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Design, synthesis and in vitro antitumor activity of 4-aminoquinoline and 4-aminoquinazoline derivatives targeting EGFR tyrosine kinase

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ABSTRACT

Two series of new 6-alkoxy-4-substituted-aminoquinazolines **(2–4f)** and their bioisoteric quinoline congeners **(5–7c)** were designed and synthesized. Virtual screening was carried out through docking the designed compounds into the ATP binding site of epidermal growth factor receptor (EGFR) to predict if these compounds have analogous binding mode to the EGFR inhibitors.

The newly synthesized compounds were tested in vitro on human breast carcinoma cell line (MCF-7) in which EGFR is highly expressed. Most of the tested compounds exploited potent antitumor activity with IC_{50} values in the nanomolar range in particular compound **3b** which displayed the highest activity among the tested compounds with IC_{50} equal to 0.13 nmol.

1. Introduction

Protein tyrosine kinases are enzymes involved in many cellular processes such as cell proliferation, metabolism, survival, and apoptosis. Several protein tyrosine kinases are known to be activated in cancer cells and to drive tumor growth and progression. Blocking tyrosine kinase activity therefore represents a rational approach to cancer therapy. Protein kinases (PTKs) catalyze the phosphorylation of tyrosine and serine/threonine residues in various proteins involved in the regulation of all functions.[1] They can be broadly classified as receptor such as EGFR, or non-receptor kinases. Inappropriate or uncontrolled activation of many of these kinases, by over-expression, constitutive activation, or mutation, has been shown to result in uncontrolled cell growth.[2] The epidermal growth factor receptor belongs to the family of transmembrane growth factor receptor PTKs. The EGFR erbB1 and erbB2 PTKs have been identified as interesting targets for medicinal chemistry programs especially in cancer therapy. Excellent descriptions of the involvement of erbB family proteins in cell physiology and disease applications have been reported and reviewed.[3–6]

Overexpression of these receptors was found in a number of cancers (e.g., breast, ovarian, colon, and prostate), their expression levels often correlate with vascularity, and is associated with poor prognosis in patients.[7,8] Inhibitors of the EGFR PTK are therefore expected to have great therapeutic potential in the treatment of malignant and nonmalignant epithelial diseases. Drug discovery efforts have targeted this aberrant kinase activity in cancer, asthma, psoriasis, and inflammation.[9] Recent advances in the identification of erbB family kinase inhibitors have created hope for the modulation of uncontrolled cell growth in cancer therapy for solid tumors.[10] This strongly suggests that these targets represent drug intervention opportunities due to pivotal role in governing cellular proliferation, survival, and metastasis.

A great number of different structural classes of tyrosine kinase inhibitors have been reported and reviewed.[3–5] The most promising small molecule selective EGFR-TK inhibitors include 4-anilinoquinazolines, anilinoquinolines, and pyrrolopyrimidines. Figure 1 includes some examples that are currently approved drugs or in clinical trials.[8]

In May 2003, the FDA approved gefitinib (ZD1839, Iressa®) as monotherapy for the treatment of patients with locally advanced or metastatic non-small cell lung cancer after chemotherapies had failed.[11]

Consequently, various approaches were adopted to enhance the potency and selectivity of these inhibitors. These efforts led to discovery of lapatinib, a dual EGFR and erbB2 inhibitor,[12] which is currently in phase III clinical trials, while EKB-569 is a potent, selective, and irreversible inhibitor of epidermal growth factor receptor (EGFR) that is being developed as an anticancer agent.[13,14]

Later, it has been reported that somatic mutations in the tyrosine kinase domain of the EGFR gene occur in a subset of patients with lung cancer who showed a dramatic response to the EGFR tyrosine kinase inhibitors gefitinib and erlotinib.[15–17]

Intensive research in the area of tyrosine kinase inhibitors led to development of enormous number of active compounds.[18–25]

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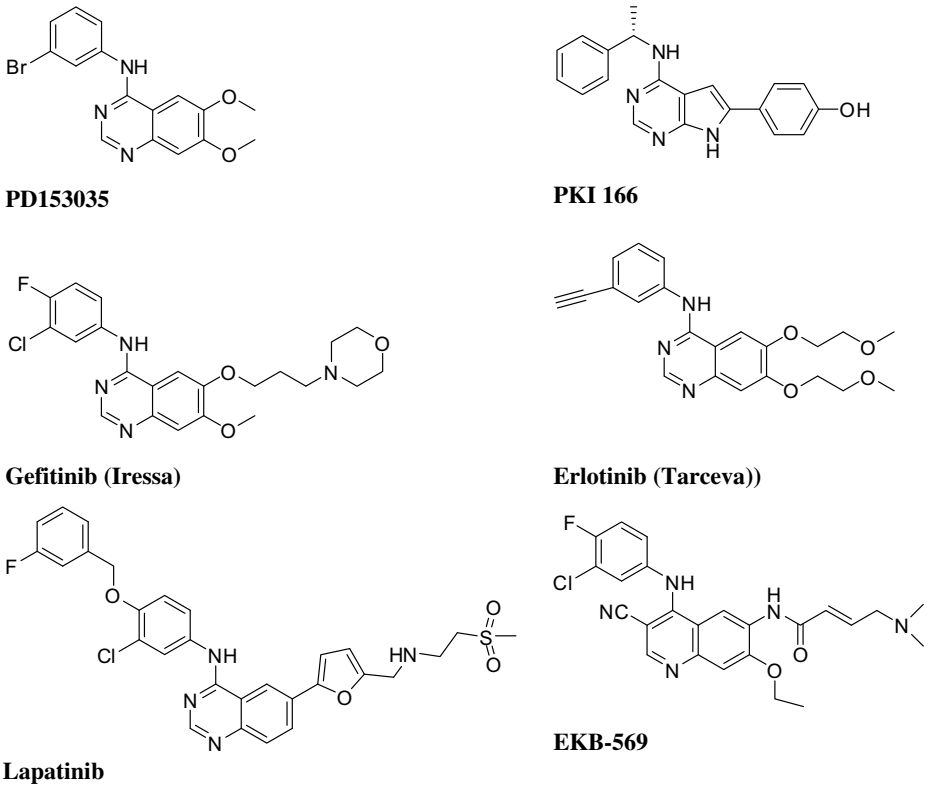


Figure 1. EGFR TK inhibitors.

In the same direction, and in continuing effort to find more potent selective lead compound, herein, we describe the design and synthesis of two series of 4-aminoquinoline and aminoquinazoline derivatives as possible antitumor agents that may act through EGFR inhibition.

