Synthesis and Biological Evaluation of the Mitochondrial Complex 1 Inhibitor 2-[4-(4-Fluorobutyl)benzylsulfanyl]-3-methylchromene-4-one as a Potential Cardiac Positron Emission Tomography Tracer

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A series of fluorinated chromone analogs with IC_{50} values ranging from 9 to 133 nM for the mitochondrial complex 1 (MC-I) has been prepared. A structure−activity relationship (SAR) study of the most potent fluorinated chromone analog **10** demonstrated the linkage heteroatom preference of the side chain region of the molecule while maintaining potent MC-I inhibitory activity. Tissue distribution studies 30 min after [^{18}F]**10** administration to Sprague−Dawley (SD) rats demonstrated high uptake of the radiotracer from the blood pool into the myocardium (2.24% ID/g), kidney (1.93% ID/g), and liver (2.00% ID/g). After 2 h about 66% of the activity in the myocardium at 30 min had been retained, whereas ∼70% had been cleared from the liver and kidney. MicroPET images of SD rats after [^{18}F]**10** administration allowed easy assessment of the myocardium through 60 min with minimal lung or liver interference.

Introduction

Coronary artery disease (CAD)[a] is currently the leading cause of morbidity and mortality in developed nations.[1] In the management of CAD, single-photon emission computed tomography (SPECT) based myocardial perfusion imaging agents (MPIA) has become an important tool used by cardiologists in the evaluation of regional myocardial blood flow and viability under rest or stress conditions.[2−4] The most widely used SPECT MPIA in clinical settings are ^{201}Tl or ^{99m}Tc complexes, which are characterized by rapid myocardial extraction and by cardiac uptake proportional to blood flow.[5−7]

More recently, positron emission tomography (PET) imaging has emerged as an alternative approach to evaluating myocardial blood flow by use of positron-emitting radionuclides.[8,9] Although SPECT constitutes the primary modality for assessing myocardial perfusion in hospital settings, PET provides several advantages over SPECT imaging.[10,11] These include higher special resolution, accurate attenuation correction, and the ability to quantify myocardial perfusion in absolute terms (milliliters per gram per minute) due to the physics inherent in the decay of positron-emitting radionuclides.[12]

Currently, three tracers are used for the assessment of myocardial perfusion with cardiac PET: ^{82}RbCl, $^{13}NH_3$, and $H_2{}^{15}O$.[13−18] Ammonia and water ($^{13}NH_3$ and $H_2{}^{15}O$) are produced using a cyclotron via the $^{16}O(p, \alpha)^{13}N$ and $^{15}N(p, n)^{15}O$ reactions, respectively, whereas ^{82}RbCl is produced from a ^{82}Sr−^{82}Rb generator. Unfortunately, the majority of currently available PET radiotracers are short-lived (<20 min). Therefore, production of $^{13}NH_3$, and $H_2{}^{15}O$ must be in close proximity to a camera facility, which requires significant investment. The development of extractable perfusion imaging agents containing positron emitters with a sufficient half-life (e.g., ^{18}F, ^{76}Br), so that commercial production and distribution would be feasible, would therefore be highly desirable.

Recent in vitro studies with ^{125}I-iodorotenone (**2**, Figure 1) demonstrated the superior extraction and retention of that agent versus ^{99m}Tc-sestamibi.[19] Rotenone (**1**) is a natural product that is widely used as an insectide, acaricide, and miticide.[20,21] It is a potent inhibitor of NADH:ubiquinone oxidoreductase complex.[22,23] This highly conserved 46-subunit heterocomplex is the first component in the electron transport chain (ETC) and is referred to as mitochondrial complex 1 (MC-I).[24] The ETC is embedded in the inner mitochondrial membrane and consists of four membrane-bound enzyme complexes, which generate the proton gradient that drives ATP synthesis and thereby the energy supply of the cell.[25] Cardiac tissue, because of its high-energy expenditure, has a very high mitochondrial content and is therefore an appealing target for the development of cardiac PET tracers.[26]

Numerous classes of MC-I inhibitors have been reported in addition to **1**.[27−31] Inhibitors of MC-I include piericidin A, ubicidin-3, rollinisatatin-1 and 2 (bullatacin), capsaicin, annonaceous acetogenins,[32,33] pyridaben, fenpyroximate, tebufenpyrad, substituted quinolones and quinolines, synthetically simplified deguelin compounds,[34] and hybrid structures of MC-I and MC-III inhibitors ("chromone derivatives").[35] The chromone derivative **6** is of particular interest because of its structural similarity to fenazaquin (**3**), pyridaben (**4**), and tebufenpyrad (**5**, Figure 2). MC-I inhibitors **3**−**6** all possess (a) a hydrophobic heterocyclic "headpiece", for example a chromone core as **6**; (b) a *p-tert*-butylphenyl moiety as the "side chain"; and (c) a heteroatom-containing linker, connecting a and b.

Our goal was to investigate radiolabeled MC-I inhibitors as potential MPIA. Therefore, PET radionuclide ^{18}F was incorporated into an analog of **6** without adversely affecting the inhibitory properties of the parent structure. The incorporation of the radioisotope was carried out by nucleophilic displacement to ensure high specific activity of the resultant radiotracer. The most amenable site for chemical modification within the chromone structure was determined to be the side chain.

Results and Discussion

Structure−Activity Relationships. Initial efforts were directed toward modification of the side chain of **6** for future

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[a] Abbreviations: CAD, coronary artery disease; SPECT, single-photon emission computed tomography; PET, positron emission tomography; ETC, electron transport chain.

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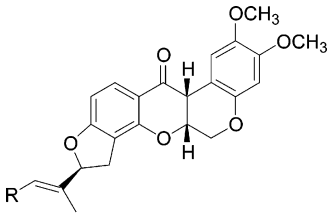


R=H, Rotenone (**1**)
R=^{125}I, Iodorotenone (**2**)

Figure 1. The natural product rotenone, a known MC-I inhibitor.

fluorine incorporation. Replacement of the *tert*-butyl moiety in the side chain with an *n*-butyl moiety yielded compound **7**, which is equal in inhibitory potency to parent chromone **6**, with an IC_{50} value of 52 nM (Table 1). Variation of the heteroatom in the linker of **7** yielded compounds **8** and **9**, with inhibitory activities of 60 and 57 nM, respectively, demonstrating that changes in the heteroatom of the linker are tolerated well.

Simultaneously, other known MC-I inhibitors were screened for activity. Rotenone (**1**) and pyridaben (**4**) were found to be more potent MC-I inhibitors than chromones (**7**−**9**), whereas fenazaquin (**3**) was less potent. Since replacement of the *tert*-butyl group of the side chain with an *n*-butyl group did not disrupt inhibitory activity against MC-I, the next phase of the program was incorporation of a fluorine atom into the side chain.

Chromone analogs containing a primary fluorine atom in the side chain were prepared, which exhibited IC_{50} values ranging from 9 to 133 nM (Table 2). The most potent analogs, **10** and **11**, exhibited IC_{50} values of 9 and 33 nM, respectively, increasing 2−5-fold in potency compared to their nonfluorinated counterparts **7** and **8** in Table 1. Conversely, the fluorinated analog **12**,with an IC_{50} value of 133 nM, was approximately 2.5-fold less active then the nonfluorinated **9** counterpart in Table 1. Due to the additional space required to accommodate the longer side chain, incorporation of sulfur or oxygen atoms in the linker as in analogs **10** and **11** is preferred because of the ability to orient the side chain for optimal binding with MC-I. The hydrogen-donating ability of the amine functionality in analog **12** may be detrimental to interactions with MC-I. Additionally, the amine linkage imparts a rigidity and a dramatic difference in orientation of the side chain due to the conjugation of the NH functionality with the chromone headpiece, compounding a less favorable interaction with MC-I.

Further SAR studies of the most potent chromone analog **10** were pursued. In the literature it has been suggested that radiotracers containing primary ^{18}F labels may be metabolically deflourinated in vivo at a faster rate than secondary or aromatic ^{18}F.[36,37] However, primary aliphatic ^{18}F atoms which are β to a heteroatom, e.g., [^{18}F]CH_2CH_2O−R, have been reported to be metabolized at a slower rate.[38] This has been termed the β-heteroatom effect. Analogs **13**−**15** in Table 3 were prepared wherein an oxygen atom was positioned in the side chain to take advantage of the β-heteroatom effect. MC-I inhibitory activities of these analogs range from 15 to 197 nM. Compound **13**, which contained a secondary fluorine atom in addition to the β-heteroatom effect, exhibited a potent inhibitory activity of 16 nM. Extension of the alkyl side chain from four carbons to seven afforded compound **14**, which had significantly less inhibitory activity (IC_{50} = 197 nM). The four-carbon side chain is optimal (by length or volume) for MC-I inhibition. Finally, replacement of a methylene unit with an oxygen atom in the original *n*-butyl side chain provides compound **15**, which is the most potent compound in this series (IC_{50} = 15 nM).

Chemistry. The syntheses of all chromone analogs discussed above are shown in Scheme 1. In general, these analogs were prepared by linking the chromone core to the aromatic moiety of the side chain via either Mitsunobu coupling (for the preparation of *S*-chromone analogs **7** and **10**) or displacement of a sulfoxide/sulfone (for the preparation for *O*-chromone analogs **8** and **11** and *N*-chromone analogs **9** and **12**).

S-Chromone analog **7** and intermediate **19** were prepared by reacting known chromone core **16** with the appropriately functionalized benzyl alcohol in THF in the presence of triphenylphosphine and diisopropyl azodicarboxylate (DIAD). It should be noted that intermediate **19** was formed exclusively under these Mitsunobu conditions when diol **18** was used. The corresponding toluenesulfonate ester **20** of compound **19** was formed by reaction with TsCl, DMAP, and TEA in anhydrous DCM. Displacement of the tosylate leaving grouping with potassium fluoride in the presence of Kryptofix222 in acetonitrile at 90 °C afforded the desired *S*-chromone analog **10**.

For the synthesis of *O*-chromone analogs, alkylation of sulfone **21** with the appropriate functionalized benzyl alcohol in the presence of NaH in DMF yielded the *O*-chromone analog **8** and intermediate **25** in good yield. Sulfone **21** was obtained by oxidizing chromone core **16** with *m*-chloroperbenzoic acid (*m*-CPBA). Deprotection of the alcohol functionality of reaction intermediate **24** with TBAF in THF followed by the prior established reaction sequence of tosylation and the subsequent fluorination renders *O*-chromone analog **11**.

