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# SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF SOME NOVEL PYRAZOLE AND ISOXAZOLE DERIVATIVES CARRYING 4-CHLORO-3-METHYL PHENOL (P-CHLORO-M-CRESOL) MOIETY

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## ABSTRACT

A new series of 3,5-disubstituted-1-phenyl pyrazoles (6a-d) and 3,5-disubstituted isoxazoles (7a-d) incorporating phenolic moiety of p-chloro-m-cresol viz. 4-chloro-3-methylphenol have been synthesized in excellent yields via interactions of corresponding substituted flavones (5a-d) with phenyl hydrazine hydrochloride and hydroxylamine hydrochloride respectively in DMF solvent with small amount of piperidine. Initially, disubstituted 2-hydroxy acetophenone (2) was prepared by an acetylation of p-chloro-m-cresol followed by Fries rearrangement which on reactions with different aromatic carboxylic acids in dry pyridine using $POCl_3$ gives substituted 2-benzoyloxyacetophenones (3a-d). A Baker-Venkataraman rearrangement (BVT) of substituted 2-benzoyloxyacetophenones (3a-d) with pulverized KOH in dry pyridine gives respective 1,3-diketone or β-diketone derivatives (4a-d) which on dehydrative cyclization in an acidic media yielded required substituted flavones (5a-d) as key intermediate for the synthesis of titled compounds. The structures of newly synthesized pyrazole and isoxazole derivatives have been confirmed by usual chemical characteristics, elemental analysis, IR, $^1$H NMR and Mass spectroscopic techniques. All the newly synthesized compounds were evaluated in *vitro* against human pathogenic microorganisms in order to assess their antibacterial and antifungal activities using disc diffusion method. They were also tested for their in *vitro* antioxidant and anti-inflammatory potential by adopting DPPH free radical scavenging and inhibition of protein denaturation method respectively with reference to standard drugs.

**KEYWORDS:** Pyrazoles, Isoxazoles, p-Chloro-m-cresol, Synthesis, Pharmacological study.

## INTRODUCTION

Heterocyclic compounds are versatile, highly valuable and unique class of compounds of chemical and biological interest. Amongst heterocyclics, the five membered heterocycles with two heteroatoms mainly pyrazoles and isoxazoles have gained considerable interest in various fields because of their wide range of pharmacological and physical applications. Pyrazole is a five membered aromatic heterocyclic moiety having two adjacent nitrogen atoms and three carbon atoms.[1] Isoxazole is also an unsaturated aromatic heterocyclic compound containing a ring with three carbon atoms and one oxygen atom next to nitrogen atom.[2] Several pyrazole and isoxazole derivatives possess antimicrobial,[3-7] analgesic and anti-inflammatory,[8-14] anticancer,[15-21] antitumor,[22-23] anti-HIV,[24-25] anticonvulsant,[26-29] antitubercular[30-31] and antioxidant[32-33] properties. Besides, pyrazole containing drugs celecoxib demonstrates anti-inflammation effect and inhibits COX-2[34] rimonabant functions as cannabinoid receptor and is utilized in obesity treatment,[35] fomepizole inhibits alcohol dehydrogenase and sildenafil inhibits phosphodiesterase.[36] Recently, pyrazole molecules have been proved to be as effective drugs for ACE2 infected human cells against COVID-19.[37] In addition, they have been found to played crucial role as agrochemicals in crop protection chemistry such as insecticidal, fungicidal and herbicidal agents.[38-40] Isoxazole motif is ubiquitous in many natural products such as ibotenic acid, muscimol, isoxazole-4-carboxylic acid and drugs like valdecoxib, leflunomide, cloxacillin, oxacillin,[41-45] dicloxacillin,[46] isocarboxazide[47] and sulfisoxazole.[48] Among the various methods for the synthesis of pyrazoles, 1,3-dipolar cycloaddition and [2+3] cyclocondensation reactions are the prominent ones.[49] Among the different methods of isoxazole synthesis, [2+3] cycloaddition of 1,3-dipoles to alkynes and the reaction of hydroxylamine with 1,3-diketone (β-diketone) or an α, β-unsaturated ketones have gained much importance.[50] Moreover, chalcones and flavones are also valuable substrates in a variety of synthetic transformations and useful as building blocks in synthesis of physiologically active pyrazole and isoxazole derivatives. Nowadays, development of pharmacologically active heterocycles adopting simple methodologies is one of the major challenges for organic chemists. On the other hand, interest towards the synthesis of novel pyrazole and isoxazole derivatives with the aim to discover their therapeutic values remains a main focus of medicinal and pharmaceutical research and much research has been carried out in this direction. Keeping in view the pharmacological profile of these two chemically distinct but pharmacologically compatible molecules, we have decided to prepare some novel 3,5-disubstituted-1-phenyl pyrazoles and 3,5-disubstituted isoxazoles bearing phenolic moiety of p-chloro-m-cresol viz. 4-chloro-3-methylphenol in order to explore their pharmacological properties. Hence, in this present communication, a new series namely 3-(5’-chloro-2’-hydroxy-4’-methylphenyl)-5-(substituted phenyl)-1-phenyl pyrazoles (6a-d) and 3-(5’-chloro-2’-hydroxy-4’-methylphenyl)-5-(substituted phenyl) isoxazoles (7a-d) has been efficiently synthesized by the interactions of newly synthesized flavone analogues (5a-d) with phenylhydrazine hydrochloride and hydroxylamine hydrochloride respectively in DMF solvent with slight amount of piperidine. The success of synthesis and constitutions of the synthesized compounds have been assigned on the basis of chemical characteristics, elemental analysis, and IR, $^1$H NMR and Mass spectral data studies. All the newly synthesized compounds have been evaluated for their in *vitro* antibacterial and antifungal activities against human pathogenic bacterial and fungal strains by using disc diffusion method. Moreover, they have been also tested for their in *vitro* antioxidant and anti-inflammatory potential against standard reference drugs by adopting DPPH free radical scavenging and inhibition of protein denaturation method respectively with slight modifications.

