



SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL PYRAZOLE DERIVATIVES

**Pagidirai Sanjeeva*, Yeragodala Narendra Reddy, Kummara Srinivasulu and
Prof. Peddakotla Venkata Ramana**

Department of Chemistry, Sri Krishnadevaraya University, Ananthapuramu, Andhra Pradesh, India.

*Corresponding Author: Pagidirai Sanjeeva

Department of Chemistry, Sri Krishnadevaraya University, Ananthapuramu, Andhra Pradesh, India.

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ABSTRACT

Novel 1,5-disubstituted pyrazole and isoxazole derivatives like, aryl 5-(3-(trifluoromethyl)phenyl)-1-(substituted phenyl)-1H-pyrazole-3-carboxylic acid 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*. Fungal strains such as *T. viride*, *A. flavus*, *A. brasillansis*, *C. albicans* by using minimum inhibitory concentration. Interestingly all the synthesized compounds exhibit good antibacterial and antifungal activity.

KEYWORDS: Pyrazole, antibacterial, antifungal.

INTRODUCTION

Pyrazoles are chemical compounds that contain a five membered heterocyclic with two nitrogen atoms and three head-to-head carbons. Pyrazole derivatives, some members of the pyrazoles class, have presented excellent pharmacological effectiveness and biological antimicrobial^[1], anti-inflammatory^[2], antihistaminic^[3], antiviral^[4], Anticonvulsant^[5], fungicidal activities^[6], herbicidal^[7-8], antitumor, cytotoxic^[9], and antiviral^[10-11], activities. Pyrazole derivatives also act as antiangiogenic agents^[12], A3 adenosine receptor antagonists^[13], neuropeptide YY5 receptor antagonists^[14], kinase inhibitor for treatment of type 2 diabetes, hyperlipidemia, obesity^[15], and thrombopiotinmimetics^[16], here in we report the synthesis of novel pyrazole derivatives and their antimicrobial activities.

METERIAL AND METHODS

Melting points of compounds were determined in open capillary tubes 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. IR spectra (KBr in cm⁻¹) were recorded on a Shimadzu Model FTIR-435. NMR spectra were recorded on a Varian Mercury TH-400 operating at 400 MHz (¹H NMR) and 100 MHz (¹³C NMR) using CDCl₃ as a solvent and TMS as an internal standard (Chemical shift in ppm). All chemicals and solvents used are of analytical grade.

EXPERIMENTAL SECTION

Synthesis of (Z)-ethyl 4-(3-(trifluoromethyl)phenyl)-4-hydroxy-2-oxobut-3-enoate 2.

To a mixture of 1-(3-(trifluoromethyl)phenyl)ethanone 1 (0.10 mol) and diethyl oxalate (0.15 mol) in 50mL of anhydrous toluene was added sodium hydride (60 %) (0.2 mol). The resulting mixture was stirred at 35-40°C for 7 hr; the solvent was evaporated under vacuum, the crude mixture was poured in 100 mL of ice cold water and acidified by dilute hydrochloric acid. Solid precipitated obtained was filtered and washed with water. Solid compound was dried under vacuum at 50°C. The crude product was purified by crystallization in methanol.

Yield 92%; m.p. 120-125°C;

IR (KBr): 1735 (C=O, ester), 1688 (C=O, ketone) 1440 (C=C);

¹H NMR (400 MHz, CDCl₃): δ 1.35 (t, 3H, -CH₃); 4.34 (q, 2H, -OCH₂); 6.82 (s, 1H, =CH-); 7.42 (s, 1H, ArH); 7.18-7.30 (m, 3H, ArH); 15.30 (bs, 1H, -OH);

¹³C NMR (100 MHz, CDCl₃): 14.23, 58.0, 94.2, 96.3, 120.8, 124.5(2C), 128.4(2C), 130.1, 131.3, 162.4, 175.6.

MS: m/z 289 (M+H).

Synthesis of ethyl 1-(substituted phenyl)-5-(3-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxylate 3a-e.

