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# Design, synthesis and biological evaluation of 4-amino-quinolines as antitumor agents

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**Abstract**: Fifteen novel 4-amino-quinolines ($I_1$-$III_3$) as antitumor agent were synthesized by *p*-nitroaniline and ethoxymethylene malonic ester (EMME) via condensation, cyclization, hydrolysis, decarboxylation, chlorination, nucleophilic substitution, reduction and amidation. The antitumor activity of compounds $I_1$-$III_3$ was evaluated on SGC-7901, BEL-7402 and A549 cancer cell lines. *In vitro* bioassay indicated that some compounds showed different degree activity against all tested cancer cell lines. Compound $I_1$, $I_4$ and $II_2$ exhibited high effects against A549 cell lines ($IC_{50}$ = 1.34μM, 1.36μM and 3.00μM, respectively). In addition, the result of molecular docking showed that compound $I_1$, $I_4$ and $II_2$ could dock into the pocket of EGFR.

**Keywords**: 4-amino-quinolines, antitumor agent, EGFR.

## INTRODUCTION

At a global level, Malignant tumour is one of the main causes of death and it is critically important to develop novel compounds with antitumor activity (Othman et al., 2019, El-Sayed et al., 2018, Zhao et al., 2019). Epidermal growth factor receptor (EGFR) belongs to ErbB lineage of proteins which has tyrosine kinase activity (Akhtar et al., 2017, Hou et al., 2016). Over expression of EGFR has been observed in a wide range of human tumours (Liu et al., 2018, Wee and Wang, 2017, Abouzid and Shouman, 2008). Small molecule EGFR inhibitors are considered as the main anticancer chemotherapeutic drugs.

Quinazolines belong to a famous class of heterocyclic compounds used in medicine due to their extensive diversity of biological activities (Zayed et al., 2018, Khan et al., 2016). A lot of small molecule inhibitors targeting EGFR have been successfully developed. Especially, the quinazoline scaffold, is widely used for the design of EGFR kinase inhibitors. Gefitinib(A) and erlotinib (B) (fig. 1) used to target therapy to NSCLC as the first generation of EGFR inhibitors were approved by the U.S. FDA in 2003 and 2004, respectively (Cohen et al., 2003, Dowell et al., 2005). Further research revealed that Acrylamide as an electrophilic Michael acceptor was introduced at position C-6 of the quinazoline ring which reacted with the sulfhydryl group of Cys797 on the catalytic site of EGFR, which inactivating the activity of tyrosine kinase, such as Afatinib (C) (fig. 1) approved by the U.S. FDA in 2013 which treated for patients with EGFR mutation-positive metastatic NSCLC (Dungo and Keating, 2013). Moreover, the quinazoline ring was replaced by quinoline through the conversion of N atom at the -3 position by C-CN group to get quinoline derivative such as neratinib (E) (fig. 1), Neratinib showed excellent inhibitory activity toward EGFR (Wissner and Mansour, 2008, Carmi et al., 2012). Daniel Rauh *et al* also demonstrated that EGFR inhibitors based on different scaffolds allow for a high degree of mutant selectivity. Quinoline scaffold can be as effective as their corresponding quinazoline (such as 4-anilinoquinoline(F) vs 4-anilinoquinazoline (D) (fig. 1)(Niggenaber et al., 2019, Pawar et al., 2010).

Based on the above mentioned results and our previous studies (Liu et al., 2019, Liu et al., 2015), a series of 6-substituted 4-amino-quinolines were designed. The nitro or amino groups are connected at C-6 position of quinoline ring as the acceptor or donor of hydrogen bond and acrylamide groups as the Michael acceptor. Additionally, different lipophilic substituents are attached on the *p*- or *m*- position of the aniline ($OCH_3$, Cl or F). In addition, 4-anilino was replaced by 3-(1*H*-imidazol-1-yl)-propyl amino group to investigate the antitumor effect of heterocycle. The design strategy for all target compounds is shown in fig. 1. The evaluation of their inhibitory activity using MTT assay and the possible EGFR binding modes were also illustrated.

## MATERIALS AND METHODS

### *Reagents and Instruments*

X-4 digital visual melting point apparatus (Tech, Beijing, China) was used to determine the melting points. Thermo-Finnigan LCQ instrument (Thermo-Finnigan, San Francisco, CA, USA) was used to acquire ESI MS. ARX-500 spectrometer (Bruker, Fallanden, Switzerland) recorded $^1$H-NMR spectra.

