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Anti-inflammatory, antitubercular screening of substituted{[3-(4-bromophenyl) -1-phenyl-1H-pyrazol-4-yl] methylene}-N-phenyl amine

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ABSTRACT

The 3-(4-Bromophenyl)-1-phenyl-1H-pyrazole-4-carboxaldehyde was prepared from phenylhydrazine and 4-bromoacetophenone via Vilsmeier-Haack reaction. This aldehyde was functionalized at 4-position by various substituted anilines gave corresponding azomethine derivatives **(2a-g)**. The newly synthesized compounds structures were established by chemical and spectral analysis (IR, NMR). Pharmacological evaluation of this series showed anti-inflammatory and antitubercular activity.

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KEYWORDS

Pyrazole;
Azomethine;
Anti-inflammatory;
Antitubercular.

INTRODUCTION

The pyrazole nucleus in general and its chemistry[1,2] has found considerable attention during decades due to outstanding biological activities as anti-inflammatory[3,4] antipyretic, analgesic, antitubercular[5-8], antianxiety[9], hypoglycemic[10] as well as its good antibacterial and antifungal properties[11-13]. The 3-(4-Bromophenyl)-1-phenyl-1H-pyrazole-4-carboxaldehyde was synthesized from 4-bromoacetophenone (25 mmol) and phenylhydrazine (27 mmol) using Vilsmeier-Haack reagent (DMF-$POCl_3$). The corresponding carboxaldehyde **(2)** (2 mmol) were functionalized by various substituted anilines (2.20 mmol) in methanol gave corresponding azomethine derivatives **(3a-g)**. Synthesized compounds **(3a-g)** were assessed for anti-inflammatory, analgesic, antianxiety, antitubercular and antibacterial. The identities of these new azomethine derivatives have been characterized by IR, ^{1}H NMR.

MATERIALS AND METHODS

Melting points were determined in open capillaries and are uncorrected. Purity of the compounds was checked by TLC using silica gel-G plates. IR spectra (KBr disc) were recorded on Jasco FTIR spectrophotometer. ^{1}H NMR spectra ($CDCl_3$) were recorded on FTNMR Varian mercury 300 MHz using TMS as internal standard.

EXPERIMENTAL

1. Synthesis of 4-bromoacetophenone phenylhydrazone (1)

A mixture of phenylhydrazine (27 mmol), 0.5 ml acetic acid and 4-bromoacetophenone (25 mmol) was added in 75 ml of freshly distilled ethanol in round bottom flask fitted with condenser. The reaction mixture was refluxed for 4-5 hours and the progress of reaction

monitored by thin layer chromatography. The reaction mixture was cooled and the precipitate was filtered, washed with cold water and recrystallized from ethanol.

2. Synthesis of 3-(4-bromophenyl)-1-phenyl-1H-pyrazole-4-carboxaldehyde (2)

A solution of 4- bromoacetophenone phenylhydra zone **(1)** (11.76 mmol) in DMF (5 ml) was prepared and added drop wise to the Vilsmeier- Haack reagent, which was then warmed at room temperature and re-fluxed for 8 hours. The reaction mixture was allowed to cool at room temperature. The cold saturated potassium carbonate solution was added until no further precipitation occurs. The precipitate was filtered, strongly washed with water and recrystallized from methanol.

3-(4-Bromophenyl)-1-phenyl-1H-pyrazole-4-carboxaldehyde (2)

Yield (60%), brown crystals, M.P.= 144-146^{0}C. IR (cm^{-1}) 3125.08(Ar C-H), 1673.91 (Ar CHO), 1597.73 (C=N), 1225.54 (C-N). ^{1}H – NMR ($CDCl_3$) δ (ppm) 7.30-7.42 (m, 4H, $H_{2,3,5,6}$; 3-phenyl), 7.45-7.61 (m, 3H, $H_{3,4,5}$ 1-phenyl), 7.79-7.91(m, 2H; $H_{2,6}$ 1-phenyl), 8.58 (s, 1H; H_5 pyrazole), 10.08 (s, 1H; CHO).

