EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 2
Issue: 2
392-401
Year: 2015

SYNTHESIS AND EVALUATION OFANTI-TUBERCULAR ACTIVITY OF BENZOTHIAZOLE DERIVATIVES

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Article Received on 23/02/2015 Article Revised on 19/03/2015 Article Accepted on 13/04/2015

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ABSTRACT

Derivatives of benzothiazoles were synthesized and evaluated for their anti-tubercular activity. 2-amino benzothiazole was first converted to 6 substituted derivatives of 2-amino benzothiazole by nitration and bromination reaction to yield 6-nitro-2-amino benzothiazole and 6-bromo-2-amino benzothiazole respectively. All the derivatives including 2-amino benzothiazole were further treated with chloroacetyl chloride to form chloroacetamido derivatives of benzothiazole. Further the product is treated with various heterocyclic and aromatic amines. The synthesized compounds were confirmed by IR, ^{1}H NMR and Mass spectral data. Synthesized substituted benzothiazole derivatives were investigated for their anti-tubercular activity using Middle brook 7H9 agar medium as described by Elmer WK et al. against H_{37}Rv Strain. It was observed that the new synthesized compounds possessing electron withdrawing group like nitro and bromo groups at 6th position of benzothiazole nucleus and chloro, fluoro substituted at 3rd position of aromatic amine exhibited higher anti-tubercular when compared to that of other synthesized compounds. The present research focus on the different methods of synthesis of substituted benzothiazoles with potential anti-tubercular activity that are now in developing phase.

KEYWORDS: Anti-tubercular, Benzothiazole; Bromination; Chloroacetyl chloride; Nitration.

INTRODUCTION

Benzothiazole is a heterocyclic compound, weak base, having varied biological activities and still of great scientific interest now a days. They are widely found in bioorganic and medicinal chemistry with application in drug discovery. Benzothiazole moites are part of compounds showing numerous biological activities such as antimicrobial[1-4] anticancer[5] anthelmintic[6], anti-diabetic[7], antitubercular[8], anticonvulsant[9], analgesic[10] and anti-inflammatory[11] activities. The benzothiazole ring is present in various marine or terrestrial natural compounds, which have useful biological activities. Thiazole is structurally related to thiophene and pyridine, but in most of its properties it resembles to the latter.

Thiazole was first described by Hantzsch and Waber in 1887. Popp confirmed its structure in 1889. Heterocycles containing the thiazole moiety are present in many natural products such as bleomycin, epothilone A, lyngbyabellin A and dolastatin. Due to their important pharmaceutical utilities, the synthesis of these compounds is of considerable interests. The basic structure of benzothiazole consist of benzene ring fused with 4, 5 position of thiazole. The two rings together constitute the basic nucleus 1, 3-benzothiazle. Being a heterocyclic compound, benzothiazole finds its use in research as a starting material for the synthesis of bioactive molecules. Its aromaticity makes it relatively stable, although as a heterocycles, it has reactive sites which allow for functionalization.

A large number of therapeutic agents are synthesized with the help of benzothiazole nucleus. During recent years there have been some interesting developments in the biological activities of benzothiazole derivatives. These compounds have special significance in the field of medicinal chemistry due to their remarkable pharmacological potentialities. Modifications on the benzothiazole nucleus have resulted in a large number of compounds having diverse pharmacological activities. The synthesis, structures and biological activities of benzothiazole derivatives have long been focused of research interest in the field of medicine due to potential activities exhibited by them. The biological profiles of these new generations of benzothiazoles represent much progress with regards to older compounds. Looking into the medicinal importance of benzothiazole moiety, it was thought worthwhile to synthesize certain newer derivatives of benzothiazole and screen them for their biological activities.

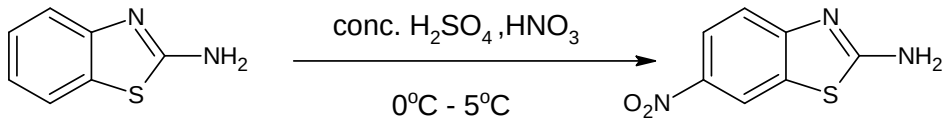
METHODOLOGY

Scheme for Synthesis (Chemistry)

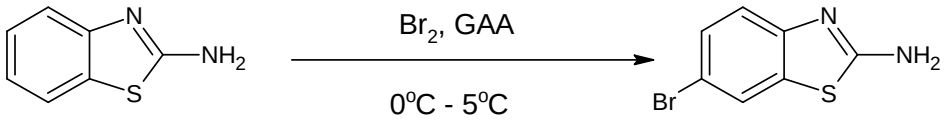
The general method of synthesis of substituted benzothiazole and its derivatives are outlined below.

