Molecular Modelling and Synthesis of Quinazoline-Based Compounds as Potential Antiproliferative Agents

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In this study, four series of 4-anilinoquinazoline derivatives were designed and synthesized as potential anti-proliferative agents. Mechanism of anticancer activity was explained through molecular docking of the target compounds into epidermal growth factor receptor tyrosine kinase (EGFR-TK) active site which displayed comparable binding mode of certain compounds to that of lapatinib. Moreover, the newly synthesized compounds were tested for their anti-proliferative activity on breast carcinoma cell line (MCF-7). 6-(4-Benzylpiperazin-1-ylsulfonyl)-4-(4-bromoanilino)quinazoline (14g) exhibited the most potent inhibitory activity (IC_{50}=5.52 μM).

Key words quinazoline; tyrosine kinase; anti-proliferative agent

Although development of novel targeted anticancer drugs have shown a great progress in recent years, cancer remains the major leading cause of death in the world.[1)] Receptor protein tyrosine kinases (RPTKs) play a vital role in signal transduction pathways that regulate cell division and differentiation by binding of the growth factors, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF). Among the known receptor tyrosine kinases that have been identified as being important in cancer is epidermal growth factor receptor (EGFR).[2)] It is a transmembrane receptor belonging to the human epidermal growth factor receptor (HER) family of receptors. To date four members of this family have been identified including EGFR (HER1/erbB-1), HER2 (erbB-2/neu), HER3 (erbB-3) and HER4 (erbB-4).[2)] The EGFR and its family members are composed of an extracellular ligand-binding domain, a transmembrane domain and an intracellular domain. When a ligand, such as EGF, binds to the ligand-binding domain, the EGFR forms a homodimer or heterodimer with other closely related receptors. Then, the intrinsic kinase domain is activated, resulting in autophosphorylation and transphosphorylation of the receptors through their tyrosine kinase domains leading to the activation of multiple downstream pathways such as protein synthesis, cell growth, survival, differentiation and apoptosis.[3)] Given this pivotal role, it is not surprising that EGFR has been implicated in several human disorder including solid tumors.[4)] Overexpression and/or coexpression of EGFR has been found in numerous cancer types, including colon, breast, ovarian, head and neck, and non-small cell lung cancers. Therefore, selective blockade of EGFR has been shown to be an effective therapeutic approach against cancers.[5)]

Many compounds with different scaffolds were reported as epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs). Among them 4-anilinoquinazoline was the most attractive scaffold to be modified for yielding effective anticancer drugs such as gefitinib, erlotinib and lapatinib. These lead compounds are selective EGFR inhibitors approved by the Food and Drug Administration (FDA) for cancer therapy[6)] (Fig. 1).

Structure–activity relationship (SAR) studies for the ability of 4-anilinoquinazoline to inhibit EGFR-TKs activity revealed that both of the quinazoline nitrogen atoms were absolutely essential for activity. The phenyl ring with small lipophilic substituents such as chloro, bromo and trifluoromethyl was also important as it occupies the lipophilic pocket. The nature of the linking group between the quinazoline ring and phenyl side chain has a great effect on the inhibitory activity. Upon replacement of nitrogen linker by oxygen or sulfur causes the reduction in the activity of EGFR but it causes increase in the activity of VEGFR especially in the compounds which possess urea or thiourea moiety on the phenyl side chain.[7)] Finally, substitution on the quinazoline ring at positions 6 or 7 was confined to substituents preferably with polar groups.[8)]

Recently, a large number of anticancer agents with diverse structures have been developed and evaluated. (*E*)-*N*-(4-(3-Chlorophenylamino)quinazolin-6-yl)-3-(2-nitrophenyl)-acrylamide (**a**) demonstrated the most potent inhibitory activity, which could be optimized as a potential EGFR inhibitor.[9)] Moreover, 4,6-substituted(diaphenylamino)quinazolines reported by Lu *et al.* displayed good antiproliferative and EGFR-TK inhibitory activities particularly, 4-(4-(3-bromophenylamino)quinazolin-6-ylamino)methyl)phenol (**b**).[10)] Furthermore, 4-anilino-6-substituted-quinazoline derivatives were evaluated for EGFR-TK and tumor growth inhibitory activities. Compound 4-(4-chlorophenylamino-6-uredioquinazoline (**c**) showed the most inhibitory activity. All of them, according to the previous SAR, were directed toward the addition of a polar moiety at position-6 and small lipophilic groups on the aniline moiety[11)] (Fig. 2).

With the aim of finding new structures which may serve as a potential chemotherapeutic agents, in our approach, we modified the molecular structure of recently reported compounds in two main sites: one was 4-anilino moiety by substituting the small lipophilic group with other large functional groups of different lipophilicity and/or steric hindrance on the

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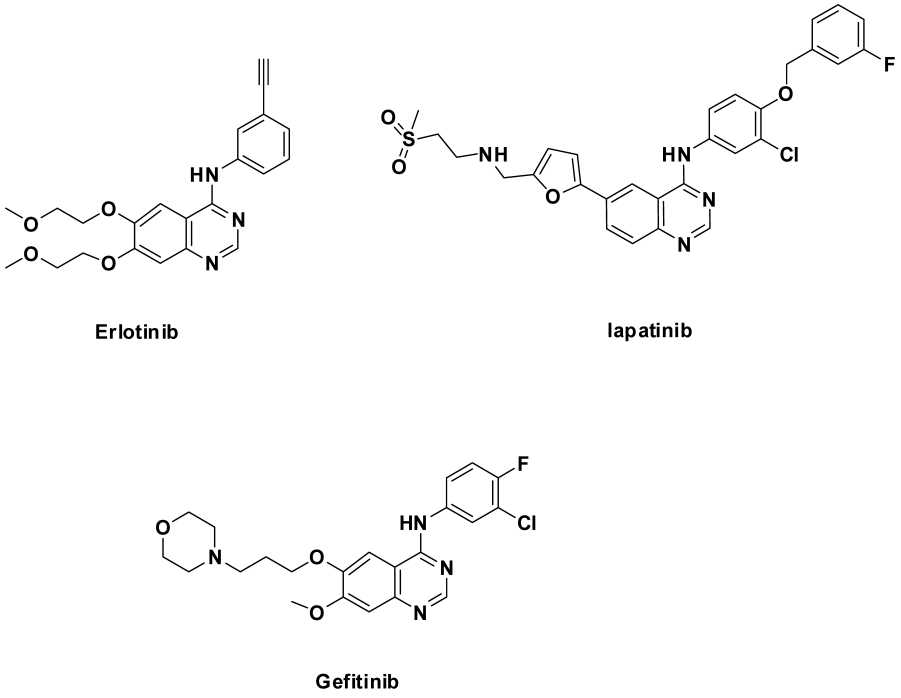


Fig. 1. Examples of EGFR Tyrosine Kinase Inhibitors

They were approved by FDA. As shown all of them possess the common structure: 4-anilinoquinazoline moiety.

