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3-Amido-4-anilinoquinolines as CSF-1R kinase inhibitors 2: Optimization of the PK profile

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ABSTRACT

The optimization of compounds from the 3-amido-4-anilinoquinolines series of CSF-1R kinase inhibitors is described. The series has excellent activity and kinase selectivity. Excellent physical properties and rodent PK profiles were achieved through the introduction of cyclic amines at the quinoline 6-position. Compounds with good activity in a mouse PD model measuring inhibition of pCSF-1R were identified.

In the preceding paper, we reported 3-amido-4-anilinoquinolines as highly selective inhibitors of CSF-1R kinase, along with our initial efforts to optimize the series.[1] Examples from the 6,7-dimethoxyquinoline series were very potent in our enzyme and cell assays, but typically had poor physical properties and rat PK, with short half-lives. Activity in our mouse PD model was achieved with compound **1** (Fig. 1), but only at the high dose of 100 mpk. With the goal of optimizing the physical properties and PK profile of the series, basic side chains were installed at the 6- and 7-positions of the quinoline scaffold, linked through oxygen and nitrogen atoms. Compounds of this type (e.g., **2**, Fig. 1) retained the good enzyme and cellular potency of the dimethoxyquinoline leads, and also possessed high aqueous solubility and low levels of plasma protein binding. For these compounds, however, the in vivo clearance in rats was extremely high, and oral exposure was minimal. The amidoquinolines displayed a remarkable kinase selectivity profile, however, with examples found to have activity against almost no kinases other than CSF-1R. This observation encouraged our continued efforts to optimize the series.

The introduction of a basic side chain has been used to improve the physical properties and PK profiles of other series of kinase inhibitors, to deliver marketed drugs such as Gleevec[2] and Iressa.[3] However, this structural feature proved to be a metabolic liability for the amidoquinoline scaffold. We next installed cyclic amines such as methylpiperazine directly at the 6- and 7-positions. Biological and pharmacokinetic data for these compounds are given in Table 1.

In the 7-MeO series, the dichloroanilino compounds, including the very potent 2,3-Cl analogue **5**, were highly cleared, but still showed an improvement over compounds in this series with basic side chains. Encouragingly, the 2,4-F example **6** had excellent rat PK, including good oral bioavailability, although it had an IC_{50} of only 1 μM in our cell proliferation assay.[4] From a small number of matched pairs, and influenced by earlier data on related compounds,[1] we felt that potency was better with the piperazine substituent at the 6-position rather than the 7-position, and focused subsequent efforts on compounds of this type.

We speculated that oxidation of the electron-rich quinoline ring might contribute to the high rate of clearance, and compounds were prepared without the 7-methoxy group, and with a 7-F substituent in its place. Compounds without the 7-MeO substituent (**7-10**) showed reduced activity in both enzyme and cell. The two 7-F compounds (**11**, **12**) did in fact show moderate in vivo clearance, but were also less potent. The 2,4-F example **12** was only weakly active

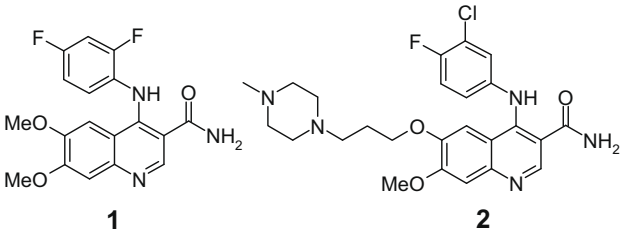

Figure 1. CSF-1R inhibitors from the amidoquinoline series.

