Synthesis and Antitumor Activity of 2-(4-Aminophenyl)-6-fluoro-N-(substituted phenyl) benzo[d]thiazol-7-amine Derivatives: A Novel Class of Anticancer Agents

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ABSTRACT

A novel series of benzothiazole derivatives were synthesized by reacting 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d] thiazole with substituted aniline followed by reduction of nitro group to yield title compounds 2-(4-aminophenyl)-6-fluoro-N-(substituted-phenyl)benzo[d]thiazol-7-amine **5a–l**. All the synthesized compounds have been confirmed by IR, ^{1}H NMR, ^{13}C NMR, and Mass spectral data. These newly synthesized compounds were screened for *in vitro* cytotoxicity against mouse Ehrlich Ascites Carcinoma (EAC) and two human cancer cell lines (MCF-7 and HeLa).

Keywords: Benzothiazole, Anticancer, Cytotoxicity, MCF-7, HeLa.

INTRODUCTION

The biomedical communities have an insatiable appetite for new anticancer drugs because the relative mortality rate caused by cancer is still very high in the developed countries, as it accounts for more than 20% of all deaths and it is the second leading cause of death in the United States after cardiovascular disease.[1] The benzothiazole derivatives have been studied extensively as a result of their important biological activities, 2-Aryl or 2-heteroaryl substituted benzothiazoles are studied as antitumor[2], series of potent and selective antitumor agents mainly from substituted 2-(4-aminophenyl) benzothiazoles was developed and examined *in vitro*, their antitumor activity to ovarian, breast, lung, renal and colon carcinoma human cell lines.[3–10] In the recent years the fluorinated benzothiazole analogues were developed as potent antitumor agent and its lysylamide derivative '*Phortress'* is in clinical studies.[11]

Several attempts were made for modifying the benzothiazole nucleus to improve their antitumor activities. Modifications on the benzothiazole nucleus have resulted in a large number of compounds having diverse pharmacological activities. Among them the 2-(4-aminophenyl) benzothiazoles was considered to be "Lead" approach to drug discovery of new anticancer agents.[12] Pertinent to the present work, a related simple benzothiazole 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole has been shown to exhibit exquisitely potent (GI_{50}<0.1 nM) and selective *in vitro* antitumor properties in human cancer cell lines of the National Cancer Institute (NCI) 60 human cancer cell line screen.[13]

In continuation of our efforts on the design and synthesis of novel anti-cancer agents[14]

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and in anticipation of better activity, herewith we report the synthesis of series of 2-(4-aminophenyl)-6-fluoro-N-(substitutedphenyl)benzo[d]thiazol-7-amine followed by preliminary cytotoxicity screening against mouse Ehrlich Ascites Carcinoma (EAC) and two human cancer cell lines (MCF-7 and HeLa) using MTT assay at different time interval of test compound treatment.

MATERIALS AND METHODS

All the chemicals were purchased from Sigma Aldrich (St. Louis, Missouri, MO, USA) and used as such for the reactions. The completion of reactions were monitored with the help of thin layer chromatography using pre-coated aluminium sheets with GF_{254} silica gel, 0.2 mm layer thickness by E.Merck (Darmstadt, Germany). Melting points (mp) were determined in Thermonik melting point apparatus and were uncorrected. The IR spectra of the compounds were recorded using KBr on Jasco FTIR spectrometer (model-4100). The ^{1}H NMR spectra of the synthesized compounds were recorded on Bruker Avance II 400 NMR spectrometer (with TMS as internal reference) and Mass spectra were recorded on Shimadzu 2010 and mass values are reported in *m/z*.

Procedure for synthesis of 7-chloro-6-fluorobenzo[d]thiazol-2-amine (1)

The starting material 7-chloro-6-fluorobenzo[d]thiazol-2-amine was prepared according to the known procedure[15,16] by treating 3-chloro-4-fluoroaniline (0.1mol) in 95% acetic acid (50mL) with a solution of KSCN (0.2mol) in 95% acetic acid (100mL). This solution was cooled to 0–5°, and the solution of Br_2 (0.1mol) in 95% acetic acid (30mL) was added slowly with stirring at 0–10° for 4h then stirring is continued at room temperature for 8h. The reaction mixture was poured in to water, heated at 80°, filtered while hot, neutralized with strong ammonia, and recrystallized with ethanol; yield: 70.5%; mp 186–188°; IR (KBr, υ_{max}, cm^{-1}): 678.45 cm^{-1} (C-Cl), 1349.01 cm^{-1} (C-F), 1637.15 cm^{-1} (-C=N), 3087.47cm^{-1} (C-H stre), 3226.20, 3386.22 cm^{-1} (-NH_2); ^{1}H NMR (400MHz, DMSO d_6) δ (ppm): 6.97 – 7.15 (m, 1*H*, Ar-H), 7.16–7.22 (m, 1*H*, Ar-H), 7.86 (s, 2*H*, NH_2); LCMS: *m/z* 202.0; calcd. 201.9.

