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Synthesis of 7-Halogenated Isatin Sulfonamides: Nonradioactive Counterparts of Caspase-3/-7 Inhibitor-Based Potential Radiopharmaceuticals for Molecular Imaging of Apoptosis

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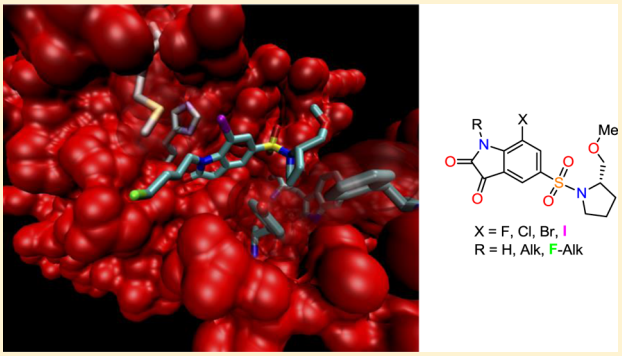
Supporting Information

ABSTRACT: *N*-Alkylated (*S*)-7-halogen-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatins were developed as a new group of nonradioactive reference compounds for future radiotracers. Inhibitor potency studies of these compounds suggest that the binding pockets readily accommodate both the 7-halogen substituents and aliphatic side chains (methyl to *n*-butyl) as well as some *ω*-fluorinated analogues (3-fluoropropyl and 4-fluorobutyl) at the isatin nitrogen. Indeed, compared to the halogen free parent compounds, some 7-halogenated derivatives exhibited slightly improved inhibitory potencies with IC_{50} values up to 2.6 nM (caspase-3) and 3.3 nM (caspase-7), respectively. Moreover, the 7-position of isatin, a potential cytochrome P450 hydroxylation site, was substituted by I, Br, Cl, and F to potentially enhance the metabolic stability of isatin sulfonamides. As an example, the radiotracer [^{18}F]**39** that was produced by ^{19}F/^{18}F isotope exchange was shown to be stable in human blood serum after incubation at 37 °C for at least 90 min.



1. INTRODUCTION

Apoptosis, or the programmed type I cell death, is the regulated destruction of overproduced or potentially dangerous and damaged cells without inflammatory responses. It is a highly conserved biological process through the evolution from hydra, nematodes, insects, and fishes to mammals and humans, and it plays vital roles in the development of cells, morphological changes, and metamorphosis in multicellular organisms.[1,2] Apoptosis is activated either by extrinsic binding of the CD95 ligand (CD95L) to the CD95 cell surface receptor (death receptor), resulting in intracellular death signaling (death receptor pathway), or by DNA damage and release of cytochrome c from the mitochondria (mitochondrial pathway).[1] The apoptotic cell death is a counter process to mitosis. The equilibrium of both processes strictly maintains the cell number, so-called "homeostasis", as well as the size and shape of organisms.[1,2] Dysregulation of apoptosis—down- or upregulated—can lead to a loss of balance, which results in the induction of pathological processes and a variety of diseases such as autoimmune and cardiovascular diseases, persistent infections, neurodegenerative disorders, developmental defects, and tumorigenesis.[1]

Although apoptosis consists of a complex network of numerous biological pathways, the process is mainly controlled by a set of intracellular cell death enzymes, so-called caspases (cysteinyl-aspartate-specific proteases), which are responsible for specific cleavage of peptide bonds after aspartate residues.[1] More than a dozen caspases have been characterized; however, it was demonstrated that inhibition of the executing caspases-3 and -7 alone is enough to slow down or even block programmed cell death.[3] Thus, targeting the executing caspases might enable novel procedures to be discovered for prevention, treatment, diagnosis, and therapy monitoring of the above-mentioned diseases by molecular imaging using positron emission tomography (PET) or single-photon emission computed tomography (SPECT). Moreover, it allows specific visualization of apoptosis at the molecular level, leading to a

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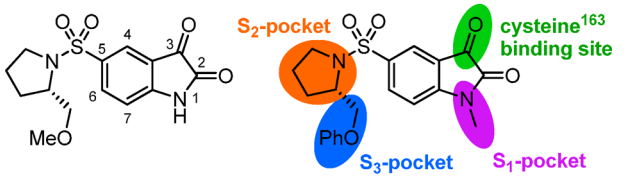
greater understanding of the mystery of the type-I cell death in more detail. Therefore, a specific imaging agent (radiotracer) is highly desirable, which possesses high affinity toward the target enzymes and is radiolabeled with a suitable positron emitter or a gamma emitter.

(*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) and (*S*)-*N*-methyl-5-[1-(2-phenoxymethylpyrrolidinyl)-sulfonyl]isatin (**2**) were identified by screening as potent and selective inhibitors of caspases-3 and -7 with K_i values in the nanomolar scale (Figure 1).[3]



1
K_i (caspase-3) = 60 nM
K_i (caspase-7) = 170 nM

2
K_i (caspase-3) = 15 nM
K_i (caspase-7) = 47 nM

Figure 1. Chemical structures and the K_i values of (*S*)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) and (*S*)-*N*-methyl-5-[1-(2-phenoxymethylpyrrolidinyl)sulfonyl]isatin (**2**) toward caspases-3 and -7. In the structure of **2**, interactions with the enzyme's pockets studied by X-ray cocrystallization are highlighted.[3]

The excellent enzyme–inhibitor interaction was confirmed by X-ray cocrystal studies of the complex of recombinant human caspase-3 with isatin sulfonamide **2** (Figure 1, right). It was revealed that the cysteine-163 of the enzyme binds the ligand covalently at the 3-keto group to form a tetrahedral thiohemiketal intermediate. While the N-methyl group putatively interacts with the S_1-pocket of the enzyme, the pyrrolidine moiety incorporates within the S_2-pocket and the 2-phenoxymethyl group of the pyrrolidine is accommodated in the enzyme's S_3-pocket.[3] Furthermore, recent metabolic stability studies using electrochemical and microsomal oxidation revealed a hydroxylation at the aromatic ring of the isatin core structure.[4] This might occur at the electronically most appropriate 7-position.

After the inhibitory properties of isatin sulfonamides have been disclosed, various structural modifications have been realized in the past decade to extend the chemical library and to study the structure–activity relationships (SAR). Moreover, the development of isatin sulfonamides as PET and SPECT imaging agents for the visualization of apoptosis was proposed.[5,6] Thus, numerous analogues of isatin sulfonamide including fluorinated ones were synthesized. N-Substitution of 2-methoxymethyl and 2-phenoxymethyl pyrrolidinyl and azetidinyl sulfonyl isatins with functionalized alkyl,[7,8] bromo-fluoroalkyl,[9] fluorohydroxyalkyl,[9,10] benzyl,[5,7,8,11,12] pyridyl,[11] and triazole groups[12,13] was confirmed to improve the biological activities against caspases-3 and -7 *in vitro*. In most cases, the compounds exhibited selective activities against caspases-3 and -7 with IC_{50} values in the nanomolar range. Compounds in the 2-phenoxymethyl class, such as **2**, tended to express superior inhibitory activities *in vitro*, while the 2-methoxymethyl versions, such as **1**, inhibited the enzymes more efficiently *in vivo*.[5] Therefore, **1** was selected as lead compound for further structural alterations. High binding affinities in the nanomolar range were maintained when 2-methoxymethyl or 2-phenoxymethyl groups of isatin sulfonamides were replaced by substituted phenoxy, pyridinylhydroxy,[11] triazolyl,[12] alkoxy, and fluoroalkoxy groups.[14,15] In contrast, introduction of functional groups such as methoxy, PEG_4yloxy (PEG_4 = tetraethylene glycol), fluoro, *gem*-difluoro, and trifluoromethyl groups at the 4-position and an extra methoxymethyl group at the 5-position of the pyrrolidine part resulted in an increase of the IC_{50} values to hundreds of nanomolar to micromolar level, and some compounds were even inactive.[16] Modification of the cysteine binding site (3-keto group) resulted in a loss or dramatic drop of binding affinity,[8,14] except for the introduction of Michael acceptors in the place of the carbonyl group.[17,18]

Since N-benzyl isatin analogues including fluorinated and methoxybenzyl ones exhibited excellent affinity, some were developed as radiotracers. For instance, the most recent isatin-based radiotracers [^{18}F]**3**,[19] [^{18}F]**4a**,[20] [^{18}F]**4b**,[6,21] [^{11}C]**5**,[20] and [^{18}F]**6**[22] (Figure 2) were prepared and their dynamics *in*

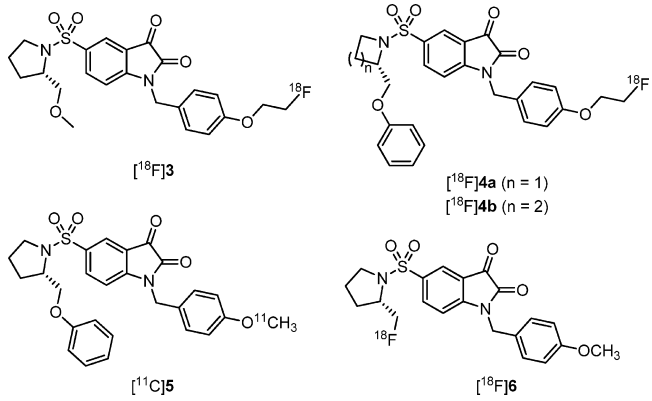


Figure 2. Most recent isatin-based radiotracers for molecular imaging of apoptosis using PET.[6,19−22]

vivo was investigated in mice using PET. However, the N-benzyl group was shown to be cleaved from the isatin core structure and the compounds are hydroxylated at the isatin aromatic ring according to electrochemical and microsomal oxidation in the metabolic stability test using EC/MS-ESI.[4] This might result in a loss of ^{18}F-activity leading to suboptimal PET imaging.

We assumed that some metabolic challenges of isatin sulfonamides could be overcome by blocking the potential aromatic hydroxylation site and replacing the exposed benzyl substituent at the isatin nitrogen with alkyl groups. There was earlier evidence from our group that a 7-brominated analogue, (*S*)-7-bromo-5-{2-[difluoro(methoxy)methyl]pyrrolidine-1-yl-sulfonyl}-1-propylindoline-2,3-dione (**9**), not only maintained the binding potency toward caspases-3 and -7 in the nanomolar scale, but even increased it. In the fluoro-desulfurization of **7** using 1,3-dibromo-5,5-dimethylhydantoin (DBH) and pyridine·9HF,[23] two products showing high binding potencies for caspases-3 and -7, respectively, were obtained. Compound **9** was even more potent than the parent **8** or the lead compound **1** (Scheme 1).[5,14]

Herein, we report the synthesis of a series of new *N*-alkyl-7-halogenated isatin sulfonamides, which are expected to be metabolically more stable, when the potential hydroxylation site is blocked by introduction of a halogen atom (I, Br, Cl, or F) and when the benzyl group at the isatin nitrogen is replaced by alkyl or fluoroalkyl substituents. In addition, the influence of the 7-halogen on the inhibition potency toward caspases-1, -3, -6, and -7 will be studied systematically. Moreover, the 7-fluoro

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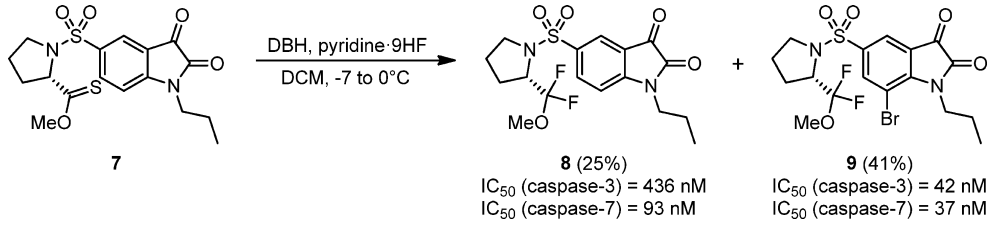
Scheme 1. Fluoro-desulfurization of 7 To Form Isatins 8 and 9 and Their *in Vitro* Binding Affinities toward Caspases-3 and -7[14]

