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Synthesis, Characterization and Biological Activity of 1, 8-Napthyridine Nucleus Linked with Pyrazolinone, Pyrazole, Isoxazolinone and Isoxazole Derivatives

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ABSTRACT

The facile synthesis of 1,8-napthyridine nucleus linked with pyrazolinone, pyrazole, Isoxazolinone , has been achieved by diazotization of 2-(p-aminophenyl)-1,8-napthyridine (1) with sodium nitrite and Con HCl at 0-5 ℃ forms diazonium salts (2). Coupling of diazonium salts (2) with active methylene compounds like Ethyl aceto acetate, Ethyl cyano acetate, Acetyl acetone and to afford the corresponding intermediate hydrazones compounds (3-5). Hydrazono Ethyl aceto acetate (3) and Ethyl cyanoacetate (4) derivatives on treatment with hydrazine and substituted hydrazine's via Cylization gave the corresponding substituted pyrazolinone (3a-e)&(4a-e) and reaction with hydroxylamine Hcl in the presence of ethanol via cyclization afforded corresponding substituted Isoxazolinones (7&8)Hydrazono acetyl acetone (5) treatment with hydrazine and substituted hydrazines via cylization gave the corresponding substituted pyrazoles (5a-e)&(6a-e) and Reaction with hydroxylamine HCl in the presence of ethanol via cyclization afforded corresponding substituted Isoxazole (9&11). All the newly synthesized compounds were purified and subjected for their spectral analysis by IR, NMR and mass spectroscopy to elucidate their structures. The absorption peaks of hydrazono and azo groups observed at frequencies 3300-3500 & 1400-1600cm^{-}1. The keto stretching vibrations were observed at 1600-1690cm^{-1}.The H^{1} NMR spectra of synthesized compounds of multiplets shows aromatic &1,8-napthyridine protons at the δ value of 6.2-8 and methyl & NH protons observed as singlet at the δ value of 2.5 & 8.2-10.1. The newly synthesized compounds were screened for their anti-inflammatory and analgesics activities. The most active compounds 3b,4b,and 5b like N-phenyl pyrazolinone & N-phenyl pyrazoles linked 1,8-napyhridine shows better anti-inflammatory &analgesic activities.

Key words: I,8-napthyridine, Pyrazolinone, Pyrazole, Isooxazolinone, Isoxazole.

INTRODUCTION

1,8-napthyridine derivatives are reported to possess a wide spectrum of biological activities such as diuretic (Gorecki DKJ *et al*., 1977), antimalarial (Balin GB *et al*., 1984), anti-inflammatory (Kuroda T *et al*., 1992), antitumor (Chen K *et al*., 1997), antihypertensive (Ferrarini M *et al*., 1998) and antibacterial activites (Tani J *et al*., 1982, Cooper C *et al*., 1992). Pyrazolone and Isoxazolone compounds are associated with broad spectrum of biological activites Antipyrine-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one,was the first pyrazolone derivative used in the management of pain and inflammation. In the view of these, we have planned to synthesis of some 1,8-napthyridine containing pyrazolinone, pyrazole, Isoxazolinone, Isoxazole and pyrimidine-2-one derivatives, which have been found to possess an interesting profile of anti-inflammatory, along with analgesic and antimicrobial activities.

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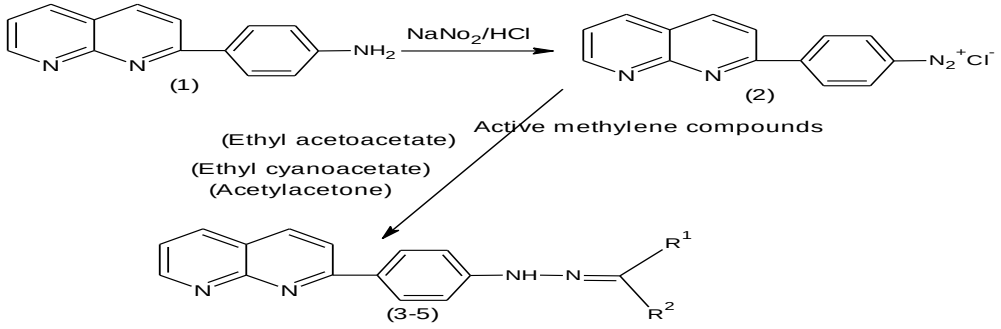
MATERIALS AND METHODS

Melting points were determined in open capillaries in electrical apparatus and are Uncorrected. IR spectra were recorded on a FT-IR spectrometer using KBR pellet. The NMR spectra were recorded in JMR spectrometer using TMS as internal standard. The Mass spectra were recorded in NCMS spectrometer. The purity of the compounds was confirmed by thin layer chromatography using silica gel glass. The spots were developed in iodine chamber and visualized under ultraviolet lamp.

