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Synthesis and Anti-cancer Studies of 2, 6-disubstituted Benzothiazole Derivatives

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ABSTRACT

On the basis of exhaustive literature review, it has been found that benzothiazole derivatives have good potential to exhibit anticancer activity[15-17]. So the present study involves the synthesis of 2,6-disubstituted benzothiazole followed by preliminary cytotoxicity screening against three human cancer cell lines(MCF-7, HeLa and MG63) using MTT assay at 48 h of exposure.

Key words: Benzothiazole, Anticancer, MCF-7, HeLa, MG63.

INTRODUCTION

The development of new anticancer therapeutic agents is one of the fundamental goals in medicinal chemistry. A dose of anticancer drug sufficient to kill tumour cells is often toxic to the normal tissue and leads to many side effects, which in turn, limits its treatment efficacy. In recent years, there has been a concerned search for the discovery and development of novel selective anti-tumor agents.

Benzothiazoles are bicyclic ring system with multiple applications. 2-substituted benzothiazole scaffold is one of the privileged structure in medicinal chemistry[1, 2] and reported cytotoxic on cancer cells[3-5]. In a recent years number of such derivatives have exhibited interesting anticancer activities[6-11]. Among the anti-tumor drugs discovered in the recent years, various benzothiazoles[12-14] possess potent anticancer properties.

MATERIAL AND METHODS

Chemistry

The synthetic starting material, reagents, and solvents were of analytical reagent grade or the highest quality commercially available and were purchased from sigma-Aldrich Chemical Co., Merck Chemical Co. Melting points were recorded by labtronics digital melting point apparatus. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $DMSOd_6$ solvent on Bruker 300 MHz spectrophotometer using tetramethylsilane as an internal reference, respectively. The apparent resonance multiplicity is described as s (singlet), d (doublet), dd (doublet of

doublets), t (triplet), q (quartet) and m(multiplet). Infrared measurements were recorded in the range 400–4000 cm^{-1} by Perkin Elmer. Elemental analysis was carried out using Perkin Elmer CHNS. Mass spectra were recorded on a Thermo LCQ Deca XP MAX at70eV. Thin layer chromatography (TLC) analysis was carried out on 5x20 cm plate coated with silica gel GF_{254}.

Synthesis of *N*-(6-nitro-1,3-benzothiazol-2-yl) acetamide (1)

To a solution of 2-amino-6-nitrobenzothiazole (10 g,0.051 mol) in 40ml acetic anhydride, pyridine(12.4 ml,0.153 mol) was added. The reaction mixture was heated to 90° C for 4 hr,and then allowed to cool. The reaction was monitored by TLC. The reaction mixture was poured on to 200 ml of 2N HCl. The solid product that formed was collected by filtration and washed with water and diethyl ether finally dried to give compound **1**. Pale yellow solid ; Yield 79%; m.p. 285-286°C;IR (KBr) ν_{max} in cm^{-1}: 3387 (NH), 3091, 2944 (CH), 1701(C=O), 1554 (NH bend), 1514, 1340 (NO_2), 1267 (C-N), 750 (CH bend); ^{1}H NMR (DMSO-d_6) δ ppm: 2.23 (s, 3H), 7.84 (d, 1H, J= 9 Hz), 8.23 (dd, 1H, J=2.4, 9 Hz), 8.98 (d, 1H, J=2.1 Hz), 12.72 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 22.7, 118.8, 120.4, 121.6, 132.0, 142.8, 153.3, 163.3, 170.1; LC-MS (ESI) m/z : 236.20 $(M-H)^-$.

Synthesis of *N*-(6-amino-1,3-benzothiazol-2-yl) acetamide (2)

A solution of compound 1(10g,0.0421mol) in 80 ml 12N HCl ,$SnCl_2$.$2H_2O$(47.4g,0.210 mol) was added at RT. The reaction mixture was stirred at RT for 2 hr. The reaction was monitored by TLC. The reaction mixture was diluted with 300 ml of cold water. The solution was basified to pH 9 with 40% NaOH and the aqueous layer was extracted with ethyl acetate (3x150 mL),washed with water (2×200 mL), Brine(1x250mL) and dried over anhydrous Na_2SO_4. The solvent was concentrated under reduced pressure to give the compound **2.** Off-white solid; Yield 82%; m.p 248-249 °C; IR (KBr) ν_{max} in cm^{-1}: 3417, 3298 (NH), 3057, 2916 (CH), 1692 (C=O), 1608 (C=N), 1556 (NH bend), 1272 (C-N),694 (CH); ^{1}H NMR (DMSO-d_6) δ ppm: 2.14 (s, 3H), 5.13 (s, 2H), 6.69 (dd, 1H, J =2.1, 8.4 Hz),6.99 (d, 1H, J =2.1 Hz), 7.39 (d,1H, J =8.4 Hz); ^{13}C NMR (DMSO-d_6) δ ppm: 22.6, 104.0, 114.3, 120.7, 132.8, 139.5, 145.6, 153.0, 168.6; LC-MS (ESI) m/z : 206.27 $(M-H)^-$.

