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Discovery of AC710, a Globally Selective Inhibitor of Platelet-Derived Growth Factor Receptor-Family Kinases

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Supporting Information

ABSTRACT: A series of potent, selective platelet-derived growth factor receptor-family kinase inhibitors was optimized starting from a globally selective lead molecule **4** through structural modifications aimed at improving the physiochemical and pharmacokinetic properties, as exemplified by **18b**. Further clearance reduction via per-methylation of the α-carbons of a solubilizing piperidine nitrogen resulted in advanced leads **22a** and **22b**. Results from a mouse tumor xenograft, a collagen-induced arthritis model, and a 7 day rat in vivo tolerability study culminated in the selection of compound **22b** (AC710) as a preclinical development candidate.

KEYWORDS: *platelet-derived growth factor receptor (PDGFR)-family kinases, feline McDonough sarcoma (FMS)-like tyrosine kinase 3 (FLT3), colony-stimulating factor-1 receptor (CSF1R) inhibitor, acute myeloid leukemia (AML), cancer bone metastasis, inflammatory arthritis, AC710*

Receptor tyrosine kinases (RTKs) are a subfamily of protein kinases that critically regulate the processes of normal cell signaling and many pathological conditions.[1] A class of RTKs known as the platelet-derived growth factor receptor (PDGFR) family,[2,3] which includes colony-stimulating factor-1 receptor (CSF1R, also known as FMS), feline McDonough sarcoma (FMS)-like tyrosine kinase 3 (FLT3), stem cell factor receptor (KIT), and PDGFR α and β, has been implicated in various proliferative and inflammatory diseases.[4]

We were particularly interested in two members of this family of kinases. Activating internal tandem duplication (ITD) mutations in FLT3 are detected in approximately 30% of acute myeloid leukemia (AML) patients and are associated with poor prognosis.[5] AC220 (**1**, quizartinib) (Figure 1), a potent FLT3 inhibitor from this laboratory,[6] has demonstrated convincing clinical activity in FLT3-ITD+ AML patients.[7]

CSF1R is the exclusive receptor for the macrophage colony-stimulating factor (M-CSF or CSF-1).[8] Activation of CSF1R leads to the proliferation, survival, motility, and differentiation of cells of the monocyte/macrophage lineage and hence plays a role in normal tissue development and immune defense.[9,10] Activation of CSF1R also leads to the proliferation and differentiation of osteoclast precursors and therefore mediates the process of bone resorption.[11]

It has been shown that M-CSF is one of several cytokines implicated in the recruitment of tumor-associated macrophages (TAMs) that contribute to tumor angiogenesis and tumor progression to metastasis.[12] More recently, the preclinical CSF1R inhibitor GW2580 (**2**) (Figure 1) inhibited tumor metastasis and angiogenesis in mouse tumor xenograft experiments.[13] JNJ-28312141 (**3**), a dual inhibitor of CSF1R and FLT3, also demonstrated suppression of solid tumor growth and preservation of bone in preclinical models.[14]

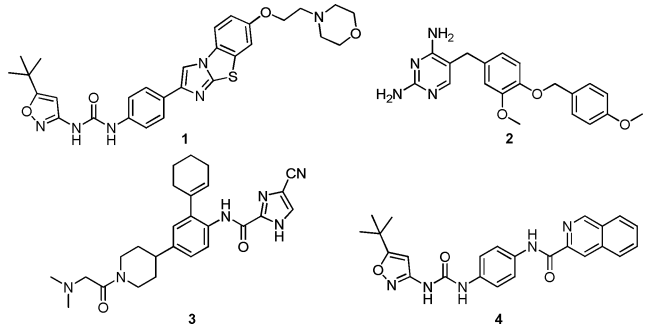

Figure 1. FLT3 inhibitor (**1**), CSF1R inhibitor (**2**), and dual inhibitors (**3** and **4**).

