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SYNTHESIS, ANTIBREAST CANCER ACTIVITY AND DOCKING STUDY OF SOME NOVEL 4-(BENZO [D] THIAZOL-2-YL) PHENYL MOIETY AS CDK2 INHIBITORS

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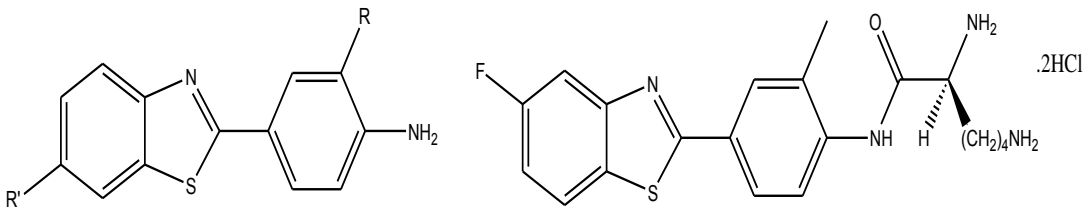
Abstract

Some novel sulfonamides, Schiff's bases and pyrrole derivatives attached to 4-benzo[d]thiazol-2-yl were prepared. Some of the newly synthesized compounds have been evaluated for their potential cytotoxicity against breast cancer cell line (MCF-7) in comparison with doxorubicin; the urea derivative **6a** exhibited the highest activity. Molecular docking into CDK2 has been done for lead optimization of the compounds in study as potential CDK2 inhibitors; compound **8b** showed the lowest binding score.

Key words 4-Benzo[d]thiazol-2-yl. Sulfonamides. Schiff's bases. Pyrrole. MCF-7. CDK2.

Introduction

Benzothiazole [1] is a core subunit in various marine compounds [2-4] and synthetic compounds [5-13] which have useful biological activities, such as neuroprotective **Riluzole** [5] as shown in figure **1** and **Pramipexole** (known commercially as Mirapex), a potent inhibitor for muscarinic receptor [6,7], and also for HIV-protease and reverse transcriptase [8] and many others. Molecules based on benzothiazole derivatives have attracted a great deal of interest due to their antitumor [9], antimicrobial [10], antiviral [11], antipyretic, analgesic, anti-inflammatory [12] and hypoglycemic activity [13].



Riluzole, R = H, R' = OCF_3 **Phortress**

A, R = H, CH_3, Cl, Br, I, R' = H

Fig. 1: The structures of Riluzole, benzothiazole derivatives A and Phortress.

Series of potent and selective anti-tumor agents derived from 2-(4-aminophenyl)benzothiazole derivatives were extensively examined during recent years [14-17]. The 3-methyl derivative **A** as shown in figure **1** showed potent growth inhibitory products against breast and ovarian cancer cell lines *in vitro* and *in vivo*. The 3-methyl analogue has consistently out-performed the 3-halogeno derivatives *in vivo* study against breast [14], ovarian [15] and colon xenografts [16]. The fluorinated analogue, 2-(4-aminophenyl)-5-fluorobenzothiazole is a novel agent with potent and selective anti-tumor properties which in the form of its L-lysylamide prodrug, **Phortress**, as illustrated in figure **1** is in early phase clinical studies [18, 19].

Cyclin-dependent kinases CDKs are one of the most important classes of protein kinases as they are involved in regulating the cell division cycle and apotosis among other activities. They exert their effects on cell cycle by phosphorylating a large number of proteins. Deregulation of activity or expression levels of CDKs is implicated and linked to different stages of tumor genesis, promotion survival and metastasis [20-22]. Within this family, CDK2, an attractive target for oncology, operates primarily for G1/S in cell cycle and undergoes a series of conformational changes upon binding to cyclin and phosphorylation to yield a fully active complex [20, 23-25].

Therefore, blocking of CDK2 activity represents a rational approach to cancer therapy and several drug discovery efforts have targeted this aberrant kinase activity in cancer.

Materials and Methods

Experimental

Melting points (˚C) were determined in open capillary tubes using silicon oil on Gallen Kamp apparatus (Ultraporter Company, Walsall, United Kingdom). ^{1}H-NMR Spectra were measured in DMSO-d_6 on JEOL-270 MHz Spectrometer (JEOL, Canada) with tetramethylsilane as an internal standard. Mass Spectra were obtained with a Schimadzu GCS-QP1000EX Spectrometer (Schimadzu Scientific Instruments, Italy) at 70 ev. The IR Spectra were recorded with a Philips Infra cord Spectrophotometer Model PU 9712 (PerkinElmer, 940 Winter Street, Waltham, Masssachusetts 02451, USA) in KBr discs. Elemental analysis was performed at the Micro analytical Laboratory of the National Research Center. All the reactions were monitored by thin layer chromatography (TLC) on silica gel with chloroform as mobile phase.

4-(Benzo[d]thiazol-2-yl)benzenamine (**1**):

A mixture of 2-(4-nitrophenyl)benzo[d]thiazole (2g, 0.015 mol) and tin(II)chloride dihydrate (8.8g, 0.075 mol) in boiling EtOH (50 mL) was stirred for 2h. EtOH was removed by vacuum evaporation, the residue was extracted into

ethyl acetate (3x10 mL), and the combined organic fractions were shaken with 2M aqueous NaOH solution (3x10 mL), followed by water (3x10 mL). The organic layer was evaporated to leave a residue of the amine which was purified by crystallization from MeOH. Yield = 1.2g (68%), mp = > 300˚C. Anal. Calcd for $C_{13}H_{10}N_2S$ (226.3): C, 69.0; H, 4.5; N, 12.4; S, 14.2. Found: C, 69.2; H, 4.6; N, 12.5; S, 14.3. IR (cm^{-1}): 3423 (NH_2); MS: *m/z* (%): 226 (M^+, 52).

General procedure for the synthesis of N-[4-(benzo[d]thiazol-2-yl)phenyl]-4-(un)substituted-benzamide (**2a**,**b**):

To a solution of compound **1** (1g, 0.15 mol) in 10 mL dry DMF, sodium hydride (0.3 mol) was added slowly and the mixture was stirred vigorously for 5 min. at room temperature. To the resulting solution, benzoyl chloride or 4-methylbenzoyl chloride (0.15 mol) in 2 mL DMF was then added, the mixture was stirred for 6h at room temperature. The reaction mixture was quenched by addition of water and diluted with ethyl acetate. The organic layer was washed with brine two times and dried over $MgSO_4$. After filtration and concentration, the crude product was purified by crystallization from DMF.

N-[4-(Benzo[d]thiazol-2-yl)phenyl]benzamide (**2a**):

Yield = 1.2g (82%), mp = 110-114˚C. Anal. Calcd for $C_{20}H_{14}N_2OS$ (330.4): C, 72.7; H, 4.3; N, 8.5; S, 9.7. Found: C, 72.9; H, 4.2; N, 8.5; S, 9.8. IR (cm^{-1}): 3171 (NH), 1687 (CO); MS: *m/z* (%): 330 (M^+, 27).

N-[4-(Benzo[d]thiazol-2-yl)phenyl]4-methyl-benzamide (**2b**):

Yield = 1.3g (85%), mp = 152-154˚C. Anal. Calcd for $C_{21}H_{16}N_2OS$ (344.4): C, 73.2; H, 4.7; N, 8.1; S, 9.3. Found: C, 73.2; H, 4.5; N, 8.0; S, 9.5. MS: *m/z* (%): 344 (M^+, 44). ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.51 (s, 3H, C<u>H</u>$_3$); 7.28-7.85 (m, 12H, Ar-H); 12.78 (s, 1H, N<u>H</u>, exchangeable with D_2O).

