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Synthesis and Evaluation of Some Novel Pyrazoles for Antinflammatory Studies

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Abstract

A series of novel pyrazoles were synthesized starting from 4-fluoro-3-chloroaniline by condensation with aromatic aldehydes to give chalcones which were cyclized with hydrazine to obtain pyrazoles. The chlorine replaced various aromatic amines to obtain the final derivatives. The final compounds evaluated for *in vitro* bovine serum denaturation assay and *in vivo* antinflammatory activity by carrageenan induced rat paw oedema. The study indicated the importance of chlorine at the para position of the aromatic ring in enhancing antiinflammatory activity. Correlation with enzyme inhibition scores also explained.

Key words : Pyrazoles, fluorine, bovine serum denaturation, antiinflammatory activity, enzyme inhibition.

Introduction

Arthritis is a very common occurrence these days. Eating habits and non-exercise have let to obesity. Lack of synovial fluids is known to increase friction resulting in the inflammation of the joints. Patients suffering from arthritis are subject to chronic medication and under such circumstances, patient safety becomes critical. The current strategies of NSAID research are to develop selective COX 2 inhibitors. Drugs like celecoxib, rofecoxib have been introduced in the past for the management of chronic inflammation. Inflammation is a complex process which involves many steps. The region of inflammation is associated with various physical changes which are visible to the unaided eye. However, these visual representations are associated with a lot of chemical changes occurring within the tissue[1]. Denatured proteins produced at the site of inflammation are also known to act as

chemo tactic factors that can prolong inflammatory process. A candidate that can inhibit denaturation of protein may be a suitable anti-inflammatory agent. Bovine serum albumin denaturation assay is a non specific *in vitro* model to evaluate anti-inflammatory agents[2]. Pyrazole based heterocycles are established drug candidates possesing a variety of pharmacological activities and are reported as antiinfectives[3,4], antiinflammatory[5,6], anticancer[7], antimycobacterial[8], nitric oxide receptor activators[9] etc.

Material and Methods

Chemistry:

The general chemicals and reagents were procured from Loba or SD Fine chem and of laboratory reagent grade. Fluoro chloroaniline was procured from a bulk drug supplier at Hyderabad. Wherever necessary, the chemicals were purified before use by distillation or recrystallization. The melting points were determined by open capillary method and are uncorrected. Infrared (IR) spectra were recorded using KBr pellets on a Shimadzu 8400S FTIR. Proton NMR were recorded on a Bruker 300 MHz instrument and with reference to internal standard tetramethyl silane (TMS).

Synthesis of Fluoro chloroacetanilide (2)

In a 100 mL round bottomed flask was placed 7.3 g (0.05 mole) of 4-fluoro-3-chloroaniline **(1)**, 25 mL acetic acid and 15 mL of acetic anhydride. The mixture was heated on a water bath for 2h, cooled and poured into 100 g of crushed ice with rapid stirring. A white solid that separated was filtered and dried over air and then in an oven at 105 p C for 1 hour. The product **(2)** was recrystallized from ethanol.

Melting range: 114 – 118 ºC; FTIR (KBr, V_{max}, cm^{-1}): 824 (C – Cl *str*), 1214(C – F *str*), 1618 (N – H *def*), 1735 (C = O *str)*, 3213 (N – H *str*). ^{1}H NMR in $CDCl_3$ (values in δ ppm): 2.15(3H, s, $COCH_3$), 4.59(1H, s, ArNH), 6.41-6.50(1H, m, Ar-H), 6.64-6.73 (1H; dd, Ar-H), 6.87-6.96(1H; app t, Ar-H).

Synthesis of Chalcone (4a and 4b)

In a 250 mL round bottomed flask was dissolved 4.7 g (0.025 mol) of 4-fluoro-3-chloro acetanilide (2) in 50 mL of rectified spirit. To the solution was added drop wise 20 mL of 30% w/v KOH solution and the resulting mixture magnetically stirred for 2h. To this was added 0.025 mole of the aldehyde (3a -3b) and the reaction refluxed between 10 -14h, cooled and poured into 100 mL of ice cold water and transferred to a separating funnel and extracted twice, each 25 mL portions of ethyl acetate to remove any unreacted aldehyde. The aqueous layer acidified to a pH of 6 with dilute hydrochloric acid during which the time a solid separated. The solution filtered and the product (4a – 4b) dried in an oven at 105 ºC for 1h, recrystallized with ethanol.

