Chapter 3: Studies on substituted 3-(benzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one derivative

8.1. Introduction

Pyrazole refers both to the class of simple aromatic ring organic compounds of the heterocyclic series characterized by a five-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions and to the unsubstituted parent compound. Being so composed and having pharmacological effects on humans and they are classified as alkaloids, although they are rare in nature.

Amongst nitrogen containing five membered heterocycles, aminopyrazolones have proved to be the most useful framework for biological activities, aminopyrazolone have attracted attention of medicinal chemists for both with regard to heterocyclic chemistry and the pharmacological activities associated with them. Aminopyrazolones have been studied extensively for their biodynamic behavior, color properties and industrial applications.

Figure 8.1: Aminopyrazolone based drugs

Metamizole sodium (analgesic, antipyretic, antiinflammatory)
Muzolimine (diuretic)
Nifedipine (antirheumatic, analgesic, antipyretic)
Aminophenazon (Amidophenazon; Amidopyrin; Aminopyrine) (analgesic, antipyretic, antiinflammatory)
8.2. Review of literature

8.2.1. Synthetic approaches

The synthesis of pyrazoles remains of great interest owing to the wide applications in pharmaceutical, dye and agrochemical industry due to their herbicidal, colorant, fungicidal, insecticidal, analgesic, antipyretic and anti-inflammatory properties\(^\text{39}\). Some methods have been developed in recent years, though the most important method is the reaction between hydrazines and β-dicarbonyl compounds\(^\text{21}\). This reaction involves the double condensation of 1,3-diketones or α, β-unsaturated ketones with hydrazine or its derivatives.\(^{10,105,312,181,115}\) However, the appealing generality of this method is somewhat vitiated by the severe reaction conditions or the multistep sequences usually required to access the starting materials. Thus, continuous efforts have been devoted to the development of more general and versatile synthetic methodologies for this class of compounds.\(^{104,96,27,110,59,106,128}\)

The chemistry of aminopyrazolones has been extensively investigated in the recent past. The considerable colorant\(^{116,313,31}\) and medicinal activities of pyrazoles\(^{203,203,223,218}\) and azolopyrazoles, for which aminopyrazoles are preferred precursors, have stimulated these investigations. Interest in aminopyrazoles synthesis and chemistry has recently been revived.\(^{211,265,266,212,277,244}\) Aminopyrazolones mainly synthesized by the reactions of cyanoaceticacid hydrazide with various substituted reagent like isocyanates, isothiocyanates, ketones, diketones, malononitriles and nitriles.\(^{225}\)

James T. Drummond, reacted cyanoaceticacid hydrazide with alkylisocyanate yields alkylcarbamoyl derivative that cyclized into pyrazole derivative (Figure 8.2) upon treatment with 2N sodium hydroxide.\(^{55}\)

**Figure 8.2**

Mohareb prepared pyrazolinone derivative refluxing of cyanoaceticacid hydrazide with phenyl isothiocyanate in basic dioxane solution (Figure 8.3).\(^{85}\)
5-Amino-3-hydroxypyrazole derivatives (Figure 8.4) were prepared from the reaction of cyanoacetic acid hydrazide with ketones in the presence of a basic catalyst via the cyclization of hydrazone derivatives. During the process of hydration of obtained hydrazone to subsequent hydrazine with sodium borohydride in water media a spontaneous reaction of heterocyclization had been observed and several 5-amino-3-hydroxypyrazoles had been obtained.

Cyanoaceto-\(N\)-arylsulfonylhydrazide on refluxing in ethanol containing a catalytic amount of piperidine, or in presence of potassium hydroxide, or in presence of sodium hydroxide undergo intramolecular cyclization to give the 5-amino-1-arylsulfonyl-4-pyrazolin-3-one or the tautomeric 5-amino-1-arylsulfonyl-3-hydroxypyrazole structure (Figure 8.5).