Procedure for synthesis of 6-amino-2-chloro-3-fluorobenzenethiol (2)

The 7-chloro-6-fluorobenzo[d]thiazol-2-amine (1a) (0.01mol) was stirred for 1h with aqueous potassium hydroxide (10M, 50mL), then the mixture was heated under reflux for 12 h with ethylene glycol (100mL), and it was left as such overnight. The reaction mixture was poured in to ice cold water (100mL), acidified with HCl, neutralized with K_2CO_3, and extracted with toluene then the organic layer is washed with water, solvent was removed under vacuum to get greenish yellow solid: yield 84%; mp 96°; IR (KBr, υ_{max}, cm^{-1}): 686.34 cm^{-1} (C-Cl), 1303.92 cm^{-1} (C-F), 2641.56 cm^{-1} (S-H), 3064.03cm^{-1} (C-H stre), 3355.29, 3443.05 cm^{-1} (-NH_2); ^{1}H NMR (400MHz, DMSO d_6) δ (ppm): 6.89 – 7.68 (m, 2*H*, Ar-H), 6.45 (s, 2*H*, NH_2), 5.52 (s, 1*H*, SH); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 112.38, 116.34, 122.23, 123.56, 149.25 (aromatic carbons), 164.02 (C-2). LCMS: *m/z* 177.1; calcd. 176.9.

Procedure for synthesis of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole (3)

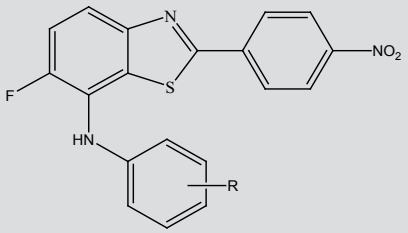
The 6-amino-2-chloro-3-fluorobenzenethiol (0.05mol) was mixed with 4-nitrobenzoic acid (0.05mol) in 85gm of polyphosphoric acid, this mixture was heated initially at 110 °C for 1 h then the temperature is raised to 210° and heated for another 3 h, then the reaction mixture is cooled and poured into ice-cold 10% sodium bicarbonate solution, precipitate was collected and purified by recrystallization from aqueous methanol as yellow solid: yield 79.4%; mp 140°; IR (KBr, υ_{max}, cm^{-1}): 688.57 cm^{-1} (C-Cl), 1345.23 cm^{-1} (C-F), 1639.34 cm^{-1} (-C=N), 3074.43cm^{-1} (C-H str); ^{1}H NMR (400MHz, DMSO d_6) δ (ppm): 7.01 – 8.12 (m, 6*H*, Ar-H); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 114.32, 122.50, 128.20, 135.62, 139.22, 145.78, 149.43 (aromatic carbons), 162.54 (C-6), 168.35 (C-2). LCMS: *m/z* 308.7; calcd. 307.9.

General procedure for the synthesis of 6-fluoro-N-(substituted-phenyl)-2-(4-nitrophenyl)benzo[d]thiazol-7-amine (4a-4l)

The 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole (0.05mol) were condensed with substituted anilines (0.05mol) in presence of dimethylsulphoxide (DMSO), the reaction mixture was heated for 2 h then allowed to cool to the room temperature and poured in to ice cold water, the precipitate is filtered, washed with water and recrystallized from hot rectified spirit. The physicochemical properties of these compounds were depicted in Table 1.

Synthesis of 2-(4-aminophenyl)-6-fluoro-N-(4-fluorophenyl)benzo[d]thiazol-7-amine (5a): This was prepared by condensation of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole with 4-fluoroaniline in presence of DMF which was then reduced in the presence of stannous chloride to yield 2-(4-aminophenyl)-6-fluoro-N-(4-fluorophenyl)benzo[d]thiazol-7-amine, yield 65.3%, mp 207-209°, IR (KBr, υ_{max}, cm^{-1}): 1348.21 cm^{-1} (C-F), 1635.82 cm^{-1} (-C=N), 2937.11cm^{-1} (C-H str), 3279.23 cm^{-1} (-NH), 3431.67 cm^{-1} (-NH_2); ^{1}H NMR (400MHz, DMSO d_6) δ (ppm): 6.43 (s, 1*H*, NH), 6.67 – 8.38 (m, 10*H*, Ar-H), 7.47 (s, 2*H*, NH_2); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 112.38, 114.34, 116.23,

Table 1 Physicochemical data of 6-fluoro-N-(substituted-phenyl)-2-(4-nitrophenyl)benzo[d]thiazol-7-amine (4a-4l)



