

## SYNTHESIS ANALGESIC, ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITIES OF SOME 1-[5-(SUBSTITUTED PHENYL)-4,5-DIHYDRO-1H-PYRAZOL-3-YL]-5-PHENYL-1H-TETRAZOLE

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Eight different derivatives of substituted 5-phenyl-1-(5-substituted phenyl) -4,5-dihydro-1H-pyrazol-3-yl)-1H-tetrazole (4a-h) were synthesized by reacting the chalcones with hydrazine hydrate in presence of glacial acetic. The chemical structures were confirmed by means of FT-IR, <sup>1</sup>H-NMR, mass spectra and elemental analysis. The compounds were screened for analgesic activity by acetic acid induced writhing method and hot plate method, antiinflammatory and antimicrobial activities. The observed increase in analgesic, anti-inflammatory and antimicrobial activities are attributed to the presence of 4-NO<sub>2</sub>, 4-OH and 4-Cl in phenyl ring at 5-position of pyrazoline ring of synthesized compounds. In some cases their activities are equal or more potent than the standard drugs.

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### 1. Introduction

Tetrazoles are an important functionality with wide- ranging applications in photography and information recording systems, pharmaceutical and material sciences and appealing ligands in coordination chemistry. All aspects of the chemistry of tetrazoles as well as medicinal application of tetrazoles were covered in the literature. The most direct method to form tetrazoles is via the formal [3 + 2] cycloaddition of azides and nitriles. Biological activity in tetrazoles is encountered due to the special metabolism of the disubstituted tetrazole system and also because in 5-substituted tetrazole compounds the tetrazole ring is isosteric with a carboxylic acid group and of comparable acidity. Hence for all biologically active molecules possessing a carboxylic group (CO<sub>2</sub>H), there is a theoretical nitrogen analogue possessing a tetrazolic group (CN<sub>4</sub>H) and since tetrazole moiety appears to be the metabolically more stable of the two a considerable exploration of these molecules is ongoing. 5-Substituted 1,2,3,4-tetrazoles are reported to possess antibacterial<sup>1-3</sup>, antifungal<sup>4</sup>, antiviral<sup>5-7</sup>, analgesic<sup>8-12</sup>, anti-inflammatory<sup>13-16</sup>, antiulcer<sup>17-19</sup> and antihypertensive<sup>20,21</sup> activities. Similarly 1,5 substituted tetrazoles have long been known for their pharmaceutical activity as stimulants or depressants on the central nervous system and are reported to show oral antidiabetic and antithrombotic and antimicrobial properties<sup>22</sup>.

Pyrazole chemically known as 1,2-diazole has become a popular topic due to its manifold uses. The chemistry of pyrazolone and its derivatives is particularly interesting because of their potential application in medicinal chemistry as analgesic<sup>23-24</sup>, anti-inflammatory<sup>24</sup>, antiparasitic<sup>25</sup>, and enzyme inhibitory agents<sup>26-27</sup>. The useful properties of pyrazole derivatives as analgesic, anti-inflammatory drew attention of many investigators. Earlier experiments showed that pyrazole derivatives can be used as blood platelet aggregation inhibitor. That is pyrazole and its derivatives

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are clearly important in the field of enzyme inhibition. showed that brominated derivatives are strikingly cytotoxic and possess more bioactivity

Encouraged by the intense recent research activity in the tetrazole field and in pursuit of our continuing interest in pyrazole and tetrazole chemistry, we envisioned the combination of these attractive functional groups by the synthesis of various 1-[5-(substituted phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-5-phenyl-1*H*-tetrazole and screened for their analgesic, anti-inflammatory and antimicrobial activity.

## 2. Experimental protocol

### Instrumentation

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. <sup>1</sup>H NMR spectra (DMSO-d<sub>6</sub>) 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.

### Chemistry

Compounds were prepared as shown in Fig. 1. The 5-substituted tetrazoles 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 Finnegan et al was adopted for the preparation of 5-phenyl-1,2,3,4-tetrazoles (**1**). This route replaces the toxic hydrazoic acid by inorganic azide to afford the titled compounds in good yield (62-78%). Compound **1** 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 (**2**) which on reaction with different aromatic aldehydes forms Chalcones (3a-h). The Chalcones further undergo cyclisation with hydrazine hydrate in presence of glacial acetic to form 5-phenyl-1-(5-(substituted phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-1*H*-tetrazole (4a-h).

