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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

EGFR tyrosine kinase targeted compounds: synthesis, docking study, and in vitro antitumor activity of some new quinazoline and benzo[*d*]isothiazole derivatives

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Abstract The preparation of new quinazoline and benzo[*d*]isothiazole-based antitumor agents is described. The target compounds fall into three groups including the N-substituted derivatives **2a–d**, the substituted amino derivatives **4–6a–d**, and the dimeric compounds **7–9a,b**. Docking study of the designed compounds into the ATP binding site of epidermal growth factor receptor (EGFR) tyrosine kinase was performed to compare the binding mode of these compounds to the known EGFR inhibitor, lapatinib. All compounds were tested, in vitro, for their activity against human mammary carcinoma cell line (MCF7) in which EGFR is highly expressed. All compounds showed significant growth inhibitory activity. The remarkable activity of the bis quinazoline derivative **8a** (IC_{50} = 0.06 μg/ml; 1.64 nmol/ml) is to be noted.

Keywords Quinazoline · Benzo[*d*]isothiazole · EGFR tyrosine kinase · Docking study · Antitumor activity · Human mammary carcinoma

Introduction

The control of disseminated tumor growth by systemically active chemotherapeutic agents remains a major challenge for cancer chemotherapy despite decades of focused efforts. Although there are some notable successes with certain forms of cancer, drug therapy has only limited impact against the three major killers: carcinoma of the lung, breast, and colorectal system (Schnur *et al.*, 1991).

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Protein tyrosine kinases (PTKs) are enzymes involved in many cellular processes such as cell proliferation, metabolism, and apoptosis. Several protein tyrosine kinases are activated in cancer cells resulting in tumor growth and progression (Jordan *et al.*, 2000). Inappropriate or uncontrolled activation of many of these kinases, by over-expression, constitutive activation or mutation has shown to result in uncontrolled cell growth. PTKs can be broadly classified as receptor (EGFR) or non-receptor kinases Blume-Jensen and Hunter, 2001). Over-expression of these receptors was found in many cancers such as breast, ovarian, colon, and prostate cancer. Therefore, blocking of tyrosine kinase activity represents a rational approach to cancer therapy and several drug discovery efforts have targeted this aberrant kinase activity in cancer (Abouzid and Shouman, 2008). Intensive research in the field of tyrosine kinase inhibitors led to the development of enormous number of active compounds among which are the FDA approved geftinib **I** (ZD1839, Iressa®) (Wakeling *et al.*, 2002) and lapatinib **II** (Higa and Abraham, 2007) (Fig. 1).

One of the major classes of benzoheterocyclic compounds that have drawn much attention in the field of cancer chemotherapy, besides other pharmacological activities, is the quinazoline ring (Morin, 2000; Bridges, 2001; Xia *et al.*, 2001; Al-Rashood *et al.*, 2006; Bavetsias *et al.*, 2007; Raghavendra *et al.*, 2009). Many 4-quinazolinone derivatives reveal potent anticancer activity mediated through the inhibition of dihydrofolate reductase enzyme (DHFR) (Masur, 1990; Berman and Werbel, 1991; Al-Rashood *et al.*, 2006; Al-Omary *et al.*, 2010) or through inhibition of Chk1 kinase (Converso *et al.*, 2009). Furthermore, 4-substituted quinazoline derivatives such as 4-pyrazolylamino-substituted quinazolines are found to be selective inhibitors of Aurora B kinase with potent antitumor activity (Foote *et al.*, 2008). In last decade, 4-

