SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME PYRAZOLE DERIVATIVES FOR ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT
This work has been undertaken for the synthesis and pharmacological evaluation of some pyrazole derivatives for anti-inflammatory activity. 3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one obtained from phenyl hydrazine and ethyl acetoacetate was acetylated and then subjected to Claisen-Schmidt condensation with 4-hydroxy benzaldehyde to get a chalcone. This chalconewas cyclized with the help of hydroxylamine hydrochloride to form an isoxazole derivatives which were further condensed with various substituted aromatic aldehydes to obtain titled compounds (4aD1-4aD15). The structures of new synthesized compounds were confirmed by IR, ¹H NMR and Mass spectral data. Synthesized derivatives were evaluated for their anti-inflammatory activity. The compound 4aD3, 4aD8, 4aD9, 4aD10 and 4aD14was found to have comparable anti-inflammatory activity with that of standard drug indomethacin.

Key words: Pyrazoles,synthesis,anti-inflammatory,indomethacin.

INTRODUCTION
Pyrazoleisa class of organic compounds of the heterocyclic series characterized by a ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions.
Pyrazolone moiety, a five-membered lactam ring containing two nitrogens and ketone group in the same molecule or alternatively a derivative of pyrazole possessing an additional carbonyl/hydroxyl group, has been the focus of medicinal chemists for over the last 100 years because of the outstanding pharmacological properties shown by several of its derivatives. Pyrazoles and their derivatives exhibit various biological activities including antimicrobial, anti-cyclooxygenase, anticonvulsant, antitubercular, antitumor, anti-inflammatory, analgesic, antidiabetic, antipshycotic, etc.

Although pain is the most common complaint in the medical field, the arsenal of effective and safe analgesics is still relatively small. Thus, the identification of compounds that can effectively treat painful states without induction of side effects remains a major challenge in biomedical research. Pyrazoles and their derivatives are also widely known for their excellent effectiveness as analgesics and antipyretics.

The existing nonsteroidal anti-inflammatory drugs have side effects like gastric or intestinal ulceration and bleeding, that sometimes can be accompanied by anemia from the resultant blood loss. Other side effects of these drugs that result from blockade of the synthesis of endogenous prostaglandins and thromboxane-A2 include disturbances in platelet functions and changes in renal function. Recently, a novel class of selective COX-2 inhibitors has been discovered. Among this class, celecoxib was shown to be a potent and gastrointestinal (GI) safe anti-inflammatory agent. It is considered a typical model of pyrazole containing, diaryl-heterocyclic template that is known to selectively inhibit COX-2.

Since most of the pyrazole derivatives show anti-inflammatory activity, the synthesized compounds are also expected to show anti-inflammatory activity. Hence, our plan is to synthesize some substituted pyrazole derivatives and subsequently screen for their anti-inflammatory activity. Anti-inflammatory activity was carried out by using carrageenan induced paw edema method in wistar albino rats for the newly synthesized compounds.

**MATERIALS AND METHODS**

**Equipments**

All TLC were performed on precoated TLC plates (Silica Gel 60 F254, Merck, Germany), visualization done under UV light. All melting points were determined in open capillaries using thermonik precision apparatus (Model-C-PMP-2, Mumbai, India) and were uncorrected. All FT-IR spectra were recorded in Tensor 27 spectrophotometer, Brukeroptik.
(Germany) using ATR technique. All ^1H NMR spectra were recorded in Bruker spectrophotometer AMX-400 (400 MHz), Brukeroptik (Germany) in CD$_3$OD using TMS as an internal standard at IISC, Bangalore. All Mass spectra were recorded using a Jeol-D-300 Mass spectrophotometer (70ev), SHIMADZU (Japan) by GC-MS-QP 2010S.

**Synthesis**

**Synthesis of 3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (1a)**$^{[17,18,19]}$

A mixture of the ethyl acetoacetate (0.00768 mol) and substituted hydrazine (0.00729 mol) was exposed to microwave irradiation (power input 20%) for 4.0 min. Upon completion, the reaction mixture was allowed to reach room temperature, diluted with ethyl acetate with vigorous stirring and the precipitate was collected by filtration. The product was isolated and recrystallized with ethanol.