#### General procedure for the synthesis of 5-Phenyl 1-Acetyl Tetrazole (**2**)

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

IR: 3445 (NH), 3054 (Ar-CH), 1608 (C=N), 1575 (-N=N-), 1569 (NH def.), 1164, 1072 (-CN)  
<sup>1</sup>H-NMR (DMSO, d) 8.80 (s, 1H, NH), 7.08 (6H, Ar-H).

#### General procedure for the synthesis of 3-phenyl -1-(5-substituted phenyl)-1*H*-tetrazol-1-yl) prop-2-en-1-one (**3a-h**)

A solution of 5-phenyl 1-acetyl tetrazole (4g, 0.005 moles) and aromatic aldehydes (0.005 mole) in ethanol (12 ml) was cooled to 5 to 10.°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 tetrazole analogues of chalcone which crystallized were collected by filtration after washing with sodium bicarbonate and water. It was further purified by crystallization from ethanol. FT-IR: 1285 (N=N=N-), 1108 and

1138(Tetrazole ring), 1735(C=O), 1630(C=C), 3054(Ar-CH). <sup>1</sup>H NMR (DMSO) 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 1-[5-(substituted phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole (4a)**

A mixture of chalcone (10.0 mmoles), hydrazine hydrate (50.0 mmoles) or phenylhydrazine (50.0 mmoles) and acetic acid (40 ml) was refluxed for 3 hours then poured into water. The precipitate was separated by filtration, washed free of acid and crystallized from methanol to afford 2-pyrazolines, dried and recrystallized from ethanol.

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

Yield 68% as a white amorphous solid: m.p. 125-126 °C. FT-IR: 3054(Ar-H), 1285(N-N=N-), 1108 and 1138(Tetrazole ring), 2966(CH str.) and 1610(C=N ring stretch), 3340(NH str.) <sup>1</sup>H NMR (DMSO) δ: 7.14-7.80 (10H, m, Ar-H), 2.20-2.25 (1H, t, CH), 7.43 (1H, d, N-H), 4.71-4.86 (2H, d, CH<sub>2</sub>). Anal. For C<sub>16</sub>H<sub>14</sub>N<sub>6</sub> cal (found)%: C 66.19(66.25) H 4.86(4.90) N 28.95(28.90) MS:(m/z) : 290(M<sup>+</sup>).

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

Yield 72% as a white amorphous solid: m.p. 148-150 °C. FT-IR: 3054(Ar-H), 1285(N-N=N-), 1108 and 1138(Tetrazole ring), 2958(CH str.) and 1606(C=N ring stretch), 3340(NH str.), 788(C-Cl). <sup>1</sup>H NMR (DMSO) δ: 7.14-7.80 (9H, m, Ar-H), 2.20-2.25 (1H, t, CH), 7.43 (1H, d, N-H), 4.71-4.86 (2H, d, CH<sub>2</sub>). Anal. For C<sub>16</sub>H<sub>13</sub>ClN<sub>6</sub> cal (found)%: C 59.17(59.23) H 4.03(4.09) N 25.88(25.90) MS:(m/z) : 324(M<sup>+</sup>).

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

Yield 73 % as a white amorphous solid: m.p. 145-146 °C. FT-IR: 3054(Ar-H), 1285(N-N=N-), 1108 and 1138(Tetrazole ring), 2958(CH str.) and 1606(C=N ring stretch), 3340(NH str.), 788(C-Cl). <sup>1</sup>H NMR (DMSO) δ: 7.14-7.80 (9H, m, Ar-H), 2.20-2.25 (1H, t, CH), 7.43 (1H, d, N-H), 4.71-4.86 (2H, d, CH<sub>2</sub>). Anal. For C<sub>16</sub>H<sub>13</sub>ClN<sub>6</sub> cal (found)%: C 59.17(59.23) H 4.03(4.09) N 25.88(25.90) MS:(m/z) : 324(M<sup>+</sup>).

