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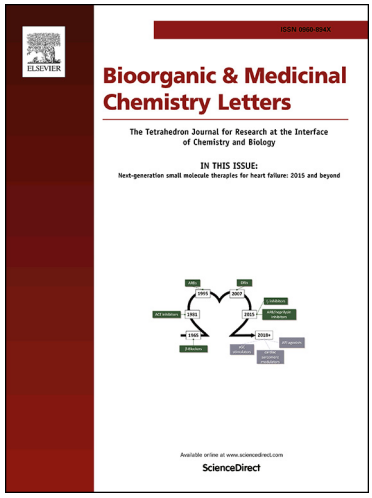

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DESIGN, SYNTHESIS AND EVALUATION OF NOVEL HYBRIDS BETWEEN 4-ANILINOQUINAZOLINES AND SUBSTITUTED TRIAZOLES AS POTENT CYTOTOXIC AGENTS

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ABSTRACT

In this research several series of novel dioxygenated ring fused 4-anilinoquinazolines (**10a-d**) and 4-anilinoquinazoline-substituted triazole hybrid compounds (**11-14**) have been designed and synthesized. Their biological significance was highlighted by evaluating *in vitro* for anticancer activities, wherein several compounds displayed excellent activity specifically against three human cancer cell lines (KB, epidermoid carcinoma; HepG2, hepatoma carcinoma; SK-Lu-1, non-small lung cancer). Especially, compound **13a** exhibited up to 100-fold higher cytotoxicity in comparison with erlotinib. Docking the most cytotoxic compounds (**11d**, **13a**, **13b**, and **14c**) into the ATP binding site of different EGFR tyrosine kinase domains was perfomed to predict the analogous binding mode of these compounds to the EGFR targets.

Keywords: 4-anilinoquinazolines; triazole; hybrids; cytotoxic agents; EGFR inhibitors

As a class of nitrogen-containing heterocyclic compounds, quinazoline derivatives have been widely known as powerful inhibitors of the epidermal growth factor receptors (EGFRs) of tyrosine kinase,[1] ligands for benzodiazepine and GABA receptors in the CNS system[2,3] or as DNA binders.[4] Quinazoline derivatives have shown remarkable biological activities, such as antiinflammatory,[5-7] antibacterial,[8] antiviral,[9] and most importantly, anticancer activities[10-14] that makes them as one important pharmacophore widely used in the developing novel anticancer drugs. Representative drugs, like gefitinib, erlotinib, and lapatinib (Fig. 1) have been approved by the FDA and were successfully applied in clinic for the treatment of multiple cancers, such as non-small cell lung cancer,[15,16] pancreatic cancer,[16] breast cancer.[17]

The structure-activity relationship (SAR) of EGFR inhibitors shows that the 4-anilinoquinazoline scaffold is crucial to EGFR inhibitory activity, and the C6- and C7-position side chains are mainly contribute to their physicochemical properties with good compatibility for bulky moieties.[18-21] As a result, in recent years, numerous 4-anilinoquinazoline derivatives have been designed and synthesized in succession. Among which, crown ether fused anilinoquinazoline analogues were synthesized as novel EGFR tyrosine kinase inhibitors.[22-24] SAR studies indicated that oxygen containing heterocycles with

ring size higher than 12 member are the favorable fused anilinoquinazolines, and the preferred substituent on the 4-anilino is a halogen such as chlorine, bromine, or phenyl group at the meta-position.[23,24] Some of them proved to be active in an EGFR-mediated intracellular tyrosine phosphorylation assay in human tumor cell line A431,[23] while another showed a very potent antiproliferative activity by the inhibition of both receptor tyrosine kinase including EGFR, VEGFR, PDGFR, and nonreceptor TKs including C-Src and Abl kinase with superior inhibition activity against EGFR.[24] In the light of the results mentioned above, and taking erlotinib as the leading compound, we have devised and synthesized the serie of dioxygenated ring fused quinazolines containing the ethynyl group at the meta-position of anilino ring, with the aim of obtaining agents displaying more potent anticancer activities.

