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Synthesis and Evaluation of Antifungal Activity of Benzothiazole Derivatives

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ABSTRACT

Derivatives of benzothiazoles were synthesized and evaluated for their antifungal 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}HNMR and Mass spectral data. Synthesized substituted benzothiazole derivatives were investigated for their antifungal activity which was evaluated by the tube dilution method (turbidimetric method). It was observed that the new synthesized compounds possessing electron withdrawing group like nitro group at 6th position of benzothiazole nucleus and chloro, fluoro substituted at 3rd position of aromatic amine exhibited moderate antifungal activity when compared to that of other synthesized compounds.

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INTRODUCTION

The small and simple benzothiazole nucleus is present in compounds involved in research aimed to evaluate new products that possess interesting biological activities like antitumor[1], antimicrobial[2], antitubercular[3], anticancer[4], anticonvulsant[5], anthelmintic[6], analgesic[7] and anti-inflammatory[8] activities. The benzothiazole ring is present in various marine or terrestrial natural compounds, which have useful biological activities. 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. 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.

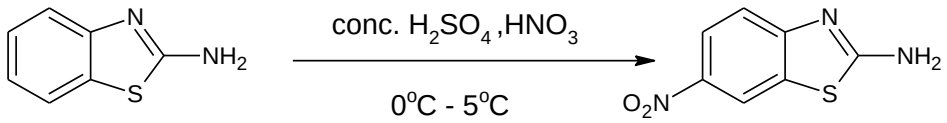
MATERIALS AND METHODS

SCHEME FOR SYNTHESIS (CHEMISTRY)