2. Rational and design

In recent years, 4-anilinoquinazolines have emerged as a versatile template for inhibition of a diverse range of receptor tyrosine kinases. The most widely studied of these is the epidermal growth factor receptor (EGFR), with the small-molecule inhibitor gefitinib being the first agent from this class to be approved for the treatment of non-small cell lung cancer refractory to prior chemotherapeutic intervention.[26,27]

Subsequent research aimed at further exploration of the SAR of this novel template has led to discovery of highly selective compounds that target EGFR. These compounds act via competing with ATP for binding at the catalytic domain of tyrosine kinase. Later on, a great structural variety of compounds of structurally diverse classes have proved to be highly potent and selective ATP-competitive inhibitors. Among them, 4-anilinoquinoline-3-carbonitriles and others provide the necessary binding properties for inhibition of the ErbB family of tyrosine as they mimic the adenine portion of ATP.[28,29]

In this study, we present a new sub-family of compounds containing 4-aminoquinazoline, and quinoline core as promising potent and selective EGFR inhibitors. Our strategy is directed toward designing a variety of ligands with diverse chemical properties hypothesizing that the potency of these molecules might be enhanced by adding alternative binding group such as sulfonamide and carboxylic group in the aniline moiety.

In this way, such substitution pattern could target different regions of the ATP-binding site of the protein kinase domain to create differentially selective molecules. The design of our ligands was done based on previous structure–activity relationship (SAR) of 4-anilinoquinazolines and earlier work with quinazoline-based inhibitors of EGFR which established that 6,7-dialkoxy substitution is compatible with good activity, and pivotal interactions between the receptor and the inhibitors.

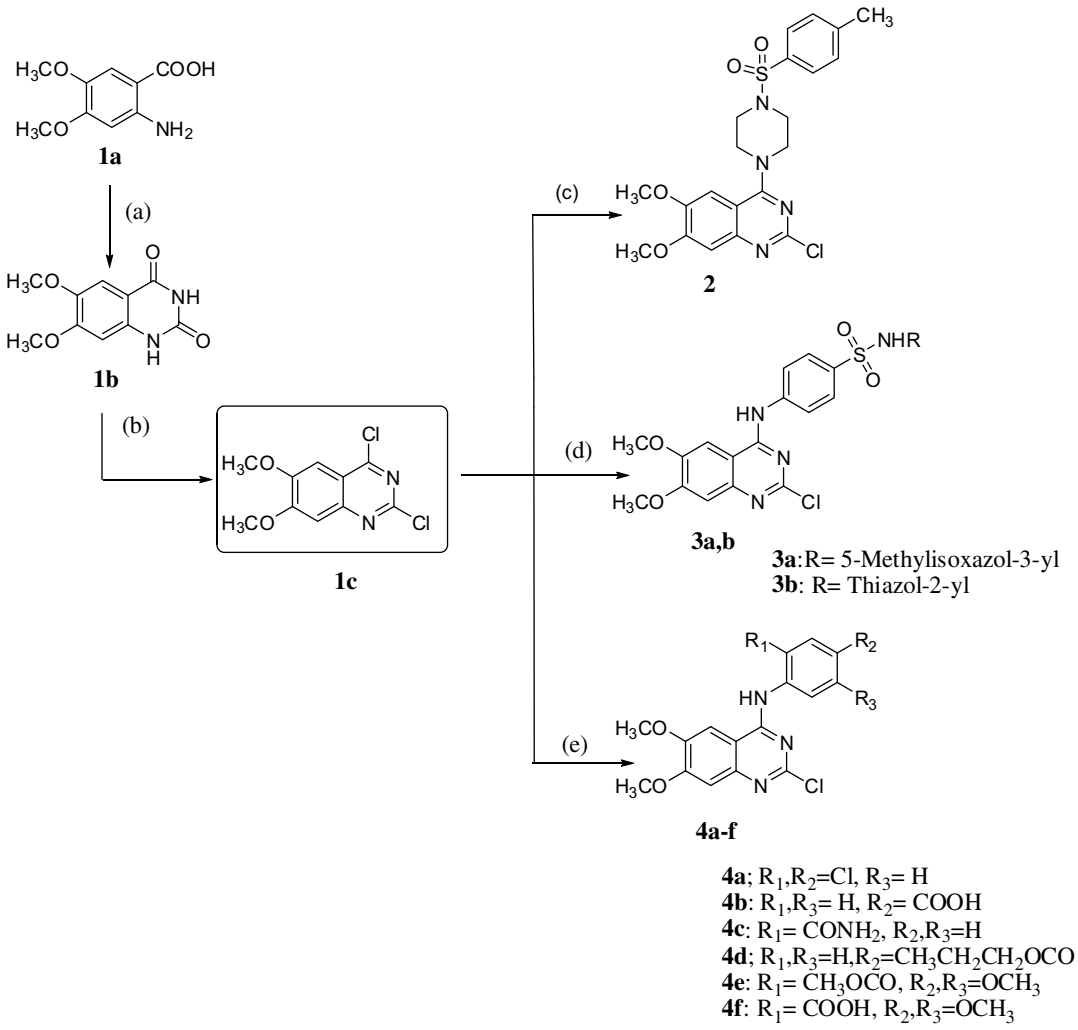
In more recent approach, it was found that dual inhibition of EGFR and ErbB-2 may offer increased activity over agents which target only one of these receptor kinases.[12,30] After discovery of lapatinib, it was claimed that 4 position of the aniline can tolerate a lot of bulky substituents; this leads to fundamental change in the pharmacophore and claims moderate ErbB-2 activity with little or no EGFR activity.

In this direction and in an approach to enhance the selectivity toward ErbB-2, we introduced larger moiety at 4 position of the aniline such as heterocyclylsulfonamido moiety in a fashion similar to lapatinib which binds in the ATP-binding cleft, so that the bulky group could be oriented deep in the back of the ATP binding site and makes predominantly hydrophobic interactions with the protein mimicking the 3′-chloro-4′-[(3-fluorobenzyl)oxy]aniline group of lapatinib. Moreover, in two compounds we replaced the aniline moiety with substituted piperazine fragment to assess if such dramatic change in the pharmacophoric model of anilinoquinolines or quinazoline will have appreciable effect on the activity.

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

3. Chemistry

General syntheses for target compounds are depicted in Schemes 1 and 2 starting from the central scaffold 2,4-quinazolindione or 4-quinolinone.