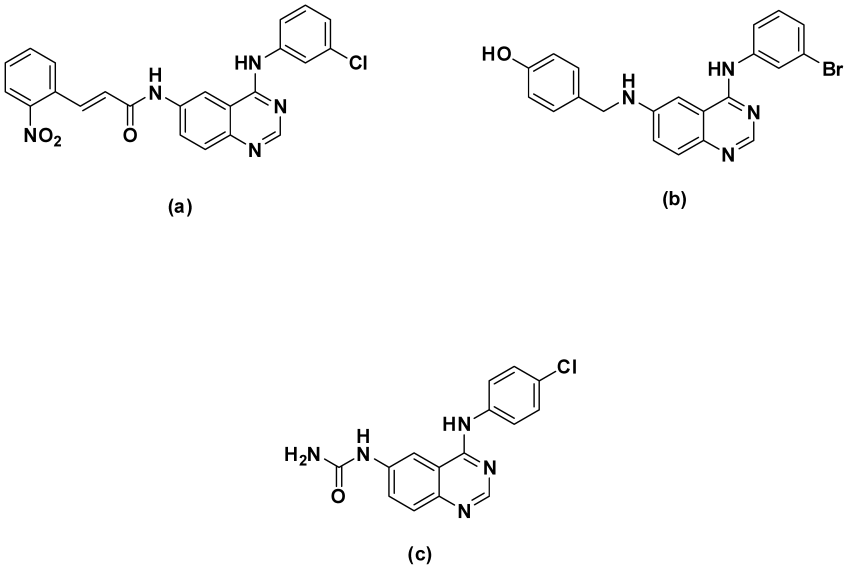


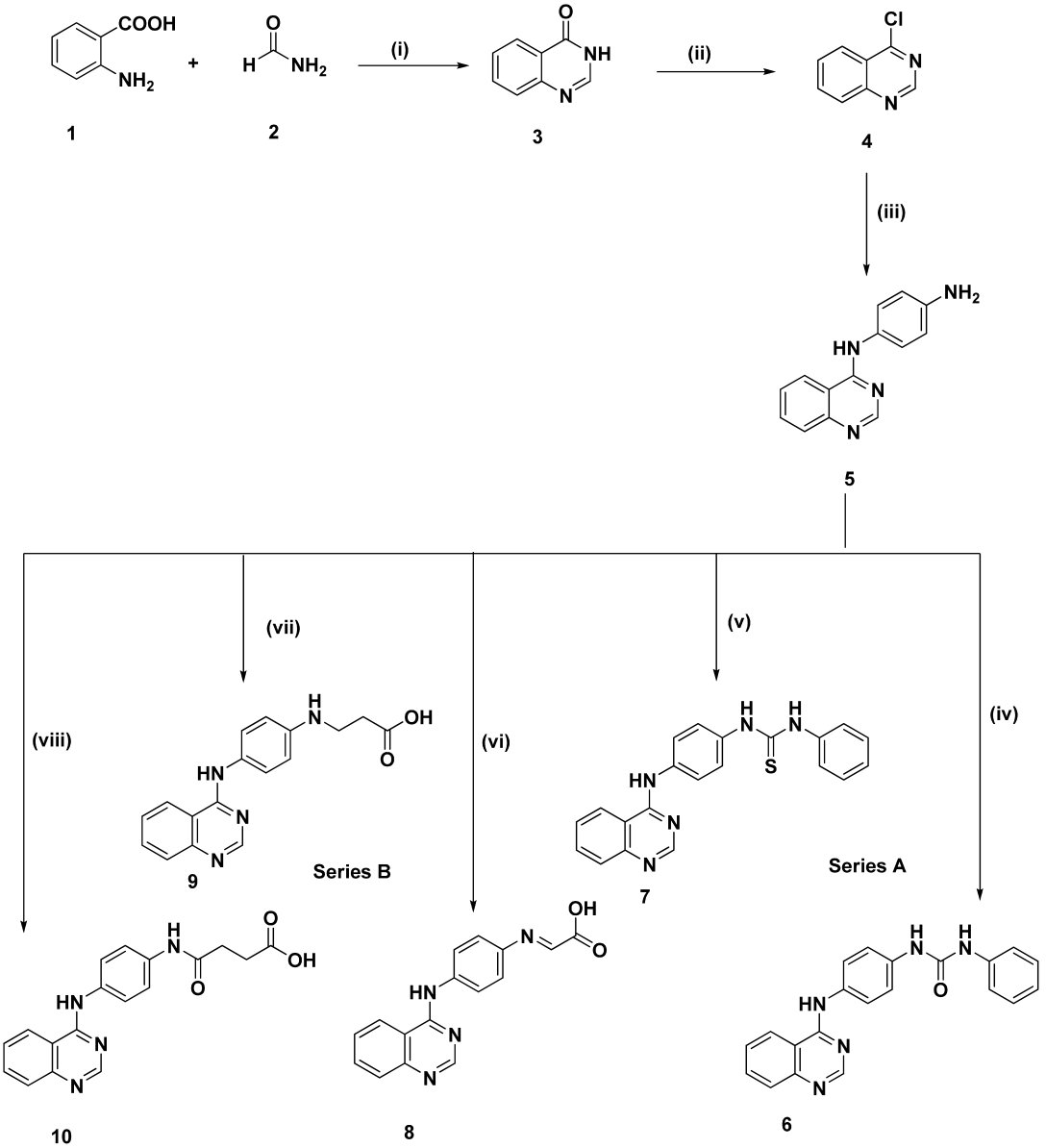
Fig. 2. Recently Reported Anticancer Agents with Novel Structures

All of them were directed toward the addition of electron donating group at position-6 and small lipophilic groups on the aniline moiety.

basis of retaining the bulky group of the structure of lapatinib (Chart 1) and the other was the position-6 by incorporating sulphonyl polar group (Chart 2, Fig. 3).

Experimental

Molecular Modeling Procedure Molecular modeling was carried out on Schrodinger computational software workstation using Maestro 9.3 graphic user interface (GUI). The atomic coordinates and the cartesian matrix of the polypeptide were obtained from the protein bank database PDB (Brookhaven protein database .2J5F). The basic and acidic amino acids were neutralized at pH 7±2 by protonation of the terminal amino groups of basic amino acids and the terminal carboxylic acid groups of acidic amino acids were deprotonated. The docking process involved the standard precision docking (SP) in which ligand poses that scored bad energies would be rejected. Partial charge cutoff was set to 0.25 for the receptor and 0.15 for the ligand to soften the potential of non-polar parts. Flexible docking was performed with no torsional constraints was applied. Twenty percent of the final poses produced from the SP docking were subjected to the Extra Precision mode of Glide docking (XP) to perform strict and



Reagents and conditions: (i) Reflux, 5–6 h. (ii) $POCl_3$, dry DMF, TEA, reflux, 7 h. (iii) *p*-NH_2PhNH_2, TEA, rt 24 h. (iv) PhNCO, C_5H_5N, THF, rt, 16 h. (v) PhNCS, C_5H_5N, THF, heat, 40°C, 2 h. (vi) Glyoxalic acid, dry EtOH, rt, 5 h. (vii) Acrylic acid, AcOH, stir at rt, 2 h. (viii) Succinic anhydride, dioxane, reflux, 5 h.

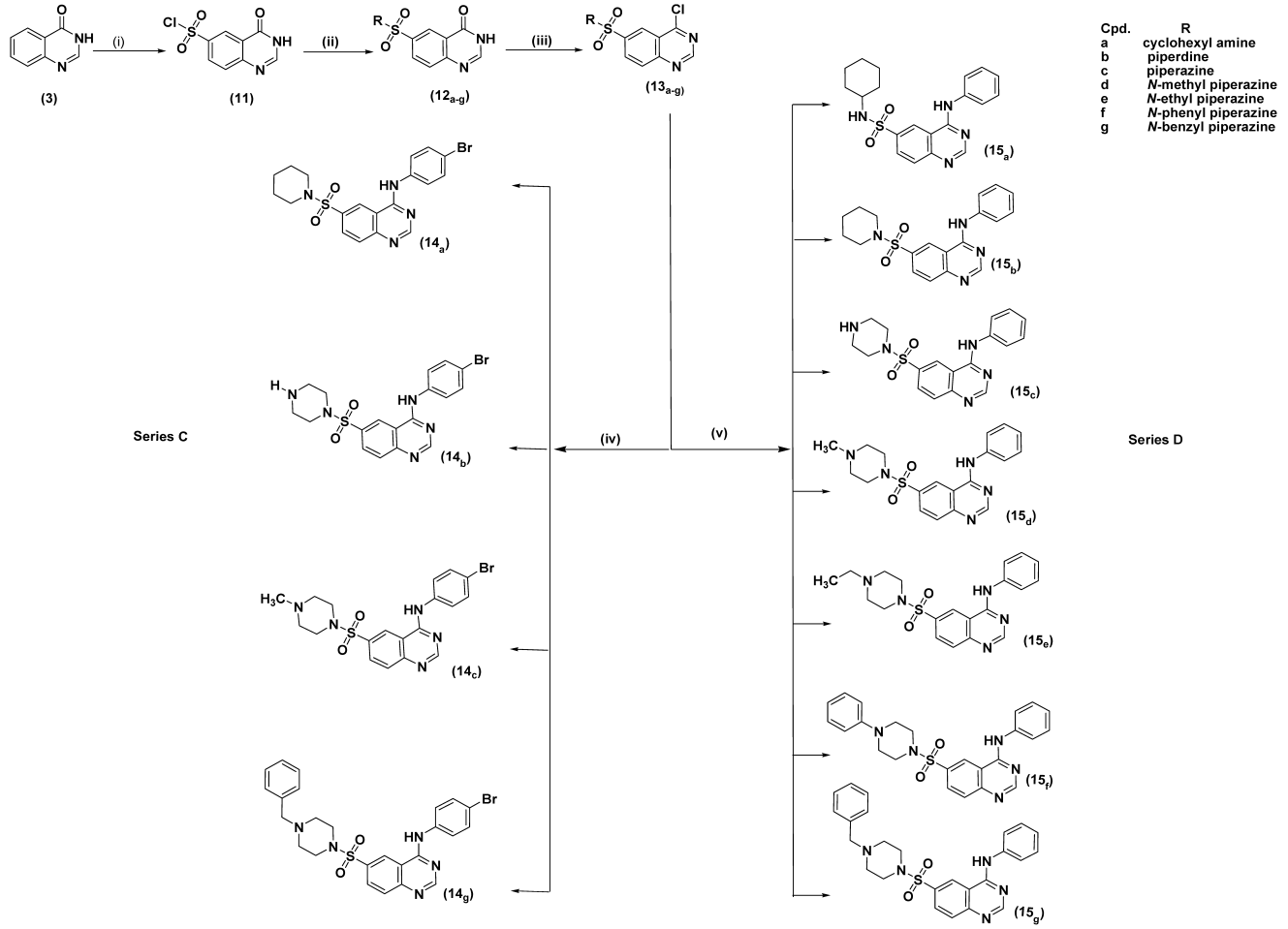
The starting material anthranilic acid (**1**) was conveniently cyclized to quinazolin-4(3*H*)-one (**3**) by heating it with formamide (**2**) at 120°C. Upon refluxing compound (**3**) with freshly distilled phosphorous oxychloride, the corresponding 4-chloroquinazoline (**4**) was obtained. 4-Chloroquinazoline was allowed to react with *p*-pheneylene diamine to produce 4-(4-aminoanilino)quinazoline (**5**). The key intermediate 4-(4-aminoanilino)quinazoline was readily converted to the title compounds (**6**), (**7**) series A, (**8**)–(**10**) series B *via* the reaction with phenyl isocyanate, phenyl isothiocyanate, glyoxalic acid, acrylic acid and succinic anhydride, respectively.

Chart 1. The Reaction Sequence for the Synthesis of the Title Compounds (**1**–**6**)

more precise docking simulation. Aromatic rings were forbidden to flip and amide bonds of the polypeptide segments with non-polar amide bonds were penalized.[12)]

Experimental Procedure All chemicals were obtained from Aldrich, Fluka and Merck chemicals. Melting points were determined on an electrothermal apparatus in open capillary tubes using Stuart melting point apparatus SMP10 and the values were uncorrected. IR spectra were determined on using KBr discs on a Vector 22 Infrared spectrophotometer (ν_{max} in cm^{-1}), with ratio (1 drug: 3 KBr). ^{1}H-NMR spectra were carried out using Varian Mercury-300 (300 MHz, DMSO-d_6). Spectrophotometer using tetramethylsilane (TMS) as internal standard. Chemical shift values are recorded in ppm on δ scale, Microanalytical Center, Cairo University, Egypt. Mass spectra were recorded on a GCMP-QP1000 EX Mass spectrometer, Microanalytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Microanalytical Center, Suez Canal University, Egypt. Reactions were monitored by thin layer chromatography (TLC) using different solvent mixtures as mobile phase and precoated aluminum sheets silica gel Merck 60 (F_{254}) as stationary phase; the spots were visualized by exposure to iodine vapor or UV light under 254 and 366 nm illumination.

