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Novel 4-anilinoquinazolines with C-6 carbon-linked side chains: Synthesis and structure–activity relationship of a series of potent, orally active, EGF receptor tyrosine kinase inhibitors

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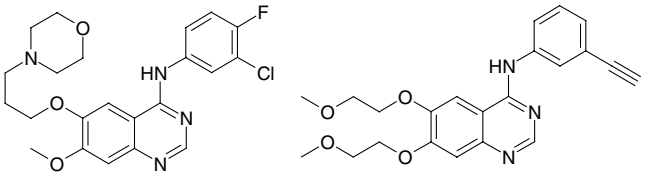
Abstract—The structure–activity relationship of a novel subseries of 4-anilinoquinazoline EGFR inhibitors substituted at the C-6 position with carbon-linked side chains has been investigated. This exploration has led to the discovery of novel aminomethyl carboxamides with good biological, pharmacokinetic and physical properties.

Approximately 20 years after the epidermal growth factor receptor (EGFR) was identified as a potential anticancer target,[1] the first small molecule ATP-competitive inhibitors of the intracellular tyrosine kinase domain of EGFR have been approved for the treatment of patients with advanced non-small-cell-lung cancer (NSCLC). Figure 1 represents the two selective EGFR tyrosine kinase inhibitors (TKIs) gefitinib (Iressa)[2a–d] and erlotinib (Tarceva)[3] currently on the market.

A number of agents which target other kinases in addition to EGFR have also been developed. For instance, lapatinib (erbB2-EGFR)[4] or ZACTIMA™ (VEGFR-EGFR)[5] is currently being evaluated in phase III clinical trials. Monoclonal antibodies targeting the extracellular ligand-binding domain are another successful approach to inhibiting the EGF signaling pathway, leading to the discovery of cetuximab (Erbitux), now approved in association with Irinotecan for advanced colorectal cancer.[6]



gefitinib, Iressa **erlotinib, Tarceva**

Figure 1. Small molecule inhibitors of EGFR on the market.

The anilinoquinazoline core has been extensively used in the design of tyrosine kinase inhibitors as a mimic of the adenine of ATP. The key role of adenine in the binding of ATP into the intracellular kinase domain is well established.[7] The positioning of the aniline into the selectivity pocket, rather small and lipophilic in the case of EGFR, is also well described.[8] In this paper, we present a new sub-family of 4-anilinoquinazolines as potent and selective EGFR inhibitors.

The synthesis of 6-aminomethyl-4-anilinoquinazolines **3**–**36** (Fig. 2) was fully described recently.[9] The amino function was introduced either by reductive amination on aldehyde **1** or by nucleophilic displacement of chloride **2**. Depending on the steric hindrance and/or the reactivity of the amine, R_1 and R_2 were either introduced in one step or sequentially.

Keywords: EGFR tyrosine kinase; Kinase inhibition; Anilinoquinazoline.
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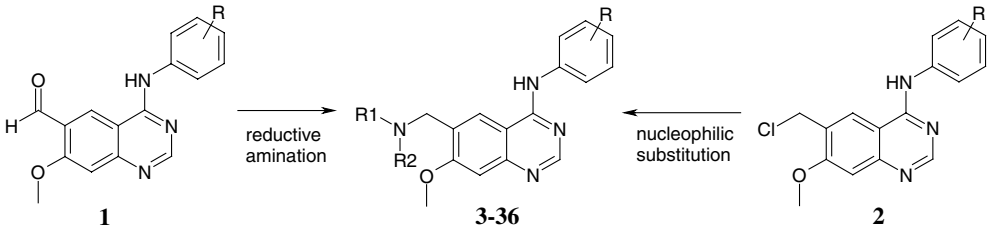


Figure 2. General synthesis of C-6 aminomethyl anilinoquinazolines.

The in vitro activity of the compounds was measured in two assays.[9] The enzyme inhibition assay reflects the ability of a compound to compete with ATP and inhibit the phosphorylation of a tyrosine containing polypeptide substrate by an EGFR tyrosine kinase enzyme. The cell activity was measured by the ability of a compound to inhibit the in vitro proliferation of EGF-stimulated human tumour cell line, KB (ATCC No. CCL-17).

Table 1 lists enzyme and cell inhibition potencies of a subset of pyrrolidines **3**–**12**. Going from the pyrrolidine **3** to the prolinamides **4**–**7** resulted in up to a 30-fold improvement in potency.