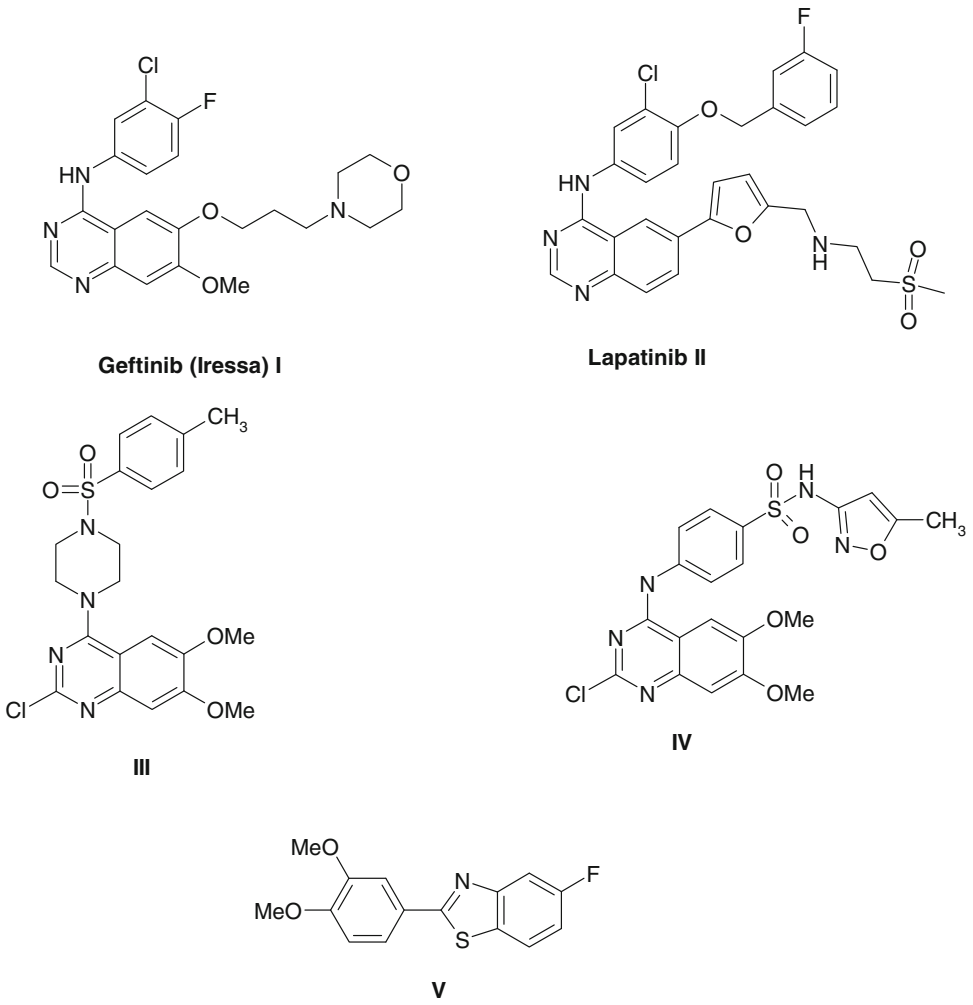


Fig. 1 Examples of some EGFR-TK inhibitors

anilinoquinazolines have emerged as a versatile template for inhibition of a diverse range of receptor tyrosine kinases (Szczepankiewicz *et al.*, 2000; Tobe *et al.*, 2003). The most widely studied of these are the inhibitors of the epidermal growth factor receptor (EGFR) tyrosine kinase and that have found wide clinical applications (Wakeling *et al.*, 1996; Grünwald and Hidalgo, 2003; Wakeling, 2005; Wedge *et al.*, 2005; Liu *et al.*, 2007; Lüth and Löwe, 2008; Li *et al.*, 2010). In a recent approach, the antitumor activity of quinazoline derivatives has shown to be enhanced by introducing sulfonamide-containing moieties substituted at position 4, as revealed in compounds **III** and **IV** which are potent inhibitors of EGFR PTK (Abouzid and Shouman, 2008) (Fig. 1). Evidently, the aforementioned geftinib and lapatinib are also derivatives of 4-anilinoquinazoline that exert their antitumor action by competing with ATP for binding at the catalytic domain of EGFR tyrosine kinase.

On the other hand, the non-classical bioisosteric benzothiazole ring system was revealed as a new promising skeleton for new anticancer agents. Many benzothiazole derivatives exhibit their antitumor activity through different mechanisms including inhibition of raf-1 (Song *et al.*, 2008), protein tyrosine phosphatase-1β (Sparks *et al.*, 2007), topoisomerase II (Choi *et al.*, 2006), and lysophosphatidic acid acyltransferase-β (Gong *et al.*, 2004). Moreover, the 2-arylbenzothiazole derivative **V** shows potent inhibitory activity against lung, colon, and breast cancer; an action that is mediated through inhibition of EGFR tyrosine kinase (Mortimer *et al.*, 2006) (Fig. 1). In addition, many 1,1-dioxobenzo[*d*]isothiazol-3(2*H*)-one (saccharin) derivatives show potent in vitro activity against various tumor cell lines (Güzel and Salamn, 2006).

In this study, we present new compounds having the quinazoline core as promising potent EGFR inhibitors. The target compounds were designed as to enhance the activity of the quinazoline nucleus by adding alternative binding groups such as the thiadiazolyl and sulfonamide moieties which are known to enhance the antitumor activity of compounds bearing them (Invidiata *et al.*, 1991; Al-Rashood *et al.*, 2006; Sławiński and Brzozowski, 2006).

In a similar approach, the 1,1-dioxobenzo[*d*]isothiazole scaffold was used as a non-classical bioisosteric substitute for the quinazoline ring in the design of analogous compounds, hoping to introduce this new ring system in the field of anticancer chemotherapy. Accordingly, 3-substituted quinazolin-4(3*H*)-ones **2a,b**, 4-substituted quinazolines **4–6a,b**, 2-substituted 1,1-dioxobenzo[*d*]isothiazol-3(2*H*)ones **2c,d**, and 3-substitued 1,1-dioxobenzo[*d*]isothiazoles **4–6c,d** were prepared. Moreover, and for further exploration of the activity of the quinazoline and benzo[*d*]isothiazole ring systems, bis compounds of these agents have been prepared using different linker groups such as 1,3-diaminophenyl **7a,b**, 1,4-diaminophenyl **8a,b**, and 1,2-diaminoethyl groups **9a,b**.

The binding mode of the designed compounds in EGFR homology model could be a helpful tool for predicting their mechanism of antitumor activity.

Results and discussion

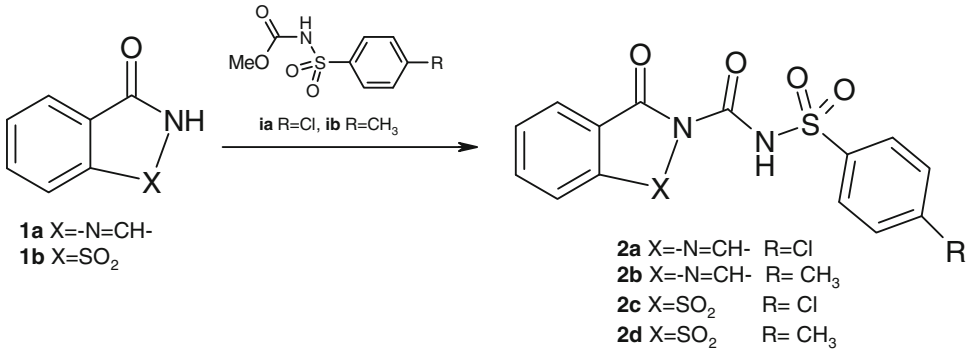
Chemistry

To verify the effects of structural modifications on the antitumor activity, substituted quinazoline and 1,1-dioxobenzo[*d*]isothiazole **2, 4–9** were synthesized.

