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SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF NOVEL ARYLAZO PYRAZOLE DERIVATIVES

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Abstract
A new series of 3,5-Dimethyl arylazo pyrazole derivatives **(3a-h)** were synthesized by the condensation of oxobutyrates **(2a-h)** (obtained by the reaction of 1,3-diketone with diazonium salts in the presence of sodium acetate in alcohol medium) with 2,4-dinitrophenyl hydrazine **(1)** in glacial acetic acid medium. All the newly synthesized compounds were assigned by ^{1}H-NMR, IR, Mass spectral data. The new compounds were evaluated for their In-*Vitro* anti-inflammatory activity by bovine serum albumin and egg albumin methods using diclofenac sodium as standard. Some of the tested compounds showed good anti-inflammatory activity.

Introduction
Nitrogen heterocycles have been explored for the last one decade in developing pharmaceutically important molecules. Pyrazole ring has attracted so much attention by the medicinal chemist, since the discovery of Antipyrine as analgesic and antipyretic drug, and thereafter a very large number of Pyrazole derivatives have been explored for various biological potency. Pyrazoles are simple aromatic heterocyclic organic compound, found as a parental structure in a wide variety of compounds. The central core consist of a five membered heterocyclic organic compound with two neighboring nitrogen atoms.
Pyrazole and its derivatives were reported to possess anti-inflammatory[I], anti-tubercular[II], antifungal[II], antipyretic[IV], analgesic[V], anxiolytic[VI], antitumour[VII], antiviral[VIII], antidepressant[IX] activities etc.
The literature survey shows that incorporation of azo substitution for different pyrazole derivatives in enhancing biological activity[X-XI]. The compounds which are having azo or hydrazono groups exhibit a wide varieties of biological activities. The diazo substitution on various heterocyclic moieties will helps in increasing the various chemotherapeutic properties. Arylazo pyrazoles exhibited a wide range of pharmacological and pharmaceutical activities, thus playing a significant role in medicinal chemistry.

Inflammation diagnosis has received considerable attention in recent decades. Pyrazoles derivatives are well known for their excellent effectiveness as an anti inflammatory agent. Anti inflammatory agents works by reducing the production of prostaglandins. Pyrazole is present as the basic structure in celecoxib, it is highly selective reversible cyclooxygenase COX- 2 inhibitor, inhibits the transformation of arachidonic acid into precursors of prostaglandin. Therefore it has antipyretic, analgesic and anti-inflammatory actions[XII].

Based on the above observations, exhibited by the pyrazoles and azo groups, it was contemplated to synthesize a new series of 3,5-arylazo pyrazole derivatives followed by In-vitro anti-inflammatory activity. The title compounds were synthesized by reacting 2,4-dinirophenyl hydrazine[12] **(1)** with hydrazono derivatives [11]**(2a-h)** in glacial acetic acid medium **(3a-h)**. The reaction sequence is given in **Scheme-01.**

Materials and Methods

All the required chemicals used were of AR grade and were procured from Spectrochem, Mumbai and solvents were further purified by suitable methods. The open capillary tube method was employed for determining the melting points. Alpha bruker FT-IR-Spectrometer was used for recording IR spectra (cm^{-1}). Bruker Avance-II NMR spectrometer (USA) was employed to record ^{1}H-NMR spectra operating at 400 MHz with DMSO/$CDCl_3$ as a solvent where TMS served as an internal standard. The recording of the mass spectra was carried out by Perkin -Elmer GC-MS. Homogeneity of the compounds was ascertained by TLC on silica gel -G plates.

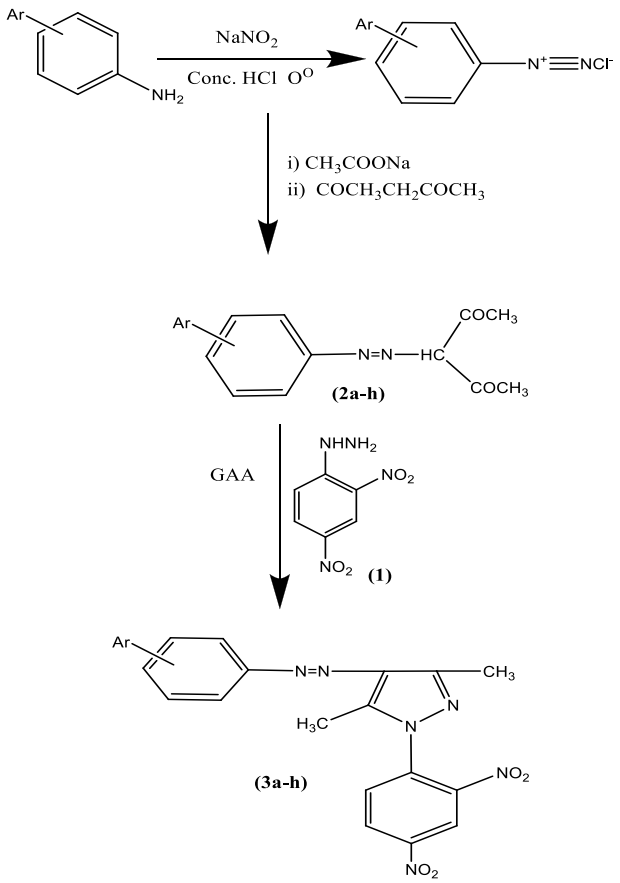
General procedure for synthesis of 3,5-dimethyl-arylazo pyrazoles (3a-h)

