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# Pyridyl and thiazolyl bisamide CSF-1R inhibitors for the treatment of cancer

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ABSTRACT

The bisamide class of kinase inhibitors was identified as being active against CSF-1R. The synthesis and SAR of pyridyl and thiazolyl bisamides is reported, along with the pharmacokinetic properties and in vivo activity of selected examples.



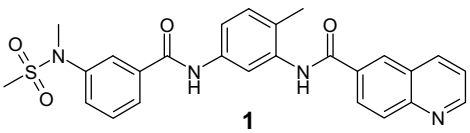
CSF-1R is a member of the class III receptor tyrosine kinases, along with c-Kit, Flt3 and PDGFR α and β. Colony stimulatory factor 1 (CSF-1), also known as macrophage/monocyte colony stimulatory factor (M-CSF), binds to CSF-1R, resulting in dimerization, autophosphorylation and activation of signal transduction.[1] CSF-1/CSF-1R signaling is essential for normal monocyte development, trophoblastic implantation and mammary gland development during pregnancy and lactation.[2,3] In cancer, pro-tumorigenic macrophages have been identified and linked to poor prognosis in breast, ovarian and prostate cancers.[4,5] Elevated levels of CSF-1 and CSF-1R have been reported in several tumor types, including breast, ovarian and endometrial cancers, and have also been linked to invasion and metastasis.[6] Inhibition of CSF-1R activity could therefore have multiple effects on the tumor through reduction in the levels of tumor-associated macrophages (TAMs) and direct effects on the tumor itself.

Compounds with activity against the other class III RTKs and KDR, such as Sutent[7] and ABT-869,[8] have been reported as inhibitors of CSF-1R. As yet, no selective small molecule has entered clinical trials, although PD0360324,[9] a monoclonal antibody from Pfizer, recently entered phase I for the treatment of rheumatoid arthritis. Other structural types have been reported as CSF-1R inhibitors, with some targeting inflammation rather than cancer indications.[10–17]

Subset screening of our compound collection identified diaminophenyl bisamides as CSF-1R inhibitors, with **1** (Fig. 1) being the most potent of these (CSF-1R $IC_{50}$ 89 nM). Within AstraZeneca, compounds from this scaffold had previously been explored as inhibitors of p38 MAP kinase.[18] Other groups have found related compounds to be inhibitors of Lck and c-Kit[19].

Activity was retained when the quinoline was replaced with other bicyclic ring systems such as quinoxaline or benzothiazole. A variety of substituents on the left-hand side aryl ring, especially at the 3-position, were also tolerated (data not included). Despite good activity in both enzyme and cell assays, compounds of this type suffered from low aqueous solubility and high plasma protein binding. The relatively high molecular weights limited options to improve the physical properties. The replacement of the quinoline with a 3-pyridyl group reduced both the MW and lipophilicity, and of the four possible configurations of the amide bonds (Fig. 2), three compounds (**2**–**4**) demonstrated good enzyme activity, with **3** having good activity in our cell proliferation assay (Table 1).[20] In our hands, Sutent had a CSF-1R enzyme $IC_{50}$ of 12 nM, and cell activity of 0.09 μM, consistent with literature data.[7,8]

Replacing methyl with chloro on the central ring of pyridyl bisamide **3** improved activity (Table 2, **6**), with small lipophilic groups at the 3-; 3,4- and 3,5-positions giving excellent cell potency (**6**, **13**–**16**). Replacing the pyridyl ring with phenyl (**17**) destroyed



**1**

**Figure 1.** Bisamide screening hit **1**.

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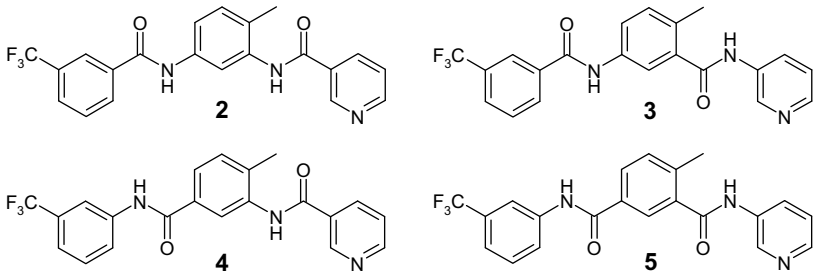
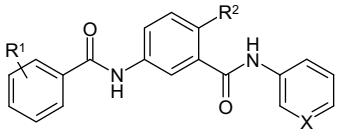



