Acridone-Based Inhibitors of Inosine 5′-Monophosphate Dehydrogenase: Discovery and SAR Leading to the Identification of *N*-(2-(6-(4-Ethylpiperazin-1-yl)pyridin-3-yl)propan-2-yl)-2-fluoro-9-oxo-9,10-dihydroacridine-3-carboxamide (BMS-566419)

Scott H. Watterson,* Ping Chen, Yufen Zhao, Henry H. Gu, T. G. Murali Dhar, Zili Xiao, Shelley K. Ballentine, Zhongqi Shen, Catherine A. Fleener, Katherine A. Rouleau, Mary Obermeier, Zheng Yang, Kim W. McIntyre, David J. Shuster, Mark Witmer, Donna Dambach, Sam Chao, Arvind Mathur, Bang-Chi Chen, Joel C. Barrish, Jeffrey A. Robl, Robert Townsend, and Edwin J. Iwanowicz

Bristol-Myers Squibb Pharmaceutical Research Institute, Post Office Box 4000, Princeton, New Jersey 08543

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Inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the de novo synthesis of guanosine nucleotides, catalyzes the irreversible nicotinamide-adenine dinucleotide dependent oxidation of inosine-5′-monophosphate to xanthosine-5′-monophosphate. Mycophenolate Mofetil (MMF), a prodrug of mycophenolic acid, has clinical utility for the treatment of transplant rejection based on its inhibition of IMPDH. The overall clinical benefit of MMF is limited by what is generally believed to be compound-based, dose-limiting gastrointestinal (GI) toxicity that is related to its specific pharmacokinetic characteristics. Thus, development of an IMPDH inhibitor with a novel structure and a different pharmacokinetic profile may reduce the likelihood of GI toxicity and allow for increased efficacy. This article will detail the discovery and SAR leading to a novel and potent acridone-based IMPDH inhibitor **4m** and its efficacy and GI tolerability when administered orally in a rat adjuvant arthritis model.

Introduction

Maintaining adequate nucleotide levels is essential for normal cellular function, including the synthesis of RNA and DNA. In mammals, nucleotides may be synthesized through one of two pathways: a de novo synthetic pathway or through a salvage pathway that utilizes existing purines and pyrimidines and their nucleotides and nucleosides.[1] The extent to which each pathway is utilized is dependent on the cell type. B- and T-lymphocytes are dependent on the de novo synthesis to generate sufficient levels of nucleotides necessary to initiate a proliferative response to mitogen or antigen.

Inosine 5′-monophosphate dehydrogenase (IMPDH[a]), a key enzyme in the de novo synthesis of guanosine nucleotides, catalyzes the irreversible nicotinamide-adenine dinucleotide (NAD) dependent oxidation of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP).[2] Two distinct cDNAs encoding IMPDH have been identified and isolated. These transcripts, labeled type I and type II, possess 84% sequence identity.[2a,3] IMPDH type II is expressed at very low levels in most resting cell types but is markedly up-regulated in actively proliferating cell types, including cancer cells and activated peripheral blood lymphocytes.[2a,4] As a result, IMPDH type II has emerged as an attractive target for selectively modulating the immune response without also inhibiting the proliferation of other cells.

Mycophenolic acid (MPA) has been shown to be a potent, uncompetitive, reversible inhibitor of human IMPDH type I and type II.[5] Mycophenolate mofetil (MMF; Figure 1), a prodrug of MPA, has clinical utility for the treatment of transplant rejection based on its inhibition of IMPDH. Unfortunately, dose-limiting gastrointestinal (GI) toxicity is observed in a clinical setting from the oral administration of either MMF or MPA.[6] MMF is rapidly and, to a large extent, completely absorbed following oral administration, whereby it is converted to MPA.[6] Three metabolites have been identified in humans: the 7-O-glucuronide (MPAG), 7-O-glucoside conjugate (MPAG1s), and the acyl glucuronide (AcMPAG).[6] MPAG, the major metabolite, is inactive against IMPDH and is found in substantially higher concentrations in the plasma of healthy subjects than MPA, contributing to the high doses required to achieve the desired plasma concentrations necessary for efficacy. Additionally, significant amounts of MPAG are excreted in the bile and

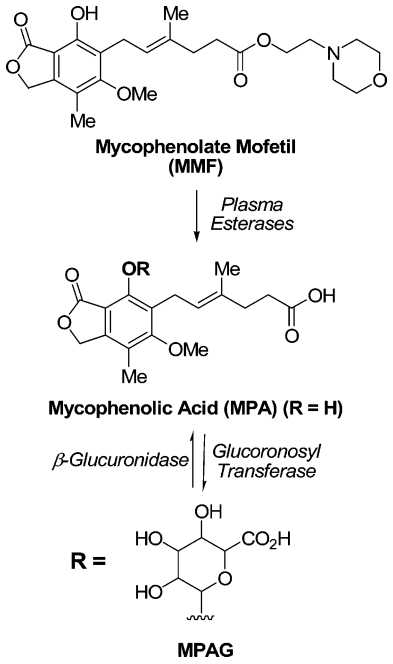

Figure 1.

* To whom correspondence should be addressed. Phone: 609-252-6778. Fax: 609-252-7410. E-mail: scott.watterson@bms.com.

[a] Abbreviations: IMPDH, inosine 5′-monophosphate dehydrogenase; MPA, mycophenolic acid; MMF, mycophenolate mofetil; HTS, high-throughput screen; CEM, human T-lymphoblast cell line; PBMC, human peripheral blood mononuclear cells; hERG, human ether-a-go-go-related gene; GI, gastrointestinal.

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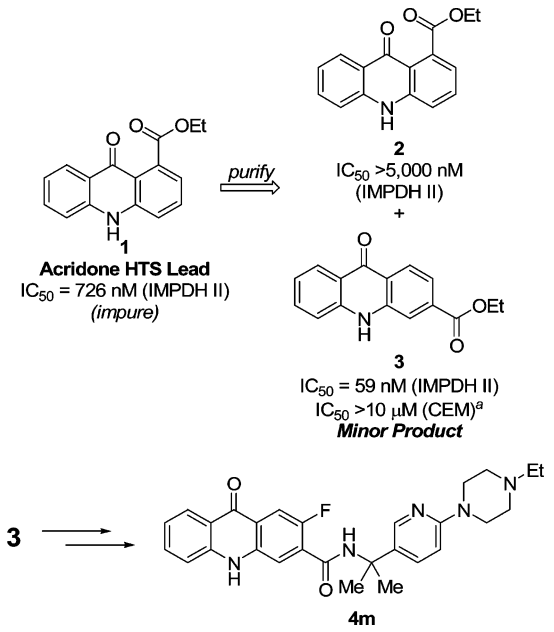


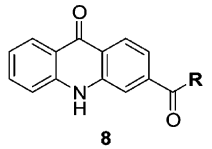
Figure 2. [a]Inhibition of T-cell proliferation in CEM lymphoblast cells (see Experimental Section for details).

converted to MPA by the action of the intestinal flora.[6] Coupled with the high doses required for efficacy, this reintroduction of MPA to the gut is likely a contributing factor to the clinically observed GI toxicity.

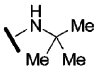
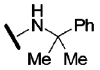
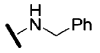
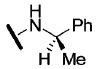
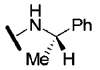
In spite of the commercial success of MMF, dose limiting GI toxicity has restricted its use primarily to the treatment of transplant rejection, even though the inhibition of IMPDH may provide a clinical benefit for other immune-mediated diseases such as rheumatoid arthritis. There is a continuing effort to identify and develop new inhibitors of IMPDH which are differentiated from MMF with improved GI tolerability. Although the GI toxicity associated with MPA is likely due to the inhibition of intestinal epithelial cell proliferation, the toxicity may be exacerbated by the unique pharmacokinetic profile (PK) of MPA, resulting from glucoronidation, excretion to the GI tract, and subsequent enterohepatic recirculation.[6] Two strategies have emerged in the literature to address the GI toxicity associated with MPA. The first is to develop inhibitors with a different PK profile that may minimize the exposure of the cells of the gut to an inhibitor, thereby reducing the likelihood of GI toxicity and allowing for increased exposure and greater efficacy. This may be accomplished by identifying inhibitors, which exclude the acid and phenolic chemical moieties implicated in the glucuronidation and subsequent enterohepatic recirculation. Several groups,[2b,7−9] including our own,[9] have focused on this approach and have identified a number of new classes of inhibitors. A second approach is to appropriately formulate MPA to reduce upper GI toxicity and improve absorption.[10] With favorable clinical data,[11] Novartis launched an enteric-coated formulation of mycophenolate sodium (ERL-80) in 2002.

In an effort to supplement our previously reported endeavors to develop IMPDH inhibitors,[9] we initiated a high-throughput screening (HTS) approach of our compound deck to find novel chemotypes. Acridone **1** (Figure 2) was identified as a potential lead with an IC_{50} of 0.726 μM against IMPDH II. Further analysis of the sample indicated the presence of an ∼12% impurity. Subsequent purification revealed that the source of the activity was a regio-isomeric acridone analogue (**3**), with an IC_{50} of 0.059 μM against IMPDH II. In this article, we outline the development of a novel acridone series and the SAR leading to the discovery of **4m** (BMS-566419;[12] Figure 2).

Table 1. In Vitro Potency of Acridone Analogues (**8**)[a]

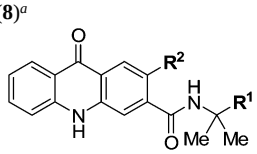


8

Cmpd	R	IMPDH II[a] IC_{50} (nM)	CEM[a,b] IC_{50} (μM)
8a		59	3.3
8b		19	2.1
8c		2,200	---
8d		95	---
8e		431	---

[a] IC_{50} values are shown as single determinations. [b] Inhibition of T-cell proliferation (see Experimental Section for details).

Table 2. In Vitro Potency of Fluoro-acridone Analogues (**4**) vs Acridone Analogues (**8**)[a]



cmpd	R^1	R^2	IMPDH II IC_{50} (nM)	CEM[c] IC_{50} (nM)	PBMC[c] IC_{50} (nM)
8[a]	Ph	H	19	2.1	0.73
8f[a]	2-pyridyl	H	53	2.2	
8g[a]	3-pyridyl	H	42	2.2	0.59
8h[a]	4-pyridyl	H	53	2.3	0.63
4a[b]	Ph	F	11 ± 5.9	0.83 ± 0.69	0.22 ± 0.07
4b[a]	2-pyridyl	F	29	1.0	
4c[b]	3-pyridyl	F	11 ± 0.8	0.42 ± 0.07	0.14 ± 0.04

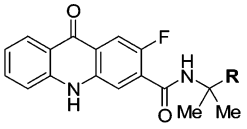
[a] IC_{50} values are shown as single determinations. [b] IC_{50} values are shown as mean values of three determinations. [c] Inhibition of T-cell proliferation (see Experimental Section for details).

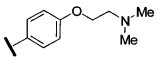
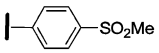
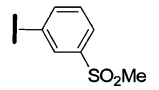
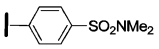
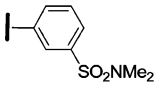
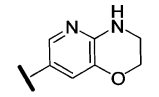
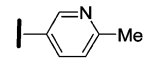
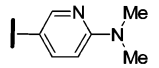
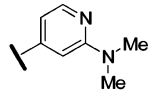
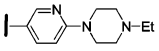
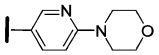
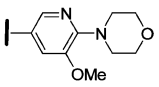
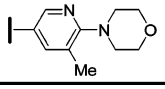
Chemistry

The synthetic pathways utilized in the preparation of the acridone inhibitors discussed in Tables 1−3 are outlined in Schemes 1−9. Acridones **8a**−**h** were prepared as shown in Scheme 1. A Buchwald−Hartwig[13] coupling of dimethyl 2-bromoterephthatate with aniline in the presence of $Pd(OAC)_2$, (*S*)-BINAP, and Cs_2CO_3 gave biphenylamine **6**. Hydrolysis of the esters with LiOH in MeOH/THF and subsequent cyclization with polyphosphoric acid at 165 °C afforded the acridone carboxylic acid **7** in high yield. Intermediate **7** was coupled with various commercially available amines using BOP-Cl and Et_3N in DMF to give acridones **8a**−**e**. The pyridyl-substituted tertiary-alkyl amines used for acridone derivatives **8f**−**h** were derived from commercially available nitriles via dimethylation using $MeCeCl_2$ in THF,[14] as outlined in Scheme 3.

