Original Article

Trends in Pharmaceutical Sciences and Technologies 2025: 11(2): 163-172.

Design, Synthesis, Molecular Docking of Novel Quinazolinone-azole Derivatives as Anticancer A gents

Leila Emami[1];Ph.D, Sara Sadeghian[1];Ph.D, Maryam Moghtader Mansouri[1];Ph.D, Razieh Sabet[1];Ph.D, Zeinab Faghih[2];Ph.D, Sedigheh Halimi[1];Ph.D, Soghra Khabnadideh[1,2*];Ph.D, Zahra Rezaei[1*];Ph.D

[1]Department of Medicinal Chemistry, School of Pharmacy, Shiraz University of Medical Sceinces, Shiraz, Iran.
[2]Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

Cancer encompasses a diverse group of diseases characterized by uncontrolled cell division, leading to immune system impairment and the potential to metastasize to other regions of the body. Globally, cancer represents a significant threat to public health, ranking as the second most prevalent cause of death following cardiovascular diseases. Quinazoline and azole derivatives are important classes of compounds in medicinal chemistry with a wide variety of biological activities. Here, five quinazoline-azole hybrids were designed and synthesized as cytotoxic agents. The chemical structures of new compounds were confirmed using spectroscopic methods. Molecular docking studies were done on epidermal growth factor receptor (EGFR) as a potential target for quinazoline and azole derivatives. The binding energies and interactions of these ligands toward the active site of EGFR were analyzed in comparison with erlotinib. Interestingly, all compounds showed lower binding energies than erlotinib. *In silico* physicochemical parameters and ADME profiling calculations were also performed.

Keywords: Quinazoline, Azole, Cytotoxic agent, EGFR.

Please cite this article as: Emami L, Sadeghian S, Moghtader Mansouri M, Sabet R, Faghih Z, Halimi S, et al. Drug Utilization Design, synthesis, molecular docking of novel quinazolinone-azole derivatives as anticancer agents. Trends in Pharmaceutical Sciences and Technologies. 2025;11(2):163-172. doi: 10.30476/tips.2025.105887.1285

1. Introduction

Cancer is generic term for many diseases that causes cells to divide without control and damage to the immune system and spread to other part of body. Cancer as the second leading causes of mortality, after cardiovascular diseases, is a great concern for human health worldwide (1, 2). Currently, chemotherapy that involves the use of cytotoxic agents to eliminate cancer cells is the major method employed in the management of numerous kinds of cancer. However, cytotoxic effect of most of the chemotherapeutic agents are not specific for cancer cells and they can also affect the normal cells and thus use of them have been associated with various organ toxicity that often limit the efficiency of chemotherapy agents (2). Therefore, the discovery and development of potent and selective anticancer agents with minimal side effects is of great value in cancer therapy (3). Quinazoline scaffolds and their bioisosteres are recognized as a useful and significant scaffold in drug design and are privilege structure to medicinal chemists, Because of broad spectrum of biological potential like antidiabetic (4), anti-cancer (5) anti-histaminic (6), anti-inflammatory (7), antibacterial (8), antifungal (9) and antiviral activities (10). Imidazole and

Corresponding Author: Soghra Khabnadideh & Zahra Rezaei, Department of Medicinal Chemistry, School of Pharmacy, Shiraz University of Medical Sceinces, Shiraz, Iran.
Email address: khabns@sums.ac.ir & rezaeiza@sums.ac.ir

Received: 16/01/2025; Accepted: 01/03/2025

triazole derivatives have been identified as a potentially advantageous class of anticancer agents with significant therapeutic potential. (11). Triazole compounds have different mechanisms of action for their anticancer effects, such as inhibition of epidermal growth factor receptor (EGFR), microtubule synthesis, aromatase, and Poly-ADP-ribose polymerase (PARP) (12). Furthermore, there are already some imidazole-based drugs including Dacarbazine, Zoledronic acid, Nilotinib, Tipifarnib, and Abemaciclib to treat neoplastic diseases (13, 14). Therefore, quinazoline and azole scaffolds could have contributed to build an enormous number of chemical compounds with cytotoxic activity particularly through EGFR inhibition (15-17). Since EGFR is frequently overexpressed in several cancer types, including non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), and colorectal cancer (18), it has rationalized the development of a number of specific targeted therapeutics. Currently, two classes of EGFR-specific cancer drugs are used in treatment of cancer: monoclonal antibodies (mAbs), which bind to the extracellular domain of the transmembrane receptor and small-molecule tyrosine kinase inhibitors (TKIs), which interact with the adenosine triphosphate (ATP) binding site (19, 20). In this study five quinazolinone-azole compounds were synthesized as EGFR inhibitors and molecular docking was done to explore interactions and binding modes of synthesized compounds against the EGFR as plausible target. Physicochemical properties and ADME profile of all synthesized compounds were also calculated.

