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

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Abstract—We have identified a new series of C-5 substituted indazolylaminoquinazolines as potent erbB2 kinase inhibitors. The lead compound **22** showed excellent in vitro potency, good physical properties, acceptable oral pharmacokinetics in rat and dog, and low human in vitro clearance. It showed at least equivalent activity dose for dose compared to lapatinib in various erbB2- or EGFR-driven xenograft models after chronic oral administration.

Following the discovery that overexpression of erbB2 and EGFR (erbB1) 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 tumours cells.[1]

Both antibody and small molecule inhibitory approaches are being developed and have demonstrated 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.[2] The mixed EGFR/erbB2 small molecule inhibitor lapatinib has just been approved in combination with capecitabine for women with metastatic erbB2-positive breast cancer who have failed to respond to trastuzumab.[3] Additional small molecule inhibitors like the erbB2 selective inhibitor CP-724714, the irreversible inhibitors HKI-272 and BIBW-2992 and the mixed EGFR–erbB2 BMS-599626[4] are undergoing clinical trials (Fig. 1).

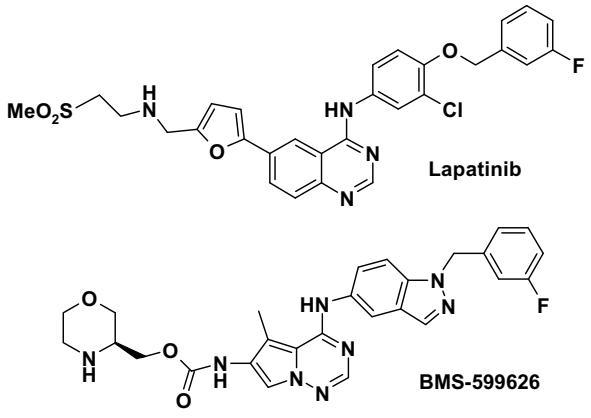

Figure 1.

The latter utilises a N^1-benzylindazolyl-5-amino as the substituent which, from the X-ray structure of lapatinib/EGFR complex,[5] has been predicted to bind to the deep hydrophobic selectivity pocket of the erbB2 kinase.[6] We[7] and others[6,8] had previously reported the use of such substituted indazolylamino groups on quinazolines or related hinge binding cores as potent erbB2 inhibitors. We also previously reported the work on anilinoquinazolines substituted at the C-5 position as inhibitors of Src,[9] EGFR[10] and erbB2.[11–13] In the latter

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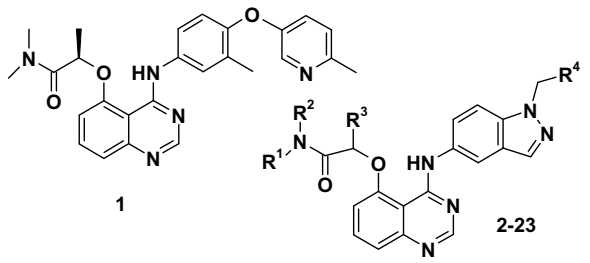


Figure 2.

publication,[13] we described a series of anilinoquinazolines bearing a C-5 substituent derived from lactamides or glycolamides. Compound **1** was described as a selective inhibitor of erbB2 which displayed good pharmacokinetics in preclinical species, and inhibited phosphorylation of erbB2 in the mouse BT474C xenograft model.

In this publication, we describe a new series of neutral indazolylaminoquinazolines **2**–**23** bearing glycolamide- and lactamide-derived side chains at C-5 as potent, orally active erbB2 inhibitors[14] (Fig. 2).

Synthetic routes to representative compounds listed in Table 1 have previously been reported.[13,14] As an illustration, the routes used for making **22** are outlined in the scheme below (Scheme 1).

The compounds were evaluated in a BT474C proliferation assay and in an erbB2 autophosphorylation assay using a MCF7 breast carcinoma cell line engineered to overexpress erbB2 as described previously.[11,14] Table 1 summarises the in vitro potency of the representative compounds prepared in the course of this work.

