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Synthesis and biological evaluation of pyrrolopyridazine derivatives as novel HER-2 tyrosine kinase inhibitors

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ABSTRACT

A series of novel pyrrolopyridazine derivatives have been discovered to be HER-2 inhibitors. These compounds selectively inhibited HER-2 kinase activity at low nanomolar concentrations. Compound **7d** was identified as a potent HER-2 inhibitor with an IC_{50} of 4 nM.

Receptor tyrosine kinases (RTKs) play a crucial role in signal transduction pathways that regulate cell differentiation, proliferation and angiogenesis. Inhibition of RTK activation has become a compelling approach in the development of anticancer agents.[1] The ErbB family of receptor tyrosine kinases comprises four closely related members: the epidermal growth factor receptor (EGFR, ErbB1 or HER-1), the human epidermal growth factor receptor 2 (ErbB2 or HER-2), ErbB3 (HER-3), and ErbB4 (HER-4).[2,3] Over expression of EGFR and HER-2 has been observed in many cancers including non-small-cell lung cancer, and prostate, breast, colon and stomach cancers. Small molecules that inhibit the kinase activity of EGFR and HER-2 are considered to be new therapeutic antitumor agents.[4,5]

Three small molecule drugs, Gefitnib (Iressa®),[6] Erlotinib (Tarceva®),[7] and Lapatinib (Tykerb®),[8] have been approved and marketed for the treatment of cancer (Fig. 1). Several other ErbB family inhibitors are currently being investigated in various phases. These quinazoline-based small molecules have a common scaffold that mimics the adenine moiety of ATP, and therefore are potent and competitive inhibitors of ATP.[9] During the course of our exploration of non-quinazoline scaffolds, pyrrolopyridazine derivatives (**I**, **II**, **III**) were found to be novel and potent HER-2 inhibitors (Fig. 2). Here, we report our progress in the synthesis and biological evaluation of these pyrrolopyridazine-based inhibitors.

As shown in Scheme 1, reaction of pyrrole diester **1** with ceric ammonium nitrate (CAN) afforded pyrrole aldehyde **2**,[10] which underwent a cyclization with hydrazine hydrate in acetic acid to give pyrrolo[2,3-*d*]pyridazin-4-one **3**. The pyrrolo[2,3-*d*]pyridazin-4-one **3** was transformed into the 4-chloro-pyridazine derivative **4** upon treatment with $POCl_3$. Acid catalysed displacement of the chloride with the appropriate aniline was performed in isopropanol to give **5**. Saponification of **5** followed by coupling with different amines afforded compounds **7a–e**.[11]

Analogs with pyrrolo[3,4-*d*]pyridazine scaffold **II** and 7-substituted pyrrolo[2,3-*d*]pyridazine scaffold **III** were synthesized by similar routes starting from different commercially available pyrroles as shown in Schemes 2 and 3. Pyrrole diester **8**[12] and **13**[13] were transformed to aldehyde **9** and **14**, respectively, under Vilsmeier–Haack reaction conditions, which were converted to **10** and **15** via intramolecular ring-closing condensations. Chlorination of **10** and **15** followed by coupling with 3-chloro-4-(3-fluoro-benzyloxy)-phenylamine in isopropanol afforded **12** and **17**. Saponification and amidation of **17** led to the desired compounds **18a–b** as shown in Scheme 3.

Inhibition of EGFR and HER-2 tyrosine kinase activity was evaluated in enzymatic and cellular assays using A431(overexpressing EGFR) and SK-BR-3 (overexpressing HER-2).[14,15] Table 1 summarises the in vitro potency of representative compounds. No significant EGFR tyrosine kinase inhibitory activity was observed in these compounds. Computational modeling studies provided a possible explanation for this result. A docking model of compound **7a** based on the crystal structure of EGFR complexed with Lapatinib[16] (PDB code 1XKK) was developed (Fig. 3). The docked models were energy minimized in Sybyl7.3. This model showed that compound **7a** bound to EGFR in definitely different fashions when compared with Lapatinib. The pyrrolopyridazine core of **7a** deviated from the quinazoline core of Lapatinib, and was oriented in the ATP binding site such that there was a hydrogen bond between N-2 and the hinge region Met769 NH. The C-4 hydrophobic

