# DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL 1, 5 DISUBSTITUTED TETRAZOLE AS POTENTIAL ANTI-INFLAMMATORY AGENTS

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In attempt to find new pharmacologically active molecules, we report here the synthesis and in vitro anti-inflammatory activity of various 1-[5-(substituted phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole (IV a-h).The anti-inflammatory activity of title compounds were examined by denaturation of proteins method. All the compounds exhibited weak to potent anti-inflammatory activity. Some derivatives bearing a methoxy group exhibited very good anti-inflammatory activity.

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*Keywords:* Tetrazole , Pyrazole ,Anti-inflammatory activity

## 1. Introduction

The chemistry of heterocyclic compounds has been an interesting field of study of long time. The synthesis of novel tetrazole derivatives and investigation of their chemical and biological behavior has gained more importance in recent decades for biological and pharmaceutical reasons.1,2,3,4-tetrazole represent important class of heterocyclic compounds. Their derivatives possess broad spectrum of biological activity in both medicinal and pharmaceutical such as antibacterial[1-3], antifungal[4], antiviral [5-7] , analgesic[8-12], anti-inflammatory[13-16], antiulcer [17-19].Pyrazole nucleus has been gaining prominence due the fact that its derivatives have been to possess wide spectrum of pharmacological properties.Pyrazole derivatives are useful heterocycles possessing potent pharmacological activities such as antifungal,antibacterial,analgesic,anti-inflamatory,antitubercular and anticonvulsant activity [20-24].

The aim of present work was to attach the substituted pyrazole residue to 5-phenyl tetrazole in order to find new pharmacologically active molecule. Thus the synthesis of novel 1, 5 disubstituted tetrazole derivatives has been achieved and evaluated for their in-vitro anti-inflammatory activity.

## 2. Experimental protocol

Melting points were determined in open capillaries and were uncorrected. Reactions were monitored by thin layer chromatography using silica gel-G as adsorbent using benzene: ethyl acetate (9:1) as eluent. IR spectra (KBr pellets) were recorded on Shimadzu FT-IR model 8010 spectrophotometer. $^1$H NMR spectra (DMSO-d6) were taken a Varian mercury spectrometer (model YH- 300 FT NMR) using TMS as internal standard and chemical shift are expressed in δ ppm. Mass spectra were taken on Jeol sx-102/PA-6000 (EI) spectrometer.

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**Chemistry**

Compounds were prepared as shown in Fig. 1. The 5-substituted tetrazole can be synthesized by number of methods, viz.reaction of hydrazoic acid or its salts with imidoyl chloride or imino ethers or diazo coupling of heterocyclic hydrazine or hydrocyanic acid. Most of these methods have limited use in preparative organic chemistry because the use of hydrazoic acid presents considerable experimental difficulties due its toxicity and tendency to explode. However, the simple route reported by Mohite P.B.et al [7] was adopted for the preparation of 5-phenyl-1,2,3,4- tetrazoles **(I).** This route replaces the toxic hydrazoic acid by inorganic azide to afford the titled compounds in good yield (58-72%). Compound **I** was cyclized using sodium azide, ammonium chloride and benzonitrile. The 5-phenyl-1,2,3,4- tetrazoles on treatment with acetic anhydride forms 5-phenyl 1-acetyl tetrazole **(II)** which on reaction with different aromatic aldehydes forms chalcones **(III a-h)** .The chalcones further undergo cyclisation with phenyl hydrazine in presence of glacial acetic to form 1-[5-(substituted phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole **(IV a-h).**

**Steps in synthesis of compounds**

**General procedure for the synthesis of 5-Phenyl 1-Acetyl Tetrazole (II):**

To a solution of 5-phenyl tetrazole (12.8 g, 0.08 mol) and acetic anhydride (0.08 mol), 2-3 drops of concentrated sulphuric acid was added .The reaction mixture was warmed for 15-20 min. on water bath. Cooled and poured into ice cold water. The product obtained was filtered, dried .It was further purified by recrystallization using ethanol and was obtained in 75% yield as a white amorphous solid: m.p. 214-215 °C.