CSF1R is also implicated in inflammatory arthritis, as M-CSF, together with RANK ligand, is required for osteoclastogenesis.[15] Elevated M-CFS signaling leads to increased osteoclast activity and bone loss attending arthritis,[16] and CSF-1 deficient (op/op) mice proved to be highly resistant to arthritis in a collagen-induced model.[17] CSF1R inhibitors, such as **2** and **3** (Figure 1), demonstrated efficacy against arthritis and inflammatory bone erosion in arthritic animal models.[18,19]

Therefore, novel small molecule inhibitors targeting both FLT3 and CSF1R can be developed for either FLT3-ITD+

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AML or cancer bone metastasis and rheumatoid arthritis. Synergy can also potentially be realized for autoimmune diseases by inhibiting both FLT3 and CSF1R simultaneously. A number of dual FLT3 and CSF1R inhibitors are being studied in clinical settings, including compound **3** (Figure 1). PLX3397 (structure undisclosed) has been reported as a FTL3, CSF1R, and KIT inhibitor and is currently in phase II clinical trial for Hodgkin's lymphoma and FLT3-mutated AML.[20]

KinomeScan technology developed in our laboratory allows for detailed interrogation of a compound's selectivity across virtually the entire kinome[21] and provides a springboard for jump starting a hit-to-lead and lead optimization process with a selective kinase inhibitor. Compound **4**, originally developed as a FLT3 inhibitor (both in vitro and in vivo), exhibited high affinity for CSF1R (Table 1) and other members of PDGFR-

Table 1. Binding, Cell Potency, and Rat PK Data of Picolinamides

compd	FLT3 K_d[a] (nM)	CSF1R K_d[a] (nM)	cell IC_{50}[a] (nM)	clearance Cl[c] (mL/min/kg)	oral AUC[c] (μM h)	F %
4	0.7	8.5	53	47	0.8	9
7	0.8	2.0	63.9	37.9	0.5	4
8	0.6	1.6	223	ND[d]	ND	ND
14a	1.2	1.8	145.0	35.8	0.4	3
14b	1.0	3.0	296	ND	ND	ND
18a	0.7	0.6	35.7	22.1	1.6	1
18b	0.5	0.8	41.0	25.8	4.8	20
18c	0.6	1.2	78	16.0	3.5	17.5
18d	3.4	3.4	169	ND	ND	ND
20a	0.4	1.12	64.4	15.0	5.0	22
20b	0.4	1.28	150	12.0	9.5	36
22a	0.5	1.0	50.4	10.9	9.1	31.5
22b	0.5	1.57	46.3	14.0	10.0	44
22c	0.3	1.8	74.7	11	2.4	8
26a	1.5	3.1	37.0	37.6	0.9	9.9
26b	0.4	1.0	67.4	24.0	1.4	12
29	0.9	4.9	109	ND	ND	ND

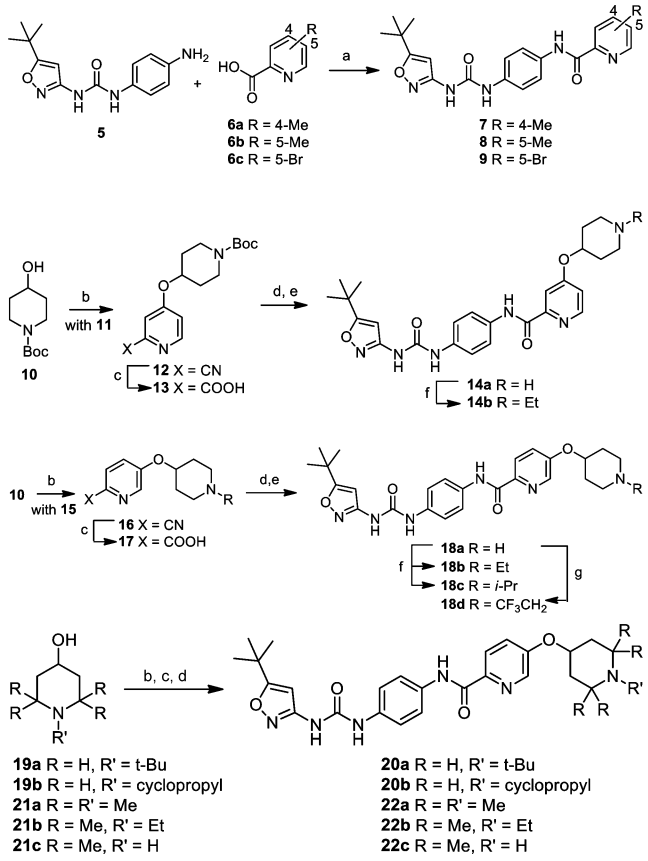
[a]Each experiment was run in duplicate, and the values shown are the averages of the two. [b]M-NFS-60 cell proliferation. [c]Sprague–Dawley rat, 1 mg/kg dosed intravenously and 10 mg/kg dosed orally. [d]ND, not determined.

family kinases.[22] Its selectivity score or S(10) of 0.033 across a panel of 386 distinct kinases established the highly desirable global kinome selectivity.[23] In M-NFS-60 cells, a murine CSF1R-driven cell system, **4** was shown to be effective in inhibiting proliferation (Table 1). Encouraged by the global selectivity and dual activity against FLT3 and CSF1R, we embarked on an optimization campaign starting from lead compound **4**.