1– Phenyl – N – (3 – chloro – 4 – fluorophenyl) propenamide 4a

Melting range: 110 – 112 ºC; FTIR (KBr, V_{max}, cm^{-1}): 1604 (N – H *def*),1630 (Ar C = C *str)*, 1742 (C = O *str)*, 3375, 3475 (N – H *str*).^{1}H NMR in $CDCl_3$ (values in δ ppm):

6.41-6.43(1H, d, vinylic-H), 6.74-6.76(1H; d, vinylic-H), 6.97-7.84 (8H; m, Ar-H), 8.04 (1H, s, ArNH).

1 – (4 – Methoxyphenyl) – N – (3 – chloro – 4 – fluorophenyl) propenamide 4b

Melting range: 141 – 142 °C; FTIR (KBr, V_{max}, cm^{-1}): 947 (C-F *str*), 1055 (C-O *str*), 1593 (Ar C = C *str)*, 1693 (N-H *def*), 1734 (C = O *str)*, 3377 (N – H *str*).^{1}H NMR in $CDCl_3$ (values in δ ppm): 3.91 (3H, s,-OCH_3), 6.38-6.40(1H, d, vinylic-H), 6.64-6.66(1H; d, vinylic-H), 6.90-7.68 (7H; Ar-H), 8.40 (1H, s, ArNH),

Construction of pyrazole ring

In a 100 mL round bottomed flask was dissolved the chalcone, 4a or 4b (0.02 mol) in 25 mL of ethanol and added 5 mL hydrazine hydrate. The resulting solution was refluxed for 6 – 10h, cooled and poured into crushed ice. The solid that separated was filtered. The reaction was monitored by TLC. The crude product was recrystallized from ethanol to obtain pyrazole intermediates 5a – 5b.

N-(3 – Chloro – 4 – fluorophenyl) – 3 – amino – 5 – phenyl pyrazole 5a

Melting range: 103 – 105 °C; FTIR (KBr, V_{max}, cm^{-1}): 841 (C-Cl *str*), 1041 (C-F *str*), 1204 (C-O *str*), 1586 (Ar C = C *str)*, 1690 (N-H *def*).^{1}H NMR in $CDCl_3$ (values in δ ppm): 4.91 (1H, s, amine NH), 5.91 (1H, s, Pyrazole NH), 6.60-7.80 (9H; Ar-H), 7.98 (1H, s, amine NH).

N-(3 – Chloro – 4 – fluorophenyl) – 3 – amino – 5 – (4 – methoxyphenyl) pyrazole 5b

Melting range: 113 – 115 °C; FTIR (KBr, V_{max}, cm^{-1}): 823 (C-Cl *str*), 1051 (C-F *str*), 1195 (C-O *str*), 1591 (Ar C = C *str)*, 1695 (N-H *def*).^{1}H NMR in $CDCl_3$ (values in δ ppm): 3.86 (3H, s,-OCH_3), 6.01 (1H, s, Pyrazole NH), 6.64-7.68 (8H; Ar-H), 7.95 (1H, s, amine NH).

General procedure for substitution of chlorine.

In a dry 25 mL 2-necked round bottom flask fitted with a condenser and a nitrogen inlet assembly was placed 5a or 5b (0.001 mol). To this was added 10 mL of dry solvent, 4 equivalents of anhydrous potassium carbonate, 1-4 equivalents of 6 (a-j), 1-2.5 mol% cuprous oxide or cuprous iodide as catalyst. The entire assembly was flushed twice with dry nitrogen and the reaction mixture was refluxed for 16 – 24h under nitrogen atmosphere. The reaction monitored by TLC. After the completion of reaction, the mixture was cooled and poured into 100 mL of ice cold water, extracted with 3 x 20 mL portions of ethyl acetate. The combined organic layer was dried over anhydrous sodium sulphate and evaporated in vacuum to obtain the desired products (7a-j & 8a -j). Purification done by column chromatography on silacgel # 80/120, gradient elution with hexane: ethyl acetate as solvent system.