## MATERIALS AND METHODS

All the chemicals and solvents were of synthetic grade procured from commercial sources and used without further purification. Melting points were determined in open glass capillaries and were uncorrected. All the newly synthesized compounds were purified by recrystallization and their purity was ascertained by TLC on silica gel (G) plates. The elemental analyses were carried out on a Thermo Scientific FLASH 2000 elemental analyzer instrument. IR spectra have been recorded on a Shimadzu IR Affinity-1 CE (Japan) spectrophotometer in KBr matrix. A Brucker Avance Neo FTNMR spectrometer of 500 MHz was used to acquire $^1$H NMR spectra with DMSO-$d_6$ as solvent and TMS as an internal reference (chemical shifts in $\delta$ ppm). Meanwhile, LC-MS spectra were recorded on the Waters Micromass Alliance 2795 Q-TOF micromass spectrometer (SAIF, PU, Chandigarh).

### General procedure for synthesis of pyrazole (6a-d) and isoxazole (7a-d) derivatives

The synthesis involves the following synthetic steps:

**1. Synthesis of p-Chloro-m-cresyl acetate (1):** A p-chloro-m-cresol (50g) was mixed with acetic anhydride (60 ml) and anhydrous sodium acetate (5g). The reaction mixture was refluxed for about 1½ hrs. The reaction mixture was allowed to cool followed by their decomposition in ice-cold water. Two layers aqueous and acetate were formed out of which lower acetate layer was separated by means of separating funnel and purified by distillation to obtained a p-chloro-m-cresyl acetate viz. 4-chloro-3-methylphenyl acetate (1) as a colourless liquid.

**2. Synthesis of 5-Chloro-2-hydroxy-4-methyl acetophenone (2):** A mixture of p-chloro-m-cresyl acetate (1) (50 ml) and anhydrous aluminium chloride (120g) in Kjeldal’s flask were heated at about 120 $^0$C for about 1 hr in an oil bath (Fries migration). The reaction mixture was cooled and decomposed with ice-cold water containing a little HCl (10%) to get crude ketone. It was purified by dissolving it in an acetic acid and allowing the solution to fall drop wise into ice-cold water with continuous stirring to get 5-chloro-2-hydroxy-4-methyl acetophenone viz. 1-(5’-chloro-2’-hydroxy-4’-methylphenyl) ethanone (2) as a white solid.

**3. Synthesis of 2-(Substituted benzoyloxy)-4-methyl-5-chloroacetophenones (3a-d):** 5-chloro-2-hydroxy-4-methylacetophenone (2) (0.04 mol) and appropriate aromatic carboxylic acids (i.e. substituted benzoic acids) (0.05 mol) were dissolved in dry pyridine and $POCl_3$ was added drop by drop with stirring and cooling simultaneously till the viscous mass was obtained. Temperature was maintained below 10 $^0$C during the addition of $POCl_3$. The reaction mixture was allowed to stand for overnight at room temperature and then decomposed by dil. HCl (10%) in an ice bath. The solid product thus separated was filtered, washed with water followed by 10% sodium bicarbonate ($NaHCO_3$) solution and then again several times with water. Finally, it was purified by recrystallization from hot ethanol to afford corresponding 2-(substituted benzoyloxy)-4-methyl-5-chloroacetophenones (3a-d) as pale-yellow solids.

**4. Synthesis of 1-(5’-Chloro-2’-hydroxy-4’-methyl phenyl)-3-(substituted phenyl) propane-1, 3-diones (4a-d) i.e. β-diketones:** 2-(substituted benzoyloxy)-4-methyl-5-chloroacetophenones (3a-d) (0.05 mol) was dissolved in dry pyridine (40 ml). The solution was warmed up to 60 $^0$C and pulverized KOH was added slowly with constant stirring (Baker-Venkataraman rearrangement). Vigorous reaction took place and mixture began to thicken when yellowish-brown mass was obtained. The reaction mixture was kept overnight and then worked up by the dilution and acidification with ice-cold dil. HCl (1:1). The yellowish-brown solid thus obtained was filtered, washed with water followed by 10% sodium bicarbonate ($NaHCO_3$) solution to remove unhydrolysed acid and then again with excess of water. The dried product was purified by recrystallization from ethanol-acetic acid mixture to get yellow crystals of respective 1-(5’-chloro-2’-hydroxy-4’-methylphenyl)-3-(substituted phenyl) propane-1,3-diones (4a-d) i.e. β-diketones with good yield.

**5. Synthesis of 2-(Substituted phenyl)-6-chloro-7-methyl-4*H*-chromen-4-ones (5a-d) i.e. substituted flavones:** To a solution of 1-(5’-chloro-2’-hydroxy-4’-methylphenyl)-3-(substituted phenyl) propane-1,3-diones (4a-d) (0.025 mol) in glacial acetic acid (30 ml), sulphuric acid (5 ml) was added. The content of reaction mixture was refluxed on water bath for 2 hrs. followed with occasional stirring. The reaction mixture was allowed to cooled at room temperature and poured into crushed ice to precipitate the product. The separated product was filtered, washed with water followed by 10% sodium bicarbonate ($NaHCO_3$) solution and then with sufficient cold water until the washings were neutral to litmus. The dried product was purified by recrystallization from hot ethanol to get shiny yellow crystals of corresponding 2-(substituted phenyl)-6-chloro-7-methyl-4*H*-chromen-4-ones (5a-d) i.e. substituted flavones with satisfactory yield.