Ethyl 4-(3-fluoro-4-methoxyphenyl)-4-hydroxy-2-oxobut-3-enoate 2 (4.85 mol) and hydrazine hydrochloride (3 mole equiv) in ethanol (20 mL) was refluxed for 8 hours. After evaporation of ethanol in vacuum, the oil obtained was poured into ice cold water. The solid obtained was filtered and purified by silica gel flash

chromatography (hexane/ethyl acetate = 8:2).

Ethyl 1-(4-chlorophenyl)-5-(3-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxylate 3a.

Yield 79%; m.p. 105-109°C;

IR (KBr): 2930 (-C-H), 1718 (C=O, ester), 1492 (C=C), 1136 cm⁻¹ (C-N);

¹H NMR (400 MHz, CDCl₃): δ 1.38 (t, 3H, -CH₃); 4.32 (q, 2H, -OCH₂); 7.15 (m, 2H, ArH); 7.20 (t, 1H, ArH); 7.32 (m, 2H, ArH); 7.37 (s, 1H, pyrazole); 7.40 (d, 1H, ArH); 7.48 (d, 1H, ArH); 7.65 (s, 1H, ArH);

¹³C NMR (100 MHz, CDCl₃): 14.5, 59.6, 110.5, 120.5(2C), 122.4, 123.2, 125.3, 128.0, 129.1(2C), 130.2, 130.9, 134.2, 135.6, 141.2, 143.3, 159.5.

MS: m/z 395 (M+H).

Ethyl 5-(3-(trifluoromethyl)phenyl)-1-(4-fluorophenyl)-1H-pyrazole-3-carboxylate 3b:

Yield 87%; m.p. 108-112°C;

IR (KBr): 2935 (-C-H), 1740 (C=O, ester), 1691 (C=O), 1487 (C=C), 1126 cm⁻¹ (C-N);

¹H NMR (400 MHz, CDCl₃): δ 1.39 (t, 3H, -CH₃); 4.30 (q, 2H, -OCH₂); 7.10 (m, 2H, ArH); 7.18 (t, 1H, ArH); 7.28 (m, 2H, ArH); 7.34 (s, 1H, pyrazole); 7.38 (d, 1H, ArH); 7.45 (d, 1H, ArH); 7.62 (s, 1H, ArH);

¹³C NMR (100 MHz, CDCl₃): 14.8, 60.2, 110.2, 114.3(2C), 119.7(2C), 122.3, 124.5, 125.1, 127.4, 129.8, 130.4, 132.5, 134.3, 142.1, 144.4, 159.7, 160.2.

MS: m/z 379 (M+H).

Ethyl 5-(3-(trifluoromethyl)phenyl)-1-(4-methoxyphenyl)-1H-pyrazole-3-carboxylate 3c:

Yield 81%; m.p. 106-110°C;

IR (KBr): 2937 (-C-H), 1738 (C=O, ester), 1695 (C=O), 1496 (C=C), 1134 cm⁻¹ (C-N);

¹H NMR (400 MHz, CDCl₃): δ 1.35 (t, 3H, -CH₃); 3.72 (s, 3H, OCH₃); 4.28 (q, 2H, -OCH₂); 6.82 (m, 2H, ArH); 7.22 (t, 1H, ArH); 7.27 (m, 2H, ArH); 7.37 (s, 1H, pyrazole); 7.43 (d, 1H, ArH); 7.49 (d, 1H, ArH); 7.64 (s, 1H, ArH);

¹³C NMR (100 MHz, CDCl₃): 14.5, 53.9, 60.5, 110.8, 114.5(2C), 120.2(2C), 122.5, 124.8, 125.6, 127.6, 129.6, 130.5, 132.6, 134.5, 142.4, 144.7, 157.8, 160.4.

MS: m/z 391 (M+H).

