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Synthesis and biological evaluation of benzamides and benzamidines: structural requirement of a pyrimidine ring for inhibition of EGFR tyrosine kinase

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Abstract—The benzamides **1** and the benzamidines **2**–**3** were synthesized as the mimics of 4-anilinoquinazolines, which possess inhibition of epidermal growth factor receptor (EGFR) tyrosine kinase, and tested for cytotoxicity toward A431 and inhibitory activity toward autophosphorylation by the enzyme assay. High cell growth inhibition was observed in a series of the cyclic benzamides **3**: the IC_{50} values are 0.09–0.32 mM. The benzamidines **3a** and **3b** exhibited high inhibition of EGFR tyrosine kinase at a 1.0 μM concentration, although the benzamides **1** and the benzamidines **2** did not show significant kinase inhibition at a 10 μM concentration.

Since the discovery of the 4-anilinoquinazoline (PD 153035), which possesses a specific inhibitory activity toward the epidermal growth factor receptor (EGFR) tyrosine kinase,[1,2] various 4-anilinoquinazoline derivatives have been synthesized.[3] Among them, ZD1839 (Iressa™) was found to be effective for lung cancer patients and approved for the clinical use in 2002.[4] Furthermore, inhibitors of vascular endothelial growth factor receptor tyrosine kinases have also been observed in 4-anilinoquinazolines.[5] According to the crystal structure of the EGFR kinase domain and in complex with erlotinib, the interplanar angle of quinazoline and aniline rings in erlotinib is 42° and hydrogen bonding between the Met^{769} amide nitrogen of the kinase domain and the nitrogen (N1) of the quinazoline ring has been observed.[6] We thought that the flexibility of the quinazoline framework would be effective for the interaction between the kinase domain and inhibitors, and therefore designed the benzamides **1** and the benzamidines **2** as the mimics of 4-anilinoquinazolines, in which the formation of a pseudocycle[7] of the quinazoline ring through the intramolecular hydrogen bonding would be expected. Herein we report the synthesis and biological evaluation of the benzamides **1** and the benzamidines **2** as well as the protected benzamidines **3**.

The 4,5-dimethoxyanthranilamides **1a**–**f** were synthesized from 4,5-dimethoxyanthranilic acid **4** by two different pathways (Scheme 1). The EDCI-promoted amidation of the N-protected benzoic acid **4** with amines (R^1NH_2) afforded the amides **5**, and the deprotection of the Boc group with trifluoroacetic acid afforded the anthranilamides **1**, quantitatively. In an alternative pathway,[8] the ring opening of the carbamates **6**[9] with amines (R^1NH_2) afforded the anthranilamides **1**. The 2-pyridylamino-4,5-dimethoxyanthranilamides **1g**–**i** were synthesized from 4,5-dimethoxy 2-nitrobenzoic acid **7** (Scheme 2). The 2-nitrobenzoic acid **7** was treated with thionyl chloride followed by the amide formation with pyridylamines to give the corresponding 2-nitrobenzylamides **8g**–**i**. The reduction of **8** with tin(II) chloride in MeOH afforded **1g**–**i** in good yields.

The 2-amino-4,5-dimethoxyphenylamidines **2a**–**d** were synthesized from 2-amino-4,5-dimethoxybenzoic acid **4** (Scheme 3).[10] The dimethoxyanthranilamide **9** derived

