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Bioorganic & Medicinal Chemistry Letters
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Bioorganic & Medicinal Chemistry Letters 14 (2004) 783–786

Omega-carboxypyridyl substituted ureas as Raf kinase inhibitors: SAR of the amide substituent

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Received 15 September 2003; accepted 6 November 2003

Abstract—Bis-aryl ureas have been disclosed previously as a potent class of Raf kinase inhibitors. Modifications in the amide portion led to an improvement in aqueous solubility, an important characteristic for an oral drug. Based on this finding, we hypothesize that this portion of the molecule is directed towards the solvent in Raf-1.

Most of the known small molecule protein kinase inhibitors bind to a highly conserved ATP-binding pocket and in general are flat, aromatic molecules that mimic the adenine portion of ATP.[1] Therefore, relatively poor drug-like properties such as high logP and low aqueous solubility are major challenges. For example, the introduction of water-solubilizing groups to the 6- and 7-positions of the quinazoline EGFR inhibitors was an important step towards the optimization of their physico-chemical properties.[2]

We have previously reported our focus on Raf-1 kinase as a validated target for the treatment of cancer.[3] The lead compound **1** [*N*-(5-*tert*-butyl-3-isoxazolyl)-*N*′-(4-phenoxyphenyl)urea, Fig. 1] was identified by screening of a combinatorial chemistry library, and exhibits an IC_{50} value of 1100 nM against recombinant human Raf-1 kinase.[4] Optimization of **1** led to a series of potent, orally active Raf-1 kinase inhibitors,[5–8] and culminated in the identification of a clinical candidate BAY 43-9006 (**5**).[3,5]

Introduction of an *N*-methyl carboxamide at the *meta* position of the distal phenyl ring (**2**) increased activity by almost 10-fold.[5] Replacement of the distal phenyl ring with a 4-pyridyl ring (**3**) also significantly improved the potency. Combination of these two structural features leads to highly potent ureas, such as **4**. An additional optimization effort where the isoxazole ring of **4** is replaced by other five-membered heterocycles as well as appropriately substituted phenyl groups led to a clinical candidate BAY 43-9006 **5**.[3,5]

We sought to identify a site for the introduction of water solubilizing groups without affecting the Raf-1 inhibitory potency. In this article, we wish to report our study of the structure–activity relationships of the amide portion of the molecule, directed towards optimizing aqueous solubility.[10]

Our group has previously disclosed synthetic routes to ureas similar to those described in this report, such as **1**–**5**.[7,8] Furthermore, the general preparation of ureas **26**–**27** and **37**–**39** is depicted in Figure 2. Reaction of 5-hydroxynicotinic acid methyl ester with 1-fluoro-4-nitrobenzene, followed by reduction of the nitro group, urea formation, hydrolysis of the ester and subsequent amide formation provides access to amide analogues.

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doi:10.1016/j.bmcl.2003.11.041

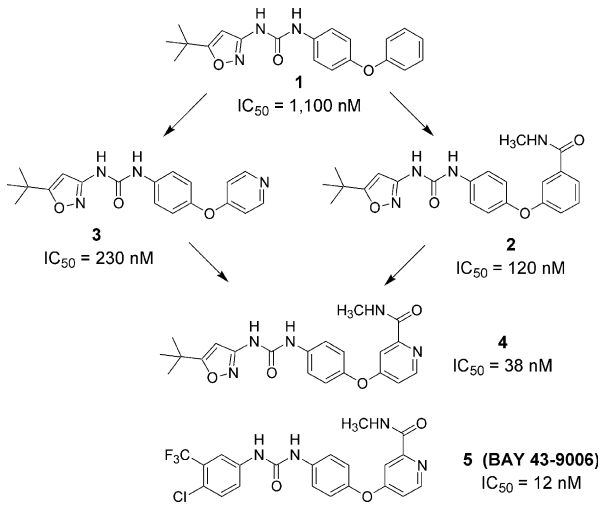


Figure 1. Urea-based Raf-1 kinase inhibitors.

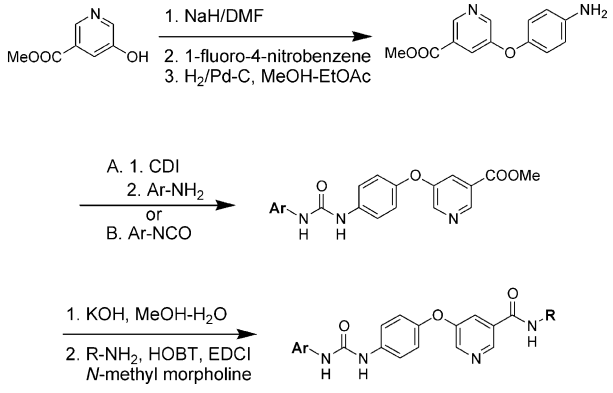
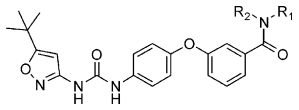


Figure 2. Preparation of ureas **26**–**27** and **37**–**39**.