**1-[5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole (4d)**

Yield 65% as a brown amorphous solid: m.p. 150-151 °C. FT-IR: 3054(Ar-H), 1285(N-N=N-), 1108 and 1138(Tetrazole ring), 2955(CH str.) and 1605(C=N ring stretch), 3345(NH str.), 674(C-Br). <sup>1</sup>H NMR (DMSO) δ: 7.14-7.80 (9H, m, Ar-H), 2.20-2.25 (1H, t, CH), 7.43 (1H, d, N-H), 4.71-4.86 (2H, d, CH<sub>2</sub>). Anal. For C<sub>16</sub>H<sub>13</sub>BrN<sub>6</sub> cal (found)%: C 52.05(52.00) H 3.55(3.58) N 22.76(22.75) MS:(m/z) : 290(M<sup>+</sup>).

**1-[5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole (4e)**

Yield 78% as a reddish brown solid: m.p. 130-132 °C. FT-IR: 3054(Ar-H), 1285(N-N=N-), 1108 and 1138(Tetrazole ring), 2968(CH str.) and 1612(C=N ring stretch), 3336(NH str.), 1251(-OCH<sub>3</sub>) 7.14-7.80 (9H, m, Ar-H), 2.20-2.25 (1H, t, CH), 7.43 (1H, d, N-H), 4.71-4.86 (2H, d, CH<sub>2</sub>), 2.37(-OCH<sub>3</sub>). Anal. For C<sub>17</sub>H<sub>16</sub>N<sub>6</sub>O cal (found)%: C 63.74(63.70) H 5.03(5.08) N 26.23(26.20) MS:(m/z) : 320(M<sup>+</sup>).

**1-[5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole (4f)**

Yield 62% as a yellowish amorphous solid: m.p. 162-163 °C. FT-IR: 3054(Ar-H), 1285(N-N=N-), 1108 and 1138(Tetrazole ring), 2958(CH str.) and 1611(C=N ring stretch), 3342(NH str.), 3412(-OH). <sup>1</sup>H NMR: 7.14-7.80 (9H, m, Ar-H), 2.20-2.25 (1H, t, CH), 7.43 (1H, d, N-H), 4.71-4.86 (2H, d, CH<sub>2</sub>) 5.35 (1H, s, Ar-OH)/ Anal. For C<sub>16</sub>H<sub>14</sub>NO cal (found)%: C 57.31(57.36) H 3.91(3.90) N 29.24(29.20) MS:(m/z) : 335(M<sup>+</sup>).

**1-[5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole (4g)**

Yield 64% as a reddish amorphous solid: m.p. 169-170 °C. FT-IR: 3054(Ar-H), 1285(N-N=N-), 1108 and 1138(Tetrazole ring), 2958(CH str.) and 1612(C=N ring stretch), 3345(NH str.), 1576(-

NO<sub>2</sub>). <sup>1</sup>H NMR: 7.14-7.80 (9H, m, Ar-H), 2.20-2.25 (1H, t, CH), 7.43 (1H, d, N-H), 4.71- 4.86 (2H, d, CH<sub>2</sub>). Anal. For C<sub>16</sub>H<sub>13</sub>N<sub>7</sub>O<sub>2</sub>. cal (found)%: C 57.31(57.36) H 3.91(3.90) N 29.24(29.20) MS:(m/z) : 335(M<sup>+</sup>).

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

Yield 67% as a reddish brown amorphous solid: m.p. 140-141 °C. FT-IR:., 3054(Ar-H), 1285(N-N=N-), 1108 and 1138(Tetrazole ring), 2958(CH str.) and 1610(C=N ring stretch), 3336(NH str.), 1365 (CH<sub>3</sub>). <sup>1</sup>H NMR: 7.14-7.80 (9H, m, Ar-H), 2.20-2.25 (1H, t, CH), 7.43 (1H, d, N-H), 4.71-4.86 (2H, d, CH<sub>2</sub>), 3.72(6H,CH<sub>3</sub>). Anal. For C<sub>18</sub>H<sub>19</sub>N<sub>7</sub> cal (found)%: C 64.85(64.90), H 5.74(5.78) N 29.41(29.38) MS:(m/z) : 333(M<sup>+</sup>).

