Novel Diphenylamine 2,4′-Dicarboxamide Based Azoles as Potential Epidermal Growth Factor Receptor Inhibitors: Synthesis and Biological Activity

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Several hybrid molecules of diphenylamine-2,4′-dicarboxamide with various azolidinones and related heterocyclic rings have been synthesized and explored as epidermal growth factor receptor (EGFR) kinase inhibitors. Most of them displayed promising *in vitro* tyrosine kinase inhibition as well as potent cellular antiproliferative activity in the EGFR over-expressing breast cancer cell line (MCF-7). Compounds 12b and 13b that exhibited the highest inhibition in the kinase assay (89, 81% inhibition at 10 μM, respectively), showed potent antiproliferative effect against MCF-7 tumor cell line (IC_{50} 1.04, 0.91 μM respectively). Molecular docking studies revealed that these compounds can bind to ATP binding site of the EGFR kinase domain and were involved in H-bonding with Met 793, in analogy to the known EGFR tyrosine kinase inhibitors. Moreover, compounds 15a—c possessed profound antitumor activity (IC_{50} 0.59—0.73 μM) and significant EGFR-TK inhibition, making them of particular interest. In summary, the newly synthesized compounds provide promising new lead for the future design and development of anticancer agents of potential EGFR-TK inhibitory activity.

Key words antiproliferative agent; azolidinone; diphenylamine; tyrosine kinase inhibitor

Breast cancer is the second leading cause of cancer deaths in women today and is the most common cancer among women, excluding nonmelanoma skin cancers.[1)] Despite the progress achieved in anticancer therapy about 1.3 million women will be diagnosed with breast cancer annually worldwide and about 465000 will die from the disease.[1)] Accordingly, novel therapeutic strategies to improve prognosis are urgently needed.

It is increasingly apparent that a number of protein tyrosine kinases, specifically epidermal growth factor receptor (EGFR) tyrosine kinase and/or its family members play a critical role in the development and progression of many solid tumors, including breast cancer.[2,3)] Over-expression of EGFR has been observed in about 70% of breast cancer[4)] and is associated with later stages of carcinogenesis[5,6)] and is implicated in the development of drug resistance with poor prognosis.[7—9)] Therefore inhibitors of EGFR kinase activity have emerged as promising new approach to cancer therapy.

During the last decade azolidinones heterocycles, particularly imidazolidinones, thiazolidinones and pyrrolidinone have gained special attention as potential lead compounds for novel anticancer agents.[10)] The antineoplastic properties of azolidinones and related heterocycles are most probably caused by their affinity to different anticancer biotargets, such as extracellular signal-regulated kinases (ERK kinases),[11,12)] JNK-stimulating phosphatase-1 (JSP-1) **1**,[13)] tumor necrosis factor-alpha (TNF-α),[14,15)] antiapoptic complex Bcl-X_L-BH3 **2**,[16)] integrin $\alpha_v\beta_3$ receptor **3**,[17)] *etc.* (Fig. 1). Recent reports showed that imidazolidinones, and thiazolidinones were found to inhibit the kinase activity of EGFR. Where, some 1-phenethyl-5-(*E*)-benzylidine hydantoins **4** inhibited EGFR autophosphorylation and polyglutamic acid/tyrosine (polyGAT) phosphorylation.[18,19)] Moreover, a series of hydrazono thiazolidin-4-one **5** that own high antineoplastic activity against breast cancer cells MCF-7 and were potent inhibitors of EGFR autophosphorylation has been reported.[20)] Furthermore, dianilinophthalimide **6** that can bind competitively with the ATP to EGFR showed good selectivity between different tyrosine kinases.[21)] Therefore, azolidinones are considered good scaffolds for future design of tyrosine kinase inhibitors. In addition, benzamides and bezamidines were synthesized as mimics of the classical EGFR inhibitors 4-anilino-quinazolines.[22)] Also, we have recently reported preliminary results on antiproliferative action of 2,4′-bis-heterocyclic diphenylamine **7** against MCF-7 cell line, that might be mediated through the inhibition EGFR kinase activity.[23)]

Promoted by these observations and in continuation to the previous work,[23)] we designed and synthesized hybrid molecules of diphenylamine-2,4′-dicarboxamide (as dibenzamide template) and different azolidinones or related heterocyclic rings **11**, **13**—**17** aiming to obtain highly active antitumor agents, with probable EGFR tyrosine kinase inhibitory activity. The new hybrids were inspired by compound **7** through replacing the diphenylamine scaffold with diphenylamine-2,4′-dicarboxamide and replacement of the thiadiazole ring with various azolidinones. Such substitution pattern could target different regions of the ATP binding site of the receptor. Molecular modeling showed that the chosen azolidinone ring systems at the 4′-position of diphenylamine-2,4′-dicarboxamide core could mimic the interaction of the adenine portion of ATP with the hinge region of the EGFR ATP binding site. Moreover, the 2-subtituent was expected to orient toward the solvent front so that, the produced interaction might gain an additional potency.

Chemistry The general methods for the synthesis of target compounds **11**—**17** are depicted in Charts 1—4. The key intermediate compounds **9** and **10** were synthesized through the reaction of diacid hyrazide **8** with the appropriate isocyanates or isothiocynates in absolute ethanol, respectively, Chart 1. Cyclization of bis-semicarbazides **9** with oxalyl chloride in benzene produced a mixture of bis-2,4,5-trioxo-

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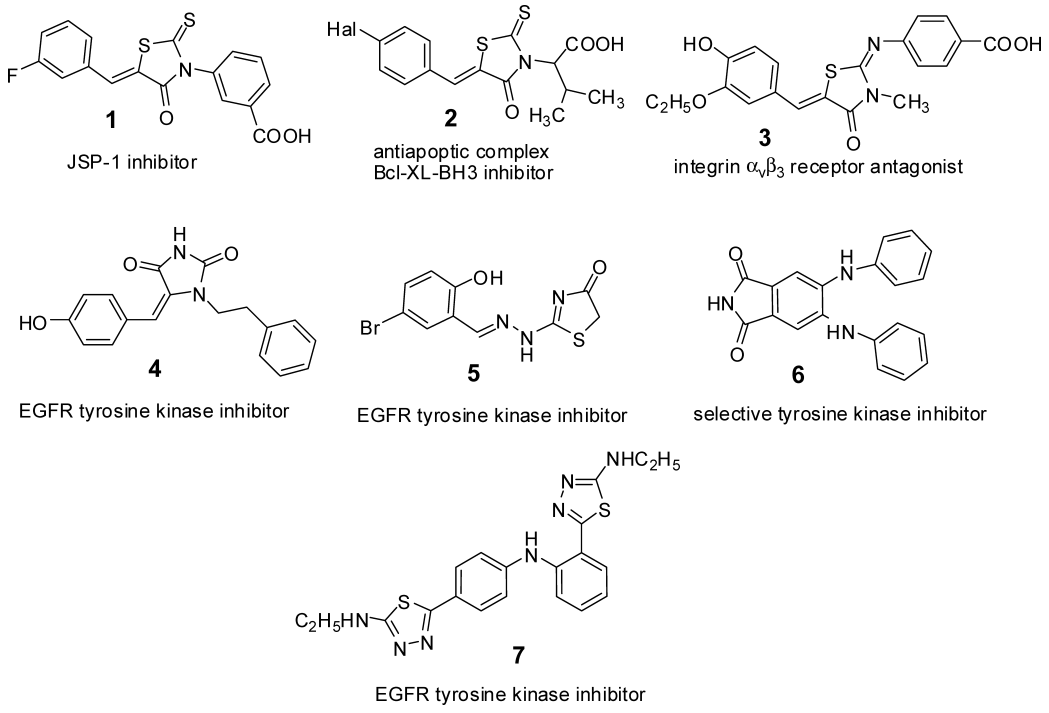
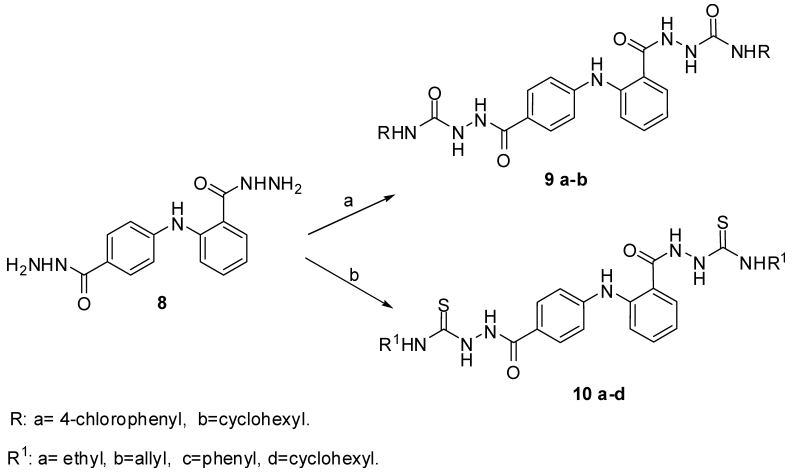


