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Synthesis and evaluation of salicylanilide derivatives as potential epidermal growth factor receptor inhibitors

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[**Abstract**] Two series of novel salicylanilide were synthesized as potential EGFR inhibitors. The enzyme inhibitory activity against EGFR of all compounds was carried out, and their anti-proliferative activities against the A549 and A431 cell lines were also evaluated. Of the compounds studied, majority of them exhibited high anti-proliferative activities compared to gefitinib. Especially, **12a** and **12b** exhibited stronger inhibitory activity against EGFR with IC_{50} values of 10.4±2.25 and

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15.4±2.33 nM, respectively, which were comparable to the positive control of gefitinib (IC_{50}=12.1±2.21 nM). Compound **12b** also showed outstanding inhibitory activity against A431 and A549 cell lines with the IC_{50} values of 0.42±0.43 μM and 0.57±0.43 μM, which was better than the positive controls. In the molecular modelling study, compound **12b** was bound into the active pocket of EGFR with two hydrogen bond and with minimum binding free energy ΔG_b=-25.1125 kcal/mol. The result also suggested that compound **12b** could bind the EGFR kinase well.

Keywords: salicylanilide derivatives; EGFR inhibitor; anti-proliferative effects; molecular modeling

1. Introduction

The protein tyrosine kinase (PTK) plays critical roles in many signal transduction processes. Deregulated PTK activity has been observed in many proliferative diseases [1]. Among the PTKs, the ErbB family, particularly epidermal growth factor receptor (EGFR) kinase has been identified as being important in cancer. The EGFR has been implicated in various tumors of epithelial origin (e.g., breast, aquamous cell carcinoma, ovarian and non-small cell lung cancer) [2-3]. Thus, the EGFR has emerged as promising anticancer target in pharmaceutical studies and a lot of EGFR inhibitors have been used in clinical. Representative small-molecular inhibitors of EGFR are the quinazoline derivatives, such as gefitinib, erlotinib and canertinib (**Fig 1**). Among them, 4-anilinoquinazoline moiety, plays a significant role in their inhibiting activity. This sort of inhibitors generally exhibit high potency *in vitro*, inhibiting EGFR at low-molar concentration [4].

Figure 1. Representative EGFR inhibitors

The salicylanilide molecule may be via OH…O=C hydrogen bond to form a pseudo six-membered ring [5]. Thus, salicylanilide is supposed to have the same

pharmacophore as 4-anilinoquinazoline, and widely used as EGFR inhibitors [6-7]. Along with a large number of salicylanilide derivatives reported, the pharmacological activity of it has been confirmed. Furthermore, their structure-activity relationship (SAR) has been clearly revealed (**Fig 2**): (1) Aniline fragment in the hydrophobic pocket of the kinase, which could accommodate large groups. (2) Introduction of the electron-donating substituent into the 4′-position showed a better activity than electron-withdrawing substituent [8-9]. (3) At the 3′-position, small, lipophilic and electron-withdrawing substituent was both well tolerated [6]. (4) The presence of electron-donating group at 4-position or 5-position of the salicylanilide moiety was favorable to the improvement of anti-tumor activity.

On this background we have recently studied a series of salicylanilide derivatives which were evaluated for their anti-tumor activities (**D**) (**Fig 3**) [10]. These compounds have been synthesized by a structural modification of 4-position, and the preliminary pharmacological showed that some of them displayed considerable anti-proliferative. Owing to our continued studies on salicylanilide as an attractive molecular skeleton as anti-tumor agents, we have designed a number of new salicylanilide derivatives containing two kinds of side chain at the 4-position and biologically evaluated them *in vitro* anti-proliferation activities and anti-EGFR activities, wish to communicate their potential as EGFR inhibitors (**Series a and Series b**) (**Fig 3**). In the present study, the substitution pattern at the 4-substituted salicylic acid was selected based on different electronic environment which would affect the hydrophobicity. The objective of forming these target compounds is an attempt to obtain potential EGFR inhibitors.

Figure 2. The structure of the salicylanilide

Figure 3. The salicylanilide derivatives reported as anti-tumor inhibitors and proposed as EGFR inhibitors

2. Chemistry

The general synthetic method for the title compound was shown in Scheme 1. Esterification of the starting material 2, 4-dihydroxybenzoic acid **1** in the presence of $NaHCO_3$, KI and methanol at 40 °C gave methyl 2, 4-dihydroxybenzoate **2**. Alkylation of intermediates **2** with 1, 3-dibromopropane in acetone afforded methyl 4-(3-bromopropoxy)-2-hydroxylbenzoate **3** which was then condensed with morpholine/piperidine **4a-4b** to obtain compounds **5a-5b**. Subsequent hydrolysis in basic condition afforded compounds **6a-6b,** and both were transformed into the corresponding acid chloride using $SOCl_2$ without purification, then reacted with an appropriate substituted amine **8-13** in the presence of triethylamine to give the title compounds **8a-13a** and **8b-13b**, respectively.

Scheme 1. Reagents and conditions: (a) CH_3I, $NaHCO_3$, DMF, 40 °C,10 h; (b) 1, 3 dibromopropane, K_2CO_3, TEBAC, acetone, 60 °C,8 h; (c) $NaHCO_3$, NaI, isopropanol, 85 °C,6 h; (d) NaOH,CH_3OH, H2O, r.t, 6 h; (e) $SOCl_2$, DMF, 85 °C, 6 h; (f) Et_3N, CH_2Cl_2, ice bath, 40 min.

3. Results and discussion

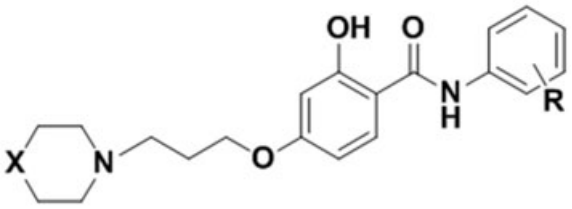
3.1. EGFR kinase inhibitory assay

The inhibitory activity of the novel salicylanilide derivatives against EGFR was evaluated, and gefitinib used as employed as positive control. IC_{50}s of the compounds were calculated according to inhibitory ratios, the *in vitro* enzymatic inhibition assay results was summarized in **Table 1**.

The data demonstrated that tested compounds exhibited potent inhibitory activity against EGFR. As shown in **Table 1**, compounds **8b-13b** showed the higher inhibitory activities than compounds **8a-13a**, possibly because the morpholine ring in

4-position side chain alkyl was high hydrophilic than piperidine ring, which decreased its interaction with EGFR protein. The IC_{50} values were different among **8a-13a** and **8b-13b**, because substituent was different in aniline. But many compounds without electron-withdrawing substituents on the 3-position of the aniline showed poor EGFR inhibitory activity which demonstrated that electron-withdrawing substituent was essential for this kind of EGFR inhibitors. Among them, compounds **8a, 13a, 9b, 8b** showed the moderate inhibitory activities, while **9a, 10a** with the lowest inhibitory activities. On the other hand, replacement of the electron-donating (CH_3) by CF_3 was favorable for the EGFR inhibitory (compounds **11a** and **11b**). Whereas, the compound **10b** was not following the rule, which suggested the substituent at the 4-position played a significant role in anti-EGFR activity. Furthermore, although the compounds of **8a**-**13a** exhibited poor activities to EGFR, it was quite interesting to note that **12a** exhibited the potent activity to EGFR (IC_{50}= 10.4±2.25 nM) and more comparable to the positive control gefitinib (IC_{50}= 12.1±2.21 nM). Meanwhile, compound **12b** displayed moderate inhibitory activity (IC_{50}= 15.4±2.33 nM). The most possible reason was that the 3-Cl, 4-F aniline moieties increased EGFR inhibition activities.

