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Neutral 5-substituted 4-anilinoquinazolines as potent, orally active inhibitors of erbB2 receptor tyrosine kinase

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Abstract—Neutral 5-substituted 4-anilinoquinazolines addressed high in vivo clearance and phospholipidosis associated with previous basic compounds. A representative compound **8a** inhibited tumor growth in a mouse xenograft model when co-administered with the cytochrome P450 inhibitor 1-aminobenzotriazole (ABT), and data are consistent with pharmacology primarily reflecting inhibition of erbB2 receptor tyrosine kinase.

With introduction into clinical practice of the EGFR (erbB1) inhibitors erlotinib and gefitinib, and the monoclonal antibody to erbB2 trastuzumab, blockade of the EGFR family signaling pathway has emerged as a leading approach to selective targeting of proliferating tumor cells.[1] The dual EGFR-erbB2 receptor tyrosine kinase inhibitor lapatinib **1**[2] (Fig. 1) has recently been approved in combination with capecitabine by the FDA for treatment of advanced metastatic breast cancer and is also undergoing clinical trials for treatment of a number of other tumor types. Other dual EGFR-erbB2 inhibitors in earlier stage trials include[1] AEE788, BMS-599626, and the irreversible inhibitors canertinib, HKI-272 and BIBW 2992. Although an erbB2-selective inhibitor may offer more flexibility in combination therapy,[1] CP-724714 **2** is the only selective small-molecule inhibitor reported to have entered the clinic.

All of these small-molecule inhibitors are based on binding of a 4-anilinoquinazoline or related template at the kinase ATP site.[1] We have previously described work leading to the 5-substituted 4-anilinoquinazoline **3**[3] (Fig. 1) a potent, selective, and orally active inhibitor

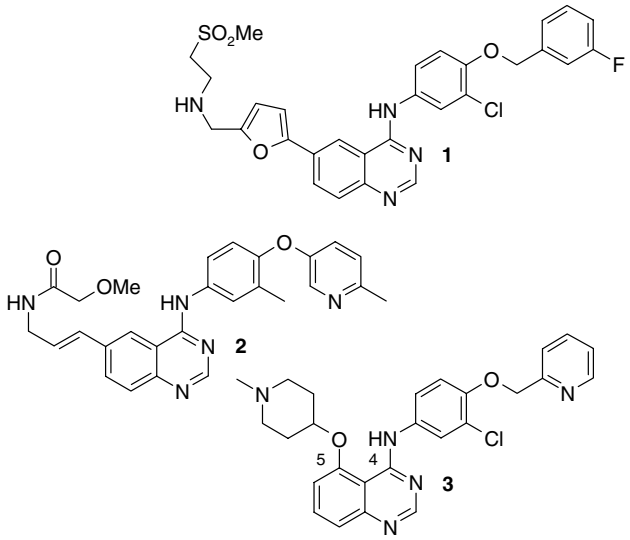

Figure 1. Lead compound **3** and examples of erbB2 receptor tyrosine kinase inhibitors undergoing clinical trials: lapatinib **1**; CP-724714 **2**.

Keywords: Anilinoquinazoline; erbB2-selective inhibitor; Tumor growth; Cytochrome p450 inhibitor.
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of erbB2. Work since published on 5-substituted quinazolines as inhibitors of EGFR[4] and c-Src[5] strongly supports the hypothesis that the 5-substituent occupies the ribose binding site of the kinase.

We were unable, however, to progress compound **3** beyond pre-clinical models for reasons attributable to the basic nature of the 5-substituent. Oral administration of **3** in rat toxicology studies[6] resulted in mild to severe effects in liver, lung, and spleen characteristic of phospholipidosis, a toxicity known to be associated with cationic amphiphilic drugs[7] (**3**: pK_a 8.1, *C*log *P* 5.1, log *D* > 3.5). In addition, a range of basic analogs generally showed moderate to high clearance in rat and dog.[6] We envisaged that hydroxyacylation of the basic nitrogen could circumvent these issues and also introduce a pendant hydroxyl group that could interact with residues located in the ribose pocket. In this publication, we describe how this work led to erbB2 inhibitors devoid of phospholipidosis and with reduced clearance.

Illustrative compounds prepared during the course of this work are listed in Table 1, and synthetic routes are outlined in Schemes 1–3.[8] Measured log *D* values for these compounds lie in the range 3–3.5 and led to comparable physicochemical properties to compound **3** (data not shown).