A mixture of hydrazono derivatives **(2a-h)** (0.01 mol) and 2-4-dinitrophenyl hydrazine **(1)** (0.01 mol) was dissolved in glacial acetic acid (30ml) and the contents were refluxed for 16-24 hrs. The reaction mixture is allowed to cool and poured into ice cold water with stirring. The solid which was formed is filtered, washed, dried and recrystallized from alcohol. The physical data of compounds **(3a-h)** is given in table-1**.**

Table 01: Physical data of compounds (3a-h)

Comp	Ar-NH_2	Molecular formula	Molecular weight	Melting Point (^OC)	Yield (%)
3a	4-Cl	$C_{17}H_{13}ClN_6O_4$	400.78	113-15	68
3b	4-F	$C_{17}H_{13}FN_6O_4$	384.10	123-25	69
3c	4-Br	$C_{17}H_{13}BrN_6O_4$	445.23	96-98	66
3d	4-NO_2	$C_{17}H_{13}N_7O_4$	411.33	136-38	72
3e	2,4-$(NO_2)_2$	$C_{17}H_{12}N_8O_8$	456.33	176-78	71
3f	2-NO_2-4-CH_3	$C_{18}H_{15}N_7O_6$	425.36	145-47	60
3g	3,4-$(Cl)_2$	$C_{17}H_{12}Cl_2N_6O_4$	435.22	201-03	62
3h	3-Cl-4-F	$C_{17}H_{13}ClFN_6O_4$	418.77	153-55	61

Scheme-01



4-((4-chlorophenyl)diazenyl)-1-(2,4-dinitrophenyl)-3,5-dimethyl-1H-pyrazole (3a): IR (KBr,cm^{-1}): 1393,1523 (NO_2), 1544 (C=N), 3099 (C-H). **^{1}H-NMR($CDCl_3$):** 2.50 (s,CH_3, 3H), 2.68 (s ,CH_3, 3H),), 7.55 - 8.31 (m, Ar-H, 8H). **MS (m/z):**400.78 (M+).

1-(2,4-dinitrophenyl)-4-((4-fluorophenyl)diazenyl)-3,5-dimethyl-1H-pyrazole (3b):IR (KBr,cm^{-1}): 1313,1511 (NO_2), 1618 (C=N), 3065 (C-H). **^{1}H-NMR(CDC_3):** 2.39 (s,CH_3, 3H), 2.50 (s ,CH_3, 3H),), 6.07-7.72 (m, Ar-H, 8H). **MS (m/z):**384.10(M+).

4-((4-bromophenyl)diazenyl)-1-(2,4-dinitrophenyl)-3,5-dimethyl-1H-pyrazole (3c): IR (KBr,cm^{-1}): 1332, 1500 (NO_2), 1607 (C=N), 2918 (C-H). **^{1}H-NMR($CDCL_3$):** 2.39 (s,CH_3, 3H), 2.50 (s ,CH_3, 3H),), 7.25 – 7.92 (m, Ar-H, 8H). **MS (m/z):**445.23 (M+).

1-(2,4-dinitrophenyl)-3,5-dimethyl-4-((4-nitrophenyl)diazenyl)-1H-pyrazole (3d): IR (KBr,cm^{-1}): 1336, 1549 (NO_2), 1611(C=N), 3027 (C-H). **^{1}H-NMR($CDCL_3$):** 2.42 (s,CH_3, 3H), 2.54(s ,CH_3, 3H),), 7.90-8.37 (m, Ar-H, 8H). **MS (m/z):**411.33 (M+).

1-(2,4-dinitrophenyl)-4-((2,4-dinitrophenyl)diazenyl)-3,5-dimethyl-1H-pyrazole (3e): IR (KBr,cm^{-1}): 1328, 1548(NO_2), 1611 (C=N), 3005 (C-H). **^{1}H-NMR($CDCL_3$):** 2.33 (s,CH_3, 3H), 2.51 (s ,CH_3, 3H),), 7.10 – 8.90 (m, Ar-H, 7H). **MS (m/z):**456 .33(M+).

1-(2,4-dinitrophenyl)-3,5-dimethyl-4-((4-methyl-2-nitrophenyl)diazenyl)-1H-pyrazole (3f) :IR (KBr,cm^{-1}): 1342, 1532 (NO_2), 1609(C=N), 3031(C-H). **^{1}H-NMR($CDCL_3$):** 2.48 (s,CH_3, 3H), 2.51 (s ,CH_3, 3H),), 7.47 – 7.81 (m, Ar-H, 7H). **MS (m/z):**425.36 (M+).