3. General procedure for preparation of azomethine derivatives (3a-g)

A mixture of compound **(2)** (2.0 mmol), 0.5 ml acetic acid and various anilines (aniline, 2-chloroaniline, 4-chloroaniline, 2-methoxyaniline, 4-methoxyaniline, 2,4-xylidine and 2,6-xylidine) (2.20 mmol) was added in 90 ml methanol in round bottom flask and reaction mixture was refluxed for 5-7 hours. After completion of reaction as monitored and confirmed by TLC, methanol was removed by rotary evaporation to obtain the crude product. The residue was recrystallized to give corresponding azomethine derivatives.

3.1. N-{[3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl]methylene}aniline (3a)

Yield (62%), M.P. = 108-110^{0}C. IR (cm^{-1}) 3065.62 (Ar C-H), 1499.38 (Ar C=C), 1621.84 (C=N), 1591.95 (ali C=N), 1344.14 (Ar C -N). ^{1}H-NMR ($CDCl_3$) δ (ppm) 7.214-7.851 (m, 14H, $H_{2,3,4,5,6}$ 1-phenyl, $H_{2,3,5,6}$ 3-phenyl and $H_{2,3,4,5,6}$ N- phenyl), 8.484 (s, 1H, H_5, pyrazole, 8.538 (s, 1H, CH=N).

3.2. N- {[3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl] methylene}-2-chloroaniline (3b)

Yield (84%), M.P.= 98-100^{0}C, I.R.(cm^{-1}) 3072.18 (Ar C-H), 1509.28 (Ar C=C), 1625.67 (C=N), 1360.25 (C-N), 1550.20 (ali CH=N). ^{1}H – NMR ($CDCl_3$) δ (ppm) 7.003-7.815 (m, 13H, $H_{2,3,4,5,6}$ 1 phenyl, $H_{2,3,5,6}$ 3- phenyl and $H_{3,4,5,6}$ N- phenyl), 8.458 (s, 1 H,H_5, pyrazole), 8.623 (s, 1 H,CH=N).

3.3. N-{[3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl] methylene}-4-chloroaniline (3c)

Yield (53%), Brown, M.P. = 218-220^{0}C, I.R. (cm^{-1}) 3062.41 (Ar C-H), 1498.42 (Ar C=C), 1618.95 (C=N), 1333.53 (C-N), 1596.77 (ali CH=N). ^{1}H – NMR ($CDCl_3$) δ (ppm) 7.029-7.844 (m, 13H, $H_{2, 3,4,5,6}$ 1 phenyl, $H_{2,3,5,6}$ 3- phenyl and $H_{2,3,5,6}$ N- phenyl), 8.460 (s, 1 H, H5, pyrazole), 8.518 (s, 1 H, CH=N).

3.4. N-{[3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl] methylene}- 2-methoxyaniline (3d)

Yield (61%), Yellow, M.P. = 133-135^{0}C, I.R. (cm^{-1}) 3050.83 (Ar C-H), 1504.20 (Ar C=C), 1640.16 (C=N), 1216.86 (C-N), 1598.70 (ali CH=N). ^{1}H – NMR ($CDCl_3$) δ (ppm) 3.816 (s, 3H, OCH_3 of N-phenyl), 6.888-7.805 (m, 13H, $H_{2, 3,4,5,6}$ 1 phenyl, $H_{2,3,5,6}$ 3-phenyl and $H_{3,4,5,6}$ N- phenyl), 8.546 (s, 1 H, H5, pyrazole), 8.618 (s, 1 H, CH=N).

