Design and Synthesis of New Templates Derived from Pyrrolopyrimidine as Selective Multidrug-Resistance-Associated Protein Inhibitors in Multidrug Resistance

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In our continued effort to identify selective MRP1 modulators, we have developed two novel templates, **3** and **4**, through rational drug design by identifying the key pharmacophore interaction at the 7-position of the pyrrolopyrimidine template **1**. Further synthesis and SAR work on these novel templates gave a number of potent MRP1 modulators with great selectivity against Pgp. Additional studies to reduce the CYP3A4 inhibition are also reported. Several compounds of these classes were subjected to in vivo xenograft studies and in vivo efficacies were demonstrated.

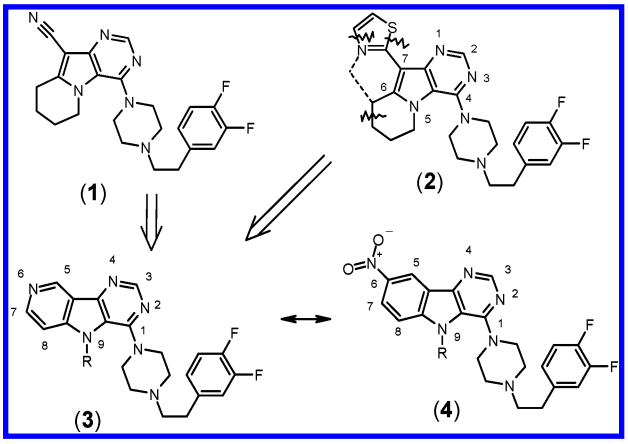
Multidrug resistance (MDR) mediated by P-glycoprotein (Pgp) or multidrug resistance associated protein (MRP) remains a major obstacle for successful treatment of cancer. Circumvention of Pgp and MRP transport is important for high efficacy of anticancer drugs. While several Pgp inhibitors have entered clinical trials, the development of specific MRP1 inhibitors is still in its infancy.[1]

Previously, we have reported the selective inhibitory activities of pyrrolopyrimidine analogues (**1**) against MRP1.[2] Our SAR studies in this series revealed an important pharmacophoric interaction of the functional groups at the 7-position as probable H-bond acceptors. However, SAR exploration of the lipophilic cyclohexyl group attached at the 5- and 6-position was not straightforward. Our new template design strategy is based on the fusion of the side chain at the 6-position, by ring-opening the cyclohexyl group, with the functional groups, such as nitrile **1** or thiazole group **2** at the 7-position, into an aromatic pyridyl analogue **3** or its isostere, the nitro analogue **4**.

This manipulation would allow the key pharmacophore interaction at the 7-position of template **1** or **2** to be retained and the conformation of the template to be more restricted. Moreover, readily amenable chemistry can be devised for templates **3** and **4** to allow us to explore the regions previously unexplored on template represented by **1** or **2**. Here, we disclose our synthesis and SAR work on templates **3** and **4**.

Chemistry

At first, we embarked on the synthesis and SAR exploration of template **4** and its analogues, starting from indole aminoesters **5** because this would allow us to adopt the protocols developed by us previously[2] (Scheme 1). Direct nitration or sulfonylation of chloropyrimidine intermediate **6** gave the functionalized intermediates **7** at the 6-position of template **6**, which were converted into sulfonamides or nitro analogues **8** as follows. Thus, the sulfonyl chloride (**7**, Y = SO_2Cl, R′ = H) was reacted with a variety of primary and secondary amines at 0 °C in the presence of triethylamine, yielding the corresponding chloropyrimidine sulfonamides, which were subsequently coupled with 3,4-difluorophenethylpiperazine (**Q**) to give a library of sulfonamides (**8**, Y = $SO_2NR_1R_2$). The 6-nitro analogue (**8**, Y = NO_2) was similarly prepared through the coupling of the 6-nitrochloropyrimidine intermediate (**7**, Y = NO_2) with the side chain **Q**. Alkylations on the indole nitrogen of analogues **9** can also be achieved successfully with NaH as base to give a number of analogues **10**. Following a literature method,[3] a number of 3-substituted indole pyrimidines **12** were prepared from the indole amino esters **5** and acetonitrile derivatives, such as chloroacetonitrile, with HCl gas and subsequent alkylations, chlorinations, and aminations.



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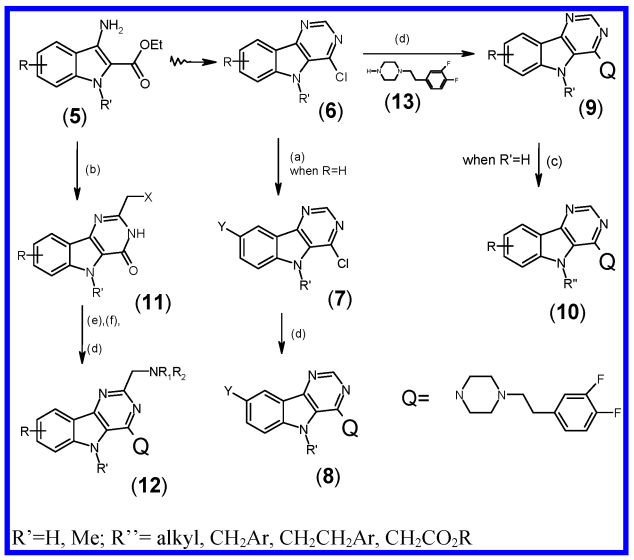
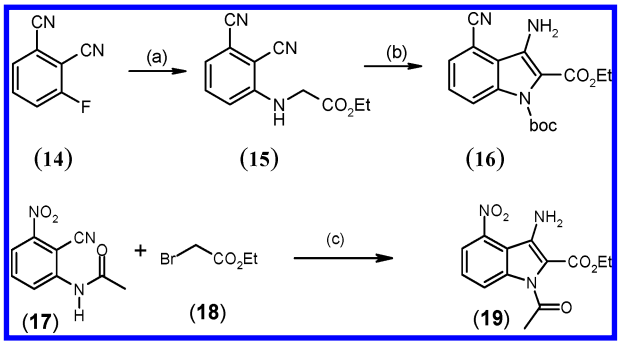
Scheme 1[a]



[a] Reagents and conditions: (a) Y = NO_2, $NaNO_3$, H_2SO_4, 0°C to room temp, 93%; Y = $SONR_1R_2$, (i) $ClSO_3H$, 0 °C to reflux, 70−95%, (ii) R_1R_2NH, DCM, TEA, 0°C, 28−77%; (b) XCH_2CN, HCl (g), dioxane, room temp, 38−87%; (c) NaH, R″X, DMF, 90%; (d) **13**, TEA, DMF, 100 °C, 27−99%; (e) X = Cl, R_1R_2NH, Na_2CO_3, EtOH, reflux, 44−77%; (f) $POCl_3$, TEA·HCl (0.4 equiv), reflux, 60−93%.

Scheme 2[a]



[a] Reagents and conditions: (a) glycine ethyl ester HCl salt, K_2CO_3, CH_3CN, reflux, 16 h, 19%; (b) $(BOC)_2O$, Et_3N, DMAP, DCM, room temp, 16 h, 100%; (c) NaH, THF, 0 °C to room temp, 80%.

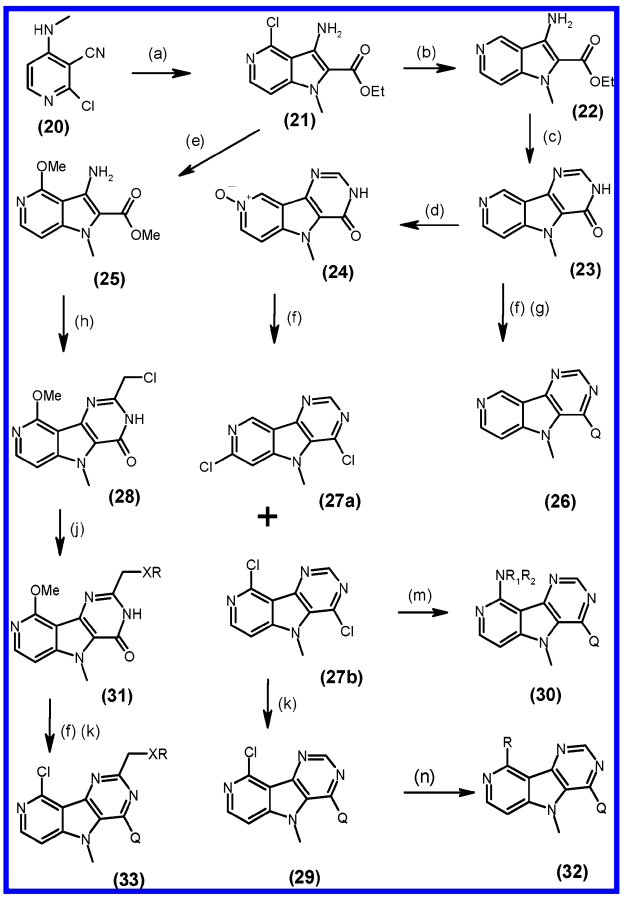
In each case, we retained the previously optimized side chain **13** in the analogue compounds **8**−**10** and **12**.

Most of the indole amino esters **5**, such as those when R = MeO at the 5- and 6-position, can be prepared from readily available starting materials with reported procedures,[4] while those with electron-withdrawing groups at the phenyl ring were prepared by novel methods as shown in Scheme 2.

Thus, 4-nitrile indole amino ester **16** was prepared from the reaction of 2,3-dicyanofluorobenzene **14** with glycine ester and base under refluxing conditions and subsequent cyclization of **15** by triethylamine. Alternatively, acetamide **17**[5] can be coupled with bromoethyl acetate **18** in the presence of NaH to give the cyclized amino ester **19** directly. Notably, both *N*-Boc and acetyl groups were cleaved during the subsequent pyrimidone formation step with ammonia under bomb conditions.[2]

Both nitrile and nitro groups in **16** and **19** served as versatile handles for further functional group manipulations using standard methodologies at the final analogue stage after the piperazine side chain was introduced into the template (see Experimental Section).

Scheme 3[a]



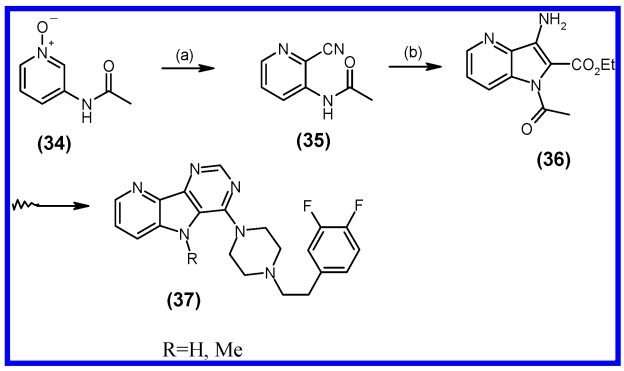
[a] Reagents and conditions: (a) **18**, NaH, DMF, 40 °C, 61%; (b) H_2, 10% Pd−C, EtOH, room temp, 96%; (c) (i) $Me_2NCH(MeO)_2$, DMF, 100 °C; (ii) NH_3, EtOH, 100 °C, 91% (two steps); (d) *m*-CPBA, $CHCl_3$, room temp, 24 h, 100%; (e) Na/MeOH, reflux, 80%; (f) $POCl_3$, Et_3N·HCl, reflux, 54−95%; (g) **13**, DMF, 100 °C, 71−95%; (h) $ClCH_2CN$, HCl(g), dioxane, 0 °C to room temp, 81%; (j) XR = OMe, Na/MeOH, reflux, 60%; XR = morpholine, K_2CO_3, EtOH, 54%; (k) **13**, DMF, 50 °C, o/n, 90−95%; (m) R_1R_2NH, DMF, 100 °C, 55−98%; (n) R = Me, MeZnCl, $Pd(Ph_3P)_4$, THF, 67 °C, 85%.

The syntheses of template **3** and its analogues are outlined in Scheme 3.

Thus, methylaminochloropyridine nitrile **20**[6] was alkylated with bromoethyl acetate **18** and simultaneous cyclization gave the amino ester **21**, which was dechlorinated by hydrogenolysis to give **22**. Methoxypyridine analogue **25** was also obtained by the displacement of the chloro group of **21** with sodium methoxide.

