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3-Amido-4-anilinocinnolines as a novel class of CSF-1R inhibitor

David A. Scott *, Les A. Dakin, David J. Del Valle, R. Bruce Diebold, Lisa Drew, Thomas W. Gero, Claude A. Ogoe, Charles A. Omer, Galina Repik, Kumar Thakur, Qing Ye, Xiaolan Zheng

Cancer Research, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451, USA

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ABSTRACT

3-Amido-4-anilinocinnolines have been identified as potent and highly selective inhibitors of CSF-1R. The synthesis and SAR of these compounds is reported, along with some physical property, pharmacokinetic and kinase selectivity data.

CSF-1R is a class III receptor tyrosine kinase, which along with its ligand CSF-1 (also known as macrophage colony-stimulating factor, M-CSF) regulates the growth, differentiation and survival of monocytes and macrophages. A recent review summarizes the biology of CSF-1R, its role in cancer and inflammation, and efforts to develop selective small molecule inhibitors.[1]

We recently reported 3-amido-4-anilinoquinolines as potent and highly selective inhibitors of CSF-1R, and described the optimization of the pharmacokinetic profile of that series through the introduction of cyclic amines.[2a,b] Several of these compounds, such as AZ683 (Fig. 1) achieved good activity in a mouse pharmacodynamic model which measured inhibition of pCSF-1R. AZ683 also demonstrated efficacy and reduced the level of tumor-associated macrophages (TAMs) in a breast cancer xenograft model.[3]

We now report the identification of a new class of CSF-1R inhibitors, the 3-amido-4-anilinocinnolines. In contrast to quinazolines and quinolines, kinase inhibitors incorporating a cinnoline scaffold are relatively rare. One reported use involved 4-anilinocinnolines, prepared as part of the program that ultimately delivered the dual VEGFR/EGFR anilinoquinazoline inhibitor ZD6474 (vandetanib).[4] In contrast, an earlier report on EGFR inhibitors found the cinnoline analogue of a potent anilinoquinazoline to be inactive.[5] 4-Anilinocinnolines and benzylideneamino cinnolines were described in an application for PLK1 inhibitors.[6] 3-Amido-4-anilinocinnolines have also been reported as inhibitors of PDE4.[7]

To establish whether cinnolines were active against CSF-1R, we prepared examples **7a–e** in the 6,7-dimethoxycinnoline scaffold (Scheme 1). Treatment of 2-amino-4,5-dimethoxyacetophenone **1** with concd HCl and sodium nitrite generated the diazonium salt,

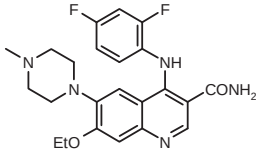

Figure 1. 3-Amidoquinoline **AZ683**.

which was trapped with pyrrolidine to give **2**.[8] Reaction of **2** with diethyl carbonate gave **3**, isolated as its sodium salt, with cyclization in TFA to give the dihydrocinnoline ester **4**. The remaining steps were similar to those employed in the synthesis of the 3-amido-4-anilinoquinolines—chlorination with $POCl_3$ to give **5**, aniline displacement, and conversion of the esters **6** to the amides **7a–e** with formamide and NaOMe. Yields are given for the synthesis of difluoro compound **7a**; comparable yields were achieved with other anilines. Compounds from this scaffold demonstrated excellent activity in both our enzyme (data not shown) and cell proliferation assays;[9] typically about twofold better in the cell than the corresponding quinoline analogues (Table 1).

Aqueous solubility and plasma protein binding data for compounds **7a–e** are reported in Table 1. With the objective of improving the physical properties of the series, we next introduced cyclic amines at the 6-position of the cinnoline scaffold. Our initial route to the *N*-methylpiperazino compounds was similar to that used for the 6,7-dimethoxycinnolines, but ran into difficulties at two points (Scheme 2). First, the acylation of the 4-bromo-3-ethoxyaniline **8** to prepare the required aminoketone **9** was relatively low yielding (42%).[10] More surprisingly, the Buchwald–Hartwig coupling step to introduce *N*-methylpiperazine onto bromoester **10** gave some of the desired ester **11** (~30% isolated material at best, which could

* Corresponding author.
E-mail address: david.scott@astrazeneca.com (D.A. Scott).