3.5. N-{[3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl] methylene}-4-methoxyaniline (3e)

Yield (80%), Brown, M.P. = 108-110^{0}C, I.R. (cm^{-1}) 3059.51 (Ar C-H), 1501.31 (Ar C=C), 1661.37 (C=N), 1362.46 (C-N), 1588.09 (ali CH=N). ^{1}H – NMR ($CDCl_3$) δ (ppm) 3.804 (s, 3H, OCH_3 of N-phenyl), 6.881-7.91 (m, 13H, $H_{2,3,4,5,6}$ 1 phenyl, $H_{2,3,5,6}$ 3- phenyl and $H_{2,3,5,6}$ N- phenyl), 8.445 (s, 1 H, H5, pyrazole), 8.599 (s, 1 H, CH=N).

3.6. N-{[3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl]methylene}-2,4-dimethylaniline (III f).

Yield (72%), Brown, M.P. = 185-187^{0}C, I.R. (cm^{-1}) 3027.69 (Ar C-H), 1504.20 (Ar C=C), 1675.84 (C=N), 1355.71 (C-N), 1596.77 (ali CH=N). ^{1}H– NMR ($CDCl_3$) δ (ppm) 2.410 (s, 6H, 2,4- CH_3 of N-phenyl), 7.226-7.922 (m, 13H, $H_{2,3,4,5,6}$ 1 phenyl, $H_{2,3,5,6}$ 3- phenyl and $H_{3,5,6}$ N- phenyl), 7.947 (s, 1 H,H_5, pyrazole), 8.310 (s, 1 H,CH=N).

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TABLE 1: Anti-inflammatory activity by carrageenan induced rat paw oedema method

Group no.	Compound no.	Mean paw oedema volume(±S.E.M.)	Percentage inhibition (%)
1	Control	2.05±0.007	-
2	Indomethacin	0.595±0.013	71.21
3	(3a)	1.20±0.011	41.46
4	(3b)	1.04±0.016	49.26
5	(3c)	0.74±0.020	63.90
6	(3d)	1.22±0.017	40.48
7	(3e)	0.71±0.008	65.36
8	(3f)	1.08±0.017	47.31
9	(3g)	0.69±0.027*	66.34

TABLE 2: Antitubercular activity by proportion method using Lowenstein- Jensen media

Sr. no.	Group	% Growth observed after 42 days		
		1	2	3
1	Control	+++	+++	+++
2	Rifampicin	-	-	-
3	Isoniazid	-	-	-
4	(3a)	++	++	+
5	(3b)	-	+	-
6	(3c)	-	+	-
7	(3d)	+	+	+
8	(3e)	+	+	+
9	(3f)	+	+	+
10	(3g)	-	-	-

Key to symbols: No growth = - (below 1%) Mild growth = + (1-50%), Moderate growth= ++ (50-100%) Severe growth= +++ (equal or ≥ 100)

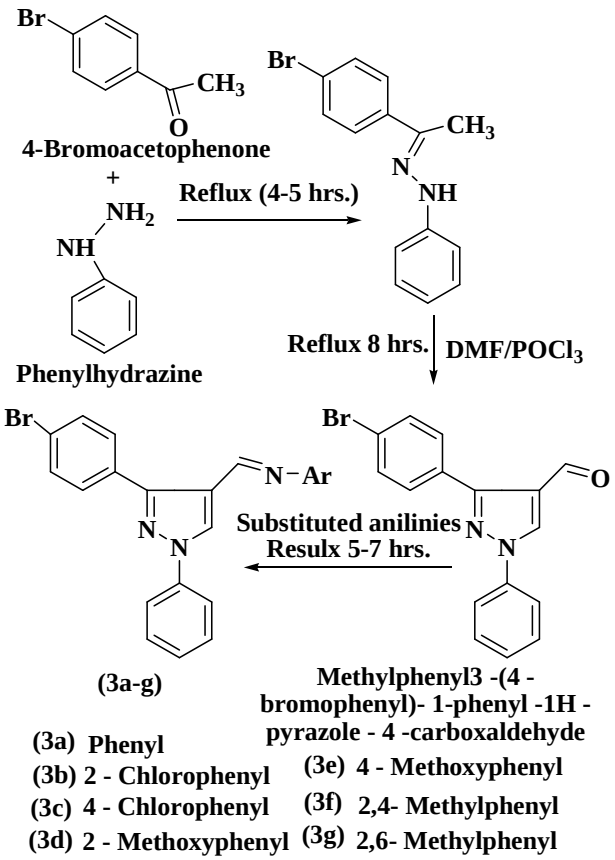
3.7. N-{[3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl]methylene}-2,6-dimethylaniline(3g)