Quinazolin-4(3*H*)-one (**3**): A mixture of anthranilic acid **1** (0.7 g, 5 mmol) and formamide **2** (2 mL) was stirred under reflux (120–140°C) for 5–6 h. The mixture was allowed to cool



Reagents and Conditions: (i) $ClSO_3H$, heat, stir at 60°C, 7 d. (ii) R_2NH, TEA, reflux, 10 h. (iii) $POCl_3$, dry DMF, TEA, reflux, 7 h. (iv) 4-Bromoaniline, TEA, stirring, reflux, 13–24 h. (v) Aniline, TEA, stirring, reflux, 3–7 h.

Compound (**3**) reacted with freshly distilled chlorosulfonic acid under dry conditions to give 6-chlorosulfonylquinazolin-4(3*H*)-one (**11**). Compound (**11**) was allowed to react with piperdine, cyclohexyl amine and substituted piperazines to produce 6-(*N*-substituted)sulfonylquinazolin-4(3*H*)-one (**12a**–**g**). Subsequently, the reaction of compounds (**12a**–**g**) with freshly distilled phosphorous oxychloride to give 4-chloro derivatives (**13a**–**g**). Finally, compounds (**13a**–**g**) underwent a nucleophilic substitution reaction with selected substituted anilines to give the title compounds (**14b**–**d**, **g**) series C and (**15a**–**g**) series D.

Chart 2. The Reaction Sequence for the Synthesis of the Title Compounds (**14b**, **c**, **d**, **g**, and **15a**–**g**)

to 100°C. Water (20 mL) was added dropwise to the mixture. The resulting precipitate was filtered, recrystallized from anhydrous ethanol to afford **3** as a white solid.[13] Yield 64%, mp 220–222°C, spectral data as reported.[14]

4-Chloroquinazoline (**4**): A mixture of quinazolin-4(3*H*)-one **3** (0.4 g, 2.5 mmol) in $POCl_3$ (25 mL) and *N*,*N*-dimethylforamide (DMF) (0.12 mL) was stirred under reflux (100–105°C) for 5–6 h. The excess $POCl_3$ was removed by vacuo. The residue was poured into a mixture of chloroform (50 mL), ice water (80 mL) and triethylamine (5 mL) then neutralized with saturated $NaHCO_3$ solution. The chloroform layer was separated, dried over Na_2SO_4 and filtered. The solvent was removed by distillation to give yellow solid of 4-chloroquinazoline **4**. The resulting compound was stored at 0°C without a further purification.[13] Yield 62.5%, mp 100–102°C, spectral data as reported.[14]

4-(4-Aminoanilino)quinazoline (**5**): A solution of 4-chloroquinazoline **4** (0.16 g, 0.95 mmol) in chloroform (10 mL) was cooled in an ice-bath for 30 min. A solution of *p*-pheneylenediamine (0.1 g, 0.95 mmol), triethylamine (1 mL) in chloroform was added dropwise to the reaction mixture in about 15 min. The mixture was allowed to reach the room temperature and left stirring overnight. The formed precipitate was filtered and recrystallized from chloroform to give 4-(4-aminoanilino)-quinazoline **5** as yellow needle crystals.[15] Yield 66%, mp >300°C, spectral as reported.[16]

1-Phenyl-3-(4-(quinazolin-4-ylamino)phenyl)urea (**6**): Phenyl isocyanate (0.15 mL, 1.26 mmol) was added dropwise to a mixture of 4-(4-aminoanilino)quinazoline **5** (0.15 g, 0.63 mmol) and pyridine (0.16 g, 1.89 mmol) in THF. The mixture was stirred for 16 h. The resulting precipitate was filtered and recrystallized from methanol to afford a greenish white crystalline powder of 1-phenyl-3-(4-(quinazolin-4-ylamino)-phenyl)urea **6**. Yield 80%, mp >300°C. ^{1}H-NMR (DMSO-d_6) δ: 12.57 (1H, s, exch with D_2O), 12.50 (1H, s, exch with D_2O), 9.18 (1H, s, exch with D_2O), 7.45–6.92 (14H, m). IR (KBr) cm^{-1}: 3298, 1637, 1561, 1505. MS *m*/*z*: 355 (M^+), 212, 125, 111, 93. *Anal.* Calcd for $C_{21}H_{17}N_5O$: C, 70.97; H, 4.82; N, 19.71. Found: C, 70.63; H, 4.54; N, 19.50.

1-Phenyl-3-(4-(quinazolin-4-ylamino)phenyl)thiourea (**7**): Phenyl isothiocyanate (0.17 mL, 1.26 mmol) was added dropwise to a mixture of 4-(4-aminoanilino)quinazoline **5** (0.15 g,

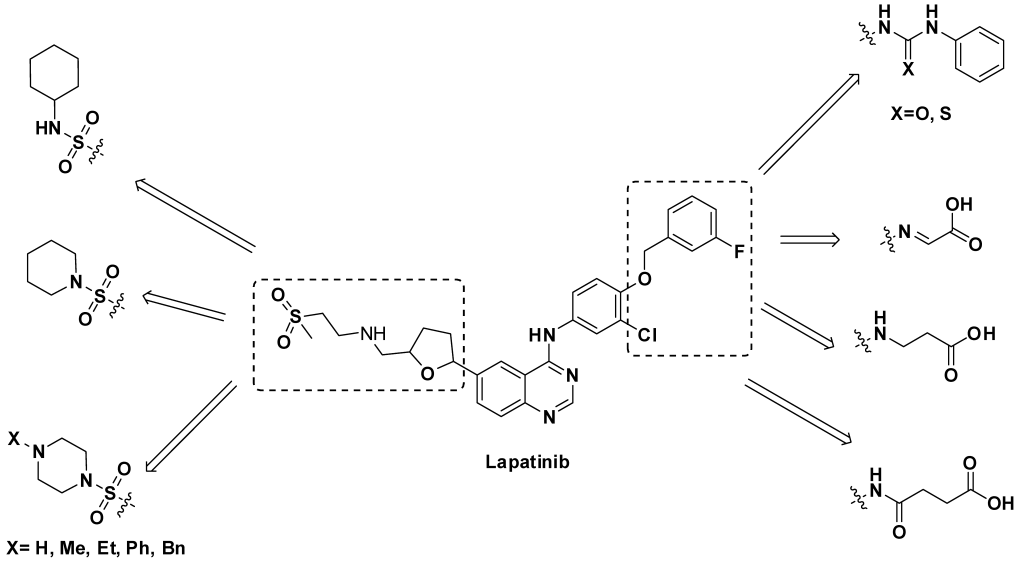


Fig. 3. Design for Lapatinib Congeners

Replacement of bulky side chain at position-4 of the aniline ring with urea, thiourea, glycine, *β*-alanine and succinamic acid. Replacement of electron donating group at position-6 with *N*-substituted sulfonyl derivatives of cyclohexyl amine, piperdine and substituted piperazines.

0.63 mmol) and pyridine (0.16 g, 1.89 mmol) in dry THF (30 mL). The mixture was heated to 40°C for 2 h. The solvent was removed under reduced pressure to give yellow residue of 1-phenyl-3-(4-(quinazolin-4-ylamino)phenyl)thiourea **7** which was purified by column chromatogrphy using 100% hexane as eluent to give white crystals. Yield 30%, mp 150–152°C. ^{1}H-NMR (DMSO-d_6) δ: 9.63 (1H, s, exch with D_2O), 8.60 (1H, s, exch with D_2O), 8.50 (1H, s, exch with D_2O), 8.49–6.61 (14H, m). IR (KBr) cm^{-1}: 3281, 1591, 1486, 1307. MS *m/z*: 371 (M^+), 294, 279, 235, 217, 71. *Anal.* Calcd for $C_{21}H_{17}N_5S$: C, 67.90; H, 4.61; N, 18.85. Found: C, 67.54; H, 4.23; N, 18.60.

2-(4-(Quinazolin-4-ylamino)phenylimino)acetic Acid (**8**): Glyoxalic acid (1.84 g, 20 mmol) in dry ethanol (100 mL) was added to a stirred solution of 4-(4-aminoanilino)quinazoline **5** (4.72 g, 20 mmol) in dry ethanol (100 mL). The mixture was stirred for 5 h. The solvent was removed under reduced pressure to give brown solid which was purified by column chromatography using chloroform–methanol (3 : 1) to give brown crystals. Yield 37%, mp >300°C. ^{1}H-NMR (DMSO-d_6) δ: 10.20 (1H, s, exch with D_2O), 9.70 (1H, s, exch with D_2O), 8.45–6.48 (9H, m), 6.5 (1H, s). IR (KBr) cm^{-1}: 3386, 2922, 1619. MS *m/z*: 292 (M^+), 291, 279, 247, 234, 97. *Anal.* Calcd for $C_{16}H_{12}N_4O_2$: C, 65.75; H, 4.14; N, 19.17. Found: C, 66.15; H, 4.52; N, 19.50.

3-(4-(Quinazolin-4-ylamino)phenylamino)propanoic Acid (**9**): Acrylic acid (0.3 mL, 4.2 mmol) and acetic acid (1 mL) were stirred for 1 h in an ice bath. 4-(4-Aminoanilino)-quinazoline **5** (1 g, 4.2 mmol) was added gradually with stirring to the reaction mixture. The mixture was allowed to reach room temperature then stirring continued for 2 h. The mixture was evaporated under vaccuo. The residue was washed with cold water (1×5 mL) and filtered to give a brown solid which was purified by column chromatography using ethanol–chloroform (4 : 1) to give brown crystals. Yield 40%, mp 240°C. ^{1}H-NMR (DMSO-d_6) δ: 11.92 (1H, s, exch with D_2O) 10.95 (1H, s, exch with D_2O), 8.08–6.86 (9H, m), 5.95 (1H, s), 3.99–2.90 (4H, m). IR (KBr) cm^{-1}: 3500, 2961, 3681, 1613, 1519. MS *m/z*: 308 (M^+), 307, 291, 263, 235, 55. *Anal.* Calcd for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.17. Found: C, 66.58; H, 5.45; N, 18.55.

