International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.5, pp 131-135, 2017

An Efficient Synthesis of Pyrazole Carboxamide Derivatives and in Vitro Evaluation for their Antimicrobial Activity

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Abstract : An efficient procedure for the synthesis of pyrazole carboxamides via Claisen-Schmidt reaction was developed. An acid catalysed reaction of chalcones and semicarbazide hydrochloride produced pyrazole carboxamides in good yields. The synthesized new compounds were characterized by spectral studies and elemental analysis; and were screened *in vitro* for their antimicrobial susceptibilities against different bacteria and fungi species.
Key words: Antibacterial, antifungal, condensation, inhibition, pyrazoline, spectral.

Introduction

Five membered nitrogen heterocycles, pyrazoles and their derivatives in particular are regarded as important molecules in organic synthesis; they serve as building blocks for the construction of varied classes of bioactive molecules. Numerous methods have been developed for synthesis of substituted pyrazoles viz. by (i) the reaction of 1, 3-diketones with hydrazines, (ii) the reaction of α, β-unsaturated aldehyde and ketones with hydrazines, 1, 3-dipolar cycloaddition reactions of nitrile imines to alkenes and alkynes.[1] Multisubstituted pyrazoles were cyclocondensation of β-thioalkyl-α,β-unsaturated ketones with hydrazines with high regioselectivity.[2] An efficient synthesis of 1, 3-diaryl-4-halo-1*H*-pyrazoles in excellent yields by 1, 3-dipolar cycloaddition of 3-arylsydnones and 2-aryl-1, 1-dihalo-1-alkenes was reported.[3]

Pyrazole analogues have been extensively used as important synthons in the field of organic chemistry and drug designing. For instance, synthesised series of 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazoles were screened for their ability to inhibit selectively monoamine oxidases, swine kidney diamine oxidase (SKDAO) and bovine serum amine oxidase (BSAO).[4] Pyrazoles have known to exhibit antimicrobial,[5] antioxidant,[6] anti-tubercular,[7] anticancer[8] activities. Prompted by the broad spectrum of synthetic and pharmacological applications associated with pyrazoles, and in continuation of our work on pyrazole carboxamides,[9] we herein report the synthesis of series of new pyrazole carboxamides and the results of their antimicrobial activities.

Materials and Methods

Melting points were determined by an open capillary tube method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates pre-coated with silica gel using solvent system ethyl acetate: dichloromethane (1:4 v/v). The spots were visualized under UV light. ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent-NMR 400 MHz and 100 MHz spectrometer respectively. The solvent $CDCl_3$ with TMS as an internal standard was used to record the spectra. The chemical shifts are expressed in δ

ppm. Mass spectra were obtained on Mass Lynx SCN781 spectrometer TOF mode. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyzer. Purification of compounds was done by column chromatography on silica gel (70-230 mesh Merck).

The synthetic method involves; the synthesis of a series of pyrazole caboxamides, **5a-g** by the cyclocondensation reaction of chalcones, **3a-f** with semicarbazide hydrochloride, **4** and few drops of concentrated hydrochloric acid in methyl alcohol under reflux conditions. The schematic diagram for the synthesis of pyrazole carboxamides is outlined in **Figure-1**.

