

## Full-length article

**Discovering novel 3-nitroquinolines as a new class of anticancer agents<sup>1</sup>**Hai-hong LI<sup>2,3</sup>, He HUANG<sup>2,3</sup>, Xiu-hua ZHANG<sup>4</sup>, Xiao-min LUO<sup>3</sup>, Li-ping LIN<sup>4,5</sup>, Hua-liang JIANG<sup>3</sup>, Jian DING<sup>4</sup>, Kai-xian CHEN<sup>3</sup>, Hong LIU<sup>3,5</sup><sup>3</sup>Drug Discovery and Design Centre, State Key Laboratory of Drug Research and <sup>4</sup>Division of Anti-Tumor Pharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China**Key words**

3-nitroquinoline; epidermal growth factor receptor; tyrosine kinase; inhibitor; molecular modeling

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**Abstract**

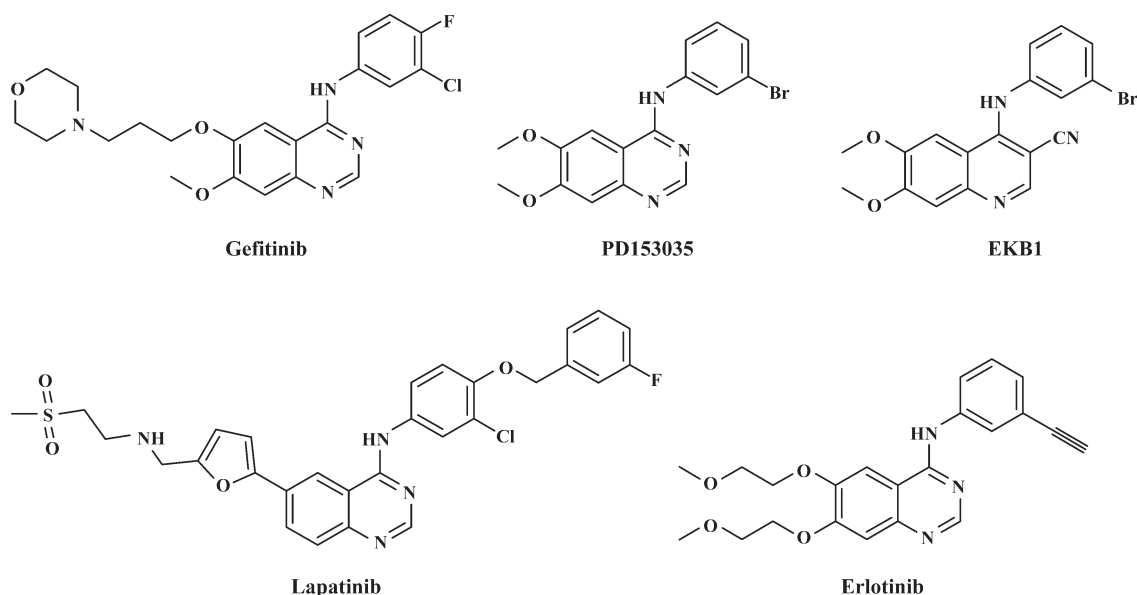
**Aim:** To design and synthesize a novel class of antitumor agents, featuring the 3-nitroquinoline framework. **Methods:** Based on the enzyme-binding features of Ekb1, introducing a nitro group at the 3-position of the quinoline core, a series of novel 3-nitroquinolines was designed and synthesized. The inhibition of epidermal growth factor receptor (EGFR) activity by these compounds was evaluated and analyzed by the sulforhodamine B assay for their inhibitory activities toward human epidermoid carcinoma (A431) cells and breast cancer (MDA-MB-468) cells, which are known to overexpress the EGFR kinase. **Results:** A series of novel 3-nitroquinoline derivatives were synthesized and evaluated for their antiproliferative effect against the EGFR-overexpressing tumor cell lines. Several compounds for concentration-response studies showed prominent inhibitory activities with IC<sub>50</sub> values in the micromolar or nanomolar range. The structure-activity relationship was discussed in terms of the inhibitory activity against the proliferation of 2 human carcinoma cell lines. **Conclusion:** This study was the first to identify new structural types of antiproliferative agents against the EGFR-overexpressing tumor cell lines by the incorporation of the nitro group at the 3-position of the quinoline core structure, providing promising new templates for the further development of anticancer agents.

**Introduction**

Receptor tyrosine kinases play crucial roles in signal transduction pathways that regulate cell differentiation and proliferation<sup>[1]</sup>. The overexpression of certain growth factor receptor kinases is strongly associated with carcinogenesis<sup>[2]</sup>. The epidermal growth factor receptor (EGFR/Her-1/ErbB-1), which belongs to the ErbB receptor family, is a 170 kDa glycoprotein that contains an extracellular ligand-binding domain, a transmembrane region, and an intracellular domain with kinase activity<sup>[3]</sup>. A strong correlation has been found between solid tumors with high levels of EGFR and poor prognosis<sup>[4]</sup>. Thus, EGFR is an attractive target for the design and development of compounds that can specifically bind to the receptor and inhibit its tyrosine kinase (TK) activity and its signal transduction pathway in cancer cells. A variety of approaches can be used to

target EGFR family members, and the most popular 2 have been extensively explored for cancer chemotherapy against cancers that overexpress EGFR family receptors: blocking ligands binding to the extracellular domain with humanized monoclonal antibodies, and using small molecule inhibitors that interact at the ATP-binding site<sup>[5]</sup>. The most promising small molecule inhibitors of the EGFR kinase are currently several scaffolds, which include quinazolines<sup>[5–10]</sup>, pyridopyrimidines<sup>[11,12]</sup>, benzamides<sup>[13–15]</sup>, indolinones<sup>[16]</sup>, and pyrrolotriazines<sup>[17]</sup>. Of these, the 4-anilinoquinazoline derivatives exhibit IC<sub>50</sub> values up to the subnanomolar range in enzymatic assays<sup>[18,19]</sup>. Figure 1 includes some representative small molecule inhibitors in the 4-anilinoquinazoline series that are potent inhibitors of the EGFR kinase, in which gefitinib<sup>[20]</sup>, erlotinib<sup>[21]</sup>, and lapatinib<sup>[22]</sup> are currently used in the market.

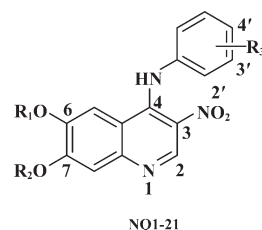
The crystal structure of OSI-774/EGFR-TK indicates that the nitrogen atom located at the 3-position of these



**Figure 1.** Representative small molecule inhibitors of the EGFR kinase.

quinazoline inhibitors is an important feature for good activity<sup>[23]</sup>. This nitrogen atom could be interacting with a water molecule, and that this water molecule could then serve as a bridge between the drug and enzyme. Replacing this atom with a carbon leads to a significant loss in the ability of the compound to inhibit the enzyme. According to this, Wissner<sup>[24]</sup> *et al* removed and replaced this nitrogen atom with a carbon atom that had an attached cyano group. A series of 4-anilinoquinoline-3-carbonitriles was then synthesized, and some of them exhibited significant ability in inhibiting the EGFR kinase.