The reaction of cyanoacetic acid hydrazide with isatin in ethanol containing a catalytic amount of triethylamine at room temperature furnished the isolated
intermediate 2-cyano-2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene) acetohydrazide which cyclized under heating to give 3-(3-amino-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene)-1,3-dihydro-2H-indol-2-one (Figure 8.6).²⁴

**Figure 8.6**

Cycloaddition of cyanoacetic acid hydrazide with arylidene of 2-cyanomethyl-1,3-benzothiazole yielded 3-aryl-2-(1,3-benzothiazol-2-yl)-3-(5-imino-3-oxopyrazolidin-1-yl)propanenitrile (Figure 8.7).⁶⁷,⁷³

**Figure 8.7**

Cyanoacetic acid hydrazide reacts with hydrazone derivatives of 2-diazoo-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide in refluxing dioxane containing a catalytic amount of triethylamine to yield fused pyrazoloazine derivatives (Figure 8.8).¹⁹⁵
It was noted, that condensation of $O$-methylbutyrolactim with cyanoacetic acid hydrazide did not produce noticeable amounts of enamine but five membered $O$-methylbutyrolactim produces 3-amino-4-(pyrrolidin-2-ylidene)-1H-pyrazol-5(4H)-one via intermediates containing a five membered saturated ring.\textsuperscript{237}

Some pyrazole derivatives were readily obtained (Figure 8.10) by the condensation of substituted ethyl 2-cyanoacetates with hydrazine.\textsuperscript{279,79,41,292} The lack of carbonyl absorption in the IR spectra of the products supported their formulation as hydroxypyrazoles rather than the alternative tautomer. However the unusual behavior of the hydroxypyrazole derivatives at their melting points, the reason for which is not yet clear, may indicate their tautomeric conversion into the pyrazolinones at elevated temperature.
1-Alkyl-5-amino-1,2-dihydro-3H-pyrazol-3-ones (Figure 8.11) could be derived from the hydrazino-Ugi reaction products cyanoacetyl hydrazine by facile removal of the trifluoroacetyl group in acidic condition, further extends the scope of the hydrazino-Ugi reaction and trifluoroacetyl group removal was attempted in basic condition, the isolated products were the unexpected 1-alkyl-3-oxo-5-(trifluoromethyl)-2,3-dihydro-1H-pyrazole-4-carbonitriles.\textsuperscript{308}

**Figure 8.11**
- Mechanism

Figure 8.12

Hydrazide derivative of 2-cyano-2-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)acetate undergoes cyclization to form 3-amino-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one.\textsuperscript{341}

8.2.2. Therapeutic importance

The chemistry of aminopyrazole has been extensively investigated in the past.\textsuperscript{82,80,69} The considerable biological and medicinal activities of aminopyrazolones for which aminopyrazolones are preferred precursors, have stimulated these investigations. Interest in aminopyrazole synthesis and its color chemistry has recently been developed.\textsuperscript{130,7,255,211} The established activity of Muzolimine, Aminophenazone, Nifenazone and Metamizole (structure shown in Figure 8.1) is surely behind this interest.\textsuperscript{107}

Muzolimine is a 1-substituted 2-pyrazolin-5-one derivative is a non-sulphonamide diuretic, differing from the structures of other diuretics since it contains neither a sulfonamide nor a carboxyl group. It has a saluretic effect similar to furosemide and acts in the proximal tubule and in the medullary portion of the ascending limb of the loop of Henle. Pharmacokinetic studies in dogs, healthy
volunteers and in patients with renal insufficiency show that the compound is readily absorbed after oral administration.\textsuperscript{35}

Dudley hart\textsuperscript{18} suggested that Nifenazone (2,3-dimethyl-4-nicotinamido-1-phenylpyrazol-5-one; "Thylin") is not of significant value in the therapy of the chronic rheumatic disorders and that side-effects may be expected to occur, particularly in those patients who give a history of abnormal reactions to phenylbutazone and oxyphenbutazone.

Metamizole was first synthesized by the German company Hoechst AG (now part of Sanofi) in 1920 and its mass production started in 1922. It remained available worldwide until the 1970s, when it was discovered that the drug carries a small risk of causing agranulocytosis, a potentially fatal condition. Several national medical authorities have since withdrawn metamizole from the market altogether or have restricted it to be available only with a prescription, although it remains available over the counter in many countries.\textsuperscript{188}

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a novel antioxidant that is currently used in Japan for the treatment of patients in the acute stage of cerebral infarction. Edaravone scavenges ROS and inhibits proinflammatory responses after brain ischemia in animals and humans. In particular, postischemic inflammation, leading to brain edema and infarction due to neuronal damage and endothelial cell death, can be ameliorated by edaravone. In addition to these antistroke effects, edaravone has also been shown to prevent oxidative damage to various extra cerebral organs. Therefore, in addition to its usefulness in the treatment of stroke, edaravone is expected to play an integral role in the treatment of many oxidative stress-related diseases.\textsuperscript{271} Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, Figure 8.13) has two tautomers; 3-hydroxy.pyrozole isoform and 1,2-dihydro-pyrazol-3-one (adapted from Watanabe\textsuperscript{187}).
A series of 2-substituted-1H-benzimidazole derivatives was synthesized and evaluated for antimicrobial, antifungal and cytotoxic activities. Compounds 4-[(1H-benzo[d] imidazol-2-yl) diazenyl]-3-amino-1-phenyl-1H-pyrazol-5(4H)-one (Figure 8.14) exerted the strongest cytotoxic effect with IC$_{50}$ 15nM.

