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Regular Article

Design, synthesis, characterization and biological evaluation of Benzothiazole-6-carboxylate derivatives

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A parent benzothiazole molecule was synthesized by Jacobson synthesis, then it is subjected to treatment with various aromatic aldehydes to get the corresponding Schiff bases followed by esterification of carboxyl group by using various alcohols. The structures of synthesized compounds were confirmed by various spectroscopic methods such as IR, NMR and mass spectroscopy. The products were evaluated for their antimicrobial activity. Some of the compounds exhibited potent activity when compared with the standards.

Key words: *Benzothiazole; Schiff base; Esterification; Antimicrobial activity.*

Benzothiazole nucleus is present in various compounds and it is responsible for various biological activities. The present work mainly focuses on the benzothiazoles with potential activities that are now in development. Benzothiazoles are bicyclic ring system with multiple applications. Amino-benzothiazoles constitute an important class of compounds. In recent year heterocyclic compounds analogues and derivatives have attracted strong interest due to their useful biological and pharmacological properties. Proposed work is based upon the development of some newer structurally related compounds of benzothiazoles and evaluation of biological activity. Benzothiazoles shows diverse biological activities ranging from antiparasitic (Alaimo, 1974), anti-inflammatory (Singh, 1986), antitumor (Schnur, 1991 and Hutchinson, 2003), immunosuppressive (Paget, 1969 and Hugershoff, 1901), anti-tubercular activity (Leon, 1951). Such wide range of activities motivated us to develop newer analogues of the benzothiazole expecting better biological activity. Synthesis of the basic nucleus is well established and the proposed derivatives can be synthesized based upon the literature available about the reaction involved. Although they have been known from long ago to be biologically active (Caleta, 2004, Bhusari, 2000 and Nargund, 1998), their varied biological features are still of great scientific interest. Benzothiazoles show antitumor activity, especially the phenyl-substituted benzothiazoles (Leong, 2004 and Bradshaw, 2002). Substituted 6-nitro- and 6-aminobenzothiazoles (Hutchinson, 2001) show antimicrobial activity. In connection with our earlier studies on the synthesis and biologic properties of substituted benzothiazoles (Prabhu, 2011 and Prabhu, 2010) we now focused our attention on the synthesis and biological evaluation of novel alkyl-2-(4-(benzylideneamino) phenyl) benzo thiazole-6-carboxylate as analogous of 2-(4-(benzylideneamino) phenyl) benzo

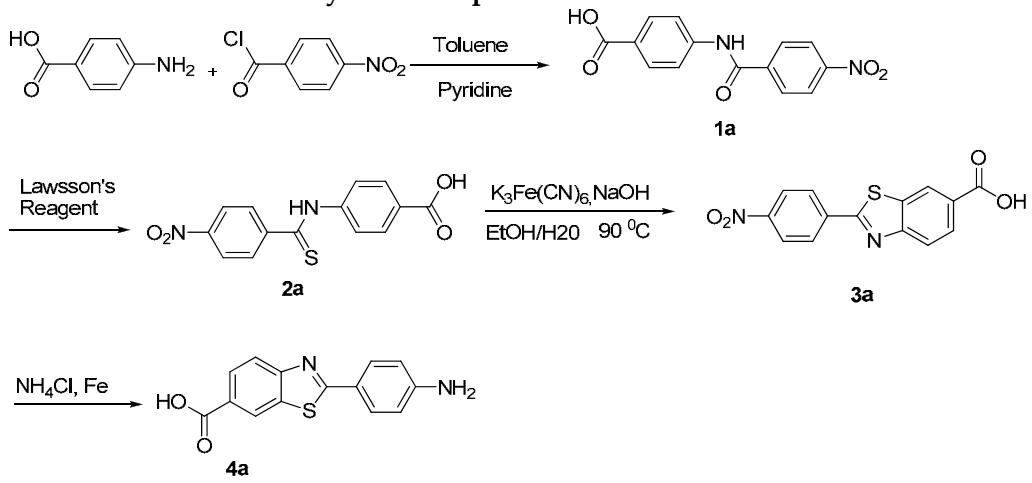
thiazole-6-carboxylic acid. We have given below a brief account of various alterations conducted on benzothiazole ring and their antimicrobial activity.