A549 cell line, SGC-7901 cell line, and BEL-7402 cell line were purchased from the Shanghai Institute of Life Sciences Cell Resource Centre (Shanghai, China). Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco Technologies, Eggenstein, Germany). 10% FBS (Hyclone, Logan, UT, USA).

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***Synthesis of intermediates 1- 5***

EMME (5.64 g, 26.07 mmol) and *p*-nitroaniline (3.00 g, 21.73 mmol) were stirred in refluxing *n*-butyl alcohol (30 mL) for 6h. After completion, *n*-butyl alcohol is evaporated by vacuum and anhydrous $C_2H_5OH$ was added to the residue to precipitate intermediate 1. A solution of intermediate 1(2.00 g, 6.50 mmol) in Dowtherm-A (40 mL) was stirred at 250°C for about one hour. The mixture was then cooled to room temperature and petroleum ether (PE) was added to obtain precipitate, which was washed with PE to produce intermediate 2. Intermediate 2 (1.02 g, 3.89 mmol) was hydrolysed in refluxing 10% (m/m) NaOH solution (30 mL) for four hours. The mixture was adjusted pH to 1 with hydrochloric acid to give a white solid and washed the solid with water (100 mL) to obtain intermediate 3. A solution of intermediate 3(0.51 g, 2.15 mmol) in Dowtherm-A (50mL) was stirred at 240-250°C for about one hour to get intermediate 4. DMF (1 drop) and $POCl_3$ (5mL) were added to a solution of intermediate 4 (0.32 g, 1.58 mmol) in $C_2H_4Cl_2$ (30 mL) and the reaction was performed under reflux temperature for three hours. Poured the solution into ice water and adjusted the pH to 3 using saturated $NaHCO_3$ solution. Collect the crude intermediate 5 (white solid) by suction filtration and the pure intermediate 5 as white solid was obtained via silica gel column chromatography (petroleum ether/ethyl acetate, 5:1, v/v).

***2-[(4-Nitrophenylamino) methylene] malonate diethyl ester (1)***

Yield 86.9%; light yellow solid, mp: 141.9-142.2°C; ESI-MS: 309.0 $[M+H]^+$.

***Ethyl 4-hydroxy-6-nitroquinoline-3-carboxylate (2)***

Yield 76.6%; white solid, mp: 352.5-352.8°C; ESI-MS: 263.0 $[M+H]^+$.

***4-Hydroxy-6-nitroquinoline-3-acetic acid (3)***

Yield 92.7%; white solid, mp: 284.5-286.4°C; ESI-MS: 235.0 $[M+H]^+$.

***4-Hydroxy-6-nitroquinoline (4)***

Yield 91.1%; yellow solid, mp: 323.7-325.5°C; ESI-MS: 191.4 $[M+H]^+$.

***4-Chloro-6-nitroquinoline (5)***

Yield 85.4%; white solid, mp: 142.2-142.8°C; ESI-MS: 209.1 $[M+H]^+$.

***Synthesis of compounds $I_1$-$I_6$***

5 (100.0mg, 0.48mmol), *m*-Chloroaniline (67.50 mg, 0.53 mmol) and pyridine (41.80mg, 0.53mmol) were dissolved in isopropanol (20mL). The reaction was allowed at reflux temperature. After completion, isopropanol was removed under vacuum condition. Ethyl ether (10mL) was added to the residue to get crude compound $I_1$ as yellow solid. Pure compound $I_1$ was obtain via silica gel column chromatography (petroleum ether/ethyl acetate, 5:1, v/v).

***6-Nitro-4-(3'-chlorobenzylamino) quinoline ($I_1$)***

Yield 87.0%; yellow solid; mp: 210.8-211.7°C; ESI-MS: 298.3$[M+H]^+$.

***6-Nitro-4-(3'-fluorobenzylamino) quinoline ($I_2$)***

$I_2$ was prepared from 5 and *m*-fluoroaniline following the similar procedure described for Compound $I_1$. Yield 90.6%; yellow solid; mp: 212.8-213.5°C; ESI-MS: 284.0 $[M+H]^+$.

