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Design, synthesis and cytotoxic activity of clinical candidate Phortress analogues of benzothiazolylpyrimidine and benzothiazolyldihydropyrimidine derivatives

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Abstract:

A series of novel Phortress analogues bearing benzothiazolyl-pyrimidine and benzothiazolyl-dihydropyrimidine analogues 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(substituted) pyrimidine-2- thiol **(4a-4e)** and 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(substituted) pyrimidin-2-ol **(3a-3e)** moieties were synthesized by reacting 1-(2-aminobenzo[*d*]thiazol-6-yl)-3-(substituted) prop- 2-en-1-one **(2a-2e)** with thiourea and urea, respectively. A total of 15 compounds were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectral techniques. In addition they were evaluated for *in vitro* cytotoxic activities. Among the screened benzothiazolyl-pyrimidine derivatives, a few compounds showed noticeable cytotoxic activity.

Keywords: Benzothiazoles, pyrimidines, Phortress analogues, cytotoxic activity.

1. INTRODUCTION

Theoretically, anti-tumor agents could be developed to target each step in the tumor activation or inhibition process and numerous new antitumor agents have been developed based on their inhibitory effects on tumor activation (1). The fluorinated benzothiazole analogue 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203, NSC 703786) exhibits selective and potent anticancer activity (2), and its lysylamide prodrug (**Phortress, NSC 710305**) recently entered clinical trials in the United Kingdom.

Toxicity and the development of resistance are major obstacles for successful therapy in the management of tumour. Multiple organ systems can be affected with both acute and chronic side effects. Cytoprotection or protection of normal cells is a strategy now being investigated in preclinical and clinical models (3). The ideal protective agent would possess selectivity (only healthy tissue protection), broad spectrum activity, and a favourable side effect profile (well tolerated)(4). The agents would prevent all toxic side effects, from nonlife threatening to potentially fatal, without diminishing the anti-tumor efficacy of the cancer therapy (5). Considering the fact that benzothiazole moiety is strongly implicated in the toxicity of chemotherapy, much effort has been focused on the research of diverse benzothiazole derivatives as potential chemotherapeutic agents (6).

Nitrogen containing heterocycles are present in a variety of biologically active compounds that can be used in a wide range of therapeutic areas. Benzothiazole contains a benzene ring fused to thiazole ring (7), these small molecules display anti-tumor properties that are modulated by substitutions at specific positions on the benzothiazole pharmacophore (8–11), and these interesting biological effects led us to synthesize novel benzothiazole containing a pyrimidine moiety which could adjoin important pharmacological activities. Indeed, pyrimidine derivatives occur widely in the living organisms. The barbiturates, valuable hypnotic drugs, a number of useful antibacterial, and antimalarial drugs also contain pyrimidine nucleus. Vitamins B1 and B2 also contain pyrimidine moiety (12). Pyrimidines are the class of drugs, known to act through the inhibition of the dihydrofolate reductase (DHFR) enzyme (13). DHFR is essential for the folate metabolism in the organism or any cell. The inhibition of this enzyme has shown to be one of the most promising ways to control the bacterial and parasitic diseases. They are also shown to possess anticancer activity, dihydrofolate reductase is the enzyme, which is essential part of any living system, and its inhibition is found to affect the growth of the cell. Some of the derivatives of pyrimidines like Trimethoprim, Methotrexate, and Pyrimethamine have shown to exhibit antimicrobial, anticancer, and antimalarial activities, respectively.

2. MATERIALS AND METHODS

2.1 Chemistry

All solvents and chemicals were of reagent grade and were used without purification. The reactions were monitored with the help of thin layer chromatography using pre-coated aluminum sheets with GF254 silica gel; 0.2mm layer thickness (E.Merck). Melting points were determined using Veego (VMP-PM) melting point apparatus and were not corrected. IR spectra were recorded on a Shimadzu (FTIR-8400S) instrument. ^{1}H and ^{13}C NMR were recorded on Bruker avance II 400 NMR spectrometer and chemical shifts (δ) were reported in parts per million (ppm) downfield from tetramethylsilane (TMS) which was used as an internal standard. The mass spectra were recorded on ESI Q-TOF Water.

