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Research Article

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Synthesis of 8-chloro/fluoro-3-aryl-10aH (1,2,4,5) Tetrazino Benzothiozole and their Analgesic and Anti-Inflammatory Activity

KM Basavaraja[1*] and B Somasekhar[2]

[1]*Department of Industrial Chemistry,Vijayanagara Shri Krishnadevaraya University, Hospet Road, Ballari(Cant)-583105, Karnataka, India*

[2]*Department of Pharmaceutical Chemistry, T.V.M. Collage of Pharmacy, Gandhinagar, Kappagal Road, Bellary-583103, Karnataka, India*

ABSTRACT

The key intermediate 6-Chloro/fluoro-2-aminobenzothiazole(1a,b) on treatment with hydrazine hydrate and HCl gave 6-chloro/fluoro-2-hydrazinobenzothiazole (2a,b), which on condensation with substituted aromatic aldehydes affords aryl substituted (6-chloro/ fluoro -1.3,-benzothiazole-2-yl)hydrazones(3a-j). The latter on nitrosation with sodium nitrite in acetic acid leading to ring closure and furnishes respective 8-chloro/fluoro-3-aryl- 10aH(1,2,4,5) tetrazinobenzothiazoles(4a-j). The analgesic and anti-inflammatory activity of these compounds have been carried out by Eddy's hot plate method on Albino mice and carrageenan induced rat paw edema method. The constitutions of the products have been elucidated by IR, NMR spectral data and elemental analysis.

Keywords: Tetrazines; Benzothiazoles; Analgesic; Anti-Inflammatory

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INTRODUCTION

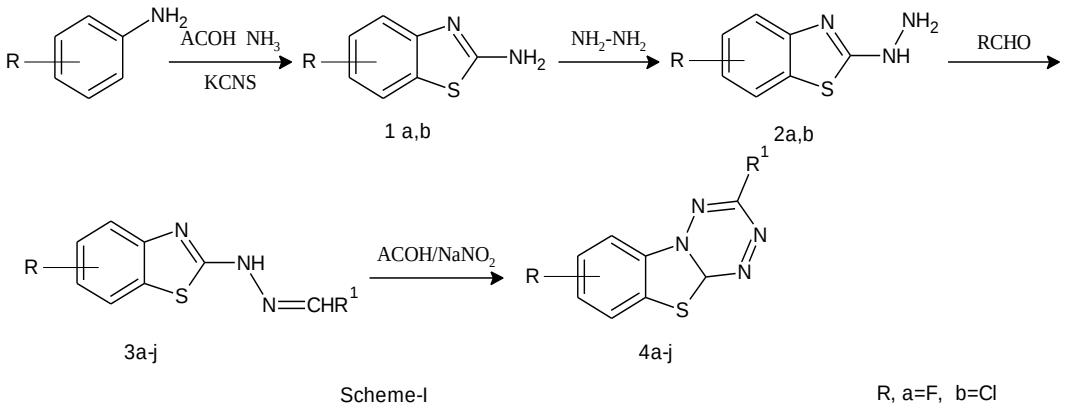
Owing to the biological importance and potency of benzothiozoles referred in the literature [1-5], in continuation of our search for synthetic biologically and pharmacologically potent heterocycles [6,7] now we are reporting the synthesis of 8-chloro/fluoro-3-aryl-10aH(1,2,4,5) tetrazino benzothiozole derivatives.

The derivatives of 1,2,4,5 tetrazines and 3,6-disubstituted 1,2,4,5-tetrazines are well known materials for the use in the area of energetic chemistry [8,9]. A literature report reveals that pharmacologically potent tetrazines were synthesized and their biological activity study shows their influence on the growth kinetic parameters of soil bacteria [10].

2-Aminobenzothiazole and its derivatives have been reported to possess antimicrobial1 and various other pharmacological activities like anti-cancer, Antihistaminic and Anti-inflammatory analgesic activities [11-13]. Further triazoles and tetrazoles are having various biological activities. Keeping this in view and in continuation of our research work for pharmacologically potential benzothiazole derivatives we now report the synthesis of tetrazino benzothiazole derivatives and their anti-inflammatory and analgesic activity.