4-((3,4-dichlorophenyl)diazenyl)-1-(2,4-dinitrophenyl)-3,5-dimethyl-1H-pyrazole (3g): IR (KBr,cm^{-1}): 1346, 1533 (NO_2), 1608 (C=N), 3056 (C-H). **^{1}H-NMR($CDCL_3$):** 2.36 (s,CH_3, 3H), 2.50 (s ,CH_3, 3H),), 7.70 – 7.90 (m, Ar-H, 7H). **MS (m/z):**435.22 (M+).

4-((3-chloro-4-fluorophenyl)diazenyl)-1-(2,4-dinitrophenyl)-3,5-dimethyl-1H-pyrazole (3h):IR (KBr,cm^{-1}): 1347, 1527 (NO_2), 1609 (C=N), 3027 (C-H). **^{1}H-NMR($CDCL_3$):** 2.42 (s,CH_3, 3H), 2.66 (s ,CH_3, 3H),), 7.67 – 8.27 (m, Ar-H, 7H). **MS (m/z):**418.77 (M+).

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In vitro anti inflammatory activity

Bovine serum albumin denaturation assay[XIII]

0.05 ml various concentration (10, 20, 30, 40, 50 µg/ml) test compounds and standard drug were taken and mixed with 0.45 ml of 5% bovine serum albumin solution. The pH 6.3 was adjusted by using a small volume of 1N HCl. The samples were then incubated for 20 minutes at 37ºC and heated at 57ºC for 3 minutes. 2.5 ml of phosphate buffer was added to the above solutions, upon cooling. The absorbance was measured at 416 nm by using UV-Visible spectrophotometer. Diclofenac sodium was used as standard drug. The percentage inhibition of protein denaturation was calculated using the following formula. Each experiment was performed in triplicates. The IC_{50} values are tabulated in Table-2.

Percentage inhibition=(($Abs_{control}$ - Abs_{sample}) / $Abs_{control}$) x100

Egg albumin Denaturation assay[XIII]

The reaction mixture (5 ml) consists of 2 ml of different concentrations of test compounds (10,20,30,40, 50 µg/ml), .0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffer (pH 6.4) and a similar volume of distilled water was used as control. The reaction mixture was incubated for 15 minutes at 37± 2ºC and then heated for five minutes at 70ºC. After cooling, the absorbance was measured at 660 nm by using UV-Visible spectrophotometer. Diclofenac sodium was used as the standard. Percentage inhibitions were calculated by using the above mentioned formula. The IC_{50} values are tabulated in Table-2.

Table -2: Data of anti-inflammatory activity of compounds (3a-h)

Comp	**IC_{50} Values**	
	Bovine serum albumin denaturation assay	**Egg serum albumin denaturation assay**
Std (Diclofenac sodium)	22.57	21.84
3a	23.28	23.41
3b	22.10	33.90
3c	33.84	21.36
3d	23.37	28.11
3e	26.44	28.10
3f	27.61	21.44
3g	44.12	27.99
3h	25.05	30.25

Results and Discussion

In the present work, a novel series of 3,5-dimethyl arylazo pyrazole derivatives was synthesized and evaluated for their In-vitro anti-inflammatory activity. The required key intermediates Oxybutyrates **(2a-h**) were prepared by converting substituted anilines into diazonium salts, followed by alcoholic condensation with acetyl acetone as per the reported procedure.**(Scheme-01)**. The physical data of the synthesized compounds is showed in table-1. All the new compounds were checked for their purity by employing TLC and further by

recrystallization technique. All the new compounds were assigned on the basis of spectral data. The new compounds were also subjected for In-silico and molecular docking studies.

In-vitro- anti- inflammatory activity of the synthesized compounds were evaluated by two methods viz, Bovine serum albumin denaturation assay and Egg albumin denaturation assay, by using diclofenac sodium as standard drug. All the compounds were tested at a concentration of 10-50 µg/ml . All the reactions were carried out in triplicates. The IC_{50} values are tabulated in Table-2.

In the bovine albumin denaturation assay the results revealed that the compounds **3a, 3b, 3d,** showed potent activity and also compounds **3e, 3f, 3h** showed good activity when compared to the standard. Similarly compounds **3a, 3c, 3f** showed potent activity and also compounds 3d, 3g showed good activity in egg albumin denaturation assay. All the other tested compounds displayed moderate activity.

Conclusion

In conclusion a new series of aryl azo pyrazole derivatives have been synthesized by the reaction of oxybutyrates with 2,4-dinitro phenyl hydrazine in glacial acetic acid medium. In the *In-vitro* anti- inflammatory activity some of the tested compounds showed very potent activity when compared to the standard diclofenac sodium. Structural modifications of these compounds are required in order to get good pharmacological profile.

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