Ethyl 5-(3-(trifluoromethyl)phenyl)-1-(3-fluorophenyl)-1H-pyrazole-3-carboxylate 3d:

Yield 87%; m.p. 102-106°C;

IR (KBr): 2969(-C-H), 1735 (C=O, ester), 1691 (C=O), 1488 (C=C), 1135 cm⁻¹ (C-N);

¹H NMR (400 MHz, CDCl₃): δ 1.33 (t, 3H, -CH₃); 4.26 (q, 2H, -OCH₂); 7.15-7.30 (m, 4H, ArH); 7.35 (s, 1H, pyrazole); 7.39 (s, 1H, ArH); 7.42 (d, 1H, ArH); 7.45 (d, 1H, ArH); 7.66 (s, 1H, ArH);

¹³C NMR (100 MHz, CDCl₃): 14.6, 59.8, 110.9, 114.5, 117.3, 123.5, 125.4, 125.9, 126.0, 129.0, 129.8, 130.2, 131.0, 132.3, 133.9, 139.8, 142.9, 144.8, 160.5.

MS: m/z 379 (M+H).

Ethyl 1-(3-chlorophenyl)-5-(3-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxylate 3e:

Yield 84%; m.p. 114-119°C;

IR (KBr): 2933 (-C-H), 1738 (C=O, ester), 1694 (C=O), 1483 (C=C), 1126 cm⁻¹ (C-N);

¹H NMR (400 MHz, CDCl₃): δ 1.32 (t, 3H, -CH₃); 4.24 (q, 2H, -OCH₂); 7.02-7.20 (m, 4H, ArH); 7.26 (t, 1H, ArH); 7.34 (s, 1H, pyrazole); 7.38 (d, 1H, ArH); 7.45 (d, 1H, ArH); 7.63 (s, 1H, ArH);

¹³C NMR (100 MHz, CDCl₃): 14.3, 60.0, 110.8, 114.2, 117.0, 121.5, 125.3, 125.9, 126.4, 129.2, 129.9, 130.0, 131.2, 132.6, 133.8, 139.7, 142.6, 144.7, 160.2.

MS: m/z 395 (M+H).

Synthesis of 1-(substitutedphenyl)-5-(3-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxylic acid 4a-e

Compound 3a-e (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-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxylic acid 4a;

Yield 87%; m.p. 158-162°C;

IR (KBr): 2934 (-C-H), 1702 (C=O), 1493 (C=C), 1132 cm⁻¹ (C-N);

¹H NMR (400 MHz, CDCl₃): δ 6.81 (s, 1H, pyrazole); 7.19 (m, 2H, ArH); 7.22 (t, 1H, ArH); 7.35 (m, 2H, ArH); 7.43 (d, 1H, ArH); 7.49 (d, 1H, ArH); 7.68 (s, 1H, ArH); 12.96 (bs, 1H, -COOH);

¹³C NMR (100 MHz, CDCl₃): 110.2, 120.3, 121.4 (2C), 124.0, 125.5, 129.0(2C), 129.4, 129.8, 130.3, 131.5, 132.4, 143.0, 145.2, 160.3.

MS: m/z 367 (M+H).

5-(3-(trifluoromethyl)phenyl)-1-(4-fluorophenyl)-1H-pyrazole-3-carboxylic acid 4b;

Yield 78%; m.p. 198-203°C;

IR (KBr): 2930 (-C-H), 1688 (C=O), 1482 (C=C), 1120 cm⁻¹ (C-N);

¹H NMR (400 MHz, CDCl₃): δ 6.79 (s, 1H, pyrazole); 7.01 (m, 2H, ArH); 7.23(m, 2H, ArH); 7.29 (t, 1H, ArH); 7.45 (d, 1H, ArH); 7.48 (d, 1H, ArH); 7.67 (s, 1H, ArH); 12.98 (bs, 1H, -COOH);

¹³C NMR (100 MHz, CDCl₃): 110.5, 114.5(2C), 119.5(2C), 122.7, 124.9, 125.8, 127.6, 129.6, 130.8, 132.7, 134.2, 142.2, 144.8, 160.2, 160.9.

MS: m/z 351 (M+H).

