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Synthesis, physicochemical, biological and pharmacological studies of some new 5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazoles

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ABSTRACT

Amination of 5-aminoarylamine-3-methyl-1-substituted pyrazole-4-carboxaldehydes with aromatic or aliphatic amines gives new ***12 (13 – 24)*** *compounds 5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazoles* ***(13 – 24)****. All the newly synthesized products were characterized by the spectral studies. The R_f, pKa and Molar absorptivity studies were carried out for all the compounds. They were screened for antibacterial, analgesic and anti-inflammatory studies. The pKa was correlated with the analgesic study and anti-inflammatory study and the plots were obtained.*

Keywords: Pyrazole, pKa, analgesic, anti-Inflammatory, antimicrobial.

INTRODUCTION

Pyrazoles refers both to the class of simple aromatic ring organic compounds of the heterocyclic series characterized by a 5-membered ring structure composed of 3-carbon atom and two nitrogen atom in adjacent position and to the unsubstituted parent compound. Being so composed and having pharmacologic effects on humans, they are classified as alkaloids, although they are rare in nature [1]. But the synthetic derivatives of Pyrazoles are used for their analgesic, anti-inflammatory, antipyretic, antiarrhythmic, and tranquilizing, muscle relaxant, psychoanaleptic, and anticonvulsant, and monoamineoxidase inhibitor, antidiabetic activities [2], for anticancer property against Human Laryngeal Carcinoma [3,4]. Several of the derivatives are used as herbicides [5], for stimulating mammalian coronary vasodilatation for therapeutic purposes [5], antibacterial activity [6] and screened against fungi [7]. Certain substituted pyrazole derivatives are also used as antiviral agents [5], antifertility agents [8], antifilarial agents [9].

The target of the present work was the synthesis of the new **12 (13 – 24)** compounds of 5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole series due to their importance in medicinal chemistry. Herein we reported the synthesis, characterization, antibacterial, analgesic and anti-inflammatory activity of all the new 12 compounds.

MATERIALS AND METHODS

All chemicals used for the synthesis were of reagent grade. The initial compounds were prepared as per known literature procedures [10, 11]. ^{1}H NMR spectra were recorded on AS 400 MHz Varian NMR spectrometer using TMS as an internal standard. IR spectra were recorded by using AFFINITY-1 FTIR SPECTROPHOTOMETER IR solution Version 1.50SU Shimadzu

Corporation. Mass spectra were recorded on QTOF – Micromass-UK. Melting points were determined by using INDO Melting Point M-AB-92 apparatus and were uncorrected. All the reactions were monitored by thin layer chromatography (TLC) using Silica gel G coated plates.

The crude compounds were purified by recrystallization from Acetone. ^{1}H NMR spectra and Mass spectra were recorded for two representative compounds **13** and **14** respectively of two series.

Physicochemical Studies

The physico-chemical studies were carried out for all the newly synthesized compounds (**13 – 24)** which includes Solubility test, pKa values and Molar absorptivity (ε_{max}) values determination.

Table 1. Solubility Test

Compounds Code	Water	Acetone	Chloroform	Ether	Benzene	Methanol
13	Insoluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Insoluble	Soluble
14	Insoluble	Soluble	Soluble	Soluble	Insoluble	Soluble
15	Insoluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Insoluble	Soluble
16	Insoluble	Sparingly Soluble	Soluble	Soluble	Insoluble	Soluble
17	Insoluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Insoluble	Soluble
18	Insoluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Insoluble	Soluble
19	Insoluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Insoluble	Soluble
20	Insoluble	Sparingly Soluble	Insoluble	Insoluble	Insoluble	Soluble
21	Insoluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Insoluble	Soluble
22	Insoluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Insoluble	Soluble
23	Insoluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Insoluble	Soluble
24	Insoluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Insoluble	Soluble

In Solubility test, the compounds were dissolved in different solvents in room temperature by taking around 10 mg in 10 ml solvent and there solubility was tabulated. Investigation on the solubility test data (Table 1) showed that the compounds were completely insoluble in **Water**. But all the compounds were soluble in **Methanol**. In **Acetone** the compounds **13, 14, 16, 18** were soluble but the compounds **15, 17, 19, 20, 21, 22, 23** and **24** were sparingly soluble. In **Chloroform** and **Ether** both the compounds **14** and **16** were soluble while all rest of the compounds **13, 15, 17, 18, 19, 20, 21, 22, 23, 24** is sparingly soluble. Finally, in **Benzene** all the compounds **13-24** were

pKa values were determined for the newly synthesized twelve compounds by the standard Potentiometric analysis [12]. This involves the measurement of the pH by pH meter (ECPC

TUTOR, Eutech instruments, Singapore, Sr.No.242510) followed by the concentration of ionized and non-ionized part of the test compound, which is neutralization procedure by a standard acidic or basic solution. All the tests are carried out at temperatures 23 ˚C using 10%

Acetone+ Water solvent system. pKa is calculated by applying the Henderson- Hasselbach equation for each addition of titrant and then the mean volume is to be expressed.

