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Research Article

Synthesis, spectral studies and biological activity of 2, 3-disubstituted imidazo [2, 1-b] benzothiazole derivatives

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ABSTRACT

Benzothiazole is a heterocyclic compound formed by the fusion of benzene and thiazole ring. The moiety had been reported to act *via* competing with ATP binding site at the catalytic domain of tyrosine kinase. The present work involves the synthesis and biological evaluation of 4, 5 disubstituted imidazo [2, 1-b] benzothiazole derivatives. The antimicrobial activity of the synthesized derivatives was carried out against Gram + ve bacteria *Staphylococcus aureus* (MTCC 3160) and Gram –ve bacteria *Bordetella bronchiseptica* (MTCC 6838), *Pseudomonas aeruginosa* (T11) and fungal strains *Candida albicans* (MTCC 1637). Ciprofloxacin and Fluconazole were used as standard drug for antibacterial and antifungal activity respectively. The compounds 6a1, 6a2, 6a3, 6b1 and 8a1 exhibited good antimicrobial activity against all the strains. The derivative 8a1 was further screened for anticancer activity against MCF-7 cell line using Doxorubicin as standard. The structures of the synthesized compounds were established by IR and NMR spectral studies.

Introduction

Benzothiazole moiety is a versatile moiety that exhibits a wide variety of biological activities including antitubercular, antimalarial and anticonvulsant, antidiabetic, antimicrobial, antifungal and antitumor. Modifications on the benzothiazole have resulted in large number of compounds having diverse biological activities [1]. Benzothiazole act *via* competing with ATP for binding at catalytic domain of tyrosine kinase. The ATP binding site has the following features: Adenine region that contains two key hydrogen bonds formed by the interaction of N-1 and N-6 amino group of the adenine ring. Many potent inhibitors use one of these hydrogen bonds. The second is sugar region which is hydrophilic region, except a few *e.g.* EGFR. The third is hydrophobic pocket which is though not used by ATP but plays an important role in inhibitor selectivity. The fourth is hydrophobic channel which is not used by ATP and may be exploited for inhibitor specificity. The fifth is phosphate binding region which is used for improving inhibitor selectivity[2]. Now days, there is growing interest in the discovery of new antibacterial agents to bate against pathogenic microorganism, especially the bacteria resistant to the current antibiotics. The 2-substituted benzthiazole derivatives have been reported to possess very good antimicrobial properties[3]. The development of anticancer drug is more difficult than discovering cures for bacterial infection as there are very few biochemical differences between cancerous cell and normal cells. Moreover, the effectiveness of many drugs is limited by their toxicity to the normal rapidly growing cells in the intestinal and bone marrow areas. Also the cancerous cells which are initially suppressed by a specific drug may develop a resistance to that drug. Among the antitumor discovered in recent years, various benzothiazole derivatives possess potent anticancer properties *e.g.* 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F203) that acts through a novel mechanism. The 3-substituted-2-imino benzothiazolines were found to be three times more potent than Riluzole, a blocker of excitatory amino acids mediated neurotransmission [4-7].

In the past few years, molecularly targeted therapeutics has become an indispensable part of medicinal chemistry research. The hybridisation of two or more bioactive drug fragments with complementary functions or different mechanisms of action into a single molecule is a novel approach that often results in synergistic activity and enhanced drug efficacy. Solomon *et al*. was the first to report this dual action/hybrid pharmacophore strategy using isatin [8]. Following the same hybrid

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pharmacophore strategy, the imidazobenzthiazole derivatives have been synthesized in the present work.

Experimental

The commercial chemicals employed for the present work were purchased from Sigma-Aldrich, Rankem and Loba Chem. All the solvents used were LR grade and were utilized in the reaction. The melting point was determined by open capillaries on Buchi-apparatus and is uncorrected. The reaction were monitored with the help of TLC using pre-coated aluminium sheets coated with $60F_{254}$ silica gel, 0.2 mm thickness from Merck. Various solvents systems used for developing chromatograms were (a) chloroform: methanol (9:0.5), (b) chloroform: ethyl acetoacetate (8.5:1.5), (c) Ethyl acetate: Pet. Ether (8:2) and U.V light chamber were used for the visualization of the TLC spots. The IR Spectra were recorded on an FT-IR Perkin-Elmer spectrophotometer (4000-400 cm^{-1}). The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance ‖ 400 spectrometer where TMS was used as internal standard and chemical shifts are expressed as δ *ppm.* The Mass spectra were run on micromas Q-T of micro spectrometer at SAIF Punjab University, Chandigarh.

