



Molecular Modeling Studies on Series of A-Aminoacid Functionalized 4-Quinazoline Derivatives Based on Cytotoxic Activity Data Against U 937 Cell Lines

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ABSTRACT

*In continuation of our efforts on synthesis and in vitro anticancer activity evaluation against U937 cell lines of series of 2-phenyl-6-substituted quinazolin-4-yl amino acetic acid derivatives **6a-6o**, the data was compared with structure-based investigations using Docking studies with the crystal structure of caspase-1 protein (1BMQ). The binding energies estimated by scoring functions were found to have a good correlation with the experimental inhibitory potencies.*

Keywords: Cytotoxic activity; amino acids; quinazolines; cell lines; modeling studies.

INTRODUCTION

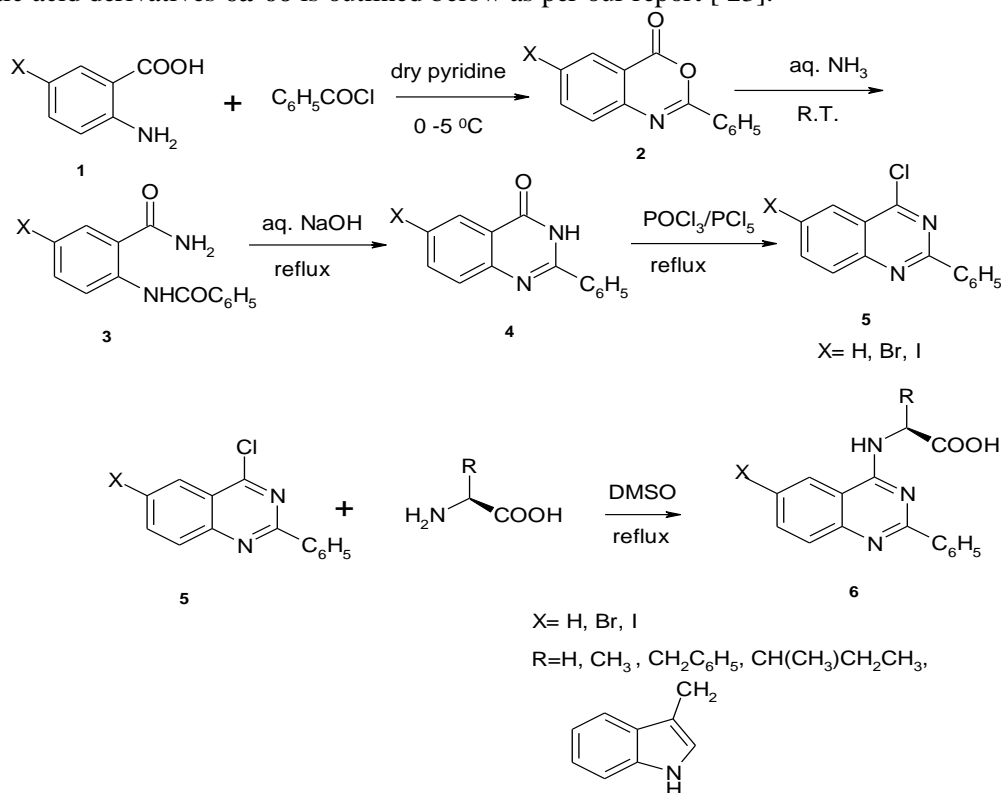
In general quinazoline derivatives are NOS-II [1], NFKB [2], TNF- α [3] IMPDH-II [4], MAPK [5], IL-6 [6], PDE-3 [7] and PDE-4 [8] inhibitors and also found to show bronchodilatory [9], anti-allergic [10] properties. Some of the known quinazoline derivatives exhibited remarkable anti-cancer activity [11-16]. More specifically trimetrexate (TMQ) and piritrexim (PTX) considered as new generation potent lipophilic DHFR inhibitors [17-19]. Further 4-anilinoquinazolines are considered to be potent and highly selective inhibitors of tyrosine kinase activity [20-22]. In view of the link between use of NSAIDs and altered cancer incidence and a growing evidence of COX-II implication in angiogenesis, a novel series of 4,6-disubstituted quinazoline derivatives [23], 2,4,6-trisubstituted quinazoline derivatives [24] as well as perfluoroalkyl-1H,1,2,3-triazol-4-yl substituted O-, N-quinazolines [25] published were synthesized by us in our laboratory. The derivatives thus prepared were screened for anti-inflammatory, anti-microbial and anti-cancer activities against various cell lines. Some of the compounds exhibited promising anti-cancer activity with reference to standard drug Etoposide.