**6. Synthesis of 3-(5’-Chloro-2’-hydroxy-4’-methyl phenyl)-5-(substituted phenyl)-1-phenyl pyrazoles (6a-d):** A mixture of 2-(substituted phenyl)-6-chloro-7-methyl-4*H*-chromen-4-ones (5a-d) (0.01 mol) and phenyl hydrazine hydrochloride (0.02 mol) was refluxed in DMF solvent with few drops or little amount of piperidine for about 1½ hrs. The reaction mixture was cooled and then acidified with ice-cold dil. HCl (1:1). The product thus separated was filtered, washed with water followed by 10% sodium bicarbonate ($NaHCO_3$) solution and then again with plenty of water. It was dried and purified by recrystallization from ethanol-acetic acid mixture to get shiny coloured crystals of corresponding 3-(5’-chloro-2’-hydroxy-4’-methyl phenyl)-5-(substituted phenyl)-1-phenyl pyrazoles (6a-d) as desired products with excellent yield.

**7. Synthesis of 3-(5’-Chloro-2’-hydroxy-4’-methyl phenyl)-5-(substituted phenyl) isoxazoles (7a-d):** A mixture of 2-(substituted phenyl)-6-chloro-7-methyl-4*H*-chromen-4-ones (5a-d) (0.01 mol) and hydroxylamine hydrochloride (0.02 mol) was refluxed in DMF solvent with few drops or little amount of piperidine for about 1½ hrs. The reaction mixture was cooled and then acidified with ice cold dil. HCl (1:1). The product thus separated was filtered, washed with water followed by 10% sodium bicarbonate ($NaHCO_3$) solution and then again with plenty of water. It was dried and purified by recrystallization from ethanol-acetic acid mixture to get shiny coloured crystals of corresponding 3-(5’-chloro-2’-hydroxy-4’-methyl phenyl)-5-(substituted phenyl) isoxazoles (7a-d) as desired products with excellent yield. The general reaction scheme outlined for the synthesis of 3,5-disubstituted-1-phenyl pyrazole (6a-d) and 3,5-disubstituted isoxazole (7a-d) derivatives is depicted below in Figure 1 and their physical characterization data is shown in Table 1.

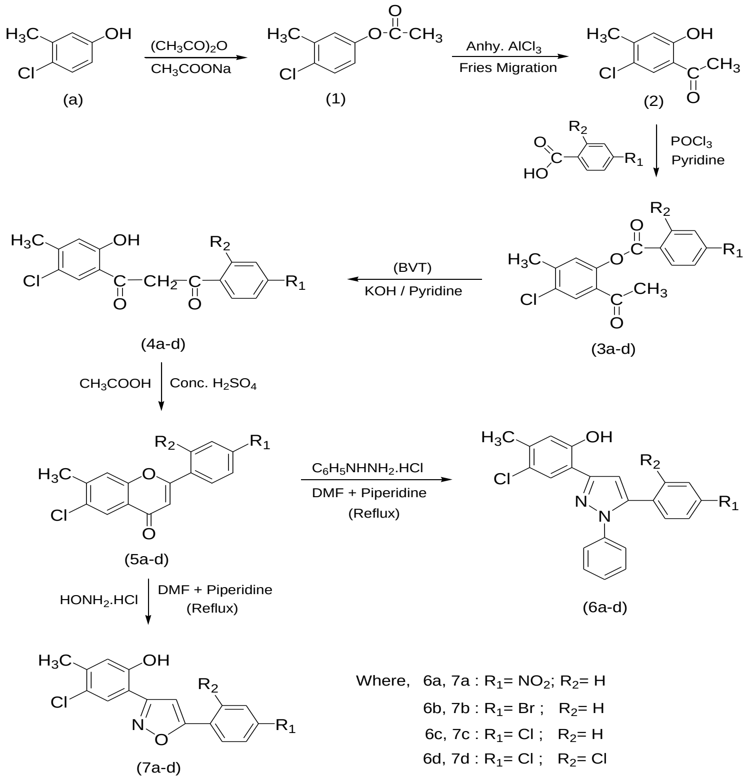


**Figure 1: The general reaction scheme for the synthesis of 3,5-disubstituted-1-phenyl pyrazoles (6a-d) and 3,5-disubstituted isoxazoles (7a-d).**

**Table 1: Physical characterization data of newly synthesized pyrazole (6a-d) & isoxazole (7a-d) derivatives.**

| Code | Compound Name | Mol. Formula | Mol.Wt. (g/mol) | M.P. ($^0$C) | Yield (%) | $R_f$ value |
|---|---|---|---|---|---|---|
| 6a | 3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(4’-nitrophenyl)-1-phenylpyrazole | $C_{22}H_{16}ClN_3O_3$ | 405.83 | 220-222 | 79 | 0.70 |
| 6b | 3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(4’-bromophenyl)-1-phenylpyrazole | $C_{22}H_{16}BrClN_2O$ | 439.73 | 218-222 | 86 | 0.67 |
| 6c | 3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(4’-chlorophenyl)-1-phenylpyrazole | $C_{22}H_{16}Cl_2N_2O$ | 395.28 | 218-224 | 84 | 0.82 |
| 6d | 3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(2’,4’-dichlorophenyl)-1-phenylpyrazole | $C_{22}H_{15}Cl_3N_2O$ | 429.73 | 238-240 | 90 | 0.80 |
| 7a | 3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(4’-nitrophenyl)isoxazole | $C_{16}H_{11}ClN_2O_4$ | 330.72 | 228-230 | 85 | 0.65 |
| 7b | 3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(4’-bromophenyl)isoxazole | $C_{16}H_{11}BrClNO_2$ | 364.62 | 254-258 | 87 | 0.76 |
| 7c | 3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(4’-chlorophenyl)isoxazole | $C_{16}H_{11}Cl_2NO_2$ | 320.17 | 218-222 | 88 | 0.82 |
| 7d | 3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(2’, 4’-dichlorophenyl)isoxazole | $C_{16}H_{10}Cl_3NO_2$ | 354.62 | 240-244 | 90 | 0.70 |

