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Research Letter

Novel Quinazoline Derivatives Bearing Various 4-Aniline Moieties as Potent EGFR Inhibitors with Enhanced Activity Against NSCLC Cell Lines

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A class of novel quinazoline derivatives bearing various C-4 aniline moieties was synthesized and biologically evaluated as potent epidermal growth factor receptor (EGFR) inhibitors for intervention of non-small-cell lung cancer (NSCLC). Most of these inhibitors are comparable to gefitinib in inhibiting these cancer cell lines, and several of them even displayed superior inhibitory activity. In particular, analogue 5b with an IC_{50} of 0.10 μM against the EGFR wild-type A431 cells and 5c with an IC_{50} of 0.001 μM against the gefitinib-sensitive HCC827 cells (EGFR del E746-A750) was identified as highly active EGFR inhibitors. It was also significant that the discovered analogue 2f, not only has high potency against the gefitinib-sensitive cells (IC_{50} = 0.031 μM), but also possesses remarkably improved activity against the gefitinib-resistant cells. In addition, the enzymatic assays and the Western blot analysis for evaluating the effects of the typical inhibitors indicated that these molecules strongly interfere with the EGFR target.

Key words: EGFR, inhibitor, NSCLC, quinazoline, T790M

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The EGFR is an important member of the erbB receptor family (erbB1/EGFR, erbB2/HER2, erbB3/HER3, and erbB4/HER4) of receptor tyrosine kinases (TKs), and often overexpressed in several human solid tumors, in particular, colorectal, pancreatic, bladder, lung, etc. (1,2) Comprehensive clinical studies showed that inhibiting EGFR family receptor TKs represents a major advance in the treatment of solid tumors (3,4). Gefitinib (**1**, Figure 1) (5,6) and erlotinib (7) were the first-generation EGFR inhibitors approved for the treatment of NSCLC in 2002 and 2004, respectively. By competitively binding to the adenosine triphosphate (ATP)-binding pocket of the intracellular EGFR TK domain, these agents effectively block the aberrant EGFR downstream signaling essential for tumor survival and proliferation (8). In general, to maintain their high antiproliferative potency, an 4-anilinoquinazoline core is essential. Until now, a family of new quinazoline derivatives, including lapatinib (9), dacomitinib (10,11), and afatinib (12), has been developed as more effective anti-NSCLC drugs. Although these agents exhibited high response rates in the treatment of NSCLC tumors, their efficacies are ultimately limited by the acquisition of drug resistance, particularly by the mutation of the gatekeeper T790M (13–15). To overcome the T790M mutation-related resistance, irreversible EGFR inhibitors containing a Michael acceptor functional group have been developed. These inhibitors form a covalent bond with the Cys-797 within the EGFR active site and have shown preclinical activity against T790M-containing mutants of EGFR. However, apart from a few examples, such as WZ4002 (16), CO-1686 (17), and AZD9291 (18,19), these irreversible inhibitors have been limited by associated skin rash and gastrointestinal toxicity (20,21). Considering that covalent inhibitors cannot be displaced by ATP and are not able to circumvent these issues, the non-covalent strategy has attracted more attention recently (22).

Gefitinib, as a reversible EGFR inhibitor, is broadly used in the treatment of NSCLC patients (5,6). The co-crystal structure of gefitinib complexed with the mutant EGFR T790M (PDB code: 4I22) indicates that the C-4 aniline side chain lies in a deep hydrophobic pocket at the back of the ATP-binding site (Figure 1) (23–25). Previous structure and activity relationship (SAR) explorations disclosed that modifying the aniline moiety is sensitive to the key mutant T790M, the inhibition of which will substantially block the drug resistance (26–31). On the basis of the quinazoline backbone, we focused on the aniline section and synthesized a number of novel 4-anilinoquinazoline derivatives bearing various new C-4 aniline moieties (**2a**–**i**, **3a**–**g**, **4a**–**c**, and **5a**–**c**, Figure 2). All these compounds were evaluated for their activities against various NSCLC cell lines ($A431^{WT}$, $HCC827^{delE746\text{-}A750}$, $H1975^{L858R/T790M}$, and

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A549$^{k\text{-}Ras}$ mutation) as well as the MCF-7 breast cancer cell line and the SGC-7901 stomach cancer cell. In addition, enzymatic assays and Western blot analysis were performed to evaluate their effects, while molecular simulation was also carried out in terms of the excellent inhibitors.

Chemistry

As illustrated in Scheme 1, amidation reaction of anilines **6a**–**b** and bromoacetyl bromide afforded the intermediate **7a**–**b**. Coupling **7a**–**b** with substituted phenol produced the nitrophenyl analogues **8a**–**i**. After reducing the nitro group in compounds **8a**–**i**, the key aniline intermediates **9a**–**i** were prepared. Finally, treating **9a**–**i** with 4-chloroquinazoline core in methanol solvents provided the title compounds **2a**–**i** with yields of 66–86%.