STEP – I

Synthesis of 6-substituted 2-amino benzothiazole from 2 amino benzothiazole



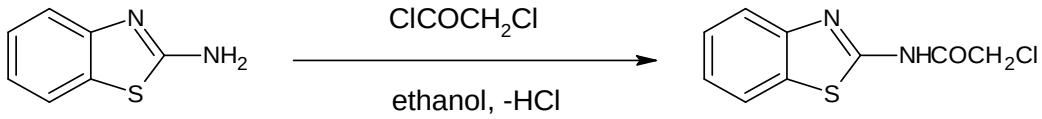
2-amino benzothiazole — 6-nitro 2-amino benzothiazole



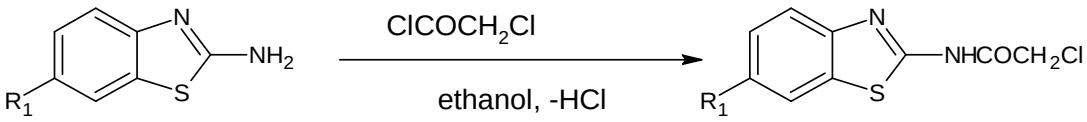
2-amino benzothiazole — 6-bromo 2-amino benzothiazole

STEP – II

Synthesis of *N*-(benzo[*d*]thiazol-2-yl)-2-chloroacetamide



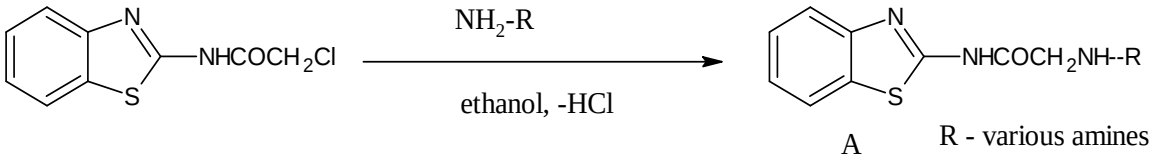
2-amino benzothiazole — *N*-(benzo[*d*]thiazol-2-yl)-2-chloroacetamide



6-substituted 2-amino benzothiazole — 2-chloro-*N*-(6-substitutedbenzo [*d*]thiazol-2-yl)acetamide

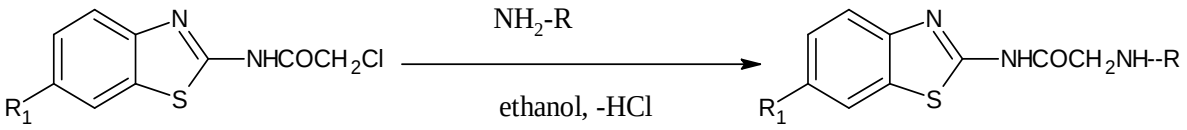
STEP – III

Incorporation of various heterocyclic and aromatic amines at 2 positions of A & B



A

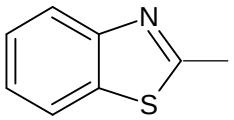
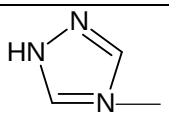
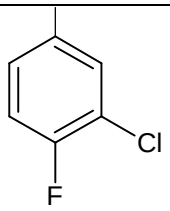
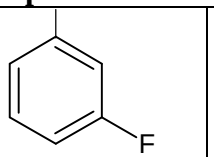
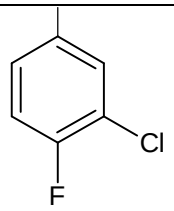
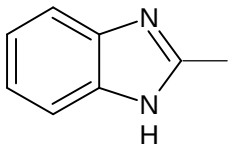
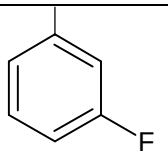
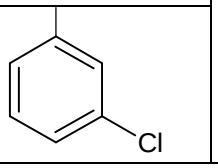
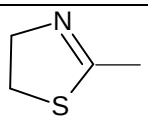
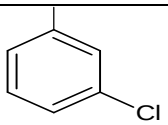
R - various amines



B

R_1 - Br, NO_2

R - various amines

Compound A -R			Compound B -R	
				
				
				