Fluoro-acridones **4a**−**p** were prepared as exemplified in the preparation of analogue **4m** depicted in Scheme 2. The biphenyl amine intermediate **11** was prepared via a Buchwald−Hartwig[13] coupling of dimethyl 2-fluoroterephthatate with aniline in the

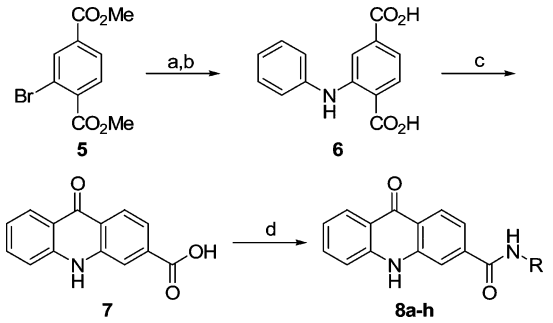
Table 3. In Vitro Potency of Fluoro-acridone Analogues[a]



Cmpd	R	IMPDH II[a] IC_{50} (nM)	CEM[a,b] IC_{50} (μM)	PBMC[a,b] IC_{50} (μM)
MPA		16 ±1.1	0.54 ±0.03	0.13 ±0.02
4d		20 ±5.2	0.56 ±0.01	0.21 ±0.04
4e		12 ±3.6	0.48 ±0.07	0.17 ±0.06
4f		12 ±3.2	0.58 ±0.13	0.28 ±0.7
4g		10 ±4.6	0.66 ±0.11	0.20 ±0.13
4h		7.9 ± 3.0	0.38 ±0.15	0.13 ±0.04
4i		17 ±4.0	0.92 ±0.12	0.32 ±0.02
4j		23 ±1.4	0.75 ±0.14	0.27 ±0.06
4k		22 ±8.3	0.85 ±0.07	0.34 ±0.06
4l		14 ±7.2	0.64 ±0.05	0.22 ±0.08
4m		17 ±2.2	0.77 ±0.08	0.22 ±0.05
4n		20 ±4.8	0.66 ±0.14	0.21 ±0.03
4o		20 ±7.5	0.81 ±0.14	0.32 ±0.07
4p		11 ±1.2	0.87 ±0.04	0.27 ±0.02

[a] IC_{50} values are shown as mean values of three determinations. [b] Inhibition of T-cell proliferation (see Experimental Section for details).

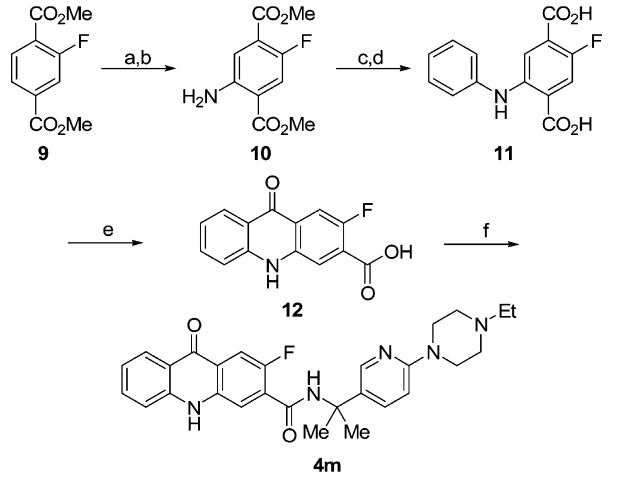
Scheme 1[a]



[a] Reagents and conditions: (a) $PhNH_2$, $Pd(OAc)_2$, (*S*)-BINAP, Cs_2CO_3, toluene, 97%; (b) LiOH, MeOH, THF, H_2O, 96%; (c) PPA, 165 °C, 98%; (d) RNH_2, BOP-Cl, Et_3N, DMF.

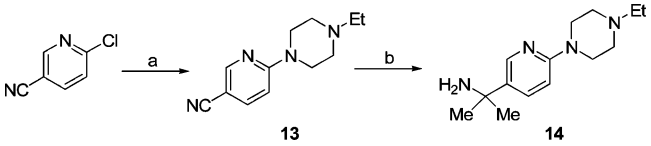
presence of $Pd(OAC)_2$, (*S*)-BINAP, and Cs_2CO_3. Hydrolysis of the esters with LiOH in MeOH/THF followed by cyclization with polyphosporic acid at 165 °C gave fluoro-acridone acid **12** in 98% yield. Acridone acid **12** was coupled with various amines using BOP-Cl and Et_3N in DMF to give fluoro-acridones **4a**−**p**. The tertiary-alkyl amines used in the final couplings were

Scheme 2[a]



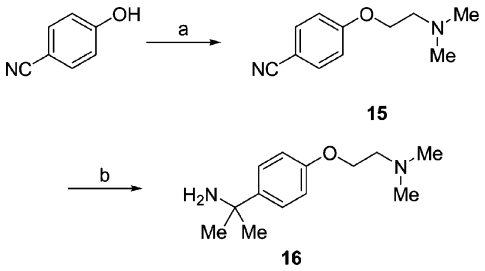
[a] Reagents and conditions: (a) HNO_3, H_2SO_4, 95%; (b) H_2, Pd/C, EtOAc, 90%; (c) PhBr, $Pd(OAc)_2$, (*S*)-BINAP, Cs_2CO_3, toluene, 80%; (d) LiOH-H_2O, H_2O, THF, MeOH, 96%; (e) PPA, 165 °C, 98%; (f) **14**, BOP-Cl, Et_3N, DMF, 75%.

Scheme 3[a]



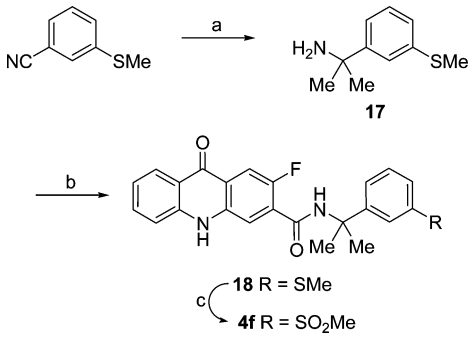
[a] Reagents and conditions: (a) *N*-ethylpiperazine, EtOH, reflux, 95%; (b) $CeCl_3$, MeLi, THF, −78 °C−rt, 90%.

Scheme 4[a]



[a] Reagents and conditions: (a) 2-dimethylaminoethylchloride hydrochloride, K_2CO_3, DMF, 33%; (b) $CeCl_3$, MeLi, THF, −78 °C−rt, 79%.

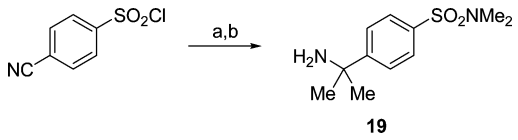
Scheme 5[a]



[a] Reagents and conditions: (a) $CeCl_3$, MeLi, THF, −78 °C−rt; (b) **12**, BOP-Cl, Et_3N, DMF, 59%; (c) mCPBA, DCM, MeOH, 56%.

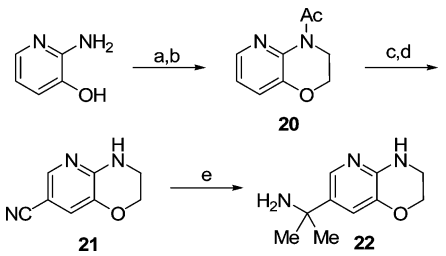
synthesized in good yield from the corresponding nitriles via dimethylation using $MeCeCl_2$ in THF,[14] as shown in Schemes 3−9. Example **4l** was prepared utilizing a similar route as outlined in Scheme 3 starting with 2-chloro-4-cyanopyridine and dimethylamine.

Scheme 6[a]



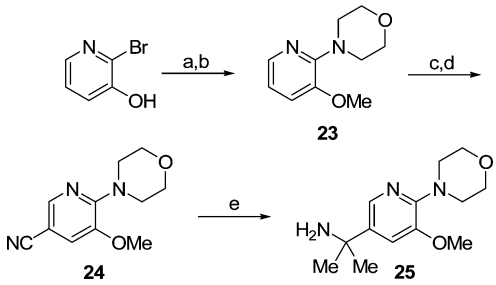
[a] Reagents and conditions: (a) dimethylamine, DCM, 0 °C, 95%; (b) $CeCl_3$, MeLi, THF, −78 °C−rt.

Scheme 7[a]



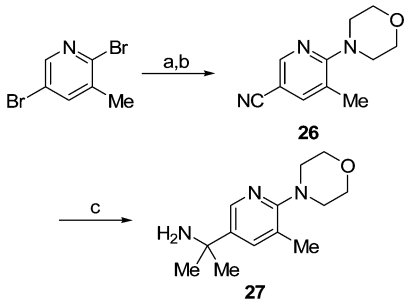
[a] Reagents and conditions: (a) Ac_2O, pyr, 1 M aq NaOH, 89%; (b) 1,2-dibromoethane, K_2CO_3, acetone, water, reflux, 99%; (c) NBS, DMF, 75 °C, 63%; (d) $Pd_2(dba)_3$, DPPF, Zn, $ZnCN_2$, DMA, 150 °C, 80%; (e) $CeCl_3$, MeLi, THF, −78 °C−rt, 95%.

Scheme 8[a]



[a] Reagents and conditions: (a) TMS-CHN_2, MeOH, 99%; (b) morpholine, 125 °C, 88%; (c) NBS, DMF, 56%; (d) $Pd_2(dba)_3$, DPPF, Zn, $ZnCN_2$, DMA, 150 °C, 87%; (e) $CeCl_3$, MeLi, THF, −78 °C−rt, 99%.

Scheme 9[a]



[a] Reagents and conditions: (a) morpholine, 140 °C, 99%; (b) $Pd_2(dba)_3$, DPPF, Zn, $ZnCN_2$, DMA, 150 °C, 77%; (c) $CeCl_3$, MeLi, THF, −78 °C−rt, 95%.

Results and Discussion

Our preliminary library work around ester **3** led to the identification of acridones **8a** and **8b** as potential leads with the phenyl substituted tertiary-alkyl amide **8b** demonstrating a 3-fold improvement in IMPDH II inhibitory activity over the simple *t*-butyl **8a**, as seen in Table 1. Further investigation of **8b** revealed that quaternary substitution adjacent to the amide nitrogen was optimal, with the benzyl amide (**8c**) having significantly reduced inhibitory activity (IMPDH II $IC_{50} = 2.2$ μM) relative to **8b** (IMPDH II $IC_{50} = 0.019$ μM). Additionally, both monomethyl enatiomers were prepared and evaluated (**8d** and **8e**), and although they showed significant improvement over **8c**, they were less potent than **8b** (5−23-fold). The (*R*)-enantiomer **8d** had an IC_{50} of 95 nM against IMPDH II, while the (*S*)-enantiomer's (**8e**) inhibitory potency was 4−5-fold less, with an IC_{50} of 431 nM. With **8b** as a viable lead, we turned our focus toward improving the cell potency (T-cell proliferation in CEM lymphoblastoid cells and in human peripheral blood mononuclear cells (PBMCs); see Experimental Section for details) and the physio-chemical properties of the series.

In exploring the structure−activity relationships (SARs) around this lead, we rationalized that a small group substituted at R^2 in structure **4** (Table 2) might provide enhancements in the in vitro potency, in particular, cell activity and physio-chemical properties, through restricted rotation of the acridone-amide carbonyl bond, forcing the carbonyl to orient 180 degrees away from the group. As anticipated, incorporation of a fluoro adjacent to the amide (**4a**; Table 2) provided a >3-fold improvement in the inhibition of T-cell proliferation in human PBMCs (see Experimental Section for details), with an IC_{50} of 0.22 μM. Interestingly, the effects were more pronounced when the amide was substituted with a 3-pryidyl (**8g** vs **4c**), resulting in a 4−5-fold improvement in the inhibition of T-cell proliferation (CEM lymphoblastoid cells and PBMCs) and the inhibition of IMPDH II. In the des-fluoro-acridone pyridyl analogues (**8f**−**h**), the positioning of the nitrogen had very little impact on either the inhibition of the enzyme or on T-cell proliferation. However, in the fluoro-acridone series, the 3-pryridyl analogue **4b** was 2−3-fold more active than the 2-pyridyl analogue **4c**. With the small but consistent improvement in in vitro potency, the fluoro-acridones became the primary focus of our effort to optimize this series of inhibitors.

With the fluoro-acridone core in hand, the optimal region for further enhancing activity and physio-chemical characteristics was the tertiary-alkyl amide substitution. We reasoned that by introducing various polar residues to the phenyl substituent of **4a** (cLogP 3.3) or to the pyridyl substituent of **4c** (cLogP 1.7) we could modulate the LogP and, consequently, modulate the potency and the physio-chemical properties of the series. As outlined in Table 3, a wide range of phenyl and pyridyl substitution was well tolerated and provided several potent analogues. The minor variations observed in the in vitro activities suggest that this region of the molecule is likely directed toward solvent. Examples **4d**−**h** represent substituted phenyl rings with sulfonamide **4g** exhibiting highly potent in vitro inhibitory activity (IMPDH $IC_{50} = 10$ nM, CEM $IC_{50} =$ 0.66 μM, PBMC $IC_{50} = 0.20$ μM). As mentioned previously, the 3-pyridyl substituted analogue **4c** was also highly potent with an IMPDH IC_{50} of 11 nM and an IC_{50} of 0.14 μM in the PBMC cell assay. To decrease any potential metabolic liability attributed to the 3-pyridyl nitrogen, substitution at the *para*-position was explored. As depicted by examples **4i**−**k** and **4m**−**p**, the substituted pyridyl analogues ranging from a simple methyl to the more elaborate methyl substituted morpholinopyridine (**4p)** provided significant in vitro potency with little differentiation in activity. Interestingly, the 4-pyridyl analogue **4l** exhibited equivalent, if not slightly better, activity overall relative to the 3-pyridyl analogue **4k**; however, the 4-pyridyl derivatives proved to be less desirable in both advanced in vitro profiling assays (e.g., CYP inhibition, hERG, metabolic stability) and pharmacokentic studies.