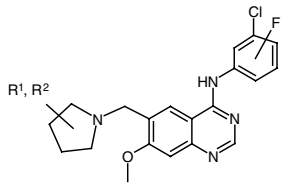
Previously reported work demonstrated that several anilines were suitable for inhibiting EGFRTK.[10] Both the 3-Cl, 4-F and the 2-F, 3-Cl anilines gave compounds more or equally potent than Iressa in this prolinamide series (**4**–**5** and **6**–**7**).[2d] The absolute configuration of the chiral centre was not crucial for potency or other properties (**6**–**7**). Substitution on the prolinamide ring was explored and tolerated (see 4-OMe example **8**). However, the nature and position of the carboxamide group were key to maintain high potency against EGFR. Primary amides were more potent than secondary and tertiary amides (**7** vs **9**–**10**). The isomer of position 3 on the pyrrolidine ring (**11**) or the homologated carboxamide **12** were both less potent than **6**.

A variety of ring systems (**13**–**17**) substituted with a carboxamide group were synthesized, as shown in Table 2. The lowest difference of potency with previous compounds was observed with piperidine **14**, which is structurally the closest to prolinamides **6**–**7**. More important modifications of the ring led to a slight decrease in potency (**13** and **15**–**17**).

Acyclic variants allowed greater SAR modifications without loss of potency, as shown in Table 3. The secondary amine with an α-methyl **18** displayed moderate in vitro activity, but the tertiary amine **19** was 7-fold more active. A combination (α-methyl + tertiary amine) led to the alanine derivatives **20**–**21** that showed potent nanomolar inhibition of EGFRTK, analogous to prolinamides **6**–**7**. Compounds **20** and **21** were also up to 5-fold more potent than the secondary and tertiary amides **22**–**23** or the cyclised amide **24**.

Table 4 shows that a variety of alkyl substituents were tolerated on nitrogen (**21** and **25**–**26**). The only

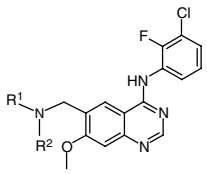
Table 1. Pyrrolidines **3**–**12**

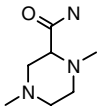
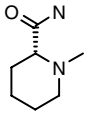
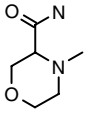
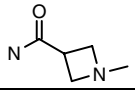


Compound	Aniline	R^1, R^2	Enzyme inhibition IC_{50}[a,b] (μM)	Inhibition of KB growth IC_{50}[b] (μM)
Iressa	2-F, 3-Cl	See Figure 1	0.033	0.054
3	2-F, 3-Cl	H,H	0.36[c]	0.30
4	3-Cl, 4-F	(*R*) 2-$CONH_2$	0.006[c]	0.04
5	3-Cl, 4-F	(*S*) 2-$CONH_2$	0.02	0.09
6	2-F, 3-Cl	(*S*) 2-$CONH_2$	0.03	0.055
7	2-F, 3-Cl	(*R*) 2-$CONH_2$	0.01	0.025
8	2-F, 3-Cl	(*R*, *R*) 2-$CONH_2$-4-OMe	0.01	0.025
9	2-F, 3-Cl	(*R*) 2-CONHMe	0.05	0.1
10	2-F, 3-Cl	(*R*) 2-$CONMe_2$	0.45	0.95
11	2-F, 3-Cl	(*S*) 3-$CONH_2$	0.08	0.15
12	2-F, 3-Cl	(*S*) 2-CH_2CONH_2	0.17[c]	0.13

[a] In all tables, the enzyme inhibition assay is run at 2 μM ATP.
[b] In all tables, numbers are a geometric mean of 2 or more values.
[c] Indicates one measurement.

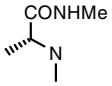
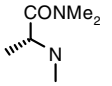
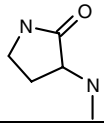
Table 2. Variety of rings **13–17**



Compound	R^1R^2 N	Enzyme inhibition IC_{50} (μM)	Inhibition of KB growth IC_{50} (μM)
13		0.03[a]	0.08
14		0.008[a]	0.075[a]
15		0.05[a]	0.09
16		0.07[a]	0.09
17		0.05	0.3[a]

[a] Indicates one measurement.