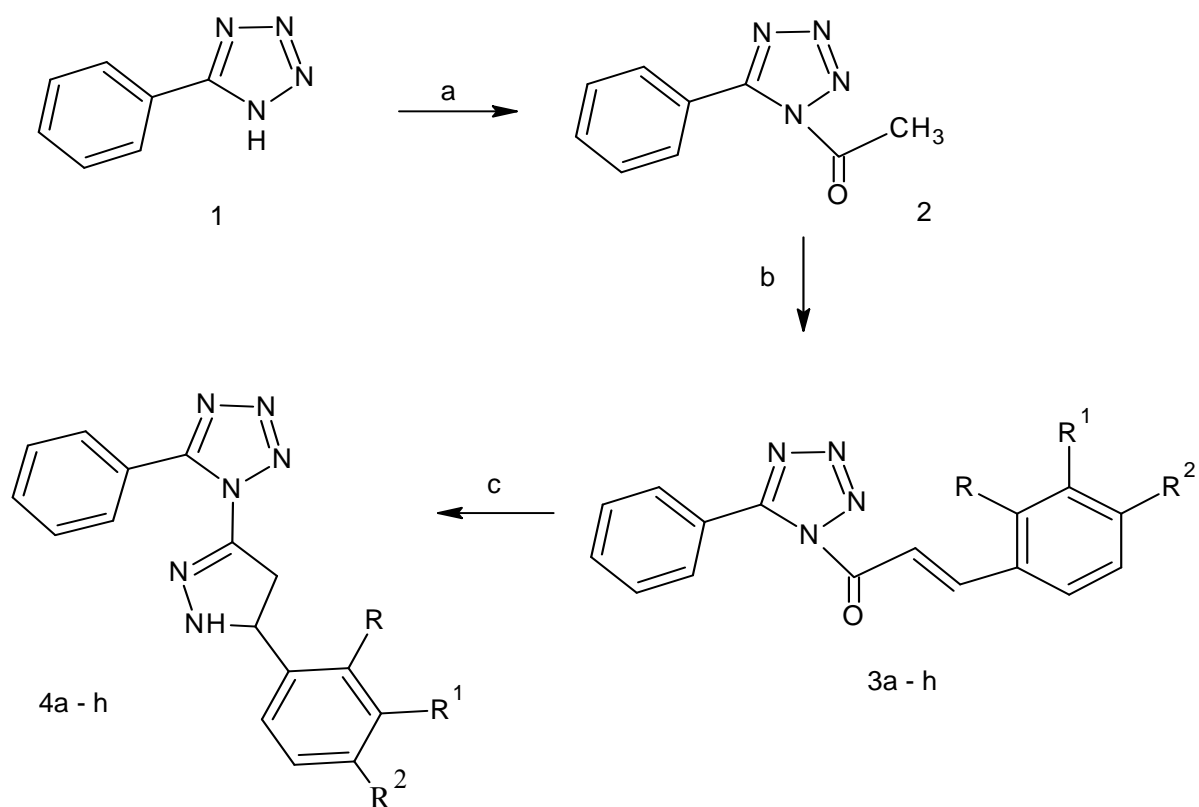


Figure 1 :Synthesis of titled compounds a. (CH<sub>3</sub>CO)<sub>2</sub>O/GAA, b. R-CHO/NaOH, c.NH<sub>2</sub>NH<sub>2</sub>.H<sub>2</sub>O

Comp.	R	R <sup>1</sup>	R <sup>2</sup>
4a	H	H	H
4b	H	Cl	H
4c	H	H	Cl
4d	H	H	Br
4e	H	H	OCH <sub>3</sub>
4f	H	H	OH
4g	H	H	NO <sub>2</sub>
4h	H	H	N(CH <sub>3</sub> ) <sub>2</sub>