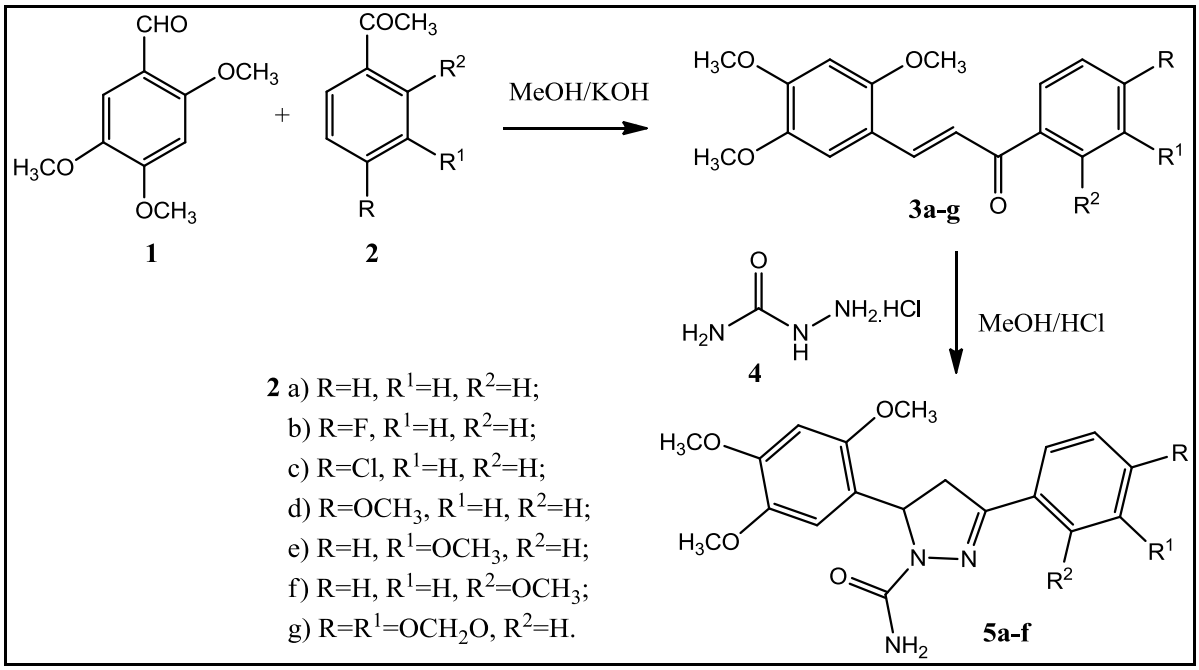

Figure. 1: Schematic diagram for the synthesis of pyrazole carboxamides, 5a-f.

Synthesis of chalcones: The intermediate chalcones, **3a-g** were obtained according to our reported procedure by Claisen-Schmidt condensation of 2,4,5-trimethoxybenzaldehyde, **1** and substituted acetophenone, **2a-g** in the presence of potassium hydroxide in 95% ethyl alcohol at room temperature.

General procedure for the synthesis of pyrazole carbixanudes, 5a-g: To a stirred solution of chalcones, **3a-g** (0.01 mol) and semicarbazide hydrochloride, **4** (0.01 mol) in methyl alcohol (15 mL), concentrated hydrochloric acid (7-8 drops) were added. The mixture was refluxed for 3-4 h and the progress of the reaction was monitored by TLC. After completion, the reaction mixture was poured in to ice cold water; solid separated was filtered, washed with ice cold water and dried. The products were purified column chromatography using silica gel (60-120 mesh) and ethyl acetate : dichloromethane (1:4 v/v) as eluent.

***3-Phenyl-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide,* 5a:** Obtained from (E)-1-phenyl-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **3a** (10 mmol) and semicabazide hydrochloride, **4** (10 mmol) in 78% yield, m.p. 109–111°C; ^{1}H NMR: δ 2.992-3.056 (dd, 1H, *J*= 7.5Hz, 13.3Hz, C_3-H_a), 3.694-3.748 (dd, 1H, *J= 8.0Hz, 14.5Hz*, C_3-H_b), 3.794 (s, 6H, OCH_3), 3.852 (s, 3H, OCH_3), 5.696-5.714 (dd, 1H, *J= 8.2Hz, 16.0Hz*, C_2-H), 6.526 (s, 2H, NH_2), 7.110–7.940 (m, 7H, Ar–H); ^{13}C NMR: δ 40.40 (1C, C-4), 59.49 (1C, C-5), 55.60 (3C), 101.84 (1C), 113.90 (1C), 121.60 (1C), 128.30 (2C), 129.10 (2C), 131.26 (1C), 135.60 (1C), 141.90 (1C), 147.28 (1C), 149.90 (1C), 152.12 (1C, C-3), 156.90 (1C, C=O); MS *m/z*: 355 (M^+, 100); Anal. calcd. for $C_{19}H_{21}N_3O_4$ (%): C, 64.21; H, 5.96; N, 11.82; Found: C, 64.11; H, 5.88; N, 11.75.

