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ARTICLE

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Synthesis and Biological Evaluation of Novel Analogues of the Pan Class I Phosphatidylinositol 3-Kinase (PI3K) Inhibitor 2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (ZSTK474)

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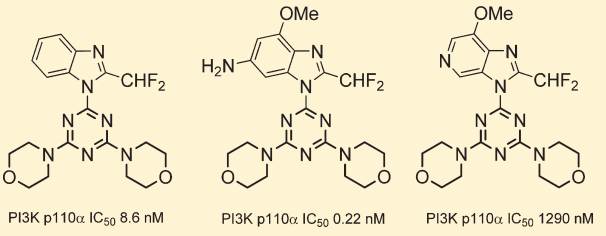
ABSTRACT: A structure−activity relationship (SAR) study of the pan class I PI 3-kinase inhibitor 2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (ZSTK474) identified substitution at the 4 and 6 positions of the benzimidazole ring as having significant effects on the potency of substituted derivatives. The 6-amino-4-methoxy analogue displayed a greater than 1000-fold potency enhancement over the corresponding 6-aza-4-methoxy analogue against all three class Ia PI 3-kinase enzymes (p110α, p110β, and p110δ) and also displayed significant potency against two mutant forms of the p110α isoform (H1047R and E545K). This compound was also evaluated in vivo against a U87MG human glioblastoma tumor xenograft model in Rag1$^{-/-}$ mice, and at a dose of 50 mg/kg given by ip injection at a qd × 10 dosing schedule it dramatically reduced cancer growth by 81% compared to untreated controls.



INTRODUCTION

Phosphoinositide 3-kinases (PI3Ks) are a family of three distinct classes (I, II, and III) of lipid kinases that play key roles in cell and tissue physiology.[1−3] The three class-Ia PI 3-kinases (p110$\alpha/\beta/\delta$) and the sole class-Ib PI 3-kinase (p110γ) couple growth factor receptors and G-protein-coupled receptors, respectively, to a wide range of downstream pathways.[4−6] These enzymes have different methods of activation and different kinetic properties,[7] but all use phosphatidylinositol 4,5-diphosphate (PIP2) to produce phosphatidylinositol 3,4,5-triphosphate (PIP3). The cellular levels of PIP3 are tightly controlled by phosphatases including PTEN which dephosphorylates PIP3 back to PIP2.[8,9] The importance of this pathway in cancer is highlighted by the fact that defects in both the kinase and phosphatase activities are commonly observed in tumors. PTEN is a tumor suppressor gene whose function is commonly lost in tumors,[8−10] while the PIK3CA gene that codes for p110α is commonly mutated in many cancers,[11,12] and there is now increasing evidence that a high proportion of human cancers depend strongly on p110α for their survival and resistance to therapy.[13−15] Therefore, the targeting of PI3K with small molecule inhibitors is one of the most promising new approaches to cancer treatment, and a number of programs to develop PI3K inhibitors are currently in progress,[5,16−19] with several inhibitors in clinical trial.[20−24]

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (ZSTK474) (**1**, Figure 1) is a potent ATP-competitive pan class I PI3K inhibitor, with high selectivity over other classes of PI3K and protein kinases.[25−29] It has demonstrated antitumor activity in vivo against human tumor xenografts[25,28−30] and is reported to be in phase I clinical trial,[31] despite its poor aqueous solubility which has required the development of an amorphous formulation containing a solid dispersion of **1**.[32] The crystal structure of **1** in complex with p110δ has been obtained[33] and shows that the oxygen of one of the morpholino groups is positioned as a hydrogen bond acceptor from the hinge residue Val828, with the morpholino ring adopting a chair conformation. The benzimidazole group extends into the affinity pocket where its nitrogen acts as a hydrogen bond acceptor for the primary amine of Lys779. The difluoromethyl group points toward Pro758 in the upper wall of the hydrophobic affinity pocket. The second morpholino group

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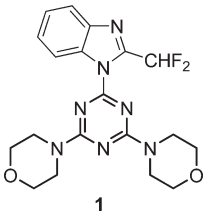

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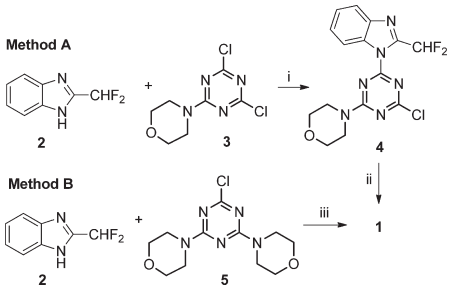
Figure 1. Structure of **1**.

Scheme 1. Synthesis of 1[a]



[a] Reagents and conditions: (i) K_2CO_3, DMF, room temp; (ii) morpholine, DMF or THF, room temp; (iii) NaH or K_2CO_3, DMF or DMSO, 120 °C.

adopts a somewhat twisted chair conformation and projects out of the ATP-binding pocket. This binding mode is flipped over relative to that originally predicted in a computational model of **1** bound to p110γ.[25]

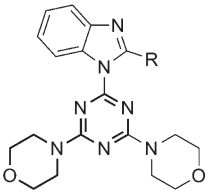
However, despite the large amount of data now available on **1**, apart from two bicyclic morpholino derivatives[34] and some early 2-unsubstituted analogues,[35] very little information regarding related analogues is currently available outside of the patent literature. In this paper we explore the structure−activity relationships for this promising series, focusing initially on changes to the benzimidazole portion of the molecule, with one of the aims being the identification of suitable changes that might lead to more soluble analogues.

CHEMISTRY

The synthesis of **1** can be performed by two main routes (Scheme 1). Method A involves the room temperature reaction of 2-(difluoromethyl)benzimidazole (**2**)[36] with 2,4-dichloro-6-morpholino-1,3,5-triazine (**3**)[37] to give the monochloro intermediate **4**[38] which is then reacted with morpholine to give **1**. Method B is a shorter procedure, involving direct reaction of **2** with 2-chloro-4,6-dimorpholino-1,3,5-triazine (**5**)[39] to give **1**, although higher temperatures are required. In our hands method B was found to be superior for the synthesis of **1**, since in addition to the shorter route it also gave a higher yield, although the same was not always true for substituted benzimidazoles where method A was often superior.

Both of the methods described in Scheme 1 were employed to produce the 2-substituted analogues **7a**−**j** of Table 1 (Scheme 2), although in the case of the 2-hydroxymethyl derivative **7e**, it was necessary to use a TBDMS protecting group to prevent concurrent ether formation. Sulfoxide **7g** and sulfones **7h** and **7j** were most efficiently prepared by oxidation of the precursor sulfides **7f** and **7i**.

Table 1. Inhibition of PI3K Isoforms and Cell Proliferation for 2-Substituted Analogues of 1



		PI3K IC_{50}[a] (nM)			cell line IC_{50}[b] (μM)	
compd	R	p110α	p110β	p110δ	NZB5[c]	NZOV9[d]
1[e]	CHF_2	8.6	4.8	0.7	0.22	0.29
7a[f]	H	265	1540	142	1.5	0.3
7b[g]	Me	143	289	35	0.28	0.6
7c	CH_2F	34	53	13	0.39	0.35
7d	CF_3	202	461	70	0.9	0.7
7e[g]	CH_2OH	$>10^3$	$>10^3$	5470	0.75	5.5
7f	CH_2SMe	170	39	41	0.083	0.086
7g	CH_2SOMe	590	230	35	0.40	0.49
7h	CH_2SO_2Me	480	250	54	0.25	0.44
7i	SMe	2900	$>10^3$	270	0.2	0.2
7j	SO_2Me	203	383	76	10	2

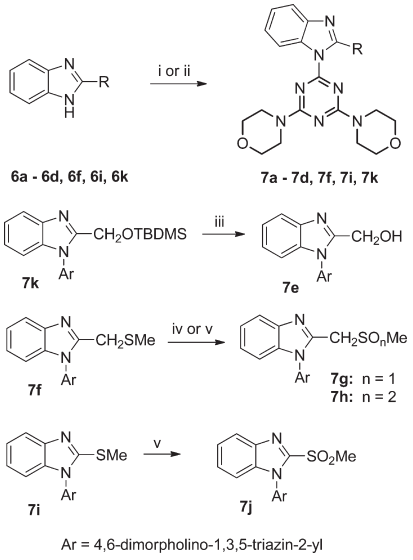
[a] Compound concentration required to produce 50% inhibition of phosphorylation in an in vitro lipid kinase assay with conditions as previously described in ref 50. [b] Compound concentration required to produce 50% inhibition of cell growth in an in vitro cell proliferation assay with conditions as previously described in ref 69. [c] Cell line derived from a medulloblastoma and expressing the wild-type gene for p110α. [d] Cell line derived from a poorly differentiated endometrioid adenocarcinoma of the ovary and expressing a mutant p110α enzyme with a single amino acid substitution in the kinase domain (Y1021C). [e] Reference 25. [f] Reference 35. [g] Reference 47.

The benzene ring substituted analogues **10a**−**y** of Table 2 were prepared using method A of Scheme 1, via the monochlorotriazines **9** and the appropriately substituted 2-(difluoromethyl)-benzimidazoles **8** (Scheme 3). The latter compounds were normally prepared from the analogous *o*-phenylenediamines and difluoroacetic acid, although compounds **8l**−**q** and **8ad**, required for the synthesis of derivatives **10l**−**q**, were prepared from the analogous 4-carboxylic acid **8ah** (Scheme 4).

It was again necessary to protect oxygen substituents as their TBDMS ethers for the successful synthesis of the 4-hydroxymethyl derivative **10l** and of the phenols **10a**, **10t**, and **10u** (Scheme 3). 4-Alkoxy derivatives **10d**−**g** were subsequently prepared by alkylation of phenol **10a**. The 4-amino derivative **10i** was prepared by reduction of the analogous nitro compound **10ae**, while the 4-methylamino derivative **10j** was prepared from **10i** via alkylation of the Boc protected intermediate. The 6-amino-4-methoxy derivatives **10w**, **10x**, and **10y** were similarly prepared from the Boc protected precursor **10af** by a combination of alkylation, reduction, and deprotection steps.

The 4-methoxy-6-aza analogue **13** of Table 2 was prepared from the imidazo[4,5-*c*]pyridine **11** via the intermediacy of chloro derivative **12**, although a higher temperature than normal was needed for the first step because of the lower nucleophilicity of **11** (Scheme 5). As with the earlier benzimidazoles, compound **11** was prepared from difluoroacetic acid by condensation with the appropriate diaminopyridine[40] (see Experimental Section).

Scheme 2. Synthesis of 2-Substituted Analogues 7[a]



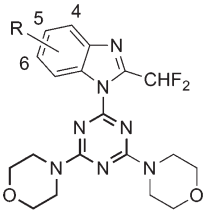
[a] Reagents and conditions: (i) (for **7a** and **7b**) **5**, NaH, DMF, 120 °C; (ii) (for **7c**, **7d**, **7f**, **7i**, **7k**) (a) **3**, K_2CO_3, DMF, room temp; (b) morpholine, THF, room temp or reflux; (iii) Bu_4NF, THF, room temp; (iv) aq $NaIO_4$, EtOH, room temp; (v) aq $KMnO_4$, HOAc, acetone, room temp.

The central ring pyrimidine analogues **16a**−**c**, **19a**, and **19b** of Table 3 were prepared as shown in Scheme 6, with a TIPS protecting group used to protect the 4-hydroxy group of **16b**. Thus, condensation of the appropriately substituted 2-(difluoromethyl)benzimidazoles **2**, **8c**, and **8ai** with 2,4,6-trichloropyrimidine (**14**) gave the analogous 2-substituted 4,6-dichloropyrimidines **15a**, **15c**, **15d**, which were then treated with morpholine at reflux to give 2-(benzimidazolyl) derivatives **16a**, **16c**, and **16d**. TIPS deprotection of **16d** then gave **16b**. The 4-(benzimidazolyl) isomers **19a** and **19b** were prepared by two different routes. For the synthesis of **19a**, 2-(difluoromethyl)-benzimidazole (**2**) was condensed with 4,6-dichloro-2-morpholinopyrimidine (**17**) at 45 °C to give intermediate **18** which was then reacted with morpholine at reflux to give **19a**. For the synthesis of **19b**, benzimidazole **8c** was reacted with 4,6-dichloro-2-(methylthio)pyrimidine **20** at room temperature to give intermediate **21** which was reacted with morpholine at room temperature to give intermediate **22**. Oxidation of **22** gave **23** which gave **19b** on treatment with morpholine at reflux.

RESULTS AND DISCUSSION

Molecular Modeling. At the commencement of this work there was no structural information available on the binding of **1** to PI3K, so to aid in the analysis of substituted analogues, we developed a model of **1** bound to the crystal structure of p110γ.[41−43] To this end, **1** was docked within the p110γ binding site (PDB code 2CHX) by means of the automated GOLD program.[44] In order to take into account protein flexibility, the conformation with the highest score (GoldScore) was further refined using the MINIMIZE module as implemented in SYBYL version 7.3 (Tripos force field and Gasteiger−Hückel charges).[45] This model (Figure 2) differed from one proposed at the time[25] and was later supported by molecular docking studies with the recently determined p110α X-ray crystal structure (PDB code 2rd0),[46] where the top ranked and most populated predicted binding mode made contacts to the active site analogous to those observed in p110γ. The binding mode predicted in both the p110γ and p110α ATP binding sites is in good agreement with the crystal structure subsequently determined for the binding of **1** to p110δ.[33] For example, our model showed hydrogen bonding between one of the morpholine oxygens and Val882 and between the benzimidazole 3-nitrogen and the amino groups of Lys833, but in addition it indicated that an appropriate electron donating substituent at the 4-position could probably participate in the hydrogen bonding to the Lys833 amino group and that a similar substituent at the 6-position might interact with the phenol group

Table 2. Inhibition of PI3K Isoforms and Cell Proliferation for 4-, 5-, and 6-Substituted Analogues of 1



		PI3K IC_{50}[a] (nM)			cell line IC_{50}[b] (μM)	
compd	R	p110α	p110β	p110δ	NZB5	NZOV9
10a[c]	4-OH	0.9	0.68	0.27	0.03	0.01
10b[d]	4-OMe	2.7	25	3.9	0.05	0.01
10c	4-OEt	2.7	104	5.5	0.07	0.04
10d	4-OPr	16	518	95	0.07	0.05
10e	4-OBu	28	2400	156	0.23	0.21
10f	4-$OCHF_2$	26	56	7.4	0.047	0.028
10g	4-$OCHMe_2$	364	2100	430	0.07	0.28
10h	4-OSO_2Me	294	570	158	0.17	0.13
10i[e]	4-NH_2	4.8	41	0.85	0.05	0.08
10j	4-NHMe	210	480	67	0.11	0.26
10k	4-NMe_2	5400	$>10^3$	539	0.06	2.54
10l	4-CH_2OH	27	68	12	0.30	0.17
10m	4-CO_2Me	9.3	310	65	2.8	0.47
10n	4-$CONH_2$	$>10^3$	$>10^3$	$\sim10^3$	0.76	0.11
10o	4-CONHMe	296	$>10^3$	$\sim10^3$	0.48	0.05
10p	4-$CONMe_2$	$>10^3$	$>10^3$	$>10^3$	0.73	0.24
10q	4-CN	61	$>10^3$	66	>20	>20
10r	4,5-OCH_2O-	550	47	39	0.13	0.04
10s	4,5-$(OMe)_2$	335	801	258	0.02	0.036
10t	4-OH, 5-OMe	37	6.7	0.3	0.334	0.13
10u	4-OMe, 5-OH	32	94	1.8	0.009	0.011
10v	4,6-$(OMe)_2$	58	118	12.5	0.095	0.059
10w	4-OMe, 6-NH_2	0.22	1.4	0.38	0.02	0.007
10x	4-OMe, 6-NHMe	2.0	0.91	0.52	0.39	0.016
10y	4-OMe, 6-NMe_2	8.0	18.5	6.8	0.13	0.15
13	4-OMe, 6-aza	1290	1030	1600	5.6	2.6

[a] Compound concentration required to produce 50% inhibition of phosphorylation in an in vitro lipid kinase assay (see Table 1 for details). [b] Compound concentration required to produce 50% inhibition of cell growth in an in vitro cell proliferation assay (see Table 1 for details). [c] Reference 47. [d] Reference 48. [e] Reference 38.

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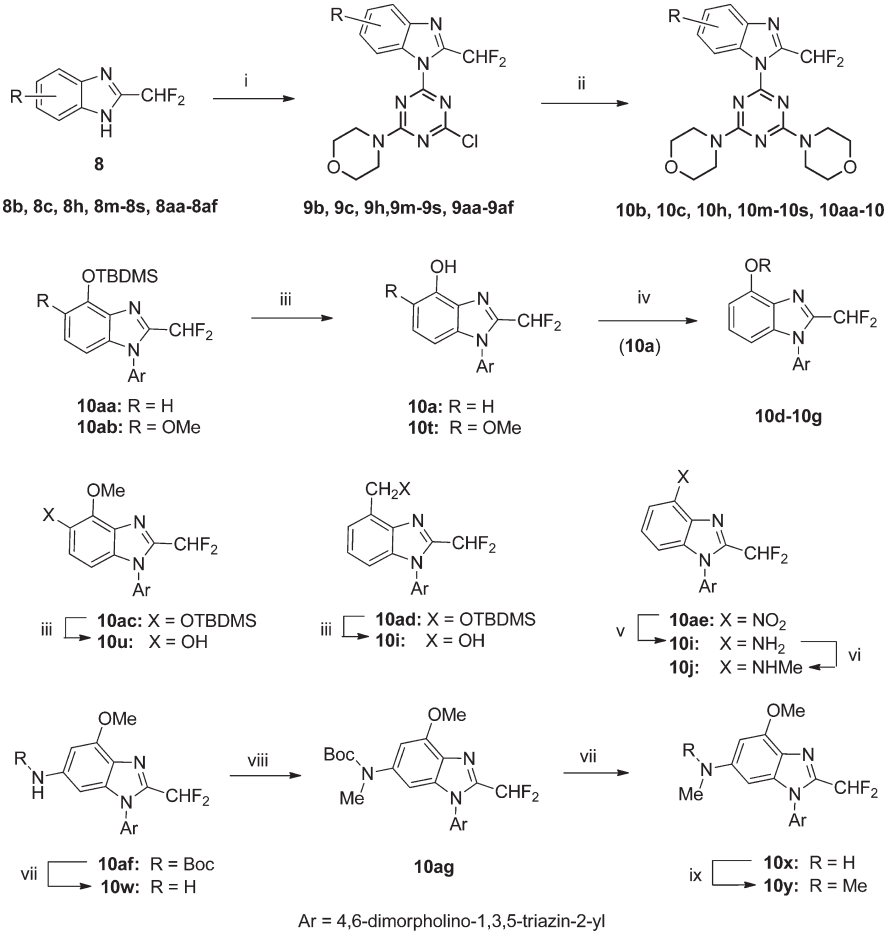
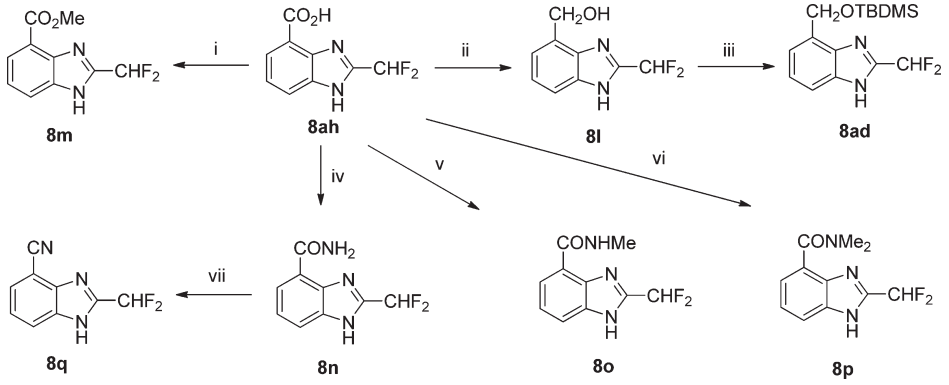
Scheme 3. Synthesis of 4-, 5- and 6-Substituted Analogues 10[a]



[a] Reagents and conditions: (i) **3**, K_2CO_3, DMF, room temp; (ii) morpholine, THF, room temp or reflux; (iii) Bu_4NF, THF, room temp; (iv) RI, K_2CO_3, DMF, room temp; (v) H_2, Pd/C, MeOH, room temp; (vi) (a) Boc_2O, dioxane, reflux; (b) NaH, MeI, DMF, room temp; (c) TFA, CH_2Cl_2, room temp; (vii) TFA, CH_2Cl_2, room temp; (viii) NaH, MeI, DMF, room temp; (ix) (a) AcOCHO, CH_2Cl_2, room temp; (b) $BH_3.SMe_2$, $(MeO)_3B$, THF, CH_2Cl_2, room temp.

