Synthesis and Biological Evaluation of Pyridazinone Analogues as Potential Cardiac Positron Emission Tomography Tracers

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A series of fluorinated pyridazinone derivatives with IC_{50} values ranging from 8 to 4000 nM for the mitochondrial complex 1 (MC1) have been prepared. Structure–activity relationship (SAR) assessment indicated preference of the fluorine label to be incorporated on an alkyl side chain rather than directly on the pyridazinone moiety. Tissue distribution studies of a series of analogues ([^{18}F] **22–28**) in Sprague−Dawley (SD) rats identified [^{18}F]**27** as the most promising radiotracer with high uptake in cardiac tissue (3.41%ID/g; 30 min post injection) in addition to favorable heart to nontarget organ distribution ratios. MicroPET images of SD rats and nonhuman primates after [^{18}F]**27** administration allowed easy assessment of the myocardium through 60 min with minimal lung or liver interference.

Introduction

Nuclear myocardial perfusion imaging agents (MPIA)[a] have been increasingly utilized in the assessment of coronary artery disease (CAD).[1] The most widely used MPIA are ^{201}Tl or cationic ^{99m}Tc complexes used with single-photon emission computed tomography (SPECT) in the determination of myocardial blood flow and viability in patients.[2,3] However, SPECT imaging has inherent limitations such as accurate attenuation correction, spatial resolution, and the ability to quantify myocardial perfusion in absolute terms (milliliters per gram per minute) compared to positron emission tomography (PET) contrast agents.[4–6] In addition, current SPECT tracers also exhibit shortcomings in their pharmacokinetic properties such as myocardial extraction, redistribution of the radiotracer to nontarget tissue over time, and linearity of uptake at elevated blood flow (the "roll-off" phenomenon).[7–9] Therefore, PET imaging has become a clinically viable alternative in evaluating myocardial blood flow by use of positron-emitting radionuclides.[10–14] The most widely used PET flow tracers in the evaluation of myocardial perfusion are: ^{82}RbCl, $^{13}NH_3$, and $H_2{}^{15}O$.[15–19] Unfortunately, their usage is limited by a short physical half-life (<10 min) and, for $^{13}NH_3$ and $H_2{}^{15}O$, the requirement of local cyclotron production. New PET tracers should therefore have greater myocardial extraction and superior linearity of uptake versus flow to existing agents, retention within the myocardium for the desired imaging window, and a sufficient half-life that single-dose commercial distribution would be feasible.

Recent studies in the isolated rabbit heart with ^{125}I-iodorotenone (**2**, Figure 1) indicated a superior extraction and retention than ^{99m}Tc-sestamibi and ^{201}Tl.[20] Rotenone (**1**), a natural product, is widely used as an insecticide, acaricide, and miticide.[21,22] Rotenone is a potent inhibitor of mitochondrial complex 1 (MC1), which is the first enzyme of four electron transport complexes embedded in the mitochondrial membrane.[23,24] Because of the very high weight percentage of mitochondria present in cardiomyocytes, MC1 may represent a new target for the development of cardiac PET tracers.[25]

Numerous classes of MC1 inhibitors have been reported in addition to **1**.[26–30] Our laboratories were particularly interested in compounds such as fenazaquin (**3**), *S*-chromone (**4**), tebufenpyrad (**5**), and pyridaben (**6**, Figure 2), which all share the same binding site of the MC1 enzyme as rotenone.[31] In addition, MC1 inhibitors **3**−**6** are structurally similar as they all possess: (a) a hydrophobic heterocyclic "headpiece", for example a pyridazinone headpiece as **6**, (b) a *p*-tert-butylphenyl moiety the "side chain", and (c) a heteroatom-containing linker, connecting (a) and (b).

Previous in vivo studies of [^{18}F] labeled analogues **7** and **8** demonstrated rapid uptake of the radiotracer into the myocardium in rats (Figure 3).[32,33] However, both radiotracers showed a significant amount of washout after 60 min post injection from the myocardium tissue. In this paper, we report our efforts toward the development of a PET tracer based on MC1 inhibitor **6**. Our goal is to incorporate PET radionuclide ^{18}F into an analogue of **6**, maintain affinity for MC1, and achieve good uptake and retention in the myocardium.

Results and Discussion

Structure–Activity Relationships. Previous structure–activity relationship studies of pyridaben have shown a significant decrease in MC1 inhibitory activity when the *t*-butyl moiety was replaced with an ethyl or methyl group demonstrating the necessity of the *t*-butyl moiety in the pyridazinone headpiece.[34] Our initial efforts focused on replacement of the chlorine moiety in the pyridazinone headpiece with other halogens such as fluorine to yield analogue **9** (Table 1). MC1 inhibitory activity significantly declined from an IC_{50} value of 8 nM of **6** to 279

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[a] Abbreviations: MPIA, myocardial perfusion imaging agent; CAD, coronary artery disease; SPECT, single-photon emission computed tomography; PET, positron emission tomography; MC1, mitochondrial complex 1; TBDMS-Cl, *tert*-butyldimethylsilyl chloride; SMP, submitochondrial particles.

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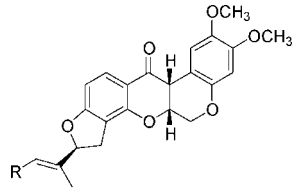


R=H, Rotenone (**1**)
R=^{125}I, Iodorotenone (**2**)

Figure 1. Natural product rotenone, a known MC1 inhibitor.

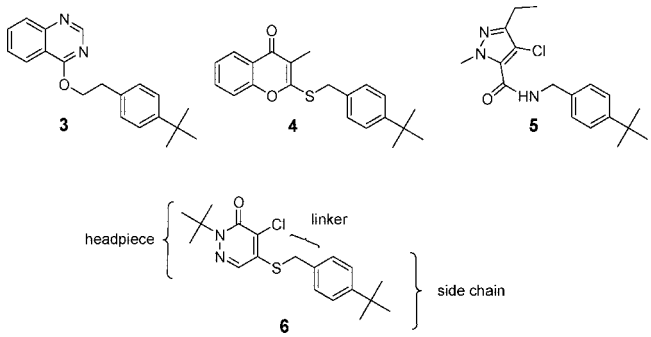


Figure 2. Various structure classes of known MC1 inhibitors.

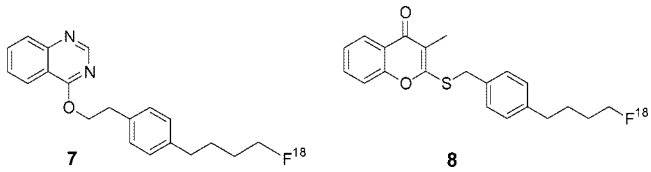
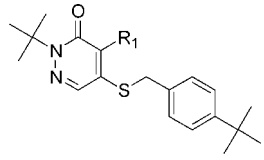


Figure 3. [^{18}F] labeled analogues of MC1 inhibitors fenazaquin (**7**) and *S*-chromone (**8**).

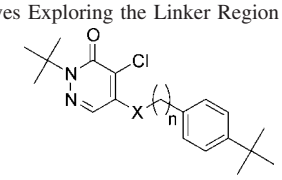
Table 1. Binding Affinity (IC_{50}, Determined in Vitro) of Known MC1 Inhibitors and Pyridaben Derivatives Exploring the Pyridazinone Headpiece



compound	R_1	IC_{50} (nM)
6	−Cl	8
9	−F	279
10	$-CH_3$	21
11	$-CH_2F$	250
1		16
3		90
4		52
7		11
8		9

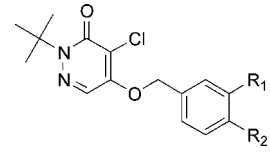
nM for **9**. Next, the chlorine moiety of **6** was replaced with a methyl group to yield compound **10**. The methyl substituent of **10** was used as an isostere for the chlorine atom of **6** but differed electronically. Here, we observed only a 3-fold decrease in activity yielding an IC_{50} value of 21 nM for **10** versus 8 nM for **6**. Unfortunately, attempts to incorporate a fluorine atom into compound **10** to yield compound **11** resulted in a significant decrease in MC1 activity. Simultaneously, known MC1 inhibitors **1**, **3**, and **4** were screened for MC1 activity and found to be less potent than **6**, whereas analogues **7** and **8** were equivalent in potency compared to **6**.

Table 2. Binding Affinity (IC_{50}, Determined in Vitro) of Nonfluorinated Pyridaben Derivatives Exploring the Linker Region



compound	X	n	IC_{50} (nM)
6	S	1	8
12	NH	1	4000
13	O	1	64
14	O	2	711

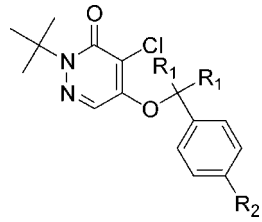
Table 3. Binding Affinity (IC_{50}, Determined in Vitro) of Nonfluorinated Pyridaben Derivatives Exploring the Side Chain Region



Compound	R_1	R_2	IC_{50} (nM)
15	-H	-H	850
16	-H	$-CH_2CH_2CH_2CH_3$	17
17	-H	$-OCH_2CH_2CH_2CH_3$	27
18	-H		31
19	$-(CH)_4-$		60
20	$-N(CH)_3-$		221

A series of analogues were prepared to investigate the heteroatom requirements in the linker as well as the optimal chain length of the linker (Table 2). When the sulfur atom in the linker of **6** was replaced with a nitrogen (**12**) or oxygen (**13**) atom, MC1 inhibitory activity decreased ~500 and 8 fold, respectively. Although sulfur as the linking atom imparts the greatest activity, the oxygen atom was preferred due to anticipated greater metabolic stability.[35] Extending the chain of the linker of **13** by one carbon to generate analogue **14** was detrimental to MC1 inhibitory activity. Therefore, compound **13** was chosen to represent the optimal compromise between MC1 potency and metabolic stability. Further synthetic efforts were directed toward modification of the side chain of **13** for future fluorine incorporation (Table 3). Initially, removal of the *t*-butyl moiety to yield compound **15** resulted in a >10 fold decrease in MC1 inhibitory activity, indicating the requirement of a para -substituted alkyl moiety in order to maintain biological activity. Therefore, replacement of the *t*-butyl moiety in the side chain with a *n*-butyl moiety as previously reported in the preparation of fluorinated analogues of **7** and **8** generated compound **16** (IC_{50} = 17 nM), which was 2-fold more potent than the parent analogue **13** (IC_{50} = 64 nM). Insertion of an oxygen atom at the benzylic position in the four-atom linker of **16** to yield five-atom linker analogue **17** resulted in a decrease in MC1 activity (IC_{50} = 27 nM). Interestingly, replacement of the alkyl moiety of the side chain in analogue **16** with an aryl substituent affording compound **18** resulted in a 2-fold decrease in MC1 binding activity (IC_{50} = 31 nM). However, replacement

Table 4. Binding Affinity (IC_{50}, Determined in Vitro) of Fluorinated Pyridaben Derivatives



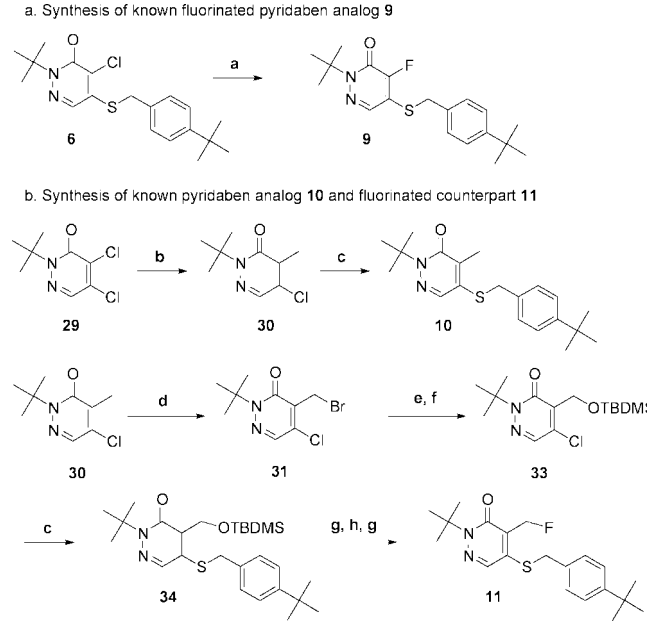
compound	R_1	R_2	IC_{50} (nM)
22	−H	$-CH_2CH_2CH_2CH_2F$	15
23	−H	$-OCH_2CH_2CH_2F$	47
24	−H	$-CH_2CH_2CHFCH_3$	10
25	−H	$-OCH(CH_2F)CH_2CH_3$	16
26	−H	$-OCH_2CHFCH_3$	8
27	−H	$-CH_2OCH_2CH_2F$	11
28	−D	$-CH_2OCD_2CD_2F$	28

of the aryl moiety in **18** with a naphthyl or quinoline group to generate compounds **19** and **20** resulted in a 2- and 7-fold loss of MC1 activity, respectively. Last, replacement of the aryl moiety in the side chain of **6** with a *n*-decyl chain to yield compound **21** resulted in complete loss of biological activity, demonstrating the need of an aryl moiety in the side chain in order to maintain MC1 activity.[36]

Next, fluorinated pyridaben analogues were prepared (Table 4) Analogue **22** possessed MC1 inhibition similar to nonfluorinated counterpart **16**, whereas compound **23** exhibited ∼3-fold decrease in activity versus analogue **22**. Further SAR studies of the most potent pyridaben analogue **22** were pursued. According to the literature, radiotracers containing primary ^{18}F labels may be metabolically defluorinated in vivo at a faster rate than secondary or aromatic ^{18}F.[37,38] However, primary aliphatic ^{18}F atoms, which are β to a heteroatom, e.g., [18F]FCH_2-CH_2O-R, have been reported to be metabolized at a slower rate. This has been termed the β-heteroatom effect.[39] In this regard, analogues **24–27** in Table 4 were prepared. Compound **24**, which contained a secondary fluorine atom, exhibited equipotent MC1 inhibitory activity compared to that of the parent compound **16** with IC_{50} values of 10 nM versus 17 nM, respectively. Introducing an oxygen atom in the side chain of compound **24** in order to take advantage of the β-heteroatom effect in addition to the secondary nature of the fluorine atom resulted in compound **26** (IC_{50} = 8 nM). Compounds **25** and **27** also contain primary fluorine labels with an oxygen atom positioned appropriately for increased metabolic stability, exhibiting potent MC1 activities of 11 and 16 nM, respectively. Lastly, deuterium atoms were substituted onto the carbon center bearing the fluorine atom of compound **27** in an attempt to further reduce in vivo defluorination. Researchers have observed an increased metabolic stability of deuterated radiotracers due to the isotope effect of deuterium on metabolism.[40] The resulting analogue **28** exhibited a ∼2.5-fold decrease in MC1 activity compared to that of the parent compound **27**. Because analogues **22–28** all exhibit good MC1 inhibitory activity, further evaluations in vivo were performed.

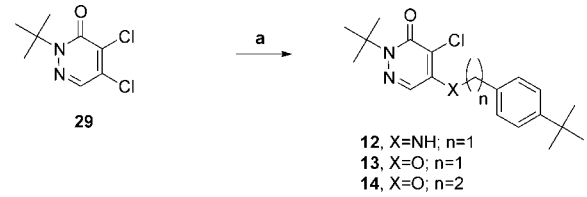
Chemistry. The synthesis for pyridaben analogues **9–11**,[41] which describe modifications of the pyridazinone headpiece, are shown in Scheme 1. Displacement of the chlorine atom in **6** using potassium fluoride and kryptofix222 at elevated temperature in DMSO afforded fluorinated analogue **9** in low yield. Analogues **10** and **11** were prepared from common pyridazinone headpiece **30**,[41] which was obtained via methylation of 3,4-dichloropyridazinone (**29**)[42] with methylmagnesium bromide in diethyl ether at 0 °C. Alkylation of **30** with commercially

Scheme 1. Alterations in the Pyridazinone Headpiece[a]



[a] Reagents: (a) KF, K222, DMSO, 120 °C; (b) MeMgBr, diethyl ether, 0 °C; (c) 4-*tert*-butylbenzyl mercaptan, Cs_2CO_3, DMF, 65 °C; (d) NBS, AIBN, CCl_4, reflux; (e) K_2CO_3, dioxane/water, 110 °C; (f) TBDMS-Cl, imidazole, DMF, r.t.; (g) TBAF, THF, 0 °C; (h) MsCl, TMPDA, ACN, 0 °C.

