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Antimicrobial Evaluation of Some New Methylene Based Schiff bases Containing Benzothiazole Derivatives

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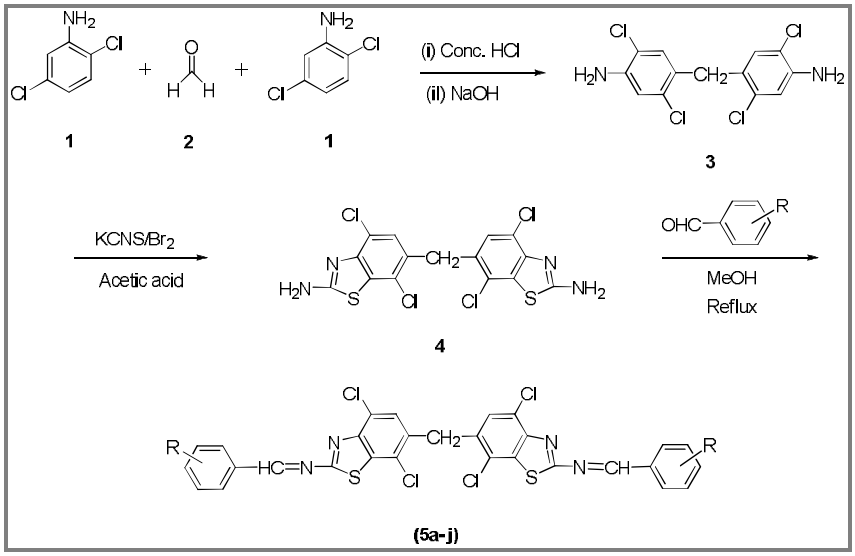
ABSTRACT

In this study, a series of 6,6'-methylene bis (N-substituted benzylidene-4,7-dichloro benzo[d]thiazole-2-amine) have been synthesized by the condensation of 6,6'-methylene bis (4,7-dichloro benzo[d]thiazol-2-amine) and various substituted aromatic aldehydes. All the synthesized compounds were characterized by elemental analysis, IR spectra and ^{1}H NMR spectra. They were screened for in vitro antibacterial (E. coli, P. aeruginosa, S. aureus, S. pyogenes) and antifungal activities (C. albicans, S. cervecieaceae and A. clavatus).

Graphical Abstract



Synthetic route for 6,6'-methylene bis (*N*-substituted benzylidene-4,7-dichloro benzo[*d*]thiazol-2-amine)from 2,5-dichloro aniline **(5a- j)**.

Keywords: 4,4'-Methylene bis (2,5-dichloro aniline), Potassium thiocyanate, Bromine, Aromatic aldehydes, Antimicrobial activity.

INTRODUCTION

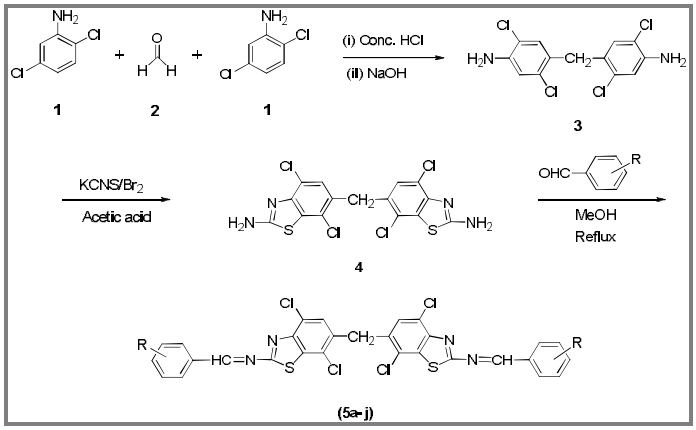
Chemistry of heterocyclic compounds is one of the leading lines of investigations in the organic chemistry. Heterocyclic compounds are widely distributed in nature and are essential for life. They play a vital role in the metabolism of all living cells. There are vast numbers of pharmacologically active heterocyclic compounds, many of which are a regular clinical use. Nitrogen, sulphur and oxygen containing five member heterocyclic compounds have occupied enormous significance in the field of drug discovery process Benzothiazole derivatives exhibit a wide range of biological activities, such as antifungal [**1**], antibacterial [**2**], antitumor [**3, 4**], anti-inflammatory [**5**], anticonvulsant [**6**], anticancer [**7**] and anti-turbercular [**8**]. We report herein the synthesis and comparative the microbial activities of five member heterocyclic derivatives (benzothiazole).

