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ChemTech

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.1, No.4, pp 1354-1358, Oct-Dec 2009

SYNTHESIS, CHARACTERISATION AND ANTI-INFLAMMATORY ACTIVITY OF SOME 2-AMINO BENZOTHIAZOLE DERIVATIVES

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ABSTRACT: A series of some novel 2-amino benzothiazole derivatives were synthesized and evaluated for anti-inflammatory activity. The titled compounds were synthesized from the substituted aromatic amines through the intermediate substituted 1-phenylthiourea oxidation by bromine water in acidic medium. The purity of the synthesized compounds were judged by their C, H and N analysis and the structure was analyzed on the basis of IR, ^{1}HNMR and Mass spectral data. The anti-inflammatory activities of new compounds were determined by λ-Carrageenan-induced mice paw edema method using diclofenac sodium as a standard. Among the compounds tested three compounds Bt2 (5-chloro-1,3-benzothiazole-2-amine), Bt (6-methoxy-1,3-benzothiazole-2-amine) and Bt7 (6-methoxy-1,3-benzothiazole-2-amine)were the most active compounds in these series when compared with diclofenac sodium. In the SAR study, the phenyl ring substituted with chloro at 5 position, methoxy substitution at 4 and 6-position in benzothiazole ring system showed better anti-inflammatory activity.
Key Words: Anti-inflammatory, bromine water, hind paw edema, benzothiazoles

INTRODUCTION

Benzothiazoles are bicyclic ring system with multiple applications which have been the subject of great interest because of their biological activities. Literature review revealed the potent inhibition of human immunodeficiency virus type 1 (HIV-1) replication by HIV-1 protease inhibition[1], anti tumor[2], analgesic and anti-inflammatory[3], antimalerial[4], antifungal[5], anticandidous activities[6] and various CNS activities [7] of benzothiazoles.

Long term therapy with nonselective NSAIDs may cause gastrointestinal complications ranging from stomach irritation to life-threatening GI ulceration and bleeding[8]. Even with selective coxibs has revealed unexpected cardiovascular adverse effects[9]. Thus there remains a compelling need for effective NSAIDs with an improved safety profile.

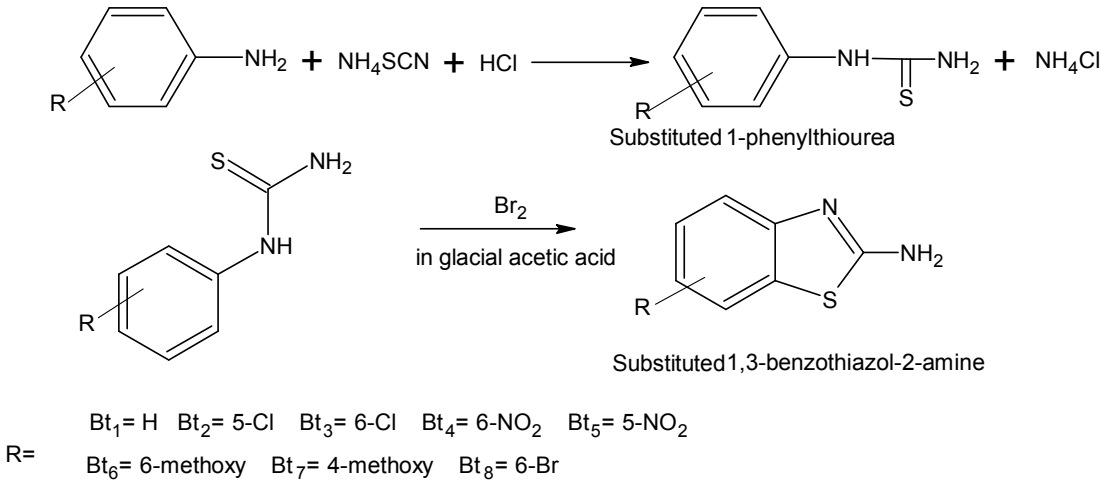
In the present study 2-aminobenzothiazole derivatives were prepared from the substituted aromatic amines, in the presence of ammonium thiocyanate forms substituted 1-phenylthiourea in acidic medium. This substituted 1-phenylthiourea in the presence of oxidizing agent like bromine is cyclised into substituted 2-aminobenzothiazoles. The titled compounds were evaluated for anti-inflammatory property by λ-Carrageenan-induced paw edema method in rats.