Comp	R	Mol formula	MP °C	R_f *	Analysis (%) Found (Calcd) in % Yield		
					C	H	N
4a	4-Fluoro	$C_{19}H_{11}F_2N_3O_2S$	267-268	0.49	59.54 (59.53)	2.90 (2.89)	10.95 (10.96)
4b	4-Chloro	$C_{19}H_{11}ClFN_3O_2S$	276-278	0.56	57.05 (57.08)	2.79 (2.77)	10.49 (10.51)
4c	4-Bromo	$C_{19}H_{11}BrFN_3O_2S$	219-221	0.72	51.37 (51.37)	2.53 (2.50)	9.45 (9.46)
4d	Un-substituted	$C_{19}H_{12}FN_3O_2S$	260-262	0.37	62.42 (68.46)	3.31 (3.31)	11.51 (11.50)
4e	4-Methyl	$C_{20}H_{14}FN_3O_2S$	236-238	0.44	63.28 (63.31)	3.69 (3.72)	11.06 (11.08)
4f	2,4-dimethyl	$C_{21}H_{16}FN_3O_2S$	270-272	0.50	64.09 (64.11)	4.13 (4.10)	10.68 (10.68)
4g	4-Methoxy	$C_{20}H_{14}FN_3O_3S$	197-199	0.61	60.71 (60.75)	3.57 (3.57)	10.64 (10.63)
4h	2,4-dichloro	$C_{19}H_{10}Cl_2FN_3O_2S$	221-223	0.80	52.53 (52.55)	2.30 (2.32)	9.70 (9.68)
4i	3,4-dimethyl	$C_{21}H_{16}FN_3O_2S$	262-264	0.55	64.09 (64.11)	4.09 (4.10)	10.61 (10.68)
4j	2,5-dichloro	$C_{19}H_{10}Cl_2FN_3O_2S$	209-211	0.85	52.55 (52.55)	2.35 (2.32)	9.72 (9.68)
4k	2-Methyl	$C_{20}H_{14}FN_3O_2S$	225-227	0.30	63.30 (63.31)	3.70 (3.72)	11.07 (11.08)
4l	3-Chloro-4-fluoro	$C_{19}H_{10}ClF_2N_3O_2S$	241-242	0.75	54.60 (54.62)	2.41 (2.41)	10.05 (10.06)

* TLC Solvent system: Acetonitrile: Methanol: Strong ammonia-(10:5:2)

123.56, 124.20, 128.60, 129.54, 135.16, 149.25, 150.31 (aromatic carbons), 164.02 (C-2). LCMS: *m/z* 353.1; calcd. 353.0.

Synthesis of 2-(4-aminophenyl)-N-(4-chlorophenyl)-6-fluorobenzo[d]thiazol-7-amine (5b): This compound was prepared by condensation of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole with 4-chloroaniline which was then reduced in the presence of stannous chloride to yield 2-(4-aminophenyl)-6-fluoro-N-(4-chlorophenyl)benzo[d]thiazol-7-amine, yield 63.1%, mp 222–224°, IR (KBr, υ_{max}, cm^{-1}): 687.09 cm^{-1} (C-Cl), 1643.11 cm^{-1} (-C=N), 3055.56 cm^{-1} (C-H stre), 3254.34(-NH), 3423.36 cm^{-1} (-NH_2); ^{1}H NMR (400MHz, DMSO d_6) δ (ppm): 6.33 (s, 1*H*, NH), 6.67 – 8.38 (m, 10*H*, Ar-H), 7.47 (s, 2*H*, NH_2); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 111.78, 114.76, 116.83, 120.34, 123.55, 124.23, 128.59, 136.39, 148.20, 150.44, 152.61 (aromatic carbons), 165.23 (C-2). LCMS: *m/z* 369.2; calcd. 369.0.

Synthesis of 2-(4-aminophenyl)-N-(4-bromophenyl)-6-fluorobenzo[d]thiazol-7-amine (5c): This was prepared by condensation of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole with 4-bromoaniline which was then reduced in the presence of stannous chloride to yield 2-(4-aminophenyl)-N-(4-bromophenyl)-6-fluorobenzo[d]thiazol-7-amine, yield 51.0%, mp 196°, IR (KBr, υ_{max}, cm^{-1}): 1645.54 cm^{-1} (-C=N), 3034.43cm^{-1} (C-H str), 3161.76, (-NH), 3239.32 cm^{-1} (-NH_2); ^{1}H

NMR (400MHz, DMSO d_6) δ (ppm): 6.72 (s, 1*H*, NH), 7.10 – 8.21 (m, 10*H*, Ar-H), 7.48 (s, 2*H*, NH_2); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 111.17, 113.96, 114.65, 123.57, 124.20, 128.65, 130.02, 134.56, 148.07, 153.21 (aromatic carbons), 164.33 (C-2). LCMS: *m/z* 414.10; calcd. 414.12.

