Design and Synthesis of Novel Epidermal Growth Factor Receptor Kinase Inhibitors

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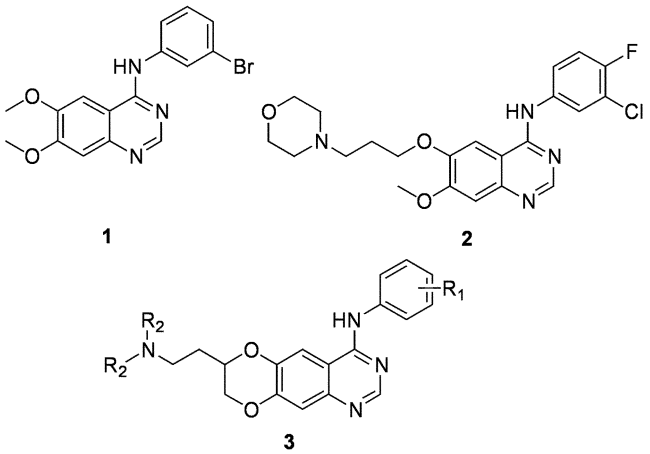
Investigation of structure-activity relationships of novel quinazolines has identified 7,8-dihydro-[1,4]dioxino-[2,3-g]quinazolines as a potent inhibitor of EGFR. These compounds have a benzodioxane framwork, which was prepared by regioselective *O*-alkylation of ethyl 3,4-dihydroxy benzoate by epoxide ring opening. Compounds **3f** and **3k** were more potent than ZD-1839 in EGF enzyme and EGFR autophosporylation inhibition assays.

Key Words : Cancer, EGFR, Tyrosine kinase, Quinazoline, Autophosphorylation assay

Introduction

Epidermal growth factor receptor (EGFR) is involved in signaling pathways controlling cell growth and differentiation.[1] Increased EGFR expression and activity are closely related with an increase in tumor malignancy.[2] Therefore, inhibitors of EGFR kinase activity may prove useful for therapeutic intervention in cancer as well as other proliferative diseases. The various 4-anilinoquinazoline derivatives[3] have been developed as a potent and selective inhibitor against various tyrosine kinase families, such as EGFR, VEGFR, and Src. However, 4-anilinoquinazoline **1**[3a-c] having dimethoxy group at C-6 and C-7 suffered from poor bioavailability *in vivo* because of their low water solubility. In order to improve the pharmacokinetic properties of compounds, a number of laboratories have extensively tried to replace the methoxy groups at C-6 or C-7 with a variety of aminoalkoxy groups, resulting in much improved bioavailability. For example, ZD-1839 (**2**),[4] having morpholino propoxy substituent at C-6 and methoxy group at C-7, is an orally active, potent, and selective EGFR inhibitor used in the clinic in the chemotherapy of non-small-cell lung cancer (NSCLC).[5] From this consideration, further increase of polarity at C-6 and (or) C-7 may enhance the physical properties of the compounds. With this in mind, we tried to find potent EGFR inhibitors and discovered novel compounds **3**, which possess a [1,4]-dioxino quinazoline structure[6] linking the alkoxy side chains together, because their structural characteristic is considered to have better solubility than dialkoxyquinazoline derivatives.



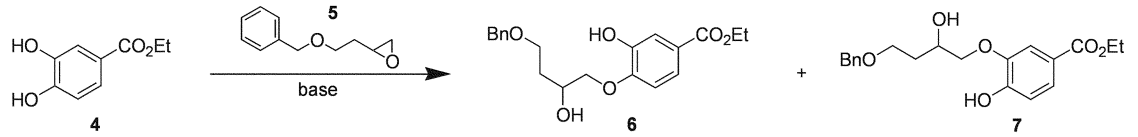
Results and Discussion

For constructing the key skeleton, [1,4]-dioxino quinazoline, an efficient method for regioselective alkylation of catechol need to be devised. Although regioselective alkylation of catechols with alkyl halides has been well studied to date,[7] there has been no report about regioselective alkylation *via* the ring opening of epoxides.[8,9] Since monoalkylation of catechol with alkyl halides under basic conditions takes place favorably at electron deficient hydroxyl group, we anticipated that alkylation of **4** with epoxides also shows the same pattern as the reaction with alkyl halides. With this in mind, we investigated the alkylation on finding effective bases under conditions as shown in Table 1.

Various base systems,[10] such as K_2CO_3, Et_3N and NaOEt were not effective for alkylation, however the use of KF and CsF (entry 1 and 3)[8b] were effective to generate the monosubstituted product as *ca.* 4 : 1 mixtures in a favor of C-4 substituted product, along with large amount of starting material (*ca.* 30%). Although the reaction occurred in the regioselective manner, the yield was poor, giving the product in less than 30% yield. Decreasing the amount of CsF (entry 4) gave improved results in terms of the yield and regioselectivity (42%, 7 : 1, respectively). Best result was obtained in the use of an excess amount of epoxide (2 eq), affording the products as 7 : 1 mixtures in 62% yield, along with small amount of dialkylated product (< 5%).

Since two regioisomers were not separated by column chromatography, the ratio of the regioisomers was deter-

Table 1.