Initial evaluation of the effect of amide substituents larger than methyl revealed no improvement, but demonstrated the existence of an extra space in this binding region of Raf-1 kinase (Table 1). For example, replacement of the *N*-methyl group of **2** with larger alkyl groups such as ethyl or *n*-propyl led to similar Raf-1 potencies[9] (entries **6** and **7**). In contrast, modification of the primary amide group to a secondary amide as in urea **8** was detrimental to the potency, suggesting the importance of a hydrogen-bond donor in this part of the molecule. Increasing the size of the *N*-substituent to benzyl or phenyl led to a less dramatic loss in potency (Table 1, entries **9** and **10**). Similar trends were observed with urea derivatives where the isoxazole heterocycle was replaced by a substituted phenyl group (Tables 2–4). Interestingly, replacement of the *N*-phenyl group in **10** with an *N*-3-pyridyl group **11** improved potency, indicating that polar amides could be beneficial to the Raf-1 potency.

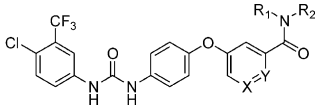
Based on this observation, various polar carboxamides were synthesized; the results are shown in Tables 2–4. In most of these examples, introduction of water-solubilizing groups provided compounds with retained Raf-1 inhibitor potency, and large groups were well tolerated. We hypothesize that this portion of the molecule is directed towards the solvent when the compound is bound to Raf-1 kinase.

Table 1. Substitution of the carboxamide group



Compd	R_1	R_2	Raf-1 kinase IC_{50} (nM)[9]
2	H	Me	120
6	H	Et	130
7	H	*n*-Pr	140
8	Me	Me	5800
9	H	CH_2Ph	460
10	H	Ph	370
11	H	3-Pyridyl	68

Table 2. Carboxamides related to diphenyl urea BAY 43-9006 (**4**)



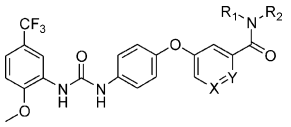
Compd	X	Y	R_1	R_2	IC_{50} (nM)[9]
12	CH	CH	H	Me	130
13	CH	CH	H	4-Morpholinyl-$(CH_2)_2$	70
14	CH	CH	H	1-Piperidinyl-$(CH_2)_2$	82
15	CH	CH	H	2-Et-pyrrolidin-1-yl-(CH_2)	270
16	CH	CH	H	3-Pyridyl	44
17	CH	CH	H	4-(Me_2N)-phenyl	230
18	CH	CH	H	PhNH-$(CH_2)_2$	160
19	CH	CH	H	MeO-$(CH_2)_2$	110
20	CH	CH	H	4-MeO-3-pyridyl	130
21	CH	CH	H	(4-Morpholinyl)phenyl	160
5	CH	N	H	Me	12
22	CH	N	H	Et	26
23	CH	N	Me	Me	300
24	CH	N	H	*i*-Pr	2300
25	CH	N	H	4-Morpholinyl-$(CH_2)_2$	73
26	N	CH	H	4-Morpholinyl-$(CH_2)_2$	140
27	N	CH	H	Me	50

Introduction of a pyridine in place of the distal phenyl ring in such analogues provided compounds with retained high potency (e.g., **25** and **26** vs **13**, Table 2); however, additive effects seen previously in the case of **3** versus **1**, **4** versus **2**, and **33** versus **28** were not realized.

Interestingly, *N*-alkylnicotinamide analogues such as **26**–**27** and **37**–**39** (Tables 2 and 3) showed Raf-1 potencies similar to those of the isomeric *N*-alkyl 2-pyridinecarboxamides.

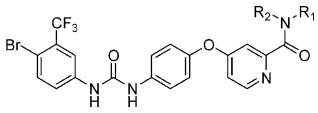
Selected examples were evaluated in an equilibrium-based aqueous solubility assay, according to a high-throughput Nuclear Magnetic Resonance (NMR) flow technology assay protocol developed in-house.[11] The effect of *N*-methyl amide substitution with polar amides on aqueous solubility is shown in Table 5. Compound **39** is more than 10-fold more soluble than the corresponding *N*-methyl amide analogue **38** at physiological pH. As expected from these basic analogues, very good aqueous solubility was observed at lower pH (Table 5), which could potentially provide improved absorption through the gastrointestinal membrane.[11]

