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Research Article

SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF SOME NEW PYRAZOLINES BEARING 6-METHYL PYRIDINE MOIETY

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ABSTRACT

New series of pyrazoline derivatives were synthesized by the cyclocondensation of 6-methylpyridine-3-carbohydrazide (**3**) with different chalcones **(4a-l).** The structures of the newly synthesized compounds were confirmed on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All the synthesized compounds were screened for their antibacterial, antioxidant and analgesic activity. The studies reveal that compounds **5b, 5e** and **5j** showed significant antibacterial property compared to the standard drug Streptomycin. Pyrazoline derivatives **5a, 5e, 5g** and **5j** exhibited significant antioxidant property when compared with the standard BHT. Compounds **5c** and **5e** showed good analgesic activity compared with the standard drug Aspirin.

Keywords: 6-Methyl nicotinate, Pyrazoline, Antibacterial, Antioxidant, Analgesic.

INTRODUCTION

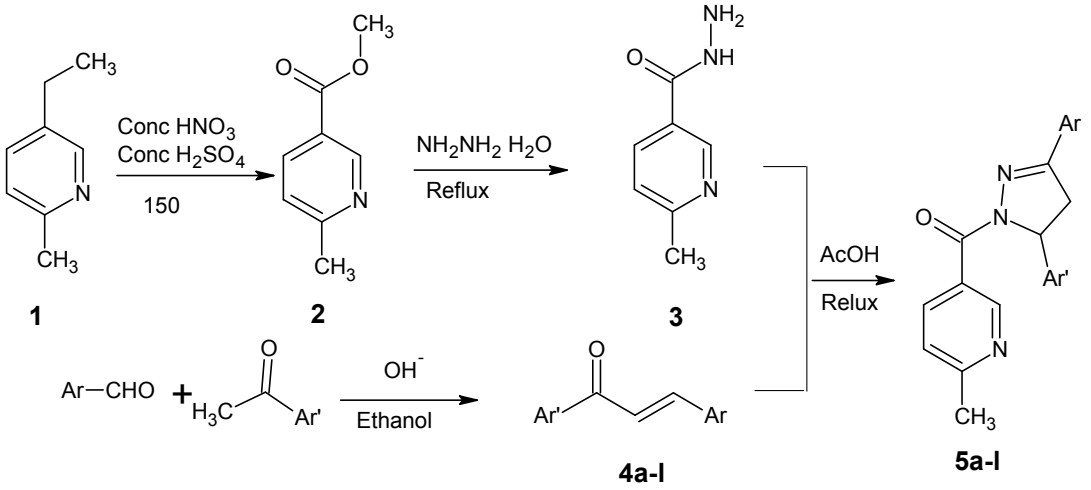
Pyrazole and its derivatives attracted organic chemists very much due to their biological and chemotherapeutic importance. The synthesis of pyrazoles and its derivatives is an interesting area of research due to the wide applications of such heterocycles in the pharmaceutical and agrochemical industry. Pyrazoles are known for their anti-inflammatory[1], analgesic, antipyretic, fungicidal[2], antibacterial[3], antitubercular[4], anticancer[5], anti-HIV and antimicrobial[6] activity. The recent success of pyrazole COX-2 inhibitor[7] has further highlighted the importance of this class of heterocycles in medicinal chemistry.

Similarly pyridine is another important heterocyclic compound showing various biological activities like antimicrobial[8,9], analgesic[10], anti-tumor[11], antioxidant[12] etc. A systematic investigation of these classes of heterocyclic compounds revealed that, pyrazole and pyridine containing pharmacoactive agents play important role in medicinal chemistry. In view of the therapeutic importance of pyrazoles and pyridines and in continuation of our search on biologically effective molecules[13-15], we herein report the synthesis, characterization, antibacterial, antioxidant and analgesic activities of a hitherto unreported pyrazoline derivatives carrying 6-methyl pyridine moiety.

RESULTS AND DISCUSSION

The reaction scheme employed for the synthesis of title compound (**5a-l**) is summarised in **Scheme-1**. The key intermediate 6-methylpyridine-3-

carbohydrazide (**3**) was obtained by the hydazinolysis of 6-methyl 3-methyl nicotinate (**2**) which in turn obtained from 2-methyl-5-ethyl pyridine (**1**)[15]. The chalcones (**4a-l**) were obtained by the condensation of aldehydes with various acetophenones in presence of sodium hydroxide (40%) in ethanol media. The chalcones (**4a-l**) on refluxing with 6-methylpyridine-3-carbohydrazide (**3**) in acetic acid yielded pyrazoline derivatives (**5a-l**). The structures of the newly synthesized compounds (**5a-l**) were established by IR, NMR, mass spectral and elemental analysis.



