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## SYNTHESIS OF SUBSTITUTED FLUORO PYRAZOLES FOR ANTI-BACTERIAL AND ANTI-OXIDANT ACTIVITY

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### ABSTRACT

A series of novel substituted pyrazoles **8(a-j)** has been synthesized by diazotization of fluoro chloro aniline (**1**) and the reaction of the corresponding diazonium salt solution (**2**) with ethyl cyanoacetate (**3**) to give the intermediate, ethyl 2-((3-chloro-4-fluorophenyl) diazenyl-2-cyanoacetate (**4**). The intermediate is then cyclised with chloroacetonitrile (**5**) using triethyl amine as the base to give the final compound, ethyl 4-amino-1-(3-chloro-4-fluorophenyl)-5-cyano-1H-pyrazole-3-carboxylate (**6**). Nucleophilic substitution group is removed from the final compound and **8(a-j)** derivatives have been synthesized. All the synthesized compounds were characterized by physical data (M.P. & TLC) and spectral Data (IR & <sup>1</sup>H NMR). The synthesized compounds were screened for their antibacterial and anti-oxidant activity.

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### Key Words

Fluoro pyrazole, antibacterial activity, antioxidant activity, DPPH.

## INTRODUCTION

Pyrazole and its derivatives are known to be biologically active compounds. And Substituted pyrazoles have shown wide range of biological activities like antioxidant<sup>1,8</sup>, antibacterial<sup>1,3,5,12</sup>, antifungal<sup>6,15</sup>, analgesic<sup>7</sup>, lipidperoxidation<sup>7</sup>, antiinflammatory<sup>1,4,7</sup>, antiatherosclerosis<sup>9</sup>, antidepressant<sup>10</sup>, antitumour activities<sup>13</sup>, antitubercular<sup>16</sup>, signal transduction therapy<sup>17</sup>, HMGCoA reductase inhibition<sup>18</sup>, anti. In view of these and our continuing interest in the synthesis of biologically active compounds. We undertook the synthesis of the title compounds and studied their antibacterial and anti oxidant activity.

## MATERIALS AND METHODS

Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded Shimadzu FTIR5400. Infrared spectrophotometer using KBr. <sup>1</sup>H NMR spectra were recorded on Perkin-Elmer, 90 MHz spectrophotometer. Using TMS as standard (chemical shift in  $\delta$  ppm)

## EXPERIMENTAL PROCEDURE:

### General procedure for Synthesis of 1-chloro-2-(3-chloro-4-fluorophenyl) diazene (2):

A mixture of (0.18 mol) of fluoro chloro aniline with 46ml of conc. HCl and 75 ml of water taken in a glass mortar. Transferred this suspension to a 500 ml RBF and stirred well. Cooled the contents of the flask in an ice bath to 0-5°C and, added a solution of 13.0g (0.19 mol) of sodium nitrite in 175 ml of water from a dropping funnel during about 20 minutes. Kept the diazonium salt solution below 5°C and, filtered it by suction through a buchner funnel just before use.

### Synthesis of ethyl 2-((3-chloro-4-fluorophenyl)diazonyl)-2-cyanoacetate(4):

Sodium acetate (20 mmol) was added to ethyl cyanoacetate (17.3 mmol), which was dissolved in ethanol (120 ml), cooled below 5°C, and the previously prepared diazonium salt was added with vigorous mechanical stirring. Stirring was continued for 2 hr at room temperature. The precipitate was filtered off, washed twice with water and finally dried at 50°C the

synthesized compounds were characterized by elemental and spectral analysis.

### FT-IR cm<sup>-1</sup> spectra of Comp No. 4

Data: 2222(CN); 2978.19(CH), 1465 (CH<sub>2</sub>) 1718 (C=O); 761(Ar- Cl); 1263(C-F).

<sup>1</sup>H NMR spectra of Comp No. 4 Data: 7.502-7.474 (d, 1H, J=5.45, CH at I)

7.2097.131(d, 2H, J=6.55, CH at II and III). 1.634(s, 1H, CH at IV). 4.431(q, 2H, CH<sub>2</sub> at V). 1.370(t, 3H, CH<sub>3</sub> at VI)

### Synthesis of substituted ethyl 4-amino-1-(3-chloro-4-fluorophenyl)-5-cyano-1H-pyrazole-3-carboxylate(6):

To the intermediate (4) 1mmol chloroacetonitrile (1ml) was added and then NEt<sub>3</sub> (5 mmol) drop wise with external cooling. Mixture was heated at 80-90°C during 1-4hours. After cooling, water was added (10-15ml) and brown oil separated. Water was decanted and the oil dissolved in ethyl acetate, dried and evaporated to produce brown oil again.