Material and methods

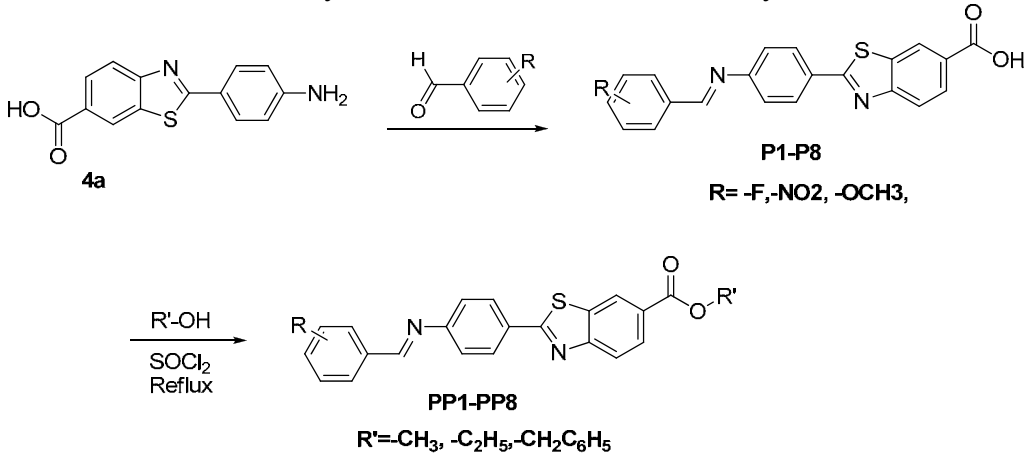
Melting points of synthesized compounds were determined by melting point apparatus and uncorrected. NMR spectra were recorded on BRUKER-spectrospin 400MHz spectrometer in $CDCl_3$, Tetra methyl silane (TMS; δ = 0.00 ppm) served as internal standards for ^1H NMR. The corresponding residual non-deuterated solvent signal ($CDCl_3$: δ = 77.00 ppm) was used as internal standards for ^{13}C NMR. IR spectra were measured using a JASCO FT/IR-410 spectrometer, and Perkin-Elmer FT/IR Spectrum BX, GX. Mass spectra were measured with Micromass Q-Tof (ESI-HRMS). Column chromatography was conducted on Silica gel 230-400 mesh (Merck) and preparative thin-layer chromatography was carried out using SILICA GEL GF-254. Spectras were obtained from IISC Bangalore. The physical data of the derivatives are listed in **table I**. The scheme of synthesis is given in **schemes 1** and **2**.

In **scheme 1** parent benzothiazole molecule was synthesized by Jacobson method using Lawsson's reagent, the product obtained was used for the synthesis of various benzothiazole-6-carboxylate derivatives as shown in the **scheme 2**.

Scheme 1: Synthesis of parent benzothiazole molecule



Scheme 2: Synthesis of benzothiazole-6-carboxylate derivatives



Procedure

Synthesis of N-(4-Carboxy phenyl)-4- nitro phenyl benzamide (1a) : To a solution of *p*-amino benzoic acid (11.5 g, 62.6 mmol) in dichloro methane (100 ml) was added pyridine (40 ml) followed by addition of mixture of *p*-nitro benzoyl chloride (8 ml, 68.9 mmol) in toluene (30 ml). Then, the mixture was heated at reflux for 5 hrs., after this, toluene was removed under vacuum, 100 ml water was added then product was extracted with dichloromethane (3×100ml) and dichloromethane layer was rinsed with 1M HCl (200 ml) followed by washing with 20% 100ml aqueous solution of sodium carbonate. The organic layer was then dried over sodium sulphate (Na_2SO_4) and concentrated to produce benzamide derivative **1a** as a purple crystalline solid. The compound was recrystallised from dichloromethane and hexane. Yield = 86 %.

Synthesis of N-(4-Carboxy phenyl)-4-nitrophenyl thio benzamide (2a): To a solution compound **1a** (1.0 g) in 40 ml dry toluene was added lawesson's reagent (0.6 molar eq). The mixture was heated under an atmosphere of nitrogen at reflux for 2 hrs. after which it was concentrated and purified by column chromatography to give yellow crystals. Yield 85 %

Synthesis of 6-carboxy-2-(P-nitrophenyl) benzothiazole (3a): To the compound **2a** (0.1g) in 0.5 ml ethanol was added 1.5 M 7 ml NaOH. The solution was cooled in an ice-bath and freshly prepared aqueous potassium ferricyanide (2-3 molar equivalents) was added. The reaction mixture was stirred at room temperature for 24 hr.; then, the mixture was neutralized with 1M HCl and extracted with ethyl acetate (3×75ml). The organic layer was dried over Na_2SO_4. Then organic layer was removed in vacuum and the residue is purified by recrystallisation from ethanol or ethyl acetate: hexanes to give white needles. Yield 79%