***6-Nitro-4-(1'-imidazolylpropylamine) quinoline($I_3$).***

$I_3$ was prepared from 5 and *N*-(3-aminopropyl)-imidazole following the similar procedure described for Compound. Yield 83.2%; yellow solid; mp: 204.3-205.9°C; ESI-MS: 298.0$[M+H]^+$; $^1$H-NMR (500 MHz, $CDCl_3$) δ 8.74 (s, 1H), 8.68 (d, *J* = 5.5 Hz, 1H), 8.42 (dd, *J* = 9.0, 1.5 Hz, 1H), 8.08 (d, *J* = 9.0 Hz, 1H), 7.56 (s, 1H), 7.20 (s, 1H), 7.03 (s, 1H), 6.46 (d, *J* = 5.5 Hz, 1H), 4.22-4.15 (m, 2H), 3.43-3.37 (m, 2H), 2.34-2.28 (m, 2H).

***6-Nitro-4-(4'-methoxybenzamine) quinoline ($I_4$).***

$I_4$ was prepared from 5 and *p*-methoxyaniline following the similar procedure described for Compound $I_1$. Yield 81.0%; yellow solid; mp: 199.7-201.2°C; ESI-MS *m/z*: 296.1 $[M+H]^+$.

***6-Nitro-4-(4'-fluorobenzylamino) quinoline ($I_5$)***

$I_5$ was prepared from 5 and *p*-fluoroaniline following the similar procedure described for Compound $I_1$. Yield 80.1%; light yellow solid; mp: 214.0-215.1°C; ESI-MS: 283.8$[M+H]^+$.

***6-Nitro-4-(3'-chloro-4'-fluorobenzamine) quinoline ($I_6$)***

$I_6$ was prepared from 5 and *m*-chloro-4-fluoroaniline following the similar procedure described for Compound $I_1$. Yield 79.6%; yellow solid; mp: 209.8-211.7°C; ESI-MS: 317.9$[M+H]^+$; $^1$H-NMR (500 MHz, $CDCl_3$) δ 9.01 (d, *J* = 2.0 Hz, 1H), 8.72 (d, *J* = 5.5 Hz, 1H), 8.48 (dd, *J* = 9.5, 2.0 Hz, 1H), 8.16 (d, *J* = 9.5 Hz, 1H), 7.46 (dd, *J* = 6.5, 2.0 Hz, 1H), 7.30-7.29 (m, 1H), 7.22-7.18 (m, 1H), 6.99 (s, 1H), 6.93 (d, *J* = 5.5 Hz, 1H).

***Synthesis of compounds $II_1$-$II_6$.***

To a solution of $I_1$ (100 mg, 0.44 mmol) in EtOH (20mL) was added $SnCl_2$ (228.80mg, 1.01mmol) at 0°C. The mixture was stirred under reflux. After that, EtOH was evaporated under vacuum and 20% NaOH (10mL) was added to the residue to give precipitate, filtered. The pure $II_1$ was obtained via silica gel column chromatography ($CHCl_3$/MeOH, 8:1, v/v).

***6-Amino-4-(3'-chlorobenzylamino) quinoline ($II_1$)***

Yield 84.5%; light green solid; mp: 110.9-111.7°C; ESI-MS 270.9$[M+H]^+$.