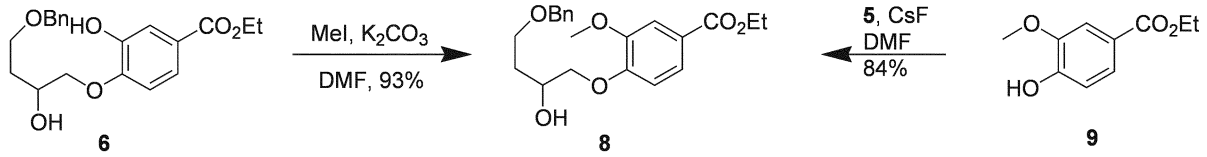
Entry	Base (eq.)	Conditions[a]	Yield (%)[b]	Ratio (**6**/**7**)
1	KF (0.3)	5h/DMF/ **5**	23	5/1
2	CsF (1.0)	24h/DMF/ **5**	21	5/1
3	CsF (0.3)	10h/DMF/ **5**	29	5/1
4	CsF (0.02)	14h/DMF/ **5**	42	7/1
5	CsF (0.02)	10h/DMSO/ **5**	40	6/1
6	CsF (0.02)	8h/NMP/ **5**	< 10	–
7	CsF (0.02)	15h/DMF/ **5**[c]	51	7/1
8	CsF (0.02)	15h/DMF/ **5**[d]	62	7/1

[a]**5** (1.1 eq), 130 °C; [b]isolated yield; [c]1.5 eq.; [d]2 eq.

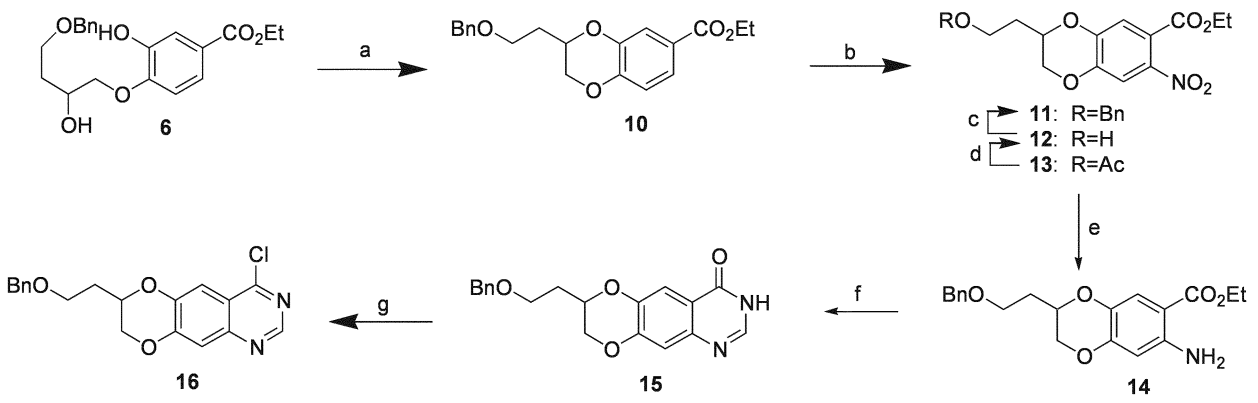
mined by ^{1}H-NMR analysis of regioisomeric mixtures after methylation of hydroxyl group of phenol. The unambiguous confirmation of structure was made by comparison of the product obtained by methylation of **6** and the product derived by methylation of a commercially available phenol **9** as described in Scheme 1. Ultimately, the desired regioisomer was obtained by crystallization of the nitro compound **11** prepared in 2 steps from the mixtures of **6** and **7**.

In order to construct the requisite benzodioxane structure **6** was treated with diisopropyl azodicarboxylate and triphenylphosphine to give the desired benzodioxane **10** in 95% yield (Scheme 2).

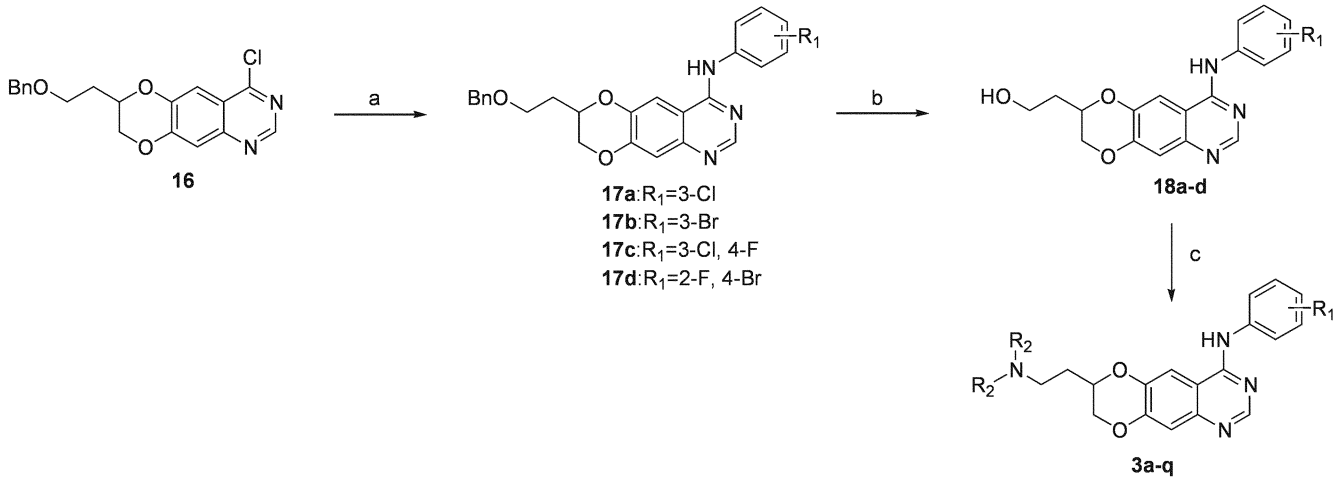
The next step, nitration of **10**, proved nontrivial. The nitration in usual condition (60% HNO_3, AcOH, 50 °C)[11] provided the desired product **11** in less than 38% yield, along with two side products, debenzylated product **12** and acetylated product **13** (16% and 15%, respectively). Although we were unable to improve the isolated yield beyond that reported here, the side products were readily converted to **11** by benzylation (BnBr, NaH) for **12**, and by deacetylation and subsequent benzylation for **13**. Reduction of **11** using Fe and NH_4Cl gave the compound **14**, which was readily converted by treatment with formamide and $(NH_4)_2CO_3$ to the 3,4-dihydroquinazoline-4-one **15**. Chlorination of **15** ($SOCl_2$-DMF) afforded the quinazoline **16**. Scheme 3 outlines the synthesis of 4-anilino-[1,4]-dioxane quinazoline derivatives from **16**. The treatment of **16** with anilines in isopropyl alcohol furnished the corresponding **17a-d**. Removal of benzyl protecting group (CF_3CO_2H, 100 °C), followed by mesylation (MsCl, Et_3N, 0 °C) and displace-