EXPERIMENTAL PROCEDURE

STEP – I

Synthesis of 6-substituted 2-amino benzothiazole[11]

i. Synthesis of 6-nitro 2-amino benzothiazole

2-amino benzothiazole (1g, 0.008 moles) and concentrated sulphuric acid (30 ml) were stirred at 0°C for 15 minutes. To the above solution a mixture of concentrated nitric acid (1.5 ml) and sulphuric acid (5 ml) were added. The reaction was kept was at 0-5°C during the period of addition, and the mixture was then continuously stirred for 2 h at 5°C. The mixture was poured into ice cold water, the precipitate formed was filtered and dried. The product was recrystallized from ethanol to get yellow coloured crystals.

ii. Synthesis of 6-bromo 2-amino benzothiazole

2-amino benzothiazole (1g, 0.008 moles) and glacial acetic acid (4 ml) were placed in conical flask. The flask was kept in an ice bath at 0-5°C. To this solution bromine (7 ml) in glacial acetic acid (14 ml) was added dropwise from dropping funnel with constant stirring. The reaction was poured into excess of water (100 ml). The precipitate was filtered and washed thoroughly with ice cold water. The product was recrystallized from ethanol.

STEP – II

iii. Synthesis of *N*-(benzo[*d*]thiazol-2-yl)-2-chloroacetamide[12]

2-amino benzothiazole (1g) was taken in 100 ml of iodine flask. To this ethanol (10 ml) was added till all benzothiazole gets dissolved. Chloroacetyl chloride (6 ml) was taken in dropping funnel and added dropwise into 2-amino benzothiazole in ice cold condition for 1 h. After complete addition of chloroacetyl chloride for 1 h the reaction mixture was stirred for additional 2 h in ice cold condition.

The reaction mixture was further refluxed at temperature 20 - 30°C for 3 h. Cool the reaction mixture and poured into ice cold water. The precipitate formed was filtered and dried. The product was recrystallized from ethanol. The same procedure was followed for 6-substituted-2-amino benzothiazole to give 6-substituted-2-chloroacetamidobenzothiazole.

STEP III

Incorporation of various heterocyclic and aromatic amines at 2 positions of A & B[13]

Equimolar mixture of compound A and various substituted aromatic and heterocyclic amines (0.1 moles) were refluxed for 6 h in presence of DMF. The reaction mixture was cooled and poured into crushed ice. The solid separated was filtered, dried and recrystallized form ethanol. The same procedure was followed for compound B with various substituted amines.

Table no. 1: Physical data for 2-substituted benzothiazole (Compound A)

Sl. No.	Comp. No.	Chemical Name	Mol. Formula	M.W. (g)	M.P (°C)	% Yield
1	**A_1**	*N*-(benzo[*d*]thiazol-2-yl)-2-(benzo[*d*]thiazol-2-ylamino) acetamide	$C_{16}H_{12}N_4OS_2$	340.42	133	63
2	**A_2**	2-(1*H*-benzo[*d*]imidazol-2-ylamino)-*N*-(benzo[*d*]thiazol-2-yl) acetamide	$C_{16}H_{13}N_5OS$	323.37	181	82

3	**A_3**	2-(4,5dihydrothiazol-2-ylamino)-*N*-(benzo[*d*]thiazol-2-yl) acetamide	$C_{12}H_{12}N_4OS_2$	292.38	111	29
4	**A_4**	2-(1, 5-dihydro-1,2,4-triazol-4-ylamino)-*N*-(benzo[*d*]thiazol-2-yl) acetamide	$C_{11}H_{12}N_6OS$	276.32	174	49
5	**A_5**	2-(4-fluorophenylamino)-*N*-(benzo[*d*]thiazol-2-yl) acetamide	$C_{15}H_{12}N_3OFS$	301.34	199	28
6	**A_6**	2-(4-chlorophenylamino)-*N*-(benzo[*d*]thiazol-2-yl) acetamide	$C_{15}H_{12}N_3OClS$	317.79	194	41
7	**A_7**	2-(3-chloro-4-fluorophenylamino)-*N*-(benzo[*d*]thiazol-2-yl) acetamide	$C_{15}H_{11}N_3OFClS$	335.78	202	25

Table no. 2: Physical data for 6-substituted 2-substituted benzothiazole (Compound B)