For an acidic drug: $pKa = pH + \log [HA]/ [A^-]$
For an basic drug: $pKa = pH + \log [BH^+]/ [B]$

where, HA = Unionized form of acid.
A^- = Ionized form of acid.
BH^+ = Ionized form of base.
B = Unionized form of the base.

Investigation on the pKa data (Table 2) revealed that the compound **13** has a less pKa value. While the compounds **14, 15, 16** has higher pKa values. Again the compounds **17** and **18** have lower pKa values. The pKa values of the compounds **21** and **23** are less in comparison to the **22** and **24**.

Table 2. pKa Values of 5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole compounds (13 – 24)

Compound Code	pKa
13	5.12
14	8.01
15	8.83
16	8.13
17	4.89
18	7.86
19	5.71
20	9.48
21	4.79
22	9.68
23	9.66
24	10.59

Molar absorptivity (ε_{max}) and Maximum Absorbance (A_{max}) at corresponding wavelength (λ_{max}) [13, 14] were determined employing the standard procedure using U.V. spectroscopy for the new compounds **(13-24)**. Absorbance values were determined using UV/VIS Spectrophotometer Model 1372. Compounds in solutions of 10 µg/ml in methanol were subjected in the analysis at 190 to 800 nm. The observations of absorbance at different wavelengths for each test compound solution were made and from these observations the wavelength corresponding to the maximum absorbance was traced out which is said to be the maximum absorbance (A_{max}) at the corresponding wavelength (λ_{max}). Then, the molar absorptivity (ε_{max}) was calculated using the equations given below:

$$\text{i. } E^{1\%}_{1cm} = \frac{A_{max}}{C.\,b} \quad ; \quad \text{ii. } \varepsilon_{max} = E^{1\%}_{1cm} \,.\, x / 10 \;;$$

where, $E^{1\%}_{1cm}$ = Absorbance at λ_{max} of 1% (w/v) solution,
A_{max} = Maximum Absorbance,
C = Concentration of solution expressed in grams per 100 ml (0.001%)
b = Path Length through the sample in centimeters (1cm) and
x = Molecular Weight of appropriate test compound

Results displayed in Table 3 revealed that Maximum absorbance (λ_{max}) and Molar Absorptivity (ε_{max}) values indicated the transition probability.

Table 3. Molar absorptivity (ε_{max}) and Maximum Absorbance (λ_{max}) data of 5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole (13-24

Compound Code	λ_{max}	A_{max}	ε_{max}	$_{1\%}E_{1cm}$
13	289	1.006	27799.98	1006
14	268	1.629	57412.443	1629
15	298.5	1.086	31534.05	1086
16	229	0.711	26655.306	711
17	303.5	0.988	24039.27	988
18	381	0.889	28395.549	889
19	290.5	0.811	20870.23	811
20	331	1.728	57616.704	1728
21	325	0.700	29135.65	700
22	405	1.848	67904.76	1848
23	314	0.840	25652.21	840
24	309	1.599	60998.98	1599

Scan Range – 190nm to 800nm
Solvent Used- Methanol

General procedure for synthesis of 5-aminoarylamine-3-methyl-1-substituted pyrazole-4-carboxaldehydes (7-12):
Equimolar ratio of compounds (**5** or **6**) and corresponding aliphatic or aromatic amines was refluxed for 6 hrs in presence of $POCl_3$ by taking dry ethanol (99% pure ethanol) as solvent (10 ml). The reaction mixture was allowed to cool and then poured onto crushed ice stirred with glass rod; solid was filtered, washed with cold water, dried and purified by recrystalisation from Acetone.

General procedure for synthesis of 5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole (13-24):
An equimolar (0.01 mol) of the mixture of substituted pyrazole-4-carboxaldehydes **(7-12)** and aniline or o-Toluidine in dry ethanol (99% pure ethanol) 50 ml was refluxed for 6 hrs. The reaction mixture was cooled and poured onto crushed ice while stirring continuously. The resultant solid was filtered, washed thoroughly with cold water, dried and purified by recrystalisation from Acetone.

5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole (13).
Yellowish White; Yield: 67%; m.p: 168^{o}C; I.R. (KBr): 974.09, 1291.09, 1617.38, 1703.11, 1365.12, 3501.28 cm^{-1}; Mass (ESI) m/z: 277 $(M+1)^{+}$; ^{1}H NMR (400 MHz,$CDCl_3$) δ 2.030(s, 3H, -CH_3), δ 2.445(s, 1H, --NH-), δ 2.45(s, 2H, Ar-NH), δ 7.236(m, 2H, Ar-H), δ 5.21(s, 1H, -CH=);

5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole (14).
Deep Brick Red; m.p. 47°C, yield 71%; I.R. (KBr): 920.26, 1281.09, 1618.35, 1704.18, 1365.12, 3559.78, 3423.8 cm^{-1}; Mass (ESI) m/z: 353 $(M+1)^{+}$; ^{1}H NMR ($CDCl_3$) δ 2.042(s, 3H, -CH_3), δ 2.493(s, 2H, -NH-Ar-), δ 7.137-7.925(m, 2H, Ar-H), δ 5.25(s, 1H, -CH=);

5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole (15).
Colourless; m.p. 171°C, yield 75%; I.R. (KBr): 917.28, 1292.06, at 1619.31, 1704.18, 1375.02, 3401.25 cm^{-1}.

