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Research Article

SYNTHESIS, ANTI-INFLAMMATORY AND ULCEROGENIC ACTIVITY OF SOME MANNICH BASES OF 6-SUBSTITUTED-2-AMINOBENZOTHIAZOLE

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ABSTRACT

Ten new mannich bases were synthesized by condensation of equimolar quantities of corresponding 6-substituted-2-aminobenzothizole derivatives with 1-(5-substituted-1*H*-1,2,4-triazol-1-yl)-ethanone derivatives in presence of formaldehyde and concentrated hydrochloric acid. The purity and homogeneity of the synthesized compounds was routinely checked by TLC using solvent systems Benzene: methanol (50:50 v/v) and Carbon tetrachloride: methanol (50:50 v/v). All the synthesized compounds were characterized by FT-IR and ^{1}H-NMR spectroscopy. The synthesized compounds were screened for anti-inflammatory and ulcerogenic activity. Few compounds exhibit excellent activities as compared to the standard drugs.

Key Words: 1*H*-1,2,4-triazole, 2-Aminobenzothiazole, Mannich base, Ulcerogenic.

INTRODUCTION

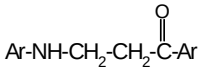
Many disease conditions and surgical procedures are associated with pain and inflammation. The currently available analgesic and anti-inflammatory agents such as aspirin, diclofenac, indomethacin, ibuprofen, naproxen and others are carboxylic acid derivatives and are associated with ulcerogenic effect. The current approaches utilizes to mask the ulcerogenic effect of these drugs includes prodrug concept and conversion of carboxylic group to some other functional groups such as amide, ester, aldehyde or ketones.

The 6-substituted-2-aminobenzothiazole derivatives and 5-substituted 1*H*-1,2,4-triazole derivatives enjoys some common therapeutic actions which includes antibacterial, analgesic and anti-inflammatory [1-3].

Number of potent medicinal agents consist of aminoalkyl chain. Major examples are from the category of antimalarials, antihistaminics, adrenergics, cholinergics, local anesthetics, non-narcotic analgesics etc. Many Mannich bases, which are identified by the presence of aminoalkyl chain, are in clinical use Major examples are atropine, cocaine, dyclonine, tutocaine, tanitidine, phenindamine, triprollidine, amodiaquin, ethacrynic acid, biperiden, procyclidine, trihexyphenidyl, molindone, zolpidem, fluoxetine and propoxyphene.

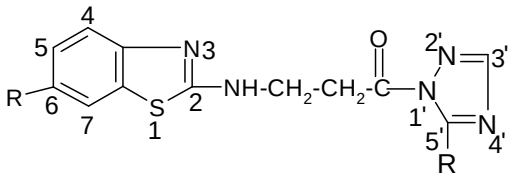
Present study focused on evaluation of anti-inflammatory activity when two different therapeutically active molecules are joined in a single one. Though presence of aminoalkyl chain is a key feature shown by no. of medicinal agents, attempts were made to link

the benzothiazole and triazole ring via this chain with use of mannich reaction.

For the compounds to acts as good analgesic, anti-inflammatory agent it should consist of a common structure i.e. 

which may consists of a secondary or tertiary nitrogen, a carbonyl carbon (whose none of the valences are satisfied by hydrogen), an ethylene bridge between the basic nitrogen and flat carbon (C=O) and aryl or heteroaryl ring substituents on nitrogen and carbonyl carbon [4,7].

From the consideration of the above factors it was planned to synthesize mannich bases of 6-substituted-2-aminobenzothiazole by carrying out reaction between 2-amino-benzothiazole, formaldehyde and 5-substituted-1*H*-1,2,4-triazole to results in molecules having the following structure:



Though position 6- on 2-aminobenzothiazole and position 5- on 1*H*-1,2,4-triazole are most active, attempts were made to synthesize the derivatives that contains the substitute groups on both of this positions.