### Spectroscopic characterization of compounds

**3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(4’-nitrophenyl)-1-phenyl pyrazole (6a):** Solid; Dark Brown colour; IR (KBr, cm$^{-1}$): 3356 (Ar-OH stretch), 3100 (Ar-C-H stretch), 2927 (C-H stretch of -CH$_3$), 1624 (C=N stretch), 1523 (-NO$_2$ asym. stretch), 1429 (C=C stretch), 1359 (-NO$_2$ sym. stretch), 1249 (C-N stretch), 1123 (C-O stretch), 841 (C-Cl stretch). $^1$H NMR (500 MHz, DMSO-d$_6$, δ ppm): δ 2.5 (s, 3H of -CH$_3$), δ 3.38 (s, 1H of Phenolic -OH), δ 6.98 (s, 1H of pyrazole-H), δ 6.71-8.06 (m, 11H of Ar-H). LC-MS (m/z): 404.05, 406.04 (M+); Anal. Calcd. for $C_{22}H_{16}ClN_3O_3$ (Mol.wt. 405.83 g/mol): C, 65.11; H, 3.97; N, 10.35; Cl, 8.74; O, 11.83%; Found: C, 65.18; H, 3.86; N, 10.45; Cl, 8.78; O, 11.73 %.

**3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(4’-bromophenyl)-1-phenyl pyrazole (6b):** Solid; Brown colour; IR (KBr, cm$^{-1}$): 3374 (Ar-OH stretch), 3055 (Ar-C-H stretch), 2927 (C-H stretch of -CH$_3$), 1631 (C=N stretch), 1430 (C=C stretch), 1248 (C-N stretch), 1091 (C-O stretch), 817 (C-Cl stretch), 654 (C-Br stretch). $^1$H NMR (500 MHz, DMSO-d$_6$, δ ppm): δ 2.5 (s, 3H of -CH$_3$), δ 3.5 (s,1H of Phenolic -OH), δ 7.11 (s, 1H of pyrazole-H), δ 7.10-8.32 (m, 11H of Ar-H). LC-MS (m/z): 439.0, 437.0 (M+); Anal. Calcd. for $C_{22}H_{16}BrClN_2O$ (Mol.wt. 439.73 g/mol): C, 60.09; H, 3.67; N, 6.37; Cl, 8.06; O, 3.64 %; Found: C, 60.32; H, 3.36; N, 6.49; Cl, 7.98; O, 3.60 %.

**3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(4’-chlorophenyl)-1-phenyl pyrazole (6c):** Solid; Brown colour; IR (KBr, cm$^{-1}$): 3380 (Ar-OH stretch), 3044 (Ar-C-H stretch), 2927 (C-H stretch of -CH$_3$), 1627 (C=N stretch), 1435 (C=C stretch), 1248 (C-N stretch), 1102 (C-O stretch), 822 (C-Cl stretch). $^1$H NMR (500 MHz, DMSO-d$_6$, δ ppm): δ 2.5 (s, 3H of -CH$_3$), δ 3.4 (s,1H of Phenolic -OH), δ 6.69 (s, 1H of pyrazole-H), δ 7.31-8.69 (m, 11H of Ar-H). LC-MS (m/z): 393.05, 395.05, 396.05 (M+); Anal. Calcd. for $C_{22}H_{16}Cl_2N_2O$ (Mol.wt. 395.28 g/mol): C, 66.85; H, 4.08; N, 7.09; Cl, 17.94; O, 4.05 %; Found: C, 66.60; H, 4.18; N, 7.20; Cl, 18.09; O, 3.93 %.

**3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(2’,4’-dichlorophenyl)-1-phenyl pyrazole (6d):** Solid; Brown colour; IR (KBr, cm$^{-1}$): 3283 (Ar-OH stretch), 3071 (Ar-C-H stretch), 2930 (C-H stretch of -CH$_3$), 1630 (C=N stretch), 1450 (C=C stretch), 1239 (C-N stretch), 1154 (C-O stretch), 857 (C-Cl stretch). $^1$H NMR (500 MHz, DMSO-d$_6$, δ ppm): δ 2.5 (s, 3H of -CH$_3$), δ 3.51 (s,1H of Phenolic -OH), δ 7.10 (s, 1H of pyrazole-H), δ 6.82-8.13 (m, 10H of Ar-H). LC-MS (m/z): 429.01, 429.06, 427.01 (M+); Anal. Calcd. for $C_{22}H_{15}Cl_3N_2O$ (Mol.wt. 429.73 g/mol): C, 61.49; H, 3.52; N, 6.52; Cl, 24.75; O, 3.72 %; Found: C, 61.38; H, 3.80; N, 6.29; Cl, 25.0; O, 3.53 %.

**3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(4’-nitrophenyl) isoxazole (7a):** Solid; Brownish-yellow colour; IR (KBr, cm$^{-1}$): 3436 (Ar-OH stretch), 2928 (C-H stretch of -CH$_3$), 1621 (C=N stretch), 1525 (N-O stretch), 1450 (C=C) stretch, 1398 (-NO$_2$ sym. stretch), 1262 (C-O-N stretch), 1102 (C-O stretch), 855 (C-Cl stretch). $^1$H NMR (500 MHz, DMSO-d$_6$, δ ppm): δ 2.5 (s, 3H of -CH$_3$), δ 3.43 (s, 1H of Phenolic -OH), δ 7.07 (s, 1H of isoxazole-H), δ 6.64-8.38 (m, 6H of Ar-H). LC- MS (m/z): 328.04, 329.03, 331.02 (M+); Anal. Calcd. for $C_{16}H_{11}ClN_2O_4$ (Mol.wt. 330.72 g/mol): C, 58.11; H, 3.35; N, 8.47; Cl, 10.72; O, 19.35 %; Found: C, 58.20; H, 3.41; N, 8.44; Cl, 10.43; O, 19.52 %.