Chen *et al* noted that when there was an indirect, water-mediated hydrogen bond from an inhibitor to the protein, there is good reason to attempt to build into the space occupied by the water molecule<sup>[25]</sup>. Wissner *et al* indicated that the space due to removal of the water molecule bound to Thr830 could accommodate a small group. On the basis of these considerations, we planned to design novel nitroquinolines related to Ekb1, in which the cyano group at the 3-position is replaced by the nitro group. The aim of the study was to find new structural types of EGFR kinase inhibitors. In this present work, we report on the synthesis and biological activity of a series of novel 3-nitroquinoline derivatives represented by the general formula of nitroquinolines in Figure 2. The cellular activity in relevant tumor cell lines will be discussed to develop the structure-activity relationship of this new series. This work was the first to explore the effect of 3-nitro group substitution on the EGFR kinase activity of the 4-anilinoquinoline



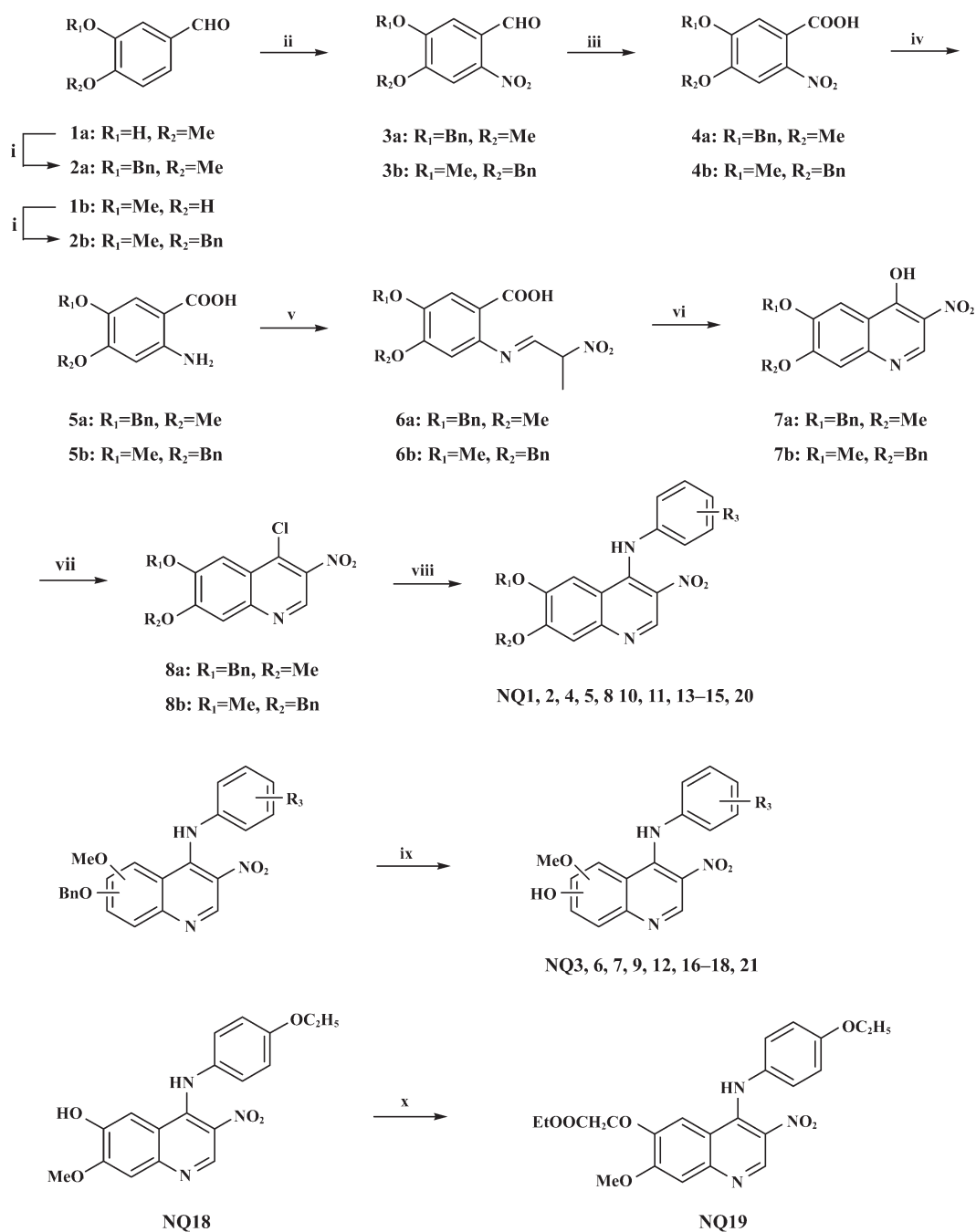
**Figure 2.** Structure of compounds NQ1-21.

series. Significantly, several of these compounds have shown promising antiproliferative effects against EGFR-overexpressing tumor cell lines.

## Materials and methods

**Synthetic procedures** An efficient and facile synthesis approach was developed to prepare a variety of 3-nitroquinoline derivatives with various C-4, C-6, and C-7 substituents. As depicted in Figure 3, the straightforward 8-step synthetic route allowed us to diversify position 4 of the quinoline moiety via the key intermediate 8 at a later stage.

Beginning with the commercially-available isovanillin or vanillin, benzylation with benzyl bromide gave aldehyde 2 in good yield. The treatment of 2 with fuming nitric acid furnished selective nitration product 3. Compound 3 was converted to the corresponding *o*-nitrobenzoic acid 4 by refluxing with 10% KMnO<sub>4</sub>. Afterwards, the nitro group was reduced and was then condensed



**Figure 3.** Reagents and conditions: (i) BnCl/K<sub>2</sub>CO<sub>3</sub>; (ii) fuming HNO<sub>3</sub>; (iii) 10% KMnO<sub>4</sub>; (iv) Fe/HOAc; (v) CH<sub>3</sub>NO<sub>2</sub>/NaOH; (vi) NaOH/(CH<sub>3</sub>O)<sub>2</sub>O; (vii) POCl<sub>3</sub>, reflux; (viii) PhNH<sub>2</sub>, Heating; (ix) TFA, reflux; (x) BrCH<sub>2</sub>COOEt, K<sub>2</sub>CO<sub>3</sub>, DMF, heating.

with nitromethane followed by thermal cyclization in refluxing acetic anhydride, giving key intermediate 7. The quinoline was converted in good yield to the corresponding chloroquinoline 8 by refluxing in an excess of POCl<sub>3</sub>. Then, refluxing a solution of a chloroquinoline and a substituted aniline derivative in DMF generated the

desired final products, 3-nitro-4-anilino-6,7-dialkoxyquinolines (NQ1, 2, 4, 5, 8, 10, 11, 13–15, 20) in good yield. Debenzylation of these benzyl group-containing compounds through hydrogenolysis catalyzed by 10% Pd/C afforded NQ3, 6, 7, 9, 12, 16–18, and NQ21 in excellent yields. The nucleophilic displacement of ethyl

bromoacetate with 4-(4-ethoxyphenylamino)-7-methoxy-3-nitroquinolin-6-ol in basic refluxing DMF solution generated NQ19.

