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SYNTHESIS, CHARACTERIZATION AND ANTI-INFLAMMATORY ACTIVITY OF NOVEL N-SUBSTITUTED TETRAZOLES

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abstract: Benzonitrile and sodium azide in presence of ammonium chloride produces 5-phenyl tetrazole; this on reaction with acetic anhydride forms 5-phenyl 1-acetyl tetrazole (2), which reacted with different aromatic aldehydes in presence of alkaline medium, to yield corresponding chalcones (3a-4h). Chalcones on further reaction with isonicotinic acid hydrazide affords pyrazolines (4a-4h). The compounds were identified by spectral data and screened for in-vitro anti-inflammatory activity.

key words: Chalcones; Tetrazole; Pyrazole; anti-inflammatory activity.

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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 an important class of heterocyclic compounds. Tetrazole and their derivatives possess broad spectrum of biological activity in both medicinal and pharmaceutical, such as antimicrobial [1], antibacterial [2], antifungal [3], analgesic [4], anti-inflammatory [5], antinociceptive [6], antitubercular activity [7], and anticancer [8].

The pyrazole ring system is a five-membered heterocyclic ring structure composed of two nitrogen atoms and used in the synthesis of pharmaceuticals. The pyrazole moiety is a versatile lead molecule in pharmaceutical development and has a wide range of biological activities. In the past few years, the therapeutic interest of pyrazole derivatives in pharmaceutical and medicinal field has been given a great attention to the medicinal chemist. Literature survey reveals that pyrazole derivatives are well known to have antibacterial [9], antifungal [10], antitubercular [11], anticancer [12], analgesic, anti-inflammatory [13], anticonvulsant [14], antidepressant [15] and anti-arrhythmic [16] activities. In recent years, the extensive studies have been focused on pyrazole derivatives because of their diverse chemical reactivity, accessibility and wide range of biological activities.

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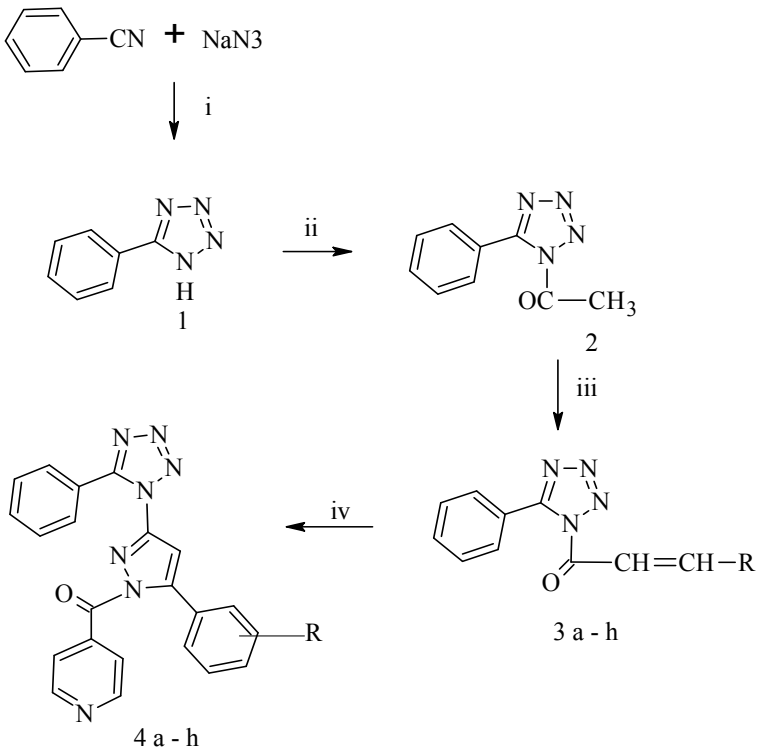
Literature survey reveals that till no tetrazole containing pyrazole has been evaluated for anti-inflammatory activity. The diverse properties of tetrazoles and pyrazoles have prompted us to synthesize them in order to study their anti-inflammatory activity which has not been reported yet. In continuation with our previous work, the present paper deals with the reaction of 5-phenyl tetrazole (1) with acetic anhydride to yield 5-phenyl 1-acetyl tetrazole (2), which on further reaction with different aromatic aldehydes forms chalcones (3a-3h) [1].

Chalcones on further reaction with isonicotinic acid hydrazide affords pyrazolines (4a-4h) (Scheme 1). The compounds were assigned on the basis of elemental analysis, IR and ^{1}H NMR spectral data. These compounds were screened for their in anti-inflammatory activity.