Theory

The required starting material 6-chloro/fluoro- 2 amino benzothiazole (1) have been prepared by the route in procedure reported in the literature [9], the compound (1) was then portion wise added to a mixture of cold solution of hydrazine hydrate, conc. Hcl, followed by ethylene glycol gave 6-chloro/fluoro-2-hydrazinobenzothiazole (2), which on condensation with substituted aromatic aldehydes affords aryl substituted (6-chloro/fluoro -3-benzothiazole -2-yl) hydrazone (3). Then nitrosation of compound (3) with sodium nitrite in acetic acid led to the ring closure and formation of tetrazino derivatives (4).



Scheme 1

Analgesic Activity

The compounds synthesized were evaluated for analgesic activity using Eddy's hot plate method. *Swiss albins* mice of either sex weighing about 25-30g were fasted for 18 hrs. The group-I received solvent control and group-II is standard and remaining groups received synthesized tetrazine derivatives at 10mg/kg dose on a rational basis. The basal reaction times of mice of all groups were recorded at 00, 30, 60 and 120 minutes intervals by keeping them individually on hot plate heated at 55^0 C. The average basal reaction time of each group is then compared statistically by using One-Way ANOVA followed by Dunnetts't' test (Results of screening are summarized in table 1).

Table 1. Analgesic activity of 8-chloro/fluoro 3 aryl 10aH(1,2,4,5) tetrazino benzothiazole(4a-j)

Time in minutes	Control	STD	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j
00 min	3.5 ± 0.428	4.00 ± 0.36	3.16 ± 0.30	3.16 ± .16	4.33 ± 0.21	4.00 ± 0.36	3.66 ± 0.21	3.16 ± .16	3.20 ± .22	3.16 ± .16	3.16 ± .16	3.16 ± .16
15min	3.33 ± 0.33	4.83 ± 0.47	3.66 ± 0.33	4.5 ± 0.22	4.16 ± 0.33	4.33 ± 0.21	4.66 ± 0.33	3.33 ± 0.33	3.33 ± 0.33	4.33 ± 0.49	3.43 ± 0.49	4.22 ± 0.49
30min	3.33 ± 0.42	5.66 ±	5.00 ±	5.5 ±	6.16 ±	5.33 ±	6.00 ±	4.40 ±	5.00 ±	5.00 ±	5.00 ±	5.00 ±

		0.42	0.25	0.22	0.40	0.55	0.36	0.25	0.25	0.25	0.25	0.25
60 min	3.5 ± 0.34	7.00 ± 0.44	6.66 ± 0.55	12.00 ± 0.85	11.66 ± 0.61	9.66 ± 0.88	5.83 ± 0.40	5.53 ± 0.40	5.83 ± 0.40	5.83 ± 0.40	5.83 ± 0.40	5.83 ± 0.40
120 min	3.66 ± 0.21	6.83 ± 0.60	5.00 ± 0.36	9.16 ± 0.60	6.33 ± 0.49	4.83 ± 0.30	4.66 ± 0.33	5.23 ± 0.45	4.33 ± 0.25	5.33 ± 0.21	5.13 ± 0.76	4.33 ± 085

Anti-Inflammatory Activity

All the newly synthesized compounds were evaluated for their anti-inflammatory activity in *Swiss albino* rats by carraginone induced rat paw edema method. Diclofinac sodium 50 mg/kg body weight was employed as standard drug and the unknown compounds at a dose level of 10mg/kg body wt. rat by intra peritoneal route. Anti-inflammatory potency was assessed by noting % inhibition of edema (Results of screening are summarized in table 2).

Table 2. Anti-Inflammatory screening of 8-chloro/fluoro 3 aryl 10aH(1,2,4,5) tetrazino benzothiazole(4a-j) compounds (% inhibition of edema)

Treatment of Drugs	**Average Reaction Time ± S E M**								
	30 min	**1 hr**	**% in h**	**2 hr**	**% in h**	**3 hr**	**% in h**	**4 h**	**% in h**
Control	0.733 ± 0.021	1.10 ± 0.036	----	1.33 ± 0.021	----	1.28 ± 0.030	---	1.33 ± 0.033	----
Diclofenac	0.734 ± 0.049	0.833 ± 0.033	27.28	0.834 ± 0.021	26.2	0.486 ± 0.021	62.4	0.883 ± 0.030	33.7
4a	0.670 ± 0.021	0.970 ± 0.041	11.9	0.966 ± 0.042	14.8	0.733 ± 0.033	42.8	0.900 ± 0.025	32.4
4b	1.164 ± 0.021	0.950 ± 0.042	13.7	0.955 ± 0.066	15.8	0.666 ± 0.042	48.0	0.866 ± 0.021	34.9
4c	0.983 ± 0.030	0.966 ± 0.042	12.2	0.967 ± 0.042	14.8	0.666 ± 0.027	48.0	1.018 ± 0.046	24.9
4d	0.966 ± 0.066	0.950 ± 0.042	13.7	0.866 ± 0.021	23.6	0.700 ± 0.025	45.4	1.067 ± 0.021	18.8
4e	1.011 ± 0.049	0.950 ± 0.022	13.7	0.866 ± 0.021	15.6	0.683 ± 0.036	46.7	1.067 ± 0.021	19.8
4f	1.167 ±	1.033 ±	6.1	1.067 ±	5.9	0.700 ±	45.4	0.900 ±	32.4