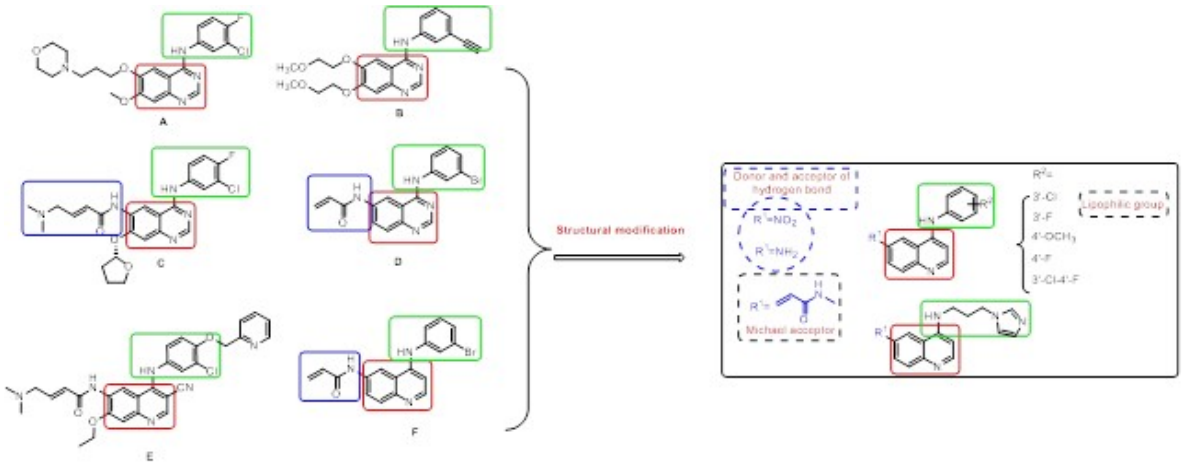
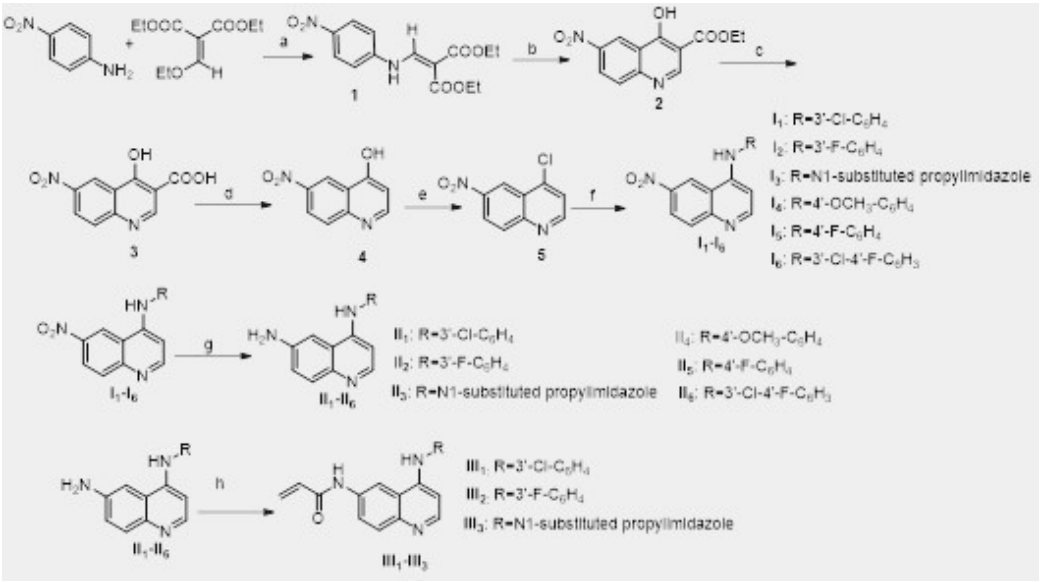



**Fig. 1**: design of 4-amino-quinolines based on chemical structures of several tyrosine kinase inhibitors containing quinazoline and quinoline moieties




Reagents and conditions: (a) *n*-BuOH, reflux, 6h; (b) Dowtherm-A, 250°C, 1h; (c) 10% NaOH aq, reflux, 4h; (d) Dowtherm-A, 240-250°C, 1h; (e) $POCl_3$, DMF, 80°C, 3h, saturated $NaHCO_3$ aq; (f) substituted aniline, pyridine, *i*-PrOH, reflux, ether, saturated $NaHCO_3$ aq, room temperature, 0.5h; (g) $SnCl_2$, anhydrous EtOH, reflux, dilute NaOH aq; (h) $Et_3N$, THF, acryloyl chloride, 0°C, saturated $NaHCO_3$ aq.

**Scheme 1**: Synthetic route of target compounds

***6-Amino-4-(3'-fluorobenzylamino) quinoline ($II_2$)***

$II_2$ was prepared from $I_2$ following the similar procedure described for $II_1$. Yield 83.3%; light green solid; mp: 114.5-116.3°C; ESI-MS: 254.7$[M+H]^+$.

***6-Amino-4-(1'-imidazolylpropylamine) quinoline ($II_3$)***

$II_3$ was prepared from $I_3$ following the similar procedure described for $II_1$. In addition, NaOH solution was replaced with anhydrous ether. Yield 88.1%; white solid; mp: 105.9-107.0°C; ESI-MS: 268.2$[M+H]^+$.

***6-Amino-4-(4'-methoxybenzamine) quinoline ($II_4$)***

$II_4$ was prepared from $I_4$ following the similar procedure described for $II_1$. Yield 91.7%; light green solid; mp: 90.9-92.6°C; ESI-MS: 266.2$[M+H]^+$.

