ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis, Spectral Studies and Biological Evaluation of Schiff Base Derivatives of Benzothiazole for Antimicrobial Activity

Rajinder Kumar, Uday Kalidhar, Amandeep Kaur* and Puneet Kaur

ASBASJSM College of Pharmacy, Bala (Ropar) 140111, Punjab, India

ABSTRACT

In the present study, we have reported the synthesis, spectral studies and biological evaluation of schiff base derivatives of benzothiazole for antimicrobial activity moiety. Thus condensation of 6-nitrobenzo[d]thiazol-2-amine (4) with appropriate aromatic aldehydes afforded N-(2, 5-dimethoxybenzylidene)-6-nitrobenzo[d]thiazol-2-amine (5 a-d). The structures of the compounds (5 a-d) were elucidated by spectral studies and screened for antibacterial activity against various strains of Staphylococcus aureus and Escherichia coli and antifungal activity against Candida albicans. The derivatives has shown good activity when compared with standard antibiotic Ampicillin and no activity when compared with standard Fluconazole.

Keywords: Benzothiazole; Schiff base; Antimicrobial activity.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Benzothiazole ring made from thiazole ring fused with benzene ring. Thiazole ring is a five-member ring consists of one nitrogen and one sulfur atom in the ring. Benzothiazoles are bicyclic ring system. In the 1950s, a number of 2-amino benzothiazoles were intensively studied as central muscle relaxants and found to interfere with glutamate neurotransmission in biochemical, electrophysiological and behavioral experiments. 2-Aminobenzothiazoloes are highly reactive compounds. They are extensively utilized as reactant or reaction intermediates since NH_2 and endocylic N functions are suitably situated to enable reaction with common electrophillic agents to form a variety of fused heterocyclic compounds [1]. Benzothiazole derivatives have been studied and found to have various chemical reactivity and biological activity. Presence of hydrophobic moieties in molecule is conductive for cytotoxic activity of benzothiazole derivative against cell lines. The NH_2,-OH,-Cl group containing benzothiazole shows better anticancer activity [2]. The substituents at the second position of benzothiazole ring like mercapto group is responsible for marked anti-inflammatory activity [3]. Introduction of ($–OCH_3$) at position 4 of 3-mercaptobenzothiazole increases antibacterial activity [4]. Minor modification of dihydroxy phenyl group, removal of –F group or its replacement with other analogues has a Profound Chemotherpeutic effect w.r.t in vitro cancer cells growth inhibitory activity. Chloro substituted amino Benzothiazole were found to have encouraging sensitivity to cancer cell lines as compared to Fluoro substituted benzothiazoles. The treatment of infectious diseases still remains an important and challenging problem because of a combination factors including emerging infectious diseases and increasing number of multi-drug resistant microbial pathogens with particular relevance for Gram-positive bacteria [5-10]. Inspite of the large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic resistant bacterial strains in the last decades constitutes a substantial need for the new class of antibacterial agents [5, 11]. Benzothiazole ring found to be possessing pharmacological activities such as anti-bacterial [12], anti-microbial [13], anti-diabetic [14], antitumor [15], anti- inflammatory [16], anthelmintic [17] activities.

We reported here a study on synthesis of some novel schiff base derivatives of benzothiazole derivatives (5a-d). These derivatives were screened for antibacterial activity against various strains of *Staphylococcus aureus* and *Escherichia coli* and antifungal activity against *Candida albicans*.

