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Research Article

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SYNTHESIS, ANALGESIC AND ANTI-INFLAMMATORY 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 analgesic and anti-inflammatory activity. Few compounds exhibit excellent activities as compared to the standard drugs.

Keywords: 1*H*-1,2,4-triazole, 2-aminobenzothiazole, Mannich base, analgesic, anti-inflammatory.

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.

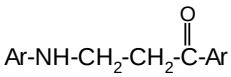
The review of literature survey reveals 6-substituted-2-aminobenzothiazole derivatives such as 6-methyl, 6-methoxy, 6-ethoxy, and 6-isopropoxy- possess potent antibacterial, analgesic and anti-inflammatory properties.[1-3]

The substituted 1*H*-1,2,4-triazole derivatives are found to possess a wide range of therapeutic applications including analgesic, anti-inflammatory, antimicrobial, antiviral, antitumor, anticonvulsant, , sedative, hypnotic and antiestrogenic effect.[4-7]

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.

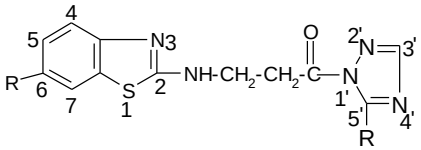
Aminoalkyl chain can be introduced in a molecule through mannich reaction, which involves use of compound containing active hydrogen, formaldehyde and a primary or secondary amine. Many mannich bases are in clinical use including atropine, cocaine, dyclonine, tutocaine, tanitidine, phenindamine, triprollidine, amodiaquin, ethacrynic acid, biperiden, procyclidine, trihexyphenidyl, molindone, zolpidem, fluoxetine and propoxyphene.

From the above mentioned factors and review of literature survey it is observed that 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.

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.

CHEMISTRY

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

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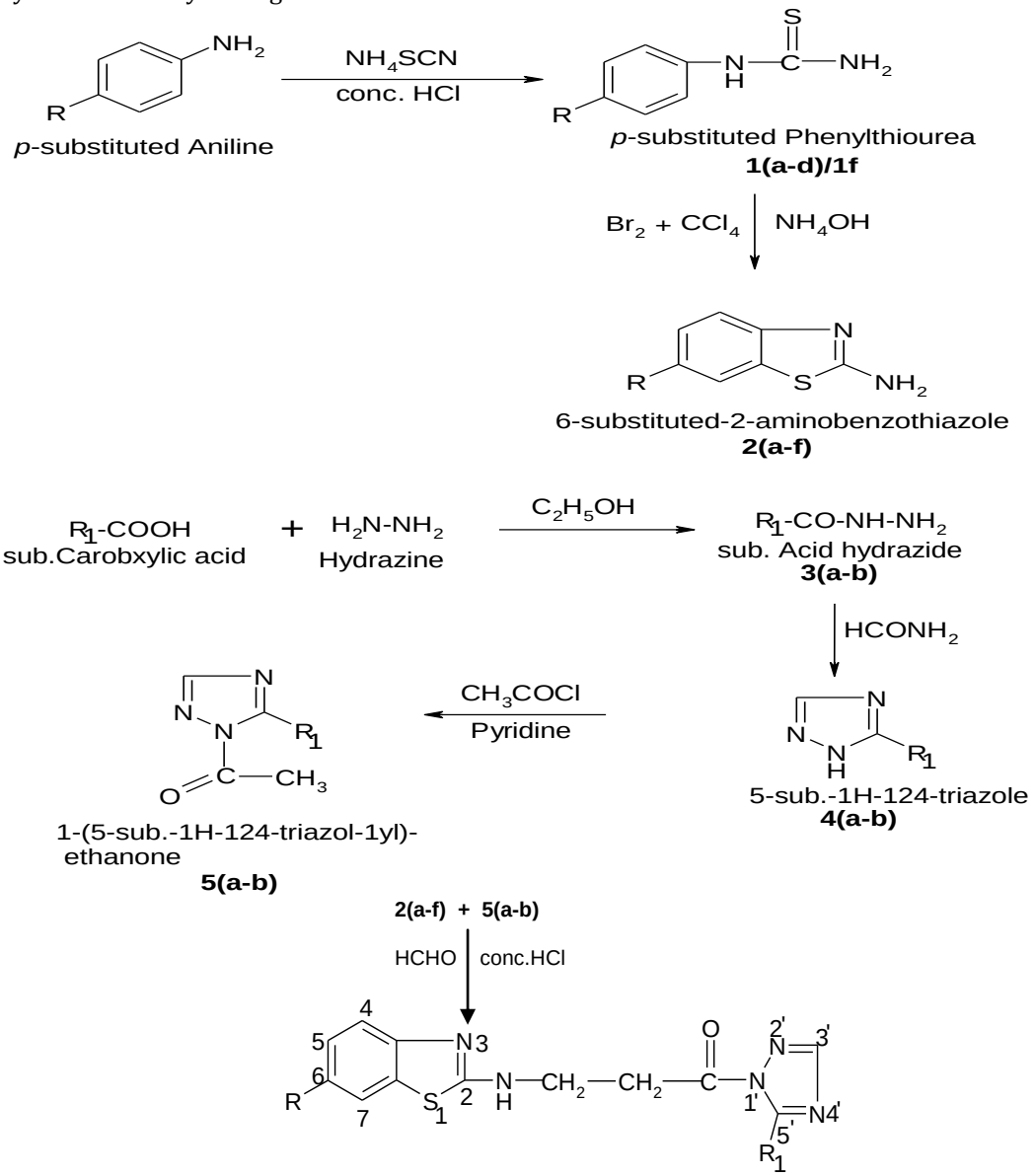
concentrated HCl. The resultant mixtures were cooled at 0°C for about 24h to yield the mannich bases. Occasionally precipitation may be achieved by adding different solvents to the final reaction mixture. All the synthesized compounds were recrystallized with rectified spirit.