Scheme 4. Synthesis of Benzimidazoles 8 with 4-Carbon Substituents[a]



[a] Reagents and conditions: (i) MeOH, H_2SO_4, reflux; (ii) $LiAlH_4$, THF, reflux; (iii) TBDMSCl, pyridine, room temp; (iv) (a) $SOCl_2$, reflux; (b) NH_3, dioxane, room temp; (v) (a) $SOCl_2$, reflux; (b) $MeNH_3Cl$, Et_3N, dioxane, room temp; (vi) (a) $SOCl_2$, reflux; (b) Me_2NH_2Cl, Et_3N, dioxane, room temp; (vii) $SOCl_2$, reflux.

of Tyr867 at the back of the binding site. Finally, comparison of the p110α binding model of **1** to the X-ray crystal structure of human p110γ with ATP bound (PDB code 1e8x)[41] indicated that substitution at the 2 position may be used to access the active site

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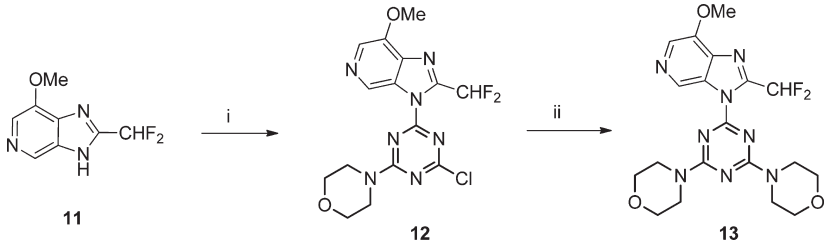
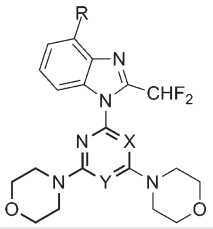
Scheme 5. Synthesis of 13[a]



[a] Reagents and conditions: (i) **3**, K_2CO_3, *t*-BuOH, reflux; (ii) morpholine, room temp.

Table 3. Inhibition of PI3K Isoforms and Cell Proliferation for Pyrimidine Analogues of 1



			PI3K IC_{50}[a] (nM)			cell line IC_{50}[b] (μM)	
compd	R	X, Y	p110α	p110β	p110δ	NZB5	NZOV9
16a[c]	H	N, CH	30	72	10	0.12	0.26
16b[d]	OH	N, CH	1.5	0.81	0.27	0.10	0.11
16c[e]	OMe	N, CH	5.1	35	18	0.06	0.03
19a	H	CH, N	88	170	13	0.42	0.3
19b	OMe	CH, N	4	40	14	0.43	0.11

[a] Compound concentration required to produce 50% inhibition of phosphorylation in an in vitro lipid kinase assay (see Table 1 for details). [b] Compound concentration required to produce 50% inhibition of cell growth in an in vitro cell proliferation assay (see Table 1 for details). [c] Reference 38. [d] Reference 47. [e] Reference 49.

region traversed by the α and β nonhydrolyzed ATP phosphate moieties. These possibilities were therefore explored in detail in our SAR study of benzimidazole-substituted analogues of **1**.

Enzyme Activity. All compounds were tested for their enzyme activity against the p110α, p110β, and p110δ isoforms of PI3K using a lipid kinase assay. Table 1 gives results for a series of 2-substituted derivatives (**7a**−**j**) compared to **1**. The known[35] unsubstituted compound **7a** was a poor PI3K inhibitor, with IC_{50} > 140 nM across the isoforms, and the 2-Me and 2-CF_3 analogues (**7b**, **7d**) were little better. The 2-CH_2F compound **7c** was considerably more active, but apart from the 2-CH_2SMe compound **7f**, which showed moderate activity, all of the other substituted 2-alkyl analogues **7d**−**h** were less active. The 2-SMe analogue **7i** also showed poor activity, although the 2-SO_2Me derivative **7j** did show improved, but still only moderate, activity. Therefore, since none of the 2-substituted analogues investigated showed superior activity to that of the 2-difluoromethyl compound **1**, this substituent group was employed in all of the subsequently studied compounds.

Compounds **10a**−**y** and **13** (Table 2) then explored the use of different substituents on the benzene ring of the benzimidazole. Since our modeling of **1** with p110γ suggested that an appropriate substituent at the 4-position could complement the binding of the benzimidazole 3-nitrogen atom to the lysine amino group (Lys833 in p110γ is equivalent to Lys802 in p110α, Lys805 in p110β, and Lys779 in p110δ), we started by exploring substituents at that position.

The known[47] 4-OH analogue **10a** showed exceptional enzyme potency against all three isoforms studied (IC_{50} < 1 nM), presumably because of tight specific binding of the OH to the lysine amino group, although with the likely pharmacologic liability of rapid glucuronic acid conjugation in an in vivo situation. The 4-OMe and 4-OEt analogues (**10b** and **10c**) were also more active than **1** against the p110α-isoform, and although the 4-OPr, 4-OBu, and 4-$OCHF_2$ compounds (**10d**−**f**) retained reasonable α-isoform potency, this was lost by the more bulky 4-$OCHMe_2$ and 4-OSO_2Me analogues **10g** and **10h**. This suggests some bulk tolerance around the 4-position for small cross-section substituents but not for those with branching close to the benzimidazole group.

A similar pattern was observed with the 4-amino derivatives **10i**−**k**, where the primary amino derivative **10i** was more active than the methylamino derivative **10j** and significantly more active than the dimethylamino derivative **10k**.

Compounds **10l**−**q** explored carbon linked subtituents, and although the 4-CH_2OH and 4-CO_2Me derivatives **10l** and **10m** showed reasonable activity, none of them displayed activity comparable to that of oxygen linked substituents.

A variety of 4,5-disubstituted compounds (**10r**−**u**) were relatively inactive, with the exception of the 4-OMe, 5-OH analogue **10u**, which was still less potent against the p110α isoform than the 5-unsubstituted 4-OMe analogue **10b**, indicating that substitution at the 5-position is sterically not preferred for this isoform.

Compounds **10v**−**y** and **13** explore 4,6-disubstitution. Since our modeling suggested that an aza atom at the 6-postion of the benzimidazole might form an H-bond to the hydroxyl group of a tyrosine residue at the back of the ATP binding site (Tyr867 in p110γ is equivalent to Tyr836 in p110α, Tyr839 in p110β, and Tyr813 in p110δ), the 4-OMe-6-aza compound **13** was designed to explore this possibility. However the dramatic loss of activity of this compound compared to 4-OMe derivative **10b** showed that electronic effects play a much more important role in the binding of these compounds, with the electron withdrawing effect of the 6-aza atom being highly detrimental to good activity. Thus, it appears that the hydrogen bonding interaction between the benzimidazole 3-nitrogen and the lysine amino group (Lys802 in p110α, Lys805 in p110β, Lys833 in p110γ, and Lys779 in p110δ) represents a major factor in determining the potency and activity of this class of inhibitors, and anything that reduces this interaction should be avoided. Conversely, increasing the

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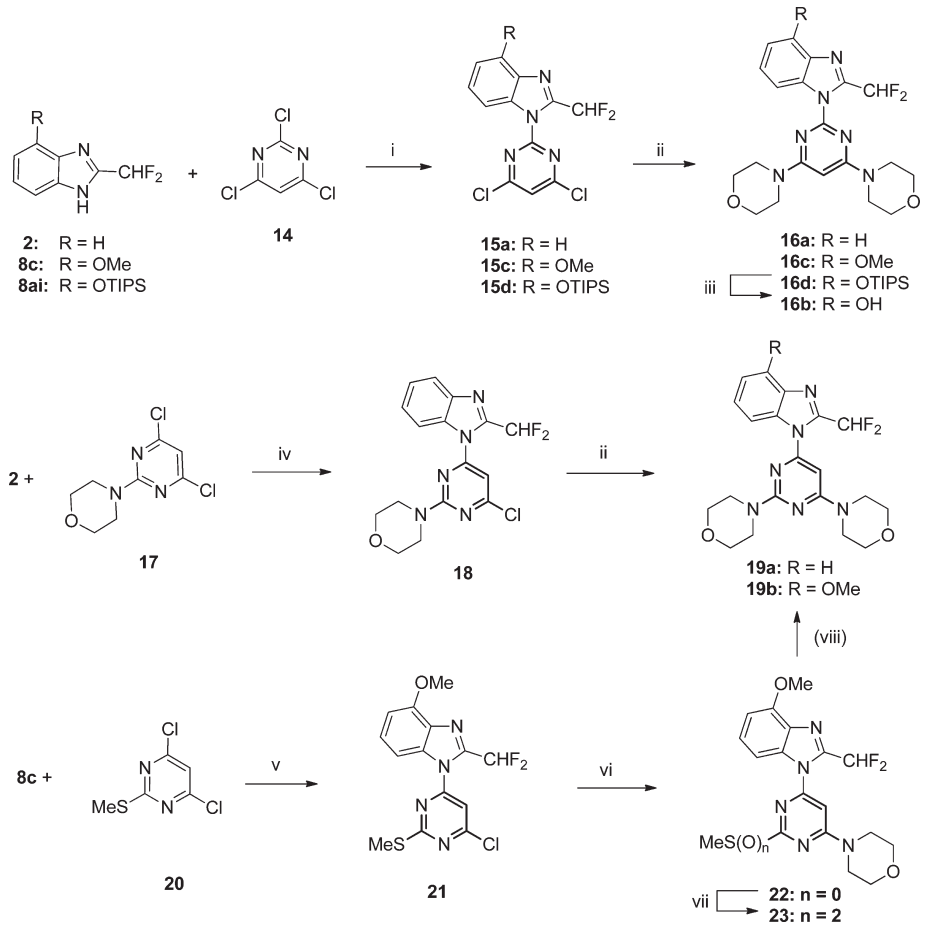
Scheme 6. Synthesis of Pyrimidinyl Analogues 16 and 19[a]



[a] Reagents and conditions: (i) NaH, DMF, room temp; (ii) morpholine, reflux; (iii) Bu_4NF, THF, room temp; (iv) K_2CO_3, DMF, 45 °C; (v) K_2CO_3, DMSO, room temp; (vi) morpholine, THF, room temp; (vii) aq $KMnO_4$, HOAc, acetone, room temp; (viii) morpholine, THF, reflux.

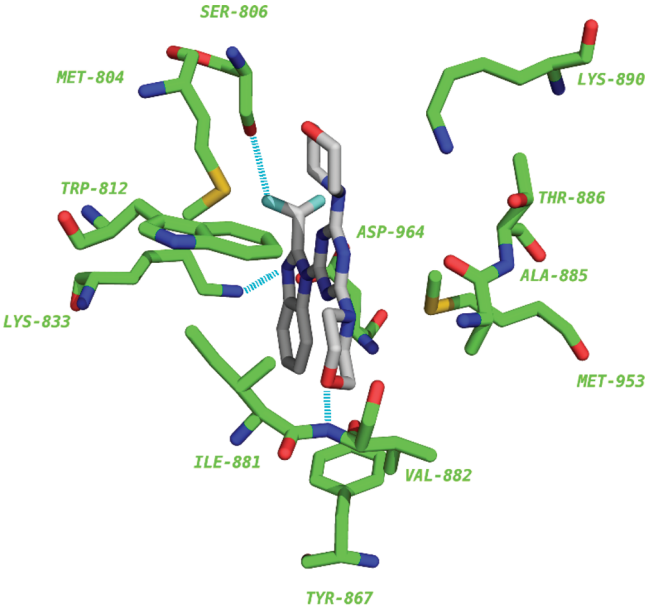


Figure 2. View of **1** docked in the ATP-binding site of PI3K p110γ.

electron density on the benzimidazole 3-nitrogen by the use of electron donating substituents should be advantageous, and thus, the 4,6-dimethoxy derivative **10v** is significantly more active than **13**, although not as active as the sterically less hindered 6-amino analogue **10w** which was the most potent new compound against the PI3K isoforms identified in this study. The *N*-methyl and *N*,*N*-dimethyl analogues of **10w** (**10x** and **10y**) also displayed very good potency, despite increased steric effects, thereby reinforcing the strong preference for electron donating substituents. Our belief that this electronic effect is due to increased electron density on the benzimidazole 3-nitrogen, resulting in increased binding to the lysine amino group, is in agreement with the fact that the very high potency of the PI3K inhibitor 2,4-difluoro-*N*-{2-(methyloxy)-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl}-benzenesulfonamide (GSK2126458) (**24**, Figure 6) is almost certainly due to its strong charged interaction with the same lysine amino group.[22]

With 2-CHF_2 and 4-OH and 4-OMe established as suitable benzimidazole substituents, Table 3 shows the results of a small study of pyrimidine analogues of the central triazine ring (compounds **16a**−**c**, **19a**,**b**). The 2-pyrimidine isomers **16a**−**c** showed only a slight reduction in potency compared to their respective triazine analogues **1**, **10a**, and **10b**, although a more pronounced effect was seen with the 4-pyrimidine isomers **19a** and **19b** where the unsubstituted benzimidazole analogue **19a** was significantly less potent than **1**, although the 4-methoxy derivative **19b** displayed similar potency compared to **10b**.

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Table 4. HTRF Assay Inhibition of PI3K Isoforms for 1, 10a, 10f, 10t, and 10w

	PI3K IC_{50} [a] (nM)					
compd	p110α	p110β	p110δ	p110γ	p110α H1047R[b]	p110α E545K[c]
1	8.9	58	38	83	25	12
10a	0.53	3.5	0.65	6	0.83	0.12
10f	4.7	>2,500[d]	34	56	8.5	3
10t	70	44	2	52	158	49
10w	3.1	27	1.7	32	0.95	1.0

[a] Compound concentration required to produce 50% inhibition of phosphorylation in a PI3-kinase (human) HTRF assay. [b] Kinase domain p110α mutant. [c] Helical domain p110α mutant. [d] Solubility problems encountered with this assay.

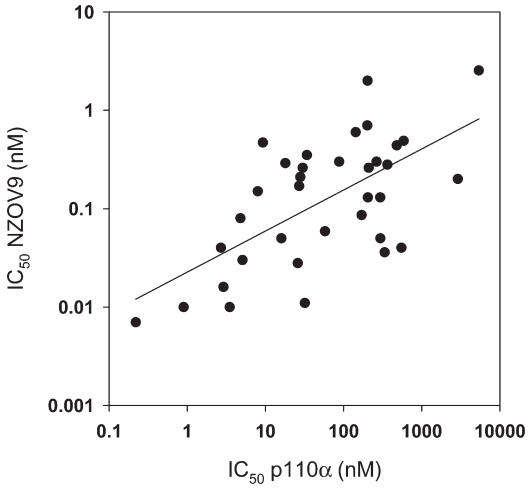


Figure 3. Correlation of NZOV9 cell line activity against p110α enzyme activity.

Compounds **10a**, **10f**, **10t**, and **10w** were additionally compared to **1**, against all four class I PI3K isoforms and two mutant isoforms of p110α (H1047R and E545K) in a homogeneous time resolved fluorescence (HTRF) assay (Table 4). Because of the different assay conditions the PI3K IC_{50} values differ from those in Tables 1 and 2 but confirm that **10a** and **10w** consistently show potency superior to that of **1** against all PI3K enzyme variants tested, including the two p110α mutant forms.

Cell Proliferation. The compounds were also evaluated in a cellular assay by measuring IC_{50} values (drug concentrations causing 50% inhibition of proliferation). Two early passage human cancer cell lines were used. NZB5 was derived from a patient with medulloblastoma and expressed the wild-type gene for p110α, while NZOV9 was derived from a poorly differentiated ovarian carcinoma and expressed a mutant p110α enzyme with a single amino acid substitution in the kinase domain (Y1021C). For all of the compounds in Tables 1−3, cell line IC_{50} values for NZB5 and NZOV9 correlated significantly with isolated enzyme IC_{50} values for p110α, as determined by Spearman rank order correlation (r = 0.53 and r = 0.40, respectively; P < 0.0001 and P < 0.001, respectively). As shown in Figure 3, a plot of those compounds having measurable values showed a significant correlation between logarithmic IC_{50} values for NZOV9 cell line (r = 0.63; p < 0.0001) but not to those for the NZB5 cell line (r = 0.29; p = 0.09). The two most potent inhibitors of p110α (**10a** and **10w**) were also the most potent inhibitors of the NZOV9 cell line. These results strongly support the hypothesis that proliferation of the NZOV9 line is driven by an activated mutant p110α PI 3-kinase in these cells. On the other hand the lower correlation found for the NZB5 cell line and the occasional large differences in IC_{50} values between the two cell lines (e.g., compounds **10o** and **10x**) suggest that proliferation of the NZB5 line is sensitive to factors additional to PI 3-kinase.

Table 5. Inhibition of Cell Signaling in U87MG (PTEN Null) Cells by 1, 10a, 10b, 10f, 10t, and 10w in Vitro

	IC_{50} [a] (nM)	
compd	pAkt/PKB Ser473	pAkt/PKB Thr308
1	111	32
10a	1.3	2.9
10b	55	28
10f	427	418
10t	583	378
10w	4.8	8.8

[a] Compound concentration required to produce 50% inhibition of phosphorylation in a cellular assay. Cells were exposed to inhibitor dissolved in DMSO for 15 min before stimulation with 500 nM insulin for 5 min. Protein isolation and immunoblotting for phospho-Akt/PKB was carried out according to the methods previously described in refs 50 and 51.

Table 6. Inhibition of Cell Signaling in HCT116+/+ (PIK3CA H1047R Mutant) Cells by 1, 10a, 10b, 10f, 10t, and 10w in Vitro

	IC_{50} [a] (nM)	
compd	pAkt/PKB Ser473	pAkt/PKB Thr308
1	97	78
10a	8.3	0.18
10b	37	25
10f	415	381
10t	507	515
10w	12	17

[a] Compound concentration required to produce 50% inhibition of phosphorylation in a cellular assay. Cells were exposed to inhibitor dissolved in DMSO for 15 min before stimulation with 500 nM insulin for 5 min. Protein isolation and immunoblotting for phospho-Akt/PKB was carried out according to the methods previously described in refs 50 and 51.

Effect on Cell Signaling. To determine whether the compounds were capable of entering cells and attenuating signaling downstream from PI 3-kinase, the effect for selective compounds on phosphorylation of Akt/PKB was determined in cell lines using previously described methods.[50,51] U87MG and HCT116 cells were chosen for these experiments, as they are commonly used in xenografts and have constitutive activation of the PI 3-kinase pathway due to PTEN deletion or activation of PIK3-CA, respectively. We determined the IC_{50} for the inhibition of phosphorylation at not only the most commonly measured

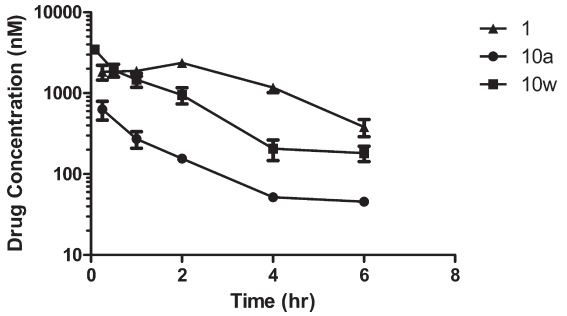


Figure 4. Plasma pharmacokinetics of **1**, **10a**, and **10w** after ip administration at 10 mg/kg to CD-1 mice.

Table 7. Plasma Pharmacokinetic Parameters for 1, 10a and 10w in Male CD-1 Mice[a]

compd	dose (mg/kg)	route	C_{max} (nM)	T_{max} (h)	AUC_{INF} (nM·h)	$T_{1/2}$ (h)
1	10	ip	2359	2	9657	1.52
10a	10	ip	628	0.25	1034	1.55
10w	10	ip	3452	0.083	5219	1.48

[a] Blood samples were centrifuged for 10 min at 6000 rpm to separate plasma, which underwent protein precipitation in MeOH. Quantitative analysis was carried out using multiple reaction monitoring and electrospray ionization. Pharmacokinetic parameters were determined by noncompartmental analysis using WinNonlin 5.3 software (Pharsight, Sunnyvale, CA, U.S.).

Ser473 site but also for Thr 308, as this site is the one most directly linked to activation of PI 3-kinase in the cell. In these studies the rank order of IC_{50} against PI 3-kinase isoforms in vitro matched with the rank order of IC_{50} against both Akt/PKB phosphorylation sites in both cell lines (Tables 5 and 6). **10a** and **10w** showed the best inhibition of phosphorylation at both the Ser473 and Thr308 sites on Akt/PKB and were selected for further evaluation.

Pharmacokinetics. On the basis of both their effects on cell signaling and exceptional potency against the isolated p110α and p110δ enzymes, compounds **10a** and **10w** were selected for pharmacokinetic evaluation in comparison with compound **1** in CD-1 mice. The pharmacokinetic profile of these compounds at a dose of 10 mg/kg by ip injection is shown in Figure 4 and Table 7. Compound **1** reached a C_{max} of 2359 nM 2 h after dosing and, despite a short $t_{1/2}$ of 1.5 h, reached an AUC of 9657 nM·h because of its delayed T_{max} (Table 7). Compound **10w** reached a higher C_{max} (3452 nM) than compound **1**, but because of its quicker T_{max} and similar $t_{1/2}$, its AUC (5219 nM·h) was lower. The pharmacokinetics of **10a** were poor comparatively, with an approximately 5-fold lower C_{max} (628 nM) and AUC (1034 nM·h) than **10w**, reflecting the liability of the phenol group of **10a** as a target for glucuronidation.