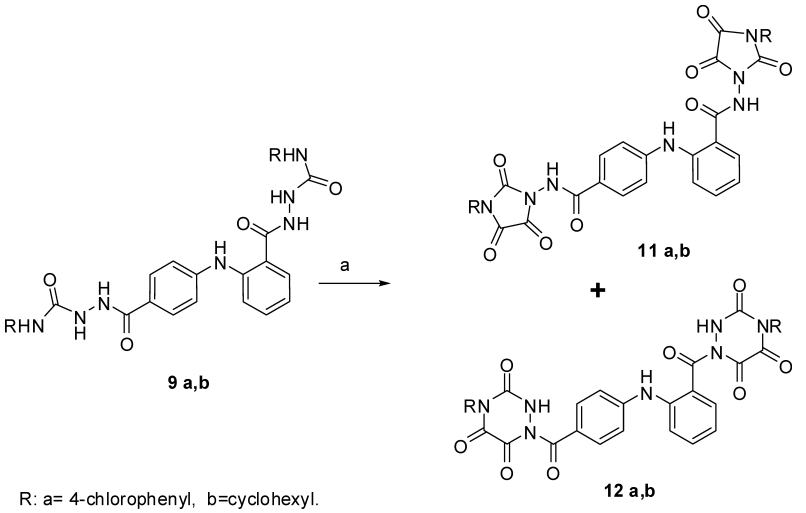
Fig. 1. Some Examples of Azolidinones with Anticancer Activity and EGFR Tyrosine Kinase Inhibition



Reagents and conditions: (a) isocyanates, absolute ethanol, reflux, (b) isothiocyantes, absolute ethanol, reflux.

Chart 1

imidazolidines **11** and bis-3,5,6-trioxotriazinanes **12**, which were separated by fractional solubility from ethanol, Chart 2. According to literature,[24] the obtained products could be differentiated based on their fragmentation in the mass spectra. The detailed mass spectrometry analysis of compounds **11** and **12** provided diagnostic fragment ions that enabled discrimination among alternative structures. The electron impact (EI) mass spectrum of compound **11b** had a measured elemental composition of $C_{32}H_{33}N_7O_8$ at *m/z* 643 designated as molecular ion peak $[M]^{\cdot +}$. It fragmented to yield product ions at *m/z* 253 corresponding to $[C_{14}H_{11}N_3O_2]^+$ and *m/z* 195 $[C_9H_{11}N_2O_3]^+$, suggesting the presence of 2,4,5-trioxoimidazolidine ring system (Fig. 2). In addition, ^{1}H-NMR spectra of compounds **11a**, **b** displayed the presence of the more deshielded amide signals resonating at 12.25—11.82 ppm. Meanwhile, compound **12b**, the molecular ion was not detected. Its EI mass spectrum showed ion peak at *m/z* 644 designated as $[M+1]^+$ and different fragmentation pattern assigned to different degradation products. It fragmented to yield product ions at *m/z* 223 corresponding to $[C_{14}H_9NO_2]^+$, *m/z* 211 and 210 corresponding to $[C_9H_{12}N_3O_3]^+$, suggesting the presence of 3,5,6-trioxotriazinane ring system (Fig. 3). Moreover, in the ^{1}H-NMR spectra of **12a**, **b** singlet signals derived from the cyclic NH were detected at 11.79—11.81 ppm. On the other hand, treatment of bis-thiosemicarbazides **10** with oxalyl chloride under the same condition gave the substituted bis-4,5-dioxo-2-thioxoimidazolidines **13** as sole product. The EI mass spectrum of **13c** revealed ion peak at *m/z* 664 assigned as $[M+1]^+$ and important fragments at *m/z* 254 and 253 corresponding to $[C_{14}H_{11}N_3O_2]^+$ and *m/z* 206 and 205 equivalent to $[C_9H_5N_2O_2S]^+$ indicating the formation of 4,5-dioxo-2-thioxoimidazolidine ring sys-



Reagents and conditions: (a) oxalyl chloride, benzene, refluxes.

Chart 2

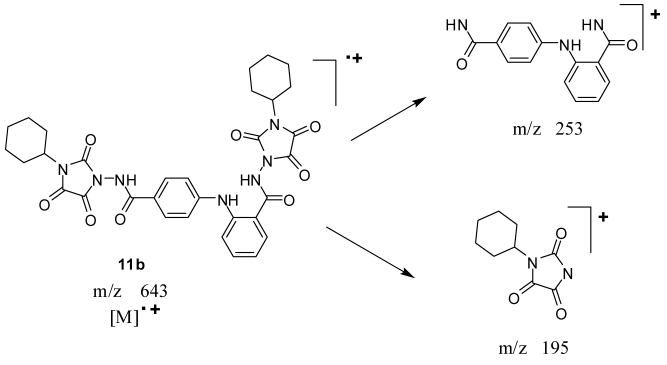


Fig. 2. The Proposed Fragmentation of Compound **11b** to Its Main Ions in EI Mass Spectrum

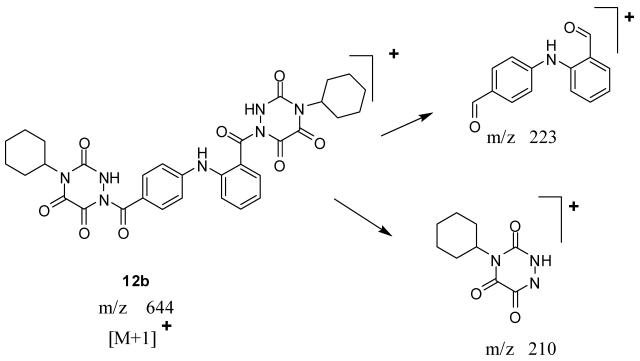


Fig. 3. The Proposed Fragmentation of Compound **12b** to Its Main Ions in EI Mass Spectrum

