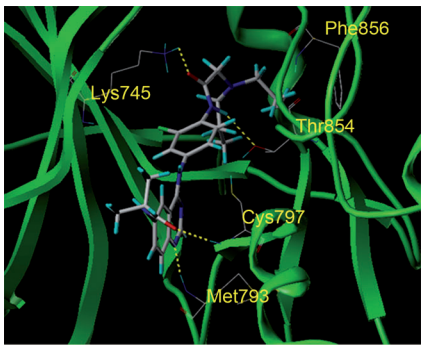
ChemPubSoc Europe

COMMUNICATIONS

Strike a pose! A series of 4-anilinoquinazolines were designed, synthesized and evaluated in vitro against lung and breast cancer cell lines. Several compounds were found to be endowed with cytotoxicity in the low micromolar range. Molecular docking suggests that these compounds bind to EGFR in a similar manner to known EGFR inhibitors.



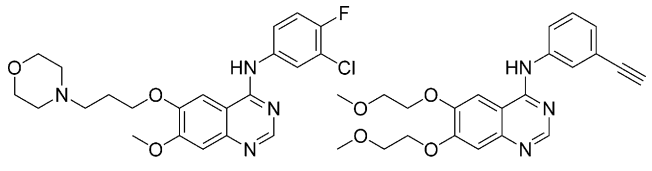
G.-W. Rao, G.-J. Xu, J. Wang, X.-L. Jiang, H.-B. Li*

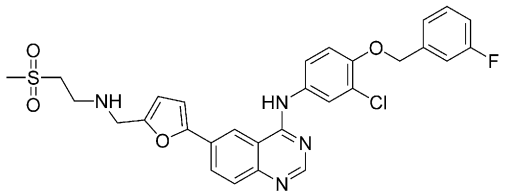
DOI: 10.1002/cmdc.201300120

Synthesis, Antitumor Evaluation and Docking Study of Novel 4-Anilinoquinazoline Derivatives as Potential Epidermal Growth Factor Receptor (EGFR) Inhibitors

Guo-Wu Rao,*[a] Geng-Jie Xu,[a] Jian Wang,[b] Xu-Liang Jiang,[b] and Hai-Bo Li[c]

Protein kinases, in particular receptor tyrosine kinases, are considered to be the second largest class of therapeutic targets by Gray et al.[1] The most extensively studied receptor tyrosine kinase is the epidermal growth factor receptor (EGFR).[2] Aberrant expression or activation of EGFR homologues has been connected with multiple human carcinomas, and drugs targeting ErbB activity have been licensed for treatment of lung, colon, breast, and head-and-neck carcinomas.[3,4] These drugs fall into two categories: monoclonal antibodies targeting ErbB extracellular regions, and small-molecule reversible tyrosine kinase inhibitors, such as erlotinib, gefitinib, and lapatinib.[4,5] These drugs are all 4-anilinoquinazoline derivatives and are among the most potent known tyrosine kinase inhibitors.[6–10] Numerous studies have aimed to identify novel compounds containing the 4-anilinoquinazoline core as small-molecule inhibitors of EGFR.[11–15]





In order to search for new promising antitumor agents that act through a mode of action similar to quinazoline-containing EGFR inhibitors, we attempted to investigate whether modifications to the 4-anilinoquinazoline structure could enhance the antitumor activities of this compound class. The co-crystal structure of the EGFR kinase domain in complex with lapatinib (PDB code 1XKK) shows that the N-1 of the quinazoline ring is hydrogen bonded to the main chain NH of Met 793,[16,17] and this interaction is presumed to exist for our design of 4-anilinoquinazoline-containing compounds. Additionally, the 4-(3-fluorobenzyloxy)-3-chlorobenzenamine moiety of lapatinib lies in a deep hydrophobic pocket at the back of the ATP binding site.[16] A hydrogen bond interaction between the CO and NH of the amide group with the backbone of Lys 745 or Thr 854 could be established by introducing an amide group on the phenyl ring of the 4-anilinoquinazoline; the hydrophobic amide group would presumably be accommodated in the deep hydrophobic pocket at the back of the ATP binding site. Taking these potential interactions into consideration, it should be possible to design compounds with strong affinities for EGFR and potent antitumor activities.

A novel series of 4-anilinoquinazoline derivatives were synthesized and evaluated for their antitumor activity. The synthetic routes are shown in Scheme 1. Early intermediates 4-chloroquinazoline (**1**) and 4-chloro-6-nitroquinazoline (**2**) were prepared by chlorination of 4-hydroxyquinazoline and 4-hydroxy-6-nitroquinazoline, respectively, according to published methods.[18,19] Key intermediates 4-(2-(substituted-amino)acetamido)aniline and 3-chloro-4-(2-(substituted-amino)acetamido)-aniline (**3a**–**g**) were synthesized from 4-nitrobenzenamine and 2-chloro-4-nitrobenzenamine, respectively, according to a published method.[20] Target compounds **4a**–**k** were synthesized from compound **3** and **1** or **2**. Intermediates **5a**–**d** were obtained by the reduction of compounds **4h**–**k**. Target compounds **4l**–**t** were prepared via the acylation of **5a**–**d**. The full structures of the final compounds and the yields are given in Table 1.