Although the phenyl series provided many highly potent analogues, substituted pyridyl acridones demonstrated optimal in vivo efficacy with improved pharmacokinetic and in vitro profiling properties. Based on this analysis, analogue **4m** was selected as our lead candidate. Steady-state enzyme kinetic studies determined that **4m** was a reversible and uncompetitive

Table 4. Partial In Vitro Profiling Data for Compound **4m**[a]

parameter	result
IMPDH type I[12] (IC_{50})	62 ± 5.0 nM
protein binding	92% in human serum
mutagenicity	ames negative
hERG (patch clamp) (IC_{50})	25 μM
CYP[a,b] inhibition (IC_{50})	>20 μM 1A2, 2C9, 2D6; 19 μM 2C19; 13 μM 3A4 BFC
LogP	2.5
Caco2 permeability	320 nm/s

[a] CYP = cytochrome P450. [b] CYP inhibition assays were performed against isolated CYP enzymes.

Table 5. Pharmacokinetic Parameters for Compound **4m**[a]

parameter	rat[a]	cyno[a]
po dose (mg/kg)	10[b]	10[b]
iv dose (mg/kg)	2	2
C_{max} (μM), PO	0.41 ± 0.21	26 ± 24
T_{max} ((μM), PO	4.0 ± 0.0	2.9 ± 2.0
AUC ((μM·h), PO	5.2 ± 2.7	102 ± 32
$T_{1/2}$ (h), iv	3.6 ± 1.8	4.0 ± 0.33
MRT (h), iv	4.4 ± 1.7	5.5 ± 0.45
Cl (mL/min/kg), iv	29 ± 3.0	3.4 ± 0.64
V_{ss} (L/kg), iv	7.4 ± 2.2	1.1 ± 0.19
F_{po} (%)	43	88

[a] Average of three animals with associated standard deviation. [b] Vehicle: 60% PEG400/40% HCl at pH 3.

inhibitor of IMPDH II with a K_i of 25 ± 3 nM with respect to IMP and 20 ± 4 nM with respect to NAD.[9j] A partial list of profiling data is presented in Table 4. In pharmocokenetic (PK) studies (Table 5), **4m** had a bioavailability of 43% in rats with a half-life of 3.6 h and a bioavailability of 88% in cynologous monkey with a half-life of 4.0 h. Through these studies, **4m** was found to be metabolically stable, and in the absence of a phenolic residue, **4m** is not expected to exhibit the corresponding enterohepatic recirculation associated with MMF.

Acridone **4m** was evaluated in the fully preventative Lewis rat adjuvant arthritis (AA rat) model, a widely utilized preclinical model of human rheumatoid arthritis,[15] to establish the effect of compound treatment on paw swelling post-inoculation with complete Freund's adjuvant.[16] The rat was chosen because of its sensitivity to the immunosuppressive effects of IMPDH inhibitors and its predisposition to the analogous mechanism-based toxicities such as anemia and GI toxicity observed clinically with MMF.

Arthritis was induced by the subcutaneous injection of Freund's complete adjuvant into the base of the tail of male Lewis rats. Acridone **4m** was administered at doses of 50 and 25 mg/kg PO/QD in the first experiment, along with MMF at 15 mg/kg (Figure 3A), and doses of 10 and 5 mg/kg PO/QD in a second experiment (Figure 3B) daily for 21 days starting at day 0. Baseline measurements of hind paw volume were obtained by plethysmometry. Additional paw volume measurements were performed over the next three weeks, and the increases in paw volume above baseline were calculated. Significant inhibition of paw swelling was seen at doses of 10, 25, and 50 mg/kg PO/QD ($p < 0.05$ vs water vehicle, Student's *t* test, Figures 3). Additionally, the rats showed no signs of GI toxicity at any dose by either gross examination or histopathology. MMF demonstrated significant inhibition at 15 mg/kg (Figure 3A) and only modest (~30%) but not statistically significant inhibition of paw swelling at a dose of 10 mg/kg PO/QD (data not shown), also showing no signs of GI toxicity at these doses. This study clearly demonstrated the efficacy of this novel, orally active acridone inhibitor (**4m**) in the rat adjuvant arthritis model.

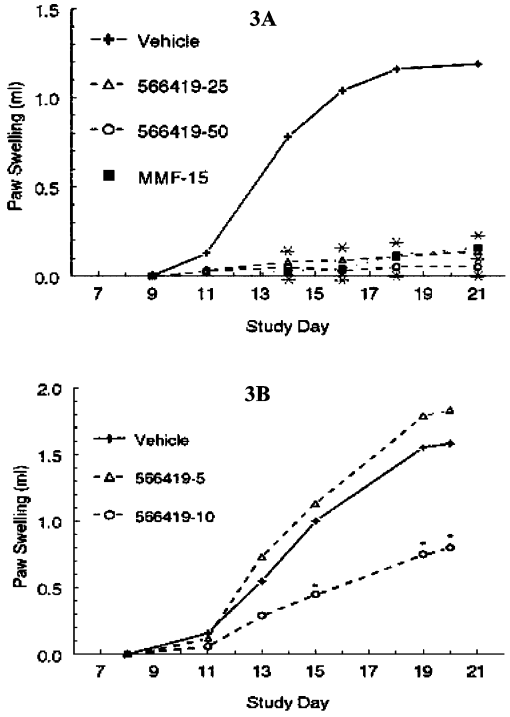


Figure 3. (A) Efficacy of **4m** (25 and 50 mg/kg) and MMF (15 mg/kg) vs vehicle in a rat adjuvant arthritis model. (B) Efficacy of **4m** (5 and 10 mg/kg) vs vehicle in a rat adjuvant arthritis model. [a]Animals per group = 8; **4m** and MMF were administered PO/QD starting on day 0. Vehicle was 60% PEG400/40% 0.01 N HCl; *p value < 0.05 compared to vehicle treatment group.

GI tolerability was assessed for both acridone **4m** and MMF in Lewis rats in the context of the rat AA model. To determine the maximum tolerated dose with respect to GI toxicity, the rats were given escalating doses of either MMF or **4m** for up to 14 days. The rats were then monitored for the development of diarrhea and weight loss. Rats receiving either a daily dose of 50 mg/kg of MMF or 125 mg/kg of **4m** survived the two week study with only mild transient weight loss and occasional soft stools observed for some rats. Higher doses of MMF and **4m** (e.g., 75 mg/kg and 150 mg/kg, respectively) resulted in moderate to severe diarrhea, confirmed by histopathology (crypt cell necrosis), leading to morbidity. As a result of these findings, doses of 50 mg/kg for MMF and 125 mg/kg for **4m** were selected as the maximum tolerated doses for GI toxicity. The therapeutic index (TI) of **4m** and MMF for GI toxicity was established based on exposure (AUC) in these rat studies. Based on the AUCs for **4m** at the lowest efficacious and maximally tolerated doses, the TI for GI toxicity in the rat AA model was determined to be 19-fold. Comparatively, the TI for MMF was determined to be 6.9-fold.

Conclusion

In summary, a novel series of acridone-based inhibitors of IMPDH II has been identified. Structure−activity relationship studies led to multiple highly potent analogues exhibiting significant IMPDH inhibition as well as the inhibition of the proliferation of CEM lymphoblastoid cells and human PBMCs. Based on its in vitro potency, in vivo activity, and pharmacokinetic and safety profiles, **4m** was selected as our lead drug candidate. Compound **4m** was efficacious at 10 mg/kg in the rat adjuvant arthritis model, a preclinical model of human rheumatoid arthritis. This compound has a different in vitro and in vivo pharmacokinetic and metabolic profile than MMF and, as a result, **4m** was evaluated in rat to establish in vivo the

relationship between efficacy and toxicity and was found to have an approximately 3-fold improvement over MMF in its therapeutic index with respect to GI toxicity. Overall, preclinical data suggest that acridone **4m** may have an improved safety window in humans over MMF with respect to GI adverse events.

Experimental Section

Proton magnetic resonance (^{1}H) spectra were recorded on either a Bruker Avance 400 or a JEOL Eclipse 500 spectrometer and are reported in ppm relative to the reference solvent of the sample in which they were run. HPLC and LCMS analyses were conducted using a Shimadzu SCL-10A liquid chromatograph and a SPD UV−vis detector at 220 or 254 nm with the MS detection performed with either a Micromass Platform LC spectrometer or a Waters Micromass ZQ spectrometer. Preparative reverse-phase HPLC purifications were performed using the following conditions: Ballistic YMC S5 ODS 20 × 100 mm column with a binary solvent system, where solvent A = 10% methanol, 90% water, 0.1% trifluoroacetic acid and solvent B = 90% methanol, 10% water, and 0.1% trifluoroacetic acid; flow rate = 20 mL/min; linear gradient time = 10 min; start %B = 20; final %B = 100. Fractions containing the product were concentrated in vacuo to remove the methanol and were neutralized with aqueous sodium bicarbonate. The products were collected by vacuum filtration or were extracted with organic solvents and concentrated under reduced pressure. All flash column chromatography was performed on EM Science silica gel 60 (particle size of 40−60 μm). All reagents were purchased from commercial sources and used without further purification unless otherwise noted. All reactions were performed under an inert atmosphere.

HPLC analyses were performed using the following conditions:

Method A. A linear gradient using 5% acetonitrile, 95% water, and 0.05% TFA (solvent A) and 95% acetonitrile, 5% water, and 0.05% TFA (solvent B); $t = 0$ min, 10% B, $t = 15$ min, 100% B (20 min) was employed on a SunFire C18 3.5u 4.6 × 150 mm column. Flow rate was 1 mL/min and UV detection was set to 254 nm. The LC column was maintained at ambient temperature.

Method B. A linear gradient using 5% acetonitrile, 95% water, and 0.05% TFA (solvent A) and 95% acetonitrile, 5% water, and 0.05% TFA (solvent B); $t = 0$ min, 10% B, $t = 15$ min, 100% B (20 min) was employed on a XBridge Ph 3.5μ 4.6 × 150 mm column. Flow rate was 1 mL/min and UV detection was set to 254 nm. The LC column was maintained at ambient temperature.

Method C. A linear gradient using 5% acetonitrile, 95% water, and 0.05% TFA (solvent A) and 95% acetonitrile, 5% water, and 0.05% TFA (solvent B); $t = 0$ min, 10% B, $t = 10$ min, 50% B (20 min) was employed on a SunFire C18 3.5μ 4.6 × 150 mm column. Flow rate was 1 mL/min and UV detection was set to 254 nm. The LC column was maintained at ambient temperature.

Method D. A linear gradient using 5% acetonitrile, 95% water, and 0.05% TFA (solvent A) and 95% acetonitrile, 5% water, and 0.05% TFA (solvent B); $t = 0$ min, 10% B, $t = 10$ min, 50% B (20 min) was employed on a XBridge Ph 3.5μ 4.6 × 150 mm column. Flow rate was 1 mL/min and UV detection was set to 254 nm. The LC column was maintained at ambient temperature.

Method E. A linear gradient using 10% methanol, 90% water, and 0.1% TFA (solvent A) and 90% methanol, 10% water, and 0.1% TFA (solvent B); $t = 0$ min, 0% B, $t = 4$ min, 100% B (5 min) was employed on a YMC ODS 4.6 × 50 mm column. Flow rate was 4 mL/min and UV detection was set to 220 or 254 nm. The LC column was maintained at ambient temperature.

Method F. A linear gradient using 10% methanol, 90% water, and 0.2% H_3PO_4 (solvent A) and 90% acetonitrile, 10% water, and 0.2% H_3PO_4 (solvent B); $t = 0$ min, 0% B, $t = 4$ min, 100% B (5 min) was employed on a YMC ODS 4.6 × 50 mm column. Flow rate was 4 mL/min and UV detection was set to 220 or 254 nm. The LC column was maintained at ambient temperature.

Method G. A linear gradient using 10% methanol, 90% water, and 0.2% H_3PO_4 (solvent A) and 90% acetonitrile, 10% water, and 0.2% H_3PO_4 (solvent B); $t = 0$ min, 0% B, $t = 8$ min, 100% B (11 min) was employed on a Zorbax Rapid Res 3.5μ 4.6 × 50 mm column. Flow rate was 2.5 mL/min and UV detection was set to 254 nm. The LC column was maintained at ambient temperature.

Method H. A linear gradient using 10% methanol, 90% water, and 0.1% TFA (solvent A) and 90% acetonitrile, 10% water, 0.1% TFA (solvent B); $t = 0$ min, 0% B, $t = 4$ min, 100% B (5 min) was employed on a Chromolith SpeedRod 4.6 × 50 mm column. Flow rate was 4 mL/min and UV detection was set to 254 nm. The LC column was maintained at ambient temperature.