In extension of previous work,[3] initial variants **5a**–**b** were prepared (Scheme 1) from fluoroquinazoline **4**[3] by a sequence involving displacement with the alkoxide derived from (*R*) or (*S*) prolinol followed by acylation of the pyrrolidine nitrogen with glycolic acid.

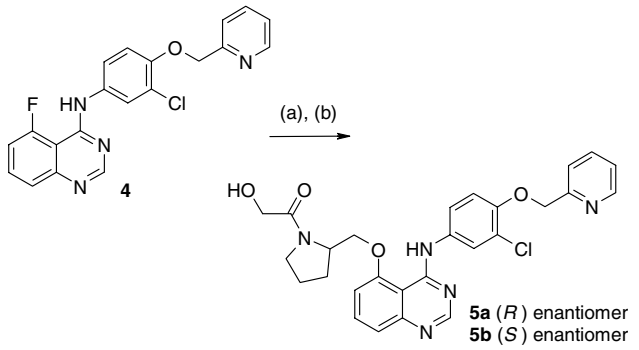
Since displacement reactions of fluoroquinazoline **4** with acyclic amino alkoxides gave low and variable yields, an alternative route was used (Scheme 2) to synthesize analogs **8a**–**c**. In contrast with **4**, base-promoted displacement of the corresponding phenol **6**[9] with the appropriate amino alcohol[10,11] gave intermediates **7a**–**c** in high yield, and *N*-acylation followed by *O*-alkylation then provided **8a**–**c**.

As part of a wider body of work,[8] this more versatile route enabled late-stage variation of both the acyl and extended aniline[3] moieties. We speculate that under strongly basic conditions the anilinoquinazoline NH of substrate **4** is deprotonated, thereby deactivating the adjacent 5-fluorine atom to nucleophilic displacement,

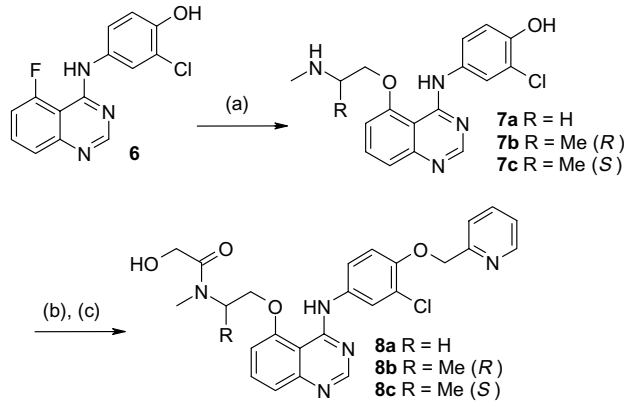
Table 1. Inhibition data[3] versus erbB2 and EGFR kinase and erbB2 cellular autophosphorylation for **5a**–**b**, **8a**–**c**, **11a**–**b**

Compound	Erb B2 IC_{50}[a] (nM)	EGFR IC_{50}[a] (nM)	Clone 24 IC_{50}[a] (nM)
5a	<2	97 (±64)	147 (±79)
5b	2	327 (±226)	259 (±53)
8a[b]	4 (±2)	35 (±6)	43 (±20)
8b	<2	3 (±1)	11 (±5)
8c	19 (±10)	2580 (±1350)	131 (±48)
11a	<2	2 (±1)	11 (±0.5)
11b	22 (±10)	191 (±137)	291 (±144)

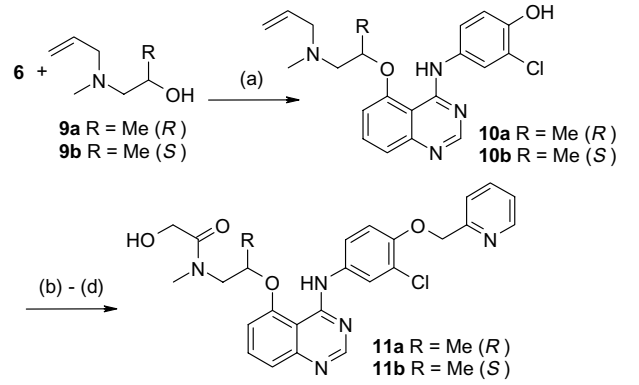
[a] $n \geqslant 2$, standard error is given in parentheses.
[b] IC_{50} > 1000 nM (Kdr, Src).