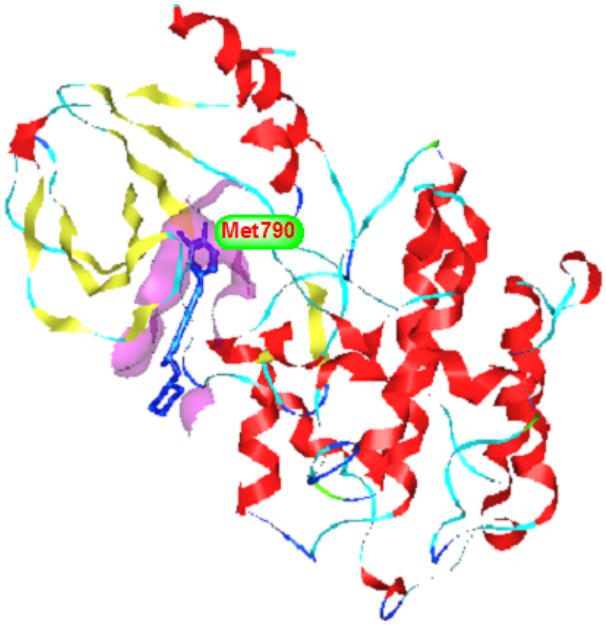


Figure 1: Structure of gefitinib and its binding sites with EGFR (PDB code: 4I22) (23).

Title compounds **3a**–**g** were synthesized starting from the material **10a**, **b** (Scheme 2). Treatment of **10a**, **b** with ethyl bromoacetate prepared compounds **11a**, **b**. After simple transformations of **11a**, **b**, the nitrophenyl intermediates **12a**–**g** were prepared in good yields (42–73%). Using the Fe-NH_4Cl condition, intermediates **12a**–**g** were reduced to aniline derivatives **13a**–**g** in good yields. Also by coupling the 4-chloroquinazoline core with the 4-substituted anilines **12a**–**g**, the title compounds **3a**–**g** were conveniently produced.

With the aim to synthesize the title compounds **4a**–**c** and **5a**–**c**, key intermediates *N*-phenyl benzenesulfonamides **15a**–**c** and **18a**–**c** were prepared in the first step (Schemes 3 and 4). By reducing the nitro group in compounds **15a**–**c** and **18a**–**c**, aniline analogues **16a**–**c** and **19a**–**c** were prepared, respectively. After installing the *N*-phenyl benzenesulfonamide moieties with the 4-chloroquinazoline core, the title compounds **4a**–**c** (Scheme 3) and **5a**–**c** (Scheme 4) were provided in yields of 63–85% and 67–81%, respectively.

Experimental Section

General procedure for the preparation of compound *N-[4-(N, N*-dibutylsulfonamide)phenyl]-7-methoxy-6-[3-(4-morpholinyl)propoxy]-4-quinazolinamine(**5c**): To a stirred mixture of aniline intermediates **19a**–c (3 mmol) in MeOH (10 mL), 4-chloroquinazoline derivatives (3.3 mmol) were added. The solution was refluxed for 2 h and then poured into H_2O (100 mL) and extracted with EtOAc (3 × 100 mL). The organic layer was collected and washed with brine. The organic layer was dried over sodium sulfate and concentrated in vacuo to obtain a crude product. The residue was purified by column chromatography on silica gel (elution with 5% DCM/MeOH) to give a white solid product. Yield 55.3%; mp 204.7–206.3 °C; ^{1}H NMR ([D]DMSO): δ = 1.02 (t, 6H, 2CH_3), 1.35 (m, 4H, 2CH_2), 1.47 (m, 4H, 2CH_2), 1.93 (m, 2H, CH_2), 2.35–2.40 (m, 6H, 3NCH_2), 3.21(t, 4H, 2NCH_2), 3.70(t, 4H, 2OCH_2), 3.75(s, 3H, OCH_3), 3.791 (t, 2H,