The starting material, 1-(2-aminobenzo[*d*]thiazol-6-yl)ethanone (**1a**), was synthesized from 4-

aminoacetophenone by reacting with potassium thiocyanate followed by oxidative cyclisation, the synthesized acetyl derivative of amino benzothiazole was treated with substituted aldehydes at alkaline condition to obtain the styryl carbonyl pharmacophore. These compounds were cyclised by using urea/thiourea in the presence of sodium hydroxide to get dihydropyrimidines, which subsequently undergo oxidation to arrive at pyrimidine derivatives of 2-amino benzothiazoles. Oxidative cyclisation of 4-aminoacetophenone takes place in competition with the possible bromination of acetyl group in the present reaction. Importantly, formation of a styryl carbonyl pharmacophore gave useful information on the way the reaction proceeds. i.e. treatment of aldehydes to 1-(2-aminobenzo[*d*]thiazol-6- yl)ethanone can result in to the formation of both arylidine as well as chalcone derivatives of benzothiazoles. In order to avoid this, acetylation of primary amine was done (protection) but in the alkaline condition free amino group was regenerated owing to hydrolysis of acetylated amino group. Details of this reaction are depicted in the Figure 1.

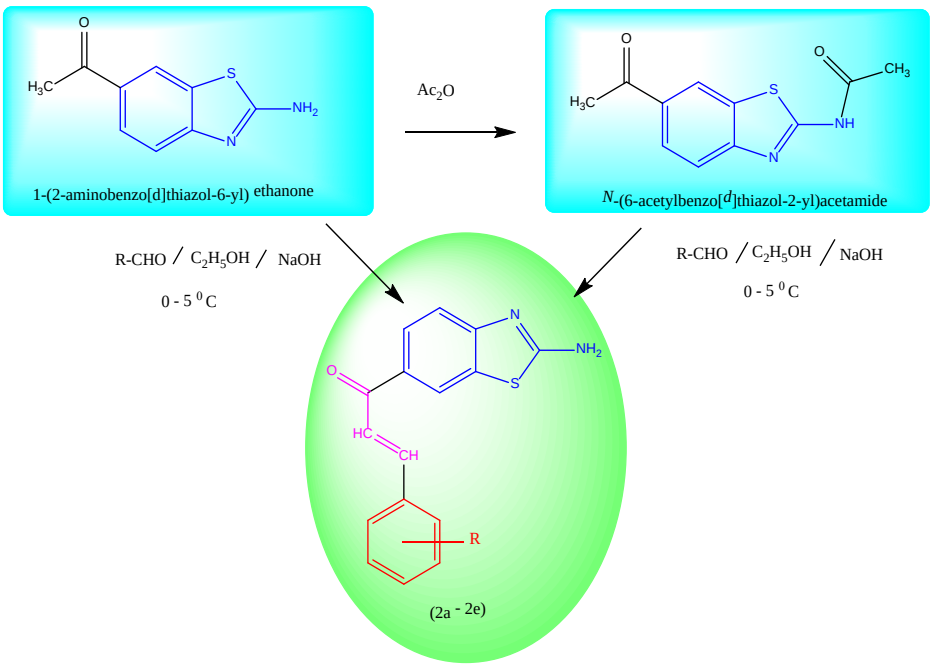
2.1.1. Synthesis of 1-(2-aminobenzo[*d*]thiazol-6-yl) ethanone (1) (14)

p-Aminoacetophenone (0.20 M), potassium thiocynate (0.55 M), and 500 mL of acetic acid were combined in a flask with continuous mechanical stirring. Bromine (0.20 M) in 500 mL of acetic acid was added drop wise and the temperature of the reaction mixture was maintained below 15°C. Stirring was continued for additional 5 h. there after the residue settled was filtered, the residual mass was boiled with water and combined with initial filtrate. The combined filtrate was neutralized with ammonia, the precipitated solid obtained on neutralization was collected and the product so obtained was purified by recrystallisation with ethyl acetate. Yield: 48.76%, mp 234°C. IR (KBr, cm^{-1}): 3356.75 (N-H), 3301.25 (C=H), 1648.60 (C=O), 1560.41 (C=N). ^{1}H NMR δ (ppm): 2.55 (s, 3H, CH_3), 7.46 (s, 2H, -NH_2), 7.82-8.87 (m, 3H, Ar-H) ^{13}C NMR (DMSO-d_6 δ ppm): 26.32, 123.21, 123.29, 127.54, 129.31, 138.97, 151.54, 165.52, 187.32. ESI-MS (m/z) 192.9, Calcd: 192.24, Anal. Found (Calcd) for $C_9H_8N_2OS$: C 56.21 (56.23), H 4.20 (4.19), N 14.56 (14.57).

