Journal of Medicinal Chemistry

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.0c00511 • Publication Date (Web): 16 Jul 2020

Discovery of Sulfonamide-Derived Agonists of SOS1-Mediated Nucleotide Exchange on RAS Using Fragment-Based Methods

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KEYWORDS: RAS, Son of Sevenless (SOS), drug discovery, Cancer, fragment-based lead discovery.

ABSTRACT: The nucleotide exchange factor Son of Sevenless (SOS) catalyzes the activation of RAS by converting it from its inactive GDP-bound state to its active GTP-bound state. Recently, we have reported the discovery of small molecule allosteric activators of SOS1 that can increase the amount of RAS-GTP in cells. The compounds can inhibit ERK phosphorylation at higher concentrations by engaging a feedback mechanism. To further study this process, we sought different chemical matter from an NMR-based fragment screen using selective methyl labeling. To aid this process, several Ile methyl groups located in different binding sites of the protein were assigned and used to categorize the NMR hits into different classes. Hit to lead optimization using an iterative structure-based design paradigm resulted in compounds with improvements in binding affinity. These improved molecules of a different chemical class increase $SOS1^{cat}$-mediated nucleotide exchange on RAS and display cellular action consistent with our prior results.

■ INTRODUCTION

The RAS family of proteins represent a target of extreme importance for cancer drug discovery.[1-3] Indeed, more than 30 % of all human cancers have activating mutations in K-RAS, H-RAS, or N-RAS, and many cancer types are driven by dysregulated signaling downstream of RAS.[4-5] Despite this disease association and recent advancements in the discovery of molecules that bind to, and affect RAS function, no agents directly acting on RAS have yet been approved for patient use.[6-7] The main reason for this is the lack of a druggable pocket on RAS proteins to which small-molecules could bind with high affinity.[8-9] However, in recent years, there has been a resurgence of research around RAS, driven by direct[10-14] and indirect[15-17] approaches of targeting RAS, in both academia and the pharmaceutical industry.

The GTPase RAS is an important signaling molecule that cycles between an inactive GDP-bound and an active GTP-bound state.[7,18] The nucleotide-bound status of RAS is controlled by various regulatory proteins: GTPase Associated Proteins (GAPs) that accelerate the hydrolysis of triphosphate nucleotides, and Guanine Exchange Factors (GEFs) that facilitate the exchange of one nucleotide to the other.[19-20] The GEF Son of Sevenless (SOS) is the primary protein responsible for this exchange process on RAS, thereby converting it to an active GTP-bound state that initiates downstream signaling pathways.[21-23]

We have previously reported on the discovery of small molecules that bind to the catalytic core of the GEF SOS and activate nucleotide exchange on RAS, increasing the amount of GTP-bound RAS, yet producing paradoxical inhibition of downstream RAS signaling in cells.[17] This paradoxical activity results from the engagement of a feedback loop in the signal transduction pathway.[24-25] We have reported several improved follow-up molecules, many of which were derived from hits discovered by a high-throughput screen[26] that were subsequently optimized using structure-based design.[27-29] To obtain additional chemical matter, we have conducted an NMR-based fragment screen on $SOS1^{cat}$, the results of which are reported herein. The same pocket was recently reported for fragment hits targeting HRAS–SOS1 using an X-ray fragment screening approach and for fragment hits targeting $KRAS^{G12C}$–SOS1 using a saturation transfer difference (STD)-NMR.[15]

Fragment-based screening methods, by virtue of the smaller size of the screened compounds, allow greater sampling of chemical space compared with traditional screening techniques, even when a modestly sized compound library is used.[30] However, due to the weaker binding affinities typically exhibited

by fragment molecules, sensitive biophysical techniques are required to detect the binding event.[31] HSQC NMR represents an ideal method for this purpose. We have previously realized great success using uniformly ^{15}N labeled protein; however, the catalytic unit of SOS1 ($SOS1^{cat}$), comprised of the CDC25 and REM domains (residues 566-1046), is relatively large.[32] Therefore, to execute an NMR-based fragment screen on the 65 kD $SOS1^{cat}$, we selectively methyl labeled the protein and used changes in the ^{13}C HMQC spectra to track spectrum changes due to ligand binding.[33-34] Using this approach, we identified 59 fragment hits that bind to $SOS1^{cat}$, representing multiple chemically distinct classes of compounds. Several of the fragment hits provide novel optimization starting points.[27-29] Using an iterative structure-based ligand design, new molecules that bind to $SOS1^{cat}$ with low micromolar affinities were obtained. High resolution X-ray structural studies of the compounds were used to understand the SAR and provide insight for the design of improved molecules. As in our prior work, we verified that these novel fragment-derived sulfonamide-based compounds can activate nucleotide exchange on RAS and affect downstream signaling.[24-25] They thus represent alternative starting points for additional compound optimization efforts

■ RESULTS AND DISCUSSION

2D HMQC NMR-Based Fragment Screening

Since SOS1 is relatively large protein (Mw ~65 kD), we used selectively labeled IVLM methyl $SOS1^{cat}$ (residues 566-1046). The protein was grown using selectively deuterated methyl precursors (i.e. 3,3 ^{2}H methyl ^{13}C alpha ketobutyric acid to label the cδ1 methyl of Ile and 3-D1 ^{2}H, ^{13}C-5 alpha ketoisovaleric acid to label cγ1,2 Val and Leu cδ1,2 methyls) and ^{13}C Cε methyl labeled Methionine. The ^{1}H-^{13}C HMQC spectra obtained from this selectively IVLM methyl-labeled $SOS1^{cat}$ was well-resolved (Figure 1A) and offers methyl reporter resonances throughout the protein (Figure S2, Supplementary information). To assign the methyl signals and to identify Ile methyl groups located in the different potential $SOS1^{cat}$ binding pockets, we used selective Ile to Ala mutations as well as ^{13}C edited nOe experiments. For example, comparison of a mutant I718A SOS1 spectra (Figure 1B, red overlay) compared to the wt-$SOS1^{cat}$ spectra (Figure 1B, blue spectra) shows the disappearance of a single Ile peak. In this case, the peak can be

assigned to the Ile 718 resonance by inspection. For spectra with multiple changes, we used ^{13}C-edited nOe studies to help assign the peaks by examination of nOe resonances to other assigned methyl groups. For example, Met 878, assigned previously, is in close proximity to Ile 839 and has shown an nOe enhancement to one of the Ile resonances that disappears in the mutant I839A spectra. To verify whether this methyl labeling pattern was sufficient to detect binding of known ligands to SOS1^{cat}, we added an activator ligand (**S1**, Supplementary information) previously found to bind to SOS1^{cat} with 0.14 μM affinity (Figure S2A, Supplementary information). This resulted in several spectral changes (Figure 1 C and D). From the known Ile and Met assignments, we identified several methyl resonances (Ile 893, Ile 839, and Met 878) that shift upon addition of the compound. The known structure of this activator ligand bound to SOS1^{cat} confirms that all of these residues are located in the activator site pocket (Figure S2B, Supplementary information).

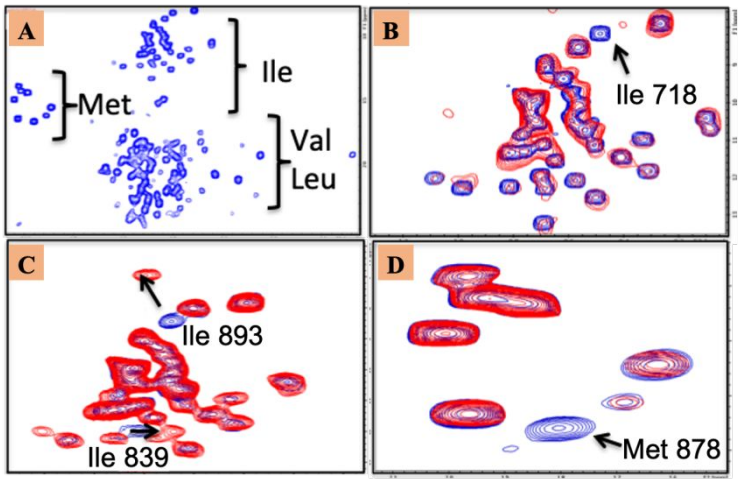


Figure 1. ^{13}C HMQC spectra of methyl labeled SOS1^{cat}. (A) Full methyl spectra of SOS1^{cat}. (B) Ile region for a Mutant I718A SOS1^{cat} protein. (C) Ile region and (D) Met region of SOS1^{cat} after addition of the activator compound **S1**.

Hit Identification

The recombinant SOS1^{cat} (residues 566-1046) selectively labeled with ^{13}C at the methyl groups was used to screen our in-house fragment library (>13,800 compounds) by recording ^{1}H-^{13}C HMQC spectra (600 MHz, 298 K)[33-34] of SOS1^{cat} (60 μM). This process yielded 59 hits that shifted activator site methyl resonances (e.g. Ile 856, Ile 839, Met 874), a calculated hit rate of 0.1%. Hits were ranked using NMR

titration experiments (Figure 2), showing affinities of 330 μM to >2 mM, with 5 hits having a K_d of less than 1 mM. Notably, fragment **F-2** is structurally similar to our earlier benzimidazole SOS1 agonist.[29] Further, we verified that these compounds bound to the activator site pocket by using a single concentration (400 μM) to test for displacement of a FITC-labeled compound from binding to SOS1^{cat} in the published fluorescence polarization anisotropy (FPA) assay.[29] Several hits that competed with the labeled probe at this site were selected for X-ray studies and chemical optimization.

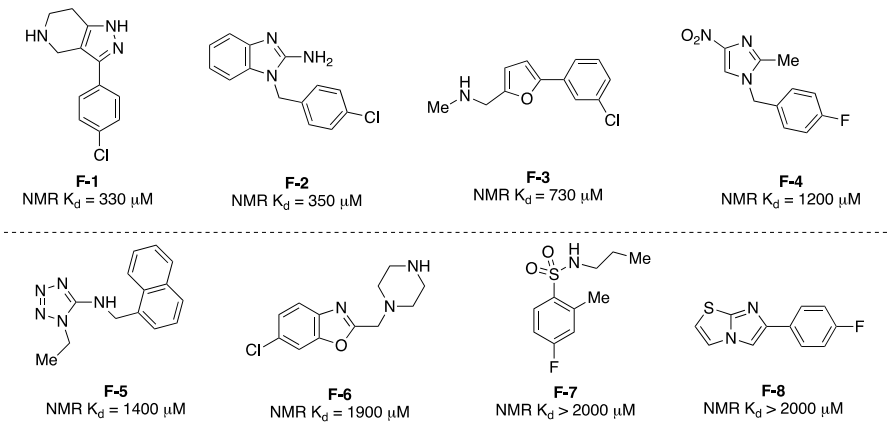


Figure 2. Representative SOS1^{cat} activator hits identified by ^{13}C HMQC NMR-based fragment screening.

