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Functionalization of Fluorinated Benzenesulfonamides and Their Inhibitory Properties toward Carbonic Anhydrases

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Substituted tri- and tetrafluorobenzenesulfonamides were designed, synthesized, and evaluated as high-affinity and isoform-selective carbonic anhydrase (CA) inhibitors. Their binding affinities for recombinant human CA I, II, VA, VI, VII, XII, and XIII catalytic domains were determined by fluorescent thermal shift assay, isothermal titration calorimetry, and a stopped-flow CO_2 hydration assay. Variation of the substituents at the 2-, 3-, and 4-positions yielded compounds with a broad range of binding affinities and isoform selectivities. Several 2,4-substituted-3,5,6-trifluorobenzenesulfonamides were effective CA XIII inhibitors with high selectivity over off-target CA I and CA II. 3,4-Disubstituted-2,5,6-trifluorobenzenesulfonamides bound CAs with higher affinity than 2,4-disubstituted-3,5,6-trifluorobenzenesulfonamides. Many such fluorinated benzenesulfonamides were found to be nanomolar inhibitors of CA II, CA VII, tumor-associated CA IX and CA XII, and CA XIII. X-ray crystal structures of inhibitors bound in the active sites of several CA isoforms provide structure–activity relationship information for inhibitor binding affinities and selectivity.

Introduction

Human carbonic anhydrases (CA) are zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide. This reaction is very important in the human body, as it regulates a broad range of physiological functions such as respiration, CO_2/bicarbonate transport between lungs and metabolizing tissues, pH and CO_2 homeostasis, and electrolyte secretion in many tissues and organs.[1,2] There are 12 active CA isoforms, which differ in cellular localization, distribution in organs and tissues, expression levels, and kinetic properties.[3–6] The structures of these isozymes are highly homologous, and only subtle differences between isozyme active sites are observed.[7,8] The increased activity or expression of various CA isoforms (e.g., CA II, IV, VA, VB, VII, IX, XII, XIII, and XIV) is often associated with different human diseases.[1,9,10] In 1954 acetazolamide, the first marketed systemic carbonic anhydrase inhibitor for the treatment of glaucoma, was reported.[11] To date, approximately 25 drugs or drug candidates are used as diuretics, antiglaucoma drugs, anticonvulsants, anti-altitude sickness, and anticancer agents. Many drugs in current use have side effects that are thought to be due to inhibition of CA isoforms not involved in the target disease.[12] Therefore, there is a need to improve the inhibition and selectivity profiles of CA inhibitors (CAIs).

The main class of CAIs is aromatic and heterocyclic sulfonamides. Benzenesulfonamides are the most investigated and effective CAIs.[4] The binding affinity of benzenesulfonamides depends highly on the pK_a of the sulfonamide group. The highest-affinity arylsulfonamides should have a pK_a near the pH of buffered medium (~7.4).[13,14] Without the electronegative groups, the pK_a of benzene sulfonamide is ~11. The presence of electronegative substituents decreases the pK_a of sulfonamide, and this correlates with an increase in CA inhibitory properties. Introduction of halogen atoms, especially fluorine, as substituents is one possible way toward better CA inhibitors.[15,16] The unique features of fluorine enable the synthesis of compounds with extraordinary properties that cannot be attained using any other element. There are numerous studies that have investigated benzenesulfonamides containing one fluorine atom in the benzene scaffold as CA inhibitors.[17–28] However, the introduction of several fluorine atoms is less common.[13,29–32]

Polyfluoroaromatic compounds such as hexafluorobenzene[33,34] or pentafluoropyridine[35] readily undergo nucleophilic aromatic substitution reactions. Even complete nucleophilic replacement of all fluorine atoms is possible. A second nucleophilic replacement for hexafluorobenzene always occurs at the position *para* to the first substituent.[33] The same reactivity profile is common for other C_6H_5X compounds.[36] However, there are exceptions for which *ortho* substitution is exclusive or predominant.[36,37] The *ortho*-directing effects depend on the

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character of the X group and the reaction conditions that allow specific interaction with the nucleophilic reagent. Exclusive *ortho* substitution was observed for substituents X (C_6F_5X) containing carbon–oxygen, carbon–nitrogen, and sulfur–oxygen double bonds in reactions with metal-containing reagents such as Grignard, organolithium, or halomagnezylamines. The interactions between the X substituent and the metal-containing reagent cause the *ortho* substitution. Analogous interactions between substituent X and the incoming nucleophiles can be enhanced in nonpolar solvents. Reactions of $C_6F_5SO_2CH_3$, $C_6F_5CO_2Et$, $C_6F_5CO_2H$, $C_6F_5COCH_3$, and $C_6F_5NO_2$ with $MeNH_2$ and Me_2NH in benzene and nitromethane have been investigated in detail.[38] A substantial shift in the *ortho*/*para* ratio was observed upon changing from a polar to a nonpolar solvent. The least common site of attack for C_6F_5X is that of *meta* substitution. Strong electron-donating substituents X (O^-, NH_2) deactivate *ortho* and *para* positions more than *meta* toward nucleophilic substitution, and replacement of *meta*-fluorine occurs.[39–41]

Owing to the high reactivity of polyfluorinated compounds (C_6F_5X), monosubstitution (C_6F_4XY) or even further substitutions are possible. An example of such compounds is pentafluoronitrobenzene. Reactions between pentafluoronitrobenzene and a variety of nucleophiles have been established and have been found to be regioselective in the majority of cases in which polar solvents were used, giving *para*-substituted products. In addition, high *ortho* selectivity was observed for cases in which nonpolar solvents were used. 4-Substituted-2,3,5,6-tetrafluoronitrobenzenes are still activated toward nucleophilic attack, and 2,4-substituted-3,5,6-trifluoronitrobenzenes can be obtained.[42] A further sequential nucleophilic substitution process affords 2,4,6-substituted-3,5-difluoronitrobenzenes.[43] The reactivity profile established for pentafluoronitrobenzene in which the 4-, 2-, and 6-positions are sequentially substituted has allowed chemists to synthesize a diverse range of compounds.[42–46]

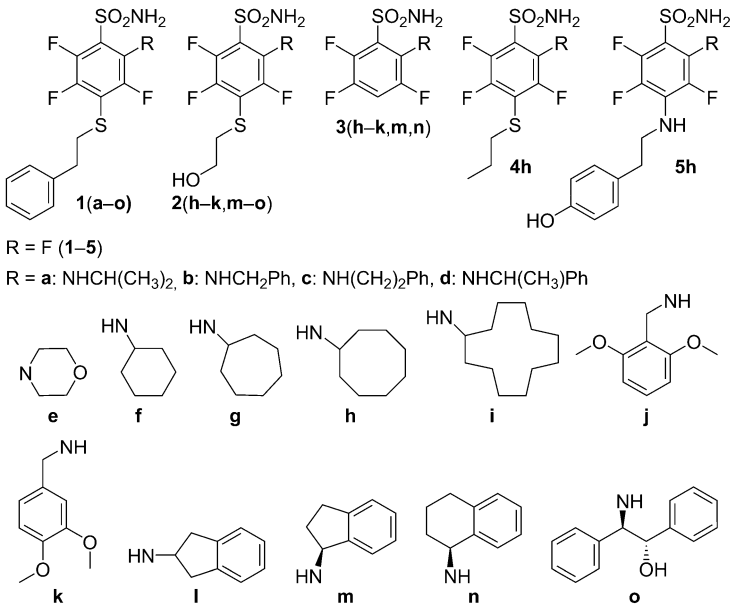
In a previous study,[47] we assayed a series of 4-substituted-2,3,5,6-tetrafluorobenzenesulfonamides as inhibitors of CA isozymes I, II, VII, XII, and XIII. All fluorinated benzenesulfonamides exhibited higher binding potency toward tested CAs (especially toward CA I) than nonfluorinated compounds. These studies suggested that a polyfluorinated benzenesulfonamide scaffold is a good tool for CA inhibitor development. To understand the contribution of substituents toward isoform selectivity and affinity, herein we focus on modifications of 4-substituted-2,3,5,6-tetrafluorobenzenesulfonamides at the *ortho* and *meta* positions.

A series of fluorinated benzenesulfonamides were synthesized, namely 2,4-substituted-3,5,6-trifluorobenzenesulfonamides, 2-substituted-3,5,6-trifluorobenzenesulfonamides, 2-substituted-3,4,5,6-tetrafluorobenzenesulfonamides, 3,4-substituted-2,5,6-trifluorobenzenesulfonamides, and 3,4,5-substituted-2,6-difluorobenzenesulfonamides, in search of CA inhibitors with high affinity and isoform selectivity.

Results and Discussion

Chemistry

A series of 2,4-substituted-3,5,6-trifluorobenzenesulfonamides, 2-substituted-3,5,6-trifluorobenzenesulfonamides, 2-substituted-3,4,5,6-tetrafluorobenzenesulfonamides, 3,4-substituted-2,5,6-trifluorobenzenesulfonamides, and 3,4,5-substituted-2,6-difluorobenzenesulfonamides were designed and synthesized (Schemes 1 and 2). The synthesis of starting compounds **1**–**5**, **9**, **10** was described previously by our research group.[47] Reactions of compounds **1**–**5** with various nitrogen-centered nucleophiles were carried out in DMSO in the presence of Et_3N. The *ortho*-substituted (according to the sulfonamide group) products **1**(**a**–**o**), **2**(**h**–**k**,**m**–**o**), **3**(**h**–**k**,**m**,**n**), **4h**, and **5h** were obtained. The substitution profile of sulfonamides **1**–**5** is similar to that of 4-substituted-2,3,5,6-tetrafluoronitrobenzenes.[42,43] Spectral elucidation of purified products proved substitution at the *ortho* position. In the ^{13}C NMR spectra of isolated products **1**(**a**–**o**) there were triplets for methylene groups (SCH_2CH_2Ph) at 35.3–35.5 ppm due to the coupling of two identical fluorines to carbon (coupling constants were 3.2–4.0 Hz). These fluorines are in the *ortho* position relative to the SCH_2CH_2Ph group. The substituents that appeared from incoming N-centered nucleophiles exhibited similar C–F couplings. The signals of carbons (N–C) appeared as doublets (due to one fluorine coupling to carbon) at 40–66 ppm with coupling constants of 10–12 Hz (6 Hz for **1e**) for compounds **1**(**a**–**o**). The H,C HETCOR spectrum of compound **1m** confirmed our assignment of carbon signals in the ^{13}C NMR spectra where the triplet at 35.5 ppm belongs to the carbon of group SCH_2CH_2Ph and the doublet at 62.3 ppm belongs to the carbon of fragment $NHCH$.



Scheme 1. Structures of compounds **1**(**a**–**o**), **2**(**h**–**k**,**m**–**o**), **3**(**h**–**k**,**m**, **n**), **4h**, and **5h**.

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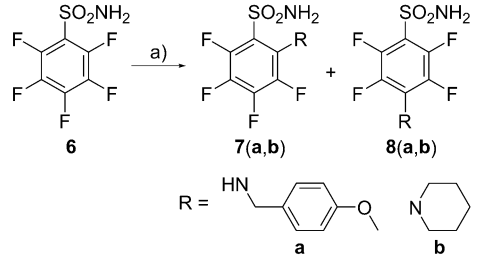
The same couplings were observed for compounds **2**(**h**–**k**,**m**–**o**) and **4h** in the ^{13}C NMR spectra.

The previously described *para*-substituted compounds **1**–**5**, **9**, and **10**[47] were synthesized from pentafluorobenzensulfonamide by nucleophilic substitution using DMSO as solvent (other solvents were not attempted). Investigation of pentafluorobenzensulfonamide nucleophilic substitution in other solvents such as dioxane and benzene showed the possibility of obtaining the *ortho*-substituted (relative to the sulfonamide group) products (Scheme 2). In the case of previously described compounds **1**(**a**–**o**), **2**(**h**–**k**,**m**–**o**), **4h**, and **5h**, *ortho* substitution was achieved after *para* substitution. The formation of *ortho*-substituted products **7**(**a**,**b**) is due to the *ortho*-directing capacity of the sulfonamide group. This feature of the sulfonamide group is readily recognizable in nonpolar solvents. The *ortho*-directing capacity of nitro[48,49] and carboxylate groups[50] in S_NAr reactions has been known for more than 50 years. The regioselectivity of 2,4-dihaloaromatic compounds with various functionalities in reactions with piperidine were investigated:[51] Wendt and Kunzer concluded that compounds which display a strong preference for *ortho* substitution in nonpolar solvents possess functionalities that can interact with the incoming nucleophile, biasing it toward *ortho* attack via a hydrogen bond or electrostatic interactions. Among other compounds, 2,4-difluorobenzenesulfonamide was investigated in reaction with piperidine using dioxane and DMSO as solvents. The significant shift in regioselectivity toward the *ortho* product was observed upon changing DMSO to dioxane (ratio from 32 in DMSO to 96 in dioxane).



Scheme 2. Synthetic route for compounds **7**(**a**,**b**) and **8**(**a**,**b**). *Reagents and conditions*: a) dioxane or C_6H_6 or DMSO, Et_3N (see Experimental Section for reaction temperatures and duration).

The substrate pentafluorobenzensulfonamide S_NA reactions with the primary amine 4-methoxybenzylamine and the secondary amine piperidine were investigated in such solvents as dioxane, C_6H_6, and DMSO (Scheme 2, Table 1). Exclusive *para* substitution with both amines was observed using DMSO. Predominant *ortho* substitution was observed in C_6H_6, and especially for primary amine. The *ortho*-*para* substitution ratios were nearly equal in dioxane for the primary amine; however, predominant *para* substitution was observed for the secondary amine. Primary amine-4-methoxybenzylamine showed a greater ability for *ortho* substitution in both solvents (dioxane and C_6H_6) than the secondary amine piperidine.

Table 1. **7a**/**8a** and **7b**/**8b** product ratios in various solvents.[a]

Solvent	**7a**/**8a**	**7b**/**8b**
dioxane	43:57	34:66
C_6H_6	84:16	71:29
DMSO	100% **8a**	100% **8b**

[a] Ratios determined by NMR peak integration of crude products.

Reactions of compounds **9** and **10** with various nitrogen-centered nucleophiles were accomplished, and *meta*-substituted (relative to sulfonamide group) products were obtained. Sulfonamides **9**(**d**,**e**,**g**–**q**) were obtained from compound **9** by using the appropriate nucleophile in DMSO in the presence of triethylamine. Compound **9a** was synthesized with excess methylamine (solution in methanol) in methanol. Fluorinated derivative **9b** was obtained by using 2 equivalents of nucleophile in DMSO. Compound **10**, bearing a (2-hydroxyethyl)sulfonyl "tail", was sensitive to such bases as Et_3N or K_2CO_3; instead of these bases the equivalent of nitrogen-centered nucleophile was used. Sulfonamides **10**(**d**,**h**–**k**,**n**–**p**) were obtained from compound **10** by using 2 equivalents of the appropriate nucleophile in DMSO. The synthesis of compounds **1i**, **2h**, and **10h** was described previously.[52]

The spectral elucidation of purified products led us to suppose that the nucleophilic substitution for compounds bearing (2-phenylethyl)sulfonyl and (2-hydroxyethyl)sulfonyl "tails" took place at the *meta* position. We expected to observe splittings for a methylene group ($SO_2CH_2CH_2Ph$ and $SO_2CH_2CH_2OH$) as in the case of compounds **1**(**a**–**o**), **2**(**h**–**k**,**m**–**o**), **4h** in the ^{13}C NMR spectra. However, such splittings were observed only for compounds **9h** (recorded in $CDCl_3$), **9p**, and **9q** (both recorded in [D_6]DMSO). In the ^{13}C NMR spectra of isolated products **9h**, **9p**, and **9q**, there were doublets for a methylene group ($SO_2CH_2CH_2Ph$) at 58.7, 58.0, and 58.0 ppm, with respective coupling constants of 4, 2, and 2 Hz.

The splitting was due to the coupling of one fluorine atom to carbon. This fluorine is at the *ortho* position relative to the $SO_2CH_2CH_2Ph$ group. The H,C HETCOR spectrum of compound **9h** confirmed our assignment of carbon signals in the ^{13}C NMR spectrum, in which the doublet at 58.7 ppm belongs to the methylene carbon of $SO_2CH_2CH_2Ph$, and the doublet at 56.2 ppm belongs to the carbon of fragment NH*CH*. The substituents that appeared from incoming N-centered nucleophiles similarly to compounds **1**(**a**–**o**), **2**(**h**–**k**,**m**–**o**), **3**(**h**–**k**,**m**,**n**), **4h**, and **5h** exhibit C–F couplings. The signals of carbons (N–C) appeared as doublets (due to one fluorine coupling to carbon) at 34–66 ppm with coupling constants of 11–13 Hz (4 Hz for **9b**,**g**,**l**) for compounds **9**(**a**,**b**,**d**,**e**,**g**–**q**) and **10**(**d**,**h**–**k**,**n**–**p**).

Furthermore, *meta* substitution for compounds **9d**, **10d**, and **10p** was demonstrated by X-ray structures of CA crystals soaked with these inhibitors. The position of the substituent was well resolved in the electron density of the compounds bound in active centers of CAs (see *Crystallography* section below). The substitution profile of compounds **9** and **10** differs from that of compounds **1** and **2**. The oxidized groups

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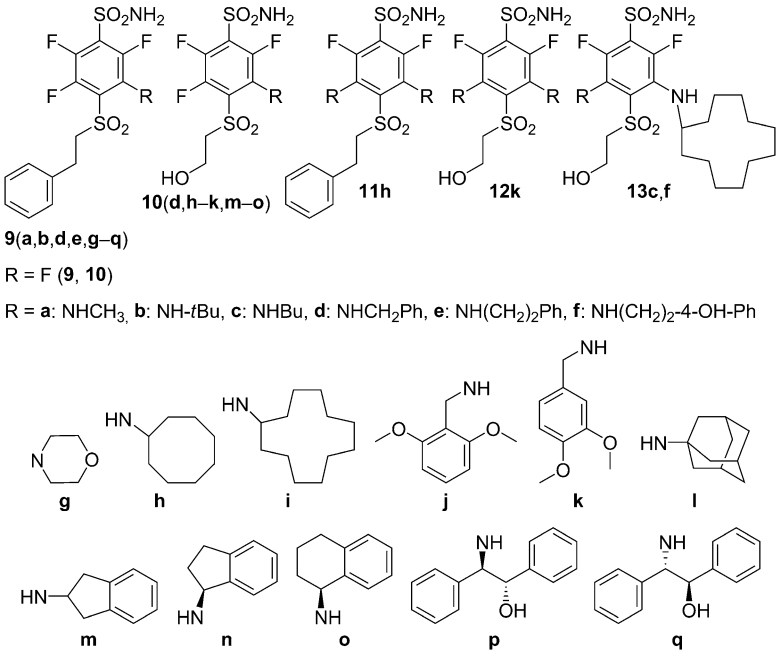
$SO_2CH_2CH_2Ph$ and $SO_2CH_2CH_2OH$ exhibit stronger electron-withdrawing properties than SO_2NH_2, and direct nucleophiles to the *meta* position relative to the SO_2NH_2 group.

The 3-substituted compounds bearing (2-phenylethyl)sulfonyl and (2-hydroxyethyl)sulfonyl "tails" are activated toward a nucleophilic attack and thus the 3,5-substituted compounds were obtained. The second nucleophilic substitution occurred at the *meta* position again. Compound **11 h** was obtained from compound **9** by using 2 equivalents of cyclooctylamine in DMSO in the presence of Et_3N. Compound **12 k** was obtained from compound **10** by using 4 equivalents of 3,4-dimethoxybenzylamine in DMSO. Compounds **13**(**c**,**f**) with different substituents at the *meta* position were obtained (Scheme 3). These compounds were synthesized from compound **9 i** by using 2 equivalents of the appropriate nucleophile in DMSO. The reactivity profile of compounds **9** and **10** is similar to that of pentafluoronitrobenzene, in which the 4-, 2-, and 6-positions were sequentially substituted. However, two similar electron-withdrawing groups were present in our case, so the asymmetric substitution was possible, as expected. Nevertheless, only symmetric substitution products were obtained.



Scheme 3. Structures of compounds **9**(**a**,**b**,**d**,**e**,**g**–**q**), **10**(**d**,**h**–**k**,**n**–**p**), **11 h**, **12 k**, **13**(**c**,**f**).

Spectral elucidation of the purified products showed a double *meta* substitution. There was one signal as singlet in the ^{19}F NMR spectra of isolated products **11 h** and **12 k**. This proved the presence of two identical fluorine atoms. The ^{13}C NMR spectra of these compounds also confirmed the double *meta* substitution. The signals of benzene ring carbons were split by fluorine coupling to carbon. The C–F couplings involved in the functional groups attached to a benzene ring were previously discussed. These couplings were most important for the determination of substitution direction. The splitting of benzene ring carbon signals is very informative in double *meta*-substituted products, and allowed us to elucidate the correct structure of the compounds. As an example, we analyzed the ^{13}C NMR spectrum of compound **12 k**. The signal of benzene ring carbon C4 appeared as a triplet at 115.5 ppm with a coupling constant of 3 Hz. The splitting was due to the coupling of two identical fluorines to *meta*-positioned carbon C4. The signal of benzene ring carbon C1 appeared as a triplet as well (127.9 ppm). However, in this case, the coupling constant was larger (16 Hz) because two identical fluorines were in the *ortho* positions relative to C1.

In the ^{13}C NMR spectrum, there was a double doublet for carbons C3 and C5 at 135.7 ppm, with respective coupling constants of 10 and 6 Hz. The splitting was due to coupling of two fluorines to *ortho*-, *para*-positioned carbons. The signal of benzene ring carbons C2 and C6 appeared as double doublet as well. However, in this case the first coupling constant was much larger (247 Hz) due to fluorines coupling to *ipso* carbons (directly substituted by fluorines). The substituents that appeared from incoming N-centered nucleophiles exhibited C–F couplings. Interestingly, the signal of carbon ($NHCH_2R$) appeared as a triplet with a coupling constant of 6 Hz instead of doublet or double doublet. The same splitting was observed for compound **11 h** as well. We assume that the triplet appeared as a result of double doublet overlapping. However, the splitting into doublets was observed for carbon signals of compounds **13**(**c**,**f**) bearing different substituents at the *meta* position. H,C HETCOR spectrum of compound **12 k** confirmed our assignment of carbon signals in the ^{13}C NMR spectrum, where the triplet at 51.2 ppm belongs to the methylene group of $NHCH_2R$, and the singlet at 58.3 ppm belongs to the methylene carbon of substituent $SO_2CH_2CH_2OH$.

Binding studies

The 4-substituted-2,3,5,6-tetrafluorobenzenesulfonamides have been previously described as potent inhibitors of CA I, II, VII, XII, and XIII, exhibiting particularly strong binding to CA I (subnanomolar affinity) and being selective for CA I.[47] The representative derivatives **1**, **2**, **9**, and **10** bound to CA I with K_d values in the range of 0.1–0.25 nM and had higher binding affinity than tetrafluorobenzenesulfonamide **3** ($K_d = 2.4$ nM) and pentafluorobenzenesulfonamide **6** (PFBSA; $K_d = 2.2$ nM). Thus, it appeared that the additional contacts between the *para* substituent on the benzenesulfonamide ring and CA have emerged. Indeed, the crystal structure of 4-phenylethylsulfon-

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yl-2,3,5,6-tetrafluorobenzenesulfonamide **9** in complex with CA II shows that the van der Waals interactions with the protein side chains participate in binding, and the sulfonyl group of the *para* "tail" also makes hydrogen bonds with the protein active site residues, which also contribute favorably to the binding affinity.[47]

Because CA I is involved in retinal cerebral edema,[53] we found such derivatives to be interesting candidates for the development of anti-edema drugs. However, for targeting of any other isozyme involved in certain pathology (for example, CA XII, which is a potential target in cancer), the inhibition of widespread cytosolic isozymes CA I and II is undesirable because it can lead to side effects.

Therefore, we focus on the modification of 4-substituted-2,3,5,6-tetrafluorobenzenesulfonamides at the *ortho* and *meta* positions in order to show the contribution of substituents to the selectivity and affinity of inhibitors. The first group of compounds were *ortho*-substituted compounds at the *para* position bearing a long-chain (phenylethylthio or hydroxyethylthio) substituent (compounds **1**(**a**–**p**), **2**(**h**–**o**), **4h**, **5h**), fluorine (**7**(**a**, **b**)) or nonsubstituted compounds (**3**(**h**–**n**)) (Schemes 1 and 2, Table 2). The second group of compounds included the *meta*-substituted ligands bearing (2-phenylethyl)sulfonyl (**9a**–**q**) and (2-hydroxyethyl)sulfonyl (**10**(**d**–**p**)) "tails" at the *para* position (Scheme 3, Table 3). Double *meta*-substituted compounds with (2-phenylethyl)sulfonyl (**11h**) and (2-hydroxyethyl)sulfonyl (**12k, 13c, 13f**) "tails" at the *para* positions were also synthesized (Scheme 3, Table 4).

The fluorinated compounds bearing substitutions at *ortho* or *meta* positions were tested for binding to seven CA isoforms: CA I, II, VA, VI, VII, XII, and XIII. The binding affinity for all CAs was determined by the fluorescent thermal shift assay (FTSA) and confirmed for some compounds (**2h** and **10d**) by ITC (Tables 2–4). In addition to the binding measurements by FTSA and ITC, IC_{50} values for compounds **2h** and **10h** for CA II were also determined by the stopped-flow CO_2 hydration assay. There was good agreement between FTSA, ITC, and inhibition data, with discrepancies between K_d and IC_{50} values not exceeding twofold. For example, the K_d values for **2h** binding to CA II were 1300 and 730 nM as determined by FTSA and ITC, respectively, and the IC_{50} was equal to 910 nM.

