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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME 2-SUBSTITUTED BENZOTHIAZOLES CONTAINING AZOMETHINE LINKAGE

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ABSTRACT

As part of ongoing studies in developing new antimicrobials, a novel series of N-(1, 3-benzothiazol-2-yl)-2-[(2Z)-2-(substituted arylidene) hydrazinyl] acetamide (5a–j) was synthesized in order to determine their antimicrobial activities. The synthesized compounds were tested in vitro against two Gram-positive, one Gram-negative bacteria and two fungal strains in comparison with control drugs. Microbiological results showed that the synthesized compounds possessed a broad spectrum of antibacterial activity against the tested microorganisms. The compounds with a 4-dimethylamino group (5j) and 4-amino group (5i) on the aromatic ring possessing azomethine linkage showed better antimicrobial activity; almost similar to that of standard drugs thus they could be promising candidates for novel drugs. The novel heterocycles were characterized by elemental analyses and spectral features.

Keywords: Benzothiazole, Azomethine, Chloroacetyl chloride, Antimicrobial activity.

INTRODUCTION

Despite a numerous attempts to develop new structural prototype in the search for more effective antimicrobials, benzothiazole still remain as one of the most versatile class of compounds against microbes[1-3] and therefore, are useful substructures for further molecular exploration. Benzothiazole derivatives have attracted continuing interest because of their varied biological activities *viz.* antitumour[4,5], antitubercular[6], anticonvulsant[7], antihelmintic[8], analgesic[9] and topoisomarase II inhibitor.[10] Azomethine derivatives have also shown an array of biological activities *viz.* antimicrobial[11] and anti-inflammatory.[12]

In previous paper, we reported the synthesis and antimicrobial activity of a series of 5-[2-(1, 3-benzothiazol-2-yl-amino) ethyl]-4-(arylideneamino)-3-mercapto-(4*H*)-1, 2, 4-triazoles (Fig. 1), against some Gram-positive, Gram-negative bacteria and fungi. The determination of the structure-activity relationships of in vitro antibacterial and antifungal activities of the previously synthesized compounds revealed that compounds II, VI, VII emerged as the most active antibacterial benzothiazoles with a MIC value of 100 µg/ml whereas compound VII showed overall maximum activity against both the *C. albicans* and *A. niger*. The influence of the substitution of position R on the benzylidene ring was found important for affecting the intensity of the activity. It was noted that activity decreases when there is o-substitution and increases with p-substitution.[13]

There is very scarce recent literature data on antimicrobial potentials of benzothiazoles containing azomethine linkage that should combine favorable structural properties of both azomethine and benzothiazole moiety. Therefore, in the present paper, we have prepared a set of ten new of N-(1, 3-benzothiazol-2-yl)-2-[(2Z)-2-(substituted arylidene) hydrazinyl] acetamide derivatives (5a–j) and evaluated for their in vitro antimicrobial activities against Gram-positive and Gram-negative bacteria and fungi.

MATERIALS AND METHODS

Chemistry

The synthetic pathway for preparation of different title compounds is shown in Scheme 1. Phenylthiourea 1 required as starting material was prepared according to the literature procedure.[14] Compound 1 on cyclization in 85% sulphuric acid in the presence of catalytical amount of ammonium bromide at 25 ^{o}C afforded 2-aminobenzothiazole[15] 2. The formation of compound 2 was evidenced by appearance of a singlet at δ 5.87 and a multiplet at δ 6.90-7.70 ppm due to NH_2 and Ar-H, respectively. Chloroacetylation of compound 2 in presence of anhydrous K_2CO_3 in chloroform as a reaction mediator afforded N-(1, 3-benzothiazol-2-yl)-2-chloroacetamide 3. In the ^{1}H NMR spectra of compound 3**,** the peak at δ 8.17 ppm was observed due to –NHCO– and a peak at δ 4.39 ppm was due to the $–CH_2$ group of compound 3. Furthermore, in the IR spectra, the bands at 1667 cm^{-1} (>C=O of amide), 2920, 2865 cm^{-1} ($-CH_2$) and 760 cm^{-1} (C-Cl) also confirmed the formation of compound 3. The amination of compound 3 with hydrazine hydrate yielded N-(1, 3-benzothiazol-2-yl)-2-hydrazinylacetamide 4. The formation of compound 4 was evidenced by appearance of a signal at δ 8.27 and 4.43 ppm due to –NH and $–NH_2$, respectively. The IR spectral bands at 3354 and 3375 cm^{-1} ($–NHNH_2$) also confirmed the formation of compound 4. Compound 4 on treatment with selected aromatic aldehydes in presence of glacial acetic acid in dimethyl formamide (DMF) as a reaction mediator afforded N-(1,3-benzothiazol-2-yl)-2-[(2Z)-2-(substituted arylidene) hydrazinyl] acetamide derivatives (5a–j). The physical characteristics of the synthesized compounds (5a–j) are presented in Table 1. The purity of the compounds was monitored by TLC and the structures of all the derivatives were assigned by IR, ^{1}H NMR spectroscopic data, which are consistent with the proposed molecular structures.