Pyrazole containing compounds (Figure 8.15) exhibited maximum zone of inhibition against *gram negative* bacteria. In addition, compound (R= -Cl) showed maximum inhibitory growth against *Aspergillus oryzae*.

The polarographical and the electrochemical behavior of 1-(toluenyl sulfonyl)-3-amino-4-(2'-nitro aryl hydrazono)-2-pyrazolin-5-one was studied in the acidic as well as in basic media. The compounds gave two well defined, diffusion controlled, irreversible waves in Britton-Robinson buffers of pH range 1.0-7.0. In alkaline media three well defined, diffusion controlled and irreversible waves were obtained. Effect of
various solvents, cations and surfactants on the reduction is presented. The effect of substituent and its correlation with the Hammett substituent constant is detailed. Based on the results, a detailed reduction mechanism in acidic as well as basic media is proposed.\textsuperscript{348,337}

**Figure 8.16**

A compound shown in Figure 8.17 is disclosed by Ulrich\textsuperscript{91} which comprises aminopyrazoles capable of inhibiting the formation of advanced glycosylation end products of target proteins by reacting with the carbonyl moiety of an early glycosylation product of such target proteins formed by their initial glycosylation. The method comprises contacting the target protein with the compound. Both industrial and therapeutic applications for the invention are envisioned, as food spoilage and animal protein aging can be treated. For example, 1-methyl-3-hydroxy-4,5-diaminopyrazole sulfate was prepared and incubated with bovine serum albumin and glucose for 1 week to evaluate the ability of the compound to inhibit glucose-mediated development of fluorescence of the albumin, a measure of crosslinking; inhibition rate of browning by the compound at 1 mM was 47.9%.

**Figure 8.17**

A series of 7-phenylazo-7H-3-(2-methyl-1H-indol-3-yl)pyrazolo[5,1-c][1,2,4]triazol-6(5H)-one (Figure 8.18) was prepared via reactions of 4-amino-3-mercapto-5-(2-methyl-1H-indol-3-yl)-1,2,4-triazole with ethyl arylhydrazonochloroacetate. A possible mechanism is also proposed to account for the formation of the products. The biological activity of some of these products was also evaluated.\textsuperscript{213}
4-[(N-Substituted-indol-3-yl)methylene]-3-amino-1H-pyrazol-5(4H)-ones were evaluated for their anti-inflammatory activity against carrageeanean-induced rat’s paw oedema by administration of 20 and 5 mg kg–1 using flufenamic acid (20 mg kg–1) and indomethacin (5 mg kg–1) as reference drugs. The presence of aminopyrazole at position 3 of indole as in compounds (Figure 8.19) showed good inhibition for their anti-inflammatory activity. \(^{347}\)

Keeping in view of wide spectrum biodynamic activities of substituted aminopyrazolones and with a view to have potent therapeutic agents, substituted 3-(benzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one derivative (Type – V) have been undertaken.
8.3. Synthesis and biological evaluation of 3-(benzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one derivative

Ethyl 2-cyano-2-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)acetate 3 was prepared from 5,6-dimethoxy-1-indanone and ethyl cyanoacetate by Knoevenagel condensation, 11,8,45,168,12,32 which was then reacted with hydrazine hydrate to form 3-amino-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one 4 via hydrazide intermediate 54 as shown in mechanism (Figure 8.12). Substituted 3-(benzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one derivatives 5a-5m (Type-V) have been prepared by reaction of 4 with corresponding aromatic aldehyde and catalytic amount of p-toluenesulfonic acid in ethanol.

Figure 8.20: Reaction scheme
The constitution of the synthesized products (Type-V) have been characterized using elemental analysis, $^1$H, $^{13}$C nuclear magnetic resonance spectroscopy and further supported by mass spectrometry. Purity of all compounds has been checked by thin layer chromatography.