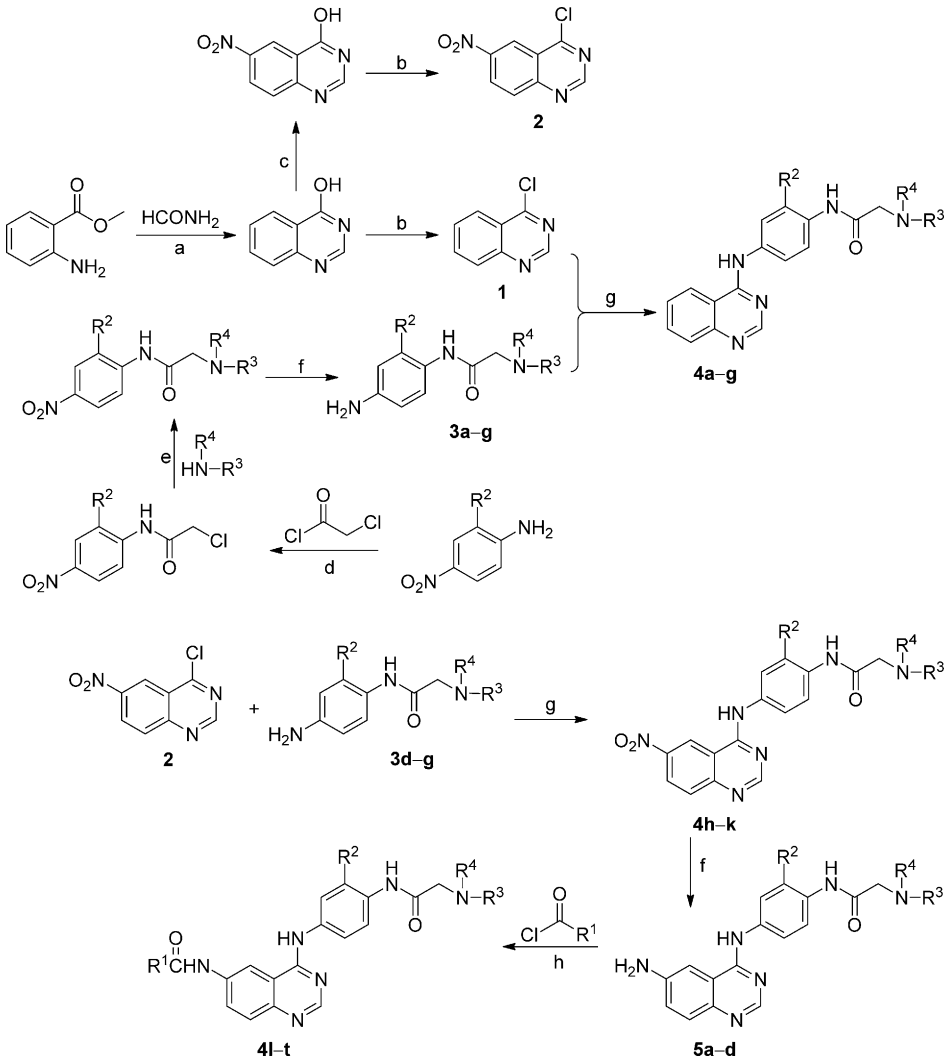
The antitumor activities of these compounds were evaluated in vitro against the growth of human lung adenocarcinoma epithelial (A-549) and breast cancer (MCF-7) cell lines with cisplatin as a positive control (Table 2). The results show that several compounds are highly effective against these two cancer cell lines. Literature reports indicate IC_{50} values for erlotinib, gefitinib, and lapatinib against A-549 cells to be 24.1, 11.8, and 2.80 μM, respectively;[21–23] in comparison, the IC_{50} values determined for compounds **4l** and **4m** against the same cell line were determined to be 16.9 and 5.38 μM, respectively. IC_{50} values for erlotinib, gefitinib, and lapatinib against the MCF-7 cell line are reported in the literature to be 10.2, 21.6, and

[a] Dr. G.-W. Rao, G.-J. Xu
College of Pharmaceutical Science
Zhejiang University of Technology
Hangzhou 310014 (P.R. China)
E-mail: rgw@zjut.edu.cn

[b] Dr. J. Wang, Dr. X.-L. Jiang
School of Pharmaceutical Engineering
Shenyang Pharmaceutical University
Shenyang 110016 (P.R. China)

[c] Dr. H.-B. Li
Laboratory of Microbiology
Nantong Centers for Disease Control and Prevention
Jiangsu 226007 (P.R. China)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201300120.