X-ray structures of fragments binding to SOS1^{cat} and fragment merging

The hits identified by screening the fragment library were tested in co-crystallization trials with SOS1^{cat} to obtain structural information on their binding modes. All 16 successfully co-crystallized fragment hits occupied the same binding site on SOS1^{cat} initially identified as the binding site of compounds discovered using an orthogonal HTS assay.[28-29] The co-crystal structure of sulfonamide fragment **F-7** bound to SOS1^{cat} (Figure 3A) shows a unique bidentate H-bonding interaction with the side chain amide of Asn879. On the other hand, fragment **F-4** (Figure 3B), containing a nitro group, picked up a similar H-bonding interaction with the side chain amide of Asn 879. Interestingly, the phenyl ring of Phe 890 is flipped up relative to its position in the co-crystal structures obtained with fragment **F-4**. A similar upward position of Phe 890 was observed in the co-crystal structures of previously reported SOS1^{cat} binding compounds.[23,28-29] Moreover, the arene ring of **F-7** and the imidazole ring of **F-4** occupied the same hydrophobic area where benzimidazole[29] and quinazoline[28] cores were previously found to bind. Based on the overlay of the crystal

structures (Figure 3C) of fragments **F-7** and **F-4** bound to SOS1^{cat}, we hypothesized that merging of these two fragments may force the Phe 890 rotation and thus open the hydrophobic core of the binding pocket under Phe 890 to enable additional optimization of **F-7**. Based on this hypothesis, as well as structural information available from previous benzimidazole[29] and quinazoline[28] work, compound **1a** was synthesized. A measurable binding affinity of **1a** (NMR K_d = 600 μM) was obtained from NMR titration experiments (Figure S3, Supplementary information). Furthermore, the X-ray co-crystal structure of **1a** bound to SOS1^{cat} (Figure 3D) confirmed that the aromatic ring of Phe 890 was flipped and the space beneath was partially occupied by the phenyl ring from compound **1a**.

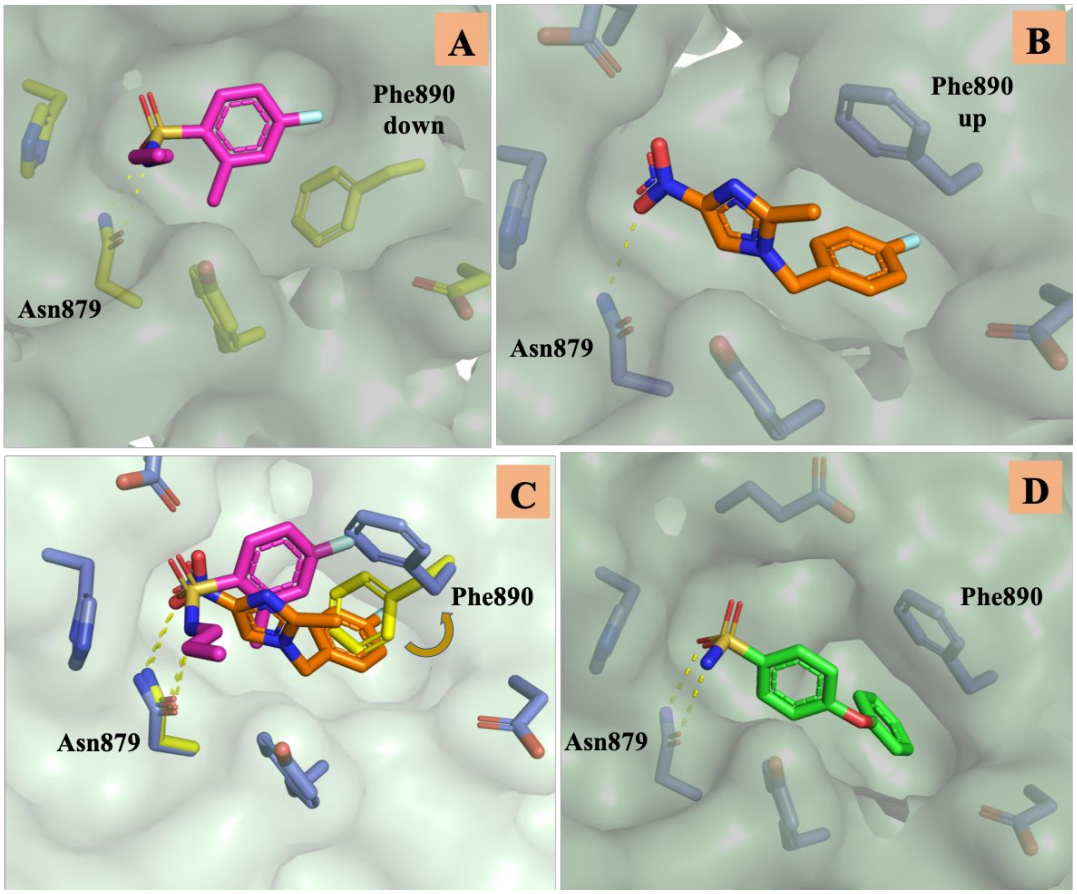
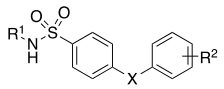


Figure 3. X-ray co-crystal structures of (A) fragment **F-7** (magenta, PDB ID code 6V9M), (B) fragment **F-4** (orange, PDB ID code 6V94), (C) overlay of **F-7** (magenta) with **F-4** (orange), and (D) compound **1a** (green, PDB ID code 6V9N) bound to SOS1^{cat}.

Aryl sulfonamide SAR at the 4-position

On the basis of the co-crystal structure of **1a** bound to SOS1^{cat} (Figure 3D) and our earlier studies, we initially focused on introducing a variety of lipophilic groups to the phenyl ring to occupy additional hydrophobic space under Phe 890. The previously reported FPA binding assay[29] was used to assess the binding affinities of the analogues (Table 1). Introduction of a lipophilic substituent at the 4-position of the phenol ring (**1b**) provided the first compound for which a binding affinity could be confidently obtained from the competition FPA assay. Encouraged from this result, we investigated alternative substitution patterns. However, 3,5-di-fluoro (**1c**) 3,5-di-methyl (**1d**), and 2,4-di-fluoro (**1e**) substituted analogues proved to be inactive in the FPA assay. Alternatively, 3,4-di-fluoro (**1f**) and 3,4-di-chloro (**1g**) derivatives restored activity and showed equal affinity. Since the aryl sulfonamide fragment (**F-7**) contained alkyl substituents on the sulfonamide, we anticipated that introduction of lipophilic substituents on the N-H of the aryl sulfonamide would boost affinity. However, alkyl substituted sulfonamides (**1h-i**) were not tolerated. Having explored the biaryl sulfonamides connected by a phenolic ether, we subsequently examined a nitrogen connection. It was found that smaller atoms, such as fluorine, at 3-, 4-, and 5-positions of the aniline ring were well tolerated (**1k-m**) and provided the tightest binding analogue (**1k**) in this initial set of compounds. Conversely, larger lipophilic groups, such as the 3-chloro group in compound **1n** and the 3- and 5-methyl groups in compound **1o**, were not well tolerated. According to these SAR trends, compounds **1g** and **1k** were identified as scaffolds for further optimization.

Table 1. SAR of aryl sulfonamide at 4-position.



Compd	X	R^1	R^2	FPA K_d (μM)[a]	LE[b]
1a	O	H	H	>100	ND
1b	O	H	4-F	86.4 ± 3.3	0.32
1c	O	H	3,5-di-F	>100	ND
1d	O	H	3-Me, 5-Me	>100	ND
1e	O	H	2,4-di-F	>100	ND
1f	O	H	3,4-di-F	85.3 ± 7.1	0.30
1g	O	H	3-Cl, 4-F	74.6 ± 4.9	0.30
1h	O	*n*-Pr	3,4-di-F	>100	ND
1i	O	*n*-Pr	3-Cl,4-F	>100	ND
1j	NH	H	4-F	>100	ND
1k	NH	H	3,4-diF	14.2 ± 2.3	0.35
1l	NH	H	2,4-diF	69.2 ± 6.1	0.34
1m	NH	H	3,5-diF	24.5 ± 4.0	0.34
1n	NH	H	3-Cl, 4-F	89.4 ± 2.8	0.29

1o	NH	H	3-Me, 4-F, 5-Me	96.4 ± 5.4	0.28

[a]Measurements are reported as the mean ± SD of two or more independent experiments, each conducted in duplicate.

[b]Ligand efficiency index, LE =1.4 × pK_d/HAC.

Aryl sulfonamide SAR at the 3-position

Analysis of the X-ray co-crystal structure of compound **1g** revealed that the side chain carboxylate group of Asp 887 lies in close proximity to the 3-position of the aryl sulfonamide (Figure 4A). Moreover, an overlay of the bound structures of fragment hit **F-2** and compound **1g** showed that both ligands rest in the same hydrophobic pocket and that the 2-position of the benzimidazole superimposes on the 3-position of the aryl sulfonamide (Figure 4B). Notably, the 2-amino group of **F-2** fragment achieved a water-mediated interaction with the side chain carboxylate of Asp 887. We therefore adopted a fragment growing approach for further optimization, anticipating that picking up an interaction with Asp 887 could be achieved from the 3-position of the aryl sulfonamide (Table 2). Engaging this residue provided a distinct affinity advantage in our optimization work in other chemical series.[29] Initial analogues evaluated the suitability of monodentate cyclic amines (**2a-b**); among them, compound **2b** showed five-fold better affinity compared with compound **2a**. With these results in hand, we sought to introduce a monodentate linear amine (**2c**). While this moiety did not show any binding affinity improvement, we were pleased to find that introduction of a linear amine (**2d**) with an additional carbon in the linker showed single-digit micromolar affinity. Encouraged from this result, we next investigated exocyclic monodentate (**2e**) and bidentate (**2f**) basic amine moieties. As might be expected, compounds **2e-f** showed comparable affinity to the linear amine (**2d**). In addition, we examined the affinity of bidentate cyclic basic amine moiety (**2g**); this compound showed low micromolar binding affinity. To improve affinity, we next examined substituted piperazines as well as additional ring sizes and topologies, including 7-membered ring amines and various spirocyclic amines. However, this set of compounds (**2h-k**) showed only weak affinity. Although spirocyclic compound **2l** was tolerated with low micromolar affinity, erosion of ligand efficiency made it less favorable for further optimization compared with other analogs, such as **2d** and **2g**. Having explored extension of phenol-containing aryl sulfonamide analogues, we turned our attention to extensions of aniline-containing aryl sulfonamide analogues toward Asp 887. Surprisingly, compound **2m** did not show any improvement in affinity relative to the parent

compound **1k** (K_d = 14.2 μM). Moreover, the 3,4-difluorophenol containing compound **2n** was roughly 2-fold worse than the corresponding 3-fluoro,4-chlorophenol aryl sulfonamide analogue (**2g**). Based on the binding affinity and ligand efficiency, we therefore selected **2g** as a starting point for additional exploration.