General procedure for synthesis of N-[4-(benzo[d]thiazol-2-yl)phenyl]-sulfonamides (**3a-c**): Compound **1** (1g, 0.016 mol) was dissolved in dry acetone (50 mL). Triethylamine (2.5 mL) was added to this solution. Then a solution of benzene sulfonyl chloride, toluene sulfonyl chloride or camphor-10-sulfonyl chloride (0.016 mol) in dry acetone was added and the mixture was stirred for 2h at room temperature. The solid formed was filtered off and the solvent was removed from the clear solution under reduced pressure. Purification of the product was carried out by preparative thin-layer chromatography using ethyl acetate as eluent. The products were recrystallized from MeOH.

N-[4-(Benzo[d]thiazol-2-yl)phenyl]-benzenesulfonamide (**3a**):

Yield = 1.4g (86%), mp = 130-134˚C. Anal. Calcd for $C_{19}H_{14}N_2O_2S_2$ (366.5): C, 62.3; H, 3.9; N, 7.6; S, 17.5. Found: C, 62.5; H, 3.8; N, 7.5; S, 17.8. IR (cm^{-1}): 3436 (NH), 1398 (SO_2).

N-[4-(Benzo[d]thiazol-2-yl)phenyl]-4-methyl-benzenesulfonamide (**3b**):

Yield = 1.3g (77%), mp = 203-206˚C. Anal. Calcd for $C_{20}H_{16}N_2O_2S_2$ (380.5): C, 63.1; H, 4.2; N, 7.4; S, 16.9. Found: C, 63.3; H; 4.2; N, 7.3; S, 16.8. IR (cm^{-1}): 3436 (NH), 1398 (SO_2). MS: m/z (%): 380 (M^+, 81); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.24 (s, 3H, C<u>H</u>$_3$); 3.13 (s, 1H, N<u>H</u>, exchangeable with D_2O); 7.08-7.43 (m, 12H, Ar-H).

N-[4-(Benzo[d]thiazol-2-yl)phenyl]-C-(7,7-dimethyl-2-oxo-bicyclo [2.2.1]hept-1-yl)-methane sulfonamide (**3c**):

Yield = 1.5g (77%), m.p. = above 300˚C. Anal. Calcd for $C_{23}H_{24}N_2O_3S_2$ (440.6): C, 62.7; H, 5.5; N, 6.4; S, 14.6. Found: C, 62.8; H; 5.3; N, 6.6; S, 14.5. IR (cm^{-1}): 3420 (NH), 1640 (CO), 1375 (SO_2). MS: *m/z* (%): 440 (M^+, 46); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.24 (s, 3H, <u>H</u>$_h$); 1.27 (s, 3H, <u>H</u>$_g$); 1.75-2.87 (m, 7H, <u>H</u>$_{c\text{-}f}$); 3.22 (d, *J* = 6.9 Hz, 1H, <u>H</u>$_a$); 3.31 (d, *J* = 6.9 Hz, 1H, <u>H</u>$_b$); 3.41 (s, 1H, N<u>H</u>, exchangeable with D_2O); 7.47-8.16 (m, 8H, Ar-H) as illustrated in fig **5**.

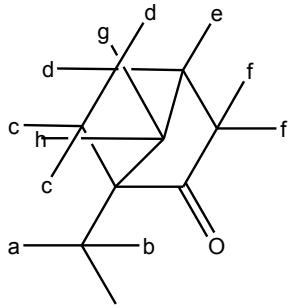


Fig. 5

{[4-(Benzo[d]thiazol-2-yl)-phenyl]ethoxycarbonylmethylamino}acetic acid ethyl ester (**4**):

A mixture of the compound **1** (1g; 0.01 mol), ethyl-2-bromoacetate (1.5 ml, 0.02 mol), anhydrous sodium carbonate (4g.) and acetone (30 ml) was heated under reflux for 8h. Most of the alcohol was distilled off, the residue was diluted with water and the obtained product was collected. Yield = 1.3g (75%); mp = 74-7˚C. Anal. Calcd for $C_{21}H_{22}N_2O_4S$ (398.5): C, 63.3; H, 5.6; N, 7.0; S, 8.0. Found: C, 63.3; H, 5.7; N, 7.2; S, 7.9. MS: *m/z* (%): 398 (M^+, 62); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.70 (t, *J* = 7.1 Hz, 3H, C<u>H</u>$_3$); 1.91 (t, *J* = 7.1 Hz, 3H, C<u>H</u>$_3$); 3.28 (s, 2H, C<u>H</u>$_2$); 3.39 (s, 2H, C<u>H</u>$_2$); 4.72 (q, 2H, C<u>H</u>$_2$); 4.82 (q, 2H, C<u>H</u>$_2$); 6.65-8.23 (m, 8H, Ar-H).

{[4-(Benzo[d]thiazol-2-yl)-phenyl] hydrazine carbonyl methyl-amino} acetic acid hydrazide (**5**):

To a solution of ester compound **4** (1g; 0.033 mol) in EtOH, hydrazine hydrate (98%; 2 mL) was added and heated on a water-bath for 5h. The reaction mixture was cooled. The crude product was filtered, washed with water and dried. It was crystallized from MeOH. Yield = 0.7g (75%); mp = 133-137˚C. Anal. Calcd for $C_{17}H_{18}N_6O_2S$ (370.4): C, 55.1; H, 4.9; N, 22.7; S, 8.7. Found: C, 55.3; H, 5.0; N, 22.9; S, 8.9. IR (cm^{-1}): 3744-3296 (NH, NH_2), 1654-1604 (CO). MS:

m/z (%): 370 (M^+, 18); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.46 (s, 2H, N<u>H</u>$_2$. exchangeable with D_2O); 2.50 (s, 2H, N<u>H</u>$_2$, exchangeable with D_2O); 3.74 (s, 2H, C<u>H</u>$_2$); 3.76 (s, 2H, C<u>H</u>$_2$); 6.64-8.24 (m, 8H, Ar-H); 8.01 (s, 1H, N<u>H</u>, exchangeable with D_2O); 8.04 (s, 1H, N<u>H</u>, exchangeable with D_2O).

General procedure for synthesis of 1-{4[-(benzo[d]thiazol-2-yl)phenyl]}-3-(un)substituted-phenylurea/thiourea (**6a**,**b**):

A mixture of **1** (1g, 0.01 mol) and 4-chloroarylisocyanate or arylisothiocyanate (0.01 mol) in dry benzene was heated under reflux for 6h. The solid material obtained on cooling was filtered off and recrystallized from MeOH.

1-{4[-(Benzo[d]thiazol-2-yl)phenyl]}-3-(4-chlorophenyl)urea (**6a**):

Yield = 1.4g (83%); mp = >300˚C. Anal. Calcd for $C_{20}H_{14}ClN_3OS$ (379.8): C, 63.2; H, 3.7; N, 11.1; S, 8.4. Found: C, 63.4; H, 3.5; N, 11.2; S, 8.5. IR (cm^{-1}): 3295-3228 (2NH), 1632 (CO); MS: *m/z* (%): 381 (M^+, 3.3); 379 (M^+, 10); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.29-8.21 (m, 12H, Ar-H); 8.91 (s, 1H, N<u>H</u>, exchangeable with D_2O); 9.07 (s, 1H, N<u>H</u>, exchangeable with D_2O).