Scheme 1. *Reagents and conditions*: a) DMF, reflux, 12 h; b) toluene, Et_3N, $POCl_3$, reflux, 4 h; c) concd HNO_3/H_2SO_4, 12 h; d) $R^2=H$, THF, 0–5 °C then RT, 20 h, or $R^2=Cl$, CH_2Cl_2, 0–5 °C then RT, 20 h; e) $R^2=H$, CH_3OH, 50 °C, 20 h, or $R^2=Cl$, CH_2Cl_2, reflux, 20 h; f) $R^2=H$, CH_3OH, $HCOONH_4$, 5% Pd, 50 °C, 4 h, or $R^2=Cl$, CH_3CH_2OH, HCl, H_2O, Fe powder, reflux, 4 h; g) $CH_3CH(CH_3)OH$, Et_3N, reflux, 8 h; h) $CHCl_3$, 4-dimethylaminopyridine, 0–5 °C then RT, 20 h. Yields are given in Table 1.

6.60 μM, respectively,[6,21,24] and the IC_{50} values for compound **4l**, **4m**, **4r**, **4s**, and **4t** against MCF-7 cells were determined to be in a comparable range (7.83, 4.18, 9.38, 9.77, and 7.45 μM, respectively). Compound **4m** displayed similar cytotoxic activities against the both cancer cell lines. In contrast, compounds **4l**–**t**, which contain nucleophilic substituents at the 6-position of the anilinoquinazoline scaffold, showed moderate to excellent in vitro antitumor activity and potential selectivity for breast over lung cancer, indicating that this type of substitution is beneficial for activity. However, compounds containing electrophilic substituents at the 6-position of the anilinoquinazoline core (**4h**–**k**) exhibit decreased activity, suggesting that electrophilic substituents at the 6-position are unfavorable.[25]

A docking analysis was carried out in an attempt to rationalize the observed biological results and to predict the potential binding modes of active compounds with their putative intracellular target, EGFR. Docking of the inhibitors into the crystal structure of the EGFR kinase domain (PDB code 1XKK)[16,17] was performed using Surflex-Dock in the Sybyl X software package.[26]

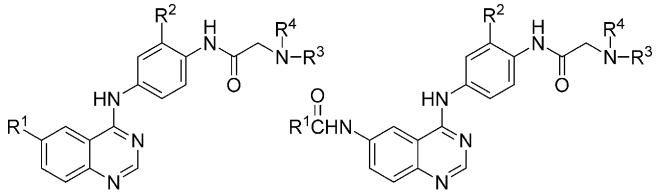
To validate our docking protocol, lapatinib was removed from the PDB structure and redocked. In agreement with the published co-crystal structure of lapatinib bound to the kinase domain of EGFR,[16] the docking result illustrates that lapatinib binds to the ATP binding cleft, with the quinazoline ring binding to the narrow hydrophobic pocket at the N terminus, the N-1 of the quinazoline ring forming a hydrogen bond to the main chain NH of Met 793, and the 4-(3-fluorobenzyloxy)-3-chlorobenzenamine lying in a deep hydrophobic pocket at the back of the ATP binding site (Figure 1 a).

Compounds **4l**, **4m**, and **4r**–**t** were submitted for docking evaluation as representative examples of active compounds (Figure 1). The docking studies suggest that the quinazoline ring binds to a narrow hydrophobic pocket in the EGFR N-terminal domain, where the N-1 of the quinazoline ring interacts with the backbone NH of Met 793 via a hydrogen bond, and similarly, that the large substituted anilino group allows for a deeper interaction and a better fit in the hydrophobic pocket at the back of the ATP binding site.[16,27]

Docking poses for compounds **4l** and **4m** predicted the formation of only one hydrogen-bonding interaction between the N-1 of the quinazoline ring and Met 793 (2.10 and 2.01 Å, respectively), and the model also suggests that the 4-(2-(diethyl amino)acetamido)anilino group of both **4l** and **4m** would probably also lie in a deep hydrophobic pocket at the back of the ATP binding site, in a manner similar to that of lapatinib (Figure 1 b,c).

The docking poses generated predict hydrogen-bonding interactions between the CO groups at the 6-position of **4r**,**s**,**t** and Cys 797 (2.16, 2.07 and 2.03 Å, respectively) (Figure 1 d–f). Furthermore, interactions were also predicted to form between the CO and NH groups at the 4-position of **4s**,**t** and Lys 745 (2.67 and 2.62 Å, respectively) and Thr 854 (2.55 and 2.57 Å, respectively) (Figure 1 e,f). Both models for compound **4s** and **4t** predicted the formation of a hydrogen bond with Met 793 (2.05 and 2.08 Å, respectively). Finally, the docked pose for compound **4r** indicates the formation of hydrogen bonds with

Table 1. Isolated yields and melting point ranges for synthesized compounds **4a–t**.



