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Article

Aryl Sulfonamide Inhibits Entry and Replication of Diverse Influenza Viruses via the Hemagglutinin Protein

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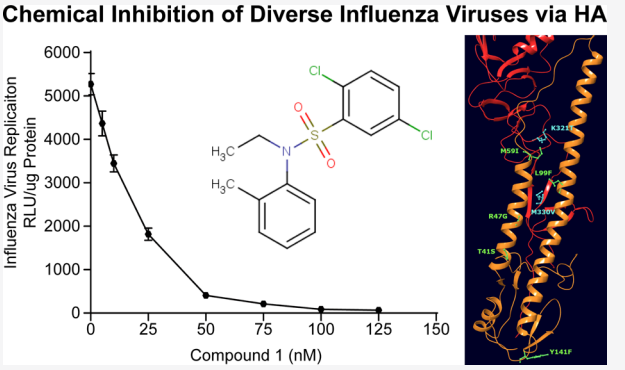
ABSTRACT: Influenza viruses cause approximately half a million deaths every year worldwide. Vaccines are available but partially effective, and the number of antiviral medications is limited. Thus, it is crucial to develop therapeutic strategies to counteract this major pathogen. Influenza viruses enter the host cell via their hemagglutinin (HA) proteins. The HA subtypes of influenza A virus are phylogenetically classified into groups 1 and 2. Here, we identified an inhibitor of the HA protein, a tertiary aryl sulfonamide, that prevents influenza virus entry and replication. This compound shows potent antiviral activity against diverse H1N1, H5N1, and H3N2 influenza viruses encoding HA proteins from both groups 1 and 2. Synthesis of derivatives of this aryl sulfonamide identified moieties important for antiviral activity. This compound may be considered as a lead for drug development with the intent to be used alone or in combination with other influenza A virus antivirals to enhance pan-subtype efficacy.



INTRODUCTION

Influenza viruses are responsible for up to 646,000 deaths worldwide per year,[1] and few therapeutic options are available to counteract the infection. These viruses enter the host cell via interactions of their hemagglutinin (HA) proteins present in the virus envelope with sialic aids that modify glycoproteins in the host plasma membrane.[2] They are internalized via endocytosis, and the eight viral ribonucleoproteins (vRNPs), which constitute the segmented viral genome, are released into the cytoplasm upon fusion of the viral and endosomal membranes. The vRNPs are subsequently imported into the nucleus through the nuclear pore complex and transcribed into mRNAs, among which are two mRNAs that undergo alternative splicing. The viral mRNAs are exported from the nucleus and translated in the cytoplasm. vRNPs are also templates for virus replication in the nucleus. vRNPs are transcribed into complementary ribonucleoproteins (cRNPs), which are then transcribed back into vRNPs. Newly generated vRNPs are exported from the nucleus to the cytoplasm where they, together with viral proteins, assemble viral particles that bud from the host cell.[2]

One key step for chemical inhibition of influenza virus replication is entry into the host cell, which involves the HA protein. The HA subtypes of influenza A virus[3] are phylogenetically divided into group 1 (H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17, and H18) and group 2 (H3, H4, H7, H10, H14, and H15). HA protein forms a trimer, and each monomer has two subunits (HA1 and HA2) linked by a disulfide bond. The HA1 subunit functions in receptor binding located in the head region of the protein. The HA2 subunit forms, together with part of the HA1 subunit, the stem region of the HA and is the mediator of the viral membrane fusion with the endosomal membrane. The low endosomal pH triggers this fusion event by inducing conformation changes in HA, that expose its fusion peptide, and culminates with the release of the viral genome into the cytoplasm. This step has been the target of several HA inhibitors[4−17] that prevent fusion of HA proteins from either group 1 or group 2 but not both. Here, we identified an aryl sulfonamide as an inhibitor of influenza A virus entry that targets the HA protein, preventing viral membrane fusion. Importantly, this compound prevents replication of influenza A viruses that encode HA proteins from both groups 1 and 2, including H1N1, H5N1, and H3N2, at non-toxic concentrations. This HA inhibitor therefore targets diverse influenza A viruses.

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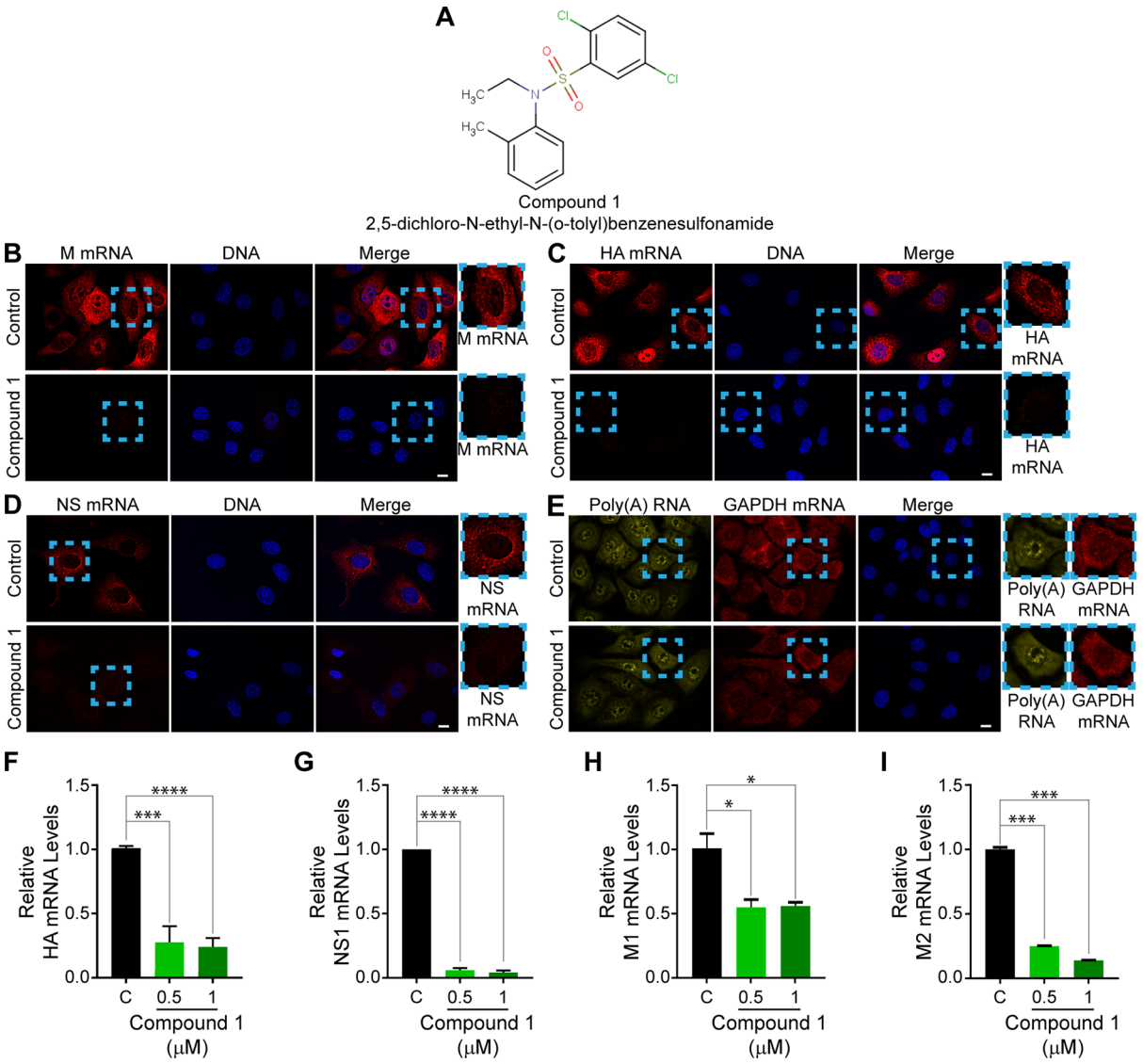