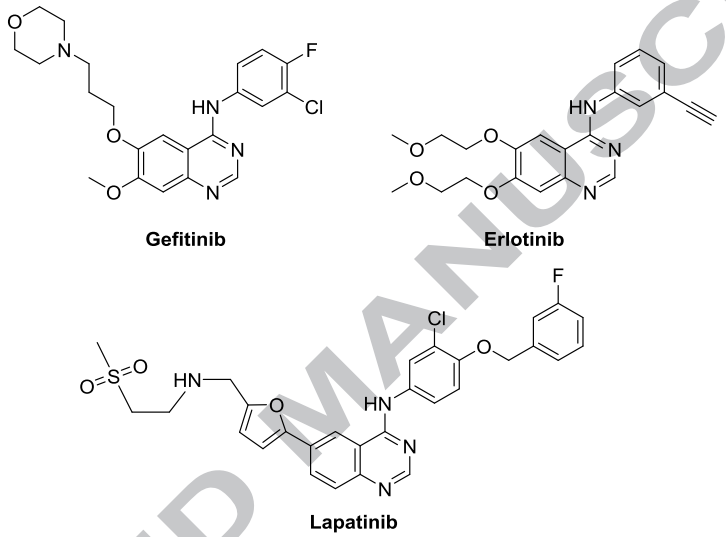
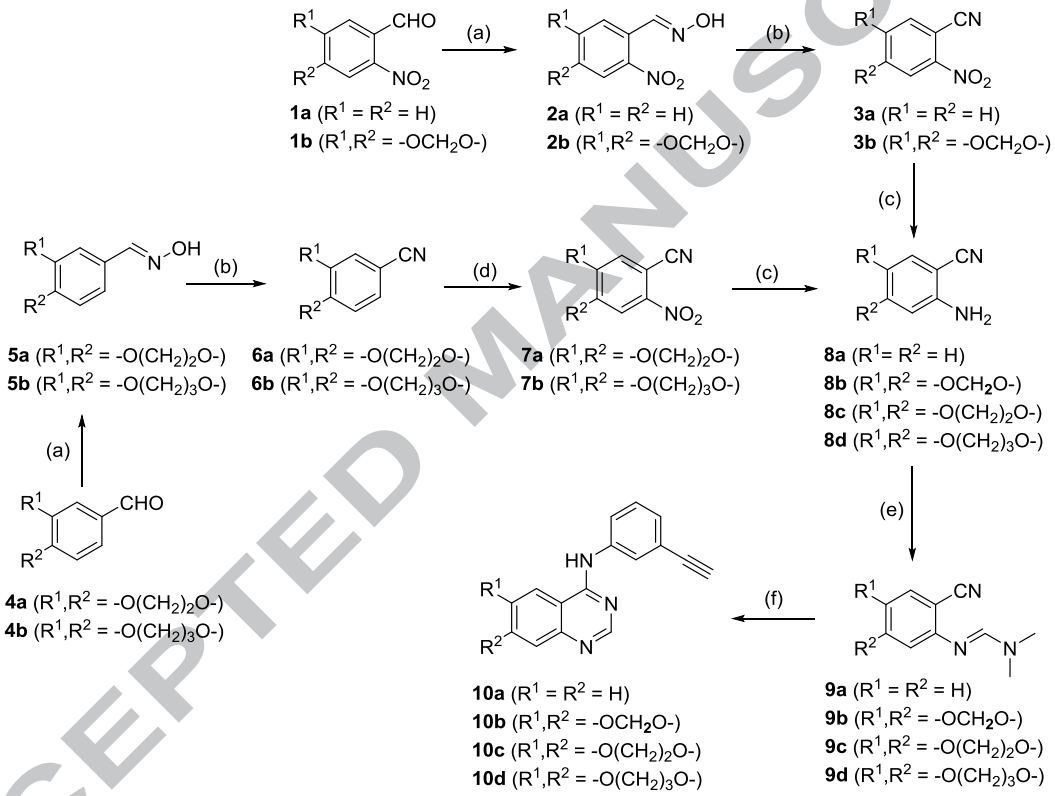


Fig 1. Structures of some EGFR inhibitors

On the other hand, 1,2,3-triazole fragment is of great importance in medicinal chemistry and can be used for the synthesis of numerous heterocyclic compounds with different biological activitives, including anticancer activities,[25-27] and antiproliferative properties.[28-30] Remarkably, this framework was widely used as a building block in the design of anticancer agents because of its ability to form hydrogen-bonding interactions with drug targets.[31] Recently, several 1,2,3-triazole-dithiocarbamate hybrids,[32] triazole-quinazolinone hybrids,[33-35] and other 1,2,3-triazole derivatives have been synthesized and demonstrated impressive biological activities.[36,37] However, the combination of two bioactive moieties 1,2,3-triazole and 4-anilinoquinazoline into single molecule has not been reported in the literature so far. These findings have led to the hypothesis that the introduction of 1,2,3-triazolyl group into targeted dioxygenated ring fused quinazolines at the anilino side chain could give the promising potent biological compounds. It was expected that this triazole scaffold would create more hydrogen interaction with the bingding pocket in the hydrophobic environment, thus increasing the EGFR inhibitory potency and improving the anticancer activities. Moreover, the ethynyl group in our designed erlotinib analogues easily takes part in the "Click" reaction with various azides to form the 1,2,3-triazole ring. In this context, in view of the above fact and to discover completely new cytotoxic agents with a novel skeleton, a relatively small library of 4-aminoquinazoline-substituted triazole hybrids was constructed via the copper(I)-catalyzed azidealkyne cycloaddition (CuAAC) reaction[38] and the biological

significance of the novel synthesized quinazolines was highlighted by evaluating them for their cytotoxic activities.

Despite the widespread utility of 4-anilinoquinazolines, the reported syntheses of these compounds require multistep and low-yielding pathways. The most common synthetic approach to these compounds involves the preparation of 4(3*H*)-quinazolinone intermediates, which are mainly obtained by Niementowski reaction between anthranilic acid derivatives and formamide.[39-42] The key 4(3*H*)-quinazolinones are then submitted to chlorination at the 4 position and finally the chlorine atom is substituted with the appropriate aniline moiety. Anilinoquinazolines can be afforded by the reaction of anthranilonitrile with *N*,*N*-dimethylformamide (DMF-DMA), followed by cyclization of produced formamidine derivatives with different amines in acetic acid.[43,44]