1-{4[-(Benzo[d]thiazol-2-yl)phenyl]}-3-phenylthiourea (**6b**):

Yield = 1.2g (75%); mp = 275-278˚C. Anal. Calcd for $C_{20}H_{15}N_3S_2$ (361.5): C, 66.5; H, 4.2; N, 11.6; S, 17.7. Found: C, 66.5; H, 4.2; N, 11.7; S, 17.8. IR (cm^{-1}): 3744-3296 (NH, NH_2). MS: *m/z* (%): 361 (M^+, 58); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.29-8.21 (m, 13H, Ar-H); 8.91 (s, 1H, N<u>H</u>, exchangeable with D_2O); 9.07 (s, 1H, N<u>H</u>, exchangeable with D_2O).

General procedure for the synthesis of [4-(benzo[d]thiazol-2-yl)-phenyl]-benzylideneamine/imino methyl-benzonitrile (**7a**,**b**):

To a solution of compound **1** (1g; 0.018 mol) dissolved in absolute EtOH (30 mL), benzaldehyde or 4-cyanobenzaldehyde (0.018 mol) was added and few drops of glacial acetic acid was added then the mixture was heated under reflux for 6h. Solvent was distilled off, white solid product crystallized from ethylacetate / petroleum ether (95:5).

[4-(Benzo[d]thiazol-2-yl)-phenyl]-benzylidene-amine (**7a**):

Yield = 0.9g (65%); mp = >300˚C. Anal. Calcd for $C_{20}H_{14}N_2S$ (314.4): C, 76.4; H, 4.5; N, 8.9; S, 10.2. Found: C, 76.8; H, 4.5; N, 8.9; S, 10.3. MS: *m/z* (%): 314 (M^+, 32); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.43-8.46 (m, 13H, Ar-H); 7.73 (s, 1H, C<u>H</u>).

4-{[4-(Benzo[d]thiazol-2-yl)phenyl imino]methyl}-benzonitrile (**7b**):

Yield = 0.8g (53%); mp = >300˚C. Anal. Calcd for $C_{21}H_{13}N_3S$ (339.4): C, 74.3; H, 3.9; N, 12.4; S, 9.5. Found: C, 74.4; H, 4.0; N, 12.5; S, 9.3. IR (cm^{-1}): 2100 (CN). MS: *m/z* (%): 339 (M^+, 42); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.43-8.46 (m, 12H, Ar-H); 7.73 (s, 1H, C<u>H</u>).

General procedure for the synthesis of (benzo[d]thiazol-2-yl)phenylimino] pentane-1,2,3,4-tetraol/hexane-1,2,3,4,5-pentaol (**8a-c**):

To a solution of compound **1** (1g, 0.1 mol) in 50 mL EtOH, the respective monosaccharide (aldoses) namely, D-(+)-xylose, D-(+)-glucose and/or D-(+)-galactose (0.1 mol) and 0.1 mL acetic acid were added. The mixture was heated under reflux on a water-bath for 2h. The solid that separated on cooling was filtered, washed with EtOH and crystallized from the appropriate solvent.

5-(Benzo[d]thiazol-2-yl)phenyl imino]pentane-1,2,3,4-tetraol (***8a***):

Yield = 1.2g (75%); mp = >300˚C. Anal. Calcd for $C_{18}H_{18}N_2O_4S$ (358.4): C, 60.3; H, 5.1; N, 7.8; S, 9.0. Found: C, 60.0; H, 5.2; N, 7.5; S, 8.8. IR (cm^{-1}): 3780-3408 (OH). MS: *m/z* (%): 358 (M^+, 74); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.49-2.53 (s, 4H, O<u>H</u>, exchangeable with D_2O); 3.29 (m, 1H, $^{'}H_5$); 4.10 (m, 1H, $^{''}H_5$); 4.20 (t, *J* = 7.1 Hz, 1H, $^1H_2$); 4.31 (t, *J* = 7.1 Hz, 1H, $^1H_4$); 5.16 (t, *J* = 7.1 Hz, 1H, $^1H_3$); 5.95 (d, *J* = 6.9 Hz, 1H, C<u>H</u>); 7.56-8.25 (m, 8H, Ar-H).

6-(Benzo[d]thiazol-2-yl)phenyl imino]hexane-1,2,3,4,5-pentaol (**8b**):

Yield = 1.3g (75%); mp = >300˚C. Anal. Calcd for $C_{19}H_{20}N_2O_5S$ (388.4): C, 58.8; H, 5.2; N, 7.2; S, 8.3. Found: C, 58.9; H, 5.2; N, 7.3; S, 8.2. IR (cm^{-1}): 3780-3409 (OH). MS: *m/z* (%): 388 (M^+, 69); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.30 (s, 5H, O<u>H</u>, exchangeable with D_2O); 3.13 (m, 1H, $^{'}H_6$); 3.29 (m, 1H, $^{''}H_6$); 4.20 (t, *J* = 7.1 Hz, 1H, $^1H_2$); 4.31 (t, *J* = 7.1 Hz, 1H, $^1H_4$); 4.89 (m, 1H, $^1H_5$); 5.16 (t, *J* = 7.1 Hz, 1H, $^1H_3$); 5.95 (d, *J* = 6.9 Hz, 1H, C<u>H</u>); 7.55-8.16 (m, 8H, Ar-H).

6-(Benzo[d]thiazol-2-yl)phenyl imino]hexane-1,2,3,4,5-pentaol (**8c**):

Yield = 1.3g (75%); mp = >300˚C. Anal. Calcd for $C_{19}H_{20}N_2O_5S$ (388.4): C, 58.8; H, 5.2; N, 7.2; S, 8.3. Found: C, 58.9; H, 5.2; N, 7.3; S, 8.2. IR (cm^{-1}): 3780-3206 (OH). MS: *m/z* (%): 388 (M^+, 69); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.30 (s, 5H, O<u>H</u>, exchangeable with D_2O); 3.13 (m, 1H, $^{'}H_6$); 3.29 (m, 1H, $^{''}H_6$); 4.20 (t, *J* = 7.1 Hz, 1H, $^1H_2$); 4.31 (t, *J* = 7.1 Hz, 1H, $^1H_4$); 4.89 (m, 1H, $^1H_5$); 5.16 (t, *J* = 7.1 Hz, 1H, $^1H_3$); 5.95 (d, *J* = 6.9 Hz, 1H, C<u>H</u>); 7.55-8.16 (m, 8H, Ar-H).

General procedure for the preparation of 1-/1-/2-[4-(benzo[d]thiazol-2-yl)phenyl]-1H-pyrrole-2,5-dione/pyrrolidine-2,5-dione/isoindoline-1,3-dione (**9a-c**):

To a stirred solution of compound **1** (1g; 0.017 mol) in glacial acetic acid (10 mL), acid anhydride (maleic anhydride, succinic anhydride or phthalic anhydride) (0.0348 mol) was added. The mixture was heated under reflux with stirring for 8h. The precipitate formed was filtered, washed with water and the crude product was crystallized from EtOH.

1-[4-(Benzo[d]thiazol-2-yl)phenyl]-1H-pyrrole-2,5-dione (**9a**): Yield = 0.7g (52%); mp = 112-115˚C. Anal. Calcd for $C_{17}H_{10}N_2O_2S$ (306.3): C, 66.7; H, 3.3; N, 9.1; S, 10.5. Found: C, 67.0; H, 3.2; N, 9.3; S, 10.4. IR (cm^{-1}): 1719 (CO); 1640 (CO). MS: *m/z* (%): 306 (M^+, 62); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 6.18 (d, *J* = 6.9 Hz, 2H, 2C<u>H</u>=); 7.21-8.06 (m, 8H, Ar-H).