MATERIALS AND METHODS

Synthesis of 4,4'-methylene bis (2, 5-dichloro aniline) (3): 4-4'-Methylene bis (2,5-dichloro aniline) (3) was synthesized by the method described in the literature [**9**].

Synthesis of the 6,6'-methylene bis (4,7-dichlorobenzo[d]thiazol-2-amine) (4): 4,4'-Methylene bis (2,5-dichloro aniline) (3.36 g, 0.01 mol) (3) and potassium thiocynate (19.44 g, 0.2 mol) were added to 40.0 mL of precooled acetic acid at 5°C temperature. To this solution, 6.0 mL of bromine in 24.0 mL of glacial acetic acid was added from addition funnel at such a rate that the temperature does not rise beyond 0°C. After all the bromine has been added (120 min), the solution was stirred for an additional 2 h at 0°C temperature. It was allowed to stand overnight to give an orange precipitates. Water (25.0 mL) was added quickly and slurry was heated at 85°C temperature on a steam bath and filtered. The orange residue was placed in a reaction flask and treated with 10.0 ml of glacial acetic acid, heated again to 85^0C temperature and filtered. The combined filtrate was cooled and neutralized with concentrated ammonia solution to pH 6 to give dark yellow precipitate of 6,6'-methylene bis (4,7-dichlorolbenzo[d]thiazol-2-amine). Recrystallized it from toluene, Yield 85%, m.p. 265°C [**10**].

General synthesis of the compounds (5a- j): 6,6'-Methylene bis (*N*-substituted benzylidene-4,7-dichloro benzo[*d*]thiazol-2-amine) were synthesized by reaction of 6,6'-methylene bis (4,7-dichlorobenzo[*d*]thiazol-2-amine) (4.50g, 0.01 mol) with various substituted aromatic aldehyde (0.02 mol), each reactant was dissolved in a minimum amount of methanol, then mixed together and followed by addition of few drops of glacial acetic acid catalyst. The solution was refluxed for 10 h then cooled to atmospheric room temperature and poured into ice cold water to give solid product. It was filtered, washed with water, dried and recrystallised from ethanol [**11**].



Where, R = a. 4-OH, b. 4-CH_3, c. H, d. 2-CH_3, e. 2-OH, f. 2-NO_2, g. 2-F, h. 4-F, i, 4-Cl, j. 2-Cl.

Scheme 1. Synthetic route for 6,6'-methylene bis (*N*-substituted benzylidene-4,7-dichloro benzo[*d*]thiazol-2-amine)from 2,5-dichloro aniline **(5a- j)**.

RESULTS AND DISCUSSION

All the synthesized compounds were recrystallization and successive purified, by using structures of the newly synthesized compounds were determined on the basis of their FTIR and ^{1}H NMR spectra data. Methyl and methylene C-H stretching vibrations observed near 2926 cm^{-1} and 2853 cm^{-1}. Broad absorption bands observed in the region between 3080-3030 cm^{-1} and 1620-1480 cm^{-1} indicates the presence of C-H stretching and C=C stretching of Aromatic. The position of various absorption bands in the spectrum is in each part. The absorption band observed in the region of 1615-1565 cm^{-1} confirm the presence of C=N stretching of benzothiazole ring. The ^{1}H NMR spectra of the synthesized compound showed chemical shifts, which are characteristics of the anticipated structure of compounds. A singlet observed at δ 3.84 attributed to the -CH_2-, δ8.60 for the-N=CH- of Schiff base observed in ^{1}H NMR.