MATERIAL AND METHODS

The titled mannich bases were synthesized by mannich reaction between 6-substitued-2-aminobenzothiazole derivatives and 5-substituted-1*H*-1,2,4-triazol-1-yl-ethanone derivatives in presence of formaldehyde and conc.HCl. The melting points of the synthesized compounds were determined on open capillary tubes and are uncorrected. The purity and homogeneity of the synthesized compounds was routinely ascertained by TLC using Benzene: Methanol (50:50 v/v) and Carbon tetrachloride: Methanol (50:50 v/v) as solvents system. The absorption maxima of the synthesized compounds were carried out in methanol (analytical grade, 1mg/100mL). The methanolic solutions of the synthesized compounds were scanned on Shimadzu UV 1700 spectrophotometer, Kyoto, Japan; in the region 200-400 nm. The infra red absorption spectra of the synthesized compounds were recorded using KBr disc on FTIR 8010 Shimadzu model. The ^{1}H-NMR spectra of the synthesized compounds were recorded on Brucker Spectrospin DPX 300 spectrophotometer. The solutions of the test compounds were prepared in dimethyl sulfoxide DMSO-d_6. Tetra Methyl Silane (TMS) was used as internal standard. Molecular weights of the synthesized compounds were determined by Rast's camphor method. The analgesic activity of the synthesized compounds was evaluated by using hot plate method using Eddy's hot plate. The anti-inflammatory activity of the standard drug naproxen and synthesized compounds was determined against carrageenan induced acute paw edema in albino rats.

RESULTS AND DISCUSSION

A. SYNTHESIS[8-10]

The corresponding equimolar solutions of 6-substituted-2-aminobenzothiazole derivatives in methanol and 5-substituted-1H-1,2,4-triazol-1-yl ethanone derivatives in methanol were refluxed for around 3-5h in presence of formaldehyde (used as formalin solution) and concentrated HCl. The resultant mixtures were cooled at 0^{0}C for about 24h to yield the mannich bases. Occasionally precipitation may be achieved by adding different solvents to the final reaction mixture. All the synthesized compounds were recrystallized with rectified spirit.

6a

3-(benzothiazol-2-yl-amino)-1-(1*H*-1,2,4-triazol-1- yl)-propan-1- one; white crystals, Yield 3.91 (43%), melting range 164 -6^{0}C, R_f 0.62, λ_{max} 239.14 (methanol), IR (KBr, V max, cm^{-1}): 3184 (NH), 3000 (-CH, Ar), 2675 (-CH_2-CH_2), 1618 (-C=O), 1236 (-C-N), ^{1}H-NMR (DMSO-*d6*, δ ppm): 2.19-2.29(t, 2H, CH_2, α to NH), 3.70-3.77 (t, 2H, CH_2, α to –C=O), 4.17 (s, 1H, NH), 7.60-7.70 (m, 4H, benzothiazole), 9.42

(s, 1H, C-3'), 10.05 (s, 1H, C-5'), M= 271.5 (Th :273).

6b

3-(benzothiazol-2-yl-amino)-1-(5-methyl-1*H*-1,2,4-triazol-1- yl)- propan-1-one, white crystals, Yield 5.5g (57.41%), melting range 182 -3^{0}C, R_f 0.84, λ_{max} 235.1 (methanol), IR (KBr, V max, cm^{-1}): 3300 (NH), 2985 (-CH, Ar), 2887 (-CH_2-CH_2), 1650 (-C=O), 1224 (-C-N),1458 (-CH_3), ^{1}H-NMR (DMSO-*d6*, δ ppm): 1.95 (s, 3H, C-5'-CH_3)2.19-2.29(t, 2H, CH_2, α to NH), 3.70-3.77 (t, 2H, CH_2, α to –C=O), 4.17 (s, 1H, NH), 7.60-7.70 (m, 4H, benzothiazole), 9.42 (s, 1H, C-3') M= 284.9 (Th:287).

6c

3-(benzothiazol-2-yl-amino)-1-[5-(*p*-nitro-phenyl)-1*H*-1,2,4-triazol-1- yl)-propan-1-one; yellow crystals, Yield 4.72g (36%), melting range 154 -6^{0}C, R_f 0.88, λ_{max} 235.93 (methanol), IR (KBr, V max, cm^{-1}): 3370 (NH), 3179 (-CH, Ar), 2964 (-CH_2-CH_2), 1680 (-C=O), 1284 (-C-N), ^{1}H-NMR (DMSO-*d6*, δ ppm): 3.70-3.77(t, 2H, CH_2, α to NH), 3.97-4.03 (t, 2H, CH_2, α to –C=O), 4.55 (s, 1H, NH), 7.62-7.89 (m, 8H, Ar), 9.42 (s, 1H, C-3') M= 397.42 (Th:394).