**3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(4’-bromophenyl) isoxazole (7b):** Solid; Brownish-yellow colour; IR (KBr, cm$^{-1}$): 3450 (Ar-OH stretch), 2930 (C-H stretch of -CH$_3$), 1629 (C=N stretch), 1571 (N-O stretch), 1404 (C=C sym. stretch), 1259 (C-O-N stretch), 1086 (C-O stretch), 648 (C-Cl stretch), 509 (C-Br stretch). $^1$H NMR (500 MHz, DMSO-d$_6$, δ ppm): δ 2.5 (s, 3H of -CH$_3$), δ 3.50 (s,1H of Phenolic -OH), δ 7.39 (s, 1H of isoxazole-H), δ 7.05-8.03 (m, 6H of Ar-H). LC-MS (m/z): 363.95, 365.95 (M+); Anal. Calcd. for

$C_{16}H_{11}BrClNO_2$ (Mol.wt. 364.62 g/mol): C, 52.70; H, 3.04; N, 3.84; Cl, 9.72; O, 8.78 %; Found: C, 52.84; H, 2.85; N, 3.97; Cl, 9.65; O, 8.60 %.

**3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(4’-chlorophenyl) isoxazole (7c):** Solid; Brownish-yellow colour; IR (KBr, $cm^{-1}$): 3399 (Ar-OH stretch), 2940 (C-H stretch of $-CH_3$), 1650 (C=N stretch), 1546 (N-O stretch), 1438 (C=C stretch), 1160 (C-O-N stretch), 1031 (C-O stretch), 875 (C-Cl stretch). $^1H$ NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 2.5 (s, 3H of $-CH_3$), δ 3.81 (s,1H of Phenolic -OH), δ 7.09 (s, 1H of isoxazole-H), δ 7.39-8.13 (m, 6H of Ar-H). LC-MS (m/z): 319.00, 320.00, 322.00 (M+); Anal. Calcd. for $C_{16}H_{11}Cl_2NO_2$ (Mol.wt. 320.17 g/mol): C, 60.02; H, 3.46; N, 4.37; Cl, 22.15; O, 9.99 %; Found: C, 59.93; H, 3.61; N, 4.54; Cl, 22.01; O, 9.91 %.

**3-(5’-Chloro-2’-hydroxy-4’-methylphenyl)-5-(2’, 4’-dichlorophenyl) isoxazole (7d):** Solid; Brown colour; IR (KBr, $cm^{-1}$): 3382 (Ar-OH stretch), 2943 (C-H stretch of $-CH_3$), 1675 (C=N stretch), 1555 (N-O stretch), 1437 (C=C stretch), 1255 (C-O-N stretch), 1036 (C-O stretch), 804 (C-Cl stretch). $^1H$ NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 2.5 (s, 3H of $-CH_3$), δ 3.71 (s,1H of Phenolic -OH), δ 6.78 (s, 1H of isoxazole-H), δ 6.67-7.99 (m, 5H of Ar-H). LC-MS (m/z): 353.96, 354.00, 355.96, 356.00 ($M^+$); Anal. Calcd. for $C_{16}H_{10}Cl_3NO_2$ (Mol.wt. 354.62 g/mol): C, 54.19; H, 2.84; N, 3.95; Cl, 29.99; O, 9.02 %; Found: C, 54.25; H, 2.77; N, 4.02; Cl, 29.82; O, 9.14 %.

## Pharmacological Evaluation

### Antibacterial and antifungal activity

All the newly synthesized 3,5-disubstituted-1-phenyl pyrazoles (6a-d) and 3,5-disubstituted isoxazoles (7a-d) bearing p-chloro-m-cresol moiety were screened for in *vitro* antibacterial and antifungal activities against the growth of two bacterial strains viz. *Escherichia coli* (gram -ve), *Staphylococcus aureus* (gram +ve) and two fungal strains viz. *Aspergillus niger*, *Candida albicans* by using disc diffusion method.[51-52] Mueller-Hinton agar (MHA) and Potato-Dextrose agar (PDA) plates were employed as culture medium respectively for bacterial and fungal sensitivity and DMSO was used as solvent control. Ofloxacin (2 µg) and amphotericin (50 µg) were used as standard drugs respectively for antibacterial and antifungal activities. The compounds were dissolved in DMSO to give 100 µg/ml, 250 µg/ml, 500 µg/ml solutions. Sterile filter paper discs (Whatmann filter paper No. 40) of 10 mm diameter were dipped in these solutions, dried, and placed on nutrient agar plates spreaded with the bacteria and fungi. The plates were further incubated for 24 hrs. at 37 $^0$C for antibacterial and 72 hrs. at 28 $^0$C for antifungal testing and the zones of inhibition were measured in mm using antibiotic zone reader (Hi-Media). The results on antibacterial and antifungal activities of newly synthesized compounds are depicted in Table 2 and Table 3 below and their effects along with zones of inhibition (mm) are shown in Figure 2 and Figure 3 respectively.

**Table 2: Antibacterial activity of newly synthesized pyrazole (6a-d) & isoxazole (7a-d) derivatives.**

| Compound code | Zone of inhibition in millimeter (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Escherichia coli* (Gram -ve) | | | | *Staphylococcus aureus* (Gram +ve) | | | |
| | Concentration of compounds (µg/ml) | | | | Concentration of compounds (µg/ml) | | | |
| | 100 | 250 | 500 | Std. (2 µg) | 100 | 250 | 500 | Std. (2 µg) |
| 6a | 10.5 | 11 | 11.5 | 20 mm | 10 | 11 | 12 | 22 mm |
| 6b | 10 | 11 | 12 | | NI | NI | NI | |
| 6c | 10 | 12 | 14 | | 10.5 | 11 | 12 | |
| 6d | NI | NI | 14 | | 10.5 | 11 | 12 | |
| 7a | NI | NI | NI | | NI | NI | NI | |
| 7b | NI | NI | NI | | 10 | 12 | 13 | |
| 7c | 10 | 11 | 12 | | 10 | 11 | 12 | |
| 7d | 11 | 12 | 14 | | 12 | 13 | 14 | |
| Control | NI | NI | NI | | NI | NI | NI | |
| *Std.: Ofloxacin drug (2 µg/ml); NI: No zone of inhibition; Control: DMSO solvent | | | | | | | | |