2.1.2. General procedure for the synthesis of 1-(2-aminobenzo[*d*]thiazol-6-yl)-3-(substituted phenyl) prop-2-en-1-one (2a-2e): 2-amino-6-acetyl benzothiazole (0.01 M) and substituted benzaldehyde (0.01 M) in absolute alcohol (20 mL) were stirred for 16 h at ice cold condition, in 4% aqueous sodium hydroxide solution (8 mL). The solution was then diluted with water, the solid separated was filtered and recrystalised from ethanol.

2.1.2.1 Synthesis of 1-(2-aminobenzo[*d*]thiazol-6-yl)-3-(4-nitrophenyl) prop-2-en-1-one (2a): This compound was prepared and purified as per the above mentioned procedure: Yield: 58.26%, mp 226-228°C. IR (KBr, cm^{-1}): 3215.24 (NH_2), 3062.15 (CH), 1684.83 (C=O), 1594.70 (C=N). ^{1}H NMR δ (ppm): 7.09 (s, 2H, NH_2), 7.17 (d, 1H, CH), 7.53 (d, 1H, CH), 7.94-8.87 (m, 7H, Ar-H) ^{13}C NMR (DMSO-d_6 δ ppm): 121.72, 123.24, 123.59, 126.01, 129.31, 134.27, 147.96, 149.42, 150.71, 154.22, 166.32, 185.41. ESI-MS (m/z) 325.19, Calcd: 325.34. Anal. Found (Calcd) for $C_{16}H_{11}N_3O_3S$: C 59.21 (59.07), H 3.35 (3.41), N 12.96 (12.92).

Figure 1 Formation of reaction mixture of arylidine as well as chalcone derivatives of benzothiazoles and their subsequent alkaline hydrolysis of acetyl group.



2.1.2.2 Synthesis of 1-(2-aminobenzo[*d*]thiazol-6-yl)-3-(3-nitrophenyl) prop-2-en-1-one

(2b): This compound was prepared and purified as per the above mentioned procedure: Yield: 65.24%, mp 202-204°C. IR (KBr, cm^{-1}): 3265.47 (NH_2), 3081.35 (CH), 1653.88 (C=O), 1586.55 (C=N). ^{1}H NMR δ (ppm): 7.19 (s, 2H, NH_2), 7.25 (d, 1H, CH), 7.68 (d, 1H, CH), 7.88-8.96 (m, 7H.Ar-H). ^{13}C NMR (DMSO-d_6 δ ppm): 121.22, 122.70, 123.47, 127.55, 130.00, 135.24, 147.41, 148.85, 150.11, 155.67, 167.02, 184.13. ESI-MS (m/z) 325.08, Calcd: 325.34. Anal. Found (Calcd) for $C_{16}H_{11}N_3O_3S$: C 59.11 (59.07), H 3.39 (3.41), N 12.01 (12.92).

2.1.2.3 Synthesis of 1-(2-aminobenzo[*d*]thiazol-6-yl)-3-(4-chlorophenyl) prop-2-en-1-one

(2c): This compound was prepared and purified as per the above mentioned procedure: Yield:
60.12%, mp 232°C. IR (KBr, cm^{-1}): 3324.05 (NH_2), 3007.24 (CH), 1655.10 (C=O). 1596.14 (C=N). ^{1}H NMR δ (ppm): 7.14 (s, 2H, NH_2), 7.24 (d, 1H, CH), 7.96 (d, 1H, CH), 7.82-8.76 (m, 7H, Ar-H) ESI-MS (m/z) 314.62, Calcd: 314.79. Anal. Found (Calcd) for $C_{16}H_{11}ClN_2OS$: C 61.14 (61.05), H 3.54 (3.52), N 8.85 (8.90).

2.1.2.4 Synthesis of 1-(2-aminobenzo[*d*]thiazol-6-yl)-3-(2-nitrophenyl) prop-2-en-1-one

(2d): This compound was prepared and purified as per the above mentioned procedure: Yield: 75.26%, mp 185-186°C. IR (KBr, cm^{-1}): 3216.88 (NH_2), 3023.15 (CH), 1676.81(C=O), 1581.52 (C=N). ^{1}H NMR δ (ppm): 7.11 (s, 2H, NH_2), 7.35 (d, 1H, CH), 8.22 (d, 1H, CH), 7.81-8.59 (m, 7H, Ar-H). ^{13}C NMR (DMSO-d_6 δ ppm): 119.41, 123.65, 124.21, 125.96, 129.10, 134.69, 137.66, 142.80, 147.73, 156.97, 165.44, 179.10. ESI-MS (m/z) 325.08, Calcd: 325.34. Anal. Found (Calcd) for $C_{16}H_{11}N_3O_3S$: C 59.14 (59.07), H 3.40 (3.41), N 12.55 (12.92).

