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Discovery of 2-aryl-8-hydroxy (or methoxy)-isoquinolin-1(2*H*)-ones as novel EGFR inhibitor by scaffold hopping

Bo-Rui Kang [a], Ai-Lin Shan [a], Yi-Ping Li [a], Jing Xu [b], She-Min Lu [b], San-Qi Zhang [a,*]

[a] Department of Medicinal Chemistry, School of Pharmacy, Health Science Center, Xi'an Jiaotong University, Xi'an 710061, PR China
[b] Department of Genetics and Molecular Biology, School of Medicine, Health Science Center, Xi'an Jiaotong University, Xi'an 710061, PR China

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ABSTRACT

2-Aryl-8-hydroxy (or methoxy)-isoquinolin-1(2*H*)-one has been proposed as a novel scaffold of EGFR inhibitor based on scaffold hoping. In the present study, a series of 2-aryl-8-hydroxy (or methoxy)-isoquinolin-1(2*H*)-one derivatives were synthesized. Their antiproliferative activities in vitro were evaluated via MTT assay against two human cancer cell lines, including A431 and A549. The SAR of the title compounds was preliminarily discussed. The compounds with ideal inhibition were evaluated through ELISA-based EGFR-TK assay. Compound **6c** showed the best activity against A431 and EGFR tyrosine kinase. These findings suggest that title compounds are EGFR inhibitors with novel structures.

1. Introduction

Receptor tyrosine kinases (RTKs) have been observed having over-expression and/or constitutive activation in numerous types of human tumor, including colon, breast, ovarian, head and neck, and nonsmall cell lung cancers. Moreover, RTKs also play key roles in proliferation, differentiation, migration, angiogenesis and evasion from apoptosis. Among the known RTKs, epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor (HER2) have been extensively studied and clinically validated as targets for chemical therapies.[1,2] Thousands of small organic molecule inhibitors against EGFR or both EGFR and HER2 were synthesized and evaluated. Gefitinib, erlotinib and lapatinib were launched successfully to treat cancer in clinical. (Fig. 1).[3,4] The shared structure of these anticancer agents is 4-arylaminoquinazoline, whose the structure activity relationships have been elucidated.[5]

The essential structure of pharmacophore in 4-arylaminoquinazolines is the two nitrogen atoms at 1-posotion, 3-position and *N*-aryl at 4-position (Fig. 2). Accordingly, pyridopyrimidines and pyrimidopyrimidines etc. were designed as new scaffolds based on bioisosterism, synthesized and evaluated against EGFR.[6–10] Fused tricyclic quinazoline, pyrimidine,[11,12] dioxolane,[13] and dioxepine quinazoline[14] derivatives were also reported as EGFR inhibitors. Among them, linear imidazo[4,5-*g*] quinazoline was the most potent compound hitherto (IC_{50} = 0.008 nM), which exhibit inhibition against phosphorylation of a fragment of phospholipase C-γ1.[15]

However, the efficacy of small organic molecules inhibitors such as gefitinib, etc. is restricted to a small subset of patients due to molecular heterogeneity among and within tumors.[16,17] The drug resistance caused by receptor mutation is another factor needed to pay attention.[18–20] Numbers of compounds with different structures have been developed as EGFR or multi-target inhibitors.[21–23] Salicylanilide molecule can construct an intramolecular hydrogen bond and form a pseudo six-membered ring to mimic the pyrimidine ring of quinazolines (Fig. 2). Thus, *N*-aryl salicylamides are supposed to have the same pharmacophore as 4-arylaminoquinazoline, and have been studied as EGFR inhibitors.[24–26] Appropriately substituted salicylanilides were shown to inhibit EGFR tyrosine kinase activity with IC_{50} values in the range of 23–71 nM. Except as drug to treat tumors, EGFR inhibitor labeled by iodine-123 was studied as potential imaging agents for EGFR-expressing prostate carcinomas.[27]

In order to seek a novel scaffold of EGFR inhibitor, we have observed that 2-aryl-8-hydroxyisoquinolin-1(2*H*)-one can construct a pseudo six-member ring via intramolecular hydrogen bond. We suspect that the two oxygen atoms at 1, 8-position in 2-aryl-8-hydroxyisoquinolin-1(2*H*)-one may serve as the two nitrogen atoms at 1, 3-position in quinazoline ring to form hydrogen bonds with EGFR-RTK. Meanwhile, the 2-aryl in 2-aryl-8-hydroxyisoquinolin-1(2*H*)-one shows the same configuration as the *N*-aryl at 4-position in 4-arylaminoquinazoline. These similar characteristics in structure might indicate that 2-aryl-8-hydroxyisoquinolin-1(2*H*)-one has the same pharmacophore (denoted as red in

* Corresponding author. Tel./fax: +86 29 82657040.
E-mail address: sqzhang@mail.xjtu.edu.cn (S.-Q. Zhang).

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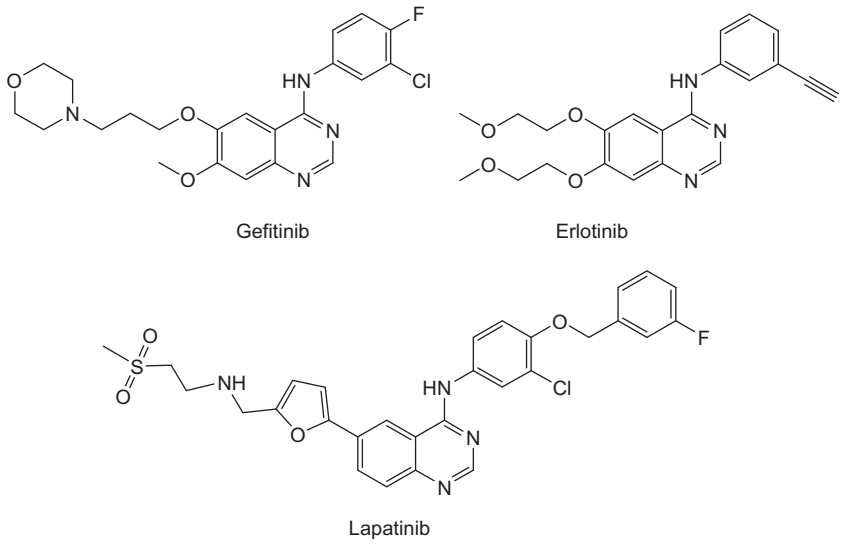


Figure 1. The chemical structures of gefitinib, erlotinib and lapatinib.

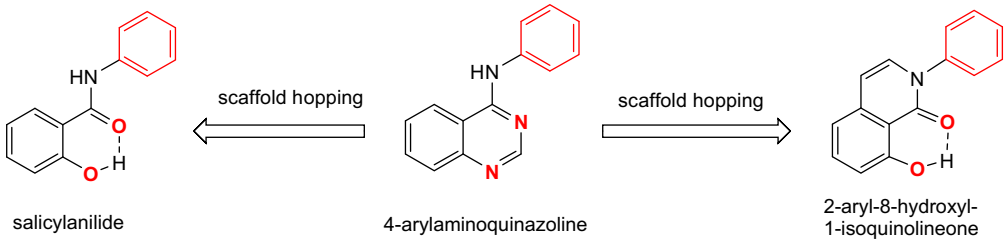


Figure 2. The discovery of the novel structures of EGFR inhibitor based on scaffold hopping.

Fig. 2) as 4-arylaminoquinazoline. Therefore, we would like to propose that 2-aryl-8-hydroxyisoquinolin-1(2*H*)-one should be novel scaffold of EGFR inhibitor on the basis of scaffold hopping. Similarly, oxygen atom in methoxy at 8-position in 2-aryl-8-methoxyisoquinolin-1(2*H*)-one may serve as a hydrogen bond acceptor. Thus, 2-aryl-8-hydroxyl (or methoxy) isoquinolin-1(2*H*)-one might be another novel scaffold of EGFR inhibitor other than 4-arylaminoquinazoline and salicylanilide etc., and display potential anti-tumor effect. Herein, we describe the synthesis of 2-aryl-8-hydroxy (or methoxy)-isoquinolin-1(2*H*)-one and the results of biological assays.

2. Results and discussion

2.1. Chemistry

The synthetic route of 2-aryl-8-hydroxy (or methoxy)-isoquinolin-1(2*H*)-one is outlined in Scheme 1.

