C=O), 1519 (N-O), 1488 (C=C), 1289 (C-F), 1130 cm⁻¹ (C-N); ¹H NMR (CDCl₃, 400 MHz): δ 2.95-3.05 (m, 4H, CH₂-pipercazine), 3.78-3.83 (m, 4H, CH₂-pipercazine), 3.80 (s, 3H, -OCH₃), 3.97 (s, 3H, -OCH₃), 6.85-7.00 (m, 4H, ArH), 7.35-7.40 (m, 2H, ArH), 7.72-8.50 (m, 2H, ArH).

[4-(Chlorobenzyl)piperciniz-1-yl] [5-(3-fluoro-4-methoxyphenyl)isoaxazol-3-yl)methanone, 9d: Yield 70%; m.p. 128-33°C; IR (KBr): 1654 (C=O), 1516 (N-O), 1482 (C=C), 1285 (C-F), 1130 cm⁻¹ (C-N); ¹H NMR (400 MHz, CDCl₃): δ 2.31-3.40 (m, 4H, CH₂-pipercazine), 3.60-3.78 (m, 4H, CH₂-pipercazine), 3.93 (s, 3H, -OCH₃), 7.20-7.25 (m, 1H, ArH), 7.27-7.48 (m, 6H, ArH), 7.71-7.82 (m, 2H, ArH); MS: m/z 430 (M+H).

[5-(Fluoro-4-methoxyphenyl)isoaxazol-3-yl[piperciniz-1-yl)methanone, 9e: Yield 79%; m.p. 155-56°C; IR (KBr): 1659 (C=O), 1513 (N-O), 1478 (C=C), 1284
General procedure for the synthesis of 5-(3-fluoro-4-methoxyphenyl)-1-(alkyl)-1H-pyrazole-3-carboxylic acid, 10a–e

Compound 7a-e (1.0 g, 1.0 mol) and NaOH (0.5 g) were taken in methanol (10 mL) and the mixture was refluxed for 8-10 hr. After completion of the reaction as indicated on TLC, the reaction mixture was poured into ice cold water. The resultant mixture was acidified with dil. HCl to obtain the product which was purified by silica column chromatography (chloroform/methanol = 8:2).

5-(3-Fluoro-4-methoxyphenyl)-1-methyl-1H-pyrazole-3-carboxylic acid, 10a: Yield 88%; m.p. 174-77°C; IR (KBr): 1681 (C=O), 1488 (C=C), 1286 (C-F), 1132 cm⁻¹ (C-N); ¹H NMR (400 MHz, CDCl₃): δ 1.55-1.59 (m, 6H, -CH₂), 2.78-2.83 (m, 1H, ArH), 7.03-7.11 (m, 2H, ArH), 7.51-7.57 (m, 2H, ArH); MS: m/z 291 (M+H).

1-(Cyanomethyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid, 10b: Yield 83%; m.p. 198-200°C; IR (KBr): 1682 (C=O), 1487 (C=C), 1287 (C-F), 1127 cm⁻¹ (C-N); ¹H NMR (400 MHz, CDCl₃): δ 3.90 (s, 3H, -OCH₃), 4.87 (s, 2H, -CH₂-CN), 6.96-7.01 (m, 2H, ArH), 7.46-7.55 (m, 2H, ArH), 11.22 (bs, 1H, -COOH); MS: m/z 251 (M+H).

1-(Cyclopropylmethyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid, 10c: Yield 90%; m.p. 210-14°C; IR (KBr): 1684 (C=O), 1480 (C=C), 1284 (C-F), 1133 cm⁻¹ (C-N); ¹H NMR (400 MHz, CDCl₃): δ 2.967 (-C=H), 2.962 (-CH₂), 3.91 (s, 3H, -OCH₃), 7.06-7.20 (m, 1H, ArH), 7.57-7.62 (m, 3H, ArH), 11.26 (bs, 1H, -COOH); MS: m/z 291 (M+H).

1- Allyl-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid, 10d: Yield 78%; m.p. 217-20°C; IR (KBr): 1680 (C=O), 1484 (C=C), 1286 (C-F), 1133 cm⁻¹ (C-N); ¹H NMR (400 MHz, CDCl₃): δ 2.967 (-C=H), 1692 (C=O), 1489 (C=C), 1262 (C-F), 1137 (C-N); ¹H NMR (CDCl₃, 400 MHz): 3.90 (s, 3H, -OCH₃), 4.45 (m, 1H, -CH₂CH₂CH₂OH), 5.15-5.19 (m, 2H, -CH₂CH₂CH₂OH), 5.76-5.87 (m, 1H, -CH₂CH₂CH₂OH), 7.06-7.19 (m, 1H, ArH), 7.55-7.65 (m, 3H, ArH), 11.12 (bs, 1H, -COOH); MS: m/z 277 (M+H).