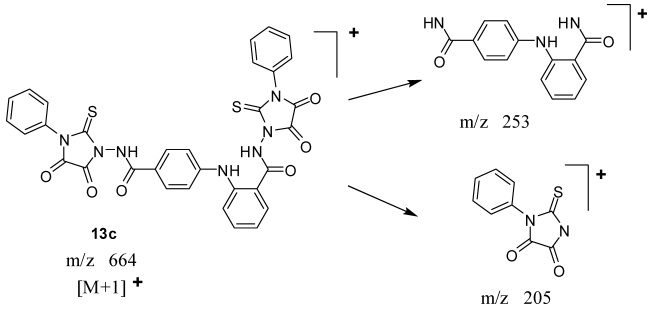


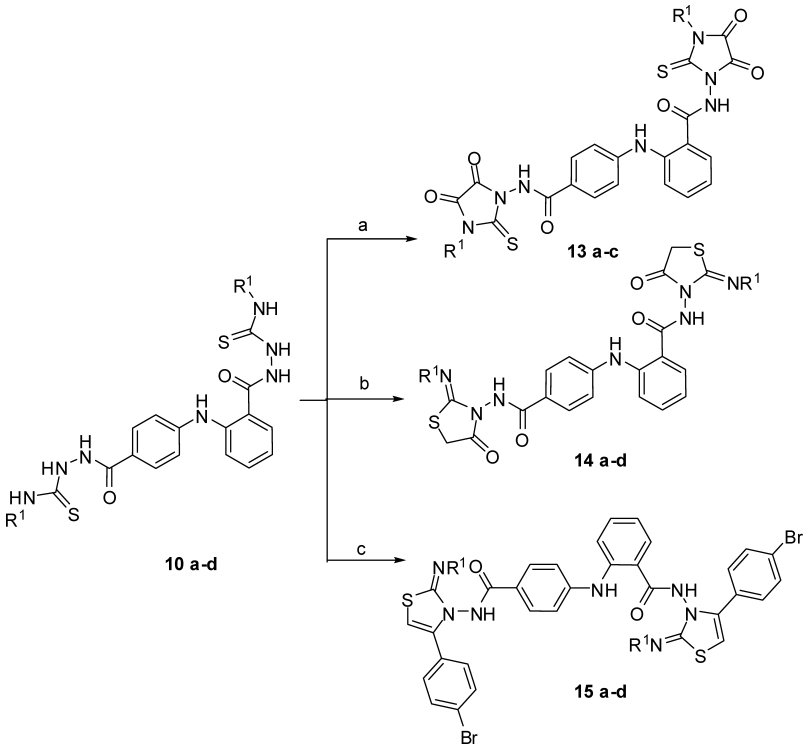
Fig. 4. The Proposed Fragmentation of Compound **13c** to Its Main Ions in EI Mass Spectrum

tem (Fig. 4). Also, in the ^{1}H-NMR spectra of compounds **13a**—**c**, downfield signals belonging to the amide proton were observed between 12.72—12.20 ppm. Alternatively, cyclocondensation of bis-thiosemicarbazides **10** with chloroacetic acid in presence of anhydrous sodium acetate in refluxing glacial acetic acid afforded the bis-2-imino-4-oxothiazolidine derivatives **14**, while their reaction with 4-bromophenacyl bromide yielded the bis-2-imino thiazolines **15**, Chart 3. Finally, condensation of diacid hydrazide **8** with succinic anhydride or phthalic anhydride in refluxing glacial acetic acid furnished the corresponding imides' analogues **16** and **17**, respectively, Chart 4. Both the analytical and spectral data (IR, ^{1}H-NMR, ^{13}C-NMR and MS) of all the newly synthesized compounds were in full agreement with the proposed structures.

Results and Discussion

***In Vitro* EGFR Kinase Inhibitory Activity** The kinase inhibitory activity of the newly synthesized compounds was evaluated using EGFR kinase activity assay by enzyme-linked immunosorbent assay (ELISA).[25,26] The assay was based on the inhibition of phosphorylation of the tyrosine kinase-specific peptide poly glutamic acid/tyrosine [poly(Glu/Tyr), 4 : 1] by EGFR. Results are illustrated in Table 1. The inhibitory activities are given as percentage inhibition at concentration of 10 μm of the inhibitor.[22,26] Most of the tested compounds exhibited significant EGFR-TK inhibitory activity with percentage inhibition ranging from 89 to 33%, where compounds **12b** and **13b** were the most active (89, 81% inhibition, respectively).

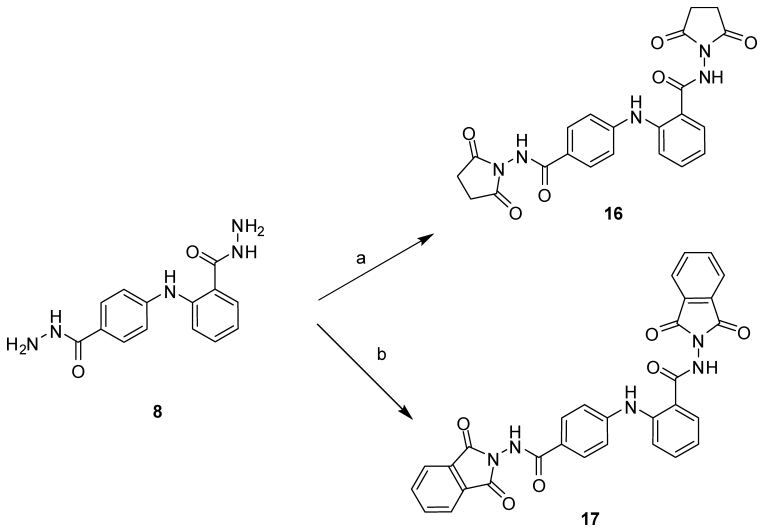
The Enzyme Assay Results Elicited That: 1) The 3,5,6-trioxotriazinanes **12a**, **b** were more potent enzyme inhibitors than the corresponding 2,4,5-trioxoimidazolidines **11a**, **b** and the compounds bearing cyclohexyl substituent **11b** and **12b** showed higher inhibitory effect than those having *p*-



R^1: a= ethyl, b=allyl, c=phenyl, d=cyclohexyl.

Reagents and conditions: (a) oxalyl chloride, benzene, reflux, (b) chloroacetic acid, anhydrous sodium acetate, glacial acetic acid, reflux, (c) 4-bromophenacyl bromide, ethanol/chloroform, reflux.

Chart 3



Reagents and conditions: (a) succinic anhydride, glacial acetic acid, reflux (b) phthalic anhydride, glacial acetic acid, reflux.

Chart 4

chlorophenyl substituent **11a** and **12a**. 2) Among the azolidinone containing compounds, the kinase inhibitory effect of the imidazolidinone derivatives **11a**, **b** and **13a**—**c** was higher than that of the 2-imino-4-oxothiazolidines **14a**—**d** and the pyrrolidinone derivatives **16** and **17**. 3) Replacement of the 2-imino-4-oxothiazolidine moiety in **14a**—**d** by 2-imino-4-bromophenyl thiazoline in **15a**—**d** produced compounds with enhanced kinase inhibition activity, except for the cyclohexyl analogue **15d**.

Regarding the effect of substituent on different series, the results showed that: 1) The cyclohexyl substituted compounds **11b**, **12b** and **14d** were more potent EGFR-TK inhibitors than the corresponding phenyl containing compounds **11a**, **12a** and **14c**. 2) For the series of 2-imino-4-bromophenyl thiazolines **15a**—**d**, it was found that the kinase inhibitory activity decreased as the size of the substituent on the imino increased from ethyl **15a** to cyclohexyl **15d**. This proved poor bulk tolerance for this class of compounds and

Table 1. EGFR Tyrosine Kinase Inhibitory Activity of the Synthesized Compounds, and Their *in Vitro* Cytotoxicity against Human Breast Cancer Cell (MCF-7)

Compound	EFGR-TK Inhibition [%] at 10 μM	*In vitro* cytotoxicity IC_{50} (μM)
7[23]	97.7±2.3***	0.94±0.05
11a	45.0±5.0*	0.96±0.08**
11b	70.0±4.2**	0.84±0.06**
12a	50.0±4.5*	1.24±0.11*
12b	89.0±8.0***	1.04±0.13*
13a	70.0±6.4**	0.71±0.05***
13b	81.0±7.1***	0.91±0.11**
13c	49.0±3.5*	0.71±0.05***
14a	52.0±5.3*	1.11±0.12*
14b	NA	ND
14c	33.0±3.1	4.44±0.30
14d	63.0±5.2**	0.62±0.05***
15a	70.0±6**	0.73±0.06***
15b	52.0±4.5*	0.64±0.05***
15c	50.0±4.2*	0.59±0.04***
15d	NA	ND
16	42.0±3.9*	0.89±0.07**
17	NA	ND
PD153035	99.6±2.0***	ND

NA=not active (<10%), ND, not determined. Significant at: $*p \leq 0.05$ $**p \leq 0.01$ $***p \leq 0.001$.