4-Oxo-4-(4-(quinazolin-4-ylamino)phenylamino)butanoic Acid (**10**): 4-(4-Aminoanilino)quinazoline **5** (9.44 g, 40 mmol) and succinic anhydride (4 g, 40 mmol) were refluxed in dioxane (20 mL) for 5 h. The mixture was cooled to room temperature and diethyl ether was added to the mixture. A white precipitate was formed, filtered and washed with cold water (3×5 mL) and subsequently dissolved in HCl (16 mL, 16% v/v). After standing at room temperature the crystallized succinic acid was removed by filtration and the pH of the mixture was adjusted to **5** by addition of 10% NaOH (12 mL). A gray precipitate was formed, filtered and purified by column chromatography using ethanol–ethyl acetate (15 : 1) to give white needle crystals. Yield 50%, mp >300°C. ^{1}H-NMR (DMSO-d_6) δ: 11.90 (1H, s, exch with D_2O), 10.90 (1H, s, exch with D_2O), 9.33 (1H, s, exch with D_2O), 8.80–6.80 (9H, m), 3.9–2.54 (4H, m). IR (KBr) cm^{-1}: 3421, 2900, 1596, 1492. MS *m/z*: 336 (M^+), 277, 235, 221, 189, 86. *Anal.* Calcd for $C_{18}H_{16}N_4O_3$: C, 64.28; H, 4.790; N, 16.66. Found: C, 64.00; H, 4.46; N, 16.26.

6-Chlorosulfonylquinazolin-4(3*H*)-one (**11**): Quinazolin-4(3*H*)-one **3** (73 g, 0.5 mol) was added gradually to cold solution of chlorosulfonic acid (174 mL, 2.49 mol) at 12–15°C. The temperature was raised to room temperature then the mixture is heated at 60°C for 7 d. The syrupy liquid was poured slowly with stirring into ice water and was neutralized with saturated $NaHCO_3$ solution. The mixture was extracted with ethyl acetate (3×10 mL). The ethyl acetate layer was separated and water was removed under reduced pressure to give a brown solid. The resulting compound 6-chlorosulfonylquinazolin-4(3*H*)-one **11** was stored at 0°C without further purification. Yield 76%, mp >300°C.

General Procedure for the Synthesis of 6-[(*N*-Substituted) sulfonyl]quinazolin-4(3*H*)-one (**12a**–**g**): 6-Chlorosulfonylquinazolin-4(3*H*)-one **11** (0.25 g, 1 mmol) was added to a stirred solution of the appropriate amine (1 mmol) and triethylamine

(1 mL) at room temperature for 1 h. The reaction mixture was heated under reflux for the appropriate time. The mixture was allowed to reach room temperature, quenched in ice with efficient stirring. The aqueous phase was extracted with dichloromethane (3×20 mL). The dichloromethane layer was dried over Na_2SO_4, filtered and the solvent was removed by distillation to give yellow solid, which was washed with cold dichloromethane to give the desired product (**12a**–**g**).

N-Cyclohexyl-4-oxo-3,4-dihydroquinazoline-6-sulfonamide (**12a**): 6-Chlorosulfonylquinazolin-4(3*H*)-one **11** (0.25 g, 1 mmol) was added to a stirred solution of cyclohexylamine (0.1 mL, 1 mmol) and triethylamine (1 mL). The reaction mixture was heated under reflux for 15 h. Yield 75% as a white crystals, mp 95–97°C. ^{1}H-NMR (DMSO-d_6) δ: 9.78 (1H, s, exch with D_2O), 7.95–7.92 (1H, d, J=9.3 Hz), 7.70–7.14 (3H, m), 4.60 (1H, s, exch with D_2O), 2.6–1 (11H, m). IR (KBr) cm^{-1}: 3390, 3295, 2931, 1623, 1552, 1327. MS *m*/*z*: 307 (M^+), 99, 70, 56, 55.

6-(Piperidin-1-ylsulfonyl)quinazolin-4(3*H*)-one (**12b**): 6-Chlorosulfonylquinazolin-4(3*H*)-one **11** (0.25 g, 1 mmol) was added to a stirred solution of piperidine (0.09 mL, 1 mmol) and triethylamine (1 mL). The reaction mixture was heated under reflux for 16 h. Yield 86% as a white crystals, mp 257–259°C. IR (KBr) cm^{-1}: 3389, 2952, 1671, 1592, 1324. MS *m*/*z*: 293 (M^+), 248, 221, 148, 136.

6-(Piperazin-1-ylsulfonyl)quinazolin-4(3*H*)-one (**12c**): 6-Chlorosulfonylquinazolin-4(3*H*)-one **11** (0.25 g, 1 mmol) was added to a stirred solution of piperazine in toluene (0.09g, 1 mmol) and triethylamine (1 mL). The reaction mixture was heated under reflux for 8 h. Yield 60% as a yellow crystals, mp 115–117°C. IR (KBr) cm^{-1}: 3418, 3208, 2956, 1624, 1556, 1363. MS *m*/*z*: 294 (M^+), 224, 206, 149, 97.

6-(4-Methylpiperazin-1-ylsulfonyl)quinazolin-4(3*H*)-one (**12d**): 6-Chlorosulfonylquinazolin-4(3*H*)-one **11** (0.25 g, 1 mmol) was added to a stirred solution of methylpiperazine (0.1 mL, 1 mmol) and triethylamine (1 mL). The reaction mixture was heated under reflux for 16 h. Yield 56% as a white crystals, mp 160–162°C. IR (KBr) cm^{-1}: 3408, 2923, 1657, 1373. MS *m*/*z*: 308 (M^+), 293, 273, 245, 219, 191.

6-(4-Ethylpiperazin-1-ylsulfonyl)quinazolin-4(3*H*)-one (**12e**): 6-Chlorosulfonylquinazolin-4(3*H*)-one **11** (0.25 g, 1 mmol) was added to a stirred solution of ethyl piperazine (0.11 mL, 1 mmol) and triethylamine (1 mL). The reaction mixture was heated under reflux for 16 h. Yield 55% as a white crystals, mp 130–132°C. IR (KBr) cm^{-1}: 3428, 2855, 1647, 1375. MS *m*/*z*: 323 (M^+), 308, 298, 244, 188.

6-(4-Phenylpiperazin-1-ylsulfonyl)quinazolin-4(3*H*)-one (**12f**): 6-Chlorosulfonylquinazolin-4(3*H*)-one **11** (0.25 g, 1 mmol) was added to a stirred solution of phenyl piperazine (0.2 mL, 1 mmol) and triethylamine (1 mL). The reaction mixture was heated under reflux for 11 h. Yield 60% as a yellow crystals, mp 200–202°C. ^{1}H-NMR (DMSO-d_6) δ: 9.36 (1H, s, exch with D_2O), 7.20–6.79 (9H, m), 3.38–3.16 (8H, m). IR (KBr) cm^{-1}: 3439, 2919, 1663, 1590, 1326. MS *m*/*z*: 370 (M^+), 344, 314, 284, 242, 76.

6-(4-Benzylpiperazin-1-ylsulfonyl)quinazolin-4(3*H*)-one (**12g**): 6-Chlorosulfonylquinazolin-4(3*H*)-one **11** (0.25 g, 1 mmol) was added to a stirred solution of benzyl piperazine (0.18 mL, 1 mmol) and triethylamine (1 mL). The reaction mixture was heated under reflux for 15 h. Yield 80% as a white crystals, mp 173–175°C. ^{1}H-NMR (DMSO-d_6) δ: 9.7 (1H, s, exch with D_2O), 7.95–7.92 (1H, d, J=9.3 Hz), 7.9–7.7 (8H, m), 4.20 (2H, s), 3.06–2.37 (8H, m). IR (KBr) cm^{-1}: 3439, 2929, 1590, 1490, 1355. MS *m*/*z*: 384 (M^+), 358, 318, 279, 217.

General Procedure for the Preparation of 4-Chloro-6-(*N*-substituted sulfonyl)quinazoline (**13a**–**g**): $POCl_3$ (25 mL) was added to a mixture of 6-[(*N*-substituted)sulfonyl]-quinazolin-4(3*H*)-one **12a**–**g** (2.5 mmol) in DMF (0.12 mL). The mixture was stirred under reflux (100–105°C) for the appropriate time. Excess $POCl_3$ was removed by *vacuo*. The residue was poured into a mixture of chloroform (50 mL), ice water (80 mL) and triethylamine (5 mL) and the mixture was neutralized with saturated $NaHCO_3$ solution. The chloroform layer was separated and the aqueous layer was extracted with chloroform (1×20 mL). The chloroform layer was dried over Na_2SO_4, filtered and the solvent was removed by distillation to give the desired compounds **13a**–**g** which were stored at 0°C and used without any further purification for the next step.

4-Chloro-*N*-cyclohexylquinazoline-6-sulfonamide (**13a**): $POCl_3$ (25 mL) was added to a mixture of *N*-cyclohexyl-4-oxo-3,4-dihydroquinazoline-6-sulfonamide **12a** (0.77 g, 2.5 mmol) in DMF (0.12 mL). The mixture was stirred under reflux for 5 h. Yield 80% as a white solid, mp 100–102°C.

4-Chloro-6-(piperidin-1-ylsulfonyl)quinazoline (**13b**): $POCl_3$ (25 mL) was added to a mixture of 6-(piperidin-1-ylsulfonyl)-quinazolin-4(3*H*)-one **12b** (0.73 g, 2.5 mmol) in DMF (0.12 mL). The mixture was stirred under reflux for 7 h. Yield 80% as a white solid, mp 120–122°C.

4-Chloro-6-(piperazin-1-ylsulfonyl)quinazoline (**13c**): $POCl_3$ (25 mL) was added to a mixture of 6-(piperazin-1-ylsulfonyl)-quinazolin-4(3*H*)-one **12c** (0.73 g, 2.5 mmol) in DMF (0.12 mL). The mixture was stirred under reflux for 6 h. Yield 55% as a yellow solid, mp 150–152°C.