Antitumor Activity. As a result of the superior pharmacokinetic profile of **10w** over **10a**, the former was selected for evaluation of in vivo antitumor activity alongside compound **1** and the pan PI3K/mTOR inhibitor 2-methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-*c*]quinolin-1-yl]-phenyl]propionitrile (NVP-BEZ235)[20] (**25**, Figure 6) at MTD in a PTEN-null U87MG human glioblastoma tumor xenograft

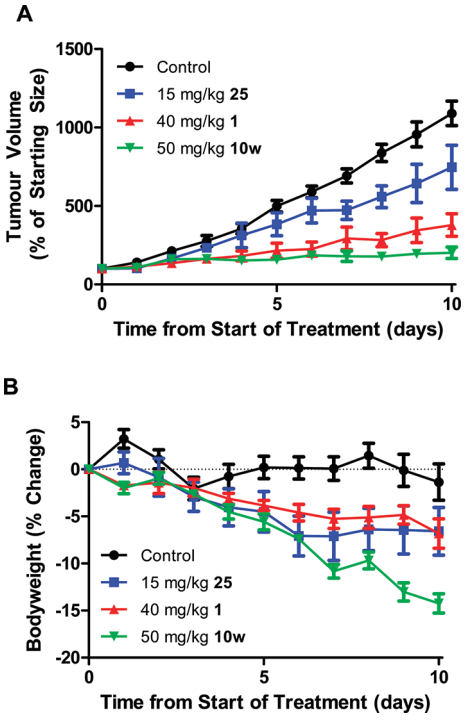


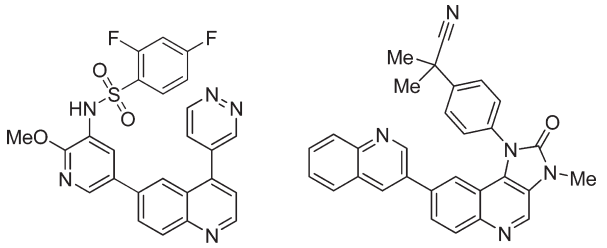
Figure 5. In vivo antitumor efficacy and bodyweight change following treatment at MTD with 15 mg/kg **25**, 40 mg/kg **1**, and 50 mg/kg **10w** in a U87MG human glioblastoma xenograft model in $Rag1^{-/-}$ mice. All treatments were administered qd × 10 by ip injection. (A) Relative mean tumor volume and (B) bodyweight change in $Rag1^{-/-}$ mice following the onset of drug treatment are shown. Bars represent the mean and standard error of 5−7 mice per group.

Table 8. Aqueous Solubility Measurements for 1, 10a, 10b, 10w, and 25[a]

compd	solubility (μg/mL)
1	0.61
10a	0.32
10b	0.08
10w[b]	4.2
25[c]	11.3

[a] Solubility was measured using an HPLC assay comparing the peak area obtained for an aqueous solution compared with that from a standard solution of the compound in DMSO. [b] Methanesulfonate salt. [c] Dimethanesulfonate salt.

model in $Rag1^{-/-}$ mice. At a dose of 50 mg/kg given by ip injection at a qd× 10 dosing schedule, **10w** reduced tumor growth by 81.5% (18.5 ± 3.4% of control; $P < 0.0001$) by day 10, compared to a 31.5% reduction by **25** at 15 mg/kg, and a 65.3% reduction by compound **1** (34.7 ± 6.6% of control; $P < 0.0001$) at 40 mg/kg (Figure 5A). All mice survived the 10-day study duration. At MTD, **1** and **25** were well tolerated over the treatment duration, but **10w** was associated with delayed bodyweight loss (Figure 5B), thought to be a result of the poor solubility properties of the compound as evidenced by the presence of drug precipitate in the IP cavity of all mice on post-mortem examination. The relative solubilities of **1**, **10a**, **10b**, **10w**, and **25** are shown in Table 8, and although **10w** was administered as the relatively soluble methanesulfonate salt, it



24 25

Figure 6. Structures of **24** and **25**.

was presumably rapidly converted to a less soluble hydrochloride salt in vivo.

CONCLUSION

Molecular modeling suggestions that the addition of appropriate substituents at the 4- and 6-positions of the benzimidazole ring of **1** could result in tighter binding derivatives were confirmed with the identification of **10a** and **10w** as very potent analogues. Phenol **10a** has poor pharmacokinetics, presumably as a result of rapid glucuronidation, although more favorable drug levels were achievable with **10w**. On the basis of cell based studies and the pharmacokinetic data, **10w** was tested in a U87MG human glioblastoma xenograft model, where it caused a significant reduction in tumor growth at MTD. However, despite the good pharmacokinetics and promising anticancer efficacy of **10w**, its poor solubility properties prevented it from being well tolerated in vivo. In conclusion, substitution at the 4 and 6 positions of the benzimidazole ring of **1** generates highly potent PI3K inhibitors that are active in vivo, although more soluble analogues are likely required before this class of compounds can progress further.

EXPERIMENTAL METHODS

Chemistry. Combustion analyses were performed by the Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal IA9100 melting point apparatus and are as read. ^{1}H NMR spectra were obtained on a Bruker Avance-400 spectrometer at 400 MHz referenced to Me_4Si. Low-resolution atmospheric pressure chemical ionization (APCI) mass spectra were measured for organic solutions on a ThermoFinnigan Surveyor MSQ mass spectrometer connected to a Gilson autosampler. Thin-layer chromatography was carried out on aluminum-backed silica gel plates (Merck 60 F254), with visualization of components by UV light (254 nm). Column chromatography was carried out on silica gel (Merck 230−400 mesh). Tested compounds were ≥95% pure, as determined by combustion analysis or by HPLC conducted on an Agilent 1100 system using a reversed-phase C8 column with diode array detection.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (1). A mixture of *o*-phenylenediamine (5.41 g, 50 mmol) and difluoroacetic acid (9.6 g, 100 mmol) in 4 M HCl (20 mL) was heated under reflux for 1 h and diluted with hot water (50 mL). The solution was treated with charcoal and filtered through Celite before being neutralized with aqueous NH_3. The resulting white precipitate was collected, washed with water, and dried to give 2-(difluoromethyl)-1*H*-benzimidazole (**2**)[36] (6.07 g, 72% yield): mp 156−158 °C; ^{1}H NMR (DMSO-d_6) δ 13.28 (br, 1H), 7.76−7.68 (m, 1H), 7.61−7.54 (m, 1H), 7.36−7.26 (m, 2H), 7.26 (t, J_{HF} = 53.3 Hz, 1H).

A mixture of **2** (0.168 g, 1 mmol), 2-chloro-4,6-di(4-morpholinyl)-1,3,5-triazine (**5**)[39] (0.286 g, 1 mmol), and powdered K_2CO_3 (0.56 g, 4 mmol) in DMSO (5 mL) was heated and stirred at 130 °C for 3 h. After cooling, the mixture was diluted with water and the white solid was collected and dried to give **1** (0.35 g, 84% yield): mp (EtOH) 217−219 °C (lit.[38] 211−214 °C); ^{1}H NMR ($CDCl_3$) δ 8.33 (dd, J = 7.3, 1.4 Hz, 1H), 7.89 (dd, J = 7.2, 1.5 Hz, 1H), 7.56 (t, J_{HF} = 53.6 Hz, 1H), 7.46−7.37 (m, 2H), 3.91−3.86 (m, 8H), 3.81−3.76 (m, 8H).

1-[4,6-Di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (7a). A solution of benzimidazole (**6a**) (0.59 g, 5 mmol) in DMF (10 mL) was treated with NaH (250 mg, 60% in oil, 6.5 mmol), and the mixture was stirred at room temperature for 30 min. Solid **5** (1.43 g, 5 mmol) in DMF (10 mL) was then added, and the resulting mixture was heated at 90 °C overnight. After cooling, the reaction mixture was diluted with water to give a solid which was collected and recrystallized from EtOH to give **7a** (1.46 g, 79% yield): mp 233−236 °C (lit.[35] 222−224 °C); ^{1}H NMR (DMSO-d_6) δ 9.15 (s, 1H), 8.39 (d, J = 7.7 Hz, 1H), 7.75 (d, J = 7.5 Hz, 1H), 7.41 (ddd, J = 8.3, 7.9, 1.2 Hz, 1H), 7.34 (ddd, J = 7.9, 7.8, 1.2 Hz, 1H), 3.91−3.75 (m, 8H), 3.73−3.64 (m, 8H).

1-[4,6-Di(4-morpholinyl)-1,3,5-triazin-2-yl]-2-methyl-1*H*-benzimidazole (7b). Similar to the synthesis of **7a**, reaction of 2-methylbenzimidazole (**6b**) (0.66 g, 5 mmol) with **5** (1.43 g, 5 mmol) gave **7b** (1.39 g, 73% yield): mp (EtOH) 222−224 °C (lit.[35] 218−220 °C); ^{1}H NMR (DMSO-d_6) δ 8.18 (dd, J = 7.0, 1.7 Hz, 1H), 7.59 (dd, J = 6.9, 1.7 Hz, 1H), 7.31−7.63 (m, 2H), 3.81−3.76 (m, 8H), 3.71−3.65 (m, 8H), 2.50 (s, 3H).

1-[4,6-Di(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(fluoromethyl)-1*H*-benzimidazole (7c). A mixture of 2-(fluoromethyl)-1*H*-benzimidazole (**6c**)[52] (0.75 g, 5 mmol), 2,4-dichloro-6-(4-morpholinyl)-1,3,5-triazine (**3**)[37] (1.17 g, 5 mmol), and powdered K_2CO_3 (2.75 g, 20 mmol) in DMF (20 mL) was stirred at room temperature overnight. After dilution with water, the solid precipitate was collected and recrystallized from EtOH to give 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(fluoromethyl)-1*H*-benzimidazole (1.08 g, 62% yield); mp 230−231 °C; ^{1}H NMR ($CDCl_3$) δ 8.42 (dd, J = 6.9, 2.0 Hz, 1H), 7.83 (dd, J = 6.8, 2.2 Hz, 1H), 7.44−7.37 (m, 2H), 5.99 (d, J_{HF} = 47.0 Hz, 1H), 3.99−3.94 (m, 4H), 3.85−3.79 (m, 4H). Anal. Calcd for $C_{15}H_{14}ClFN_6O$: C, 51.7; H, 4.05; N, 24.1. Found: C, 51.8; H, 3.9; N, 24.4%.

A mixture of the above chloro compound (0.75 g, 5 mmol) and morpholine (4.35 g, 50 mmol) in THF (20 mL) was heated under reflux for 1 h, cooled, and diluted with water to give **7c** (0.39 g, 98% yield): mp (EtOH) 229−231 °C; ^{1}H NMR ($CDCl_3$) δ 8.34 (dd, J = 7.4, 1.6 Hz, 1H), 7.82 (dd, J = 7.2, 1.7 Hz, 1H), 7.39−7.32 (m, 2H), 5.98 (d, J_{HF} = 47.3 Hz, 1H), 3.91−3.84 (m, 8H), 3.81−3.75 (m, 8H). Anal. Calcd for $C_{19}H_{22}FN_7O_2$: C, 57.1; H, 5.55; N, 24.55. Found: C, 57.1; H, 5.3; N, 24.8%.

1-[4,6-Di(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(trifluoromethyl)-1*H*-benzimidazole (7d). A mixture of 2-(trifluoromethyl)-1*H*-benzimidazole (**6d**)[52] (4.86 g, 26 mmol), **3** (5.11 g, 22 mmol), and powdered K_2CO_3 (14 g, 0.1 mol) in DMF (100 mL) was stirred at room temperature overnight. After dilution with water the solid was collected by filtration and washed successively with water and then EtOH to give 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(trifluoromethyl)-1*H*-benzimidazole (6.01 g, 71%): mp (EtOH) 239−241 °C; ^{1}H NMR ($CDCl_3$) δ 8.39 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 7.9 Hz, 1H), 7.54 (ddd, J = 8.4, 7.8, 1.2 Hz, 1H), 7.46 (ddd, J = 8.3, 7.7, 1.2 Hz, 1H), 3.99−3.93 (m, 4H), 3.82−3.78 (m, 4H). Anal. Calcd for $C_{15}H_{12}ClF_3N_6O$: C, 46.8; H, 3.1; N, 21.8. Found: C, 46.9; H, 3.3; N, 22.1%.

A mixture of the above chloro compound (0.386 g, 1 mmol) and morpholine (0.435 g, 5 mmol) in THF (5 mL) was heated at 70 °C for 1 h. After cooling, the mixture was diluted with water to give a solid

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which was recrystallized from EtOH to give **7d** (0.295 g, 68%): mp 197−200 °C; ^{1}H NMR ($CDCl_3$) δ 8.15 (d, J = 7.7 Hz, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.46 (ddd, J = 7.7, 7.3, 1.4 Hz, 1H), 7.41 (ddd, J = 7.7, 7.3, 1.3 Hz, 1H), 3.91−3.87 (m, 8H), 3.79−3.75 (m, 8H). Anal. Calcd for $C_{19}H_{20}F_3N_7O_2$: C, 52.4; H, 4.6; N, 22.5. Found: C, 52.1; H, 4.6; N, 22.7%.

1-[4,6-Di(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(hydroxymethyl)-1*H*-benzimidazole (7e). A mixture of 2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-1*H*-benzimidazole (**6e**)[53] (1.31 g, 5 mmol), **3** (1.175 g, 5 mmol), and powdered K_2CO_3 (5.5 g, 40 mmol) in DMF (50 mL) was stirred at room temperature overnight and diluted with water. The precipitate was collected by filtration and washed with water and then with EtOH to give 2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (0.49 g, 20%): ^{1}H NMR ($CDCl_3$) δ 8.29−8.25 (m, 1H), 7.79−7.75 (m, 1H), 7.37−7.33 (m, 2H), 5.33 (s, 2H), 3.99−3.94 (m, 4H), 3.83−3.78 (m, 4H), 0.85 (s, 9H), 0.08 (s, 6H).

A mixture of the above chloro compound (0.31 g, 0.67 mmol) and morpholine (0.59 g, 6.7 mmol) in THF (10 mL) was stirred at room temperature for 1 h and diluted with water. Extraction with CH_2Cl_2 followed by chromotography on silica, eluting with CH_2Cl_2/EtOAc (95:5), gave 2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (**7k**) (0.25 g, 73%): ^{1}H NMR ($CDCl_3$) δ 8.18−8.14 (m, 1H), 7.79−7.75 (m, 1H), 7.32−7.28 (m, 2H), 5.33 (s, 2H), 3.91−3.85 (m, 8H), 3.79−3.75 (m, 8H), 0.85 (s, 9H), 0.07 (s, 6H).

Reaction of the above crude silyl ether **7k** with Bu_4NF in THF gave **7e** (0.123 g, 63%): mp (EtOH) 271−273 °C (lit.[47] 208−210 °C dec); ^{1}H NMR ($CDCl_3$) δ 12.49 (br, 1H), 7.54−7.49 (m, 2H), 7.19−7.14 (m, 2H), 5.48 (s, 2H), 3.70−3.65 (m, 8H), 3.60−3.54 (m, 8H).

1-[4,6-Di(4-morpholinyl)-1,3,5-triazin-2-yl]-2-[(methylsulfanyl)methyl]-1*H*-benzimidazole (7f). A mixture of 2-[(methylsulfanyl)methyl]-1*H*-benzimidazole (**6f**)[54] (7.13 g, 40 mmol), **3** (9.40 g, 40 mmol), and powdered K_2CO_3 (22 g, 0.16 mol) in DMF (250 mL) was stirred at room temperature for 2 h and diluted with water. The precipitate was collected by filtration and washed successively with water and EtOH to give 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-[(methylsulfanyl)-methyl]-1*H*-benzimidazole (13.7 g, 91%): mp (CH_2Cl_2/MeOH) 213−215 °C; ^{1}H NMR ($CDCl_3$) δ 8.39−8.34 (m, 1H), 7.75−7.70 (m, 1H), 7.40−7.33 (m, 2H), 4.41 (s, 2H), 4.00−3.91 (m, 4H), 3.86−3.78 (m, 4H), 2.14 (s, 3H). Anal. Calcd for $C_{16}H_{17}ClN_6OS$: C, 51.0; H, 4.55; N, 22.3. Found: C, 51.1; H, 4.85; N, 22.6%.

A mixture of the above chloro compound (3.769 g, 10 mmol) and morpholine (4.4 g 50 mmol) in THF (250 mL) was heated under reflux for 10 min, and the solvent was concentrated. Dilution with water gave **7f** (3.91 g, 91%): mp (CH_2Cl_2/MeOH) 195−197 °C; ^{1}H NMR ($CDCl_3$) δ 8.27−8.23 (m, 1H), 7.75−7.71 (m, 1H), 7.34−7.29 (m, 2H), 4.14 (s, 2H), 3.93−3.85 (m, 8H), 3.82−3.76 (m, 8H), 2.16 (s, 3H). Anal. Calcd for $C_{20}H_{25}N_7O_2S$: C, 56.2; H, 5.9 N, 22.9. Found: C, 56.5; H, 6.2; N, 23.2%.

1-[4,6-Di(4-morpholinyl)-1,3,5-triazin-2-yl]-2-[(methylsulfinyl)methyl]-1*H*-benzimidazole (7g). A solution of **7f** (0.43 g, 1 mmol) in EtOH (500 mL) was treated with a solution of $NaIO_4$ (1.6 g, 7.5 mmol) in water (75 mL), and the mixture was stirred at room temperature overnight. The solution was decolorized with aqueous Na_2SO_3, and the EtOH was removed under vacuum. The residue was diluted with water and extracted with EtOAc. Chromatography on alumina, eluting with EtOAc, followed by recrystallization from MeOH gave **7g** (0.29 g, 65%): mp 208−210 °C; ^{1}H NMR ($CDCl_3$) δ 8.29−8.23 (m, 1H), 7.76−7.71 (m, 1H), 7.39−7.31 (m, 2H), 5.29 (dd, J = 12.6, 0.5 Hz, 1H), 4.66 (d, J = 12.6 Hz, 1H), 3.94−3.86 (m, 8H), 3.82−3.76 (m, 8H), 2.78 (s, 3H). Anal. Calcd for $C_{20}H_{25}N_7O_3S$: C, 54.2; H, 5.7; N, 22.1. Found: C, 54.4; H, 5.9; N, 22.4%.

1-[4,6-Di(4-morpholinyl)-1,3,5-triazin-2-yl]-2-[(methylsulfonyl)methyl]-1*H*-benzimidazole (7h). A solution of **7f** (0.43 g, 1 mmol) in a mixture of acetone (130 mL) and HOAc (20 mL) was treated with a solution of $KMnO_4$ (1 g) in water (10 mL) at room temperature. After 10 min the solution was decolorized with aqueous Na_2SO_3 and the acetone was removed under vacuum. The residue was diluted with aqueous NH_3 to give a solid which was collected and dried. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (2:1), gave **7h** (0.426 g, 92%): mp (MeOH) 232−234 °C; ^{1}H NMR ($CDCl_3$) δ 8.28−8.23 (m, 1H), 7.78−7.73 (m, 1H), 7.40−7.33 (m, 2H), 5.47 (d, J = 0.4 Hz, 2H), 3.92−3.85 (m, 8H), 3.81−3.75 (m, 8H), 3.09 (s, 3H). Anal. Calcd for $C_{20}H_{25}N_7O_4S$: C, 52.3; H, 5.5; N, 21.3. Found: C, 52.5; H, 5.2; N, 21.6%.

1-[4,6-Di(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(methylsulfanyl)-1*H*-benzimidazole (7i). A mixture of 2-(methylsulfanyl)-1*H*-benzimidazole (**6i**) (1.64 g, 10 mmol), **3** (2.35 g, 10 mmol), and powdered K_2CO_3 (11 g, 80 mmol) in DMF (50 mL) was stirred at room temperature for 1 h. The mixture was diluted with water and the resulting precipitate was collected, washed with water and then EtOH, and dried to give 3.56 g (98% yield) of 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(methylsulfanyl)-1*H*-benzimidazole: mp ($CHCl_3$/EtOH) 260−261 °C; ^{1}H NMR ($CDCl_3$) δ 8.42 (br dd, J = 7.0, 1.9 Hz, 1H), 7.65 (br dd, J = 6.8, 1.9 Hz, 1H), 7.30 (m, 2H), 4.06 (m, 2H), 3.95 (m, 2H), 3.84 (m, 2H), 3.80 (m, 2H), 2.74 (s, 3H). Anal. Calcd for $C_{15}H_{15}ClN_6OS$: C, 49.65; H, 4.2; N, 23.2. Found: C, 49.8; H, 4.1; N, 23.1%.

A solution of the above chloro compound (0.363 g, 1 mmol) and morpholine (0.87 g, 10 mmol) in $CHCl_3$ (50 mL) was stirred at room temperature for 1 h. The solution was washed with water, and after being dried (Na_2SO_4), the solvent was removed to give **7i** (0.41 g, 100% yield): mp ($CHCl_3$/EtOH) 249−252 °C; ^{1}H NMR ($CDCl_3$) δ 8.30 (dd, J = 7.3, 1.5 Hz, 1H), 7.66 (dd, J = 7.4, 1.7 Hz, 1H), 7.29−7.21 (m, 2H), 4.01−3.83 (m, 8H), 3.81−3.75 (m, 8H), 2.72 (s, 3H). Anal. Calcd for $C_{19}H_{23}N_7O_2S$: C, 55.2; H, 5.6; N, 23.7. Found: C, 55.1; H, 5.7; N, 23.7%.

1-[4,6-Di(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(methylsulfonyl)-1*H*-benzimidazole (7j). A solution of **7i** (0.41 g, 1 mmol) in a mixture of acetone (250 mL) and HOAc (35 mL) was treated with a solution of $KMnO_4$ (1 g) in water (50 mL), and the resulting mixture was stirred for 15 min. After dilution with water and decolorization with aqueous Na_2SO_3, the product was extracted into $CHCl_3$, washed with aqueous NH_3, and dried (Na_2SO_4). Chromatography on alumina, eluting with CH_2Cl_2, followed by recrystallization from MeOH gave **7j** (0.112 g, 25% yield): mp (MeOH) 174−177 °C; ^{1}H NMR ($CDCl_3$) δ 8.25 (br d, J = 8.4 Hz, 1H), 7.82 (br d, J = 8.0 Hz, 1H), 7.48 (ddd, J = 8.4, 7.3, 1.3 Hz, 1H), 7.48 (ddd, J = 8.0, 7.3, 1.3 Hz, 1H), 4.41−3.96 (m, 4H), 3.90−3.83 (m, 4H), 3.79−3.75 (m, 8H), 3.58 (s, 3H). Anal. Calcd for $C_{19}H_{23}N_7O_4S$: C, 51.2; H, 5.2; N, 22.0. Found: C, 51.25; H, 5.25; N, 22.05%.