Scheme 2. Variations in Chain Length and Hetero Atoms within the Side Chain[a]

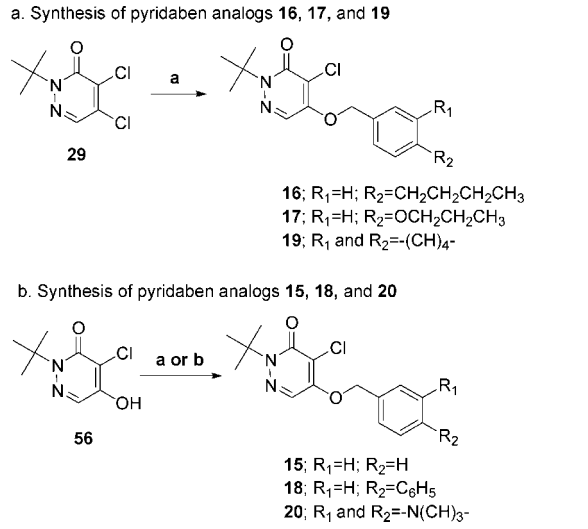


[a] Reagents: (a) **37**, **38**, or **39**, Cs_2CO_3, DMF, 55-80 °C.

available 4-*tert*-butylbenzyl mercaptan in the presence of cesium carbonate in DMF at 65 °C rendered final compound **10**, whereas the synthesis of **11** required the introduction of a fluorine moiety in the pyridazinone headpiece. Bromination of **30** using *N*-bromosuccinimide in the presence of AIBN in carbon tetrachloride under reflux condition afforded headpiece **31**. Hydrolysis of the bromide moiety in **31** with potassium carbonate in a mixture of dioxane and water at 110 °C introduced the hydroxyl moiety in the headpiece, which was protected with TBDMS-Cl to afford intermediate **33** in good yield. Introduction of the side chain using standard alkylation conditions (4-*tert*-butylbenzyl mercaptan, Cs_2CO_3, DMF, 65 °C) yielded intermediate **34**. Deprotection of the alcohol moiety in headpiece **34** followed by subsequent mesylation afforded fluorination precursor **36**. Fluorination of **36** using TBAF in THF at 0 °C afforded final compound **11**.

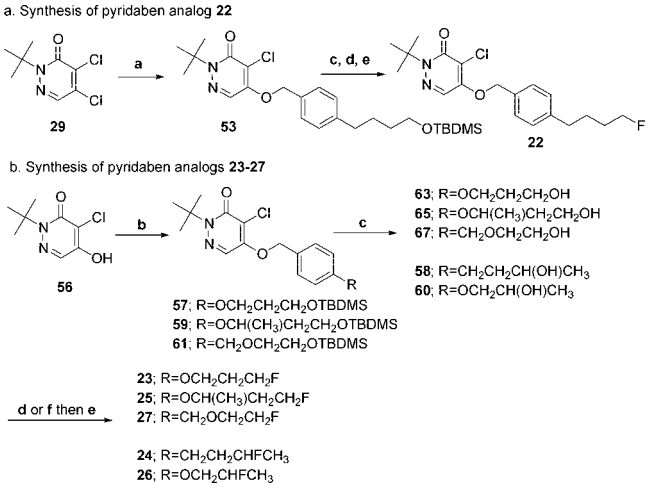
Next, the length of the linker and heteroatom preference within the linker of the side chain was investigated. In this regard, analogues **12–14** were prepared as shown in Scheme 2. Alkylation of headpiece **29** in the presence of cesium carbonate in DMF at temperatures ranging from 55 to 80 °C with the appropriate alcohol or amine (**37–39**) afforded the desired final compounds. Similar alkylation conditions were also utilized when preparing compounds **16, 17**, and **19** (Scheme 3a) to investigate the substitution patterns of the phenyl moiety in the

Scheme 3. SAR Studies of the Side Chain Substitution Pattern[a]



[a] Reagents: (a) **40**, **41**, **42**, **43**, or **44**, Cs_2CO_3, DMF, 25-75 °C; (b) **45**, DIAD, PPh_3, THF, 0 °C.

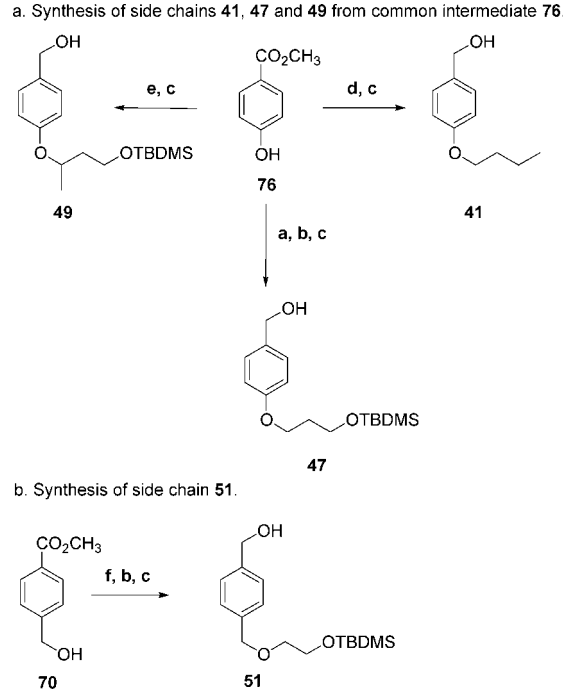
Scheme 4. Synthesis of Fluorinated Pyridaben Analogues **22**−**27**[a]



[a] Reagents: (a) **46**, Cs_2CO_3, DMF, 68 °C; (b) **47**, **48**, **49**, **50**, or **51**, DIAD, PPh_3, THF; (c) TBAF, THF, r.t.; (d) TsCl, DMAP, DIEA, DCM; (e) KF, K222, ACN, 90 °C; (f) TsCl, pyridine, r.t.

side chain. Additional functionalized side chains were installed using pyridazinone headpiece 3-hydroxy-4-chloropyridazinone (**56**)[43] and functionalized benzyl bromides in the presence of cesium carbonate in DMF at 25 and 70 °C to afford analogues **15** and **18**, respectively (Scheme 3b). Coupling of **56** with functionalized benzyl alcohols via Mitsunobu conditions could also be used to afford pyridaben analogues such as **20** and intermediates **57–61** for fluorinated pyridaben analogues **22–27** (Scheme 4). Reaction intermediates **57**, **59**, and **61** contained a protected hydroxyl moiety at various positions within the side chain. Deprotection of the alcohol moiety of these intermediates with TBAF in THF led to pyridazinone intermediates **63**, **65**, and **67**. Formation of the toluenesulfonate ester of alcohols **58**, **60**, **63**, **65**, and **67** affords fluorination precursors **64**, **66**, **69**, **71**, and **73.** Fluorination of these sulfonates at 90 °C in acetonitrile for time intervals ranging from 10 to 40 min rendered final pyridaben analogues **23–27**. Final pyridaben analogue **22** in this series was the only analogue prepared using

Scheme 5. Synthesis of Oxygen Containing Side Chains for Pyridaben Analogues **23**, **25**−**28**[a]



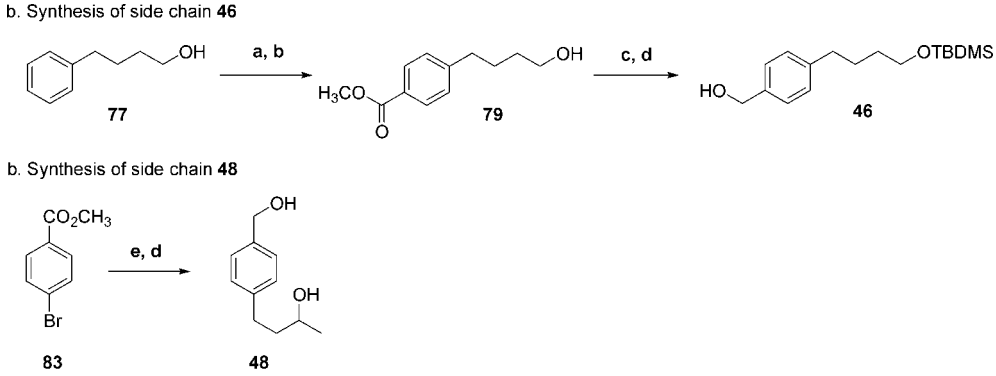
[a] Reagents: (a) 1-bromo-3-propanol, K_2CO_3, DMF, 50 °C; (b) TBDMS-Cl, imidazole, DMF, r.t.; (c) LAH, THF or ether, 0−25 °C; (d) 1-bromobutane, Cs_2CO_3, DMF, r.t.; (e) 1-*tert*-butyldimethylsilyloxy-2-hydroxybutane, DIAD, PPh_3, THF, 0 °C; (f) ethylene oxide, BF_3 etherate, ether, DCM, −10 °C.

alkylation conditions to couple the pyridazinone headpiece with the appropriate side chain (Scheme 4a).

When pyridaben analogues **17** and **22–27** were prepared according to the reaction sequences shown in Schemes 3 and 4, the required functionalized benzyl alcohols used as reaction partners in Mitsunobu reactions or alkylations had to be synthesized separately. Scheme 5 shows the preparation of side chain precursors **41**, **47**, **49**, and **51** required in the preparation of fluorinated pyridaben analogues **17**, **23**, **25**, and **27.**

Commercially available methyl-4-hydroxybenzoate (**76**) was used as a common intermediate to prepare side chain precursor **41**, **47**, and **49**. Alkylation of **76** with 1-bromobutane in the presence of cesium carbonate in DMF at room temperature yielded alkylation product **75**. Reduction of the ester moiety in **75** with LAH in ether at 0 °C afforded side chain precursor **41** in good yield. Side chain precursor **47** was prepared in a similar fashion. Modified alkylation conditions (K_2CO_3, DMF, 50 °C) using 1-bromo-3-propanol to afford alkylation product **81** followed by protection of the alcohol moiety with TBDMS-Cl and imidazole in DMF at room temperature yielded **82**. Reduction of the ester moiety in **82** with LAH in ether at 0 °C afforded functionalized benzyl alcohol **47**. Side chain **49** was prepared using Mitsunobu conditions to couple **76** with 1-*tert*-butyldimethylsiloxy-2-hydoxybutane followed by reduction of the ester moiety with LAH in ether at 0 °C to afford benzyl alcohol **49**. Commercially available methyl 4-(hydoxymethyl) benzoate (**70**) was exposed to ethylene oxide in the presence of a Lewis acid to afford intermediate **72**. Protection of the primary alcohol moiety in **72** with TBDMS-Cl and imidazole in DMF to yield **86** followed by reduction of the ester moiety in **86** with LAH in THF afforded final side chain precursor **51**. The synthesis of the side chain precursor for the deuterated

Scheme 6. Synthesis of All Carbon Side Chains **46** and **48**[a]



[a] Reagents: (a) butyryl chloride, DCM, r.t.; (b) i. oxalyl chloride, $AlCl_3$, DCM; ii. MeOH, 48 h. (c) TBDMS-Cl, imidazole, DMF, r.t.; (d) LAH, THF or diethyl ether, 0 °C; (e) 3-butene-2-ol, $Pd(OAc)_2$, PPh_3, TEA.

Table 5. Biodistribution of [^{18}F]Pyridaben Analogues in Sprague−Dawley Rats[a]

	heart uptake		tissue ratios[b]			
compounds	30 min	120 min	heart:blood	heart:lung	heart:liver	heart:femur
22	2.94 ± 0.24	2.99 ± 0.99	60.67	14.93	1.45	6.84
23	1.31 ± 0.09	0.86 ± 0.43	9.27	5.40	0.77	2.00
24	2.25 ± 0.04	2.14 ± 0.26	20.76	12.49	1.78	1.98
25	1.05 ± 0.13	1.24 ± 0.06	6.05	1.37	1.11	0.89
26	1.15 ± 0.36	1.14 ± 0.17	13.17	11.41	2.62	0.40
27	3.41 ± 0.27	3.47 ± 0.37	30.02	16.05	2.74	7.51
28	1.09 ± 0.06	1.60 ± 0.24	12.74	7.33	1.70	4.60

[a] Data are expressed as the %ID/g ± SD with three animals per data point. [b] Ratios are taken from 30 min post injection data points.

analogue **28** was prepared in an analogous fashion. The alcohol **70** was exposed to deuterated ethylene oxide in the presence of a Lewis acid to afford intermediate **87**. Subsequent protection of the alcohol **87** (TBDMS-Cl, imidazole, DMF) yielded **88**. Reduction of the ester moiety in **88** with LAD in THF afforded deuterated side chain precursor **52**.

The preparation of side chain precursors **46** and **48**, which were used to prepare analogues **22** and **24**, are shown in Scheme 6. Commercially available alcohol **77** was esterified using butyryl chloride in DCM to afford intermediate **78**. Introduction of the benzyl ester moiety in **78** was accomplished via a two-step procedure to afford intermediate **79** in good yield. Protection of the primary alcohol moiety in **79** with TBDMS-Cl and imidazole in DMF and subsequent reduction of the benzyl ester moiety with LAH in ether at 0 °C afforded side chain precursor **46**, which was coupled with **29** to yield final analogue **22**. Side chain precursor **48** was obtained from 4-bromomethylbenzoate **83** via a Heck coupling with 2-butene-2-ol, $Pd(OAc)_2$, PPh_3 in TEA to afford intermediate **84**, followed by reduction of the ketone moiety of **84** with LAH in THF.

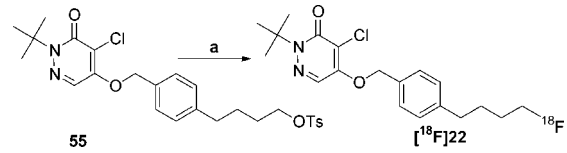
In Vitro Experiments. The inhibitory activities of compounds **1**, **3**, **4**, and **6–28** for the MC1 enzyme were determined by measuring the rate of NADH oxidation in the presence of decyl ubiquinone at 340 nm with a UV–vis spectrophotometer over 120 s.[44] Submitochondrial particles (SMP) were isolated from bovine heart mitochondria according to Lester and Matsuno-Yagi et al.[45,46] The SMP were used as a suspension, and rotenone was run as a reference standard versus each compound.

Radiosynthesis. Compounds **22–28** within this series were radiolabeled with the PET isotope ^{18}F. Nucleophilic radiofluorination methods require activated leaving groups such as sulfonates (e.g., tosylates, mesylate) in order to generate alkylfluoride containing radioligands.[35] Primary sulfonates are often the preferred leaving group because of their ease of displacement with nucleophilic ^{18}F, compared with secondary sulfonate leaving groups, which often eliminate under fluorination conditions to yield olefinic byproducts. With this in mind, the [^{18}F]**22–28** were prepared with chemical yields ranging from 8.2 to 35% (Table 6) by the reaction of their corresponding tosylate precursors with kryptofix222/K^{18}F complex and potassium carbonate in acetonitrile at 90 °C for 30 min followed by preparative HPLC separation of the reaction mixture (Scheme 7). The appropriate fractions were concentrated and analyzed for radiochemical yield and purity. The radioactivity of the final product was ∼25 mCi with radiochemical purity >99% when prepared with 500 mCi [^{18}F]fluoride, with a total time of synthesis of 90 min. The specific activity of the material ranged from 750 to 2000 Ci/mmol depending upon the quantity of ^{19}F present in the system.

Table 6. Radiochemical and Reaction Yield for the Synthesis of [^{18}F]**22**−**28**

compounds	RCP (%)	yield (%)
22	100	12.3
23	100	10.2
24	91.5	8.2
25	100	13.4
26	100	17.6
27	100	35
28	100	18.2

Scheme 7. Radiosynthesis of [^{18}F]**22**[a]



[a] (a) K^{18}F/K222, ACN, 90 °C, 30 min. Analogues [^{18}F]**23**−**28** were prepared in an analogous fashion from the appropriate sulfonate precursor.

Table 7. Biodistribution of [^{18}F]Pyridaben Analogues in Sprague−Dawley Rats

	tissue distribution[a]			
compounds	lung	liver	femur	blood
22	0.01 ± 0.18[b]	2.03 ± 0.11[b]	0.43 ± 0.06[b]	0.11 ± 0.01[b]
	0.18 ± 0.03[c]	0.62 ± 0.08[c]	1.16 ± 0.12[c]	0.04 ± 0.01[c]
28	0.24 ± 0.03[b]	1.70 ± 0.31[b]	0.65 ± 0.09[b]	0.15 ± 0.00[b]
	0.08 ± 0.00[c]	0.55 ± 0.05[c]	0.76 ± 0.71[c]	0.07 ± 0.01[c]
27	0.18 ± 0.02[b]	1.27 ± 0.10[b]	1.14 ± 0.13[b]	1.15 ± 0.36[b]
	0.14 ± 0.08[c]	0.63 ± 0.16[c]	2.38 ± 0.28[c]	3.41 ± 0.27[c]
26	0.79 ± 0.33[b]	0.98 ± 0.10[b]	1.23 ± 0.23[b]	0.18 ± 0.03[b]
	0.22 ± 0.02[c]	0.39 ± 0.01[c]	3.00 ± 0.43[c]	0.06 ± 0.00[c]
25	0.10 ± 0.00[b]	0.44 ± 0.07[b]	2.91 ± 0.56[b]	0.09 ± 0.01[b]
	0.06 ± 0.01[c]	0.40 ± 0.04[c]	3.91 ± 0.78[c]	0.04 ± 0.00[c]
24	0.21 ± 0.03[b]	1.25 ± 0.17[b]	0.45 ± 0.04[b]	0.11 ± 0.01[b]
	0.28 ± 0.04[c]	0.55 ± 0.12[c]	0.75 ± 0.08[c]	0.19 ± 0.01[c]
23	0.15 ± 0.00[b]	0.64 ± 0.07[b]	0.23 ± 0.03[b]	0.08 ± 0.00[b]
	0.13 ± 0.01[c]	0.44 ± 0.04[c]	0.78 ± 0.07[c]	0.10 ± 0.01[c]

[a] Data are expressed as the %ID/g ± SD with three animals per data point. [b] Percent ID/g determined after 30 min post injection. [c] Percent ID/g determined after 120 min post injection.