**Table 3: Antifungal activity of newly synthesized pyrazole (6a-d) & isoxazole (7a-d) derivatives.**

| Compound code | Zone of inhibition in millimeter (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Aspergillus niger* | | | | *Candida albicans* | | | |
| | Concentration of compounds (µg/ml) | | | | Concentration of compounds (µg/ml) | | | |
| | 100 | 250 | 500 | Std. (50 µg) | 100 | 250 | 500 | Std. (50 µg) |
| 6a | NI | NI | 11 | 15 mm | 10.5 | 11 | 12 | 14 mm |
| 6b | NI | NI | NI | | NI | NI | NI | |
| 6c | NI | NI | NI | | 10 | 11 | 12 | |
| 6d | NI | NI | NI | | NI | NI | 12 | |
| 7a | NI | NI | NI | | 11 | 12 | 13 | |
| 7b | NI | NI | NI | | 10 | 11 | 12 | |
| 7c | NI | NI | NI | | 10 | 11 | 12 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7d | 10 | 11 | 12 | | 12 | 17 | 18 | |
| Control | NI | NI | NI | | NI | NI | NI | |
| *Std.: Amphotericin drug (50 µg/ml); NI: No zone of inhibition; Control: DMSO solvent | | | | | | | | |

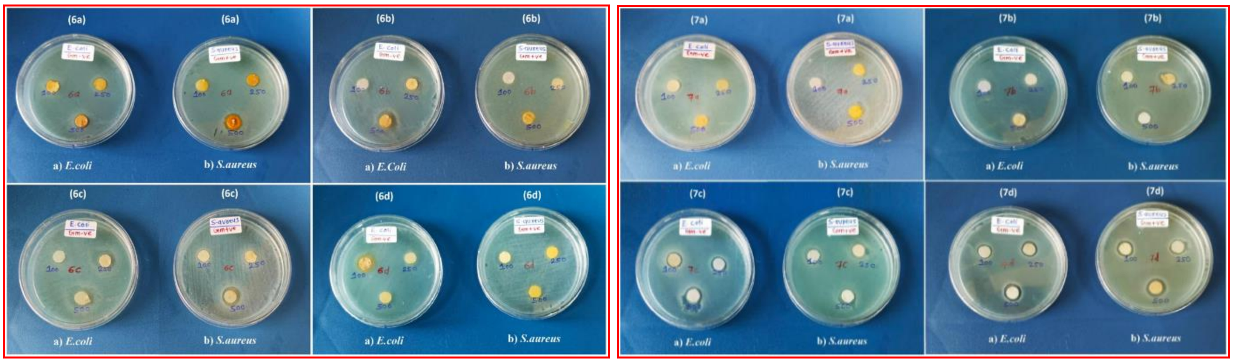


**Figure 2: Effects of newly synthesized pyrazole (6a-d) & isoxazole (7a-d) derivatives on the growth response of bacterial strains viz. a) *E. coli* b) *S. aureus.***

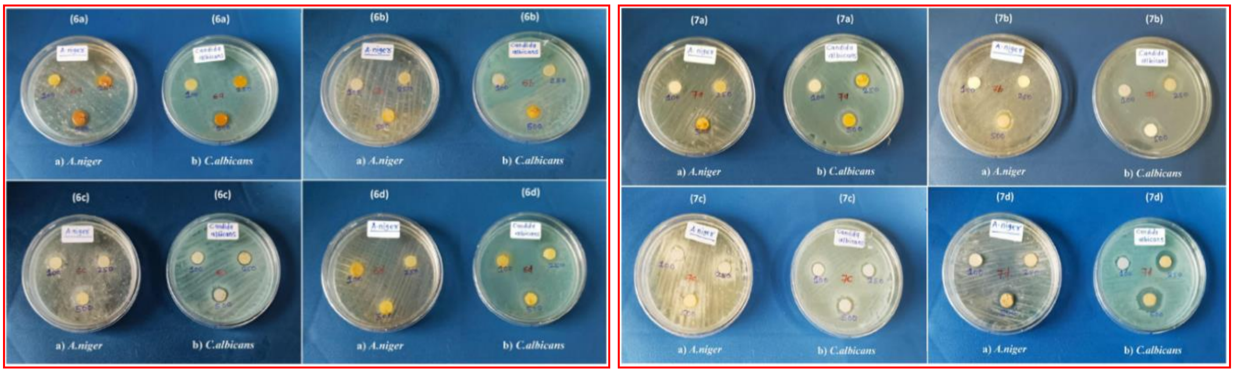


**Figure 3: Effects of newly synthesized pyrazole (6a-d) & isoxazole (7a-d) derivatives on the growth response of fungal strains viz. a) *A. niger* b) *C. albicans.***

**Antioxidant activity**

The in *vitro* antioxidant activity of newly synthesized compounds was performed based on DPPH radical scavenging assay with free radical scavenging effect of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) with slight modifications.[53-56] A stock solution of DPPH (1.3 mg/ml) was prepared by dissolving 13.0 mg in 10 ml of methanol. The stock solutions of test compounds 6a-d & 7a-d take (100 µg/ml) were prepared by dissolving 1mg of sample in 10ml of methanol. From this stock solution, further dilutions were prepared of various concentrations (10, 20, 30, 40 and 50 µg/ml) using methanol. Similarly, a stock solution of standard ascorbic acid (1mg/ml) was prepared by dissolving 10 mg ascorbic acid in 10 ml of methanol. From this stock solution further dilutions of various concentrations (10, 20, 30, 40, and 50 µg/ml) were prepared. Further, 1 ml of DPPH solution was mixed with 1 ml of different concentration of test solutions and standard (ascorbic acid). These solutions were kept for 30 min in dark and absorbance was measured at 517 nm using methanol (5 ml) with DPPH (1 ml) solution as blank (positive control). The percentage of scavenging activity was calculated by following formula, whose results are summarized in Table 4 and shown in Figure 4.

