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SYNTHESIS AND IDENTIFICATION OF NEW AMINOACETYLENIC 1,2,3,4-TETRAHYDROISOQUINOLINE DERIVATIVES OF SIGNAL TRANSDUCTION INHIBITOR IN CANCER TREATMENT

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ABSTRACT

Small molecule tyrosine kinase inhibitors of the epidermal growth receptor (EGF-R) are of pharmaceutical interest because these proteins kinases (PKs) are considered validated drug targets. In cancer, protein tyrosine kinase plays a key role in regulating numerous cellular functions including increased proliferation, decreased apoptosis, increased invasion, metastasis, and promote angiogenesis. Because signal transduction pathways are regulated in many tumor cells, targeting and inhibiting these kinases enzymes became attractive candidates for cancer therapy. The targeting of human epidermal growth factor receptor 2(HER2 or ErB-2/ neu) and epidermal growth factor receptor (EGFR or HER1-ErbB-1) by tyrosine kinase inhibitors (KIs) represent one such therapeutic approach. The new novel 2-[4-(amino-1-yl)but-2-yn]-1,2,3,4-

tetrahydroisoquinoline (**ZI 1-6**) provide effective overlap with (EGFR or HER2) through ionic, hydrogen bonding, charge transfer and hydrophobicity. These speculations are supported by molecular docking, structural elucidation were consistent with IR, NMR, and elemental analysis of the prepared compounds.

Keywords: Tetrahydroisoquinoline; Alkylation; Mannich reaction; Molecular docking

INTRODUCTION

Epidermal growth factor (EGFR) is a membrane-bound tyrosine kinase receptor having an extracellular binding site of epidermal growth factor (EGF), and an intracellular kinase active site. Several agents have been studied as kinase inhibitors and the first of these to reach the clinic was gefitinib (Iressa; Figure 1) [1, 2]. Gefitineb was developed by AstraZeneca and belongs to a group of structures known as the 4-anilinoquinazolines. Gefitinib was found to mimic ATP and bind to the ATP-binding region of the kinase active site. The binding of ATP itself involves two important hydrogen bonding interactions between the purine base of ATP and the protein backbone between amino acids Gln-767 and Met-769. One of these interactions involves the purine group acting as a hydrogen bond donor, while the other involves purine acting as a hydrogen bond acceptor. Gefitinib possess two nitrogen atoms in the quinazoline ring which both act as hydrogen bond acceptors.

Erlotinib [Figure 2] and Iapatinib bind like gefitinib; the acetylene group fits into the hydrophobic pocket quard by the gatekeeper residue threonine, which leads to inhibition of tyrosine kinase of EGFR [3, 4].

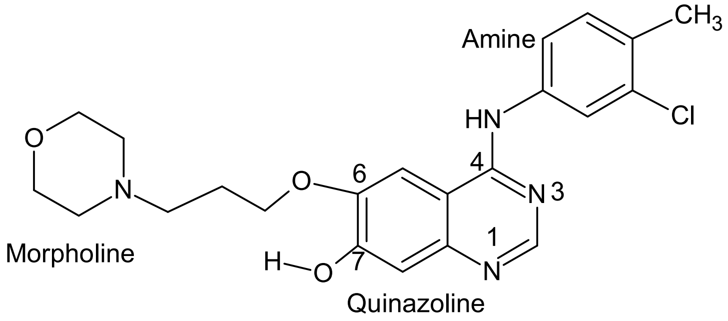


Figure 1: N-(3-chloro-4-fluoro-Phenyl)-7-methoxy-6-(3-morpholine-4-ylpropoxy)quinazoline-4-amine[1]

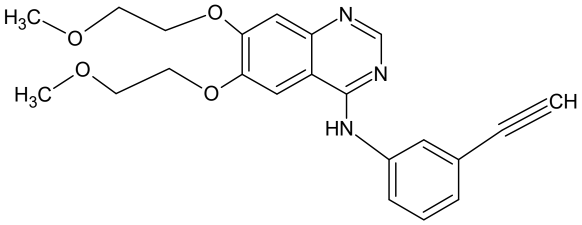


Figure 2: N-(3-ethnylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazoline amine[3]