Scheme 1



Scheme 2. (a) DIAD, TPP/benzene, r.t, 95% (b) 60% HNO_3, AcOH, 50 °C; (c) NaH, BnBr/THF, 0 °C, 92%; (d) EtONa/ EtOH, r.t, 91%; (e) Fe, NH_4Cl/MeOH-H_2O, reflux, 89%; (f) $HCONH_2$, ammonium carbonate, 180 °C, 88%; (g) $SOCl_2$-DMF, 94%



Scheme 3. (a) anilines, IPA, reflux, 68-82%; (b) BBr_3, reflux, 75-95%; (c) (i) MsCl, Et_3N, 0 °C; (ii) amines, KI, 80 °C.

Table 2.

Compounds	R_1	R_2	IC_{50} (nM)	Compounds	R_1	R_2	IC_{50} (nM)
3a	4-Br, 2-F	*N*-methylpiperazine	120	**3k**	3-Cl	morpholine	22
3b	4-Br, 2-F	morpholine	99	**3l**	3-Cl	piperidine	58
3c	4-Br, 2-F	piperidine	122	**3m**	3-Cl	diethylamine	66
3d	4-Br, 2-F	diethylamine	120	**3n**	3-Cl	pyrrolidine	70
3e	3-Br	*N*-methylpiperazine	36	**3o**	3-Cl, 4-F	*N*-methylpiperazine	72
3f	3-Br	morpholine	42	**3p**	3-Cl, 4-F	morpholine	66
3g	3-Br	piperidine	52	**3q**	3-Cl, 4-F	piperidine	66
3h	3-Br	diethanolamine	100	**18a**	3-Cl	OH	52
3i	3-Br	pyrrolidine	62	**18b**	4-Br, 2-F	OH	120
3j	3-Cl	*N*-methylpiperazine	64	ZD-1839			56

ment of mesylate by various amines afforded the compound **3a-q**.

The compounds were tested for their *in vitro* inhibitory activity using the Alpha Screen™ P-Tyr-100 assay kit against EGF kinase. The results are given in IC_{50} values as shown in Table 2. In this assay, ZD-1839 was used as a reference and showed a potent inhibitory activity (IC_{50} = 56 nM) in our system.

Table 2 shows compounds derived by combination between 4 different anilines and various amine derivatives at R_2. Activity of compounds largely depends on variation of the C-4 anilines, however, varying amine derivatives at R_2 showed a minimal effect for *in vitro* activity. Compounds (**3a-d**) with 4-bromo-2-fluoro aniline were 2-fold less potent than ZD-1839. On the other hand, other anilines (*e.g.*, 3-bromo, 3-chloro, and 3-chloro-4-fluoro anilines) showed comparable enzyme activities, having IC_{50} values ranging from 22 to 72 nM, to that of ZD-1839, except for **3h**. Of those compounds, **3e** and **3k** were among the most potent in EGF enzyme assay, IC_{50}'s of 36 nM and 22 nM, respectively. It is interesting that when the amino group at R_2 was replaced with the hydroxy (**18a**), the activity was comparable to ZD-1839 in enzyme assay.

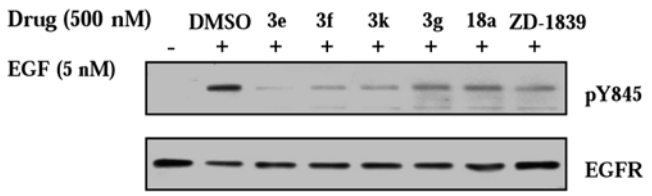


Figure 1. Effect of selective inhibitors on EGFR phosphorylation in A431 cells. Cells were harvested 10 min after addition of EGF (5 nM). Cells were treated with 500 nM of each compounds as indicated, 45 min before EGF addition. Equal amounts of protein were loaded in each lane. Blots were first probed with antibody against p-EGFR and then stripped, reprobed with antibody against total EGFR.

For inhibition studies of EGFR autophosphorylation[11] for selective inhibitors, A431cells were incubated with **3e**, **3f**, **3k**, **3g**, and **18a** at 500 nM concentration and then stimulated with EGF. Each compound considerably inhibited autophosphorylation of EGFR in A431 cells, as shown by Western blot analysis (Figure 1).

For inhibition studies of EGFR autophosphorylation[12] for selective inhibitors, A431cells were incubated with **3e**, **3f**, **3k**, **3g**, and **18a** at 500 nM concentration and then stimulated with EGF. Each compound considerably inhibited autophosphorylation of EGFR in A431 cells, as shown by Western blot analysis (Figure 1). The compounds **3e** and **3f** with 3-bromo aniline are much more potent than ZD-1839.