4a–k **4l–t**

Compd	R^1	R^2	NR^3R^4	Yield[a] [%]	Mp[b] [°C]
4a	H	H	$N(CH_3)_2$	46	207–210
4b	H	H	morpholino	43	222–224
4c	H	H	*m*-$CH_3C_6H_4NH$	61	221–223
4d	H	H	$N(CH_2CH_3)_2$	66	145–148
4e	H	Cl	$N(CH_2CH_3)_2$	76	208–210
4f	H	H	$N(CH_2CH_2CH_3)_2$	89	113–115
4g	H	Cl	$N(CH_2CH_2CH_3)_2$	91	184–186
4h	NO_2	H	$N(CH_2CH_3)_2$	78	235–238
4i	NO_2	Cl	$N(CH_2CH_3)_2$	90	218–220
4j	NO_2	H	$N(CH_2CH_2CH_3)_2$	89	174–176
4k	NO_2	Cl	$N(CH_2CH_2CH_3)_2$	83	224–226
4l	$(CH_3)_2CHCH_2O$	H	$N(CH_2CH_3)_2$	40	179–181
4m	cyclohexylmethoxyl	H	$N(CH_2CH_3)_2$	46	216–218
4n	$(CH_3)_2CHCH_2O$	Cl	$N(CH_2CH_3)_2$	65	235–238
4o	cyclohexylmethoxyl	Cl	$N(CH_2CH_3)_2$	71	272–274
4p	$(CH_3)_2CHCH_2O$	H	$N(CH_2CH_2CH_3)_2$	63	105–107
4q	$(CH_3)_2CH$	H	$N(CH_2CH_2CH_3)_2$	60	191–193
4r	$(CH_3)_2CHCH_2O$	Cl	$N(CH_2CH_2CH_3)_2$	88	241–243
4s	CH_3CH_2	Cl	$N(CH_2CH_2CH_3)_2$	73	112–114
4t	$(CH_3)_2CH$	Cl	$N(CH_2CH_2CH_3)_2$	74	116–118

[a] Isolated yield of the final step, determined after purification by column chromatography. [b] Melting point (mp) values were determined on a X-4 melting point apparatus and uncorrected.

Table 2. Antitumor activities of 4-anilinoquinazolines **4a–t** against a human lung adenocarcinoma epithelial (A-549) and a breast cancer (MCF-7) cell line in vitro.[a]

Compd	IC_{50} [μM] A-549	MCF-7	Compd	IC_{50} [μM] A-549	MCF-7
4a	>100	>100	**4m**	**5.38**	**4.18**
4b	>100	>100	**4n**	94.6	77.5
4c	>100	>100	**4o**	44.0	22.3
4d	79.4	175	**4p**	>100	34.1
4e	>100	55.4	**4q**	52.6	18.2
4f	68.6	43.0	**4r**	30.3	**9.38**
4g	>100	>100	**4s**	54.6	**9.77**
4h	>100	>100	**4t**	>100	**7.45**
4i	>100	>100	Cisplatin	20.3	23.0
4j	114	49.1	Erlotinib[21]	24.1	10.2
4k	89.4	36.8	Gefitinib[22,24]	11.8	21.6
4l	**16.9**	**7.83**	Lapatinib[6,23]	2.80	6.60

[a] IC_{50} values were determined as described in the Experimental Section.

Met 793 and Thr 854 (1.99 Å and 2.40 Å, respectively) (Figure 1 d).

In the computational study, stronger binding energies were calculated for compounds **4m** and **4t** than for compounds **4l**, **4r**, and **4s**. Calculated Helmholtz free energies of interactions for protein–ligand atom pairs rank **4r** over lapatinib followed by **4m** and **4t**. Charge and van der Waals interactions between the protein and ligand suggest that **4s** and **4t** are superior ligands for EGFR than compounds **4l**, **4m**, and **4r**. Scoring of the compounds with respect to the reward for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept terms revealed that compound **4s** and **4t** are calculated to have more interactions with EGFR than **4l**, **4m**, and **4r**. The consensus score (C-score) summarizes all of the calculated forces of interaction between the ligand and receptor (EGFR); both the C-score and crash score predict lapatinib to be the best ligand, followed by compounds **4m**, **4r**, **4l**, **4s** and **4t** (Table 3); these compounds are calculated to have high C-scores, which is in keeping with their observed potent antitumor activity in vitro.

In conclusion, a novel series of 4-anilinoquinazoline derivatives has been synthesized and tested for their antitumor activities against A-549 and MCF-7 cell lines. The results showed several compounds to be endowed with cytotoxicity in the low micromolar range. Compounds **4l**, **4m, 4r–t** exhibited potent antitumor activity with IC_{50} values in the range of 4.18–9.77 μM against a human breast cancer (MCF-7) cell line. Molecular docking studies supported the postulation that the active compounds bind to EGFR in the same manner as known EGFR inhibitors.[27] Together, these results will facilitate and guide the design of novel and more potent quinazoline derivatives.

Experimental Section

Chemistry

Full details about the instruments and reagents used are given in the Supporting Information along with characterisation data for all

Table 3. Surflex dock scores of compounds **4l**, **4m**, **4r–t**, and reference agent lapatinib.