![Chemical structure of 3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (1a)](image)

**Synthesis of 4-acetyl-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (2a)**$^{[20]}$

Compound 1a (0.00862 mol) was dissolved in 15-20 ml of 1,4- dioxan by heating followed by 1.2 g of Calcium hydroxide and 0.9 ml of acetyl chloride added drop wise within 1 min. The reaction mixture became a thick paste and the temperature increased during first few minutes. The mixture was refluxed for 30 min. The calcium complex in the flask was decomposed by pouring the mixture into dil. HCl which caused separation of brown color solid product to separate. The product was collected on funnel. They were recrystallized from methanol-water slightly acidify to destroy any undecomposed calcium complex to yield pure product 2a.
Synthesis of 4-[(2E)-3-(4-hydroxyphenyl)prop-2-enoyl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (3a) [21,22]

4-hydroxy benzaldehyde (0.01 mol) and compound 2a (0.01 mol) were mixed together in 30 ml 95% ethanol and then added 70% aq. NaOH drop wise (10 ml). Resulting mixture was stirred for 2 h at 5-10°C, poured into crushed ice and acidified with dil.HCl. The precipitate obtained was washed and filtered with cold water, filtered and recrystallized from ethanol.

Synthesis of 4-[5-(4-hydroxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one(4a) [23,24, 25]

A mixture of 3a (0.01mol) in ethanol (20 ml), hydroxylamine hydrochloride (0.01 mol) and a drop of HCl was heated under reflux for 6 h, the reaction mixture was cooled, the solid separated and recrystallized from ethanol.
Synthesis of 4-[(4Z)-5-(4-hydroxyphenyl)-4-(substituted phenyl methyldiene)-4,5 dihydro-1,2-oxazol-3-yl]3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (4aD1-4aD15)\(^{[26,27]}\)

Equimolar solution of 4a (0.02 mol) and substituted benzaldehydes (0.02 mol) in ethanol in the presence of sodium ethoxide was refluxed for about 5 h on water bath and solvent was removed by evaporation. The resulting solid was recrystallized from methanol.

Melting point determination\(^{[28]}\)

The melting point of a substance is the temperature at which the material changes from a solid to a liquid state. Pure crystalline substances have a clear, sharply defined melting point. During the melting process, all of the energy added to a substance is consumed as heat of
fusion, and the temperature remains constant. The determination of melting point is the most important and easy way of differentiating this physical constant of one compound from other. All melting points were determined in open capillaries using thermonik precision apparatus (Model-C-PMP-2, Mumbai, India).

**Thin layer chromatography (TLC)**[28]

TLC is an important method for synthetic chemistry to infer the formation of compound based on the \( R_f \) values. It also helps in confirming the progress of the reaction. The precoated TLC plate made of silica gel F\(_{254}\) was used to known the \( R_f \) value.

**Infra-red spectroscopy (IR)**[29,30]

Infrared spectroscopy (IR spectroscopy) is the spectroscopy that deals with the infrared region of the electromagnetic spectrum, that is light with a longer wavelength and lower frequency than visible light. It covers a range of techniques, mostly based on absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify and study chemicals. A common laboratory instrument that uses this technique is a Fourier transform infrared (FTIR) spectrometer. IR is one of the most important tools for determining the various functional groups and the possible chemical structure. This technique is based upon the molecular vibration of the compound such that each and every bond will vibrate at the different frequency and this vibration frequency corresponds to IR frequency. Thus spectra of each and every bond will be formed. The important advantage of IR over the other technique is that it gives fingerprints (1300-650 cm\(^{-1}\)) information about the structure (functional group, bonding with each other) of molecules easily. No two compounds have identical fingerprint region. The Infrared Spectra were recorded by FT-IR technique in Tensor 27 spectrophotometer, Bruckeroptik (Germany) using ATR technique.