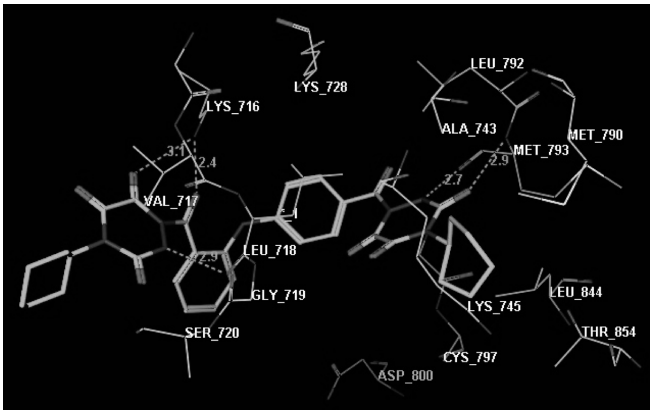


Fig. 5. Molecular Docking of Compound **12b** in EGFR Kinase Domain in 3D Diagram

could justify the inactivity of the cyclohexyl congener **15d** compared to the other cyclohexyl bearing compounds.

Molecular Modeling To rationalize the obtained biological results and to describe the binding mode of the active compounds with their predicted intracellular target, docking analysis was carried out using Molecular Operating Environment MOE version 2008.10.[27] Molecular docking of the most potent inhibitors **12b** and **13b** into the ATP binding site of the EGFR kinase domain was performed on the X-ray crystal structure of EGFR complex (3IKA.pdb).[28] The binding model of compound **12b** showed that the 4′-[(4-cyclohexyl-3,5,6-trioxo-1,2,4-triazinane-1-yl)carbonyl] moiety bound to the narrow hydrophobic pocket in the N-terminal of EGFR, which is the binding site of adenine base of ATP (Fig. 5). A bidentate H-bonding interaction was observed between the 3,5,6-trioxo-1,2,4-triazinane ring and the main chain NH and C=O of Met 793 (2.9, 2.7 Å, respectively), hydrogen bonding with this amino acid helps to fix the adenine base in

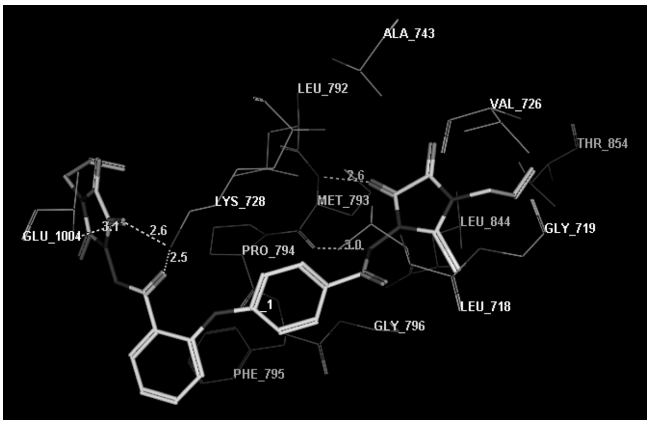


Fig. 6. Compound **13b** Docked in EGFR Kinase Domain in 3D Diagram

the binding pocket and is considered crucial for the activity of the known EGFR inhibitors.[29,30] Meanwhile the cyclohexyl substituent made a predominant hydrophobic interaction with the adjacent lipophilic pocket lined with Ala 743, Met 790, Leu 844 and Thr 854. The additional binding energy provided by the interaction of the cyclohexyl with this pocket may be responsible for the high binding affinity of this molecule. Also the 2-[(4-cyclohexyl-3,5,6-trioxo-1,2,4-triazinane-1-yl)carbonyl]phenyl moiety was direct to the entrance of the active site, with phenyl extended to the solvent exposed region. Where, the triazinane ring nitrogen N-2 and the 6-oxo group together with carbonyl bridge were involved in H-bonding with Leu 718 and Lys 716 (2.9, 3.1, 2.4 Å).

Docking of compound **13b**—as an example of the azolidinone derivatives—is depicted in Fig. 6. The model revealed a similar orientation to **12b**. The 4′-[(4,5-dioxo-2-thioxoimidazolidin-1-yl)carbamoyl] moiety bound in the adenine binding pocket, where the 5-oxo and the carbamoyl NH were engaged in H-bonds with the backbone amide and carbonyl atoms of Met 793 (2.6, 3.0 Å, respectively). The allyl group in **13b** was inserted in the adjacent lipophilic pocket defined by Val 726, Met 790, Leu 844 and Thr 854, in analogy to the cyclohexyl substituent in **12b**. Moreover, the 2-[(3-allyl-4,5-dioxo-2-thioxoimidazolidin-1-yl)carbamoyl]phenyl moiety was oriented toward the entrance of binding site, so that the oxygen atoms of the carbamoyl and the 5-oxo functionalities were involved in three H-bonds with the side chain NH of Lys 728 and the backbone NH of Glu 1004 (Fig. 6). The molecular docking results, along with the enzyme assay data suggesting that compounds **12b** and **13b** are potential inhibitors of EGFR.

Also, in order to clarify the reason for the lack of activity of cyclohexyl substituted compound **15d**. Comparison of the docked poses of **14d** and **15d** was performed (Figs. 7a, b). In **14d** the 2-[2-cyclohexylimino-4-oxothiazolidine] ring occupied the adenine binding pocket with the 4-oxo group H-bonded to Met 793 (2.6 Å) and 2-cyclohexylimino projecting away from the pocket (Fig. 7a). On the other hand, **15d** revealed flipping of the 2-cyclohexylimino-4-bromophenyl thiazoline moiety, locating the 2-cyclohexylimino in the narrow adenine binding pocket. Therefore, compound **15d** was too sterically hindered to effectively occupy this pocket and to make the necessary interaction with Met 793 (Fig. 7b).

On the other hand, no conclusive reason could account for

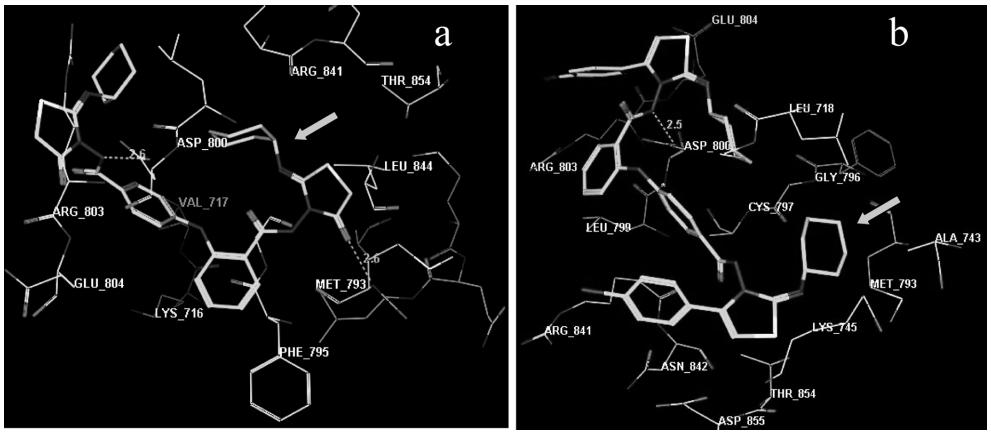


Fig. 7. The Docking Poses of Compounds **14d** (a) and **15d** (b) in EGFR Kinase Domain, Showing the Different Positions of the 2-Cyclohexylimino Group as Indicated by Green Arrows

the difference in activity between compound **13b** and **14b**. It could be referred to difference in substitution pattern and relative position of the ally substituent that was reflected as bad docking scores of **14b** (−7.26) compared to **13b** (−15.07). This indicated the formation of unstable receptor-ligand complex and low binding affinity of **14b**.