Ar = 4-Chlorophenyl, 2,4-Dimethoxyphenyl, 3-Chloro-2-Flourophenyl, 4- Methoxyphenyl
Ar' =Phenyl, 2,4-Dichlorophenyl, 4-Hydroxyphenyl, 3-Bromophenyl, 3-Flouro-4-methoxyphenyl
Scheme. 1: Synthetic route for the compounds 5a-l

In the IR spectrum of 5-{[5-(4-chlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl] carbonyl}-2-methylpyridine **5a**, the carbonyl absorption band was observed at 1635 cm^{-1}. Further in the ^1H NMR spectrum of **5a**, the methyl group attached to the pyridine moiety came into resonance as a singlet at δ 2.63 integrating for three protons. The diastereotropic protons of pyrazoline CH_2 appeared as doublet of doublet at δ 3.20 and 3.81 each integrating for one protons. The CH proton of pyrazoline resonated as doublet of doublet centred at δ 5.76. The signals due to five aromatic protons of phenyl moiety overlapped with each other and appeared as multiplet in the region of δ 7.23-7.33. The aromatic protons of 4-chlorophenyl moiety appeared as two doublet at δ 7.45 and 7.73 corresponding to two protons each. The pyridine 6H proton appeared as a singlet at δ 9.24, while the pyridine 3H and 4H protons appeared as two doublets centred at δ 7.41 and 8.21 respectively. Further the mass spectrum of compound **5a** showed the molecular ion peak at m/z 376 (M + 1 peak, 100%) consistent with the molecular formula $C_{22}H_{18}ClN_3O$, the isotope peak was seen at m/z 378 (M + 3). The characterisation data of the newly synthesised compounds are given in Table-1.

Pharmacology

Antibacterial studies

The *in vitro* antibacterial activity (zone of inhibition) of newly synthesized compounds **5a-l** were determined by well plate method in Muller Hinton Agar[16,17]. The newly synthesized compounds were screened for their antibacterial activity against *E. coli* (ATCC 25922), *B. subtilis* (MTCC- 441) and *P. aeruginosa* (ATCC 27853). All bacterial strains were maintained on nutrient agar medium at ± 37°C. The cultures were inoculated in fresh 10 mL nutrient broth to yield an initial suspension of approximately 10–100 cfu/mL. All broths were then incubated statically at ± 37°C for 18–24 hr. The bacterial suspensions were diluted tenfold in distilled water and 0.1 mL of the bacterial suspension was spread on nutrient agar in order to give a population of approximately 10^6 cfu/plate. The wells were dug in each petri plate by sterilized cork borer. The compounds were dissolved in DMSO and appropriate dilutions were made (1mg/mL and 0.5mg/mL). Each experiment was carried out in triplicate. After the inoculation of organism and compound, the petri plates were incubated for 24 hrs at 37°C. The diameter of zone of inhibition was measured. Streptomycin was taken as the standard drug for antibacterial screening.

The result indicated that among the tested compounds, **5b, 5e** and **5j** showed good activity against all the tested microbial strains *E. coli*, *B. subtilis* and *P. aeruginosa* at concentrations of 1 and 0.5 mg/mL comparable with standard drug streptomycin. Compounds **5d, 5h, 5i, 5k** and **5l** showed moderate antimicrobial activity against all the tested microbial strains. All the three bacterial strains are resistant towards the compounds **5a**, **5c**, **5g** and **5f**. The results are summarized in Table-2.

Antioxidant studies: DPPH radical scavenging assay

Free radical-scavenging capacities of the compounds **5a-l** were determined using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH)[18]. The stock solution of the test compounds (1 mg/mL) and DPPH (0.004%) were prepared using 95% methanol. Freshly prepared DPPH solution were taken in test tubes and organic compounds were then added (100 µg) to every test tube. The absorbance was measured after 10 minutes at 517 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). BHT was used as a reference standard. Control sample was prepared without any extract or BHT. 95% Methanol was used as blank.

Pyrazoline derivatives **5a, 5e, 5g** and **5j** showed significant antioxidant property when compared with the standard BHT. Results are presented in Table-3 and Fig. 1 shows the graphical representation of antioxidant activity of compounds **5a-l**.

Analgesic activity (Acetic acid-induced writhing test in mice)

The Analgesic activity of synthesized compounds was carried out by following the method of Koster *et al*[19] as modified by Dambisya and Lee[20]. The required number of albino mice was selected and grouped. Each group consisting of 6 mice and these mice was starved for 16 hours and treated as follows: the 1st group which served as positive control and received 1 ml saline orally. The second group received the standard drug Aspirin in a dose of 100 mg/kg body weight of mice. The remaining groups received aqueous suspension of synthesized compounds orally in a dose of 100 mg/kg body weight of mice. After 30 min, each mice was administered 0.6% of an aqueous solution of acetic acid (10 ml/kg) and mice were then placed in transparent boxes for observation. The number of writhes was counted for 20 min after acetic acid injection. The analgesic activity was expressed in terms of % inhibition.

