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A new series of neutral 5-substituted 4-anilinoquinazolines as potent, orally active inhibitors of erbB2 receptor tyrosine kinase

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Abstract—Starting from initial lead **1** containing a basic 5-substituent, optimisation of the glycolamide-derived neutral 5-substituent led to potent inhibitors of erbB2 with good pharmacokinetics. Representative compounds **19** and **21** inhibited phosphorylation of erbB2 in a mouse BT474C xenograft model after oral administration.

Aberrant function of the erbB family of receptor tyrosine kinases and their ligands has been described in many human cancers.[1] Within this family, erbB2 plays a central role: mis-regulation of erbB2, for example by over-expression/gene amplification, has been observed to varying degrees in a range of tumours including those of the prostate, cervix, ovary, endometrium, gastrointestinal tract and, in particular, the breast.[2]

With such a large body of evidence implicating erbB2 in tumour progression to date,[3] both antibody and small molecule inhibitory approaches are being developed and have proven to possess anti-tumour activity in the clinic. The monoclonal antibody trastuzumab is now approved for use in erbB2-overexpressing metastatic breast cancer, either as monotherapy or in combination with chemotherapy.[4] The mixed EGFR/erbB2 small molecule inhibitor lapatinib has recently been approved in combination with capecitabine for women with metastatic erbB2-positive breast cancer who have failed to respond to trastuzumab.[5] Additional small molecule inhibitors[1b] such as the erbB2 selective inhibitor CP-724714, and the irreversible inhibitors HKI-272 and BIBW-2992 are undergoing clinical trials (Fig. 1).

We previously reported work on anilinoquinazolines substituted at the C-5 position, as inhibitors of Src,[6] EGFR[7] and erbB2.[8] In the latter publication, compound **1** was described as a potent inhibitor of erbB2 that displayed good pharmacokinetics in mouse and inhibited tumour growth in the mouse BT474C xenograft model.

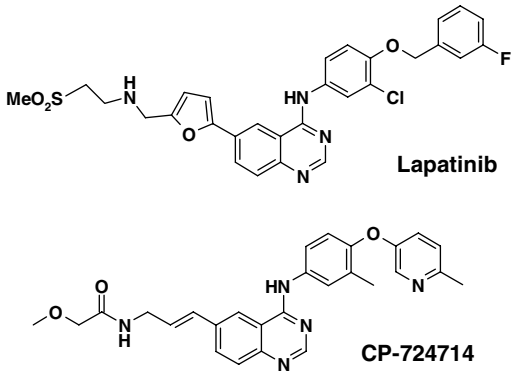

Figure 1.

Keywords: Anilinoquinazoline; C-5 substitution; erbB2 kinase inhibitor.
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However, this compound showed potent inhibition of the hERG channel (IC_{50} = 5 μM), modest pharmacokinetics in other species (especially dog) and mild to severe effects in liver and lung in rat toxicological studies attributed to phospholipidosis, which precluded its progression beyond pre-clinical models. We reasoned that the basicity of the *N*-methyl piperidine (pK_a = 8.1) and the high lipophilicity of **1** could be responsible for its affinity to the hERG channel and phospholipidosis. Work on neutral 5-substituted 4-anilinoquinazolines derived from **1** has shown that good pharmacokinetics in rat and dog and absence of phospholipidosis after chronic dosing in the rat could be achieved in this series.[9]

In this publication, we describe a new series of neutral 5-substituted anilinoquinazolines as potent and orally active erbB2 inhibitors (Fig. 2). Representative compounds prepared during the course of this work are listed in Table 1, and synthetic routes are outlined in the following Schemes.[10]

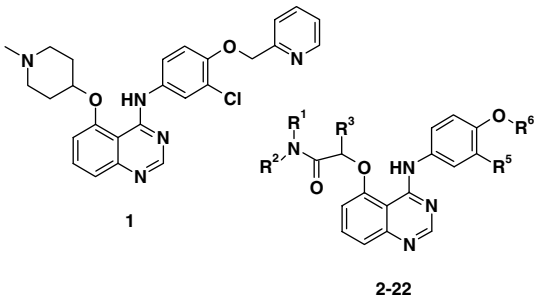


Figure 2.

