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EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE: STRUCTURE-ACTIVITY RELATIONSHIPS AND ANTITUMOUR ACTIVITY OF NOVEL QUINAZOLINES.

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Abstract: Investigation of structure-activity relationships of novel quinazolines has identified a 4-(4-*iso*-quinolylamino)-quinazoline and a 4-(*trans*-2-phenylcyclopropylamino)-quinazoline as potent inhibitors of EGF-receptor tyrosine kinase *in vitro*. Further modifications of the latter compound have identified a derivative which shows anti-tumour activity against a tumour xenograft model when dosed orally once per day.

Introduction

Overexpression of the epidermal growth factor receptor (EGF-R) is common in a wide variety of major human solid tumours of epithelial origin including breast, colorectal, non-small cell lung, head and neck, ovarian, bladder and prostate carcinomas. Additionally, EGF receptors are implicated in invasion, metastasis and poor prognosis in a number of these diseases. The tyrosine kinase activity associated with the EGF-R (EGF-RTK) is believed to be a key component of the signalling pathway whereby mitogenic signals are transduced through the membrane to the nucleus. Consequently, inhibition of EGF-RTK is an attractive target as an approach to the potential treatment of cancers which have an EGF-R dependence.

The anilinoquinazolines are a class of compounds with well-established activity against the EGF-RTK enzyme.[1-7] Many compounds of this class inhibit the EGF-RTK *in vitro*, inhibit EGF-stimulated receptor autophosphorylation, inhibit EGF-stimulated growth of tumour cell lines and some have been shown also to inhibit the growth of certain tumour xenografts. For example, compound 1 (Figure 1) is a potent inhibitor of the EGF-RTK *in vitro* (IC_{50} = 0.009μM; against the enzyme from A431 vulval squamous carcinoma cells) and of the EGF-stimulated proliferation of KB (oral carcinoma) cells in culture (IC_{50} = 0.08μM); furthermore, this compound shows modest activity in a xenograft model and will inhibit (37% by weight; $p < 0.05$) the growth of KB xenografts in nude mice when dosed orally at 50mg/kg twice per day for 21 days (days 0-20 after tumour implantation).

In order to try to find different (i.e. non-anilinoquinazoline) structural types of tyrosine kinase inhibitors we have systematically replaced the aniline moiety of the anilinoquinazoline type with a wide range of other moieties whereby the distance and orientation of a benzenoid ring relative to the quinazoline have been modified by the

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use of different linking groups and additional rings. This approach is shown schematically in Figure 1. We chose to include the 6,7-dimethoxyquinazoline moiety as an integral part of our synthetic targets because electron donor substituents in the 6- and 7-positions of the quinazoline are known to confer potent *in vitro* activity on the anilinoquinazoline series.[4,5]

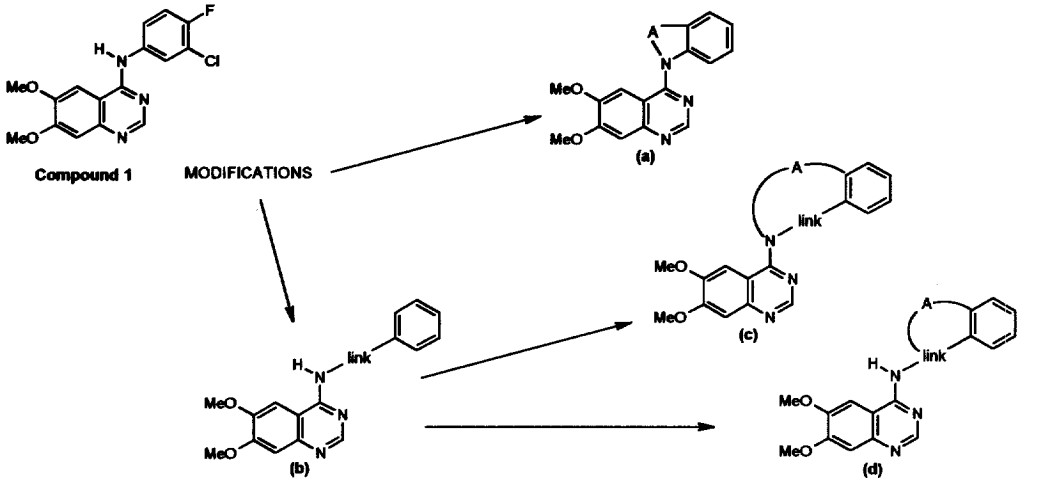
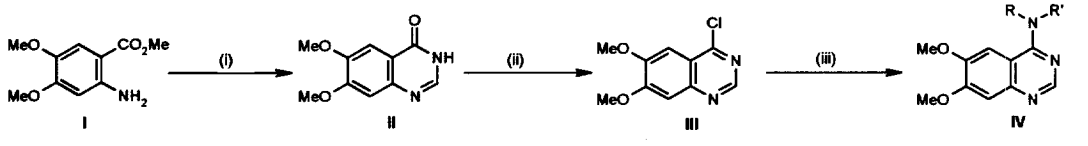