Synthesis of Substituted 2-Amino Benzothiazole

The appropriately substituted aniline (0.1mol) in 95% acetic acid (50 ml) was added to a solution of KSCN (0.2mol) in 95% acetic acid (100 ml). The mixture was cooled to 0°C, and a solution of Br_2 (0.1 mol) in acetic acid (30 ml) was added slowly with vigorous stirring so that the temperature remained between 0 and 10°C. After the addition was complete, the stirring was continued for 1h at 5°C and then the mixture was poured into water. The solid was collected and recrystallized from ethanol to yield substituted-2-thiocyanatoaniline. The product (0.036 mol), concentrated HCl (27 ml) and water (54 ml) were refluxed for 2 h. The solution was cooled, and the product was filtered off, washed with water, and recrystallized from ethanol to yield the derivatives of 2-amino-4-(5-or 6-) substituted benzothiazole[9-10].

2-Amino benzothiazole (3a): Yield: 70%; State: Yellow powder; Melting point: 226-228℃; R_f 0.71 (Ethyl acetate: Pet. Ether 8:2).

6-Methoxy-2-amino benzothiazole (3b): Yield: 64%; State: Grey powder; Melting point: 228-232℃; R_f 0.65 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 1661 (C=N), 609 (C-S-C), 3388 (NH_2); ^{1}H NMR (DMSO-d^6, 400 MHz): δ 3.72 (s, 3H, CH_3), 6.75 (m, 1H, Ar), 7.23 (s, 2H, Ar).

Synthesis of Substituted Imidazo-Benzthiazole Derivatives

A mixture of equimolar quantities of substituted 2-amino benzothiazole (2.5 mmol) and substituted phenacyl bromide was heated to reflux on a water bath for 6-8 h, phosphorus pentoxide (0.75 mmol) was then added, and refluxing was continued for another 4-6 h. The reaction mixture was cooled overnight at room temperature. Excess of solvent was removed under reduced pressure and the crude product was filtered, washed with cold ethanol and dried. Neutralization of crude product with cold aqueous solution of Na_2CO_3 yielded the corresponding free bases, which were purified by recrystallization from dry ethanol.

2-(4′-Bromo) phenyl imidazo [2, 1-b] benzothiazole 5(a1): Yield: 60%; State: Yellow powder; Melting point: 290-294℃; R_f 0.6 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2977 (Ar-H), 1691 (C=N), 748 (C-S-C); ^{1}H NMR (DMSO-d^6, 400 MHz): δ 8.69 (s, 1H, imidazole-H), 7.3 -7.9 (m, 8H, Ar-H).

2-(4′-Fluoro) phenyl imidazo [2, 1-b] benzothiazole 5(a2): Yield: 64%; State: Yellow powder; Melting point: 290-295℃; R_f 0.63 (Ethyl acetate: Pet. Ether 8:2). ^{1}H NMR (DMSO-d^6, 400 MHz): δ 8.73 (s, 1H, imidazole-H), 8.0-8.3(m, 8H, Ar-H).

2-(4′-Methoxy) phenyl imidazo [2, 1-b] benzothiazole 5(a3): Yield: 55%; State: Light yellow powder; Melting point: 270-273℃; R_f 0.54 (Ethyl acetate: Pet. Ether 8:2). ^{1}H NMR (DMSO-d^6, 400 MHz): δ 8.71 (s, 1H, imidazole-H), 7.9-8.2 (m, 8H, Ar-H).

2-(4′-Nitro) phenyl imidazo [2, 1-b] benzothiazole 5(a4): Yield: 70%; State: Red powder; Melting point: 283-287℃; R_f 0.67 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 1685 (C=N), 746 (C-S-C), 1517 (NO_2), 2970 (Ar-H); ^{1}H NMR (DMSO-d^6, 400 MHz): δ 8.96 (s, 1H, imidazole-H), 7.4-8.4 (m, 8H, Ar-H).

6-Methoxy-2-(4′-bromo) phenyl imidazo [2, 1-b] benzothiazole 5(b1): Yield: 53%; State: Light yellow powder; Melting point: 130-135℃; R_f 0.5 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}):1695 (C=N), 752 (C-S-C), 2972 (Ar-H), 1592 (C-O); ^{1}H NMR (DMSO-d^6, 400 MHz): δ 8.84 (s, 1H, imidazole-H), 7.3-8.0 (m, 7H, Ar), 3.7- 3.9 (m, 3H, CH_3).

6-Methoxy-2-(4′-fluoro) phenyl imidazo [2, 1-b] benzothiazole 5(b2): Yield: 57%; State: Pale yellow powder; Melting point: 140-145℃; R_f 0.48 (Ethyl acetate: Pet. Ether 8:2). IR (KBr, ν, cm^{-1}):1698 (C=N), 758 (C-S-C), 2989 (Ar-H), 1598 (C-O);^{1}H NMR (DMSO-d^6, 400 MHz): δ 9.1 (s, 1H, imidazole-H), 7.7-8.3 (m, 7H, Ar), 3.7- 4.3 (m, 3H, CH_3).