Compd	C-score[a]	Crash score[b]	Polar score[c]	G-score[d]	PMF score[e]	D-score[f]	Chem score[g]
Lapatinib	7.43	−0.89	2.13	−237	−10.4	−699	−43.8
4l	5.21	−1.37	1.12	−203	−2.63	−645	−33.5
4m	6.08	−0.94	1.13	−224	−14.7	−675	−35.7
4r	5.66	−1.27	2.30	−197	−15.1	−663	−39.5
4s	5.35	−1.84	2.18	−199	−9.17	−687	−40.3
4t	5.40	−1.88	2.22	−212	−11.7	−690	−40.1

[a] Consensus score (C-score) reports the output of the total score. [b] Crash score reveals inappropriate penetration into the binding site. [c] Polar region of the ligand. [d] G-score shows hydrogen bonding, complex (ligand–protein), and internal (ligand–ligand) energies. [e] Potential of mean force (PMF) score indicates the Helmholtz free energies of interactions for protein–ligand atom pairs. [f] D-score for charge and van der Waals interactions between the protein and ligand. [g] Chem score points to hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept term.

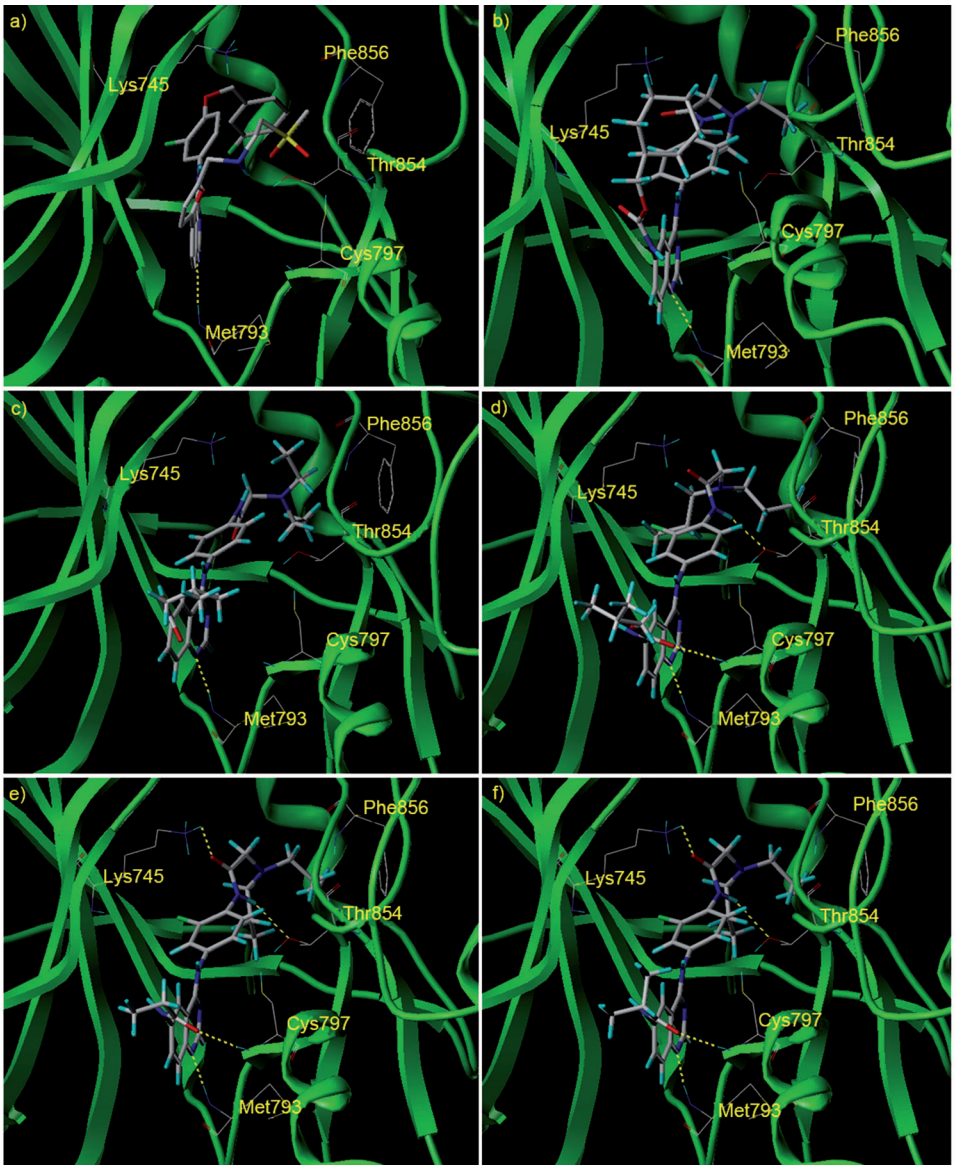


Figure 1. The docking models of 4-anilinoquinazoline inhibitors a) lapatinib, b) **4m**, c) **4l**, d) **4r**, e) **4s**, and f) **4t** with EGFR. Inhibitors are shown in stick model colored by atom type. Hydrogen bonds are depicted as yellow dashed lines. The receptor is shown in thin stick and ribbon style (green).

