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DESIGN AND SYNTHESIS OF BENZOTHIAZOLE SCHIFF BASES OF POTENTIAL ANTITUMOR ACTIVITY

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Abstract – In an attempt to develop a new class of selective antitumor agents, a novel series of benzothiazole derivatives was prepared *via* the condensation of 5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine with aromatic aldehydes. The preliminary bioassay reveals that (4-fluorobenzylidene)-[5-fluoro-6-(4-methylpiperazin-1-yl)-benzothiazol-2-yl]-amine show specific anticancer cytotoxicity.

Structural modification of heterocyclic compounds may alter the biological properties *via* introduction of potent moieties. For instance, Schiff bases have been used extensively in drug design and proven to be outstanding moieties with a wide spectrum of biological activities.[1]

Moreover, benzothiazole scaffold serve as a useful constituent for many natural products and pharmaceutical candidates which are known to possess a wide spectrum of biological activities. They attracted much attention in recent years due to their wide range of biological activities and they have shown significant antimicrobial,[2] anticancer,[3] anti-AD (Alzheimer's disease), anti-inflammatory,[4] anti-HIV (human immunodeficiency virus),[5] antioxidant,[6] anticonvulsant,[7] antidiabetic,[8] antitubercular,[9] antidepressant,[10] antiviral,[11] and antimalarial[12] activities.

Incorporation of fluorine and piperazine in bioactive molecules imparts special characteristic properties that enhance therapeutic efficacy and improve pharmacological properties.[13] Ciprofloxacin® (**1**) remains the most potent antibiotic among the quinolone drugs. It turns out that the presence of both fluorine and piperazine moieties in positions 6 and 7 respectively, plays a vital role and provides an optimum activity.[14]