Introduction of substituents at the *ortho* position (compounds **1**(**a**–**o**), **2**(**h**–**o**), **3**(**h**–**n**), **4h**, **5h, 7**(**a,b**)) in most cases lowered the affinity for all CA isozymes, especially for CA I (Table 2). The binding of all these compounds to CA I was in the micromolar or millimolar range (or even lower affinity), significantly weaker than their analogues without *ortho* substituents (compounds **1**–**3, 6**) which bound to CA I in the nanomolar range. Most of the compounds were also weak inhibitors of CA II, VA, and VI (K_d in the micromolar range). *ortho*-Substituted compounds were the most effective and selective inhibitors toward CA XIII. Compound **1d** was the most potent inhibitor of CA XIII (K_d = 40 nM) in the series of **1**(**a**–**p**), **2i** (K_d = 11 nM) was most potent among the compounds of series **2**(**h**–**o**), and **3k** (K_d = 140 nM) was the most potent of the series **3**(**h**–**n**). Figure 1 A shows CA I and CA XIII stabilization by **2k**, resulting in the >100-fold differences in their K_d values.

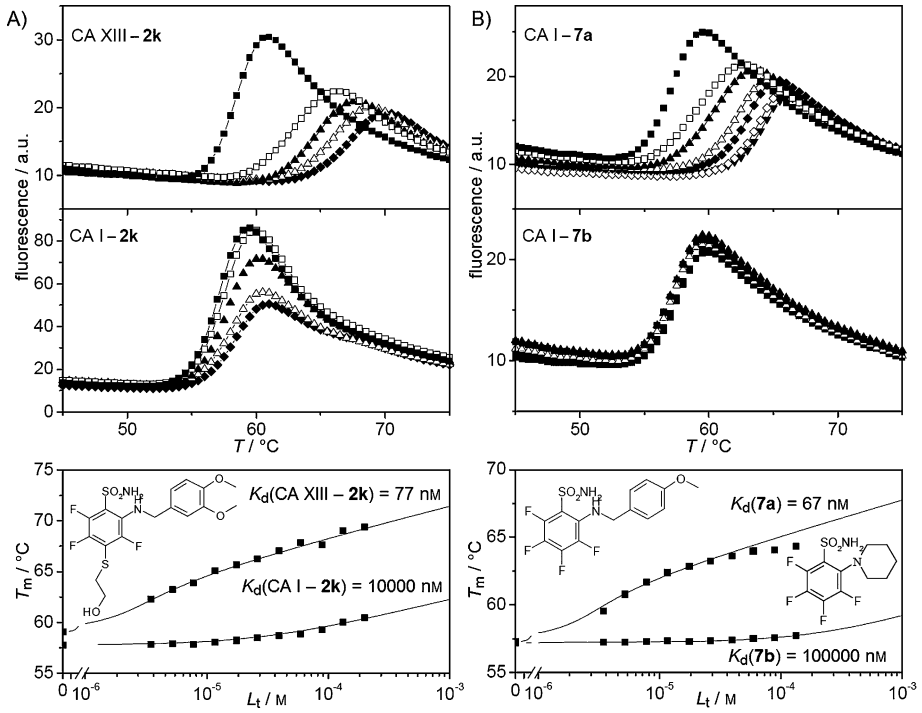

Figure 1. Selected compound binding curves as determined by fluorescent thermal shift assay. A) Compound **2k** binding to CA isozymes I and XIII. B) Compounds **7a** and **7b** binding to CA I. Panels at top show the protein raw melting curves at several added ligand concentrations. Panels on the bottom show the dependence of the protein melting temperatures (T_m) on ligand concentrations. Data points are from the experimental values of the raw melting curves (upper panels) and the curve fits are simulated according to the model.[55]

Compounds of the series **1**(**a**–**o**) with a phenylethylthio group at the *para* position were, in most cases, weaker inhibitors of CAs than compounds **2**(**h**–**o**), bearing the same substituents at the *ortho* position, but a different (hydroxyethylthio) substituent at the *para* position. By comparing two compounds bearing SCH_2CH_2Ph (compound **1h**) and SCH_2CH_2OH (compound **2h**) groups at the *para* position with the same dodecylamino substituent at the *ortho* position, the latter displayed higher binding affinity for CA VII, XII, and XIII. Compounds **2h** and **3h** bound CA VA, VI, VII, XII, and XIII similarly,

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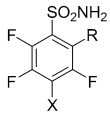
but the binding with CA I and II differed (for example, the K_d for binding of CA II with **3h** is ~5-fold lower than with **2h**). Similarly, the addition of a *para*-phenylethylthio group weakened the binding to all isoforms more strongly, as compared with the SCH_2CH_2OH group at the *para* position (compare **1h** and **2h** vs. **3h**). On the other hand, the absence of a *para* group (compounds of series **3**) causes less selectivity across various isoforms due to better binding of CA I and CA II (compound **3h**, **3i** and **3k** vs. **2h**, **2i** and **2k**).

Substitution of the cyclohexylamino group in **1f** with bulkier groups in **1g–i** resulted in a significant decrease in binding affinities for all CAs. Compound **1i**, bearing the largest cyclo-

Table 2. 2,4-Disubstituted-3,5,6-trifluorobenzenesulfonamide dissociation constants for seven human recombinant CA isoform catalytic domains.



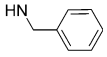
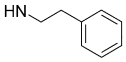
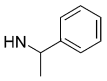
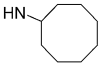
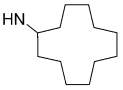
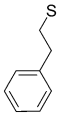
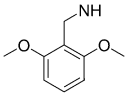
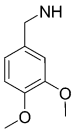
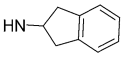
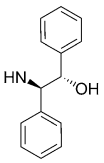
Compd	X	R	K_d [nM] for CA isoforms[a]						
			CA I	CA II	CA VA	CA VI	CA VII	CA XII	CA XIII
1		F	0.20	1.7	200	200	0.83	110	0.22
1a		$NHCH(CH_3)_2$	3300	500	670	3300	130	2900	67
1b			5000	2500	1000	2500	1700	2900	110
1c			10000	2200	20000	>200000	14000	1300	330
1d			1700	3300	200	1400	4000	5000	40
1e			>200000	4000	33000	>200000	>200000	>200000	>200000
1f			25000	250	20000	100000	170	500	500
1g			>200000	1400	>200000	10000	1000	1300	500
1h			>200000	>200000	>200000	>200000	>200000	4000	710
1i[52]			>200000	>200000	>200000	>200000	>200000	>200000	1700
1j			6700	>200000	>200000	>200000	>200000	>200000	3300
1k			1700	1700	1700	20000	25000	20000	67
1l			5000	3300	>200000	6700	4000	1700	100
1m			10000	5000	>200000	>200000	>200000	>200000	670
1n			2500	>200000	>200000	>200000	>200000	>200000	330
1o			14000	>200000	>200000	>200000	>200000	>200000	>200000
1p			>200000	1100	33000	3300	2500	4000	50

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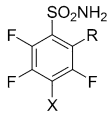
Table 2. (Continued)



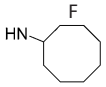
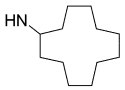
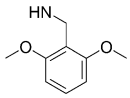
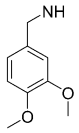
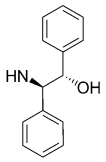
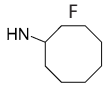
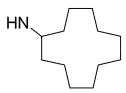
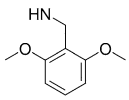
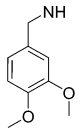
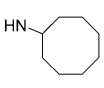
Compd	X	R	K_d [nM] for CA isoforms[a]						
			CA I	CA II	CA VA	CA VI	CA VII	CA XII	CA XIII
2		F	0.11	6.7	330	200	46	220	8.3
2h[52]			50000 (> 10000)	1300 (580)	3300 (ND)[b]	4300 (ND)[b]	330 (900)	330 (180)	140 (370)
2i			20000	1700	> 200000	25000	3300	330	11
2j			20000	6600	33000	100000	42000	13000	1200
2k			10000	4000	670	11000	6700	10000	77
2m			8300	3000	10000	2900	1700	1100	33
2n			14000	10000	33000	25000	17000	2000	120
2o			> 200000	> 200000	> 200000	33000	> 200000	6700	5600
3		F	2.4	29	290	670	11	330	20
3h			420	250	670	4000	330	450	200
3i			2500	4000	14000	> 200000	111000	6700	1400
3j			1000	4000	20000	11000	33000	20000	4300
	H		560	670	5000	13000	6700	5000	140
3m			14000	3500	10000	3600	12000	2900	390
3n			10000	2200	20000	7700	21000	10000	450
4h			25000	2000	25000	20000	1000	1000	290

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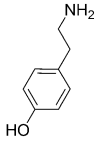
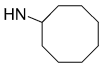
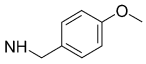
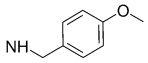
Table 2. (Continued)

Compd	X	R	K_d [nM] for CA isoforms[a] CA I	CA II	CA VA	CA VI	CA VII	CA XII	CA XIII
5h			> 200 000	10 000	> 200 000	100 000	1700	1400	770
6	F	F	2.2	27	400	450	25	500	40
7a	F		67	200	4000	710	560	1000	140
7b	F		100 000	29 000	14 000	> 200 000	> 200 000	> 200 000	14 000
8a		F	0.14	10	500	1800	20	330	2.5
8b		F	0.03	13	670	1700	14	100	13

[a] Determined by FTSA at 37 °C, pH 7.0 (ITC values are given in parentheses); uncertainties in FTSA and ITC measurements do not exceed 1.6-fold K_d. [b] Not determined.

dodecylamino group, bound only CA XIII isoform with micromolar affinity (K_d = 1.7 μM).

Compounds **2**(**h**–**o**) exhibited remarkable selectivity toward CA XIII against CA I, II, VI, VII, and XII. For example, **2i** bound to CA XIII ~1800-fold stronger than to CA I, 154-fold stronger than to CA II, 300-fold stronger than to CA VII, and 30-fold stronger than to CA XII. Comparison of compounds **2** and **2i** showed that fluorine substitution at the *ortho* position with a bulky cyclododecylamino group does not affect the binding affinity for CA XIII (K_d: 8.3 and 11.0 nM, respectively), but weakened the binding to other CA isoforms, especially with CA I (K_d from 0.1 nM for **2** to 20 μM for **2i**).

Compounds **3**(**h**–**n**) have no substituent at the *para* position, but the fluorine atom at the *ortho* position is substituted by a bulky group. They bound with considerably higher affinity to CA I than the same compounds with a substituent at the *para* position (**1**(**a**–**o**), 2(**h**–**o**)). For example, **3h**, with a cyclooctylamino group at the *ortho* position, was found to be a nanomolar inhibitor of CA I (K_d = 420 nM), but the introduction of phenylethylthio (compound **1h**) and hydroxyethylthio (compound **2h**) groups at the *para* position weakened binding to CA I significantly ($K_d \geq 200$ and 50 μM, respectively). Interestingly, the compounds **3**(**h**–**n**) bound CA XIII weaker than **2**(**h**–**o**) (contrary to CA I), showing that the SCH_2CH_2OH group in **2**(**h**–**o**) compounds interacts with the protein and enhances binding affinity (Figure 2A).

The nature of the *para* substituent on the benzenesulfonamide ring with a cyclododecylamino moiety at the *ortho* position (compounds **1h**, **2h**, **4h**, **5h**) influenced the binding affinity for all CAs. Compound **2h**, with a hydroxyethylthio group, was found to be the most potent inhibitor among the compounds of this group.

2,3,4,5-Tetrafluoro-6-[(4-methoxybenzyl)amino]benzenesulfonamide **7a** was most similar to **3k** (hydrogen at the *para* position), and both compounds showed the same affinity for

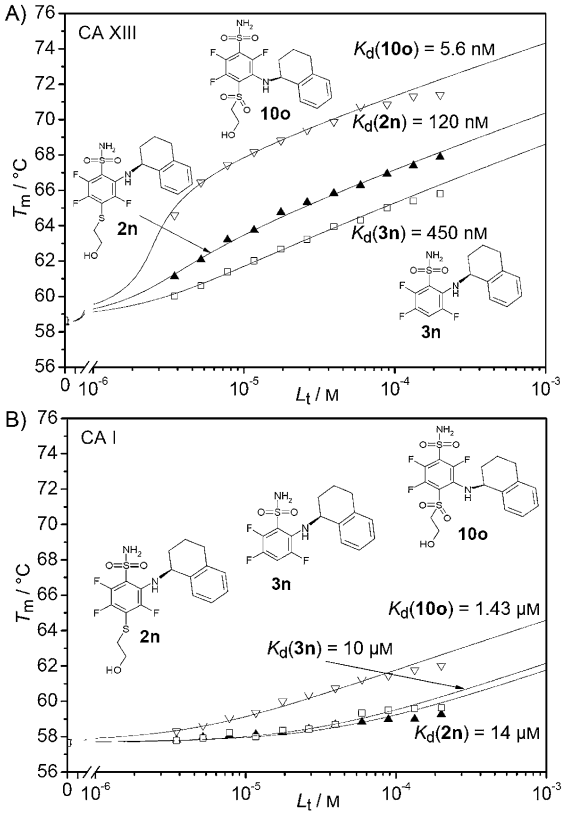


Figure 2. FTSA data for the binding of **10o**, **2n**, and **3n** to A) CA XIII and B) CA I.

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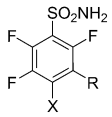
CA XIII (K_d = 140 nM), but with **7a** being more active for other CAs than **3k**. Interestingly, an inflexible piperidine group at the *ortho* position led to a sharp decrease in binding affinity for all tested CAs, especially CA I (Figure 1B). Compounds **8a** and **8b**, with 4-methoxybenzylamine or piperidine tails, bound CA I with sub-nanomolar affinity (K_d: 0.14 and 0.03 nM, respectively) showing that an inflexible piperidine group is more favorable for CA I binding.

Overall, most of the 2,4-disubstituted-3,5,6-trifluorobenzenesulfonamides (Table 2) are potent and highly selective CA XIII inhibitors, being weak inhibitors of cytosolic isozymes CA I and CA II. As CA XIII is involved in the sperm motility processes,[4]

Table 3. 3,4-Disubstituted-2,5,6-trifluorobenzensulfonamide dissociation constants for seven human recombinant CA isoform catalytic domains.



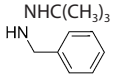
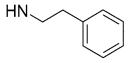
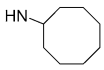
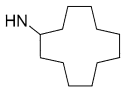
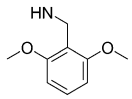
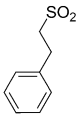
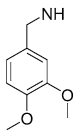
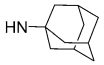
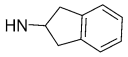
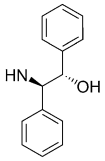
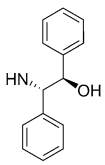
Compd	X	R	K_d [nM] for CA isoforms[a]						
			CA I	CA II	CA VA	CA VI	CA VII	CA XII	CA XIII
9		F	0.25	1.3	140	100	1.3	77	0.40
9a		$NHCH_3$	67	5.9	500	100	8.3	290	1.3
9b		$NHC(CH_3)_3$	670	1.7	770	45	0.22	50	3.6
9d			56	6.7	250	110	5.0	40	2.5
9e			500	17	500	670	4.0	33	6.7
9g			100	1.3	500	40	2.5	150	5.0
9h			330	13	2500	200	3.3	6.7	3.3
9i			2500	670	6700	2500	25000	50	3.3
9j			59	22	1000	220	670	170	8.3
9k			210	22	200	400	6.7	250	3.3
9l			500	50	14000	330	5.0	17	5.6
9m			500	20	830	180	13	36	1.0
9n			1000	6.7	3300	330	2.0	40	2.5
9o			1000	6.7	1000	130	13	50	6.7
9p			1700	33	2000	830	10	250	5.6
9q			770	91	3300	290	40	400	6.7

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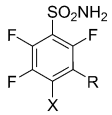
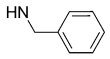
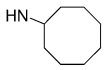
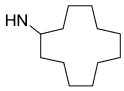
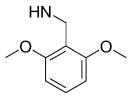
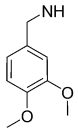
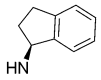
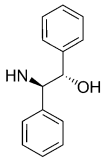
Table 3. (Continued)



Compd	X	R	K_d [nM] for CA isoforms[a]						
			CA I	CA II	CA VA	CA VI	CA VII	CA XII	CA XIII
10		F	0.20	17	290	67	7.1	250	29
10d			200 (320)	83 (120)	330 (ND)[b]	100 (220)	130 (350)	25 (16)	14 (52)
10h[52]			710 (710)	60 (62)	2500 (ND)[b]	95 (ND)[b]	9.8 (140)	3.3 (16)	3.6 (39)
10i			400	36	2900	200	50	5.0	0.8
10j			200	17	1000	1000	40	67	25
10k			83	25	67	910	14	67	4.3
10n			1000	17	3300	100	5.0	8.3	8.3
10o			1400	67	1000	100	10	13	5.6
10p			20000	50	5000	370	33	NA[c] (48)	7.1

[a] Determined by FTSA at 37 °C, pH 7.0 (ITC values are given in parentheses for several compounds); uncertainties in FTSA and ITC measurements do not exceed 1.6-fold K_d. [b] Not determined. [c] Not available due to high compound fluorescence.

such trifluorobenzensulfonamides could be good lead compounds for the development of CA XIII inhibitors as contraceptive agents. Our newly synthesized compounds **8a** and **8b**, similar to the previously described 4-substituted-2,3,5,6-tetrafluorobenzenesulfonamides,[47] are very effective (sub-nanomolar affinity) and highly selective CA I inhibitors.

The effect of the substitution at the *meta* position of 4-phenylethylsulfonyl-2,3,5,6-tetrafluorobenzenesulfonamide **9** and 4-hydroxyethylsulfonyl-2,3,5,6-tetrafluorobenzenesulfonamide **10** was also investigated. It was observed for the series of **9**(**a**–**q**) and **10**(**d**–**p**) that even a slight structural modification at the *meta* position causes significant variations in binding affinity for CA I as well as for other isoforms. For example, fluorine substitution in compound **9** with an $NHCH_3$ group (compound **9a**) resulted in a >260-fold lower K_d value for CA I, 4.5-fold for CA II, 3.5-fold for CA VA, 6.4-fold for CA VII, 3.8-fold for CA XII, and 3.3-fold for CA XIII. The binding affinity for CA VI did not change upon introduction of the $NHCH_3$ group. In general, the bulkier substituent R at the *meta* position caused weaker binding to CA I.

Interestingly, the K_d value for CA XIII did not significantly depend on the size of the substituent at the *meta* position; the K_d values for all tested compounds of series **9**(**a**–**q**) ranged in the narrow nanomolar affinity range (K_d: 1.0–8.3 nM). The compounds of series **10**(**d**–**p**) bound CA XIII with K_d values of 0.8–25 nM. In this series of derivatives, compounds **9h** and **10h**, which bound CA XIII (K_d: 3.3 and 3.6 nM, respectively), also strongly inhibited the cancer-associated isoform CA XII with K_d values of 6.7 and 3.3 nM, respectively. Compound **9h** bound the cytosolic isoform CA I 49-fold weaker, whereas **10h** binding to CA I was more than 215-fold weaker than for CA XII. However, the most interesting feature of compounds **10**(**d**–**p**) was that they were much better inhibitors of CA XII and XIII than nonsubstituted compound **10**. Compound **10i** was the most potent and selective inhibitor of CA XIII (K_d = 0.8 nM), whereas **9b** was most potent and selective for CA VII (K_d = 0.22 nM).

CA II inhibition (IC_{50}) showed that the position of the substituent R on the 4-substituted benzenesulfonamide ring strongly affects enzyme activity (Figure 3). The IC_{50} value for compounds **2h** and **10h** were 910 and 29 nM, respectively.

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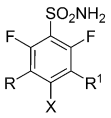
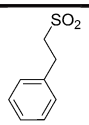
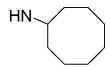
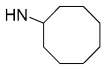
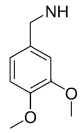
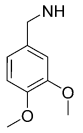
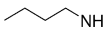
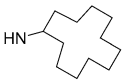
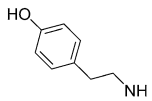
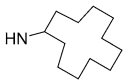
Table 4. 3,4,5-trisubstituted-2,6-difluorobenzenesulfonamide dissociation constants for seven human recombinant CA isoform catalytic domains.



Compd	X	R	R¹	K_d [nM] for CA isoforms[a]						
				CA I	CA II	CA VA	CA VI	CA VII	CA XII	CA XIII
11h				> 200 000	> 200 000	25 000	> 200 000	> 200 000	> 200 000	> 200 000
12k				5000	5000	2500	17 000	3300	10 000	250
13c				33 000	1400	33 000	10 000	> 200 000	140	330
13f				> 200 000	> 200 000	33 000	5600	> 200 000	500	330

[a] Determined by FTSA at 37 °C, pH 7.0; uncertainties of the FTSA measurements do not exceed 1.6-fold K_d.

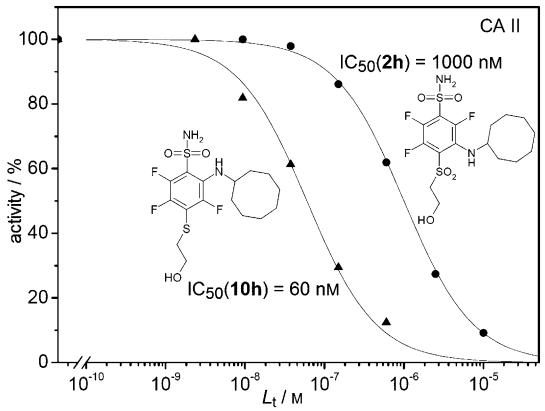


Figure 3. Inhibition curves of CA II catalytic activity by **2h** and **10h** as determined by stopped-flow CO_2 hydration assay.

These values are very close to those determined by FTSA (1300 and 59 nM, respectively). CA inhibition assay confirmed that *ortho*-substituted compounds are less potent inhibitors of CAs than the *meta*-substituted benzenesulfonamides.

Several double *meta*-substituted compounds bearing the same (**11h**, **12k**) or different (**13c**, **13f**) substituents at these positions were synthesized and evaluated (Table 4). The double substitution at the *meta* position resulted in much weaker-binding compounds relative to their single *meta*-substituted analogues. Steric reasons may play a role in the significantly decreased activity of these compounds. For example, compound **10h** was found to be a nanomolar inhibitor of CAs (except CA VA), whereas introduction of the second cyclooctylamine group at the *meta* position resulted in a completely ineffective compound **11h** ($K_d \geq 200$ μM for all tested CA isoforms). Compound **12k**, bearing a more flexible group at the *meta* position than **11h**, showed micromolar binding affinity for all CAs (K_d from 17 μM for CA VI to 2.5 μM for CA VA), whereas its affinity for CA XIII was notably higher (K_d = 250 nM). Compounds **13c** and **13f** bound CA XII and XIII significantly better (K_d values in the range of 140–500 nM) than the other tested CAs. Compound **13c** could be distinguished as the most effective and selective CA XII inhibitor in this group of compounds.

Crystal structures

The binding modes of three inhibitors, **9d**, **10d**, and **10p**, in the active centers of CA II, XII, and XIII were determined by X-ray crystallography. The CA II–**10p** as CA XII complexes had four, whereas CA XIII usually had two protein chains in the asymmetric unit, and binding of the ligand was slightly different between subunits. For subsequent analysis, the protein chains that represent different ligand positions or where the ligand has alternate orientations, as in the CA XII–**10d** complex, were used. Selected protein chains are shown in bold in Supporting Information (SI) Table S1. The residues are numbered as in the corresponding crystal structures. The structurally coinciding residues in CA II, XII, and XIII ligand binding pockets can be found in SI Table S2.