EXPERIMENTAL

The melting points of the compounds were determined in open capillary tubes on a Thomas hoover melting point apparatus (Perfit) and are uncorrected. IR spectra were recorded in KBr pellets on JASCO FT IR-5300 infrared spectrophotometer (Japan). ^{1} H-NMR spectra were determined at 300.40 MHZ JEOL-AL 300 (Fourier Transformer, Japan) and mercury plus Varian (400MHZ) spectrometers with tetramethyl silane as internal standard. The FT 13C NMR recorded in $CDCl_3$ at 25.2MHZ. Mass spectra were recorded on JOEL SX 102/DA -6000 Mass spectrometer (Japan). U.V/Visible spectra were taken in the region of 200-600nm, on Jasco UV-Visible spectrophotometer (Japan). The elemental analysis of the compounds was performed by Perkin Elmer model 240C analyzer (U.S.A).

Synthesis: General method of synthesis

Equimolar quantities aniline, substituted aniline (0.02mol), and ammonium thiocyanate (1.5g, 0.02mol) were dissolved in ethanol containing 2ml of Conc. Hydrochloric acid. To this bromine in glacial acetic acid (2.7ml, 0.05mol) was added and the reaction mixture was refluxed for 1hr. Then, it was cooled in ice-water mixture. The precipitate obtained, strained well, filtered washed with cold water and dried. The crude product was recrystallised from rectified spirit (Scheme-1).



Scheme-1 : Synthesis of benzothiazoles

Compound (Bt1): Solid, mp 163^{0}C, **UV (λ_{max}) in ethanol:** 244 nm, **(IR) υ_{max} (KBr/cm^{-1}):** 3339(NH), 3038(Ar=C-H) 1610(C=N), 1473(CN), 1270 (C-S), 928, 738(Ar-H bending viberation), **^{1}H-NMR (δ-ppm):** 3.61(s, 2H, NH_2), 7.17 (dd, aryl H adjuscent to nitrogen), 7.46 (dd, aryl H, adjuscent to sulphur J=4.8 Hz).

Compound (Bt2): Solid, mp 186^{0}C**, UV (λ_{max}) in ethanol:** 249 nm**, (IR) υ_{max} (KBr/cm^{-1}):** NH(3325), 3034(Ar=C-H) 1597(C=N),1465(C-N), 1255(C-S), 916,725(Ar-H bending viberation), 780(C-Cl)**, ^{1}H-NMR (δ-ppm):** 3.65(s,2H,NH_2), 6.74 (s,1H,Ar-ortho-H toCl , J= 4.7Hz), 7.15 (t,1H,ortho, meta hydrogen coupling), 7.26 (m,Ar-H adjuscent to sulphur and meta chlorine)

Compound ((Bt3): Solid, mp 192^{0}C, **UV (λ_{max}) in ethanol :** 263 nm, **(IR) υ_{max} (KBr/cm^{-1}):** NH(3330), 3036(Ar=CH), 1602(C=N),1470(C-N), 1262(C-S), 920,730(Ar-H bending viberation), 785(C-Cl)**, ^{1}H-NMR (δ-ppm):** 3.62(s, 2H, NH2), 6.78 (dd, 1H, Ar-ortho-H toCl J= 4.9Hz), 7.19(t, 1H, ortho to chlorine atom, ortho, meta hydrogen coupling), 7.36(m, Ar-H ortho to chlorine and adjuscent to sulphur)

Compound (Bt4): Solid, mp 123^{0}C **UV (λ_{max}) in ethanol:** 247 nm, **(IR) υ_{max} (KBr/cm^{-1}):** NH(3335), 3031(Ar=C-H) 614(C=N), 1476(C-N), 1356(C-NO_2), 1265(C-S), 924,735(Ar-H bending viberation)**, 1H-NMR (δ-ppm):** 3.90 (s,2H,NH_2), 7.59 (dd,1H,Ar-ortho-H's to NO_2 J= 4.7Hz), 7.36(t, 1H,ortho,meta hydrogen coupling), 6.96(s,Ar-H meta to NO_2), 7.66(dd,1H, ortho,meta long range coupling J=4.9 Hz).