3 – [N – (4 – fluoro – 3 – phenylamino) phenyl]amino – 5 – phenylpyrazole.7a

Melting range: 112 – 114 °C; FTIR (KBr, V_{max}, cm^{-1}): 1001 (C – F *str*): 1369 (C – N *str*), 1626 (N – H *def*), 1637 (Ar C = C

str), 3412 (N – H *str*).^{1}H NMR in $CDCl_3$ (values in δ ppm): 6.99 (1H, s, Ar-NH), 7.09 – 8.05 (14H; m, Ar-H), 8.27 (1H; s, NH), 8.41 (1H; s, NH).

3 – [N – (4 – fluoro – 3 – (2-chlorophenyl) amino)phenyl]amino – 5 – phenylpyrazole.7b

Melting range: 124 – 126 °C; FTIR (KBr, V_{max}, cm^{-1}): 980 (C – F *str*): 1371(C – N *str*), 1636 (N – H *def*), (Ar 3401 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (2-nitrophenyl) amino)phenyl]amino – 5 – phenylpyrazole.7c

Melting range: 150 – 152 °C; FTIR (KBr, V_{max}, cm^{-1}): 1045 (C – F *str*): 1385 (C – N *str*), 1430 (NO_2 *asym.str*), 1543 (NO_2 *sym.str*),1626 (N – H *def*), 3385 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (2-methylphenyl) amino)phenyl]amino – 5 – phenylpyrazole.7d

Melting range: 160 – 162 °C; FTIR (KBr, V_{max}, cm^{-1}): 1103 (C – F *str*), 1608 (N – H *def*), 2960 (C – H *str)*, 3299 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (3-chlorophenyl) amino)phenyl]amino – 5 – phenylpyrazole.7e

Melting range: 210 – 212 °C; FTIR (KBr, V_{max}, cm^{-1}): 1115 (C – F *str*), 1645 (N – H *def*), 2960 (C – H *str)*, 3301 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (3-nitrophenyl) amino)phenyl]amino – 5 – phenylpyrazole.7f

Melting range: 200 – 202 °C; FTIR (KBr, V_{max}, cm^{-1}): 1110 (C – F *str*), 1445 (NO_2 *asym.str*) 1551 (NO_2 *sym.str*), 1608 (N – H *def*), 2967 (C – H *str)*, 3250 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (3-methylphenyl) amino)phenyl]amino – 5 – phenylpyrazole.7g

Melting range: 208 – 210 °C; FTIR (KBr, V_{max}, cm^{-1}): 980 (C – F *str*), 1665 (N – H *def*), 2942 (C – H *str)*, 3345 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (4-chlorophenyl) amino)phenyl]amino – 5 – phenylpyrazole.7h

Melting range: sticky mass; FTIR (KBr, V_{max}, cm^{-1}): 1080 (C – F *str*), 1635 (N – H *def*), 2954 (C – H *str)*, 3345 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (4-nitrophenyl) amino)phenyl]amino – 5 – phenylpyrazole.7i

Melting point: 150 °C; FTIR (KBr, V_{max}, cm^{-1}): 1070 (C – F *str*), 1390 (NO_2 *asym.str*), 1516 (NO_2 *sym.str*), 1647 (N – H *def*), 3475 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (4-methylphenyl) amino)phenyl]amino – 5 – phenylpyrazole.7j

Melting range: sticky mass; FTIR (KBr, V_{max}, cm^{-1}): 1074 (C – F *str*), 1645(N – H *def*), 2995 (C – H *str)*, 3331 (N – H *str*).