**Figure 2.** Pyridyl bisamides **2**–**5**.

**Table 1**
CSF-1R enzyme and cell activity for **2**–**5**

| Compound | $IC_{50}$ (μM) | Cell (μM) |
|---|---|---|
| **2** | 0.011 | 2.15 |
| **3** | 0.037 | 0.23 |
| **4** | 0.032 | 1.34 |
| **5** | 6.9 | ND |

**Table 2**
CSF-1R enzyme and cell activity—pyridyl bisamides




| Compound | $R^1$ | $R^2$ | X | $IC_{50}$ (μM) | Cell (μM) |
|---|---|---|---|---|---|
| **3** | 3-$CF_3$ | Me | N | 0.037 | 0.23 |
| **6** | 3-$CF_3$ | Cl | N | 0.016 | 0.14 |
| **7** | 3-Me | Cl | N | 0.010 | 0.24 |
| **8** | 3-F | Cl | N | 0.025 | 1.64 |
| **9** | 3-Cl | Cl | N | 0.010 | 0.33 |
| **10** | 3-Br | Cl | N | 0.014 | 0.26 |
| **11** | 3-OMe | Cl | N | 0.009 | 0.94 |
| **12** | 3-$NMe_2$ | Cl | N | 0.016 | 0.77 |
| **13** | 3,5-Me | Cl | N | 0.008 | 0.05 |
| **14** | 3,4-Me | Cl | N | 0.005 | 0.15 |
| **15** | 3,5-Cl | Cl | N | 0.003 | 0.13 |
| **16** | 3,4-Cl | Cl | N | 0.003 | 0.09 |
| **17** | 3-$CF_3$ | Me | CH | >50 | >10 |

activity, suggesting that the pyridine N makes a key H-bond interaction with the protein.

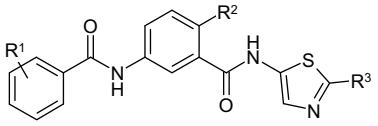
Examples from this set of compounds were found to have acceptable oral PK properties in rats, with moderate or low in vivo clearance and good bioavailability (Table 3).

Compounds from this series were also active against another AstraZeneca kinase target, B-Raf. Replacing the 3-pyridyl with other six-membered heterocycles gave less potent compounds for both CSF-1R and B-Raf.[21] However, modeling studies within our B-Raf program suggested a thiazole ring as an alternative to the pyridine. Compounds of this type (Table 4, **18**–**20**) also had good in vitro potency for CSF-1R; about twofold less active in the cell assay than the corresponding pyridyl examples (Table 2, **6**, **9** and **13**). 2-Methylthiazole derivatives were more potent than the corresponding pyridyl compounds; isopropyl and cyclopropyl groups were also tolerated at the thiazole 2-position. The SAR established for the pyridyl series transferred closely to the thiazolyl bisamides.

**Table 3**
Rat pharmacokinetic data of pyridyl bisamides upon iv (3 mpk) and po (10 mpk) dosing

| Compound | *F* (%) | Cl (ml/min/kg) | Vss (L/kg) | $T_{1/2}$ (h) |
|---|---|---|---|---|
| **3** | 40 | 16 | 1 | 1 |
| **6** | 76 | 20 | 1.5 | 1 |
| **7** | 28 | 30 | 1.3 | 0.8 |
| **9** | 33 | 16 | 1.4 | 1.1 |