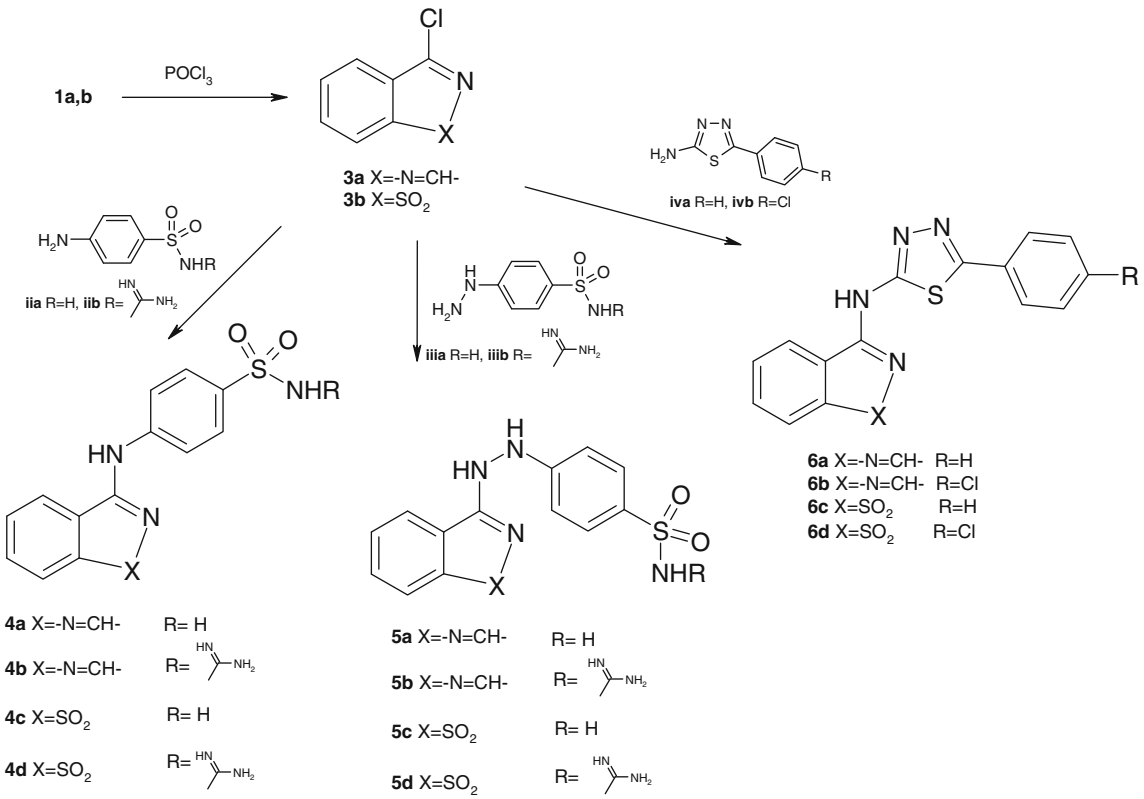
Compounds **2a–d** were synthesized via the reaction of **1a**; previously synthesized according to the literature method (Endicott *et al.*, 1946), or the commercially available saccharine **1b** with *N*-[(4-chloro/methyl phenyl)sulfonyl]carbamic acid methyl ester **ia,b** in a suitable solvent (Scheme 1). The structure of the obtained compounds was established through spectroscopic (IR, ^{1}H-NMR, Mass) as well as elemental analyses data. IR spectra revealed the presence of an additional carbonyl group (=1722–1660 cm^{-1}). ^{1}H-NMR showed the presence of singlet signal integrated for three protons of CH_3 at 2.18 and 2.50 ppm for **2b** and **2d**, respectively. Mass spectra of compounds **2a**, **2b**, and **2d** showed the molecular ion peaks of the compounds (=364 (M^+), 341 (M − 2), and 382 (M + 2), respectively).

On the other hand, the chloro compounds **3a,b** were prepared according to the previously reported procedure using phosphorous oxychloride (Endicott *et al.*, 1946; Meadoe and Reid, 1943). Reaction of the chloro derivatives with an appropriate sulfonamide **iia,b**, 4-hydrazinosulfonamide **iiia,b**, and 2-amino-5((4-chlorophenyl/phenyl) 1,3,4-thiadiazole **iva,b** afforded **4a–d**, **5a–d**, and **6a–d**, respectively (Scheme 2). IR spectra revealed the appearance of NHs in **4a–d** and **5a–d** (=3461–3121 cm^{-1}) and NH in **6a–d** (=3276–3267 cm^{-1}). ^{1}H-NMR revealed, in addition to the corresponding integration for the aromatic protons, the presence of NHs or NH protons. Also, mass spectra are concomitant with the molecular weight of the synthesized compounds.

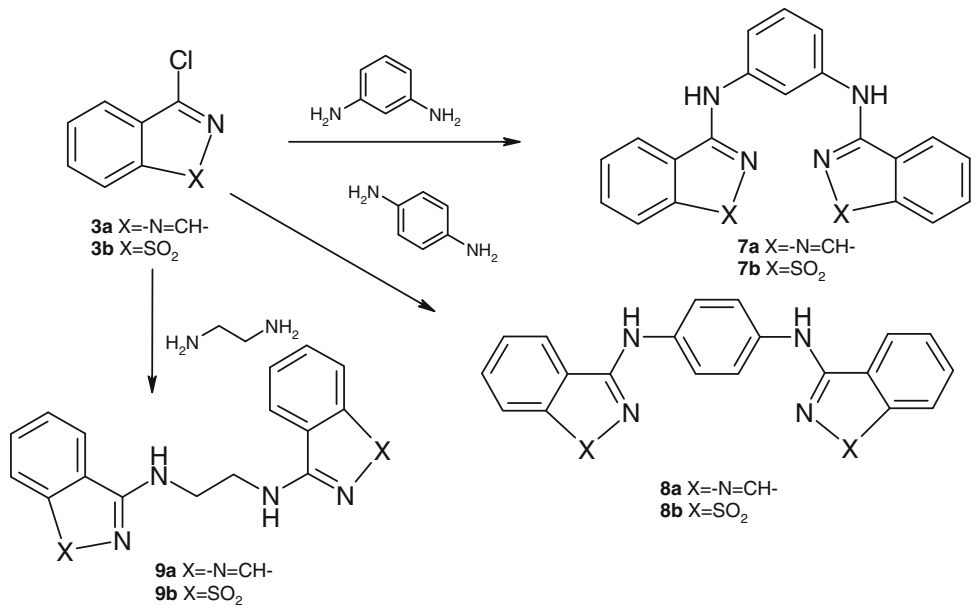
Moreover, the bis diamino derivatives **7a,b**, **8a,b**, and **9a** were synthesized by reacting one mole equivalent of 1,3-phenylenediamine, 1,4-phenylenediamine, or 1,2-ethylenediamine with two mole equivalents of **3a,b** in a suitable solvent in the presence of a basic catalyst as depicted in Scheme 3. IR revealed the presence of NH group (=3364–3285 cm^{-1}). ^{1}H-NMR naked, besides the corresponding integration for the aromatic protons, the presence of NH protons. In addition, mass spectra are concomitant with the calculated molecular weights of the compounds. In an attempt to prepare **9b** by reacting one mole equivalent of 1,2-ethylenediamine with two mole equivalents of **3b**, an un-separated water soluble product was obtained. Trials to isolate the product by extracting with different organic solvents failed. Alternatively, fusion of the two reactants with the same mentioned ratio followed by neutralization with sodium carbonate solution, extraction with chloroform to remove any organic impurities and evaporation of the aqueous solution afforded **9b** as an oily product together with the inorganic impurities. The pure product **9b** was obtained by centrifugation to leave out the inorganic impurities as a residue from which the product was decanted. ^{1}H-NMR was performed in D_2O and it revealed the presence of singlet signal at 3.40 ppm integrated for four protons corresponding to the CH_2CH_2 protons.