In Vivo Studies. Biodistribution Study of [^{18}F]Pyridaben-Analogues 22–28 in Sprague−Dawley (SD) Rats. Biodistribution studies were performed with [^{18}F]pyridazinone derivatives [^{18}F]**22–28** in SD rats (see Table 5).

High uptake of radiotracers [^{18}F]**22**, [^{18}F]**24**, and [^{18}F]**27** from the blood pool into the myocardium was observed at 30 min post injection (>2.0%ID/g; >20:1 heart:blood pool ratio). The concentration of radiotracers [^{18}F]**22** and [^{18}F]**27** residing in the heart remained constant from 30 to 120 min post injection, and [^{18}F]**24** exhibited minimal wash-out at 120 min post injection, at which point approximately 95% of the activity present at 30 min post injection was still present.

The uptake of radiotracers [^{18}F]**22–28** in nontarget tissues was measured as well. At 30 min post injection, radiotracers [^{18}F]**22**, [^{18}F]**24**, and [^{18}F]**27** exhibited heart:lung tissue ratios with >10 fold selectivity while maintaining high uptake in myocardium tissue (>2.0%ID/g). However, [^{18}F]**27** exhibited the best heart to lung ratio (16.05) while exhibiting the highest uptake in myocardium tissue (3.41%ID/g) compared to [^{18}F]**22** (heart:lung ratio 14.93, 2.94%ID/g) and [^{18}F]**24** (heart:lung ratio 12.49, 2.25%ID/g) at 30 min post injection. Low uptake of the radiotracer in lung tissue suggests an excellent signal-to-noise ratio for future imaging studies with [^{18}F]**27**. When measuring the ratio of radiotracer in heart:liver tissue, [^{18}F]**27** showed a selectivity of 2.74 fold for the myocardium, which is more selective than [^{18}F]**22** (heart:liver ratio 1.45) and [^{18}F]**24** (heart:liver ratio 1.78). Interestingly, the heart:liver ratio of [^{18}F]**26** was measured to be 2.62, which is comparable to [^{18}F]**27**. However the heart uptake of [^{18}F]**26** is only 1.15%ID/g at 30 min post injection.

Addressing the previous concerns regarding in vivo defluorination and subsequent accumulation of the free fluoride in bone, compounds [^{18}F]**22**, [^{18}F]**27**, and [^{18}F]**28** exhibited femural uptake of less than 0.5%ID/g after 30 min post injection (Table 7). Introduction of an oxygen atom in the side chain of [^{18}F]**22** to generate the β-heteroatom effect did not reduce in vivo defluorination (0.43%ID/g for [^{18}F]**22** vs 0.45%ID/g for [^{18}F]**27**) as previously suggested. On the other hand, introduction of deuterium labels in [^{18}F]**27** to yield [^{18}F]**28** did result in a 2-fold decrease of in vivo defluorination at 30 min post injection. When the fluorine label was secondary as in [^{18}F]**24**, femur uptake of the radiotracer at 30 min post injection was measured to be 1.13%ID/g. Compounds [^{18}F]**25** and [^{18}F]**26**, which also contain a secondary fluorine label in addition to the β-heteroatom, exhibited even higher uptake of free fluoride in femur than [^{18}F]**24**. Compounds [^{18}F]**22** and [^{18}F]**27** were identified as the radiotracers with high uptake in heart tissue (>2%ID/g) in addition to exhibiting low in vivo defluorination (<0.5%ID/g). Heart:femur ratios of compounds [^{18}F]**22** and [^{18}F]**27** possessed the highest selectivity with ratios of 6.84 and 7.51, respectively (Table 7) within this series of fluorinated pyridaben analogues. Although [^{18}F]**28** exhibited a 2-fold decrease of in vivo defluorination compared to [^{18}F]**27**, the heart uptake was only 1.09%ID/g, resulting in a heart:femur ratio of 4.60. Analogously, [^{18}F]**24** exhibited high heart uptake (2.25%ID/g) but also exhibited high femural uptake (1.13%ID/g), resulting in a heart:femur ratio of 1.98. Stability toward in vivo defluorination of [^{18}F]**27** did not improve with the introduction of deuterium atoms on the fluorine bearing carbon (heart:femur ratio 4.60).

These results suggest that although there is literature precedence on strategies for minimization of in vivo defluorination of radiotracers in general, our compound series does not follow these literature observations. This suggests that the phenomena of in vivo defluorination is highly substrate dependent as only few records in the literature successfully address reducing in vivo defluorination using the above-mentioned strategies.[47] In addition, the metabolism of any compound may be species dependent and therefore in vivo defluorination may occur to a more significant extent in one species than the other.[48] Nevertheless, this series does demonstrate that the alkyl portion of the side chain can tolerate structural variation such as incorporation of heteroatoms and alternative placement of the fluorine label without profoundly affecting the MC1 activity. Tables 3 and 4 show that five out of the seven analogues synthesized exhibit an MC1 inhibitory activity of less than 20 nM. However, biodistribution studies of these compounds demonstrated significant differences in uptake of the individual radiotracers in various tissues. Thus [^{18}F]**27** was chosen as the lead structure in this series because it exhibited the highest uptake in the heart at 30 min post injection (3.41%ID/g) with essentially no washout over 120 min and superior target-to-background tissue ratios compared to other compounds within this series. Comparison studies with ^{99m}Tc-Sestamibi demonstrated [^{18}F]**27** to be superior in heart uptake, myocardium retention, and target-to-background tissue ratios.[49,50] Overall, these results suggest [^{18}F]**27** to be a suitable agent for pursuing PET imaging studies.

Imaging Studies of [^{18}F]27 in SD Rats and Nonhuman Primates. Dynamic PET Imaging studies were carried out on SD rats and nonhuman primates. The PET images obtained on SD rats complemented the results obtained in the above tissue distribution study. [^{18}F]**27** accumulated within minutes after the

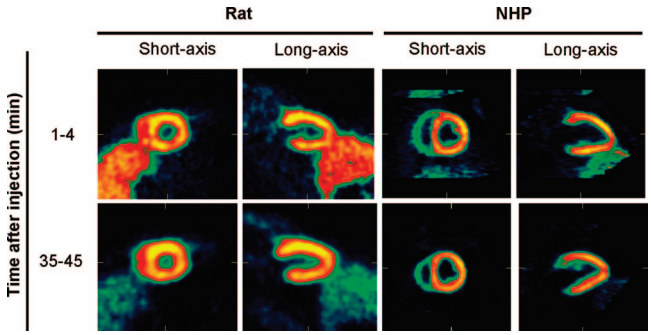


Figure 4. Images of a rat and nonhuman primate (NHP) heart at two different time points using [^{18}F]**27**.

injection of the rat in cardiac and liver tissue as is depicted in Figure 4. Significant uptake of the radiotracer is visible in the images of the rat heart, which was maintained over a 120 min imaging period with minimal loss of signal intensity. Sufficient clearance of compound [^{18}F]**27** from the liver was observed by 35–45 min post injection to obtain good myocardial images and was complete by 55–60 min post injection. No interference from lung uptake of the radiotracer was detected, as demonstrated by the high quality images obtained from this study. However, some in vivo bone uptake of [^{18}F] could be detected in images after 30 min post injection.

Observations made in the PET imaging studies of nonhuman primates were similar to the PET imaging studies of rats. Rapid accumulation of the radiotracer in cardiac tissue was observed within minutes after the injection of the animal. Uptake of [^{18}F]**27** in liver tissue was seen as well, although to a much lesser extent than that in the PET images of rats. Direct comparison among disparate species is possible via scaling to quantified external activity sources placed beside the subject animals in each study; normalization to these reference sources gives comparable uptake values. Uptake levels of [^{18}F]**27** in the myocardium allowed clear visualization of the right and left ventricle walls. This signal intensity was maintained over a 120 min imaging period. At 35–45 min post injection, clearance of the radiotracer from liver tissue was complete. As was observed in the PET imaging studies of the rat, no interference from lung uptake of [^{18}F]**27** was detected. However, unlike in the rat imaging studies, no in vivo bone uptake of [^{18}F]could be detected in nonhuman primate images up to 120 min post injection, confirming a more favorable metabolic profile in higher species.

Conclusion

A series of fluorinated pyridaben derivatives with high affinity for MC1 have been prepared. SAR studies were focused on addressing metabolic stability of the linker region of **6** as well as the optimal location of the fluorine label. Combined SAR studies of **6** and **13** have shown that incorporation of a fluorine label in the pyridaben alkyl side chain such as compounds **22–28** exhibit more potent binding affinities for the MC1 complex (IC_{50} < 50 nM) than attachment of a fluorine label directly onto the pyridazinone ring (compounds **9** and **11**, IC_{50} > 200 nM). Introduction of an oxygen atom in the alkyl side chain or changing the location of the fluorine label within the alkyl side chain did not disrupt MC1 inhibition (IC_{50} = 8–47 nM).

Biodistribution studies of [^{18}F]**22–28** in rats identified [^{18}F]**27** as the radiotracer with the highest absolute heart uptake at 30 min post injection (3.41%ID/g) in addition to the largest uptake ratios of heart to nontarget organs such as liver, lung, femur, and blood. No washout of the radiotracer was observed in the myocardium at 120 min post injection.

Imaging studies of rats and nonhuman primates demonstrated a high initial uptake of [^{18}F]**27** in the liver (more uptake was seen in PET images with rats compared to nonhuman primates), which cleared by 25 min post injection sufficiently to afford high quality images of the heart. While some in vivo bone uptake of [^{18}F] was observed in rat images 30 min after [^{18}F]**27** administration, no in vivo [^{18}F] bone accumulation was observed in nonhuman primate PET images up to 120 min. This observation strongly suggests that the in vivo defluorination observed in biodistribution and imaging studies of compounds [^{18}F]**22–28** is a highly species-dependent phenomenon. Future studies will therefore be directed toward examining the metabolic profile and potential utility of these compounds in other animal species.

Experimental Section

General Methods and Materials. All reagents used in synthesis were commercial products and were used without further purification unless otherwise indicated. All solvents used were ACS or HPLC grade. ^{1}H NMR spectra were obtained in a Bruker DRX 600 MHz FTNMR spectrometer in $CDCl_3$ unless otherwise indicated. Chemical shifts are reported as δ values (parts per million) relative to solvent peak. Coupling constants are reported in Hz. The multiplicity is defined by a s (singlet), d (doublet), t (triplet), br (broad), or m (multiplet).

High-performance-liquid chromatography (HPLC) analysis and purification were performed on a Varian/PrepStar model SD-1 at 220 and 254 nm. The instrument was equipped with the ProStar/Dynamax.24 software program on a Dell/Optiplex GX 1 computer system. Water used in the preparation of the mobile phases was purified using a Millipore/Milli-Q Gradient A10 system. Flash chromatography was conducted using silica gel 230–400 mesh (60 Å), Merck. LCMS and TOF analysis were carried out on an Agilent LC/MSD instrument (model G1969A), which is connected to an 1100 Series HPLC system. For radiolabeling studies, F-18 was obtained from PETNET Pharmaceutical Services, Cummings Park, Woburn, MA, and the synthesis was carried out in Wheaton vials using a customized robotic synthesis platform. Biodistribution studies were measured in an autogamma counter (Wallac Wizard 1480), while cardiac imaging was carried out using a microPET camera (Focus220, CTI Molecular Imaging, Inc. Knoxville, TN). All study protocols were approved by the Institutional Animal Care and Use Committee.

Synthesis of 2-*tert*-Butyl-4-fluoro-5-(4-*tert*-butylbenzyl)mercapto-3-(2*H*)-pyridazinone (9).[41] To a solution of KF (23.8 mg, 0.41 mmol) and kryptofix222 (154 mg, 0.41 mmol) in DMSO (4.0 mL) was added pyridaben (**6**, 150 mg, 0.41 mmol). The reaction mixture was heated at 120 °C. After 2.5 h, the reaction mixture was cooled to room temperature and diluted with ethyl acetate (10 mL). The organic layer was separated, washed with water (40 mL), brine (40 mL), dried over Mg_2SO_4, filtered, and concentrated. The crude brown oil was purified by HPLC (Phenomenex Luna C18(2) column 10 μ, 21.2 mm × 250 mm; gradient: 0–90% B over 30 min at 20 mL/min; Mobile phase A = water with 0.1% TFA modifier and B = 90% acetonitrile in water with 0.1% TFA modifier) to obtain compound **9** (37 mg, 25% yield) as a fluffy white solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.57 (d, 1H, J = 7.5 Hz), 7.34 (d, 2H, J = 8.3 Hz), 7.27 (d, 2H, J = 8.3 Hz), 4.23 (s, 2H), 1.61 (s, 9H), 1.29 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 155.2, 154.3, 153.4, 151.1, 133.4, 132.3, 128.4, 125.8, 65.9, 36.0, 34.5, 31.2, 27.7; ^{19}F ($CDCl_3$, 564 MHz) δ: –119.69 (m, 1F).

Synthesis of 2-*tert*-Butyl-4-methyl-5-chloro-3-(2*H*)-pyridazinone (30).[41] To a cooled (0 °C) solution of MeMgBr (6.02 mL of 3.0 M solution in ether, 18.1 mmol) was added dropwise a solution of **29** (2.0 g, 2.27 mmol)[42] dissolved in Et_2O (10 mL). The resulting dark-red solution was stirred at 0 °C for 2 h and then quenched by the dropwise addition of 5 N HCl (aq) (5 mL). The resulting yellow

solution was poured into a separatory funnel to separate the layers. The aqueous layer was extracted with ethyl acetate (2 × 15 mL), and the combined organic layers were washed with water (10 mL), brine (10 mL), dried over Mg_2SO_4, filtered, and concentrated. The crude oil was purified by silica gel chromatography (hexanes:ether gradient, 99:1 to 97:3) to obtain compound **30** (0.55 g, 31% yield) as a thick oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.52 (s, 1H), 2.21 (s, 3H), 1.60 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 157.4, 137.9, 135.4, 66.1, 27.7, 16.7.

Synthesis of 2-*tert*-Butyl-4-methyl-5-(4-*tert*-butylbenzyl)mercapto-3-(2*H*)-pyridazinone (10).[41] A suspension of compound **30** (64 mg, 0.32 mmol), 4-*tert*-butylbenzyl mercaptan (180.3 mg, 0.64 mmol), Cs_2CO_3 (207.8 mg, 0.64 mmol) in DMF (1.5 mL) was heated at 65 °C. After 2.5 h, the reaction mixture was diluted with water (1 mL) and ethyl acetate (3 mL). The organic layer was separated and washed with water (4 × 4 mL), brine (4 mL), dried over Mg_2SO_4, filtered, and concentrated. The crude oil was purified by silica gel chromatography (hexanes:ethyl acetate gradient, 98:2 to 96:4) to obtain compound **10** as a white solid (60 mg, 55% yield). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.63 (s, 1H), 7.35 (d, 2H, J = 8.4 Hz), 7.27 (d, 2H, J = 8.4 Hz), 4.16 (s, 2H), 2.13 (s, 3H), 1.61 (s, 9H), 1.30 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 160.2, 150.9, 138.8, 135.6, 132.5, 132.0, 128.4, 125.8, 64.8, 36.1, 34.5, 31.2, 27.9, 13.4.

Synthesis of 2-*tert*-Butyl-4-bromomethyl-5-chloro-3-(2*H*)-pyridazinone (31). A solution of compound **30** (0.4 g, 1.99 mmol), *N*-bromosuccinimide (1.42 g, 7.99 mmol), and azobisisobutyronitrile (AIBN, 16.40 mg, 0.1 mmol) in CCl_4 (55 mL) was refluxed for 16 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated in vacuo to obtain a crude oil, which was dissolved in ethyl acetate (50 mL) and washed with water (2×15 mL), brine (15 mL), and dried over Mg_2SO_4. A white crystalline solid (0.48 g, 86% yield) was obtained upon concentration of the organic layer and was used in the next step without further purification; mp 99 °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.70 (s, 1H), 4.47 (s, 2H), 1.62 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 158.8, 137.4, 135.7, 134.3, 66.6, 28.0, 22.8; HRMS-TOF (*m*/*z*): $[M + H]^+$ calcd for $C_9H_{12}BrClN_2O$, 278.9894; found, 278.9891.