Procedure for synthesis *N*-(2-acetamido-1,3-benzothiazol-6-yl)-2-(1*H*-indol-3-yl)acetamide (3a)

To a solution of compound 2 (0.4g,.0019mol) in 12 mL THF ,indole-3-aceticacid (0.45g,0.0023), EDC.HCl(0.489g,0.0025m0l)and HOBt(0.260,0.0019 mol) was added. The reaction mixture was cooled to 0°C and triethylamine was added then reaction mixture was allowed to RT and stirred for 8 hr.the reaction was monitored by TLC. The reaction solution was concentred under reduced pressure and partianated between ethylacetate (50mL) and water(40mL). The combined ethylacetate layer was washed with 2N HCl(1X25),10%NaHCO3(1X25,Brine(1x25mL) and dried over anhydrous Na_2SO_4.the resulting ethylacetate layer was washed and concentred under reduced pressure to afford compound **3a**. Orange solid; yield 40%; m.p.254-256 °C; IR (KBr) ν_{max} in cm^{-1}: 3358 (NH), 1673 (C=O), 1575 (NH bend), 1273 (C-N), 731 (CH); ^{1}H NMR (DMSO-d_6) δ ppm: 2.18 (s,1H), 3.76 (s, 2H), 6.98 (t, 1H J =7.5 Hz), 7.07 (t,1H, J =7.5 Hz), 7.29 (d, 1H, J =1.5 Hz), 7.36 (d, 1H, J =8.1 Hz),7.55 (dd, 1H, J =1.5, 8.7 Hz),7.63 (d, 1H, J =7.5 Hz), 7.65 (d, 1H, J =8.7 Hz), 8.30 (d, 1H, J =1.5 Hz), 10.27 (s, 1H), 10.93 (s, 1H), 12.28(s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 22.6, 33.7, 108.4, 111.2, 111.3, 118.3, 118.4, 120.3, 120.9, 123.7, 127.13, 131.7, 135.2, 136.0, 144.3, 156.7, 169.1,169.5; LC-MS (ESI) m/z : 365.27 $(M+H)^+$; Anal. calcd. for $C_{19}H_{16}N_4O_2S$ (364.42) %: C, 62.62; H,4.43; N, 15.37; Found: C, 62.72; H, 4.48; N, 15.22.

Procedure for synthesis *N*-(2-acetamido-1,3-benzothiazol-6-yl)-3-methoxybenzamide (3b)

Prepared as reported above for **3a** starting from compound 2 and 3-methoxybenzoic acid. This reaction was carried out at room temperature for 6hr. Paleyellow solid; Yield 20%; m.p.257-258 °C; IR (KBr) ν_{max} in cm^{-1}: 3347 (NH), 3074, 2984 (CH), 1698 (C=O), 1621 (C=N), 1575 (NH bend), 1322 (C-N), 1273, 1039 (C-O), 710 (CH); ^{1}H NMR (DMSO-d_6) δ ppm: 2.19 (s, 3H), 3.84 (s, 3H), 7.16 (dd, 1H, J =2.1,7.8 Hz), 7.45 (t, 1H, J =7.8 Hz), 7.51 (d,1H, J =2.1 Hz),7.56 (d,1H, J =7.5 Hz), 7.74 (d, 1H, J =7.8 Hz), 7.70(d, 1H J =7.8 Hz), 8.41 (d, 1H), 10.36 (s, 1H), 12.30(s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 22.7, 55.3, 112.8, 112.9, 117.2, 199.8, 119.9, 120.2, 129.5, 131.6, 134.9, 136.2, 144.8, 157.1, 159.1, 165.1, 169.2. LC-MS (ESI) m/z : 340.60 $(M-H)^-$; Anal. calcd. for $C_{17}H_{15}N_3O_3S$ (341.38) %: C, 59.81; H,4.43; N, 12.31; Found: C, 59.66; H, 4.49; N, 12.11.

Procedure for synthesis *N*-(2-acetamido-1,3-benzothiazol-6-yl)-2-(3-fluorophenyl)acetamide (3c)

Prepared as reported above for 3a starting from compound **2** and 3-fluorophenylacetic acid. This reaction was carried out at room temperature for 10 hr. Light brown solid; Yield 21%; m.p.274-275 °C;IR (KBr) ν_{max} in cm^{-1}: 3324 (NH), 3256, 3073, 3006 (CH), 1658 (C=O), 1565 (NH bend), 1235(C-F), 1283 (C-N), 754 (CH); ^1H NMR (DMSO-d_6) δ ppm: 2.18 (s, 3H), 3.75 (s, 2H), 8.27 (d, 1H, J =1.8 Hz), 7.66 (d, 1H, J =8.7 Hz), 7.52 (dd, 1H, J =1.8, 9 Hz), 7.40 (t, 1H, J =7.8 Hz), 7.31 (s, 1H), 7.18 (d, 1H, J =7.2 Hz),8.27(s, 1H), 10.37 (s, 1H), 12.28 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 22.6, 36.16, 111.4, 115.0, 118.4, 120.3, 122.7, 122.9, 124.1, 124.2, 128.6, 128.7, 131.8, 134.9,144.4, 156.8, 167.8, 169.1; LC-MS (ESI) m/z : 344.20 $(M+H)^+$; Anal. calcd. for $C_{17}H_{14}FN_3O_2S$ (343.08) %: C, 59.46; H, 4.11; N, 12.24; Found: C, 59.72; H, 4.05; N, 12.27.