The oil was scratched with 10 percent solution of ethyl acetate in ether, solidified and the product was filtered and washed with ether, which yielded a brown solid.

FT-IR cm<sup>-1</sup> spectra of Comp No. 6 Data: 3390(NH<sub>2</sub>); 2216(CN) 1691 (C=O); 827(Ar- Cl); 1263(C-F); 1454(CH<sub>2</sub>)1527(Ar C=C); 2982(CH).

<sup>1</sup>H NMR spectra of Comp No. 6 Data: 7.822-7.778 (m, 1H, CH at I). 7.359-7.317 (d, 1H, CH at II). 7.644-7.567 (m, 1H, CH at III). 4.527-4.414 (m, 2H, NH<sub>2</sub> at IV). 1.589 (m, 2H, CH<sub>2</sub> at V). 1.475-1.404(t, 3H, CH<sub>3</sub> at VI)

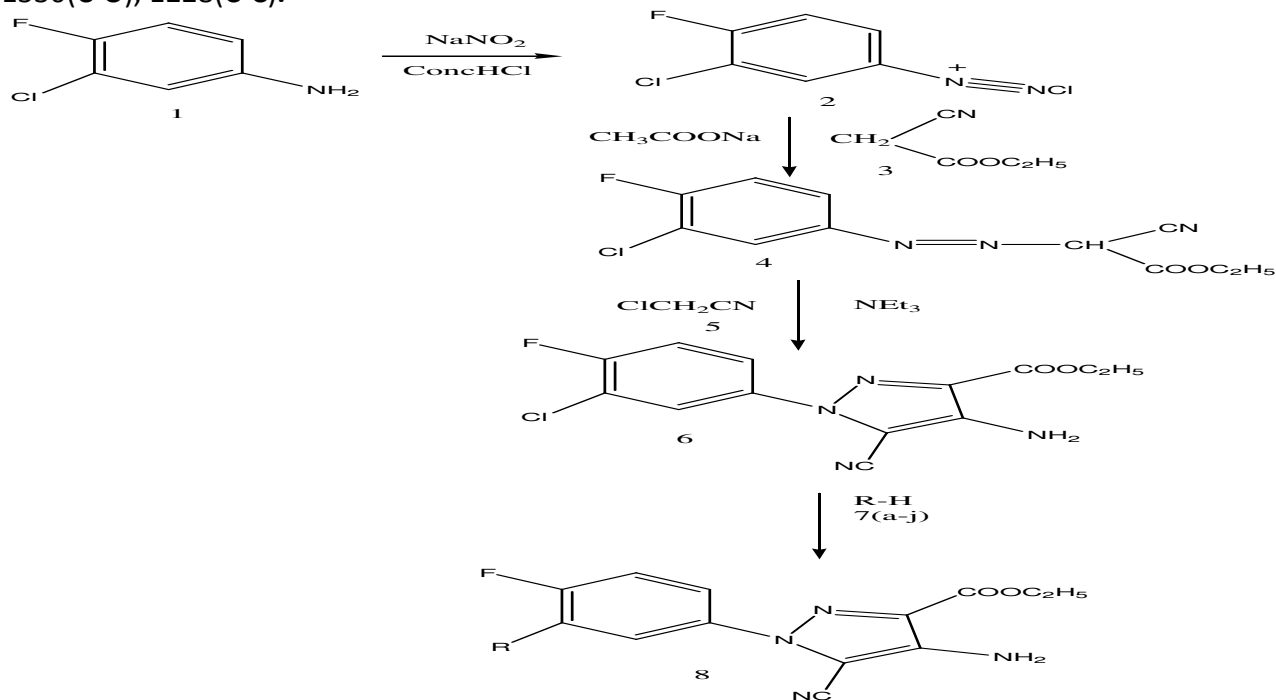
### Synthesis of substituted ethyl 4-amino-1-(3-substituted-4-fluorophenyl)-5-cyano-1H-pyrazole-3-carboxylate (8a-j):

To the above ethyl 4-amino-1-(3-chloro-4-fluorophenyl)-5-cyano-1H-pyrazole-3-carboxylate derivative (1mol), different types anilines( 3-Nitro aniline, 4-chloro aniline) or phenol (*o*- cresol, *p*- cresol ) (1mol), 1,4-dioxane 10 ml and 2-3 drops of triethylamine was taken in a 100 ml RBF fitted with a reflux condenser. Refluxed the reaction mixture 12-14 hr and monitored by TLC, then transferred to ice cold water and filtered the solution to get the desired products.