Figure 1. Strategy for the design of modifications to the structure of compound 1.

Synthesis

Compounds were synthesised by the route shown in Scheme 1.



Scheme 1. Reagents: (i) $HCONH_2$/180°C; (ii) $SOCl_2$; (iii) RR^1NH/heat/base.

Results

The results are shown in Tables 1-3. The 'EGF-RTK' result shows the IC_{50} (μM) for the inhibition of the EGF-RTK from A431 vulval squamous carcinoma cells. Compounds were tested to a maximum concentration of 1μM. If the IC_{50} had not been reached by 1μM then the result is quoted as >1 if the % inhibition at 1μM was

Table 1. Results for type **(a)** compounds.

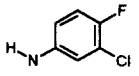
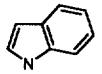
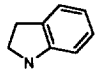
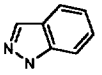
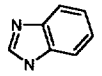
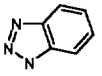
Compound (type)	IV; RNR[1]	EGF-RTK IC_{50} (μM)	KB cells IC_{50} (μM)
1 (reference)		0.009	0.08
2 **(a)**		>>1 (10%)	
3 **(a)**		0.82	4.1
4 **(a)**		>>1 (7%)	
5 **(a)**		>>1 (0%)	
6 **(a)**		>>1 (14%)	

Table 2. Results for compounds where link = one atom, including types **(b)**, **(c)** and **(d)**.

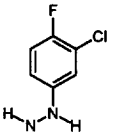
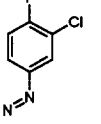
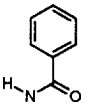
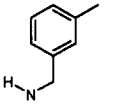
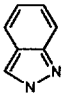
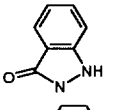
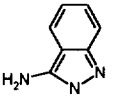
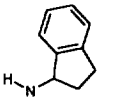
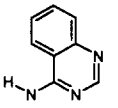
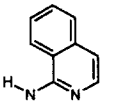
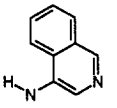
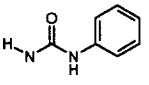
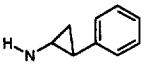
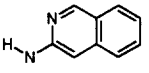
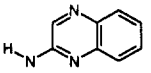
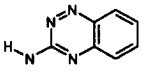
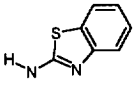
Compound (type)	IV; RNR[1]	EGF-RTK IC_{50} (μM)	KB cells IC_{50} (μM)
7 **(b)**		0.43	0.78
8 **(b)**		0.73	3.93
9 **(b)**		>>1 (1%)	
10 **(b)**		0.17	1.6
11 **(c)**		>>1 (0%)	
12 **(c)**		>>1 (14%)	
13 **(c)**		>>1 (13%)	
14 **(d)**		0.91	1.73
15 **(d)**		>1 (28%)	
16 **(d)**		0.2	1.8
17 **(d)**		0.0064	0.36

Table 3. Results for compounds where link = two atoms, including types **(b)** and **(d)**.