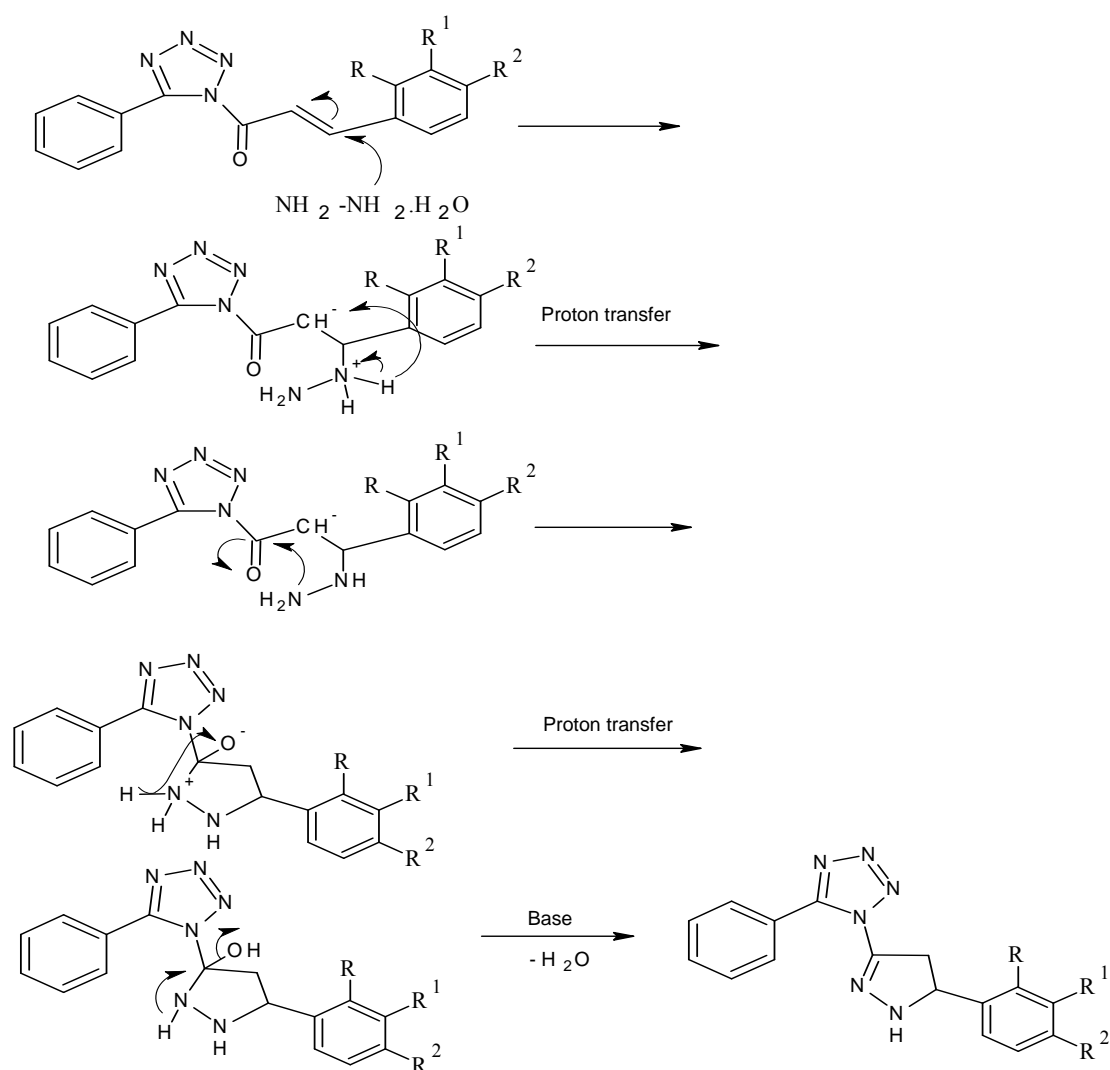


Fig. 2. Mechanism Of Synthesis Of Titled Compounds

### Biological Evaluation:

#### Evaluation of analgesic activity

Swiss strain albino mice of either sex weighing 25–30 g were used for this study. The test compounds (in 1/10th dose of the average LD<sub>50</sub> values of titled compounds) were administered intraperitoneally in 10% v/v Tween 80 suspensions. LD<sub>50</sub> of the newly synthesized compounds were determined by Miller and Tainter method administering the compounds intraperitoneally.

#### Behavioral test

Minimum motor impairment was measured in mouse by Rota rod test showed that all synthesized compounds have no effect which was indicated by their ability to maintain the equilibrium on the Rota rod for more than 1 min.