Synthesis of 6-carboxy-2-(*p*-aminophenyl) benzothiazole (4a): To 6-carboxy-2-(*p*-nitrophenyl)-benzothiazole **3a** (5g, 25 mmol) in 20ml ethanol: 10ml water was added iron powder (4.1g, 75 mmol) (325 mesh) and ammonium chloride (0.7g, 12.5 mmol).The reaction was stirred at 85°C for one hr., cooled to room temperature, and filtered through celite. The filter cake was then washed with 100ml toluene and the filtrate and filtrate was washed with water (2 ×100 ml). The toluene layer was dried over $MgSO_4$ or Na_2SO_4 and concentrated to a solid that was triturated with hexane (25 ml). The solid was separated by filtration. Yield = 86%

General procedure for the synthesis of Schiff bases of benzothiazole (5a-5c):

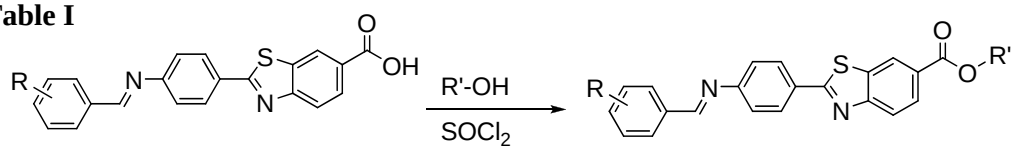
6-carboxy-2-(P-aminophenyl) benzothiazole **4a** (0.025 moles) was dissolved in 20 ml ethanol, followed by drop wise addition of substituted aromatic aldehyde (0.030 moles) dissolved in 10ml ethanol at room temperature. The reaction mixture was stirred for 24 hr at room temperature. Then, ethanol was evaporated and product was recrystallized from ethyl acetate: hexane mixture.

General Procedure for the synthesis of corresponding esters of benzothiazoles (P5a-P5h): To the corresponding compound (5a-5c) (5.58 mmoles) added corresponding alcohol (27.9 mmoles). Followed by the addition of thionyl chloride (6.69 mmoles). Then reaction mixture was refluxed for 5 hrs. The excess of thionyl chloride and the alcohol was removed under reduced pressure. The product washed with sufficient cold Petroleum ether, dried and weighted. **Table I** indicates yield and M.P. of various corresponding esters synthesized

Spectral data for various synthesized compounds

Methyl 2-(4-(3-fluorobenzylideneamino) phenyl) benzo [d] thiazole-6-carboxylate (5a): IR (neat) cm^{-1}: 1742(C=O), 1630(C=N), 1714(C=C); **^{1}HNMR ($CDCl_3$):** δ 3.9 (s, 3H, -CH3), δ 6.9-8.7 (m, 12H, aromatic); **^{13}CNMR** δ 55.06, 119.7, 120.0, 120.1, 124.2, 127.6, 127.7, 130.3, 131.4, 134.0, 136.4, 141.1 , 141.7, 141.8, 157.2, 164.1, 165.4, 168, 169.8. ***HR-ESIMS:*** Calculated for $C_{22}H_{15}FN_2O_2S$ (390.43010): found 390.42020.

Table I



R= -F,-NO2, -OCH3,

R'= -CH3, -CH2CH3, -CH2C6H5 etc

Sr No.	Code	-R	-R'	Physical nature	% Yield	M.P.
1	P5a	*m*-F	$-CH_3$	light yellow crystals	72	270-272
2	P5b	*m*-F	$-C_2H_5$	light Yellow crystals	75	280-282
3	P5c	*m*-F	$-CH_2C_6H_5$	Light yellow crystals	76	290-292
4	P5d	*p*-NO_2	$-CH_3$	yellow crystals	71	261-264
5	P5e	*p*-NO_2	$-C_2H_5$	yellow crystals	72	228-231
6	P5f	*p*-NO_2	$-CH_2C_6H_5$	yellow crystals	75	272-274
7	P5g	*p*-OCH_3	$-C_2H_5$	colourless crystals	74	246-248
8	P5h	*p*-OCH_3	$-CH_2C_6H_5$	colourless crystals	76	249-251