Figure 1. Compound **1** reduces viral mRNA levels. (A) Structure of compound **1**. (B) smRNA-FISH followed by fluorescence microscopy was performed with probes to detect M mRNA in A549 cells infected with WSN at multiplicity of infection (MOI) 2 for 8 h. (C) Cells were treated as in **B** except that smRNA-FISH was performed with probes to detect HA mRNA. (D) Cells were treated as in **B** except that smRNA-FISH was performed with probes to detect NS mRNA. (E) RNA-FISH and smRNA-FISH followed by fluorescence microscopy were performed in cells treated with 0.1% DMSO or 1 μM compound **1** to detect poly(A) RNA and GAPDH mRNA, respectively, in uninfected cells. (F–I) A549 cells were treated with 0.1% DMSO or 0.5 or 1 μM compound **1** for 1 h before infection with A/WSN/33 at MOI 2. Infection was performed for 8 h in the absence and presence of compound. Purified RNA from total cell lysates was collected and subjected to qPCR to measure viral HA (F), NS1 (G), M1 (H), and M2 (I) mRNA levels. Three independent experiments were performed for all data sets. Graphs are mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

RESULTS AND DISCUSSION

We have recently reported a chemical screen that identified inhibitors of influenza A virus mRNA processing and nuclear export.[18] Here, we reveal a hit in the screen, a tertiary aryl sulfonamide (2,5-dichloro-*N*-ethyl-*N*-(*o*-tolyl)-benzenesulfonamide) (Figure 1A), also termed here compound **1**. This aryl sulfonamide reduced influenza virus (A/WSN/33) mRNA levels, as detected by single-molecule RNA in situ hybridization (smRNA-FISH) (Figure 1B–D). We assessed the viral M, HA, and NS mRNAs. HA mRNA, as mentioned above, encodes the HA protein involved in viral entry into the host cell.[2] M mRNA encodes the M1 and M2 proteins, which are involved in viral trafficking and budding.[2,19,20] M2 has also a role in the inhibition of autophagy during infection.[21] The NS mRNA encodes the NS1 and NS2 proteins. NS1 protein is a multifunctional virulence factor that inhibits host gene expression and immunity,[22] whereas NS2 functions in nuclear export of viral RNAs[2] and in viral RNA replication.[23] While this compound decreased viral mRNA levels (Figure 1B–D), it did not have a significant effect on cellular bulk poly(A) RNA or GAPDH, as shown by RNA-FISH and smRNA-FISH, respectively (Figure 1E). The effect of compound **1** on viral mRNA levels detected by imaging was further corroborated by quantitative real-time PCR (qPCR) (Figure 1F–I), which again detected reduced levels of viral mRNAs. The experiments mentioned above were performed in A549 cells, which are lung epithelial cells derived from adenocarcinoma. Thus, we have also tested the effect of compound **1** in primary human bronchial epithelial cells

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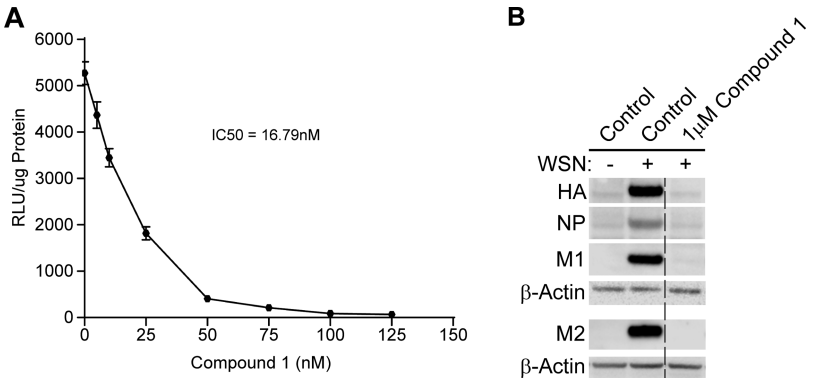

Figure 2. Compound **1** decreases viral protein levels. (A) Dose-dependent inhibition of WSN-PB2-Nanoluc virus by compound **1**. HCT116 cells were incubated with compound **1** for 2 h and then infected with WSN-PB2-Nanoluc with a MOI of 0.02. After 24 h of infection, cells were harvested in Renilla lysis buffer, and luminescence was measured using the Nano-Glo substrate. Luminescence was normalized to protein levels, and IC_{50} was calculated using GraphPad Prism 7. (B) A549 cells were pre-treated with either 0.1% DMSO or 1 μM compound **1** before infection with A/WSN/33 at MOI 2 for 8 h. Cell lysates were subjected to western blot analysis to detect the depicted viral proteins. β-Actin was used as a loading control. This blot is a representative of three independent experiments.

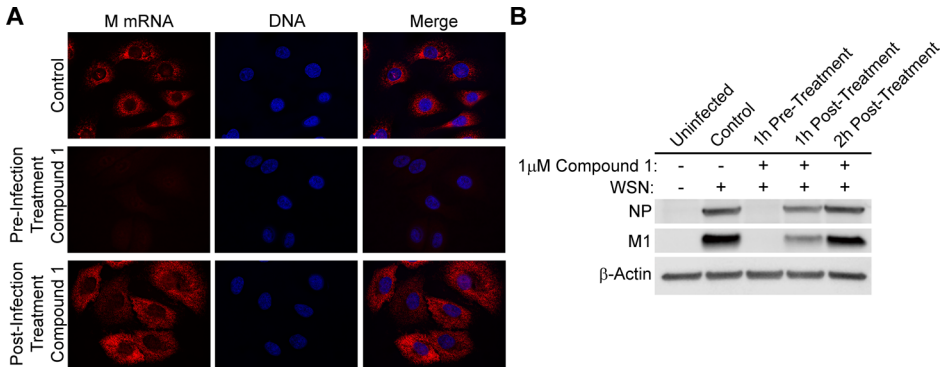

Figure 3. Influenza virus entry is inhibited by compound **1**. (A) A549 cells were incubated with 1 μM compound **1** for 1 h before infection or for 1 h post-infection. Infection was performed with WSN at MOI 2 for 8 h. Cells were then subjected to smRNA-FISH with probes to detect viral M mRNA. (B) A549 cells were incubated with 1 μM compound **1** for 1 h before infection or for 1 h or 2 h post-infection. Infection was performed with WSN at MOI 2 for 8 h. Cells were lysed and then subjected to western blot analysis to detect the depicted viral proteins.

infected with influenza virus. As shown in Figure S1, compound **1** also robustly decreased viral mRNA levels in primary cells. Taken together, these results suggest that this compound could be interfering with viral entry or viral mRNA transcription and/or processing. Consequently, less viral proteins were expressed. In fact, we show that cells infected with WSN-PB2-Nanoluc virus, in which Nano luciferase is fused with the viral polymerase subunit PB2 protein and then released by cleavage at a T2A site, show decreased levels of Nanoluc in a dose-dependent manner with an IC_{50} = 16.79 nM (Figure 2A). Additionally, western blot analysis of cells infected with WSN also depicted reduced levels of influenza virus proteins (Figure 2B).