6-Methoxy-2-(4′-methyl) phenyl imidazo [2, 1-b] benzothiazole 5(b3): Yield: 60%; State: Off white powder; Melting point: 137-140℃; R_f 0.59 (Ethyl acetate: Pet. Ether 8:2). IR (KBr, ν, cm^{-1}):1691 (C=N), 748 (C-S-C), 2964 (Ar-H), 1573 (C-O);^{1}H NMR (DMSO-d^6, 400 MHz): δ 8.2 (s, 1H, imidazole-H), 7.3-7.9 (m, 7H, Ar), 3.4-3.6 (m, 3H, CH_3).

6-Methoxy-2-(4′-nitro) phenyl imidazo [2, 1-b] benzothiazole 5(b4): Yield: 56%; State: Brown powder; Melting point: 155-159℃; R_f 0.52 (Ethyl acetate: Pet. Ether 8:2). IR (KBr, ν, cm^{-1}):1700 (C=N), 767 (C-S-C), 3014 (Ar-H), 1612 (C-O);^{1}H NMR (DMSO-d^6, 400 MHz): δ 9.14 (s, 1H, imidazole-H), 7.7-8.3 (m, 7H, Ar), 3.9-4.2 (m, 3H, CH_3).

Synthesis of 2, 3-Disubstituted Imidazo [2, 1-b] Benzthiazole

Vilsmeir Haack reagent was prepared by adding phosphoryl-chloride (3ml) in dimethyl formamide (20 ml) at 0℃ with stirring. Then 5-(4-substituted)-phenyl imidazo-benzothiazole (0.16 mg) 0.5 mmol was added to the reagent and stirred for 2 h at room temperature and at 60℃ for additional 2 h. The mixture was then poured in sodium carbonate solution and stirred at 90℃ for 2 h. after cooling the mixture was diluted with water and extracted with chloroform. The residue obtained was recrystallized from ethanol solvent to get crystalline solid.

2-(4′-Bromo) phenyl imidazo [2, 1-b] benzothiazole-3-carbaldehyde 6(a1): Yield: 62%; State: Off white powder; Melting point: 100-105℃; R_f 0.6 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2970 (Ar-H), 1685 (C=O), 1602 (C=N), 746 (C-S-C); ^1H NMR ($CDCl_3$,400 MHz): δ 7.9 (s, 1H, CHO), 7.2-7.7 (m, 8H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 148.26 (1C), 146.47 (1C), 132.75 (1C), 131.98 (1C), 131.79 (1C), 130.22 (1C), 126.64 (1C), 126.28 (1C), 125.04 (1C), 124.41 (1C), 121.22 (1C), 112.67 (1C), 107.06 (1C), 77.41 (1C), 77.09 (1C), 76.77 (1C); MS (ESI): m/z 329.158 $(M+H)^+$.

2-(4′-Fluoro) phenyl imidazo [2, 1-b] benzothiazole-3-carbaldehyde 6(a2): Yield: 68%; State: Brown crystalline powder; Melting point: 220-224℃; R_f 0.65 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2885 (Ar-H), 1690 (C=O), 1619 (C=N), 710 (C-S-C). ^1H NMR ($CDCl_3$,400 MHz): δ 8.2 (s, 1H, CHO), 7.9-8.2 (m, 8H, Ar-H);

2-(4′-Methoxy) phenyl imidazo [2, 1-b] benzothiazole-3-carbaldehyde 6(a3): Yield: 50%; State: Brown crystal; Melting point: 135-140℃; R_f 0.49 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2965 (Ar-H), 1705 (C=O), 1630 (C=N), 730 (C-S-C). ^1H NMR ($CDCl_3$,400 MHz): δ 8.4 (s, 1H, CHO), 8.1-8.5 (m, 8H, Ar-H).

2-(4′-Nitro) phenyl imidazo [2, 1-b] benzothiazole-3-carbaldehyde 6(a4): Yield: 53%; State: Red colour powder; Melting point: 218-223℃; R_f 0.54 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2972 (Ar-H), 1683 (C=O), 1597 (C=N), 751 (C-S-C). ^1H NMR ($CDCl_3$,400 MHz): δ 9.1 (s, 1H, CHO), 8.4-8.8 (m, 8H, Ar-H).