3 – [N – (4 – fluoro – 3 – phenylamino) phenyl]amino – 5 – (4 – methoxyphenyl) pyrazole.8a

Melting point: 180 °C; FTIR (KBr, V_{max}, cm^{-1}): 1008 (C – F *str*), 1130 (C – O

str), 1647 (N – H *def*), 3475 (N – H *str*). ^{1}H NMR in $CDCl_3$ (values in δ ppm): 3.90 (3H, s, OCH3), 6.82 - 6.84 (2H, d, Ar-H), 7.29 – 7.37 (4H; m, Ar-H), 7.70 – 7.76 (4H; m, Ar-H), 7.88 – 7.92 (4H; m, Ar-H), 7.99 (1H; s, Ar-NH), 8.27 (1H; s, NH), 8.41 (1H; s, NH)

3 – [N – (4 – fluoro – 3 – (2-chlorophenyl) amino)phenyl]amino – 5 – (4 – methoxyphenyl) pyrazole.8b

Melting point: 195 ^{o}C; FTIR (KBr, V_{max}, cm $^{-1}$): 1035 (C– F *str*), 1095 (C –O)*str*), 1647 (N – H *def*), 3475 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (2-nitrophenyl) amino)phenyl]amino – 5 – (4 – methoxyphenyl) pyrazole.8c

Melting point: 220 ^{o}C; 1035 (C – F *str*), 1095 (C –O)*str*), 1386 (NO_2 *asym.str*), 1535 (NO_2 *sym.str*), 1647 (N – H *def*), 3475 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (2-methylphenyl) amino)phenyl]amino – 5 – (4 – methoxyphenyl) pyrazole.8d

Melting range: 110 – 112 ^{o}C; FTIR (KBr, V_{max}, cm $^{-1}$): 1008 (C – F *str*), 1140 (C – O)*str*), 1640(N – H *def*), 3358 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (3-chlorophenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8e

Melting range: low melting; FTIR (KBr, V_{max}, cm $^{-1}$): 1005 (C – F *str*), 1121 (C – O)*str*), 1610 (N – H *def*), 3300 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (3-nitrophenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8f

Melting range: 163 – 165 ^{o}C; FTIR (KBr, V_{max}, cm $^{-1}$): 1021 (C – F *str*), 1087 (C – O)*str*), 1412 (NO_2 *asym.str*), 1541 (NO_2 *sym.str*), 1635 (N – H *def*), 3387 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (3-methylphenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8g

Melting range: 93 - 95 ^{o}C; FTIR (KBr, V_{max}, cm $^{-1}$): 1005 (C – F *str*), 1087 (C – O)*str*), 1605 (N – H *def*), 3289 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (4-chlorophenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8h

Melting range: 135 ^{o}C; FTIR (KBr, V_{max}, cm^{-1}): 1021 (C – F *str*), 1104 (C –O)*str*), 1687 (N – H *def*), 3357 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (4-nitrophenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8i

Melting range: 136 – 138 ^{o}C; FTIR (KBr, V_{max}, cm $^{-1}$): 1047 (C – F *str*), 1115 (C – O)*str*), 1335 (NO_2 *asym.str*), 1535 (NO_2 *sym.str*), 1637 (N – H *def*), 3410 (N – H *str*).

3 – [N – (4 – fluoro – 3 – (4-methylphenyl) amino)phenyl]amino– 5 – (4 – methoxyphenyl) pyrazole.8j

Melting range: 162 – 166 ^{o}C; FTIR

(KBr, V_{max}, cm^{-1}): 1001 (C – F *str*), 1098 (C – O)*str*), 1641 (N – H *def*), 3365 (N – H *str*). ^1H NMR in $CDCl_3$ (values in δ ppm): 1.52 (3H, s, Ar-CH_3), 3.77 (1H; s, NH), 4.18 (3H, s, OCH3), 5.30 (1H; s, NH), 6.61 - 6.84 (6H, m, Ar-H), 7.17- 7.25 (6H; m, Ar-H), 8.02 (1H; s, NH)