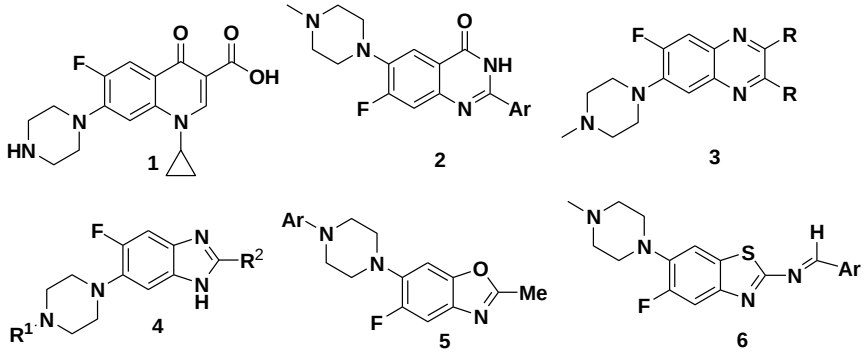


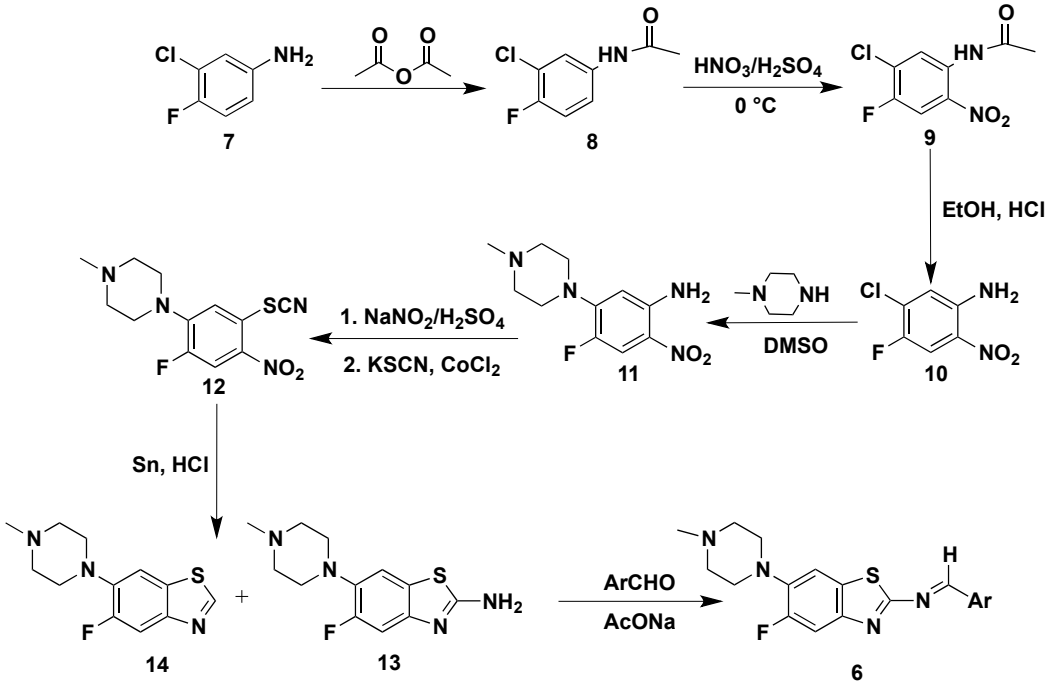
Figure 1

On the other hand, cytotoxic agents have the potential of causing damage to normal and healthy cells. Thus, upon designing new potential antitumor agents, the balance of developing high cytotoxicity towards carcinogenic cells without affecting healthy cells is highly needed in the development of new antitumor agents. Our strategy in designing cytotoxic agents takes advantage of the reported high therapeutic profile of fluorine and piperazine appendages and their proven safety profile. In recent years we have successfully designed and synthesized various heterocyclic systems (**2-5**) incorporating fluorine and piperazine appendages (Figure 1) and some systems showed a promising biological activities[15] which were attributed to the presence of both appendages.

To the best of our knowledge, the synthesis of benzothiazole Schiff bases bearing the combination of both appendages (fluorine and piperazine at positions 5 and 6, respectively) is not reported in literature. Based on the above findings and as part of our ongoing research devoted towards the synthesis of potential bioactive heterocyclic systems, herein we report the synthesis and cytotoxicity of a novel series of benzothiazole Schiff bases of 5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (**13**).

The new series of benzothiazole Schiff base derivatives were prepared following a multi-step synthetic strategy as depicted in Scheme 1.[15b] The foregoing standard methodology used the commercially available 3-chloro-4-fluoroaniline (**7**) and involved acylation, nitration, de-acylation and piperazinylation. The key intermediate (**12**) was prepared by the diazotization of nitroaniline (**11**) using sodium nitrite in sulfuric acid to form the diazonium salt followed by a Sandmeyer reaction using potassium thiocyanate. The *in situ* reductive cyclization of **12** affords the key intermediate 5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (**13**) as a major product.[16] Surprisingly, 5-fluoro-6-(4-methylpiperazin-1-yl)-benzothiazole (**14**) was isolated and characterized as a minor product in the latter reaction. The proposed mechanism leading to the formation of **13** and **14** is depicted in Figure 2. To explain the formation of the unexpected compound (**14**), we believe that the 1-(2-fluoro-4-nitro-5-thiocyanatophenyl)-4-methylpiperazine **12** has been reduced in two ways: selective

reduction of the nitro group in **12** and selective reduction of the cyanide group as shown in the Figure 2. The selective reduction of the nitro group yielded (**E**, Figure 2) in a higher yield than the reduction of the cyano group that yielded (**A**, Figure 2). The latter, underwent intramolecular nucleophilic aromatic substitution to furnish the benzothiazolidine derivative (**D**) which leads to the formation of **14** by the oxidation driven by aromatic ring formation.



Scheme 1

Comp.	6a	6b	6c	6d	6e	6f	6g	6h	6i	6j	6k	6l
Ar	4-Me-ph	4-MeO-ph	4-F-ph	4-Cl-ph	4-Br-ph	4-NO_2-ph	3-F-ph	3-Br-ph	3-NO_2-ph	2-Br-ph	3,4-MeO-ph	furan
Yield %	20	47	43	20	55	62	71	20	71	25	10	40

The benzothiazole Schiff bases series **6a-l** is achieved by the treatment of 2-aminobenzothiazole (**13**) with several substituted aromatic aldehydes in the presence of sodium acetate which acts as base catalyst.
The formation of 1-(2-fluoro-4-nitro-5-thiocyanatophenyl)-4-methylpiperazine (**12**) was confirmed by the disappearance of exchangeable amine proton signals of **11** at 6.12 ppm in ^{1}H NMR and the appearance of a new signal of the thiocyanate carbon at 112.1 ppm in ^{13}C NMR of **12**.

