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Synthesis, Characterization and Antimicrobial Activity Studies of Novel Pyrazole Derivatives

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ABSTRACT

An efficient and accessible procedure for the synthesis of substituted pyrazoles was described. The method involves cyclocondensation reaction of chalcones with 2,4-dimethylphenylhydrazine hydrochloride in the presence of catalytic amount of hydrochloric acid to obtain pyrazole derivatives in good yields. The synthesized new compounds were characterized by spectral studies and elemental analysis; and evaluated in vitro for their antimicrobial susceptibilities.

Keywords: Antibacterial, Antifungal, Cyclocondensation, Inhibition, Pyrazoline, Spectral

INTRODUCTION

Chalcones form the central core for the construction of a variety of bioactive compounds. These were extensively used as key intermediates in the synthesis of bioactive heterocycles such as cyclopropyl derivatives [1], pyrrolines [2], isoxazoles [3], benzothiazepines [4], pyrazolines [5] etc. Amongst the heterocycles, pyrazole and its derivatives have drawn a great attention due to their applications in biological and pharmacological fields. Usual methods for the construction of pyrazoles consist of the reaction of α, β-unsaturated aldehyde and ketones with hydrazines [6], 1, 3-dipolar cycloaddition reactions of nitrile imines to alkenes and alkynes [7,8].

Pyrazole and their derivatives have been extensively tested for their biological potencies such as, antimicrobial [9], analgesic and anti-inflammatory [10] activities. A much attention was given to pyrazoles as antimicrobial agents after the discovery of the natural pyrazole C-glycoside pyrazofurin; 4-hydroxy-3-β-D-ribofuranosyl-1*H*-pyrazole-5-carboxamide [11]. With these wide spectrums of applications associated with pyrazoles in the background and in search of new antimicrobial agents, we herein report the synthesis and antimicrobial evaluation results of series of new pyrazoles.

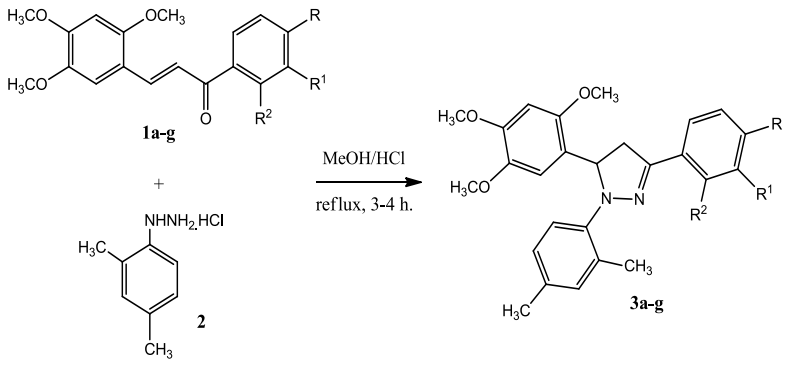
MATERIALS AND METHODS

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. Proton Nuclear Magnetic Resonance (^{1}H-NMR) and Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) spectra were recorded on Agilent-NMR 400 MHz and 100 MHz spectrometer respectively. The solvent Deuterated Chloroform ($CDCl_3$) with Tetramethylsilane (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.

The synthetic strategy involves; the cyclocondensation reaction of chalcones, 1a-g with 2,4-dimethylphenylhydrazine hydrochloride, 2 and 6-8 drops of conc. hydrochloric acid in methyl alcohol to obtain pyrazole derivatives, 3a-g in good yields. The schematic diagram for the synthesis of pyrazole derivatives is outlined in Figure 1.

Procedure for preparation of chalcones, 1a-g

The intermediate chalcones 1a-g were obtained according to our earlier reported procedure [12-15], which involves the reaction of mixture of 2,4,5-trimethoxybenzaldehyde, substituted acetophenone and sodium hydroxide in 95% ethyl alcohol at room temperature for 3 h.