Scheme.1 Synthesis of intermediate hydrazono compounds (3-5)

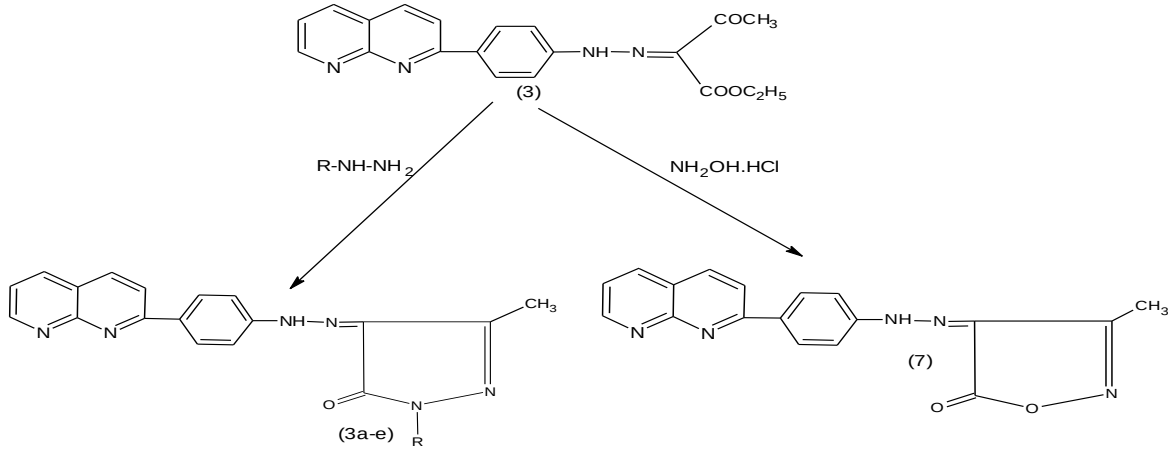
2-(P-aminophenyl)-1,8-napthyridine (1) was prepared according to the literature procedure. The diazonium salt (II) was synthesized by diazotization of 2-(P-aminophenyl)-1,8-napthyridine (I) using a mixture of sodium nitrite and HCl at 0-5 the diazomnium salt(II) was treated in ethanol in the presence of sodium acetate with active methylene compoundes ,namely Ethyl aceto acetate, Ethl cyano acetate and acetyl acetone afford the corresponding hydrazono derivatives 3-5(scheme.I).

Scheme.1 Synthesis of intermediate hydrazono compounds (3-5)



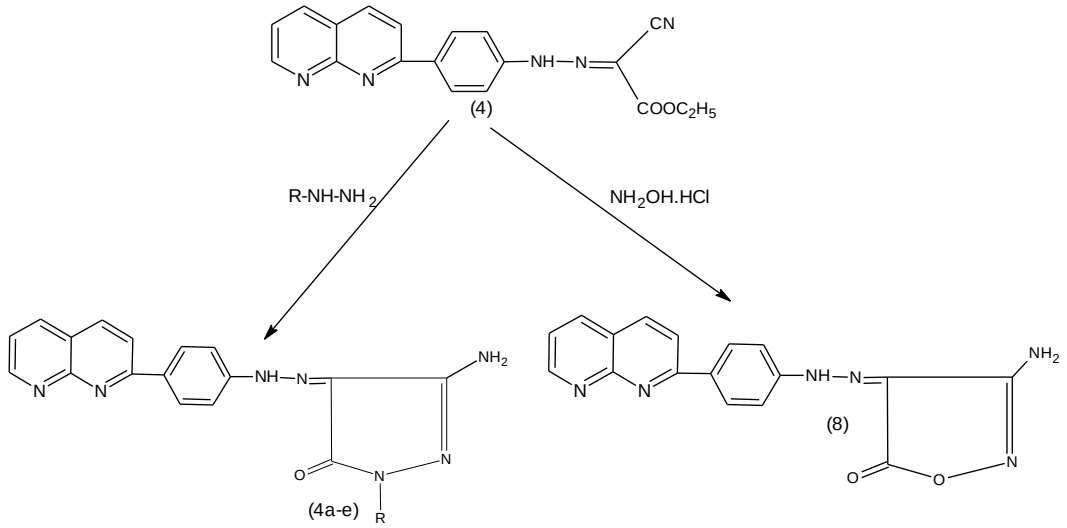
Compounds	Active methylene compounds	R^1	R^2
3	Ethyl acetoacetate	$COCH_3$	$COOC_2H_5$
4	Ethyl cyanoacetate	CN	$COOC_2H_5$
5	Acetyl acetone	$COCH_3$	$COCH_3$