Scheme 1 Preparation of compounds 2**a–d**



Scheme 2 Preparation of compounds **4a–d**, **5a–d** and **6a–d**



Scheme 3 Preparation of compounds **7a,b**, **8a,b** and **9a,b**

Antitumor activity

The newly synthesized compounds were tested for their antitumor activity against human mammary carcinoma cell line (MCF7) in the National Cancer Institute, Cairo University. The screening involved calculation of the percentage growth of surviving fraction of the compound-treated cell lines compared by untreated control using Sulforhodamie B (SRB) colorimetric assay. Sulforhodamie B is a bright pink aminoxanthene anionic dye with two sulfonic acid groups that bind electrostatically to protein basic amino acid residues of trichloroacetic acid fixed cells under mild acidic conditions. Cultures fixed

with trichloroacetic acid were stained for 30 min with 0.4% w/v Sulforhodamie B dissolved in 1% acetic acid, and protein-bound dye was extracted with 10 mM tris base for determination of optical density in a computer-interfaced, 96-well microtiter plate reader (Skehan *et al.*, 1990).

The optical density measured is linear to the cell number of the surviving fraction. Therefore, the assay is a sensitive measure of compound-induced cytotoxicity with the best signal to noise ratio. The assay also, provides a colorimetric end point that is non-destructive, indefinitely stable and visible to naked eye.

Data were collected, revised, and analyzed by SPSS statistical package version 11. Excel computer program was used to tabulate the results (Table 1). Probit regression analysis procedure had been introduced to select the best model that described the relationship between the probit (percentages of protection) as a dependant variable in order to be used for prediction of the concentration of the compound that caused inhibition of 50% (IC_{50}) of cancer cells. The in vitro growth inhibition properties of each compound were described by IC_{50} and the degree of inhibition of cancer cell line was described by the equation:

$$\text{The probit (P)} = \text{intercept} + (\text{regression coefficient} \times \text{conc})$$

Table 1 Antitumor activity of the newly synthesized compounds

Compound	IC_{50} (μg/ml)	IC_{50}(nmol/ml)
2a	3.74	10.27
2b	3.96	11.52
2c	6.03	15.04
2d	5.65	14.85
4a	2.40	7.98
4b	1.85	5.39
4c	4.17	12.35
4d	4.30	11.33
5a	1.13	3.58
5b	1.65	4.61
5c	3.73	10.58
5d	4.35	11.02
6a	3.61	11.82
6b	2.04	6.00
6c	2.92	8.52
6d	2.92	7.74
7a	2.04	5.59
7b	3.14	7.16
8a	0.06	1.64
8b	2.76	6.29
9a	2.04	6.44
9b	3.69	9.30

The results of the preliminary antitumor activity against human mammary carcinoma cell line (Table 1) revealed that all newly synthesized derivatives showed significant antitumor activity.

In general, it could be noticed that the activity of the quinazoline derivatives was superior to that of the benzo[*d*]isothiazole ones. The most active compounds are among the quinazoline series especially compounds **5a**, **5b**, and **8a** with IC_{50} equal to 3.58, 4.61, and 1.64 nmol, respectively. In the benzoisothiazole series, the most active compound is **8b** having an IC_{50} equal to 6.29 nmol. It could also be revealed that the *N*-substituted derivatives **2a–d** were less active than the substituted amino derivatives **4–6a–d**.

Regarding the effect of the substituent on the amino derivatives **4–6a–d**, the results showed that the sulfamoylphenylamino derivatives **4a–d** were less active than their hydrazino analogs **5a–d**. Moreover, substitution at position 4 of the quinazoline ring with the thiadiazolylamino group, as in compounds **6a,b**, led to decrease in activity compared to compounds **4a,b**; while the reverse is true for the 3-thiadiazolylamino substituted benzo[*d*]isothiazoles **6c,d** which are more active than **4c,d**.

As for the bis products **7–9a,b**, the derivatives having the 1,4-diaminophenyl linker group **8a,b** were found to be more active than those with the 1,3-diaminophenyl **7a,b** and 1,2-diaminoethyl spacers **9a,b**. It is noteworthy that compound **8a** exhibited the highest activity, having an IC_{50} of 0.06 μg/ml; 1.64 nmol/ml.

Docking study

Docking study of the designed compounds into EPFR tyrosine kinase was performed using "Molecular Operating Environment (MOE) version 2008.10 release of Chemical Computing Group's". The crystal structure of the enzyme with lapatinib was obtained from the protein data bank; PDB code: 1XKK.

To study the binding interactions of the reference ligand, redocking of lapatinib in the ATP binding region of the kinase active site was carried out, revealing that two amino acids are involved in the interaction: Thr-830 and Met-769. The quinazoline ring binds to a narrow hydrophobic pocket in the N-terminal domain of EGFR TK where N-1 of the quinazoline ring interacts with the backbone NH of Met-769 via a hydrogen bond (H-acceptor; bond length 2.42 Å), and similarly, a water molecule-mediated hydrogen bonding interaction was observed with the S=O and Thr-830 (H-acceptor; bond length 3.03 Å).

The target compounds were modeled by positioning them in the lapatinib binding site. The results for the most active compound **8a** showed two hydrogen bonding interactions with Thr-830: one with N-3 of the quinazoline ring

(H-acceptor; bond length 2.57 Å) and the other with the 4-NH group of the same ring (H-donor; bond length 0.91 Å). Additional hydrogen bonding interaction of N-3 of the same quinazoline ring with Thr 766 (H-acceptor; bond length 2.18 Å) was observed. The 4-NH group of the other quinazoline ring interacts with the backbone Met-769 (H-donor; bond length 1.38 Å) (Fig. 2).

The binding mode of compound **9b**, revealed the involvement of the S=O moiety of both benzoisothiazole rings by hydrogen bonding interaction with both amino acids. The study revealed a backbone hydrogen bonding interaction between S=O of one ring and Met-769 (H-acceptor; bond length 2.40 Å). The S=O of the other ring was found to be involved into two hydrogen bonding interactions: one with Thr-830 (H-acceptor; bond length 2.25 Å) and the other with the backbone Asp 831 (H-acceptor; bond length 2.71 Å) (Fig. 3).

Comparing the docking modes of our compounds with lapatinib, it could be postulated that the designed compounds might act on the same enzyme target where lapatinib acted.