1-Benzyl-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid, 10e: Yield 81%; m.p. 170-72°C; IR (KBr): 1681 (C=O), 1488 (C=C), 1289 (C-F), 1132 cm⁻¹ (C-N); ¹H NMR (CDCl₃, 400 MHz): δ 3.88 (s, 3H, -OCH₃), 5.02 (s, 2H, -CH₂Ph), 7.07-7.32 (m, 1H, ArH), 7.56-7.74 (m, 8H, ArH), 11.18 (bs, 1H, -COOH).

Antimicrobial screening

The antibacterial activity of all the newly synthesized compounds was done by the agar-well diffusion assay technique. Twenty four hour old bacterial cultures of all test microorganisms were used as inoculums, which was adjusted to 0.5 McFarland standard, that is, 1.0 × 10⁸ CFU/mL. The stock solutions of all test compounds (100 µg/mL) were prepared by dissolving 100 µg of the test compound in DMSO (1 mL). Chloramphenicol and DMSO were used as positive and negative controls, respectively. Twenty milliliter of molten and cooled MHA and 320 µL of each test bacterial culture were mixed (separate flasks were used for each bacterial culture) and poured in sterilized and labeled petri plates. The wells of 6 mm were punched in the solidified petri plates, aseptically. Fifty microlitres from stock solutions of all compounds as well as controls was added to each well of labeled petri plates and incubated at 35°C for 24 hr. The diameter of the zone of growth inhibition around each well was measured after incubation using vernier caliper.

The minimum inhibitory concentration (MIC) of compounds against Gram-positive and Gram-negative test bacteria was determined by the method of NCCLS. All the test cultures were streaked on SCDA and incubated overnight at 37°C. Turbidity of all the bacterial cultures was adjusted to 0.5 McFarland standard by preparing bacterial suspension of 3-5 well isolated colonies of the same morphological type selected from an agar plate culture. The cultures were further diluted to get an inoculum size of 1.0 × 10⁸ CFU/mL. Stock solutions of 100 µg/mL of each compound was prepared in DMSO and was appropriately diluted to get a final concentration of 90, 80, 70, 60, 50, 40 µg/mL. Standard antibiotic chloramphenicol were also diluted to get a final concentration in the same manner. Three hundred and twenty micro liters of each dilution was added to 20 mL molten and cooled MHA (separate flasks was taken for each dilution). After thorough mixing, the medium was poured in sterilized petri plates. The test bacterial cultures were spotted in a predefined pattern by ascetically transferring 5 mL of each bacterial culture.
on the surface of solidified agar plates and incubated at 35°C for 24 hr. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as MIC.

For the antifungal activity, Potato dextrose agar (Hi media) medium was used. This sterilized hot medium (15 mL) was pipetted out into flat petri plates. When it solidified 15 mL of warm seeded agar was applied over it. The seeded agar was made by cooling the medium to 40°C and then adding spore suspension to seeded medium. The spores were obtained from 10 days culture of C. albicans and A. niger species. The final inoculum size was adjusted to $1 \times 10^6$ spore mL$^{-1}$. Nystatin and DMSO were used as positive and negative controls, respectively. Before the solidification of agar, the plate was tilted to ensure that coverage should be even. These petri plates were then put into the refrigerator upside down to prevent condensation of moisture. Concentration 100 µg/mL of the synthesized compounds were prepared by dissolving the required quantity of compounds in DMSO, sterilized Whatman filter paper number 541 discs were prepared by cutting 6 mm diameter were spread individually with needle and planted upon the chilled seeded medium. The culture plates were then incubated for 24-72 hr at 37°C and inhibition zone around each disc was measured from the centre of the discs. The diameter of growth inhibition zone was calculated by vernier caliper. For concentration 100 to 40 µg/mL. The standardized micro broth dilution methods, were used according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical and Laboratory Standards NCCLS) 43.

Conclusions

All the target compounds 8a-e, 9a-e and 10a-e exhibited moderate to good antibacterial as well as antifungal activities as compared to the standard drugs. The aryl substitutions and alkyl substitutions on the nitrogen of pyrazole ring, did not affect the antibacterial activities of the compounds 8a-e and 10a-e respectively. However, with phenyl substitution 8a-e, the antifungal activity was better than those with alkyl substitution 10a-e. It is noteworthy that the isoxazole-piperazinyl amide derivatives 9a-e exhibited slightly better activities than their pyrazole analogues. It is still premature to arrive at any meaningful conclusion regarding the effect of substitution on antimicrobial activities. However, the promising activity of these molecules make them good lead molecules for further exploration in terms of structure activity relationship for which further studies are warranted.

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References

43 NCCLS Approval Standard Document M2–A7. National Committee for Clinical Laboratory Standards, (Vilanova, PA, USA) **2000**.