***3-(4-Fluorophenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide,* 5b:** Obtained from (E)-1-(4-fluorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **3b** (10 mmol) and semicarbazide hydrochloride, **4** (10 mmol) in 80% yield; ^{1}H NMR: δ 2.990-3.049 (dd, 1H, *J*= 7.0Hz, 13.0Hz, C_3-H_a), 3.691-3.743 (dd, 1H, *J= 8.1Hz, 13.8Hz*, C_3-H_b), 3.789 (s, 6H, OCH_3), 3.850 (s, 3H, OCH_3), 5.692-5.710 (dd, 1H, *J= 7.4Hz, 14.0Hz*, C_2-H), 6.544 (s, 2H, NH_2), 7.111–7.935 (m, 6H, Ar–H); ^{13}C NMR: δ 40.46 (1C, C-4), 59.66

(1C, C-5), 55.55 (3C), 100.23 (1C), 113.96 (1C), 115.30 (2C), 121.88 (1C), 129.16 (2C), 132.20 (1C), 141.78 (1C), 147.74 (1C), 148.12 (1C), 152.66 (1C, C-3), 157.33 (1C, C=O), 182.22 (1C); MS *m/z*: 373 (M^+, 100); Anal. calcd. for $C_{19}H_{20}FN_3O_4$ (%): C, 61.12; H, 5.40; N, 11.25; Found: C, 61.02; H, 5.31; N, 11.18.

***3-(4-Chlorophenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide,* 5c:** Obtained from (E)-1-(4-chlorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **3c** (10 mmol) and semicarbazide hydrochloride, **4** (10 mmol) in 81% yield; ^{1}H NMR: δ 2.989-3.047 (dd, 1H, *J*= 7.2Hz, 13.5Hz, C_3-H_a), 3.695-3.740 (dd, 1H, *J= 8.2Hz, 14.4Hz*, C_3-H_b), 3.781 (s, 6H, OCH_3), 3.847 (s, 3H, OCH_3), 5.688-5.702 (dd, 1H, *J= 7.0Hz, 14.1Hz*, C_2-H), 6.512 (s, 2H, NH_2), 7.103–7.946 (m, 6H, Ar–H); ^{13}C NMR: δ 41.33 (1C, C-4), 59.88 (1C, C-5), 55.45 (3C), 101.65 (1C), 113.54 (1C), 122.14 (1C), 128.20 (2C), 129.05 (2C), 133.21 (1C), 136.70 (1C), 141.22 (1C), 148.20 (1C), 149.47 (1C), 152.47 (1C, C-3), 156.32 (1C, C=O); MS *m/z*: 391 (M^+, ^{37}Cl, 33), 389 (M^+, ^{35}Cl, 100); Anal. calcd. for $C_{19}H_{20}ClN_3O_4$ (%): C, 58.54; H, 5.17; N, 10.78; Found: C, 58.46; H, 5.06; N, 10.65.

***3-(4-Methoxyphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide,* 5d:** Obtained from (E)-1-(4-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **3d** (10 mmol) and semicarbazide hydrochloride, **4** (10 mmol) in 79% yield; ^{1}H NMR: δ 2.987-3.028 (dd, 1H, *J*= 7.1Hz, 14.6Hz, C_3-H_a), 3.694-3.740 (dd, 1H, *J= 7.9Hz, 15.6Hz*, C_3-H_b), 3.810 (s, 6H, OCH_3), 3.855 (s, 6H, OCH_3), 5.695-5.708 (dd, 1H, *J= 7.0Hz, 15.0Hz*, C_2-H), 6.510 (s, 2H, NH_2), 7.112–7.880 (m, 6H, Ar–H); ^{13}C NMR: δ 40.98 (1C, C-4), 59.26 (1C, C-5), 55.50 (4C), 101.55 (1C), 113.56 (1C), 114.60 (2C), 122.30 (1C), 128.24 (2C), 129.10 (1C), 141.72 (1C), 148.20 (1C), 148.84 (1C), 152.33 (1C, C-3), 155.98 (1C, C=O), 161.12 (1C); MS *m/z*: 385 (M^+, 100); Anal. calcd. for $C_{20}H_{23}N_3O_5$ (%): C, 62.33; H, 6.01; N, 10.90; Found: C, 62.21; H, 5.93; N, 10.81.

