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RESEARCH ARTICLE

Anti oxidant and Anti-Inflammatory activities of novel pyrazole derivatives

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ABSTRACT

Series of 5-amino-N-(substituted phenyl)-1-(substituted)-3-[(3-chloro-4-fluorophenyl) amino]-1H-pyrazole-4-carboxamide. Were synthesized from N-(3-chloro-4-fluorophenyl)-2-cyanoa 3, 3 Bis methyl sulfonyl acrylamide using various hydrazines. All the synthesized compounds were characterized by physical data (M.P. & TLC) and spectral Data (IR & ^{1}H NMR). The synthesized compounds were screened for *in vitro* anti-inflammatory activity by using Diclofenac sodium as standard of concentration (100µg/0.1mL). And *in vitro* antioxidant activity (300µg/mL). A few of the compounds gave promising results.

INTRODUCTION

Synthesis of nitrogen containing heterocyclic compounds has been a subject of great interest due to the wide application in pharmaceutical fields. Pyrazoles and its derivatives represent one of the most active classes of compounds possess in wide range of biological activities like Antibacterial[1-2], anti-inflammatory[3-6], antioxidant[7,8], anti diabetic[9], antitumor[10], antituberculosis[11], antimalarial[12], anti hypertension[13], antidepressant[14], antiobesity[15], anticoagulant[16], kinase inhibitor[17], insecticidal[18]. Reactive Oxygen Species termed as free radicals which play havoc with the body. These free radicals can cause lipid peroxidation in foods, which leads to their deterioration[19-20]. In addition, reactive oxygen species have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer[21-24]. When produced in excess, ROS can cause tissue injury[25]. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system that functions interactively and synergistically to neutralize free radicals. Thus, antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. 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, *in vitro* anti oxidant activity and Anti inflammatory activity.

MATERIALS AND METHODS

Melting points were determined in open capillaries and are uncorrected.IR spectra were recorded Shimadzu FTIR5400. Infrared spectrophotometer using KBr.1HNMR spectra were recorded on Perkin-Elmer, 90 MHz spectrophotometer. Using TMS as standard (chemical shift in δ ppm)

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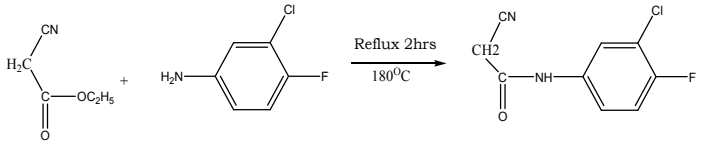
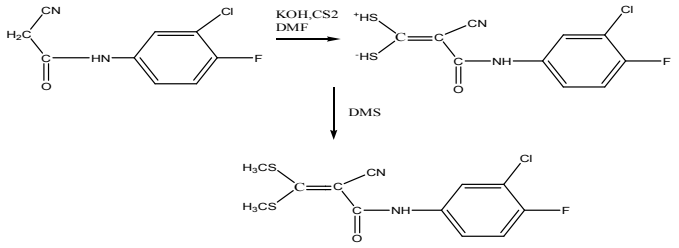
EXPERIMENTAL PROCEDURE

Step 1. Synthesis of N-(3-chloro-4-fluorophenyl)-2-cyanoacetamide



A mixture of 3-chloro-4-fluoroaniline (0.05M), ethyl cyano acetate (5.75ml: 0.05M) refluxed at 180°C for 2 hrs, cooled and stirred overnight at room temperature. The solid product that was obtained was washed with ethanol, dried and recrystallized from ethanol: water mixture.

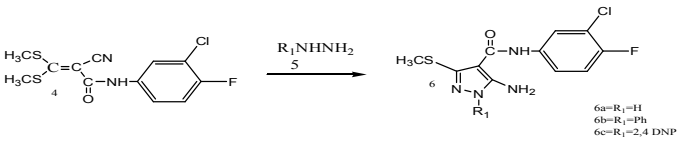
Step 2. Synthesis of N-(3-chloro-4-fluorophenyl)-2-cyanoa 3, 3Bis methyl sulfonyl acrylamide.