On the other hand, **3k**, having the most potent enzyme activity in these series, resulted in a similar inhibitory activity in autophosphorylation of EGFR compared to ZD-1839. Interestingly, the OH compound (**18a**) at R_2 retained their activity as shown in enzyme assay.

In summary, a new series of potent EGFR kinase inhibitors has been synthesized. Regioselective alkylation of catechol with epoxide using CsF as base can be rapidly accessable to [1,4]-dioxine intermediate, which was readily converted to [1,4]-dioxino quinazoline structure. The compounds **3f** and **3k** were more potent than ZD-1839 in EGF enzyme and EGFR autophosporylation inhibition assays.

Experimental Sections

4-(4-Benzyloxy-2-hydroxybutoxy)-3-hydroxybenzoic acid ethyl ester (6): To a solution of ethyl 3,4-dihydroxybenzoate **4** (7.5 g, 41 mmol) and CsF (0.14 g, 0.9 mmol) in DMF (100 mL) was added 2-(2-benzyloxyethyl)oxirane **5** (7.3 g, 41 mmol), and the reaction mixture was stirred for 7 h at 130 °C. After additional amount of 2-(2-benzyloxyethyl)oxirane **5** (7.3 g, 41 mmol) was added, the resulting mixture was stirred for 10 h at 130 °C. Ethyl acetate and water were added and the organic layer was separated. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine and dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (3 : 1 hexane-EtOAc) to afford an inseparable mixture of **6** and **7** (7.1g, 62%, 7 : 1), along with a small amount of dialkylated product (< 5%). Spectral data for **6**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.59 (d, J = 2.1 Hz, 1H), 7.54 (dd, J = 8.3, 2.1 Hz, 1H), 7.25-7.37 (m, 5H), 6.82 (d, J = 8.3 Hz, 1H), 4.54 (s, 2H), 4.32 (q, J = 7.2 Hz, 2H), 4.08-3.92 (m, 3H), 3.76-3.69 (m, 2H), 1.90-1.76 (m, 2H), 1.37 (t, J = 7.2 Hz, 3H); MS-ESI 360 (M^+, 1), 179 (5), 137 (6), 91 (100).

3-(2-Benzyloxyethyl)-2,3-dihydrobenzo[1,4]dioxine-6-carboxylic acid ethyl ester (10): To a solution of a crude mixture of **6** (8.5 g, 23.5 mmol) in benzene (200 mL) were added diisopropyldiazodicarboxylate (DIAD, 4.1 mL, 25.9 mmol) and triphenylphosphine (6.8 g, 25.9 mmol), and the resulting mixture was stirred for 4 h at room temperature. The mixture was concentrated, and the residue was purified by column chromatography (5 : 1 hexane-EtOAc) to give an inseparable benzodioxine mixture (7.9 g, 98%). Spectral data for **10**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.57-7.54 (m, 2H), 7.34-7.25 (m, 5H), 6.87 (d, J = 9.0 Hz, 1H), 4.53 (s, 2H), 4.31 (q, J = 6.9 Hz, 2H), 3.97 (dd, J = 11.6, 7.8 Hz, 1H), 3.72-3.64 (m, 2H), 1.98 (m, 2H), 1.37 (t, J = 6.9 Hz, 3H); MS-ESI 342 (M^+, 6), 161 (4), 91 (100).

3-(2-Benzyloxyethyl)-7-nitro-2,3-dihydrobenzo[1,4]dioxine-6-carboxylic acid (11): To a solution of benzodioxine **10** (4.2 g, 11.7 mmol) in acetic acid (30 mL) was added nitric acid (fuming, 10 mL) and the mixture was stirred for 3 h at 50 °C. After cooling, the reaction mixture was poured into ice water and extracted with EtOAc (2 × 50 mL). The combined extracts were washed with water and aqueous saturated $NaHCO_3$ solution, followed by brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (3 : 1 hexane-EtOAc) to give the benzyl ether **11** (38%), along with two side products **12** (16%) and **13** (15%). Spectral data for **11**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.32-7.25 (m, 5H), 7.13 (s, 1H), 4.53 (s, 2H), 4.43-4.31 (m, 2H), 4.33 (q, J = 7.1 Hz, 2H), 4.00-3.94 (m, 1H), 3.70-3.64 (m, 2H), 1.95 (m, 1H), 1.33 (t, J = 7.1 Hz, 3H). Transformations of **12** and **13** to **11** were followed as described below. To a solution of the acetate **13** in EtOH (10 mL) was added cat. NaOEt, and the reaction mixture was stirred for 2 h at room temperature. After evaporating the solvent, the residue was dissolved in EtOAc, washed with brine, dried over $MgSO_4$. Concentration and purification by column chromatography (3 : 1 hexane-EtOAc) gave the alcohol **12** (91%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.15 (s, 1H), 4.47-4.39 (m, 2H), 4.33 (q, J = 7.1 Hz, 2H), 4.02 (dd, J = 11.6, 7.7 Hz, 1H), 3.90-3.85 (m, 2H), 2.36 (brs, 1H), 1.91 (m, 2H), 1.34 (t, J = 7.1 Hz, 3H); MS-ESI 297 (M^+, 10), 153 (8), 71 (92), 43 (100). To a solution of the alcohol **12** (1.0g, 3.36 mmol) in THF (30 mL) were added benzyl bromide (0.48 mL, 4.03 mmol) and tetrabutylammonium iodide (0.12 g, 0.33 mmol). After 60% NaH (0.16 g, 4.04 mmol) was added slowly at 0 °C, the reaction mixture was stirred for 1 h at 0 °C. The solution was quenched with addition of EtOH and diluted with EtOAc. The solution was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified with column chromatography (3 : 1 hexane-EtOAc) to give the nitrobenzodioxine **11** (1.18 g, 91%).