The general synthesis of representative compounds described herein is outlined in Scheme 1. Condensation of known aniline **5**[24] with commercially available pyridine carboxylic acids **6a–c** provided **7**, **8**, and **9**. For the more complicated 4-pyridine-substituted analogues, nucleophilic substitution of 4-chloropicolinonitrile **11** with alcohol **10** yielded the pyridine ether **12**, which can be hydrolyzed under basic conditions to give the acid **13**. Coupling of acid **13** with **5** followed by acid treatment revealed compound **14a**. Subsequent reductive alkylation of **14a** with acetaldehyde afforded compound **14b**.

The 5-substituted picolinamide analogues **18a**, **20a,b**, and **22a–c** can be prepared in a three-step sequence similar to that

Scheme 1. General Synthesis of Picolinamides[a]



[a]Reagents and conditions: (a) 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI), *N*-hydroxybenzotriazole, *i*-Pr_2NEt, DMF, rt, 20–86%. (b) NaH, DMF, rt, 21–85%. (c) 3 N NaOH, EtOH, reflux, 78–90%. (d) Compound **5**, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU), Et_3N, DMF, rt, 61–90%. (e) 4 N HCl in 1,4-dioxane, rt, 77–95%. (f) CH_3CHO or acetone, $NaCNBH_3$, MeOH buffer (pH ~4), 0 °C–rt or reflux, 50–68%. (g) CF_3CH_2OTf, *i*-Pr_2NEt, DMF, rt–85 °C, 46%.

described for compound **14a**, using 4-fluoropicolinonitrile **15** as the starting material (Scheme 1). Reductive alkylation of **18a** with either acetaldehyde or acetone yielded **18b** and **18c**, respectively. Alkylation of **18a** with trifluoroethyl triflate provided **18d**.

In Scheme 2, the carbon-linked pyridine carboxylate **25** can be prepared using a Suzuki–Miyaura coupling[25] to form the requisite C–C bond. Subsequent straightforward manipulations (hydrogenation and saponification) yielded acid **25** for the coupling with aniline **5**. Deprotection and reductive alkylation provided **26a**. Analogously, a Negishi coupling[26] step, followed by a four-step sequence, generated analogue **26b**. Compound **29** was synthesized using a Molander-modified Suzuki coupling[27] from bromide **9**.

Despite the attractive in vitro profile of compound **4**, it did suffer from a number of issues, including poor aqueous solubility (<5 μg/mL pH 2–7.4), high rat clearance, and poor oral absorption when dosed at 10 mg/kg in a liposome formulation (Table 1). It was suspected that dissolution-limited absorption was partially contributing to the poor bioavailability.

Our initial modification centered on downsizing compound **4** to improve its physiochemical properties. On the basis of the putative binding mode for a type II adenosine triphosphate

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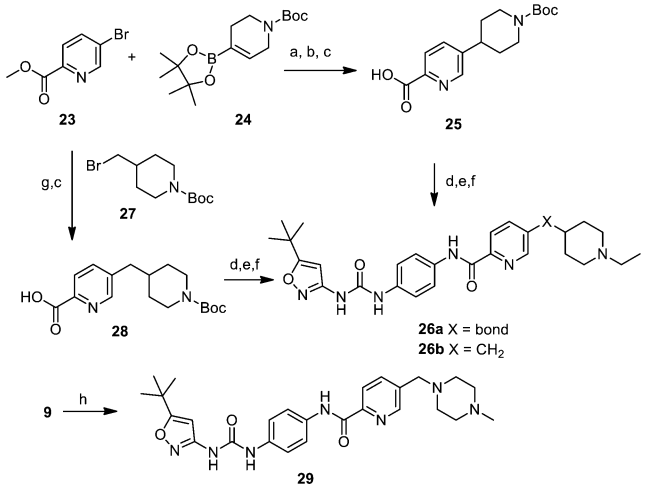
Scheme 2. Synthesis of 5-Pyridine Carbon-Substituted Analogues[a]