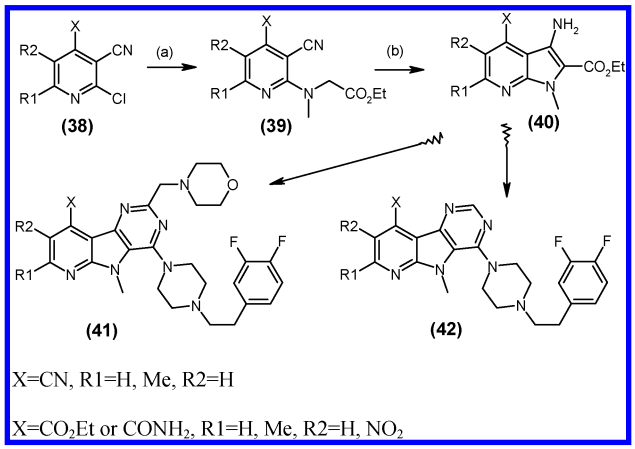
Following the protocols described earlier, amino esters **22** and **25** were converted into the corresponding pyrimidones **23** and **28**, respectively. *m*-CPBA oxidation of pyrimidone **23** gave the *N*-oxide intermediate **24**, which was converted into the regioisomeric dichloro intermediates **27a** (minor) and **27b** (major) upon treatment with $POCl_3$. The chloro group on the pyrimidine ring of **27b** was selectively replaced by the piperazine amine side chain **13** at 50 °C to give the chloropyridine analogue **29**. This was then used as a key intermediate for reaction with a diverse set of amines to give a series of analogues **30**. Direct alkylation of compound **29** was also achieved with palladium-catalyzed couplings,[7] yielding analogues **32**. Intermediates with improved aqueous solubility (**31**) were achieved by the displacement of the chloro group of **28** with amines, such as morpholine, or

Scheme 4[a]



[a] Reagents and conditions: (a) Me_2NCOCl, TMSCN, DCM, room temp, 92%; (b) NaH, **18**, THF, 0 °C to room temp, 88%.

Scheme 5[a]



[a] Reagents and conditions: (a) sarcosine ethyl ester·HCl, K_2CO_3, DMF, 85 °C, 32−52%; (b) Cs_2CO_3 or KO-*t*-Bu, CH_3CN, room temp to 50 °C, 48-70%.

alkoxide groups. Analogues **26** and **33** were obtained by the standard protocols described earlier from pyrimidones **23** and **31**, respectively.

To extend the SAR studies on the 6-pyridyl template **3**, the corresponding 5-pyridyl and 8-pyridyl analogues were also synthesized according to Schemes 4 and 5.

Thus, the key 5-pyridyl amino ester **36** was synthesized from the acetamidopyridine *N*-oxide[8] **34** with its cyanation to compound **35**, following a literature procedure.[9] The subsequent alkylation and cyclization of **35** to **36** and eventual synthesis of the analogue **37** was carried out by the protocols described earlier. Similarly, 8-pyridyl analogues **41** and **42** were prepared from the readily accessible starting materials **38** (X = CN,[10] X = CO_2Et[11]). The key intermediate esters **36** and **40** were reacted with *N*,*N*-dimethylformamide dimethyl acetal and subsequent heating with ammonia in ethanol to give the corresponding pyrimidones, which were converted into chloropyrimidines with $POCl_3$. These were then reacted with the side chain **Q** to give **37** and **42**, using the same conditions as described in Schemes 1−3. Analogues **41** were prepared using the same synthetic route as for compound **33** in Scheme 3.

Results and Discussion

Our initial SAR study was focused on the substitution of the phenyl ring of the indolopyrimidine template **4**. The compounds were evaluated in drug accumulation assays both in the MRP1 expressing cell line COR.L23/R (human non-small-cell lung carcinoma) and in the Pgp expressing cell line EMT6/AR1.0 (murine mammary carcinoma). Most of the active compounds were then subjected to the secondary single-dose potentiation assay.

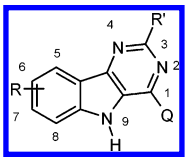
To screen out compounds that inhibit P450 enzymes to avoid any potential drug−drug interactions, selective potent compounds were also subjected to CYP 3A4 inhibition studies. The CYP 3A4 isozyme was chosen initially for this study because it is one of the major oxidative enzymes among the family of P450 enzymes.[12] Table 1 highlights the results of the MRP1 and Pgp accumulation and MRP1 and Pgp single-dose potentiation (sdpa) and CYP 3A4 assays.

Compound **43**, bearing no H-bond acceptor functional group, showed poor or moderate activity against MRP1 and Pgp, whereas 6-nitro analogue **44** showed potent inhibitory activity against MRP1 in both accumulation and single-dose potentiation assays with good selectivity against Pgp. These results validated our hypothesis on template **4**. Further library syntheses on alkoxy, acetamidoyl, amide, or sulfonamide linkages did not show improved activity as exemplified by compounds **45**, **46**, **48**, and **49**, although 6-carboxy ester analogue **47** was equipotent with **44**. Further SAR studies on the 5-position revealed a number of compounds with good activity (**50**−**54**); in particular, the 5-nitro analogue (**54**) and carboxyamide analogue (**52**) are as potent as compound **44** with good selectivity against Pgp. Further derivatization of amide **52** to secondary amides such as **53** or analogues with electron-donating groups such as **55** did not give improved potency. Analogues with 7- or 8-substitutions showed reduced activity (**56**, **57**). The additive substitution effect at the 5- and 6-position is not very significant (**58**).

Many of the above active compounds were evaluated in CYP 3A4 assays. Unfortunately, nearly all the compounds so far showed significant inhibitory activity against CYP 3A4 as shown in Table 1. Previously, we have demonstrated potent MRP1 activity in the pyrrolopyrimidine series,[2] and here, CYP 3A4 activity was not a serious problem in general. Close comparison of the two templates **1** and **4** revealed that the latter has a much flatter conformation, while the template **1** has a puckered cyclohexyl ring (Figure 1). It was tentatively assumed that this particular steric group might be responsible for the general inactivity of the template **1** against CYP 3A4. Having observed that introducing substituents at the 3-position of the pyrimidine ring was not detrimental to MRP1 activity,[2] we sought to introduce a steric group at the 3-position of the pyrimidine ring as shown in Scheme 1. Indeed, compounds **59** and **60** did not show any significant CYP3A4 activity, but much of the MRP1 activity was retained. This finding was also applied successfully to overcome CYP 3A4 activity for most of the active compounds later. To summarize the above results, it is clear that H-bond acceptors at the 5- or 6-position are important for pharmacophore interactions.

Next we turned our attention to the SAR studies on the indole N-substitutions at the 9-position with either 5- or 6-nitro analogues **54** or **44** or 5-amide analogue **52** as the parent molecules. A library of compounds was prepared via a parallel synthesis approach. Table 2

Table 1. Inhibitory Activities[a] of Indolopyrimidine Analogues in MRP1, Pgp, and CYP 3A4 Assays



compd	R	R′	IC_{50} (μM)[a] MRP1 (acc[b])	IC_{50} (μM)[a] Pgp (acc[b])	IC_{50} (μM)[a] MRP1 (sdpa[c])	IC_{50} (μM)[a] Pgp (sdpa[c])	IC_{50} (μM)[a] CYP3A4
43	5-CH_3	H	1.235	21.6	0.632	nd[d]	9.25
44	6-NO_2	H	0.133	9.23	0.051	1.738	12.58
45	6-MeO	H	0.82	8.3	0.608	nd[d]	nd[d]
46	6-$NHCOCH_3$	H	2.71	>40	nd[d]	nd[d]	nd[d]
47	6-CO_2CH_3	H	0.113	>40	0.059	0.731	2.53
48	6-$CONH_2$	H	3.44	>40	nd[d]	nd[d]	nd[d]
49	6-SO_2-N(CH$_2$)$_4$	H	0.235	3.31	0.542	nd[d]	nd[d]
50	5-Cl	H	0.367	17.2	0.167	>1.0	nd[d]
51	5-CN	H	0.277	11.6	0.169	>1.0	nd[d]
52	5-$CONH_2$	H	0.183	28.3	0.065	3.32	1.36
53	5-CONHPy-4	H	0.65	24.55	0.708	nd[d]	nd[d]
54	5-NO_2	H	0.183	18.04	0.059	0.78	6.62
55	5-NH_2	H	1.9	27.86	0.708	nd[d]	nd[d]
56	7-MeO	H	3.15	>40	nd[d]	nd[d]	1.53
57	7-MeO,8-NO_2	H	3.45	>40	nd[d]	nd[d]	1.79
58	5-NO_2,6-MeO	H	0.078	>40	0.156	>1.0	nd[d]
59	6-NO_2		0.672	14.3	0.107	>1.0	>100
60	6,8-di-NO_2		0.118	8.22	0.019	1.48	42.26

[a] All the IC_{50} values in this text represent the mean of a minimum of three experiments. Variations among the results were less than 10%. [b] acc: accumulation assay. [c] sdpa: single-dose potentiation assay. [d] nd: not determined.

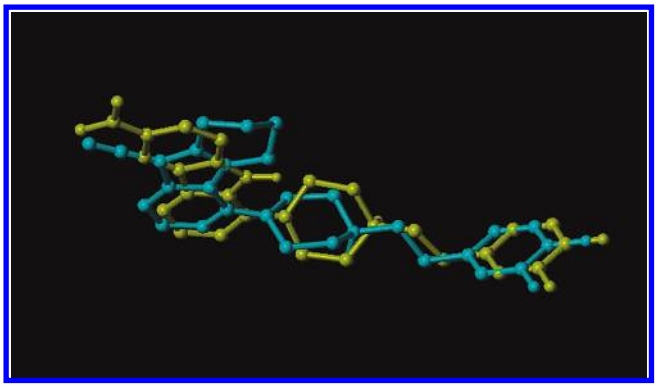


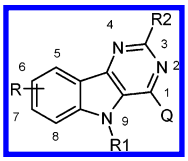
Figure 1. Superimposition of templates **1** (green) and **4** (yellow) carried out in Sybyl, version 6.9, using the default setting of the GASP module.

shows the assay results on some of the typical examples. N-alkylations of the 5-nitro analogue **54** did not result in loss of potency (**61**−**63**). The 4-pyridylmethylene alkylated analogue **64** showed much improved MRP1 activity in both accumulation and single-dose potentiation assays; its MRP1 selectivity over Pgp is maintained in these assays as well. N-alkylations of the 5-amide analogue **52** gave consistently more active compounds **65**−**67**, whereas N-alkylations of the 6-nitro analogue **44** did not yield compounds with much improved activity (**68**). Unfortunately, most of the above compounds showed significant inhibitory activity against CYP 3A4 except compound **61**, which showed no activity in this assay. The reason for this is not yet understood. However, by use of the same strategy as previously with the introduction of a morpholine group at the 3-position, the CYP 3A4 activity was significantly diminished to an acceptable level (**69**).

A library of 6-pyridylindolopyrimidine compounds was prepared according to the chemistry shown in Scheme 3. As shown in Table 3, compound **70** is the most simple analogue of template **3**, which showed very good MRP1 inhibitory activity with remarkable selectivity against Pgp in both accumulation and single-dose potentiation assays. More lipophilic benzyl substitution at the 9-N position gave compounds with slightly reduced activity (**71**). Therefore, we focused the rest of our SAR studies on the 9-*N*-methyl analogues. The key intermediate 5-chloro compound **29** showed potent activity against MRP1 with a significant level of CYP3A4 activity, while a similar magnitude of potency was observed in the corresponding alkoxy analogues **72** and **73**; these analogues did not show any level of CYP3A4 activity at 100 μM. Replacement of the 5-chloro group with diverse amines gave a number of very potent analogues (**74**−**77**) with acceptable levels of CYP 3A4 activity; even the 5-methyl analogue **78** showed a similar in vitro profile. However, the 7-chloro-substituted analogue **79** resulted in reduction of MRP1 activity. For those compounds with a certain degree of CYP 3A4 activity like **29**, introduction of a steric group at the 3-position of the pyrimidine ring gave compounds with a much reduced level of CYP 3A4 activities while the MRP1 activities were retained (**80**, **81**).