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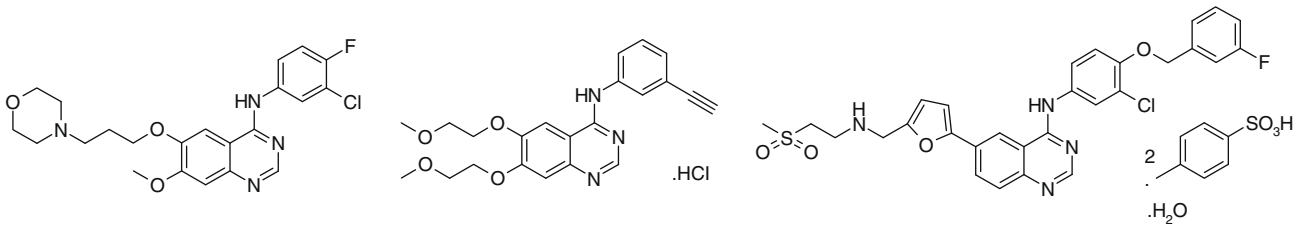


Gefitinib (Iressa®) Erlotinib (Tarceva®) Lapatinib (Tykerb®)

Figure 1. Examples of launched ErbB family inhibitors.

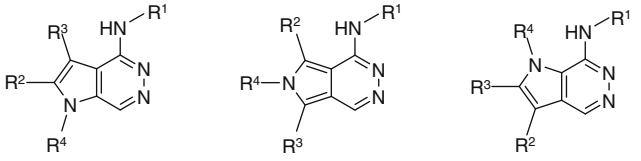
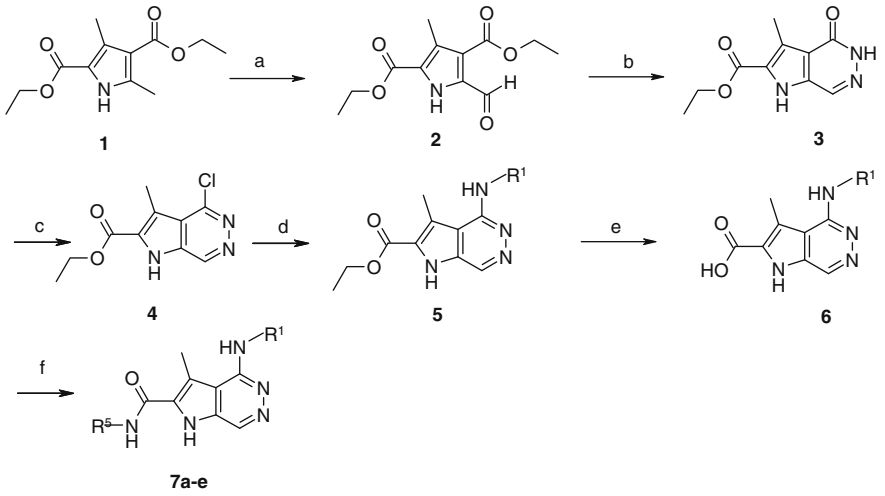


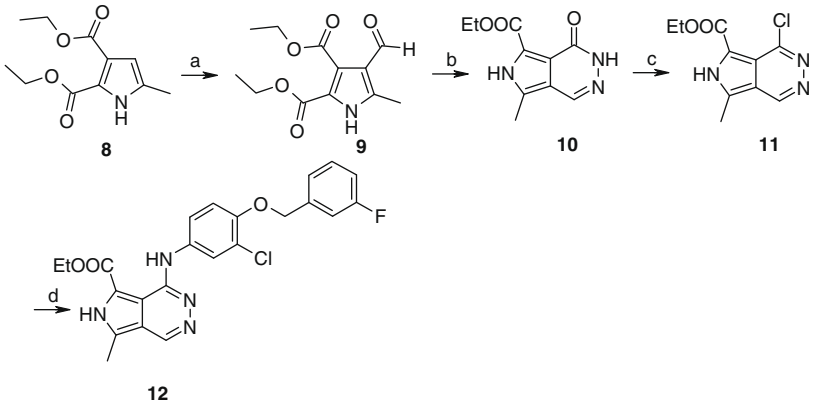
Figure 2. General structure of the pyrrolopyridazine-based compounds.

group extended out into the aqueous phase instead of extending into the deep hydrophobic pocket. This might explain why compound **7a** was almost devoid of EGFR activity.