7-Amino-3-(2-benzyloxyethyl)-2,3-dihydrobenzo[1,4]-dioxine-6-carboxylic acid ethyl ester (14): A suspension of NH_4Cl (4 g, 73.8 mmol) and Fe (2.5 g, 44.8 mmol) in MeOH : H_2O (2 : 1, 60 mL) was heated at reflux for 15 min. The nitrobenzodioxine **11** (6.2 g, 16.0 mmol) was added, and the resulting mixture was heated at reflux for 3 h. After cooling, the solid material was filtered and the filtrate was extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated to give the aminobenzodioxine (4.7 g, 82%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.4-7.25 (m, 6H), 6.13 (s, 1H), 4.52 (s, 2H), 4.40-4.1 (m, 5H), 3.89-3.7 1(m, 2H), 1.97-1.86 (m, 2H), 1.39-1.3 (m, 3H); MS-ESI 357 (M^+, 52), 196 (32), 167 (13), 91 (100).

7-(2-Benzyloxyethyl)-7,8-dihydro-3H-[1,4]dioxino[2,3-g]quinazolin-4-one (15): A solution of the aminobenzodioxine **14** (2.2 g, 6.2 mmol) and ammonium carbonate (0.99 g, 9.8 mmol) in formamide (25 mL) was heated at reflux for 3h. After cooling, water was added and the precipitate was filtered, washed with water, and dried *in vacuo* to give the dioxinoquinazoline **15** (1.5 g, 72%): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.83 (brs, 1H), 7.95 (s, 1H), 7.45 (s, 1H), 7.35-7.26 (m, 5H), 7.09 (s, 1H), 4.51 (s, 2H), 4.49-4.37 (m, 2H), 4.08 (dd, J = 11.7, 7.7 Hz, 1H), 3.65 (m, 2H), 1.94 (m, 2H); MS-ESI 338 (M^+, 6), 229 (3), 202 (2), 174 (1), 91 (100).

7-(2-Benzyloxyethyl)-4-chloro-7,8-dihydro-[1,4]dioxino-

[2,3-g]quinazoline (16): A solution of the quinazolin-4-one **15** (0.9 g, 2.7 mmol) and DMF (2 drops) in $SOCl_2$ (20 mL) was heated at reflux for 2 h. After cooling, the solvent was evaporated and the residue was dissolved with CH_2Cl_2, washed with aqueous sat. $NaHCO_3$ solution (3 × 15 mL), dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (3 : 1 hexane-EtOAc) to give the 4-chloroquinazoline **16** (0.7g, 74%): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.85 (s, 1H), 7.51 (s, 1H), 7.34-7.26 (m, 6H), 4.55-4.42 (m, 2H), 4.50 (s, 2H), 4.14 (d, J = 11.7, 7.7 Hz, 1H), 3.68-3.61 (m, 2H), 1.99-1.90 (m, 2H); MS-ESI 356 (M^+, 2), 338 (15), 174 (11), 91 (100).

[7-(2-Benzyloxyethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]-quinazolin-4-yl]-(3-chlorophenyl)-amine (17a): A solution of the 4-quinazoline **16** (50 mg, 0.14 mmol) and 3-chlorophenylamine (0.018 mL, 0.17 mmol) in 2-propanol (2 mL) was heated at reflux for 3 h. After cooling, the precipitate was filtered, washed with ether, and dried to give the 4-anilinoquinazoline **17a** (42 mg, 68%): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.0 (brs, 1H), 8.88 (s, 1H), 8.38 (s, 1H), 7.94 (brs, 1H), 7.74 (d, J = 9.9 Hz, 1H), 7.52 (t, J = 8.1 Hz, 1H), 7.40-7.28 (m, 7H), 4.63 (m, 2H), 4.54 (s, 2H), 4.24 (dd, J = 11.7, 7.8 Hz, 1H), 3.68 (m, 2H), 2.07-1.97 (m, 2H); MS-ESI 447 (M^+, 9), 354 (1), 104 (2), 91 (100).

[7-(2-Benzyloxyethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]-quinazolin-4-yl]-(3-bromophenyl)-amine (17b): Yield: 71%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.9 (brs, 1H), 8.87 (s, 1H), 8.36 (s, 1H), 8.07 (s, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.51-7.27 (m, 8H), 4.63 (m, 2H), 4.53 (s, 2H), 4.23 (dd, J = 11.7, 7.8 Hz, 1H), 3.71-3.67 (m, 2H), 2.06-1.96 (m, 2H); MS-ESI 492 (M^+, 0.2), 340 (2), 291 (12), 220 (10), 164 (100).

[7-(2-Benzyloxyethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]-quinazolin-4-yl]-(3-chloro-4-fluorophenyl)-amine (17c): Yield: 82%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.0 (brs, 1H), 8.88 (s, 1H), 8.35 (s, 1H), 8.06 (dd, J = 6.6, 2.7 Hz, 1H), 7.74 (m, 1H), 7.54 (t, J = 9.0 Hz, 1H), 7.39-7.28 (m, 6H), 4.59 (m, 2H), 4.50 (s, 2H), 4.20 (dd, J = 11.7, 7.8 Hz, 1H), 3.66 (m, 2H), 2.05-1.91 (m, 2H); MS-ESI 465 (M^+, 6), 274 (1), 157 (2), 91 (100).