Procedure for synthesis *N*-(2-acetamido-1,3-benzothiazol-6-yl)cyclopentanecarboxamide (3f)

Prepared as reported above for 3a starting from compound 2 and cyclopentane carboxylic acid This reaction was carried out at room temperature for 7 hr. White solid; Yield 27%;m.p.281-282 °C; IR(KBr) ν_{max}in cm^{-1}: 3361 (NH), 3082, 2951, 2867 (CH), 1688 (C=O), 1610 (C=N), 1523 (NH bend), 1320 (C-N), 1464, 734 (CH bend); ^1H NMR (DMSO-d_6) δ ppm: 1.56-1.86 (m, 8H), 2.18 (s, 1H), 2.76-2.81 (m, 1H), 7.51 (d, 1H, J =8.4 Hz), 7.63(d, 1H, J =8.7 Hz),8.30 (s, 1H), 10.0 (s, 1H), 12.2 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 22.3, 25.2, 29.7, 44.8, 110.9, 118.1, 119.9, 131.4, 135.0, 143.8, 156.33, 168.7, 173.8; LC-MS (ESI) m/z : 304.27 $(M+H)^+$; Anal. calcd. for $C_{15}H_{17}N_3O_2S$ (303.01) %: C, 59.38; H, 5.65; N, 13.85; Found: C, 59.36; H, 5.23; N, 13.82.

Procedure for synthesis *N*-(2-acetamido-1,3-benzothiazol-6-yl)-3,5-difluorobenzamide (3g)

Prepared as reported above for 3a starting from compound 2 and 3,5-difluorobenzoic acid, This reaction was carried out at room temperature for 6hr. Pale yellow solid; Yield 20%; m.p.282-283 °C; IR (KBr) ν_{max} in cm^{-1}: 3268 (NH), 3080 (CH), 1655 (C=O), 1592 (C=N), 1279 (C-N), 1123 (C-F),722 (CH bend); ^1H NMR (DMSO-d_6) δ ppm: 2.20 (s, 3H), 7.51-7.58 (m, 1H), 7.68 (s, 2H), 7.72 (d, 2H), 8.41(s, 1H), 10.51 (s, 1H), 12.33 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 22.6, 110.8, 111.1, 133.0, 119.7, 120.2, 131.6, 134.3, 138.2, 145.1, 157.3, 160.4, 160.5, 162.6, 162.8, 169.2; LC-MS (ESI) m/z : 348.20 $(M+H)^+$; Anal. calcd. for $C_{16}H_{11}F_2N_3O_2S$ (347.34) %: C, 55.33; H, 3.19; N, 12.10; Found: C, 55.32; H, 3.27; N, 12.54.

Procedure for synthesis *N*-(2-acetamido-1,3-benzothiazol-6-yl)-2-furamide (3d)

To the solution compound 2 (0.4g,0019 mol) in 12 mL THF,Triethylamine(0.8mL,0.0057mol) was added at 0°C. To the reaction mixture 2-furoylchloride was added. The reaction mixture was allowed to RT and stirred for 4hr. The reaction was monitored by TLC. The reaction solution was concentrated under reduced pressure and partitioning between ethylacetate (50mL) and water (40mL). The combined ethylacetate layer was washed with 2N HCl (1X30), 10%$NaHCO_3$ ((1X40), Brine (1x250mL) and dried over anhydrous Na_2SO_4. The resulting ethylacetate layer was washed concentrated under reduced pressure to afford compound 3d. Pale yellow solid; Yield 28%; m.p.287-289 °C; IR (KBr) ν_{max} in cm^{-1}: 3347 (NH), 3207, 3087 (CH), 1644 (C=O), 1603 (C=N), 1530 (NH bend), 1275 (C-N), 1163 (C-O), 734 (CH); ^1H NMR (DMSO-d_6) δ ppm: 2.19 (s, 3H), 6.71 (dd, 1H J =1.5, 3.3 Hz), 7.35 (1H, d, J =3.3Hz), 7.69 (d,1H, J=8.7 Hz), 7.73 (d, 1H, J =8.7Hz), 7.95 (s,1H, J=1.5 Hz), 10.34 (s, 1H),12.31 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 22.6, 112.0, 112.8, 114.6, 119.7, 120.2, 131.6, 134.2, 144.8, 145.6, 147.4, 156.0, 157.0, 169.1; LC-MS (ESI) m/z : 302.20 $(M+H)^+$; Anal. calcd. for $C_{14}H_{11}N_3O_3S$ (301.32) %: C, 55.80; H, 3.68; N, 13.95; Found: C, 55.68; H, 3.45; N, 13.27.