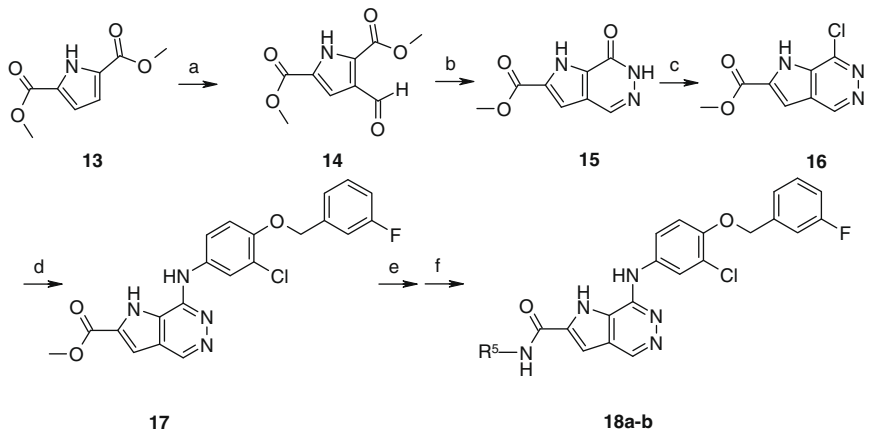
Moderate growth inhibitions toward A431 cells in compounds **7b**, **7c** and **7e** might be caused by the inhibition of other kinases. It was noticeable that compounds **7a** and **7d** exhibited similar potency as Lapatinib (0.003 μM) against HER-2 kinase, but was found to be >100 times more selective than the closely related EGFR ki-



Scheme 1. Synthesis of type **I** derivatives. Reagents and conditions: (a) 4.0 equiv CAN, THF/HOAc/H_2O, rt, 1.5 h, 65%; (b) 1.1 equiv $NH_2NH_2{\cdot}H_2O$, HOAc, 100 °C, 2 h, 79%; (c) 2.0 equiv $POCl_3$, CH_3CN, 80 °C, 1.5 h, 95%; (d) 1.5 equiv R^1NH_2, 2.5 equiv Et_3N, *i*-PrOH, aq HCl, 80 °C, 4 h, 50%; (e) 1 *N* NaOH, EtOH, reflux, then 1 *N* HCl, pH 1, 91%; (f) 1.5 equiv R^5NH_2, Et_3N, EDCI, HOBt, DMF, rt, 17–58%.

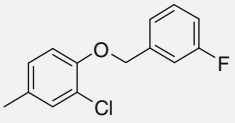
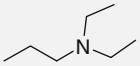
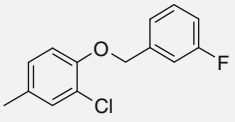
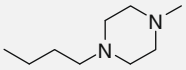
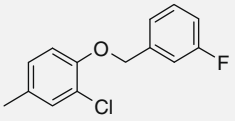
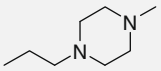
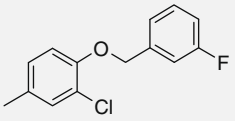
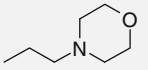
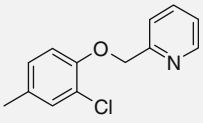
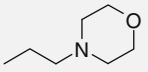
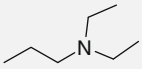
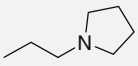


Scheme 2. Synthesis of type **II** derivatives. Reagents and conditions: (a) 2.0 equiv $POCl_3$, DMF, 100 °C, 2 h, 77%; (b) 1.5 equiv $NH_2NH_2{\cdot}H_2O$, EtOH, 90 °C, 3 h, 72%; (c) 2.0 equiv $POCl_3$, CH_3CN, 80 °C, 5 h; (d) 1.1 equiv 3-chloro-4-(3-fluoro-benzyloxy) -phenylamine, *i*-PrOH, 90 °C, 7 h, 51%.



Scheme 3. Synthesis of type **III** derivatives. Reagents and conditions: (a) 2.0 equiv $POCl_3$, DMF, 100 °C, 3 h, 21%; (b) 1.5 equiv $NH_2NH_2{\cdot}H_2O$, EtOH 90 °C, 1.5 h, 44%; (c) 2.0 equiv $POCl_3$, CH_3CN, 80 °C, 3 h; (d) 1.1 equiv 3-chloro-4-(3-fluoro-benzyloxy)-phenylamine, *i*-PrOH, 90 °C, 3 h, 34%; (e) 1 *N* NaOH, EtOH, reflux, then 1 *N* HCl, pH 1, 85%; (f) 1.5 equiv R^5NH_2, Et_3N, EDCI, HOBt, DMF, rt, 62–85%.