With the aim of rationalizing the biological data obtained for the synthesized compounds, 2-phenyl-6-substituted quinazolin-4-yl amino acetic acid derivatives **6a-6o** [23], a Molecular Modeling study was carried out in order to investigate the possible interaction of such compounds with caspase-1 protein.

Molecular Docking methodologies ultimately seek to predict the best binding mode by which a compound will fit into a site of a macro molecular target. The docking studies have been made to explain the observed variance in biological activity which predicts the best drug candidate providing an insight into substitutional and configurational requirements for optimum receptor pit that leads to the development of best pharmacophore activity and reporting here for the first time.

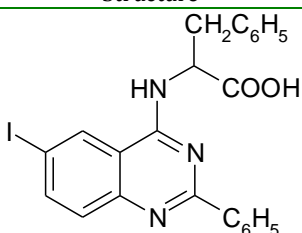
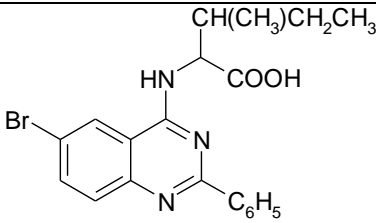
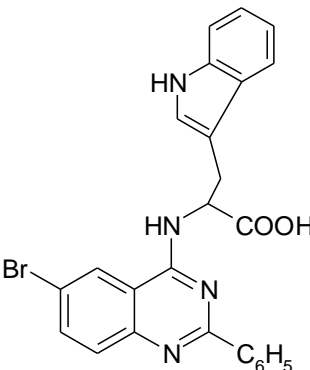
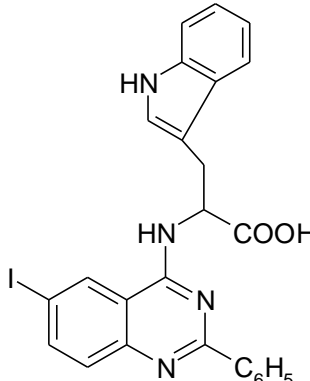
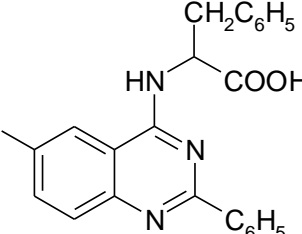
MATERIALS AND METHODS

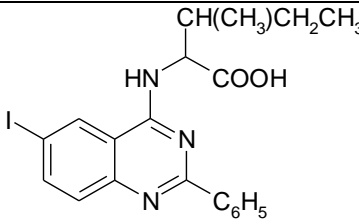
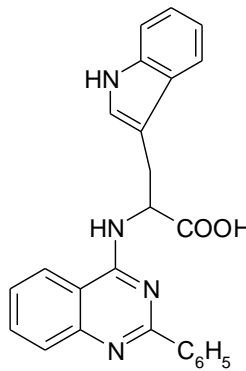
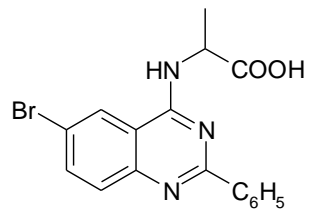
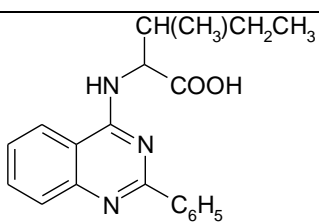
Chemistry: The synthetic sequence involved in preparation of 2-phenyl-6-substituted quinazolin-4-yl amino acetic acid derivatives **6a-6o** is outlined below as per our report [23].