Table 3. Carboxamides in the diphenyl urea class



Compd	X	Y	R_1	R_2	IC_{50} (nM)[9]
28	CH	CH	H	Me	130
29	CH	CH	H	3-Pyridyl	100
30	CH	CH	H	4-(Me_2N)-Phenyl	410
31	CH	CH	H	6-MeO-3-Pyridyl	150
32	CH	CH	H	(4-Morpholinyl)phenyl	210
33	CH	N	H	Me	53
34	CH	N	H	Et	460
35	CH	N	Me	Me	330
36	CH	N	H	*i*-Bu	500
37	N	CH	H	4-Morpholinyl-$(CH_2)_2$	63
38	N	CH	H	Me	61
39	N	CH	H	Me_2N-$(CH_2)_2$	100

Table 4. Carboxamides in the diphenyl urea class



Compd	R_1	R_2	IC_{50} (nM)[9]
40	H	Me	6
41	H	Et	27
42	Me	Me	170
43	H	4-Morpholinyl-$(CH_2)_2$	60

Table 5. Aqueous solubility of selected ureas

Compd	Solubility, pH 2.7 (μg/mL)	Solubility, pH 7.2 (μg/mL)
38	≤ 4	≤ 4
39	129	41
25	141	≤ 5
43	39	≤ 5
26	36	≤ 5

Conditions: (a) pH 7.2, 0.5×PBS; or pH 2.7, 3 mM citrate, 1.4 mM KCl, 68 mM NaCl; (b) agitate at rt for 3 h; (c) centrifuge and analyze by NMR.[11]

In summary, novel Raf-1 kinase inhibitors from the urea class have been prepared. The carboxamide group of BAY 43-9006 and its analogues was shown to be a suitable position for the introduction of water-solubilizing groups. Improvements of aqueous solubilities by up to 10-fold were realized without significant impact on Raf-1 kinase potency.

Acknowledgements

We would like to thank our colleagues at Onyx Pharmaceuticals for their support in this collaborative effort, particularly Dr. John Lyons and Dr. Vivienne Marsh, who developed the Raf-1 kinase assay. We also would like to thank Mr. Anthony Paiva and Mr. Stuart Coleman for mass spectrometry support.

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9. In vitro Raf Kinase activity screen: Human Raf-1 (c-Raf) and Mek were cloned, expressed and purified at Onyx Pharmaceuticals. Briefly, Sf-9 cells were infected with baculovirus encoding epitope-tagged c-Raf or Mek (EYMPME; EE-tag on the C-terminus). Epitope-tagged Raf and Mek were then purified from infected Sf-9 cell lysates by immuno-affinity chromatography, and were stored at −80 °C in the following storage buffers. Raf kinase: 20 mM Tris pH 8.0, 1mM EDTA, 1mM DTT, 20 μM Leupeptin, 1% v/v NP40, 50% glycerol. Mek kinase: 25 mM Tris pH 7.8, 10 mM NaCl, 1 mM EDTA, 1 mM DTT, 4 μM Leupeptin, 50% glycerol. In order to assay for Raf-1 activity, Raf and Mek were diluted together with reaction buffer (200 mM Tris pH 8.2, 100 mM NaCl and 20 mM 2-mercaptoethanol) to 4 and 20 μg/mL, respectively, and 20 μL of this enzyme-substrate mixture was added to each well of a 96-well plate. The kinase reaction was initiated by adding 25 μL of 10 μM γ-[^{33}P]ATP (sp. Act. 400 Ci/mol) for incubation at 32 °C for 25 min. Filtration onto a phosphocellulose mat was used to harvest protein, and 1% phosphoric acid was used to wash away unbound radio-nucleotide. Following drying by microwave heating, the filter was enclosed in a plastic sample bag, scintillation fluid was added, and a b-plate counter was used to measure filter-bound radioactivity. To screen for inhibitors, test compounds were serially diluted from 10 mM stock solutions in DMSO, using a liquid handling robot, into 10% DMSO in water to 10 times the final desired concentrations (1% final DMSO concentration). Five microliters of these serially diluted stocks or matching DMSO containing vehicle was added to the enzyme-substrate mixture, prior to the addition of radiolabelled ATP. IC_{50} values were calculated using a four-parameter non-linear curve-fitting program. At least two independent IC_{50} determinations

were performed on each compound, and the mean value is reported. In all cases, standard deviations were less than 50% of the mean IC_{50} value.
10. For water solubilizing groups we specifically focused on substituents with heteroatoms that could add hydrophilicity or provide a site for salt formation. For a discussion on importance of the aqueous solubility as a drug-like property: Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3, and references cited therein. Huuskonen, J. *Combin. Chem., High Throughput Screen.* **2001**, *4*, 311.
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