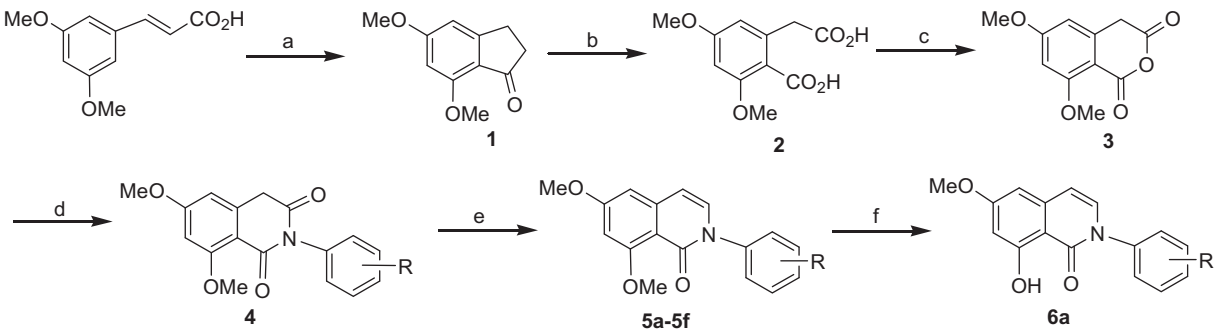
The commercially available (*E*)-3-(3,5-dimethoxyphenyl)acryl acid was reduced by hydrogenation in the catalysis of Pd–C (5%) to give 3-(3,5-dimethoxyphenyl) propanoic acid which was cyclized in polyphosphoric acid to give 2,3-dihydroinden-1-one (**1**) without further purification. Acylation of **2** with diethyl oxalate, subsequent oxidation with alkaline hydrogen peroxide, according to Bhakta's conditions,[28] afforded homophthalic acid (**2**). Cyclization of **2** into **3** was completed with acetic anhydride in hot toluene. Compound **3** was isolated directly from the reaction mixture as a white powder. The synthesis of compounds **4** was achieved by condensation of homophthalic anhydride **3** with aniline or substituted aniline. The yields of compounds **4** were distinctly improved when the reaction was carried out in glacial acetic acid. Reduction of carbonyl group at 3-position of **4** with sodium borohydride in dichloromethane produced compounds **5**.[29] Removal of methyl at 8-methoxy of compounds **5** with lithium chloride afforded compounds **6**.[30]

The yield is not high in the step of conversion of compound **4** to compound **5**. Therefore, an alternative synthetic route to the 2-aryl-8-hydroxy (or methoxy)-isoquinolin-1(2*H*)-one was applied (Scheme 2).

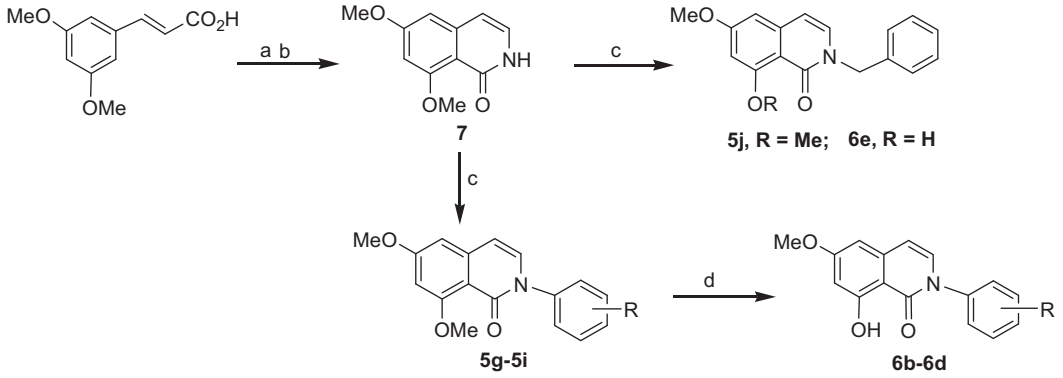
(*E*)-3-(3,5-Dimethoxyphenyl)acryl acid was converted into corresponding acyl azide, which can be transformed into 6,8-dimethoxyisoquinolin-1-one **7** via Curtius rearrangement and intramolecular electrophilic substitution. The presence of tertiary amine and high temperature are favorable for the process. In the presence of potassium carbonate and cuprous iodide, the mixture of **7** and aryl iodide was stirred in DMSO at 140 °C to yield **5**. Methyl at 8-position of compound **5** was removed by stirring a mixture of **5** and lithium chloride in DMF at 140 °C for 5 h. Compounds **6b**–**e** were synthesized according to this route.

To explore the influence of substituted group at 5-position on activity, compounds **10**–**12** were synthesized according to Scheme 3. Nitration of compounds **5g** or **6c** afforded compound **8**. Reduction of nitro group produced corresponding amine. Without further purification, the amine was converted to compound **9** or **10** by acylation, converted to compound **11** by the reaction with isocyanate. Compound **12** was produced by the nucleophilic substitution of **9** with morpholine.

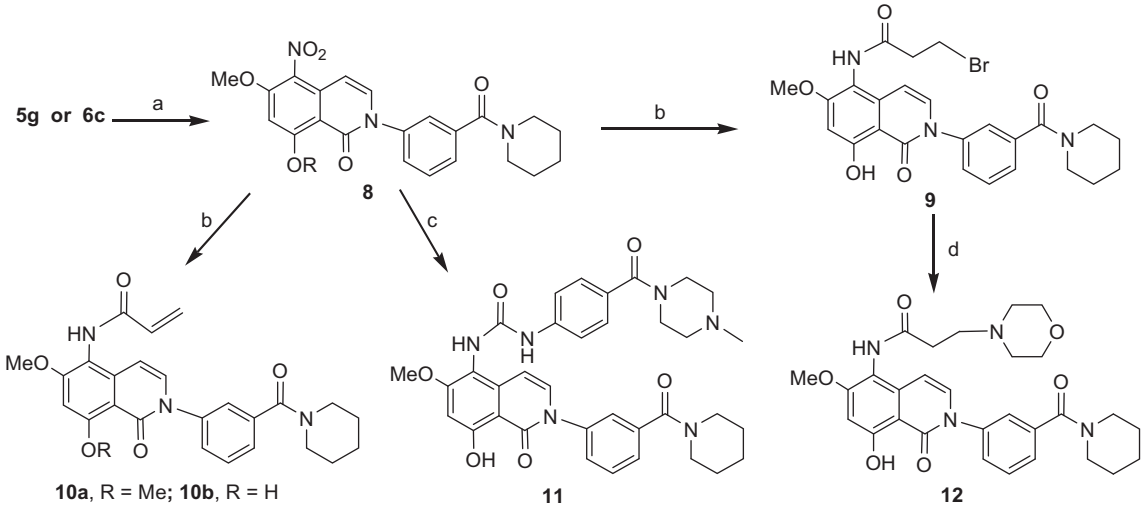
To expand the structural diversity of the target compounds, we synthesized 2-aryl-3,4-dihydro-8-hydroxy (or methoxy)-6-methoxyisoquinolin-1(2*H*)-ones from compound **1** (Scheme 4). Compound **1** was converted to **13** by Schmidt rearrangement.



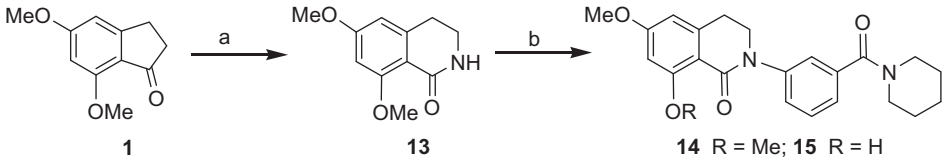
Scheme 1. Reagents and conditions: (a) (i) 5% Pd–C, MeOH, THF, rt; (ii) PPA, 80 °C; (b) (i) diethyl oxalate, sodium methoxide, toluene, 0 °C–rt; (ii) KOH, H_2O_2. Rt–50 °C; (c) acetic anhydride, toluene, reflux; (d) anilines, acetic acid, reflux; (e) $NaBH_4$, CH_2Cl_2, MeOH, THF, 0–2 °C, then, con. HCl, rt; (f) $LiCl{\cdot}H_2O$, DMA, 140 °C.



Scheme 2. Reagents and conditions: (a) (i) oxalyl chloride; (ii) sodium azide; (b) *N*,*N*-diethylaniline, reflux; (c) ArI or $BnCl/K_2CO_3/CuI/DMSO$; (d) LiCl/DMA, 140 °C.