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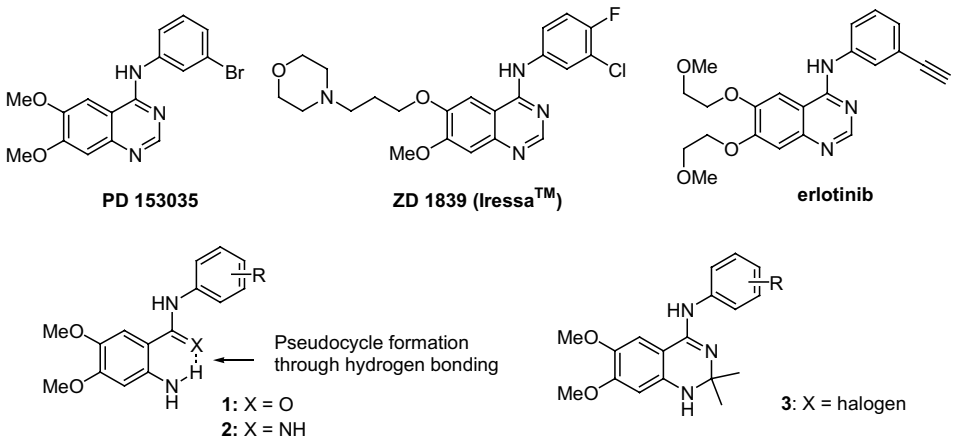
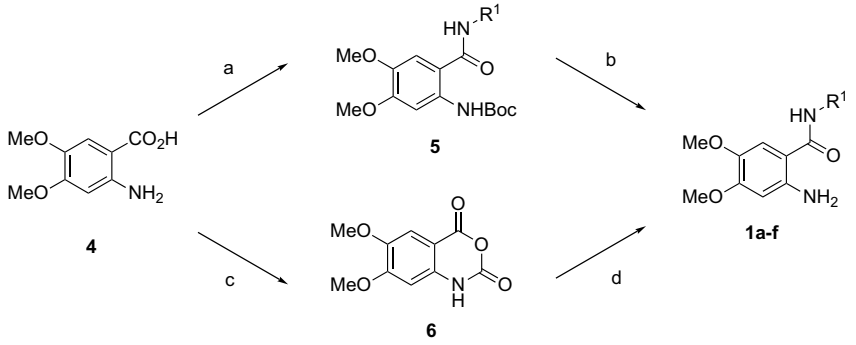
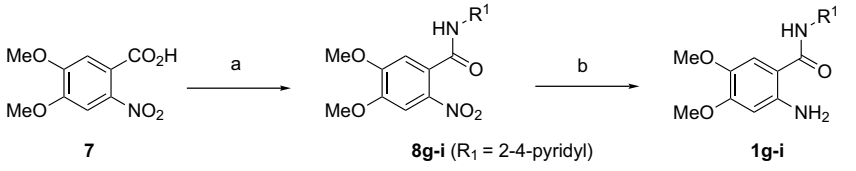


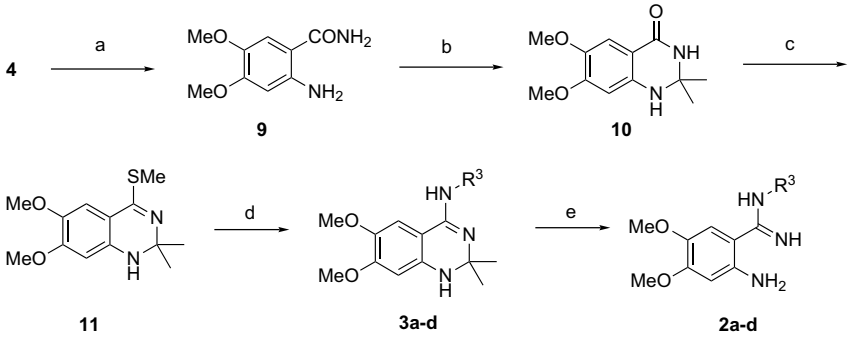
from **4** with ammonia by DCC coupling was converted to **10** in the presence of a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) in acetone, and **10** was treated with Lawesson's reagent followed by methylation with iodomethane afforded the thioamidate **11**. Displacement of methanethiol by amines gave the protected



Scheme 1. Reagents and conditions: (a) i. $(Boc)_2O$, NaOH, H_2O; ii R^1NH_2, EDCI, HOBt, DMF, 35–71%; (b) TFA, CH_2Cl_2, >99%; (c) i. ethyl chloroformate, THF; ii. PBr_3, Et_2O, >99%; (d) R^1NH_2, DMF, 100 °C, 10–80%.