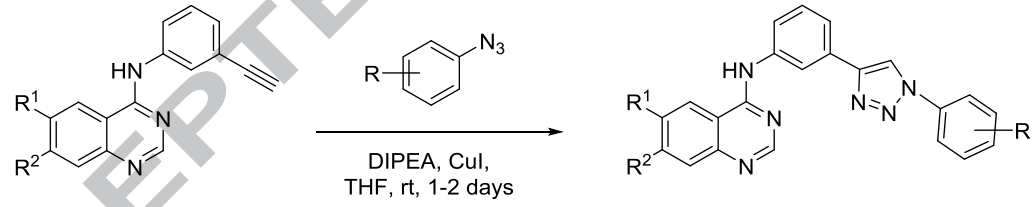


Scheme 1: Preparation of dioxygenated ring fused quinazolines **10a-d**. Reagents and conditions: (a) $NH_2OH.HCl$, NaOH, MeOH, H_2O, rt, 30-60 min, 95-98%; (b) Ac_2O, reflux, 8-12 h, 90-95%; (c) $Na_2S_2O_4$, H_2O, 50-65°C, 3-4 h, 80-85%; (d) HNO_3, CH_3COOH, 50°C, 4 h, 90-95%; (e) DMF-DMA, acetic acid, toluene, reflux, 4-6 h; (f) 3-ethynylaniline, acetic acid, toluene, 60°C-110°C, 4-6 h, 50-63%.

In this study, the synthesis of dioxygenated ring fused quinazolines **10a-d** were achieved in 5-6 steps from various benzaldehydes as depicted in Scheme 1. The aldoximes **2a**,**b** and **5a**,**b**, prepared by the treatment of benzaldehydes **1a**,**b** or **4a**,**b** with hydroxylamine, were subjected to intramolecular dehydration with anhydride acetic to afford the benzonitriles **3a**,**b** and **6a**,**b**, respectively (up to quantitative yields). Reduction of the nitro group of **3a**,**b** using sodium dithionite in acidic solution gave the corresponding amines **8a**,**b**. In the case of compounds containing two or three methylene groups in the dioxygenated ring fused, the nitrile compounds **6a**,**b** were nitrated with 65% nitric acid in acetic acid at 50°C and the resulted nitroderivatives **7a**,**b** were subsequently submitted to reduction with sodium dithionite in acidic solution to furnish the corresponding amines **8c**,**d**. Afterwards, the coupling of amines **8a-d** with DMF-DMA gave the formamidine intermediates **9a-d**, which

were subjected to cyclization with 3-ethynylanilines to afford the target quinazolines **10a-d** in 50-63% yields. The structures of the synthesized compounds **10a-d** were determined straightforwardly based on analysis of spectroscopic data, including IR and ^{1}H NMR.

The second section of the work deals with the syntheses of 4-anilinoquinazoline–substituted triazole hybrids, showing different quinazolines and constant triazole substitution patterns. Most of the EGFR-tyrosine kinase inhibitors have the same 4-anilinoquinazoline skeleton, only the substituents and the side chains are variable. Therefore, the replacement of the acetylene moiety at the C3 position of the phenyl ring by a triazole nucleus could rigid the resulting structure by only one single bonding between the triazole and the quinazoline nucleus. Moreover, hydrogen bonding between the triazole ring and the peptide backbone of the EGF receptor could afford specific conformations, improving the inhibitory activities of the resulting derivatives. Besides, with respect to the triazolyl substituent, we consider bioactive functionalization including nitrophenyl and cyanotrifluoromethylphenyl. Due to the specific chemical and physical properties of nitrogen and fluorine, the introduction of a NO_2, CF_3, and CN moieties in pharmacologically active compounds is known to convey beneficial biological effects to the resulting molecules, hence the increasing interest from organic and medicinal chemists in polyfunctional NO_2-, CF_3-, and CN-substituted scaffolds.[45-52] In that respect, copper(I) catalyzed click reaction (CuAAC) of the alkyne key intermediates **10a-d** with nitrophenyl- and cyanotrifluoromethylphenylazides generated the target 4-anilinoquinazoline–substituted triazole hybrid compounds **11-14** in 70-90% yields (Scheme 2). The structure of hybrid compounds **11-14** was determined by their ^{1}H NMR, ^{13}C NMR, and MS (ESI) spectra. Notably, the ^{1}H NMR spectrum showed a singlet at 9.17-9.65 ppm corresponding to the triazolyl proton, while the ^{13}C NMR spectrum showed peaks at 120-123 ppm and 147-149 ppm corresponding to CH and Cq characteristic to the triazole core unit.



10a (R^1 = R^2 = H)
10b (R^1,R^2 = -OCH_2O-)
10c (R^1,R^2 = -$O(CH_2)_2O$-)
10d (R^1,R^2 = -$O(CH_2)_3O$-)

11 (R^1 = R^2 = H)
12 (R^1,R^2 = -OCH_2O-)
13 (R^1,R^2 = -$O(CH_2)_2O$-)
14 (R^1,R^2 = -$O(CH_2)_3O$-)
R = 2-NO_2, 3-NO_2, 4-NO_2, 3-CN-4-CF_3.

Scheme 2. Synthesis of 4-anilinoquinazoline–substituted triazole hybrids. Reagents and conditions: 1 equiv 4-anilinoquinazoline **10**, 1.1 equiv azide, 12 equiv DIPEA, 0.2 equiv CuI, THF, rt, 1-2 days, 70-90%.

The synthesized compounds were evaluated for their cytotoxicity against three human cancer cell lines, including KB (epidermoid carcinoma cancer), HepG2 (hepatoma carcinoma cancer) and SK-Lu-1 (non-small lung cancer). Erlotinib, erlotinib hydrochloride and ellipticine were used as positive controls. The results are presented in Table 1. As shown in Table 1, in general, the synthesized compounds exhibited good cytotoxic inhibitory effects with IC_{50} values in submicromolar range in most cases. Most of the active compounds exhibited higher inhibitory activity than those of the reference drugs erlotinib and erlotinib hydrochloride.