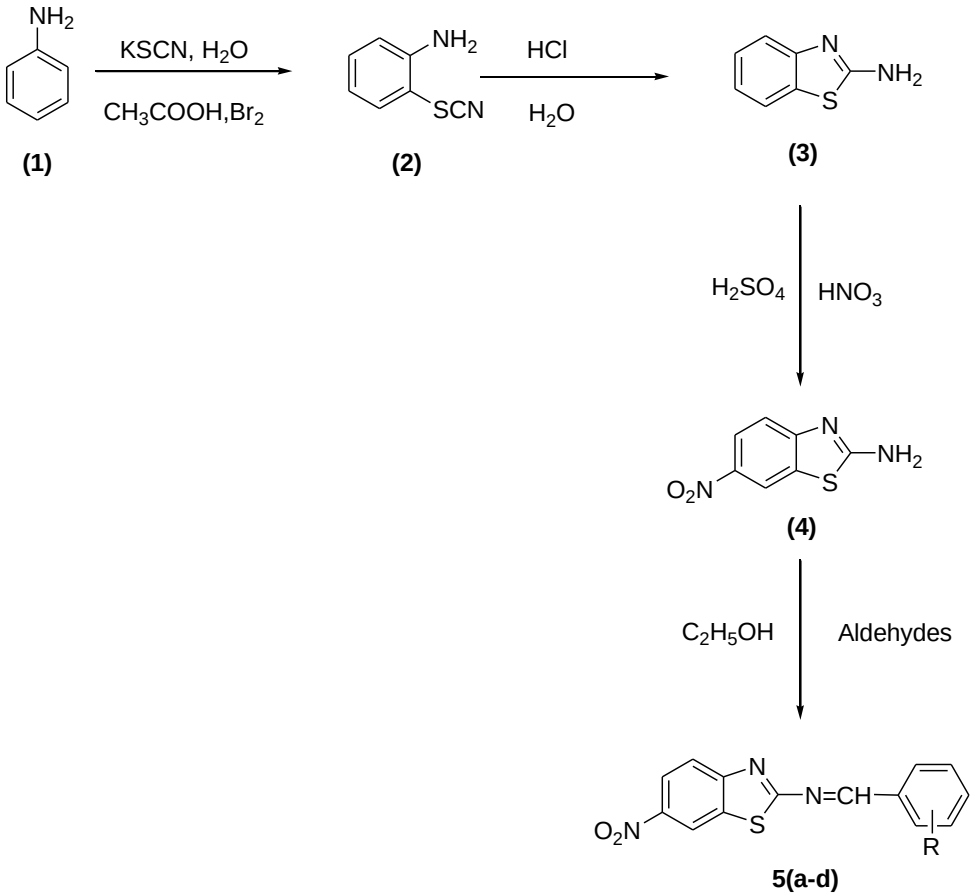
MATERIALS AND METHODS

Chemistry

Most of the solvents used were of LR grade and were purified before use in different reactions. Chemicals used were of LR grade and obtained from Merck India, Loba chem., Central Drug House ltd, S. D. Fine chemicals ltd. and Alpha aesar etc. The solvents used throughout the experiment for running TLC were chloroform and methanol in the ratio of 9:1 and 95:05; toluene, ethyl acetate and formic acid in ratio of 5:4:1 (T:E:F) and benzene and ethanol in the ratio of 7:3. Iodine chambers were used for visualization of TLC spots. Melting

ISSN: 0975-8585

points were determined on an electrothermal capillary melting point apparatus and were uncorrected. All the Infra red (IR) spectra were recorded by KBr pellets technique using Perkin Elmer IR spectrophotometer 4000-400 (ν_{max} in cm^{-1}). Proton magnetic resonance (^1H NMR) spectra were recorded on Bruker Advance II 400 (400 MHz) NMR spectrometer (chemical shift in ppm) in $CDCl_3$ and DMSO using Tetramethylsilane (TMS) as standard. Mass spectra of the synthesized compounds were recorded in MAT 120.



R = **a**-H, **b**-2',5'OCH_3, **c**-3'NO_2, **d**-4'OCH_3

Scheme 1: Various schiff base derivatives of benzothiazole were synthesized using reaction scheme 1.

Synthesis of 2-thiocyanatoaniline (2) General procedure

The appropriately aniline (0.1mol) in acetic acid was added to a solution of KSCN (0.2mol) in acetic acid (100 ml). The mixture was cooled to 0 to 5^0C, and a solution of Br_2 (0.1mol) in acetic acid (30 ml) was added slowly with vigorous stirring so that the temperature remained between 0 and 10^o C. After the addition was complete, the stirring was continued for 1h at 5^0C. And then the mixture was poured into water. The solid was collected and recrystallized from ethanol to get 2-thiocyanatoaniline [18].

ISSN: 0975-8585

Synthesis of 2-aminobenzothiazole (3) General procedure

The 2-thiocyanatoaniline (0.036 mol), concentrated HCl (27 ml) and water (54 ml) were refluxed for 2h.The solution was cooled, and product was filtered off, washed with water, recrystallized from ethanol to yield 2-aminobenzothiazole [18].