	0.049	0.034		0.049		0.025		0.025	
4g	0.985 ± 0.021	0.958 ± 0.029	12.2	0.900 ± 0.162	20.6	0.700 ± 0.025	45.4	0.983 ± 0.040	26.1
4h	1.033 ± 0.030	0.950 ± 0.030	13.7	0.983 ± 0.064	13.3	0.716 ± 0.040	44.1	0.950 ± 0.034	28.6
4i	1.017 ± 0.030	0.816 ± 0.147	16.8	0.766 ± 0.142	32.4	0.683 ± 0.025	46.7	0.933 ± 0.033	21.9
4j	0.900 ± 0.051	0.816 ± 0.047	25.9	0.933 ± 0.021	17.7	0.700. ± 0.025	45.4	0.966 ± 0.042	27.4

Data were analyzed by one-way ANOVA followed by Dunnet's Test $P<0.05$ values are mean ± SEM of six animals in each group.

EXPERIMENTAL SECTION

Melting points were determined in open capillaries and are uncorrected. The purity of all the synthesized compounds was checked by TLC, IR spectra were recorded on Shimadzu FTIR 8400S by using KBr disc method and the NMR spectra were recorded on AV-400 Instrument.

Synthesis of 6-chloro-2-hydrazino benzothiazole (2):

Concentrated HCl (10 ml) was added drop wise to hydrazine hydrate (0.2 mole) at 5-10 0c followed by ethylene glycol (40ml). To the above solution 0.1mole of 2-aminobenzothiazole was added in portion wise and refluxed for 3-4 hrs, cooled in ice, the separated solid is filtered , dried and recrystallized from ethanol (Figures 1-15).

Synthesis of aryl substituted (6-chloro-1, 3-benzothiazole-2-yl) hydrazone (3):

Aryl substituted (6-chloro-1,3-benzothiazole-2-yl) hydrazone (3) (0.1mole) and substituted aldehydes (0.1mole) were refluxed in ethanol in presence of acetic acid on a water bath for 4hrs. The resultant solution were cooled and poured on to crushed ice. The solid that separated was filtered and recrystalised from ethanol to yield compounds 3a-j. Melting point and % yields are given in table 3.

Synthesis of 8-chloro/fluoro 3 aryl 10aH (1,2,4,5) tetrazinobenzothiazole (4)

A solution of hydrazone (3) (0.01mole) in acetic acid (10-15ml), sodium nitrite (0.03mole) and water (2 ml) was refluxed for an appropriate time , cooled and poured in to ice cold water. The separated solid was filtered and recrystallized from acetic acid to afford the titled compounds (4a-j). Melting point and % yields are given in table 4

IR data of 3c; 3300(N-H), 1680(C=N) and 1085cm-1 (-OCH3):

3d; 3300 (N-H), 1670, (C-N) and 1530 and 1350 cm-1 (NO_2):

4a; 1615 (C-N), 1690(N=N) and 725 cm-1, (Ar-F)

4e; 3500-3580(Ar-OH), 1650(C-N), 1690(N-N), 1070(-OCH3), 725 cm-1(Ar-F)

NMR data of representative compound-4a; 1.6(s-1H, ΞC-H), 7.2-8.2 δ(m,8H, Ar-H)

MASS data of representative compound- 4a; M+1 peak at 285.9

Table 3. Physical data of aryl substituted (6-chloro-1,3-benzothiazole-2-yl) hydrazine (3a-j)