5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole (16).
Brown; m.p. 64°C, yield 72%; I.R. (KBr): 939.37, 1300, 1613.52, 1593.27, 1380, 3655.26, 3462.37 cm^{-1}.

5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole (17).
Whitish Brown; m.p. 172°C, yield 60%; I.R. (KBr): 973.13, 1616.42, 1670, 2876.95, 758.06, 3390, 1230.03 cm^{-1}.

5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole, (18).
Brick Red; m.p. 65°C, yield 69%; I.R. (KBr): 916, 1620, 1597, 2895, 757.09, 3385, 1030, 1362.77 cm^{-1}.

5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole, (19).
Colourless; m.p. 172°C, yield 75%; I.R. (KBr): 974.09, 1601.23, 1617.38, 2885, 1355 cm^{-1}.

5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole (20).
Coffee brown; m.p. 48°C, yield 78%; I.R. (KBr): 913.12, 1602.12, 1597.13, 2873.17, 757.09, 3385.65, 1375.2 cm^{-1}.

5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole (21).
Black; m.p. 263°C, yield 80%; I.R. (KBr): 920.4, 1284, 1600, 1685, 1375, 3481.66 cm^{-1}.

5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole (22).
Black; m.p. 124°C, yield 79%; I.R. (KBr): 920, 1282.78, 1600, 1374.2, 3501 cm^{-1}.

5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole (23).
Black; m.p. 163°C, yield 82%; I.R. (KBr): 976.02, 1292.78, 1599, 1376.2, 3509.63, 1625, 3362.07 cm^{-1}.

5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole (24).
Black; m.p. 163°C, yield 82%; I.R. (KBr): 914.6, 1280.78, 1601.73, 1382.2, 3470.76, 1598.09 cm^{-1}.

Biological Activity

The newly synthesized pyrazole compounds **(13-24)** have been screened for antibacterial activity against *Staphylococcus aureus* (ATTC-25923), *Bacillus pumillus* (ATCC-7061), *Escherichia coli* (ATTC-25922) and *Vibrio cholarae* (recultured) by the paper disc method [15, 16]. Tetracycline was used as standard for antibacterial activities and Dimethyl formamide (DMF) was used as a solvent. The compounds were used in a concentration of 200 μg/ml.

Investigation on antibacterial screening data (Table 3) showed some of the compounds were active against four human pathogenic bacteria. The results of antibacterial activity reveal that all the compounds were not active against all the four organisms. Out of the newly synthesized twelve compounds only four compounds showed good activity. The compound **13** was active against Gram Negative strain *Vibrio cholera*. The compound **18** was active against Gram positive strain *Staphylococcus aureus*. While compound no. **16**, was moderately active against *Staphylococcus aureus*, another compound **20** was active against both the gram positive bacteria *Bacillus pumillus* and gram negative bacteria *Escherichia coli*. Other than these all the compounds were showing lower antibacterial activity in respect to the standard.

Table 4. Antibacterial activity data for the compounds (13-24)

Compounds Code	Dose (µg/ml)	Zone of Inhibition (mm)			
		+ve Strains		-ve Strains	
		S. aureus	*B. pumillus*	*E. coli*	*V. cholarae*
Tetracycline	200	23	24	24	23
13	200	15	18	10	22
14	200	16	15	11	14
15	200	13	14	12	13
16	200	19	10	14	16
17	200	11	16	14	14
18	200	22	11	16	13
19	200	13	12	15	15
20	200	12	19	19	12
21	200	14	17	13	14
22	200	16	12	21	11
23	200	12	14	14	13
24	200	10	15	16	9

Anti-inflammatory activity:

Anti-inflammatory activity by Carrageenan induced rat paw edema method

Anti-inflammatory activity was assessed by the method described by Winter *et al* [17]. Albino rats of either sex weighing 200 – 250 g were divided in 5 groups (N=5). Group-1 received 1ml distilled water(control), Group-2 received 0.1ml of 1% w/v suspension of carrageenan sodium salt in normal distilled water (Toxicant control), Group-3 received Diclofenac Sodium (reference standard 8mg/kg p.o.) and Group-5 were given the compounds **13-24** (200mg/kg paw edema). The standard Diclofenac Sodium and synthesized compounds under study were administered orally to all the rats. After 1 hour 0.1ml of 1% w/v suspension of carrageenan sodium salt in normal distilled water was injected into the sub plantar region of the right hind paw of the each rat to induce edema. The edema volumes of the injected paw were measured at 0hrs, 1 hrs, 2 hrs, 3 hrs and 5 hrs plethysmometrically. The difference between the paw volumes of treated animals were compared with that of the control group and the mean edema volume was calculated. % protection or inhibition in edema volume was calculated by using the formula.