Scheme 1 *Synthesis of some N-substituted tetrazoles.*

***Reagents and conditions**:* **i**. DMF/ammonium chloride **ii.** acetic anhydride, 20 min.; **iii.** R-CHO, 50% KOH, ethanol; **iv**. Isonicotinic acid hydrazide/GAA, reflux 3h.

2. Experimental

Melting points were determined with open capillary and were uncorrected. FTIR spectra were recorded on a Shimadzu FT-IR model 8010 spectrophotometer, ^{1}H-NMR spectra were recorded in deuterated DMSO on a Varian mercury FT-NMR model YH- 300 instrument, using TMS as internal standard.

2.1. Synthesis of 5-phenyl tetrazole (1):

A mixture of benzonitrile (3.3 g, 0.10 mol), sodium azide (0.65 g, 0.10 mol) dimethylformamide (10 mL) and ammonium chloride (5.3 g, 0.10 mol) was heated in an oil bath for 7 h at 125 °C. The solvent was removed under reduced pressure. The residue was dissolved in 100 mL of water and carefully acidified with concentrated hydrochloric acid to pH 2. The solution was cooled to 5°C in ice bath. Compound 1 has been recrystallized from aqueous methanol.

2.2. Synthesis of 5-Phenyl 1-Acetyl Tetrazole (2):

A solution of 5-phenyl tetrazole (12.8g, 0.08 moles), acetic anhydride (0.08 moles) and 2-3 drops of concentrated sulphuric acid was heated for 15-20 min. on a water bath, then cooled and poured into ice cold water. The product separated was filtered and dried. It was further purified by crystallization from ethanol.

2.3.General procedure for the preparation of chalcones(3a-4h):[1]

A solution of 5-phenyl 1-acetyl tetrazole (8.5g, 0.005 moles) and aromatic aldehydes (0.005 mole) 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 potassium 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 chalcone which crystallized was collected by filtration and washed with aqueous sodium bicarbonate and water. It was further purified by crystallization from ethanol.

2.4. General procedure for synthesis of pyrazolines (4a-4h):

A mixture of 3 a-f (0.001 moles), isonicotinic acid hydrazide (0.005 moles) 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. The physical data of compounds 4(a-h) reported in Table 1.

2.5. Spectral data of compounds [IR (KBr), ν cm^{-1} and ^{1}H-NMR (DMSO), δ ppm]

4a:[5-phenyl-3-(5-phenyl-1H-tetrazol-1-yl)-4,5-dihydro-1H-pyrazol-1-yl](pyridin-4-yl) methanone. FT-IR:1280(N-N=N-),1108 and 1140(tetrazole), 1720(C=O), 1625(C=C), 3050(Ar-CH). ^{1}H-NMR: 2.3 (2H, s, CH2 pyrazole), 3.2(1H, s, CH pyrazole), 7.1(1H, d, =CH-Ar), 6.9-7.8 (14H, m, Ar-H).

4b:[5-(2-chlorophenyl)-3-(5-phenyl-1H-tetrazol-1-yl)-4,5-dihydro-1H-pyrazol-1-yl](pyridin-4-yl)methanone. FT-IR:1282(N-N=N),1110 and 1138(tetrazole), 1717(C=O), 1622(C=C), 3050(Ar-CH),785(C-Cl). ^{1}H-NMR: 2.35(2H,s, CH2, pyrazole),3.2(1H,s, CH pyrazole), 7.1 (1H, d,=CH-Ar), 6.9-7.8 (13H, m, Ar-H).

4c:[5-(4-chlorophenyl)-3-(5-phenyl-1H-tetrazol-1-yl)-4,5-dihydro-1H-pyrazol-1-yl](pyridin-4-yl)methanone. FT-IR:1278 (N-N=N-),1112 and 1136(tetrazole), 1718(C=O), 1622(C=C), 3045(Ar-CH), 780(C-Cl). ^{1}H-NMR: 2.35(2H,s,CH2, pyrazole), 3.2(1H,s,CH pyrazole), 7.1(1H,d,=CH-Ar), 6.9-7.8 (13H, m, Ar-H).

Table 1 Physical Data of Compounds.