PHARMACOLOGY

Ten mannich bases of 6-substituted-2-aminobenzothiazole have been synthesized. Analgesic activity of these compounds was evaluated by using Eddy's hot plate method. Anti-inflammatory activity was examined against carrageenan induced acute paw edema in albino rats.

RESULTS AND DISCUSSION

Biological assay

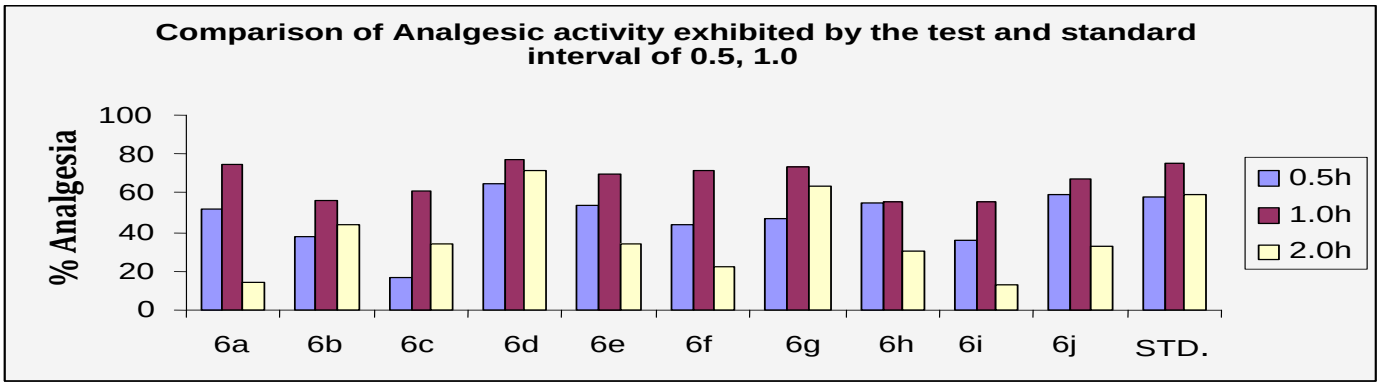
All the compounds (**6a-j**) were evaluated for analgesic and anti-inflammatory activity with aspirin, naproxen respectively as standards.