Next, we tested whether viral entry is inhibited by compound **1**. Cells were incubated with compound for 1 h before infection, or compound **1** was added 1 h post-infection. After 8 h post-infection, cells were subjected to smRNA-FISH to detect viral M mRNA (Figure 3A). We found that the viral M mRNA was expressed at very low levels when compound **1** was added prior to infection, whereas viral M mRNA was robustly expressed when cells were incubated with compound **1** after infection (Figure 3A). The lack of antiviral activity of the compound when used shortly post-infection was confirmed by western blot against the viral proteins NP and M1 (Figure 3B). These results indicate that compound **1** likely inhibits viral entry. Then, we tested whether compound **1** would target a viral protein. We therefore sought to determine whether compound **1** would yield viral mutants that could resist infection in the presence of the highest compound concentration that still generates enough viruses for passaging at the same MOI. After four passages at MOI 0.01, resistant WSN viruses were identified by increases in viral titers in the presence of compound **1** similar to dimethyl sulfoxide (DMSO) controls and isolated in seven independent experiments. Three virus plaques were purified from each independent resistance experiment, and viral RNA was purified and submitted for Illumina sequencing (Figure 4A). We found that all sequenced viruses contained mutations in the HA gene, indicating that HA is likely the target of compound **1** (Figure 4B). Interestingly, the identified mutations in the resistant virus were spread across the HA stem region formed by part of HA1 and by the ectodomain of HA2 and did not highlight a single binding pocket (Figure 4C). This scattered resistance mutation phenotype in the HA gene is actually well established in our previous work[24] and work from several other groups[8−11,14] to result from escape from the antiviral activity of HA fusion inhibitors. In fact, in our previous work, we identified the same exact M59I (HA2) and K321T (HA1) mutations to cause resistance to the fusion inhibitor S20.[24] These fusion inhibitors are shown to stabilize the prefusion

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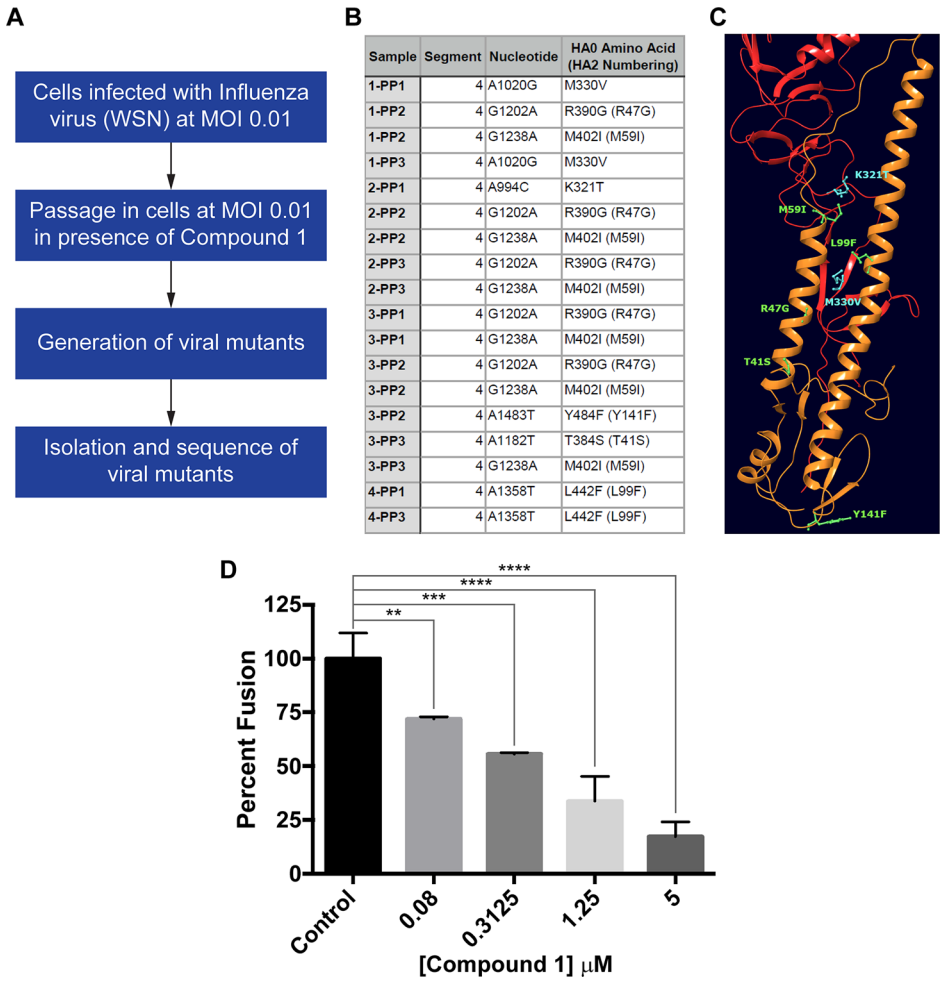

Sample	Segment	Nucleotide	HA0 Amino Acid (HA2 Numbering)
1-PP1	4	A1020G	M330V
1-PP2	4	G1202A	R390G (R47G)
1-PP2	4	G1238A	M402I (M59I)
1-PP3	4	A1020G	M330V
2-PP1	4	A994C	K321T
2-PP2	4	G1202A	R390G (R47G)
2-PP2	4	G1238A	M402I (M59I)
2-PP3	4	G1202A	R390G (R47G)
2-PP3	4	G1238A	M402I (M59I)
3-PP1	4	G1202A	R390G (R47G)
3-PP1	4	G1238A	M402I (M59I)
3-PP2	4	G1202A	R390G (R47G)
3-PP2	4	G1238A	M402I (M59I)
3-PP2	4	A1483T	Y484F (Y141F)
3-PP3	4	A1182T	T384S (T41S)
3-PP3	4	G1238A	M402I (M59I)
4-PP1	4	A1358T	L442F (L99F)
4-PP3	4	A1358T	L442F (L99F)

Figure 4. Mutations within the HA protein cause resistance to the inhibitory effects of compound **1**. (A) Schematic representation of the assays for the identification of viral mutants resistant to compound **1** (0.5 μM) effect on viral replication. (B) Table depicting the viral RNA segment in which the mutations where identified, showing that the HA RNA was the target of mutations at the positions listed. (C) Crystal structure of A/PR/8/34 HA (PDB 1RU7) protein showing the HA1 (red) and HA2 (orange) subunits. Residue positions where compound **1** escape mutations occurred are indicated in teal (HA1) and green (HA2). (D) Suspension of chicken erythrocytes was incubated with WSN on ice in the absence of presence of compound **1** at the indicated concentrations. The mixture was then acidified with a pH of 5. The suspension was incubated at 37 °C for 30 min and assayed at 340 nM for NADH released upon lysis of the erythrocytes, as a measurement of fusion. Data are expressed as percentage relative to the DMSO control, and means of triplicates ±SD are shown.

conformation of HA[24] and prevent fusion from occurring, and it is thought that these resistance mutations counteract the inhibition through destabilization of the prefusion complex. Mutations in similarly scattered, but not identical, positions were also found to cause resistance to the well-established fusion inhibitor arbidol,[25] which also was shown to stabilize the prefusion conformation of the HA protein. This indicates that this resistance pattern is a paradigm of this class of fusion inhibitors, and it can be considered as strong evidence of the HA prefusion stabilizing mechanism. In support of this hypothesis, a resistance mutation identified in this study through selective pressure from compound **1**, R47G (HA2), occurs in the same position where a positively charged amino acid has been associated with the prefusion stability of the HA protein.[26] Therefore, this set of resistance mutations induced by compound **1** is suggestive of an HA fusion inhibitor that may stabilize the prefusion conformation of influenza HA, preventing the HA conformational change required for viral entry. To test this possibility, we performed a hemolysis assay, which measures the ability of influenza virus to induce membrane fusion and lysis of red blood cells under low-pH conditions. We found that compound **1** displayed a robust inhibition of fusion induced at a pH of 5 (Figure 4D). Together these results demonstrate that compound **1** targets the HA protein and thereby prevents HA-mediated membrane fusion.

