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Studies Leading to the Identification of ZD1839 (Iressa^{TM}): An Orally Active, Selective Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Targeted to the Treatment of Cancer

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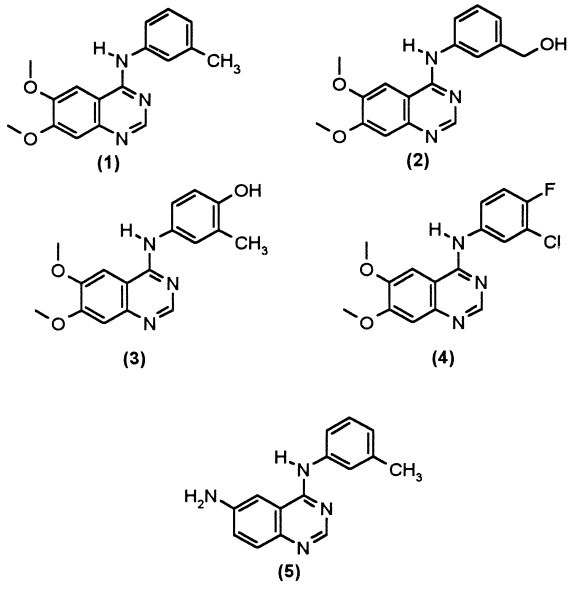
Abstract—This paper describes the development of the epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 from a lead series of 4-anilinoquinazoline compounds. ZD1839 has suitable properties for use as a clinically effective drug and shows activity against human tumours. In particular, the use of pharmacokinetic data in the development of ZD1839 is discussed.

Introduction

The discovery of 4-anilinoquinazolines as a lead series for the inhibition of epidermal growth factor receptor tyrosine kinase (EGFR-TK) activity,[1,2] and the rationale behind the inhibition of this enzyme as a potential method for treating human cancer,[3,4] are well documented. Conventional structure–activity relationship studies have established the substitution patterns within anilinoquinazolines which provide the most potent compounds against EGFR-TK in vitro.[5,6] Compound **1**, identified early in these studies, is a potent inhibitor of EGFR-TK ($IC_{50} = 5$ nM) and a very potent inhibitor of EGF-stimulated human tumour cell growth [$IC_{50} = 50$ nM (KB oral carcinoma cells)]. Compound **1** contains all of the elements required in an anilinoquinazoline for highly potent enzyme inhibition: electron-donating substituents at the 6- and 7-positions of the quinazoline; a small lipophilic substituent at the *meta*-position of the aniline; a free NH at the 4-position and a free CH at the 2-, 5- and 8-positions. On oral dosing, compound **1** showed evidence of antitumour activity against A431 human tumour xenografts in nude mice, but appeared to be rapidly metabolised (half-life approximately 1 h). Two major metabolites were identified; both were oxidation products. Oxidation of the methyl group produced the corresponding benzyl alcohol **2** and oxidation at the *para*-position of the aniline moiety produced the corresponding phenol metabolite **3**.



Substitution with chlorine in place of the methyl group and introduction of a fluorine at the *para*-position of the aniline gave compound **4**, which was resistant to these routes of metabolism. Although this compound did

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show a slight loss of potency in vitro, relative to compound **1** (EGFR-TK enzyme test, $IC_{50}=9$ nM; EGF-stimulated KB cell growth, $IC_{50}=80$ nM), it nevertheless showed a better in vivo profile, having improved efficacy on oral dosing and also reduced clearance. No major metabolites were detected and the half-life in mice improved to approximately 3 h. A related compound **5**, although weaker in the in vitro assays, revealed significant antitumour activity for the first time, at well-tolerated doses in a related model (the KB xenograft).[3] This was associated with a much improved pharmacokinetic profile which highlighted the importance of inhibiting EGFR-TK for a large part of the 24 h in a day, when using once-daily dosing, in achieving good activity in this model. A key structural change in this regard was the substitution at the 6-position of the quinazoline.