6,6'-Methylene bis(N-4-hydroxybenzylidene-4,7-dichlorobenzo[d]thiazole-2-amine) 5a: Brown colour solid powder, mp106°C, yield 71%; IR (KBr, cm^{-1}): 3400 (O-H stretching, Ar-OH), 3060 (C-H stretching, aromatic), 2935, 2850 (C-H stretching, -CH_2- group), 1635 (C=N stretching, Schiff base), 1585 (C=N stretching, benzothiazole), 1505 (C=C stretching, aromatic), 1470, 1430 (C-H bending, -CH_2- group), 1330 (O-H bending, Ar-OH), 720 (C-Cl stretching, chloro); ^{1}H NMR (400.1 MHz, DMSO): δ_H3.84 (s, 2H, CH_2), 6.85-7.79 (m, 10H, Ar-H), 8.50 (s, 2H, HC=N), 9.80 (s, 2H, OH); Anal. Calcd for: $C_{29}H_{16}Cl_4N_4O_2S_2$ (685.40); Found (C, 52.97), requires (C, 52.90); Found (H, 2.38), requires (H, 2.45); Found (N, 8.45), requires (N, 8.51).

6,6'-Methylene bis(N-4-methylbenzylidene-4,7-dichlorobenzo[d]thiazole-2-amine) 5b: Light yellow colour solid powder, mp126°C, yield 68%; IR (KBr, cm^{-1}): 3060 (C-H stretching, aromatic), 2930, 2855 (C-H stretching, -CH_2- group), 2920, 2870 (C-H stretching, -CH_3 group), 1620 (C=N stretching, Schiff base), 1590 (C=N stretching, benzothiazole), 1510 (C=C stretching, aromatic), 1475, 1430 (C-H bending, -CH_2- group), 1380 (C-H bending, -CH_3),740 (C-Cl stretching, chloro); ^{1}H NMR (400.1 MHz, DMSO): δ_H2.23 (s, 6H, -CH_3), 3.92(s, 2H, CH_2), 6.90-7.69 (m, 10H, Ar-H), 8.60 (s, 2H, HC=N); Anal. Calcd for: $C_{31}H_{20}Cl_4N_2S_2$ (654.46); Found (C, 56.83), requires (C, 56.89); Found (H, 3.14), requires (H, 3.08); Found (N, 8.49), requires (N, 8.56).

6,6'-Methylene bis(N-benzylidene-4,7-dichlorobenzo[d]thiazole-2-amine) 5c: Orange colour solid powder,mp144°C, yield 72%; IR (KBr, cm^{-1}): 3060 (C-H stretching, aromatic), 2930, 2855 (C-H stretching, -CH_2- group), 1628 (C=N stretching, Schiff base), 1600 (C=N stretching, benzothiazole), 1515 (C=C stretching, aromatic), 1470, 1430 (C-H bending, -CH_2- group), 745 (C-Cl stretching, chloro); ^{1}H NMR (400.1 MHz, DMSO): δ_H3.95 (s, 2H, CH_2), 6.95-7.83 (m, 12H, Ar-H), 8.50 (s, 2H, HC=N); Anal. Calcd for: $C_{29}H_{16}Cl_4N_4S_2$ (626.41); Found (C, 55.65), requires (C, 55.60); Found (H, 2.51), requires (H, 2.57); Found (N, 8.89), requires (N, 8.94).

6,6'-Methylene bis(N-2-methylbenzylidene-4,7-dichlorobenzo[d]thiazole-2-amine) 5d: Light yellow colour solid powder, mp123°C, yield 68%; IR (KBr, cm^{-1}): 3065 (C-H stretching, aromatic), 2930, 2850 (C-H stretching, -CH_2- group), 2925, 2870 (C-H stretching, -CH_3 group), 1625 (C=N stretching, Schiff base), 1595 (C=N stretching, benzothiazole), 1515 (C=C stretching, aromatic), 1475, 1440 (C-H bending, -CH_2- group), 1385 (C-H bending, -CH_3), 760 (C-Cl stretching, chloro); ^{1}H NMR (400.1 MHz, DMSO): δ_H2.25 (s, 6H, -CH_3), 3.90 (s, 2H, CH_2), 6.92-7.69 (m, 10H, Ar-H), 8.75 (s, 2H, HC=N); Anal. Calcd for: $C_{31}H_{20}Cl_4N_2S_2$ (654.46); Found (C, 56.94), requires (C, 56.89); Found (H, 3.15), requires (H, 3.08); Found (N, 8.50), requires (N, 8.56).