Synthesis of 2-(4-aminophenyl)-6-fluoro-N-phenylbenzo[d]thiazol-7-amine (5d): This was prepared by condensation of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole with aniline which was then reduced in the presence of stannous chloride to yield 2-(4-aminophenyl)-6-fluoro-N-phenylbenzo[d] thiazol-7-amine, yield 58.2%, mp 213–214°, IR (KBr, υ_{max}, cm^{-1}): 1653.12 cm^{-1} (-C=N), 3052.28 cm^{-1} (C-H str), 3243.76(-NH), 3425.23 cm^{-1} (-NH); ^{1}H NMR (400MHz, DMSO d_6) δ (ppm): 6.35 (s, 1*H*, NH), 7.67 – 8.31 (m, 11*H*, Ar-H), 7.48 (s, 2*H*, NH_2); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 112.06, 114.71, 116.00, 119.34, 123.61, 124.21, 130.50, 135.30, 136.87, 148.13, 149.40, 151.83 (aromatic carbons), 166.73 (C-2). LCMS: *m/z* 335.1; calcd. 335.0.

Synthesis of 2-(4-aminophenyl)-6-fluoro-N-p-tolylbenzo[d]thiazol-7-amine (5e): This was prepared by condensation of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole with p-toluidine which was then reduced in the presence of stannous chloride to yield 2-(4-aminophenyl)-6-fluoro-N-p-tolylbenzo[d]thiazol-7-amine, yield 73.7%, mp 174–176°, IR (KBr, υ_{max}, cm^{-1}): 1636.42 cm^{-1} (-C=N), 2944.28cm^{-1} (C-H str), 3282.45 cm^{-1} (-NH), 3378.54 (NH_2); ^{1}H NMR (400MHz, DMSO d_6) δ (ppm): 3.12 (s, 3*H*, CH_3), 6.65 (s, 1*H*, NH), 6.97 – 7.98 (m, 10*H*, Ar-H), 7.46 (s, 2*H*, NH_2); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 30.21, 112.17, 114.06, 116.20, 123.38, 124.20, 128.73, 130.76, 135.49, 149.41, 150.69 (aromatic carbons), 165.56 (C-2). LCMS: *m/z* 349.5; calcd. 349.4.

Synthesis of 2-(4-aminophenyl)-6-fluoro-N-(2,4-dimethylphenyl)benzo[d]thiazol-7-amine (5f): This was prepared by condensation of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole with 2,4-dimethylaniline which was then reduced in the presence of stannous chloride to yield 2-(4-aminophenyl)-6-fluoro-N-(2,4-dimethylphenyl)benzo[d]thiazol-7-amine, yield 81.7%, mp 162–164°, IR (KBr, υ_{max}, cm^{-1}): 1655.54 cm^{-1} (-C=N), 2952.06 cm^{-1} (C-H str), 3258.22(NH), 3432.05 cm^{-1} (-NH_2); ^{1}H NMR (400MHz, DMSO d_6) δ (ppm): 2.34 (s, 6*H*, CH_3), 6.61 (s, 1*H*, NH), 7.07 – 8.52 (m, 9*H*, Ar-H), 7.44 (s, 2*H*, NH_2); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 29.36, 112.70, 114.24, 115.62, 120.04, 124.84, 125.33, 128.57, 135.48, 148.20, 151.60, 152.64 (aromatic carbons), 166.47 (C-2). LCMS: *m/z* 363.4; calcd. 363.4.

Synthesis of 2-(4-aminophenyl)-6-fluoro-N-(4-methoxyphenyl)benzo[d]thiazol-7-amine (5g): This was prepared by condensation of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole with 4-methoxyaniline which was then reduced in the presence of stannous chloride to yield 2-(4-aminophenyl)-6-fluoro-N-(4-methoxyphenyl)benzo[d]thiazol-7-amine, yield 68.7%, mp 186-188°, IR (KBr, υ_{max}, cm^{-1}): 1652.47 cm^{-1} (-C=N), 2984.14 cm^{-1} (C-H stre), 3267.61 cm^{-1} (-NH), 3416 cm^{-1} (-NH_2); ^{1}H NMR (400MHz, DMSO d_6) δ (ppm): 4.11 (s, 3H, OCH_3), 6.93 (s, 1*H*, NH), 6.66 - 8.12 (m, 10*H*, Ar-H), 7.51 (s, 2*H*, NH_2); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 60.03, 111.47, 114.66, 116.07, 123.37, 124.21, 128.51, 130.04, 134.94, 149.66, 150.42 (aromatic carbons), 165.78 (C-2). LCMS: *m/z* 365.3; calcd. 365.4.

