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Novel benzothiazole hybrids targeting EGFR: Design, synthesis, biological evaluation and molecular docking studies

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ABSTRACT

Novel benzo[*d*] thiazole-based analogues were synthesized with the aim of screening their *in vitro* anticancer activity. All the new derivatives **4**–**21** were evaluated against human hepatocellular carcinoma (HepG-2) and breast cancer cells (MCF-7) using doxorubicin as a reference drug. All compounds exhibited excellent potency against MCF-7 (IC_{50} values ranging from 0.71 ± 0.4 to 1.04 ± 0.7 μM) and variable promising potency against HepG-2 (IC_{50} ranged from 2.53 ± 2.5 to 3.47 ± 3.4 μM) comparing with the standard (IC_{50} = 1.03 ± 0.8 μM and 2.85 ± 1.9 μM, respectively) in addition to their safety towards the normal cell line. Compounds **5, 6, 7, 13** and **16** having the highest cytotoxic activity, were further evaluated for their EGFR inhibitory activity using Erlotinib as a reference drug. Molecular docking studies were performed for the promising compounds **5, 6** and **7** to interpret their detected enzymatic activities based upon their binding interactions with the receptor. Moreover, cell cycle analysis and detection of apoptosis induction illustrated that compounds **5** and **6** exhibited a significant pre G_1 and G_2/M cell cycle arrest, in comparison with the untreated MCF-7 cells. In addition, compounds **5** and **6** elevated the levels of the oncogenic parameters; Bax, p53 and caspase-3 with decreased level of Bcl-2. These previous encouraging results of biological evaluation of the newly synthesized benzothiazoles could recommend an excellent framework toward the detection of new potent antitumor leads.

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1. Introduction

Of various types of remediations applicable to treat cancer, chemotherapy is the most common strategy [1] which includes the use of chemotherapeutics to prevent tumor cells from growing or kill them by various mechanisms [2]. The discovery of novel, safe and effective chemotherapeutic entities for treatment of cancer is considered a challenge due to several limitations like drug resistance, poor bioavailability and solubility, and toxicity to normal cells [3–6].

Kinases are overexpressed in many types of malignancies and are considered as viable targets for development of anticancer drugs [7]. The epidermal growth factor receptor tyrosine kinase (EGFR-TK) has a vital role in signal transduction pathways and regulation of multiple bioactivities, like adhesion, regulation of the cell cycle and motility of the cell [8]. Mutations or over-expression of EGFR prompts cell proliferation, angiogenesis, anti-apoptosis and metastasis leading to a variety of epidermal carcinomas, especially breast, bladder and colon cancers [9–11]. Therefore, the design of EGFR inhibitors is an attractive strategy for the development of new therapeutic agents [12]. Erlotinib bearing 4-anilinoquinazoline scaffold, is a common example of clinical EGFR inhibitors used in treating breast and lung cancers [13–15].

Benzothiazoles are versatile and unique scaffolds employed in the design of numerous analogs of pharmacological benefits [16]. They revealed diverse pharmacological activities as anticancer [17], analgesic, anti-inflammatory [18], anticonvulsant [19], antitubercular [20], antiviral [21], antimalarial [22], antidiabetics [23], antimicrobial [24] and antioxidant [25]. It was reported that several benzothiazole derivatives (**I**-**V**) (Fig. 1) exhibited a unique profile of cytotoxicity especially against MCF-7 cell lines [6,26–28]. Addition-

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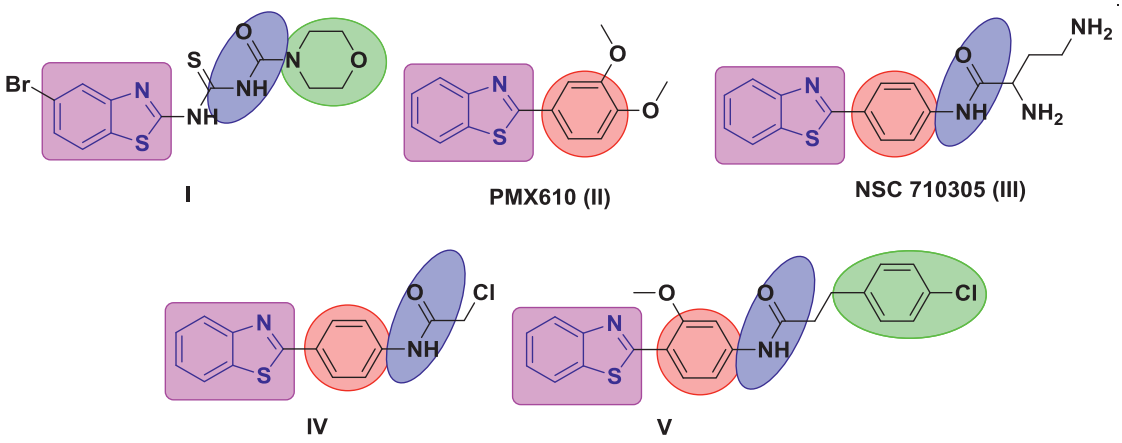


Fig. 1. Some benzothiazole hybrid molecules as anticancer agents.

ally, the benzothiazole containing cores (**VI–IX**) were found to be active as anticancer through inhibition of EGFR [27,29–31] (Fig. 2).

Guided by the afore-mentioned considerations, and upon continuance of our research plan in the area of the detection of biologically active anticancer targets [32–39], the work depicted herein reports the design of benzothiazole-based derivatives designed *via* bioisosterism to the quinazoline ring system in Erlotinib and molecular hybridization with other moieties linked to active benzothiazoles (Figs. 1 and 2) with the proposed binding mode within the EGFR receptor. The synthesized prototypes comprising benzothiazole motif were subjected to *in vitro* antitumor screening against HepG-2 and MCF-7 cancer cell lines, in addition to the assessment of the inhibitory activity of the most active anticancer derivatives against the EGFR kinase. Molecular docking studies were also done to describe and rationalize the binding modes of the most potent compounds into the active site of EGFR kinase. Moreover, the cell cycle analysis apoptosis, the levels of Bax, Bcl2, p-53 and caspase-3 were evaluated in this study.

2. Results and discussion

2.1. Chemistry

This study aims to synthesis of novel benzo[d]thiazole derivatives (**4-21**), which may be expected to possess various anticancer properties. Accordingly, a rational design, synthesis, purification, and structural characterization of novel derivatives (**4-21**), were optimally synthesized. The compounds 4-(benzo[d]thiazol-2-yl)-2-methoxyphenol (**1**); ethyl 2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy) acetate (**2**) and 2-[4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy]acetohydrazide (**3**) were composed in accordance with the methods reported beforehand [40,41]. The compound 2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)-N'-(2-chloroacetyl)acetohydrazide (**4**) was synthesized by the addition of chloroacetylchloride drop wisely to a well stirred solution of 2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy) acetohydrazide (**3**) at room temperature, (Scheme **1**). IR of compound **4** showed absorption bands at 3396 cm^{-1} due to NH in addition to absorptions of C=O amide at 1680 and 1620 cm^{-1}, respectively. ^{1}H-NMR revealed two signals at δ 10.48, 10.42 of 2NH in addition to the D_2O exchangeable signals of amidic, as well as, ^{13}C-NMR revealed two signals at δ166.60 and 165.50 of 2CONH. The mass spectrum of compound **4** showed a peak equal to its molecular weight at m/z = 406 (M+).

On the other hand, 2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetohydrazide (**3**) was refluxed with the appropriate acid anhydride in glacial acetic acid, which produced the novel derivatives (**5–10**), (Scheme **2**). The structure of compounds **5-10** was established under the basis of their spectral data. IR of compounds **5–10** showed absorption bands in the region at 3385-3400 cm^{-1} due to NH in addition to absorptions of C=O amide groups in the region at 1640-1612 cm^{-1}. ^{1}H-NMR revealed signals in the region at δ 10.52-11.16 of NH in addition to the D_2O exchangeable signals of amidic, in addition to signals in the region at δ 3.45-3.96 of OCH_3 group, as well as, multiples at 2.51 of $2CH_2$, pyrrolidine-2, 5-dione. ^{13}C-NMR revealed signals in the region at δ 180.55-164.65 of CONH, in addition to signals in the region at δ 54.25-61.75 of OCH_3 group. The mass spectrum of compounds **5–10** showed a peak equal to its molecular weight at m/z = 497 (M^+, 1.09), 409 (M^+, 1.12), 411 (M^+, 0.22), 459 (M^+, 3.22), 775 (M^+, 3.03) and 437 (M^+, 1.12), respectively.

The novel conjugated amino acid derivatives **11–21**, were synthesized by a stirred compound **4** in ice bath in presence of triethylamine with different amino acids (Scheme **3**). IR of Compounds **11–21** showed absorption bands in the region at 3371-3400 cm^{-1} due to NH, as well as, absorptions of C=O acid in the region at 1689-1676 cm^{-1}, in addition to absorptions of C=O amide groups in the region at 1632-1600 cm^{-1}. ^{1}H-NMR revealed signals in the region at δ 10.35-10.52 of NH in addition to the D_2O exchangeable signals of amidic, signals in the region at δ 12.50-10.65 of COOH group. ^{13}C-NMR revealed signals in the region at δ 167.51-171.19 of (COOH) in addition to signals in the region at δ 154.02-167.52 of CONH. The mass spectrum of compounds **11–21** showed a peak equal to its molecular weight at m/z = 458 (M^+, 0.09), 474 (M^+, 0.03), 543 (M^+, 0.11), 501 (M^+, 0.03), 515 (M^+, 0.08), 488 (M^+, 0.03), 502 (M^+, 0.52), 486 (M^+, 2.50), 504 (M^+, 3.05), 480 (M^+, 1.50) and 534 (M^+, 2.60), respectively, as well as, showed a base peak at m/z =257 (100).

2.2. Biological activity

2.2.1. Anticancer evaluation

The cytotoxic activity of all synthesized benzothiazole derivatives **4–21** was evaluated by MTT assay method [42] using doxorubicin as standard reference. The cytotoxic screening was done *in-vitro* for all derivatives against HepG-2 and MCF-7 cancer cell lines. By investigation of the obtained data (Table 1, Fig. 3), it was noticed that all compounds displayed excellent potency against MCF-7 with IC_{50} values ranging from 0.71 ± 0.4 to 1.04 ± 0.7 μM comparable with the reference drug (IC_{50} = 1.03 ± 0.8 μM). Also, all the screened derivatives illustrated promising and variable strength of potency (IC_{50} ranged from 2.53 ± 2.5 to 3.47 ± 3.4 μM) in comparison with the standard (IC_{50} = 2.85 ± 1.9 μM). The deterioration of the normal cells by anticancer drugs is one of the most important drawbacks in cancer treatment strategy. Thus, the cytotoxic activity of the promising tested compounds (**5** and **6**)