Compound **10d** in CA XII in all subunits is in a similar position, except for protein chain A, where it has two alternate positions that differ with respect to the orientation of the *para* substituent (2-hydroxyethyl)sulfonyl group (Figure 4B). One of the alternative positions allows a hydrogen bond between the hydroxy group and Pro200 and with Gln89. The most remarkable feature of the CA XII active site is the absence of Phe131

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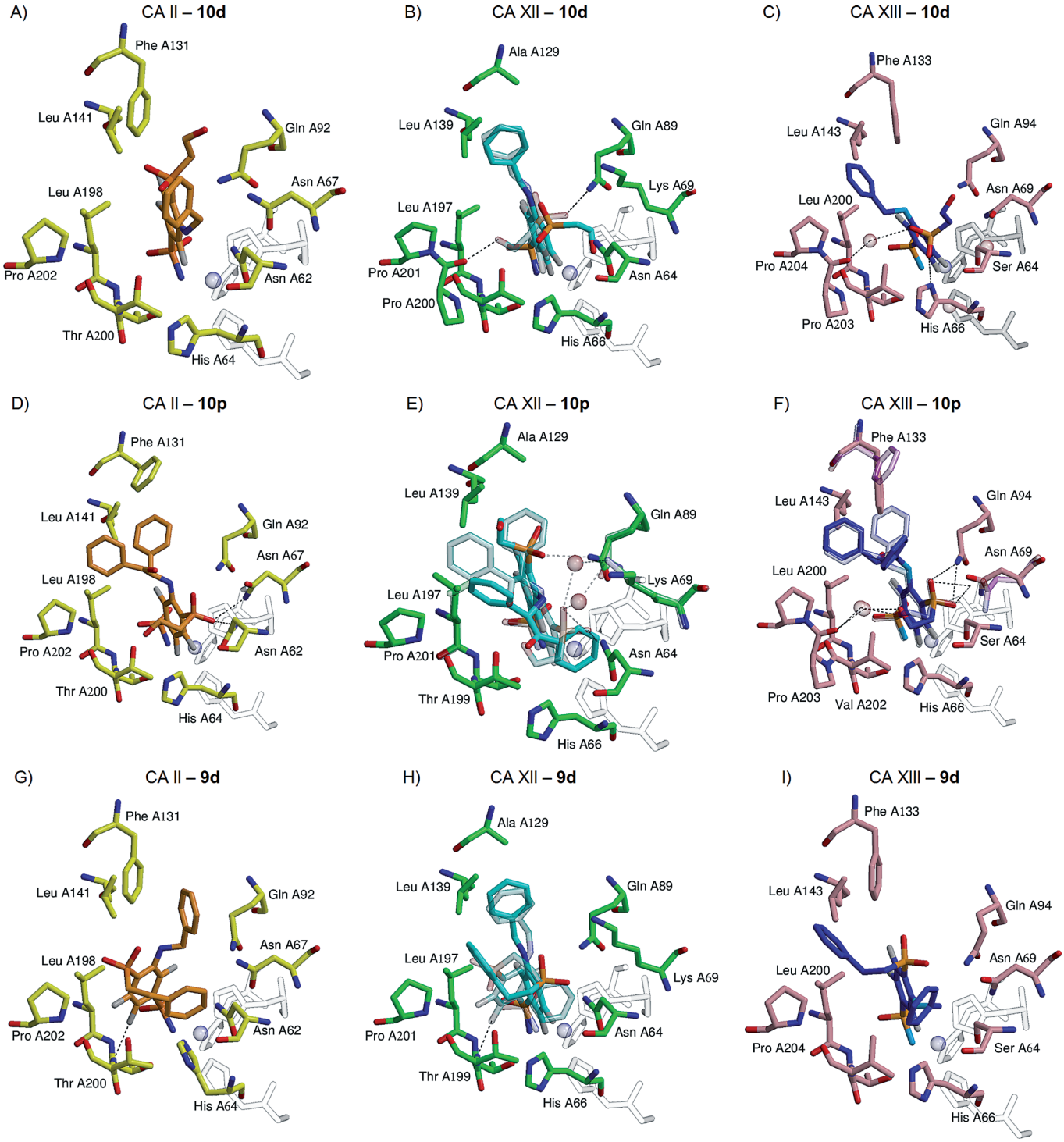

Figure 4. Binding of **10d** (A–C), **10p** (D–F), and **9d** (G–I) in the active sites of CA II (A, D, G), CA XII (B, E, H), and CA XIII (C, F, I). Histidine residues that coordinate Zn^{2+} ions are transparent, while the Zn^{2+} ions themselves are shown as grey semitransparent spheres. Protein residues are shown in yellow for CA II, green for CA XII, and pink for CA XIII. Alternate conformations of the protein amino acid side chains are rendered transparent. Inhibitors bound CA II are colored orange. In the crystal structures of CA XII, the alternate conformations in chain A (crystal CA XII–**10d**) are colored cyan and transparent cyan. Positions of ligands **10p** and **9d** from both representative protein chains of CA XII are also shown in cyan and transparent cyan for the protein chains A and B, respectively. Ligands bound to CA XIII are colored blue. In panel F, the positions of **10p** are shown for both protein chains of CA XIII (blue for chain A and transparent blue for chain B). Hydrogen bonds are shown as dashed lines.

analogue, which is replaced by an alanine residue (Ala129). Therefore, bulky substituents at the *meta* position of the fluorobenzene ring are often oriented in CA XII toward the alanine,[47] as in this case. In CA II and XIII, where Phe131 is present, the fluorobenzene rings of **10d** are shifted; they are in the same plane as in CA XII (Figure 4A,C, SI Figure S1). In contrast to CA XII and XIII, the *N*-benzylamine group of **10d** in CA II is rotated toward the opposite side of the ligand binding pocket

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(SI Figure S1 and Figure 4A). In CA XIII, this group interacts hydrophobically with Pro204 and Phe133, whereas the *para* substituent (2-hydroxyethyl)sulfonyl group makes a hydrogen bond with His66 (Figure 4C) and water-mediated hydrogen bond with Pro203.

Compound **10p** carries the bulkiest *meta* substituent [(1*R*,2*S*)-2-hydroxy-1,2-diphenylethyl]amino group. The K_d values of both compounds **10d** and **10p** in CA II (K_d: 83 nM for **10d** and 50 nM for **10p**), XII (25 nM for **10d** and 48 nM for **10p**) and XIII (14 nM for **10d** and 7.1 nM for **10p**) were similar (Table 3). The electron density of this compound is very good in all crystal structures (SI Figure S2, D–F). In the CA II–**10p** crystal structure in all protein subunits, the ligand was found in the same orientation that coincides with **10p** in CA XII protein chain B, and CA XIII protein chain B (Figure 4D–F and SI Figure S3B). This orientation is considered to be the main one. Here, the phenyl of the bulky *meta* group is fixed by van der Waals contacts between Phe131 (in CA II, Ala129 in CA XII, and Phe133 in CA XIII) and Pro202 (in CA II, Pro201 in CA XII, and Pro204 in CA XIII) at the entrance into the active site of all three isoforms. In CA II and CA XIII, Phe131 (Phe133 in CA XIII) is turned away from the active site, making more space for the *meta* group. The second phenyl of the *meta* group is fixed between the same phenylalanine residue (Ala129 in CA XII) and hydrophobic side chains of Val121 (Val119 in CA XII and Val123 in CA XIII), Leu198 (Leu197 in CA XII and Leu200 in CA XIII), and Leu141 (Leu139 in CA XII and Leu143 in CA XIII). The flexible (2-hydroxyethyl)sulfonyl group at the *para* position is directed in a similar way in this orientation (it could not be bound in the opposite orientation due to steric clash with the wall of the binding pocket in all three isoforms; SI Figure S3B). Interestingly, in the CA XIII chain A, the *meta* group is found to face the solvent, allowing Phe133 to adopt its usual position (Figure 4F). An alternate position of **10p** is found in the A chain of CA XII (SI Figure S3A). The *meta* group is directed toward the opposite wall of the ligand binding pocket and has van der Waals contacts with His91, Asn64, Ser67, Lys69, Leu197, Thr199, Pro201, and Trp4. These interactions push the fluorobenzene ring in the opposite direction, in the same plane toward Leu139 (SI Figure S3A).

From comparison of these compounds one can come to the conclusion that the interaction between ligands and CAs is mostly hydrophobic; the substituents at the *meta* position define the position of the first ring. In most cases, the first ring is positioned in the same plane with the nitrogen atom of sulfonamide (these are referred to as "N-main" and "N-shift" in SI Table S1). In the case of such an orientation of the fluorobenzene ring the *meta* group of **10p** pushes Phe131 (Phe133 in CA XIII, chain B) away from the active center (Figure 4F). The group at the *para* position does not influence binding, although it can make occasional hydrogen bonds with the protein moiety (with Asn64 or Gnl89 in CA XII, Figure 4E) and it interacts mostly with solvent.

To evaluate the influence of the *para* substituent on the positioning of the ligand in the active sites of CA isoforms the binding of **10d** was compared with that of **9d** (Figure 4G–I, SI Figure S4), which has a large phenyl group instead of a small hydroxy group. With respect to the orientation of the fluorobenzene ring, **9d** shows remarkable orientational flexibility (SI Table S1).

The position of the fluorobenzene ring of **9d** and **10d** in CA II differs (SI Figure S4A). Compound **9d** makes hydrogen bonds with the protein main chain and Thr200. In the crystal structure with CA XII (Figure 4H), which has four protein chains per asymmetric unit, in two of them (protein chains B and D) the fluorobenzene ring of **9d** is found in the same orientation as in CA II ("O–H bond" position in SI Table S1). In CA XII (chains A and C) the fluorobenzene rings are oriented along the nitrogen atom of sulfonamide and shifted toward His66 (Figure 4H), similar to **9d** in CA XIII (Figure 4I), which is bound uniformly in both protein chains. The orientation of the compound itself when bound in CA II and CA XII (chains B and D) exhibits stacking interactions between the *para*-phenyl and fluorobenzene ring (Figure 4G–H). The *para* phenyl in this orientation makes van der Waals contacts with the ligand binding pocket (His66, Ser67, Asn64 in CA XII). The *meta* substituent interacts with the loop carrying Ala129 in CA XII (Figure 4H), whereas in CA II it is shifted to avoid a steric clash with Phe131 (Figure 4G). In CA XIII, the first ring of both **10d** and **9d** is positioned in the same plane with the nitrogen atom of the sulfonamide, and **10d** is shifted toward His66 (SI Table S1, Figure S4B). These two crystal structures nicely illustrate two interesting points: First, the presence of valine residue in CA XIII instead of threonine in CA II and CA XII could be the reason why none of the compounds was found with its fluorobenzene ring positioned along sulfonamide oxygen (the orientation that allows formation of hydrogen bonds between the fluorobenzene ring and protein, "O–H bond" in SI Table S1). Such an orientation was found in **9d**–CA II and in **9d**–CA XII crystal structures (Figure 4G–H). Second, the *para*-phenyl group of **9d** is located in the same position as the *meta*-phenyl in **10d**, that is, between Pro204 and Phe133. The *meta*-phenyl of **9d** overlaps in space with the hydroxy group of **10d** and is located between His66, Ser64, and Asn69. In common, **9d** undergoes more van der Waals contacts with the protein moiety than **10d**, which could explain the better binding of this ligand with CA II (K_d: 6.7 nM for **9d** and 83 nM for **10d**) and CA XIII (K_d by FTSA: 2.5 nM for **9d** and 14 nM for **10d**).

Compounds **10d** and **10p** carry different *meta* substituents but the same group at the *para* position. In CA XII–**10p**, the *meta* group is found in two orientations (SI Figure S5A–B). In the protein chain A of CA XII–**10p**, the *meta* substituents of **10p** are oriented toward Ala129, opposite to **10d** bearing *N*-benzylamine at the *meta* position. The bulky group displaces the fluorobenzene ring of **10p** toward Ala129 ("N-shift" position, SI Table S1). In the protein chain B of the **10p**–CA XII complex, the *meta* group is oriented similarly to **10d** in CA XII (SI Figure S5B). No significant displacement of the fluorobenzene ring occurs in this case, as there are no steric conflicts of the *meta* substituent with protein residues. Interestingly, this location of **10p** in CA XII nearly coincides with that in the protein chain B of the **10p**–CA XIII complex. In this case, one of the phenyl groups of **10p** overlaps with the *N*-benzylamine group of **10d** in CA XIII (SI Figure S5C).

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Both the *meta*- and *para*-position substituents were found to influence compound affinities and selectivities toward a target CA isoform. No general tendencies could be drawn based on three compound structures with three isoforms, as every substituent had a particular effect on each CA isoform. However, *ortho* substituents significantly diminish affinities toward most CA isoforms relative to *meta* substituents.

Conclusions

The *ortho*-substituted compounds bearing a *para*-phenylethylthio group (compounds of series **1**) bound CA I, II, VA, VI, VII, XII, and XIII with lower affinity than ligands carrying *para*-hydroxyethylthio (series **2**) or no *para* group (series **3**). Both *para* groups had a moderate effect on the selectivity toward CA XIII, which is a common feature of all *ortho*-substituted ligands, because the absence of *para* substitution results in stronger binding of CA I and II, thus leading to the loss of observed selectivity.

The chemical nature of *ortho* substituents influenced both affinity and selectivity. The bulkier substituents usually decreased binding affinity and could lead to better selectivity toward CA XIII, and in few cases for other isoforms.

The nature and size of the substituent at the *meta* position of the fluorinated benzenesulfonamide ring did not significantly influence binding affinity for CA XIII, but had a strong effect on the binding to other tested CA isozymes. 3,4-Disubstituted-2,5,6-trifluorobenzensulfonamides showed moderate-to-weak inhibition of CA I, but nanomolar binding to CA II, VII, tumor-associated XII, and CA XIII.

The crystal structures of **9d**, **10d**, and **10p** in CA II, CA XII, and CA XIII showed that all ligands were bound in the active site mostly hydrophobically. The fluorobenzene ring of **9d** in CA II and XII was found in two alternate positions, one of which allows the formation of hydrogen bonds between fluorine and the protein (residues 199–200 in CA II). In CA XIII, there is a Val202 instead of Thr200, and therefore this orientation was not observed in CA XIII.

Experimental Section

Chemistry

All starting materials and reagents were commercial products and were used without further purification. Melting points of the compounds were determined in open capillaries on a Thermo Scientific 9100 Series, and are uncorrected. Column chromatography was performed using silica gel 60 (0.040–0.063 mm, Merck). ^{1}H and ^{13}C NMR spectra were recorded on Varian Unity Inova (300 and 75 MHz, respectively) or Bruker (400 and 100 MHz, respectively) spectrometers with TMS as an internal standard; chemical shifts are expressed in parts per million (ppm) in the indicated solvent. ^{19}F NMR spectra were recorded on Varian Unity Inova (282 MHz) or Bruker (376 MHz) spectrometers with $CFCl_3$ as an internal standard, and fluorine chemical shifts are expressed in ppm in the indicated solvent. Multiplicity is defined as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), ddd (double double doublet), m (multiplet), br s (broad singlet), br d (broad doublet) or br t (broad triplet). TLC was performed with silica gel 60 F_{254} aluminum plates (Merck) and visualized with UV light. High-resolution mass spectra (HRMS) were recorded on a Dual-ESI Q-TOF 6520 mass spectrometer (Agilent Technologies). The purity of final compounds was verified by HPLC to be >95% using an Agilent 1290 Infinity instrument with a Poroshell 120 SB-C_{18} (2.1 mm×100 mm, 2.7 μm) reversed-phase column. Analytes were eluted using a linear gradient of H_2O/MeOH (20 mM ammonium formate in both phases) from 60:40 to 30:70 over 12 min, then from 30:70 to 20:80 over 1 min, and then 20:80 over 5 min at a flow rate of 0.2 mL min^{-1}. UV detection was at λ 254 nm.

General procedure for the syntheses of 1(a–o). A mixture of 2,3,5,6-tetrafluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (compound **1**) (0.20 g, 0.55 mmol), Et_3N (0.080 mL, 0.57 mmol), DMSO (1 mL) and appropriate nucleophile (0.57 mmol) was stirred at 60 °C for 16 h, compounds **1n**,**o** were obtained after 40 h, compound **1e** was obtained after stirring at 70 °C for 26 h. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (3× 10 mL). The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure.

2-(Isopropylamino)-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1a). The product was purified by chromatography on a column of silica gel with EtOAc (5%)/$CHCl_3$, R_f=0.51; yield: 0.12 g, 55%; mp: 47–48 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.23 (6H, dd, ^1J=6.3 Hz, ^2J=1.2 Hz, $2CH_3$), 2.94 (2H, t, J=7.8 Hz, SCH_2CH_2), 3.28 (2H, t, J=7.8 Hz, SCH_2CH_2), 3.84–3.95 (1H, m, CH), 5.52 (2H, br s, SO_2NH_2), 7.17–7.44 ppm (5H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=24 ($2CH_3$), 35.4 (SCH_2CH_2, t, $J_{^{19}F-^{13}C}$=3.7 Hz), 36.8 (SCH_2CH_2), 48.4 (CH, d, $J_{^{19}F-^{13}C}$=11 Hz), 117.4 (C1, dd, $^1J_{^{19}F-^{13}C}$=12 Hz, $^2J_{^{19}F-^{13}C}$=5 Hz), 119.9 (C4, t, $J_{^{19}F-^{13}C}$=21 Hz), 127.0 (Ar), 128.8 (Ar), 132.6 (C2, d, $J_{^{19}F-^{13}C}$=16 Hz), 139.4 (Ar), 142.2 (C5, ddd, $^1J_{^{19}F-^{13}C}$=240 Hz, $^2J_{^{19}F-^{13}C}$=16 Hz, $^3J_{^{19}F-^{13}C}$=5 Hz), 145.0 (C6, ddd, $^1J_{^{19}F-^{13}C}$=247 Hz, $^2J_{^{19}F-^{13}C}$=16 Hz, $^3J_{^{19}F-^{13}C}$=5 Hz), 148.6 ppm (C3, d, $J_{^{19}F-^{13}C}$=243 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ=−125.1 (C3-F, d, J=11 Hz), −143.4 (C5-F, dd, ^1J=27 Hz, ^2J=12 Hz), −148.5 ppm (C6-F, d, J=26 Hz); HRMS for $C_{17}H_{19}F_3N_2O_2S_2$ $[M+H]^+$: calcd 405.0913, found 405.0918.

2-(Benzylamino)-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1b). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:14), R_f=0.45; yield: 0.15 g, 60%; mp: 94–95 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ=2.74 (2H, t, J=7.5 Hz, SCH_2CH_2), 3.19 (2H, t, J=7.5 Hz, SCH_2CH_2), 4.51 (2H, dd, ^1J=6.3 Hz, ^2J=4.2 Hz, $NHCH_2$), 6.81 (1H, td, ^1J=6.3 Hz, ^2J=1.8 Hz, NH), 7.07–7.43 (10H, m, ArH), 8.20 (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D_6]DMSO): δ=35.3 (SCH_2CH_2, t, $J_{^{19}F-^{13}C}$=3.2 Hz), 36.3 (SCH_2CH_2), 50.5 ($NHCH_2$, d, $J_{^{19}F-^{13}C}$=12 Hz), 118.0 (C4, t, $J_{^{19}F-^{13}C}$=19 Hz), 119.2 (C1, dd, $^1J_{^{19}F-^{13}C}$=12 Hz, $^2J_{^{19}F-^{13}C}$=4.5 Hz), 127.1 (Ar), 127.9 (Ar), 128.1 (Ar), 129.0 (Ar), 129.1 (Ar), 129.2 (Ar), 132.9 (C2, d, $J_{^{19}F-^{13}C}$=14 Hz), 139.8 (Ar), 140.1 (Ar), 141.8 (C5, d, $J_{^{19}F-^{13}C}$=234 Hz), 144.8 (C6, d, $J_{^{19}F-^{13}C}$=261 Hz), 148.1 (C3, d, $J_{^{19}F-^{13}C}$=242 Hz); ^{19}F NMR (282 MHz, [D_6]DMSO): δ=−121.2 (C3-F, d, J=9 Hz), −138.1 (C5-F, dd, ^1J=27 Hz, ^2J=12 Hz), −145.4 ppm (C6-F, d, J=27 Hz); HRMS $C_{21}H_{19}F_3N_2O_2S_2$ $[M+H]^+$: calcd 453.0913, found 453.0917.

2-[(2-Phenylethyl)amino]-3,5,6-trifluoro-4-[(2-phenylethyl)thio]-benzenesulfonamide (1c). The product was purified by chromatography on a column of silica gel with EtOAc (10%)/$CHCl_3$, R_f=0.53; yield: 0.24 g, 92%; mp: 90–91 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.89–3.01 (4H, m, SCH_2CH_2, $NHCH_2CH_2$), 3.29 (2H, t, J=7.8 Hz, SCH_2CH_2), 3.62–3.75 (2H, m, $NHCH_2$), 5.21 (2H, s, SO_2NH_2), 7.17–7.42 ppm (10H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=35.4 (SCH_2CH_2, t, $J_{^{19}F-^{13}C}$=4 Hz), 36.8 (SCH_2CH_2), 37.1 ($NHCH_2CH_2$), 48.2 ($NHCH_2$, d, $J_{^{19}F-^{13}C}$=11.5 Hz), 116.3 (C1, dd, $^1J_{^{19}F-^{13}C}$=12 Hz, $^2J_{^{19}F-^{13}C}$=

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5 Hz), 120.0 (C4, t, $J_{^{19}F-^{13}C}$ = 22 Hz), 126.9 (Ar), 127 (Ar), 128.8 (Ar), 128.9 (Ar), 129.2 (Ar), 133.1 (C2, d, $J_{^{19}F-^{13}C}$ = 14 Hz), 139.0 (Ar), 139.4 (Ar), 141.9 (C5, ddd, $^1J_{^{19}F-^{13}C}$ = 242 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz, $^3J_{^{19}F-^{13}C}$ = 5 Hz), 145.0 (C6, ddd, $^1J_{^{19}F-^{13}C}$ = 253 Hz, $^2J_{^{19}F-^{13}C}$ = 11 Hz, $^3J_{^{19}F-^{13}C}$ = 4 Hz), 148.2 ppm (C3, d, $J_{^{19}F-^{13}C}$ = 242 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −126.5 (C3-F, d, J = 11 Hz), −143.6 (C5-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −149.1 ppm (C6-F, d, J = 28 Hz); HRMS for $C_{22}H_{21}F_3N_2O_2S_2$ $[M+H]^+$: calcd 467.1069, found 467.1077.

2-[(1-Phenylethyl)amino]-3,5,6-trifluoro-4-[(2-phenylethyl)thio]-benzenesulfonamide (1 d). The product was purified by chromatography on a column of silica gel with EtOAc (5%)/$CHCl_3$, R_f = 0.75; yield: 0.15 g, 58%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.59 (3H, dd, ^1J = 6.6 Hz, ^2J = 1.2 Hz, CH_3), 2.73–2.82 (2H, m, SCH_2CH_2), 3.13 (2H, t, J = 8.4 Hz, SCH_2CH_2), 4.85–4.95 (1H, m, CH), 5.33 (2H, s, SO_2NH_2), 7.07–7.38 ppm (10H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.6 (CH_3), 35.5 (SCH_2CH_2, t, $J_{^{19}F-^{13}C}$ = 3.5 Hz), 36.7 (SCH_2CH_2), 56.4 (NHCH, d, $J_{^{19}F-^{13}C}$ = 12 Hz), 117.5 (C1, dd, $^1J_{^{19}F-^{13}C}$ = 12 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 119.8 (C4, t, $J_{^{19}F-^{13}C}$ = 19.5 Hz), 126.3 (Ar), 126.9 (Ar), 127.7 (Ar), 128.8 (Ar), 128.9 (Ar), 132.3 (C2, d, $J_{^{19}F-^{13}C}$ = 13 Hz), 139.4 (Ar), 142.4 (C5, ddd, $^1J_{^{19}F-^{13}C}$ = 240 Hz, $^2J_{^{19}F-^{13}C}$ = 15 Hz, $^3J_{^{19}F-^{13}C}$ = 5 Hz), 144.9 (C6, ddd, $^1J_{^{19}F-^{13}C}$ = 248 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz, $^3J_{^{19}F-^{13}C}$ = 4 Hz), 148.8 ppm (C3, d, $J_{^{19}F-^{13}C}$ = 244 Hz);^{19}F NMR (282 MHz, $CDCl_3$): δ = −122.5 (C3-F, d, J = 11 Hz), −143.2 (C5-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −147.4 ppm (C6-F, d, J = 26 Hz); HRMS for $C_{22}H_{21}F_3N_2O_2S_2$ $[M+H]^+$: calcd 467.1069, found 467.1069.

2-Morpholin-4-yl-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1 e). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:3), R_f = 0.38; yield: 0.10 g, 42%; mp: 149–150 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.82–3.05 (4H, m, SCH_2CH_2, morpholine), 3.33 (2H, t, J = 7.8 Hz, SCH_2CH_2), 3.48 (2H, t, J = 11.4 Hz, morpholine), 3.73 (2H, t, J = 11.4 Hz, morpholine), 4.00 (2H, d, J = 11.4 Hz, morpholine), 6.12 (2H, s, SO_2NH_2), 7.15–7.35 ppm (5H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 35.3 (SCH_2CH_2, t, $J_{^{19}F-^{13}C}$ = 4 Hz), 36.9 (SCH_2CH_2), 51.3 (morpholine, d, $J_{^{19}F-^{13}C}$ = 6 Hz), 67.7 (morpholine), 120.3 (C4, t, $J_{^{19}F-^{13}C}$ = 21 Hz), 127.1 (Ar), 128.8 (Ar), 128.9 (Ar), 129.5 (C2, d, $J_{^{19}F-^{13}C}$ = 7 Hz), 131.7 (C1, dd, $^1J_{^{19}F-^{13}C}$ = 16 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 139.1 (Ar), 143.9 (C5, ddd, $^1J_{^{19}F-^{13}C}$ = 260 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz, $^3J_{^{19}F-^{13}C}$ = 5 Hz), 149.6 (C6, ddd, $^1J_{^{19}F-^{13}C}$ = 250 Hz, $^2J_{^{19}F-^{13}C}$ = 17 Hz, $^3J_{^{19}F-^{13}C}$ = 6 Hz), 157.8 ppm (C3, d, $J_{^{19}F-^{13}C}$ = 251 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −118.8 (C3-F, d, J = 13 Hz), −131.5 (C6-F, d, J = 25 Hz), −143.0 ppm (C5-F, d, ^1J = 24 Hz, ^2J = 13 Hz); HRMS for $C_{18}H_{19}F_3N_2O_3S_2$ $[M+H]^+$: calcd 433.0862, found 433.0863.

2-(Cyclohexylamino)-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1 f). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:9), R_f = 0.63; yield: 0.12 g, 50%; mp: 62–63 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.14–1.47 (5H, m, cyclohexane), 1.57–1.69 (1H, m, cyclohexane), 1.72–1.85 (2H, m, cyclohexane), 1.92–2.05 (2H, m, cyclohexane), 2.93 (2H, t, J = 7.8 Hz, SCH_2CH_2), 3.27 (2H, t, J = 7.8 Hz, SCH_2CH_2), 3.6–3.7 (1H, m, CH of cyclohexane), 5.57 (2H, s, SO_2NH_2), 6.16 (1H, br s, NH), 7.15–7.38 ppm (5H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.0 (cyclohexane), 25.8 (cyclohexane), 34.4 (cyclohexane), 35.4 (SCH_2CH_2, t, $J_{^{19}F-^{13}C}$ = 4 Hz), 36.8 (SCH_2CH_2), 55.3 (CH of cyclohexane, d, $J_{^{19}F-^{13}C}$ = 11 Hz), 117.2 (C1, dd, $^1J_{^{19}F-^{13}C}$ = 12 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 119.6 (C4, t, $J_{^{19}F-^{13}C}$ = 22 Hz), 127.0 (Ar), 128.8 (Ar), 132.6 (C2, d, $J_{^{19}F-^{13}C}$ = 15 Hz), 139.4 (Ar), 142.0 (C5, ddd, $^1J_{^{19}F-^{13}C}$ = 240 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz, $^3J_{^{19}F-^{13}C}$ = 5 Hz), 145.0 (C6, ddd, $^1J_{^{19}F-^{13}C}$ = 248 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz, $^3J_{^{19}F-^{13}C}$ = 4 Hz), 148.5 ppm (C3, d, $J_{^{19}F-^{13}C}$ = 243 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −125.1 (C3-F, d, J = 10 Hz), −143.5 (C5-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −149 ppm (C6-F, d, J = 27 Hz); HRMS for $C_{20}H_{23}F_3N_2O_2S_2$ $[M+H]^+$: calcd 445.1226, found 445.1235.