6d

3-(benzothiazol-2-yl-amino)-1-[5-(*p*-methoxy-phenyl)-1*H*-1,2,4- triazol-1- yl)-propan-1-one; black crystals, Yield 8.08g (64%), melting range164 -5^{0}C, R_f 0.91, λ_{max} 293.14 (methanol), IR (KBr, V max, cm^{-1}): 3462 (NH), 3179 (-CH, Ar), 2963 (-CH_2-CH_2), 1741 (-C=O), 1290 (-C-N), ^{1}H-NMR (DMSO-*d6*, δ ppm): 3.15 (s, 3H, C5'-Ar-OCH_3), 3.70-3.77 (t, 2H, CH_2, α to NH), 3.97-4.03 (t, 2H, CH_2, α to –C=O), 4.60 (s, 1H, NH), 7.62-7.89 (m, 8H, Ar), 9.36 (s, 1H, C-3') M= 376.25 (Th:379).

6e

3-(6-methoxy-benzothiazol-2-yl-amino)-1-(1*H*-1,2,4-triazol-1-yl)- propan-1-one, white crystals, Yield 3.86g (46%), melting range 208 -10^{0}C, R_f 0.71, λ_{max} 222.6 (methanol), IR (KBr, V max, cm^{-1}): 3208 (NH), 2961 (-CH, Ar), 2872 (-CH_2-CH_2), 1718 (-C=O), 1458 (-CH_3), 1324 (-C-N), 1093(-OCH_3), ^{1}H-NMR (DMSO-*d6*, δ ppm): 3.45-3.61 (t, 2H, CH_2, α to NH), 4.21-4.41 (t, 2H, CH_2, α to –C=O), 4.49 (s, 3H, C6-OCH_3), 4.54 (s, 1H, NH), 6.42-7.77 (m, 3H, Benzothiazole), 9.39 (s, 1H, C-3'), 10.05 (s, 1H, C-5') M= 306.64 (Th:303).

6f

3-(6-methoxy-benzothiazol-2-yl-amino)-1-[5-(*p*-nitro-phenyl)-1*H*-1,2,4- triazol-1- yl)-propan-1-one, yellow crystals, Yield 3.64g (31%), melting range 209 -11^{0}C, R_f 0.86, λ_{max} 252.34 (methanol), IR(KBr,V max, cm^{-1}): 3200 (NH), 3100 (-CH, Ar), 2983 (-CH_2-CH_2), 1718 (-C=O), 1458 (-CH_3), 1240 (-C-N), 1084 (-OCH_3), ^{1}H-NMR (DMSO-*d6*, δ ppm): 3.45-3.64(t, 2H, CH_2, α to NH), 4.21-4.41 (t, 2H, CH_2, α to –C=O), 4.49 (s, 3H, -OCH_3), 4.54 (s, 1H, NH), 6.84-7.77 (m, 7H, Ar), 9.39 (s, 1H, C-3') M= 427.04 (Th:424).

6g

3-(6-methoxy-benzothiazol-2-yl-amino)-1-[5-(*p*-methoxy-phenyl)-1*H*-1,2,4- triazol-1- yl)-propan-1-one, white crystals, Yield 5.89g (52%), melting range 264 -5^{0}C, R_f 0.63, λ_{max} 232.15 (methanol), IR (KBr, V max, cm^{-1}): 3200 (NH), 3054 (-CH, Ar), 2841 (-CH_2-CH_2), 1630 (-C=O), 1458 (-CH_3), 1240 (-C-N), 1093 (-OCH_3), ^{1}H-NMR (DMSO-*d*6, δ ppm): 3.16 (s, 6H, C6-OCH_3 and C5'Ar-OCH_3), 3.45-3.64(t, 2H, CH_2, α to NH), 4.21-4.44 (t, 2H, CH_2, α to –C=O), 4.54 (s, 1H, NH), 6.84-7.77 (m, 7H, Ar), 9.42 (s, 1H, C-3') M= 406.79 (Th:409).