Yield (78%), Brown, M.P. =76-78^{0}C, I.R.(cm^{-1}) 3064. 33(Ar C-H), 1504.20 (Ar C=C), 1633.41 (C=N), 1347.03 (C-N), 1596.77(ali CH=N). ^{1}H-NMR ($CDCl_3$) δ (ppm) 2.167 (s, 6H, 2,6- CH_3 of N- phenyl), 7.225-7.823 (m, 13H, $H_{2,3,4,5,6}$ 1 phenyl, $H_{2,3,5,6}$ 3-phenyl and $H_{3,4,5}$ N- phenyl), 8.499 (s, 1 H, H5, pyrazole), 8.667 (s, 1 H, CH=N).

Biological activity

1. Anti-inflammatory activity against carrageenan induced rat paw oedema

Wistar rat weighing in the range of 180-220 gm were used for the experimental work. The animals were fed with standard diet and water *ad libitum*. The animals were acclimatized for one week under standard laboratory conditions prior to experimental work. Adult wistar rats of either sex were divided into three groups each of six animals. The test compounds, indomethacine



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(10 mg/kg) and the control rats received the equivalent amount of DMSO solution. Inflammation was induced by injecting 0.1 ml of 1% w/v of solution of carrageenan into the subplanter surface of right hind paw. Paw edema was measured with a plethysmometer (model UGO-Basile 7140, Itely), at 3 hours after the injection of carrageenan and the inhibitory activity was calculated and reported in TABLE 1

2. Antitubercular activity (Lowenstein-Jensen medium)

The antitubercular screening of synthesized compounds was carried out by proportion method using Lowenstein-Jensen egg medium (L J medium) as per modification introduced and recommended by the International Union Against Tuberculosis and Lung Disease (IUATLD) against H_{37} RV strain. Test compounds 100μg/ml, standard drug rifampicin 40μg/ml in DMSO, isoniazid 0.2μg/ml in sterile distilled water as well as control L J medium was inoculated with mycobacterium tuberculosis of H_{37} RV strain. The medium inocu-

lated was incubated for 37^{0}C for 6 weeks. Total number of colonies in control, test compound and standard drug counted on the 42nd day and the percentage resistance was calculated and reported in TABLE 2

RESULT AND DISCUSSION

The most widely used primary test to screen new anti-inflammatory agents measures the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent. This edema depends on the precipitation of kinins and polymorphonuclear leukocytes with their pro-inflammatory factors including prostaglandins. The initial phase observed around one hour is attributed to the release of histamine and serotonins; the second, accelerating phase of swelling is due to the release of prostaglandin like substitutes. The present result indicated that the compounds **(3c, 3e** and **3g)** showed maximum inhibition of rat paw edema at the end of three hours, similar to the standard drug indomethacine. The edema supressant effects were found to be significant as compared to control.

Antitubercular activity of synthesized compounds **(3a-g)** was screened by proportion method using Lowenstein- Jensen media. Test compounds 100μg/ml in DMSO, standard drug isoniazid 0.2μg/ml in sterile distilled water and rifampicin 40μg/ml in DMSO were used for activity. The average number of colonies obtained for the tests compound containing slopes indicates the number of resistant bacilli contained in the inoculum. Total number of colonies in control, test compound and standard drug counted on the 42nd day and the percentage resistance was calculated as the ratio of number of colonies on the tests compound containing medium to those on the control medium multiply by hundred.

It was observed that compounds **(3b)** and **(3g)** showed below 1% growth. These compounds were classified as resistant. The sensitive class of compounds was **(3a)** observed severe growth.

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