IR (KBr),v,$cm^{-1}$: 3054 (Ar-CH), 1735(C=O), 1285(N-N=N-),1108 and 1138(Tetrazole ring),1608(C=N),1575(-N=N-),1164,1072(-CN). $^1$H NMR (400.0MHz,DMSO-$d_6$) δ :8.80(s,1H,NH),7.08(6H,Ar-H).

**General procedure for the synthesis of compounds (III a-h):**

A solution of 5-phenyl 1-acetyl tetrazole (4g, 0.005 mol) and aromatic aldehydes (0.005 mol) in ethanol (12 ml) was cooled to 5 to 10$^o$C in an ice bath. The cooled solution was treated with drop wise addition of aqueous sodium hydroxide (2.5 ml, 50%). The reaction mixture was magnetically stirred for 30 min and then left over night. The resulting dark solution was diluted with ice water and carefully acidified using diluted hydrochloric acid. The product obtained were collected by filtration after washing with water. It was further purified by crystallization from ethanol. IR ( KBr),v,$cm^{-1}$:1285(N-N=N-),1108 and 1138(Tetrazole ring) ,1735(C=O), 1630(C=C), 3054(Ar-CH). $^1$H NMR (400.0MHz,DMSO-$d_6$) δ:6.61(1H,d,-CO-CH=),7.05(1H,d,=CH-Ar),7.14-7.80 (10H, m, Ar-H).

**General procedure for the synthesis of compound (IV a-h):**

A mixture of III a-f (0.001 mol), phenyl hydrazine (0.005 mol) and acetic acid (40 ml) was refluxed for 3 h. Then poured into ice cold water. The precipitate was separated by filtration, washed free of acid to afford 2-pyrazolines, dried and recrystalised from ethanol.

**IVa: 1-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)-5-phenyl-1H-tetrazole**

Yield:68%,m.p:125-126°C.IR(KBr),v,$cm^{-1}$:3054(Ar-H),1285(N-N=N-),1108 and 1138 (Tetrazole ring) ,2966 (CH str.) and 1610(C=N ring stretch).$^1$H NMR (400.0MHz,DMSO-$d_6$) δ: 6.90-8.20 (15H, m, Ar-H) , 4.80(1H, t, CH), 2.90-3.10 (2H, d,CH2). $^{13}$CNMR (100.0MHz,DMSO$d_6$) δ:163(C-6),161.10(C-3),145.15(C-23),142.20(C-17),130.10(C-16),129(C-18,19,20,21),128.90(C-14,15),128.50(C-26,27),128.40(C-12,13),127.30(C-22), 27(C-11),118(C-28),113.25(C-24,25),64.70(C-9),36.40(C-7).Anal.For $C_{22}H_{18}N_6$ ,cal (found)%: C ,72.11(72.04) ,H, 4.95(4.90),N ,22.94(22.90) MS:(m/z) : 366($M^+$).

**IVb:1-[5-(2-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole**

Yield: 65%,m.p:140-141°C.IR(KBr),v,$cm^{-1}$: 3050(Ar-H),1285(N-N=N-),1108 and 1138 (Tetrazole ring) ,2966(CH str.) and 1610(C=N ring stretch).$^1$H NMR (400.0MHz,DMSO-$d_6$) δ: 6.90-8.20

(15H, m, Ar-H) ,4.80(1H, t, CH), 2.90-3.10 (2H, d, CH2). $^{13}$CNMR (100.0MHz, DMSO$d_6$)δ:163(C-6),161.10(C-3),145.15(C-23),143.20(C-17),137.4(C-23), 133.10 (C-22), 131.10(C-16),129.90(C-20,21),128.50(C-14,15),128.40(C-26,27),128.30(C-12,13), 127.10 (C-11),118(C-28),113.25(C-24,25),64.70(C-9),36.40(C-7).Anal. For $C_{22}H_{17}ClN_6$ ,cal (found)%: C, 65.92(65.85) ,H ,4.27,(4.20), N 20.96(20.90) MS:(m/z) : 400($M^+$).

