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Enantioselective Inhibition of the Epidermal Growth Factor Receptor Tyrosine Kinase by 4-(α-Phenethylamino)quinazolines

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Abstract—4-Benzylaminoquinazolines can be potent reversible inhibitors of the EGFR tyrosine kinase at the ATP binding site. Examination of benzylic methylation reveals that an (*R*)-methyl group is four- to six-fold activating, with an optimal K_i of 630 pM for compound **11**. In sharp contrast, (*S*)-methylation causes a > 30 to 500-fold loss of inhibitory activity, showing that the ATP-binding site of the receptor has very low tolerance for even moderate out-of-plane bulk in certain directions. It is suggested that the best of these inhibitors can induce a conformation of the kinase not available to poorer inhibitors.

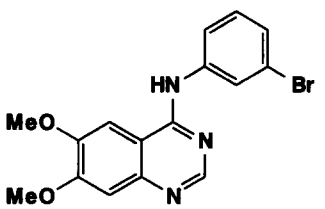
Introduction

Cancer appears to be mainly a disease of unchecked cellular proliferation, whereby certain cells pick up by mutations the unusual ability to reproduce uncontrollably without the normal appropriate growth signals from the surrounding medium. One of the commonest defects seen in such transformed cells is the overexpression of growth factor receptors on the cell surface, so that very weak signals can be amplified inappropriately, allowing the cells to grow autonomously.[1] These cell-surface receptors bind extracellular ligands and transduce a growth signal into the cell by activating a tyrosine kinase domain inside the cell. Phosphorylation of proteins on tyrosine in the cell then becomes the initial growth signal in the cell. A large percentage of all cancers have mutations which lead to excessive tyrosine kinase activity in the cell by growth factor receptors, especially of the Epidermal Growth Factor Receptor (EGFR) family.[2,3] The EGFR and the highly related erb-B2,[4] erb-B3[5] and erb-B4 receptors[6] are overexpressed in a large number of different types of important cancers[7–10] and are oncogenic in nude mice.

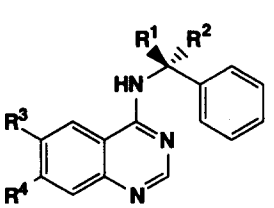
We and others have recently revealed that a family of 4-amino-substituted quinazolines are potent and selective inhibitors of the EGFR tyrosine kinase.[11–13] The inhibition is reversible and competitive for ATP in the examples that have been analysed.[12,14] As the ATP-binding domain performs an identical function in all kinases, tyrosine or otherwise, and in many other ATP-utilising enzymes, we initially did not expect much selectivity between kinases, but the quinazoline inhibitors and other series[15,16] proved to be highly selective inhibitors of the EGFR-family kinases. This culminated in the discovery that appropriately synergistic substitutions on the quinazoline and aniline rings of 4-anilinoquinazoline can generate extraordinarily potent inhibitors of the EGFR, with K_i values down to the low picomolar, as exemplified by example 18.[14] In this study we examined a series of compounds with the general structure shown below, looking at substitution on the benzylic methylene group of 4-benzylaminoquinazolines.

Chemistry

4-Chloroquinazoline[17] and 4-chloro-7-methoxyquinazoline[18] were prepared by literature procedures. 4-Chloro-6,7-dimethoxyquinazoline[18] was prepared from 4,5-dimethoxyanthranilic acid by fusion with formamidine, followed by chlorination with oxalyl chloride/DMF and is described in detail elsewhere as part of the synthesis of example 18.[19] All the inhibitors were synthesized by displacement on the corresponding 4-chloroquinazolines with the desired aniline or benzylamine derivative, in refluxing isopropanol or another appropriate alcohol solvent. Anilines could be made without adding any extra base, but best results for benzylamines were obtained by adding an exogenous base such as triethyl-