9-Oxo-9,10-dihydro-acridine-3-carboxylic Acid (7). To a mixture of dimethyl bromoterephthalate (4.5 g, 16.0 mmol), aniline (2.2 mL, 24.0 mmol), (*S*)-(−)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (922 mg, 1.48 mmol), and toluene (50 mL) was added cesium carbonate (7.53 g, 23.1 mmol), followed by palladium(II) acetate (0.22 g, 1.0 mmol). The mixture was heated to 100 °C for 24 h. HPLC indicated that the reaction was complete. The reaction mixture was cooled to room temperature, diluted with diethyl ether, and filtered under reduced pressure through a pad of Celite. The filtrate was concentrated under reduced pressure and purified by silica gel chromatography using a 1:10 mixture of ethyl acetate and hexane to give 4.58 (97%) of 5-phenylamino-terephthalic acid dimethyl ester as a bright yellow solid. HPLC t_r = 3.74 min (condition E); LCMS (EI) *m*/*z* calcd for $C_{16}H_{15}NO_4$ $[M + H]^+$, 286.11; found, 286^+. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.88 (s, 3H), 3.93 (s, 3H), 7.13 (m, 1H), 7.25 (m, 1H), 7.32−7.40 (m, 4H), 7.91 (d, $J = 1.50$, 1H), 8.01 (d, $J = 8.30$, 1H), 9.50 (bs, 1H).

A mixture of 5-phenylamino-terephthalic acid dimethyl ester (4.56 g, 16.0 mmol) and lithium hydroxide monohydrate (2.0 g, 48.0 mmol) in methanol (32 mL), tetrahydrofuran (32 mL), and water (16 mL) was heated to reflux for 0.5 h. HPLC indicated that the reaction was complete. The organic solvents were removed under reduced pressure, and the aqueous residue was diluted with water. The pH was adjusted to 3.0 with 6 N aqueous hydrochloric acid. The resulting precipitate was collected by vacuum filtration, rinsed with water, and dried under reduced pressure to provide 3.95 g (96%) of 5-phenylamino-terephthalic acid (**6**) as a bright yellow solid. HPLC t_r = 2.75 min (condition E); LCMS (EI) *m*/*z* calcd for $C_{14}H_{11}NO_4$ $[M + H]^+$, 258.08; found, 258^+.

To a round-bottom flask containing polyphosphoric acid (35 g) at 165 °C was added finely ground 5-phenylamino-terephthalic acid (**6**; 3.0 g, 1.17 mmol) over 10 min. After the addition was complete, the reaction mixture was stirred for 30 min at 165 °C. HPLC indicated that the reaction was complete. While at 165 °C, the mixture was *slowly* added to a mixture of ice and sodium hydroxide. The pH was adjusted to 3.0 with additional sodium hydroxide, and the resulting mixture was filtered through a medium porosity fritted funnel to give a yellow paste that was rinsed with methanol and dichloromethane into a round-bottom flask. The organic solvents were removed under reduced pressure, and the residue was azeotroped several times with methanol and dichloromethane to give 2.75 g (98%) of 9-oxo-9,10-dihydro-acridine-3-carboxylic acid (**7**) as a yellow solid. HPLC t_r = 2.45 min (condition E); LCMS (EI) *m*/*z* calcd for $C_{14}H_9NO_3$ $[M + H]^+$, 240.07; found, 240^+. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.30 (t, $J = 7.55$ Hz, 1H), 7.55 (d, $J = 8.20$ Hz, 1H), 7.72 (d, $J = 8.42$ Hz, 1H), 7.77 (t, $J = 7.55$ Hz, 1H), 8.18 (s, 1H), 8.24 (d, $J = 8.20$ Hz, 1H), 8.31 (d, $J = 8.42$ Hz, 1H), 11.96 (s, 1H), 13.13 (br s, 1H).

2-Fluoro-9-oxo-9,10-dihydro-acridine-3-carboxylic Acid (12). To a mixture of dimethyl 2-fluoroterephthalate (42.0 g, 0.198 mol) in concentrated sulfuric acid (200 mL) at 0 °C was added 36 mL of a 1:1 mixture of nitric acid and sulfuric acid dropwise. The mixture was stirred at 0 °C for 15 min, the ice-bath was removed, and the reaction mixture was stirred for an additional 45 min. HPLC indicated that the starting material had been consumed. The reaction mixture was poured over ice and extracted with ethyl acetate. The organic layer was collected, washed with brine, and dried over anhydrous sodium sulfate. Concentration under reduced pressure followed by recrystallization from methanol afforded 48.5 g (95%) of 2-fluoro-5-nitro-terephthalic acid dimethyl ester as a white solid.

HPLC purity >98%, t_r = 2.69 min (condition B). ^{1}H NMR (500 mHz, $CDCl_3$) δ 3.95 (s, 3H), 3.98 (s, 3H), 7.44 (d, 1H), 8.59 (d, 1H).

A mixture of 2-fluoro-5-nitro-terephthalic acid dimethyl ester (48.5 g, 0.178 mol) and palladium/carbon (4.6 g) in ethyl acetate (450 mL) was evacuated under reduced pressure and charged with hydrogen (3×). The reaction mixture was subsequently stirred under a steady stream of nitrogen for 1 h. The reaction mixture was filtered through a pad of Celite, concentrated, and recrystallized from a mixture of methanol and dichloromethane to provide 36.2 g (90%) of 2-amino-5-fluoro-terephthalic acid dimethyl ester (**10**) as a bright yellow solid. HPLC purity >98%, t_r = 2.68 min (condition B); LCMS (EI) *m*/*z* calcd for $C_{10}H_{10}FNO_4$ $[M + H]^+$, 228.07; found, 228$^+$. ^{1}H NMR (500 mHz, $CDCl_3$) δ 3.89 (s, 3H), 3.92 (s, 3H), 7.20 (d, 1H), 7.61 (d, 1H).

To a mixture of 2-amino-5-fluoro-terephthalic acid dimethyl ester (**10**; 10.0 g, 44.0 mmol), bromobenzene (4.6 mL, 44.0 mmol), (*S*)-(−)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (2.06 g, 3.30 mmol), and toluene (250 mL) was added cesium carbonate (20.0 g, 44.0 mmol) followed by palladium(II) acetate (0.494 g, 2.20 mmol). The mixture was heated at 90 °C for 24 h. HPLC indicated that the reaction was complete. The reaction mixture was cooled to room temperature and filtered under reduced pressure through a pad of Celite topped with a pad of silica gel, and the Celite/silica gel pads were rinsed with ethyl acetate. The filtrate was concentrated under reduced pressure and purified by silica gel chromatography using a 1:10 mixture of EtOAc and hexane to give 10.7 g (80%) of 2-fluoro-5-phenylamino-terephthalic acid dimethyl ester as a pale yellow solid. HPLC purity > 99%, t_r = 3.72 min (condition F). ^{1}H NMR (400 mHz, $CDCl_3$) δ 3.89 (s, 3H), 3.93 (s, 3H), 7.12 (m, 1H), 7.22 (m, 2H), 7.37 (m, 2H), 7.74 (m, 2H), 9.22 (br s, 1H).

A mixture of 2-fluoro-5-phenylamino-terephthalic acid dimethyl ester (30.0 g, 9.89 mmol) and lithium hydroxide monohydrate (12.5 g, 29.7 mmol) in MeOH (200 mL), tetrahydrofuran (200 mL), and water (100 mL) was heated at 75 °C for 0.5 h. HPLC indicated that the reaction was complete. The organic solvents were removed under reduced pressure, and the aqueous residue was diluted with water. The pH was adjusted to 3.0 with 6 N aqueous hydrochloric acid, which resulted in a precipitate. The solid was collected by vacuum filtration and dried under reduced pressure to provide a quantitative yeild of compound 2-fluoro-5-phenylamino-terephthalic acid (**11**) as an orange-yellow solid. HPLC purity 99%, t_r = 3.02 min (condition F). ^{1}H NMR (400 mHz, DMSO) δ 7.11 (m, 1H), 7.25 (m, 2H), 7.39 (m, 2H), 7.60 (m, 1H), 7.68 (m, 1H), 9.32 (br s, 1H), 13.55 (br s, 2H).

To a round-bottom flask containing polyphosphoric acid (254 g) at 165−172 °C was added finely ground 2-fluoro-5-phenylamino-terephthalic acid (**11**; 15.0 g, 55.6 mmol) over 30 min. After the addition was complete, the reaction mixture was stirred for 10 min at 165 °C. HPLC indicated that the reaction was complete. While at 165 °C, the reaction mixture was slowly added to a mixture of ice and sodium bicarbonate. The pH was adjusted to 3.0 with additional solid sodium bicarbonate, and the resulting mixture was filtered through a medium porosity fritted funnel to give a greenish-yellow paste that was washed with water (3×) and air-dried under vacuum. The resulting paste was rinsed with methanol into a flask. The paste/methanol mixture was then sonicated to disperse the paste. Dichloromethane was added, and the mixture was concentrated under reduced pressure. The azeotrope procedure was repeated 2× to give 12.0 g (84%) of 2-fluoro-9-oxo-9,10-dihydro-acridine-3-carboxylic acid (**12**) as a yellow solid. HPLC retention time = 2.35 min (condition A); LCMS (EI) *m*/*z* calcd for $C_{14}H_8FNO_3$ $[M + H]^+$, 258.06; found, 258.03. ^{1}H NMR (500 MHz, DMSO) δ 7.30 (t, J = 7.6 Hz, 1H), 7.56 (d, J = 7.6 Hz, 1H), 7.78 (t, J = 7.6 Hz, 1H), 7.93 (d, J = 11.0 Hz, 1H), 8.11 (d, J = 6.1 Hz, 1H), 8.22 (d, J = 7.6 Hz, 1H), 12.06 (s, 1H), 13.69 (br s, 1H).

9-Oxo-*N*-(2-phenylpropan-2-yl)-9,10-dihydroacridine-3-carboxamide (8b). To a mixture of 9-oxo-9,10-dihydroacridine-3-carboxylic acid (**7**, 0.050 g, 0.209 mmol), cumylamine (0.036 mL, 0.251 mmol), and triethylamine (0.087 mL, 0.627 mmol) in *N*,*N*-dimethylformamide (5.0 mL) at room temperature was added *N*,*N*-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride (0.064 g, 0.251 mmol). The reaction mixture was stirred at room temperature overnight, diluted with dichloromethane, washed with a 1.0 N aqueous solution of sodium hydroxide (3×), and dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded a yellow solid which was triturated with methanol with sonication and filtered to give 9-oxo-*N*-(2-phenylpropan-2-yl)-9,-10-dihydroacridine-3-carboxamide (**8b**, 0.054 g, 73%) as a pale yellow solid (Alternatively, all compounds could be purified by flash silica gel chromatography or preparative HPLC). HPLC purity 99.6%, t_r = 10.94 min (method A); 99.6%, t_r = 12.43 min (method B); LCMS (EI) *m*/*z* calcd for $C_{23}H_{20}N_2O_2$ $[M + H]^+$, 357.16; found, 357.14. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.69 (s, 6H), 7.18 (t, J = 7.42 Hz, 1H), 7.27−7.32 (m, 3H), 7.40 (s, 1H), 7.42 (s, 1H), 7.55 (d, J = 8.80 Hz, 1H), 7.64 (d, J = 7.15 Hz, 1H), 7.75 (t, J = 7.70 Hz, 1H), 7.91 (s, 1H), 8.23 (d, J = 7.15 Hz, 1H), 8.27 (d, J = 8.25 Hz, 1H), 8.73 (s, 1H), 11.90 (s, 1H).

***N*-*tert*-Butyl-9-oxo-9,10-dihydroacridine-3-carboxamide (8a).** Acridone **8a** was prepared as a pale yellow solid following the procedure described for **8b**. HPLC purity 99.4%, t_r = 10.94 min (method A); 99.5%, t_r = 12.94 min (method B); LCMS (EI) *m*/*z* calcd for $C_{18}H_{18}N_2O_2$ $[M + H]^+$, 295.14; Found, 295.17. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.41 (s, 9H), 7.28 (m, 1H), 7.55−7.60 (m, 2H), 7.70 (m, 1H), 7.89 (s, 1H), 8.07 (s, 1H), 8.23−8.25 (m, 2H), 11.89 (s, 1H).

***N*-Benzyl-9-oxo-9,10-dihydroacridine-3-carboxamide (8c).** Acridone **8c** was prepared as a pale yellow solid following the procedure described for **8b**. HPLC purity 99.2%, t_r = 11.33 min (method A); 99.1%, t_r = 13.58 min (method B); LCMS (EI) *m*/*z* calcd for $C_{21}H_{16}N_2O_2$ $[M + H]^+$, 329.13; found, 329.19. ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.52 (d, J = 6.05 Hz, 2H), 7.23−7.30 (m, 2H), 7.32−7.37 (m, 4H), 7.55 (d, J = 8.25 Hz, 1H), 7.69 (d, J = 8.25 Hz, 1H), 7.75 (t, J = 7.70 Hz, 1H), 8.03 (s, 1H), 8.23 (d, J = 7.15 Hz, 1H), 8.28 (d, J = 8.25 Hz, 1H), 9.31 (t, J = 5.77 Hz, 1H), 11.91 (s, 1H).