2-(Difluoromethyl)-4-hydroxy-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (10a). 2-Amino-3-nitrophenol (7.71 g, 50 mmol) in EtOH (100 mL) was hydrogenated over 5% Pd on carbon, and the solution was filtered through Celite and acidified with concentrated HCl. The solvents were evaporated to dryness, and the residue was combined with difluoroacetic acid (9.6 g, 100 mmol) in 4 M HCl (30 mL). The mixture was heated under reflux for 3 h before being cooled and basified with aqueous NH_3. Extraction with EtOAc, followed by chromatography on silica, eluting with CH_2Cl_2/EtOAc (7:3), gave 2-(difluoromethyl)-4-hydroxy-1*H*-benzimidazole (**8a**) (7.68 g, 83%) as an oil: ^{1}H NMR ($CDCl_3$) δ 9.80 (br, 1H), 9.46 (br, 1H), 7.28 (t, J = 8.0 Hz, 1H), 7.08 (t, J_{HF} = 53.4 Hz, 1H), 7.05 (br, 1H), 6.88 (d, J = 7.9 Hz, 1H).

A mixture of **8a** (7.68 g, 42 mmol) and TBDMSCl (10.7 g, 71 mmol) in pyridine (100 mL) was stirred at room temperature overnight. The pyridine was removed under vacuum, and the residue was diluted with

water and extracted with CH_2Cl_2. Chromatography on alumina, eluting with CH_2Cl_2, gave 4-(*tert*-butyldimethylsilyloxy)-2-(difluoromethyl)-1*H*-benzimidazole (**8aa**) (11.9 g, 96%): mp (CH_2Cl_2/hexanes) 139−141 °C; ^{1}H NMR ($CDCl_3$) δ 9.60 (br, 1H), 7.50−7.30 (m, 1H), 7.19 (t, J = 8.0 Hz, 1H), 6.89 (t, J_{HF} = 53.9 Hz, 1H), 6.79 (d, J = 8.3 Hz, 1H), 1.04 (s, 9H), 0.29 (s, 6H).

A mixture of **8aa** (1.49 g, 5 mmol), **3** (1.175 g, 5 mmol), and powdered K_2CO_3 (5.5 g, 40 mmol) in acetone (40 mL) was stirred at room temperature for 2 h and diluted with water. Extraction with CH_2Cl_2 and chromatography on silica, eluting with CH_2Cl_2/hexanes (3:1), gave 4-(*tert*-butyldimethylsilyloxy)-1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-1*H*-benzimidazole (**9aa**) (1.27 g, 51%): mp (hexanes) 143−145 °C; ^{1}H NMR ($CDCl_3$) δ 7.99 (d, J = 8.4 Hz, 1H), 7.46 (t, J_{HF} = 53.5 Hz, 1H), 7.32 (t, J = 8.2 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 3.98−3.93 (m, 3.5H), 3.89−3.86 (m, 0.5H), 3.84−3.78 (m, 3.5H), 3.76−3.73 (m, 0.5H), 1.05 (s, 9H), 0.29 (s, 6H). Anal. Calcd for $C_{21}H_{27}ClF_2N_6O_2Si$: C, 50.75; H, 5.5; N, 16.9. Found: C, 50.7; H, 5.6; N, 17.0%.

A solution of **9aa** (0.25 g, 0.5 mmol) and morpholine (0.44 g, 5 mmol) in THF (10 mL) was stirred at room temperature for 30 min and diluted with water. Extraction with EtOAc and chromatography on silica, eluting with CH_2Cl_2/hexanes (19:1), gave 4-{[*tert*-butyl(dimethyl)-silyl]oxy}-2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (**10aa**) (0.25 g, 91%): ^{1}H NMR ($CDCl_3$) δ 7.89 (dd, J = 8.3, 0.8 Hz, 1H), 7.45 (t, J_{HF} = 53.6 Hz, 1H), 7.26 (t, J = 8.1 Hz, 1H), 6.84 (dd, J = 7.8, 0.8 Hz, 1H), 3.90−3.85 (m, 8H), 3.80−3.75 (m, 8H), 1.05 (s, 9H), 0.30 (s, 6H).

A solution of **10aa** (0.25 g, 0.46 mmol) in THF (10 mL) was treated with an excess of Bu_4NF (1 M in THF) at room temperature for 30 min, and the solvent was removed under vacuum. Addition of water gave a white solid which was collected and dried to give **10a** (0.195 g, 98%): mp (EtOH) 288−290 °C (lit.[47] mp >250 °C); ^{1}H NMR ($CDCl_3$) δ 7.81 (d, J = 8.2 Hz, 1H), 7.55 (t, J_{HF} = 53.6 Hz, 1H), 7.31 (t, J = 8.2 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 3.90−3.85 (m, 8H), 3.81−3.75 (m, 8H).

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-methoxy-1*H*-benzimidazole (10b). 2-Amino-3-methoxynitrobenzene[55] (15.10 g, 0.09 mol) was hydrogenated over palladium on carbon in MeOH, and the solution was filtered through Celite into a methanolic HCl solution. The solvent was removed under vacuum, and the resulting hydrochloride salt was combined with difluoroacetic acid (19.2 g, 0.18 mol) and 4 M HCl (100 mL). The mixture was heated under reflux for 3 h, diluted with water, decolorized with charcoal, and filtered through Celite. Neutralization with aqueous ammonia gave 2-difluoromethyl-4-methoxy-1*H*-benzimidazole[56] (**8b**) (15.2 g, 84%) as a solid: ^{1}H NMR ($CDCl_3$) (tautomeric mixture) δ 9.95−9.70 (m, exchangeable with D_2O, 1H), 7.44 (br d, J = 7.9 Hz, 0.4H), 7.31−7.24 (m, 1H), 7.12 (br d, J = 8.0 Hz, 0.5H), 6.89 (t, J_{HF} = 53.8 Hz, 1H), 6.82−6.74 (m, 1H), 4.03 and 3.98 (2s, 3H).

A mixture of **8b** (3.96 g, 20 mmol), **3** (4.70 g, 20 mmol), and powdered K_2CO_3 (22 g, 80 mmol) in DMF (150 mL) was stirred rapidly for 3 h and then diluted with water. The resulting precipitate was collected by filtration, washed with water and then with EtOH, and oven-dried to give 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-4-methoxy-1*H*-benzimidazole (**9b**) (6.82 g, 86%): mp ($CHCl_3$/EtOH) 263−266 °C; ^{1}H NMR ($CDCl_3$) δ 7.99 (d, J = 8.4 Hz, 1H), 7.48 (t, J_{HF} = 53.4 Hz, 1H), 7.40 (t, J = 8.3 Hz, 1H), 6.86 (d, J = 8.1 Hz, 1H), 4.05 (s, 3H), 3.99−3.93 (m, 4H), 3.84−3.78 (m, 4H). Anal. Calcd for $C_{16}H_{15}ClF_2N_6O_2$: C, 48.4; H, 3.8; N, 21.2. Found: C, 48.3; H, 3.8; N, 21.1%.

A suspension of **9b** (0.199 g, 0.5 mmol) in morpholine (0.87 g, 10 mmol) was heated at 70 °C for 1 h and diluted with water. The resulting solid was collected by filtration and recrystallized from CH_2Cl_2/EtOH to give **10b** (0.188 g, 84%): mp 270−273 °C (lit.[48] > 250 °C); ^{1}H NMR ($CDCl_3$) δ 7.88 (d, J = 8.4 Hz, 1H), 7.47 (t, J_{HF} = 53.5 Hz, 1H), 7.35 (t, J = 8.2 Hz, 1H), 6.81 (d, J = 8.1 Hz, 1H), 4.05 (s, 1H), 3.90−3.85 (m, 8H), 3.80−3.76 (m, 8H). Anal. Calcd for $C_{20}H_{23}F_2N_7O_3$: C, 53.7; H, 5.2; N, 21.9. Found: C, 53.75; H, 5.1; N, 22.05%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-ethoxy-1*H*-benzimidazole (10c). A solution of 2-amino-3-ethoxynitrobenzene[55] (1.64 g, 9 mmol) in MeOH (100 mL) was hydrogenated over 5% Pd on carbon and filtered through Celite. The solution was acidified with concentrated HCl, and the solvents were removed under vacuum. The residue was combined with difluoroacetic acid (1.73 g, 18 mmol) in 4 M HCl (10 mL), and the mixture was heated under reflux for 3 h. After cooling, the solution was neutralized with aqueous NH_3 to give 2-difluoromethyl-4-ethoxy-1*H*-benzimidazole (**8c**) (1.29 g, 68% yield): mp (aqueous MeOH) 185−187 °C; ^{1}H NMR (DMSO-d_6) δ 13.30 (br, 1H), 7.20 (t, J_{HF} = 53.3 Hz, 1H), 7.22−7.14 (m, 2H), 6.78 (br d, J = 7.5 Hz, 1H), 4.24 (q, J = 7.0 Hz, 2H), 1.41 (t, J = 7.0 Hz, 3H). Anal. Calcd for $C_{10}H_{10}F_2N_2O$: C, 56.6; H, 4.75; N, 13.2. Found: C, 56.9; H, 4.8; N, 13.4%.

A mixture of **8c** (0.85 g, 4 mmol), **3** (0.49 g, 4 mmol), and powdered K_2CO_3 (4.4 g, 32 mmol) in DMF (25 mL) was stirred overnight and then diluted with water. The resulting precipitate was collected by filtration, washed with water and then with EtOH, and dried to give 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-4-ethoxy-1*H*-benzimidazole (**9c**) (1.48 g, 90%): mp ($CHCl_3$/EtOH) 272−275 °C; ^{1}H NMR ($CDCl_3$) δ 7.88 (d, J = 8.4 Hz, 1H), 7.47 (t, J_{HF} = 53.4 Hz, 1H), 7.38 (t, J = 8.3 Hz, 1H), 6.86 (d, J = 8.1 Hz, 1H), 4.33 (q, J = 7.0 Hz, 2H), 3.99−3.93 (m, 4H), 3.84−3.78 (m, 4H), 1.56 (t, J = 7.0 Hz, 3H). Anal. Calcd for $C_{17}H_{17}ClF_2N_6O_2$: C, 49.7; H, 4.2; N, 20.5. Found: C, 49.8; H, 4.4; N, 20.6%.

A mixture of **9c** (0.205 g, 0.5 mmol) and morpholine (0.44 g, 0.5 mmol) in $CHCl_3$ (25 mL) was stirred at room temperature for 30 min and washed with water. The solvent was then evaporated to dryness to give **10c** (0.201 g, 98%); mp ($CHCl_3$/EtOH) 225−228 °C; ^{1}H NMR ($CDCl_3$) δ 7.87 (d, J = 8.4 Hz, 1H), 7.47 (t, J_{HF} = 53.5 Hz, 1H), 7.32 (t, J = 8.2 Hz, 1H), 6.81 (d, J = 8.1 Hz, 1H), 4.33 (q, J = 7.0 Hz, 2H), 3.90−3.85 (m, 8H), 3.80−3.76 (m, 8H), 1.55 (t, J = 7.0 Hz, 3H). Anal. Calcd for $C_{21}H_{25}F_2N_7O_3$: C, 54.7; H, 5.5; N, 21.25. Found: C, 54.85; H, 5.7; N, 21.5%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-propoxy-1*H*-benzimidazole (10d). A mixture of **10a** (108 mg, 0.25 mmol), 1-iodopropane (0.47 mg, 0.28 mmol), and powdered K_2CO_3 (0.40 g, 2.9 mmol) in DMF (5 mL) was stirred at room temperature overnight and diluted with water to give **10d** (102 mg, 86%): mp (CH_2Cl_2/MeOH) 192−194 °C; ^{1}H NMR ($CDCl_3$) δ 7.87 (d, J = 8.3 Hz, 1H), 7.47 (t, J_{HF} = 53.5 Hz, 1H), 7.32 (t, J = 8.2 Hz, 1H), 6.81 (d, J = 8.0 Hz, 1H), 4.21 (t, J = 6.9 Hz, 2H), 3.90−3.85 (m, 8H), 3.80−3.76 (m, 8H), 1.97 (sextet, J = 7.2 Hz, 2H), 1.08 (t, J = 7.4 Hz, 3H). Anal. Calcd for $C_{22}H_{27}F_2N_7O_3$: C, 55.6; H, 5.7; N, 20.6. Found: C, 55.8; H, 5.8; N, 20.8%.

4-Butoxy-2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (10e). A mixture of **10a** (108 mg, 0.25 mmol), 1-iodobutane (51 mg, 0.28 mmol), and powdered K_2CO_3 (0.40 g, 2.9 mmol) in DMF (5 mL) was stirred at room temperature overnight and diluted with water to give **10e** (95 mg, 69%): mp (CH_2Cl_2/MeOH) 211−213 °C; ^{1}H NMR ($CDCl_3$) δ 7.86 (d, J = 8.1 Hz, 1H), 7.46 (t, J_{HF} = 53.5 Hz, 1H), 7.32 (t, J = 8.2 Hz, 1H), 6.81 (d, J = 8.0 Hz, 1H), 4.26 (t, J = 6.8 Hz, 2H), 3.90−3.85 (m, 8H), 3.80−3.76 (m, 8H), 1.93 (m, 2H), 1.55 (m, 2H), 0.99 (t, J = 7.4 Hz, 3H). Anal. Calcd for $C_{23}H_{29}F_2N_7O_3$: C, 56.4; H, 6.0; N, 20.0. Found: C, 56.7; H, 5.9; N, 20.2%.

4-(Difluoromethoxy)-2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (10f). A mixture of **10a** (228 mg, 0.52 mmol) and dry powdered K_2CO_3 (1.8 g, 13.6 mmol) in DMF (5 mL) was stirred at 20 °C for 30 min. Then sodium chloro(difluoro)acetate (396 mg, 2.6 mmol) was added and the

mixture was stirred at 80 °C for 20 h. The reaction mixture was cooled to room temperature and diluted with water. The resulting precipitate was collected by filtration, washed with water, and dried. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (9:1), gave **10f** (105 mg, 41%): mp (CH_2Cl_2/hexanes) 231−233 °C; ^{1}H NMR ($CDCl_3$) δ 8.14 (dd, J = 8.4, 0.8 Hz, 1H), 7.49 (t, J_{HF} = 53.3 Hz, 1H), 7.32 (t, J_{HF} = 74.6 Hz, 1H), 7.17 (dd, J = 8.0, 0.3 Hz, 1H), 3.89−3.87 (m, 8H), 3.80−3.77 (m, 8H). Anal. Calcd for $C_{20}H_{21}F_4N_7O_3$: C, 49.7; H, 4.4; N, 20.3. Found: C, 49.7; H, 4.4; 20.3%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-isopropoxy-1*H*-benzimidazole (10g). A mixture of **10a** (108 mg, 0.25 mmol), 2-iodopropane (0.47 mg, 0.28 mmol), and powdered K_2CO_3 (0.40 g, 2.9 mmol) in DMF (5 mL) was stirred at 20 °C overnight and diluted with water to give **10g** (121 mg, 100%): mp (CH_2Cl_2/MeOH) 218−220 °C; ^{1}H NMR ($CDCl_3$) δ 7.85 (d, J = 8.2 Hz, 1H), 7.46 (t, J_{HF} = 53.5 Hz, 1H), 7.31 (t, J = 8.2 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 4.96 (septet, J = 6.1 Hz, 1H), 3.90−3.85 (m, 8H), 3.80−3.76 (m, 8H), 1.47 (d, J = 6.1 Hz, 6H). Anal. Calcd for $C_{22}H_{27}F_2N_7O_3$: C, 55.6; H, 5.7; N, 20.6. Found: C, 55.6; H, 5.85; N, 20.7%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazol-4-yl Methanesulfonate (10h). A solution of 2-amino-3-nitrophenol (1.54 g, 1 mmol) in pyridine (20 mL) was cooled to 0 °C, and methanesulfonyl chloride (1.73 g, 1.5 mmol) was added with stirring. The temperature was allowed to rise to room temperature as stirring was continued overnight. Water was added to give a solid which was collected and dried. The solid was dissolved in CH_2Cl_2 and eluted through a short column of alumina to remove polar impurities. Removal of the solvent gave 2-amino-3-nitrophenyl methanesulfonate (2.06 g, 89% yield): mp (CH_2Cl_2/MeOH) 128−130 °C; ^{1}H NMR ($CDCl_3$) δ 8.12 (dd, J = 8.8, 1.4 Hz, 1H), 7.50 (dd, J = 7.9, 1.3 Hz, 1H), 6.73 (dd, J = 8.6, 8.1 Hz, 1H), 6.35 (br, 2H), 3.27 (s, 3H). Anal. Calcd for $C_7H_8N_2O_5S$: C, 36.2; H, 3.5; N, 12.1. Found: C, 36.5; H, 3.7; N, 12.1%.

A solution of the above compound (1.16 g, 5 mmol) in MeOH (50 mL) was hydrogenated over 5% Pd on carbon, and after filtration through Celite, the solution was acidified with concentrated HCl. The solvent was evaporated to dryness, and the residue was combined with difluoroacetic acid (0.96 g, 10 mmol) in 4 M HCl (20 mL). The mixture was heated under reflux for 4 h, cooled, and neutralized with aqueous NH_3. Extraction with EtOAc gave 2-(difluoromethyl)-1*H*-benzimidazol-4-yl methanesulfonate (**8h**) (0.71 g, 54% yield): mp (MeOH/*i*-Pr_2O) 160−162 °C; ^{1}H NMR (DMSO-d_6) δ 13.76 (br, 1H), 7.65−7.59 (m, 1H), 7.38 (t, J = 8.0 Hz, 1H), 7.31 (t, J_{HF} = 53.1 Hz, 1H), 7.28 (d, J = 7.7 Hz, 1 H), 3.53 (s, 3H). Anal. Calcd for $C_9H_8F_2N_2O_3S$: C, 41.2; H, 3.1. Found: C, 41.1; H, 3.3%.

A mixture of **8h** (1.57 g, 6 mmol), **3** (1.41 g, 6 mmol), and powdered K_2CO_3 (6.6 g, 48 mmol) in acetone (50 mL) was stirred at room temperature for 3 h and then diluted with water. Extraction with EtOAc, followed by chromatography on silica, eluting with CH_2Cl_2/EtOAc (9:1), gave 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-1*H*-benzimidazol-4-yl methanesulfonate (**9h**) (1.70 g, 61% yield): mp (CH_2Cl_2/EtOH) 208−210 °C; ^{1}H NMR ($CDCl_3$) δ 8.38 (dd, J = 8.4, 0.8 Hz, 1H), 7.52 (t, J_{HF} = 53.2 Hz, 1H), 7.50 (t, J = 8.2 Hz, 1H), 7.41 (dd, J = 8.1, 0.7 Hz, 1H), 4.10−3.97 (m, 2H), 3.95−3.92 (m, 2H), 3.85−3.80 (m, 4H), 3.50 (s, 3H). Anal. Calcd for $C_{16}H_{15}ClF_2N_6O_4S$: C, 41.7; H, 3.3; N, 13.2. Found: C, 42.0; H, 3.3; N 13.4%.

A mixture of **9h** (0.23 g, 0.5 mmol) and morpholine (0.22 g, 6 mmol) in THF (20 mL) was stirred at room temperature for 15 min and diluted with water to give a solid which was recrystallized from $CHCl_3$/EtOH to give **10h** (0.25 g, 97% yield): mp 249−251 °C; ^{1}H NMR ($CDCl_3$) δ 8.27 (dd, J = 8.3, 0.9 Hz, 1H), 7.50 (t, J_{HF} = 53.3 Hz, 1H), 7.44 (t, J = 8.2 Hz, 1H), 7.37 (dd, J = 8.0, 0.9 Hz, 1H), 3.89−3.85 (m, 8H), 3.80−3.76 (m, 8H), 3.51 (s, 3H). Anal. Calcd for $C_{20}H_{23}ClF_2N_7O_5S$: C, 47.0; H, 4.5; N, 19.2. Found: C, 46.6; H, 4.6; N 19.0%.

4-Amino-2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (10i). A mixture of 1,2-diamino-3-nitrobenzene (5 g, 33 mmol) and difluoroacetic acid (15 g, 0.156 mol) in 4 M HCl (75 mL) was heated under reflux for 4 days and cooled to give 2-(difluoromethyl)-4-nitro-1*H*-benzimidazole (**8ae**)[57] (4.74 g, 68%): ^{1}H NMR (DMSO-d_6) δ 14.00 (br, 1H), 8.26 (d, J = 8.1 Hz, 1H), 8.23 (d, J = 8.0 Hz, 1H), 7.53 (t, J = 8.1 Hz, 1H), 7.31 (t, J_{HF} = 52.7 Hz, 1H).