Sl. No.	Comp No.	Chemical name	Mol. Formula	M.W (g)	M.P (°C)	% Yield
9	**A_8**	2-(4-chlorophenylamino)-*N*-(6-nitrobenzo[*d*]thiazol-2-yl) acetamide	$C_{15}H_{11}N_4O_3ClS$	362.79	168	59
10	**A_9**	2-(4-fluorophenylamino)-*N*-(6-nitrobenzo[*d*]thiazol-2-yl) acetamide	$C_{15}H_{11}N_4O_3FS$	346.34	171	48
11	**A_{10}**	2-(4-chloro-3-fluorophenylamino)-*N*-(6-nitrobenzo[*d*]thiazol-2yl) acetamide	$C_{15}H_{10}N_4O_3ClFS$	380.78	184	56
12	**A_{11}**	2-(4-chloro-3-fluorophenylamino)-*N*-(6-bromobenzo[*d*]thiazol-2-yl) acetamide	$C_{15}H_{10}N_2OClFBrS$	412.68	210	66

Table no. 3: Spectra characterization of derivatives A_1-A_{11}

Comp. No.	IR spectra (cm^{-1})	^1H NMR δ (ppm)	MS m/z
A_1	Aromatic C-H stretch (3060 cm^{-1}), C=N strech (1600 cm^{-1}), C-N strech (1316 cm^{-1}), C-S strech (1106 cm^{-1}), NH strech (3288 cm^{-1}), C=O strech (1656 cm^{-1}), CH aliphatic (2805 cm^{-1})	8.5 (m, aromatic); 8.2 (m, aromatic); 3.3 (s, 1H CH_2); 7.3 (s, NH), 4.0 (s, NH)	341 (M^+)
A_2	Aromatic C-H stretch (3053 cm^{-1}), C=O stretch (1657 cm^{-1}), N-H stretch (3248 cm^{-1}), C=S stretch (1163 cm^{-1}), C-N stretch (1314 cm^{-1}).	-------	--------

A_3	Aromatic C-H stretch (3050-3150 cm^{-1}), C=O stretch (1658 cm^{-1}), N-H stretch (3289 cm^{-1}), C-N stretch (1318 cm^{-1}), C=N stretch (1615 cm^{-1}), CH aliphatic (2830 cm^{-1})	8.5 (m, aromatic); 3.3 (s, 1H CH_2); 7.3 (s, NH), 4.0 (s, NH)	292 (M^+)
A_4	Aromatic C-H stretch (3050-3150 cm^{-1}), C=O stretch (1669 cm^{-1}), C=S stretch (1159 cm^{-1}), C=N stretch (1604 cm^{-1}), N-H stretch (3286 cm^{-1}), CH aliphatic (2830cm^{-1})	-------	-------
A_5	Aromatic C-H stretch (3057 cm^{-1}), N-H stretch (3270 cm^{-1}), C=O stretch (1634 cm^{-1}), C-F stretch (1110 cm^{-1}), aliphatic C-H (2853 cm^{-1})	8.23-8.12 (m aromatic), 7.3 (s NH), 3.2 (d CH_2), 4.0 (s NH)	302 (M^+)
A_6	Aromatic C-H stretch (3067 cm^{-1}), N-H stretch (3290 cm^{-1}), C=O stretch (1657 cm^{-1}), C-Cl (691 cm^{-1}), C=N (1601 cm^{-1}), C-N stretch (1257 cm^{-1}), aliphatic C-H (2801 cm^{-1})	-------	_____
A_7	Aromatic C-H stretch (3059 cm^{-1}), N-H stretch (3263 cm^{-1}), C=O stretch (1692 cm^{-1}), C-Cl stretch (676 cm^{-1}), C-F stretch (1131 cm^{-1}), C-N stretch (1274 cm^{-1}), aliphatic C-H (2851 cm^{-1})	8.2-8.4 (m aromatic), 7.3 (s NH), 3.2 (d CH_2)	335 (M^+)
A_8	Aromatic C-H stretch (3096 cm^{-1}), N-H stretch (3293 cm^{-1}), C=O stretch (1648 cm^{-1}), C-NO_2 stretch (1531 cm^{-1}) C-Cl stretch (696 cm^{-1})	-------	-------
A9	Aromatic C-H stretch (3037 cm^{-1}), N-H stretch (3297 cm^{-1}), C=O stretch (1650 cm^{-1}), C-F stretch (1109 cm^{-1}), C-NO_2 stretch (1502 cm^{-1})	8.4 (m, aromatic); 8.2 (m, aromatic); 3.3 (s, 1H CH2); 7.3 (s, NH)	362 (M^+)
A10	Aromatic C-H stretch (3078 cm^{-1}), N-H stretch (3200-3350 cm^{-1}), C=O stretch (1646 cm^{-1}), C-NO_2 stretch (1528 cm^{-1}), C-F stretch (1123 cm^{-1}), C-Cl stretch (696 cm^{-1}).	-------	-------
A11	Aromatic C-H stretch (3081 cm^{-1}), N-H stretch (3278 cm^{-1}), C=O stretch (1591 cm^{-1}), C-Br stretch (1055 cm^{-1}), C-Cl stretch (688 cm^{-1}), aliphatic C-H (2831 cm^{-1})	-------	-------