Percentage protection or inhibition in edema volume was calculated by using the formula.

$$\% \text{ Protection} = 100 - [(PV5-PV0/PV5) \text{ X } 100],$$

where,

PV5 = paw volume at 5^{th} hours,
PV0 = paw volume at 0 hour

From the data obtained the mean edema volume and % protection in edema was calculated and results were displayed in Table- 5.

Table 5: Anti-inflammatory Activity of synthesized compounds (13-24) in carrageenan induced paw edema model in rats

Sl.No.	Treatments	Mean ± SEM of Paw Volume (ml) 0 hrs	1 hrs	2 hrs	3 hrs	5 hrs	% Protection
1	Normal control (1ml dist. Water (p.o.)	0.24± 0.2449	0.36± 0.2915	0.55± 0.02739	0.63± 0.3742	0.59± 0.4000	41.3
2	Standard (Diclofenac Sodium8mg/kg p.o.)	0.26 ± 0.02915	0.37 ± 0.02550	0.44 ± 0.01871	0.40 ± 0.01581	0.32 ± 0.02550	82.3
3	**13**	0.28 ± 0.03391	0.36 ± 0.03317	0.40 ± 0.02739	0.35 ± 0.2236	0.31 ± 0.02915	91.9
4	**14**	0.27 ± 0.03742	0.41 ± 0.03674	0.50 ± 0.03536	0.46 ± 0.02916	0.37 ± 0.03742	75
5	**15**	0.26 ± 0.02915	0.29 ± 0.02715	0.36 ± 0.01581	0.32 ± 0.01281	0.30 ± 0.01581	97.3
6	**16**	0.27 ± 0.02000	0.34 ± 0.02449	0.39 ± 0.02449	0.38 ± 0.02550	0.34 ± 0.03317	97.04
7	**17**	0.18 ± 0.01674	0.28 ± 0.01859	0.30 ± 0.02001	0.24 ± 0.02212	0.22 ± 0.02187	81.3
8	**18**	0.02000 0.23 ± 0.02000	0.32 ± 0.02550	0.38 ± 0.02550	0.33 ± 0.03000	0.30 ± 0.03162	75.7
9	**19**	0.19 ± 0.01868	0.24 ± 0.02231	0.28 ± 0.03123	0.32 ± 0.01668	0.33 ± 0.02876	57.33
10	**20**	0.24 ± 0.01871	0.31 ± 0.01871	0.38 ± 0.02550	0.44 ± 0.02449	0.38 ± 0.02550	62.5
11	**21**	0.24 ± 0.02449	0.31 ± 0.02786	0.38 ± 0.02550	0.40 ± 0.02210	0.42 ± 0.01876	53.5
12	**22**	0.15 ± 0.01875	0.14 ± 0.01332	0.22 ± 0.01235	0.28 ± 0.01654	0.20 ± 0.01123	79.8
13	**23**	0.17 ± 0.01998	0.20 ± 0.01700	0.25 ± 0.01565	0.22 ± 0.01465	0.20 ± 0.01576	88.05
14	**24**	0.23 ± 0.02000	0.36 ± 0.01581	0.35 ± 0.01581	0.32 ± 0.01225	0.27 ± 0.01225	89.2

% Protection = 100 – [(PV5-PV0/PV5) X 100],
PV5 = paw volume at 5th hours, PV0 = paw volume at 0 hour
Values are expressed as mean ±Standard Error Mean (SEM).
*Statistically significant ***P < 0.001, **P<0.01, *P<0.05 (Vs. Control) (n=5).*

