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Synthesis and biological evaluation of a series of novel salicylanilides as inhibitors of EGFR protein tyrosine kinases

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Abstract

The synthesis and biological evaluation of two series of salicylanilide derivatives on the EGFR and ErbB-2 tyrosine kinases inhibitory activities were conducted. Of the tested compounds those having an additional aryl group substituted on the anilino ring were active on the EGFR tyrosine kinase inhibition (**7a–c** and **13a**, **13c**, **13d**, **13f**). The inhibitory activities were all in the low micromolar or submicromolar range. In addition, compound **13a** was found to have dual inhibitory activities both on EGFR and ErbB-2 tyrosine kinases (1.654 ± 1.280 and 7.134 ± 1.265 μmol/L).

Keywords: Salicylanilides; EGFR; HER2; Tyrosine kinase inhibitor

ErbB family type I receptor tyrosine kinases (EGFR, HER2, ErbB-3 and ErbB-4) play a critical role in mediating growth factor signaling [1]. Overexpression of EGFR and/or HER-2 (ErbB-2) has been associated with oncogenic activity such as unregulated cell growth, proliferation, differentiation and survival [2]. For this reason, they become attractive targets for the design of novel anticancer drugs [3–6]. Two main approaches, humanized monoclonal antibodies and tyrosine kinase inhibitors, have been developed. The ability to disrupt the ErbB-family signaling pathway either by monoclonal antibody therapy [7] or small molecule ATP-competitive kinase inhibitors has yielded novel anticancer agents [8]. In the last few years, a large structural variety of compounds, such as 4-anilinoquinazolines [9–11], 4-anilinopyrazolo[3,4-d]pyrimidines [12], 4-anilinoquinoline-3-carbonitriles [13], 4-anilinopyrazolo- and 4-anilinopyrroloquinazolines [14], were reported as EGFR tyrosine kinase inhibitors. As we know, Iressa (**1**) [15] and Tarceva (**2**) [16] (Fig. 1) have been developed and approved for the chemotherapeutic treatment of patients with advanced non-small-cell-lung cancer. The dual EGFR/HER-2 inhibitor Lapatinib (**3**) [17] was recently approved for the treatment of HER2-positive metastatic breast cancer [18]. The latter possesses a novel anilino-aryl head-group that yields dual EGFR/ErbB-2 potency (Fig. 1).

These agents all belong to the 4-anilinoquinazoline class of inhibitors and the key features between the receptor and this template have been revealed as follows [19,20]. The quinazoline moiety fits into the ATP binding pocket of the kinase domain, where the *N*-1 nitrogen of the quinazoline nucleus interacts with the backbone NH of Met-769 *via* a

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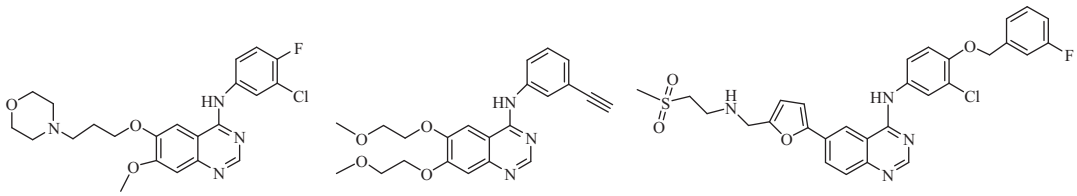


Fig. 1. Structures of Iressa, Tarceva and Lapatinib.

hydrogen bond, and water mediated hydrogen bonding is observed between the *N*-3 of the quinazoline and the Thr-766 side chain. In addition, the aniline ring fills an adjacent lipophilic pocket. Furthermore, the solubilizing side chains at C-6 and/or C-7 of the quinazoline core act to improve physical properties and confer a more favorable pharmacokinetic profile. Many more compounds of this type are still under evaluation in clinical trials for the treatment of cancer [21].

According to the results of Hodge and Traxler that revealed a bioisosteric relationship between the salicylic group and the quinazoline (illustrated in Fig. 2) [22,23], the *pseudo* six-membered ring formed by the intramolecular hydrogen bridge between OH and the carbonyl oxygen would function as a mimic of the pyrimidine ring of the quinazoline [24]. The biological activities of salicylanilides are usually positively related to the electron-withdrawing ability of the substituent at the anilino moiety [25], because the substituent of higher electron-withdrawing ability facilitates the closed-ring conformation of salicylanilides required to be functional [26]. So far many salicylanilides derivatives were synthesized using salicylic acid core structure function as an important pharmacophore group [25,27].