Example 18 K_i = 6 pM



General structure

Table 1. EGFR tyrosine kinase IC_{50} values for substituted quinazolines

Entry	B-ring R^3/R^4	4-Side chain	IC_{50} nM
1	None	Anilino	344
2	7-Methoxy	Anilino	120
3	6,7-Dimethoxy	Anilino	29
4	None	Benzylamino	320
5	7-Methoxy	Benzylamino	58
6	6,7-Dimethoxy	Benzylamino	10
7	None	(*R*)-1-Phenylethylamino	86
8	None	(*S*)-1-Phenylethylamino	>10,000
9	7-Methoxy	(*R*)-1-Phenylethylamino	29
10	7-Methoxy	(*S*)-1-Phenylethylamino	4000
11	6,7-Dimethoxy	(*R*)-1-Phenylethylamino	1.6
12	6,7-Dimethoxy	(*S*)-1-Phenylethylamino	3760
13	None	2-Phenylethylamino	4100
14	6,7-Dimethoxy	Naphth-1-ylmethylamino	148
15	6,7-Dimethoxy	(*R*)-1-Naphth-1-ylethylamino	139
16	6,7-Dimethoxy	(*R*)-1-Phenylprop-1-ylamino	234
17	6,7-Dimethoxy	(*R,S*)-Indan-1-ylamino	1000
18	6,7-Dimethoxy	3-Bromoanilino	0.029

amine. Most compounds either precipitated from the reaction mixture pure enough to be used directly or could be purified by recrystallisation. Several of the compounds were obtained as solvates or partial HCl salts, but with very clean NMR spectra. These compounds were not purified further. Entry 16 was purified by preparative TLC on silica gel.

Results

Compounds **1–18** were examined for their ability to inhibit the kinase activity of the EGF-stimulated EGFR. All proved to be kinase inhibitors of good to excellent potency, as shown in Table 1. A small series of 4-anilino- and 4-benzylamino-substituted quinazoline inhibitors is shown in entries 1–6. The two side chains are equipotent in the parent quinazoline, (entries 1 and 4). Addition of a 7-methoxy group is modestly favourable in both series (entries 2 and 5) and the 6,7-dimethoxy analogues (entries 3 and 6) are more activated, benzyl being the best, with an impressive 10 nM IC_{50} against the enzyme. However, when the aromatic side chain nucleus was substituted, the anilines were capable of great enhancements of activity, exemplified by compound **18**,[12,19] whereas the benzylamino side chains in this series,[12] and a closely related series,[15] revealed that nuclear substitution on the benzylic side chain was almost always detrimental. This left the benzylic position to explore and it could also be used to probe the enzyme binding site for stereochemical preferences.

Three enantiomeric pairs of 4-α-phenethylaminoquinazolines, with the same B-ring substitution patterns as previously, were prepared. The (*R*)-methyl substituent in each case (entries 7, 9 and 11) had a moderate enhancement of inhibitory activity compared with the corresponding parent benzylamine (Table 2). Entry 11 with an IC_{50} of 1.6 nM for the EGFR TK is 200-fold more potent than the parent (entry 4). In sharp contrast, the (*S*)-enantiomers (entries 8, 10 and 12) were all considerably weaker inhibitors than the corresponding benzyl compounds, with > 30 to 400-fold losses of inhibitory activity upon adding the methyl group in this orientation. When comparing pairs of enantiomers the contrast became sharper with the (*R*)-enantiomers being from > 100 to 2350 times as potent as their (*S*)-enantiomers.

Table 2. Effects of multiple substitution on quinazoline EGFR tyrosine kinase inhibition

B-Ring	Benzylamino Side Chains		
Substituent	Unsub	(*R*)-Me	Un/(*R*)-Me
None	320	86	3.72
7-MeO	58	29	2.0
6,7-diMeO	10	1.6	6.25
	Unsub	(*S*)-Me	Un/(*S*)-Me
None	320	>10,000	<0.031
7-MeO	58	4,000	0.0145
6,7-diMeO	10	3760	0.00266
	(*S*)-Me	(*R*)-Me	(*S*)/(*R*)-Me
None	>10,000	86	>116
7-MeO	4000	29	138
6,7-diMeO	3760	1.6	2350

All inhibition values are IC_{50}s in nanomolar.