In reviewing various structural features of different compounds in use or under investigation as EGFR inhibitors, and considering the fractional base analysis in drug design, promoted our interest in the design and synthesis of 2-[4-(amino-1-yl) but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (**ZI1-6**). These new novel derivatives presumably inhibit EGFR, for the following reason: 1,2,3,4-tetrahydroisoquinoline is an isostere or fractional base analogue to quinazoline found in many EGFR inhibitors as in gefitineb, erlotinib and others; aminoacetylenic moiety provide the appropriate functional groups required to ensure the inhibition activity of EGFR. The basic amino group is required for hydrogen bonding with the corresponding groups in EGFR to induce the blockade activity, 2-butye group to provides the electrostatic interaction and to provide the appropriate distance between the nitrogen of cyclic amine and 1,2,3,4-tetrahydroisoquinoline; aromatic ring which initiate π-overlap with aromatic amino acid on the EGFR; molecular docking results showed the effective interaction with EGFR and the ease for free energy requirement to cross the mouth of the receptor to induce inhibitory activity as shown in molecular docking in table 1.

MATERIALS AND METHODS

Chemicals

The following chemicals were used: sodium metal (S&C chemicals), 1,2,3,4-tetrhydroisoquinoline 95% (Sigma-Aldrich, USA), propargyl bromide (Sigma-Aldrich, USA), piperidine 99% reagent plus (Sigma-Aldrich, USA), 2-methylpiperidine 99% (Alfa Aesar USA), 2,6-dimethylpiperidine (Sigma-Aldrich, USA), pyrrolidine 98% (Sigma-Aldrich, USA), N-methylpiperazine 99% (Sigma-Aldrich, USA), hexamethyleneimine 98% (Sigma –Aldrich, USA), tetrahydrofuran (Acros UK), 1,4-dioxane (Fulltime, China), paraformaldehyde (BDH chemicals, UK), cuprous chloride(East Anglia Chemicals, Hadleigh Ipswich), DMSO (BBC chemicals for lab, EU), TMS (Sigma-Aldrich, USA).

Instrumentation

Infrared spectra (IR) were recorded using Bruker FT-IR spectrophotometer (Evisa, Poland), H^1 and C^{13}-NMR were acquired with the aid of Varian 300 MHz spectrophotometer (USA) and DMSO-d_6 as solvent. And TMS as standard. The analysis was indicated by EuroEA elemental analyzer. The result obtained had a maximum deviation of (+2.59 to -2.95) from the theoretical value, which is considered within the acceptable variation range in results (± 0.4%). This variation range is set according to the accuracy of EuroEA elemental analyzer device in the faculty of Pharmacy at the University of Jordan.

Molecular docking

The EGFR kinase domain used in the docking study was downloaded from the protein data bank (PDB: 1XKK) then the co-crystallized ligand and all water molecules were removed from the protein structure. Protein atoms were given partial charges using Kollman united atom modelin the Autodock tool program. The ATP binding site of the EFGR kinase domain was identified by its own co-crystalized ligand (Iapatinib) then a grid box of a 60 X 50 X 60 Å size was created using the Autogrid module with a grid spacing of 0.375 Å [5-9].

Ligand 3D structures were built then were energy minimized using the Maestro program and the OPLS force field, respectively. Atomic partial charges were given for all ligands by Gasteiger-Marsili model (tertiary amine groups were assigned as protonated) (Maestro, version9.2) [10-12].

Next, ligands were docked into the ATP binding site using the Autodock software (version 4.2) where the Lamarckian Genetic Algorithm was employed for the conformational sampling process. Subsequently, docked poses were scored via the Autodock scoring function which includes terms for vander Waals, hydrogen bond, electrostatic interactions, and the ligand internal energy [8, 9].