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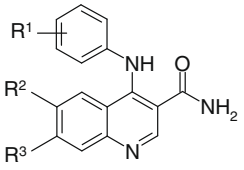
Table 1
Potency, physical property data and rat PK upon iv (3 mpk) and po (10 mpk) dosing for **1** and **3–17**



Compound	R^1	R^2	R^3	IC_{50} (μM)	Cell (μM)	Sol (μM)	PPB (% free)	*F* (%)	Cl (ml/min/kg)	Vss (L/kg)	$T_{1/2}$ (h)
1	2,4-F	MeO	MeO	0.025	0.40	54	5.6	100	23	0.9	0.6
3	2,4-Cl	*N*-Methylpiperazine	MeO	0.003	0.64	160	3.2	ND	72	8.7	1.7
4	3,4-Cl	*N*-Methylpiperazine	MeO	0.002	0.38	ND	3.6	ND	50	3.8	1.2
5	2,3-Cl	*N*-Methylpiperazine	MeO	<0.003	0.06	810	4.5	110	76	6.8	2.5
6	2,4-F	*N*-Methylpiperazine	MeO	0.006	1.0	>1000	25	90	15	2.3	1.8
7	2,4-Cl	*N*-Methylpiperazine	H	0.129	8.9	180	1.8				
8	3,4-Cl	*N*-Methylpiperazine	H	0.059	2.7	720	5.8				
9	2,3-Cl	*N*-Methylpiperazine	H	0.008	0.71	44	4.6	ND	190	5.9	0.7
10	2,4-F	*N*-Methylpiperazine	H	0.110	3.9	970	26	21	45	7.6	1.9
11	2,3-Cl	*N*-Methylpiperazine	F	0.003	0.44	8	4.4	ND	23	1.1	0.8
12	2,4-F	*N*-Methylpiperazine	F	0.054	4.2	71	24	13	20	1.4	1.1
13	3,4-Cl	MeO	*N*-Methylpiperazine	0.024	0.55	ND	ND				
14	2,3-Cl	MeO	*N*-Methylpiperazine	0.023	0.17	220	ND				
15	2,4-F	MeO	*N*-Methylpiperazine	0.011	0.72	>1000	ND				
16	2,3-Cl	*N*-Methylpiperazine	EtO	<0.003	0.06	50	2.0	100	57	3.5	0.9
17	2,4-F	*N*-Methylpiperazine	EtO	0.006	0.23	290	11	100	12	2.5	2.1

in our cell assay (4.2 μM). The 2,3-Cl example **11** was closer to the level of potency we felt was required (0.44 μM in the cell), but still significantly less active than the corresponding 7-MeO compound **5**.

We had observed previously improved potency for 6-aminoquinolines when 7-MeO was replaced by 7-EtO. The 2,4-F analogue **17** achieved both good in vitro activity, 0.23 μM in the cell, and excellent in vivo PK. The 2,3-Cl compound **16** had higher clearance in rats than the 2,4-F example, but was very potent in the cell assay (0.06 μM).

With a combination of good cell potency and encouraging PK, compounds **16** and **17** represented excellent advanced leads for further optimization of the series. We next explored a range of cyclic amines at the 6-position, with the goal of identifying compounds with the best overall balance of potency and PK, as determined by our mouse PD model. Sets of compounds were prepared with both 2,3-Cl aniline **18–25** (best in vitro potency) and 2,4-F aniline **26–32** (best physical properties and good PK). Data for these compounds is shown in Table 2.

To assess the in vivo CSF-1R activity of the amidoquinolines, 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-$1R_{MT}$) in which the kinase activity was constitutively on. Female nude mice were implanted with 5×10^6 3T3/CSF-$1R_{MT}$ cells subcutaneously and grown in vivo until tumors were >250 mm^3 in size. Tumors were analyzed for pCSF-1R levels by ELISA 2 and 6 h after dosing, and blood plasma samples were assessed for drug concentrations. Earlier compounds had been dosed at 50 or 100 mpk, but the potency and other properties of these amidoquinolines allowed us to drop the screening dose to 25 mpk. Compounds with 2,3-Cl aniline were typically ~10-fold more potent in the cell assay than the 2,4-F aniline examples, but had higher levels of plasma protein binding, and higher rat in vivo clearance. The choice of amine at the 6-position had relatively little effect on cell potency, but a significant impact on physical properties and PK. Basic groups here typically resulted in compounds with a superior profile to those with neutral substituents such as morpholine (**29**), or the piperazine amides (**24**, **25**). Examples with both 2,3-Cl and 2,4-F anilines showed good PD activity out to 6 h.