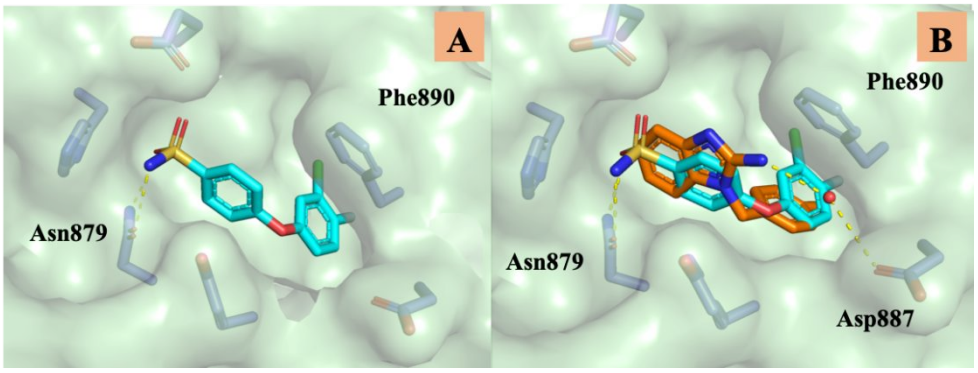
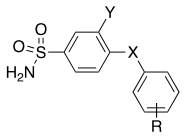
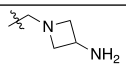
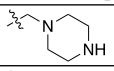
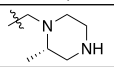
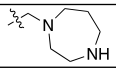
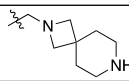
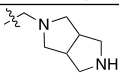
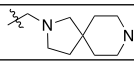
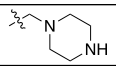
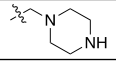


Figure 4. (A) X-ray co-crystal structures of **1g** (cyan, PDB ID code 6V9J); (B) Overlay of **1g** (cyan) with **F-2** (orange, PDB ID code 6V9F) bound to SOS1^{cat}.

Table 2. SAR of aryl sulfonamide at 3-position.



Compd	X	R	Y	FPA K_d (μM)[a]	LE[b]
2a	O	3-Cl, 4-F		87.1 ± 6.2	0.19
2b	O	3-Cl, 4-F		13.3 ± 0.6	0.26
2c	O	3-Cl, 4-F		62.0 ± 4.4	0.20
2d	O	3-Cl, 4-F		4.51 ± 0.04	0.34
2e	O	3-Cl, 4-F		14.1 ± 1.3	0.28
2f	O	3-Cl, 4-F		8.10 ± 0.65	0.30
2g	O	3-Cl, 4-F		6.42 ± 0.95	0.28
2h	O	3-Cl, 4-F		>100	ND
2i	O	3-Cl, 4-F		>100	ND
2j	O	3-Cl, 4-F		73.4 ± 6.0	0.22
2k	O	3-Cl, 4-F		24.5 ± 4.3	0.23
2l	O	3-Cl, 4-F		3.12 ± 0.95	0.24

2m	NH	3-F, 4-F		19.1 ± 1.1	0.26
2n	O	3-F, 4-F		10.1 ± 1.1	0.24

[a]Measurements are reported as the mean ± SD of two or more independent experiments, each conducted in duplicate.

[b]Ligand efficiency index, LE =1.4 × pK_d/HAC.

SAR of the extension of 4-piperazine.

Inspection of the X-ray co-crystal structure (Figure 5) of **2d** bound to SOS1^{cat} confirmed the water-mediated interaction between the ethyl amine moiety and Asp887, and also revealed regions that are solvent-exposed, occupied by molecules of water. We hypothesized that these regions would allow considerable tolerance for functional groups that may offer the ability to both tune the overall molecular properties of the analogues and also improve the binding affinity. To exploit these opportunities, we explored a wide range of chemical diversity at the piperazine nitrogen (Table 3). Expectedly, *N*-methyl piperazine analogue **3a** showed comparable binding affinity to the parent compound **2g**. However, introduction of additional lipophilic substituents did not improve affinity (**3b-d**) compared to the parent compound **2g**. Moreover, cyclic analogues (**3e-f**) were not tolerated, and exhibited diminished affinity. Linear amine **3g** also exhibited 2-fold less affinity than the parent compound and a longer chain (**3h**) was not tolerated. Furthermore, compounds bearing cyclic ether (**3i**) or alcohol (**3j**) moieties exhibited comparable ligand efficiency (LE) and AlogP values, but marginal improvement in the FPA assay relative to the parent compound **2g** (K_d = 6.42 μM, LE = 0.28, and AlogP = 2.46).

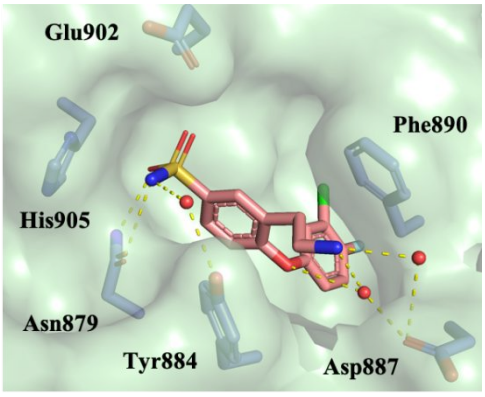
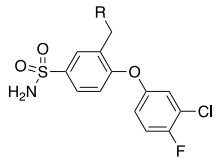
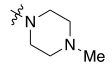
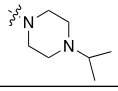
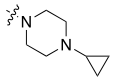
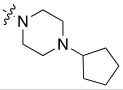
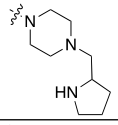
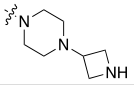
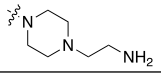
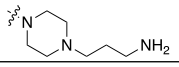
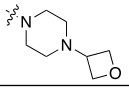
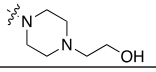
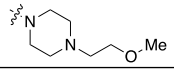


Figure 5. X-ray co-crystal structure of **2d** (salmon, PDB ID code 6V9L) bound to SOS1^{cat}.

Table 3. SAR of the extended amines.



Compd	R	FPA K_d (μM)[a]	LE[b]	AlogP
3a		6.51 ± 1.65	0.27	3.2
3b		80.3 ± 5.5	0.20	3.72
3c		23.0 ± 4.9	0.22	3.48
3d		30.5 ± 2.2	0.20	3.94
3e		>100	ND	3.09
3f		79.8 ± 5.2	0.19	2.44
3g		16.5 ± 0.8	0.23	2.17
3h		63.4 ± 4.1	0.19	2.23
3i		5.69 ± 1.76	0.24	2.71
3j		3.08 ± 0.52	0.27	2.45
3k		28.4 ± 4.0	0.21	2.86

[a]Measurements are reported as the mean ± SD of two or more independent experiments, each conducted in duplicate.

[b]Ligand efficiency index, LE =1.4 × pK_d/HAC.

Nucleotide Exchange Assay

We evaluated the ability of selected compounds to affect SOS1^{cat}-mediated nucleotide exchange on RAS (Table 4). To modulate RAS−GTP signaling and achieve potency in cancer cells, the compounds need to elicit a marked increase in the maximum rate of nucleotide exchange. Compound efficacy was expressed in terms of the maximal percent activation (defined as the maximum exchange rate elicited by an individual compound at 100 μM relative to the DMSO vehicle control), normalized to the effect of the

published compound **S3** (Supplementary information).[17] Compound potency was defined as the half maximal effective concentration (EC_{50}), calculated from the concentration−response curve (0−100 μM compound) generated from the nucleotide exchange assay. A general agreement between nucleotide exchange EC_{50} and FPA K_d was observed, and moderate to good efficacy was observed for selected compounds relative to the control compound (**S3**). Compound **2d** displayed the most potent exchange activation yet observed in this series; its 1.32 μM EC_{50} is comparable to many of the molecules identified from the medicinal chemistry campaigns that began with molecules from our earlier HTS.[26]

Table 4. Nucleotide Exchange Results.

Compd	FPA K_d (μM)[a]	EC_{50} (μM)[a]	Rel. Act. (%)[b]
2d	4.51 ± 0.04	1.32 ± 0.15	69.9 ± 7.1
2e	14.1 ± 1.3	4.32 ± 2.39	67.0 ± 13.4
2g	6.42 ± 0.46	8.30 ± 0.92	68.1 ± 2.5
2l	3.12 ± 0.95	3.31 ± 0.86	89.3 ± 7.4
3g	16.5 ± 0.8	16.3 ± 0.4	53.0 ± 1.4
3i	5.69 ± 1.76	7.88 ± 2.72	91.6 ± 9.1
3j	3.08 ± 0.52	4.76 ± 0.98	84.6 ± 9.2

[a]Measurements are reported as the mean ± SD of two or more independent experiments, each conducted in duplicate. [b]Activation values calculated as the percentage activation for each compound at 100 μM relative to the activation of control compound (**S3**) at 100 μM.

Cellular Assessment

To verify that the sulfonamide series compounds discovered from the NMR screen can elicit the same signaling changes in cells as our prior series discovered from an HTS, we investigated the effect of compounds **2d**, **2g**, and **3g** in cancer cells, using western blotting for p-ERK (Figure 6). The levels of p-ERK1/2$^{T202/Y204}$ and the total ERK1/2 protein levels were measured in response to compound treatment. Previously discovered SOS1 activator indole compound **S3** (Supplementary information) was also tested and served as a control compound.[17] Importantly, all three of these compounds (**2d**, **2g**, and **3g**) elicited the expected biphasic modulation of ERK1/2 phosphorylation at higher concentrations. Notably, the rank order of ERK1/2 phosphorylation (**2d** > **2g** > **3g**) is consistent with the rank order in binding affinity (FPA K_d), nucleotide exchange EC_{50}, and *in vitro* efficacy (relative activation). Although the cellular activity of the sulfonamides **2d**, **2g**, and **3g** is weaker than the previously reported SOS1 activator indole compounds (**S3**), the trend of modulation of ERK1/2 phosphorylation data is consistent and exemplifies the

characteristic activity of our compounds that bind to this pocket on SOS1. Together, these results suggest that the sulfonamide series compounds likely act via the same biological mechanism as previously described, wherein compound-induced increases in the levels of RAS−GTP produce biphasic modulation of ERK1/2 phosphorylation via negative feedback on SOS1 by pERK1/2$^{T202/Y204}$.[24,27]

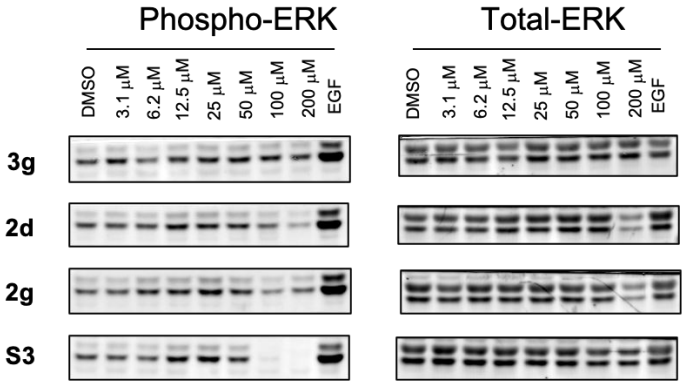
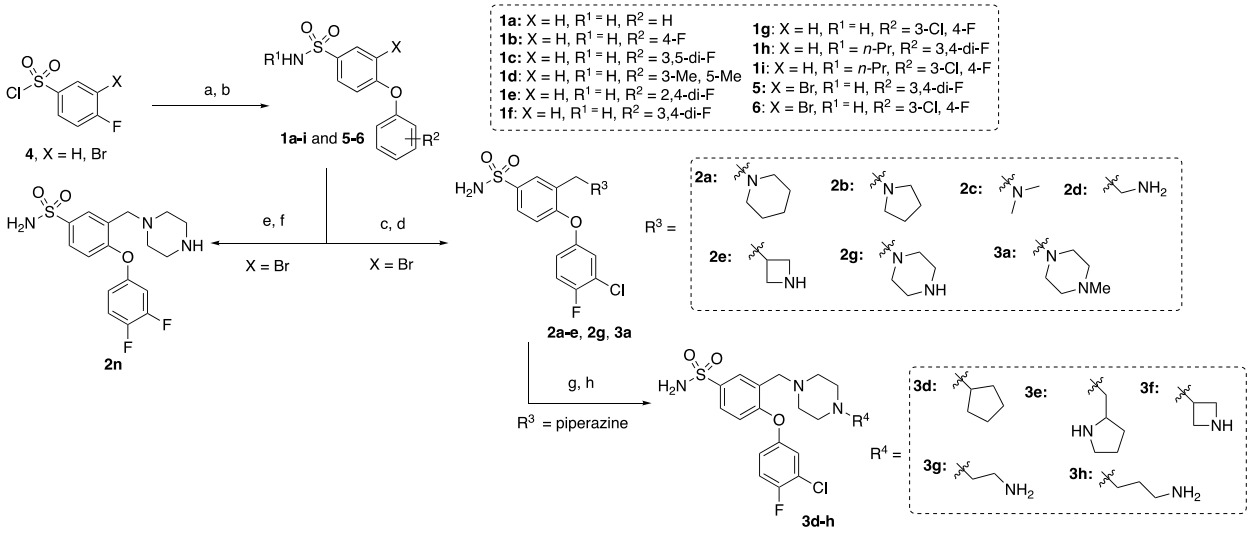


Figure 6. pERK1/2$^{T202/Y204}$ levels from HeLa cells that were treated for 30 min with up to 200 μM of compound **2d**, **2g**, **3g**, or **S3**. EGF (50 ng/mL for 5 min) was used as a positive control for pathway activation. Data are representative of two independent experiments.