Table 1

Cytotoxicity of the synthesized compounds against three human cancer cell lines

Entry	R^1,R^2	R	Compound	IC_{50} (KB), μM	IC_{50} (HepG2), μM	IC_{50} (Lu), μM
1		-	**10a**	5.46 ± 0.14	4.16 ± 0.09	4.16 ± 0.08
2		2-NO_2	**11a**	5.50 ± 0.19	4.64 ± 0.12	4.76 ± 0.12
3	H	3-NO_2	**11b**	104.20 ± 3.20	286.75 ± 4.70	259.74 ± 1.35
4		4-NO_2	**11c**	5.47 ± 0.24	8.11 ± 0.37	9.16 ± 0.42
5		3-CN-4-CF_3	**11d**	1.46 ± 0.07	1.86 ± 0.07	4.50 ± 0.21
6		-	**10b**	75.60 ± 2.30	26.17 ± 0.69	64.88 ± 1.93
7		2-NO_2	**12a**	193.33 ± 6.42	207.01 ± 4.31	216.84 ± 5.78
8	-OCH_2O-	3-NO_2	**12b**	6.35 ± 0.02	6.88 ± 0.02	6.66 ± 0.02
9		4-NO_2	**12c**	30.10 ± 2.04	11.29 ± 0.56	44.48 ± 2.62
10		3-CN-4-CF_3	**12d**	4.59 ± 0.01	6.10 ± 0.03	27.46 ± 0.15
11		-	**10c**	2.60 ± 0.81	2.84 ± 0.09	3.36 ± 0.08
12		2-NO_2	**13a**	0.04 ± 0.01	0.14 ± 0.03	1.03 ± 0.03
13	-$O(CH_2)_2O$-	3-NO_2	**13b**	3.51 ± 0.12	0.88 ± 0.03	5.67 ± 0.18
14		4-NO_2	**13c**	79.09 ± 0.43	230.02 ± 0.85	54.77 ± 0.32
15		3-CN-4-CF_3	**13d**	0.27 ± 0.83	6.09 ± 2.27	4.44 ± 0.94
16		-	**10d**	54.83 ± 1.85	69.89 ± 2.34	80.67 ± 2.14
17	-$O(CH_2)_3O$-	2-NO_2	**14a**	20.44 ± 1.21	43.35 ± 1.95	14.75 ± 0.81
18		3-NO_2	**14b**	53.17 ± 2.37	76.81 ± 3.94	230.40 ± 7.91
19		3-CN-4-CF_3	**14c**	1.49 ± 0.09	1.61 ± 0.08	1.81 ± 0.09
20		Erlotinib		13.01 ± 0.61	25.01 ± 1.24	99.76 ± 4.21
21		Erlotinib.HCl		49.62 ± 0.16	14.17 ± 0.05	31.15 ± 0.09
22		Ellipticine		1.95 ± 0.05	2.72 ± 0.04	1.38 ± 0.04

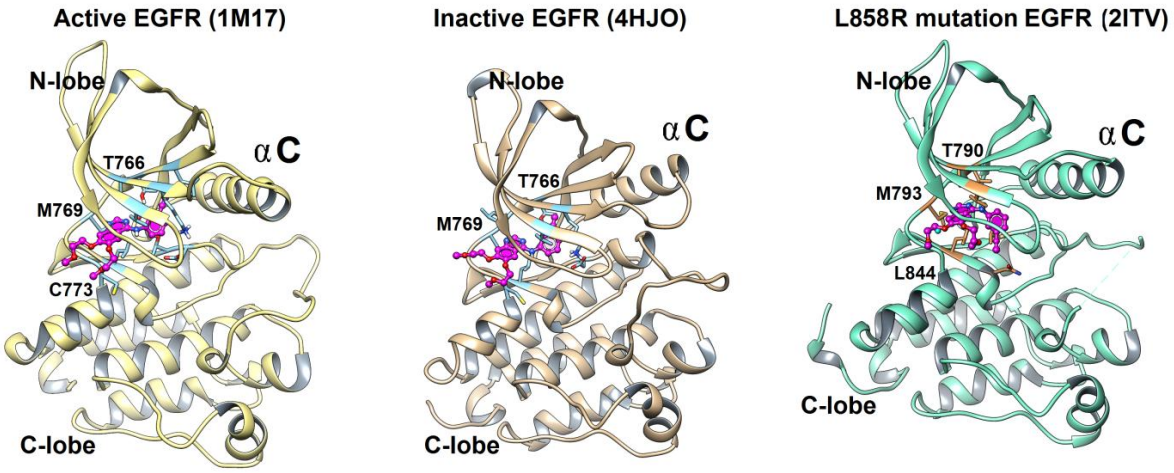
Substitution by different oxygen substituent heterocycles on the positions 6 and 7 of quinazolines skeleton affected the cytotoxic inhibition differently. Compound **10a** and the dioxane derivative **10c** are clearly more preferred than dioxolane and dioxepine derivatives **10b**, **10d**. The effect of these unfavorable substitutions may result from steric hindrance. Moreover, compounds **10a** and **10c** were found to be more potent cytotoxic inhibitors against all three cancer cell lines than erlotinib and erlotinib hydrochloride with IC_{50}-values ranging from 2 to 6 μM.