Analgesic activity (%)=
(Mean control group--Mean treated group/Mean control group) X 100

The results of analgesic activity are presented in Table-4. Compounds **5c** and **5e** displayed good analgesic activity compared with the standard drug Aspirin. Compounds **5g**, **5h** and **5l** did not show any analgesic activity. The remaining compounds are showed moderate activity.

CONCLUSION

New series of substituted pyrazoline derivatives containing 6-methyl pyridine moiety were synthesized in reasonably good yields. The synthesized compounds were evaluated for their antibacterial, antioxidant and analgesic activities. The compounds **5b, 5e** and **5j** have emerged as most active against all tested bacterial strains. The enhanced antibacterial activity of compounds **5b, 5e** and **5j** may be due to the presence of 4-chlorophenyl, 2,4-dichlorophenyl, 3-flouro-4-methoxy phenyl moiety and 3-chloro-2-flouro-phenyl moiety on pyrazole ring.

Pyrazoline derivatives **5a, 5e, 5g** and **5j** showed significant antioxidant property when compared with the standard BHT. Compounds **5a, 5e, 5g** and **5j** are having 4-chlorophenyl, 3-flouro-4-methoxyphenyl, 2,4-dimethoxyphenyl, 2,4-dichlorophenyl, 3-chloro-2-fluorophenyl substituents on pyrazole moiety which is accounted for the enhanced activity. Compounds **5c** and **5e** exhibited moderate analgesic activity compared with the standard drug Aspirin. The improved activity of **5c** and **5e** may be due to the presence of 4-chlorophenyl, 3-bromophenyl, 3-flouro-4-methoxy phenyl substituents on pyrazole ring.

Experimental Section

Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr pellets) were recorded on a JASCO FT/IR-4100 spectrophotometer. ^{1}H NMR spectra were recorded ($CDCl_3$) on a Bruker (400 MHz) using TMS as internal standard. Chemical shift values are given in δ (ppm) scales. The mass spectra were recorded on a JEOL JMS-D 300 mass spectrometer operating at 70 eV. Elemental analyses were performed on a Flash EA 1112 series CHNS-O Analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254) obtained from Merck. Commercial grade solvents and reagents were used without further purification.

Synthesis of 6-methylpyridine-3-carbohydrazide *(3)*

6-Methyl-3-metyl nicotinate (**2**) was prepared from 2-methyl 5-ethyl pyridine as per the literature procedure[15]. 6-Methyl-3-methyl nicotinate (**2**) (0.05 mmol) and hydrazine hydrate (99%) (0.05 mmol) were dissolved in ethanol (10 mL) and the clear solution was refluxed for 2 hrs. The reaction mass was then reduced to half of its volume and allowed to cool. The solid mass thus separated out was filtered, washed with small amount of chilled ethanol and dried. (Yield = 90%).

General procedure for the synthesis of chalcones (4a-l)

To a solution of aromatic aldehyde (0.02 mol) in ethanol substituted acetophenone (0.02 mol) was added in presence of 40% sodium hydroxide solution and the reaction mixture was stirred for 24 hr. The reaction mixture was poured into crushed ice and acidified with dilute hydrochloric acid. The precipitated solid was filtered and purified by recrystallization from hot ethanol.

(2*E*)-3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (4a)

Yield 82%, m.p. 112- 115 ºC [Lit ; m.p. 113-117 ºC] IR (KBr) γ/cm^{-1}: 2935 (C-H), 1637 (C=O), 1529 (C=C); Anal. Calcd for $C_{15}H_{11}ClO$: C, 74.23 ; H, 5.37 Found : C, 74.24 ; H, 5.38.

(2*E*)-3-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)prop-2-en-1-one (4b)

Yield 86%, m.p. 118-120 ºC; Anal. Calcd for $C_{15}H_9Cl_3O$, 57.82 ; H, 2.91. Found: C, 57.84 ; H, 2.90.

1-(3-Bromophenyl)-3-(4-chlorophenyl)prop-2-en-1-one (4c)

Yield 80%, m.p. 102-104 ºC Anal. Calcd for $C_{15}H_9Cl_3O$, 56.02 ; H, 3.13. Found: C, 55.97 ; H, 3.09.

3-(4-Chlorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (4d)

Yield 82%, m.p. 145-147 ºC [Lit ; m.p. 145 ºC] Anal. Calcd for $C_{15}H_{11}ClO_2$: C, 69.64 ; H, 4.29 ; Found: C, 69.53 ; H, 4.26.