**IVc: 1-[5-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole**
Yield: 66 %,m.p:144-145 °C.IR(KBr),v,cm$^{-1}$: 3052(Ar-H),1285(N-N=N-),1108 and 1138 (Tetrazole ring) ,2966(CH str.) and 1610(C=N ring stretch).$^1$H NMR (400.0MHz,DMSO-$d_6$) δ: 6.90-8.20 (15H, m, Ar-H) , 4.80(1H, t, CH), 2.90-3.10 (2H, d, CH2). $^{13}$CNMR (100.0 MHz,DMSO$d_6$)δ:163(C-6),161.10(C-3),145.15(C-23),143.20(C-17), 137.4 (C-23),133.10(C-22),131.10(C-16),129.90(C-20,21),128.50(C-14,15),128.40(C-26,27),128.30(C-12,13),127.10 (C-11),118(C-28),113.25(C-24,25),64.70(C-9),36.40(C-7).Anal. For $C_{22}H_{17}ClN_6$ ,cal (found)%: C, 65.92(65.85) ,H ,4.27,(4.20), N 20.96(20.90) MS:(m/z) : 400($M^+$).

**IVd: 1-[5-(4-bromophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole**
Yield: 68%,m.p:148-150 °C.IR(KBr),v,cm$^{-1}$: 3052(Ar-H),1285(N-N=N-),1108 and 1138 (Tetrazole ring) ,2966(CH str.) and 1610(C=N ring stretch). $^1$H NMR (400.0MHz,DMSO-$d_6$) δ: 6.90-8.20 (15H, m, Ar-H) , 4.80(1H, t, CH), 2.90-3.10 (2H, d, CH2). $^{13}$CNMR (100.0MHz,DMSO$d_6$)δ:163(C-6),161.10(C-3),145.15(C-23),143.20(C-17),133.10(C-22), 131.10 (C-16),129.90(C-20,21),128.50(C-14,15),128.40(C-26,27),128.30(C-12,13),127.10(C-11), 122.15(C-22),118(C-28),113.25(C-24,25),64.70(C-9),36.40(C-7).Anal. For $C_{22}H_{17}BrN_6$ cal (found)%: C,59.34(59.25), H, 3.85(3.79), N, 18.87,(18.90) MS:(m/z) : 445($M^+$).

**IVe:1-[5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole**
Yield: 58%,m.p:161-162 °C.IR(KBr),v,cm$^{-1}$: 3050(Ar-H),1285(N-N=N-),1108 and 1138 (Tetrazole ring) ,2966(CH str.) and 1610(C=N ring stretch) .$^1$H NMR (400.0MHz,DMSO-$d_6$) δ: 6.90-8.20 (15H, m, Ar-H) , 4.80(1H, t, CH), 2.90-3.10 (2H, d, CH2).$^{13}$C NMR (100.0MHz,DMSO-$d_6$)δ:163(C-6),161.10(C-3),158.40(C-22),145.15(C-23),142.20(C-17), 130.10(C-16),128.90(C-14,15),128.50(C-26,27),128.40(C-12,13),127.30(C-18),127.10(C-19), 118(C-28),114.80(C-20,21),113.25(C-24,25),64.70(C-9),52.30(C-30), 36.40 (C-7).Anal. For $C_{24}H_{22}N_6$ cal (found)%: C ,73.07(73.01), H, 5.62(5.64), N, 21.30 (21.24), MS:(m/z) : 394($M^+$).