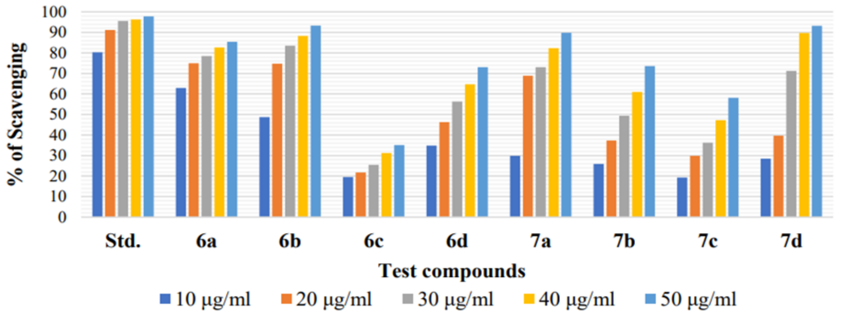
% Scavenging = [Abs. of control – Abs. of test sample/Abs. of control] × 100

**Table 4: Antioxidant activity of newly synthesized pyrazole (6a-d) & isoxazole (7a-d) derivatives.**

| Sr. No. | Compound code | % Scavenging of synthesized compounds and std. Ascorbic acid | | | | |
|---|---|---|---|---|---|---|
| | | Concentration of compounds (µg/ml) | | | | |
| | | 10 µg/ml | 20 µg/ml | 30 µg/ml | 40 µg/ml | 50 µg/ml |
| 1. | 6a | 62.93 | 75.00 | 78.50 | 82.64 | 85.46 |
| 2. | 6b | 48.68 | 74.78 | 83.55 | 88.33 | 93.36 |
| 3. | 6c | 19.51 | 21.71 | 25.43 | 31.22 | 35.09 |
| 4. | 6d | 34.86 | 46.27 | 56.35 | 64.70 | 73.00 |
| 5. | 7a | 29.82 | 68.85 | 73.02 | 82.34 | 89.76 |
| 6. | 7b | 25.87 | 37.28 | 49.34 | 61.03 | 73.56 |
| 7. | 7c | 19.29 | 29.82 | 36.26 | 47.22 | 58.09 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8. | 7d | 28.50 | 39.69 | 71.19 | 89.70 | 93.19 |
| 9. | Std. Ascorbic acid | 80.26 | 91.22 | 95.61 | 96.27 | 97.80 |

**Figure-4: Antioxidant activity of synthesized compounds and std. Ascorbic acid by DPPH radical scavenging method**



## Anti-Inflammatory activity

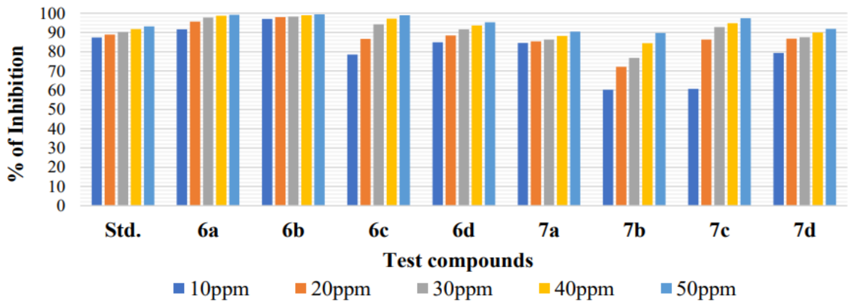
The in *vitro* anti-inflammatory activity of newly synthesized compounds was performed by employing inhibition of protein denaturation assay.[57,58] Stock solution of NSAID Ibuprofen as a reference drug (positive control) was prepared by dissolving 10mg of Ibuprofen in 10 ml of distilled water. Serial dilution from above stock solution takes 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml and prepare 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm and also it was performed for samples 6a-d & 7a-d. The reaction mixtures were prepared using 2.8 ml of phosphate-buffered saline (pH 6.4) and 0.2 ml of egg-albumin. Then take 2 ml of sample from each different concentration were mixed gently with reaction mixtures. All samples contain 5 ml of total volume. A similar procedure was used for reference Ibuprofen drug solution. The absorbance of these solutions was determined by spectrophotometer at the wavelength of 660 nm. The percentage inhibition of protein denaturation was calculated by following the formula, whose results are summarized in Table 5 and shown in Figure 5.

% Inhibition = [Abs. of blank – Abs. of test sample/Abs. of blank] × 100

**Table 5: Anti-inflammatory activity of newly synthesized pyrazole (6a-d) & isoxazole (7a-d) derivatives.**

| Sr. No. | Compound code | % Inhibition of synthesized compounds and std. Ibuprofen drug | | | | |
|---|---|---|---|---|---|---|
| | | Concentration of compounds (ppm) | | | | |
| | | 10 ppm | 20 ppm | 30 ppm | 40 ppm | 50 ppm |
| 1. | 6a | 91.61 | 95.58 | 97.79 | 98.65 | 99.13 |
| 2. | 6b | 97.01 | 97.95 | 98.23 | 98.95 | 99.36 |
| 3. | 6c | 78.53 | 86.69 | 94.20 | 97.21 | 98.98 |
| 4. | 6d | 84.87 | 88.41 | 91.66 | 93.67 | 95.22 |
| 5. | 7a | 84.60 | 85.32 | 86.31 | 88.15 | 90.43 |
| 6. | 7b | 60.32 | 72.18 | 76.71 | 84.45 | 89.76 |
| 7. | 7c | 60.70 | 86.33 | 92.77 | 94.78 | 97.38 |
| 8. | 7d | 79.30 | 86.81 | 87.47 | 89.91 | 91.87 |
| 9. | Std. Ibuprofen | 87.36 | 88.90 | 90.23 | 91.74 | 93.10 |

**Figure-5: Anti-Inflammatory activity of synthesized compounds and std. Ibuprofen by Inhibition of protein denaturation assay**