RESULTS

Synthesis of 2-(prop-2-yn-1-yl)-1,2,3,4-tetrahydroisoquinoline (ZI-0)

A solution of 1,2,3,4-tetrahydroisoquinoline and sodium (1.5 g 0.01 mol) in 40 ml tetrahydrofuran was refluxed up to 65-67°C for 1 h. 3-bromoprop-1-yne (3 g 0.02 mol) was added drop wise to the above solution for 15 min. the mixture was stirred for 45 min. after cooling, the insoluble residue was removed by filtration to give orange brown solution. The filtrate then concentrated under reduced pressure rotary evaporator yielding the desired brown semi-solid compound (**ZI-0**) fig. 3). The instrumental and analytical data support the structures of our designed compounds. The IP spectra of 2-(prop-2-yn-1-yl)-1,2,3,4-tetrahydroisoquinoline (**ZI-0**), showed the terminal acetylenic proton at 3300 cm$^-$, acetylenic C≡C at 2200-2150 cm^{-1}, the aryl double bond stretch at 1550-1600 cm^{-1}, and the aliphatic carbon at 1465 cm^{-1} and 2900 cm^{-1}, the ^{1}H-NMR results showed each proton in our compound as numbered below: yield: 2 g,80%, IR spectra: 3300 (acetylenic C-H stretch),2900 (C-H stretch), 2150 (C≡C stretch), 1600 (Ar C=C stretch),1465 (CH_2 bend), 780 (Ar C-H bend). 1H-NMR (DMSO-D_6): δ, 2.71 (q,H,H14), 3.24 (s, H, H20), 3.56 (q, H, H19), 3.64 (s, H, H21), 7.1 (t, Ar-H, H1-4). Elemental analysis Calcd: ($C_{12}H_{13}N$) C:84.21%, H: 7.60%, N: 8.18%,Found: C: 84.27%, H: 7.33%, N: 8.46%.

Synthesis of 2-[4-(amino-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI 1-6)

A mixture of 2-(prop-2-yn-1-yl)-1,2,3,4-tetrahydroisoquinoline (**ZI-0**) (2 g 0.01 mol), paraformaldehyde (0.5 g 0.01 mol) and the cyclic amine around (0.01 mol), catalytic amount of cuprous chloride (0.03 g), in peroxide-free dioxin 40 ml was refluxed for 1h. Charcoal was added, Filtered and evaporated under reduced pressure to afford the desired brown semi-solid compounds, (**ZI 1-6**). The IR spectra of 2-[4-(amino-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (**ZI 1-6**), showed the disappearance of terminal acetylenic proton at 3300 cm^{-1}, which conclude that the right path for the synthesis of compounds (**ZI 1-6**) as shown in results. The 1H-NMR results showed each proton in our compound as numbered below.

Preparation of 2-[4-(2,6-dimethyl piperidin-1-yl) but-2-yn-1-yl] - 1,2,3,4-tetrahydroiso quinoline (ZI-1)

The titled compound was prepared following the general procedure for synthesis of 2-[4-(amino-1-yl)but-2-yn-1-yl]-1,2,3,4-tetra hydro iso quinoline compounds (**ZI 1-6**). Yield: 2.5 g, 84.4%. IR spectra: 2915 (C-H stretch), 2800 (C-H stretch), 1480 (Ar C=C stretch), 1250 (C-N stretch), 1025 (C-N stretch), 850 (Ar C-H bend), 780 (Ar C-H bend), 750 (Ar C-H bend), 650 (acetylenic C-H bend). ^{1}H-NMR (DMSO-d_6): δ, 1.19 (m, various protons of cyclic amine, H 23, 26), 1.21 (q, various protons of cyclic amine, H 21, H 22), 1.56 (m, various protons of cyclic amine, H24, H 27), 2.68 (q, H, H 30), 3.50 (q, H, H 35), 3.50 (s, H, H 39), 3.51 (s, H,H37), 7.10 (t, Ar-H, H 1-4), (Fig 3.5, 3.6). Elemental analysis: Calcd($C_{20}H_{28}N_2$) C: 80.88%, H: 9.21%, N: 9.19%, Found: C: 80.53%, H: 9.50%, N: 9.12%.