**Cell growth inhibition assay** Human breast adenocarcinoma cancer cell MDA-MB-468 and epidermoid carcinoma cancer cell A431 were obtained from ATCC (Manassas, VA, USA) and used for the cell proliferation assay. Both cell lines were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol/L glutamine, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin (Gibco, Grand Island, NY, USA) in a highly-humidified atmosphere of 95% air with 5% CO<sub>2</sub> at 37 °C. The cytotoxicity of the compounds was analyzed by the sulforhodamine B (SRB; Sigma, St Louis, MO, USA) assay. Briefly, the cells were seeded at 6000 cells/well in 96-well plates (Falcon, San Mateo, CA, USA) and allowed to attach overnight. The cells were treated in triplicate with graded concentrations of compounds at 37 °C for 72 h. After being fixed with 10% trichloroacetic acid at 4 °C for 1 h, the cells were stained with 100  $\mu$ L SRB solution (0.4% w/v in 1% acetic acid) for 15 min and washed with 1% acetic acid to remove any unbound dye. Bound dye was solubilized with 10 mmol/L Tris base (pH 10.5). The absorbance values of the plates were measured using a multiwell spectrophotometer (VERSA<sub>max</sub>; Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 515 nm. The growth inhibitory rate of treated cells was calculated by the following formula:  $(1 - [A_{515 \text{ treated}}/A_{515 \text{ control}}]) \times 100\%$ . The results were also expressed as IC<sub>50</sub> (the compound concentration required for 50% growth inhibition of tumor cells), which was calculated by the Logit method.

**Molecular docking** To explore the interaction mechanism between the novel 3-nitroquinoline derivatives and the EGFR kinase, molecular docking was carried out with the AutoDock 3.0.5 program<sup>[26,27]</sup>. The 3-D structures of the target proteins of human EGFR are from the Protein Data Bank (entry N<sub>Q</sub> 1M17). The ligand and solvent molecules were removed from the crystal structure to obtain the docking grid, and the active site was defined using AutoGrid. The grid size was set to 50×50×50 points with grid spacing of 0.375 Å. The grid box was centered on the center of the ligand from the corresponding crystal structure complexes. The Lamarckian genetic algorithm was applied to account for protein-ligand interactions. Finally, the conformation with the lowest predicted binding free energy of the most occurring binding modes in the EGFR active pocket was selected.

## Results

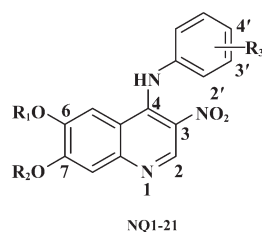
**Analog design and synthesis** On the basis of the enzyme-binding features of Ekb1, introducing a nitro group at the 3-position of the quinoline core, a series of novel 3-nitroquinolines were designed and synthesized; their chemical structures are shown in Table 1. These compounds were synthesized through the route outlined in Scheme 1, and the details for the synthetic procedures have been previously described.

**Biology assay** The inhibition of EGFR activities by NQ1–21 was evaluated and analyzed by SRB assay for their inhibitory activities toward human epidermoid carcinoma (A431) cells and breast cancer (MDA-MB-468) cells. These cells are known to overexpress EGFR, which leads to the continuous activation of the EGFR pathway involved in cell proliferation. For the primary assay, the percentage of inhibition of the compounds at the 10  $\mu$ mol/L concentration against A431 and MDA-MB-468 was measured. The biological results for the 3-nitro-4-anilino-6,7-dialkoxyquinolines inhibitors are shown in Table 1.

## Discussion

Due to earlier work by some research groups, who found that the 4-anilinoquinazoline-based inhibitors of EGFR established that a meta-substituted electro-withdraw group in the aniline moiety is compatible with good activity, we decided to retain this feature in our initial compounds. As shown in Table 1, the initial compound NQ2 with an attached 3'-ethynyl group exhibited potential inhibitory activities towards the A431 cell line, with an 87.3% inhibition at the 10  $\mu$ mol/L concentration, while replacing the ethynyl group with the bromo atom did not improve the inhibitory activities. Substitution of the bromo atom with the chloro atom or fluoro atom resulted in a sharp loss of inhibitory activities to the A431 cell line. The 3-fluoro-4-chlorobenzenamine substituted nitroquinoline derivative NQ11 presented potent inhibitory effects against the A431 cell line, but changing the substituted groups at position 6 resulted in a clear decrease in the ability to inhibit EGFR. These findings indicated that both the aniline moiety and the 6,7-dialkoxy substitution play important roles in the inhibitory activities. Thus, compound NQ11 was chosen as the benchmark compound for subsequent optimization studies. Compounds NQ13–15, which retained the 6,7-dialkoxy substitution of NQ11, were first investigated. Among them, compound NQ14 was a little more active than the initial compounds, with 50% inhibition against MDA-MB-468 at 10  $\mu$ mol/L. However, their inhibitory