Scheme.2 Synthesis of 1,8 –Napthyridine linked with 3-methyl Pyrazolinone (3a-e) and Isoxazolinone (7)



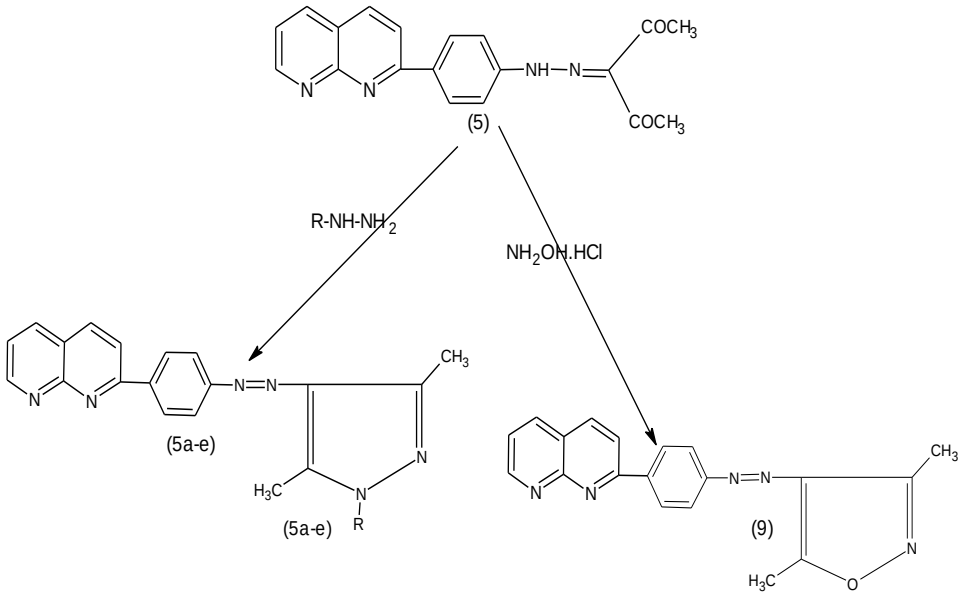
Title compounds	R-NH-NH_2 (Chemical name)	R
3a	Hydrazine hydrate	H
3b	phenylhydrazine	C_6H_5
3c	Thiosemicarbazide	$CSNH_2$
3d	chloro phenylhydrazine	Cl-C_6H4
3e	Isoniazide	C_5H_4N Co

Scheme.3 Synthesis of 1,8 –Napthyridine linked with 3-Amino Pyrazolinone (4a-e) and Isoxazolinone (8)



Title compounds	R-NH-NH_2 (Chemical name)	R
4a	Hydrazine hydrate	H
4b	phenylhydrazine	C_6H_5
4c	Thiosemicarbazide	$CSNH_2$
4d	chloro phenylhydrazine	Cl-C_6H4
4e	Isoniazide	C_5H_4N Co

Scheme.4 Synthesis of 1,8- Napthyridine linked with 3,5-dimethyl pyrazole &- 3,5-dimethyl Isoxazole (5a-e)&(9)



Title compounds	R-NH-NH_2 (Chemical name)	R
5a	Hydrazine hydrate	H
5b	phenylhydrazine	C_6H_5
5c	Thiosemicarbazide	$CSNH_2$
5d	chloro phenylhydrazine	Cl-C_6H4
5e	Isoniazide	C_5H_4N Co

Synthesis of title compounds

Hydrazono derivatives (3-5) were treatment of hydrazine or substituted hydrazine in acetic acid to afford the corresponding 1,8-napthyridine liked with substituted Pyrazolinones (3a-e&4a-e) and Isoxazolinone (7&9) (scheme.2&3) and with hydroxylamine HCl afforded corresponding substituted Pyrazole & Isoxazole (5a-e & 9) (scheme.4).