We also investigated the SAR of the corresponding 5- or 8-pyridyl templates. The results on selected examples are illustrated in Table 4. The 5-pyridyl analogue **82** showed slightly reduced activity compared with the 6-pyridyl analogue **70** in both MRP1 accumulation and potentiation assays. Active functional groups, such as nitrile and amides identified in template **4**, were introduced into the 5-position of the 8-pyridyl template, resulting in a number of potent MRP1 inhibitors (**83**, **85**, **86**). Further introduction of substitutents

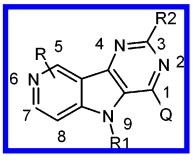
Table 2. SAR Studies on Analogues with 9-N Substitutions



compd	R	R1	R2	IC_{50} (μM)[a] MRP1 (acc[b])	IC_{50} (μM)[a] Pgp (acc[b])	IC_{50} (μM)[a] MRP1 (sdpa[c])	IC_{50} (μM)[a] Pgp (sdpa[c])	IC_{50} (μM)[a] CYP3A4
61	5-NO_2	CH_3	H	0.147	10.0	0.231	>1.0	>100
62	5-NO_2	$Ph(CH_2)_2$	H	0.222	6.94	0.0409	0.443	11.79
63	5-NO_2	CH_2Py-3	H	0.621	6.94	0.0265	0.294	8.10
64	5-NO_2	CH_2Py-4	H	0.08	6.14	0.0137	0.236	5.78
65	5-$CONH_2$	CH_3	H	0.062	>40	0.024	1.2	0.26
66	5-$CONH_2$	CH_2CO_2Et	H	0.122	9.2	0.045	0.651	nd
67	5-$CONH_2$	CH_2Py-4	H	0.27	>40	0.02	0.85	0.20
68	6-NO_2	CH_2CO_2Et	H	0.138	10.5	0.078	1.02	15.0
69	5-$CONH_2$	CH_3		0.23	>40	0.029	0.608	31.31

[a] All the IC_{50} values in this text represent the mean of a minimum of three experiments. Variations among the results were less than 10%. [b] acc: accumulation assay. [c] sdpa: single-dose potentiation assay.

Table 3. SAR Studies on 6-Pyridineindolopyrimidine Series



compd	R	R1	R2	IC_{50} (μM)[a] MRP1 (acc[b])	IC_{50} (μM)[a] Pgp (acc[b])	IC_{50} (μM) MRP1 (sdpa[c])	IC_{50} (μM)[a] Pgp (sdpa[c])	IC_{50} (μM)[a] CYP3A4
70	H	CH_3	H	0.044	22.5	0.024	4.7	17.85
71	H	CH_2Ph	H	0.545	8.4	0.048	1.11	nd[d]
29	5-Cl	CH_3	H	0.13	nd[d]	0.041	1.22	9.44
72	5-MeO	CH_3	H	0.172	nd[d]	0.063	1.71	>100
73	5-^{i}PrO	CH_3	H	0.265	nd[d]	0.027	0.95	>100
74	5-NMe_2	CH_3	H	0.166	nd[d]	0.012	1.34	26.41
75	5-NHMe	CH_3	H	0.115	nd[d]	0.028	1.90	53.81
76	5-$NHCH_2$Py-2	CH_3	H	0.106	nd[d]	0.015	0.78	18.48
77	morpholine	CH_3	H	0.071	nd[d]	0.010	2.1	20.80
78	5-CH_3	CH_3	H	0.033	nd[d]	0.021	1.2	>100
79	7-Cl	CH_3	H	1.20	nd[d]	0.087	>10	nd[d]
80	5-Cl	CH_3	CH_2OMe	0.244	nd[d]	0.013	2.0	36.82
81	5-Cl	CH_3		0.09	nd[d]	0.017	2.47	20.03

[a] All the IC_{50} values in this text represent the mean of a minimum of three experiments. Variations among the results were less than 10%. [b] acc: accumulation assay. [c] sdpa: single-dose potentiation assay. [d] nd: not determined.

into the pyridine ring had some effects; however, they were not dramatic (**84**, **87**). The morpholine methylene side chain was introduced at the 3-position of the pyrimidine ring of analogue **86** to overcome its potent activity against CYP3A4 with retained MRP1 activity (**88**).

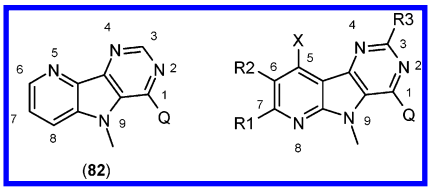
On the basis of the SAR obtained from the two template series **3** and **4**, a number of compounds were subjected to pharmacokinetics studies. This study was carried out with Balb/C mice dosed at 20 mg/kg intravenously (iv) or 50 mg/kg intraperitoneally (ip) or orally (po). Table 5 illustrates the results of some early examples (more detailed studies will be published elsewhere).

As shown in Table 5, **52** showed a good level of absorption following ip or po administration. Compounds **52**, **60**, **69**, and **77** showed a range of half-lives from 2 to 7 h depending on the routes of administration. A wide range of C_{max} and AUC values was observed with the above compounds.

To understand the compounds' characteristics and to demonstrate the in vivo efficacy for these new classes of compounds in our xenograft models in the early stage of the project, we selected analogues **52** (XR13097) and **60** (XR13287) for in vivo xenograft studies. The significant level of CYP 3A4 activity of compound **52** was not a major concern for us at this stage, although CYP activity remains a major criterion for selecting clinical candidates to avoid potential drug−drug interactions. The study was carried out with CD1 athymic mice bearing subcutaneous COR L23/R tumors (n = 6). Compounds **52** (XR13097) and **60** (XR13287) were administered ip (50 mg/kg) 30 min before and 4 h after vincristine (0.6 mg/kg iv) on days 0 and 5 (Figure 2).

As shown in Figure 2, **52** significantly enhanced the antitumor efficacy of vincristine when compared with vincristine alone [from day 5 onward for **52** and day 10 onward for **60** (graft not shown)]. This was reflected in an optimal *T*/*C* % ratio of 19.3 for compound **52** plus vincristine (compared with 83.4 and 84.2 for vincristine alone and compound **52** alone, respectively) and 45.7 for compound **60** plus vincristine (compared with 73.6

Table 4. SAR Studies on 5- and 8-Pyridylindolopyrimidine Analogues



compd	R1	R2	R3	X	IC$_{50}$ (μM)[a] MRP1 (acc[b])	IC$_{50}$ (μM)[a] MRP1 (sdpa[c])	IC$_{50}$ (μM)[a] Pgp (sdpa[c])	IC$_{50}$ (μM)[a] CYP3A4
82					0.27	0.094	7.56	19.3
83	H	H	H	CN	0.355	0.045	>10	nd[d]
84	CH_3	H	H	CN	1.255	0.19	0.356	>100
85	CH_3	H	H	$CONH_2$	0.223	0.067	>10	0.21
86	H	H	H	$CONH_2$	0.11	0.012	>10	1.23
87	CH_3	NO_2	H	$CONH_2$	0.088	0.011	6.04	nd[d]
88	H	H		$CONH_2$	0.097	0.010	1.91	33.69

[a] All the IC$_{50}$ values in this text represent the mean of a minimum of three experiments. Variations among the results were less than 10%. [b] acc: accumulation assay. [c] sdpa: single-dose potentiation assay. [d] nd: not determined.

Table 5. Pharmacokinetic Studies in Mice[a]

dose (mg/kg)	route	C_{max} (μM)	AUC (μg·h/mL)	$t_{1/2}$ (h)	F (%)
		Compound **52**			
20	iv	37	94	4.6	
50	ip	84	76	2.6	81
50	po	13	123	6.0	52
		Compound **60**			
20	iv	9	5.1	2.4	
50	ip	26.43	30.57	6.86	
		Compound **69**			
20	iv	15	14	3.03	
		Compound **77**			
20	iv	11.88	6.32	1.97	

[a] C_{max}, maximum plasma concentration; AUC, area under the concentration−time curve; F, % bioavailability.

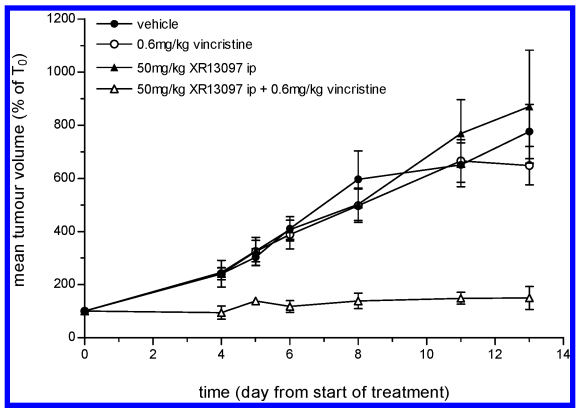


Figure 2. Xenograft curves depicting tumor growth inhibition of subcutaneous COR L23/R cell in CD1 NUDE mice under coadminstration of compound **52** or XR13097 with vincristine.

and 94.0 for vincristine alone and compound **60** alone, respectively). In contrast, vincristine, **52**, or **60** alone had no significant effect on tumor growth. In addition, the treatments with combination schedules were well tolerated (loss of body weight less than 13%).

Conclusions

Through rational drug design by identifying the key pharmacophore interactions in the pyrrolopyrimidine template series exemplified by **1** and **2**, we have synthesized and investigated two novel template series **3** and **4**, which showed very potent MRP1 modulating activity with good selectivity against Pgp. The amenable chemistry developed allowed the use of parallel synthesis techniques to generate a library of analogues to explore the templates on positions previously difficult to access in the pyrrolopyrimidine series. We have identified a solution to overcome the CYP 3A4 inhibition problem on some of the active compounds by introducing steric groups at the 3-position of the pyrimidine ring of templates **3** and **4**. Many compounds from these series demonstrated good pharmacokinetic profiles and in vivo efficacies in our xenograft models, exemplified by our early compounds **52** and **60**. More detailed efficacy studies on some more analogues will be published elsewhere.

Experimental Section

Methods and Materials. Reagents, starting materials, and solvents were purchased from common commercial suppliers and used as received or distilled from the appropriate drying agent. Reactions requiring anhydrous conditions were performed under an atmosphere of nitrogen or argon. Precoated aluminum-backed silica gel 60 F_{254} plates with a layer thickness of 0.25 mm were used for thin-layer chromatography, and the stationary phase for preparative column chromatography using medium pressure was silica gel 60, mesh size 40−60 μm from E. Merck, Darmstadt, Germany.

NMR spectra were obtained using a Bruker ACF 400 operating at 400 MHz and the ^{1}H shifts in ppm were calibrated to that of residual $CHCl_3$ in $CDCl_3$ at 7.26 ppm. Mass spectra were obtained in the indicated mode using a Finnigan SSQ 710L machine. Melting points were determined using an electrothermal 9100 series apparatus.

The compound purity of all compounds tested in biological systems was assessed as being >95% using HPLC using both a water/acetonitrile gradient containing 0.02% TFA at 30 °C on a Waters symmetry C_{18}, 5 μM, 150 mm × 3.9 mm column and a water/actonitrile gradient containing 0.05% phosphoric acid at 30 °C on a LiChrospher RP-8, 5 μM, 250 mm × 4.6 mm column. UV photodiode array detection was applied. Microanalyses were performed on a representation of compounds by MEDAC Ltd. U.K. Where analyses are indicated only by the symbols of the elements, results obtained were within 0.4% of the theoretical values.

General Procedure for Preparation of Analogues 8−10.[2,13] A mixture of 1-chloro-9*H*-2,4,9-triazafluorene **6** (1.0 equiv), 1-[2-(3,4-difluorophenyl)ethyl]piperazine (1.2 equiv), and triethylamine (1.1 equiv) was heated in dimethylformamide at 80 °C overnight. The reaction mixture was cooled and poured onto ice/water and the precipitated solid was collected

by filtration and triturated from hot ethyl acetate to yield the desired title compounds **8** and **9**.

General Procedure for N-9 Alkylation of 1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluorene (9). To a suspension of NaH (1.1 equiv) in DMF cooled to 0 °C was added a solution of the corresponding triazafluorene derivatives **9** (1.0 equiv) in DMF dropwise over 10 min. The resultant mixture was stirred at 0 °C for a further 40 min before addition of an alkyl halide (1.1 equiv), and the mixture was stirred at room temperature for 2 h. Purification by flash chromatography with EtOAc/petrol/EtOH gave **10**.