6h

3-(6-chloro-benzothiazol-2-yl-amino)-1-[5-(*p*-methoxy-phenyl)- 1*H*-1,2,4- triazol-1- yl)-propan-1-one, white crystals, Yield 3.8g (34%), melting range.247 -8^{0}C, R_f 0.68, λ_{max} 217.0 (methanol), IR (KBr, V max, cm^{-1}): 3208 (NH), 3049 (-CH, Ar), 2934(-CH_2-CH_2), 1718(-C=O), 1356 (-C-N), 1263 (-OCH_3), 702 (C-Cl), ^{1}H-NMR (DMSO-*d6*, δ ppm): 2.51 (s, 3H, -OCH_3), 3.45-3.64 (t, 2H, CH_2, α to NH), 4.21-4.41 (t, 2H, CH_2, α to –C=O), 4.54 (s, 1H, NH), 7.60-7.89 (m, 7H, Ar), 9.42 (s, 1H, C-3'), M= 412.11(Th:413.5)

6i

3-(6-nitro-benzothiazol-2-yl-amino)-1-(1*H*-1,2,4-triazol-1-yl)- propan-1-one, yellow crystals, Yield 2.85g (35%), melting range 264-5^{0}C, R_f 0.90, λ_{max} 205.67 (methanol), IR (KBr, V max, cm^{-1}): 3200 (NH),3100 (-CH, Ar), 2916 (-CH_2-CH_2), 1650 (-C=O), 1560 (C-NO_2), 1224 (-C-N), ^{1}H-NMR (DMSO-*d6*, δ ppm): 3.70-3.77 (t, 2H, CH_2, α to NH), 3.97-4.03 (t, 2H, CH_2, α to –C=O), 4.60 (s, 1H, NH), 7.62-7.73 (m, 3H, Benzothiazole), 9.39 (s, 1H, C-3'), 10.05 (s, 1H, C-5') M= 315.5 (Th:318)

6j
3-(6-nitro-benzothiazol-2-yl-amino)-1-[5-(*p*-nitro-phenyl)-1*H* 1,2,4-triazol-1-yl]-propan-1-one, yellowish white crystals, Yield 4.16g (37%), melting range 266 -7^{0}C, R_f 0.68, λ_{max} 324.3 (methanol), IR (KBr, V max,cm^{-1}): 3268(NH), 3179 (-CH, Ar), 2963(-CH_2-CH_2), 1643(-C=O), 1529 (-C-NO_2),1284 (-C-N), ^{1}H-NMR (DMSO-*d6,* δ ppm): 3.45-3.64 (t, 2H, CH_2, α to NH), 4.21-4.41 (t, 2H, CH_2, α to –C=O), 4.49 (s, 1H, NH), 6.80-7.44 (m, 7H, Ar), 8.90 (s, 1H, C-3'), M= 435.98 (Th:439).

B. Biological activity

1. Anti-inflammatory activity[10-14]

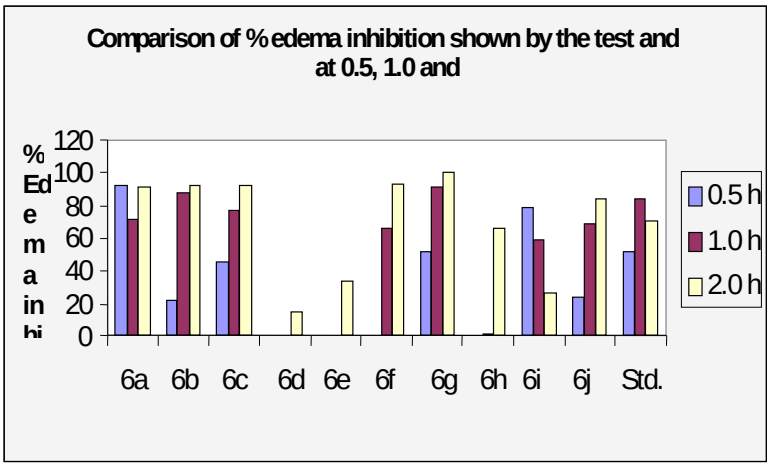
The anti-inflammatory activity of the standard drug naproxen and synthesized compounds, (**6a-j),** was determined against carrageenan induced acute paw edema in albino rats (72 no. Weighing 200-225g). The 1% w/v solution of carrageenan for injection was prepared in normal saline (0.9% NaCl) and 0.1 ml was injected underneath planter region. The dose, of standard drug and synthesized compounds, administered, in animals was 50 mg/ kg, by oral route using oral feeding tube through tuberculin syringe. The stock suspensions of standard and synthesized compound were prepared in concentration of 10 mg/ml of 2% w/v CMC in distilled water. Results of anti-inflammatory activity are shown in Table 1, Table 2 and Graph 1.