[a]Reagents and conditions: (a) Pd(1,1′-bis(diphenylphosphino)-ferrocene)Cl_2, K_2CO_3, DMF, 140 °C, 10 min, 68%. (b) 10% Pd/C, H_2, EtOAc, 100%. (c) NaOH, MeOH/THF, 100%. (d) Compound **5**, 2-(7-aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), Et_3N, DMF, rt, 75−80%. (e) 4 N HCl in 1,4-dioxane, rt, 100%. (f) CH_3CHO, $NaCNBH_3$, MeOH buffer (pH ∼4), 0 °C−rt, 83−87%. (g) Rieke zinc, THF, rt, 3 h; then $Cl_2Ni(Ph_2PCH_2CH_2PPh_2)$, rt, 17%. (h) Potassium 1-methyl-4-trifluoroboratomethylpiperazine, $Pd(OAc)_2$, 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl, Cs_2CO_3, THF/H_2O, 140 °C, 38%.

(ATP)-competitive inhibitor such as **4**,[28] it was hypothesized that the isoquinoline carboxamide portion of **4** is involved in interacting with the hinge region of CSF1R, and the extensive hydrophobic motif within the isoquinoline ring may not be needed. Toward that end, the benzo portion of the isoquinoline was replaced with a simple methyl group at either position of the ring junction. As shown in Table 1, the resulting analogues **7** and **8** retained good binding affinity for CSF1R with somewhat reduced cell activity in the case of **8**. The FLT3 K_d values for these two compounds were also maintained.

In many cases of kinase inhibitor design, introduction of solubilizing groups can improve cell permeability and activity. Therefore, a piperidinyl ether as a prototype solubilizing group was incorporated at either the 4- or the 5-position of the pyridine. When such a group was introduced at the 4-position of the pyridine ring, the binding affinity of resulting analogues (**14a** and **14b**) did not change much as compared to **7**; however, the cell activity decreased 2−4-fold (Table 1). Interestingly, when the same solubilizing groups were placed at the 5-position of the pyridine, a noticeable 3-fold binding affinity improvement and a 5-fold cell potency improvement were observed with analogues **18a** and **18b** (as compared to **8**). Rat PK studies were used to assess the change in clearance for select analogues. Both **7** and **14a** still showed high clearance, while **18a** and **18b** showed a moderate improvement in clearance, consistent with our initial design. In addition, the mesylate salt of **18b** exhibited good aqueous solubility (1134 μg/mL in water), reasonable oral exposure, and bioavailability, which prompted evaluation of all of the ensuing analogues as their mesylate salts.

Metabolite profiling studies indicated that the major route of metabolism for **18b** appeared to be de-ethylation at the piperidine nitrogen (data not shown). Unfortunately, the dealkylated product **18a** had much lower bioavailability than **18b** (Table 1). A number of approaches for slowing down the dealkylation were pursued. Increasing steric hindrance with an *i*-propyl group (**18c**) retained cell potency and slightly lowered the clearance (Table 1). Incorporation of a trifluoroethyl group (**18d**) led to 3-fold drop of cell potency in comparison to **18b**. Analogue **20a** with an *N*-*t*-butyl group exhibited slightly reduced cell activity and lower clearance. Even though the *N*-cyclopropyl group (**20b**) resulted in low clearance and high oral exposure, the 3-fold drop of potency over **18b** made it less appealing.

It was then hypothesized that bulking up the α-positions of the piperidine nitrogen might slow down the N-dealkylation, due to increased steric hindrance.[29] Therefore, 1,2,2,6,6-pentamethyl piperidine analogue **22a** was prepared. We were delighted by the cellular activity of this compound (Table 1). More importantly, as compared to **18b**, rat clearance was substantially lower, and oral bioavailability was higher. Encouraged by this result, the *N*-ethyl analogue **22b** was also synthesized and was found to be active in cells with a very similar rat PK profile to **22a**. The mesylate salts of **22a** and **22b** were shown to have solubility of greater than 1 mg/mL in water. The N−H analogue **22c** was also shown to have similar cell potency and clearance. Unfortunately, it exhibited much lower oral bioavailability and oral exposure, which could be attributed to the increased number of hydrogen bond donors.

In the meantime, we also surveyed other types of solubilizing elements at the 5-position of the pyridine ring. As shown in Table 1, as compared to **18b**, removal of the ether linkage led to an equipotent compound **26a** with higher clearance. Insertion of a methylene linker (**26b**) led to roughly 2-fold reduction of cell activity and similar clearance to **18b**. *N*-Methylpiperazine methylene-substituted compound **29** was about 3-fold less potent than **18b**.