Experimental

Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Melting points were performed on Stuart SMP3 version 5

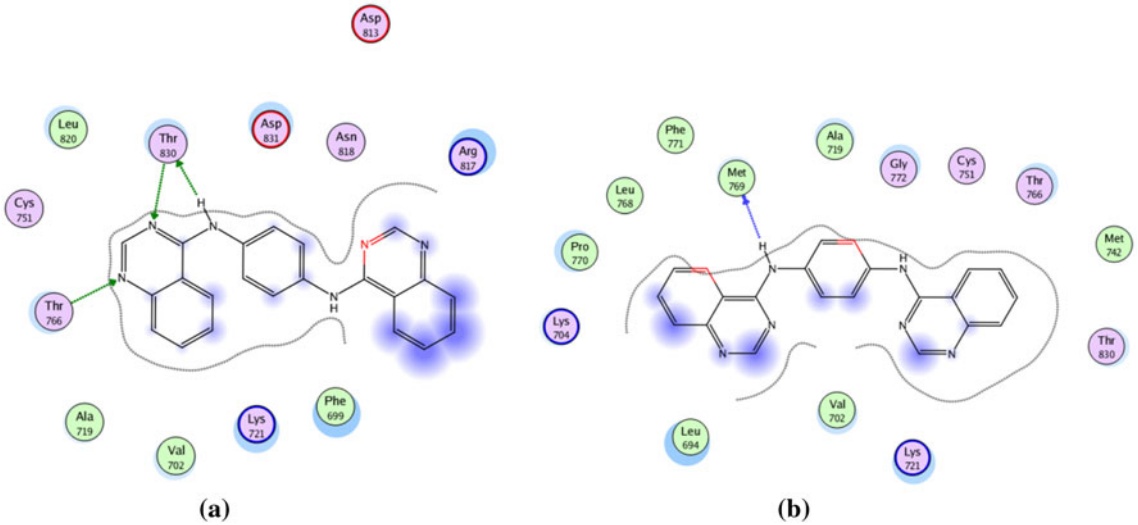


Fig. 2 Simplified structure of **8a** docked in the ATP binding site of EGFR TK. **a** interaction with Thr-830; (**b**) interaction with Met-769

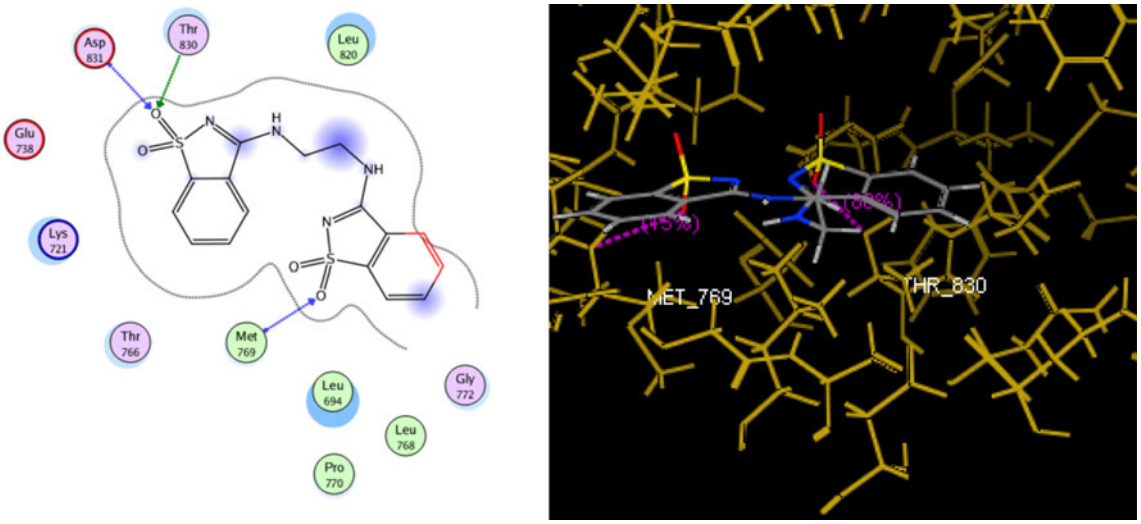


Fig. 3 Binding mode of compound **9b** in the ATP binding site of EGFR TK

digital melting point apparatus and were uncorrected. Elemental microanalyses were performed at the microanalytical center, Faculty of Science, Cairo University. ^{1}H-NMR spectra were recorded on Varian Gemini 200 spectrophotometer at 200 MHz in DMSO-d_6, unless otherwise noted; using tetramethylsilane (TMS) as internal reference. Chemical shift values were given in ppm. Mass spectra were performed on Fennigan MAT, SSQ 7000 mass spectrophotometer at 70 eV. IR spectra were recorded on Bruker FT-IR spectrophotometer as potassium bromide disc. Sulfanilamide **iia** and sulfaguanidine **iib** were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Compounds **1a**, **3a** (Endicott *et al.*, 1946), **3b** (Meadoe and Reid, 1943), **ia**, **ib** (Lange *et al.*, 2004), **iiia** (Itano, 1955), **iiib** (Takeda, 1954), and **iva**, **ivb** (Jatav *et al.*, 2008) were prepared according to the reported procedure.

General methods for the preparation of the target compounds

Method A A mixture of the chloro derivative **3a** (5 mmol), the appropriate amine (5 mmol), and anhydrous potassium carbonate (10 mmol) in dry acetone (15 ml) was heated under reflux for 24 h. The reaction mixture was filtered while hot, and the filtrate was evaporated till dryness under vacuum. The residue was treated with cold water, and the suspension was filtered to yield the crude product which was dried and recrystallized from the appropriate solvent.

Method B A solution of **1b** or **3a**/**3b** (5 mmol) in dry dimethyl formamide (DMF) (5 ml) was heated at 120° with the appropriate ester or amine (5 mmol), respectively, in the presence of triethylamine (3 ml) for 10 h. The solution was concentrated under vacuum and the residue was triturated with cold water. The precipitated solid was filtered, dried, and recrystallized from the appropriate solvent.

Method C A solution of **1a** or the chloro derivative **3a** (5 mmol) and the appropriate ester or amine (5 mmol), respectively, in dry toluene (15 ml) was refluxed for 8 h. The solvent was removed under vacuum and the residue was recrystallized from the appropriate solvent.

4-Chloro-*N*-((4-oxo-4*H*-quinazolin-3-yl)carbonyl)benzenesulfonamide (**2a**)

This compound was prepared according to method C. m.p. 128–129°C (from ethanol), yield 78%. IR (KBr, cm^{-1}): 3203 (NH), 3039 (CH aromatic), 1702 (CO), 1663 (CO), 1610 (NH bending), 1323, 1169 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm):7.54–8.12 (9H, m, aromatic H), 8.75 (1H,s, NH). MS: *m/z* (%) 364(M$^+$, 100). *Anal.* Calcd. for $C_{15}H_{10}ClN_3O_4S$ (363.95): C, 49.50; H 2.70; N, 11.55. Found: C, 49.80; H, 2.70; N, 11.55.