Reagents, Instrumentation and Measurements

All reagents, solvents and catalyst were of LR grade (Loba Chemie Ltd., India) and used directly. All the melting points were determined in open glass capillary tubes and are uncorrected. The completion of reaction was monitored by thin layer chromatography (TLC) using silica gel-G coated glass plates (0.3 cm thickness) and spots were visualized by exposing the dry plates to iodine vapour. IR spectra (υ_{max} in cm^{-1}) were recorded on a FT-IR 8300 spectrophotometer (Schimadzu) using KBr pellet technique. ^{1}H

NMR spectra were recorded on a 400 MHz NMR spectrometer (Brucker Avance II) in $CDCl_3$ or DMSO-d6 as the solvent and TMS as an internal standard. All chemical shifts were reported as δ (ppm) values. Elemental analysis for carbon, hydrogen and nitrogen was performed on elemental analyzer (Elementer-Vario EL III). Where analyses are indicated only as symbols of elements, analytical results obtained are within 0.4% of the theoretical values.

Synthesis

Synthesis of 2-aminobenzothiazole (2)

Phenylthiourea (0.144 mol, 22.0 g) was dissolved in sulphuric acid (45.39 ml) during half of an hour at 20 to 25 ^{0}C. In the period of 3 h, 8.64 g of 40% NH_4Br solution was then continuously metered in the resulting solution at 25 to 30 ^{0}C. The solution was then added to water, clarified by filtration and adjusted to pH 8 with sodium hydroxide solution. The mixture was stirred for half an hour at 40 ^{0}C and filtered using suction. The residue was washed free of sulfate with water, dried and recrystallized from ethanol-water mixture to afford compound 2. Yield: 84%. m.p.: 130-132 ^{0}C. R_f : 0.74. IR (KBr): 3440, 3433, 1590, 900 (-NH_2), 3060, 1540, 1500, 1480, 768 (aromatic ring), 1650 (-C=N-), 721 (C-S-C) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.87 (s, 2H, NH2), 6.90-7.70 (m, 4H, ArH); Anal. Calcd for $C_7H_6N_2S$: C, 55.97; H, 4.03; N, 18.65. Found: C, 55.93; H, 4.07; N, 18.63 %.

Synthesis of N-(1, 3-benzothiazol-2-yl)-2-chloroacetamide (3)

A mixture of compound 2 (0.133 mol), chloroacetyl chloride (0.133 mol, 10.6 ml) and potassium carbonate (2.0 g) in chloroform was refluxed for 12 h. After completion of reaction (monitored by TLC), the reaction mixture was allowed to attain room temperature and treated with cold water. The solid thus obtained was collected by vacuum filtration, dried and recrystallized from methanol to furnish compound 3. Yield: 76%. m.p.: 141-143 ^{0}C. R_f : 0.91. IR (KBr): 3315 (-NH-), 1667 (>C=O of amide), 2920, 2865 (-CH_2), 760 (C-Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 6.93-7.74 (m, 4H, ArH), 8.17 (s, 1H, -CONH), 4.39 (s, 2H, -CH_2); Anal. Calcd for $C_9H_7ClN_2OS$: C, 47.69; H, 3.11; N, 12.36. Found: C, 47.67; H, 3.08; N, 12.36 %.