Compound Synthesis

To access various O-linked aryl sulfonamide derivatives, commercially available 4-fluorobenzenesulfonyl chlorides **4** were treated with ammonium hydroxide or alkyl amines. Subsequent displacement of the 4-fluoro atom with an array of phenols via an S_NAr-type reaction furnished compounds **1a-i** and **5-6** (Scheme 1). From there, alkylation of the 3-bromosulfonamides (**5-6**) via a Suzuki-Miyaura cross-coupling reaction with alkyltrifluoroboronates and Boc removal (if required) furnished analogue **2a-e**, **2g**, **2n**, and **3a**. Subsequently, a reductive amination or *N*-alkylation of compounds **2g**, followed by acid-mediated removal of the *N*-Boc protecting group (if required) gave compounds **3d-h**.

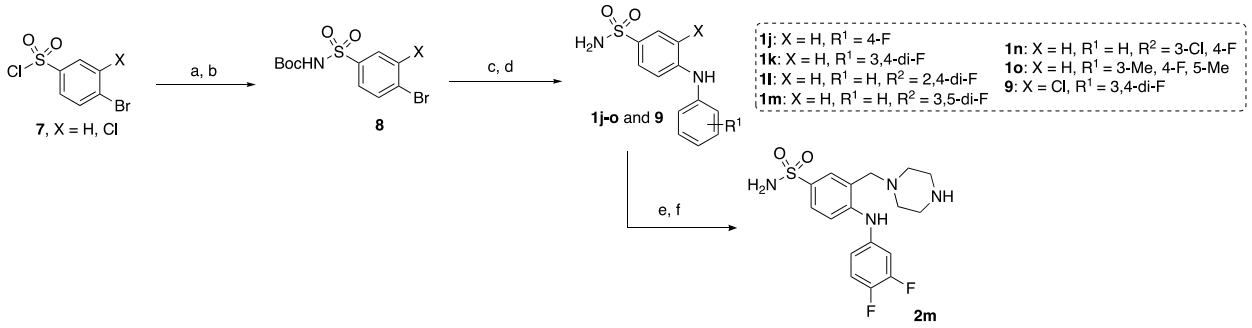
Scheme 1. Synthesis of O-linked aryl sulfonamide derivatives.[a]



[a]Reaction conditions: (a) NH_4OH, H_2O, CH_2Cl_2, rt; or *n*-propylamine, Et_3N, CH_2Cl_2, rt; (b) phenol derivatives, Cs_2CO_3, DMSO, 120 °C; (c) potassium alkyl trifluoroborate, $Pd(OAc)_2$, XPhos, Cs_2CO_3, THF/ H_2O, 80 °C; or potassium alkyl trifluoroborate, $PdCl_2(dppf).CH_2Cl_2$, Cs_2CO_3, toluene/ H_2O, 80 °C; (d) TFA, CH_2Cl_2, rt when Boc removal necessary; (e) alkyl trifluoroborate, $Pd(OAc)_2$, XPhos, Cs_2CO_3, THF/ H_2O, 80 °C; (f) TFA, CH_2Cl_2, rt; (g) alkyl ketone or aldehyde derivatives, $NaBH(OAc)_3$, CH_2Cl_2, rt; or bromoalkylamines, K_2CO_3, NaI, MeCN, reflux; (h) TFA, CH_2Cl_2, rt when Boc removal necessary.

To synthesize various *N*-linked aryl sulfonamide derivatives, commercially available 4-fluorobenzenesulfonyl chlorides **7** were treated with ammonia, followed by Boc protection of the sulfonamide nitrogen atom to afford intermediate **8** (Scheme 2). Subsequently, a Buchwald-Hartwig cross-coupling reaction of intermediates **8** with anilines and Boc removal afforded compounds **1j-o** and **9**. Finally, alkylation of the 3-bromosulfonamide (**9**) via a Suzuki-Miyaura cross-coupling reaction with alkyltrifluoroboronates and Boc-removal (when required) furnished analogue **2m**.

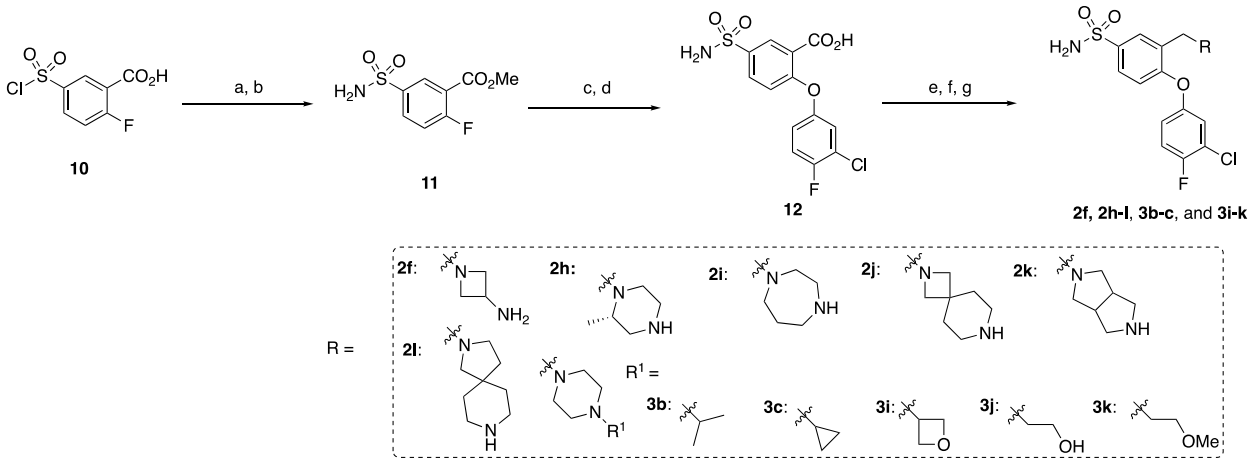
Scheme 2. Synthesis of N-linked aryl sulfonamide derivatives.[a]



[a]Reaction conditions: (a) NH_3, MeOH, rt; (b) Boc_2O, Et_3N, 4-DMAP, CH_2Cl_2, rt; (c) anilines, $Pd(OAc)_2$, BINAP, Cs_2CO_3, dioxane, 100 °C; (d) TFA, CH_2Cl_2, rt; (e) potassium alkyl trifluoroborate, $Pd(OAc)_2$, XPhos, Cs_2CO_3, THF/ H_2O, 100 °C; (f) TFA, CH_2Cl_2, rt.

To access various O-linked aryl sulfonamide derivatives in an alternative route, commercially available 5-(chlorosulfonyl)-2-fluorobenzoic acid **10** was treated with ammonium hydroxide and esterified with ethyl iodide. Then an S_NAr-type reaction with 3-chloro-4-fluorophenol was used to produce the intermediate **11** (Scheme 3). Subsequently, saponification of ester **11**, followed by coupling with amines afforded intermediate **12**. Lastly, reduction of **12** with borane and Boc removal (when required) furnished analogues **2f**, **2h-l**, **3b-c**, and **3i-k**.

Scheme 3. Synthesis of O-linked aryl sulfonamides using alternative route.[a]



[a]Reaction conditions: (a) NH_4OH, H_2O, EtOAc, rt; (b) methyl iodide, K_2CO_3, DMF, rt; (c) phenols, Cs_2CO_3, DMSO, 120 °C; (d) LiOH, H_2O, MeOH, rt; (e) alkyl amines, HATU, N-methylmorpholine, CH_2Cl_2, DMF, rt; (f) BH_3-THF, THF, reflux; (g) TFA, CH_2Cl_2, rt when Boc removal necessary.

■ CONCLUSION

We have utilized a fragment screening approach, based on selectively labeled $SOS1^{cat}$ and ^{13}C HMQC spectra, to discover small-molecules that bind to $SOS1^{cat}$. X-ray co-crystal structures of multiple fragment hits confirmed that they bind to the same hydrophobic pocket of $SOS1^{cat}$ as previously reported compounds, but in a distinctive manner. Beginning with a weakly binding sulfonamide fragment (**F-7**), we used a structure-guided fragment merging approach to attach a phenol group to the 4-position, and a subsequent fragment-growing approach to tether a basic amine. These strategies improved the affinity of the compounds by > 500-fold. Compounds in the series exhibit low micromolar activation of $SOS1^{cat}$-catalyzed nucleotide exchange on RAS and also display biphasic modulations in ERK1/2 phosphorylation at higher concentrations. These observations are in agreement with our previously proposed mechanism in which compound-mediated agonism of SOS1 can modulate RAS signaling via negative feedback on SOS1 by active $pERK1/2^{T202/Y204}$. It is well precedented that sulfonamide-based compounds can show a range of biological activities, including antiviral, antibacterial, antiallergic, antimalarial, and antitumor properties.[35-37] Indeed, more than 72 sulfonamide-containing small molecule drugs have been approved to date by the FDA.[38] In preliminary cellular experiments, the sulfonamide-based compounds described here display on-target SOS1 agonist activity identical to multiple unrelated chemical series. Thus, these compounds, which are derived from a fragment-based approach, provide an excellent starting point within a different chemical series for the discovery and design of future modulators of RAS signaling via binding to SOS1.

