Synthesis and Biological Evaluation of Bis(methoxy-methyl)-7,8-dihydro-[1,4]dioxino[2,3-*g*]quinazolines as EGFR Tyrosine Kinase Inhibitors

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A series of 7,8-bis(methoxymethyl)-7,8-dihydro-[1,4]dioxino[2,3-*g*]quinazolines were prepared and evaluated for their inhibition of phosphorylation of the isolated epidermal growth factor receptor (EGFR) enzyme and for their growth inhibition of the A431 cell line. Among them, compound **3c** having a 3-iodophenyl ring was most potent ($IC_{50} = 1.66$ nM) against the isolated EGFR enzyme and also showed meaningful potency ($GI_{50} = 1.99$ μM) against the A431 cell line, although less than PD153035 ($GI_{50} = 1.03$ μM). However, compound **3e** as the exact rigidified analogue of Erlotinib (Tarceva™) was inferior to the original compound when compared to its reported data.

Introduction

The epidermal growth factor receptor (EGFR) plays a central role in growth signaling and regulation of mammalian cells [1]. Like other protein kinases, it mainly transduces signals to a target cell by catalyzing the transfer of the terminal phosphate of ATP to tyrosine residues in protein substrates. Overexpression of EGFR has been implicated in a significant proportion of human tumors and other proliferative diseases [2]. EGFR is therefore expected to be an attractive target for cancer chemotherapy. Due to the relative conservation of the ATP binding site of protein kinases, the development of selective inhibitors of protein kinases of the EGFR has been an important concern. As a result of extensive researches, 4-anilinoquinazoline (PD153035) has recently proven to be a potent and selective inhibitor of tyrosine kinase activity of EGFR *via* competitive binding at the ATP site of the enzyme [3]. Since then, various 4-anilinoquinazoline derivatives have been synthesized [4−8]. We have recently reported that the 4-anilino-[1,4]dioxino[2,3-*g*]quinazoline (**1**, **2**) ring could be considered as a new scaffold for EFGR tyrosine kinase inhibitors when considered with regard to the inhibitory activity against both the EGFR kinase and the growth of human tumor cell lines (Figure 1) [9−10]. Among them, ZD1839 (Iressa™) and Erlotinib (Traceva™) were found to be effective for lung cancer patients and approved for clinical use in 2002 and 2004, respectively (Figure 1) [11−13].

In further development of this class, herein, we wish to report the synthesis and biological activity of a series of 7,8-bis(methoxymethyl)-7,8-dihydro-[1,4]dioxino[2,3-*g*]-quinazolines (**3a−c** and **3e**) as the rigidified analogues of Erlotinib (Traceva™), with the aim of studying the effect of rigidification of the original structure on the biological activity.

Results and discussion

Our synthetic tactic was to prepare a key intermediate having C_2-symmetry, 2,3-dihydro-2,3-bis(methoxymethyl)-benzo[1,4]dioxane (**10**), starting from diethyl *L*-tartarate (**4**) as shown in Scheme 1. Using this key intermediate (**10**), the 7,8-bis(methoxymethyl)-7,8-dihydro-[1,4]dioxino[2,3-*g*]-quinazoline ring (**14**) was easily prepared by the known procedures, as shown in Scheme 2 [9−10, 14−16].

First, the dihydroxyl groups of diethyl *L*-tartarate (**4**) were protected with dimethoxypropane (DMP) and *p*-TsOH to provide an acetonide **5**, which was reduced with LiAlH₄ to afford 1,2-bis(hydroxymethyl)acetonide (**6**). The dimethylation of compound **6** with CH₃I/NaH and the following deprotection of the acetonide group of compound **7** with HCl/MeOH provided *trans*-3,4-dihydroxy-1,2-bis(methoxymethyl)butane **8**. The tosylation of the dihydroxyl groups and the subsequent substitution with cathecol under basic