Scheme 3. Reagents and conditions: (a) urea nitrate, con. H_2SO_4; (b) (i) Fe, NH_4Cl, ethanol, reflux; (ii) RCOCl, TEA, (c) 4-(4-methylpiperizin-1-yl)carbonyl) phenylisocyanate, toluene; (d) (i) KI, CH_3CN, reflux; (ii) morpholine, CH_3CN, DMAP, reflux.



Scheme 4. Reagents and conditions: (a) NaN_3, TFA; (b) *N*-(3-iodobenzoyl)piperidine/K_2CO_3/CuI/DMSO, 140 °C.

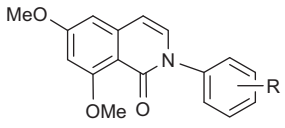
Compounds **14** and **15** were prepared according the procedures described in Scheme 2.

2.2. Antiproliferative assay in vitro

We evaluated antiproliferative activities of synthesized compounds against human epithelial carcinoma cell line (A431) and lung adenocarcinoma epithelial cell line (A549) by applying the MTT colorimetric assay. EGFR inhibitor gefitinib was used as the positive control. The results are summarized in Table 1.

As expected, the compounds exhibited distinct effects on antiproliferative activities against A431 and A549 in vitro, especially against A431, which may be related to the overexpression of EGFR in A431. Compound **6c** (IC_{50} = 2.25 μM) showed the most potent inhibitory activity against A431. This datum was close to that of positive control (IC_{50} = 1.98 μM). In the case of compound **5**, the substituted group at 2-phenyl plays some roles in activity. Compounds with small substituted groups at 2-phenyl (**5a**, IC_{50} = 5.35 μM; **5c**, IC_{50} = 3.95 μM; **5d**, IC_{50} = 4.17 μM against A431) exhibited better potency. On the contrary, a large substituted group at 2-phenyl in compound **6** (**6a**, IC_{50} = 4.44 μM; **6c**, IC_{50} = 2.25 μM) is beneficial to the activity. This may be related to different interaction of small molecule with EGFR. The activity of compounds **6** with 8-hydroxyl group (**6a**, IC_{50} = 4.44 μM and **6c**, IC_{50} = 2.25 μM) was more potent than compounds **5** with 8-methoxy group (**5b**, IC_{50} = 7.13 μM and **5g**, IC_{50} = 15.27 μM) against A431. The similar data can be observed in the activity against A549. These results indicate that a pseudo six member ring in title compounds is more favorable for the activity. When the substituted group at 2-position is benzyl (**5i** and **6e**), the activity was not changed distinctly against A431. The activities of compounds **5g** and **14**, **6c** and **15** were close to each other against A431 and A549, which suggest that isoquinolin-1(2*H*)-one scaffold and 3,4-dihydroisoquinolin-1(2*H*)-one scaffold play a similar effect on the antiproliferative activity. Compounds **10**–**12** were synthesized, respectively, from **5g** or **6c** which are easy to prepare. Compared with compound **5g** (IC_{50} = 15.27 μM), compound **10a** (IC_{50} = 6.23 μM) was more potent against A431. However, the activity of compound **11** declined. The activity of compound **12** (IC_{50} = 13.05 μM against A549) was close to that of gefitinib (IC_{50} = 12.92 μM). These results suggest that the proper side chain at 5-position may improve the activity.

Table 1
Antiproliferative activities of synthesized compounds against A431 and A549 ($\bar{x} \pm s$, $n = 3$)



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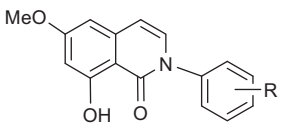

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Compds	R	IC_{50} (μM)	
		A431	A549
5a	H	5.35 ± 0.94	44.49 ± 12.24
5b	3-Ethynyl	7.13 ± 1.84	25.23 ± 3.27
5c	3-Cl-4-F	3.95 ± 0.43	17.81 ± 1.66
5d	3-F	4.17 ± 0.98	15.25 ± 1.94
5e	3,4-diF	3.79 ± 0.85	nt
5f	3-CF_3	14.84 ± 1.34	23.31 ± 2.59
5g	3-$CONC_5H_{10}$	15.27 ± 2.16	18.64 ± 2.60
5h	3-$CONH_2$	28.25 ± 3.56	31.26 ± 3.60
5i		3.88 ± 0.52	nt
6a	3-Ethynyl	4.44 ± 2.27	15.86 ± 1.12
6b	H	nt	27.90 ± 3.12
6c	3-$CONC_5H_{10}$	2.25 ± 0.21	14.23 ± 1.54
6d	3-$CONH_2$	15.16 ± 3.22	24.80 ± 3.07
6e		8.29 ± 1.27	nt
14		10.82 ± 2.28	23.44 ± 0.35
15		5.32 ± 1.31	13.23 ± 1.28
10a		nt	14.65 ± 3.02
10b		5.30 ± 0.87	14.68 ± 2.19
11		19.51 ± 1.30	>100
12		11.36 ± 3.11	13.05 ± 1.52
Gefitinib		1.98 ± 0.60	12.92 ± 1.86

Nt: not tested.

2.3. EGFR inhibitory activity assay

To study mechanism of antiproliferative activity of synthesized compounds, some compounds with potent antiproliferative activities were evaluated against EGFR kinase activity assays with gefitinib as positive control. EGFR was prepared from human A431 carcinoma cell. Compounds were initially screened at final concentrations of 100.0, 10.0, 1.0 and 0.1 μM by using ELISA-based EGFR-TK assay. IC_{50}s of compounds were calculated according to inhibitory ratios, the in vitro enzymatic inhibition assay results are summarized in Table 2.

The data in Table 2 demonstrated that tested compounds exhibited the inhibitory activity against EGFR. The activities of compounds **6b**, **14** and **15** against EGFR were close to that of gefitinib. The activities of rest compounds against EGFR were found to be less potent than gifitinib. In most cases, the activities of tested compounds against EGFR are consistent with their antiproliferative activities against A431. The results suggest that title compounds are potential EGFR inhibitors.

2.4. Docking studies

We performed docking analysis by utilizing the C-DOCKER program within Discovery Studio 2.5 software package to further look into the binding mode of the title compounds. Docking simulations were performed on human EGFR kinase domain (PDB code 1M17)[31] (Fig. 3).

The docking results of three compounds with EGFR (Fig. 3) have the following three indications: (a) the title compounds can interact with the catalytic domain of EGFR. (b) A pseudo six-member ring in compounds **6a** and **15** can mimic pyrimidine ring in erlotinib as initial assumption (Fig. 3B and C). The oxygen atom of 8-hydroxy can form a hydrogen bond with MET769. The oxygen atom of carbonyl in **15** can form a hydrogen bond with LYS721. (c) The oxygen atom of 8-methoxy in compound **5b** can form a hydrogen bond with CYS773, which may explain that the activities of compounds **5** are less potent than that of compounds **6**.

3. Conclusions

2-Aryl-8-hydroxy (or methoxy)-isoquinolin-1(2*H*)-one derivatives have been proposed as a novel structure of EGFR inhibitor

Table 2
The inhibitory activities of compounds against EGFR

Compds	IC_{50}[a] (μM)
5b	3.51
5c	5.49
5g	2.12
6a	3.06
6c	1.38
14	1.23
15	1.18
Gefitinib	1.10

[a] The dates given are mean values derived from three replicates in two independent experiments.