Bovine Serum Denaturation Study[2] *:*

In a 10 mL volumetric flask were mixed 1 mL of bovine serum albumin solution (70 mg bovine serum albumin in 1000 mL phosphate buffer pH 7.4), 1 mL of test/ standard/control solution (concentration of 1 millimolar/ mL prepared in a solution containing 60 mg tween 20 in 1000 mL phosphate buffer.) and volume made up to 10 mL using phosphate buffer. The contents were transferred to a test tube which was loosely plugged with non absorbable cotton. The test tube was incubated at 37 ± 2 °C for 15 minutes using an incubator to induce the reaction. Subsequently, the test tube was transferred to a water bath and heated to 60 ± 1 °C for 10 minutes which induced denaturation. The mixture was cooled and the absorbance of the solution was measured at 660 nm using a spectrophotometer. The percentage inhibition of denaturation was calculated from the formula:

$$\% \text{ Inhibition} = 100 \times (1 - A_T/A_C)$$

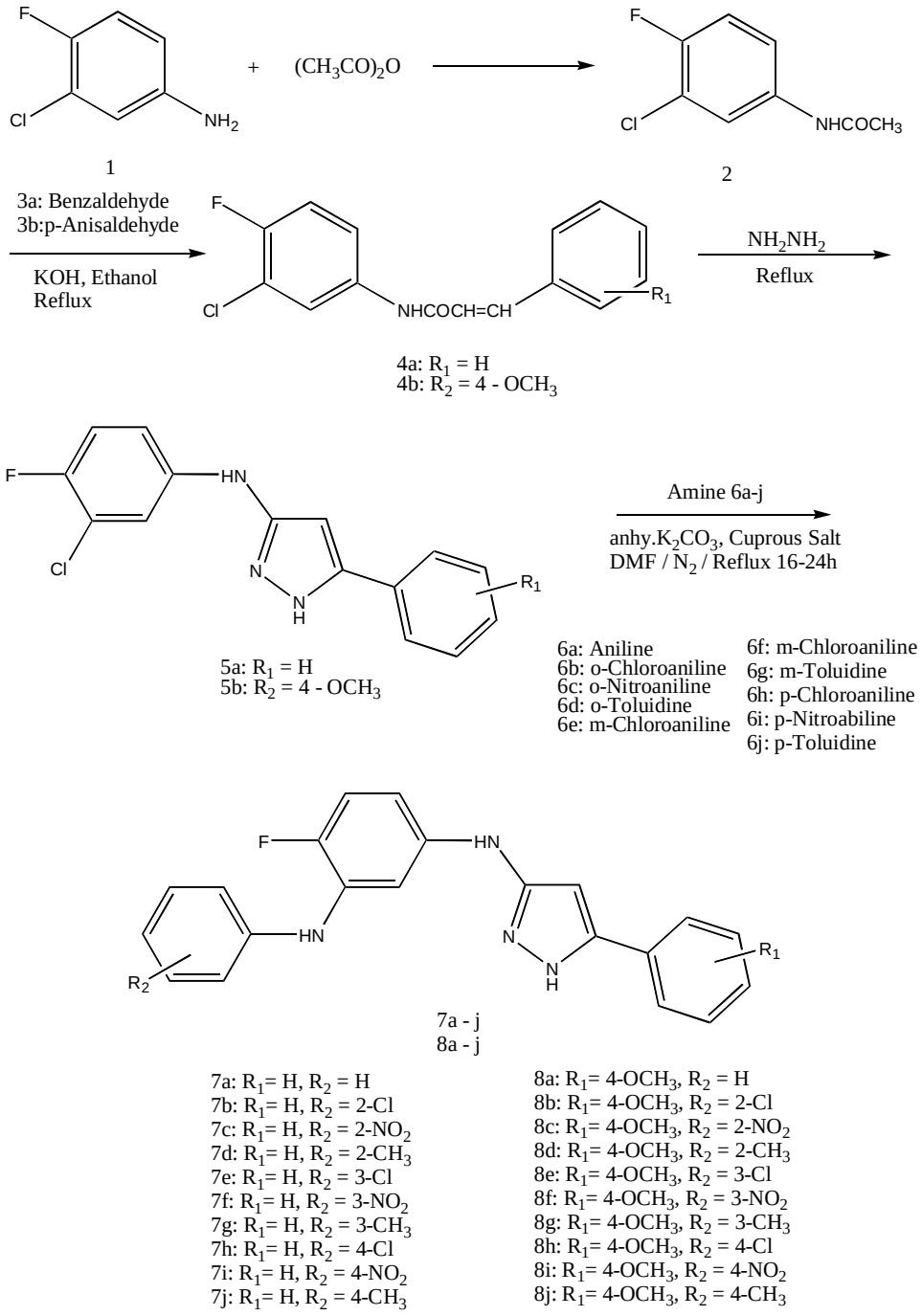
Where A_T is absorbance of test or standard and A_C is absorbance of control. The values indicated are mean values of three readings (table 1).