2.1.2.5 Synthesis of 1-(2-aminobenzo[*d*]thiazol-6-yl)-3-(2, 4-dichlorophenyl) prop-2-en-1-one (2e): This compound was prepared and purified as per the above mentioned procedure: Yield: 76.28%, mp 285°C. IR (KBr, cm^{-1}): 3304.74 (NH_2), 2998.44 (CH), 1730.21 (C=O), 1582.32 (C=N). ^{1}H NMR δ (ppm): 6.98 (s, 2H, NH_2), 7.25 (d, 1H, CH), 7.52 (d, 1H, CH), 7.88-8.96 (m, 6H, Ar-H) ESI-MS (m/z) 349.09, Calcd: 349.23. Anal. Found (Calcd) for $C_{16}H_{10}Cl_2N_2OS$: C 55.14 (55.03), H 2.82 (2.89), N 8.05 (8.02).

2.1.3. General procedure for the synthesis of 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(substituted phenyl) pyrimidin-2-ol (3a-3e): A mixture of 1-(2-aminobenzo[*d*]thiazol-6-yl)-3-(substituted phenyl)prop-2-en-1-one (**2a-2e**) (0.01 M) and urea (0.01 M) in ethanol (50 mL) was refluxed with aqueous solution of sodium hydroxide (0.005 M) for 6 h. The reaction mixture was poured in to 250 mL of water, the product obtained was filtered, and recrystalised from ethanol.

2.1.3.1. Synthesis of 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(4-nitrophenyl) pyrimidin-2-ol

(3a): This derivative was prepared and purified as per the above mentioned procedure: Yield: 42.15%, mp 223-224°C. IR (KBr, cm^{-1}): 3316.88 (NH_2), 3029.11 (CH), 1581.95 (C=N). ^{1}HNMR δ (ppm): 6.91 (s, 2H, NH_2), 7.95-8.25 (m, 8H, Ar-H), 9.29 (s, 1H, OH). ^{13}C NMR ($CDCl_3$ δ ppm): 85.75, 115.28, 121.01, 122.05, 124.21, 125.96, 129.10, 134.61, 137.77, 148.26, 153.71, 159.69, 166.04. ESI-MS (m/z) 365.19, Calcd: 365.37. Anal. Found (Calcd) for $C_{17}H_{11}N_5O_3S$: C 55.81 (55.88), H 3.09 (3.03), N 19.85 (19.26).

2.1.3.2. Synthesis of 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(3-nitrophenyl) pyrimidin-2-ol

(3b): This derivative was prepared and purified as per the above mentioned procedure: Yield: 79.21%, mp 196°C. IR (KBr, cm^{-1}): 3266.72 (NH_2), 3068.49 (CH), 1588.95 (C=N). ^{1}H NMR δ (ppm): 7.05 (s, 2H, NH_2), 7.25-8.65 (m, 8H, Ar-H), 9.34 (s, 1H, OH). ^{13}C NMR ($CDCl_3$ δ ppm): 84.63, 114.89, 119.09, 123.02, 124.11, 126.92, 128.19, 135.51, 139.75, 147.63, 158.04, 160.60, 169.55. ESI-MS (m/z) 365.02, Calcd: 365.37. Anal. Found (Calcd) for $C_{17}H_{11}N_5O_3S$: C 55.86 (55.88), H 3.10 (3.03), N 19.39 (19.26).

2.1.3.3. Synthesis of 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(4-chlorophenyl) pyrimidin-2-ol

(3c): This derivative was prepared and purified as per the above mentioned procedure: Yield: 84.00%, mp 204-206°C. IR (KBr, cm^{-1}): 3234.70 (NH_2), 2919.70 (CH), 1586.51 (C=N). ^{1}HNMR δ (ppm): 7.06 (s, 2H, NH_2), 7.01-8.14 (m, 8H, Ar-H), 9.64 (s, 1H, OH). ^{13}C NMR ($CDCl_3$ δ ppm): 79.31, 119.64, 120.19, 122.96, 123.92, 124.62, 128.16, 135.87, 141.71, 148.11, 160.58, 161.31, 165.50. ESI-MS (m/z) 354.56, Calcd: 354.81. Anal. Found (Calcd) for $C_{17}H_{11}ClN_4OS$: C 57.14 (57.55), H 3.10 (3.12), N 15.39 (15.79).