Analgesic activity[11-13]**:** The analgesic activity of the test compounds **6(a-j)** was evaluated by using Eddy's hot plate method. The albino mice (55 no., weighing 20-25 g) were divided into eleven groups with five animals per cage. The first group was for standard drug (Aspirin) and rest of ten groups were for the synthesized compounds **6(a-j)**. The solutions of the test compounds were prepared in carboxy methyl cellulose (CMC) (2% w/v). Results of analesic activity are shown in Table **1** and Graph **1**.

Table 1. Percent analgesia of the test and standard drug with mean and SEM values

Compound no.	Dose (mg/kg, *p.o.*)	% Analgesia (Mean ± SEM) at		
		0 h	1.0 h	2.0 h
6a	100	51.42 ± 1.90	74.71 ± 1.17	14.00 ± 2.89
6b	100	37.66 ± 2.06	56.42 ± 2.98	43.42 ± 3.23
6c	100	17.00 ± 3.84	61.26 ± 1.77	34.42 ± 3.97
6d	100	65.33 ± 1.32	76.99 ± 0.83	71.27 ± 1.06
6e	100	53.83 ± 2.63	69.56 ± 2.10	34.00 ± 5.51
6f	100	43.92 ± 4.20	71.61 ± 1.80	22.0 ± 5.0
6g	100	47.14 ± 2.67	73.80 ± 1.47	63.39 ± 1.74
6h	100	54.99 ± 1.90	55.87 ± 2.50	30.33 ± 3.16
6i	100	35.49 ± 2.72	55.43 ± 3.63	13.0 ± 4.15
6j	100	59.16 ± 2.74	67.54 ± 2.24	32.33 ± 2.43
Std. (Aspirin)	100	58.33 ± 1.95	75.09 ± 1.19	59.83 ± 2.27

Graph 1. Comparison of the analgesic activity exhibited by the test and standard compounds at time interval of 0.5, 1.0 and 2.0h



Anti-inflammatory activity[14-16]**:** 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 **2**, Table **3** and Graph **2**.

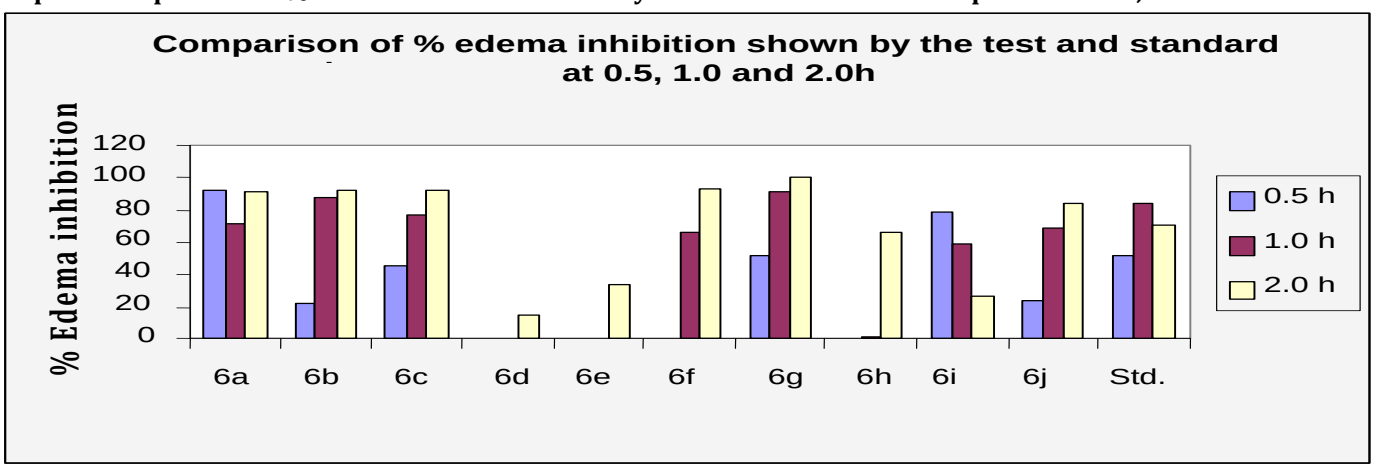
Table 2. Effect of the Test and Standard compounds on carrageenan-induced rat paw Edema