#### Acetic acid induced writhing method

The method suggested by Witkin et al. was adopted for the study. The animals were divided into 14 groups of mice each. The control group of animals was administered with 10% v/v Tween 80 (0.5 ml) suspension. The standard drug ibuprofen, was administered intraperitoneally in a dose of 10 mg kg<sup>-1</sup> to other group of animals. After 20 min of the administration the test compounds, all the groups of mice were given with the writhing agent 3% v/v aqueous acetic acid

in a dose of 2 ml kg<sup>-1</sup> intraperitoneally. The writhing produced in these animals were counted visually for 15 min and the numbers of writhings produced in treated groups were compared with those in the control group. The results are analyzed statistically by Student's *t*-test and recorded in Table 1.

### Hot plate method

The method of Eddy and Leimbach was adopted for the study. The pain threshold of the animals was measured on a hot plate before treatment of the test and reference compounds, and the animals that showed more than 10 s of reaction time were rejected. The reference compound Pentazocine was administered intraperitoneally in a dose of 5 mg kg<sup>-1</sup>. After the treatment of test and reference compounds, the pain threshold of the animals is presented in Table 2.

Table 1 Evaluation of analgesic activity by acetic acid induced writhing method

Compound	Dose mg kg <sup>-1</sup>	Writhing episodes in 15 min. (Mean ±SEM)	Percent protection
Control	-	52.83±0.00	-
Ibuprofen	2.5	09.17±0.83**	82
4a	25	28.83±0.81**	50
4b	25	27.50±0.88**	47
4c	25	14.17±0.79**	73
4d	25	34.17±0.94**	36
4e	25	18.83±0.81**	65
4f	25	20.67±0.86**	61
4g	25	35.42±0.96**	32
4h	25	27.55±0.89**	48

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

Table 2 Evaluation of analgesic activity by hot plate method

Compound	Before treatment	Reaction time in sec. after treatment		
		15	30	60
Control	4.95	4.95	4.95	4.95
Pentazocine	4.90±0.40**	7.45±0.32**	9.12±0.21**	10.22±0.35**
4a	5.41±0.20**	7.25±0.84**	8.95±0.42**	9.45±0.22**
4b	6.55±0.28**	6.75±0.42**	8.45±0.84**	10.18±0.49**
4c	6.25±0.35**	6.84±0.54**	7.65±0.72**	11.95±0.54**
4d	5.84±0.21**	10.11±0.75**	10.45±0.69**	9.25±0.45**
4e	5.63±0.42**	5.86±0.35**	7.27±0.40**	10.84±0.12**
4f	6.15±0.35**	6.75±0.40**	8.68±0.38**	11.12±0.18**
4g	6.28±0.36**	6.82±0.48**	9.25±0.39**	9.98±0.52**
4h	5.35±0.48**	6.55±0.49**	8.48±0.22**	9.15±0.31**

Dose 25 mg/kg<sup>-1</sup> for all test compound and comparison with control

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

**Evaluation of Anti-inflammatory Activity:** A number of agents caused marked reduction of the carrageenan induced edema of the rat foot, however, with exception of compounds 2f (R =p-Nitro phenyl). In this test also only analogs with a p-Methoxy phenyl group in R (2d) showed equal to that exhibited by the standard paracetamol. Compounds 2f, in addition to being the most potent

agents of this series against rat-foot inflammation, were also found to be among the most active analgesic when assayed in Glassman's analgesic model. The results of which are summarized in Table 3.

Table 3: Anti-inflammatory activity (carrageenan induced rat paw oedema method) of Compounds 4a-h.

Comp.	Dose mg/kg	Percentage inhibition			
		30 min	1 hour	2 hour	3 hour
4a	100	26± 0.10 *	32± 0.62 *	39± 0.10 *	33± 0.07 *
4b	100	28± 0.19 *	37± 0.17 *	43± 0.78 *	36± 0.17 *
4c	100	27± 0.41 **	33± 0.81 *	38± 0.67 *	29± 0.24 *
4d	100	26± 0.40 *	32± 0.36**	35± 0.96 **	27± 0.66 *
4e	100	28± 0.27 **	35± 0.49 **	41± 0.11 *	32± 0.53 *
4f	100	29± 0.78 **	33± 0.27**	34±0.42 **	41± 0.62 **
4g	100	28± 0.27 **	35± 0.49 **	42± 0.11 *	32± 0.53 *
4h	100	27± 0.27 **	35± 0.49 **	40± 0.11 *	32± 0.53 *
Control	-	5.11± 0.28	6.13± 0.26	5.68± 0.36	3.30± 0.91
Paracetamol	100	26± 0.29 **	30± 0.22 **	34± 0.91 **	28± 0.62

Results are expressed in mean ± SEM. (n=6) significance levels \*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$  as compared with the respective control.