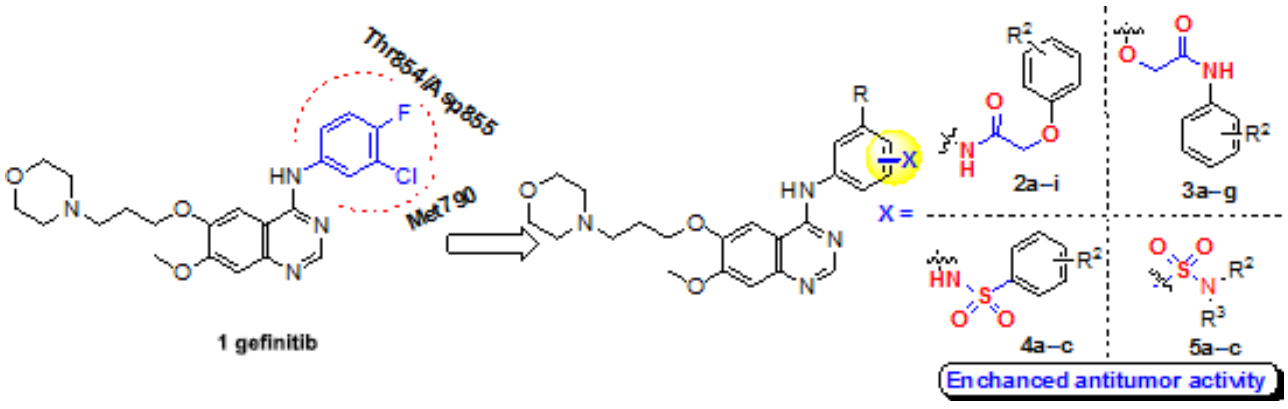
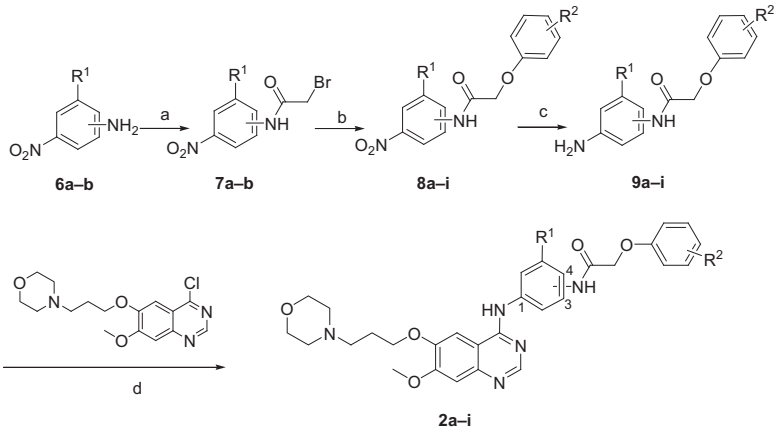
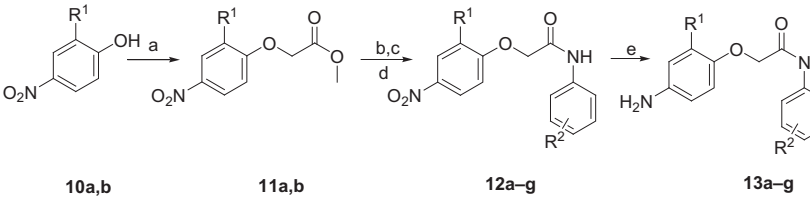


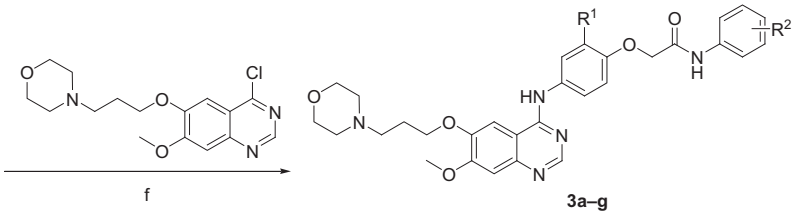
Figure 2: Structures of the designed compounds bearing various 4-aniline moieties.



2	R^1	R^2	$ArOCH_2CONH$
a	H	2-Me	3-position
b	H	2-Cl	3-position
c	H	4-*t*-Bu	3-position
d	H	4-Cl	3-position
e	H	2,4-$DiMe_2$	3-position
f	H	2-Me	4-position
g	H	4-*t*-Bu	4-position
h	H	4-Cl	4-position
i	MeO	2,4-$DiMe_2$	4-position

Scheme 1: Synthetic route of the title compounds **2a**–**i**. Reagents and conditions: (a) $BrCH_2COBr$, $NaHCO_3$, CH_3CN, r.t, 0.5 h. (b) Substituted phenol, K_2CO_3, KI, CH_3CN, 2 h, 80 °C. (c) Fe-NH_4Cl, EtOH-H_2O, 2 h, 80 °C. (d) MeOH, reflux, 2 h.