Preparation of 2-[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI-2)

The titled compound was prepared following the general procedure for synthesis of 2-[4-(amino-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline compounds (**ZI 1-6**). Yield: 2.4 g, 85.1%. IR spectra: 2915 (C-H stretch), 2800 (C-H stretch), 1480 (Ar C=C stretch), 1250 (C-N stretch), 1025 (C-N stretch), 850 (Ar C-H bend), 780 (Ar C-H bend), 750 (Ar C-H bend), 650 (acetylenic C-H bend). ^{1}H-NMR (DMSO-d_6): δ, 1.22 (d, various protons of cyclic amine, H 21), 1.53 (m, various protons of cyclic amine, H22), 2.49 (q, H 24), 2.72 (q, H, H 30), 2.82 (q, H, H 31), 3.1 (s, H,H39), 3.48 (q, H, H 37), 3.94 (s, H, H32), 7.1 (t, H, H 1-4) (Fig 3.8, 3.9). Elemental analysis: Calcd ($C_{19}H_{26}N_2$), C: 80.85%, H: 9.22%, N: 9.31, Found: C: 80.62%, H: 9.50%, N: 9.55%.

Preparation of 2-[4-(piperidin-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI-3)

The titled compound was prepared following the general procedure for synthesis of 2-[4-(amino-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline compounds (**ZI 1-6**). Yield: 2.2 g, 82.08%. IR spectra: 2915 (C-H stretch), 2800 (C-H stretch), 1600 (Ar C=C stretch), 1480 (Ar C=C stretch), 1250 (C-N stretch), 850 (Ar C-H bend), 750 (Ar C-H bend), 650 (acetylenic C-H bend). ^{1}H-NMR (DMSO-d_6): δ, 1.79 (m, various protons of cyclic amine, H 28), 2.7 (q, H 29), 3.17 (s, H,H35), 3.56 (s, H, H 36), 3.75 (s, H, H37), 4.4 (s, H, H 32), 7.1 (t, Ar-H, H 1-4) (Fig 3.11, 3.12). Elemental analysis: Calcd ($C_{18}H_{24}N_2$), C: 80.62%, H: 8.05%, N: 10.45%. Found: C: 80.83%, H: 8.01%, N: 10.80%.

Preparation of 2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI-4)

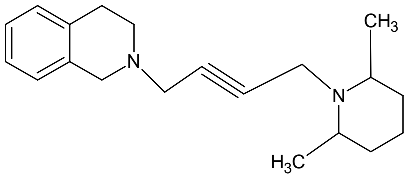
The titled compound was prepared following the general procedure for synthesis of 2-[4-(amino-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline compounds (**ZI 1-6**). Yield: 2.3 g, 90.55%. IR spectra: 2915 (C-H stretch), 2800 (C-H stretch), 1600 (Ar C=C stretch), 1480 (Ar C=C stretch), 1250 (C-N stretch), 1025 (C-N stretch), 850 (Ar C-H bend), 750 (Ar C-H bend), 650 (acetylenic C-H bend). ^{1}H-NMR (DMSO-d_6): δ, 1.71 (m, 2H, various protons of cyclic amine, H 20, H21), 2.65 (q, H 26), 2.9 (q, H, H27), 3.19 (s, H,H 32), 3.53 (s, H, H 33), 3.65 (s, H, H34), 7.1 (t, Ar-H, H 1-4) (Fig 3.14, 3.15). Elemental analysis: Calcd ($C_{17}H_{22}N_2$), C: 80.31%, H: 8.66%, N: 11.02%, Found: C: 80.1%, H: 8.86%, N: 11.42%.