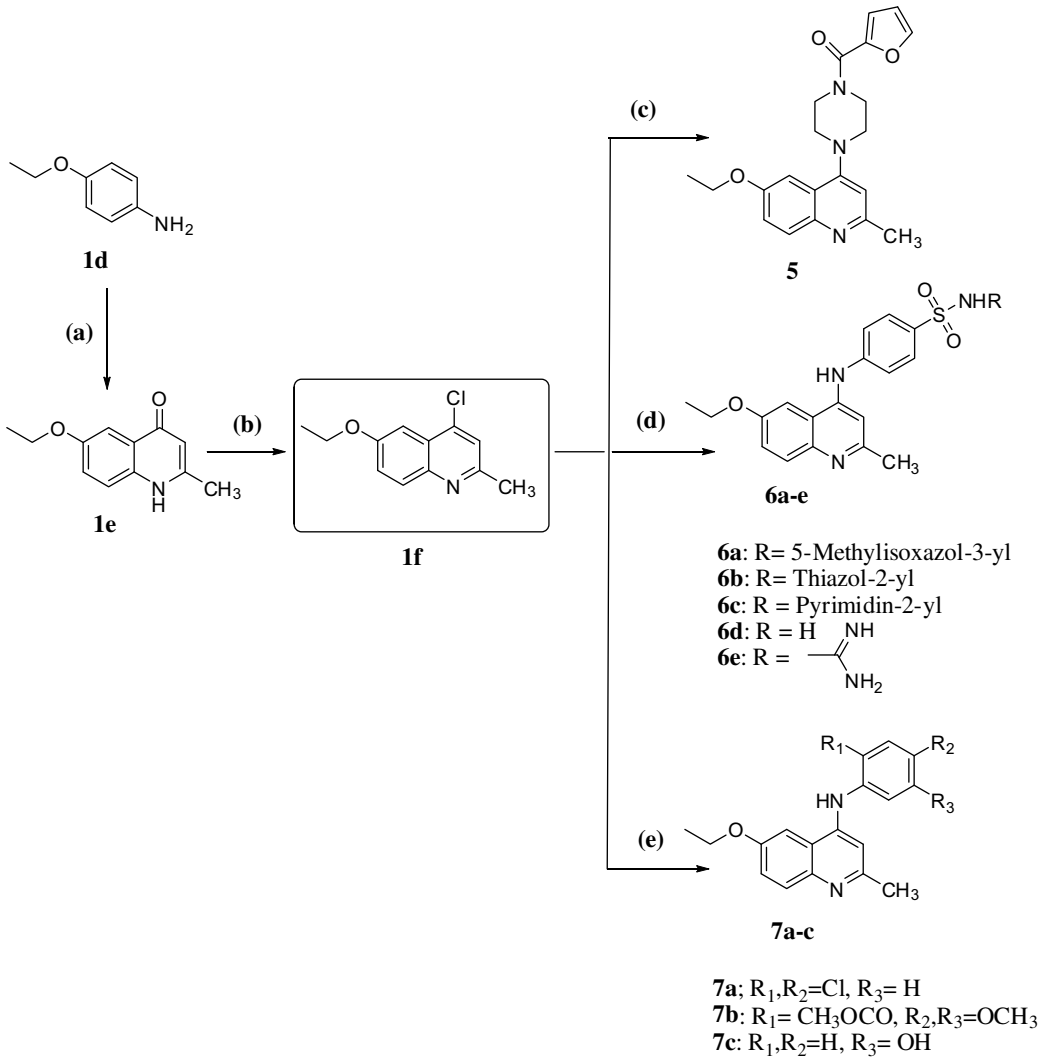
Scheme 1. Reagents and conditions: (a) KCNO, AcOH then NaOH; (b) $POCl_3$, *N*,*N*-dimethylaniline; (c) *p*-toluenesulfonylpiperazine THF, rt 24 h; (d) sulfamethoxazole or sulfathiazole, 2-propanol, reflux; (e) substituted-anilines, ethanol, reflux.

In the first scheme, the synthesis of final 4-quinazolin-4-amines (**2–4**) was described. Accordingly, the quinazolinedione **(1b)** was synthesized from 4,5-dimethoxyanthranilic acid **(1a)** and potassium cyanate in acetic acid followed by NaOH and acidification according to reported method.[31a] Chlorination of the dione with phosphoryl chloride in the presence *N*,*N*-dimethylaniline gave the key starting material, 2,4-dichloro-6,7-dimethoxyquinazoline **(1c)**.[31b] The 4-substituted-aminoquinazoline compounds were synthesized via the reaction of **(1c)** with different nucleophilic amine derivatives under carefully controlled conditions to prevent disubstitution. The piperazine derivative (**2**) was obtained by reacting the N^1-*p*-toluenesulfonylpiperazine at room temperature overnight in THF followed by liberation of the free base with sodium carbonate. The sulfonamide derivatives **3a,b** were smoothly obtained as their hydrochloride salt via nucleophilic substitution of the more reactive chlorine at the 4 position of the dichloroquinazoline **1c** with the respective sulfonamide under reflux in 2-propanol for 2 h. Furthermore, anilinoquinazoline derivatives (**4a–f)** were obtained by reaction between different aniline derivatives and the dichloroquinazoline derivative **1c** under careful reaction condition.

On the other hand, Scheme 2 describs the synthetic pathway of various quinolinamine compounds **5–7**. The key intermediate **1f** was synthesized by standard methods starting from *p*-phenetidine and ethyl acetoacetate to give the condensation product which was cyclized in diphenyl ether affording 6-ethoxy-4-hydroxy-2-methylquinoline **(1e).**[32,33] The latter compound was treated with phosphorus oxychloride to afford the desired 4-chloro derivative **1f**. Furoylpiperazinylquinoline **5** was obtained by coupling 4-chloroquinoline derivative **1f** with *N*-furoylpiperazine in ethanol under reflux, while the reaction between **1f** and either sulfonamide or the requisite aniline derivatives proceeds preferably in presence of catalytic amount of HCl. The structures of all the newly synthesized compounds were elucidated with H^1 NMR, EIMs, and elemental analyses.

4. Experimental

Melting points were determined using a Stuart Scientific apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR series using KBr cell. ^{1}H NMR spectra were recorded on Brücker 300 MHz. in δ scale. All the spectra were obtained on solutions in DMSO-d_6 with TMS as internal standard; the values of the chemical shifts (δ) are given in ppm, and coupling constants (J) are given in Hz. The electron impact (EI) mass spectra were recorded on Finnigan Mat SSQ 7000 (70 ev) mass spectrometer. Analytical thin layer chromatography (TLC) on silica gel plates containing UV indicator was employed routinely to follow the course of



Scheme 2. Reagents and conditions: (a) Ethyl acetoacetate, reflux then diphenyl ether reflux, Dean stark; (b) $POCl_3$, reflux; (c) 2-furoylpiperazine reflux; (d) sulfonamides, 2-propanol, reflux; (e) substituted-anilines, ethanol, reflux, few drops HCl.

reactions and to check the purity of products. All reagents and solvents were purified and dried by standard techniques. Elemental microanalyses were performed at Microanalytical Center, Cairo University, and were within ±0.4% unless otherwise stated. The chemicals used in the synthesis are purchased from Aldrich and Merck and are of analytical grades. *p*-Toluenesulfonylpiperazine was not commercially available and was synthesized according to reported method.[34]

4.1. 2-Chloro-6,7-dimethoxy-4-(4-tosylpiperazin-1-yl)quinazoline hydrochloride (2)

A mixture of equimolar amounts of 2,4-dichloro-6,7-dimethoxyquinazoline **(1c)** (0.181 g, 0.7 mmol) and 1-tosylpiperazine (0.168 g, 0.7 mmol) in THF (15 mL) was stirred at ambient temperature overnight. The white solid formed was filtered, washed with diethyl ether, and recrystallized from methanol to give **2** as buff solid. Yield: 0.234 g, 67%; mp 196–198 C; ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 2.4 (s, 3H, CH_3), 3.13 (m, 4H, CH_2, piperazine), 3.76 (m, 4H, CH_2, piperazine), 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 7.05–7.66 (m, 6H, Ar H).