Compound (type)	IV; RNR[1]	EGF-RTK IC_{50} (μM)	KB cells IC_{50} (μM)
18 **(b)**		>>1 (0%)	
19 **(b)**		0.01	0.14
20 **(d)**		0.66	0.81
21 **(d)**		0.78	>25
22 **(d)**		0.82	>25
23 **(d)**		>1 (24%)	

20-49%, or as >>1 if the % inhibition at 1μM was 0-19% and the percent inhibition is shown in brackets in Tables 1-3. The 'KB cells' result shows the IC_{50} (μM) for the inhibition of EGF-stimulated proliferation of KB cells in culture. The details of these test systems have been described previously.[2]

Table 1 shows the results for compounds which fall into type (a; Figure 1). Table 2 shows the results for those compounds which have as a common feature a 'link' (Figure 1) consisting of only one atom (which can be either carbon or nitrogen); these compounds can fall into types (b), (c) or (d) depending on the nature of additional rings created by 'A' (Figure 1; (c) and (d)). Similarly, Table 3 shows the results for compounds which have as a common feature a 'link' consisting of two atoms (which can be either carbon or nitrogen); likewise, these compounds can fall into types (b) or (d) depending on the nature of 'A'.

Discussion

The results in Tables 1-3 show that only two compounds have potency comparable to that of compound 1 against both the isolated enzyme and the cell proliferation test. These are a 4-aminoisoquinoline derivative, compound 17, and a *trans*-2-phenylcyclopropyl derivative, compound 19.

Of the type (a) compounds (Table 1, compounds 2-6) only the indoline, compound 3,[8] shows any significant activity at <1μM in the EGF-RTK test and this is about 100 fold less potent than compound 1. Type (a) compounds do not have a hydrogen atom on N-4; large detrimental effects on the inhibitory potency of anilinoquinazolines on EGF-RTK have been reported[5,9] when the hydrogen atom on N-4 has been replaced by a methyl group and evidence for a conformational effect of N-4 substitution has been presented.[9] Type (c) compounds (Table 2, compounds 11-13), which again do not have a hydrogen atom attached to N-4, are also inactive at <1μM and this is also consistent with substitution on N-4 being detrimental to potency.

Type (b) compounds (Table 2, compounds 7-10; Table 3, compounds 18 and 19) show a wide range of potencies in the EGF-RTK test; compound 19 is very potent whereas compounds 9 and 18 are inactive at <1μM. Compounds 9 and 18 have a carbonyl group adjacent to N-4 and this will have a significant effect on the pKa's of the compounds as well as on the preferred conformations. The benzylic derivative, compound 10 has moderate activity. Structure-activity relationships have been reported previously for 4-benzylaminoquinazolines.[5] Also, substitution on the benzylic carbon adjacent to N-4 in 4-benzylaminoquinazolines has been reported[10] by the same group, who showed that the potency of the substituted products depends strongly on the chirality at the benzylic centre thus indicating that inhibitor potency is quite sensitive to even moderate out of plane bulk in certain directions. Interestingly, compounds 7 and 8 have similar potency in the EGF-RTK test which could indicate that, although substitution on N-4 is detrimental to potency in the EGF-RTK test, this is not necessarily due to the hydrogen on N-4 being involved in a beneficial interaction because compound 8 has neither a hydrogen nor an additional substituent on N-4.

Type (d) compounds (Table 2, compounds 14-17; Table 3, compounds 20-23) also show a wide range of potencies in the EGF-RTK test. Compounds 20-22 have very similar potency in the EGF-RTK test indicating the acceptability of nitrogen or carbon at the positions between N-4 and the benzenoid ring; however, it is

interesting to note that only compound 20 shows activity in the KB cell test thus indicating that activity in cells has additional requirements. Of the closely related compounds 15-17, compound 17 is very potent in the EGF-RTK test but compound 15 is inactive below 1μM. As compound 16 is moderately active in the EGF-RTK test, there is a strong indication that with this group of compounds a nitrogen atom *para* to the N-4 is detrimental to potency in this test.