2.1.3.4. Synthesis of 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(2-nitrophenyl) pyrimidin-2-ol

(3d): This derivative was prepared and purified as per the above mentioned procedure: Yield:
62.56%, mp 189-190°C. IR (KBr, cm^{-1}): 3368.10 (NH_2), 2939.03 (CH), 1587.02 (C=N). ^{1}HNMR δ (ppm): 7.31 (s, 2H, NH_2), 7.02-8.92 (m, 8H, Ar-H), 9.92 (s, 1H, OH). ^{13}C NMR ($CDCl_3$ δ ppm): 98.13, 108.46, 110.09, 122.46, 125.09, 128.62, 129.03, 131.01, 133.55, 135.79, 146.53, 153.47 150.64, 154.45, 161.07, 168.70. ESI-MS (m/z) 365.36, Calcd: 365.37. Anal. Found (Calcd) for $C_{17}H_{11}N_5O_3S$: C 55.01 (55.88), H 3.16 (3.03), N 19.87 (19.17).

2.1.3.5. Synthesis of 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(2,4-dichlorophenyl) pyrimidin-2-ol (3e): This derivative was prepared and purified as per the above mentioned procedure:
Yield: 59.25%, mp 199-201°C. IR (KBr, cm^{-1}): 3292.72 (NH_2), 3045.26 (CH), 1585.51 (C=N), ^{1}H NMR δ (ppm): 7.86 (s, 2H, -NH_2), 7.67-8.14 (m, 7H.Ar-H), 10.02 (s.1H, OH). ^{13}CNMR ($CDCl_3$ δ ppm): 89.48, 109.78, 111.63, 122.06, 124.06, 127.11, 129.92, 133.67, 135.95, 148.17, 156.51, 160.44, 162.32. ESI-MS (m/z) 389.11, Calcd: 389.26, Anal. Found (Calcd) for $C_{17}H_{10}Cl_2N_4OS$: C 52.85 (52.45), H 2.61 (2.59) N 14.42 (14.39).

2.1.4. General procedure for the synthesis of 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(substituted phenyl) pyrimidine-2-thiol (4a-4e): A mixture of 1-(2-aminobenzo[*d*]thiazol-6-yl)-3-(substituted

phenyl)prop-2-en-1-one (2a-2e) (0.01 M) and thiourea (0.01 M) in ethanol (50 mL) was refluxed with aqueous solution of sodium hydroxide (0.005 M) for 6 h. The reaction mixture was poured in to 250 mL of water. The product obtained was filtered and recrystalised from ethanol.

2.1.4.1. Synthesis of 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(4-nitrophenyl) pyrimidine-2-thiol (4a): This derivative was prepared and purified as per the above mentioned procedure: Yield: 61.32%, mp 201°C. IR (KBr, cm^{-1}): 3326.45 (NH_2), 3062.43 (CH), 1589.54 (C=N), ^{1}HNMR δ (ppm): 2.89 (s, 1H, SH), 7.01 (s, 2H, -NH_2), 6.62-8.06 (m, 8H.Ar-H), ^{13}C NMR ($CDCl_3$ δ ppm): 103.63, 105.47, 110.99, 121.86, 122.42, 125.31, 128.36, 133.75, 139.04, 148.71, 149.53, 161.86, 163.17, 165.39, 179.23, ESI-MS (m/z) 381.06, Calcd: 381.43, Anal. Found (Calcd) for $C_{17}H_{11}N_5O_2S_2$: C 53.32 (53.53), H 3.02 (2.91) N 18.61 (18.36).

2.1.4.2. Synthesis of 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(3-nitrophenyl) pyrimidine-2-thiol (4b): This derivative was prepared and purified as per the above mentioned procedure: Yield: 50.96%, mp 212-214°C. IR (KBr, cm^{-1}): 3352.47 (NH_2), 3062.63 (CH), 1594.74 (C=N), ^{1}H NMR δ (ppm): 2.92 (s, 1H, SH), 7.23 (s, 2H, -NH_2), 6.68-8.16 (m, 8H.Ar-H), ^{13}CNMR ($CDCl_3$ δ ppm): 104.21, 107.56, 114.06, 121.33, 122.25, 125.17, 130.61, 133.52, 133.96, 148.33, 149.36, 161.03, 162.10, 166.47, 180.03, ESI-MS (m/z) 381.52, Calcd: 381.43, Anal. Found (Calcd) for $C_{17}H_{11}N_5O_2S_2$: C 53.01 (53.53), H 2.96 (2.91) N 18.11 (18.36).