***6-Amino-4-(4'-fluorobenzamine) quinoline ($II_5$)***

$II_5$ was prepared from $I_5$ following the similar procedure described for $II_1$. Yield 92.4%; light green solid; mp: 113.5-115.3°C; ESI-MS: 254.1$[M+H]^+$.

***6-Amino-4-(3'-chloro-4'-fluorobenzamine)quinoline ($II_6$)***

$II_6$ was prepared from $I_6$ following the similar procedure described for $II_1$. Yield 92.0%; light green solid; mp: 110.4-111.6°C; ESI-MS: 288.2 $[M+H]^+$.

***Synthesis of compounds $III_1$-$III_3$***

A solution of $Et_3N$ (22.60 mg, 0.22 mmol) and $II_1$ (50 mg, 0.19 mmol) in THF (5mL) was stirred in an ice-water bath and Acryloyl chloride (20.20 mg, 0.22 mmol) was

**Table 1**: Anti-proliferative activity of target compounds against human cancer cell lines.

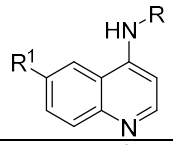
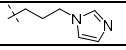
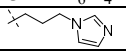
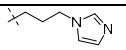
| Compound |  | | | | |
|---|---|---|---|---|---|
| | $IC_{50}(\mu M^a)$ | | | | |
| | $R^1$ | R | SGC-7901 | BEL-7402 | A549 |
| $I_1$ | $NO_2$ | 3'-Cl-$C_6H_4$ | >100 | >100 | 1.34 |
| $I_2$ | $NO_2$ | 3'-F-$C_6H_4$ | 86.80 | 51.82 | >100 |
| $I_3$ | $NO_2$ |  | >100 | 49.77 | >100 |
| $I_4$ | $NO_2$ | 4'-$OCH_3$-$C_6H_4$ | 35.11 | >100 | 1.36 |
| $I_5$ | $NO_2$ | 4'-F-$C_6H_4$ | 88.42 | 83.51 | 92.98 |
| $I_6$ | $NO_2$ | 3'-Cl-4'-F -$C_6H_3$ | >100 | >100 | 31.76 |
| $II_1$ | $NH_2$ | 3'-Cl-$C_6H_4$ | >100 | >100 | >100 |
| $II_2$ | $NH_2$ | 3'-F-$C_6H_4$ | >100 | >100 | 3.00 |
| $II_3$ | $NH_2$ |  | >100 | >100 | >100 |
| $II_4$ | $NH_2$ | 4'-$OCH_3$-$C_6H_4$ | 69.06 | >100 | 22.10 |
| $II_5$ | $NH_2$ | 4'-F-$C_6H_4$ | 75.82 | 55.20 | 84.63 |
| $II_6$ | $NH_2$ | 3'-Cl-4'-F -$C_6H_3$ | 70.23 | 72.60 | 72.98 |
| $III_1$ |  | 3'-Cl-$C_6H_4$ | 51.41 | >100 | >100 |
| $III_2$ |  | 3'-F-$C_6H_4$ | >100 | >100 | >100 |
| $III_3$ |  |  | >100 | 41.20 | >100 |
| Gefitinib | | | 11.01 | 18.91 | 14.77 |

**Table 2**: The interacting residues for the best-docked conformation and binding energy of the three target compounds as well as Gefitinib

| Compound | Protein | Protein interacting residue | Binding energy (*KJ/mol*) |
|---|---|---|---|
| Gefitinib | EGFR | THR A:854[a] ; MET A:793[a]; THR A:790[a]; | -103.409 |
| $I_1$ | EGFR | THR A:854[a]; LYS A:745[a]; ALA A:743[a] | -87.038 |
| $I_4$ | EGFR | THR A:854[a]; MET A:793[a]; ASP A:855[a] | -91.350 |
| $II_2$ | EGFR | THR A:854[a]; GLU A:762[a] | -88.285 |

a: Conventional hydrogen bond

dropwise. After completion, THF was evaporated in vacuum, followed by the addition of saturated $NaHCO_3$ solution (10mL) to precipitate solid, filtered and neutralized by washing, then purified via silica gel column chromatography ($CHCl_3$/MeOH, 10:1, v/v) to obtain the pure product $III_1$.