3-(2,4-Chlorophenyl)-1-(3-flouro-4-methoxyphenyl)prop-2-en-1-one (4e)

Yield 84%, m.p. 136-138 ºC Anal. Calcd for $C_{16}H_{12}ClFO_2$: C, 66.10 ; H, 4.16 ; Found: C, 66.13 ; H, 4.17.

3-(2,4-Dimethoxyphenyl)-1-phenyl-prop-2-en-1-one (4f)

Yield 78%, m.p. 136-138 ºC Anal. Calcd for $C_{17}H_{16}O_3$: C, 76.10 ; H, 6.01 ; Found: C, 76.12 ; H, 6.02.

1-(2,4-Dichlorophenyl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (4g)

Yield 78%, m.p. 126-128 ºC Anal. Calcd for $C_{17}H_{14}Cl_2O_3$: C, 60.55; H, 4.18 ; Found: C, 60.53 ; H, 4.17.

3-(2,4-Dimethoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (4h)

Yield 72%, m.p. 142-144 ºC Anal. Calcd for $C_{17}H_{16}O_4$: C, 71.82 ; H, 5.67 ; Found: C, 71.85 ; H, 5.69.

3-(3-Chloro-2-flourophenyl)-1-phenyl-prop-2-en-1-one (4i)

Yield 80%, m.p. 122-124 ºC Anal. Calcd for $C_{10}H_{15}ClFO$: C, 69.11 ; H, 3.87 ; Found: C, 69.12 ; H, 3.89.

3-(3-Chloro-2-flourophenyl)-1-(2,4-dichlorophenyl)prop-2-en-1-one (4j)

Yield 86%, m.p. 132-134 ºC Anal. Calcd for $C_{15}H_8Cl_3FO$: C, 54.66; H, 2.45 ; Found: C, 54.68 ; H, 2.47.

3-(4-Methoxyphenyl)-1-phenyl-prop-2-en-1-one (4k)

Yield 93%, m.p. 74-76 ºC [Lit ; m.p. 73-76ºC] Anal. Calcd for $C_{16}H_{14}O_2$: C, 80.65; H, 5.92 ; Found: C, 80.63 ; H, 5.94.

3-(4-Methoxyphenyl)-1-(3-flouro-4-methoxyphenyl)prop-2-en-1-one (4l)

Yield 76%, m.p. 110-112 ºC Anal. Calcd for $C_{17}H_{15}FO_3$: C, 71.32; H, 5.28 ; Found: C, 71.34 ; H, 5.27.

General procedure for the synthesis of pyrazolines *(5a-l)*

A mixture of **4a-l** (0.01mmol) and 6 -methylpyridine-3-carbohydrazide (**3**) (0.01 mmol) were refluxed in acetic acid for 3-4hrs. After the completion of the reaction, the contents were poured into ice cold water and kept overnight. The solid thus separated was filtered, washed with water and dried. The crude product was purified by column chromatography (Mobile phase: 9:1; n-Hexane: Ethyl acetate) to get pure compounds **5a-l.**

5-{[5-(4-Chlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]carbonyl}-2-methylpyridine *(5a)*

White microcrystals; IR (KBr) γ/cm^{-1}: 3046 (ArC-H), 2924 (C-H), 1635 (C=O), 1594 (C=N);

^{1}H NMR ($CDCl_3$, 400MHz): δ 2.63 (s, 3H, CH_3), 3.20 (dd, 1H, pyrazoline CH_2), 3.81 (dd, 1H, pyrazoline CH_2), 5.76 (dd, 1H, pyrazoline CH), 7.23-7.33 (m, 5H, Ar-H of phenyl), 7.41 (d, 1H, pyridine ring 4H), 7.45 (d, 2H, Ar-H of 4-chlorophenyl), 7.73 (d, 2H, Ar-H of 4-chlorophenyl), 8.21 (d, 1H, pyridine 3H), 9.24 (s, 1H, pyridine 6H); ^{13}C NMR ($CDCl_3$, 100MHz): δ 24.60 (CH_3), 41.47 (C_4 of pyrazoline), 60.81 (C_5 of pyrazoline), 122.43 (C_5 of pyridine), 127.11 (C_3 of pyridine), 126.85, 127.26, 128.86, 130.84 (C of phenyl), 138.14 (C_4 of pyridine), 129.22, 130.80, 133.70, 140.03 (C of 4-chlorophenyl), 150.85 (C_2 of pyridine), 155.33 (C_3 of pyrazoline), 161.00 (C_6 of pyridine ring), 164.28 (C=O); DEPT: CH and CH_3 δ 24.59, 41.47, 60.80, 122.44, 126.85, 127.26, 128.86, 129.22, 130.80, 138.15, 150.85; LC-MS (m/z): 376 (M + 1, 100%) , (M.F: $C_{22}H_{18}ClN_3O$).