2. Experimental

2.1. Chemistry

All solvents and chemical substance were purchased from Merck Company (analytical grade) and used without further purification. Melting points and IR spectra of all compounds were determined using Electrothermal 9200 apparatus and a VERTEX70 spectrometer, respectively. ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER DRX-400 AVANCE (Bruker, Germany) instrument using $CDCl_3$ as deuterated solvent and with an internal standard of tetramethylsilane at 500 and 125 MHz, respectively. Mass spectra were recorded on Mass instrument using (M++1) mode.

2.1.1. General procedure for the synthesis of 2-(chloromethyl)-4H-benzo[d][1,3]oxazin-4-one (3).

A solution of anthranilic acid (1) (1 mmol) in dichloromethane (10 mL) was added to diisopropylethylamine (DIPEA) (1.5 mmol) and then 1.2 mmol of chloroacetyl chloride (2) was added dropwise for 20 minutes at room temperature, and the reaction mixture was stirred for 2 hours. The reaction mixture was washed with water and extracted with ethyl acetate (2 × 20 mL) and the organic layers dried with anhydrous Na_2SO_4. The filtered evaporated by rotary evaporator (Scheme 1).

2.1.2. General procedure for the synthesis of 2-(chloromethyl)-3-substituted quinazoline-4(3H)-one (5).

Intermediate 5 was synthesized using 3-chloroanilines (4) and compound 3 (1 mmol) in the presence of PCl_3 (1.5 mmol) in acetonitrile (CH_3CN) at 60 °C for 2 hours. After completion of the reaction, a saturated $NaHCO_3$ solution was added and then the product (5) extracted with ethyl acetate (3 × 20 mL). The organic layers were dried with anhydrous Na_2SO_4, and the crude products were purified by recrystallization with ethanol (Scheme 1).

2.1.3. General procedure for the synthesis of 2-((1H-azolyl)methyl)-3-(3-chlorophenyl) quinazolin-4(3H)-one (7a-7e).

One mmol of azole compounds (6a-6e) were added to a solution of trimethylamine 1mmol, anhydrous potassium carbonate 1mmol and 1 mmol of compound 5 in acetonitrile and then stirred for 24 hours at reflux temperature. After completion of the reaction, the solvent was evaporated and purified by

column chromatography using chloroform/n-hexane (25/75) as an eluent.

2.1.4. Spectral data

2.1.4.1. 2-((1H-imidazol-1-yl)methyl)-3-(3-chlorophenyl)quinazolin-4(3H)-one (7a)

Chemical Formula: $C_{18}H_{13}C_1N_4O$, Molecular Weight: 336.78, Yield: 78%. ^{1}H-NMR (500 MHz,$CDCl_3$) δ(ppm): 8.25 – 8.19 (m, 1H), 7.79 (td, *J*= 8.0, 7.4, 1.5 Hz, 1H), 7.71–7.69 (m, 1H), 7.54–7.48 (m, 21H), 7.43 (d, *J*=8.0 Hz, 1H), 7.13 – 7.11 (m, 2H), 7.00 (s, 1H), 6.99 – 6.96 (m, 1H), 6.75 (s, 1H), 4.88 (s, 2H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ(ppm): 161.68, 149.35, 146.54, 137.95, 137.40, 136.53, 135.82, 135.11, 131.16, 130.27, 128.89, 128.47, 128.18, 127.84, 127.09, 126.45, 119.21, 50.33. MS (EI), m/z (%): 336.5(60), 316.6(29), 284.6(19), 255.4(68), 149.3(23), 11.6(35), 71.3(56), 57.3(100), 43.3(90). IR (KBr)Umax (cm^{-1}): Ar-H aromatic: 3125, 3065 cm^{-1}; CH2: 2923, 2853 cm^{-1}; C=O amide: 1678 cm^{-1}; C=N: 1611cm^{-1}; C-C aromatic: 1585, 1421 cm^{-1}; CH2:1472 cm^{-1}; Ar-N: 1351, 1272 cm^{-1}; C-N: 1229, 1076 cm^{-1}, C-Cl: 770 cm^{-1}.