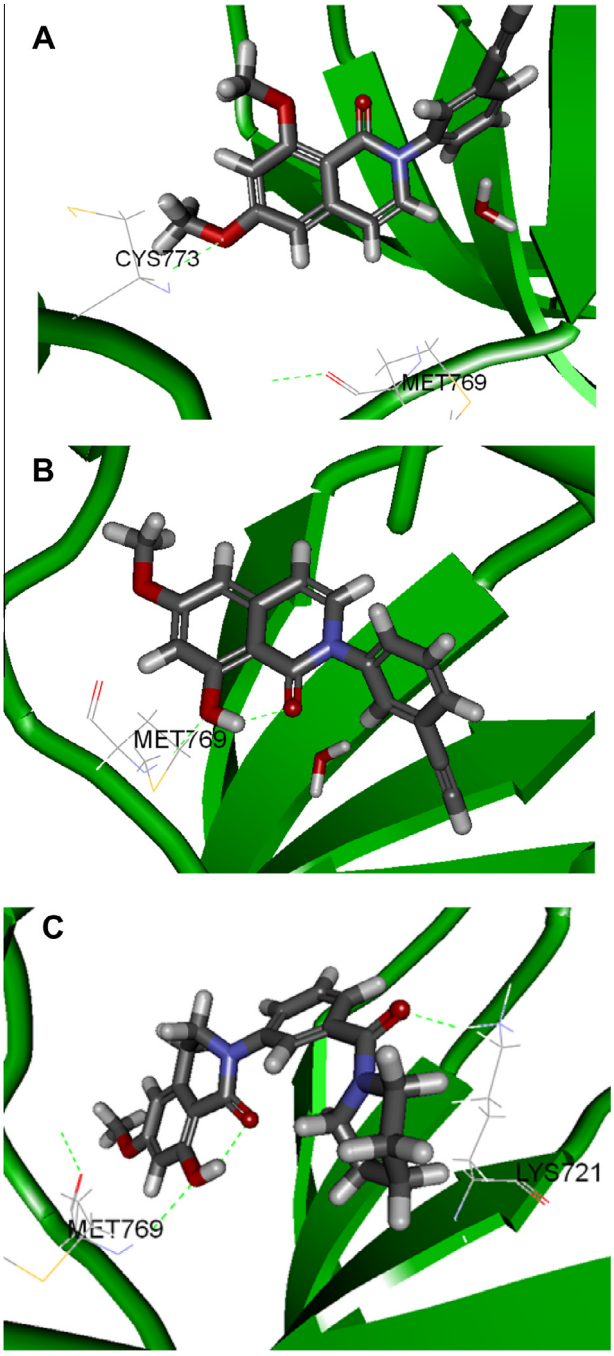

Figure 3. Docking mode comparison between compounds **5b**, **6a** and **15**. (A) Ribbon show of compound **5b** bound to EGFR; (B) ribbon show of compound **6a** bound to EGFR; (C) ribbon show of compound **15** bound to EGFR. The green dashed lines indicate hydrogen bonds.

based on scaffold hoping. Twenty compounds were synthesized and characterized by ^{1}H NMR and HRMS. Antiproliferative activities of synthesized compounds against A431 and A549 were evaluated by MTT assay. The inhibitory activity of compounds with good antiproliferative activity against EGFR were evaluated by using ELISA-based EGFR-TK assay. Compound **6c** displayed good activity against A431 and EGFR tyrosine kinase. These findings suggest that title compounds are EGFR inhibitors with novel structure.

4. Experimental

4.1. Chemistry

Unless specified otherwise, all starting materials, reagents and solvents were commercially available. All reactions were monitored by thin-layer chromatography on silica gel plates (GF-254) and visualized with UV light. All the melting points were determined on a Beijing micromelting-point apparatus and thermometer was uncorrected. ^{1}H NMR spectra were recorded in DMSO-d_6 on a 300 or 400 Bruker NMR spectrometer with tetramethylsilane (TMS) as an internal reference. All chemical shifts are reported in parts per million (ppm). High-resolution exact mass measurements were performed using electrospray ionization (positive mode) on a quadrupole time-of-flight (QTOF) mass spectrometer (Maxis Q-TOF, Bruker Inc.).

4.1.1. Compound 1 5,7-dimethoxy-2,3-dihydroinden-1-one

The mixture of 3-(3,5-dimethoxyphenyl)propionic acid (10.0 g, 47.6 mmol) and poly phosphoric acid (120 g) was stirred at 80 °C for 5 h under nitrogen, cooled to ambient temperature and poured onto crushed-ice (300 ml) slowly with stirring. The mixture was extracted with ethyl acetate (150 ml × 4). Combined organic layers were washed with saturated sodium carbonate aqueous solution (50 ml × 2), brine (50 ml) and dried over anhydrate sodium sulfate. After removal of solvent, 9.35 g of crude product was obtained. Yield 97.4%; ^{1}H NMR (300 MHz, DMSO-d_6), δ: 2.50 (t, 2H, J = 12.0 Hz, –CH_2), 2.95 (t, 2H, J = 12.0 Hz, –CH_2), 3.79 (s, 3H, –OCH_3), 3.84 (s, 3H, –OCH_3), 6.42 (s, 1H, Ar-H), 6.62 (s, 1H, Ar-H). MS: 193.0 $[M+H]^+$.

4.1.2. Compound 2 2-carboxymethyl-4,6-dimethoxybenzoic acid

To a freshly prepared solution of sodium methoxide (0.46 g, 20.0 mmol of sodium in 40 ml of absolute methanol) were added a solution of **1** and diethyl oxalate (2.19 g, 15.0 mmol) in toluene (20 ml) dropwise below 2 °C. The mixture was stirred at ambient temperature overnight. After evaporating solvent, the residue was soaked in 5% hydrochloric acid (200 ml). The precipitate was obtained by filtering, recrystallized from methanol–water (70 ml, 2:1 v/v) to yield yellow intermediate (2.27 g). To a suspension of intermediate in water (30 ml) was added dropwise freshly prepared KOH aqueous solution (2.24 g, 40.0 mmol of KOH in 10 ml of water) below 2 °C. After the addition, hydrogen peroxide (18.0 g, 30%) was added dropwise below 5 °C and the mixture was stirred at ambient temperature for 2 h. Then the temperature was generally raised to 50 °C and the mixture was stirred at this temperature for another 3 h. The cooled solution was acidified with concentrate hydrochloric acid and extracted with ethyl acetate (100 ml, 50 ml × 3). The combined organic layers were washed with brine (100 ml), dried over anhydrate sodium sulfate. After evaporating solvent, the crude product was recrystallized from dichloromethane (30 ml) to yield **2** (1.81 g). Yield 75.0%; ^{1}H NMR (300 MHz, DMSO-d_6), δ: 3.57 (s, 2H, CH_2), 3.76 (s, 3H, –OCH_3), 3.77 (s, 3H, –OCH_3), 6.47 (s, 1H, Ar-H), 6.53 (s, 1H, Ar-H).

4.1.3. Compound 3 6,8-dimethoxy-4*H*-isochromene-1,3-dione

The suspension of **2** (5.20 g, 21.6 mmol) and acetic anhydrate (3.33 g, 32.4 mmol) in toluene (14 ml) was refluxed under nitrogen for 3 h and cooled to ambient temperature. The cooled solution was standing at 2 °C for 4 h to give a yellow needle crystal. The crystal was collected by filtration to produce 4.05 g of **3**. Yield 84.4%; ^{1}H NMR (300 MHz, DMSO-d_6), δ: 3.88 (s, 6H, –OCH_3), 4.16 (s, 2H, –CH_2–), 6.58 (s, 1H, Ar-H), 6.62 (s, 1H, Ar-H).

4.1.4. General synthesis for 2-aryl-6,8-dimethoxyisoquinozoline-1,3-dione

The mixture of **3** (10.0 mmol), aniline (10.0 mmol) and glacial acetic acid (5 ml) was refluxed under nitrogen for 5 h, cooled to ambient temperature, poured into water slowly with stirring. The solid was collected, recrystallized from methanol to give a off-white solid.

4.1.5. Compound 4a 6,8-dimethoxy-2-phenylisoquinoline-1,3(2*H*,4*H*)-dione

Yield 52.4%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.91 (s, 6H, $-OCH_3$), 4.13 (s, 2H, $-CH_2-$), 6.38 (s, 1H, Ar-H), 6.46 (s, 1H, Ar-H), 7.19 (b, 2H, Ar-H), 7.44 (b, 1H, Ar-H), 7.46 (b, 2H, Ar-H). HRMS: Calcd for $C_{17}H_{15}NNaO_4$ $[M+Na]^+$: 320.0889; Found: 320.0880.

4.1.6. Compound 4b 6,8-dimethoxy-2-(3-ethynylphenyl) isoquinoline-1,3(2*H*,4*H*)-dione

Yield 78.8%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.07 (s, 1H, ≡CH), 3.90 (s, 3H, $-OCH_3$), 3.91 (s, 3H, $-OCH_3$), 4.12 (s, 2H, $-CH_2-$), 6.38 (s, 1H, Ar-H), 6.46 (s, 1H, Ar-H), 7.17 (d, 1H, J = 7.8 Hz, Ar-H), 7.33 (s, 1H, Ar-H), 7.40 (t, 1H, J = 7.6 Hz, Ar-H), 7.51 (d, 1H, J = 7.8 Hz, Ar-H). HRMS: Calcd for $C_{19}H_{15}NNaO_4$ $[M+Na]^+$: 344.0899, Found: 344.0880.