intermediates and additional final compounds. Given below are two representative protocols for the synthesis of **4f** and **4o**. Compounds **4a**–**k** and **4l**–**t** were synthesized in the same manner as **4f** and **4o**, respectively. 4-Chloroquinazoline (**1**) was prepared from 4-hydroxyquinazoline according to a literature procedure,[18,19] and 4-(2-(dipropylamino)acetamido) aniline (**3e**) was prepared as described by Moses et al.[20]

4-(4-(2-(Dipropylamino)acetamido)anilino)quinazoline (4f): A mixture of **1** (0.59 g, 3.6 mmol), **3e** (1.00 g, 4.0 mmol), and Et_3N (0.61 g, 6.0 mmol) was dissolved in isopropanol (20 mL), and the mixture was heated at reflux for 8 h with stirring. The solvent was removed in vacuo, and the residue was purified by column chromatography (petroleum ether/EtOAc, gradient elution, 8:1, 4:1, 2:1, 1:1, 0:1) to give **4f** as an off-white solid (1.20 g, 88.7%): mp: 113–115 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 0.89$ (t, $J = 7.4$ Hz, 6H), 1.44–1.52 (m, 4H), 2.49–2.52 (m, 4H), 3.18 (s, 2H), 7.61–7.66 (m, 3H), 7.77–7.79 (m, 3H), 7.84–7.87 (m, 1H), 8.54–8.56 (m, 2H), 9.59 (s, 1H), 9.79 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 11.7$, 19.9, 56.5, 58.5, 115.1, 119.1, 122.9, 123.1, 126.1, 127.7, 132.8, 134.4, 134.5, 149.6, 154.5, 157.7, 169.4 ppm; IR (KBr): $\tilde{\nu} = 3381$, 3256, 2958, 1667, 1618, 1605, 682 cm^{-1}; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{28}N_5O$: 378.2288, found: 378.2291; HPLC: $t_R = 5.27$ min (>95%).

4-(3-Chloro-4-(2-(diethylamino)acetamido)anilino)-6-(cyclohexylmethoxylformamido)quinazoline (4o): 4-(3-Chloro-4-(2-(diethylamino)acetamido)anilino)-6-nitroquinazoline (**4i**, 0.80 g, 1.9 mmol) was dissolved in anhydrous EtOH (30 mL) with stirring. Fe powder (2.00 g, 35.7 mmol), concd HCl (0.5 mL) and H_2O (2.0 mL) were added to the mixture. The reaction was stirred at RT for 30 min, then heated at reflux for 4 h and monitored to completion by TLC (petroleum ether/EtOAC, 1:1). Upon completion, the reaction mixture was filtered under suction. The filtrate was neutralized with saturated aq Na_2CO_3 and then filtered again. The filtrate was concentrated in vacuo, and the residue was dissolved in EtOAc (30 mL) and then extracted with 3% aq NH_3 (30 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give compound **5b** as a yellow solid (0.58 g, 77.9%).

Compound **5b** (0.26 g, 0.65 mmol) and DMAP (0.15 g, 1.2 mmol) were dissolved in $CHCl_3$ (10 mL) with stirring. A solution of cyclohexylmethyl chloroformate (0.70 g, 4.0 mmol) and $CHCl_3$ (5 mL) was added dropwise to the mixture at 0–5 °C. The mixture was stirred at 0–5 °C for 20 min and then stirring was continued at RT while monitoring by TLC (petroleum ether/EtOAc, 1:1). Upon completion, the reaction mixture was basified with 3% aq NH_3 in an ice bath. The water phase was extracted with CH_2Cl_2 (3×10 mL), and the combined organic phase was washed with water and dried over anhydrous $MgSO_4$. The solvent was removed in vacuo, and the yellow residue was further purified by column chromatography (petroleum ether/EtOAc, gradient elution, 8:1, 4:1, 2:1, 1:1, 0:1) to give **4o** as a light yellow solid (0.25 g, 71.1%): mp: 272–274 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.04–1.05 (m, 2H), 1.07 (t, J = 7.1 Hz, 6H), 1.21–1.26 (m, 3H), 1.66–1.78 (m, 6H), 2.64 (q, J = 7.1 Hz, 4H), 3.20 (s, 2H), 3.98 (d, J = 6.6 Hz, 2H), 7.74–7.77 (m, 3H), 8.15 (d, J = 2.4 Hz, 1H), 8.30 (d, J = 9.0 Hz, 1H), 8.54 (d, J = 6.3 Hz, 2H), 9.86 (s, 1H), 9.95 (s, 1H), 10.01 ppm (s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 12.3, 25.1, 25.9, 29.1, 36.9, 48.1, 57.9, 69.3, 110.4, 115.5, 120.6, 121.5, 121.9, 122.4, 126.6, 128.4, 129.9, 136.0, 137.1, 146.1, 152.8, 153.9, 157.1, 169.6 ppm; IR (KBr): $\tilde{\nu}$ = 3428, 3287, 2970, 2927, 1726, 1681, 1633, 1609, 693 cm^{-1}; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{28}H_{36}ClN_6O_3$: 539.2532, found: 539.2534; HPLC: t_R = 33.10 min (> 99%).