**Nuclear Magnetic Resonance spectroscopy (NMR)**[29,30]

Nuclear magnetic resonance spectroscopy, most commonly known as NMR spectroscopy, is the name given to a technique that exploits the magnetic properties of certain nuclei. The interaction between matter and electromagnetic forces can be observed by subjecting a substancesimultaneously to 2 magnetic forces, one other varying at some radio frequency. At a particular combination of fields, energy is absorbed by the sample and absorption can be observed as a change in signal developed by a radio frequency detector and amplifier. This energy of absorption can be related to a magnetic dipolar nature of the spinning nuclei. This technique is known as Nuclear Magnetic Resonance. The technique is useful in assuming the
structure of the molecule. The NMR Spectra of selected compounds were recorded in CD3OD (internal standard TMS-tetra methyl silane) at 400 MHz on the AMX 400 at the Sophisticated Instruments Facility (SIF), Dept. of Physics, Indian Institute of Sciences, Bangalore and interpreted for the aromatic/heterocyclic protons (delta values).

Mass spectroscopy\[^{29,30}\]
Mass spectroscopy is a technique through which molecular characterization is carried out according to the manner in which they fragment when bombardment with high energy electrons. In addition to elucidation or interpretation of molecular structure, mass spectra’s are useful for determining molecular weight. The physics behind mass spectrometry is that a charged particle passing through a magnetic field is deflected along a circular path on a radius that is proportional to the mass to charge ratio, m/z. In an electron impact mass spectrometer, a high energy beam of electrons is used to displace an electron from the organic molecule to form a radical cation known as the molecular ion. If the molecular ion is too unstable then it can fragment to give other smaller ions. The collection of ions is then focused into a beam and accelerated into the magnetic field and deflected along circular paths according to the masses of the ions. By adjusting the magnetic field, the ions can be focused on the detector and recorded.

Anti-inflammatory
Anti-inflammatory activity was carried out by using carrageenan induced paw edema method in wistar albino rats for the newly synthesized compounds.

Inflammation by Carrageenan induced paw edema Method \[^{31,32}\]
The inflammatory reaction is readily produced in rats in the form of paw edema with the help of irritants or inflammation. Carrageenan induced paw edema is the most commonly used experimental method. Carrageenan is a sulphated polysaccharide obtained from seaweed (Rhodophyceae) causing the release of histamine, 5-HT, bradykinin and prostaglandins. It produces inflammation and edema.

The rats were weighed and numbered. Mark was made on both hind paws (right and left) just beyond tibiotarsal junction, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume. The initial paw volume (both right and left) of each rat was noted by mercury displacement method. Animals were divided into different groups, each containing at least six rats. The first group of rats was treated with 0.1 mL of
1% Carboxy methyl cellulose suspension orally (control), second group was administered with a dose of 20 mg/kg of the suspension of indomethacin (standard) and the other groups were treated with 50 mg/kg of the suspension of test compounds (group 1-group 12).

After 30 min 0.1 mL of 1% (w/v) carrageenan was injected in the plantar region of the left paw of control, standard and test group 1-12. The right paw will serve as reference non-inflamed paw for comparison. The paw volume of both legs was noted for 30, 60 and 90 min after carrageenan challenge. The percent difference in the right and left paw volumes of each animal of control in paw volumes was calculated for each animal and expressed as percent edema inhibition by the drugs.

\[
\% \text{ inhibition} = \left\{1 - \frac{V_t}{V_c}\right\} \times 100
\]

Where, Vt: is edema volume in drug treated group.
Vc: is edema volume in the control group.

The results of anti-inflammatory activity of synthesized compounds are tabulated in Table 1 and Table 2.

RESULTS AND DISCUSSION

Synthesis of 3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (1a)

Yield 82.4%; m.p. 115 °C; Rf. 0.81; FT-IR 3260 (N-H, Pyrazole), 3054 (C-H, Ar-H), 1706 (C=O, Pyrazole); \(^1\)H NMR (400 MHz, DMSO): \(\delta\) 2.144 (s, CH\(_3\), 3H), 2.561 (s, CH\(_2\), 2H), 7.13-7.87 (m, Ar-H, 5H).