All of the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method with two Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pyogenes*, two Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and three fungal strains *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus* taking Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin and Greseofulvin as standard drugs. Showed activity against the reference strains with the MIC = 62.5 to >1000 μg/mL.
8.4. Experimental

Melting points were determined in open capillary tubes and are uncorrected. NMR were recorded in either CDCl$_3$ or DMSO-d$_6$ on a Bruker Avance 400 Hz and signal are given in ppm (δ) relative to TMS. Elementary analyses were taken on Euro EA 3000 elementary analysis instrument. LCMS were measured on Agilent 1100 Series MS spectrometer. TOF MS were measured on a Waters ZQ 2000 spectrometer. All the solvent and materials are reagent grades and purified before use. Purity of all reagent and product were checked by TLC (hexane: ethylacetate; 60:40).

5,6-Dimethoxy-1-indanone 1 was prepared according to the literature procedure$^{148}$ in 60.0% overall yield based on 3,4-dimethoxybenzaldehyde purity was confirmed by TLC and mp.

8.4.1. Synthesis of ethyl 2-cyano-2-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)acetate (3)

A suspension of, 2.57 gm (0.03 mole) of piperidine and 1.56 gm (0.03 mole) of acetic acid in 100.0 mL of toluene was stirred for 10 min. To this 25.0 gm (0.130 mole) of 5,6-dimethoxy-1-indanone 1 and 17.95gm (0.158 mole) of ethylcyanoacetate 2 were charged and heated the reaction mixture to reflux temperature to remove water for 24 hr. TLC shown presence of some 5,6-dimethoxy-1-indanone, reaction mixture was cooled to 20 °C and filtered the solid and washed with 5 mL toluene and 10 mL. 10.0 gm of 3 was obtained as yellowish solid; yield 12.0 gm (32.11%); mp 192-194°C (lit,$^{45}$ 193-194°C); MS(Cl) : m/z 288 (M+1); TLC, single spot (ethyl acetate).

8.4.2. Synthesis of 3-amino-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one (4)

A solution of 10.0 gm (0.034 mole) of ethyl 2-cyano-2-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)acetate 3 and 2.4 gm (0.047 mole) of hydrazine hydrate (80% in water) in 100 mL ethanol was stirred for 18 hr at room temperature. Water (200 mL) was added and the solid was filtered. The filter cake was washed thoroughly with water and the solid purified by chromatography on silica gel (hexane/ethylacetate 3-8%) to yield a beige solid; (4.2 g, 44.15 %): mp 240°C (dec); ESI MS: m/z 273.3 (43,M+), 569.1 (100, 2M+22).
8.4.3. General procedure for the preparation of Substituted 3-(benzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-ones (5a-5m)

A solution of 500 mg (1.830 mmole) of 3-amino-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one 4 and 1.4 equivalent (2.560 mmole) of corresponding aromatic aldehyde and approximately 2-5 mg of p-toluenesulfonic acid 5.0 mL of ethanol was heated at reflux temperature. After 4 hr, the mixture was cooled to 10 °C and the solid was collected by filtration and recrystallized by 2-propanol to yield 30–68 % of pure 5a-5m. TLC has checked for purity of all the compounds.

8.4.4. Experimental data of 3-(benzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one

3-(Benzyldieneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one (5a)

Orange solid, 280 mg (37.61 %), mp 160-161°C; $^1$H NMR (CDCl$_3$, 400MHz) $\delta = 9.43$ (s, 1 H), 8.84 (s, 1 H), 8.05 (s, 2 H), 7.54 (s, 1 H), 7.47 (s, 1 H), 7.35 (s, 2 H), 6.91 (s, 1 H), 3.89 (s, 3 H), 3.91 (s, 3 H), 3.49 (s, 2 H), 3.14 (s, 2 H) ppm; $^{13}$C NMR (CDCl$_3$, 40MHz): $\delta = 168.6, 162.5, 158.9, 153.1, 151.2, 148.7, 145.7, 135.6, 135.3, 134.3, 132.8, 130.0, 121.6, 110.3, 109.3, 56.5, 56.0, 38.8, 30.1 ppm; MS: m/z 361.97 (M+); Anal. Calcd. for C$_{21}$H$_{19}$N$_3$O$_3$: C,69.79; H,5.30; N,11.63; O,13.28. Found: C, 69.86; H, 5.28; N, 11.55.
3-(2-Chlorobenzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one (5b)