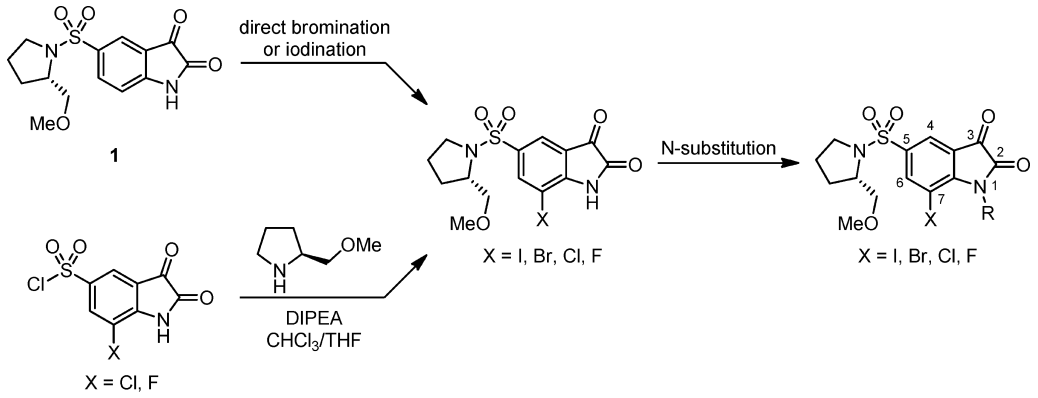


7

8 (25%)
IC_{50} (caspase-3) = 436 nM
IC_{50} (caspase-7) = 93 nM

9 (41%)
IC_{50} (caspase-3) = 42 nM
IC_{50} (caspase-7) = 37 nM

Scheme 2. Synthetic Routes towards 7-Halogenated Isatin Sulfonamides



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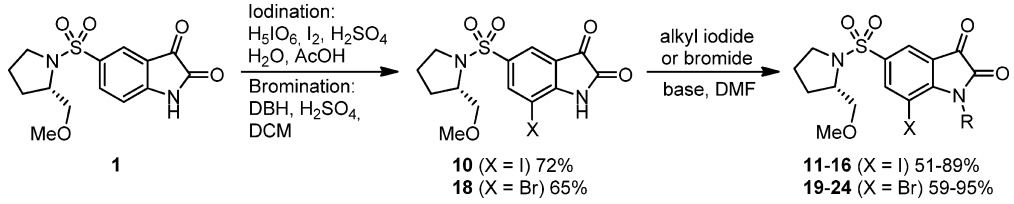
Scheme 3. Preparation of 7-Iodo and 7-Bromo Isatin Analogues 10−16 and 18−24



1

10 (X = I) 72%
18 (X = Br) 65%

11-16 (X = I) 51-89%
19-24 (X = Br) 59-95%

and 7-iodo analogues might allow future labeling with fluorine-18 and iodine-124 for PET application and with iodine-123 and -131 for SPECT apoptosis imaging *in vivo*. Furthermore, for a model compound, $^{19}F/^{18}F$ isotope exchange and blood serum stability tests were carried out.

2. RESULTS AND DISCUSSION

2.1. Chemistry. A series of 7-halogenated isatin sulfonamides was prepared following two methodologies depending on the halogen atom to be attached: (i) direct bromination and iodination of the lead compound **1** and (ii) coupling of 7-chloro- and 7-fluoroisatin-5-sulfonyl chloride with 2-methoxymethylpyrrolidine as depicted in Scheme 2.

The syntheses of the 7-iodinated and 7-brominated series started from the lead compound **1**,[7] and iodine or bromine was attached to the aromatic ring first by direct electrophilic aromatic substitution and the N-substitutions were performed subsequently (Scheme 3). In analogy to a literature precedent,[24] the direct iodination was successful by treatment of **1** with H_5IO_6 and I_2 under strong acidic conditions to give the 7-iodoisatin **10** in 72% yield, while using NaI and $NaClO_2$ analogously to a literature procedure[25] did not lead to formation of the desired product. Similarly to a literature protocol,[14] a bromine atom was attached to the 7-position of the aromatic ring using DBH in the presence of concentrated H_2SO_4 to give **18** in 65% yield. The structures of the new products were confirmed by ^1H and ^{13}C NMR spectroscopy. The two doublet ^1H NMR signals with *meta*-coupling ($^4J_{H,H}$ = 1.3−1.6 Hz) of 4-CH and 6-CH were observed for both compounds. In addition, ^{13}C NMR signals of 7-C bearing either iodine (**10**) or bromine (**18**) were detected at δ = 78.1 and 106.2 ppm (δ = 112.7 ppm for 7-CH of **1**), respectively.

As mentioned above, N-substitution significantly improved the affinities. Thus, various aliphatic chains, such as methyl, ethyl, propyl, 3-fluoropropyl, butyl, 4-fluorobutyl, and 4-hydroxybutyl groups, were attached to the 7-iodinated and 7-brominated isatins **10** and **18** to yield the corresponding derivatives **11−16** and **19−24**, as listed in Table 1. The N-methylation took place using MeI and NaH in DMF after stirring at 0 °C for 30−60 min and at room temperature for another 30 min to give products **11** and **19** in 82% and 81% yields, respectively. N-Propylation of **18** using propyl bromide and K_2CO_3 in DMF at room temperature needed 4 days to obtain **21** in good yield. For other N-alkylations, the reaction efficacy was therefore enhanced using Cs_2CO_3[26] as base and microwave irradiation[27] (at 90−100 °C for 2−10 min). The desired products **12−16** and **20**, **22−24** were obtained within a short time with moderate to excellent yields (Table 1).

In contrast to the mentioned N-alkylations, the 7-iodinated and 7-brominated (*S*)-*N*-(4-hydroxybutyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatins **17** and **25** were obtained in two consecutive steps by coupling of **10** and **18** with 4-bromobutyl pivalate using Cs_2CO_3 in DMF under microwave irradiation followed by acidic hydrolysis as shown in

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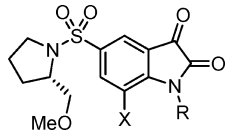
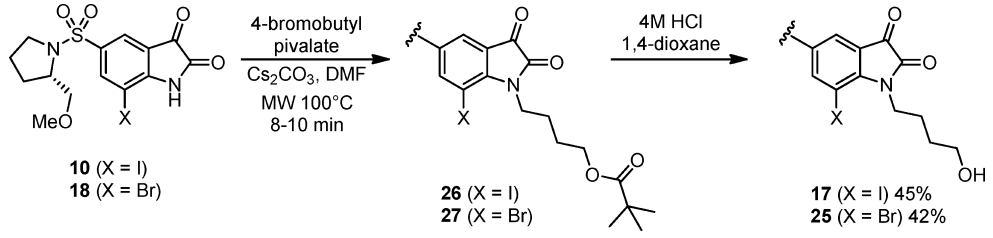
Table 1. *In Vitro* Inhibitory Potencies of New 7-Halogenated Isatin Sulfonamides 10−17, 18−25, 31−33, and 37−39 with Standard Deviation of Three Independent Experiments Expressed as IC_{50} Values



				Inhibitory potencies IC_{50} (nM)			
Inhibitor	X	R	Yield [%]	caspase-1	caspase-3	caspase-6	caspase-7
1[5]	H	H	47	>500,000	84.9 ± 25.6	>500,000	1,290 ± 466
1a[5]	H	Me	82	n.d.[a]	2.4 ± 0.3	n.d.	304 ± 73
1b[5]	H	Et	59	n.d	183 ± 48	n.d.	319 ± 60
1c[5]	H	*n*-C_3H_7	65	n.d.	5.3 ± 3.8	n.d.	58 ± 15
1d[5]	H	*n*-C_4H_9	86	n.d.	29 ± 13	n.d.	8.1 ± 1.1
1e[5]	H	*n*-C_4H_8F	70	n.d.	41 ± 17	n.d.	28 ± 6
10	I	H	72	n.d.	>500,000	n.d.	>500,000
11	I	Me	82	n.d.	1,190 ± 194	n.d.	10,500 ± 2,020
12	I	Et	79	n.d.	210 ± 44	n.d.	20.1 ± 10.0
13	I	*n*-C_3H_7	51	n.d.	124 ± 10	n.d.	42.4 ± 9.8
14	I	*n*-C_4H_9	89	>500,000	17.4 ± 1.6	>500,000	907 ± 844
15	I	*n*-C_3H_6F	57	>500,000	20.1 ± 0.3	28,500 ± 6,710	123 ± 13
16	I	*n*-C_4H_8F	84	>500,000	50.7 ± 0.9	>500,000	68.1 ± 0.5
17	I	*n*-C_4H_8OH	45	>500,000	33.2 ± 3.6	>500,000	279 ± 11
18	Br	H	65	n.d.	>500,000	n.d.	>500,000
19	Br	Me	81	n.d.	353 ± 80	n.d.	553 ± 55
20	Br	Et	62	n.d.	133 ± 41	n.d.	135 ± 35
21	Br	*n*-C_3H_7	72	n.d.	26.2 ± 12.6	n.d.	157 ± 53
22	Br	*n*-C_4H_9	59	n.d.	46.9 ± 4.3	n.d.	3.3 ± 0.3
23	Br	*n*-C_3H_6F	65	n.d.	81.4 ± 24.6	n.d.	20.1 ± 9.1
24	Br	*n*-C_4H_8F	95	n.d.	31.5 ± 6.2	n.d.	5.95 ± 2.55
25	Br	*n*-C_4H_8OH	42	>500,000	2.6 ± 0.7	>500,000	22.7 ± 1.3
31	Cl	H	60	>500,000	>500,000	>500,000	>500,000
32	Cl	*n*-C_4H_9	97	>500,000	57.7 ± 15.3	>500,000	263 ± 104
33	Cl	*n*-C_4H_8F	95	2,590 ± 383	4.8 ± 2.3[b]	>500,000	55.7 ± 30.6
37	F	H	43	>500,000	>500,000	>500,000	6,620 ± 234
38	F	*n*-C_4H_9	82	9,970 ± 4,250	2.9 ± 0.8	78,900 ± 69,700	9.7 ± 5.4
39	F	*n*-C_4H_8F	78	7,430 ± 3,520	41.0 ± 19.0	12,900 ± 5,160	39.3 ± 11.2

[a]n.d. = not determined. [b]Determined using a different bach of caspase-3.

Scheme 4. Synthetic Route to 7-Iodinated and 7-Brominated (*S*)-*N*-(4-hydroxybutyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatins 17 and 25



Scheme 4. The *N*-(4-hydroxybutyl)isatins **17** and **25** were formed in 45% and 42% yields (over two steps), respectively.