Table 1. EGFR inhibition of the salicylanilide derivatives **8a-13a** and **8b-13b** (n=3, $\bar{x} \pm s$)



Compounds	X	R	IC_{50}(nM)
8a	O	3-Br	85.8±6.41
9a	O	3-Cl	195.8±8.76
10a	O	3-CH_3	142.1±8.34
11a	O	3-CF_3	34.6±3.22

12a	O	3-Cl,4-F	10.4±2.25
13a	O	2-Cl,4-NO_2	84.8±4.23
8b	C	3-Br	45.7±2.66
9b	C	3-Cl	63.9±6.44
10b	C	3-CH_3	22.4±2.49
11b	C	3-CF_3	35.2±4.68
12b	C	3-Cl,4-F	15.4±2.33
13b	C	2-Cl,4-NO_2	23.0±3.65
Gefitinib	-	**-**	12.1±2.21

3.2 Inhibitory activity on A549 and A431

The anti-proliferative effects of the novel salicylanilide derivatives on human pulmonary carcinoma A549 and squamous cell carcinoma cell line A431 were investigated with gefitinib as controls by applying the 3-(4, 5-dimethylthylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The A431 and A549 cell lines were known to over express EGFR, which was related to abnormal activity of EGFR pathway in many carcinogenesis. Generally, the activity of inhibiting A431 and A549 cell proliferation were determined after 72 h of treatment with various concentrations (0.003-50 μM) of the tested compounds. The results were expressed as IC_{50} values (**Table 2**).

As shown in **Table 2**, when treated of A431 cells and A549 with compounds **8a-13a** and **8b-13b**, which made the cell proliferation decrease. Among the two series of novel salicylanilide derivatives, SAR studies demonstrated that almost all the compounds belonging to the **series b** showed good activity both with anti-proliferative activity and EGFR inhibition. The results also indicated that the substituented piperidine ring substituent at the 4-position could significantly increase the activity. Here, compound **12b** displayed the most potent inhibitory activity (IC_{50}= 0.42±0.43 μM for A431 and IC_{50}= 0.57±0.43 μM for A549), and comparable to the

positive control gefitinib (IC_{50}= 4.28±3.93 μM against A431 and IC_{50}= 15.56±3.93 μM against A549).

In the assay, anti-proliferative activities of some compounds were inconsistent with their kinase assay data. Compound **8b** (IC_{50}= 2.07±1.47 μM against A431 and IC_{50}= 6.96±1.47 μM against A549) was found to have about 2-3 times than gefitinib in activity against all of the tested cancer cells. These implied that compound **8b** may exert inhibition on the proliferation not only *via* blocking the EGFR signaling, but also *via* other pathways. Compound **12a** did not exhibit excellent anti-proliferative potency even though it had the most potent inhibitory activity against EGFR. Nonetheless, it also displayed equivalent inhibition activity compared to gefitinib. Once again, the excellent anti-proliferation activity of compounds **12a** and **12b** proved the positive impact of the 3-Cl, 4-F aniline moieties. In the case of all the compounds, compounds **8a-10a** and **9b** showed almost no inhibitory activities (IC_{50}>50 μM). Results for the **11a** (37.53±4.77 μM against A549) have shown equivalent potencies in inhibition compared to the **11b** (16.28±1.82 μM against A549). Whereas the compound **11b** showed weaker inhibitory activity against A431, comparing stronger inhibitory activity against A549. Consistent with their kinase inhibition, compounds **10b** and **13b** displayed moderate anti-proliferative activities on A549 and A431.

Table 2. Anti-proliferative activity of title salicylanilide compounds

Compounds	**IC_{50}(μM)**	
	A431	**A549**
8a	> 50	> 50
9a	> 50	> 50
10a	> 50	> 50
11a	9.32±4.77	37.53±4.77
12a	6.06±1.24	14.42±1.24

13a	29.86±4.77	> 50
8b	2.07±1.47	6.96±1.47
9b	> 50	> 50
10b	59.71±2.87	18.15±2.87
11b	> 50	16.28±1.82
12b	0.42±0.43	0.57±0.43
13b	10.58±1.32	21.12±1.32
Gefitinib	4.28±3.93	15.56±3.93

3.3 Molecular docking study with EGFR

To give structural insight into the ligand/enzyme interactions, and to give an explanation and understanding of good activity observed, we proceeded to examine the interaction of those with EGFR complex structure (PDB code: 2ITO) by molecular docking, of compounds into the ATP binding site in EGFR. The binding free energy of all the compounds was mentioned in **Table 3**. Of the compounds studied, compound **12b** was nicely bound into the active site of EGFR with minimum binding free energy ΔG_b= -25.1125 kcal/mol. The binding model of compound **12b** with EGFR was depicted in **Figures 4a** and **4b**. In general, the aniline of compound 12b could fit well into the hydrophobic pocket. In the binding mode, compound **12b** was nicely bound to the ATP binding site of EGFR through hydrophobic interaction and the binding was stabilized by two hydrogen bonds. Among them one hydrogen bond formed H atom of -OH group and MET793, second one between O atom of carbonyl group and MET793. From this binding model, it could be concluded that two hydrogen bonds were responsible for the effective EGFR inhibition.

Table 3. Binding free energy of compounds **8a-13a** and **8b-13b** with EGFR

Compounds	Binding free energy ΔGb (kcal/mol)	Compounds	Binding free energy ΔGb (kcal/mol)
8a	-21.1105	**8b**	-19.1034
9a	-19.0346	**9b**	-16.1552
10a	-18.1034	**10b**	-22.5462
11a	-17.5341	**11b**	-22.1031
12a	-24.1109	**12b**	-25.1125
13a	-18.0346	**13b**	-21.1325
Gefitinib	-24.1523		

Figure 4. (a) Compound **12b** (light blue) docked into the EGFR binding domain. (b) Dock structure of compound **12b** with EGFR. Hydrogen bonds towards MET793 were highlighted.

4. Conclusion

In general, the aim of the present investigation was to design two series of new salicylanilide derivatives by introduction of substituted side chain at the 4-position for evaluating their anti-EGFR activities. The anti-proliferative activities against the human cancer cell line A431 and A549 were evaluated by MTT assay, which had almost the same trend as the anti-EGFR activities assay. Some magnificent biological results have been obtained and it has been concluded that majority of the compounds showed effective both against anti-EGFR and anti-proliferative activity. EGFR kinase assay and MTT assay showed that an electron-withdrawing group in 3′-position or pyridine ring in 4-position would maintain the inhibitory activity of the salicylanilides pharmacophore at a high level. In the case of inhibitory activities, compounds **12a** and

12b against EGFR had been found to be the most effective members of the two series. But, the compound **12a** did not show excellent activity against EGFR. Compound **12b** demonstrated the most potent inhibitory activity that inhibited the growth of tumor cell, which was almost 10 times compared to the positive control gefitinib. Molecular docking of the most potent inhibitor **12b** into ATP binding site of EGFR kinase was performed. The docking study also revealed that compound **12b** nicely bound into the active site of EGFR with minimum binding free energy. So, it is worthy of elucidate the molecular mechanism of compound **12b** and **12a** in detail**.** These results are of help in the rational design of higher selectivity EGFR inhibitors in further.