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

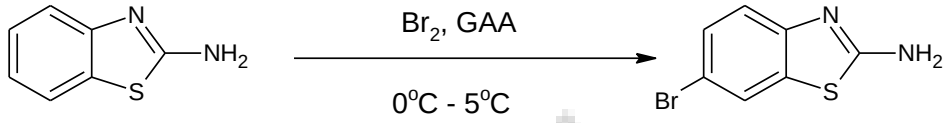
STEP – I

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



2-amino benzothiazole

6-nitro 2-amino benzothiazole

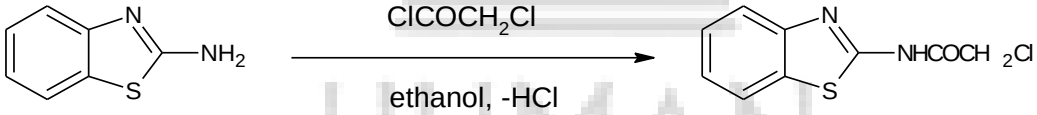


2-amino benzothiazole

6-bromo 2-amino benzothiazole

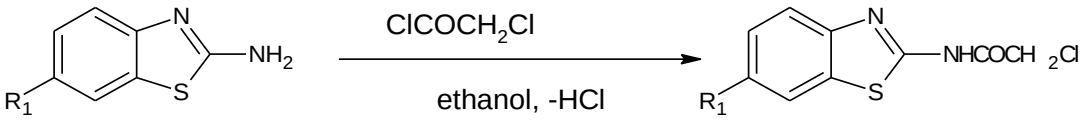
STEP – II

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



2-amino benzothiazole

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

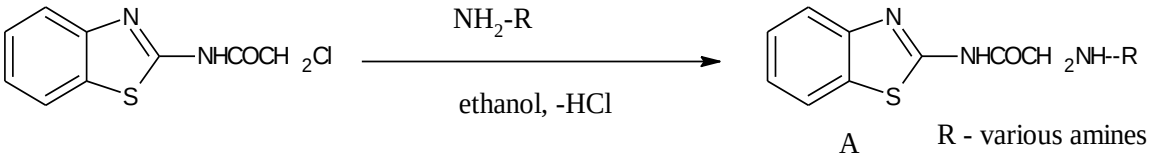


6-substituted 2-amino benzothiazole

2-chloro-*N*-(6-substitutedbenzo [*d*]thiazol-2-yl)acetamide

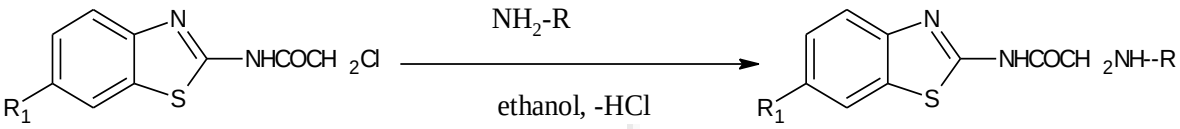
STEP – III

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



A

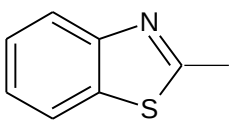
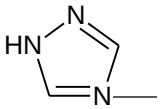
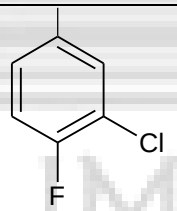
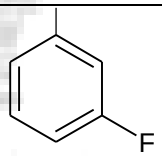
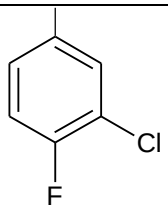
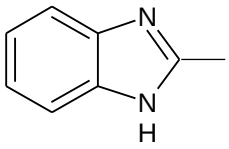
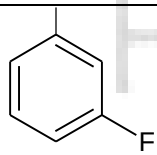
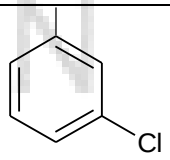
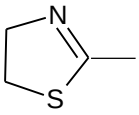
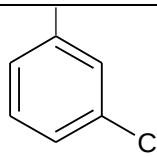
R - various amines



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[9]:

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 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. Bromine (7 ml) in glacial acetic acid (14 ml) was added dropwise in solution by using 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[10]:

2-amino benzothiazole (1g) was taken in 100 ml of iodine flask. Ethanol (10 ml) was added into the solution till all benzothiazole gets dissolved. By using dropping funnel Chloroacetyl chloride (6 ml) was 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[11]:

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 separated solid was filtered, dried and recrystallized form ethanol. The same procedure was followed for compound B with various substituted amines.

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 1600-1690 cm^{-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}HNMR 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^+.

ANTIFUNGAL ACTIVITY[12]

The targeted compounds synthesized were screened for the antifungal potential against *Candida albicans*, isolated from sputum sample of patient and was found to be sensitive to Itraconazole, Fluconazole, Ketoconazole and Clotrimazole but developed resistance against common antifungal antibiotics such as Nystatin and Amphoterecin-B. For convenience the synthesized compounds were coded by alphabets (RG). A stock solution of 0.250 µmol/ml of each compound was prepared.

The fungistatic assay was carried out using Sabouraud's liquid medium. The media used for the microorganism was the double strength Sabouraud's broth. The composition of the Sabouraud,s broth is given below.

Composition of Sabouraud's broth:

1. Glucose 40 gm
2. Peptone 10 gm
3. Water q.s. to 1000ml

Glucose and peptone were dissolved in water with heating, cooled and the pH was adjusted to 5.4 with lactic acid and filtered. Total matrix was sterilized at 120^0C for 15 minutes.

Evaluation of Antifungal Activity

The antifungal activity was evaluated by the tube dilution method (turbidimetric method). The turbidimetric method depends upon the inhibition of growth of a microbial culture in a uniform solution of drug in a fluid medium that is favorable to its rapid growth. In this method, minimal inhibitory concentration (MIC) of the antifungal agent is determined. The MIC is the lowest concentration of an antimicrobial agent that inhibits the test organism.

The growth in the tube was observed visually for turbidity and inhibition was determined by the absence of growth. MIC was determined by the lowest concentration of the sample that prevented the development of turbidity.

Procedure:

The stock solution of (1 µmol/ml) of compounds was prepared in DMSO and water. To each tube containing sterilized Sabouraud's liquid medium (1 ml), 1 ml of drug solution were added. Each tube was inoculated with the microorganism and was kept at 30^0C for 14 days. The serial dilutions were made to obtain concentrations (in µmol/ml) such as 0.125, 0.0625 and 0.0314. Positive control tubes (organism + broth + DMSO) and negative control tubes (broth + drug) were also prepared. Fungal strain used was *Candida albicans*. All the tubes were incubated at 30^0C for 14 days. The readings were taken and expressed as (-), if inhibition of growth is seen and (+), if inhibition of growth is not seen.