![Fig:1. FT-IR and \(^1\)H NMR Spectrum of 3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (1a)](image)
Synthesis of 4-acetyl-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (2a)

Yield 76%; m.p. 90 °C; Rf. 0.56; FT-IR 3019 (C-H, Ar-H), 1803 (C=O, Pyrazole), 1595 (C=N, Pyrazole); $^1$H NMR (400 MHz, DMSO): δ 2.367 (s, CH$_3$, 3H), 2.502 (s, CH$_3$, 3H), 3.5 (s, CH, 1H), 7.13-7.87 (m, Ar-H, 5H).

Fig:2. FT-IR and $^1$H NMR Spectrum of 4-acetyl-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one(2a)

Synthesis of 4-[(2E)-3-(4-hydroxyphenyl)prop-2-enoyl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (3a)

Yield 80%; m.p. 80 °C; Rf. 0.83; FT-IR 3054 (C-H, Ar-H), 1693 (C=O, Pyrazole), 1605 (C=N, Pyrazole); $^1$H NMR (400 MHz, DMSO): δ 2.374 (s, CH$_3$, 3H), 3.292 (s, CH, 1H), 5.341 (s, OH, 1H), 6.74-6.98 (d, CH, 2H), 7.19-7.32 (d, CH, 2H), 7.43-7.786 (m, Ar-H, 9H).
Fig:3. FT-IR and $^1$H NMR Spectrum of 4-[(2E)-3-(4-hydroxyphenyl)prop-2-enoyl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (3a)

Synthesis of 4-[(5-(4-hydroxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (4a)

Yield 76%; m.p. 106 °C; Rf. 0.67; FT-IR 3063 (C-H, Ar-H), 1708 (C=O, Pyrazole), 1600 (C=N, Pyrazole), 3470(OH); $^1$H NMR (400 MHz, DMSO): $\delta$ 2.167 (s, CH$_3$, 3H), 2.383 (d, CH$_2$, 2H), 2.467 (t, CH, 1H), 3.341 (s, CH, 1H), 5.42 (s, OH, 1H), 7.1-7.82 (m, Ar-H, 9H).
Fig: 4. FT-IR and $^1$H NMR Spectrum of 4-[5-(4-hydroxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (4a)

Synthesis of 4-[(4Z)-5-(4-hydroxyphenyl)-4-(substituted phenyl methylidene)-4,5-dihydro-1,2-oxazol-3-yl]3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (4aD1-4aD15)

4-[(4Z)-5-(4-hydroxyphenyl)-4-(phenylmethylidene)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C_{26}H_{21}N_{3}O_{3}] (4aD1)

Yield 65%; m.p. 90 °C; Rf. 0.45; FT-IR 3063 (C-H, Ar-H), 1704 (C=O, Pyrazole), 1596 (C=N, Pyrazole), 3378(OH, Stretching); $^1$H NMR (400 MHz, DMSO): $\delta$ 2.350 (s, CH$_3$, 3H), 2.672 (s, CH, 1H), 3.392 (s, CH, 1H), 6.698 (s, CH, 1H), 5.41 (s, OH, 1H), 7.1-7.82 (m, Ar-H, 13H).

Fig: 5. FT-IR and $^1$H NMR Spectrum of 4-[(4Z)-5-(4-hydroxyphenyl)-4-(phenylmethylidene)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (4aD1)
4-[(4Z)-5-(4-hydroxyphenyl)-4-[(4-nitrophenyl)methylidene]-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C_{26}H_{26}N_{4}O_{5}](4aD2)

Yield 74%; m.p. 83°C; Rf. 0.66; FT-IR 3024(C-H, Ar-H), 1643(C=O, Pyrazole), 1510 (C=N, Pyrazole), 3496 (OH, Stretching); \(^1\)H NMR (400 MHz, DMSO): \(\delta\) 2.42 (s, CH\(_3\), 3H), 2.84 (s, CH, 1H), 3.34 (s, CH, 1H), 6.54 (s, CH, 1H), 5.64 (s, OH, 1H), 6.98-8.69 (m, Ar-H, 13H).