Differing from the synthesis of the 7-iodinated and 7-brominated isatin sulfonamides **11**−**17** and **19**−**25**, the 7-chloro and 7-fluoroisatin analogues **31**−**33** and **37**−**39** were constructed commencing from the commercially available 7-chloro- and 7-fluoroisatins **28** and **34**, as depicted in Scheme 5. Similar to the preparation of the parent system **1**,[7] **28** and **34** were sulfonated using oleum followed by treatment with NaOH, giving the 7-halogenated sodium sulfonates **29** and **35** in quantitative yields. The *meta*-coupling of protons at the 4- and 6-positions in ^{1}H NMR spectra revealed the addition at the 5-position for both compounds. Subsequent chlorination using $POCl_3$ resulted in the formation of 7-chloro and 7-fluoro-5-chlorosulfonyl isatins **30** and **36** in 80% and 66% yields, respectively. The couplings of **30** and **36** with 2-methoxymethylpyrrolidine were performed in the presence of diisopropyl ethyl amine (DIPEA) to give isatin sulfonamides **31** and **37** in 60% and 43% yields. The ^{13}C NMR spectra indicated signals of 7-C bearing chlorine (δ = 118.9 ppm) and fluorine (δ = 146.7 ppm), respectively. Since the *N*-butyl- and *N*-fluorobutyl-7-iodo and 7-bromoisatins exhibited superior

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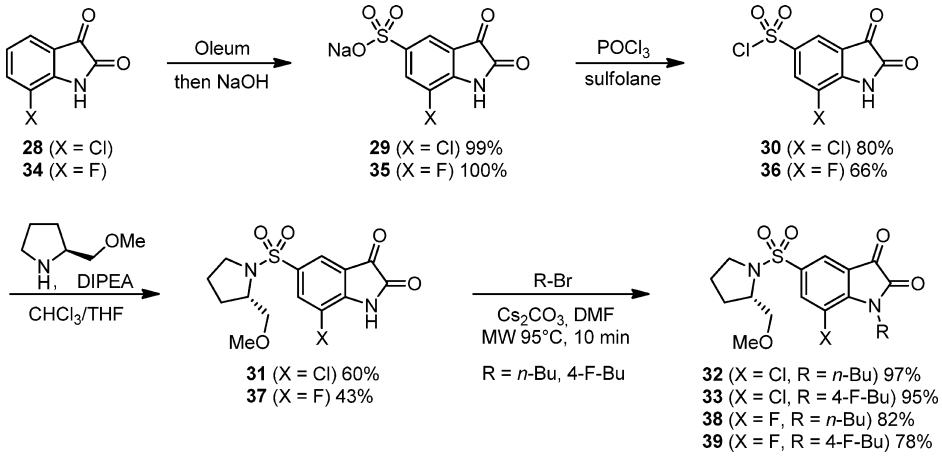
Scheme 5. Synthetic Pathways to N-Substituted 7-Chloro 32, 33 and 7-Fluoroisatin Sulfonamides 38, 39



potencies toward caspases-3 and -7 over the shorter chains (see section 2.2), compounds **31** and **37** were *N*-alkylated only with *n*-butyl and 4-fluorobutyl groups using similar conditions as described in the 7-iodine and 7-bromine series to obtain the target compounds **32**, **33**, **38**, and **39** in good to excellent yields (Table 1).

2.2. *In Vitro* Caspase Inhibition Potencies. The inhibitory potencies of all synthesized 7-halogenated isatin sulfonamides toward caspases-3 and -7 were evaluated *in vitro* and compared to those of the lead compound **1**. Some selected compounds were additionally evaluated toward caspases-1 and -6. The IC_{50} values in nanomolar scale are listed in Table 1.

All N-unsubstituted 7-halogenated isatins **10**, **18**, **31**, and **37** did not inhibit caspases-3 and -7 on the 500 μM scale, except compound **37**, which showed no caspase-3 inhibition and reduced caspase-7 inhibition with IC_{50} = 6.62 μM. However, after N-substitution, the inhibition potencies toward caspases-3 and -7 were improved significantly to nanomolar level. These findings revealed that N-substitution has a crucial impact on binding of 7-halogenated isatins. It was also observed that 7-iodo and 7-bromo N-substituted isatin sulfonamides with *n*-propyl and in particular *n*-butyl, 4-fluorobutyl, and 4-hydroxybutyl groups showed considerably superior potencies over the shorter chain analogues. Thus, *n*-butyl and 4-fluorobutyl groups were selected to be appropriate candidates for N-substitutions of 7-chloro- and 7-fluoroisatins.

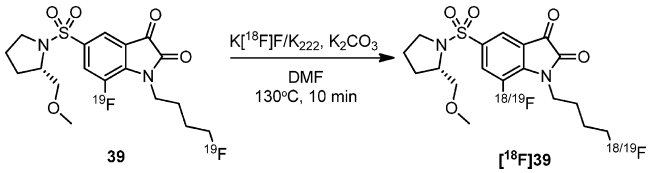
Among the compounds in this class, the 7-fluoro derivative **38** was among the most potent inhibitor for caspase-3 (IC_{50} = 2.9 nM), i.e. 10 times more potent than the parent compound **1d**. The 7-bromo compound **22** was most potent against caspase-7 (3.3 nM), which means 2.5 more potent than **1d**. Furthermore, compound **38** was equally as potent as compound **1d** with respect to caspase-7. Compared to the lead compound **1**, this is a 20- and 400-fold improvement, respectively. In contrast, the binding potencies toward caspases-1 and -6 were weak, in most cases with IC_{50} > 500 μM. Therefore, only a selection of compounds was assayed for caspases-1 and -6.

According to the above results, several compounds, such as **12**−**17**, **23**, **24**, **33**, **38**, and **39**, which possess high potencies to the target enzymes and contain fluorine and/or iodine, can be considered as potential radiotracers for PET after labeling with fluorine-18 or iodine-124, and for SPECT when labeled with iodine-123. Besides ^{18}F for ^{19}F isotope exchange,[28] also aromatic substitution of iodonium salts[29] and aliphatic bimolecular nucleophilic substitution of sulfonate esters[30] using no-carrier-added [^{18}F]fluoride should be suitable for these inhibitors. For ^{123}I and ^{124}I labeling, the iodinated precursors can be isotopically exchanged with radioactive iodides.[31,32] Alternatively, the ^{123}I and ^{124}I labeled tracers should be available by iododemetalation reactions as precedented in the literature.[5,33]

In order to get the first information on stability, compound **39** was chosen for ^{19}F/^{18}F isotope exchange and the subsequent blood serum stability test.

2.3. Radiosynthesis and Serum Stability Test. One possible method for [^{18}F]-radiolabeling is the isotope exchange either in the aromatic position by nucleophilic aromatic substitution[28] or in the aliphatic position by an S_N2 process.[30] Therefore, compound **39** is an attractive candidate offering both possibilities. As a result, its reaction with K[^{18}F]/K_{222} in DMF at 130 °C for 10 min provided [^{18}F]**39** with 0.2% (d.c.) radiochemical yield (r.y.) and 99% radiochemical purity by either one or the other of the mentioned reactions (Scheme 6).

Scheme 6. Radiosynthesis of [^{18}F]39



Unfortunately, all further variations of the solvent, stoichiometry, reaction time, and temperature did not improve the r.y. The radio-HPLC is shown in Figures S1 and S2 in the Supporting Information (SI).

The *in vitro* stability study of [^{18}F]**39** was carried out using human blood serum. After the long-term incubation for up to 90 min at 37 °C, only the parent compound [^{18}F]**39** was detected by radio-HPLC as shown in Figure 3.

Oxidative metabolism tests using electrochemistry/mass spectrometry of compounds **24**, **33**, and **39** showed monooxygenation in the heterocyclic part of the isatin core and dehydrogenation of the pyrrolidine ring as major processes. Oxygenation of the benzene moiety, which was a major process in electrochemical and liver microsome oxidation in similar 7-unsubstituted isatins,[4] was not observed. The methodological

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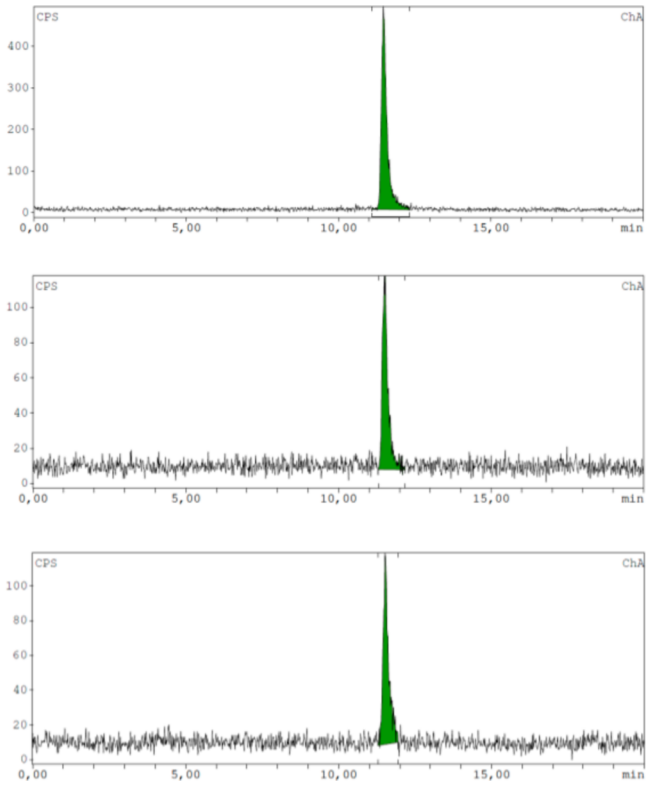

Figure 3. Radio-HPLC chromatograms of a typical quality control (QC) of a [^{18}F]**39** batch (top) and of the *in vitro* stability of [^{18}F]**39** (t_R = 11.38−11.67 min) after incubation in human blood serum at 37 °C after 10 min (middle) and 90 min (bottom) measured at analytical HPLC. Radiometabolites or decomposition products were not observed. The full radio-HPLC chromatograms are attached to the SI (Figure S3−S6).

developments and detailed results of this study will be reported elsewhere.[34]

3. CONCLUSION

A complete series of new 7-halogenated and N-alkylated isatin sulfonamides was synthesized by direct electrophilic aromatic substitution (for iodine and bromine) or starting from commercially available 7-halogenated isatins (for chlorine and fluorine). Inhibitor potency studies of these compounds suggest that the binding pockets readily accommodate both the 7-halogen substituents and aliphatic side chains (methyl to *n*-butyl) as well as some analogues fluorinated in the terminal position (3-fluoropropyl and 4-fluorobutyl) at nitrogen in the 1-position (interacting with the S_1 pocket). Most of these compounds exhibited excellent binding potencies toward caspases-3 and -7 in the nanomolar range, similar to the earlier reported parent compounds with hydrogen in the 7-position. Although attachment of halogens at this position did increase the inhibitory activities only in some cases, these compounds provide the possibility to be labeled with positron emitters (fluorine-18 and iodine-124) and gamma emitters (iodine-123 and -131) for PET and SPECT imaging of apoptosis. Compounds **12**−**17**, **23**, **24**, **33**, **38**, and **39** were discovered to be appropriate candidates for these purposes. Moreover, the fluoroalkyl groups at nitrogen might provide another position for fluorine-18 labeling. Furthermore, the 7-halogenated isatin compounds are expected to resist a potential cytochrome P450 metabolic hydroxylation at this position, and hence, an improved *in vivo* stability is predicted. The *in vitro* metabolic stability of compound [^{18}F]**39** prepared by ^{19}F/^{18}F isotope exchange revealed the resistance to the hydrolytic metabolism after incubation in human blood serum at 37 °C for at least 90 min.

4. EXPERIMENTAL SECTION

4.1. Materials and Methods. All reagents and solvents for reactions were analytical grade and were used as delivered without further purification. 1-Bromo-4-fluorobutane was purchased from Apollo Scientific. 1-Bromo-3-fluoropropane was prepared by treating 3-bromopropanol with DAST in dry THF. 4-Bromobutyl pivalate was obtained by reaction of 4-bromobutanol and pivaloyl chloride in the presence of TEA in dry DCM.[8] The lead compound (*S*)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) was synthesized using the protocol published by Lee et al.[7] For reactions under anhydrous conditions, the glassware was heated under vacuum and flushed with argon gas prior to use. The reactions were performed under argon atmosphere. To characterize the synthesized compounds, melting points (if the products were solid at r.t.), NMR, and ESI-MS were exploited. ^{1}H NMR (300 and 400 MHz), ^{13}C NMR (75 and 100 MHz), and ^{19}F NMR (282 MHz) spectra were recorded in $CDCl_3$, CD_3CN, or DMSO-d_6 with TMS and $CHCl_3$ as the internal standards for ^{1}H NMR, and $CDCl_3$, CD_3CN, or DMSO-d_6 for ^{13}C NMR, and $CFCl_3$ for ^{19}F NMR. DEPT and two-dimensional NMR techniques (COSY, HMQC, and HMBC) were used to assign the signals of some complex structures. All chemical shifts were indicated in ppm. Exact mass analyses were recorded with a MicroTOF apparatus. All spectroscopic and analytical investigations were performed by staff members of Organisch-Chemisches Institut, Westfälische Wilhelms-Universität Münster. Thin layer chromatography (TLC) analyses were performed on silica coated aluminum foils (Silica Gel 60 F_{254}) with 0.02 mm layer thickness from Merck. Silica gel (60−120 mesh) used for column chromatography was obtained from Merck. The purity of all compounds tested *in vitro* or *in vivo* was proved by HPLC to be >95%. Reactions under microwave irradiation were performed in sealed glass vessels using a CEM Discovery microwave machine.