Procedure for synthesis (2*E*)-*N*-(2-acetamido-1,3-benzothiazol-6-yl)-3-(2-furyl)acrylamide (3e)

Prepared as reported above for 3g starting from compound 2 and (2E)-3-(furan-2-yl)acryloyl chloride . This reaction was carried out at room temperature for 3hr. yellow solid;yield 35%; m.p.257-258 °C; IR (KBr) ν_{max} in cm^{-1}: 3351 (NH), 1669 (C=O), 1605 (C=N), 1469 (CH bend), 1273 (C-N), 1173 (C-O), 748(CH); ^1H NMR (DMSO-d_6) δ ppm: 2.19 (s, 3H), 6.62 (d, 1H, J =3.6 Hz), 6.65 (1H, d, J =15.5 Hz), 6.86 (d, 1H J =3.3 Hz), 7.40 (d, 1H, J =15.3 Hz), 7.56 (dd, 1H, J =1.5, 8.7 Hz), 7.68 (d,1H, J =8.7 Hz), 7.83 (s, 1H), 8.42 (d, 1H, J =1.5 Hz), 10.37 (s, 1H), 12.29 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 22.7, 111.4, 112.5, 114.5, 118.4, 119.3, 120.5, 127.2, 131.9, 135.2, 144.5, 145.5, 145.1, 150.9, 156.9, 163.2, 169.2;

LC-MS (ESI) m/z : 328.27 $(M+H)^+$; Anal. calcd. for $C_{16}H_{13}N_3O_3S$ (327.36) %: C, 58.70; H, 4.00; N, 12.84; Found: C, 58.88; H, 3.97; N, 12.90.

Procedure for synthesis *N*-(6-{[(4-*tert*-butylphenyl) sulfonyl]amino}-1,3-benzothiazol-2-yl)acetamide(4a)

To the solution compound 2 (0.4g,0019 mol) in 10 mL 1,2-dichloroethane,pyridine (0.46mL, 0.0057mol) and 4-tert-butylbenzene-1-sulfonylchloride (0.45g,0.0019 mol) was added. The reaction mixture was heated to 90°C for 7 hr. reaction was monitored by TLC. The reaction mixture was cooled to RT ,diluted with 30 mL of ethyl acetate. The combined ethylacetate layer was washed with 2N HCl(1X30),10%NaHCO3(1X40,Brine(1x50mL) and dried over anhydrous Na_2SO_4.the resulting ethylacetate layer was concentrated under reduced pressure to afford compound 4a.white solid;yield 39%; m.p.265-266 °C; IR (KBr) ν_{max} in cm^{-1}: 3310 (NH), 2961(CH), 1690 (C=O), 1606 (C=N), 1546 (NH bend), 1275 (C-N), 1328, 1158 (S=O), 734 (CH bend); ^1H NMR (DMSO-d_6) δ ppm: 1.23 (s, 9H), 2.17 (s, 3H), 7.16 (dd, 1H, J =2.1, 8.7 Hz), 7.57 (d, 1H, J =8.7 Hz), 7.54 (d, 2H, J =8.4 Hz), 7.69 (d, 2H J =8.4 Hz), 7.66 (s, 1H), 10.32 (s, 1H), 12.29 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 20.6, 30.6, 34.7, 113.0, 119.6, 120.7, 125.9, 126.4, 132.1, 133.4, 136.6, 145.2, 155.7, 157.3, 169.2; LC-MS (ESI) m/z : 404.20 $(M+H)^+$; Anal. calcd. for $C_{19}H_{21}N_3O_3S_2$ (403.52) %: C, 56.55; H, 5.25; N, 10.41; Found: C, 56.63; H, 5.79; N, 10.32.

Procedure for synthesis *N*-(6-{[(4-cyclohexylphenyl)sulfonyl]amino}-1,3-benzothiazol-2-yl)acetamide (4b)

Prepared as reported above for 4a starting from compound 2 and 4-cyclohexylbenzene-1-sulfonylchloride. This reaction was carried out at 90°C for 10hr. White solid; Yield 39%; m.p.267-268°C; IR (KBr) ν_{max} in cm^{-1}: 3312 (NH), 2924, 2852 (CH), 1689 (C=O), 1605 (C=N), 1549 (NH bend), 1470 (CH bend), 1275 (C-N), 1327, 1154 (S=O); ^1H NMR (DMSO-d_6) δ ppm: 1.29-1.32 (m, 5H), 1.64-1.73 (m, 5H), 2.16 (s, 3H), 2.51 (m, 1H), 7.13 (dd, 1H, J =2.1, 8.7 Hz), 7.36 (d, 2H J =8.4 Hz), 7.57 (d, 1H, J =8.7 Hz), 7.64 (s, 1H), 7.66 (d, 2H J =8.4 Hz), 10.28 (s,1H), 12.28 (s,1H); ^{13}C NMR (DMSO-d_6) δ ppm: 22.6, 25.3, 26.0, 33.3, 43.4, 113.2, 119.8, 120.7, 126.7, 127.4, 132.1, 133.4, 136.9, 145.3, 152.6, 157.4, 169.3; LC-MS (ESI) m/z : 430.27 $(M+H)^+$; Anal. calcd. for $C_{21}H_{23}N_3O_3S_2$ (429.56) %: C, 58.72; H, 5.40; N, 9.78; Found: C, 58.45; H, 5.92; N, 9.68.