IR (KBr) cm^{-1} 1570, 1484, 1347 (S=O). MS *m/z* 462 (M^+, 2.9%), 464 (M+2, 0.8%). Anal. Calcd for $C_{21}H_{23}ClN_4O_4S{\cdot}HCl$: C, 50.50; H, 4.84; N, 11.22. Found: C, 50.95; H, 5.29; N, 11.10.

4.2. 4-(2-Chloro-6,7-dimethoxyquinazolin-4-ylamino)-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide hydrochloride (3a)

A mixture of 2,4-dichloro-6,7-dimethoxyquinazoline **(1c)** (0.181 g, 0.7 mmol) and 4-amino-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (sulfamethoxazole) (0.177 g, 0.7 mmol) in isopropanol (20 mL) was heated at reflux and stirred for 2 h. The white solid formed was filtered while hot and recrystallized from methanol/DMF to give **3a**. Yield: 0.28 g, 88%; mp 310–312 °C, ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 2.30 (s, 3H, CH_3), 3.93 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.16 (s, 1H, isoxazole), 7.20 (s, 1H, quinazoline), 7.8–8.06 (m, 5H, ArH), 10.16 (s, 1H, NH, D_2O exchangeable), 11.36 (s, 1H, NH, D_2O exchangeable).

IR (KBr) cm^{-1}. 3275 (NH), 1625, 1514, 1428, 1336, 3293; MS *m/z* 478 (M+3, 100%). Anal. Calcd for $C_{22}H_{22}N_4O_4S{\cdot}HCl$: C, 46.88; H, 3.74; N, 13.67. Found: C, 46.48; H, 3.91; N, 13.89.

4.3. 4-(2-Chloro-6,7-dimethoxyquinazolin-4-ylamino)-N-(thiazol-2-yl)benzene-sulfonamide hydrochloride (3b)

Compound **3b** was prepared as described for **3a** from **1c** and 4-amino-*N*-(thiazol-2-yl)benzenesulfonamide (sulfathiazole) and recrystallized from Methanol/DMF. Yield: 91%; mp 290–292 °C (decomp), ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 3.39 (s, 3H, OCH_3),

3.97 (s, 3H, OCH_3), 6.82–7.97 (m, 8H, Ar H), 10.19 (s, 1H, NH, D_2O exchangeable), 11.24 (s, 1H, NH, D_2O exchangeable).

IR (KBr) cm^{-1}, 3286 (NH), 1631 (C=N), 1568, 1435, 1328 (S=O); MS *m/z* 477 (M^+, 22.4%). Anal. Calcd for $C_{19}H_{16}ClN_5O_4S_2{\cdot}HCl$: C, 44.36; H, 3.33; N, 13.61. Found: C, 44.77; H, 3.55; N, 13.81.

4.4. 2-Chloro-4-[*N*-(2,4-dichlorophenyl)amino]-6,7-dimethoxyquinazoline hydrochloride (4a)

Compound **4a** was prepared as described for **3a** from **1c** and 2,4-dichloroaniline, and the product obtained was recrystallized from methanol. Yield: 74%; mp 264–266 °C, ^1H NMR (DMSO-d_6) (300 MHz) δ: 3.93 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 7.16 (s, 1H, Ar H), 7.50–7.59 (m, 2H, Ar H), 7.76 (s, 1H, Ar), 7.90 (s, 1H, ArH) 10.09 (s, 1H, NH, D_2O exchangeable).

IR (KBr) cm^{-1} 3430, 3230 (NH), 1627 (C=N), 1590. MS *m/z* 383 (M^+, 19.4%), 385 (M+2, 7.6%). Anal. Calcd for $C_{16}H_{12}Cl_3N_3O_2{\cdot}HCl$: C, 49.96; H, 3.14; N, 10.92. Found: C, 49.64; H, 3.44; N, 11.22.

4.5. 4-(2-Chloro-6,7-dimethoxyquinazolin-4-ylamino)benzoic acid hydrochloride (4b)

Compound **4b** was prepared as described for **3a** from **1c** and 4-aminobenzoic acid and the product was recrystallized from DMF as off-white solid. Yield: 79%; mp 322–324 °C (decomp), ^1H NMR (DMSO-d_6) (300 MHz) δ: 3.86 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 7.05 (s, 1H, Ar H), 7.70 (s, 1H, Ar H), 7.84 (d, 2H, Ar), 7.92 (d, 2H, ArH), 10.10 (s, 1H, NH, D_2O exchangeable), 11.3 (br s, 1H, COOH, D_2O exchangeable).

IR (KBr) cm^{-1} 3425 (OH), 1694 (C=O), 1629. MS *m/z* 359 (M^+, 100%), 361 (M+2, 52.1%). Anal. Calcd for $C_{17}H_{14}ClN_3O_4{\cdot}HCl$: C, 56.75; H, 3.92; N, 11.68. Found: C, 56.66; H, 4.11; N, 11.88.

4.6. 2-(2-Chloro-6,7-dimethoxyquinazolin-4-ylamino)benzamide hydrochloride (4c)

Compound **4c** was prepared as described for **3a** from **1c** and 2-aminobenzamide, and the product was recrystallized from methanol as buff solid. Yield: 65%; mp 214–216 °C, ^1H NMR (DMSO-d_6) (300 MHz) δ: 3.92 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 7.18 (s, 1H, Ar H), 7.42 (s, 1H, Ar H), 7.60–8.80 (m, 4H, Ar), 11.6 (s, 1H, NH, D_2O exchangeable), 13.08 (s, 2H, $CONH_2$, D_2O exchangeable).

IR (KBr) cm^{-1} 3386 (NH), 1625 (amide C=O), 1545. MS *m/z* 358 (M^+, 52.8%), 360 (M+2, 21.5%). Anal. Calcd for $C_{17}H_{15}ClN_4O_3{\cdot}HCl$: C, 51.66; H, 4.08; N, 14.18. Found: C, 51.62; H, 4.98; N, 14.28.

4.7. Propyl 4-(2-chloro-6,7-dimethoxyquinazolin-4-ylamino)benzoate hydrochloride(4d)

Compound **4d** was prepared as described for **3a** from **1c** and propyl 4-aminobenzoate, and the product was recrystallized from methanol as beige solid. Yield: 74%; mp 258–260 °C, ^1H NMR (DMSO-d_6) (300 MHz) δ: 1.01 (t, 3H, CH_3), 1.74 (m, 2H, CH_2), 3.93 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.2 (t, 2H, OCH_2), 7.18 (s, 1H, Ar H), 7.97–7.99 (m, 5H, ArH), 10.17 (s, 1H, NH, D_2O exchangeable).