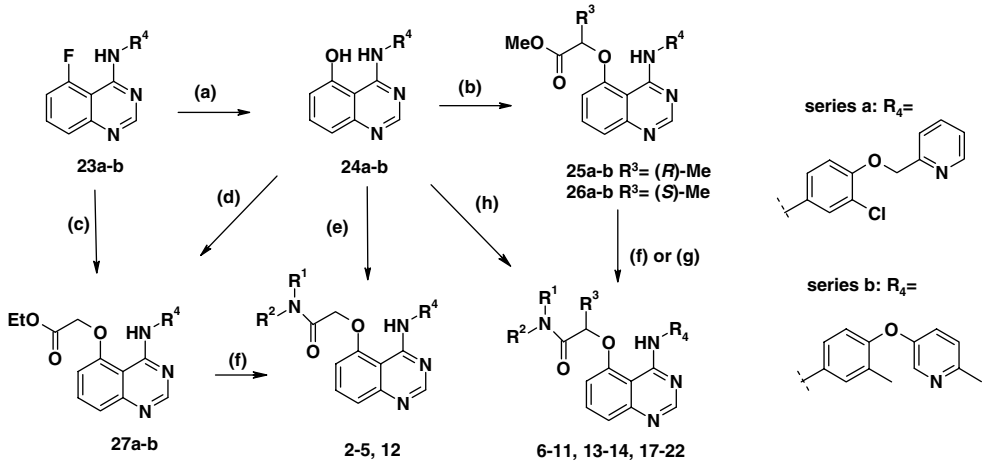
Compounds **2**–**14** and **17**–**22** were prepared from the key fluoroanilinoquinazolines **23a**[8] and **23b** (made from 5-fluoro-4-chloroquinazoline[8]). The key sequence (Scheme 1) involves nucleophilic displacement of the fluoro group with 2-acetamidoethoxide[11] or with methoxide followed by demethylation with pyridine hydrochloride under mild conditions to give the corresponding phenols **24a** and **24b**, Mitsunobu reaction with the corresponding lactate or glycolate and transformation of the ester into the amide directly or via the carboxylic acid.[12,13] Other analogues were synthesised using similar approaches, for example: Mitsunobu reaction of **24b** with (*S*)-*N*,*N*-dimethyl lactamide to give **21**; alkylation of the phenol **24a** with the corresponding haloacetamide (illustrated by **2** and **5**); nucleophilic displacement of the 5-fluoro group from **23a** with ethyl glycolate to give ester **27a** which can be readily transformed into the corresponding amides. An efficient method (Scheme 2) for variation of the aniline at the final step was also developed: **32** which was made from **28**[6a] in four steps was activated with $POCl_3$ and reacted with the corresponding anilines[10] to give **15**–**16**.

The compounds listed in Table 1 were evaluated in an erbB2 autophosphorylation assay using a MCF7 breast carcinoma cell line engineered to overexpress erbB2 as described previously.[8,10] Initial glycolamide-derived compounds **2**–**5** showed moderate activity in this assay. Introduction of a methyl on the position α to the amide significantly improved potency as illustrated by the following pairs (**8** vs **4**, **6** and **7** vs **3**).