5. Experimental section

5.1 General

All analytical grade chemicals and solvents were purchased from commercial sources and used without further purification in our laboratory. Melting points were determined by a WRS-1B apparatus and were reported without any correction. IR spectra were recorded on a Nicolet Avatar 370DTGS spectrometer using KBr film. The ^{1}H and ^{13}C NMR spectra were collected on Bruker Anance-400 MHz instruments using deuterated solvents with tetramethylsilane (TMS) as internal standard. EI-MS was recorded on FINNIGA LCQ ADVANTAGE MAX apparatus. TLCs and preparative TLC were performed on silica gel GF/UV 254, and the chromatograms were conducted on silica gel (10-20 mesh) and visualized under UV light at 254 and 365 nm.

5.1.1 Synthesis of 2, 4-dihydroxy benzoic acid methyl ester (2) [11]

2, 4-dihydroxy benzoic acid (10 g, 0.065 mol) was dissolved in 100 mL of dry DMF, $NaHCO_3$ (6.5 g, 0.077 mol) was added and stirred for several minutes at room temperature. Then, CH_3I (13.8 g, 0.097 mol) was added. The reaction mixture was

stirred at 40 °C and monitored by TLC. Upon completion, 100 mL of water was poured into the reaction mixture and extracted with ethyl acetate (3×100 mL). The organic layer was subsequently washed with 5 % of $NaHCO_3$ (3×50 mL) and 5 % of NaCl (3×50 mL) solution, and dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford compound **8** as a white powder (9.2 g, 84.4 %): R_f=0.6 (Pe/EtOAc 3:1); mp:110.9-113.2 °C. The melting point for compound **8** is consistent with previous reports [12].

5.1.2 Synthesis of 4-(3-bromo-propoxy)-2-hydroxy benzoic acid methyl ester (3) [13]

To a solution of compound **2** (8.0 g, 0.047 mol) in 80 mL of acetone was added potassium carbonate (6.5 g, 0.047 mol) and, benzyl triethyl ammonium chloride (BTEAC) (0.1 g) followed by stirring at 60 °C for 0.5 h. Then 1, 3-dibromopropane (19.0 g, 0.095 mol) was added and stirred for another 8 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled and the solid inside was removed by filtration. The filtrate was concentrated to afford a crude product, which was further purified with a silica gel column eluted with petroleum ether-chloroform(2:1, v/v) give compound **3** as a white solid (4.8 g, 55.5 %): R_f=0.8 (Pe/EtOAc 10:1); mp: 72.8-74.5 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.97 (s, 1H), 7.77 (d, *J*=8.8 Hz, 1H), 6.48 (d, *J*=2.4 Hz, 1H), 6.46 (dd, *J*=8.8 Hz, 2.4 Hz, 1H), 4.16 (t, *J*=5.6 Hz, 2H), 3.93 (s, 3H), 3.62 (t, *J*=6.4 Hz, 2H), 2.37-2.31 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 170.3, 164.6, 163.7, 161.4, 131.3, 107.7, 101.3, 65.5, 52.0, 32.0, 29.7; IR (KBr) υ˙==3016, 2949, 1673, 1623, 1585, 1509, 1467, 1438.3, 1351, 1256, 1222, 1187, 1138, 1022, 986, 952, 917, 832 cm^{-1}; MS (ESI) m/z 294.26 $[M+H]^+$.

5.1.3 Synthesis of 4-(3-morpholinopropoxy)-2-hydroxy benzoic acid methyl ester (5a) [14]

A 50 mL of isopropanol solution of compound **3** (7.0 g, 0.024 mol), morpholine (3.1 g, 0.036 mol) and $NaHCO_3$ (3.1 g, 0.037 mol) was heated at 85 °C. The progress of the reaction was followed by TLC. After completion of the reaction, the reaction mixture was cooled and the solid was filtered. The filtrate was concentrated to afford mixture**.** The mixture was purified through silica gel column separation (ethyl acetate) to obtain compound **5a** as light yellow oil (4.8 g, 67.6 %): R_f=0.3 (EtOAc); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.95 (s, 1H, OH), 7.73 (d, *J*=8.8 Hz, 1H, ArH), 6.45 (d, *J*=2.4 Hz, 1H, ArH), 6.44 (dd, *J*=8.8 Hz, 2.4 Hz, 1H, ArH), 4.07 (t, *J*=6.4 Hz, 2H, $NCH_2CH_2CH_2O$), 3.91 (s, 3H, $COOCH_3$), 3.73 (t, *J*=4.8 Hz, 4H, morpholine ring CH_2OCH_2), 2.52-2.46 (m, 6H, morpholine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 2.00-1.93 (m, 2H, $NCH_2CH_2CH_2O$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 170.4, 165.0, 163.7, 131.2, 107.8, 105.3, 101.2, 67.0, 66.3, 55.3, 53.7, 51.9, 26.2; IR (KBr) υ˜==3416, 3066, 2956, 2861, 2824, 2782, 1663, 1626, 1582, 1508, 1470, 1436, 1402, 1354, 1306, 1259, 1222, 1191, 1144, 1113, 1028, 999, 973, 951, 915, 860, 832, 779 cm^{-1}; MS (ESI) m/z 294.26 $[M-H]^+$.

Compound **5b** was synthesized according to the procedure described above.

5.1.4 4-(3-(piperidin-1-yl)propoxy)-2-hydroxy benzoic acid methyl ester (5b)

Compound **5b** was obtain as a light yellow oil (4.9 g, 70.5 %): R_f=0.3 (EtOAc); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 10.93 (s, 1H, OH), 7.71 (d, *J*=8.8 Hz, 1H, ArH), 6.42 (d, *J*=9.2 Hz, 1H, ArH), 4.03 (t, *J*=6.4 Hz, 2H, $NCH_2CH_2CH_2O$), 3.89 (s, 3H, $COOCH_3$), 2.51-2.43 (m, 6H, piperidine ring $NCH_2CH_2CH_2O$), 2.02~1.96 (m, 2H, $NCH_2CH_2CH_2O$), 1.64~1.58 (m, 4H, piperidine ring), 1.46-1.44 (m, 2H, piperidine ring); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 170.3, 165.0, 163.7, 131.1, 107.7, 105.3, 101.2, 66.6, 55.6, 54.5, 51.8, 26.4, 25.7, 24.2; IR (KBr) υ˜==3430, 2955, 2874, 2647,

1627, 1587, 1502, 1439, 1364, 1227, 1188, 1149, 1089, 975, 905, 865 cm^{-1}; MS (ESI) m/z 294.19 $[M+H]^+$.