4-Chloro-6-(4-methylpiperazin-1-ylsulfonyl)quinazoline (**13d**): $POCl_3$ (25 mL) was added to a mixture of 6-(4-methylpiperazin-1-ylsulfonyl)quinazolin-4(3*H*)-one **12d** (0.77g, 2.5 mmol) in DMF (0.12 mL). The mixture was stirred under reflux for 7 h. Yield 50% as a yellow solid, mp 130–132°C.

4-Chloro-6-(4-ethylpiperazin-1-ylsulfonyl)quinazoline (**13e**): $POCl_3$ (25 mL) was added to a mixture of 6-(4-ethylpiperazin-1-ylsulfonyl)quinazolin-4(3*H*)-one **12e** (0.8 g, 2.5 mmol) in DMF (0.12 mL). The mixture was stirred under reflux for 6 h. Yield 50% as a yellow solid, mp 110–112°C.

4-Chloro-6-(4-phenylpiperazin-1-ylsulfonyl)quinazoline (**13f**): $POCl_3$ (25 mL) was added to a mixture of 6-(4-phenylpiperazin-1-ylsulfonyl)quinazolin-4(3*H*)-one **12f** (0.9 g, 2.5 mmol) in DMF (0.12 mL). The mixture was stirred under reflux for 6 h. Yield 60% as a white solid, mp 120–122°C.

6-(4-Benzylpiperazin-1-ylsulfonyl)-4-chloro-quinazoline (**13g**): $POCl_3$ (25 mL) was added to a mixture of 6-(4-benzylpiperazin-1-ylsulfonyl)quinazolin-4(3*H*)-one **12g** (0.96 g, 2.5 mmol) in DMF (0.12 mL). The mixture was stirred under reflux for 7 h. Yield 70% as a yellow solid, mp 140–142°C.

General Procedure for the Preparation of 4-(4-Bromoanilino)-6-(*N*-substituted sulfonyl)]quinazoline (**14b**–**d**, **g**): 4-Chloro-6-(*N*-substitutedsulfonyl)quinazoline **13b**–**d**, **g** (0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of 4-bromoaniline (0.34 g,

1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0°C. The mixture was allowed to reach room temperature then heated to reflux for appropriate time. The solvent was removed under reduced pressure and the residue was washed with dichloromethane (2×10 mL) and purified with column chromatography using ethyl acetate–petroleum ether (3 : 1) to give the listed compounds **14b**–**d**, **g**.

4-(4-Bromoanilino)-6-(piperidin-1-ylsulfonyl)quinazoline (**14b**): 4-Chloro-6-(piperidin-1-ylsulfonyl)quinazoline **13b** (0.29 g, 0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of 4-bromoaniline (0.34 g, 1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0°C. The mixture was allowed to reach room temperature then heated to reflux for 24 h. Yield 60% as a white needle crystals, mp 140–142°C. ^{1}H-NMR (DMSO-d_6) δ: 9.10 (1H, s, exch with D_2O), 7.28–6.62 (8H, m), 3.26–1.54 (10H, m). IR (KBr) cm^{-1}: 3472, 2983, 1620, 1356. MS *m/z*: 447 (M^+), 414, 374, 286, 228, 86. *Anal.* Calcd for $C_{19}H_{19}BrN_4O_2S$: C, 51.01; H, 4.28; N, 12.52. Found: C, 50.62; H, 3.97; N, 12.29.

4-(4-Bromoanilino)-6-(piperazin-1-ylsulfonyl)quinazoline (**14c**): 4-Chloro-6-(piperazin-1-ylsulfonyl)quinazoline **13c** (0.29 g, 0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of 4-bromoaniline (0.34 g, 1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0°C. The mixture was allowed to reach room temperature then heated to reflux for 13 h. Yield 50% as a yellow crystals, mp 180–182°C. ^{1}H-NMR (DMSO-d_6) δ: 9.36 (1H, s, exch with D_2O), 7.95–7.92 (1H, d, J=9.3 Hz), 7.70–6.96 (7H, m), 4.13–1.31 (8H, m), 2.20 (1H, s, exch with D_2O). IR (KBr) cm^{-1}: 3467, 2923, 1624, 1463, 1178. MS *m/z*: 447 (M^+), 414, 379, 336, 289, 57. *Anal.* Calcd for $C_{18}H_{18}BrN_5O_2S$: C, 48.22; H, 4.05; N, 15.62. Found: C, 48.34; H, 4.30; N, 15.98.

4-(4-Bromoanilino)-6-(4-methylpiperazin-1-ylsulfonyl)-quinazoline (**14d**): 4-Chloro-6-(4-methylpiperazin-1-ylsulfonyl)quinazoline **13d** (0.3 g, 0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of 4-bromoaniline (0.34 g, 1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0°C. The mixture was allowed to reach room temperature then heated to reflux for 19 h. Yield 49% as a white crystals, mp 180–182°C. ^{1}H-NMR (DMSO-d_6) δ: 9.18 (1H, s, exch with D_2O), 7.95–7.92 (1H, d, J=9.3 Hz), 7.8–7.4 (1H, dd, J=9.3, 2.4 Hz), 7.4–6.6 (6H, m), 3.3–2.03 (11H, m). IR (KBr) cm^{-1}: 3395, 2923, 1623, 1461, 1370. MS *m/z*: 460 (M^+), 432, 390, 295, 86. *Anal.* Calcd for $C_{19}H_{20}BrN_5O_2S$: C, 49.36; H, 4.36; N, 15.15. Found: C, 49.68; H, 4.72; N, 15.15.

6-(4-Benzylpiperazin-1-ylsulfonyl)-4-(4-bromoanilino)-quinazoline (**14g**): 6-(4-Benzylpiperazin-1-ylsulfonyl)-4-chloro-quinazoline **13g** (0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of 4-bromoaniline (0.34 g, 1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0°C. The mixture was allowed to reach room temperature then heated to reflux for 20 h. Yield 65% as a white needle crystals, mp 150–152°C. ^{1}H-NMR (DMSO-d_6) δ: 9.3 (1H, s, exch with D_2O), 7.95–7.92 (1H, d, J=9.3 Hz), 7.89–7.13 (12H, m), 4.15 (2H, s), 3.5–3.2 (8H, m). IR: (KBr) cm^{-1}: 3396, 2923, 1623, 1370. MS *m/z*: 537 (M^+), 513, 495, 458, 365, 58. *Anal.* Calcd for $C_{25}H_{24}BrN_5O_2S$: C, 55.76; H, 4.49; N, 13.01. Found: C, 55.49; H, 4.12; N, 12.68.

General Procedure for the Preparation of 4-Anilino-6-(*N*-substituted sulfonyl)quinazoline (**15a**–**g**): 4-Chloro-6-(*N*-substituted sulfonyl)quinazoline **13a**–**g** (0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of aniline (0.18 mL, 1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0 °C. The mixture was allowed to reach room temperature then heated under reflux for the appropriate time. The solvent was removed under reduced pressure and the residue was washed with dichloromethane (2×10 mL) and purified with column chromatography using the appropriate eluent to give the desired compounds **15a**–**g**.

N-Cyclohexyl-4-(phenylamino)quinazoline-6-sulfonamide (**15a**): 4-Chloro-*N*-cyclohexylquinazoline-6-sulfonamide **13a** (0.3 g, 0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of aniline (0.18 mL, 1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0°C. The mixture was allowed to reach room temperature then heated under reflux for 5 h. The residue was purified with column chromatography using ethyl acetate–petrolum ether (3 : 1) as eluent. Yield 49% as a white crystals, mp 110–112°C. ^{1}H-NMR (DMSO-d_6) δ: 9.36 (1H, s, exch with D_2O), 7.95–7.92 (1H, d, J=9.3 Hz), 7.80–6.80 (8H, m), 4.50 (1H, s, exch with D_2O), 2.99–1.11 (11H, m). IR (KBr) cm^{-1}: 3428, 2922, 1642, 1548, 1375. MS *m/z*: 382 (M^+), 354, 312, 270, 231, 71. *Anal.* Calcd for $C_{20}H_{22}N_4O_2S$: C, 62.80; H, 5.80; N, 14.65. Found: C, 63.10; H, 6.05; N, 14.87.

4-Anilino-6-(piperidin-1-ylsulfonyl)quinazoline (**15b**): 4-Chloro-6-(piperidin-1-ylsulfonyl)quinazoline **13b** (0.29 g, 0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of aniline (0.18 mL, 1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0°C. The mixture was allowed to reach room temperature then heated under reflux for 7 h. The residue was purified with column chromatography using ethyl acetate–petrolum ether (3 : 1) as eluent. Yield 55% as a white needle crystals, mp 140–142°C. ^{1}H-NMR (DMSO-d_6) δ: 9.36 (1H, s, exch with D_2O), 7.28–6.82 (9H, m), 3.26–1.54 (10H, m). IR (KBr) cm^{-1}: 3471, 2984, 1620, 1356. MS *m/z*: 368 (M^+), 315, 280, 235, 138, 121, 86. *Anal.* Calcd for $C_{19}H_{20}N_4O_2S$: C, 61.94; H, 5.47; N, 15.21. Found: C, 61.58; H, 5.21; N, 15.06.

4-Anilino-6-(piperazin-1-ylsulfonyl)quinazoline (**15c**): 4-Chloro-6-(piperazin-1-ylsulfonyl)quinazoline **13c** (0.29 g, 0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of aniline (0.18 mL, 1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0°C. The mixture was allowed to reach room temperature then heated under reflux for 4 h. The residue was purified with column chromatography using ethyl acetate–petrolum ether (3 : 1) as eluent. Yield 50% as a yellow crystals, mp 110–112°C. ^{1}H-NMR (DMSO-d_6) δ: 9.18 (1H, s, exch with D_2O), 7.95–7.92 (1H, d, J=9.3 Hz), 8.51–6.81 (8H, m), 4.70–1.31 (8H, m), 2.0 (1H, s, exch with D_2O). IR (KBr) cm^{-1}: 3425, 2922, 1597, 1493, 1375. MS *m/z*: 369 (M^+), 343, 309, 281, 266, 57. *Anal.* Calcd for $C_{18}H_{19}N_5O_2S$: C, 58.52; H, 5.18; N, 18.96. Found: C, 58.25; H, 4.91; N, 18.80.