Biological evaluation

The cytotoxic activities of compounds **4a**–**t** were determined against A-549 and MCF-7 cell lines, obtained from the Shanghai Institutes for Biological Science, Chinese Academy of Sciences, using an MTT assay.[28] In brief, tumor cells were cultivated at 37 °C, 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 100 U mL^{-1} penicillin, 0.1 mg mL^{-1} streptomycin, 10% *v/v* fetal bovine serum for 3–5 d. Then, tumor cells were treated with trypsin–EDTA solution and then seeded into 96-well plates at 5×10^3 cells/well and incubated in a 5% CO_2 incubator at 37 °C for 24 h. The cells were treated with the synthesized compounds at different concentrations in DMEM for 72 h. Mitochondrial metabolism was measured as a marker for cell growth by adding 10 μL/well MTT (5 mg mL^{-1} in medium, Sigma) with 3 h of incubation at 37 °C. Crystals formed were dissolved in 150 μL DMSO. The absorbance was determined using a microplate reader at 490 nm. The absorbance data were converted into a cell proliferation percentage, compared with DMSO-only treated control cells, to determine growth inhibition. Each assay was performed in triplicate.

Molecular modeling

All calculations were carried out using the Sybyl X molecular modeling package.[26] The X-ray crystal structure of the EGFR kinase domain in complex with GW572016 (lapatinib) (PDB code: 1XKK) was obtained from the RCSB Protein Data Bank.[24] The enzyme was prepared for docking as follows: (1) the co-crystallized ligand was extracted as reference to identify the active site; (2) all phosphate ions and water molecules were removed; (3) hydrogen atoms were added to the receptor, partial charges were computed using the Amber method, protonation states were set, and side chain amides and bumps were fixed. Finally, the receptor was subjected to energy minimization using the Powell algorithm (with Amber force field and with gradient 0.05 kcal mol^{-1} Å). The most active compounds were constructed with Sybyl X molecular sketcher, energy minimized, and then molecular docking was performed. The ProtoMol was generated using standard fully automated Surflex-Dock procedures to characterize the surface properties of the EGFR active site, including steric effects, hydrogen bond acceptor groups, and hydrogen bond donor groups. During docking, ligands were aligned to the ProtoMol based on surface shape, with each pose bedding scored based on hydrophobic (HY) and polar contacts between atoms. The reference ligand was redocked into the binding pocket to reproduce the binding mode observed in the crystal structure; afterwards, the molecules in the data set were docked into the active site to investigate the binding modes and affinities. Docked poses in the active site were visualized using Sybyl X.[26]

Acknowledgements

The authors are very grateful for financial support from the National Natural Science Foundation of China (grant no. 20802069), the Natural Science Foundation of Zhejiang Province (P.R. China) (grant no. Y2090985), and the Education Department of Liaoning Province (P.R. China) (grant no. L2011174). The authors also thank Tripos, Inc. (St. Louis, USA) for kindly providing free access to their software package for evaluation.

Keywords: 4-anilinoquinazolines · antitumor agents · epidermal growth factor receptor (EGFR) · molecular docking

