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Acryloylamino-salicylanilides as EGFR PTK inhibitors

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Abstract—A series of acryloylamino-salicylanilides were synthesized as inhibitors of EGFR PTK. A strategy of pseudo six-membered ring formed through intramolecular hydrogen bonding in salicylanilides is employed to mimic the planar pyrimidine ring of quinazoline EGFR inhibitors. Acrylamido moiety is incorporated to target the Cys-773 of EGFR specifically. Some of the obtained compounds exhibited good activity as EGFR inhibitors.

The protein tyrosine kinase (PTK) plays critical roles in many of the signal transduction processes that control cell growth, differentiation, mitosis and apoptosis. The epidermal growth factor receptor (EGFR) belongs to the family of transmembrane growth factor receptor PTKs. The EGFR and its ligands (EGF, TGF-α) have been implicated in various tumors of epithelial origin (e.g., aquamous cell carcinoma, breast, ovarian and NSC lung cancer).[1,2] Thus, inhibitors of the EGFR have emerged as promising anticancer agents and two main approaches, humanized monoclonal antibodies and tyrosine kinase inhibitors, have been developed.

In the last few years, a large structural variety of compounds, such as 4-anilinoquinazolines,[3–5] 4-anilinopyrazolo[3,4-*d*]pyrimidines,[6] 4-anilinoquinoline-3-carbonitriles,[7] 4-anilinopyrazolo- and 4-anilinopyrroloquinazolines,[8] were reported as EGFR tyrosine kinase inhibitors. Representative inhibitors of the quinazoline series are illustrated in Figure 1.[9]

According to the crystal structure of the AQ4774 (Tarceva™)–EGFR complex (Fig. 2)[10] and previous structure–activity relationship (SAR) of 4-anilinoquinazolines,[3–8,11–19] pivotal interactions between the receptor and the inhibitors have been revealed as follows: (1) The quinazoline ring binds to a narrow hydrophobic pocket in the N-terminal domain of EGFR TK. (2) The N-1 of the quinazoline ring interacts with the backbone NH of Met-769 via a hydrogen bond, and similarly, a water molecule-mediated hydrogen bonding interaction is observed between the N-3 of the quinazoline ring and the Thr-766 side chain. These interactions underscore the importance of both nitrogen atoms for binding and the subsequent inhibitory capacity. In fact, replacement of a carbon atom for either nitrogen atom results in drastic loss in inhibitory activity. (3) The aniline moiety lies in a deep and hydrophobic pocket. (4) The acrylamido moiety at C-6 or C-7 of the quinazoline ring conveys an irreversible inhibition and a more potent antitumour activity as compared to the congeners in the absence of this unique moiety. Michael addition occurred between the acrylamido moiety of the inhibitor and sulfhydryl group of the Cys-773 residue is believed to be responsible for the distinct activity profile.

Herein, we report the synthesis and biological activity of a series of EGFR PTK inhibitors with an acrylamido group at the 4- or 5-position of salicylanilides. A dock model and preliminary SAR based on in vitro enzyme assay will also be discussed.

The salicylanilide molecules may assume alternative conformations via either NH···O hydrogen bonding (I) or OH···O=C hydrogen bonding (II). Intramolecular hydrogen bonding stabilizes both conformations and makes the molecule considerably rigid. By NMR spectroscopy, Suezawa et al. have shown that almost all salicylanilide derivatives without 3- or 2′(6′)-substituent form a pseudo six-membered ring via a strong hydrogen bond between OH and O=C, and take a fairly rigid and planar conformation (II) (Fig. 3).[20] The conformational similarity of II to quinazoline led to the