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 3. 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 2. Comparison of % edema inhibition shown by the test and standard compounds at 0.5, 1.0 and 2.0 h.



CONCLUSION

In overall the compounds **6d** and **6g** exhibit very markable analgesic and anti-inflammatory respectively than standard drugs (aspirin and naproxen). On the basis of above observations it is assumed that, among the series of tested derivatives, the analgesic and anti-inflammatory activity was exhibited excellently by the compounds bearing substitutions with electron releasing groups like –OCH_3 on C6 of benzothiazole ring and C5 of triazole ring.

EXPERIMENTAL

Chemistry

All the starting materials and reagents were obtained from commercial sources and were used without further purification. 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 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.

3-(benzothiazol-2-yl-amino)-1-(1*H*-1,2,4-triazol-1- yl)-propan-1- one (6a); white crystals, Yield 3.91 (43%), melting range 164 -6°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-*d*6, 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).

3-(benzothiazol- 2-yl- amino)- 1-(5-methyl -1*H*-1,2,4-triazol-1- yl)- propan-1-one (6b); white crystals, Yield 5.5g (57.41%), melting range 182 -3°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-*d*6, 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).

3-(benzothiazol- 2-yl-amino)- 1-[5-(*p*-nitro- phenyl)-1*H* -1,2,4-triazol-1- yl)-propan-1-one (6c); yellow crystals, Yield 4.72g (36%), melting range 154 -6°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-*d*6, 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).

3-(benzothiazol- 2-yl-amino)-1-[5- (*p*-methoxy-phenyl) -1*H*-1,2,4- triazol-1- yl)-propan-1-one (6d); black crystals, Yield 8.08g (64%), melting range164 -5°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-*d*6, 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).

3-(6-methoxy -benzothiazol-2-yl-amino) -1-(1*H*-1,2,4-triazol-1-yl)- propan-1-one (6e); white crystals, Yield 3.86g (46%), melting range 208 -10°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-*d*6, 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).

3-(6-methoxy -benzothiazol-2-yl-amino)-1 -[5-(*p*-nitro-phenyl)- 1*H*-1,2,4- triazol-1- yl)-propan-1-one (6f); yellow crystals, Yield 3.64g (31%), melting range 209 -11°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-*d*6, 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).

3-(6-methoxy -benzothiazol- 2-yl-amino)- 1-[5-(*p*-methoxy-phenyl)- 1*H*-1,2,4- triazol-1- yl)-propan-1-one (6g); white crystals, Yield 5.89g (52%), melting range 264 -5°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).

3-(6-chloro- benzothiazol-2 -yl-amino)- 1-[5-(*p*-methoxy -phenyl)- 1*H*- 1,2,4- triazol-1- yl)-propan-1-one (6h); white crystals, Yield 3.8g (34%), melting range.247 -8°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-*d*6, 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).

3-(6-nitro- benzothiazol- 2-yl-amino)-1-(1*H*-1,2,4-triazol-1-yl)- propan-1-one (6i); yellow crystals, Yield 2.85g (35%), melting range 264-5°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-*d*6, 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).

3-(6-nitro- benzothiazol- 2-yl-amino)-1- [5-(*p*-nitro-phenyl)-1*H* 1,2,4-triazol-1-yl]-propan-1-one (6j); yellowish white crystals, Yield 4.16g (37%), melting range 266 -7°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-*d*6, 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).

Biological Assays

The new molecules were evaluated for analgesic and anti-inflammatory activity with aspirin, naproxen as standards.

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.

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