Preparation of 2-[4-(4-methylpiperazin-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI-5)

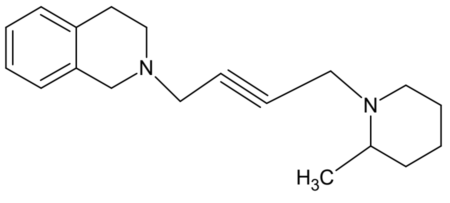
The titled compound was prepared following the general procedure for synthesis of 2-[4-(amino-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline compounds (**ZI 1-6**). Yield: 2.5 g, 88.33%. IR spectra: 2915 (C-H stretch), 2800 (C-H stretch), 1600 (Ar C=C stretch), 1480 (Ar C=C stretch), 1250 (C-N stretch), 1025 (C-N stretch), 850 (Ar C-H bend), 750 (Ar C-H bend), 650 (acetylenic C-H bend). ^{1}H-NMR (DMSO-d$_6$): δ, 2.139 (o, 2H, various protons of cyclic amine, H 22, H26), 2.8 (o, 2H various protons of cyclic amine, H 24, H38), 3 (q, H, H32), 3.12 (s, H, H 34), 3.65 (s, H, H 35), 4.13 (s, H, H37), 7.17 (t, Ar-H, H 1-4) (Fig 3.17, 3.18). Elemental analysis: Calcd ($C_{18}H_{25}N_3$), C:76.28%, H: 8.89%, N: 14.83, Found: C: 79.99%, H: 9.04%, N: 14.01.

Preparation of 2-[4-(azepan-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI-6)

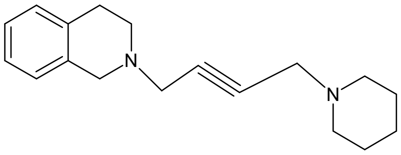
The titled compound was prepared following the general procedure for synthesis of 2-[4-(amino-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline compounds (**ZI 1-6**). Yield: 2.4 g, 85.1%. IR spectra: 2915 (C-H stretch), 2800 (C-H stretch), 1600 (Ar C=C stretch), 1480 (Ar C=C stretch), 1250 (C-N stretch), 1025 (C-N stretch), 850 (Ar C-H bend), 750 (Ar C-H bend), 650 (acetylenic C-H bend). ^{1}H-NMR (DMSO-d$_6$): δ, 1.48 (m, 2H, various protons of cyclic amine, H 27, H31), 2.4 (o, 2H various protons of cyclic amine, H 22, H24), 2.65 (q, H, H32), 3.1 (s, H, H 38), 3.65 (s, H, H 39), 3.77 (s, H, H40), 7.10 (t, Ar-H, H 1-4) (Fig 3.20, 3.21).^{13}C-NMR, (DMSO-d6): δ, 20, 26, 28, 39, 40, 41, 72, 74, 80, 121, 122, 124, 128, 130. Elemental analysis: Calcd ($C_{19}H_{26}N_2$), C: 80.8%, H: 9.28%, N: 9.92, Found: C: 80.52%, H: 9.43%, N: 9.65.



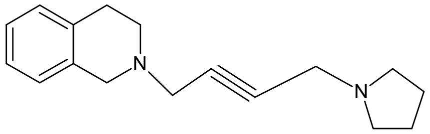
2-[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI-1)



2-[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI-2)



2-[4-(piperidin-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI-3)



2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI-4)

2-[4-(4-methylpiperazin-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI-5)

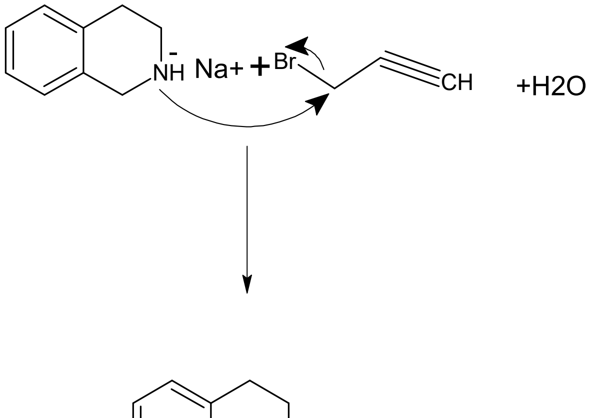
2-[4-(azepan-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI-6)