We next determined the breadth of compound **1** inhibition against multiple subtypes of influenza A virus. We tested an inhibitory, but non-toxic, concentration of compound **1** for its ability to reduce the viral titers from infections performed with wild-type A/WSN/1933 (H1N1), A/Vietnam/2506/2004 (H5N1), or A/Panama/1999 (H3N2) in A549 cells. We found that compound **1** was capable of reducing viral titers by a minimum of four logs against all three subtypes of influenza A virus (Figure 5), indicating that it has antiviral activity against influenza viruses with either group 1 or group 2 HAs. Additionally, we used smRNA-FISH to test the effect of compound **1** and two of its active analogues (**12** and **21**) on different H1N1 (A/California/07/2009) and H3N2 (A/Wyoming/03/2003) strains from the ones mentioned above. Cells were infected at MOI of 1. We found that these three compounds robustly reduced the number of cells infected with

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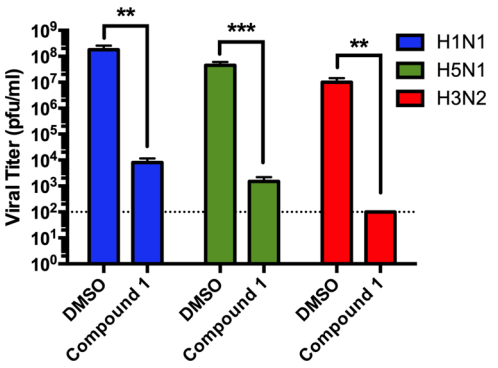

Figure 5. Compound **1** inhibits replication of diverse influenza viruses. A549 cells were infected with influenza H1N1 A/WSN/1933 virus (MOI = 0.01, 24 h), H5N1 A/Vietnam/1203/2004 HaLo virus (MOI = 0.01, 24 h), or H3N2 A/Panama/1999 (MOI = 0.1, 48 h) in the absence or presence of 25 μM of compound **1**, and viral titers were determined by standard plaque assay. The limit of detection for viral titers is indicated with a dotted line. Means ± SD (error bars) of three independent experiments are indicated (**$p < 0.01$ and ***$p < 0.001$).

A/California/07/2009 (Figure S2A). In the case of A/Wyoming/03/2003, while compounds **1** and **21** slightly inhibited the number of infected cells, compound **12** more effectively decreased infection (Figure S2B) at non-toxic concentrations (Figure S2C). Taken together, compound **1** and its analogues can inhibit influenza viruses from group 1 and group 2 HAs with different potency.

Structure−activity relationship: To explore the structural requirements for activity, we prepared a series of derivatives of compound **1**, starting with modifications to the aniline moiety (Table 1). Removing or shrinking the *N*-ethyl group substantially diminished activity (**2**, **3**). Allyl and propargyl groups maintained activity (**4**, **5**), albeit at a decreased level, and an *N*-isopropyl analogue (**6**) had greater than 10-fold loss of activity compared to compound **1**. On the aryl ring, removing the 2-methyl group cost >10× (**7**), but moving it to the para position was well tolerated (**11**). Chloride and nitrile were less effective substituents than the methyl group (**8**, **9**), but fluorine was similarly potent (**10**). Disubstituted aryl rings proved to be among the most active analogues. For example, the 2-methyl-4-chlorophenyl analogues **12**, **13**, and **14** were generally more potent than the corresponding mono-substituted matched pairs (**1** vs **12**; **4** vs **13**; **5** vs **14**). In an attempt to remove potential metabolic liabilities, we synthesized the N−CH_2-cPr derivative **15**, but it was >10× less active than the *N*-ethyl version (**12**). Similarly, we attempted to introduce polarity on the *N*-alkyl group to reduce log *P* and improve drug-like properties. However, analogues **16**, **17**, **18**, and **19** displayed lower activity. Among dihalogenated analogues, the difluoro analogue **21** proved equipotent to compound **1** while replacing or blocking potential sites of metabolism. Trisubstituted derivatives (**22** and **23**) were less active. Finally, we attempted to introduce polarity and functional handles at the para position of the phenyl ring with mixed success. An ester maintained activity (**23**), whereas the corresponding acid, alcohol, and amides were inactive (**24**, **26**−**29**).

Building off of compound **21**, we explored modification of the aryl sulfonamide (Table 2). The two chlorides were important as the phenyl sulfonamide (**30**) and 2-Cl (**31**) analogues were inactive. The 3-Cl derivative maintained activity (**32**) but at a 10-fold lower level than 21. The positioning of the halides was also important as other arrangements were either inactive (**33**, **35**, **36**, and **37**) or >10-fold less active than 1−55 (e.g., **34**). Similarly, a brief exploration of other halogen combinations did not identify analogues with improved potency relative to dichloride 1−55 (see **38**, **39**, and **40**).

Table 3 highlights our efforts to improve metabolic stability and solubility of compound **1** analogues by replacing the N-aryl ring with an N-heteroaryl substituent. Unfortunately, analogues **41**−**48** proved inactive.

Finally, several analogues were synthesized to make substantial changes to the scaffold or to provide reagents for affinity purification (Figure 6). For example, **49** and **50** cyclized the N-alkyl and N-aryl ring in the context of an indoline or tetrahydroquinoline, respectively. Analogue **51** replaced the sulfonyl linker with an amide. All of these were inactive under the assay conditions. Likewise, a pair of alkyne-diazarines (**52** and **53**) as potential photocross-linkers were not active. Encouragingly, however, a simple analogue featuring an aryl azide and an alkyne (**54**) retained robust activity and could provide a tool for photocross-linking experiments in future studies. In summary, the initial analogues demonstrate (a) a steep structure−activity relationship with regard to the aryl sulfonyl group, (b) the requirement for both N-alkyl and N-aryl groups on the central nitrogen, (c) the incompatibility of heteroaryl rings or highly polar groups as nitrogen substituents, and (d) the beneficial effects of 1,4-disubstitution on the N-aryl ring.

CONCLUSIONS

We identified an inhibitor of influenza A virus replication that targets the HA protein of both group 1 (H1 and H5) and group 2 (H3). H1N1 and H3N2 strains co-circulate during the same season and can cause severe disease. Moreover, sporadic human infections with highly pathogenic avian H5 viruses resulting in severe disease are still taking place since their emergence in 1997. What separates compound **1** from those fusion inhibitors identified previously in the literature is that compound **1** shows potent antiviral activity against both group 1 and group 2 influenza viruses, while most other HA fusion inhibitors have been group specific. Therefore, this compound warrants further study as a lead for drug development of anti-influenza therapy and may also be used in combination with other antivirals for increasing pan-subtype efficacy. Future mechanistic studies may broaden our understanding on the activity of this compound toward its target and provide a platform for additional therapeutic strategies.