Compound	R	R'	MP(^{o}C)	Yield (%)
3a	F	-C_6H_5	205	80
3b	F	-C_6H_4.OH(o)	210	78
3c	F	-C_6H_4.OCH_3(p)	175	82
3d	F	-C_6H_4.NO_2(o)	195-197	70
3e	F	-C_6H_3.OCH_3(m).OH(p)	190	75
3f	Cl	-C_6H_5	210	80
3g	Cl	-C_6H_4.OH(o)	170	72
3h	Cl	-C_6H_4.OCH_3(p)	160	78
3i	Cl	-C_6H_4.NO_2(o)	185	75
3j	Cl	-C_6H_3.OCH_3(m).OH(p)	200	70

Table 4. Physical data of 8-chloro/fluoro 3 aryl 10aH(1,2,4,5) tetrazino benzothiazole(4a-j)

Compound	R	R'	MP (^{o}C)	Yield (%)
4a	F	-C_6H_5	200	80
4b	F	-C_6H_4.OH(o)	203	78
4c	F	-C_6H_4.OCH_3(p)	165	82
4d	F	-C_6H_4.NO_2(o)	180	70
4e	F	-C_6H_3.OCH_3(m).OH(p)	198	75
4f	Cl	-C_6H_5	203	83
4g	Cl	-C_6H_4.OH(o)	208	82
4h	Cl	-C_6H_4.OCH_3(p)	178	80
4i	Cl	-C_6H_4.NO_2(o)	192-194	68
4j	Cl	-C_6H_3.OCH_3(m).OH(p)	199	73

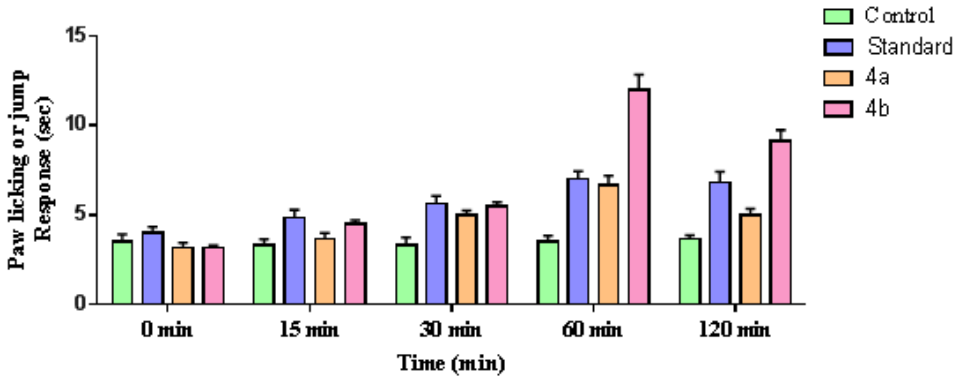


Figure 1. Effect of 8-chloro/fluoro 3 aryl 10aH (1,2,4,5)tetrazino benzothiazole (4a & 4b) on analgesic activity (Eddy's hot plate method) in mice

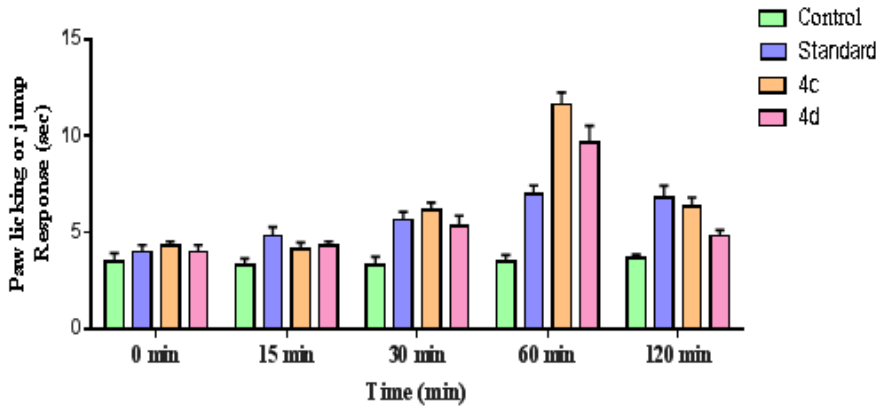


Figure 2. Effect of 8-chloro/fluoro 3 aryl 10aH (1,2,4,5) tetrazino benzathiazole (4c & 4d) on analgesic activity (Eddy's hot plate method) in mice