Next, *N*-chromone analogs **9** and intermediate **29** were obtained using modified alkylation conditions. Here, sulfoxide **26** reacted with the appropriate functionalized benzyl amine at 50 °C in acetonitrile to afford final compound **9** and reaction intermediate **29**. Again, formation of the toluenesulfonate ester of the alcohol moiety of intermediate **29** with TsCl, DMAP, and TEA in dichloromethane followed by displacement of the leaving group using previous fluorination conditions renders the desired *N*-chromone analog **12**.

When chromone analogs **10**−**12** were prepared according to the reaction sequence shown in Scheme 1, the required functionalized benzyl alcohol or amine used as reaction partners in Mitsunobu reactions or alkylations had to be synthesized separately. Scheme 2 shows the preparation of benzyl alcohols **18** and **23** and benzyl amine **28** required in the preparation of **10**, **11**, and **12**. Sonogashira coupling of methyl 4-bromobenzoate with 3-butyn-1-ol in the presence of palladium chloride, triphenylphosphine, and a catalytic amount of copper iodide in diethylamine yielded desired intermediate **30**, which was reduced with hydrogen at 50 psi using palladium on carbon as a catalyst to afford key intermediate **31**.[39] Reduction of the ester functionality of **31** with LAH in THF afforded diol **18** in high yield. To obtain desired benzyl alcohol **23**, protection of the alcohol moiety of **31** with TBDMS−Cl followed by LAH reduction in THF was carried out. The functionalized benzyl amine substrate **28** was obtained in a similar fashion. Alkylation of 4-iodobenzyl bromide with phthalic anhydride in the presence of cesium carbonate in DMF introduced the nitrogen moiety masked as a protecting group. Sonogashira coupling of intermediate **32** with 3-butyn-1-ol in the presence of palladium chloride, triphenylphosphine, and a catalytic amount of copper iodide in diethylamine yielded desired intermediate **33**, which was reduced with hydrogen at 50 psi in the presence of palladium on carbon to yield **34**. Deprotection of the nitrogen functionality in **34** with hydrazine in refluxing butanol afforded the desired functionalized benzyl amine **28** used in Scheme 1.

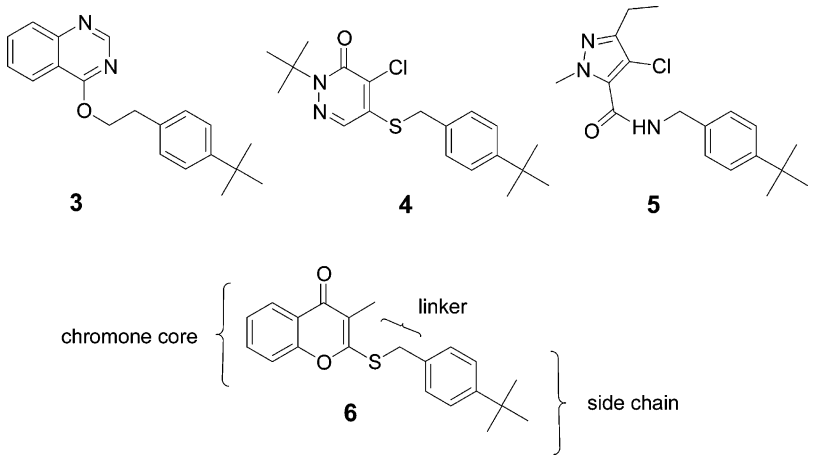
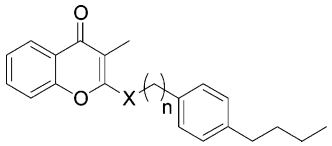


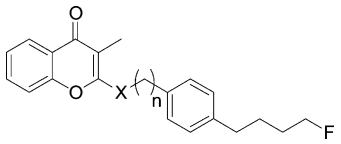
Figure 2. Various structure classes of MC-I inhibitors.

Table 1. Binding Affinity (IC_{50} Determined in Vitro) and Lipophilicity (clog $D_{7.4}$) of Nonfluorinated Chromone Derivatives and Known MC-I Inhibitors



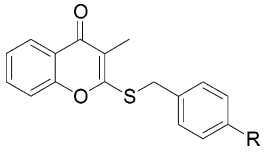
compd	X	n	IC_{50} (nM)	clog $D_{7.4}$
7	S	1	52	6.3
8	O	1	60	5.7
9	NH	1	57	5.5
1			16	4.7
3			90	5.5
4			8	4.7
6			52	5.9

Table 2. Binding Affinity (IC_{50} Determined in Vitro) and Lipophilicity (clog $D_{7.4}$) of Fluorinated Chromone Derivatives



compd	X	n	IC_{50} (nM)	clog $D_{7.4}$
10	S	1	9	5.6
11	O	1	33	5.0
12	NH	1	133	4.8

Table 3. Binding Affinity (IC_{50} Determined in Vitro) and Lipophilicity (clog $D_{7.4}$) of Additional Fluorinated *S*-Chromone Derivatives



compd	R	IC_{50} (nM)	clog $D_{7.4}$
13	$OCH_2CH(CH_3)F$	16	4.7
14	$CH_2(CH_2)_3OCH_2CH_2F$	197	5.1
15	$CH_2OCH_2CH_2F$	15	4.2

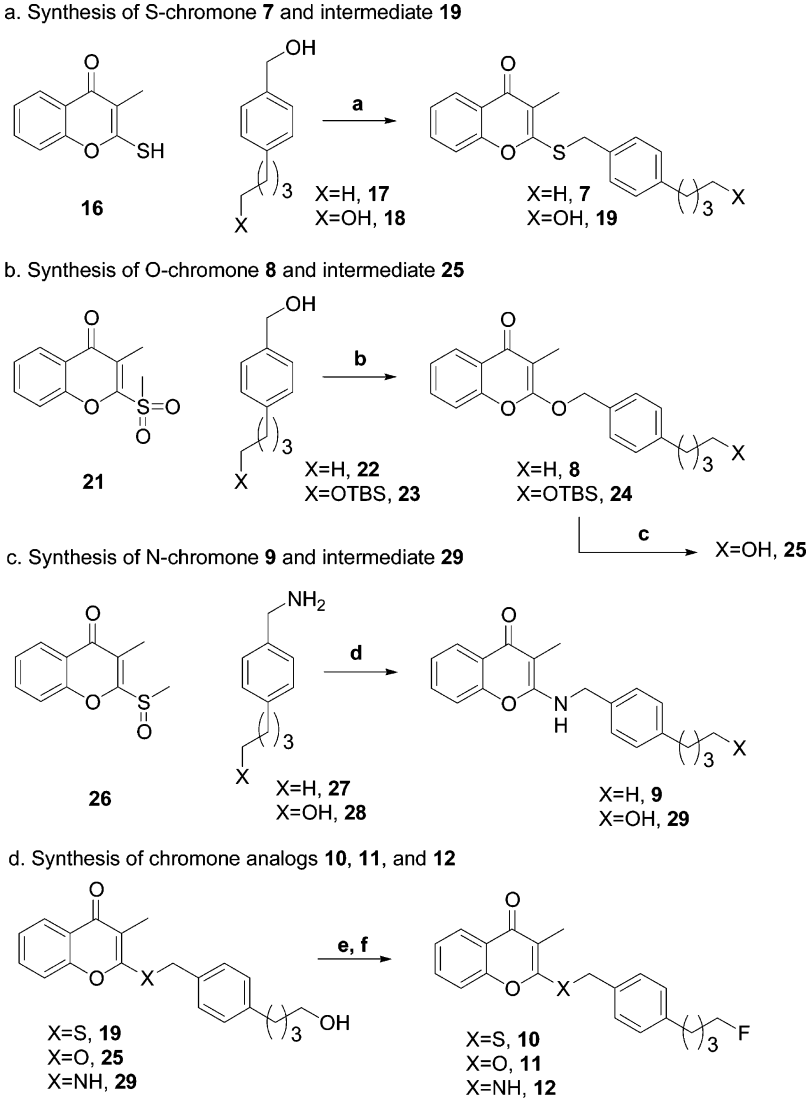
Compounds containing variations in the side chain of *S*-chromone analog **10** were prepared in order to investigate the effect of a heteroatom-containing alkyl side chain on the current MC-I inhibition activity of **10**. Analogs **13** and **15** were prepared using Mitsunobu conditions, which required prior synthesis of the appropriate functionalized benzyl alcohol. For analog **13**, the appropriate benzyl alcohol **36** was obtained in two steps. Alkylation of 4-hydroxy benzyl alcohol with chloroacetone in the presence of potassium carbonate in acetone afforded the ketone intermediate **35**, which was reduced with sodium borohydride in methanol to the diol **36**. Mitsunobu coupling of **36** with **16** afforded analog **37** as the only product. Formation of the toluenesulfonate ester of **37** with TsCl, DMAP, and TEA in DCM followed by fluorination with KF and Kryptofix222 in acetonitrile afforded final analog **13**. For analog **15** the appropriate benzyl alcohol **37** was obtained in one step: alkylation of 1,4-benzenedimethanol with bromofluoroethane in the presence of KHMDS in THF/toluene. Coupling under Mitsunobu conditions with chromone **16** yielded **15**. Last, analog **14** was prepared via alkylation of intermediate **19** with bromofluoroethane in the presence of NaH in DMF.

In Vitro Experiments. The inhibitory activities of analogs **1**, **3**, **4**, **6**, and **10**−**15** for the MC-I 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.[40] Submitochondrial particles (SMP) were isolated from bovine heart mitochondria[41] according to the method of Lester and Matsuno-Yagi et al.[42] The SMP were used as a suspension and each determination was referred to a concurrent rotenone standard, ensuring MC-I activity in the SMP preparation.

Lipophilicity. log $D_{7.4}$ is a partition coefficient for charged species between octanol and water at pH = 7.4. In general, the log D_{pH} is defined as $\log D_{pH} = \log(\sum a_i^{org}/\sum a_i^{water})$, where a_i^{org} is the concentration of the *i*th microspecies in octanol and a_i^{water} is the concentration of the *i*th microspecies in water. The computed lipophilicity, clog $D_{7.4}$, was calculated with ACD/LogD Suite Software (Advanced Chemistry Development Inc., Toronto, Canada) for all the compounds in this study (Table 1−3).

Radiosynthesis. Compound **10** manifests the highest inhibition constant within the series and was therefore chosen as a candidate for radiolabeling with the PET isotope ^{18}F. Nucleophilic radiofluorination methods require activated leaving groups, such as sulfonates (e.g., tosylates, mesylate), in order to generate alkyl fluoride containing radioligands.[45] Primary sulfonates are often the preferred leaving group because of their ease of displacement with nucleophilic ^{18}F versus secondary sulfonate leaving groups, which often eliminate under fluorination conditions to yield undesired olefin byproducts. With this in mind, the [^{18}F]**10** was prepared with 6% chemical yield (decay corrected) by the reaction of tosylate precursor **20** with Kryptofix222/K^{18}F complex and potassium carbonate in aceto-

Scheme 1. Synthesis of Chromone Analogs[a]



[a] Reagents: (a) PPh_3, DIAD, THF, rt; (b) NaH, DMF, rt; (c) TBAF, THF, rt; (d) ACN, 50 °C; (e) TsCl, DMAP, TEA, DCM, rt; (f) KF, K222, ACN, 90 °C.