6-Methoxy-2-(4′-bromo) phenyl imidazo [2, 1-b] benzothiazole-3-carbaldehyde 6(b1): Yield: 53%; State: Red colour powder; Melting point: 220-224℃; R_f 0.5 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2875 (Ar-H), 1715 (C=O), 1607 (C=N), 1205 (C-O), 742 (C-S-C). ^1H NMR ($CDCl_3$,400 MHz): δ 9.4(s, 1H, CHO), 8.3-8.8 (m, 8H, Ar-H); 3.5-3.7 (m, 3H, CH_3).

6-Methoxy-2-(4′-fluoro) phenyl imidazo [2, 1-b] benzothiazole-3-carbaldehyde 6(b2): Yield: 72%; State: Yellow powder; Melting point: 130-135℃; R_f 0.63 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2895 (Ar-H), 1715 (C=O), 1610 (C=N), 1262 (C-O), 715 (C-S-C). ^1H NMR ($CDCl_3$,400 MHz): δ 9.6 (s, 1H, CHO), 8.5-8.9 (m, 8H, Ar-H); 3.6-3.9 (m, 3H, CH_3).

6-Methoxy-2-(4′-methyl) phenyl imidazo [2, 1-b] benzothiazole-3-carbaldehyde 6(b3): Yield: 51%; State: Red powder; Melting point: 218-223℃; R_f 0.59 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2972 (Ar-H), 1720 (C=O), 1645 (C=N), 1190 (C-O), 730 (C-S-C). ^1H NMR ($CDCl_3$,400 MHz): δ 9.2 (s, 1H, CHO), 8.4-8.8 (m, 8H, Ar-H); 3.4-3.6 (m, 3H, CH_3).

6-Methoxy-2-(4′-nitro) phenyl imidazo [2, 1-b] benzothiazole-3-carbaldehyde 6(b4): Yield: 63%; State: Red powder; Melting point: 130-135℃; R_f 0.58 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2878 (Ar-H), 1710 (C=O), 1623 (C=N), 1195 (C-O), 720 (C-S-C). ^1H NMR ($CDCl_3$,400 MHz): δ 9.7 (s, 1H, CHO), 8.8-9.1 (m, 8H, Ar-H); 3.7-3.9 (m, 3H, CH_3).

3-Nitroso-2-(4′-Substituted) Phenyl Imidazo-Benzthiazole

To a well stirred solution of 5-(4-substituted) phenyl-imidazo benzothiazole (0.16 mg 0.5 mmol) in acetic acid (10 ml) was added sodium nitrite solution (0.075 mg, in 5 ml water) dropwise at room temperature. After the addition, stirring was continued for 30 min and then mixture was refluxed for 2h. The mixture was poured in to ice cold water; separated crude solid was collected, washed with water, dried and recrystallized from ethanol.

2-(4′-Bromo) phenyl-3-nitroso imidazo [2, 1-b] benzothiazole 7(a1): Yield: 60%; State: Red powder; Melting point: 160-164℃; R_f 0.56 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2945 (Ar-H), 1630 (C=N), 1514 (N-O), 748 (C-S-C); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.5-8.0 (m, 8H, Ar-H).

2-(4′-Fluoro) phenyl-3-nitroso imidazo [2, 1-b] benzothiazole 7(a2): Yield: 62%; State: Pink powder; Melting point: 149-154℃; R_f 0.6 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2972 (Ar-H), 1647 (C=N), 1498 (N-O), 725 (C-S-C). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.7-8.2 (m, 8H, Ar-H).

2-(4′-Methoxy) phenyl-3-nitroso imidazo [2, 1-b] benzothiazole 7(a3): Yield: 60%; State: Red powder; Melting point: 140-144℃; R_f 0.52 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2975 (Ar-H), 1649 (C=N), 1535 (N-O), 728 (C-S-C). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.8-8.3 (m, 8H, Ar-H).

2-(4′-Nitro) phenyl-3-nitroso imidazo [2, 1-b] benzothiazole 7(a4): Yield: 55%; State: Light pink powder; Melting point: 147-153℃; R_f 0.6 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν, cm^{-1}): 2985 (Ar-H), 1677 (C=N), 1498 (N-O), 755 (C-S-C). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.9-8.4 (m, 8H, Ar-H).

3-Bromo-2-(4-Bromo) Phenyl Imidazo-Benzothiazole

To a well stirred solution of 2(4-substituted) imidazo [2, 1-b] benzothiazole (0.01 mol) in glacial acetic acid (5 ml) and anhydrous sodium acetate (0.02 mol), Bromine (0.1 ml) was added dropwise with stirring at room temperature. After the addition, stirring was continued for 2h. The reaction mixture was poured on to ice cold water and basified with ammonia solution. The separated solid was collected, washed with water and recrystallized with ethanol.