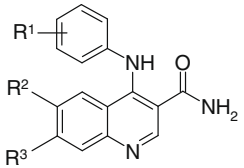
Compounds were screened for their activity against the hERG channel,[5] with the 2,3-Cl compounds more active here than the 2,4-F compounds. The homopiperazine examples (**20, 21, 28, 32**) showed increased hERG activity relative to the corresponding piperazines.

The cell data for the 6,7-dimethoxyquinoline compounds[1] showed that other anilines were also tolerated. In addition to determining potency, the choice of aniline also influenced the overall physical properties and PK. For example, compounds with a 2,4-substituted aniline typically had the best PK profiles. To explore further the relative importance of in vitro potency, physical properties (especially protein binding) and PK on in vivo activity, the aniline substituent was re-evaluated with the 6-methylpiperazine, 7-ethoxy scaffold (**33–38**, Table 2).

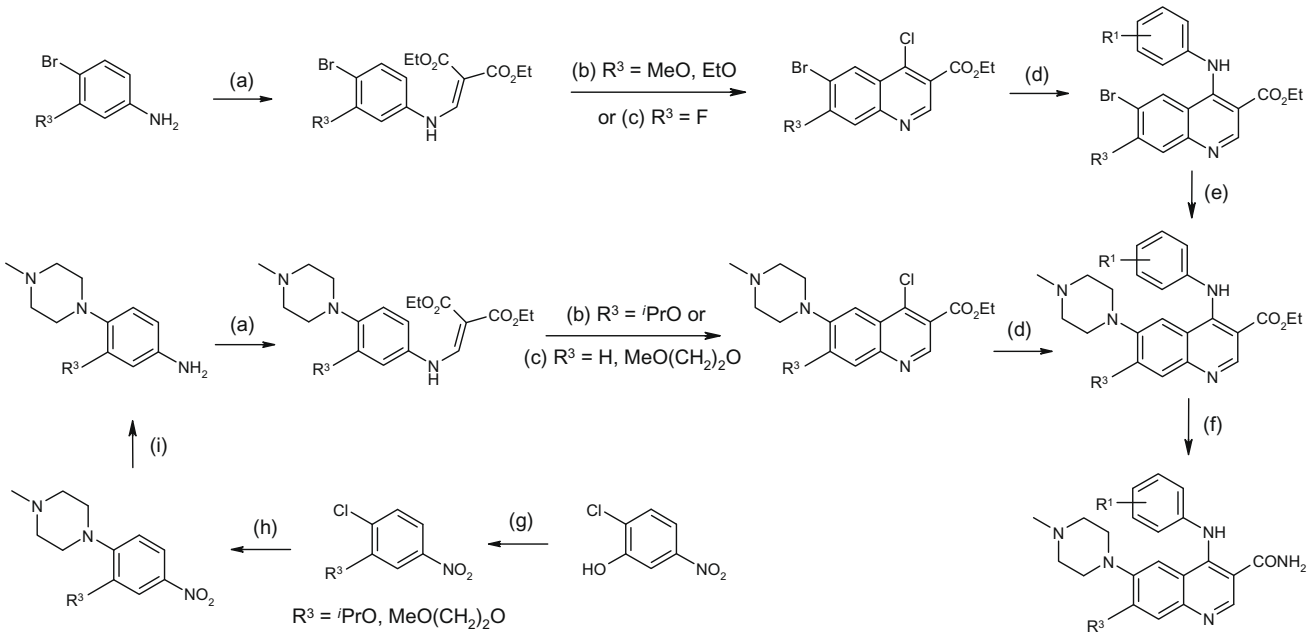
Enhanced in vitro potency had been observed upon moving from 7-MeO to 7-EtO, and a set of 7-^{i}PrO examples (**39–42**) was also prepared, to see if this trend would continue. While tolerated in terms of potency, there was no further improvement to justify the increase in lipophilicity. Introducing a 7-methoxyethoxy group (**43–47**), however, gave compounds that retained the potency of the 7-EtO analogues, but with higher aqueous solubility and reduced plasma protein binding. Several examples from Table 2, including the 7-methoxyethoxy compounds, showed excellent activity in the PD model at both 2 and 6 h.