PHARMACOLOGICAL ACTIVITY

Anti-tubercular[14]

The antitubercular screening was carried out by Middle brook 7H9 agar medium against $H_{37}Rv$ Strain. Middle brook 7H9 agar medium containing different derivatives **(A_1-A_{11})**, standard drug as well as control, Middle brook 7H9 agar medium was inoculated with *Mycobacterium tuberculosis* of $H_{37}Rv$ Strain. The inoculated bottles were incubated for 37°C for 4 weeks. At the end of 4 weeks they were checked for growth.

Table 4: Antitubercular activity of the synthesized compounds (A_1-A_{11})

SL. No.	*Compounds*	25 mcg/ml	50mcg/ml	100mcg/ml
1.	A_1	R	**S**	**S**
2.	A_2	R	R	S
3.	A_3	R	R	R
4.	A_4	R	R	S
5.	A_5	R	R	S
6.	A_6	R	R	R
7.	A_7	R	S	R
8.	A_8	R	**S**	**S**
9.	A_9	R	**S**	**S**
10.	A_{10}	R	**S**	**S**
11.	A_{11}	R	**S**	**S**
STD.	**Streptomycin**	**S**	**S**	**S**

R -denotes Resistance and S -denote Sensitive.

RESULTS AND DISCUSSION

Several aromatic and heterocyclic acetamido benzothiazole derivatives were synthesized from 2-amino benzothiazole by treating with chloroacetyl chloride and further treatment with various aromatic and heterocyclic amines. The progress of reaction was monitored using precoated TLC plates. The absence of TLC spots for starting materials and appearance of new TLC spot at different R_f value were ensured to declare completion of reaction. The TLC plates were visualized either by iodine vapors or by viewing in UV-visible chamber. The reaction products of all the reactions were purified initially by different workup processes to remove unreacted starting materials if any and then by recrystallization using suitable solvents. Most of the steps were optimized in order to achieve quantitative yields. The physical data of synthesized derivatives are reported in **Table no. 1 and 2** respectively.

The FTIR spectra of final derivatives showed the expected bands for the characteristic groups which are present in the compounds such as C=O at 1600-1690cm^{-1}. The N-H stretching bands at 3343 -3185 cm^{-1} show the presence of –NH group. The presence of aliphatic CH_2 stretch was observed bands at 2800-2950 cm^{-1}. The IR spectral studies are reported in **Table no. 3.** In ^{1}H NMR spectra of some derivatives, band was observed at δ 8.2-8.4 which showed the presence of aromatic ring and bands around δ 3.2 showed the presence of CH_2. The mass spectra of one compound was taken and found to have 346 M^+.

All the compounds were screened for antitubercular activity by Middle brook 7H9 agar medium as described by Elmer WK et al. against H_{37}Rv Strain. Compounds **A_8 – A_{11}** has

shown promising antitubercular activity and **A_1, A_2, A_4, A_5, A_7** have shown moderate antitubercular activity. H_{37}Rv strain was used as standard tubercular organism. Streptomycin was used as standard drug. However Streptomycin has shown antitubercular activity at 25 µcg/ml.

CONCLUSION

The present work is a bonafide and novel for the synthesis of Sulphur and Nitrogen containing heterocycles like benzothiazole of biological interest. In this view we have made extensive review and literature survey on substituted thiazole and benzothiazole derivatives for their medicinal uses with the help of chemical abstracts, journals, internet surfing and text books etc. The chemical and medicinal significance of title compounds were studied. 11 new derivatives of benzothiazole were synthesized with the standard chemicals and by established procedures. The synthesized compounds were tested for their preliminary tests, physical constants, TLC etc. The structures of the final compounds were confirmed by IR for all the compounds. However **^{1}H-NMR spectra, MS m/z and CHN** analysis were carried out for prototype of compounds. The proposed compounds were screened for their antitubercular with the standard drugs in the well-equipped microbiology lab by using standard methods. The proposed work has given out many active antitubercular agents. Some of the compounds have showed moderate activities. These compounds with suitable modification can be explored better for their therapeutic activities in the future.

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