Fig: 6. FT-IR and \(^1\)H NMR Spectrum of 4-[(4Z)-5-(4-hydroxyphenyl)-4-[(4-nitrophenyl)methylidene]-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H pyrazol-5-one(4aD2)

4-[(4Z)-4-[(4-chlorophenyl)methylidene]-5-(4-hydroxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C_{26}H_{20}ClN_{3}O_{3}](4aD3)

Yield 60%; m.p. 114°C; Rf. 0.58; FT-IR 3024(C-H, Ar-H), 1704(C=O, Pyrazole), 1510 (C=N, Pyrazole), 3378(OH, Stretching); \(^1\)H NMR (400 MHz, DMSO): \(\delta\) 2.36 (s, CH\(_3\), 3H), 2.78 (s, CH, 1H), 3.34 (s, CH, 1H), 6.42 (s, CH, 1H), 5.74 (s, OH, 1H), 6.92-8.64 (m, Ar-H, 13H); Mass 457.9(M+).
Fig:7. FT-IR, $^1$H NMR and Mass Spectrum of 4-[(4Z)-4-[(4-chlorophenyl)methylidene]-5-[4- hydroxyphenyl]-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-
pyrazol-5 one(4aD3)

4-[(4Z)-5-(4-hydroxyphenyl)-4-[(4-methoxyphenyl)methylidene]-4,5-dihydro-1,2-oxazol-
3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C$_{27}$H$_{23}$N$_3$O](4aD4)

Yield 77%; m.p. 126°C; Rf. 0.76; FT-IR 3024(C-H, Ar-H), 1704(C=O, Pyrazole), 1643
(C=N, Pyrazole); $^1$H NMR (400 MHz, DMSO): $\delta$1.25 (s, CH$_3$, 3H), 1.87 (s, CH, 1H),2.372
(s, CH, 1H), 3.194 (s, CH, 1H), 5.61 (s, OH, 1H), 6.87 (s, CH, 1H), 6.92-7.94(m, Ar-H, 13H).
Fig: 8. FT-IR and $^1$H NMR Spectrum of 4-[(4Z)-5-(4-hydroxyphenyl)-4-[(4-methoxyphenyl)methylidene]-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5dihydro-1H-pyrazol-5-one (4aD4)

4-[(4Z)-4-(anthracen-2-ylmethylidene)-5-(4-hydroxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C$_{34}$H$_{27}$N$_3$O$_3$] (4aD5)

Yield 81%; m.p. 119°C; R$_f$. 0.83; FT-IR 3064 (C-H, Ar-H), 1706 (C=O, Pyrazole), 1583 (C=N, Pyrazole); $^1$H NMR (400 MHz, DMSO): δ 1.97 (s, CH$_3$, 3H), 2.413 (s, CH, 1H), 3.27 (s, CH, 1H), 6.92 (s, CH, 1H), 5.24 (s, OH, 1H), 7.227-9.34 (m, Ar-H, 18H).

Fig: 9. FT-IR and $^1$H NMR Spectrum of 4-[(4Z)-4-(9,10-dihydroanthracen-1-ylmethylidene)-5-(4-hydroxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (4aD5)

4-[(4Z)-4-[(4-hydroxy-3-methoxyphenyl)methylidene]-5-(4-hydroxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C$_{27}$H$_{23}$N$_3$O$_5$] (4aD6)

Yield 66%; m.p. 80°C; R$_f$. 0.49; FT-IR 3024 (C-H, Ar-H), 1643 (C=O, Pyrazole), 1511 (C=N, Pyrazole), 3445 (OH, Stretching); $^1$H NMR (400 MHz, DMSO): δ 2.12 (s, CH$_3$, 3H), 2.34 (s,
CH, 1H), 2.72 (s, CH, 1H), 3.34 (s, CH, 1H), 6.46 (s, CH, 1H), 5.32 (s, OH, 1H), 5.64 (s, OH, 1H), 6.92-8.64 (m, Ar-H, 12H).