Cytotoxic activity: The compounds 2-phenyl-6-substituted quinazolin-4-yl amino acetic acid derivatives **6a-6o** were tested against U937 leukemia human lymphoma cancer cell lines [23] and shown significant decrease in cell viability with reference to concentration. Among all, the compound **6i** exhibited maximum cytotoxic activity and is close to standard positive control (Etoposide). The activity presumed to be due to the presence of iodine on 6th position with L-phenylalanine on 4th position. If iodine is replaced with bromine **6h**, the activity is drastically reduced. Alternately on comparison of compounds **6k**, **6l** and **6j** with L-isoleucin on fourth position and Br, I and H on sixth position showed descending order of activity. Similar trend was observed in compounds **6n**, **6o** and **6m** with L-tryptophan and compounds **6e**, **6f** and **6d** with L-methyl glycine. The bromine on 6th position is considered to be optimum to show anticancer activity except in compound **6i**, which showed highest activity with iodine on 6th position. The cytotoxic activity of test compounds with decreasing order of **6i** > **6k** > **6n** > **6o** > **6h** > **6l** > **6m** > **6e** > **6j** is tabulated in **Table- 1**.

Table- 1: Cytotoxic activity of Compounds 6a-6o

Test compound	Structure	IC ₅₀ (µg/ml) †
6i: 2-(6-Iodo-2-phenyl quinazolin-4-yl amino)-3-phenyl propanoic acid		16.11±1.12
6k: 2-(6-Bromo-2-phenyl quinazolin-4-yl amino)-3-methyl pentanoic acid		32.02±1.73
6n: 6-Bromo-2-phenyl quinazolin-4-(2-amino-3-(1H-indol-3-yl) propanoic acid)		37.90±1.10
6o: 6-Iodo-2-phenyl quinazolin-4-(2-amino-3-(1H-indol-3-yl) propanoic acid)		46.11±1.21
6h: 2-(6-Bromo-2-phenyl quinazolin-4-yl amino)-3-phenyl propanoic acid		50.38±0.92

6l: 2-(6-Iodo-2-phenylquinazolin-4-yl amino)-3-methyl pentanoic acid		52.36±0.61
6m: 2-Phenyl quinazolin-4-(2-amino-3-(1H-indol-3-yl) propanoic acid		74.78±1.80
6e: (6-Bromo-2-phenyl quinazolin-4-yl amino) propanoic acid		104.24±1.85
6j: 3-Methyl-2-(2-phenyl quinazolin-4-yl amino)pentanoic acid		120.71±6.09
6a: (2-Phenyl quinazolin-4-yl amino) acetic acid 6b: (6-Bromo-2-phenyl quinazolin-4-yl amino) acetic acid 6c: (6-Iodo-2-phenyl quinazolin-4-yl amino) ethane peroxy acid 6d: (2-Phenyl quinazolin-4-yl amino) propanoic acid 6f: (6-Iodo-2-phenyl quinazolin-4-yl amino) propanoic acid 6g: 3-Phenyl-2-(2-phenyl quinazolin-4-yl amino) propanoic acid		ns
Etoposide*		10.56±0.70

Docking studies: In order to elucidate possible interactions for the cytotoxic activity, we have performed docking studies using FRED (Openeye Scientific Software, Inc) [26]. Conformation and minimization of the compounds was performed using Omega (Openeye Scientific Software, Inc) [26]. After this the output file is used for docking. Docking was carried out against caspase-1 protein (1BMQ) which is the possible anticancer target of quinazoline derivatives. Caspase-1 protein was chosen as a target for docking because the sensitivity of U937 monocytic cells to apoptosis induced by Etoposide and that the apoptotic process involves the activity of members of the caspase-1 subfamily. Selected target protein was retrieved from PDB. (www.rcsb.org/pdb).

RESULTS AND DISCUSSION

The predicted binding energies of 2-phenyl-6-substituted quinazolin-4-yl amino acetic acid derivatives **6a-6o** have shown good correlation with the respective inhibitory activities, i.e. **6i** has lowest scoring values and higher inhibitory activity than all analogs which are synthesized but the control Etoposide has lowest scoring value and higher inhibitory activity than all analogs. To visualize the binding conformations of these analogs with in the active site of caspase-1 protein are displayed in **Figure- 1**. In the active site region (5 \AA^0) the caspase-1 protein twenty amino acids can play an important role are shown in **Figure-2** (namely Pro 177, Arg 178, Arg 179, Thr 180, Ser 236, His 237, Gly 238, Gln 283, Ala 284, Cys 285, Gly 287, Asp 288, Val 338, Ser 339, Thp 340, Arg 341, His 342, Pro 343, Ser 347 and Arg 383). As observed in **Figure-3**, **6i** and **6k** were docked in the active site of protein with a significant binding mode compared with Etoposide molecule.