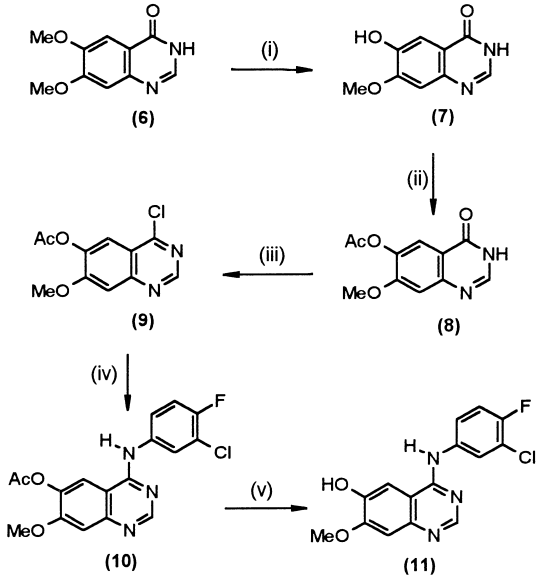
Compound **4** became the focus of further attempts to improve in vivo activity because of its potency and metabolic stability, and also provided a useful starting point for the discovery of different structural types of EGFR-TK inhibitors.[7] Efforts to synthesise irreversible inhibitors have used similar starting points and strategies.[8,9] One approach aimed at improving in vivo activity, which eventually proved successful, involved modification of the alkyl groups of the methoxy side chains. This method of varying the physical properties of the compounds was predicted to favourably influence their pharmacokinetic properties. In particular, basic groups were introduced into the alkoxy side chains, as this was a versatile way to modify physical properties over a wide range of basicity, lipophilicity and solubility. This approach had the advantage of retaining both the potency-enhancing features of the 6- and 7-alkoxy groups, and the metabolic stability associated with the 3-chloro-4-fluoroaniline moiety. In this paper, the effects of modifications to the 6-alkoxy group, involving the addition of nitrogen-containing side chains are reported.

It was believed that good activity in an in vivo tumour model (following once-daily, oral dosing) would require concentrations of drug in the blood sufficient to provide sustained 24-h inhibition of EGFR-TK. Therefore, studies were designed to investigate the blood concentrations in mice at various times following oral doses of the candidate drugs.

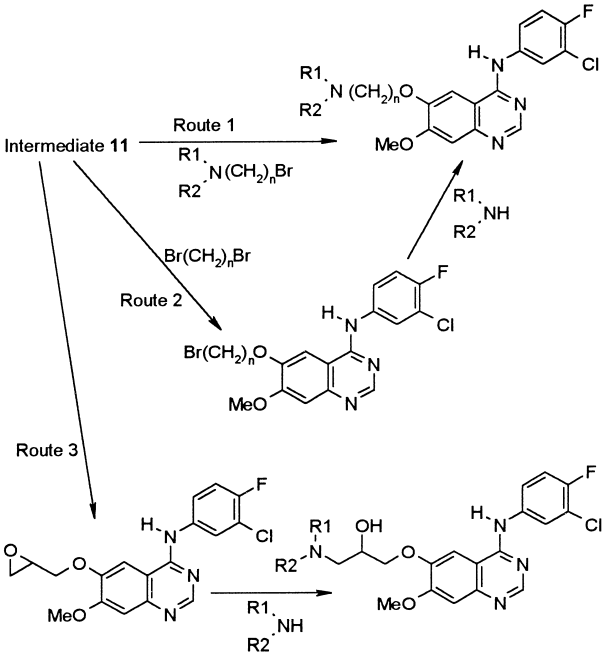
Chemistry

The efficient synthesis of a wide range of 6-alkoxy analogues required a suitable late-stage intermediate, the 6-hydroxy derivative **11** (Scheme 1); details of this synthesis have been reported previously.[7] The key step was the selective removal of the methyl group from the 6-methoxy group of **6**, by means of the methionine in methanesulphonic acid, to give **7**. In the next step, the free phenol group of the intermediate **7** was protected as the acetyl derivative **8**, which was then chlorinated with thionyl chloride to give **9**, followed by reaction with 3-chloro-4-fluoroaniline to give **10** and finally deprotection with methanolic aqueous ammonia to remove the acetyl group and give the required intermediate **11**.