2.1.4.2. 2-((1H-1,2,4-triazol-1-yl)methyl)-3-(3-chlorophenyl)quinazolin-4(3H)-one (7b)

Chemical Formula: $C_{17}H_{12}C_1N_5O$, Molecular Weight: 337.77, yield: 62%, MP: 161-163 °C. ^{1}H- NMR (500 MHz, $CDCl_3$) δ(ppm): 8.29 - 8.27 (m, 1H), 7.98 (s, 1H), 7.96 (s, 1H), 7.85 – 7.77 (m, 1H), 7.69 (d, *J* = 8.1 Hz, 1H), 7.56 (d, *J* = 7.6 Hz, 1H), 7.54 (s, 1H), 7.50 (t, *J* = 7.9 Hz, 1H), 7.26 (t, *J* = 2.0 Hz, 1H), 7.18 – 7.10 (m, 1H), 5.17 (s, 2H).^{13}C- NMR (125 MHz, $CDCl_3$) δ(ppm): 161.65, 151.97, 148.43, 146.55, 144.39, 136.62, 135.99, 135.06, 131.21, 130.39, 128.59, 128.15, 127.92, 127.13, 126.47, 121.01, 52.29. MS (EI), m/z (%): 377.5(82), 316.6(53), 268.4(13), 255.4(100), 191.5(13), 111.3(34), 57.3(100). IR (KBr)Umax (cm^{-1}): Ar-H aromatic: 3120 cm^{-1}; CH2: 2985, 2954, 2921, 2852 cm^{-1}; C=O amide: 1682 cm^{-1}; C=N: 1609cm^{-1}; C-C aromatic: 1586, 1505, 1438 cm^{-1}; CH_2: 1471 cm^{-1}; Ar-N: 1348, 1276 cm^{-1}; C-N: 1211, 1111, 1015 cm^{-1}, C-Cl: 775 cm^{-1}.

2.1.4.3 2-((1H-benzo[d]imidazol-1-yl)methyl)-3-(3-chlorophenyl)quinazolin-4(3H)-one (7c)

Chemical Formula: $C_{22}H_{15}C_1N_4O$, Molecular Weight: 386.84, Yield: 81%, MP: 174-177 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 8.24 - 8.23 (m, 1H), 7.81 – 7.77 (m, 2H), 7.71 (d, *J* = 8.1 Hz, 1H), 7.53 (t, *J* = 7.5 Hz, 1H), 7.48 – 7.45 (m, 1H), 7.40 – 7.36 (m, 2H), 7.26 (dd, *J* = 8.0, 4.1 Hz, 1H), 7.21 (s, 1H), 7.20 (s, 1H), 7.10 (s, 1H), 6.92 (d, *J* = 7.8 Hz, 1H), 5.16 (d, *J* = 2.2 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 161.71, 149.05, 146.49, 143.22, 143.03, 136.62, 135.95, 135.09, 133.54, 131.19, 130.27, 128.33, 128.17, 127.90, 127.13, 126.39, 123.44, 122.64, 121.02, 120.40, 109.76, 48.91. MS (EI), m/z (%): 386.6 (100), 387.6 (42), 255.4(30), 131.4(30). IR (KBr)Umax (cm^{-1}): Ar-H aromatic: 3062 cm^{-1}; CH_2: 2921, 2851 cm^{-1}; C=O amide: 1678 cm^{-1}; C=N: 1610cm^{-1}; C-C aromatic: 1585, 1498, 1430 cm^{-1}; CH_2:1462 cm^{-1}; Ar-N: 1335, 1262 cm^{-1}; C-N: 1207, 1182 cm^{-1}, C-Cl: 767cm^{-1}.