2-(Cycloheptylamino)-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1 g). The product was purified by chromatography on a column of silica gel with EtOAc (5%)/$CHCl_3$, R_f = 0.63; yield: 0.12 g, 48%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37–1.82 (10H, m, cycloheptane), 1.89–2.06 (2H, m, cycloheptane), 2.94 (2H, t, J = 7.8 Hz, SCH_2CH_2), 3.27 (2H, t, J = 7.8 Hz, SCH_2CH_2), 3.67–3.83 (1H, m, CH of cycloheptane), 5.57 (2H, s, SO_2NH_2), 6.01 (1H, br s, NH), 7.17–7.38 ppm (5H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.1 (cycloheptane), 28.3 (cycloheptane), 35.4 (SCH_2CH_2, t, $J_{^{19}F-^{13}C}$ = 4 Hz), 36.1 (cycloheptane), 36.8 (SCH_2CH_2), 57.5 (CH of cycloheptane, d, $J_{^{19}F-^{13}C}$ = 10 Hz), 117.2 (C1, dd, $^1J_{^{19}F-^{13}C}$ = 12 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 119.7 (C4, t, $J_{^{19}F-^{13}C}$ = 19 Hz), 127.0 (Ar), 128.8 (Ar), 132.5 (C2, d, $J_{^{19}F-^{13}C}$ = 15 Hz), 139.5 (Ar), 142.0 (C5, ddd, $^1J_{^{19}F-^{13}C}$ = 240 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz, $^3J_{^{19}F-^{13}C}$ = 5 Hz), 145.0 (C6, ddd, $^1J_{^{19}F-^{13}C}$ = 250 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz, $^3J_{^{19}F-^{13}C}$ = 4 Hz), 148.5 ppm (C3, d, $J_{^{19}F-^{13}C}$ = 243 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −125.1 (C3-F, d, J = 11 Hz), −143.4 (C5-F, dd, ^1J = 26 Hz, ^2J = 12 Hz), −148.9 ppm (C6-F, d, J = 25 Hz); HRMS for $C_{21}H_{25}F_3N_2O_2S_2$ $[M+H]^+$: calcd 459.1382, found 459.1388.

2-(Cyclooctylamino)-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1 h). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:6), R_f = 0.8; yield: 0.14 g, 54%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43–1.81 (12H, m, cyclooctane), 1.85–2.01 (2H, m, cyclooctane), 2.94 (2H, t, J = 7.8 Hz, SCH_2CH_2), 3.27 (2H, t, J = 7.8 Hz, SCH_2CH_2), 3.75–3.92 (1H, m, CH of cyclooctane), 5.45 (2H, s, SO_2NH_2), 7.08–7.41 ppm (5H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.7 (cyclooctane), 25.8 (cyclooctane), 27.5 (cyclooctane), 33.1 (cyclooctane), 35.4 (SCH_2CH_2, t, $J_{^{19}F-^{13}C}$ = 4 Hz), 36.8 (SCH_2CH_2), 56.3 (CH of cyclooctane, d, $J_{^{19}F-^{13}C}$ = 10 Hz), 117.0 (C1, dd, $^1J_{^{19}F-^{13}C}$ = 12 Hz, $^2J_{^{19}F-^{13}C}$ = 6 Hz), 119.8 (C4, t, $J_{^{19}F-^{13}C}$ = 18 Hz), 127.0 (Ar), 128.8 (Ar), 132.6 (C2, d, $J_{^{19}F-^{13}C}$ = 15 Hz), 139.5 (Ar), 141.8 (C5, ddd, $^1J_{^{19}F-^{13}C}$ = 240 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz, $^3J_{^{19}F-^{13}C}$ = 5 Hz), 145.1 (C6, ddd, $^1J_{^{19}F-^{13}C}$ = 245 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz, $^3J_{^{19}F-^{13}C}$ = 4 Hz), 148.5 ppm (C3, d, $J_{^{19}F-^{13}C}$ = 243 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −124.9 (C3-F, d, J = 11 Hz), −143.4 (C5-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −149.2 ppm (C6-F, d, J = 25 Hz); HRMS for $C_{22}H_{27}F_3N_2O_2S_2$ $[M+H]^+$: calcd 473.1539, found 473.1548.

2-(Cyclododecylamino)-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1 i). The synthesis of compound **1 i** was described previously.[52]

2-[(2,6-Dimethoxybenzyl)amino]-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1 j). The product was purified by chromatography on a column of silica gel with EtOAc (5%)/$CHCl_3$, R_f = 0.53; yield: 0.20 g, 71%; mp: 118–119 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.96 (2H, t, J = 7.8 Hz, SCH_2CH_2), 3.30 (2H, t, J = 7.8 Hz, SCH_2CH_2), 3.79 (6H, s, $2CH_3$), 4.53 (2H, d, J = 1.5 Hz, $NHCH_2$), 5.25 (2H, s, SO_2NH_2), 6.55 (2H, d, J = 8.4 Hz, ArH), 7.18–7.40 ppm (6H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 35.5 (SCH_2CH_2, t, $J_{^{19}F-^{13}C}$ = 4 Hz), 36.8 (SCH_2CH_2), 40.3 ($NHCH_2$, d, $J_{^{19}F-^{13}C}$ = 11 Hz), 56.0 ($2CH_3$), 104.0 (Ar), 115.3 (Ar), 119.1 (C4, t, $J_{^{19}F-^{13}C}$ = 19 Hz), 119.6 (C1, dd, $^1J_{^{19}F-^{13}C}$ = 11 Hz, $^2J_{^{19}F-^{13}C}$ = 4 Hz), 127.0 (Ar), 128.8 (Ar), 128.9 (Ar), 129.6 (Ar), 133.6 (C2, dd, $^1J_{^{19}F-^{13}C}$ = 15 Hz, $^2J_{^{19}F-^{13}C}$ = 3.3 Hz), 139.5 (Ar), 143.3 (C5, ddd, $^1J_{^{19}F-^{13}C}$ = 242 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz, $^3J_{^{19}F-^{13}C}$ = 5 Hz), 144.4 (C6, ddd, $^1J_{^{19}F-^{13}C}$ = 251 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz, $^3J_{^{19}F-^{13}C}$ = 4 Hz), 150.5 (C3, d, $J_{^{19}F-^{13}C}$ = 245 Hz), 158.8 ppm (Ar); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −122.4 (C3-F, d, J = 12 Hz), −144.2 (C5-F, dd, ^1J = 24 Hz, ^2J = 12 Hz), −146.3 ppm (C6-F, d, J = 25 Hz); HRMS for $C_{23}H_{23}F_3N_2O_4S_2$ $[M+H]^+$: calcd 513.1124, found 513.1122.

2-[(3,4-Dimethoxybenzyl)amino]-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1 k). The product was purified by chromatography on a column of silica gel with EtOAc (5%)/$CHCl_3$, R_f = 0.43; yield: 0.13 g, 46%; mp: 104–105 °C; ^{1}H NMR (300 MHz,

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$CDCl_3$): $\delta = 2.87$ (2H, t, $J = 7.8$ Hz, SCH_2CH_2), 3.22 (2H, t, $J = 8.1$ Hz, SCH_2CH_2), 3.81 (3H, s, CH_3), 3.88 (3H, s, CH_3), 4.43 (2H, d, $J = 3.3$ Hz, $NHCH_2$), 5.41 (2H, s, SO_2NH_2), 6.76 (1H, d, $J = 8.1$ Hz, ArH), 6.83–6.92 (2H, m, ArH), 7.14–7.36 ppm (5H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 35.4$ (SCH_2CH_2, t, $J_{^{19}F-^{13}C} = 3.5$ Hz), 36.8 (SCH_2CH_2), 51.0 ($NHCH_2$, d, $J_{^{19}F-^{13}C} = 12$ Hz), 56.0 (CH_3), 56.1 (CH_3), 111.2 (Ar), 117.6 (C1, dd, $^1J_{^{19}F-^{13}C} = 12$ Hz, $^2J_{^{19}F-^{13}C} = 5$ Hz), 119.8 (C4, t, $J_{^{19}F-^{13}C} = 21$ Hz), 120.4 (Ar), 127.0 (Ar), 128.8 (Ar), 131.6 (Ar), 132.8 (C2, d, $J_{^{19}F-^{13}C} = 15$ Hz), 139.4 (Ar), 142.4 (C5, ddd, $^1J_{^{19}F-^{13}C} = 240$ Hz, $^2J_{^{19}F-^{13}C} = 16$ Hz, $^3J_{^{19}F-^{13}C} = 5$ Hz), 144.8 (C6, ddd, $^1J_{^{19}F-^{13}C} = 250$ Hz, $^2J_{^{19}F-^{13}C} = 16$ Hz, $^3J_{^{19}F-^{13}C} = 4$ Hz), 148.6 (Ar), 148.9 (C3, d, $J_{^{19}F-^{13}C} = 243$ Hz), 149.3 ppm (Ar); ^{19}F NMR (282 MHz, $CDCl_3$): $\delta = -123.9$ (C3-F, d, $J = 13$ Hz), -143.5 (C5-F, dd, $^1J = 27$ Hz, $^2J = 12$ Hz), -147.7 ppm (C6-F, d, $J = 26$ Hz); HRMS for $C_{23}H_{23}F_3N_2O_4S_2$ $[M-H]^-$: calcd 511.0979, found 511.0982.

2-(2,3-Dihydro-1*H*-inden-2-ylamino)-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1l). The product was purified by chromatography on a column of silica gel with EtOAc (10%)/$CHCl_3$, $R_f = 0.55$; yield: 0.19 g, 73%; mp: 73–74 °C; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 2.85$–3.05 (4H, m, SCH_2CH_2, CH_2 of indane), 3.25–3.39 (4H, m, SCH_2CH_2, CH_2 of indane), 4.58–4.68 (1H, m, NHCH), 5.12 (2H, s, SO_2NH_2), 7.19–7.41 ppm (9H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 35.4$ (SCH_2CH_2, t, $J_{^{19}F-^{13}C} = 4$ Hz), 36.9 (SCH_2CH_2), 41.2 (CH_2 of indane), 57.8 (NHCH, d, $J_{^{19}F-^{13}C} = 11$ Hz), 117.2 (C1, dd, $^1J_{^{19}F-^{13}C} = 12$ Hz, $^2J_{^{19}F-^{13}C} = 5.5$ Hz), 120.1 (C4, t, $J_{^{19}F-^{13}C} = 22$ Hz), 125.2 (Ar), 127 (Ar), 127.2 (Ar), 128.8 (Ar), 132.2 (C2, d, $J_{^{19}F-^{13}C} = 15$ Hz), 139.4 (Ar), 141.1 (Ar), 142.2 (C5, ddd, $^1J_{^{19}F-^{13}C} = 239$ Hz, $^2J_{^{19}F-^{13}C} = 16$ Hz, $^3J_{^{19}F-^{13}C} = 5$ Hz), 145.0 (C6, ddd, $^1J_{^{19}F-^{13}C} = 251$ Hz, $^2J_{^{19}F-^{13}C} = 12$ Hz, $^3J_{^{19}F-^{13}C} = 4$ Hz), 148.2 ppm (C3, d, $J_{^{19}F-^{13}C} = 242$ Hz); ^{19}F NMR (282 MHz, $CDCl_3$): $\delta = -126$ (C3-F, d, $J = 12$ Hz), -143.3 (C5-F, dd, $^1J = 27$ Hz, $^2J = 12$ Hz), -148.3 ppm (C6-F, d, $J = 25$ Hz); HRMS for $C_{23}H_{21}F_3N_2O_2S_2$ $[M+H]^+$: calcd 479.1069, found 479.1077.

2-[(1*S*)-2,3-Dihydro-1*H*-inden-1-ylamino]-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1m). The product was purified by chromatography on a column of silica gel with EtOAc (5%)/$CHCl_3$, $R_f = 0.53$; yield: 0.08 g, 31%; mp: 101–102 °C; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 2.05$ (1H, sext, $J = 6.6$ Hz, indane), 2.56 (1H, sext, $J = 6.9$ Hz, indane), 2.85–2.97 (1H, m, indane, signal overlaps with signal of SCH_2CH_2), 2.99 (2H, t, $J = 7.8$ Hz, SCH_2CH_2), 3.07–3.19 (1H, m, indane), 3.33 (2H, t, $J = 7.8$ Hz, SCH_2CH_2), 5.18 (3H, brs, SO_2NH_2, NHCH), 6.34 (1H, brs, NH), 7.18–7.41 ppm (9H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 30.3$ (indane), 35.0 (indane), 35.5 (SCH_2CH_2, t, $J_{^{19}F-^{13}C} = 3.8$ Hz), 36.9 (SCH_2CH_2), 62.3 (NHCH, d, $J_{^{19}F-^{13}C} = 11$ Hz), 117.6 (C1, dd, $^1J_{^{19}F-^{13}C} = 12$ Hz, $^2J_{^{19}F-^{13}C} = 5$ Hz), 120.1 (C4, t, $J_{^{19}F-^{13}C} = 22$ Hz), 124.3 (Ar), 125.3 (Ar), 126.9 (Ar), 127.0 (Ar), 128.4 (Ar), 128.8 (Ar), 132.9 (C2, dd, $^1J_{^{19}F-^{13}C} = 15$ Hz, $^2J_{^{19}F-^{13}C} = 3$ Hz), 139.4 (Ar), 142.5 (C5, ddd, $^1J_{^{19}F-^{13}C} = 240$ Hz, $^2J_{^{19}F-^{13}C} = 15$ Hz, $^3J_{^{19}F-^{13}C} = 4$ Hz), 143.7 (Ar), 144.3 (Ar), 145.0 (C6, ddd, $^1J_{^{19}F-^{13}C} = 248$ Hz, $^2J_{^{19}F-^{13}C} = 16$ Hz, $^3J_{^{19}F-^{13}C} = 5$ Hz), 148.8 ppm (C3, d, $J_{^{19}F-^{13}C} = 242$ Hz); ^{19}F NMR (282 MHz, $CDCl_3$): $\delta = -124.1$ (C3-F, d, $J = 12$ Hz), -143.1 (C5-F, dd, $^1J = 26$ Hz, $^2J = 12$ Hz), -147.9 ppm (C6-F, d, $J = 25$ Hz); H,C HETCOR (300 MHz, $CDCl_3$): $\delta = 35.5$–3.33 (SCH_2CH_2Ph), 62.3–5.18 ppm (NHCH); HRMS for $C_{23}H_{21}F_3N_2O_2S_2$ $[M+H]^+$: calcd 479.1069, found 479.1063.

2-[(1*S*)-1,2,3,4-Tetrahydronapthalen-1-ylamino)-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1n). The product was purified by chromatography on a column of silica gel with EtOAc (5%)/$CHCl_3$, $R_f = 0.79$; yield: 0.14 g, 52%; mp: 123–124 °C; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.78$–2.12 (4H, m, tetrahydronapthalene), 2.71–2.93 (2H, m, tetrahydronapthalene), 2.99 (2H, t, $J = 7.5$ Hz, SCH_2CH_2), 3.33 (2H, t, $J = 7.5$ Hz, SCH_2CH_2), 4.83 (1H, brs, NHCH), 5.10 (2H, s, SO_2NH_2), 6.26 (1H, brs, NH), 7.11–7.40 ppm (9H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 19.0$ (tetrahydronapthalene), 29.3 (tetrahydronapthalene), 30.3 (tetrahydronapthalene), 35.5 (SCH_2CH_2, t, $J_{^{19}F-^{13}C} = 4$ Hz), 36.9 (SCH_2CH_2), 54.7 (NHCH, d, $J_{^{19}F-^{13}C} = 11$ Hz), 118.2 (C1, dd, $^1J_{^{19}F-^{13}C} = 12$ Hz, $^2J_{^{19}F-^{13}C} = 5$ Hz), 120.0 (C4, t, $J_{^{19}F-^{13}C} = 21$ Hz), 126.2 (Ar), 127.0 (Ar), 127.7 (Ar), 128.9 (Ar), 129.1 (Ar), 129.7 (Ar), 132.6 (C2, d, $J_{^{19}F-^{13}C} = 15$ Hz), 137.57 (Ar), 137.6 (Ar), 139.4 (Ar), 142.7 (C5, ddd, $^1J_{^{19}F-^{13}C} = 240$ Hz, $^2J_{^{19}F-^{13}C} = 16$ Hz, $^3J_{^{19}F-^{13}C} = 5$ Hz), 145.0 (C6, ddd, $^1J_{^{19}F-^{13}C} = 249$ Hz, $^2J_{^{19}F-^{13}C} = 12$ Hz, $^3J_{^{19}F-^{13}C} = 4$ Hz), 149.3 ppm (C3, d, $J_{^{19}F-^{13}C} = 243$ Hz); ^{19}F NMR (282 MHz, $CDCl_3$): $\delta = -123.1$ (C3-F, d, $J = 11$ Hz), -142.9 (C5-F, dd, $^1J = 27$ Hz, $^2J = 12$ Hz), -147.4 ppm (C6-F, d, $J = 26$ Hz); HRMS for $C_{24}H_{23}F_3N_2O_2S_2$ $[M+H]^+$: calcd 493.1226, found 493.1222.

2-{[(1*R*,2*S*)-2-Hydroxy-1,2-diphenylethyl]amino}-3,5,6-trifluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (1o). The product was purified by chromatography on a column of silica gel with EtOAc (10%)/$CHCl_3$, $R_f = 0.37$; yield: 0.11 g, 35%; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 2.73$ (2H, t, $J = 7.5$ Hz, SCH_2CH_2), 3.08 (2H, m, SCH_2CH_2), 5.01 (1H, dd, $^1J = 5.4$ Hz, $^2J = 1.8$ Hz, CH), 5.06 (1H, d, $J = 4.8$ Hz, CH), 5.58 (2H, s, SO_2NH_2), 7.05–7.32 ppm (15H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 35.5$ (SCH_2CH_2, t, $J_{^{19}F-^{13}C} = 3.4$ Hz), 36.6 (SCH_2CH_2), 66.1 (NHCH, d, $J_{^{19}F-^{13}C} = 11$ Hz), 77.3 (CHOH, signal overlaps with $CDCl_3$ signal), 117.7 (C1, dd, $^1J_{^{19}F-^{13}C} = 12$ Hz, $^2J_{^{19}F-^{13}C} = 5$ Hz), 119.8 (C4, t, $J_{^{19}F-^{13}C} = 21$ Hz), 126.8 (Ar), 126.9 (Ar), 128.2 (Ar), 128.3 (Ar), 128.5 (Ar), 128.7 (Ar), 128.8 (Ar), 131.6 (C2, d, $J_{^{19}F-^{13}C} = 15$ Hz), 138.1 (Ar), 139.3 (Ar), 140.4 (Ar), 142.5 (C5, ddd, $^1J_{^{19}F-^{13}C} = 240$ Hz, $^2J_{^{19}F-^{13}C} = 16$ Hz, $^3J_{^{19}F-^{13}C} = 5$ Hz), 144.8 (C6, ddd, $^1J_{^{19}F-^{13}C} = 248$ Hz, $^2J_{^{19}F-^{13}C} = 12$ Hz, $^3J_{^{19}F-^{13}C} = 4$ Hz), 148.6 ppm (C3, d, $J_{^{19}F-^{13}C} = 243$ Hz); ^{19}F NMR (282 MHz, $CDCl_3$): $\delta = -122.6$ (C3-F, d, $J = 12$ Hz), -143.2 (C5-F, dd, $^1J = 25$ Hz, $^2J = 12$ Hz), -147.1 ppm (C6-F, d, $J = 26$ Hz); HRMS for $C_{28}H_{25}F_3N_2O_3S_2$ $[M+H]^+$: calcd 559.1331, found 559.1331.

General procedure for the syntheses of 2(h–k,m–o). A mixture of 2,3,5,6-tetrafluoro-4-[(2-hydroxyethyl)thio]benzenesulfonamide (**2**) (0.20 g, 0.65 mmol), Et_3N (0.093 mL, 0.67 mmol), DMSO (1 mL) and appropriate nucleophile (0.67 mmol) was stirred at 60 °C for 16 h. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (3×10 mL). The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure.

2-(Cyclooctylamino)-3,5,6-trifluoro-4-[(2-hydroxyethyl)thio]benzenesulfonamide (2h). The synthesis of compound **2h** was described previously.[52]

2-(Cyclododecylamino)-3,5,6-trifluoro-4-[(2-hydroxyethyl)thio]-benzenesulfonamide (2i). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:1), $R_f = 0.65$; yield: 0.17 g, 55%; mp: 113–114 °C; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.28$–1.76 (22H, m, cyclododecane), 2.31 (1H, brs, OH), 3.16 (2H, t, $J = 6.0$ Hz, SCH_2CH_2), 3.75 (2H, t, $J = 6.0$ Hz, SCH_2CH_2), 3.78–3.86 (1H, m, CH of cyclododecane, signal overlaps with signal of SCH_2CH_2), 5.59 (2H, s, SO_2NH_2), 6.12 ppm (1H, brs, NH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 21.3$ (cyclododecane), 23.3 (cyclododecane), 23.4 (cyclododecane), 24.3 (cyclododecane), 24.6 (cyclododecane), 30.9 (cyclododecane), 37.6 (SCH_2CH_2, t, $J_{^{19}F-^{13}C} = 3$ Hz), 53.6 (CH of cyclododecane, d, $J_{^{19}F-^{13}C} = 11$ Hz), 61.1 (SCH_2CH_2), 117.5 (C1, dd, $^1J_{^{19}F-^{13}C} = 12$ Hz, $^2J_{^{19}F-^{13}C} = 5$ Hz), 118.4 (C4, t, $J_{^{19}F-^{13}C} = 20$ Hz), 133.3 (C2, d, $J_{^{19}F-^{13}C} = 15$ Hz), 141.9 (C5, ddd, $^1J_{^{19}F-^{13}C} = 239$ Hz, $^2J_{^{19}F-^{13}C} = 16$ Hz, $^3J_{^{19}F-^{13}C} = 5$ Hz), 145.1 (C6, ddd, $^1J_{^{19}F-^{13}C} = 248$ Hz, $^2J_{^{19}F-^{13}C} = 16$ Hz, $^3J_{^{19}F-^{13}C} = 4$ Hz), 148.9 ppm (C3, d, $J_{^{19}F-^{13}C} = 243$ Hz); ^{19}F NMR (282 MHz, $CDCl_3$): $\delta = -124.5$ (C3-F, d, $J = 11$ Hz), -142.9 (C5-F, dd, $^1J = 27$ Hz, $^2J = 12$ Hz), -149.4 ppm (C6-F, d, $J = 24$ Hz); HRMS for $C_{20}H_{31}F_3N_2O_3S_2$ $[M+H]^+$: calcd 469.1801, found 469.1804.

2-[(2,6-Dimethoxybenzyl)amino]-3,5,6-trifluoro-4-[(2-hydroxyethyl)thio]benzenesulfonamide (2j). The product was purified by

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chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:1), R_f = 0.54; yield: 0.10 g, 34%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.15 (2H, t, J = 6.0 Hz, S$CH_2$$CH_2$), 3.72 (2H, t, J = 6.0 Hz, SCH_2CH_2), 3.79 (6H, s, $2CH_3$), 4.51 (2H, s, CH_2), 5.44 (2H, brs, SO_2NH_2), 6.55 (2H, d, J = 8.4 Hz, ArH), 7.22 ppm (1H, t, J = 8.4 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 37.4 (S$CH_2$$CH_2$, brt), 40.3 ($NHCH_2$, d, $J_{^{19}F-^{13}C}$ = 11 Hz), 56.0 (CH_3), 61.2 (SCH_2CH_2), 104.0 (Ar), 115.0 (Ar), 117.9 (C4, t, $J_{^{19}F-^{13}C}$ = 19 Hz), 120.3 (C1, dd, $^1J_{^{19}F-^{13}C}$ = 11 Hz, $^2J_{^{19}F-^{13}C}$ = 4 Hz), 129.6 (Ar), 133.4 (C2, d, $J_{^{19}F-^{13}C}$ = 16 Hz), 143.7 (C5, dd, $^1J_{^{19}F-^{13}C}$ = 240 Hz, $^2J_{^{19}F-^{13}C}$ = 15 Hz), 144.4 (C6, dd, $^1J_{^{19}F-^{13}C}$ = 248 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz), 150.9 (C3, d, $J_{^{19}F-^{13}C}$ = 244 Hz), 158.8 ppm (Ar); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −121.9 (C3-F, d, J = 12 Hz), −143.7(C5-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −145.9 ppm (C6-F, d, J = 25 Hz); HRMS for $C_{17}H_{19}F_3N_2O_5S_2$ $[M+H]^+$: calcd 453.0760, found 453.0752.

2-[(3,4-Dimethoxybenzyl)amino]-3,5,6-trifluoro-4-[(2-hydroxyethyl)thio]benzenesulfonamide (2k). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:1), R_f = 0.45; yield: 0.27 g, 91%; mp: 73–74 °C;. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.08 (2H, t, J = 6.0 Hz, S$CH_2$$CH_2$), 3.63 (2H, t, J = 6.0 Hz, SCH_2CH_2), 3.84 (3H, s, CH_3), 3.86 (3H, s, CH_3), 4.41 (2H, d, J = 3.3 Hz, CH_2), 5.67 (2H, s, SO_2NH_2), 6.75–6.87 ppm (3H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 37.4 (S$CH_2$$CH_2$, t, $J_{^{19}F-^{13}C}$ = 3 Hz), 51.0 ($NHCH_2$, d, $J_{^{19}F-^{13}C}$ = 12 Hz), 56.1 (CH_3), 56.2 (CH_3), 61.1 (SCH_2CH_2), 111.3 (Ar), 118.3 (C1, dd, $^1J_{^{19}F-^{13}C}$ = 11 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz signal overlaps with signal of C4), 118.5 (C4, t, $J_{^{19}F-^{13}C}$ = 21 Hz, signal overlaps with signal of C1), 120.4 (Ar), 131.5 (Ar), 132.9 (C2, d, $J_{^{19}F-^{13}C}$ = 16 Hz), 142.6 (C5, ddd, $^1J_{^{19}F-^{13}C}$ = 242 Hz, $^2J_{^{19}F-^{13}C}$ = 15 Hz, $^3J_{^{19}F-^{13}C}$ = 4 Hz), 144.8 (C6, ddd, $^1J_{^{19}F-^{13}C}$ = 249 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz, $^3J_{^{19}F-^{13}C}$ = 5 Hz), 148.6 (Ar), 149.2 (Ar), 149.3 ppm (C3, d, $J_{^{19}F-^{13}C}$ = 243 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −123.6 (C3-F, d, J = 12 Hz), −143.1 (C5-F, dd, ^1J = 25 Hz, ^2J = 12 Hz), −147.6 ppm (C6-F, d, J = 26 Hz); HRMS for $C_{17}H_{19}F_3N_2O_5S_2$ $[M-H]^-$: calcd 451.0615, found 451.0621.