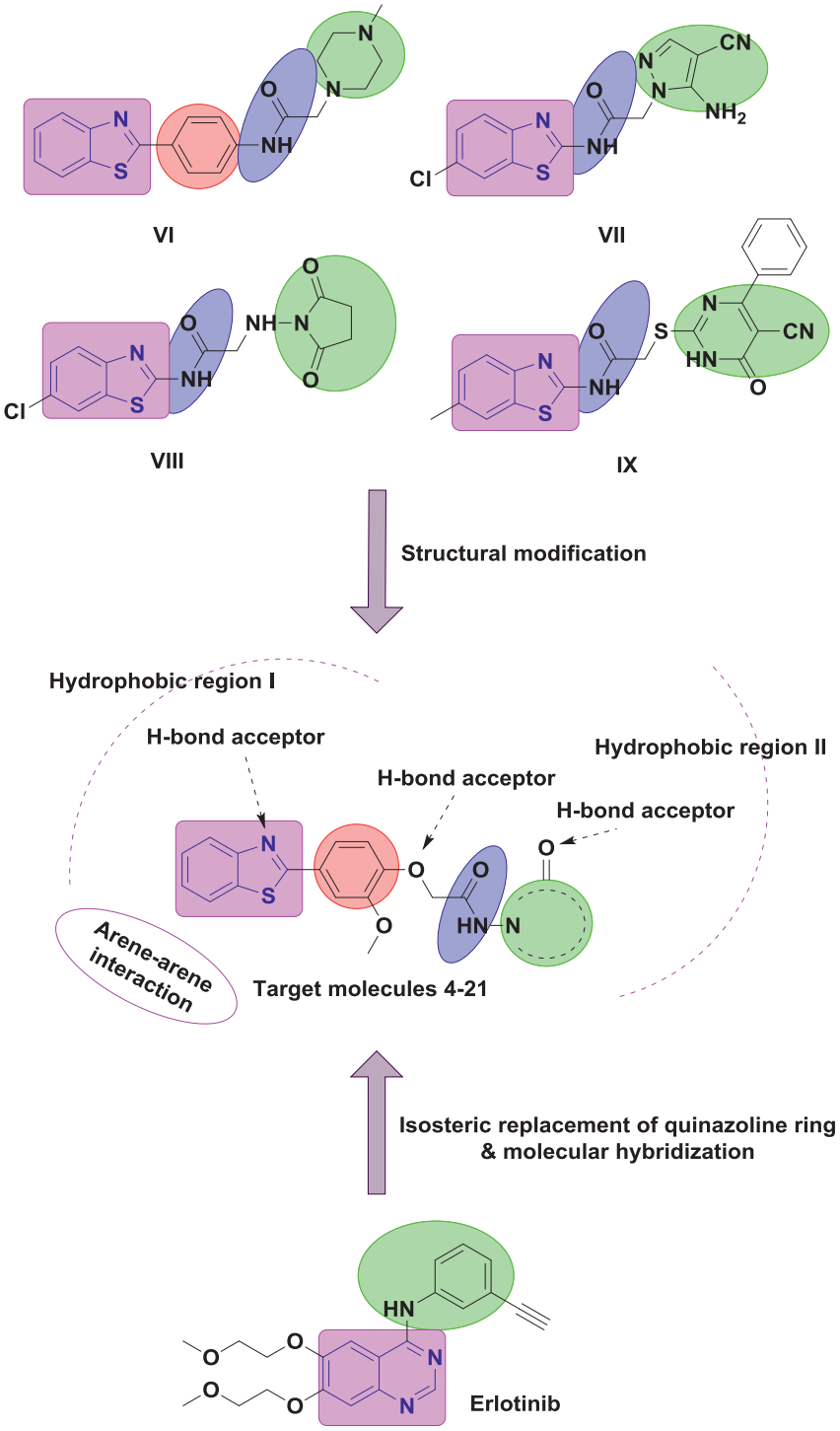
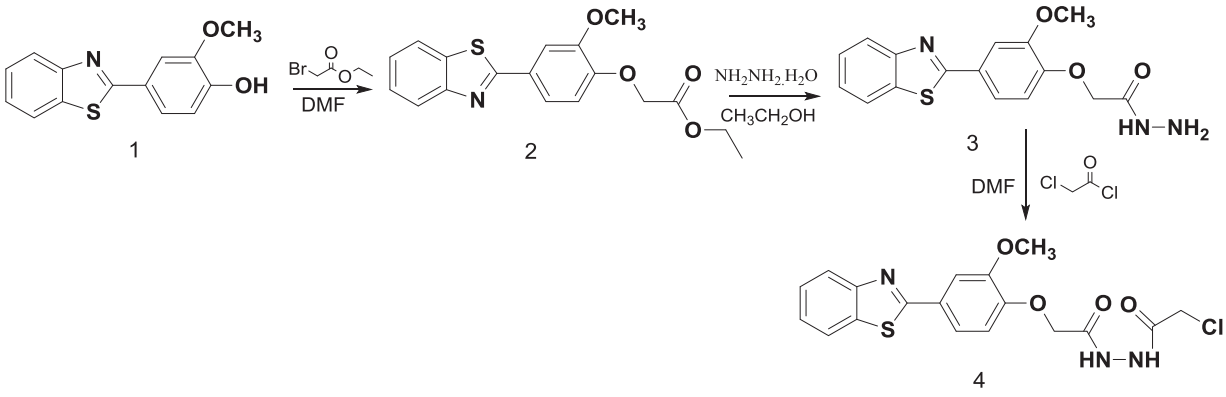


Fig. 2. Reported benzothiazoles and the design strategy for the new hybrids **4**–**21** targeting EGFR TK with their proposed hypothetic model.

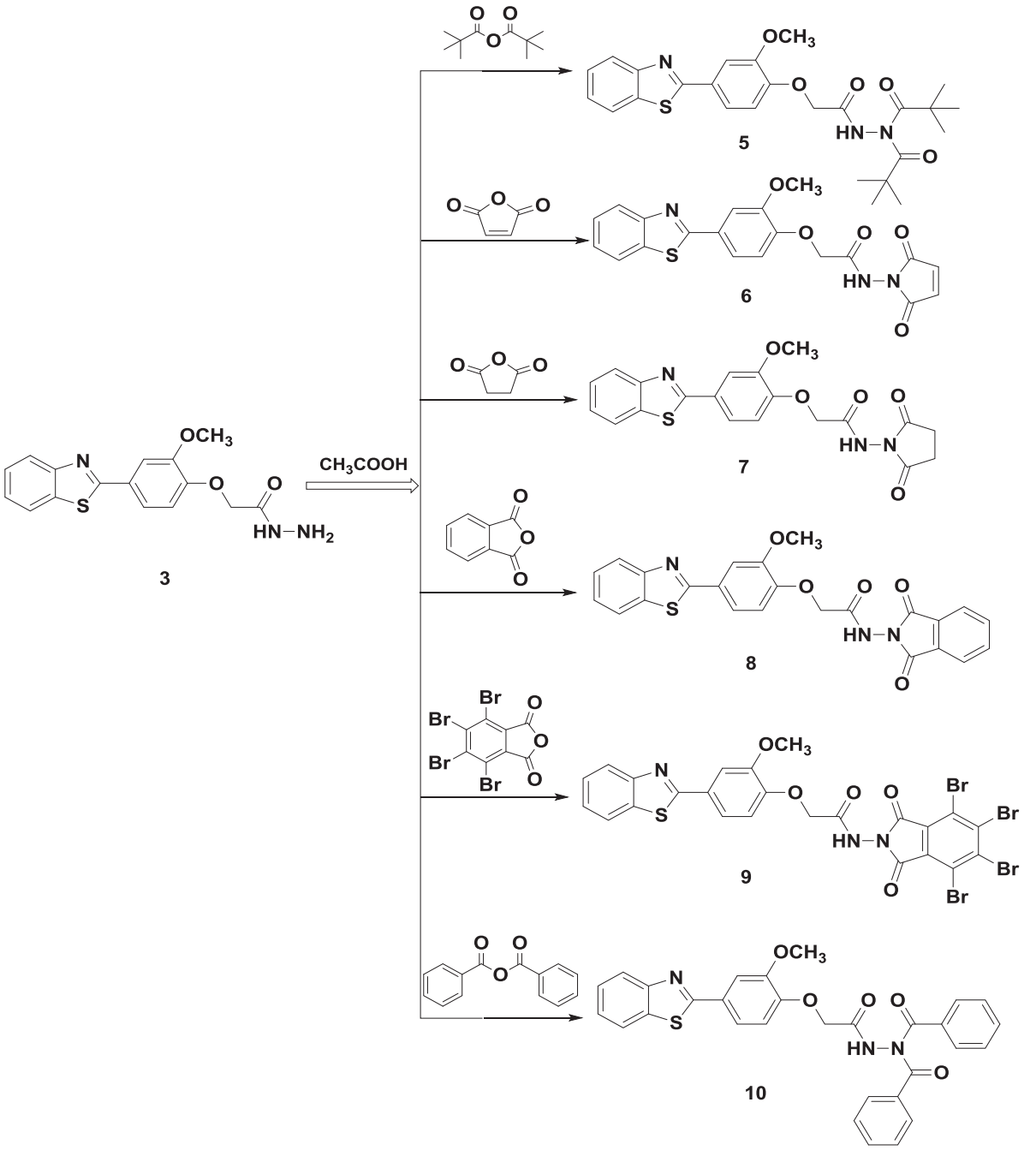
against tested cancer cells was also examined against the human normal fibroblast cells (WI-38) relative to Doxorubicin as a reference drug, and their IC_{50} values were determined by the MTT assay. The IC_{50} values of all tested compounds against WI-38 were higher than their IC_{50} values against the tested cell lines (Table 1). The most potent cytotoxic candidates **5** and **6** exhibited significant cytotoxic activity against HepG-2 and MCF-7 cancer cell lines and also showed safety profile against the normal cells.

Structure–activity relationship (SAR) study

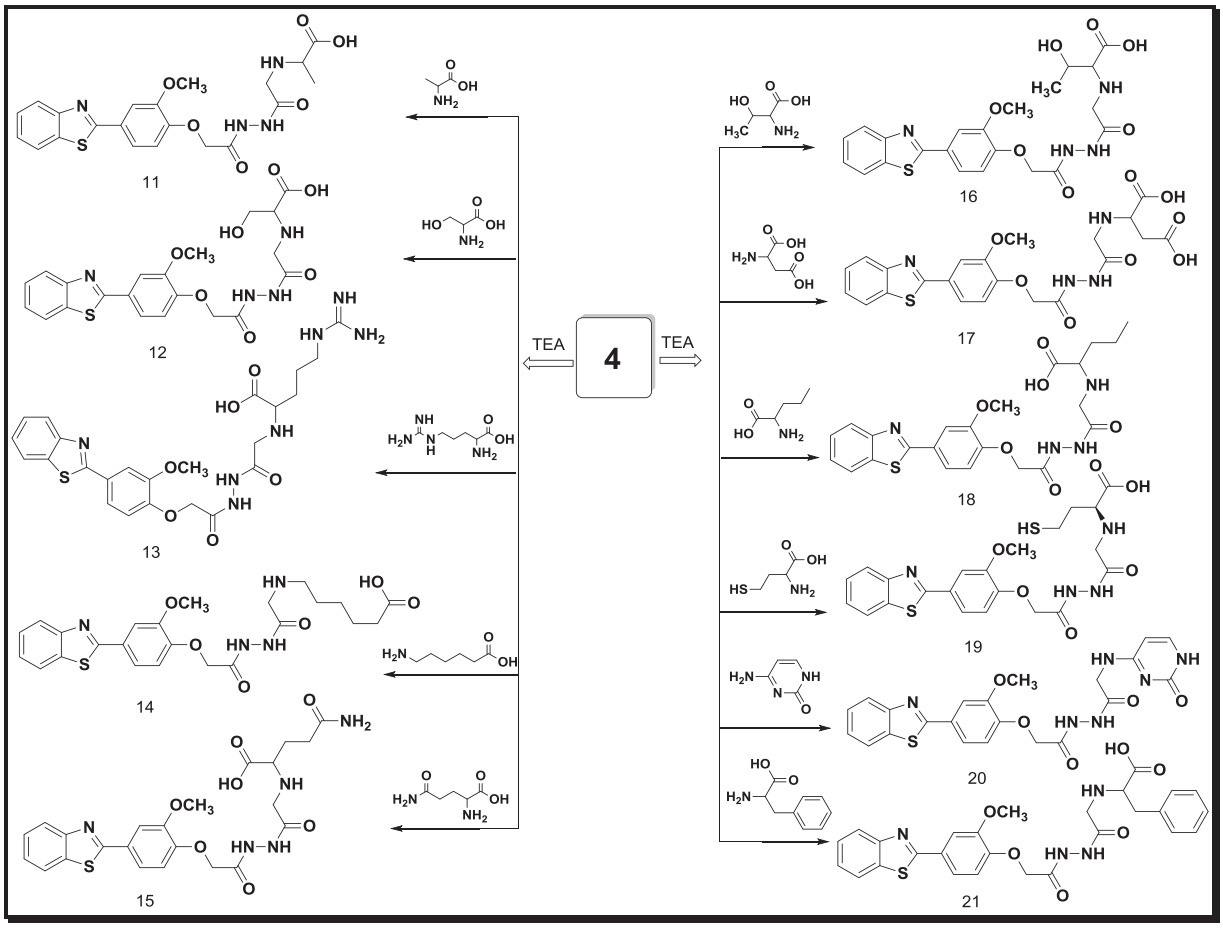
It was clear that all derivatives are bearing 4-(benzo[*d*]thiazol-2-yl)-2-methoxyphenoxy)acetamide nucleus in their structures. Regarding to the gained data against HepG-2 cell line, the open chain derivative **4** having chloroacetamide moiety revealed equipotency with the reference (IC_{50} = 2.89 ± 3.1 µM). Replacement with N-pivaloylpivalamide **5**, pyrrole-2,5-dione **6** or pyrrolidine-2,5-dione **7** displayed increase in the potency (IC_{50} = 2.53 ± 2.5, 2.56 ± 3.1 and 2.58 ± 2.3 µM, respectively). Ring fusion *via* replacement of pyrrole-2,5-dione with isoindoline-1,3-dione **8**, afforded slight decrease in the activity but still equipotent with the reference (IC_{50} = 2.86 ± 3.1 µM). Substitution with electron withdrawing groups (i.e., Br) at positions-4, 5, 6 and 7 of isoindoline-1,3-dione moiety in compound **9**, improved the anti-



Scheme 1. Synthesis of parent benzothiazole derivatives **3** and **4**.



Scheme 2. Synthesis of benzothiazole derivatives **5–10**.



Scheme 3. Synthesis of benzothiazole derivatives **11–21**.