5.1.5 Synthesis of 4-(3-morpholinopropoxy)-2-hydroxy benzoic acid (6a) [15]

To a solution of **6a** (0.014 mol) in methanol (20 mL) and pure water (10 mL) was added NaOH (0.8 g, 0.020 mmol). The reaction mixture was stirred at room temperature. After the reaction was completed (monitored by TLC), the mixture was concentrated and acidified with HCl solution (3 $mol \cdot L^{-1}$) till pH to be 5~6, the white precipitation appeared immediately. Then precipitation was filtered and washed with water, dried to afford the **6a** (3.1 g, 81.6 %): R_f=0.58 ($CHCl_3$); white solid; mp: 199.8-201.1 °C; ^{1}H-NMR (400MHz, DMSO-d_6) δ: 7.65 (d, *J*=8.8 Hz, 1H, ArH), 6.38 (s, 2H, ArH), 4.08 (t, *J*=5.6 Hz, 2H, $NCH_2CH_2CH_2O$), 3.78 (s, 4H, morpholine ring CH_2OCH_2), 2.98 (s, 6H morpholine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 2.07 (s, 2H, $NCH_2CH_2CH_2O$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 172.4, 164.0, 163.8, 131.9, 106.8, 106.7, 101.5, 65.7, 64.6, 54.2, 52.1, 24.2; IR (KBr) υ==2949, 2872, 1591, 1504, 1436, 1369, 1263, 1184, 1112, 1083, 983, 932, 866, 830, 779 cm^{-1}; MS (ESI) m/z 280.19 $[M-H]^+$.

Compounds **6b** was synthesized according to the procedure described above.

5.1.6 4-(3-(piperidin-1-yl)propoxy)-2-hydroxy-benzoic acid (6b) Compound **6a** was obtained as white solid (3.2 g, 82.1 %): R_f=0.58 ($CHCl_3$); mp: 180.5-182.2 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 12.89 (s, 1H, COOH), 10.87 (s, 1H, OH), 7.71 (d, *J*=9.6 Hz, 1H, ArH), 6.47 (d, *J*=9.6 Hz, 2H, ArH), 4.12 (t, *J*=6.0 Hz, 2H, $NCH_2CH_2CH_2O$), 3.42 (s, 2H, $NCH_2CH_2CH_2O$), 3.17 (t, J=7.8 Hz, 2H, piperidine ring CH_2N), 2.87 (s, 2H, piperidine ring CH_2N), 2.23-2.18 (m, 2H, $NCH_2CH_2CH_2O$), 1.79 (s, 4H, piperidine ring CH_2), 1.69~1.40 (m, 2H,piperidine ring CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 172.4, 164.0, 163.8, 131.9, 106.8, 106.7, 101.5, 65.7, 64.6,

54.2, 52.1, 24.2; IR (KBr) υ=3373, 2954, 2873, 1591, 1505, 1438, 1363, 1265, 1185, 1089, 1049, 978, 907, 830, 786 cm^{-1}; MS (ESI) m/z 278.40 $[M-H]^+$.

5.1.7 Synthesis of 4-(3-morpholinopropoxy)-2-hydroxy benzoyl chloride (7a) [16]

To a solution of **6a** (7.1 mmol) in excess thionyl chloride (15 mL), DMF (3 drops) was added. Then, the solution stirred at 50 °C for 3 h. After completion of the reaction, the solvent was evaporated and the crude was dissolved with CH_2Cl_2 (10mL) and cooled for the next step.

Compounds **7b** was synthesized according to the procedure described above.

5.1.8 Synthesis of 4-(3-morpholinopropoxy)-*N*-(3-bromophenyl)-2-hydroxy-benzamide (8a)

The compound **7a** was added dropwise to a cooled solution of dichloromethane (20 mL) (ice bath) of 3-bromoaniline (7.6 mmol), triethylamine (2 mL, 14.4 mmol), and stirred for 10 minutes at 0-5 °C. After completion of the reaction and monitored by TLC, the resulted mixture was added to 10mL of water and extracted with ethyl dichloromethane (3×20 mL). The organic phase was dried over Na_2SO_4 and filtered to afford the mixture of **8a**, which was concentrated and purified by column chromaogrtaphy ($CHCl_3$:CH_3OH=20:1) to afford **8a** as a white solid (1.0 g, 36.7 %): R_f=0.38 ($CHCl_3$:CH_3OH 15:1); mp: 167.3-168.4 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.15 (s, 1H, NH), 10.39 (s, 1H, OH), 8.04 (s, 1H, ArH), 7.95 (d, *J*=8.8 Hz, 1H, ArH), 7.65 (s, 1H, ArH), 7.32 (s, 1H, ArH), 7.31 (s, 1H, ArH), 6.55 (d, *J*=8.8 Hz, 1H, ArH), 6.49 (s, 1H, ArH), 4.06 (t, *J*=6.4 Hz, 2H, $NCH_2CH_2CH_2O$), 3.57 (s, 4H, morpholine ring CH_2OCH_2), 2.44-2.37 (m, 6H, morpholine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 1.91-1.84 (m, 2H, $NCH_2CH_2CH_2O$); ^{13}C-NMR (100MHz, DMSO-d_6) δ: 167.5, 163.7, 162.0, 140.3, 131.1, 130.8, 126.9, 123.6, 121.8, 120.1, 109.5, 107.1, 102.2, 66.6, 66.5, 55.1, 53.7, 26.1; IR (KBr) υ=3438, 2923, 2867, 1648,

1588, 1530, 1470, 1439, 1234, 1211, 1113, 1058, 988, 781 cm^{-1}; MS (ESI) m/z 433.39 $[M-H]^+$.

Compound **9a-13a** and **8b-13b** were synthesized according to the procedure described above.

5.1.9 4-(3-morpholinopropoxy)-*N*-(3-chlorophenyl)-2-hydroxy-benzamide (9a)

Compound **9a** was obtained as a white solid (0.95, 36.5 %): R_f=0.39 ($CHCl_3$:CH_3OH 15:1); mp: 167.3-168.4 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 12.15 (s, 1H, NH), 10.36 (s, 1H, OH), 7.95 (d, *J*=8.8 Hz, 1H, ArH), 7.90 (s, 1H, ArH), 7.62 (t, *J*=7.6 Hz, 1H, ArH), 7.41 (t, *J*=8.4 Hz, 1H, ArH), 7.19 (d, *J*=7.6 Hz, 1H, ArH), 6.57 (dd, *J*=8.8 Hz, 1.6 Hz, 1H, ArH), 6.49 (d, *J*=1.6 Hz, 1H, ArH), 4.07 (t, *J*=6.4 Hz, 2H, $NCH_2CH_2CH_2O$), 3.58 (s, 4H, morpholine ring CH_2OCH_2), 2.45-2.39 (m, 6H, morpholine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 1.93-1.85 (m, 2H, $NCH_2CH_2CH_2$-O); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 167.5, 163.7, 161.9, 140.2, 133.4, 130.8, 124.1, 120.8, 119.7, 119.6, 109.5, 107.2, 102.2, 66.5, 55.1, 55.0, 53.7, 26.0; IR (KBr) υ==3422, 2867, 1650, 1588, 1534, 1469, 1432, 1236, 1112, 1056, 990, 689 cm^{-1}; MS (ESI) m/z 389.23 $[M-H]^+$.