(2,3-Dicyanophenyl)carbamic Acid Ethyl Ester (15). 3-Flouro-1,2-dicyanobenzene (2.48 g, 17.03 mmol) and glycine ethyl ester HCl salt (1.05 equiv, 2.49 g, 17.88 mmol) were dissolved in acetonitrile (25 mL). To this was added potassium carbonate (2.2 equiv, 5.18 g, 37.47 mmol), and the reaction mixture was refluxed overnight. The volatiles were removed in vacuo, and the residue was taken up in DCM and washed with water. The organic layer was dried ($MgSO_4$), filtered, and evaporated in vacuo. Subsequent purification by flash chromatography afforded the title compound as a beige solid (0.84 g, 19%). ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3H, J = 7.1 Hz, CH_3), 3.90 (d, 2H, J = 5.4 Hz, CH_2), 4.23 (q, 2H, J = 7.1 Hz, CH_2), 5.35 (br, 1H, NH), 6.70 (d, 1H, J = 8.7 Hz, ArH), 6.98 (d, 1H, J = 8.7 Hz, ArH), 7.39 (t, 1H, J = 8.8 Hz, ArH).

3-Amino-4-cyanoindole-1,2-dicarboxylic Acid 1-*tert*-Butyl Ester 2-Ethyl Ester (16). (2,3-Dicyanophenyl)carbamic acid ethyl ester (453 mg, 1.71 mmol) was dissolved in anhydrous DCM (5 mL), and to this was added triethylamine (1 equiv, 240 μL), BOC_2O (1.2 equiv, 447 mg, 2.05 mmol), and DMAP (0.1 equiv, 20 mg, 0.17 mmol). The reaction mixture was stirred at room temperature overnight, diluted with DCM, and washed with water. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography to give the title compound as a light-yellow oil (606 mg, 100%), which crystallized on standing. ^{1}H NMR ($CDCl_3$) δ 1.18 (t, 3H, J = 7.2 Hz, CH_3), 1.20 (s, 9H, *t*-BOC), 4.05 (q, 2H, J = 7.1 Hz, CH_2), 4.12−4.20 (br, 2H, NH_2), 7.60−7.64 (m, 2H, ArH), 7.72−7.82 (m, 1H, ArH).

1-Acetyl-3-amino-4-nitro-1*H*-indole-2-carboxylic Acid (19). To a suspension of NaH (21 mg, 1.1 equiv) in THF (10 mL) at 0 °C was added a solution of *N*-(2-cyano-3-nitrophenyl)-acetamide (100 mg, 0.49 mmol) in THF (10 mL) dropwise over 15 min. The resultant mixture was stirred at 0 °C for a further 40 min before addition of ethyl bromoacetate (0.06 mL, 1.1 equiv), and the mixture was allowed to warm to room temperature and stirred for 2 h. Brine was added to the mixture, the organic phase was collected, the aqueous layer was extracted with EtOAc, and the combined organic fractions were dried ($MgSO_4$) and concentrated to give a red solid (126 mg, 88%). ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, J = 7.1 Hz, CH_3), 2.41 (s, 3H, CH_3), 4.34 (q, 2H, J = 7.1 Hz, CH_2), 5.60 (br s, 2H, NH_2) 7.49 (dd, 1H, J = 7.9 Hz, J = 8.5 Hz, ArH), 7.90 (d, 1H, J = 7.9 Hz, ArH), 7.76 (d, 1H, J = 8.5 Hz, ArH).

3-Amino-4-chloro-1-methyl-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic Acid Ethyl Ester (21). 21 was synthesized from 2-chloro-4-methylaminopyridine-3-nitrile (**20**) according to a reported method.[6]

3-Amino-1-methyl-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic Acid Ethyl Ester (22). To a solution of 3-amino-4-chloro-1-methyl-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid ethyl ester (9.95 g, 39.2 mmol) in ethanol (400 mL) was added 10% palladium on carbon (800 mg) as a slurry in ethanol under argon. The mixture was purged with hydrogen and stirred for 24 h at atmospheric pressure. The mixture was purged with argon and filtered through Celite, and the ethanol was removed in vacuo. The residue was partitioned between saturated aqueous sodium hydrogen carbonate (200 mL) and ethyl acetate (3 × 250 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo to give the title compound as a yellow solid (8.28 g, 96%). ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, J = 7.1 Hz, CH_3), 4.35 (q, 2H, J = 7.1 Hz, CH_2), 3.95 (s, 3H, CH_3), 5.19 (br s, 2H, NH_2), 7.30 (d, 1H, PyH), 7.25 (s, 1H, PyH), 8.38 (d, 1H, PyH), 8.95 (s, 1H, PymH).

3-Amino-1-methyl-4-methoxy-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic Acid Ethyl Ester (25). Sodium (0.417 g, 18.14 mmol, 3 equiv) was dissolved in methanol (20 mL) at room temperature, to which was added 3-amino-4-chloro-1-methyl-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid ethyl ester (**21**) (1.533 g, 6.05 mmol, 1.0 equiv). The mixture was heated at reflux overnight. The solvent was removed, and water was added to the residue. The resulting precipitate was filtered, washed with water, and dried in vacuo to give the title compound **25** (1.128 g, 79%). ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3H, CH_3O), 4.83 (s, 3H, CH_3O), 4.0 (s, 3H, CH_3N), 5.40 (br s, 2H, NH_2), 6.60 (d, 1H, PyH), 7.75 (d, 1H, PyH).

1,5-Dichloro-9-methyl-9*H*-2,4,6,9-tetraazafluorene (27b) and 1,7-Dichloro-9-methyl-9*H*-2,4,6,9-tetraazafluorene-(27a). To a suspension of 9-methyl-2,9-dihydro-2,4,6,9-tetraazafluoren-1-one (**23**) (6.40 g, 32.0 mmol), prepared from 3-amino-1-methyl-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid ethyl ester (**22**) by standard protocol,[2] in chloroform (500 mL) at 0 °C was added *m*-chlorobenzoic acid (16.6 g, purity 77% max), and the mixture was stirred at room temperature for 16 h. The material was then collected by filtration and washed thoroughly with ether to give the *N*-oxide as pale-cream solid **24** (6.90 g, 99%). To this material was added triethylamine hydrochloride (2.94 g, 21.3 mmol) and phosphorus oxychloride (70 mL), and the mixture was heated under reflux for 2 h. The majority of the phosphorus oxychoride was then removed by distillation at reduced pressure. The residue was diluted with toluene, and the solid was collected by filtration. The residue was suspended in water, and the pH was adjusted to 7 by the addition of saturated aqueous sodium hydrogen carbonate. The white solid was collected by filtration, washed with water, and dried to give compound **27b** (3.62 g, 54%). ^{1}H NMR ($CDCl_3$) δ 4.20 (s, 3H, NCH_3), 7.38 (d, 1H, J = 6.0 Hz, PyH), 8.50 (d, 1H, J = 6.0 Hz, PyH), 9.03 (s, 1H, PymH).

The toluene phosphorus oxychloride residue was poured into a vigorously stirred ice/water mixture, the pH was adjusted to 7, and the mixture was then extracted with ethyl acetate to give traces of the 5- and 7-chloropyridines. These were purified by chromatography (50% ethyl acetate/petrol) to afford compound **27a** (145 mg, 1.8%). ^{1}H NMR ($CDCl_3$) δ 4.14 (s, 3H, CH_3), 7.42 (s, 1H, PyH), 8.88 (s, 1H, PymH), 9.44 (s, 1H, PyH).

5-Chloro-1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-9*H*-2,4,6,9-tetraazafluorene (29). To a solution of 1,5-dichloro-9-methyl-9*H*-2,4,6,9-tetraazafluorene **27b** (1.52 g. 6.01 mmol, 1.0 equiv) in DMF (30 mL) was added triethylamine (0.92 mL, 6.61 mmol. 1.1 equiv) and the piperazine side chain (1.68 g, 6.61 mmol, 1.1 equiv). The solution was heated at 50 °C for 20 h, and the solvent was removed in vacuo. The residue was partitioned between saturated aqueous sodium hydrogen carbonate (60 mL) and chloroform (3 × 80 mL). The combined organic layers were washed with brine (50 mL), separated, and dried ($MgSO_4$). The residue was triturated in ethyl acetate to give a pale-cream solid (2.10 g, 79%). ^{1}H NMR ($CDCl_3$) δ 2.59 (t, 2H, J = 7.6 Hz, CH_2), 2.66 (br s, 4H, 2 × CH_2), 2.73 (t, 2H, J = 7.6 Hz, CH_2), 3.45 (br s, 4H, 2 × CH_2), 3.97 (s, 3H, CH_3), 6.85 (m, 1H, ArH), 7.03−6.91 (m, 2H, ArH), 7.25 (d, 1H, J = 5.8 Hz, PyH), 8.36 (d, 1H, J = 5.8 Hz, PyH), 8.83 (s, 1H, PymH); MS (DCI/NH_3) m/z 443 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-5-methyl-9*H*-2,4,9-triazafluorene (43). 43 was synthesized by the standard protocols[2,13] from 3-amino-1-benzoyl-4-methyl-1*H*-indole-2-carboxylic acid ethyl ester, which was prepared from 2-amino-6-methylbenzonitrile using a reported procedure.[14] Mp 180−180.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.6−2.74 (m, 6H, 3 × CH_2), 2.81−2.85 (m, 2H, CH_2), 3.05 (s, 3H, CH_3), 3.87−3.89 (m, 4H, 2 × CH_2), 6.93−6.96 (m, 1H, ArH), 7.03−7.12 (m, 3H, 3 × ArH), 7.33 (d, 1H, J = 8.2 Hz, ArH), 7.44 (t, 1H, J = 7.7 Hz, ArH), 7.96 (s, 1H, NH), 8.75 (s, 1H, PymH); MS m/z 408.5 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-6-nitro-9*H*-2,4,9-triazafluorene (44). Sodium nitrate (0.26 g, 3.08 mmol, 1.1 equiv) was dissolved in concentrated sulfuric acid (3 mL) with a few drops of water. This solution was added dropwise to a stirring solution of commercially available

1-chloro-9*H*-2,4,9-triazafluorene (0.57 g, 2.8 mmol, 1.0equiv) in concentrated sulfuric acid (20 mL) at 0 °C. The reaction mixture was allowed to warm slowly to room temperature and stirred overnight. The reaction mixture was poured onto ice, and the precipitated solid was collected by filtration to yield 1-chloro-6-nitro-9*H*-2,4,9-triazafluorene (0.68 g, 98%). This compound (0.265 g, 1.07 mmol) was treated according to the general procedure to give **44** (0.351 g, 75%). ^{1}H NMR (DMSO-d_6) δ 2.60 (m, 6H, 3 × CH_2), 2.80 (m, 2H, CH_2), 3.85 (m, 4H, 2 × CH_2), 7.10 (m, 1H, ArH), 7.35 (m, 2H, ArH), 7.75 (d, 1H, J = 9.1 Hz, ArH), 8.40 (dd, 1H, J = 9.0, 2.3 Hz, ArH), 8.55 (s, 1H, PymH), 8.90 (d, 1H, J = 2.3 Hz, ArH), 12.05 (s, br, 1H, NH); MS *m*/*z*: 439.3 (M + H)$^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-6-methoxy-9*H*-2,4,9-triazafluorene (45). Compound **45** was prepared by treatment of **6** (when R = 6-MeO) the side chain **13** according to a general procedure.[2] ^{1}H NMR ($CDCl_3$) δ 2.65 (m, 6H, 3 × CH_2), 2.80 (m, 2H, CH_2), 3.90 (s, 3H, OCH_3), 3.95 (m, 4H, 2 × CH_2), 6.95 (m, 1H, ArH), 7.05 (m, 2H, ArH), 7.20 (m, 1H, ArH), 7.40 (d, 1H, J = 8.9 Hz, ArH), 7.70 (s, 1H, ArH), 8.20 (s, 1H, NH), 8.70 (s, 1H, PymH); MS *m*/*z* 424.1 (M + H)$^+$.