We briefly examined the effects of making the side chain larger. Lengthening the link to ethyl (entry 13) leads to a 12-fold loss of activity, in marked contrast to the introduction of the first methylene spacer. On the more active 6,7-dimethoxyquinazoline nucleus, increasing the size of the aromatic ring from phenyl to naphthyl is *ca* 15-fold detrimental (entry 14) and in this

case the addition of the (*R*)-methyl group (entry 15) had a negligible effect on potency. We also examined the (*R*)-ethyl substituted analogue (entry 16), where it can be seen that addition of any extra bulk at the methyl position is strongly disfavoured. Therefore, the 1 μM potency of the (*R,S*)-1-indanyl side chain (entry 17) was unsurprising.

The most potent compound, 4-[(*R*)-1-phenylethylamino]quinazoline, was subjected to a Lineweaver–Burke kinetic analysis, shown graphically in Figure 1. Analysis of the data revealed that the compound is a very clean, reversible, ATP-competitive inhibitor and a K_i of 627 pM was obtained by fitting the data to the equations of Cleland,[20] derived for competitive inhibition through non-linear regression analysis using the program 'GraFit' (Erithacus Software). This result is completely consistent with kinetic analyses of other compounds of this type.[12,14]

Discussion

As 4-aminoquinazoline appears to be the major binding determinant for these inhibitors and chirality is determined by a methyl group, pairs of enantiomers probably bind in the same orientation with respect to the enzyme. If so, the difference in binding affinities is determined by differential interactions of the epimeric methyl groups with the enzyme. As the (*R*)-methyl substituents increase inhibitory potency by up to the amount derived from a 1 kcal mol^{-1} increase in binding energy, about the maximum one would expect for a hydrophobic interaction of a single carbon atom, this is suggestive that the (*R*)-methyl group of **11** binds rather precisely in a small hydrophobic depression on the enzyme. The tightness of this fit would explain the major loss in binding energy (*ca* 3 kcal) when this methyl is enlarged to ethyl. In sharp contrast, the (*S*)-methyl substituents cause at least a 2-kcal mol^{-1} loss in binding affinity for the enzyme. These results are suggestive that the inhibitor binding pocket is a rather narrow cleft, with the (*R*)-methyl fitted tightly to one surface and the (*S*)-methyl group sticking 'up' from this surface and causing quite unfavourable steric interactions with the other surface of the cleft.

Kinase catalytic domains have highly conserved structures, consisting of a small N-terminal lobe largely made up of β-sheets, which binds ATP on its underside, connected by a rather flexible linking section to a C-terminal lobe, largely made up of α-helical segments, which binds substrate and contains almost all the catalytic residues. The X-ray structure of the insulin receptor TK[21] shows the lobes spread apart and the ATP binding site open to the solvent and similar structures are seen for MAP kinase[22] and PKA,[23] but these structures are all of inactive forms of the enzymes. In contrast, the X-ray structure of the catalytic subunit of PKA complexed to ATP and an inhibitory substrate-mimicking peptide shows a closed binding site for ATP, formed by surfaces from both lobes,[24] with all the cata-