4.1.7. Compound 4c 6,8-dimethoxy-2-(3-chloro-4-fluorophenyl) isoquinoline-1,3(2*H*,4*H*)-dione

Yield 63.6%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.81 (s, 2H, $-CH_2-$), 3.90 (s, 3H, $-OCH_3$), 3.92 (s, 3H, $-OCH_3$), 6.38 (s, 1H, Ar-H), 6.46 (s, 1H, Ar-H), 7.08 (d, 1H, J = 7.1 Hz, Ar-H), 7.19 (d, 1H, J = 7.1 Hz, Ar-H), 7.22 (s, 1H, Ar-H). HRMS: Calcd for $C_{17}H_{13}ClFNNaO_4$ $[M+Na]^+$: 372.0415; Found: 372.0402.

4.1.8. Compound 4d 6,8-dimethoxy-2-(3-fluorophenyl) isoquinoline-1,3(2*H*,4*H*)-dione

Yield 70.5%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.90 (s, 3H, $-OCH_3$), 3.92 (s, 3H, $-OCH_3$), 4.12 (s, 2H, $-CH_2-$), 6.38 (s, 1H, Ar-H), 6.46 (s, 1H, Ar-H), 6.98 (t, 1H, J = 8.0 Hz, Ar-H), 7.11 (s,1H, Ar-H), 7.13 (d, 1H, J = 8.2 Hz, Ar-H), 7.42 (d, 1H, J = 8.0 Hz, Ar-H). HRMS: Calcd for $C_{17}H_{14}FNNaO_4$ $[M+Na]^+$: 338.0805; Found: 338.0790.

4.1.9. Compound 4e 6,8-dimethoxy-2-(3,4-difluorophenyl)isoquinoline-1,3(2*H*,4*H*)-dione

Yield 53.4%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.91 (s, 3H, $-OCH_3$), 3.92 (s, 3H, $-OCH_3$), 4.12 (s, 2H, $-CH_2-$), 6.38 (s, 1H, Ar-H), 6.47 (s, 1H, Ar-H), 6.94 (d, 1H, J = 5.7 Hz, Ar-H), 7.05 (d, 1H, J = 5.7 Hz, Ar-H), 7.27 (s, 1H, Ar-H). HRMS: Calcd for $C_{17}H_{13}F_2NNaO_4$ $[M+Na]^+$: 356.0710; Found: 356.0709.

4.1.10. Compound 4f 6,8-dimethoxy-2-(3-trifluoromethylphenyl)isoquinoline-1,3(2*H*,4*H*)-dione

Yield 36.5%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.91 (s, 3H, $-OCH_3$), 3.92 (s, 3H, $-OCH_3$), 4.15 (s, 2H, $-CH_2-$), 6.39 (s, 1H, Ar-H), 6.47 (s, 1H, Ar-H), 7.40 (d, 1H, J = 5.85 Hz, Ar-H), 7.48 (s, 1H, Ar-H), 7.60 (t, 1H, J = 7.17 Hz, Ar-H), 7.67 (d, 1H, J = 5.85 Hz, Ar-H). HRMS: Calcd for $C_{18}H_{14}F_3NNaO_4$ $[M+Na]^+$: 388.0773; Found: 388.0749.

4.1.11. General synthesis for 2-aryl-6,8-dimethoxyisoquinozoline-1(2*H*)-one

To a solution of **4** (1.00 g) in dichloromethane (100 ml) was added sodium borohydride (1.1 fold of **4** in mole), then the mixture was cooled to −15 °C. Methanol and tetrahydrofuran mixture (40 ml, 1:1, v/v) was added to reaction mixture dropwise and stirred at this temperature for 20 h. Then concentrated hydrochloric acid (20 ml) was added. The solid was obtained by filtering and purified though silica gel column chromatography.

4.1.12. Compound 5a 6,8-dimethoxy-2-phenylisoquinolin-1(2*H*)-one

Yield 33.0%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.91 (s, 3H, $-OCH_3$), 3.93 (s, 3H, $-OCH_3$), 6.56 (d, 1H, J = 7.2 Hz, –CH═), 6.49 (s, 2H, Ar-H), 7.13 (d, 1H, J = 7.2 Hz, ═CH–), 7.40–7.48 (m, 5H, Ar-H). HRMS: Calcd for $C_{17}H_{16}NO_3$ $[M+H]^+$: 282.1130; Found: 282.1143.

4.1.13. Compound 5b 6,8-dimethoxy-2-(3-ethynylphenyl) isoquinolin-1(2*H*)-one

Yield 64.5%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.09 (s, 1H, ≡CH), 3.91 (s, 3H, $-OCH_3$), 3.94 (s, 3H, $-OCH_3$), 6.37 (d, 1H, J = 7.2 Hz, –CH═), 6.49 (s, 2H, Ar-H), 7.09 (d, 1H, J = 7.2 Hz, ═CH–), 7.42 (s, 2H, Ar-H), 7.48 (b, 1H, Ar-H), 7.54 (s, 1H, Ar-H). HRMS: Calcd for $C_{19}H_{16}NO_3$ $[M+H]^+$: 306.1130; Found: 306.1149.

4.1.14. Compound 5c 6,8-dimethoxy-2-(3-chloro-4-fluorophenyl)isoquinolin-1(2*H*)-one

Yield 44.5%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.91 (s, 3H, $-OCH_3$), 3.94 (s, 3H, $-OCH_3$), 6.38 (d, 1H, J = 7.2 Hz, –CH═), 6.50 (s, 2H, Ar-H), 7.06 (d, 1H, J = 7.2 Hz, ═CH–), 7.22–7.26 (m, 2H, Ar-H), 7.52 (d, 1H, J = 6.6 Hz, Ar-H). HRMS: Calcd for $C_{17}H_{14}ClFNO_3$ $[M+H]^+$: 334.0646; Found: 334.0659.

4.1.15. Compound 5d 6,8-dimethoxy-2-(3-fluorophenyl) isoquinolin-1(2*H*)-one

Yield 29.3%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 3.94 (s, 3H, $-OCH_3$), 3.96 (s, 3H, $-OCH_3$), 6.41 (d, 1H, J = 7.2 Hz, –CH═), 6.52 (s, 2H, Ar-H), 7.11 (s, 1H, Ar-H), 7.12 (d, 1H, J = 8.0 Hz, ═CH–), 7.20 (s, 1H, Ar-H), 7.22 (s, 1H, Ar-H), 7.44 (q, 1H, J = 5.6 Hz, Ar-H). HRMS: Calcd for $C_{17}H_{15}FNO_3$ $[M+H]^+$: 300.1036; Found: 300.1048.

4.1.16. Compound 5e 6,8-dimethoxy-2-(3,4-difluorophenyl) isoquinolin-1(2*H*)-one

Yield 22.3%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 3.90 (s, 3H, $-OCH_3$), 3.92 (s, 3H, $-OCH_3$), 6.45 (d, 1H, J = 7.5 Hz, –CH═), 6.48 (s, 2H, Ar-H), 7.15 (d, 1H, J = 7.5 Hz, ═CH–), 7.15–7.20 (m, 2H, Ar-H), 7.60 (d, 1H, J = 6.0 Hz, Ar-H). HRMS: Calcd for $C_{17}H_{14}F_2NO_3$ $[M+H]^+$: 318.0942; Found: 318.0953.

4.1.17. Compound 5f 6,8-dimethoxy-2-(3-trifluoromethylphenyl)isoquinoline-1(2*H*)-dione

Yield 12.3%; ^{1}H NMR (400 MHz, DMSO-d_6), δ: 3.89 (s, 3H, $-OCH_3$), 3.92 (s, 3H, $-OCH_3$), 6.39 (d, 1H, J = 7.2 Hz, –CH═), 6.50 (s, 2H, Ar-H), 7.12 (d, 1H, J = 8.0 Hz, ═CH–), 7.20 (b, 1H, Ar-H), 7.24 (b, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.58 (q, 1H, J = 5.6 Hz, Ar-H). HRMS: Calcd for $C_{18}H_{15}F_3NO_3$ $[M+Na]^+$: 372.0823; Found: 372.8010.

4.1.18. General synthesis for 2-aryl-8-hydroxy-6-methoxyisoquinozoline-1(2*H*)-one

The mixture of **5** (1.00 mmol) and lithium chloride monohydrate (20.00 mmol) in DMA (10 ml) was heated to 140 °C for 6 h under nitrogen with stirring, cooled to room temperature, poured into a solution of hydrochloric acid (2 M, 50 ml) at about 5 °C. The off-white solid was collected by filtering and refined though silica gel column chromatography.