Synthesis of N-(1, 3-benzothiazol-2-yl)-2-hydrazinylacetamide (4)

A mixture of compound **3** (0.048 mol) and hydrazine hydrate (0.12 mol, 6 ml) in ethanol (50 ml) was refluxed for 7 h on a water bath. After completion of reaction (monitored by TLC), the mixture was concentrated and cooled. The solid thus obtained was filtered, dried and recrystallized from methanol to afford compound 4. Yield: 74%. m.p.: 149-151 ^{0}C. R_f : 0.88. IR (KBr): 3317 (-NH-), 1665 (>C=O of amide), 2928, 2867 (-CH_2), 3354, 3375 (-$NHNH_2$) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 6.87-7.72 (m, 4H, ArH), 8.15 (s, 1H, -CONH), 4.37 (s, 2H, -CH_2), 8.27 (s, 1H, -NH), 4.43 (s, 2H, -NH_2); Anal. Calcd for $C_9H_{10}N_4OS$: C, 48.64; H, 4.53; N, 25.21. Found: C, 48.63; H, 4.51; N, 25.20 %.

General procedure for the synthesis of N-(1, 3-benzothiazol-2-yl)-2-[(2Z)-2-(substituted arylidene) hydrazinyl] acetamide (5a–j)

A mixture of compound 4 (0.01 mol), corresponding aldehyde (0.01 mol) and 2-3 drops of glacial acetic acid in dimethyl formamide (50 ml) were refluxed for 7 h at 154 ^{0}C. After completion of reaction (monitored by TLC), the excess solvent was distilled off and the resulting residue was cooled and poured in cold water. The solid thus obtained was filtered, dried and recrystallized from chloroform-methanol mixture to afford the desired compounds (5a–j).

N-(1,3-benzothiazol-2-yl)-2-[(2Z)-2-benzylidenehydrazinyl]acetamide (5a)

IR (KBr): 3065, 1542, 1504, 1476, 770 (aromatic ring),1652 (-C=N in ring), 720 (C-S-C), 3315 (-NH-), 1667 (>C=O of amide), 2950, 2860 (-CH_2), 1595 (-CH=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.13-7.90 (m, 9H, ArH), 8.18 (s, 1H, -NHCO), 4.36 (s, 2H, -CH_2), 8.23 (s, 1H, -NHN), 4.94 (s, 2H, -N=CH).

N-(1,3-benzothiazol-2-yl)-2-[(2Z)-2-(2-hydroxybenzylidene)hydrazinyl]acetamide (5b)

IR (KBr): 3064, 1544, 1500, 1475, 776 (aromatic ring), 1654 (-C=N in ring), 724 (C-S-C), 3318 (-NH-), 1666 (>C=O of amide), 2951, 2862 (-CH_2), 1594 (-CH=N), 3595 (Ar-OH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.20-7.89 (m, 8H, ArH), 8.16 (s, 1H, -NHCO), 4.37 (s, 2H, -CH_2), 8.20 (s, 1H, -NHN), 4.93 (s, 2H, -N=CH), 3.64 (s, 1H, -OH).

N-(1,3-benzothiazol-2-yl)-2-[(2Z)-2-(4-hydroxybenzylidene)hydrazinyl]acetamide (5c)

IR (KBr): 3068, 1546, 1502, 1478, 780 (aromatic ring), 1655 (-C=N in ring), 725 (C-S-C), 3320 (-NH-), 1670 (>C=O of amide), 2955, 2865 (-CH_2), 1597 (-CH=N), 3598 (Ar-OH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.25-7.95 (m, 8H, ArH), 8.19 (s, 1H, -NHCO), 4.39 (s, 2H, -CH_2), 8.25 (s, 1H, -NHN), 4.95 (s, 2H, -N=CH), 3.67 (s, 1H, -OH).

N-(1,3-benzothiazol-2-yl)-2-[(2Z)-2-(2-chlorobenzylidene)hydrazinyl]acetamide (5d)

IR (KBr): 3070, 1548, 1505, 1480, 775 (aromatic ring), 1658 (-C=N in ring), 728 (C-S-C), 3326 (-NH-), 1672 (>C=O of amide), 2956, 2870 (-CH_2), 1600 (-CH=N), 750 (C-Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.30-7.98 (m, 8H, ArH), 8.20 (s, 1H, -NHCO), 4.51 (s, 2H, -CH_2), 8.24 (s, 1H, -NHN), 4.98 (s, 2H, -N=CH).

N-(1,3-benzothiazol-2-yl)-2-[(2Z)-2-(4-chlorobenzylidene)hydrazinyl]acetamide (5e)

IR (KBr): 3071, 1546, 1504, 1483, 778 (aromatic ring), 1654 (-C=N in ring), 725 (C-S-C), 3325 (-NH-), 1670 (>C=O of amide), 2953, 2869 (-CH_2), 1599 (-CH=N), 752 (C-Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.28-7.97 (m, 8H, ArH), 8.19 (s, 1H, -NHCO), 4.50 (s, 2H, -CH_2), 8.23 (s, 1H, -NHN), 4.95 (s, 2H, -N=CH).