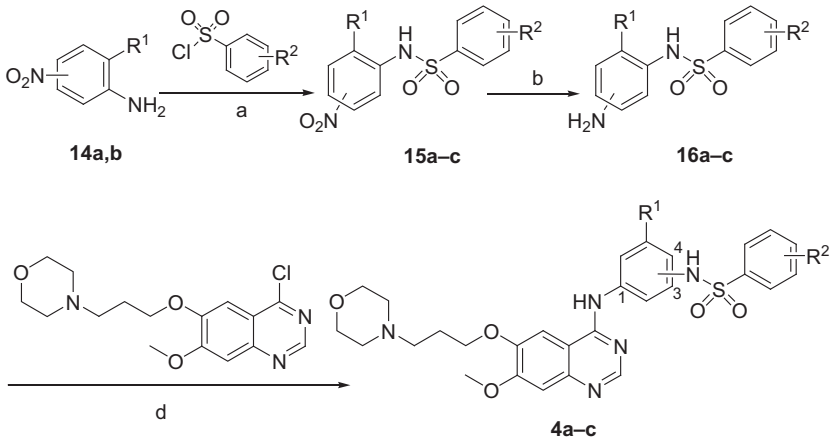
3	R^1	R^2
a	H	3-CF_3
b	H	4-Cl
c	H	2-Cl
d	H	2-Me
e	H	4-Me
f	H	3-Cl-4-F
g	Cl	H

Scheme 2: Synthetic route of the title compounds **3a**–**g**. Reagents and conditions: (a) $BrCH_2COOMe$, K_2CO_3, KI, CH_3CN, 2 h, 80 °C. (b) NaOH, H_2O-MeOH, 2 h, 60 °C. (c) $(COCl)_2$, 1 h, 60 °C. (d) aniline, $NaHCO_3$, CH_3CN, 2 h, 60 °C. (e) Fe-NH_4Cl, EtOH-H_2O, 2 h, 80 °C. (f) MeOH, reflux, 2 h.

OCH_2), 4.02(br, H, N*H*), 7.65–7.31 (m, 6H, Ar*H*), 8.41(s, H, N=C*H*); ^{13}C NMR ([D]DMSO): δ = 15.1(2C), 20.3(2C), 28.1, 29.3(2C), 49.3(2C), 50.2, 53.1(2C), 57.2, 67.1, 67.4 (2C), 100.1, 110.2, 113.2, 117.5(2C), 129.1(2C), 129.9, 145.9, 146.7, 152.3, 154.9, 159.1, 170.1; MS (ESI^+) *m/z* 586 $[M + H]^+$; Anal. calcd for $C_{30}H_{43}N_5O_5S$: C 61.49, H 7.38, N 11.95, O 13.69, S 5.49.

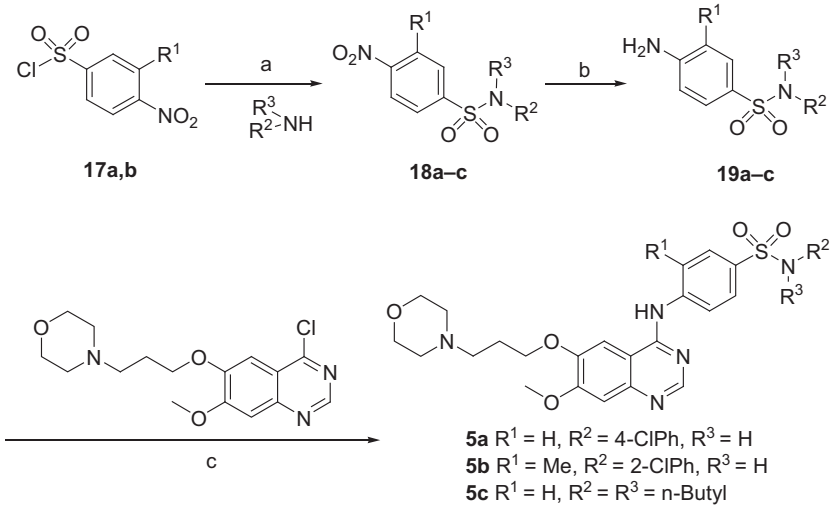
Results and Discussion

To evaluate the bioactivity of the synthesized compounds, the typical NSCLC cell lines $A431^{WT}$, $HCC827^{delE746\text{-}A750}$, $H1975^{L858R/T790M}$, and $A549^{K\text{-}ras\ mutant}$ were used in this study. Additionally, the EGFR-overexpressing MCF-7 cell line and SGC-7901 cell line were also chosen for further biological investigation. All theses cell-based evaluations were performed using the MTT assay (32). The test results are summarized in Table 1. In addition, the analogues **2a**, **2g**, **3a**, and **5c** which showed promising activity against the NSCLC cell lines were also investigated for their interference effects on the EGFR enzymatic activity. The enzymatic activity data are shown in Table 2. As a control, the effect of gefitinib was also evaluated. The results from these assays revealed that the quinazoline derivatives synthesized by us were as potent as gefitinib in inhibiting the proliferation of the tested cell lines, and several analogues even displayed higher antitumor activity than the lead compound gefitinib. Importantly, analogues **2f**–**h** and **5c** also showed enhanced antigefitinib-resistant H1975 cells,



4	R^1	R^2	$ArSO_2NH$
a	MeO	3-MeO	4-position
b	MeO	4-Cl	4-position
c	H	3-MeO	3-position

Scheme 3: Synthetic route of the title compounds **4a**–**c**. Reagents and conditions: (a) $ArSO_2Cl$, $NaHCO_3$, CH_3CN, reflux, 2 h. (b) Fe-NH_4Cl, EtOH-H_2O, 2 h, 80 °C. (c) MeOH, reflux, 2 h.