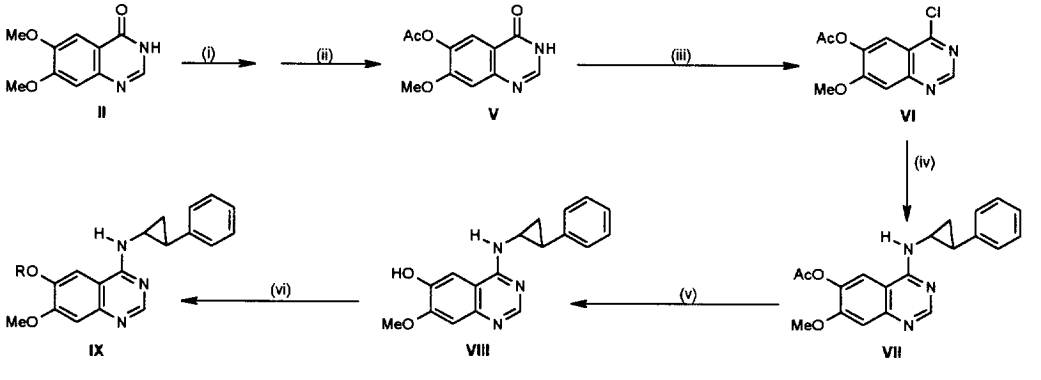
The Parke-Davis/Univ. of Auckland group have reported[10] that, when the quinazoline is unsubstituted at positions 6 and 7, the 4-(2-phenethylamino)-derivative was much less effective than either the corresponding 4-benzylamino- or the 4-anilino- compound; reported IC_{50}'s 4100nM compared to 320nM and 344nM respectively. This group also reported the 4-indan-1-ylamino-analogue, compound 14, to be about 100 fold less potent than the corresponding benzyl analogue; reported IC_{50}'s 1000nM and 10nM respectively.

Clearly, the introduction of the cyclopropyl moiety into the type (b) compound has had a dramatic effect on the inhibitory potency against the EGF-RTK *in vitro*.

Xenograft Studies

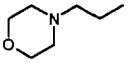
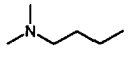
The 4-(*trans*-2-phenylcyclopropylamino)-quinazoline, compound 19, was evaluated in a tumour xenograft model (A431 vulval carcinoma in nude mice) by daily oral dosing at 200mg/kg but failed to show significant activity with this dosing regime against this tumour.

Because related work had shown[11] that the *in vivo* activity of compounds such as compound 1 could be greatly improved by a variety of modifications of the 6,7-dialkoxy groups, we prepared compounds of the 4-(*trans*-2-phenylcyclopropylamino)-quinazoline series with modifications of the 6-substituent. The synthetic route to these compounds is shown in Scheme 2; the compounds and their *in vitro* results are shown in Table 4.



Scheme 2. Reagents: (i) Methionine/$MeSO_3H$/100°C/3hr; (ii) Ac_2O/pyridine; (iii) $SOCl_2$; (iv) 2-*trans*-phenylcyclopropylamine; (v) NH_4OH/MeOH ; (vi) RCl/K_2CO_3/80°C.

Table 4. Results for various 6-derivatives of 4-(*trans*-2-phenylcyclopropylamino)-quinazolines.

Compound	IX; R	EGF-RTK IC_{50} (μM)	KB cells IC_{50} (μM)
19	Me	0.01	0.14
24	CH_3CO	0.084	11.4
25	H	0.39	>25
26		0.3	0.57
27		0.027	0.31

As can be seen from the *in vitro* results in Table 4, compound 27 is slightly less potent than compound 19 in both the enzyme and cell assays. However, when evaluated in the A431 xenograft model, compound 27 on once daily oral dosing at 50mg/kg showed a statistically significant 36% ($p < 0.05$) inhibition of tumour growth after 21 days dosing (days 7-27 after implantation).

Conclusion

We have identified the 4-(4-*iso*-quinolylamino)-quinazoline derivative, compound 17, and the 4-(*trans*-2-phenylcyclopropylamino)-quinazoline, compound 19, as novel types of EGF-RTK inhibitors. Further modification of the latter compound has led to the identification of compound 27 which shows significant inhibitory activity against the growth of a xenograft tumour when dosed orally once per day.

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