Synthesis of 2-(4-aminophenyl)-N-(3,4-dichlorophenyl)-6-fluorobenzo[d]thiazol-7-amine (5h): This was prepared by condensation of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole with 2,4-dichloroaniline which was then reduced in the presence of stannous chloride to yield 2-(4-aminophenyl)-N-(3,4-dichlorophenyl)-6-fluorobenzo[d]thiazol-7-amine, yield 60.3%, mp 245-247°, IR (KBr, υ_{max}, cm^{-1}): 688.10 cm^{-1} (C-Cl), 1645.46 cm^{-1} (-C=N), 3106.19 cm^{-1} (C-H stre), 3293.34, cm^{-1} (-NH), 3457.81 cm^{-1} (-NH_2); ^{1}H NMR (400MHz, DMSO d_6) δ (ppm): 6.61 (s, 1*H*, NH), 7.14 – 7.78 (m, 9*H*, Ar-H), 7.36 (s, 2*H*, NH_2); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 112.71, 114.09, 116.63, 120.37, 123.50, 124.22, 128.11, 136.57, 148.20, 150.16, 152.97 (aromatic carbons), 166.03 (C-2). LCMS: *m/z* 404.2; calcd. 404.2.

Synthesis of 2-(4-aminophenyl)-6-fluoro-N-(3,4-dimethylphenyl)benzo[d]thiazol-7-amine (5i): This was prepared by condensation of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole with 3,4-dimethylaniline which was then reduced in the presence of stannous chloride to yield 2-(4-aminophenyl)-6-fluoro-N-(3,4-dimethylphenyl)benzo[d]thiazol-7-amine, yield 76.2%, mp 237-239 °, IR (KBr, υ_{max}, cm^{-1}): 1635.82 cm^{-1} (-C=N), 2937.11cm^{-1} (C-H str), 3279.23 cm^{-1} (-NH); ^{1}H NMR (400MHz, DMSO d_6) δ (ppm): 2.33 (s, 6H, CH_3), 6.62 (s, 1*H*, NH), 7.05 – 7.92 (m, 9*H*, Ar-H), 7.42 (s, 2*H*, NH_2); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 30.02, 112.71, 114.20, 115.67, 120.00, 124.81, 125.13, 128.58, 135.48, 148.20, 151.63, 152.65 (aromatic carbons), 166.45. LCMS: *m/z* 363.3; calcd. 363.4.

Synthesis of 2-(4-aminophenyl)-N-(2,5-dichlorophenyl)-6-fluorobenzo[d]thiazol-7-amine (5j): This was prepared by condensation of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole with 2,5-dichloroaniline which was then reduced in the presence of stannous chloride to yield 2-(4-aminophenyl)-N-(2,5-dichlorophenyl)-6-fluorobenzo[d]thiazol-7-amine, yield 72.1%, mp 241–243°, IR (KBr, υ_{max}, cm^{-1}): 687.96 cm^{-1} (C-Cl), 1650.17 cm^{-1} (-C=N), 3105.56 cm^{-1} (C-H

str), 3274.33(NH), 3453.41 cm^{-1} (-NH_2); ^1H NMR (400MHz, DMSO d_6) δ (ppm): 6.62 (s, 1*H*, NH), 7.26 – 7.81 (m, 9*H*, Ar-H), 7.37 (s, 2*H*, NH_2); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 112.42, 114.07, 116.07, 120.31, 124.00, 124.74, 128.14, 136.23, 148.21, 150.19, 153.05 (aromatic carbons), 165.81 (C-2). LCMS: *m/z* 404.3; calcd. 404.2.

Synthesis of 2-(4-aminophenyl)-6-fluoro-N-o-tolylbenzo[d]thiazol-7-amine (5k): This was prepared by condensation of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole with o-toluidine which was then reduced in the presence of stannous chloride to yield 2-(4-aminophenyl)-6-fluoro-N-o-tolylbenzo[d]thiazol-7-amine, yield 75.4%, mp 278–280°, IR (KBr, v_{max}, cm^{-1}): 1656.77 cm^{-1} (-C=N), 3011.74 cm^{-1} (-CH str), 3272.69 cm^{-1} (-NH), 3362.33 (NH_2); ^1H NMR (400MHz, DMSO d_6) δ (ppm): 3.16 (s, 3*H*, CH_3), 6.64 (s, 1*H*, NH), 7.01 – 8.01 (m, 10*H*, Ar-H), 7.44 (s, 2*H*, NH_2); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 30.24, 112.19, 114.06, 116.23, 123.32, 124.21, 128.66, 130.77, 135.51, 150.02, 150.54, (aromatic carbons), 165.74. LCMS: *m/z* 349.3; calcd. 349.4