Table 4. Biodistribution of [^{18}F]**10**[a] and ^{99m}Tc-Sestamibi[b] in Sprague−Dawley Rats

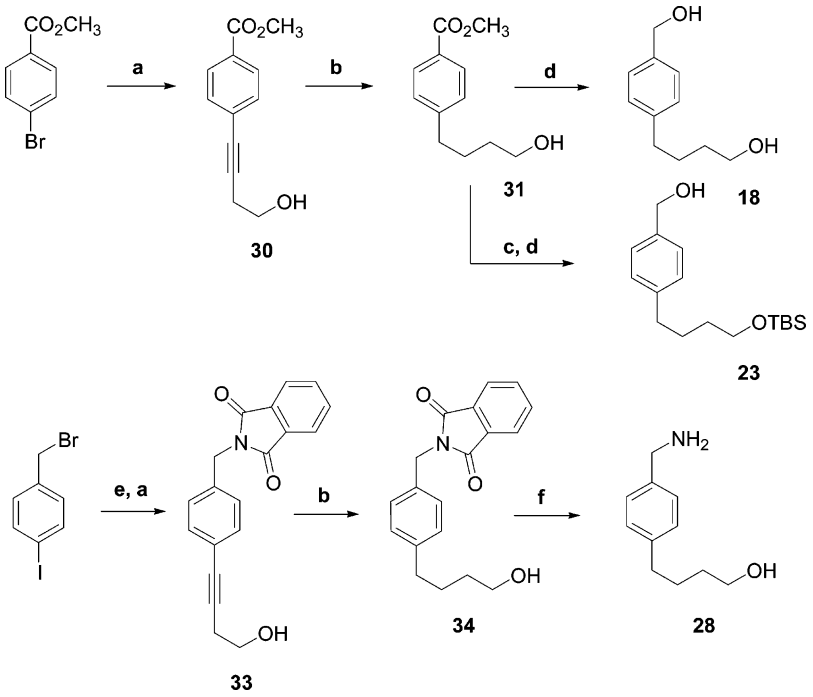
	[^{18}F]**10**		
organ	30 min	120 min	^{99m}Tc-sestamibi: 30 min
blood	0.11 ± 0.02	0.06 ± 0.01	0.02 ± 0.00
heart	2.24 ± 0.27	1.52 ± 0.19	1.59 ± 0.11
lung	0.32 ± 0.07	0.12 ± 0.03	0.51 ± 0.02
liver	2.00 ± 0.31	0.60 ± 0.12	0.82 ± 0.08
spleen	0.41 ± 0.05	0.09 ± 0.08	0.89 ± 0.07
kidney	1.93 ± 0.14	0.59 ± 0.08	2.52 ± 0.39
femur	0.36 ± 0.04	0.74 ± 0.06	0.22 ± 0.02
muscle	0.06 ± 0.02	0.09 ± 0.01	0.05 ± 0.01
brain	0.26 ± 0.03	0.17 ± 0.02	-

[a] Data are expressed as the %ID/g ± SD with five animals per data point. [b] Data are expressed as the %ID/g ± SD with six animals per data point.

nitrile at 90 °C for 30 min followed by preparative HPLC separation of the reaction mixture. The appropriate fractions were concentrated and analyzed for radiochemical yield and purity. The radioactivity of the final product was on average 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.

In Vivo Studies. Biodistribution Study of [^{18}F]10 and ^{99m}Tc-Sestamibi in Sprague−Dawley (SD) Rats. Table 4 shows the results of the biodistribution study carried out with [^{18}F]**10** in SD rats. The widely used clinical SPECT MPIA, ^{99m}Tc-sestamibi, was used as a reference compound for this study. High uptake of radiotracer [^{18}F]**10** from the blood pool into the myocardium was observed at 30 min postinjection (2.24% ID/g; ∼20:1 heart:blood pool ratio). During this 30 min time frame, uptake of [^{18}F]**10** was also elevated in liver and kidney tissue, 2.00% and 1.93% ID/g, respectively. Retention of [^{18}F]**10** in the heart was good and 66% of the activity at 30 min was present at 120 min. In contrast, levels in the liver and kidney decreased by 120 min to approximately 30% of the activity at 30 min. [^{18}F]**10** had heart levels at 30 min after administration 40% higher than ^{99m}Tc-sestamibi. Both radiotracers exhibit a blood:heart ratio >20:1, which should be more than sufficient to obtain clear images of the myocardium using SPECT or PET imaging technology. In addition, heart:lung ratio of [^{18}F]**10** compared to ^{99m}Tc-sestamibi was significantly improved, 1:7 versus 1:3, respectively. Low uptake of the radiotracer in lung tissue suggests an excellent signal: noise ratio in future imaging

Scheme 2. Synthesis of Side Chains **18**, **23**, and **28**[a]



[a] Reagents: (a) PPh_3, $PdCl_2$, CuI, 3-butyn-1-ol, DEA; (b) H_2, Pd/C, EtOH; (c) TBDMS−Cl, imidazole, DMF; (d) LAH, THF; (e) phthalic anhydride, Cs_2CO_3, DMF; (f) hydrazine, BuOH, reflux.

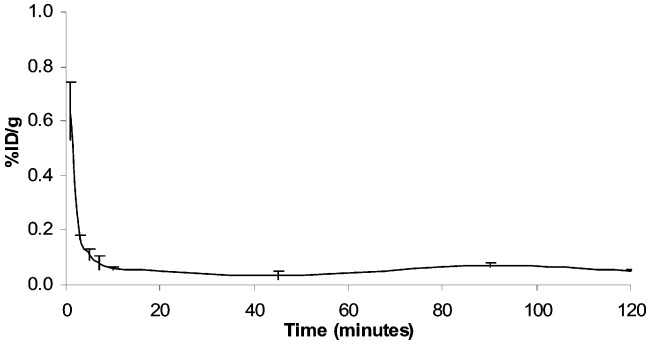


Figure 3. Blood clearance curve of [^{18}F]**10**.

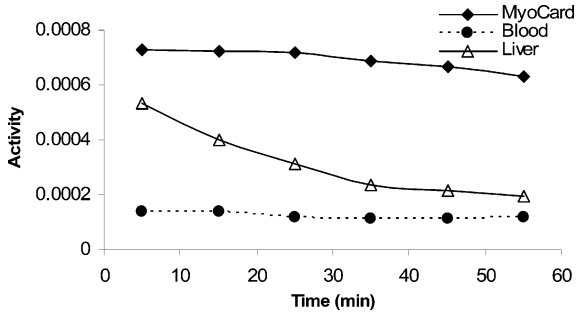


Figure 4. Time−activity curves from rat imaging data of blood, myocardium, and liver tissue.

studies with [^{18}F]**10**. However, both [^{18}F]**10** and ^{99m}Tc-sestamibi exhibit low selectivity toward liver tissue (1:1 and 1:2, respectively). The level of radiotracer [^{18}F]**10** in the blood initially decreased rapidly (within 5−7 min; Figure 3) to well below that of the heart and was then maintained throughout the remainder of the study (120 min).

With regards to the previous concern of in vivo defluorination and subsequent accumulation of free fluoride in bone, periodic measurements of the femur detected an approximate 2-fold increase of free fluoride from 30 to 120 min for [^{18}F]**10**, which

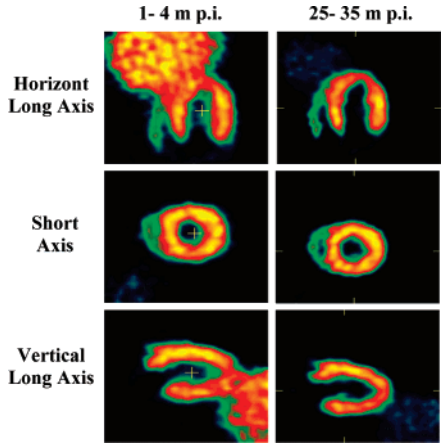


Figure 5. Images of a rat heart at two different time points.

corresponds to approximately 7% and 15% of injected dose at 30 and 120 min postinjection, respectively.[43] The overall ratio of radioactivity measured in the femur versus cardiac tissue decreased from 1:6 femur:heart at 30 min postinjection to 1:2 femur:heart after 120 min for [^{18}F]**10**. It should be noted that ^{99m}Tc-sestamibi exhibited a 1:7 femur:heart at 30 min postinjection as well. It has been our experience that more defluorination is seen with some compounds in the rat than occurs in higher species.

In summary, the biodistribution data of [^{18}F]**10** is superior to that of ^{99m}Tc-sestamibi with regards to uptake of the radiotracer in the myocardium. Furthermore, the organ selectivity of [^{18}F]**10** is comparable to that of ^{99m}Tc-sestamibi and therefore represents a suitable agent for pursuing PET imaging studies.

Imaging Studies in SD Rats. Dynamic PET imaging studies carried out on SD rats complemented the results obtained in the above distribution study. The radiotracer [^{18}F]**10** accumulated within minutes after the injection of the animal in cardiac and liver tissue, as is depicted in Figure 5. Time−activity

plots generated from the rat imaging studies show a steady blood level from 5 to 55 min postinjection, whereas heart levels decreased slightly and liver levels decreased markedly (Figure 4). These findings are consistent with those of the biodistribution study. Images of the rat heart show sufficient clearance of the radiotracer from the liver was observed by 25−35 min postinjection to obtain good myocardial images (Figure 5) and was complete by 55−60 min postinjection. No interference of the radiotracer from lung uptake 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 postinjection.

Conclusion

A series of fluorinated chromone derivatives with high affinity for MC-I have been prepared. SAR studies have shown that chromone analogs containing a sulfur atom in the linker moiety such as **10** exhibit the highest binding affinities for the MC-I complex versus oxygen- or nitrogen-containing chromone analogs **11** and **12**, respectively. In addition, introduction of an oxygen atom in the alkyl side chain of **10** does not disrupt MC-I inhibition, provided the chain length remains at four atoms. In vivo evaluation of [^{18}F]**10** demonstrated high uptake of the radiotracer by 30 min postinjection and minimal wash-out over 2 h. Initial high uptake of the radiotracer in the liver was seen in biodistribution and imaging studies. However, clearance of the radiotracer from the liver was sufficient by 25 min postinjection to afford high-quality images of the heart. Some in vivo bone uptake of ^{18}F was observed after [^{18}F]**10** administration in both in vivo studies. Biodistribution studies indicated higher myocardium uptake of [^{18}F]**10** than ^{99m}Tc-sestamibi, while maintaining a similar nontarget organ distribution. Future imaging studies will be directed toward investigating in vivo defluorination of [^{18}F]**10** in other animal species in addition to chromone derivatives **13**−**15**. Furthermore, additional studies will be directed toward determining the linearity of myocardial extraction of [^{18}F]**10** at high blood flow rates.