2.1.4.3. Synthesis of 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(4-chlorophenyl) pyrimidine-2-thiol (4c): This derivative was prepared and purified as per the above mentioned procedure: Yield: 59.28%, mp 154-155°C. IR (KBr, cm^{-1}): 3256.40 (NH_2), 3066.32 (CH), 1595.19 (C=N), ^{1}H NMR δ (ppm): 2.89 (s, 1H, SH), 7.84 (s, 2H, -NH_2), 7.23-8.34 (m, 8H.Ar-H), ^{13}C NMR ($CDCl_3$ δ ppm): 103.22, 105.09, 111.56, 122.71, 125.11, 128.24, 129.63, 131.06, 133.69, 135.96, 149.76, 161.88, 162.52, 165.13, 178.01, ESI-MS (m/z) 370.63, Calcd: 370.88, Anal. Found (Calcd) for $C_{17}H_{11}ClN_4S_2$: C 55.36 (55.05), H 3.05 (2.99) N 15.19 (15.11).

2.1.4.4. Synthesis of 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(2-nitrophenyl) pyrimidine-2-thiol (4d): This derivative was prepared and purified as per the above mentioned procedure: Yield: 58.48%, mp 188-189°C. IR (KBr, cm^{-1}): 3279.23 (NH_2), 3081.79 (CH), 1578.19 (C=N), ^{1}H NMR δ (ppm): 3.15 (s, 1H, SH), 7.45 (s, 2H, -NH_2), 7.27-8.45 (m, 8H.Ar-H), ^{13}C NMR ($CDCl_3$ δ ppm): 103.15, 106.24, 115.41, 121.00, 123.95, 129.03, 130.10, 132.41, 133.52, 136.16, 150.69, 160.41, 162.44, 164.12, 176.47, ESI-MS (m/z) 381.04, Calcd: 381.41. Anal. Found (Calcd) for $C_{17}H_{11}N_5S_2$: C 53.11 (53.53), H 3.01 (2.91) N 18.66 (18.36).

2.1.4.5. Synthesis of 4-(2-aminobenzo[*d*]thiazol-6-yl)-6-(2, 4-dichlorophenyl) pyrimidine-2-thiol (4e): This derivative was prepared and purified as per the previously described procedure: Yield: 68.59%, mp 244-246°C. IR (KBr, cm^{-1}): 3239.41 (NH_2), 2999.24 (CH), 1586.21 (C=N), ^{1}H NMR δ (ppm): 3.19 (s, 1H, SH), 7.81 (s, 2H, -NH_2), 7.22-8.36 (m, 7H.Ar-H), ^{13}CNMR ($CDCl_3$ δ ppm): 103.21, 105.51, 111.86, 121.62, 125.37, 127.03, 129.63, 133.06, 135.24, 150.63, 162.01, 163.58, 166.25, 176.07, ESI-MS (m/z) 404.09, Calcd: 405.32, Anal. Found (Calcd) for $C_{17}H_{10}Cl_2N_4S_2$: C 51.41 (50.37), H 2.55 (2.49) N 13.96 (13.82).

2.2 Cytotoxicity Activity

The cell viability was investigated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (15). This reaction depends on the mitochondrial dependant reduction of yellow MTT into purple formazan, where in metabolically active cells can form insoluble purple formazan crystals, which would be solubilised by the addition of dimethylsulphoxide (DMSO). The color can then be quantified by spectrophotometric measurement. All the compounds were tested for their cytotoxicity by MTT assay. The assay was performed on ELISA plate reader 96 well micro plates (16–20).