3-Bromo-2-(4′-bromo) phenyl imidazo [2, 1-b] benzothiazole 8(a1): Yield: 64%; State: Yellow brown powder; Melting point: 180-184℃; R_f 0.71 (Ethyl acetate: Pet. Ether 8:2); IR (KBr, ν,

cm^{-1}): 2972 (Ar-H), 1645 (C=N), 738 (C-S-C); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.6-8.1 (m, 8H, Ar-H).

BIOLOGICAL SCREENING OF THE SYNTHESIZED COMPOUNDS

Antimicrobial screening by agar diffusion method

The antimicrobial screening of the synthesized compounds was carried out by cup plate agar diffusion method. All the synthesized compounds are evaluated to antibacterial and antifungal activity against Gram + ve bacteria *Staphylococcus aureus* (MTCC 3160) and Gram –ve bacteria *Bordetella bronchiseptica* (MTCC 6838), *Pseudomonas aeruginosa* (T11) and fungal strains *Candida albicans* (MTCC 1637) at IMTECH Chandigarh. The standards Ciprofloxacin and Fluconazole were dissolved in DMSO to get a concentration 100µg/ml for testing antibacterial and antifungal activity respectively. Each test compounds was dissolved in DMSO to get a concentration of 100µg/ml for testing antibacterial activity. The zone of inhibition were observed and measured in mm.

In vitro Anticancer screening

In vitro anticancer screening was carried out using MTT assay method. For this firstly, the MTT solution (stock solution) was prepared by dissolving 5 mg MTT in 1 ml of PBS (Phosphate Buffer Saline). The cell line used for the study was MCF-7. TheMCF-7 cell line was maintained in 96 wells microliter plate containing MEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of Gentamicin (10ug), Penicillin (100 Units/ml) and Streptomycin (100µg/ml) in presence of 5% CO_2 incubated at 37ºC for 48-72 hours. *In-vitro* growth inhibition effect of test compound was assessed by calorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells. The final test concentrations of 10, 20, 25, 30 and 50µg/ml were added to the media. After 48 hrs incubation at 37ºC in a humidified atmosphere of 5% CO_2, stock solution of MTT was added into each well (20µl, 5mg per ml in sterile (PBS) for further 4 hrs incubation. The supernatant carefully aspirated, the precipitated crystals of "Formazan blue' were solubilized by adding DMSO (100µl) and optical density was measured at wavelength of 570 nm by using ELISA plus. The results represent the mean of five readings. The concentration in which the OD of treated cells was reduced by 50% with respect to the untreated control, it is known as IC_{50} value. The % of Surviving cells which calculated by the formula that given below.

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound} \times 100}{\text{Mean OD at control}}$$

The % of Inhibition cells which calculated by the formula that given below

Inhibition cells (%) = 100 - Surviving cells (%)

IC_{50} value was calculated by using graph pad software. A graph presenting concentration (x-axis) vs % inhibition (y-axis) can be plotted to calculate the IC_{50} value.

Results and Discussion

The synthesis of 2,3-disubstituted imidazo [2, 1-b] benzthiazole was carried out as per the proposed protocol (Figure 1). The 2-amino benzothiazole was synthesized in good yields by reacting substituted aniline with potassium thiocyanate and bromine. The reaction proceed *via* the intermediate substituted-2-thiocyanatoaniline which refluxed for 2h in presence of HCl/H_2O and form 2-substituted imidazo [2, 1-b] benzothiazole. Then prepared imidazo [2, 1-b] benzothiazole was treated with Vilsmeier hack reagent, nitration, bromination reaction to form 3-substituted carbaldehyde, nitroso and bromine derivatives respectively. All the reactions were monitored through TLC observation till the completion using suitable mobile phase each time. After completion of the reaction, the products were purified by using suitable solvents *e.g* ethanol. The outcome of the present work has been summarized in Table 1.

The purity of the compound was confirmed by TLC using precoated silica gel as a stationary phase, using appropriate solvent system as mobile phase and visualized under UV-light as well as analyzed. Structures of the title compounds were confirmed by FT-IR and ^1H NMR spectral studies. The R_f for the title compounds was observed between 0.49-0.71 using different solvent system and detecting agent. The R_f for different imidazo-benzothiazole was found to be higher than the synthesized compounds. The melting point of all the compounds are in range between 115–295℃ and uncorrected. The melting point for all the precursors was found to be in agreement with the literature. The yield of all the synthesized derivatives was found in between 45-72%. The formation of 2–Aminobenzothiazole by the reaction between aniline and potassium thiocyanate and bromine was confirmed by its IR spectrum. The formation of 2-aminobenzothiazole (3a, 3b) was confirmed by its IR spectrum that indicated the presence of C-N, N-H stretch complied with the spectra reported in the literature. Further the ^{1}HNMR spectra displayed aromatic proton at δ 6.73 and NH_2 proton at δ 7.21. The structure of different 2-substituted imidazo [2, 1-b] benzothiazole derivatives were established by the absence of N-H stretch in the IR spectra between 1650-1580 cm^{-1} region and appearance of singlet of imidazole proton (C_5-H) around δ 8.69 in the ^1H NMR confirms the formation of final ring system. The 2, 3-disubstituted imidazo [2, 1-b] benzothiazole derivatives clearly indicated the absence of imidazole proton (C_5-H) around δ 8.0-9.0 in the ^1H NMR.