The coupling of substituted 1,2,3-triazolyl groups with targeted dioxygenated ring fused quinazolines **10a-d** at the anilino side chain was found to greatly enhance the cytotoxicity of the resulting hybrid compounds **11-14**. It is important to note that these separate pharmacophores and the reference drugs display considerably less potent cytotoxic activities (IC_{50}-values ranging from 2 to 100 μM) as compared to the most promising conjugates **11a**,**c**,**d**, **12b**,**d**, **13a**,**b**,**d** and **14a**,**c** (IC_{50}-values ranging from 0.04 μM to 25 μM) showing a reasonable activity against these cancer cell lines. Preliminary investigation of the structure-activity relationships (SARs) of these synthesized hybrid compounds **11-14** revealed that the nature of the oxygen substituent heterocycles and the aryl group which connected to the triazole influenced the cytotoxicity activity remarkably. For instance, the result revealed that the cytotoxic activity of dioxane substituted analogues **13a-d** are more potent than those of the corresponding dioxolane, dioxepine counterparts, and the analogues without oxygen substituent heterocycles (IC_{50}: **13a** > **11a** > **14a** > **12a**, **13b** > **12b** > **14b** > **11b**, **13d** > **14c** ≈ **11d** > **12d**). Especially, compound **13a** displayed the most potent inhibitory activity against KB, HepG2, and Lu with IC_{50}-values of 0.04 μM, 0.14 μM, and 1.03 μM, respectively, which was up to 100 fold higher than those of erlotinib. The introduction of an electron-withdrawing groups such as NO_2, CF_3, and CN of the aryl which connected to the triazole can remarkable improve the cytotixic activities. Substitution by a trifluoromethyl group of the aryl seemed to be better than a nitro (IC_{50}: **11d** > **11a** > **11c** > **11d**, **12d** > **12b** > **12c** > **12b**, **14c** > **14a** >

14b). In addition, the effects of the substituent position seemed to be also dependent on the substituent nature. In fact, with a nitro substituent, substitution at *orto*-position was better than at *meta*- or *para*-positions in most cases.

Among the 4-anilinoquinazoline derivatives designed, compounds **11d**, **13a-b**, and **14c** were identified as novel hits given their excellent *in vitro* citotoxicity against three human cancer cell lines. In particular, **13a** exhibited up to 100-fold higher cytotoxicity in comparison with erlotinib, a potent inhibitor that bind both active and inactive conformations of the EGFR tyrosine kinase domain.[53] In order to explore the structure-activity relationships at molecular target, docking simulation of **11d**, **13a-b**, and **14c** into ATP binding site of different conformations of EGFR were performed. To this end, three common protein structures of EGFR were modelled, including active (1M17), inactive (4HJO), and L858R mutant (2ITV) conformations. The docking protocol was evaluated by using the initial conformation of Erlotinib obtained from 1M17.

As can be visualized in Fig. 2, the catalytic domains of these EGFR kinase proteins comprise two lobes: an NH_2-terminal lobe (N-lobe) formed from mostly β-trands and one α-helix, and a larger COOH-terminal lobe (C-lobe) mostly consisted of α-helical. The binding site is positioned between these two lobes and comprises two regions: the front cleft which contains ATP-binding site, and the back cleft that contains important elements for the regulation of kinase catalysis.[54] The main difference between active and inactive conformations relies on the rotation of the α-helix by 45° away from the active site in the N-lobe and the switch of Asp-Phe-Gly (DFG) motif in the activation loop in the C-lobe that significantly alter the conformation of the active site.[54,55] However, the re-docked orientations of erlotinib on PDB entries 1M17 and 4HJO revealed that this drug similarly bound to active and inactive EGFR conformations with binding affinities of -7.8 and -7.1 kCal/mol, respectively. The same hydrogen-bonding network was observed in two systems, including quinazoline N-1 and Met769 (distance: 2.3-2.9 Å), and a water-mediated H-bond between quinazoline N-3 and Thr766 side chain. These findings were in agreement with the crystallographic coordinates found for erlotinib binding.[53] On the other hand, erlotinib was docked into the L858R mutant domains of EGFR using the crystal structures established by Yun et al.[56] As shown in amino acid alignment (Fig. 2), this point mutation made a leucine-to-arginine substitution at position 858, and would lead to enhance the stabilization of active conformation of EGFR.[57] The docking result of erlotinib on PDB entry 2ITV revealed that this drug stricly bound to the L858R mutant with dG of -9.9 kCal/mol, significantly higher than affinity calculated with non-mutated active conformation. Three H-bonds were formed between erlotinib and the "gatekeeper" residues Thr790, Met793, and Gly796. In addition, the drug was mainly accomodated in the ATP-binding site which is a small hydrophobic pocket, and form multiple pi-alkyl stacking interactions involving quinazoline moiety of erlotinib, toward Leu718, Val726, Ala743 and Leu844 of L858R mutant. Given the selectivity of erlotinib toward L858R mutant over other wild-type EGFRs in clinical trials,[58] current docking protocol provided acceptable accuracy of the binding mode predictions.