**IVf:1-[5-(3-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole**
Yield: 72%,m.p:150-151 °C.IR(KBr),v,cm$^{-1}$: 3053(Ar-H),1285(N-N=N-),1108 and 1138 (Tetrazole ring) ,2966(CH str.) and 1610(C=N ring stretch) .$^1$H NMR (400.0MHz,DMSO-$d_6$) δ: 6.90-8.20 (15H, m, Ar-H) , 4.80(1H, t, CH), 2.90-3.10 (2H, d, CH2). $^{13}$CNMR (100.0MHz,DMSO$d_6$)δ:163(C-6),161.10(C-3),148.50(C-20),145.15(C-23),141.20(C-17), 130.10 (C-16),129(C-21),128.90(C-14,15),128.50(C-26,27),128.40(C-12,13),127.30(C-19) 122.60(C-18), 122.10(C-22),118.25(C-28),113.25(C-24,25),64.70(C-9), 36.40 (C-7).Anal. For $C_{22}H_{17}N_7O_2$ cal (found)%: C, 64.23(64.20) ,H ,4.16(4.10), N ,23.83(23.80) MS:(m/z) : 411($M^+$).

**IVg:1-[5-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole**
Yield: 70%,m.p:152-153 °C.IR(KBr),v,cm$^{-1}$: 3053(Ar-H),1285(N-N=N-),1108 and 1138 (Tetrazole ring) ,2966(CH str.) and 1610(C=N ring stretch) .$^1$H NMR (400.0MHz,DMSO-$d_6$) δ: 6.90-8.20 (15H, m, Ar-H) , 4.80(1H, t, CH), 2.90-3.10 (2H, d, CH2). $^{13}$CNMR (100.0MHz,DMSO$d_6$)δ:163(C-6),161.10(C-3),148.50(C-22),145.15(C-23),141.20(C-17), 130.10 (C-16),129(C-21),128.90(C-14,15),128.50(C-26,27),128.40(C-12,13),127.30(C-18,19), 127.10 (C-11),122.10(C-20.21),118.25(C-28),113.25(C-24,25),64.70(C-9), 36.40 (C-7) .Anal. For $C_{22}H_{17}N_7O_2$ cal (found)%: C, 64.23(64.20) ,H ,4.16(4.10), N ,23.83(23.80) MS:(m/z) : 411($M^+$).

**IVh: 1-[5-(4-dimethylaminophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole**

Yield: 60%,m.p:155-156 °C.IR(KBr),v,$cm^{-1}$: 3050(Ar-H),1285(N-N=N-),1108 and 1138 (Tetrazole ring) ,2966(CH str.) and 1610(C=N ring stretch) .$^{1}$H NMR (400.0MHz,DMSO-$d_6$) δ: 6.90-8.20 (15H, m, Ar-H) , 4.80(1H, t, CH), 2.90-3.10 (2H, d, CH2). $^{13}$CNMR (100.0MHz,DMSO$d_6$)δ:163(C-9),161.10(C-3),148.20(C-22),145.15(C-23),142.20(C-17), 131.10 (C-16),129(C-14,15),128.90(C-26,27),128.50(C-12,13),128.40(C-18,19),127.10 (C-11), 118(C-28),113.25(C-24,25),112.45(C-20,21),64.70(C-9),41.50(C-30,31), 36.40 (C-7). Anal. For $C_{24}H_{23}N_7$ cal (found)%: C, 70.39(70.31), H, 5.66(5.61), N, 23.94 (23.90) MS:(m/z) : 409($M^+$).