2.1.4.4. 3-(3-chlorophenyl)-2-((2-methyl-1H-benzo[d]imidazol-1-yl)methyl)quinazolin-4(3H)-one (7d)

Chemical Formula: $C_{23}H_{17}C_1N_4O$, Molecular Weight: 400.87, yield: 82.5%, MP: 179-182 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 8.27 – 8.21 (m, 1H), 7.77 – 7.72 (m, 1H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.57 (d, *J* = 8.2 Hz, 1H), 7.52 – 7.49 (m, 2H), 7.47 (d, *J* = 7.7 Hz, 1H), 7.26 (t, *J* = 1.7 Hz, 1H), 7.23 – 7.19 (m, 1H), 7.16 – 7.12 (m, 1H), 7.07 – 7.04 (m, 1H), 7.01 (d, *J* = 8.0 Hz, 1H), 4.95 (d, *J* = 1.5 Hz, 2H), 2.38 (s, 3H).^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 174.43, 161.81, 152.26, 148.90, 146.51, 141.71, 136.72, 136.13, 135.03, 134.78, 131.39, 130.40,

128.28, 127.99, 127.95, 127.03, 126.27, 122.55, 122.43, 120.84, 118.94, 108.88, 46.93, 13.42. MS (EI), m/z (%): 400.6(8), 256.5(10), 132.3(26), 60.3(80), 43.3(100) IR (KBr)Umax (cm^{-1}): Ar-H aromatic: 3061 cm^{-1}; CH_2: 2925, 2853 cm^{-1}; C=O amide: 1680 cm^{-1}; C=N: 1610cm^{-1}; C-C aromatic: 1585, 1531, 1406 cm^{-1}; CH_2:1466 cm^{-1}; Ar-N: 1333, 1269 cm^{-1}; C-N: 1255, 1227, 1078 cm^{-1}, C-Cl: 769 cm^{-1}.

3.1.4.5 3-(3-chlorophenyl)-2-((5,6-dimethyl-1H-benzo[d]imidazol-1-yl)methyl)quinazolin-4(3H)-one (7e)

Chemical Formula: $C_{24}H_{19}C_1N_4O$, Molecular Weight: 414.89, Yield: 82%, MP: 180-184 °C ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 8.29 – 8.21 (m, 1H), 7.86 – 7.81 (m, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.59 – 7.55 (m, 1H), 7.54 (s, 1H), 7.50 – 7.47 (m, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.30 (s, 1H), 7.12 (s, 1H), 6.93 (s, 1H), 6.91 (s, 1H), 5.13 (s, 2H), 2.36 (s, 3H), 2.31 (s, ^{3}H). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 161.77, 150.52, 149.33, 146.57, 142.12, 136.69, 135.97, 135.09, 132.56, 132.05, 131.49, 131.15, 130.14, 128.38, 128.14, 127.87, 127.16, 126.49, 121.06, 120.38, 109.81, 48.86, 20.58, 20.22. MS (EI), m/z (%): 414.6(100), 255.4(14), 159.4(30). IR (KBr)Umax (cm^{-1}): Ar-H aromatic: 3072, 3029 cm^{-1}; CH2: 2971, 2942, 2917 cm^{-1}; C=O amide: 1679 cm^{-1}; C=N: 1612cm^{-1}; C-C aromatic: 1586, 1493, 1434 cm^{-1}; CH_2:1471 cm^{-1}; Ar-N: 1315, 1277 cm^{-1}; C-N: 1257, 1224, 1183 cm^{-1}, C-Cl: 778 cm^{-1}.

2.2. Molecular Docking

Molecular docking was done using Autodock Vina software. In order to docking procedure EGFR was selected as a target. EGFR with erlotinib (PDB ID: 1M17) was obtained from Protein Data Bank (PDB database; http://www.rcsb.org). In the first step water molecules and cocrystal ligand (erlotinib) were removed, and the enzyme save as PDBQT format using MGLTools 1.5.6 software. All new synthesized compounds as well as erlotinib and nilotinib were drawn and optimized in HyperChem Professional version 8 (Hypercube Inc., Gainesville, FL, USA) and then geometry optimization was done by Molecular Mechanic (MM+) method. The optimized structures were converted to PDBQT using MGLTools. Finally, all five investigated compounds were characterized by a docking mode in the active site of EGFR. The docking mod was performed by an in-house batch script (DOCK-FACE), based on the Lamarckian genetic algorithm, in a parallel mode, using all system resources, as described in our recent studies. The grid box of 70 × 70 × 70 Å was centered with a spacing of 0.375 Å to accommodate ligand in a respectable orientation. For internal validation, erlotinib was excluded from the PDB structure of receptor and was docked the same as examined ligands. Root-mean-square deviation (RMSD) value obtained below of 2 Å showed the validity of docking (33).