N-4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluoren-6-yl)acetamide (46). To a stirring suspension of 1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluoren-6-ylamine (0.05 g, 0.12 mmol) [prepared from 1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-6-nitro-9*H*-2,4,9-triazafluorene **44** using hydrogenolysis (see method for **55**)] in anhydrous dichloromethane (3 mL) was added acetic anhydride (11.5 μL, 0.12 mmol) followed by triethylamine (17 μL, 0.12 mmol). The mixture was stirred overnight at room temperature under a nitrogen atmosphere. The reaction mixture was filtered to collect a white solid, which was further purified by flash chromatography (silica gel, 5% MeOH/$CHCl_3$) to give product **46** (40 mg, 67%). Mp 234.5 °C; ^{1}H NMR (DMSO-d_6) δ 2.18 (s, 3H, CH_3), 2.56−2.72 (m, 6H, 3 × CH_2), 2.80 (t, 2H, J = 7.4 Hz, CH_2), 3.70−3.88 (m, 4H, 2 × CH_2), 7.08−7.18 (m, 1H, CH), 7.28−7.42 (m, 2H, 2 × CH), 7.53 (d, 1H, CH, J = 8.8 Hz), 7.64 (dd, 1H, CH, J = 1.8, 7.0 Hz), 8.45 (s, 2H, 2 × CH), 9.98 (s, 1H, NH), 11.18 (s, 1H, NH); MS *m*/*z* 451 (M + H)$^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluorene-6-carboxylic Acid Methyl Ester (47). 2-Fluoro-5-formylbenzonitrile (9.22 g, 61.83 mmol) and pyridinium dichromate (2 equiv, 123.65 mmol, 46.52 g) were stirred in DMF (90 mL) at room temperature overnight. The reaction mixture was diluted with ethyl acetate and washed with water (×3), the organic layer was dried ($MgSO_4$) and filtered, and the volatiles were removed in vacuo to give 3-cyano-4-fluorobenzoic acid as a grayish solid (8.21 g, 80%). 3-Cyano-4-fluorobenzoic acid (8.21 g, 49.70 mmol) was suspended in methanol (80 mL). To this was added acetyl chloride (4 equiv, 14 mL), and the reaction mixture was stirred at room temperature for 2 days. Then the reaction volume was reduced in vacuo, and the precipitate was collected by filtration, washed with water, and air-dried to give 3-cyano-4-fluorobenzoic acid methyl ester as a white solid (7.25 g, 82%). Subsequent treatment of 3-cyano-4-fluorobenzoic acid methyl ester according to Scheme 2 and standard protocols[2,13] afforded **47** (375 mg, 98%). Mp. 260−261 °C; ^{1}H NMR ($CDCl_3$/MeOD-d_4) δ 2.56−2.68 (m, 6H, 3 × CH_2), 2.72−2.80 (m, 2H, CH_2), 3.84−3.90 (m, 4H, 2 × CH_2), 3.90 (s, 3H, MeO), 6.82−6.90 (m, 1H, ArH), 6.94−7.05 (m, 2H, ArH), 7.47 (d, 1H, J = 8.6 Hz, ArH), 8.14 (d, 1H, J = 8.6 Hz, ArH), 8.52 (s, 1H, ArH), 8.93 (s, 1H, PymH); MS *m*/*z* 452 (M + H)$^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluorene-6-carboxylic Acid Amide (48). To a stirring solution of 1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluorene-6-carboxylic acid methyl ester (**47**) (0.3 g, 0.66 mmol) in ethanol (3 mL) was added a solution of sodium hydroxide (53.2 mg, 1.32 mmol, 2 equiv). The mixture was heated to reflux for 5 days before concentration in vacuo. The resulting solid was dissolved in water, and the solution was acidified by dropwise addition of concentrated hydrochloric acid until a precipitate formed. The solid was collected and dried to give 1-{4-[2-(3,4-difluorophenyl)ethyl]-piperazin-1-yl}-9*H*-2,4,9-triazafluorene-6-carboxylic acid as a white solid (89 mg, 31%).

To a suspension of 1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluorene-6-carboxylic acid (85 mg, 0.19 mmol) in DMF (3 mL) was added 1′,1-carbonyldiimidazole (63 mg, 0.36 mmol, 2 equiv). The mixture was stirred for 1 h at room temperature before addition of concentrated aqueous ammonia (18.2 μL, 0.46 mmol, 2.4 equiv) and stirring overnight. Water was added and extracted with ethyl acetate. The organic layer was washed with brine and dried (Na_2SO_4), and the solvent was removed in vacuo to give a crude product. Purification by flash chromatography (2−20% MeOH/CH_2Cl_2) gave **48** as a white solid (25 mg, 30%). Mp 303−304 °C; ^{1}H NMR (DMSO-d_6) δ 2.42−2.52(m, 6H, 3 × CH_2), 2.64 (t, 2H, J = 7.5 Hz, CH_2), 3.64 (t, 4H, J = 4.5 Hz, 2 × CH_2), 6.95 (m, 1H, CH), 7.08 (s, br, 1H, NH_2), 7.28 (m, 2H, 2 × CH), 7.5 (d,-1H, CH, J = 8.7 Hz), 7.90 (br dd, 2H, CH + NH_2), 8.34 (s, 1H, CH), 8.57 (s, 1H, CH), 11.38 (br, s, 1H, NH); MS *m*/*z* 437 (M + H)$^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-6-(pyrrolidine-1-sulfonyl)-9*H*-2,4,9-triazafluorene (49). 1-Chloro-9*H*-2,4,9-triazafluorene **6** (when R = R′ = H) (183 mg, 0.76 mmol) was added portionwise to chlorosulfonic acid (2 mL) at 0 °C. After 2 h, the mixture was heated to 100 °C and maintained at this temperature for 1 h. The reaction mixture was then cooled and poured onto ice/water carefully and the resulting precipitate was collected by filtration to give 1-chloro-9*H*-2,4,9-triazafluorene-6-sulfonyl chloride (221 mg, 96%). ^{1}H NMR (DMSO-d_6) δ 7.55 (d, 1H, J = 8.5 Hz, ArH), 7.88 (d, 1H, J = 8.5 Hz, ArH), 8.37 (s, 1H, PymH), 8.79 (s, 1H, ArH), 12.37 (s br, 1H, NH).

1-Chloro-9*H*-2,4,9-triazafluorene-6-sulfonyl chloride (83.6 mg, 0.28 mmol), pyrrolidine (44 μL, 0.54 mmol, 2 equiv), and triethylamine (39 μL, 0.28 mmol, 1 equiv) were stirred together in dichloromethane (3 mL) at room temperature. After the mixture was stirred overnight, the reaction was quenched with water. The mixture was then extracted into dichloromethane and dried (Na_2SO_4), and the solvent was removed in vacuo to yield a residue that was triturated with diethyl ether to give 1-chloro-6-(pyrrolidine-1-sulfonyl)-9*H*-2,4,9-triazafluorene (37.6 mg, 38%). ^{1}H NMR ($CDCl_3$/MeOD-d_4) δ 1.67−1.72 (m, 4H, 2 × CH_2), 3.22−3.27 (m, 4H, 2 × CH_2), 7.68 (d, 1H, J = 8.7 Hz, ArH), 8.04 (d, 1H J = 8.7 Hz, ArH), 8.82 (s, 1H, NH), 8.87 (s, 1H, PymH).

Subsequent treatment of 1-chloro-6-(pyrrolidine-1-sulfonyl)-9*H*-2,4,9-triazafluorene according to standard protocol[2] gave product **49** (37 mg, 42%). Mp 238−239 °C; ^{1}H NMR ($CDCl_3$) δ 1.68−1.75 (m, 4H, 2 × CH_2), 2.58−2.76 (m, 8H, 4 × CH_2), 3.00−3.06 (m, 4H, 2 × CH_2), 3.69−3.73 ((m, 4H, 2 × CH_2), 6.82 (m, 1H, ArH), 6.90−7.00 (m, 2H, 2 × ArH), 7.44 (d, 1H, J = 8.6 Hz, ArH), 7.80 (d, 1H, J = 8.6 Hz, ArH), 8.56 (s, 1H, PymH), 8.63 (s, 1H, ArH), 8.88 (s, 1H, NH); MS *m*/*z* 527.4 (M + H)$^+$.

5-Chloro-1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluorene (50). 50 was synthesized from 2-chloro-6-fluorophenylnitrile according to Scheme 2 and a general protocol.[2] ^{1}H NMR (DMSO-d_6) δ 2.65 (m, 6H, 3 × CH_2), 2.75 (t, 2H, CH_2), 3.75 (t, 4H, 2 × CH_2), 7.05 (m, 1H, ArH), 7.21 (d, 1H, J = 6.5 Hz, ArH), 7.30 (m, 2H, ArH), 7.50 (dd, 1H, J = 6.5 Hz, 6.5 Hz, ArH), 7.55 (d, 1H, J = 8.0 Hz, ArH), 8.50 (s, 1H, PymH), 11.60 (s, 1H, NH); MS *m*/*z* 428.1 (M + H)$^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluorene-5-carbonitrile (51). 51 was synthesized from 3-amino-4-cyanoindole-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-ethyl ester (**16**) according to the general protocol.[2] Mp 233−234 °C; ^{1}H NMR ($CDCl_3$/MeOD-d_4) δ 2.75−2.88 (m, 6H, 3 × CH_2), 2.90−3.00 (m, 2H, CH_2), 4.00−4.08 (m, 4H, 2 × CH_2), 7.00−7.04 (m, 1H, ArH), 7.12−7.20 (m, 2H, 2 × ArH), 7.63 (t, 1H, J = 7.7 Hz, ArH), 7.2 (d, 1H, J = 8.3 Hz, ArH), 7.89 (d, 1H, J = 8.3 Hz, ArH), 8.80 (s, 1H,); MS *m*/*z* 419 (M + H)$^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluorene-5-carboxylic Acid Amide (52). A mix-