■ EXPERIMENTAL

General Procedures. All chemical reagents and reaction solvents were purchased from commercial suppliers and used as received. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded at 400 MHz on a Bruker spectrometer. For ^1H NMR spectra, chemical shifts are reported in parts per million (ppm) and are reported relative to residual non-deuterated solvent signals. Coupling constants are reported in hertz (Hz). The following abbreviations (or a combination, thereof) are used to describe splitting patterns: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; septate, sept; m, multiplet; comp, overlapping multiplets of non-magnetically equivalent protons; br, broad. All final compounds were of 95% purity or

higher, unless otherwise noted, as measured by analytical reversed-phase HPLC. Analytical HPLC was performed on an Agilent 1200 series system with UV detection at 214 and 254 nm, along with evaporative light scattering detection (ELSD). Low-resolution mass spectra were obtained on an Agilent 6140 mass spectrometer with electrospray ionization (ESI). LC-MS experiments were performed with the following parameters: Phenomenex Kinetex 2.6 µm XB-C18 100 Å, LC column 50 x 2.1 mm; 2 min gradient, 5%–95% MeCN in H_2O, and 0.1% TFA or 0.1% formic acid. Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F_{254} glass plates precoated with a 0.25 mm thickness of silica gel. TLC plates were visualized with UV light and iodine. Silica gel chromatography was performed using a Teledyne ISCO Combiflash® Rf system, eluting with varying concentrations of EtOAc in hexanes or MeOH in CH_2Cl_2. Preparative reversed-phase HPLC was performed on a Gilson instrument equipped with a Phenomenex Kinetex C18 column, using varying concentrations of MeCN in H_2O, and 0.1% TFA. Starting materials **4**, **7**, **10**, and phenol derivatives, aniline derivatives, potassium alkyl trifluoroborates, alkyl ketones, and alkyl aldehydes were purchased from commercial suppliers and used as received.

General Procedure A: Synthesis of 1a–i and **5–6**. *Step 1:* A stirred solution **4** (10 mmol) in dichloromethane (10 ml) at room temperature, the ammonium hydroxide (30% aqueous solution) (10 ml) was added dropwise. The reaction mixture was stirred at room temperature for 1-2 h. The product had formed as a white solid precipitate. The solid was collected by filtration, and washed with cold water (3 x 10 mL). The obtained crude was used for next step without further purification. Or, to a solution of *n*-propylamine (0.6 mmol) in CH_2Cl_2 (6 ml) was added Et_3N (0.8 mmol). After 10 minutes of stirring, 4-fluorobenzene sulfonyl chloride (0.5 mmol) was added and the mixture was allowed to stir for overnight at room temperature. The reaction mixture was quenched with water and the organic layer was washed with HCl (1N), dried over magnesium sulfate and then concentrated in vacuo. The obtained crude was used for next step without further purification. *Step 2:* To a solution of aryl phenol (0.6 mmol) and arylsulfonamide (0.18 g, 0.5 mmol) in dimethylsulfoxide (3 mL) was added Cesium carbonate (1.2 mmol) and the reaction mixture was stirred at 120 °C for 8 h and allowed to cool to ambient temperature. The mixture was diluted with ethyl acetate (80 mL) and washed with water (2x10 mL) and brine (10 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The crude solid was purified by silica gel chromatography.

4-phenoxybenzenesulfonamide (1a). The title compound was prepared from 4-fluorobenzenesulfonamide and phenol in similar fashion to general procedure A in 31% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92-7.88 (m, 2H), 7.46-7.42 (m, 2H), 7.27-7.23 (m, 1H), 7.10-7.05 (m, 4H), 4.82 (s, 2H). MS (ESI) *m/z* = 267.1 $[M+NH_4]^+$. Purity (AUC), ≥95%.

4-(4-fluorophenoxy)benzenesulfonamide (1b). The title compound was prepared from 4-fluorobenzenesulfonamide and 4-fluorophenol in similar fashion to general procedure A in 41% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82-7.79 (m, 2H), 7.05-6.92 (comp, 6H), 4.82 (s, 2H). MS (ESI) *m/z* = 285.1 $[M+NH_4]^+$. Purity (AUC), ≥95%.

4-(3,5-difluorophenoxy)benzenesulfonamide (1c). The title compound was prepared from 4-fluorobenzenesulfonamide and 3,5-difluorophenol in similar fashion to general procedure A in 35% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90-7.86 (m, 2H), 7.08-7.05 (m, 2H), 6.61-6.55 (comp, 1H), 6.53-6.48 (comp, 2H), 4.76 (s, 2H). MS (ESI) *m/z* = 303.1 $[M+NH_4]^+$. Purity (AUC), ≥95%.

4-(3,5-dimethylphenoxy)benzenesulfonamide (1d). The title compound was prepared from 4-fluorobenzenesulfonamide and 3,5-dimethylphenol in similar fashion to general procedure A in 30% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80-7.78 (m, 2H), 6.97-7.95 (m, 2H), 6.78 (s, 1H), 6.61 (s, 2H), 4.70 (br, 2H), 2.25 (s, 6H). MS (ESI) *m/z* = 295.1 $[M+NH_4]^+$. Purity (AUC), ≥95%.

4-(2,4-difluorophenoxy)benzenesulfonamide (1e). The title compound was prepared from 4-fluorobenzenesulfonamide and 2,4-difluorophenol in similar fashion to general procedure A in 35% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83-7.79 (m, 2H), 7.11-7.06 (comp, 1H), 6.95-6.84 (comp, 4H), 4.77 (s, 2H). MS (ESI) *m/z* = 303.1 $[M+NH_4]^+$. Purity (AUC), ≥95%.

4-(3,4-difluorophenoxy)benzenesulfonamide (1f). The title compound was prepared from 4-fluorobenzenesulfonamide and 3,4-difluorophenol in similar fashion to general procedure A in 38% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86-7.7.82 (m, 2H), δ 7.16-7.09 (comp, 1H), 7.00-6.96 (m, 2H), 6.87-6.82 (comp, 1H), 6.76-6.72 (comp, 1H), 4.85 (s, 2H). MS (ESI) *m/z* = 303.1 $[M+NH_4]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (1g). The title compound was prepared from 4-fluorobenzenesulfonamide and 4-chloro-3-fluorophenol in similar fashion to general procedure A in 31%

yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93-7.90 (m, 2H), 7.20-7.12 (comp, 2H), 7.06-7.03 (m, 2H), 6.97-6.94 (comp, 1H), 4.85 (s, 2H). MS (ESI) *m/z* = 319.1 $[M+NH_4]^+$. Purity (AUC), ≥95%.

4-(3,4-difluorophenoxy)-N-propylbenzenesulfonamide (1h). The title compound was prepared from 4-fluoro-*N*-propylbenzenesulfonamide and 3,4-difluorophenol in similar fashion to general procedure A in 38% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79-7.76 (m, 2H), 7.16-7.09 (comp, 1H), 6.99-6.95 (m, 2H), 6.87-7.82 (comp, 1H), 6.76-6.72 (comp, 1H), 4.41 (s, 1H), 2.86 (t, *J* = 7.3 Hz, 2H), 1.44 (sept, *J* = 7.3 Hz, 2H), 0.82 (t, *J* = 7.3 Hz, 3H). MS (ESI) *m/z* = 328.2 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)-*N*-propylbenzenesulfonamide (1i). The title compound was prepared from 4-fluoro-*N*-propylbenzenesulfonamide and 3-chloro-4-fluorophenol in similar fashion to general procedure A in 38% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85-7.84 (m, 2H), 7.23-7.15 (comp, 2H), 7.08-7.04 (m, 2H), 7.00-6.96 (comp, 1H), 4.42 (s, 1H), 2.96 (q, *J* = 7.3 Hz, 2H), 1.53 (sept, *J* = 7.3 Hz, 2H), 0.91 (t, *J* = 7.3 Hz, 3H). MS (ESI) *m/z* = 344.1 $[M+H]^+$. Purity (AUC), ≥95%.

3-bromo-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (5). The title compound was prepared from 3-bromo-4-fluorobenzenesulfonamide and 3-chloro-4-fluorophenol in similar fashion to general procedure A in 42% yield over two steps.

3-bromo-4-(3,4-difluorophenoxy)benzenesulfonamide (6). The title compound was prepared from 3-bromo-4-fluorobenzenesulfonamide and 3,4-difluorophenol in similar fashion to general procedure A in 35% yield over two steps.

General Procedure B: Synthesis of 2a–c, 2g, 2n and 3a. *Step 1:* A mixture of an potassium alkyl trifluoroborate (0.75 mmol), $Pd(OAc)_2$ (0.05 mmol), XPhos (0.15 mmol), Cs_2CO_3 (1.5 mmol), and **5** or **6** (0.5 mmol) under argon was charged with dry THF/ H_2O (4:1, 2 mL) and heated to a temperature of 80 °C. After 12 h, the reaction mixture was cooled to rt, diluted with sat. aqueous ammonium chloride, and extracted with CH_2Cl_2 (5 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography/ reversed-phase preparative HPLC. *Step 2:* The compounds obtained from step 1 (0.2 mmol) was dissolved in CH_2Cl_2 (0.06 M) and stirred at rt. TFA (0.02 M) was added and the mixture was stirred for 2 h before being concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC and the pure fractions were concentrated under a stream of warm air to afford the target compound as a TFA salt.

4-(3-chloro-4-fluorophenoxy)-3-(piperidin-1-ylmethyl)benzenesulfonamide (2a as TFA salt). The title compound was prepared as the corresponding TFA salt from 3-bromo-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (**5**) and potassium 1-trifluoroboratomethylpiperidine in similar fashion to general procedure B in 51% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.15 (d, *J* = 2.2 Hz, 1H), 7.98 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.45-7.38 (comp, 2H), 7.22-7.18 (m, 1H), 7.01 (d, *J* = 8.5 Hz, 1H), 4.52 (s, 2H), 3.50 (br, 2H), 3.15 (br, 2H), 1.96-1.59 (m, 6H). MS (ESI) *m/z* = 399.1 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)-3-(pyrrolidin-1-ylmethyl)benzenesulfonamide (2b as TFA salt). The title compound was prepared as the corresponding TFA salt from 3-bromo-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (**5**) and potassium 1-trifluoroboratomethylpyrrolidine in similar fashion to general procedure B in 48% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.15 (d, *J* = 2.2 Hz, 1H), 7.97 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.46-7.37 (comp, 2H), 7.23-7.19 (comp, 1H), 7.00 (d, *J* = 8.5 Hz, 1H), 4.62 (s, 2H), 3.66 (br, 2H), 3.34 (br, 2H), 2.23 (br, 2H), 2.19 (br, 2H). MS (ESI) *m/z* = 385.1 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)-3-((dimethylamino)methyl)benzenesulfonamide (2c as TFA salt). The title compound was prepared as the corresponding TFA salt from 3-bromo-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (**5**) and potassium N,N-dimethylaminomethyltrifluoroborate in similar fashion to general procedure B in 41% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.13 (d, *J* = 2.2 Hz, 1H), 7.99 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.46-7.38 (comp, 2H), 7.24-7.20 (comp, 1H), 7.00 (d, *J* = 8.5 Hz, 1H), 4.57 (s, 2H), 3.53 (s, 6H). MS (ESI) *m/z* = 359.1 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)-3-(piperidin-1-ylmethyl)benzenesulfonamide (2g as TFA salt). The title compound was prepared as the corresponding TFA salt from 3-bromo-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (**5**) and potassium 1-trifluoroboratomethyl-4-(*N*-Boc) piperazine in similar fashion to general procedure B in 26% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.06 (d, *J* = 2.3 Hz, 1H), 7.86 (dd, *J* = 2.3 Hz, *J* = 8.7 Hz, 1H), 7.34-7.30 (comp, 1H), 7.23-7.21 (comp, 1H), 7.05-7.01 (m, 2H), 3.26-3.23 (m, 4H), 2.85-2.83 (br, 4H), 3.15 (br, 2H). MS (ESI) *m/z* = 399.9 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)-3-((4-methylpiperazin-1-yl)methyl)benzenesulfonamide (3a as TFA salt). The title compound was prepared as the corresponding TFA salt from 3-bromo-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (**5**) and potassium 1-trifluoroboratomethyl-4-(*N*-Boc) piperazine in