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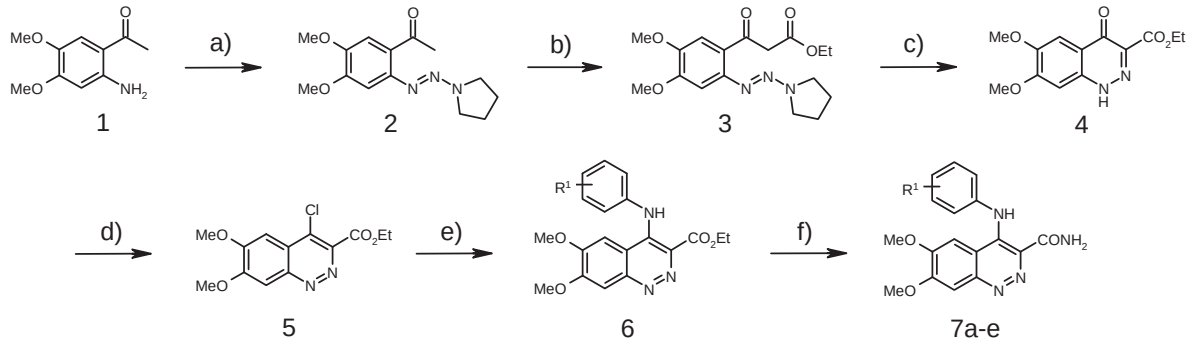
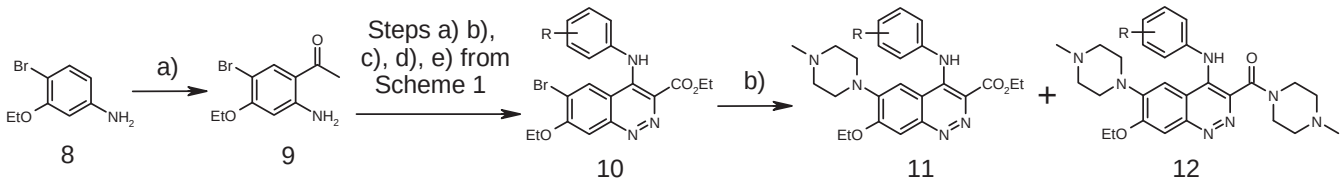


Scheme 1. Reagents and conditions: (a) concd HCl, $NaNO_2$, H_2O, 0 °C then pyrrolidine, 0.4 N aq NaOH, 85%; (b) $CO(OEt)_2$, NaH, THF, reflux; (c) TFA, 0 °C, 50% over two steps; (d) $POCl_3$, reflux, 88%; (e) aniline, EtOH, cat. AcOH, 75 °C, 88%; (f) NH_2CHO, 0.5 M NaOMe in MeOH, DMF, 100 °C, 96%.

Table 1
Cell potency and physical property data for cinnolines **7a–e**

Compound	R^1	Cell (μM)	Quinoline potency cell[2a] (μM)	Sol (μM)	PPB (% free)
7a	2,4-F	0.16	0.40	3	4.5
7b	2-F,4-Me	0.11	0.25	<1	3.3
7c	2-F,5-Me	0.20	0.21	<1	3.1
7d	2-F,3-Cl	0.08	0.12	<1	2.2
7e	2,3-Cl	0.05	0.09	<1	1.2

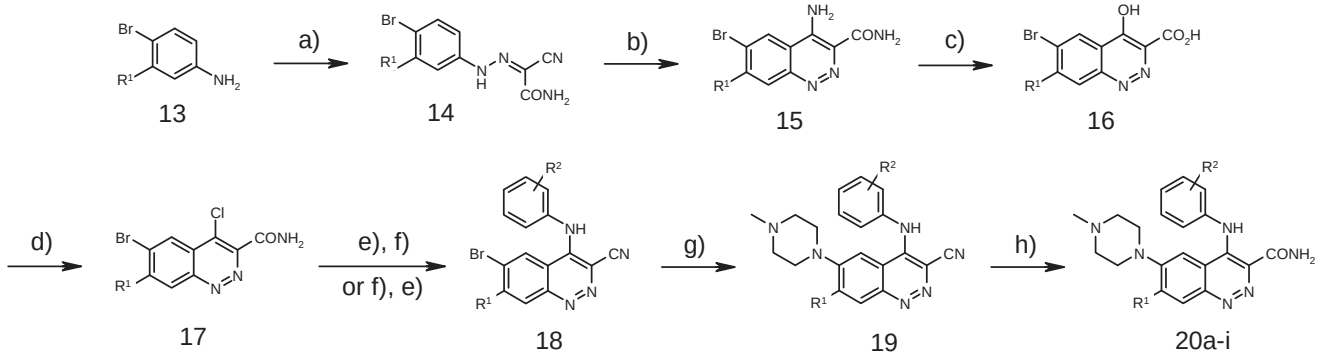


Scheme 2. Reagents and conditions: (a) 1.0 M BCl_3 in DCM, CH_3CN, 1.0 M $TiCl_4$ in DCM, reflux, 42%; (b) *N*-methylpiperazine, $Pd_2(dba)_3$, BINAP, Cs_2CO_3, DMA, 90 °C.

then be converted to the primary amide) but also amide **12** as the major product. This contrasted with our synthesis of the corresponding quinolines, in which a variety of amines were routinely introduced at the 6-position of the quinoline esters.[2b]

An alternative synthetic route avoided aminoketone **9**, and enabled amine couplings to the bromocinnoline nitrile **18**, rather than the ester **10** (Scheme 3).[11] Alkoxybromoaniline **13** in a suspension of concd HCl was treated with sodium nitrite, and then 2-cyanoacetamide and aqueous sodium acetate to give intermediate **14**. Cyclization to the 3-amido-4-aminocinnoline **15** was performed with $TiCl_4$ in refluxing toluene, followed by hydrolysis to the hydroxy acid **16**. Hydroxy acid **16** was converted to chloroamide **17** with thionyl chloride and then aqueous ammonia in acetone. Aniline displacement and amide dehydration with phosphorus oxychloride gave bromocinnoline nitrile **18**. These two steps could also be reversed, to introduce the required aniline slightly later in the sequence. Buchwald–Hartwig couplings introduced *N*-methylpiperazine to give **19**, and hydrolysis of the nitrile under basic conditions gave final compounds **20a–i**. Yields for Scheme 3 are for **20g**, but comparable yields were achieved for other anilines and the 7-EtO compounds.