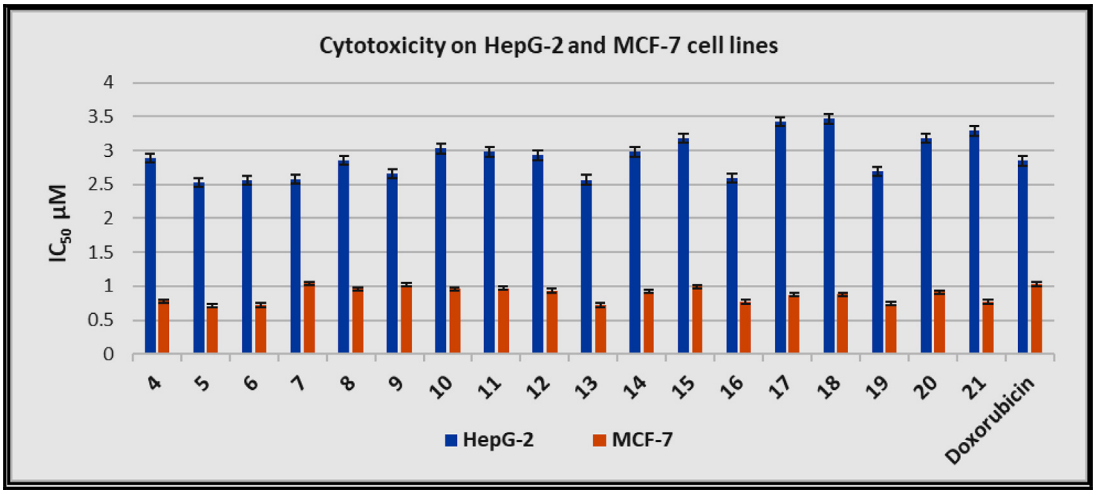


Fig. 3. IC_{50} (µM) data of benzothiazole derivatives **4–21** in comparison with doxorubicin against human HepG-2 and MCF-7 cancer cell lines according to the MTT assay.

cancer activity (IC_{50} = 2.66 ± 2.4 µM). Isosteric replacement of N-pivaloylpivalamide with N-benzoylbenzamide **10**, exhibited marked decrease in the potency (IC_{50} = 3.03 ± 3.3 µM). As demonstrated in Scheme 3, there was open chain elongation with different amino acids that gave slight decrease in the potency in almost derivatives in comparison with reference drug (IC_{50} ranged from 2.93 ± 3.1 to 3.47 ± 3.4 µM). With exception of the derivatives bearing arginine **13**, 2-amino-3-hydroxybutanoic acid **16** and L-homocysteine **19**, an excellent and a higher cytotoxic activity was obtained comparing with the standard (IC_{50} = 2.57 ± 3.5, 2.59 ± 2.9 and 2.69 ± 3.6 µM, respectively).

2.2.2. In vitro *inhibitory assay of EGFR*

Cancer is usually associated with excessive activation of receptor tyrosine kinase (RTK) signaling pathways, so blocking of these receptors gives significant inhibitors with therapeutic potential in treatment of cancer [7]. Regarding to the well-balanced cytotoxic potency, the benzothiazole targets **5, 6, 7, 13** and **16** were selected for further elucidation of their EGFR inhibitory activity using Erlotinib as a reference drug. As depicted in Table 2, compounds **5, 6** and **7** displayed the highest potential EGFR inhibitory activity with IC_{50} values of 0.09 ± 0.42, 0.12 ± 0.22 and 0.16 ± 0.30 µM, respectively but slightly less than that of Erlotinib (IC_{50} = 0.088 ± 0.25

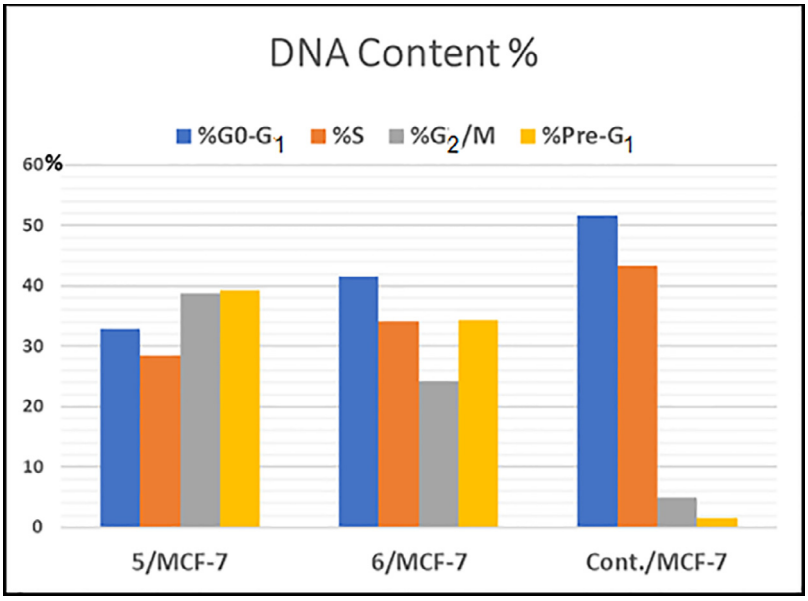


Fig. 4. Cell cycle analysis of the promising derivatives **5** and **6**.

Table 1
The cytotoxic IC_{50} values of the newly synthesized compounds **4**–**21** against HepG-2, MCF-7 cancer cell lines and normal cell line WI-38 according to the MTT assay.

Compound	IC_{50} (µM) ± SD		
	HepG-2	MCF-7	WI-38
4	2.89 ± 3.1	0.78 ± 0.5	-
5	2.53 ± 2.5	0.71 ± 0.4	88.5 ± 1.4
6	2.56 ± 3.1	0.72 ± 0.6	72.5 ± 1.1
7	2.58 ± 2.3	1.04 ± 0.7	-
8	2.86 ± 3.1	0.96 ± 0.6	-
9	2.66 ± 2.4	1.02 ± 0.8	-
10	3.03 ± 3.3	0.96 ± 0.5	-
11	2.98 ± 3.1	0.97 ± 1.1	-
12	2.93 ± 3.1	0.93 ± 0.5	-
13	2.57 ± 3.5	0.72 ± 0.9	-
14	2.98 ± 3.1	0.92 ± 0.7	-
15	3.18 ± 2.9	0.99 ± 1.1	-
16	2.59 ± 2.9	0.77 ± 1.1	-
17	3.43 ± 3.6	0.87 ± 0.5	-
18	3.47 ± 3.4	0.88 ± 0.5	-
19	2.69 ± 3.6	0.74 ± 0.5	-
20	3.18 ± 3.1	0.91 ± 0.9	-
21	3.29± 3.5	0.77 ± 0.7	-
Doxorubicin	2.85 ± 1.9	1.03 ± 0.8	78.9 ± 0.8

IC_{50}: Compound concentration required to inhibit the cell viability by 50%, SD = standard deviation mean; each value is the mean of three values.

Table 2
In vitro inhibitory activities of the newly synthesized derivatives **5, 6, 7, 13** and **16** against EGFR enzyme.

Compd.No.	IC_{50} (Mean ± SEM) (µM) EGFR
5	0.09±0.42
6	0.12±0.22
7	0.16±0.30
13	17.04±0.11
16	23.80±0.26
Erlotinib	0.088±0.25

IC_{50}: Compound concentration required to inhibit EGFR enzyme activity by 50%, SEM = standard error mean; each value is the mean of three values.

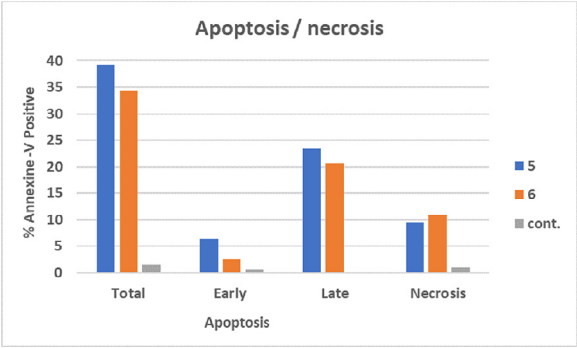


Fig. 5. Apoptotic activity of benzothiazole derivatives **5** and **6**.

µM). However, the rest derivatives **13** and **16** revealed drastic decrease in the suppression effect (IC_{50} = 17.04 ± 0.11 and 23.80 ± 0.26 µM, respectively) which might be attributed to the improper fitting in the EGFR binding pocket.

2.2.3. Flow cytometry analysis and apoptosis detection

To investigate the molecular mechanism of the benzo[*d*]thiazole analogues against cancerous cells, the highly potent compounds **5** and **6** were chosen to evaluate their effect on the cell cycle progression through flow cytometry. In current work, MCF-7 cells were treated with 0.71 and 0.72 µM concentrations of the analogues **5** and **6** for 24 h, respectively to study their consequence on the normal profile of cell cycle and apoptosis. As illustrated in Fig. 4, compounds **5** and **6** demonstrated cell accumulations of 39.27 % and 34.28 % at pre G_1 phase and 38.78 % and 24.3 % at G_2/M phase, respectively, regarding to the control values (untreated cells) 1.61 % and 4.99 % for pre G_1 and G_2/M phases, respectively. These obtained findings clearly revealed that derivatives **5** and **6** prompted a significant pre G_1 and G_2/M cell cycle arrest, in comparison with the untreated MCF-7 cells.

2.2.4. Annexin V-FITC apoptosis study

The MCF-7 cells treated with the compounds **5** and **6** were double stained with Annexin V/PI (annexin V-FITC and propidium iodide) and tested through flow cytometry technique comparing

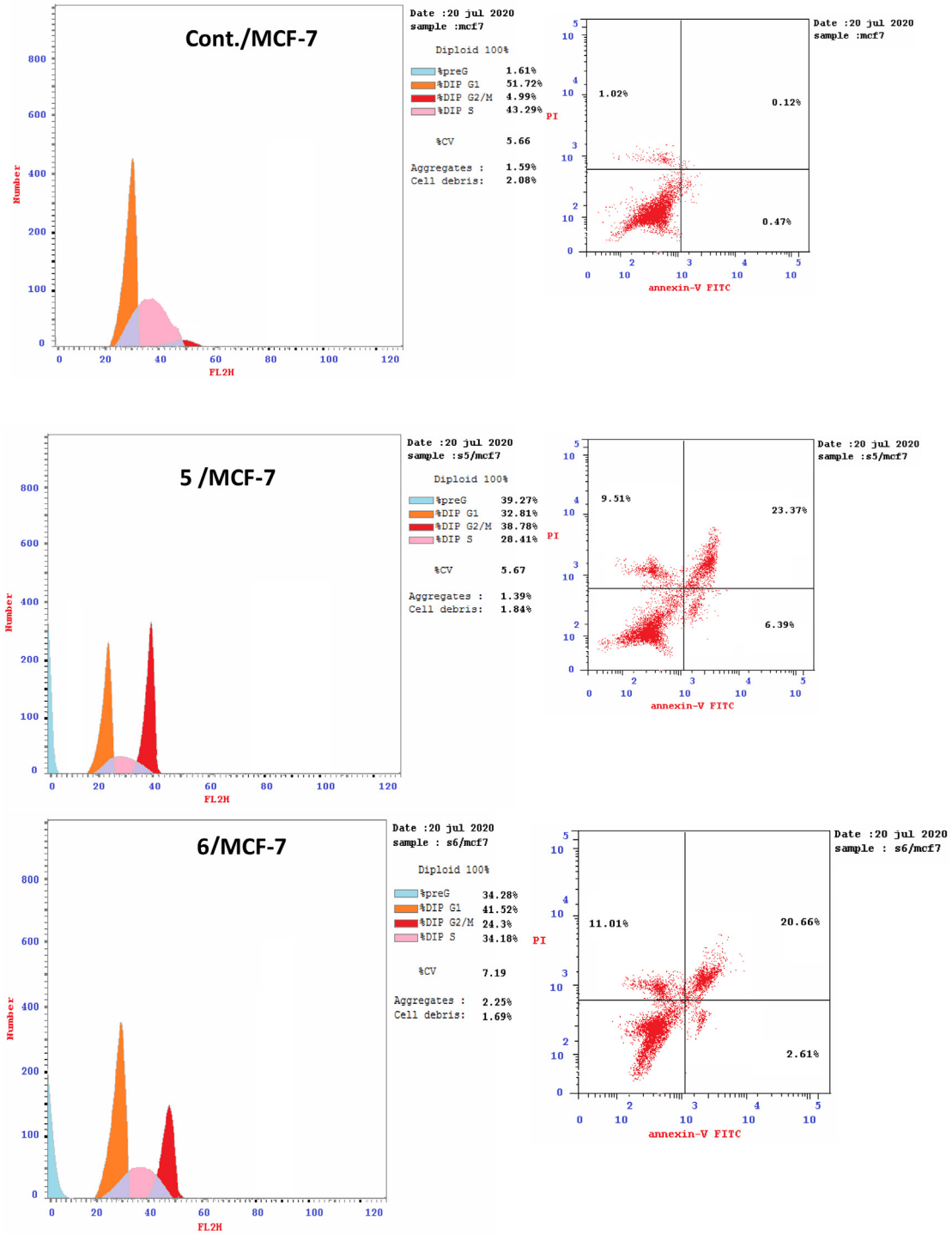


Fig. 6. Cell cycle analysis and the effect of the compounds **5** and **6** on the percentage of V-FITC-positive annexin staining in MCF-7 cells in comparison with the control.

with the control cells to validate the ability of these derivatives to cause apoptosis (Table 3, Figs. 5 and 6). It was noted that there was an increase to 23.37 % and 20.66 % created by the investigated compounds **5** and **6** from 0.12 % (DMSO control) in the late apoptosis and an increase to 6.39 % and 2.61 % in the early apoptosis from 0.47 % (DMSO control), respectively. Also, these analogues produced necrosis percent of 9.51 and 11.01 *versus* 1.02 % caused by DMSO control, respectively. Thus, the marked increase in the apoptotic cells indicated the ability of the compounds **5** and **6** to afford apoptosis induction.

Table 3
Apoptosis and necrosis results on MCF-7 cells induced by **5** and **6**.