5-{[5-(4-Chlorophenyl)-3-(2,4-dichloro phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]carbonyl}-2-methylpyridine*(5b)*

White microcrystals; IR (KBr) γ/cm^{-1}: 3032 (ArC-H), 2930 (C-H), 1646 (C=O), 1601 (C=N); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.60 (s, 3H, CH_3), 3.18 (dd, 1H, pyrazoline CH_2), 3.75 (dd, 1H, pyrazoline CH_2), 5.75 (dd, 1H, pyrazoline CH), 6.86 (m, 3H, Ar-H), 7.45-7.67 (m, 5H, Ar-H and pyridine ring 4H), 8.19 (d, 1H, pyridine 3H), 9.18 (s, 1H, pyridine 6H); LC-MS (m/z): 445 (M + 1, 100%) , (M.F: $C_{22}H_{16}Cl_3N_3O$).

5-{[3-(3-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]carbonyl}-2-methylpyridine *(5c)*

White microcrystals; IR (KBr) γ/cm^{-1}: 3041 (ArC-H), 2938 (C-H), 1642 (C=O), 1598 (C=N); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.64 (s, 3H, CH_3), 3.17 (dd, 1H, pyrazoline CH_2), 3.77 (dd, 1H, pyrazoline CH_2), 5.77 (dd, CH, pyrazoline CH), 7.26-7.32 (m, 6H, Ar-H), 7.56 (d, 1H, pyridine ring 4H), 7.66 (1H, Ar-H), 7.81 (1H, Ar-H), 8.18 (d, 1H, pyridine 3H), 9.20 (s, 1H, pyridine 6H); LC-MS (m/z): 455 (M + 1, 100%) , (M.F: $C_{22}H_{17}BrClN_3O$).

5-{[3-(4-Hydroxyphenyl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]carbonyl}-2-methylpyridine *(5d)*

Brown microcrystals; IR (KBr) γ/cm^{-1}: 3052 (Ar C-H), 2926 (C-H), 1646 (C=O), 1594 (C=N); ^{1}H NMR ($CDCl_3$, 400M): δ 2.63 (s, 3H, CH_3), 3.18 (dd, 1H, pyrazoline CH_2), 3.74 (dd, 1H, pyrazoline CH_2), 5.75 (dd, CH, pyrazoline CH), 6.81-7.03. (m, 4H, Ar-H), 7.58 (d, 1H, pyridine ring 4H),7.64-7.81 (m, 4H, Ar-H), 8.23 (d, 1H, pyridine 3H), 9.25 (s, 1H, pyridine 6H); LC-MS (m/z): 392 (M + 1, 100%) , (M.F: $C_{22}H_{18}ClN_3O_2$).

5-{[3-(3-Flouro-4-methoxyphenyl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]carbonyl}-2-methylpyridine *(5e)*

Brown microcrystals; IR (KBr) γ/cm^{-1}: 3050 (Ar C-H), 2924 (C-H), 1645 (C=O), 1592 (C=N); ^{1}H NMR ($CDCl_3$, 400M): δ 2.65 (s, 3H, CH_3), 3.17 (dd, 1H, pyrazoline CH_2), 3.75 (dd, 1H, pyrazoline CH_2), 3.80 (s, 3H, -OCH_3), 5.77 (dd, CH, pyrazoline CH), 6.91-7.15 (m, 4H, Ar-H), 7.60 (d, 1H, pyridine ring 4H),7.80-7.92 (m, 4H, Ar-H), 8.26 (d, 1H, pyridine 3H), 9.27 (s, 1H, pyridine 6H); LC-MS (m/z): 425 (M + 1, 100%), (M.F: $C_{23}H_{19}FClN_3O_2$).

5-{[3-Phenyl-5-(2,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]carbonyl}-2-methyl pyridine *(5f)*