5.1.10 4-(3-morpholinopropoxy)-*N*-(m-tolyl)-2-hydroxy-benzamide (10a)

Compound **10a** was obtained as a white solid (1.1 g, 37.9 %): R_f=0.37 ($CHCl_3$:CH_3OH 15:1); mp: 166.8-168.7°C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 12.41 (s, 1H, NH), 10.17 (s, 1H, OH), 7.99 (d, *J*=8.8 Hz, 1H, ArH), 7.51 (s, 1H, ArH), 7.48 (d, *J*=8.0 Hz, 1H, ArH), 7.26 (t, *J*=7.6 Hz, 1H, ArH), 6.96 (d, *J*=7.6 Hz, 1H, ArH), 6.55 (d, *J*=8.8 Hz, 1H, ArH), 6.48 (d, *J*=1.6Hz, 1H, ArH), 4.07 (t, *J*=6.0 Hz, 2H, $NCH_2CH_2CH_2O$), 3.58 (s, 4H, morpholine ring CH_2OCH_2), 2.45-2.39 (m, 6H, morpholine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 2.31 (s, 3H, CH_3),1.93-1.85 (m, 2H, $NCH_2CH_2CH_2O$); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 167.5, 163.5, 162.1, 138.4,

138.3, 130.5, 128.9, 125.2, 122.1, 118.8, 109.4, 107.0, 102.2, 66.6, 66.5, 55.1, 53.7, 26.1, 21.6; IR (KBr) υ˜==3423, 2921, 2871, 1649, 1592, 1555, 1489, 1439, 1240.9, 1216, 1169, 1110, 1056, 986, 900, 840, 782 cm^{-1}; MS (ESI) m/z 369.37 $[M-H]^+$.

5.1.11 4-(3-morpholinopropoxy)-*N*-(3-(trifluoromethyl)phenyl)-2-hydroxy-benz - amide (11a) Compound **11a** was obtained as a white solid (1.0 g, 38.9 %): R_f=0.38 ($CHCl_3$:CH_3OH 15:1); mp: 159.2-160.8 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 12.07 (s, 1H, NH), 10.48 (s, 1H, OH), 8.17 (s, 1H, ArH), 7.97 (t, *J*=8.8 Hz, 2H, ArH), 7.62 (t, *J*=8.0 Hz, 1H, ArH), 7.48 (d, *J*=7.6 Hz, 1H, ArH), 6.58 (d, *J*=8.8 Hz, 1H, ArH), 6.50 (s, 1H, ArH), 4.07 (t, *J*=6.0 Hz, 2H, $NCH_2CH_2CH_2O$), 3.58 (s, 4H, morpholine ring CH_2OCH_2), 2.45-2.38 (m, 6H, morpholine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 1.90~1.87 (m, 2H, $NCH_2CH_2CH_2O$); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 167.7, 163.8, 162.0, 139.5, 130.8, 130.3, 124.9, 120.8, 120.7, 117.5, 117.4, 109.4, 107.2, 102.2, 66.6, 66.5, 55.1, 53.7, 26.1; IR (KBr) υ˜==3298, 2963, 2862, 1662, 1601, 1558, 1508, 1446, 1332, 1289, 1244, 1192, 1161, 1120, 1068, 998, 759, 705 cm^{-1}; MS (ESI) m/z 423.37 $[M-H]^+$.

5.1.12 4-(3-morpholinopropoxy)-*N*-(3-chloro-4-fluorophenyl)-2-hydroxy-benzam-

ide (12a) Compound **12a** was obtained as a white solid (1.2 g, 37.2 %): R_f=0.36 ($CHCl_3$:CH_3OH 15:1); mp: 168.3-170.1 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.14 (s, 1H, NH), 10.39 (s, 1H, OH), 8.01 (dd, *J*=6.8 Hz, 2.0 Hz, 1H, ArH), 7.94 (d, *J*=9.2 Hz, 1H, ArH), 7.65~7.61 (m, 1H, ArH), 7.44 (t, *J*=8.8 Hz, 1H, ArH), 6.56 (dd, *J*=9.2 Hz, 2.0 Hz, 1H, ArH), 6.49 (d, *J*=2.0 Hz, 1H, ArH), 4.06 (t, *J*=6.4 Hz, 2H, $NCH_2CH_2CH_2O$), 3.57 (s, 4H, morpholine ring CH_2OCH_2), 2.44-2.38 (m, 6H, morpholine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 1.91-1.84 (m, 2H, $NCH_2CH_2CH_2O$); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 167.5, 163.7, 162.0, 135.9,

130.7, 129.7, 123.0, 121.9, 121.8, 117.4, 117.1, 107.2, 102.2, 66.5, 55.1, 55.0, 53.7, 26.0; IR (KBr) υ˜=3413, 2955, 2862, 1658, 1600, 1546, 1502, 1460, 1433, 1393, 1325.3, 1238, 1115, 1052, 997, 817 cm^{-1}; MS (ESI) m/z 407.33 $[M-H]^+$.

5.1.13 4-(3-morpholinopropoxy)-*N*-(2-chloro-4-nitrophenyl)-2-hydroxy-benzamide (13a) Compound **13a** was obtained as a white solid (1.2 g, 39.2 %): R_f=0.38 ($CHCl_3$:CH_3OH 15:1); mp: 176.9-178.2 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 11.65 (s, 1H, NH), 8.87 (d, *J*=9.2 Hz, 1H, ArH), 8.41 (d, *J*=2.0 Hz, 1H, ArH), 8.28 (dd, *J*=9.2 Hz, 2.0 Hz, 1H, ArH), 7.96 (d, *J*=9.2 Hz, 1H, ArH), 6.58 (d, *J*=8.8 Hz, 1H, ArH), 6.52 (s, 1H, ArH), 4.04 (t, *J*=6.4 Hz, 2H, $NCH_2CH_2CH_2O$), 3.58 (s, 4H, morpholine ring CH_2OCH_2), 2.47-2.43 (m, 6H, morpholine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 1.92-1.86 (m, 2H, $NCH_2CH_2CH_2O$); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 164.4, 163.8, 159.6, 142.5, 142.3, 133.1, 125.2, 124.3, 122.4, 120.8, 111.4, 107.3, 102.3, 66.4, 66.3, 55.0, 53.6, 25.8; IR (KBr) υ˜=3408, 3288, 2918, 1678.6, 1607, 1546, 1503, 1468, 1397, 1337, 1236, 1114 cm^{-1}; MS (ESI) m/z 434.22 $[M-H]^+$.