4.2. General Procedure for N-Alkylation using Microwave Irradiation. In a microwave compatible glass vessel, a stirred solution of isatin sulfonamides (1 equiv) dissolved in dry DMF (3−5 mL) was treated with 60% NaH (1.5 equiv in mineral oil) or Cs_2CO_3 (2−3 equiv) under argon atmosphere at room temperature. After stirring for 10 min, the yellow mixture turned blue-purple. Methyl iodide or the respective alkyl bromide (3−10 equiv) was added slowly into the blue mixture. Subsequently, the vessel was sealed and the mixture was stirred at 90−100 °C for 2−10 min using microwave irradiation (maximum power = 150 W). Then, the precipitate was removed by filtration through Celite, and the filtrate was concentrated under reduced pressure. Finally, the residue was purified by flash column chromatography to obtain the corresponding final N-substituted isatin sulfonamides.

4.3. (*S*)-7-Iodo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]-isatins. *4.3.1. (S)-7-Iodo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (**10**).* Similar to a literature procedure,[24] a mixture of the lead compound **1**[7] (162 mg, 0.50 mmol, 1.00 equiv), H_5IO_6 (50 mg, 0.22 mmol, 0.44 equiv), and I_2 (102 mg, 0.40 mmol, 0.80 equiv) was treated with a solution of concentrated H_2SO_4 (0.70 mL) and H_2O (0.42 mL) in acetic acid (2.10 mL) at room temperature. The resulting mixture was stirred at 90 °C under microwave irradiation for 10 min. The reaction mixture was allowed to cool down to room temperature, quenched with sat. aq. $Na_2S_2O_3$ (30 mL), and extracted with EtOAc (3 × 30 mL). The combined organic phase was washed with H_2O (20 mL) and brine (20 mL). After removal of the solvent under reduced pressure, the product was purified by flash column chromatography (silica gel, 40% EtOAc in toluene) to obtain a yellow solid (162 mg, 72%). Mp. 195 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.50 (br s, 1H), 8.39 (d, $^4J_{H,H}$ = 1.6 Hz, 1H), 8.03 (d, $^4J_{H,H}$ = 1.3 Hz, 1H), 3.84−3.75 (m, 1H), 3.54 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.47−3.38 (m, 1H), 3.40 (dd, $^2J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 3.7 Hz, 1H), 3.36

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(s, 3H), 3.20−3.12 (m, 1H), 2.00−1.84 (m, 2H), 1.80−1.64 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.0, 157.9, 154.0, 145.0, 136.0, 124.1, 118.7, 78.1, 74.7, 59.4, 59.2, 49.3, 28.8, 24.2 ppm. HRMS (ESI+, MeOH): m/z = 472.9637, $[M + Na]^+$; calcd. 472.9639 for $C_{14}H_{15}IN_2O_5S$ + Na.

4.3.2. (S)-N-Methyl-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (11). Under argon, a stirred solution of (*S*)-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**10**) (25 mg, 55.5 μmol, 1.00 equiv) in dry DMF (3 mL) at 0 °C was treated with 60% NaH (7 mg, 167 μmol, 3.00 eq. in mineral oil). After stirring for 10 min, MeI (10.4 μL, 167 μmol, 3.00 equiv) was added to the stirred mixture at the same temperature. The resulting reaction mixture was stirred further at 0 °C for 1 h and at room temperature for another 30 min. The solvent was completely removed under reduced pressure, and the residue was purified by flash column chromatography (silica gel, 25% EtOAc in toluene) to furnish an orange solid (21 mg, 82%). Mp. 140 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.47 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 7.99 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 3.84−3.75 (m, 1H), 3.73 (s, 3H), 3.54 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.45−3.35 (m, 1H), 3.39 (dd, $^2J_{H,H}$ = 9.6 Hz, $^3J_{H,H}$ = 3.4 Hz, 1H), 3.36 (s, 3H), 3.21−3.10 (m, 1H), 2.00−1.83 (m, 2H), 1.80−1.65 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.2, 158.3, 153.9, 149.0, 135.7, 123.7, 119.6, 74.7, 72.7, 59.4, 59.2, 49.3, 29.9, 28.8, 24.2 ppm. HRMS (ESI+, MeOH): m/z = 486.9786, $[M + Na]^+$; calcd. 486.9795 for $C_{15}H_{17}IN_2O_5S$ + Na.

4.3.3. (S)-N-Ethyl-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (12). (*S*)-7-Iodo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (**10**) (25 mg, 55.5 μmol, 1.00 equiv) was converted to (*S*)-*N*-ethyl-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**12**) by stirring with Cs_2CO_3 (54 mg, 167 μmol, 3.00 equiv) and ethyl bromide (12.4 μL, 167 μmol, 3.00 equiv) in dry DMF (3 mL) at 95 °C for 10 min under microwave irradiation as described in the general procedure (section 4.2). The residue was purified by flash column chromatography (silica gel, 20% EtOAc in toluene) to yield an orange-yellow solid (21 mg, 79%). Mp. 117 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.42 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 7.93 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 4.25 (q, $^3J_{H,H}$ = 7.1 Hz, 2H), 3.78−3.70 (m, 1H), 3.48 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.39−3.31 (m, 1H), 3.34 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 3.3 Hz, 1H), 3.30 (s, 3H), 3.16−3.04 (m, 1H), 1.94−1.79 (m, 2H), 1.75−1.61 (m, 2H), 1.32 (t, $^3J_{H,H}$ = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.5, 158.3, 153.5, 149.3, 135.4, 123.8, 120.0, 74.7, 72.3, 59.4, 59.2, 49.3, 35.9, 28.8, 24.2, 15.0 ppm. HRMS (ESI+, MeOH): m/z = 500.9946, $[M + Na]^+$; calcd. 500.9952 for $C_{16}H_{19}IN_2O_5S$ + Na.

4.3.4. (S)-N-Propyl-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (13). (*S*)-7-Iodo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (**10**) (25 mg, 55.5 μmol, 1.00 equiv) was converted to (*S*)-*N*-propyl-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]-isatin (**13**) by stirring with Cs_2CO_3 (54 mg, 167 μmol, 3.00 equiv) and 1-bromopropane (15 μL, 167 μmol, 3.00 equiv) in dry DMF (3 mL) at 95 °C for 10 min under microwave irradiation as described in the general procedure (section 4.2). The residue was purified by flash column chromatography (silica gel, 20% EtOAc in toluene) to obtain a sticky orange-yellow solid (14 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.47 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 7.99 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 4.15 (t, $^3J_{H,H}$ = 7.7 Hz, 2H), 3.80 (tt, $^3J_{H,H}$ = 7.3 Hz, $^3J_{H,H}$ = 3.8 Hz, 1H), 3.54 (dd, $^2J_{H,H}$ = 9.6 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.46−3.37 (m, 1H), 3.40 (dd, $^2J_{H,H}$ = 9.9 Hz, $^3J_{H,H}$ = 2.9 Hz, 1H), 3.36 (s, 3H), 3.22−3.10 (m, 1H), 2.00−1.85 (m, 2H), 1.81−1.68 (m, 2H), 1.77 (sextet, $^3J_{H,H}$ = 7.5 Hz, 2H), 1.01 (t, $^3J_{H,H}$ = 7.4 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.4, 158.4, 153.7, 149.3, 135.5, 123.8, 119.9, 74.7, 72.4, 59.4, 59.2, 49.3, 42.0, 28.8, 24.2, 23.1, 10.5 ppm. HRMS (ESI+, MeOH): m/z = 515.0108, $[M + Na]^+$; calcd. 515.0108 for $C_{17}H_{21}IN_2O_5S$ + Na.

4.3.5. (S)-N-Butyl-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (14). (*S*)-7-Iodo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (**10**) (80 mg, 178 μmol, 1.00 equiv) was converted to (*S*)-*N*-butyl-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**14**) by stirring with Cs_2CO_3 (117 mg, 355 μmol, 2.00 equiv) and 1-bromobutane (57 μL, 533 μmol, 3.00 equiv) in dry DMF (5 mL) at 95 °C for 10 min under microwave irradiation as described in the general procedure (section 4.2). The residue was purified by flash column chromatography (silica gel, 30% EtOAc in cyclohexane) to obtain a yellow solid (80 mg, 89%). Mp. 99 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.47 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 7.99 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 4.19 (t, $^3J_{H,H}$ = 7.5 Hz, 2H), 3.85−3.74 (m, 1H), 3.54 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.46−3.34 (m, 2H), 3.36 (s, 3H), 3.22−3.10 (m, 1H), 2.01−1.83 (m, 2H), 1.83−1.65 (m, 4H), 1.46 (sextet, $^3J_{H,H}$ = 7.3 Hz, 2H), 0.99 (t, $^3J_{H,H}$ = 7.3 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.4, 158.4, 153.7, 149.3, 135.4, 123.8, 119.9, 74.7, 72.5, 59.3, 59.2, 49.3, 40.5, 31.7, 28.8, 24.2, 19.6, 13.8 ppm. HRMS (ESI+, MeOH): m/z = 529.0252 $[M + Na]^+$, 561.0517 $[M + Na + MeOH]^+$; calcd. 529.0265 for $C_{18}H_{23}IN_2O_5S$ + Na, 561.0527 for $C_{18}H_{23}IN_2O_5S$ + Na + MeOH.

4.3.6. (S)-N-(3-Fluoropropyl)-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (15). (*S*)-7-Iodo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**10**) (80 mg, 178 μmol, 1.00 equiv) was converted to (*S*)-*N*-(3-fluoropropyl)-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**15**) by stirring with Cs_2CO_3 (117 mg, 355 μmol, 2.00 equiv) and 1-bromo-3-fluoropropane (75 mg, 533 μmol, 3.00 equiv) in dry DMF (5 mL) at 95 °C for 10 min under microwave irradiation as described in the general procedure (section 4.2). The residue was purified by flash column chromatography (silica gel, 25% EtOAc in cyclohexane) to obtain a sticky orange-yellow solid (52 mg, 57%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.48 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 8.00 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 4.62 (dt, $^2J_{H,F}$ = 46.9 Hz, $^3J_{H,H}$ = 5.6 Hz, 2H), 4.43 (t, $^3J_{H,H}$ = 7.4 Hz, 2H), 3.85−3.75 (m, 1H), 3.54 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.47−3.34 (m, 2H), 3.37 (s, 3H), 3.23−3.11 (m, 1H), 2.31−2.11 (m, 2H), 2.00−1.84 (m, 2H), 1.84−1.65 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.0, 158.6, 153.3, 149.2, 135.6, 123.9, 120.0, 81.5 (d, $^1J_{C,F}$ = 166.4 Hz), 74.7, 72.6, 59.4, 59.1, 49.3, 37.9 (d, $^3J_{C,F}$ = 4.6 Hz), 30.5 (d, $^2J_{C,F}$ = 19.7 Hz), 28.8, 24.2 ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −221.5 (s, 1F) ppm. HRMS (ESI+, MeOH): m/z = 533.0027 $[M + Na]^+$, 565.0285 $[M + Na + MeOH]^+$; calcd. 533.0014 for $C_{17}H_{20}FIN_2O_5S$ + Na, 565.0276 for $C_{17}H_{20}FIN_2O_5S$ + Na + MeOH.