Procedure for synthesis *N*-(6-{[(2,5-dichloro-3-thienyl)sulfonyl]amino}-1,3-benzothiazol-2-yl) acetamide (4c)

Prepared as reported above for 4a starting from compound 2 and 2,5-Dichlorothiophene-3-sulfonyl chloride. This reaction was carried out at 90°C for 8 hr. White solid; Yield 30%; m.p.262-263 °C; IR (KBr) ν_{max} in cm^{-1}: 3410 (NH), 3062, 2973(CH), 1675 (C=O), 1561 (NH bend), 1276(C-N), 1345, 1163 (S=O), 1044(C-Cl), 725(CH bend); ^1H NMR (DMSO-d_6) δ ppm: 2.18 (s, 3H), 7.16 (dd, 1H, J =2.1,8.7 Hz), 7.31 (s, 1H), 7.64 (d, 1H, J =8.7 Hz), 7.72 (d, 1H, J =2.1 Hz), 10.70 (s, 1H), 12.35 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 22.6, 114.4, 120.5, 120.8, 126.3, 126.7, 129.5, 131.9, 132.1, 135.4, 145.9, 157.8, 169.3; LC-MS (ESI) m/z : 423.87 $(M+H)^+$; Anal. calcd. for $C_{13}H_9Cl_2N_3O_3S_3$ (422.33) %: C, 36.97; H, 2.15; N, 9.95; Found: C, 36.92; H, 2.23; N, 9.92.

Procedure for synthesis *N*-(6-{[(4-fluorophenyl) carbamoyl]amino}-1,3-benzothiazol-2-yl)acetamide (5a)

To the solution compound 2 (0.3g,0015 mol) in 6 mL THF, 4-fluorophenylisocyanate (0.24g,0.0017mol) in 3 mL dichloromethane was added at 0°C. The reaction mixture was allowed to RT and stirred for 1 hr.The reaction mixture was concentrated under reduced pressure and partitioning between ethyl acetate (50mL) and water (40mL). The ethylacetate layer was washed with Brine(1x250mL) and dried over anhydrous Na_2SO_4. The resulting ethylacetate layer was washed concentrated under reduced pressure. The residue formed was washed with 20mL of diethyl ether and dried under vacuum to afford 5a. Light brown solid; Yield 25%; m.p.262-263 °C; IR (KBr) ν_{max} in cm^{-1}: 3278 (NH), 3068, 2997 (CH), 1635 (C=O), 1557 (NH bend), 1216 (C-F), 1270 (C-N), 719 (CH bend); ^1H NMR (DMSO-d_6) δ ppm: 2.21(s, 3H), 7.14 (t, 2H, J=9Hz), 7.44 (dd, 1H, J =3,9Hz), 7.49 (1H, d , J =9 Hz), 7.52 (1H, d, J =9Hz), 8.15 (d, 1H, J =3Hz), 8.77 (s, 1H), 8.83 (s, 1H), 12.27 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 23.1, 110.9, 115.7, 118.4, 120.4, 120.9, 132.6, 136.9, 136.50, 144.18, 153.2, 156.5, 159.3, 169.6; LC-MS (ESI) m/z : 345.27 $(M+H)^+$; Anal. calcd. for $C_{16}H_{13}FN_4O_2S$ (344.36) %: C, 55.80;

H, 3.81; N, 16.27; Found: C, 55.72; H, 3.77; N, 16.54.

Procedure for synthesis *N*-(6-{[(4-fluorophenyl) carbamothioyl]amino}-1,3-benzothiazol-2-yl)acetamide (5b)

Prepared as reported above for 5a starting from compound 2 and 4-fluorophenylisothiocyanate. This reaction was carried out at RT for 1 hr. White solid; Yield 36%; m.p.231-232 ^{o}C; IR (KBr) ν_{max} in cm^{-1}: 3330 (NH), 2931 (CH), 1688 (C=O), 1600 (C=N), 1552 (NH bend), 1265 (C-N), 1220 (C-F), 723(CH bend); ^{1}H NMR (DMSO-d_6) δ ppm: 2.21 (s, 3H), 7.17 (t, 2H, J =9Hz), 7.43 (d,1H J =9Hz), 7.48 (d, 2H, J =9Hz), 7.69 (d, 1H, J =9Hz), 8.03 (s, 1H), 9.75 (s, 1H), 9.90 (s,1H); ^{13}C NMR (DMSO-d_6) δ ppm: 27.9, 120.3, 122.5, 125.3, 128.9, 131.6, 136.7, 140.1, 141.0, 151.1, 162.8, 162.9, 174.5, 185.5; LC-MS (ESI) m/z : 361.27 $(M+H)^{+}$; Anal. calcd. for $C_{16}H_{13}FN_4OS_2$ (360.43) %: C, 53.32; H, 3.64; N, 15.54; Found: C, 53.38; H, 3.48; N, 15.77.