***3-(3-Methoxyphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide,* 5e:** Obtained from (E)-1-(3-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **3e** (10 mmol) and semicarbazide hydrochloride, **4** (10 mmol) in 84% yield; ^{1}H NMR: δ 2.992-3.044 (dd, 1H, *J*= 7.8Hz, 14.2Hz, C_3-H_a), 3.699-3.752 (dd, 1H, *J= 8.8Hz, 15.2Hz*, C_3-H_b), 3.796 (s, 6H, OCH_3), 3.850 (s, 6H, OCH_3), 5.690-5.712 (dd, 1H, *J= 7.6Hz, 14.9Hz*, C_2-H), 6.531 (s, 2H, NH_2), 7.110–7.942 (m, 6H, Ar–H); ^{13}C NMR: δ 41.30 (1C, C-4), 60.12 (1C, C-5), 55.48 (4C), 101.40 (1C), 113.76 (1C), 114.88 (2C), 122.26 (1C), 128.21 (2C), 129.18 (1C), 141.35 (1C), 148.06 (1C), 148.45 (1C), 152.18 (1C, C-3), 157.40 (1C, C=O), 161.10 (1C); MS *m/z*: 385 (M^+, 100); Anal. calcd. for $C_{20}H_{23}N_3O_5$ (%): C, 62.33; H, 6.01; N, 10.90; Found: C, 62.18; H, 5.91; N, 10.77.

***3-(2-Methoxyphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide,* 5f:** Obtained from (E)-1-(2-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **3f** (10 mmol) and semicarbazide hydrochloride, **4** (10 mmol) in 76% yield; ^{1}H NMR: δ 2.996-3.050 (dd, 1H, *J*= 6.8Hz, 13.1Hz, C_3-H_a), 3.689-3.738 (dd, 1H, *J= 7.5Hz, 15.0Hz*, C_3-H_b), 3.806 (s, 6H, OCH_3), 3.854 (s, 6H, OCH_3), 5.688-5.703 (dd, 1H, *J= 7.2Hz, 15.8Hz*, C_2-H), 6.524 (s, 2H, NH_2), 7.109–7.884 (m, 6H, Ar–H); ^{13}C NMR: δ 41.44 (1C, C-4), 60.16 (1C, C-5), 55.53 (4C), 102.10 (1C), 113.32 (1C), 114.93 (2C), 122.35 (1C), 128.22 (2C), 129.44 (1C), 141.90 (1C), 148.17 (1C), 148.90 (1C), 152.66 (1C, C-3), 156.40 (1C, C=O), 161.46 (1C); MS *m/z*: 385 (M^+, 100); Anal. calcd. for $C_{20}H_{23}N_3O_5$ (%): C, 62.33; H, 6.01; N, 10.90; Found: C, 62.20; H, 5.89; N, 10.80.

***3-(Benzo[d][1,3]dioxol-5-yl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide,* 5g:** Obtained from (E)-1-(benzo[d][1,3]dioxol-5-yl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, **3g** (10 mmol) and semicabazide hydrochloride, **4** (10 mmol) in 87% yield; ^{1}H NMR: δ 2.986-3.038 (dd, 1H, *J*= 7.9Hz, 13.8Hz, C_3-H_a), 3.692-3.747 (dd, 1H, *J= 6.8Hz, 12.1Hz*, C_3-H_b), 3.798 (s, 6H, OCH_3), 3.847 (s, 3H, OCH_3), 5.699-5.717 (dd, 1H, *J= 8.8Hz, 16.8Hz*, C_2-H), 6.028 (s, 2H, OCH_2O), 6.518 (s, 2H, NH_2), 7.114–7.956 (m, 5H, Ar–H); ^{13}C NMR: δ 40.08 (1C, C-4), 59.85 (1C, C-5), 55.46 (3C), 101.20 (1C), 102.56 (1C), 110.20 (1C), 113.71 (1C), 114.26 (1C), 122.10 (1C), 126.10 (1C), 141.69 (1C), 147.20 (1C), 148.70 (1C), 149.96 (1C), 150.18 (1C), 150.60 (1C), 151.76 (1C, C-3), 156.28 (1C, C=O); MS *m/z*: 399 (M^+, 100); Anal. calcd. for $C_{20}H_{21}N_3O_6$ (%): C, 60.14; H, 5.30; N, 10.52; Found: C, 60.04; H, 5.25; N, 10.40.