Experimental studies

Scheme.1 Procedure for synthesis of intermediate hydrazono compounds

Procedure for ethyl 2-{[4-(1,8-naphthyridin-2-yl)phenyl]hydrazono}-3- butanoate(3)

To 0.01 mole of compound 2- (P-aminoPhenyl)-1,8- napthyridine (1)was dissolved in a mixture of concentrated Hcl and water (30ml) and then cooled to 05c in ice-bath. A cold solution of aqueous sodium nitrite was added slowly maintaining the temperature at 0C. The diazonium salt solution was filtered directly to a cold solution of ethyl aceto acetate and sodium acetate in 50ml ethanol. The solid was filtered and washed with water and dried. Then the compound was re-crystallized from ethanol.

Procedure for Ethyl cyano{[4-(1,8-naphthyridin-2-l)phenyl]hydrazono}acetate (4)

To 0.05 mole of compound 2- (P-aminoPhenyl)-1,8- napthyridine (1)was dissolved in a mixture of concentrated Hcl and water (50ml) amd then cooled to 05c in ice-bath. A cold solution of aqueous sodium nitritewas added slowly maintaining the temperature at 0C. The diazonium salt solution was filtered directly to a cold solution of ethyl cyanoacetate and sodium acetate in 100ml ethanol. The solid was filtered and washed with water and dried. Then the compound was re-crystallized from ethanol.

Procedure for 3-{[4-(1,8-naphthyridin-2-yl)phenyl]hydrazono}pentane-2,4-dione (5)

To 0.05 mole of compound 2- (P-aminoPhenyl)-1,8- napthyridine (1)was dissolved in a mixture of concentrated Hcl and water (50ml) and then cooled to 05c in ice-bath. A cold solution of aqueous sodium nitrite was added slowly maintaining the temperature at 0C. The diazonium salt solution was filtered directly to a cold solution of Acetyl acetone and sodium acetate in 100ml ethanol. The solid was filtered and washed with water and dried. Then the compound was recrystallized from ethanol.

Scheme.2

General procedure for synthesis of 1,8 –Napthyridine linked with 3-methyl Pyrazolinones (3a-e)

Ethyl-2-{[4-(1,8-naphthyridin-2-yl)phenyl]hydrazono}-3-butanoate(3) (0.01 mole) was dissolved in glacial acetic acid and is treated with hydrazine hydrate & its derivatives (0.02 mole) in round bottom flask. The mixture was refluxed for 4 hrs. The mixture then was cooled by pouring in 100ml of chilled water and then allowed to stand overnight. The solid was filtered, dried and recrystallised with ethanol.

Procedure for synthesis of 1,8 –Napthyridine linked with 3-methyl Isoxazolinone (7)

Ethyl 2-{[4-(1,8-naphthyridin-2-yl)phenyl]hydrazono}-3-butanoate (3) (.01 mole) was dissolved in ethanol. A solution of sodium acetate (2 g) and hydroxyl amine hydrochloride (0.01 mole) in water was added. Then it was refluxed for 3 hrs. The resulting solution then poured onto crushed ice to get solid product.

Scheme.3

General procedure for synthesis of 1,8 –Napthyridine linked with 3-Amino Pyrazolinones (4a-e)

Ethyl cyano{[4-(1,8-naphthyridin-2-l)phenyl]hydrazono}acetate (4) (0.01 mole) was dissolved in glacial acetic acid and is treated with hydrazine hydrate&its derivatives (0.02 mole) in round bottom flask. The mixture was refluxed for 4 hrs. The mixture then was cooled by pouring in 100ml of chilled water and then allowed to stand overnight. The solid was filtered, dried and recrystallised with ethanol.

Procedure for synthesis of 1,8 –napthyridine linked with 3-amino Isoxazolinone (9)

Ethyl cyano{[4-(1,8-naphthyridin-2-l)phenyl]hydrazono}acetate (4) (.01 mole) was dissolved in ethanol. A solution of sodium acetate (2 g) and hydroxyl amine hydrochloride (.01 mole) in water was added. Then it was refluxed for 3 hrs. The resulting solution then poured onto crushed ice to get solid product.