N-(1,3-benzothiazol-2-yl)-2-[(2Z)-2-(2-nitrobenzylidene)hydrazinyl]acetamide (5f)

IR (KBr): 3077, 1550, 1500, 1486, 780 (aromatic ring), 1652 (-C=N in ring), 724 (C-S-C), 3328 (-NH-), 1668 (>C=O of amide), 2957, 2880 (-CH_2), 1602 (-CH=N), 1525, 1353 (-NO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.31-8.02 (m, 8H, ArH), 8.17 (s, 1H, -NHCO), 4.56 (s, 2H, -CH_2), 8.27 (s, 1H, -NHN), 4.98 (s, 2H, -N=CH).

N-(1,3-benzothiazol-2-yl)-2-[(2Z)-2-(3-nitrobenzylidene)hydrazinyl]acetamide (5g)

IR (KBr): 3075, 1551, 1507, 1487, 779 (aromatic ring), 1653 (-C=N in ring), 727 (C-S-C), 3330 (-NH-), 1672 (>C=O of amide), 2958, 2876 (-CH_2), 1605 (-CH=N), 1527, 1358 (-NO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.35-8.10 (m, 8H, ArH), 8.20 (s, 1H, -NHCO), 4.58 (s, 2H, -CH_2), 8.30 (s, 1H, -NHN), 5.02 (s, 2H, -N=CH).

N-(1,3-benzothiazol-2-yl)-2-[(2Z)-2-(2-aminobenzylidene)hydrazinyl]acetamide (5h)

IR (KBr): 3077, 1556, 1504, 1483, 781 (aromatic ring), 1651(-C=N in ring), 725 (C-S-C), 3332 (-NH-), 1668 (>C=O of amide), 2954, 2872 (-CH_2), 1600 (-CH=N), 3380, 3300 (-NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.40-8.15 (m, 8H, ArH), 8.17 (s, 1H, -NHCO), 4.60 (s, 2H, -CH_2), 8.31 (s, 1H, -NHN), 5.00 (s, 2H, -N=CH), 4.73 (s, 2H, -NH_2).

N-(1,3-benzothiazol-2-yl)-2-[(2Z)-2-(4-aminobenzylidene)hydrazinyl]acetamide (5i)

IR (KBr): 3075, 1558, 1502, 1487, 785 (aromatic ring), 1645 (-C=N in ring), 730 (C-S-C), 3335 (-NH-), 1672 (>C=O of amide), 2958, 2880 (-CH_2), 1607 (-CH=N), 3320, 3310 (-NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.36-8.20 (m, 8H, ArH), 8.25 (s, 1H, -NHCO), 4.63 (s, 2H, -CH_2), 8.36 (s, 1H, -NHN), 5.12 (s, 2H, -N=CH), 4.78 (s, 2H, -NH_2).

*N-(1,3-benzothiazol-2-yl)-2-{(2Z)-2-[4-(dimethylamino)benzylidene]hydrazinyl}acetamide **(5j)***

IR (KBr): 3078, 1560, 1500, 1490, 791 (aromatic ring), 1648 (-C=N in ring), 732 (C-S-C), 3338 (-NH-), 1670 (>C=O of amide), 2960, 2882 (-CH_2), 1605 (-CH=N), 1350-1190 (C-N, -N(CH_3)$_2$) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.28-8.16 (m, 8H, ArH), 8.27 (s, 1H, -NHCO), 4.68 (s, 2H, -CH_2), 8.28 (s, 1H, -NHN), 5.14 (s, 2H, -N=CH), 3.02 (s, 6H, -N(CH_3)$_2$).

ANTIMICROBIAL ACTIVITY

The synthesized compounds (5a–j) were tested in vitro for antibacterial activity against Gram-positive *Bacillus subtilis* (MTCC 441), *Streptomyces griseus* (MTCC 1540), Gram-negative *Escherichia coli* (MTCC 443) and antifungal activity against *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282) by measuring the zone of inhibition in mm. They were collected form MTCC Chandigarh. The antimicrobial activity was carried out by using cup-plate agar diffusion method.[16, 17]

Preparation of sample solution

The sample solution was prepared by dissolving 10 mg of each of the compound in 10 ml of dimethyl formamide (DMF). Further dilutions were made to get concentrations of 50, 100, 150 and 200 µg/ml of each compound.