(*R*)-9-Oxo-*N*-(1-phenylethyl)-9,10-dihydroacridine-3-carboxamide (8d). Acridone **8d** was prepared as a pale yellow solid following the procedure described for **8b**. HPLC purity 98.8%, t_r = 11.83 min (method A); 99.0%, t_r = 14.10 min (method B); LCMS (EI) *m*/*z* calcd for $C_{22}H_{18}N_2O_2$ $[M + H]^+$, 343.14; found, 343.18. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.50 (d, J = 7.15 Hz, 3H), 5.20 (qd, J = 7.33, 7.15 Hz, 1H), 7.25 (ddd, J = 18.01, 7.56, 7.42 Hz, 2H), 7.34 (t, J = 7.70 Hz, 2H), 7.42 (d, J = 7.70 Hz, 2H), 7.55 (d, J = 8.25 Hz, 1H), 7.69 (d, J = 7.15 Hz, 1H), 7.74 (t, J = 7.70 Hz, 1H), 7.98 (s, 1H), 8.23 (d, J = 6.60 Hz, 1H), 8.28 (d, J = 8.25 Hz, 1H), 9.10 (d, J = 7.70 Hz, 1H), 11.92 (s, 1H).

(*S*)-9-Oxo-*N*-(1-phenylethyl)-9,10-dihydroacridine-3-carboxamide (8e). Acridone **8e** was prepared as a pale yellow solid following the procedure described for **8b**. HPLC purity 98.2%, t_r = 11.86 min (method A); 98.4%, t_r = 14.16 min (method B); LCMS (EI) *m*/*z* calcd for $C_{22}H_{18}N_2O_2$ $[M + H]^+$, 343.14; found, 343.15. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.50 (d, J = 7.15 Hz, 3H), 5.20 (qd, J = 7.33, 7.15 Hz, 1H), 7.25 (dt, J = 18.70, 7.42 Hz, 2H), 7.34 (t, J = 7.70 Hz, 2H), 7.42 (d, J = 7.70 Hz, 2H), 7.55 (d, J = 8.25 Hz, 1H), 7.69 (d, J = 9.90 Hz, 1H), 7.74 (t, J = 6.87 Hz, 1H), 7.98 (s, 1H), 8.23 (d, J = 7.15 Hz, 1H), 8.28 (d, J = 8.25 Hz, 1H), 9.10 (d, J = 7.70 Hz, 1H), 11.91 (s, 1H).

9-Oxo-*N*-(2-(pyridin-2-yl)propan-2-yl)-9,10-dihydroacridine-3-carboxamide (8f). Acridone **8f** was prepared as a pale yellow solid following the procedure described for **8b** using acridone acid (**7**) and 2-(pyridin-2-yl)propan-2-amine. The amine was prepared from picolinonitrile as described in the preparation of **4i**. HPLC purity 98.2%, t_r = 6.97 min (method A); 99.1%, t_r = 10.56 min (method B); LCMS (EI) *m*/*z* calcd for $C_{22}H_{19}N_3O_2$ $[M + H]^+$, 358.16; found, 358.17. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.71 (s, 6H), 7.24 (d, J = 6.60 Hz, 1H), 7.28 (t, J = 7.15 Hz, 1H), 7.47 (d, J = 8.25 Hz, 1H), 7.55 (d, J = 8.80 Hz, 1H), 7.66 (d, J = 9.90 Hz, 1H), 7.73−7.77 (m, 2H), 7.94 (s, 1H), 8.23 (d, J = 7.15 Hz, 1H), 8.28 (d, J = 8.25 Hz, 1H), 8.52 (d, J = 3.85 Hz, 1H), 8.91 (s, 1H), 11.91 (s, 1H).

9-Oxo-*N*-(2-(pyridin-3-yl)propan-2-yl)-9,10-dihydroacridine-3-carboxamide (8g). Acridone **8g** was prepared as a pale yellow solid following the procedure described for **8b** using acridone acid (**7**) and 2-(pyridin-3-yl)propan-2-amine. The amine was prepared from nicotinonitrile as described in the preparation of **4i**. HPLC purity 97.3%, t_r = 7.02 min (method A); 98.0%, t_r = 10.59 min (method B); LCMS (EI) *m*/*z* calcd for $C_{22}H_{19}N_3O_2$ $[M + H]^+$, 358.16; found, 358.17. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.72 (s, 6H), 7.20−7.26 (m, 1H), 7.33 (dd, J = 7.97, 4.67 Hz, 1H) 7.54 (d, J = 8.25 Hz, 1 H), 7.58−7.61 (m, 1H), 7.68−7.72 (m, 1H), 7.77 (d, J = 7.70 Hz, 1H), 7.94 (s, 1 H), 8.22 (d, J = 7.70 Hz, 1H), 8.26 (d, J = 8.3 Hz, 1H), 8.40 (d, J = 4.40 Hz, 1H), 8.64 (s, 1H), 8,81 (s, 1H), 11.95 (s, 1H).

9-Oxo-*N*-(2-(pyridin-4-yl)propan-2-yl)-9,10-dihydroacridine-3-carboxamide (8h). Acridone **8h** was prepared as a pale yellow solid following the procedure described for **8b** using acridone acid (**7**) and 2-(pyridin-4-yl)propan-2-amine. The amine was prepared from isonicotinonitrile as described in the preparation of **4i**. HPLC purity 97.6%, t_r = 6.90 min (method A); 95.9%, t_r = 10.49 min (method B); LCMS (EI) *m*/*z* calcd for $C_{22}H_{19}N_3O_2$ $[M + H]^+$, 358.16; found, 358.17. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.67 (s, 6H), 7.27 (t, J = 7.42 Hz, 1H), 7.37 (d, J = 6.05 Hz, 2H), 7.55 (d, J = 8.25 Hz, 1H), 7.66 (d, J = 8.25 Hz, 1H), 7.74 (t, J = 7.70 Hz, 1H), 7.92 (s, 1H), 8.23 (d, J = 8.25 Hz, 1H), 8.28 (d, J = 8.80 Hz, 1H), 8.49 (d, J = 6.05 Hz, 2H), 8.87 (s, 1H), 11.92 (s, 1H).

2-Fluoro-9-oxo-*N*-(2-phenylpropan-2-yl)-9,10-dihydroacridine-3-carboxamide (4a). Acridone **4a** was prepared as a bright yellow solid following the procedure described for **8b** starting with fluoro-acridone acid **12**. HPLC purity 99.1%, t_r = 13.39 min (method A); 98.7%, t_r = 15.38 min (method B); LCMS (EI) *m*/*z* calcd for $C_{23}H_{19}FN_2O_2$ $[M + H]^+$, 375.15; found, 375.15. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.67 (s, 6H), 7.21 (t, J = 7.42 Hz, 1H), 7.29 (t, J = 7.42 Hz, 1H), 7.34 (t, J = 7.70 Hz, 2H), 7.45 (d, J = 7.15 Hz, 2H), 7.54 (d, J = 8.25 Hz, 1H), 7.68 (d, J = 5.50 Hz, 1H), 7.76 (t, J = 7.70 Hz, 1H), 7.92 (d, J = 9.90 Hz, 1H), 8.22 (d, J = 6.60 Hz, 1H), 8.85 (s, 1H), 11.94 (s, 1H).

2-Fluoro-9-oxo-*N*-(2-(pyridin-2-yl)propan-2-yl)-9,10-dihydroacridine-3-carboxamide (4b). Acridone **4b** was prepared as a bright yellow solid following the procedure described for **8b** using fluro-acridone acid (**12**) and 2-(pyridin-2-yl)propan-2-amine. The amine was prepared from picolinonitrile as described in the preparation of **4i**. HPLC purity 98.2%, t_r = 4.83 min (method G); 99%, t_r = 1.85 min (method H); LCMS (EI) *m*/*z* calcd for $C_{22}H_{18}FN_3O_2$ $[M + H]^+$, 376.15; found, 376.15. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.70 (s, 6H), 7.24−7.31 (m, 2H), 7.54 (d, J = 8.25 Hz, 2H), 7.73−7.83 (m, 3H), 7.94 (d, J = 10.45 Hz, 1H), 8.22 (d, J = 7.70 Hz, 1H), 8.54 (d, J = 3.85 Hz, 1H), 9.08 (d, J = 2.20 Hz, 1H), 11.99 (s, 1H).

2-Fluoro-9-oxo-*N*-(2-(pyridin-3-yl)propan-2-yl)-9,10-dihydroacridine-3-carboxamide (4c). Acridone **4c** was prepared as a bright yellow solid following the procedure described for **8b** using fluro-acridone acid (**12**) and 2-(pyridin-3-yl)propan-2-amine. The amine was prepared from nicotinonitrile as described in the preparation of **4i**. HPLC purity 96.4%, t_r = 3.86 min (method G); 99%, t_r = 1.78 min (method H); LCMS (EI) *m*/*z* calcd for $C_{22}H_{18}FN_3O_2$ $[M + H]^+$, 376.15; found, 376.14. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.70 (s, 6H), 7.29 (t, J = 7.42 Hz, 1H), 7.33−7.39 (m, 1H), 7.54 (d, J = 8.25 Hz, 1H), 7.69 (d, J = 5.50 Hz, 1H), 7.76 (t, J = 6.87 Hz, 1H), 7.81 (d, J = 8.25 Hz, 1H), 7.93 (d, J = 9.90 Hz, 1H), 8.22 (d, J = 8.25 Hz, 1H), 8.44 (d, J = 6.05 Hz, 1H), 8.68 (d, J = 2.20 Hz, 1H), 8.97 (s, 1H), 11.94 (s, 1H).

***N*-(2-(4-(2-(Dimethylamino)ethoxy)phenyl)propan-2-yl)-2-fluoro-9-oxo-9,10-dihydroacridine-3-carboxamide (4d).** A mixture of 4-cyanophenol (1.19 g, 10 mmol), 2-dimethylaminoethyl-chloride hydrochloride (2.16 g, 15 mmol), and potassium carbonate (5.53 g, 40 mmol) in DMF (20 mL) was heated at 80 °C for 2 h and then filtered. The filtrate was concentrated under reduced pressure, and the resulting residue was purified by flash silica gel chromatography to give 0.628 g of 4-(2-(dimethylamino)ethoxy)-benzonitrile (**15**). 2-(4-(2-(Dimethylamino)-ethoxy)phenyl)propan-2-amine (**16**) was then prepared as a colorless oil by a route analogous to that used for the preparation of compound **4i**. HPLC t_r = 0.203 min (condition E); LCMS (EI) *m*/*z* calcd for $C_{13}H_{22}N_2O$ $[M + H]^+$, 223.18; found, 223$^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 6H), 2.31 (s, 6H), 2.70 (t, J = 5.72 Hz, 2H), 4.02 (t, J = 5.72 Hz, 2H), 6.85 (d, J = 8.79 Hz, 2H), 7.39 (d, J = 8.79 Hz, 2H).

Fluoro-acridone **4d** was prepared as a yellow solid from **12** and **16** by a route analogous to that used for the preparation of **8b**. HPLC purity 96.9%, t_r = 8.96 min (method A); 96.7%, t_r = 9.76 min (method B); LCMS (EI) *m*/*z* calcd for $C_{27}H_{28}FN_3O_3$ $[M + H]^+$, 462.22; found, 462.16. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.65 (s, 6H), 2.37 (s, 6H), 2.83 (s, 2H), 4.10 (t, J = 5.50 Hz, 2H), 6.91 (d, J = 8.80 Hz, 2H), 7.29 (t, J = 7.42 Hz, 1H), 7.35 (d, J = 8.80 Hz, 2H), 7.55 (d, J = 8.25 Hz, 1H), 7.68 (d, J = 5.50 Hz, 1H), 7.76 (t, J = 7.70 Hz, 1H), 7.91 (d, J = 9.90 Hz, 1H), 8.21 (d, J = 8.25 Hz, 1H), 8.76 (s, 1H), 11.97 (s, 1H).

2-Fluoro-*N*-(2-(4-(methylsulfonyl)phenyl)propan-2-yl)-9-oxo-9,10-dihydroacridine-3-carboxamide (4e). Acridone **4e** was prepared as a bright yellow solid following the procedure described for **4f**, starting with 4-bromophenyl-methylsulfide. HPLC purity 98.0%, t_r = 11.34 min (method A); 98.0%, t_r = 13.77 min (method B); LCMS (EI) *m*/*z* calcd for $C_{24}H_{21}FN_2O_4S$ $[M + H]^+$, 453.13; found, 453.11. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.69 (s, 6H), 3.22 (s, 3H), 7.28 (t, J = 7.42 Hz, 1H), 7.55 (d, J = 8.25 Hz,1 H), 7.70 (d, J = 8.25 Hz, 3H), 7.75 (t, J = 7.42 Hz, 1H), 7.90 (d, J = 8.80 Hz, 2H), 7.93 (d, J = 10.45 Hz, 1H), 8.21 (s, 1H) 9.03 (s, 1H), 11.97 (s, 1H).

2-Fluoro-*N*-(2-(3-(methylsulfonyl)phenyl)propan-2-yl)-9-oxo-9,10-dihydroacridine-3-carboxamide (4f). To a solution of 3-bromophenyl-methylsulfide (1.624 g, 8.0 mmol) in dimethylformamide (30 mL) was added cuprous cyanide (0.788 g, 8.8 mmol). The reaction mixture was heated at 150 °C overnight. The solvent was removed under reduced pressure, and the residue was diluted wth dichloromethane, washed with water, and dried over anhydrous sodium sulfate. Concentration under reduced pressure followed by purification by flash silica gel chromatography afforded 3-(methylthio)-benzonitrile as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.53 (s, 3H), 7.44 (m, 4H).