Pharmacology

Cell lines

The human breast adenocarcinoma (MCF 7), human cervical adenocarcinoma (HeLa) and human osteosarcoma (MG63) cell lines were obtained from National Centre for Cell Science (NCCS), Pune and grown in Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

In vitro cytotoxic activities (MTT assay)

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspension and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity. After 24 hr, the cells were treated with serial concentrations of the test samples. They were initially dissolved DMSO, and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum-free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^{0}C, 5% CO_2, 95% air, and 100% relative humidity. The medium containing without samples were served as control and triplicate were maintained for all the concentrations. After 48 h of incubation, 15 µl of MTT (5mg/ml) solution was added to each well and incubated at 37^{0}C for 4 h. The medium with MTT was then flicked off, and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570 nm using a micro plate reader. The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

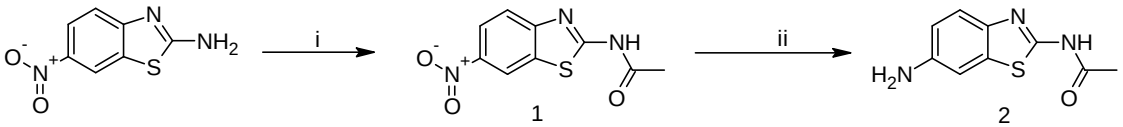
RESULTS AND DISCUSSION

Chemistry

The route for the synthesis of the intermediates and target compounds are shown in the scheme (1-3). The Intermediate 1 was prepared by using the reagent acetic anhydride and pyridine. The FT-IR value at 1701 cm^{-1}(for C=O), ^{1}H NMR value δ 2.23 (3H, singlet) and ^{13}C NMR δ 170.1(C=O) which confirms that there is the formation of the acetylated product. The intermediate 2 was synthesized by using the reagent stannous chloride and Con.HCl. The FT-IR value at 3417,3298(for NH2) and ^{1}H NMR δ 5.13 (2H, broad peak) which confirm that there is the formation of reduction product. The resulting primary amine also confirmed by ninhydrin activity in TLC. The compound 2 was taken as a common scaffold for the synthesis of the new series of 2, 6-disubstituted benzothiazole derivatives.

First the amide derivatives 3a,3b,3c,3f,3g was prepared by using the reagent 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide(EDCI). The corresponding acid chloride was used for making of amide derivatives for 3d,3e. For all the amide derivatives, the two amide NH observed at δ 10-10.5 and δ 12-12.5 as a broad singlet in ^{1}H NMR.

The sulfonamide derivatives 4a, 4b, 4c was prepared by using corresponding sulfonyl chloride and compound 2. The reaction was carried out in the presence of pyridine base at 90°C. The FT-IR stretching frequency 1320-1350 cm^{-1} and 1150-170 cm^{-1} (S=O) confirm that there is the formation of sulfonamide derivatives. The sulfonamide NH chemical shift value in ^1H NMR observed at δ10-10.5 as a broad singlet. The corresponding isocyanate and isothiocyanate respectively was used to prepare urea and thiourea derivative 5a and 5b.

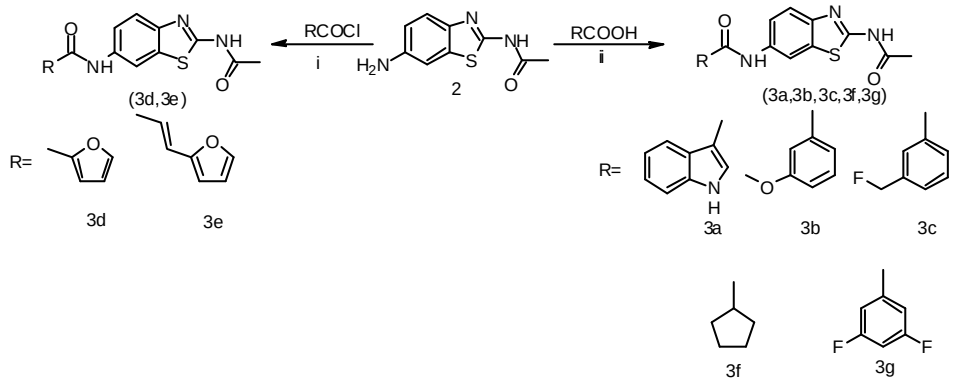


Scheme 1: Reagents and conditions: (i). $(ACO)_2O$, Pyridine, 4h,90°C; (ii). $SnCl_2$.$2H_2O$, HCl,2h, RT