4.1.19. Compound 6a 8-hydroxy-6-methoxy-2-(3-ethynylphenyl)isoquinolin-1(2*H*)-one

Yield 94.0%; ^{1}H NMR (300 MHz, DMSO-d_6), δ: 3.36 (s, 1H, ≡CH), 3.86 (s, 3H, $-OCH_3$), 6.47 (s, 1H, –CH═), 6.71 (s, 2H, Ar-H), 7.42 (d, 1H, J = 7.4 Hz, ═CH–), 7.56 (s, 3H, Ar-H), 7.63 (s, 1H, Ar-H), 12.81 (s, 1H, Ar-OH). HRMS: Calcd for $C_{18}H_{14}NO_3$ $[M+H]^+$: 292.0974; Found: 292.0986.

4.1.20. Compound 7 6,8-dimehoxyisoquinolin-1(2*H*)-one

3-(3,5-Dimethoxyphenyl)acryl azide was prepared from 3-(3,5-dimethoxy phenyl)acryl acid. The mixture of the azide (13.0 g, 55.7 mmol) in *N*,*N*-diethylaniline (70 ml) was refluxed for 1.5 h under nitrogen, cooled to about 50 °C, added petroleum ether (60–90 °C, 100 ml), kept at rt over night. The solid was collected with suction, recrystallized from ethyl acetate and methanol (9:1) to produce a white crystal (8.06 g). Yield 70.5%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.89 (s, 3H, O–CH_3), 3.99 (s, 3H, O–CH_3), 6.38 (d, J = 6.96 Hz, Ar-CH),6.49 (s, 2H, Ar-H), 7.16 (d, J = 6.63 Hz, Ar-CH). MS: 205.1 $[M]^+$, 176.1.

4.1.21. Compound 5g 6,8-dimethoxy-2-(3-(piperidin-1-ylcarbonyl)phenyl) isoquinoline-1(2*H*)-one

The mixture of **7** (3.00 g, 14.63 mmol), *N*-(3-iodobenzoyl)piperidine (5.53 g, 17.56 mmol), L-proline (1.76 g, 14.63 mmol), K_2CO_3 (4.00 g, 29.26 mmol), CuI (1.50 g) and DMSO (50 ml) was heated at 120 °C for 24 h with stirring, cooled. The solvent was removed under vacuum and the residue was dissolved in chloroform (50 ml). The organic layer was washed with saturated Na_2CO_3 solution (20 ml × 3), hydrochloric acid solution (1 M, 20 ml × 3) and brine (20 ml), dried over anhydrous sodium sulfate. The crude product was purified though silica gel column chromatograph (chloroform/methanol = 30:1, v/v) to give a solid (2.70 g). Yield 47.0%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.67 (s, 6H, –CH_2–), 3.39 (s, 2H, –NCH_2–), 3.70 (s, 2H, –NCH_2–), 3.92 (s, 3H, –OCH_3), 3.94 (s, 3H, –OCH_3), 6.39 (d, 1H, J = 7.4 Hz, –CH═), 6.49 (s, 2H, Ar-H-5,7), 7.13 (d, 1H, J = 7.3 Hz, ═CH–), 7.38 (d, 1H, J = 6.8 Hz, Ar-H-4′), 7.43 (s, 1H, Ar-H-2′), 7.47 (m, 1H, Ar-H-5′), 7.49 (m, 1H, Ar-H-6′). HRMS: Calcd for $C_{23}H_{25}N_2O_4$ $[M+H]^+$: 393.1814; Found: 393.1824.

4.1.22. Compound 5h 6,8-dimethoxy-2-(3-aminocarbonylphenyl)isoquinoline-1(2*H*)-one

Yield 65.4%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.93 (s, 6H, –OCH_3), 6.40 (d, 1H, J = 7.0 Hz, Ar-H), 6.50 (s, 2H, Ar-H), 7.13 (d, 1H, J = 7.3 Hz, Ar-H), 7.57 (m, 2H, Ar-H), 7.87 (m, 2H, Ar-H). HRMS: Calcd for $C_{18}H_{17}N_2O_4$ $[M+H]^+$: 325.1188; Found: 325.1195.

4.1.23. Compound 5i 2-benzyl-6,8-dimethoxyisoquinoline-1(2*H*)-one

Yield 54.7%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 3.90 (s, 3H, –OCH_3), 3.99 (s, 3H, –OCH_3), 5.17 (s, 2H, –CH_2–), 6.32 (d, J = 7.2 Hz, 1H, –CH═), 6.45 (s, 1H, Ar-H), 6.49 (s, 1H, Ar-H), 7.07 (d, J = 7.2 Hz, 1H, ═CH–), 7.32 (d, J = 8.0 Hz, 2H, Ar-H), 7.35 (s, 2H, Ar-H), 7.37 (br, 1H, Ar-H). HRMS: Calcd for $C_{17}H_{16}NO_3$ $[M+H]^+$: 282.1130; Found: 282.1143.

4.1.24. Compound 6b 8-hydroxy-6-methoxy-2-phenylisoquinolin-1(2*H*)-one

Prepared from **5a**. Yield 42.1%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.89 (s, 3H, O–CH_3), 6.47 (d, 1H, J = 6.2 Hz, Ar-H), 6.52 (s, 2H, Ar-H), 7.07 (d, J = 5.8 Hz, 1H, Ar-H), 7.43–7.53 (m, 5H, Ar-H). HRMS: Calcd for $C_{16}H_{14}NO_3$ $[M+H]^+$: 268.0974; Found: 268.0982.

4.1.25. Compound 6c 8-hydroxy-6-methoxy-2-(3-(piperidin-1-ylcarbonyl)phenyl)isoquinolin-1(2*H*)-one

Prepared from **5g**. Yield 77.7%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.71 (s, 6H, –CH_2–), 3.46 (s, 2H, –NCH_2–), 3.74 (s, 2H, –NCH_2–), 3.91 (s, 3H, –OCH_3), 6.49 (s, 1H, –CH═), 6.54 (s, 2H, Ar-H-5,7), 7.10 (d, 1H, J = 6.4 Hz, ═CH–), 7.49 (s, 2H, Ar-H), 7.52 (s, 1H, Ar-H), 7.57 (d, 1H, J = 7.2 Hz, Ar-H), 12.75 (s, 1H, Ar-OH). HRMS: Calcd for $C_{22}H_{22}N_2NaO_4$ $[M+Na]^+$: 401.1477; Found: 401.1488.

4.1.26. Compound 6d 8-hydroxy-6-methoxy-2-(3-aminocarbonylphenyl)isoquinolin-1(2*H*)-one

Prepared from **5h**. Yield 57.5%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.93 (s, 3H, O–CH_3), 6.40 (d, 1H, J = 7.2 Hz, Ar-H), 6.50 (s, 2H, Ar-H), 7.13 (d, 1H, J = 7.3 Hz, Ar-H), 7.57 (m, 2H, Ar-H), 7.87 (m, 2H, Ar-H). HRMS: Calcd for $C_{17}H_{14}N_2NaO_4$ $[M+Na]^+$: 33.0851; Found: 333.0860.

4.1.27. Compound 6e 2-benzyl-8-hydroxy-6-methoxyisoquinoline-1(2*H*)-one

Prepared from **5j**. Yield 78.4%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 3.90 (s, 3H, –OCH_3), 5.27 (s, 2H, –CH_2–), 6.30 (d, J = 7.0 Hz, 1H, –CH═), 6.45 (s, 1H, Ar-H), 6.63 (s, 1H, Ar-H), 7.12 (d, J = 7.0 Hz, 1H, ═CH–), 7.22 (d, J = 8.0 Hz, 2H, Ar-H), 7.35 (s, 2H, Ar-H), 7.47 (b, 1H, Ar-H), 12.84 (s, 1H, Ar-OH). HRMS: Calcd for $C_{17}H_{16}NO_3$ $[M+H]^+$: 282.1130; Found: 282.1143.

4.1.28. Compound 8a 6,8-dimethoxy-5-nitro-2-(3-(piperidin-1-ylcarbonyl)phenyl)isoquinoline-1(2*H*)-one

Compound **5g** (0.39 g, 1.0 mmol) was dissolved in con. H_2SO_4 (8 ml) at 0 °C. Then urea nitrate (0.11 g, 1.0 mmol) was carefully added in portions at 0 °C with stirring. The mixture was stirred at 0 °C for 30 min, poured onto crashed ice (50 ml) with stirring. The solid was obtained by filtering and recrystallized from ethyl acetate to produce 0.29 g of product. Yield 65.61%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.57 (s, 2H, –CH_2–), 1.71 (s, 4H, –CH_2–), 3.46 (s, 2H, –NCH_2–), 3.74 (s, 2H, –NCH_2–), 3.91 (s, 3H, –OCH_3), 3.94 (s, 3H, –OCH_3), 6.49 (s, 1H, –CH═), 6.54 (s, 1H, Ar-H), 7.10 (d, 1H, J = 6.4 Hz, ═CH–), 7.49 (b, 2H, Ar-H), 7.52 (s, 1H, Ar-H), 7.57 (d, 1H, J = 7.2 Hz, Ar-H). HRMS: Calcd for $C_{23}H_{23}N_3NaO_6$ $[M+Na]^+$: 460.1485; Found: 460.1496.