Enlightened by the recently reported small molecule EGFR-tyrosine kinases inhibitor such as Tarceva (**2**) and Lapatinib (**3**), we would like to report here the design, synthesis and biological activity of a series of salicylanilide derivatives as new tyrosine kinase inhibitors which specifically target EGFR and/or ErbB-2 tyrosine kinases. We reserved the solubilizing side chains of **2** and **3** and put them into the salicylic acid core structure to construct two series of salicylanilides derivatives **7a–c** and **13a–h** (Scheme 1), respectively. At the same time, different groups were introduced to 3′- and/or 4′-positions of the anilino portion to see if they could keep or enhance the interaction with the EGFR and/or HER-2 tyrosine kinase.

The synthetic routes towards the designed two series compounds are depicted in Scheme 1. Compounds **7a–c** were prepared from **4** [28] in three steps. Compound **4** was converted to **5** by treated with 10% NaOH aqueous solution and then the amino-group of **5** was transformed into hydroxyl group according to the methodology reported by Cohen et al. [29] to afford compound **6**. Acylations of various of anilines by 2-hydroxy-4,5-bis(2-methoxy-ethoxy)benzoic acid **6** were achieved in the presence of EDC·HCl and HOAt (1-Hydroxy-7-azabenzotriazole) in DMF/THF (1:8) co-solvent and afforded the corresponding target compounds **7a–c** in good yield, respectively.

The synthetic route towards **13a–m** is also depicted in Scheme 1. The first step was the coupling of commercially available 2-hydroxy-5-iodobenzoic acid **8** with various anilines in the presence of EDC·HCl and HOAt in DMF/THF (1:8) co-solvent to provide amides **9a–m**. Then the palladium-catalyzed Suzuki coupling reaction [30] was adopted to introduce the 5-formylfuran-2-yl into the amides **9a–m** and to afford **11a–m**. Finally, reductive amination of the aldehyde with 2-aminoethylmethylsulfone **12** yielded the desired analogs **13a–m**.

Target compounds **7a–c** and **13a–m** were evaluated for their EGFR and ErbB-2 tyrosine kinase activity following a method described by Brignola and co-workers [31]. The IC_{50} values are listed in Table 1. It is interesting that of the

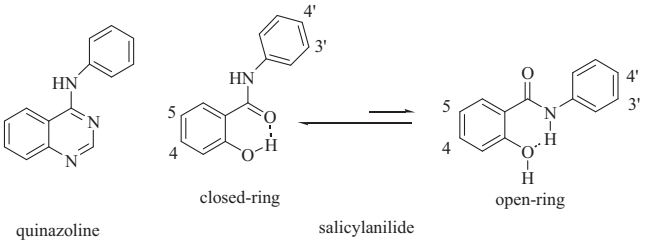
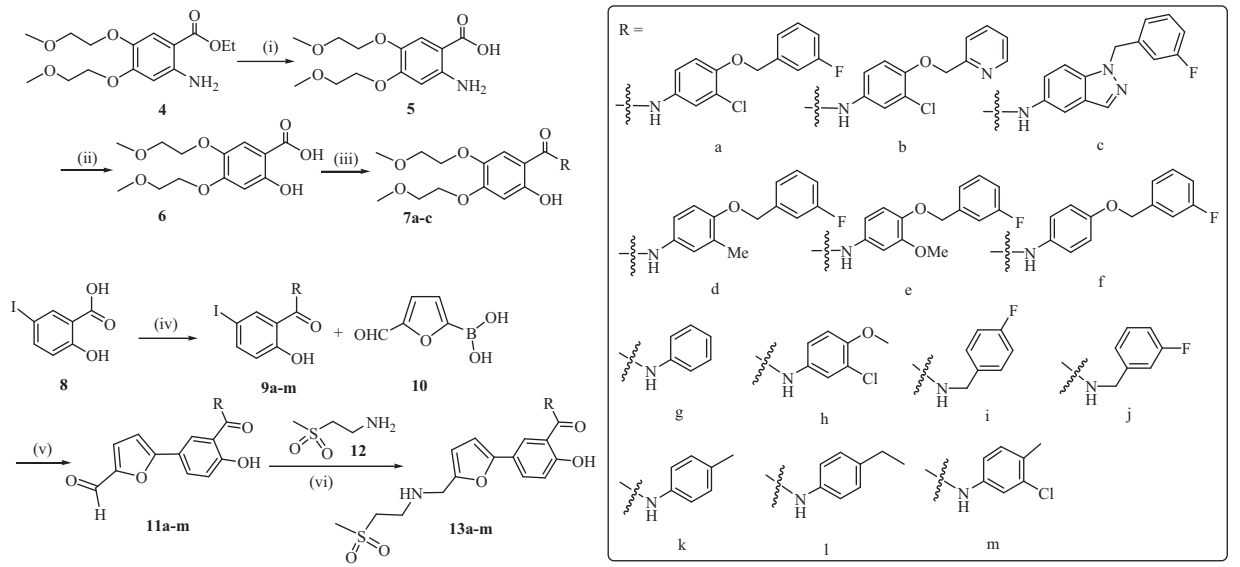