Figure 10: FT-IR and $^1$H NMR Spectrum of 4-[(4Z)-4-[(4-hydroxy-3-methoxy phenyl)methylidene]-5-(4-hydroxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (4aD6)

4-[(4Z)-5-(4-hydroxyphenyl)-4-[(4-methylphenyl)methylidene]-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C$_{27}$H$_{23}$N$_3$O$_3$] (4aD7)

Yield 78%; m.p. 88°C; Rf. 0.55; FT-IR 3063 (C-H, Ar-H), 1704 (C=O, Pyrazole), 1643 (C=N, Pyrazole), 3378 (OH, Stretching); $^1$H NMR (400 MHz, DMSO): 52.09 (s, CH$_3$, 3H), 2.34 (s, CH, 1H), 2.75 (s, CH, 1H), 3.12 (s, CH, 1H), 6.94 (s, CH, 1H), 4.53 (s, OH, 1H), 6.99-7.92 (m, Ar-H, 13H).

Figure 11: FT-IR and $^1$H NMR Spectrum of 4-[(4Z)-5-(4-hydroxyphenyl)-4-[(4-methylphenyl)methylidene]-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (4aD7)

4-[(4Z)-4-[(3,4-dimethoxyphenyl)methylidene]-5-(4-hydroxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C$_{28}$H$_{25}$N$_3$O$_5$] (4aD8)
Yield 82%; m.p. 106°C; Rf. 0.64; FT-IR 3024(C-H, Ar-H), 1643(C=O, Pyrazole), 1437 (C=N, Pyrazole), 3421(OH, Stretching); 1H NMR (400 MHz, DMSO): δ1.19 (s, CH3, 3H), 1.76 (s, CH, 1H), 2.339 (s, CH, 1H), 2.876 (s, CH, 1H), 3.29 (s, CH, 1H), 5.29 (s, OH, 1H), 6.94-7.78 (m, Ar-H, 12H); Mass 483.5 (M+).

Fig: FT-IR, 1H NMR and Mass Spectrum of 4-[(4Z)-4-[(3,4-dimethoxyphenyl)methylidene]-5-(4-hydroxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl]-3 methyl-1-phenyl-4,5- dihydro-1H-pyrazol-5-one(4aD8)

Yield 62%; m.p. 84°C; Rf. 0.71; FT-IR 3024(C-H, Ar-H), 1680(C=O, Pyrazole), 1585 (C=N, Stretching), 3405 (OH, Stretching); 1H NMR (400 MHz, DMSO): δ2.19 (s, CH3, 3H), 2.64 (s, CH, 1H), 2.92 (s, CH, 1H), 3.11 (s, CH, 1H), 3.32 (s, CH, 1H), 4.64 (s, OH, 1H), 6.97-7.85 (m, Ar-H, 11H).
Fig:13. FT-IR and $^1$H NMR Spectrum of 4-[(4Z)-5-(4-hydroxyphenyl)-4-[(3,4,5-
trimethoxyphenyl)methylidene]-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-
dihydro-1H-pyrazol-5-one(4aD9)

4-[(4Z)-5-(4-hydroxyphenyl)-4-[(4-hydroxyphenyl)methylidene]-4,5-dihydro-1,2-oxazol-
3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C$_{26}$H$_{21}$N$_3$O$_4$](4aD10)

Yield 70%; m.p. 89°C; Rf. 0.76; FT-IR 3024(C-H, Ar-H), 1643(C=O, Stretching), 1509
(C=N, Stretching), 3435 (OH, Stretching); $^1$H NMR (400 MHz, DMSO): δ1.24 (s, CH$_3$, 3H),
2.89 (s, CH, 1H), 3.36 (s, CH, 1H), 6.76 (s, CH, 1H), 5.94 (s, OH, 1H), 6.92 (s, CH, 1H), 6.21
(s, OH, 1H), 6.98-7.92(m, Ar-H, 13H).

Fig:14. FT-IR and $^1$H NMR Spectrum of 4-[(4Z)-5-(4-hydroxyphenyl)-4-[(4-
hydroxyphenyl)methylidene]-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-
dihydro-1H-pyrazol-5-one(4aD10)

4-[(4Z)-5-(4-hydroxyphenyl)-4-[(2-hydroxyphenyl)methylidene]-4,5-dihydro-1,2-oxazol-
3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C$_{26}$H$_{21}$N$_3$O$_4$](4aD11)
Yield 75%; m.p. 80°C; Rf. 0.54; FT-IR 3024(C-H, Ar-H), 1646(C=O, Stretching), 1507 (C=N, Stretching), 3448 (OH, Stretching).