The ^{1}H NMR spectrum of 2-aminobenzothiazole revealed an exchangeable proton at δ 5.09 ppm which was assigned to NH_2 protons. The aromatic protons resonate at δ 7.24 ppm and δ 7.16 ppm and appear as two doublets respectively. The proton ortho to the fluorine shows a higher coupling constant J = 12.92 Hz compared to the proton meta to the fluorine J = 8.04 Hz. The formation of **14** with **13** (confirmed by the

HRMS and ^{1}H NMR) was proven by the appearance of a new aromatic proton as a sharp singlet at 8.87 ppm. The methyl protons of the piperazine moieties in **11-14** and **6a-l** appear as sharp singlets around δ 2.31-3.38 ppm whereas the piperazine methylene proton signals appear as two broad singlets around δ 4.03-3.10 ppm and δ 2.64-3.87 ppm.

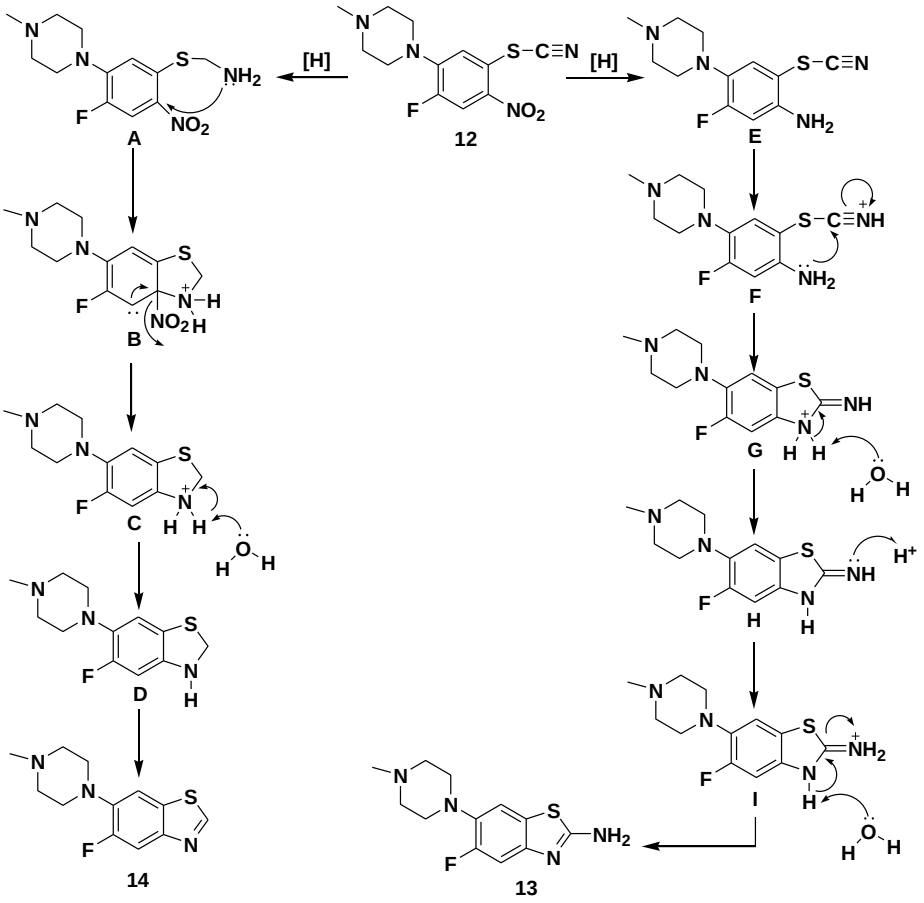


Figure 2

The Schiff bases' ^{1}H NMR spectra show that there is only one set of isomers, existing as the *E*-isomer. The characteristic azomethine proton resonates around δ 8.90-9.15 ppm and other protons appear within the expected chemical shift. Carbon 2 of the benzothiazole derivatives appear downfield around δ 170.3-172.0 ppm and those of the azomethine carbon around δ 160.8-165.6 ppm.