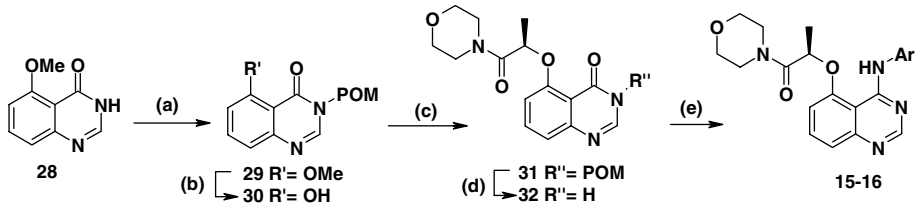
Table 1. Inhibition data versus erbB2 cellular autophosphorylation and hERG for compounds **2**–**22**

Compound	R^5	R^6	R^3	NR^1R^2	Synthetic route[a] (for Scheme 1)	p-erbB2 IC_{50}[b] (μM)	hERG IC_{50}[c] (μM)
2	Cl	CH_2-(2-Pyridyl)	H	NH_2	a,e	0.180	
3	Cl	CH_2-(2-Pyridyl)	H	$N(Me)CH_2CH_2OH$	c,f	0.120	
4	Cl	CH_2-(2-Pyridyl)	H	4-Morpholine	c,f	0.140	
5	Cl	CH_2-(2-Pyridyl)	H	4-Me-Piperazine	a,e	0.350	
6	Cl	CH_2-(2-Pyridyl)	(*R*)-Me	$N(Me)CH_2CH_2OH$	a,b,g	0.031	12
7	Cl	CH_2-(2-Pyridyl)	(*S*)-Me	$N(Me)CH_2CH_2OH$	a,b,g	0.079	11
8	Cl	CH_2-(2-Pyridyl)	(*R*)-Me	4-Morpholine	a,b,f	0.019	10
9	Cl	CH_2-(2-Pyridyl)	(*R*)-Me	NMe_2	a,b,f	0.034	14
10	Cl	CH_2-(2-Pyridyl)	(*S*)-Me	NMe_2	a,b,f	0.043	10
11	Cl	CH_2-(2-Pyridyl)	(*R*)-Me	NHMe	a,b,g	0.066	32
12	Me	3-Pyridyl-6-Me	H	$N(Me)CH_2CH_2OH$	a,d,f	0.130	
13	Me	3-Pyridyl-6-Me	(*R*)-Me	4-Morpholine	a,b,f	0.022	
14	Me	3-Pyridyl-6-Me	(*S*)-Me	4-Morpholine	a,b,f	0.035	
15	Cl	3-Pyridyl-6-Me	(*R*)-Me	4-Morpholine		0.027	
16	OMe	3-Pyridyl-6-Me	(*R*)-Me	4-Morpholine		0.023	
17	Me	3-Pyridyl-6-Me	(*R*)-Me	$NHCH_2CH_2OH$	a,b,f	0.200	
18	Me	3-Pyridyl-6-Me	(*R*)-Me	$N(Me)CH_2CH_2OMe$	a,b,f	0.120	
19	Me	3-Pyridyl-6-Me	(*R*)-Me	$N(Me)CH_2CH_2OH$	a,b,f	0.069	12
20	Me	3-Pyridyl-6-Me	(*S*)-Me	$N(Me)CH_2CH_2OH$	a,b,f	0.078	
21	Me	3-Pyridyl-6-Me	(*R*)-Me	NMe_2	a,b,f or a,h	0.023	8
22	Me	3-Pyridyl-6-Me	(*S*)-Me	NMe_2	a,b,f or a,h	0.030	11

[a] Steps used for the synthesis (as referred to in Scheme 1).
[b] $n \geqslant 2$, standard error is typically 0.3 log unit.
[c] $n \geqslant 2$.