4.3.7. (S)-N-(4-Fluorobutyl)-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (16). (*S*)-7-Iodo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**10**) (80 mg, 178 μmol, 1.00 equiv) was converted to (*S*)-*N*-(4-fluorobutyl)-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**16**) by stirring with Cs_2CO_3 (117 mg, 355 μmol, 2.00 equiv) and 1-bromo-4-fluorobutane (82 mg, 533 μmol, 3.00 equiv) in dry DMF (5 mL) at 95 °C for 10 min under microwave irradiation as described in the general procedure (section 4.2). The residue was purified by flash column chromatography (silica gel, 10% EtOAc in toluene) to obtain a brown-yellow solid (78 mg, 84%). Mp. 111 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.48 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 8.00 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 4.52 (dt, $^2J_{H,F}$ = 47.4 Hz, $^3J_{H,H}$ = 5.5 Hz, 2H), 4.26 (t, $^3J_{H,H}$ = 7.4 Hz, 2H), 3.85−3.75 (m, 1H), 3.54 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.46−3.33 (m, 2H), 3.36 (s, 3H), 3.22−3.11 (m, 1H), 2.03−1.57 (m, 8H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.2, 158.4, 153.4, 149.3, 135.6, 123.9, 119.9, 83.2 (d, $^1J_{C,F}$ = 165.8 Hz), 74.7, 72.5, 59.4, 59.2, 49.3, 40.2, 28.8, 27.3 (d, $^2J_{C,F}$ = 20.3 Hz), 26.0 (d, $^3J_{C,F}$ = 4.4 Hz), 24.2 ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −217.9 (s, 1F) ppm. HRMS (ESI+, MeOH): m/z = 547.0166 $[M + Na]^+$, 579.0430 $[M + Na + MeOH]^+$; calcd. 547.0170 for $C_{18}H_{22}FIN_2O_5S$ + Na, 579.0432 for $C_{18}H_{22}FIN_2O_5S$ + Na + MeOH.

4.3.8. (S)-N-(4-Hydroxybutyl)-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (17). Under argon, a stirred solution of (*S*)-7-iodo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (**10**) (60 mg, 0.133 mmol, 1.00 equiv) in dry DMF (4 mL) was treated with Cs_2CO_3 (87 mg, 0.267 mmol, 2.00 equiv) at ambient temperature. After stirring for 10 min, the yellow mixture turned to purple-blue and 4-bromobutyl pivalate (63 mg, 0.267 mmol, 2.00 equiv) was added to this mixture. The reaction mixture was stirred in a sealed glass vessel at 100 °C for 10 min using microwave irradiation, and then the solvent was removed completely under reduced pressure. The residue was dissolved in 1,4-dioxane (2 mL),

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treated with 4 M HCl (1 mL), and stirred at 85 °C for 15 h. The resulting mixture was neutralized by dropwise addition of sat. aq. $NaHCO_3$ until no more gas evolved and extracted with EtOAc (3 × 10 mL). The combined organic phase was concentrated under reduced pressure, and the residue was purified by flash column chromatography (silica gel, 70% EtOAc in toluene) to obtain the required product as a yellow wax (31 mg, 45% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.48 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 8.00 (d, $^4J_{H,H}$ = 1.9 Hz, 1H), 4.26 (t, $^3J_{H,H}$ = 7.4 Hz, 2H), 3.85−3.75 (m, 1H), 3.73 (t, $^3J_{H,H}$ = 6.2 Hz, 2H), 3.55 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.47−3.34 (m, 1H), 3.39 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 7.2 Hz, 1H), 3.37 (s, 3H), 3.22−3.11 (m, 1H), 2.02−1.63 (m, 8H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.3, 158.5, 153.5, 149.3, 135.4, 123.8, 120.0, 74.7, 72.6, 62.1, 59.4, 59.2, 49.3, 40.4, 29.2, 28.8, 26.4, 24.2 ppm. HRMS (ESI+, MeOH): m/z = 545.0217 $[M + Na]^+$, 577.0482 $[M + Na + MeOH]^+$; calcd. 545.0214 for $C_{18}H_{23}IN_2O_6S$ + Na, 577.0476 for $C_{18}H_{23}IN_2O_6S$ + Na + MeOH.

4.4. (*S*)-7-Bromo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatins. *4.4.1. (S)-7-Bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**18**).* According to a modified literature procedure,[14] a stirred solution of (*S*)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**)[7] (250 mg, 0.771 mmol, 1.00 equiv) in dry DCM (200 mL) was cooled to 0 °C and conc. H_2SO_4 (30 drops) was added. After stirring for 5 min, the solution was treated slowly with DBH (441 mg, 1.54 mmol, 2.00 equiv) at the same temperature. The resulting solution was stirred at 0 °C for 20 min and refluxed in the dark for 3 h. Then it was allowed to cool to room temperature before water (50 mL) was added. The organic phase was separated and the aq. phase was extracted with DCM (3 × 100 mL). The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 40% EtOAc in toluene) to obtain the title product as a yellow solid (202 mg, 65% yield). Mp. 158 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.74 (s, 1H), 8.17 (d, $^4J_{H,H}$ = 1.6 Hz, 1H), 7.96 (dd, $^4J_{H,H}$ = 1.6 Hz, 1H), 3.78−3.69 (m, 1H), 3.49 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.40−3.30 (m, 1H), 3.33 (dd, $^2J_{H,H}$ = 9.5 Hz, $J_{H,H}$ = 7.2 Hz, 1H), 3.30 (s, 3H), 3.16−3.06 (m, 1H), 1.94−1.78 (m, 2H) 1.74−1.60 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.2, 157.9, 150.8, 139.4, 135.7, 123.4, 118.9, 106.2, 74.7, 59.4, 59.1, 49.3, 28.8, 24.2 ppm. HRMS (ESI+, MeOH): m/z = 424.9776, 426.9753 $[M + Na]^+$; calcd. 424.9777, 426.9757 for $C_{14}H_{15}BrN_2O_5S$ + Na.

*4.4.2. (S)-N-Methyl-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (**19**).* Under argon, a stirred solution of (*S*)-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**18**) (30 mg, 74.4 μmol, 1.00 equiv) in dry DMF (2 mL) was cooled to 0 °C and treated with a 60% NaH dispersion in mineral oil (4.46 mg, 112 μmol, 1.50 equiv). After stirring for 10 min, the solution turned blue-purple and MeI (6.95 μL, 112 μmol, 1.50 equiv) was added dropwise. The reaction mixture was stirred at 0 °C for 30 min and at ambient temperature for 30 min. The solvent was removed completely under reduced pressure, and the crude was purified by flash column chromatography (silica gel, 35% EtOAc in toluene) to furnish the desired product as an orange-yellow solid (25 mg, 81% yield). Mp. 111 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.20 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 7.96 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 3.83−3.75 (m, 1H), 3.70 (s, 3H), 3.53 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.45−3.36 (m, 1H), 3.39 (dd, $^2J_{H,H}$ = 9.9 Hz, $^3J_{H,H}$ = 7.2 Hz, 1H), 3.35 (s, 3H), 3.21−3.11 (m, 1H), 1.99−1.84 (m, 2H), 1.79−1.65 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.1, 158.2, 151.0, 142.2, 135.4, 123.0, 119.9, 104.5, 74.7, 59.4, 59.1, 49.3, 29.9, 28.8, 24.2 ppm. HRMS (ESI+, MeOH): m/z = 438.9941, 440.9913 $[M + Na]^+$, 471.0193, 473.0171 $[M + Na + MeOH]^+$; calcd. 438.9934, 440.9914 for $C_{15}H_{17}BrN_2O_5S$ + Na, 471.0196, 473.0176 for $C_{15}H_{17}BrN_2O_5S$ + Na + MeOH.

*4.4.3. (S)-N-Ethyl-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (**20**).* Under argon, a stirred solution of (*S*)-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**18**) (30 mg, 74.4 μmol, 1.00 equiv) in dry DMF (3 mL) was treated with a 60% NaH dispersion in mineral oil (4.5 mg, 112 μmol, 1.50 equiv). After stirring for 10 min, the yellow solution turned to blue-purple mixture and 1-bromoethane (17 μL, 223 μmol, 3.00 equiv) was added. The reaction mixture was stirred in a sealed glass vessel at 100 °C for 3 min using microwave irradiation. After that the solvent was removed completely under reduced pressure and the residue was purified by flash column chromatography (silica gel, 40% EtOAc in toluene) to obtain a yellow solid (20 mg, 62% yield). Mp. 145 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.22 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 7.98 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 4.26 (q, $^3J_{H,H}$ = 7.1 Hz, 2H), 3.84−3.76 (m, 1H), 3.54 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.46−3.36 (m, 1H), 3.39 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 7.2 Hz, 1H), 3.36 (s, 3H), 3.17 (dt, $^2J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 7.3 Hz, 1H), 2.00−1.84 (m, 2H), 1.81−1.68 (m, 2H), 1.39 (t, $^3J_{H,H}$ = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.5, 158.1, 150.6, 142.5, 135.3, 123.2, 120.2, 103.9, 74.7, 59.4, 59.1, 49.3, 36.9, 28.8, 24.2, 14.9 ppm. HRMS (ESI+, MeOH): m/z = 455.0062 $[M + Na]^+$, 487.0331 $[M + Na + MeOH]^+$; calcd. 455.0070 for $C_{16}H_{19}BrN_2O_5S$ + Na, 487.0332 for $C_{16}H_{19}BrN_2O_5S$ + Na + MeOH.

*4.4.4. (S)-N-Propyl-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (**21**).* Under argon, a stirred solution of (*S*)-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**18**) (45 mg, 0.112 mmol, 1.00 equiv) in dry DMF (3 mL) was treated with anhydrous K_2CO_3 (31 mg, 0.223 mmol, 2.00 equiv). After stirring for 30 min, the yellow solution turned to a purple-blue mixture and then 1-bromopropane (30 μL, 0.335 mmol, 3.00 equiv) was added. The reaction mixture was stirred at room temperature for 4 days (or until the starting material was completely disappeared). The solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography (silica gel, 40% EtOAc in toluene) to yield a sticky red-yellow solid (36 mg, 72% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.22 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 7.99 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 4.15 (t, $^3J_{H,H}$ = 7.8 Hz, 2H), 3.85−3.77 (m, 1H), 3.55 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.48−3.37 (m, 1H), 3.40 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 7.2 Hz, 1H), 3.37 (s, 3H), 3.19 (dt, $^2J_{H,H}$ = 9.9 Hz, $^3J_{H,H}$ = 7.3 Hz, 1H), 2.01−1.87 (m, 2H), 1.85−1.69 (m, 2H), 1.80 (sextet, $^3J_{H,H}$ = 7.6 Hz, 2H), 1.01 (t, $^3J_{H,H}$ = 7.4 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.4, 158.3, 150.7, 142.5, 135.3, 123.2, 120.2, 104.0, 74.7, 59.4, 59.1, 49.3, 43.1, 28.9, 24.2, 23.0, 10.8 ppm. HRMS (ESI+, MeOH): m/z = 467.0245, 469.0232 $[M + Na]^+$; calcd. 467.0247, 469.0227 for $C_{17}H_{21}BrN_2O_5S$ + Na.

*4.4.5. (S)-N-Butyl-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (**22**).* (*S*)-7-Bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**18**) (30 mg, 74.4 μmol, 1.00 equiv) was converted to (*S*)-*N*-butyl-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**22**) by stirring with Cs_2CO_3 (73 mg, 0.223 mmol, 3.00 equiv) and 1-bromobutane (24 μL, 0.223 mmol, 3.00 equiv) in dry DMF (3 mL) at 100 °C for 3 min under microwave irradiation as described in the general procedure (section 4.2). The residue was purified by flash column chromatography (silica gel, 20% EtOAc in toluene) to obtain the required product as a yellow solid (20 mg, 59% yield). Mp. 104 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 7.91 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 4.11 (t, $^3J_{H,H}$ = 7.6 Hz, 2H), 3.78−3.70 (m, 1H), 3.48 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.40−3.31 (m, 1H), 3.33 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 7.2 Hz, 1H), 3.30 (s, 3H), 3.11 (dt, $^2J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 7.2 Hz, 1H), 1.94−1.80 (m, 2H), 1.76−1.62 (m, 2H), 1.73−1.62 (m, 2H), 1.37 (sextet, $^3J_{H,H}$ = 7.5 Hz, 2H), 0.92 (t, $^3J_{H,H}$ = 7.4 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.4, 158.2, 150.7, 142.5, 135.3, 123.2, 120.3, 104.0, 74.7, 59.4, 59.1, 49.3, 41.5, 31.6, 28.8, 24.2, 19.8, 13.7 ppm. HRMS (ESI+, MeOH): m/z = 483.0370 $[M + Na]^+$, 515.0633 $[M + Na + MeOH]^+$; calcd. 483.0383 for $C_{18}H_{23}BrN_2O_5S$ + Na, 515.0646 for $C_{18}H_{23}BrN_2O_5S$ + Na + MeOH.