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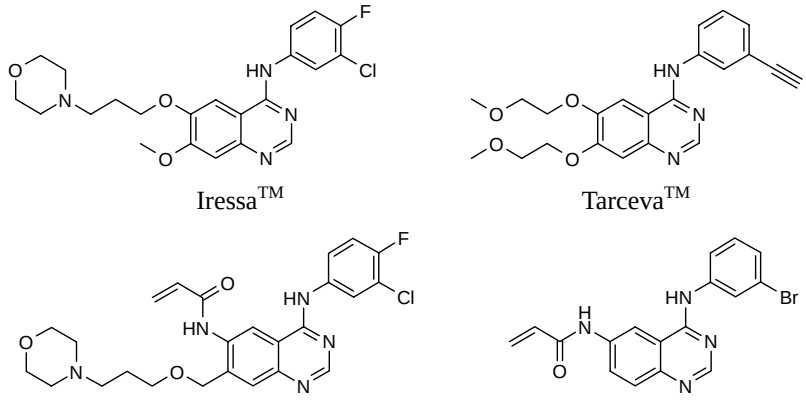


Figure 1. Representative EGFR PTK inhibitors.

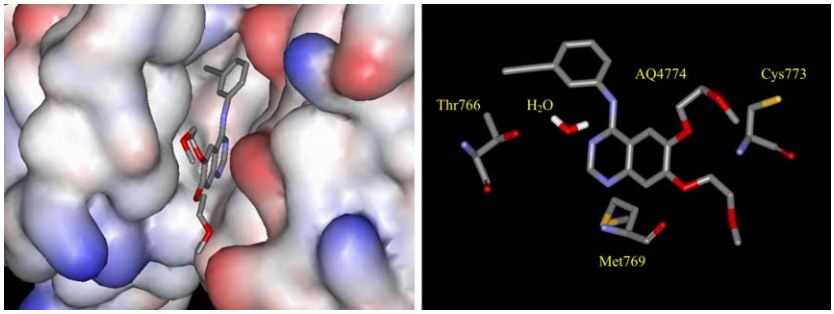


Figure 2. Crystal structure of AQ4774 (Tarceva™)–EGFR complex.

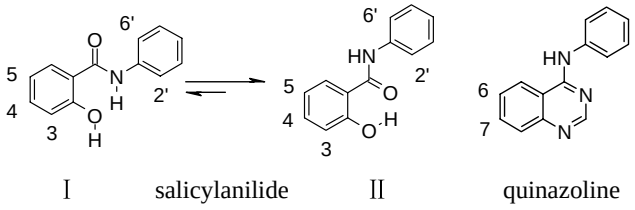


Figure 3. Salicylanilide and quinazoline.

hypothesis that the pseudo six-membered ring may function as a mimic of the pyrimidine ring of the quinazolines. The acrylamide side chain at the 5- or 4- position of salicylanilide corresponds to that of the 6- or 7-position of the quinazoline core. The presence of the acrylamido moiety favors the trapping of the Cys-773 of EGFR.

Docking studies were performed to fit salicylanilides to the enzyme. The structure of salicylanilides and Tarceva™ are in good spatial match (Fig. 4). The pseudo six-membered ring and pyrimidine ring orient in a similar manner and superpose at the same site. The oxygen atom of the phenolic group forms a hydrogen bond with the backbone NH of Met-769 as the N1 atom of the quinazoline does. The oxygen of the carbonyl group binds to a structural water molecule via hydrogen bonding similar to the way N3 atom of the quinazoline functions. This water molecule, in turn, associates with the OH of Thr-766 by another hydrogen bond. The aniline moiety situates into the same hydrophobic pocket. The acrylamido side chain extrudes from the enzyme surface towards the solvent. The γ-carbon is positioned 4.11 Å from the sulfhydryl group of Cys-773, which is favorable for the occurrence of the Michael addition.