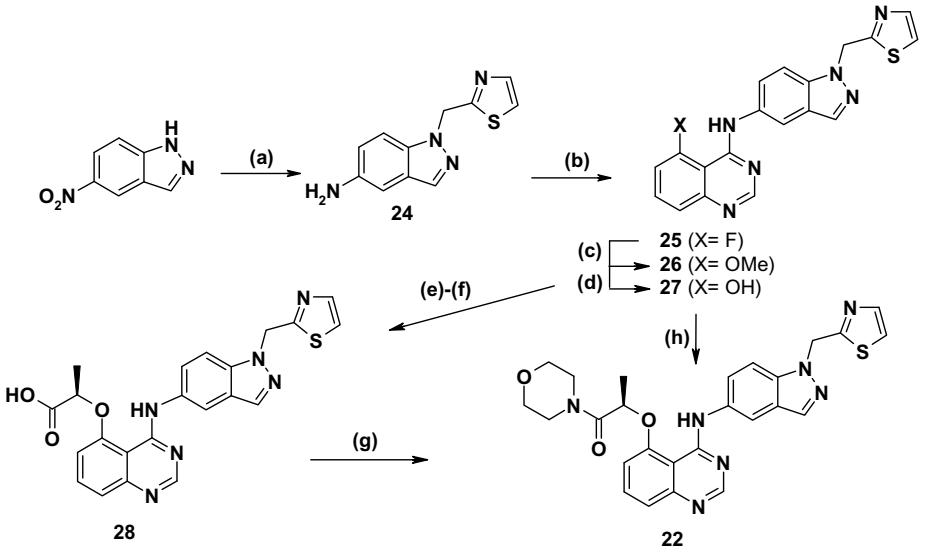
Indazolylaminoquinazoline **2** exhibited good potency in vitro (Table 2), good physical properties and acceptable pharmacokinetics in rat and dog (Table 3), no CYP450 mediated drug–drug interaction potential (inhibition of 5 major isoforms of P450: $IC_{50} > 10$ μM) and low hERG activity (IC_{50}: 25 μM). It was evaluated for the inhibition of phosphorylation of erbB2 in BT474C xenograft in the athymic mice. When dosed at 100 mg/kg orally, it showed 47%, 52% and 49% inhibition, respectively, at 1, 4 and 16 h. However, it showed much higher intrinsic clearance in human microsomes and hepatocytes compared to rat (respectively, microsomal Cl_{int} 183 vs 10 μl/min/mg and hepatocyte Cl_{int} 132 vs 19 μl/min/10^6 cell), which led to a high risk of poor human pharmacokinetics.

We explored the variations of both substituents on the indazole and at the C-5 position with the aim of keeping the good in vitro potency and physical properties, and reducing the in vitro human microsomal intrinsic clearance. The presence of a methyl on the position α to the amide increased potency (as shown by comparison of **2**

Table 1. Inhibition data versus BT474C cell proliferation and erbB2 cellular autophosphorylation and human and rat microsomal intrinsic clearance data for compounds **1**–**23**