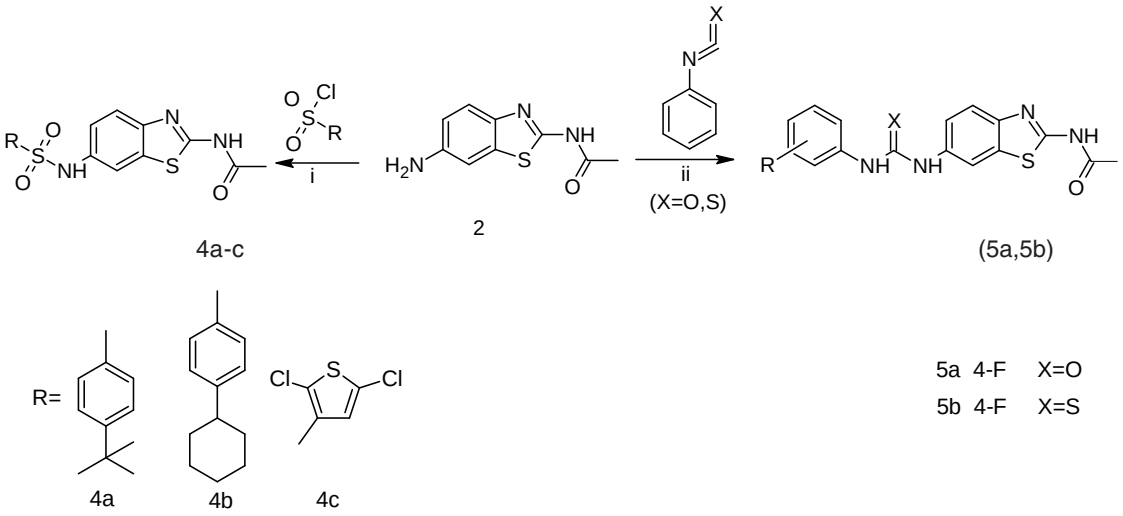
Biology

Cytotoxicity

In vitro cytotoxicity of the synthesized compounds were assessed by standard 3-(4, 5-imethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in MCF-7, HeLa, and MG63 cell lines. Among the synthesized compounds 3a, 3f, 3g, and 4b showed cytotoxic effects on all the three cell lines



Scheme 2: Reagents and conditions: (i).TEA,THF,O°C; (ii). THF,EDCI,HOBt,TEA



Scheme 3: Reagents and conditions: (i).Pyridine,90°C;(ii).MDC,0°C.

after 48 h exposure. Maximum cytotoxicity was obtained for the sulfonamide derivative 4b in all the three cell lines when compared to others, and the IC_{50} was 36, 44 and 34 µM against MCF7, HeLa and MG63 respectively. The amide derivative 3a also showed cytotoxicity in all the three cell lines and the IC_{50} was in the range of 48 to 53 µM. The remaining urea and thiourea derivatives did not show any cytotoxicity activity at the tested concentrations. For standard cisplatin was used. All the results are showed in **Table 1.**

Table 1: Preliminary cytotoxicity screening of synthesized benzothiazole derivatives against human cancer cell lines at 48h exposure

Compound	IC50 (µM)		
code	MCF-7	HeLa	MG63
3a	53.3	48.4	50.86
3b	>100	>100	>100
3c	>100	>100	>100
3d	>100	>100	>100
3e	>100	>100	>100
3f	74.5	77.4	74.83
3g	66.5	99.01	119.2
4a	100	>100	>100
4b	34.5	44.15	36.1
4c	>100	>100	>100
5a,5b	>100	>100	>100
Cisplatin	3.5	3.5	3.5

The known numbers of cells ($1x10^5$ cells/ml) were incubated for 24 h in a 5% CO2 Incubator at 37°C in the presence of different concentrations of test compounds. The medium with MTT was then flicked off, and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570 nm using a micro plate reader.

CONCLUSIONS

A series of new 2,6-disubstitued benzothiazol derivatives were synthesized, and their anti-cancer activities were evaluated *in vitro*. The results showed that the cyclohexyl benzene sulphonamide derivative of benzothiazole(4b) showed good activity against MCF-7, HeLa, and MG63 cells. Further amide derivatives 3a,3g,3h also show good activity against MCF7, HeLa, and MG63. These data suggested that the compound **4b** may be powerful anticancer agent and be worth being further investigated as a potential of an anticancer agent.