Cytotoxicity

Cytotoxicity assays were performed in order to test the newly synthesized benzothiazole Schiff bases for anticancer activities and to evaluate their safety to human primary non-transformed cells. Thus, initial screening of cell viability was performed for the Schiff bases **6a-l** at concentrations ranging from 1 μg/mL to 0.01 ng/mL on DMS-53 human small cell lung cancer cell line and on primary human lung

microvascular endothelial cells (HLMVECs) using prestoBlue® Cell Viability Reagent (Life Technologies). The initial screening (Figure 3) revealed that the compounds (**6c, 6d**) are especially promising as they seem to display specific anticancer cytotoxicity while leaving primary non-transformed cells unharmed. Therefore, further experiments will be performed to confirm the cytotoxic activity of **6c** on killing cancer cells. Other compounds, such as **6e**, **6j** also showed cytotoxic activities, but could not differentiate between tumor and non-tumor cells.

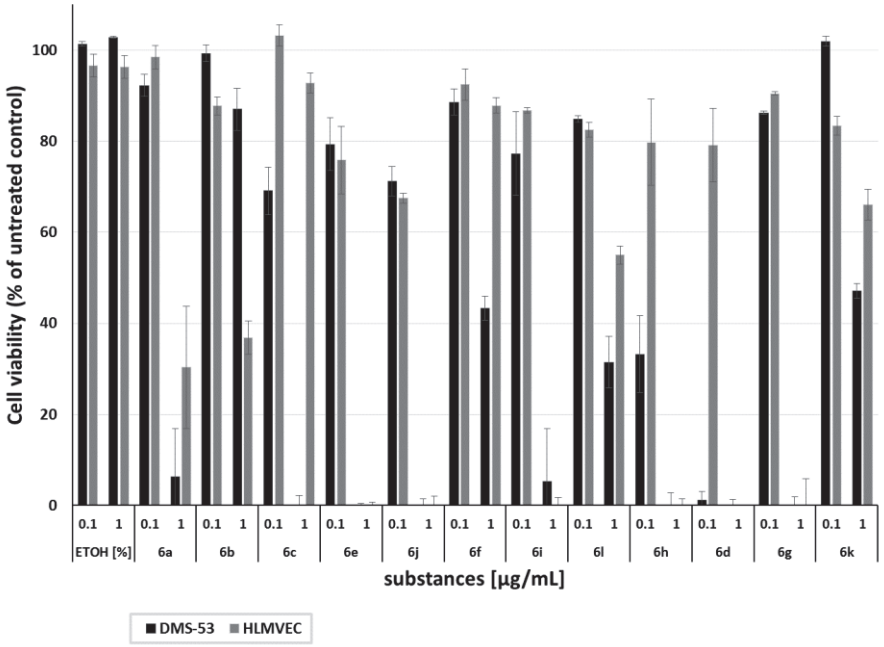


Figure 3

In this study, a new series of benzothiazole Schiff bases was successfully prepared and their structures have been successfully confirmed. Some of the newly synthesized compounds show promising cytotoxicity specifically against the lung cancer cell line in comparison to primary HLMVECs. We are aiming by this approach to develop novel antitumor agents with the lowest cytotoxicity possible against human primary cells. The detailed results of further bioassays analyzing cytotoxicity of the new anti-cancer candidates against additional cancer cell lines and primary cells will be published separately in due course.

EXPERIMENTAL

Chemistry. The ^{1}H NMR spectra were measured on BRUKER 400 MHz NMR spectrometer and ^{13}C NMR spectra were recorded at 100 MHz using TMS as internal standard and the chemical shifts δ are given in parts per million (ppm) downfield from TMS. The EI and FAB high resolution mass spectra were recorded on a Finnigan MAT 312 mass spectrometer. Melting points were determined on SMP 10 Stuart Scientific

apparatus. The reactions were monitored by thin layer chromatography (TLC) using silica gel plates and the components visualized by UV light.