Fig. 2. Salicylanilide and quinazoline.



Scheme 1. Reagents and conditions: (i) 10% NaOH, 92%; (ii) $NaNO_2$, concentrated H_2SO_4, 120 °C, 43%; (iii) RNH_2, EDC·HCl, HOAt, DMF, 59% for **7a**, 49% for **7b**, 67% for **7c**; (iv) RNH_2, EDC·HCl, HOAt, DMF/THF, 95% for **9a**, 50% for **9b**, 79% for **9c**, 79% for **9d**, 83% for **9e**, 80% for **9f**, 50% for **9g**, 94% for **9h**, 94% for **9i**, 96% for **9j**, 85% for **9k**, 94% for **9l**, 94% for **9m**; (v) KOH, 5 mol% $Pd(OAc)_2$, acetone/H_2O, 35 °C, 66% for **11a**, 29% **11b**, 73% for **11c**, 86% for **11d**, 54% for **11e**, 94% for **11f**, 73% for **11g**, 39% for **11i**, 31% for **11j**, 71% for **11k**, 59% for **11l**, 85% for **11m**; (vi) $MgSO_4$, $NaBH_4$, dry THF, reflux, 70% for **13a**, 19% for **13b**, 55% for **13c**, 19% for **13d**, 29% for **13e**, 49% for **13f**, 36% for **13g**, 35% for **13h**, 30% for **13i**, 29% for **13j**, 33% for **13k**, 36% for **13l**, 38% for **13m**.

Table 1
EGFR and ErbB-2 tyrosine kinases inhibition activity.[a]

Compound	EGFR IC_{50} (μmol/L)	ErbB-2 IC_{50} (μmol/L)	Compound	EGFR IC_{50} (μmol/L)	ErbB-2 IC_{50} (μmol/L)
7a	0.780 ± 0.380	>10	**13f**	4.928 ± 5.366	>10
7b	0.990 ± 0.400	>10	**13g**	>10	>10
7c	0.300 ± 0.218	>10	**13h**	>10	>10
13a	1.654 ± 1.280	7.134 ± 1.265	**13i**	>10	>10
13b	>10	>10	**13j**	>10	>10
13c	0.740 ± 0.380	>10	**13k**	>10	>10
13d	2.522 ± 0.783	>10	**13l**	>10	>10
13e	>10	>10	**13m**	>10	>10
Lapatinib	0.006 ± 0.001	0.044 ± 0.016			

[a] IC_{50} values are shown as the mean ± SD from three separate experiments.

tested compounds only those having an additional aryl group substituted on the anilino ring were active on the EGFR tyrosine kinase inhibition (**7a–c** and **13a**, **13c**, **13d**, **13f**). The inhibitory activities were all in the low micromolar or submicromolar range. In contrast, compounds **13f** and **g** with no substituted aniline or a simply substituted anilino moiety had no inhibitory activities on EGFR tyrosine kinase. In addition, of the active compounds **7a–c** with two side chains on the salicylic acid core structure were shown to be more active than those bearing a 5-methylsulfonylethylamino-2-furanyl group (**13a**, **13c**, **13d**, **13f**) generally. However, only compound **13a** has dual inhibitory activities both on EGFR and ErbB-2 tyrosine kinases (1.654 ± 1.280 and 7.134 ± 1.265 μmol/L). Although the activity of **13a** is lower than Lapatinib, it can be a start lead compound for these types of compounds at the very beginning and could be further optimized.

In summary, we have conducted the synthesis and biological evaluation of two series of salicylanilide derivatives on the EGFR and ErbB-2 tyrosine kinases inhibitory activities. The preliminary structure–activity relationships were discussed and several compounds were found to be potent inhibitors of EGFR protein tyrosine kinases. All the results

obtained here will render new clues to the understanding of the tyrosine kinases inhibitory profiles for these types of compounds.

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