[a]Comp. no.	R	Formula	MW	[b]Yield (%)	[c]mp ^{0}C	R[f].	Calcd (Found)%		
							C	H	N
4a	H	$C_{22}H_{17}N_70$	395	65	204	0.57	66.82 (66.78)	4.33 (4.30)	24.80 (24.78)
4b	2-Cl	$C_{22}H_{16}ClN_70$	429	62	180	0.68	61.47 (61.44)	3.75 (3.67)	22.81 (22.75)
4c	4-Cl	$C_{22}H_{16}ClN_70$	429	60	186	0.58	61.47 (61.44)	3.75 (3.67)	22.81 (22.76)
4d	4- Br	$C_{22}H_{16}BrN_70$	474	58	174	0.70	55.71 (55.68)	3.40 (3.36)	20.67 (20.65)
4e	4-OCH3	$C_{23}H_{19}N_70_2$	425	72	198	0.62	64.93 (64.90)	4.50 (4.45)	23.05 (23.00)
4f	4-NO2	$C_{23}H_{22}N_80_3$	440	74	154	0.61	60.00 (59.96)	3.66 (3.61)	25.44 (25.40)
4g	4-$(CH_3)_2$N-	$C_{24}H_{22}N_80$	438	70	184	0.57	65.74 (65.71)	5.06 (5.02)	25.55 (25.51)
4h	4-CH_3	$C_{23}H_{19}N_70$	409	58	160	0.65	67.47 (67.42)	4.68 (4.63)	23.95 (23.90)

[a] Products were analysed by IR, NMR and elemental analysis.
[b] synthesized yields
[c] uncorrected melting points

4d:[5-(4-bromophenyl)-3-(5-phenyl-1H-tetrazol-1-yl)-4,5-dihydro-1H-pyrazol-1-yl](pyridin-4-yl)methanone

FT-IR:1288(N-N=N-),1112 and 1145(tetrazole), 1725(C=O), 1628(C=C), 3058(Ar-CH), 658(C-Br), ^{1}H-NMR: 2.25(2H,s,CH2 pyrazole),3.2(1H,s,CH, pyrazole), 7.05(1H,d,=CH-Ar), 6.9-7.8 (13H, m, Ar-H).

4e:[5-(4-methoxyphenyl)-3-(5-phenyl-1H-tetrazol-1-yl)-4,5-dihydro-1H-pyrazol-1-yl](pyridin-4-yl)methanone. FT-IR: 1275(N-N=N-),1118 and 1144(tetrazole), 1722(C=O), 1626(C=C), 3048(Ar-CH), 1245(-OCH3) ^{1}H-NMR: 2.30(2H, s, CH2 pyrazole), 3.2(1H, s, CH pyrazole), 7.05(1H, d, =CH-Ar), 6.9-7.8 (13H, m, Ar-H).

4f:[5-(4-nitrophenyl)-3-(5-phenyl-1H-tetrazol-1-yl)-4,5-dihydro-1H-pyrazol-1-yl](pyridin-4-yl)methanone. FT-IR: 1278(N-N=N-),1108 and 1134(tetrazole), 1722(C=O), 1625(C=C), 3040(Ar-CH), 1560(-NO2). ^{1}H-NMR: 2.35(2H, s, CH2 pyrazole), 3.2(1H, s, CH pyrazole), 7.05(1H,d,=CH-Ar), 6.9-7.8 (13H, m, Ar-H).

4g:[5-(4-dimethylaminophenyl)-3-(5-phenyl-1H-tetrazol-1-yl)-4,5-dihydro-1H-pyrazol-1-yl](pyridin-4-yl)methanone. FT-IR: 1288(N-N=N-),1112 and 1132(tetrazole), 1722(C=O), 1628(C=C), 3050(Ar-CH), 1331(-N(CH3)2. ^{1}H-NMR: 2.35(2H, s, CH2 pyrazole), 3.2(1H, s, CH pyrazole), 7.05 (1H, d,=CH-Ar), 6.9-7.8 (13H, m, Ar-H).

4h:[5-(4-methylphenyl)-3-(5-phenyl-1H-tetrazol-1-yl)-4,5-dihydro-1H-pyrazol-1-yl](pyridin-4-yl)methanone. FT-IR: 1278(N-N=N-),1110 and 1134 (Tetrazole), 1718(C=O), 1622(C=Cstr.), 3055(Ar-CH), 1355(CH3). ^{1}H-NMR: 2.25(2H, s, CH2 pyrazole), 3.2(1H, s, CH pyrazole), 7.05(1H,d,=CH-Ar), 6.9-7.8 (13H, m, Ar-H).