3 a) R=H, R^1=H, R^2=H; b) R=F, R^1=H, R^2=H; c) R=Cl, R^1=H, R^2=H; d) R=OCH_3, R^1=H, R^2=H; e) R=H, R^1=OCH_3, R^2=H; f) R=H, R^1=H, R^2=OCH_3; g) R=R^1=OCH_2O, R^2=H.

Figure 1: Schematic diagram for the synthesis of dihydropyrazoles, 3a-g

General procedure for the synthesis of dihydropyrazoles, 3a-g

To a stirred solution of chalcones, 1a-g (0.01 mol) and 3-chlorophenylhydrazine hydrochloride, 2 (0.01 mol) in methyl alcohol (15 ml), concentrated hydrochloric acid (7-8 drops) was 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.

1-(2,4-Dimethylphenyl)-3-phenyl-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole, 3a

Obtained from 1-phenyl-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1a (10 mmol) and 2,4-dimethylphenylhydrazine hydrochloride, 2 (10 mmol) in 66% yield. ^{1}H-NMR (δ ppm): 2.320 (s, 6H, CH_3), 3.546-3.592 (dd, 1H, J=7.1, 13.0Hz, C_4-H_a), 3.770-3.786 (dd, 1H, J=6.0, 12.5Hz, C_4-H_b), 3.892 (s, 9H, OCH_3), 4.882-4.991 (dd, 1H, J=6.8, 14.0Hz, C_5-H), 6.428-7.375 (m, 10H, Ar-H); ^{13}C-NMR (δ ppm): 17.50 (1C), 20.32 (1C), 40.35 (1C, C-4), 55.81 (1C, C-5), 56.55 (3C), 100.90 (1C), 112.50 (1C), 113.25 (1C), 122.50 (1C), 126.60 (1C), 127.34 (1C), 128.21 (2C), 128.96 (2C), 130.72 (1C), 131.24 (1C), 135.10 (1C), 136.20 (1C), 140.94 (1C), 141.20 (1C), 148.86 (1C), 149.40 (1C), 151.90 (1C, C-3). MS *m/z*: 417 (MH^+, 100); Anal. Calcd. for $C_{26}H_{28}N_2O_3$ (%): C, 74.97; H, 6.78; N, 6.73; Found: C, 74.87; H, 6.71; N, 6.59.

1-(2,4-Dimethylphenyl)-3-(4-fluorophenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole, 3b

Obtained from 1-(4-fluorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1b (10 mmol) and 2,4-dimethylphenylhydrazine hydrochloride, 2 (10 mmol) in 69% yield. ^{1}H-NMR (δ ppm): 2.326 (s, 6H, CH_3), 3.550-3.595 (dd, 1H, J=8.0, 12.7Hz, C_4-H_a), 3.772-3.789 (dd, 1H, J=9.0, 16.5Hz, C_4-H_b), 3.856 (s, 9H, OCH_3), 4.876-4.960 (dd, 1H, J=6.5, 13.0Hz, C_5-H), 6.878-7.765 (m, 9H, Ar-H); ^{13}C-NMR (δ ppm): 17.56 (1C), 20.30 (1C), 40.66 (1C, C-4), 55.75 (1C, C-5), 56.50 (3C), 100.90 (1C), 112.56 (1C), 113.20 (1C), 115.20 (2C), 122.33 (1C), 125.61 (1C), 127.36 (1C), 129.90 (2C), 131.28 (1C), 132.70 (1C), 136.12 (1C), 140.92 (1C), 141.26 (1C), 148.85 (1C), 149.44 (1C), 151.86 (1C, C-3), 165.12 (1C). MS *m/z*: 435 (MH^+, 100); Anal. Calcd. For $C_{26}H_{27}FN_2O_3$ (%): C, 71.87; H, 6.26; N, 6.45; Found: C, 71.80; H, 6.10; N, 6.35.