Result and Discussion

Structure proof of synthesized compounds, **5a-g** were provided by ^{1}H NMR, ^{13}C NMR, Mass spectral studies and elemental analysis. The structural assignments were made by NMR analysis by considering compound, **5c** as the representative compound among the series. In ^{1}H NMR spectra, two methylene protons designated as C_4-H_a and C_4-H_b of the newly formed pyrazoline ring is diastereotopic. The C_4-H_a, C_4-H_b and C_3-

H protons appeared as a doublet of doublets. The doublet of doublet for C_4-H_a appeared in the region δ 2.989-3.047 (*J*=7.2, 13.5*Hz*) ppm; doublet of doublet for C_4-H_b appeared in the region δ 3.695-3.740 (*J=8.2, 14.4Hz*) ppm; and that of C_3-H in the region δ 5.688-5.702 (*J=7.0, 14.1Hz*) ppm. Among C_4-H_a, C_4-H_b and C_3-H protons, C_3-H is the most deshielded due to its close proximity to benzene ring and electronegative nitrogen. C_3-H couples not only with C_4-H_a but also with C_4-H_b and appears as doublet of doublet instead of a triplet. A signal appeared as singlet for two protons at δ 6.512 was assigned to NH_2 protons. A collection of signal observed singlet for six and three protons each at δ 3.781 and δ 3.847 ppm; a multiplet for six protons in the region δ 7.103–7.946 ppm were assigned to OCH_3 and aromatic protons respectively.

In ^{13}C NMR spectrum, compound **5c** showed a signal at δ 41.33, 59.88 and 152.47 ppm due to C-4, C-5 amd C-3 carbons of the pyrazole ring. A signal appeared for three carbons at δ 55.45 ppm was assigned to three OCH_3 carbons. A signal for carbonyl carbon of an amide function appeared at δ 156.32 ppm. An array of signals one carbon each appeared at δ 101.65, 113.54, 122.14, 133.21, 136.70, 141.22, 148.20, 149.47 ppm and for two carbons each at δ 128.20, 129.05 ppm were ambiguously assigned to aromatic carbons. Compound **5c** showed molecular mass peak at m/z 391 with a relative abundance of 33% corresponding to corresponding to its molecular mass and ^{37}Cl isotope, and a base peak at m/z 389 corresponds to ^{35}Cl isotope. Further, satisfactorily elemental analysis data obtained for the compound were in good agreement with theoretically calculated values. Similar and consistent pattern signals were observed in the ^{1}H NMR, ^{13}C NMR and Mass spectra of the synthesized series of compounds **5a-g**, which strongly supports the structure proof for the synthesized compounds.

Antimicrobial activity

Antimicrobial studies of synthesized compounds **5a-g** were assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method [14,15]. The compounds were screened for their antimicrobial activities against Gram-negative bacteria *Escherichia coli,* Gram-positive bacteria *Staphylococcus aureus,* fungi species *Aspergillus nigar* and *Aspergillus flavus.* The experiments were carried out in triplicate; the results were taken as a mean of three determinations. The antibiotics Ciprofloxacin and Nystatin were used as standard drugs for antibacterial and antifungal studies respectively. The results of MIC's were tabulated in **Table 1**.

Table 1: Antimicrobial activities of the compounds 3a-g and 5a-g against bacterial and fungal stains

Compound	Minimum inhibitory concentration (MIC's) in µg/mL*			
	S.aureus	***E.coli***	***A.niger***	***A.flavus***
5a	50	25	25	25
5b	12.5	12.5	12.5	12.5
5c	12.5	12.5	12.5	12.5
5d	50	50	50	50
5e	50	50	50	50
5f	50	50	50	50
5g	25	12.5	50	100
Ciprofloxacin	25	12.5	----	----
Nystatin	----	----	12.5	25
*The results are expressed as mean of three determinations (n=3)				

The synthesized pyrazoles **5a-g** exerted a wide range of *in vitro* antimicrobial activities against the tested organisms. Results of the study reveal that, among the synthesized series, compounds **5b** and **5c** having fluoro and chloro substitution showed an excellent inhibition potential against all the tested species. Compounds **5d**, **5e** and **5f** with methoxy substitutions in the aromatic ring found moderately active; while compounds **5a** and **5g** showed good inhibitory effect against the testes organisms.

Conclusions

The simple and accessible procedure for the synthesis of pyrazole carboxamides was reported. Preliminary investigations on *in vitro* antimicrobial activity studies of the synthesized pyrazole carboxamides validate the significance of the study. Among the synthesized series of compounds, the pyrazole carboxamides

with fluoro and chloro substitutions have demonstrated potent antimicrobial activity against the tested microorganisms.

Acknowledgement:

One of the authors Anada is grateful to the Institution of Excellence, University of Mysore, Mysuru, for spectral analysis.

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