1-[4-(Benzo[d]thiazol-2-yl)phenyl]-pyrrolidine-2,5-dione (**9b**):

Yield = 0.8g (59%); mp = 122-125˚C. Anal. Calcd for $C_{17}H_{12}N_2O_2S$ (308.3): C, 66.2; H, 3.9; N, 9.1; S, 10.4. Found: C, 66.3; H, 3.9; N, 9.3; S, 10.5. IR (cm^{-1}): 1704 (CO); 1620 (CO). MS: *m/z* (%): 308 (M^+, 62); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.34 (t, *J* = 7.1 Hz, 2H, C<u>H</u>$_2$); 2.41 (t, *J* = 7.1 Hz, 2H, C<u>H</u>$_2$); 7.47-8.23 (m, 8H, Ar-H).

2-[4-(Benzo[d]thiazol-2-yl)phenyl]-isoindoline-1,3-dione (**9c**):

Yield = 1.0g (64%); mp = 186-188˚C. Anal. Calcd for $C_{21}H_{12}N_2O_2S$ (356.4): C, 70.8; H, 3.4; N, 7.9; S, 9.0. Found: C, 71.0; H, 3.4; N, 8.1; S, 9.3. MS: *m/z* (%): 356 (M^+, 10); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.47-8.23 (m, 12H, Ar-H).

3[4-(Benzothiazol-2-yl)phenyl imino]-5-nitroindolin-2-one (**10**):

To a solution of compound **1** (1g; 0.018 mol) in absolute EtOH (30 mL), 5-nitroisatin (0.85g, 0.018 mol) and few drops of glacial acetic acid were added then the mixture was heated under reflux for 6h. Solvent was distilled off, white solid product crystallized from ethylacetate/petroleum ether (95:5). Yield = 1.5g (85%); mp = >300˚C. Anal. Calcd for $C_{21}H_{12}N_4O_3S$ (400.4): C, 63.0; H, 3.0; N, 14.0; S, 8.0. Found: C, 63.3; H, 3.0; N, 14.2; S, 8.3. IR (cm^{-1}): 3335 (NH); 1735 (CO). MS: *m/z* (%): 400 (M^+, 69); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.06-8.41 (m, 11H, Ar-H); 11.70 (s, 1H, N<u>H</u>, exchangeable with D_2O).

2-(4-Cyanophenyl)benzo[d]thiazole (**11**):

4-Cyanobenzaldehye (1.05 g, 0.21 mol) and o-aminothiophenol **1** (1 mL, 0.21 mol) were dissolved in EtOH. This mixture was heated under reflux for 5 h and cooled to room temperature. Then, water was added slowly to the mixture with stirring. The suspension was maintained at -5 ºC overnight. The product was washed repeatedly with EtOH-water (1:1) mixture and then recrystallized from acetone. Yield = 1.2 g (64 %), mp = 150-152 ˚C. Anal. Calcd for $C_{14}H_8N_2S$ (236.2): C, 69.0; H, 4.5; N, 12.4; S, 14.2. Found: C, 69.2; H, 4.6; N, 12.5; S, 14.3. MS: *m/z* (%): 236 (M^+, 69).

4-(Benzo[d]thiazol-2-yl)benzoic acid (**12**):

A mixture of **11** (2g, 0.01 mol) and 15 mL 70% sulfuric acid was stirred at 140ºC for 5h, then suspended in 150 mL water and the resulting precipitate was filtered off. Recrystallization from diluted EtOH afforded white crystals. Yield = 0.8g (74%); mp = 250-253˚C. Anal. Calcd for $C_{14}H_9NO_2S$ (255.3): C, 65.9; H, 3.6; N, 5.5; S, 12.6. Found.: C, 66.0; H, 3.6; N, 5.6; S, 12.8. MS: *m/z* (%): 255 (M^+, 100).

General procedure for the preparation of ethyl 2[4-(benzo[d]thiazol-2-yl)benzamido]propanoate/4-(methylthio)butanoate (**13a**,**b**):

A stirring mixture of D-alanine or L-methionine ethyl ester hydrochloride (0.038 mol) and compound **12** (1g, 0.038 mol) in anhydrous methylene chloride (30 mL) was cooled to 0˚C. Diisopropylethylamine (1.86g, 0.14 mol) was then slowly added to the mixture, followed by the addition of the coupling reagent benzotriazol-1-yloxytris(dimethylamino)phosphonimhexafluorophosphate reagent (BOP) (1.91g, 0.042 mol) dissolved in 5 mL of anhydrous methylene chloride. The reaction was stirred for 12h at 20˚C. Ethyl acetate (50 mL) was added to the reaction mixture and the organic layer was successively washed with a 1N hydrochloric acid solution (2x35 mL), a 20% sodium carbonate solution (2x30 mL) and brine. The organic layer was dried over magnesium sulfate and concentrated in vacuum. The crude residue was purified by chromatography on a silica gel column using ethyl acetate/petroleum ether as eluent (1:1).

Ethyl 2[4-(benzo[d]thiazol-2-yl)benzamido]propanoate (**13a**):

Yield = 1.2g (86%); mp = 204-207˚C. Anal. Calcd for $C_{19}H_{18}N_2O_3S$ (354.4): C, 64.4; H, 5.1; N, 7.9; S, 9.1. Found: C, 64.5; H, 5.2; N, 8.0; S, 9.3. IR (cm^{-1}): 3430 (NH), 1786 (CO), 1745 (CONH). MS: *m/z* (%): 354 (M^+, 41); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.17 (t, *J* = 7.1 Hz, 3H, C$\underline{H}_3$); 2.48 (d, *J* = 6.9 Hz, 3H, C$\underline{H}_3$); 4.15 (q, 2H, C$\underline{H}_2$CH$_3$); 4.97 (q, 1H, C$\underline{H}$); 7.49-8.29 (m, 8H, Ar-H); 8.30 (s, 1H, NH, exchangeable with D_2O). ^{13}C-NMR (270 MHz, DMSO-d_6) δ 13.9 ($\underline{C}$H$_3$CH$_2$), 30.5 ($\underline{C}$H$_3$CH), 60.9 ($\underline{C}$HCH$_3$), 61.4 ($\underline{C}$H$_2$CH$_3$), 122.4-130.7 (Ar-8$\underline{C}$H), 134.7 ($\underline{C}$=C), 137.0 ($\underline{C}$S), 153.3 ($\underline{C}$-N), 164.5 ($\underline{C}$=C), 165.7 ($\underline{C}$=N) and 167.5 (2$\underline{C}$O).

Ethyl 2[4-(benzo[d]thiazol-2-yl)benzamido]-4-(methylthio)butanoate (**13b**):

Yield = 1.45g (89%); mp = 240-243˚C. Anal. Calcd for $C_{21}H_{22}N_2O_3S_2$ (414.5): C, 60.9; H, 5.4; N, 6.8; S, 15.5. Found: C, 61.0; H, 5.6; N, 7.0; S, 15.7. IR (cm^{-1}): 3400 (NH), 1740 (CO), 1705 (CONH). MS: *m/z* (%): 414 (M^+, 51); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.02 (t, *J* = 7.1 Hz, 3H, C$\underline{H}_3$); 1.05 (t, *J* = 7.1 Hz, 2H, C$\underline{H}_2$S); 1.33 (q, 2H, C$\underline{H}_2$); 2.50 (s, 3H, C$\underline{H}_3$); 3.33 (t, *J* = 7.1 Hz, 1H, C$\underline{H}$); 4.32 (q, 2H, C$\underline{H}_2$CH$_3$); 7.48-8.26 (m, 8H, Ar-H); 11.27 (s, 1H, N$\underline{H}$,

exchangeable with D_2O). ^{13}C-NMR (270 MHz, DMSO-d_6) δ 14.0 ($\underline{C}H_3CH_2$), 19.5 ($\underline{C}H_3S$), 38.6 ($\underline{C}H_2CH$), 40.2 ($\underline{C}H_2CH_2$), 58.0 ($\underline{C}HCH_2$), 61.0 ($\underline{C}H_2CH_3$), 122.4-132.8 (Ar-8$\underline{C}$H), 134.6 ($\underline{C}$=C), 136.5 ($\underline{C}$S), 153.4 ($\underline{C}$-N), 164.9 ($\underline{C}$=C), 165.8 ($\underline{C}$=N) and 166.0-166.5 (2$\underline{C}$O).