Compd.	Apoptosis %			Necrosis %
	Total %	Early %	Late %	
5 / MCF-7	39.27	6.39	23.37	9.51
6 / MCF-7	34.28	2.61	20.66	11.01
Cont. / MCF-7	1.61	0.47	0.12	1.02

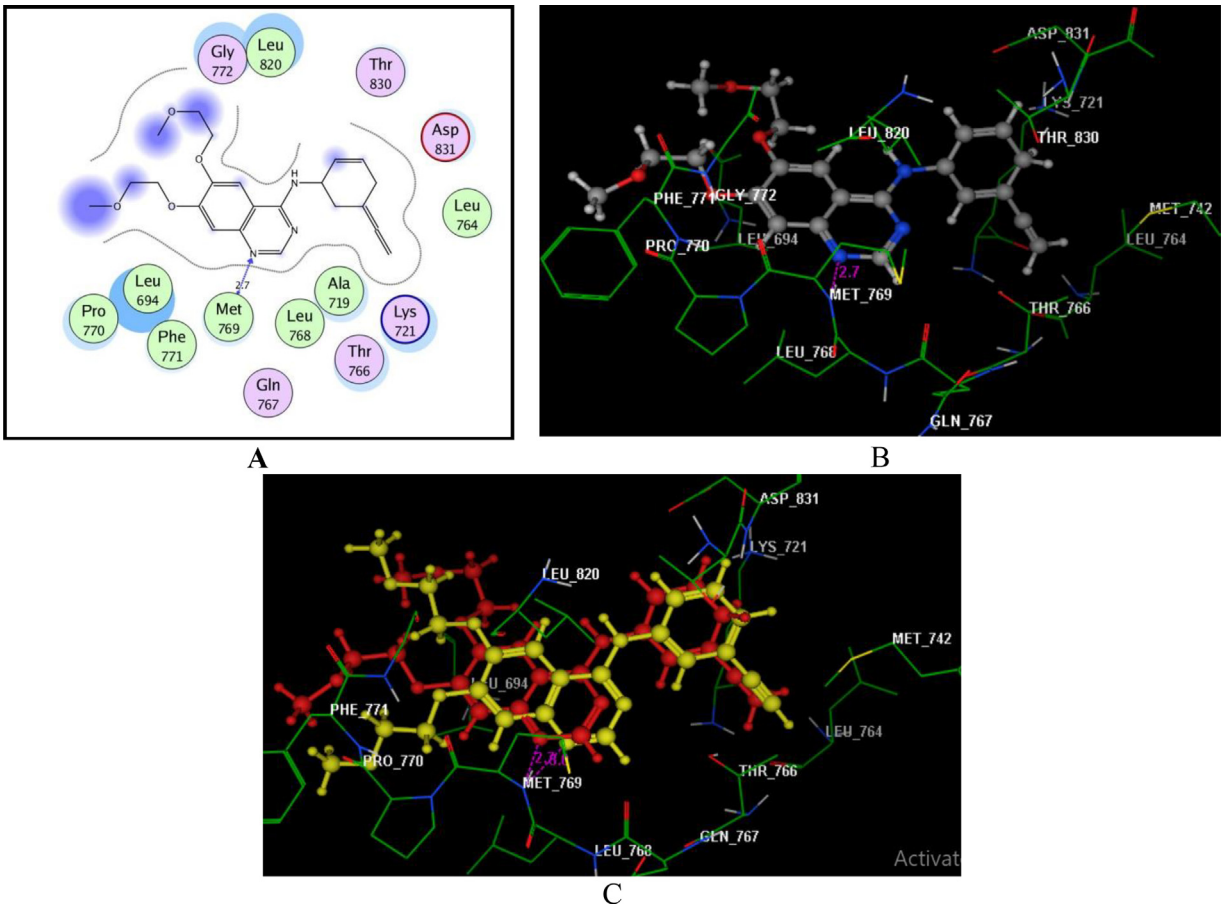


Fig 7. **A** & **B** illustrating 2D & 3D Views of the native ligand, erlotinib re-docked in the active site of EGFR (PDB ID: 1M17) using MOE software. **C** illustrating 3D representation of the superimposition of the co-crystallized (yellow) and the docking pose (red) of **Erlotinib** with an RMSD value of 0.85 Å.

2.2.5. The impact of the derivatives 5 and 6 on Bax / Bcl-2 / p53 / caspase-3 levels

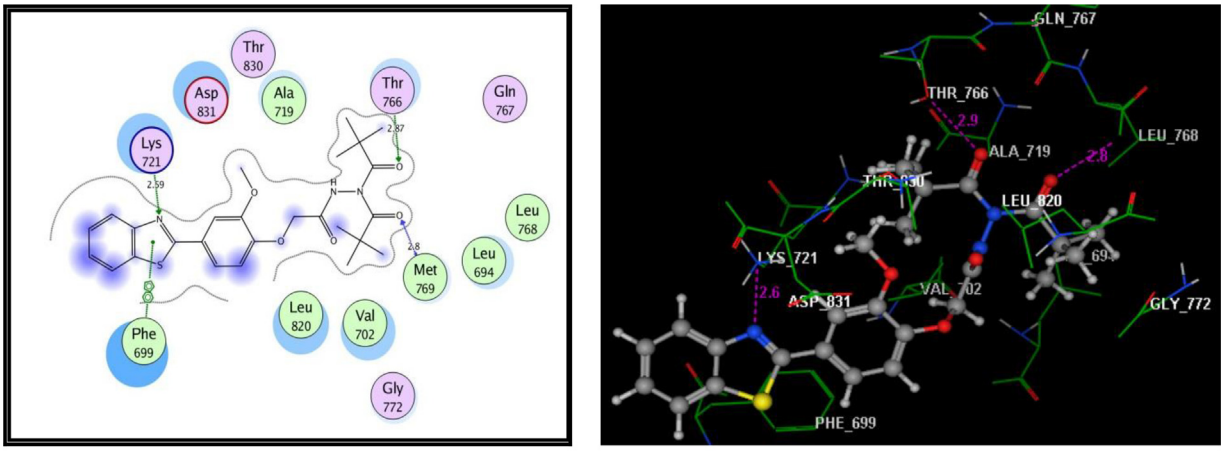
There are two different pathways that control the cell apoptotic process: the intrinsic mitochondrial pathway and the extrinsic death receptor pathway [43]. The anti-apoptotic Bcl-2 protein overexpresses in the tumor cell boosting the cell survival *via* inhibition of apoptosis, while the pro-apoptotic Bax performs a vital role in cell apoptosis. So, the balance between these two proteins controls the cell fate [44]. Moreover, the protein p53 is a tumor suppressor acting as a transcription factor that is involved in the regulation of the G_2 to M transition preventing mitosis, thus leads to either apoptosis or growth arrest. Also, it controls the expression of Bax and Bcl-2 genes [45]. Caspase-3 is a member in the family of cysteine proteases and its induction catalyzes apoptosis process [45]. In this study, the MCF-7 cells were exposed to the derivatives **5** and **6** at their IC_{50} concentrations of 0.71 and 0.72 µM, respectively for 24 h to evaluate the levels of Bax, Bcl-2, p53 and caspase-3. The obtained results exhibited that the screened compounds **5** and **6** boosted the levels of Bax by 6.02 and 4.90 folds, p53 by 6.03 and 5.04 folds, and caspase-3 by 9.45 and 7.41 folds, respectively comparing with the untreated cells. Reversely, **5** and **6** reduced the protein Bcl-2 level by 0.23 and 0.30 folds, respectively in comparison with the control cells (Table 4).

2.3. Molecular docking study

The docking study of the promising benzo[*d*]thiazole analogues **5, 6** and **7** was performed to rationalize the gained *in vitro* enzyme assessment data on EGFR enzyme. The domain of EGFR kinase complexed with 4-anilinoquinazoline inhibitor (Erlotinib, 4AQ) (PDB ID: IM17) [45] was downloaded from the protein data bank. The docking calculations were done using MOE (Molecular Operating Environment) software 10.2008 [46]. The original ligand was extracted from the X-ray co-crystallized structure and re-docked in the ATP-active site of EGFR to validate the docking approach. This validation was confirmed through the small RMSD value (root mean standard deviation, 0.83 Å) between the experimental and the docked poses of co-crystallized inhibitor, the score energy of -12.66 kcal/mol, and the highly observed superimposition between them (Fig. 7c).

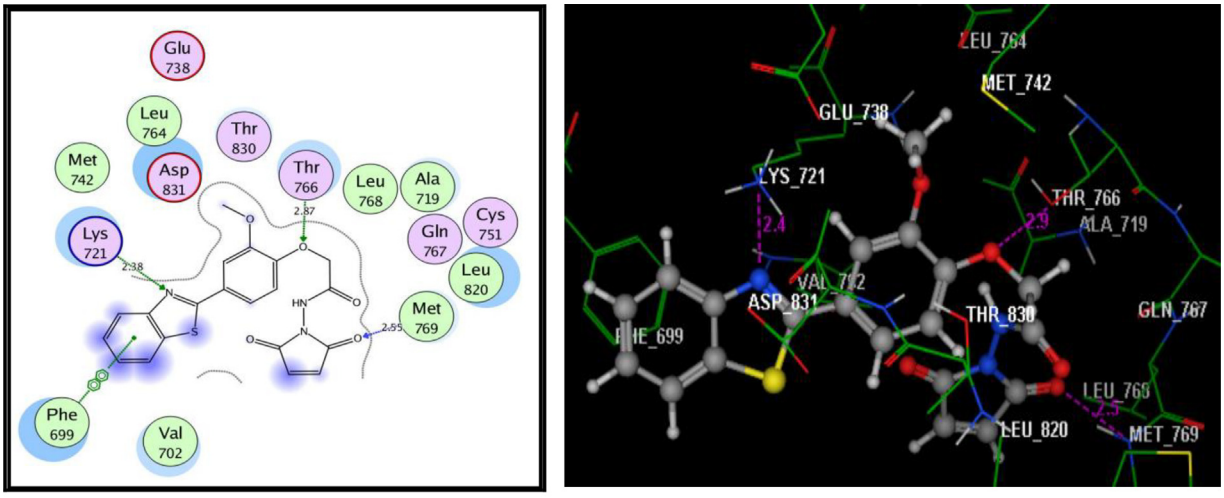
As previously described in the docking of Erlotinib [47], the quinazoline scaffold interacted with the active site of EGFR *via* hydrogen bonding between the N1 atom and the backbone of **Met769**. Moreover, the methoxyethoxy group formed hydrophobic interaction within the hydrophobic region II (**Gly772, Leu768** and **Leu694** residues). While the ethynylphenyl moiety inserted in the hydrophobic pocket I and exhibited hydrophobic interactions with **Asp831, Thr830, Leu820, Thr766, Lue764, Lys721** and **Ala719**. (Fig. 7a, b)

The docked benzo[*d*]thiazole targets **5, 6** and **7** displayed higher negative energy scores of −13.55, 13.25 and -12.83 kcal/mol, and respectively suggesting higher expected binding affinity than the original co-crystalized ligand. It was observed that the benzo[*d*]thiazole scaffold of all these analogues were fitted in the active site of EGFR in a similar manner through two different interactions: hydrogen bonding between nitrogen atom and the sidechain of **Lys721** and hydrophobic arene-arene interaction with **Phe699** (Figs. 8–10).



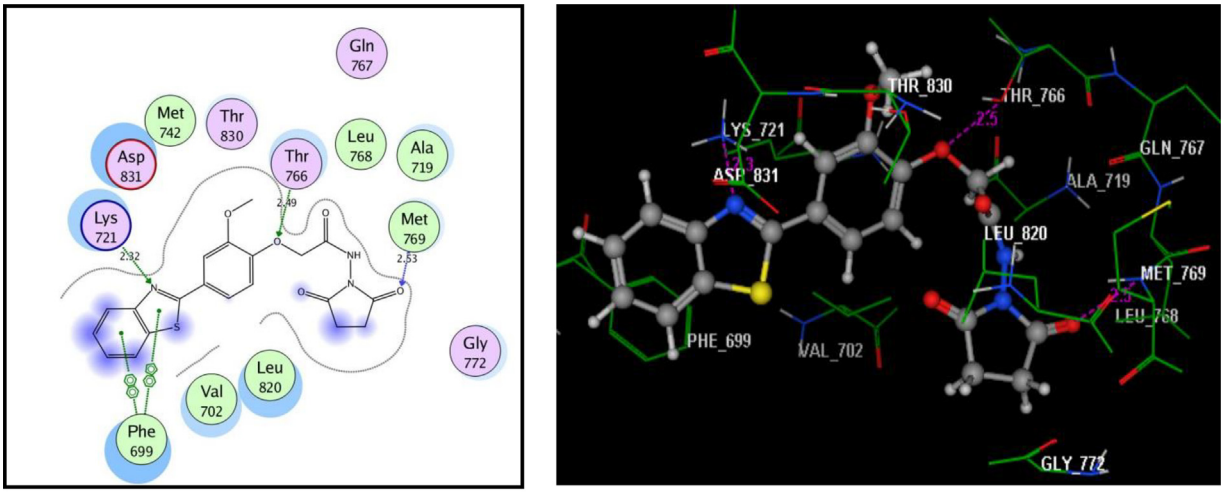
A **B**

Fig. 8. **A** & **B** illustrating 2D & 3D Views of the compound **5** docked in the active site of EGFR (PDB ID: 1M17) using MOE software.



A **B**

Fig. 9. **A** & **B** illustrating 2D & 3D Views of the compound **6** docked in the active site of EGFR (PDB ID: 1M17) using MOE software.