A mixture of **8ae** (1.07 g, 5 mmol), **3** (1.29 g, 5.5 mmol), and powdered K_2CO_3 (6.5 g, 47 mmol) in THF (50 mL) was stirred at room temperature overnight and then diluted with water to give a solid, which was collected and dried. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (19:1), gave 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-4-nitro-1*H*-benzimidazole (**9ae**) (0.90 g, 44%): mp (CH_2Cl_2/EtOH) 236−238 °C; ^{1}H NMR ($CDCl_3$) δ 8.83 (dd, J = 8.4, 1.0 Hz, 1H), 8.24 (dd, J = 8.1, 1.0 Hz, 1H), 7.62 (t, J = 8.3 Hz, 1H), 7.50 (t, J_{HF} = 53.0 Hz, 1H), 4.02−3.99 (m, 2H), 3.97−3.94 (m, 2H), 3.86−3.81 (m, 4H). Anal. Calcd for $C_{15}H_{12}ClF_2N_7O_3$: C, 43.75; H, 2.9; N, 23.8. Found: C, 44.0; H, 3.1; N, 24.0%.

A mixture of **9ae** (0.90 g, 2.2 mmol) and morpholine (0.95 g, 11 mmol) in THF (20 mL) was stirred at room temperature for 1 h and diluted with water to give 2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-nitro-1*H*-benzimidazole (**10ae**) (1.01 g, 99%): mp (CH_2Cl_2/EtOH) 292−295 °C; ^{1}H NMR ($CDCl_3$) δ 8.66 (dd, J = 8.4, 0.9 Hz, 1H), 8.21 (dd, J = 8.1, 0.9 Hz, 1H), 7.55 (t, J = 8.2 Hz, 1H), 7.51 (t, J_{HF} = 53.1 Hz, 1H), 3.90−3.85 (m, 8H), 3.80−3.76 (m, 8H). Anal. Calcd for $C_{19}H_{20}F_2N_8O_4$: C, 49.35; H, 4.4; N, 24.2. Found: C, 49.55; H, 4.5; N, 24.4%.

A suspension of **10ae** (0.925 g, 2 mmol) in MeOH (100 mL) was hydrogenated over 5% Pd on C to give a solution, which was filtered through Celite to remove the catalyst. Removal of the solvent, followed by chromatography on alumina, eluting with CH_2Cl_2/EtOAc (19:1), gave **10i** (0.79 g, 91%): mp (MeOH) 229−231 °C (lit.[38] mp 214−216 °C); ^{1}H NMR (DMSO-d_6) δ 7.69 (t, J_{HF} = 53.0 Hz, 1H), 7.44 (dd, J = 8.2, 0.8 Hz, 1H), 7.13 (t, J = 8.1 Hz, 1H), 6.55 (dd, J = 7.8, 0.8 Hz, 1H), 5.59 (br s, 2H), 3.81−3.77 (m, 8H), 3.70−3.65 (m, 8H). Anal. Calcd for $C_{19}H_{22}F_2N_8O_2$: C, 52.8; H, 5.1; N, 25.9. Found: C, 52.9; H, 5.3; N, 26.2%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-(methylamino)-1*H*-benzimidazole (10j). A mixture of **10i** (0.23 g, 0.53 mmol) and di-*tert*-butyl dicarbonate (0.35 g, 1.6 mmol) in dioxane (50 mL) was heated under reflux for 2 days. Dilution with water gave a white solid which was collected and dried. Chromatography on alumina, eluting with CH_2Cl_2, gave *tert*-butyl 2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazol-4-ylcarbamate (0.21 g, 55% yield): mp (CH_2Cl_2/MeOH) 248−251 °C; ^{1}H NMR ($CDCl_3$) δ 8.05 (d, J = 8.0 Hz, 1H), 7.90 (dd, J = 8.3, 0.6 Hz, 1H), 7.80 (br s, exchangeable with D_2O, 1H), 7.56 (t, J_{HF} = 53.6 Hz, 1H), 7.38 (t, J = 8.2 Hz, 1H), 3.89−3.85 (m, 8H), 3.80−3.75 (m, 8H), 1.55 (s, 9H). Anal. Calcd for $C_{24}H_{30}F_2N_8O_9$: C, 54.2; H, 5.7; N, 21.0. Found: C, 54.0; H, 5.7; N, 21.3%.

The above carbamate (0.20 g, 0.38 mmol) in DMF (20 mL) was treated with NaH (60%, 24 mg, 6 mmol), and the mixture was stirred at room temperature for 15 min before iodomethane (82 mg, 0.58 mmol) was added. The resulting mixture was stirred for 30 min, and water was added. The solid was collected and dried. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (9:1), gave *tert*-butyl 2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazol-4-yl(methyl)-carbamate (0.14 g, 67%): ^{1}H NMR ($CDCl_3$) δ 8.17 (dd, J = 8.3, 0.9 Hz, 1H), 7.51 (t, J_{HF} = 49.2 Hz, 1H), 7.37 (t, J = 8.1 Hz, 1H), 7.90 (dd, J = 8.3, 0.6 Hz, 1H), 7.26 (m, 1H), 3.90−3.85 (m, 8H), 3.81−3.76 (m, 8H), 3.45 (s, 3H), 1.42 (s, 9H).

The above carbamate (0.14 g, 0.26 mmol) was treated with TFA in CH_2Cl_2 to give **10j** (0.11 g, 99%): mp (CH_2Cl_2/MeOH) 285−288 °C; ^{1}H NMR ($CDCl_3$) δ 7.56 (d, J = 8.3 Hz, 1H), 7.53 (t, J_{HF} = 53.8 Hz, 1H), 7.28 (t, J = 8.1 Hz, 1H), 6.47 (d, J = 7.9 Hz, 1H), 5.06 (br d, J = 4.3 Hz, 1H), 3.89−3.84 (m, 8H), 3.79−3.75 (m, 8H), 3.00 (d, J = 4.5 Hz, 3H). Anal. Calcd for $C_{20}H_{24}F_2N_8O_2$: C, 53.8; H, 5.4; N, 25.1. Found: C, 53.9; H, 5.5; N, 25.3%.

2-(Difluoromethyl)-4-(dimethylamino)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (10k). N^1,N^1-dimethyl-2-nitro-1,3-benzenediamine[58] (1.09 g, 6 mmol) in MeOH (50 mL) was hydrogenated over Pd on carbon, and the solution was filtered through Celite and acidified with concentrated HCl. The solvent was evaporated to dryness, and the residue was combined with difluoroacetic acid (3 g, 31 mmol) in 4 M HCl (20 mL). The mixture was heated under reflux for 3 h, clarified with charcoal, cooled and neutralized with aqueous NH_3. Extraction with EtOAc, followed by chromatography on silica, eluting with CH_2Cl_2/EtOAc (9:1), gave 2-(difluoromethyl)-4-(dimethylamino)-1*H*-benzimidazole (**8k**) (0.77 g, 61% yield): mp (CH_2Cl_2/hexanes) 100−101 °C; ^{1}H NMR ($CDCl_3$) δ 9.49 (br, 1H), 7.22 (t, J = 8.0 Hz, 1H), 6.94 (d, J = 8.0 Hz, 1H), 6.86 (t, J_{HF} = 53.9 Hz, 1H), 6.54 (d, J = 7.9 Hz, 1H), 3.21 (s, 6H). Anal. Calcd for $C_{10}H_{11}F_2N_3$: C, 56.9; H, 5.25; N, 19.9. Found: C, 57.0; H, 5.3; N, 20.1%.

A mixture of **8k** (0.422 g, 2 mmol), **3** (0.564 g, 2.4 mmol), and powdered K_2CO_3 (2.8 g, 20 mmol) in THF (20 mL) was stirred at room temperature for 3 days and then diluted with water. Extraction with CH_2Cl_2 followed by chromatography on silica, eluting with CH_2Cl_2/EtOAc (49:1), gave 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-4-(dimethylamino)-1*H*-benzimidazole (**9k**) (0.50 g, 61%): mp (CH_2Cl_2/MeOH) 200−201 °C; ^{1}H NMR ($CDCl_3$) δ 7.82 (dd, J = 8.3, 0.7 Hz, 1H), 7.48 (t, J_{HF} = 53.7 Hz, 1H), 7.31 (t, J = 8.2 Hz, 1H), 6.94 (d, J = 7.8 Hz, 1H), 3.98−3.94 (m, 4H), 3.83−3.78 (m, 4H), 3.24 (s, 6H). Anal. Calcd for $C_{17}H_{18}ClF_2N_7O$: C, 49.8; H, 4.4; N, 23.9. Found: C, 50.4; H, 4.4; N, 24.0%.

A mixture of **9k** (0.10 g, 0.25 mmol) and morpholine (0.22 g, 2.5 mmol) in THF (10 mL) was stirred at room temperature for 30 min and diluted with water to give **10k** (97 mg, 84%): mp (CH_2Cl_2/MeOH) 231−233 °C; ^{1}H NMR ($CDCl_3$) δ 7.74 (dd, J = 8.3, 0.6 Hz, 1H), 7.46 (t, J_{HF} = 53.7 Hz, 1H), 7.27 (t, J = 8.1 Hz, 1H), 6.61 (d, J = 8.0 Hz, 1H), 3.89−3.85 (m, 8H), 3.79−3.75 (m, 8H), 3.23 (s, 6H). Anal. Calcd for $C_{21}H_{26}F_2N_8O_2$: C, 54.8; H, 5.7; N, 24.3. Found: C, 54.8; H, 5.7; N, 24.1%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-(hydroxymethyl)-1*H*-benzimidazole (10l). A mixture of 2,3-diaminobenzoic acid 3.42 g, 18.13 mmol) and difluoroacetic acid (6.96 g, 4.6 mL, 72.5 mmol) in 4 M HCl (20 mL) was refluxed for 16 h and cooled to room temperature. The reaction mixture was diluted with water (20 mL) and basified with aqueous NH_3. The resulting clear solution was filtered and neutralized with aqueous HOAc to produce a precipitate, which was collected and dried to give 2-(difluoromethyl)-1*H*-benzimidazole-4-carboxylic acid (**8ah**) (3.7 g, 96% yield): mp (aq MeOH) 280−282 °C; ^{1}H NMR (DMSO-d_6) δ 13.39−13.10 (br, 2H), 8.01 (d, J = 8.0 Hz, 1H), 7.93 (dd, J = 7.6, 0.7 Hz, 1H), 7.40 (t, J = 7.7 Hz, 1H), 7.25 (J_{HF} = 53.3 Hz, 1H). Anal. Calcd for $C_9H_6F_2N_2O_2$: C, 51.0; H; 2.9; F, 17.9; N, 13.2. Found: C, 50.9; H, 3.0, F, 18.2; N, 13.3%.

To a solution of **8ah** (1.0 g, 4.71 mmol) in dry THF (20 mL) at 0 °C was added $LiAlH_4$ (268 mg, 7.05 mmol) in portions. The mixture was stirred at this temperature for 5 min and then heated under reflux for 20 min. After the mixture was cooled to 20 °C, another portion of $LiAlH_4$ (200 mg) was added, and the resulting mixture was heated under reflux for 4 h. The mixture was cooled to 20 °C and slightly acidified by the careful addition of dilute HCl. The mixture was extracted into EtOAc and dried (Na_2SO_4). Removal of the solvents gave 2-(difluoromethyl)-4-(hydroxymethyl)-1*H*-benzimidazole (**8l**) (306 mg, 33%): ^{1}H NMR ($CDCl_3$) δ 7.52 (d, J = 7.6 Hz, 1H), 7.32−7.27 (m, 2H), 7.25 (t, J_{HF} = 53.3 Hz, 1H), 5.19 (br s, 1H), 4.87 (s, 2H), 4.05 (br s, 1H).

A mixture of **8l** (300 mg, 1.51 mmol) and TBDMSCl (456 mg, 3.2 mmol) in pyridine (5 mL) was stirred at room temperature for 3 h. The mixture was diluted with water and extracted into EtOAc. After the mixture was washed with water and dried with Na_2SO_4, the solvent was removed. Chromatography of the residue on silica, eluting with CH_2Cl_2/MeOH (99:1), gave 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-(difluoromethyl)-1*H*-benzimidazole (**8ad**) (443 mg, 94%): ^{1}H NMR ($CDCl_3$) δ 10.22 (br, 1H), 7.73 (d, J = 8.2 Hz, 1H), 7.26 (t, J = 7.8 Hz, 1H), 7.09 (d, J = 7.3 Hz, 1H), 6.89 (t, J_{HF} = 54.0 Hz, 1H), 5.10 (s, 2H), 0.97 (s, 9H), 0.14 (s, 6H).

A mixture of **8ad** (295 mg, 0.94 mmol), **3** (332 mg, 1.5 equiv), and K_2CO_3 (389 mg, 3 equiv) in dry THF (10 mL) was stirred under reflux for 20 h. The mixture was poured into water (100 mL), and the resulting precipitate was collected by filtration and dried. Chromatography on silica, eluting with CH_2Cl_2/hexanes (3:1), gave 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-1*H*-benzimidazole (**9ad**) (225 mg, 47%): ^{1}H NMR ($CDCl_3$) δ 8.29 (dd, J = 8.2, 0.6 Hz, 1H), 7.60 (dd, J = 7.6, 1.0 Hz, 1H), 7.49 (t, J = 7.9 Hz, 1H), 7.56 (t, J_{HF} = 53.5 Hz, 1H), 5.31 (s, 2H), 4.00−3.95 (m, 4H), 3.85−3.80 (m, 4H), 0.99 (s, 9H), 0.15 (s, 6H).

A mixture of **9ad** (220 mg, 0.43 mmol) and morpholine (0.5 mL, excess) in THF (5 mL) was stirred at 20 °C for 18 h, and the mixture was poured into water. The resulting precipitate was collected by filtration, washed with water, and oven-dried to give 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (**10ad**) (241 mg, 100%): ^{1}H NMR ($CDCl_3$) δ 8.19 (d, J = 7.8 Hz, 1H), 7.56 (dd, J = 7.7, 1.1 Hz, 1H), 7.55 (t, J_{HF} = 48.9 Hz, 1H), 7.45−7.41 (m, 1H). 5.32 (s, 2H), 3.88−3.82 (m, 8H), 3.80−3.77 (m, 8H), 0.99 (s, 9H), 0.16 (s, 6H).

To a solution of **10ad** (241 mg, 0.43 mmol) in THF (10 mL) was added TBAF (1 M in THF, 3 mL), and the reaction mixture was stirred at 20 °C for 1 h and diluted with water. Chromatography of the resulting precipitate on silica, eluting with CH_2Cl_2/MeOH (9:1), gave **10l** (102 mg, 53%): mp (CH_2Cl_2/hexanes) 266−270 °C; ^{1}H NMR ($CDCl_3$) δ 8.24 (d, J = 7.6 Hz, 1 H), 7.55 (t, J = 49.9, 1 H), 7.40 (d, J = 8.4 Hz, 1 H), 7.33 (d, J = 6.6 Hz, 1 H), 5.17 (d, J = 6.3 Hz, 2 H), 3.89−3.87 (m, 8 H), 3.80−3.78 (m, 8 H), 3.25 (t, J = 6.4 Hz, 1 H). Anal. Calcd for $C_{20}H_{23}F_2N_7O_3$: C, 53.7; H; 5.2; N, 21.9. Found: C, 53.7; H, 5.2; N, 22.0%.

Methyl 2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole-4-carboxylate (10m). A mixture of **8ah** (223 mg, 1.05 mmol), 2,2-dimethoxypropane, and concentrated H_2SO_4 (1 mL) in MeOH (20 mL) was heated under reflux for 16 h, and the solvent was concentrated. The residue was basified with aqueous NH_3 and extracted with CH_2Cl_2. After the mixture was dried with Na_2SO_4, the solvent was evaporated to dryness, and the resulting residue was chromatographed on silica, eluting with CH_2Cl_2 followed by CH_2Cl_2/MeOH (99.5:0.5) to give methyl 2-(difluoromethyl)-1*H*-benzimidazole-4-carboxylate (**8m**) (237 mg 100%): ^{1}H NMR ($CDCl_3$) δ 10.74 (br s, 1H), 8.04 (t, J = 7.7 Hz, 2H), 7.40 (t, J = 7.9 Hz, 1H), 6.90 (t, J_{HF} = 53.7 Hz, 1H), 4.03 (s, 3H).

A mixture of **8m** (237 mg, 1.05 mmol), **3** (296 mg, 1.26 mmol), and powdered dry K_2CO_3 (290 mg, 2.15 mmol) in THF (20 mL) was stirred at 20 °C for 16 h. The solvent was evaporated to dryness and the residue was chromatographed on silica, eluting with CH_2Cl_2/EtOAc (9:1), to give methyl 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-1*H*-benzimidazole-4-carboxylate (**9m**) (358 mg, 80%): mp (CH_2Cl_2−hexanes) 233−235 °C; ^{1}H NMR ($CDCl_3$) δ 8.69 (dd, J = 8.4, 1.1 Hz, 1H), 8.09 (dd, J = 7.7, 1.0 Hz, 1H), 7.56 (t, J = 8.0 Hz, 1H), 7.50 (t, J_{HF} = 53.2 Hz, 1H), 4.05 (s, 3H), 4.00−3.95 (m, 4H), 3.84−3.80 (m, 4H). Anal. Calcd for $C_{17}H_{15}ClF_2N_6O_3$: C, 48.1; H, 3.6; N, 19.8. Found: C, 48.1; H, 3.4; N, 19.9%.

To a suspension of **9m** 10 (337 mg, 0.79 mmol) in THF (30 mL) was added morpholine (350 mg, 4 mmol), and the reaction mixture was stirred at room temperature for 20 h. The solvent was concentrated, and the residue was diluted with water. The resulting precipitate was collected by filtration, washed with water and hexanes, and recrystallized from CH_2Cl_2/hexanes to give **10m** (361 mg, 96%): mp >290 °C; ^{1}H NMR ($CDCl_3$) δ 8.55 (dd, J = 8.37, 1.14 Hz, 1H), 8.06 (dd, J = 7.64, 1.09 Hz, 1H), 7.50 (t, J_{HF} = 53.3 Hz, 1H), 7.49 (t, J = Hz, 1H), 4.06 (s, 3H), 3.89−3.87 (m, 8H), 3.79−3.77 (m, 8H). Anal. Calcd for $C_{21}H_{23}F_2N_7O_4$: C, 53.1; H, 4.6; N, 20.6. Found: C, 53.0; H, 4.6; N, 20.7%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole-4-carboxamide (10n). A mixture of **8ah** (1.0 g, 4.7 mmol), thionyl chloride (10 mL), and a trace of DMF was refluxed for 1 h and cooled to 20 °C, and the excess thionyl chloride was removed under vacuum. The resulting residue was suspended in 1,4-dioxane (10 mL) and cooled in ice. NH_3 was bubbled through the suspension, and the resulting mixture was stirred at room temperature for 72 h and poured into water (50 mL). The resulting precipitate was collected by filtration, washed with water, and dried to give 2-(difluoromethyl)-1*H*-benzimidazole-4-carboxamide (**8n**) (543 mg, 56% yield): mp (H_2O) 238−241 °C; ^{1}H NMR (DMSO-d_6) δ 13.85 and 13.03 (2 br s, 1H), 7.89−7.25 (m, 6H). Anal. Calcd for $C_9H_7F_2N_3O$: C, 51.2; H, 3.3; N, 19.9. Found: C, 51.3; H, 3.5; H, 19.4%.

Reaction of **8n** and **3** as before gave 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-1*H*-benzimidazole-4-carboxamide (**9n**) in 78% yield: ^{1}H NMR (DMSO-d_6) δ 8.70 (br, 1H), 8.56 (dd, J = 8.4, 1.1 Hz, 1H), 8.09 (dd, J = 7.6, 1.1 Hz, 1H), 7.96 (br, 1H), 7.73 (t, J_{HF} = 52.3 Hz, 1H), 7.70−7.67 (m, 1H), 3.90−3.86 (m, 4H), 3.78−3.72 (m, 4H).

Reaction of **9n** with morpholine gave **10n** in 82% yield: mp (H_2O) >295 °C; ^{1}H NMR ($CDCl_3$) δ 9.36 (s, 1 H), 8.50 (dd, J = 8.3, 1.0 Hz, 1H), 8.29 (dd, J = 7.6, 1.0 Hz, 1H), 7.55 (t, J_{HF} = 53.3 Hz, 1H), 7.77 (t, J = 8.0 Hz, 1H), 5.92 (s, 1H), 3.90−3.87 (m, 8H), 3.80−3.79 (m, 8H). Anal. Calcd for $C_{20}H_{22}F_2N_8O_3$: C, 52.2; H, 4.8, N, 24.3; F, 8.3%. Found: C, 52.0; H, 4.8; N, 24.4; F, 8.4%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-*N*-methyl-1*H*-benzimidazole-4-carboxamide (10o). Similar to the above, treatment of **8ah** with thionyl chloride followed by addition of dry methylamonium chloride and Et_3N in 1,4-dioxane gave 2-(difluoromethyl)-*N*-methyl-1*H*-benzimidazole-4-carboxamide (**8o**) in 75% yield: ^{1}H NMR (DMSO-d_6) δ 13.81 (br, 1H), 9.20 (br, 1H), 7.93 (d, J = 6.9 Hz, 1H), 7.81 (d, J = 8 Hz, 1H), 7.48−7.22 (m, 2H), 2.94 (s, 3H).