Synthesis of 2-(4-aminophenyl)-N-(3-chloro-4-fluorophenyl)-6-fluorobenzo[d]thiazol-7-amine (5l): This was prepared by condensation of 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole with 3-chloro-4-fluoroaniline which was then reduced in the presence of stannous chloride to yield 2-(4-aminophenyl)-N-(3-chloro-4-fluorophenyl)-6-fluorobenzo[d]thiazol-7-amine, yield 63.1%, mp 222-224°, IR (KBr, v_{max}, cm^{-1}): 690.12 cm^{-1} (C-Cl), 1635.78 cm^{-1} (-C=N), 3082.46 cm^{-1} (C-H str), 3232.30(NH), 3373.34 cm^{-1} (-NH_2); ^1H NMR (400MHz, DMSO d_6) δ (ppm): 6.72 (s, 1*H*, NH), 6.68 – 7.56 (m, 9*H*, Ar-H), 7.45 (s, 2*H*, NH_2); ^{13}C NMR (400MHz, DMSO d_6) δ (ppm): 112.31, 114.45, 116.80, 120.11, 124.23, 128.52, 136.41, 148.21, 150.41, 152.75, (aromatic carbons), 166.03. LCMS: *m/z* 387.7; calcd. 387.8.

In Vitro Cytotoxic Activity

Cell lines

Human cancer cell lines, MCF-7 and HeLa cells were cultured in MEM medium supplemented with 10% FBS, 1% L-glutamine and 50 mg/mL gentamicin sulphate in a CO_2 incubator in a humidified atmosphere of 5% CO_2 and 95% air. The EAC cells were maintained for 12-14 days in the peritoneal cavity of Swiss albino mice. The tumor cell cultures were started from mouse Ehrlich Ascites with at least one passage *in vitro* prior to use.

MTT assay

In vitro cytotoxicity was determined using a standard MTT assay[17] with protocol appropriate for the individual test system. In brief, exponentially growing cells were plated in 96-well plates (10^4 cells/well in 100 mL of medium) and incubated for 24 h for attachment. Test compounds were prepared prior to the experiment by dissolving in 0.1% DMSO and diluted with medium. The cells were then exposed to different concentrations of the drugs (1–100 μM) in the volume of 100 mL/well. Cells in the control wells received the same volume of medium containing 0.1% DMSO. After 24 h, the medium was removed and cell cultures were incubated with 100 mL MTT reagent (1 mg/mL) for 4 h at 37°. The formazan produced by the viable cells was solubilized by addition of 100 mL DMSO. The suspension was placed on micro-vibrator for 5 min and absorbance was recorded at 540 nm by the ELISA reader. The experiment was performed in triplicate. The percentage cytotoxicity was calculated using the formula

$$\% \text{ Cytotoxicity} = \frac{(Ca - Ba) - (Ta - Ba)}{(Ca - Ba)} \times 100$$

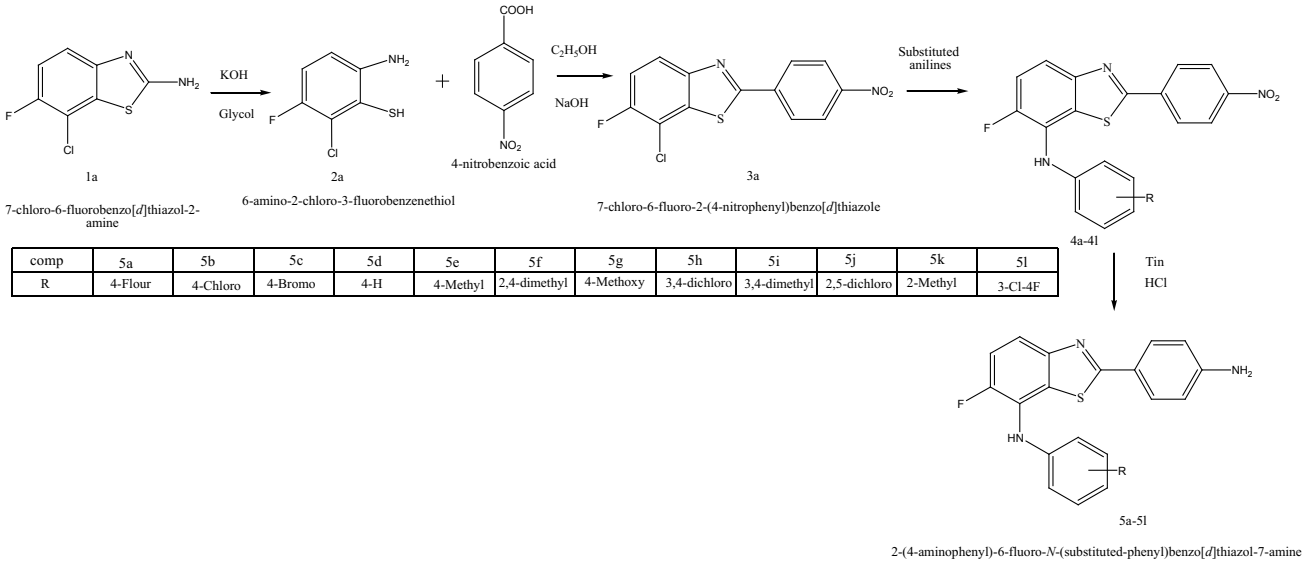
Where, Ca = Control absorbance; Ba = Blank absorbance; Ta = Test absorbance

For MTT assay, MCF-7 cells (5 x 10^3cells/well) and HeLa cells (5 x 10^3cells/well) seeded in 96-wells plates were exposed to different concentrations of test compounds (1–100 μM). The percentage cytotoxicity and IC_{50} values were determined at 24, 48, and 72 h of drug incubation.