4-Methyl-*N*-((4-oxo-4*H*-quinazolin-3-yl)carbonyl)benzenesulfonamide (**2b**)

This compound was prepared according to method C. m.p. 175–177°C (from ethanol), yield 83%. IR (KBr, cm^{-1}): 3203 (NH), 3038 (CH aromatic), 2976–2923 (CH aliphatic), 1702 (CO), 1661 (CO), 1610 (NH bending), 1321, 1167 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.18 (3H, s, CH_3), 7.30–8.13 (9H, m. aromatic H), 8.17 (1H, s, NH). MS: *m/z* (%) 341(M-2, 65), 91 (100). *Anal.* Calcd. for $C_{16}H_{13}N_3O_4S$ (343.53): C, 55.94; H, 3.81; N, 12.28. Found: C, 56.06; H, 3.70; N, 12.20.

4-Chloro-*N*-((1,1,3-trioxo-2,3-dihydrobenzo[*d*]isothiazol-2yl)carbonyl)benzenesulfonamide (**2c**)

This compound was prepared according to method B. m.p. 145–147°C (from DMF), yield 55%. IR (KBr, cm^{-1}): 3335 (NH), 3118 (CH aromatic), 1660 (CO), 1630 (CO), 1572 (NH bending), 1331, 1152 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 7.50–8.18 (8H, m, aromatic H), 8.21 (1H, s, NH). *Anal.* Calcd. for $C_{14}H_9ClN_2O_6S_2$ (400.82): C, 41.95; H, 2.26; N, 6.99. Found: C, 42.34; H, 2.41; N, 7.22.

4-Methyl-*N*-((1,1,3-trioxo-2,3-dihydrobenzo[*d*]isothiazol-2yl)carbonyl)benzenesulfonamide (**2d**)

This compound was prepared according to method B. m.p. 215–218°C (from DMF), yield 59%. IR (KBr, cm^{-1}): 3361 (NH), 3093 (CH aromatic), 2926–2855 (CH aliphatic), 1722 (CO), 1654 (CO), 1592 (NH bending), 1337, 1178 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 2.50 (3H, s, CH_3), 6.97–8.11 (8H, m, aromatic H), 8.14 (1H, s, NH). MS: *m/z* (%) 382 (M + 2, 4.9), 64(100). *Anal.* Calcd. for $C_{15}H_{12}N_2O_6S_2$ (380.40): C, 47.36; H; 3.18; N, 7.36. Found: C, 47.60; H, 3.50; N, 7.81.

4-(Quinazolin-4-ylamino)benzenesulfonamide (**4a**)

This compound was prepared according to method A. m.p. 217–218°C (from ethanol), yield 62%. IR (KBr, cm^{-1}): 3181–3121 (NH), 3061 (CH aromatic), 1609 (NH bending), 1309, 1149 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 6.57–8.11 (10H, m, aromatic H and aniline NH), 12.2 (2H, s, NH_2). MS: *m/z* (%) 303 (M + 3, 3.25), 63.9 (100). *Anal.* Calcd. for $C_{14}H_{12}N_4O_2S$ (300.57): C, 55.94; H, 4.02; N, 18.72. Found: C, 56.30; H, 4.00; N, 18.70.

1-(4-(Quinazolin-4-ylamino)phenylsulfonyl)guanidine (**4b**)

This compound was prepared according to method A. m.p. 161–163°C (from ethanol), yield 60%. IR (KBr, cm^{-1}): 3428–3340 (NH), 3040 (CH aromatic), 1620 (NH bending), 1307, 1132 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 6.53–8.08 (10H, m, aromatic H and aniline NH), 10.10 (4H, br s, NHs, NH_2). MS: *m/z* (%) 341 (M^+, 36), 63.9 (100). *Anal.* Calcd. for $C_{15}H_{14}N_6O_2S$ (342.73): C, 52.56; H, 4.11; N, 24.62. Found: C, 52.63; H, 4.10; N, 25.32.

4-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)benzenesulfonamide (**4c**)

This compound was prepared according to method B. m.p. >350°C (from DMF), yield 65%. IR (KBr, cm^{-1}): 3332–3246 (NH), 3090 (CH aromatic), 1619 (NH bending), 1319, 1161 (SO_2). ^{1}H-NMR (200 MHz, $CDCl_3$: δ, ppm):5.02 (1H, s, NH), 7.26–7.80 (8H, m, aromatic H), 11.85 (2H, br s, NH_2). MS: *m/z* (%) 337 (M^+, 0.98), 57 (100). *Anal.* Calcd. for $C_{13}H_{11}N_3O_4S_2$ (337.38): C, 46.28; H, 3.29; N, 12.45. Found: C, 46.74; H, 4.06; N, 12.25.

1-(4-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)phenylsulfonyl)guanidine (**4d**)

This compound was prepared according to method B. m.p. >350°C (from DMF), yield 43%. IR (KBr, cm^{-1}): 3461–3344 (NH), 3050 (CH aromatic), 1623 (NH bending), 1301, 1162 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 6.73–8.52 (11H, m, aromatic H and NHs), 11.03 (2H, br s, NH_2). MS: *m/z* (%) 379 (M^+, 64.71), 97.4 (100). *Anal.* Calcd. for $C_{14}H_{13}N_5O_4S_2$ (379.42): C, 44.32; H, 3.45; N, 18.46. Found: C, 44.00; H, 3.20; N, 17.92.

4-(*N*-(Quinazolin-4-yl)hydrazino)benzenesulfonamide (**5a**)

This compound was prepared according to method B. m.p. 295–297°C (from ethanol), yield 56%. IR (KBr, cm^{-1}): 3340–3187 (NH), 3063 (CH aromatic), 1606 (NH bending), 1311, 1152 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 6.80 (2H, br s, NHNH), 7.29–8.62 (9H, m, aromatic H), 12.10 (2H, br s, NH_2). MS: *m/z* (%) 315 (M^+, 0.17), 73 (100). *Anal.* Calcd. for $C_{14}H_{13}N_5O_2S$ (315.65): C, 53.27; H, 4.15; N, 22.28. Found: C, 53.10; H, 4.01; N, 23.10.

1-(4-(*N*-(Quinazolin-4-yl)hydrazino)phenylsulfonyl)guanidine (**5b**)

This compound was prepared according to method B. m.p. 297–299°C (from ethanol), yield 51%. IR (KBr, cm^{-1}): 3420–3187 (NH), 3062 (CH aromatic), 1614 (NH bending), 1313, 1137 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 6.77 (2H, br s, NHNH), 7.26–8.69 (9H, m, aromatic H), 12.01 (4H, br s, NHs, NH_2). MS: *m/z* (%) 356 (M-1, 38), 57 (100) *Anal.* Calcd. for $C_{15}H_{15}N_7O_2S$ (357.81): C, 50.35; H, 4.22; N, 27.51. Found: C, 50.59; H, 4.09; N, 27.51.