Antibacterial and antifungal agents

Ciprofloxacin and Fluconazole were used as standard for antibacterial and antifungal activity, respectively.

Medium

For antibacterial activity, nutrient broth and nutrient agar media were prepared. The medium was adjusted to pH 6.8 at 25 ^{o}C. The sterility of medium was performed before it was used.

For antifungal activity, Sabouraud's dextrose agar media was prepared. The medium was adjusted to pH 5.6 at 25 ^{o}C. The sterility of medium was performed before it was used.

Preparation of inoculum

Vial containing lactose dilution of *E.coli* was broken using sterile scalpel in aseptic condition in flask containing 100 ml of nutrient broth. The flask was incubated for 24 h at 37 ^{o}C. Same procedure was applied for the preparation of inoculum for *B.subtilis* and *S.griseus*. For the preparation of inoculum for *A.niger* and *C.albicans*, the flasks were incubated for 48 h at 28 ^{o}C.

Standardization

24 h old culture of bacteria in 100 ml of nutrient broth and 48 h old culture of *A.niger* in 100 ml of Sabouraud's dextrose broth were serially diluted in 10 folds. The bacterial and fungal suspensions were adjusted with sterile water to a concentration of $6*10^6$ CFU/ml.

Antibacterial/antifungal assay

The compounds were screened for antibacterial activity in nutrient agar medium and for antifungal activity in Sabouraud's dextrose agar medium. These sterilized agar media were poured into Petri dishes and allowed to solidify. On the surface of the media, microbial suspensions were spread with the help of sterilized triangular loop. A stainless steel cylinder of 8 mm diameter (pre sterilized) was used to bore the cavities. All synthesized compounds 5a-j (50, 100, 150 and 200 µg/ml) were placed serially in the cavities with the help of micropipette and allowed to diffuse for one h. These plates were incubated at 37 ^{o}C for 24 h and 28 ^{o}C for 48 h for antibacterial and antifungal activity, respectively. The zone of inhibition observed around the cups after respective incubation was measured.Ciprofloxacin and Fluconazole were also screened under similar conditions for comparison.

RESULTS AND DISCUSSION

Antibacterial screening results (the zone of inhibition), presented in Table 2, revealed that all compounds tested showed some degree of antibacterial activity. The minimum activity was shown by the compound 5a having an unsubstituted benzylideneamino group. When substitution was made in the benzylideneamino group, activity started increasing. Compounds 5i and 5j exhibited more pronounced activity against Gram positive bacteria whereas compounds 5c and 5e exhibited better activity against Gram negative bacteria. The antibacterial activity of compounds 5d and 5f were 60% lower than the standard against the Gram positive bacteria and 5b and 5h against Gram negative species. Moreover, the compound 5g was moderately active against all bacterial species used. From the above discussion, it is evident that compounds 5c, 5e, 5i and 5j emerged as the most active antibacterial benzothiazoles.

The results of antifungal activity of the test compounds (5a–j) were found to be quite different from their antibacterial activity. For all drugs, the minimum inhibitory concentration (MIC) of the compounds was defined as the lowest concentration at which there was 100% inhibition of growth compared with the growth for a drug free control. Sensitivity of the selected fungal pathogens to some synthetic compounds (5a–j) was determined in vitro at four concentrations (50, 100, 150 and 200 µg/ml). Standard minimum inhibitory concentration for Fluconazole is (++++) at ≤50 µg/ml against all microbes. The antifungal screening results (MIC), presented in Table 3, it is evident from the screening data that the compound 5j showed maximum activity against both the *C.albicans* and *A.niger* with a MIC of 100 µg/ml. Compounds 5c and 5e were more effective against *C.albicans* (100 µg/ml) and *A.niger* (100 µg/ml), respectively, compared with the other derivatives. Compounds 5b and 5g showed weak antifungal activity against *C.albicans* and *A.niger*, respectively, with a MIC of 200 µg/ml, but compounds 5a, 5d and 5h showed moderate antifungal activity against all fungal species with a MIC of 150 µg/ml. Compounds 5b and 5c showed moderate antifungal activity against *A.niger* with a MIC of 150 µg/ml, compounds 5e and 5i showed moderate antifungal activity against *C.albicans* with a MIC of 150 µg/ml. Compound 5g was not active against *C.albicans*, whereas compound 5f was not active against both the organisms with MIC values up to ≥ 200 µg/ml.