Table 1: Effect of the Test and Standard compounds on carrageenan-induced rat paw Edema

Compd. No.	Dose (mg/kg, *p.o*)	Increase in paw volume (mean ± SEM) in ml at			
		0h	0.5h	1.0h	2.0h
Control (N/saline)	2.5 ml/kg	0.0	0.83 ± 0.13	1.25 ± 0.13	1.73 ± 0.13
6a	50 mg/kg	0.0	0.06 ± 0.13	0.36 ± 0.13	0.16 ± 0.13
6b	50 mg/kg	0.0	0.65 ± 0.13	0.16 ± 0.13	0.13 ± 0.13
6c	50 mg/kg	0.0	0.46 ± 0.14	0.28 ± 0.14	0.13 ± 0.13
6d	50 mg/kg	0.0	1.46 ± 0.14	1.25 ± 0.14	1.48 ± 0.14
6e	50 mg/kg	0.0	1.46 ± 0.13	1.95 ± 0.13	1.16 ± 0.13
6f	50 mg/kg	0.0	1.63 ± 0.13	0.43 ± 0.13	0.11 ± 0.13
6g	50 mg/kg	0.0	0.4 ± 0.13	0.11 ± 0.13	0.0 ± 0.13
6h	50 mg/kg	0.0	1.5 ± 0.13	1.23 ± 0.14	0.6 ± 0.13
6i	50 mg/kg	0.0	0.18 ± 0.14	0.51 ± 0.13	1.28 ± 0.13
6j	50 mg/kg	0.0	0.63 ± 0.13	0.38 ± 0.13	0.28 ± 0.13
Std. (Naproxen)	50 mg/kg	0.0	0.4 ± 0.14	0.2 ± 0.13	0.51 ± 0.14

Table 2: Percentage inhibition of carrageenan induced rat paw edema, exhibited by the Test and Standard compounds

Compound No.	% Inhibition of carageenan induced rat paw edema at		
	0.5 h	1.0 h	2.0 h
6a	92.77	71.2	90.75
6b	21.68	87.2	92.48
6c	44.57	77.6	92.48
6d	0.0	0.0	14.45
6e	0.0	0.0	32.94
6f	0.0	65.6	93.64
6g	51.80	91.2	100
6h	0.0	1.6	65.31
6i	78.31	59.2	26.01
6j	24.09	69.6	83.81
Std. (Naproxen)	51.80	84.00	70.52



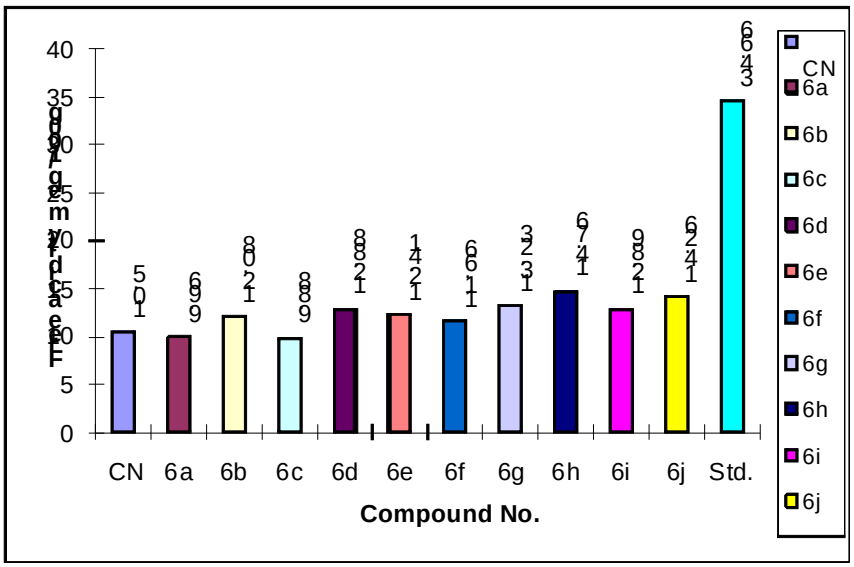
Graph 1: Comparison of % edema inhibition shown by the test and standard compounds at 0.5, 1.0 and 2.0 h.