2.3. Physicochemical parameter (ADME) prediction

The pharmacokinetic parameters are important to determine safety of drugs and are described by main physicochemical characteristics such as Absorption, Distribution, Metabolism, and Excretion (ADME). These parameters were predicted using the http://www.swissadme.ch/ server.

3. Results and Discussion

3.1. Design Approach

Erlotinib and Gefitinib are the most potent and selective EGFR inhibitor which have quinazoline scaffold (Figure 1), In recent years, azole derivatives have attracted much interest as potent anticancer agents (21). Among the various azole derivatives, triazole, tetrazol and pyrazole indicated the best anticancer activity (22, 23). Nilotinib contains an imidazole component and acts as a potent Bcr-Abl tyrosine kinase inhibitor (24-26). Different stud-

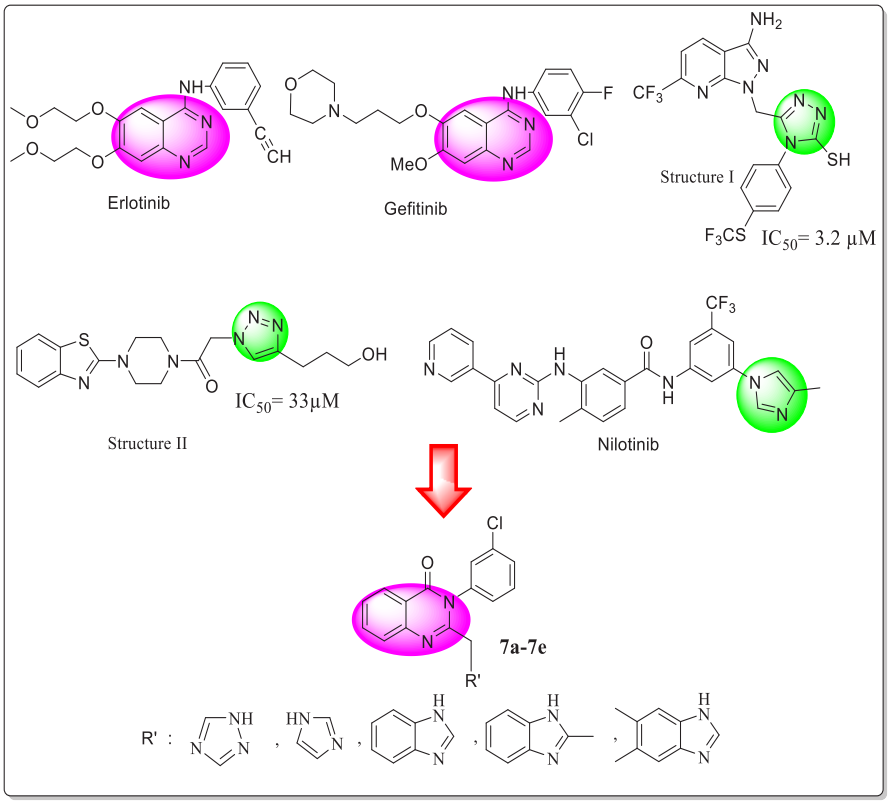

Figure 1. Schematic of designed compounds.

ies reported the significant of azole scaffold as novel anticancer agents (27-29). For example, structure I (30) exhibited promising cytotoxicity against A549 (human lung carcinoma), MCF7 breast carcinoma, Structure II, a benzothiazole-piperazine-1,2,3-triazole hybrid, demonstrated promising anticancer activity against DU145 prostate carcinoma and HeLa cervical carcinoma cell lines (IC50 ≤ 50 µM) values, these results support its potential for further development as a therapeutic agents. (31).

Recently, pharmaceutical researchers have drawn attention to the design of more effective anticancer agents in order to save time and money based on hybridization of potent scaffold. In this regard, there is growing interest in quinazoline scaffolds and azole compounds as anticancer agents. Given the increasing focus on hybridization approaches, we introduced the recent hybridization of the quinazolinone scaffold with azole moieties (7a-7e).