Compound (Bt5): Solid, mp 141^{0}C**, UV (λ_{max}) in ethanol:** 300nm, **(IR) υ_{max} (KBr/cm^{-1}):** NH(3338), 3033(Ar=C-H) 1617(C=N), 1480(C-N),1266(C-S), 1359(C-NO_2), 925, 736(Ar-H bending viberation), **^{1}H-NMR (δ-ppm):** 3.81(s, 2H, NH2), 7.57(dd, 1H, Ar-ortho-H's to NO_2 J= 4.6Hz), 7.274(t, 1H meta to–NO_2 group, ortho, meta hydrogen coupling), 7.48(s, Ar-H meta to NO_2) 6.87 (dd, 1H, near to sulphur ortho, meta long range coupling J=5 Hz).

Compound (Bt6): Solid, mp 186^{0}C**, UV (λ_{max}) in ethanol :** 333 nm, **(IR) υ_{max} (KBr/cm^{-1}):** NH(3320), 3034(Ar=C-H) 2951(C-CH_3), 1625 (C=N), 1470(C-N), 1260 (C-S), 1291 (C-O), 920,7 34(Ar-H bending viberation), **1H-NMR (δ-ppm):** 1.81(s, 3H, of O-CH_3) 3.62 (s, 2H, NH_2), 6.78 (dd, 2H, Ar-ortho-H's to –OCH_3J= 4.9Hz), 7.19(t, 1H, ortho to methoxy, ortho,meta hydrogen coupling), 7.06(s,Ar-H ortho to –OCH_3 and adjacent to sulphur), 7.34 (dd,1H, meta to methoxy long range coupling).

Compound (Bt7): Solid, mp 193^{0}C**, UV (λ_{max}) in ethanol :** 274 nm, **(IR) υ_{max} (KBr/cm^{-1}):** NH(3315), 3033(Ar=C-H) 2955(C-CH_3), 1621 (C=N), 1479(C-N), 1265 (C-S), 1289 (C-O), 925, 732(Ar-H bending vibrations), **1H-NMR (δ-ppm):** 1.86(s, 3H, of O-CH_3), 3.22(s, 2H, NH_2), 6.45 (dd, 1H, phenyl hydrogen meta to OCH_3, J= 4.6Hz), 7.34 [t, 1H (ortho to –OCH_3), ortho, meta hydrogen coupling)], 7.46(dd, 1H, para to –OCH_3ortho, meta long range coupling).

Compound (Bt8): Solid, mp 203^{0}C**, UV (λ_{max}) in ethanol:** 276.5 nm, **(IR) υ_{max} (KBr/cm^{-1}):** NH(3325), 3034 (Ar=C-H) 1595 (C=N), 1468 (C-N), 1265 (C-S), 923,735 (Ar-H bending viberation), 508 (C-Br)**1H-NMR (δ-ppm):** 3.65(s, 2H, NH2), 6.81 (dd, 1H, Ar-ortho-H to Br J= 4.8Hz), 7.26 (t, 1H, ortho to bromine atom, ortho, meta hydrogen coupling), 7.44 (m, Ar-H ortho to bromine and adjuscent to sulphur)

Biological investigation

Materials and methods

Experimental protocols and procedures used in this study were approved by the Animal Ethics Committee of the Allahabad Agricultural Institute-Deemed University, Allahabad.

Animals

Wister rats of both sexes weighing 250–300g were used. The animals were kept and maintained under laboratory conditions of temperature, humidity, and light; and were allowed free access to food (standard pellet diet) and water ad libitum. The animals were divided into benzothiazoles (BT), reference drug treated 'test', and distilled water-treated 'control' groups of six animals per group.