5-(3-(trifluoromethyl)phenyl)-1-(4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid 4c;

Yield 85%; m.p. 140-145°C;

IR (KBr): 2938 (-C-H), 1694 (C=O), 1495 (C=C), 1134

cm⁻¹ (C-N);

¹H NMR (400 MHz, CDCl₃): □ 3.75 (s, 3H, OCH₃), 6.72 (s, 1H, pyrazole); 6.85 (m, 2H, ArH); 7.25 (m, 3H, ArH); 7.43 (d, 1H, ArH); 7.47 (d, 1H, ArH); 7.66 (s, 1H, ArH); 13.12 (bs, 1H, -COOH);

¹³C NMR (100 MHz, CDCl₃): 54.6, 111.2, 113.9(2C), 120.5(2C), 122.7, 124.6, 125.7, 127.4, 129.5, 130.3, 132.4, 134.6, 142.6, 145.0, 158.0, 160.5.

MS: *m/z* 363 (M+H).

5-(3-(trifluoromethyl)phenyl)-1-(3-fluorophenyl)-1H-pyrazole-3-carboxylic acid 4d;

Yield 78%; m.p. 168-172°C;

IR (KBr): 2969(-C-H), 1692 (C=O), 1487 (C=C), 1133 cm⁻¹ (C-N);

¹H NMR (400 MHz, CDCl₃): □ 6.75 (s, 1H, pyrazole); 7.0-7.25 (m, 5H, ArH); 7.44 (d, 1H, ArH); 7.46 (d, 1H, ArH); 7.67 (s, 1H, ArH); 13.25 (bs, 1H, -COOH);

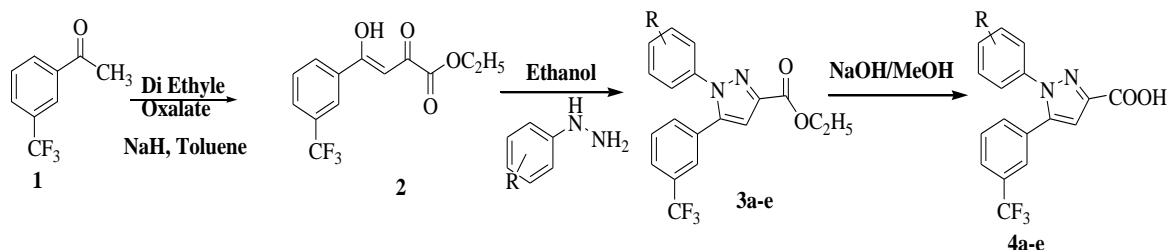
¹³C NMR (100 MHz, CDCl₃): 102.6, 110.8, 112.7, 115.0, 123.5, 124.3, 125.4, 128.3, 129.9, 130.8, 131.1, 133.0, 141.2, 142.2, 145.0, 160.4, 162.4.

MS: *m/z* 351 (M+H).

1-(3-chlorophenyl)-5-(3-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxylic acid 4e;

Yield 81%; m.p. 152-157°C;

IR (KBr): 2934 (-C-H), 1696 (C=O), 1480 (C=C), 1125 cm⁻¹ (C-N);



R= 4a= 4-Cl
 4b = 4-F
 4c = 4-OMe
 4d = 3-F
 4e = 3-Cl

Physical data of the final compounds are presented in **Table 1**.

Table 1: Physical characterization data of synthesized compounds.

| Entry | R | Melting point (°C) | Reaction Time (hr) | Yield (%) |
|-------|------------------------|--------------------|--------------------|-----------|
| 4a | 4-Cl Phenyl hydrazine | 158-162 | 9.0 | 87 |
| 4b | 4-F Phenyl hydrazine | 198-203 | 9.5 | 78 |
| 4c | 4-OMe Phenyl hydrazine | 140-145 | 8.5 | 85 |
| 4d | 3-Cl phenyl hydrazine | 168-172 | 10.0 | 78 |
| 4e | 3-F Phenyl hydrazine | 152-157 | 8.0 | 81 |

Antibacterial and Antifungal Activity

All the synthesized pyrazole derivatives (**4a**, **4b**, **4c**, **4d** and **4e**) were tested for their *in vitro* anti-bacterial activity against clinically isolated bacterial strains such as *S.aureus*, *E.coli*, *B.subtilis*, *P.aeruginosa*, *S.pyogens*, *K.terrigena* and *K.pneumonia* by using minimum inhibitory

¹H NMR (400 MHz, CDCl₃): □ 6.78 (s, 1H, pyrazole); 7.15-7.25 (m, 5H, ArH); 7.34 (m, 2H, ArH); 7.43 (d, 1H, ArH); 7.49 (d, 1H, ArH); 7.65 (s, 1H, ArH); 13.25 (bs, 1H, -COOH);

¹³C NMR (100 MHz, CDCl₃): 110.4, 114.6, 117.4, 123.3, 124.4, 126.3(2C), 128.7, 130.3(2C), 131.4, 132.7, 134.5, 140.5, 142.8, 144.7, 160.3.