Orange solid, 250 mg (30.66 %), mp 156-157°C; \(^1\)H NMR (CDCl\(_3\), 400MHz): \(\delta = 9.43\) (s, 1 H), 9.02 (s, 1 H), 8.38 (s, 1 H), 7.55 - 7.45 (m, 3 H), 7.41 (s, 1 H), 6.91 (s, 1 H), 3.89 (s, 3 H), 3.91 (s, 3 H), 3.49 (s, 2 H), 3.14 (s, 2 H) ppm; \(^{13}\)C NMR (CDCl\(_3\), 40MHz): \(\delta = 168.6, 160.5, 158.7, 153.3, 151.2, 148.7, 145.7, 135.8, 135.6, 135.5, 133.5, 131.8, 130.8, 130.2, 121.1, 110.3, 109.3, 56.5, 56.0, 38.8, 30.1\) ppm; MS: m/z 396.97 (M\(^+\)); Anal. Calcd. for C\(_{21}\)H\(_{18}\)ClN\(_3\)O\(_3\): C, 63.72; H, 4.58; Cl, 8.96; N, 10.62; O, 12.13. Found: C, 63.82; H, 4.58; N, 10.64.

3-(3-Chlorobenzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one (5c)

Orange solid, 400 mg (49.05 %), mp 155-156°C; \(^1\)H NMR (CDCl\(_3\), 400MHz): \(\delta = 9.43\) (s, 1 H), 9.02 (s, 1 H), 8.38 (s, 1 H), 7.55 - 7.45 (m, 3 H), 7.41 (s, 1 H), 6.91 (s, 1 H), 3.89 (s, 3 H), 3.91 (s, 3 H), 3.49 (s, 2 H), 3.14 (s, 2 H) ppm; \(^{13}\)C NMR (CDCl\(_3\), 40MHz): \(\delta = 168.6, 161.7, 158.9, 152.4, 151.2, 148.7, 145.7, 137.0, 135.6, 134.2, 130.3, 130.3, 129.2, 129.1, 121.6, 110.3, 109.3, 56.5, 56.0, 38.8, 30.1\) ppm; MS: m/z 396.97 (M\(^+\)); Anal. Calcd. for C\(_{21}\)H\(_{18}\)ClN\(_3\)O\(_3\): C, 63.72; H, 4.58; Cl, 8.96; N, 10.62; O, 12.13. Found: C, 63.84; H, 4.58; N, 10.63.
3-(4-Chlorobenzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one (5d)

Orange solid, 500 mg (61.32 %), mp 130-131°C; \(^1\)H NMR (CDCl\(_3\), 400MHz): \(\delta=\) 9.43 (s, 1 H), 8.90 (s, 1 H), 7.74 (s, 2 H), 7.54 (s, 1 H), 7.31 (s, 2 H), 6.91 (s, 1 H), 3.89 (s, 3 H), 3.91 (s, 3 H), 3.49 (s, 2 H), 3.14 (s, 2 H) ppm; \(^{13}\)C NMR (CDCl\(_3\), 40MHz): \(\delta = \) 168.6, 160.8, 158.9, 153.3, 151.2, 148.7, 145.7, 137.2, 135.6, 134.3, 129.8, 129.5, 121.6, 110.3, 109.3, 56.5, 56.0, 38.8, 30.1 ppm; MS: m/z 396.97 (M+); Anal. Calcd. for C\(_{21}\)H\(_{18}\)ClN\(_3\)O\(_3\): C, 63.72; H, 4.58; Cl, 8.96; N, 10.62; O, 12.13. Found: C, 63.80; H, 4.59; N, 10.65.

4-(5,6-Dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(2-methoxybenzylidene amino)-1H-pyrazol-5(4H)-one (5e)

Pale yellow solid, 550 mg (68.21 %), mp 145-146°C; \(^1\)H NMR (CDCl\(_3\), 400MHz): \(\delta=\) 8.52 (s, 1 H), 7.78-7.77 (d, 1 H), 7.26 (s, 1 H), 6.95 (d, 1 H), 6.84 (d, 1 H), 3.96-3.93 (s, 3 H, 2OCH\(_3\)), (s, 3 H), 3.85 (s, 3 H, OCH\(_3\)), 3.19-3.17 (t, 2 H), 3.05-3.02 (t, 2 H) ppm; MS: m/z 391.97 (M+); Anal. Calcd. for C\(_{22}\)H\(_{21}\)N\(_3\)O\(_4\): C, 67.51; H, 5.41; N, 10.74; O, 16.35. Found: C, 67.66; H, 5.37; N, 10.75.
4-(5,6-Dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(3-methoxybenzyldiene amino)-1H-pyrazol-5(4H)-one (5f)