**Table 4**
CSF-1R enzyme and cell activity—thiazolyl bisamides



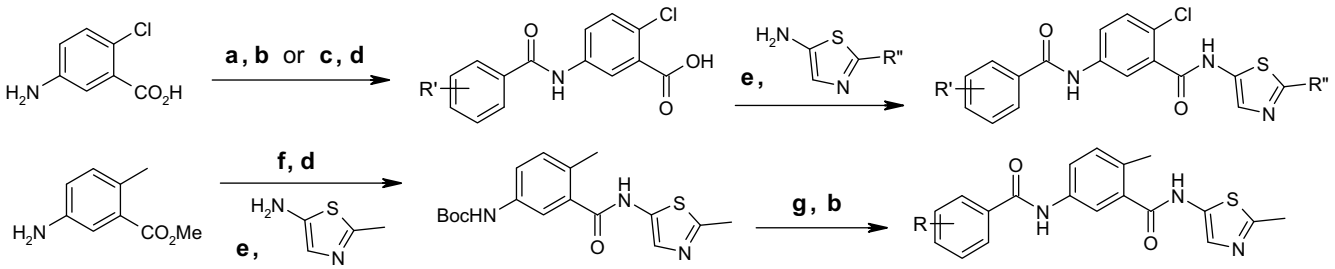

| Compound | $R^1$ | $R^2$ | $R^3$ | $IC_{50}$ (μM) | Cell (μM) |
|---|---|---|---|---|---|
| **18** | 3-$CF_3$ | Cl | H | 0.003 | 0.27 |
| **19** | 3-Cl | Cl | H | 0.005 | 0.52 |
| **20** | 3,5-Me | Cl | H | 0.003 | 0.11 |
| **21** | 3-$CF_3$ | Me | Me | 0.010 | 0.11 |
| **22** | 3-$CF_3$ | Cl | Me | 0.007 | 0.05 |
| **23** | 3-Cl | Me | Me | 0.007 | 0.32 |
| **24** | 3-Cl | Cl | Me | 0.004 | 0.18 |
| **25** | 3,5-Me | Me | Me | 0.004 | 0.10 |
| **26** | 3,5-Me | Cl | Me | 0.006 | 0.06 |
| **27** | 3-$CF_3$,5-F | Cl | Me | 0.007 | 0.11 |
| **28** | 3-Cl,5-F | Cl | Me | 0.011 | 0.13 |
| **29** | 3-$CF_3$ | Cl | $^i$Pr | 0.013 | 0.15 |
| **30** | 3,5-Me | Cl | $^i$Pr | 0.031 | 0.11 |
| **31** | 3-$CF_3$ | Cl | cPr | 0.14 | 0.10 |

Compounds were prepared via the routes shown in Scheme 1, varying the sequence of reactions to install the thiazole or the phenyl ring at the end of the synthesis.[22] (Pyridyl examples were prepared using similar chemistry).

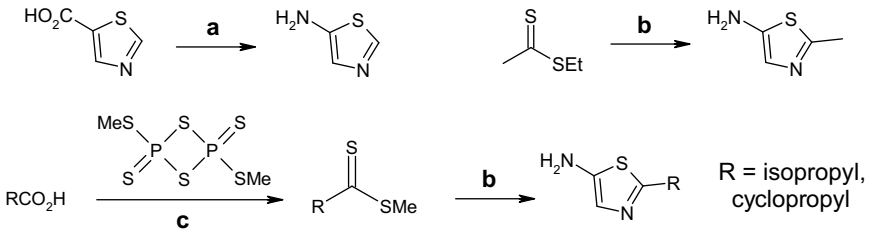
The aminothiazole building blocks were prepared as shown in Scheme 2. 5-Aminothiazole was prepared by a Curtius rearrangement of the thiazole acid and deprotection of the Boc-protected intermediate. 2-Methyl-5-aminothiazole was prepared via condensation and subsequent cyclization of aminoacetonitrile and ethyl dithioacetate.[23] The isopropyl and cyclopropyl thiazoles were prepared in a similar fashion, after first converting the carboxylic acid to the appropriate dithioate with the Davy reagent.[24]

Compound **22** was screened against a diverse panel of kinases at 1 μM (Table 5) and found to have good general kinase selectivity, including the other class III RTKs: c-Kit, Flt3 and PDGFRβ.[25]

Compounds with an unsubstituted thiazole (Table 6, **18**, **19**) were found to have higher in vivo rat clearance than the pyridyl analogues (Table 3, **6**, **9**). However, the more potent 2-methyl thiazoles also had improved PK profiles (**22**, **24**). In vivo clearance could be reduced further with the introduction of fluorine on the phenyl ring (**27**). Acceptable rat PK was also achieved when the methyl group on the thiazole ring was replaced with isopropyl (**29**).