Compound	R3	R4	NR1R2	BT474C IC_{50}[a] (μM)	p-erbB2 IC_{50}[a,b] (μM)	Microsome Cl_{int}[c] (μl/min/mg)	
						Human	Rat
1				0.32	0.023		
2	(*R*)-Me	2-Pyridyl	NMe_2	0.29	0.090	183	10
3	(*S*)-Me	2-Pyridyl	NMe_2	0.31	0.080	33	26
4	(*R*)-Me	2-Pyridyl	NH_2	0.15	0.032	27	9
5	(*R*)-Me	2-Pyridyl	NHMe	0.56	0.059	27	13
6	(*R*)-Me	2-Pyridyl	1-Pyrrolidinyl	0.28		75	19
7	(*R*)-Me	2-Pyridyl	4-Morpholinyl	0.10	0.051	23	<3
8	(*S*)-Me	2-Pyridyl	4-Morpholinyl	0.25	0.14	21	9
9	H	2-Pyridyl	NMe_2	0.82			
10	H	2-Pyridyl	4-Morpholinyl	0.61		7	
11	(*R*)-Me	3-F–Ph	NMe_2	0.029	0.008	305	34
12	(*S*)-Me	3-F–Ph	NMe_2	0.035	0.006	77	77
13	(*R*)-Me	3-F–Ph	NHMe	0.029	0.057	36	34
14	(*R*)-Me	3-F–Ph	4-Morpholinyl	0.005	0.022	46	40
15	(*R*)-Me	4-Thiazolyl	NMe_2	0.35	0.23[d]	264	18
16	(*S*)-Me	4-Thiazolyl	NMe_2	0.50	0.13	26	35
17	(*R*)-Me	4-Thiazolyl	NH_2	0.20	0.14	20	22
18	(*R*)-Me	4-Thiazolyl	4-Morpholinyl	0.16	0.16[d]	16	10
19	(*R*)-Me	2-Thiazolyl	NMe_2	0.14	0.026	146	14
20	(*S*)-Me	2-Thiazolyl	NMe_2	0.19	0.024	36	46
21	(*R*)-Me	2-Thiazolyl	NHMe	0.47	0.24	16	<6
22	(*R*)-Me	2-Thiazolyl	4-Morpholinyl	0.037	0.043	20	<5
23	(*S*)-Me	2-Thiazolyl	4-Morpholinyl	0.13	0.012[d]	28	14

[a] $n \geqslant 2$, standard error is typically 0.3 log unit.
[b] erbB2 cellular autophosphorylation assay in an erbB2 overexpressing MCF7 breast carcinoma engineered cell ('Clone 24').
[c] $n \geqslant 2$, tested at 1 μM concentration, using female rat microsome.
[d] $n = 1$.



Scheme 1. Synthesis of compound **22**. Reagents and conditions: (a) 2-($ClCH_2$)-thiazole,[15] K_2CO_3, DMF, 75 °C, H_2, PtO_2, MeOH, 20 °C; (b) 5-F–4-Cl-quinazoline[11] (1 equiv), N*i*-Pr_2Et (1 equiv), *i*-PrOH, 80 °C; (c) MeONa (3 equiv), MeOH, reflux; (d) pyridine·HCl (5 equiv), pyridine, reflux; (e) (*t*-$BuO_2C)_2N_2$, PPh_3, (*S*)-methyl lactate, CH_2Cl_2, 20 °C; (f) NaOH, THF/MeOH, 20 °C; (g) 2-OH–pyridine-N-oxide, N*i*-Pr_2Et, morpholine, EDCI, DMF, 20 °C; (h) (*t*-$BuO_2C)_2N_2$, PPh_3, 4-((*S*)-2-hydroxypropionyl)morpholine,[16] CH_2Cl_2, 20 °C.

Table 2. Inhibition data versus erbB2 and EGFR kinase, erbB2 and EGFR cellular autophosphorylation, cell proliferation assays for compounds **2** and **22**

Compound	erbB2 enzyme[a] (μM)	EGFR enzyme[a] (μM)	p-erbB2 IC_{50}[a] (μM)	p-EGFR IC_{50}[a,b] (μM)	BT474C IC_{50}[a] (μM)	Clone 24 IC_{50}[a,c] (μM)	KB IC_{50}[a,d] (μM)
2	IC_{50} 0.008	IC_{50} 0.18	0.09		0.29	0.36	1.1
22	K_i 0.0027	K_i 0.0016	0.043	0.366	0.037	0.093	0.40

[a] $n \geqslant 2$.
[b] EGFR cellular autophosphorylation assay in KB cell following EGF stimulation.
[c] 'Clone 24' proliferation assay.
[d] KB cell proliferation assay following EGF stimulation.

Table 3. Pharmacokinetic parameters, plasma protein binding, solubility and log *D* for compounds **2** and **22**

Compound	Rat/dog Cl[a] %hbf	Rat/dog Vdss[a] (L/kg)	Rat/dog *F*%[a]	Rat/dog/mouse/human[b] % free	Solubility[c] (μM)	log *D*[d]
2	22/77	2.1/2.3	36/40	11/25/8.6/13	270	2.9
22	24/217	2/3.1	50/36	14/25/10/22	101	2.6

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

and **3** vs **9**; **7** and **8** vs **10**). Primary, secondary or tertiary amides were tolerated, including cyclic versions (**6**–**8**), with morpholine **7** being the most potent substituent.