White microcrystals; IR (KBr) γ/cm^{-1}: 3053 (Ar C-H), 2931 (C-H), 1634 (C=O), 1592 (C=N); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.63 (s, 3H, CH_3), 3.24 (dd, 1H, pyrazoline CH_2), 3.79 (dd, 1H, pyrazoline CH_2), 3.85 (s, 6H, -OCH_3), 5.75 (dd, CH, pyrazoline CH), 6.81-6.88 (m, 3H, Ar-H), 7.25(d, 1H, pyridine ring 4H),7.39-7.44 (d, 3H, Ar-H), 7.73 (d, 2H, Ar-H), 8.21 (d, 1H, pyridine 3H), 9.24 (s, 1H, pyridine 6H); ^{13}C NMR ($CDCl_3$, 100MHz): δ 24.57 (CH_3), 41.69 (C_4 of pyrazoline), 55.94 (-OCH_3), 61.17 (C_5 of pyrazoline),109.60, 111.60, 117.78 (C of benzene), 122.41 (C_5 of pyridine), 126.84,128.81, 130.66,131.07,134.20 (C of benzene), 127.42 (C_3 of pyridine), 138.11 (C_4 of pyridine), 148.67, 149.34 (C of benzene), 150.81 (C_2 of pyridine), 155.45 (C_3 of pyrazoline), 160.81 (C_6 of pyridine ring), 164.32 (C=O); DEPT: CH and CH_3 δ 24.57, 41.69, 55.94, 61.17, 109.13, 111.60, 117.78, 122.42, 126.85, 128.81,130.66, 138.12, 150.80; LC-MS (m/z): 402 (M + 1, 100%), (M.F: $C_{24}H_{23}N_3O_3$).

5-{[3-(2,4-Dichlorophenyl)-5-(2,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]carbonyl} -2-methylpyridine *(5g)*

Brown microcrystals; IR (KBr) γ/cm^{-1}: 3050 (Ar C-H), 2927 (C-H), 1650 (C=O), 1599 (C=N); ^{1}H NMR ($CDCl_3$, 400M): δ 2.61 (s, 3H, CH_3), 3.41 (dd, 1H, pyrazoline CH_2), 3.86 (s, 6H, OCH_3), 3.93 (dd, 1H, pyrazoline CH_2), 5.75 (dd, CH, pyrazoline CH), 6.86. (m, 3H, Ar-H), 7.23-7.69 (m, 3H, Ar-H and pyridine ring 4H), 8.19 (d, 1H, pyridine 3H), 9.18 (s, 1H, pyridine 6H); LC-MS (m/z): 471 (M + 1, 100%) , (M.F: $C_{24}H_{21}Cl_2N_3O_3$).

5-{[3-(4-Hydroxyphenyl)-5-(2,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl] carbonyl}-2-methylpyridine *(5h)*

Off white microcrystals; IR (KBr) $\gamma_i cm^{-1}$: 3051 (Ar C-H), 2932 (C-H), 1645 (C=O), 1594 (C=N); ^{1}H NMR ($CDCl_3$, 400M): δ 2.64 (s, 3H, CH_3), 3.18 (dd, 1H, pyrazoline CH_2), 3.79 (dd, 1H, pyrazoline CH_2), 3.86 (s, 6H, OCH_3), 5.76 (dd, CH, pyrazoline CH), 6.92-6.99 (m, 4H, Ar-H), 7.24-7.31 (m, 3H, Ar-H), 7.34 (d, 1H, pyridine ring 4H),8.23 (d, 1H, pyridine 3H), 9.26 (s, 1H, pyridine 6H); LC-MS (m/z): 418 (M + 1, 100%), (M.F: $C_{24}H_{23}N_3O_4$).

5-{[5-(3-Chloro-2-flourophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]carbonyl}-2-methyl pyridine*(5i)*

White microcrystals; IR (KBr) $\gamma_i cm^{-1}$: 3047 (ArC-H), 2926 (C-H), 1642 (C=O), 1595 (C=N); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.61 (s, 3H, CH_3), 3.17 (dd, 1H, pyrazoline CH_2), 3.79 (dd, 1H, pyrazoline CH_2), 5.75 (dd, CH, pyrazoline CH), 6.78-6.84 (m, 5H, Ar-H), 7.28-7.34 (m, 3H, Ar-H), 7.37 (d, 1H, pyridine ring 4H), 8.20 (d, 1H, pyridine 3H), 9.26 (s, 1H, pyridine 6H); LC-MS (m/z): 395 (M + 1, 100%), (M.F: $C_{22}H_{17}FClN_3O$).

5-{[5-(3-Chloro-2-fluorophenyl)-3-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl] carbonyl}-2-methylpyridine *(5j)*

White microcrystals; IR (KBr) $\gamma_i cm^{-1}$: 3032 (ArC-H), 2934 (C-H), 1640 (C=O), 1599 (C=N); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.60 (s, 3H, CH_3), 3.24 (dd, 1H, pyrazoline CH_2), 3.80 (dd, 1H, pyrazoline CH_2), 5.80 (dd, CH, pyrazoline CH), 7.26-7.30 (m, 3H, Ar-H of 2,4-dichlorophenyl), 7.39-7.444 (m, 3H, Ar-H), 7.45 (d, 1H, pyridine ring 4H), 8.21 (d, 1H, pyridine 3H), 9.20 (s, 1H, pyridine 6H); LC-MS (m/z): 463 (M + 1, 100%) , (M.F: $C_{22}H_{15}FCl_3N_3O$).