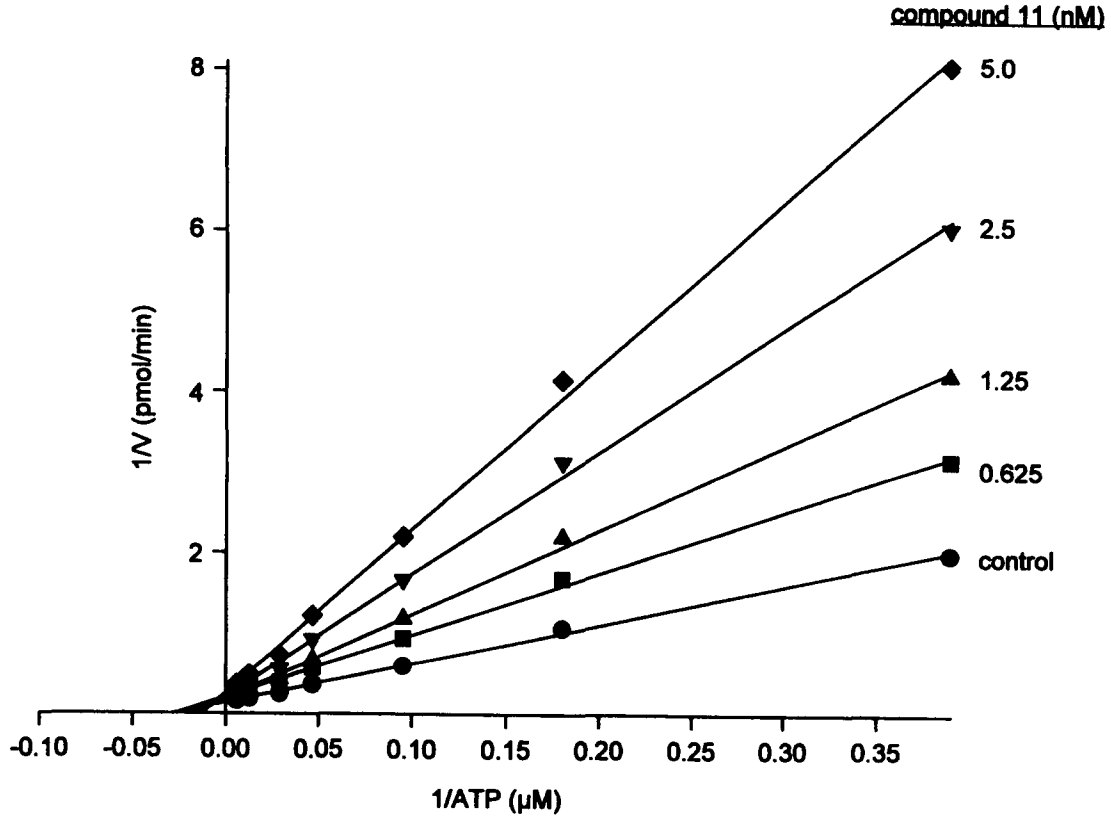


Figure 1. Double reciprocal plot for inhibition of EGFR tyrosine kinase by compound **11**. Enzyme activity was determined as described in Biology, Experimental, except with the indicated concentrations of compound **11** and ATP. Lines were determined by least-squares analysis.

lytic residues in the correct orientation for phosphate transfer. Probably, when a kinase is activated, it binds both ATP and substrate when in a partially open conformation[21] and then the two domains close together around the hinge domain[25,26] pulling the substrate and ATP into the correct orientation for phosphotransfer to occur.

A good inhibitor of kinases may well induce a partial or complete closure of the lobes around it and provide a hydrophobic core about which residues on both domains can pack. This could partially mimic the active form of the enzyme, as has been shown for peptidic inhibitors of the structurally analogous (two-lobe) aspartic protease endothiapepsin, where inhibitors induce domain movements similar to those seen during the catalytic cycle of that enzyme.[27] The large loss of binding energy with the (*S*)-methyl compounds suggests that the (*S*)-methyl probably prevents the domains from closing together as far as they can with other inhibitors and, therefore, obtain less binding energy from hydrophobic packing.

Conclusion

In this paper we have demonstrated that the stereochemistry of substitution on the benzyl carbon of 4-benzylaminoquinazolines can have major consequences for inhibition of the EGFR tyrosine kinase. We have also found an inhibitor with a subnanomolar K_i in this series and demonstrated that the SAR around it is rather tight, with all side chain substitutions which add further bulk being rather detrimental. We suggest that the optimal inhibitors may well allow or induce the enzyme to change its conformation in a similar manner to that which occurs when the enzyme is passing through its catalytic cycle.

Experimental

Chemistry

A typical experimental is given below.