Statistical analysis

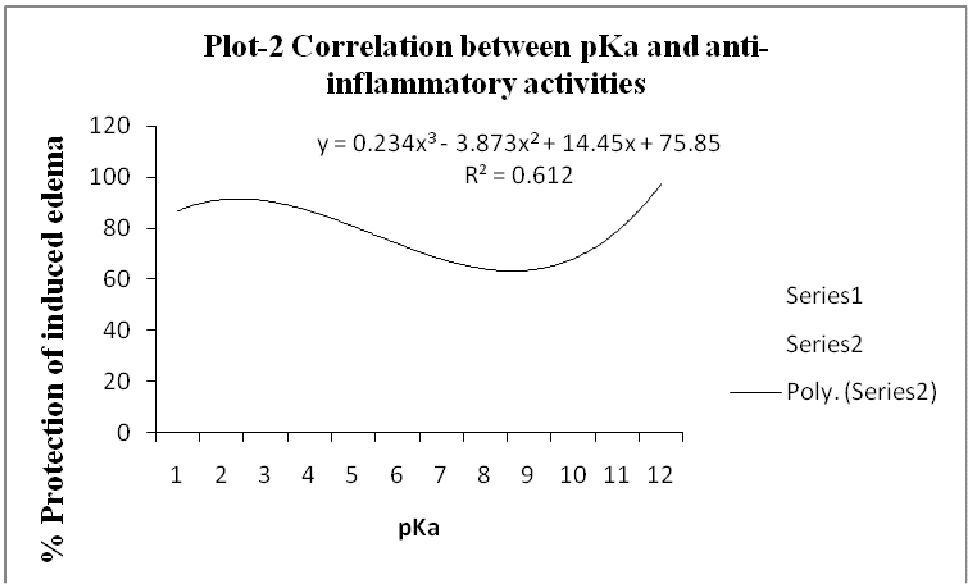
Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. P< ***0.001 was considered statistically significant.

The results of the study indicated that the compound no. **13, 15** and **16** were highly active than the standard Diclofenac Sodium, while the compound no. **23** and **24** were also comparatively more active than the standard drug. But the compound no. **17** is similar in efficacy to that of the standard drug used. The compound no. **14**, **18 and 20** showed less significant results, while compound no. **19** and **21** showed no significance at all.

Correlation of pKa values with the anti-inflammatory activity of the test compounds were studied as presented in Table 6 and plot 2 and the observations were made out. The value of R^2 is 0.612 and this establishes relationship still not the perfect one. So, we can conclude that correlation of pKa with anti-inflammatory activities is established but only to an extent.

Table 6:Correlation between pKa and Anti-inflammatory activities (Plot – 2) of synthesized compounds (13-24)

Sl. No.	Compound	pKa	% protection of produced edema
1	**13**	5.12	91.9
2	**14**	8.01	75
3	**15**	8.83	97.3
4	**16**	8.13	97.04
5	**17**	4.89	81.3
6	**18**	7.86	75.7
7	**19**	5.71	57.33
8	**20**	9.48	62.5
9	**21**	4.79	53.5
10	**22**	9.68	79.8
11	**23**	9.66	88.05
12	**24**	10.58	89.2



Analgesic activity
Analgesic Activity by Acetic acid induced Writhing Method

The compounds (13-24) all are tested for their analgesic activity using standard procedures of Acetic acid induced writhing Method [18]. Mice of either sex weighing 25 – 30 g were divided

in 3 groups (N=5). Group-1 received water and acetic acid (control), Group-2 received Acetic acid 1% v/v (10 ml/kg of body wt - Toxicant control), Group-3 received Aspirin (reference standard 200mg/kg .) and Group-5 were given the compounds **13-24** (200mg/kg).

Table 7: Analgesic Activity of synthesized compounds (13-24) in Acetic acid induced writhing model

Sl. No.	Treatment	Dose	No. of Writhing (10 min duration)	Responders	% Protection
1	**Control (water) + Acetic Acid**		14.8 ± 0.8602	5/5	0
2	**13 + Acetic Acid**	200mg + 0.1ml/kg	3.4 ± 0.2449	5/5	77
3	**14 + Acetic Acid**	200mg + 0.1ml/kg	3.4 ± 0.5099	5/5	75
4	**15 + Acetic Acid**	200mg + 0.1ml/kg	5.0 ± 0.5332	5/5	64.6
5	**16 + Acetic Acid**	200mg + 0.1ml/kg	5.0 ± 0.7746	5/5	63
6	**17 + Acetic Acid**	200mg + 0.1ml/kg	7.8 ± 0.4223	5/5	47.4
7	**18 + Acetic Acid**	200mg + 0.1ml/kg	7.8 ± 0.6633	5/5	46
8	**19 + Acetic Acid**	200mg + 0.1ml/kg	2.4 ± 0.2211	5/5	83.2
9	**20 + Acetic Acid**	200mg + 0.1ml/kg	2.4 ± 0.4000	5/5	82
10	**21 + Acetic Acid**	200mg + 0.1ml/kg	7 ± 0.7112	5/5	45
11	**22 + Acetic Acid**	200mg + 0.1ml/kg	7 ± 0.9002	5/5	45.7
12	**23 + Acetic Acid**	200mg + 0.1ml/kg	5.2 ± 0.6114	5/5	62.8
13	24 + Acetic Acid	200mg + 0.1ml/kg	5.2 ± 0.8602	5/5	62
14	Aspirin Standard	200mg + 0.1ml/kg	2.5 ± 0.8602	5/5	78.5

Values are expressed as mean ± Standard Error Mean (SEM).
****Statistically significant P < 0.001 (Vs. Control) (n=5).*

The standard Aspirin and synthesized compounds under study were administered intraperitoneally to all the mice. After that Acetic acid 1% v/v (10 ml/kg of body wt) was administered to induce writhing. Mice are then placed individually into large plastic trays and numbers of writhes are recorded for 10 min beginning from 5 min after the injection . The time

period with the greatest percent of protection of writhing is considered the peak time. Percentage protection or inhibition in edema volume was calculated by using the formula.

$$\% \text{ Protection} = 100 - \left[\frac{\text{No. of writhes of Tests/ Standard}}{\text{No. of writhes in Control}} \times 100\right]$$

From the data obtained the mean no. of writhes and % protection in writhing was calculated and results were displayed in Table- 7.