EXPERIMENTAL SECTION

Cell Culture. Human lung adenocarcinoma epithelial cells (A549) and MDCK cells, obtained from American Type Culture Collection (ATCC), were maintained in high-glucose Dulbecco's modified Eagle medium (Gibco), 10% FBS (Sigma), and 100 units/mL Pen/Strep antibiotics at 37 °C with 5% CO_2. HCT116 cells were obtained from ATCC and cultured in McCoy's 5a medium modified with 10% FBS and antibiotics as mentioned above. Primary human bronchial epithelial cells were cultured, as previously described.[27]

Viruses. Influenza A viruses (A/WSN/33, A/Vietnam/1203/04, A/Panama/99, A/California/07/2009, and A/Wyoming/03/2003) were generated in embryonated eggs or in MDCK cells after growth from a clonal population of virus at low MOI to avoid accumulation of defective virus particles. A/Vietnam/1203/2004 (H5N1) has a mutated polybasic cleavage site in the HA segment (HAlo).[28] In

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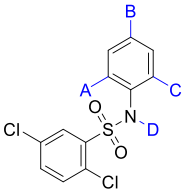
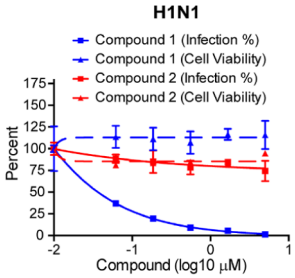
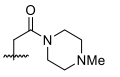
Table 1. Aniline Modifications of Compound 1[a]





cmpd	A	B	C	D	viral replication		Cytotoxicity (MTT)		selectivity index
					IC_{50} (μM)	IC_{90} (μM)	CC_{10} (μM)	CC_{50} (μM)	
1	Me	H	H	Et	0.040	1.2	>25	>25	>625
2	Me	H	H	H	>5	>5	>5	>5	
3	Me	H	H	Me	>5	>5	>5	>5	
4	Me	H	H	Allyl	0.12	0.57	>5	>5	>42
5	Me	H	H	Propargyl	0.38	5.1	>5	>5	>13
6	Me	H	H	iPr	1.8	>5	>5	>5	>3
7	H	H	H	Et	0.86	6.9	>5	>5	>6
8	Cl	H	H	Et	0.15	1.5	>5	>5	>33
9	CN	H	H	Et	0.17	2.1	>5	>5	>29
10	H	F	H	Et	0.068	0.94	>5	>5	>74
11	H	Me	H	Et	0.084	1.8	>25	>25	>298
12	Me	Cl	H	Et	0.034	0.50	>5	>5	>147
13	Me	Cl	H	Allyl	0.037	0.43	>5	>5	>135
14	Me	Cl	H	Propargyl	0.12	3.4	>5	>5	>42
15	Me	Cl	H	$CH_2{}^cPr$	0.49	2.8	>5	>5	>10
16	Me	Cl	H	$(CH_2)_2OMe$	1.2	6.0	>5	>5	>4
17	Me	Cl	H	$(CH_2)_2OH$	>5	>5	>5	>5	
18	Me	Cl	H	$(CH_2)_2F$	0.55	>5	>5	>5	>9
19	Me	Cl	H	CHF_2	1.3	>5	>5	>5	>4
20	F	Cl	H	Et	0.15	1.8	>5	>5	>33
21	F	F	H	Et	0.052	0.90	>5	>5	>96
22	Me	Cl	Cl	Et	0.39	>5	>5	>5	>13
23	F	Cl	F	Et	0.41	>5	0.025	>5	>12
24	H	CH_2CO_2Et	H	Et	0.080	5.7	>5	>5	>63
25	H	CH_2CO_2H	H	Et	>25	>25	>25	>25	
26	H	$(CH)_2OH$	H	Et	>25	>25	>25	>25	
27	H	$(CH_2)_2NH_2$	H	Et	>25	>25	>25	>25	
28	H	$(CH_2)_2NHAc$	H	Et	>25	>25	>25	>25	
29	H		H	Et	>25	>25	>25	>25	

[a]A549 cells were infected with A/WSN/33 at MOI 0.01. After 24 h post-infection, cells were subjected to immunofluorescence microscopy using antibody against the influenza virus NP protein. Percent infection was quantified by dividing the number of NP-positive cells by the total number of cells. Cytotoxicity was also performed using MTT assay concurrent with immunostaining.

MDCK cells, virus was amplified at MOI 0.1−0.001 in infection media containing EMEM (ATCC, 30-2003), 10 mM HEPES (Gibco), 0.125% bovine serum albumin (BSA) (Gibco), and 0.5 μg/mL TPCK trypsin (Worthington Biomedical Corporation). Cells were incubated with virus for 1 h at 37 °C and then washed before amplification in infection media. After cell death was observed at 48−72 h post-infection, supernatants were centrifuged at 1000*g* for 10 min to remove cell debris, aliquoted, and stored at −80 °C. All virus stocks are controlled for an appropriate ratio of HA/PFU titer and sequenced by RNAseq to confirm the full sequence of the virus. WSN PB2-WSN-Nanoluc encodes Nano luciferase fused with PB2.[29]

Viral Replication and Cytotoxicity Assays. A549 cells were infected with each viral strain at MOI 0.01. After 24 h post-infection with A/WSN/33 and A/Vietnam/1203/04, or 48 h with A/Panama/99, cells were fixed with 4% formaldehyde for 30 min. Cells were briefly washed with phosphate buffered saline (PBS) and then permeabilized with 0.1% Triton X-100 in PBS for 15 min. Blocking occurred at room temperature for 1 h with 0.5% BSA in PBS followed by incubation with the NP antibody (HT103, a gift from Thomas Moran) in 0.5% BSA in PBS for 1 h at room temperature. Cells were washed with PBS 2× and incubated with a fluorescently labeled secondary antibody, Alexa-fluor-488 (Invitrogen), in 0.5% BSA in PBS with DAPI for 45 min at room temperature. Two washes with PBS

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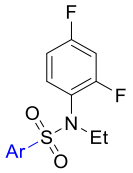
Table 2. Sulfonyl Modifications[a]

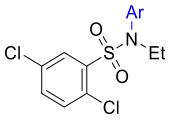


cmpd	Ar	viral replication		CC_{50} (μM)	selectivity index
		IC_{50} (μM)	IC_{90} (μM)		
21		0.052	0.90	>5	>96
30	Ph	>25	>25	>25	
31	2-Cl-C_6H_4	>25	>25	>25	
32	3-Cl-C_6H_4	0.49	10.3	>25	>51
33		>25	>25	>25	
34		0.75	>25	>25	>33
35		>25	>25	>25	
36		>25	>25	>25	
37		>25	>25	>25	
38		0.22	9.8	>25	>114
39		0.82	18.5	>25	>30
40		3.4	>25	>25	>7

[a]A549 cells were infected with A/WSN/33 at MOI 0.01. After 24 h post-infection, cells were subjected to immunofluorescence microscopy using antibody against the influenza virus NP protein. Percent infection was quantified by dividing the number of NP-positive cells by the total number of cells. Cytotoxicity was also performed using MTT assay concurrent with immunostaining.