Synthesis of 1-(2-fluoro-4-nitro-5-thiocyanatophenyl)-4-methylpiperazine (12)

4-Fluoro-5-(4-methylpiperazin-1-yl)-2-nitrobenzenamine (1 mmol) was dissolved in 25 mL mixture of 50% water/concentrated H_2SO_4 and the solution was cooled to 0 °C. Then the chilled solution was diazotized with a solution of sodium nitrite (1.2 mmol) in 20 mL of water, dropwise addition while maintaining the temperature not higher than 5 °C. The resulting solution was added with vigorous stirring to a solution of cobalt chloride (3 mmol) and potassium thiocyanate (2 mmol) in 50 mL of water. The resultant mixture was stirred overnight. The green precipitate was collected by suction filtration, washed with water and dried under vacuum.

Green solid; mp 129-130 °C; yield 75%; ^{1}H NMR (400 MHz, CD_3COCD_3) δ 8.16 (s, 1H, H-3), 7.42 (s, 1H, H-4), 4.03 (bs, 4H, piperazine), 3.87 (bs, 4H, piperazine), 3.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, CD_3COCD_3) δ 153.9, 145.0, 137.4, 123.8, 117.6, 115.6, 112.1, 54.6, 47.5, 44.1; HRMS (EI) [M] *m/z* Calcd for $C_{12}H_{13}FN_4O_2S$ 296.3206 found 296.0743.

Synthesis of 5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (13) 5-fluoro-6-(4-methylpiperazin-1-yl)benzothiazole (14)

To concentrated HCl (25 mL) were added 1-(2-fluoro-4-nitro-5-thiocyanatophenyl)-4-methylpiperazine (2 g, 7.9 mmol) and tin (6 g, 47.2 mmol), and the mixture was stirred at room temperature overnight. After stirring, the mixture was diluted with water and basified by addition of aqueous ammonium solution. The precipitate was filtered and dried before extraction with hot EtOAc (5×25 mL). The organic extracts were collected, dried by Na_2SO_4 and evaporated under reduced pressure. The residue was chromatographed (1% MeOH/CH_2Cl_2) to yield the titled compounds.

5-Fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (13)

Pale yellow crystals; mp 223-224 °C; yield 45%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 12.92 Hz, 1H, H-3), 7.16 (d, *J* = 8.04, 1H, H-4), 5.09 (bs, 2H, NH_2), 3.10 (bs, 4H, piperazine), 2.64 (bs, 4H, piperazine), 2.37 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 155.0, 147. 0, 135.1, 126.0, 110.8, 105.6, 54.7, 50.7, 49.5; HRMS (EI) [M] *m/z* Calcd for $C_{12}H_{15}FN_4S$ 266.1001 found 266.0986.

5-Fluoro-6-(4-methylpiperazin-1-yl)benzothiazole (14)

Purple solid; yield 15%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H, H-C=N), 7.77 (d, *J* = 12.72 Hz, 1H, H-3), 7.44 (d, *J* = 7.96 Hz, 1H, H-4), 3.20 (bs, 4H, piperazine), 2.70 (bs, 4H, piperazine), 2.41(s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 153.5, 148.5, 139.6, 129.9, 110.3, 110.0, 54.9, 50.6, 45.9; HRMS (EI) [M] *m/z* Calcd for $C_{12}H_{16}FN_3S$ 251.0892 found 251.0912.

General procedure for the synthesis of (*E*)-*N*-(substituted benzylidene)-5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6a-l)

To a solution of 2-aminobenzothiazole (**13**) (1 mmol) in toulene, sodium acetate (1 mmol) and substituted aromatic aldehydes (2 mmol) were added and the reaction mixture refluxed for 24 h. Upon cooling, a precipitate formed. The precipitate was collected by suction filtration, washed with water and dried under reduced pressure.