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 the solvent peak. Coupling constants are reported in hertz. The multiplicity is defined by an 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. The clog $D_{7.4}$ values were calculated using ACD/LogD Suite software (Advanced Chemistry Development Inc., Toronto, Canada). For radiolabeling studies, ^{18}F 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-(4-Butyl)benzylsulfanyl]-3-methylchromen-4-one (7). To a solution of 2-mercaptochromen-4-one (**16**, prepared according to the method of Lindell et al.)[35] (0.5 g, 2.6 mmol) and (4-butylphenyl)methanol (0.64 g, 3.90 mmol) dissolved in anhydrous THF (20 mL) were added solid PPh_3 (1.02 g, 3.90 mmol) and DIAD (757 μL, 3.90 mmol). After complete addition the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was concentrated to yield a crude yellow oil. The crude material was treated with diethyl ether (10 mL), and the precipitated phosphine byproducts were removed via filtration. The filtrate was concentrated to yield a yellow oil, which was purified using silica gel chromatography (4:1 pentane:EtOAc) followed by HPLC purification using a Phenomenex Luna C-18 (2) column (10 μm, 250 × 21.2 mm, gradient method of 40−60% B over 25 min, where B = 90% ACN in water using 0.1% TFA as a modifier and A = water using 0.1% TFA as a modifier) with a flow rate of 20 mL/min to afford compound **7** (571 mg, 1.69 mmol, 65%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.18 (dd, J = 8.0, 1.3 Hz, 1H), 7.60 (ddd, J = 8.4, 7.3, 1.8 Hz, 1H), 7.34−7.40 (m, 2H), 7.29 (d, J = 8.0 Hz, 2H), 7.13 (d, J = 8.0 Hz, 2H), 4.36 (s, 2H), 2.57 (t, J = 7.8 Hz, 2H), 2.04 (s, 3H), 1.53−1.59 (m, 2H), 1.31 (m, 2H), 0.90 (t, J = 7.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 175.4, 162.2, 156.7, 142.6, 133.4, 132.8, 128.9, 128.7, 126.2, 125.0, 122.7, 117.2, 116.8, 35.3, 35.2, 33.5, 22.3, 13.9 10.8. HRMS calcd for $C_{21}H_{22}O_2S$: 339.1413, found 339.1416.

Synthesis of 2-(4-Butylbenzyloxy)-3-methylchromen-4-one (8). Solid NaH (10.8 mg, 0.45 mmol) was placed in a reaction flask and cooled at 0 °C in an ice bath while a solution of (4-butylphenyl)-methanol (81.2 mg, 0.50 mmol) in dry THF (1 mL) was added dropwise with stirring. After complete addition, the reaction mixture was stirred at 0 °C for an additional hour. Solid 2-methanesulfinyl-3-methylchromen-4-one (**21**) (100 mg, 0.45 mmol) was added. After complete addition, the reaction mixture was stirred at room temperature for 6 h. The reaction mixture was cooled to 0 °C and quenched with methanol. The crude material was concentrated to yield a solid, which was redissolved in ACN/H_2O (3.5 mL, 1:1). HPLC purification using a Phenomenex Luna C-18 (2) column (10 μm, 250 × 21.2 mm, gradient method of 40−100% B over 15 min, where B = 90% ACN in water using 0.1% TFA as a modifier and A = water using 0.1% TFA as a modifier) with a flow rate of 20 mL/min afforded compound **8** (71 mg, 0.22 mmol, 49%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 8.00 (dd, J = 7.8, 1.7 Hz, 1H), 7.75 (ddd, J = 8.5, 7.3, 1.9 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.48 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.0 Hz, 1H), 7.24 (d, J = 8.0 Hz, 2H), 5.53 (s, 2H), 2.58 (t, J = 7.8 Hz, 2H), 1.83 (s, 3H), 1.50−1.57 (m, 2H), 1.26−1.32 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 176.7, 162.2, 152.2, 142.9, 133.0, 132.6, 128.5, 128.3, 125.4, 125.0, 121.7, 117.3, 97.2, 70.6, 34.5, 33.0, 21.7, 13.7, 7.2. HRMS calcd for $C_{21}H_{22}O_3$: 323.1641, found 323.1642.

Synthesis of 2-(4-Butylbenzylamino)-3-methylchromen-4-one (9). To a solution of 2-methanesulfinyl-3-methylchromen-4-one (**26**) (100 mg, 0.45 mmol) in ACN (5 mL) was added 4-butylbenzylamine (78 mg, 0.50 mmol). The reaction was stirred in a 50 °C oil bath overnight under N_2 atmosphere. The reaction mixture was allowed to cool to room temperature, concentrated to yield a yellow oil, and redissolved in ACN/H_2O (3.5 mL, 1:1). HPLC purification using a Phenomenex Luna C-18 (2) column (10 μm, 250 × 21.2 mm, gradient method of 60−90% B over 30 min, where B = 90% ACN in water using 0.1% TFA as a modifier and A = water using 0.1% TFA as a modifier) with a flow rate of 20 mL/min afforded compound **9** (81 mg, 0.25 mmol, 55%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.95 (t, J = 6.1 Hz, 1H), 7.92 (dd, J = 7.8, 1.8 Hz, 1H), 7.57 (ddd, J = 8.4, 6.6, 1.3 Hz, 1 H), 7.41 (d, J = 8.3 Hz, 1 H), 7.37−7.29 (m, 3H), 7.26 (d, J = 8.3 Hz, 2 H), 4.55 (d, J = 6.2 Hz, 2 H), 2.52 (m, 2H), 1.88 (s, 3H), 1.56−1.46 (m, 2H), 1.33−1.21 (m, 2H), 0.86 (t, J = 7.3 Hz, 3H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 172.6, 160.5, 152.1, 141.1, 136.7, 131.4,

128.3, 127.2, 124.7, 124.4, 122.1, 116.4, 92.0, 44.1, 34.4, 33.1, 21.7, 13.7, 7.7. HRMS calcd for $C_{21}H_{23}NO_2$: 322.1801, found 322.1800.

Synthesis of 2-[4-(4-Hydroxybutyl)benzylsulfanyl]-3-methylchromen-4-one (19). To a solution of 2-mercaptochromen-4-one (**16**) (1.52 g, 7.90 mmol) and 4-(4-hydroxymethylphenyl)butan-1-ol (**18**) (1.90 g, 9.90 mmol) dissolved in anhydrous THF (80 mL) were added solid PPh_3 (3.11 g, 11.90 mmol) and DIAD (2.30 mL, 11.90 mmol). After complete addition, the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with water (80 mL). The aqueous layer was separated and extracted with ethyl acetate (3 × 100 mL). All combined organic layers were dried over Na_2SO_4 and concentrated to yield an oil. The crude material was purified using silica gel chromatography (1:1 pentane:EtOAc) to yield compound **19** (1.29 g, 3.64 mmol, 46%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 8.01 (dd, J = 7.9, 2.2 Hz, 1H), 7.78 (ddd, J = 8.6, 7.0, 1.6 Hz, 1H), 7.70 (d, J = 8.5 Hz, 2H), 7.49−7.44 (m, 2H), 7.38 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 8.0 Hz, 2H), 4.50 (s, 2H), 3.37 (t, J = 6.5 Hz, 2H), 2.55−2.49 (m, 2H), 1.91 (s, 3H), 1.60−1.49 (m, 2H), 1.43−1.34 (m, 2H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 173.8, 161.9, 156.0, 141.6, 134.3, 133.5, 128.8, 128.5, 125.4, 125.2, 121.8, 117.5, 115.9, 60.4, 34.5, 34.1, 32.0, 27.3, 10.2. HRMS calcd for $C_{21}H_{22}O_3S$: 355.1362, found 355.1363.

Synthesis of 2-[4-(4-Fluorobutyl)benzylsulfanyl]-3-methylchromen-4-one (10). To a solution of 2-[4-(4-hydroxybutyl)-benzylsulfanyl]-3-methylchromen-4-one (**19**) (300 mg, 0.85 mmol) dissolved in anhydrous DCM (8.0 mL) were added TsCl (194 mg, 1.01 mmol), DMAP (124 mg, 1.01 mmol), and TEA (0.213 mL, 1.52 mmol). The reaction mixture was stirred at room temperature for 3 h and was then diluted with water (4 mL). The aqueous layer was separated and extracted with ethyl acetate (3 × 10 mL). All combined organic layers were dried over Na_2SO_4 and concentrated to yield an oil. The crude material was purified using silica gel chromatography (1:1 pentane:EtOAc) to yield **20** (280 mg, 0.55 mmol, 65%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.20 (dd, J = 7.8, 1.70 Hz, 1H), 7.80−7.76 (m, 2H), 7.62 (ddd, J = 8.6, 6.9, 1.6 Hz, 1H), 7.41−7.29 (m, 6H), 7.08 (d, J = 8.2 Hz, 2H), 4.38 (s, 2H), 4.03 (t, J = 6.0 Hz, 2H), 2.55 (t, J = 7.2 Hz, 2H), 2.45 (s, 3H), 2.05 (s, 3H), 1.65 (m, 4H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 175.5, 162.1, 156.7, 144.9, 141.5, 134.1, 133.4, 133.0, 130.0, 129.1, 129.0, 128.1, 126.5 125.3, 122.9, 117.5, 117.0, 70.5, 35.3, 34.9, 28.6, 27.2, 21.8, 10.8. HRMS calcd for $C_{28}H_{28}O_5S_2$: 509.1450, found 509.1441.