RESULTS AND DISCUSSION

A novel series of 2-(4-aminophenyl)-6-fluoro-N-(substituted-phenyl)benzo[d]thiazol-7-amine (**5a-5l**) were synthesized as shown in Figure 1. The starting compound 7-chloro-6-fluorobenzo[d]thiazol-2-amine (**1**) was prepared and characterized by spectroscopic techniques. The IR Spectra of compound (**1**) showed a characteristic absorption band 3226.20 cm^{-1} to 3386.22 cm^{-1} was attributed to the amino (-NH_2) group of benzothiazole and two absorption bands, one of which, appearing at 3087.47 cm^{-1} was due to the aromatic C-H stretching and other observed 1637.15 cm^{-1} was assigned to C=N stretching which confers the formation of benzothiazole ring. The ^1H NMR spectrum of compound (**1**) showed a characteristic signals between δ 7.86 as a singlet were attributed to –NH_2 and signals of aromatic proton of compounds were observed at δ 6.97–7.22, the structure of (**1**) were further supported by the molecular ion peak at 202.0 in the mass spectrum.

The 7-chloro-6-fluorobenzo[d]thiazol-2-amine (**1**) was subjected for hydrolysis to give 6-amino-2-chloro-3-fluorobenzenethiol (**2**) the structure of this compound was ascertained by absence of C=N stretching



comp	5a	5b	5c	5d	5e	5f	5g	5h	5i	5j	5k	5l
R	4-Flour	4-Chloro	4-Bromo	4-H	4-Methyl	2,4-dimethyl	4-Methoxy	3,4-dichloro	3,4-dimethyl	2,5-dichloro	2-Methyl	3-Cl-4F

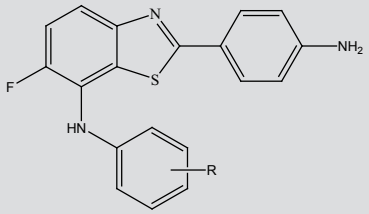
Figure 1: Synthesis of 2-(4-aminophenyl)-6-fluoro-***N***-(substituted phenyl)benzo[*d*]thiazol-7-amine

at 1637.27 cm^{-1} and presence of new peak at 2641.56 cm^{-1} for free –S-H group. The ^{1}H NMR spectrum of compound (**2**) showed a characteristic signals at δ 5.52 and 6.45 as a singlet were attributed to –SH and $–NH_2$ respectively, the formation of 6-amino-2-chloro-3-fluorobenzenethiol (**2**) was confirmed by molecular ion peak at 177.1 in the mass spectral studies. So formed 6-amino-2-chloro-3-fluorobenzenethiol (**2**) was subjected for cyclization by treating with p-nitrobenzoic acid in polyphosphoric acid to yield 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole (**3**). The spectral data of compound (**3**) exhibit IR band at 1639.34 cm^{-1} band for C=N stretching which confirms the reformation of benzothiazole ring and absence of amino stretching gives support for the formation of compound (**3**). The ^{1}H NMR spectrum of compound (**3**) showed absence of amino protons and showed a characteristic signal at δ 7.01 – 8.12 indicate the presence of only aromatic protons, further structure of (**3**) was substantiated by mass spectral data.

The title compounds (**5a-l**) was obtained by condensing 7-chloro-6-fluoro-2-(4-nitrophenyl)benzo[d]thiazole (**3**) with substituted anilines followed by catalytic reduction of 6-fluoro-N-(substituted-phenyl)-2-(4-nitrophenyl) benzo[d]thiazol-7-amine (**4a–4l**). The spectral data of compound (**5a**) exhibit IR band at 1635.82 cm^{-1} was due to the C=N stretching which confirms the formation of benzothiazole ring, the band at 3279.23–3431.67 cm^{-1} was due the primary amine ($-NH_2$) group, which gives the evidence of nitro reduction. The ^{1}H NMR data of (**5a**) reveals the presence of primary amino protons by giving strong singlet peak at δ 7.47, peaks at δ 6.67 – 8.38 confirms the aromatic protons, the structure of **5a** was further substantiated with ^{13}C NMR data, and a molecular ion peak at 353.1 in mass spectrum.