2-[(1*S*)-2,3-Dihydro-1*H*-inden-1-ylamino]-3,5,6-trifluoro-4-[(2-hydroxyethyl)thio]benzenesulfonamide (2m). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:1), R_f = 0.71; yield: 0.13 g, 48%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.02 (1H, sext, J = 6.9 Hz, indane), 2.54 (1H, sext, J = 6.6 Hz, indane), 2.88 (1H, quint, J = 8.1 Hz, indane), 3.04–3.16 (1H, m, indane), 3.19 (2H, t, J = 6.0 Hz, S$CH_2$$CH_2$), 3.77 (2H, t, J = 6.0 Hz, SCH_2CH_2), 5.12–5.21 (1H, m, NH*CH*), 5.35 (2H, s, SO_2NH_2), 7.15–7.35 ppm (4H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 30.3 (indane), 35 (indane), 37.6 (S$CH_2$$CH_2$, t, $J_{^{19}F-^{13}C}$ = 2.5 Hz), 61.3 (SCH_2CH_2), 62.2 (NHCH, d, $J_{^{19}F-^{13}C}$ = 10 Hz), 118.3 (C1, dd, $^1J_{^{19}F-^{13}C}$ = 12 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 118.8 (C4, t, $J_{^{19}F-^{13}C}$ = 21 Hz), 124.2 (Ar), 125.3 (Ar), 126.9 (Ar), 128.4 (Ar), 133.0 (C2, d, $J_{^{19}F-^{13}C}$ = 15 Hz), 142.7 (C5, ddd, $^1J_{^{19}F-^{13}C}$ = 242 Hz, $^2J_{^{19}F-^{13}C}$ = 15 Hz, $^3J_{^{19}F-^{13}C}$ = 4 Hz), 143.7 (Ar), 144.2 (Ar), 145.1 (C6, ddd, $^1J_{^{19}F-^{13}C}$ = 251 Hz, $^2J_{^{19}F-^{13}C}$ = 12 Hz, $^3J_{^{19}F-^{13}C}$ = 4 Hz), 149.2 ppm (C3, d, $J_{^{19}F-^{13}C}$ = 242 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −123.8 (C3-F, d, J = 12 Hz), −142.7 (C5-F, dd, ^1J = 25 Hz, ^2J = 12 Hz), −147.7 ppm (C6-F, d, J = 25 Hz); HRMS for $C_{17}H_{17}F_3N_2O_3S_2$ $[M+H]^+$: calcd 419.0705, found 419.0714.

2-[(1*S*)-1,2,3,4-Tetrahydronapthalen-1-ylamino)-3,5,6-trifluoro-4-[(2-hydroxyethyl)thio]benzenesulfonamide (2n). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:1), R_f = 0.66; yield: 0.10 g, 36%; mp: 94–95 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.81–2.05 (4H, m, tetrahydronapthalene), 2.37 (1H, brs, OH), 2.68–3.02 (2H, m, tetrahydronapthalene), 3.19 (2H, t, J = 6.0 Hz, S$CH_2$$CH_2$), 3.78 (2H, t, J = 6.0 Hz, SCH_2CH_2), 4.76–4.86 (1H, m, NH*CH*), 5.28 (2H, brs, SO_2NH_2), 6.28 (1H, d, J = 9.0 Hz, NH), 7.1–7.3 ppm (4H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.9 (tetrahydronapthalene), 29.2 (tetrahydronapthalene), 30.3 (tetrahydronapthalene), 37.6 (S$CH_2$$CH_2$, brt, $J_{^{19}F-^{13}C}$ = 3 Hz), 54.7 (NHCH, d, $J_{^{19}F-^{13}C}$ = 11 Hz), 61.3 (SCH_2CH_2), 118.7 (C4, t, $J_{^{19}F-^{13}C}$ = 22 Hz, signal overlaps with signal of C1), 118.9 (C1, dd, $^1J_{^{19}F-^{13}C}$ = 12 Hz, $^2J_{^{19}F-^{13}C}$ = 4 Hz, signal overlaps with signal of C4), 126.1 (Ar), 127.7 (Ar), 129.1 (Ar), 129.7 (Ar), 132.7 (C2, d, $J_{^{19}F-^{13}C}$ = 14 Hz), 137.5 (Ar), 137.6 (Ar), 142.9 (C5, ddd, $^1J_{^{19}F-^{13}C}$ = 240 Hz, $^2J_{^{19}F-^{13}C}$ = 15 Hz, $^3J_{^{19}F-^{13}C}$ = 5 Hz), 145.1 (C6, ddd, $^1J_{^{19}F-^{13}C}$ = 250 Hz, $^2J_{^{19}F-^{13}C}$ = 12 Hz, $^3J_{^{19}F-^{13}C}$ = 5 Hz), 149.7 ppm (C3, d, $J_{^{19}F-^{13}C}$ = 243 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −122.7 (C3-F, d, J = 11 Hz), −142.5 (C5-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −147.3 ppm (C6-F, d, J = 26 Hz); HRMS for $C_{18}H_{19}F_3N_2O_3S_2$ $[M-H]^-$: calcd431.0716, found 431.0719.

2-{[(1*R*,2*S*)-2-Hydroxy-1,2-diphenylethyl]amino}-3,5,6-trifluoro-4-[(2-hydroxyethyl)thio]benzenesulfonamide (2o). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:1), R_f = 0.43; yield: 0.13 g, 39%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.38 (1H, brs, OH), 2.88 (2H, t, J = 6.0 Hz, S$CH_2$$CH_2$), 3.09 (1H, brs, OH), 3.35–3.45 (2H, m, SCH_2CH_2), 4.97 (1H, brt, CH), 5.05 (1H, d, J = 5.1 Hz, CH), 5.97 (2H, brs, SO_2NH_2), 7.04–7.32 ppm (10H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 37.1 (S$CH_2$$CH_2$, brt, $J_{^{19}F-^{13}C}$ = 2 Hz), 60.9 (SCH_2CH_2), 66.1 (NHCH, d, $J_{^{19}F-^{13}C}$ = 12 Hz), 77.0 (CHOH, signal overlaps with $CDCl_3$ signal), 118.4 (C1, dd, $^1J_{^{19}F-^{13}C}$ = 11 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz, signal overlaps with C4 signal), 118.3 (C4, t, $J_{^{19}F-^{13}C}$ = 20 Hz, signal overlaps with C1 signal), 126.8 (Ar), 128.0 (Ar), 128.2 (Ar), 128.3 (Ar), 128.4 (Ar), 128.5 (Ar), 131.7 (C2, d, $J_{^{19}F-^{13}C}$ = 15 Hz), 138.1 (Ar), 140.4 (Ar), 142.7 (C5, ddd, $^1J_{^{19}F-^{13}C}$ = 241 Hz, $^2J_{^{19}F-^{13}C}$ = 15 Hz, $^3J_{^{19}F-^{13}C}$ = 4 Hz), 144.9 (C6, ddd, $^1J_{^{19}F-^{13}C}$ = 251 Hz, $^2J_{^{19}F-^{13}C}$ = 12 Hz, $^3J_{^{19}F-^{13}C}$ = 4 Hz), 148.8 ppm (C3, d, $J_{^{19}F-^{13}C}$ = 243 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −122.2 (C3-F, d, J = 11 Hz), −142.9 (C5-F, dd, ^1J = 26 Hz, ^2J = 12 Hz), −147.3 ppm (C6-F, d, J = 26 Hz); HRMS for $C_{22}H_{21}F_3N_2O_4S_2$ $[M+H]^+$: calcd 499.0968, found 499.0967.

General procedure for the syntheses of 3(h–k,m,n). A mixture of 2,3,5,6-tetrafluorobenzenesulfonamide (**3**) (0.2 g, 0.87 mmol), Et_3N (0.124 mL, 0.89 mmol), DMSO (1 mL) and appropriate nucleophile (0.93 mmol) was stirred at 60 °C for 8 h, compound **3k** was obtained after 16 h, compound **3n** was obtained after stirring at 70 °C for 16 h. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (3×10 mL). The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure.

2-(Cyclooctylamino)-3,5,6-trifluorobenzenesulfonamide (3h). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:20), R_f = 0.32; yield: 0.15 g, 52%; mp: 117–118 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 1.37–1.89 (14H, m, cyclooctane), 3.72 (1H, brs, CH of cyclooctane), 6.35 (1H, brs, NH), 7.65–7.82 (1H, m, ArH), 8.11 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 23.6 (cyclooctane), 25.7 (cyclooctane), 27.6 (cyclooctane), 32.8 (cyclooctane), 55.7 (CH of cyclooctane, d, $J_{^{19}F-^{13}C}$ = 10 Hz), 110.2 (C4, t, $J_{^{19}F-^{13}C}$ = 25 Hz), 120.6 (C1, dd, $^1J_{^{19}F-^{13}C}$ = 12 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 132.9 (C2, dd, $^1J_{^{19}F-^{13}C}$ = 13 Hz, $^2J_{^{19}F-^{13}C}$ = 3 Hz), 141.1 (C5, dt, $^1J_{^{19}F-^{13}C}$ = 240 Hz, $^2J_{^{19}F-^{13}C}$ = 14 Hz), 145.0 (C6, ddd, $^1J_{^{19}F-^{13}C}$ = 248 Hz, $^2J_{^{19}F-^{13}C}$ = 14 Hz, $^3J_{^{19}F-^{13}C}$ = 5 Hz), 148.3 ppm (C3, dd, $^1J_{^{19}F-^{13}C}$ = 236 Hz, $^2J_{^{19}F-^{13}C}$ = 6 Hz); ^{19}F NMR (282 MHz, [D_6]DMSO): δ = −120.6 (C3-F, t, J = 13 Hz), −133.35–−133.56 (C5-F or C6-F, m), −145.2 ppm (C5-F or C6-F, dd, ^1J = 25 Hz, ^2J = 11 Hz); HRMS for $C_{14}H_{19}F_3N_2O_2S$ $[M+H]^+$: calcd 337.1192, found 337.1195.

2-(Cyclododecylamino)-3,5,6-trifluorobenzenesulfonamide (3i). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:20), R_f = 0.49; yield: 0.10 g, 29%; mp: 113–114 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 1.18–1.67 (22H, m, cyclododecane), 3.71 (1H, brs, CH of cyclododecane), 6.22 (1H, *d* = 7.8 Hz, NH), 7.64–7.78 (1H, m, ArH), 8.09 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 21.4 (cyclododecane), 23.4 (cyclododecane), 23.6 (cyclododecane), 24.1 (cyclododecane), 24.4 (cyclo-

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dodecane), 31.1 (cyclododecane), 52.8 (CH of cyclododecane, d, $J_{^{19}F-^{13}C}=11$ Hz), 110.2 (C4, t, $J_{^{19}F-^{13}C}=25$ Hz), 120.6 (C1, dd, $^{1}J_{^{19}F-^{13}C}=12$ Hz, $^{2}J_{^{19}F-^{13}C}=5$ Hz), 133.4 (C2, dd, $^{1}J_{^{19}F-^{13}C}=12$ Hz, $^{2}J_{^{19}F-^{13}C}=2$ Hz), 141.1 (C5, dt, $^{1}J_{^{19}F-^{13}C}=238$ Hz, $^{2}J_{^{19}F-^{13}C}=13$ Hz), 145.0 (C6, ddd, $^{1}J_{^{19}F-^{13}C}=249$ Hz, $^{2}J_{^{19}F-^{13}C}=14$ Hz, $^{3}J_{^{19}F-^{13}C}=4$ Hz), 148.1 ppm (C3, dd, $^{1}J_{^{19}F-^{13}C}=245$ Hz, $^{2}J_{^{19}F-^{13}C}=9$ Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): $\delta=-125.4$ (C3-F, t, $J=13$ Hz), −138–138.3 (C5-F or C6-F, m), −150.1 ppm (C5-F or C6-F, dd, $^{1}J=25$ Hz, $^{2}J=11$ Hz); HRMS for $C_{18}H_{27}F_3N_2O_2S$ $[M+H]^+$: calcd 393.1818, found 393.1816.

2-[(2,6-Dimethoxybenzyl)amino]-3,5,6-trifluorobenzenesulfonamide (3j). The product was purified by chromatography on a column of silica gel with EtOAc (5%)/CHCl$_3$, $R_f=0.4$; yield: 0.16 g, 48%; mp: 137–138 °C; ^{1}H NMR (300 MHz, CD$_3$OD): $\delta=3.79$ (6H, s, 2CH$_3$), 4.51 (2H, d, $J=1.5$ Hz, CH$_2$), 4.91 (2H, s, SO$_2$NH$_2$), 6.61 (2H, d, $J=8.4$ Hz, ArH), 7.22 (1H, t, $J=8.4$ Hz, ArH), 7.29–7.41 ppm (1H, m, ArH); ^{13}C NMR (75 MHz, CD$_3$OD): $\delta=39.3$ (CH$_2$, d, $J_{^{19}F-^{13}C}=12$ Hz), 55.0 (CH$_3$), 103.6 (Ar), 108.7 (C4, t, $J_{^{19}F-^{13}C}=25$ Hz), 115.1 (Ar), 121.4 (C1, dd, $^{1}J_{^{19}F-^{13}C}=12$ Hz, $^{2}J_{^{19}F-^{13}C}=4$ Hz), 129.2 (Ar), 134.3 (C2, dd, $^{1}J_{^{19}F-^{13}C}=13$ Hz, $^{2}J_{^{19}F-^{13}C}=3$ Hz), 142.2 (C5, d, $J_{^{19}F-^{13}C}=241$ Hz), 144.6 (C6, d, $J_{^{19}F-^{13}C}=248$ Hz), 149.9 (C3, d, $J_{^{19}F-^{13}C}=249$ Hz), 158.8 ppm (Ar); ^{19}F NMR (282 MHz, CD$_3$OD): $\delta=-124.1$ (C3-F, t, $J=13$ Hz), −141.5−141.7 (C5-F or C6-F, m), −150.3 ppm (C5-F or C6-F, dd, $^{1}J=25$ Hz, $^{2}J=10$ Hz); HRMS for $C_{15}H_{15}F_3N_2O_4S$ $[M-H]^-$: calcd 375.0632, found 375.0634.

2-[(3,4-Dimethoxybenzyl)amino]-3,5,6-trifluorobenzenesulfonamide (3k). The product was purified by chromatography on a column of silica gel with EtOAc (10%)/CHCl$_3$, $R_f=0.29$. Recrystallization was accomplished from EtOH/H$_2$O (2:1) after chromatography; yield: 0.10 g, 33%; mp: 115–116 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=3.73$ (3H, s, CH$_3$), 3.75 (3H, s, CH$_3$), 4.37–4.45 (2H, m, CH$_2$), 6.67–6.76 (1H, m, NH), 6.82–6.98 (3H, m, ArH), 7.65–7.78 (1H, m, ArH), 8.14 ppm (2H, s, SO$_2$NH$_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=50.3$ (CH$_2$, d, $J_{^{19}F-^{13}C}=12$ Hz), 56.0 (CH$_3$), 56.1 (CH$_3$), 110.9 (C4, t, $J_{^{19}F-^{13}C}=24$ Hz), 111.9 (Ar), 112.2 (Ar), 120.3 (Ar), 120.4 (C1, signal overlaps with Ar signal), 132.3 (Ar), 133.5 (C2, d, $J_{^{19}F-^{13}C}=13$ Hz), 141.2 (C5 dt, $^{1}J_{^{19}F-^{13}C}=238$ Hz, $^{2}J_{^{19}F-^{13}C}=12$ Hz), 144.8 (C6, d, $J_{^{19}F-^{13}C}=252$ Hz), 148.2 (C3, d $J_{^{19}F-^{13}C}=240$ Hz), 148.6 (Ar), 149.2 ppm (Ar); ^{19}F NMR (282 MHz, [D$_6$]DMSO): $\delta=-124.9$ (C3-F, t, $J=13$ Hz), −138.6–138.8 (C5-F or C6-F, m), −149.6 ppm (C5-F or C6-F, dd, $^{1}J=25$ Hz, $^{2}J=10$ Hz); HRMS for $C_{15}H_{15}F_3N_2O_4S$ $[M-H]^-$: calcd 375.0632, found 375.0631.

2-[(1*S*)-2,3-Dihydro-1*H*-inden-1-ylamino]-3,5,6-trifluorobenzenesulfonamide (3m). The product was purified by chromatography on a column of silica gel with EtOAc/CHCl$_3$ (1:10), $R_f=0.38$; yield: 0.09 g, 30%; mp: 103–104 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=$ 1.79–1.95 (1H, m, indane), 2.38–2.49 (1H, m, indane), 2.82 (1H, pent, $J=7.8$ Hz, indane), 2.91–3.06 (1H, m, indane), 5.09–5.21 (1H, m, NH*CH*), 6.59 (1H, dd, $^{1}J=8.4$ Hz, $^{2}J=1.8$ Hz, NH), 7.19–7.38 (4H, m, ArH), 7.75–7.88 (1H, m ArH), 8.13 ppm (2H, s, SO$_2$NH$_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=30.2$ (indane), 35.2 (indane), 61.6 (CH of indane, d, $J_{^{19}F-^{13}C}=11$ Hz), 110.5 (C4, t, $J_{^{19}F-^{13}C}=25$ Hz), 120.5 (C1, dd, $^{1}J_{^{19}F-^{13}C}=12$ Hz, $^{2}J_{^{19}F-^{13}C}=5$ Hz), 124.7 (Ar), 125.5 (Ar), 127.3 (Ar), 128.6 (Ar), 133.2 (C2, dd, $^{1}J_{^{19}F-^{13}C}=13$ Hz, $^{2}J_{^{19}F-^{13}C}=3$ Hz), 141.4 (C5, dt, $^{1}J_{^{19}F-^{13}C}=238$ Hz, $^{2}J_{^{19}F-^{13}C}=14$ Hz), 143.6 (Ar), 144.7 (Ar), 144.9 (C6, ddd, $^{1}J_{^{19}F-^{13}C}=233$ Hz, $^{2}J_{^{19}F-^{13}C}=14$ Hz, $^{3}J_{^{19}F-^{13}C}=4$ Hz), 148.2 ppm (C3, d, $J_{^{19}F-^{13}C}=226$ Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): $\delta=-120.0$ (C3-F, t, $J=13$ Hz), −133.35–133.55 (C5-F or C6-F, m), −144.7 ppm (C5-F or C6-F, dd, $^{1}J=25$ Hz, $^{2}J=11$ Hz); HRMS for $C_{15}H_{13}F_3N_2O_2S$ $[M-H]^-$: calcd 341.0577, found 341.0580.

2-[(1*S*)-1,2,3,4-Tetrahydronapthalen-1-ylamino)-3,5,6-trifluorobenzenesulfonamide (3n). The product was purified by chromatography on a column of silica gel with EtOAc (10%)/CHCl$_3$, $R_f=$ 0.64; yield: 0.10 g, 32%; mp: 124–125 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): 1.60–2.01 (4H, m, tetrahydronapthalene), 2.61–2.92 (2H, m, tetrahydronapthalene), 4.81 (1H, brs, NH*CH*), 6.53 (1H, d, $J=8.7$ Hz, NH), 7.11–7.25 (3H, m, ArH), 7.41 (1H, d, $J=7.5$ Hz, ArH), 7.73–7.85 (1H, m, ArH), 8.12 ppm (2H, s, SO$_2$NH$_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=19.3$ (tetrahydronapthalene), 29.3 (tetrahydronapthalene), 30.2 (tetrahydronapthalene), 53.8 (CH of tetrahydronapthalene, d, $J_{^{19}F-^{13}C}=12$ Hz), 110.4 (C4, t, $J_{^{19}F-^{13}C}=25$ Hz), 121.0 (C1, dd, $^{1}J_{^{19}F-^{13}C}=12$ Hz, $^{2}J_{^{19}F-^{13}C}=5$ Hz), 126.6 (Ar), 127.9 (Ar), 129.6 (Ar), 129.7 (Ar), 132.7 (C2, dd, $^{1}J_{^{19}F-^{13}C}=12$ Hz, $^{2}J_{^{19}F-^{13}C}=2$ Hz), 137.6 (Ar), 137.9 (Ar), 141.5 (C5, dt, $^{1}J_{^{19}F-^{13}C}=239$ Hz, $^{2}J_{^{19}F-^{13}C}=13$ Hz), 145.0 (C6, d, $J_{^{19}F-^{13}C}=247$ Hz), 148.4 ppm (C3, d, $J_{^{19}F-^{13}C}=239$ Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): $\delta=-123.8$ (C3-F, t, $J=13$ Hz), −137.9–−138.14 (C5-F or C6-F, m), −149.2 ppm (C5-F or C6-F, dd, $^{1}J=$ 25 Hz, $^{2}J=10$ Hz); HRMS for $C_{16}H_{15}F_3N_2O_2S$ $[M-H]^-$: calcd 355.0734, found 355.0733.

2-(Cyclooctylamino)-3,5,6-trifluoro-4-(propylthio)benzenesulfonamide (4h). A mixture of 2,3,5,6-tetrafluoro-4-(propylthio)benzenesulfonamide (**4**) (0.20 g, 0.66 mmol), Et$_3$N (0.095 mL, 0.68 mmol), DMSO (1 mL) and cyclooctylamine (0.10 mL, 0.72 mmol) was stirred at 60 °C for 12 h. The mixture was then diluted with H$_2$O (20 mL) and extracted with EtOAc (3×10 mL). The combined organic phase was dried over MgSO$_4$ and evaporated under reduced pressure. The product was purified by chromatography on a column of silica gel with EtOAc (5%)/CHCl$_3$, $R_f=0.64$; yield: 0.16 g, 59%; ^{1}H NMR (300 MHz, CDCl$_3$): $\delta=1.03$ (3H, t, $J=7.2$ Hz, CH$_3$), 1.43–1.75 (14H, m, *CH*$_2$CH$_3$, cyclooctane), 1.78–1.95 (2H, m, cyclooctane), 2.98 (2H, t, $J=7.2$ Hz, SCH$_2$), 3.72–3.85 (1H, m, CH of cyclooctane), 5.62 ppm (3H, brs, NH, SO$_2$NH$_2$); ^{13}C NMR (75 MHz, CDCl$_3$): $\delta=13.3$ (CH$_3$), 23.5 (cyclooctane), 23.7 (cyclooctane), 25.8 (cyclooctane), 27.5 (cyclooctane), 33.0 (CH$_2$), 36.3 (SCH$_2$, t, $J_{^{19}F-^{13}C}=3.6$ Hz), 56.5 (CH of cyclooctane, d, $J_{^{19}F-^{13}C}=11$ Hz), 117.3 (C1, dd, $^{1}J_{^{19}F-^{13}C}=12$ Hz, $^{2}J_{^{19}F-^{13}C}=$ 6 Hz), 120.0 (C4, t, $J_{^{19}F-^{13}C}=21$ Hz), 132.4 (C2, d, $J_{^{19}F-^{13}C}=13$ Hz), 142.2 (C5, ddd, $^{1}J_{^{19}F-^{13}C}=239$ Hz, $^{2}J_{^{19}F-^{13}C}=16$ Hz, $^{3}J_{^{19}F-^{13}C}=5$ Hz), 145.0 (C6, ddd, $^{1}J_{^{19}F-^{13}C}=249$ Hz, $^{2}J_{^{19}F-^{13}C}=17$ Hz, $^{3}J_{^{19}F-^{13}C}=4$ Hz), 148.9 ppm (C3, d, $J_{^{19}F-^{13}C}=243$ Hz); ^{19}F NMR (282 MHz, CDCl$_3$): $\delta=$ −124.8 (C3-F, d, $J=11$ Hz), −143.5 (C5-F, dd, $^{1}J=27$ Hz, $^{2}J=12$ Hz), −149.0 ppm (C6-F, d, $J=26$ Hz); HRMS for $C_{17}H_{25}F_3N_2O_2S_2$ $[M+H]^+$: calcd 411.1382, found 411.1388.

2-(Cyclooctylamino)-3,5,6-trifluoro-4-{[2-(4-hydroxyphenyl)ethyl]amino}benzenesulfonamide (5h). A mixture of 2,3,5,6-tetrafluoro-4-{[2-(4-hydroxyphenyl)ethyl]amino}benzenesulfonamide (**5**) (0.20 g, 0.55 mmol), Et$_3$N (0.085 mL, 0.61 mmol), DMSO (1 mL) and cyclooctylamine (0.085 mL, 0.61 mmol) was stirred at 70 °C for 28 h. The mixture was then diluted with H$_2$O (20 mL) and extracted with EtOAc (3×10 mL). The combined organic phase was dried over MgSO$_4$ and evaporated under reduced pressure. The product was purified by chromatography on a column of silica gel with EtOAc/CHCl$_3$ (1:3), $R_f=0.60$; yield: 0.13 g, 50%; ^{1}H NMR (300 MHz, CDCl$_3$): $\delta=1.38$–1.94 (14H, m, cyclooctane), 2.82 (2H, t, $J=6.6$ Hz, NHCH$_2$*CH*$_2$), 3.61–3.75 (3H, m, CH of cyclooctane, NH*CH*$_2$CH$_2$), 5.59 (2H, s, SO$_2$NH$_2$), 6.04 (2H, brs, 2NH), 6.79 (2H, d, $J=8.4$ Hz, ArH), 7.04 ppm (2H, d, $J=8.4$ Hz, ArH); ^{13}C NMR (75 MHz, CDCl$_3$): $\delta=23.8$ (cyclooctane), 25.8 (cyclooctane), 27.5 (cyclooctane), 32.7 (cyclooctane), 36.6 (NHCH$_2$*CH*$_2$), 46.9 (NH*CH*$_2$CH$_2$), 56.4 (CH of cyclooctane, d, $J_{^{19}F-^{13}C}=10$ Hz), 106.3 (C1, dd, $^{1}J_{^{19}F-^{13}C}=12$ Hz, $^{2}J_{^{19}F-^{13}C}=5$ Hz), 115.7 (C4, signal overlaps with Ar signal), 115.8 (Ar), 130.2 (Ar), 130.3 (Ar), 132.0 (C2, d, $J_{^{19}F-^{13}C}=13$ Hz, signal overlaps with C5 signal), 133.8 (C6, ddd, $^{1}J_{^{19}F-^{13}C}=239$ Hz, $^{2}J_{^{19}F-^{13}C}=18$ Hz, $^{3}J_{^{19}F-^{13}C}=6$ Hz), 138.5 (C3, d, $J_{^{19}F-^{13}C}=233$ Hz), 146.5(C6, ddd, $^{1}J_{^{19}F-^{13}C}=243$ Hz, $^{2}J_{^{19}F-^{13}C}=14$ Hz, $^{3}J_{^{19}F-^{13}C}=3$ Hz), 154.8 ppm (Ar); ^{19}F NMR (282 MHz, CDCl$_3$): $\delta=$

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−144.8 (C5-F, dd, ^1J=23 Hz, ^2J=9 Hz), −154.1 (C3-F, s), −171.4 ppm (C6-F, d, J=23 Hz); HRMS for $C_{22}H_{28}F_3N_3O_3S$ $[M+H]^+$: calcd 472.1876, found 472.1877.