[1] W. Zhou, D. Ercan, P. A. Jänne, N. S. Gray, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 638–643.
[2] P. Ballard, R. H. Bradbury, C. S. Harris, L. F. A. Hennequin, M. Hickinson, P. D. Johnson, J. G. Kettle, T. Klinowska, A. G. Leach, R. Morgentin, M. Pass, D. J. Ogilvie, A. Olivier, N. Warin, E. J. Williams, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1633–1637.
[3] N. E. Hynes, H. A. Lane, *Nat. Rev. Cancer* **2005**, *5*, 341–354.
[4] C. Qiu, M. K. Tarrant, T. Boronina, P. A. Longo, J. M. Kavran, R. N. Cole, P. A. Cole, D. J. Leahy, *Biochemistry* **2009**, *48*, 6624–6632.
[5] J. B. Johnston, S. Navaratnam, M. W. Pitz, J. M. Maniate, E. Wiechec, H. Baust, J. Gingerich, G. P. Skliris, L. C. Murphy, M. Los, *Curr. Med. Chem.* **2006**, *13*, 3483–3492.
[6] X. Cai, H.-X. Zhai, J. Wang, J. Forrester, H. Qu, L. Yin, C.-J. Lai, R. Bao, C. Qian, *J. Med. Chem.* **2010**, *53*, 2000–2009.
[7] E. Mishani, G. Abourbeh, O. Jacobson, S. Dissoki, R. B. Daniel, Y. Rozen, M. Shaul, A. Levitzki, *J. Med. Chem.* **2005**, *48*, 5337–5348.
[8] H.-R. Tsou, N. Mamuya, B. D. Johnson, M. F. Reich, B. C. Gruber, F. Ye, R. Nilakantan, R. Shen, C. Discafani, R. DeBlanc, R. Davis, F. E. Koehn, L. M. Greenberger, Y.-F. Wang, A. Wissner, *J. Med. Chem.* **2001**, *44*, 2719–2734.
[9] A. Wissner, D. M. Berger, D. H. Boschelli, M. B. Floyd, L. M. Greenberger, B. C. Gruber, B. D. Johnson, N. Mamuya, R. Nilakantan, M. F. Reich, R. Shen, H.-R. Tsou, E. Upeslacis, Y. F. Wang, B. Wu, F. Ye, N. Zhang, *J. Med. Chem.* **2000**, *43*, 3244–3256.
[10] J. B. Smaill, G. W. Rewcastle, J. A. Loo, K. D. Greis, O. H. Chan, E. L. Reyner, E. Lipka, H. D. H. Showalter, P. W. Vincent, W. L. Elliott, W. A. Denny, *J. Med. Chem.* **2000**, *43*, 1380–1397.
[11] Y.-Y. Ke, H.-Y. Shiao, Y. C. Hsu, C.-Y. Chu, W.-C. Wang, Y.-C. Lee, W.-H. Lin, C.-H. Chen, J. T. A. Hsu, C.-W. Chang, C.-W. Lin, T.-K. Yeh, Y.-S. Chao, M. S. Coumar, H.-P. Hsieh, *ChemMedChem* **2013**, *8*, 136–148.
[12] A. Garofalo, A. Farce, S. Ravez, A. Lemoine, P. Six, P. Chavatte, L. Goossens, P. Depreux, *J. Med. Chem.* **2012**, *55*, 1189–1204.
[13] A. Garofalo, L. Goossens, P. Six, A. Lemoine, S. Ravez, A. Farce, P. Depreux, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2106–2112.
[14] A. Garofalo, L. Goossens, A. Lemoine, S. Ravez, P. Six, M. Howsam, A. Farce, P. Depreux, *MedChemComm* **2011**, *2*, 65–72.
[15] A. Garofalo, L. Goossens, A. Lemoine, A. Farce, Y. Arlot, P. Depreux, *J. Enzyme Inhib. Med. Chem.* **2010**, *25*, 158–171.
[16] S. M. Abou-Seri, *Eur. J. Med. Chem.* **2010**, *45*, 4113–4121.

[17] E. R. Wood, A. T. Truesdale, O. B. McDonald, D. Yuan, A. Hassell, S. H. Dickerson, B. Ellis, C. Pennisi, E. Horne, K. Lackey, K. J. Alligood, D. W. Rusnak, T. M. Gilmer, L. Shewchuk, *Cancer Res.* **2004**, *64*, 6652 – 6659.
[18] C. Fernandes, C. Oliveira, L. Gano, A. Bourkoula, I. Pirmettis, I. Santos, *Bioorg. Med. Chem.* **2007**, *15*, 3974 – 3980.
[19] R. O. Bora, I. S. Rathod, S. S. Toshniwal, M. Farooqui, *Int. J. Chem. Sci.* **2005**, *3*, 469 – 474.
[20] A. D. Moorhouse, A. M. Santos, M. Gunaratnam, M. Moore, S. Neidle, J. E. Moses, *J. Am. Chem. Soc.* **2006**, *128*, 15972 – 15973.
[21] D.-D. Li, F. Fang, J.-R. Li, Q.-R. Du, J. Sun, H.-B. Gong, H.-L. Zhu, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5870 – 5875.
[22] M. Zuo, Y.-W. Zheng, S.-M. Lu, Y. Li, S.-Q. Zhang, *Bioorg. Med. Chem.* **2012**, *20*, 4405 – 4412.
[23] D. M. Collins, J. Crown, N. O'Donovan, A. Devery, F. O'Sullivan, L. O'Driscoll, M. Clynes, R. O'Connor, *Invest. New Drugs* **2010**, *28*, 433 – 444.
[24] T. Kitazaki, M. Oka, Y. Nakamura, J. Tsurutani, S. Doi, M. Yasunaga, M. Takemura, H. Yabuuchi, H. Soda, S. Kohno, *Lung Cancer* **2005**, *49*, 337 – 343.
[25] A. J. Bridges, H. Zhou, D. R. Cody, G. W. Rewcastle, A. McMichael, H. D. H. Showalter, D. W. Fry, A. J. Kraker, W. A. Denny, *J. Med. Chem.* **1996**, *39*, 267 – 276.
[26] SYBYL-X version 1.2, released October 26, 2010, Tripos Inc., St. Louis, MO, USA.
[27] A. S. El-Azab, M. A. Al-Omar, A. A.-M. Abdel-Aziz, N. I. Abdel-Aziz, M. A.-A. El-Sayed, A. M. Aleisa, M. M. Sayed-Ahmed, S. G. Abdel-Hamide, *Eur. J. Med. Chem.* **2010**, *45*, 4188 – 4198.
[28] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55 – 63.

Received: March 13, 2013
Published online on ■■ ■■, 0000