(*E*)-*N*-(4-Methylbenzylidene)-5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6a)

Yellow crystals; mp 166-167 °C; yield 20%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H, N=CH), 7.91 (d, *J* = 8.08 Hz, 2H, H-Ar), 7.61 (d, *J* = 12.84 Hz, 1H, H-3), 7.33 (d, *J* = 8.04 Hz, 1H, H-4), 7.31 (d, *J* = 5.16 Hz, 2H, H-Ar), 3.18 (bs, 4H, piperazine), 2.64 (bs, 4H, piperazine), 2.44 (s, 3H, CH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 165.6, 156.12, 147.2, 139.6, 132.7, 131.0, 130.6, 130.1, 110.7, 110.2, 55.5, 51.5, 46.5, 22.2; HRMS (ESI) Calcd. for $C_{20}H_{21}FN_4S$ [$(M+H)^+$] 369.1544 found 369.1542.

(*E*)-*N*-(4-Methoxybenzylidene)-5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6b)

Orange crystals; mp 159-160 °C; yield 47%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H, N=CH), 7.97 (d, *J* = 8.80 Hz, 2H, H-Ar), 7.60 (d, *J* = 12.80 Hz, 1H, H-3), 7.32 (d, *J* = 8.08 Hz, 1H, H-4), 7.00 (d, *J* = 8.84 Hz, 2H, H-Ar), 3.89 (s, Ar-OCH_3), 3.18 (bs, 4H, piperazine), 2.66 (bs, 4H, piperazine), 2.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 165.0, 164.3, 156.1, 147.2, 139.6, 132.7, 130.8, 128.1, 114.8, 110.7, 110.2, 56.0, 55.5, 51.3, 46.5; HRMS (EI) [M] *m/z* Calcd for $C_{20}H_{21}FN_4OS$ 384.14201found 384.14095.

(*E*)-*N*-(4-Fluorobenzylidene)-5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6c)

Orange crystals; mp 183-184 °C; yield 43%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (s, 1H, N=CH), 8.03 (dd, J_1 = 5.48 Hz, J_2 = 5.48 Hz, 2H, H-Ar), 7.61 (d, *J* = 12.80 Hz, 1H, H-3), 7.33 (d, *J* = 8.04 Hz, 1H, H-4), 7.20 (dd, J_1 = 8.56 Hz, J_2 = 8.60 Hz, 2H, H-Ar); 3.18 (bs, 4H, piperazine), 2.65 (bs, 4H, piperazine), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 164.9, 164.0, 156.1, 147.0, 139.9, 132.3, 131.6, 131.2, 116.7, 110.7, 110.2, 55.5, 51.2, 46.5; HRMS (EI) [M] *m/z* Calcd for $C_{19}H_{18}F_2N_4S$ 372.1220 found 372.1245.

(*E*)-*N*-(4-Chlorobenzylidene)-5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6d)

Orange crystals; mp 164-165 °C; yield 20%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (s, 1H, N=CH), 7.95 (d, *J* = 8.52 Hz, 2H, H-Ar), 7.61 (d, *J* = 12.80 Hz, 1H, H-3), 7.49 (d, *J* = 8.52 Hz, 2H, H-Ar), 7.33 (d, *J* = 8.08 Hz, 1H, H-4), 3.18 (bs, 4H, piperazine), 2.64 (bs, 4H, piperazine), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 163.6, 156.2, 147.1, 140.9, 139.9, 133.7, 131.6, 131.3, 129.8, 110.7, 110.3, 55.5, 51.3, 46.5; HRMS (ESI) [$(M+H)^+$] *m/z* Calcd for $C_{19}H_{18}ClFN_4S$ 389.0997 found 389.1001.

(*E*)-*N*-(4-Bromobenzylidene)-5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6e)

Orange crystals; mp 179-181 °C; yield 55%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H, N=CH), 7.88 (d, *J* = 8.48 Hz, 2H, H-Ar), 7.66 (d, *J* = 7.04 Hz, 2H, H-Ar), 7.60 (d, *J* = 12.76 Hz, 1H, H-3), 7.33 (d, *J* = 8.08 Hz,

1H, H-4), 3.18 (bs, 4H, piperazine), 2.66 (bs, 4H, piperazine), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 164.2, 156.2, 147.1, 139.9, 134.1, 132.8, 131.7, 131.3, 128.5, 110.7, 110.3, 55.5, 51.3, 46.5; HRMS (ESI) [$(M+H)^+$] *m/z* Calcd for $C_{19}H_{18}BrFN_4S$ 433.0492 found 433.0494.