IR (KBr) cm^{-1}; 3217 (NH), 2970 (CH aliphatic), 1720 (C=O, ester). MS *m/z* 401 (M^+, 100%), 403 (M+2, 33.8%). Anal. Calcd for $C_{20}H_{20}ClN_3O_4{\cdot}HCl$: C, 59.78; H, 5.02; N, 10.46. Found: C, 59.52; H, 5.32; N, 10.71.

4.8. Methyl 2-(2-chloro-6,7-dimethoxyquinazolin-4-ylamino)-4,5-dimethoxybenzoate (4e)

Compound **4e** was prepared as described for **3a** from **1c** and methyl 4,5-dimethoxyanthranilate, and the product was suspended in saturated solution of Na_2CO_3 and stirred, filtered, and recrystallized from ethanol as faint yellow crystals. Yield: 67%; mp 278–280 °C; ^1H NMR (DMSO-d_6) (300 MHz) δ: 3.81 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 7.18–8.15 (m, 4H, Ar H), 11.08 (s, 1H, NH, D_2O exchangeable).

IR (KBr) cm^{-1} 2950 (CH aliphatic), 1673 (C=O, ester). MS *m/z* 433 (M^+, 56.8%), 435 (M+2, 18.9%), 374 (100%). Anal. Calcd for $C_{20}H_{20}ClN_3O_6$. C, 55.37; H, 4.65; N, 9.69. Found: 55.81; H, 4.70; N, 9.93.

4.9. 2-(2-Chloro-6,7-dimethoxyquinazolin-4-ylamino)-4,5-dimethoxybenzoic acid (4f)

Compound **4f** was prepared as described for **3a** from **1c** and 4,5-dimethoxyanthranilic acid, and the product was stirred in a solution of sodium acetate, filtered, and recrystallized from methanol to give yellow solid. Yield: 52%; mp 296–298 °C; ^1H NMR (DMSO-d_6) (300 MHz) δ: 3.75 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.98 (s, 1H, Ar H), 7.05 (s, 1H, Ar H), 7.29 (s, 1H, Ar), 8.61 (s, 1H, Ar H), 11.98 (s, 1H, NH, D_2O exchangeable), 12.3 (br s, 1H, COOH, D_2O exchangeable).

IR (KBr) cm^{-1} 3436 (NH), 2928 (CH aliphatic), 1732 (C=O, acid). MS *m/z* 419 (M^+, 45.2%), 421 (M+2, 21%), Anal. Calcd for $C_{19}H_{18}ClN_3O_6.0.25\ H_2O$. C, 53.78; H, 4.39; N, 9.90. Found: C, 53.67; H, 4.89; N, 9.88.

4.10. 6-Ethoxy-4-[4-(2-furoyl)piperazin-1-yl]-2-methylquinoline hydrochloride monohydrate (5)

A mixture of equimolar amount of 4-chloro-6-ethoxy-2-methylquinoline **(1f)** and 1-(2-furoyl) piperazine (1 mmol) each in ethanol (15 mL) was heated at reflux and stirred for 6 h. The solution was cooled, diluted with diethyl ether, and the solid formed was filtered and crystallized from absolute ethanol to give buff solid. Yield: 45%; mp 266–268 °C; ^1H NMR (DMSO-d_6) (300 MHz) δ: 1.41 (t, 3 H, J = 6.9 Hz, OCH_2CH_3) 2.72 (s, 3H, CH_3), 3.82 (m, 4H, CH_2, piperazine), 3.98 (m, 4H, CH_2, piperazine), 4.21 (q, 2H, J = 6.9 Hz, OCH_2CH_3), 6.61 (s, 1H, quinoline C-3), 7.08–7.11 (m, 2H, Ar H), 7.37 (s, 1H, Ar H), 7.6 (d, 1H, J = 9.3 Hz, Ar H), 7.88 (s, 1 H, Ar H), 8.08 (d, 1H, J = 9.3 Hz, furan).

IR (KBr) cm^{-}1627,1592. MS *m/z* 365 (M^+, 67.6%,). Anal. Calcd for $C_{21}H_{23}N_3O_3{\cdot}HCl{\cdot}H_2O$: C, 60.07; H, 6.24; N, 10.01. Found: C, 59.81; H, 6.14; N, 10.22.

4.11. 4-(6-Ethoxy-2-methylquinolin-4-ylamino)-*N*-(5-methylisoxazol-3-yl)benzene sulfonamide hydrochloride (6a)

A mixture of equimolar amounts of 4-chloro-6-ethoxy-2-methylquinoline **(1f)** (0.7 mmol), and 4-amino-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (sulfamethoxazole) in methoxyethanol (15 mL) was heated at reflux and stirred for 6 h. A white solid was formed, which was filtered and recrystallized from methanol/DMF to give **6a** as white powder. Yield: 87%; mp 298–300 °C, ^1H NMR (DMSO-d_6) (300 MHz) δ: 1.41 (t, 3H, J = 6.9, Hz OCH_2CH_3), 2.31 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 4.26 (q, 2H, J = 6.9 Hz, OCH_2CH_3), 6.16 (s, 1H, isoxazole), 7.02 (s, 1H, quinoline C-3), 7.62–8.1 (m, 7H, ArH), 10.69 (s, 1H, NH, D_2O exchangeable), 11.39 (s, 1H, NH, D_2O exchangeable).

IR (KBr) cm^{-1} 1608, 1585,1457, 1396 (S=O). MS *m/z* 438 (M^+, 100%). Anal. Calcd for $C_{22}H_{22}N_4O_4S{\cdot}HCl$: C, 55.63; H, 4.88; N, 11.80. Found: C, 55.53; H, 5.13; N, 11.77.

4.12. 4-(6-Ethoxy-2-methylquinolin-4-ylamino)-*N*-(thiazol-2-yl)benzenesulfonamide hydrochloride (6b)

Compound **6b** was prepared as described for **6a** from **1f** and 4-amino-*N*-(thiazol-2-yl)benzenesulfonamide (sulfathiazole), and

recrystallized from Methanol/DMF. Yield: 86%; mp 320–322 °C (decomp), ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 1.368 (t, 3H, J = 6.9, Hz OCH_2CH_3) 2.54 (s, 3H, CH_3), 4.26 (q, 2H, J = 6.9 Hz, OCH_2CH_3), 6.84 (d, 1H, J = 4.5 Hz, thiazole), 6.85 (s, 1H, ArH), 7.24 (d, 1H, J = 4.5 Hz, thiazole), 7.56–7.94 (m, 7H, ArH), 8.17 (s, 1H, NH, D_2O exchangeable), 10.67 (s, 1H, NH, D_2O exchangeable).