Scheme 1. Synthesis of compounds **5a**–**b**. Reagents and conditions: (a) (*R*) or (*S*) prolinol, NaH (2.5 equiv), 15-crown-5, DMA, 95 °C; (b) $HOCH_2CO_2H$, HATU, *i*-Pr_2NEt, DMF, 20 °C.



Scheme 2. Synthesis of compounds **8a**–**c**. Reagents and conditions: (a) MeNHCH(R)CH_2OH,[9,10] NaH (2.5 equiv), 15-crown-5, DMA, 110 °C; (b) $HOCH_2CO_2H$, HATU, *i*-Pr_2NEt, DMF, 20 °C; (c) 2-picolyl chloride·HCl, K_2CO_3, 18-crown-6, DMF, 20 °C.



Scheme 3. Synthesis of compounds **11a**–**b**. Reagents and conditions: (a) NaH (2.5 equiv), 15-crown-5, DMA, 110 °C; (b) $(Ph_3P)_3RhCl$, $MeCN/H_2O$, microwave, 110 °C; (c) $HOCH_2CO_2H$, HATU, *i*-Pr_2NEt, DMF, 20 °C; (d) 2-picolyl chloride·HCl, K_2CO_3, 18-crown-6, DMF, 20 °C.

whereas for substrate **6** it is the phenol which is deprotonated, leaving the free anilinoquinazoline NH to assist the neighboring 5-fluoro leaving group.

As the chiral amino alcohol precursors of analogs **11a**–**b** were not readily accessible, a modified route was em-

ployed (Scheme 3) whereby precursor **6** underwent base-promoted displacement with *N*-protected amino alcohols **9a**–**b**, readily obtained by ytterbium triflate catalyzed ring opening[12] of the corresponding chiral oxirane with *N*-methylallylamine. De-allylation of intermediates **10a**–**b** and functionalization as detailed earlier then gave **11a**–**b**.

The compounds listed in Table 1 were evaluated in erbB2 and EGFR kinase assays and in a cellular erbB2 autophosphorylation assay ('Clone 24') as cited previously.[3] Evolution of this series of compounds led to neutral acyclic variants **8a**–**b** and **11a** with enhanced cellular potency relative to compound **3**.[3] Significant potency differences were also seen between pairs of enantiomers (**8b** vs **8c**, **11a** vs **11b**). Compound **8a** showed a clean selectivity profile versus in-house and external kinase panels (data not shown).[13] Thus, for example, **8a** gave selectivity ratios of >333 versus the tyrosine kinases Kdr and Src (Table 1, footnote b).

Antiproliferative effects were determined in assays using BT474C and KB cell lines (Table 2), known to respond, respectively, to stimulation by both erbB2 and EGFR, and EGFR alone.[3] Good separation was seen between antiproliferative activity and effect on basal growth in KB cells, but in contrast with lead compound **3**[3] a less marked difference was observed between the magnitude of the antiproliferative effect in the two cell lines. As discussed below, however, we believe that antitumor effects in vivo continue to primarily reflect inhibition of erbB2.

Pharmacokinetic parameters were determined in mouse, rat, and dog, the species used for in vivo models and preclinical toxicology studies. Based on these parameters, compound **8a** (Table 3) was selected for further evaluation. In contrast to compound **3**, the neutral 5-substituent in **8a** led to low to moderate clearance in rat and dog. Oral exposure in rat scaled well to higher doses,

Table 2. Cellular antiproliferative data[3] for **8a** and **11a**

Compound	BT474C IC_{50}[a] (nM)	KB IC_{50}[a] (nM)	KB basal[a] (nM)
8a	130 (±20)	621 (±350)	8145 (±3770)
11a	34 (±17)	141 (±44)	6936 (±3500)

[a] $n \geqslant 2$, standard error is given in parentheses.

Table 3. Mouse, rat and dog pharmacokinetic parameters for **8a**

Species	Vdss (l/kg)	Cl (ml/min/kg)	AUC[d] (μM h)	Bioavailability %
Mouse[a]	0.7	72	0.02	4
Rat[b]	0.9	10	5.5	32
Dog[c]	1.0	10	32	51

[a] Immunocompetent female Alderley Park mice dosed at 2 mg/kg iv and 5 mg/kg p.o.
[b] Female Alderley Park rats dosed at 2 mg/kg iv and 5 mg/kg p.o.
[c] Mean values for male Alderley Park beagle administered doses of 50 mg iv and 250 mg p.o.
[d] AUC_{0-24} from p.o. dosing.