**Scheme 1.** Preparation of examples **18**–**31**. Reagents and conditions: (a) $SOCl_2$, MeOH, rt; (b) $ArCO_2H$, HATU, $^iPr_2NEt$, DMF, rt; (c) ArCOCl, $Et_3N$, $CH_2Cl_2$, 0 °C; (d) LiOH, THF/MeOH/$H_2O$ 3:1:1, rt; (e) HATU, $^iPr_2NEt$, DMF, rt; (f) $O(CO_2{}^tBu)_2$, $K_2CO_3$, THF/$H_2O$ 4:1, rt; (g) HCl gas, MeOH, rt.




**Scheme 2.** Preparation of aminothiazoles. Reagents and conditions: (a) i—DPPA, $Et_3N$, $^t$BuOH, Δ, 8 h; ii—4 N HCl in dioxane, MeOH, 0 °C, then rt 2 h; (b) $NH_2CH_2CN{\cdot}H_2SO_4$, $Et_3N$, MeOH, 0 °C, then dithioate rt, 2 h; (c) 1,2,4-trichlorobenzene, 130 °C, 10 min.

**Table 5**
Kinase selectivity of thiazolyl bisamide **22**

| Kinase | % Kinase activity remaining |
|---|---|
| CSF-1R | 1 |
| EphA2 | 5 |
| Hck | 6 |
| Fyn | 7 |
| c-Raf | 9 |
| Src | 10 |
| KDR | 10 |
| PDGFRβ | 45 |
| c-Kit | 57 |
| GSK3β | 91 |
| CDK2/cyclinA | 95 |
| IGF-1R | 107 |
| IR | 108 |
| Met | 109 |
| FGFR1 | 111 |
| Tie2 | 111 |
| IKKβ | 112 |
| JAK2 | 112 |
| Flt3 | 114 |
| MAPK1 | 116 |
| Pim-1 | 116 |
| EGFR | 118 |

**Table 6**
Rat pharmacokinetic data upon iv (3 mpk) and po (10 mpk) dosing, and PD activity, of thiazolyl bisamides

| Compound | *F* (%) | Cl (ml/min/kg) | Vss (L/kg) | $T_{1/2}$ (h) | % Inhibition of pCSF-1R at 2 and 6 h |
|---|---|---|---|---|---|
| **18** | 40 | 58 | 4.2 | 1.2 | |
| **19** | 20 | 54 | 1.8 | 0.5 | |
| **22** | 62 | 13 | 1.1 | 1.2 | 70, 35 |
| **24** | 43 | 12 | 0.9 | 0.9 | |
| **26** | 27 | 14 | 1.2 | 1.3 | 90, 60 |
| **27** | 31 | 2 | 1.4 | 7.3 | 100, 100 |
| **29** | 65 | 37 | 2.2 | 1.0 | |

To assess the in vivo CSF-1R activity of the thiazolyl bisamides, compounds were dosed orally in a mouse pharmacodynamic (PD) model. 3T3 cells were engineered to express human mutant full length CSF-1R (301–969) (3T3/CSF-1$R_{MT}$) in which the kinase activity was constitutively on. Female nude mice were implanted with $5 \times 10^6$ 3T3/CSF-1$R_{MT}$ cells subcutaneously and grown in vivo until tumors were >250 $mm^3$ in size. After dosing, tumors were analyzed for pCSF-1R levels by ELISA, and blood plasma samples assessed for drug concentrations. Examples from the thiazolyl bisamide series showed excellent inhibition of pCSF-1R in vivo at 2 and 6 h after dosing at 50 mpk (Table 6).

In conclusion, pyridyl and thiazolyl bisamides have excellent in vitro activity against CSF-1R, with an example from the latter series demonstrating good selectivity against other class III RTKs. Members of both series have good oral PK profiles, and compounds from within the thiazolyl class effectively inhibit CSF-1R phosphorylation in an orally dosed mouse PD model. These compounds may therefore have potential utility in the treatment of diseases driven by the involvement of CSF-1 dependent macrophages.

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