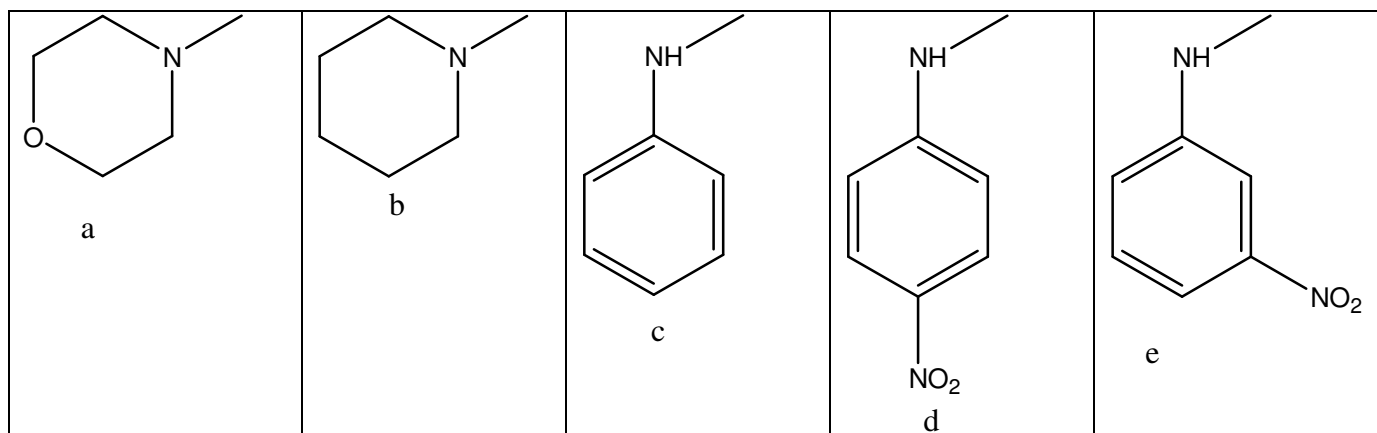
**FT-IR  $\text{cm}^{-1}$  spectra of Comp No. 8a**

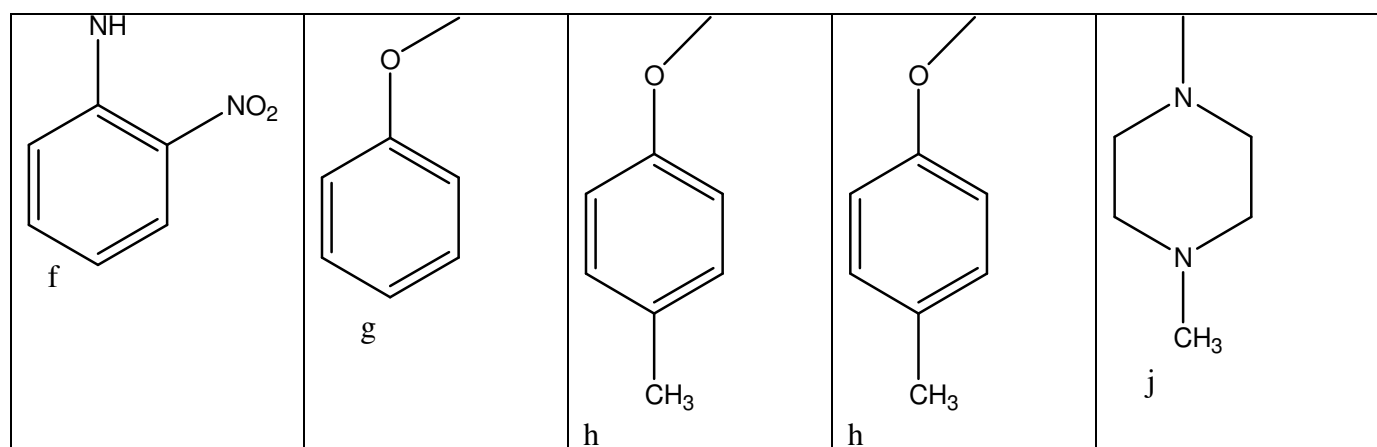
Data: 3232( $\text{NH}_2$ ); 2216( $\text{CN}$ ); 1703 ( $\text{C=O}$ ); 1271( $\text{C-F}$ );  
1444( $\text{CH}_2$ ); 1558(Ar  $\text{C=C}$ );  
2985(  $\text{CH}$ ); 1330( $\text{C-O}$ ), 1228( $\text{C-C}$ ).

**FT-IR spectra of Comp No. 8i** Data: 3367( $\text{NH}_2$ );  
2360( $\text{CN}$ ); 1718 ( $\text{C=O}$ ); 1261( $\text{C-F}$ ); 1438( $\text{CH}_2$ ); 1535(Ar  
 $\text{C=C}$ ); 2935( $\text{CH}$ ); 1103( $\text{C-O}$ ), 1230( $\text{C-C}$ ).