IR (KBr) cm^{-1}, 1606, 1557,1457, 1396 (S=O) MS m/z 440 (58.6% M^+). Anal. Calcd for $C_{21}H_{20}N_4O_3S_2$·HCl: C, 51.89; H, 4.14; N, 12.10. Found: C, 51.62; H, 4.29; N, 12.02.

4.13. 4-(6-Ethoxy-2-methylquinolin-4-ylamino)-*N*-(pyrimidin-2-yl)benzenesulfonamide hydrochloride (6c)

Compound **6c** was prepared as described for **6a** from **1f** and 4-amino-*N*-(pyrimidin-2-yl) benzenesulfonamide (sulfadiazine), and recrystallized from methanol/DMF. Yield: 78%; mp 316–318 °C (decomp), ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 1.39 (t, 3H, J = 6.9, Hz OCH_2CH_3) 2.62 (s, 3H, CH_3), 4.25 (q, 2H, J = 6.9 Hz, OCH_2CH_3), 7.0 (s, 1H, ArH), 7.07 (t, 1H, J = 2 Hz, pyrimidine), 7.65–8.1 (m, 7H, ArH), 8.53 (d, 2H, J = 2 Hz, pyrimidine), 10.64 (s, 1H, NH, D_2O exchangeable), 11.55 (s, 1H, NH, D_2O exchangeable).

IR (KBr) cm^{-1}, 1611, 1586,1458, 1399 (S=O) MS m/z 435 (M^+, 31.2%). Anal. Calcd for $C_{22}H_{21}N_5O_3S$·HCl. C, 55.99; H, 4.70; N, 14.84. Found: C, 55.76; H, 4.68; N, 14.64.

4.14. 4-(6-Ethoxy-2-methylquinolin-4-ylamino)benzenesulfonamide hydrochloride (6d)

Compound **6d** was prepared as described for **6a** from **1f** and 4-aminobenzenesulfonamide (sulfanilamide) and recrystallized from methanol/DMF. Yield: 66%; mp 314-316 °C (decomp), ^{1}H NMR (DMSO-d_6) (200 MHz) δ: 1.42 (t, 3H, J = 6.9, Hz OCH_2CH_3), 2.64 (s, 3H, CH_3), 3.07 (s, 2H, SO_2NH_2), 4.25 (q, 2H, J = 6.9 Hz, OCH_2CH_3), 6.61 (s, 1H, quinoline at C3), 7.49–8.25 (m, 7H, ArH), 10.87 (s, 1H, NH).

IR (KBr) cm^{-1}, 3320 (NH), 1611, 1588, 1458, 1390 (S=O), Anal. Calcd for $C_{18}H_{19}N_3O_3S$·HCl: C, 53.75; H, 4.78; N, 11.06. Found: 53.67; H, 4.98; N, 11.22.

4.15. 6-Ethoxy-4-[N-(4-guanidosulfonylphenyl)amino]-2-methylquinoline hydrochloride (6e)

Compound **6e w**as prepared as described for **6a** from **1f** and sulfaguanidine, and crystallized from methanol. Yield: 83%; mp 302–304 °C, ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 1.42 (t, 3H, J = 6.9, Hz OCH_2CH_3), 2.63 (s, 3H, CH_3), 4.25 (q, 2H, J = 6.9 Hz, OCH_2CH_3), 6.83–6.86 (m, 4H, 2 NH, NH_2 guanido, D_2O exchangeable), 6.92 (s, 1H, quinoline C-3), 7.60–8.17 (m, 7H, ArH), 10.68 (s, 1H, NH, D_2O exchangeable).

IR (KBr) cm^{-1} 3436, 3340, (NH, NH_2) 1612, 1585, 1459, 1397 (S=O). MS m/z 399 (M^+, 100%). Anal. Calcd for $C_{19}H_{21}N_5O_3S$·HCl: C, 52.35; H, 5.09, N, 16.07. Found: C, 52.39; H, 5.14, N, 16.21.

4.16. 4-[*N*-(2,4-Dichlorophenyl)amino]-6-ethoxy-2-methylquinoline (7a)

A mixture of equimolar quantities of **1f** and 2,4-dichloroaniline (0.7 mmol for each) and 3 drops of conc. HCl in *n*-butanol (15 mL) was heated under reflux and stirring for 8 h, and the mixture was left at room temperature overnight where a fluffy solid is formed. The solid was triturated in Na_2CO_3 solution, stirred, filtered, and crystallized from ethanol. Yield: 48%; mp 248–250 °C. ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 1.43 (t, 3H, J = 6.9, Hz OCH_2CH_3), 2.58 (s, 3H, CH_3), 4.26 (q, 2H, J = 6.9 Hz, OCH_2CH_3), 6.29 (s, 1H, Ar H), 7.6–8.0 (m, 5H, ArH), 8.12 (s, 1H, ArH), 10.57 (s, 1H, NH, D_2O exchangeable).

IR (KBr) cm^{-1} 3302 (NH), 1609 (C=N), 1578. MS m/z 346 (M^+, 100%), 348 (M+2, 56%), 350 (M+4, 13.3%). Anal. Calcd for $C_{18}H_{16}Cl_2N_2O$.0.5 H_2O. C, 60.69; H, 4.81, N, 7.86. Found: C, 60.49; H, 5.12, N, 7.77.

4.17. Methyl 2-(6-ethoxy-2-methylquinolin-4-ylamino)-4,5-dimethoxybenzoate hydrochloride (7b)

A mixture of equimolar quantities of **1f** (0.22 g, 1 mmol) and methyl 4,5-dimethoxyanthranilate (0.21 g, 1 mmol) and 3 drops conc. HCl in *n*-pentanol (12 mL) was heated under reflux and stirring for 6 h, and the mixture was left at room temperature overnight. The yellow solid formed was filtered washed with diethyl ether and recrystallized from ethanol/DMF. Yield: 0.2 g, 47:%; mp 252–254 °C; ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 1.43 (t, 3H, J = 6.9, Hz OCH_2CH_3), 2.56 (s, 3H, CH_3), 3.66 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.25 (q, 2H, J = 6.9 Hz, OCH_2CH_3), 6.43 (s, 1H, Ar H), 7.15 (s, 1H, Ar H), 7.45 (s, 1H, Ar H), 7.6 (d, 1H, ArH), 8.04–8.07 (m, 2H, ArH), 10.40 (s, 1H, NH, D_2O exchangeable).

IR (KBr) cm^{-1} 3442, 2945 (CH aliphatic), 1697(C=O, ester), 1609,1457, 1396. MS m/z 396 (M^+, 100%). Anal. Calcd for $C_{22}H_{24}N_2O_5$·HCl: C, 61.04; H, 5.82; N, 6.47. Found: C, 60.49; H, 5.55; N, 6.37.