4.1.29. Compound 8b 8-hydroxy-6-methoxy-5-nitro-2-(3-(piperidin-1-ylcarbonyl)phenyl)isoquinoline-1(2*H*)-one

From **6c**. Yield 87.9%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.58 (s, 2H, –CH_2–), 1.71 (s, 4H, –CH_2–), 3.56 (s, 2H, –NCH_2–), 3.70 (s, 2H, –NCH_2–), 3.91 (s, 3H, –OCH_3), 6.49 (s, 1H, –CH═), 6.54 (s, 1H, Ar-H), 7.10 (d, 1H, J = 6.4 Hz, ═CH–), 7.49 (s, 2H, Ar-H), 7.52 (s, 1H, Ar-H), 7.57 (d, 1H, J = 7.2 Hz, Ar-H), 12.75 (s, 1H, Ar-OH). HRMS: Calcd for $C_{22}H_{21}NNaO_6$ $[M+Na]^+$: 446.1328; Found: 446.1335.

4.1.30. Compound 9 5-(3-bromopropionylamino)-8-hydroxy-6-methoxy-2-(3-(piperidin-1-ylcarbonyl)phenyl)isoquinoline-1(2*H*)-one

The mixture of **8b** (0.40 g, 0.94 mmol), iron powder (1.04 g, 1.84 mmol) and ammonium chloride (0.3 g) in ethanol (30 ml) and water (60 ml) was refluxed with stirring for 4.5 h. The iron mud was removed by filtering and the filtrate was concentrated and the residue was dissolved in chloroform (50 ml). The organic phase was washed with saturated Na_2CO_3 solution (20 ml × 3) and brine (20 ml), dried over anhydrous sodium sulfate. A dark-green solid (0.28 g) was obtained by removing the solvent and used without further purification. The solid was dissolved in anhydrate THF (10 ml) and TEA (0.2 ml) was added. The mixture was cooled to 0 °C, added dropwise a solution of 3-bromopropionyl chloride (2 mmol) in dry THF (5 ml), stirred at this temperature for 2 h. The solvent was removed under vacuum and the residue was dissolved in chloroform (20 ml). The organic layer was washed with saturated Na_2CO_3 solution (20 ml × 3), hydrochloric acid solution (1 M, 20 ml × 3) and brine (20 ml), dried over anhydrous sodium sulfate. The crude product was refined though silica gel column chromatograph (chloroform/methanol = 30:1, v/v) to give a solid of 0.14 g. Yield 28.3%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.57 (b, 2H, –CH_2–), 1.71 (b, 4H, –CH_2–), 2.19 (b, 2H, –CH_2–), 3.07 (t,

2H, J = 6.2 Hz, –CH_2–), 3.45 (b, 2H, –NCH_2–), 3.73(b, 2H, –NCH_2–), 3.79 (t, 2H, J = 6.2 Hz, –CH_2–), 3.92 (s, 3H, –OCH_3), 6.59 (s, 1H, Ar-H), 6.62 (d, 1H, J = 7.6 Hz, –CH=), 7.00 (s, 1H, Ar-H), 7.12 (d, 1H, J = 7.6 Hz, =CH–), 7.47 (s, 1H, Ar-H), 7.50 (s, 1H, Ar-H), 7.57 (t, 1H, Ar-H), 13.07 (s, 1H, Ar-OH). HRMS: Calcd for $C_{25}H_{26}N_3NaO_5$ $[M+Na]^+$: 550.0954; Found: 550.0966.

4.1.31. Compound 10a 5-acryloylamino-6,8-dimethoxy-2-(3-(piperidin-1-ylcarbonyl)phenyl)isoquinoline-1(2*H*)-one

The nitro group in **8a** was reduced with iron powder. The obtained amine (0.07 g, 0.16 mmol) was dissolved in dried THF (5 ml) and TEA (0.1 ml) was added. The solution was cooled to 0 °C, added dropwise acryloyl chloride (0.05 ml, 0.30 mmol) in anhydrate THF (5 ml). The resulted mixture was stirred at 0 °C for 30 min, then at rt for another 4 h. The volatile was removed under vacuum and the residue was dissolved in chloroform (20 ml). The organic layer was washed with saturated Na_2CO_3 solution (20 ml × 3), hydrochloric acid solution (2 M, 20 ml × 3) and brine (20 ml), dried over anhydrous sodium sulfate. The crude product was purified though silica gel column chromatograph ($CHCl_3$/MeOH = 30:1, v/v) to give a light-yellow solid (0.04 g). Yield 54.2%; ^{1}H NMR (300 MHz, $CDCl_3$), δ: 1.42–1.80 (m, 6H, 3× CH_2), 3.40 (b, 2H, N–CH_2), 3.61 (b, 2H, N–CH_2), 3.90 (s, 6H, O–CH_3), 5.81 (dd, 1H, J = 10.2, 1.2 Hz, CH=), 6.26 (dd, 1H, J = 16.9, 10.2 Hz, =CH_2), 6.45 (dd, 1H, J = 16.9, 1.2 Hz, =CH_2), 6.50 (m, 1H, Ar-CH), 6.61(s, 1H, Ar-H), 7.12 (d, 1H, J = 7.6 Hz, Ar-CH), 7.38–7.55 (m, 4H, Ar-H). HRMS: Calcd for $C_{26}H_{27}N_3NaO_5$ $[M+Na]^+$: 484.1848; Found: 484.1856.

4.1.32. Compound 10b 5-acryloylamino-8-hydroxy-6-methoxy-2-(3-(piperidin-1-ylcarbonyl)phenyl)isoquinoline-1(2*H*)-one

Yield 71.5%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.58 (b, 2H, –CH_2–), 1.71 (b, 4H, –CH_2–), 3.45 (b, 2H, N–CH_2), 3.76 (b, 2H, N–CH_2), 3.92 (s, 3H, –OCH_3), 5.80 (dd, 1H, J = 10.2, 1.2 Hz, CH=), 6.22 (dd, 1H, J = 16.9, 10.2 Hz, =CH_2), 6.47 (dd, 1H, J = 16.9, 1.2 Hz, CH=CH_2), 6.60 (s, 1H, Ar-H), 6.66 (d, 1H, J = 7.6 Hz, –CH=), 7.00 (s, 1H, Ar-H), 7.12 (b, 1H, J = 7.2 Hz, =CH–), 7.48 (d, 1H, J = 3.2 Hz, Ar-H), 7.50 (s, 1H, Ar-H), 7.56 (q, 1H, Ar-H), 13.07 (s, 1H, Ar-OH). HRMS: Calcd for $C_{25}H_{25}N_3NaO_5$ $[M+Na]^+$: 470.1692; Found: 470.1711.

4.1.33. Compound 11 5-[4-(4-methylpiperazin-1-ylcarbonyl)phenyl]urea-8-hydroxy-6-methoxy-2-(3-(piperidin-1-ylcarbonyl)phenyl)isoquinoline-1(2*H*)-one

4-(4-Methylpiperazin-1-ylcarbonyl)phenylisocyanate was prepared as our reported method.[32] Arylamine, reduced from **8b**, was directly added to the isocyanate, and the mixture was stirred at room temperature for 2 h. The solvent was removed and flash chromatograph of the residue over silica gel, using chloroform/methanol (10:1), produce **11** as white solids. Yield 44.6%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.59 (s, 2H, –CH_2–), 1.72 (s, 4H, –CH_2–), 2.40 (s, 3H, NCH_3), 2.54 (b, 4H, –NCH_2–), 3.46 (b, 2H, –NCH_2–), 3.67 (d, 1H, J = 10.8 Hz, =CH–), 3.76 (b, 4H, –NCH_2–), 3.79 (s, 2H, –NCH_2–), 3.87 (s, 3H, –OCH_3), 6.55 (s, 1H, Ar-H), 6.67 (d, 1H, J = 6.4 Hz, –CH=), 6.96 (s, 1H, Ar-H), 7.31 (d, 2H, J = 8.8 Hz, Ar-H), 7.41 (d, 2H, J = 8.8 Hz, Ar-H), 7.45 (d, 1H, J = 9.2 Hz, Ar-H), 7.49 (s, 1H, Ar-H), 7.55 (m, 1H, Ar-H), 12.96 (s, 1H, Ar-OH). HRMS: Calcd for $C_{35}H_{39}N_6O_6$ $[M+H]^+$: 639.2931; Found: 639.2957.