Figure 3: Synthesized compounds (ZI 1-6)

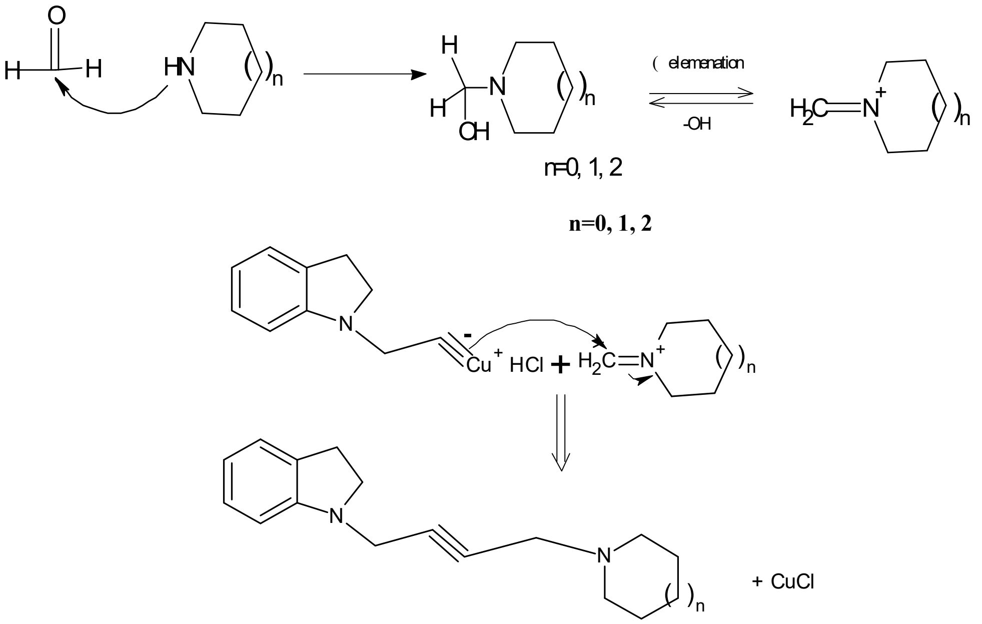
DISCUSSION

The designed compounds were prepared according to **scheme 1** as mentioned in the synthesis of 2-(prop-2-yn-1-yl)-1,2,3,4-tetrahydroisoquinoline (**ZI-0**). (**ZI-0)** was generated through nucleophilic displacement of the bromine located at 3-bromoprop-1-yne. The mannich reaction of 2-(prop-2-yn-1-yl)-1,2,3,4,-tetrahydroisoquinoline (**ZI-0**)with paraformaldehyde, appropriate cyclic amine and catalytic amount of cuprous chloride, generated the desired compounds (**ZI 1-6**). Their structure was verified through IR, 1H-NMR, 13C-NMR, and elemental analysis as illustrated in the results. The proposed mechanism for mannich reaction is outlined in **Scheme 2**.

In order for mannich reaction to proceed, a reactive immonium cations intermediates should be formed from the condensation of the formaldehyde and the appropriate amines (Schiff base formation). The attack of the carbanion in 2-(prop-2-yn-1-yl)-1,2,3,4-tetrahydroisoquinoline cuprous salt on the Schiffbase generates the desired mannich products (**ZI 1-6**), **scheme 1**.



2-(prop-2-yn-1-yl)-1,2,3,4-tetrahydroisoquinoine (IZ-0)
Scheme 1: Alkylation reaction of 1,2,3,4-tetrahydroisoquinoine



Scheme 2: Mannich reaction, (ZI 1-6)

The design of these compounds as EGFR antagonists was based on our rationalization for the important criteria required to overlap effectively with EGFR to induce antagonistic activity. These criteria are: 1,2,3,4-tetrahydrophalate as an isoster in fractional base approach in drug design to quinazoline ring moiety found in effective EGFR antagonists, a basic amino group for ionic interaction or hydrogen bonding, the acetylenic group in 2-butyne provide electrostatic interaction and the appropriate distance for antagonistic interaction with EGFR. Molecular docking results supported our assumption in the design of new and novel EGFR antagonist.