A solution of 1.3 gm of KOH in 5 mL ice cold water was placed in a 50 mL RBF which was immersed in a freezing bath. To this was added DMF (3ml), 0.01 mol of N-(3-chloro-4-fluorophenyl)-2-cyanoacetamide and treated drop wise with carbon disulphide (0.01mol) with the temperature being maintained below 0ºC during addition. The mixture further stirred for 3 hrs. To this was added drop wise 2.5 ml of dimethylsulphate while the mixture was stirred and maintained at 10-15ºC during addition, allowed to stand further for

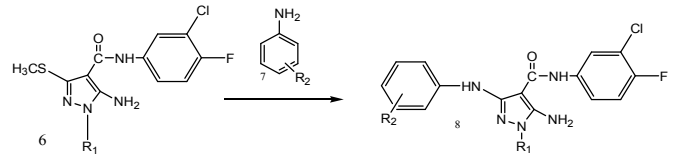
12 hrs and poured into 25 mL ice water. The solid that separated was filtered, washed with water, dried and recrystallized using ethanol.

Step 3. 5 Amino-3-methyl sulfonyl-1H-pyrazole-4-carboxylic acid (3-chloro-4-fluorophenyl) Amide.



The product obtained in step 2 (0.05mol) hydrazines (0.05mol) in ethanol was taken in round bottom flask. Added glacial acetic acid drop wise, after addition the mixture was refluxed for 12 hrs. The mixture was cooled and poured into 100 mL of ice water, extracted with ethyl acetate. The combined organic layer was dried over anhydrous sodium sulphate and evaporated in vacuum to obtain the desired product. The product so obtained was recrystallized from ethanol.

Step 4. 5-amino-N-(substituted phenyl)-1-(substituted)-3-[(3-chloro-4-fluorophenyl) amino]-1H-pyrazole-4-carboxamide



Into a 250 mL 3-necked round bottom flask fitted with a mechanical stirrer, were placed aniline substitutes (0.01mol) and product obtained in step 4 in ethanol was taken in round bottom flask and refluxed for 12 hrs. Cooled, poured into ice water. The solid product that was obtained was filtered, washed with cold ethanol and recrystalized with ethanol-water mixture.

BIOLOGICAL EVALUATION

Anti-Oxidant activity

The reaction mixture containing o-phenanthroline (0.5m), ferric chloride (0.2mM) and different type fractions of test compound in a volume of 5 ml was incubated for 15-20 min at ambient temperature. The absorbance at 630 was measured. In other set, sodium dithionite (0.3mM) was added instead of the extract and the absorbance was taken as equivalent to 100% reduction of all the ferric ions present. Anti-oxidant activity can be calculated by the following formula:
% Activity=[A_t /A_s] × 100 Where, A_t= absorbance of sample. A_s= absorbance of standard

ANTI-INFLAMMATORY ACTIVITY

Inhibition of bovine serum albumin denaturation

The test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer (0.2M, pH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test solution (1ml) containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 27° ± 1°C for 15 min. Denaturation was induced by keeping the reaction mixture at 60° ± 1°C in a water bath for 10 min. After cooling the turbidity was measured at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and the average was taken. The percentage of inhibition was calculated from the following

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

RESULT AND DISCUSSION

Spectral Analysis

IR Spectroscopy (values in cm^{-1}):
IR spectrum of (6b): Data: 3421.8.cm^{-1}(NH_2).3321.53cm^{-1}(NH str), 3057.27cm^{-1}(C-H str Aromatic), 2918.4cm^{-1}(C-H str aliphatic) 2354.21cm^{-1}(C-S str) 1688.48cm^{-1}(C=0 str).

^{1}H NMR Spectroscopy in $CDCl_3$ (values in δ ppm):
^{1}H NMR spectrum (6b) 1H singlet9.207δ-CONH, 8HMultiplet 7.076δ-7.851δ (aromatic proton)
2 Hsinglet 5.754., NH_2 Aliphatic, 3Hsinglet 2.630δ-SCH_3.
IR spectrum of S2/1: 3470.06 cm^{-1}(NH_2 str)., 3348.54 & 3250.16 cm^{-1}(N-H str), 3041.84 cm^{-1} (C-H str Aromatic),2993.62(C-H str aliphatic),1656.91 cm^{-1}(C=0 str).
IR spectrum of S2/3 :3470.06 cm^{-1}(NH_2 str)., 3279.10 cm^{-1} (N-H str), 3041.84 cm^{-1} (C-H str Aromatic), 2978.19 (C-H str aliphatic),1656.91 cm^{-1}(C=0 str ketone).
^{1}H NMR spectrum of S2/3: 7.78(2H at H_1 & H_3), 9.923 (2H at H_4 &H_6), 7.26(3H at H_2 &H_{10}),
5.604(3H at H_5, H_7 &H_8), 7.827(2H at H_{11} &H_{12})
IR spectrum of S2/8: 3346.51 $cm^{-1,}$ (NH_2 str)., 3250.16.10 cm^{-1} (N-H str),3041.84 cm^{-1} (C-H str Aromatic), 2978.04 (C-Hstr aliphatic),1656.91 cm^{-1}(C=0 str aliphatic ketone).
IR spectrum of S2/9: 3568.43, 3392.90 cm^{-1}(str NH2), 3246.31 (str NH).3076.56cm^{-1}(C-H str Aromatic),1672.34 cm^{-1}(C=0 str aliphatic ketone)
IR spectrum of S2/13: 3392.50,3360 $cm^{-1,}$ (3321.53 $cm^{-1,}$ (NH str Primary amine) 3101.64 cm^{-1} (C-H str Aromatic), 1605 cm^{-1}(C=0 str ketone).
IR spectrum of S2/15: Data: 3353.97cm^{-1}(NH_2 str).3321.53cm^{-1}(NH str), 3101.64cm^{-1}(C-H str Aromatic), 2950cm^{-1}(C-H str aliphatic) 1650cm^{-1}(C=0 str aliphatic ketone