To a solution of **20** (10 mg, 0.020 mmol) in anhydrous ACN (0.2 mL) were added KF (2.28 mg, 0.04 mmol) and Kryptofix222 (14.8 mg, 0.04 mmol). After complete addition, the reaction mixture was heated at 90 °C for 25 min. The resultant mixture was allowed to cool to room temperature and diluted with water (1 mL). The aqueous layer was separated and extracted with ethyl acetate (3 × 2 mL). All combined organic layers were dried over Na_2SO_4 and concentrated to yield an oil. Purification by HPLC using a Phenomenex Luna C-18 (2) column (10 μm, 250 × 21.2 mm, gradient method of 20−80% B over 30 min, where B = 90% ACN in water using 0.1% TFA as a modifier and A = water using 0.1% TFA as a modifier) with a flow rate of 20 mL/min afforded compound **10** (3.3 mg, 0.01 mmol, 46%). ^{19}F NMR ($CDCl_3$, 564 MHz): δ −218.67 (m, 1F). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.18 (dd, J = 8.0, 1.3 Hz, 1H), 7.60 (ddd, J = 8.5, 6.7, 1.3 Hz, 1H), 7.39−7.35 (m, 4H), 7.13 (d, J = 8.2 Hz, 2H), 4.47 (m, 1H), 4.39 (t, J = 5.7 Hz, 1H), 4.36 (s, 2H), 2.63 (t, J = 7.5 Hz, 2H), 2.03 (s, 3H), 1.73−1.67 (m, 4H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 175.3, 162.0, 156.5, 141.7, 133.8, 132.7, 128.8 (2C), 126.2, 125.2, 122.6, 117.3, 116.8, 84.4 (83.3), 35.1, 35.0, 29.9 (29.8), 26.9, 10.5. HRMS calcd for $C_{21}H_{21}FO_2S$: 357.1319, found 357.1324.

Synthesis of 2-[4-(4-Hydroxybutyl)benzyloxy]-3-methylchromen-4-one (25). Solid NaH (37 mg, 1.5 mmol) was placed in a reaction flask and cooled to 0 °C in an ice bath. A solution of {4-[4-(*tert*-butyldimethylsilanyloxy)butyl]phenyl}methanol (**23**) (377 mg, 1.28 mmol) in dry DMF (23 mL) was added to the reaction flask dropwise with stirring. After complete addition, the reaction mixture was stirred at 0 °C for an additional hour. A solution of 2-methanesulfonyl-3-methylchromen-4-one (**21**) (0.92 g, 3.84 mmol) dissolved in dry DMF (20 mL) was added dropwise and the resultant mixture was stirred at room temperature overnight. The next day, the mixture was cooled to 0 °C and quenched with water (10 mL). The aqueous layer was separated and extracted with ethyl acetate (3 × 50 mL). All combined organic layers were dried over Na_2SO_4 and concentrated to yield an oil. The crude material was partially purified using silica gel chromatography (1:1 hexanes: EtOAc) to yield 2-{4-[4-(*tert*-butyldimethylsilanyloxy)butyl]-phenyloxy}-3-methylchromen-4-one (**24**) in addition to a minor amount of {4-[4-(*tert*-butyldimethylsilanyloxy)butyl]phenyl}methanol. HRMS calcd for $C_{27}H_{36}O_4Si$: 453.2455, found 453.2457.

To a solution of the above product mixture (258 mg, 0.57 mmol) dissolved in anhydrous THF (5 mL) was added a solution of TBAF (1.0 M solution in THF, 1.15 mL, 1.15 mmol). After complete addition, the reaction was stirred at room temperature for 1 h and then quenched with water (5 mL). The aqueous layer was separated and extracted with ethyl acetate (3 × 10 mL). All combined organic layers were dried over Na_2SO_4 and concentrated to yield an oil. The crude material was partially purified using silica gel chromatography (1:2 hexanes:EtOAc) to afford compound **25**, which was contaminated with 4-(4-hydroxymethylphenyl)butan-1-ol (40 %mol according to ^{1}H NMR), in moderate yield (101 mg of impure product mixture, 23.3% yield of impure product). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.21 (dd, J = 8.1, 1.4 Hz, 1H), 7.61 (m, 1H), 7.41−7.37 (m, 4H), 7.23 (d, J = 8.2 Hz, 2H), 5.44 (s, 2H), 3.67 (m, 2H), 2.65 (m, 2H), 1.99 (s, 3H), 1.72−1.68 (m, 2H), 1.64−1.60 (m, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 178.7, 162.3, 152.7, 143.2, 132.5, 132.3, 128.8, 128.2, 126.1, 125.1, 122.6, 116.6, 99.0, 70.7, 62.7, 35.3, 32.3, 27.4, 7.2. HRMS calcd for $C_{21}H_{22}O_4$: 339.1590, found 339.1591.

Synthesis of 2-[4-(4-Fluorobutyl)benzyloxy]-3-methylchromen-4-one (11). To a solution of 2-[4-(4-hydroxybutyl)benzyloxy]-3-methylchromen-4-one (**25**) (101 mg, 0.30 mmol) dissolved in anhydrous DCM (3.0 mL) were added TsCl (68 mg, 0.36 mmol), DMAP (55 mg, 0.45 mmol), and TEA (0.050 mL, 0.36 mmol). The reaction mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with water (3 mL), and the aqueous layer was separated and extracted with ethyl acetate (3 × 10 mL). All combined organic layers were dried over Na_2SO_4 and concentrated to yield an oil. The crude material was purified using silica gel chromatography (4:1 pentane:EtOAc) to yield toluene-4-sulfonic acid 4-[4-(3-methyl-4-oxo-*4H*-chromen-2-yloxymethyl)-phenyl]butyl ester **38** (75.2 mg, 0.15 mmol, 51%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.22 (dd, J = 8.2, 1.8 Hz, 1H), 7.77 (d, J = 8.4 Hz, 2H). 7.61 (m, 1H), 7.41−7.33 (m, 6H), 7.16 (d, J = 8.0 Hz, 2H), 5.44 (s, 2H), 4.04 (t, J = 5.9 Hz, 2H), 2.59 (t, J = 6.8 Hz, 2H), 2.44 (s, 3H), 2.00 (s, 3H), 1.68 (m, 4H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 178.2, 161.8, 152.2, 144.2, 142.0, 132.7, 132.2, 131.9, 129.3, 128.3, 127.7, 127.4, 125.6, 124.7, 122.1, 116.1, 98.5, 70.1, 69.8, 34.3, 27.9, 26.5, 21.1, 6.7. HRMS calcd for $C_{28}H_{28}O_6S$: 515.1498, found 515.1493.

To a solution of **38** (20 mg, 0.04 mmol) in anhydrous ACN (0.5 mL) were added KF (4.72 mg, 0.08 mmol) and Kryptofix222 (30.6 mg, 0.08 mmol). After complete addition, the reaction mixture was heated at 90 °C for 15 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 and concentrated to yield an oil. Purification by HPLC using a Phenomenex Luna C-18 (2) column (10 μm, 250 × 21.2 mm, isocratic method of 70% B over 30 min, where B = 90% ACN in water using 0.1% TFA as a modifier and A = water using 0.1% TFA as a modifier) with a flow rate of 20 mL/min afforded compound **11** (6.8 mg, 0.02 mmol, 13.6%).^{19}F NMR ($CDCl_3$, 564 MHz): δ −218.72 (m, 1F). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.21 (dd, J = 8.2, 1.7 Hz, 1H), 7.59 (m, 1H), 7.39 (m, 4H), 7.21 (d, J = 8.0 Hz, 2H), 5.43 (s, 2H), 4.48 (m,1H), 4.40 (t, J = 5.6 Hz, 1H), 2.67 (t, J = 7.8 Hz, 2H), 1.98 (s, 3H), 1.76−1.69 (m, 4H); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 178.7, 162.3, 152.2, 142.9, 132.6, 132.3, 128.8, 128.2,

126.1, 125.2, 122.6, 116.1, 99.0, 84.4 (83.3), 70.6, 35.1, 23.0 (29.9), 26.9, 7.2. HRMS calcd for $C_{21}H_{21}FO_3$: 341.1547, found 341.1547.

Synthesis of 2-[4-(4-Hydroxybutyl)benzylamino]-3-methylchromen-4-one (29). A solution of 2-[4-(4-hydroxybutyl)benzyl]-isoindole-1,3-dione (**34**) (964 mg, 3.12 mmol) and hydrazine (215 μL, 6.86 mmol) in n-butanol (59 mL) was heated at reflux for 1 h. Upon cooling to room temperature, a precipitate formed which was removed by filtration and washed with *n*-butanol. The filtrate was then concentrated in vacuo to obtain 4-(4-aminomethylphenyl)butan-1-ol (**28**) as a yellow solid (0.47 g, 2.61 mmol, 84%), which was used in the next step without any further purification.

To a solution of 2-methanesulfinyl-3-methylchromen-4-one (**26**) (0.35 g, 1.78 mmol) in ACN (37 mL) were added 4-(4-aminomethylphenyl)butan-1-ol (**28**) (0.47 g, 2.13 mmol) and DMF (18 mL). The reaction was heated with stirring at 50 °C in an oil bath overnight under N_2 atmosphere. The reaction mixture was allowed to cool to room temperature and concentrated to yield an oil. Purification of the oil by silica gel chromatography (100% EtOAc) afforded the desired product **29** (120 mg, 0.32 mmol, 18%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.21 (d, J = 7.7 Hz, 1H), 7.53 (t, J = 8.3 Hz, 1H), 7.35 (t, J = 7.4 Hz, 1H), 7.31 (d, J = 8.3 Hz, 1H), 7.29 (d, J = 7.9 Hz, 2H), 7.21 (d, J = 7.9 Hz, 2H), 4.83 (br t, 1H), 4.66 (m, 2H), 3.67 (m, 2H), 2.67 (t, J = 7.7 Hz, 2H), 1.98 (s, 3H), 1.74−1.69 (m, 2H), 1.64−1.60 (m, 2H), 1.24 (t, J = 5.4 Hz, 1H). ^{13}C NMR (DMSO-d_6/$CDCl_3$, 150 MHz): δ 174.0, 160.3, 152.2, 141.4, 135.5, 130.7, 128.2, 126.9, 125.0, 123.8, 122.3, 115.8, 92.7, 61.5, 44.6, 34.8, 31.8, 27.2, 7.3. HRMS calcd for $C_{21}H_{23}NO_3$: 380.1751, found 338.1753.