4-Anilino-6-(4-methylpiperazin-1-ylsulfonyl)quinazoline (**15d**): 4-Chloro-6-(4-methylpiperazin-1-ylsulfonyl)quinazoline **13d** (0.3 g, 0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of aniline (0.18 mL, 1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0°C. The mixture was allowed to reach room temperature then heated under reflux for 3 h. The residue was pu-

rified with column chromatography using ethyl acetate–hexane (1 : 1) as eluent. Yield 57% as a white crystals, mp 140–142°C. ^{1}H-NMR (DMSO-d_6) δ: 9.9 (1H, s, exch with D_2O), 7.95–7.92 (1H, d, J=9.3 Hz), 7.80–7.42 (1H, dd, J=9.3, 2.4 Hz), 7.40–6.60 (7H, m), 3.32–2.03 (11H, m). IR (KBr) cm^{-1}: 3175, 2972, 1599, 1494, 1379. MS m/z: 382 (M^+), 348, 318, 249, 164, 79. *Anal.* Calcd for $C_{19}H_{21}N_5O_2S$: C, 59.51; H, 5.52; N, 18.26. Found: C, 59.32; H, 5.19; N, 17.86.

4-Anilino-6-(4-ethylpiperazin-1-ylsulfonyl)quinazoline (**15e**): 4-Chloro-6-(4-ethylpiperazin-1-ylsulfonyl)quinazoline **13e** (0.3 g, 0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of aniline (0.18 mL, 1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0°C. The mixture was allowed to reach room temperature then heated under reflux for 3 h. The residue was purified with column chromatography using ethyl acetate–hexane (1 : 1) as eluent. Yield 53% as a white crystals, mp 135–137°C. ^{1}H-NMR (DMSO-d_6) δ: 9.18 (1H, s, exch with D_2O), 7.36–7.24 (9H, m), 3.5–2.4 (13H, m). IR (KBr) cm^{-1}: 3175, 2921, 1598, 1494, 1376. MS m/z: 397 (M^+), 374, 316, 287, 217, 56. *Anal.* Calcd for $C_{20}H_{23}N_5O_2S$: C, 60.43; H, 5.83; N, 17.62. Found: C, 60.67; H, 6.17; N, 17.84.

4-Anilino-6-(4-phenylpiperazin-1-ylsulfonyl)quinazoline (**15f**): 4-Chloro-6-(4-phenylpiperazin-1-ylsulfonyl)quinazoline **13f** (0.37 g, 0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of aniline (0.18 mL, 1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0°C. The mixture was allowed to reach room temperature then heated under reflux for 4 h. The residue was purified with column chromatography using ethyl acetate–petrolum ether (3 : 1) as eluent. Yield 54% as a yellow crystals, mp 110–112°C. ^{1}H-NMR (DMSO-d_6) δ: 9.78 (1H, s, exch with D_2O), 7.95–7.92 (1H, d, J=9.3 Hz), 7.7–7.2 (14H, m), 3.4–2.1 (8H, m). IR (KBr) cm^{-1}: 3742, 2913, 1598, 1494, 1411. MS m/z: 445 (M^+), 417, 435, 266, 168, 93. *Anal.* Calcd for $C_{24}H_{23}N_5O_2S$: C, 64.70; H, 5.20; N, 15.72. Found: C, 65.00; H, 5.55; N, 15.87.

4-Anilino-6-(4-benzylpiperazin-1-ylsulfonyl)quinazoline (**15g**): 6-(4-Benzylpiperazin-1-ylsulfonyl)-4-chloro-quinazoline **13g** (0.38 g, 0.95 mmol) in dichloromethane (10 mL) was added gradually with stirring to a solution of aniline (0.18 mL, 1.95 mmol) and triethylamine (1 mL) in dichloromethane (10 mL) at 0°C. The mixture was allowed to reach room temperature then heated under reflux for 8 h. The residue was purified with column chromatography using ethyl acetate–petrolum ether (3 : 1) as eluent. Yield 60% as a white needle crystals, mp 170–172°C. ^{1}H-NMR (DMSO-d_6) δ: 9.186 (1H, s, exch with D_2O), 7.95–7.92 (1H, d, J=9.3 Hz), 7.91–7.10 (13H, m), 4.15 (2H, s), 3.30–2.37 (8H, m). IR (KBr) cm^{-1}: 3396, 2913, 1625, 1365. MS m/z: 459 (M^+), 308, 290, 279, 203, 57. *Anal.* Calcd for $C_{25}H_{25}N_5O_2S$: C, 65.34; H, 5.48; N, 15.24. Found: C, 65.00; H, 5.09; N, 14.91.

Biological Evaluations Sensitivity Test: Cells were plated in 96-multiwellplate (104 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. A single concentration of the compounds under test was added to the cell monolayer. Monolayer cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with Sulforhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris ethylenediaminetetraacetic acid (EDTA) buffer. Color intensity was measured in an enzyme-linked immunosorbent assay (ELISA). Percentage of the surviving and inhibition were tabulated.[17]

Cytotoxicity Test: Cell Culture: MCF-7 human breast cancer cells was grown in RPMI-1640 medium, supplemented with 10% heat inactivated fetal bovine serum (FBS), 50 units/mL of penicillin and 50 g/mL of streptomycin and maintained at 37°C in a humidified atmosphere containing 5% CO_2. The cells were maintained as "monolayer culture" by serial subculturing.

Sulforhodamine B Colorimetric Assay (SRB) Cytotoxicity Assay: Cytotoxicity was determined using SRB method as previously described by Skehan *et al.*[17] Exponentially growing cells were collected using 0.25% trypsin–EDTA and seeded in 96-well plates at 1000–2000 cells/well in RPMI-1640 supplemented medium. After 24 h, cells were incubated for 72 h with various concentrations of the tested compounds. Following 72 h treatment, the cells will be fixed with 10% trichloroacetic acid for 1 h at 4°C. Wells were stained for 10 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The plates were air dried for 24 h and the dye was solubilized with Tris–HCl for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was measured spectrophotometrically at 564 nm with an ELISA microplate reader (ChroMate-4300, FL, U.S.A.). The IC_{50} values were calculated according to the equation for Boltzman sigmoidal concentration–response curve using the nonlinear regression fitting models (Graph Pad, Prism Version 5).

Results and Discussion

Chemistry The synthetic pathway of the title compounds is outlined in Charts 1 and 2. The starting material anthranilic acid (**1**) was conveniently cyclized to quinazolin-4(3*H*)-one (**3**) by heating with formamide (**2**) at 120°C.[14] Upon refluxing compound (**3**) with phosphorous oxychloride gave compound 4-chloroquinazoline (**4**).[13] 4-Chloroquinazoline was allowed to react with *p*-pheneylene diamine to produce 4-(4-aminoanilino)quinazoline (**5**).[15] Compounds 1-phenyl-3-(4-(quinazolin-4-ylamino) phenyl)-urea (**6**), 1-phenyl-3-(4-(quinazolin-4-ylamino) phenyl)thiourea (**7**), 2-(4-(quinazolin-4-ylamino)phenylimino)acetic acid (**8**), 3-(4-(quinazolin-4-ylamino)phenylamino)propanoic acid (**9**) and 4-oxo-4-(4-(quinazolin-4-ylamino)phenylamino)butanoic acid (**10**) were synthesized from intermediate compound 4-(4-aminoanilino)quinazoline using different reagents as reported in Chart 1.

Moreover, compound 6-chlorosulfonylquinazolin-4(3*H*)-one (**11**) was synthesized from compound (**3**) by reacting with chlorosulfonic acid under dry conditions. Subsequently compound (**11**) reacted with piperidine, cyclohexyl amine and substituted piperazines to produce 6-(*N*-substituted)-sulfonylquinazolin-4(3*H*)-one (**12a**–**g**). This series then reacted with phosphorous oxychloride to produce 4-chloro derivatives (**13a**–**g**).

Finally, compounds (**13a**–**g**) underwent a nucleophilic substitution reaction in the presence of substituted anilines to give the title compounds (**14b**–**d**, **g**) and (**15a**–**g**) in Chart 2.

The structure of the synthesized final products was confirmed by elemental analyses, IR, ^{1}H-NMR and mass spectral data. Chart 1, IR spectra, generally, showed a peak of secondary amine at 3300–3500 cm^{-1} due to the coupling of

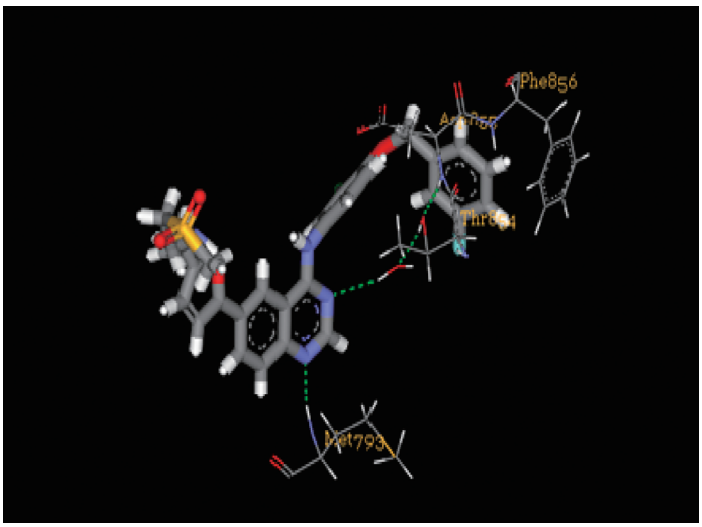


Fig. 4. Docking of Lapatinib in the ATP Binding Site of EGFR-TK

Lapatinib docking pose showed a maximum fitting to the receptor cavity where the quinazoline ring be inserted nicely inside the pocket. The anilino portion is oriented deep in the hydrophobic region I. The modeling also suggested that there were also two hydrogen bonds observed. One between N-1 of quinazoline ring and Met-793 and the other between N-3 of quinazoline ring and a water molecule that bridged the gap between N-3 and Asp-855.