5.1.14 4-(3-(piperidin-1-yl)propoxy)-*N*-(3-bromophenyl)-2-hydroxy-benzamide (8b) Compound **8b** was obtained as a white solid (1.0 g, 32.7 %): R_f=0.36 ($CHCl_3$:CH_3OH 15:1); mp: 220.5-222.3 °C; 1H-NMR (DMSO-d_6, 400 MHz) δ: 10.74 (s, 1H, OH), 8.03 (s, 1H, ArH), 7.90 (d, J=9.2 Hz, 1H, ArH), 7.61-7.59 (m, 1H, ArH), 7.30~7.26 (m, 2H, ArH), 6.48-6.46 (m, 1H, ArH), 6.43 (d, J=2.4 Hz, 1H, ArH), 4.02 (t, J=6.0 Hz, 2H, $NCH_2CH_2CH_2O$), 3.37~3.29 (m, 6H, piperidine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 1.91~1.84 (m, 2H, $NCH_2CH_2CH_2O$), 1.53-1.48 (m, 4H, piperidine ring CH_2), 1.38 (s, 2H, piperidine ring CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 166.9, 163.0, 162.1, 138.1, 137.8, 130.1, 128.4, 124.5, 121.4, 118.1, 109.2, 106.1, 101.8, 65.8, 55.3, 44.8, 26.4, 21.1; IR (KBr) υ˜=2947, 1647, 1578.2, 1528, 1473, 1435, 1225, 986, 779.7 cm^{-1}; MS (ESI) m/z 431.37 $[M-H]^+$.

5.1.15 4-(3-(piperidin-1-yl)propoxy)-*N*-(3-chlorophenyl)-2-hydroxy-benzamide (9b) Compound **9b** was obtained as a white solid (1.1 g, 36.8 %): R_f=0.39 ($CHCl_3$:CH_3OH 15:1); mp:215.4-217.3 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 11.59 (s, 1H, NH), 10.75 (s, 1H, OH), 7.93(s, 1H, ArH), 7.91 (t, *J*=1.8 Hz, 1H, ArH), 7.59 (t, *J*=8.1 Hz, 1H, ArH), 7.40 (t, *J*=8.1 Hz, 1H, ArH), 7.18 (d, *J*=7.5 Hz, 1H, ArH), 6.52 (dd, *J*=9.0, 2.4 Hz, 1H, ArH), 6.54 (d, *J*=2.1 Hz, 1H, ArH), 4.05 (t, *J*=6.3 Hz, 2H, $NCH_2CH_2CH_2O$), 3.34 (m, 6H, piperidine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 1.92-1.87 (m, 2H, $NCH_2CH_2CH_2O$), 1.54-1.51 (m, 4H, piperidine ring CH_2), 1.41 (s, 2H, piperidine ring CH_2); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ: 167.6, 163.6, 162.4, 160.1, 136.5, 133.4, 132.1, 130.8, 124.0, 120.8, 119.7, 106.9, 102.4, 66.4, 55.0, 54.1, 25.8, 25.3, 23.8; IR (KBr) υ˜==3412, 2945, 1646, 1588, 1532, 1475, 1433, 1327, 1230, 1135, 1096, 986, 871, 690 cm^{-1}; MS (ESI) m/z 387.29 $[M-H]^+$.

5.1.16 4-(3-(piperidin-1-yl)propoxy)-*N*-(m-tolyl)-2-hydroxy-benzamide (10b) Compound **10b** was obtained as a white solid (0.9 g, 35.6 %): R_f=0.37 ($CHCl_3$:CH_3OH 15:1); mp: 169.8-171.6 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 11.48 (s, 1H, NH), 10.26 (s, 1H, OH), 8.02 (d, *J*=8.7 Hz, 1H, ArH), 7.51 (t, *J*=8.7 Hz, 2H, ArH), 7.27 (t, *J*=7.5 Hz, 1H, ArH), 6.96 (d, *J*=7.5 Hz, 1H, ArH), 6.56 (t, *J*=8.7 Hz, 2H, ArH), 4.07 (t, *J*=5.7 Hz, 2H, $NCH_2CH_2CH_2O$), 2.83 (m, 6H, piperidine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 2.31 (s, 3H, CH_3), 2.09~2.03 (m, 2H, $NCH_2CH_2CH_2O$), 1.66~1.64 (m, 4H, piperidine ring CH_2), 1.47 (s, 2H, piperidine ring CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 167.4, 163.2, 162.1, 138.4, 138.3, 130.7, 129.0, 125.3, 122.1, 118.8, 109.7, 106.9, 102.3, 65.8, 54.0, 52.8, 24.1, 23.4, 22.2, 21.6; IR (KBr) υ˜==2945, 1645, 1597, 1549, 1488, 1438, 1380, 1330, 1244, 1145, 1093, 982, 850, 788, 694 cm^{-1}; MS (ESI) m/z 367.33 $[M-H]^+$.

5.1.17 4-(3-(piperidin-1-yl)propoxy)-*N*-(3-(trifluoromethyl)phenyl)-2-hydroxybe-nzamide (11b) Compound **11b** was obtained as a white solid (1.1 g, 37.5 %): R_f=0.37 ($CHCl_3$:CH_3OH 15:1); mp: 196.7-198.0 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz)

δ: 11.31 (s, 1H, NH), 10.61 (s, 1H, OH), 8.20 (s, 1H, ArH), 8.05 (d, *J*=8.4 Hz, 1H, ArH), 7.97 (d, *J*=8.4 Hz, 1H, ArH), 7.62 (t, *J*=7.6 Hz, 1H, ArH), 7.48 (d, *J*=7.6 Hz, 1H, ArH), 6.58 (d, *J*=9.2 Hz, 2H, ArH), 4.12 (t, *J*=5.6 Hz, 2H, $NCH_2CH_2CH_2O$), 3.08 (m, 6H, piperidine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 2.18-2.14 (m, 2H, $NCH_2CH_2CH_2O$), 1.77-1.74 (m, 4H, piperidine ring CH_2), 1.52 (s, 2H, piperidine ring CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 167.7, 163.4, 161.9, 139.5, 131.0, 130.3, 125.0, 120.8, 120.7, 117.6, 117.5, 109.8, 107.0, 102.4, 65.8, 53.8, 52.7, 23.9, 23.1, 22.1; IR (KBr) υ~=3283, 2947, 2635, 1648, 1598, 1554, 1494, 1447, 1379, 1332, 1249, 1154, 1122, 1071, 801 cm^{-1}; MS (ESI) m/z 421.38 $[M-H]^+$.

5.1.18 4-(3-(piperidin-1-yl)propoxy)-*N*-(3-chloro-4-fluorophenyl)-2-hydroxy-benzamide (12b) Compound **12b** was obtained as a white solid (1.0 g, 37.2 %): R_f=0.37 ($CHCl_3$:CH_3OH 15:1); mp: 214.4-215.7 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.81(s, 1H, O**H**), 8.03(m, 1H, Ar**H**), 7.91(d, *J*=8.7 Hz, 1H, Ar**H**), 7.63-7.58(m, 1H, Ar**H**), 7.44(t, *J*=8.7 Hz, 1H, Ar**H**), 6.51(dd, *J*=8.7, 2.1 Hz, 1H, Ar**H**), 6.45(s, 1H, Ar**H**), 4.05(t, *J*=6.0 Hz, 2H, $NCH_2CH_2C\mathbf{H}_2O$), 3.36(m, 6H, piperidine ring $C\mathbf{H}_2NC\mathbf{H}_2$ and $NC\mathbf{H}_2CH_2CH_2O$), 1.93~1.85(m, 2H, $NCH_2C\mathbf{H}_2CH_2O$), 1.54-1.51(m, 4H, piperidine ring $C\mathbf{H}_2$), 1.41(s, 2H, piperidine ring $C\mathbf{H}_2$); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ: 180.6, 169.4, 167.5, 163.6, 136.1, 130.7, 125.4, 122.8, 121.7, 117.4, 117.1, 106.7, 102.4, 66.4, 55.1, 54.2, 26.1, 25.5, 24.0; IR (KBr) υ~=3416, 2944, 2867, 1646, 1594, 1546, 1498, 1436, 1324, 1243, 1212, 1140, 1051, 982, 859 cm^{-1}; MS (ESI) m/z 405.39 $[M-H]^+$.