**Table 1.** Inhibitory effect of compounds NQ1–21 on the growth of tumor cell lines.

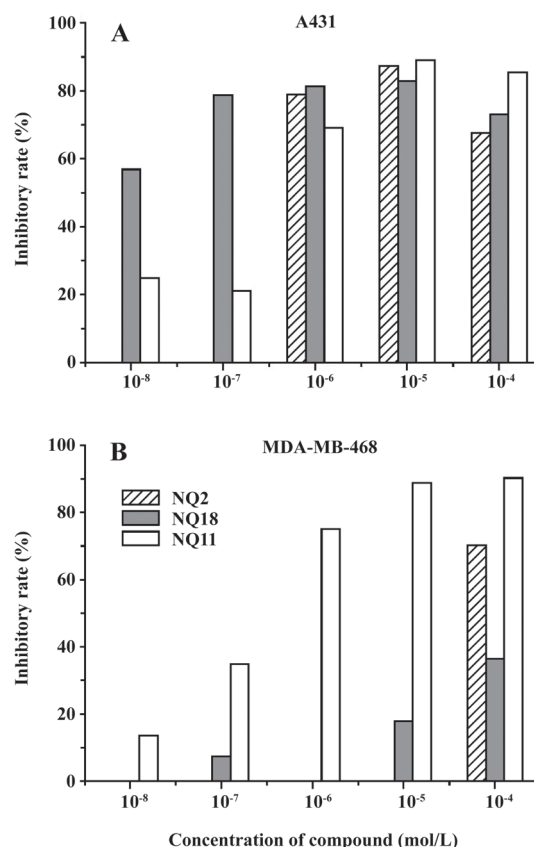


| Com-<br>pound | R <sub>1</sub>        | R <sub>2</sub>  | R <sub>3</sub>                      | Tumor cell inhibition<br>rate at 10 μmol/L (%) |                |
|---------------|-----------------------|-----------------|-------------------------------------|--|----------------|
|               |                       |                 |                                     | A431   | MDA-MB<br>-468 |
| NQ1           | Bn                    | CH <sub>3</sub> | 3'-C≡CH                             | 34.0   | 3.4            |
| NQ2           | CH <sub>3</sub>       | Bn              | 3'-C≡CH                             | 87.3   | 0              |
| NQ3           | H                     | CH <sub>3</sub> | 3'-C≡CH                             | 0  | 28.8           |
| NQ4           | Bn                    | CH <sub>3</sub> | 3'-Br                               | 11.7   | 31.4           |
| NQ5           | CH <sub>3</sub>       | Bn              | 3'-Br                               | 47.6   | 0              |
| NQ6           | CH <sub>3</sub>       | H               | 3'-Br                               | 22.7   | 0              |
| NQ7           | H                     | CH <sub>3</sub> | 3'-Br                               | 0  | 14.7           |
| NQ8           | Bn                    | CH <sub>3</sub> | 3'-F                                | 0  | 44.3           |
| NQ9           | H                     | CH <sub>3</sub> | 3'-F                                | 30.0   | 12.7           |
| NQ10          | Bn                    | CH <sub>3</sub> | 3'-Cl                               | 7.0  | 17.8           |
| NQ11          | Bn                    | CH <sub>3</sub> | 3'-F, 4'-Cl                         | 82.8   | 17.9           |
| NQ12          | H                     | CH <sub>3</sub> | 3'-F, 4'-Cl                         | 26.5   | 27.5           |
| NQ13          | Bn                    | CH <sub>3</sub> | 2'-OCF <sub>3</sub>                 | 12.7   | 33.9           |
| NQ14          | Bn                    | CH <sub>3</sub> | 3'-OCH <sub>2</sub> CH <sub>3</sub> | 14.6   | 50.0           |
| NQ15          | Bn                    | CH <sub>3</sub> | 4'-OCH <sub>2</sub> CH <sub>3</sub> | 0  | 18.8           |
| NQ16          | H                     | CH <sub>3</sub> | 2'-OCF <sub>3</sub>                 | 4.8  | 41.2           |
| NQ17          | H                     | CH <sub>3</sub> | 3'-OCH <sub>2</sub> CH <sub>3</sub> | 29.0   | 14.5           |
| NQ18          | H                     | CH <sub>3</sub> | 4'-OCH <sub>2</sub> CH <sub>3</sub> | 89.0   | 88.8           |
| NQ19          | CH <sub>2</sub> COOEt | CH <sub>3</sub> | 4'-OCH <sub>2</sub> CH <sub>3</sub> | 24.0   | 9.9            |
| NQ20          | CH <sub>3</sub>       | Bn              | 4'-OCH <sub>2</sub> CH <sub>3</sub> | 61.0   | 0              |
| NQ21          | CH <sub>3</sub>       | H               | 4'-OCH <sub>2</sub> CH <sub>3</sub> | 0  | 11.7           |

activities toward A431 were decreased. Surprisingly, when the benzyl group was removed from NQ15, compound NQ18 exhibited high inhibitory activity toward both A431 and MDA-MB-468, with 89% and 88.8% inhibition at 10 μmol/L, respectively. Subsequently, derivatives NQ19–21 were synthesized, which were designed based on potent inhibitor NQ18. Disappointingly, all of these compounds showed decreased inhibitory activities toward both A431 and MDA-MB-468, and a few proved to have completely lost inhibitory activity. To some extent, NQ20 exhibited a better ability to inhibit A431 than the other compounds, whereas all were poor inhibitors of MDA-MB-468.

To determine the potency of the compounds that exhibited significant inhibition toward A431 or MDA-MB-468 at 10 μmol/L, 3 compounds (NQ2, NQ11, and NQ18) were further investigated in concentration-response studies, and

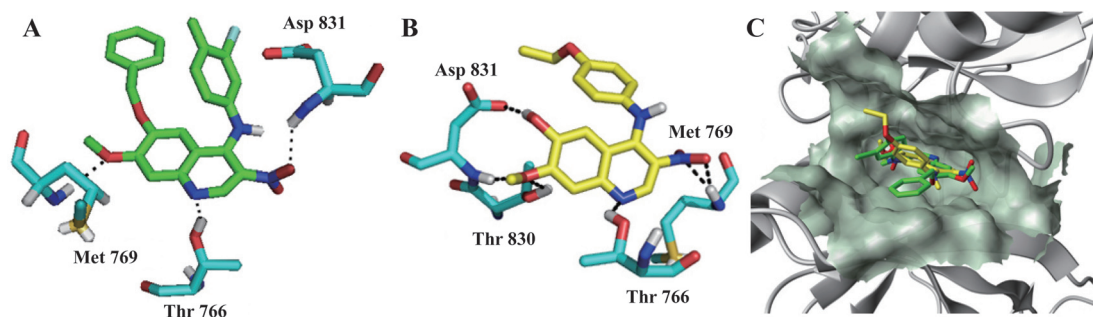
the results are summarized in Figure 4. Compound NQ2 displayed good activity for the cell line A431 (IC<sub>50</sub>=0.49 μmol/L), but was much less effective in inhibiting the MDA-MB-468 cell line. Encouragingly, compound NQ11 showed a remarkably positive response on the both cell lines (IC<sub>50</sub>=0.40 and =0.22 μmol/L, for A431 and MDA-MB-468, respectively). Even more remarkable is that compound NQ18 showed prominent inhibitory activities against the A431 cell line with IC<sub>50</sub> values up in the nano-molar range. Compound NQ18 exhibits inhibitory activity as high as 56.9% against A431, even at 10 nmol/L.



**Figure 4.** Inhibitory effect of selected compounds on the growth of tumor cell lines.

Molecular modeling experiments were carried out to investigate the binding interactions between this series of compounds and the active site of EGFR. The conformation with the lowest predicted binding free energy of the most occurring binding modes in the EGFR active pocket was selected. In the final model with compound NQ11 (Figure 5), the N1 atom of the quinoline forms a hydrogen bond with the hydroxyl group of Thr766, and the 3-nitro





**Figure 5.** Binding models of compounds NQ11 and NQ18 in the binding cleft of EGFR. (A) NQ11 and (B) NQ18 depict the detailed interactions. (C) docking models of NQ11 (green) and NQ18 (yellow) into the active sites of EGFR. Images were generated using the Pymol program (<http://pymol.sourceforge.net>).

group, extending deep into the cleft, forms a hydrogen bond interaction with the backbone NH of Asp831. As for the 6,7-dialkoxy moiety, the 6-benzyloxy group points to the entrance of the active pocket. Interestingly, the oxygen atom at the 7-methoxy group forms a hydrogen bond with the NH of Met769. For the orientation of NQ18, its interactions with the protein are not similar to that observed in the NQ11 model. The hydrogen bond of the N1 atom to the hydroxyl group of Thr766 is retained. In this model, the 3-nitro group displaces the 7-methoxy group of NQ11 that was previously hydrogen bonded to the protein, and instead forms 2 hydrogen bonds with the backbone NH of Met769. The significance of this interaction was particularly reinforced by the performance of the 6-hydroxy group and the 7-methoxy group which form 3 hydrogen bonds with Asp831 and Thr830, respectively. From the binding modes of NQ11 and NQ18 with EGFR, we found that although different conformations were adopted for the 2 compounds in the EGFR active pocket, both formed favorable hydrogen bonds with the hydroxyl group of Thr766 and the backbone NH of Met769. As reported previously, the interaction with the backbone NH of the Met769 is important for binding to the ATP site, both for ATP and inhibitors, which can explain why NQ11 and NQ18 are potent with respect to their ability to inhibit the growth of EGFR-overexpressing cell lines. This model will be helpful for our further structural elaboration of the novel nitroquinolin series to improve kinase activity.