Table 1
Enzyme and cellular inhibitory activity assay results

No.	R^1	R^5	EGFR IC_{50}[a] (μM)	A431 IC_{50}[a] (μM)	HER-2 IC_{50}[a] (μM)	SK-BR-3 IC_{50}[a] (μM)
7a			1.28	1.16	0.007	0.96
7b			>10	0.98	ND	2.24
7c			>10	0.257	ND	0.843
7d			5–10	12.65	0.004	>5
7e			>10	0.867	ND	>5
12	—	—	>10	1.21	ND	0.934
18a	—		>10	77.22	ND	3.84
18b	—		>10	15.14	ND	0.62
Tarceva®[b]	—	—	0.015	0.42	1.52	1.89
Tykerb®[b]	—	—	0.019	0.14	0.003	0.124

ND: not determined.
[a] Average values (at least two experiments).
[b] Tarceva® and Tykerb® were prepared according to the literatures.[7,8]

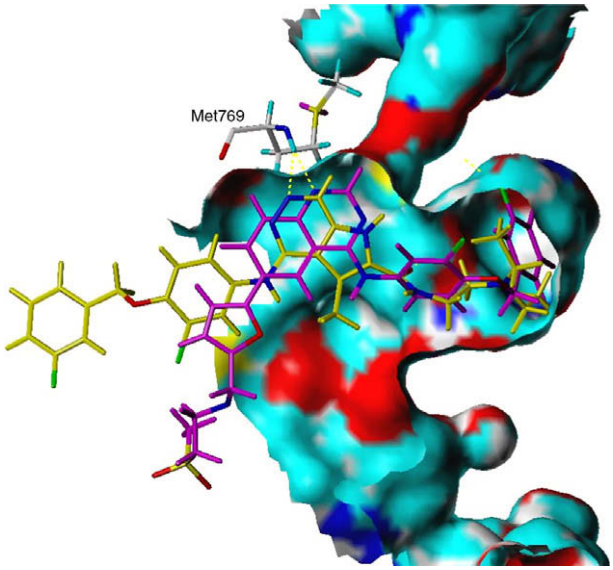


Figure 3. Predicted binding mode of compound **7a** (yellow) modeled in the X-ray structure of the Lapatinib/EGFR kinase complex. The Lapatinib molecule is shown in magenta.

nase. The HER-2 selectivities of the compounds **7a** and **7d** cannot be explained adequately due to lack of an HER-2 X-ray structure. Further studies are planned to investigate these selectivities. Compounds **7a**, **7c**, **12** and **18** were also found to be low-micromolar inhibitors toward SK-BR-3 cells.

In conclusion, pyrrolopyridazine derivatives were prepared and found to be low nanomolar HER-2 selective inhibitors over EGFR. Further studies of these compounds are in progress.

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11. All compounds were characterized by ^{1}H NMR and MS etc. Preparation of compound **7d** is described herein. A stirred solution of 4-[3-chloro-4-(3-fluoro-benzyloxy)-phenylamino]-3-methyl-1*H*-pyrrolo[2,3-*d*] pyridazine-2-carboxylic acid **6** (1 mmol) in *N*,*N*-dimethylformamide (15 mL) was added *N*-ethyl-*N*′-(dimethylamino propyl)-carbodiimide hydrochloride (1.5 mmol), 1-hydroxy-benzotriazol (1.5 mmol) and triethylamine (2.5 mmol), the mixture was stirred for 5 min at room temperature, then 2-morpholin-4-yl-ethylamine (1.5 mmol) was added, the reaction mixture was stirred overnight at this temperature until the start material disappeared as monitored by TLC. The mixture was poured into ice water (80 mL) and extracted with ethyl acetate (4 × 30 mL). The combined organic extracts were washed with saturated sodium chloride aqueous solution (50 mL), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure, the residue is purified by column chromatography to give 4-[3-chloro-4-(3-fluoro-benzyloxy)-phenyl amino]-3-methyl-1*H*-pyrrolo[2,3-*d*]-pyridazine-2-(2-morpholin-4-yl-ethyl)-formamide **7d** (176 mg, yellow solid, yield 30.2%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.87 (s, 1H), 7.89 (s, 1H), 7.55 (d, 1H), 7.40 (m, 1H), 7.30 (m, 2H), 7.17 (m, 2H), 5.22 (s, 2H), 3.59 (t, 4H), 3.44 (t, 2H), 2.71 (s, 3H), 2.49 (t, 2H), 2.40 (m, 4H). MS *m*/*z* (ESI): 539 [M+1].
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