were performed before imaging the cells on a Celigo Image cytometer. Percent infection was quantified by dividing the number of NP-positive cells by the total number of cells. Cytotoxicity was also performed using MTT assay (Roche), according to the manufacturer's instructions, concurrent with immunostaining. IC_{50}, as shown in Figures 2A and 6, was estimated using WSN-PB2-Nanoluc virus. Cells were incubated with compounds for 2 h and then infected with WSN-PB2-Nanoluc with a MOI of 0.02. After 24 h of infection, cells were harvested in Renilla lysis buffer (Promega), and luminescence was measured using the Nano-Glow substrate (Promega) in a microplate reader (BMG Labtech). The lysate protein concentration was measured using the Bradford reagent (Bio-Rad). Luminescence readings were normalized to protein levels wherever mentioned. Using GraphPad Prism 7, (inhibitor) versus response-variable slope (four parameters) equation was selected for nonlinear regression analysis to determine the IC_{50}. As shown in Figure 6, CC_{50} was calculated using CellTiter-Glo (Promega), according to the manufacturer's instructions. As shown in Figure S1, CellTiter-Glo (Promega) was used to assess cell viability by measuring cellular ATP levels.

Table 3. Heterocyclic Aniline Replacements[a]



cmpd	Ar	IC_{50} (μM) viral replication
41		>5
42		>5
43		>5
44		>5
45		>5
46		>5
47		>5
48		>5

[a]A549 cells were infected with A/WSN/33 at MOI 0.01. After 24 h post-infection, cells were subjected to immunofluorescence microscopy using antibody against the influenza virus NP protein. Percent infection was quantified by dividing the number of NP-positive cells by the total number of cells. Cytotoxicity was also performed using MTT assay concurrent with immunostaining.

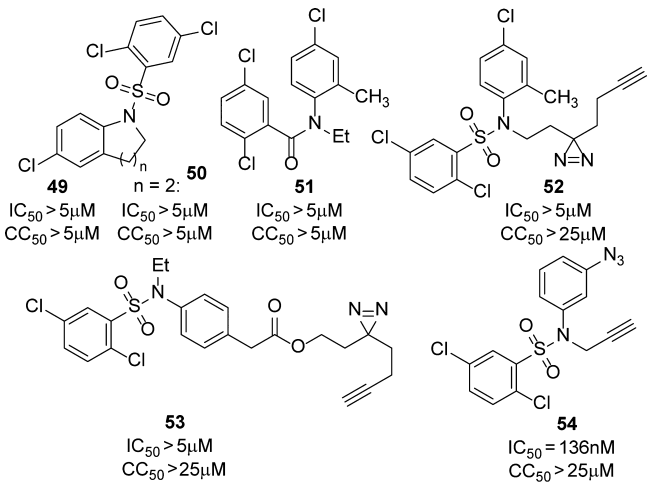


Figure 6. Modified scaffolds and probes for target identification. A549 cells were treated with various concentrations of compound **1** and its analogues before infection. After 2 h of compound treatment, cells were infected with WSN-PB2-Nanoluc virus at MOI 0.02 for 24 h and then harvested in Renilla lysis buffer. Luminescence was measured for equal volume of lysates using the Nano-Glo substrate, and IC_{50} was determined. CC_{50} was determined using CellTiter-Glo.

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smRNA-FISH. smRNA-FISH was performed, as previously described,[30] which includes the sequences of M1 and NS1 probes except for the HA probes that are listed below. Briefly, cells were grown on glass coverslips (Fisherbrand, Fisher Scientific) coated with 1 mL of 0.1% gelatin (Sigma-Aldrich). Cells were fixed with 4% paraformaldehyde (PFA, Electron Microscopy Sciences) in PBS for 15 min before incubation in 70% ethanol for 12 h at 4 °C. Coverslips were then placed in wash buffer for 5 min, containing nuclease free water, 2× SSC Buffer (Sigma), and 10% formamide (Sigma). The coverslips were then removed and incubated in hybridization buffer containing the FISH probe. Hybridization occurred at 37 °C for 4 h, then cells were washed with wash buffer for 30 min at 37 °C. Coverslips were then washed twice for 5 min in PBS and stained with 1 μg/mL Hoechst 33258 (Molecular Probes/Life Technologies) for 10 min. Coverslips were briefly washed with PBS before mounting in the ProLong Gold antifade reagent (Life Technologies).

RNA Purification and RT-qPCR. Total RNA was isolated from A549 cells using the RNeasy Plus Mini kit (Qiagen) and reverse transcribed into cDNA by SuperScript II reverse transcriptase (Invitrogen), each according to the manufacturers' protocols. Samples were then amplified in a LightCycler 480 qPCR system (Roche) using SYBR Green I (Roche) and sequence-specific primers.

RT-PCR primer sequences:
M1 Forward-ATCAGACATGAGAACAGAATGG.
Reverse-TGCCTGGCCTGACTAGCAATATC.
M2 Forward: CGAGGTCGAAACGCCTATCAGAAAC.
Reverse: CCAATGATATTTGCTGCAATGACGAG.
18S Forward: GTAACCCGTTGAACCCCATT.
Reverse: CCATCCAATCGGTAGTAGCG.

Selection of Compound 1-Resistant Influenza Viruses. The concentration of compound **1** required for maximum virus inhibition (three logs), while maintaining enough virus production for subsequent passages, was determined (0.5 μM compound **1**). A549 cells were infected with WSN at an MOI of 0.01 for 24 h at 37 °C under compound **1** treatment. The supernatant was then collected and titered by plaque assay. If the recovered compound **1**-treated virus did not show increased viral titer similar to that of the DMSO-treated control, the virus was passaged again by using the same method. Once increased titers in the presence of compound **1** were detected for two consecutive passages, the viruses were plaque purified. Following plaque purification, all eight genome segments were sequenced using the libraries prepared with the NEBNext Ultra II RNA Library Prep Kit for Illumina (New England BioLabs) and sequenced on a NextSeq 550 system (Illumina). Sequencing from the DMSO-passaged control virus was used to generate the FASTA file to which all compound **1**-treated samples were compared using the Integrative Genomics Viewer (Version 2.5.0) to detect escape mutations.

Hemolysis Inhibition Assay. Fresh chicken erythrocytes (RBCs) were used in hemolysis inhibition assay, as previously described.[24]

Compounds. Compound **1** was initially purchased from ChemDiv (Y030-0868) and synthesized in-house.

Chemical Methods. *General.* All tested compounds have purity of >95%, as judged by HPLC analysis (UV detection at 210 nM). Chemical shifts δ are measured in parts per million, and spectra were referenced using the residual solvent peak. The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), double doublet (dd), quintet (quin), multiplet (m), and broad signal (bs). Mass spectra (m/z) were recorded on an Agilent LC−MS 1290 Infinity using ESI ionization. All chemicals were used as received unless otherwise noted. Compounds **1** and **10** were purchased from ChemDiv.

General Procedure for the Synthesis of N-Alkyl-N-aryl-sulfonamides. Step 1. Pyridine (87.0 mg, 3.3 mmol, 1.1 equiv) was added to a mixture of aryl sulfonyl chloride (3.0 mmol, 1.0 equiv) and substituted aniline (3.0 mmol, 1.0 equiv) in 9 mL of anhydrous dichloromethane (DCM). The mixture was sealed in a 20 mL scintillation vial and stirred at room temperature overnight. DCM and excess pyridine were removed under vacuum. The crude white powder was resuspended in 10 mL of DCM and washed with 5 mL of saturated ammonium chloride solution three times and then washed with 5 mL of distilled water. The DCM layer was dried over sodium sulfate, filtered, and concentrated. The residue was purified by filtration through a small plug of silica gel, eluting with DCM.