Synthesis of 2-amino-6-nitobenzothiazole (4-d) General procedure

2-aminobenzothiazole (0.157 mol) was dissolved in sulphuric acid (36 ml) below 5°C with vigorous stirring, Nitric acid (19 ml) was added dropwise so that temperature was maintained at 20°C. Reaction mixture was stirred 4-5 hrs. The mixture was then poured on ice with stirring, and aqueous ammonia added until the solids became slightly orange. The solids were filtered, washed with water and dried. The crude product was recrystallized from ethanol and 2-amino-6-nitrobenzothiazole was obtained [19].

Synthesis of N-Substituted-6-nitro[d]thiazol-2-amine 5(a-d) General procedure

0.01 mol of 2-amino-6-nitrobenzothiazole was added to solution of 0.015 mol of benzaldehyde, added 40 ml of ethanol and 4-5 drops of glacial acetic acid and refluxed for 8-10 hrs. The reaction progress was monitored with the help of TLC. It was eluted with Toluene: Ethyl acetate: formic acid (5:4:1) and recrystallised with ethanol [20].

N-benzylidene-6-nitro[d]thiazol-2-amine (5a): Yield 64%; m.p. 278-280 $^{\circ}$C; IR (KBr) cm^{-1}: 3072 (Ar-CH stretch), 1644 (N=CH), 1328 (NO_2); ^{1}H NMR (TMS) δ ppm: 8. 7 (s, H, N=CH), 7.00-8.12 (m, 8H, Ar-H); MS m/z: 284.1 (M+1).

N-(2,5-dimethoxybenzylidene)-6-nitrobenzo[d]thiazol-2-amine (5b): Yield 61%; m.p. 307-309 $^{\circ}$C; IR (KBr) cm^{-1}: 3073 (Ar-CH stretch), 1676 (N=CH), 1328 (NO_2); ^{1}H NMR (TMS) δ ppm: 8.42(s, H, N=CH), 6.96-8.10 (m, 6H, Ar-H), 3.83 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3); ^{13}C NMR (TMS) δ ppm: 171.39, 158.25, 156.05, 152.95, 140.71, 131.40, 124.22, 122.52, 121.51, 116.74, 116.58, 113.99, 113.34, 110.07, 55.79, 55.21.

6-nito-N-(3-nitrobenzylidene)benzo[d]thiazol-2-amine (5c): Yield 57%; m.p. 287-289 $^{\circ}$C; IR (KBr) cm^{-1}: 3073 (Ar-CH stretch), 1635 (N=CH), 1324 (NO_2); ^{1}H NMR (TMS) δ ppm: 8. 7 (s, H, N=CH), 7.40-8.50 (m, 7H, Ar-H);

N-(4-methoxybenzylidene)-6-nitro[d]thiazol-2-amine (5d): Yield 68%; m.p. 294-296 $^{\circ}$C; IR (KBr) cm^{-1}: 3071 (Ar-CH stretch), 1645 (N=CH), 1325 (NO_2); ^{1}H NMR (TMS) δ ppm: 8.42 (s, H, N=CH), 6.90-8.10 (m, 7H, Ar-H), 3.88 (s, 3H, OCH_3).

Antimicrobial susceptibility test

The newly synthesized compounds were screened for their antibacterial and antifungal screening using agar cup plate method.

The antibacterial activity of test compounds were evaluated against Gram-positive bacteria, *Staphylococcus aureus* and Gram-negative bacteria, *Escherichia coli*.

Antifungal activity was screened against fungal strain, *Candida albicans*.

The bacterial cultures were inoculated on Nutrient Agar and fungal culture was inoculated on Sabouraud dextrose agar.

The nutrient agar media was taken in a 1000ml beaker and made up the volume to 1000ml with water then the media was sterilized by autoclaving at 121 ˚C for 15 min at 15-*psi* pressure. Afterwards the mixture was cooled to 45 ˚C and then inoculums were added to the above cooled media, mixed properly and poured into the sterile petridishes for solidifying. Bores were made on the medium using sterile borer. 0.1 ml of test solution and standard solution at a concentration of 100 µg/ml were taken. The standard antibiotic (Ampicillin) for bacteria and (Fluconazole) for fungal was maintained with same concentration in each plate and a control having only DMSO in one plate. Then petridishes were incubated at 37 ˚C for 24 hrs and zones of inhibition were observed and measured.