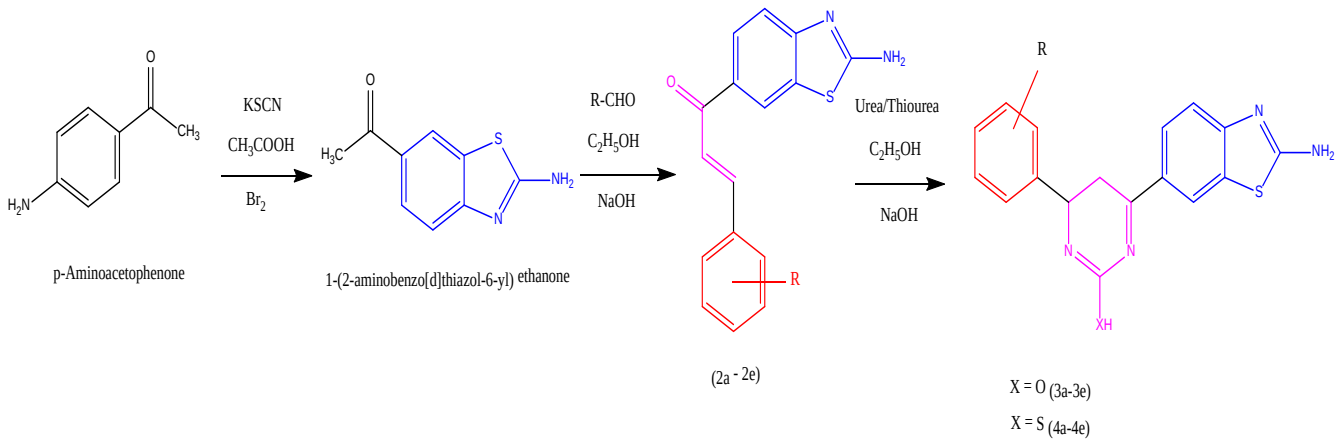
2.2.1 Procedure

Ehlrich Asiatic Carcinoma (EAC) cells were taken at a concentration of 5000 cells/ well. Concentration of the cells was fixed by examining the count of cells with the help of tryphon blue. All dilutions were done by phosphate buffered saline (PBS) of pH 7.4 and 200 µL of the suspension was introduced to the wells. MTT assay was performed on cells cultured in 96- well plates. The cells were seeded at density of 5 X 10^3cells/well. After 24 h, pre-incubation period needed for cells to attach to the bottom and achieve exponential growth, the cells were treated with different concentrations of the freshly prepared test compounds in DMSO and PBS mixture (three wells per concentration) for 12 h. Control wells contained 0.2 % DMSO as vehicle control which had no influence on the growth of the cells compared with cells cultured in normal complete medium. After 48 h, the solutions were removed from all the plates and 50 µL of MTT solution (0.5 mg/mL MTT in PBS medium) was added to each well. Plates were incubated for 4 h following which the formed purple formazan crystals were dissolved in DMSO. The complete dissolving required 4 h incubation at room temperature. The absorbance was measured with a spectrophotometric microplate reader Power WaveXS (Bio-Tek, Winooski, VT, USA) at the wavelength of 540 nm. The optical density (OD), proportional to the number of viable cells inside the well, was calculated by comparing the OD of control (21,22). For each concentration tested, wells containing all reagents except cells served as reference blank. The percentage of viable cells within the well as compared with the control wells was calculated as (OD of drug-treated sample - OD of blank / OD of control - OD of blank) X 100. Results were expressed as %viability and % inhibition and the values are given in Table 1.

Table 1 Cytotoxic activity of synthesized benzothiazole derivatives (Phortress analogues).

Comp	R	% Viability/Well			% Inhibition/Well		
		1µM	10µM	100µM	1µM	10µM	100µM
2a	4-NO_2-C_6H_5	92.02	98.11	97.25	07.99	01.88	02.75
2b	3-NO_2-C_6H_5	24.17	17.39	16.87	75.82	82.62	83.12
2c	4-Cl-C_6H_5	24.24	12.40	11.98	75.76	87.75	88.03
2d	2-NO_2-C_6H_5	94.22	98.63	97.84	05.80	01.36	02.16
2e	2,4-dichloro-C_6H_5	96.45	92.78	94.47	03.54	07.30	05.59
3a	4-NO_2-C_6H_5	93.85	97.35	95.24	06.13	02.72	05.36
3b	3-NO_2-C_6H_5	96.45	92.34	94.16	04.08	08.41	06.55
3c	4-Cl-C_6H_5	93.62	92.55	55.02	07.71	08.14	44.97
3d	2-NO_2-C_6H_5	35.13	15.60	11.28	64.48	84.39	88.72
3e	2,4-dichloro-C_6H_5	69.68	73.46	73.46	26.53	26.58	26.34
4a	4-NO_2-C_6H_5	73.65	73.66	66.44	26.53	26.53	26.33
4b	3-NO_2-C_6H_5	75.46	75.46	68.42	25.00	24.34	31.53
4c	4-Cl-C_6H_5	67.25	33.71	30.79	32.74	66.29	69.21
4d	2-NO_2-C_6H_5	70.55	75.93	75.36	29.44	24.06	24.63
4e	2,4-dichloro-C_6H_5	77.54	75.14	70.69	22.45	24.85	29.30

Figure 2 Synthesis of 4-(2-aminobenzo[d]thiazol-6-yl)-6-(substituted) pyrimidine-2-thiol (4a-4e) and 4-(2-aminobenzo[d]thiazol-6-yl)-6-(substituted) pyrimidin-2-ol (3a-3e)