Example 11. 4-[(R)-1-Phenylethylamino]-6,7-dimethoxyquinazoline. A mixture of 4-chloro-6,7-dimethoxyquinazoline[19] (224 mg, 1.0 mmol), (*R*)(+)-phenethylamine (133 mg, 1.1 mmol) and triethylamine (212 mg, 2.1 mmol) in ethanol (2 mL) was refluxed under N_2 with stirring for 7 h. The reaction mixture was cooled to 0 °C and the solid was collected by Buchner filtration. This material was recrystallised from ethanol to give 4-[(*R*)-1-phenylethylamino]-6,7-dimethoxyquinazoline (211 mg, 60%) as pale magnolia glistening plates; mp 195.5–197 °C. Calcd for $C_{18}H_{19}N_3O_2 \cdot 0.87C_2H_6O$: C, 67.73; H, 6.94; N, 12.05; found: C, 67.62; H, 7.01; N, 11.71. ^{1}H NMR [$(CD_3)_2SO$] δ: 8.27 (*s*, 1H, H-2), 8.09 (*d*, *J* = 8 Hz, NH), 7.76 (*s*, 1H, H-5), 7.43 (*d*, *J* = 7.5 Hz, 2H, H-2'), 7.31 (*t*, *J* = 7.6 Hz, 2H, H-3'), 7.21 (*t*, *J* = 7.6 Hz, 1H, H-4'), 7.08 (*s*, 1H, H-8), 5.61 (*t*, *J* = 7.2 Hz, 2H, NCH_2), 3.93, 3.89 (2*s*, 3H, 3H, OCH_3). MS (EI), 309 (32, M^+), 120 (100).

Example 2. 4-(Phenylamino)-7-methoxyquinazoline hydrochloride. Mp 265–267 °C. Cald for $C_{15}H_{13}N_3O \cdot HCl$: C, 62.61; H, 4.90; N, 14.60; found: C, 62.65; H, 4.87; N, 14.44. ^{1}H NMR [$(CD_3)_2SO$] δ: 11.38 (*sl br s*, 1H, NH), 8.86 (*s*, 1H, H-2), 8.87 (*d*, *J* = 9.2 Hz, H-5), 7.69 (*d*, *J* = 7.6 Hz, 2H, H-2'), 7.53–7.46 (*m*, 3H, H-3', H-6), 7.37–7.30 (*m*, 2H, H-4', H-8), 3.99 (*s*, 3H, OCH_3). MS (CI): 252 (100, MH^+).

Example 3. 4-(Phenylamino)-6,7-dimethoxyquinazoline. Mp 271 °C. Calcd for $C_{16}H_{15}N_3O_2 \cdot HCl$: C, 60.48; H, 5.08; N, 13.22; found: C, 60.42; H, 5.12; N, 13.07. ^{1}H NMR [$(CD_3)_2SO$] δ: 11.31 (*s*, 1H, NH), 8.81 (*s*, 1H, H-2), 8.27 (*s*, 1H, H-5), 7.69 (*d*, *J* = 7.5 Hz, 1H, H-2'), 7.52 (*t*, *J* = 7.8 Hz, 1H, H-3'), 7.35 (*m*, 2H, H-4', H-8), 4.02 (*s*, 3H, OCH_3), 4.00 (*s*, 3H, OCH_3). MS (EI) m/z 281 (55, M^+), 280 (100).

Example 4. 4-(Phenylmethylamino)quinazoline. Mp 169–170 °C. $C_{15}H_{13}N_3$ requires: C, 75.99; H, 5.67; N, 17.26; found: C, 76.57; H, 5.57; N, 17.86 ^{1}H NMR [$(CD_3)_2SO$] δ: 8.68 (*s*, 1H, H-2), 7.89 (*d*, *J* = 8.2 Hz, 1H, H-5), 7.82 (*d*, *J* = 8.2 Hz, 1H, H-8), 7.76 (*t*, *J* = 7.7 Hz, 1H, H-7), 7.48 (*m*, 6H, H-6, H-2', H-3', H-4'), 6.47 (*br s*, 1H, NH), 4.90 (*d*, *J* = 5.3 Hz, 2H, CH_2). MS (EI) m/z 235 (37, M^+), 106 (100).