MS: *m/z* 367 (M+H).

RESULTS AND DISCUSSION

The target compounds **4a-e** was synthesized according to **Scheme**. Initially, 1-(3-(trifluoromethyl)phenyl)ethanone **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 presence of ethyl group □ 1.35 (-CH₃) and 4.34 (-OCH₂-). Further Compound **2** was also treated with substituted phenyl hydrazines to obtain substituted pyrazole esters **3a-e** whose structure was established by IR and ¹H NMR. Pyrazole ester **3a-e** was then hydrolyzed using sodium hydroxide to obtain compounds **4a-e**. Structures of compounds **4a-e** were established by ¹H NMR where in absence of ester peaks at δ 1.35 and 4.34 confirmed their formation. Similarly, the structures of all the other derivatives were confirmed similarly and the results are presented in the experimental part.

concentration (MIC). Fungal strains such as *T. viride*, *A. flavus*, *A. brasillensis*, *C. albicans* by using minimum inhibitory concentration. Antibacterial and Antifungal results are summarized in **Table 2** and **Table 3** respectively. Which indicated that, most of the synthesized compounds registered the MIC at 100g/mL

or less, that is, these compounds exhibited comparable or at most twofold less activity against all bacterial strains as compared with standard drug Chloramphenicol and

fungal strains such as compared with standard drug Nystatin.

Table 2: Minimum bacterial inhibitory concentrations (MIC $\mu\text{g/mL}$) of the compounds 4a-e.

| Entry | <i>S.aureus</i> | <i>E.coli</i> | <i>B.subtilis</i> | <i>P.aeruginosa</i> | <i>S.pyogenes</i> | <i>K.terrigena</i> | <i>K.pneumoniae</i> |
|-----------------|-----------------|---------------|-------------------|---------------------|-------------------|--------------------|---------------------|
| 4a | -- | 85 | 90 | 85 | -- | 75 | 90 |
| 4b | 90 | 75 | 75 | -- | 90 | 65 | 75 |
| 4c | 75 | -- | 65 | 75 | 65 | -- | -- |
| 4d | -- | 60 | 70 | 90 | -- | 90 | -- |
| 4e | 70 | 70 | -- | 70 | 60 | 60 | 80 |
| Chloramphenicol | 50 | 50 | 50 | 40 | 50 | 40 | 50 |

Table 3: Minimum fungal inhibitory concentrations (MIC $\mu\text{g/mL}$) of the compounds 4a-e.

| Entry | <i>T. viride</i> | <i>A. flavus</i> | <i>A. brasillansis</i> | <i>C. albicans</i> |
|----------|------------------|------------------|------------------------|--------------------|
| 4a | 80 | -- | 90 | 80 |
| 4b | 80 | -- | 100 | -- |
| 4c | 80 | 70 | -- | 60 |
| 4d | -- | 60 | 60 | 80 |
| 4e | 70 | 70 | 70 | -- |
| Nystatin | 60 | 50 | 40 | 40 |

CONCLUSIONS

We have successfully synthesized (4a-e) compounds in good yield. The synthesized compounds structures were confirmed by their spectral data. Newly synthesized compounds were screened for their Minimum bacterial/fungal inhibitory concentrations. All the target compounds 4a, 4b, 4c, 4d and 4e exhibited modest to acceptable 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 4a-e. However, with phenyl substitution 4a-e, the antifungal activity was better than those with alkyl substitution. It is still untimely to arrive at any meaningful conclusion concerning the effect of substitution on antimicrobial activities. However, the activity of these molecules fabricate them good lead molecules for further exploration in terms of structure activity relationship for which further studies are justify.

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