3.2. Chemistry

Synthesis of the quinazoline-azole hybrids (7a–7e) were carried out via three steps (Scheme 1). In the first step, anthranilic acid (1) with chloroacetyl chloride (2) reacted to 2-chloromethyl benzoxazine-4-one (3) in the presence of a catalytic amount of diisopropyl ethyl amine (DIPEA) in dichloromethane (DCM) for 2 hours at room temperature. Next, a nucleophilic attack occurred between 3 and 3-chloroanilines (4) to give 2-(chloromethyl)-3-m-chloroaniline substituted quinazoline-4(3H)-one derivatives (5) under acidic conditions at 60 °C. The third step of the reaction proceeded by replacing the chlorine atom at the side chain with azole derivatives (6a-6e) in the presence of K_2CO_3 and thriethylamine in acetonitrile for 24 hours to obtain final products (7a-7e). The products (7a-7e) were purified on a chromatography plate, using silicagel and 25% chloroform in n-hexane as an eluent (yield 62-82.5 %). The chemical structures of all compounds were confirmed by IR, ^{1}H-

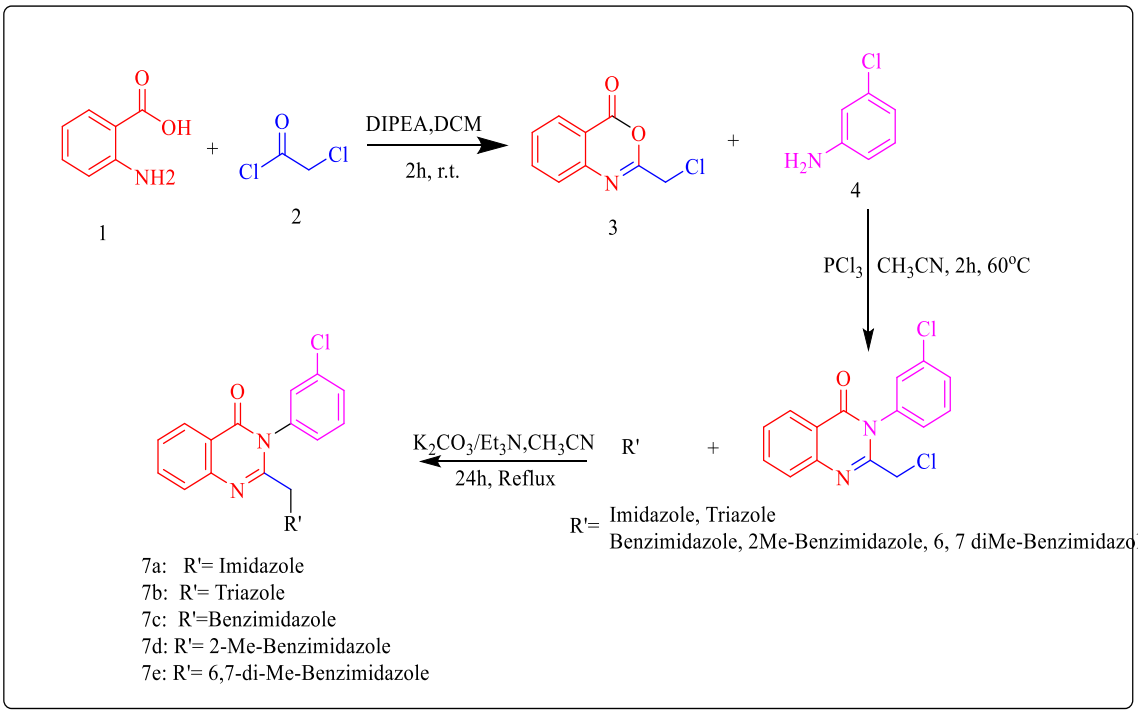

Figure 2. Scheme route of synthesized compounds (7a-7e).

NMR, ^{13}C-NMR, and Mass Spectrometry. Spectroscopic data are presented in experimental section.

3.3. Molecular docking studies

As previously mentioned, EGFR is the main target of quinazoline derivatives as cytotoxic agents, molecular docking studies was performed to evaluate their interactions and conformations of compounds against EGFR enzyme. The results including the estimated free binding energy (Kcal.mol^{-1}) of ligand-complex, and also possible interactions with the main amino acid residues at the active site of the enzyme are shown in Table 1, Figure 3 and 4.