Scheme 4: Synthetic route of the title compounds **5a**–**c**. Reagents and conditions: (a) RNH_2, $NaHCO_3$, CH_3CN, reflux, 2 h. (b) Fe-NH_4Cl, EtOH-H_2O, 2 h, 80 °C. (c) MeOH, reflux, 2 h.

with IC_{50} values of 17.9, 12.5, 18.3 and 18.9 μM, respectively.

Inhibitory activity on cancer cells

Regarding the effects on the wild-type A431 cells, more than half of the tested molecules have IC_{50} values in the range of 0.07–7.01 μM. Obviously, introducing a 2-phenoxyacetamide molecule into the 4-aniline ring does not favor the improvement of the activity against the A431 cells. Among the analogues **2a**–**i**, the most active inhibitor **2b** (IC_{50} = 0.42 μM) is still less potent than the lead compound gefitinib. Also, the benzenesulfonamide substituent (analogues **4a**–**c**, IC_{50} > 9.31 μM) did not favor the suppression of the proliferation of the wild-type cells. On the other hand, fortunately, the set of analogues **5a**–**c** bearing an *N*-substituent benzenesulfonamide moiety displayed a powerful inhibitory potency (IC_{50} < 0.70 μM). The peculiar analogue **5b** (IC_{50} = 0.07 μM) bearing an *N*-(2-chlorophenyl)-sulfonamide substituent showed approximately 19 times higher activity than gefitinib. In contrast, replacing the 2-chlorophenyl group with a dibutyl substituent (analogue **5c**) resulted in about a 10-fold loss of anti-wild-type potency.

In the particular case of the activity against the gefitinib-sensitive cells, all the tested molecules were able to suppress HCC827 cells proliferation at nanomolar concentrations. The most active inhibitors, namely **2c** and **5c**, showed the same IC_{50} value of 0.001 μM. Admittedly, the

Table 1: Cellular antiproliferative activities of the title compounds[a]

	Cellular antiproliferative activity (IC_{50}, μM)					
Compound	A431	HCC827	H1975	A549	MCF-7	SGC-7901
2a	6.69 ± 0.12	0.003 ± 0.0009	31.4 ± 3.3	35.1 ± 3.4	11.9 ± 2.2	10.0 ± 3.1
2b	4.97 ± 0.56	0.018 ± 0.0001	19.5 ± 5.2	34.2 ± 4.6	12.5 ± 4.2	18.1 ± 1.2
2c	19.7 ± 1.1	0.001 ± 0.0006	29.0 ± 3.4	38.3 ± 8.1	17.3 ± 1.2	14.2 ± 1.1
2d	>10.0	>0.040	26.3 ± 2.3	24.5 ± 5.5	15.1 ± 2.1	20.1 ± 6.1
2e	7.01 ± 0.74	>0.040	26.0 ± 6.1	28.27 ± 4.3	10.5 ± 4.1	14.5 ± 2.0
2f	6.42 ± 0.32	>0.040	17.9 ± 3.3	36.5 ± 7.7	17.8 ± 2.2	19.6 ± 7.2
2g	17.8 ± 1.60	0.031 ± 0.007	12.5 ± 4.3	39.3 ± 6.4	18.7 ± 4.1	17.9 ± 3.3
2h	>10.0	>0.040	18.3 ± 5.5	39.1 ± 7.2	15.2 ± 1.1	16.7 ± 1.2
2i	5.80 ± 0.45	>0.040	19.7 ± 3.3	>40.0	18.3 ± 3.3	15.2 ± 3.1
3a	2.85 ± 0.65	0.005 ± 0.001	29.7 ± 4.6	18.3 ± 3.5	6.8 ± 1.1	5.7 ± 1.0
3b	0.33 ± 0.74	0.006	34.01 ± 4.1	26.7 ± 4.6	24.5 ± 2.1	12.1 ± 1.1
3c	0.42 ± 0.42	0.006 ± 0.0008	24.7 ± 3.2	25.3 ± 3.3	29.6 ± 5.1	15.2 ± 2.3
3d	1.54 ± 0.33	0.009 ± 0.0005	31.1 ± 4.2	24.7 ± 4.5	14.3 ± 2.1	14.5 ± 2.0
3e	1.03 ± 0.51	0.006 ± 0.0001	38.2 ± 9.1	23.0 ± 5.1	>40.0	17.5 ± 3.1
3f	8.33 ± 0.21	0.004 ± 0.0004	29.7 ± 6.3	26.3 ± 6.1	13.2 ± 3.1	16.9 ± 4.3
3g	0.10 ± 0.01	0.006 ± 0.0009	33.2 ± 7.1	25.8 ± 4.1	18.6 ± 1.2	13.7 ± 2.0
4a	>10.0	0.005 ± 0.0007	28.5 ± 8.1	38.6 ± 6.2	13.7 ± 2.2	15.6 ± 1.2
4b	9.31 ± 0.33	0.017 ± 0.0021	39.4 ± 9.1	>40.0	14.2 ± 3.3	13.5 ± 2.0
4c	10.30 ± 0.70	0.017 ± 0.0010	>40.0	32.5 ± 5.4	30.8 ± 8.1	>40.0
5a	0.20 ± 0.02	0.033 ± 0.0074	35.9 ± 3.5	32.2 ± 6.1	22.0 ± 6.4	13.1 ± 2.1
5b	0.07 ± 0.01	0.020 ± 0.0010	35.4 ± 4.3	>40.0	23.8 ± 4.4	38.4 ± 6.2
5c	0.70 ± 0.02	0.001 ± 0.0004	18.9 ± 5.5	22.7 ± 5.6	9.7 ± 1.2	6.4 ± 1.1
Gefitinib	1.33 ± 0.54	0.013 ± 0.07	26.9 ± 8.7	39.7 ± 9.1	13.1 ± 4.1	27.6 ± 4.2