Kinases inhibitors are known to have a cyclic system which has a nitrogen atom that is able to make an electrostatic interaction with the backbone amide of the ATP-binding site hinge region. Another amino group could exist in the kinase inhibitor which makes a water-mediated hydrogen bond with the Thr 845 hydroxyl group (also exists in the purine binding region). Additionally, they usually have a hydrophobic cyclic system that fits in a hydrophobic specificity determinant pocket on the other side of the binding site [5].

Accordingly, our compounds were designed to have a two heterocyclic system linked with each other via rigid spacer (i.e. an acetylenic group). Such a design should offer these compounds the ability to bind with the purine binding region via the first cyclic system and with the specificity binding region via the second cyclic group. The rigid linker should boost compound binding via decreasing the entropic penalty (usually associated with flexible linkers).

The **ZI** compounds have a heterocyclic system linked to a tetrahydroisoquinoline ring via an aceytelenic spacer. Docking these compounds into the ATP-binding site of the EGFR kinase domain has resulted in energetically favorable binding modes (Table 1) although none of them were able to make the usual hydrogen bond with the hinge region backbone amide. However, some of them were still capable of making strong electrostatic interactions via the piperdine protonated nitrogen whilst others were able to make a weak water-mediated hydrogen bond via the same atom. For instance, the best binder**ZI-1** (binding energy = -8.5 kcal/mol) had the piperdine ring with two different binding modes (figure 4), (the PyMOL Molecular Graphics System, Version 1.8); in the first mode it was able to make strong electrostatic interactions with Asp 855 and Asn 842 side chains; in the second mode it is able to make the common water-mediated hydrogen bond with Thr 845. On the other hand, the terahydroisoquinoline ring had a perfect fit in the specificity binding region which should attribute a lot in the favorable binding energy obtained by these compounds. Thus, these compounds have the potential to inhibit the EGFR kinase enzyme with a new unusual binding mode. **ZI-2** has the best score among the tetrahydroisoquinoline compounds 9-8.5 Kcal/mol).

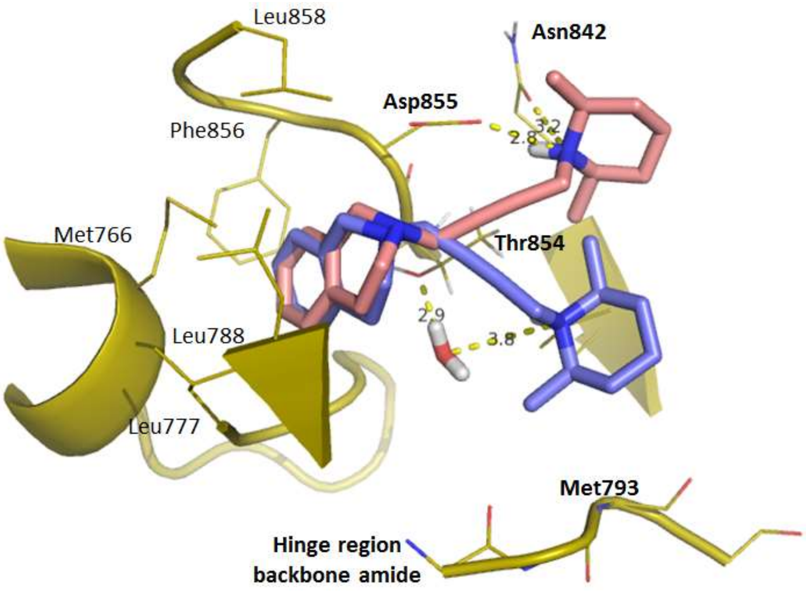
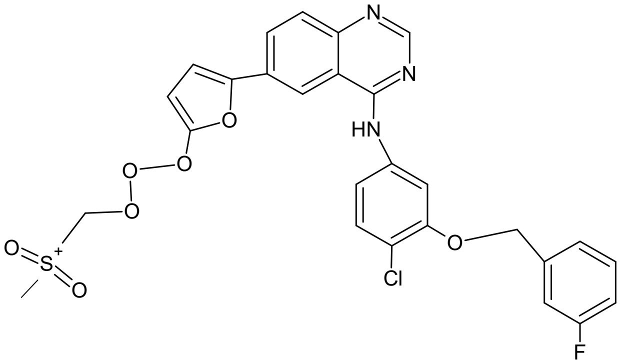