Step 2. The required alkyl bromide (0.30 mmol, 3.0 equiv) was added to a mixture of *N*-aryl sulfonamide from step 1 (0.10 mmol, 1.0 equiv) and potassium carbonate (16.6 mg, 0.12 mmol, 1.2 equiv) in 1 mL of acetone. The mixture was sealed in a 20 mL scintillation vial and stirred at 60 °C overnight. Acetone and excess bromide were removed under vacuum. The crude residue was resuspended in 10 mL of DCM and washed with 2 mL of saturated ammonium chloride solution three times and then washed with 2 mL of distilled water. The DCM layer was dried over sodium sulfate, filtered, and concentrated. The resulting residue was either loaded to a silica gel plug and eluted by DCM only or further purified by silica gel chromatography using DCM and hexane to afford the desired product.

*2,5-Dichloro-N-(o-tolyl)benzenesulfonamide (**2**).* 2,5-Dichloro-*N*-(*o*-tolyl)benzenesulfonamide (**2**) was prepared according to the general procedure using *o*-toluidine (0.43 mL, 4.0 mmol). Purification by automated flash chromatography in 100% DCM gave 1.19 g of the title compound in 93% yield. ^{1}H NMR (400 MHz, chloroform-*d*): δ 8.00 (dd, *J* = 1.8, 1.0 Hz, 1H), 7.50−7.44 (m, 2H), 7.19−7.13 (m, 1H), 7.09 (td, *J* = 5.7, 4.0 Hz, 3H), 6.79 (s, 1H), 2.30 (s, 3H). ESI-MS (m/z): 316.0 $[M + H]^+$.

*2,5-Dichloro-N-methyl-N-(o-tolyl)benzenesulfonamide (**3**).* 2,5-Dichloro-*N*-methyl-*N*-(*o*-tolyl)benzenesulfonamide (**3**) was prepared according to the general procedure from **2** (77.3 mg, 0.24 mmol) to give 77.1 mg of the title compound in 96% yield. ^{1}H NMR (400 MHz, chloroform-*d*): δ 7.82 (d, *J* = 2.5 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 1H), 7.44 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.26 (dd, *J* = 7.7, 1.9 Hz, 1H), 7.22 (td, *J* = 7.4, 1.3 Hz, 1H), 7.06 (td, *J* = 7.5, 1.9 Hz, 1H), 6.81 (dd, *J* = 7.9, 1.3 Hz, 1H), 3.40 (s, 3H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*): δ 139.2, 138.8, 138.7, 133.5, 133.2, 133.1, 132.3, 131.8, 130.4, 129.0, 128.8, 127.0, 40.4, 18.1. ESI-MS (m/z): 330.0 $[M + H]^+$.

*N-Allyl-2,5-dichloro-N-(o-tolyl)benzenesulfonamide (**4**).* *N*-Allyl-2,5-dichloro-*N*-(*o*-tolyl)benzenesulfonamide (**4**) was prepared according to the general procedure from **2** (74.7 mg, 0.24 mmol) to give 81.5 mg of the title compound in 97% yield. ^{1}H NMR (400 MHz, chloroform-*d*): δ 7.83 (d, *J* = 2.4 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.47 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.28−7.22 (m, 2H), 7.11 (ddd, *J* = 8.7, 6.1, 2.9 Hz, 1H), 6.92−6.87 (m, 1H), 5.90 (ddt, *J* = 17.0, 10.0, 6.9 Hz, 1H), 5.15−5.01 (m, 2H), 4.44 (d, *J* = 6.5 Hz, 2H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*): δ 139.4, 139.30, 136.4, 133.5, 133.1, 133.1, 132.8, 132.3, 131.6, 130.7, 130.3, 128.9, 126.5, 119.9, 56.0, 18.3. ESI-MS (m/z): 356.0 $[M + H]^+$.

*2,5-Dichloro-N-(prop-2-yn-1-yl)-N-(o-tolyl)benzenesulfonamide (**5**).* 2,5-Dichloro-*N*-(prop-2-yn-1-yl)-*N*-(*o*-tolyl)benzenesulfonamide (**5**) was prepared according to the general procedure from **2** (75 mg, 0.24 mmol) to give 80.5 mg of the title compound in 94% yield. ^{1}H NMR (400 MHz, chloroform-*d*): δ 7.85 (d, *J* = 2.4 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.46 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.30−7.25 (m, 2H), 7.12 (dt, *J* = 8.8, 4.3 Hz, 1H), 7.05−7.00 (m, 1H), 4.77 (s, 1H), 4.51 (s, 1H), 2.31 (s, 3H), 2.26 (t, *J* = 2.5 Hz, 1H). ^{13}C NMR (101 MHz, chloroform-*d*): δ 139.38, 139.01, 136.2, 133.8, 133.2, 133.1, 132.3, 131.7, 130.7, 130.5, 129.5, 126.7, 78.1, 74.1, 42.5, 18.1. ESI-MS (m/z): 354.0 $[M + H]^+$.

*2,5-Dichloro-N-isopropyl-N-(o-tolyl)benzenesulfonamide (**6**).* 2,5-Dichloro-*N*-isopropyl-*N*-(*o*-tolyl)benzenesulfonamide (**6**) was prepared according to the general procedure from **2** (75 mg, 0.24 mmol). After overnight stirring, the conversion to the desired product was slow when monitored by LC−MS. The reaction was condensed, and acetonitrile (2.4 mL) and K_2CO_3 (42.2 mg, 0.31 mmol) were added, and the reaction was heated to 80 °C overnight and went to completion. The crude reaction mixture was condensed to dryness and purified by automated flash chromatography in 100% DCM to give 73.8 mg of the title compound in 87% yield. ^{1}H NMR (400 MHz, chloroform-*d*): δ 7.75 (d, *J* = 2.4 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.39 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.27−7.20 (m, 2H), 7.13−7.06 (m,

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1H), 6.95−6.90 (m, 1H), 4.84 (p, J = 6.7 Hz, 1H), 2.15 (s, 3H), 1.12 (dd, J = 14.1, 6.7 Hz, 6H). ^{13}C NMR (101 MHz, chloroform-d): δ 141.0, 140.1, 133.34, 133.31, 133.13, 133.07, 132.96, 132.1, 131.7, 130.3, 129.1, 126.0, 53.5, 22.8, 21.7, 18.9. ESI-MS (m/z): 358.0 $[M + H]^+$.

*2,5-Dichloro-N-ethyl-N-phenylbenzenesulfonamide (**7**).* Step 1. 2,5-Dichloro-*N*-phenylbenzenesulfonamide was prepared according to the general procedure using aniline (0.37 mL, 4.1 mmol). The title compound was isolated from flash chromatography in 100% DCM to give 1.17 g in 94% yield. ^{1}H NMR (400 MHz, chloroform-d): δ 7.98 (dd, J = 1.9, 1.0 Hz, 1H), 7.43 (d, J = 1.8 Hz, 2H), 7.26 (dd, J = 8.8, 6.9 Hz, 2H), 7.18−7.10 (m, 3H), 7.04 (s, 1H). ESI-MS (m/z): 300.0 $[M-H]^-$.