Antiinflammatory activity[10,11]*:*

In vivo antiinflammatory activity was evaluated by carrageenan induced rat paw method. Wistar rats of either sex weighing between 150 – 170 g were starved overnight with access to water ad libidum. The test compounds or standard indomethacin was administered orally at a dose of 100mg/kg body weight as a suspension. Inflammation induced in the left hind paw by injecting 0.05 ml of 1% carrageenan in the sub plantar region at 30 mins after oral dosage and the time noted as 0 h. The paw volume measured by a plethysmograph at 0 h, 1h, 3h and 6h respectively with reference to a mark just above the tibio-tarsal junction. The percentage inhibition of oedema calculated as $100(1-V_t/V_C)$, where V_t and V_c are volume of test/ standard and control respectively. The values expressed as mean ± SEM, n = 6 and statistical significance determined by one way ANOVA with significance $p < 0.05$ (table 2)

Computational studies :

Structures of test molecules were drawn by molecular editor at web based molinspiration.com and corresponding 'Smiles' for each molecule determined. The drug likeness scores: G-protein coupled receptor ligand (GPCR-L), ion channel modulation (ICM), kinase inhibition (KI), nuclear receptor –ligand (NR-L), protease inhibition (PI), enzyme inhibition scores (EI) for each molecule found using the 'smiles.'(table 3).



7a: R_1= H, R_2 = H
7b: R_1= H, R_2 = 2-Cl
7c: R_1= H, R_2 = 2-NO_2
7d: R_1= H, R_2 = 2-CH_3
7e: R_1= H, R_2 = 3-Cl
7f: R_1= H, R_2 = 3-NO_2
7g: R_1= H, R_2 = 3-CH_3
7h: R_1= H, R_2 = 4-Cl
7i: R_1= H, R_2 = 4-NO_2
7j: R_1= H, R_2 = 4-CH_3

8a: R_1= 4-OCH_3, R_2 = H
8b: R_1= 4-OCH_3, R_2 = 2-Cl
8c: R_1= 4-OCH_3, R_2 = 2-NO_2
8d: R_1= 4-OCH_3, R_2 = 2-CH_3
8e: R_1= 4-OCH_3, R_2 = 3-Cl
8f: R_1= 4-OCH_3, R_2 = 3-NO_2
8g: R_1= 4-OCH_3, R_2 = 3-CH_3
8h: R_1= 4-OCH_3, R_2 = 4-Cl
8i: R_1= 4-OCH_3, R_2 = 4-NO_2
8j: R_1= 4-OCH_3, R_2 = 4-CH_3

Scheme 1: Synthesis of substituted pyrazoles from 4-fluoro-3-chloroaniline.

Table 1. Bovine serum albumin denaturation activity of synthesized pyrazoles.

Compound Code	Absorbanceat 660 nm	% Inhibition ofdenaturation
7a	0.058	27.5
7b	0.074	7.5
7c	0.057	28.75
7d	0.066	17.5
7e	0.051	36.25
7f	0.048	40
7g	0.072	10
7h	0.058	27.5
7i	0.047	41.25
7j	0.057	28.75
8a	0.047	41.25
8b	0.049	38.75
8c	0.062	22.5
8d	0.065	18.75
8e	0.070	12.5
8f	0.043	46.25
8g	0.051	36.25
8h	0.045	43.75
8i	0.044	45
8j	0.050	37.5
Control	0.080	-
Standard (Diclofenac Sodium)	0.042	47.5

Table 2. Anti-inflammatory activity of tested compounds using acute carrageenan induced rat paw oedema in rats at a concentration of 100 mg/kg body weight.