RESULTS AND DISCUSSION

Benzothiazole is a privileged bicyclic ring system. Due to its potent and significant biological activities it has great pharmaceutical importance; hence, synthesis of this compound is of considerable interest. The small and simple benzothiazole nucleus if present in compounds involved in research aimed at evaluating new products that possess interesting biological activities. 2-substitued benzothiazole has emerged in its usage as a core structure in the diversified therapeutical applications. The studies of structure–activity relationship interestingly reveal that change of the structure of substituent group at C-2 position commonly results the change of its bioactivity. Among those 2-substituted benzothiazole derivatives with fluorine substituted molecules have already received considerable attention due to their potential bioactivities. Since most of the benzothiazole derivatives were reported for their diversified activity viz., antitumor, antitubercular, antimalarial, anticonvulsant, anthelmintic, analgesic, anti-inflammatory, antifungal, a typical carbonic anhydrase inhibitor. Benzothiazole derivatives have much better chemical, thermal and photochemical stabilities than structurally similar compounds. The aromatic benzothiazole nucleus is associated with a variety of antihistaminic activity. These activities are due to the presence of the –N=C-S group. Substituted benzothiazole have reported to display diverse applications as photostablizer and metal complexing agents. A heterocyclic compound is one which possesses a cyclic structure with at least two different kinds of heteroatoms. Benzothiazole are fused membered rings, which contains the heterocycles bearing thiazole. Sulphur and Nitrogen atoms constitute the core structure of thiazole and many pharmacologically and biologically active compounds. Thiazole is structurally releated to thiophene and pyridine. Thiazole was first described by Hantzsch ana Waber in 1887. The numbering in thiazole starts from sulphur atom. The basic structure of benzothiazole consist of benzene ring fused with 4,5 position of thiazole. The two ring together constitute the basic nucleus 1,3 benzothiazole.

In first step aniline react with potassium thiocyanate to prepare 2-thiocyanatoaniline. This prepared 2-thiocyanatoaniline yield 2-aminobenzothiazole on nitration with nitric acid in the presence of sulphuric acid. Condensation of 2-amino-6-nitrobenzothiazole by using ethanol results in the synthesis of various Schiff-bases of benzothiazole. All the reactions were monitored through TLC observation till their completion using suitable mobile phase each time. After the completion of the reaction, the products were purified by using appropriate sovents e.g. ethanol. The structures of all the synthesized compounds were characterized on the basis of their physical, Spectral (NMR, IR, MS) and Analytical data. Schiff base (N=C) were confirmed by IR spectra showing peak at 1635-1680 cm^{-1}. Absorption at 3071-3073 cm^{-1}, confirmed presence of –CH-.The -NO_2 showed a sharp absorption band at 1324.18-1328. NMR data shows Ar, m, H at δ 3.77-8.50 ppm. Spectra had singlet peaks indicating N=CH at δ 8.40-8.70.The derivatives were also confirmed by ^{13}C NMR spectra, peaks obtained from 171.39, 158.25, 156.05, 152.95, 140.71, 131.40, 124.22, 122.52, 121.51, 116.74, 116.58, 113.99, 113.34, 110.07, 55.79 and 55.21 ppm. The R_f was observed in the range 0.56-0.73 and the melting point observed in between 278-309 ^oC. The physical data and the yield of the synthesized compounds are given in Table-1.

Table:1 Physical data of compounds (5 a-d)

Compounds	Yield (%)	m.p. (ºC)	Molecular Formula
5 a	64	278-280	$C_{14}H_9N_3O_2S$
5 b	61	307-309	$C_{16}H_{13}N_3O_4S$
5 c	57	287-289	$C_{14}H_8N_4O_4S$
5 d	68	294-296	$C_{15}H_{11}N_3O_3S$

Table:2 Antibacterial and antifungal activities of compounds (5 a-d)

Compound No.	Diameter of zone of inhibition (mm)		
	Gram-postive bacteria	Gram-negative bacteria	Fungi tested
	S.aureus	*E.coli*	*C.albicans*
5a	10.2 ± 0.3	12.4 ± 0.2	NA
5b	13.6 ± 0.2	12.8 ± 0.3	NA
5c	11.6 ± 0.1	10.8 ± 0.4	NA
5d	12.9 ± 0.2	12.2 ± 0.3	NA
Ampicillin	17.2 ± 0.2	18.9 ± 0.2	-
Fluconazole	-	-	15.1 ± 0.1

(-) activity not evaluated, (NA) no activity observed * compound number
± SD (n=3) of inhibition zone was taken as mean.