A **B**

Fig. 10. **A** & **B** illustrating 2D & 3D Views of the compound **7** docked in the active site of EGFR (PDB ID: 1M17) using MOE software.

Table 4
Effect of the compounds **5** and **6** on Bax, Bcl-2, p53 and caspase-3 levels.

Compd.	Bax		Bcl-2		P53		Casp-3	
	Conc. Pg/mL	FLD	Conc. ng/mL	FLD	Conc. Pg/mL	FLD	Conc. ng/mL	FLD
5 / MCF-7	181.90±9.60	6.02	1.95±0.16	0.23	882.60±20.80	6.03	24.95±1.14	9.45
6 / MCF-7	148.10±4.80	4.90	2.57±0.11	0.30	737±22	5.04	19.56±0.77	7.41
Cont./MCF-7	30.24±1.90	1.00	8.43±0.21	1.00	146.30±2.80	1.00	2.64±0.10	1.00

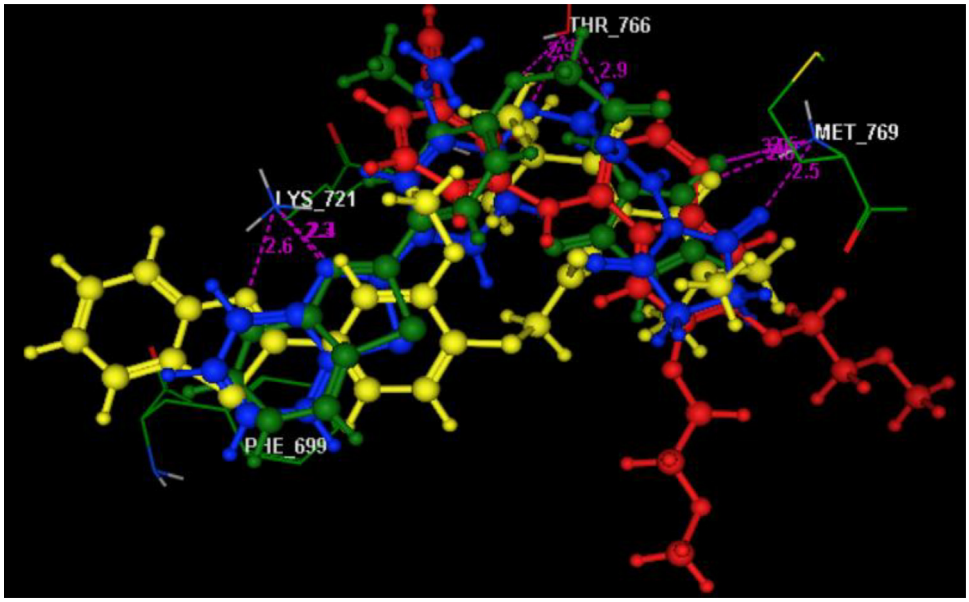


Fig. 11. 3D diagram of the superimposition of the original ligand **Erlotinib** (red), **5** (yellow), **6** (green) and **7** (blue) within the ATP-binding pocket of EGFR (PDB code: 1M17) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

By inspection of Fig. 8, the two oxygens of N-pivaloylpivalamide moiety in compound **5** formed two H-bond acceptors with sidechain of the gatekeeper residue **Lys766** and the backbone of the key residue **Met769** (distance: 2.87 and 2.80 Å, respectively).

On the other hand, the sidechain of **Lys766** was bound to the phenoxy oxygen in the compounds **6** and **7** (distance: 2.87 and 2.49 Å, respectively). Moreover, the backbone of **Met769** enforced fixation through hydrogen bond acceptor with the oxygen of pyrrole-2,5-dione in **6** or pyrrolidine-2,5-dione in **7** (distance: 2.55 and 2.53 Å, respectively) (Figs. 9 and 10).

Furthermore, the superimposition diagram between the native ligand Erlotinib and the new targets **5, 6** and **7** within ATP-binding pocket of EGFR confirmed that all of them fitted in the same active site through the same hydrophilic interactions with the key residue **Met769**. The targets improved their binding *via* hydrophobic interactions with **Phe699** and hydrophilic interactions with **Lys721** and **Lys766** (Fig. 11).

Finally, it could be assumed that the existence of the benzo[*d*]thiazole scaffold bearing N-pivaloylpivalamide, pyrrole-2,5-dione or pyrrolidine-2,5-dione and separated with phenoxy fragment allowed the chance for excellent binding profile and inhibitory activities against EGFR.

3. Conclusion

In summary, a Novel series of benzo[*d*]thiazole derivatives **4**–**21** bearing various heterocyclic scaffolds was designed and synthesized. All newly synthesized targets were examined for their *in vitro* cytotoxicity against HepG-2 and MCF-7 cancerous cell lines in comparison with doxorubicin as a reference. The highest cytotoxic compounds **5, 6, 7, 13** and **16** were further screened for their EGFR inhibitory activity using erlotinib as a standard drug. Molecular docking studies were done to confirm the promising enzymatic results of the compounds **5, 6** and **7**. Furthermore, mechanistic studies revealed that compounds **5** and **6** arrested cell cycle at pre G_1 and G_2/M phases and induced apoptosis through upregulating p53, caspase-3 and Bax and downregulating Bcl-2 levels, in comparison with the untreated MCF-7 cells.

4. Experimental

4.1. Chemistry

These companies: Sigma (Ronkonkoma, NY, USA), Fluka (Buchs, Switzerland), and E. Merck (Hohenbrunn, Germany) were used to import all chemical and related materials. All “melting points; M.P.” uncorrected which gained from " Digital melting point apparatus; model: IA9100”. The “Micro-Analytical center” in Cairo University of Egypt were used for measuring the following spectroscopic analysis; (i) Elemental Micro-analyses for C, N, and H, which gained through a good restriction of the theoretical values (±0.4%). (ii) “Potassium bromide; KBr” disks were handled for preparation tested samples to implement into the “Fourier transforms infrared spectrophotometer; IR Shimadzu; Model: with 1S affinity”. (iii) Mass spectra measured by “Shimadzu gas chromatograph–spectrometer, Model: QP2010ultra; Kyoto, Japan”. ^{1}H- and ^{13}C NMR spectra were recorded at 400 (100) MHz using Jeol ECA 500 MHz Spectrometer and performed at Micro Analytical Laboratory Center, Faculty of Pharmacy, Cairo University, Cairo, Egypt. DMSO-*d*6 was used as a solvent and chemical shifts were given in ppm relative to TMS as internal standard.

*4.1.1. Synthesis of 4-(benzo[d]thiazol-2-yl)-2-methoxyphenol (**1**)*

This compound was composed in accordance with the methods reported beforehand [40].

*4.1.2. Synthesis of ethyl 2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetate (**2**) and 2-[4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy]acetohydrazide (**3**)*

These compounds were composed in accordance with the methods reported beforehand [41].

*4.1.3. Synthesis of 2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)-N'-(2-chloroacetyl) acetohydrazide (**4**)*

To a well stirred solution of 2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetohydrazide (**3**) (1mmol, 0.33 g) in dry dimethylformamide (10 ml), chloroacetylchloride (1 mmol, 0.11 ml) was drop wisely added with continuous stirring, then stir at room temperature for 6 h. The reaction mixture was subsequently poured over ice with continues stirring. The precipitated solid was filtered and dried to give the crude product which was further purified by crystallization from ethanol to give analytically pure product as a white powder.

Yield: 80%; melting point: 207–209°C, IR (cm^{-1}): (KBr): γ = 3396 (NH stretching), 3160, 3050 (CH, aromatic), 2920 (CH, aliphatic), 1680, 1620 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.48, 10.42 (s, 2H, 2NH, D_2O exchangeable, amide, hydrazide), 8.12-7.02 (s, 7H, aromatic H), 4.80, 4.74 (s, 2H, OCH_2), 3.93, 3.92 (s, 2H, CH_2Cl), 3.38 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 166.60, 165.50 (2CONH), 154.00-110.00 (13 C, aromatic), 66.75 (OCH_2), 60.90 (OCH_3), 50.25 ($COCH_2Cl$). MS (EI, 70 eV): m/z (%) = 406 (M^+, 0.13), 405 374 (4.10), 270 (100), 258 (31.60), 256 (77.00), 185 (18.48), 57 (30.30), 51 (8.00). Molecular formula (M.wt.): $C_{18}H_{16}ClN_3O_4S$ (405.9). Calculated analysis: C, 53.27; H, 3.97; N, 10.35; found: C, 53.26; H, 3.97; N, 10.35.

*4.1.4. Synthetic routes for compounds **5–10***

2-(4-(Benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetohydrazide (**3**) (1mmol, 0.33 g) and the appropriate acid anhydride (10 mmol) were refluxed in least amount of glacial acetic acid (5 ml) for 5 h. The reaction mixture was subsequently poured over ice with continuous stirring. The precipitated solid was filtered and dried to give the crude product which was further purified by crystallization from methanol to give analytically pure products.

*4.1.4.1. N'-(2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetyl)-N-pivaloyl pivalohydrazide (**5**).* Yield: 65 %; melting point: 120–122 °C, IR (cm^{-1}): (KBr): γ = 3397 (NH stretching), 3167, 3049 (CH, aromatic), 2925 (CH, aliphatic), 1625 (C=O amide). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.52 (s, 1H, 1NH, D_2O exchangeable, amide, hydrazide), 8.15-7.22 (s, 7H, aromatic H), 4.90, 4.85 (s, 2H, OCH_2), 3.45 (s, 3H, OCH_3), 1.40-1.20 (s, 18H, CH_3). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 180.55, 180.00 (2C, 2 NCO, aliphatic), 166.15 (CONH), 154.05-111.00 (13 C, aromatic), 66.60 (OCH_2), 61.75 (OCH_3), 40.25 (2C, 2COCH), 27.50 (6C, 6 $CHCH_3$). MS (EI, 70 eV): m/z (%) = 498 (M^+ +1, 0.19), 497 (M^+ +1, 1.09), 415 (10.39), 314 (09.88), 270 (17.06), 258 (22.60), 257 (100), 185 (33.55), 69 (40.75), 57 (40.80), 51 (5.30). Molecular formula (M.wt.): $C_{26}H_{31}N_3O_5S$ (497.6). Calculated analysis: C, 62.76; H, 6.28; N, 8.44; found: C, 62.75; H, 6.28; N, 8.44.

*4.1.4.2. 2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)-N-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (**6**).* Yield: 80%; melting point: 240–242 °C, IR (cm^{-1}): (KBr): γ = 3400 (NH stretching), 3170, 3050 (CH, aromatic), 2930 (CH, aliphatic), 1620 (C=O amide). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.73 (s, 1H, 1NH, D_2O exchangeable, amide, hydrazide), 8.13-7.02 (s, 7H, aromatic H), 4.89, 4.80 (s, 2H, OCH_2), 3.95-3.92 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 166.60 (CONH), 162.55, 160.00 (2C, 2 NCO, aromatic), 153.65-112.20 (15 C, aromatic), 66.25 (OCH_2), 58.05 (OCH_3). MS (EI, 70 eV): m/z (%) = 409 (M^+, 1.12), 258 (50.06), 257 (100), 256 (44.00), 109 (10.70), 69 (30.32), 57 (34.80), 55 (10.75), 51 (3.00). Molecular formula (M.wt.): $C_{20}H_{15}N_3O_5S$ (409.4). Calculated analysis: C, 58.67; H, 3.69; N, 10.26; found: C, 58.66; H, 3.69; N, 10.26.

*4.1.4.3. 2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)-N-(2,5-dioxopyrrolidin-1-yl)acetamide (**7**).* Yield: 90 %; melting point: 180–182 °C, IR (cm^{-1}): (KBr): γ = 3395 (NH stretching), 3170, 3045 (CH, aromatic), 2935 (CH, aliphatic), 1630 (C=O amide). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.80 (s, 1H, 1NH, D_2O exchangeable, amide, hydrazide), 8.10-7.22 (s, 7H, aromatic H), 4.80, 4.65 (s, 2H, OCH_2), 3.80-3.75 (s, 3H, OCH_3), 2.51 (m, 4H, $2CH_2$, pyrrolidine-2, 5-dione). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 172.05, 170.00 (2C, 2 NCO), 165.00 (CONH), 153.50-112.02 (15 C, aromatic), 65.00 (OCH_2), 58.05 (OCH_3), 33.25 (2C, CH_2CH_2). MS (EI, 70 eV): m/z (%) = 411 (M^+, 0.22), 355 (11.00), 300 (19.70), 257 (100), 69 (40.10), 57 (22.33), 55 (7.00). Molecular formula (M.wt.): $C_{20}H_{17}N_3O_5S$ (411.4). Calculated analysis: C, 58.38; H, 4.16; N, 10.21; found: C, 58.38; H, 4.16; N, 10.21.