1-Methyl-1-(3-methylthiophenyl)ethyl-amine (**17**) was prepared from 3-bromophenyl methylsulfide as described in the preparation of **4i**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 6H), 1.60 (br s, 2H), 2.50 (s, 3H), 7.12 (m, 1H), 7.27 (m, 2H), 7.44 (s, 1H).

2-Fluoro-*N*-(2-(3-(methylthio)phenyl)propan-2-yl)-9-oxo-9,10-dihydroacridine-3-carboxamide (**18**) was prepared as a bright yellow solid following the procedure described for **8b** using fluro-acridone (**12**) and 1-methyl-1-(3-methylthiophenyl)ethyl-amine (**17**). HPLC purity 99%, t_r = 3.28 min (method E); LCMS (EI) *m*/*z* calcd for $C_{24}H_{21}FN_2O_2S$ $[M + H]^+$, 421.14; found, 421$^+$.

2-Fluoro-*N*-(2-(3-(methylthio)phenyl)propan-2-yl)-9-oxo-9,10-dihydroacridine-3-carboxamide (**18**) was dissolved in a mixture of dichloromethane and methanol. To the solution was added excess mCPBA, and the reaction mixture was stirred overnight. The solvents were removed under reduced pressure, and the resulting residue was dissolved in dichloromethane, washed sequentially with aqueous $Na_2S_2O_8$ and aqueous sodium bicarbonate, and dried over sodium sulfate. Concentration under reduced pressure followed by purification by flash silica gel chromatography afforded (2-fluoro-*N*-(2-(3-(methylsulfonyl)phenyl)propan-2-yl)-9-oxo-9,10-dihydroacridine-3-carboxamide (**4f**; 56%) as a bright yellow solid. HPLC purity 98.0%, t_r = 11.37 min (method A); 98.2%, t_r = 13.92 min (method B); LCMS (EI) *m*/*z* calcd for $C_{24}H_{21}FN_2O_4S$ $[M + H]^+$, 453.13; found, 453.15. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.71 (s, 6H), 3.22 (s, 3H), 7.29 (t, J = 7.15 Hz, 1H), 7.55 (d, J = 8.25 Hz, 1H), 7.62−7.68 (m, 2H), 7.75−7.82 (m, 3H), 7.92−7.96 (m, 2H), 8.22 (d, J = 7.15 Hz, 1H), 9.06 (s, 1H), 11.97 (s, 1H).

***N*-(2-(4-(*N*,*N*-Dimethylsulfamoyl)phenyl)propan-2-yl)-2-fluoro-9-oxo-9,10-dihydroacridine-3-carboxamide (4g).** To a suspension of 4-cyanobenzenesulfonyl chloride (0.402 g, 1.99 mmol) in dichloromethane (3 mL) was added dimethylamine (3 mL, 2 M in tetrahydrofuran). The reaction mixture was stirred for 30 min, diluted with water, and extracted with dichloromethane. The organic

layer was collected, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give 0.409 g (98%) of 4-cyano-*N*,*N*-dimethylbenzenesulfonamide as a white solid. 4-(2-Aminopropan-2-yl)-*N*,*N*-dimethylbenzene-sulfonamide (**19**) was prepared from 4-cyano-*N*,*N*-dimethylbenzenesulfonamides, as described in the preparation of **4i**.

Acridone **4g** was prepared as a bright yellow solid following the procedure described for **8b** using fluro-acridone (**12**) and 4-(2-aminopropan-2-yl)-*N*,*N*-dimethylbenzenesulfonamide (**19**). HPLC purity 97.3%, t_r = 12.52 min (method A); 97.7%, t_r = 14.97 min (method B); LCMS (EI) *m*/*z* calcd for $C_{25}H_{24}FN_3O_4S$ $[M + H]^+$, 482.15; found, 482.18. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.70 (s, 6H), 2.63 (s, 6H), 7.29 (t, J = 7.42 Hz, 1H), 7.54 (d, J = 8.25 Hz, 1H), 7.68−7.70 (m, 3H), 7.71−7.78 (m, 3H), 7.93 (d, J = 10.45 Hz, 1H), 8.22 (d, J = 8.25 Hz, 1H), 9.03 (s, 1H), 11.94 (s, 1H).

***N*-(2-(3-(*N*,*N*-Dimethylsulfamoyl)phenyl)propan-2-yl)-2-fluoro-9-oxo-9,10-dihydroacridine-3-carboxamide (4h).** Acirdone **4h** was prepared as a bright yellow solid following the procedure described for **8b** using fluro-acridone (**12**) and 3-(2-aminopropan-2-yl)-*N*,*N*-dimethylbenzenesulfonamide. The amine was prepared from 3-cyano-*N*,*N*-dimethylbenzene-sulfonamide as described in **4g**. HPLC purity 93.5%, t_r = 12.47 min (method A); 94.2%, t_r = 14.73 min (method B); LCMS (EI) *m*/*z* calcd for $C_{25}H_{24}FN_3O_4S$ $[M + H]^+$, 482.15; found, 482.19. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.70 (s, 6H), 2.57 (s, 6H), 7.29 (t, J = 7.97 Hz, 1H), 7.55 (d, J = 8.25 Hz, 1H), 7.59−7.66 (m, 3H), 7.77 (q, J = 7.15 Hz, 3H), 7.93 (d, J = 10.45 Hz, 1H), 8.22 (d, J = 7.15 Hz, 1H), 9.07 (s, 1H), 11.98 (s, 1H).

***N*-(2-(3,4-Dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazin-7-yl)propan-2-yl)-2-fluoro-9-oxo-9,10-dihydroacridine-3-carboxamide (4i).** A mixture of 2-amino-3-hydroxypyridine (3.0 g, 27.2 mmol) and 2 mL of pyridine in 141 mL of acetic anhydride was heated at reflux for 5 min, cooled to room temperature, and concentrated under reduce pressure. The oily residue was then stirred in a 1 M aqueous solution of sodium hydroxide (75 mL) until the mixture became homogeneous. The solution was poured into a mixture of crushed ice and 6 M aqueous hydrochloric acid, and the pH was adjusted to ∼4.0. The aqueous mixture was extracted with dichloromethane, washed with water, dried over anhydrous sodium sulfate, and concentrated to give *N*-(3-hydroxypyridin-2-yl)acetamide (3.70 g, 89%) as an off-white solid. HPLC purity 99%, t_r = 0.193 min (method F); LCMS (EI) *m*/*z* calcd for $C_7H_8N_2O_2$ $[M + H]^+$, 153.07; found, 153.07.

To a mixture of *N*-(3-hydroxypyridin-2-yl)acetamide (3.70 g, 24.3 mmol), 1,2-dibromoethane (8.4 mL, 97.2 mmol), water (30 mL), and acetone (120 mL) was added potassium carbonate (13.4 g, 97.2 mmol. The reaction mixture was heated at reflux overnight and concentrated. The residue was dissolved in dichloromethane, washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude solid was dissolved in ether, and the undissolved material was removed by vacuum filtration. The filtrate was concentrated to give 4.28 g (99%) of 1-(2*H*-pyrido-[3,2-*b*][1,4]oxazin-4(3*H*)-yl)ethanone (**20**) as an off-white solid. HPLC t_r = 0.720 min (method F); LCMS (EI) *m*/*z* calcd for $C_9H_{10}N_2O_2$ $[M + H]^+$, 179.08; found, 179.10.

A mixture of **20** (0.438 g, 2.46 mmol) and *N*-bromosuccinimide (0.438 g, 2.46 mmol) in 6 mL of dimethylformamide was heated at 75 °C for 1 h. The solvent was removed under reduced pressure, and the residue was dissolved in dichloromethane, washed with water, and dried over anhydrous sodium sulfate. Concentration under reduced pressure followed by purification by flash silica gel chromatography afforded 0.315 g of 1-(7-bromo-2*H*-pyrido[3,2-b]-[1,4]oxazin-4(3*H*)-yl)ethanone as a white solid. HPLC purity ∼95%, t_r = 2.99 min (method F); LCMS (EI) *m*/*z* calcd for $C_9H_9BrN_2O_2$ $[M + H]^+$, 256.99; found, 256.90 and 258.90. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.58 (s, 3H), 4.04−4.07 (m, 2H), 4.25−4.27 (m, 2H), 7.37 (d, J = 2.2 Hz, 1H), 7.51 (d, J = 2.2 Hz, 1H).

A mixture of 1-(7-bromo-2*H*-pyrido[3,2-*b*][1,4]oxazin-4(3*H*)-yl)-ethanone (0.290 g, 1.13 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.062 g, 0.068 mmol), 1,1′-bis(diphenylphosphino)-ferrocene (0.075 g, 0.136 mmol), zinc powder (9.0 mg, 0.136 mmol), and zinc cyanide (0.080 g, 0.678 mmol) was flushed with nitrogen. Dimethyl acetamide (4 mL) was added, and the resulting mixture was heated at 150 °C overnight. HPLC indicated that the reaction was complete. The reaction mixture was cooled to room temperature, diluted with ethyl acetate, and washed with 2 N aqueous ammonium hydroxide, washed with brine, and dried over anhydrous sodium sulfate. Concentration under reduced pressure followed by purification by flash silica gel chromatography afforded 3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-7-carbonitrile (**21**; 0.183 g, 80%) as an off-white solid. HPLC purity 98%, t_r = 0.997 min (method F); LCMS (EI) *m*/*z* calcd for $C_8H_7N_3O$ $[M + H]^+$, 162.07; found, 162.03.

To a flame-dried flask under nitrogen was added cerium chloride (0.918 g, 3.73 mmol) followed by anhydrous tetrahydrofuran (11 mL). The mixture was stirred vigorously for 45 min, during which time the cerium chloride became suspended. The suspension was cooled to −78 °C, and methyl lithium (2.66 mL, 3.73 mmol, 1.4 M in ether) was added dropwise. After the addition was complete, the reaction mixture was stirred for 0.5 h at −78 °C. Compound **21** (0.120 g, 0.745 mmol) in tetrahydrofuran (4 mL) was added via cannula to the −78 °C solution. The dry ice bath was removed, and the reaction mixture was stirred at room temperature overnight. The mixture was quenched with a few drops of a saturated aqueous solution of ammonium chloride, and a 50% aqueous solution of ammonium hydroxide was added dropwise until a precipitate formed and settled to the bottom of the flask. The mixture was filtered through Celite under reduced pressure, diluted with dichloromethane, washed with water, and dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded 2-(3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazin-7-yl)propan-2-amine (**22**; 95%). HPLC t_r = 0.197 min (method F); LCMS (EI) *m*/*z* calcd for $C_{10}H_{15}N_3O$ $[M + H]^+$, 194.13; found, 194.13. The amine was used without any further purification.

To a mixture of **12** (0.020 g, 0.078 mmol), **22** (0.017 g, 0.086 mmol), and triethylamine (0.033 mL, 0.233 mmol) was added *N*,*N*-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride (0.024 g, 0.093 mmol) in 4 mL of anhydrous dimethylformamide at room temperature. The reaction mixture was stirred at room temperature overnight. The mixture was diluted with dichloromethane, washed with water, washed with 1 N aqueous sodium hydroxide (3×), and dried over anhydrous sodium sulfate. Concentration under reduced pressure followed by purification by preparative HPLC afforded compound **4i** (17.0 mg) as a bright yellow solid. HPLC purity 98.5%, t_r = 7.80 min (method A); 98.4% t_r = 11.80 min (method B); LCMS (EI) *m*/*z* calcd for $C_{24}H_{21}FN_4O_3$ $[M + H]^+$, 433.17; found, 433.27. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.62 (s, 6H), 3.38 (br s, 2H), 4.11−4.12 (m, 2H), 6.54 (s, 1H), 6.98 (d, J = 2.2 Hz, 1H), 7.29 (t, J = 7.7 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.65 (t, J = 2.2 Hz, 2H), 7.76 (t, J = 7.7 Hz, 1H), 7.91 (d, J = 9.9 Hz, 1H), 8.21 (d, J = 8.3 Hz, 1H), 8.72 (s, 1H), 11.94 (s, 1H).

2-Fluoro-*N*-(2-(6-methylpyridin-3-yl)propan-2-yl)-9-oxo-9,10-dihydroacridine-3-carboxamide (4j). Acridone **4j** was prepared as a bright yellow solid following the procedure described for **8b** using fluro-acridone (**12**) and 2-(6-methylpyridin-3-yl)propan-2-amine. The amine was prepared from 6-methylnicotinonitrile as described in the preparation of **4i**. HPLC purity 96%, t_r = 3.97 min (method G); 97%, t_r = 1.84 min (method H); LCMS (EI) *m*/*z* calcd for $C_{23}H_{20}FN_3O_2$ $[M + H]^+$, 390.16; found, 390.20. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.67 (s, 6H), 2.44 (s, 3H), 7.21 (d, J = 7.70 Hz, 1H), 7.28 (t, J = 7.15 Hz, 1H), 7.54 (d, J = 8.25 Hz, 1H), 7.64−7.71 (m, 2H), 7.75 (t, J = 6.87 Hz, 1H), 7.92 (d, J = 9.90 Hz, 1H), 8.21 (d, J = 6.60 Hz, 1H), 8.53 (d, J = 2.75 Hz, 1H), 8.92 (s, 1H), 11.95 (s, 1H).