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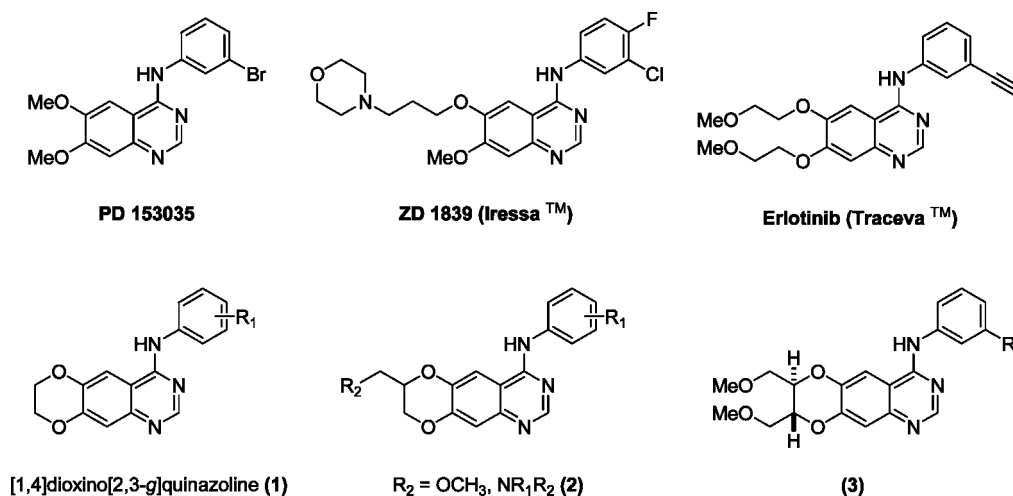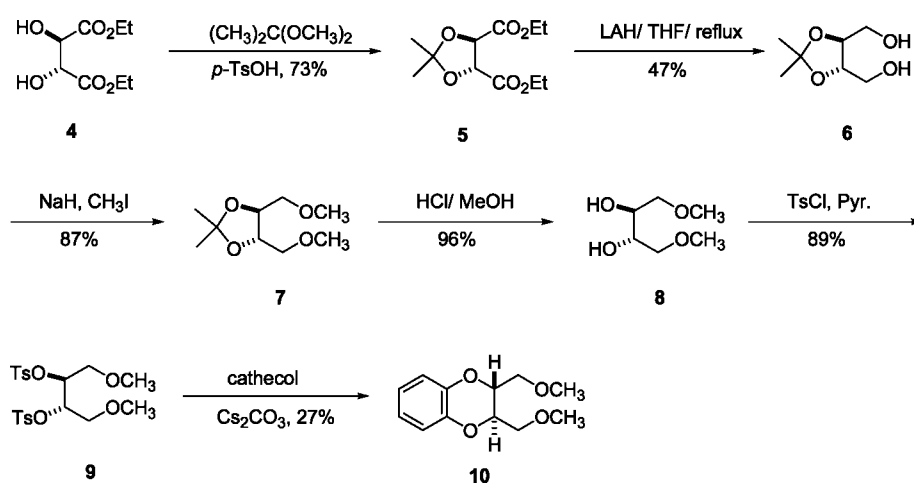
Figure 1. EGFR tyrosine kinase inhibitors.

Scheme 1. Synthesis tactic of key intermediate 2,3-dihydro-2,3-bis(methoxymethyl)-benzo[1,4]dioxane (**10**).

conditions (Cs$_2$CO$_3$) gave key intermediate **10**, as depicted in Scheme 1.

