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5-Substituted 4-anilinoquinazolines as potent, selective and orally active inhibitors of erbB2 receptor tyrosine kinase

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Abstract—Starting from a 6,7-substituted quinazoline lead **4**, optimisation of 5-substituted quinazolines containing an extended aniline motif led to potent and selective inhibitors of erbB2 receptor tyrosine kinase, and a representative compound **12a** inhibited tumour growth in a mouse xenograft model.
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Following the discovery that over-expression of the EGFR (erbB1) and erbB2 receptor tyrosine kinases is found in a number of cancers and is associated with poor prognosis in patients, blockade of this signalling pathway has emerged as a promising approach to selective targeting of tumour cells.[1] The clinical utility of the EGFR inhibitor gefitinib **1** (Fig. 1) in non-small cell lung cancer has been demonstrated,[2] while the monoclonal antibody trastuzumab has been shown to increase survival time in patients with metastatic breast tumours that over-express erbB2.[3] Among compounds currently undergoing clinical trials are the mixed EGFR-erbB2 receptor tyrosine kinase inhibitor lapatinib (GW2016) **2**,[4] the erbB2-selective inhibitor CP-724714 **3**,[5] and the irreversible inhibitors canertinib (CI-1033),[6] EKB-569[7] and HKI-272.[8]

X-ray crystal structures of EGFR enzyme-inhibitor complexes provide clear evidence that the mechanism of action of receptor tyrosine kinase inhibitors such as **1**–**3** involves reversible displacement of ATP at the kinase active site, through binding to either the active[9] or the inactive[10] form of the kinase. As part of the programme of work that led to the discovery of gefitinib, we found 6,7-substituted quinazoline **4**[11] (Fig. 1) to be a moderately potent inhibitor of erbB2 receptor tyrosine kinase. Like other documented quinazoline-based erbB2 inhibitors, compound **4** contains an extended aniline motif, which we assumed to interact with the selectivity pocket at the erbB2 active site.[10] From consideration of an in-house homology model[12] and related work on inhibitors of c-Src,[13] we reasoned that switching of the quinazoline substituent from the 6- to the 5-position could improve erbB2 affinity through occupation of the kinase sugar pocket. In this publication, we describe

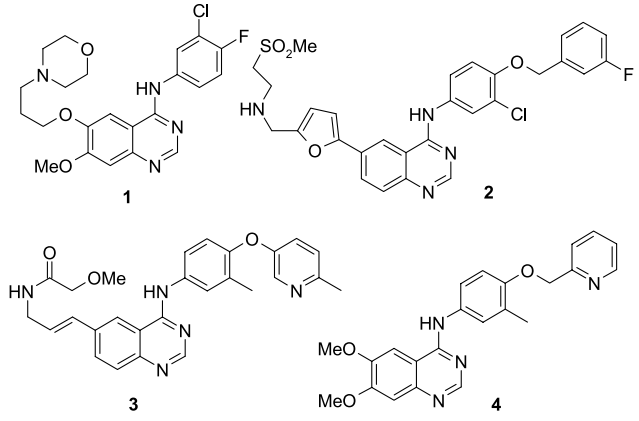

Figure 1. Screening lead **4** and examples of EGFR and erbB2 receptor tyrosine kinase inhibitors in clinical use or undergoing trials: gefitinib **1**; lapatinib **2**; CP-724714 **3**.

Keywords: Anilinoquinazoline; erbB2; Receptor tyrosine kinase.
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how this work led to potent, selective and orally active inhibitors of erbB2.

Representative compounds prepared during the course of this work are listed in Table 1, and synthetic routes are outlined in Scheme 1–4.[14] Initial variant **7** was prepared (Scheme 1) from the appropriate extended aniline[11] and chloroquinazoline **6**, in turn obtained from 5,7-dimethoxy-4(3*H*)-quinazolone **5**[14b] via a sequence involving as the key steps selective demethylation,[13] protection of quinazolone nitrogen and Mitsunobu alkylation.