[7-(2-Benzyloxyethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]-quinazolin-4-yl]-(2-bromo-4-fluorophenyl)-amine (17d): Yield: 68%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.3 (brs, 1H), 8.79 (s, 1H), 8.31 (s, 1H), 7.77 (d, J = 9.9 Hz, 1H), 7.49-7.40 (m, 2H), 7.31 (m, 5H), 7.29 (m, 1H), 4.59 (m, 2H), 4.53 (s, 2H), 4.25 (dd, J = 11.7, 7.8 Hz, 1H), 3.71-3.63 (m, 2H), 2.05-1.95 (m, 2H); MS-ESI 510 (M^+, 3), 430 (5), 269 (7), 131 (4), 91 (100).

2-[4-(3-Chlorophenylamino)-7,8-dihydro-[1,4]dioxino-[2,3-g]quinazolin-7-yl]-ethanol (18a): To a solution of the 4-anilinoquinazoline **17a** (0.42 g, 0.94 mmol) in CH_2Cl_2 (20 mL) was added 1 N-BBr_3 (1.4 mL) at −30 °C and the mixture was stirred for 2 h at the same temperature. The reaction mixture was quenched with addition of MeOH, and concentrated *in vacuo*. The residue was partitioned with CH_2Cl_2 and 2 N-NaOH solution, and the organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated to give the alcohol **18a** (0.3 g, 75%): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.5 (brs, 1H), 8.50 (s, 1H), 8.15 (s, 1H), 7.88 (d, J = 7.3 Hz, 1H), 7.40-7.36 (m, 2H), 7.14 (d, J = 7.5 Hz, 1H), 7.29 (m, 1H), 4.78 (m, 1H), 4.52-4.48 (m, 2H), 4.24 (dd, J = 11.7, 7.8 Hz, 1H), 3.66-3.63 (m, 2H), 1.86-1.82 (m, 2H).

2-[4-(3-Bromophenylamino)-7,8-dihydro-[1,4]dioxino-[2,3-g]quinazolin-7-yl]-ethanol (18b): Yield: 81%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.61 (brs, 1H), 8.57 (s, 1H), 8.33 (s, 1H), 8.17 (s, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.56-7.26 (m, 3H), 4.79 (m, 1H), 4.52-4.48 (m, 2H), 4.24 (dd, J = 11.7, 7.8 Hz, 1H), 3.66-3.63 (m, 2H), 1.86-1.82 (m, 2H); MS-ESI 402 (M^+, 100), 275 (38), 251 (47), 193 (42), 114 (29), 43 (4).

2-[4-(3-Chloro-4-fluorophenylamino)-7,8-dihydro-[1,4]-dioxino[2,3-g]quinazolin-7-yl]-ethanol (18c): Yield: 67%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.62 (brs, 1H), 8.55 (s, 1H), 8.30 (dd, J = 6.9, 2.4 Hz, 1H), 8.11 (s, 1H), 7.92 (m, 1H), 7.49 (t, J = 9.1 Hz, 1H), 7.27 (s, 1H), 4.79 (m, 1H), 4.49-4.59 (m, 2H), 4.17 (dd, J = 11.4, 7.9 Hz, 1H), 3.71 (m, 2H), 1.88 (q, J = 6.3 Hz, 2H); MS-ESI 375 (M^+, 99), 374 (100), 44 (34).

2-[4-(4-Bromo-2-fluorophenylamino)-7,8-dihydro[1,4]-dioxino[2,3-g]quinazolin-7-yl]-ethanol (18d): Yield: 75%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.58 (brs, 1H), 8.39 (s, 1H), 7.96 (s, 1H), 7.63 (dd, J = 9.9, 1.3 Hz, 1H), 7.44-7.54 (m, 2H), 7.20 (s, 1H), 4.71 (m, 1H), 4.51-4.53 (m, 2H), 4.12 (dd, J = 11.4, 7.8 Hz, 1H), 3.65 (m, 2H), 1.81 (q, J = 6.3 Hz, 2H); MS-ESI 420 (M^+, 55), 403 (46), 269 (88), 103 (39), 91 (76), 71 (81), 43 (100).

(4-Bromo-2-fluorophenyl)-{7-[2-(4-methyl-piperazin-1-yl)-ethyl]-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl}-amine(3a): To a solution of 2-[4-(4-bromo-2-fluoro-phenylamino)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-7-yl]-ethanol (0.11 g, 0.26 mmol) in CH_2Cl_2 (10 mL) were added Et_3N (0.17 mL, 1.12 mmol) and MsCl (0.043 mL, 0.56 mmol) at 0 °C and the mixture was stirred for 1 h. at room temperature. The reaction mixture was diluted with EtOAc, and washed with 0.5 N-NaOH and brine. The organic layer was dried over Na_2SO_4 and concentrated to give a mesylate (0.12 g, 93%) as a yellow solid, which was used for next reaction without further purification. A mixture of methanesulfonic acid 2-[4-(4-bromo-2-fluoro-phenylamino)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-7-yl]-ethyl ester (55 mg, 0.13 mmol), 1-methyl-piperazine (1 mL), and a catalytic amount of NaI was stirred at 80 °C for overnight. The mixture was diluted with EtOAc, and washed with sat. $NaHCO_3$ solution and brine, and dried over Na_2SO_4 and concentrated to give **3a**: Yield: 55%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.50 (brs, 1H), 8.32 (s, 1H), 7.95 (s, 1H), 7.62 (d, J = 9.9 Hz, 1H), 7.50 (m, 1H), 7.40-7.26 (m, 2H), 7.01 (s, 1H), 4.51 (d, J = 10.5 Hz, 1H), 4.44 (m, 1H), 4.11 (dd, J = 11.4, 7.0 Hz, 1H), 2.48 (m, 8H), 2.37 (m, 2H), 2.24 (s, 3H), 1.83 (m, 2H).

