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SYNTHESIS, CHARACTERIZATION AND ANALGESIC ACTIVITY OF SOME NOVEL SUBSTITUTED 2-AMINO BENZOTHIAZOLE DERIVATIVES

D. Nooka Raju, D.V.R. Subhadra Devi, D. Grace Aghastina, K. Govinda Rao* and K. Sathish Kumar

Department of Pharmaceutical Chemistry, Aditya Institute of Pharmaceutical Sciences, Surampalem, Dist- East Godavari, Andhra Pradesh- 533437, India.

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***Correspondence for Author**
K. Govinda Rao
Department of Pharmaceutical Chemistry, Aditya Institute of Pharmaceutical Sciences, Surampalem, Dist- East Godavari, Andhra Pradesh- 533437, India.

ABSTRACT

The present study deals with synthesis of some novel substituted 2-amino benzothiazole and their spectral characterization by means of UV, IR and ^{1}H NMR. The compounds were screened for analgesic activity. Results obtained established compounds J3 and J1 to have highly significant analgesic activity with reference to the standard aspirin and consequently further exploration of these benzothiazole derivatives should make these molecules accessible for widespread use as potent analgesic agents.

KEYWORDS: Benzothiazole, aniline derivatives, Ammonium thiocyanate, Analgesic activity, Tail flick method.

INTRODUCTION

Benzothiazole is revealed to have shown potential for application in a variety of pharmacological targets and is a privileged bicyclic ring System.[1] It contains a benzene ring fused to a thiazole ring.[2] The small and simple benzothiazole nucleus is present in compounds involved in research aimed at evaluating new products that possess interesting biological activities like- antimicrobial[3-7] antitubercular,[8] antitumour,[9,10] local anaesthetic activity,[11] anticonvulsant,[12,13] antifungal,[14] anthelmintic,[15] analgesic and anti-inflammatory activity.[16,17] Most alarming of all are diseases where resistance is developing for all currently available drugs; current trends suggest that some diseases will have no effective therapies within the next ten years. So, there is a requirement to develop new replacement drug immediately which is effective against

resistant bacteria having lesser toxicity as well as economical also.[18] In view of the biological importance of the benzothiazole nucleus containing compounds, in the present work, it is plan to synthesize substituted 2-amino benzothiazoles by developing novel methodology. Different synthetic methods are reported for the synthesis of benzothiazole and its derivatives which include processes like aniline was treated with KH_4SCN in presence of and glacial acetic acid in bromine.[19] The present study utilizes the same reaction phenomenon of substituted aniline were treated with NH_4SCN in presence of chloroform and bromine to get substituted 2-amino benzothiazole derivatives followed by their analgesic screening using tail flick method.[20]

MATERIALS AND METHODS

All the reagents used for synthesis were of analytical grade commercial products and used without further purification. The melting points of the synthesized compounds were determined using an electric melting point apparatus by open capillary method. (Expressed in degree Celsius) and are uncorrected. The progress of reactions and purity of synthesized compounds were checked on silica gel-G TLC plates using various solvent combinations of different polarity. The spots were detected with iodine vapors as visualizing agent. The λ_{max} (in nm) of the synthesized compounds was recorded on *Elico SL 164* UV-visible spectrophotometer using alcohol as solvent. The FT-IR spectra of the synthesized compounds were recorded on a FT-IR *Perkin Elmer Spectrum RX-I* spectrometer using KBr disc in the range of 4000-400 $cm^{-1.}$ The Proton NMR (^1H NMR) spectra were recorded in *Bruker AC-F 400* FT-NMR spectrometer at a frequency of 400 MHz. Spectra were obtained in deuterated acetone (acetone-d_6) using TMS (δ 0.00 ppm) as an internal standard at room temperature. Chemical shift (δ) values are expressed in ppm relative to internal standard.

Synthetic Scheme

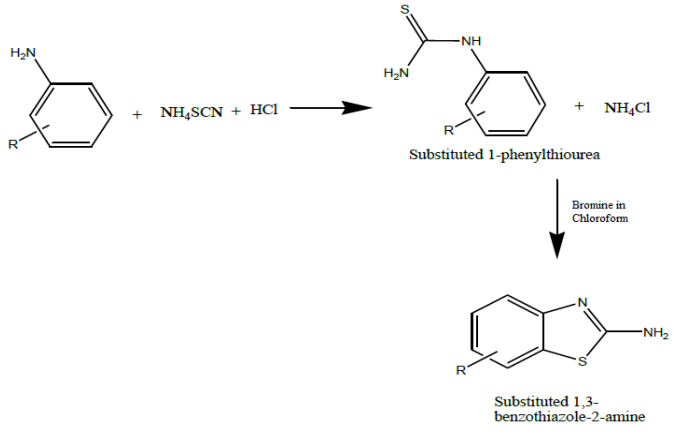
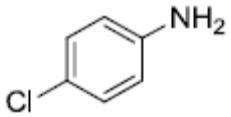
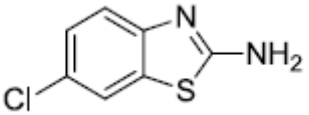
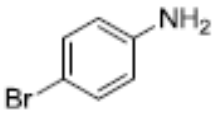
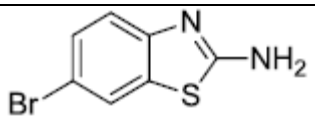
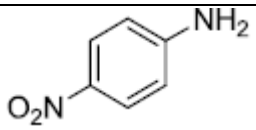
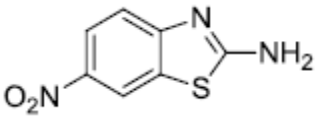
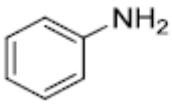
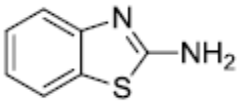
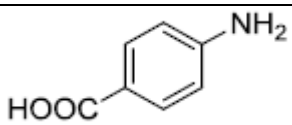
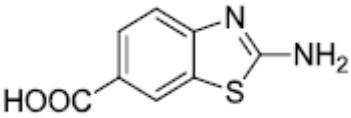