In summary, a series of novel 3-nitroquinoline derivatives was synthesized. All of the compounds were evaluated for their antiproliferative effect against the EGFR-overexpressing tumor cell lines. Several compounds for concentration–response studies showed prominent inhibitory activities with  $IC_{50}$  values in the micromolar or nanomolar range. The SAR was discussed in terms of the inhibitory

activity against the proliferation of the 2 human carcinoma cell lines. The results suggest that both the aniline portion and the 6,7-dialkoxy substituents may play strong roles in determining the potency of the 3-nitroquinolin series as kinase inhibitors. It is noteworthy that the substitution in the aniline moiety need not be an electro-withdraw group at the meta position. This study was the first to identify new structural types of EGFR kinase inhibitors by the incorporation of the nitro group at position 3 of the quinoline core structure, providing promising new templates for the further development of potent inhibitors targeting EGFR kinase. There is an urgent need to discover small molecule EGFR inhibitors, so the new chemical structures produced in this study are of significance.

## Appendix

The reagents (chemicals) were purchased from commercial sources (Alfa, Acros, Sigma-Aldrich and Shanghai Chemical Reagent Company), and used without further purification. Analytical-thin layer chromatography was HSGF254 (0.15–0.2 mm thickness; Yantai Huiyou Company, Yantai, China). Yields were not optimized. Melting points were measured in a capillary tube on a SGW X-4 melting point apparatus (Shanghai Precision & Scientific Instrument Co, Ltd) without correction. Nuclear magnetic resonance (NMR) spectra were given on a Bruker AMX-400 and AMX-300 (Bruker, Fällanden, Switzerland; internal standard as tetramethylsilane). Chemical shifts were reported in parts per million (ppm,  $\delta$ ) downfield from tetramethylsilane. Proton coupling patterns were described as singlet, doublet, triplet, quartet, multiplet, and broad. Low- and high-resolution mass spectra were given with an electric ionization (EI) and electrospray and a LCQ-DECA spectrometer produced by Finnigan MAT-95 (Finnigan, Santa Clara, CA, USA).

**3-(Benzyloxy)-4-methoxybenzaldehyde (2a)** A mixture of 3-hydroxy-4-methoxybenzaldehyde (9 g, 59 mmol), Na<sub>2</sub>CO<sub>3</sub> (8.1 g), benzylchloride (11.4 g, 90 mmol), and 40 mL of ethanol was stirred at reflux for 5 h. The resulting mixture was filtered and the filtrate was evaporated. The crude product was recrystallized from EtOH to give 10.3 g (76%) of a white solid. Mp 59 °C (lit<sup>[28]</sup>, mp 63.5 °C). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 9.8 (s, 1H, CHO), 7.3–7.5 (m, 5H, Ph-H), 7.25–7.28 (m, 2H, Ph-H), 7.0 (s, 1H, Ph-H), 5.25 (s, 2H, PhCH<sub>2</sub>O), 4.0 (s, 3H, CH<sub>3</sub>O).

**4-(Benzyloxy)-3-methoxybenzaldehyde (2b)** The compound was prepared in 69.4% yield according to the procedure for 2a using 4-hydroxy-3-methoxybenzaldehyde. Mp 55 °C (lit<sup>[28]</sup>, mp 53.5 °C). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 9.8 (s, 1H, CHO), 7.3–7.5 (m, 5H, Ph-H), 7.25–7.28 (m, 2H, Ph-H), 7.0 (s, 1H, Ph-H), 5.25 (s, 2H, PhCH<sub>2</sub>O), 4.0 (s, 3H, CH<sub>3</sub>O).

**5-(Benzyloxy)-4-methoxy-2-nitrobenzaldehyde (3a)** 3-(Benzyloxy)-4-methoxybenzaldehyde (10 g, 41 mmol) was added cautiously to 40 mL of concentrated nitric acid at 0 °C. The mixture was then stirred at 15 °C for 40 min. On pouring the reaction mixture into ice water, the precipitate was filtrated to afford 5-(benzyloxy)-4-methoxy-2-nitrobenzaldehyde (10.4 g, 93%) as a yellow solid. Mp 131 °C (lit<sup>[28]</sup>, mp 133 °C). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 10.4 (s, 1H, CHO), 7.6 (s, 1H, Ph-H), 7.3–7.5 (m, 5H, Ph-H), 7.20 (s, 1H, Ph-H), 5.25 (s, 2H, PhCH<sub>2</sub>O), 4.0 (s, 3H, CH<sub>3</sub>O).

**4-(Benzyloxy)-5-methoxy-2-nitrobenzaldehyde (3b)** The compound was prepared in 91% yield according to the procedure for 3a using 4-(benzyloxy)-3-methoxybenzaldehyde. Mp 131 °C (lit<sup>[28]</sup>, mp 133 °C). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 10.4 (s, 1H, CHO), 7.60 (s, 1H, Ph-H), 7.3–7.5 (m, 5H, Ph-H), 7.2 (s, 1H, Ph-H), 5.25 (s, 2H, PhCH<sub>2</sub>O), 4.0 (s, 3H, CH<sub>3</sub>O).

**5-(Benzyloxy)-4-methoxy-2-nitrobenzoic acid (4a)** In total, 10% KMnO<sub>4</sub> was added to a stirred mixture of 5-(benzyloxy)-4-methoxy-2-nitrobenzaldehyde (10 g, 47 mmol) and 120 mL of acetone in hot water (100 mL). The resulting mixture was stirred for 1 h, and the reaction mixture was filtered. The filtrate was concentrated to remove the acetone. Then 4 mol/L HCl was added slowly with cooling until the insoluble material precipitated. The product was collected to give 10.2 g (58%) of a white solid. Mp 192 °C (lit<sup>[28]</sup>, mp 195 °C). <sup>1</sup>H NMR (DMCO-d<sub>6</sub>): δ 7.60 (s, 1H, Ph-H), 7.52 (s, 1H, Ph-H), 7.30–7.41 (m, 5H, Ph-H), 5.35 (s, 2H, PhCH<sub>2</sub>O), 4.0 (s, 3H, CH<sub>3</sub>O).

**4-(Benzyloxy)-5-methoxy-2-nitrobenzoic acid (4b)** The compound was prepared in 60% yield according to the procedure for 4a using 4-(benzyloxy)-5-methoxy-2-nitrobenzaldehyde. Mp 192 °C (lit<sup>[28]</sup>, mp 195 °C). <sup>1</sup>H NMR (DMCO-d<sub>6</sub>): δ 7.59 (s, 1H, Ph-H), 7.52 (s, 1H, Ph-H), 7.30–7.41 (m, 5H, Ph-H), 5.35 (s, 2H, PhCH<sub>2</sub>O), 4.0 (s, 3H, CH<sub>3</sub>O).

**2-Amino-5-(benzyloxy)-4-methoxybenzoic acid (5a)** Iron powder (3 g, 188 mmol) was partially added to a stirred mixture of 5-(benzyloxy)-4-methoxy-2-nitrobenzoic acid (10 g, 33.3 mmol) and 300 mL of acetic acid at 90 °C in 20 min. The resulting mixture was stirred for 45 min, and the reaction mixture was filtered. The filtrate was poured into 10% aqueous hydrochloric

acid solution (500 mL), and the insoluble material precipitated. The residue was dissolved in hot water, and 15% sodium hydroxide solution was carefully added until the pH was 12. After cooling to room temperature, the insoluble material precipitated. The product was filtered, recrystallized from isopropanol, and dried to give 6.1 g (70%) of a white solid. Mp 108 °C. <sup>1</sup>H NMR (DMCO-d<sub>6</sub>): δ 7.30–7.41 (m, 5H, Ph-H), 7.02 (s, 1H, Ph-H), 6.82 (s, 1H, Ph-H), 5.35 (s, 2H, PhCH<sub>2</sub>O), 4.0 (s, 3H, CH<sub>3</sub>O).