Data analysis

Experimental data obtained from 'test' rats treated compounds (BT) (A1-A7), diclofenac sodium alone, as well as those obtained from sodium carboxy methyl cellulose-treated 'control' mice and rats, were pooled and expressed as means (±S.E.M.). The differences standard drug treated - or synthesized compounds - treated 'test' rats means, and sodium carboxy methyl cellulose (NaCMC) treated' control' rats means, Statistical comparisons were performed using Students ' t' test, to assess the level of significance of the differences between the 'test' and 'control' group data means. Values of $P \leq 0.05$ were taken to imply statistical significance.

Method

The rats used were divided into three broad (A, B and C) experimental groups of six rats per group. Group A rats were used as control and each animal in this group (A) received sodium carboxy methyl cellulose (Sodium CMC) (0.1% 3ml / kg i.p.) only. Group B 'test rats received the benzothiazoles (Bt1-Bt7) (100mg/kg, i.p.). Group C 'test' rats received diclofenac sodium (DIC, 100 mg/kg i.p.). The rat hind paw oedema was used as a model of acute inflammation. Acute inflammation of the hind paw was induced in each of the rat by injecting carrageenan (0.1ml (3%)/kg) into the sub plantar surface of the right hind paw. Pedal inflammation (oedema) was always evident within 5–8 min following fresh carrageenan (0.1ml (3%) / kg) injection. Linear diameter of the injected paw was measured (with a screw gauze) for 3 h at 30 min intervals after the administration of the phlogistic agent. Increases in the linear diameter of the right hind paws were taken as indicators of paw oedema. Oedema was assessed in terms of the difference in the 'zero time' ($C0$) linear diameter of the injected right hind paw, and its linear diameter at 'time t' [(Ct)—that is, 30, 60, 90, 120, 150 and 180 min] following fresh carrageenan administration. At the doses tested (30, 100 and 300mg/kg) all the compounds possessed activity at 100mg.

The increases in the right hind paw diameters induced by injections of fresh carrageenen were compared with those of the contra-lateral, non-injected left hind paw diameters. Benzothiazoles (100mg/kg i.p.) were separately administered to each of the rats in the 'test' Group B, 30 min before inducing inflammation with the injection of fresh carrageenan. Similarly other groups also received the respective drugs. Percentage inflammation (oedema) was calculated from the formula: $C0/Ct \times 100$; while percentage inhibition of the oedema was calculated from the formula: $C0–Ct/C0 \times 100$ [where $C0$ is the average inflammation (hind paw oedema) of the 'control' Group A rats at a given time; and Ct is the average inflammation of the (Group B) Compounds (td) B1-B8- or (Group C) diclofenac-treated rats at the same time]. At the doses tested 30 and 100 /kg all the compounds possessed activity at 100mg. (Table-I)

Results and Discussion

2-aminobenzothiazole derivatives were prepared from the substituted aromatic amines which in the presence of ammonium thiocyanate forms substituted 1-phenylthiourea in acidic medium. This substituted 1-phenylthiourea cyclised into substituted 2-aminobenzothiazoles in the presence of oxidizing agent bromine water (scheme V). All the compounds obtained were good yield ranging from 63-85%. The homogeneity of the compounds was monitored by performing TLC by which Rf and Rm values were calculated. The solvent system used for all the compounds was Toluene: Methanol (8:2).

Compounds Bt3, Bt4, Bt7 and Bt8 were found to be more lipophylic indicated by their higher Rm values. Compounds Bt1, Bt3, Bt4 and Bt5 were obtained in good yields (74-85%)

Sub plantar injections of carrageenan (0.1 ml (3%) /kg i.p) provoked marked, time-related, progressive increases in the hind paw diameters of the 'control', untreated rats. Although pedal inflammation (oedema) was always evident within 5–8 min following fresh carrageenan (0.1 ml (3%) /kg i.p) injection, maximal swelling and/or oedema occurred approximately 90 min following administration.

All the tested synthesized 2-amino benzothiazole derivatives showed significant ($P < 0.05–0.001$) anti-inflammatory activity against carrageenan induced paw edema. The protection against the carrageenan induced oedema showed by benzothiazole derivatives (Bt1-Bt7), Dilclofenac sodium (100mg/kg i.p) given in the table 1. Sodium carboxy methyl cellulose (3 ml/kg i.p.) alone neither modified responses to nociceptive stimuli in the rat pedal oedema induced by fresh carrageenan administration. Compounds Bt2 (5-chloro-1,3-benzothiazole-2-amine), Bt (6-methoxy-1,3-benzothiazole-2-amine) and Bt7 (6-methoxy-1,3-benzothiazole-2-amine)were the most active compounds in this series when compared with diclofenac sodium (table-1).