Synthesis of 2-*tert*-Butyl-4-hydroxymethyl-5-chloro-3-(2*H*)-pyridazinone (32). A suspension of compound **31** (0.48 g, 1.73 mmol), K_2CO_3 (1.20 g, 8.7 mmol) in dioxane (3.2 mL), and water (3.2 mL) was heated at 110 °C. After 4.5 h, the reaction mixture was cooled to room temperature and diluted with DCM (20 mL). The organic layer was separated and washed with water (10 mL), brine (10 mL), and dried over Mg_2SO_4. Concentration of the organic layer in vacuo afforded **32** (0.3 g, 80% yield) as a pale-yellow solid, which was used in the next step without further purification. ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.04 (s, 1H), 4.65 (s, 2H), 1.67 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.0, 143.4, 135.0, 67.8, 59.7, 28.0; HRMS-TOF (*m*/*z*): $[M + H]^+$ calcd for $C_9H_{13}ClN_2O_2$, 217.0734; found 217.0734.

Synthesis of 2-*tert*-Butyl-4-(*tert*-butyldimethylsilyloxy)methyl-5-chloro-3-(2*H*)-pyridazinone (33). A solution of compound **32** (0.3 g, 1.38 mmol), *tert*-butyldimethylsilyl chloride (0.42 g, 2.76 mmol) and imidazole (0.19 g, 2.76 mmol) dissolved in DMF stirred for 16 h. The reaction mixture was diluted with ethyl acetate (15 mL). The organic layer was separated, washed with water (5 × 20 mL), brine (20 mL), dried over Mg_2SO_4, filtered, and concentrated. The crude oil was purified by silica gel chromatography (hexanes: diethyl ether gradient, 99:1 to 95:5) to obtain compound **33** (0.36 g, 80% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.64 (s, 1H), 4.72 (s, 2H), 1.61 (s, 9H), 0.88 (s, 9H), 0.10 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.7, 137.7, 137.1, 134.6, 65.7, 56.9, 27.7, 25.8, 18.4, –5.3; HRMS-TOF (*m*/*z*): $[M + H]^+$ calcd for $C_{15}H_{27}ClN_2O_2Si$, 331.1603; found, 331.1602.

Synthesis of 2-*tert*-Butyl-4-(*tert*-butyldimethylsilyloxy)methyl-5-(4-*tert*-butylbenzyl)mercapto-3-(2*H*)-pyridazinone (34). 4-*tert*-Butylbenzylmercaptan (0.4 g, 2.26 mmol) and Cs_2CO_3 (0.74 g, 2.26 mmol) were added to a solution of compound **33** (0.25 g, 0.76 mmol) and dissolved in DMF (9 mL). The reaction mixture was stirred for 1.5 h at 65 °C before being filtered at room temperature. The filtrate was diluted with ethyl acetate (20 mL), washed with water (5 × 20 mL) and brine (20 mL), and then dried over Mg_2SO_4. The crude oil obtained upon concentration in vacuo was purified by silica gel chromatography (hexanes:diether gradient, 100 to 95:5) to obtain **34** (280 mg, 78% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.84 (s, 1H), 7.23 (d, 2H, J = 8.4 Hz), 7.13 (d, 2H, J = 8.4 Hz), 4.52 (s, 2H), 4.34 (s, 2H), 1.69 (s, 9H), 1.27 (s, 9H), 0.9 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.4, 149.9, 145.7, 135.2, 132.6, 132.3, 128.5, 125.2, 65.5, 60.8, 35.7, 34.4, 31.2, 27.9, 25.8, –5.5; HRMS-TOF (*m*/*z*): $[M + H]^+$ calcd for $C_{26}H_{42}N_2O_2SSi$, 475.2809; found, 475.2813.

Synthesis of 2-*tert*-Butyl-4-hydroxymethyl-5-(4-*tert*-butylbenzyl)mercapto-3-(2*H*)-pyridazinone (35). To a cooled (0 °C) solution of compound **34** (0.12 g, 0.25 mmol) in THF (0.25 mL) was added TBAF (0.5 mL of 1.0 M solution in THF, 0.5 mmol) dropwise. After completion of addition the reaction mixture stirred for 3 h followed by concentration in vacuo to obtain a crude oil. Purification by silica gel chromatography (hexanes:ethyl acetate gradient, 100 to 92:8) of the crude material afforded compound **35** (73 mg, 80% yield) in good yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.71 (s, 1H), 7.35 (d, 2H, J = 8.4 Hz), 7.26 (d, 2H, J = 8.4 Hz), 4.64 (d, 2H, J = 6 Hz), 4.36 (t, 1H, J = 6 Hz), 4.17 (s, 2H), 1.61 (s, 9H), 1.30 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 160.4, 151.1, 139.2, 136.0, 133.1, 132.1, 128.4, 125.8, 65.4, 59.3, 36.5, 34.5, 31.2, 27.8; HRMS-TOF (*m*/*z*): $[M + H]^+$ calcd for $C_{20}H_{28}N_2O_2S$, 361.1944; found, 361.1942.

Synthesis of 2-*tert*-Butyl-4-(methanesulfonyloxy)methyl-5-(4-*tert*-butylbenzyl)mercapto-3-(2*H*)-pyridazinone (36). To a cooled (0 °C) solution of compound **35** (11 mg, 0.03 mmol) in ACN (0.2 mL) and tetramethylenepropylenediamine (TMPDA, 5.96 mg, 0.045 mmol) was added MsCl (5.24 mg, 3.56 μL, 0.045 mmol). After 5 min, the reaction mixture was diluted with ethyl acetate (1 mL) and water (0.5 mL). The organic layer was separated and washed with water (0.5 mL) and brine (0.5 mL), dried over Mg_2SO_4, filtered, and then concentrated. Compound **36** was obtained as a crude oil (14 mg, 100% yield), which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.70 (s, 1H), 7.24 (d, 2H, J = 8.2 Hz), 7.12 (d, 2H, J = 8.2 Hz), 5.06 (s, 2H), 4.39 (s, 2H), 2.93 (s, 3H), 1.68 (s, 9H), 1.26 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 158.7, 149.9, 137.6, 136.6, 134.2, 131.9, 128.1, 125.0, 65.7, 64.9, 59.9, 35.7, 34.0, 30.8, 27.4; HRMS-TOF (*m*/*z*): $[M + H]^+$ calcd for $C_{21}H_{30}N_2O_4S_2$, 439.1719; found, 439.1721.

Synthesis of 2-*tert*-Butyl-4-(fluoromethyl)-5-(4-*tert*-butylbenzyl)mercapto-3-(2*H*)-pyridazinone (11). To a cooled (0 °C) solution of compound **36** (14 mg, 0.03 mmol) dissolved in THF (0.2 mL) was added TBAF (95.8 μL of 1.0 M solution in THF, 0.10 mmol) dropwise. After completion of addition the reaction mixture was stirred for 40 min. Concentration of the reaction mixture in vacuo afforded a crude oil, which was purified by preparative silica gel chromatography (hexanes:ethyl acetate, 9:1) to obtain analogue **11** (7 mg, 50% yield) in moderated yield. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.76 (s, 1H), 7.25 (d, 2H, J = 8.4 Hz), 7.10 (m, 2H), 5.22 (d, 2H, J = 47.2 Hz), 4.26 (s, 2H), 1.68 (s, 9H), 1.26 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ: 161.1, 151.5, 144.0, 136.1, 133.4, 129.6, 126.3, 81.3 (79.7), 67.2, 36.9, 35.3, 31.6, 28.1; HRMS-TOF (*m*/*z*): $[M + H]^+$ calcd for $C_{20}H_{27}FN_2OS$, 363.1900; found, 363.1906.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(4-*tert*-butylbenzyl)amino-3-(2*H*)-pyridazinone (12). A suspension of compound **29** (0.1 g, 0.45 mmol), 4-*tert*-butylbenzylamine (**37**, 0.15 g, 0.91 mmol), and Cs_2CO_3 (0.44 g, 1.36 mmol) in DMF (3 mL) was heated at 80 °C. After 16 h, the reaction mixture was cooled to room temperature and filtered. The filtrate was diluted with ethyl acetate (10 mL), washed with water (2×5 mL) and brine (5 mL), dried over Mg_2SO_4, filtered, and then concentrated. The crude material was purified by silica gel chromatography (hexanes:ethyl acetate gradient, 100 to 80:20) to obtain analogue **12** (78 mg, 50% yield) in moderate yield. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.67 (s, 1H), 7.35 (d, 2H, J = 8 Hz), 7.25 (d, 2H, J = 8 Hz), 4.48 (d, 2H, J = 6.4 Hz), 3.30 (br,

1H), 1.49 (s, 9H), 1.24 (s, 9H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 157.4, 149.9, 144.1, 136.6, 127.0, 125.8, 125.0, 64.6, 45.1, 34.6, 31.5, 28.2; HRMS-TOF (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{26}ClN_3O$, 348.1837; found, 348.1843.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(4-*tert*-butylbenzyl)oxy-3-(2*H*)-pyridazinone (13).[41] A suspension of compound **29** (0.1 g, 0.45 mmol), 4-*tert*-butylbenzylalcohol (**38**, 0.15 g, 0.91 mmol), and cesium carbonate (0.29 g, 0.9 mmol) dissolved in DMF (3 mL) was heated at 75 °C. After 16 h, the reaction mixture was cooled to room temperature and filtered. The filtrate was diluted with ethyl acetate (10 mL) and washed with water (2 × 5 mL) and brine (5 mL). The organic layer was dried over Mg_2SO_4 and concentrated in vacuo to obtain an oil. The crude material was purified by silica gel chromatography (hexanes:ethyl acetate gradient, 100 to 94:6) to obtain analogue **13** (143 mg, 63% yield) in moderate yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.73 (s, 1H), 7.42 (d, 2H, J = 8.4 Hz), 7.33 (d, 2H, J = 8.4 Hz), 5.27 (s, 2H), 1.62 (s, 9H), 1.31 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.0, 153.8, 151.9, 131.8, 126.9, 125.8, 125.1, 118.2, 71.8, 66.3, 34.6, 31.2, 27.8.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(2-(4-*tert*-butylphenyl)-ethyl)oxy-3-(2*H*)-pyridazinone (14). A suspension of compound **29** (0.08 g, 0.34 mmol), 2-(4-*tert*-butylphenyl) ethyl alcohol (**39**, 90 mg, 0.51 mmol), and Cs_2CO_3 (166 mg, 0.51 mmol) in DMF (3 mL) was heated at 55 °C. After 16 h, the reaction mixture was cooled to room temperature and filtered. The filtrate was diluted with ethyl acetate (10 mL) and washed with water (2 × 5 mL) and brine (5 mL). The organic layer was dried over Mg_2SO_4 and concentrated in vacuo to obtain an oil. The crude material was purified by silica gel chromatography (hexanes:ethyl acetate, 90:10) to obtain analogue **14** (28 mg, 23% yield) in moderate yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.16 (s, 1H), 7.31 (d, 2H, J = 8 Hz), 7.24 (d, 2H, J = 8 Hz), 4.1 (t, 2H, J = 4 Hz), 3.0 (t, 2H, J = 4 Hz), 1.56 (s, 9H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 158.2, 154.4, 149.3, 134.7, 129.2, 126.4, 125.5, 115.5, 71.3, 65.7, 34.9, 34.5, 31.6, 27.9; HRMS-TOF (*m/z*): $[M + H]^+$ calcd for $C_{20}H_{27}ClN_2O_2$, 363.1833; found, 363.1836.

Synthesis of 2-*tert*-Butyl-4-chloro-5-benzyloxy-3-(2*H*)-pyridazinone (15).[41] A suspension of compound **56** (0.03 g, 0.15 mmol), benzyl bromide (**43**, 22.9 mg, 15.87 μL, 0.13 mmol), and Cs_2CO_3 (48.3 mg, 0.14 mmol) in DMF (1 mL) was stirred at room temperature. After 2 h, the reaction mixture was concentrated in vacuo to obtain a crude material, which was redissolved in a mixture of ethyl acetate (5 mL) and water (2 mL). The organic layer was separated and washed with water (2 × 2 mL), brine (2 mL), dried over Mg_2SO_4, filtered, and concentrated. The crude material was purified by preparative silica gel chromatography (hexanes:ethyl acetate, 4:1) to obtain analogue **15** (19 mg, 44% yield) as a viscous oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.71 (s, 1H), 7.37 (m, 5H), 5.30 (s, 2H), 1.61 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.0, 153.6, 134.8, 128.9, 128.7, 127.0, 125.1, 118.2, 71.8, 66.3, 27.8; HRMS-TOF (*m/z*): $[M + H]^+$ calcd for $C_{15}H_{17}ClN_2O_2$, 292.0979; found, 315.0874 $[M + Na]^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(4-butylbenzyl)oxy-3-(2*H*)-pyridazinone (16). A suspension of compound **29** (0.1 g, 0.45 mmol), 4-butylbenzyl alcohol (**40**, 0.14 g, 0.90 mmol), and Cs_2CO_3 (0.29 g, 0.99 mmol) in DMF (2.0 mL) was stirred at 65 °C. After 16 h, the reaction mixture was cooled to room temperature and diluted with ethyl acetate (10 mL). The organic layer was washed with water (2 × 10 mL) and brine (10 mL), dried over Mg_2SO_4, filtered, and then concentrated. The crude oil was purified by silica gel chromatography (hexanes:ethyl acetate gradient, 100 to 96:4) to obtain analogue **16** (118 mg, 75% yield) as a viscous oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.71 (s, 1H), 7.30 (d, 2H, J = 8.4 Hz), 7.2 (d, 2H, J = 8.4 Hz), 5.20 (s, 2H), 2.61 (t, 2H, J = 7.6, 7.8 Hz), 1.62 (s, 9H), 1.59 (m, 2H), 1.34 (m, 2H), 0.91 (m, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.2, 154, 143.8, 132.2, 129.1, 127.4, 125.4, 118.4, 72.2, 66.5, 35.5, 33.8, 28, 22.5,14.1; HRMS-TOF (*m/z*): $[M + H]^+$ for $C_{19}H_{25}ClN_2O_2$, 349.1677; found, 349.1674.

Synthesis of Methyl-4-butoxybenzoate (75). A suspension of methyl-4-hydroxybenzoate (**76**, 1.0 g, 6.57 mmol), 1-bromobutane (0.9 g, 6.57 mmol), and Cs_2CO_3 in DMF (15 mL) was stirred at room temperature. After 16 h, the reaction mixture was diluted with ethyl acetate (30 mL) and water (30 mL). The organic layer was separated and washed with water (4 × 30 mL), 1 M NaOH (aq) (10 mL), and brine (30 mL), dried over Mg_2SO_4, filtered, and then concentrated. Compound **75** was obtained as a crude oil (1.20 g, 87% yield), which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.98 (d, 2H, J = 9 Hz), 6.90 (d, 2H, J = 9 Hz), 4.02 (t, 2H, J = 6.6 Hz), 3.90 (s, 3H), 1.79 (m, 2H), 1.50 (m, 2H), 0.99 (t, 3H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 166.3, 163.0, 131.6, 122.1, 114.8, 67.9, 52.1, 31.0, 19.1, 14.0.