Scheme.4

General procedure for synthesis of 1,8 –Napthyridine linked with 3,5-Dimethyl Pyrazoles (5a-e)

3-{[4-(1,8-naphthyridin-2-yl)phenyl]hydrazono}pentane-2,4-dione (5) (.01 mole) and hydrazine hydrateand its derivatives (0.01mol) in ethanol was heated under reflux for 4-6 hours. The solvent was concentrated and the reaction product was allowed to cool. The separated product was filtered off, washed with water, dried and recrystallized from ethanol.

Procedure for synthesis of 1,8 –Napthyridine linked with 3,5-Dimethyl Isoxazole(9)

1,3-diketo-1,3-diphenyl-2-3-{[4-(1,8-naphthyridin-2-yl)phenyl]hydrazono}-propane (6) (.01 mole) was dissolved in glacial acetic acid and is treated with hydroxyl amine (.02 mole) in round bottom flask. The mixture was refluxed for 4 hrs. The mixture then was cooled by pouring in 100ml of chilled water and then allowed to stand overnight. The solid was filtered, dried and recrystallised with ethanol.

RESULT & DISCUSSION

Spectral data of 5-methyl-4-{[4-(1,8-naphthyridin-2-yl)phenyl]hydrazono}-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (3a)

IR (cm^{-1}) 3309.70(NH), 2917.10(AromaticC-H (strecth), 1590.90 (C=N), 1656.70 Pyrazolinone(C=O) NMR δ: 8.4 (1H, M, C3-H), δ 8.49 (1H,m, C_4-H), δ 8.63 (1H, m, C_5-H), δ 8.2 (1H, m, C_6-H) 9.1(1H, m, C_7-

H)(1,8-napthyridine), 7.5-7.8 (4H, m, Ar-H), 9.8 (1H, s, NH-N=C), 2.45 (3H, s, CH_3) Mass: (M/Z) 330.

(4*Z*)-5-methyl-4-[(4-naphthalen-2-yl phenyl) hydrazono]-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (3b)

IR (cm^{-1}) 3417.70(NH), 3005.50(AromaticC-H (strecth), 1585.50 (C=N), 1667.20 Pyrazolinone(C=O) NMR δ: 8.32 (1H, M, C3-H), δ 8.4 (1H,m, C_4-H), δ 8.52 (1H, m, C_5-H), 8.1 (1H, m, C_6-H) 9.1(1H, m, C_7-H)(1,8-napthyridine), 7.5-7.8 (4H, m, Ar-H), 10.1 (1H, s, NH-N=C), 2.45 (3H, s, CH_3) Mass: (M/Z) 406.

Spectral data of (4*Z*)-3-methyl-4-[(4-naphthalen-2-ylphenyl) hydrazono]isoxazol -5(4*H*)-one (7)

IR (cm^{-1}) 3426.50(NH), 2917.10(AromaticC-H (strecth), 1594.50 (C=N), 1698.30 Isoxazolin-5-one(C=O) NMR δ: 8.41 (1H, M, C3-H), δ 8.49 (1H,m, C_4-H), δ 8.63 (1H, m, C_5-H), 8.38 (1H, m, C_6-H) 9.09(1H, m, C_7-H)(1,8-napthyridine), 7.4-7.92 (4H, m, Ar-H), 10.1 (1H, s, NH-N=C), 2.42 (3H, s, CH_3) Mass: (M/Z) 331

(4*Z*)-5-amino-4-[(4-naphthalen-2-ylphenyl) hydrazono]-2,4-dihydro-3*H*-pyrazol-3-one (4a)

IR (cm^{-1}) 3483.10(NH), 3317,3292.(NH_2),2918.10 (AromaticC-H (strecth), 1603.70 (C=N), 1688.70 Pyrazolinone (C=O) NMR δ: 8.1 (1H, M, C3-H), δ 8.2(1H,m, C_4-H), δ 8.3 (1H, m, C_5-H), 7.9 (1H, m, C_6-H), 9.2(1H, m, C_7-H)(1,8-napthyridine), 7.1-7.6 (4H, m, Ar-H), 6.3 (1H, s, NH_2), 9.9 (1H, s, NH-N=C) Mass: (M/Z) 331

(4*Z*)-5-amino-4-[(4-naphthalen-2-ylphenyl) hydrazono]-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (4b)