[a]The IC_{50} values were obtained by the Logit method based on the data obtained from three separate experiments and expressed as mean ± SD ($p < 0.05$).

Table 2: Inhibitory activity of compounds **2a**, **2g**, **3a**, and **5c** against wild-type and mutant EGFR receptor kinases[a]

	EGFR IC_{50} [nM]	
Compound	WT	L858R/T790M
2a	11.49 ± 0.23	10.79 ± 0.65
2g	4.51 ± 0.07	5.32 ± 1.02
3a	6.53 ± 1.10	11.52 ± 0.96
5c	8.06 ± 0.78	71.08 ± 1.56
Gefitinib	5.94 ± 1.11	832.3 ± 101.7

[a]IC_{50} values were obtained by the Logit method based on the data obtained from three separate experiments and expressed as mean ± SD ($p < 0.05$).

four designed moieties appear to be greatly effective. Notably, HCC827 cells are far sensitive to the class of compounds **2a**–**i** bearing a 2-phenoxyacetamide moiety, and a small structural change in the 2-phenoxyacetamide moiety did significantly affect its capability. Also as indicated by the activity data of compounds **2a**–**d** and **2h**–**i** ($IC_{50} > 0.040$ μM), a small substituent, such as Cl, and Me, at the *C*-4 position of the 2-phenoxyacetamide moiety is not suitable.

As expected, by modifying the 4-aniline group, we produced several inhibitors that exhibited improved activity against drug-resistant cells, such as the 2-(2-methylphenoxy)acetamide-substituted **2f** (IC_{50} = 17.9 μM), the 2-(4-*t*-butylphenoxy)acetamide-substituted **2g** (IC_{50} = 12.5 μM), and the 2-(4-chlorophenoxy)acetamide-substituted **2h** (IC_{50} = 18.3 μM). Among the four series of analogues, the class of inhibitors **2a**–**i** bearing a 2-phenoxyacetamide moiety displayed considerable activity against the H1975 cells. Notably, the moiety at the para position of the 4-aniline group (analogues **2f**–**g**) is actually advantageous, indicating that a substituent at the para position will be in close contact with key mutant amino acid T790M and thus form strong binding forces.

To further evaluate their bioactivity, the A549 cells, the MCF-7 cells, and the SGC-7901 cells were also used in the subsequent biological evaluation. Regarding the effects on the EGFR-negative A549 cells, most of the synthesized compounds had IC_{50} values ranging from 20 to 40 μM. Moreover, these molecules also showed approximately twofold increase in inhibitory activity against the MCF-7 and SGC-7901 cells proliferation. In particular, the 2,4-dimethylphenoxyacetamide-subsituted **2i** and the *N,N*-dibutyl-benzenesulfonamide-substituted **5c** exhibited a superior potency for inhibiting both the MCF-7 cells and SGC-7901 cells at concentrations of 9.7 μM and below.