2,3,4,5-Tetrafluoro-6-[(4-methoxybenzyl)amino]benzenesulfonamide (7a) and 2,3,5,6-tetrafluoro-4-[(4-methoxybenzyl)amino]-benzenesulfonamide (8a). *Method A*: A mixture of 2,3,4,5,6-pentafluorbenzenesulfonamide (**6**) (0.20 g, 0.81 mmol), Et_3N (0.11 mL, 0.81 mmol), C_6H_6 (3 mL) and 4-methoxybenzylamine (0.11 mL, 0.81 mmol) was stirred at 80 °C for 7 h. C_6H_6 was evaporated under reduced pressure and the resultant precipitate was washed with H_2O. A mixture of **7a** and **8a** was obtained. The compounds were separated by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:5); yield: 0.14 g, 47% (7a); 0.04 g, 12% (**8a**).

2,3,4,5-Tetrafluoro-6-[(4-methoxybenzyl)amino]benzenesulfonamide (7a). R_f=0.62 (EtOAc/$CHCl_3$, 1:5); mp: 97–98 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=2.87 (3H, s, CH_3), 3.59 (2H, dd, ^1J=6.4 Hz, ^2J=4.4 Hz, CH_2), 6.02 (1H, brs, NH signal overlaps with signal of Ar), 6.04 (2H, d, J=8.8 Hz, Ar), 6.41 (2H, d, J=8.8 Hz, Ar), 7.34 ppm (2H, s, SO_2NH_2); ^{13}C NMR (100 MHz, [D_6]DMSO): δ=49.4 (CH_2, $J_{^{19}F-^{13}C}$=11.8 Hz), 55.5 (CH_3), 114.4 (Ar), 114.5 (C1), 129.2 (Ar), 131.3 (Ar), 131.4 (Ar), 131.8 (C3 or C4, dt, ^1J=243 Hz, ^2J=16 Hz), 133.5 (C6, dd, ^1J=9 Hz, ^2J=3 Hz), 137.9 (C2 or C5, dd, ^1J=243 Hz, ^2J=13 Hz), 143.5 (C3 or C4, d, J=249 Hz), 145.6 (C2 or C5, dd, ^1J=247 Hz, ^2J=9 Hz), 159.0 ppm (Ar); ^{19}F NMR (376 MHz, [D_6]DMSO): δ=−136.2 (C2-F or C5-F, dt, ^1J=26 Hz, ^2J=7.5 Hz), −152.0 (C3-F or C4-F, td, ^1J=23 Hz, ^2J=7.5 Hz), −154.3 (C2-F or C5-F, dt, ^1J=23 Hz, ^2J=7.5 Hz), −172.6 ppm (C3-F or C4-F, td, ^1J=23 Hz, ^2J=4 Hz); HRMS for $C_{14}H_{12}F_4N_2O_3S$ $[M+H]^+$: calcd 365.0578, found 365.0581.

2,3,5,6-Tetrafluoro-4-[(4-methoxybenzyl)amino]benzenesulfonamide (8a). R_f=0.45 (EtOAc/$CHCl_3$, 1:5); mp: 161–162 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=3.72 (3H, s, OCH_3), 4.47 (2H, d, J=4.0 Hz, CH_2), 6.89 (2H, d, J=9.2 Hz, Ar), 7.23 (2H, d, J=9.2 Hz, Ar), 7.31 (1H, brt, NH), 7.91 ppm (2H, s, SO_2NH_2); ^{13}C NMR (100 MHz, [D_6]DMSO): δ=47.4 (CH_2, t, $J_{^{19}F-^{13}C}$=4 Hz), 55.4 (OCH_3), 108.8 (C1, t, J=16 Hz), 114.3 (Ar), 128.4 (Ar), 131.4 (C4, t, J=11 Hz), 132.0 (Ar), 136.3 (C3, C5, d, $J_{^{19}F-^{13}C}$=243 Hz), 144.0 (C2, C6, d, $J_{^{19}F-^{13}C}$=251 Hz), 158.7 ppm (Ar); ^{19}F NMR (376 MHz, [D_6]DMSO): δ=−142.1 (2F, d, J=17 Hz), −159.6 ppm (2F, d, J=17 Hz); HRMS for $C_{14}H_{12}F_4N_2O_3S$ $[M+H]^+$: calcd 365.0578, found 365.0581.

2,3,5,6-Tetrafluoro-4-[(4-methoxybenzyl)amino]benzenesulfonamide (8a). *Method B*: A mixture of **6** (0.20 g, 0.81 mmol), Et_3N (0.11 mL, 0.81 mmol), DMSO (1 mL) and 4-methoxybenzylamine (0.11 mL, 0.81 mmol) was stirred at ambient temperature for 24 h. The mixture was then diluted with H_2O (20 mL). The resultant precipitate was filtered, washed with H_2O. Recrystallization was accomplished from EtOH/H_2O (2:1); yield: 0.25 g, 86%; mp: 161–162 °C.

2,3,4,5-Tetrafluoro-6-piperidin-1-ylbenzenesulfonamide (7b) and 2,3,5,6-tetrafluoro-4-piperidin-1-ylbenzenesulfonamide (8b). *Method A*: A mixture of **6** (0.20 g, 0.81 mmol), Et_3N (0.11 mL, 0.81 mmol), C_6H_6 (3 mL) and piperidine hydrochloride (0.10 g, 0.81 mmol) was stirred at 80 °C for 12 h. C_6H_6 was evaporated under reduced pressure and the resultant precipitate was washed with H_2O. A mixture of **7b** and **8b** was obtained. The compounds were separated by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:5); yield: 0.09 g, 37% (7b); 0.04 g, 17% (**8b**).

2,3,4,5-Tetrafluoro-6-piperidin-1-ylbenzenesulfonamide (7b). R_f=0.62 (EtOAc/$CHCl_3$, 1:5); mp: 175–176 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=1.63 (4H, brs, $2CH_2$), 1.72 (2H, brs, CH_2), 3.02 (4H, brs, $2CH_2$), 7.59 ppm (2H, s, SO_2NH_2); ^{13}C NMR (100 MHz, [D_6]DMSO): δ=23.7 (CH_2), 26.1 (CH_2), 52.2 (CH_2, $J_{^{19}F-^{13}C}$=3 Hz), 127.2 (C1), 135.2 (C6, dd, ^1J=11 Hz, ^2J=4 Hz), 138.9 (C3 or C4, dt, ^1J=254 Hz, ^2J=14 Hz), 142.8 (C3 or C4, dt, ^1J=257 Hz, ^2J=16 Hz), 144.1 (C2 or C5, dd, ^1J=252 Hz, ^2J=12 Hz), 148.0 ppm (C2 or C5, dd, ^1J=250 Hz, ^2J=10 Hz); ^{19}F NMR (376 MHz, [D_6]DMSO): δ=−137.4 (d, J=26 Hz), −144.0, −151.0, −157.9 ppm; HRMS for $C_{11}H_{12}F_4N_2O_2S$ $[M+H]^+$: calcd 313.0628, found 313.0631.

2,3,5,6-Tetrafluoro-4-piperidin-1-ylbenzenesulfonamide (8b). R_f=0.48 (EtOAc/$CHCl_3$, 1:5); mp: 174–175 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=1.59 (6H, s, $3CH_2$), 3.26 (4H, s, $2CH_2$), 8.14 ppm (2H, s, SO_2NH_2); ^{13}C NMR (100 MHz, [D_6]DMSO): δ=23.9 (CH_2), 26.4 (CH_2), 51.8 (CH_2, t, $J_{^{19}F-^{13}C}$=3 Hz), 114.3 (C1, t, J=15 Hz), 134.1 (C4, t, J=11 Hz), 141.2 (C3, C5, dd, ^1J=245 Hz, ^2J=14 Hz), 144.0 ppm (C2, C6, dd, ^1J=252 Hz, ^2J=14 Hz); ^{19}F NMR (376 MHz, [D_6]DMSO): δ=−141.1−−141.3 (2F, m), −150.9−−151.1 ppm (2F, m); HRMS for $C_{11}H_{12}F_4N_2O_2S$ $[M+H]^+$: calcd 313.0628, found 313.0630.

2,3,5,6-Tetrafluoro-4-piperidin-1-ylbenzenesulfonamide (8b). *Method B*: A mixture of **6** (0.20 g, 0.81 mmol), Et_3N (0.11 mL, 0.81 mmol), DMSO (1 mL) and piperidine hydrochloride (0.10 g, 0.81 mmol) was stirred at ambient temperature for 24 h. The mixture was then diluted with H_2O (20 mL). The resultant precipitate was filtered, washed with H_2O. Recrystallization was accomplished from EtOH/H_2O (2:1); yield: 0.22 g, 88%; mp: 174–175 °C.

3-(Methylamino)-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9a). A mixture of 2,3,5,6-tetrafluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (**9**) (0.20 g, 0.50 mmol), MeOH (10 mL) and methylamine (2 M in MeOH) (0.75 mL, 1,50 mmol) was held at reflux for 7 h. MeOH was evaporated under reduced pressure and the resultant precipitate was filtered, washed with H_2O. Recrystallization was accomplished from EtOH/H_2O (2:1); yield: 0.12 g, 57%; mp: 152–153 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ=3.01 (3H, dd, ^1J=7.5 Hz, ^2J=5.1 Hz, CH_3), 3.08 (2H, t, J=7.5 Hz, $SO_2CH_2CH_2$), 3.89 (2H, t, J=7.5 Hz, $SO_2CH_2CH_2$), 6.62 (1H, brs, NH), 7.13–7.33 (5H, m, ArH), 8.31 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D_6]DMSO): δ=28.6 ($SO_2CH_2CH_2$), 34.1 (CH_3, d, $J_{^{19}F-^{13}C}$=13 Hz), 57.5 ($SO_2CH_2CH_2$), 114.4 (C4, dd, $^1J_{^{19}F-^{13}C}$=12 Hz, $^2J_{^{19}F-^{13}C}$=5 Hz), 127.5 (Ar), 128.0 (C1, t, $J_{^{19}F-^{13}C}$=18 Hz), 128.9 (Ar), 129.1 (Ar), 137.4 (C3, d, $J_{^{19}F-^{13}C}$=12 Hz), 137.6 (Ar), 136.7 (C6, d, $J_{^{19}F-^{13}C}$=244 Hz), 144.4 (C2, d, $J_{^{19}F-^{13}C}$=251 Hz), 146.1 ppm (C5, d, $J_{^{19}F-^{13}C}$=240 Hz); ^{19}F NMR (282 MHz, [D_6]DMSO): δ=−127.5 (C2-F, s), −135.9 (C6-F, dd, ^1J=25 Hz, ^2J=12 Hz), −152.6 ppm (C5-F, dd, ^1J=26 Hz, ^2J=7 Hz); HRMS $C_{15}H_{15}F_3N_2O_4S_2$ $[M+H]^+$: calcd 409.0498, found 409.0505.

3-(*tert*-Butylamino)-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9b). A mixture of compound **9** (0.20 g, 0.50 mmol), DMSO (1 mL) and *tert*-butylamine (0.11 mL, 0.105 mmol) was stirred at ambient temperature for four days. The mixture was then diluted with H_2O (20 mL) and the resultant precipitate was filtered, washed with H_2O. The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:4), R_f=0.62; yield: 0.04 g, 18%; mp: 127–128 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.38 (9H, d, J=2.1 Hz, $3CH_3$), 3.14 (2H, t, J=7.8 Hz, $SO_2CH_2CH_2$), 3.65 (2H, t, J=7.8 Hz, $SO_2CH_2CH_2$), 5.74 (2H, s, SO_2NH_2), 6.63 (1H, s, NH), 7.12–7.43 ppm (5H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=28.8 ($SO_2CH_2CH_2$), 30.9 (CH_3, d, J=7 Hz), 55.7 ($SO_2CH_2CH_2$), 58.8 (NHC, d, $J_{^{19}F-^{13}C}$=4 Hz), 118.5 (C4, dd, $^1J_{^{19}F-^{13}C}$=12 Hz, $^2J_{^{19}F-^{13}C}$=6 Hz), 126.3 (C1, t, $J_{^{19}F-^{13}C}$=16 Hz), 127.6 (Ar), 128.5 (Ar), 129.2 (Ar), 135.8 (C3, dd, $^1J_{^{19}F-^{13}C}$=18 Hz, $^2J_{^{19}F-^{13}C}$=3 Hz), 136.4 (Ar), 138.3 (C6, ddd, $^1J_{^{19}F-^{13}C}$=252 Hz, $^2J_{^{19}F-^{13}C}$=18 Hz, $^3J_{^{19}F-^{13}C}$=5 Hz), 145.8 (C2, d, $J_{^{19}F-^{13}C}$=254 Hz), 146.1 ppm (C5, ddd, $^1J_{^{19}F-^{13}C}$=253 Hz, $^2J_{^{19}F-^{13}C}$=16 Hz, $^3J_{^{19}F-^{13}C}$=4 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ=

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−122.2 (C2-F, s), −137.6 (C6-F, dd, ^1J=25 Hz, ^2J=12 Hz), −152.9 ppm (C5-F, dd, ^1J=26 Hz, ^2J=6 Hz); HRMS for $C_{18}H_{21}F_3N_2O_4S_2$ $[M+H]^+$: calcd 451.0968, found 451.0969.

General procedure for the syntheses of 9d,e,g–k,m,n,o. A mixture of **9** (0.20 g, 0.50 mmol), Et_3N (0.071 mL, 0.51 mmol), DMSO (1 mL) and appropriate nucleophile (0.51 mmol) was stirred at ambient temperature. The reaction was monitored by TLC. The reaction proceeded completion after 4–24 h. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (3×10 mL). The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure.

3-(Benzylamino)-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9d). Recrystallization was accomplished from EtOH; yield: 0.21 g, 57%; mp: 59–61 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=3.00 (2H, t, J=7.5 Hz, $SO_2CH_2CH_2$), 3.82 (2H, t, J=7.5 Hz, $SO_2CH_2CH_2$), 4.54 (2H, dd, J=6.0 Hz, J=4.2 Hz, $NHCH_2$), 7.01 (1H, t, J=6.0 Hz, NH), 7.14–7.45 (10H, m, ArH), 8.34 ppm (2H, br s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=28.5 ($SO_2CH_2CH_2$), 50.5 ($NHCH_2$, d, $J_{^{19}F-^{13}C}$=13 Hz), 57.7 ($SO_2CH_2CH_2$), 115.6 (C4, dd, $^1J_{^{19}F-^{13}C}$=13 Hz, $^2J_{^{19}F-^{13}C}$=5 Hz), 127.5 (Ar), 128.2 (Ar), 128.4 (Ar), 129 (Ar), 129.1 (Ar), 129.3 (Ar), 136.0 (C3, d, $J_{^{19}F-^{13}C}$=14 Hz), 137.5 (Ar), 139.7 (Ar), 139.4 (C6, d, $J_{^{19}F-^{13}C}$=244 Hz), 144.9 (C2, d, $J_{^{19}F-^{13}C}$=253 Hz), 146.0 ppm (C5, d, $J_{^{19}F-^{13}C}$=253 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ=−124.7 (C2-F, s), −134.9 (C6-F, dd, ^1J=25 Hz, ^2J=12 Hz), −150.4 ppm (C5-F, dd, ^1J=26 Hz, ^2J=7 Hz); HRMS for $C_{21}H_{19}F_3N_2O_4S_2$ $[M+H]^+$: calcd 485.0811, found 485.0814.

3-[(2-Phenylethyl)amino]-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9e). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:6), R_f=0.48; yield: 0.18 g, 72%; mp: 141–142 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=2.88 (2H, t, J=7.2 Hz, $NHCH_2CH_2$), 2.98 (2H, t, J=7.5 Hz, $SO_2CH_2CH_2$), 3.60 (2H, br t, $NHCH_2CH_2$), 3.77 (2H, t, J=7.2 Hz, $SO_2CH_2CH_2$), 6.68 (1H, br s, NH), 7.05–7.42 (10H, m, ArH), 8.33 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=28.6 ($SO_2CH_2CH_2$), 36.8 ($NHCH_2CH_2$), 48.3 ($NHCH_2CH_2$, d, $J_{^{19}F-^{13}C}$=13 Hz), 57.5 ($SO_2CH_2CH_2$), 114.6 (C4, d, $J_{^{19}F-^{13}C}$=12 Hz), 127.1 (Ar), 127.5 (Ar), 128.1 (C1, t, $J_{^{19}F-^{13}C}$=16 Hz), 128.9 (Ar), 129.0 (Ar), 129.2 (Ar), 129.5 (Ar), 136.1 (C3, d, $J_{^{19}F-^{13}C}$=13 Hz), 137.5 (Ar), 139.3 (Ar), 137.0 (C6, d, $J_{^{19}F-^{13}C}$=244 Hz), 144.3 (C2, d, $J_{^{19}F-^{13}C}$=250 Hz), 145.7 ppm (C5, d, $J_{^{19}F-^{13}C}$=233 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ=−127.0 (C2-F, s), −135.3 (C6-F, dd, ^1J=27 Hz, ^2J=12 Hz), −152.0 ppm (C5-F, dd, ^1J=26 Hz, ^2J=7 Hz); HRMS for $C_{22}H_{21}F_3N_2O_4S_2$ $[M+H]^+$: calcd 499.0968, found 499.0971.

3-Morpholin-4-yl-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9g). Recrystallization was accomplished from EtOH; yield: 0.11 g, 46%; mp: 198–199 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=2.90 (2H, d, J=10.8 Hz, morpholine), 3.12 (2H, t, J=7.8 Hz, $SO_2CH_2CH_2$), 3.21 (2H, t, J=11.1 Hz, morpholine), 3.57 (2H, t, J=11.1 Hz, morpholine), 3.79 (2H, d, J=10.8 Hz, morpholine), 4.06 (2H, t, J=7.8 Hz, $SO_2CH_2CH_2$), 7.15–7.37 (5H, m, ArH), 8.48 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=33.3 ($SO_2CH_2CH_2$), 56.6 (morpholine, d, $J_{^{19}F-^{13}C}$=4 Hz), 62.3 ($SO_2CH_2CH_2$), 71.8 (morpholine), 132.2 (Ar), 133.8 (Ar), 134.1 (Ar), 136.1 (C1, t, $J_{^{19}F-^{13}C}$=6 Hz), 139.7 (C4, dd, $^1J_{^{19}F-^{13}C}$=16 Hz, $^2J_{^{19}F-^{13}C}$=5 Hz), 142.9 (Ar), 150.7 (C5, C6, dd, $^1J_{^{19}F-^{13}C}$=261 Hz, $^2J_{^{19}F-^{13}C}$=17 Hz), 159.3 ppm (C2, d, $J_{^{19}F-^{13}C}$=259 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ=−119.1 (C2-F, d, J=14 Hz), −132.4 (C5-F, d, J=25 Hz), −136.7 ppm (C6-F, dd, ^1J=25 Hz, ^2J=14 Hz); HRMS for $C_{18}H_{19}F_3N_2O_5S_2$ $[M+H]^+$: calcd 465.0760, found 465.0765.

3-(Cyclooctylamino)-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9h). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:9), R_f=0.50; yield: 0.22 g, 88%; mp: 90–92 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.43–1.99 (14H, m, cyclooctane), 3.14 (2H, t, J=7.8 Hz, $SO_2CH_2CH_2$), 3.64 (2H, t, J=7.8 Hz, $SO_2CH_2CH_2$), 3.85–3.95 (1H, m, cyclooctane), 5.68 (2H, s, SO_2NH_2), 6.91 (1H, d, J=8.7 Hz, NH), 7.14–7.35 ppm (5H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=23.5 (cyclooctane), 25.7 (cyclooctane), 27.5 (cyclooctane), 28.7 ($SO_2CH_2CH_2$), 33.2 (cyclooctane), 56.2 (CH of cyclooctane, d, $J_{^{19}F-^{13}C}$=11 Hz), 58.7 ($SO_2CH_2CH_2$, d, $J_{^{19}F-^{13}C}$=4 Hz), 114.7 (C4, dd, $^1J_{^{19}F-^{13}C}$=12 Hz, $^2J_{^{19}F-^{13}C}$=7 Hz), 126.4 (C1, t, $J_{^{19}F-^{13}C}$=16 Hz), 127.6 (Ar), 128.5 (Ar), 129.1 (Ar), 136.0 (C3, d, $J_{^{19}F-^{13}C}$=13 Hz), 136.5 (Ar), 136.7 (C6, d, $J_{^{19}F-^{13}C}$=251 Hz), 145.8 (C2, d, $J_{^{19}F-^{13}C}$=252 Hz), 146.2 ppm (C5, dd, $^1J_{^{19}F-^{13}C}$=252 Hz, $^2J_{^{19}F-^{13}C}$=16 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ=−131.0 (C2-F, s), −138.2 (C6-F, dd, ^1J=25 Hz, ^2J=12 Hz), −156.9 ppm (C5-F, dd, ^1J=26 Hz, ^2J=7 Hz); H,C HETCOR (300 MHz, $CDCl_3$): δ=56.2–3.89 ($NHCH$) 58.7–3.64 ppm ($SO_2CH_2CH_2Ph$); HRMS for $C_{22}H_{27}F_3N_2O_4S_2$ $[M+H]^+$: calcd 505.1437, found 505.1439.

3-(Cyclododecylamino)-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9i). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:10), R_f=0.37; yield: 0.13 g, 46%; mp: 130–131 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=1.21–1.74 (22H, m, cyclododecane), 3.07 (2H, t, J=7.5 Hz, $SO_2CH_2CH_2$), 3.78 (1H, br s, CH of cyclododecane), 3.88 (2H, t, J=7.5 Hz, $SO_2CH_2CH_2$), 6.55 (1H, d, J=8.1 Hz, NH), 7.14–7.37 (5H, m, ArH), 8.36 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=21.2 (cyclododecane), 23.3 (cyclododecane), 23.4 (cyclododecane), 24.5 (cyclododecane), 24.7 (cyclododecane), 28.5 ($SO_2CH_2CH_2$), 30.8 (cyclododecane), 53.5 (CH of cyclododecane, d, $J_{^{19}F-^{13}C}$=12 Hz), 58.0 ($SO_2CH_2CH_2$), 115.4 (C4, dd, $^1J_{^{19}F-^{13}C}$=13 Hz, $^2J_{^{19}F-^{13}C}$=4 Hz), 127.5 (Ar), 128.2 (C1, t, $J_{^{19}F-^{13}C}$=16 Hz), 129.0 (Ar), 135.8 (C3, d, $J_{^{19}F-^{13}C}$=16 Hz), 137.6 (Ar), 137.5 (C6, dd, $^1J_{^{19}F-^{13}C}$=246 Hz, $^2J_{^{19}F-^{13}C}$=17 Hz), 144.7 (C2, d, $J_{^{19}F-^{13}C}$=250 Hz), 146.2 ppm (C5, dd, $^1J_{^{19}F-^{13}C}$=249 Hz, $^2J_{^{19}F-^{13}C}$=17 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ=−125.4 (C2-F, s), −134.5 (C6-F, dd, ^1J=27 Hz, ^2J=12 Hz), −151.0 ppm (C5-F, dd, ^1J=27 Hz, ^2J=6 Hz); HRMS $C_{26}H_{35}F_3N_2O_4S_2$ $[M+H]^+$: calcd 561.2063, found 561.2071.

3-[(2,6-Dimethoxybenzyl)amino]-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9j). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:5), R_f=0.47; yield: 0.13 g, 48%; mp: 133–137 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=2.76 (2H, t, J=8.1 Hz, $SO_2CH_2CH_2$), 3.47 (2H, t, J=8.1 Hz, $SO_2CH_2CH_2$), 3.74 (6H, s, $2CH_3$), 4.52 (2H, d, J=5.4 Hz, $NHCH_2$), 6.62 (2H, d, J=8.4 Hz, ArH), 6.69 (1H, br t, NH), 7.05–7.32 (6H, m, ArH), 8.40 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=28.2 ($SO_2CH_2CH_2$), 39.4 ($NHCH_2$, d, $J_{^{19}F-^{13}C}$=13 Hz, signal overlaps with signal of DMSO), 56.4 (CH_3), 57.6 ($SO_2CH_2CH_2$), 104.7 (Ar), 114.7 (Ar), 116.5 (C4, dd, $^1J_{^{19}F-^{13}C}$=13 Hz, $^2J_{^{19}F-^{13}C}$=5 Hz), 127.4 (Ar), 127.9 (C1, t, $J_{^{19}F-^{13}C}$=16 Hz), 129.0 (Ar), 129.1 (Ar), 130.4 (Ar), 136.9 (C3, d, $J_{^{19}F-^{13}C}$=13 Hz), 137.5 (Ar), 138.1 (C6, d $^1J_{^{19}F-^{13}C}$=251 Hz), 145.5 (C5, d, $J_{^{19}F-^{13}C}$=253 Hz), 146.1 (C2, d, $J_{^{19}F-^{13}C}$=254 Hz), 158.7 ppm (Ar); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ=−121.8 (C2-F, dd, ^1J=11 Hz, ^2J=5 Hz), −135.5 (C6-F, dd, ^1J=27 Hz, ^2J=12 Hz), −149.6 ppm (C5-F, dd, ^1J=27 Hz, ^2J=5 Hz); HRMS $C_{23}H_{23}F_3N_2O_6S_2$ $[M-H]^-$: calcd 543.0877, found 543.0881.