Secondly, Friedel-Crafts acylation of compound **10** with AcCl/AlCl$_3$, oxidation with NaOCl and nitration and reduction provided a [1,4]dioxino-fused anthranilic acid (**13**) as a precursor for cyclization. The cyclization of **13** with triazine was conducted in the presence of piperazine to afford 4-hydroxyquinazoline (**14**), which was easily converted into 4-chloroquinazoline (**15**) with POCl$_3$, quantitatively. The coupling reactions between 4-chloroquinazoline (**15**) and appropriate anilines in *iso*-propanol under reflux were finally conducted to provide 4-anilino-7,8-bis(methoxymethyl)-7,8-dihydro-[1,4]dioxino[2,3-*g*]quinazolines (**3a**−**c**)

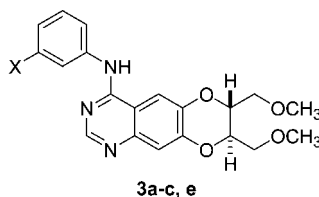in good yields. The structures and yields of these compounds are summarized in Table 1.

Finally, for the effect of the rigidification of the original structure on the biological activity, the rigidified analogue **3e** of Erlotinib (Traceva™) was easily prepared *via* the coupling reaction of **3c** with TMS-acetylene using Pd catalyst and the deprotection of the TMS group in resultant compound **3d** under basic conditions (K$_2$CO$_3$, MeOH), as shown in Scheme 2 [17−18].

The compounds (**3a**−**c**) shown in Table 1 were evaluated for their ability to inhibit the phosphorylation of tyrosine kinase substrate 2 by EGFR and the growth of the A431

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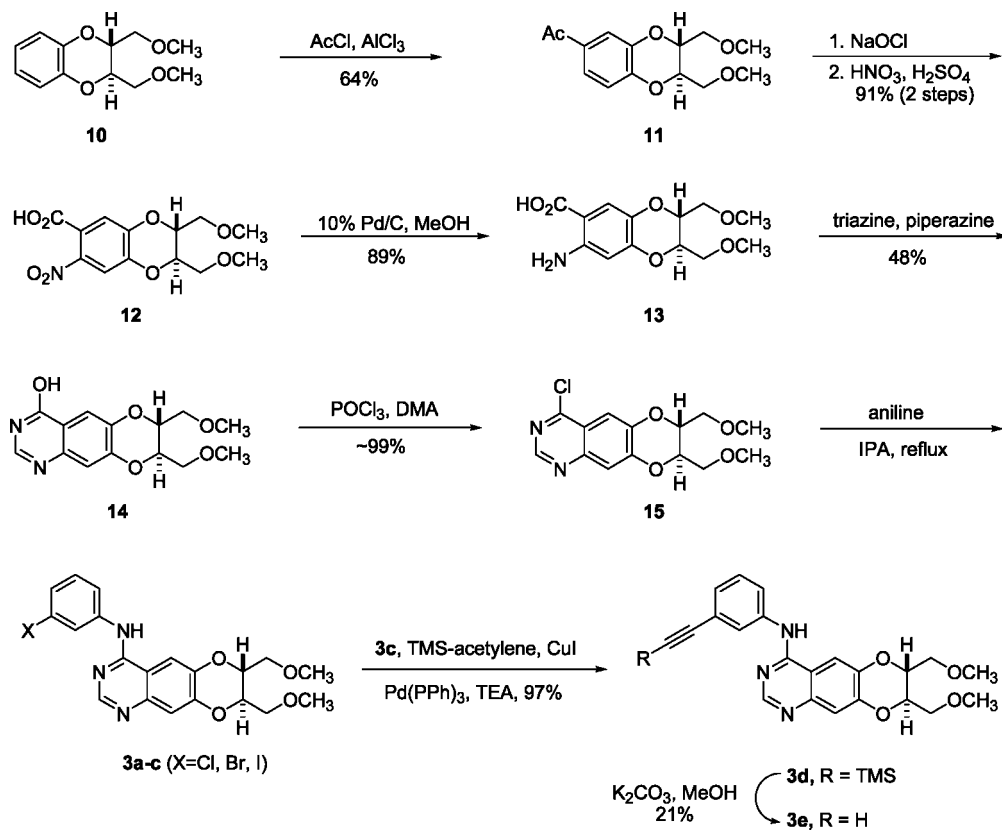
Scheme 2. Synthesis of the 7,8-bis(methoxymethyl)-7,8-dihydro-[1,4]dioxino[2,3-*g*]quinazoline ring (**14**) using intermediate **10**.