4.18. 6-Ethoxy-4-[N-(3-hydroxyphenyl)amino]-2-methylquinoline hydrochloride (7c)

Compound **7c** was prepared as described for **7b** from **1f** and 3-aminophenol, and crystallized from ethanol. Yield: 68%; mp 324–326 °C; ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 1.42 (t, 3H, J = 6.9, Hz OCH_2CH_3), 2.58 (s, 3H, CH_3), 4.25 (q, 2H, J = 6.9 Hz, OCH_2CH_3), 6.67 (s, 1H, Ar H), 6.80–6.88 (m, 3H, Ar H), 7.34 (t, 1H, Ar H), 7.61 (d, 1H, ArH), 8.0 (d, 1H, ArH), 8.12 (s, 1H, ArH), 9.94 (s, 1H, phenolic OH, D_2O exchangeable), 10.47 (s, 1H, NH, D_2O exchangeable).

IR (KBr) cm^{-1}, 3368 (OH), 3234 (NH), 2944 (CH aliphatic), 1613, 1591, 1457, 1396. MS m/z 294 (M^+, 100%). Anal. Calcd for $C_{18}H_{18}N_2O_2$·HCl: C, 65.35; H, 5.79; N, 8.47. Found: C, 65.75; H, 5.82; N, 8.33.

5. Molecular modeling study

Docking study was carried out for the target compounds into EGFR using SYBYL version 7.3, Tripos Inc and Molegro virtual docker version 2007. The crystal structure of the enzyme with lapatinib (1XKK) was obtained from protein data bank PDB.[35]

Since it was found that gefitinib mimic ATP and bind to the ATP binding region of the kinase active site. The binding of ATP itself involves two important hydrogen bonding interactions between the purine base of ATP and the protein backbone between amino acids Gln-767 and Met-769 and have two nitrogen atoms in the quinazoline ring which both act as hydrogen bond acceptors. One of these interactions involves the purine group acting as a hydrogen bond donor, while the other involves purine acting as hydrogen bond acceptor.

Our compounds were modeled by positioning them in the lapatinib binding site in accordance with the published crystal structures of quinazoline derivatives bound to kinase.[36] The entire complex was then subjected to alternate cycles of minimization and dynamics. The intent was to get a satisfactory structure for the complex that was consistent with the published crystal structure.[37,38]

From the comparative docking study of our compounds with many structurally related lead compounds such as lapatinib and gefitinib we could observe how our compounds might bind to the

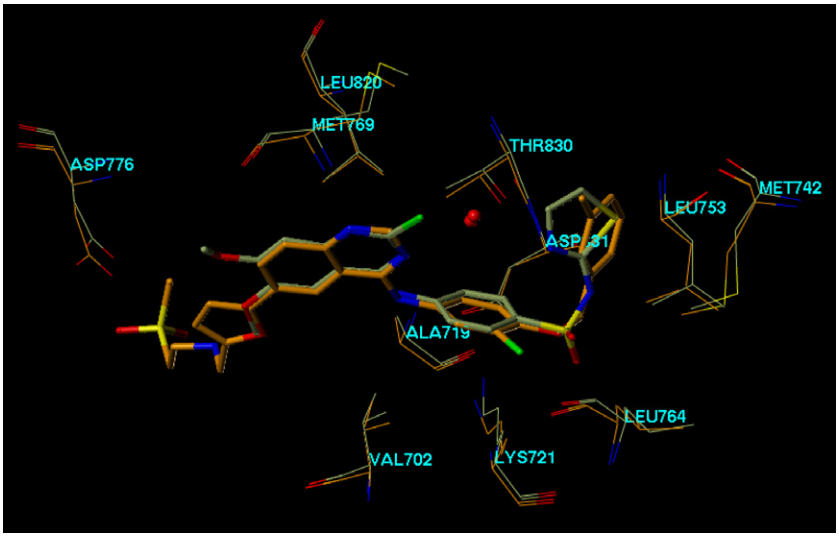


Figure 2. Overlying of **3b** and Lapatinib in the ATP binding site of EGFR-TK shows amino acids in contact in the same position. This picture was created with SYBYL version 7.3.

kinase binding site, based on the knowledge of the structure of similar active sites. We redocked lapatinib into the active site of the enzyme (Fig. 2), then we replaced with our compounds in order to compare the binding mode of both ligand and the test compound. These docking studies have revealed that 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, and similarly, a water molecule-mediated hydrogen bonding interaction is observed between the N-3 of the quinazoline ring and the Thr-830 side chain. These interactions underscore the importance of both nitrogen atoms for binding and the subsequent inhibitory capacity. The aniline moiety lies in a deep and hydrophobic pocket. The bulky sulfamoyl group at the C-4 of aniline moiety lies at the same position of the 3′-chloro-4′-(3-fluorobenzyl)oxy moiety of lapatinib.

The results of this virtual screening could support the postulation that our active compounds may act on the same enzyme target where EGFR inhibitor acts. Figure 2 shows optimum overlay of lapatinib with **3b** with amino acids in vicinity kept in place, while Figures 3 and 4 demonstrate binding models of quinazoline in the ATP binding site. In each case, the N-1 atom of the quinazoline is hydrogen bonded to the backbone nitrogen of Met 769, while the N-3 atom is hydrogen bonded to the side chain hydroxyl of Thr 830 via a water molecule, and in case of compound **3b** we noticed that an additional H bond is formed between thiazole N and water molecule. On the other hand, in the quinoline counterparts, the N-1 atom is similarly hydrogen bonded to Met 769 as shown in Figure 5.

6. Biological testing

6.1. Materials and methods

The human tumor cell lines (MCF-7) were obtained as a gift from NCI, MD, USA.

All chemicals and solvents were purchased from Sigma–Aldrich.

6.1.1. Measurement of potential cytotoxicity

The cytotoxic activity was measured in vitro for the newly synthesized compounds using the Sulfo-Rhodamine-B stain **(SRB)** assay using the method of Skehan.[39]

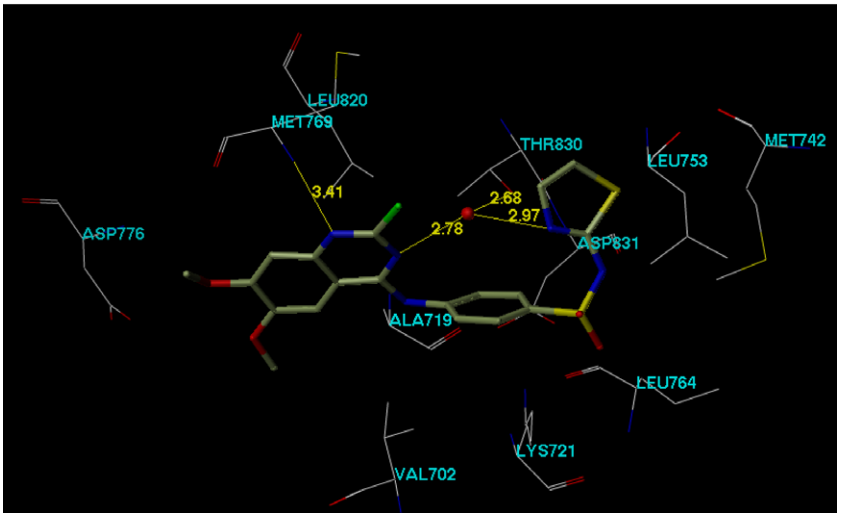


Figure 3. Binding mode of compound **3b** in the ATP binding site of EGFR-TK showing two H bonds between N-1 and Met 769 and another H-bonds between N-3 of the quinazoline and N of thiazole ring to Thr 830 through water bridge.