ture of compound **51** (0.5 g, 1.19 mmol), sodium hydroxide solution (1 M, 1.2 mL), and 30% hydrogen peroxide solution (0.7 mL) was stirred in methanol (30 mL) for 3 days. Saturated sodium carbonate solution was added, resulting in a precipitate, which was collected by filtration to yield a crude product that was purified using flash chromatography to yield the title compound (0.475 g, 90%). Mp 274−276 °C; ^{1}H NMR (DMSO-d_6) δ 2.54−2.68 (m, 6H, 3 × CH_2), 2.72−2.80 (m, 2H, CH_2), 3.28−3.35 (m, 4H, 2 × CH_2), 7.04−7.10 (m, 1H, ArH), 7.25−7.34 (m, 2H, 2 × ArH), 7.60 (t, 1H, J = 7.8 Hz, ArH), 7.72 (s, br, 1H, NH), 7.80 (d, 1H, J = 7.8 Hz, ArH), 8.10 (d, 1H, J = 7.8 Hz, ArH), 8.50 (s, 1H, PymH), 11.70 (s br, 1H, NH), 11.72 (s br, 1H, NH); MS m/z 437 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluorene-5-carboxylic Acid Pyridin-4-ylamide (53). 1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluorene-5-carboxylic acid amide **52** (100 mg, 0.28 mmol) and 4-bromopyridine hydrochloride (1.1 equiv, 49 mg) were suspended in 1,4-dioxane (1.5 mL). To this was added cesium carbonate (2.5 equiv, 185 mg), $Pd_2(dba)_3$ (2 mol % Pd, 0.0023 mmol, 2 mg), and xanthphos (1.5 equiv to Pd, 0.0035 mmol, 2 mg). After 3 days at 100 °C, the reaction mixture was diluted with DCM, washed with water (×2), and dried (Na_2SO_4), and the solvent was removed in vacuo to yield a residue, which was purified by column chromatography to give the title compound as a light-yellow solid (16 mg, 11%). Mp 262−263 °C; ^{1}H NMR ($CDCl_3$/MeOD-d_4) δ 2.45−2.52 (m, 6H, 3 × CH_2), 2.60−2.63 (m, 2H, CH_2), 3.75−3.80 (m, 4H, 2 × CH_2), 6.68−6.71 (m, 1H, ArH), 6.80−6.95 (m, 2H, 2 × ArH), 7.45 (t, 1H, J = 8.0 Hz, ArH), 7.60 (d, 1H, J = 8.2 Hz, ArH), 7.78 (d, 2H, J = 5.0 Hz, ArH), 8.18 (d, 1H, J = 7.5 Hz, ArH), 8.25 (d, 2H, J = 6.5 Hz, ArH), 8.45 (s, 1H,PymH), 14.95 (s br, 1H, CONH); MS m/z 514 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9*H*-5-nitro-9*H*-2,4,9-triazafluorene (54). 54 was synthesized from compound **19** according to the standard protocols.[2,13] ^{1}H NMR (DMSO) δ 2.75−2.85 (m, 6H, 3 × CH_2), 2.95 (m, 2H, CH_2), 4.00 (m, 4H, 2 × CH_2), 7.26 (m, 1H, ArH), 7.50 (m, 2H, 2 × ArH), 7.84 (dd, 1H, J = 8.1 Hz, J = 7.5 Hz, ArH), 7.94 (d, 1H, J = 7.5 Hz, ArH), 8.14 (d, 1H, J = 8.1 Hz, ArH), 8.64(s, 1H, ArH), 12.14 (br s, 1H, NH); MS m/z 439.3 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,9-triazafluoren-5-ylamine (55). Compound **54** (70 mg, 0.16 mmol) was stirred in MeOH (4 mL) in the presence of 10% Pd/C (cat.) under H_2 balloon for 2 h. The mixture was filtered through a pad of Celite and evaporated to dryness to give the title compound **55** (30 mg, 46%). ^{1}H NMR ($CDCl_3$) δ 2.72−2.81 (m, 6H, 3 × CH_2), 2.89 (m, 2H, CH_2), 3.93 (br s, 4H, 2 × CH_2), 5.46 (br s, 2H, NH_2), 6.54 (d, 1H, J = 7.7 Hz, ArH), 6.86 (d,1H, J = 8.0 Hz, ArH), 7.01 (m, 1H, ArH), 7.17 (m, 2H, 2 × ArH), 7.38 (dd, 1H, J = 7.7 Hz, J = 8.0 Hz, ArH), 7.83 (br s, 1H, NH), 8.70 (s, 1H, ArH); MS m/z 409.3 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-7-methoxy-9*H*-2,4,9-triazafluorene (56). 56 was synthesized from compound **6** (when R = 7-MeO, R′ = H) according to general protocol.[2] ^{1}H NMR ($CDCl_3$) δ 2.70 (m, 2H, CH_2), 2.75 (m, 4H, 2 × CH_2), 2.85 (m, 2H, CH_2), 3.90 (m, 4H, 2 × CH_2), 3.98 (s, 3H, CH_3), 6.95 (m, 3H, 3 × ArH), 7.10 (m, 2H, ArH), 8.0 (s, 1H, NH), 8.18 (d, 1H, J = 8.5 Hz, ArH), 8.70 (s, 1H, PymH); MS m/z 424.4 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-7-methoxy-8-nitro-9*H*-2,4,9-triazafluorene (57). 57 was synthesized from 1-chloro-7-methoxy-9*H*-2,4,9-triazafluorene using the nitration method described for compound **44**. ^{1}H NMR (DMSO-d_6) δ 2.45 (m, 6H, 3 × CH_2), 2.70 (m, 2H, CH_2), 3.70 (m, 4H, 2 × CH_2), 3.90 (s, 3H, CH_3O), 6.90 (m, 1H, ArH), 7.12 (d, 1H, J = 8.9 Hz, ArH), 7.20 (m, 2H, ArH), 8.20 (d, 1H, J = 8.9 Hz, ArH), 8.38 (s, 1H, PymH); MS m/z 424.4 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-6-methoxy-5-nitro-9*H*-2,4,9-triazafluorene (58). 1-Chloro-6-methoxy-9*H*-2,4,9-triazafluorene (300 mg, 1.288 mmol) was nitrated (99%) (see **59**). The resulting 1-chloro-6-methoxy-5-nitro-9*H*-2,4,9-triazafluorene was then treated per the general protocol[2] to give **58** (83.7 mg, 66%). Mp 276−277 °C; ^{1}H NMR (DMSO-d_6) δ 2.63−2.70 (m, 6H, 3 × CH_2), 2.77−2.81 (m, 2H, CH_2), 3.78 (m, 4H, 2 × CH_2), 3.92 (s, 3H, CH_3), 7.10−7.15 (m, 1H, ArH), 7.23−7.32 (m, 2H, 2 × ArH), 7.52 (d, 1H, J = 8.9 Hz, ArH), 7.72 (d, 1H, J = 9.3 Hz, ArH), 8.32 (s, 1H, PymH), 11.52 (s, 1H, NH); MS m/z 469.3 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-3-morpholin-4-ylmethyl-6-nitro-9*H*-2,4,9-triazafluorene (59). To a solution of 3-chloromethyl-2,9-dihydro-2,4,9-triazafluoren-1-one **11** (when X = Cl, R = R′ = H) (502 mg, 2.15 mmol, 1.0 equiv) in concentrated sulfuric acid (2 mL) at 0 °C was added a solution of sodium nitrate (206.4 mg, 2.36 mmol, 1.1 equiv) in concentrated sulfuric acid (2 mL) and water (1 drop) dropwise. The resultant was stirred at 0 °C for 2 h before ice was added. The precipitate obtained was collected by filtration, washed with water, and dried to give 3-chloromethyl-6-nitro-2,9-dihydro-2,4,9-triazafluoren-1-one as a yellow solid (560 mg, 93%). ^{1}H NMR (DMSO-d_6) δ 4.62 (s, 2H, CH_2), 7.62 (d, 1H, J = 9.1 Hz, ArH), 8.24 (d, 1H, J = 9.2 Hz, ArH), 8.75 (s, 1H, ArH), 8.95 (s, 1H, NH), 9.07 (s, 1H, NH).

3-Chloromethyl-6-nitro-2,9-dihydro-2,4,9-triazafluoren-1-one (296 mg,1.06 mmol), morpholine (0.14 mL, 1.6 mmol, 1.5 equiv), and anhydrous sodium carbonate (99 mg, 0.94 mmol, 1.4 equiv) were refluxed in absolute ethanol (5 mL) for 1.5 h. The reaction mixture was cooled to room temperature, water was added, and the resultant was extracted into ethyl acetate. The organic layer was dried (Na_2SO_4), and the solvent was removed in vacuo. 3-Morpholin-4-ylmethyl-6-nitro-2,9-dihydro-2,4,9-triazafluoren-1-one (175 mg, 50%) was isolated and used without further purification. ^{1}H NMR ($CDCl_3$/MeOD-d_4) δ 2.59−2.62 (m, 4H, 2 × CH_2), 3.64 (s, 2H, CH_2), 3.74−3.77 (m, 4H, 2 × CH_2), 7.55 (d, 1H, J = 9.2 Hz, ArH), 8.32 (d, 1H, J = 9.2 Hz, ArH), 9.07 (s, 1H, ArH), 9.27 (s, 1H, NH), 9.38 (s, 1H, NH).

Following the general protocols,[2,3] the title compound **59** was obtained (34%). Mp 110−111 °C; ^{1}H NMR ($CDCl_3$) δ 2.48−2.68 (m, 12H, 6 × CH_2), 3.60−3.63 (m, 4H, 2 × CH_2), 3.77 (s, 2H, CH_2), 3.89−3.91 (m, 4H, 2 × CH_2), 6.78−6.80 (m, 1H, ArH), 6.86−6.98 (m, 2H, 2 × ArH), 7.29 (d, 1H, J = 9.1 Hz, ArH), 8.06 (d, 1H, J = 9.1 Hz, ArH), 8.92 (d, 1H, J = 2.0 Hz, ArH), 10.45 (s br, 1H, NH); MS m/z 538.4 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-3-morpholin-4-ylmethyl-6,8-dinitro-9*H*-2,4,9-triazafluorene (60). Compound **60** was prepared in an analogous manner to **59** except 3-chloromethyl-6,8-dinitro-2,9-dihydro-2,4,9-triazafluoren-1-one was prepared in 92% yield from 3-chloromethyl-2,9-dihydro-2,4,9-triazafluoren-1-one (2.32 g, 9.94 mmol) using 2.1 equiv of sodium nitrate (2.0 equiv, 20.23 mmol, 1.72 g) and stirring overnight at room temperature. ^{1}H NMR (DMSO-d_6) δ 4.84 (s, 2H, CH_2), 9.18 (s, 1H, ArH), 9.30 (s, 1H, ArH), 13.32 (s, br, 1H, NH), 13.45 (s, br, 1H, NH).

Subsequent displacement of chloride with morpholino group (as per **59**) and further reactions using general protocols[2,3] as above gave product **60** (80% overall yield). Mp 69−70 °C; ^{1}H NMR ($CDCl_3$) δ 2.60−2.68 (m, 10H, 5 × CH_2), 2.74−2.78 (m, 2H, CH_2), 3.73−3.75 (m, 4H, 2 × CH_2), 3.80 (s, 2H, CH_2), 3.91−3.93 (m, 4H, 2 × CH_2), 6.85−6.92 (m, 1H, ArH), 6.96−7.04 (m, 2H, 2 × ArH), 9.25 (d, 1H, J = 2.0 Hz, ArH), 9.51 (d, 1H, J = 2.0 Hz, ArH), 10.03 (s br, 1H, NH); MS m/z 583.4 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-5-nitro-9*H*-2,4,9-triazafluorene (61). Compound **61** was prepared by treatment of compound **54** with iodomethane under the general alkylation procedure. ^{1}H NMR ($CDCl_3$) δ 2.84 (m, 2H, CH_2), 2.92 (m, 4H, 2 × CH_2), 2.99 (m, 2H, CH_2), 3.75 (m, 4H, 2 × CH_2), 4.26 (s, 3H, CH_3), 7.11 (m, 1H, ArH), 7.24 (m, 2H, ArH), 7.85 (dd, 1H, J = 7.8 Hz, J = 8.1 Hz, ArH), 7.94 (d, 1H, J = 8.1 Hz, ArH), 8.07 (d, 1H, J = 7.8 Hz, ArH), 9.01 (s, 1H, ArH); MS m/z 453.1 $(M + H)^+$

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-5-nitro-9-phenethyl-9*H*-2,4,9-triazafluorene (62). Compound **62** was prepared by treatment of compound **54** with (2-bromoethyl)benzene following the general alkylation procedure. ^{1}H NMR ($CDCl_3$) δ 2.55 (m, 2H, CH_2), 2.66 (m, 4H, 2 ×

CH_2), 2.73 (m, 2H, CH_2), 3.34 (m, 6H, 3 × CH_2), 4.7 (t, 2H, J = 6.9 Hz, CH_2), 6.63 (m, 2H, 2 × ArH), 6.85 (m, 1H, ArH), 6.98 (m, 5H, 5 × ArH), 7.61 (dd, 1H, J = 8.2 Hz, J = 7.9 Hz, ArH), 7.74 (d, 1H, J = 8.2 Hz, ArH), 7.81 (d, 1H, J = 7.65, ArH), 8.69 (s, 1H, ArH); MS m/z 543.5 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-5-nitro-9-pyridin-3-ylmethyl-9*H*-2,4,9-triazafluorene (63). Compound **63** was prepared by treatment of compound **54** with 3-(iodomethyl)pyridine following the general alkylation procedure. ^{1}H NMR ($CDCl_3$) δ 2.56 (m, 6H, 3 × CH_2), 2.70 (m, 2H, CH_2), 5.71 (s, 2H, CH_2), 6.85 (m, 1H, ArH), 6.9−7.2 (m, 4H, 4 × ArH), 7.5−7.6 (m, 2H, ArH), 7.75 (m, 1H, ArH), 8.47 (m, 1H, ArH), 8.83 (s, 1H, ArH); MS m/z 530.5 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-5-nitro-9-pyridin-4-ylmethyl-9*H*-2,4,9-triazafluorene (64). Compound **64** was prepared by treatment of compound **54** with 4-(chloromethyl)pyridine in the presence of KI following the general alkylation procedure. ^{1}H NMR ($CDCl_3$) δ 2.53 (m, 6H, 3 × CH_2), 2.69 (m, 2H, CH_2), 3.37 (m, 4H, 2 × CH_2), 5.71 (s, 2H, CH_2), 6.68 (m, 3H, ArH and d, J = 4.5 Hz, 2 × PyH), 6.97 (m, 2H, ArH), 7.40 (d, 1H, J = 8.3 Hz, ArH), 7.53 (dd, 1H, J = 8.3 Hz, J = 7.3 Hz, ArH), 7.80 (d, 1H, J = 7.3 Hz, ArH), 8.48 (d, 2H, J = 4.5 Hz, PyH), 8.83 (s, 1H, ArH); MS m/z 530.5 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-9*H*-2,4,9-triazafluorene-5-carboxylic Acid Amide (65). Compound **65** was prepared by treatment of compound **51** with iodomethane following the general alkylation procedure and then hydrolysis with NaOH/MeOH following the procedure similar to that for **52**. Mp 234−235 °C; ^{1}H NMR ($CDCl_3$) δ 2.57 (m, 2H, CH_2), 2.66 (m, 4H, 2 × CH_2), 2.75 (m, 2H, CH_2), 3.43 (m, 4H, 2 × CH_2), 4.00 (s, 3H, CH_3), 7.06 (m, 1H, ArH), 7.22−7.37 (m, 2H, 2 × ArH), 7.74 (dd, 1H, J = 7.7 Hz, J = 8.1 Hz), 7.83 (br s, 1H, NH), 7.91 (d, 1H, J = 8.1 Hz, ArH), 8.14 (d, 1H, J = 7.8 Hz, ArH), 8.64 (s, 1H, ArH), 11.48 (br s, 1H, NH); MS m/z 451.1 $(M + H)^+$.