***In Vitro* Cytotoxicity** The compounds that showed EGFR inhibitory activity were evaluated for their antiproliferative activity on human breast cancer cell line (MCF-7) by Skehan's method.[31] MCF-7 is known to over-express EGFR and provides a good measure to determine the effectiveness of EGFR-TK inhibition. As shown in Table 1, all of the tested compounds exhibited potent activity against MCF-7 cell line, indicating that these compounds are potential inhibitors of EFGR-TK. The majority of compounds (**11a**, **b**, **13a**—**c**, **14d**, **15a**—**c**, **16**) showed significant antiproliferative effect at sub-micromolar level with IC_{50} ranging from 0.59 to 0.97 μM. On the other hand, compounds **12a**, **b** and **14a** possessed remarkable antitumor activity at micromolar level with IC_{50} ranging from 1.04 to 1.24 μM, while compound **14c** demonstrated the least activity with IC_{50} of 4.44 μM. Analysis of the data in Table 1 illustrated that the nature of the ring system on the diphenylamine-2,4′-dicarboxamide core affected the antitumor activity. In general, 4,5-dioxo-2-thioxoimidazolidines **13a**—**c** exhibited better cytotoxic activity than the 2,4,5-trioxoimidazolidine analogues **11a**, **b**. With respect to the thiazole containing compounds **14a**—**d** and **15a**—**c**, it can be observed that the 2-imino-4-bromophenyl thiazolines **15a**—**c** had superior anticancer effect compared to the corresponding 2-imino-4-oxothiazolidine derivatives **14a**—**d** [c.f. **14c**, **15c**]. Moreover, the potency of the 2-imino-4-bromophenyl thiazoline derivatives **15a**—**c** was found to increase parallel to the increase in lipophilicity of the 2-imino substituent. Finally, the pyrrolidinone derivative **16** revealed antiproliferative activity comparable to that of the 2,4,5-trioxoimidazolidine derivatives **11a**, **b**.

Conclusion

Several hybrid molecules of diphenylamine-2,4′-dicarboxamide with various azolidinones and related heterocyclic rings have been synthesized and investigated for their ability to inhibit EGFR tyrosine kinase activity. Most of them displayed promising EGFR-TK inhibitory activity at various levels. Compounds **12b** and **13b** exploited the most potent kinase inhibitory activity (89, 81% at 10 μM, respectively). Molecular docking models of these compounds indicated that they bound to the ATP binding site of EGFR forming a bidentate H-bond interaction with Met 793. Therefore, the molecular docking results, along with the enzyme assay data suggested that compounds **12b** and **13b** are potential inhibitors of EGFR. In addition, the cytotoxicity assay results indicated that all evaluated compounds own high antiproliferative activity against EGFR over-expressing breast cancer cells (MCF-7) at micromolar and submicromolar levels. In particular, the 2-imino-4-bromophenyl thiazoline derivatives **15a**—**c** which possessed moderate EGFR-TK inhibition showed unexpected high potencies as antitumor agents (IC_{50} 0.59—0.73 μM), making them of special interest. In summary, even if further structure–activity investigation is needed for potency optimization of this class of compounds with respect to the enzyme test, they seem potentially attractive as antiproliferative agents.

Experimental

Chemistry Melting points were determined on Galle Kamp and Kofler melting point apparatus and are uncorrected. Elemental analyses (C, H, N) were performed by Micro Analytical Center, Faculty of Science, Cairo University. Infrared spectra were recorded on Shimadzu IR 435 Spectrophotometer or on a Genesis II FT-IR TM, Mattson, 5225, Verona Road, Madison wi. 53711 U.S.A., using KBr discs. ^{1}H-NMR spectra were scanned on Varian XL-300 MHz, Varian XL-200 MHz or Joel FX 90Q-90 MHz (chemical shifts are given in part per million ppm downfield from TMS), ^{13}C-NMR spectrum was scand on Varian XL-300 MHz . Mass spectra were made on a Finnigan MAT, SSQ 7000 spectrophotometer at 70 eV. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel plates 60-F-254 (Merck; 0.25 mm), developing with chloroform. Compounds **8** and **10a**—**c** were prepared according to the reported procedure.[23]

General Procedure for Preparation of 2,4′-Bis-(4-substituted semicarbazidocarbonyl)diphenylamine (9a, b) A mixture of the diacid hydrazide **8** (1.0 g, 3.5 mmol) and the appropriate isocyanate (10.5 mmol) in absolute ethanol (20 ml) was refluxed for 8 h the reaction mixture was concentrated and left to cool. The separated solid was filtered and dried. The product was crystallized from ethanol.

2,4′-Bis-[4-(4-chlorophenyl)semicarbazidocarbonyl)]diphenylamine (**9a**): Yield, 95%; mp 264—266 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 6.97—7.82 (16H, m, Ar-H), 9.61 (4H, s, 2×*p*-chlorophenyl–NHC=ONH, D_2O exch.), 9.88 (3H, s, NH and 2×CONH, D_2O exch.). IR (KBr) cm^{-1}: 3290.3, 3219.3 and 3183.7 (NH)s, 305.61 (CH aromatic), 1663.9 and 1623.1 (C=O)s. *Anal.* Calcd for $C_{28}H_{23}Cl_2N_7O_4.H_2O$: C, 55.09; H, 4.13; N, 16.06. Found: C, 54.71; H, 4.51; N,15.70.

2,4′-Bis-(4-cyclohexylsemicarbazidocarbonyl)diphenylamine (**9b**): Yield,

85%; mp 241—242 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 1.01—1.24 (10H, m, cyclohexyl), 1.67—1.79 (10H, m, cyclohexyl), 3.35 (2H, br s, 2×CH-1 cyclohexyl), 6.22 (2H, s, 2×cycolhexyl-NHC=O, D_2O exch.), 6.97—7.84 (8H, m, Ar-H), 9.48 (2H, s, 2×NHC=O, D_2O exch.), 9.93 (1H, s, NH, D_2O exch.), 10.12 (2H, s, 2×CONH, D_2O exch.). IR (KBr) cm^{-1}: 3348.7 and 3247.7 (NH)s, 3064.3 (CH aromatic), 2927.7 and 2853.5 (CH cyclohexyl), 1683 and 1634 (C=O)s. *Anal.* Calcd for $C_{28}H_{37}N_7O_6S_2$: C, 62.78; H, 6.96; N, 18.30. Found: C, 62.70; H, 7.00; N, 18.00.

2,4′-Bis-(4-cyclohexylthiosemicarbazidocarbonyl)diphenylamine (10d) A mixture of the diacid hydrazide **8** (1.0 g, 3.5 mmol) and cyclohexyl isothiocyanate (1.48 g, 1.5 ml, 10.5 mmol) in absolute ethanol (20 ml) was refluxed for 8 h. The reaction mixture was concentrated and left to cool. The separated solid was filtered and dried (yield 95%), the product was crystallized from ethanol, mp 184—186 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 1.01—1.24 (10H, m, cyclohexyl), 1.67—1.79 (10H, m, cyclohexyl), 4.12 (2H, br s, 2×CH-1 cyclohexyl), 6.97—7.86 (8H, m, Ar-H), 9.12 (2H, s, 2×cyclohexyl-NHC=S, D_2O exch.), 9.48 (1H, s, NH, D_2O exch.), 10.07 (2H, s, 2×NHC=S, D_2O exch.), 10.33 (2H, s, 2×CONH, D_2O exch.). IR (KBr) cm^{-1}: 3269.3, br (NH)s, 2928.4 and 2852.3 (CH cyclohexyl), 1649.9 (C=O)s, 1253.9 (C=S)s *Anal.* Calcd for $C_{28}H_{37}N_7O_2S_2$: C, 59.23; H, 6.57; N, 17.27. Found: C, 58.90; H, 6.30; N, 17.58.