1-(2,4-Dimethylphenyl)-3-(4-chlororophenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole, 3c

Obtained from 1-(4-chlorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1c (10 mmol) and 2,4-dimethylphenylhydrazine hydrochloride, 2 (10 mmol) in 76% yield. ^{1}H-NMR (δ ppm): 2.316 (s, 6H, CH_3), 3.534-3.585 (dd, 1H, J=8.0, 14.1Hz, C_4-H_a), 3.765-3.795 (dd, 1H, J=6.6, 12.9Hz, C_4-H_b), 3.845 (s, 9H, OCH_3), 4.867-4.937 (dd, 1H, J=6.5, 14.4Hz, C_5-H), 6.920-7.670 (m, 9H, Ar-H); ^{13}C-NMR (δ ppm): 17.14 (1C), 20.30 (1C), 40.22 (1C, C-4), 55.85 (1C, C-5), 56.51 (3C), 100.56 (1C), 112.52 (1C), 113.24 (1C), 122.67 (1C), 126.63 (1C), 127.30 (1C), 128.20 (2C), 128.94 (2C), 130.64 (1C), 131.20 (1C), 134.80 (1C), 136.08 (1C), 140.56 (1C), 141.27 (1C), 148.80 (1C), 149.78 (1C), 152.44 (1C, C-3). MS *m/z*: 452 (M^+, ^{37}Cl, 33), 450 (M^+, ^{35}Cl, 100); Anal. Calcd. for $C_{26}H_{27}ClN_2O_3$ (%): C, 69.25; H, 6.03; N, 6.21; Found: C, 69.11; H, 6.00; N, 6.07.

1-(2,4-Dimethylphenyl)-3-(4-methoxyphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole, 3d

Obtained from 1-(4-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1d (10 mmol) and 2,4-dimethylphenylhydrazine hydrochloride, 2 (10 mmol) in 80% yield. ^{1}H-NMR (δ ppm): 2.316 (s, 6H, CH_3), 3.544-3.5902 (dd, 1H, J=9.0, 14.6Hz, C_4-H_a), 3.760-3.785 (dd, 1H, J=8.0, 12.9Hz, C_4-H_b), 3.860 (s, 12H, OCH_3), 4.890-4.976 (dd, 1H, J=6.9, 14.3Hz, C_5-H), 6.788-7.673 (m, 9H, Ar-H); ^{13}C-NMR (δ ppm): 17.22 (1C), 20.30 (1C), 40.65 (1C, C-4), 55.84 (1C, C-5), 56.49 (4C), 100.21 (1C), 112.33 (1C), 113.22 (1C), 122.40 (1C), 126.65 (1C), 127.39 (1C), 128.20 (2C), 128.95 (2C), 130.76 (1C), 131.27 (1C), 135.00 (1C), 136.26 (1C), 140.88 (1C), 141.26 (1C), 148.80 (1C), 149.66 (1C), 151.95 (1C, C-3). MS *m/z*: 447 (MH^+, 100); Anal. Calcd. for $C_{27}H_{30}N_2O_4$ (%): C, 72.62; H, 6.77; N, 6.27; Found: C, 72.50; H, 6.66; N, 6.17.

1-(2,4-Dimethylphenyl)-3-(3-methoxyphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole, 3e

Obtained from 1-(3-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1e (10 mmol) and 2,4-dimethylphenylhydrazine hydrochloride, 2 (10 mmol) in 73% yield. ^{1}H-NMR (δ ppm): 2.320 (s, 6H, CH_3), 3.538-3.867 (dd, 1H, J=9.5, 14.3Hz, C_4-H_a), 3.762-3.794 (dd, 1H, J=8.5, 13.2Hz, C_4-H_b), 3.847 (s, 12H, OCH_3), 4.893-4.945 (dd, 1H, J=7.5, 14.3Hz, C_5-H), 6.775-7.552 (m, 9H, Ar-H); ^{13}C-NMR (δ ppm): 17.20 (1C), 20.45 (1C), 40.62 (1C, C-4), 54.85 (1C, C-5), 55.48 (4C), 100.34 (1C), 112.36 (1C), 113.20 (1C), 122.47 (1C), 126.62 (1C), 127.40 (1C), 128.26 (2C), 128.98 (2C), 130.74 (1C), 131.22 (1C), 135.03 (1C), 136.28 (1C), 140.80 (1C), 141.23 (1C), 148.77 (1C), 149.60 (1C), 151.94 (1C, C-3). MS *m/z*: 447 (MH^+, 100); Anal. Calcd. for $C_{27}H_{30}N_2O_4$ (%): C, 72.62; H, 6.77; N, 6.27; Found: C, 72.46; H, 6.65; N, 6.12.