Scheme 1. Synthesis of compounds **2**–**14** and **17**–**22**. Reagents and conditions: (a) $AcNHCH_2CH_2OH$ (2.5 eq), NaH (6 equiv), DMA, 120 °C (series a) or MeONa (3 equiv), MeOH, reflux, then pyridine·HCl (4 equiv), pyridine, reflux (series b); (b) $(t\text{-}BuO_2C)_2N_2$ or $(EtO_2C)_2N_2$, PPh_3, (*R*) or (*S*)-methyl lactate, CH_2Cl_2, 20 °C; (c) EtONa, ethyl glycolate, reflux (series a); (d) $(EtO_2C)_2N_2$, PPh_3, ethyl glycolate, CH_2Cl_2, 20 °C (series b); (e) $BrCH_2CONH_2$, K_2CO_3, DMA, 20 °C (for **2**); $ClCH_2CO$-*N*-piperazine-*N*′-BOC, KI, K_2CO_3, DMA, 60 °C, then TFA, 20 °C, then 37% aq CH_2O, HCO_2H, DMSO, 180 °C μw heating (for **5**); (f) NaOH, THF/MeOH or EtOH, 20 °C; HATU, N*i*-Pr_2Et, NHR^1R^2, DMF, 20 °C (for **3** and **4**) or HATU, N*i*-Pr_2Et, DMA, 50–70 °C, then NHR^1R^2, 20 °C (for **8**–**10**, **12**) or HOBT, EDCI, NHR^1R^2, DMF, 20 °C (for **13**–**14**, **17**–**22**); (g) THF or EtOH, NHR^1R^2, μw heating (for **6**–**7**, **11**); (h) $(t\text{-}BuO_2C)_2N_2$, PPh_3, (*R*) or (*S*)-*N*,*N*-dimethyl lactamide, CH_2Cl_2, 20 °C (for **21**–**22**).



Scheme 2. Synthesis of compounds **15**–**16**. Reagents and conditions: (a) NaH, DMF, 25 °C, then $ClCH_2OC(O)t$-Bu; (b) $MgBr_2$, pyridine, 120 °C; (c) $(t\text{-}BuO_2C)_2N_2$, PPh_3, 4-((*S*)-2-hydroxypropionyl)morpholine,[14] CH_2Cl_2, 20 °C; (d) 7 N NH_3, MeOH, 20 °C; (e) $POCl_3$ (1.2 equiv), N*i*-Pr_2Et (2.5 equiv), $ClCH_2CH_2Cl$, 80 °C; $ArNH_2$ (1.05 equiv), CH_3CN, 80 °C.

A similar SAR for the C-5 substitution was observed when using the aniline from series b at C-4 (compounds **12**–**14**, **17**–**22**): lactamides were preferred over glycolamides. The level of activity between the 2 anilines was similar. Exploration of the SAR around the aniline from series b was studied (**15**–**16** vs **13**). The 3-methyl on the aniline could be replaced by a chlorine or a methoxy without significant loss of activity.

Selectivity versus EGFR was evaluated against EGFR and erbB2 isolated enzymes,[8,10] and in BT474C and KB cell lines, known to respond, respectively, to stimulation of both erbB2 and EGFR, and EGFR alone.[8] Excellent separation between erbB2 activity and EGFR activity was seen with compounds bearing the aniline from series b whereas a less marked difference was seen with the aniline of series a (see **19** and **21** vs **9** in Table 2). Compounds **9**, **19** and **21** showed a clean selectivity profile versus in-house and external kinase panels (data not shown).[15]

Inhibition of the hERG channel was evaluated[10] on a selection of compounds (see Table 1). Slightly reduced hERG activity was seen compared to **1**, as previously observed with neutral compounds.[16]

Physical properties and pharmacokinetic parameters of selected compounds were evaluated further (see Table 3). Although being more lipophilic than **9**, **21** displayed better solubility and higher levels of free drug. Excellent pharmacokinetic parameters in rat and dog were seen for both compounds, with low clearance and high bioavailability.[17]

Compounds **19** and **21** were evaluated for inhibition of phosphorylation of erbB2 in BT474C[18] xenograft in athymic mice. Compound **19**, dosed at 100 mg/kg orally with coadministration of ABT[9,20] (an irreversible inhibitor[19] of cytochrome P450 known to reduce P450 mediated clearance in vivo), gave 78% and 36% inhibition, respectively, at 1 and 8 h post dose, which correlates with free exposure over the in vitro BT474C cell IC_{50} (2.3-fold at 1 h; 0.6-fold at 8 h). Compound **21**, dosed at 100 mg/kg orally without ABT, gave 37% and 55% inhibition, respectively, at 1 and 8 h post dose.