IR (cm^{-1}) 3475.80(NH), 3217,3192.(NH_2),2925.80 (AromaticC-H (strecth), 1604.30 (C=N), 1689.40 Pyrazolinone (C=O) NMR δ: 8.41 (1H, M, C3-H), δ 8.49(1H,m, C_4-H), 8.63 (1H, m, C_5-H), 8.2 (1H, m, C_6-H), 9.1(1H, m, C_7-H)(1,8-napthyridine), 7.2-7.5 (4H, m, Ar-H), 6.5 (1H, s, NH_2), 9.9(NH-N=C) Mass: (M/Z) 331

2-{4-[(3,5-dimethyl-1*H*-pyrazol-4-yl)diazenyl]phenyl}-1,8-naphthyridine.(5a)

IR (cm^{-1}) 3421.10(NH), 3017.54 (AromaticC-H (strecth), 1602.52(C=N), 1471.37 Azogroup NMR δ: 7.52 (1H, M, C3-H), δ 7.72 (1H,m, C_4-H), 7.92 (1H, m, C_5-H), 7.42 (1H, m, C_6-H) 8.12(1H, m, C_7-H)(1,8-napthyridine), 6.9-7.2 (4H, m, Ar-H), 2.3 (3H, s,CH_3), 2.6 (3H, s, CH_3)(pyrazole)

Pharmacological screening

Acute toxicity Studies

The acute toxic study was done according to the OECD guidelines on Acute Oral Toxicity under a computer guided statistical programme-AOT423 stat programme, The animals were monitored for the behavioural changes, weight variation, toxicity and death rate.

Anti-inflammatory Activity

Carrageenan-Induced Rat Paw Oedema

Experimental Procedure

Oedema was induced by sub planter injection of 0.1 ml of 1% freshly prepared suspension of carrageenan into the right hind paws of the rats of eleven groups of six animals each. The volume of the injected and contra-lateral paws were measured 1,2,3 and 4 h after induction of inflammation using a plethysmometer according to the method described by Winter et al. (1962) The test groups received the synthesized compounds (3a,3b.4a,4b,5a,5b,5c,5d,7) (200mg/kg), the standard group received phenylbutazone (100 mg:kg), and the control animals received the vehicle only alone (3% V/V tween-80 10 ml/kg) p.o. All the treatments were given intraperitoneally 30 min prior to the injection of carrageenen an except for the synthesized compounds. Increase of paw oedema thickness was calculated The results are expressed as mean ± S.E.M. Dennett's t-test was used to verify the statistical significance at $p<0.05$ between the treated and control groups. For comparison purpose, the volume of oedema at various prefixed time intervals was measured. The difference between paw volumes of the treated animals was measured and the mean oedema volume was calculated.

Percentage reduction in oedema volume was calculated by using the formula,

$$\text{Percentage reduction} = \frac{Vo - V_t}{Vo} \times 100$$

Where, Vo = Volume of the paw of control at time't'.

V_t = Volume of the paw of drug treated at time't'.

From the data obtained, the mean oedema volume and percentage reduction in oedema was calculated.

Analgesic Activity

The writhing test described by Koster et al. (1959) was adopted. A total of 66 mice divided into 11 groups (n=6) were used and treated as follows; group 1 served as control and received vehicle alone (3% V/V tween-80 10 ml/kg) p.o., groups 3 to 11 received 200 mg/kg p.o. of the synthesized compounds like 3a,3b.4a,4b,5a,5b,5c,5d, and 7 respectively, while group 2 received 100 mg/kg of acetyl salicylic acid (standard drug) p.o. Ten ml/kg of 0.7% aqueous solution of acetic acid were given to all mice i.p. 30 min later. Each mouse was placed in a transparent observation cage and abdominal constriction resulting from injection of acetic acid for the period of 20 minutes was counted. Results were presented as percent inhibition of analgesia, calculated as the reduction in the number of writhes between control animals and those pre-treated with either the synthesized compounds or acetyl salicyclic acid. The values were expressed as mean ± SEM from 6 animals. The results are expressed as mean ± S.E.M. Dennett's t-test was used to verify the statistical significance at $p<0.05$ between the treated and control groups.