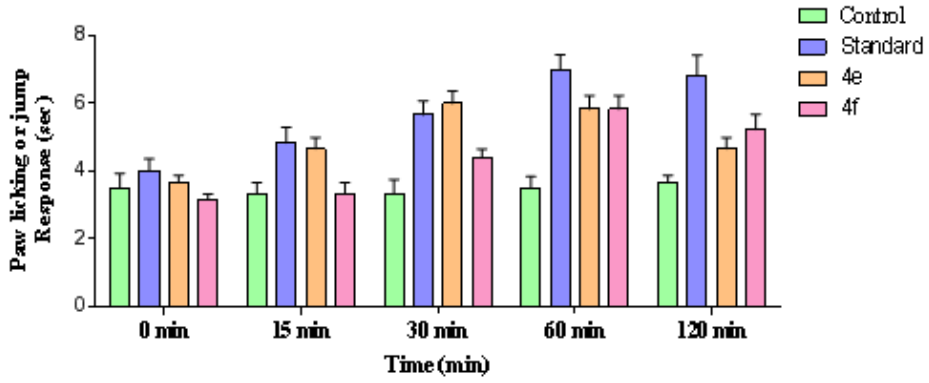


Figure 3. Effect of 8-chloro/fluoro 3 aryl 10aH (1,2,4,5) tetrazino benzathiazole (4e & 4f) on analgesic activity (Eddy's hot plate method) in mice

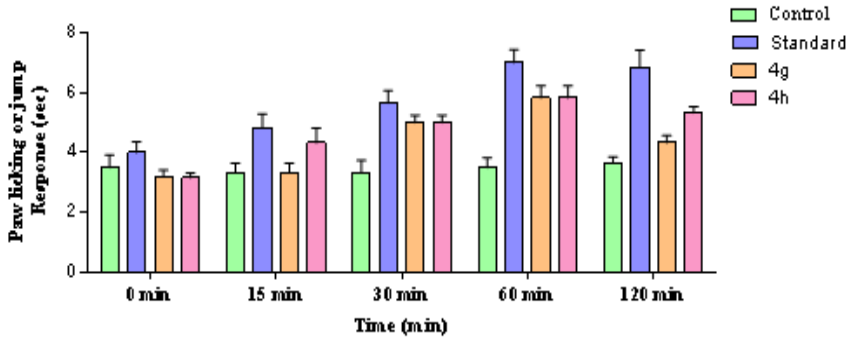


Figure 4. Effect of 8-chloro/fluoro 3 aryl 10aH (1,2,4,5) tetrazino benzathiazole (4g & 4h) on analgesic activity (Eddy's hot plate method) in mice

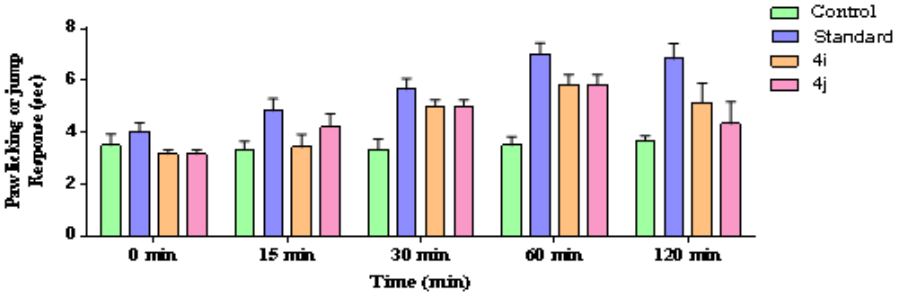


Figure 5. Effect of 8-chloro/fluoro 3 aryl 10aH (1,2,4,5) tetrazino benzathiazole (4i & 4j) on analgesic activity (Eddy's hot plate method) in mice