Most of the 5-substituted salicylanilides (**5**–**14**) listed in Table 1 were obtained from 2-methoxyl-5-nitro-benzoic acid. The acid was transformed into the corresponding acid chloride using $SOCl_2$, which reacted with substituted aniline to give the corresponding anilide. Ether cleavage with BCl_3 gave 5-nitro-salicylanilide, and subsequent reduction of the nitro group and acylation of the resulting amino group with acryloyl chloride or propionyl chloride yielded 5-acryloylamino-salicylanilides (**5**, **7**, **9**–**14**). To identify the role of the *o*-hydroxyl group, 5-acryloylamino-2-methoxyl-benzanilides (**6** and **8**) were prepared. The intermediate 5-nitro-salicylanilide could also be synthesized through the reaction of the corresponding salicylic acid with substituted aniline with phosphorus trichloride in boiling toluene (**1** and **3**) (Scheme 1).

To explore the regioisomeric effect of the 4-acryloylamino moiety on the activity, 4-substituted salicylanilides (**2** and **4**) were prepared in a different approach as shown in Scheme 2. *p*-Aminosalicylic acid (PAS) reacting with acryloyl chloride gave 4-acryloylamino-salicylic acid, which was converted to 4-acryloylamino-salicylanilide by acylation with substituted aniline.

The synthesized target compounds were tested for their inhibitory activity toward the EGFR tyrosine kinase. The results are listed in Table 1. Inhibitory activities are given as percentage inhibition at four geometric concentrations of 10, 1.0, 0.1, and 0.01 μM.

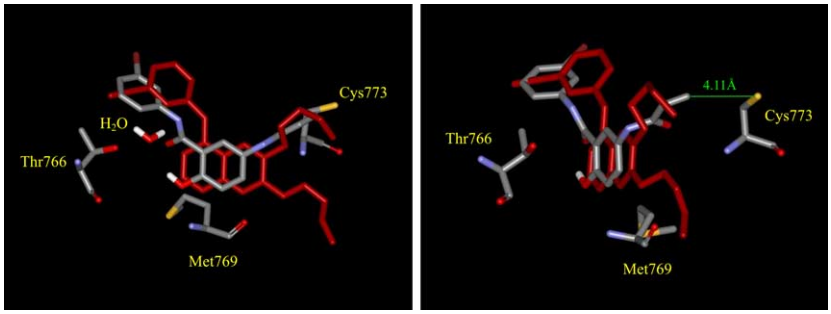
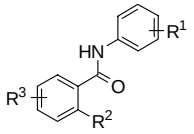


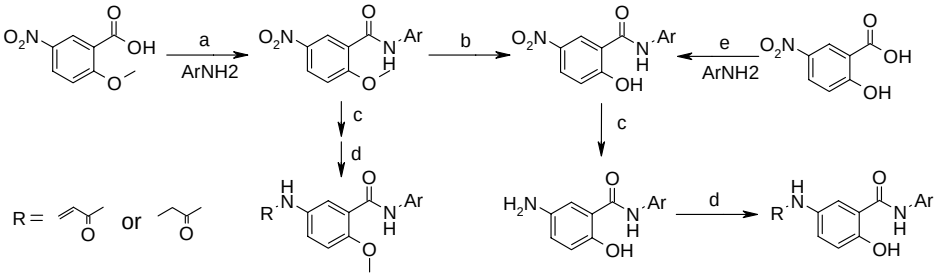
Figure 4. Compound **3** docked (gray) and X-ray crystallographic conformations of AQ4774 (red).