Figure.1 Anti-inflammatory Activity

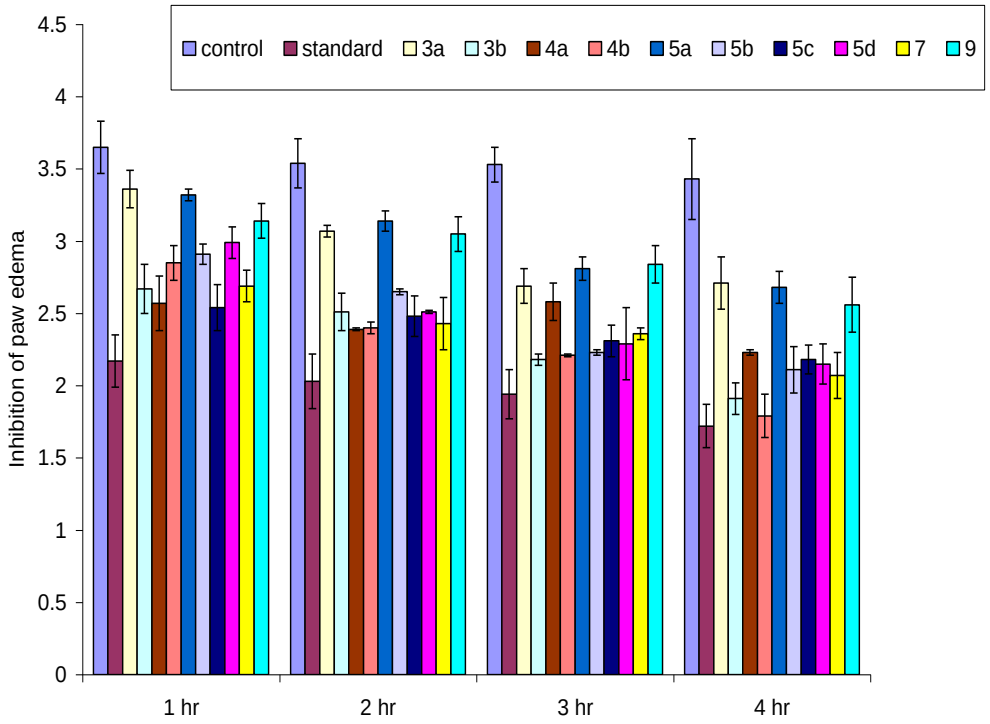
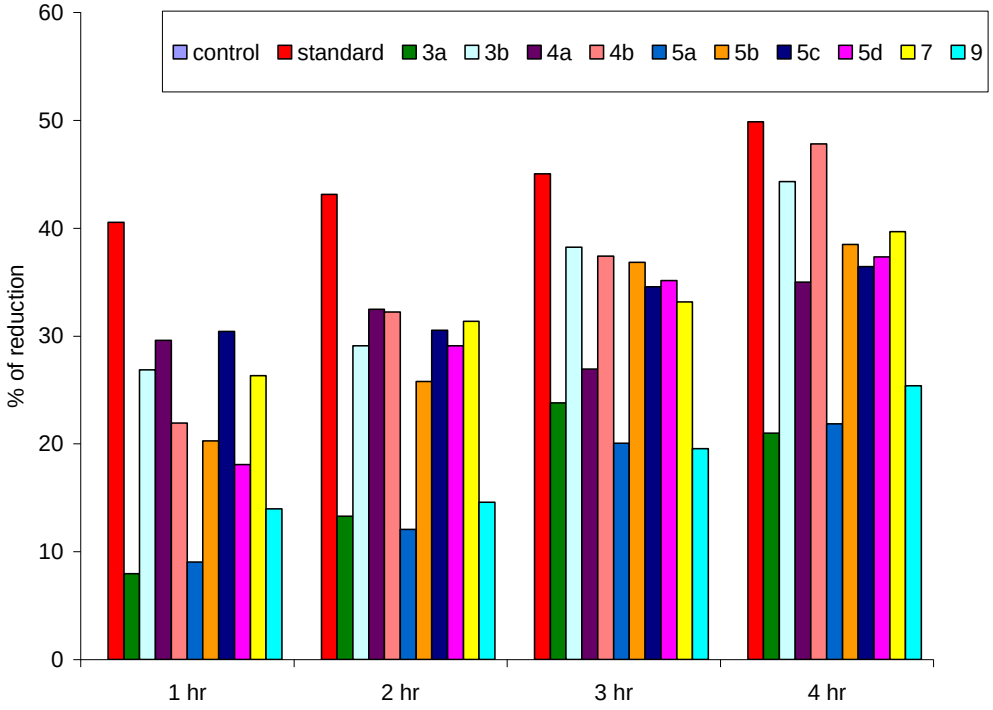