*4.1.4.4. 2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)-N-(1,3-dioxoisoindolin-2-yl) acetamide (**8**).* Yield: 75 %; melting point: 100–102 °C, IR (cm^{-1}): (KBr): γ = 3397 (NH stretching), 3152, 3033 (CH, aromatic), 2902 (CH, aliphatic), 1628 (C=O amide). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.98 (s, 1H, 1NH, D_2O exchangeable, amide, hydrazide), 8.14-7.02 (s, 11H, aromatic H), 4.96, 4.80 (s, 2H, OCH_2), 3.96-3.92 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 165.15 (CONH), 164.55 (2C, 2 NCO, aromatic), 155.02-112.00 (19 C, aromatic), 65.20 (OCH_2), 56.00 (OCH_3). MS (EI, 70 eV): m/z (%) = 460 (M^+ +1, 1.01), 459 (M^+, 3.22), 317 (19.70), 258 (100), 109 (31.31), 69 (50.10), 57 (34.90). Molecular formula (M.wt.): $C_{24}H_{17}N_3O_5S$ (459.5). Calculated analysis: C, 62.74; H, 3.73; N, 9.15; found: C, 62.74; H, 3.73; N, 9.15.

*4.1.4.5. 2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)-N-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)acetamide (**9**).* Yield: 75 %; melting point: 166–168 °C, IR (cm^{-1}): (KBr): γ = 3385 (NH stretching), 3162, 3030 (CH, aromatic), 2922 (CH, aliphatic), 1622 (C=O amide). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.85 (s, 1H, 1NH, D_2O exchangeable, amide, hydrazide), 8.20-7.10 (s, 7H, aromatic H), 4.90, 4.70 (s, 2H, OCH_2), 3.90-3.88 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 164.65 (CONH), 163.05, 162.70 (2C, 2 NCO, aromatic), 154.22-110.02 (19 C, aromatic), 63.25 (OCH_2), 54.25 (OCH_3). MS (EI, 70 eV): m/z (%) = 776 (M^+ +1, 0.11), 775 (M^+, 3.03), 630 (20.90), 510 (10.70), 458 (18.25), 305 (46.80), 258 (20.60), 257 (100), 110 (60.11), 69 (20.00), 57 (44.85), 55 (10.70), 51 (5.55). Molecular formula (M.wt.): $C_{24}H_{13}Br_4N_3O_5S$ (775.1). Calculated analysis: C, 37.19; H, 1.69; N, 5.42; found: C, 37.18; H, 1.68; N, 5.42.

*4.1.4.6. N'-(2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetyl)-N-benzoylbenzohydrazide (**10**).* Yield: 70 %; melting point: 248–250 °C, IR (cm^{-1}): (KBr): γ = 3386 (NH stretching), 3166, 3047 (CH, aromatic), 2920 (CH, aliphatic), 1689 (C=O, acid), 1640, 1612 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 11.16 (s, 1H, 1NH, D_2O exchangeable, amide, hydrazide), 8.14-7.16 (s, 17H, aromatic H), 4.97 (s, 2H, OCH_2), 3.96 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 163.70 (NCOPhe), 163.60 (CONH), 155.20-111.00 (25 C, aromatic), 62.20 (OCH_2), 54.00 (OCH_3). MS (EI, 70 eV): m/z (%) = 437 (M^+, 1.12), 314 (19.19), 257 (100), 256 (20.66), 109 (11.00), 57 (24.75), 55

(7.70), 51 (6.80). Molecular formula (M.wt.): $C_{30}H_{23}N_3O_5S$ (537.6). Calculated analysis: C, 67.03; H, 4.31; N, 7.82; found: C, 67.00; H, 4.27; N, 7.81.

*4.1.5. Synthetic routes for compounds **11–21***

A dichloromethane solution of compound **4** (1mmol, 50 ml) was drop wisely added to a cold and stirred dichloromethane solution (50 ml) of free amino acid (1mmol, obtained by the addition of two equivalents amount of triethylamine (2mmol) to the amino acid to a stirred and cold dichloromethane, 50 ml) in ice bath. The obtained mixture was additionally stirred for extra 3 h in ice bath, then for 24 h at room temperature, washed with distilled water, 1N sodium bicarbonate, 1N potassium hydrogen sulphate and distilled water then dried for (24 h at 0°C) over sodium sulphate anhydrous. The volatile materials were evaporated till drought then triturated with petroleum ether (B.P. = 40-60 °C) to get residual material. The obtained precipitate was collected, and then recrystallized from methanol to gain the compounds **11–21**.

*4.1.5.1. 2-(2-(2-(2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetyl)hydrazinyl)-2-oxoethylamino)propanoic acid (**11**).* Yield: 80%; melting point: 213–215°C, IR (cm^{-1}): (KBr): γ = 3400 (NH stretching), 3167, 3047 (CH, aromatic), 2925 (CH, aliphatic), 1679 (C=O, acid), 1632, 1612 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.45, 10.37 (s, 2H, 2NH, D_2O exchangeable, amide, hydrazide), 8.11-7.10 (s, 7H, aromatic H), 4.79, 4.73 (s, 2H, OCH_2), 4.18 (s, 2H, $\underline{CH_2}NH$), 3.93 (s, 3H, OCH_3), 3.44 (m, 1H, $\underline{CH}CH_3$), 1.27-1.25 (d, 3H, $CH\underline{CH_3}$). ^{13}C-NMR (500 MHz, δ, ppm, DMSO-d_6): δ = 167.51 ($\underline{C}OOH$), 166.62, 165.32 (2$\underline{C}$ONH), 154.02-110.25 (13 C, aromatic), 66.99 (OCH_2), 56.20 (CH_2NH), 7.88 (CH_3). MS (EI, 70 eV): m/z (%) = 458 (M^+, 0.09), 405 (26.91), 314 (9.79), 258 (20.66), 257 (100), 256 (62.00), 109 (11.71), 69 (20.12), 57 (14.80), 55 (17.75), 51 (6.95). Molecular formula (M.wt.): $C_{21}H_{22}N_4O_6S$ (458.5). Calculated analysis: C, 55.01; H, 4.84; N, 12.22; found: C, 55.00; H, 4.79; N, 12.20.

*4.1.5.2. 2-(2-(2-(2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetyl)hydrazinyl)-2-oxoethylamino)-3-hydroxypropanoic acid (**12**).* Yield: 65 %; melting point: 202-205 °C, IR (cm^{-1}): (KBr): γ = 3395 (NH stretching), 3168, 3049 (CH, aromatic), 2920 (CH, aliphatic), 1689 (C=O, acid), 1622, 1612 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.44, 10.37 (s, 2H, 2NH, D_2O exchangeable, amide, hydrazide), 8.12-7.10 (s, 7H, aromatic H), 4.75 (s, 2H, OCH_2), 4.19, 4.17 (s, 2H, $\underline{CH_2}NH$), 3.93 (s, 3H, OCH_3), 3.48 (m, 1H, $\underline{CH}CH_2$), 3.44, 3.42 (d, 3H, $CH\underline{CH_2}$). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 167.54 ($\underline{C}OOH$), 166.61, 165.31 (2$\underline{C}$ONH), 154.05-110.29 (13 C, aromatic), 66.83 (3C, $O\underline{C}H_2$, $NH\underline{C}H\underline{C}H_2$), 56.04 (2C, $O\underline{C}H_3$, $CO\underline{C}H_2NH$), 7.88 (CH_3). MS (EI, 70 eV): m/z (%) = 474 (M^+, 0.03), 405 (14.39), 374 (4.11), 314 (11.28), 270 (7.46), 258 (21.64), 257 (100), 256 (67.29), 228 (32.83), 185 (17.44), 69 (20.75), 57 (10.30), 51 (8.36). Molecular formula (M.wt.): $C_{21}H_{22}N_4O_7S$ (474.5). Calculated analysis: C, 53.16; H, 4.67; N, 11.81; found: C, 53.12; H, 4.66; N, 11.80.

*4.1.5.3. 2-(2-(2-(2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetyl)hydrazinyl)-2-oxoethylamino)-5-guanidinopentanoic acid (**13**).* Yield: 85%; melting point: 165-168 °C, IR (cm^{-1}): (KBr): γ = 3397 (NH stretching), 3167, 3049 (CH, aromatic), 2922 (CH, aliphatic), 1679 (C=O, acid), 1632, 1612 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.65 (s, 1H, OH, acid), 10.45, 10.37 (s, 2H, 2NH, D_2O exchangeable, amide, hydrazide), 8.12-7.12 (s, 7H, aromatic H), 4.87 (s, 2H, OCH_2), 3.93 (s, 3H, OCH_3), 3.55 (s, 2H, $\underline{CH_2}NH$), 3.47 (t, $\underline{CH}CH_2CH_2$), 1.71-1.26 (6H, $3CH_2$, aliphatic). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 171.19 ($\underline{C}OOH$), 167.52, 166.62 (2$\underline{C}$ONH), 165.32 (CNH_2), 162.37-110.29 (13 C, aromatic), 67.02 ($O\underline{C}H_2$), 66.91 ($NH\underline{C}HCOOH$), 61.21 ($O\underline{C}H_3$), 56.20 ($CO\underline{C}H_2NH$), 54.61 ($CH_2CH_2\underline{C}H_2NH$), 25.45 (2C, $\underline{C}H_2\underline{C}H_2CH_2NH$). MS (EI, 70 eV): m/z (%) = 543 (M^+, 0.11), 424 (6.84), 328 (9.48), 314 (19.61), 282 (11.03), 258 (21.49), 257 (100), 256 (74.33), 228 (27.20), 185 (19.73), 86 (31.13), 69 (58.18), 57 (50.56), 55 (60.23). Molecular formula (M.wt.): $C_{24}H_{29}N_7O_6S$ (543.6). Calculated analysis: C, 53.03; H, 5.38; N, 18.04; found: C, 52.99; H, 5.37; N, 18.04.

*4.1.5.4. 6-(2-(2-(2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetyl)hydrazinyl)-2-oxoethylamino)hexanoic acid (**14**).* Yield: 80%; melting point: 202-205 °C, IR (cm^{-1}): (KBr): γ = 3389 (NH stretching), 3167, 3049 (CH, aromatic), 2920 (CH, aliphatic), 1679 (C=O, acid), 1632, 1612 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.82 (s, 1H, OH, acid), 10.44, 10.36 (s, 2H, 2NH, D_2O exchangeable, amide, hydrazide), 8.12-7.10 (s, 7H, aromatic H), 4.75 (s, 2H, OCH_2), 3.93, 3.92 (s, 3H, OCH_3), 3.46 (s, 2H, $\underline{CH_2}NH$), 2.51-1.25 (10H, $5CH_2$, aliphatic). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 167.53 ($\underline{C}OOH$), 166.64, 165.35 (2$\underline{C}$ONH), 154.00-110.31 (13 C, aromatic), 66.93 ($O\underline{C}H_2$), 56.21 ($O\underline{C}H_3$), 41.28 (2C, $NH\underline{C}H_2CH_2CH_2CH_2\underline{C}H_2$ COOH), 39.00 (3C, $NHCH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2CH_2COOH$). MS (EI, 70 eV): m/z (%) = 501 (M^+, 0.03), 404 (12.64), 314 (16.34), 270 (7.22), 258 (21.16), 257 (100), 256 (67.70), 228 (37.12), 185 (23.19), 86 (56.74), 69 (37.46), 57 (27.97). Molecular formula (M.wt.): $C_{24}H_{28}N_4O_6S$ (500.6). Calculated analysis: C, 57.59; H, 5.64; N, 11.19; found: C, 57.59; H, 5.65; N, 11.18.

*4.1.5.5. 5-amino-2-(2-(2-(2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetyl) hydrazinyl)-2-oxoethylamino)-5-oxopentanoic acid (**15**).* Yield: 75 %; melting point: 207–209°C, IR (cm^{-1}): (KBr): γ = 3396 (NH stretching), 3168, 3049 (CH, aromatic), 2920 (CH, aliphatic), 1678 (C=O, acid), 1625, 1615 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.65 (s, 1H, OH, acid), 10.45, 10.37 (s, 2H, 2NH, D_2O exchangeable, amide, hydrazide), 8.12-7.10 (s, 7H, aromatic H), 4.75 (s, 2H, OCH_2), 3.93 (s, 3H, OCH_3), 3.44 (3H, $\underline{CH_2}NH$ and $\underline{CH}CH_2CH_2$), 2.51 (q, 2H, CH_2, $CHCH_2\underline{CH_2}$), 1.27-1.25 (m, 2H, CH_2, $CH\underline{CH_2}CH_2$). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 167.50 ($\underline{C}OOH$), 166.62 ($CONH_2$), 165.32, 154.02 (2$\underline{C}$ONH), 150.50-110.29 (13 C, aromatic), 66.96 (2C, $O\underline{C}H_2$, $NH\underline{C}HCOOH$), 56.20 (2C, $O\underline{C}H_3$, $CO\underline{C}H_2NH$), 41.30, 39.56 ($CH_2CH_2CONH_2$). MS (EI, 70 eV): m/z (%) = 515 (M^+, 0.08), 406 (12.60), 317 (26.33), 257 (100), 228 (77.10), 185 (73.10), 69 (30.06), 57 (20.90). Molecular formula (M.wt.): $C_{23}H_{25}N_5O_7S$ (515.5). Calculated analysis: C, 53.58; H, 4.89; N, 13.58; found: C, 53.57; H, 4.88; N, 13.60.