Table 3. Linear chains **18–24**

Compound	R^1R^2 N	Enzyme inhibition IC_{50} (μM)	Inhibition of KB growth IC_{50} (μM)
18		0.15[a]	0.12
19		0.02[a]	0.045
20		0.009	0.03
21		0.008	0.025
22		0.03[a]	0.02
23		0.04[a]	0.09
24		0.02	0.07

[a] Indicates one measurement.

noticeable exception was when we introduced a basic group on the side chain as shown by compound **27**. Substitution of the alanine methyl group with ether chains of variable length (**28–30**) or an amine (**31**) sustained an excellent level of potency. We nevertheless reached a limitation when introducing longer basic chains.

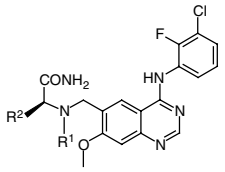
In parallel with this weakly basic series (pK_a of **20** = 6.8), we also explored compounds of higher pK_a in order to modulate the pharmacokinetic profile of the series. As shown in Table 5, cyclic basic amines (pK_as from 7.1 to 9.1) generally displayed excellent potency in both in vitro assays. Piperidines **32–33** and azetidines **34–36** were of interest in view of their larger volume of distribution (>1 L/kg in rat) compared to their neutral counterparts (cf. Table 6). However, they tended to suffer from higher clearance, some efflux or P450 inhibition (data not shown) which in turn led to bioavailability below 50%.

This series of 6-aminomethyl quinazolines proved to be very selective for EGFR over other erbB family members (erbB2 and erbB4 selectivities > 20-fold) as well as over a wide panel of different kinases (IC_{50} > 10 μM against more than 20 representatives of different kinase classes).[11] Moreover, the best neutral compounds, either proline or alanine derivatives, exhibited good physical properties, as represented in Table 6 with compounds **6**, **20** and **21**. The solubilities of these derivatives were high for weakly basic quinazolines, probably due to the reduction of log *P* generated by incorporating a carboxamide group. Levels of unbound drug in plasma were in a range 2–10% in rodent, 1–3% in dog and ~5% in human. The neutral compounds also proved devoid of significant P450 inhibition (IC_{50} > 10 μM), showing good absorption ($CaCO_2$ Papp A-B > 13 cm $s^{-1} \cdot 10^{-6}$) and no efflux.

Pharmacokinetics evaluated in rat and dog showed in general low clearances (<10 ml/min/kg), small volumes of distribution (~1 L/kg) and moderate plasma half lives. Combined with good physical properties, this led to good bioavailabilities (>50%) in both species. The antitumour activity of these anilinoquinazolines was examined using a human tumour xenograft model in athymic mice (Lovo, colorectal tumour).[9] As shown in Table 6, once daily oral administration of 25–50 mg/kg/day for up to 18 days significantly inhibited (37–60%) the growth of these established LoVo tumours, which compares favourably with previously published activity for Iressa.[2d] At the doses used, the percentage of inhibition observed was in good agreement with sustained free plasma exposure (free C_{min} ~ cell IC_{50}).

In conclusion, anilinoquinazolines possessing C-6 aminomethyl side chains proved to be potent and selective inhibitors of EGFR tyrosine kinase. Moreover, the most interesting compounds displayed excellent physical properties and high bioavailabilities in rat and dog. In vivo, they showed significant antitumour activity in xenograft model.

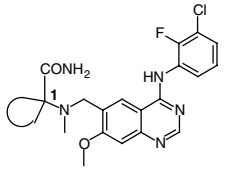
Table 4. Chain variations **25–31**

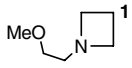


Compound	R^1	R^2	Enzyme inhibition IC_{50} (μM)	Inhibition of KB growth IC_{50} (μM)
21	Me	Me	0.008	0.025
25[a]	*i*-Pr	Me	< 0.001	0.05
26	CH_2CH_2OMe	Me	0.01[b]	0.055
27	$CH_2CH_2NMe_2$	Me	0.12[b]	0.09
28	Me	CH_2OH	0.02	0.03
29	Me	CH_2OMe	0.03[b]	0.035
30	Me	CH_2CH_2OMe	0.01	0.05
31	Me	CH_2NMe_2	0.05[b]	0.075

[a] Compound **25** was very potent but also poorly soluble (1 μM at pH 7.4 in phosphate buffer).
[b] Indicates one measurement.