CONCLUSION

Summarizing, a series of benzothiazole derivatives have been synthesized successfully in appreciable yields and screened for their in vitro antimicrobial activity. From the antibacterial activity study, it was observed that compound **(5a)** having an unsubstituted benzylideneamino group showed minimum activity. When the substitution was made in the benzylideneamino group, activity started increasing. Benzylideneamino group having substitution at p-position by chloro (5e), hydroxy (5b), amino (5i) and N,

N-dimethylamino group (5j) showed an increase in the activity in comparison to o-substitution by chloro, hydroxy and nitro group. Thus, it was concluded that among all benzothiazole derivatives, antibacterial activity decreases when there is an o-substitution and it increases with p-substitution showing maximum activity by amino (5i) and N, N-dimethylamino group (5j) attached to p-position of benzylideneamino group.

From the antifungal activity study, it was observed that compound (5a) having an unsubstituted benzylideneamino group showed moderate activity against all fungal strains used. Substitution at o-position by chloro (5d) or amino group (5h) retains the activity whereas substitution by 4-hydroxy group (5c) resulted in an increase in activity against *C.albicans*. Substituition by 4-chloro (5e) or 4-amino group (5i) resulted in an increase in activity against *A.niger*. It was concluded that N, N-dimethylamino substituent (5j) showed overall maximum antifungal activity against both the strains.

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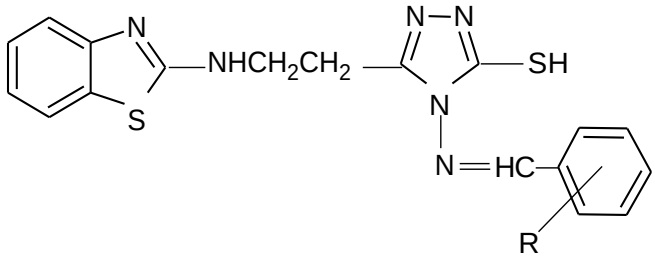
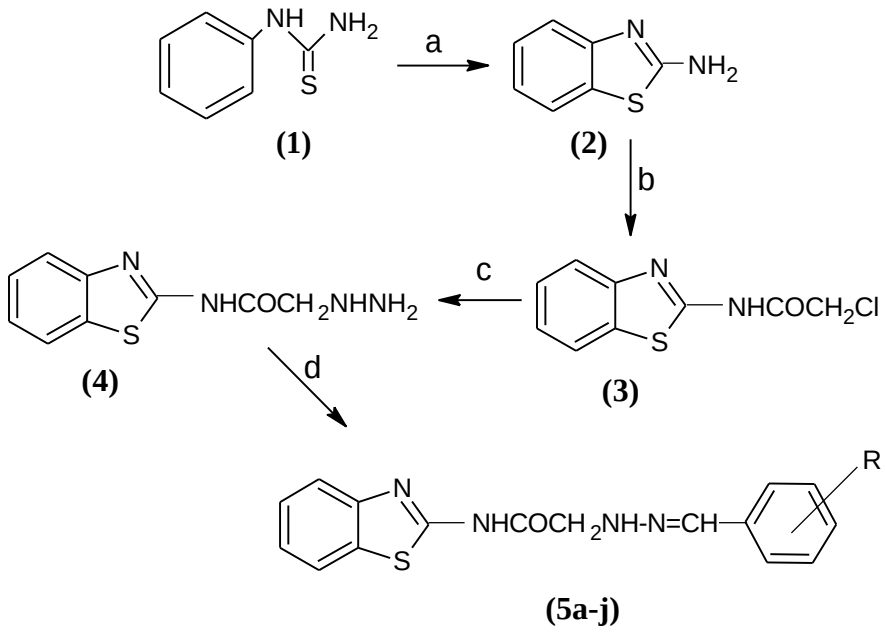


Compound	R
I	H
II	4-OH
III	2-NO_2
IV	3-NO_2
V	2-Cl
VI	4-$N(CH_3)_2$
VII	3,4-OCH_3

Figure1: Previously synthesized 5-[2-(1, 3-benzothiazol-2-yl-amino) ethyl]-4-(arylideneamino) - 3-mercapto-(4*H*)-1, 2, 4-triazoles (Compounds **I-VII**)



Scheme 1

Figure 2: Synthesis of the compounds. Reagents and conditions: (a) H_2SO_4, NH_4Br, stir 3h; (b) $ClCOCH_2Cl$, K_2CO_3, $CHCl_3$, reflux 12h; (c) $NH_2NH_2.H_2O$ (99%), EtOH, reflux 7h; (d) RC_6H_4CHO, MeCOOH, DMF, reflux 7h, 154 ^oC.