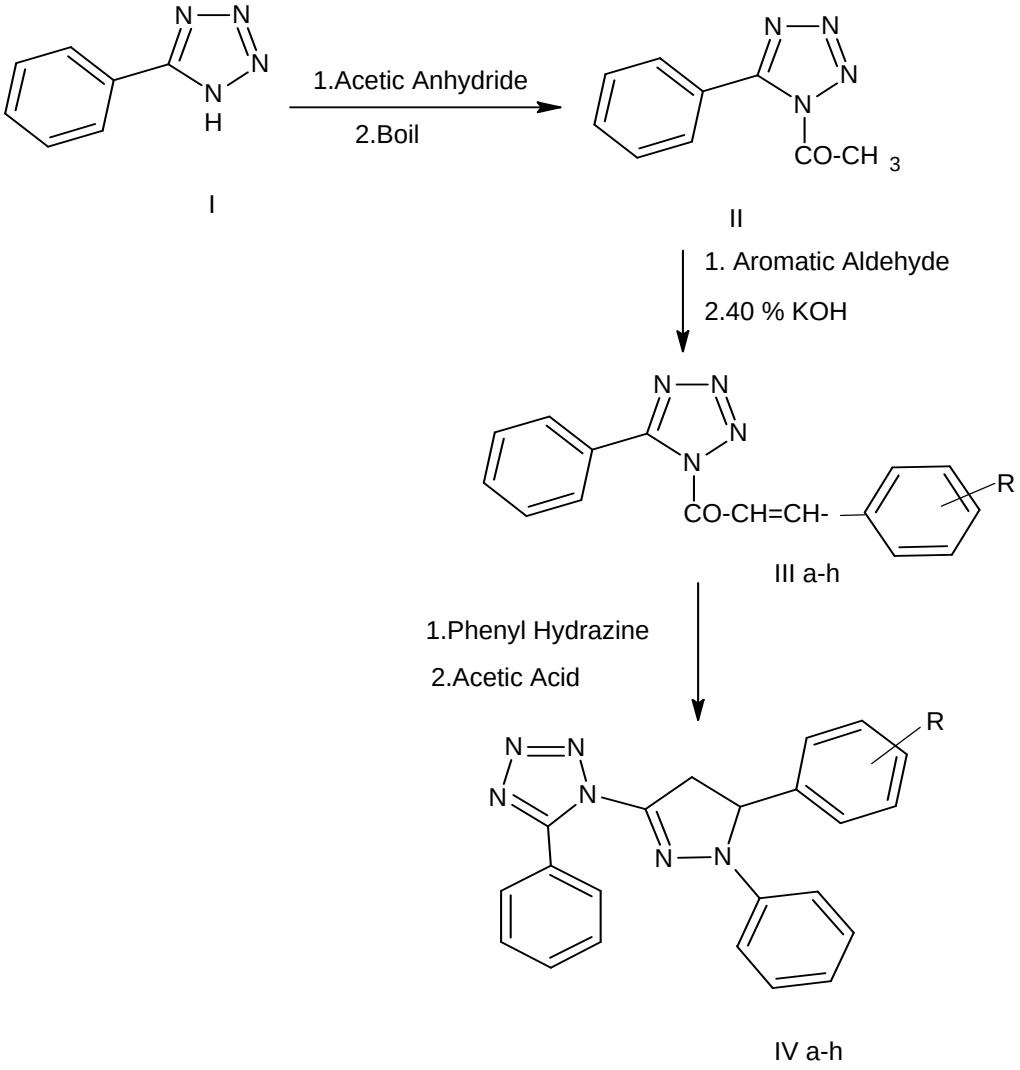


*Fig.1.Synthesis protocol of titled compounds*
*Where R= 2-Cl, 4-Cl ,4 –Br,,4-$OCH_3$, 3-$NO_2$, 4-$NO_2$,4-$N(CH_3)_2$.*

**In –Vitro Anti-inflammatory activity [25-32].**

Many in vitro assays, each based on a specific biochemical or cellular mechanism have been developed for the initial screening of the anti-inflammatory compounds. A number of anti-inflammatory drugs are known to inhibit the denaturation of proteins as an in vitro screening model for anti-inflammatory compounds.The synthesized compounds are screened for anti-inflammatory activity by using inhibition of albumin denaturation technique which was studied according to Muzushima and Kabayashi with slight modification.The standard drug ibuprofen and

test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.0%. Test solution (1 ml) containing different conc. of drugs was mixed with 1 ml of 1% mM albumin solution in phosphate buffer and incubated at $27^0 \pm 1^0$C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at $60^0 \pm 1^0$C water bath for 10 min. After cooling the turbidity was measured at 660 nm (UV-Visible Spectrophotometer Jasco V-630). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The ibuprofen was used as standard drug. The percent of inhibition was calculated in comparison with control data, Vt.