Kinase inhibitory activity

The 4-aniline quinazoline scaffold has long been recognized as a classical EGFR kinase inhibitor motif (3–6,

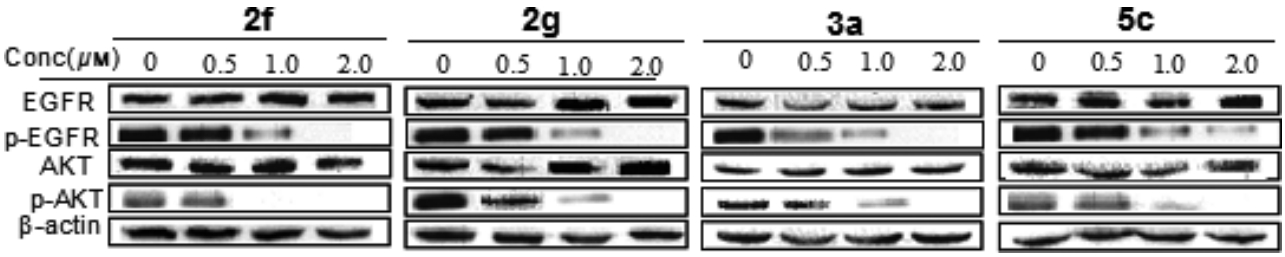


Figure 3: Compound **2f**, **2g**, **3a**, and **5c** inhibited the activation of EGFR and downstream signaling in H1975 NSCLC cell harboring EGFR L858R/T790M.

9–12). To further confirm the targeted active site of our synthesized compounds, the activities of the selected representative inhibitors compounds **2a**, **2g**, **3a**, and **5c** against both the wild-type EGFR and the mutant EGFRT790M enzymes were evaluated. The results, shown in Table 2, revealed that all the four active inhibitors were able to inhibit EGFR enzymes at nanomolar concentrations. Specifically, compound **2g** which bears a bulky *t*-butyl group was very effective, showing a stronger activity against the wild-type EGFR kinase than gefitinib. However, an even more exciting result was the inhibitory activity it displayed against the drug-resistant mutant cells with the EGFR harboring the T790M mutation. Compared with the control (IC_{50} = 832.3 nM), all the selected compounds (IC_{50} < 71.08 nM) showed greatly improved potency against the cells expressing EGFRT790M. Additionally, the *t*-butyl-substituted analogue **2g** was the most potent inhibitor against the