5-{[3-(4-Methoxyphenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]carbonyl}-2-methylpyridine *(5k)*

White microcrystals; IR (KBr) $\gamma_i cm^{-1}$: 3035 (ArC-H), 2932 (C-H), 1648 (C=O), 1600 (C=N); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.61 (s, 3H, CH_3), 3.26 (dd, 1H, pyrazoline CH_2), 3.61 (dd, 1H, pyrazoline CH_2), 3.86 (s, 3H, OCH_3), 5.85 (dd, CH, pyrazoline CH), 6.98 (d, 2H, Ar H), 7.04 -7.08 (d, 2H, Ar H), 7.28-7.36 (m, 3H, Ar-H), 7.48 (d, 1H, pyridine ring 4H), 7.57-7.55 (d, 2H, Ar-H), 8.25 (d, 1H, pyridine 3H), 9.20 (s, 1H, pyridine 6H); LC-MS (m/z): 372 (M + 1, 100%) , (M.F: $C_{23}H_{21}N_3O_2$).

5-{[5-(4-Methoxyphenyl)-3-(3-flouro-4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl] carbonyl}-2-methylpyridine *(5l)*

White microcrystals; IR (KBr) $\gamma_i cm^{-1}$: 3032 (ArC-H), 2930 (C-H), 1646 (C=O), 1601 (C=N); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.63 (s, 3H, CH_3), 3.26 (dd, 1H, pyrazoline CH_2), 3.72 (dd, 1H, pyrazoline CH_2), 3.92 (s, 6H, OCH_3), 5.85 (dd, CH, pyrazoline CH), 7.02-7.05 (2H, Ar-H), 7.33-7.48 (m, 4 H, Ar-H and pyridine ring 4H), 7.57- 7.55 (2H, Ar-H), 8.24 (d, 1H, pyridine 3H), 9.18 (s, 1H, pyridine 6H); LC-MS (m/z): 420 (M + 1, 100%) , (M.F: $C_{24}H_{22}FN_3O_3$).

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Table 1: Characterization data of synthesized compounds (5a-l)

Comp. No	Ar	Ar'	M.P. (°C)	Yield (%)	Molecular formula (Molecular weight)	Elemental analysis found(calculated) (%)		
						C	H	N
5a	4-Chlorophenyl	Phenyl	140-142	76	$C_{22}H_{18}ClN_3O$ (375.85)	70.36 (70.30)	4.86 (4.83)	11.22 (11.18)
5b	4-Chlorophenyl	2,4-Dichlorophenyl	146-148	80	$C_{22}H_{16}Cl_3N_3O$ (444.74)	59.45 (59.41)	3.67 (3.63)	9.48 (9.45)
5c	4-Chlorophenyl	3-Bromophenyl	160-163	69	$C_{22}H_{17}BrClN_3O$ (454.75)	58.16 (58.11)	3.81 (3.77)	9.29 (9.24)
5d	4-Chlorophenyl	4-Hydroxy phenyl	138-140	72	$C_{22}H_{18}ClN_3O_2$ (391.85)	67.45 (67.43)	4.67 (4.63)	10.76 (10.72)
5e	4-Chlorophenyl	3-Fluoro-4-methoxy phenyl	170-172	58	$C_{23}H_{19}FClN_3O_2$ (423.86)	65.19 (65.17)	4.55 (4.52)	9.93 (9.91)
5f	2,4-Dimethoxyphenyl	Phenyl	102-105	84	$C_{24}H_{23}N_3O_3$ (401.45)	71.84 (71.80)	5.81 (5.77)	10.50 (10.47)
5g	2,4-Dimethoxyphenyl	2,4-Dichlorophenyl	124-126	76	$C_{24}H_{22}Cl_2N_3O_3$ (470.35)	61.33 (61.29)	4.53 (4.50)	8.97 (8.93)
5h	2,4-Dimethoxyphenyl	4-Hydroxy phenyl	131-134	69	$C_{24}H_{23}N_3O_4$ (417.45)	69.07 (69.05)	5.58 (5.55)	10.11 (10.07)
5i	3-Chloro-2-fluoro phenyl	Phenyl	156-158	79	$C_{22}H_{17}FClN_3O$ (393.84)	67.07 (67.09)	4.32 (4.35)	10.65 (10.67)
5j	3-Chloro-2-fluoro phenyl	2,4-Dichlorophenyl	144-146	68	$C_{22}H_{15}FCl_3N_3O$ (462.73)	57.13 (57.10)	3.30 (3.27)	9.12 (9.08)
5k	4-Methoxy phenyl	Phenyl	120-122	66	$C_{23}H_{21}N_3O_2$ (371.43)	74.39 (74.37)	5.72 (5.70)	8.63 (8.61)
5l	4-Methoxy phenyl	3-Fluoro-4-methoxy phenyl	118-120	62	$C_{24}H_{22}FN_3O_3$ (419.45)	68.74 (68.72)	5.28 (5.29)	10.00 (10.02)