Synthesis of 2-[4-(4-Fluorobutyl)benzylamino]-3-methylchromen-4-one (12). To a solution of 2-[4-(4-hydroxybutyl)-benzylamino]-3-methylchromen-4-one (**29**) (100 mg, 0.30 mmol) in DCM (37 mL) cooled at 0 °C in an ice bath were added TsCl (68 mg, 0.36 mmol), DMAP (43.4 mg, 0.36 mmol), and TEA (62 μL, 0.44 mmol). The resultant slurry was stirred overnight under N_2 atmosphere, slowly warming to room temperature. The reaction mixture was concentrated and purified by flash column chromatography (3:1 hexane:EtOAc increasing gradient to 100% EtOAc) to yield toluene-4-sulfonic acid 4-{4-[(3-methyl-4-oxo-4*H*-chromen-2-ylamino)methyl]phenyl}butyl ester **39** as an oil (45 mg, 0.091 mmol, 31%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.19 (dd, J = 8.1, 1.1 Hz, 1H), 7.53 (m, 1H), 7.36−7.29 (m, 4H), 7.22 (d, J = 7.9 Hz, 2H), 4.83 (br t, 1H), 4.67 (d, J = 5.6 Hz, 2H), 3.68 (m, 2H), 2.67 (t, J = 7.7 Hz, 2H), 1.98 (s, 3H), 1.73−1.69 (m, 2H), 1.64−1.60 (m, 2H), 1.24 (t, J = 5.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 178.6, 162.6, 152.6, 140.7, 135.5, 132.3, 129.0, 128.6, 126.1, 125.6, 122.6, 116.6, 98.3, 84.1 (81.9), 68.9, 36.3, 35.0, 30.8 (30.6), 28.7, 27.4, 7.2. HRMS calcd for $C_{28}H_{29}NO_5S$: 492.1839, found 492.1846.

To a solution of **39** (24 mg, 0.049 mmol) in ACN (1.1 mL) was added Kryptofix222 (37 mg, 0.098 mmol) followed by KF (6 mg, 0.098 mmol). The reaction was heated at 90 °C for 30 min with stirring under nitrogen atmosphere. The reaction was then cooled to room temperature, filtered, and injected directly onto the preparative HPLC coloum chromatography (Luna, 10 μm, C18, 250 × 21.2 mm 10 micro, 60% B isocratic method over 40 min, where B = 90% ACN in water using 0.1% TFA as a modifier and A = water with 0.1% TFA as the modifier). The desired fractions were collected and neutralized to pH 7.6 and then lyophilized. The material was repurified by flash column chromatography (3:1 hexanes: EtOAc) to obtain the desired product **12** (0.3 mg, < 2%). ^{19}F NMR ($CDCl_3$, 564 MHz): δ −178.2 (m, 1F). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.21−8.17 (m, 1H), 7.53−7.48 (m, 1H), 7.35−7.28 (m, 4H), 7.19 (d, J = 9.0 Hz, 2H), 4.88 (br t, 1H), 4.65 (d, J = 6.0 Hz, 2H), 4.52 (m, 1H), 4.37 (m, 1H), 2.66 (m, 2H), 1.96 (s, 3H), 1.76−1.67 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 175.0, 160.2, 152.8, 141.9, 135.3, 131.4, 129.0, 127.6, 125.9, 124.6, 122.9, 116.2, 93.4, 85.0 (82.77), 45.5, 35.0, 30.1 (29.8), 27.0 (26.9), 7.6. HRMS calcd for $C_{21}H_{22}FNO_2$: 340.1707, found 340.1701.

Synthesis of 2-Methanesulfonyl-3-methylchromen-4-one (21). To a solution containing 2-mercapto-3-methylchromen-4-one (**16**) (2.26 g, 11.76 mmol) and potassium carbonate (1.62 g, 11.76 mmol) in acetone (120 mL) was added iodomethane (807 μL, 12.93 mmol). The reaction was stirred under nitrogen atmosphere at room temperature for 16 h and then was concentrated to yield a crude oil. The residue was taken up in water (50 mL) and adjusted to pH 7 with 5% HCl. The resulting aqueous layer was extracted with ethyl acetate (3 × 200 mL). The organic layer was then washed with water (1 × 200 mL) and brine (1 × 200 mL), dried over sodium sulfate, and concentrated to yield 3-methyl-2-methylsulfanylchromen-4-one as a yellow solid (1.95 g, 9.45 mmol, 80%), which was taken on to the next step without further purification. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.03−7.99 (m, 1H), 7.77−7.71 (m, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.48−7.43 (m, 1H), 2.69 (d, J = 0.8 Hz, 3H), 1.94 (d, J = 0.8 Hz, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.0, 163.2, 156.4, 133.7, 125.8, 125.6, 122.3, 118.0, 115.4, 13.3, 10.5. HRMS calcd for $C_{11}H_{10}O_2S$: 207.04743, found 207.04743.

To a solution containing 3-methyl-2-methylsulfanylchromen-4-one (0.97 g, 4.70 mmol) dissolved in DCM (19 mL) and cooled at 0 °C in an ice bath was added mCPBA (2.03 g, 11.75 mmol) dissolved in DCM (19 mL). The resultant mixture was stirred for 2 h at 0 °C and then warmed up to room temperature overnight. The next day, the reaction mixture was filtered and the resulting filtrate was washed with 1 N NaOH (2 × 100 mL), dried over sodium sulfate, and concentrated to obtain compound **21** as a white solid (0.84 g, 3.53 mmol, 75%), which was taken on to the next step without further purification. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.08 (dd, J = 8.0, 1.4 Hz, 1H,), 7.90 (m, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.56 (m, 1H), 3.52 (s, 3H), 2.30 (s, 3H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 177.3, 156.2, 154.6, 135.3, 126.3, 125.3, 121.5, 119.8, 118.5, 41.2, 8.5. HRMS calcd for $C_{11}H_{10}O_4SNa$: 261.0192, found 261.0196.

Synthesis of 2-Methanesulfinyl-3-methylchromen-4-one (26). To a solution containing 3-methyl-2-methylsulfanylchromen-4-one (1.95 g, 9.45 mmol) in DCM (75 mL) at 0 °C was added *m*-CPBA (2.0 g, 11.82 mmol) and the resultant mixture was stirred for 2 h. Upon consumption of the starting material, the solids were removed by filtration, and the resulting filtrate was washed with cold 5% sodium carbonate (1 × 150 mL), water (1 × 150 mL), and saturated sodium bisulfate (1 × 150 mL). The organic layer was dried over sodium sulfate and concentrated to obtain the desired product as a light yellow solid (1.74 g, 7.84 mmol, 83%), which was taken on to the next step without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.21−8.17 (m, 1H,), 7.75−7.69 (m, 1H), 7.60−7.57 (m, 1H), 7.46−7.40 (m, 1H), 2.97 (s, 3H), 2.23 (s, 3H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 177.2, 160.8, 156.3, 134.5, 126.1, 125.8, 122.5, 120.9, 118.1, 37.9, 9.3. HRMS calcd for $C_{11}H_{10}O_3S$: 223.04234, found 223.04217.

Synthesis of 4-(4-Hydroxybut-1-ynyl)benzoic Acid Methyl Ester (30). Compound **30** was prepared according to the method of Taylor et al.[39] To a stirred solution of methyl 4-bromobenzoate (13.4 g, 0.62 mmol) in diethyl amine (200 mL) were added palladium chloride (0.55 g, 3.06 mmol) and triphenylphosphine (0.16 g, 0.62 mmol). The solution was degassed, and copper iodide (0.12 g, 0.62 mmol) and 3-butyn-1-ol (4.34 g, 62 mmol) were added. The reaction mixture was stirred at room temperature for 48 h, during which time an additional 0.5 mol % palladium chloride, 1.0 mol % triphenyl phosphine, and 12 mol % 3-butyn-1-ol were added. Once the reaction was complete as judged by LCMS, the reaction mixture was concentrated and the crude material was taken up in a slurry of silica gel and EtOAc. The organic solvent was removed and the remaining dried silica gel was packed in a fritted funnel. Extensive washes with a hexane:ethyl acetate mixture (1:4) followed by ethyl acetate (100%) washes yielded compound **30** (11.9 g, 0.58 mmol, 94%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.97 (d, J = 8.58 Hz, 2H), 7.47 (d, J = 8.7 Hz, 2H), 3.92 (s, 3H), 3.83 (t, J = 6.4 Hz, 2H), 2.73 (t, J = 6.3 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.8, 131.8, 129.6, 129.5, 128.4, 90.0, 82.0, 61.2, 52.4, 24.1. ^{1}H NMR data corresponds to published data.

Synthesis of 4-(4-Hydroxybutyl)benzoic Acid Methyl Ester (31). To a stirring solution of 4-(4-hydroxybut-1-ynyl)benzoic acid methyl ester (**30**) (6.29 g, 0.031 mol) in ethanol (60 mL) was added

palladium on carbon (5 g, 10% on carbon), and the reaction mixture pressurized to 50 psi under an atmosphere of hydrogen. After 20 h, the reaction mixture was filtered through Celite to remove the catalyst and the filtrate was concentrated to afford compound **31** (5.67 g, 0.027 mol, 89%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.96 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.6 Hz, 2H), 3.91 (s, 3H), 3.68 (t, J = 6.6 Hz, 2H), 2.71 (t, J = 7.5 Hz, 2H), 1.76−1.71 (m, 2H), 1.64−1.59 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 167.2, 147.9, 129.8, 128.4, 127.8, 62.7, 52.0, 35.7, 32.2, 27.2.

Synthesis of 4-(4-Hydroxymethylphenyl)butan-1-ol (18). To a stirred solution of 4-(4-hydroxybutyl)benzoic acid methyl ester (**31**) (2.24 g, 0.01 mol) in THF (100 mL) was added dropwise a solution of lithium aluminum hydride (8.0 mL, 1 M in THF). After complete addition the reaction mixture was stirred at room temperature for 6 h. The reaction mixture was quenched with water (50 mL), the layers were separated, and the aqueous layer was extracted with ethyl acetate (3 × 150 mL). All combined organic layers were dried over Na_2SO_4 and concentrated to afford compound **18** (1.90 g, 0.01 mol, 98%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.27 (d, J = 8.3 Hz, 2H), 7.17 (d, J = 8.0 Hz, 2H), 4.64 (s, 2H), 3.64 (t, J = 6.4 Hz, 2H), 2.64 (t, J = 7.6 Hz, 2H), 1.71−1.66 (m, 2H), 1.62−1.57 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 141.9, 138.4, 128.6, 127.2, 65.3, 62.8, 35.3, 32.3, 27.5.

Synthesis of {4-[4-(*tert*-Butyldimethylsilanyloxy)butyl]phenyl}-methanol (23). To a solution of 4-(4-hydroxybutyl)benzoic acid methyl ester (**31**) (300 mg, 1.44 mmol) in DMF (4 mL) was added imidazole (147 mg, 2.16 mmol) followed by TBDMS−Cl (324 mg, 2.16 mmol). The reaction was stirred at room temperature for 2 h, with monitoring by TLC (3:1 hexane:EtOAc). Upon consumption of the starting material, the reaction was diluted with EtOAc (25 mL) and washed with water (3 × 50 mL) and saturated sodium bicarbonate (1 × 50 mL). The organic layer was dried over sodium sulfate and concentrated down to obtain 4-[4-(*tert*-butyldimethylsilanoxy)butyl]benzoic acid methyl ester (360 mg, 1.12 mmol, 77%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.87 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 8.6 Hz, 2H), 3.83 (s, 3H), 3.50 (t, J = 6.5 Hz, 2H), 2.66 (t, J = 7.5 Hz, 2H), 1.66−1.61 (m, 2H), 1.49−1.45 (m, 2H), 0.85 (s, 9H), 0.01 (s, 6H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 166.1, 148.0, 129.1, 128.6, 127.2, 62.1, 51.9, 34.7, 31.7, 26.8, 25.7, 17.8, −5.4.