4-(*N*-(1,1-Dioxobenzo[*d*]isothiazol-3-yl)hydrazino)benzenesulfonamide (**5c**)

This compound was prepared according to method B. m.p. 136–137°C (from ethanol), yield 66%. IR (KBr, cm^{-1}): 3351–3258 (NH), 3067 (CH aromatic), 1628 (NH bending), 1334, 1159 (SO_2). ^{1}H-NMR (200 MHz, $CDCl_3$: δ, ppm): 5.40 (2H, br s, NHNH), 7.48–8.10 (10H, m, aromatic H and NH_2). MS: *m/z* (%) 353 (M + 1, 0.26), 237 (100). *Anal.* Calcd. for $C_{13}H_{12}N_4O_4S_2$ (352.39): C, 44.31; H, 3.43; N, 15.90. Found: C, 44.50; H, 4.20; N, 15.80.

1-(4-(*N*-(1,1-Dioxobenzo[*d*]isothiazol-3-yl)hydrazino)phenylsulfonyl) guanidine (**5d**)

This compound was prepared according to method B. m.p. 165–168°C (from ethanol), yield 72%. IR (KBr, cm^{-1}): 3422–3220 (NH), 3050 (CH aromatic), 1625 (NH bending), 1338, 1142 (SO_2). ^{1}H-NMR (200 MHz, $CDCl_3$: δ, ppm): 5.60 (2H, br s, NHNH), 7.35–8.15 (12H, m, aromatic H, NHs and NH_2). MS: *m/z* (%) 394 (M^+, 3.76), 76 (100). *Anal.* Calcd. for $C_{14}H_{14}N_6O_4S_2$ (394.43): C, 42.63; H, 3.58; N, 21.31. Found: C, 42.50; H, 4.10; N, 21.77.

4-(5-Phenyl-1,3,4-thiadiazol-2-ylamino)quinazoline (**6a**)

This compound was prepared according to method A. m.p. 216–217°C (from ethanol), yield 81%. IR (KBr, cm^{-1}): 3275 (NH), 3085 (CH aromatic), 1631 (NH bending). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 7.43–8.38 (11H, m, aromatic H and NH). MS: *m/z* (%) 305 (M^+, 5.8), 60 (100). *Anal.* Calcd. for $C_{16}H_{11}N_5S$ (305.37): C, 62.93; H, 3.63; N, 22.93. Found: C, 62.58; H, 3.34; N, 22.60.

4-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-ylamino)quinazoline (**6b**)

This compound was prepared according to method A. m.p. 339–340°C(from ethanol), yield 84%. IR (KBr, cm^{-1}): 3267 (NH), 3087 (CH aromatic), 1613 (NH bending). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 7.50–8.29 (9H, m, aromatic H), 9.30 (1H, s, NH). *Anal.* Calcd. for $C_{16}H_{10}ClN_5S$ (339.80): C, 56.55; H, 2.97; N, 20.61. Found: C, 56.64; H, 3.57; N, 20.20.

3-(5-Phenyl-1,3,4-thiadiazol-2-ylamino)-1, 1-dioxobenzo[*d*]isothiazole (**6c**)

This compound was prepared according to method B. m.p. 229–231°C (from DMF), yield 76%. IR (KBr, cm^{-1}): 3276 (NH), 3086 (CH aromatic), 1631 (NH bending), 1331, 1136 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 7.41–7.77 (10H, m, aromatic H and NH). MS: *m/z* (%) 342 (M^+, 1.84), 60 (100). *Anal.* Calcd. for $C_{15}H_{10}N_4O_2S_2$ (342.40): C, 52.62; H, 2.94; N, 16.36. Found: C, 52.76; H, 2.92; N, 16.06.

3-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-ylamino)-1, 1-dioxobenzo[*d*]isothiazole (**6d**)

This compound was prepared according to method B. m.p. 213–215°C (from DMF), yield 77%. IR (KBr, cm^{-1}): 3272 (NH), 3084 (CH aromatic), 1631 (NH bending), 1333, 1136 (SO_2). ^{1}H-NMR (200 MHz, $CDCl_3$: δ, ppm): 7.40–8.00 (8H, m, aromatic H), 8.30(1H, s, NH). MS: *m/z* (%) 379 (M^++3, 18), 57 (100). *Anal.* Calcd. for $C_{15}H_9ClN_4O_2S_2$ (376.85): C, 47.81; H, 2.41; N, 14.87. Found: C, 47.89; H, 2.69; N, 14.99.

N,*N*′-Bis-quinazolin-4-ylbenzene-1,3-diamine (**7a**)

This compound was prepared according to method B but using 10 mmol of **3a** and 5 mmol of *m*-phenylenediamine. m.p. 183–185°C (from ethanol), yield 56%. IR (KBr, cm^{-1}): 3342 (NH), 3059 (CH aromatic), 1610 (NH bending). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 6.62–8.74 (14H, m, aromatic H), 10.20 (2H, br s, NHs). MS: *m/z* (%) 364(M+, 57.5), 84 (100). *Anal.* Calcd. for $C_{22}H_{16}N_6$ (364.40): C, 72.51; H, 4.43; N, 23.06. Found: C, 72.40; H, 3.73; N, 22.74.

N,*N*′-Bis-1,1-dioxobenzo[*d*]isothiazol-3-ylbenzene-1, 3-diamine (**7b**)

This compound was prepared according to method B but using 10 mmol of **3b** and 5 mmol of *m*-phenylenediamine. m.p. >350°C (from ethanol), yield 48%. IR (KBr, cm^{-1}): 3351 (NH), 3060 (CH aromatic), 1623 (NH bending), 1300, 1159 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 7.81–8.25 (12H, m, aromatic H), 10.31 (2H, br s, NHs). *Anal.* Calcd. for $C_{20}H_{14}N_4O_4S_2$ (438.49): C, 54.78; H, 3.22; N, 12.78. Found: C, 54.99; H, 3.60; N, 13.12.