Fig.1: Synthetic scheme for 2-substituted benzothiazole

Substituted aniline used for synthesis

Table.1: Substrate used for the reaction process to form target compound

Sample Code	Substrate	Compound
J1 Mol. Wt = 185		
J2 Mol. Wt = 214		
J3 Mol. Wt = 195		
J4 Mol. Wt = 150		
J5 Mol. Wt = 194		

General method for synthesis of Substituted 2-amino benzothiazole

In 1st step, the substituted aniline (0.01mol) was taken in a 250 ml beaker with magnetic stirrer and covered with watch glass. It was placed into Hotplate, a mixture of 5ml of HCl & 20ml of water was added & then it was stirring at temperature 80°C for 15min. The solution of aniline HCl obtained & then ammonium thiocyanate (0.01mol) was slowly added. The reaction mixture was again stirring to continue 1 hr. The solid separated out on cooling was filtered, washed with water, dried and crystallized from absolute alcohol.

In 2nd step, above obtained compound [0.01mol] in 20 ml chloroform was taken a beaker & then it was stirring at temperature 50°C for 20min. To this reaction mixture bromine [0.01mol] in 20ml chloroform was added with stirring over a period of 1hr, during the addition of bromine, a temp of reaction mixture was maintained below 10°C. It was refluxed until the evaluation of HBr ceased [about 30min] chloroform was removed by filtration. The filtrate was neutralized with aq. ammonia. The precipitate of substituted 2-amino benzothiazole was filtered washed with water & recrystallised from absolute alcohol.

Analgesic Activity

Albino mice of either sex as experimental models were maintained at Aditya Institute of Pharmaceutical Sciences & Research, Surampalem. The animals were maintained in an

animal house under standard environmental conditions. The acute oral toxicity of the synthesized compounds was performed as per OECD guidelines (OECD guideline 423). Animals were observed individually after dosing at least once during the first 30 min; periodically during the first 24 hr with special attention given during the first 4 hr and daily thereafter, for a total of 14 days. As no mortality was observed with the administered dose, a dose of 200 mg/Kg body weight was selected for the pharmacological screening. Aspirin at the dose of 10 mg/kg was administered as standard drug for comparison. The test compounds were administered by the oral routes using an animal feeding needle at the dose level of 100 mg/kg body weight. The control group received appropriate volume of vehicle (distilled water, oral). The analgesic activity was tested using analgesiometer.[21] The instrument is fitted with hot-plate maintained at constant temperature (adjustable with front panel controls both coarse and fine). The temperature to be maintained is 55° C and the acrylic box is fitted to place the mice on hot-plate. This box is fitted with hinge arrangement and consists of lid over it. Analgesiometer operates at 220-230 Volts, 50 Hz. The reaction time (in seconds) of animals to radiant heat was recorded by taking the tail flick from the radiant heat source as end point for every 0, 30, 60 and 90 min time intervals. A cut-off point of 15 sec was imposed to avoid the tail damage.

RESULTS AND DISCUSSON

Physico-chemical properties and spectral data of the synthesized compounds

The yields of all the synthesized compounds were found to be satisfactory within the range of 67 to 78%. The spectral data generated upon analysis were found in accordance with the anticipated structure of the synthesized compounds.

J1: ***6-chloro-2-aminobenzthiazole derivative***

Yield: 75%; **Melting point**: 194-196°C; **R_f value**: 0.81; **λ_{max}**: 445; **IR** (KBr cm^{-1}): 3420, 3240, 3025, 1627, 1503, 1425, 1323, 1250, 740; **^{1}H NMR** (400 MHz, acetone-d_6), δ (ppm): 7.50-7.28(m, 3H, Ar-H), 5.36-5.29(S, 2H,-C-NH_2).