Compound	1 hour		3 hour		6 hour	
	Mean swelling volume (mL) [a,b]	% inhibition of oedema	Mean swelling volume (mL)[a,b]	% inhibition of oedema	Mean swelling volume (mL)[a,b]	% inhibition of oedema
Control	0.1683 ± 0.0047		0.5567 ± 0.0111		0.6250 ± 0.0423	
Indomethacin	0.0983 ± 0.0030	41.58	0.1167 ± 0.0042	79.04	0.0900 ± 0.0044	85.6
7a	0.1367 ± 0.0067	18.81	0.3217 ± 0.0040	42.21	0.4200 ± 0.0190	32.8
7h	0.1133 ± 0.0042	32.67	0.2167 ± 0.0117	61.07	0.2767 ± 0.0125	55.73
7i	0.1383 ± 0.0030	17.82	0.2833 ± 0.0092	49.10	0.3683 ± 0.0147	41.06
7j	0.1233 ± 0.0042	26.73	0.3133 ± 0.0120	43.71	0.4983 ± 0.0113	20.26
8a	0.1417 ± 0.0060	15.84	0.3367 ± 0.0133	39.52	0.4917 ± 0.0091	21.33
8h	0.1217 ± 0.0070	27.72	0.2133 ± 0.0067	61.67	0.3367 ± 0.0105	46.13
8i	0.1483 ± 0.0095	11.88	0.3700 ± 0.0173	33.53	0.5117 ± 0.0114	18.13
8j	0.1283 ± 0.0079	23.76	0.3233 ± 0.0184	41.90	0.3983 ± 0.0119	36.26

[a] Values expressed as mean ± SEM [b] Statistically significant from control at $p < 0.05$

Table 3. Computational Data from Molinispiration Studies

Comp Code	Drug Likeness Scores					
	GPCR-L	ICM	KI	NR-L	PI	EI
7a	- 0.09	- 0.22	0.69	- 0.37	- 0.21	- 0.05
7b	- 0.10	- 0.22	0.75	- 0.40	- 0.28	- 0.10
7c	- 0.19	- 0.33	0.49	- 0.59	- 0.40	- 0.15
7d	- 0.14	- 0.27	0.69	- 0.40	- 0.27	- 0.10
7e	- 0.10	- 0.22	0.65	- 0.38	- 0.27	- 0.10
7f	- 0.21	- 0.24	0.48	- 0.42	- 0.31	- 0.14
7g	- 0.13	- 0.29	0.62	- 0.39	- 0.26	- 0.13
7h	- 0.09	- 0.22	0.64	- 0.38	- 0.24	- 0.09
7i	- 0.21	- 0.24	0.48	- 0.42	- 0.31	- 0.14
7j	- 0.12	- 0.28	0.62	- 0.38	- 0.26	- 0.11
8a	- 0.13	- 0.28	0.60	- 0.36	- 0.25	- 0.10
8b	- 0.13	- 0.28	0.66	- 0.38	- 0.31	- 0.14
8c	- 0.22	- 0.37	0.42	- 0.56	- 0.43	- 0.18
8d	- 0.17	- 0.32	0.61	- 0.39	- 0.31	- 0.14
8e	- 0.13	- 0.28	0.57	- 0.37	- 0.31	- 0.14
8f	- 0.23	- 0.29	0.41	- 0.41	- 0.34	- 0.18
8g	- 0.17	- 0.34	0.54	- 0.38	- 0.30	- 0.16
8h	- 0.13	- 0.27	0.56	- 0.37	- 0.28	- 0.13
8i	- 0.23	- 0.29	0.42	- 0.40	- 0.34	- 0.18
8j	- 0.16	- 0.33	0.54	- 0.37	- 0.29	- 0.15

Results and Discussion

All the synthesized compounds were characterized by IR and proton NMR techniques. Moderate to good activity was observed in the bovine serum denaturation assay. Series 8 molecules were better active in the *in vitro* assay. However, no specific activity could be attributed to the presence of a particular substituent on the benzene ring. Results from the *in vivo* carrageenan induced rat paw oedema model for antiinflammatory activity in rats indicate good to moderate activity, though not comparable to standard indomethacin. The molecules were effective upto 3rd hour with a subsequent plateauing of activity. The presence of chlorine (7h, 8h) remarkably increased the antiinflammatory activity. The relatively better enzyme inhibition(EI) scores (table 3) suggests an interaction at molecular level with various active sites in the enzyme with a possibility of hydrogen bonding. An in depth study with more molecules and better models maybe needed to ascertain the hypothesis.