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1M17 ACT_EGFR    672  GEAPNQALLR ILKETEFKKI KVLGSGAFGT VYKGLWIPEG EKVKIPVAIK ELREATSPKA NKEILDEAYV  741
2ITV L858R_EGFR  696  GEAPNQALLR ILKETEFKKI KVLGSGAFGT VYKGLWIPEG EKVKIPVAIK ELREATSPKA NKEILDEAYV  765
4HJO INACT_EGFR  679  -------LLR ILKETEFKKI KVLGSGAFGT VYKGLWIP-- --VKIPVAIK ELREATSPKA NKEILDEAYV  741

1M17 ACT_EGFR    742  MASVDNPHVC RLLGICLTST VQLITQLMPF GCLLDYVREH KDNIGSQYLL NWCVQIAKGM NYLEDRRLVH  811
2ITV L858R_EGFR  766  MASVDNPHVC RLLGICLTST VQLITQLMPF GCLLDYVREH KDNIGSQYLL NWCVQIAKGM NYLEDRRLVH  835
4HJO INACT_EGFR  742  MASVDNPHVC RLLGICLTST VQLITQLMPF GCLLDYVREH KDNIGSQYLL NWCVQIAKGM NYLEDRRLVH  811

1M17 ACT_EGFR    812  RDLAARNVLV KTPQHVKITD FGLAKLLGAE EKEYHAEGGK VPIKWMALES ILHRIYTHQS DVWSYGVTVW  881
2ITV L858R_EGFR  836  RDLAARNVLV KTPQHVKITD FGRAKLLGAE E--------- VPIKWMALES ILHRIYTHQS DVWSYGVTVW  905
4HJO INACT_EGFR  812  RDLAARNVLV KTPQHVKITD FGLAKLLGAE EKEYHAEGGK VPIKWMALES ILHRIYTHQS DVWSYGVTVW  881

1M17 ACT_EGFR    882  ELMTFGSKPY DGIPASEISS ILEKGERLPQ PPICTIDVYM IMVKCWMIDA DSRPKFRELI IEFSKMARDP  951
2ITV L858R_EGFR  906  ELMTFGSKPY DGIPASEISS ILEKGERLPQ PPICTIDVYM IMVKCWMIDA DSRPKFRELI IEFSKMARDP  975
4HJO INACT_EGFR  882  ELMTFGSKPY DGIPASEISS ILEKGERLPQ PPICTIDVYM IMRKCWMIDA DSRPKFRELI IEFSKMARDP  951

1M17 ACT_EGFR    952  QRYLVIQGDE RMHLMDEEDM DDVVDADEYL IP                                          995
2ITV L858R_EGFR  976  QRYLVIQGDE RMHLMDEEDM DDVVDADEYL IP                                         1019
4HJO INACT_EGFR  952  QRYLVIQGD- ---------- ---------- --                                          960
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Fig 2. Docking poses of erlotinib with protein structures (top panel) and amino acid alignment (bottom panel) of different conformations of EGFR. In black, the residues are conserved between all kinases. Residues in red refer to the amino acid replacements observed in L858R mutant and inactive EGFRs.

The next steps involved in docking selected hit compounds **11d**, **13a-b**, and **14c** into binding sites of difference EGFR conformations. The topological docking conformation and interactions between these compounds and three PDB entries were depicted in Fig. 3. To better depict the role of 1,2,3-triazolyl group and dioxygenated ring fused quinazolines in targeting EGFR proteins, table 2 was added with special focus on the interactions of these moieties.

According to the docking results in the active conformation (1M17), compounds **13a-b** and **14c** showed higher affinity (-10.0 to -10.6 kCal/mol) compared to **11d** and erlotinib (Table 2). Visual inspection of the interactions of **14c** with the binding pocket of active EGFR revealed that two H-bonds were formed in the similar way to erlotinib between dioxygenated ring fused quinazoline moiety and Thr766 (through a water bridge) and Met769. A H-bond was also generated between 3-CN substituent and the Lys721 side chain. The 4-anilinoquinazoline rings deeply inserted into the hydrophobic pocket of active EGFR and showed multiple pi-alkyl stacking interactions with Leu694, Val702, Ala719, Lys721, and Leu820.

To the inactive conformation of EGFR (4HJO), all the compounds are more strongly bound than erlotinib, with **13b** and **14c** having the highest binding energies of -12.3 and -12.1 kCal/mol, respectively. In cases of **11d** and **13a-b**, the quinazoline moieties can make one or two H-bonds with Met769, and the 1,2,3-triazole ring can form an additional water-mediated

H-bond with Thr766 or Lys721. For **13a-b** and **14c**, the dioxygenated fused 4-anilinoquinazoline structures assembled multiple interactions with the residues in the hydrophobic ATP-binding cleft and plays a significant role in the binding to the target. Interestingly, the docking orientations of **13b** and **14c** extended to outside the hydrophobic ATP-binding cleft and formed three H-bonds with the side chain residues Thr830, Asp831, and Phe832. These interactions are quite different from those of erlotinib.