Table 2: Antibacterial activity of title compounds 5a-l

Organic compound	*E.coli*		*B.subtilis*		*P.aeruginosa*	
Concentration In mg/ml	1	0.5	1	0.5	1	0.5
Streptomycin	19±0.2	17±0.3	22±0.6	19±0.6	19±0.5	15±0.3
Control	00	00	00	00	00	00
5a	-	-	-	-	-	-
5b	12±0.2	09±0.4	11±0.1	08±0.7	12±0.5	10±0.3
5c	-	-	-	-	-	-
5d	06±0.4	03±0.5	04±0.3	03±0.5	03±0.2	02±0.3
5e	10±0.2	07±0.6	13±0.5	10±0.2	13±0.1	09±0.4
5f	-	-	-	-	-	-
5g	-	-	-	-	-	-
5h	04±0.3	02±0.2	03±0.8	01±0.1	04±0.2	02±0.1
5i	06±0.4	04±0.2	05±0.1	03±0.4	07±0.6	05±0.3
5j	10±0.6	08±0.2	11±0.3	09±0.7	12±0.2	09±0.1
5k	06±0.6	04±0.2	05±0.6	03±0.3	03±0.6	02±0.3
5l	06±0.3	04±0.5	05±0.3	03±0.3	05±0.6	04±0.3

Table 3: DPPH scavenging activity of title compounds 5a-l

Compounds	DPPH Assay in %
5a	64.48
5b	27.95
5c	25.86
5d	24.50
5e	71.74
5f	27.7
5g	63.12
5h	25.36
5i	26.37
5j	68.14
5k	44.74
5l	54.12
BHT	90.4

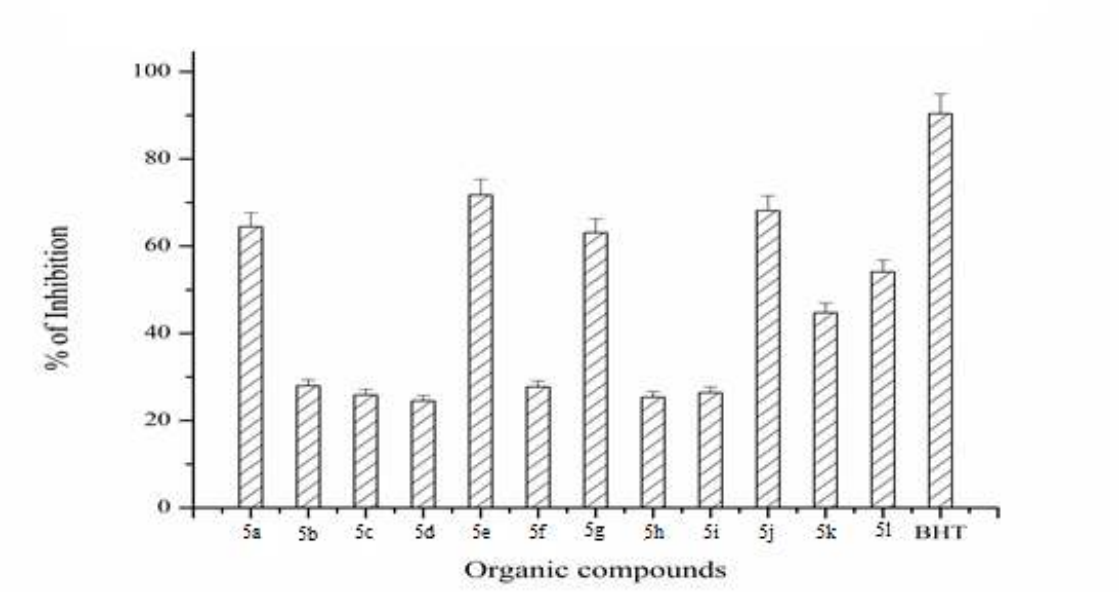

Fig. 1: DPPH scavenging activity of title compounds 5a-l

Table 4: Effect of synthesized compounds (5a-l) on acetic acid-induced writhing test in mice

Compounds	% of inhibition
Control	----
Aspirin(100 mg/kg body weight)	71.1
5a	16.0
5b	30.2
5c	47.4
5d	29.3
5e	51.3
5f	10.4
5g	-----
5h	-----
5i	8.4
5j	36.2
5k	34.7
5l	----

Values are mean± S.E.M., n=6, p<0.05, Significant compare to control

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