$$\% \text{ of inhibition} = 100 \times \frac{1}{Vc}$$

*Tab .1. In-vitro anti-inflammatory activity of compounds IVa-h.*

| Sr.No. | Name of compound | Absorbance at 660 nm (Mean±S.E.) | Inhibition of denaturation in( %) |
|---|---|---|---|
| 1 | Control | 0.120±0.003 | - |
| 2 | Ibuprofen | 0.226±0.005** | 91.66 |
| 3 | IVa | 0.205±0.006** | 70.83 |
| 4 | IVb | 0.185±0.002** | 54.16 |
| 5 | IVc | 0.201±0.002** | 67.75 |
| 6 | IVd | 0.198±0.005** | 65.00 |
| 7 | IVe | 0.207±0.010** | 72.50 |
| 8 | IVf | 0.164±0.004** | 36.66 |
| 9 | IVg | 0.155±0.003** | 29.16 |
| 10 | IVh | 0.145±0.005** | 20.83 |

** All the compounds tested at 0.2 mM concentration*

*** p<0.01 represent the significant difference when compared with control group.*

## 3. Results and discussion

The titled compounds were synthesized according to scheme 1.The structures of all synthesized compounds were confirmed by spectral data. Compound (I) was prepared by the reaction of benzonitrile with sodium azide in presence of ammonium chloride. 5-phenyl tetrazole (I) was converted to 5-phenyl,1-acetyl tetrazole (2) by reaction with acetic anhydride using catalytic amount of sulphuric acid. Compound III a-h was obtained by treatment of (II) with aromatic aldehydes in presence of 40% KOH. The IR spectra of compound III a-h shows absorption bands at 1735 due to (C=O str.) , 1630 due to (C=C str.) which is characteristics of chalcones. Compound III a-h on treatment with phenyl hydrazine in presence of acetic acid yielded compound IV a-h. The IR spectra of compounds IV a-h shows absorption bands at 3054 due to (Ar-H str.),1610 due to C=N ring stretch. Similarly absorption also occurs at 1285(N-N=N-), 1108 and 1138(Tetrazole ring).The $^1$H NMR spectra shows chemical shift at 6.90-8.20 due to aromatic protons, 4.80 (1H,s,CH of pyrazole), 2.90-3.10 (2H, d, CH2 of pyrazole).The results of spectral data are in good agreement with the structure of synthesized compounds.

***In vitro Anti-inflammatory activity***

The results of in vitro antiinflammatory activity are depicted in Tab.1.reveals that all compounds could inhibit the denaturation of albumin in comparison with control. Standard drug Ibuprofen exhibited 91.66% inhibition of albumin denaturation. The compounds IVa and IVe inhibit the denaturation of albumin in 70.83% and 72. 50% respectively when compared with

control possess potent anti-inflammatory activity. Other compounds like IVb, IVc, IVd inhibit the denaturation of proteins by 54.16%, 67. 75% and 65% respectively. It means these compounds possess good anti-inflammatory activity. The rest of compounds tested were found to possess weak anti-inflammatory activity.

## 4. Conclusions

1,5 disubstituted tetrazole derivatives were synthesized from 5-phenyl tetrazole in good yields.1,5 disubstituted tetrazole containing pyrazolyl derivatives at first position are found to possess good anti-inflammatory activity. The compound IVa and IVe with no substitution and methoxy substitution possess potent anti-inflammatory activity in comparison with control. The compounds IVb, IVc, IVd containing 2-Cl,4-Cl,4-Br substitution produces moderate anti-inflammatory activity. The compounds containing 3-$NO_2$, 4-dimethylamino substitution produces minimum anti-inflammatory activity.

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