Figure 4: Shows multiple binding modes demonstrated by ZI-1 (blue or pink sticks) in the ATP-binding site of the EGFR kinase domain (gold). The picture was generated by PyMol[13]. Electrostatic interactions are shown as yellow dotted lines. Some protein chains are not shown for clarity

Table 1: Docking scores of our compounds into the EGFR kinase enzyme

Molecule (Kcal/mol)	Autodock score
	**-13.8**
ZI-1	**-8.5**
ZI-2	**-8.4**
ZI-3	**-8.0**
ZI-4	**-7.5**
ZI-5	**-8.1**
ZI-6	**-8.0**

CONCLUSION

The synthesis and characterization of the new series of **2-[4-(amino-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI 1-6)** were accomplished. Molecular docking of the **2-[4-(amino-1-yl)but-2-yn-1-yl]-1,2,3,4-tetrahydroisoquinoline (ZI 1-6)** compounds showed promising approach in treatment of cancer, through inhibition of EGFR. Further modifications to enhance the activity are in progress.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SOURCE OF FUNDING

Self

REFERENCES

[1] Baker AJ, Gibson KH, Grundy W, et al., Studies leading to the identification of ZD1839 (Iressa^{TM}): an orally active, selective epidermal growth factor receptor tyrosine kinase inhibitor targeted to the treatment of cancer, Bioorg Med Chemlett. 11(14), 2001: 1911-4.

[2] Patrick GL, An introduction to medicinal chemistry, Oxford university press, UK, 2013.

[3] Dancey J, and Sausvile EA, Issues and progress with protein kinase inhibitors for cancer treatment, Nat Rev Drug Discov. 2(4), 2003:296-313.

[4] Capdeville R, Buchdunger E, Zimmermann J, & Matter A, Glivec (ST1571, imatinib), a rationally developed, targeted anticancer drug, Nat Rev Drug Discov.1(7), 2002:493-502.

[5] Wood ER, Truesdale AT, McDonald OB et al., A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib) relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. Cancer research, 2004; 64(18), 6652-6659.

[6] Sanner MF, Python: a programming language for software integration and development, J Mol Graph model. 17(1), 1999:57-61.

[7] Weiner SJ, Kollman PA, Case DA et al., A new force field for molecular mechanical simulation of nucleic acids and proteins, JACS. 106(3), 1984:765-84.

[8] Morris GM, Goodsell DS, Halliday RS et al., Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, J Comput Chem.19(14), 1998:1639-1662.

[9] Morris GM, Huey R, Lindstrom W et al., AutoDock4 and AutoDocktools4: Automated docking with selective receptor flexibility, J Comput Chem. 30(16), 2009:2785-2791.

[10] Maestro V (2011), Schrodinger, LLC, New York, NY.

[11] Jorgensen WL, and Tirado-Rives J, The OPLS (optimized potentials for liquid simulations) potential function for proteins, energy minimizations for crystals of cyclic peptides and crambin. J A C. 110(6), 1988:1657-1666.

[12] Gasteiger J, and Marsili M, Iterative partial equalization of orbital electronegativity- a rapid access to atomic charges. Tetrahedron, 36(22), 1980:3219-3228.

[13] Schrödinger*, LLC,* The PyMOL Molecular Graphics System, version 1.8, New York, NY, 2015. available from: https://pymol.org/2/support.html.