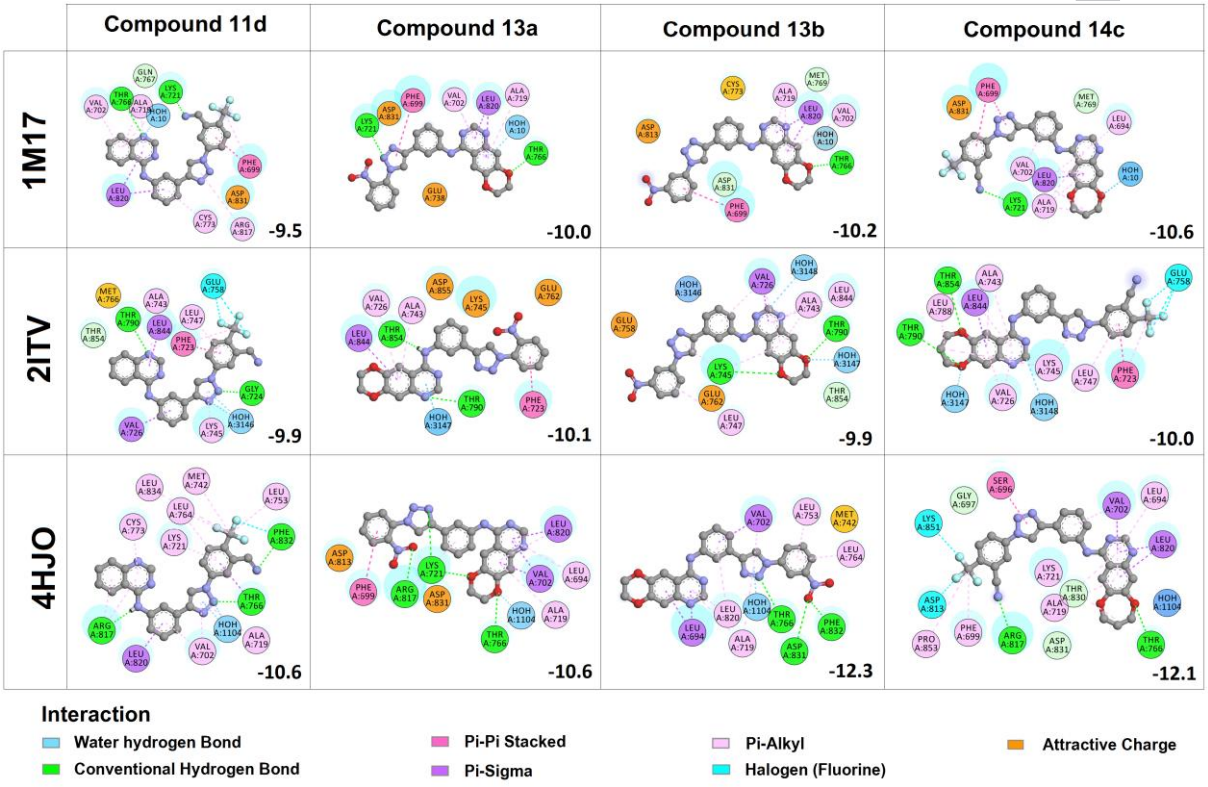

Fig 3. 2D diagrams of docking interactions of compounds **11d**, **13a-b** and **14c** (atoms colored by element type) in the active sites of three conformations of EGFR protein. Number in windows represent the free binding energies (kCal/mol) of the compounds.

On the other hands, all four compounds displayed similar affinity to erlotinib (dG ranges from -9.9 to -10.1 kCal/mol) when bound to L858R mutant domains, with the highest value corresponding to **13a**. The quinazoline and triazole moieties received two hydrogen bonds from two water molecules which took part in the H-bonding network with Thr790, Thr854, Ser720, and Phe723. In cases of compounds **13a** and **13b** an additional H-bond was formed between triazole ring and Gly724. The docking results also revealed the favourable van der Waals interactions between all four compounds and the residues in the back of the ATP-binding cleft (Val726, Ala743, Lys745, and Leu844) of L858R mutant. In general, compounds **13a** and **13b** are in very similar positions to the crystal structures of erlotinib determined by Yun et al.[56] These findings suggested that our hit compounds may have less activity against L858R mutated domain compared to the other conformations analyzed herein. However, more extensive analysis is required in order to identify the difference between synthesized quinazolines and erlotinib, which ultimately should aid in rationalizing the design of potent and selective kinase inhibitors.

Table 2.

Docking results of binding energies and interactions between quinazoline/triazole moieties and residues in the active site of different EGFR conformations

Cpd. code	Active EGFR (1M17)			Inactive EGFR (4HJO)			L858R Mutation EGFR (2ITV)		
	Interactions with Quinazoline system[1]	Interactions with 1,2,3-Triazole[2]	dG[3]	Interactions with Quinazoline system[1]	Interactions with 1,2,3-Triazole[2]	dG[3]	Interactions with Quinazoline system[1]	Interactions with 1,2,3-Triazole[2]	dG[3]
11d	Thr766^{HB*}, Val702, Ala719(2), Lys721, Leu820(2)	Arg817	-9.5	Cys773, Arg817(2)	Thr766^{HB*}, Val702, Ala719	-10.6	Thr790^{HB}, Ala743, Leu844	Gly724^{HB}, Lys745	-9.9
13a	Thr766$^{HB\$}$, Val702(2) Ala719, Leu820(2)	Lys721^{HB}, Phe699	-10.0	Thr766$^{HB*\$}$, Lys721$^{HB\$}$, Leu694, Val702(2), Ala719, Leu820(2)	Lys721^{HB}, Asp831	-10.6	Thr790^{HB}, Thr854^{HB}, Val726, Ala743, Leu844	NE	-10.1
13b	Thr766$^{HB\$*}$, Val702, Ala719(2), Leu820(2)	NE	-10.2	Leu694(3), Ala719, Leu820	Thr766^{HB*}, Thr830^{HB*,} Val702, Ala719,	-12.3	Lys745$^{HB\$}$, Thr790$^{HB\$}$, Val726(2), Ala743(2), Leu844	Lys745	-9.9
14c	Thr766$^{HB*\$}$, Met769 ^{HB}, Leu694, Val702(2)$^{\$}$, Ala719(2), Lys721$^{\$}$, Leu820(2),	Phe699	-10.6	Thr766$^{HB\$*}$, Leu694, Val720(2), Ala719(2), Lys721$^{\$}$, Leu820(2),	Ser696	-12.1	Thr790$^{HB\$}$, Thr854$^{HB\$}$, Val726(2), Ala743(2), Lys745$^{\$}$, Leu788$^{\$}$, Leu844(2)	Lys745	-10.0
Erlotinib	Thr766^{HB*}, Met769 ^{HB}, Leu694, Ala719(2), Leu820(2)	NE	-7.8	Thr766^{HB*}, Met769 ^{HB}, Leu694, Ala719, Leu820(2)	NE	-7.1	Thr790^{HB*}, Met793^{HB}, Leu718, Val726(2), Ala743(2), Leu844(2)	NE	-9.9