Compounds in Tables 1 and 2 were prepared as shown in Scheme 1. For the 7-MeO (**3–6**) and 7-EtO compounds (**16–17** and **33–38**) condensation of the appropriate 4-bromoaniline with diethylethoxymethylene malonate followed by a one-pot cyclization/chlorination gave the chloroquinoline esters. For the 7-F quinolines (**11–12**), it was necessary to perform the cyclization step at 250 °C in diphenyl ether, followed by a separate chlorination step. Aniline additions were typically straightforward, but required higher temperatures for the less nucleophilic anilines such as 2,3-Cl. The methylpiperazine was introduced under standard Buchwald–Hartwig conditions,[6] and final compounds were prepared by conversion of the quinoline ester to the amide by treatment with formamide and then methoxide.[7] 6-Methoxy, 7-piperazine compounds (**13–15**) were prepared in a similar fashion from 3-bromo-4-methoxyaniline.[8] An alternative route was used to prepare compounds with either no substituent (**7–10**), isopropoxy (**39–42**) or methoxyethoxy (**43–47**)

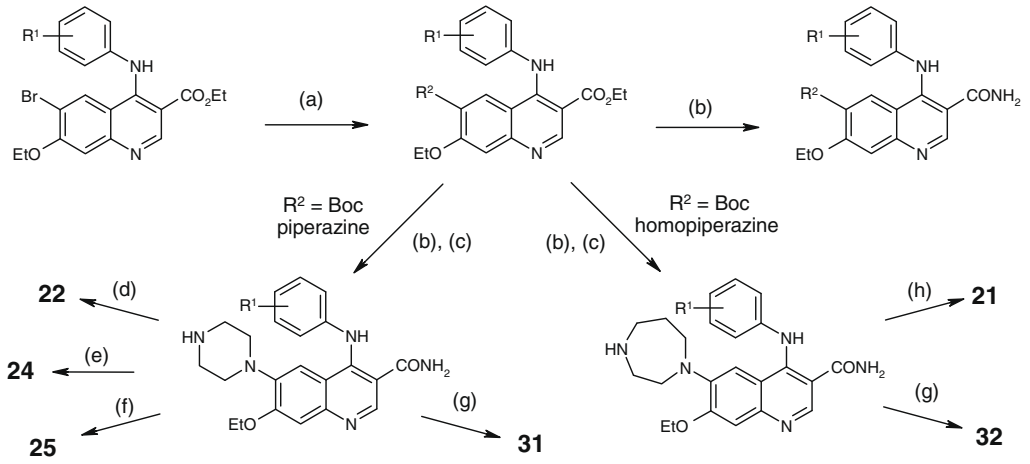
Table 2
Cell potency, physical property data, rat PK upon iv (3 mpk) and po (10 mpk) dosing, mouse PD and hERG activity for **16**–**47**