2.6. In vitro anti-inflammatory activity:[5,17-19]

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 dimethylformamide (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 0.2 mM 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. Results are tabulated in Table 2.

$$\%\,\text{of inhibition} = 100 \times \left(\frac{V_t}{V_c}\right) - 1$$

Where V_t = Absorbance of test compounds

V_c = Absorbance of control

3. Results and Discussions

The titled compounds were synthesized according to Scheme 1.The structures of all synthesized compounds were confirmed by spectral data. Compound (1) was prepared by the reaction of benzonitrile with sodium azide in presence of ammonium chloride. 5-phenyl tetrazole (1) was converted to 5-phenyl-1-acetyl tetrazole (2) by reaction with acetic anhydride using catalytic amount of sulphuric acid. Compound 3a-3h was obtained by treatment of (2) with aromatic aldehydes in presence of 40% KOH. The IR spectra of compound 3a-h shows absorption bands at 1735 due to (C=O) , 1630 due to (C=C) which is characteristics of chalcones. Compound (3a-3h) on treatment with isonicotinic acid hydrazide in presence of acetic acid yielded compound (4a-4h) respectively. The IR spectra of compounds IV a-h shows absorption bands at 3050 due to (Ar-H), 1625 due to C=N ring stretch. Similarly absorption also occurs at 1280(N-N=N-), 1108 and 1140 (tetrazole ring). The ^{1}H-NMR spectra shows chemical shift at 6.9-7.8 due to aromatic protons, 3.2(1H, s, CH of pyrazole), 2.3(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 anti-inflammatory activity are depicted in Tab. 2 and Fig. 1, revealing that all compounds could inhibit the denaturation of albumin in comparison with control. Standard drug Ibuprofen exhibited 90.00% inhibition of albumin denaturation. The

compounds 4a and 4e inhibit the denaturation of albumin in 68.33% and 70.00% respectively when compared with control possess potent anti-inflammatory activity. Other compounds like 4b, 4c, 4d inhibit the denaturation of proteins by 54.16%, 65.00% and 62.50% respectively. It means these compounds possess good anti-inflammatory activity. The compound 4b with 2-Cl substitution shows 50.83%. The rest of compounds tested were found to possess weak anti-inflammatory activity.

Table 2 *In-vitro* anti-inflammatory activity of pyrazoles.

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**	90.00
3	4a	0.202±0.006**	68.33
4	4b	0.181±0.002**	50.83
5	4c	0.198±0.002**	65.00
6	4d	0.195±0.005**	62.50
7	4e	0.207±0.010**	70.00
8	4f	0.164±0.004**	40.00
9	4g	0.158±0.003**	31.66
10	4h	0.150±0.005**	25.00

* All the compounds tested at 0.2 mM concentration
** $p<0.01$ represent the significant difference when compared with control group.

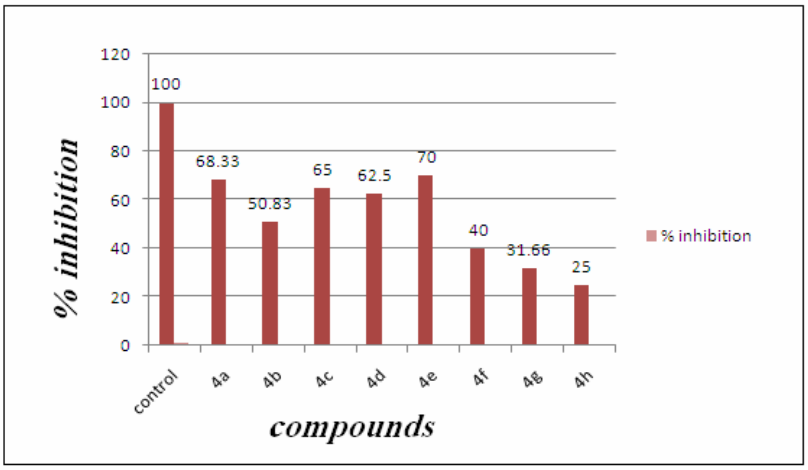


Fig. 1 *Percentage anti-inflammatory activity of compounds in comparison with control*
** Percentage are avg. of three determinations*

4. Conclusions

Tetrazole derivatives were synthesized from 5-phenyl-tetrazole which was synthesized from benzonitrile and sodium azide in good yields.1,5-disubstituted tetrazole containing

substituted pyrazolyl derivatives at first position by simple, rapid and high yield synthetic route and are found to possess good anti-inflammatory activity by denaturation of proteins mechanism. The compound 4a and 4e with no substitution and 4-methoxy substitution possess potent anti-inflammatory activity in comparison with control. The compounds 4c, 4d containing 4-Cl, 4-Br substitution produces moderate anti-inflammatory activity. The compounds containing 4-NO_2, 4-dimethylamino substitution produces weak anti-inflammatory activity.

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