Ethyl 4-(benzo[d]thiazol-2-yl)benzoate (**14**): To a solution of compound **12** (2g; 0.073 mol) in absolute EtOH, few drops of concentrated sulfuric acid were added and the mixture was heated under reflux for 4h. The crude product was filtered, air-dried and crystallized from EtOH. Yield = 2.1g (94%), mp = 102-106˚C. Anal. Calcd for $C_{16}H_{13}NO_2S$ (283.3): C, 67.8; H, 4.6; N, 4.9; S, 11.3. Found: C, 68.0; H, 4.6; N, 5.0; S, 11.5. MS: *m/z* (%): 283 (M^+, 45); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.32 (t, *J* = 7.1 Hz, 3H, C$\underline{H}_3$); 4.32 (q, 2H, C$\underline{H}_2$CH$_3$); 7.48-8.22 (m, 8H, Ar-H).

Furan-2-carboxylic acid-N'[4-(benzo[d]thiazol-2-yl)-benzoyl]hydrazide (**15**):

To a solution of ester compound **14** (1g; 0.033 mol) in EtOH, 2-furoichydrazide (0.033 mol) was added and heated on a water-bath for 5h. The reaction mixture was cooled. The crude product was filtered, washed with water and dried. It was crystallized from MeOH. Yield = 1.1g (86%); mp = 112-115˚C. Anal. Calcd for $C_{19}H_{13}N_3O_3S$ (363.4): C, 62.8; H, 3.6; N, 11.6; S, 8.8. Found: C, 63.0; H, 3.6; N, 11.8; S, 9.0. IR (cm^{-1}): 3310-3270 (NH), 1706-1650 (CO). MS: *m/z* (%): 363 (M^+, 31); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.47-8.24 (m, 11H, Ar-H); 8.81 (s, 1H, N$\underline{H}$, exchangeable with D_2O); 8.83 (s, 1H, N$\underline{H}$, exchangeable with D_2O).

{[4-(Benzo[d]thiazol-2yl)phenyl]1H-imidazol-1-yl}methanone (**16**):

A mixture of compound **14** (1g, 0.002 mol), 1*H*-imidazole (0.23g, 0.002 mol) and 20 mL of EtOH were heated under reflux for 10h. The reaction mixture was then cooled and the precipitate was filtered off, dried and recrystallized from MeOH. Yield = 0.9g (84%), mp = 83-86˚C. Anal. Calcd for $C_{17}H_{11}N_3OS$ (305.3): C, 66.9; H, 3.6; N, 13.8; S, 10.5. Found: C, 66.9; H, 3.6; N, 13.9; S, 10.7. IR (cm^{-1}): 1633 (CO). MS: *m/z* (%): 305 (M^+, 35); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.47-8.33 (m, 11H, Ar-H).

General procedure for the preparation of 4-benzo[d]thiazol-2-yl-N-(4-sulfamoylphenyl)/[4-(thiazol-2-yl-sulfamoyl)phenyl]benzamide (**17a**,**b**):

Compound **14** (1g, 0.0034 mol) was dissolved in EtOH (20 mL) and 10% aqueous NaOH solution, the sulfa drug (sulfanilamide or sulfathiazole) (0.0034 mol) was added dropwisely and the reaction mixture was stirred for 4h at room temperature. The mixture was then poured onto ice/cold water and neutralized with dilute HCL. The crude product was filtered and crystallized from petroleum ether.

4-(Benzo[d]thiazol-2-yl)-N-(4-sulfamoylphenyl)benzamide (**17a**):

Yield = 1.2g (83%), mp = 247-249˚C. Anal. Calcd for $C_{20}H_{15}N_3O_3S_2$ (409.5): C, 58.7; H, 3.7; N, 10.3; S, 15.7. Found: C, 58.9; H, 3.8; N, 10.5; S, 15.7. MS: *m/z* (%): 409 (M^+, 45); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.32 (s, 2H, N<u>H</u>$_2$, exchangeable with D_2O); 6.85 (s, 1H, N<u>H</u>, exchangeable with D_2O); 7.27-8.18 (m, 12H, Ar-H).

4-(Benzo[d]thiazol-2-yl)-N-[4-(thiazol-2-yl-sulfamoyl)phenyl]benzamide (**17b**):

Yield = 1.3g (75%), mp = >300˚C. Anal. Calcd for $C_{23}H_{16}N_4O_3S_3$ (492.6): C, 56.1; H, 3.3; N, 11.4; S, 19.5. Found: C, 56.2; H, 3.6; N, 11.5; S, 19.7. MS: *m/z* (%): 492 (M^+, 72); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.46 (s, 1H, N<u>H</u>, exchangeable with D_2O); 3.47 (s, 1H, N<u>H</u>, exchangeable with D_2O); 7.42-8.29 (m, 12H, Ar-H)**;** 8.01 (d, *J* = 6.9 Hz, 1H, C<u>H</u>); 8.09 (d, *J* = 6.9 Hz, 1H, C<u>H</u>).

Bioactivity materials and methods

Cytotoxicity against human breast cancer cell line MCF-7

Ten of the newly synthesized compounds have been evaluated for their potential cytotoxicity testing against breast cancer (MCF7) using the method of Skehan *et al.* [27]. Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the tested compounds to allow attachment of cells to the wall of the plate. For each tested compound, a solution of the compound was prepared by dissolving 1 µg of each compound in 1mL DMSO. After being incubated for 24 h, cells were treated with 5, 12.5, 25 and 50 µg/ml different concentration of compounds in triplicate and were being further incubated for 48 h at 37 ºC and in atmosphere of 5% CO_2.

Control cells were treated with vehicle alone. After 48 h, cells were fixed, washed and stained with 0.4% (wt/vol) SRB dissolved in 1 % acetic acid, for 30 min. Excess unbound dye was removed by four washes with 1 % acetic acid and attached stain was recovered with tris-EDTA buffer. Color intensity was measured in an ELISA reader at a wave length of 570 nm.

Molecular docking study

All molecular modeling studies were carried out on an Intel Pentium 1.6 GHz processor, 512 MB memory with Windows XP operating system using MOE, 10.2008 software [25, 28]. All the minimizations were performed with MOE until a RMSD gradient of 0.05 kcal $mol^{-1}A^{o-1}$ with MMFF94X forcefield and the partial charges were automatically calculated.

The X-ray crystal structure of the enzyme with benzothiazole ligand (PDB code: 1FVV) [25, 29], was obtained from the protein data bank in PDF format.

The enzyme was prepared for docking studies; Ligand molecule was removed from the enzyme active site, hydrogen atoms were added to the isolated target with their standard geometry, a connect and type procedure was run for automatic completion of missed bonds during isolation and crystallization.

The target was fixed to be dealt as a rigid structure, the active site was isolated by the Alpha Site Finder tool using the binding amino acids as key elements in isolation and dummies were created around the active site.

Preparation of the synthesized compounds

The 2D structures of the compounds were built using Chem Sketch and were saved as mol file; the latter was subjected to energy minimization and conformational search using hyperchem 8.

The lowest energy conformation was selected for docking studies. Torsion angles of these conformers were measured.