As shown in Scheme 2, the 6-hydroxy intermediate (**11**) was used as the starting point for three different routes by which the required side chains were introduced. First, direct alkylation introduced the preformed side chain in one step (Route 1). Second, introduction of a bromoalkyl group (with dibromoethane or dibromopropane), followed by displacement of the second bromine by an appropriate amine, produced the required compound (Route 2). Third, for side chains containing a 2-hydroxypropyl group, introduction of an epoxy-



Scheme 1. Synthesis of intermediate **11**. Reagents: (i) methionine/$MeSO_3H$/100 °C/3 h; (ii) Ac_2O/pyridine; (iii) $SOCl_2$; (iv) 3-chloro-4-fluoroaniline; (v) NH_4OH/MeOH.



Scheme 2. Routes to final products from intermediate **11**.

propyl group followed by opening of the epoxide with the appropriate amine was used (Route 3).

Results and Discussion

The results are shown in Tables 1–3. The 'TKI' result is the IC_{50} (μM) for the inhibition of EGFR-TK from A431 vulval squamous carcinoma cells. The 'KB2' result is the IC_{50} (μM) for the inhibition of EGF-stimulated proliferation of KB cells in culture. Details of these tests have been described previously.[1]

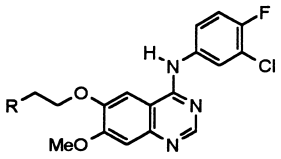
Blood levels of candidate drugs in mice were estimated by a bioassay procedure. Mice (male Alderley Park mice, 30–40 g, three per time point) were dosed orally with 200 mg/kg of compound and blood samples were collected at one or more time points (2, 6 or 24 h) post-dosing. Samples were assayed for EGFR-TK inhibitory activity in the TKI assay. The concentration of compound in the blood was estimated in μM by interpolation from a standard curve, relating percentage inhibition to compound concentration, prepared with 'spiked' blood.

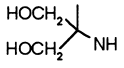
Tables 1–3 show different sub-classes of structural types. Table 1 shows compounds with two carbon atoms between the oxygen atom and the nitrogen atom of the R group. Table 2 shows compounds with three carbon atoms between the oxygen atom and the nitrogen atom of the R group. Table 3 shows compounds with three atoms between the oxygen atom and the nitrogen atom of the R group but with a hydroxyl group at the 2-position of the carbon chain.

The parent compound **4**, the 6,7-dimethoxy analogue, gave blood concentrations in the TKI assay of 38, 16 and 0.3 μM, at the 2-, 6- and 24-h time points, respectively (Table 1).

The TKI results show that all of the compounds reported here are potent enzyme inhibitors (IC_{50} <400 nM); compound **17** (IC_{50} = 2 nM) was the most potent inhibitor. In the EGF-stimulated cell proliferation test, all of the compounds had IC_{50} <1 μM; compound **29** (IC_{50} = 80 nM) was the most potent inhibitor of tumour cell growth.

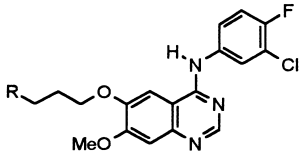
Table 1. Compounds with two carbon atoms between the 6-oxygen and the side-chain nitrogen atom



		In vitro[a]		In vivo[b]		
Compd	R	TKI	KB2	2 h	6 h	24 h
4		0.009	0.08	38	16	0.3
12		0.069	0.91	—	0.1	0.3
13	$(HOCH_2CH_2)_2N$	0.035	0.46	—	1.0	—
14	MeNH	0.008	0.35	—	1.6	0.1
15	$MeOCH_2CH_2NH$	0.008	0.18	—	11	—
16		0.066	0.64	—	164	7.9
17	$Me_2NCH_2CH_2NH$	0.002	0.15	—	1.1	0.4
18	Me_2N	0.007	0.12	82	52	—
19		0.01	0.38	—	156	5.6
20	$(MeOCH_2CH_2)_2N$	0.061	0.17	—	7.2	1.7
21		0.31	0.22	—	0.4	4.7
22		0.01	0.09	—	7.1	2.4
23		0.1	0.32	—	0.1	0.1
24		0.087	0.73	—	8.2	3.5
25	Et_2N	0.093	0.31	—	2.8	2.3
26		0.098	0.19	—	16	35