Further more, differences in the docking studies were observed that the Etoposide is having the lowest scoring values than all molecules like steric interactions, ligand donor interaction with protein acceptor, lipophilic-lipophilic interactions, AMBIG, Acc/Metal interactions and Shape similarity between ligand and protein **Figure-4**. To further validate the binding mode best scored molecule in surface form is given in **Figure-5**.

Docking functions [27-29] for analysis are given in table- 2. The predicted binding energies of novel (6-substituted-2-phenyl quinazolin-4-yl amino) substituted acetic acid derivatives **6(a-o)** have shown good correlation with the respective inhibitory activities, i.e. **6i** has lowest scoring values and higher inhibitory activity than all analogs which are synthesized but the positive control Etoposide has lowest scoring value and higher inhibitory activity than all analogs. Further more, differences in the docking studies were observed that the Etoposide is having the lowest scoring values than all molecules like steric interactions, ligand donor interaction with protein acceptor, lipophilic-lipophilic interactions, Ambig, Acc/Metal interactions and Shape, having less interactions of Ambig and Acc/metal are given in table- 3.

Figure Legends: To visualize the binding conformations of these analogs with in the active site of caspase-1 protein are displayed in **figure- 1**.

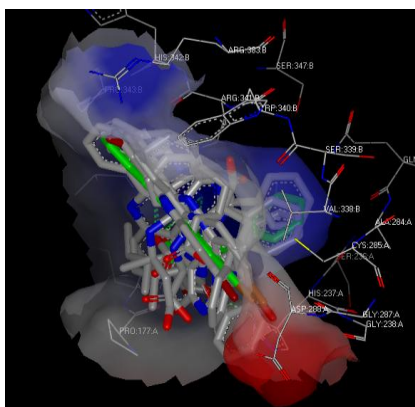


Figure- 1: All molecules in the protein active-site

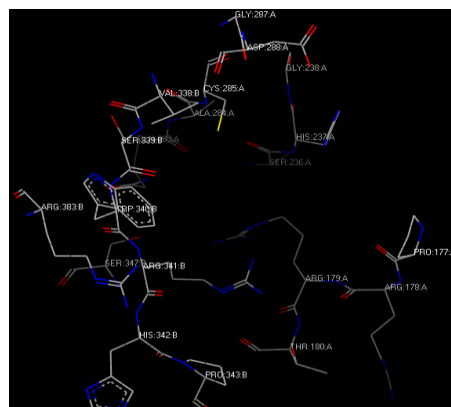


Figure- 2: Active-site Amino-acids in ball and stick model

Table - 2: Docking functions

Entry	Descriptor code	Description
1	Steric	Steric interactions
2	Donor	Contribution from ligand donor interaction with protein acceptor
3	Rb	Rotatable bond penalty
4	Aromatic	Phenyl interactions with amides, methyl and aryl CH groups
5	Plp	Piecewise linear potential
6	Clash/ss	Clash penalty / screenscore
7	Lipo	Lipophilic - lipophilic interactions
8	Ambig	Lipophilic - polar and polar - polar interactions
9	Np	All interactions of ligand non-polar atoms
10	Acc/Metal	Ligand acceptor - protein donor and all metal interactions
11	Shape	Shape complementarity between ligand and protein

Table- 3: Docking scores

Descriptor code	Etoposide	6i	6k
Steric	-63.46	-50.47	-38.59
Donor	-0.11	-0.10	0.00
Rb	10.93	12.31	12.86
Aromatic	-2.05	-2.12	-3.46
Plp	-16.40	-25.11	-8.33
Clash/ss	28.66	12.53	12.17
Lipo	-59.39	-49.33	-43.23
Ambig	-43.02	-16.71	-21.42
Np	-30.19	-23.38	-10.23
Acc/Metal	-3.76	-2.78	-1.96
Total	-178.8	-145.2	-102.2
Shape	-529.61	-365.20	-301.07