Conclusion

Suitably substituted pyrazoles could be ideal candidates for antiinflammatory activity. The presence of chlorine at the para position

is of great significance for better enzyme inhibition and better antiinflammatory activity. Though *in vitro* bovine serum denaturation assay indicated moderate activity, the data could neither be directly correlated to the *in vivo* antiinflammatory activity nor attributed to any specific substitution on the aromatic ring. More halogenated derivatives need to be synthesized and evaluated for antiinflammatory activity to validate its role in enhancing activity.

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References

1. Dey N.C., Debashish Sinha and P.K. Dey.eds, Text Book of Pathology. New central book agency (pvt) ltd. Calcutta 2nd edn.; pp 6.1 - 6.59 (1995).
2. Williams L.A., O'Connar A., Latore L., Dennis O., Ringer S., Whittaker J.A., Conrad J., Vogler B., Rosner H., Kraus W. *West Indian Med J. 57(4),* 327-31 (2008).
3. Sharma P.K. Chandak N., Pawan Kumar, Sharma C. and Aneja K. R. Synthesis and biological evaluation of some 4-functionalized pyrazoles as antimicrobial agents. *Eur.J. Med.Chem. 46,* 1425–1433 (2011).
4. Dung-Jia Wang, Ling Fan, Chu-Yang Zheng Zhang-Dong Fang. Synthesis and antimicrobial activity of some new fluorinated 1H-pyrazoles. *J.Fluorine.Chem. 131,* 584–586 (2010).
5. Menozzi G., Mosti L., Schenone P, D'Amico M., Falzarano C., Rossi F. N-substituted 4-carboxy-1-phenyl-1H-pyrazole-5-propanamides with antiinflammatory, analgesic, antipyretic and platelet antiaggregating activities. *II Farmaco.* 48, 539-549 (1993).
6. G. Lavanya, L. Mallikarjuna Reddy, V. Padmavathi and A. Padmaja. Synthesis and antimicrobial activity of (1,4-phenylene) bis (aryl sulfonyl) pyrazoles and isoxazoles. *Eur. J. Med.Chem. 73,* 187–194 (2014).
7. Castagnolo D., De Logu A, Marco Radi, Bechi B, Manetti F, Pagano M, Supino S, Rita M, Saddi M and Botta M. Synthesis, biological evaluation, and SAR study of novel pyrazole analogues as inhibitors of *Mycobacterium tuberculosis*: Part 2. Synthesis of rigid pyrazolones. *Bioorg. Med. Chem. 19,* 2203–2205 (2009).
8. Brasca M. G., Albanese C., Alzani R., Amici R., Avanzi N., Ballinari D. *et al.* Optimization of 6,6 – dimethyl pyrallo[3,4-c]pyrazoles:identification of PHA-793887, a potent CDK inhibitor suitable for intravenous dosing. *Bioorg. Med. Chem. 18,* 1844–1853 (2010).
9. David L. Selwood, David G. Brummett, Joanna Budworth, Guillame E. Brutin, Richard O Campbell, Surinder S. Chana *et al.* Synthesis and biological evaluation of novel pyrazoles and indazoles as activators of the nitric oxide receptor, soluble guanylate. *J. Med. Chem. 44,* 78-93 (2001).
10. Sheth U.K., Dadkar N.K., Kamat U. G.eds in Selected topics in Experimental Pharmacology. pp 21-230 (1987).
11. S. K. Kulkarni, Ed. Hand book of experimental pharmacology, 4e.Vallabhai Prakashan, New Delhi, pp 128-130 (2004).