*4.4.6. (S)-N-(3-Fluoropropyl)-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**23**).* (*S*)-7-Bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**18**) (50 mg, 0.124 mmol, 1.00 equiv) was converted to (*S*)-*N*-(3-fluoropropyl)-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**23**) by stirring with Cs_2CO_3 (80 mg, 0.248 mmol, 2.00 equiv) and 1-bromo-3-fluoropropane (52 mg, 0.372 mmol, 3.00 equiv) in dry DMF (4 mL) at 90 °C for 5 min under microwave irradiation as described in the general procedure (section 4.2). The residue was purified by flash column chromatography (silica gel, 20% EtOAc in toluene) to obtain

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the required product as an orange-yellow oil (37 mg, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.24 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 8.00 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 4.61 (dt, $^2J_{H,F}$ = 46.9 Hz, $^3J_{H,H}$ = 5.5 Hz, 2H), 4.41 (t, $^3J_{H,H}$ = 7.3 Hz, 2H), 3.86−3.78 (m, 1H), 3.55 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.47−3.39 (m, 1H), 3.40 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 7.1 Hz, 1H), 3.36 (s, 3H), 3.19 (dt, $^2J_{H,H}$ = 9.1 Hz, $^3J_{H,H}$ = 7.0 Hz, 1H), 2.29−2.13 (m, 2H), 2.02−1.85 (m, 2H), 1.83−1.68 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.0, 158.4, 150.4, 142.5, 135.7, 123.3, 120.3, 104.0, 81.6 (d, $^1J_{C,F}$ = 166.4 Hz), 74.7, 59.4, 59.1, 49.3, 38.9 (d, $^3J_{C,F}$ = 4.4 Hz), 30.5 (d, $^2J_{C,F}$ = 19.7 Hz), 28.9, 24.2 ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −221.5 (s, 1F) ppm. HRMS (ESI+, MeOH): m/z = 485.0148, 487.0130 $[M + Na]^+$, 517.0413, 519.0391 $[M + Na + MeOH]^+$; calcd. 485.0153, 487.0133 for $C_{17}H_{20}BrFN_2O_5S$ + Na, 517.0415, 519.0395 for $C_{17}H_{20}BrFN_2O_5S$ + Na + MeOH.

4.4.7. (S)-N-(4-Fluorobutyl)-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (24). (*S*)-7-Bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**18**) (50 mg, 0.124 mmol, 1.00 equiv) was converted to (*S*)-*N*-(4-fluorobutyl)-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**24**) by stirring with Cs_2CO_3 (80 mg, 0.248 mmol, 2.00 equiv) and 1-bromo-4-fluorobutane (40 μL, 0.372 mmol, 3.00 equiv) in dry DMF (3 mL) at 100 °C for 2 min under microwave irradiation as described in the general procedure (section 4.2). The residue was purified by flash column chromatography (silica gel, 30% EtOAc in toluene) to obtain the required product as a yellow solid (56 mg, 95% yield). Mp. 114 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.29 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 8.05 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 4.58 (dt, $^2J_{H,F}$ = 47.4 Hz, $^3J_{H,H}$ = 5.7 Hz, 2H), 4.31 (t, $^3J_{H,H}$ = 7.6 Hz, 2H), 3.92−3.83 (m, 1H), 3.61 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.53−3.45 (m, 1H), 3.46 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 7.2 Hz, 1H), 3.43 (s, 3H), 3.25 (dt, $^2J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 7.1 Hz, 1H), 2.05−1.92 (m, 4H), 1.95−1.76 (m, 2H), 1.88−1.76 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.2, 158.3, 150.5, 142.5, 135.5, 123.2, 120.3, 104.0, 83.2 (d, $^1J_{C,F}$ = 165.8 Hz), 74.7, 59.4, 59.1, 49.3, 41.2, 28.8, 27.5 (d, $^2J_{C,F}$ = 20.3 Hz), 26.0 (d, $^3J_{C,F}$ = 4.2 Hz), 24.2 ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −218.6 (s, 1F) ppm. HRMS (ESI+, MeOH): m/z = 499.0317, 501.0302 $[M + Na]^+$, 531.0578, 533.0558 $[M + Na + MeOH]^+$; calcd. 499.0309, 501.0289 for $C_{18}H_{22}BrFN_2O_5S$ + Na, 531.0571, 533.0551 for $C_{18}H_{22}BrFN_2O_5S$ + Na + MeOH.

4.4.8. (S)-N-(4-Hydroxybutyl)-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (25). Under argon, a stirred solution of (*S*)-7-bromo-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (**18**) (50 mg, 0.124 mmol, 1.00 equiv) in dry DMF (4 mL) was treated with Cs_2CO_3 (80 mg, 0.248 mmol, 2.00 equiv) at ambient temperature. After stirring for 10 min, the yellow mixture turned to purple-blue and 4-bromobutyl pivalate (59 mg, 0.248 mmol, 2.00 equiv) was added. This mixture was stirred in a sealed glass vessel at 100 °C for 8 min using microwave irradiation, and then the solvent was removed completely under reduced pressure. The residue was dissolved in 1,4-dioxane (1.5 mL), treated with 4 M HCl (0.80 mL), and stirred at 85 °C for 15 h. The resulting mixture was neutralized by dropwise addition of sat. aq. $NaHCO_3$ until no more gas evolved and extracted with EtOAc (3 × 5 mL). The combined organic phase was concentrated under reduced pressure, and the residue was purified by flash column chromatography (silica gel, 90% EtOAc in toluene) to obtain the required product as a yellow gummy-solid (25 mg, 42% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.22 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 7.98 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 4.24 (t, $^3J_{H,H}$ = 7.6 Hz, 2H), 3.85−3.77 (m, 1H), 3.72 (t, $^3J_{H,H}$ = 6.3 Hz, 2H), 3.55 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.48−3.37 (m, 1H), 3.40 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 7.2 Hz, 1H), 3.37 (s, 3H), 3.18 (dt, $^2J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 7.2 Hz, 1H), 1.99−1.90 (m, 2H), 1.94−1.84 (m, 2H), 1.82−1.72 (m, 2H), 1.73−1.63 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.3, 158.3, 150.6, 142.5, 135.4, 123.2, 120.3, 104.0, 74.7, 62.2, 59.4, 59.1, 49.3, 41.5, 29.4, 28.9, 26.4, 24.2 ppm. HRMS (ESI+, MeOH): m/z = 497.0359, 499.0345 $[M + Na]^+$, 529.0611, 531.0596 $[M + Na + MeOH]^+$; calcd. 497.0352, 499.0332 for $C_{18}H_{23}BrN_2O_6S$ + Na, 529.0615, 531.0595 for $C_{18}H_{23}BrN_2O_6S$ + Na + MeOH.

4.5. (*S*)-7-Chloro-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatins. *4.5.1. Sodium 7-chloro-2,3-dioxoindoline-5-sulfonate (29).* A dried round-bottom flask containing oleum (3.1 mL) was cooled to −20 °C, and 7-chloroisatin (**28**) (2.00 g, 11.0 mmol, 1.00 equiv) was slowly added in portions over 10 min. The suspension was allowed to stir below −10 °C for 30 min and then was heated to 70 °C and further stirred for 45 min. The reaction mixture was cooled and poured on ice (3 g). The mixture was neutralized using 20% aq. NaOH solution (30−40 mL) to approximately pH 7.5 and completely evaporated under reduced pressure. The resulting dry crude product was suspended in MeOH (30 mL) and stirred for 30 min. The obtained suspension was filtered and the solid was washed with MeOH (30 mL). The combined filtrate was evaporated under reduced pressure to afford an orange-red solid (3.09 g, 99% yield). The product was used in the next step without further purification. Mp. >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.55 (s, 1H), 7.78 (d, $^4J_{H,H}$ = 1.5 Hz, 1H), 7.57 (d, $^4J_{H,H}$ = 1.5 Hz, 1H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 183.3, 160.0, 147.8, 144.0, 134.1, 120.0, 119.4, 115.5 ppm. HRMS (ESI+, MeOH): m/z = 305.9206 $[M + Na]^+$, 337.9480 $[M + Na + MeOH]^+$; calcd. 305.9210 for $C_8H_3ClNO_5SNa$ + Na, 337.9473 for $C_8H_3ClNO_5SNa$ + Na + MeOH. HRMS (ESI-, MeOH): m/z = 259.9429 $[M\text{-}Na]^-$, 291.9691 $[M - Na + MeOH]^-$; calcd. 259.9426 for $C_8H_3ClNO_5S$−Na, 291.9688 for $C_8H_3ClNO_5S$ − Na + MeOH.

4.5.2. 5,7-Dichlorosulfonyl Isatin (30). To a suspension of sodium 7-chloro-2,3-dioxoindoline-5-sulfonate (**29**) in sulfolane (14.5 mL) was added $POCl_3$ (2.9 mL, 31.7 mmol, 4.50 equiv), dropwise at 40 °C. The reaction mixture was stirred at 60 °C for 4 h followed by cooling to 0 °C and very slow quenching with ice−water (50 mL). The precipitate was filtered and washed with cold water (20−30 mL). The resulting solid was suspended in EtOAc (30 mL) and washed with water (3 × 10 mL) and brine (20 mL). The orange solution was dried over $MgSO_4$ and concentrated under reduced pressure to obtain a yellow solid (1.58 g, 80% yield). The product was used in the next step without further purification. Mp. 178 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.52 (s, 1H), 7.72 (d, $^4J_{H,H}$ = 1.5 Hz, 1H), 7.51 (d, $^4J_{H,H}$ = 1.5 Hz, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 183.4, 160.2, 147.9, 144.2, 134.1, 120.0, 119.6, 115.7 ppm. HRMS (ESI+, MeOH): m/z = 329.9809 $[M - Cl + MeO + Na + MeOH]$+; calcd. 329.9810 for $C_8H_3Cl_2NO_4S$−Cl + MeO + Na + MeOH.

Note: In the ESI-MS measurement MeOH was used as the solvent, which reacted with product **30** to form the corresponding methyl sulfonate. The latter compound was detected in the ESI-MS spectrum.

4.5.3. (S)-7-Chloro-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]-isatin (31). Under argon, a stirred solution of *N*-Boc-2-methoxymethylpyrrolidine (699 mg, 3.25 mmol, 1.00 equiv) in dry DCM (17.5 mL) was cooled to 0 °C and treated slowly with TFA (2.40 mL, 32.5 mmol, 10.00 equiv). The resulting solution was stirred at 0 °C for 30 min and at ambient temperature for 2 h. Then, the solution was poured into cooled 10% aq. NaOH (50 mL) and extracted with DCM (3 × 50 mL). The solution was dried over $MgSO_4$ and concentrated under reduced pressure to obtain the deprotected pyrrolidine as a colorless oil. A solution of this pyrrolidine and DIPEA (1.13 mL, 6.49 mmol, 2.00 equiv) in $CHCl_3$ (10 mL) was added dropwise to a stirred solution of 5,7-dichlorosulfonyl isatin (**30**) (1.00 g, 3.57 mmol, 1.10 equiv) in $CHCl_3$/THF (1:1, 46 mL) at room temperature. It was stirred at this temperature for 1 h, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 60% EtOAc in cyclohexane) to furnish a yellow solid (700 mg, 60% yield). Mp. 172 °C. ^{1}H NMR (300 MHz, CD_3CN): δ = 9.64 (br s, 1H), 8.04 (d, $^4J_{H,H}$ = 1.7 Hz, 1H), 7.84 (d, $^4J_{H,H}$ = 1.7 Hz, 1H), 3.80−3.66 (m, 1H), 3.46 (dd, $^2J_{H,H}$ = 9.6 Hz, $^3J_{H,H}$ = 4.1 Hz, 1H), 3.38−3.27 (m, 1H), 3.36 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 7.1 Hz, 1H), 3.30 (s, 3H), 3.23−3.11 (m, 1H), 1.90−1.47 (m, 4H) ppm. ^{13}C NMR (75 MHz, CD_3CN): δ = 183.2, 159.6, 151.6, 137.2, 134.7, 123.1, 120.4, 118.9, 75.7, 60.4, 59.3, 50.3, 29.3, 24.8 ppm. HRMS (ESI+, MeOH): m/z = 381.0289 $[M + Na]^+$, 413.0546 $[M + Na + MeOH]^+$; calcd. 381.0282 for $C_{14}H_{15}ClN_2O_5S$ + Na, 413.0545 for $C_{14}H_{15}ClN_2O_5S$ + Na + MeOH.