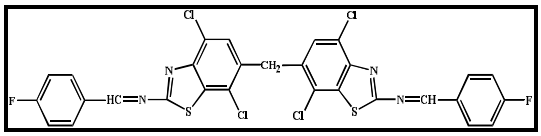
6,6'-Methylene bis(N-2-hydroxybenzylidene-4,7-dichlorobenzo[d]thiazole-2-amine) 5e: Brown colour solid powder,mp134°C, yield 73%; IR (KBr, cm^{-1}): 3405 (O-H stretching, Ar-OH), 3065 (C-H stretching, aromatic), 2935, 2855 (C-H stretching, -CH_2- group), 1620 (C=N stretching, Schiff base), 1590 (C=N stretching, benzothiazole), 1505 (C=C stretching, aromatic), 1470, 1435 (C-H bending, -CH_2- group), 1335 (O-H bending, Ar-OH), 750 (C-Cl stretching, chloro); ^{1}H NMR (400.1 MHz,

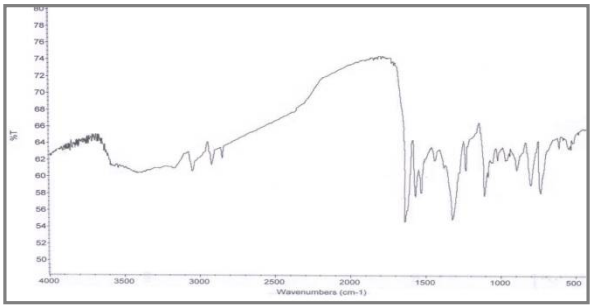
DMSO): δ_H3.91 (s, 2H, CH_2), 6.85-7.70 (m, 10H, Ar-H), 8.60 (s, 2H, HC=N), 9.85 (s, 2H, OH); Anal. Calcd for: $C_{29}H_{16}Cl_4N_4O_2S_2$ (685.40); Found (C, 52.95), requires (C, 52.90); Found (H, 2.38), requires (H, 2.45); Found (N, 8.57), requires (N, 8.51).

6,6'-Methylene bis(N-2-nitrobenzylidene-4,7-dichlorobenzo[d]thiazole-2-amine) 5f: Yellow colour solid powder, mp96°C, yield 69%; IR (KBr, cm^{-1}): 3055 (C-H stretching, aromatic), 2925, 2845 (C-H stretching, -CH_2- group), 1627 (C=N stretching, Schiff base), 1605 (C=N stretching, benzothiazole), 1580, 1350 (N=O stretching, -NO_2), 1525 (C=C stretching, aromatic), 1477, 1440 (C-H bending, -CH_2- group), 760 (C-Cl stretching, chloro); ^{1}H NMR (400.1 MHz, DMSO): δ_H3.96 (s, 2H, CH_2), 6.89-7.85 (m, 10H, Ar-H), 8.80 (s, 2H, HC=N); Anal. Calcd for: $C_{29}H_{14}Cl_4N_6O_4S_2$ (716.40); Found (C, 48.56), requires (C, 48.62); Found (H, 1.90), requires (H, 1.97); Found (N, 11.79), requires (N, 11.73).

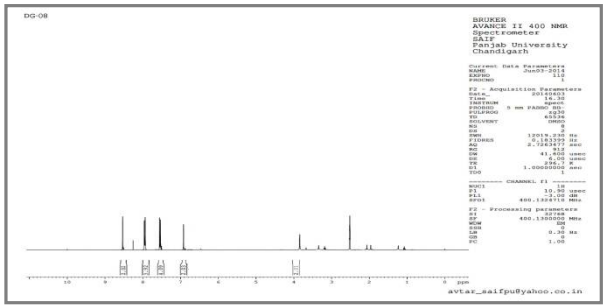
6,6'-Methylene bis(N-2-fluorobenzylidene-4,7-dichlorobenzo[d]thiazole-2-amine) 5g: Brown colour solid powder,mp128°C, yield 67%; IR (KBr, cm^{-1}): 3060 (C-H stretching, aromatic), 2930, 2855 (C-H stretching, -CH_2- group), 1635 (C=N stretching, Schiff base), 1610 (C=N stretching, benzothiazole), 1520 (C=C stretching, aromatic), 1465, 1435 (C-H bending, -CH_2- group), 1105 (C-F stretching, Fluoro), 760 (C-Cl stretching, chloro); ^{1}H NMR (400.1 MHz, DMSO): δ_H3.95 (s, 2H, CH_2), 6.90-7.86 (m, 10H, Ar-H), 8.90 (s, 2H, HC=N); Anal. Calcd for: $C_{29}H_{14}Cl_4F_2N_2S_2$ (662.38); Found (C, 52.65), requires (C, 52.58); Found (H, 2.21), requires (H, 2.13); Found (N, 8.53), requires (N, 8.46).