Compound	R^1	R^2	R^3	Cell (μM)	Sol (μM)	PPB (% free)	*F* (%)	Cl (ml/min/kg)	Vss (L/kg)	$T_{1/2}$(h)	% Inhibition of pCSF-1R at 2 and 6 h	hERG (μM)
16	2,3-Cl	*N*-Methylpiperazine	EtO	0.06	50	2.0	100	57	3.5	0.9	75, 45	8.4
18	2,3-Cl	*N*-Ethylpiperazine	EtO	0.02	11	1.9	19	86	6.8	1.2	95, 75	3.2
19	2,3-Cl	*N*-Isopropylpiperazine	EtO	0.02	250	1.5	66	120	11	1.2	85, 65	7.6
20	2,3-Cl	*N*-Methylhomopiperazine	EtO	0.05	130	1.9	100	63	40	8.6	90, 95	4.0
21	2,3-Cl	*N*-Isopropylhomopiperazine	EtO	0.02	420	4.9					90, 75	3.1
22	2,3-Cl	*N*-Hydroxyethylpiperazine	EtO	0.03	67	4.8						8.1
23	2,3-Cl	*N*-Methoxyethylpiperazine	EtO	0.03	13	1.7						—
24	2,3-Cl	*N*-Acetylpiperazine	EtO	0.07	2	9.6	11	62	2.3	0.65	65, 30	8.2
25	2,3-Cl	*N*-Hydroxyacetylpiperazine	EtO	0.09	29	16	6	72	2.2	0.66		—
17	2,4-F	*N*-Methylpiperazine	EtO	0.23	290	11	100	12	2.5	2.1	90, 65	23
26	2,4-F	*N*-Ethylpiperazine	EtO	0.17	320	4.6	46	19	3.6	2.3		19
27	2,4-F	*N*-Isopropylpiperazine	EtO	0.17	>1000	14					70, 50	—
28	2,4-F	*N*-Methylhomopiperazine	EtO	0.26	530	31	16	32	16	6.6	90, 55	5.9
29	2,4-F	Morpholine	EtO	0.48	64	8.4	25	36	1.1	0.6		>30
30	2,4-F	4-Hydroxypiperidine	EtO	0.31	2	8.9						17
31	2,4-F	*N*-Cyclopropylpiperazine	EtO	0.66	150	1.4	34	21	0.6	0.9	70, 10	20
32	2,4-F	*N*-Cyclopropylhomopiperazine	EtO	0.27	100	2.2						6.9
33	3-Cl, 4-F	*N*-Methylpiperazine	EtO	0.13	ND	3.4	55	53	9.3	2.2	70, 55	6.1
34	3,4-Cl	*N*-Methylpiperazine	EtO	0.06	3	0.8	42	47	3.7	1.2	80, 40	4.3
35	2-F, 3-Cl	*N*-Methylpiperazine	EtO	0.11	10	7.1	54	24	1.9	1.1	100, 90	8.4
36	2-F, 4-Me	*N*-Methylpiperazine	EtO	0.05	54	6.0	46	10	1.8	2.3	85, 60	15
37	2-F, 5-Me	*N*-Methylpiperazine	EtO	0.09	66	6.0	88	28	5.3	2.3	90, 80	13
38	2,5-F	*N*-Methylpiperazine	EtO	0.11	71	7.3	33	28	2.9	1.3	75, 45	26
39	2,3-Cl	*N*-Methylpiperazine	^{i}PrO	0.05	200	1.4					70, 35	4.9
40	2,4-F	*N*-Methylpiperazine	^{i}PrO	0.17	52	1.7	40	7	1.4	2.3	100, 50	28
41	2-F, 3-Cl	*N*-Methylpiperazine	^{i}PrO	0.21	790	4.9						
42	3,4-Cl	*N*-Methylpiperazine	^{i}PrO	0.12	250	1.0						6.8
43	2,3-Cl	*N*-Methylpiperazine	$MeO(CH_2)_2O$	0.01	380	10	32	290	21	0.83	100, 80	7.6
44	2,4-F	*N*-Methylpiperazine	$MeO(CH_2)_2O$	0.24	720	46	100	68	18	3.8	100, 70	>30
45	2-F, 3-Cl	*N*-Methylpiperazine	$MeO(CH_2)_2O$	0.10	910	39					80, 75	22
46	2-F, 4-Me	*N*-Methylpiperazine	$MeO(CH_2)_2O$	0.14	63	16	100	33	8.6	3.4	100, 80	>30
47	2-F, 5-Me	*N*-Methylpiperazine	$MeO(CH_2)_2O$	0.17	ND	16	45	60	4.7	4.2	85, 70	21