— indicates measurement not taken or replicates were considered unsuitable to give a reliable value.
[a]Values are IC_{50} in μM.
[b]Values are concentrations in blood (μM) and are the average of three separate determinations.

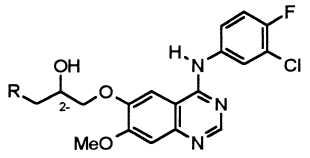
Table 2. Compounds with three carbon atoms between the 6-oxygen and the side-chain nitrogen atom



		In vitro[a]		In vivo[b]		
Compd	R	TKI	KB2	2 h	6 h	24 h
27	Me_2N	0.049	0.15	—	34	—
28		0.084	0.16	—	24	1.3
29		0.023	0.08	6.8	38	5.7
30	Et_2N	0.057	0.16	—	5.4	0.9
31		0.071	0.12	—	8.8	3.7
32		0.079	0.12	—	5.2	2.3

— indicates measurement not taken or replicates were considered unsuitable to give a reliable value.
[a]Values are IC_{50} in μM.
[b]Values are concentrations in blood (μM) and are the average of three separate determinations.

Table 3. Compounds with three carbon atoms between the 6-oxygen and the side-chain nitrogen atom, and containing a 2-hydroxy substituent



Compd	R	In vitro[a] TKI	In vitro[a] KB2	In vivo[b] 2 h	In vivo[b] 6 h	In vivo[b] 24 h
33	$(HOCH_2CH_2)_2N$	0.005	0.85	—	0.2	—
34	H_2N	0.006	0.39	—	0.1	—
35	MeNH	0.005	0.22	1.1	0.5	—
36		0.008	0.38	—	0.04	0.03
2-S-37		0.095	0.16	—	23	3.0
2-R-38		0.067	0.13	—	15	0.43

— indicates measurement not taken or replicates were considered unsuitable to give a reliable value.
[a]Values are IC_{50} in μM.
[b]Values are concentrations in blood (μM) and are the average of three separate determinations.

Drug concentrations in blood varied over a wide range (0.1–164 μM at 6 h, Tables 1–3). The imidazo-ethyl derivative **16**, and the morpholino-ethyl derivative **19** produced the highest concentrations at the 6-h time point. Amongst the structural types described here, the morpholino derivatives consistently produced high blood concentrations at the 6-h time point. These compounds also sustained a higher concentration at 24 h than the parent compound **4**. Compounds containing a 2-hydroxy substituent (Table 3) generally achieved low blood concentrations.

Table 2 includes the compound that was eventually chosen as a drug development candidate (**29**; ZD1839). In vitro, ZD1839 is not the most potent compound reported here, but achieves high and sustained blood levels, in vivo, over a 24-h period. At the 24-h time point, the concentration of drug in the blood (5.7 μM) from this dosing regime (200 mg/kg po) is approximately 70 times that of the IC_{50} in the KB2 cell test (0.08 μM). As well as ZD1839, several other compounds reported here showed high and sustained blood concentrations; these results were used to prioritise candidates for further antitumour testing in xenograft models.

ZD1839 was identified as being of particular interest. As well as having good oral bioavailability, ZD1839 inhibits the growth of a broad range of human solid tumour xenografts in a dose-dependent manner (range 12.5–200 mg/kg, po once daily) with marked regressions seen in some tumours. Treatment for up to 4 months in nude mice was well tolerated.[10] ZD1839 has demonstrated a long half-life in humans compatible with once-daily oral dosing and extensive Phase I clinical trials have shown biomarker evidence for inhibition of the EGFR signal transduction pathway and antitumour activity.

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