The synthesized novel compounds 2-(4-aminophenyl)-6-fluoro-N-(substituted-phenyl)benzo[d]thiazol-7-amine (**5a-l**) were screened for *in vitro* cytotoxicity against mouse Ehrlich Ascites Carcinoma (EAC) and two human cancer cell lines (MCF-7 and HeLa) by standard MTT assay. The preliminary cytotoxicity study of benzothiazole derivatives was found more effective on both MCF-7 and HeLa cell lines. On the MCF-7 the IC_{50} for the title compounds (**5a–5l**) were in the range of 20.31 - 71.75 μM at 24 h of drug exposure, whereas on HeLa cell lines the IC_{50} value were in the range of 25.59 - 86.55 μM at 24 h of drug exposure (Table 2). This preliminary cytotoxicity reveals that MCF-7 cells are more sensitive to all the tested compounds than HeLa cells. Many of the chemotherapeutic agents effective against MCF-7 and HeLa cells causing apoptosis through the expression of caspase-3, generating reactive oxygen species (ROS), and damaging DNA.[18] Cisplatin causes cytotoxicity in MCF-7 and HeLa cells by a similar mechanism.[19] The anticancer drugs such as doxorubicin, mitoxantrone and bleomycin cause cytotoxicity by generating ROS.[20]P revious studies have shown that strong electronegative atom substitution such as fluoro/chloro/bromo on the 4th position of the aromatic ring increases the lipophilicity of molecules and is responsible for enhanced cytotoxicity in MTT bioassay.[21] Similar substitutions are incorporated in the present work (**5a**, **5b**, **5h** and **5l**.); we have also observed enhanced cytotoxicity in these newly synthesized molecules. Hence these molecules were taken up to assess the cytotoxic potency at different intervals in MCF-7

Table 2 Cytotoxic activity of synthesized compounds 5a–3l in EAC, MCF-7 and HeLa cells at different time points of drug exposure by MTT assay



Compounds	R	IC_{50} (μm) in EAC			IC_{50} (μm) in MCF-7 cells			IC_{50} (μm) in HeLa cells		
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
5a	4-Fluoro	31.44	29.63	36.41	27.30	22.34	19.43	30.32	26.06	22.54
5b	4-Chloro	40.60	35.61	33.01	21.43	18.89	16.39	39.22	35.25	34.74
5c	4-Bromo	43.45	40.56	38.78	31.67	26.80	24.57	51.05	47.36	42.56
5d	Un-substituted	49.20	43.32	42.65	35.06	31.76	28.89	51.02	47.90	45.25
5e	4-Methyl	67.23	55.65	51.18	51.28	48.17	45.31	43.22	42.30	37.61
5f	2,4-dimethyl	80.32	78.21	75.31	71.75	68.40	63.86	86.55	79.34	77.34
5g	4-Methoxy	33.38	30.67	29.30	43.36	39.07	37.42	30.28	27.43	26.33
5h	2,4-dichloro	31.30	27.20	25.90	20.31	17.56	15.43	25.59	21.08	19.06
5i	3,4-dimethyl	77.56	72.36	68.86	44.43	40.12	37.32	36.02	32.37	28.56
5j	2,5-dichloro	34.23	27.80	22.53	26.21	20.12	18.24	44.21	31.24	26.33
5k	2-Methyl	72.67	63.34	60.30	38.09	29.08	20.52	48.34	38.56	32.69
5l	3-Chloro-4-fluoro	26.17	22.43	18.34	20.43	17.08	15.32	35.65	27.09	20.31
Doxorubicin	-	5.23	4.98	4.76	3.88	3.21	2.92	5.01	4.65	4.20

The IC_{50} values of tested compounds at 48 h and 72 h were significantly reduced in comparison with 24 h values

cells and HeLa cells. Finally it is concluded that in the synthesis of novel antitumor agents the introduction of halogen substituents in benzothiazole ring could open the door to promising and selective antitumor agents.

CONCLUSION

In the present paper, we report the synthesis, spectral characterization, and *in vitro* antitumor activity of new series of 2-(4-aminophenyl)-6-fluoro-N-(substituted-phenyl) benzo[d]thiazol-7-amine (**5a–l**). These novel heterocyclic compounds were synthesized by condensation of different substituted anilines with 6-fluoro-N-(substituted-phenyl)-2-(4-nitrophenyl)benzo[d]thiazol-7-amine (**4a–4l**) followed by catalytic reduction of nitro group in presence of ethanol. The results of *in vitro* preliminary MTT assay showed significant antitumor activity, as out of twelve title compounds (**5a–5l**) tested, compounds **5a**, **5b**, **5h, 5j** and **5l** exhibited promising activity. The overall outcome of this study revealed that the benzothiazole ring is the suitable backbone for antitumor activity and fluoro/chloro/bromo substituents showed significant activity, as reported for fluorinated benzothiazole (5F 203) which produces apoptosis and DNA damage in MCF-7 cells,[22,23] which is characteristic of cytotoxic activity.

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