[1]Residues interacting with dioxygenated fused quinazoline moieties; [2]residues interacting with 1,2,3-triazole ring; [3]free energy binding (kCal/mol) of the ligands and EGFR proteins computed by affinity scoring function; ^{HB}hydrogen bonding interaction; *Water mediated hydrogen bonds; $^{\$}$interaction with oxygen heterocycles; NE: not exists; in parenthesis (): number of interactions.

In summary, we have reported a serie of dioxygenated fused 4-anilinoquinazolines and three series of 4-anilinoquinazoline–substituted triazole hybrid compounds with potent cytotoxicity against three human cancer cell lines, including KB (epidermoid carcinoma cancer), HepG2 (hepatoma carcinoma cancer), and SK-Lu-1 (non-small lung cancer). Several compounds, e.g. **10a**,**c**, **11a**,**c**,**d**, **12b**,**d**, **13a**,**b**,**d** and **14a**,**c** displayed up to 100-fold more potent than reference drugs erlotinib and erlotinib hydrochloride in term of cytotoxicity. Preliminary investigation of the structure-activity relationships (SARs) of these synthesized hybrid compounds **11-14** revealed that the nature of the oxygen substituent heterocycles and the aryl group which connected to the triazole influenced the cytotoxicity activity remarkably. The size of the fused dioxygenated ring was crucial for the biological activity, the dioxane derivatives being the most promissing class of these series. The different substitutents and the

effects of the substituent position substantially influenced cytotoxicity of the resulting compounds. The molecular docking studies showed that **13a-b** and **14c** are more strongly bound than erlotinib to the ATP binding site of the active and inactive conformations of EGFR, meanwhile they are equally potent binders to the L858R mutated EGFR. In particular, the H-bonding interactions of 1,2,3-triazole ring as well as the dense hydrophobic interaction network of dioxygenated ring fused quinazolines with the residues in the ATP pocket play an important role in EGFR binding. The results we obtained from this study suggest that the introduction of 1,2,3-triazolyl group into dioxygenated ring fused quinazolines at the anilino side chain increases the EGFR inhibitory potency and improves the anticancer properties.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.

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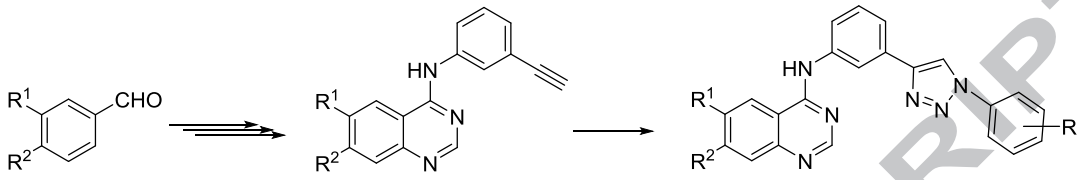
GRAPHICAL ABSTRACT

DESIGN, SYNTHESIS AND EVALUATION OF NOVEL HYBRIDS BETWEEN 4-ANILINOQUINAZOLINES AND SUBSTITUTED TRIAZOLES AS POTENT CYTOTOXIC AGENTS



$R^1 = R^2 = H$

R^1,R^2 = -O(CH_2)O-, -O(CH_2)$_2$O-, -O(CH_2)$_3$O-

15 examples

R^1,R^2 = -O(CH_2)$_2$O-; R = 2-NO_2

IC_{50} (KB) = 0.04 μM

IC_{50} (HepG2) = 0.14 μM

IC_{50} (Lu) = 1.03 μM

R^1,R^2 = -O(CH_2)$_3$O-; R = 3-CN-4-CF_3

IC_{50} (KB) = 1.49 μM

IC_{50} (HepG2) = 1.61 μM

IC_{50} (Lu) = 1.81 μM

DESIGN, SYNTHESIS AND EVALUATION OF NOVEL HYBRIDS BETWEEN 4-ANILINOQUINAZOLINES AND SUBSTITUTED TRIAZOLES AS POTENT CYTOTOXIC AGENTS

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- Fifteen new 4-anilinoquinazoline-substituted triazole hybrid compounds were designed and synthesized.
- Their anticancer activities against human cancer cell lines were evaluated.
- Compound **13a** exhibited up to 100-fold higher cytotoxicity in comparison with erlotinib.
- Molecular docking suggests greater affinity of **13a-b** and **14c** than erlotinib for the ATP binding sites of the active and inactive conformations of EGFR.