Example 5. 4-(Phenylmethylamino)-7-methoxyquinazoline. Mp 181 °C. $C_{16}H_{15}N_3O \cdot 0.1HCl$ requires: C, 71.45 ; H, 5.66; N, 15.62; found: C, 71.53; H, 5.45; N, 15.67. ^{1}H NMR [$(CD_3)_2SO$] δ: 8.38 (*s*, 1H, H-2), 8.21 (*d*, *J* = 9.2 Hz, 1H, H-6), 7.36–7.09 (*m*, 6H, H-5 and H-2'–H-6'), 5.20 (*sl br t*, *J* = 6 Hz, 1H, NH), 4.77 (*d*, *J* = 6.0 Hz, 2H, CH_2), 3.89 (*s*, 3H, OCH_3). MS (CI) m/z 266 (100, MH^+).

Example 6. 4-(Phenylmethylamino)-6,7-dimethoxyquinazoline. Mp 226–229 °C. Calcd for $C_{17}H_{17}N_3O_2 \cdot 0.1HCl$: C, 68.29; H, 5.76; N, 14.08; found: C, 68.62; H, 5.69; N, 14.00. ^{1}H NMR [$(CD_3)_2SO$] δ: 8.59 (*s*, 1H, H-2), 7.42 (*d*, *J* = 7.4 Hz, H-2'), 7.36 (*t*, *J* = 7 Hz, 2H, H-3'), 7.31 (*t*, *J* = 7 Hz, 1H, H-4'), 7.20 (*s*, 1H, H-5), 6.93 (*s*, 1H, H-8), 5.97 (*br t*, *J* = 5 Hz, 1H, NH), 4.87 (*d*, *J* = 5.3 Hz, CH_2N), 3.98, 3.92 (2*s*, 3H, 3H, OCH_3). MS (CI): 295 (100, M^+).

Example 7. 4-[(R)-1-Phenylethylamino]quinazoline. Mp 108 °C. Calcd for $C_{16}H_{15}N_3 \cdot 0.2HCl$: C, 74.89; H, 5.97; N, 16.38; found: C, 74.77; H, 5.70; N, 16.17. ^{1}H NMR [$(CD_3)_2SO$] δ: 8.47 (*d*, *J* = 8.2 Hz, 1H, H-5), 8.40 (*s*, 1H, H-2), 7.79 (*dt*, *Jd* = 1.2 Hz, *Jt* = 7.0 Hz, 1H, H-7), 7.69 (*d*, *J* = 7.7 Hz, 1H, H-8), 7.54 (*dt*, *Jd* = 1.2 Hz, *Jt* = 8 Hz, 1H, H-6), 7.44 (*d*, *J* = 7.5 Hz, H-2'), 7.31 (*t*, *J* = 7.5 Hz, 2H, H-3'), 7.21 (*t*, *J* = 7.5 Hz, 1H, H-4'), 5.60 (*p*, *J* = 7.2 Hz, 1H, CHN), 1.59 (*s*, 3H, CH_3). MS (CI): 250 (100, MH^+).

Example 8. 4-[(S)-1-Phenylethylamino]quinazoline. Mp 106.5–108.5 °C. Calcd for $C_{16}H_{15}N_3 \cdot 0.5CH_3OH$: C, 74.96;

H, 6.48; N, 15.89; found: C, 74.86; H, 6.11; N, 16.17. ^{1}H NMR [$(CD_3)_2SO$] and MS (CI): identical to example 7.

Example 9. 4-[(R)-1-Phenylethylamino]-7-methoxyquinazoline. Mp 126.5–127 °C. $C_{17}H_{17}N_3O{\cdot}0.1HCl$ requires: C, 72.15; H, 6.09; N, 14.85; found: C, 72.26; H, 6.04; N, 14.86. ^{1}H NMR [$(CD_3)_2SO$]: 8.37 (*d*, 1H, *J* = 9.2 Hz, H-5), 8.33 (*s*, 1H, H-2), 8.31 (*d*, *J* = 8.0 Hz, 1H, NH), 7.42 (*d*, *J* = 7.2 Hz, 2H, H-2'), 7.30 (*t*, *J* = 7.7 Hz, 2H, H-3'), 7.20 (*t*, *J* = 7.2 Hz, 1H, H-4'), 7.13 (*dd*, *J* = 2.5, 9.0 Hz, 1H, H-6), 7.07 (*d*, *J* = 2.6 Hz, 1H, H-8), 5.58 (*p*, *J* = 7.5 Hz, 1H, CHN), 3.88 (*s*, 3H, OCH_3), 1.56 (*d*, *J* = 7.0 Hz, 3H, CH_3). MS (CI) m/z: 279 (100, MH^+).