1-(2,4-Dimethylphenyl)-3-(2-methoxyphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole, 3f

Obtained from 1-(2-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1f (10 mmol) and 2,4-dimethylphenylhydrazine hydrochloride, 2 (10 mmol) in 81% yield. ^{1}H-NMR (δ ppm): 2.310 (s, 6H, CH_3), 3.540-3.5910 (dd, 1H, J=8.3, 14.7Hz, C_4-H_a), 3.761-3.782 (dd, 1H, J=8.5, 13.5Hz, C_4-H_b), 3.857 (s, 12H, OCH_3), 4.882-4.965 (dd, 1H, J=6.1, 14.8Hz, C_5-H), 6.782-7.655 (m, 9H, Ar-H); ^{13}C-NMR (δ ppm): 17.10 (1C), 20.31 (1C), 40.62 (1C, C-4), 54.89 (1C, C-5), 55.48 (4C), 100.40 (1C), 112.26 (1C), 113.10 (1C), 122.46 (1C), 126.73 (1C), 127.30 (1C), 128.23 (2C), 128.94 (2C), 130.70 (1C), 131.30 (1C), 135.44 (1C), 136.21 (1C), 140.90(1C), 141.23 (1C), 148.65 (1C), 149.89 (1C), 151.70 (1C, C-3). MS *m/z*: 447 (MH^+, 100); Anal. Calcd. for $C_{27}H_{30}N_2O_4$ (%): C, 72.62; H, 6.77; N, 6.27; Found: C, 72.55; H, 6.64; N, 6.10.

3-(Benzo[d][1,3]dioxol-5-yl)-1-(2,4-dimethylphenyl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole, 3g

Obtained from 1-(benzo[d][1,3]dioxol-5-yl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 1g (10 mmol) and 2,4-dimethylphenylhydrazine hydrochloride, 2 (10 mmol) in 70% yield. ^{1}H-NMR (δ ppm): 2.318 (s, 6H, CH_3), 3.556-3.599 (dd, 1H, J=6.8, 12.1Hz, C_4-H_a), 3.782-3.797 (dd, 1H, J=6.5, 13.0Hz, C_4-H_b), 3.890 (s, 9H, OCH_3), 4.878-4.998 (dd, 1H, J=6.7, 14.5Hz, C_5-H), 6.012 (s, 2H, OCH_2O), 6.678-7.685 (m, 8H, Ar-H); ^{13}C-NMR (δ ppm): 17.45 (1C), 21.30 (1C), 40.24 (1C, C-4), 54.84 (1C, C-5), 55.85 (3C), 100.12 (1C), 112.22 (1C), 113.08 (1C), 122.80 (1C), 126.48 (1C), 127.30 (1C), 128.32 (2C), 128.90 (2C), 130.71 (1C), 131.26 (1C), 135.70 (1C), 136.39 (1C), 140.87 (1C), 141.26 (1C), 148.80 (1C), 149.44 (1C), 151.95 (1C, C-3). MS *m/z*: 460 (MH^+, 100); Anal. Calcd. for $C_{27}H_{28}N_2O_5$ (%): C, 70.42; H, 6.13; N, 6.08; Found: C, 70.30; H, 6.01; N, 5.90.