(*E*)-*N*-(4-Nitrobenzylidene)-5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6f)

Red crystals; mp 236-237 °C; yield 62%; ^1H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H, N=CH), 8.36 (d, *J* = 8.72 Hz, 2H, H-Ar), 8.19 (d, *J* = 8.76 Hz, 1H, H-Ar), 7.64 (d, *J* = 12.72 Hz, 1H, H-3), 7.35 (d, *J* = 8.04 Hz, 2H, H-4), 3.20 (bs, 4H, piperazine), 2.66 (bs, 4H, piperazine) 2.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 162.3, 156.8, 150.5, 147.0, 140.4, 140.3, 131.9, 131.0, 124.6, 110.7, 110.5, 55.5, 51.2, 46.5; HRMS (ESI) [$(M+H)^+$] *m/z* Calcd for $C_{19}H_{18}FN_5O_2S$ 400.1238 found 400.1243.

(*E*)-*N*-(3-Fluorobenzylidene)-5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6g)

Orange crystals; mp 175-176 °C; yield 71%; ^1H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 1H, N=CH), 7.77 (d, *J* = 9.28 Hz, 1H, H-Ar), 7.75 (d, *J* = 8.08, 1H, H-Ar), 7.63 (d, *J* = 12.76 Hz, 1H, H-3), 7.50 (dd, J_1 = 5.92 Hz, J_2 = 13.36 Hz, 1H, H-Ar), 7.34 (d, *J* = 8.04 Hz, 1H, H-4), 7.28 (s, 1H, H-Ar), 3.18 (bs, 4H, piperazine), 2.66 (bs, 4H, piperazine), 2.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 164.0, 162.2, 156.2, 147.1, 140.0, 137.4, 131.4, 130.9, 126.8, 120.4, 116.1, 110.6, 110.2, 55.5, 51.3, 46.5; HRMS (EI) [M] *m/z* Calcd for $C_{19}H_{18}F_2N_4S$ 372.1220 found 372.1245.

(*E*)-*N*-(3-Bromobenzylidene)-5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6h)

Orange crystals; mp 138-140 °C; yield 20%; ^1H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H, N=CH), 8.21 (s, 1H, H-Ar), 7.90 (d, *J* = 7.76 Hz, 1H, H-Ar), 7.69 (d, *J* = 8.84 Hz, 1H, H-Ar), 7.63 (d, *J* = 12.76 Hz, 1H, H-3), 7.39 (t, J_1 = 15.64 Hz, J_2 = 7.84 Hz, 1H, H-Ar), 7.34 (d, *J* = 8.04 Hz, 1H, H-4), 3.16 (bs, 4H, piperazine), 2.65 (bs, 4H, piperazine), 2.39 (CH_3, CH_3-piperazine); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 163.7, 156.2, 148.3, 147.1, 140.0, 137.2, 136.2, 132.8, 131.4, 130.9, 129.3, 110.7, 110.3, 55.5, 51.3, 46.5; HRMS (ESI) [$(M+H)^+$] *m/z* Calcd for $C_{19}H_{18}BrFN_4S$ 433.0492 found 433.0495.

(*E*)-*N*-(3-Nitrobenzylidene)-5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6i)

Orange solid; mp 180-181 °C; yield 71%; ^1H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H, N=CH), 8.85 (s, 1H, H-Ar), 8.40 (d, *J* = 8.16 Hz, 1H, H-Ar), 8.34 (d, *J* = 7.76 Hz, 1H, H-Ar), 7.71 (t, J_1 = 7.92 Hz, J_2 = 8.00 Hz, 1H, H-Ar), 7.64 (d, *J* = 12.76 Hz, 1H, H-3), 7.35 (d, *J* = 8.04 Hz, 1H, H-4), 3.20 (bs, 4H, piperazine), 2.65 (bs, 4H, piperazine), 2.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 162.2, 156.3, 149.2, 147.0, 140.3, 136.9, 135.5, 131.8, 130.5, 127.4, 124.9, 110.6, 110.4, 55.5, 51.2, 46.5; HRMS (ESI) [$(M+H)^+$] *m/z* Calcd for $C_{19}H_{18}FN_5O_2S$ 400.1238 found 400.1242.