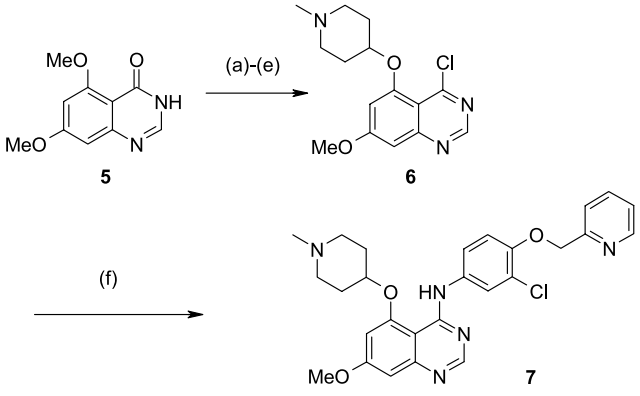
Compounds **10a**–**c** were likewise prepared (Scheme 2) from the appropriate anilines[11,15] and the corresponding chloroquinazolines **9a**–**b**, readily accessible in two steps from commercially available 5-fluoro-4(3*H*)-quinazolone **8** via alkoxide displacement and chlorination.

More versatile routes enabled introduction of the aniline para substituent or the quinazoline 5-substituent at the last stage of the synthesis. Reaction of chloroquinazoline **9a** with the appropriate amino phenol gave intermediates **11a**–**b**, which were then alkylated to provide

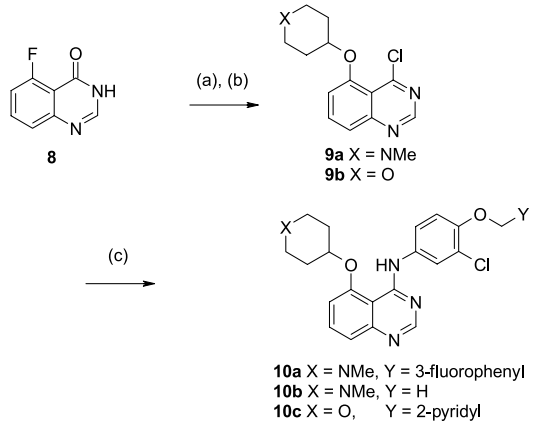
Table 1. erbB2 and EGFR kinase inhibition data[18] for compounds **4**, **7**, **10a**–**c**, **12a**–**d**, **14**

Entry	IC_{50} (μM)[a]	
	erbB2	EGFR
4	0.056 (±0.003)	0.059 (±0.003)
7	0.005 (±0)	0.005 (±0.003)
10a	<0.002	0.005 (±0.002)
10b	1.1 (±0.085)	1.8 (±0.25)
10c	0.024 (±0.001)	2.4 (±0.41)
12a	<0.002[b]	0.14 (±0.048)
12b	<0.002	0.69 (±0.18)
12c	<0.002	0.28(±0.08)
12d	0.070 (±0.014)	2.1 (±0.11)
14	0.024 (±0.010)	0.44 (±0.026)

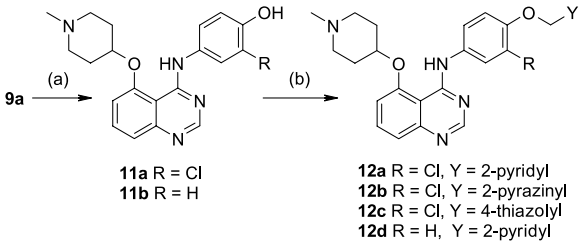
[a] $n \geqslant 3$, standard error is given in parentheses.
[b] IC_{50} 7.6 μM (Kdr), 23 μM (Src).



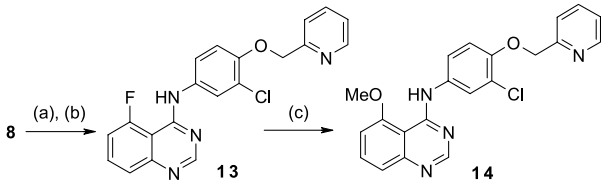
Scheme 1. Synthesis of compound **7**. Reagents and conditions: (a) $MgBr_2$, pyridine, reflux; (b) NaH, chloromethyl pivalate, DMF, 0–20 °C; (c) 4-hydroxy-*N*-methylpiperidine, Ph_3P, $(t\text{-}BuO_2C)_2N_2$, CH_2Cl_2, 20 °C; (d) NH_3, MeOH, 20 °C; (e) $POCl_3$, *i*-Pr_2NEt, $ClCH_2CH_2Cl$, reflux; (f) H_2NAr,[11] *i*-Pr_2NEt, *i*-PrOH, reflux.