Antimicrobial activity

The synthesized derivatives 6a1, 6a2, 6a3, 6b1, 7a3 and 8a1 exhibited antimicrobial potential against *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (T11) and *Candida albicans* (MTCC 1637) during the primary screening of the compounds (Table 3). The active compounds were further screened at a concentration of 100µg/ml for test and standard against the same strains. The compound 2-(4-bromo) phenyl imidazo [2, 1-b] benzothiazole-3-carbaldehyde (6a1) and 3-

bomo-2-(4-bromo) phenyl imidazo [2, 1-b] benzothiazole (8a1) had marked antimicrobial activity against all the strains. The compound 2-(4-fluoro) phenyl imidazo [2, 1-b] benzothiazole-3-carbaldehyde **(**6a2), 2-(4-methoxy) phenyl imidazo [2, 1-b] benzothiazole-3-carbaldehyde(6a3), 6-methoxy-2-(4'-bromo) phenyl imidazo [2, 1-b] benzothiazole-3-carbaldehyde (6b1) had exhibited moderate activity. The detail with regard to zone of inhibition (in mm) has been summarized in Table 4.

Anticancer screening using *MTT Assay*

Biological (anticancer) activities of 2,3-disubstituted imidazo benzothiazole derivative was carried out on MCF-7 cancer cell-line at IMTECH Chandigarh. Synthesized compounds **8a1** was screened for anticancer activity on MCF-7 cancer cell-line (Table 4 and figures 2-4).

The synthesized compound possess better antimicrobial activity in comparison to the 2-substituted imidazo [2, 1-b] benzothiazole derivatives. Their better activity can be attributed to the presence of various electron withdrawing group substituted at position 3 in the ring system. However, the synthesized derivatives can be further explored for better antimicrobial activity by studying their structure activity relationship. The compound 8a have exhibited encouraging activity on MCF-7 cell lines using Doxorubicin as standard indicating its potential as anticancer agent.

Conclusion

In the present study a total of 12 imidazo [2, 1-b] benzothiazole derivatives were synthesized. The compounds 6a1 and 8a1 exhibited good antimicrobial activity as compared with standard drugs. The compound 8a1 have exhibited marked activity in both the antimicrobial as well as anticancer assays. The synthesized derivatives can be further explored for better antimicrobial activity by studying their structure activity relationship. The compound 8a can be utilized further to develop potential antimicrobial and anticancer agents.

Table 1: Physical characteristic of synthesized compounds

Sr. No	Compounds	Molecular Formula	Molecular weight	Melting point(℃)	Yield(%)	R_f^*
1.	6a1	$C_{16}H_9FN_2OS$	296.2	100 – 105	62	0.6
2.	6a2	$C_{16}H_9BrN_2OS$	358.2	220 – 224	68	0.65
3.	6a3	$C_{17}H_{12}N_2O_2S$	308.3	135 – 140	50	0.49
4.	6a4	$C_{16}H_9N_3O_3S$	323.3	218 - 223	53	0.54
5.	6b1	$C_{17}H_{11}FN_2O_2S$	326.3	130 - 135	72	0.63
6.	6b2	$C_{17}H_{11}BrN_2O_2S$	387.2	220 - 224	53	0.5
7.	6b3	$C_{18}H_{14}N_2O_2S$	322.3	218 - 223	51	0.59
8.	6b4	$C_{17}H_{11}N_3O_4S$	353.3	130 - 135	63	0.58
9.	7a1	$C_{15}H_8FN_3O_2S$	313.2	160 - 164	60	0.56
10.	7a2	$C_{15}H_8BrN_3O_2S$	374.2	149 - 154	62	0.6
11.	7a3	$C_{16}H_{11}N_3O_4S$	325.3	140 - 144	60	0.5
12.	7a4	$C_{15}H_8BrN_3O_2S$	340.2	147 - 153	55	0.6
13.	8a1	$C_{15}H_8Br_2N_2S$	408.8	180 - 184	64	0.7

*Ethyl acetate: Pet. Ether (8:2)