Fig:15. FT-IR Spectrum of 4-[(4Z)-5-(4-hydroxyphenyl)-4-[(4-hydroxyphenyl)methylidene]-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (4aD11)

Yield 67%; m.p. 175°C; Rf. 0.69; FT-IR 3045(C-H, Ar-H), 1653(C=O, Stretching), 1515 (C=N, Stretching), 3422 (OH, Stretching).
Fig:16. FT-IR spectrum of 4-[(4Z)-5-(4-hydroxyphenyl)-4-[(2-nitrophenyl)methylidene]-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one(4aD12)

4-[(4Z)-5-(4-hydroxyphenyl)-4-[(2-nitrophenyl)methylidene]-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C_{26}H_{20}N_{4}O_{5}](4aD13)

Yield 73%; m.p. 172°C; Rf. 0.61; FT-IR 3021(C-H, Ar-H), 1670(C=O, Stretching), 1525 (C=N, Stretching), 3405 (OH, Stretching); 1H NMR (400 MHz, DMSO): δ1.22 (s, CH₃, 3H), 2.65 (s, CH, 1H), 2.83 (s, CH, 1H), 6.86 (s, CH, 1H), 4.23 (s, OH, 1H), 4.64 (s, OH, 1H), 7.22-8.96(m, Ar-H, 13H); Mass 467.5 (M-1).

Fig:17. FT-IR, 1H NMR and Mass Spectrum of 4-[(4Z)-5-(4-hydroxyphenyl)-4-[(3-nitrophenyl)methylidene]-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one(4aD13)

4-[(4Z)-5-(4-hydroxyphenyl)-4-(naphthalen-1-ylmethylidene)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C_{30}H_{23}N_{3}O_{3}](4aD14)
Yield 63%; m.p. 154°C; Rf. 0.51; FT-IR 3022(C-H, Ar-H), 1683(C=O, Stretching), 1437 (C=N, Stretching), 3412 (OH, Stretching).

Fig:18. FT-IR Spectrum of 4-[(4Z)-5-(4-hydroxyphenyl)-4-(naphthalen-1-ylmethylidene)-4,5-dihydro-1,2-oxazol-3-yl]-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (4aD14)

4-[(4E)-5-(4-hydroxyphenyl)-4-(thiophen-2-ylmethylidene)-4,5-dihydro-1,2-oxazol-3-yl]-1-phenyl-4,5-dihydro-1H-pyrazol-5-one [C23H17N3O3S](4aD15)

Yield 64%; m.p. 133°C; Rf. 0.48; FT-IR 3063(C-H, Ar-H), 1704(C=O, Stretching), 1562 (C=N, Stretching), 3378 (OH, Stretching).

Fig:19. FT-IR Spectrum of 4-[(4E)-5-(4-hydroxyphenyl)-4-(thiophen-2-ylmethylidene)-4,5-dihydro-1,2-oxazol-3-yl]-1-phenyl-4,5-dihydro-1H-pyrazol-5-one (4aD15)

Anti-inflammatory activity

Acute toxicity study was done according to Organization for economic co-operation and development (OECD) guidelines and LD50 was found to be 2g/kg body weight of animal. Anti-inflammatory activity of the synthesized compounds was evaluated by carrageenan induced rat paw edema method. The significant (p<0.01) reduction of rat paw edema was observed in most of the test compounds at 4 h compared to control group (Table 8.5 and 8.6). Out of all synthesized compounds only one compound 4aD8 at a dose of 200 mg/Kg
has shown more anti-inflammatory activity than indomethacin at a dose of 20 mg/Kg. Other compounds like 4aD9, 4aD3 and 4aD14 showed significant anti-inflammatory activity.