4.1.34. Compound 12 5-(3-(4-morpholinyl)propionylamino)-8-hydroxy-6-methoxy-2-(3-(piperidin-1-ylcarbonyl)phenyl)isoquinoline-1(2*H*)-one

The mixture of **9** (0.13 g, 0.25 mmol), potassium iodide (0.083 g, 0.50 mmol) and acetonitrile (10.0 ml) was refluxed for 2 h under nitrogen and cooled to room temperature. Then morpholine (0.04 ml, 0.50 mmol) was added. The mixture was refluxed for 6 h under nitrogen. The solvent was removed under vacuum. The residual was purified though silica gel column chromatography ($CHCl_3$/MeOH = 20:1, v/v) to produce 0.13 g of white solid. Yield 80.0%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.71 (b, 6H, –CH_2–), 2.67 (d, 6H, J = 4.0 Hz, –NCH_2–), 2.85 (b, 2H, –NCH_2–), 3.45 (b, 2H, –NCH_2–), 3.73 (d, 2H, J = 5.6 Hz, –$COCH_2$–), 3.81 (b, 4H, –CH_2O), 3.91 (s, 3H, –OCH_3), 6.53 (d, 1H, J = 8.0 Hz, –CH=), 6.61 (s, 1H, Ar-H), 7.09 (d, 1H, J = 8.0 Hz, =CH–), 7.47 (d, 1H, J = 1.2 Hz, Ar-H), 7.48 (s, 1H, Ar-H), 7.50 (s, 1H, Ar-H), 7.57 (t, 1H, J = 7.8 Hz, Ar-H), 13.02 (s, 1H, Ar-OH). HRMS: Calcd for $C_{29}H_{35}N_4O_6$ $[M+H]^+$: 535.2557; Found: 535.2576.

4.1.35. Compound 13 6,8-dimethoxy-3,4-dihydro-isoquinolin-1-(2*H*)-one

To the solution of compound **1** (2.00 g, 10.4 mmol) in TFA (30 ml) was added to NaN_3 (1.95 g, 30.0 mmol) in portions. The reaction mixture was refluxed with stirring for 10 h. The solvent was removed and the residue was dissolved in ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate. The crude product was refined though silica gel column chromatography (chloroform/methanol = 40:1, v/v) to give brown solid (1.08 g). Yield 50.1%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 2.91 (t, 2H, J = 6.4 Hz, –CH_2–), 3.45 (t, 2H, J = 5.2 Hz, –CH_2–), 3.86 (s, 3H, –OCH_3), 3.92 (s, 3H, –OCH_3), 6.33 (s, 1H, Ar-H), 6.42 (s, 1H, Ar-H), 6.83 (s, 1H, –NH). MS: 208.1 $[M+H]^+$.

4.1.36. Compound 14 3,4-dihydro-6,8-dimethoxy-2-(3-(piperidin-1-ylcarbonyl) phenyl)-isoquinolin-1-(2*H*)-one

From **13**. Yield 73.6%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.67 (b, 6H, –CH_2–), 3.05 (t, J = 6.0 Hz, 2H, –CH_2), 3.42 (br, 2H, N–CH_2), 3.72 (br, 2H, N–CH_2), 3.88 (s, 3H, –OCH_3), 3.90 (s, 3H, –OCH_3), 3.95 (t, 2H, J = 6.4 Hz, –CH_2), 6.35 (s, 1H, Ar-H), 6.44 (s, 1H, Ar-H), 7.21 (d, J = 7.2 Hz, 1H, Ar-H), 7.39 (d, J = 7.6 Hz, 1H, Ar-H), 7.34 (s, 1H, Ar-H), 7.46–7,42 (t, J = 7.2 Hz, 1H, Ar-H). HRMS: Calcd for $C_{23}H_{26}N_2NaO_4$ $[M+Na]^+$: 417.1790; Found: 417.1803.

4.1.37. Compound 15 3,4-dihydro-8-hydroxy-6-methoxy-2-(3-(piperidin-1-ylcarbonyl)phenyl)-isoquinolin-1-(2*H*)-one

From **14**. Yield 90.0%; ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.71 (b, 6H, –CH_2–), 3.09 (t, 2H, J = 6.4 Hz, –CH_2–), 3.47 (b, 2H, –NCH_2–), 3.71 (b, 2H, –NCH_2–), 3.85 (s, 3H, –OCH_3), 3.97 (t, 2H, J = 6.4 Hz, –CH_2–), 6.29 (s, 1H, Ar-H), 6.37 (d, J = 2.0 Hz, 1H, Ar-H), 7.32 (d, J = 7.2 Hz, 1H, Ar-H), 7.42 (s, 1H, Ar-H), 7.45 (d, J = 7.2 Hz, 1H, Ar-H), 7.48 (t, J = 7.6 Hz, 1H, Ar-H), 12.54 (br, 1H, Ar-OH). HRMS: Calcd for $C_{22}H_{24}N_2NaO_4$ $[M+Na]^+$: 403.1634; Found: 403.1643.

4.2. Biological materials and methods

4.2.1. In vitro antiproliferative assay

Cellular chemosensitivity was determined by using a modified MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2*H*-tetrazolium bromide) method assay in vitro. In brief, A431 or A549 cells in 100 μl culture medium were seeded into 96-well microplates respectively and incubated at 37 °C for 24 h prior to drug exposure. Cell numbers were titrated to keep control cells growing in the exponential phase throughout the 72 h incubation period. Cells were treated with final concentrations of 100.0, 10.0, 1.0, and 0.1 μM of tested compounds simultaneously and incubated for 72 h and then 20 μl of MTT solution (5 mg/ml in PBS) was added to each well and incubated for 4 h. The formed blue formazan crystals were pelleted to the bottom of the well by centrifugation, separated from the supernatant, and dissolved in 200 μl of DMSO. The optical density at 570 nm was determined by an ELISA reader. Three separate experiments with triplicate data were performed to obtain mean cell viability. The IC_{50} values were calculated according to the inhibition ratios.

4.2.2. ELISA-based EGFR-TK assay

In vitro EGFR-TK inhibition assays were carried out as described in reference.[33] EGFR-TK was prepared from shed membrane vesicles of human A431 carcinoma cell. Briefly, 96-well plates were precoated with a synthetic substrate poly-Glu-Tyr (Sigma, 0.25 mg/ml) overnight at 37 °C. 15 μg/μl of EGFR-TK extract, reaction medium and tested compound (20 μl) were added. The reaction mixtures were incubated for 30 min at room temperature while being shaken. Kinase reaction was quenched by removal of the reaction mixture, then the wells were washed with washing buffer for three times. Phosphorylated tyrosine substrate was blocked in PBS containing 3% BSA for 30 min, washed with washing buffer (PBS containing 0.1% Tween 20) for three times, detected by adding anti-phosphotyrosine antibody for 1 h. Then antibody was removed, and wells were washed with washing buffer for three times. Anti-mouse lgG (ZSGB-BIO; ZB-2305; 100 μl/well) coupled with horseradish peroxidase (HRP) was added and incubated for 1 h. HRP substrate was added (100 μl/well) and incubated for 10−20 min. The TMB reaction was quenched by addition of 50 μl of 0.1 M H_2SO_4. The optical density was measured at 450 nm by an ELISA reader. Experiment with triplicate data were performed. IC_{50} values were calculated for test compounds by using a regression analysis of the concentration/inhibition data.

4.3. Molecular modeling

The protein–ligand complex crystal structure of erlotinib bound to EGFR was chosen as the template to compare the docking mode between compound **5b**, **6a** and **15** bound to EGFR. The molecular docking procedure was performed by using C-DOCKER protocol within Discovery Studio 2.5. For enzyme preparation, the hydrogen atoms were added. The whole EGFR enzyme was defined as a receptor and the site sphere was selected on the basis of the ligand binding location of erlotinib. Erlotinib was removed and compound **5b** or **6a** or **15** was placed. After end of molecular docking, ten docking poses was scored and selected based on calculated C-DOCKER energy.

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