***6-Acrylamide-4-(3'-chlorobenzylamino) quinoline ($III_1$)***

Yield 89.7%; white solid; mp: 172.0-173.4°C; ESI-MS: 324.9$[M+H]^+$.

***6-Acrylamide-4-(3'-fluorobenzylamino) quinoline ($III_2$)***

$III_2$ was prepared from $II_2$ following the similar procedure described for $III_1$. Yield 76.7%; white solid; mp: 164.5-166.1°C; ESI-MS: 309.1$[M+H]^+$.

***6-Acrylamide-4-(1'-imidazolylpropylamine)quinoline ($III_3$)***

$III_3$ was prepared from $II_3$ following the similar procedure described for $III_1$. In the reaction process, $Et_3N$ and THF were replaced by pyridine as organic base and solvent. Yield 77.1%; white solid; mp: 172.3-174.1°C; ESI-MS: 322.0$[M+H]^+$.

***Anti-tumour activities***

The antiproliferative activities of target compounds were evaluated against a panel of three human cancer cell lines over expressing EGFR, including gastric (SGC-7901), liver (BEL-7402) and lung (A549) using colorimetric MTT cytotoxicity screening assay, with Gefitinib as the positive control (Wang et al., 2016, Hou et al., 2016, Li et al., 2019). Briefly, logarithmic-phase tumour cells were plated onto 96-well culture plates ($5\times10^3$ cells/well) for twenty-four hours. Cells were treated with the target compounds and Gefitinib at different concentrations (0.4μg/mL, 1.2μg/mL, 3.6μg/mL, 11μg/mL and 33μg/mL) for forty-eight hours. MTT solution was added to each well and incubate four hours in atmosphere with 5% $CO_2$ at 37°C. Measured absorbance at 490 nm using Microplate Reader (Rayto RT-6000) and calculated $IC_{50}$.