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REFERENCES

1. Reddy, P.; Lin, Y.; Chang, H. Synthesis of novel benzothiazole compounds with an extended conjugated system. *Arcivoc.* **2007**; *xvi*: 113-122.
2. Piscitelli, F.; Ballatore, C.; Smith, A. Solid Phase synthesis of 2- aminobenzothiazoles. *Bioorg.Med Chem Lett.***2010**; *(20)*:644-648.
3. Shi, D.; Bradshaw, T. D.; Wrigley, S.; McCall, C. J.; Lelieveld, P.; Fichtner, I.; Stevens, M.F G. *J. Med. Chem.***1996**, *37*, 3375.
4. Bradshaw, T. D.; Shi, D. F.; Schultz, R. J.; Paull, K. D.; Kelland. L.; Wilson, A.; Garner, C.; Fiebig, H. H.; Wrigley, S.; Stevens, M. F. G. Br. *J. Cancer* **1998**, *77*, 421.
5. Kashiyama, E.; Hutchinson, I.; Chua, M.S.; Stinson, S. F.; Phillips, L. R.; Kuar, G.;Sausville, E. A.; Bradshaw, T. D.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem*. **1999**,*42*, 4172.
6. Quiroga, J.; Hernandez, P.; Insuasty, B.; Abon1a, R.; Cobo, J.; Sanchez, A.; Nogueras, M.; Low, J.N. Control of the reaction between 2-aminobenzothiazoles and Mannich bases.Synthesis of pyrido[2,1-*b*][1,3]benzothiazoles *versus* [1,3]benzothiazolo[2,3-*b*]quinazolines.*J. Chem. Soc.Perkin Trans*.*I* **2002**, *1*, 555–559.
7. Kok, S.H.L.; Chui, C.H.; Lam, W.S.; Chen, J.; Lau, F.Y.; Wong, R.S.M.; Cheng, G.Y.M.; Lai, P.B.S.; Leung,R.W.T.; Tang, J.C.O.; *et al*. Synthesis and structure evaluation of a Novel

cantharimide and its cytotoxicity on SK-Hep-1 hepatoma cells. *Bioorg. Med. Chem. Lett*.**2007**,*17*, 1155–1159.

8. Kok, S.H.L.; Chui, C.H.; Lam, W.S.; Chen, J.; Lau, F.Y.; Wong, R.S.M.; Cheng, G.Y.M.; Tang, W.K.; Teo, I.T.N.; Cheung, F.; *et al*. Apoptogenic activity of a synthetic cantharimide in leukaemia: Implication on its structural activity relationship. *Int. J. Mol. Med*. **2006**, *18*, 1217–1221.
9. Xuan-Hong, Shi.; Zhao, Wang.; Yong Xia, Ting-Hong, Ye.; Mei, Deng.; You-Zhi, Xu.; Yu-Quan, Wei and Luo- Ting Yu .Synthesis and Biological Evaluation of Novel Benzothiazole-2-thiol Derivatives as Potential Anticancer Agents *Molecules* **2012**, *17*,3933-3944.
10. Sohail Saeed, a.; Naghmana Rashid, a.; Peter, G.; Jones, b.; Muhammad Ali, c.;, Rizwan Hussain Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole Moiety as potential antimicrobial and anticancer agents, *European Journal of Medicinal Chemistry 45* **(2010)**1323–31.
11. Dao Thi Kim Oanh, a.; Hoang Van Hai, a.; Sang Ho Park, b.; Hyun-Jung Kim, c.; Byung-Woo Han, b.;Hyung- Sook Kim, d.; Jin-Tae Hong, d.; Sang-Bae Han, d.; Van Thi My Hue, a.;Nguyen-Hai Nama,Benzothiazole-containing hydroxamic acids as histone deacetylase inhibitors and antitumor agents, *Bioorganic & Medicinal Chemistry Letters 21* ***2011*** *7509–7512*.
12. Mortimer, C.; Wells, G.; Crochard, J.P .; Stone, E.L.; Bradshaw, T.D.; Stevens, M.G.F.; West well, A.D. *J. Med. Chem. 49* **(2006)** 179–185.
13. Yoshida, M.; Hayakawa, I.; Hayashi, N.; Agatsuma, T.; Oda, Y.; Tanzawa, F.; Iwasaki, S.; Koyama, K.; Furukawa, H.; Kurakata, Y.; Sugano, Y. Bioorg. *Med. Chem. Lett.* **2005,** *15*, 3328–3332.
14. Vicini, P.; Gernonikaki, A.; Incerti, M.; Busonera, B.; Poni, G.; Cabras, C.A.; Colla, P.L. *Bioorg. Med. Chem.11* **(2003)** 4785–4789.
15. Pattan, S.R,; Narendra babu, S.N. Synthesis and antifungal activity of 2-amino [5'-(4-sulphonyl benzylidene)-2, 4- thiazolidene Dione] -7- (substituted)-6-fluro benzothiazoles, *Ind J of Het. Chem*., **2002**, *11*,333-334.
16. Huang, S.T.; IJen, H.; Synthesis and anticancer evaluation of bis (benzimidazoles), bis (benzoxazoles), and benzothiazoles, *Bio. and Med. Chem*., **2006**, *146*, 106-6119.
17. Kok, S.H.L.; Gambari, R.; Chui, C.H.; Yuen, M.C.W.; Lin, E.; Wong, R.S.M.; Lau, F.Y.; Cheng, G.Y.M.; Lam, W.S.; Chan, S.H.; et al. Synthesis and anti-cancer activity of benzothiazole containing phthalimide on human carcinoma cell lines. *Bioorg. Med. Chem*. **2008**, *16*, 3626–3631.