***N*-(2-(6-(Dimethylamino)pyridin-3-yl)propan-2-yl)-2-fluoro-9-oxo-9,10-dihydroacridine-3-carboxamide (4k).** 6-(Dimethylamino)nicotinonitrile was prepared from 6-chloronicotinonitrile and dimethylamine as described for **4n**. The nitrile was then dimethylated as described for **4n** to give 5-(2-aminopropan-2-yl)-*N*,*N*-dimethylpyridin-2-amine. Acridone **4k** was prepared as a bright yellow solid from **12** and 5-(2-aminopropan-2-yl)-*N*,*N*-dimethylpyridin-2-amine as described in the preparation of **8b**. HPLC purity

91.8%, $t_r = 8.16$ min (method A); 93.2%, $t_r = 15.38$ min (method B); LCMS (EI) *m/z* calcd for $C_{24}H_{23}FN_4O_2$ $[M + H]^+$, 419.19; found, 419.18. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.64 (s, 6H), 3.00 (s, 6H), 6.61 (d, $J = 8.80$ Hz, 1H), 7.26 (t, $J = 7.42$ Hz, 1H), 7.52−7.57 (m, 2H), 7.66 (d, $J = 5.50$ Hz, 1H), 7.74 (t, $J = 7.15$ Hz, 1H), 7.90 (d, $J = 9.90$ Hz, 1H), 8.17 (d, $J = 2.20$ Hz, 1H), 8.21 (d, $J = 8.25$ Hz, 1 H), 8.75 (s, 1H), 11.94 (s, 1H).

***N*-(2-(2-(Dimethylamino)pyridin-4-yl)propan-2-yl)-2-fluoro-9-oxo-9,10-dihydroacridine-3-carboxamide (4l).** 2-(Dimethylamino)isonicotinonitrile was prepared from 2-chloro-4-cyanopyridine and dimethylamine as described for **4n**. The nitrile was then dimethylated as described for **4n** to give 4-(2-aminopropan-2-yl)-*N*,*N*-dimethylpyridin-2-amine. Acridone **4k** was prepared as a bright yellow solid from **12** and 4-(2-aminopropan-2-yl)-*N*,*N*-dimethylpyridin-2-amine as described in the preparation of **8b**. HPLC purity 95%, $t_r = 4.06$ min (method G); 98%, $t_r = 2.01$ min (method H); LCMS (EI) *m/z* calcd for $C_{24}H_{23}FN_4O_2$ $[M + H]^+$, 419.19; found, 419.23. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.62 (s, 6H), 3.02 (s, 6H), 6.61 (s, 1H), 6.63 (d, $J = 5.50$ Hz, 1H), 7.29 (t, $J = 7.15$ Hz, 1H), 7.55 (d, $J = 8.25$ Hz, 1H), 7.67 (d, $J = 5.50$ Hz, 1H), 7.76 (t, $J = 6.87$ Hz, 1H), 7.93 (d, $J = 10.45$ Hz, 1H), 8.02 (d, $J = 5.50$ Hz, 1H), 8.22 (d, $J = 8.25$ Hz, 1H), 8.90 (s, 1H), 11.99 (s, 1H).

***N*-(2-(6-(4-Ethylpiperazin-1-yl)pyridin-3-yl)propan-2-yl)-2-fluoro-9-oxo-9,10-dihydroacridine-3-carboxamide (4m).** To 2-chloropyridine-5-carbonitrile (100 g, 0.72 mol) in 400 mL of ethanol was added *N*-ethylpiperazine (250 mL). The mixture was stirred at 80 °C for 3.5 h under nitrogen. The solvent was removed under reduced pressure, and the resulting residue was cooled to room temperature and diluted with 1 L of water. The aqueous mixture was stirred overnight, and the resulting precipitate was collected by vacuum filtration, washed with water (3×), and dried under reduced pressure to give 6-(4-ethylpiperazin-1-yl)nicotinonitrile (**13**; 141 g, 91%) as an off-white solid. HPLC purity >99%, $t_r = 0.580$ min (method F); LCMS (EI) *m/z* calcd for $C_{12}H_{16}N_4$ $[M + H]^+$, 217.15; found, 217.12. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13 (t, $J = 7.2$ Hz, 3H), 2.47 (q, $J = 14.5$, 7.2 Hz, 2H), 2.52−2.54 (m, 4H), 3.69−3.71 (m, 4H), 6.59 (d, $J = 9.0$ Hz, 1H), 7.60 (dd, $J = 9.0$, 2.3 Hz, 1H), 8.40 (d, $J = 2.3$ Hz, 1H).

To a flame dried flask under nitrogen was added cerium chloride (Alfa Aesar) (68.4 g, 0.28 mol) and 450 mL of anhydrous tetrahydrofuran. The mixture was stirred for 45 min at room temperature and cooled to −78 °C. Methyl lithium (173 mL of a 1.6 M solution in ether, 0.28 mol) was added dropwise with stirring. The reaction mixture was stirred for 1 h at −78 °C. 6-(4-Ethylpiperazin-1-yl)nicotinonitrile (**13**; 20.0 g, 0.093 mol) in 50 mL of anhydrous tetrahydrofuran was added slowly via cannula at −78 °C, and the mixture was stirred overnight at room temperature. The reaction was quenched with a small volume of water (∼ 5 mL) at 0 °C, and then a 50% aqueous solution of ammonium hydroxide was added until a precipitate formed and settled to the bottom of the flask. The mixture was filtered through a pad of Celite, concentrated, and purified by silica gel chromatography using CH_2Cl_2/MeOH/TEA (100:2:1) to give 2-(6-(4-ethylpiperazin-1-yl)-pyridin-3-yl)propan-2-amine (**14**; 16.5 g, 72%) as a pale yellow solid. HPLC purity 86%, $t_r = 0.183$ min (method F); LCMS (EI) *m/z* calcd for $C_{14}H_{24}N_4$ $[M + H]^+$, 249.21; found, 249.20. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13 (t, $J = 7.2$ Hz, 3H), 1.47 (s, 6H), 2.47 (q, $J = 14.4$, 7.2 Hz, 2H), 2.55−2.59 (m, 4H), 3.54−3.56 (m, 4H), 3.75 (m, 2H), 6.64 (d, $J = 8.8$ Hz, 1H), 7.65 (dd, $J = 8.8$, 2.6 Hz, 1H), 8.33 (d, $J = 2.6$ Hz, 1H).

To a mixture of the F-acridone acid (**7**; 7.76 g, 30.2 mmol), 2-(6-(4-ethylpiperazin-1-yl)pyridin-3-yl)propan-2-amine (**14**; 9.0 g, 36.2 mmol), and triethylamine (9.2 g, 90.6 mmol) in 160 mL of anhydrous dimethylformamide was added *N*,*N*-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride (9.2 g, 36.2 mmol). The reaction mixture was stirred at room temperature overnight. To the mixture was added 400 mL of a 5% aqueous solution of sodium bicarbonate, and the mixture was stirred for 1 h. The resulting precipitate was collected by vacuum filtration and washed with water (2×). The wet solid was collected and crystallized from methanol to provide pure *N*-[1-[6-(4-ethyl-1-piperazinyl)-3-pyridinyl]-1-methylethyl]-2-fluoro-9,10-dihydro-9-oxo-3-acridinecarboxamide (**4m**; 8.2 g, 56%) as a bright yellow solid. HPLC purity 99.5%, $t_r = 6.65$ min (method A); 99.7%, $t_r = 10.53$ min (method B); LCMS (EI) *m/z* calcd for $C_{28}H_{30}FN_5O_2$ $[M + H]^+$, 488.25; found, 488.22. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.03 (t, 3H, $J = 7.2$ Hz), 1.66 (s, 6H), 2.36 (q, $J = 14.3$,7.2 Hz, 2H), 2.45 (m, 4H), 3.45 (m, 4H), 6.80 (d, $J = 8.8$, 1H), 7.29 (t, $J = 7.5$ Hz, 1H), 7.53 (d, $J = 8.2$ Hz, 1H), 7.57 (d, $J = 8.8$ Hz, 1H), 7.66 (d, $J = 5.5$ Hz, 1H), 7.76 (t, $J = 7.5$ Hz, 1H), 7.91 (d, 9.9 Hz, 1H), 8.20 (s, 1H), 8.21 (d, $J = 8.2$ Hz, 1H), 8.78 (s, 1H), 11.93 (s, 1H). Anal. Calcd for $C_{28}H_{30}FN_5O_2$, 1.1% H_2O: C, 68.22; H, 6.26; N, 14.21; F, 3.85. Found: C, 67.83; H, 6.21; N, 14.06; F, 3.76.

2-Fluoro-*N*-(2-(6-morpholinopyridin-3-yl)propan-2-yl)-9-oxo-9,10-dihydroacridine-3-carboxamide (4n). A mixture of 6-chloronicotinonitrile (0.139 g, 1.00 mmol) and morpholine (0.35 mL, 4.00 mmol) in 2 mL of ethanol was heated to reflux for 1 h. Upon cooling to room temperature, the solvent was removed under reduced pressure and the residue was diluted with water. The resulting white solid was collected by filtration, rinsed thoroughly with water, and dried under high vacuum overnight to afford 6-morpholinonicotinonitrile as a white crystalline solid (36% yield). HPLC $t_r = 1.59$ min (condition E); LCMS (EI) *m/z* calcd for $C_{10}H_{11}N_3O$ $[M + H]^+$, 190.10; found, 190$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.65 (t, $J = 4.92$, 4H), 3.80 (t, $J = 4.92$, 4H), 6.59 (d, $J = 8.74$, 1H), 7.64 (dd, $J = 2.41$, $J = 8.74$, 1H), 8.42 (d, $J = 2.41$, 1H).

2-(6-Morpholinopyridin-3-yl)propan-2-amine was prepared from 6-morpholinonicotinonitrile as described in the preparation of **4m** to give the product as a pale yellow solid. HPLC retention time = 0.203 min (condition E); LCMS (EI) *m/z* calcd for $C_{12}H_{19}N_3O$ $[M + H]^+$, 222.16; found, 222$^+$.

2-(6-Morpholinopyridin-3-yl)propan-2-amine was coupled with **12** following the procedure described in **4m** to give **4n** as a pale yellow solid. HPLC purity 97.0%, $t_r = 7.59$ min (method A); 97.6%, $t_r = 11.65$ min (method B); LCMS (EI) *m/z* calcd for $C_{26}H_{25}FN_4O_3$ $[M + H]^+$, 461.20; found, 461.26. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.65 (s, 6H), 3.40 (s, 4H), 3.69 (s, 4H), 6.81 (d, $J = 8.80$ Hz, 1H), 7.28 (t, $J = 7.42$ Hz, 1H), 7.53 (d, $J = 8.25$ Hz, 1H), 7.61 (dd, $J = 8.80$, 2.75 Hz, 1H), 7.66 (d, $J = 5.50$ Hz, 1H), 7.76 (t, $J = 6.87$ Hz, 1H), 7.91 (d, $J = 9.90$ Hz, 1H), 8.20−8.23 (m, 2H), 8.80 (s, 1H), 11.94 (s, 1H).

2-Fluoro-*N*-(2-(5-methoxy-6-morpholinopyridin-3-yl)propan-2-yl)-9-oxo-9,10-dihydroacridine-3-carboxamide (4o). To 2-bromo-3-hydroxypyridine (8.20 g, 47.1 mmol) in 200 mL of dichloromethane and 100 mL of methanol was added trimethylsilyl diazomethane dropwise (2.0 M solution in hexane, 47.1 mL, 94.2 mmol). After 30 min at room temperature, an extra equivalent of trimethylsilyl diazomethane was added, and the mixture was stirred for 2.5 h. The solvent was removed under reduced pressure to give a quantitative yield of 2-bromo-3-methoxypyridine as a light-brown solid. HPLC purity 85%, $t_r = 1.81$ min (method F); LCMS (EI) *m/z* calcd for C_6H_6BrNO $[M + H]^+$, 187.97; found,187.93, 189.93.

A mixture of 2-bromo-3-methoxypyridine (8.50 g, 45.2 mmol) and morpholine (60 mL) in a sealed tube was heated at 125 °C for 4.5 h. The reaction mixture was diluted with dichloromethane, washed with water, and dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded 5.10 g (58%) of 4-(3-methoxypyridin-2-yl)morpholine (**23**) as a brownish oil. HPLC $t_r = 0.603$ min (method F); LCMS (EI) *m/z* calcd for $C_{10}H_{14}N_2O_2$ $[M + H]^+$, 195.11; found, 195.14. The compound was used without any further purification.

A mixture of **23** (5.05 g, 26.0 mmol) and *N*-bromosuccinimide (4.80 g, 27.0 mmol) in 60 mL of dimethylformamide was heated at 85 °C for 3 h. The solvent was removed under reduced pressure, and the residue was dissolved in dichloromethane, washed with water, and dried over anhydrous sodium sulfate. Concentration under reduced pressure followed by purification by flash silica gel chromatography afforded 4.43 g (62%) of 4-(5-bromo-3-methoxypyridin-2-yl)morpholine as a white solid. HPLC purity 99%, $t_r = 2.72$ min (method F); LCMS (EI) *m/z* calcd for $C_{10}H_{13}BrN_2O_2$ $[M + H]^+$, 273.02; found, 274.15. ^{1}H NMR (500 MHz, $CDCl_3$) δ

3.36−3.38 (m, 4H), 3.83−3.85 (m, 7H), 7.13−7.14 (m, 1 H), 7.90−7.91 (m, 1H).