Statistical analysis

Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. P< ***0.001 was considered statistically significant.

The observations made on the analgesic studies revealed that compound no. **13** and **14** are almost on par with that of the standard drug Aspirin in their analgesic activity. But the compound no. **19** and **20** were showing activities more than the **standard**. But compound no. **15** and **16** are having comparatively less activity than the previous two compounds. Similarly two compounds **23** and **24** were also showing activity like **15** and **16**. While the compound no. **17, 18, 21** and **22** are showing less significant activities.

Correlation of pKa values with the analgesic activity of the test compounds were studied as presented in Table 8 and plot 1 and the observations were made out. The value of R^2 is 0.165 which does not establish any relationship.

$$\% \text{ Protection} = 100 - \left[\frac{\text{No. of writhes of Tests/ Standard}}{\text{No. of writhes in Control}} \times 100\right]$$

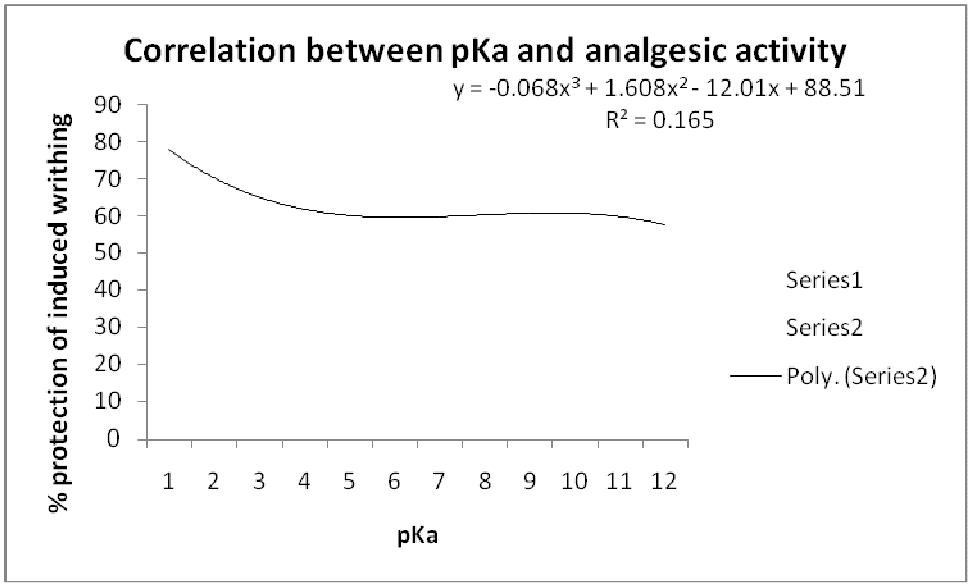


Table 8: Correlation between pKa and Analgesic activities (Plot – 2) of synthesized compounds (13-24)