*4.1.5.6. 2-(2-(2-(2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetyl)hydrazinyl)-2-oxoethylamino)-3-hydroxybutanoic acid (**16**).* Yield: 70%; melting point: 209-212 °C, IR (cm^{-1}): (KBr): γ = 3371 (NH stretching), 3167, 3049 (CH, aromatic), 2921 (CH, aliphatic), 1679 (C=O, acid), 1622, 1612 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.43, 10.36 (s, 2H, 2NH, D_2O exchangeable, amide, hydrazide), 8.12-7.10 (s, 7H, aromatic H), 4.74 (s, 2H, OCH_2), 4.17 (s, 2H, $\underline{CH_2}NH$), 3.93 (s, 3H, OCH_3), 3.46 (d, 1H, $NHCH\underline{CH}$), 3.18 (d, 1H, $NH\underline{CH}CH$). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 167.53 ($\underline{C}OOH$), 166.64, 165.35 (2$\underline{C}$ONH), 154.00-110.29 (13 C, aromatic), 66.95 ($NH\underline{C}HCH$), 66.92 (2C, OCH_2, $NHCH\underline{C}H$), 56.21 (2C, $O\underline{C}H_3$, $CO\underline{C}H_2NH$), 7.86 (CH_3). MS (EI, 70 eV): m/z (%) = 488 (M^+, 0.03), 400 (22.04), 314 (10.30), 270 (77.25), 257 (100), 256 (60.60), 86 (54.78), 57 (7.90). Molecular formula (M.wt.): $C_{22}H_{24}N_4O_7S$ (488.5). Calculated analysis: C, 54.09; H, 4.95; N, 11.47; found: C, 54.08; H, 4.92; N, 11.47.

*4.1.5.7. 2-(2-(2-(2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetyl)hydrazinyl)-2-oxoethylamino)succinic acid (**17**).* Yield: 85%; melting point: 200-203 °C, IR (cm^{-1}): (KBr): γ = 3397 (NH stretching), 3168, 3048 (CH, aromatic), 2921 (CH, aliphatic), 1679 (C=O, acid), 1620, 1602 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.44, 10.36 (s, 2H, 2NH, D_2O exchangeable, amide, hydrazide), 8.11-7.10 (s, 7H, aromatic H), 4.74 (s, 2H, OCH_2), 4.17 (s, 2H, CH_2NH), 3.93 (s, 3H, OCH_3), 3.49 (m, 1H, CHCH$_2$), 2.51 (d, 2H, CHCH$_2$). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 167.59, 166.64 (2C, 2COOH), 165.35 (2CONH), 154.35-110.29 (13 C, aromatic), 66.95 (2C, OCH_2, NHCHCH), 56.20 (2C, OCH_3, CH_2NH), 40.12 (CH_2COOH). MS (EI, 70 eV): m/z (%) = 502 (M^+, 0.52), 409 (1.53), 314 (22.71), 258 (21.91), 257 (100), 256 (84.27), 228 (42.89), 185 (26.49), 86 (37.08), 57 (30.01), 52 (55.15). Molecular formula (M.wt.): $C_{22}H_{22}N_4O_8S$ (502.5). Calculated analysis: C, 52.58; H, 4.41; N, 11.15; found: C, 52.58; H, 4.40; N, 11.14.

*4.1.5.8. 2-(2-(2-(2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetyl)hydrazinyl)-2-oxoethylamino)pentanoic acid (**18**).* Yield: 90%; melting point: 197-199 °C, IR (cm^{-1}): (KBr): γ = 3396 (NH stretching), 3168, 3048 (CH, aromatic), 2921 (CH, aliphatic), 1679 (C=O, acid), 1622, 1602 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.47, 10.38 (s, 2H, 2NH, D_2O exchangeable, amide, hydrazide), 8.10-7.10 (s, 7H, aromatic H), 4.75 (s, 2H, OCH_2), 4.18 (s, 2H, CH_2NH), 3.93 (s, 3H, OCH_3), 3.48 (m, 1H, CHCH$_2$), 2.51 (t, 2H, CHCH$_2$), 1.20-1.18 (m, 2H, CH_2CH_2), 0.92-0.82 (t, 3H, CH_2CH_3). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 167.52 (COOH), 166.64, 165.35 (2CONH), 154.02-110.24 (13 C, aromatic), 66.96 (2C, OCH_2, NHCHCOOH), 56.19 (2C, OCH_3, $COCH_2NH$), 41.30-8.90 (3C, $CH_2CH_2CH_3$). MS (EI, 70 eV): m/z (%) = 487 (M^+ +1, 0.52), 486 (M^+, 2.50), 319 (20.70), 258 (20.90), 257 (100), 256 (94.27), 228 (40.80), 185 (36.40), 86 (47.18), 57 (40.04). Molecular formula (M.wt.): $C_{23}H_{26}N_4O_6S$ (486.5). Calculated analysis: C, 56.78; H, 5.39; N, 11.52; found: C, 56.78; H, 5.38; N, 11.53.

*4.1.5.9. 2-(2-(2-(2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetyl)hydrazinyl)-2-oxoethylamino)-4-mercaptobutanoic acid (**19**).* Yield: 80%; melting point: 195-197 °C, IR (cm^{-1}): (KBr): γ = 3371 (NH stretching), 3164, 3046 (CH, aromatic), 2919 (CH, aliphatic), 1676 (C=O, acid), 1623 and 1602 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.37, 10.28 (s, 2H, 2NH, D_2O exchangeable, amide, hydrazide), 8.12-7.02 (s, 7H, aromatic H), 4.80 (s, 2H, OCH_2), 4.20 (s, 2H, CH_2NH), 3.93 (s, 3H, OCH_3), 3.48 (m, 1H, CHCH$_2$), 2.53, 2.51 (t, 2H, CHCH$_2$), 2.20-2.00 (m, 2H, CH_2CH_2), 1.5 (s, 1H, CH_2SH). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 167.51 (COOH), 166.62, 165.33 (2CONH), 154.01-110.24 (13 C, aromatic), 66.98, 66.95 (2C, OCH_2, NHCHCOOH), 56.20 (2C, OCH_3, $COCH_2NH$), 41.30, 27.78 (2C, CH_2CH_2SH). MS (EI, 70 eV): m/z (%) = 505 (M^+ +1, 0.75), 504 (M^+, 3.05), 429 (51.50), 344 (20.00), 258 (27.71), 257 (100), 256 (74.77), 227 (32.89), 187 (16.40), 87 (47.00), 57 (30.00), 50 (50.10). Molecular formula (M.wt.): $C_{22}H_{24}N_4O_6S_2$ (504.6). Calculated analysis: C, 52.37; H, 4.79; N, 11.10; found: C, 52.36; H, 4.77; N, 11.10.

*4.1.5.10. 2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)-N'-(2-(2-oxo-2,3-dihydropyrimidin-4-ylamino)acetyl)acetohydrazide (**20**).* Yield: 85%; melting point: 118-120 °C, IR (cm^{-1}): (KBr): γ = 3400 (NH stretching), 3100 (CH, aromatic), 2910 (CH, aliphatic), 1630 and 1600 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 10.52, 10.45 (s, 2H, 2NH, D_2O exchangeable, amide, hydrazide), 8.15 (s, H, NH, D_2O exchangeable, pyrimidine moiety), 8.02-7.25 (s, 9H, 7 H aromatic H, 2 H pyrimidine moiety), 4.80, 4.70 (s, 2H, OCH_2), 3.93, 3.90 (s, 2H, CH_2NH), 3.40 (s, 3H, OCH_3), 3.30 (s, 2 H, CH_2-Phe). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 167.00, 164.20 (2CONH), 163.00 (NHCCHCHN, pyrimidine moiety), 155.20 (CO, pyrimidine moiety), 150.00 (NHCCHCHN, pyrimidine moiety), 149.00-109.00 (13 C, aromatic), 91.00 (NHCCHCHN, pyrimidine moiety), 68.55 (OCH_2), 69.50 (OCH_3), 49.00 ($COCH_2NH$). MS (EI, 70 eV): m/z (%) = 480 (M^+, 1.50), 459 (11.50), 330 (42.72), 257 (100), 256 (80.20), 228 (62.80), 187 (66.00), 86 (47.18), 57 (60.11). Molecular formula (M.wt.): $C_{22}H_{20}N_6O_5S$ (480.5). Calculated analysis: C, 54.99; H, 4.20; N, 17.49; found: C, 54.98; H, 4.21; N, 17.49.

*4.1.5.11. 2-(2-(2-(2-(4-(benzo[d]thiazol-2-yl)-2-methoxyphenoxy)acetyl)hydrazinyl)-2-oxoethylamino)-3-phenylpropanoic acid (**21**).* Yield: 75%; melting point: 256-258 °C, IR (cm^{-1}): (KBr): γ = 3390 (NH stretching), 3170, 3070 (CH, aromatic), 2900 (CH, aliphatic), 1680 (C=O, acid), 1630 and 1610 (C=O amide I and II, respectively). ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): δ = 12.50 (s, H, COOH), 10.48-10.35 (s, 3H, 3NH, D_2O exchangeable), 8.12-7.02 (s, 12H, aromatic H), 4.75, 4.65 (s, 2H, OCH_2), 3.97, 3.95 (s, 2H, CH_2NH), 3.87 (s, H, CHCOOH), 3.35 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): δ = 170.00 (COOH), 165.75, 164.00 (2CONH), 152.00-110.50 (19 C, aromatic), 67.85 (OCH_2), 62.00 (OCH_3), 60.00 (CH_2-Phe), 51.05 ($COCH_2NH$). MS (EI, 70 eV): m/z (%) = 535 (M^+ +1, 0.50), 534 (M^+, 2.60), 439 (11.50), 321 (20.70), 257 (100), 230 (52.80), 185 (36.00), 87 (30.28), 57 (30.21). Molecular formula (M.wt.): $C_{27}H_{26}N_4O_6S$ (534.6). Calculated analysis: C, 60.66; H, 4.90; N, 10.48; found: C, 60.65; H, 4.90; N, 10.50.

4.2. Biological activity

4.2.1. Cytotoxicity assay

The cytotoxic activities of the newly synthesized derivatives **4–21** on the HepG-2, MCF-7 human cancer cell lines and normal fibroblast cell line (WI-38) were assessed, employing the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay according to the reported method [42]. More details were indicated in the supplementary file.

4.2.2. EGFR inhibitory assay

The promising cytotoxic targets **5, 6, 7, 13** and **16** were estimated for their *in vitro* inhibitory activity against epidermal growth factor receptor (EGFR). Erlotinib was allowed as a reference following the previously mentioned process [45]. More details were indicated in the supplementary file.

4.2.3. Flow cytometry analysis and annexin V-FITC apoptosis study

Cell cycle analysis and apoptosis investigation were done by flow cytometry [45]. MCF-7 cells were incubated at 37°C and treated with the tested compounds, for 24 h. More details were indicated in the supplementary file.

4.2.4. The impact of the derivatives 5 and 6 on Bax / Bcl-2 / p53 / caspase-3 levels

The levels of the apoptotic marker Bax, anti-apoptotic marker Bcl-2 and the tumor suppressor gene p53 were estimated using BIO RAD iScript TM One-Step RT-PCR kit with SYBR® Green. The procedure of the used kit was done according to the manufacturer's instructions. The activity of caspases-3 was assessed using DRG Caspase-3 (human) ELISA (EIA-4860) kit (DRG International Inc., USA) according to the manufacturer instructions.

4.3. Molecular docking study

The molecular docking is a powerful instrument to rationalize the obtained biological data. The interactions of the newly synthesized compounds **5, 6**, and **7** having the highest EGFR inhibitory

activity were examined with the active site of the target enzyme to study their binding modes and orientations using (PDB ID: 1M17) [45] and MOE, 10.2008 software [46]. More details were indicated in the supplementary file.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.130595.