6,6'-Methylene bis(N-4-fluorobenzylidene-4,7-dichlorobenzo[d]thiazole-2-amine) 5h: Brown colour solid powder,mp141°C, yield 65%; IR (KBr, cm^{-1}): 3050 (C-H stretching, aromatic), 2935, 2850 (C-H stretching, -CH_2- group), 1630 (C=N stretching, Schiff base), 1585 (C=N stretching, benzothiazole), 1520 (C=C stretching, aromatic), 1465, 1435 (C-H bending, -CH_2- group), 1100 (C-F stretching, Fluoro), 730 (C-Cl stretching, chloro); ^{1}H NMR (400.1 MHz, DMSO): δ_H3.84 (s, 2H, CH_2), 6.92-7.95 (m, 10H, Ar-H), 8.53 (s, 2H, HC=N); Anal. Calcd for: $C_{29}H_{14}Cl_4F_2N_2S_2$ (662.38); Found (C, 52.50), requires (C, 52.58); Found (H, 2.20), requires (H, 2.13); Found (N, 8.38), requires (N, 8.46).





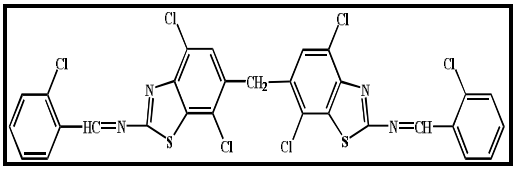
FTIR spectrum of compound (5h)

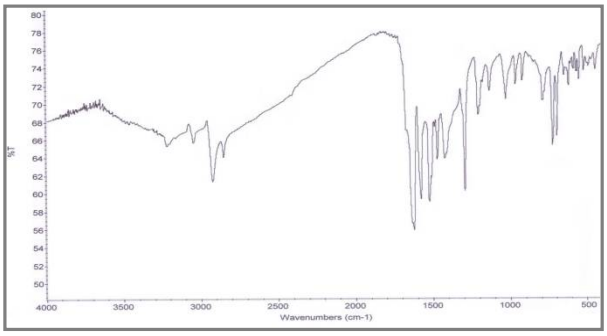


NMR spectrum of compound (5h)

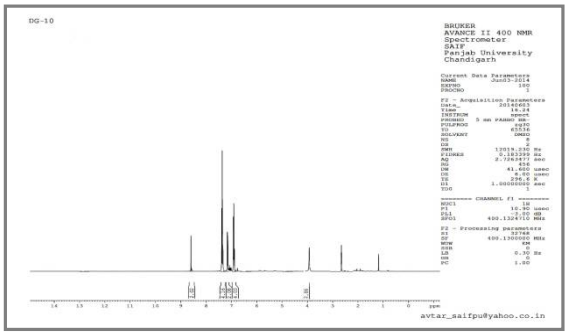
6,6'-Methylene bis(N-4-chlorobenzylidene-4,7-dichlorobenzo[d]thiazole-2-amine) 5i: Green colour solid powder,mp130°C, yield 68%; IR (KBr, cm^{-1}): 3055 (C-H stretching, aromatic), 2945, 2850 (C-H stretching, -CH_2- group), 1628 (C=N stretching, Schiff base), 1585 (C=N stretching, benzothiazole), 1525 (C=C stretching, aromatic), 1460, 1435 (C-H bending, -CH_2- group), 750 (C-Cl stretching, chloro); ^{1}H NMR (400.1 MHz, DMSO): δ_H3.89 (s, 2H, CH_2), 6.89-7.55 (m, 10H, Ar-H), 8.65 (s, 2H, HC=N); Anal. Calcd for: $C_{29}H_{14}Cl_6N_4S_2$ (695.30); Found (C, 50.18), requires (C, 50.10); Found (H, 2.09), requires (H, 2.03); Found (N, 8.12), requires (N, 8.06).