To a stirred solution of 4-[4-(*tert*-butyldimethylsilanoxy)butyl]-benzoic acid methyl ester (670 mg, 2.18 mmol) in THF (22 mL) was added dropwise a solution of lithium aluminum hydride (2.18 mL, 1 M in THF). After complete addition, the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was quenched with water (50 mL), the layers were separated, and the aqueous layer was extracted with ethyl acetate (3 × 150 mL). All combined organic layers were dried over Na_2SO_4 and concentrated to afford compound **23** (586.8 mg, 2.00 mmol, 92%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.29 (d, J = 8.0 Hz, 2H), 7.19 (d, J = 8.0 Hz, 2H), 4.67 (s, 3H), 3.64 (t, J = 6.3 Hz, 2H), 2.65 (t, J = 7.9 Hz, 2H), 1.74−1.64 (m, 2H), 1.62−1.52 (m, 2H), 0.91 (s, 9H), 0.06 (s, 6H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 142.4.0, 138.5, 128.8, 127.3, 65.4, 63.2, 35.6, 32.6, 27.9, 26.2, 18.6, −5.1.

Synthesis of 2-[4-(4-Hydroxybut-1-ynyl)benzyl]isoindole-1,3-dione (33). To a solution of 4-iodobenzyl bromide (9.04 g, 30.4 mmol) in DMF (316 mL) were added phthalimide (4.47 g, 30.4 mmol) and cesium carbonate (14.86 g, 45.6 mmol). The reaction was stirred at room temperature overnight under nitrogen atmosphere. The reaction mixture was diluted with water (500 mL) and 2-(4-iodobenzyl)isoindole-1,3-dione precipitated out of solution. The white solid was collected by filtration and washed with water to afford the desired compound (9.50 g, 26.15 mmol, 86% yield), which was used in the next step without additional purification. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.83 (m, 2H), 7.71 (m, 2H), 7.63 (m, 2H), 7.17 (m, 2H), 4.77 (s, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 168.1, 138.0, 136.2, 134.5, 132.2, 130.8, 123.7, 93.7, 41.3.

To a slurry of 2-(4 iodobenzyl)isoindole-1,3-dione (2 g, 5.51 mmol), triphenylphosphine (14.4 mg, 0.055 mmol), and palladium chloride (5 mg, 0.028 mmol) in diethylamine (20 mL) were added DMF (4 mL) and copper iodide (11 mg, 0.055 mmol) followed by 3-butyn-1-ol (417 μL, 5.51 mmol). The reaction was stirred at room temperature overnight under nitrogen atmosphere. The reaction mixture was concentrated and purified by flash column chromatography (2:1 hexane:EtOAc) to yield compound **33** as a yellow solid (0.76 g, 2.49 mmol, 45% yield). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.86 (m, 2H), 7.72 (m, 2H), 7.36 (s, 4H), 4.83 (s, 2H), 3.80 (m, 2H), 2.68 (t, J = 6.0 Hz, 2H), 1.78 (t, J = 6.6 Hz, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.6, 136.4, 134.6, 131.5, 131.4, 127.5, 123.2, 122.3, 88.7, 80.6, 59.7, 40.6, 23.2. HRMS calcd for $C_{19}H_{15}NO_3Na$: 328.0944, found 328.0947.

Synthesis of 2-[4-(4-Hydroxybutyl)benzyl]isoindole-1,3-dione (34). To a solution of 2-[4-(4-hydroxybut-1-ynyl)benzyl]isoindole-1,3-dione (**33**) (2 g, 6.55 mmol) in EtOH/EtOAc (3:1, 163 mL) was added palladium on carbon (1.04 g, 10 wt %). The reaction was stirred at room temperature overnight under 50 psi of hydrogen. The reaction was monitored by ^{1}H NMR for conversion to product. Upon completion, the reaction mixture was filtered through Celite, washed with EtOAc, and concentrated to obtain compound **34** as a yellow oil (1.88 g, 6.14 mmol, 93% yield). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.90−7.85 (4H, m), 7.21 (d, J = 8.0 Hz, 2H), 7.14 (d, J = 7.8 Hz, 2H), 6.51 (br s, 1H), 4.73 (s, 2H), 3.38 (t, J = 6.6 Hz, 2H), 2.53 (t, J = 7.5 Hz, 2H), 1.57−1.52 (m, 2H), 1.42−1.37 (m, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 167.7, 141.5, 134.5, 133.9, 131.5, 128.4, 127.3, 60.4, 34.5, 32.0, 27.3.

Synthesis of 1-(4-Hydroxymethylphenoxy)propan-2-ol (36). To a suspention of 4-hydroxybenzyl alcohol (1 g, 8.06 mmol) and K_2CO_3 (1.34 g, 9.68 mmol) in acetone (80 mL) was added chloroacetone (0.771 mg, 9.68 mmol). After complete addition, the reaction mixture was heated at reflux overnight. The reaction mixture was concentrated to yield a crude oil, which was partitioned between EtOAc (100 mL) and water (100 mL). The aqueous layer was separated and extracted with EtOAc (2 × 150 mL). Combined organic layers were dried over Na_2SO_4 and concentrated. The resulting crude material was partially purified using silica gel chromatography (4:1 pentane:EtOAc to 1:1 pentane:EtOAc) to yield 1-(4-hydroxybenzyloxy)propan-2-one (**35**) in addition to 4-hydroxybenzyl alcohol (10% mol present according to ^{1}H NMR, 981 mg of product mixture obtained). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.32−7.29 (m, 2H), 6.90−6.85 (m, 2H), 4.54 (s, 2H,), 2.28 (s, 3H), ^{13}C NMR ($CDCl_3$, 75 MHz): δ 201.0, 157.6, 134.5, 129.0, 114.8, 73.4, 65.1, 26.8.

To a solution of the partially purified **35** (1.26 g) dissolved in MeOH (60 mL) was added solid $NaBH_4$ (0.32 g, 8.39 mmol). After complete addition, the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with water (30 mL), the layers were separated, and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). All combined organic layers were dried over Na_2SO_4 and concentrated to yield **36** as an oil (1.24 g, 6.81 mmol, 98% yield). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.21 (m, 2H), 6.87 (m, 2H), 5.00 (br s, 1H), 4.8 (br s, 1H), 4.41 (s, 2H,), 3.95−3.91 (m, 1H), 3.82−3.73 (m, 2H), 1.14 (d, J = 6.32 Hz, 3H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 157.6, 134.4, 127.9, 114.1, 73.2, 64.5, 62.5, 20.1.

Synthesis of 2-[4-(4-Hydroxypropoxy)benzylsulfanyl]-3-methylchromen-4-one (37). To a solution of 2-mercapto-3-methylchromen-4-one (**16**) (115.4 mg, 0.60 mmol), 1-(4-hydroxymethylphenoxy)propan-2-ol (**36**) (131.3 mg, 0.72 mmol) and PPh_3 (236.4 mg, 0.90 mmol) dissolved in THF (6 mL) was added DIAD (174.6 μL, 0.90 mmol) dropwise. After complete addition, the reaction was stirred at room temperature overnight. The reaction mixture was concentrated in vacuo to yield a yellow oil, which was purified using silica gel chromatography (3:2 pentane:EtOAc) to obtain compound **37** (190 mg, 0.534 mmol, 89%).^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.18 (dd, J = 8.03,1.63 Hz, 1H), 7.61 (ddd, J = 8.6, 6.9, 1.6 Hz, 1H), 7.40−7.30 (m, 4H), 6.86 (d, J = 8.7 Hz, 2H), 4.35 (s, 2H), 4.22−4.12 (m, 1H), 3.91 (dd, J = 9.2, 3.3 Hz, 1H), 3.77 (dd, J = 9.3, 7.6 Hz, 1H), 2.04 (s, 3H), 1.27 (d, J = 6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 174.8, 161.5, 157.7, 156.0, 132.3, 129.6, 128.2, 125.7, 124.6, 122.1, 116.8, 116.3, 114.4, 72.9, 65.7, 34.4, 18.3, 10.1. HRMS calcd for $C_{20}H_{20}O_4S$: 357.1155, found 357.1157.

Synthesis of 2-[4-(2-Fluoropropoxy)benzylsulfanyl]-3-methylchromen-4-one (13). To a solution of 2-[4-(4-hydroxypropoxy)-benzylsulfanyl]-3-methylchromen-4-one (**37**) (345 mg, 0.97 mmol) dissolved in anhydrous DCM (10.0 mL) were added TsCl (222 mg, 1.16 mmol), DMAP (178 mg, 1.45 mmol), and TEA (0.162 mL, 1.16 mmol). The reaction mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with water (10 mL), and the aqueous layer was separated and extracted with ethyl acetate (3 × 20 mL). All combined organic layers were dried over Na_2SO_4 and concentrated to yield an oil. The crude material was purified using silica gel chromatography (4:1 pentane:EtOAc) to yield toluene-4-sulfonic acid 1-methyl-[4-(3-methyl-4-oxo-*4H*-chromen-2-ylsulfanylmethyl)phenoxy]ethyl ester (387 mg, 0.75 mmol, 78%).^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.21 (dd, J = 8.2,1.5 Hz, 1H), 7.78 (d, 2H, J = 8.3 Hz), 7.62 (m, 1H), 7.39 (m, 2H), 7.31 (m, 2H), 7.30 (m, 2H), 6.67 (m, 2H), 4.87 (m, 1H), 4.35 (s, 2H), 4.00 (m, 1H), 2.89 (m, 1H), 2.44 (s, 3H), 2.05 (s, 3H), 1.41 (m, 3H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 175.5, 162.0, 157.9, 156.7, 144.9, 134.3, 133.0, 130.2, 129.9, 129.1, 128.1, 126.5, 125.3, 122.9, 117.7, 117.0, 115.0, 70.1, 35.1, 21.8, 18.0, 10.8. HRMS calcd for $C_{27}H_{26}O_6S$: 511.1243, found 511.1247.