References

[1] V.T. DeVita, E. Chu, A history of cancer chemotherapy, Cancer Res. 68 (21) (2008) 8643–8653, doi:10.1158/0008-5472.
[2] E. Dickens, S. Ahmed, Principles of cancer treatment by chemotherapy, Surgery (Oxford) 36 (3) (2018) 134–138 https://doi.org/, doi:10.1016/j.mpsur.2017.12.002.
[3] G. Housman, S. Byler, S. Heerboth, K. Lapinska, M. Longacre, N. Snyder, S. Sarkar, Drug resistance in cancer: an overview, Cancers 6 (3) (2014) 1769–1792, doi:10.3390/cancers6031769.
[4] J. Zugazagoitia, C. Guedes, S. Ponce, I. Ferrer, S. Molina-Pinelo, L. Paz-Ares, Current challenges in cancer treatment, Clinical Ther. 38 (7) (2016) 1551–1566, doi:10.1016/j.clinthera.2016.03.026.
[5] J. Boström, D.G. Brown, R.J. Young, G.M. Keserü, Expanding the medicinal chemistry synthetic toolbox, Nat. Rev. Drug Discov. 17 (10) (2018) 709–727, doi:10.1038/nrd.2018.116.
[6] V.G. Reddy, T.S. Reddy, C. Jadala, M.S. Reddy, F. Sultana, R. Akunuri, S.K. Bhargava, D. Wlodkowic, P. Srihari, A. Kamal, Pyrazolo-benzothiazole hybrids: synthesis, anticancer properties and evaluation of antiangiogenic activity using in vitro VEGFR-2 kinase and in vivo transgenic zebrafish model, Eur. J. Med. Chem. 182 (2019) 111609, doi:10.1016/j.ejmech.2019.111609.
[7] A. Levitzki, Tyrosine kinase inhibitors: views of selectivity, sensitivity, and clinical performance, Annu. Rev. Pharmacol. Toxicol. 53 (2013) 161–185, doi:10.1146/annurev-pharmtox-011112-140341.
[8] P.C. Lv, C.F. Zhou, J. Chen, P.G. Liu, K.R. Wang, W.J. Mao, H.Q. Li, Y. Yang, J. Xiong, H.L. Zhu, Design, synthesis and biological evaluation of thiazolidinone derivatives as potential EGFR and HER-2 kinase inhibitors, Bioorg. Med. Chem. 18 (1) (2010) 314–319, doi:10.1016/j.bmc.2009.10.051.
[9] J. Capdevila, E. Elez, T. Macarulla, F.J. Ramos, M. Ruiz-Echarri, J. Tabernero, Anti-epidermal growth factor receptor monoclonal antibodies in cancer treatment, Cancer Treat. Rev. 35 (4) (2009) 354–363, doi:10.1016/j.ctrv.2009.02.001.
[10] D.A. Reardon, P.Y. Wen, I.K. Mellinghoff, Targeted molecular therapies against epidermal growth factor receptor: past experiences and challenges, Neuro Oncol. 16 (suppl_8) (2014) viii7–viii13, doi:10.1093/neuonc/nou232.
[11] P. Seshacharyulu, M.P. Ponnusamy, D. Haridas, M. Jain, A.K. Ganti, S.K. Batra, Targeting the EGFR signaling pathway in cancer therapy, Expert Opin. Ther. Targets 16 (1) (2012) 15–31, doi:10.1517/14728222.2011.648617.
[12] Y. Yardern, An. Ullrich, Development variable receptor tyrosine kinases, Annu. Rev. Biochem. 57 (1988) 443–478, doi:10.1146/annurev.bi.57.070188.002303.
[13] A. Petrelli, S. Giordano, From single to multi-target drugs in cancer therapy: when specificity becomes an advantage, Curr. Med. Chem. 15 (2008) 422–432, doi:10.2174/092986708783503212.
[14] W. Pao, V. Miller, M. Zakowski, J. Doherty, K. Politi, I. Sarkaria, B. Singh, R. Heelan, V. Rusch, L. Fulton, E. Mardis, D. Kupfer, R. Wilson, M. Kris, H. Varmus, EGF receptor gene mutations are common in lung cancers from 'never smokers' and are associated with sensitivity of tumors to gefitinib and erlotinib, Proc. Natl. Acad. Sci. USA 101 (2004) 13306–13311, doi:10.1073/pnas.0405220101.
[15] H.W. Lo, S.C. Hsu, M.C. Hung, EGFR signaling pathway in breast cancers: from traditional signal transduction to direct nuclear translocalization, Breast Cancer Res. Treat. 95 (2006) 211–218, doi:10.1007/s10549-005-9011-0.
[16] A. Gupta, S. Rawat, Synthesis and cyclization of benzothiazole, J. Curr. Pharm. Res. 3 (2010) 13–23.
[17] M. Chhabra, S. Sinha, S. Banerjee, P. Paira, An efficient green synthesis of 2-arylbenzothiazole analogues as potent antibacterial and anticancer agents, Bioorg. Med. Chem. Lett. 26 (2016) 213–217, doi:10.1016/j.bmcl.2015.10.087.
[18] S. Shafi, M.M. Alam, N. Mulakayala, C. Mulakayala, G. Vanaja, A.M. Kalle, R. Pallu, M.S. Alam, Synthesis of novel 2-mercapto benzothiazole and 1,2,3-triazole based bis-heterocycles: Their anti-inflammatory and anti-nociceptive activities, Eur. J. Med. Chem. 49 (2012) 324–333, doi:10.1016/j.ejmech.2012.01.032.
[19] N. Siddiqui, S.N. Pandeya, S.A. Khan, J. Stables, A. Rana, M. Alam, M.F. Arshad, M.A. Bhat, Synthesis and anticonvulsant activity of sulfonamide derivatives-hydrophobic domain, Bioorg. Med. Chem. Lett. 17 (2007) 255–259, doi:10.1016/j.bmcl.2006.09.053.
[20] L. Katz, Antituberculous compounds. iii. benzothiazole and benzoxazole derivatives, J. Am. Chem. Soc. 75 (3) (1953) 712–714, doi:10.1021/ja01099a059.
[21] Y.I. Asiri, A. Alsayari, A.B. Muhsinah, et al., Benzothiazoles as potential antiviral agents, J. Pharmacy Pharmacol. 72 (2020) 1459–1480, doi:10.1111/jphp.13331.
[22] A. Burger, S.N. Sawhney, Antimalarials. III. benzothiazole amino alcohols, J. Med. Chem. 11 (2) (1968) 270–273 https://doi.org/, doi:10.1021/jm00308a018.
[23] H. Moreno-Diaz, R. Villalobos-Molina, R. Ortiz-Andrade, et al., Antidiabetic activity of N-(6-substituted-1,3-benzothiazol-2-yl)benzenesulfonamides, Bioorg. Med. Chem. Lett. 18 (9) (2008) 2871–2877, doi:10.1016/j.bmcl.2008.03.086.
[24] P.K. Sahu, P.K. Sahu, P. Samadhiya, P.L. Sahu, D.D. Agarwal, POM analyses and evaluation of *in vitro* antimicrobial, antitumor activity of 4H-Pyrimido[2,1-b]benzothiazole derivatives, Med. Chem. Res. 25 (8) (2016) 1551–1563 https://doi.org/, doi:10.1007/s00044-016-1589-8.
[25] P.C. Sharma, A. Sinhmar, A. Sharma, H. Rajak, D.P. Pathak, Medicinal significance of benzothiazole scaffold: an insight view, J. Enzyme Inhib. Med. Chem. 28 (2) (2013) 240–266, doi:10.3109/14756366.2012.720572.
[26] S. Saeed, N. Rashid, P.G. Jones, M. Ali, R. Hussain, Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents, Eur. J. Med. Chem. 45 (4) (2010) 1323–1331, doi:10.1016/j.ejmech.2009.12.016.
[27] M.A. Abdelgawad, A. Belal, H.A. Omar, L. Hegazy, M.E. Rateb, Synthesis, antibreast cancer activity, and molecular modeling of some benzothiazole and benzoxazole derivatives, Archiv der Pharmazie. 346 (7) (2013) 534–541 https://doi.org/, doi:10.1002/ardp.201300044.
[28] A. Irfan, F. Batool, S.A. Zahra Naqvi, A. Islam, S.M. Osman, A. Nocentini, S.A. Alissa, C.T. Supuran, Benzothiazole derivatives as anticancer agents, J. Enzyme Inhib. Med. Chem. 35 (1) (2020) 265–279, doi:10.1080/14756366.2019.1698036.
[29] M.T. Gabr, N.S. El-Gohary, E.R. El-Bendary, M.M. El-Kerdawy, EGFR tyrosine kinase targeted compounds: in vitro antitumor activity and molecular modeling studies of new benzothiazole and pyrimido [2, 1-b] benzothiazole derivatives, EXCLI J. 13 (2014) 573 https://www.excli.de/index.php/excli/article/view/723.
[30] K.R. Abdellatif, A. Belal, M.T. El-Saadi, N.H. Amin, E.G. Said, L.R. Hemeda, Design, synthesis, molecular docking and antiproliferative activity of some novel benzothiazole derivatives targeting EGFR/HER2 and TS, Bioorg. Chem. 101 (2020) 103976, doi:10.1016/j.bioorg.2020.103976.
[31] M.T. Gabr, N.S. El-Gohary, E.R. El-Bendary, M.M. El-Kerdawy, New series of benzothiazole and pyrimido [2, 1-b] benzothiazole derivatives: synthesis, antitumor activity, EGFR tyrosine kinase inhibitory activity and molecular modeling studies, Med. Chem. Res. 24 (2) (2015) 860–878, doi:10.1007/s00044-014-1114-x.
[32] W.S. El-Hamouly, K.M. Amin, S.A. El-Assaly, E.A. Abdel-Meguid, Synthesis and antitumor activity of some new N-substituted-sulfonyl, 1,2,4-triazole, N-substituted-benzylidene and pyrrole derivatives attached to 4-(benzo[d]thiazol-2-yl)benzohydrazide, Der Pharma Chemica. 3 (6) (2011) 293–306.
[33] E.A. Abd El-Meguid, M.M. Ali, Synthesis and biological evaluation of some new substituted-pyrazole attached to 4-(benzothiazol-2-yl) moiety as anti-angiogenic agents, Res. J. Pharm. Biol. Chem. Sci. 5 (6) (2014) 888–897.
[34] E.A. Abd El-Meguid, M.M. Ali, Anticancer evaluation of some newly synthesized oxadiazol-2-yl-pyrazole derivatives attached to 4-benzothiazol-2-yl moiety, Res. J. Pharm. Biol. Chem. Sci. 6 (2) (2015) 524–532.
[35] E.A. Abd El-Meguid, E.R. Kotb, S.F. Mohamed, Synthesis and antibreast cancer evaluation of some novel schiff's bases and their c-nucleoside derivatives incorporating a 4-benzothiazol-2-yl moiety, Int. J. Pharm. Technol. 7 (1) (2015) 8363–8382.
[36] E.A. Abd El-Meguid, M.M. Ali, Synthesis of some novel 4-benzothiazol-2-yl-benzoyl-1H-pyrazoles, and evaluation as antiangiogenic agents, Res. Chem. Intermed. 42 (2) (2016) 1521–1536 https://doi.org/, doi:10.1007/s11164-015-2100-8.
[37] K.M. Amin, E.A. Abd El-Meguid, S.A. El-Assaly, W.S. El-Hamouly, Synthesis, antibreast cancer activity and docking study of some novel 4-(benzo[d]thiazol-2-yl)phenyl moiety as CDK2 inhibitors, Int. J. Pharm. Technol. 8 (1) (2016) 10853–10871.
[38] A.F. Kassem, G.O. Moustafa, E.S. Nossier, H.S. Khalaf, M.M. Mounier, S.A. Al-Yousef, S.Y. Mahmoud, In vitro anticancer potentiality and molecular modelling study of novel amino acid derivatives based on N1, N3-bis-(1-hydrazinyl-1-oxopropan-2-yl) isophthalamide, J. Enzyme Inhib. Med. Chem. 34 (1) (2019) 1247–1258, doi:10.1080/14756366.2019.1613390.
[39] M.H. Abo-Ghalia, G.O. Moustafa, A.E. Amr, A.M. Naglah, E.A. Elsayed, A.H. Bakheit, Anticancer activities of newly synthesized chiral macrocyclic heptapeptide candidates, Molecules 25 (5) (2020) 1253, doi:10.3390/molecules25051253.
[40] I.A.Y. Ghannam, E.A. Abd El-Meguid, I.H. Ali, D.H. Sheir, A.M. El Kerdawy, Novel 2-arylbenzothiazole DNA gyrase inhibitors: Synthesis, antimicrobial evaluation, QSAR and molecular docking studies, Bioorg. Chem. 93 (2019) 103373 https://doi.org/, doi:10.1016/j.bioorg.2019.103373.
[41] H.T. Abdel-Mohsen, E.A. Abd El-Meguid, A.M. El Kerdawy, A.E.E. Mahmoud, M.M. Ali, Design, synthesis, and molecular docking of novel 2-arylbenzothiazole multiangiokinase inhibitors targeting breast cancer, Arch. Pharm. Chem. Life Sci. 353 (2020) e1900340, doi:10.1002/ardp.201900340.

[42] E.S. Nossier, A. El-Karim, S. Somaia, N.M. Khalifa, A.S. El-Sayed, E.S. Hassan, S.M. El-Hallouty, Kinase inhibitory activities and molecular docking of a novel series of anticancer pyrazole derivatives, Molecules 23 (12) (2018) 3074, doi:10.3390/molecules23123074.
[43] C.M. Pfeffer, A.T.K. Singh, Apoptosis: a target for anticancer therapy, Int. J. Mol. Sci. 19 (2) (2018) 448, doi:10.3390/ijms19020448.
[44] K.W. Yip, J.C. Reed, BCL-2 family proteins and cancer, Oncogene 77 (2008) 6398–6406 https://doi.org/, doi:10.1038/onc.2008.307.
[45] A.M. Srour, N.S. Ahmed, S.S. Abd El-Karim, M.M. Anwar, S.M. El-Hallouty, Design, synthesis, biological evaluation, QSAR analysis and molecular modelling of new thiazol-benzimidazoles as EGFR inhibitors, Bioorg. Med. Chem. 28 (18) (2020) 115657, doi:10.1016/j.bmc.2020.115657.
[46] I.M. Othman, M.A. Gad-Elkareem, A.E.G.E. Amr, M.A. Al-Omar, E.S. Nossier, EA. Elsayed, Novel heterocyclic hybrids of pyrazole targeting dihydrofolate reductase: design, biological evaluation and in silico studies, J. Enzyme Inhib. Med. Chem. 35 (1) (2020) 1491–1502, doi:10.1080/14756366.2020.1791842.
[47] S.Y. Abbas, R.A.K. Al-Harbi, M.A.M.Sh El-Sharief, Synthesis and anticancer activity of thiourea derivatives bearing a benzodioxole moiety with EGFR inhibitory activity, apoptosis assay and molecular docking study, Eur. J. Med. Chem. 198 (2020) 112363, doi:10.1016/j.ejmech.2020.112363.