Pale yellow solid, 420 mg (52.09 %), mp 148-149°C; \(^1\)H NMR (CDCl\(_3\), 400MHz): \(\delta = 8.46\) (s, 1 H), 7.77 - 7.74 (d, 2 H), 7.33 (s, 1 H), 7.12 (s, 1 H), 6.94-6.91, (d, 2 H), 6.82 (s, 1 H), 3.95 - 3.92 (d, 6 H), 3.84 (s, 3 H), 3.18 - 3.15 (m, 2 H), 3.05 - 3.02 (m, 2 H) ppm; MS: m/z 392.13 (M+); Anal. Calcd. for C\(_{22}\)H\(_{21}\)N\(_3\)O\(_4\): C,67.51; H,5.41; N,10.74; O,16.35. Found: C, 67.62; H, 5.38; N, 10.77.

4-(5,6-Dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(4-methoxybenzyldiene amino)-1H-pyrazol-5(4H)-one (5g)

Pale yellow solid, 290 mg (35.96 %), mp 140-141°C; \(^1\)H NMR (CDCl\(_3\), 400MHz): \(\delta = 8.46\) (s, 1 H), 7.77 - 7.74 (d, 2 H), 7.33 (s, 1 H), 7.12 (s, 1 H), 6.94-6.91, (d, 2 H), 6.82 (s, 1 H), 3.95 - 3.92 (d, 6 H), 3.84 (s, 3 H), 3.18 - 3.15 (m, 2 H), 3.05 - 3.02 (m, 2 H) ppm; \(^13\)C NMR (CDCl\(_3\), 40MHz): \(\delta = 168.6, 163.2, 162.1, 158.9, 153.3, 151.2, 148.7, 145.7, 135.6, 133.4, 129.8, 121.6, 114.3, 110.3, 109.3, 56.5, 56.2, 56.0, 38.8, 30.1 ppm; MS: m/z 392.13 (M+); Anal. Calcd. for C\(_{22}\)H\(_{21}\)N\(_3\)O\(_4\): C,67.51; H,5.41; N,10.74; O,16.35. Found: C, 67.66; H, 5.37; N, 10.75.
3-(3-Bromobenzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one (5h)

Yellow solid, 300 mg (33.08 %), mp 167-169°C; $^1$H NMR (CDCl$_3$, 400MHz):
δ = 9.43 (s, 1 H), 8.81 (s, 1 H), 8.34 (s, 1 H), 7.97 (s, 1 H), 7.73 (s, 1 H), 7.54 (s, 1 H), 7.33 (s, 1 H), 6.91 (s, 1 H), 3.89 (s, 3 H), 3.91 (s, 3 H), 3.49 (s, 2 H), 3.14 (s, 2 H) ppm; $^{13}$C NMR (CDCl$_3$, 40MHz): δ = 168.6, 162.5, 158.9, 153.5, 151.2, 148.7, 145.7, 137.3, 135.6, 132.3, 131.3, 130.9, 130.3, 123.1, 121.6, 110.3, 109.3, 56.5, 56.0, 38.8, 30.1 ppm; MS: m/z 440.88 (M+); Anal. Calcd. for C$_{21}$H$_{18}$BrN$_3$O$_3$: C, 57.29; H, 4.12; Br, 18.15; N, 9.54; O, 10.90. Found: C, 57.28; H, 4.12; N, 9.55.

4-(5,6-Dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(2-ethoxybenzylidene amino)-1H-pyrazol-5(4H)-one (5i)

Yellow solid, 320 mg (38.31 %), mp 128-129°C; $^1$H NMR (CDCl$_3$, 400MHz):
δ = 9.43 (s, 1 H), 9.25 - 7.43 (m, 3 H), 7.32 (s, 1 H), 7.26 - 3.69 (m, 10 H), 3.49 (s, 2 H), 3.14 (s, 2 H), 1.54 (s, 4 H) ppm; $^{13}$C NMR (CDCl$_3$, 40MHz): δ = 167.9, 152.8, 147.8, 130.2, 125.2, 120.7, 110.0, 108.6, 107.9, 69.3, 61.1, 55.67, 55.5, 30.4, 13.9 ppm; MS: m/z 405.97 (M+); Anal. Calcd. for C$_{23}$H$_{25}$N$_3$O$_4$: C, 68.13; H, 5.72; N, 10.36; O, 15.78. Found: C, 68.16; H, 5.78; N, 10.35.
4-(5,6-Dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(3-hydroxy-4-methoxy benzylideneamino)-1H-pyrazol-5(4H)-one (5j)

Yellow solid, 290 mg (34.55 %), mp >260°C; MS: m/z 408.02 (M+); Anal. Calcd. for C_{22}H_{21}N_{3}O_{5}: C, 64.86; H, 5.20; N, 10.31; O, 19.64. Found: C, 64.89; H, 5.22; N, 10.33.