R=



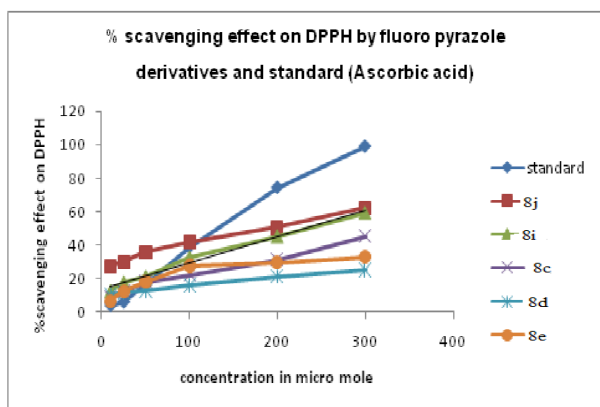
**BIOLOGICAL ACTIVITY.**

**ANTI-MICROBIAL ACTIVITY:** The antimicrobial activity was carried out by the agar diffusion method. Against *Escherichia coli* And *Staphylococcus aureus* and The sensitivity of organisms to the synthesized compounds were measured and compared with that of the standard reference drug. The standard reference drug used in the present work was Amoxicillin. Each test compound was dissolved in DMF to get a concentration of 500 µg/ml.

**Table 1:** Anti-bacterial activity of substituted fluoro pyrazoles (8a-j)

This concentration was used for testing antibacterial activity. 0.1 ml of test solution and standard solution at a concentration of (50 µg) were placed in petriplates containing 30-50 ml nutrient agar, inoculated with test culture.. Then the petriplates were incubated at 37 °C for 24 hr and after incubation, the zones of inhibition was observed, measured and compared with standard drug. The zone of inhibition listed in table-1

compounds	Zone of inhibition (mm)	
	Staphylococcus aureus	Escherichia coli
8a	4.2	3.7
8b	3.6	3.3
8c	2.5	3.1
8d	3.9	3.6
8e	4.3	3.9
8f	4.8	4.0
8g	2.4	3.0
8h	2.6	2.7
8i	2.8	2.9
8j	2.7	3.0
Control	NA	NA
standard	8.2	8.5



**Fig 1:** % scavenging effect on DPPH by fluoro pyrazole derivatives and standard (Ascorbic acid)

#### ANTI-OXIDANT ACTIVITY:

Free radical scavenging activity by DPPH method: Free radical scavenging potentials of the synthesized compounds were tested against a methanolic solution of  $\alpha, \alpha$  diphenyl- $\beta$ -picryl hydrazyl (DPPH). Antioxidant reacts with DPPH and converts it to  $\alpha, \alpha$  diphenyl- $\beta$ -picryl hydrazide. The degree of discoloration indicates the scavenging potentials of the antioxidant activity. The changes in the absorbance produced at 517 nm were used to measure antioxidant activity.

#### PREPARATION OF SOLUTION:

DPPH stock solution (100  $\mu$ M): 39.4 mg of DPPH was dissolved in analytical grade methanol; 10 mg of each synthesized compound was dissolved separately in 10 ml of analytical grade methanol. BHT (Butylated hydroxy toluene) and ascorbic acid were taken as standard. 10 mg of each standard compound was dissolved in 10ml of methanol.

#### PROCEDURE:

10 to 300  $\mu$ l (10 to 300  $\mu$ g) of ascorbic acid, BHT and synthesized compounds were taken in different test tubes. Then the volume was adjusted to 1000  $\mu$ l with methanol. To this 4 ml of methanolic solution of DPPH was added, shaken well and the mixture was allowed to stand at room temperature for 20 minutes. The control was prepared as above without compound. Absorbance measured at 517 nm. Scavenging activity was expressed

as the inhibition percentage calculated using the following formula,

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

#### RESULTS AND DISCUSSION

Antibacterial activity of synthesized compounds was performed by agar diffusion method. Compounds **8a**, **8b**, **8d**, **8e** and **8f** showed good antibacterial activity. **8c**, **8g**, **8h**, **8i** and **8j** compounds exhibited weak antibacterial activity. Finally it can be inferred from the above result that newly synthesized compound possessing electron withdrawing group at ortho, Meta or Para position showed better antibacterial activity than compound with electron donating group. Antioxidant activity was done by free radical scavenging DPPH method. **8i** and **8j** compounds resulted better antioxidant activity. **8d** and **8e** exhibited weak antioxidant activity. This shows that electron donating group at ortho or Para position gives better antioxidant activity as compared to electron withdrawing group.

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