(4-Bromo-2-fluorophenyl)-[7-(2-morpholin-4-yl-ethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl]-amine (3b): Yield: 56%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.51 (brs, 1H), 8.31 (s, 1H), 7.95 (s, 1H), 7.62 (d, J = 9.8 Hz, 1H),

7.51 (m, 1H), 7.42-7.26 (m, 2H), 7.02 (s, 1H), 4.51 (d, J = 10.5 Hz, 1H), 4.42 (m, 1H), 4.10 (dd, J = 11.3, 7.8 Hz, 1H), 3.56 (m, 4H), 2.47 (m, 2H), 2.35 (m, 4H), 1.82 (m, 2H).

(4-Bromo-2-fluorophenyl)-[7-(2-piperidin-4-yl-ethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl]-amine (3c): Yield: 49%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.50 (brs, 1H), 8.31 (s, 1H), 7.96 (s, 1H), 7.62 (d, J = 9.9 Hz, 1H), 7.53 (m, 1H), 7.41-7.27 (m, 2H), 7.02 (s, 1H), 4.52 (d, J = 10.5 Hz, 1H), 4.45 (m, 1H), 4.11 (dd, J = 11.3, 7.0 Hz, 1H), 2.36 (m, 2H), 2.28 (m, 4H), 1.83 (m, 2H), 1.72-1.50 (m, 6H).

(4-Bromo-2-fluorophenyl)-[7-(2-diethylaminoethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl]-amine (3d): Yield: 48%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.51 (brs, 1H), 8.31 (s, 1H), 7.96 (s, 1H), 7.63 (d, J = 9.9 Hz, 1H), 7.52 (m, 1H), 7.39-7.25 (m, 2H), 7.01 (s, 1H), 4.51 (d, J = 10.5 Hz, 1H), 4.41 (m, 1H), 4.10 (dd, J = 11.3, 7.9 Hz, 1H), 2.43 (q, J = 7.1 Hz, 4H), 2.34 (m, 2H), 1.82 (m, 2H), 0.99 (t, J = 7.1 Hz, 6H).

(3-Bromophenyl)-{7-[2-(4-methyl-piperazin-1-yl)-ethyl]-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl}-amine (3e): Yield: 42%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.50 (brs, 1H), 8.48 (s, 1H), 8.26 (s, 1H), 8.10 (s, 1H), 7.91 (d, J = 7.1 Hz, 1H), 7.34-7.16 (m, 3H), 4.50 (d, J = 10.3 Hz, 1H), 4.39 (m, 1H), 4.11 (dd, J = 11.4, 7.9 Hz, 1H), 2.47 (m, 8H), 2.37 (m, 2H), 2.25 (s, 3H), 1.82 (m, 2H); MS-ESI 484 (M^+, 16), 415 (16), 113 (100), 84 (72), 70 (99).

(3-Bromophenyl)-[7-(2-morpholin-4-yl-ethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl]-amine (3f): Yield: 61%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.51 (brs, 1H), 8.48 (s, 1H), 8.25 (s, 1H), 8.09 (s, 1H), 7.91 (d, J = 7.1 Hz, 1H), 7.34-7.17 (m, 3H), 4.51 (d, J = 10.5 Hz, 1H), 4.39 (m, 1H), 4.10 (dd, J = 11.4, 7.9 Hz, 1H), 3.56 (m, 4H), 2.48 (m, 2H), 2.37 (m, 4H), 1.83 (m, 2H).

(3-Bromophenyl)-[7-(2-piperidin-4-yl-ethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl]-amine (3g): Yield: 65%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.58 (brs, 1H), 8.50 (s, 1H), 8.26 (s, 1H), 8.11 (s, 1H), 7.94 (d, J = 7.1 Hz, 1H), 7.39-7.20 (m, 3H), 4.52 (d, J = 10.5 Hz, 1H), 4.48 (m, 1H), 4.11 (dd, J = 11.4, 7.9 Hz, 1H), 2.37 (m, 2H), 2.28 (m, 4H), 1.84 (m, 2H), 1.72-1.49 (m, 6H).

2-[{2-[4-(3-Bromophenylamino)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-7-yl]-ethyl}-(2-hydroxy-ethyl)-amino]-ethanol (3h): Yield: 56%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.53 (brs, 1H), 8.51 (s, 1H), 8.28 (s, 1H), 8.10 (s, 1H), 7.94 (d, J = 7.1Hz, 1H), 7.39-7.21 (m, 3H), 4.52 (d, J = 10.5 Hz, 1H), 4.48 (m, 1H), 4.11 (dd, J = 11.4, 7.9 Hz, 1H), 3.49 (m, 4H), 2.59 (m, 4H), 2.49 (m, 2H), 1.82 (m, 2H).

(3-Bromophenyl)-[7-(2-pyrrolidin-1-yl-ethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl]-amine (3i): Yield: 68%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.52 (brs, 1H), 8.50 (s, 1H), 8.27 (s, 1H), 8.11 (s, 1H), 7.94 (d, J = 7.1 Hz, 1H), 7.36-7.20 (m, 3H), 4.51 (d, J = 10.5 Hz, 1H), 4.42 (m, 1H), 4.11 (dd, J = 11.4, 7.9 Hz, 1H), 2.62 (m, 2H), 2.48 (m, 4H), 1.85 (m, 2H), 1.69 (m, 4H).