#### Evaluation of Antimicrobial Activity:

The in-vitro antimicrobial activity of compounds (4a-h) were determined by agar cup plate method, The results of which are summarized in Table 4.

Table 4-Antibacterial and Antifungal data of compound (4a-h)

Compound	Zone of inhibition in mm							
	S.aureus		E. coli		C. albicans		A.niger	
	50 ug	100ug	50ug	100ug	50ug	100ug	50 ug	100ug
4a	13	15	10	12	12	15	10	12
4b	15	16	15	17	18	20	15	17
4c	15	16	14	15	18	20	13	15
4d	11	14	10	12	16	18	11	13
4e	12	17	08	10	19	22	20	22
4f	12	15	08	11	12	15	11	15
4g	13	15	10	11	13	15	10	12
4h	12	13	10	12	15	17	09	11
Ciprofloxacin	20	24	20	24	-	-	-	-
Griseofulvin	-	-	-	-	20	24	20	24

### 3. Results and discussion

#### Analgesic activity Acetic acid induced writhing method

All compounds tested exhibited activity in a dose of 25 mg/kg. The analgesic activity of compound 4c was found to be superior compared to other synthesized compounds. Introduction of chloro, nitro group, hydroxyl, bromo group, dimethylamino group, methyl group, methoxyl group showed almost equivalent analgesic activity as that of Ibuprofen. 5-phenyl Tetrazole showed moderate analgesic activity. Introduction of 4-methoxy, 4-dimethylamino group group in 1-[5-(substituted phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-5-phenyl-1H-tetrazole have shown minimum or no analgesic activity.

#### Hot plate method.

All compounds tested by Eddy's hot plate method exhibited activity in a dose of 25 mg/kg. The analgesic activity of compounds 4c, 4f and 4e is found to be superior compared to other synthesized compounds. 2-chloro, 4-bromo and 4-methoxy analogs exhibited moderate analgesic activity.

#### Anti-inflammatory Activity:

A number of agents caused marked reduction of the carrageenan induced edema of the rat foot, however, with exception of compounds 4g (R = p-Nitro phenyl). In this test also only analogs with a p-Methoxy phenyl group in R (4e) showed equal to that exhibited by the standard paracetamol. Compounds 4f, in addition to being the most potent agents of this series against rat-foot inflammation, were also found to be among the most active analgesic when assayed in Glassman's analgesic model.

#### Antimicrobial Activity:

The in-vitro antimicrobial activity of compounds (4a-h) were determined by agar cup plate method, The results of which are summarized in Table 4. The antimicrobial data in table 4 clearly showed that the halogen, nitro & hydroxyl phenyl groups is by far the most active substituted R group. The methoxy group generally confers weak antimicrobial activity. Phenyl substitution are weakly active to inactive among the synthesized compounds. Compounds 4c, 4e and 4f showed good activity against *S. aureus* and *E. coli*. The compound 4b & 4c exhibit promising activity against *C. albicans* and *A. niger*. However, the tested compounds were less active in comparison to Ciprofloxacin and Griseofulvin (standard Drugs).

### 4. Conclusions

In conclusion, the results of this investigation revealed that the observed increase in analgesic, anti-inflammatory and antimicrobial activities are attributed to the presence of 4-NO<sub>2</sub>, 4-OH and 4-Cl in phenyl ring at 5-position of pyrazoline ring of synthesized compounds containing tetrazole. Obviously, the comparative evaluation of active compounds will require further studies; the data reported in this article may be helpful guide for the medicinal chemist who are working in this area.