**2-Amino-4-(benzyloxy)-5-methoxybenzoic acid (5b)** The compound was prepared in 68% yield according to the procedure for 5a using 4-(benzyloxy)-5-methoxy-2-nitrobenzoic acid. Mp 102 °C. <sup>1</sup>H NMR (DMCO-d<sub>6</sub>): δ 7.30–7.41 (m, 5H, Ph-H), 7.10 (s, 1H, Ph-H), 6.79 (s, 1H, Ph-H), 5.35 (s, 2H, PhCH<sub>2</sub>O), 4.0 (s, 3H, CH<sub>3</sub>O).

**5-(Benzyloxy)-4-methoxy-2-(2-nitropropylideneamino)benzoic acid (6a)** Nitromethane (1.34 g, 22 mmol) was added to a stirred mixture of NaOH (2.68 g, 67 mmol) and 10 mL of water at 0 °C. The mixture was stirred at 40 °C until the solid dissolved. Then nitromethane (1.34 g, 22 mmol) was added, and the mixture was stirred at 50 °C for 15 min. The reaction mixture was poured into ice water, and concentrated hydrochloric acid was carefully added until the pH was 2. The brown mixture was added to a solution of 2-amino-5-(benzyloxy)-4-methoxybenzoic acid (5.48 g, 20 mmol) and concentrated hydrochloric acid (1 mL) in water (50 mL). The reaction mixture was stirred at room temperature for 24 h, and insoluble material precipitated. The product was filtered and dried to give 6.3 g (90%) of a yellow solid. Mp 159 °C. EI-MS *m/z* 358 [M]<sup>+</sup>.

**4-(Benzyloxy)-5-methoxy-2-(2-nitropropylideneamino)benzoic acid (6b)** The compound was prepared in 85% yield according to the procedure for 6a using 2-amino-4-(benzyloxy)-5-methoxybenzoic acid. Mp 165 °C. EI-MS *m/z* 358 [M]<sup>+</sup>.

**7-(Benzyloxy)-6-methoxy-2-nitronaphthalen-1-ol (7a)** A stirred mixture of 5-(benzyloxy)-4-methoxy-2-(2-nitropropylideneamino)benzoic acid (3.58 g, 10 mmol) and 10 mL of acetic anhydride was heated at 110 °C until the solid dissolved. After cooling to room temperature, NaOH (400 mg, 10 mmol) was added cautiously. Then the reaction mixture was stirred at 100 °C for 5 h, allowed to cool to room temperature, and filtered. The residue was filtered off and dried to give 1.3 g (40%) of solid. Mp >300 °C. EI-MS *m/z* 326 [M]<sup>+</sup>.

**6-(Benzyloxy)-7-methoxy-2-nitronaphthalen-1-ol (7b)** The compound was prepared in 37% yield according to the procedure for 7a using 4-(benzyloxy)-5-methoxy-2-(2-nitropropylideneamino)benzoic acid. Mp >300 °C. EI-MS *m/z* 326 [M]<sup>+</sup>.

**7-(Benzyloxy)-1-chloro-6-methoxy-2-nitronaphthalene (8a)** A stirred mixture of 7-(benzyloxy)-6-methoxy-2-nitronaphthalen-1-ol (1 g, 3 mmol) and 15 mL of fresh POCl<sub>3</sub> was heated at reflux for 18 h. After cooling to room temperature, the POCl<sub>3</sub> was removed under vacuum. The product was recrystallized from ethanol, and dried to give 1.1 g (97%) of a brown solid. Mp >300 °C. EI-MS *m/z* 344 [M]<sup>+</sup>.

**6-(Benzyloxy)-1-chloro-7-methoxy-2-nitronaphthalene**

**(8b)** The compound was prepared in 90% yield according to the procedure for 8a using 6-(benzyloxy)-7-methoxy-2-nitronaphthalen-1-ol. Mp >300 °C. EI-MS  $m/z$  344 [M]<sup>+</sup>.

**6-(Benzyloxy)-N-(3-ethynylphenyl)-7-methoxy-3-nitroquinolin-4-amine (NQ1)** A mixture of 8a (1 g, 2.9 mmol), 3-ethynylbenzenamine (340 mg, 2.9 mmol), and 20 mL of DMF was stirred at 100 °C for 24 h. The solvent was removed under vacuum. The crude product was recrystallized from methanol to give 1.1 g (89%) of a yellow solid. Mp 222 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 9.87 (s, 1H, quinoline-2-H), 7.19–7.68 (m, 11H, Ph-H), 5.20 (s, 2H, CH<sub>2</sub>Ph), 4.0 (s, 3H, OCH<sub>3</sub>), 3.8 (s, 1H, C≡CH). EI-MS  $m/z$  425 [M]<sup>+</sup>. HR-MS Calcd. For C<sub>25</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>: 425.1376; found: 425.1225.

**7-(Benzyloxy)-N-(3-ethynylphenyl)-6-methoxy-3-nitroquinolin-4-amine (NQ2)** Using the procedure described earlier for NQ1 using 8b, the title compound was obtained in 87% yield. Mp 223 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 9.87 (s, 1H, quinoline-2-H), 7.19–7.68 (m, 11H, Ph-H), 5.20 (s, 2H, CH<sub>2</sub>Ph), 4.0 (s, 3H, OCH<sub>3</sub>), 3.8 (s, 1H, C≡CH). EI-MS  $m/z$  425 [M]<sup>+</sup>. HR-MS Calcd. For C<sub>25</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>: 425.1376; found: 425.1325.

**4-(3-Ethynylphenylamino)-7-methoxy-3-nitroquinolin-6-ol (NQ3)** A mixture of NQ1 (1 g, 2.35 mmol) and 15 mL trifluoroacetic acid was stirred at reflux for 1 h. The solvent was removed under vacuum. Then ammonium hydroxide was carefully added until the pH was 7, and the solvent was removed under vacuum again. The crude product was washed by water and recrystallized from methanol to give the title compound (95%). Mp 156 °C. <sup>1</sup>H NMR (DMCO-*d*<sub>6</sub>): δ 9.27 (s, 1H, quinoline-2-H), 7.19–7.68 (m, 6H, Ph-H), 4.0 (s, 3H, OCH<sub>3</sub>), 3.8 (s, 1H, C≡CH). EI-MS  $m/z$  425 [M]<sup>+</sup>. Element analysis: Calcd. For C<sub>23</sub>H<sub>18</sub>BrN<sub>3</sub>O<sub>4</sub>: C, 57.51; H, 3.78; N, 8.75; found: C, 57.01; H, 3.58; N, 8.55. HR-MS Calcd. For C<sub>18</sub>H<sub>13</sub>N<sub>3</sub>O<sub>4</sub>: 335.0906; found: 335.0902.