The PDGFR-family kinase activity and selectivity for the three promising lead compounds, **18b**, **22a**, and **22b**, are shown in Table 2. First of all, the compounds were very selective across the entire panel of 386 unique kinases as demonstrated by the very low S(10) scores and more than 30-fold selectivity over any other non-PDGFR kinases.[30] Second, their binding affinities for the PDGFR-family kinases were very similar. In the relevant cell assays, all three compounds potently inhibited the phosphorylation of these kinases. FLT3 appeared to be the

Table 2. Kinome Selectivity, Binding Affinity, and Cell Activity for PDGFR-Family Kinases

		nM							
compd	S(10)[a] score	pCSF1R IC_{50}	FLT3 K_d	pFLT3 IC_{50}[b]	KIT K_d	pKIT IC_{50}[c]	PDGFRα K_d	PDGFRβ K_d	pPDGFRβ IC_{50}[d]
18b	0.063	6.9	0.5	1	0.6	7.9	2.3	1.3	6.2
22a	0.049	15	0.3	2	1.1	8	2.5	2.5	9.2
22b	0.034	10.5	0.6	2	1.0	1.2	1.3	1.0	7.7

[a]Selectivity determined against a panel of 386 unique kinases. [b]In MV4-11 cells. [c]In H526 cells. [d]In MG-63 cells.

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target that was more potently inhibited by these three compounds. KIT, PDGFR, and CSF1R were equally inhibited by these three compounds, with the exception of **22b**, being slightly more potent in inhibiting KIT phosphorylation. All three compounds also showed clean cytochrome P450 inhibition profiles (IC_{50} values > 40 μM) against a panel of five isoforms (1A2, 2C9, 2C19, 2D6, and 3A4). No Cyp induction was observed for **22b** when tested at 3 and 30 μM concentrations against three isoforms (1A2, 2D6, and 3A4).

In a CD-1 mouse PK study with compounds **18b**, **22a**, and **22b**, oral AUCs for **22a** and **22b** were 3−4-fold higher than those observed in rat (Table 3). To better characterize and

Table 3. Mouse PK and Dose−Response PD

		% inhibition			
compd	mouse AUC[a] (μM h)	3 mg/kg[b]	10 mg/kg[b]	30 mg/kg[b]	100 mg/kg[b]
18b	9.9	ND[c]	31	46	73
22a	65.8	ND	ND	82	92
22b	56.4	29	54	79	ND

[a]Dosed orally in CD-1 mice using Pharmatek#6 as vehicle. [b]Proliferation of M-NFS-60 cells in IP cavity in *Nu/Nu* mice. [c]ND, not determined.

compare these three compounds, more detailed dose−response pharmacodynamic (PD) studies in a CSF1R driven system were performed. M-NFS-60 cells are murine leukemic cells dependent upon CSF1R for survival and proliferation. As shown in Table 3, all three compounds dose dependently inhibited M-NFS-60 cell proliferation in the intraperitoneal (IP) cavity of mice when dosed orally.[31] Maximal effect was observed at 100 mg/kg for **18b** and at 30 mg/kg for **22a** and **22b**, consistent with the exposure difference among them.

Because of the close clustering of the three compounds in their in vitro and in vivo properties, a 7 day in vivo tolerability study in rat was conducted at 200 and 800 mg/kg. None of the compounds was tolerated at the 800 mg/kg dose due to diarrhea, body weight loss, and death, with much earlier onset of death for **22a**. All animals were clinically normal when dosed at 200 mg/kg, where **22b** gave approximately 5 times higher exposure than that of **18b**, suggesting better tolerability for **22b** in vivo at equivalent dose in comparison to **18b** and **22a**.

The antitumor efficacy of **22b** was assessed in a subcutaneous flank-tumor xenograft model in athymic nude mice using the MV4-11cell line, a human leukemia cell line that is FLT3-dependent and harbors a homozygous FLT3-ITD mutation. Compound **22b** was dosed at 0.3, 3, and 30 mg/kg for 2 weeks (Figure 2A). Compound **1**, used as a positive control, was dosed at 1 mg/kg. At 0.3 mg/kg of **22b**, tumor growth was temporally inhibited, and growth resumed quickly thereafter. At 3 and 30 mg/kg of **22b**, tumors regressed completely, and the tumor volume stayed suppressed for an extended period after dosing was halted. No body weight loss was observed in animals treated with **22b** at all doses, indicating that it is well tolerated in mice at efficacious doses.