Docking running

Prior to the docking of the benzothiazole derivatives, redocking of the native ligand bound in the CDK2 active site was performed to validate the docking protocol. The generated most stable conformer of each compound was virtually docked into the predefined active site of the CDK2.

The developed dock models were energetically minimized and were then used to predict the interactions of the ligand with the amino acids in the active site of the enzyme.

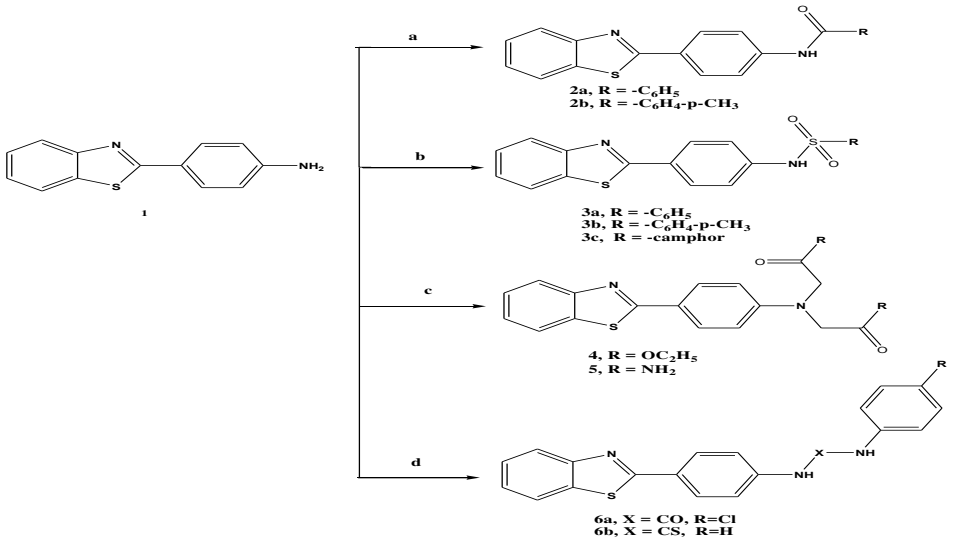
Results and discussion

Chemistry

4-(Benzo[d]thiazol-2-yl)benzenamine **1** was prepared according to reported procedure [26]. Reacting **1** with benzoyl chloride or 4-methylbenzoyl chloride, in 10 mL DMF and sodium hydride form the following products:

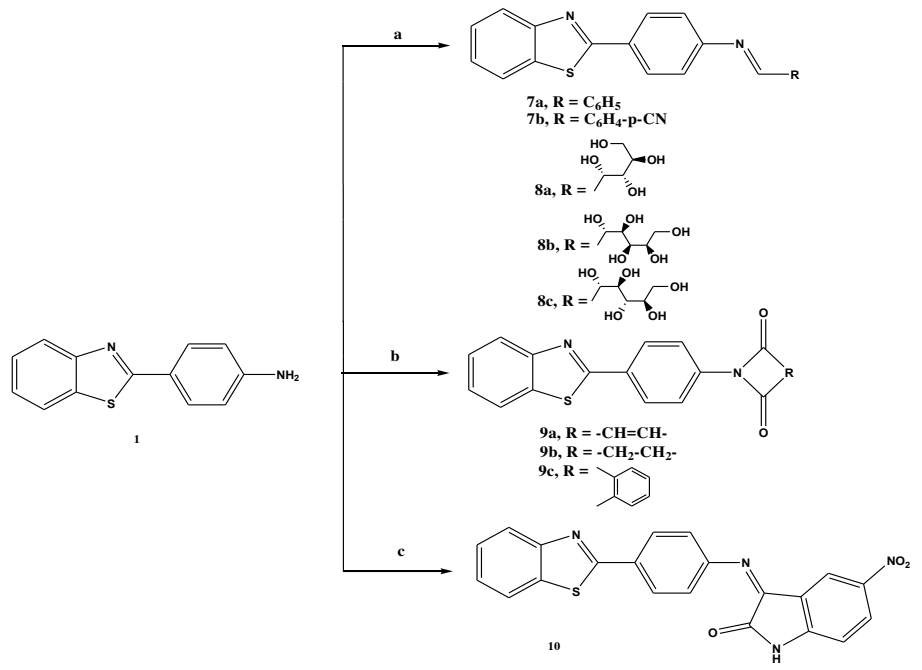
N-[4-(benzo[d]thiazol-2-yl)phenyl]benzamide (**2a**) and N-[4-(benzo[d]thiazol-2-yl)phenyl]-4-methylbenzamide (**2b**), in addition, stirring benzenamine **1** with sulfonyl chloride, toluene sulfonyl chloride or camphor-10-sulfonyl chloride in dry acetone and triethylamine, yielded the corresponding sulfonamides **3a-c** as illustrated in Scheme **1**.

Compounds **4** and **5** were obtained through esterification of **1** with two equivalent ethyl-2-bromoacetate followed by hydrazine hydrate. Compounds **6a**,**b** were synthesized *via* the reaction of benzenamine **1** with 4-chloroarylisocyanate or arylisothiocyanate in dry benzene as illustrated in Scheme **1**.



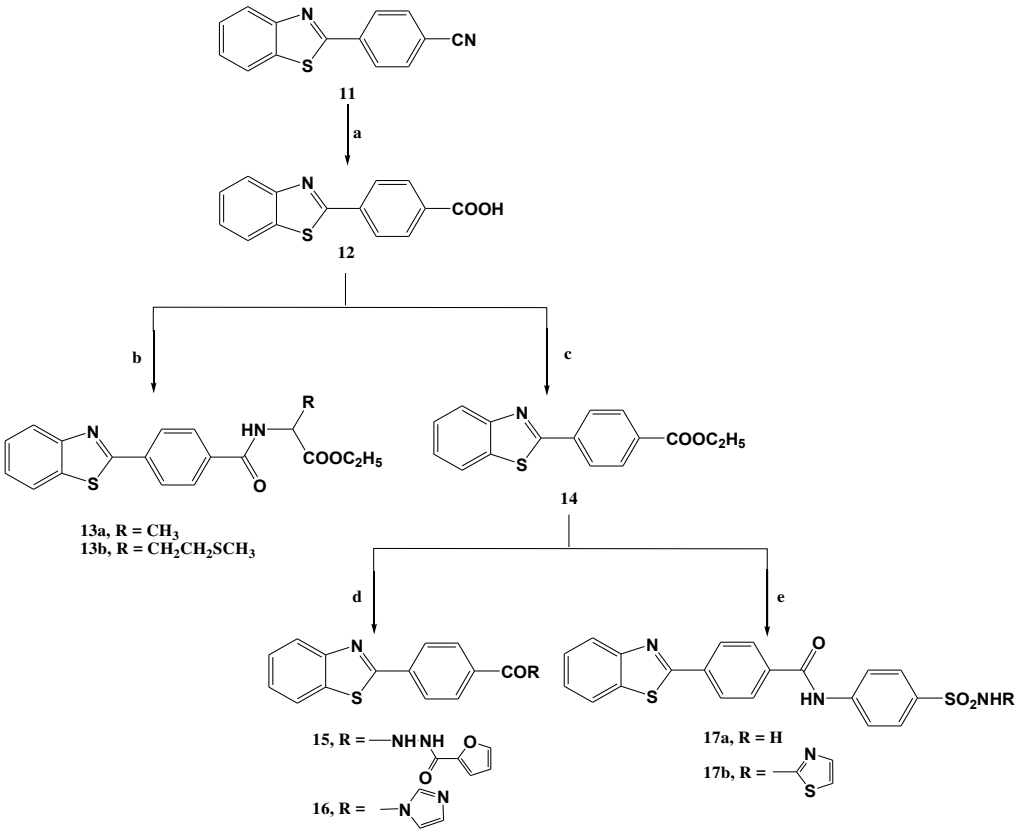
Scheme-1: Reagents: (a) benzoyl chloride or 4-methylbenzoyl chloride, DMF, sodium hydride; **(b)** sulfonyl chloride, toluene sulfonyl chloride or camphor-10-sulfonyl chloride, dry acetone, triethylamine; **(c)** i) two equivalent ethyl-2-bromoacetate, acetone, Na_2CO_3; ii) NH_2NH_2, EtOH; **(d)** 4-chloroarylisocyanate or arylisothiocyanate, dry benzene.