**6-(Benzyloxy)-N-(3-bromophenyl)-7-methoxy-3-nitroquinolin-4-amine (NQ4)** Using the procedure described earlier for NQ1 with 3-bromobenzenamine, the title compound was obtained in 91% yield. Mp 268 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 9.77 (s, 1H, quinoline-2-H), 7.19–7.68 (m, 11H, Ph-H), 5.20 (s, 2H, CH<sub>2</sub>Ph), 4.0 (s, 3H, OCH<sub>3</sub>). EI-MS  $m/z$  480 [M+1]<sup>+</sup>. Element analysis: Calcd. For C<sub>23</sub>H<sub>18</sub>BrN<sub>3</sub>O<sub>4</sub>: C, 57.51; H, 3.78; N, 8.75; found: C, 57.01; H, 3.58; N, 8.55.

**7-(Benzyloxy)-N-(3-bromophenyl)-6-methoxy-3-nitroquinolin-4-amine (NQ5)** Using the procedure described earlier for NQ2 with 3-bromobenzenamine, the title compound was obtained in 91% yield. Mp 201 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 9.87 (s, 1H, quinoline-2-H), 7.19–7.68 (m, 11H, Ph-H), 5.20 (s, 2H, CH<sub>2</sub>Ph), 4.0 (s, 3H, OCH<sub>3</sub>). EI-MS  $m/z$  480 [M+1]<sup>+</sup>. Element analysis: Calcd. For C<sub>23</sub>H<sub>18</sub>BrN<sub>3</sub>O<sub>4</sub>: C, 57.51; H, 3.78; N, 8.75; found: C, 57.41; H, 3.28; N, 8.65.

**4-(3-Bromophenylamino)-6-methoxy-3-nitroquinolin-7-ol (NQ6)** Using the procedure described earlier for NQ3 with NQ5, the title compound was obtained in 95% yield. Mp 254 °C. <sup>1</sup>H NMR (DMCO-*d*<sub>6</sub>): δ 9.27 (s, 1H, quinoline-2-H), 7.12–7.42 (m, 6H, Ph-H), 4.0 (s, 3H, OCH<sub>3</sub>). EI-MS  $m/z$  390 [M+1]<sup>+</sup>. HR-MS

Calcd. For C<sub>16</sub>H<sub>12</sub>BrN<sub>3</sub>O<sub>4</sub>: 355.0011; found: 355.0005.

**4-(3-Bromophenylamino)-7-methoxy-3-nitroquinolin-6-ol (NQ7)** Using the procedure described earlier for NQ3 with NQ4, the title compound was obtained in 95% yield. Mp 254 °C. <sup>1</sup>H NMR (DMCO-*d*<sub>6</sub>): δ 9.27 (s, 1H, quinoline-2-H), 7.12–7.42 (m, 6H, Ph-H), 4.0 (s, 3H, OCH<sub>3</sub>). EI-MS  $m/z$  389 [M]<sup>+</sup>. HR-MS Calcd. For C<sub>16</sub>H<sub>12</sub>BrN<sub>3</sub>O<sub>4</sub>: 355.0011; found: 355.0005.

**6-(Benzyloxy)-N-(3-fluorophenyl)-7-methoxy-3-nitroquinolin-4-amine (NQ8)** Using the procedure described earlier for NQ1 with 3-fluorobenzenamine, the title compound was obtained in 91% yield. Mp 256 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 9.0 (s, 1H, quinoline-2-H), 6.60–7.62 (m, 11H, Ph-H), 5.20 (s, 2H, CH<sub>2</sub>Ph), 4.0 (s, 3H, OCH<sub>3</sub>). EI-MS  $m/z$  419 [M]<sup>+</sup>. HR-MS Calcd. For C<sub>23</sub>H<sub>18</sub>FN<sub>3</sub>O<sub>4</sub>: 419.1281; found: 419.1236.

**4-(3-Fluorophenylamino)-7-methoxy-3-nitroquinolin-6-ol (NQ9)** Using the procedure described earlier for NQ3 with NQ8, the title compound was obtained in 95% yield. Mp 164 °C. <sup>1</sup>H NMR (DMCO-*d*<sub>6</sub>): δ 9.17 (s, 1H, quinoline-2-H), 7.00–7.42 (m, 6H, Ph-H), 4.0 (s, 3H, OCH<sub>3</sub>). EI-MS  $m/z$  329 [M]<sup>+</sup>. HR-MS Calcd. For C<sub>16</sub>H<sub>12</sub>FN<sub>3</sub>O<sub>4</sub>: 329.0812; found: 329.0803.

**6-(Benzyloxy)-N-(3-chlorophenyl)-7-methoxy-3-nitroquinolin-4-amine (NQ10)** Using the procedure described earlier for NQ1 with 3-chlorobenzenamine, the title compound was obtained in 90% yield. Mp 286 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 9.89 (s, 1H, quinoline-2-H), 7.19–7.68 (m, 11H, Ph-H), 5.20 (s, 2H, CH<sub>2</sub>Ph), 4.0 (s, 3H, OCH<sub>3</sub>). EI-MS  $m/z$  435 [M]<sup>+</sup>. HR-MS Calcd. For C<sub>23</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>4</sub>: 435.0986; found: 435.0956.

**6-(Benzyloxy)-N-(4-chloro-3-fluorophenyl)-7-methoxy-3-nitroquinolin-4-amine (NQ11)** Using the procedure described earlier for NQ1 with 4-chloro-3-fluorobenzenamine, the title compound was obtained in 87% yield. Mp 284 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 9.0 (s, 1H, quinoline-2-H), 6.60–7.62 (m, 9H, Ph-H), 5.20 (s, 2H, CH<sub>2</sub>Ph), 4.0 (s, 3H, OCH<sub>3</sub>). EI-MS  $m/z$  453 [M]<sup>+</sup>. Element analysis: Calcd. For C<sub>23</sub>H<sub>17</sub>ClFN<sub>3</sub>O<sub>4</sub>: C, 52.83; H, 3.05; N, 11.55; found: C, 52.40; H, 3.97; N, 11.52.

**4-(3-Chloro-4-fluorophenylamino)-7-methoxy-3-nitroquinolin-6-ol (NQ12)** Using the procedure described earlier for NQ3 with NQ11, the title compound was obtained in 95% yield. Mp 178 °C. <sup>1</sup>H NMR (DMCO-*d*<sub>6</sub>): δ 9.17 (s, 1H, quinoline-2-H), 7.00–7.42 (m, 4H, Ph-H), 4.0 (s, 3H, OCH<sub>3</sub>). EI-MS  $m/z$  363 [M]<sup>+</sup>. Element analysis: Calcd. For C<sub>16</sub>H<sub>11</sub>ClFN<sub>3</sub>O<sub>4</sub>: C, 52.83; H, 3.05; N, 11.55; found: C, 52.43; H, 3.00; N, 11.05.

**6-(Benzyloxy)-7-methoxy-3-nitro-N-(2-(trifluoromethoxy)phenyl)quinolin-4-amine (NQ13)** Using the procedure described earlier for NQ1 using 2-(trifluoromethoxy)benzenamine, the title compound was obtained in 92% yield. Mp 201 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 9.0 (s, 1H, quinoline-2-H), 6.60–7.62 (m, 11H, Ph-H), 5.20 (s, 2H, CH<sub>2</sub>Ph), 4.0 (s, 3H, OCH<sub>3</sub>). EI-MS  $m/z$  485 [M]<sup>+</sup>. Element analysis: Calcd. For C<sub>24</sub>H<sub>18</sub>F<sub>3</sub>N<sub>3</sub>O<sub>5</sub>: C, 59.38; H, 3.74; N, 8.66; found: C, 59.18; H, 3.61; N, 8.12.