Reaction of **8o** with **3** as before gave 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-*N*-methyl-1*H*-benzimidazole-4-carboxamide (**9o**) in 32% yield: mp (CH_2Cl_2/hexanes) 277−281; ^{1}H NMR ($CDCl_3$) δ 9.29 (d, J = 3.4 Hz, 1 H), 8.56 (dd, J = 8.4, 1.1 Hz, 1 H), 8.35 (dd, J = 7.7, 1.1 Hz, 1 H), 7.58 (t, J_{HF} = 53.2 Hz, 1H), 7.60 (t, J = 8.0 Hz, 1H), 4.00 (t, J = 4.9 Hz, 2H), 3.96 (t, J = 4.9 Hz, 2H), 3.86−3.81 (m, 4H), 3.11 (d, J = 4.8 Hz, 3H). Anal. Calcd for $C_{17}H_{16}F_2N_7O_2$: C, 48.2; H, 3.8; N, 23.1. Found: C, 48.2; H, 3.9; N, 23.2%.

Reaction of **9o** with morpholine gave **10o** in 99% yield: mp (CH_2Cl_2/hexanes) 291−293 °C; ^{1}H NMR ($CDCl_3$) δ 9.43 (q, J = 4.5 Hz, 1H, NH), 8.45 (dd, J = 8.4, 1.0 Hz, 1H), 8.31 (dd, J = 7.6, 1.1 Hz, 1H), 7.55 (t, J_{HF} = 53.3 Hz, 1H), 7.54 (dd, J = 10.2, 5.6 Hz, 1H), 3.90−3.87 (m, 8H), 3.80−3.79 (m, 8H), 3.15 (d, J = 4.8 Hz, 3H). Anal. Calcd for $C_{21}H_{24}F_2N_8O_3$: C, 53.2; H, 5.1; N, 23.6. Found: C, 53.1; H, 5.1; N, 23.7%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-*N*,*N*-dimethyl-1*H*-benzimidazole-4-carboxamide (10p). Similar to the above, treatment of **8ah** with thionyl chloride followed by addition of dry dimethylammonium chloride and Et_3N in 1,4-dioxane gave 2-(difluoromethyl)-*N*,*N*-dimethyl-1*H*-benzimidazole-4-carboxamide (**8p**) in 28% yield: ^{1}H NMR ($CDCl_3$) δ 10.62 (br, 1H), 7.92 (d, J = 7.7 Hz, 1H), 7.48 (d, J = 7.3 Hz, 1H), 7.33 (dd, J = 8.1, 7.6 Hz, 1H), 6.85 (t, J_{HF} =53.8 Hz, 1H), 3.25 (s, 6H).

Reaction of **8p** with **3** as above gave 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-*N*,*N*-dimethyl-1*H*-benzimidazole-4-carboxamide (**9p**) in 58% yield: ^{1}H NMR ($CDCl_3$) δ 8.47 (dd, J = 7.9, 1.6 Hz, 1 H), 7.48 (t, J = 53.3 Hz, 1 H, C*H*F$_2$), 7.61−7.48 m, 2 H), 4.00−3.94 (m, 4 H), 3.84−3.80 (m, 4 H), 3.23 (s, 3 H), 2.92 (s, 3 H).

Reaction of **9p** with morpholine gave **10p** in 100% yield: mp (CH_2Cl_2/hexanes) 262−266 °C; ^{1}H NMR ($CDCl_3$) δ 8.35 (quintet, J = 3.6 Hz, 1H), 7.49 (t, J_{HF} = 53.4 Hz, 1H), 7.47−7.45 (m, 2H), 3.89−3.87 (m, 8H), 3.80−3.78 (m, 8H), 3.23 (s, 3H), 2.93 (s, 3H). Anal. Calcd for $C_{21}H_{24}F_2N_8O_3$: C, 54.1; H, 5.4; N, 22.9. Found: C, 54.3; H, 5.5; N, 23.0%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole-4-carbonitrile (10q). A mixture of **8n** (204 mg, 0.97 mmol) and thionyl chloride (1 mL) was refluxed for 20 h and cooled to 20 °C, and the excess thionyl chloride was removed under vacuum. The residue was treated with water, and the mixture was filtered to remove an insoluble solid. The filtrate was neutralized with aqueous NH_3 and extracted into EtOAc. The solution was dried (Na_2SO_4), and the solvent was removed. The resulting residue was chromatographed on silica, eluting with CH_2Cl_2/EtOAc (17:3), to give 2-(difluoromethyl)-1*H*-benzimidazole-4-carbonitrile (**8q**) (101 mg, 54% yield): mp (CH_2Cl_2/hexanes) 180−182 °C; ^{1}H NMR (DMSO-d_6) δ 10.03 (br, 1H), 7.97 (d, J = 8.1 Hz, 1H), 7.82 (dd, J = 7.5, 0.7 Hz, 1H), 7.45 (t, J = 7.9, Hz, 1H), 7.36 (t, J_{HF} = 53.1 Hz, 1H). Anal. Calcd for $C_9H_5F_2N_3$: C, 56.0; H, 2.6, N, 21.8: Found: C, 56.2; H, 2.7; N, 21.9%.

A mixture of **8q** (100 mg, 0.52 mmol), **3** (182 mg, 0.78 mmol), and dry K_2CO_3 (214 mg, 1.56 mmol) in THF (10 mL) was refluxed for 20 h. The resulting mixture was diluted with water and extracted into CH_2Cl_2. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (9:1), gave 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-1*H*-benzimidazole-4-carbonitrile (**9q**) (108 mg, 54% yield): mp (CH_2Cl_2/hexanes) 215−218 °C; ^{1}H NMR ($CDCl_3$) δ 8.72 (dd, J = 8.5, 1.0 Hz, 1H), 7.78 (dd, J = 7.6, 1.0 Hz, 1H), 7.49 (t, J_{HF} = 53.0 Hz, 1H), 7.57 (dd, J = 8.4, 7.4 Hz, 1H), 4.01−3.99 (m, 2H), 3.96−3.94 (m, 2H), 3.85−3.81 (m, 4H). Anal. Calcd for $C_{16}H_{12}ClF_2N_2O$: C, 49.1; H, 3.1: N, 25.0. Found: C, 49.3; H, 3.2; N, 25.1%.

A mixture of **9q** (100 mg, 0.26 mmol) and morpholine (0.25 mL, excess) in THF (10 mL) was stirred at room temperature for 3 h and then diluted with water. The resulting precipitate was collected and dried. Recrystallization from CH_2Cl_2/hexanes gave **10q** (112 mg, 97% yield): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 8.66 (dd, J = 8.4, 0.8 Hz, 1H), 7.97 (dd, J = 7.6, 0.9 Hz, 1H), 7.67 (dd, J = 8.3, 7.8 Hz, 1H), 7.76 (t, J_{HF} = 52.4 Hz, 1 H), 3.81−3.0 (br m, 8H), 3.69 (br, 8H). Anal. Calcd for $C_{20}H_{20}F_2N_8O_2$: C, 54.3; H, 4.6; N, 25.33. Found: C, 54.5; H, 4.6; N, 25.5%.

7-(Difluoromethyl)-6-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-6*H*-[1,3]dioxolo[4,5-*e*]benzimidazole (10r). A mixture of 6-bromo-4,5-dinitro-1,3-benzodioxole[59] (2.25 g, 7.7 mmol) and Et_3N (1.2 g, excess) in MeOH (100 mL) was hydrogenated over 5% Pd on carbon, and the mixture was filtered through Celite into methanolic HCl. The solvent was evaporated to dryness, and the residue was combined with difluoroacetic acid (3.7 g, 39 mmol) in 4 M HCl (20 mL). The mixture was heated under reflux for 30 min before being cooled and neutralized with aqueous NH_3. The product was extracted into EtOAc and dried. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (4:1), gave 7-(difluoromethyl)-6*H*-[1,3]dioxolo[4,5-*e*]benzimidazole (**8r**) (1.23 g, 75%): mp (*i*-Pr_2O) 149−150 °C; ^{1}H NMR (DMSO-d_6) δ 13.33 (br, 1H), 7.22 (t, J_{HF} = 53.2 Hz, 1H), 7.14−7.05 (m, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.11 (s, 2H). Anal. Calcd for $C_9H_6F_2N_2O_2$: C, 50.95; H, 2.85; N, 13.2. Found: C, 51.1; H, 2.9; N, 13.2%.

A mixture of **8r** (1.06 g, 5 mmol), **3** (1.17 g, 5 mmol), and powdered K_2CO_3 (3.5 g, 25 mmol) in DMF (50 mL) was stirred at room temperature for 30 min and then diluted with water. The crude product was collected by filtration, washed with water, and then with EtOH. Recrystallization from $CHCl_3$/EtOH gave 6-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-7-(difluoromethyl)-6*H*-[1,3]dioxolo[4,5-*e*]benzimidazole (**9r**) (1.65 g, 80%); mp >300 °C; ^{1}H NMR ($CDCl_3$) δ 7.95 (d, J = 8.8 Hz, 1H), 7.50 (t, J_{HF} = 53.3 Hz, 1H), 7.04 (d, J = 8.8 Hz, 1H), 6.16 (s, 2H), 3.99−3.96 (m, 2H), 3.95−3.92 (m, 2H), 3.84−3.79 (m, 4H). Anal. Calcd for $C_{16}H_{13}ClF_2N_6O_3$: C, 46.8; H, 3.2; N, 20.5. Found: C, 47.1; H, 3.3; N, 20.5%.

A mixture of **9r** (0.206 g, 0.5 mmol) and morpholine (0.22 g, 2.5 mmol) in THF (20 mL) was heated at 50 °C for 30 min and cooled. Dilution with water gave **10r** (0.23 g, 99%): mp (CH_2Cl_2/MeOH) > 300 °C; ^{1}H NMR ($CDCl_3$) δ 7.83 (d, J = 8.7 Hz, 1H), 7.49 (t, J_{HF} = 53.4 Hz, 1H), 7.00 (d, J = 8.7 Hz, 1H), 6.13 (s, 2H), 3.88−3.84 (m, 8H), 3.81−3.76 (m, 8H). Anal. Calcd for $C_{20}H_{21}F_2N_7O_4$: C, 52.1; H, 4.6; N, 21.25. Found: C, 52.2; H, 4.7; N, 21.5%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4,5-dimethoxy-1*H*-benzimidazole (10s). A solution of 3,4-dimethoxy-2-nitroaniline[60] (0.92 g, 4.6 mmol) in MeOH (100 mL) was hydrogenated over Pd on carbon, and the solution was filtered through Celite and acidified with concentrated HCl. The solvent was evaporated to dryness and the residue was combined with difluoroacetic acid (0.89 g, 2 equiv) in 4 M HCl (10 mL). The mixture was heated under reflux for 2 h, cooled, and neutralized with aqueous NH_3. Extraction with EtOAc, followed by chromatography on silica, eluting with CH_2Cl_2/EtOAc (4:1), gave 2-(difluoromethyl)-4,5-dimethoxy-1*H*-benzimidazole (**8s**) (0.97 g, 92% yield): mp (*i*-Pr_2O) 110−111 °C; ^{1}H NMR (DMSO-d_6) δ 13.13 (br, 1H), 7.30−7.20 (m, 1H), 7.20 (t, J_{HF} = 53.2 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 4.08−3.99 (m, 3H), 3.82 (s, 3H). Anal. Calcd For $C_{10}H_{10}F_2N_2O_2$: C, 52.6; H, 4.4; N, 12.3. Found: C, 52.9; H, 4.5; N, 12.25%.

A mixture of **8s** (0.57 g g, 2.5 mmol), **3** (0.59 g, 2.5 mmol), and powdered K_2CO_3 (1.7 g, 12.5 mmol) in DMF (35 mL) was stirred at room temperature for 30 min and then diluted with water. The crude product was collected by filtration, washed with water and then with EtOH, and dried. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (9:1), gave 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-4,5-dimethoxy-1*H*-benzimidazole (**9s**) (0.71 g, 67%): mp (CH_2Cl_2/EtOH) 209−211 °C; ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 9.0 Hz, 1H), 7.49 (t, J_{HF} = 53.4 Hz, 1H), 7.13 (d, J = 9.0 Hz, 1H), 4.35 (s, 3H), 3.99−3.92 (m, 4H), 3.94 (s, 3H), 3.84−3.78 (m, 4H). Anal. Calcd For $C_{17}H_{17}ClF_2N_6O_3$: C, 47.8; H, 4.0; N, 19.7. Found: C, 47.8; H, 3.9; N, 19.9%.

A mixture of **9s** (0.213 g, 5 mmol) and morpholine (0.217 g, 25 mmol) in THF (10 mL) was heated to 50 °C for 30 min and diluted with water to give **10s** (0.23 g, 96%): mp (CH_2Cl_2/MeOH) 242−245 °C; ^{1}H NMR ($CDCl_3$) δ 7.92 (d, J = 9.0 Hz, 1H), 7.49 (t, J_{HF} = 53.5 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 4.34 (s, 3H), 3.93 (s, 3H), 3.88−3.85 (m, 8H), 3.79−3.76 (m, 8H). Anal. Calcd for $C_{21}H_{25}F_2N_7O_4$: C, 52.8; H, 5.3; N, 20.5. Found: C, 52.9; H, 5.3; N, 20.5%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-hydroxy-5-methoxy-1*H*-benzimidazole (10t). 3-Methoxy-6-nitro-2-[(phenylsulfonyl)oxy]benzoic acid[61] (3.5 g, 9.9 mmol) in $SOCl_2$ (10 mL) with a trace of DMF was refluxed for 1 h and cooled to 20 °C, and the excess $SOCl_2$ was removed under vacuum. The resulting solid residue was dissolved in acetone (15 mL) and added slowly to a solution of NaN_3 (2 g, 30.8 mmol) in H_2O (10 mL) at 0 °C. The reaction mixture was stirred for 20 h and cooled to 0 °C, and the resulting precipitate was collected by filtration. The solid was suspended in aqueous HOAc (72%) (10 mL), and the mixture was refluxed for 2 h, cooled, and diluted with H_2O. The resulting precipitate was filtered, washed with water and dried to give 2-amino-6-methoxy-3-nitrophenyl benzenesulfonate (2.9 g, 90% yield): mp (H_2O) 140−142 °C; ^{1}H NMR ($CDCl_3$) δ 10.13 (s, 1H), 8.16 (d, J = 9.2 Hz, 1H), 7.94 (dd, J = 8.5, 1.7 Hz, 2H), 7.76−7.71 (m, 1H), 7.62−7.58 (m, 2H), 7.07 (d, J = 9.3 Hz, 1H), 3.75 (s, 3H). Anal. Calcd for $C_{13}H_{12}N_2O_6S$: C, 48.2; H, 3.7; N, 8.6. Found: C, 48.3; H, 3.9; N, 8.7%.

A solution of the above compound (4.0 g, 12.33 mmol) in aqueous 2 N NaOH (20 mL) and EtOH (20 mL) was refluxed for 30 min, and the EtOH was removed. The resulting aqueous solution was neutralized with concentrated HCl to give 2-amino-6-methoxy-3-nitrophenol (2.04 g, 90%) yield: ^{1}H NMR ($CDCl_3$) δ 7.79 (d, J = 9.6 Hz, 1H), 6.36 (d, J = 9.65 Hz, 1H), 6.14 (br, 2H), 5.52 (s, 1H), 3.96 (s, 3H).

The above compound (2.0 g, 0.87 mmol) was hydrogenated over Pd/C in MeOH. This was filtered into a solution of concentrated HCl (3 mL) in MeOH (10 mL) and evaporated to dryness. The resulting residue was refluxed in 4 N HCl (20 mL) and difluoroacetic acid (5 mL) for 20 h. The reaction mixture was diluted with H_2O, neutralized with aqueous NH_3, and stirred overnight. The resulting precipitate was collected, washed with H_2O, and recrystallized from aqueous MeOH to give 2-(difluoromethyl)-4-hydroxy-5-methoxy-1*H*-benzimidazole (**8t**) (2.29 g, 98% yield), which was used directly.

A mixture of **8t** (2.29 g, 10.6 mmol) and TBDMSCl (4.79 g, 3 equiv) in pyridine (10 mL) was stirred at 20 °C for 4 days, diluted with water, extracted in EtOAc (3 × 50 mL), and dried (Na_2SO_4), and the solvent was evaporated. The residue was chromatographed on silica, eluting with CH_2Cl_2/EtOAc (9:1) to give a viscous oil, which was stirred in hexanes to give 4-{[*tert*-butyl(dimethyl)silyl]oxy}-2-(difluoromethyl)-5-methoxy-1*H*-benzimidazole (**8ab**) (1.96 g, 56% yield) as a solid: ^{1}H NMR ($CDCl_3$) δ 9.56 and 9.20 (2 br, 1H, NH), 7.38 (d, J = 8.8 Hz, 1H), 7.06−7.99 (m, 1H), 6.84 (t, J_{HF} = 54.0 Hz, 1H), 3.88 and 3.85 (2s, 3H), 1.08 and 1.06 (2s, 9H), 0.24 and 0.22 (2s, 6H).

Reaction of **8ab** with **3** gave 4-{[*tert*-butyl(dimethyl)silyl]oxy}-1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-5-methoxy-1*H*-benzimidazole (**9ab**) which was used without further purification. Reaction with morpholine in THF gave 4-{[*tert*-butyl(dimethyl)-silyl]oxy}-2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-5-methoxy-1*H*-benzimidazole (**10ab**) in 68% yield over two steps: ^{1}H NMR ($CDCl_3$) δ 7.83 (d, J = 8.9 Hz, 1H), 7.45 (t, J_{HF} = 53.6 Hz, 1H), 7.07 (d, J = 8.9 Hz, 1H), 3.89−3.86 (m, 8H), 3.87 (s, 3H), 3.78−3.77 (m, 8H), 1.06 (s, 9H), 0.26 (s, 6H).

Deprotection of **10ab** with TBAF in THF gave **10t** in 92% yield: mp (CH_2Cl_2/MeOH) 294−300 °C; ^{1}H NMR (DMSO-d_6) δ 9.67 (br, 1H), 7. 70 (d, J = 8.0 Hz, 1H), 7.70 t, J_{HF} = 53.0 Hz, 1H), 7.18 (d, J = 9.0 Hz, 1H), 3.82 (s, 3H), 3.81−3.79 (m, 8H), 3.69 (br, 8H). Anal. Calcd for $C_{20}H_{23}F_2N_7O_4$: C, 51.8; H, 5.0; N, 21.2. Found: C, 52.1; H, 5.0; N, 21.2%

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-5-hydroxy-4-methoxy-1*H*-benzimidazole (10u). A solution of 4-(benzyloxy)-3-methoxy-2-nitrobenzaldehyde[62] (2.87 g, 10 mmol) in a mixture of acetone (110 mL) and DMSO (45 mL) was combined with a solution of sulfamic acid (1.65 g, 17 mmol) in water (33 mL), and the resulting mixture was cooled to 0 °C. A solution of $NaClO_2$ (1.75 g, 19 mmol) in water (75 mL) was added slowly with stirring, and after a further 30 min at 0 °C, the mixture was diluted with water and extracted with EtOAc. Removal of the solvent gave 4-(benzyloxy)-3-methoxy-2-nitrobenzoic acid[63] (2.93 g, 97%): ^{1}H NMR (DMSO-d_6) δ 13.75 (br, 1H), 7.78 (d, J = 8.8 Hz, 1H), 7.51 (br d, J = 8.4 Hz, 2H), 7.47−7.41 (m, 3H), 7.40−7.35 (m, 1H), 5.33 (s, 2H), 3.84 (s, 3H).

A mixture of the above crude acid (1 g, 3.3 mmol) and DPPA (1.0 g, 4 mmol) in *t*-BuOH (2.5 g, 33 mmol) was heated under reflux for 1 h. After cooling, the mixture was diluted with water, and bascified with aq. NH_3 to give a solid, which was collected and dried. Chromatography on silica, eluting with CH_2Cl_2/hexanes (3:1), gave *tert*-butyl 4-(benzyloxy)-3-methoxy-2-nitrophenylcarbamate (0.68 g, 55%): mp (aqueous MeOH) 94−96 °C; ^{1}H NMR ($CDCl_3$) δ 7.67 (d, J = 9.3 Hz, 1H), 7.43−7.31

(m, 5H), 7.13 (br, 1H), 7.06 (d, J = 9.3 Hz, 1H), 5.12 (s, 2H), 4.00 (s, 3H), 1.49 (s, 9H). Anal. Calcd for $C_{19}H_{22}N_2O_6$: C, 60.95; H, 5.9; N, 7.5. Found: C, 61.0; H, 5.8; N, 7.6%.

Treatment of the above carbamate with TFA in CH_2Cl_2 gave 4-(benzyloxy)-3-methoxy-2-nitroaniline: mp (i-Pr_2O) 70−71 °C; ^{1}H NMR ($CDCl_3$) δ 7.42−7.35 (m, 5H), 6.94 (d, J = 9.0 Hz, 1H), 6.42 (d, J = 9.0 Hz, 1H), 5.03 (s, 2H), 4.48 (br, 1H), 4.00 (s, 3H). Anal. Calcd for $C_{14}H_{14}N_2O_4$: C, 61.3; H, 5.1; N, 10.2. Found: C, 61.4; H, 5.2; N, 10.15%.

A solution of the above compound (1.10 g, 4 mmol) in MeOH (100 mL) was hydrogenated over Pd on carbon, and the mixture was filtered through Celite into a mixture of MeOH and concentrated HCl. The solution was evaporated to dryness, and the residue was combined with difluoroacetic acid (3.84 g, 40 mmol) and 4 M HCl (20 mL) and heated under reflux for 2 h. After cooling, the mixture was bascified with aqueous NH_3 and extracted with EtOAc. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (7:3), gave 2-(difluoromethyl)-5-hydroxy-4-methoxy-1*H*-benzimidazole (**8u**) (0.7 g, 82%) as an oil: ^{1}H NMR (DMSO-d_6) δ 13.10 (br, 1H), 9.18 and 8.61 (2 br, 1H), 7.35−6.96 (m, 2H), 6.87 (d, J = 8.5 Hz, 1H), 4.07 and 3.89 (2 br, 3H).