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## References

- [1] Demarinis R.M. ; Hoover J.R.E.; Dunn G.L.; Actor P.; Uri J.V. ; Weisbach J.A.; J. Antibiot.**28**, 463–465, (1975).
- [2] Essey J.M. ; J. Med. Chem. **12**.703,(1969).
- [3] Okabayashi T.; Kano H. ; Makisumi Y. ;Chem. Pharm. Bull. **8**,157,(1990).
- [4] Sangal S.K.; Ashok Kumar A.; J. Indian Chem. Soc. **63** ,351,(1986).
- [5] Witkowski J.K.; Robins R.K.; Sidwell R.W. ; Simon L.N. ; J. Med. Chem.,**15**,1150 (1972).
- [6] Tsov K.C.; Su H.C.F.; J. Med. Chem., **6**, 693–699,(1963).
- [7] Bary V.C.; Conalty M.C.; O’Sullivan J.P.; Twomey D.; Chemother. , Chemother. Proc. 9th Int. Congr. **8**, 103,(1977).
- [8] Mungnaini A.; Friz L.P.; Provinciali E.; Rugarli P.; Olivi A.; San- filippo E.; Almirante L.; Murmann N.; Bull. Chim. Farm. ,**105**,596,(1966).
- [9] J.R. Maxwell, D.A. Wasdahl, A.C. Wolfson, V.I. Stenberg, J. Med. Chem. **27** 1565, (1984).
- [10] Kumar P.; Knaus E.E.; Drug Des. Discov. **11**, 15,(1994).
- [11] Vicini P.; Amoretti L.; Barocelli E.; Chiavarini M.; Impicciatore M.; Farmaco, **41**, 111,(1986).
- [12] Stewart K.D.; Bioorg. Med. Chem. Lett. **12**, 529(1998).
- [13] Shukla J.S.; Ahmed J., Saxena S.; Indian Chem. Soc. ,**41**, 70,(1979).
- [14] Shishoo C.J.; Devani M.B.; Karvekar M.D.; Vilas G.V.; Anantham S.; Bhaati V.S.; Ind. J. Chem. , **21B**, 666,(1982).
- [15] Pande K.; Tandon M. ; Bhalla T.N.; Parmar S.S.; Barthwal J.P.;Pharmacology, **35**, 333,(1987).
- [16] Ray S.M.; Lahiri S.C.; Ind. Chem. Soc.,**67**, 324,(1990).
- [17] Uchida M. ; Komatsu M.; Morita S.; Kanbe T.;Yamasaki K.; Nakagawa K.;Chem. Pharm. Bull. ,**37**, 958,(1989).
- [18] Ueda I.; Ishii K.; Sinozaki K.; Htanaka M.; Chem. Pharm. Bull.,**39**, 1430,(1991).
- [19] Terashima K.; Tanimura T.; Shimamura H.; Kawase A.; Uenishi K.; Tanaka Y., Kamisaki I.; Ishizuka Y. ; Sato M.; Chem. Pharm. Bull. **43**, 1042,(1995).
- [20] Cosgrove C.E.; Laforge R.A.; J. Org. Chem., **21**, 197,(1956).
- [21] Hayao S.; Havera H.J. ; Strycker W.G.; Leipzig T.J.; Rodriguez R.; J.Med. Chem.,**10**,400,(1965).
- [22] Esplin D.W. ; Woodbuy D.M. ;J. Pharmacol. Exp. Ther.**118**,129–133,(1956).
- [23] GÜrsoy A; Demiravak S; Capan G; Erol K, Vural K; Eur J Med Chem., **35**, 359,(2000).
- [24] Amir M.; Kumar H.; Khan. S.A.; Bioorg Med Chem Lett.,**18**,918-22,(2008).
- [25] Kuettel S.; Zambon A.; Kaiser A.; Brun R.; Scapozza L.; Perozzo R.; J Med Chem., **50**,5833,(2007).
- [26] Li J, DeMello KM, Cheng H, Sakya SM, Bronk BS, Rafka RJ, Jaynes BH, Ziegler CB, Kilroy C, Mann DW, Nimz EL, Lynch MP, Haven ML, Kolosko NL, Minich ML, Li C, Dutra JK, Rast B, Crosson RM, Morton BJ, Kirk GW, Callaghan KM, Koss DA, Shavnya A, Lund LA, Seibel SB, Silvia, Bioorg Med Chem Lett. **14**. 95,(2004).
- [27] Regan J; Capolino A;Cirillo PF; Gilmore T; Graham AG; Hickey E; Kroe RR; Madwed J; Moriak M; Nelson R; Pargellis CA; Swinamer A; Torcellini C; Tsang M, Moss N. (BIRB 796). J Med Chem. **46**, 4676,(2003).