**6-(Benzyloxy)-N-(3-ethoxyphenyl)-7-methoxy-3-nitroquinolin-4-amine (NQ14)** Using the procedure described earlier for NQ1 with 3-ethoxybenzenamine, the title compound was



obtained in 93% yield. Mp 219 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 9.77 (s, 1H, quinoline-2-H), 7.19–7.88 (m, 11H, Ph-H), 5.20 (s, 2H, CH<sub>2</sub>Ph), 4.31 (q, 2H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 4.0 (s, 3H, OCH<sub>3</sub>), 2.1 (t, 3H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>). EI-MS *m/z* 445 [M]<sup>+</sup>. Element analysis: Calcd. For C<sub>25</sub>H<sub>23</sub>N<sub>3</sub>O<sub>5</sub>: C, 67.41; H, 5.20; N, 9.43; found: C, 67.31; H, 5.10; N, 9.01.

**6-(Benzyloxy)-N-(4-ethoxyphenyl)-7-methoxy-3-nitroquinolin-4-amine (NQ15)** Using the procedure described earlier for NQ1 with 4-ethoxybenzenamine, the title compound was obtained in 94% yield. Mp 211 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 9.77 (s, 1H, quinoline-2-H), 7.19–7.88 (m, 11H, Ph-H), 5.20 (s, 2H, CH<sub>2</sub>Ph), 4.31 (q, 2H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 4.0 (s, 3H, OCH<sub>3</sub>), 2.1 (t, 3H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>). EI-MS *m/z* 445 [M]<sup>+</sup>. HR-MS Calcd. For C<sub>25</sub>H<sub>23</sub>N<sub>3</sub>O<sub>5</sub>: 445.1638; found: 445.1614.

**7-Methoxy-3-nitro-4-(2-(trifluoromethoxy)phenylamino)quinolin-6-ol (NQ16)** Using the procedure described earlier for NQ3 with NQ13, the title compound was obtained in 96% yield. Mp 143 °C. <sup>1</sup>H NMR (DMCO-*d*<sub>6</sub>): δ 9.17 (s, 1H, quinoline-2-H), 7.00–7.42 (m, 6H, Ph-H), 4.00 (s, 3H, OCH<sub>3</sub>). EI-MS *m/z* 395 [M]<sup>+</sup>. Element analysis: Calcd. For C<sub>17</sub>H<sub>12</sub>F<sub>3</sub>N<sub>3</sub>O<sub>5</sub>: C, 51.65; H, 3.06; N, 10.63; found: C, 51.32; H, 3.02; N, 9.84.

**4-(3-Ethoxyphenylamino)-7-methoxy-3-nitroquinolin-6-ol (NQ17)** Using the procedure described earlier for NQ3 with NQ14, the title compound was obtained in 95% yield. Mp 189 °C. <sup>1</sup>H NMR (DMCO-*d*<sub>6</sub>): δ 9.27 (s, 1H, quinoline-2-H), 7.10–7.48 (m, 6H, Ph-H), 4.0 (s, 3H, OCH<sub>3</sub>). EI-MS *m/z* 355 [M]<sup>+</sup>. Element analysis: Calcd. For C<sub>18</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>: C, 60.84; H, 4.82; N, 11.83; found: C, 60.81; H, 4.56; N, 11.12.

**4-(4-Ethoxyphenylamino)-7-methoxy-3-nitroquinolin-6-ol (NQ18)** Using the procedure described earlier for NQ3 with NQ15, the title compound was obtained in 95% yield. Mp 181 °C. <sup>1</sup>H NMR (DMCO-*d*<sub>6</sub>): δ 9.17(s, 1H, quinoline-2-H), 7.09–7.38 (m, 6H, Ph-H), 4.31 (q, 2H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 4.0 (s, 3H, OCH<sub>3</sub>), 2.1 (t, 3H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>). EI-MS *m/z* 355 [M]<sup>+</sup>. HR-MS Calcd. For C<sub>18</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>: 355.1168; found: 355.1125.

**Ethyl 2-(4-(4-ethoxyphenylamino)-7-methoxy-3-nitroquinolin-6-yloxy)acetate (NQ19)** A mixture of NQ8 (700 mg, 1.97 mmol), K<sub>2</sub>CO<sub>3</sub> (500 mg, 3.62 mmol), and 10 mL DMF was stirred at 80 °C for 1 h. Then ethyl 2-bromoacetate (400 mg, 2.22 mmol) was added, and the mixture was stirred for another 1 h. The solvent was removed under vacuum. The crude product was recrystallized from ethyl acetate to give the title compound (95%). Mp 195 °C. <sup>1</sup>H NMR (DMCO-*d*<sub>6</sub>): δ 9.27(s, 1H, quinoline-2-H), 7.19–7.88 (m, 6H, Ph-H), 4.90 (s, 2H, COCH<sub>2</sub>O), 4.31 (q, 2H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 4.20 (q, 2H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 4.0 (s, 3H, OCH<sub>3</sub>), 2.3 (t, 3H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.0 (t, 3H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>). EI-MS *m/z* 441 [M]<sup>+</sup>. Element analysis: Calcd. For C<sub>22</sub>H<sub>23</sub>N<sub>3</sub>O<sub>7</sub>: C, 59.86; H, 5.25; N, 9.52; found: C, 59.63; H, 5.55; N, 9.55.

**7-(Benzyloxy)-N-(4-ethoxyphenyl)-6-methoxy-3-nitroquinolin-4-amine (NQ20)** Using the procedure described earlier for NQ2 with 4-ethoxybenzenamine, the title compound was obtained in 93% yield. Mp 274 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 9.77 (s, 1H, quinoline-2-H), 7.19–7.88 (m, 11H, Ph-H), 5.20 (s, 2H,

CH<sub>2</sub>Ph), 4.31 (q, 2H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 4.0 (s, 3H, OCH<sub>3</sub>), 2.1 (t, 3H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>). EI-MS *m/z* 445 [M]<sup>+</sup>. HR-MS Calcd. For C<sub>25</sub>H<sub>23</sub>N<sub>3</sub>O<sub>5</sub>: 445.1638; found: 445.1614.

**4-(4-Ethoxyphenylamino)-6-methoxy-3-nitroquinolin-7-ol (NQ21)** Using the procedure described earlier for NQ3 with NQ20, the title compound was obtained in 94% yield. Mp 185 °C. <sup>1</sup>H NMR (DMCO-*d*<sub>6</sub>): δ 9.17 (s, 1H, quinoline-2-H), 7.09–7.38 (m, 6H, Ph-H), 4.31 (q, 2H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 4.0 (s, 3H, OCH<sub>3</sub>), 2.1 (t, 3H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>). EI-MS *m/z* 355 [M]<sup>+</sup>. HR-MS Calcd. For C<sub>18</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>: 355.1168; found: 355.1125.

## Author contribution

Hong LIU, Li-ping LIN, Hua-liang JIANG, Jian DING, and Kai-xian CHEN designed research; Xiao-min LUO designed the research about molecular modeling; Hai-hong LI, He HUANG, and Xiu-hua ZHANG performed research; Hai-hong LI analyzed data; He HUANG wrote the paper.

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