Other substituents than the 2-picolyl group on the indazole were accepted. The 3-fluorobenzyl group showed an increased potency compared to the 2- or 4-thiazolylmethyl or the 2-picolyl (see **11** vs **2**, **15** and **19** or **14** vs **7**, **18** and **22**). However, better aqueous solubility and lower plasma protein binding were seen with these more hydrophilic groups compared to the 3-fluorobenzyl group (see Table 4: **2**, **15** and **19** vs **11**). Better solubility was seen with tertiary amides in contrast to the primary or secondary amides (see **2** vs **4**, **5**).

We next looked at the influence of C-5 substitution on human microsomal intrinsic clearance (see Table 1). All (*R*)-dimethyl lactamides (**2**, **11**, **15** and **19**) showed a high clearance. However, the (*S*) enantiomers (**3**, **12**, **16** and **20**) had a much reduced clearance. Reduced clearance was also observed with primary amides (**4** and **17**), *N*-methyl amides (**5**, **13** and **21**) and cyclic ter-

Table 4. Plasma protein binding and solubility for selected compounds

Compound	Rat % free[a]	Solubility[b] (μM)
2	11	270
11	1	3
15	14	44
19	14	29
4		3
5		4

[a] Protein binding of compound in plasma, expressed in % free.
[b] Solubility in aqueous phosphate buffer, pH 7.4 at 24 h.

tiary amides, especially for morpholines (**7**, **8**, **14**, **18**, **22** and **23**).

Interestingly, most compounds, including the (*R*)-dimethyl lactamides **2**, **11**, **15** and **19** which showed a high human microsomal intrinsic clearance, exhibit low to moderate rat microsomal intrinsic clearance (Table 1).

Compound **22** was identified as a highly potent erbB2 inhibitor with lower metabolic clearance both in microsomes and in hepatocytes (intrinsic clearance in human and rat hepatocytes: respectively, 33 and 29 μl/min/10^6 cell) than **2**. Compound **22** also exhibited some EGFR activity, but its erbB2 activity appears to be the primary pharmacology in vitro, based on a phosphorylation endpoint (p-erbB2 in 'Clone 24' vs p-EGFR in EGF-stimulated KB cell) or a proliferation endpoint ('Clone 24' vs EGF-stimulated KB cell proliferation) (Table 2). Good selectivity versus other kinases was observed for **22** in in-house and external kinase panels.[17] Compound **22** displayed good physical properties (solubility, plasma protein binding, log *D*), acceptable pharmacokinetics in rat and dog (Table 3), no CYP450 mediated drug–drug interaction potential (inhibition of 5 major isoforms of P450: $IC_{50} > 10$ μM), low hERG activity (IC_{50}: 23 μM) and high permeability across membranes (P_{app} measured in MDCK-MDR[18] cell line: 10×10^{-6} cm s^{-1}).