General Procedure for Preparation of 2,4′-Bis-[*N*-(3-substituted-2,4,5-trioxoimidazolidin-1-yl)carbamoyl]diphenylamine (11a, b) and 2,4′-Bis-[(4-substituted-3,5,6-trioxo-1,2,4-triazinane-1-yl)carbonyl]-diphenylamine (12a, b) To a solution of **9a**, **b** (1.0 mmol), in dry benzene (20 ml), oxalyl chloride (0.17 ml, 2.0 mmol) was added drop wise while stirring. The reaction mixture was refluxed at 60—65 °C for 2 h, the solvent was distilled under vacuum. The obtained residue was treated with cold ethanol then filtered. The filtrate was evaporated under vacuum, and the obtained residue was crystallized from benzene–pet ether (9 : 1) to give **11a**, **b**. The residual product insoluble in cold ethanol was crystallized from ethanol to yield **12a**, **b**.

2,4′-Bis-{*N*-[3-(4-chlorophenyl)-2,4,5-trioxoimidazolidin-1-yl]carbamoyl}diphenylamine (**11a**): Yield 37%, mp 167—169 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 6.82—8.00 (16H, m, Ar-H), 11.19 (1H, s, NH, D_2O exch.), 12.25 (2H, s, 2×NHC=O, D_2O exch.). IR (KBr) cm^{-1}: 3506.7 and 3450.0 (NH)s, 1702.0 and 1605.0 (C=O)s. MS *m*/*z*: 700 (M^+). *Anal.* Calcd for $C_{32}H_{19}Cl_2N_7O_8$: C, 54.78; H, 2.73; N, 14.00. Found: C, 54.76; H, 2.73; N, 13.9.

2,4′-Bis-[*N*-(3-cyclohexyl-2,4,5-trioxoimidazolidin-1-yl)carbamoyl]-diphenylamine (**11b**): Yield 45%, mp 155—157 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 1.24—1.37 (10H, m, cyclohexyl), 1.62—1.86 (10H, m, cyclohexyl), 4.02 (2H, br s, 2×CH-1 cyclohexyl), 6.63—8.24 (8H, m, Ar-H), 11.55 (1H, s, NH, D_2O exch.), 11.82 (2H, s, 2×CONH, D_2O exch.). IR (KBr) cm^{-1}: 3507.5, br (NH)s, 2926.4 and 2845.6 (CH cyclohexyl), 1703.0 and 1606.7 (C=O)s. MS *m*/*z*: 643 (M^+). *Anal.* Calcd for $C_{32}H_{33}N_7O_8$: C, 59.71; H, 5.17; N, 15.23. Found: C, 59.80; H, 5.20; N, 15.22.

2,4′-Bis-{[4-(4-Chlorophenyl)-3,5,6-trioxo-1,2,4-triazinane-1-yl]carbonyl}diphenylamine (**12a**): Yield 32%, mp 237—239 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 7.10—7.95 (16H, m, Ar-H), 11.58 (2H, s, 2×NH of triazinanetrione, D_2O exch.), 11.79 (1H, s, NH, D_2O exch.). IR (KBr) cm^{-1}: 3493.0 and 3307.3 (NH)s, 1753.9, 1702.2 and 1673.7 (C=O)s. *Anal.* Calcd for $C_{32}H_{19}Cl_2N_7O_8$: C, 54.82; H, 2.73; N, 14.00. Found: C,55.03; H, 2.81; N, 14.11.

2,4′-Bis-[(4-cyclohexyl-3,5,6-trioxo-1,2,4-triazinane-1-yl)carbonyl]-diphenylamine (**12b**): Yield 40%, mp 224—226 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 1.24—1.37 (10H, m, cyclohexyl), 1.62—1.86 (10H, m, cyclohexyl), 4.03 (2H, br s, 2×CH-1 cyclohexyl), 6.62—8.24 (8H, m, Ar-H), 11.81 (3H, s, 2×NH of triazinanetrione and NH, D_2O exch.). IR (KBr) cm^{-1}: 3491.1 and 3395.5 (NH)s, 2932.6 and 2858.5 (CH cyclohexyl), 1753.5, 1702.2 and 1675.4 (C=O)s. MS *m*/*z*: 644 (M+1). *Anal.* Calcd for $C_{32}H_{33}N_7O_8$: C, 59.71; H, 5.17; N, 15.21. Found: C, 59.60; H, 5.00; N, 14.94.

General Procedure for Preparation of 2,4′-Bis[*N*-(3-substituted-4,5-dioxo-2-thioxoimidazolidin-1-yl)carbamoyl]diphenylamine (13a—c) To a solution of **10a**—**c** (1.0 mmol), in dry benzene (20 ml), oxalyl chloride (0.17 ml, 2.0 mmol) was added drop wise while stirring. The reaction mixture was refluxed at 60—65 °C for 2 h, the solvent was distilled under reduced pressure. The obtained residue was crystallized from benzene.

2,4′-Bis[*N*-(4,5-dioxo-3-ethyl-2-thioxoimidazolidin-1-yl)carbamoyl]-diphenylamine (**13a**): Yield 55%, mp 130—132 °C. ^{1}H-NMR (300 MHz) (DMSO-d_6) δ: 1.10—1.28 (6H, t, 2×CH_2CH_3), 3.77—3.99 (4H, q, 2×CH_2CH_3), 6.62—8.21 (8H, m, Ar-H), 11.35 (1H, s, NH), 12.72 (2H, s, 2×CONH). ^{13}C-NMR (300 MHz) (DMSO-d_6) δ: 12.29 (CH_3), 40.71 (CH_2), 113.32, 116.27, 124.52, 128.59, 129.45, 133.97, 137.01, 139.01, 140.82, 151.8, 153.00, 157.92 (C=O), 160.86 (C=O), 166.41 (C=O), 178.91 (C=S). IR (KBr) cm^{-1}: 3243.1, br (NH)s, 2980.6 and 2935.0 (CH), 1797.9, 1740.0 and 1702.8 (C=O)s, 1240.6 (C=S). *Anal.* Calcd for $C_{24}H_{21}N_7O_6S_2$: C, 50.79; H, 3.73; N, 17.27. Found: C, 50.53; H, 3.56; N,17.32.

2,4′-Bis[*N*-(3-allyl-4,5-dioxo-2-thioxoimidazolidin-1-yl)carbamoyl]-diphenylamine (**13b**): Yield 60%, mp 92—94 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 4.57 (4H, br s, 2×CH_2-CH=CH_2), 5.17—5.26 (4H, t, 2×CH_2-CH=CH_2), 5.83 (2H, m, 2×CH_2-CH=CH_2), 6.65—8.22 (8 H, m, Ar-H), 11.44 (1H, s, NH, D_2O exch.), 12.22 (2H, s, 2×CONH, D_2O exch.). IR (KBr) cm^{-1}: 3221.2, br (NH)s, 2926.4 (CH), 1798.2, 1740.9 and 1704.8 (C=O)s, 1271,4 (C=S). *Anal.* Calcd for $C_{26}H_{21}N_7O_6S_2$: C, 52.78; H, 3.58; N, 16.57. Found: C, 52.60; H, 3.40; N, 16.32.

2,4′-Bis[*N*-(4,5-dioxo-3-phenyl-2-thioxoimidazolidin-1-yl)carbamoyl]-diphenylamine (**13c**): Yield 62%, mp 165—167 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 7.42—8.29 (18H, m, Ar-H), 11.97 (1H, s, NH, D_2O exch.), 12.20 (2H, s, 2×CONH, D_2O exch.). IR (KBr) cm^{-1}: 3261.7, br (NH)s, 3064.1 (CH aromatic), 1796.5, 1738.2 and 1701.5 (C=O)s, 1340.4 (C=S). MS *m*/*z*: 664 (M+1). *Anal.* Calcd for $C_{26}H_{21}N_7O_6S_2$: C, 57.91; H, 3.19; N, 14.77. Found: C, 57.90; H, 3.59; N, 14.44.