The above crude **8u** (0.7 g, 3.3 mmol) was combined with TBDMSCl (0.84 g, 5.6 mmol) in pyridine (10 mL), and the resulting mixture was stirred at room temperature overnight. After removal of the pyridine under vacuum, the residue was diluted with water and extracted with CH_2Cl_2. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (95:5), gave 5-{[*tert*-butyl(dimethyl)silyl]oxy}-2-(difluoromethyl)-4-methoxy-1*H*-benzimidazole (**8ac**) (0.335 g, 31%) as an oil: ^{1}H NMR ($CDCl_3$) δ 10.5−9.0 (br 1H), 7.29−7.20 (m, 1H), 6.91 (d, J = 8.7 Hz, 1H), 6.86 (t, J_{HF} = 53.9 Hz, 1H), 4.05 (br, 3H), 1.03 (s, 9H), 0.20 (s, 6H).

A mixture of crude **8ac** (0.33 g, 1 mmol), **3** (0.23 g, 1 mmol), and powdered K_2CO_3 (0.56 g, 4 mmol) in THF (20 mL) was stirred at room temperature for 5 days and diluted with water. The product was extracted in to CH_2Cl_2 and dried. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (97:3), gave 5-{[*tert*-butyl(dimethyl)silyl]oxy}-1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-4-methoxy-1*H*-benzimidazole (**9ac**) (0.26 g, 49%): mp (hexanes) 140−142 °C; ^{1}H NMR ($CDCl_3$) δ 7.93 (d, J = 8.9 Hz, 1H), 7.48 (t, J_{HF} = 53.5 Hz, 1H), 7.03 (d, J = 8.9 Hz, 1H), 4.26 (s, 3H), 3.99−3.92 (m, 4H), 3.84−3.78 (m, 4H), 1.03 (s, 9H), 0.19 (s, 6H). Anal. Calcd for $C_{22}H_{29}ClF_2N_6O_3Si$: C, 50.1; H, 5.6; N, 15.95. Found: C, 50.15; H, 5.6; N, 16.1%.

A mixture of **9ac** (0.21 g, 0.40 mmol) and morpholine (0.35 g, 4.0 mmol) in THF (10 mL) was stirred at room temperature for 30 min and diluted with water to give 5-{[*tert*-butyl(dimethyl)silyl]oxy}-2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-methoxy-1*H*-benzimidazole (**10ac**) (0.22 g, 97%): mp (hexanes) 212−214 °C; ^{1}H NMR ($CDCl_3$) δ 7.83 (d, J = 8.9 Hz, 1H), 7.48 (t, J_{HF} = 53.6 Hz, 1H), 6.99 (d, J = 8.9 Hz, 1H), 4.25 (s, 3H), 3.88−3.84 (m, 8H), 3.80−3.76 (m, 8H), 1.03 (s, 9H), 0.19 (s, 6H). Anal. Calcd for $C_{26}H_{37}F_2N_7O_4Si$: C, 54.1; H, 6.5; N, 17.0. Found: C, 54.1; H, 6.6; N, 17.1%.

A solution of the above compound (0.14 g, 0.35 mmol) in THF (5 mL) was treated with a slight excess of Bu_4NF in THF at room temperature for 5 min. The solvent was removed under vacuum and the residue was diluted with water to give a solid, which was collected by filtration and recrystallized from MeOH, to give **10u** (76 mg, 68%): mp (MeOH) 253−256 °C; ^{1}H NMR ($CDCl_3$) δ 7.87 (d, J = 8.9 Hz, 1H), 7.49 (t, J_{HF} = 53.5 Hz, 1H), 7.07 (d, J = 8.9 Hz, 1H), 5.66 (s, 1H), 4.42 (s, 3H), 3.88−3.84 (m, 8H), 3.79−3.75 (m, 8H). Anal. Calcd for $C_{20}H_{23}F_2N_7O_4$: C, 51.8; H, 5.0; N, 21.2. Found: C, 51.9; H, 4.9; N, 21.0%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4,6-dimethoxy-1*H*-benzimidazole (10v). A solution of 3,5-dimethoxy-2-nitrobenzoic acid[64] (7.95 g, 35 mmol) in $SOCl_2$ (100 mL) was heated under reflux for 2 h, and the solvent was evaporated to dryness. The residue was dissolved in dry acetone (65 mL) and combined with a solution of NaN_3 (13 g) in water (25 mL) with rapid stirring. After 10 min the mixture was poured into water and the precipitate was collected and washed with water. The solid was suspended in a mixture of acetic acid (400 mL) and water (40 mL), and the resulting mixture was heated slowly to reflux and maintained at that temperature for 2 h. After cooling, the mixture was neutralized with aqueous NH_3 to give 3,5-dimethoxy-2-nitroaniline (6.06 g, 87%): mp (aqueous i-PrOH) 112−114 °C; ^{1}H NMR ($CDCl_3$) δ 5.58 (d, J = 2.5 Hz, 1H), 5.79 (d, J = 2.5 Hz, 1H), 5.52 (br, 2H), 3.86 (s, 3H), 3.80 (s, 3H). Anal. Calcd for $C_8H_{10}N_2O_4$: C, 48.5; H, 5.1; N, 14.1. Found: C, 48.65; H, 5.2; N, 14.3%.

A solution of the above compound (1.98 g, 10 mmol) in MeOH (100 mL) was hydrogenated over 5% Pd on carbon and filtered through Celite into a mixture of MeOH and concentrated HCl. The solvents were removed under vacuum and the residue was combined with difluoracetic acid (1.92 g, 20 mmol) in 4 M HCl (20 mL). The mixture was heated under reflux for 2 h, cooled, and neutralized with aqueous NH_3. Extraction with EtOAc, followed by chromatography on silica, eluting with CH_2Cl_2/EtOAc (4:1), gave 2-(difluoromethyl)-4,6-dimethoxy-1*H*-benzimidazole (**8v**) (1.83 g, 80%): mp (aqueous MeOH) 165−167 °C; ^{1}H NMR (DMSO-d_6) δ 13.12 (br, 1H), 7.14 (t, J_{HF} = 53.4 Hz, 1H), 6.65−6.57 (m, 1H), 6.44−6.38 (m, 1H), 3.91 (m, 3H), 3.79 (s, 3H). Anal. Calcd for $C_{10}H_{10}F_2N_2O_2$: C, 52.6; H, 4.4; N, 12.3. Found: C, 52.85; H, 4.45; N, 12.3%.

A mixture of 2-(difluoromethyl)-4,6-dimethoxy-1*H*-benzimidazole (**8v**) (0.57 g g, 2.5 mmol), **3** (0.59 g, 2.5 mmol), and powdered K_2CO_3 (1.7 g, 12.5 mmol) in DMF (35 mL) was stirred at room temperature for 30 min and then diluted with water. The crude product was collected by filtration and washed with water and then with EtOH to give 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-4,6-dimethoxy-1*H*-benzimidazole (**9v**) (0.79 g, 74%): mp (CH_2Cl_2/EtOH) 261−263 °C; ^{1}H NMR ($CDCl_3$) δ 7.58 (d, J = 2.1 Hz, 1H), 7.40 (t, J_{HF} = 53.6 Hz, 1H), 6.49 (d, J = 2.1 Hz, 1H), 4.01 (s, 3H), 3.99−3.93 (m, 4H), 3.89 (s, 3H), 3.83−3.78 (m, 4H). Anal. Calcd for $C_{17}H_{17}ClF_2N_6O_3$: C, 47.8; H, 4.0; N, 19.7. Found: C, 47.8; H, 3.9; N, 19.9%.

A mixture of the above chloro compound (0.213 g, 5 mmol) and morpholine (0.217 g, 25 mmol) in THF (10 mL) was heated to 50 °C for 30 min and diluted with water to give **10v** (0.215 g, 90%): mp (CH_2Cl_2/MeOH) 296−299 °C; ^{1}H NMR ($CDCl_3$) δ 7.49 (d, J = 2.2 Hz, 1H), 7.44 (t, J_{HF} = 53.7 Hz, 1H), 6.47 (d, J = 2.2 Hz, 1H), 4.02 (s, 3H), 3.92−3.87 (m, 8H), 3.86 (s, 3H), 3.80−3.77 (m, 8H). Anal. Calcd for $C_{21}H_{25}F_2N_7O_4$: C, 52.8; H, 5.3; N, 20.5. Found: C, 52.95; H, 5.3; N, 20.5%.

6-Amino-2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-methoxy-1*H*-benzimidazole (10w). A mixture of 2,3-diamino-5-nitroanisole[65] (1.10 g, 6 mmol) and difluoroacetic acid (2.31 g, 24 mmol) in polyphosphoric acid (PPA) (50 g) was heated at 130 °C in an oil bath for 1 h. The hot solution was poured into water and, with cooling, the pH was adjusted to neutral with aqueous NH_3 to give 2-(difluoromethyl)-4-methoxy-6-nitro-1*H*-benzimidazole (1.33 g, 91%): mp (EtOH/H_2O) 192−194 °C; ^{1}H NMR (DMSO-d_6) δ 14.18 (br, exchangeable with D_2O, 1H), 8.18 (br, 1H), 7.65 (dd, J = 1.4 Hz, 1H), 7.30 (t, J_{HF} = 52.9 Hz, 1H), 4.07 (s, 3H). Anal. Calcd for $C_9H_7F_2N_3O_3$: C, 44.45; H, 2.9; N, 17.3. Found: C, 44.75; H, 3.0; N, 17.3%.

A solution of the above compound (1.22 g, 5 mmol) in MeOH (50 mL) was hydrogenated over 10% Pd on carbon (50 mg). After filtration to remove the catalyst, the solution was evaporated to dryness. The residue was combined with di-*tert*-butyl dicarbonate (3.2 g, 15 mmol) in dioxane (20 mL), and the mixture was heated under reflux for 5 h. The solvent was removed under vacuum, and the residue was dissolved in MeOH (30 mL) containing aqueous NaOH (2 M, 12.5 mL, 5 equiv). The mixture was stirred at room temperature for 1 h,

neutralized with HOAc, and evaporated to dryness. The residue was extracted with EtOAc, washed with $NaHCO_3$ solution, and dried over Na_2SO_4. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (9:1), gave 1.54 g (98% yield) of *tert*-butyl 2-(difluoromethyl)-4-methoxy-1*H*-benzimidazol-6-ylcarbamate (**8af**): mp (*i*-Pr_2O) 189−191 °C; ^{1}H NMR (DMSO-d_6) δ 13.0 (br, exchangeable with D_2O, 1H), 9.31 (br s, exchangeable with D_2O, 1 H), 7.42 (br s, 1H), 7.15 (t, J_{HF} = 53.4 Hz, 1H), 6.90 (br, 1H), 3.90 (s, 3H), 1.49 (s, 9H). Anal. Calcd for $C_{14}H_{17}F_2N_3O_3$: C, 53.7; H, 5.5; N, 13.4. Found: C, 53.9; H, 5.6; N, 13.4%.

A mixture of **8af** (0.47 g, 1.5 mmol), **3** (0.35 g, 1.5 mmol), and powdered K_2CO_3 (0.83 g, 6 mmol) in DMF (10 mL) was stirred at room temperature for 30 min. The reaction mixture was then diluted with water. The resulting precipitate was collected, washed with water and then MeOH, and dried to give *tert*-butyl 1-[4-chloro-6-(4-morpholinyl)-1,3,5-triazin-2-yl]-2-(difluoromethyl)-4-methoxy-1*H*-benzimidazol-6-ylcarbamate (**9af**) (0.45 g, 59% yield): mp (CH_2Cl_2/MeOH) > 300 °C; ^{1}H NMR ($CDCl_3$) δ 8.45 (d, J = 0.6 Hz, 1H), 7.57 (t, J_{HF} = 53.6 Hz, 1H), 6.67 (br, exchangeable with D_2O, 1H), 6.63 (d, J = 0.9 Hz, 1H), 4.11 (m, 2H), 4.02 (s, 3H), 3.97 (m, 2H), 3.88 (m, 2H), 3.82 (m, 2H), 1.52 (s, 9H). Anal. Calcd for $C_{21}H_{24}ClF_2N_7O_4$: C, 49.3; H, 4.7; N, 19.15. Found: C, 49.4; H, 4.8; N, 19.2%.

A mixture of **9af** (205 mg, 0.4 mmol) and morpholine (0.35 g, 4.0 mmol) in THF (20 mL) was stirred at room temperature for 1 h and diluted with water to give *tert*-butyl 2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-methoxy-1*H*-benzimidazol-6-ylcarbamate (**10af**) (0.22 g, 98%): ^{1}H NMR ($CDCl_3$) δ 8.63 (br s, 1H), 7.48 (t, J_{HF} = 53.6 Hz, 1H), 6.60 (br s, 1H), 6.36 (d, J = 1.6 Hz, 1H), 3.99 (s, 3H), 3.98−3.91 (m, 4H), 3.90−3.84 (m, 4H), 3.83−3.75 (m, 8H), 1.51 (s, 9H).

Treatment of **10af** with TFA in CH_2Cl_2, followed by chromatography on silica, eluting with CH_2Cl_2/EtOAc (3:2), gave **10w** (88% yield): mp (MeOH) 277−280 °C; ^{1}H NMR ($CDCl_3$) δ 7.39 (t, J_{HF} = 53.7 Hz, 1H), 7.18 (d, J = 1.8 Hz, 1H), 6.21 (d, J = 1.8 Hz, 1H), 3.98 (s, 3H), 3.88−3.84 (m, 8H), 3.82 (m, exchangeable with D_2O, 2H), 3.78−3.75 (m, 8H). Anal. Calcd for $C_{20}H_{24}F_2N_8O_3$: C, 51.9; H, 5.2; N, 24.2. Found: C, 52.1; H, 5.3; N, 24.5%.

Methanesulfonate: mp (MeOH/EtOAc) 260−264 °C; ^{1}H NMR (DMSO-d_6) δ 10.50−8.00 (br, exchangeable with D_2O, 2H), 7.71 (d, J = 1.2 Hz, 1H), 7.67 (t, J_{HF} = 52.9 Hz, 1H), 6.77 (d, J = 1.5 Hz, 1H), 3.97 (s, 3H), 3.83−3.78 (m, 8H), 3.73−3.67 (m, 8H), 2.34 (s, 3H). Anal. Calcd for $C_{21}H_{28}F_2N_8O_6S$: C, 45.2; H, 5.05; N, 20.1. Found: C, 45.4; H, 5.1; N, 20.4%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-methoxy-6-(methylamino)-1*H*-benzimidazole (10x). A solution of **10af** (0.25 g, 0.44 mmol) in DMF (20 mL) at room temperature was treated with 60% NaH in oil (27 mg, 0.67 mmol) to give a red solution. Iodomethane (86 mg, 0.55 mmol) was added, and after 5 min the reaction mixture was diluted with water to give *tert*-butyl 2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-methoxy-1*H*-benzimidazol-6-yl(methyl)carbamate (**10ag**) (0.25 g, 97%): ^{1}H NMR ($CDCl_3$) δ 7.89 (br s, 1H), 7.46 (t, J_{HF} = 53.5 Hz, 1H), 6.73 (d, J = 1.8 Hz, 1H), 4.03 (s, 3H), 3.89−3.84 (m, 8H), 3.79−3.75 (m, 8H), 3.32 (s, 3H), 1.46 (s, 9H).

A solution of **10ag** (0.17 g, 0.3 mmol) in CH_2Cl_2 (10 mL) was treated with TFA (1 mL), and the mixture was stirred at room temperature for 4 h and diluted with water. The mixture was basified with aqueous NH_3, and the organic layer was separated and dried. Removal of the solvent gave **10x** (0.14 g, 99%): mp (CH_2Cl_2/MeOH) 277 °C dec; ^{1}H NMR ($CDCl_3$) δ 7.41 (t, J_{HF} = 53.8 Hz, 1H), 7.14 (d, J = 1.9 Hz, 1H), 6.13 (d, J = 1.9 Hz, 1H), 3.98 (s, 3H), 3.91−3.84 (m, 9H), 3.78−3.75 (m, 8H), 2.87 (s, 3H). Anal. Calcd for $C_{21}H_{26}F_2N_8O_3$: C, 52.9; H, 5.5; N, 23.5. Found: C, 53.0; H, 5.5; N, 23.8%.

2-(Difluoromethyl)-6-(dimethylamino)-1-(4,6-dimorpholino-1,3,5-triazin-2-yl)-4-methoxy-1*H*-benzimidazole (10y). A solution of **10x** (0.163 g, 0.34 mmol) in CH_2Cl_2 (30 mL) was treated with an excess of acetic formic anhydride at room temperature for 3 h. Dilution with water and recrystallization from CH_2Cl_2/MeOH gave 2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-methoxy-1*H*-benzimidazol-6-yl(methyl)formamide (0.16 g, 93%): mp 255−257 °C; ^{1}H NMR ($CDCl_3$) δ 8.54 (s, 1H), 7.77 (d, J = 1.9 Hz, 1H), 7.46 (t, J_{HF} = 53.4 Hz, 1H), 6.63 (d, J = 1.9 Hz, 1H), 4.07 (s, 3H), 3.89−3.85 (m, 8H), 3.79−3.76 (m, 8H), 3.38 (s, 3H). Anal. Calcd for $C_{22}H_{26}F_2N_8O_4$: C, 52.4; H, 5.2; N, 22.2. Found: C, 52.4; H, 5.5; N, 22.3%.

A suspension of the above compound (151 mg, 0.3 mmol) and $(MeO)_3B$ (186 mg, 1.8 mmol) in THF (100 mL) was treated with $BH_3 \cdot SMe_2$ (136 mg, 1.8 mmol), and the mixture was diluted with CH_2Cl_2 until a clear solution was obtained. The solution was stirred at room temperature overnight, and MeOH (1 mL) was added. The solvents were evaporated to dryness, and water was added. The solid was collected, washed with aqueous $NaHCO_3$ and dried. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (3:2), gave **10y** (116 mg, 79%); mp (CH_2Cl_2/MeOH) 280−282 °C; ^{1}H NMR ($CDCl_3$) δ 7.42 (t, J_{HF} = 53.8 Hz, 1H), 7.27 (d, J = 2.1 Hz, 1H), 6.32 (d, J = 2.1 Hz, 1H), 4.03 (s, 3H), 3.92−3.85 (m, 8H), 3.78−3.74 (m, 8H), 3.02 (s, 6H). Anal. Calcd for $C_{22}H_{28}F_2N_8O_3$: C, 53.9; H, 5.75; N, 22.8. Found: C, 54.15; H, 5.8; N, 22.9%.

2-(Difluoromethyl)-3-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-7-methoxy-3*H*-imidazo[4,5-*c*]pyridine (13). A solution of 4-amino-3-methoxy-5-nitropyridine[40] (1.08 g, 6.4 mmol) in MeOH (50 mL) was hydrogenated over 5% Pd on C, and after filtration to remove the catalyst the solution was acidified with concentrated HCl. The solvent was removed under vacuum, and the residue was combined with difluoroacetic acid (3.1 g, 32 mmol) in PPA (40 g). The mixture was stirred at 100 °C for 1 h and poured into water. The solution was neutralized with aqueous NH_3 and extracted with EtOAc to give 2-(difluoromethyl)-7-methoxy-3*H*-imidazo[4,5-*c*]pyridine (**11**) (0.87 g, 68% yield): mp (EtOH) 234−238 °C; ^{1}H NMR ($CDCl_3$) δ 14.0−12.0 (br, 1H), 8.69 (s, 1H), 6.04 (s, 1H), 7.22 (t, J_{HF} = 53.3 Hz, 1H), 4.06 (s, 3H). Anal. Calcd for $C_8H_7F_2N_3O$: C, 48.25; H, 3.5; N, 21.1. Found: C, 48.15; H, 3.4; N, 20.9%.

A mixture of **11** (0.21 g, 1 mmol), **3** (0.32 g, 1.37 mmol), and powdered K_2CO_3 (0.69 g, 5 mmol) in *t*-BuOH (25 mL) was heated under reflux for 5 h and cooled to room temperature. Morpholine (0.87 g, 10 mmol) was added, and the resulting mixture was stirred at room temperature for 1 h before being diluted with water. Extraction with CH_2Cl_2, followed by chromatography on alumina, eluting with CH_2Cl_2/EtOAc (3:1), gave 2-(difluoromethyl)-3-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-7-methoxy-3*H*-imidazo[4,5-*c*]pyridine (**13**) (58 mg, 13% yield): mp (CH_2Cl_2/MeOH) 262−264 °C; ^{1}H NMR ($CDCl_3$) δ 9.88 (d, J = 0.8 Hz, 1H), 8.72 (d, J = 1.4 Hz, 1H), 6.92 (t, J_{HF} = 54.5 Hz, 1H), 4.22 (s, 3H), 3.96−3.87 (m, 8H), 3.82−3.76 (m, 8H). Anal. Calcd for $C_{19}H_{22}F_2N_8O_3$: C, 50.9; H, 4.9; N, 25.0. Found: C, 51.2; H, 4.9; N, 25.0%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-2-pyrimidinyl]-1*H*-benzimidazole (16a). A solution of **2** (0.84 g, 5 mmol) in DMF (25 mL) was treated with 60% NaH (0.24 g, 6 mmol), and the mixture was stirred at room temperature for 30 min. 2,4,6-Trichloropyrimidine (**14**) (0.92 g, 5 mmol) was added, and the resulting mixture was stirred for 2 h. Dilution with water gave a solid which was collected and dried. Chromatography on silica, eluting with CH_2Cl_2/hexanes (4:1), gave mixed fractions which were recrystallized from CH_2Cl_2−hexanes to give 1-(4,6-dichloro-2-pyrimidinyl)-2-(difluoromethyl)-1*H*-benzimidazole[38] (**15a**) (0.54 g, 34%): mp 131−132 °C; ^{1}H NMR ($CDCl_3$) δ 8.48 (br d, J = 8.3 Hz, 1H), 7.92 (br d, J = 7.6 Hz, 1H), 7.67 (t, J_{HF} = 53.5 Hz, 1H), 7.52 (td, J = 8.3, 1.3 Hz, 1H), 7.46 (td, J = 7.9, 1.3 Hz, 1H), 7.35 (s, 1H).