6,6'-Methylene bis(N-2-chlorobenzylidene-4,7-dichlorobenzo[d]thiazole-2-amine) 5j: Light yellow colour solid powder,mp145°C, yield 71%; IR (KBr, cm^{-1}): 3060 (C-H stretching, aromatic), 2935, 2845 (C-H stretching, $-CH_2-$ group), 1620 (C=N stretching, Schiff base), 1580 (C=N stretching, benzothiazole), 1520 (C=C stretching, aromatic), 1460, 1430 (C-H bending, $-CH_2-$ group), 740 (C-Cl stretching, chloro); ^1H NMR (400.1 MHz, DMSO): δ_H3.90 (s, 2H, CH_2), 6.88-7.47 (m, 10H, Ar-H), 8.60 (s, 2H, HC=N); Anal. Calcd for: $C_{29}H_{14}Cl_6N_4S_2$ (695.30); Found (C, 50.04), requires (C, 50.10); Found (H, 2.07), requires (H, 2.03); Found (N, 8.14), requires (N, 8.06).





FTIR spectrum of compound (5j)



NMR spectrum of compound (5j)

APPLICATION

Antibacterial activity: For the antibacterial activity, the newly synthesized compounds were screened for their antibacterial activity against gram positive bacteria *S. aureus* (MTCC-96) and *Streptococcus pyogenes* (MTCC-443) and gram negative *E.Coli* (MTCC-442) and *Pseudomonas aeruginosa* (MTCC-2488)]. Antibacterial activity was carried out by serial broth dilution method [**12-13**]. The standard strains used for the antimicrobial activity was procured from Institute of Microbial Technology, Chandigarh. The compounds (5a-j) were screened for their antibacterial activity in triplicate against *E. coli*, *S. aureus, P. aeruginosa*, and *S. pyogenes* at different concentrations of 1000, 500, 250, 125, 62.5 µg mL^{-1} as shown in (Table 1). The drugs which were found to be active in primary screening were similarly diluted to obtain 125, 62.5 µg mL^{-1} concentrations.

Table-1. Antibacterial activity of compounds [5a to 5j]

Compound	Minimum Inhibitory Concentrations (µg mL^{-1})			
	Gram negative bacteria		Gram positive bacteria	
	E. coli	*P. aeruginosa*	*S. aureus*	*S. pyogenus*
5a	250	500	125	250
5b	250	62.5	250	250
5c	125	250	500	250
5d	250	250	500	250
5e	250	250	500	250
5f	500	125	250	250
5g	500	250	500	250
5h	250	500	250	250
5i	500	500	125	250
5j	125	250	250	500
Norfloxacin	50	50	50	50
Ciprofloxacin	50	50	50	50
Chloramphenicol	50	50	50	50

The lowest concentration, which showed no growth after spot subculture was considered as MIC for each drug. The highest dilution showing at least 99% inhibition is taken as (MIC). The test mixture should contain 10^8 cells mL^{-1}. The standard drug used in this study was 'Norfloxacin, Ciprofloxacin and Chloramphenicol' for evaluating antibacterial activity which showed (100, 50, 25, and 12.5 µg mL^{-1}) MIC against *E. coli, P. aeruginosa, S. aureus*, and S. *pyogenes* respectively.

Antifungal activity: While for the antifungal activity, same compounds were tested for antifungal activity in triplicate against *C. albicans*, *S. Cervecieaceae* and *A. clavatus* at various concentrations of 1000, 500, 200 and 100 µg mL^{-1} as shown in (Table 2). The results were recorded in the form of primary and secondary screening. The synthesized compounds were diluted at 1000 µg mL^{-1} concentration, as a stock solution. The synthesized compounds which were found to be active in this primary screening were further tested in a second set of dilution against all microorganisms. The lowest concentration, which showed no growth after spot subculture was considered as (MIC) for each drug. The highest dilution showing at least 99% inhibition is taken as MIC. The test mixture should contain 10^8 spores mL^{-1} MIC. "Nystatin-B" and "grisiofulvin" was used as a standard drug for antifungal activity, which showed MIC against *C. albicans*, *S. cervecieaceae*, and *A. clavatus*, respectively. The results of antimicrobial evaluation of derivatives (5a-j) are collected in (Table 2).