All of the compounds showed comparable docking scores with that of the co-crystallized ligand, (Erlotinib) with binding energies lower than -7.1 kcal/mol, indicating their favorable binding to the EGFR target. The range of the binding energy values were showed between -7.4 to -8.5 Kcal.mol^{-1}.

2D and 3D interaction of the Erlotinib is shown in Figure 3. The nitrogen atom of quinazoline ring and the oxygen atoms of the ether chains involved in hydrogen bond interactions with the residues of Lys721, Cys733, Met769. Other important interactions of Erlotinib are π-sigma, π-stacking and π-anion bonds of quinazoline motif rings with Val702, Phe699, and Asp831 residues (Figure 3). 2D interactions of all synthesized compounds (7a-7e) are shown in Figure 4. For compound 7a, quinazoline moiety formed hydrogen bond and π-sigma with Ser 223, and the imidazole ring involved in π-anion with Asp 227 residue. 7b and 7c have similar interaction like, the quinazoline moiety formed three interactions such as hydrogen bond, π-sigma and π-anion with Lys 56, Phe 34 and Asp 166 residue amino acid. Also, the triazole ring of 7b forms hy-

Table 1. The binding energies kcal.mol-1 of the tested compounds on EGFR (1M17) using AutoDock vina.

Entry	7a	7b	7c	7d	7e	Erlotinib
1M17	-7.4	-7.9	-8.4	-8.1	-8.5	-7.1

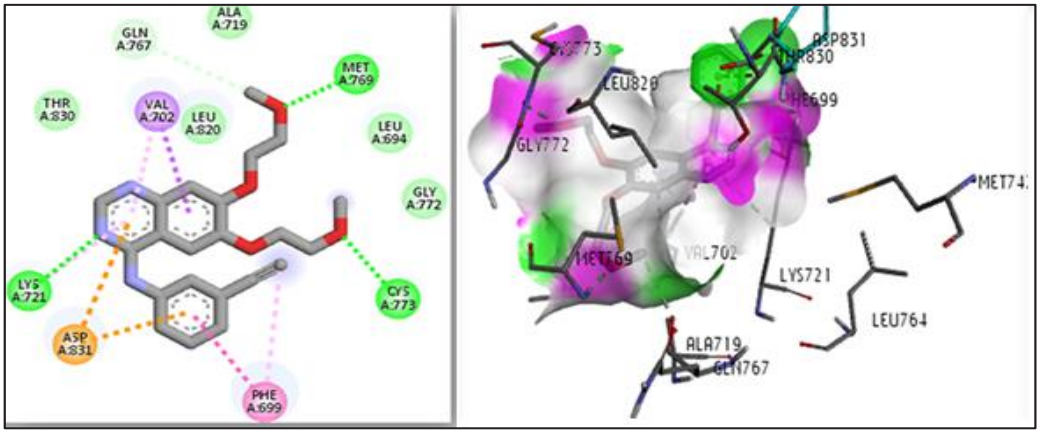

Figure 3. 2D and 3D interaction of the Erlotinib as the co-crystal ligand of IM17.

drogen bond interaction with the Lys 56 and benzimidazole ring of 7c involved in π-anion interaction with Glu 69. On the other hand, compound 7d formed hydrogen bond through the carbonyl group of quinazoline moiety with Gln 293. Also, the quinazoline ring of 7e involved in π-anion interactions with Asp 166 and π-sigma with Phe 34 and hydrogen bond with Lys 56. Finally, the 6, 7 di Me benzimidazole have π-anion interaction with Glu 69.

3.4. ADME properties

A valuable tool to the design and discovery of new candidate as drug is Lipinski's Rule of 5. Oral bioavailability is a key determinant of drug efficacy, and therefore, accurately assessing it is essential for selecting compounds with the potential to achieve therapeutic concentrations *in vivo* (32). The