Compound **22b** was further evaluated prophylactically in a mouse collagen-induced arthritis (CIA) model. As shown in Figure 2B, compound **22b** exhibited a significant impact on disease in a dose-dependent fashion, at a dose as low as 3 mg/kg for 15 days (day 0−14) as measured by paw clinical scores. At 10 and 30 mg/kg, **22b** demonstrated equivalent or slightly better efficacy in reducing the joint swelling and inflammation

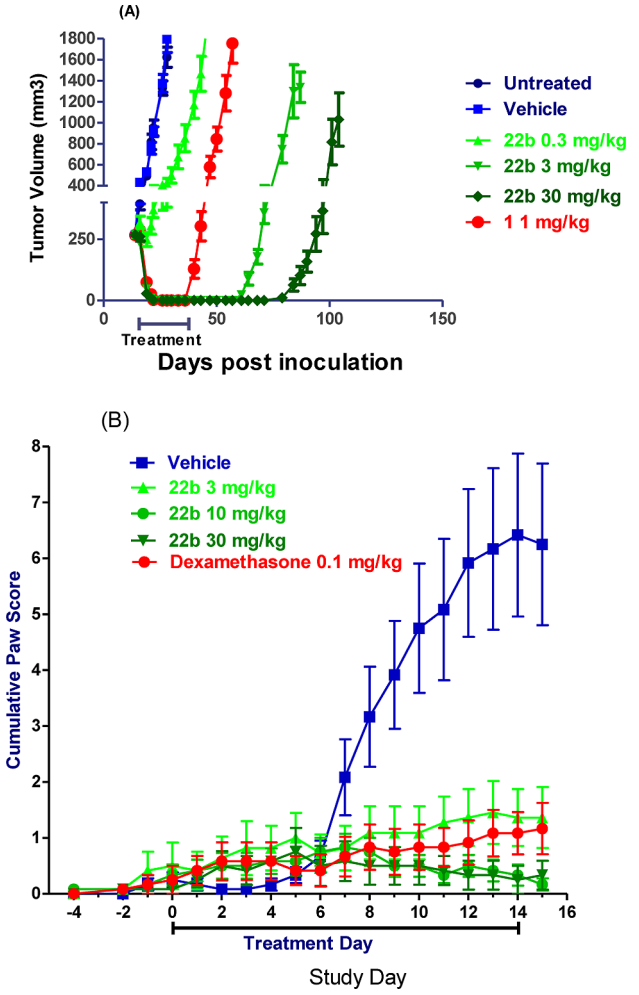

Figure 2. In vivo efficacy of compound **22b** in mouse models.

than dexomethasone administered at a safe dose. Histology analysis proved consistent with clinical score (data not shown). Compound **22b** was well tolerated at the tested doses based on body weight and body condition. On the basis of the overall better in vivo tolerability and the mouse efficacy data, compound **22b** (AC710) was selected as a preclinical development candidate.

In summary, starting from a globally selective, PDGFR-family kinase inhibitor **4** that exhibited poor aqueous solubility, high clearance, and low oral bioavailability, initial optimization through molecular weight reduction and introduction of solubilizing groups led to an advanced lead compound **18b** with an improved rat PK profile. Further reduction of clearance and PK improvement were achieved by per-methylating the α-positions of piperidine nitrogen. In vivo tolerability results in rat, coupled with in vivo efficacy in a mouse xenograft tumor model and a CIA model, precipitated the selection of compound **22b** (AC710) as a preclinical development candidate. Further effort in achieving greater selectivity within the PDGFR-family kinases will be reported in due course.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full experimental details for all final compounds synthesized (**7**, **8**, **14a,b**, **18a−d**, **20a,b**, **22a−c**, **26a,b**, and **29**), description of representative assays, rat PK protocol, and animal efficacy

studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

RTK, receptor tyrosine kinase; PDGFR, platelet-derived growth factor; CSF1R, colony-stimulating factor-1 receptor; FMS, feline McDonough sarcoma; FLT3, FMS-like tyrosine kinase 3; KIT, stem cell factor receptor; AML, acute myeloid leukemia; ITD, internal tandem duplication; M-CSF, macrophage colony-stimulating factor; TAM, tumor-associated macrophage; ATP, adenosine triphosphate; PD, pharmacodynamic; CIA, collagen-induced arthritis

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