Synthesis of (4-Butoxyphenyl)-methanol (41). To a solution of compound **75** (15 mg, 0.59 mmol) in ether at 0 °C was added LAH (45 mg, 1.18 mmol). The resulting suspension was stirred for 3 h before water (45 μL), 15% NaOH (aq) (45 μL), and water (3 × 45 μL) were added sequentially. After completion of all the additions, the reaction mixture stirred for an additional 15 min before being filtered and concentrated to obtain compound **41** (110 mg, 85% yield) as an oil. **41** was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.25 (d, 2H, J = 7.8 Hz), 6.87 (d, 2H, J = 7.8 Hz), 4.59 (s, 2H), 3.95 (t, 2H, J = 7.8 Hz), 1.76 (m, 2H), 1.49 (m, 2H), 0.96 (t, 3H, J = *7.8* Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 158.8, 132.9, 128.6, 114.5, 67.1, 65.1, 31.3, 19.2, 13.8.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(4-butoxy-benzyloxy)-3(2*H*)-pyridazinone (17). A suspension of compound **29** (100 mg, 0.45 mmol), compound **41** (51 mg, 0.28 mmol), and Cs_2CO_3 (110 mg, 0.34 mmol) in DMF (5 mL) was heated at 75 °C. After 16 h, an additional portion of compound **29** (0.05 g, 0.225 mmol) and Cs_2CO_3 (0.05 g, 0.17 mmol) was added. The reaction mixture stirred at 75 °C for another 3 h before being diluted with water (10 mL). The aqueous layer was separated and extracted with DCM (2 × 10 mL). All of the combined organic layers were washed with water (4 × 10 mL) and brine (10 mL), dried over Mg_2SO_4, filtered, and then concentrated. The crude oil obtained was purified by silica gel chromatography (hexanes:ethyl acetate gradient, 99:1 to 9:1) to afford analogue **17** (28 mg, 25% yield) in moderate yield. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.26 (s, 1H), 7.37 (d, 2H, J = 8.4 Hz), 6.95 (d, 2H, J = 8.4 Hz), 5.35 (s, 2H), 3.95 (t, 2H, J = 7.8 Hz), 1.67 (m, 2H), 1.55 (s, 9H), 1.41 (m, 2H), 0.92 (t, 3H, J = 7.8 Hz); ^{13}C (DMSO-d_6, 100 MHz) δ: 159.4, 158.3, 154.3, 130.2, 127.5, 126.7, 116.0, 115.0, 71.8, 67.6, 65.8, 31.1, 27.9, 19.1, 14.2; HRMS-TOF (*m/z*): $[M + H]^+$ $C_{19}H_{25}ClN_2O_3$: 364.1554, found 387.1446 $[M + Na]^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(4-biphenylmethyl)oxy-3-(2*H*)-pyridazinone (18). A suspension of compound **56** (0.20 g, 1.01 mmol), 4-bromomethyl biphenyl (**44**, 0.25 g, 1.01 mmol), and Cs_2CO_3 (0.39 g, 1.2 mmol) in DMF (20 mL) was heated at 70 °C. After 2 h, the reaction mixture was concentrated to obtain a crude oil, which was redissolved in ethyl acetate (50 mL) and water (20 mL). The organic layer was separated and washed with water (2 × 20 mL) and brine (20 mL), dried over Mg_2SO_4, filtered, and then concentrated. The crude material obtained was purified by preparative silica gel chromatography (hexanes:ethyl acetate, 4:1) to obtain analogue **18** (0.11 g, 30% yield) as a thick oil. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.20 (s, 1H), 7.70 (m, 4H), 7.47 (m, 5H), 5.50 (s, 2H), 1.57 (s, 9H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 158.3, 154.3, 140.8, 140.0, 135.0, 129.4, 128.9, 128.1, 127.4, 127.1, 126.7, 117.0, 71.6, 65.8, 27.9; HRMS-TOF (*m/z*): $[M + H]^+$ for $C_{21}H_{21}ClN_2O_2$, 368.1292; found, 391.1178 $[M + Na]^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(2-naphthylmethyl)oxy-3-(2*H*)-pyridazinone (19). A suspension of compound **29** (100 mg, 0.50 mmol), 2-naphthyl methanol (**42**, 118 mg. 0.75 mmol), and Cs_2CO_3 (240 mg, 0.75 mmol) in DMF (2 mL) was heated at 65 °C. After 16 h, the reaction mixture was concentrated to obtain a crude oil, which was redissolved in ethyl acetate (10 mL). The organic layer was washed with water (2 × 5 mL) and brine (5 mL), dried over Mg_2SO_4, filtered, and then concentrated. The crude oil was purified by silica gel chromatography (hexanes:ethyl acetate, 100 to 80:20) to obtain analogue **19** (35 mg, 25% yield) in moderate yield. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.30 (s, 1H), 7.96 (m,

4H), 7.55 (m, 3H), 5.60 (s, 2H), 1.56 (s, 9H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 157.9, 153.9, 133.0, 132.7, 128.5, 128.0, 127.7, 126.8, 126.6, 126.3, 125.5, 71.6, 65.4, 27.5; HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{19}H_{19}ClN_2O_2$, 342.1135; found, 365.1031 $[M + Na]^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(6-quinolinylmethyl)oxy-3-(2*H*)-pyridazinone (20). To a cooled (0 °C) solution of compound **56** (49 mg, 0.24 mmol) in THF (2 mL) was added 6-quinolinyl methanol (**45**, 25 mg, 0.15 mmol), PPh_3 (61 mg, 0.23 mmol), and diisopropylazodicarboxylate (DIAD, 48 mg, 0.23 mmol). After completion of addition, the reaction mixture was stirred at 0 °C for 2 h and then concentrated to afford a yellow oil, which was purified by preparative thin layer chromatography (silica gel, hexanes:ethyl acetate, 1:1) to obtain analogue **20** (18 mg, 43% yield) as a white crystalline solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.90 (s, 1H), 8.40 (d, 1H, J = 8 Hz), 8.30 (s, 1H), 8.08 (d, 1H, J = 7.8 Hz), 8.07 (s, 1H), 7.82 (m, 1H), 7.56 (m, 1H), 5.60 (s, 2H), 1.56 (s, 9H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 158.3, 154.3, 151.5, 147.9, 136.6, 134.1, 130.0, 129.4, 128.0, 127.3, 126.7, 122.4, 116.2, 71.6, 65.9, 27.9; HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{18}H_{18}ClN_3O_2$, 358.1846; found, 344.1160 $[M + Na]^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-decylsulfanyl-3-(2*H*)-pyridazinone (21). A suspension of compound **29** (100 mg, 0.45 mmol), decanethiol (158 mg, 0.90 mmol), and Cs_2CO_3 (290 mg, 0.90 mmol) in DMF (1.5 mL) was stirred at room temperature for 3 h. The reaction mixture was diluted with water (1 mL) and ethyl acetate (2 mL). The organic layer was separated and washed with water (3 × 5 mL) and brine (5 mL), dried over Mg_2SO_4, filtered, and then concentrated. The crude oil was purified by silica gel chromatography (hexanes:diethyl ether gradient, 100 to 96:4) to obtain analogue **21** as a viscous oil (140 mg, 86% yield). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.58 (s, 1H), 2.99 (t, 2H, J = 7.2 Hz), 1.72 (m, 2H), 1.63 (s, 9H), 1.44 (m, 2H), 1.25 (br m, 12H), 0.8 (t, 3H, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 156.0, 141.4, 129.9, 129.6, 66.1, 31.8, 31.1, 29.4, 29.3, 29.2, 29.1, 29.0, 28.6, 27.8, 22.6, 14.0; HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{18}H_{31}ClN_2OS$, 358.1846; found, 381.1741 $[M + Na]^+$.

Synthesis of 4-Phenylbutyl Butyrate (78). To a solution of 4-phenyl-1-butanol (**77**, 7.0 g, 47 mmol) dissolved in DCM (20 mL) was added a solution of butyryl chloride (4.79 g, 45 mmol) in DCM (20 mL) dropwise. After completion of addition, the reaction mixture stirred for 36 h. The reaction mixture was then concentrated in vacuo to yield a crude oil, which was purified by silica gel chromatography (hexanes:ethyl acetate, 3:1) to afford compound **78** (9.8 g, 94% yield) as a clear viscous liquid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.27 (m, 2H), 7.17 (m, 3H), 4.08 (t, 2H, J = 7.2 Hz), 2.64 (t, 2H, J = 7.2 Hz), 2.27 (t, 2H, J = 7.8 Hz), 1.65 (m, 6H), 0.94 (t, 3H, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 173.7, 142.0, 128.3, 125.6, 64.0, 36.2, 35.4, 28.2, 27.7, 18.1, 13.6; HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{14}H_{20}O_2$, 220.1463; found, 243.1353 $[M + Na]^+$.

Synthesis of Methyl-4-(4-hydroxybutylbenzoate) (79). To a cooled (0 °C) solution of $AlCl_3$ (6.70 g, 0.05 mol) in DCM (100 mL) was added oxalyl chloride (6.4 g, 0.05 mol) dropwise. After completion of addition, the mixture was stirred for 5 min before the dropwise addition of a solution of compound **78** (9.80 g, 44 mmol) in DCM (50 mL). After completion of addition, the reaction mixture was stirred at 0 °C for 4 h. The reaction mixture was poured into a separatory funnel containing ice and brine. The organic layer was separated and washed with brine (100 mL), dried over Mg_2SO_4, filtered, and then concentrated. Then 9.0 g of the resulting yellow oil was suspended in methanol and stirred for 48 h. The reaction mixture was concentrated in vacuo, and the resulting crude material was purified by silica gel chromatography (hexanes:ethyl acetate, 2.5:1) to provide compound **79** (2.80 g, 31% yield) as a clear viscous liquid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.93 (d, 2H, J = 7.8 Hz), 7.23 (d, 2H, J = 7.8 Hz), 3.88 (s, 3H), 3.64 (t, 2H, J = 7.2 Hz), 2.67 (t, 2H, J = 7.2 Hz), 1.66 (m, 2H), 1.56–1.61 (m, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 167.3, 148.0, 129.8, 128.6, 62.8, 52.1, 35.8, 32.4, 27.6.

Synthesis of Methyl-4-[4-(*tert*-butyldimethylsilyloxy)butyl]-benzoate (80). A solution of compound **79** (1.0 g, 4.8 mmol), imidazole (0.5 g, 7.2 mmol), and *tert*-butyldimethylsilyl chloride (1.08 g, 7.3 mmol) dissolved in DMF (10 mL) was stirred at room temperature. After 2 h, the reaction mixture was diluted with ethyl acetate and washed with water (5 × 20 mL) and saturated $NaHCO_3$ (aq) (2 × 20 mL). The organic layer was dried over Mg_2SO_4, filtered, and concentrated to give compound **80** as an oil (1.17 g, 75% yield). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.05 (s, 6H), 0.90 (s, 9H), 1.49–1.74 (m, 4H), 2.65–2.70 (t, 2H, J = 7.2 Hz), 3.59–3.64 (t, 2H, J = 7.2 Hz), 3.89 (s, 3 H), 7.22–7.25 (m, 2 H), 7.93–7.96 (m, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 167.1, 148.1, 129.6, 128.4, 127.7, 62.8, 51.9, 35.6, 32.3, 27.4, 25.9, 18.3, –5.3; HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{18}H_{30}O_3Si$, 322.1964; found, 345.1865 $[M + Na]^+$.

Synthesis of (4-(4-(*tert*-Butyldimethylsilyloxy)butyl)phenyl)-methanol (46). To a cooled (0 °C) solution of compound **80** (1.17 g, 3.6 mmol) in Et_2O (14 mL) was added portionwise solid LAH (0.28 g, 7.2 mmol). After completion of addition, the reaction mixture was stirred for 1 h. Sequential addition of water (0.28 mL), 15% NaOH (aq) (0.28 mL), and water (0.84 mL) resulted in a cloudy solution, which stirred for an additional 15 min. The white solid was removed via filtration, and the filtrate was dried over Mg_2SO_4, filtered, and concentrated. The crude material was purified by silica gel chromatography (hexanes:ethyl acetate, 4:1) to afford compound **46** (1.02 g, 96% yield) as a clear viscous liquid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.27 (d, 2H, J = 6 Hz), 7.17 (d, 2H, J = 6 Hz), 4.60 (s, 2H), 3.60 (t, 2H, J = 6 Hz), 2.60 (t, 2H. J = 6 Hz), 1.61 (m, 4H), 0.89 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 142.2, 138.2, 128.6, 127.1, 65.8, 65.3, 62.9, 35.3, 32.3, 27.6, 25.7, 18.3, –5.2.

Synthesis of 2-*tert*-Butyl-4-chloro-5-[4-(4-hydroxybutyl)benzyloxy]-3-(2*H*)-pyridazinone (54). A suspension of compound **46** (0.41 g, 1.4 mmol), compound **29** (0.93 g, 4.2 mmol), and Cs_2CO_3 (1.37 g, 4.2 mmol) in DMF (11 mL) was heated at 68 °C. After 12 h, the reaction mixture was cooled down to room temperature and filtered. The filtrate was diluted with ethyl acetate (20 mL) and washed with water (5 × 25 mL) and brine (25 mL). The organic layer was dried over Mg_2SO_4, filtered, and concentrated. The crude material was purified using silica gel chromatography (hexanes: ethyl acetate, 9:1) to afford compound **53** (594 mg, 89% yield) as an oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.74 (s, 1 H), 7.33 (d, 2H, J = 8.4 Hz), 7.23 (d, 2H, J = 8.4 Hz), 5.23 (s, 2H), 3.64 (t, 2H, J = 6.6 Hz), 2.65 (t, 2H, J = 6.6 Hz), 1.64 (s, 9H), 0.90 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.3, 154.0, 143.7, 132.3, 129.2, 127.4, 125.4, 118.4, 72.1, 66.5, 63.1, 35.6, 32.5, 28.0, 27.7, 26.1, 18.5.

To a solution of compound **53** (594 mg, 1.45 mmol) dissolved in THF (3 mL) was added a solution of TBAF (2.9 mL of 1 M solution in THF, 2.9 mmol). After 1 h, the reaction mixture was concentrated under reduced pressure. The resulting crude material was purified using silica gel chromatography (pentane:ethyl acetate, 1.8:1) to afford compound **54** (410 mg, 77% yield) in good yield. ^{1}H ($CDCl_3$, 600 MHz) δ: 1.61–1.64 (m, 11H), 1.67–1.74 (m, 2H), 2.68 (t, 2H, J = 6.6 Hz), 3.68 (t, 2H, J = 6.6 Hz), 5.23 (s, 2H), 7.23 (d, 2H, J = 7.8 Hz), 7.33 (d, 2H, J = 7.8 Hz), 7.74 (s, 1H); ^{13}C ($CDCl_3$, 150 MHz) δ: 159.0, 153.7, 143.1, 132.2, 128.9, 127.2, 125.1, 118.2, 71.8, 66.3, 62.7, 35.3, 32.5, 27.8, 27.4; HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{19}H_{25}ClN_2O_3$, 364.1554; found, 387.1449 $[M + Na]^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(4-(4-*p*-toluenesulfonyloxy butyl)benzyloxy]-3-(2*H*)-pyridazinone (55). A solution of compound **54** (200 mg, 0.55 mmol), TsCl (125 mg, 0.66 mmol), DMAP (80 mg, 0.66 mmol), and DIEA (85 mg, 0.66 mmol) in DCM (2 mL) was stirred at room temperature. After 2 h, the reaction mixture was diluted with ethyl acetate (10 mL) and washed with 0.1 N HCl (aq) (3 mL) and brine (3 mL). The organic layer was dried over Mg_2SO_4, filtered, and concentrated. The resulting crude oil was purified by silica gel chromatography (pentane:ethyl acetate, 3:1) to provide compound **55** (197 mg, 69% yield). ^{1}H ($CDCl_3$, 600 MHz) δ: 1.62–1.70 (m, 13H), 2.43 (s, 3H), 2.58 (t,

2H, J = 7.2 Hz), 4.03 (t, 2H, J = 6.0 Hz), 7.15 (d, 2H, J = 7.4 Hz), 7.29–7.33 (m, 4H), 7.72 (s, 1H), 7.77 (d, 2H, J = 6.6 Hz); ^{13}C ($CDCl_3$, 150 MHz) δ: 159.0, 153.7, 144.7, 142.4, 133.1, 132.4, 129.8, 128.9, 127.8, 127.3, 125.1, 118.2, 71.8, 70.2, 66.3, 34.8, 28.3, 27.8, 26.9, 21.6; HRMS-TOF (m/z): $[M + H]^+$ for $C_{18}H_{30}O_3Si$, 518.1642; found, 541.1529 $[M + Na]^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(4-(4-fluorobutyl)benzyl)-oxy-3-(2*H*)-pyridazinone (22). Compound **55** (57 mg, 0.10 mmol) dissolved in ACN (1 mL) was added to a mixture of KF-kryptofix222 (1:1; 0.164 mmol) in ACN (1 mL). After completion of addition, the reaction mixture was heated at 90 °C for 15 min. The reaction mixture was concentrated in vacuo, and the resulting crude oil was purified by silica gel chromatography (hexanes:ethyl acetate, 4:1) to afford analogue **22** (28 mg, 70% yield) as an oil that solidified upon standing. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.71 (s, 1H), 7.30 (d, 2H, J = 8.4 Hz), 7.20 (d, 2H, J = 8.4 Hz), 5.20 (s, 2H), 4.44 (dt, 2H, J = 47.4, 6 Hz), 2.60 (t, 2H, J = 7.2 Hz), 1.70 (m, 4H), 1.60 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.0, 153.0, 142.8, 132.3, 128.9, 127.2, 125.1, 118.2, 84.3 (83.3), 71.8, 66.3, 35.1, 29.9 (29.8), 27.8, 26.8; ^{19}F ($CDCl_3$, 564 MHz) δ: −18.6 (m, 1F); HRMS-TOF (m/z): $[M + H]^+$ for $C_{19}H_{24}ClFN_2O_2$, 367.1583; found, 367.1587.

Synthesis of Methyl-4-(3-hydroxy-propoxy)benzoate (81). A suspension of 4-hydroxy methyl benzoate (**76**, 3.0 g, 0.02 mol), 3-bromo-1-propanol (4.17 g, 0.03 mol), and K_2CO_3 (4.15 g, 0.03 mol) in DMF (40 mL) was heated at 50 °C. After 12 h, the reaction mixture was diluted with ethyl acetate (50 mL) and washed with 0.1N HCl (aq) (2 × 15 mL), water (8 × 50 mL), and brine (50 mL). The organic layer was dried over Mg_2SO_4, filtered, and then concentrated to yield a crude oil, which was purified by silica gel chromatography (hexanes:ethyl acetate, 1.68:1) to afford compound **81** (1.25 g, 30% yield) as a white powder. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.98 (m, 2 H), 6.91 (m, 2H), 4.17 (t, 2H, J = 6.0 Hz), 3.87 (m, 5H), 2.04–2.08 (m, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 166.8, 162.6, 11.5, 122.6, 114.0, 65.5, 59.8, 51.8, 31.8; HRMS-TOF (m/z): $[M + H]^+$ for $C_{11}H_{14}O_4$, 210.0892; found, 233.0785 $[M + Na]^+$.