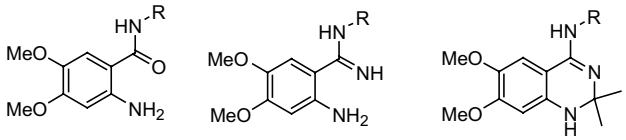


Scheme 2. Reagents and conditions: (a) i. $SOCl_2$, CH_2Cl_2; ii. pyridylamine, CH_2Cl_2, 10–67%; (b) $SnCl_2$, MeOH, 70–89%.

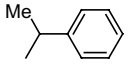
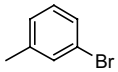
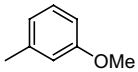
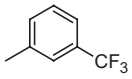
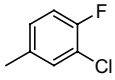
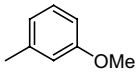
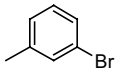
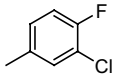
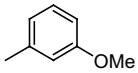
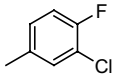


Scheme 3. Reagents and conditions: (a) NH_3, DCC, HOBt, THF, 97%; (b) acetone, *p*-TsOH, 71%; (c) i. Lawesson's reagent, 60%; ii. MeI, 98%; (d) amine, 110 °C, 26–56%; (e) HCl, reflux, 34–82%.

Table 1. Cytotoxicity of the benzamides **1a–i**, and the benzamidines **2a–d** and **3a–d** toward A431 cells



1 **2** **3**

	R	IC_{50} (mM)[a]		R	IC_{50} (mM)
1a		>1	**2a**		0.30
1b		>1	**2b**		0.46
1c		0.34	**2c**		0.34
1d		0.47	**2d**		0.20
1e		0.20	**3a**		0.13
1f		>1	**3b**		0.20
1g		>1	**3c**		0.32
1h		>1	**3d**		0.09
1i		>1			

[a] An IC_{50} of >1 indicates that no curve was noted in the dose response up to 1 mM.