The percentage of protection against the carrageenan induced inflammation shown at 30, 60, 90, 120, 150 and 180 minutes interval diclofenac was 58.38, 67.04, 78.13, 85.25, 94.28 and 99.56. The protection shown by the compounds, E2 and E7 were comparable

with diclofenac. Compounds E6 also showed significant anti inflammatory activity when compared to control and diclofenac treated group, although the compounds E3, E4 and E5. E7 exhibit significant anti inflammatory activity however, is lesser than other compounds in comparison between the negative control group and the positive control group-treated with diclofenac.

Further, when the 2-amino benzothiazole, is substituted at 4 or 5 position with electron withdrawing groups like –Cl, NO_2, $-OCH_3$ increased in the anti inflammatory activity was found, which was equivalent to the anti inflammatory activity of diclofenac whereas theses substituents are shifted to 6 or 7 position decreased activity was found.

Table:1 Anti-inflammatory activity of Benzothiazoles (BT1-Bt8)

Experimental Group & Dose DoseDose	Time(in min) and paw diameter(in mm)					
	30	**60**	**90**	**120**	**150**	**180**
Group A 0	10.38±0.40	12.44±0.32	15.36 ± 0.44	13.56 ± 0.34	12.36 ± 0.38	11.48 ± 0.31
Group B 3ml/Kg	10.38±0.35 (0.00%)	12.43 ± 0.36 (0.08%)	15.34 ± 0.41 (0.13%)	13.53 ± 0.40 (0.22%)	12.34 ± 0.40 (0.22%)	11.46 ± 0.37 (0.16%)
Bt1 100mg/Kg	5.84 ± 0.38** (43.74%)	5.66 ± 0.41** (54.5 %)	5.60 ± 0.45** (63.5%)	4.05 ±0.15*** (70.06%)	3.03 ±0.23*** (77.06%)	2.34±0.21*** (79.08%)
Bt2 100mg/Kg	4.34± 0.31** (58.18%)	4.20 ± 0.41** (66.21%)	3.48 ± 0.18*** (77.31%)	2.75± 0.27** (79.67%)	1.88 ± 0.34** (86.10%)	0.06 ±0.38** (97.5%)
Bt3 100mg/Kg	6.68± 0.29** (35.64%)	6.54± 0.42** (47.38%)	5.82 ± 0.38*** (62.05%)	5.44±0.24*** (60.01%)	5.21±0.36** (61.49%)	4.23± 0.36** (63.72%)
Bt4 100mg/Kg	6.48± 0.37** (37.57%)	6.21±0.42** (50.04%)	5.64±3.51** (63.23%)	4.84±0.37** (64.22%)	4.12±0.44** (66.612%)	3.67± 0.45** (67.97%)
Bt5 100mg/Kg	5.65± 0.35** (45.56%)	5.12 ± 0.38** (58.80%)	4.92±0.43** (67.92%)	4.18±0.29*** (69.33%)	3.61±0.39*** (70.74%)	3.22± 0.21** (72.02%)
Bt6 100mg/Kg	4.36±0.44** (58.1%)	4.42± 0.32** (64.41%)	3.84± 0.40** (75.09%)	2.81 ± .40*** (79.23%)	2.11 ±0.11 *** (82.90%)	1.86 ±0.26** (83.76%)
Bt7 100mg/Kg	4.34± 0.39** (58.18 %)	4.26±0.32** (65.72%)	3.51±0.24*** (77.70%)	1.91±0.26*** (85.88%)	1.76±0.38** (86.26%)	0.07±0.18*** (99.32%)
DIC 100mg/Kg	4.22 ± 0.31** (58.38%)	4.00 ± 0.40** (67.04%)	3.26 ± 0.35*** (78.13%)	2.10 ± .11*** (85.25%)	0.73 ± 10*** (94.28%)	0.05 ± 24*** (99.56%)

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