Synthesis of Methyl-4-(3-(*tert*-butyldimethylsilyloxy)propoxy)-benzoate (82). A solution of compound **81** (300 mg, 1.4 mmol), *tert*-butyldimethylsilyl chloride (317 mg, 2.1 mmol), and imidazole (146 mg, 2.1 mmol) in DMF (4 mL) was stirred at room temperature. After 2 h, the reaction mixture was diluted with ethyl acetate (20 mL) and washed with 0.1 N HCl (aq) (2 × 5 mL), water (4 × 10 mL), and brine (10 mL) and then dried over Mg_2SO_4, filtered, and concentrated. The resulting crude material was purified using silica gel chromatography (hexanes:ethyl acetate, 9.5:1) to afford compound **82** (413 mg, 91% yield) in high yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.97 (d, 2H, J = 8.4 Hz), 6.90 (d, 2H, J = 9.0 Hz), 4.11 (t, 2H, J = 6.0 Hz), 3.87 (s, 3H), 3.79 (t, 2H, J = 6.0 Hz), 1.97–2.01 (m, 2H), 0.87 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 166.9, 162.9, 131.5, 122.4, 114.0, 64.6, 59.2, 51.7, 32.3, 25.8, 18.3; HRMS-TOF (m/z): $[M + H]^+$ for $C_{17}H_{28}O_4Si$, 324.1757; found, 347.1651 $[M + Na]^+$.

Synthesis of (4-(3-(*tert*-Butyldimethylsilyloxy)propoxy) benzyl alcohol (47). To a cooled (0 °C) solution of compound **82** (396 mg, 1.22 mmol) in Et_2O (10 mL) was added portionwise solid LAH (93 mg, 2.44 mmol). After completion of addition, the reaction mixture was stirred for 2 h. Sequential addition of water (0.093 mL), 15% NaOH (aq) (0.093 mL), and water (0.28 mL) resulted in a cloudy solution. The white solid was removed by filtration, and the filtrate was dried over Mg_2SO_4, filtered, and concentrated to yield compound **47** (291 mg, 80% yield). **47** was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.26 (m, 2H), 6.88 (m, 2H), 4.60 (s, 2H), 4.05 (t, 2H, J = 6.6 Hz), 3.79 (t, 2H, J = 6.6 Hz), 1.97 (m, 2H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 158.7, 132.9, 128.6, 114.5, 65.1, 64.5, 59.5, 32.4, 25.9, 18.3; HRMS-TOF (m/z): $[M + H]^+$ for $C_{16}H_{28}O_3Si$, 297.1880; found, 297.1884.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(4-(3-(*tert*-butyl-dimethylsilyloxy)propoxy)benzyloxy)-3-(2*H*)-pyridazinone (57). To a cooled (0 °C) solution of compound **47** (211 mg, 0.71 mmol) in THF (3 mL) was added PPh_3 (187 mg, 0.71 mmol), compound **56** (142 mg, 0.71 mmol), and DIAD (144 mg, 0.71 mmol). The reaction mixture was stirred at 0 °C for 1 h and was then diluted with diethyl ether (6 mL). The organic solution was washed with water (3 mL) and brine (3 mL), dried over Mg_2SO_4, filtered, and then concentrated. Silica gel chromatography (hexanes:ethyl acetate, 9:1) of the crude material afforded compound **57** (106 mg, 31% yield). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.72 (s, 1H), 7.30 (d, 2H, J = 6.6 Hz), 6.91 (d, 2H, J = 6.6 Hz), 5.23 (s, 2 H), 4.06 (t, 2H, J = 6.6 Hz), 3.79 (t, 2H, J = 6.0 Hz), 1.97 (m, 2H), 1.62 (s, 9H), 0.87 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.5, 159.0, 153.7, 128.9, 125.3, 118.3, 114.9, 71.8, 66.0, 64.6, 59.4, 32.3, 27.8, 25.9, 18.2; HRMS-TOF (m/z): $[M + H]^+$ for $C_{24}H_{37}Cl\ N_2O_4Si$, 481.2283; found, 481.2287.

Synthesis of 2-*tert*-Butyl-4-chloro-5-[4-(3-hydroxypropoxy)-benzyloxy]-3-(2*H*)-pyridazinone (63). A solution of compound **57** (100 mg, 0.21 mmol) and TBAF (0.42 mL of 1 M solution in THF, 0.42 mmol) dissolved in THF (2 mL) was stirred at room temperature. After 2 h, the reaction mixture was concentrated in vacuo to yield a crude oil, which was purified by preparative thin layer chromatography (silica gel, hexanes:ethyl acetate, 1:1) to afford compound **63** (57.8 mg, 76% yield) in good yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.71 (s, 1H), 7.31 (d, 2H, J = 9.0 Hz), 6.92 (d, 2H, J = 9.0 Hz), 5.30 (s, 2H), 4.13 (t, 2H, J = 6.0 Hz), 3.86 (t, 2H, J = 6.0 Hz), 2.04 (m, 2H), 1.62 (s, 9H) ; ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.2, 159.0, 153.7, 128.9, 127.0, 125.3, 118.3, 114.9, 71.8, 66.3, 65.5, 60.2, 31.9, 27.8; HRMS-TOF (m/z): $[M + H]^+$ for $C_{18}H_{23}Cl\ N_2O_4$, 367.1419; found, 367.1422.

Synthesis of 2-*tert*-Butyl-4-chloro-5-[4-(3-(*p*-toluenesulfonyloxy)propoxy)benzyloxy]-3-(2*H*)-pyridazinone (69). A solution of compound **63** (40 mg, 0.11 mmol), TsCl (31 mg, 0.16 mmol), DMAP (20 mg, 0.16 mmol), and DIEA (16.6 mg, 0.16 mmol) dissolved in DCM (0.6 mL) was stirred at room temperature. After 1 h, the reaction mixture was concentrated in vacuo and the resulting crude material was purified by preparatory thin layer chromatography (silica gel, pentane:ethyl acetate, 3:2) to provide compound **69** (18.6 mg, 33% yield) in moderate yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.73–7.75 (m, 3H), 7.29 (d, 2H, J = 8.4 Hz), 7.23 (d, 2H, J = 8.4 Hz), 6.78 (d, 2H, J = 9.0 Hz), 5.22 (s, 2H), 4.23 (t, 2H, J = 6.0 Hz), 3.95 (t, 2H, J = 6.0 Hz), 2.37 (s, 3H), 2.11 (m, 2H), 1.62 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.0, 158.9, 153.7, 144.8, 132.7, 129.8, 128.9, 127.8, 127.1, 125.1, 118.2, 114.7, 71.7, 66.8, 66.3, 63.1, 28.8, 27.8, 21.6; HRMS-TOF (m/z): $[M + H]^+$ for $C_{25}H_{29}Cl\ N_2O_6S$, 520.1435; found, 543.1319 $[M + Na]^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-[4-(3-fluoropropoxy)benzyloxy]-3-(2*H*)-pyridazinone (23). To a solution of compound **69** (4.5 mg, 8.64×10^{-3} mmol) dissolved in ACN (0.25 mL) was added a solution of KF (1.6 mg, 4.07×10^{-2} mmol) and kryptofix222 (15.0 mg, 4.07×10^{-2} mmol) in ACN (0.25 mL). After completion of addition, the reaction vial was capped and partially immersed in a 90 °C oil bath. The reaction mixture was stirred for 40 min and was then concentrated under reduced pressure to yield a crude oil. Purification of the crude material by preparative thin layer chromatography (silica gel, pentane:ethyl acetate, 3:2) provided analogue **23** (0.8 mg, 25% yield) in moderate yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.20 (s, 1H), 7.39 (d, 2H, J = 8.4 Hz), 6.98 (d, 2H, J = 8.4 Hz), 5.36 (s, 2H), 4.59 (m, 2H), 4.07 (t, 2H, J = 6.4 Hz), 4.09–4.11 (m, 2H), 2.09 (m, 2H, J = 25.6 Hz), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 158.7, 157.9, 153.9, 129.9, 127.4, 126.3, 114.6, 81.6 (80.0), 71.4, 65.4, 63.6, 63.5, 29.7, 27.5; ^{19}F NMR ($CDCl_3$, 564.6 MHz) δ: –222.66 (m, 1F); HRMS-TOF (m/z): $[M + H]^+$ for $C_{18}H_{22}ClFN_2O_3$, 387.1481; found, 387.1486 $[M + NH_4]^+$.

Synthesis of Methyl-4-(3-oxobutyl)benzoate (84). To a solution of methyl-4-bromobenzoate (**83**, 1.0 g, 4.65 mmol) in TEA (13 mL) was added 3-buten-2-ol (1 mL, 11.63 mmol), $Pd(OAc)_2$ (104 mg, 0.465 mmol), and PPh_3 (244 mg, 0.93 mmol). The reaction mixture was stirred at 75 °C overnight. The reaction mixture was cooled to room temperature and concentrated in vacuo to yield a crude oil. The crude material was partitioned between water (20 mL) and ethyl acetate (20 mL). The organic layer was separated

and washed with water (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and then concentrated. The crude product was purified by silica gel chromatography (hexane:ethyl acetate gradient, 5:1 to 3:1) to obtain compound **84** (250 mg, 26% yield) in moderate yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.95 (d, 2H, J = 8.4 Hz), 7.25 (d, 2H, J = 8.4 Hz), 3.90 (s, 3H), 2.95 (t, 2H, J = 7.5 Hz), 2.77 (t, 2H, J = 7.7 Hz), 2.14 (s, 3H).

Synthesis of 2-*tert*-Butyl-4-chloro-5-[4-(3-hydroxybutyl)benzyloxy]-3-(2*H*)-pyridazinone (58). To a solution of compound **84** (505 mg, 2.45 mmol) in THF (19 mL) at 0 °C was added LAH (12.2 mL of 1 M THF solution, 12.24 mmol) dropwise. After completion of addition the reaction mixture was stirred at room temperature. After 1 h, water (183 μL), 15% NaOH (aq) (183 μL), and water (548 μL) was added sequentially. The reaction mixture was stirred for an additional 15 min before being filtered. The filtrate was concentrated under reduced pressure to obtain compound **48** (314 mg, 71% yield), which was used in the next step without further purification.

To a solution of compound **56** (234 mg, 1.16 mmol) in THF (45 mL) was added compound **48** (312 mg, 1.73 mmol), PPh_3 (454 mg, 1.73 mmol), and DIAD (335 μL, 1.73 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated in vacuo, and the crude material was purified by silica gel chromatography (hexanes:ethyl acetate gradient, 4:1 to 100% ethyl acetate) to obtain compound **58** (200 mg, 48% yield) as a clear oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.73 (s, 1H), 7.32 (d, 2H, J = 8.0 Hz), 7.24 (d, 2H, J = 8.0 Hz), 5.30 (s, 1H), 5.27 (s, 2H), 3.83 (m, 1H), 2.80–2.76 (m, 1H), 2.71–2.66 (m, 1H), 1.63 (s, 9H), 1.23 (d, 3H, J = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.3, 153.9, 143.2, 132.5, 129.2, 127.6, 125.4, 118.5, 73.4, 67.6, 66.6, 40.9, 32.0, 28.1, 23.9; HRMS calcd for $C_9H_{25}ClN_2O_3$, 365.1626; found, 365.1624.

Synthesis of 2-*tert*-Butyl-4-chloro-5-[4-(3-fluorobutyl)benzyloxy]-3-(2*H*)-pyridazinone (24). To a solution of compound **58** (200 mg, 0.55 mmol) in pyridine (10 mL) was added TsCl (209 mg, 1.10 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate (30 mL) and washed repeatedly with 5% $CuSO_4$ (200 mL) until a light-blue aqueous solution was maintained. The organic layer was then dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by silica gel chromatography (hexanes: ethyl acetate gradient, 3:1 to 100% ethyl acetate) to recover the unreacted starting material (90 mg) and compound **64** as a clear oil (74 mg, 47% yield based on recovered starting material). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.80 (d, 2H, J = 8.3 Hz), 7.72 (s, 1H), 7.33 (d, 2H, J = 8.0 Hz), 7.30 (d, 2H, J = 8.1 Hz), 7.13 (d, 2H, J = 8.1 Hz), 5.27 (s, 2H), 4.66 (m, 1H), 2.65 (m, 1H), 2.54 (m, 1H), 2.45 (s, 3H), 1.94 (m, 1H), 1.81 (m, 1H), 1.63 (s, 9H), 1.26 (s, 3H).

To a solution of compound **64** (18.2 mg, 0.035 mmol) in ACN (400 μL) was KF (4.1 mg, 0.070 mmol) and kryptofix222 (26.4 mg, 0.070 mmol). The reaction mixture was stirred at 90 °C. After 20 min, the reaction was cooled to room temperature and concentrated under reduced pressure. The crude material was purified by preparative thin layer chromatography (silica gel, hexanes:ethyl acetate, 4:1) to obtain analogue **24** (5 mg, 39% yield). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.70 (s, 1H), 7.34 (d, 2H, J = 7.9 Hz), 7.24 (d, 2H, J = 8.0 Hz), 5.28 (s, 2H), 4.71–4.60 (m, 2H), 2.84–2.80 (m, 1H), 2.73–2.69 (m, 1H), 2.02–1.93 (m, 1H), 1.87–1.77 (m, 1H), 1.63 (s, 9H), 1.35 (m, 3H, J = 6.2, 23.9 Hz);^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.1, 153.8, 142.4, 132.5, 129.0, 127.4, 125.2, 118.3, 90.4 (89.3), 71.9, 66.3, 38.5 (38.4), 31.1 (31.0), 27.9, 21.1 (21.0);^{19}F NMR ($CDCl_3$, 564 MHz) δ: –174.7 (1F, m); HRMS-TOF (m/z): [M + H]$^+$for $C_{19}H_{23}ClFN_2O_2$, 367.1583; found, 367.1582.

Synthesis of Methyl-4-(1-*tert*-butyldimethylsilyloxy-but-2-oxy)-benzoate (85). To a solution of 1,2-butanediol (1 g, 11.09 mmol) in DMF (8 mL) was added TBDMS-Cl (2.5 g, 16.64 mmol) and imidazole (1.88 g, 27.7 mmol). The reaction mixture was stirred for 10 h before being diluted with DCM (20 mL). The organic layer was washed with water (4 × 20 mL) and brine (20 mL), dried over Mg_2SO_4, filtered, and then concentrated. The crude oil was purified by silica gel chromatography (hexanes:ethyl acetate gradient, 100:0 to 94:6) to obtain 1-*tert*-butyldimethylsilyloxy-2-hydroxybutane (1 g, 45% yield). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 3.60 (m, 1H), 3.50 (m, 1H), 3.40 (m, 1H), 2.40 (s, 1H), 1.44 (m, 2H), 0.99 (m, 3H), 0.90 (s, 9H), 0.06 (s, 6H).

A solution of methyl-4-hydroxybenzoate (**76**, 1.10 g, 7.34 mmol), 1-*tert*-butyldimethylsilyloxy-2-hydroxybutane (0.75 g, 3.67 mmol), and PPh_3 (1.97 g, 7.34 mmol) dissolved in THF (8 mL) was cooled to 0 °C. DIAD (1.49 g, 7.34 mmol) was added dropwise The reaction mixture was stirred for 2 h before being concentrated in vacuo. The crude material was purified using silica gel chromatography (hexanes:diethyl ether gradient, 100 to 99:1) to obtain compound **85** (1.0 g, 83% yield) as a viscous oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.90 (m, 2H), 6.90 (m, 2H), 4.30 (m, 1H), 3.90 (s, 3H), 3.70 (2H), 1.78 (m, 1H), 1.70 (m, 1H), 0.90 (t, 3H, J = 7.8 Hz), 0.89 (s, 9H), 0.05 (s, 3H), 0.01 (s, 3H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 166.8, 162.8, 131.5, 122.3, 115.2, 80.0, 64.5, 51.7, 25.8, 24.1, 18.2, 9.5, –5.3.