Table 1. Inhibitory activity of salicylanilides toward EGFR

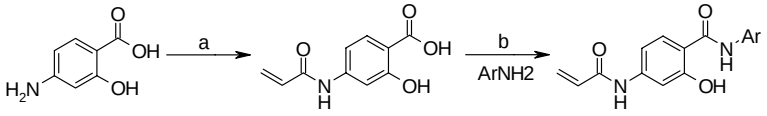


Compound	R^1	R^2	R^3	EGFR PTK (μM)			
				10	1	0.1	0.01
1	3-Cl	OH	5-CH_2=CHC(O)NH	62.6	46.3	21.6	—
2	3-Cl	OH	4-CH_2=CHC(O)NH	73.1	38.3	23.3	—
3	3-Br	OH	5-CH_2=CHC(O)NH	91.3	89.9	88.2	76.0
4	3-Br	OH	4-CH_2=CHC(O)NH	93.0	90.6	89.5	52.9
5	3-PhO	OH	5-CH_2=CHC(O)NH	91.2	89.3	86.6	18.8
6	3-PhO	OMe	5-CH_2=CHC(O)NH	1.59	—	—	—
7	3-Ph(OH)CH	OH	5-CH_2=CHC(O)NH	93.5	91.7	84.8	49.3
8	3-Ph(OH)CH	OMe	5-CH_2=CHC(O)NH	5.84	—	—	—
9	3-Et_2NC(O)	OH	5-CH_2=CHC(O)NH	63.9	54.4	40.4	14.1
10	3-Et_2NC(O)	OH	5- CH_3CH_2C(O)NH	46.6	—	—	—
11	4-PhO	OH	5-CH_2=CHC(O)NH	62.2	28.1	0.8	0
12	4-PhO	OH	5-CH_3CH_2C(O)NH	48.5	—	—	—
13	3-$(CH_2)_4$NC(O)	OH	5-CH_2=CHC(O)NH	91.0	88.6	84.2	26.0
14	3-$(CH_2)_5$NC(O)	OH	5-CH_2=CHC(O)NH	91.9	91.2	88.0	75.1

Enzyme tests were performed using ELISA. '—' not determined.



Scheme 1. Reagents and conditions: (a) $SOCl_2$, reflux, then $ArNH_2$, Et_3N, THF, rt; (b) BCl_3, dichloromethane, rt; (c) H_2, Raney-Ni, methanol, 1 atm, rt; (d) Acryloyl chloride or propionyl chloride, Et_3N, THF, rt; (e) PCl_3, toluene, reflux.



Scheme 2. Reagents and conditions: (a) Acryloyl chloride, NaOH (aqueous), rt; (b) PCl_3, toluene, reflux.

The data presented in Table 1 clearly show that most compounds exhibit high activities toward EGFR. The percentage inhibitions of the three compounds (**3**, **4** and **14**) at a concentration of 0.01 μM are more than 50%. When the phenolic hydroxyl groups of the active compounds **5** and **7** are methylated, the resulting molecules **6** and **8** are abolished from the ability to form the intramolecular hydrogen bond and are devoid of the

activity (inactive at 10 μM), indicating that the pseudo six-membered ring formed through the intramolecular hydrogen bond between OH and O=C in the salicylic acid part is important for the inhibition.

The presence of acrylamido group at position **4** or **5** of the salicylic acid moiety is another prerequisite for the inhibition, as shown in compounds **1**–**5**, **7**, **9**, **11**, **13**, and **14**. However, the replacement of a saturated propionamido group (compounds **10** and **12**) for the acrylamido group (compounds **9** and **11**) leads to significant decrease in activity, which agrees with the hypothesis that acrylamido group is critical for the inhibitory activity by alkylation reaction with Cys-773 as Michael acceptor.

Compounds with bulky substituents such as phenoxy and benzyl in the 3-position of the aniline moiety (compounds **5**, **7**, **9**, **11**, **13**, and **14**) were shown to be active, suggesting that the hydrophobic pocket of the kinase can accommodate large groups.

The structural features of acryloylamino-salicylanilides as EGFR PTK inhibitors include: (1) Salicylanilide is implicitly necessary as the structure framework, and the phenolic hydroxyl group is very important for the pseudo six-membered ring. (2) The acrylamido side chain as Michael acceptor increases the inhibitory activity. (3) The substituent at the aniline moiety is favorable for activity.

It could be verified that the pseudo six-membered ring formed by the intramolecular hydrogen bond in salicylanilides can isosterically replace pyrimidine ring of the quinazolines. Acrylamido side chain enhances inhibition activity of EGFR PTK. Not only halogen but bulky substituents at the aniline moiety are beneficial for the increase in the inhibitory activity.

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