Anal. Calcd for $C_{12}H_6Cl_2F_2N_4$: C, 45.7; H, 1.9; N, 17.8. Found: C, 46.0; H, 2.0; N, 17.7%.

A solution of the above compound (0.33 g, 1.05 mmol) in morpholine (0.91 g, 10.5 mmol) was heated under reflux for 1 h, cooled, and diluted with water to give **16a** (0.47 g 100%): mp (EtOH) 205−208 °C (lit.[38] mp 201−202 °C); ^{1}H NMR ($CDCl_3$) δ 8.22 (br d, J = 8.2 Hz, 1H), 7.89 (br d, J = 7.1 Hz, 1H), 7.51 (t, J_{HF} = 53.6 Hz, 1H), 7.43−7.35 (m, 2H), 5.51 (s, 1H), 3.84−3.80 (m, 8H), 3.65−3.61 (m, 8H). Anal. Calcd for $C_{20}H_{22}F_2N_6O_2$: C, 57.7; H, 5.3; N, 20.2. Found: C, 57.9; H, 5.3; N, 20.1%.

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-4-hydroxy-1*H*-benzimidazole (16b). A mixture of **8a** (7.0 g, 38 mmol), imidazole (50 mL), and TIPSCl (15 g, 78 mmol) in DMF was stirred at room temperature for 2 h and diluted with water. Extraction with EtOAc and chromatography on silica, eluting with hexanes/EtOAc (9:1), gave 2-(difluoromethyl)-4-[(triisopropylsilyl)oxy]-1*H*-benzimidazole (**8ai**) (10.85 g, 84%): mp (hexanes) 120−121 °C; ^{1}H NMR (DMSO-d_6) δ 13.21 (br, 1H), 7.24 (t, J_{HF} = 53.2 Hz, 1H), 7.19−7.13 (m, 2H), 6.73−6.68 (m, 1H), 1.36 (septet, J = 7.5 Hz, 3H), 1.08 (d, J = 7.5 Hz, 18H). Anal. Calcd for $C_{17}H_{26}F_2N_2OSi$: C, 60.0; H, 7.7; N, 8.2. Found: C, 59.8; H, 7.5; N, 8.3%.

A mixture of **8ai** (3.405 g, 10 mmol), **14** (2.75 g, 15 mmol), and powdered K_2CO_3 (5.5 g, 40 mmol) in THF (25 mL) was stirred at room temperature for 1 week and filtered to remove all solids. Morpholine (8.7 g, 0.1 mol) was added to the solution, and the resulting mixture was heated under reflux for 8 h and diluted with water. The product was extracted with CH_2Cl_2, and after drying, the solvent was removed and the crude residue of **16d** was dissolved in THF and treated with a slight excess of 1 M Bu_4NF in THF. Dilution with water gave a solid which was collected and dried. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (3:1), gave **16b** (2.30 g, 53%): mp (CH_2Cl_2/EtOH) 272−275 °C (lit.[47] mp >250 °C); ^{1}H NMR (DMSO-d_6) δ 10.16 (br, 1H), 7.65 (t, J_{HF} = 53.0 Hz, 1H), 7.62 (dd, J = 8.3, 0.7 Hz, 1H), 7.22 (t, J = 8.1 Hz, 1H), 6.72 (dd, J = 7.9, 0.7 Hz, 1H), 5.97 (s, 1H), 3.72−3.67 (m, 8H), 3.65−3.60 (m, 8H).

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-2-pyrimidinyl]-4-methoxy-1*H*-benzimidazole (16c). A mixture of **8b** (0.396 g, 2 mmol), **14** (0.37 g, 2 mmol), and powdered K_2CO_3 (1.1 g, 8 mmol) in DMSO (10 mL) was stirred at room temperature for 3 h and diluted with water. The solid was dried and chromatographed on silica, eluting first with CH_2Cl_2/hexanes (4:1) and then with CH_2Cl_2, to give 1-(4,6-dichloro-2-pyrimidinyl)-2-(difluoromethyl)-4-methoxy-1*H*-benzimidazole[49] (**15c**) (0.475 g, 69%): mp (*i*-Pr_2O) 158−159 °C; ^{1}H NMR ($CDCl_3$) δ 8.01 (dd, J = 8.4, 0.6 Hz, 1H), 7.59 (t, J_{HF} = 53.5 Hz, 1H), 7.41 (t, J = 8.3 Hz, 1H), 7.33 (s, 1H), 6.87 (d, J = 7.9 Hz, 1H), 4.06 (s, 3H). Anal. Calcd for $C_{13}H_8Cl_2F_2N_4O$: C, 45.2; H, 2.3; N, 16.2. Found: C, 45.3; H, 2.55; N, 16.3%.

A solution of **15c** (0.172 g, 0.5 mmol) in morpholine (10 mL) was heated under reflux for 30 min, cooled, and diluted with water to give **16c** (0.21 g, 94%): mp (MeOH) 209−211 °C (lit.[49] mp 203−205 °C); ^{1}H NMR ($CDCl_3$) δ 7.78 (dd, J = 8.4, 0.6 Hz, 1H), 7.42 (t, J_{HF} = 53.5 Hz, 1H), 7.32 (t, J = 8.2 Hz, 1H), 6.78 (d, J = 7.8 Hz, 1H), 5.50 (s, 1H), 4.05 (s, 3H), 3.83−3.80 (m, 8H), 3.64−3.61 (m, 8H). Anal. Calcd for $C_{21}H_{24}F_2N_6O_3$: C, 56.5; H, 5.4; N, 18.8. Found: C, 56.7; H, 5.4; N, 19.0%.

2-(Difluoromethyl)-1-[2,6-di(4-morpholinyl)-4-pyrimidinyl]-1*H*-benzimidazole (19a). A mixture of **2** (0.336 g, 2 mmol), 4-(4,6-dichloro-2-pyrimidinyl)morpholine (**17**) (0.367 g, 2 mmol), and powdered K_2CO_3 (2 g, 15 mmol) in DMF (10 mL) was heated at 45 °C overnight and diluted with water. The precipitate was collected by filtration and dried. Chromatography on silica, eluting with CH_2Cl_2/EtOAc (49:1), gave 1-[6-chloro-2-(4-morpholinyl)-4-pyrimidinyl]-2-(difluoromethyl)-1*H*-benzimidazole[66] (**18**) (0.17 g, 23%): mp (MeOH) 159−161 °C; ^{1}H NMR ($CDCl_3$) δ 7.92 (dd, J = 7.9, 1.5 Hz, 1H), 7.67 (dd, J = 7.2, 1.6 Hz, 1H), 7.49−7.41 (m, 2H), 7.19 (t, J_{HF} = 53.3 Hz, 1H), 6.84 (s, 1H), 3.91−3.84 (m, 4H), 3.81−3.77 (m, 4H). Anal. Calcd for $C_{16}H_{14}ClF_2N_5O$: C, 52.5; H, 3.9; N, 19.15. Found: C, 52.8; H, 3.8; N, 19.4%.

A solution of **18** (0.16 g, 0.44 mmol) in morpholine (10 mL) was heated under reflux for 30 min and diluted with water to give **19a** (0.175 g, 96%): mp (aqueous MeOH) 172−174 °C; ^{1}H NMR ($CDCl_3$) δ 7.92−7.88 (m, 1H), 7.67−7.62 (m, 1H), 7.42−7.36 (m, 2H), 7.24 (t, J_{HF} = 53.5 Hz, 1H), 6.09 (s, 1H), 3.83−3.76 (m, 12H), 3.68−3.63 (m, 4H). Anal. Calcd for $C_{20}H_{22}F_2N_6O_2$: C, 57.7; H, 5.3. Found: C, 57.3; H, 5.3%.

2-(Difluoromethyl)-1-[2,6-di(4-morpholinyl)-4-pyrimidinyl]-4-methoxy-1*H*-benzimidazole (19b). A mixture of 4,6-dichloro-2-(methylsulfanyl)pyrimidine (**20**) (7.72 g, 0.04 mol), **8b** (7.93 g, 0.04 mol), and powdered K_2CO_3 (22 g, 0.26 mol) in DMSO (100 mL) was stirred at room temperature for 3 days and diluted with water. The solid was collected, washed with water, and dried. Chromatography on silica, eluting with CH_2Cl_2-EtOAc (95:5), gave 1-[6-chloro-2-(methylsulfanyl)-4-pyrimidinyl]-2-(difluoromethyl)-4-methoxy-1*H*-benzimidazole (**21**) (5.91 g, 41% yield): mp (*i*-Pr_2O/hexanes) 120−121 °C; ^{1}H NMR ($CDCl_3$) δ 7.40 (t, J = 8.2 Hz, 1H), 7.32 (s, 1H), 7.26 (dd, J = 8.4, 0.7 Hz, 1H), 7.18 (t, J_{HF} = 53.3 Hz, 1H), 6.85 (d, J = 7.9 Hz, 1H), 4.07 (s, 3H), 2.62 (s, 3H). Anal. Calcd for $C_{14}H_{11}ClF_2N_4OS$: C, 47.1; H, 3.1; N, 15.7. Found: C, 47.3; H, 3.4; N, 15.7%.

Further elution with CH_2Cl_2−EtOAc (9:1) gave the bis-addition byproduct 2-(difluoromethyl)-1-[6-[2-(difluoromethyl)-4-methoxy-1*H*-benzimidazol-1-yl]-2-(methylsulfanyl)-4-pyrimidinyl]-4-methoxy-1*H*-benzimidazole (4.16 g, 20% yield): ^{1}H NMR ($CDCl_3$) δ 7.64 (s, 1H), 7.45−7.38 (m, 4H), 7.26 (t, J_{HF} = 53.3 Hz, 2H), 6.87 (dd, J = 6.9, 2.0 Hz, 2H), 4.07 (s, 6H), 2.62 (s, 3H).

A mixture of **21** (3.57 g, 10 mmol) and morpholine (2.18 g, 25 mmol) in THF (50 mL) was stirred at room temperature for 30 min and diluted with water to give 2-(difluoromethyl)-4-methoxy-1-[2-(methylsulfanyl)-6-(4-morpholinyl)-4-pyrimidinyl]-1*H*-benzimidazole (**22**) (3.85 g, 94% yield): mp (MeOH) 169−171 °C; ^{1}H NMR ($CDCl_3$) δ 7.32 (t, J = 8.2 Hz, 1H), 7.22 (dd, J = 8.4, 0.7 Hz, 1H), 7.18 (t, J_{HF} = 53.4 Hz, 1H), 6.79 (d, J = 7.5 Hz, 1H), 6.42 (s, 1H), 4.06 (s, 3H), 3.82 (m, 4H), 3.71 (m, 4H), 2.54 (s, 3H). Anal. Calcd for $C_{18}H_{19}F_2N_5O_2S$: C, 53.1; H, 4.7; N, 17.2. Found: C, 53.1; H, 4.7; N, 17.3%.

A solution of **22** (2.04 g, 5 mmol) in a mixture of acetone (500 mL) and acetic acid (50 mL) was combined with a solution of $KMnO_4$ (5 g) in water (100 mL), and the resulting mixture was stirred at room temperature for 1 h. Dilution with water and decolorization with $NaHSO_3$ gave 2-(difluoromethyl)-4-methoxy-1-[2-(methylsulfonyl)-6-(4-morpholinyl)-4-pyrimidinyl]-1*H*-benzimidazole (**23**) (1.80 g, 82% yield) as a white solid: mp (MeOH) 190−191 °C; ^{1}H NMR ($CDCl_3$) δ 7.38 (t, J = 8.2 Hz, 1H), 7.26 (dd, J = 8.4, 0.7 Hz, 1H), 7.13 (t, J_{HF} = 53.2 Hz, 1H), 6.86 (s, 1H), 6.83 (d, J = 7.8 Hz, 1H), 4.07 (s, 6H), 3.85 (m, 4H), 3.31 (s, 3H). Anal. Calcd for $C_{18}H_{19}F_2N_5O_4S$: C, 49.2; H, 4.4; N, 15.9. Found: C, 49.4; H, 4.25; N, 15.9%.

A mixture of **23** (0.439 g, 1 mmol) and morpholine (0.87 g, 10 mmol) in THF (20 mL) was heated under reflux for 3 h before being cooled, and diluted with water, to give **19b** (0.41 g, 92% yield): mp (CH_2Cl_2/MeOH) 180−182 °C; ^{1}H NMR ($CDCl_3$) δ 7.30 (t, J = 8.9 Hz, 1H), 7.22 (dd, J = 8.3, 0.8 Hz, 1H), 7.15 (t, J_{HF} = 54.1 Hz, 1H), 6.78 (dd, J = 7.9, 0.7 Hz, 1H), 6.09 (s, 1H), 4.05 (s, 3H), 3.82−3.74 (m, 12H), 3.66−3.62 (m, 4H). Anal. Calcd for $C_{21}H_{24}F_2N_6O_3$: C, 56.5; H, 5.4; N, 18.8. Found: C, 56.3; H, 5.7; N, 19.1%.

Molecular Modeling. Molecular modeling studies were carried out using SYBYL software, version 7.3, running on a Linux workstation.[45] Compound **1** was built using the Sketch module and optimized using the Tripos force field. The protein structures were prepared for docking using SYBYL7.3, and this included stripping all waters and adding hydrogens consistent with the changes proposed after analysis of the structure with Molprobity.[67] Docking simulations were performed

on human p110γ (PDB code 2CHX) and human p110α (2rd0) using the automated GOLD program.[44] Twenty conformations were generated. In order to take into account protein flexibility, the conformation with the best score (GoldScore) was further refined using the MINIMIZE module. The minimization process used the Powell method with the Tripos force field (dielectric constant 1r) to reach a final convergence of 0.01 kcal mol^{-1}.[68]

Enzyme Assays. The class Ia PI3K lipid kinase assays were performed using recombinant PI 3-kinase and phosphatidylinositol as a substrate and [^{33}P]ATP tracer with a final ATP concentration of 10 μM, as described previously.[50,69] For the HTRF assay p85α/p110δ was obtained from Invitrogen while all other isoforms were produce in house by coexpressing full length human p85α with the indicated human full length catalytic subunit containing a His tag at the amino terminus to allow purification. IC_{50} values were evaluated using the PI 3-kinase (human) HTRF assay (Millipore no. 33-016). The PI 3-kinases were titrated and used at a concentration between their EC_{65} and EC_{80}. The protocol was carried out according to the manufacturer's recommendations, with the final mixture being incubated for 2 h prior to reading in a BioTek Synergy 2 plate reader equipped with excitation filter (360/40) and emission filters (620/40 and 665/7). IC_{50} data are normally the average of at least two determinations.

Cell Proliferation Assay. The early passage cell lines used in this study were NZB5, a cell line derived from a medulloblastoma and expressing the wild-type gene for p110α, and NZOV9 a cell line derived from a poorly differentiated endometrioid adenocarcinoma of the ovary and expressing a mutant p110α enzyme with a single amino acid substitution in the kinase domain (Y1021C). The derivation of the latter line has been described previously.[70] Cells were cultured in the presence of drugs in a 5-day assay as previously described, and proliferation was measured by incorporation of [^{3}H]thymidine.[69] IC_{50} data are normally the average of at least two determinations.

Inhibition of Cell Signaling. HCT116 and U87MG cells were grown in MEM α supplemented with 10% (v/v) FBS (fetal bovine serum), 100 units/mL penicillin, and 100 μg/mL streptomycin (all from Invitrogen). For inhibition studies, cells were seeded in 12-well plates and grown for 1 day before overnight starvation in serum-free medium. Cells were then exposed to varying concentrations of inhibitor dissolved in DMSO or DMSO alone (final concentration of DMSO in media 0.1%) for 15 min before stimulation with 500 nM insulin for 5 min. Protein isolation and immunoblotting for phospho-PKB were carried out according to the methods previously described, with antibodies from Cell Signaling Technology (Ser473 catalogue no. 9271, Thr308 catalogue no. 9275).[50]

Pharmacokinetics. Age-matched specific pathogen-free male CD-1 mice were administered a single 10 mg/kg dose of **1** in 5% DMSO, 10% Cremophor EL, 10% EtOH, 75% saline, **10a** in 20% DMSO, 70% H_2O, 10% NaOH or **10w** in 10% DMSO, 40% PEG-400, 50% D5W (5% dextrose). Mice were culled at multiple time points after dosing, and blood was removed by cardiac puncture into EDTA collection tubes (Becton Dickinson, Auckland, New Zealand). Blood samples were centrifuged for 10 min at 6000 rpm to separate plasma, which underwent protein precipitation in MeOH. Quantitative analysis was carried out on an Agilent 6460 triple quadrupole LC−MS/MS (Agilent Technologies, Forest Hill, Victoria, Australia) using multiple reaction monitoring and electrospray ionization. An Agilent Zorbax SB-C18 column (2.1 mm × 50 mm, 5 μm) column was used for chromatographic separation with a mobile phase gradient of 20−100% MeOH in 0.1% formic acid and 5 mM ammonium formate at a flow rate of 0.4 mL/min. Quantitation was achieved against a calibration curve from 10 to 10 000 nM, and quality controls were included at 65, 650, and 6500 nM. A MeOH slug was run between each sample to prevent contamination from previous samples. Pharmacokinetic parameters were determined by noncompartmental analysis using WinNonlin 5.3 software (Pharsight, Sunnyvale, CA, U.S.).

Tumor Xenografts. Age-matched specific pathogen-free female Rag1$^{-/-}$ mice were subcutaneously inoculated on the right flank with 5×10^6 U87MG cells in phosphate buffered saline (PBS). Tumor volume (mm^3) was calculated using the formula $(Lw^2)(\pi/6)$ (where L is the longest tumor diameter and w is the perpendicular diameter). Dosing began when tumors were well established, averaging approximately 7 mm in diameter. Compound **10w** was administered as a suspension in D5W, **25** was administered in 10% EtOH and **1** in 10% DMSO, 15% Cremophor EL, 15% EtOH, and 60% saline. All drugs were dosed by ip injection at MTD as the free base equivalent at a dosing volume of 10 mL/kg. Tumor volume and animal body weight were measured daily. Mice were culled if they developed signs of toxicity or if bodyweight loss exceeded 20% of starting weight. All animal experiments followed protocols approved by the Animal Ethics Committee of The University of Auckland.

Aqueous Solubility Determinations. The solid compound sample was mixed with water (enough to make a 2 mM solution) in an Eppendorf tube, and the suspension was sonicated for 15 min and then centrifuged at 13 000 rpm for 6 min. An aliquot of the clear supernatant was diluted 2-fold with water and then centrifuged again at 13 000 rpm for 6 min. A 100 μL aliquot of the clear supernatant was injected into the HPLC and the peak area measured. The solubility was calculated by comparing the peak area obtained with that from a standard solution of the compound in DMSO (after allowing for varying dilution factors and injection volumes). HPLC was conducted on an Agilent 1100 system using an Altima C18 column with a gradient elution from an organic phase of 80% v/v acetonitrile and water (40%), and an aqueous mobile phase of 45 mM ammonium formate solution (pH 3.5) (60%), to 100% organic phase.

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ABBREVIATIONS USED

AKT, v-akt murine thymoma viral oncogene homologue 1; ATP, adenosine triphosphate; AUC, area under the curve; AUC_{INF}, area under the curve to time infinity; Boc, *tert*-butoxycarbonyl; C_{max}, maximum concentration; D5W, 5% dextrose in water; DMF, *N*, *N*-dimethylformamide; DMSO, dimethylsulfoxide; DPPA, diphenylphosphorylazide; E545K, somatic mutation replacing glutamic acid by lysine at position 545 of p110α; EtOAc, ethyl acetate; EDTA, ethylenediaminetetraacetic acid; FBS, fetal bovine serum; H1047R, somatic mutation replacing histidine by arginine at position 1047 of p110α; HOAc, acetic acid; HTRF, homogeneous time-resolved fluorescence; ip, intraperitoneal; MEM, minimum

essential medium; mTOR, mammalian target of rapamycin; PBS, phosphate buffered saline; PEG, polyethylene glycol; PFK, perfluorokerosene; PIK3CA, phosphoinositide 3-kinase, catalytic, α polypeptide; PIP2, phosphatidylinositol 4,5-biphosphate; PIP3, phosphatidylinositol 3,4,5-triphosphate; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; pPKB, phosphoprotein kinase B; PTEN, phosphatase and tensin homologue gene; PPA, polyphosphoric acid; qd, every day; SAR, structure—activity relationship; $t_{1/2}$, biological half-life; TBAF, tetrabutylammonium fluoride; TBDMS, *tert*-butyldimethylsilyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TIPS, triisopropylsilyl; T_{max}, time to maximum plasma concentration; Y1021C, somatic mutation replacing tyrosine by cysteine at position 1021 of p110α

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