Table 2: *In vitro* antimicrobial activity of synthesized compounds

Compounds	Bacterial Strains			Fungal Strain
1&2 mg/mL	*Staphylococcus aureus* (MTCC 3160)	*Pseudomonas aeruginosa* (T11)	*Bordetella bronchiseptica* (MTCC 6838)	*Candida albicans* (MTCC 1637)
6 a1	+	+	+	+
6 a2	+	+	+	+
6 a3	+	+	+	+
6 b1	+	+	+	+
6 b2	-	-	-	-
6 b3	-	-	-	-
7 a1	-	-	-	-
7a2	-	-	-	-
7 a3	+	+	+	+
8 a1	+	+	+	+

Table 3: *In vitro* antimicrobial activity against standard drug

Compounds	**Zone of Inhibition (mm)**			
Concentration	**Bacterial Strains**			**Fungal Strain**
100 μg/mL	*Staphylococcus aureus* (MTCC 3160)	*Pseudomonas aeruginosa* (T11)	*Bordetella bronchiseptica* (MTCC 6838)	*Candida albicans* (MTCC 1637)
6a1	21±1	20±1	18±3	18±2
6a2	20±2	19±1	17±2	19±1
6a3	19±1	18±2	15±2	17±2
6b1	21±1	18±1	17±1	13±2
7a3	20±2	17±2	14±1	15±1
8a1	22±3	21±2	19±1	19±2
St.	23±1*	28±2*	24±2*	22±2*

St., Standard (Ciprofloxacin and Fluconazole); *± SD (n=3) mean of zone of inhibition in mm; NA, No activity.

Table 4: *In vitro* anticancer activity of compound 8a in comparison to doxorubicin

Sr. No.	Compound	CONCENTRATION(μg/ml)	ABSORBANCE(nm)	OBSERVATION	IC_{50} (μg)
1	**Standard (Doxorubicin)**	2.5	0.610	No lysis	10
2		5	0.452	No lysis	
3		7.5	0.438	25% lysis	
4		10	0.358	50% lysis	
5		Control	0.668	No lysis	
1	**8a1**	10	0.446	50% lysis	10
2		20	0.420	50% lysis	
3		25	0.416	>50% lysis	
4		30	0.415	>50% lysis	
5		50	0.413	>50% lysis	

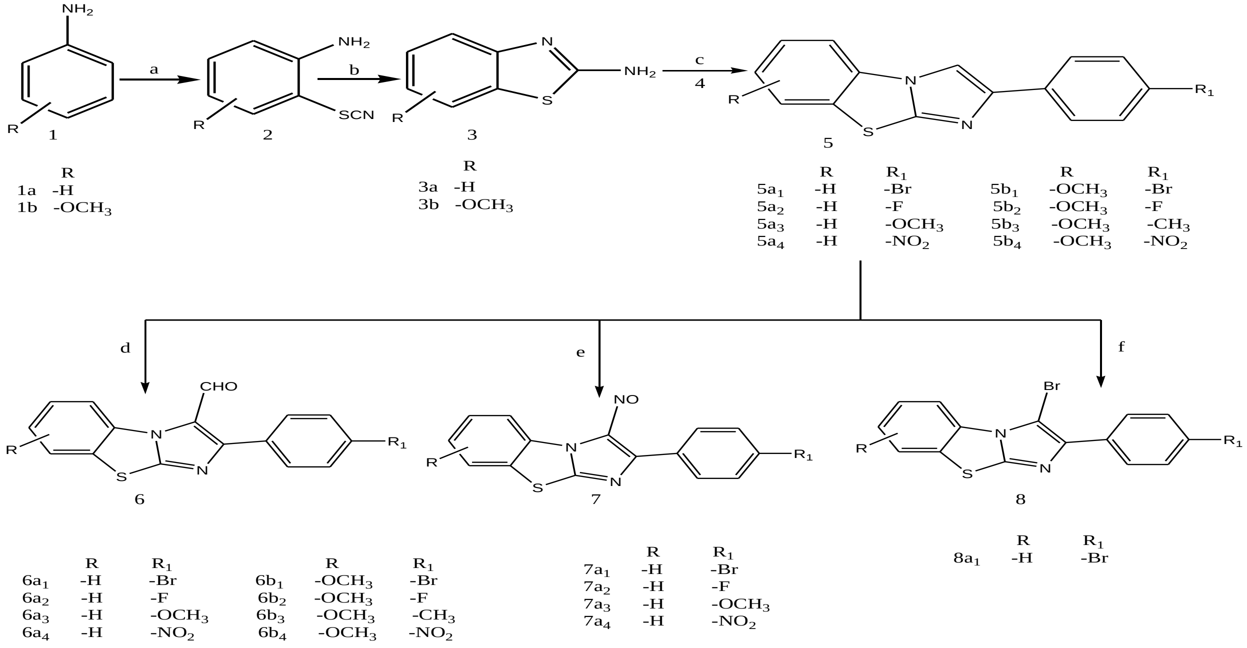


Figure 1:Scheme for the synthesis of 2,3-disubstituted imidazo [2, 1-b] benzthiazole derivatives