Table 1: Physical and analytical characteristics of synthesized compounds (5a-j)

Compd.	R	Yield (%)	mp (^oC)	M.F.	R_f	Elemental analyses[a] (calculated % / found %)		
						C	H	N
5a	H	68	152-154	$C_{16}H_{14}N_4OS$	0.76	(61.92/61.89)	(4.55/4.58)	(18.05/18.00)
5b	2-OH	73	158-160	$C_{16}H_{14}N_4O_2S$	0.90	(58.88/58.82)	(4.32/4.37)	(17.17/17.15)
5c	4-OH	71	164-167	$C_{16}H_{14}N_4O_2S$	0.91	(58.88/58.84)	(4.32/4.40)	(17.17/17.19)
5d	2-Cl	74	161-162	$C_{16}H_{13}ClN_4OS$	0.87	(55.73/55.78)	(3.80/3.89)	(16.25/16.19)
5e	4-Cl	70	171-174	$C_{16}H_{13}ClN_4OS$	0.85	(55.73/55.80)	(3.80/3.85)	(16.25/16.27)
5f	2-NO_2	69	168-170	$C_{16}H_{13}N_5O_3S$	0.78	(54.08/54.16)	(3.69/3.61)	(19.71/19.65)
5g	3-NO_2	70	170-172	$C_{16}H_{13}N_5O_3S$	0.80	(54.08/54.18)	(3.69/3.65)	(19.71/19.80)
5h	2-NH_2	72	175-177	$C_{16}H_{15}N_5OS$	0.73	(59.06/59.13)	(4.65/4.72)	(21.52/21.50)
5i	4-NH_2	73	178-180	$C_{16}H_{15}N_5OS$	0.82	(59.06/59.18)	(4.65/4.68)	(21.52/21.58)
5j	4-$N(CH_3)_2$	75	221-223	$C_{18}H_{19}N_5OS$	0.86	(61.17/61.10)	(5.42/5.33)	(19.81/19.88)

[a] Elemental analyses for C, H and N were within $\pm$ 0.4% of the theoretical value.

Table 2: The zone of inhibition values (mm) of compounds (5a-j)

	Antibacterial activity (mm)		
	Gram positive (+ve)		Gram negative (-ve)
Compounds	B.s. (MTCC 441)	S.g. (MTCC 1540)	E.c. (MTCC 443)
5a	–	–	–
5b	+	–	–
5c	+	++	+++
5d	–	–	+
5e	+	++	+++
5f	–	–	+
5g	++	++	++
5h	+	+	–
5i	+++	+++	++
5j	+++	+++	++
Zone of inhibition of standard drug (mm)			
Ciprofloxacin	++++	++++	++++

Diameter of the zone of inhibition: (-) 6 mm; (+) 7-9 mm; (++) 10-15 mm; (+++) 16-22 mm; (++++) 23-28 mm.

B.s., *Bacillus subtilis*; S.g., *Streptomyces griseus*; E.c., *E.coli*

Table 3: The MIC values (µg/ml) of compounds (5a-j)

	Antifungal activity (µg/ml)	
Compounds	C.a. (MTCC 227)	A.n. (MTCC 282)
5a	++	++
5b	+	++
5c	+++	++
5d	++	++
5e	++	+++
5f	–	–
5g	–	+
5h	++	++
5i	++	+++
5j	+++	+++
MIC of standard drug (µg/ml)		
Fluconazole	++++	++++

50 µg/ml = ++++, 100 µg/ml = +++, 150 µg/ml = ++, 200 µg/ml = +, Not active upto 200 µg/ml = –, Fluconazole is (++++) at ≤50 µg/ml.

Diameter of the MIC: (-) 6 mm; (+) 7-9 mm; (++) 10-15 mm; (+++) 16-22 mm; (++++) 23-28 mm.

C.a., *Candida albicans*; A.n., *Aspergillus niger*.