Ethyl-2-(4-(3-fluorobenzylideneamino) phenyl) benzo[d] thiazole-6-carboxylate (5b):IR (neat) cm^{-1}: 1746(C=O), 1634(C=N), 1710(C=C) **HNMR ($CDCl_3$):** δ 1.37-1.40 (t, 3H, -CH3, J=5.6), δ 4.3-4.36 (q, 2H, -CH2, J=5.5), δ 7.0-9.0 (m, 12H, aromatic; **^{13}CNMR** δ 14.8, 61.4, 116.9, 117.1, 118.1, 118.9, 121.4, 124.7, 124.8, 127.9, 127.7, 128.5, 129.9, 130.0, 130.1, 133.5, 138.4, 139.0, 154.3, 161.2, 162.5, 165.1, 166.7. ***HR-ESIMS:*** Calculated for $C_{23}H_{17}FN_2O_2S$ (404.45668): found 404.44433.

Benzyl 2-(4-(3-fluorobenzylideneamino) phenyl)benzo[d]thiazole-6-carboxylate (5c):IR (neat) cm^{-1}: 1736(C=O), 1710(C=C), 1622(C=N); **^{1}HNMR ($CDCl_3$):** δ 5.4 (s, 2H, -CH2), δ 7.0-8.8 (m, 17H, aromatic); **^{13}CNMR** δ 66.8, 117.9, 118.5, 118.7, 121.3, 124.6, 127.8, 128.1, 128.3, 130.0, 133.4, 137.0, 138.2, 138.7, 154.1, 161.4, 162.4, 165.0, 166.9. ***HR-ESIMS:*** Calculated for $C_{28}H_{19}FN_2O_2S$ (466.52606): found 466.51334.

Methyl 2-(4-(4-nitrobenzylideneamino) phenyl)benzo[d]thiazole-6-carboxylate (5d):IR (neat) cm^{-1}: 1754(C=O), 1710(C=C), 1622(C=N); **^{1}HNMR ($CDCl_3$):** δ 3.9 (s, 3H, -CH3), δ 7.4-8.8 (m, 12H, aromatic); **^{13}CNMR** δ 124.6, 128.7, 132.0, 132.2, 134.7, 135.9, 138.5, 138.9, 140.9, 145.9, 147.4, 159.2, 161.7, 168.6, 169.8, 170.5, 174.3, 187.5. ***HR-ESIMS:*** Calculated for $C_{22}H_{15}N_3O_4S$ (417.43720): found 417.42620.

Ethyl 2-(4-(4-nitrobenzylideneamino) phenyl)benzo[d]thiazole-6-carboxylate (5e):IR (neat) cm^{-1}: 1742(C=O), 1710(C=C), 1620(C=N); **^{1}HNMR ($CDCl_3$):** δ 1.36-1.39 (t, 3H, -CH3, J=5.7), δ 4.31-4.35 (q, 2H, -CH2, J=5.6), δ 7.4-8.8 (m, 12H, aromatic); **^{13}CNMR** δ 14.4, 61.1, 117.8, 121.1, 124.4, 124.4, 127.6, 128.2, 129.8, 129.8, 131.3, 133.8, 139.8, 151.5, 154.0, 161.0, 162.2, 162.9, 166.4. ***HR-ESIMS:*** Calculated for $C_{23}H_{17}N_3O_4S$ (431.46378): found 431.45541.

Benzyl 2-(4-(4-nitrobenzylideneamino) phenyl)benzo[d]thiazole-6-carboxylate (5f):IR (neat) cm^{-1}: 1741(C=O), 1710(C=C), 626(C=N); **^{1}HNMR ($CDCl_3$):** δ 5.4 (s, 2H, -CH2), δ 7.1-8.8 (m, 17H, aromatic); **^{13}CNMR** δ 61.1, 117.2, 120.5, 123.8, 127.0, 127.4, 127.5,

127.6, 129.2, 130.7, 1323.6, 136.3, 137.5, 139.2, 150.9, 153.4, 160.4, 161.6, 162.2, 166.1. ***HR-ESIMS:*** Calculated for $C_{28}H_{19}N_3O_4S$ (493.53316): found 493.52206.