General Procedure for Preparation of 2,4′-Bis-{*N*-[2-(substituted imino)-4-oxothiazolidin-3-yl]carbamoyl}diphenylamine (14a—d) To a suspension of the appropriate thiosemicarbazide **10a**—**d** (1.0 mmol) in glacial acetic acid (10 ml), anhydrous sodium acetate (0.33 g, 4.0 mmol) and monochloroacetic acid (0.38 g, 4.0 mmol) were added. The reaction was refluxed for 5 h. After cooling the mixture was diluted with ice water and allowed to stand overnight in the fridge. The product was filtered, washed with water and crystallized from the appropriate solvent.

2,4′-Bis-[*N*-[2-(ethylimino)-4-oxothiazolidin-3-yl]carbamoyl]diphenylamine (**14a**): Yield 83%, crystallized from ethanol, mp 228—230 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 1.16—1.22 (6H, t, 2×CH_2CH_3), 3.74—3.77 (4H, q, 2×CH_2CH_3), 4.04 (4H, s, 2×CH_2 of oxothiazolidine), 6.99—7.81 (8H, m, Ar-H), 9.43 (1H, s, NH, D_2O exch.), 10.67 (2H, s, 2×CONH, D_2O exch.). IR (KBr) cm^{-1}: 3326.4 and 3164.7 (NH)s, 2980.9 (CH), 1717.5 and 1638.4 (C=O)s. *Anal.* Calcd for $C_{24}H_{25}N_7O_4S_2$: C, 53.42; H, 4.67; N, 18.17. Found: C, 53.70; H, 4.78; N, 18.30.

2,4′-Bis-{*N*-[2-(allylimino)-4-oxothiazolidin-3-yl]carbamoyl}diphenylamine (**14b**): Yield 70%, crystallized from acetone, mp 218—220 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 3.77 (4H, br s, 2×CH_2-CH=CH_2), 4.09 (4H, s, 2×CH_2 of oxothiazolidine), 5.14—5.20 (4H, m, 2×CH_2-CH=CH_2), 5.84 (2H, m, 2×CH_2-CH=CH_2), 7.04—8.19 (8H, m, Ar-H), 9.38 (1H, s, NH, D_2O exch.), 10.68 (2H, s, 2×CONH, D_2O exch.). IR (KBr) cm^{-1}: 3377.9 and 3170.8 (NH)s, 2972.2 and 2841.2 (CH), 1725.7 and 1636.0 (C=O)s. *Anal.* Calcd for $C_{26}H_{25}N_7O_4S_2.H_2O$: C, 53.69; H, 4.68; N, 16.86. Found: C,53.70; H, 4.80; N, 17.00.

2,4′-Bis-{*N*-[2-(phenylimino)-4-oxothiazolidin-3-yl]carbamoyl}diphenylamine (**14c**): Yield 93%, crystallized from acetone, mp 176—178 °C. ^{1}H-NMR (300 MHz) (DMSO-d_6) δ: 4.18 (4H, s, 2×CH_2 of oxothiazolidine), 6.89—7.95 (18H, m, Ar-H), 9.39 (1H, s, NH, D_2O exch.), 10.74 (2H, s, 2×CONH, D_2O exch.). IR (KBr) cm^{-1}: 3285.1, br (NH)s, 3063.9 (CH aromatic), 1727.2 and 1644.2 (C=O)s. *Anal.* Calcd for $C_{32}H_{25}N_7O_4S_2$: C, 60.42; H, 3.96; N, 15.42. Found: C, 60.09; H, 4.33; N, 15.15.

2,4′-Bis-{*N*-[2-(cyclohexylimino)-4-oxothiazolidin-3-yl]carbamoyl}-diphenylamine (**14d**): Yield 82%, crystallized from acetone/water, mp 140—142 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 1.10—1.31 (10H, m, cyclohexyl), 1.60—1.82 (10H, m, cyclohexyl), 3.99 (4H, s, 2×CH_2 of oxothiazolidine), 4.20—4.50 (2H, m, 2×CH-1 cyclohexyl), 7.28—7.85 (8H, m, Ar-H), 9.36 (1H, s, NH, D_2O exch.), 11.00 (2H, s, 2×CONH, D_2O exch.). IR (KBr) cm^{-1}: 3278 (NH)s, 2930 and 2855 (CH), 1719 and 1630 (C=O)s, 1254 (C=S)s. *Anal.* Calcd for $C_{32}H_{37}N_7O_4S_2$: C, 59.33; H, 5.76; N, 15.14. Found: C, 59.20; H, 5.50; N, 14.80.

General Procedure for Preparation of 2,4′-Bis-{*N*-[4-(4-bromophenyl)-2-(substituted imino)thiazol-3(2*H*)-yl]carbamoyl}diphenylamine (15a—d) A mixture of the appropriate thiosemicarbazide **10a**—**d** (1.0 mmol) and 4′-bromophenacyl bromide (0.56 g, 2.0 mmol) in ethanol–chloroform (7 : 3) mixture (20 ml) was refluxed for 3 h. The mixture was concentrated. After cooling the precipitate was filtered, washed several times with ether and crystallized from the appropriate solvent.

2,4′-Bis-{*N*-[4-(4-bromophenyl)-2-(ethylimino)thiazol-3(2*H*)-yl]carbamoyl}diphenylamine (**15a**): Yield 65%, crystallized from acetone/water, mp 210—212 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 1.05—1.12 (6H, t, 2×CH_2CH_3), 3.32—3.43 (4H, q, 2×CH_2CH_3), 6.91(2H, s, 2×CH of thiazoline), 7.13—7.81 (16H, m, Ar-H), 9.46 (1H, s, NH, D_2O exch.), 11.62 (1H, s, CONH, D_2O exch.), 11.71 (1H, s, CONH, D_2O exch.). IR (KBr) cm^{-1}:

3182.3, br (NH)s, 3109.0 (CH aromatic), 2971.2 and 2929.6 (CH), 1654.1 (C=O)s. *Anal.* Calcd for $C_{36}H_{31}Br_2N_7O_2S_2$: C, 52.88; H, 3.82; N, 11.99. Found: C, 52.59; H, 3.82; N, 11.69.

2,4′-Bis-{*N*-[4-(4-bromophenyl)-2-(allylimino)thiazol-3(2*H*)-yl]carbamoyl}diphenylamine (**15b**): Yield 60%, crystallized from acetone/water, mp 196—198 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 4.51 (4H, d, 2×C$\underline{H}_2$-CH=CH$_2$), 4.91—5.24 (4H, m, 2×CH$_2$-CH=C$\underline{H}_2$), 5.75—5.84 (2H, m, 2×CH$_2$-C$\underline{H}$=CH$_2$), 6.40 (2H, s, 2×C$\underline{H}$ of thiazoline), 7.10—7.95 (16H, m, Ar-$\underline{H}$), 9.41 (1H, s, N$\underline{H}$), 11.34 (1H, s, CON$\underline{H}$, D$_2$O exch.), 11.44 (1H, s, CON$\underline{H}$, D$_2$O exch.). IR (KBr) cm^{-1}: 3283.3, br (NH)s, 3096.7 (CH aromatic), 2955.5, 2895.7 and 2820.3 (CH), 1659.0 (C=O)s. *Anal.* Calcd for $C_{38}H_{31}Br_2N_7O_2S_2$: C, 54.23; H, 3.71; N, 11.56. Found: C,54.33; H,3.54; N,11.66.