Table 5. Basic series **32–36**[a]



Compound	Ring	Enzyme inhibition IC_{50} (μM)	Inhibition of KB growth IC_{50} (μM)	pK_a	Rat Cl[b]	Rat Vd[c]
32		0.009	0.04	8.3	55	7.5
33		0.025[d]	0.095	9.1	114	4.9
34		0.02[d]	0.01	7.9	36	9.4
35		0.009[d]	0.035	7.8	61	7.2
36		0.006[d]	0.045	7.1	35	4.2

[a] Rings are attached by the quaternary carbon **1**.
[b] Expressed in ml/min/kg.
[c] Expressed in L/kg.
[d] Indicates one measurement.

Table 6. Pharmacokinetic, in vivo efficacy and physical properties

Compound	Physical properties		Pharmacokinetics[a]					LoVo xenograft	
	Sol[b] (μM)	Log *D*	Rat free drug (%)	Rat/dog Cl (ml/min/kg)	Rat/dog Vd (L/kg)	Rat/dog $T_{1/2}$ (h)	Rat/dog F (%)	Dose (mg/kg/day)	% inhibition tumour
6	260	3.6	3.7	3/5	0.7/0.4	6.6/0.7	111/88	25	43[c,d]
20	40	3.4	6.0	6/—	0.6/—	4.3/—	58/—	25	37[d]
21	40	3.4	6.5	12/14	0.8/0.4	4.8/0.4	75/63	50	60[c,e]

[a] Oral:iv dose = 5:2 mg/kg for rat, 2:1 mg/kg for dog.
[b] Measured at pH 7.4 in phosphate buffer.
[c] Average of two distinct experiments.
[d] % inhibition of the tumour volume by caliper measurement after 18 days.
[e] % inhibition after 13 days.

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References and notes

1. Woodburn, J. R. *Pharmacol. Ther.* **1999**, *82*, 241.
2. (a) Muhsin, M.; Graham, J.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2003**, *2*, 515; (b) Herbst, R. S.; Fukuoka, M.; Baselga, J. *Nat. Rev. Cancer* **2004**, *4*, 956; (c) Barker, A. J.; Gibson, K. H.; Grundy, W.; Godfrey, A. A.; Barlow, J. J.; Woodburn, J. R.; Ashton, S. E.; Curry, B. J.; Scarlett, L.; Henthorn, L.; Richards, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1911; (d) Wakeling, A. E.; Guy, S. P.; Woodburn, J. R.; Ashton, S. E.; Curry, B. J.; Barker, A. J.; Gibson, K. H. *Cancer Res.* **2002**, *62*, 5749.
3. Dowell, J.; Minna, J. D.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2005**, *4*, 13.
4. Wood, E. R.; Truesdale, A. T.; McDonald, O. B.; Yuan, D.; Hassell, A.; Dickerson, S. H.; Ellis, B.; Pennisi, C.; Horne, E.; Lackey, K.; Alligood, K. J.; Rusnak, D. W.; Gilmer, T. M.; Shewchuk, L. *Cancer Res.* **2004**, *64*, 6652.
5. Hennequin, L. F.; Stokes, E. S. E.; Thomas, A. P.; Johnstone, C.; Plé, P. A.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Kendrew, J.; Curwen, J. O. *J. Med. Chem.* **2002**, *45*, 1300.
6. Graham, J.; Muhsin, M.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2004**, *3*, 549.
7. (a) Traxler, P. *Exp. Opin. Ther. Patents* **1997**, *7*, 571; (b) Traxler, P. *Exp. Opin. Ther. Patents* **1998**, *8*, 1599.
8. Stamos, J.; Sliwkowski, M. X.; Eigenbrot, C. *J. Biol. Chem.* **2002**, *277*, 46265.
9. For a full description of synthesis and biological assays, see: (a) Hennequin, L. F. A.; Halsall, C. T. WO2005/026156; 2005; (b) Delouvrié, B.; Harris, C. S.; Hennequin, L. F. A.; Halsall, C. T.; Pease, J. E.; Smith, P. M. WO2005/075439; **2005**.
10. Ballard, P.; Bradbury, R. H.; Harris, C. S.; Hennequin, L. F. H.; Hickinson, M.; Johnson, P. D.; Kettle, J. G.; Klinoswka, T.; Leach, A. G.; Morgentin, R.; Pass, M.; Ogilvie, D. J.; Olivier, A.; Warin, N.; Williams, E. J. *Bioorg. Med. Chem. Lett.*, in press.
11. Examples of kinases tested in our selectivity panels: MAP kinase, JNK, PKA, PDK1, SGK, CDKs, Src, KDR, GSK3b, AMPK, CHK1, SAP kinase, ROCK-ll and LCK.