4-(5,6-Dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(3-nitrobenzylideneamino)-1H-pyrazol-5(4H)-one (5k)

Yellow solid, 450 mg (53.75 %), mp >260°C; \(^1\)H NMR (CDCl\(_3\), 400MHz): \(\delta=\) 89.43 (s, 1 H), 9.11 (s, 1 H), 8.74 (s, 1 H), 8.35 (s, 1 H), 8.03 (s, 1 H), 7.55 (d, J = 3.2 Hz, 2 H), 6.91 (s, 1 H), 3.89 (s, 3 H), 3.91 (s, 3 H), 3.49 (s, 2 H), 3.14 (s, 2 H) ppm; \(^{13}\)C NMR (CDCl\(_3\), 40MHz): \(\delta = \) 168.6, 162.1, 158.9, 152.7, 151.2, 148.7, 148.2, 145.7, 137.9, 137.5, 135.6, 130.6, 126.3, 124.5, 121.6, 110.3, 109.3, 56.5, 56.0, 38.8, 30.1ppm; TOF MS: m/z 407.09 (M+); Anal. Calcd. for C_{21}H_{18}N_{4}O_{5}: C, 62.06; H, 4.46; N, 13.79; O, 19.68. Found: C, 62.11; H, 4.45; N, 13.80.

4-(5,6-Dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(3-fluorobenzylidene amino)-1H-pyrazol-5(4H)-one (5l)

Yellow solid, 340 mg (43.50 %), mp 130-132°C; \(^1\)H NMR (CDCl\(_3\), 400MHz): \(\delta=\) 9.43 (s, 1 H), 9.02 (s, 1 H), 8.38 (s, 1 H), 7.55 - 7.45 (m, 3 H), 7.41 (s, 1 H), 6.91 (s,
1 H), 3.89 (s, 3 H), 3.91 (s, 3 H), 3.49 (s, 2 H), 3.14 (s, 2 H) ppm; $^{13}$C NMR (CDCl$_3$, 40MHz): $\delta =$ 168.6, 161.7, 158.9, 152.4, 151.2, 148.7, 145.7, 137.0, 135.6, 134.2, 130.3, 130.3, 129.2, 129.1, 121.6, 110.3, 109.3, 56.5, 56.0, 38.8, 30.1 ppm; MS: m/z 380.27 (M$^+$); Anal. Calcd. for C$_{21}$H$_{18}$FN$_3$O$_3$: C, 66.48; H, 4.78; F, 5.01; N, 11.08; O, 12.65. Found: C, 66.46; H, 4.76; N, 11.10.

4-(5,6-Dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(2-hydroxybenzylidene amino)-1H-pyrazol-5(4H)-one (5m)

Pale yellow solid, 300 mg (38.59 %), mp 252-253°C, MS: m/z 378.81 (M$^+$); Anal. Calcd. for C$_{21}$H$_{19}$N$_3$O$_4$: C, 66.83; H, 5.07; N, 11.13; O, 16.96; Found: C, 66.84; H, 5.02; N, 11.12.
8.5. Chromatographic and spectroscopic data

8.5.1. LCMS spectrum of 3-amino-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one (4) (MW = 273.29)

8.5.2. LCMS spectrum of 3-(benzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one (5a) (MW = 361.97)
8.5.3. $^1$H NMR spectrum of 3-(benzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one (5a)

8.5.4. $^1$H NMR spectrum of 4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(4-methoxybenzylidene amino)-1H-pyrazol-5(4H)-one (5g)
8.5.5. $^1$H NMR spectrum of 4-(5,6-Dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(2-methoxybenzylidene amino)-1H-pyrazol-5(4H)-one (5e)

8.5.6. $^{13}$C NMR of 4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(2-ethoxybenzylidene amino)-1H-pyrazol-5(4H)-one (5i)
8.5.7. APT $^{13}$C NMR of 4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(2-ethoxybenzylidene amino)-1H-pyrazol-5(4H)-one (DMSO-$d^6$) (5i)

8.5.8. IR spectrum of 4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-3-(2-ethoxybenzylidene amino)-1H-pyrazol-5(4H)-one (5i)
8.6. Biological evaluation

All of the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) \textit{in vitro} by broth dilution method with two Gram-positive bacteria \textit{Staphylococcus aureus} and \textit{Streptococcus pyogenes}, two Gram-negative bacteria \textit{Escherichia coli} and \textit{Pseudomonas aeruginosa} and three fungal strains \textit{Candida albicans}, \textit{Aspergillus Niger} and \textit{Aspergillus clavatus} taking Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin and Greseofulvin as standard drugs.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to Clinical and Laboratory Standards Institute (formerly NCCLS).