Ethyl 2-(4-(4-methoxy benzylidene amino) phenyl) benzo[d] thiazole-6-carboxylate (5g): IR (neat) cm^{-1}: 1745(C=O), 1623(C=N), 1710(C=C); **^{1}HNMR ($CDCl_3$):** δ 1.37-1.40 (t, 3H, -CH3, J=5.6), δ 3.8 (s, 3H, -OCH3), δ 4.32-4.36 (q, 2H, -CH2, J=5.5), δ 6.9-8.9 (m, 12H, aromatic); **^{13}CNMR** δ 13.5, 54.9, 60.1, 113.8, 116.8, 120.2, 123.5, 126.7, 127.2, 127.3, 128.9, 131.2, 132.1, 137.1, 153.0, 160.0, 161.3, 161.9, 162.9, 165.5. ***HR-ESIMS:*** Calculated for $C_{24}H_{20}N_2O_3S$ (416.49220): found 416.48210.

Benzyl 2-(4-(4-methoxybenzylideneamino) phenyl)benzo[d] thiazole-6-carboxylate (P5h): IR (neat) cm^{-1}: 1741(C=O), 1622(C=N), 1710(C=C) **^{1}HNMR ($CDCl_3$):** δ 3.80 (s, 3H, -OCH3,), δ 5.4 (s, 2H, -CH2), δ 6.8-8.9 (m, 17H, aromatic).**^{13}CNMR** δ 40.5, 51.3, 98.9, 102.4, 105.8, 109.1, 112.3, 112.6, 112.8, 112.9, 114.5, 116.8, 117.9, 121.5, 122.7, 145.6, 146.9, 147.5, 148.5, 151.4. ***HR-ESIMS:*** Calculated for $C_{29}H_{22}N_2O_3S$ (478.56158): found 478.55238.

Biological evaluation

***In vitro* antimicrobial study**

Benzothiazoles show a wide spectrum of chemotherapeutic activity and a considerable amount of work has been done on the synthesis of new potent antibacterial and antifungal benzothiazoles.

Synthesized compounds were screened for their *in-vitro* antibacterial activity against *P.aeruginosa, E.coli, S.aureus, B.subtilis* at 100 µg/ml and *in-vitro* antifungal activity against *Candida albicans and Aspergillus Niger* activities at 100 µg/ml concentration. Standard antibacterial ampicillin and standard antifungal ketoconazole were also screened under similar conditions for comparison. DMSO was used as a solvent control. The culture media was nutrient agar and method employed was cup plate method (Cruichshank, 1975). All the compounds showed varying degree of antibacterial activity. All the compounds were far less active than the standard drug taken.

Results and discussion

Amongst the synthesized compounds several compounds exhibit antifungal and anti-bacterial activity. Compounds **P5d, P5e, P5f, P5g** have shown significant antibacterial activity Compound **P5e& P5f** was most significant We were pleased to observe significant activity of compound **P5e, P5f** &**P5g** against *Candida albicans* and *Aspergillus niger*. While other compound shown less significant activity against bacteria and fungi. The antimicrobial activity and antifungal activity studies have shown in **table II** and **table III** respectively.

Table II: Results of anti-bacterial activity

Sl.No	code	Diameter of zone of inhibition (in mm)			
		P.aeruginos	***E.coli***	***S.aureus***	***B.subtilis***
01	**P5a**	15	13	14	16
02	**P5b**	10	10	13	12
03	**P5c**	12	10	12	13
04	**P5d**	16	16	15	16
05	**P5e**	18	16	17	16
06	**P5f**	17	16	18	16
07	**P5g**	16	15	16	16
08	**P5h**	15	12	11	14
09	**Ampicillin**	21	20	22	20
10	**DMSO**	-	-	-	-

Table III: Results of Anti-Fungal Activity

Sl. No	Code	Diameter of zone of inhibition (in mm)	
		Candida albicans	*Aspergillus niger*
1	**P5a**	07	06
2	**P5b**	08	05
3	**P5c**	06	08
4	**P5d**	07	08
5	**P5e**	13	14
6	**P5f**	15	12
7	**P5g**	14	13
8	**P5h**	14	14
9	**Keto conazole**	18	18
10	**DMSO**	-	-

Conclusion

Various benzothiazole derivatives having 4-(benzylideneamino) phenyl substituents at position 2 and different carboxylate substituents at position 6 were synthesized with a view of enhancing the biological activity. The structure of newly synthesized compounds was confirmed by IR, ^{1}H NMR, Mass spectra. Further evaluation of antimicrobial activity was carried out. The synthesis of various 2-(4-(benzylideneamino) phenyl) benzothiazole-6-carboxylate derivatives by the described method resulted in products with good yield. Micro b2iological evaluation of the synthesized compounds showed good to moderate activity.

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