Example 10. 4-[(S)-1-Phenylethylamino]-7-methoxyquinazoline. Mp 144–146 °C. Calcd for $C_{17}H_{17}N_3$: C, 73.10; H, 6.13; N, 15.04; found: C, 72.70; H, 6.12; N, 14.95. ^{1}H NMR and MS (CI): identical to example 9.

Example 12. 4-[(S)-1-Phenylethylamino]-6,7-dimethoxyquinazoline. Mp 195–196.5 °C. Calcd for $C_{18}H_{19}N_3O_2{\cdot}0.8$ C_2H_6O: C, 67.99; H, 6.93; N, 12.14; found: C, 67.64; H, 7.01; N, 11.93. ^{1}H NMR [$(CD_3)_2SO$] and MS (CI): identical to example 11.

Example 13. 4-(2-Phenylethylamino)quinazoline. Mp 167–170 °C. Calcd for $C_{16}H_{15}N_3{\cdot}0.4$ H_2O requires: C, 74.86; H, 6.21; N, 16.37; found: C, 75.26; H, 6.07; N, 15.80. ^{1}H NMR [$(CD_3)_2SO$] δ: 8.47 (*s*, 1H, H-2), 8.37 (*br s*, 1H, NH), 8.620 (*d*, *J* = 8.4 Hz, 1H, H-5), 7.76 (*t*, *J* = 7.0 Hz, 1H, H-6), 7.67 (*d*, *J* = 7.5 Hz, 1H, H-8), 7.50 (*t*, *J* = 8.2 Hz, 1H, H-7), 7.30 (*m*, 4H, H-2', H-3'), 7.19 (*t*, *J* = 6.1 Hz, 1H, H-4'), 3.77 (*m*, 2H, CH_2), 2.97 (*t*, *J* = 7.5 Hz, 2H, CH_2). MS (EI) m/z 249 (3, M^+), 84 (100).

Example 14. 4-(Naphth-1-ylmethylamino)-6,7-dimethoxyquinazoline. Mp 244–246 °C. Calcd for $C_{21}H_{19}N_3O_2$: C, 73.03: H, 5.54; N, 12.17; found: C, 73.18; H, 5.76; N, 11.85. ^{1}H NMR [$(CD_3)_2SO$] δ: 8.45 (*t*, *J* = 5 Hz, 1H, NH), 8.37 (*s*, 1H, H-2), 8.24–8.16 (*m*, 1H, H-8'), 8.02–7.94 (*m*, 1H, H-5'), 7.86 (*dd*, *J* = 2.3, 7.0 Hz, H-4'), 7.72 (*s*, 1H, H-5), 7.62–7.42 (*m*, 4H, H-2', H-3', H-6', H-7'), 7.13 (*s*, 1H, H-8), 5.25 (*d*, *J* = 5.1 Hz, NCH_2), 3.91, 3.86 (2*s*, 3H, 3H, OCH_3). MS (CI), 346 (100, MH^+).

Example 15. 4-[(R)-1-Naphth-1-ylethylamino]-6,7-dimethoxyquinazoline. Mp 223–224 °C. $C_{22}H_{21}N_3O_2{\cdot}0.1HCl$ requires: C, 72.78; H, 5.86; N, 11.57; found: C, 72.85; H, 5.77; N, 11.34. ^{1}H NMR [$(CD_3)_2SO$] δ: 8.28 (*s*, 1H, H-2), 8.24 (*d*, *J* = 7.7 Hz, 1H, NH), 8.21 (*d*, *J* = 8.0 Hz, H-8'), 7.94 (*dd*, *J* = 7.5, 1.9 Hz, 1H, H-5'), 7.83 (*d*, 1H, *J* = 8.2 Hz, H-4'), 7.79 (*s*, 1H, H-5), 7.66 (*d*, 1H, *J* = 7.0 Hz, 1H, H-2'); 7.58–7.47 (*m*, 3H, H-3, H-6', H-7'), 7.09 (*s*, 1H, H-8), 6.37 (*t*, 1H, *J* = 7.0 Hz, 1H, CHN), 3.91 (*s*, 3H, OCH_3), 3.89 (*s*, 3H, OCH_3), 1.72 (*d*, *J* = 6.8 Hz, 3H, CH_3). MS (CI) m/z 360 (100, MH^+).