- **Minimal inhibition concentration (MIC)**

  The main advantage of the \textbf{Broth Dilution Method} for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

  - Serial dilutions were prepared in primary and secondary screening.
  - The control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 0C overnight.
  - The MIC of the control organism is read to check the accuracy of the drug concentrations.
  - The lowest concentration inhibiting growth of the organism is recorded as the MIC.
  - The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

- **Methods used for primary and secondary screening**

  Each synthesized compounds were diluted in DMSO to obtain 2000 \(\mu\)g mL\(^{-1}\) concentration, as a stock solution. Inoculum size for test strain was adjusted to \(10^8\) cfu (colony forming unit) per milliliter by comparing the turbidity.
**Primary screen:** In primary screening 1000 μg mL⁻¹, 500 μg mL⁻¹ and 250 μg mL⁻¹ concentrations of the synthesized compounds were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

**Secondary screen:** The compounds found active in primary screening were similarly diluted to obtain 200 μg mL⁻¹, 100 μg mL⁻¹, 50 μg mL⁻¹, 25 μg mL⁻¹, 12.5 μg mL⁻¹ and 6.250 μg mL⁻¹ concentrations.

**Reading Result:** The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculums. The test mixture should contain 10⁸ organism/mL.

The results obtained from antimicrobial susceptibility testing are depicted in Table 8.3.

**Table 8.1: Minimal fungicidal concentration**

<table>
<thead>
<tr>
<th>MINIMAL FUNGICIDAL CONCENTRATION</th>
<th>Standard Drugs</th>
<th>C.Albicans</th>
<th>A.Niger</th>
<th>A.Clavatus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nystatin</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Greseofulvin</td>
<td>500</td>
<td>100</td>
<td>100</td>
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</table>

**Table 8.2: Minimal inhibition concentration**

<table>
<thead>
<tr>
<th>MINIMAL INHIBITION CONCENTRATION</th>
<th>Standard Drugs</th>
<th>S.aureus</th>
<th>S.pyogenus</th>
<th>E.coli</th>
<th>P.aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamycin</td>
<td>0.25</td>
<td>0.5</td>
<td>0.05</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>250</td>
<td>100</td>
<td>100</td>
<td>100</td>
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</tr>
<tr>
<td>Chloramphenicol</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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### Table 8.3: Antimicrobial activity

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimal bactericidal concentration μg/mL</td>
<td>Minimal fungicidal concentration μg/mL</td>
</tr>
<tr>
<td></td>
<td>Gram +ve Bacteria</td>
<td>Gram –ve Bacteria</td>
</tr>
<tr>
<td></td>
<td><em>S.aureus</em></td>
<td><em>S.pyogenus</em></td>
</tr>
<tr>
<td>5a</td>
<td>125</td>
<td>200</td>
</tr>
<tr>
<td>5b</td>
<td>62.5</td>
<td>125</td>
</tr>
<tr>
<td>5c</td>
<td>62.5</td>
<td>125</td>
</tr>
<tr>
<td>5d</td>
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</tr>
<tr>
<td>5e</td>
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<td>125</td>
</tr>
<tr>
<td>5f</td>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
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<tr>
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### 8.7. Summary

Substituted 3-(benzylideneamino)-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one **5a-5m** derivatives have been prepared by reaction of 3-amino-4-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)-1H-pyrazol-5(4H)-one with corresponding aromatic aldehyde and catalytic amount of p-toluenesulfonic acid in ethanol in 30–68% isolated yields. The constitution of all the synthesized compounds have been characterized by using elemental analysis, FT-IR and $^1$HNMR, $^{13}$CNMR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatography. All of the synthesized...
compounds were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method with two Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pyogenes*, two Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and three fungal strains *Candida albicans*, *Aspergillus Niger* and *Aspergillus clavatus* taking Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacain, Nystatin and Greseofulvin as standard drugs. None of the compounds showed significant antibacterial and antifungal activities.