N,*N*′-Bis-quinazolin-4-ylbenzene-1,4-diamine (**8a**)

This compound was prepared according to method B but using 10 mmol of **3a** and 5 mmol of *p*-phenylenediamine. m.p. 303–305°C (from ethanol), yield 72%. IR (KBr, cm^{-1}): 3364 (NH), 3060 (CH aromatic), 1600 (NH bending). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 6.88–8.67 (14H, m, aromatic H), 10.02 (2H, br s, NHs). MS: *m/z* (%) 367 (M + 3, 72), 300(100). *Anal.* Calcd. for $C_{22}H_{16}N_6$ (364.40): C, 72.51; H, 4.43; N, 23.06. Found: C, 72.40; H, 4.57; N, 23.61.

N,*N*′-Bis-1,1-dioxobenzo[*d*]isothiazol-3-ylbenzene-1, 4-diamine (**8b**)

This compound was prepared according to method B but using 10 mmol of **3b** and 5 mmol of *p*-phenylenediamine. m.p. 188–190°C (from ethanol), yield 82%. IR (KBr, cm^{-1}): 3335 (NH), 3050 (CH aromatic), 1622 (NH bending), 1300, 1142 (SO_2). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 6.47–8.79 (12H, m, aromatic H), 10.16 (2H, br s, NHs). MS: *m/z* (%) 415 (M + 1, 64), 71(100). *Anal.* Calcd. for $C_{20}H_{14}N_4O_4S_2$ (438.49): C, 54.78; H, 3.22; N, 12.78. Found: C, 54.33; H, 3.15; N, 12.24.

N,*N*′-Bis-quinazolin-4-ylethylene-1,2-diamine (**9a**)

This compound was prepared according to method A but using 10 mmol of **3a** and 5 mmol of 1,2-ethylenediamine. m.p. 207–208°C (from ethanol), yield 68%. IR (KBr, cm^{-1}): 3285 (NH), 3060 (CH aromatic), 2922–2852 (CH aliphatic), 1619 (NH bending). ^{1}H-NMR (200 MHz, DMSO-d_6: δ, ppm): 3.06 (4H, s, CH_2CH_2), 6.60–8.77 (10H, m, aromatic H), 12.2 (2H, br s, NHs). MS: *m/z* (%) 314(M-2, 4.03), 174(100). *Anal.* Calcd. for $C_{18}H_{16}N_6$ (316.35): C, 68.34; H, 5.10; N, 26.56. Found: C, 68.49; H, 5.44; N, 26.23.

N,*N*′-Bis-1,1-dioxobenzo[*d*]isothiazol-3-ylethylene-1, 2-diamine (**9b**)

This compound was prepared by fusion of 1,2-ethylenediamine (5 mmol, 0.30 g, 0.33 ml) and **3b** (10 mmol; 2.01 g) at 120° for 5 h. The reaction mixture was dissolved in water, neutralized with sodium carbonate solution and extracted with chloroform (3 × 20 ml). The aqueous layer was evaporated under reduced pressure. The oily residue left was cooled and then centrifuged. The supernatant oil was decanted and dried in an oven under reduced pressure to give compound **9b**. Oil, yield 88%. IR (KBr, cm^{-1}): 3302 (NH), 3072 (CH aromatic), 2952–2860 (CH aliphatic), 1622 (NH bending), 1305, 1158 (SO_2). ^{1}H-NMR (200 MHz, D_2O: δ, ppm): 3.40 (4H, s, CH_2CH_2), 7.72–8.20 (8H, m, aromatic H). MS: *m/z* (%) 391(M + 1, 3.16), 76(100). *Anal.* Calcd. for $C_{16}H_{14}N_4O_4S_2$ (390.44): C, 49.22; H, 3.61; N, 14.35. Found: C, 49.49; H, 3.59; N, 14.99.

In vitro antitumor activity measurement

Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5, and 10 μg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were then incubated with the compounds for 48 h at 37°C and in atmosphere of 5% CO_2. After this time, cells were fixed, washed, and stained with Sulforhodamie B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. The color intensity was measured in an ELISA reader. Finally, the relation between surviving fraction and drug concentration was plotted to get the survival curve of the tumor cell line after the specific compound (Skehan *et al.*, 1990).

Docking study

All molecular modeling calculations and docking studies were performed using "Molecular Operating Environment (MOE) version 2008.10 release of Chemical Computing Group's". The program operated under "Windows XP" operating system installed on an Intel Pentium IV PC with a 2.8 MHz processor and 512 RAM.

The target compounds were built using the MOE builder interface and subjected to energy minimization using the included MOPAC. The produced model was subjected to Systematic Conformational Search where all items were set as default with RMS gradient of 0.01 kcal/mol and RMS distance of 0.1 Å.

The X-ray crystallographic structure of the Tyrosine Kinase Domain from Epidermal Growth Factor Receptor (EGFR TK) complexed with lapatinib was obtained from the Protein Data Bank; code "1XKK". The enzyme was prepared for docking studies as follows:

a. The ligand molecule was removed from the enzyme active site.
b. Hydrogen atoms were added to the isolated target with their standard geometry.
c. A connect and type procedure was run for automatic completion of missed bonds during isolation and crystallization.
d. The target was fixed to be dealt as a rigid structure.
e. The active site was isolated by the Alpha site finder tool using the binding amino acids as key elements in isolation.
f. Dummies were created around the active site.

Conclusion

Different substituted quinazoline and benzo[*d*]isothiazole derivatives **2a–d**, **4a–d**, **5a–d**, **6a–d**, **7a,b**, **8a,b**, and **9a,b** were synthesized and their structures were established by spectral (IR, ^{1}H-NMR, and MS) as well as elemental microanalytical methods. All compounds were screened for their antitumor activity against human mammary carcinoma cell line (MCF7) and they were found to possess significant antitumor activity.

In general, the quinazoline derivatives were more active than their benzo[*d*]isothiazole bioisosteres. Also, the substituted amino derivatives **4–6a–d** were more active than the *N*-substituted ones **2a–d**. Within the substituted amino derivatives, the sulfamoylphenylhydrazino compounds **5a–d** were the most active. The bis compounds with the 1,4-diaminophenyl spacer group **8a,b** were more active than the other derivatives; compound **8a** being the most active one (IC_{50} = 1.64 nmol).

In conclusion, the bis quinazoline derivative **8a** has succeeded in providing antitumor activity comparable to the lead compounds **III** and **IV** exhibiting IC_{50} values of 1.08 and 1.98 nmol, respectively (Abouzid and Shouman, 2008). The other quinazoline derivatives have also exhibited promising activities that may be subjected for future structure optimization.

Docking study into the ATP binding domain of the active site of EGFR-TK crystal structure could support the postulation that our active compounds may act on the same enzyme target as does the reference ligand, lapatinib.

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