4.5.4. (S)-N-Butyl-7-chloro-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (32). (*S*)-7-Chloro-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**31**) (100 mg, 0.279

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mmol, 1.00 equiv) was converted to (*S*)-*N*-butyl-7-chloro-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**32**) by stirring with Cs_2CO_3 (182 mg, 0.557 mmol, 2.00 equiv) and 1-bromobutane (298 μL, 2.79 mmol, 10.00 equiv) in dry DMF (4 mL) at 95 °C for 10 min under microwave irradiation as described in the general procedure (section 4.2). The crude product was purified by flash column chromatography (silica gel, 30% EtOAc in cyclohexane) to obtain a yellow solid (112 mg, 97% yield). Mp. 103 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.03 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 7.93 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 4.15 (t, $^3J_{H,H}$ = 7.4 Hz, 2H), 3.85−3.73 (m, 1H), 3.55 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.48−3.34 (m, 1H), 3.43−3.35 (m, 1H), 3.36 (s, 3H), 3.24−3.10 (m, 1H), 2.04−1.64 (m, 4H), 1.84−1.64 (m, 2H), 1.43 (sextet, $^3J_{H,H}$ = 7.3 Hz, 2H), 0.99 (t, $^3J_{H,H}$ = 7.3 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.5, 158.1, 149.3, 139.2, 134.9, 122.6, 120.0, 117.5, 74.7, 59.3, 59.1, 49.3, 42.0, 31.6, 28.8, 24.2, 19.8, 13.7 ppm. HRMS (ESI+, MeOH): *m*/*z* = 437.0913 $[M + Na]^+$, 469.1177 $[M + Na + MeOH]^+$; calcd. 437.0908 for $C_{18}H_{23}ClN_2O_5S$ + Na, 469.1171 for $C_{18}H_{23}ClN_2O_5S$ + Na + MeOH.

4.5.5. (S)-N-(4-Fluorobutyl)-7-chloro-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (33). (*S*)-7-Chloro-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**31**) (100 mg, 0.279 mmol, 1.00 equiv) was converted to (*S*)-*N*-(4-fluorobutyl)-7-chloro-5-[1-(2-methoxymethyl-pyrrolidinyl)sulfonyl]isatin (**33**) by stirring with Cs_2CO_3 (182 mg, 0.557 mmol, 2.00 equiv) and 1-bromo-4-fluorobutane (299 μL, 2.79 mmol, 10.00 equiv) in dry DMF (4 mL) at 95 °C for 10 min under microwave irradiation as described in the general procedure (section 4.2). The crude product was purified by flash column chromatography (silica gel, 50% EtOAc in cyclohexane) to obtain a yellow solid (115 mg, 95% yield). Mp. 115 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 7.93 (d, $^4J_{H,H}$ = 1.8 Hz, 1H), 4.51 (dt, $^2J_{H,F}$ = 47.4 Hz, $^3J_{H,H}$ = 5.7 Hz, 2H), 4.21 (t, $^3J_{H,H}$ = 7.4 Hz, 2H), 3.85−3.75 (m, 1H), 3.54 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 3.49−3.34 (m, 1H), 3.39 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 7.3 Hz, 1H), 3.36 (s, 3H), 3.24−3.12 (m, 1H), 2.04−1.64 (m, 8H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.3, 158.2, 149.0, 139.2, 135.2, 122.7, 120.1, 117.5, 83.3 (d, $^1J_{C,F}$ = 165.6 Hz), 74.7, 59.4, 59.1, 49.3, 41.7, 28.8, 27.5 (d, $^2J_{C,F}$ = 20.2 Hz), 26.0 (d, $^3J_{C,F}$ = 4.2 Hz), 24.2 ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −219.1 (s, 1F) ppm. HRMS (ESI+, MeOH): *m*/*z* = 455.0821 $[M + Na]^+$, 487.1080 $[M + Na + MeOH]^+$; calcd. 455.0814 for $C_{18}H_{22}ClFN_2O_5S$ + Na, 487.1076 for $C_{18}H_{22}ClFN_2O_5S$ + Na + MeOH.

4.6. (S)-7-Fluoro-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatins. *4.6.1. Sodium 7-Fluoroisatin-5-sulfonate (35).* Oleum (4.0 mL) was cooled to −20 °C and treated portionwise with 7-fluoroisatin (**34**) (2.00 g, 12.1 mmol, 1.00 equiv) over 10 min. The suspension was stirred vigorously below −10 °C for 45 min and then at 70 °C for 1 h. After cooling to r.t., the mixture was neutralized with 20% aq. NaOH to approximately pH 7.5 and concentrated to dryness under reduced pressure. The residue was suspended in MeOH (40 mL) and stirred for 30 min. The precipitate was filtered and washed with MeOH (10 mL). The combined filtrate was evaporated to obtain a red-colored solid (quantitative). Mp. >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.65 (br s, 1H), 7.64 (dd, $^3J_{H,F}$ = 10.0 Hz, $^4J_{H,H}$ = 1.3 Hz, 1H), 7.50 (d, $^4J_{H,H}$ = 1.4 Hz, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 182.8, 159.4, 146.1 (d, $^1J_{C,F}$ = 247.4 Hz), 143.8 (d, $^3J_{C,F}$ = 3.0 Hz), 137.4 (d, $^2J_{C,F}$ = 13.7 Hz), 121.4 (d, $^2J_{C,F}$ = 18.8 Hz), 119.7 (d, $^3J_{C,F}$ = 3.9 Hz), 117.2 (d, $^4J_{C,F}$ = 3.1) ppm. ^{19}F NMR (282 MHz, DMSO-d_6): δ = −132.9 (s, 1F) ppm. HRMS (ESI+, MeOH): *m*/*z* = 289.9511 $[M + Na]^+$, 321.9769 $[M + Na + MeOH]^+$; calcd. 289.9511 for $C_8H_3FNO_5SNa$ + Na, 321.9774 for $C_8H_3FNO_5SNa$ + Na + CH_3OH. HRMS (ESI-, MeOH): *m*/*z* = 243.9725 $[M - Na]^-$, 275.9988 $[M - Na + MeOH]^-$, calcd. 243.9710 for $C_8H_3FNO_5S$, 275.9973 for $C_8H_3FNO_5S$ + CH_3OH.

4.6.2. 5-Chlorosulfonyl-7-fluoroisatin (36). A stirred suspension of sodium 7-fluoroisatin-5-sulfonate (**35**) (2.00 g, 7.49 mmol, 1.00 equiv) in sulfolane (15 mL) was treated dropwise with $POCl_3$ (3.07 mL, 33.7 mmol, 4.50 equiv) at 40 °C over 10 min. The reaction mixture was then heated at 60 °C for 4 h followed by cooling to 0 °C and very slow quenching with ice−water (30 mL). The mixture was stirred at 0 °C for 30 min, filtered, and washed with a small amount of water. The resulting crude product was suspended in EtOAc (30 mL) and warmed to 40 °C (for complete dissolution). The solution was cooled to ambient temperature and washed with water (3 × 10 mL) and brine (10 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness to yield a yellow-brown solid (1.30 g, 66% yield). Mp. 199 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.68 (s, 1H), 7.73 (dd, $^3J_{H,F}$ = 9.8 Hz, $^4J_{H,H}$ = 1.4 Hz, 1H), 7.70−7.66 (m, 1H) ppm. ^{13}C NMR (75 MHz, CDCl3): δ = 183.0, 159.2, 146.5 (d, $^1J_{C,F}$ = 248.9 Hz), 143.5 (d, $^3J_{C,F}$ = 3.1 Hz), 138.1 (d, $^2J_{C,F}$ = 13.9 Hz), 122.3 (d, $^2J_{C,F}$ = 19.1 Hz), 119.2 (d, $^3J_{C,F}$ = 4.0 Hz), 118.0 (d, $^4J_{C,F}$ = 3.3 Hz) ppm. ^{19}F NMR (282 MHz, DMSO-d_6): δ = −133.0 (s, 1F) ppm. HRMS (ESI+, MeOH): *m*/*z* = 281.9852 $[M + Na]^+$, 314.0110 $[M + Na + MeOH]^+$; calcd. 281.9848 for $C_9H_6FNO_5S$ + Na, 314.0111 for $C_9H_6FNO_5S$ + Na + MeOH.

Note: 5-Chlorosulfonyl-7-fluoroisatin was not stable in MeOH under the ESI-MS measurement. Methyl 7-fluoroisatin-5-sulfonate was exclusively observed in the MS-spectrum.

4.6.3. (S)-7-Fluoro-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]-isatin (37). Under argon, a stirred solution of *N*-Boc-2-methoxymethylpyrrolidine (200 mg, 0.929 mmol, 1.00 equiv) in dry DCM (5 mL) was treated dropwise with TFA (690 μL, 9.29 mmol, 10.00 equiv) at 0 °C. The solution was stirred at 0 °C for 30 min and at ambient temperature for 2 h. Then, the resulting mixture was poured into ice-cooled 10% aq. NaOH (25 mL) and extracted with DCM (3 × 25 mL). The combined organic phase was dried over $MgSO_4$, and solvent was removed under reduced pressure. The residue was taken up in $CHCl_3$ (3.0 mL) and treated with DIPEA (324 μL, 1.86 mmol, 2.00 equiv). The above solution was added dropwise to a stirred solution of 5-chlorosulfonyl-7-fluoroisatin (**36**) (367 mg, 1.39 mmol, 1.50 equiv) in $CHCl_3$/THF (1:1, 18 mL) at room temperature. The reaction mixture was stirred at this temperature for 1 h and then concentrated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 65% EtOAc in cyclohexane) to obtain a yellow solid (138 mg, 43% yield). Mp. 174 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.99 (br s, 1H), 8.01 (dd, $^3J_{H,F}$ = 9.5 Hz, $^4J_{H,H}$ = 1.6 Hz, 1H), 7.66 (d, $^4J_{H,H}$ = 1.6 Hz, 1H), 3.81−3.68 (m, 1H), 3.44 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 3.9 Hz, 1H), 3.37−3.30 (m, 1H), 3.31−3.25 (m, 1H), 3.28 (s, 3H), 3.14 (dt, $^2J_{H,H}$ = 10.2 Hz, $^3J_{H,H}$ = 7.1 Hz, 1H), 1.90−1.65 (m, 2H), 1.65−1.43 (m, 2H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 181.7, 159.2, 146.7 (d, $^1J_{C,F}$ = 250.3 Hz), 141.2 (d, $^2J_{C,F}$ = 13.6 Hz), 131.5 (d, $^3J_{C,F}$ = 4.0 Hz), 123.0 (d, $^2J_{C,F}$ = 20.2 Hz), 120.6 (d, $^3J_{C,F}$ = 4.5 Hz), 119.0 (d, $^4J_{C,F}$ = 3.1 Hz), 74.4, 58.7, 58.4, 48.9, 28.1, 23.5 ppm. ^{19}F NMR (282 MHz, DMSO-d_6): δ = −130.5 (s, 1F) ppm. HRMS (ESI+, MeOH): *m*/*z* = 365.0570 $[M + Na]^+$, 397.0830 $[M + Na + MeOH]^+$; calcd. 365.0578 for $C_{14}H_{15}FN_2O_5S$ + Na, 397.0840 for $C_{14}H_{15}FN_2O_5S$ + Na + MeOH.