Reagents and conditions (a) KSCN, Br_2, H_2O, stirring (b) HCl, H_2O, reflux 2h (c) C_2H_5OH, P_2O_5, Na_2CO_3, reflux (d) DMF/$POCl_3$, Na_2CO_3, stirring (e) $NaNO_2$, AcOH, reflux 2h (f) Br_2/AcOH, sodium acetate, stirring

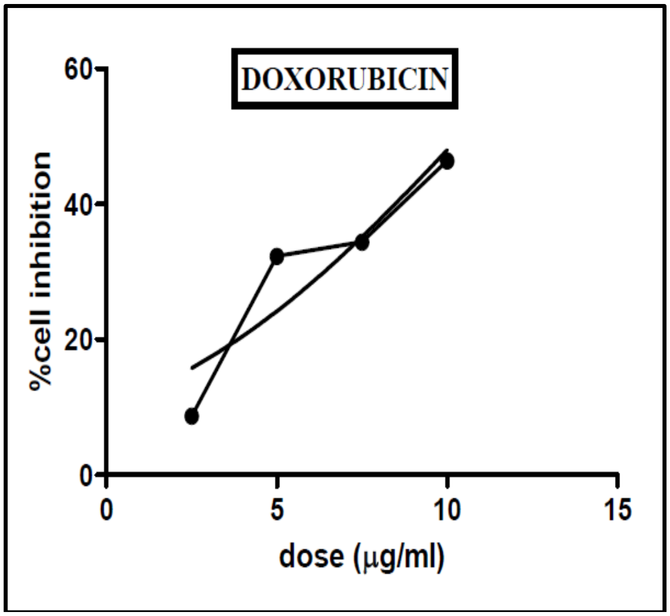


Figure 2: Variation of cell inhibition (%) *vs* dose (µg/ml) for Doxorubicin

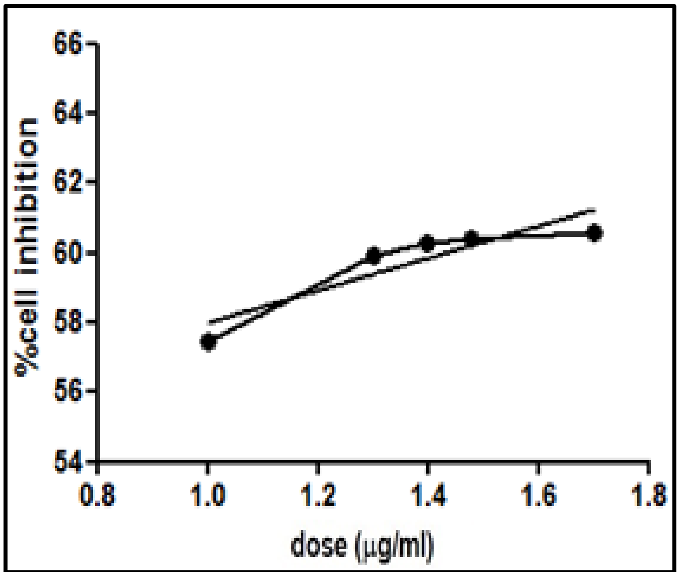
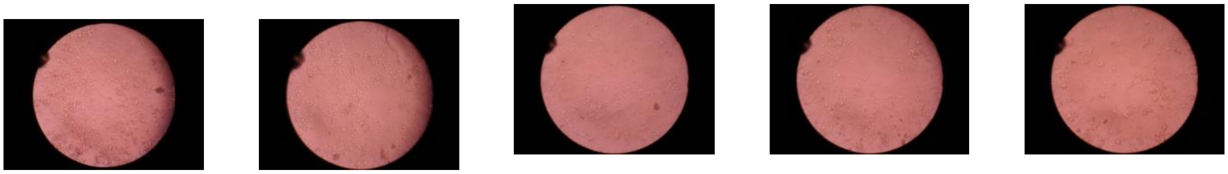


Figure 3:Variation of cell inhibition (%) *vs* dose (µg/ml) for Compound 8a1



10 µg/ml **20 µg/ml** **25 µg/ml** **30 µg/ml** **50 µg/ml**

Figure 4: Photographs showing cell Lysis with different concentration of Compound 8a1

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