Scheme 2. Synthesis of compounds **10a**–**c**. Reagents and conditions: (a) NaH, 4-hydroxy-*N*-methylpiperidine or 4-hydroxytetrahydropyran, $MeCONMe_2$, 80 °C (work up: DOWEX H+/MeOH/NH_3, 20 °C); (b) $POCl_3$, *i*-Pr_2NEt, CH_2Cl_2, reflux (work up: aq $NaHCO_3$, 0 °C); (c) H_2NAr,[11,15] *i*-PrOH, reflux.



Scheme 3. Synthesis of compounds **12a**–**d**. Reagents and conditions: (a) 4-amino-2-chlorophenol or 4-aminophenol, *i*-PrOH, reflux; (b) $ClCH_2Y$,[16] K_2CO_3, 18-crown-6, MeCN, reflux.



Scheme 4. Synthesis of compound **14**. Reagents and conditions: (a) $SOCl_2$, cat $HCONMe_2$, reflux (work up: aq $NaHCO_3$, 0 °C);[17] (b) H_2NAr,[11] *i*-PrOH, reflux; (c) NaOMe, 15-crown-5, MeOH, dioxan, reflux.

extended aniline variants **12a**–**d** (Scheme 3). Alternatively (Scheme 4) the fluoroquinazoline **13**, readily obtained from fluoroquinazolone **8**,[17] underwent microwave assisted displacement by alkoxide to give 5-methoxy quinazoline derivative **14**.

The compounds listed in Table 1 were evaluated in erbB2 and EGFR kinase assays measuring inhibition of phosphorylation of a synthetic peptide substrate at K_m ATP concentration for each enzyme.[18] Lead compound **4** showed moderate inhibition in the erbB2 kinase assay (IC_{50} 0.056 μM). Transposition of the quinazoline substituent from the 6- to the 5-position, introduction of a cyclic amine and changing the meta substituent in the extended aniline from methyl to chlorine led to

compound **7** with significantly improved affinity for erbB2 and EGFR. Removal of the 7-methoxy substituent then provided compound **12a** with favourable erbB2 kinase selectivity versus EGFR (IC_{50} 0.002 μM vs 0.11 μM). Compound **12a** also showed a clean selectivity profile versus in-house and external kinase panels (data not shown).[19] Thus, for example, **12a** gave selectivity ratios of >3800 and >11,500, respectively, versus the receptor tyrosine kinases Kdr and Src (Table 1, footnote b).

The remaining compounds included in Table 1 illustrate SAR for the series of 5-substituted quinazolines. Alternative extended anilines (**10a**, **12b–c**) gave comparable ErbB2 activity but differing selectivity versus EGFR. More drastic aniline modifications (**10b**, **12d**) gave reduced affinity, consistent with interaction of the extended aniline with the selectivity pocket at the erbB2 active site[10] and with published work highlighting the importance of the meta substituent.[20] Alternative 5-substituted quinazolines (**10c**, **14**) also showed reduced affinity relative to **12a**.

A number of assays[18,21–24] were used to determine the cellular profile of selected compounds (Table 2). ErbB2-selective inhibitor **12a** and mixed erbB2-EGFR inhibitor **10a** were shown to inhibit erbB2 autophosphorylation in a MCF7 breast carcinoma cell line engineered to overexpress erbB2 ('Clone 24' assay).[21] The cellular activity of compounds **10a** and **12a** was confirmed in a proliferation assay using the BT474C cell line.[22,23] Compounds **10a** and **12a** were shown to be only weak to moderate inhibitors of growth of the KB cell line when stimulated with EGF,[18] and good separation was also seen between BT474C antiproliferative activity and effect on basal growth in KB cells,[18] consistent with a cellular mode of action involving inhibition of erbB2. The moderate EGFR cellular potency of compound **10a** was confirmed using an EGFR autophosphorylation assay,[24] reflecting contrasting enzyme and cellular profiles for this compound.