J2: ***6-bromo-1, 3-benzothiazol-2-amine***

Yield: 72%; **Melting point**: 204-206ºC; **R_f value**: 0.78; **λ_{max}**: 460; **IR** (KBr cm^{-1}): 3430, 3250, 3060, 1626, 1520, 1435, 1300, 1270, 752; **^{1}H NMR** (400 MHz, acetone-d_6), δ (ppm): 8.52 (s, 1H, Ar-H), 7.70-7.77 (m, 2H, Ar-H), 6.85-6.11 (S, 2H,-C-NH2).

J3: *6-nitro-1, 3-benzothiazol-2-amine*:

Yield: 70%; **Melting point**: 162-165^{O}C; **R_f value**: 0.90; **λ_{max}**: 450; **IR** (KBr cm^{-1}): 3425, 3226, 3029, 1642, 1555, 1530, 1473, 1322; **^{1}H NMR** (400 MHz, acetone-d_6), δ (ppm): 8.50-8.12(m, 3H, Ar-H), 5.90-5.86 (s, 2H,-C-NH2).

J4: *1, 3-benzothiazol-2-amine:*

Yield: 67%; **Melting point**: 128-130°C; **R_f value**: 0.915; **λ_{max}**: 430; **IR** (KBr cm^{-1}): 3446, 3230, 3030, 1620, 1510, 1440, 1302, 1110; **^{1}H NMR** (400 MHz, acetone-d_6), δ (ppm): 7.73-7.50(m, 4H, Ar-H), 5.70-5.63 (S, 2H,-C-NH2).

J5: *2-amino-1, 3-benzothiazole-6-carboxylic acid*:

Yield: 78%; **Melting point**: 156-158°C; **R_f value**: 0.82; **λ_{max}**: 425; **IR** (KBr cm^{-1}): 3430, 3220, 3050, 1695, 1625, 1530, 1420, 1306, 1260, 758; **^{1}H NMR** (400 MHz, acetone-d_6), δ (ppm): 10.92 (m, 1H, -OH), 8.58 (s, 1H, Ar-H), 8.10-7.68 (m, 2H,-Ar-H), 5.92-5.58 (bs, 2H,-C-NH2).

Analgesic activity data of the synthesized compounds

Analgesic screening of the synthesized compounds show J3, J1 and J4 exhibiting marked analgesic activity in comparison to standard aspirin whereas compound J2 too showed good analgesic activity. Compound J5 showed the least activity amongst the series. The results are statistically expressed in terms of Mean ± SEM as depicted in Table.2. $P < 0.05$ was considered significant.

Table.2 Analgesic activity of synthesized compounds

COMPOUND	DOSE (in mg/kg body weight)	TAIL FLICK METHOD Reaction time (in seconds)			
		0 min	30 min	60 min	90 min
Control	1 ml	3.64 ± 0.14	3.81 ± 0.10	3.95 ± 0.68	4.00 ± 0.10
Standard (Asprin)	10	3.66 ± 0.19	7.40 ± 0.36	12.68 ± 0.40	10.22 ± 0.22
J1	200	3.63 ± 0.52	8.38 ± 0.22	8.62 ± 0.42	7.44 ± 0.24
J2	200	3.70 ± 0.20	7.39 ± 0.28	5.58 ± 0.40	4.85 ± 0.28
J3	200	3.70 ± 0.60	6.40 ± 0.30	11.82 ± 0.21	10.42 ± 0.20
J4	200	3.74 ± 0.10	7.38 ± 0.14	7.68 ± 0.30	6.74 ± 0.22
J5	200	3.40 ± 0.28	3.60 ± 0.34	4.42 ± 0.50	4.74 ± 0.11

Reaction time expressed statistically in terms of Mean ± SEM

CONCLUSION

In this study, we have synthesized five derivatives of substituted 2- amino benzothiazole by the scheme depicted in Figure 1. The test compounds were synthesized in good percentage of yield, their physical and analytical determination was done by using melting point apparatus, purification of compounds by TLC, and the structural assignments of new compounds were made on the basis of UV-visible spectrophotometer, IR and $_1$HNMR data. This scheme of reaction went to completion within 2 hr. After completion of reaction and work up the products were identified and characterized by using UV-visible spectrophotometer, IR and $_1$HNMR techniques and their structures were elucidated as 6-*chloro-2-aminobenzthiozole*, *6-bromo-1, 3-benzothiazol-2-amine*, *6-nitro-1, 3-benzothiazol-2-amine*, *1, 3-benzothiazol-2-amin, 2-amino-1, 3-benzothiazole-6-carboxylic acid*. The isolated yield was 75%, 72%, 70%, 67%, 78%. From our present investigation, it can be concluded that substituted 2- amino benzothiazole are formed by fusion of aryl ring with thiazole and the condensing ring containing at 5^{th} position are various substituent's such as - Cl, - Br, - NO_2, - H, and -COOH the structural assignments of these compounds are made on the basis of UV-visible spectrophotometer, IR and $_1$HNMR data. Further exploration with this series can prove to be instrumental in the field of analgesic drug development.

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