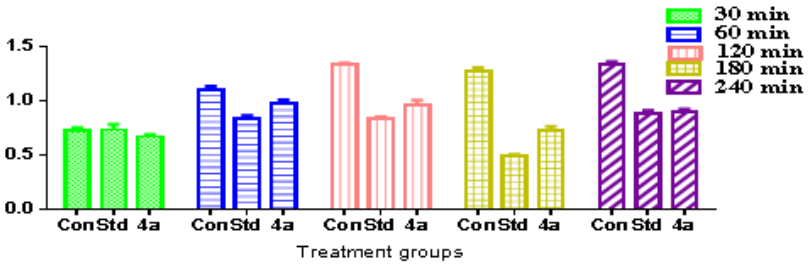


Figure 6. Effect of compound 4a on carrageenan induced paw edema in rats

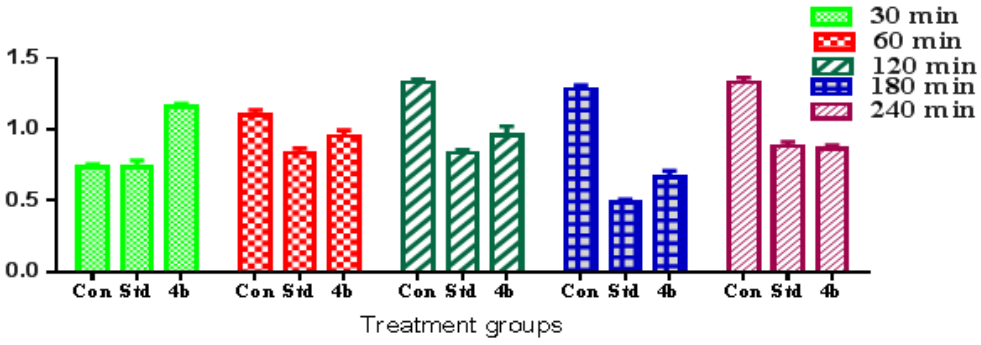


Figure 7. Effect of compound 4b on carrageenan induced paw edema in rats

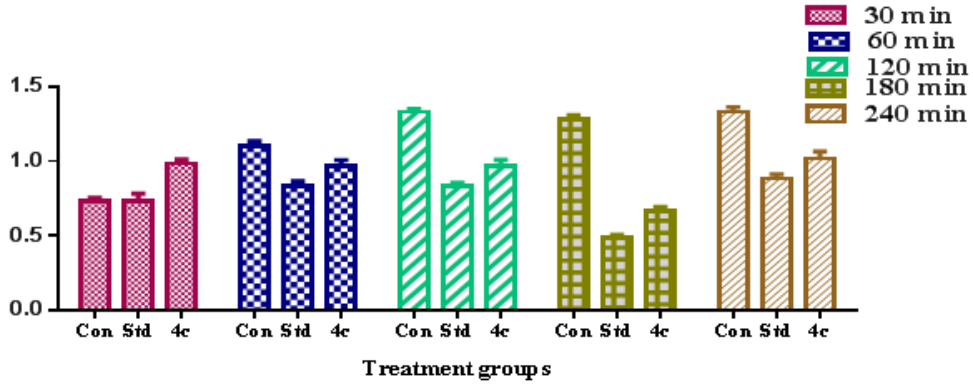


Figure 8. Effect of compound 4c on carrageenan induced paw edema in rats

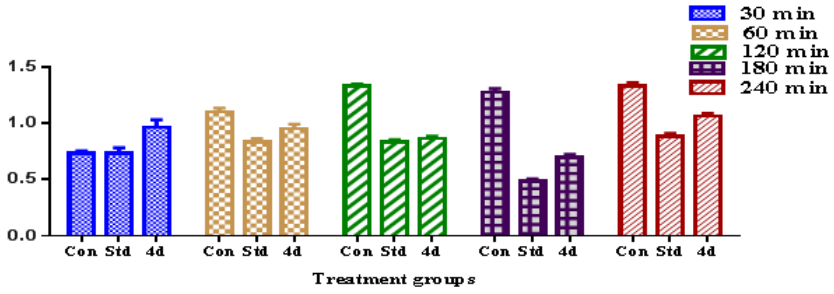


Figure 9. Effect of compound 4d on carrageenan induced paw edema in rats

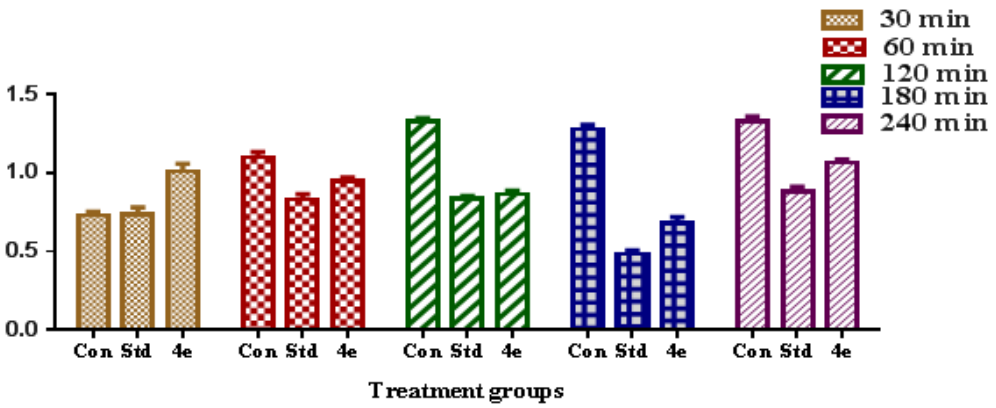


Figure 10. Effect of compound 4e on carrageenan induced paw edema in rats

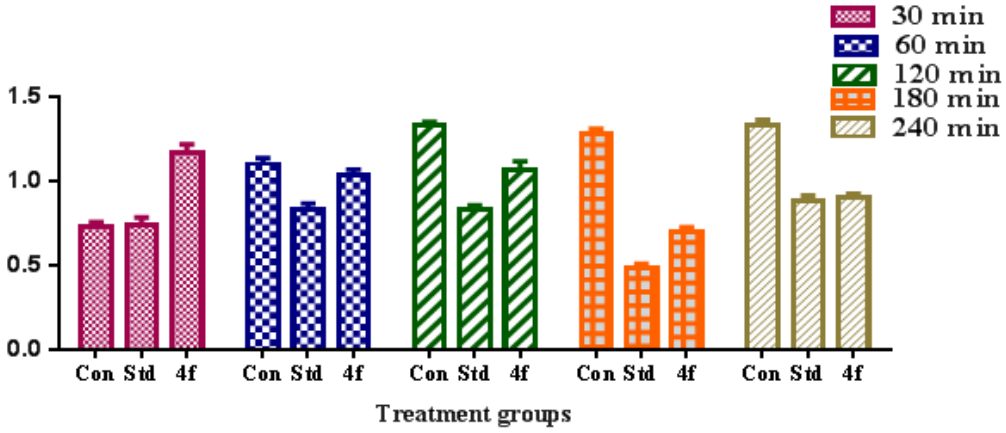


Figure 11. Effect of compound 4f on carrageenan induced paw edema in rats

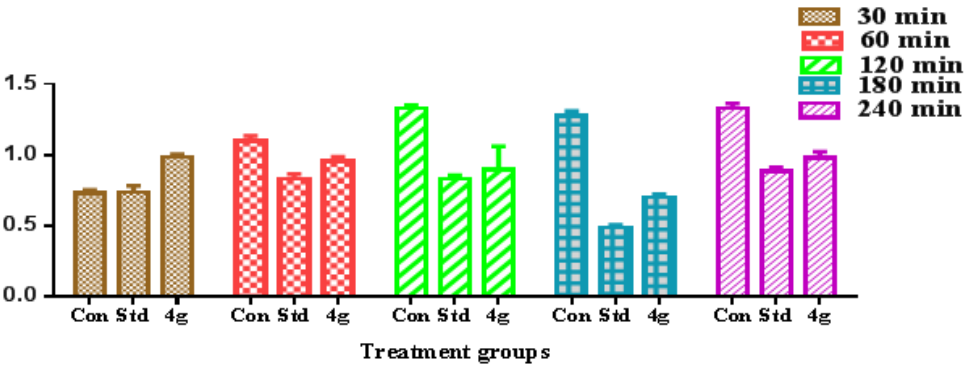


Figure 12. Effect of compound 4g on carrageenan induced paw edema in rats

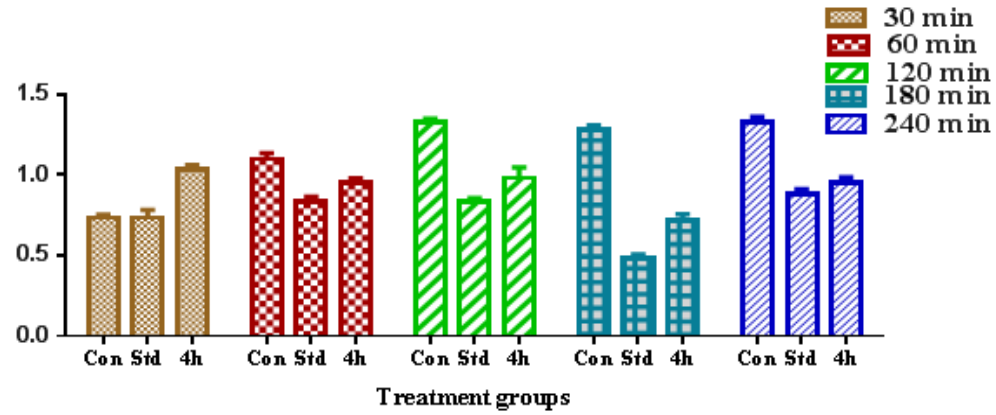