In summary, we have discovered a new series of 5-substituted anilinoquinazolines displaying good activity and good pharmacokinetics compared to the initial lead **1** containing a basic 5-substituent. Representative compounds **19** and **21** inhibited phosphorylation of erbB2

Table 2. Inhibition data versus erbB2 and EGFR kinases, erbB2 cellular autophosphorylation, BT474C and KB cell proliferation assays for compounds **9**, **19** and **21**

Compound	erbB2 enz. IC_{50}[a] (μM)	EGFR enz. IC_{50}[a] (μM)	p-erbB2 IC_{50}[a] (μM)	BT474C IC_{50}[a] (μM)	KB IC_{50}[a,b] (μM)
9	<0.001	0.022	0.034	0.100	1.0[c]
19	0.002	2.0	0.069	0.270	23[c]
21	<0.001	1.1	0.023	0.320	2.7

[a] $n \geqslant 2$, standard error is typically 0.3 log unit.
[b] KB cell proliferation end point after EGF stimulation.
[c] $n = 1$.

Table 3. Pharmacokinetic parameters, plasma protein binding, solubility and log *D* for compounds **9**, **19** and **21**

Compound	Rat/dog Cl %hbf[a]	Rat/dog V_{dss}[a] (L/kg)	Rat/dog *F*%[a]	Rat/dog/mouse %free[b]	Solubility[c] (μM)	log *D*[d]
9	4.3/24	0.5/1	75/89	0.4/1.1/—	6	3.7
19	23/34	1/0.9	34/33	2.9/3.8/2.5	66	3.8
21	12/48	0.9/1.8	67/47	1.7/2.5/1.1	28	>4.3

[a] Female Han Wistar rats dosed at 2 mg/kg iv and 5 mg/kg po except for **9** dosed at 0.4 mg/kg iv and 1 mg/kg po; mean values for male and female beagle dogs dosed at 0.2 mg/kg iv and 0.4 mg/kg po except for **21** dosed at 1 mg/kg iv and 2 mg/kg po; Cl expressed as % of hepatic blood flow.
[b] Protein binding of compound in plasma, expressed as %free.
[c] Solubility in aqueous phosphate buffer, pH 7.4, at 24 h.
[d] Measured from octanol/water, pH 7.4.

in a mouse BT474C xenograft model after oral administration. Further evolution of 5-substituted 4-anilinoquinazolines will be reported in due course.

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10. For experimental procedures and hERG assay protocol, see: Bradbury, R. H.; Kettle, J. G.; Scott, J. S.; Barlaam, B. C. PCT Int. Appl. WO 2005118572. In particular, the synthesis of the aniline precursor of compounds **15**–**16** is described herein.
11. The phenol **24a** resulted from the intramolecular nucleophilic attack of the anion of the acetamide (formed in the reaction conditions because of excess of sodium hydride) onto the ether adduct. This intermediate is formed by displacement of the fluoro group of **23a** with sodium 2-acetamidoethoxide.
12. In the absence of amine, activation of the carboxylic acid with EDCI or HATU gave the activated anilide resulting from the intramolecular attack of the NH of the aniline onto the activated carboxylic acid; this activated anilide can be isolated and reacted with an amine to form the expected amide. Alternatively in the presence of the amine, activation of the carboxylic acid gave the expected amides with minor amounts of the activated anilide.
13. Good enantiomeric purity was observed with these methods: **19** and **21** made as described in Scheme 1 gave, respectively, 99.6% ee and >99.6% ee measured by chiral HPLC; **21** made in a similar manner as compounds in Scheme 2 gave 98.6% ee; however attempts to displace **23b** with the (*R*)-*N*,*N* dimethyl lactamide in the presence of sodium hydride in THF at reflux gave partial racemisation.

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20. Increased blood levels of **19** dosed at 100 mg/kg orally were seen after oral coadministration of 100 mg/kg of ABT (AUC 670 μM h vs 130 μM h without ABT and concentration at 8 h 23 μM vs 2 μM).