Benzenamine **1** was heated under reflux with the appropriate arenaldehydes (benzaldehyde or 4-cyanobenzaldehyde) in the presence of EtOH and few drops of glacial acetic acid to give the corresponding Schiff's bases **7a**,**b**. Also, heating under reflux compound **1** with various monosaccharide (aldoses) namely, D-(+)-xylose, D-(+)-glucose and/or D-(+)-galactose yielded the following products: 5-(benzo[d]thiazol-2-yl)phenylimino]pentane-1,2,3,4-tetraol (**8a**), 6-(benzo[d]thiazol-2-yl)phenylimino]hexane-1,2,3,4,5-pentaol (**8b**) and 6-(benzo[d]thiazol-2-yl)phenylimino]hexane-1,2,3,4,5-pentaol (**8c**). Products **9a-c** formed *via* the reaction of benzenamine **1** with the appropriate acid anhydride. 3[4-(Benzo[d]thiazol-2-yl)phenylimino]-5-nitroindolin-2-one (**10**) was prepared by reacting **1** with 5-nitroisatin dissolved in EtOH and few drops of glacial acetic acid as shown in Scheme **2**.



Scheme-2: Reagents: (a) i) benzaldehyde or 4-cyanobenzaldehyde, EtOH, AcOH; ii) D-(+)-xylose, D-(+)-glucose and/or D-(+)-galactose, EtOH, AcOH; (**b**) maleic anhydride, succinic anhydride and/or phthalic anhydride, AcOH; (**c**) 5-nitroisatin, EtOH, AcOH.

2-(4-Cyanophenyl)benzo[d]thiazole **11** was prepared according to reported procedure [26]. Acid oxidation of cyano group by stirring with 70% sulfuric acid to give 4-(benzo[d]thiazol-2-yl)benzoic acid (**12**). Products **13a**,**b** were prepared from coupling compound **12** with D-alanine or L-methionine ethyl ester hydrochloride in presence of coupling reagent BOP and diisopropylethylamine as illustrated in Scheme **2**. The derivatives **15** and **16** and sulfa compounds **17a**,**b** were obtained from compound **12** through esterification and reacting with the following reagents: (2-furoichydrazide, 1*H*-imidazole, sulfanilamide or sulfathiazole) as shown in Scheme **3**.



Scheme-3: Reagents: (a) i) o-aminothiophenol, 4-cyanobenzaldehyde, EtOH**;** ii) 70% H_2SO_4; **(b)** D-alanine or L-methionine ethyl ester HCL, BOP, diisopropylethylamine; **(c)** EtOH, H_2SO_4; **(d)** 2-furoichydrazide or 1*H*-imidazole, EtOH; **(e)** sulfanilamide or sulfathiazole, EtOH, 10% NaOH.

Biological evaluation

Cytotoxicity against human breast cancer cell line MCF-7

Potential cytotoxicty of ten representative compounds prepared in this study was measured *in vitro* by the SRB assay [27]. The selected compounds represent various classes of the new target (4-substituedphenyl)-benzothiazol-2-yl

derivatives. The inhibitory activity of the selected compounds (**3c**, **6a**,**b**, **8a**,**b**, **9a**, **13a**,**b**, **15** and **17b**) was tested against the human breast carcinoma cell line (MCF-7) in comparison to the known anticancer drug: doxorubicin as reference drug. The ten compounds selected were carefully chosen to be representatives for all the newly synthesized twenty four derivatives and covering all structural variations in these analogs, being of camphor sulphonyl (**3c**), urea and thiourea derivatives (**6a**,**b**), sugar [D(+)xylose and D(+)glucose] derivatives (**8a**,**b**) Figure **2**, pyrrole derivative (**9a**), alanine and methionine ethyl ester derivatives (**13a**,**b**), 2-furoic hydrazide (**15**), sulfamoyl benzamide derivative (**17b**). It has been noticed from Table **1** that most of the tested compounds showed antibreast cancer activities.

Table-1: IC_{50} (µg/ml) of some selected new compounds against MCF-7.

Compound	IC_{50} (µg/ml)
3c	> 50
6a	**8.4**
6b	20.9
8a	21.2
8b	**13.0**
9a	22.8
13a	> 50
13b	18.3
15	17.0
17b	19.5
DOX	4.5

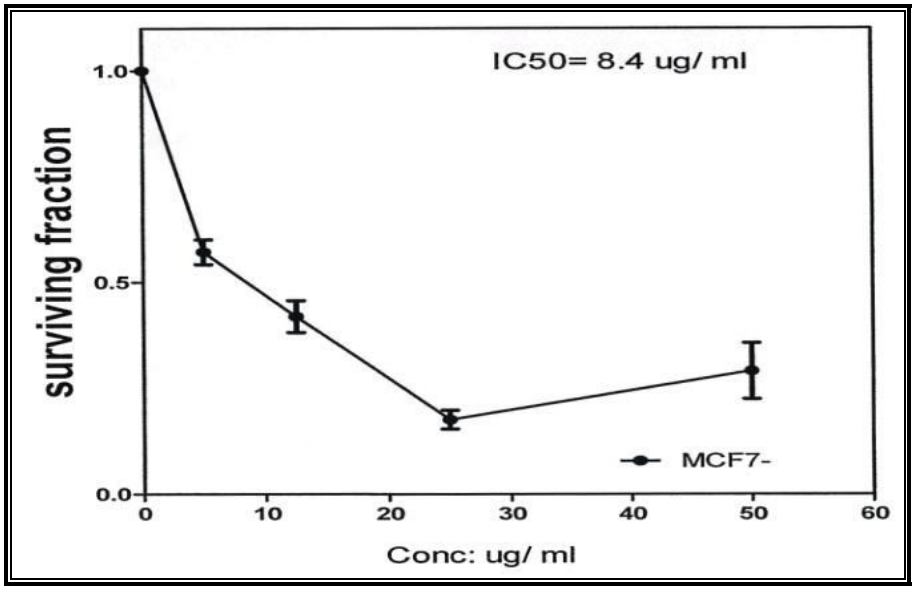


Fig. 2: IC_{50} (µg/ml) of 6a compound against MCF-7.

Molecular docking study

Docking our compounds on the active site of CDK2 were evaluated and determined their interactions with amino acids on the active site of this enzyme in order to better understand their biological activity as cytotoxic agents. A protein data bank file with the code 1FVV was selected for this purpose. The file contains the CDK2 enzyme co-crystallized with a benzothiazole ligand [27]. All docking procedures were achieved using MOE software 10.2008 provided by chemical computing group, Canada. Docking on the active site of CDK2 was performed for ten compounds which showed cytotoxic activity.

Validation of the accuracy of MOE

The inhibitor-CDK2 complex was precisely reproduced by the docking procedure as demonstrated by low (3.1726 A˚) root-mean-square deviation and dock score (-18.1741 Kcal/mol, Table **2**), i.e. the docking protocol was valid. As shown in Figure **3**, the inhibitor nearly fits in the active site forming various hydrogen bonding interactions with the active site residues: O=S=O with Lys A 89 (3.13 A˚) and Asp A86 (3.22 A˚), CO of pyrrole with Leu A83 (2.96 A˚) and NH of pyrrole with Glu A81 (1.98 A˚).