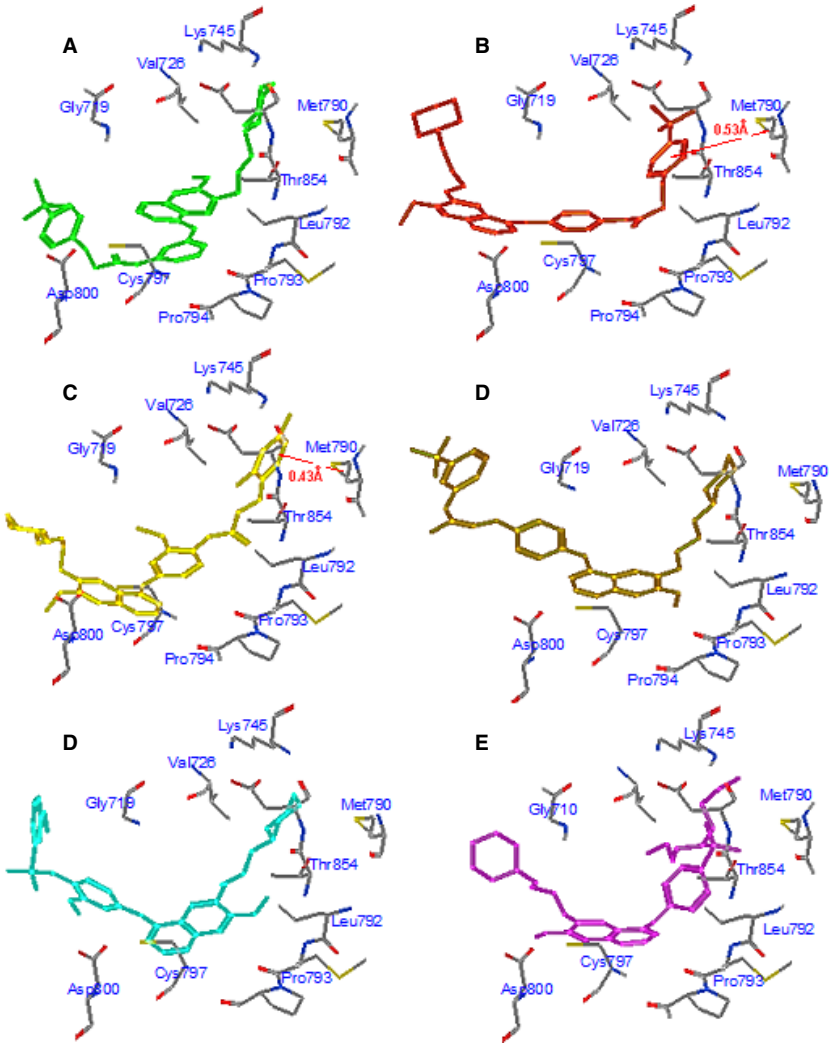


Figure 4: (A) Putative binding mode of analogue **2c** within the active pocket of the EGFR T790M (PDB code: 4I22). (B) Putative binding mode of **2g** within the active pocket of the EGFRT790M (PDB code: 4I22). (C) Putative binding mode of **2i** within the active pocket of the EGFRT790M (PDB code: 4I22). (D) Putative binding mode of **3a** within the active pocket of the EGFRT790M (PDB code: 4I22). (E) Putative binding mode of **4a** within the active pocket of the EGFRT790M (PDB code: 4I22). (F) Putative binding mode of **5c** within the active pocket of the EGFRT790M (PDB code: 4I22) (23).

$EGFR^{T790M}$ enzyme, exhibiting an IC_{50} value of 5.32 nM. These results directly corroborated that these analogues are effective EGFR inhibitors.

Effects on EGFR activation and downstream signaling

To get an insight into the mechanisms underlying the activity of these inhibitors, the four active representative compounds **2a**, **2g**, **3a**, and **5c** were assessed for their inhibitory effects on the activation of EGFR and the downstream signaling in H1975 cell. The expression and activation of proteins involved in the EGFR-mediated AKT pathway were assessed by immunoblotting, and the results are presented in Figure 3. These assessments demonstrated that treatment with these inhibitors greatly repressed the phosphorylation of the EGFR and downstream signaling proteins of the AKT pathway in a dose-dependent manner achieving complete inhibition at a concentration of 1.0 μM, while the total protein levels of EGFR, AKT, and β-actin remain unchanged.

Molecular simulation

In addition, a set of typical analogues **2g**, **2i**, and **5c**, which were effective at inhibiting the H1975 cells, were chosen to dock into the ATP-binding site in the model of EGFR kinase harboring the T790M mutation (PDB code: 4I22). The program AUTODOCK 4.2 in parallel with the default parameters was applied (33,34). For comparison, three less potent inhibitors **2c**, **3a**, and **4a** were also evaluated using same procedure. The results revealed that all the selected compounds were able to interact with the ATP-binding cleft of the enzyme through hydrophobic forces and/or hydrogen bond forces, as shown in Figure 4. Clearly, in the less potent compounds **2c**, **3a**, and **4a**, the newly introduced 4-aniline groups were so bulky that they were squeezed out of the hydrophilic pocket containing amino acids Lys745, Asp855, Thr854, Leu788, while they remained close to the amino acids Phe795, Cys796, Asp800, and Cys797. Instead, the hydrophilic morphine side chain fully occupied the hydrophobic region of the pocket. Consistent with the less potent activity, this binding site model is less effective at binding with $EGFR^{T790M}$. The binding sites of the inhibitors **2c**, **2f**–**i**, and **5c** in the $EGFR^{T790M}$ enzyme were very similar to those of gefitinib. The introduced 4-aniline moieties tightly interact with the hydrophobic pocket, whereas the morphine chain reaches to the tip of the pocket, and forms strong polar interactions with Asp800 and Gly719. After introducing a 2-phenoxyacetamide group into the 4-aniline moiety, the additional phenyl ring is closer to Met790 (0.5–0.6 Å) compared with that of gefitinib (0.67 Å). These closer contacts may result in the stronger capacity to overcome the drug resistance. Concerning the enhanced potency of inhibitor **5c**, its improved activity may be mainly generated from the interactions between the Met790 with the dibutyl substituent. Two important hydrogen bonds between the N-1 group of quinazoline core and the amino acid Met793 (NH), and the N-3 group of quinazoline core and the amino acid Thr854 (NH) disappeared in the active inhibitors. Nevertheless, there are several new interactions, such as the carbonyl group (C=O) with MET793 (NH), a water molecule-mediated hydrogen bond between quinazoline N3 and the side chain of Pro794. Taken together, the results of all these analyses were consistent with the test results.

Conclusion

In summary, a series of 4-anilinoquinazoline derivatives containing various *C*-4 aniline moieties were synthesized and biologically evaluated. Most of these compounds exhibited moderate to good inhibitory effects toward the EGFR-overexpressing cell lines, and several analogues even demonstrated higher potency than gefitinib. Furthermore, we also identified four analogues **2f**, **2g**, **2h**, and **5c** with enhanced inhibitory activity against drug-resistant cancer cells. Western blot analysis indicated that the compounds **2f**, **2g**, **3a**, and **5c** inhibited EGFR activation and downstream signaling in H1975 cells at a concentration of 0.5 μM. Overall, this study provided a number of new valuable 4-anilinoquinazoline derivatives worthy of further development as potentially more effective EGFR inhibitors.

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