(5-Carbamoyl-1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-2,4,9-triazafluoren-9-yl)acetic Acid Ethyl Ester (66). Compound **66** was prepared by treatment of compound **52** with ethyl bromoacetate following the general alkylation procedure. ^{1}H NMR ($CDCl_3$) δ 1.12 (t, 3H, J = 6.9 Hz, CH_3), 2.52−2.68 (m, 6H, 3 × CH_2), 2.70−2.73 (m, 2H, CH_2), 3.36−3.43 (m, 4H, 2 × CH_2), 4.12 (q, 2H, J = 6.9 Hz, CH_2), 5.18 (s, 2H, CH_2), 6.05 (br s, 1H, NH), 6.82−6.87 (m, 1H, ArH), 6.93−7.01 (m, 2H, 2 × ArH), 7.41 (d, 1H, J = 7.5 Hz, ArH), 7.64 (t, 1H, J = 7.5 Hz, ArH), 8.40 (d, 1H, J = 7.5 Hz, ArH), 8.69 (s, 1H, ArH), 12.15 (br s, 1H, NH); MS m/z 523.0 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-pyridin-4-ylmethyl-9*H*-2,4,9-triazafluorene-5-carboxylic Acid Amide (67). Compound **67** was prepared by treatment of compound **52** with 4-(chloromethyl)pyridine in the presence of KI following the general alkylation procedure. ^{1}H NMR ($CDCl_3$) δ 2.50−2.58 (m, 6H, 3 × CH_2), 2.68−2.71 (m, 2H, CH_2), 3.37−3.41 (m, 4H, 2 × CH_2), 5.70 (s, 2H, CH_2), 6.10 (br s, 1H, NH), 6.82−6.86 (m, 3H, 3 × ArH), 6.95−7.05 (m, 2H, 2 × ArH), 7.38 (d, 1H, J = 8.2 Hz, ArH), 7.60 (t, 1H, J = 8.2 Hz), 8.42 (d, 1H, J = 8.2 Hz, ArH), 8.46 (d, 2H, J = 4.5 Hz, 2 × ArH), 8.72 (s, 1H, ArH), 12.24 (br s, 1H, NH); MS m/z 528.5 $(M + H)^+$.

(1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-6-nitro-2,4,9-triazafluoren-9-yl)acetic Acid Ethyl Ester (68). Compound **68** was prepared by treatment of the 6-nitro derivative of compound **6** (R = 6-NO_2, R′ = H) with ethyl bromoacetate following the general alkylation procedure and subsequent coupling with **13** using the general protocol.[2] ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, J = 6.9 Hz, CH_3), 2.70−2.85 (m, 8H, 4 × CH_2), 3.50 (m, 4H, 2 × CH_2), 4.25 (q, 2H, J = 6.9 Hz, CH_2), 5.25 (s, 2H, CH_2), 6.95 (m, 1H, ArH), 7.10 (m, 2H, ArH), 7.40 (d, 1H, J = 9.1 Hz, ArH), 8.50 (dd, 1H, J = 9.1 Hz, 2.2 Hz, ArH), 8.90 (s, 1H, PrmH), 9.30 (d, 1H, J = 2.2 Hz, ArH); MS m/z 525.5 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-3-morpholin-4-ylmethyl-9*H*-2,4,9-triazafluorene-5-carboxylic Acid Amide (69). Compound **69** was prepared by treatment of the 5-cyano-3-methylmorpholine derivative of compound **12** (R = 5-NO_2, R′ = CH_3), followed by NaOH/MeOH hydrolysis. ^{1}H NMR (acetone-d_6) δ 2.62−2.89 (m, 12H, 6 × CH_2, including HOD), 3.60 (m, 4H, 2 × CH_2), 3.66−3.68 (m, 4H, 2 × CH_2), 3.84 (s, 2H, CH_2), 4.14 (s, 3H, CH_3), 7.13 (m, H, ArH), 7.19−7.31 (m, 2H, 2 × ArH), 7.75 (t, H, ArH), 7.88 (d, H, J = 8.3 Hz, ArH), 8.33 (d, H, ArH, J = 7.5 Hz); MS m/z 550.5 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-9*H*-2,4,6,9-tetraazafluorene (70). 70 was synthesized from compound **22** using the general protocols.[2,13] ^{1}H NMR ($CDCl_3$) δ 2.65 (m, 8H, 4 × CH_2), 3.41 (br s, 4H, 2 × CH_2), 3.91 (s, 3H, CH_3), 6.94 (m, 3H, ArH), 7.27 (d, 1H, J = 5.9 Hz, PyH), 8.58 (d, 1H, J = 5.9 Hz, PyH), 8.9 (s, 1H, PymH), 9.47 (s, 1H, PyH); MS m/z 409.4 $(M + H)^+$.

9-Benzyl-1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-9*H*-2,4,6,9-tetraazafluorene (71). 71 was synthesized as **70**. ^{1}H NMR ($CDCl_3$) δ 2.50 (m, 6H, 3 × CH_2), 2.65 (m, 2H, CH_2), 3.35 (m, 4H, 2 × CH_2), 5.58 (s, 2H, CH_2), 6.75 (m, 1H, ArH), 6.90 (m, 4H, ArH), 7.10 (d, 1H, J = 5.9 Hz, PyH), 7.15 (m, 3H, ArH), 8.45 (d, 1H, J = 5.9 Hz, PyH,), 8.70 (s, 1H, PymH), 9.45 (s, 1H, PyH); MS m/z 485.4 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-5-methoxy-9-methyl-9*H*-2,4,6,9-tetraazafluorene (72). Sodium (0.021 g, 0.91 mmol) was dissolved in MeOH (5 mL), to which was added compound **29** (0.06 g, 0.135 mmol). The mixture was then heated at reflux for 2 h. The solvent was removed under vacuum, water was added to the residue, and the resulting solid was filtered and dried to give the title compound **72** (0.0409 g, 69% yield). ^{1}H NMR ($CDCl_3$) δ 2.60 (m, 2H, CH_2), 2.67 (m, 4H, 2 × CH_2), 2.72 (m, 2H, CH_2), 3.40 (m, 4H, 2 × CH_2), 3.95 (s, 3H, NCH_3), 4.20 (s, 3H, CH_3O), 6.88 (m, 1H, ArH), 6.92 (d, 1H, J = 6.0 Hz, PyH), 7.0 (m, 2H, ArH), 8.15 (d, 1H, J = 6.0 Hz, PyH), 8.78 (1H, s, PymH); MS (DCI/NH_3) m/z 439.1 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-5-isopropoxy-9-methyl-9*H*-2,4,6,9-tetraazafluorene (73). 73 was synthesized as **72**. ^{1}H NMR ($CDCl_3$) δ 1.50 (d, 6H, J = 6.2 Hz, 2 × CH_3), 2.60 (m, 2H, CH_2), 2.65 (m, 4H, 2 × CH_2), 2.72 (m, 2H, CH_2), 3.40 (m, 4H, 2 × CH_2), 3.92 (s, 3H, CH_3), 5.60 (m, 1H, CH), 6.82 (m, 1H, ArH), 6.85 (d, 1H, J = 6.0 Hz, PyH), 7.0 (m, 2H, ArH), 8.12 (d, 1H, J = 6.0 Hz, PyH), 8.80 (s, 1H, PymH); MS (DCI/NH_3) m/z 467.5 $(M + H)^+$.

General Procedure for Reactions of Amines with 29 (Scheme 3, Step m). Preparation of 74−77. To the amine (10 equiv) in *n*-butanol (2 mL) (gaseous amines were presaturated in *n*-butanol) was added 5-chloro-1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-9*H*-2,4,6,9-tetraazafluorene (29) (50 mg, 0.11 mmol) and triethylamine (0.023 mL, 0.165 mmol, 1.5 equiv). The reaction mixture was heated at 100 °C for 48 h. After the mixture was cooled to room temperature, volatiles were removed in vacuo. The residue was partitioned between water (60 mL) and chloroform (3 × 80 mL). The combined organic layers were washed with brine (50 mL), separated, and dried ($MgSO_4$). The residue was either triturated or columned to give target compounds in 34−98% yields.

(1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-9*H*-2,4,6,9-tetraazafluoren-5-yl)dimethylamine (74). This compound was prepared using the general method above. The crude material was purified by chromatography (5% → 10% methanol in chloroform) to give the title compound as a white solid (38 mg, 74%). Mp 166−168 °C; ^{1}H NMR ($CDCl_3$) δ 2.68 (t, 2H, J = 6.3 Hz, CH_2), 2.75 (br s, 4H, 2 × CH_2), 2.82 (t, 2H, J = 7.7 Hz, CH_2), 3.3 (s, 6H, 2 × NCH_3), 3.50 (br s, 4H, 2 × CH_2), 3.96 (s, 3H, CH_3), 6.73 (d, 1H, J = 6.1 Hz, CH), 6.89 (m, 1H, ArH), 7.11−7.00 (m, 2H, ArH), 8.2 (d, 1H, J = 6.3 Hz, PyH), 8.7 (s, 1H, PymH); MS (DCI/NH_3) m/z 452.1 $(M + H)^+$.

(1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-9*H*-2,4,6,9-tetraazafluoren-5-yl)methylamine (75). This compound was prepared using the general method above. The crude material was purified by trituration with diethyl ether to give the title compound as a white solid (42 mg, 85%). Mp 166−168 °C; ^{1}H NMR ($CDCl_3$) δ 2.68 (t, 2H, J = 6.3 Hz,

CH_2), 2.75 (br s, 4H, 2 × CH_2), 2.82 (t, 2H, J = 7.7 Hz, CH_2), 3.15 (d, 3H, J = 5.1 Hz, NCH_3), 3.40 (br s, 4H, 2 × CH_2), 3.96 (s, 3H, NCH_3), 6.6 (d, 1H, J = 6.2 Hz, PyH), 6.7 (m, 1H, NH), 6.9 (m, 1H, ArH), 7.0−7.11 (m, 2H, ArH), 8.2 (d, 1H, J = 6.0 Hz, PyH), 8.65 (s, 1H, PymH); MS (DCI/NH_3) m/z 438.3 $(M + H)^+$.

(1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-9*H*2,4,6,9-tetraazafluoren-5-yl)pyridin-2-ylmethylamine (76). This compound was prepared using the general method above. The crude material was purified by chromatography (4% methanol in chloroform) to give the title compound as a white solid (20 mg, 34%). Mp 174−176 °C; ^{1}H NMR ($CDCl_3$) δ 2.68 (t, 2H, J = 6.3 Hz, CH_2), 2.75 (br s, 4H, 2 × CH_2), 2.82 (t, 2H, J = 7.7 Hz, CH_2), 3.40 (br s, 4H, 2 × CH_2), 3.96 (s, 3H, NCH_3), 5.08 (d, 2H, J = 5.9 Hz, CH_2), 6.68 (d, 1H, J = 5.9 Hz, PyH), 6.9 (m, 1H, ArH), 6.98−7.11 (m, 2H, ArH), 7.12−7.21 (m, 1H, PyH), 7.4 (d, 1H, J = 7.9 Hz, PyH), 7.6 (t, 2H, J = 5.9 Hz, PyH), 8.17 (d, 1H, J = 6.4 Hz, PyH), 8.63 (d, 1H, J = 5.9 Hz, PyH), 8.7 (s, 1H, PymH); MS (DCI/NH_3) m/z 515.4 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-5-morpholin-4-yl-9*H*-2,4,6,9-tetraazafluorene (77). This compound was prepared using the general method above. The crude material was purified by chromatography (4% methanol in chloroform) to give the title compound as a white solid (35 mg, 62%). Mp 138−140 °C; ^{1}H NMR ($CDCl_3$) δ 2.68 (t, 2H, J = 6.3 Hz, CH_2), 2.75 (br s, 4H, 2 × CH_2), 2.82 (t, 2H, J = 7.7 Hz, CH_2), 3.40 (br s, 4H, 2 × CH_2), 3.85 (br s, 4H, 2 × CH_2), 3.96 (s, 3H, NCH_3), 4.01 (m, 4H, 2 × CH_2), 6.85 (d, 1H, J = 5.9 Hz, PyH), 6.9 (m, 1H, ArH), 6.98−7.11 (m, 2H, ArH), 8.2 (d, 1H, J = 5.9 Hz, PyH), 8.65 (s, 1H, PymH); MS (DCI/NH_3) m/z 494.5 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-5,9-dimethyl-9*H*-2,4,6,9-tetraazafluorene (78). To a solution of 5-chloro-1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-9*H*-2,4,6,9-tetraazafluorene **29** (50 mg, 0.11 mmol) in THF (1.5 mL) was added palladium tetrakistriphenylphosphine (5 mol %, 6.5 mg) followed by methylzinc chloride (0.34 mL, 0.68 mmol, 6 equiv, 2.0 M solution in THF). The solution was heated at reflux for 24 h and then poured into an aqueous solution of EDTA (300 mg) in water (10 mL), and the pH was adjusted to 7 by addition of potassium carbonate powder. The solution was extracted with ethyl acetate (3 × 20 mL), the combined organic layers were separated and dried ($MgSO_4$), and the solvent was removed in vacuo. The crude material was purified by chromatography (5% → 10% methanol in chloroform) to give the title compound as a white solid (41 mg, 85%). Mp 168−170 °C; ^{1}H NMR ($CDCl_3$) δ 2.59 (m, 2H, CH_2), 2.68 (br, s, 4H, 2 × CH_2), 2.75 (t, 2H, J = 7.7 Hz, CH_2), 3.14 (s, 3H, CH_3), 3.45 (br, s, 4H, 2 × CH_2), 3.94 (s, 3H, NCH_3), 6.86 (m, 1H, ArH), 6.99 (m, 2H, ArH), 7.15 (d, 1H, J = 5.9 Hz, PyH), 8.48 (d, 1H, J = 5.9 Hz, PyH), 8.76 (s, 1H, PymH); MS (DCI/NH_3) m/z 423 $(M + H)^+$.