Schiff base derivatives of benzothiazole were assayed *in vitro* for their antimicrobial activity against a panel of selected Gram-positive, Gram-negative bacteria and fungi in Table-2, in comparison with those of the standard drugs ampicillin and Fluconazole. Among the synthesized compounds 5a-5d, good antibacterial activity was compared to the standard drug. The zone of inhibition was observed between 10.2-13.6mm. However the derivatives showed little or no antifungal activity.

ISSN: 0975-8585

The synthesized compounds were screened for their *in vitro* antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* and antifungal activity against *Candida albicans* by measuring the zone of inhibition in mm. The antimicrobial activity was performed by agar cup plate method at concentration 100 µg/ml. Nutrient agar & Sabouraud dextrose agar were employed as culture medium and DMSO was used as solvent control for antimicrobial activity. Ampicillin and Fluconazole were used as standard for antibacterial and antifungal activities of test and standard compounds. The mean ± SD (n=3) of inhibition zone was taken for evaluating the antimicrobial activity of test and standard compounds. A total of four benzothiazole derivatives were synthesized. The colour of synthesized derivatives was observed from yellow to orange red.

CONCLUSION

All the newly synthesized schiff base derivatives of benzothiazole were analysed with different spectral techniques and screened *in vitro* for their antibacterial activity against both Gram-positive and Gram-negative strains of bacteria and also subjected for the antifungal activity. The results of antimicrobial screening reveals all compounds exhibited good activity against all strains and no activity against *Candida albicans* strain.

ACKNOWLEDGMENTS

The authors would like to thank Chairman, Captain M.P. Singh and Sardar Bhag Singh Bola, President ASBASJSM College of Pharmacy, Bela for providing the necessary facilities.

REFERENCES

[1] Vogel AI. Text Book of Practical Organic Chemistry. 2005; 4thed.
[2] Prabhu PP, Panneerselvam T, Shastry CS, Sivakumar A, Pande SS. J Saudi Chem Soc 2012; doi:10.1016/j.jscs.2012.02.001.
[3] Shafi S, Alam MM, Mulakayala N, Mulakayala C, Vanaja G, Kalle AM, Pallu R, Alam MS. Eur J Med Chem 2012; 49: 324-333.
[4] [4] Soni B, Ranawat MS, Sharma R, Bhandari A, Sharma S. Eur J Med Chem 2010; 45: 2938-2942.
[5] Alagawadi KR, Alegaon SG. Arabian J Chem 2010: doi:10.1016/j.arabjc.2010.07.012.
[6] Tenover FC, McDonald LC. Curr Opin Infect Dis 2005; 18: 300-305.
[7] Pfeltz RF, Wilkinson BJ. Curr Drug Targets Infect Disord 2004; 4: 273-294.
[8] Roberts MC. Curr Drug Targets Infect Disord 2004; 4: 207-215.
[9] Dessen A, Di Guilmi AM, Vernet T, Dideberg O. Curr Drug Targets Infect Disord 2001; 1: 63-77.
[10] Muroi H, Nihei K, Tsujimoto K, Kubo I. Biorg Med Chem 2004; 12: 583-587.
[11] Chopra I, Schofield C, Everett M, O'Neill K, Miller K, Wilcox M. Lancet Infect Dis 2008; 8: 133-139.
[12] Russo F, Santagati M. Farmaco Ed Sci 1976; 31: 41.

[13] Ghoneim KM, Basil S, Osman AN, Said MM, Megahed SA. Rev Roum Chim 1991; 36: 1355.
[14] Pattan SR, Suresh C, Pujar VD, Reddy VVK, Rasal VP, Kotti BC. Indian J Chem 2005; 4B: 2404.
[15] Yoshida M, Hayakawa I, Hyashi N, Agatsuma T, Oda Y, Tanzawa F et al. Bioorg Med Chem Letters 2005; 15: 3328.
[16] Sawhney SN, Bansal OP. Indian J Chem 1977; 15B: 121.
[17] Brown HD. Chem Abstr 65: 18593.
[18] Malik JK, Noolvi MN, Manvi FV, Nanjwade BK, Patel HM, NS Manjula, CR Mallikarjuna, Barve Ashutosh. Letter in Drug Design & Discovery. 2011; 8: 1-8.
[19] Caleta I, Grdisa M, Mrvos-Sermek D, Cetina M, Tralic-Kulenovic V, Pavelic K, Karminski-Zamola G. IL FARMACO 2004; 59:297-305.
[20] Sathe SB, Jaychandran E, Jagtap AV, Sreenivasa GM. IJPRD 2011; 3(3): 164-169.