similar fashion to general procedure B in 32% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.06 (d, *J* = 2.4 Hz, 1H), 7.85 (dd, *J* = 2.4 Hz, *J* = 8.7 Hz, 1H), 7.35-7.30 (comp, 1H), 7.23-7.20 (comp, 1H), 7.05-7.00 (m, 2H), 3.79 (s, 2H), 3.50 (br, 2H), 3.15 (br, 2H), 3.12 (br, 2H), 2.90 (s, 3H), 2.52 (br, 2H). MS (ESI) *m/z* = 414.1 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3,4-difluorophenoxy)-3-(piperazin-1-ylmethyl)benzenesulfonamide (2n as TFA salt). The title compound was prepared as the corresponding TFA salt from 3-bromo-4-(3,4-difluorophenoxy)benzenesulfonamide (**6**) and potassium 1-methyl-4-trifluoroboratomethylpiperidine in similar fashion to general procedure B in 32% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.07 (d, *J* = 2.4 Hz, 1H), 7.85 (dd, *J* = 2.4 Hz, *J* = 8.6 Hz, 1H), 7.37-7.30 (comp, 1H), 7.07-7.02 (m, 2H), 6.87-6.84 (comp, 1H), 3.78 (s, 2H), 3.24-3.21 m, 4H), 2.79-2.77 (m, 4H). MS (ESI) *m/z* = 384.1 $[M+H]^+$. Purity (AUC), ≥95%.

General Procedure C: Synthesis of 2d–e. *Step 1:* A mixture of an potassium alkyl trifluoroborate (0.75 mmol), $Pd(OAc)_2$ (0.05 mmol), XPhos (0.15 mmol), Cs_2CO_3 (1.5 mmol), and **5** (0.5 mmol) under argon was charged with dry Toluene/ H_2O (8:1, 2 mL) and heated to a temperature of 80 °C. After 12 h, the reaction mixture was cooled to rt, diluted with sat. aqueous ammonium chloride, and extracted with CH_2Cl_2 (5 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography. *Step 2:* The compounds obtained from step 1 (0.2 mmol) was dissolved in CH_2Cl_2 (0.06 M) and stirred at rt. TFA (0.02 M) was added and the mixture was stirred for 2 h before being concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC and the pure fractions were concentrated under a stream of warm air to afford the target compound as a TFA salt.

3-(2-aminoethyl)-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (2d as TFA salt). The title compound was prepared as the corresponding TFA salt from 3-bromo-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (**5**) and potassium t-butyl N-[2-(trifluoroboranuidyl)ethyl] carbamate in similar fashion to general procedure C in 21% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 7.93 (d, *J* = 2.3 Hz, 1H), 7.83 (dd, *J* = 2.2 Hz, *J* = 8.4 Hz, 1H), 7.37-7.30 (comp, 2H), 7.12-7.08 (comp, 1H), 6.96-6.94 (d, *J* = 8.4 Hz, 1H), 3.28 (t, *J* = 6.3 Hz, 2H), 3.16 (t, *J* = 6.3 Hz, 2H). MS (ESI) *m/z* = 345.1 $[M+H]^+$. Purity (AUC), ≥95%.

3-(azetidin-3-ylmethyl)-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (2e as TFA salt). The title compound was prepared as the corresponding TFA salt from 3-bromo-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (**5**) and potassium (1-Boc-azetidin-3-yl)methylfluoroborate in similar fashion to general procedure C in 24% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 7.85 (d, *J* = 2.4 Hz, 1H), 7.79 (dd, *J* = 2.4 Hz, *J* = 8.8 Hz, 1H), 7.37-7.32 (comp, 1H), 7.29-7.26 (comp, 1H), 7.09-7.05 (comp, 1H), 6.93 (d, *J* = 8.8 Hz, 1H), 4.18-4.13 (m, 2H), 3.99-3.95 (m, 2H), 3.40-3.34 (m, 1H), 3.15 (d, *J* = 7.8 Hz, 2H). MS (ESI) *m/z* = 371.1 $[M+H]^+$. Purity (AUC), ≥95%.

General Procedure D: Synthesis of 3d–f. *Step 1:* A mixture of **2g** (0.2 mmol), and alkyl aldehydes or ketones (0.4 mmol) in CH_2Cl_2 (3 mL) was stirred for 1h, then $NaBH(OAc)_3$ (0.5 mmol) was added. The resulting mixture was stirred for 2h, then was poured into 2 N aqueous KOH solution (15 mL) and extracted with EtOAc. The organic phases were combined, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC and the pure fractions were concentrated under a stream of warm air to afford the target compound as a TFA salt. *Step 2:* The compound obtained from step 1 (0.1 mmol) was dissolved in CH_2Cl_2 (0.06 M) and stirred at rt. TFA (0.02 M) was added and the mixture was stirred for 2 h before being concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC and the pure fractions were concentrated under a stream of warm air to afford the target compound as a TFA salt.

4-(3-chloro-4-fluorophenoxy)-3-((4-cyclopentylpiperazin-1-yl)methyl)benzenesulfonamide (3d as TFA salt). The title compound was prepared as the corresponding TFA salt from 4-(3-chloro-4-fluorophenoxy)-3-(piperazin-1-ylmethyl)benzenesulfonamide (**2g**) and cyclopentanone in similar fashion to general procedure D in 26% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.06 (d, *J* = 2.4 Hz, 1H), 7.85 (dd, *J* = 2.4 Hz, *J* = 8.6 Hz, 1H), 7.35-7.30 (comp, 1H), 7.23-7.20 (comp, 1H), 7.05-7.00 (m, 2H), 3.81 (s, 2H), 3.56-3.52 (m, 3H), 3.16-3.12 (m, 4H), 2.60-2.57 (m, 2H), 2.18-2.15 (m, 2H), 1.85-1.84 (m, 2H), 1.74-1.71 (m, 4H). MS (ESI) *m/z* = 468.1 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)-3-((4-(pyrrolidin-2-ylmethyl)piperazin-1-yl)methyl) benzenesulfonamide (3e as TFA salt). The title compound was prepared as the corresponding TFA salt from 4-(3-chloro-4-fluorophenoxy)-3-(piperazin-1-ylmethyl)benzenesulfonamide (**2g**) and *tert*-butyl 2-formylpyrrolidine-1-carboxylate in similar fashion to general procedure D in 31% yield over two steps. ^{1}H

NMR (400 MHz, CD_3OD): δ 8.16 (d, *J* = 2.3 Hz, 1H), 7.98 (dd, *J* = 2.3 Hz, *J* = 8.8 Hz, 1H), 7.44-7.38 (comp, 2H), 7.22-7.18 (comp, 1H), 7.01 (d, *J* = 8.8 Hz, 1H), 4.58 (s, 2H), 3.86-3.80 (m, 2H), 3.57-3.52 (m, 2H), 3.39-3.35 (m, 2H), 3.31-3.27 (m, 2H), 2.74-2.68 (m, 5H), 2.22-2.05 (m, 4H). (ESI) *m/z* = 483.2 $[M+H]^+$. Purity (AUC), ≥95%.

3-((4-(azetidin-3-yl)piperazin-1-yl)methyl)-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (3f as TFA salt). The title compound was prepared as the corresponding TFA salt from 4-(3-chloro-4-fluorophenoxy)-3-(piperazin-1-ylmethyl)benzenesulfonamide (**2g**) and *tert*-butyl 3-oxoazetidine-1-carboxylate in similar fashion to general procedure D in 15% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.16 (d, *J* = 2.3 Hz, 1H), 7.98 (dd, *J* = 2.3 Hz, *J* = 8.7 Hz, 1H), 7.44-7.37 (comp, 2H), 7.22-7.18 (comp, 1H), 7.05-7.00 (d, *J* = 8.7 Hz, 1H), 4.56 (s, 2H), 4.17-4.12 (m, 2H), 4.05-4.00 (m, 2H), 3.56-3.37 (m, 5H), 2.85-2.71 (m, 2H), 2.50-2.39 (m, 2H). MS (ESI) *m/z* = 455.2 $[M+H]^+$. Purity (AUC), ≥95%.

General Procedure E: Synthesis of 3g–h. *Step 1:* A mixture of **2g** (0.2 mmol), alkyl bromide (75 mg, 0.4 mmol), KI (0.4 mmol), and K_2CO_3 (0.4 mmol), in MeCN (3 mL) was stirred and heated to 80 °C for 15 h. The reaction mixture was cooled to rt, diluted with sat. aqueous ammonium chloride, and extracted with CH_2Cl_2 (5 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography. *Step 2:* The compound obtained from step 1 (0.1 mmol) was dissolved in CH_2Cl_2 (0.06 M) and stirred at rt. TFA (0.02 M) was added and the mixture was stirred for 2 h before being concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC and the pure fractions were concentrated under a stream of warm air to afford the target compound as a TFA salt.

3-((4-(2-aminoethyl)piperazin-1-yl)methyl)-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (3g as TFA salt). The title compound was prepared as the corresponding TFA salt from 4-(3-chloro-4-fluorophenoxy)-3-(piperazin-1-ylmethyl)benzenesulfonamide (**2g**) and *tert*-butyl (2-bromoethyl)carbamate in similar fashion to general procedure E in 41% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.05 (d, *J* = 2.3 Hz, 1H), 7.86 (dd, *J* = 2.3 Hz, *J* = 8.8 Hz, 1H), 7.33-7.26 (comp, 2H), 7.10-7.06 (comp, 1H), 6.99 (d, *J* = 8.8 Hz, 1H), 4.45 (s, 2H), 3.49-3.14 (m, 8H), 2.99 (t, *J* = 5.6 Hz, 2H), 2.63 (t, *J* = 5.6 Hz, 2H). MS (ESI) *m/z* = 443.1 $[M+H]^+$. Purity (AUC), ≥95%.

3-((4-(3-aminopropyl)piperazin-1-yl)methyl)-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (3h as TFA salt). The title compound was prepared as the corresponding TFA salt from 4-(3-chloro-4-fluorophenoxy)-3-(piperazin-1-ylmethyl)benzenesulfonamide (**2g**) and *tert*-butyl (3-bromopropyl)carbamate in similar fashion to general procedure E in 38% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.10 (d, *J* = 2.3 Hz, 1H), 7.91 (dd, *J* = 2.3 Hz, *J* = 8.8 Hz, 1H), 7.37-7.7.32 (comp, 2H), 7.22-7.20 (comp, 1H), 7.02 (d, *J* = 8.8 Hz, 1H), 4.08 (s, 2H), 3.23 (t, *J* = 5.6 Hz, 2H), 3.15-3.00 (m, 8H), 2.82-2.79 (m, 2H), 2.07-1.98 (m, 2H). MS (ESI) *m/z* = 457.0 $[M+H]^+$. Purity (AUC), ≥95%.