REFERENCES

1. Alang, G; Budhlakoti, P; Kaur, R; Singh, A *et al.* (2010), "Synthesis, characterization and antifungal activity of certain (E)-1-(1-(substituted phenyl) ethylidene)-2-(6-methylbenzo[d]thiazol-2-yl) hydrazine analogues", ***Int. J. Pharma. Bio. Arc.***, Vol. 1 (1), 56 – 61.
2. Bujdakova, H; Kova, MM (1994), "Antifungal activity of a new benzothiazole derivative against *Candida* in vitro and in vivo", ***Int. J. Antimicrob. Agents.***, Vol. 4 (4), 303-308.
3. Franco, M; Latrofa, A; Lopedota, A; Rosato, A *et al.* (2005), "Structural modifications and antimicrobial activity of *N*-cycloalkenyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles", ***IL Farmaco.***, Vol. 60 (4), 291-297.
4. Devmurari, VP; Goyani, MB; Nandanwar, RR; Pandey, S (2010), "Synthesis and anticancer activity of some novel 2-substituted benzothiazole derivatives", ***Int. J. Chem. Tech. Res.***, Vol. 2 (1), 681-689.
5. Crochard, JP; Mortimer, CG; Stone, EL; Wells, G *et al.* (2006), "Antitumor benzothiazoles", ***J. Med. Chem.***, Vol. 49 (1), 179-185.
6. Jagtap, VA; Jaychandran, E; Sathe, BS; Sreenivasa, GM. (2010), "Anti-mycobacterial activity of some synthesized 2[2'-phenyl-4'-benzidinyl-5'-oxo-imidazoline-1yl-amino]-6-fluoro-7-substituted (1, 3) benzothiazoles", ***Int. J. Pharma. Bio. Sci.***, Vol. 1 (4), 139-143.
7. Amnerkar, ND; Bhusari, KP; Chakole, RD; Khedekar, PB (2005), "Synthesis and substituted benzothiazole derivatives of thioquinazoline as anticovulsant agents", ***Indian J. Heterocycl. Chem.***, Vol. 15 (1), 27-30.
8. Bhatia, K; Jayachandran, E; Naragud, LVG; Roy, A (2003), "Anthelmintic activity of 2-[3-amino, 5-*S*-methyl 4-carboxamido pyrazol-1-yl] 6-fluoro, 7-substituted (1, 3) benzothiazoles on *Perituma Posthuma*", ***Indian Drugs.***, Vol. 40 (7), 408-11.
9. Alam, M; Siddiqui, AA; Siddiqui, N (2004), "Synthesis and analgesic activity of some 2-[{4-(Alkyl thioureido) phenyl} sulphonamido]-6-substituted benzothiazoles", ***Asian J. Chem.***, Vol. 16, 1005-1008.
10. Choi, S; Lee, SK; Park, HJ; Kim, SW *et al.* (2006), "Solid phase combinatorial synthesis of benzothiazoles and evaluation of topoisomarase II inhibitory activity", ***Bioorg. Med. Chem.***, Vol. 14, 1229-1235.
11. Ahmad, M; Ashraf, CM; Iqbal, A; Siddiqui, HL *et al.* (2007), "Synthesis, characterization and antibacterial activity of azomethine derivatives derived from 2-formylphenoxyacetic acid", ***Molecules***, 12, 245-254.
12. Alagwadi, KR; Bhanushali, MD; Manisha, VS; Reyee, N *et al.* (2010), "Synthesis and antimicrobial activity of some substituted azomethine derivatives", ***Int. J. Pharm. Res. Dev.***, Vol. 2 (9), 63-68.
13. Bhandari, A; Ranawat, MS; Sharma, S; Soni, B *et al.* (2010), "Synthesis and evaluation of some benzothiazole derivatives as potential antimicrobial agents", ***Eur. J. Med. Chem.,*** Vol. 45 (7), 2938-2942.
14. Horning, EC (1995), "Synthesis of phenylthiourea", ***Organic Synthesis***, Vol. 3, John Willey & Sons, Chapman & Hall Limited, London, 735-736.
15. Dapperheld, S (1994), "Process for preparation of 2-aminobenzothiazoles", U. S. patent 5,374,737.
16. Harley, JP; Klein, DA; Prescott, LM (1990), "***Microbiology***", 2, The Mc Graw-Hill Companies, Oxford, England, 328.
17. Amir, A; Azam, F (2004), "Synthesis and antimicrobial activities of some 5-(4'-pyridyl)-4-substituted benzylideneamino-3-mercapto (4H)-1, 2, 4-triazoles", ***Indian J. Pharm. Sci.***, 818-821.