5.1.19 4-(3-(piperidin-1-yl)propoxy)-*N*-(2-chloro-4-nitrophenyl)-2-hydroxy-benzamide (13b) Compound **13b** was obtained as a yellow solid (1.1 g, 37.2 %): R_f=0.37 ($CHCl_3$:CH_3OH 15:1); mp:.231.5-232.7; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.90 (d, *J*=9.2 Hz, 1H, ArH), 8.38 (d, *J*=2.8 Hz, 1H, ArH), 8.26 (dd, *J*=9.2, 2.4 Hz,

1H, ArH), 7.93 (d, *J*=9.2 Hz, 1H, ArH), 6.47 (s, 1H, ArH), 6.45 (s, 1H, ArH), 4.04 (t, *J*=6.0 Hz, 2H, $NCH_2CH_2CH_2O$), 2.96 (m, 6H, piperidine ring CH_2NCH_2 and $NCH_2CH_2CH_2O$), 2.06-2.02 (m, 2H, $NCH_2CH_2CH_2O$), 1.69-1.65 (m, 4H, piperidine ring CH_2), 1.49 (s, 2H, piperidine ring CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 170.0, 168.2, 148.1, 146.7, 137.5, 129.9, 129.0, 127.1, 125.5, 116.6, 115.9, 110.6, 107.5, 70.3, 59.0, 57.8, 29.2, 28.5, 27.2; IR (KBr) υ==2966, 2703, 1658, 1618, 1587, 1513, 1470, 1413, 1344, 1296, 1220, 1155, 1098, 981, 846 cm^{-1}; MS (ESI) m/z 434.11$[M-H]^+$.

5.2 Pharmacology

5.2.1 *In vitro* EGFR tyrosine kinase activity.

In vitro EGFR-TK inhibition assays were carried out as described in reference. EGFR and EGF were purchased from Wuhan Boster Biological Technology, *Ltd*, and the ADP-Glo™ Kinase was purchased from Promega. The experiments were performed according to the instructions of the manufacturer. The EGFR tyrosine kinase activity was performed using Kinase-Glo Plus luminescence kinase assay kit (Promega). It measures kinase activity by quantitating the amount of ATP remaining in solution following a kinase reaction. The luminescent signal from the assay is correlated with the amount of ATP present and is inversely correlated with the amount of kinase activity. Briefly, 96-well plates were precoated with EGFR, EGF and tested compound in a ratio of 2:2:1 were added. The reaction mixtures were incubated for 40 min at room temperature while being shaken. After completion of the reaction, 20 μL of ADP-Glo™ Plus Luminescence kinase assay solution were added, incubate the plate for 30 min at room temperature. The corrected activity for each protein kinase target was determined by removing the blank control value. Luminescence signal was measured using a Tecan Infinite Spectramax M2e microplate reader.

5.2.2 Experimental method for cell proliferation assay

The anti-proliferative activities of salicylanilide derivatives **8a-13a** and **8b-13b** were determined using a standard (MTT)-based colorimetric assay. Briefly, cell lines were seeded at a density 10^4 cells/well in 96-well microtiter plates. The cells were then treated in triplicate with various concentration of each compound and cultured in 5 % medium for 72 h. Control cells were treated with vehicle alone. During the last 4 h of incubation, the cells were exposed to tetrazolium dye (MTT) Solution (5 mg/mL, 20 µL per well). The generated formazan crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO), and the absorbance was read spectrophotometrically at 570 nm using an enzyme-linked immunosorbent assay plate reader. The data was calculated using Graph Pad Prism version 5.0. The IC_{50} were fitted using a non-linear regression model with a sigmodial dose response.

5.2.3 Molecular modeling study

The docking was performed using a receptor from the 2ITO structure from RCSB Protein Data Bank [17], and Auto Dock Vina. The co-crystal inhibitor (gefitinib) was removed from the initial X-ray structure, water molecules were then removed and polar hydrogens and gastieger charges were added by using Autodock Tool. Ligands were optimized for the energy and geometry using MM2 force fields. In the structures of the ligands, all bonds were treated as rotatable, except for the aromatic bonds. The force field for protein is “Semiempirical Free Energy Force Field with Charge-Based Desolvation”. Lamarckian genetic algorithmwas used for all molecular docking simulations. Population size of 300, mutation rate of 0.02, and crossover rate of 0.8 were set as the parameters. Simulations were performed using up to 2.5 million energy evaluations with a maximum of 27,000 generations. Docking proceeded with an exhaustiveness value of 100 and a maximum output of 10 structures. The lowest energy conformations were regarded as the binding conformations between the ligands and the proteins. Visualization was done using the PyMOL software (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC).

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Figure 1. Representative EGFR inhibitors

Figure 2. The structure of the salicylanilide

Figure 3. The salicylanilide derivatives reported as anti-tumor inhibitors and proposed as EGFR inhibitors

Scheme 1. Reagents and conditions: (a) CH_3I, $NaHCO_3$, DMF, 40 °C,10 h; (b) 1, 3 dibromopropane, K_2CO_3, TEBAC, acetone, 60 °C,8 h; (c) $NaHCO_3$, NaI, isopropanol, 85 °C,6 h; (d) NaOH,CH_3OH, H2O, r.t, 6 h; (e) $SOCl_2$, DMF, 85 °C, 6 h; (f) Et_3N, CH_2Cl_2, ice bath, 40 min.

Table 1. EGFR inhibition of the salicylanilide derivatives **8a-13a** and **8b-13b** (n=3, $\bar{x} \pm s$)

Figure 4. (a) Compound **12b** (light blue) docked into the EGFR binding domain. (b) Dock structure of compound **12b** with EGFR. Hydrogen bonds towards MET793 were highlighted.