(*E*)-*N*-(2-Bromobenzylidene)-5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6j)

Orange crystals; mp 161-162 °C; yield 25%; ^1H NMR (400 MHz, $CDCl_3$) δ 9.32 (s, 1H, N=CH), 8.29 (d, *J* = 7.60 Hz, 1H, H-Ar), 7.60 (m, 1H, H-Ar), 7.58 (m, 1H, H-3), 7.35 (m, 2H, H-Ar), 7.26 (d, *J* = 8.08 Hz, 1H, H-4), 3.14 (bs, 4H, piperazine), 2.59 (bs, 4H, piperazine), 2.31 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$)

δ 171.1, 164.4, 155.2, 150.0, 147.2, 140.0, 134.4, 133.9, 131.3130.6, 130.3, 128.3, 127.9, 110.6, 110.4, 55.5, 51.3, 46.5; HRMS (ESI) [(M+H)$^+$] *m/z* Calcd for $C_{19}H_{18}FN_5O_2S$ 433.0493 found 433.0494.

(*E*)-*N*-(3,4-Dimethoxybenzylidene)-5-fluoro-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6k) Yellow solid; mp 152-155 °C; yield 10%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H, N=CH), 7.72 (s, 1H, H-Ar), 7.60 (d, *J* = 12.76 Hz, 1H, H-3), 7.44 (d, *J* = 8.32 Hz, 1H, H-Ar), 7.32 (d, *J* = 8.04 Hz, 1H, H-4), 6.96 (d, *J* = 8.24 Hz, 1H, H-Ar), 3.99 (s, 3H, Ar-OCH_3), 3.97 (s, 3H, Ar-OCH_3), 3.19 (bs, 4H, piperazine), 2.67 (bs, 4H, piperazine), 2.66 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 165.2, 156.1, 153.8, 150.1, 147.2, 139.5, 130.6, 128.5, 127.4, 110.9, 110.7, 110.3, 110.0, 56.5, 55.5, 51.3, 46.5; HRMS (ESI) [(M+H)$^+$] *m/z* Calcd for $C_{19}H_{18}FN_5O_2S$ 415.1599 found 415.1603.

(*E*)-5-Fluoro-*N*-((furan-2-yl)methylene)-6-(4-methylpiperazin-1-yl)benzo[*d*]thiazol-2-amine (6l) Brown solid; mp 141-142 °C; yield 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1H, N=CH), 7.72 (bs, 1H, H-O-Ar), 7.58 (d, *J* = 12.88 Hz, 1H, H-3), 7.32 (d, *J* = 8.08 Hz, 1H, H-4), 7.23 (d, *J* = 3.44 Hz, 1H, H-Ar), 6.64 (dd, J_1 = 1.64 Hz, J_2 = 3.48 Hz, 1H, H-Ar), 3.17 (bs, 4H, piperazine), 2.64 (bs, 4H, piperazine), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 160.8, 154.9, 151.9, 148.1, 147.1, 139.9, 128.5, 120.9, 113.6, 110.7, 110.3, 55.5, 51.3, 46.5; HRMS (ESI) [(M+H)$^+$] *m/z* Calcd for $C_{17}H_{17}FN_4OS$ 345.1180 found 345.1181.

Cytotoxicity. The viability assays were performed in triplicate in 96-well plates and reproduced in three independent experiments. Cell activity was measured with PrestoBlue® cell viability reagents (Life Technologies) according to the manufacturer's protocol. 5x104 Cells (DMS-53 purchased from ATCC; or primary HLMVECs purchased from LONZA) were seeded into 96-well plates and incubation over night at 37 °C, 5% CO_2 and 95% humidified air. Serial dilutions of the substances were added to the cells in a total volume of 90 µL. After an incubation for another 24 h, 10 µL of Prestoblue® reagent were added, after 30 min fluorescence was measured using the SpectraMax Paradigm Modular Multi-Mode Reader (Molecular Devices).

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