Example 16. 4-[(R)-1-Phenylpropylamino]-6,7-dimethoxyquinazoline. Mp 194.5–195.5 °C. Calcd for $C_{19}H_{21}N_3O_2{\cdot}$ $0{\cdot}4H_2O$: C, 69.02; H, 6.45; N, 12.71; found: C, 69.34; H, 6.45; N, 12.51. ^{1}H NMR [$CDCl_3$] δ: 8.53 (*s*, 1H, H-8), 7.43 (*d*, *J* = 7.2 Hz, 2H, H-2'), 7.36 (*t*, *J* = 7.5 Hz, 2H, H-3'), 7.29 (*t*, *J* = 7.3 Hz, 1H, H-4'), 7.19 (*s*, 1H, H-5), 6.86 (*s*, 1H, H-8), 5.55 (*sl br d*, *J* = 7.2 Hz, 1H, NH), 5.43 (*q*, *J* = 7.4 Hz, 1H, NCH), 4.00, 3.99 (2*s*, 3H, 3H, OCH_3), 2.18–1.97 (*m*, 2H, CH_2), 1.00 (*t*, *J* = 7.4 Hz, 3H, CH_3). MS (EI) 324 (100, MH^+).

Example 17. 4[(R,S)-Indan-1-ylamino]-6,7-dimethoxyquinazoline. Mp 223–224 °C. $C_{19}H_{19}N_3O_2{\cdot}0.1HCl$ requires: C, 70.22; H, 5.92; N, 12.93; found: C, 70.44; H, 5.72; N, 12.19. ^{1}H NMR [$(CD_3)_2SO$] δ: 8.38 (*s*, 1H, H2), 8.15 (*d*, *J* = 8.2 Hz, 1H, NH), 7.68 (*s*, 1H, H-5), 7.26–7.10 (*m*, 4H, H-4'–H-7'), 7.11 (*s*, 1H, H-8), 6.04 (*q*, *J* = 8.2 Hz, 1H, H-1'), 3.90 (*s*, 3H, OCH_3), 3.87 (*s*, 3H, OCH_3), 3.07–2.86 (*m*, 2H, H-3'), 2.61–2.52 (*m*, 1H, H-2'), 2.08–1.98 (*m*, 1H, H-2'). MS (CI) m/z: 322 (100, MH^+).

Biology

Enzyme assay. Epidermal growth factor receptor was prepared from human A431 carcinoma cell shed membrane vesicles by immunoaffinity chromatography as previously described.[28] The assays were carried out as reported previously.[14] Reactions were carried out in a total volume of 0.1 mL of 25 mM HEPES buffer (pH 7.4) containing 5 mM $MgCl_2$, 2 mM $MnCl_2$, 50 μM sodium vanadate, 0.5–1.0 ng EGFR (which contained enough EGF to make a final concentration of 2 μg mL^{-1}) and 10 μM ATP containing 1 μCi of [^{32}P]ATP, varying concentrations of the drug under test and 200 μM of the substrate. The latter was based on a portion of phospholipase C-1, having the sequence Lys-His-Lys-Lys-Leu-Ala-Glu-Gly-Ser-Ala-Tyr^{472}-Glu-Glu-Val. The reaction was allowed to proceed for 10 min at room temperature, then stopped by the addition of 2 mL of 75 mM phosphoric acid. The solution was then passed through a 2.5 cm phosphocellulose disk which bound the peptide. This filter was washed with 75 mM phosphoric acid (5×) and incorporated label was assessed by scintillation counting in an aqueous fluor. Control activity (no drug) gave a count of approximately 100,000 cpm. At least two independent dose–response curves were carried out and the IC_{50} values computed. The reported values are averages; variation was generally ± 15%.

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