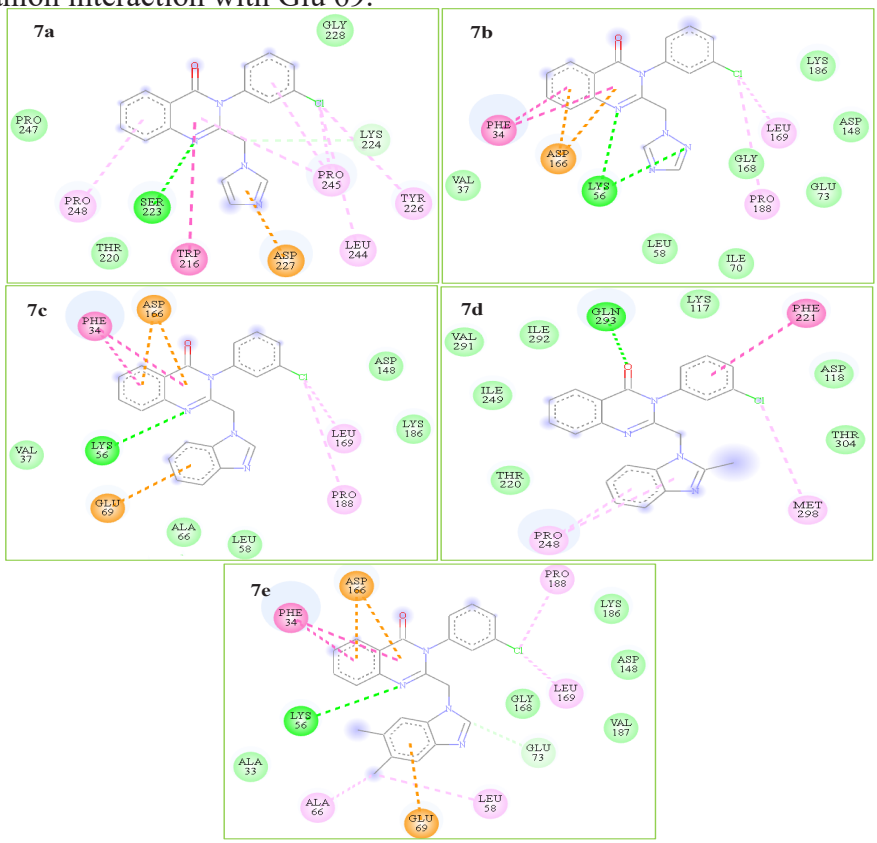

Figure 4. The interaction of the synthesized compounds in the active site of EGFR (PDB code 1M17).

Table 2. Physicochemical properties of studied ligands.

Compounds	MW (g/mol)	LogP	HBD	HBA	TPSA (Å2)	n-RB	Lipinski
7a	violation						
7b	336.78	2.52	0	3	52.71	3	0
7c	337.76	2.69	0	4	65.60	3	0
7d	386.83	3.50	0	3	52.71	3	0
7e	400.86	4.38	0	3	52.71	3	0
Rule of Lipinski	414.89	4.59	0	3	52.71	3	0
	≤500	≤5	≤5	≤10	≤140	≤10	≤1

molecular weight must be less than 500 Daltons to permeability across cell membranes. The number of hydrogen bond acceptor and donor atoms should be fewer than 5 to exist well absorbability. The logarithm of the octanol-water partition coefficient (LogP) should be less than 5 to show desire oral absorption. Although Total Polar Surface Area (TPSA) showed improved in oral absorption. As shown in Table 2, all synthesized compounds confirm to Lipinski's Rule of Five and can be administer as oral candidate.

4. Conclusion

Some of the new quinazoline-azole hybrids were successfully synthesized and characterized by ^{1}H-NMR, ^{13}CNMR IR and MASS spectroscopy. Molecular docking studies of the synthesized compounds on EGFR (1M17) revealed that they form more favorable interactions within the active site than Erlotinib, including key hydrogen bonds, hydrophobic contacts, and Van der Waals forces.All of the compounds comply the lipiniski rule. These findings suggest that quinazolinone-azole hybrids, such as 7c and 7e, have potential for further development as novel anticancer agents.

Acknowledgements

Financial support of Shiraz University of Medical Sciences (Shiraz, I.R. Iran) through the Grant No.16987 is appreciated.

Authors' Contributions

The study conception and design were performed by Leila Emami, Soghra Khabnadideh, and Zahra Rezaei. Data analysis was conducted by Sara Sadeghian and Zeinab Faghih. Maryam Moghtader Mansouri and Sedigheh Halimi conducted the analysis spectra data and biological section. Razieh Sabet and all authors, contributed to the manuscript's drafting and critical review.

Conflict of Interest

The authors declare that they have no conflict of interest.

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