4.6.4. (S)-N-Butyl-7-fluoro-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (38). (*S*)-7-Fluoro-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**37**) (100 mg, 0.292 mmol, 1.00 equiv) was converted to (*S*)-*N*-butyl-7-fluoro-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**38**) by stirring with Cs_2CO_3 (190 mg, 0.584 mmol, 2.00 equiv) and 1-bromobutane (313 μL, 2.92 mmol, 10.00 equiv) in dry DMF (4.0 mL) at 95 °C for 10 min under microwave irradiation as described in the general procedure (section 4.2). The residue was purified by flash column chromatography (silica gel, 40% EtOAc in cyclohexane) to afford a yellow waxy solid (95 mg, 82% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.85 (dd, $^3J_{H,F}$ = 11.2 Hz, $^4J_{H,H}$ = 1.5 Hz, 1H), 7.85 (d, $^4J_{H,H}$ = 1.5 Hz, 1H), 3.91 (td, $^3J_{H,H}$ = 7.4 Hz, $^5J_{H,F}$ = 1.6 Hz, 2H), 3.82−3.73 (m, 1H), 3.56 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 3.9 Hz, 1H), 3.48−3.37 (m, 1H), 3.43−3.35 (m, 1H), 3.36 (s, 3H), 3.22−3.10 (m, 1H), 2.03−1.64 (m, 4H), 1.81−1.64 (m, 2H), 1.42 (sextet, $^3J_{H,H}$ = 7.3 Hz, 2H), 0.98 (t, $^3J_{H,H}$ = 7.3 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.4 (d, $^4J_{C,F}$ = 3.0 Hz), 157.5, 147.2 (d, $^1J_{C,F}$ = 253.1 Hz), 140.5 (d, $^2J_{C,F}$ = 8.8 Hz), 134.7 (d, $^3J_{C,F}$ = 4.1 Hz), 125.5 (d, $^2J_{C,F}$ = 22.8 Hz), 120.3 (d, $^4J_{C,F}$ = 3.4 Hz), 120.0 (d, $^3J_{C,F}$ = 2.8 Hz), 74.7, 59.3, 59.1, 49.4, 42.7 (d, $^4J_{C,F}$ = 4.8 Hz), 30.8 (d, $^5J_{C,F}$ = 2.6 Hz), 28.8, 24.2, 19.9, 13.6 ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −130.7 (s, 1F) ppm. HRMS (ESI+, MeOH): *m*/*z* = 421.1197 $[M + Na]^+$, 453.1456 $[M + Na + MeOH]^+$;

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calcd. 421.1204 for $C_{18}H_{23}FN_2O_5S$ + Na, 453.1466 for $C_{18}H_{23}FN_2O_5S$ + Na + MeOH.

4.6.5. (S)-N-(4-Fluorobutyl)-7-fluoro-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (39). (*S*)-7-Fluoro-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**37**) (100 mg, 0.292 mmol, 1.00 equiv) was converted to (*S*)-*N*-(4-fluorobutyl)-7-fluoro-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**39**) by stirring with Cs_2CO_3 (190 mg, 0.584 mmol, 2.00 equiv) and 1-bromo-4-fluorobutane (314 μL, 2.92 mmol, 10.00 equiv) in dry DMF (4.0 mL) at 95 °C for 10 min under microwave irradiation as described in the general procedure (section 4.2). The residue was purified by flash column chromatography (silica gel, 50% EtOAc in cyclohexane) to afford a yellow waxy solid (95 mg, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.86 (dd, $^3J_{H,F}$ = 11.1 Hz, $^4J_{H,H}$ = 1.5 Hz, 1H), 7.85 (d, $^4J_{H,H}$ = 1.5 Hz, 1H), 4.50 (dt, $^2J_{H,F}$ = 47.6 Hz, $^3J_{H,H}$ = 5.6 Hz, 2H), 3.97 (t, $^3J_{H,H}$ = 6.7 Hz, 2H), 3.81−3.73 (m, 1H), 3.55 (dd, $^2J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 3.9 Hz, 1H), 3.47−3.38 (m, 1H), 3.40 (dd, $^2J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 2.5 Hz, 1H), 3.36 (s, 3H), 3.21−3.12 (m, 1H), 2.02−1.64 (m, 8H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.2 (d, $^4J_{C,F}$ = 3.1 Hz), 157.5, 147.2 (d, $^1J_{C,F}$ = 252.9 Hz), 140.3 (d, $^2J_{C,F}$ = 8.8 Hz), 135.0 (d, $^3J_{C,F}$ = 4.1 Hz), 125.5 (d, $^2J_{C,F}$ = 22.7 Hz), 120.3 (d, $^4J_{C,F}$ = 3.4 Hz), 120.0 (d, $^3J_{C,F}$ = 2.8 Hz), 83.2 (d, $^1J_{C,F}$ = 165.5 Hz), 74.7, 59.4, 59.1, 42.4 (d, $^4J_{C,F}$ = 4.8 Hz), 49.3, 28.8, 27.5 (d, $^2J_{C,F}$ = 20.2 Hz), 25.0 (dd, $^3J_{C,F}$ = 4.3 Hz, $^5J_{C,F}$ = 2.7 Hz), 24.2 ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −131.0 (s, 1F), −219.5 (s, 1F, CH_2F) ppm. HRMS (ESI +, MeOH): *m*/*z* = 439.1108 $[M + Na]^+$, 471.1368 $[M + Na + MeOH]^+$; calcd. 439.1110 for $C_{18}H_{22}F_2N_2O_5S$ + Na, 471.1372 for $C_{18}H_{22}F_2N_2O_5S$ + Na + MeOH.

4.7. *In Vitro* Enzyme Inhibition Assay. The inhibitory activities toward caspases of the target isatin sulfonamides **10**−**25**, **31**−**33**, and **37**−**39** are determined as IC_{50} values. The recombinant human caspases-1, -3, -6, and -7, including their peptide-specific substrate Ac-YVAD-AMC (Ac-Tyr-Val-Ala-Asp-AMC) for caspase-1, Ac-DEVD-AMC (Ac-Asp-Glu-Val-Asp-AMC) for caspases-3 and -7, and Ac-VEID-AMC (Ac-Val-Glu-Ile-Asp-AMC) for caspase-6, were purchased from Alexis Biochemicals (Switzerland). As already described,[5,11] reaction rates showing inhibitory potencies of the inhibitors were assessed by measuring the accumulation of the cleaved fluorogenic product 7-amino-4-methylcoumarin (AMC) with a Fusion universal microplate analyzer (PerkinElmer) at excitation and emission wavelengths of 360 and 460 nm, respectively. All assays were performed at a volume of 200 μL at 37 °C in reaction buffer.[5] Buffers contained the target compounds **10**−**25**, **31**−**33**, and **37**−**39** in DMSO (1−5%) in single doses (end concentrations 500 μM, 50 μM, 5 μM, 500 nM, 50 nM, 5 nM, 500 pM, 50 pM, or 5 pM). Recombinant caspases were diluted into the appropriate buffer to a concentration of 0.5 units per assay (=500 pmol substrate conversion after 60 min). After 10 min incubation time, the peptide substrates (end concentration 10 μM) were added and reacted for further 10 min. The IC_{50} values were determined by nonlinear regression analysis using the *XMGRACE* program (Linux software).

4.8. General Procedure for Radiochemistry. The radiosyntheses were carried out on a modified PET tracer radiosynthesizer (TRACERLab Fx_{FDG}, GE Healthcare). The recorded data were processed by the TRACERLab Fx software (GE Healthcare). Separation and purification of the radiosynthesized compounds were performed on the following semipreparative radio-HPLC-system A: K-500 and K-501 pump, K-2000 UV detector (Herbert Knauer GmbH), NaI(TI) Scintibloc 51 SP51 γ-detector (Crismatec), and an ACE 5 AQ column (250 mm × 10 mm). Method A started with a linear gradient from 30% to 90% CH_3CN in water (0.1% TFA) over 30 min, holding for 5 min, followed by a linear gradient from 90% to 30% CH_3CN in water (0.1% TFA) over 5 min, with λ = 254 nm and a flow rate of 5.5 mL·min^{-1}. Radiochemical purities were determined using the analytical radio-HPLC-system B: Two Smartline 1000 pumps and a Smartline UV detector 2500 (Herbert Knauer GmbH), a GabiStar γ-detector (Raytest Isotopenmessgeräte GmbH), and a Nucleosil 100-5 C-18 column (250 mm × 4 mm). Method B started with a linear gradient from 10% to 100% CH_3CN in water (0.1% TFA) over 15 min, holding for 3 min, followed by a linear gradient from 100% to 10% CH_3CN in water (0.1% TFA) over 2 min, with λ = 254 nm and a flow rate of 1.0 mL·min^{-1}. The recorded data of both HPLC-systems were processed by the GINA Star software (Raytest Isotopenmessgeräte GmbH). No-carrier-added aqueous $[^{18}F]$fluoride was produced on a RDS 111e cyclotron (CTI-Siemens) by irradiation of a 2.8 mL water target using 10 MeV proton beams on 97.0% enriched $[^{18}O]H_2O$ by the $^{18}O(p,n)^{18}F$ nuclear reaction. After the production of $[^{18}F]$fluoride, the 2.8 mL water target was unloaded. To remove residual $[^{18}F]$fluoride activity from the target, it was rinsed with 2.0 mL of water. These rinsing water batches were used for the radiosyntheses.

4.9. Radiosynthesis of [^{18}F]39 by Isotopic Exchange. In a computer controlled TRACERLab Fx_{FDG} Synthesizer the batch of aqueous $[^{18}F]$fluoride ions (336−12780 MBq) from the rinsing water was passed through an anion exchange resin (Sep-Pak Light Waters Accell Plus QMA cartridge, preconditioned with CO_3^{2-} as counterions). $[^{18}F]$Fluoride ions were eluted from the resin with a mixture of 36 μL of 1 M K_2CO_3, 200 μL of water for injection, and 800 μL of DNA-grade CH_3CN containing 23.8 mg (63 μmol) of Kryptofix2.2.2 (K_{222}) in the reactor. Subsequently, the aqueous $K[^{18}F]F/K_{222}$ solution was carefully evaporated to dryness *in vacuo*. An amount of 2.1 mg (5.3 μmol) of precursor **39** in 0.5 mL of dry DMF was added, and the mixture was heated at 130 °C for 10 min. After cooling to 55 °C, 10 mL of water was added and the mixture was passed through a Waters Sep-Pak C18 Light cartridge (preconditioned with 10 mL of ethanol and 10 mL of water). The cartridge was washed with 10 mL of water and eluted with 0.5 mL of DMF that was preheated to 90 °C. After addition of 0.5 mL of water, the raw product solution was purified by gradient-radio-HPLC-system A (method A). The product fraction of compound $[^{18}F]$**39** (retention time t_R($[^{18}F]$**39**) = 10.47 min) was evaporated to a volume of 40 μL *in vacuo* at <50 °C. Product compound $[^{18}F]$**39** was obtained in a radiochemical yield (r.y.) of ≤0.2% (decay-corrected based on cyclotron-derived $[^{18}F]$fluoride ions (d.c.)) in 93 min. The target compound was isolated in radiochemical purities of >99%. The radiochemical identity of $[^{18}F]$**39** was proven by coinjection and coelution of $[^{18}F]$**39** and **39** on analytical radio-HPLC B (method B, retention time t_R = 11.38−11.67 min). The radiochemical purities of $[^{18}F]$**39** were determined with the same HPLC-system and -method.

4.10. Stability Test of the Radiotracer [^{18}F]39 in Human Serum. The serum stability of radioligand $[^{18}F]$**39** was evaluated by incubation in human serum at 37 °C for up to 90 min. An aliquot of $[^{18}F]$**39** (20 μL, 5.8 MBq) was added to a sample of human serum (200 μL), and the mixture was incubated at 37 °C. Samples of 20 μL each were taken after periods of 10, 30, 60, and 90 min and quenched in $MeOH/CH_2Cl_2$ (1:1 (v/v), 100 μL) followed by centrifugation for 2 min. The clear solution was analyzed by analytical radio-HPLC.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Radio-HPLC chromatograms, ^{1}H, ^{13}C, ^{19}F NMR spectral data with assignment of signals and copies of ^{1}H, ^{13}C, ^{19}F NMR spectra of all inhibitors. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CD95, cell death receptor 95; DBH, *N*,*N*′-dibromo-5,5-dimethylhydanthoin; d.c., decay corrected; DIPEA, diisopropylethyl amine; EC/MS, electrochemistry/mass spectrometry; K_{222}, Kryptofix 222; MW, microwave irradiation; QC, quality control; r.y., radiochemical yield; TEA, triethyl amine

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