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	**A1**	*N*-(benzo[*d*]thiazol-2-yl)-2-(benzo[*d*]thiazol-2-ylamino) acetamide	$C_{16}H_{12}N_4OS_2$	340.42	134	62
2	**A2**	2-(1*H*-benzo[*d*]imidazol-2-ylamino)-*N*-(benzo[*d*]thiazol-2-yl) acetamide	$C_{16}H_{13}N_5OS$	323.37	180	81
3	**A3**	2-(4,5dihydrothiazol-2-ylamino)-*N*-(benzo[*d*]thiazol-2-yl) acetamide	$C_{12}H_{12}N_4OS_2$	292.38	110	28
4	**A4**	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	175	46

5	**A5**	2-(4-fluorophenylamino)-*N*-(benzo[*d*]thiazol-2-yl) acetamide	$C_{15}H_{12}N_3OFS$	301.34	198	27
6	**A6**	2-(4-chlorophenylamino)-*N*-(benzo[*d*]thiazol-2-yl) acetamide	$C_{15}H_{12}N_3OClS$	317.79	195	38
7	**A7**	2-(3-chloro-4-fluorophenylamino)-*N*-(benzo[*d*]thiazol-2-yl) acetamide	$C_{15}H_{11}N_3OFClS$	335.78	201	19

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	**A8**	2-(4-chlorophenylamino)-*N*-(6-nitrobenzo[*d*]thiazol-2-yl) acetamide	$C_{15}H_{11}N_4O_3ClS$	362.79	165	57
10	**A9**	2-(4-fluorophenylamino)-*N*-(6-nitrobenzo[*d*]thiazol-2-yl) acetamide	$C_{15}H_{11}N_4O_3FS$	346.34	170	46
11	**A10**	2-(4-chloro-3-fluorophenylamino)-*N*-(6-nitrobenzo[*d*]thiazol-2yl) acetamide	$C_{15}H_{10}N_4O_3ClFS$	380.78	185	55
12	**A11**	2-(4-chloro-3-fluorophenylamino)-*N*-(6-bromobenzo[*d*]thiazol-2-yl) acetamide	$C_{15}H_{10}N_2OClFBrS$	412.68	209	65

Table no. 3: Spectra characterization of derivatives A1-A11

Comp. No.	IR spectra (cm^{-1})	^1H NMR δ (ppm)	MS m/z
A1	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^+)
A2	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}).	-------	-------
A3	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^+)
A4	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})	-------	-------
A5	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^+)
	Aromatic C-H stretch (3067 cm^{-1}), N-H stretch (3290 cm^{-1}), C=O stretch (1657 cm^{-1}), C-Cl (691	-------	-------

A6	cm^{-1}), C=N (1601 cm^{-1}), C-N stretch (1257 cm^{-1}), aliphatic C-H (2801 cm^{-1})		
A7	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^+)
A8	Aromatic C-H stretch (3096 cm^{-1}), N-H stretch (3293 cm^{-1}), C=O stretch (1648 cm^{-1}), C-NO_2stretch (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})	-------	-------

Table no. 4: Results of serial dilutions for *Candida albicans*

Sr. No	Compounds	Concentration of compound required for inhibition (µmol/ml)				
		0.125	0.0625	0.0312	0.015	0.007
1	A1	+	+	+	+	+
2	A2	+	+	+	+	+
3	A3	+	+	-	-	-
4	A4	+	+	+	+	+
5	A5	+	+	+	+	-
6	A6	+	+	-	-	-
7	A7	+	+	+	-	-
8	A8	+	+	-	-	-
9	A9	+	-	-	-	-
10	A10	+	+	+	-	-
11	A11	+	+	+	+	+
12	Ketoconazole	-	-	-	-	-

(-) Indicates absence of growth.

(+) Indicates presence of growth.

CONCLUSION

Research program for the discovery of new antitumor, antimicrobial, antitubercular, anticancer, anticonvulsant, anthelmintic, antifungal, analgesic and anti-inflammatory drug for improving the evaluation criteria are under way of many laboratories. Small and simple heterocyclic structures often have surprising complex biological properties. Benzothiazole plays a vital role in the field of medicinal chemistry. The literature study reveals that benzothiazole moiety is an important pharmacophore and exhibits outstanding biological activities. Benzothiazole moiety is an important pharmacophore and exhibits outstanding biological activities. Anti-fungal activity was carried out for synthesized compounds. It was observed that the new synthesized compounds possessing electron withdrawing groups like nitro, chloro, fluoro exhibits better activity than the

compounds with electron donating groups. It can act as an important tool for medicinal chemists to develop newer compounds possessing benzothiazole moiety that could be better agents in terms of efficacy and safety.

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