A mixture of 4-(5-bromo-3-methoxypyridin-2-yl)morpholine (4.40 g, 16.0 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.885 g, 0.966 mmol), 1,1′-bis(diphenylphosphino)ferrocene (1.07 g, 1.93 mmol), zinc powder (0.124 g, 1.93 mmol), and zinc cyanide (1.89 g, 16.0 mmol) was flushed with nitrogen. Dimethyl acetamide (60 mL) was added, and the resulting mixture was heated at 150 °C overnight. HPLC indicated that the reaction was complete. The reaction mixture was cooled to room temperature, diluted with ethyl acetate, washed with 2 N aqueous ammonium hydroxide, washed with brine, and dried over anhydrous sodium sulfate. Concentration under reduced pressure followed by purification by flash silica gel chromatography afforded 5-methoxy-6-morpholinonicotinonitrile (**24**; 3.37 g, 96%) as an off-white solid. HPLC purity >99%, t_r = 2.26 min (method F); LCMS (EI) *m*/*z* calcd for $C_{11}H_{13}N_3O_2$ [M + H]$^+$, 220.11; found, 220.05. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.65−3.67 (m, 4H), 3.80−3.82 (m, 4H), 3.86 (s, 3H), 7.10 (s, 1H), 8.12 (s, 1H).

To a flame-dried flask under nitrogen was added cerium chloride (18.8 g, 76.4 mmol) followed by 150 mL of anhydrous tetrahydrofuran. The mixture was stirred vigorously for 45 min, during which time the cerium chloride became suspended. The suspension was cooled to −78 °C, and methyl lithium (54.6 mL, 76.4 mmol, 1.4 M in ether) was added dropwise. After the addition was complete, the reaction mixture was stirred for 0.5 h at −78 °C. Compound **24** (3.35 g, 15.3 mmol) in tetrahydrofuran was added via cannula to the −78 °C solution. The dry ice bath was removed, and the reaction mixture was stirred at room temperature overnight. The mixture was quenched with a few drops of a saturated aqueous solution of ammonium chloride, and a 50% aqueous solution of ammonium hydroxide was added dropwise until a precipitate formed and settled to the bottom of the flask. The mixture was filtered through Celite under reduced pressure, diluted with dichloromethane, washed with water, and dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded 3.41 g (89%) 2-(5-methoxy-6-morpholinopyridin-3-yl)propan-2-amine (**25**) as a yellowish-orange oil. HPLC purity 88%, t_r = 0.603 min (method F); LCMS (EI) *m*/*z* calcd for $C_{13}H_{21}N_3O_2$ [M + H]$^+$, 252.17; found, 252.20. The compound was used without any further purification.

To a mixture of **12** (2.78 g, 10.8 mmol), **25** (3.40 g, 13.5 mmol), and triethylamine (4.52 mL, 32.4 mmol) was added *N*,*N*-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride (3.44 g, 13.5 mmol) in 90 mL of anhydrous dimethylformamide at room temperature. The reaction mixture was stirred at room temperature overnight. The mixture was diluted with dichloromethane, washed with water, washed with 1 N aqueous sodium hydroxide (2×), washed with brine (2×), and dried over anhydrous sodium sulfate. Concentration under reduced pressure followed by recrystallization from methanol afforded 4.42 g (83%) of 2-fluoro-*N*-(2-(5-methoxy-6-morpholinopyridin-3-yl)propan-2-yl)-9-oxo-9,10-dihydroacridine-3-carboxamide (**4o**) as a bright yellow solid. HPLC purity 99.4%, t_r = 9.61 min (method C); 99.3%, t_r = 14.10 min (method D); LCMS (EI) *m*/*z* calcd for $C_{27}H_{27}FN_4O_4$ [M + H]$^+$, 491.21; found, 491.30. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.86 (s, 6H), 3.26−3.28 (m, 4H), 3.71−3.73 (m, 4H), 3.83 (s, 3H), 7.27 (d, J = 2.0 Hz, 1H), 7.31 (t, J = 7.4 Hz, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.68 (d, J = 10.0 Hz, 1H), 7.77−7.79 (m, 1H), 7.90 (d, J = 2.0 Hz, 1H), 7.94 (d, J = 10 Hz, 1H), 8.23 (d, 7.4 Hz, 1H), 8.88 (s, 1H), 11.95 (s, 1H).

2-Fluoro-*N*-(2-(5-methyl-6-morpholinopyridin-3-yl)propan-2-yl)-9-oxo-9,10-dihydroacridine-3-carboxamide (4p). To a heterogeneous suspension of 2-amino-5-bromo-3-methylpyridine (5.0 g, 26.7 mmol) in 17 mL of hydrobromic acid at 0 °C was added bromine (4.25 mL). To this mixture was added sodium nitrite (5.25 g) in 7.5 mL of water slowly via pipet. The mixture was stirred for 15 min, and 11.3 g of sodium hydroxide in 29 mL of water was slowly added. The resulting oil was extracted with ether, and the organic layer was collected, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give 2,5-dibromo-3-methylpyridine as a yellowish-orange solid. HPLC purity 99%, t_r = 3.19 min (method F); LCMS (EI) *m*/*z* calcd for $C_6H_5Br_2N$ [M + H]$^+$, 249.89; found, 249.89.

A mixture of the 2,5-dibromo-3-methylpyridine (3.3 g, 13.2 mmol) and morpholine (30 mL) in a sealed tube was heated at 140 °C for 6 h. The reaction mixture was cooled to room temperature, diluted with water, and extracted with dichloromethane. The organic layer was collected, washed with water, and dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded a quantitative yield (3.67 g) of 4-(5-bromo-3-methylpyridin-2-yl)-morpholine. HPLC t_r = 3.03 min (method F); LCMS (EI) *m*/*z* calcd for $C_{10}H_{13}BrN_2O$ [M + H]$^+$, 257.03; found, 257.05. The compound was used without any further purification.

A mixture of 4-(5-bromo-3-methylpyridin-2-yl)morpholine (3.65 g, 14.2 mmol), tris-(dibenzylideneacetone)dipalladium(0) (0.78 g, 0.852 mmol), 1,1′-bis(diphenylphosphino)-ferrocene (0.945 g, 1.70 mmol), zinc powder (0.111 g, 1.70 mmol), and zinc cyanide (1.67 g, 14.2 mmol) was flushed with nitrogen. Dimethyl acetamide (50 mL) was added, and the resulting mixture was heated at 150 °C overnight. HPLC indicated that the reaction was complete. The reaction mixture was cooled to room temperature, diluted with ethyl acetate, and washed with 2 N aqueous ammonium hydroxide, washed with brine, and dried over anhydrous sodium sulfate. Concentration under reduced pressure followed by purification by flash silica gel chromatography afforded 5-methyl-6-morpholinonicotinonitrile (**26**; 2.7 g, 94%) as an off-white solid. HPLC purity >99%, t_r = 2.33 min (method F); LCMS (EI) *m*/*z* calcd for $C_{11}H_{13}N_3O$ [M + H]$^+$, 204.11; found, 204.09. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.29 (s, 3H), 3.33−3.35 (m, 4H), 3.82−3.84 (m, 4H), 7.57 (s, 1H), 8.40 (s, 1H).

To a flame-dried flask under nitrogen was added cerium chloride (16.4 g, 66.4 mmol) followed by 150 mL of anhydrous tetrahydrofuran. The mixture was stirred vigorously for 45 min, during which time the cerium chloride became suspended. The suspension was cooled to −78 °C, and methyl lithium (47.4 mL, 66.4 mmol, 1.4 M in ether) was added dropwise. After the addition was complete, the reaction mixture was stirred for 0.5 h at −78 °C. Compound **26** (2.70 g, 13.3 mmol) in tetrahydrofuran was added via cannula to the −78 °C solution. The dry ice bath was removed, and the reaction mixture was stirred at room temperature overnight. The mixture was quenched with a few drops of a saturated aqueous solution of ammonium chloride, and a 50% aqueous solution of ammonium hydroxide was added dropwise until a precipitate formed and settled to the bottom of the flask. The mixture was filtered through Celite under reduced pressure, diluted with dichloromethane, washed with water, and dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded 3.10 g (99%) of 2-(5-methyl-6-morpholinopyridin-3-yl)propan-2-amine (**27**) as a yellowish-orange oil. HPLC purity 90%, t_r = 0.39 min (method F); LCMS (EI) *m*/*z* calcd for $C_{13}H_{21}N_3O$ [M + H]$^+$, 236.18; found, 236.21. The compound was used without any further purification.

To a mixture of **12** (2.67 g, 10.4 mmol), **27** (3.05 g, 13.0 mmol), and triethylamine (5.44 mL, 39.0 mmol) was added *N*,*N*-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride (3.30 g, 13.0 mmol) in 80 mL of anhydrous dimethylformamide at room temperature. The reaction mixture was stirred at room temperature overnight. The mixture was diluted with dichloromethane, washed with water, washed with 1 N aqueous sodium hydroxide (2×), washed with brine, and dried over anhydrous sodium sulfate. Concentration under reduced pressure followed by recrystallization from methanol afforded 3.31 g (67%) of 2-fluoro-*N*-(2-(5-methyl-6-morpholinopyridin-3-yl)propan-2-yl)-9-oxo-9,10-dihydroacridine-3-carboxamide (**4p**) as a bright yellow solid. HPLC purity 98.9%, t_r = 9.39 min (method C); 99.4%; t_r = 13.95 min (method D); LCMS (EI) *m*/*z* calcd for $C_{27}H_{27}FN_4O_3$ [M + H]$^+$, 475.21; found, 475.54. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.66 (s, 6H), 2.26 (s, 3H), 3.02−3.04 (m, 4H), 3.72−3.73 (m, 4H), 7.28 (t, J = 7.5 Hz, 1H), 7.53−7.55 (m, 2H), 7.68 (d, J = 5.5 Hz, 1H), 7.76 (t, J = 7.5 Hz, 1H), 7.92 (d, J = 10.4 Hz, 1H), 7.93 (s, 1H), 8.20−8.22 (m, 2H), 8.86 (s, 1H), and 11.94 (s, 1H).

IMPDH Type I and II Enzymatic Activity. The enzymatic activity of human IMPDH type I or II was measured using a

procedure similar to that previously reported.[17] The conversion of NAD^+ to NADH was followed spectrophotometrically at 340 nm. A reaction mixture containing 0.1 M Tris, 0.1 M KCl, 3 mM EDTA, pH 8.0, 400 μM IMP, 2 mM DTT, and 40 nM of either IMPDH I or II was added to the wells of flat bottom UV-transparent 96-well plates (Costar 3635). To test inhibitors, **4m** dissolved in DMSO was diluted in the reaction to give a final DMSO concentration of 2.5%. IMPDH I and II used in these assays was purified from *E. coli* expressing the gene for the human Type I or Type II enzyme, respectively. The reaction was initiated by addition of NAD^+ to a final concentration of 400 μM. After a 2-hour incubation at 25 °C, readings were taken at 340 nM. The concentrations of compound required to inhibit NADH accumulation by 50% (IC_{50}) were calculated using a four-parameter logistic plot.

Human T-Lymphoblast (CEM) Proliferation Inhibition Assay. The human T-lymphoblast CEM cell line (ATCC) was cultured in RPMI 1640 (Gibco) containing 10% heat inactivated FBS and 100 units/mL of penicillin and streptomycin. Cells were seeded in 96-well Costar flat bottom tissue culture plates at a concentration of 3000 cells/well in the presence of 0.5% DMSO. Test compounds were added in triplicate at a final concentration of 10 μM with 3-fold serial dilutions. Cell cultures were maintained in a 5% CO_2 humidified atmosphere for 72 h. Cell viability was measured after a final 5 h incubation with 10% (v/v) Alamar Blue dye. The fluorometric conversion of Alamar Blue was read on a Cytoflour II multi-well plate reader with excitation/emission settings of 530/590 nm, respectively. The IC_{50} values were calculated using a four-parameter logistic plot.

Human Peripheral Blood Mononuclear Cells Proliferation Inhibition Assay. Human PBMCs were isolated from heparinized whole blood by density gradient centrifugation according to ICN/Cappel LSM specifications. PBMCs were maintained in RPMI 1640 medium containing 10% heat inactivated FBS and 100 units/mL of penicillin and streptomycin. Directly following whole blood isolation, 5×10^4 cells/well were plated into 96-well tissue culture plates and stimulated with anti-CD3 mAb OKT3 at 200 ng/mL and 1 μg/mL soluble anti-CD28 antibody. Test compounds were added in triplicate at a final starting concentration of 3.3 μM with 3-fold serial dilutions. All culture wells included 0.5% DMSO. Cell cultures were maintained in a 5% CO_2 humidified atmosphere for 72 h followed by the addition of 1 μCi ^{3}H-thymidine/well. Radiolabeled thymidine [^{3}H] (Amersham) incorporation was determined after a 5 h incubation. The concentrations of compound required to inhibit PBMC proliferation by 50% (IC_{50}) were calculated using a four-parameter logistic plot.

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