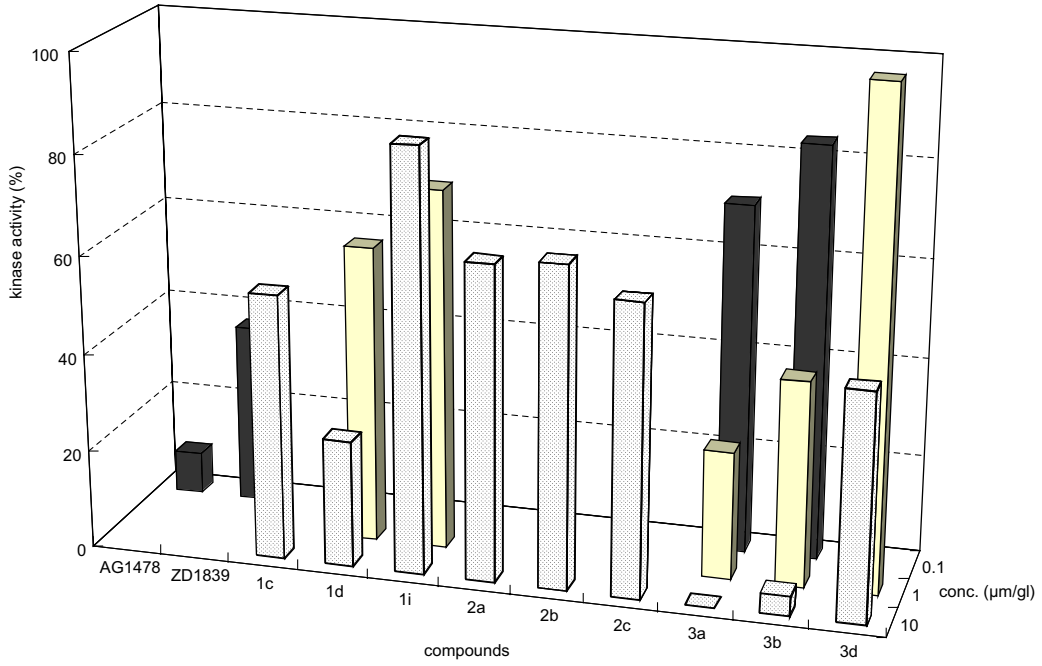


Figure 1. Inhibition of EGF-stimulated EGFR phosphorylation by compounds. EGFR tyrosine kinase activities are expressed as a percentage of the maximal phosphorylation induced by EGF.

benzamidines **3a**–**d**. The deprotection of **3a**–**d** was carried out under the acidic condition to give the 2-amino-4,5-dimethoxyphenylamidines **2a**–**d**.

The cytotoxicity of the benzamides **1a**–**i**, and the benzamidines **2a**–**d** and **3a**–**d** toward A431 human epidermoid carcinoma cells was determined. The concentration of compounds, which exhibited the 50% cell growth inhibition is shown as an IC_{50} value in Table 1. The benzamides **1c**–**e**, which have an aniline group in the molecules, exhibited the cell growth inhibition and the IC_{50} values are 0.34, 0.47, and 0.20 mM, respectively, although the 50% cell growth inhibition was not observed at a 1.0 mM concentration of compounds in the case of the other benzamides **1a**,**b** and **1f**–**i**. The benzamidines **2a**–**d** exhibited the similar cell growth inhibitions as the corresponding benzamides and the IC_{50} values are 0.20–0.46 mM. Interestingly, the protected benzamidines **3a**–**d** exhibited relatively higher cell growth inhibitions in comparison with the corresponding benzamidines **2a**–**d** and the IC_{50} values are 0.09–0.32 mM.

The benzamides **1a**–**i**, and the benzamidines **2a**–**d** and **3a**–**d** were tested for inhibition of EGF-stimulated EGFR tyrosine kinase phosphorylation according to assay conditions described in detail in the literatures.[11] As a preliminary experiment, the inhibition assay of EGFR tyrosine kinase was carried out at a 10 μg/mL concentration of compounds. Among the compounds synthesized, **3a**,**b** exhibited higher than 50% inhibition of the kinase at this concentration and therefore tested for the assay at lower concentrations (10–0.1 μg/mL) as shown in Figure 1. The maximum phosphorylation activity to the peptide substrate by the EGF-stimulated EGFR tyrosine kinase is plotted as 100% activity after reduction of the kinase activity without stimulated by EGF. AG1478 and ZD1839, which have been reported to be potent inhibitors of EGFR tyrosine kinase,[4] showed high inhibition of the kinase activity: the phosphorylation activities were 10% and 35% at a 0.1 μg/mL concentration of compounds, respectively. The compounds **1a**–**i**, **3c**, and **2a**–**d** did not show significant tyrosine kinase inhibition at a 10 μg/mL concentration, unexpectedly. Interestingly, the compounds **3a**,**b**, which have the cyclic framework by conjunction with a ketal formation between an amine and an amidine moiety in the molecule, showed a high inhibitory activity at 10 and 1.0 μg/mL.

Although we succeeded in the synthesis of the benzamides **1** and the benzamidines **2** as the mimics of 4-anilinoquinazolines to expect the formation of a pseudocycle of the quinazoline ring through the intramolecular hydrogen bonding, significant enhancement of EGFR tyrosine kinase inhibition was not observed. Since the cyclic compounds **3a**,**b** exhibited higher inhibitory activities, it may be concluded that the rigid conformation such as a quinazoline ring is essential for the suitable interaction to EGFR tyrosine kinase.

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