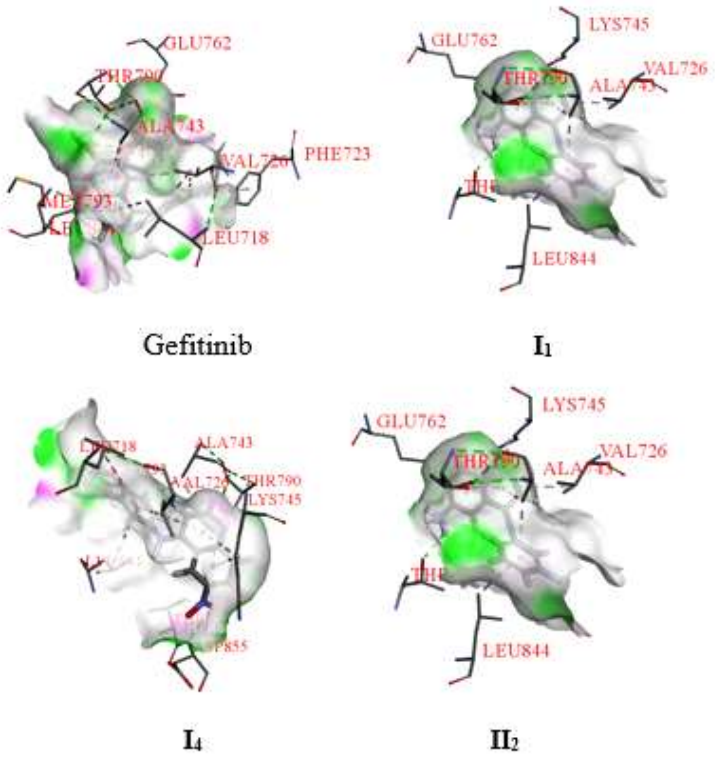



**Fig. 3**: 3D Diagram of compound $I_1$, $I_4$, $II_2$ and Gefitinib into the kinase domain of EGFR (PDB code: 5CAV).

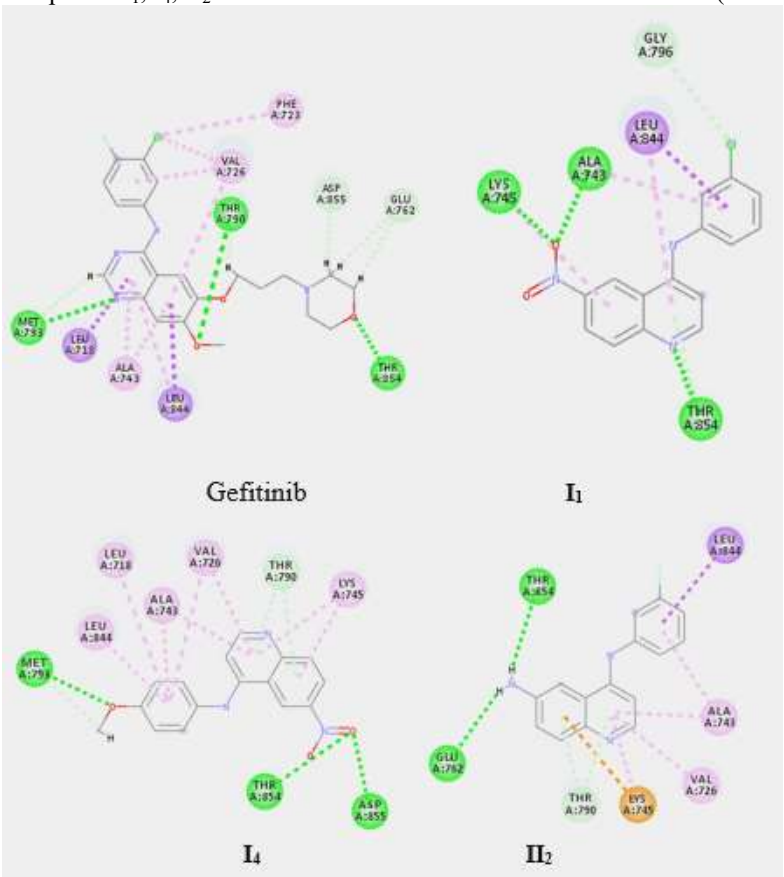



**Fig. 4**: 2D Diagram of interaction modes of $I_1$, $I_4$, $II_2$ and Gefitinib inside EGFR.The hydrogen bonds are displayed as green dotted lines.

***Molecular docking***

EGFR (PDB ID: 5CAV) (Heald et al., 2015) was obtained from the protein database (PBD), all binding water and ligands were removed from the protein. The molecular docking of the protein with the target compounds $I_1$, $I_4$, $II_2$ and Gefitinib were completed by Molegro Virtual Docker (MVD) software and the binding energy was calculated. The conformation with the lowest energy was selected and the interaction mode between the compounds and protein was analysed by Discovery Studio 2016 Client (2016) (DS) software.

## RESULTS

Fifteen target quinoline derivatives ($I_1$-$III_3$) were designed and synthesized. The reaction route is shown in Scheme 1.

As depicted in table 1, the anti- proliferative activity results indicated that most compounds showed different degree activity against all tested cell lines with $IC_{50}$ values in the μM range, while compounds $II_1$, $II_3$ and $III_2$ had no inhibitory effect against the three cell lines ($IC_{50}$ >100μM). All the compounds displayed poor activities in SGC-7901 cell line ($IC_{50}$: 35.11μM ->100μM) and BEL-7402 cell line ($IC_{50}$:41.20μM ->100μM), as compared with gefitinib. Compounds $I_1$, $I_4$ and $II_2$ exhibited higher selective antiproliferative activities with $IC_{50}$ values ranging from 1.34μM -3.00μM than that of gefitinib ($IC_{50}$: 14.77μM) against A549 cell line. In addition, molecular docking between $I_1$, $I_4$, $II_2$ and EGFR was performed and the result showed that compound $I_1$, $I_4$, $II_2$ could dock into the EGFR enzyme pocket and form two or three hydrogen bonds with amino acids residues.

## DISCUSSION

***Chemistry***

Compound 1 was obtained via a condensation reaction between *p*-nitroaniline and ethoxymethylene malonic ester (EMME) (Price and Roberts, 1946, Gould and Jacobs, 1939). The nucleophilic ability of the amino group decreased due to the presence of $NO_2$ group, *n*-Butanol was selected as the solvent and condensation reaction was allowed at the reflux temperature to get compound 1. To produce compound 2, cyclization should be induced in Dowtherm-A at 250°C. Hydrolysis compound 2 with 10% aqueous NaOH yielded compound 3. Compound 4 was obtained by decarboxylation of compound 3 at 240-250°C in Dowtherm-A. Chlorination of compound 4 resulted in compound 5, which reacted with 3-chloroaniline in isopropyl alcohol at reflux temperature to produce compound $I_1$.