Table 3 summarises pharmacokinetic parameters for compound **12a** determined following dosing to immunocompetent Alderley Park mice at 2 mg/kg iv and 5 mg/kg po. Exposure of the compound scaled well on dosing at 100 mg/kg po, and calculation of plasma free concentrations showed the free levels of **12a** to compare favourably with the IC_{50} value in the Clone 24 phosphorylation assay (Table 4).[25]

Encouraged by these pharmacokinetic data, we evaluated compound **12a** at 100 mg/kg po q.d. and b.i.d. and at 200 mg/kg po q.d. for inhibition of growth of a BT474C tumour xenograft in a 28-day study in athymic mice.[22] No significant reduction in body weight was observed for any animal group in this study. As shown in Figure 2, compound **12a** gave statistically significant inhibition of tumour growth with all dosing schedules. Analysis of excised tumour samples confirmed significant inhibition of phospho-erbB2 (e.g., >90% inhibition 4 and 24 h after the last dose at 100 mg/kg b.i.d.).

Although the BT474C mouse xenograft model responds to both erbB2[22] and EGFR inhibitions,[26] the weak activity of compound **12a** in the KB cell assay (Table 2) is consistent with xenograft activity reflecting inhibition of erbB2. Further support for this hypothesis is provided by the lack of activity of **12a** at 100 mg/kg po in the EGFR-driven mouse LoVo xenograft model[18] (data not shown).

In summary, optimisation of 5-substituted quinazolines containing an extended aniline motif led to potent and selective inhibitors of erbB2 receptor tyrosine kinase, and a representative compound **12a** inhibited tumour growth in the mouse BT474C xenograft model. Further work describing a wider range of 5-substituted quinazolines as selective inhibitors of erbB2 will be reported in due course.

Table 2. Cell assay data[18,21–23] for compounds **10a** and **12a**

Entry	IC_{50} (μM)[a]			
	Clone 24	BT474C	KB	KB Basal
10a	0.056 (±0.007)	0.027 (±0.007)	0.46[b](±0.10)	1.7 (±0.46)
12a	0.061 (±0.009)	0.041 (±0.004)	2.6 (±0.81)	11.9 (±4.1)

[a] $n \geqslant 3$, standard error is given in parentheses.
[b] IC_{50} 2.5 (±0.75) μM for inhibition of phospho-EGFR in KB cells.[24]

Table 3. Pharmacokinetic parameters for compound **12a**, determined by dosing to immunocompetent mice at 2 mg/kg iv and 5 mg/kg po

Entry	V_{dss} (l/kg)	Cl (ml/min/kg)	po AUC (μM.h)	Bioavailability %
12a	2.6	15	5.5	50

Table 4. Plasma concentrations for compound **12a** after dosing at 100 mg/kg po,[a] and ratios of free plasma levels[b] to IC_{50} value in Clone 24 cellular assay

	Time (h)				
	2	4	6	16	24
Plasma concentration **12a** (μM)	9.2	7.2	6.9	2.2	1.1
Ratio free **12a**/Clone 24 IC_{50}	4.1	3.2	3.1	0.97	0.48

[a] AUC 98.5 μM.h.
[b] Mouse free drug 2.72%.

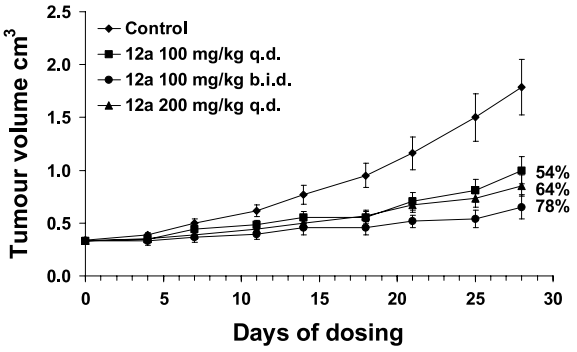


Figure 2. Inhibition of growth of BT474C xenograft[22] in athymic mice dosed po with compound **12a**.

Acknowledgments

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