Synthesis of 4-(1-*tert*-Butyldimethylsilyloxy-but-2-oxy) benzyl alcohol (49). To a solution of compound **76** (1 g, 2.95 mmol) in ether (15 mL) at 0 °C was added LAH (336 mg, 8.8 mmol). After completion of addition, the reaction mixture was stirred for 1.5 h. Addition of water (0.336 mL), 15% NaOH (aq) (0.336 mL), and water (1.00 mL) in succession quenched the reaction mixture. The resulting cloudy mixture was stirred for an additional 20 min before being filtered. The filtrate was dried over Mg_2SO_4, filtered, and concentrated to yield compound **49** (0.50 g, 54% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.24 (d, 2H, J = 8.4 Hz), 6.92 (d, 2H, J = 8.4 Hz), 4.60 (d, 2H, J = 5.4 Hz), 4.20 (m, 1H), 3.76 (dd, 1H, J = 5.4, 10.8 Hz), 3.66 (dd, 1H, J = 5.4, 10.8 Hz), 1.74 (m, 1H), 1.65 (m, 1H), 1.50 (t, 1H, J = 5.4 Hz), 0.90 (t, 3H, J = 7.8 Hz), 0.89 (s, 9H), 0.04 (s, 3H), 0.01 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 158.5, 133.0, 128.4, 116.1, 80.1, 65.0, 64.5, 25.8, 24.1, 18.2, 9.5, –5.3; HRMS-TOF (m/z): [M + H]$^+$ for $C_{17}H_{30}O_3Si$, 310.1964; found 333.1867 [M + Na]$^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(4-(1-hydroxy-but-2-oxy)-benzyl)oxy-3-(2*H*)-pyridazinone (65). To a cooled (0 °C) solution of compound **56** (0.48 g, 2.42 mmol) dissolved in THF (40 mL) was added compound **49** (500 mg, 1.61 mmol), PPh_3 (633 mg, 2.42 mmol), and DIAD (488 mg, 2.42 mmol). After completion of addition, the reaction mixture was stirred for 2 h and then concentrated in vacuo to obtain a crude oil. Purification of the crude material by silica gel chromatography (hexanes:ethyl acetate gradient, 100 to 94:6) afforded compound **59** (330 mg, 41% yield) as an oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.72 (s, 1H), 7.20 (d, 2H, J = 9 Hz), 6.90 (d, 2H, J = 8.4 Hz), 5.20 (s, 2H), 4.20 (m, 1H, J = 5.4 Hz), 3.75 (dd, 1H, J = 6 Hz), 3.68 (dd, 1H, J= 6 Hz), 1.75 (m, 1H), 1.65 (m, 1H), 1.60 (s, 9H), 0.99 (m, 3H), 0.85 (s, 9H), 0.02 (s, 3H), –0.01 (s, 3H); ^{13}C NMR ($CDCl_3$, 600 MHz) δ: 159.6, 159.3, 154.0, 129.0, 126.9, 125.0, 118.5, 116.5, 80.3, 72.1, 66.5, 64.8, 28.1, 26.0, 24.4, 18.4, 9.6, –5.3.

To a solution of compound **59** (0.3 g, 0.6 mmol) in THF (2 mL) was added TBAF (1.8 mL of 1.0 M solution in THF, 1.8 mmol) dropwise. After completion of addition, the reaction mixture was stirred for 1.5 h before being concentrated in vacuo. The crude oil was purified by silica gel chromatography (hexanes:ethyl acetate gradient, 100 to 4:1) to obtain compound **65** (185 mg, 80% yield) in good yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.74 (s, 1H), 7.30 (d, 2H, J = 8.4 Hz), 6.90 (d, 2H, J = 8.4 Hz), 5.20 (s, 2H), 4.30 (m, 1H), 3.81–3.77 (br m, 2H), 1.84 (br m, 1H), 1.72 (m, 2H), 1.64 (s, 9H), 0.98 (m, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.2, 158.9, 153.9, 129.2, 127.5, 125.4, 116.6, 80.4, 71.9, 66.5, 64.2, 28.0, 23.5, 9.7; HRMS-TOF (m/z): [M + H]$^+$ for $C_{19}H_{25}ClN_2O_4$, 380.1503; found, 403.1394 [M + Na]$^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(4-(1-tosyloxy-but-2-oxy)-benzyl)oxy-3-(2*H*)-pyridazinone (71). A solution of compound **65** (50 mg, 0.13 mmol), TsCl (75 mg, 0.39 mmol), DMAP (48 mg, 0.39 mmol), and DIEA (50 mg, 0.39 mmol) in DCM (2 mL) was stirred at room temperature. After 35 min, the reaction mixture was diluted with water (1 mL). The organic layer was separated and washed with water (1 mL) and brine (1 mL), dried over Mg_2SO_4,

filtered, and then concentrated. The crude oil was purified by silica gel chromatography (hexanes:ethyl acetate gradient, 90:10 to 70:30) to obtain compound **71** (54 mg, 77% yield) as a viscous colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.75 (s, 1H), 7.74 (s, 2H), 7.30 (m, 4H), 6.84 (d, 2H, J = 9 Hz), 5.20 (s, 2H), 4.38 (m, 1H), 4.15 (m, 2H), 2.44 (s, 3H), 1.72 (m, 2H), 1.60 (s, 9H), 0.95 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.2, 158.5, 153.9, 145.1, 133.0, 130.0, 129.0, 128.1, 127.2, 125.4, 118.5, 116.5, 71.9, 70.2, 66.6, 28.1, 24.2, 21.8, 9.4; HRMS-TOF (m/z): $[M + H]^+$ for $C_{26}H_{31}ClN_2O_6S$, 534.1591; found, 557.1490 $[M + Na]^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-(4-(1-fluoro-but-2-oxy)-benzyl)oxy-3-(2*H*)-pyridazinone (25). To a solution of compound **71**(28 mg, 52.4 μmol) dissolved in ACN (0.5 mL) was added a solution of KF (4.5 mg, 78.6 μmol) and kryptofix222 (29.6 mg, 78.6 μmol) in 0.5 mL ACN. The flask was fitted with a reflux condenser, and the solution was then immersed in an oil bath preheated at 90 °C. The reaction mixture was stirred for 90 min, after which all the volatiles were removed under reduced pressure. The crude oil was purified by preparative thin layer chromatography (silica gel, hexanes:ethyl acetate, 7:3) to obtain compound **25** (13 mg, 65% yield) in good yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.72 (s, 1H), 7.30 (m, 2H), 6.90 (m, 2H), 5.23 (s, 2H), 4.54 (dd, 2H, J = 4.8, 46.2 Hz), 4.40 (m, 1H), 1.74 (m, 2H), 1.60 (s, 9H), 1.0 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.0, 158.7, 153.7, 129.0, 127.5, 125.2, 118.3, 116.4, 83.9 (82.7), 78.0, 71.1, 66.3, 27.8, 23.2, 9.5. ^{19}F NMR ($CDCl_3$, 564 MHz) δ: –228 (1F, m); HRMS-TOF (m/z): $[M + H]^+$ for $C_{19}H_{24}ClFN_2O_3$, 382.1459; found, 405.1350 $[M + Na]^+$.

Synthesis of Toluene-4-sulfonic acid-2-[4-(1-*tert*-butyl-5-chloro-6-oxo-1,6-dihydro-pyridazin-4-yloxymethyl)-phenoxy]-1-methyl-ethyl ester (66). To a solution of compound **50**[33] (269 mg, 1.48 mmol) and compound **56** (250 mg, 1.23 mmol) dissolved in anhydrous THF (18.5 mL) was added PPh_3 (485 mg, 1.85 mmol) and DIAD (0.358 mL, 1.85 mmol). After completion of addition, the reaction mixture stirred for 20 h before being diluted with water. The aqueous layer was separated and extracted with ethyl acetate (3 × 50 mL). All combined organic layers were dried over Na_2SO_4, filtered, and concentrated to provide an oil. The crude material was purified using silica gel chromatography (pentane:ethyl acetate, 1:1) to provide compound **60** (234 mg, 51% yield) in moderate yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.71 (s, 1H), 7.33 (d, 2H, J = 8.7 Hz), 6.94 (d, 2H, J = 8.7 Hz), 5.24 (s, 2H), 4.19 (m, 1H), 3.95 (dd, 1H, J = 9.2, 3.1 Hz), 3.81 (dd, 1H, J = 9.2, 7.7 Hz), 1.62 (s, 9H) 1.29 (d, 3H, J = 6.4 Hz).

To a solution of compound **60** (200 mg, 0.55 mmol) dissolved in DCM (6.0 mL) was added TsCl (125 mg, 0.66 mmol), DMAP (100 mg, 0.82 mmol), and TEA (0.0914 mL, 0.66 mmol). The reaction mixture stirred for 22 h, before being diluted with water. The aqueous layer was separated and extracted with ethyl acetate (3 × 20 mL). All combined organic layers were dried over Na_2SO_4, filtered, and concentrated to provide an oil. The crude material was purified using silica gel chromatography (pentane:ethyl acetate, 70:30) to provide compound **66** (166 mg, 58% yield) in good yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.80 (d, 2H, J = 8.3 Hz), 7.72 (s, 1H), 7.32 (d, 2H, J = 7.9 Hz), 7.29 (d, 2H, J = 8.7 Hz), 6.74 (d, 2H, J = 8.7 Hz), 5.22 (s, 2H), 4.19 (m, 1H), 4.02 (dd, 1H, J = 10.4, 6.0 Hz), 3.93 (dd, 1H, J = 10.4, 4.5 Hz), 2.44 (s, 3H), 1.63 (s, 9H) 1.42 (d, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 158.9, 158.3, 153.6, 144.6, 133.8, 129.6, 128.8, 127.8, 127.4, 125.1, 118.0, 114.7, 76.8, 71.5, 69.7, 66.2, 27.7, 21.5, 17.6; HRMS-TOF (m/z): $[M + H]^+$ for $C_{25}H_{29}ClN_2O_6S$, 521.1507; found, 521.1505.

Synthesis of 2-*tert*-Butyl-4-chloro-5-[4-(2-fluoropropoxy)benzyloy]-3-(2*H*)-pyridazinone (26). To a solution of compound **66** (50 mg, 0.10 mmol) in ACN (1.0 mL) was added KF (11.2 mg, 0.19 mmol) and kryptofix222 (72.4 mg, 0.19 mmol). After completion of addition, the reaction mixture was heated at 90 °C. After 40 min, the reaction mixture was cooled to room temperature and diluted with water (1.0 mL). The aqueous layer was separated and extracted with ethyl acetate (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to provide an oil. The crude material was purified using a preparative silica gel thin layer chromatography plate (pentane:ethyl acetate, 4:1) to isolate unreacted starting material (5.8 mg, 0.011 mmol) in addition to analogue **26** (12.5 mg, 41% yield based on recovered starting material). ^{1}H NMR ($CDCl_3$600 MHz) δ: 7.73 (s, 1H) 7.34 (d, 2H, J = 8.6 Hz), 6.95 (d, 2H, J = 8.6 Hz), 5.25 (s, 2H), 5.06–4.96 (m, 1H), 4.06 (2H, m), 1.63 (s, 9H) 1.47 (dd, 3H, J = 6.4, 23.6 Hz); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 158.4, 157.8, 153.9, 129.8, 127.6, 126.2, 115.5, 114.6, 89.0 (88.0), 71.2, 70.4 (70.3), 65.3, 27.4, 16.9 (16.8); ^{19}F(DMSO-d_6, 564 MHz) δ: –178.20 (1F, m); HRMS-TOF (m/z): $[M + H]^+$ $C_{18}H_{22}ClFN_2O_3$, 369.1375; found, 369.1370.

Synthesis of Methyl-4-(2-hydroxyethoxymethyl)benzoate (72). A solution of methyl 4-hydroxymethylbenzoate (**70**, 2.50 g, 0.015 mol) in DCM (30 mL) was charged into a two-neck round-bottom flask that was equipped with a Dewar condenser and cooled at –10 °C in a salt/ice bath. Ethylene oxide (1.10 mL) was added to the cooled stirring solution dropwise, followed by the addition of boron trifluoride etherate (0.51 mL). The reaction mixture was stirred for 45 min and then warmed to room temperature for 30 min to distill out any excess of ethylene oxide in the reaction mixture. The reaction mixture was then diluted with brine (50 mL). The aqueous layer was extracted with dichloromethane (3 × 50 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated to provide an oil. The crude material was purified using silica gel chromatography (pentane:ethyl acetate, 4:1) to provide compound **72** (537 mg, 17% yield) in low yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 8.36 (d, 2H, J = 8.4 Hz), 7.41 (d, 2H, J = 8.5 Hz), 4.62 (s, 3H), 3.92 (s, 2H), 3.78 (m, 2H), 3.63 (m, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 167.1, 143.5, 130.0, 129.8, 127.5, 72.9, 72.0 62.1, 52.3.

Synthesis of 4-[2-(*tert*-Butyldimethylsilanyloxy)ethoxymethyl]-benzoic acid methyl ester (86). To a solution of the compound **72** (544.5 mg, 2.59 mmol) in DMF (26 mL) was added imidazole (264 mg, 3.89 mmol) and TBDMS-Cl (586 mg, 3.89 mmol). The reaction mixture was stirred at room temperature overnight, then quenched with water. The aqueous layer was extracted with ethyl acetate (3 × 100 mL). All combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude material was purified using silica gel chromatography (pentane:ethyl acetate, 4:1) to provide compound **86** (677.5 mg, 84% yield) in good yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 8.01 (d, 2H, J = 8.3 Hz), 7.42 (d, 2H, J = 8.4 Hz), 4.63 (s, 2H), 3.91 (s, 2H), 3.82 (t, 2H, J = 5.0 Hz), 3.58 (t, 2H, J = 5.1 Hz), 0.91 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$,150 MHz) δ: 166.5, 143.5, 129.2, 128.8, 126.5, 72.1, 71.6, 62.3, 51.5, 25.4, 17.9, –5.8.

Synthesis of {4-[2-(*tert*-Butyldimethylsilanyloxy)ethoxymethyl]-phenyl}methanol (51). To a solution of compound **86** (670 mg, 2.18 mmol) dissolved in THF (22 mL) was added LAH (2.18 mL of 1.0 M solution in THF, 2.18 mmol) dropwise. After completion of addition the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with water, and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to provide compound **51** as an oil (587 mg, 91% yield), which was used in the next step without any further purification. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.34 (s, 4H), 4.68 (s, 2H), 4.57 (s, 2H), 3.80 (t, 2H, J = 5.2 Hz), 3.56 (t, 2H, J = 5.3 Hz), 1.69 (br, s, 1H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 140.4, 138.3, 128.0, 127.2, 73.2, 71.9, 65.4, 63.0, 26.2, 18.6, –5.0.

Synthesis of 2-*tert*-Butyl-5-{4-[2-(*tert*-butyldimethylsilanyloxy)-ethoxymethyl]benzyloxy}-4-chloro-3-(2*H*)-pyridazinone (61). To a solution of compound **51** (437 mg, 1.48 mmol) and compound **56** (250 mg, 1.23 mmol) dissolved in THF (12 mL) was added PPh_3 (485 mg, 1.85 mmol) and DIAD (0.358 mL, 1.85 mmol). After completion of addition, the reaction mixture was stirred at room temperature for 20 h and then diluted with water (20 mL). The aqueous layer was separated and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to provide an oil. The crude material was purified using silica gel chromatography (pentane:ethyl acetate, 4:1) to provide compound **61** (528 mg, 89% yield) in good yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.70 (s, 1H), 7.38 (m, 4H), 5.30 (s, 2H), 4.58 (s, 2H), 3.80 (t, 2H, J = 5.4 Hz), 3.57 (t, 2H, J = 5.4

Hz), 1.63 (br s, 9H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.0, 153.7, 138.8, 134.4, 128.3, 127.3, 125.1, 118.5, 72.8, 71.7, 71.6, 66.4, 61.9, 29.7, 27.9, 25.6, –5.1; HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{24}H_{37}ClN_2O_4Si$, 481.2283; found, 481.2282.

Synthesis of 2-*tert*-Butyl-4-chloro-5-[4-(2-hydroxyethoxymethyl)benzyloxy]-3-(2*H*)-pyridazinone (67). To a solution of compound **61** (528 mg, 1.09 mmol) dissolved in THF (11 mL) was added a solution of TBAF (1.65 mL of 1.0 M solution in THF, 1.65 mmol) dropwise. After completion of addition, the reaction was stirred at room temperature for 1 h and then quenched with water (10 mL). The aqueous layer was separated and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to provide an oil. The crude material was purified using silica gel chromatography (hexanes: ethyl acetate, 4:1) to provide compound **67** (311 mg, 78% yield). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.70 (s, 1H), 7.38 (m, 4H), 5.30 (s, 2H), 4.56 (s, 2H), 3.76 (t, 2H, J = 4.9 Hz), 3.60 (t, 2H, J = 4.8 Hz), 2.00 (br s, 1H), 1.61 (br s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.0, 153.6 138.8, 134.4, 128.2, 127.2, 125.1, 118.3, 72.8, 71.6, 71.6, 66.4, 61.9, 27.8; HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{18}H_{23}ClN_2O_4$, 367.1419; found, 367.1419.

Synthesis of Toluene-4-sulfonic acid-2-[4-(1-*tert*-butyl-5-chloro-6-oxo-1,6-dihydro-pyridazin-4-yloxymethyl)-benzyloxy]-ethyl ester (73). To a solution of compound **67** (200 mg, 0.55 mmol) dissolved in DCM (5.50 mL) was added TsCl (125 mg, 0.66 mmol), DMAP (100 mg, 0.82 mmol), and TEA (0.091 mL, 0.66 mmol). The reaction mixture was stirred at room temperature for 22 h and then was diluted with water (10 mL). The aqueous layer was separated and extracted with ethyl acetate (3 × 20 mL). All combined organic layers were dried over Na_2SO_4, filtered, and concentrated to provide an oil. The crude material was purified using silica gel chromatography (pentane:ethyl acetate, 3:2) to provide compound **73** (232 mg, 82% yield) in good yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.79 (d, 2H, J = 8.3 Hz), 7.71 (s, 1H), 7.38 (d, 2H, J = 8.2 Hz), 7.32 (m, 4H), 5.30 (s, 2H), 4.50 (s, 2H), 4.21 (m, 2H), 3.69 (m, 2H), 2.43 (s, 3H), 1.63 (br s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.0, 153.7, 144.8, 138.8, 134.4, 133.1, 129.8, 128.1, 128.0, 127.2, 125.1, 118.4, 72.8, 71.7, 69.2, 67.8, 66.4, 27.9, 21.6; HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{25}H_{29}ClN_2O_6$, 521.1507; found, 521.1503.