2,4′-Bis-{*N*-[4-(4-bromophenyl)-2-(phenylimino)thiazol-3(2*H*)-yl]-carbamoyl}diphenylamine (**15c**): Yield 73%, crystallized from ethanol/water, mp 236—238 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 6.58 (2H, s, 2×C$\underline{H}$ of thiazoline), 7.02—7.73 (26 H, m, Ar-$\underline{H}$), 9.06 (1H, s, N$\underline{H}$, D$_2$O exch.), 11.36 (1H, s, CON$\underline{H}$, D$_2$O exch.), 11.54 (1H, s, CON$\underline{H}$, D$_2$O exch.). IR (KBr) cm^{-1}: 3150.0, br (NH)s, 3056.9 (CH aromatic), 2964.8 and 2922.7 (CH), 1674.6 (C=O)s. *Anal.* Calcd for $C_{44}H_{31}Br_2N_7O_2S_2$: C, 57.84; H, 3.42; N, 10.73. Found: C, 57.70; H, 3.30; N, 11.08.

2,4′-Bis-{*N*-[4-(4-bromophenyl)-2-(cyclohexylimino)thiazol-3(2*H*)-yl]-carbamoyl}diphenylamine (**15d**): Yield 68%, crystallized from acetone, mp 182—184 °C. ^{1}H-NMR (200 MHz) (DMSO-d_6) δ: 1.06—1.71 (20H, m, 2×cyclohexyl), 3.56 (2H, br s, overlapped, 2×C$\underline{H}$-1 of cyclohexyl), 6.24 (1H, s, C$\underline{H}$ of thiazoline), 6.26 (1H, s, C$\underline{H}$ of thiazoline), 7.02—7.73 (16 H, m, Ar-$\underline{H}$), 9.16 (1H, s, N$\underline{H}$), 11.99 (1H, s, CON$\underline{H}$), 12.05(1H, s, CON$\underline{H}$). IR (KBr) cm^{-1}: 3199.1 and 3111.3 (NH)s, 2930.8 and 2854.7 (CH), 1678.0 (C=O)s. *Anal.* Calcd for $C_{44}H_{43}Br_2N_7O_2S_2$: C, 57.08; H, 4.68; N, 10.59. Found: C, 57.30; H, 4.60; N, 10.40.

General Procedure for the Preparation of 2,4′-Bis-(*N*-substituted carbamoyl)-diphenylamine (16) and (17) A solution of the diacid hydrazide **8** (1.0 g, 3.5 mmol) and the appropriate acid anhydride (7.0 mmol) in glacial acetic acid (10 ml) was refluxed for 3 h. The reaction mixture was concentrated and poured over crushed ice. The product was filtered, washed several times with cold water then crystallized from the appropriate solvent.

2,4′-Bis-[*N*-(2,5-dioxopyrrolidin-1-yl)carbamoyl]diphenylamine (**16**): Yield 60%, crystallized from ethanol/water, mp 178—180 °C. ^{1}H-NMR (90 MHz) (DMSO-d_6) δ: 2.84 (8H, s, 4×C$\underline{H}_2$ of dioxopyrolidine), 7.03—7.93 (8H, m, Ar-$\underline{H}$), 9.46 (1H, s, NH), 10.90 (1H, s, CON$\underline{H}$), 11.30 (1H, s, CON$\underline{H}$). IR (KBr) cm^{-1}: 3276.1, br (NH)s, 2988.9 and 2943.7 (CH), 1730.3 and 166.20 (C=O)s. *Anal.* Calcd for $C_{22}H_{19}N_5O_6$: C, 58.80; H, 4.26; N, 15.58. Found: C, 58.70; H, 4.52; N, 15.55.

2,4′-Bis(*N*-phthalimidocarbamoyl)diphenylamine (**17**): Yield 59%, crystallized from ethanol/water, mp 166—8 °C. ^{1}H-NMR (90 MHz) (DMSO-d_6) δ: 7.19—7.90 (16H, m, Ar-$\underline{H}$), 9.44 (1H, s, N$\underline{H}$, D$_2$O exch.), 11.01 (1H, s, CON$\underline{H}$, D$_2$O exch.), 11.42 (1H, s, CON$\underline{H}$, D$_2$O exch.). IR (KBr) cm^{-1}: 3300 (NH)s, 1740 and 1640 (C=O)s. *Anal.* Calcd for $C_{30}H_{19}N_5O_6$: C, 66.05; H, 3.51; N, 12.84. Found: C, 65.80; H, 3.80; N, 12.86.

Biological Testing. *In Vitro* EGFR Inhibitory Activity Assays by ELISA[25,26] The assay was performed in 96-well plates pre-coated with 20 μg/ml poly(Glu, Tyr) 4 : 1 (Sigma) as a substrate. In each well, 85 μl of 8 μM ATP solutions and 100 μl of the title compound were added at 10 μM concentrations.[22,26] PD153035 was used as a positive control for EGFR kinase and 0.1% (v/v) dimethyl sulfoxide (DMSO) was the negative control. Triplicate wells were prepared each compound. The reaction was initiated by adding 5 μl of EGFR kinase. After incubation for 1 h at 37 °C, the plate was washed three times with phosphate buffered saline (PBS) containing 0.1% Tween 20 (T-PBS). Next, 100 μl HRP-conjugate antiphosphotyrosine antibody was added. After 1 h of incubation at room temperature, the plate was washed three times. Tetramethylbenzidine (TMB) substrate solution (100 μl) diluted in T-PBS containing 5 mg/ml bovine serum albumin (BSA) was added. The plate was reincubated at 37 °C for 15 min, and washed as before. Finally, a 100 μl solution (0.03% H_2O_2, 2 mg/ml *o*-phenylenediamine in citrate buffer 0.1 M, pH 5.5) was added and incubated at room temperature until color emerged. The reaction was terminated by the addition of 100 μl of 2 M H_2SO_4, and A_{492} was measured using an ELISA reader. Results should be read immediately after addition of the stop solution or within one hour if the microwell strips are stored at 4 °C in the dark. The inhibition rate (%) was calculated using the equation:

$$\text{the inhibition } \% = [1 - (A_{492}/A_{492}\ \text{control})] \times 100$$

***In Vitro* Cytotoxic Assay**[31] The human tumor cell lines (MCF-7) were obtained as a gift from NCI, MD, U.S.A. All chemicals and solvents were purchased from Sigma-Aldrich. The cytotoxic activity was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay using the method of Skehan.[31] Cells were inoculated in 96-well 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, 10 mmol/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% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris-ethylenediaminetetraacetic acid (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.

Molecular Modeling All molecular modeling calculation and docking studies were carried out using Molecular Operating Environment MOE version 2008.10.[27] The target compounds were drawn on MOE. The structures were subjected to energy minimization using Hamiltonian-Force Field-MMFF94x. The most stable conformers for each compound were retained and partial charges were calculated. The X-ray crystal structure of the kinase domain of EGFR in complex with WZ4002 PDB ID code 3IKA was recovered RSCB protein data bank.[28] The enzyme was prepared for docking as follows: 1) The Co-crystallized ligand, and water molecules were removed. 2) The enzyme was 3D protonated, where hydrogen atoms were added at their standard geometry, the partial charges were computed and the system was optimized. Flexible ligand-rigid receptor docking of the most stable conformers was done with MOE-DOCK using triangle matcher as placement method and London dG as a scoring function. The obtained poses were subjected to force field refinement using the same scoring function. Ten conformers of the ligand were retained with the highest and best score. In order to validate the docking procedure, WZ4002 was docked into the active site of 1XKK. The docking results show that the compound exhibit similar interaction reported in literature.[27]

Acknowledgment The authors are grateful for Dr. Nadia Hamdy, Department of Biochemistry, Faculty of Pharmacy, Ain Shams University, for conducting the *in vitro* EGFR inhibitory activity assays. The authors thank the National Cancer Institute, Cairo University, for performing the *in vitro* cytotoxicity.

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