(3-Chlorophenyl)-{7-[2-(4-methyl-piperazin-1-yl)-ethyl]-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl}-amine (3j): Yield: 55%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.55 (brs, 1H), 8.51 (s, 1H), 8.16 (s, 1H), 8.11 (s, 1H), 7.80 (d, J = 7.1 Hz, 1H), 7.42-7.34 (m, 2H), 7.14 (d, J = 8.0 Hz, 1H), 4.53 (d, J = 10.5 Hz, 1H), 4.49 (m, 1H), 4.11 (dd, J = 11.4, 7.9 Hz, 1H), 2.47 (m, 8H), 2.37 (m, 2H), 2.25 (s, 3H), 1.82 (m, 2H).

(3-Chlorophenyl)-[7-(2-morpholin-4-yl-ethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl]-amine (3k): Yield: 56%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.53 (brs, 1H), 8.50 (s, 1H), 8.16 (s, 1H), 8.10 (s, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.36 (t, J = 8.0 Hz, 1H), 7.32 (m, 1H), 7.10 (d, J = 8.0 Hz, 1H), 4.53 (d, J = 10.5 Hz, 1H), 4.38 (m, 1H), 4.11 (dd, J = 11.3, 7.9 Hz, 1H), 3.59 (m, 4H), 2.39-2.33 (m, 6H), 1.83 (m, 2H).

(3-Chlorophenyl)-[7-(2-piperidin-4-yl-ethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl]-amine (3l): Yield: 57%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.54 (brs, 1H), 8.50 (s, 1H), 8.17 (s, 1H), 8.11 (s, 1H), 7.85 (d, J = 7.4 Hz, 1H), 7.37-7.32 (m, 2H), 7.12 (d, J = 8.0 Hz, 1H), 4.52 (d, J = 10.5 Hz, 1H), 4.48 (m, 1H), 4.10 (dd, J = 11.3, 7.9 Hz, 1H), 2.36 (m, 2H), 2.28 (m, 4H), 1.82 (m, 2H), 1.73-1.48 (m, 6H).

(3-Chlorophenyl)-[7-(2-diethylaminoethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl]-amine (3m): Yield: 56%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.54 (brs, 1H), 8.51 (s, 1H), 8.16 (s, 1H), 8.10 (s, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.36-7.32 (m, 2H), 7.10 (d, J = 8.0 Hz, 1H), 4.52 (d, J = 10.5 Hz, 1H), 4.38 (m, 1H), 4.11 (dd, J = 11.3, 7.9 Hz, 1H), 2.42 (q, J = 7.1 Hz, 4H), 2.36 (m, 2H), 1.83 (m, 2H), 0.96 (t, J = 7.1 Hz, 6H).

(3-Chlorophenyl)-[7-(2-pyrrolidin-1-yl-ethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl]-amine (3n): Yield: 68%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.53 (brs, 1H), 8.50 (s, 1H), 8.16 (s, 1H), 8.10 (s, 1H), 7.86 (d, J = 7.9 Hz, 1H), 7.37-7.33 (m, 2H), 7.10 (d, J = 7.9 Hz, 1H), 4.50 (d, J = 10.5 Hz, 1H), 4.43 (m, 1H), 4.10 (dd, J = 11.4, 7.9 Hz, 1H), 2.62 (m, 2H), 2.47 (m, 4H), 1.83 (m, 2H), 1.70 (m, 4H).

(3-Chloro-4-fluorophenyl)-{7-[2-(4-methyl-piperazin-1-yl)-ethyl]-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl}-amine (3o): Yield: 55%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.58 (brs, 1H), 8.49 (s, 1H), 8.25 (dd, J = 6.9, 2.6 Hz, 1H), 8.09 (s, 1H), 7.86 (m, 1H), 7.43 (t, J = 9.1 Hz, 1H), 7.21 (s, 1H), 4.52 (d, J = 10.6 Hz, 1H), 4.39 (m, 1H), 4.11 (dd, J = 11.4, 7.9 Hz, 1H), 2.49 (m, 8H), 2.37 (m, 2H), 2.24 (s, 3H), 1.86 (m, 2H); MS-ESI 458 (M^+, 19), 276 (21), 152 (20), 113 (89), 70 (52), 40 (100).

(3-Chloro-4-fluorophenyl)-[7-(2-morpholin-4-yl-ethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl]-amine (3p): Yield: 66%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.62 (brs, 1H), 8.50 (s, 1H), 8.24 (dd, J = 6.9, 2.6 Hz, 1H), 8.09 (s, 1H), 7.93 (m, 1H), 7.44 (t, J = 9.1 Hz, 1H), 7.27 (s, 1H), 4.58 (d, J = 10.5 Hz, 1H), 4.45 (m, 1H), 4.18 (dd, J = 11.3, 7.8 Hz, 1H), 3.65 (m, 4H), 2.56 (m, 2H), 2.46 (m, 4H), 1.92 (m, 2H).

(3-Chloro-4-fluorophenyl)-[7-(2-piperidin-4-yl-ethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-yl]-amine (3q): Yield: 62%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.62

(brs, 1H), 8.50 (s, 1H), 8.24 (dd, J = 6.9, 2.6 Hz, 1H), 8.09 (s, 1H), 7.93 (m, 1H), 7.44 (t, J = 9.1 Hz, 1H), 7.27 (s, 1H), 4.52 (d, J = 11.0 Hz, 1H), 4.48 (m, 1H), 4.14 (dd, J = 11.4, 6.9 Hz, 1H), 2.36 (m, 2H), 2.28 (m, 4H), 1.83 (m, 2H), 1.72-1.50 (m, 6H).

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