2. Ulcerogenic activity[15-17]

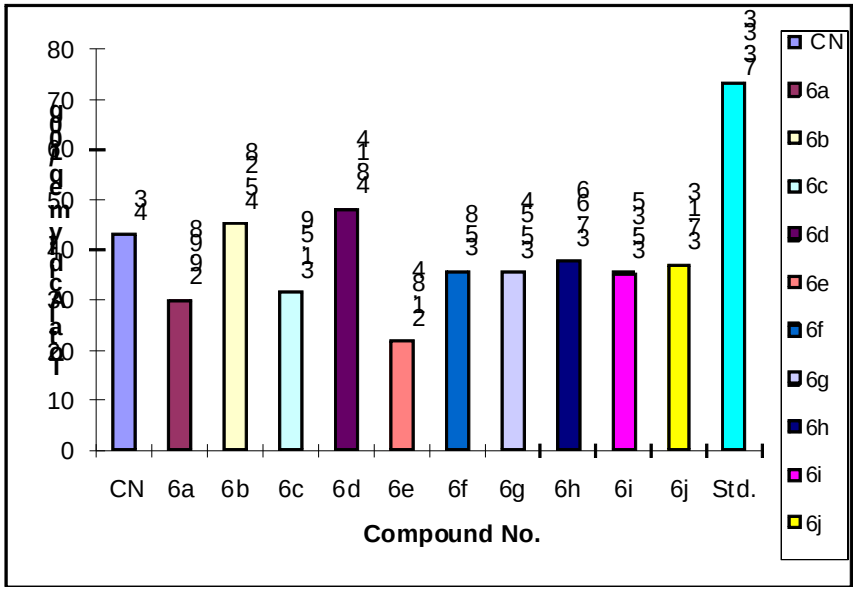
The Ulcerogenic activity of the standard drug naproxen and synthesized compounds **6(a-j)** was determined by 14 days chronic studies using pyloric ligation-induced acid secretion and ulcer formation technique in albino rats. The procedure produces reliable number of gastric ulcers. Diethyl ether (anesthetic ether) as inhalant was used as for anesthesia. The standard and test compounds were administered at dose of 50 mg/ kg[27] by oral route using oral feeding tube through tuberculin syringe. The stock suspension of the standard and test compounds was and prepared in concentration of 10 mg/ml of 2% w/v CMC in distilled water. The 0.01 N NaOH solution was used to determine acidity of gastric fluid using topfer's reagent for free acidity and phenolphthalein indicator for total acidity. The gastric volume, acidity and ulcer index were compared for control and drug treated animals and are shown in Table 3, Graph 2 and Graph 3.

Table 3: Ulcerogenic profile of the standard and test compounds

Compound No.	Volume of gastric secretion (ml/100g) ± SEM	Ulcer Score	Acidity of Gastric Secretion	
			Free acidity ± SEM	Total acidity ± SEM
Control	1.90 ± 0.12	0	10.5 ± 0.20	43.0 ± 0.36
6a	1.21 ± 0.12	0	9.96 ± 0.12	29.98 ± 0.12
6b	1.37 ± 0.12	0.5	12.08 ± 0.12	45.28 ± 0.43
6c	1.14 ± 0.12	0	9.88 ± 0.12	31.59 ± 0.48
6d	1.45 ± 0.12	0	12.88 ± 0.31	48.14 ± 0.49
6e	1.33 ± 0.12	0	12.41 ± 0.20	21.84 ± 0.35
6f	1.57 ± 0.12	0	11.66 ± 0.12	35.80 ± 0.25
6g	1.46 ± 0.12	0.5	13.23 ± 0.17	35.54 ± 0.34
6h	1.76 ± 0.12	0	14.76 ± 0.35	37.66 ± 0.39
6i	1.42 ± 0.12	0	12.89 ± 0.14	35.35 ± 0.38
6j	1.36 ± 0.12	0	14.26 ± 0.19	37.13 ± 0.24
Std.	2.46 ± 0.12	2	34.66 ± 0.41	73.33 ± 0.41