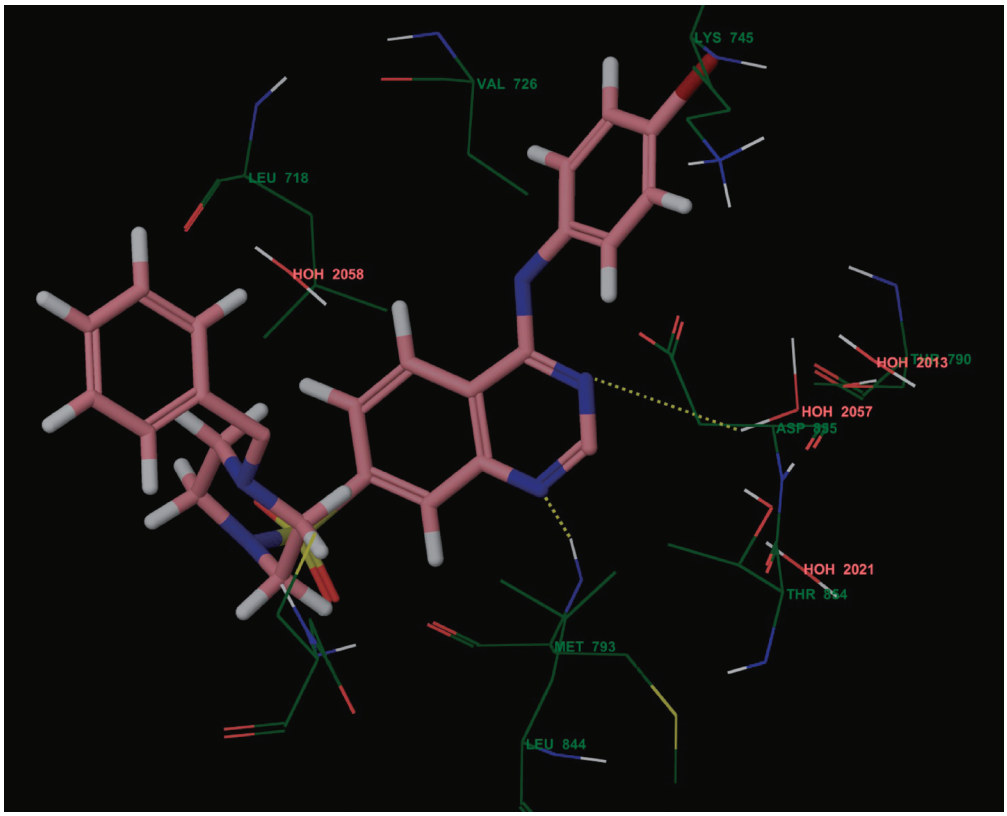


Fig. 5. Docking of Compound (**14g**) in the ATP Binding Site of EGFR-TK (PDB Code: 2J5F)

Compound (**14g**) showed a docking pose similar to the docking pose of the lapatinib in the receptor. Two hydrogen bonds were observed, one between N-1 of quinazoline and Met-793 while, the other between N-3 of quinazoline and a water molecule of crystallization.

4-chloroquinazoline (**4**) with *p*-phenylene diamine. Mass spectra showed the major fragments according to their expected molecular formula. ^{1}H-NMR spectra of compounds (**6**) and (**7**) displayed the presence of two downfield exchangeable singlets with D_2O corresponding to two NH of urea and thiourea moiety respectively. ^{1}H-NMR spectrum of compound (**8**) showed the presence of a downfield exchangeable singlet with D_2O corresponding to OH of the carboxylic group (COOH). One singlet shielded proton was also observed upfield corresponding to N=CH of the imine group. ^{1}H-NMR Spectra of compounds (**9**) and (**10**) showed the presence of three downfield exchangeable singlets with D_2O corresponding to OH of the

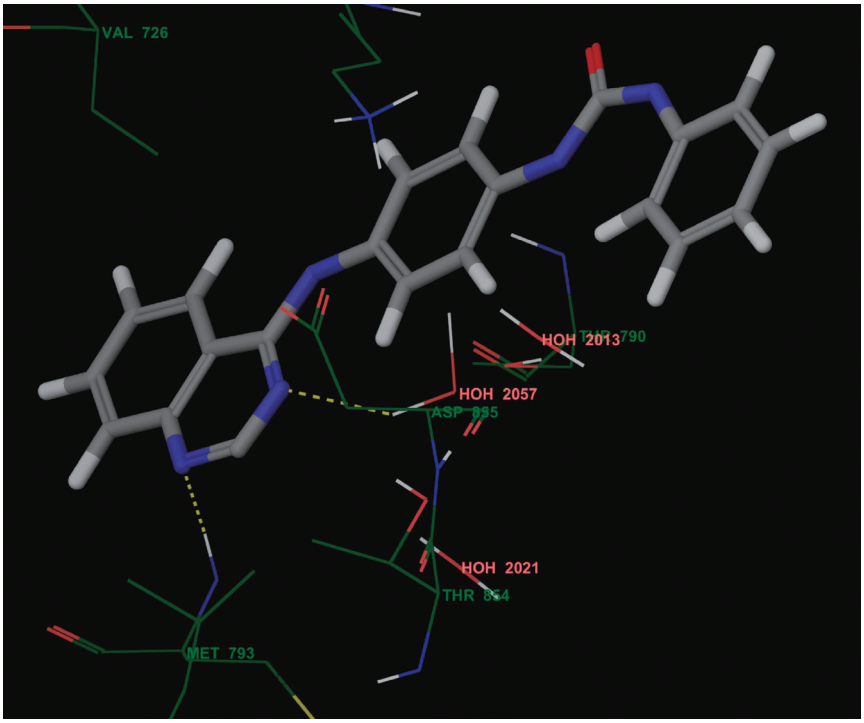


Fig. 6. Docking of Compound (**6**) in the ATP Binding Site of EGFR-TK (PDB Code: 2J5F)

Compound (**6**) showed a docking pose similar to the docking pose of the lapatinib in the receptor. Two hydrogen bonds were observed, one between N-1 of quinazoline and Met-793 while, the other between N-3 of quinazoline and a water molecule of crystallization.

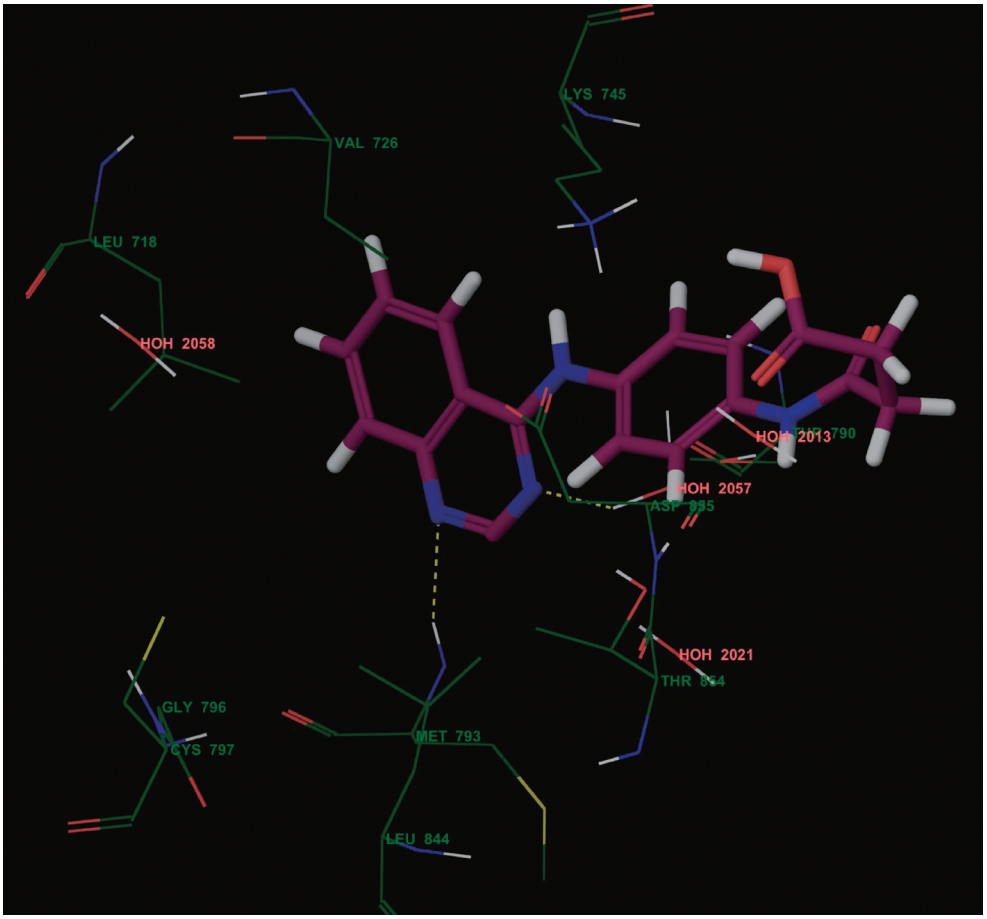


Fig. 7. Docking of Compound (**10**) in the ATP Binding Site of EGFR-TK (PDB Code: 2J5F)

Compound (**10**) showed a docking pose similar to the docking pose of the lapatinib in the receptor. Two hydrogen bonds were observed, one between N-1 of quinazoline and Met-793 while, the other between N-3 of quinazoline and a water molecule of crystallization.

carboxylic group (COOH) and NH of $NH–CH_2–CH_2$ group in compound (**9**) and NH of the amide group in compound (**10**). A set of multiplets was also observed upfield corresponding to aliphatic side chain. Chart 2, IR spectra, generally, showed a peak of sulfonamide moiety at 1363 cm^{-1} and a peak of secondary amine at 3300–3500 cm^{-1} due to the coupling of 4-chloro-derivatives (**13a**–**g**) with primary aromatic amines. Mass spectra showed the major fragment according to their expected molecular formula. ^{1}H-NMR Spectra were characterized by two important regions: a highly shielded region consisting of aliphatic multiplet signals corresponding to cyclohexane or piperdine ring or substituted piperazine ring at δ 1–4 ppm and deshielded region consisting of aromatic and/or heteroaromatic multiplets corresponding to quinazoline ring, 4-anilino moiety and benzene ring at δ 6–9 ppm.