Table 1: Effect of Percentage inhibition in edema by Pyrazole derivatives

<table>
<thead>
<tr>
<th>Compounds</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>38.50**</td>
</tr>
<tr>
<td>4aD1</td>
<td>5.31**</td>
</tr>
<tr>
<td>4aD2</td>
<td>10.00**</td>
</tr>
<tr>
<td>4aD3</td>
<td>20.70**</td>
</tr>
<tr>
<td>4aD4</td>
<td>10.91**</td>
</tr>
<tr>
<td>4aD5</td>
<td>9.97**</td>
</tr>
<tr>
<td>4aD6</td>
<td>8.10*</td>
</tr>
<tr>
<td>4aD7</td>
<td>10.50**</td>
</tr>
<tr>
<td>4aD8</td>
<td>39.40**</td>
</tr>
<tr>
<td>4aD9</td>
<td>29.00**</td>
</tr>
<tr>
<td>4aD10</td>
<td>17.90*</td>
</tr>
<tr>
<td>4aD11</td>
<td>7.20*ss</td>
</tr>
<tr>
<td>4aD12</td>
<td>6.60*ss</td>
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<tr>
<td>4aD13</td>
<td>11.90**</td>
</tr>
<tr>
<td>4aD14</td>
<td>15.40**</td>
</tr>
<tr>
<td>4aD15</td>
<td>10.30**</td>
</tr>
</tbody>
</table>

(ANOVA) followed by Dunnett’s t-test for multiple comparisons. P < 0.05 (*) and P < 0.01 (**) were taken as significant; a= Standard Error Mean.; ns= non-significant.
Table 2: Anti-inflammatory activity of Pyrazole derivatives

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Paw Edema volume in mL (Mean ± SEMa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Control</td>
<td>0.03516 ±0.0897</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.02166 ±0.0016</td>
</tr>
<tr>
<td>4aD1</td>
<td>0.0333 ±0.0021</td>
</tr>
<tr>
<td>4aD2</td>
<td>0.03166 ±0.0016</td>
</tr>
<tr>
<td>4aD3</td>
<td>0.02789 ±0.0667</td>
</tr>
<tr>
<td>4aD4</td>
<td>0.03133±0.0016</td>
</tr>
<tr>
<td>4aD5</td>
<td>0.03166 ±0.0021</td>
</tr>
<tr>
<td>4aD6</td>
<td>0.03233 ±0.0016</td>
</tr>
<tr>
<td>4aD7</td>
<td>0.0315 ±0.0022</td>
</tr>
<tr>
<td>4aD8</td>
<td>0.02133 ±0.0016</td>
</tr>
<tr>
<td>4aD9</td>
<td>0.025 ±0.0022</td>
</tr>
<tr>
<td>4aD10</td>
<td>0.0289 ±0.0021</td>
</tr>
<tr>
<td>4aD11</td>
<td>0.03266 ±0.0021</td>
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<tr>
<td>4aD12</td>
<td>0.03288 ±0.0021</td>
</tr>
<tr>
<td>4aD13</td>
<td>0.03099 ±0.0016</td>
</tr>
<tr>
<td>4aD14</td>
<td>0.02976 ±0.0016</td>
</tr>
<tr>
<td>4aD15</td>
<td>0.0315 ±0.0021</td>
</tr>
</tbody>
</table>

% inhibition = \{1 - Vt/Vc\} \times 100

Where, Vt: - is edema volume in drug treated group.
Vc: - is edema volume in the control group

CONCLUSION

A series of chalcone derivatives having pyrazole and isoxazole nuclei were synthesized by various steps and characterized by FT-IR, H\textsuperscript{1} NMR and MASS spectral data. Synthesized compounds were evaluated for anti-inflammatory activity. For anti-inflammatory activity studies we have observed that derivatives having +I groups like -OCH\textsubscript{3}, -OH in phenyl ring have shown significant anti-inflammatory activity whereas compounds with -I groups like –
NO₂, -Cl were not active. Out of all synthesized compounds only one compound 4aD8 at a dose of 200mg/Kg has shown more anti-inflammatory activity than indomethacin at a dose of 20 mg/Kg. Other compounds like 4aD3, 4aD9 and 4aD14 showed significant anti-inflammatory activity. The mechanism of action of these derivatives for their anti-inflammatory activity might be through cyclooxygenase inhibition.

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REFERENCES