General Procedure F: Synthesis of 8. *Step 1*: A stirred solution **7** (8 mmol) in methanol (10 ml) at room temperature, the methanol/ammonia solution (10 ml, excess) was added dropwise. The reaction mixture was stirred at room temperature for 10 h. The reaction mixture was concentrated under reduced vacuum to yield intermediate arylsulfonamide as a white solid. This material was used without further purification. *Step 2*: A mixture of aryllsulfonamide (6 mmol) and di-tert-butyl dicarbonate (6.9 mmol) in CH_2Cl_2 (40 mL) under nitrogen was treated with triethylamine (7.2 mmol) and DMAP (0.6 mmol) and stirred at room temperature for 12 h. The crude residue was purified by silica gel chromatography.

General Procedure G: Synthesis of 1j-o and 9. *Step 1:* A mixture of a aniline (0.5 mmol), $Pd(OAc)_2$ (0.05 mmol), BINAP (0.1 mmol), Cs_2CO_3 (1.5 mmol), and **8** (0.5 mmol) under argon was charged with degassed doixane (2 mL) and heated to a temperature of 100 °C. After 12 h, the reaction mixture was cooled to rt, diluted with sat. aqueous ammonium chloride, and extracted with CH_2Cl_2 (10 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography. *Step 2:* The compound obtained from step 1 (0.2 mmol) was dissolved in CH_2Cl_2 (0.06 M) and stirred at rt. TFA (0.02 M) was added and the mixture was stirred for 2 h before being concentrated under reduced pressure. The residue was purified by silica gel chromatography.

4-((4-fluorophenyl)amino)benzenesulfonamide (1j). The title compound was prepared from *tert*-butyl ((4-bromophenyl)sulfonyl)carbamate (obtained from general procedure F) and 4-fluoroaniline in similar fashion to general procedure G in 42% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70-7.67 (m, 2H), 7.10-7.07 (comp, 2H), 7.02-6.98 (comp, 2H), 6.85-6.82 (m, 2H), 5.86 (s, 1H), 4.59 (s, 2H). MS (ESI) *m/z* = 267.1 $[M+H]^+$. Purity (AUC), ≥95%.

4-((3,4-difluorophenyl)amino)benzenesulfonamide (1k). The title compound was prepared from *tert*-butyl ((4-bromophenyl)sulfonyl)carbamate (obtained from general procedure F) and 3,4-difluoroaniline in similar fashion to general procedure G in 45% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 7.75 (d, *J* = 8.5 Hz, 2H), 7.24-7.17 (comp, 1H), 7.11-7.04 (m, 3H), 6.98-6.95 (comp, 1H). MS (ESI) *m/z* = 285.1 $[M+H]^+$. Purity (AUC), ≥95%.

4-((2,4-difluorophenyl)amino)benzenesulfonamide (1l). The title compound was prepared from *tert*-butyl ((4-bromophenyl)sulfonyl)carbamate (obtained from general procedure F) in similar fashion to general procedure G in 38% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 7.71 (d, *J* = 8.5 Hz, 2H), 7.41-7.35 (comp, 1H), 7.10-7.04 (comp, 1H), 7.00-6.96 (comp, 1H), 6.90 (d, *J* = 8.5 Hz, 2H). MS (ESI) *m/z* = 285.1 $[M+H]^+$. Purity (AUC), ≥95%.

4-((3,5-difluorophenyl)amino)benzenesulfonamide (1m). The title compound was prepared from *tert*-butyl ((4-bromophenyl)sulfonyl)carbamate (obtained from general procedure F) and 3,5-difluoroaniline in similar fashion to general procedure G in 31% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (m, 2H), 7.09-7.04 (comp, 1H), 6.97-6.93 (m, 3H), 6.82-6.80 (m, 1H), 5.89 (s, 1H), 4.62 (s, 2H). MS (ESI) *m/z* = 285.1 $[M+H]^+$. Purity (AUC), ≥95%.

4-((3-chloro-4-fluorophenyl)amino)benzenesulfonamide (1n). The title compound was prepared from *tert*-butyl ((4-bromophenyl)sulfonyl)carbamate (obtained from general procedure F) and 4-chloro-3-fluoroaniline in similar fashion to general procedure G in 38% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 7.64 (d, *J* = 8.6 Hz, 2H), 7.15-7.13 (comp, 1H), 7.10-7.01 (comp, 2H), 6.96 (d, *J* = 8.6 Hz, 2H). MS (ESI) *m/z* = 302.2 $[M+H]^+$. Purity (AUC), ≥95%.

4-((4-fluoro-3,5-dimethylphenyl)amino)benzenesulfonamide (1o). The title compound was prepared from *tert*-butyl ((4-bromophenyl)sulfonyl)carbamate (obtained from general procedure F) and 4-fluoro-3,5-dimethylaniline in similar fashion to general procedure G in 49% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, *J* = 8.8 Hz, 2H), 6.81 (d, *J* = 8.8 Hz, 2H), 6.77-6.75 (comp, 2H), 5.76 (s, 1H), 4.56 (s, 2H), 2.19 (s, 6H). MS (ESI) *m/z* = 295.2 $[M+H]^+$. Purity (AUC), ≥95%.

3-chloro-4-((3,4-difluorophenyl)amino)benzenesulfonamide (9). The title compound was prepared from *tert*-butyl ((3-chloro-4-fluorophenyl)sulfonyl)carbamate (obtained from general procedure F) and 3,4-difluoroaniline in similar fashion to general procedure G in 49% yield over two steps.

Synthesis of 3-chloro-4-((3,4-difluorophenyl)amino)benzenesulfonamide (2m as TFA salt). *Step 1:* A mixture of an potassium alkyl trifluoroborate (0.3 mmol), $Pd(OAc)_2$ (0.02 mmol), XPhos (0.03 mmol), Cs_2CO_3 (0.6 mmol), and **9** (0.2 mmol) under argon was charged with dry THF/ H_2O (4:1, 1 mL) and heated to a temperature of 100 °C. After 12 h, the reaction mixture was cooled to rt, diluted with sat. aqueous ammonium chloride, and extracted with CH_2Cl_2 (3 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography. *Step 2:* The compounds obtained from step 1 (0.1 mmol) was dissolved in CH_2Cl_2 (0.06 M) and stirred at rt. TFA (0.02 M) was added and the mixture was stirred for 2 h before being concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC and the pure fractions were concentrated under a stream of warm air to afford the target compound as a TFA salt with 30% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 7.62-7.60 (m, 2H), 7.15-7.09 (comp, 2H), 7.04-6.99 (m, 1H), 6.89-6.85 (comp, 1H), 3.63 (s, 2H), 3.22-3.19 (m, 4H), 2.63 (br, 4H). MS (ESI) *m/z* = 383.2 $[M+H]^+$. Purity (AUC), ≥95%.

Synthesis of methyl 2-fluoro-5-sulfamoylbenzoate (11). *Step 1:* To a solution of 5-(chlorosulfonyl)-2-fluorobenzoic acid (16.76 mmol) in CH_2Cl_2 (20 mL), the ammonium hydroxide (30% aqueous solution) (20 mL) was added dropwise at 0 °C and the mixture was stirred overnight at room temperature. After consumption of the starting material, the reaction mixture was concentrated to half volume with a rotary evaporator and acidified with 6 M HCl to ~pH 5. The resulting solution was left at room temperature until the product precipitated. The precipitate was filtered, washed with water, and dried under vacuum to give 2-fluoro-5-sulfamoylbenzoic acid (yield 52%) as a white solid. *Step 2:* 2-fluoro-5-sulfamoylbenzoic acid (8.67 mmol) in anhydrous DMF (15 mL) were added to iodomethane (9 mmol) and potassium carbonate (5 mmol). The reaction mixture was stirred overnight at room temperature. The reaction was quenched with water, extracted with ethyl acetate (50 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography to afford methyl 2-fluoro-5-sulfamoylbenzoate (yield 56%) as a white solid.

Synthesis of ethyl 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoate (12). *Step 1:* To a solution of 3-chloro-4-fluorophenol (5.82 mmol), and **11** (4.85 mmol) in dimethylsulfoxide (20 mL) was added Cesium carbonate (14.55 mmol) and the reaction mixture was stirred at 120 °C for 12 h and allowed to cool to

ambient temperature. The mixture was diluted with ethyl acetate (30 mL) and washed with water (2x10 mL) and brine (10 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The crude solid was purified by silica gel chromatography to afford methyl 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoate (yield 61%) as a white solid. *Step 2:* To a solution of 2 methyl 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoate (2.94 mmol) in 10% H_2O in methanol was added to lithium hydroxide (81.6 mmol). The reaction mixture was stirred for 4 h at room temperature. The reaction was quenched with water, extracted with ethyl acetate (20 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography to afford 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid (yield 78 %) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 8.35 (d, *J* = 2.5 Hz, 1H), 7.92 (dd, *J* = 2.5 Hz, *J* = 8.7 Hz, 1H), 7.21-7.17 (comp, 1H), 7.11-7.09 (comp, 1H), 7.01 (d, *J* = 8.7 Hz, 1H), 6.93-6.89 (comp, 1H), 4.10 (s, 2H) 3.38-3.33 (m, 4H), 3.23-3.21 (m, 2H), 3.08-3.05 (m, 2H), 2.12-2.10 (m, 2H). MS (ESI) *m/z* = 346.1 $[M+H]^+$.

General Procedure H: Synthesis of 2f, 2h–l, **3b-c,** and **3i-k**. *Step 1:* To a mixture of 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid (0.2 mmol) and HATU (0.3 mmol) in 3:1 anhydrous DCM: DMF (2 ml) was added to the corresponding alkyl amines (0.25 mmol) and 4-methylmorpholine (0.75 mmol). The reaction mixture was stirred for 18 h at room temperature. The reaction was diluted with water, extracted with ethyl acetate (3 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography. *Step 2:* The compound obtained from step 1 (0.1 mmol) was dissolved in THF (0.05 M), was treated with 1 M BH_3.THF complex (0.4 mmol) at room temperature, and was refluxed at 100°C for 12 hours. The mixture was then cooled to room temperature, was treated with 6 M HCl solution (1 ml), and was refluxed at 100°C for 30 minutes. The mixture was cooled to room temperature, diluted with water and basified by addition of K_2CO_3 to pH 9. The mixture was extracted with ethyl acetate (2 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC and the pure fractions were concentrated under a stream of warm air to afford the target compound as a TFA salt. *Step 3:* The compound obtained from step 2 (0.05 mmol) was dissolved in CH_2Cl_2 (0.06 M) and stirred at rt. TFA (0.02 M) was added and the mixture was stirred for 2 h before being concentrated under reduced pressure. The residue was purified by reversed-phase

preparative HPLC and the pure fractions were concentrated under a stream of warm air to afford the target compound as a TFA salt.

3-((3-aminoazetidin-1-yl)methyl)-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (2f as TFA salt). The title compound was prepared as the corresponding TFA salt from 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid and *tert*-butyl azetidin-3-ylcarbamate in similar fashion to general procedure H in 19% yield over three steps.^{1}H NMR (400 MHz, CD_3OD): δ 8.16 (d, *J* = 2.3 Hz, 1H), 7.97 (dd, *J* = 2.3 Hz, *J* = 8.8 Hz, 1H), 7.44-7.37 (comp, 2H), 7.21-7.17 (comp, 1H), 7.00 (d, *J* = 8.8 Hz, 1H), 4.54 (s, 2H), 3.49-3.43 (m, 3H), 3.35-3.20 (m, 2H). MS (ESI) *m/z* = 386.1 $[M+H]^+$. Purity (AUC), ≥95%.