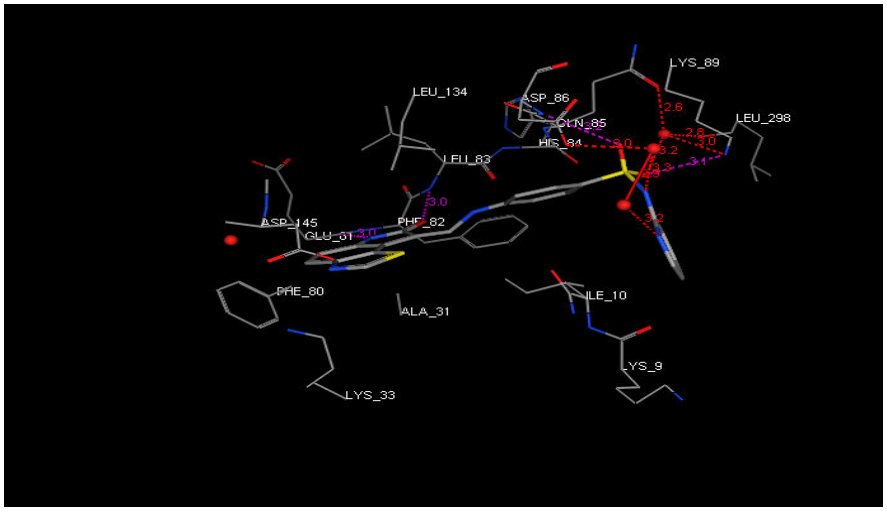


Fig. 3: **Interaction map of Ligand with CDK2 3D.**

Table-2: Binding scores and interaction of the amino acids on the active site of CDK2 with the docked compounds.

Compound	S (kcal/mol)	Amino acid	Groups interacted	Length (A˚)
3c	-26.2594	Lys A89	O=C-NH	2.44
6a	-26.4755	Asp A86	O=C	2.63
		Lys A89	Phenyl	Arene cation
6b	-22.0941	Lys A89	Phenyl	Arene cation
		His A84	H-N-phenyl	2.06

8a	-27.3361	Lys A89	O-H	2.73
			O-H	2.00
8b	-28.3717	Lys A89	O-H	2.46
		Lys A89	N-benzylidene	3.04
		Glu A8	O-H	1.47
9a	-19.4778	Lys A89	O=C	2.57
13a	-26.1825	Lys A89	O-S	2.94
				2.67
13b	-23.9036	Lys A89	O=C-NH	2.85
		Asp A36	H-N	1.78
15	-25.7646	Lys A89	H-N-furan	2.46
17b	-26.8268	Lys A89	O-S	3.07
		Glu A8	H-N-SO	1.31
		Ile A10	H-N-CO	2.17
Ligand	-18.174	Asp A86	O=S=O	3.22
		Lys A89	O=S=O	3.13
		Leu A83	C=O pyrrole	2.96
		Glu A81	NH pyrrole	1.98

MOE binding affinities of the synthesized compounds into CDK2

The binding affinity of the ligand was evaluated with the energy score, dock score (S, Kcal/mol). The compound which revealed the highest binding affiniy, minimum dock score, is the one forming the most stable ligand–enzyme complex. Length of hydrogen bond and arene cation interaction were also used to assess the binding models. Binding scores and interaction of the amino acids on the active site of CDK2 with the docked compounds are represented in Table **2**. The sugar derivative **8b** with the minimum energy score (-28.3717 Kcal/mol) mediated two strong hydrogen bonds with Lys A89 (2.46 and 3.04 A˚) through the O-H of D(+)glucose and N-benzylidene group and another one with Glu A8 (1.47 A˚) Figure **4**, while compound **8a** with minimum energy score (-27.3361 Kcal/mol) mediated two strong hydrogen bonds with Lys A89 (2.73 and 2.00 A˚) through the O-H of D(+)glucose. The observed binding mode of compound **6a** (-26.4755 Kcal/mol), revealed a strong hydrogen bonding interaction with CO of urea and Asp A86 (2.63 A˚) and arene cation interaction between the phenyl and Lys A89. In addition, the thiazole sulfonamide analogue **17b** (-26.8268 Kcal/mol), the third one in score ranking, showed three hydrogen bonding interactions with S=O and Lys A89 (3.07 A˚), SO_2N-H and Glu A8 (1.31 A˚) and CON-H with Ile A10 (2.17 A˚), while compound **15** mediated a strong hydrogen bond with furan side chain NH and the backbone CO of Lys A89 (2.46 A˚).

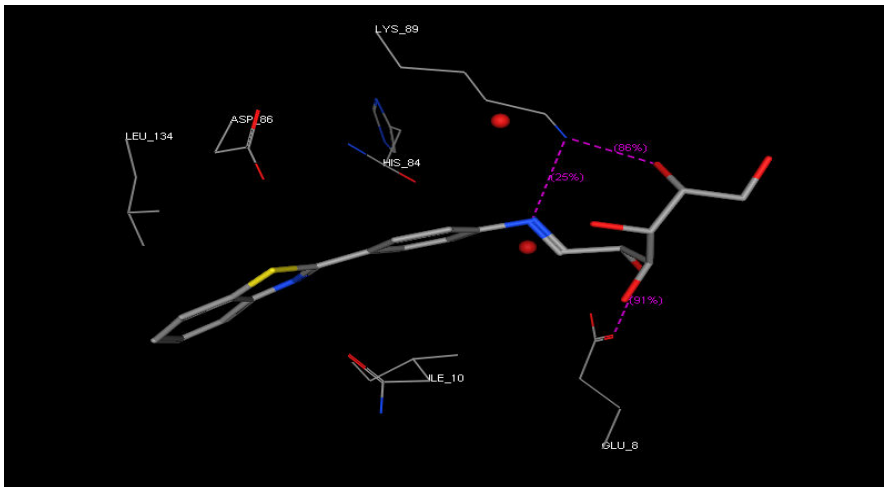


Fig. 4: Interaction map of 8b with CDK2 3D.

Conclusion

Our main goal throughout this study was the synthesis of some new sulfonamides, Schiff's bases, pyrrole and other derivatives attached to 4-benzo[d]thiazol-2-yl moiety as anticancer agents working *via* inhibiting MCF-7 cell line and CDK2.

In vitro growth inhibitory activities of compounds **3c**, **6a**,**b**, **8a**,**b**, **9a**, **13a**,**b**, **15** and **17b** against MCF-7 cell line revealed potential antibreast cancer activity. Best results were gained by compound **6a** since it showed IC_{50} = 8.4 µg/ml compared to DOX (IC_{50} = 4.5 µg/ml). The MOE investigation of the synthesized analogs, **3c**, **6a**, **6b**, **8a**, **8b**, **9a**, **13a**, **13b**, **15** and **17b** was carried out for molecular docking study. Thus were docked on the active site of CDK2. The overall correlation between the growth inhibitory activities (IC_{50}, µg/ml) of the synthesized compounds against tumor cells and the binding affinities predicted by MOE was fairly good for most compounds. Derivative **8b** showed lowest energy score (S = -28.3717 kcal/mol) compared to ligand (S = -18.1741 kcal/mol) and also exhibited moderate *in vitro* antibreast cancer activity while the urea derivative **6a** showed low energy score (S = -26.4755 Kcal/mol) and exhibited the highest *in vitro* antibreast cancer activity.

Acknowledgments

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The authors have declared no conflicts of interest.

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