Table 1. EGFR kinase inhibitory properties and cancer cell line growth inhibition assay results.

3a-c, e

Entry	X	Yield (%)	EGFR assay CI$_{50}$, nM[†]	Cell-based assay GI$_{50}$, μM[‡] A431	HCT116	SNU638
3a	Cl	71	10.08	2.58	10.37	5.25
3b	Br	71	28.89	2.63	17.79	16.98
3c	I	70	1.66	1.99	6.66	11.51
PD153035[§]	–		6.10	1.03	19.50	11.48
Erlotinib	–		3.69[d]	1.55[#]	–	–

[†] Concentration to inhibit by 50% the phosphorylation of the tyrosine kinase substrate 2 by EGFR, as determined from the dose-response curve.

[‡] GI$_{50}$, concentration needed to inhibit cell growth by 50%, as determined from the dose-response curve.

[§] The standard compound was made for comparison of activities and the value was determined by using our assay protocol.

[#] [18].

cell line, which highly overexpresses EGFR. For comparison, the inhibitory activity of PD153035 under our assay conditions was inserted into the data set, and the biological data of Erlotinib (Traceva^{TM}) from the previous literature were also inserted into Table 1 for indirect comparison [18].

Among the 4-haloanilinoquinazolines, at first, 4-iodoanilinoquinazoline (**3c**) showed better potencies (CI_{50} = 1.66 nM) and the other two compounds (**3a** and **3b**) showed less inhibitory activities (IC_{50} = 10.08 and 28.89 nM, respectively) than both PD153035 and Erlotinib (CI_{50} = 6.10 and 3.69 nM, respectively) against the isolated enzyme. Against the growth of the A431 cell line, all 4-haloanilinoquinazolines (**3a–c**) showed relatively potent growth inhibition (GI_{50} = 1.99–2.63 μM), whereby 4-iodoanilinoquinazoline (**3c**, GI_{50} = 1.99 μM) appears to be the best inhibitor and was as potent as Erlotinib (GI_{50} = 1.55 μM) although less potent than PD153035 (GI_{50} = 1.03 μM). However, the 4-ethynylanilino compound (**3e**), the rigidified analogue of Erlotinib, showed less potency (GI_{50} = 4.74 μM) against the growth of the A431 cell line. It was, however, not tested against the isolated enzyme.

All 4-haloanilinoquinazolines (**3a–c**) were also evaluated for their ability to inhibit two other human cancer cell lines, for their selectivity: HCT116 (colon cancer) and SNU638 (stomach cancer), which express low levels of EGFR; the data was also inserted in Table 1. With respect to the cell assays, it is evident that each compound is a better inhibitor of the A431 than the HCT116 and SNU638 cell lines, as shown in Table 1. This is consistent with the mechanism of growth inhibition being largely due to the targeting of the EGFR. Of all compounds tested, 4-bromoanilinoquinazoline (**3b**) showed better selectivity (more than six-fold) for A431 than the two other compounds (**3a** and **3c**), while it is a less potent inhibitor against the isolated EGFR enzyme.

In conclusion, we prepared the 7,8-bis(methoxymethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolines (**3a–c**) and the rigidified analogue (**3e**) of Erlotinib (Traceva^{TM}) to probe the effect of rigidification of substituents on the inhibition of EGFR tyrosine kinase and on the growth inhibition of three human tumor cell lines. Among them, 4-iodoanilinoquinazoline (**3c**) was most potent against the isolated enzyme and showed potent antitumor activity nearly comparable to that of Erlotinib, but the rigidified analogue (**3e**) was found to be less potent than expected. This result implicates that the rigidification of substituents of Erlotinib (Traceva^{TM}) resulted in the decreased effect on the growth inhibition of the tumor cell lines.

Acknowledgments

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