3-[(3,4-Dimethoxybenzyl)amino]-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9k). Recrystallization was accomplished from EtOH; yield: 0.18 g, 67%; mp: 167–168 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=2.95 (2H, t, J=7.8 Hz, $SO_2CH_2CH_2$), 3.69 (3H, s, CH_3), 3.73 (3H, s, CH_3), 3.82 (2H, t, J=7.8 Hz, $SO_2CH_2CH_2$), 4.45 (2H, t, J=4.7 Hz, $NHCH_2$), 6.78–6.99 (4H, m, ArH, NH), 7.10–7.32 (5H, m, ArH), 8.38 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=28.5 ($SO_2CH_2CH_2$), 50.3 ($NHCH_2$, d,

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$J_{^{19}F-^{13}C}$ = 12.5 Hz), 56.0 (CH_3), 56.1 (CH_3), 57.7 ($SO_2CH_2CH_2$), 112.3 (Ar), 112.4 (Ar), 115.8 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 13 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 120.8 (Ar), 127.5 (Ar), 128.1 (C1, t, $J_{^{19}F-^{13}C}$ = 16 Hz), 128.9 (Ar), 129.0 (Ar), 131.9 (Ar), 136.0 (C3, d, $J_{^{19}F-^{13}C}$ = 14 Hz), 137.5 (Ar), 137.7 (C6, dd, $^1J_{^{19}F-^{13}C}$ = 249 Hz, $^2J_{^{19}F-^{13}C}$ = 18 Hz), 145.1 (C2, d, $J_{^{19}F-^{13}C}$ = 256 Hz), 146.0 (C5, d, $J_{^{19}F-^{13}C}$ = 250 Hz), 148.9 (Ar), 149.4 ppm (Ar); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = −123.7 (C2-F, s), −134.8 (C6-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −150.4 ppm (C5-F, dd, ^1J = 27 Hz, ^2J = 6 Hz); HRMS for $C_{23}H_{23}F_3N_2O_6S_2$ [M−H]$^-$: calcd 543.0877, found 543.0875.

3-(2,3-Dihydro-1*H*-inden-2-ylamino)-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9m). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:4), R_f = 0.60. Recrystallization was accomplished from EtOH after chromatography; yield: 0.12 g, 45%; mp 155–156 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 2.77–2.95 (4H, m, $SO_2CH_2CH_2$ and CH_2 of indane), 3.26 (1H, d, J = 6.3 Hz, indane), 3.31 (1H, d, J = 6.3 Hz, indane), 3.67 (2H, t, J = 7.8 Hz, $SO_2CH_2CH_2$), 4.45–4.55 (1H, m, CH of indane), 6.87 (1H, d, J = 6.6 Hz, NH), 7.07–7.30 (10H, m, ArH), 8.38 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 28.4 ($SO_2CH_2CH_2$), 41.1 (CH_2 of indane, signal overlaps with signal of [D$_6$]DMSO), 57.6 ($SO_2CH_2CH_2$), 57.7 (CH of indane, d, $J_{^{19}F-^{13}C}$ = 11 Hz), 115.3 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 13 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 125.4 (Ar), 127.4 (Ar), 127.5 (Ar), 128.2 (C1, t, $J_{^{19}F-^{13}C}$ = 16 Hz), 129.0 (Ar), 129.1 (Ar), 135.4 (C3, d, $J_{^{19}F-^{13}C}$ = 14 Hz), 137.5 (Ar), 137.6 (C6, dd, $^1J_{^{19}F-^{13}C}$ = 247 Hz, $^2J_{^{19}F-^{13}C}$ = 17 Hz), 141.1 (Ar), 144.4 (C2, d, $J_{^{19}F-^{13}C}$ = 252 Hz), 146.2 ppm (C5, dd, $^1J_{^{19}F-^{13}C}$ = 250 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = −126.1 (C2-F, s), −134.7 (C6-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −150.8 ppm (C5-F, dd, ^1J = 27 Hz, ^2J = 7 Hz); HRMS for $C_{23}H_{21}F_3N_2O_4S_2$ [M+H]$^+$: calcd 511.0968, found 511.0972.

3-[(1*S*)-2,3-Dihydro-1*H*-inden-1-ylamino]-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9n). The product was purified by chromatography on a column of silica gel with EtOAc (5%)/$CHCl_3$, R_f = 0.38; yield: 0.17 g, 66%; mp: 90 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 2.01 (1H, sext, J = 6.0 Hz, indane), 2.49 (1H, sext, indane, signal overlaps with signal of [D$_6$]DMSO), 2.78–3.23 (4H, m, CH_2 of indane, $SO_2CH_2CH_2$), 3.67–3.89 (2H, m, $SO_2CH_2CH_2$), 5.18 (1H, brs, indane), 6.89 (1H, d, J = 7.8 Hz, NH), 7.13–7.44 (9H, m, ArH), 8.43 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 28.3 ($SO_2CH_2CH_2$), 30.2 (indane), 35.3 (indane), 57.8 ($SO_2CH_2CH_2$), 61.7 (CH of indane, d, $J_{^{19}F-^{13}C}$ = 11.4 Hz), 115.6 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 14 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 124.6 (Ar), 125.7 (Ar), 127.4 (Ar), 127.5 (Ar), 128.2 (C1, t, $J_{^{19}F-^{13}C}$ = 16 Hz), 128.9 (Ar), 129.0 (Ar), 129.1 (Ar), 135.7 (C3, d, $J_{^{19}F-^{13}C}$ = 15 Hz), 137.5 (Ar), 137.8 (C6, d, $J_{^{19}F-^{13}C}$ = 246 Hz), 143.7 (Ar), 144.2 (Ar), 144.8 (C2, d, $J_{^{19}F-^{13}C}$ = 252 Hz), 146.1 ppm (C5, d, $J_{^{19}F-^{13}C}$ = 251 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = −124.2 (C2-F, s), −134.5 (C6-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −150.2 ppm (C5-F, dd, ^1J = 27 Hz, ^2J = 6 Hz); HRMS for $C_{23}H_{21}F_3N_2O_4S_2$ [M+H]$^+$: calcd 511.0968, found 511.0964.

3-[(1*S*)-1,2,3,4-Tetrahydronapthalen-1-ylamino)-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9o). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:10), R_f = 0.37; yield: 0.13 g, 50%; mp: 116–119 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.71–2.09 (4H, m, tetrahydronapthalene), 2.61–2.89 (2H, m, tetrahydronapthalene), 2.94 (2H, t, J = 7.8 Hz, $SO_2CH_2CH_2$), 3.74 (2H, t, J = 7.8 Hz, $SO_2CH_2CH_2$), 4.78–4.92 (1H, m, CH of tetrahydronapthalene), 6.82 (1H, d, J = 9.0 Hz, NH), 7.11–7.43 (9H, m, ArH), 8.41 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 19.1 (tetrahydronapthalene), 28.3 ($SO_2CH_2CH_2$), 29.1 (tetrahydronapthalene), 30.5 (tetrahydronapthalene), 54.1 (CH of tetrahydronapthalene, d, $J_{^{19}F-^{13}C}$ = 12 Hz), 58.0 ($SO_2CH_2CH_2$), 116.1 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 13 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 126.8 (Ar), 127.5 (Ar), 128.2 (Ar), 128.2 (C1, t, $J_{^{19}F-^{13}C}$ = 18 Hz, signal overlaps with signal of Ar), 129.0 (Ar), 129.1 (Ar), 129.5 (Ar), 129.9 (Ar), 135.3 (C3, d, $J_{^{19}F-^{13}C}$ = 11 Hz), 137.4 (Ar), 137.5 (Ar), 137.6 (Ar), 138.0 (C6, d, $J_{^{19}F-^{13}C}$ = 238 Hz), 145.1 (C2, d, $J_{^{19}F-^{13}C}$ = 254 Hz), 146.1 ppm (C5, d, $J_{^{19}F-^{13}C}$ = 254 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = −123.5 (C2-F, s), −134.3 (C6-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −149.9 ppm (C5-F, dd, ^1J = 27 Hz, ^2J = 5 Hz); HRMS for $C_{24}H_{23}F_3N_2O_4S_2$ [M−H]$^-$: calcd 523.0979, found 523.0983.

General procedure for the syntheses of 9l,p,q. A mixture of **9** (0.20 g, 0.50 mmol), Et_3N (0.071 mL, 0.51 mmol), DMSO (1 mL) and appropriate nucleophile (0.52 mmol) was stirred at ambient temperature for three days, compound **9l** was obtained after stirring for five days. The mixture was then diluted with H_2O (20 mL).

3-(1-Adamantylamino)-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]-benzenesulfonamide (9l). The resultant precipitate was filtered, washed with H_2O. The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:4), R_f = 0.75; yield: 0.02 g, 8%; mp: 155–156 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.69 (6H, brs, adamantane), 1.91 (6H, brs, adamantane), 2.15 (3H, brs, adamantane), 3.16 (2H, m, $SO_2CH_2CH_2$), 3.67 (2H, m, $SO_2CH_2CH_2$), 5.53 (2H, s, SO_2NH_2), 6.41 (1H, s, NH), 7.11–7.40 ppm (5H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.7 ($SO_2CH_2CH_2$), 30.2 (adamantane), 36.2 (adamantane), 43.4 (adamantane), 43.5 (adamantane), 56.6 ($SO_2CH_2CH_2$), 58.9 (adamantane, d, $J_{^{19}F-^{13}C}$ = 4 Hz), 119.6 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 12 Hz, $^2J_{^{19}F-^{13}C}$ = 6 Hz), 126.0 (C1, t, $J_{^{19}F-^{13}C}$ = 16 Hz), 127.6 (Ar), 128.5 (Ar), 129.2 (Ar), 135.3 (C3, dd, $^1J_{^{19}F-^{13}C}$ = 18 Hz, $^2J_{^{19}F-^{13}C}$ = 3 Hz), 137.0 (Ar), 139.0 (C6, d, $J_{^{19}F-^{13}C}$ = 252 Hz), 146.0 (C5, d, $J_{^{19}F-^{13}C}$ = 254 Hz), 146.4 ppm (C2, d, $J_{^{19}F-^{13}C}$ = 253 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −120.3 (C2-F, s), −137.6 (C6-F, dd, ^1J = 25 Hz, ^2J = 12 Hz), −152.0 ppm (C5-F, dd, ^1J = 26 Hz, ^2J = 6 Hz); HRMS for $C_{24}H_{27}F_3N_2O_4S_2$ [M+H]$^+$: calcd 529.1437, found 529.1440.

3-{[(1*R*,2*S*)-2-Hydroxy-1,2-diphenylethyl]amino}-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9p). The mixture was then extracted with EtOAc (3×10 mL). The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. Recrystallization was accomplished from EtOH/H_2O = 2:1; yield: 0.12 g, 40%; mp: 175–176 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.06 (2H, t, J = 7.5 Hz, $SO_2CH_2CH_2$), 3.75–3.95 (2H, m, $SO_2CH_2CH_2$), 4.89–4.98 (1H, m, CH), 5.10 (1H, d, J = 4.5 Hz, CH), 6.01 (1H, brs, OH), 7.05–7.31 (15H, m, ArH), 7.87 (1H, d, J = 9.0 Hz, NH), 8.30 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 28.5 ($SO_2CH_2CH_2$), 58.0 ($SO_2CH_2CH_2$, d, $J_{^{19}F-^{13}C}$ = 2 Hz), 65.4 (CH, d, $J_{^{19}F-^{13}C}$ = 12.8 Hz), 75.6 (CH), 115.3 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 13 Hz, $^2J_{^{19}F-^{13}C}$ = 6 Hz), 127.2 (Ar), 127.4 (Ar), 127.8 (Ar), 127.9 (Ar), 128.2 (Ar), 128.3 (Ar), 128.9 (Ar), 129.0 (Ar), 129.1 (Ar), 135.2 (C3, d, $J_{^{19}F-^{13}C}$ = 12 Hz), 137.2 (C6, d, $J_{^{19}F-^{13}C}$ = 250 Hz), 137.5 (Ar), 139.7 (Ar), 142.9 (Ar), 144.5 (C2, d, $J_{^{19}F-^{13}C}$ = 254 Hz), 146.0 ppm (C5, d, $J_{^{19}F-^{13}C}$ = 249 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = −123.3 (C2-F, s), −134.7 (C6-F, dd, ^1J = 25 Hz, ^2J = 12 Hz), −150.8 ppm (C5-F, dd, ^1J = 26 Hz, ^2J = 7 Hz); HRMS for $C_{28}H_{25}F_3N_2O_5S_2$ [M+H]$^+$: calcd 591.1230, found 591.1220.

3-{[(1*S*,2*R*)-2-Hydroxy-1,2-diphenylethyl]amino}-2,5,6-trifluoro-4-[(2-phenylethyl)sulfonyl]benzenesulfonamide (9q). The resultant precipitate was filtered, washed with H_2O. Recrystallization was accomplished from EtOH/H_2O = 2:1; yield: 0.12 g, 40%; mp: 176–177 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.06 (2H, t, J = 7.5 Hz, $SO_2CH_2CH_2$), 3.75–3.95 (2H, m, $SO_2CH_2CH_2$), 4.90–5.01 (1H, m, CH), 5.10 (1H, t, J = 4.2 Hz, CH), 6.01 (1H, d, J = 4.2 Hz, OH), 7.02–7.31 (15H, m, ArH), 7.87 (1H, d, J = 8.4 Hz, NH), 8.25 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 28.5 ($SO_2CH_2CH_2$), 58.0 ($SO_2CH_2CH_2$, d, $J_{^{19}F-^{13}C}$ = 2 Hz), 65.4 (CH, d, $J_{^{19}F-^{13}C}$ = 12.8 Hz), 75.6 (CH), 115.3 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 13 Hz, $^2J_{^{19}F-^{13}C}$ = 6 Hz), 127.2 (Ar), 127.4 (Ar), 127.8 (Ar), 127.9 (Ar), 128.2 (Ar), 128.3 (Ar), 128.9 (Ar), 129.0

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(Ar), 129.1 (Ar), 135.2 (C3, d, $J_{^{19}F-^{13}C}$ = 12 Hz), 137.2 (C6, d, $J_{^{19}F-^{13}C}$ = 250 Hz), 137.5 (Ar), 139.7 (Ar), 142.9 (Ar), 144.5 (C2, d, $J_{^{19}F-^{13}C}$ = 254 Hz), 146.0 ppm (C5, d, $J_{^{19}F-^{13}C}$ = 249 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = −123.3 (C2-F, s), −134.7 (C6-F, dd, ^1J = 25 Hz, ^2J = 12 Hz), −150.8 ppm (C5-F, dd, ^1J = 26 Hz, ^2J = 7 Hz); HRMS for $C_{28}H_{25}F_3N_2O_5S_2$ [M+H]$^+$: calcd 591.1230, found 591.1221.

General procedure for the syntheses of 10 (d,h-k,n–p). A mixture of 2,3,5,6-tetrafluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide (**10**) (0.20 g, 0.59 mmol), DMSO (1 mL) and appropriate nucleophile (1.20 mmol) was stirred at ambient temperature for 24 h. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (3×10 mL). The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure.

3-(Benzylamino)-2,5,6-trifluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide (10 d). The product was purified by chromatography on a column of silica gel with EtOAc/CHCl$_3$ (1:2), R_f = 0.19; yield: 0.11 g, 35%; mp 127–128 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.65 (2H, t, J = 5.4 Hz, $SO_2CH_2CH_2$), 3.83 (2H, k, J = 5.4 Hz, $SO_2CH_2CH_2$), 4.45–4.55 (2H, m, NHCH_2), 5.03 (1H, t, J = 5.1 Hz, OH), 6.96 (1H, brt, NH), 7.28–7.52 (5H, m, ArH), 8.36 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 50.7 (NHCH_2, d, $J_{^{19}F-^{13}C}$ = 13 Hz), 55.8 ($SO_2CH_2CH_2$), 60.1 ($SO_2CH_2CH_2$), 117.6 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 13 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 127.9 (C1 signal overlaps with signal of Ar), 128.1 (Ar), 128.3 (Ar), 129.3 (Ar), 136.1 (C3, d, $J_{^{19}F-^{13}C}$ = 13 Hz), 137.8 (C6, d, $J_{^{19}F-^{13}C}$ = 246 Hz), 139.6 (Ar), 144.9 (C2, d, $J_{^{19}F-^{13}C}$ = 252 Hz), 146.2 ppm (C5, d, $J_{^{19}F-^{13}C}$ = 252 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = −125.1 (C2-F, s), −135.3 (C6-F, dd, ^1J = 25 Hz, ^2J = 13 Hz), −150.7 ppm (C5-F, dd, ^1J = 26 Hz, ^2J = 7 Hz); HRMS for $C_{15}H_{15}F_3N_2O_5S_2$ [M+H]$^+$: calcd 425.0447, found 425.0439.

3-(Cyclooctylamino)-2,5,6-trifluoro-4-[(2-hydroxyethyl)sulfonyl]-benzenesulfonamide (10 h). The synthesis of compound **10 h** was described previously.[52]

3-(Cyclododecylamino)-2,5,6-trifluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide (10 i). The product was purified by chromatography on a column of silica gel with EtOAc/CHCl$_3$ (1:1), R_f = 0.50; yield: 0.26 g, 88%; mp: 143–144 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.23–1.74 (22H, m, cyclododecane), 3.68 (2H, t, J = 5.4 Hz, $SO_2CH_2CH_2$), 3.78 (1H, brs, CH of cyclododecane, signal overlaps with signal of $SO_2CH_2CH_2$), 3.83 (2H, t, J = 5.4 Hz, $SO_2CH_2CH_2$), 5.01 (1H, t, J = 5.4 Hz, OH), 6.55 (1H, d, J = 8.7 Hz, NH), 8.36 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 21.3 (cyclododecane), 23.4 (cyclododecane), 23.5 (cyclododecane), 24.4 (cyclododecane), 24.6 (cyclododecane), 30.8 (cyclododecane), 53.4 (CH of cyclododecane, d, $J_{^{19}F-^{13}C}$ = 12 Hz), 55.8 ($SO_2CH_2CH_2$), 60.3 ($SO_2CH_2CH_2$), 117.4 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 13 Hz, $^2J_{^{19}F-^{13}C}$ = 6 Hz), 127.9 (C1, t, $J_{^{19}F-^{13}C}$ = 16 Hz), 135.7 (C3, d, $J_{^{19}F-^{13}C}$ = 13 Hz), 137.4 (C6, dd, $^1J_{^{19}F-^{13}C}$ = 246 Hz, $^2J_{^{19}F-^{13}C}$ = 19 Hz), 144.7 (C2, d, $J_{^{19}F-^{13}C}$ = 253 Hz), 146.3 ppm (C5, d, $J_{^{19}F-^{13}C}$ = 247 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = −125.4 (C2-F, s), −134.8 (C6-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −151.4 ppm (C5-F, dd, ^1J = 27 Hz, ^2J = 6 Hz); HRMS for $C_{20}H_{31}F_3N_2O_5S_2$ [M+H]$^+$: calcd 501.1699, found 501.1701.

3-[(2,6-Dimethoxybenzyl)amino]-2,5,6-trifluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide (10 j). The product was purified by chromatography on a column of silica gel with EtOAc (60%)/CHCl$_3$, R_f = 0.45; yield: 0.15 g, 52%; mp: 164–165 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.35 (2H, t, J = 5.4 Hz, $SO_2CH_2CH_2$), 3.63 (2H, t, J = 5.4 Hz, $SO_2CH_2CH_2$), 3.75 (6H, s, 2CH$_3$), 4.48 (2H, d, J = 5.4 Hz, NHCH_2), 4.93 (1H, brs, OH), 6.58 (1H, brt, NH), 6.66 (2H, d, J = 8.4 Hz, ArH), 7.26 (1H, t, J = 8.4 Hz, ArH), 8.42 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 39.5 (NHCH_2), 55.4 ($SO_2CH_2CH_2$), 56.3 (2CH$_3$), 59.9 ($SO_2CH_2CH_2$), 104.7 (Ar), 114.6 (Ar), 118.2 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 12 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 127.7 (C1, t, $J_{^{19}F-^{13}C}$ = 16 Hz), 130.3 (Ar), 136.8 (C3, d, $J_{^{19}F-^{13}C}$ = 11 Hz), 137.9 (C6, d, $^1J_{^{19}F-^{13}C}$ = 228 Hz), 144.8 (C5, d, $J_{^{19}F-^{13}C}$ = 242 Hz), 146.0 (C2, d, $J_{^{19}F-^{13}C}$ = 253 Hz), 158.7 ppm (Ar); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = −122.1 (C2-F, s), −135.9 (C6-F, dd, ^1J = 26 Hz, ^2J = 13 Hz), −150.1 ppm (C5-F, dd, ^1J = 27 Hz, ^2J = 6 Hz); HRMS for $C_{17}H_{19}F_3N_2O_7S_2$ [M−H]$^-$: calcd 483.0513, found 483.0517.

3-[(3,4-Dimethoxybenzyl)amino]-2,5,6-trifluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide (10 k). The product was purified by chromatography on a column of silica gel with EtOAc/CHCl$_3$ (2:1), R_f = 0.38. Recrystallization was accomplished from EtOH after chromatography; yield: 0.10 g, 29%; mp: 164–165 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.65 (2H, t, J = 5.7 Hz, $SO_2CH_2CH_2$), 3.74 (3H, s, CH$_3$), 3.76 (3H, s, CH$_3$), 3.82 (2H, brt, $SO_2CH_2CH_2$), 4.43 (2H, brs, NHCH_2), 6.78–7.03 (3H, m, ArH), 6.99 (1H, s, NH), 8.38 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 50.6 (NHCH$_2$, d, $J_{^{19}F-^{13}C}$ = 12 Hz), 55.8 (CH$_3$), 56.0 (CH$_3$), 56.1 ($SO_2CH_2CH_2$), 60.1 ($SO_2CH_2CH_2$), 112.2 (Ar), 112.3 (Ar), 117.7 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 13 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 120.7 (Ar), 127.8 (C1, t, $J_{^{19}F-^{13}C}$ = 16 Hz), 131.8 (Ar), 136.0 (C3, d, $J_{^{19}F-^{13}C}$ = 16 Hz), 137.8 (C6, d, $J_{^{19}F-^{13}C}$ = 252 Hz), 145.1 (C2, d, $J_{^{19}F-^{13}C}$ = 253 Hz), 146.1 (C5, dd, $^1J_{^{19}F-^{13}C}$ = 253 Hz, $^2J_{^{19}F-^{13}C}$ = 16 Hz), 148.9 (Ar), 149.5 ppm (Ar); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = −124.4 (C2-F, s), −135.2 (C6-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −150.7 ppm (C5-F, dd, ^1J = 27 Hz, ^2J = 6 Hz); HRMS for $C_{17}H_{19}F_3N_2O_7S_2$ [M−H]$^-$: calcd 483.0513, found 483.0515.

3-[(1*S*)-2,3-Dihydro-1*H*-inden-1-ylamino]-2,5,6-trifluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide (10 n). The product was purified by chromatography on a column of silica gel with EtOAc/CHCl$_3$ (1:1), R_f = 0.38; yield: 0.16 g, 60%; mp: 131–132 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.95 (1H, sext, J = 6.6 Hz, indane), 2.51 (1H, sext, indane, signal overlaps with signal of [D$_6$]DMSO), 2.78–3.08 (2H, m, indane), 3.55–3.68 (2H, m, $SO_2CH_2CH_2$), 3.71–3.85 (2H, m, $SO_2CH_2CH_2$), 5.02 (1H, t, J = 5.4 Hz, OH), 5.12–5.25 (1H, m, CH of indane), 6.88 (1H, d, J = 7.8 Hz, NH), 7.21–7.42 (4H, m, ArH), 8.43 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 30.2 (indane), 35.2 (indane), 55.8 ($SO_2CH_2CH_2$), 60.2 ($SO_2CH_2CH_2$), 61.9 (CH of indane, d, $J_{^{19}F-^{13}C}$ = 12 Hz), 117.6 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 13 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 124.7 (Ar), 125.6 (Ar), 127.4 (Ar), 128 (C1, t, $J_{^{19}F-^{13}C}$ = 16 Hz), 128.9 (Ar), 135.6 (C3, d, $J_{^{19}F-^{13}C}$ = 12 Hz), 137.8 (C6, d, $J_{^{19}F-^{13}C}$ = 253 Hz), 144.4 (Ar), 144.5 (Ar), 144.8 (C2, d, $J_{^{19}F-^{13}C}$ = 251 Hz), 146.3 ppm (C5, d, $J_{^{19}F-^{13}C}$ = 258 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = −124.6 (C2-F, s), −134.8 (C6-F, dd, ^1J = 27 Hz, ^2J = 12 Hz), −150.7 ppm (C5-F, dd, ^1J = 27 Hz, ^2J = 6 Hz); HRMS for $C_{17}H_{17}F_3N_2O_5S_2$ [M−H]$^-$: calcd 449.0458, found 449.0461.