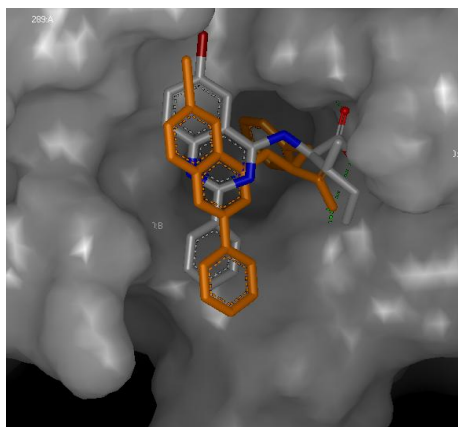


Figure- 3: 6i and 6k in active site (6i in brown color and 6k in normal color)

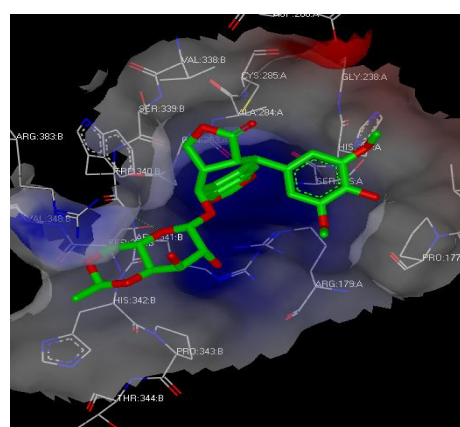


Figure- 4: Etoposide molecule in the Active-site

To further validate the binding mode best scored molecule in surface form is given in **Figure- 5**.

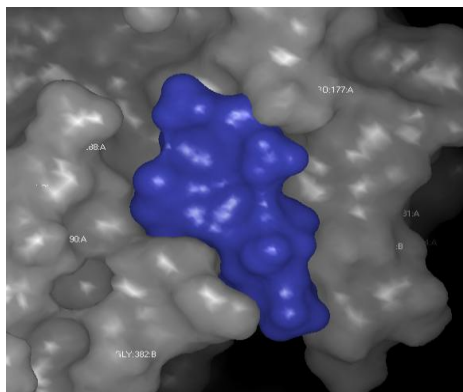


Figure 5: Etoposide in the Active-site area with surface contact in blue color

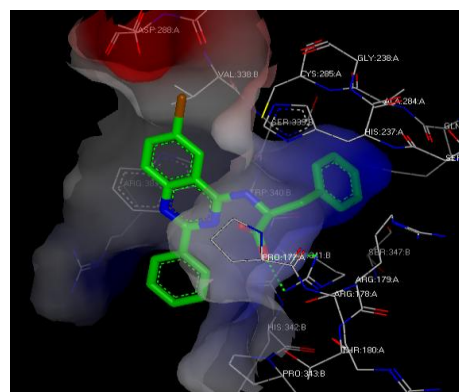


Figure- 6: 6i molecule in the Active-site

APPLICATIONS

The optimum steric, lipophilic and lipophilic-polar and polar-polar interactions are important to fit in the active site of the target. The binding energies estimated by scoring functions, were found to have a good correlation with the experimental inhibitory potencies. Based on the binding conformations from molecular docking, it is applicable that the steric and lipophilic interactions play major role to promote cytotoxic activity against U 937 cell lines.

CONCLUSIONS

The cytotoxic activity data against U937 cell lines of all the compounds **6a-6o** was correlated with structure based investigations using Docking studies. Compound **6i** has lowest scoring values and higher inhibitory activity than all analogs which are synthesized but the positive control Etoposide has lowest scoring value and higher inhibitory activity than all analogs. The optimum steric, lipophilic and lipophilic-polar and polar-polar interactions are important to fit in the active site of the target. The binding energies estimated by scoring functions, were found to have a good correlation with the experimental inhibitory potencies. Based on the binding conformations from molecular docking, it is concluded that the steric and lipophilic interactions plays major role to promote high activity.

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