Table 4. Plasma concentrations for **8a** after dosing in female mice at 50 mg/kg p.o., and ratios of free plasma levels to IC_{50} value in the Clone 24 and BT474C cellular assays

Time (h)	2	4	6	16	24
Plasma concentration **8a**[a] (μM)	0.35	0.09	0.02	ND[b]	ND[b]
Plasma concentration **8a**[c] (μM)	16.5	13.8	6.3	0.026	0.007
Ratio free **8a**/Clone 24 IC_{50}[c,d]	7.6	6.4	2.9	0.012	0.003
Ratio free **8a**/BT474C IC_{50}[c,d]	2.5	2.1	0.97	0.004	0.001

[a] Without co-administered ABT, AUC 0.89 μM h.
[b] Not detected.
[c] With ABT co-administered at 100 mg/kg, AUC 99 μM h.
[d] Measured free drug in mouse plasma is 2.0% total concentration.

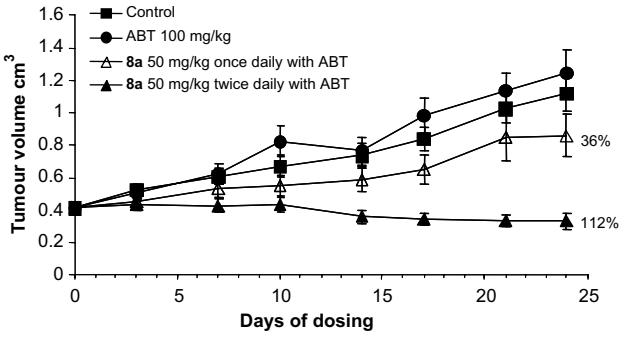


Figure 2. Inhibition of growth of BT474C xenograft[15] in athymic mice dosed orally with compound **8a** and ABT.

and no evidence of phospholipidosis was seen in 14-day rat toxicology studies at up to 600 mg/kg q.d.

Compound **8a** and analogs were, however, more highly cleared and poorly bioavailable in mouse, and consequently at an oral dose of 50 mg/kg minimal exposure was seen (Table 4). To increase exposure, the compound was co-administered with pre-dosed 1-aminobenzotriazole (ABT), an irreversible inhibitor of cytochrome P450 metabolizing enzymes known to reduce P450-mediated clearance in vivo.[14] This regimen gave approximately 100-fold increase in exposure, and calculation of mouse plasma concentrations showed the free levels of **8a** to compare favorably with IC_{50} values in the Clone 24 and BT474C assays up to the 6-h time point (Table 4).

Compound **8a** was evaluated at 50 mg/kg p.o. q.d. and b.i.d. for inhibition of growth of a BT474C tumor xenograft in a 28-day study in athymic mice.[15] No significant reduction in body weight was observed for any animal group in the study. As shown in Figure 2, compound **8a** gave statistically significant inhibition with both dosing schedules, and twice daily dosing gave better efficacy, in accord with more prolonged cover relative to cellular IC_{50}.[16] Analysis of excised tumor samples confirmed significant inhibition of phospho-erbB2 (e.g. 70% inhibition 1 h after the last dose at 50 mg/kg b.i.d.).

Although the BT474C mouse xenograft model responds to both erbB2[15] and EGFR inhibition,[6] the modest activity of compound **8a** in the KB cell assay (Table 2) is consistent with xenograft activity primarily reflecting inhibition of erbB2. In accord with this proposal, **8a**

showed no growth or phospho-EGFR inhibition in mouse and rat LoVo xenograft models[17,18] at oral doses of 50 mg/kg b.i.d. and 300 mg/kg q.d., respectively (data not shown).

In summary, replacement of the basic 5-substituent in this anilinoquinazoline subseries with a neutral hydroxyacylamino moiety circumvented high in vivo clearance and phospholipidosis attributable to the basicity of the 5-substituent, and a representative compound **8a** inhibited tumor growth in a mouse BT474C xenograft model when co-administered with ABT. Further evolution of 5-substituted 4-anilinoquinazolines as inhibitors of erbB2 receptor tyrosine kinase will be reported in due course.

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