Scheme 1. Preparation of examples **3**–**17** and **33**–**47**. Reagents and conditions: (a) diethylethoxymethylene malonate, CH_3CN, rt; (b) $POCl_3$, Tol, 100 °C; (c) Ph_2O, 250 °C; then $POCl_3$, 105 °C; (d) aniline, cat. AcOH, EtOH, 80 °C or DMF, 100 °C; (e) *N*-methylpiperazine, $Pd_2(dba)_3$, BINAP, Cs_2CO_3, Tol, 100 °C; (f) $HCONH_2$, DMF, NaOMe, 100 °C; (g) $MeO(CH_2)_2Br$, or ^iPrI, DMF, K_2CO_3, 40 °C; (h) *N*-methylpiperazine, DMF, 130 °C; (i) 10% Pd/C, H_2 (50 psi), MeOH.



Scheme 2. Preparation of examples **16**–**32**. Reagents and conditions: (a) amine, $Pd_2(dba)_3$, BINAP, Cs_2CO_3, Tol, 100 °C; (b) $HCONH_2$, DMF, NaOMe, 100 °C; (c) TFA/DCM; (d) $HCOCH_2OH$, Tol/MeOH, Δ, then $NaBH(OAc)_3$, AcOH, THF, Δ; (e) Ac_2O, Et_3N, DCM; (f) $HOCH_2CO_2H$, HATU, $^iPr_2NEt$, DMF; (g) $(CH_2)_2C(OEt)OSiMe_3$, AcOH, 4 Å mol. Sieves, $NaBH_3CN$, MeOH, Δ; (h) $(CH_3)_2CO$, $NaBH(OAc)_3$, AcOH, THF, Δ.

at the 7-position, in which the amine was installed prior to the cyclization step. Quinolines unsubstituted at the 7-position were prepared from commercially available 4-(4-methylpiperazino)aniline. The other alkoxy substituents were derived from 2-chloro-5-nitrophenol, via alkylation, nucleophilic substitution of the chloronitrobenzene with *N*-methylpiperazine, and reduction of the nitro group to give the required aniline.

Where the amine substituent was commercially available, compounds in Table 2 were prepared from the appropriate 6-bromoquinoline ester by standard Buchwald–Hartwig coupling conditions, followed by amidation of the ester. Where the amine substituents were either unavailable, or unsuitable for the Pd-coupling step, compounds were prepared by derivatization of the unsubstituted piperazine or homopiperazine amides, accessed in turn from coupling of the Boc protected amines, as shown in Scheme 2. A reductive amination reaction with [(1-ethoxycyclopropyl)oxy]trimethylsilane[9] gave the cyclopropyl derivatives **31** and **32**.

As with example **1**,[1] compound **17** was found to have an extremely selective profile when evaluated in a panel of ~85 kinases (Table 3).[10] Apart from moderate activity versus ARK5, the compound was essentially inactive against all the other kinases screened, including the other class III RTKs: c-Kit, Flt3 and PDGFR α.

Table 3
Kinase selectivity of **17** at 1 μM

Kinase	% Inhibition
CSF-1R	96
ARK5	51
ALK	23
Rsk1	21
80 kinases	<15

In conclusion, 3-amido-4-anilinoquinolines are potent inhibitors of CSF-1R, with excellent kinase selectivity. The introduction of cyclic amines such as *N*-methylpiperazine at the 6-position gives compounds with attractive physical properties and PK profiles. Several of these compounds show good activity in our mouse PD model. The amidoquinoline series may therefore be able to test the hypothesis that a CSF-1R inhibitor will impact tumor progression through an effect on tumor-associated macrophages. Examples have been selected for further biological profiling, the results of which will be published in due course.

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