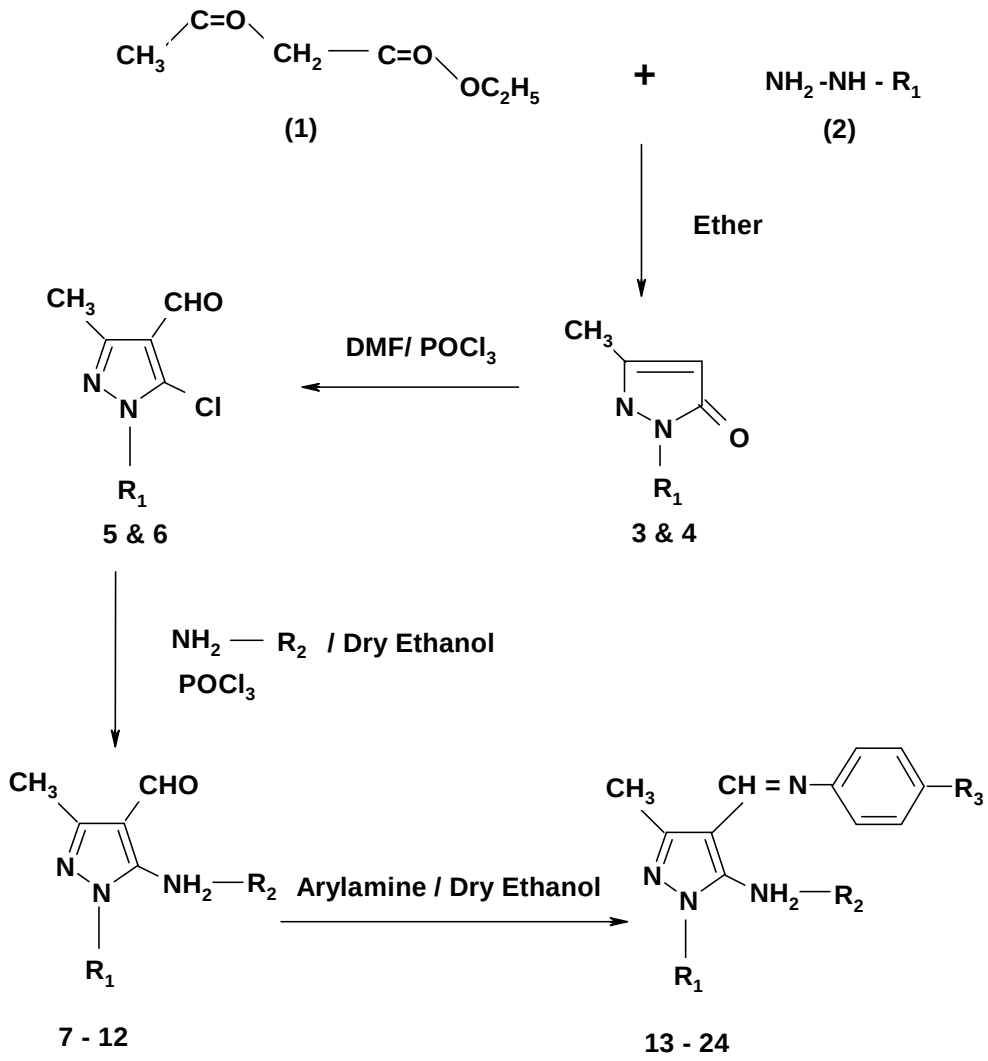
Sl.No.	Compound	pKa	% protection of produced writhing
1	**13**	5.12	77
2	**14**	8.01	75
3	**15**	8.83	64.6
4	**16**	8.13	63
5	**17**	4.89	47.4
6	**18**	7.86	46
7	**19**	5.71	83.2
8	**20**	9.48	82
9	**21**	4.79	45
10	**22**	9.68	45.7
11	**23**	9.66	62.8
12	**24**	10.58	62

RESULTS AND DISCUSSION

The initial compounds **3,4, 5** and **6** were prepared by the known literature as per the scheme I [10,11]. The compounds **3** and **4** were prepared by a general reaction. This is done by 49 ml of Ethylacetoacetate and 36.5 ml of Hydrazine Hydrate or Phenyl Hydrazine were taken in a 1000 ml beaker and heated on hot water bath for 2 hrs with continuous stirring by glass rod. 100 ml of ether was added slowly with stirring and the solidification was followed within 15 minutes, filtered, washed with ether. Resultant colorless solid was obtained [10]. The products were recrystallized from Acetone to obtain pure products. These compounds **(3&4)** were then treated with Dimethyl Formamide (DMF) of 1mole in Phosphorus Oxychloride ($POCl_3$), stirred at 60°C for 6 hrs and neutralized with Sodium Hydroxide (NaOH) giving the formylated product [11]. The products were recrystallized from Acetone to obtain pure compounds **(5&6)**. The synthesized compounds **(5&6)** and corresponding aliphatic or aromatic amines was refluxed for 6 hrs in presence of $POCl_3$ by taking dry ethanol (99% pure ethanol) as solvent (10 ml). The reaction mixture was allowed to cool and then poured onto crushed ice stirred with glass rod; solid was filtered, washed with cold water, dried and purified by recrystalisation from Acetone to obtain pure compounds **(7-12)**. An equimolar (0.01 mol) of the mixture of substituted pyrazole-4-carboxaldehydes **(7-12)** and aniline or o-Toluidine in dry ethanol (99% pure ethanol) 50 ml was refluxed for 6 hrs. The reaction mixture was cooled and poured onto crushed ice while stirring continuously. The resultant solid was filtered, washed thoroughly with cold water, dried and purified by recrystalisation from Acetone to obtain pure compounds **(13-24)**. The novel compounds were characterized by their spectral data like MASS, IR and NMR.

Physicochemical Studies

Solubility studies of the test compounds (**13-24**) were performed. It could be observed from (Table1**)** the compounds were completely insoluble in **Water**. But all the compounds (**13-24**) were soluble in **Methanol**. In **Acetone** the compounds **13,14,16,18** were soluble but the compounds **15,17,19,20,21,22,23** and **24** were sparingly soluble. In **Chloroform** and **Ether** both the compounds **14** and **16** were soluble while all rest of the compounds **13,15,17,18,19,20,21,22,23,24** is sparingly soluble. Finally, in **Benzene** all the compounds **13-24** were sparingly soluble.