Comp 2a 2b 2c 2d 2e 3a 3b 3c 3d 3e 4a 4b 4c 4d 4e
R 4-NO_2-C_6H_5 3-NO_2-C_6H_5 4-Cl-C_6H_5 2-NO_2-C_6H_5 2,4-dichloro-C_6H_5 4-NO_2-C_6H_5 3-NO_2-C_6H_5 4-Cl-C_6H_5 2-NO2-C_6H_5 2,4-dichloro-C_6H_5 4-NO_2-C_6H_5 3-NO_2-C_6H_5 4-Cl-C_6H_5 2-NO_2-C_6H5 2,4-dichloro-C_6H_5

3. Results and Discussion:

A novel series of benzothiazolylpyrimidine **(3a-3e)** and benzothiazolyldihydropyrimidine **(4a-4e)** analogues were synthesized as outlined in the graphical abstract. The starting material 1-(2-aminobenzo[*d*]thiazol-6-yl)ethanone **(1)** was prepared by reacting 4-aminoacetophenone with potassium thiocyanate, in presence of bromine in acetic acid. In usual reaction conditions, bromination of acetyl group is much expected but by using potassium thiocyanate at a higher concentration and its action as a thiocynogen makes the reaction to proceed in other way resulting in to cyclisation of the aminoacetophenone yielding 1-(2- aminobenzo[*d*]thiazol-6-yl)ethanone. Being a pseudo-halogen, it behaves as an electrophile by attacking the carbon atom adjacent to amino group of 4-aminoacetophenone. Synthesis of 1-(2-aminobenzo[*d*]thiazol-6-yl)ethanone was ascertained by a peak at 1560.41 cm^{-1} in IR spectroscopy, which correlates to –C=N bond of benzothiazole. Further, 1H NMR spectroscopy clearly reveals the presence of –$COCH_3$ with peaks corresponding to –CH_3 protons at δ 2.55, aromatic and –NH_2 protons were observed at δ 7.82-8.87 and δ 7.46, respectively. Importantly, mass spectrum gave clear confirmation of the product by m/z value of 192.9.

Synthesized benzothiazole was acetylated in order to protect the amino terminal of benzothiazole to facilitate the synthesis of chalcone analogues. Acetylated product on reaction with substituted aldehydes yields chalcones (styryl ketones). It was observed that acetylated amino group of benzothiazole converted back in to free amine moiety due to the possible hydrolysis of – $NHCOCH_3$ in the presence of aqueous sodium hydroxide. This indicates that the synthesis of chalcones is not interfered by the free amino group of benzothiazoles. Interestingly, selective synthesis of chalcones took place instead of the expected mixture of chalcones and Schiff's bases, possibility of this type of reaction could be the basic conditions and the temperature (0-4 °C) of the reaction, the reaction between aldehydes and acetophenones, takes place by well known crossed aldol condensation reaction. The IR spectrum of compound **2a** showed a characteristic absorption bands at

3215.24 cm^{-1} and 1684.83 cm^{-1} which were attributed to the free amino group and carboxyl functionalities, respectively. The ^1H NMR spectrum of **2a** showed signal at δ 7.09 as singlet was attributed to $–NH_2$ group and signals of aromatic protons were observed at δ 7.94-8.87. Synthesized chalcones were treated with urea and thiourea, in the presence of bases to get hydroxypyrimidine and thiopyrimidine derivatives of benzothiazoles, as depicted in the Figure 2. Spectral data of the synthesized compounds revealed the structural features, where in the resolution between $–NH_2$ group of amino chalcones and the hydroxyl group of cyclised hydroxy pyrimidines was overlapping in IR spectra, but in the NMR spectra of 3a showed characteristic peak at δ 6.91 singlet of free amino group and peak at δ 9.29 singlet of hydroxyl group which gives an indication of cyclization, further mass spectral data of the compounds give clear indication on completion of the reaction.

The synthesized compounds **2a-2e**, **3a-3e**, and **4a-4e** were screened for preliminary cytotoxicity by MTT assay. % Viability and % inhibition were measured at 1μM, 10 μM, and 100 μM concentrations. The compounds **2a**, **2c**, and **3d** showed significant cytotoxicity.

4. CONCLUSION:

The present study describes the synthesis of series of benzothiazolylpyrimidine **(3a-3e)** and benzothiazolyldihydropyrimidine **(4a-4e)**. These synthesized compounds were evaluated for cytotoxicity study and antimicrobial activity. Among the compounds tested, **2a**, **2c**, and **3d** showed significant cytotoxicity. These preliminary encouraging results of biological screening of the tested compounds could offer an excellent framework in this field that may lead to discovery of potent anticancer agent.

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