The nitro group in compound $I_1$ was reduced using $SnCl_2$ in anhydrous alcohol to give the amine $II_1$. The amino group in compound $II_1$ was acylated with the acryloyl chloride to obtain the 6-acrylamide-4-anilinoquinoline derivative $III_1$.

***Biological Evaluation***

As shown in table 1, among the 6-nitro-4-anilino-quinolines, compounds $I_1$ and $I_4$, with 3'-Cl or 4'-$OCH_3$ substitution on the 4-aniline ring exhibited higher potency against A549 cells ($IC_{50}$=1.34$\mu$M and 1.36 $\mu$M, respectively) than that of the reference drug (gefitinib, $IC_{50}$=14.77$\mu$M). While, among the 6-amino-4-anilino-quinolines, compounds $II_2$ and $II_4$, with 3'-F or 4'-$OCH_3$ substitution also showed good selective activity against A549 cells ($IC_{50}$=3.00$\mu$M, 22.10$\mu$M, respectively). The above outcomes also illustrated that the lipophilic group (chlorine atom/fluorine atom/methoxy group) at the C-4 or C-3 position of the 4-aniline ring and hydrogen-bond acceptor/donor group (nitro group/amino group) at C-6 position of the quinoline ring may be a factor contributing to cytotoxicity. Compounds $I_3$, $II_3$ and $III_3$ contained the same group (namely N1-imidazolylamino) at C-4 position of the quinoline ring showed no antitumor activity against SGC-7901 and A549 cells ($IC_{50}$>100$\mu$M, respectively). $I_3$ and $III_3$ showed moderate inhibitory activity against BEL-7402 cells ($IC_{50}$=49.77$\mu$M and 41.20$\mu$M, respectively). It revealed that N1-imidazolylamino is unfavourable for the increasing activity. Acrylamide fragment, commonly employed in designing irreversible inhibitors that was able to bind with cysteine residues of EGFR (Carmi et al., 2012). We synthesized the compounds containing an acrylamide attached C-6 position of 4- amino-quinolines. Unfortunately, among the 6-acrylamide series, compounds $III_1$, $III_2$ and $III_3$ showed poor antitumor activity against the three test cells ($IC_{50}$: 41.20μM ->100$\mu$M). The results indicated that introduction of the acrylamide group into the C-6 position of the quinoline ring did not obtain the anticipated effect as we hypothesized, led to loss the activity of the compounds ($I_1$ vs $III_1$ and $II_2$ vs. $III_2$).

***Molecular docking***

As shown in table 2, fig. 3 and fig.4, the nitro group of compound $I_1$ formed hydrogen bond with LYS745 and ALA743, respectively. The nitrogen atom on the C-1 position of compound $I_1$ formed one hydrogen bond with THR854. While the nitro group of compound $I_4$ formed hydrogen bond with ASP855 and THR854, respectively. The methoxy group of compound $I_4$ formed one hydrogen bond with MET 793. The amino group of compound $II_2$ formed hydrogen bond with GLU762 and THR854, respectively. The interaction highlights the importance of nitro group and amino group as an acceptor or donor of hydrogen bond. The substituent group at C-4 of the quinoline ring was also the main moiety affecting the binding mode. Moreover, compounds $I_1$, $I_4$, $II_2$ bind in a similar manner to gefitinib where strong hydrogen interaction was clearly observed with amino acid residue THR854, respectively.

## CONCLUSION

A novel series of 4-amino-quinoline derivatives ($I_1$-$III_3$) were designed and synthesized for evaluation as anticancer agents against A549 cell line, SGC-7901 cell line, and BEL-7402 cell line. Activity data showed that some of the prepared compounds displayed different levels of antitumor activity in the three cell lines tested. Especially, compounds $I_1$, $I_4$ and $II_2$ showed greater activities against A549 cells than that of the reference drug Gefitinib. The inhibition of the compounds with nitro group is favoured over that with amino group or acrylamide group. Molecular docking studies revealed that nitro or amino group as hydrogen-bond acceptor or donor are beneficial to improve the activity. Compounds $I_1$, $I_4$, $II_2$ and Gefitinib bind EGFR in a similar manner, forming hydrogen bonds with the amino acid residue THR854 of EGFR, respectively. The results could help us obtain more potent quinoline derivatives as anti-tumour agents.

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