Synthesis of 2-*tert*-Butyl-4-chloro-5-[4-(2-fluoro-ethoxymethyl)-benzyloxy]-3-(2*H*) -pyridazinone (27). To a solution of compound **73** (50 mg, 0.10 mmol) in ACN (1.0 mL) was added KF (11.2 mg, 0.19 mmol) and kryptofix222 (72.4 mg, 0.19 mmol). After completion of addition, the reaction mixture was heated at 90 °C. After 10 min, the reaction mixture was cooled to room temperature and diluted with water (1 mL). The aqueous layer was separated and extracted with ethyl acetate (3 × 5 mL). All combined organic layers were dried over Na_2SO_4, filtered, and concentrated to provide an oil. The crude material was purified using silica gel chromatography (pentane:ethyl acetate, 4:1) to provide analogue **27** (28 mg, 79% yield) in good yield. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.22 (s, 1H), 7.45 (d, 2H, J = 8.2 Hz), 7.39 (d, 2H, J = 8.2 Hz), 5.42 (s, 2H), 4.60 (m, 1H), 4.54 (s, 2H), 4.52 (m, 1H), 3.71 (m, 1H), 3.66 (m, 1H), 1.57 (s, 9H); ^{13} C NMR (DMSO-d_6, 150 MHz) δ: 157.8, 153.8, 138.6, 134.6, 127.8, 127.7, 126.2, 115.6, 83.5 (82.4), 71.6, 71.2, 69.1 (69.0), 65.3, 27.4; ^{19}F NMR (DMSO-d_6, 564 MHz) δ: –221.74 (1F, m); HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{18}H_{22}ClFN_2O_3$, 369.1375; found, 369.1377.

Synthesis of Methyl-4-[2-hydroxy-d_4-ethoxymethyl]benzoate (87). A solution of methyl 4-hydroxymethylbenzoate (**70**, 2.5 g, 15 mmol) in DCM (30 mL) was added to a two-neck round-bottom flask, which was equipped with a Dewar condenser and cooled at –10 °C in a salt/ice bath. d_4-Ethylene oxide (1.10 mL) was added to the cooled stirring solution dropwise followed by the addition of boron trifluoride etherate (0.51 mL). The reaction mixture was stirred for 45 min and then warmed to room temperature for 30 min to distill out any excess of d_4-ethylene oxide in the reaction mixture. The reaction mixture was then diluted with brine (50 mL). The aqueous layer was extracted with dichloromethane (3 × 50 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated to provide an oil. The crude material was purified using silica gel chromatography (pentane:ethyl acetate, 4:1) to provide compound **87** (520 mg, 16% yield) in low yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 8.02 (d, 2H, J = 8.2 Hz), 7.41 (d, 2H, J = 8.1 Hz), 4.62 (s, 2H), 3.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 167.1, 143.5, 130.8, 129.9, 127.5, 72.8, 52.4.

Synthesis of Methyl-4-[2-(*tert*-butyldimethylsilanyloxy)-d_4-ethoxymethyl]benzoate (88). To a solution of compound **87** (500 mg, 2.33 mmol) in DMF (23 mL) was added TBDMS-Cl (528 mg, 3.50 mmol) and imidazole (238 mg, 3.50 mmol). The reaction was stirred at room temperature overnight and then diluted with water (30 mL) and extracted with ethyl acetate (2×50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to obtain a crude oil. The oil was purified bypassage through a thick pad of silica gel (pentane:ethyl acetate, 3:1) to obtain the compound **88** as a clear oil (602 mg, 79% yield). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 8.00 (d, 2H, J = 8.3 Hz), 7.40 (d, 2H, J = 8.5 Hz), 4.62 (s, 2H), 3.90 (s, 3H), 0.90 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$,150 MHz) δ: 167.2, 144.2, 129.9, 129.5, 127.3, 72.8, 52.3, 26.1, 18.6, –5.8.

Synthesis of {4-[2-(*tert*-Butyldimethylsilanyloxy)-d_4-ethoxy-d_2-methyl]phenyl}methanol (52). To a solution of compound **88** (610 mg, 1.86 mmol) in THF (19 mL) at 0 °C was added LAD (1.9 mL of 1 M solution in THF, 1.86 mmol) dropwise. After completion, the reaction was stirred at room temperature for 3.5 h before being diluted with water and extracted with ethyl acetate (2 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to obtain compound **52** as a clear oil (482 mg, 86% yield). The material was used in the next step without further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.33 (s, 4H), 4.56 (s, 2H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 140.3, 138.4, 128.1, 127.3, 73.1, 29.9, 26.2, 18.6, –5.0.

Synthesis of 2-*tert*-Butyl-4-chloro-5-{4-[2-(*tert*-butyldimethylsilanyloxy)-d_4-ethoxy-d_2-methyl]benzyloxy}-3-(2*H*)-pyridazinone (62). To a solution of compound **56** (212 mg, 1.05 mmol) in THF (15 mL) was added compound **52** (475 mg, 1.57 mmol), PPh_3 (412 mg, 1.57 mmol), and DIAD (304 μL, 1.57 mmol). The reaction mixture was stirred at room temperature for 2 h. After concentration of the reaction mixture in vacuo, the crude material was purified by silica gel chromatography (pentane:ethyl acetate, 9:1) to obtain compound **62** as a clear oil (336 mg, 66% yield). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.70 (s, 1H), 7.39 (m, 4H), 4.58 (s, 2H), 1.63 (s, 9H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 158.5, 153.2, 139.0, 133.4, 127.5, 126.7, 124.6, 117.8, 72.2, 65.8, 27.3, 25.4, 17.8, –5.1; HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{24}H_{31}D_6ClN_2O_4Si$, 486.2588; found, 509.2480 $[M + Na]^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-[4-(2-hydroxy-d_4-ethoxy-d_2-methyl)benzyloxy]-3-(2*H*)-pyridazinone (68). To a solution of compound **62** (330 mg, 0.68 mmol) in THF (7 mL) was added TBAF (1 mL of 1 M solution in THF, 1.02 mmol) dropwise. The reaction mixture was stirred at room temperature for 2 h followed by solvent removal under reduced pressure to yield a crude oil. The crude material was dissolved in DCM and passed through a thick pad of silica (100% ethyl acetate) to obtain compound **68** (250 mg, 99% yield) containing a minor percentage of the corresponding silanol. The material was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.72 (s, 1H), 7.41 (s, 4H), 4.59 (s, 2H), 1.64 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.2, 153.9, 139.5, 134.5, 128.5, 127.5, 125.3, 118.6, 73.0, 66.6, 28.1; HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{18}H_{17}D_6ClN_2O_4$, 395.1609; found, 395.1615 $[M + Na]^+$.

Synthesis of 2-*tert*-butyl-4-chloro 5-[4-(2-*p*-toluenesulfonyloxy-d_4-ethoxy-d_2-methyl)benzyl]oxy-3-(2*H*)-pyridazinone (74). To a solution of compound **68** (250 mg, 0.67 mmol) in DCM (7 mL) was added TsCl (153 mg, 0.81 mmol), DMAP (98 mg, 0.81 mmol), and TEA (140 μL, 1.01 mmol). The reaction was stirred at room temperature overnight and was then concentrated under reduced pressure. The crude material was purified by silica gel chromatography (hexanes:ethyl acetate gradient, 2:1 to 100% ethyl acetate) to recover the unreacted starting material **68** (9 mg) and the product **74** (261 mg, 77% yield based on recovered starting

material) as a clear oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.76 (d, 2H, J = 8.3 Hz), 7.73 (s, 1H), 7.36 (d, 2H, J = 8.1 Hz), 7.29 (m, 4H), 4.47 (s, 2H), 2.40 (s, 3H), 1.61 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 159.0, 153.8, 145.0, 138.5, 134.4, 133.1, 129.9, 128.1, 128.0, 127.3, 125.2, 118.1, 72.7, 71.0, 37.0, 63.4, 28.0, 21.7; HRMS-TOF (*m*/*z*): $[M + H]^+$ for $C_{25}H_{23}D_6ClN_2O_6S$, 526.1811; found, 549.1705 $[M + Na]^+$.

Synthesis of 2-*tert*-Butyl-4-chloro-5-[4-(2-fluoro-d_4-ethoxy-d_2-methyl)-benzyloy]-3-(2*H*)-pyridazinone (28). To a solution of compound **74** (14 mg, 0.03 mmol) in ACN (300 μL) was added KF (3.1 mg, 0.05 mmol) and kryptofix222 (20 mg, 0.05 mmol). The reaction mixture was stirred at 90 °C for 10 min. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. The crude material was purified by preparative TLC (hexanes:ethyl acetate, 2:1) to obtain analogue **28** (6.2 mg, 62% yield) as an oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.70 (s, 1H), 7.40 (s, 4H), 4.61 (s, 2H), 1.63 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 158.5, 153.1, 138.2, 133.8, 127.7, 126.8, 124.6, 117.8, 72.4, 65.9, 27.3; ^{19}F NMR ($CDCl_3$, 564 MHz) δ: –225.2 (m, 1F).

General Procedure for the Radiosynthesis of F-18 Labeled Pyridazinone Analogues 22–28. A 500 mCi lot of aqueous F-18 was made by the ^{18}O(p,n)^{18}F reaction (PETNET Pharmaceutical Services, Cummings Park, Woburn, MA) and applied to a previously activated MP1 anion exchange resin (BioRad) cartridge. This cartridge was placed into an elution loop contained within a remotely controlled radiosynthesis system. The radioactivity was eluted from the cartridge and collected into a silanized 25 mL pear-shaped flask by the addition of 1 mL of a solution prepared as follows: K_2CO_3 (15 mg) was dissolved in deionized water (1 mL), and kryptofix222 (90 mg) was dissolved in ACN (4 mL); the two solutions were combined, and the appropriate aliquot was used for elution of the column. The eluate from the MP1 anion exchange resin was then concentrated to dryness by applying a gentle stream of heated He and a slight vacuum. ACN (0.5 mL) was added, and the solvent was again removed by the same vacuum heated He procedure to ensure removal of all water. The material was reconstituted with ACN (0.5 mL). The ^{18}F solution with the remaining constituents (K, CO_3^{2-}, K222) was transferred to a conical bottomed 5 mL Wheaton vial containing 3.0 mg of the desired tosylate precursor **55**, **64**, **66**, **69**, **71**, **73**, or **74** (3.0 mg) dissolved in ACN (0.5 mL). The vial and contents were heated at 90 °C for 30 min. The reaction solution was transferred to a 25 mL pear-shaped flask and diluted with water (18.5 mL). The resultant mixture was passed through a Sep Pak C18 cartridge, then rinsed with water (5 mL). The Sep Pak C18 cartridge was then eluted with ACN (3 mL) and the column dried with $N_{2(g)}$. The collected acetonitrile fraction was purified via HPLC (Phenomenex LUNA C-18 column 250 mm × 10 mm, 5 μm particle size, 100 Å pore, mobile phases A: 90/10 H_2O/CH_3CN; B: CH_3CN, both containing 0.1%TFA as stabilizer; gradient: 0–100/15 sustained at 100% B to 20 min, flow rate 2.5 mL/min) ^{18}F analogues **22–28** eluted from the column and were collected as single fractions (Table 6). The solvent was then removed via rotary evaporation. Upon drying, the contents of the flask were reconstituted with a 10% ethanol solution. The final product yield was ~25 mCi, with a radiosynthesis and purification time of 90 min. Aliquots of this material were used for biodistribution and PET imaging studies in rats.

Preparation of Submitochondrial Particles from Bovine Hearts. Bovine heart mitochondria were prepared as described by Lester et al.[44] A brief description of the procedure follows: bovine heart was minced, and 200 g of ground heart tissue was suspended in 400 mL of 0.25 M sucrose, 0.01 M Tris-Cl, 1 mM Tris-succinate, and 0.2 mM ethylenediamine tetra-acetic acid (EDTA) and homogenized in a Waring blender. The homogenate was centrifuged for 20 min at 1200*g*, and the supernatant was centrifuged for 15 min at 26000*g*, resulting in a mitochondrial pellet. The protein concentration of the mitochondrial samples as measured by a BioRad Protein Assay Kit (BioRad Life Science Research, Hercules, CA) was adjusted to 20 mg/mL using 0.25 M sucrose, 10 mM Tris-acetate pH 7.5, 1.5 mM adenosine triphosphate (ATP), and 10 mM $MgCl_2$. The samples were stored at −80 °C.

Bovine submitochondrial particles (SMPs) were prepared from mitochondria as described by Matsuno-Yagi et al.[45] Isolated bovine heart mitochondria were sonicated in batches of 15 mL for 1 min with a digital Branson sonifier (Branson, Danbury, CT) at 70% maximum output in an ice bath. The sonicated suspension was centrifuged at 16000*g* for 10 min, and the supernatant was centrifuged at 150000*g* for 45 min at 4 °C. The submitochondrial pellet was resuspended in buffer containing 0.25 M sucrose, 10 mM Tris-acetate, pH 7.5. The protein concentration was determined using the BioRad Protein Assay Kit (BioRad Life Science Research, Hercules CA), and the samples were stored at –80 °C, at a concentration of 20 mg/mL.

Submitochondrial Particle (Bovine) Catalytic Activity and Compound Inhibition Assay. The procedure for determining the catalytic activity of submitochondrial particles was adapted from Satoh et al.[46] NADH-DB reductase activity was measured using a stirred cuvette in a spectrophotometer (Hewlett-Packard, Houston TX) at 37 °C, as the rate of NADH oxidation at 340 nm (ϵ = 5.4 $mM^{-1} \times cm^{-1}$) for 120 s. The final volume of the reaction was 2.5 mL, containing 50 mM K_2HPO_4 (pH 7.4), 0.4 μM Antimycin A, and 2 mM KCN. The final SMP concentration was 45 μg/mL. The enzyme reaction was initiated by the addition of 100 μM decyl ubiquinone (DB) and 50 μM NADH. Inhibitors at varying concentrations were preincubated with the reaction mixture containing SMPs for 4 min prior to initiation of the reaction. The IC_{50} value was determined as the concentration of the inhibitor required for 50% inhibition of NADH oxidation. The IC_{50} value was calculated using GraphPad Prism Version 4 (GraphPad, San Diego, CA).

Tissue Biodistribution of [^{18}F]**22–28** in rats (300–400 g male Sprague–Dawley rats). Tissue biodistribution of [^{18}F]**22–28** was examined in sodium pentobarbital (50 mg/kg, ip) anesthetized Sprague–Dawley rats. After anesthesia, the left femoral vein of rats was canulated and [^{18}F]**22–28** at a dose of about 15 μCi in 0.3 mL 10% ethanol saline per rat was injected intravenously. At 30 or 120 min after the injections, rats (n = 3/each time point) were euthanized in a CO_2 chamber and tissue samples of blood, heart, lung, liver, spleen, kidney, femur, muscle, and brain were collected, weighed, and counted in an autogamma counter (Wallac Wizard 1480, PerkinElmer Life and Analytical Sciences, Shelton, CT) for radioactivity. The radioactivity measurements were decay corrected and the net injected dose was calulcated by substracting residual activities in the syringe and venous catheter. The tissue biodistribution of [^{18}F]**22–28** was expressed as % injected dose per gram of tissue (%ID/g) (Table 7).

Cardiac Imaging with [^{18}F]27 in Rats (300–400 g Male Sprague–Dawley rats) and Nonhuman Primate (Male and Female Rhesus Monkeys, 4–6 kg, Covenace). Rats were anesthetized with pentobarbital (50 mg/kg ip) and the left femoral vein was canulated for imaging agent injection. Nonhuman primates were anesthetized with acepromazine (0.3 mg/kg, i.m) and ketamine (10 mg/kg, i.m) in addition to being orally intubated and maintained with isoflurane 0.4–1.5%). The saphenous vein was canulated for drug injection. Cardiac imaging was performed using a microPET camera (Focus220, CTI Molecular Imaging, Inc. Knoxville, TN), which provides 95 transaxial slices in 22 cm operational field of view. After the animal was positioned for cardiac imaging, about 1 mCi of [^{18}F]**27** was injected intravenously into the rat, whereas 3 mCi of [^{18}F]**27** was injected into the nonhuman primate via the canulated saphenous vein. Image acquisition was started 5 min post injection and performed for the next 2 h in dynamic mode (10 min × 12 frames). The images were reconstructed using OSEM2D algorithm with 256 × 256 matrix at zoom 2, no attenuation correction. Image visualization, ROI placement, and quantification were performed using ASIPRO software developed by the manufacturer.

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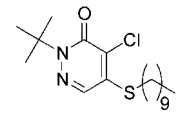
(36) Compound **21** (IC_{50} > 4000 nM):



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