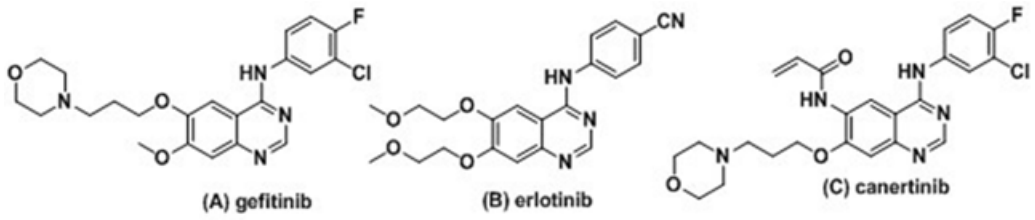


Figure 1. Representative EGFR inhibitors

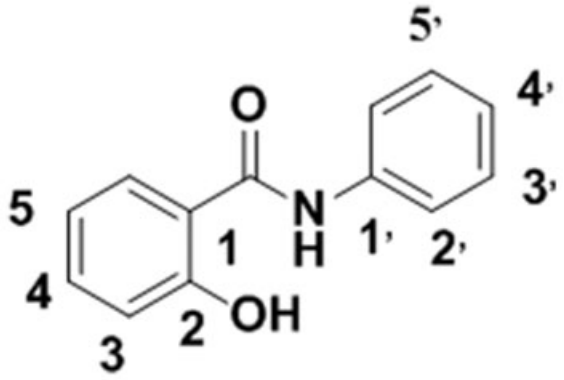


Figure 2. The structure of the salicylanilide

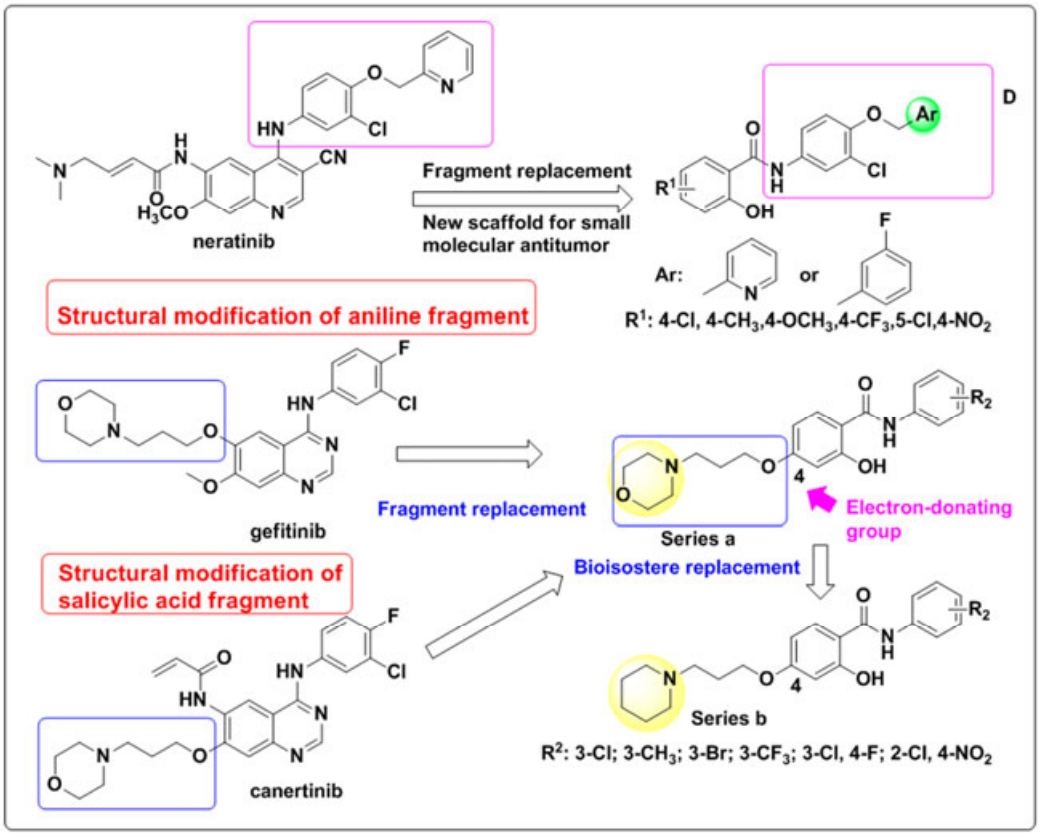


Figure 3. The salicylanilide derivatives reported as anti-tumor inhibitors and proposed as EGFR inhibitors

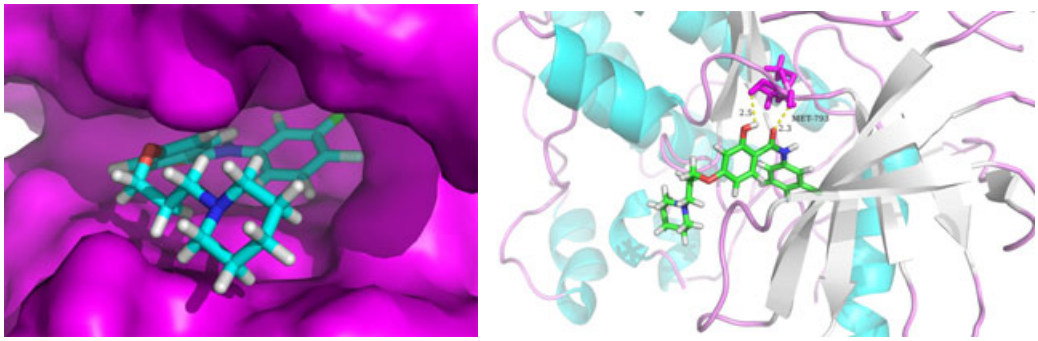
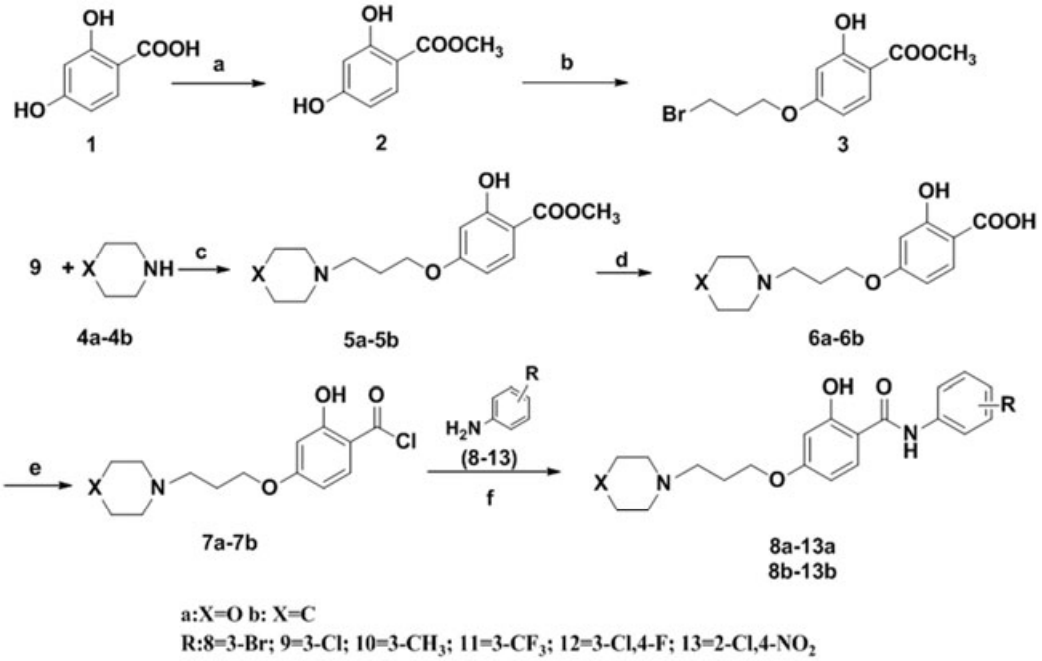


Figure 4. (a) Compound **12b** (light blue) docked into the EGFR binding domain. **(b)** Dock structure of compound **12b** with EGFR. Hydrogen bonds towards MET793 were highlighted.



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