Figure 13. Effect of compound 4h on carrageenan induced paw edema in rats

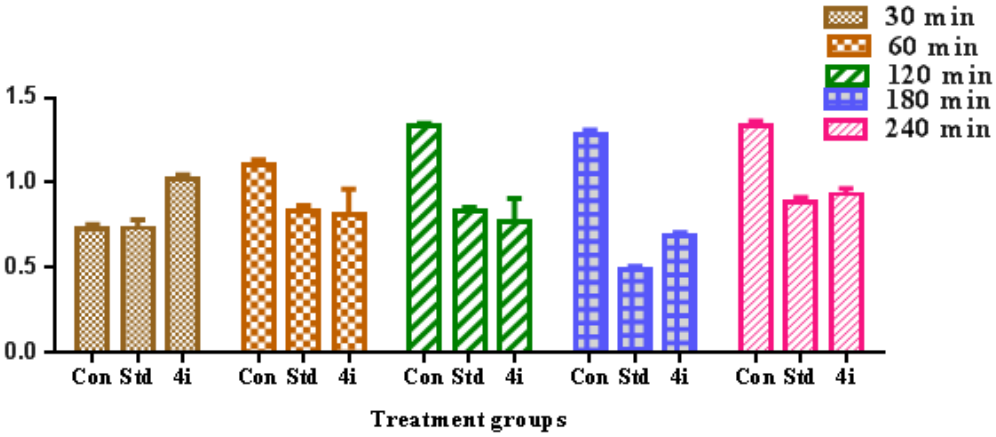


Figure 14. Effect of compound 4i on carrageenan induced paw edema in rats

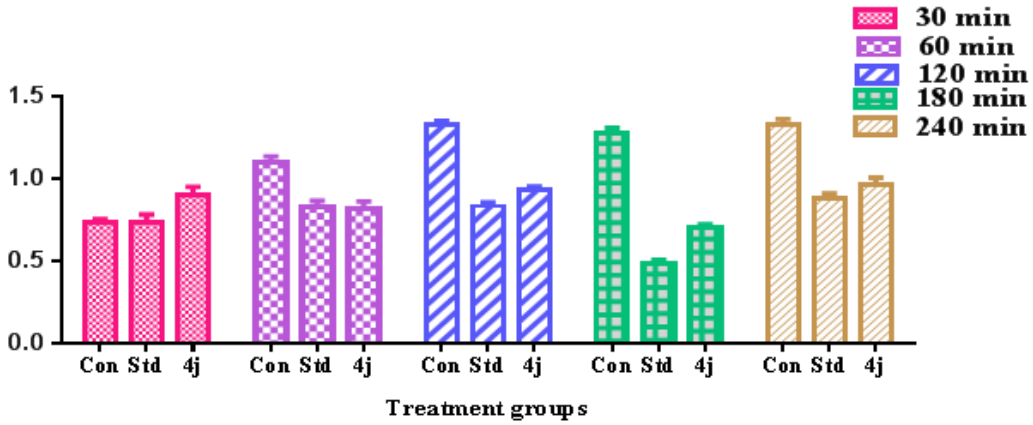


Figure 15. Effect of compound 4j on carrageenan induced paw edema in rats

RESULT AND DISCUSSION

The carrageenan induced paw edema is a most commonly used as the standard techniques to screen anti-inflammatory activity. The carrageenan induced paw edema is a biphasic response. The first phase (0-2h) is associated with the release several of chemical mediators such as histamine, 5-HT and kinins. The second phase (3h onwards) inflammation is sensitive to most clinically effective anti-inflammatory drugs which are primarily due to

the enhancement of individual cycloxygenaseiso-enzyme (Cox –2) and subsequent formation of prostaglandins. The new synthesized tetrazine derivatives have showed significant anti-inflammatory ($P<0.05$) activity at 3 hrs so that the above mentioned compounds () exhibited as Cox -2 inhibitors.

The same compounds were also subjected for screening analgesic activity. The same compounds were also subjected for screening of analgesic activity. The compounds II, III and IV inhibit the pain in the thermal method to lower extent compared to standard. The analgesic activity also exhibited by the compounds after 1h. These results encouraging clearly indicate that, the synthesized analogues of ibuprofen can be better examples of novel analgesics and anti-inflammatory agents and may be explored further the detailed pharmacological screening there by for inflammatory diseases.

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