(S)-4-(3-chloro-4-fluorophenoxy)-3-((2-methylpiperazin-1-yl)methyl)benzenesulfonamide (2h as TFA salt). The title compound was prepared as the corresponding TFA salt from 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid and *tert*-butyl (S)-3-methylpiperazine-1-carboxylate in similar fashion to general procedure H in 21% yield over three steps.^{1}H NMR (400 MHz, CD_3OD): δ 7.97 (d, *J* = 2.3 Hz, 1H), 7.73 (dd, *J* = 2.3 Hz, *J* = 8.7 Hz, 1H), 7.23-7.19 (comp, 1H), 7.12-7.10 (comp, 1H), 4.21-4.17 (m, 1H), 3.53-3.49 (m, 1H), 3.05-2.98 (m, 4H), 2.85-2.76 (m, 4H), 1.19-1.18 (m, 3H). MS (ESI) *m/z* = 414.0 $[M+H]^+$. Purity (AUC), ≥95%.

3-((1,4-diazepan-1-yl)methyl)-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (2i as TFA salt). The title compound was prepared as the corresponding TFA salt from 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid and *tert*-butyl 1,4-diazepane-1-carboxylate in similar fashion to general procedure H in 31% yield over three steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.13 (d, *J* = 2.3 Hz, 1H), 7.88 (dd, *J* = 2.3 Hz, *J* = 8.7 Hz, 1H), 7.36-7.32 (comp, 1H), 7.27-7.26 (comp, 1H), 7.09-7.06 (comp, 1H), 7.07 (d, *J* = 8.7 Hz, 1H), 4.10 (s, 2H) 3.38-3.33 (m, 4H), 3.23-3.21 (m, 2H), 3.08-3.05 (m, 2H), 2.12-2.10 (m, 2H). MS (ESI) *m/z* = 414.0 $[M+H]^+$. Purity (AUC), ≥95%.

3-((2,7-diazaspiro[3.5]nonan-7-yl)methyl)-4-(3-chloro-4-fluorophenoxy)benzenesulfonamide (2j as TFA salt). The title compound was prepared as the corresponding TFA salt from 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid and *tert*-butyl 2,7-diazaspiro[3.5]nonane-2-carboxylate in similar fashion to general procedure H in 21% yield over three steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.14 (d, *J* = 2.4 Hz, 1H), 7.97 (dd, *J* = 2.4 Hz, *J* = 8.8 Hz, 1H), 7.43-7.41 (comp, 1H), 7.38-7.36 (comp, 1H), 7.20-7.16

(comp, 1H), 7.01 (d, *J* = 8.8 Hz, 1H), 4.45 (s, 2H) 3.99-3.36 (m, 4H), 3.22-3.18 (m, 4H), 2.23-2.18 (m, 4H). MS (ESI) *m/z* = 439.9 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)-3-((hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)methyl) benzenesulfonamide (2k as TFA salt). The title compound was prepared as the corresponding TFA salt from 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid and *tert*-butyl hexahydropyrrolo[3,4-*c*]pyrrole-2(1*H*)-carboxylate in similar fashion to general procedure H in 25% yield over three steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.13 (d, *J* = 2.3 Hz, 1H), 7.92 (dd, *J* = 2.3 Hz, *J* = 8.7 Hz, 1H), 7.39-7.35 (m, 2H), 7.14-7.12 (comp, 1H), 7.00 (d, *J* = 8.8 Hz, 1H), 4.34 (s, 2H) 3.54-3.51 (m, 2H), 3.36-3.3.33 (m, 2H), 2.29-2.18 (m, 6H). MS (ESI) *m/z* = 426.0 $[M+H]^+$. Purity (AUC), ≥95%.

3-((2,8-diazaspiro[4.5]decan-2-yl)methyl)-4-(3-chloro-4-fluorophenoxy)benzenesulfonami (2l as TFA salt). The title compound was prepared as the corresponding TFA salt from 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid and *tert*-butyl 1,7-diazaspiro[3.5]nonane-7-carboxylate in similar fashion to general procedure H in 23% yield over three steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.06 (d, *J* = 2.3 Hz, 1H), 7.5 (dd, *J* = 2.3 Hz, *J* = 8.8 Hz, 1H), 7.34-7.30 (comp, 1H), 7.22-7.19 (comp, 1H), 7.04-7.02 (m, 2H), 3.81 (s, 2H), 3.40-3.37 (m, 3H), 3.16-3.10 (m, 4H), 2.53 (t, *J* = 11.2 Hz, 2H), 2.31 (t, *J* = 11.2 Hz, 2H), 1.32-1.30 (m, 3H). MS (ESI) *m/z* = 453.9 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)-3-((4-isopropylpiperazin-1-yl)methyl)benzenesulfonamide (3b as TFA salt). The title compound was prepared as the corresponding TFA salt from 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid and *1*-isopropylpiperazine in similar fashion to general procedure H in 38% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.05 (d, *J* = 2.3 Hz, 1H), 7.86 (dd, *J* = 2.3 Hz, *J* = 8.7 Hz, 1H), 7.34-7.30 (comp, 1H), 7.21-7.19 (comp, 1H), 7.05-7.01 (m, 2H), 3.81 (s, 2H), 3.55-3.44 (m, 5H), 3.15-3.13 (m, 4H), 1.37 (d, *J* = 6.7 Hz, 6H). MS (ESI) *m/z* = 441.9 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)-3-((4-cyclopropylpiperazin-1-yl)methyl)benzenesulfonamide (3c as TFA salt). The title compound was prepared as the corresponding TFA salt from 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid and 1-cyclopropylpiperazine in similar fashion to general procedure H in 31% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.10 (d, *J* = 2.4 Hz, 1H), 7.90 (dd, *J* = 2.4 Hz, *J* = 8.6 Hz, 1H), 7.37-7.29 (comp, 2H), 7.12-7.09 (comp, 1H), 7.01 (d, *J* = 8.6 Hz, 1H), 4.10 (s,

2H), 3.29-3.24 (m, 4H), 3.06-3.02 (m, 4H), 2.48-2.44 (m, 1H), 0.83-0.79 (m, 4H). MS (ESI) *m/z* = 439.9 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)-3-((4-(oxetan-3-yl)piperazin-1-yl)methyl)benzenesulfonamide (3i as TFA salt). The title compound was prepared as the corresponding TFA salt from 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid and 1-(oxetan-3-yl)piperazine in similar fashion to general procedure H in 27% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.07 (d, *J* = 2.4 Hz, 1H), 7.86 (dd, *J* = 2.4 Hz, *J* = 8.6 Hz, 1H), 7.35-7.30 (comp, 1H), 7.23-7.21 (comp, 1H), 7.06-7.01 (m, 2H), 3.92-3.85 (m, 3H), 3.71-3.66 (m, 1H), 3.46-3.40 (m, 7H), 2.99-2.91 (m, 4H). MS (ESI) *m/z* = 458.1 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)-3-((4-(2-hydroxyethyl)piperazin-1-yl)methyl)benzenesulfonamide (3j as TFA salt). The title compound was prepared as the corresponding TFA salt from 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid and 2-(piperazin-1-yl)ethan-1-ol in similar fashion to general procedure H in 27% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.07 (d, *J* = 2.4 Hz, 1H), 7.86 (dd, *J* = 2.4 Hz, *J* = 8.6 Hz, 1H), 7.35-7.30 (comp, 1H), 7.24-7.21 (comp, 1H), 7.06-7.00 (m, 2H), 3.89 (t, *J* = 5.3 Hz, 2H), 3.84 (s, 2H), 3.37-3.36 (m, 4H), 3.27 (t, *J* = 5.3 Hz, 2H), 2.94-2.87 (m, 4H). MS (ESI) *m/z* = 444.9 $[M+H]^+$. Purity (AUC), ≥95%.

4-(3-chloro-4-fluorophenoxy)-3-((4-(2-methoxyethyl)piperazin-1-yl)methyl)benzenesulfonamide (3k as TFA salt). The title compound was prepared as the corresponding TFA salt from 2-(3-chloro-4-fluorophenoxy)-5-sulfamoylbenzoic acid and 1-(2-methoxyethyl)piperazine in similar fashion to general procedure H in 37% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 8.06 (d, *J* = 2.5 Hz, 1H), 7.85 (dd, *J* = 2.5 Hz, *J* = 8.8 Hz, 1H), 7.35-7.30 (comp, 1H), 7.23-7.21 (comp, 1H), 7.05-7.00 (m, 2H), 3.82 (s, 2H), 3.72 (t, *J* = 5.3 Hz, 2H), 3.42 (s, 3H), 3.36 (t, *J* = 5.3 Hz, 2H), 3.33-3.28 (m, 4H), 2.90-2.85 (m, 4H). MS (ESI) *m/z* = 457.9 $[M+H]^+$. Purity (AUC), ≥95%.

■ ASSOCIATED CONTENT

Supplementary Information. The Supplementary information associated with this manuscript is available free of charge on the ACS Publications website. Files include: 2D HMQC NMR-based fragment screening details, identified fragment hits at the activator site of SOS1, biochemical and cellular assay

conditions; details regarding protein expression and purification; details regarding crystallization, X-Ray data collection, structure solution, and refinement; and a table containing X-Ray data collection and refinement statistics.

Molecular formula strings (CSV)

Accession Codes. Atom coordinates and structure factors for the following SOS1-ligand complexes will be released to the PBD upon article publication: Compound **F-2** (PDB ID code 6V9F)**,** Compound **F-4** (PDB ID code 6V94), Compound **1g** (PDB ID code 6V9J), Compound **F-7** (PDB ID code 6V9M), Compound **2d** (PDB ID code 6V9L), Compound **1a** (PDB ID code 6V9N).

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The authors declare the following competing financial interest(s): RAS activator compounds have been licensed to Boehringer Ingelheim.

■ ACKNOWLEDGEMENTS

Funding for this work came from the following sources: U.S.National Institutes of Health, NIH Director's Pioneer Award (DP1OD006933/DP1CA174419) to S. W. Fesik; Lustgarten Foundation Research Investigator Grant to S. W. Fesik; National Cancer Institute SPORE Grant in GI Cancer (5P50A095103-09) to R. J. Coffey. The authors thank the Vanderbilt Institute of Chemical Biology (VICB) High-Throughput Screening Core Facility at Vanderbilt University for chemical management of our in-house fragment library. We also thank the Vanderbilt University Biomolecular NMR Facility, with assistance provided by Donald Stec. This facility receives support from an NIH SIG grant (1S-10RR025677-01) and Vanderbilt University

matching funds. Finally, we thank the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences for use of the Advanced Photon Source (contract: DE-AC02-06CH11357).

■ ABBREVIATIONS USED

EGF, epidermal growth factor; ERK1/2, extracellular regulated kinases 1 and 2; GEF, guanine nucleotide exchange factor; LE, ligand efficiency; pERK1/2, phosphorylated ERK1/2; Act., relative percent activation; SOS1, son of sevenless homologue 1; HMQC, heteronuclear multiple quantum coherence; HSQC, heteronuclear single quantum coherence; PDB, Protein Data Bank.

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