SCHEME - I

The study of the pKa values given in Table 2 indicated that the compound **13** has a less pKa value which is due to the absence of any positive inductive effect. While the compounds **14, 15, 16** has higher pKa values due to the presence of positive inductive group. Again the compounds **17** and **18** have lower pKa values due to the absence of positive inductive phenyl group. The pKa values of the compounds **21** and **23** are less in comparison to the **22** and **24**, only because of the absence of methyl and phenyl group in **21** and only phenyl group in the compound **23**, respectively.

Correlation of pKa values with the pharmacological properties of the test compounds were also studied as presented in Table 23 and 24 and plots 1 & 2 and the observations were made out. It can be clearly understood from the Plot 1 that correlation of pKa with analgesic activities is not established at all. The value of R^2 is 0.165 which does not establish any relationship. But in Plot 2 the value of R^2 is 0.612 and this establishes relationship still not the perfect one. So, we can

conclude that correlation of pKa with anti-inflammatory activities is established but only to an extent.

Molar Absorptivity Studies show the Maximum absorbance (λ_{max}) and Molar Absorptivity values (ε_{max}) which were given in the Table 3 indicated the transition probability. From the λ_{max} and ε_{max} values it is clear that all the compounds exhibited allowed transitions. All the compounds showed n $\rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions [19].

Antimicrobial studies

The results of antibacterial activity of all the title compounds were given in Table 4. The results indicated that the compound **13**, which is a Schiff's base of Aniline, was active against Gram Negative strain *Vibrio cholera* showing a zone of inhibition of 22 mm, with respect to the standard Tetracycline. The compound **18**, which is a Schiff's base of Aniline having a phenyl and ethylene diamine substitution in the pyrazole ring, was active against Gram positive strain *Staphylococcus aureus* showed a zone of inhibition of 22 mm. While compound no. **16**, which is a Schiff's base of o-Toluidine and having a phenyl substitution in the pyrazole ring was moderately active against *Staphylococcus aureus* showed a zone of inhibition of 19 mm. Another compound which also showed a zone of inhibition of 19 mm is **20**, which is a Schiff's base of o-Toluidine and ethylene diamine group is present as a substitution the pyrazole ring and was active against the gram positive bacteria *Bacillus pertusis* and gram negative bacteria *Escherichia coli*. Other than these all the compounds were having comparatively lower antibacterial activity in respect to the standard.

Analgesic studies

The observations made on the analgesic studies as given in the **Table no. 20** revealed that compound no. **13** and **14** are almost on par with that of the standard drug Aspirin in their analgesic activity. But the compound no. **19** and **20** were showing activities more than the **standard**. But compound no. **15** and **16** are having comparatively less activity than the previous two compounds.

Similarly two compounds **23** and **24** were also showing activity like **15** and **16**. While the compound no. **17, 18, 21** and **22** are showing less significant activities. The variation in the results might be due to variation in the presence of the positive inductive group in the compounds. Studies revealed that when one $-CH_3$ group is attached to the compound no. **13** and **14**, compound no. **15** and **16** is obtained. It is observed that there was a decrease in analgesic activity. On the contrary, compound no. **19** and **20** showed increase in activity although both of them contain the $-CH_3$ group. This might be due to the steric hindrance which occurs due to the presence of two bulky phenyl groups along with the methyl group in compound no. **15** and **16**. The compound no. **23** and **24** also showed a good analgesic activity.

CONCLUSION

In conclusion, a series of novel 5-aminoarylamine-3-methyl-1-substituted-4-(aryliminomethyl)-pyrazole analogues were synthesized and their physico-chemical, antimicrobial and anti-inflammatory activities were evaluated. The physicochemical studies revealed that some of the new compounds posses higher pKa values while some posses lower pKa values. Molar Absorptivity Studies show the Maximum absorbance (λ_{max}) and Molar Absorptivity values (ε_{max}) indicated that all the compounds showed n π^* and π π^* transitions. The observations made on the analgesic studies revealed that compound no. **13** and **14** are almost on par with that of the standard drug

Aspirin in their analgesic activity. But the compound no. **19** and **20** were showing activities more than the **standard**. But compound no. **15** and **16** are having comparatively less activity than the previous two compounds. Similarly two compounds **23** and **24** were also showing activity like **15** and **16**. While the compound no. **17, 18, 21** and **22** are showing less significant activities. The anti-inflammatory activity studies were carried out indicated that the compound no. **13, 15** and **16** were highly active than the **standard Diclofenac Sodium**, while the compound no. **23** and **24** were also comparatively more active than the standard drug. But the compound no. **17** is similar in efficacy to that of the standard drug used. The compound no. **14**, **18 and 20** showed less significant results, while compound no. **19** and **21** showed no significance at all. Based on the present observations, the selected compounds which were found to be subjected for extensive pharmacological studies can be made to exploit the pharmacodynamic nucleus in conjugation with the different heterocyclic moieties which may result in the new molecules with quite promising microbiological and pharmacological properties.

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