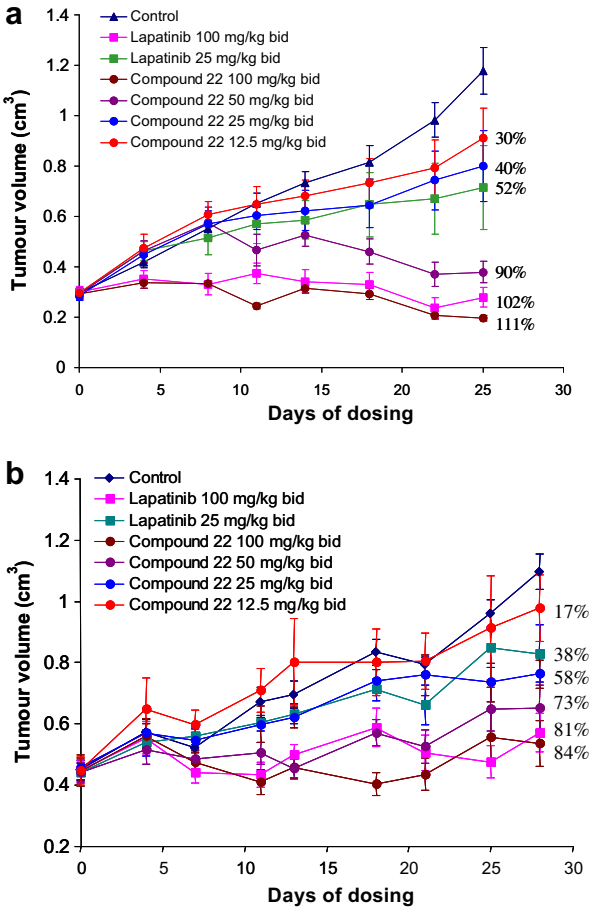


Figure 3. Inhibition of growth of erbB2 dependant xenografts (a, BT474C; b, Calu3), respectively, in nude or SCID mice dosed orally with **22** and lapatinib.

Compound **22** showed good exposure orally in mouse and was evaluated in erbB2-driven xenograft models. A single oral administration of **22** inhibited p-erbB2 levels in the BT474C[19] xenograft model in nude mice (respectively, 90% and 49% inhibition 1 and 8 h postdose at 100 mg/kg). Chronic oral administration of **22** inhibited the growth of BT474C and Calu3[20] xenografts, respectively, in nude and SCID mice (Fig. 3) in a dose dependant manner: compound **22** showed at least equivalent activity dose to dose in these two models compared to lapatinib (Fig. 3).

The EGFR inhibition component of **22** was also evaluated in vivo using the LoVo xenograft model[21] in nude mice. Compound **22** significantly inhibited the growth of the tumour when dosed at 100 mg/kg twice daily, whereas lapatinib was not active in this model at the same dose (Fig. 4).

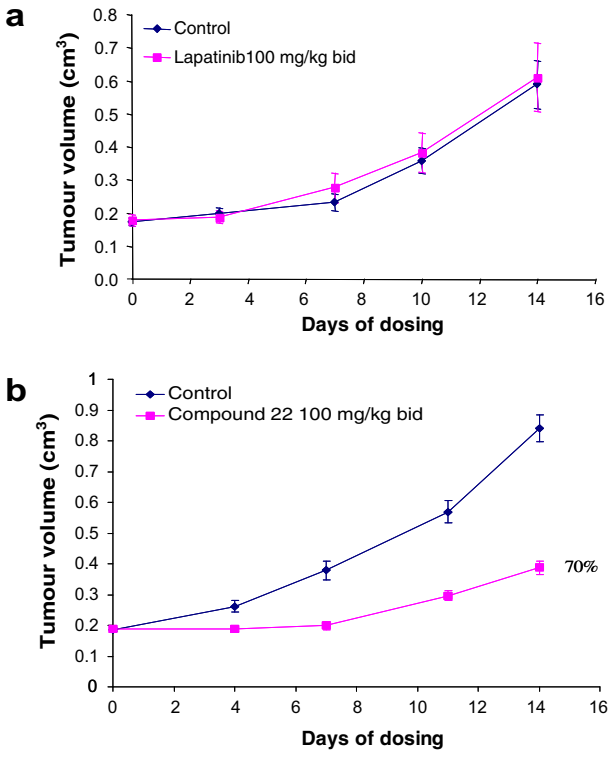


Figure 4. Inhibition of growth of LoVo xenografts in nude mice dosed orally with lapatinib (a) and **22** (b).

Toxicological evaluation in rats after chronic dosing showed no evidence of phospholipidosis at the maximum tolerated dose, in contrast to an earlier lipophilic anilinoquinazoline containing a basic 5-substituent.[12]

In summary, we have identified a new series of C-5 substituted indazolylaminoquinazolines as potent erbB2 kinase inhibitors. The lead compound **22** showed excellent in vitro potency, good physical properties, acceptable pharmacokinetics in rat and dog, and low human in vitro clearance. It showed at least equivalent activity dose to dose compared to lapatinib in various erbB2- or EGFR-driven xenograft models after chronic oral administration. Compound **22** represents an excellent candidate drug for clinical evaluation.

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