Graph 2: Comparison of Free acidity

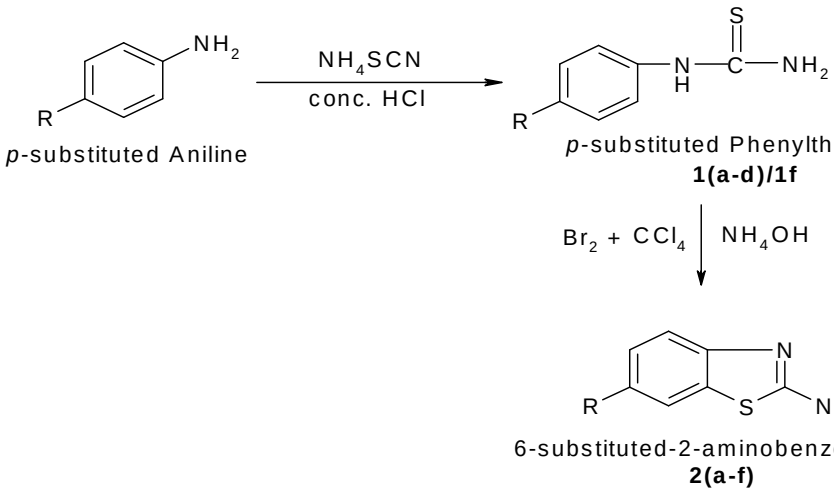


Graph 3: Comparison of Total Acidity

CONCLUSION

The determination of anti-inflammatory activity inferred that compounds 6a, 6b, 6c and 6g possess more potent anti-inflammatory activity than the standard drug. The activity shown by these compounds is attributed to the presence of electron releasing groups on C6 of Benzothiazole and C5 to triazole ring system. None of the tested compounds exhibited ulcerative effect as that was shown by the standard drug naproxen. Thus it can be assumed that the further exploration on this heterocycles may produce a good, yet devoid of ulcerative effect, anti-inflammatory agent.

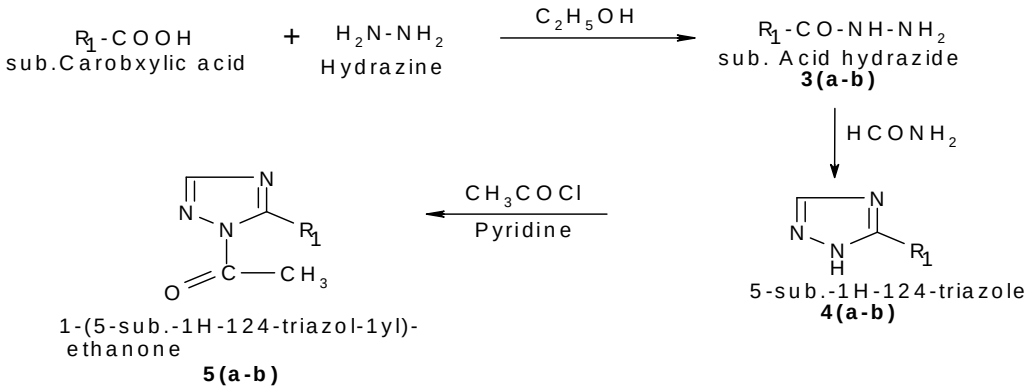
SYNTHETIC SCHEME



p-substituted Aniline

p-substituted Phenylthiourea
1(a-d)/1f

6-substituted-2-aminobenzothiazole
2(a-f)



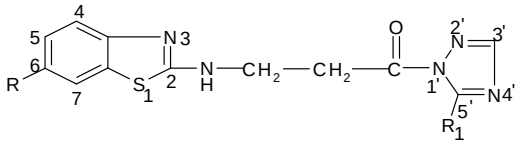
3(a-b)

5-sub.-1H-124-triazole
4(a-b)

1-(5-sub.-1H-124-triazol-1yl)-ethanone
5(a-b)

2(a-f) + 5(a-b)

HCHO conc.HCl



3-(6-substituted-benzothiazol-2-yl-amino)-1-(5-substituted-1H-1,2,4-triazol-1-yl)-propan-1-one

6 (a-j)

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