7-Chloro-1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-9*H*-2,4,6,9-tetraazafluorene (79). This compound was prepared from **27a** using the same method as for **29**. ^{1}H NMR ($CDCl_3$) δ 2.59 (m, 2H, CH_2), 2.67 (br s, 2H, CH_2), 2.75 (br s, 4H, 2 × CH_2), 3.47 (br s, 4H, 2 × CH_2), 3.92 (s, 3H, CH_3), 6.87 (m, 1H, ArH), 7.04−6.93 (m, 2H, ArH), 7.34 (s, 1H, PyH), 8.70 (s, 1H, PrmH), 9.23 (s, 1H, PyH); MS (DCI/NH_3) m/z 442 $(M + H)^+$.

5-Chloro-1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-3-methoxymethyl-9-methyl-9*H*-2,4,6,9-tetraazafluorene (80). 80 was synthesized from compound **25** using the literature protocol.[3] ^{1}H NMR ($CDCl_3$) δ 2.60 (m, 2H, CH_2), 2.70 (m, 4H, 2 × CH_2), 2.80 (m, 2H, CH_2), 3.50 (m, 4H, 2 × CH_2), 3.58 (s, 3H, CH_3O), 4.0 (s, 3H, CH_3N), 4.75 (s, 2H, CH_2), 6.90 (m, 1H, ArH), 7.05 (m, 2H, ArH), 7.30 (d, 1H, J = 5.8 Hz, PyH), 8.40 (d, 1H, J = 5.8 Hz, PyH); MS m/z 488.1 $(M + H)^+$

5-Chloro-1-{4-[2-(3,4-difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-3-morpholin-4-ylmethyl-9*H*-2,4,6,9-tetraazafluorene (81). 81 was synthesized from **25** using the literature protocol.[3] ^{1}H NMR ($CDCl_3$) δ 2.51 (m, 2H, CH_2), 2.60 (m, 4H, 2 × CH_2), 2.65 (m, 2H, CH_2), 2.70 (m, 4H, 2 × CH_2), 3.40 (m, 4H, 2 × CH_2), 3.70 (m, 4H, 2 × CH_2), 3.80 (s, 2H, CH_2), 3.85 (s, 3H, CH_3), 6.70 (m, 1H, ArH), 6.90 (m, 2H, ArH), 7.18 (d, 1H, J = 5.88 Hz, PyH), 8.30 (d, 1H, J = 5.88 Hz, PyH); MS m/z 543.2 $(M + H)^+$.

1-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-9*H*-2,4,5,9-tetraazafluorene (82). 82 was prepared from compound **36** by standard protocols described in Schemes 2 and 4 and in the literature.[2,13] Mp 195−197 °C; ^{1}H NMR (MeOD-d_4) δ 2.86 (m, 4H, 2 × CH_2), 2.96 (m, 4H, 2 × CH_2), 3.63 (m, 4H, 2 × CH_2), 3.97 (s, 3H, CH_3), 7.05 (m, 1H, ArH), 7.10 (m, 2H, 2 × ArH), 7.54 (dd, 1H, J = 4.5 Hz, J = 8.4 Hz, ArH), 8.04 (d, 1H, J = 8.4 Hz, ArH), 8.35 (d, 1H, J = 4.5 Hz, ArH), 8.58 (s, 1H, ArH); MS m/z 409.4 $(M + H)^+$.

8-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-9*H*-1,5,7,9-tetraazafluorene-4-carbonitrile (83). [(3,4-Dicyanopyridin-2-yl)methylamino]acetic acid ethyl ester (**39**)[11] was prepared from 2-chloro-3,4-dicyanopyridine (**38**)[10] (2.8 g, 17.09 mmol). A mixture of the above ester **39** and cesium carbonate (3.07 g, 2.0 equiv) was heated in dry acetonitrile (20 mL) at 50 °C. After 2 h, ice was added and the orange precipitate that formed was collected, washed with water, and dried to give 3-amino-4-cyano-1-methyl-1*H*-pyrrole[2,3-*b*]pyridine-2-carboxylic acid ethyl ester **40** (1.07 g, 4.36 mmol, 92%). Treatment of **40** according to general protocols[2,13] gave the title compound **83** (347 mg, 67%). Mp 180−181 °C; ^{1}H NMR ($CDCl_3$) δ 2.49−2.53 (m, 2H, CH_2), 2.59 (m, 4H, 2 × CH_2), 2.63−2.67 (m, 2H, CH_2), 3.41 (m, 4H, 2 × CH_2), 3.97 (s, 3H, CH_3), 6.74−6.77 (m, H, ArH), 6.85−6.93 (m, 2H, 2 × ArH), 7.37 (d, H, J = 4.9 Hz, ArH,), 8.61 (d, H, J = 4.9 Hz, ArH,), 8.70 (s, H, ArH); MS m/z 435 $(M + H)^+$.

8-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-2,9-dimethyl-9*H*-1,5,7,9-tetraazafluorene-4-carbonitrile (84). 84 was synthesized as **83**. Mp 178−179 °C; ^{1}H NMR ($CDCl_3$/D_2O) δ 2.66−2.83 (m, 11H, CH_3 + 4 × CH_2), 3.55 (m, 4H, 2 × CH_2), 4.10 (s, 3H, CH_3), 4.72 (s br, 2H, NH_2), 6.93 (m, H, ArH), 7.02−7.10 (m, 2H, 2 × ArH), 7.40 (s, H, ArH), 8.85 (s, H, ArH); MS m/z 448 $(M + H)^+$.

8-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-2,9-dimethyl-9*H*-1,5,7,9-tetraazafluorene-4-carboxylic Acid Amide (85). 85 was synthesized from **84** using the procedure described for **52**. Mp 259−260 °C; ^{1}H NMR ($CDCl_3$) δ 2.57−2.63 (m, 2H, CH_2), 2.70−2.89 (m, 9H, 3 × CH_2 + CH_3), 3.50 (m, 4H, 2 × CH_2), 4.05 (s, 3H, CH_3), 6.17 (s, br, H, NH), 6.84−6.86 (m, H, ArH), 6.94−7.03 (m, 2H, 2 × ArH), 8.00 (s, H, ArH), 8.61 (s, H, ArH), 11.97 (s, H, NH); MS m/z 466 $(M + H)^+$.

8-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-9*H*-1,5,7,9-tetraazafluorene-4-carboxylic Acid Amide (86). 86 was synthesized from **83** using the procedure described for **52.** Mp 253−254 °C; ^{1}H NMR ($CDCl_3$) δ 2.60−2.63 (m, 2H, CH_2), 2.70 (m, 4H, 2 × CH_2), 2.73−2.77 (m, 2H, CH_2), 3.53 (m, 4H, 2 × CH_2), 4.09 (s, 3H, CH_3), 66.12 (s, br, H, NH), 6.84−6.87 (m, H, ArH), 6.97−7.03 (m, 2H, 2 × ArH), 8.15 (d, H, J = 5.0 Hz, ArH), 8.64 (s, H, NH), 8.77 (d, H, J = 5.0 Hz, ArH), 12.01 (s, H, NH); MS m/z 452 $(M + H)^+$.

8-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-2,9-dimethyl-3-nitro-9*H*-1,5,7,9-tetraazafluorene-4-carboxylic Acid Amide (87). 87 was prepared from 2-chloro-6-methyl-5-nitropyridine-3,4-dicarbonitrile in a manner analogous to that of **83**, followed by the procedure for **52** (46 mg, 70%). Mp 217−219 °C; ^{1}H NMR ($CDCl_3$) δ 2.60−2.64 (m, 2H, CH_2), 2.68 (s, 3H, CH_3), 2.68−2.72 (m, 4H, 2 × CH_2), 2.74−2.78 (m, 2H, CH_2), 3.50−3.55 (m, 4H, 2 × CH_2), 4.10 (s, 3H, CH_3), 6.10 (s, br, 1H, NH), 6.83−6.88 (m, 1H, ArH), 6.98−7.04 (m, 2H, 2 × ArH), 8.67 (s, 1H, ArH), 11.67 (br s, 1H, NH); MS m/z 511 $(M + H)^+$.

8-{4-[2-(3,4-Difluorophenyl)ethyl]piperazin-1-yl}-9-methyl-6-morpholin-4-ylmethyl-9*H*-1,5,7,9-tetraazafluorene-4-carboxylic Acid Amide (88). 88 was prepared from compound **40** (Scheme 5) according to standard protocol.[3] Mp 195−196 °C; ^{1}H NMR ($CDCl_3$) δ 2.56−2.63 (m, 6H, 3 × CH_2), 2.69 (m, 4H, 2 × CH_2), 2.73−2.73 (m, 2H, CH_2), 3.52 (m, 4H, 2 × CH_2), 3.68−3.70 (m, 4H, 2 × CH_2), 3.81 (s, 2H, CH_2), 4.04 (s, 3H, CH_3), 6.12 (s, br, H, NH), 6.84−6.86 (m, H, ArH), 6.95−

7.03 (m, 2H, 2 × ArH), 8.13 (d, H, J = 5.0 Hz, ArH), 8.75 (d, H, J = 5.0 Hz, ArH), 12.27 (br s, H, NH); MS m/z 551 $(M + H)^+$.

Biology Assays.[2] In Vitro Assays. Drug accumulation assays (MRP1 and Pgp) have been described and referenced in detail in our previous communication.[2]

CYP 3A4 Inhibition Assay. This was carried out according to a literature protocol.[2,15]

Single-Dose Potentiation Assay (MRP1). The ability of modulators to potentiate drug cytotoxicity in COR.L23 cells was assessed at a single dose of the cytotoxic drug doxorubicin, selected to provide a differential window of activity between the parental cell line COR.L23/P (80−100% growth inhibitory activity) and the drug-selected resistant cell line COR.L23/R (0−10% growth inhibitory activity). Addition of the MRP-1 modulator to the resistant cell line effected a dose-dependent potentiation of drug cytotoxicity to levels observed with the parental cell line.

Briefly, cells were seeded into 96-well plates at (2−4) × 10^2 cells per 100 μL/well and incubated for 2 h at 37 °C. Varying concentrations of modulator (or solvent control) were subsequently added at 50 μL/well, in quadruplicate for each concentration, and incubated for an additional hour before addition of 50 μL/well doxorubicin to a final concentration of 100 nM. After incubation for 5 days, cell proliferation was assessed by addition of 10 μL/well alamarBlue. Viable cell number, proportional to fluorescence (excitation λ 560/15 nm; emission λ 590/35 nm), was determined after further incubation for 5 h. IC_{50} values were calculated for the modulator in the presence of a single dose of cytotoxic, using media-only wells as background and untreated, resistant cells as being representative of 100% viability.

Similar protocols were applied for Pgp single-dose potentiation assays with the use of Pgp expressing murine mammary carcinoma EMT6/AR1.0 subline.

Pharmacokinetic Studies and in Vivo Efficacy Studies. Protocols for these studies have been described in detail in our early communication.[2]

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