RESULTS AND DISCUSSION

Structure proof of synthesized compounds, 3a-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, 3a 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 and exhibits typical ABX spin. The C_4-H_a, C_4-H_b and C_5-H protons appeared as a doublet of doublets. The doublet of doublet for C_4-H_a appeared at δ=3.546-3.592 (J=7.1, 13.0Hz) ppm; doublet of doublet for C_4-H_b appeared at δ=3.770-3.786 (J=6.0, 12.5Hz) ppm; and that of C_5-H at δ=4.882-4.991 (J=6.8, 14.0Hz) ppm. Among C_4-H_a, C_4-H_b and C_5-H protons, C_5-H is the most deshielded due to its close proximity to benzene ring and electronegative nitrogen atom. C_5-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 collection of signal observed singlet for nine protons at δ=2.320 ppm and δ=3.892 ppm were due to two methyl and three OCH_3 group protons. A collection of signals observed as multiplet for at δ=6.428-7.375 ppm were assigned to aromatic protons.

In the ^{13}C-NMR, compound 3a showed a signal at δ=40.35, 55.81 and 151.90 ppm due to C-4, C-5 and C-3 carbons of the pyrazole ring. Signals appear for one carbon each at δ=17.50, 20.32 ppm and for three carbons at δ=56.55 ppm were assigned to methyl and methoxy carbons. An array of signals appeared at δ=100.90, 112.50, 113.25, 122.50, 126.60, 127.34, 128.21, 128.96, 130.72, 131.24, 135.10, 136.20, 140.94, 141.20, 148.86 and 149.40 ppm were ambiguously assigned to aromatic carbons. Compound 3a showed molecular ion peak at m/z 417 as base peak corresponding to its molecular mass (MH^+). Further elemental analysis showed that the analytical 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 3b-g, which strongly supports the structure proof for the synthesized compounds.

Antimicrobial activity

Antimicrobial studies of synthesized compounds 3a-g were assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method [16,17]. The compounds were screened for their antimicrobial activities against Gram-negative bacteria *Escherichia coli*, Gram-positive bacteria *Staphylococcus aureus*, fungi species *Aspergillus niger* and *Aspergillus flavus*. The experiments were carried out in triplicate; the results were taken as a mean of three determinations. Antibiotics ciprofloxacin and nystatin were used as standard drugs for antibacterial and antifungal studies respectively. The results of MIC's were depicted in Figure 2.

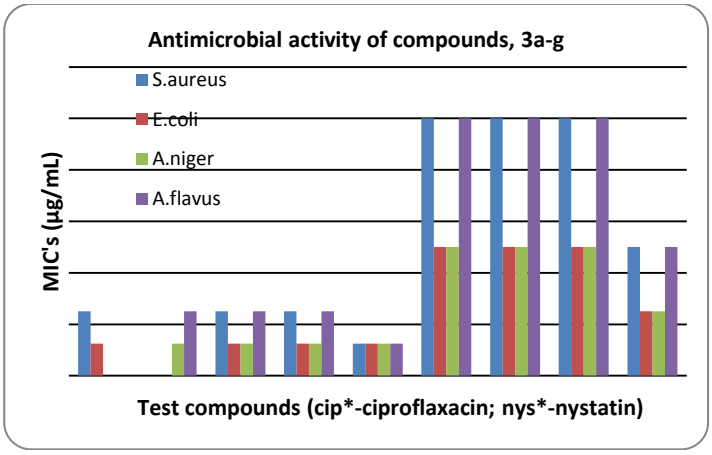


Figure 2: Minimum Inhibitory Concentrations (MIC) of the synthesized compounds 3a-g against bacterial and fungal stains (the values are expressed as mean of three determinations)

Preliminary investigation on the antimicrobial activities reveals that the synthesized pyrazoles 3a-g shows a wide range of *in vitro* antimicrobial activities against the tested organisms. Amongst the series, compound 3c having chloro substitution showed excellent antimicrobial activity by inhibiting spore germination of all the tested organisms. Compounds, 3a and 3b have displayed greater potency against tested species. Compounds 3d, 3e and 3f having methoxy substitutions on the aromatic ring showed poorer activities. Compound 3g having methelenedioxy substitution displayed moderate activities against the testes organisms.

CONCLUSIONS

In search of new antimicrobial agents, we have synthesized pyrazole analogues by the simple easy accessible procedure. Synthesized compounds were evaluated for antimicrobial activity. Among the series, compound 3c demonstrated potent antibacterial and antifungal activities.

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