Cell potency and physical property data for compounds **20a–i** are shown in Table 2. The 7-ethoxy-6-*N*-methylpiperazinocinnolines **20a–e** were more potent than both the corresponding



Scheme 3. Reagents and conditions: (a) concd HCl, aq $NaNO_2$, 0 °C then $NCCH_2CONH_2$, aq NaOAc, rt, 94%; (b) $TiCl_4$, toluene, reflux, 87%; (c) KOH, EtOH/H_2O, reflux, 86%; (d) $SOCl_2$, cat. DMF, reflux, then acetone, 0 °C, aq NH_3, 94%; (e) aniline, EtOH, cat. AcOH, 75 °C; (f) $POCl_3$, Et_3N, DCM, reflux, 43–64% over two steps; (g) *N*-methylpiperazine, $Pd_2(dba)_3$, BINAP, Cs_2CO_3 (or XPHOS, Na^{t}BuO), DMA, reflux, 69%; (h) KOH, ^{t}BuOH, reflux, 49%.

Table 2
Cell potency and physical property data for cinnolines **20a–i**

Compound	R^1	R^2	Cell (μM)	Quinoline potency cell[2b] (μM)	Sol (μM)	PPB (% free)	hERG (μM)
20a	EtO	2,4-F	0.048	0.23	8	3.9	16
20b	EtO	2-F,4-Me	0.013	0.05	27	1.6	13
20c	EtO	2-F,5-Me	0.040	0.09	120	11	—
20d	EtO	2-F,3-Cl	0.026	0.11	18	1.9	12
20e	EtO	2,3-Cl	0.014	0.06	10	<1.7	5
20f	MeO	2,4-F	0.048	1.03	290	9.6	20
20g	MeO	2-F,4-Me	0.025	—	87	6.3	>30
20h	MeO	2-F,5-Me	0.057	—	22	7.1	18
20i	MeO	2-F,3-Cl	0.021	—	110	7.8	10

Table 3
Mouse, rat and dog PK data for **20b**

Species	Cl (ml/min/kg)	$T_{1/2}$ (h)	Vss (L/kg)	*F* (%)
Mouse	12	0.9	0.6	82
Rat	10	9.7	2.8	90
Dog	2	12.6	1.8	78

IV dosing at 3 mpk; oral dosing at 10 mpk for mice, rats, 5 mpk for dogs.

Table 4
Kinase selectivity of **20a** at 1 mM and 0.1 mM

% Inhibition at 1 μM		% Inhibition at 0.1 μM	
CSF-1R	95	CSF-1R	100
cKit	90	cKit	35
ARK5	78	ARK5	23
Rsk1	44	Rsk1	7
MELK	41	MELK	15
78 Kinases	<35	78 Kinases	<30

6,7-dimethoxycinnoline compounds **7a–e** and the corresponding quinoline analogues; several different anilines gave compounds with a cell IC_{50} <0.05 μM. The 7-methoxycinnolines **20f–i** were also extremely potent in our cell assay. The introduction of *N*-methylpiperazine significantly improved the physical properties of this series relative to the 6,7-dimethoxy compounds. In particular, the 7-methoxy examples **20f–i** combined excellent cell potency with good aqueous solubility and plasma protein binding data. Compounds were screened against the hERG ion channel,[12] with a trend towards the more lipophilic examples showing increased levels of activity.

An excellent in vivo PK profile was achieved for some examples of this class of cinnoline. Data for **20b**, upon IV and oral dosing in mice, rats and dogs, are shown in Table 3. Low rates of clearance were observed in all three species, accompanied by good bioavailability.

We previously reported the kinase selectivity profile of the 3-amido-4-anilinoquinoline compound AZ683.[2b] Cinnoline **20a** was also highly selective for CSF-1R. In a panel of 83 kinases,[13] cKit and ARK5 were the only kinases other than CSF-1R against which it showed significant (IC_{50} <1 μM) levels of activity (Table 4). Compound **20a** had an IC_{50} <4 nM in our CSF-1R enzyme assay.[14]

In conclusion, the reported 3-amido-4-anilinocinnoline compounds are very potent inhibitors of CSF-1R. The introduction of *N*-methylpiperazine at the 6-position delivers compounds with good physical properties, and an excellent PK profile was observed for **20b** in multiple species. Examples in both the 6,7-dimethoxy and 7-alkoxy-6-*N*-methylpiperazino scaffolds demonstrated enhanced cellular potency relative to the corresponding quinoline compounds. Cinnoline **20a** also showed an excellent kinase selectivity profile. Our efforts to develop further CSF-1R inhibitors from the cinnoline class will be reported in due course.

Acknowledgments

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