To a solution of toluene-4-sulfonic acid 1-methyl-[4-(3-methyl-4-oxo-*4H*-chromen-2-ylsulfanylmethyl)phenoxy]ethyl ester (100 mg, 0.20 mmol) in anhydrous ACN (2.8 mL) were added KF (22.8 mg, 0.39 mmol) and Kryptofix222 (146.8 mg, 0.39 mmol). After complete addition, the reaction mixture was heated at 90 °C for 30 min. The reaction mixture was cooled to room temperature and concentrated to yield a dark orange oil. The crude material was redissolved in DCM (2.0 mL) and purified using a preparative TLC plate (4:1 hexane:EtOAc) to obtain compound **13** (43.0 mg, 0.12 mmol, 60%).^{19}F NMR (DMSO-d_6, 564 MHz): δ −178.2 (m, 1F). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 8.00 (dd, J = 8.3,1.6 Hz, 1H), 7.77 (m, 1H), 7.72 (d, J = 8.3 Hz, 1H), 7.47 (m, 1H), 7.40 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.8, 2H), 5.02−4.91 (m, 1H), 4.50 (s, 2H), 4.41−3.98 (m, 2H), 1.91 (s, 3H), 1.35 (dd, J = 6.6, 23.7 Hz, 3H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 173.8, 161.9, 157.5, 156.0, 133.5, 131.7, 130.2, 129.3, 125.4, 125.1, 121.8, 117.5, 116.0, 115.0, 114.6, 89.0 (87.9), 70.4 (70.2), 33.9, 16.8, 10.2. HRMS calcd for $C_{20}H_{19}FO_3$: 359.1111, found 359.1114.

Synthesis of 2-{4-[4-(2-Fluoroethoxy)butyl]benzylsulfanyl}-3-methylchromen-4-one (14). To a solution of 2-[4-(4-fluorobutyl)-benzylsulfanyl]-3-methylchromen-4-one (**10**) (50 mg, 0.15 mmol) dissolved in DMF (1.0 mL) was added solid NaH (4.26 mg, 0.18 mmol). After 15 min of stirring at room-temperature, 1-bromo-2-fluoroethane (13.18 μL, 0.18 mmol) was added, and the reaction was stirred at room temperature overnight. The reaction mixture was quenched with water (0.5 mL), the layers were separated, and the aqueous layer was extracted with ethyl acetate (3 × 2 mL). All combined organic layers were dried over Na_2SO_4 and concentrated to yield a crude oil. Purification by HPLC using a Phenomenex Luna C-18 (2) column (10 μm, 250 × 21.2 mm, gradient method of 35−95% B over 33 min, where B = 90% ACN in water using 0.1% TFA as a modifier and A = water using 0.1% TFA as a modifier) with a flow rate of 20 mL/min afforded compound **14** (10.2 mg, 0.025 mmol, 6.0%). ^{19}F NMR (DMSO-d_6, 564 MHz): δ −212.53 (m, 1F). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.18 (dd, J = 6.0, 9.0 Hz, 1H, m), 7.61−7.55 (m, 1H), 7.40−7.32 (m, 2H), 7.21 (d, J = 6.0 Hz, 2H), 7.14 (d, J = 6.0 Hz, 2H), 4.55 (t, J = 6.0 Hz, 1H), 4.44 (t, J = 6.0 Hz, 2H), 4.37 (d, J = 9.0 Hz, 1H), 2.74−2.63 (m, 4H), 1.97 (s, 3H), 1.85−1.80 (m, 4H). HRMS calcd for $C_{19}H_{15}NO_3Na$: 401.1581, found 401.1583.

Synthesis of 2-[4-(2-Fluoroethoxymethyl)benzylsulfanyl]-3-methylchromen-4-one (15). To a stirred solution of 1,4-benzenedimethanol (81.5 g, 0.59 mol) in DMF (236 mL) was added dropwise a solution of KHMDS (0.5 M in toluene, 283.2 mL, 0.14 mol). After complete addition, the reaction mixture was stirred at room temperature for 2 h. 1-Bromo-2-fluoroethane (8.80 mL, 0.12 mol) was added dropwise and the reaction was stirred at room temperature overnight. The reaction mixture was poured over ice, and the organic layer was separated and concentrated in vacuo to yield an oily solid. The crude material was redissolved in a minimal amount of toluene and cooled at −20 °C for several hours to precipitate excess 1,4-benzenedimethanol from the solution, which was removed via filtration. The filtrate collected was washed multiple times with water to remove remaining 1,4-benzenedimethanol and the resultant organics were concentrated to yield a yellow oil. The remaining excess 1,4-benzenedimethanol was removed via liquid−liquid extraction over 24 h using water and DCM to obtain [4-(2-fluoroethoxymethyl)phenyl]methanol as a white solid (10.4 g, 56.5 mmol, 47%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.29 (m, 4H), 5.15 (m, 1H), 4.59 (m, 1H), 4.51−4.48 (m, 5H), 3.68 (m, 1H), 3.63 (m, 1H).

To a solution of 2-mercapto-3-methylchromen-4-one (80.9 mg, 0.42 mmol), 4-(2-fluoroethoxymethyl)phenyl]methanol (93 mg, 0.51 mmol), and PPh_3 (165 mg, 0.63 mmol) dissolved in THF (1.0 mL) was added DIAD (122.4 μL, 0.63 mmol) dropwise. After complete addition, the reaction was stirred at room temperature overnight. The reaction mixture was concentrated in vacuo to yield a yellow oil, which was purified using silica gel chromatography (4:1 pentane:EtOAc) to obtain the desired product mixed with DIAD byproducts. Repurification via silica gel chromatography (4:1 DCM: hexane to 100% DCM) afforded the desired compound in low yield (3.5 mg, 9.75 mmol, 23%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.18 (dd, J = 8.0, 2.2 Hz, 1H), 7.60 (ddd, J = 8.7, 7.1, 1.4 Hz, 1H), 7.39−7.35 (m, 4H), 7.31 (d, J = 8.5 Hz, 2H), 4.60 (m, 1H), 4.56 (s, 2H), 3.72 (m, 1H), 3.67 (m, 1H), 2.03 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 175.3, 161.6, 156.5, 137.5, 136.0, 132.8, 128.9, 128.1, 126.2, 125.0, 122.6, 117.4, 116.8, 83.6 (82.5), 72.9, 69.4 (69.3), 35.1, 10.6. HRMS calcd for $C_{20}H_{19}FO_3S$: 359.1111, found 359.1112.

Preparation of Submitochondrial Particles from Bovine Hearts. Bovine heart mitochondria were prepared as described by Lester et al.[41] 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 ethylenediaminetetraacetic 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 26 000*g*, resulting in a mitochondrial pellet. The protein concentration of the mitochondrial samples as measured by a Bio-Rad Protein Assay Kit (Bio-Rad 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.[43] 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 16 000*g* for 10 min, and the supernatant was centrifuged at 150 000*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 Bio-Rad Protein Assay Kit (Bio-Rad 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.[40] 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} 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).

Procedure for the Radiosynthesis of ^{18}F-Labeled *S*-Chromone 10. A 500 mCi sample of aqueous ^{18}F 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 (Bio-Rad) cartridge. This cartridge was placed into an elution loop contained within a remote control 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 anhydrous acetonitrile (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. An aliquot of acetonitrile (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 acetonitrile (0.5 mL). The solvated ^{18}F with the remaining constituents (K, CO_3^{2-}, K222) was transferred to a conical bottomed 5 mL Wheaton vial containing 3.0 mg of tosylate precursor **20** (3.0 mg) dissolved in acetonitrile (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, rinsed with water (5 mL). The Sep Pak C18 cartridge was then eluted with acetonitrile (3 mL) and the column dried with N_2(g). The collected acetonitrile fraction was purified via HPLC (Phenomenex LUNA C-18 column 250 × 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). The ^{18}F-chromone analog **10** eluted from the column in 16−17 min and was collected as a single fraction. The solvent was then concentrated via rotary evaporation. Upon drying, the contents of the flask were reconstituted with a 10% ethanol solution. The final product yield was on average 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. The specific activity of the material ranged from 750 to 2000 Ci/mmol depending upon the quantity of ^{19}F present in the system.

Tissue Biodistribution of [^{18}F]10 and ^{99m}Tc-Sestamibi in Rats. Tissue biodistribution of [^{18}F]**10** and ^{99m}Tc-sestamibi was examined in sodium pentobarbital (50 mg/kg, ip) anesthetized Sprague−Dawley (300-400 g, male) rats. After anesthesia, the left femoral vein of rats was canulated and [^{18}F]**10** at a dose of about 15 μCi (range of specific activity, 750−2000 Ci/mmol) in 0.3 mL of 10% ethanol saline per rat was injected intravenously. At 30 and 120 min after the injections, rats (n = 5/each time point) were sacrificed in a CO_2 chamber, and blood, heart, lung, liver, spleen, kidney, femur, muscle, and brain were collected, weighed, and counted in an autogamma counter (Wallac Wizard 1480, Perkin-Elmer Life and Analytical Sciences, Shelton, CT) for radioactivity. The radioactivity measurements were decay-corrected, and the tissue biodistribution of [^{18}F]**10** was expressed as percent injected dose per gram of tissue (%ID/g). The same protocol was followed for ^{99m}Tc-sestamibi, where 30 min postinjection rats (n = 6) were sacrificed in a CO_2 chamber, and blood, heart, lung, liver spleen, kidney, femur, and muscle were collected, weighed, and counted in an autogamma counter for radioactivity. The radioactivity measurements were decay-corrected, and the tissue biodistribution of ^{99m}Tc-sestamibi was expressed as percent injected dose per gram of tissue (%ID/g).

Blood Clearance Curve of [^{18}F]10 in Rats. Blood clearance of [^{18}F]**10** was examined in sodium pentobarbital (50 mg/kg, ip) anesthetized Sprague−Dawley (300-400 g, male) rats. After anesthesia, the left femoral vein of the rat was canulated and [^{18}F]-**10** at a dose of about 15 μCi (range of specific activity: 750−2000 Ci/mmol) in 0.3 mL of 10% ethanol saline per rat was injected intravenously. Blood samples were collected from these rats by the bleeding tail veins at 1, 3, 5, 7, 10, 45, and 90 min or puncturing the heart at the time of tissue harvesting at 120 min after the injection. Rats (n = 3/each time point) were sacrificed in a CO_2 chamber and blood samples were counted in an autogamma counter (Wallac Wizard 1480, Perkin-Elmer Life and Analytical Sciences, Shelton, CT) for radioactivity. The radioactivity measurements were decay-corrected, and the blood clearance of [^{18}F]**10** was expressed as percent injected dose per gram (%ID/g).

Cardiac Imaging with [^{18}F]10 in Rats. Cardiac imaging was performed in anesthetized (pentobarbital, 50 mg/kg ip) rats (300-400 g, male, SD) 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]**10** (range of specific activity: 750−2000 Ci/mmol) was injected intravenously into the rat via a catheter precatheterized in femoral vein. Image acquisition was started 5 min postinjection 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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