3-[(1*S*)-1,2,3,4-Tetrahydronapthalen-1-ylamino)-2,5,6-trifluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide (10 o). The product was purified by chromatography on a column of silica gel with EtOAc/CHCl$_3$ (1:1), R_f = 0.41; yield: 0.14 g, 51%; mp: 103–105 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.68–2.11 (4H, m, tetrahydronapthalene), 2.63–2.92 (2H, m, tetrahydronapthalene), 3.61 (2H, t, J = 5.4 Hz, $SO_2CH_2CH_2$), 3.76 (2H, brt, $SO_2CH_2CH_2$), 4.77–4.90 (1H, m, CH of tetrahydronapthalene), 5.01 (1H, brs, OH), 6.82 (1H, d, J = 9.0 Hz, NH), 7.11–7.28 (3H, m, ArH), 7.39 (1H, d, J = 6.9 Hz, ArH), 8.42 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 19.3 (tetrahydronapthalene), 29.2 (tetrahydronapthalene), 30.6 (tetrahydronapthalene), 54.3 (CH of tetrahydronapthalene, d, $J_{^{19}F-^{13}C}$ = 12 Hz), 55.7 ($SO_2CH_2CH_2$), 60.3 ($SO_2CH_2CH_2$), 117.9 (C4, dd, $^1J_{^{19}F-^{13}C}$ = 13 Hz, $^2J_{^{19}F-^{13}C}$ = 5 Hz), 126.8 (Ar), 128.0 (C1, t, $J_{^{19}F-^{13}C}$ = 16 Hz), 128.2 (Ar), 129.5 (Ar), 129.8 (Ar), 135.1 (C3, d, $J_{^{19}F-^{13}C}$ = 15 Hz), 137.48 (Ar), 137.56 (Ar), 137.9 (C6, d, $J_{^{19}F-^{13}C}$ = 245 Hz), 145.1 (C2, d, $J_{^{19}F-^{13}C}$ = 255 Hz), 146.2 ppm (C5, d, $J_{^{19}F-^{13}C}$ = 254 Hz); ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = −123.9 (C2-F, s), −134.5 (C6-F, dd, ^1J = 27 Hz, ^2J =

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12 Hz), −150.5 ppm (C5-F, dd, ^1J=27 Hz, ^2J=6 Hz); HRMS for $C_{18}H_{19}F_3N_2O_5S_2$ $[M+H]^+$: calcd 465.076, found 465.0760.

3-{[(1*R*,2*S*)-2-Hydroxy-1,2-diphenylethyl]amino}-2,5,6-trifluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide (10 p). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (2:1), R_f=0.53; yield: 0.12 g, 39%; ^{1}H NMR (300 MHz, CD_3OD): δ=3.62 (2H, t, J=5.4 Hz, $SO_2CH_2CH_2$), 4.04 (2H, t, J=5.4 Hz, $SO_2CH_2CH_2$), 4.88 (SO_2NH_2, NH, OH signals overlap with signal of H_2O), 5.07 (1H, dd, ^1J=5.1 Hz, ^2J=2.1 Hz, CH), 5.14 (1H, d, J=4.8 Hz, CH), 7.11–7.35 ppm (10H, m, ArH); ^{13}C NMR (75 MHz, CD_3OD): δ=55.5 ($SO_2CH_2CH_2$), 59.8 ($SO_2CH_2CH_2$), 65.7 (CH, d, $J_{^{19}F-^{13}C}$=12.5 Hz), 76.3 (CH), 116.8 (C4, dd, $^1J_{^{19}F-^{13}C}$=13 Hz, $^2J_{^{19}F-^{13}C}$=5.4 Hz), 126.8 (Ar), 127.3 (Ar), 127.4 (Ar), 127.7 (Ar), 127.8 (Ar), 128.5 (Ar), 135.0 (C3, d, $J_{^{19}F-^{13}C}$=14 Hz), 137.7 (C6, d, $J_{^{19}F-^{13}C}$=250 Hz), 139.1 (Ar), 141.7 (Ar), 144.8 (C2, d, $J_{^{19}F-^{13}C}$=257 Hz), 146.1 ppm (C5, d, $J_{^{19}F-^{13}C}$=247 Hz); ^{19}F NMR (282 MHz, CD_3OD): δ=−123.9 (C2-F, s), −136.4 (C6-F, dd, ^1J=25 Hz, ^2J=12 Hz), −152.3 ppm (C5-F, dd, ^1J=24 Hz, ^2J=6 Hz); HRMS for $C_{22}H_{21}F_3N_2O_6S_2$ $[M+H]^+$: calcd 531.0866, found 531.0865.

3,5-Bis(cyclooctylamino)-2,6-difluoro-4-[(2-phenylethyl)sulfonyl]-benzenesulfonamide (11 h). A mixture of **9** (0.20 g, 0.50 mmol), Et_3N (0.142 mL, 1.02 mmol), DMSO (1 mL) cyclooctylamine (0.142 mL, 1.02 mmol) was stirred at 60 °C for 32 h. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (3×10 mL). The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The product was purified by chromatography on a column of silica gel with EtOAc (10%)/$CHCl_3$, R_f=0.72; yield: 0.15 g, 48%; ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.43–1.98 (28H, m, cyclooctane), 3.05–3.15 (2H, m, $SO_2CH_2CH_2$), 3.51–3.62 (2H, m, $SO_2CH_2CH_2$), 3.88 (2H, brs, 2×CH of cyclooctane), 5.58 (2H, s, SO_2NH_2), 6.43 (2H, brs, 2NH), 7.14–7.38 ppm (5H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=23.8 (cyclooctane), 25.8 (cyclooctane), 27.4 (cyclooctane), 28.5 ($SO_2CH_2CH_2$), 33.5 (cyclooctane), 55.9 ($SO_2CH_2CH_2$), 56.2 (CH of cyclooctane, t, J=6 Hz), 111.1 (C4, t, $J_{^{19}F-^{13}C}$=5 Hz), 126.3 (C1, t, $J_{^{19}F-^{13}C}$=16 Hz), 127.4 (Ar), 128.6 (Ar), 129.2 (Ar), 135.3 (C3, dd, $^1J_{^{19}F-^{13}C}$=10 Hz, $^2J_{^{19}F-^{13}C}$=6 Hz), 137.3 (Ar), 139.4 ppm (C2, dd, $^1J_{^{19}F-^{13}C}$=244 Hz, $^2J_{^{19}F-^{13}C}$=4.5 Hz); ^{19}F NMR (282 MHz, $CDCl_3$): δ=−144.1 ppm (2F, s); HRMS for $C_{30}H_{43}F_2N_3O_4S_2$ $[M+H]^+$: calcd 612.2736, found 612.2729.

3,5-Bis[(3,4-dimethoxybenzyl)amino]-2,6-difluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide (12 k). A mixture of **10** (0.20 g, 0.59 mmol), DMSO (1 mL) and 3,4-dimethoxybenzylamine (0.359 mL, 2.38 mmol) was stirred at ambient temperature for five days. The mixture was then diluted with H_2O (20 mL), the resultant precipitate was filtered, washed with H_2O. Recrystallization was accomplished from EtOH; yield: 0.20 g, 53%; mp: 99–102 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ=3.41 ($SO_2CH_2CH_2$, signal overlaps with signal of H_2O), 3.65 (2H, k, J=5.4 Hz, $SO_2CH_2CH_2$), 3.75 (12H, s, 4CH_3), 4.35 (4H, d, J=5.7 Hz, 2NHCH_2), 5.04 (1H, t, J=5.4 Hz, OH), 6.38 (2H, t, J=5.7 Hz, 2NH), 6.85–7.05 (6H, m, ArH), 8.15 ppm (2H, s, SO_2NH_2); ^{13}C NMR (75 MHz, [D_6]DMSO): δ=51.2 ($NHCH_2$, t, $J_{^{19}F-^{13}C}$=6 Hz), 55.5 ($SO_2CH_2CH_2$), 56.1 (CH_3), 56.2 (CH_3), 58.3 ($SO_2CH_2CH_2$), 112.4 (Ar), 116.0 (C4, t, $J_{^{19}F-^{13}C}$=3 Hz), 120.8 (Ar), 127.9 (C1, t, $J_{^{19}F-^{13}C}$=16 Hz), 132.3 (Ar), 135.7 (C3, dd, $^1J_{^{19}F-^{13}C}$=10 Hz, $^2J_{^{19}F-^{13}C}$=6 Hz), 141.7 (C2, dd, $^1J_{^{19}F-^{13}C}$=247 Hz, $^2J_{^{19}F-^{13}C}$=4 Hz), 148.8 (Ar), 149.4 ppm (Ar); ^{19}F NMR (282 MHz, [D_6]DMSO): δ=−133.47 ppm (2F, s); H,C HETCOR (300 MHz, [D_6]DMSO): δ=51.2–4.35 (NHCH_2), 56.2–3.75 (CH_3), 55.5–3.65 ($SO_2CH_2CH_2$), 58.3–3.41 ppm ($SO_2CH_2CH_2$); HRMS for $C_{26}H_{31}F_2N_3O_9S_2$ $[M+H]^+$: calcd 632.1543, found 632.1548.

General procedure for the syntheses of 13 c and 13 f. A mixture of **10 i** (0.20 g, 0.40 mmol), DMSO (1 mL) and appropriate nucleophile (0.82 mmol) was stirred, compound **13 c** was obtained after stirring at 50 °C for 14 h, compound **13 f** was obtained after stirring at 60 °C for 22 h. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (3×10 mL). The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure.

3-(Butylamino)-5-(cyclododecylamino)-2,6-difluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide (13 c). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:2), R_f=0.66; yield: 0.09 g, 42%; ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.93 (3H, t, J=7.2 Hz, CH_3), 1.22–1.51 (22H, m, cyclododecane), 1.54–1.71 (4H, m, $NHCH_2CH_2CH_2CH_3$), 2.56 (1H, brs, OH), 3.31–3.41 (2H, m, NHCH_2), 3.48 (3H, t, J=5.2 Hz, $SO_2CH_2CH_2$), 3.84 (1H, brs, NHCH), 4.06 (3H, t, J=5.2 Hz, $SO_2CH_2CH_2$), 5.58 (2H, brs, SO_2NH_2), 6.12 (1H, brs, NH), 6.35 ppm (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.7 (CH_3), 20.0 (CH_3CH_2), 20.9 (cyclododecane), 23.0 (cyclododecane), 23.1 (cyclododecane), 24.2 ($SO_2CH_2CH_2$), 24.4 (cyclododecane), 30.6 (cyclododecane), 32.8 ($NHCH_2CH_2$, d, $J_{^{19}F-^{13}C}$=3.2 Hz), 47.1 ($NHCH_2$, d, $J_{^{19}F-^{13}C}$=12.7 Hz), 53.5 (CH of cyclododecane, d, $J_{^{19}F-^{13}C}$=12.5 Hz), 56.2 ($SO_2CH_2CH_2$), 57.0 ($SO_2CH_2CH_2$), 111.2 (C4, t, $J_{^{19}F-^{13}C}$=5 Hz), 126.3 (C1, t, $J_{^{19}F-^{13}C}$=16 Hz), 135.5 (C3, dd, $^1J_{^{19}F-^{13}C}$=12.5 Hz, $^2J_{^{19}F-^{13}C}$=3 Hz), 136.0 (C5, dd, $^1J_{^{19}F-^{13}C}$=12.7 Hz, $^2J_{^{19}F-^{13}C}$=3 Hz), 139.3 ppm (C2, C6 dd, $^1J_{^{19}F-^{13}C}$=243 Hz, $^2J_{^{19}F-^{13}C}$=4.3 Hz); ^{19}F NMR (376 MHz, $CDCl_3$): δ=−156.44 (C2-F or C6-F, d, J=11 Hz), −158.1 ppm (C2-F or C6-F, d, J=10.5 Hz); HRMS for $C_{24}H_{41}F_2N_3O_5S_2$ $[M+H]^+$: calcd 554.2528, found 554.2530.

3-(Cyclododecylamino)-2,6-difluoro-4-[(2-hydroxyethyl)sulfonyl]-5-{[2-(4-hydroxyphenyl)ethyl]amino}benzenesulfonamide (13 f). The product was purified by chromatography on a column of silica gel with EtOAc/$CHCl_3$ (1:2), R_f=0.25; yield: 0.05 g, 18%; mp: 158–159 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=1.22–1.73 (22H, m, cyclododecane), 2.75 (2H, brt, CH_2Ph), 3.44 (4H, brt, NHCH_2, SO_2CH_2), 3.73 (3H, brs, NHCH, $SO_2CH_2CH_2$), 5.02 (1H, t, J=5.4 Hz, OH), 5.94 (1H, brs, NH), 6.28 (1H, brs, NH), 6.70 (2H, d, J=7.6 Hz, ArH), 7.06 (2H, d, J=8.4 Hz, ArH), 8.08 (2H, s, SO_2NH_2), 9.23 ppm (1H, s, OH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ=21.2 (cyclododecane), 23.2 (cyclododecane), 23.3 (cyclododecane), 24.1 (cyclododecane), 24.3 (cyclododecane), 30.6 (cyclododecane), 35.9 (CH_2Ph), 49.2 ($NHCH_2$, $J_{^{19}F-^{13}C}$=11.4 Hz), 53.0 (NHCH, $J_{^{19}F-^{13}C}$=11.9 Hz), 55.2 (SO_2CH_2), 57.7 ($SO_2CH_2CH_2$), 113.9 (C4), 115.7 (Ar), 127.7 (C1, t, $J_{^{19}F-^{13}C}$=16 Hz), 129.3 (Ar), 130.1 (Ar), 135.3 (C3, dd, $^1J_{^{19}F-^{13}C}$=12.2 Hz, $^2J_{^{19}F-^{13}C}$=3 Hz),135.5 (C5, dd, $^1J_{^{19}F-^{13}C}$=12.9 Hz, $^2J_{^{19}F-^{13}C}$=3 Hz), 140.3 (C2, C6 d, $J_{^{19}F-^{13}C}$=243.4 Hz), 156.3 ppm (Ar); ^{19}F NMR (376 MHz, [D_6]DMSO): δ=−135.3 (C2-F or C6-F, s), −136.9 ppm (C2-F or C6-F, s); HRMS for $C_{28}H_{41}F_2N_3O_6S_2$ $[M+H]^+$: calcd 586.2757, found 586.2761.

Protein preparation

The expression and purification of CA I,[54] II,[55] VII,[56] XII,[57] and XIII[56] has been described previously. A DNA oligomer encoding full-length CA VA was amplified by PCR from the *CA VA* gene purchased from the Deutsches Ressourcenzentrum für Genomforschung (RZPD; Berlin, Germany), using forward primer with NdeI recognition site: 5′-GGG *CATATG* TGT TCT CAG CGT TCC TG-3′ and reverse primer with XhoI recognition site: 5′-CTA ATG *CTCGAG* GGA CCT TGT GCC CTC-3′ (recognition sites in italics). The PCR product was cloned into the bacterial expression vector pET21a (Novagen) via NdeI and XhoI restriction sites fusing a 6×His tag into the C terminus of the protein.

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Expression of recombinant CA VA was done in *Escherichia coli* BL21(DE3) Codon Plus-RIL strain (Stratagene). Transformed cells were transferred to LB medium containing 100 μg mL^{-1} ampicillin, 34 μg mL^{-1} chloramphenicol and grown at 37 °C and 220 rpm for 16 h. Then the saturated culture was diluted (1:50) in fresh LB medium containing 100 μg mL^{-1} ampicillin, 34 μg mL^{-1} chloramphenicol, 0.05 mM $ZnSO_4$ and grown to $OD_{600} \approx 0.8$. The expression of recombinant CA VA was induced with 1 mM isopropyl-β-D-thiogalactoside (IPTG) and 0.5 mM $ZnSO_4$. The culture was grown for 4 h at 30 °C and 250 rpm. The cells were harvested by centrifugation at 4000 *g* for 20 min at 4 °C.

The pellet was suspended in lysis buffer (25 mM Tris, 1% Triton X-100, 0.1 M NaCl, 2 mM 2-mercaptoethanol, 0.1 M imidazole (pH 7.5) and 1 mM PMSF) containing protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA). The cells were incubated at 4 °C for 60 min and then disrupted by sonication. The supernatant, containing soluble proteins, was obtained after centrifugation at 20000 *g* for 25 min. Soluble protein was purified using a Sepharose-IDA-Ni^{+2} affinity column (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Eluted protein was dialyzed against storage buffer (25 mM Tris, 0.1 M NaCl, 1 mM DTT, 10% glycerol pH 7.5) and stored at −80 °C.

The purity of CA VA preparations was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein concentrations were determined by UV/Vis spectrophotometry using an extinction coefficient $\varepsilon_{280} = 71515\ M^{-1}\,cm^{-1}$ and confirmed by the standard Bradford method.

Determination of compound binding to CAs

Fluorescent thermal shift assay: Fluorescent thermal shift assay (also termed ThermoFluor and differential scanning fluorimetry) experiments were performed in a Corbett Rotor-Gene 6000 (Qiagen Rotor-Gene Q) instrument using the blue channel (excitation λ 365±20, detection λ 460±15 nm). Protein samples containing various concentrations of inhibitor were heated from 25 to 99 °C while recording extrinsic fluorescence and determining the protein melting temperatures at each inhibitor concentration. Data analysis was performed as previously described.[54] Samples contained 10 μM protein, 0–200 μM ligand, 50 μM solvatochromic dye 8-anilino-1-naphthalene sulfonate (ANS) and 50 mM sodium phosphate buffer containing 100 mM NaCl at pH 7.0, with a final DMSO concentration of 2%. The applied heating rate was 1 °C min^{-1}.

Isothermal titration calorimetry: ITC experiments were performed using VP-ITC instruments (Microcal Inc., Northampton, USA) with 5–10 μM protein solution in the cell and 50–100 μM of the ligand solution in the syringe. Syringe volume was 250 μL, cell volume was 1.4315 mL. A typical experiment consisted of 25 injections (initial injection of 3 μL followed by 24 injections of 10 μL) within 3- or 4-min intervals, a stirring rate of 260 rpm, and a reference power of 4 μcal s^{-1}. The pre-titration delay was set to 200–600 s. Experiments were carried out at 37 °C in a 50 mM sodium phosphate or Tris chloride buffer containing 100 mM NaCl at pH 7.0, with a final DMSO concentration of 1–2%. Protein stock solutions were dialyzed against buffers that were used to prepare ligand solutions. ITC data were analyzed using MicroCal Origin software. The first point from the 3 μL injection in the integrated data graph was deleted. The binding constants, enthalpies, and entropies of binding were estimated after fitting the data with the single binding site model. The Wiseman *c* parameter for all ITC binding measurements was in the range of 7–900. Experiments were repeated at least twice (except for CA VI due to the lack of protein). Raw and integrated data for each ITC run is provided in the Supporting Information in the ITC Origin Final Figure format.

CO_2 hydration assay: The catalytic activity of the recombinant human CA II catalyzing carbon dioxide hydration was measured using an Applied Photophysics SX.18MV-R stopped-flow spectrometer as previously described.[47,58] The reaction of CO_2 hydration leads to a decrease in pH of the medium due to the release of bicarbonate and protons. The acidification velocities were measured by recording the drop in absorbance of bromothymol blue indicator (40 μM, 615 nm). The sample consisted of 20 nM CA II, 0.0–0.6 μM **10h** or 0–10 μM **2h** inhibitor (in <0.1% DMSO) and 10 mM HEPES buffer (pH 7.4) containing 10 mM NaCl. Saturated CO_2 solution was prepared by bubbling CO_2 gas in Milli-Q water at 25 °C for 1 h. The experiments were repeated twice.

Crystallization and structure determination

CAs in 20 mM Na-HEPES pH 7.5 and 50 mM NaCl were concentrated by ultrafiltration to a concentration of 20–60 mg mL^{-1}. Crystallization in sitting drop was started by mixing equal volumes (0.5–2 μL) of protein solution with reservoir buffers. Crystallization buffers are presented in SI Table S3. Complexes of ligands with CA isoforms were prepared by soaking of CA crystal with 0.5 mM solution of ligand prepared by mixing of 50 mM stock solution of ligand in DMSO with 50 μL of corresponding reservoir solution. Soaked crystals were measured after several days.

Crystal diffraction data were collected at EMBL beam lines on DESY synchrotron on the PETRA III and DORIS storage ring (Hamburg, Germany) and on X-ray diffractometer MicroMax 007-HF (Rigaku, Japan) at the Institute of Biotechnology (Vilnius, Lithuania), as listed in Table 5. Data collection statistics and refinement details are presented in Table 5.

Datasets for all CAs with **10p** and CA XIII with **9d** were processed using XDS,[59] datasets of CA XII and CA II with **9d** and **10d** as well CA XIII–**10d** were processed by MOSFLM.[60] All structures were solved by molecular replacement using MOLREP.[61] Initial phases for protein structures were obtained using the following PDB entries: 3HLJ for CA II, 1JD0 for CA XII, and 2NNO for CA XIII. A single protein chain stripped of all ligands was used as initial model in all molecular replacement procedures. Inhibitor 3D models were created using AVOGADRO,[62] and molecule geometry description was generated using LIBREFMAC.[63] Protein models were refined and manually remodeled using REFMAC[64] and COOT.[65] All graphic representations were made with MOLSCRIPT,[66] BOBSCRIPT,[67] and RASTER3D.[68] Coordinates and structure factors were submitted to the RCSB Protein Data Bank (PDB) with access codes listed in Table 5.

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Table 5. X-ray crystallographic data collection and refinement statistics.[a]

CA isoform–ligand:	XII–**10d**	XII–**9d**	XII–**10p**	II–**10d**	II–**9d**	II–**10p**	XIII–**10d**	XIII–**9d**	XIII–**10p**
PDB ID:	4QJW	4QJO	4QJ0	4QTL	4QJM	4QIY	4QJX	4QJP	4QIZ
Data collection statistics									
Space group	$P1$	$P1$	$P12_11$	$P12_11$	$P12_11$	$P1$	$P4_32_12$	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters [Å]	$a=46.5$ $b=66.8$ $c=80.5$ $\alpha=81.6$ $\beta=84.2$ $\gamma=86.7$	$a=46.5$ $b=66.6$ $c=80.3$ $\alpha=820$ $\beta=84.3$ $\gamma=86.5$	$a=77.5$ $b=74.2$ $c=91.4$ $\alpha=\gamma=90.0$ $\beta=108.9$	$a=42.0$ $b=40.9$ $c=71.7$ $\alpha=\gamma=90.0$ $\beta=103.9$	$a=42.2$ $b=41.1$ $c=720$ $\alpha=\gamma=90.0$ $\beta=104.1$	$a=73.6$ $b=41.2$ $c=84.0$ $\alpha=\gamma=90.0$ $\beta=109.3$	$a=56.8$ $b=56.8$ $c=159.6$ $\alpha=\beta=\gamma=90.0$	$a=56.0$ $b=57.5$ $c=159.5$ $\alpha=\beta=\gamma=90.0$	$a=56.6$ $b=159.6$ $c=57.1$ $\alpha=\beta=\gamma=90.0$
Resolution [Å]	41.66–1.55	23.59–1.80	86.50–1.55	16.16–1.80	20.62–1.75	79.32–1.30	56.82–1.95	159.52–1.620	57.10–1.55
Wavelength [Å]	0.82	1.54	0.83	1.54	1.54	0.83	0.82	0.83	0.83
Radiation source	EMBL/DESY X11	Rigaku MM007-HF	EMBL/DESY P14	Rigaku MM007-HF	Rigaku MM007-HF	EMBL/DESY P13	EMBL/DESY X11	EMBL/DESY P13	EMBL/DESY P13
No. unique reflections	133601	81977	141122	22117	23685	206964	19979	65652	75960
R_{merge} overall	0.058	0.050	0.069	0.094	0.057	0.032	0.095	0.038	0.037
(outer shell)	(0.319)	(0.153)	(0.371)	(0.380)	(0.177)	(0.286)	(0.537)	(0.141)	(0.405)
I/σ overall	41.6	15.3	13.1	19.9	12.6	13.0	20.9	26.3	27.2
(outer shell)	(1.7)	(4.0)	(4.7)	(5.6)	(5.1)	(3.4)	(5.8)	(8.1)	(4.4)
Multiplicity overall	1.9	2.0	5.2	5.4	3.6	3.8	14	6.5	6.6
(outer shell)	(1.5)	(2.0)	(5.2)	(5.2)	(3.6)	(3.8)	(14.2)	(5.1)	(6.4)
Completeness [%]	96.8	93.3	99.4	99.7	97.4	90.0	100.0	98.5	99.9
overall (outer shell)	(92.2)	(89.8)	(99.7)	(99.5)	(95.4)	(89.6)	(100.0)	(90.0)	(100.0)
Refinement statistics									
R_{work}	0.185	0.161	0.18	0.161	0.161	0.151	0.19	0.168	0.175
R_{free}	0.222	0.206	0.225	0.203	0.212	0.193	0.237	0.205	0.209
RMSD bond lengths [Å]	0.021	0.019	0.023	0.018	0.019	0.023	0.018	0.025	0.025
RMSD bond angles [°]	2.174	1.968	2.237	2.011	2.127	2.286	1.89	2.441	2.332
Average B factors [Å^2]									
all	15.8	19.0	19.0	17.3	17.0	16.6	20.6	21.0	19.5
main chain	12.6	16.2	16.2	14.6	14.0	13.4	18.1	17.9	16.6
side chain	15.8	19.3	19.5	17.4	16.8	17.2	20.9	21.5	20.1
inhibitors	26.5	43.3	22.4	35.7	23.3	19.2	28.4	34.8	25.7
waters	25.2	27.6	27.3	27.8	27.1	26.7	28.0	29.6	28.6
zinc	7.8	10.6	10.2	7.4	8.7	8.0	12.9	11.8	10.9
other molecules	28.9	34.7	33.8	35.7	33.5	36.0	40.9	39.0	31.4
Number of atoms									
all	9883	9590	9838	2376	2453	9716	2410	4818	4865
protein	8510	8628	8593	2126	2111	8499	2118	4233	4297
inhibitor	135	128	175	27	32	140	27	64	70
water	1206	818	1042	211	286	1013	253	483	484
zinc	4	4	4	1	1	4	1	2	2
other molecules	28	12	24	11	23	60	11	36	12
Ramachandran statistics [%]									
most favored regions	97.8	97.1	97.1	96.8	96.6	97.0	97.3	96.2	96.8
additionally allowed regions	2.2	2.9	2.9	3.2	3.4	3.0	2.7	3.8	3.2
outliers	0	0	0	0	0	0	0	1	0

[a] All datasets were collected at 100 K; test set size was 10%.

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