^{1}H NMR spectrum of S2/15:1H singlet broad peak 9.44δ-CONH, 1HMultiplet 7.13δ-8.34δ (aromatic proton) 1Hsinglet 6.52δ NH Aromatic, 2H singlet 4.00δ-NH_2
IR spectrum of S2/16:3568.43 & 3394.83(NH_2 str), 3246.31 (NH str), 3078.49 cm^{-1}(C-H str Aromatic), 1647.27 cm^{-1}(C=0 str aliphatic ketone)

^{1}H NMR spectrum of S2/16: 7.78-7.96(4H at H_1 H2, H3 & H4), 5.84(1H at H5), 9.09-9.13 (2H at H6 &H7), 8.81(1H at H8), 7.06-7.13(2H at H9, & H10), 9.45(1H at H_{11}), 7.25(1H at H12), 8.71(1H at H13), 8.31(1H at H14)
IR spectrum of S2/20: 3568.43 & 3394.83 (NH str), 3246.31(NH2 str), 3107.43 cm^{-1}(C-H str Aromatic), 1672.34 cm^{-1}(C=0 str aliphatic ketone)

A series of targeted compounds were synthesized (Table 1) and all derivatives were identified and characterized by physical method like melting point, and thin layer chromatography and spectral analysis like UV, IR and NMR. All compounds were screened for biological activities like anti-inflammatory activity test *in vitro* by using Bovine serum albumin denaturation and antioxidant activity by Ferric ion reduction method. Antioxidant activity for the tested compounds S2/1 to S2/21 exhibited moderate to good activity. Results are given in Table 2. A clear look at the data shows that the presence of electron withdrawing, 2, 4 dinitrophenyl substitutes on the pyrazole was very much beneficial as indicated by the activity of /S2/16, S2/17, S2/18. A possible reason for this could be the electron withdrawing nitro substitutes could be instrumental in making the molecule accept an electron thereby increasing reductive properties.

Anti-inflammatory

The synthesized molecules were screened for *in vitro* anti-inflammatory activity by Bovine serum albumin denaturation activity. An inhibitory effect on protein denaturation by synthesized compounds is shown in table 3. From results observed that aromatic ring on pyrazole nitrogen (S2/16, S2/17, S2/18) exhibited very good anti-inflammatory activity.