## RESULTS AND DISCUSSION

A total four derivatives of each pyrazole and isoxazole bearing p-chloro-m-cresol moiety have been synthesized with excellent yield, purified by recrystallization, characterized and further used individually to analyze its in *vitro* antibacterial, antifungal, antioxidant and anti-inflammatory activity. The IR and $^1$H NMR spectra of all the compounds showed expected signals which corresponds to various groups present in each compound. Also, the mass spectra of compounds were found in full agreement with the proposed structures and showed expected peaks which confirms the molecular weights of compounds. The results on antibacterial activities (Table 2) reveals that, compounds 6a, 6b, 6c, 7c and 7d showed (10.5,11,11.5), (10,11,12), (10,12,14), (10,11,12) and (11,12,14) mm of zones of inhibition respectively at 100 µg/ml, 250 µg/ml and 500 µg/ml concentrations while compound 6d showed zone of 14 mm at 500 µg/ml against bacteria *E. coli*. The compounds 7a and 7b showed either less or no any zone of inhibition against the growth of *E. coli* strain at all the tested concentrations. In case of pathogen *S. aureus*, only the compounds 6a, 6c, 6d, 7b, 7c and 7d showed respectively (10,11,12), (10.5,11,12), (10.5,11,12), (10,12,13), (10,11,12) and (12,13,14) mm of zones of inhibitions at 100 µg/ml, 250 µg/ml and 500 µg/ml concentrations respectively while compounds 6b and 7a were found to have either less or zero zones of inhibition against the growth response of *S. aureus*. According to the obtained results on antifungal activity (Table 3), it was observed that, only the compounds 6a and 7d were showed 11 mm and (10,11,12) mm of zones respectively at 500 µg/ml and 100 µg/ml, 250 µg/ml and 500 µg/ml concentrations against fungus *A. niger* while other compounds did not show any zones of inhibition against the growth of *A. niger* fungal strain. As well *C. albicans* is concern, compounds 6a, 6c, 7a, 7b, 7c and 7d showed respectively (10.5,11,12), (10,11,12), (11,12,13), (10,11,12), (10,11,12) and (12,17,18) mm of zones at all the tested concentrations while compound 6d showed 12 mm of zone at the concentration of only 500 µg/ml. Only the compound 6b did not show any zone against the growth response of *C. albicans* at all the tested concentrations. Standard ofloxacin (2 µg/ml) and amphotericin (50 µg/ml) showed inhibition zones of diameter 20, 22 and 15, 14 mm against pathogens *E. coli*, *S. aureus*, *A. niger* and *C. albicans* respectively. The experimental data on antioxidant activities (Table 4 & Figure 4) reveals that, compounds 6a, 6b, 6d, 7a, 7b,7d showed good to excellent scavenging activity at all the tested concentrations while only the compound 6c and 7c showed low to mild scavenging activity with reference to standard antioxidant ascorbic acid. From the results on antioxidant activity, it was observed that all the pyrazole derivatives showed 6b > 6a > 6d > 6c and isoxazole derivatives showed 7d > 7a > 7b > 7c order of scavenging power and their scavenging strength was increased with increasing concentration. The results on anti-inflammatory activity (Table 5 & Figure 5) reveals that, all the newly synthesized compounds exhibit excellent and notable inhibition to protein (egg-albumin) denaturation with comparison to standard NSAID ibuprofen drug. The pyrazole derivatives have shown 6b > 6a > 6c > 6d and isoxazole derivatives shown 7c > 7d > 7a > 7b order of inhibition of protein denaturation. Moreover, it is surprising that their inhibition power was also increased as we increased their concentrations. By comparing their inhibition strength with standard reference, it was observed that the inhibition power of all the synthesized compounds was found to be in good agreement with the standard ibuprofen as well as their values were found to be comparable with it.

## CONCLUSION

A new class of 3,5-disubstituted-1-phenyl pyrazoles and 3,5-disubstituted isoxazoles incorporating phenolic p-chloro-m-cresol moiety were successfully synthesized with high yield by refluxing substituted flavones with nucleophiles phenylhydrazine hydrochloride and hydroxylamine hydrochloride respectively in DMF-piperidine solvent media and screened for their pharmacological properties such as antibacterial, antifungal, antioxidant and anti-inflammatory in *vitro*. Among all the tested compounds, 6a, 6b, 6c, 6d, 7c and 7d displayed promising but low to moderate antibacterial activity particularly against *E. coli* while 7a and 7b were found to be inactive against *E. coli* pathogen. In case of *S. aureus* bacteria, compounds 6a, 6c, 6d, 7b, 7c and 7d displayed low to moderate antibacterial sensitivity whereas 6b and 7a were found to be inactive. Also, amongst the all compounds, only the compounds 6a and 7d were found to be active and showed low to mild activity against *A. niger* while other compounds were found to be inactive against it. In addition, except compound 6b, all the other compounds showed promising but less to mild or moderate antifungal activity against *C. albicans.* Besides, the compounds 6a, 6b, 6d, 7a, 7b,7d exhibited promising and excellent antioxidant potential due to the presence of electron withdrawing substituents on the aromatic ring while compound 6c and 7c showed promising but low to mild antioxidant activity with respect to ascorbic acid. Furthermore, the results on anti-inflammatory were surprising because all the compounds exhibited superior inhibition to protein denaturation due to an incorporation of p-chloro-m-cresol moiety in the pyrazole and isoxazole nucleus and were found to be potent anti-inflammatory agents. Overall, the experimental findings on anti-inflammatory and antioxidant studies may be implicated as an informative resource for pharmaceutical industries engaged in the drugs manufacturing. In conclusion, all the synthesized novel p-chloro-m-cresol incorporated pyrazole and isoxazole derivatives can be further exploited as lead compounds for drug discovery research in pharmaceutical industries and veterinary sciences as well as future studies. Also, it is conceivable that these newly synthesized titled compounds may be further modified to achieve marketable antioxidants and NSAID agents for welfare of society.

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