Table.1 Anti-inflammatory Activity

Compound	1hr		2hr		3hr		4hr	
	Mean ± SEM	%red	Mean ± SEM	%red	Mean ±SEM	%red	Mean ± SEM	%red
Control	3.65 ± 0.18	NA	3.54 ± 0.17	NA	3.53 ± 0.12	NA	3.43 ± 0.28	NA
Standard	2.17 ± 0.18	40.54	2.03 ± 0.19	43.13	1.94 ± 0.17	45.04	1.72 ± 0.15	49.85
3a	3.36 ± 0.13	7.94	3.07 ± 0.04	13.27	2.69 ± 0.12	23.79	2.71 ± 0.18	20.99
3b	2.67 ± 0.17	26.84	2.51 ± 0.13	29.09	2.18 ± 0.04	38.24.	1.91 ± 0.11	44.31
4a	2.57 ± 0.19	29.58	2.39 ± 0.01	32.48	2.58 ± 0.73	26.91	2.23 ± 0.02	34.98
4b	2.85 ± 0.12	21.91	2.40 ± 0.04	32.20	2.21 ± 0.01	37.39	1.79 ± 0.15	47.81
5a	3.32 ± 0.04	9.04	3.14 ± 0.07	12.04	2.81 ± 0.08	20.03	2.68 ± 0.11	21.86
5b	2.91 ± 0.07	20.27	2.65 ± 0.02	25.77	2.23 ± 0.02	36.82	2.11 ± 0.16	38.48
5c	2.54 ± 0.16	30.41	2.48 ± 0.14	30.53	2.31 ± 0.11	34.56	2.18 ± 0.10	36.44
5d	2.99 ± 0.11	18.08	2.51 ± 0.01	29.09	2.29 ± 0.25	35.12	2.15 ± 0.14	37.31
7	2.69 ± 0.11	26.30	2.43 ± 0.18	31.35	2.36 ± 0.04	33.14	2.07 ± 0.16	39.65
9	3.14 ± 0.12	13.97	3.05 ± 0.12	14.56	2.84 ± 0.13	19.54	2.56 ± 0.19	25.36

Figure.2 Graphical representation of effect of compounds 3a,3b.4a,4b,5a,5b,5c,5d, and 7 standard drug in acetic acid-induced writhing test on mice



***p<0.001; ** p< 0.01; *p< 0.05*

Table.2 Analgic Activity- acetic acid induced writhing test

Compound	Abdominal constriction		Trunk twisting		Extention of hind limbs	
	Mean ± SEM	% red	Mean ± SEM	% red	Mean ± SEM	% red
control	30.1 ± 0.11	NA	18.4 ± 0.41	NA	11.6 ± 0.07	NA
standard	14 ± 0.13	53.48	7.11 ± 0.02	61.35	5.18 ± 0.01	55.34
3a	2 8.06 ± 0.02	6.67	15.8 ± 0.06	14.13	10.77 ± 0.07	7.15
3b	17.19 ± 0.07	42.89	13.25 ± 0.11	27.98	7.14 ± 0.17	38.44
4a	21.04 ± 0.18	30.09	16.11 ± 0.09	12.44	8.05 ± 0.14	30.60
4b	19.11 ± 0.18	36.51	14.33 ± 0.16	22.11	7.51 ± 0.05	35.25
5a	28.96 ± 0.13	3.78	16.79 ± 0.14	8.75	10.28 ± 0.17	11.37
5b	21.7 ± 0.15	27.90	11.3 ± 0.08	38.58	9.10 ± 0.81	21.55
5c	22.03 ± 0.01	26.81	14.15 ± 0.12	23.09	8.26 ± 0.02	28.79
5d	27.50 ± 0.11	8.63	17.04 ± 0.11	7.39	11.11 ± 0.13	4.22
7	22.78 ± 0.04	24.31	15.97 ± 0.13	13.20	8.19 ± 0.15	29.39
9	25.37 ± 0.16	15.71	16.17 ± 0.11	12.11	9.71 ± 0.03	16.29

CONCLUSION

All the test compounds were showed potent to weak anti-inflammatory analgesic activities. The most active compounds 3b,4b,and 5b like N-phenyl pyrazolinone & N-phenyl pyrazoles linked 1,8-napyhridine shows better anti-inflammatory &analgesic activities.

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