General scheme for synthesis of fluoro pyrazole

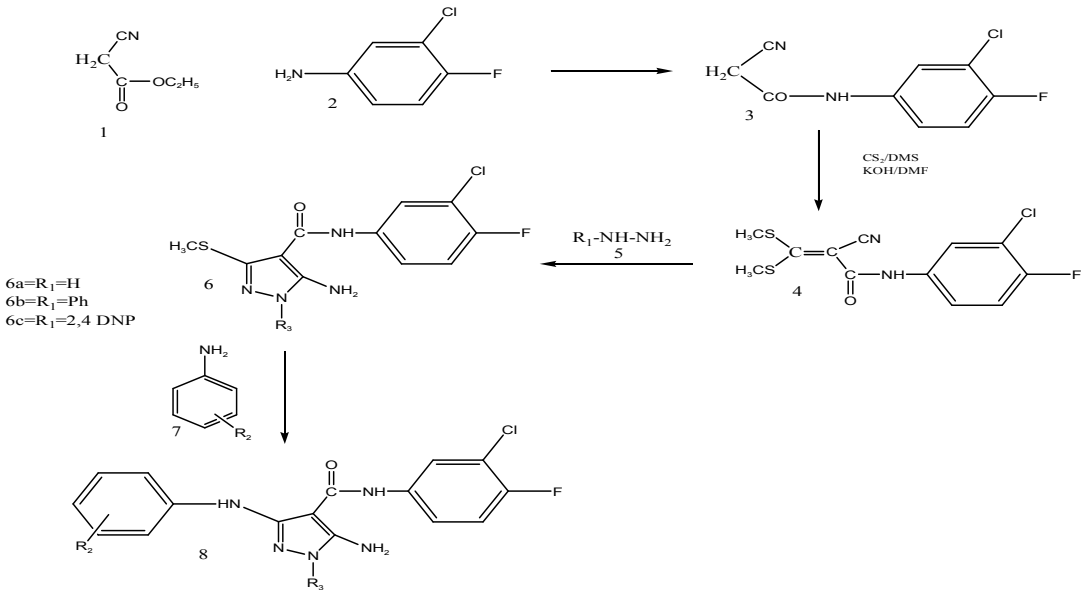
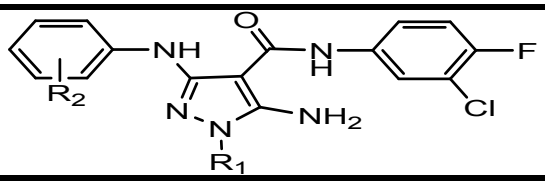


Table 1. List of synthesized compounds



Comp. Code

	R1	R2		R1	R2
S2/1	H	H	S2/12	Ph	2-Cl
S2/2	H	2-NO_2	S2/13	Ph	3-Cl
S2/3	H	3-NO_2	S2/14	Ph	4-Cl
S2/4	H	4-NO_2	S2/15	2,4 DNP	H
S2/5	H	2-Cl	S2/16	2,4 DNP	2-NO_2
S2/6	H	3-Cl	S2/17	2,4 DNP	3-NO_2
S2/7	H	4-Cl	S2/18	2,4 DNP	4-NO_2
S2/8	Ph	H	S2/19	2,4 DNP	2-Cl
S2/9	Ph	2-NO_2	S2/20	2,4 DNP	3-Cl
S2/10	Ph	3-NO_2	S2/21	2,4 DNP	4-Cl
S2/11	Ph	4-NO_2			

Table 2. Ferric ion reduction activity (antioxidant activity)

Sl. No	Comp code	Absorbance 510 nM	Antioxidant activity (%)
1	S2/1	0.182	33.7
2	S2/2	0.281	52.1
3	S2/3	0.287	53.2
4	S2/4	0.279	51.7
5	S2/5	0.203	37.6
6	S2/6	0.217	40.2
7	S2/7	0.229	42.4
8	S2/8	0.237	43.9
9	S2/9	0.291	53.9
10	S2/10	0.286	53.06
11	S2/11	0.284	52.6
12	S2/12	0.239	44.3
13	S2/13	0.224	41.5
14	S2/14	0.194	35.9
15	S2/15	0.290	53.8
16	S2/16	0.392	72.7
17	S2/17	0.384	71.2
18	S2/18	0.372	69.01
19	S2/19	0.285	52.8
20	S2/20	0.284	52.6
21	S2/21	0.289	53.6
	Sodium dithionite	0.539	100

Table 3. Bovine serum albumin denaturation activity

Sl. No	Comp. Code	Absorbance at 660 nm	% Denaturation activity
1	S2/1	0.064	18.9
2	S2/2	0.52	34.1
3	S2/3	0.049	37.9
4	S2/4	0.054	31.6
5	S2/5	0.068	13.9
6	S2/6	0.071	10.1
7	S2/7	0.063	20.2
8	S2/8	0.064	18.9
9	S2/9	0.053	32.9
10	S2/10	0.062	21.5
11	S2/11	0.058	26.5
12	S2/12	0.074	6.32
13	S2/13	0.069	12.6
14	S2/14	0.073	7.5
15	S2/15	0.069	12.6
16	S2/16	0.046	41.2
17	S2/17	0.043	45.5
18	S2/18	0.045	43.03
19	S2/19	0.056	29.11
20	S2/20	0.051	35.4
21	S2/21	0.054	31.6
	control	0.079	---
	Std Diclofenac sodium	0.040	49.3

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