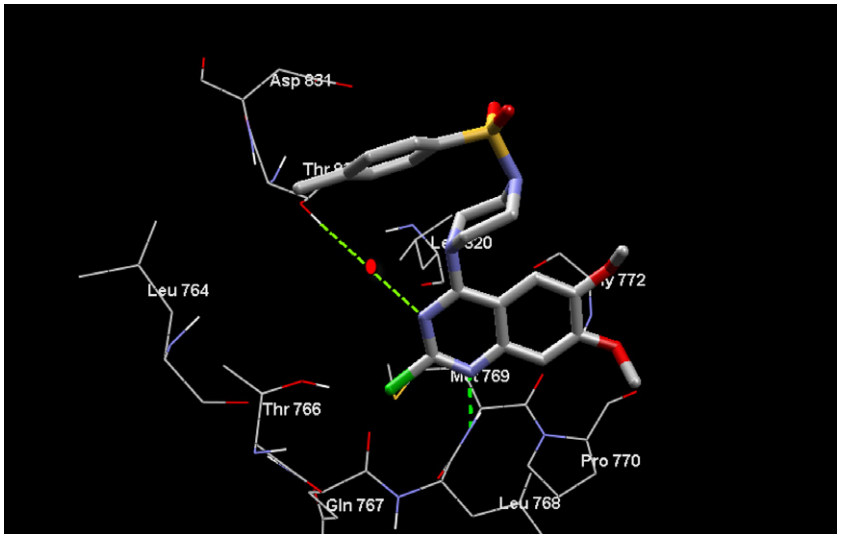


Figure 4. Binding model of compound **2** in the ATP binding site of EGFR showing H bonding between N-1 of the quinazoline and Met 769 and the water-bridged H-bond to Thr 830. The hydrogen bonds are indicated as green dashed lines. This picture was created with Molegro, virtual docker program.

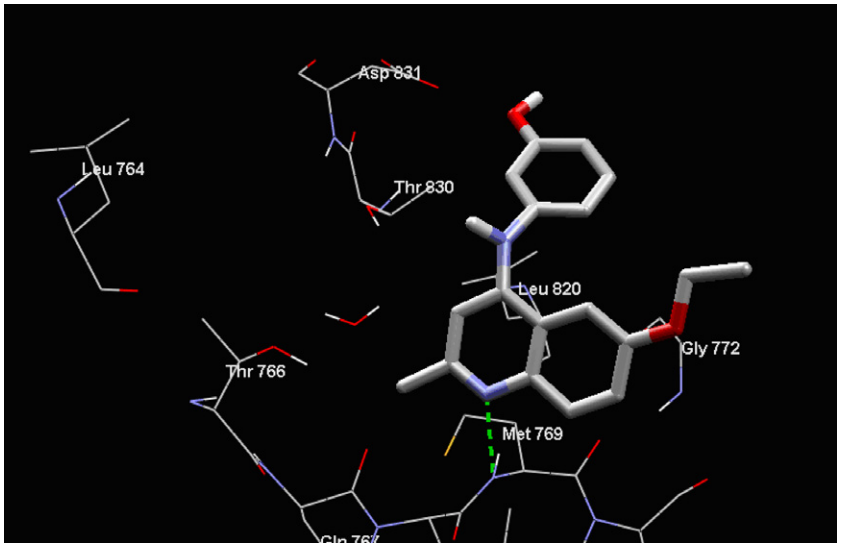


Figure 5. Binding of 6-Ethoxy-4-[*N*-(3-hydroxyphenyl)amino]-2-methylquinoline (**7c**) to ATP binding site of EGFR showing H bonding between N-1 of the quinazoline and Met 769. This picture was created with Molegro, virtual docker program.

Cells were plated in 96-multiwell microtiter plate (10^4 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compound under test (0.1, 2.5, 5, and 10 nmol/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained for 30 min with 0.4% (wt/vol) with SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 1. The inhibition curve for the most potent compound **(3b)** is presented in Figure 6.

Table 1
In vitro cytotoxic activities of the synthesized compounds against human breast cancer cell (MCF-7)

Compound	Cytotoxicity (IC_{50})[a,b] (nmol)
2	1.08
3a	1.98
3b	0.13
5	4.31
6a	13.85
6b	NA
6c	3.96
6e	19.25
7a	4.38
7b	1.67
7c	2.84

NA, means the IC_{50} not achieved at 20 nmol.
[a] IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.
[b] Values are means of three experiments.

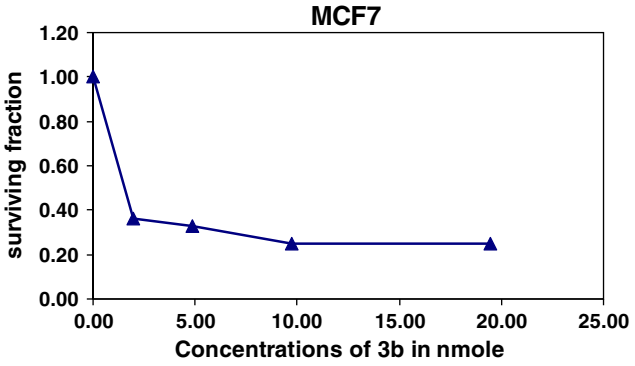


Figure 6. Effect of different concentrations of **3b** on the viability of MCF-7 cell line.

7. Conclusions

Since breast cells are known to overexpress EGFR, which leads to continuous activation of the EGFR pathway involved in cell proliferation, therefore, we measured antitumor activity of the compounds in vitro on human breast carcinoma cell line (MCF-7). Most of the tested compounds exhibited potent inhibitory activity against MCF-7 cell line.

In the quinazoline series, compounds **2**, **3a,** and **3b** displayed the most potent cytotoxic activity with IC_{50} equal to 1.08, 1.98, and 0.13 nmol, respectively. On the other hand, in the quinoline series, compounds **7b** and **7c** are the most potent ones with IC_{50} equal to 1.67 and 2.84, respectively. From the biological testing results, we concluded that the quinazoline derivatives with five-membered hetrocyclysulfonamido moiety at C-4 of anilino group are more active than their quinoline analogues. It was also noticed that the activity is retained or enhanced in compounds **2** and **5** which have piperazine moiety in place of aniline.

These preliminary encouraging results of biological screening of the tested compounds could offer an excellent framework in this field that may lead to discovery of potent antitumor agent.

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