Molecular Docking Molecular docking was performed on the binding model based on the EGFR-lapatinib complex structure (2J5F.pdb). There is a direct proportionality between the free energy of binding of the inhibitors (anticancer agents) and their activity. The smaller the free energy of binding, the more tight is the binding of the inhibitors to their binding domain with the inhibition of cancer cell growth.[12] Lapatinib was docked inside the receptor as illustrated in Fig. 4. Lapatinib docking pose showed a maximum and plausible fitting to the receptor cavity where the quinazoline ring was inserted nicely inside the pocket. The anilino portion was oriented deep inside the hydrophobic region I. The model also suggested that there were also two hydrogen bonds observed between N-1 of quinazoline ring and Met-793 and the other between N-3 of quinazoline ring and a water molecule that bridged the gap between N-3 and Asp-855. The binding models of the most active inhibitor in each series and EGFR were shown in Figs. 5–7. Compound (**14g**) showed low free energy of binding −6 kJ/mol and a high inhibition in the cancer cell growth (IC_{50}=6.735 μM). The root mean square deviation (RMSD) for the compound was 1.6 Å. Compound (**14g**) showed a docking pose similar to the docking pose of the lapatinib in the receptor. Two hydrogen bonds were observed, one between N-1 of quinazoline and Met-793 while, the other between N-3 of quinazoline and a water molecule of crystallization. Compound (**6**) showed a moderate free energy of binding −4.75 kJ/mol and a moderate inhibition in the cancer cell growth (IC_{50}=21.71 μM). The RMSD value was 1.9 Å. Compound (**6**) also showed a docking pose similar to the docking pose of the lapatinib in the receptor. Two hydrogen bonds were also observed, one between N-1 of quinazoline and Met-793 while, the other between N-3 of quinazoline and a water molecule of crystallization. Compound (**10**) also showed a moderate free energy of binding −3 kJ/mol and a weak to moderate inhibition in the cancer cell growth (IC_{50}=34.91 μM). The RMSD value was 1.9 Å. Compound (**10**) also showed a docking pose similar to the docking pose of the lapatinib in the receptor. Two hydrogen bonds were observed between N-1 of quinazoline and Met-793 while, the other between N-3 of quinazoline and a water molecule of crystallization.

Biological Activity The anticancer activity was evaluated *via* a two-stage process, beginning with measurement of the sensitivity of series A, B, C and D *via* selection of one compound representing each series from our newly synthesized compounds against a panel of eight human cancer cell lines

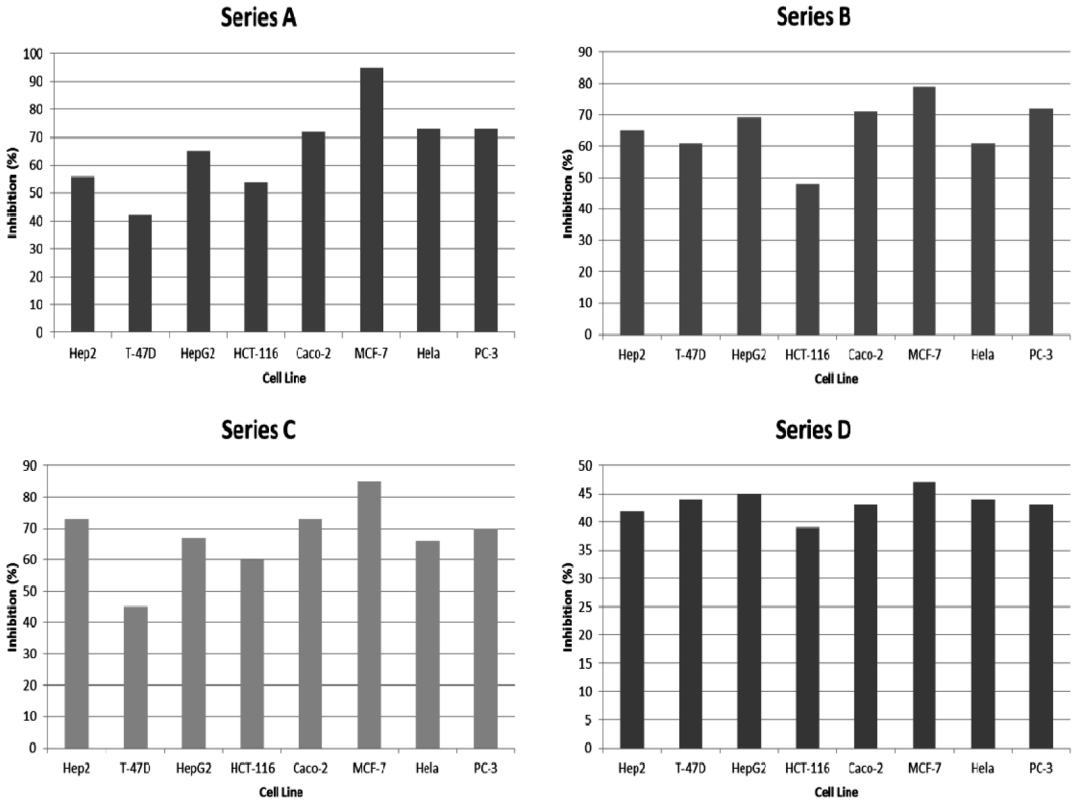


Fig. 8. Sensitivity Test of Series A, B, C and D against 8 Human Carcinoma Cell Lines

Most of the newly synthesized derivatives displayed a significant inhibitory activity against breast carcinoma cell line (MCF-7).

Table 1. *In Vitro* Cytotoxic Activity of the Synthesized Compounds against MCF-7

Compound	Series	IC_{50} (μM)
6	A	21.71
7	A	24.25
8	B	64.09
9	B	45.87
10	B	34.91
14b	C	9.48
14c	C	6.735
14d	C	18.26
14g	C	5.52
15a	D	82.48
15b	D	>100
15c	D	>100
15d	D	>100
15e	D	>100
15f	D	>100
15g	D	>100
Lapatinib	Reference	3

Series C was the most active series followed by series A, then series B. Series D was the least cytotoxic as it showed high IC_{50}.

representing different tumor types, namely liver carcinoma cell line (Hep G2), cervical carcinoma cell line (Hela), colon carcinoma cell line (HCT-116), breast carcinoma cell line (MCF-7), larynx carcinoma cell line (Hep2), intestinal carcinoma cell line (Caco-2), breast carcinoma cell line (T-47D) and prostate carcinoma cell line (PC-3) at a single dose of 100 μg/mL, followed by the evaluation of potential cytotoxicity of the new compounds at five different concentrations (0.1, 1, 10, 100, 1000 μg/mL) against the most promising cell lines, corresponding to the maximum percentage of inhibition achieved at the single dose experiment. The initial screening effect *i.e.*, sensitivity test indicated that, most of the newly synthesized derivatives displayed a significant inhibitory activity against breast carcinoma cell line (MCF-7) (Fig. 8), so the cytotoxic activity of target compounds against (MCF-7) cells was determined using Sulforhodamine-B assay using labatinib as a reference. Compounds were tested over a range of concentrations from 0.1 to 1000 μg/mL, and the calculated IC_{50} values, that is, the concentration (μg/mL) of a compound that was able to cause 50% cell death with respect to the control, were reported. The results were summarized in Table 1. As shown in Table 1. Among the compounds tested for their cytotoxicity against the breast carcinoma cell line (MCF-7), compound (**14g**) showed the best inhibitory activity and its inhibitory rate was 85% at 100 μg/mL and 64% at 10 μg/mL. Compounds (**14c**) and (**14b**) also displayed a remarkable activity at micromole order of magnitude, with IC_{50} values of 6.735 and 9.48, respectively, followed by compounds (**14d**), (**6**) and (**7**). The remaining compounds were the least cytotoxic as they showed high IC_{50}. The cytotoxic activity of the tested compounds could be correlated to structural modification. Chart 1, the quinazoline core was modified at the position-4 of the aniline ring with different bulky substituents such as urea, thiourea, amide or glycine, with the aim of strengthening the ligand receptor interaction and therefore increasing affinity. In series A, there was no obvious difference between urea and thiourea moiety both showed good activities. On the other hand, in series B, It was noticed that elongation and branching of the investigated substituents increased their ability to inhibit EGFR. Elongation allowed deep penetration in the hydrophobic region I while branching suggested several possibilities for hydrogen bonds. Therefore, compound (**10**) showed a higher activity than its congeners (**8**) and (**9**). Chart 2, the quinazoline core was modified at position-6 of the quinazoline ring with *N*-substituted sulfonyl derivatives, with the aim of improving physical properties (solublization) and conferring a more favorable pharmacokinetic profile.[18] It was noticed that series C showed a higher cytotoxic activity than series D. This was because that the phenyl ring of the aniline was tilted out of the plane from the quinazoline allowing the bromine atom to fit more precisely into the hydrophobic region I. We may conclude that compound containing halogen at aniline ring showed a better cytotoxic activity than those without halogen. In series C, it was also noticed that the presence of one or more rotamer allowed a free rotation of this side chain so as to be close to the amino acid residue to increase the chance of the interaction. Therefore, compound (**14g**) showed the highest cytotoxic activity in series C.

Conclusion

The present work led to the development of four series of novel anticancer molecules containing 4-anilinoquinazoline pharmacophore. Eight cell lines were used to measure cytotoxic sensitivity of the proposed quinazoline derivatives. Most of tested compounds showed good activity against breast cancer (MCF-7) with IC_{50} range of 5.52–34.91 μg/mL. The best cytotoxic result was obtained with series C especially; compound (**14g**) that showed the most potent inhibitory activity. Molecular docking studies supported the strong inhibitory activity of compound (**14g**) and help to design novel potent inhibitors.

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