Table 2. Antifungal activity of compounds [5a to 5j].

Compound	Minimum Inhibitory Concentrations (µg mL^{-1})		
	Fungus		
	C. albicans	*S. cervecieaceae*	*A. clavatus*
5a	1000	1000	1000
5b	1000	500	1000
5c	500	500	1000
5d	1000	500	1000
5e	500	500	1000
5f	500	500	500
5g	500	250	1000
5h	500	500	500
5i	1000	500	1000
5j	1000	1000	500
Nystatin-B	100	100	100
Gresiofulvin	100	100	100

CONCLUSION

A variety of benzothiazole derivatives have been successfully synthesized in excellent appreciable yields and screened in vitro for their antimicrobial activities against both strains of Gram-positive, Gram-negative bacteria and fungal strains. All spectral analysis data confirmed the proposed structures for these newly synthesized compounds.

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REFERENCES

[1]. C. Alessia, D. Ivana, M. Marilena, C. Antonio, V. B. Françoise, R. Antonio, C. Filomena, 2-Amino benzothiazole derivatives: Search for new antifungal agent, *Euro. J. of Med. Chem.*, **2013**, 64, 357-364.

[2]. Russo F., Romeo G., Santagati N. A., Caruso A., Cutuli V., Amore D., Synthesis of new thienopyrimidobenzothiazole and thienopyrimidobenzoxazoles with analgesic and anti-inflammatory properties, *Eur. J. Med. Chem.*, **1994**, 29, 569-578.
[3]. C. J. Lion, C. S. Matthews, G. Wells, T. D. Bradshaw, A. D. Westwell, Antitumour Properties of Fluorinated Benzothiazole-Substituted Hydroxycyclohexa-2,5-Dienones ('Quinols'), *Bioorg. Med. Chem. Lett*., **2006**, 16, 5005-5008.
[4]. L. Racane, R. Stojkovic, V. Kulenovic, G. K. Zamola, Synthesis and antitumor evaluation of novel derivatives of 6-amino-2-phenylbenzothiazoles, *Molecules*, **2006,** 11, 325-333.
[5]. N. K. Sharma, K. K. Jha, Benzothiazole: The Molecule of Diverse Biological Activities, *Int. J. Curr. Pharma. Res.*, **2010**, 2, 01-06.
[6]. S. T. Huang, I. J. Hsei, Synthesis and anticancer evaluation of bis(benzimidazoles), bis(benzoxazoles) and benzothiazoles, *Bioorg. Med. Chem*., **2006**, 14, 6106-6119.
[7]. S. Xuan-Hong, W. Zhao, X. Yong, Y. Ting-Hong, D. Mei, Synthesis and Biological evaluation of Novel Benzothiazole-2-thiol Derivatives as Potential Anticancer Benzothiazole agents, *Molecules*, **2012**, 17,3933-3944.
[8]. K. G. Desai, J. P. Raval, K. R. Desai, Neat reaction technology for the synthesis of 4-oxo-thiazolidines derived from 2-SH-benzothiazole and antimicrobial screening of some synthesized 4-thiazolidinones, *J. Iranian Chem. Soc*., **2006**, 3, 233.
[9]. D. D. Patel, M. S. Patel, Synthesis of benzothiazole derivative, Their Schiff bases and its Anti-infective biological activities, *Int. J. Adv. Res.*, **2014**, 2, 1048-1054.
[10]. D. Shashank, T. Vishavnath, V. Balasubramaniam, A. Nagendra, R. Suthakaran, Synthesis of some substituded benzothiazole derivatives and its biological activities, *Int. J. Chem. Res*., **2009**, 1, 1224-1231.
[11]. M. N Ibrahim, K. J Hamad, S. H. Al-Joroshi, Synthesis and characterization of some Schiff Bases, *Asian J. Chem.*, **2006**, 18, 2404-2406.
[12]. B. RGhalem, B. Mohamed, Antimicrobial evaluation of the oleoresin oil of *Pistaciavera*, L. *African J Pharm Pharmacol*, **2009**, 3, 92-96.
[13]. N. C. Desai, P. Trivedi, Synthesis and antimicrobial activity of some heterocyclic compounds, *Ind. J. Chem.,* 33B, **1993**, 497-500.