Step 2. Compound 7 was prepared according to the general procedure using the sulfonamide from step 1 (76.1 mg, 0.25 mmol) to give 81.1 mg of the title compound in 98% yield. ^{1}H NMR (400 MHz, chloroform-d): δ 7.78 (d, J = 2.4 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.36 (dd, J = 8.5, 2.4 Hz, 1H), 7.31−7.22 (m, 3H), 7.19−7.13 (m, 2H), 3.88 (q, J = 7.1 Hz, 2H), 1.13 (dd, J = 7.5, 6.7 Hz, 3H). ^{13}C NMR (101 MHz, chloroform-d): δ 138.6, 137.7, 133.53, 133.01, 132.95, 132.3, 130.4, 129.5, 129.4, 128.4, 47.7, 14.8. ESI-MS (m/z): 330.0 $[M + H]^+$.

*2,5-Dichloro-N-(2-chlorophenyl)-N-ethylbenzenesulfonamide (**8**).* 2,5-Dichloro-*N*-(2-chlorophenyl)-*N*-ethylbenzenesulfonamide (**8**) was prepared according to the general procedure to give 78.2 mg of the title compound in 95% yield. ^{1}H NMR (400 MHz, chloroform-d): δ 7.71−7.66 (m, 1H), 7.36−7.22 (m, 4H), 7.21−7.14 (m, 2H), 3.85 (d, J = 171.6 Hz, 2H), 1.05 (td, J = 7.1, 1.2 Hz, 3H). ^{13}C NMR (101 MHz, chloroform-d): δ 139.7, 134.9, 134.54, 134.46, 133.45, 133.1, 132.8, 131.7, 130.8, 130.8, 130.2, 127.5, 47.0, 14.6. ESI-MS (m/z): 364.0 $[M + H]^+$.

*2,5-Dichloro-N-(2-cyanophenyl)-N-ethylbenzenesulfonamide (**9**).* Step 1. 2,5-Dichlorobenzenesulfonyl chloride (1.0 g, 4.1 mmol) was slowly added to a solution of 2-aminobenzonitrile (0.484 g, 4.1 mmol) in pyridine (14 mL) with a catalytic amount of dimethylaminopyridine. The reaction was heated to 60 °C overnight. The cooled reaction mixture was diluted with Et_2O and washed several times with water and then brine. The organic layer was dried over Na_2SO_4, filtered, and condensed. The crude mixture was purified by automated flash chromatography in 100% DCM to give 345.8 mg of 2,5-dichloro-*N*-(2-cyanophenyl)benzenesulfonamide in 47% yield. ^{1}H NMR (400 MHz, chloroform-d): δ 8.10−8.05 (m, 1H), 7.62 (dt, J = 8.1, 1.1 Hz, 1H), 7.59−7.44 (m, 5H), 7.19 (td, J = 7.6, 1.1 Hz, 1H). ESI-MS (m/z): 325.0 $[M-H]^-$.

Step 2. Compound **9** was prepared following the general procedure using 2,5-dichloro-*N*-(2-cyanophenyl)benzenesulfonamide (75.0 mg, 0.23 mmol) to give 48.0 mg of the title compound in 59% yield. ^{1}H NMR (400 MHz, chloroform-d): δ 7.82 (d, J = 2.3 Hz, 1H), 7.68−7.60 (m, 2H), 7.51−7.42 (m, 4H), 3.98 (q, J = 7.1 Hz, 2H), 1.23−1.14 (m, 3H). ^{13}C NMR (101 MHz, chloroform-d): δ 140.3, 138.5, 134.2, 134.0, 133.71, 133.66, 133.2, 133.13, 133.09, 131.7, 131.0, 129.3, 115.9, 114.5, 48.0, 14.6. ESI-MS (m/z): 355.0 $[M + H]^+$.

*2,5-Dichloro-N-ethyl-N-(p-tolyl)benzenesulfonamide (**11**).* Step 1. 2,5-Dichloro-*N*-(*p*-tolyl)benzenesulfonamide was prepared according to the general procedure using *p*-toluidine (111.9 mg, 1.0 mmol). The crude product was further purified by trituration in DCM and hexane to afford 286 mg of white solid as the product, yield 90%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.93 (d, J = 2.1 Hz, 1H), 7.46−7.37 (m, 2H), 7.06−6.93 (m, 5H), 2.25 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 137.6, 136.4, 133.9, 133.5, 132.5, 132.4, 131.7, 130.1, 129.4, 122.6, 20.9. ESI-MS m/z = 314.0 $[M-H]^+$.

Step 2. Compound **11** was prepared from the sulfonamide from step 1 (32.0 mg, 0.10 mmol) following the general procedure to provide 33.7 mg of yellow oil as the product, yield 97%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.81 (d, J = 2.5 Hz, 1H), 7.46−7.34 (m, 2H), 7.07 (q, J = 8.4 Hz, 4H), 3.87 (q, J = 7.1 Hz, 2H), 2.31 (s, 3H), 1.14 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 138.7, 138.4, 134.8, 133.4, 132.9, 132.8, 132.2, 130.3, 130.0, 129.3, 47.7, 21.1, 14.7. ESI-MS m/z = 344.0 $[M + H]^+$.

*2,5-Dichloro-N-(4-chloro-2-methylphenyl)-N-ethylbenzenesulfonamide (**12**).* 2,5-Dichloro-*N*-(4-chloro-2-methylphenyl)-*N*-ethylbenzenesulfonamide (**12**) was prepared according to the general procedure as an oil, 78.1 mg; 92% yield. ^{1}H NMR (400 MHz, chloroform-d): δ 7.78 (d, J = 2.3 Hz, 1H), 7.49−7.40 (m, 2H), 7.23 (d, J = 2.5 Hz, 1H), 7.07 (dd, J = 8.5, 2.5 Hz, 1H), 6.82 (d, J = 8.5 Hz, 1H), 3.82 (dp, J = 20.7, 6.9 Hz, 2H), 2.23 (s, 3H), 1.10 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, chloroform-d): δ 141.5, 139.0, 134.9, 134.5, 133.6, 133.3, 133.2, 132.2, 131.7, 131.5, 130.3, 126.9, 47.9, 18.2, 14.3. ESI-MS (m/z): 378.0 $[M + H]^+$.

*N-Allyl-2,5-dichloro-N-(4-chloro-2-methylphenyl)-benzenesulfonamide (**13**).* *N*-Allyl-2,5-dichloro-*N*-(4-chloro-2-methylphenyl)benzenesulfonamide (**13**) was prepared according to the general procedure as an oil, 79.3 mg; 93% yield. ^{1}H NMR (400 MHz, chloroform-d): δ 7.79 (d, J = 2.3 Hz, 1H), 7.50−7.42 (m, 2H), 7.21 (d, J = 2.5 Hz, 1H), 7.04 (dd, J = 8.5, 2.5 Hz, 1H), 6.80 (d, J = 8.5 Hz, 1H), 5.82 (ddt, J = 17.0, 10.0, 6.9 Hz, 1H), 5.09−4.95 (m, 2H), 4.35 (d, J = 6.7 Hz, 2H), 2.21 (s, 3H). ^{13}C NMR (101 MHz, chloroform-d): δ 141.3, 139.1, 135.0, 134.6, 133.8, 133.3, 133.2, 132.4, 132.3, 132.0, 131.5, 130.3, 126.8, 120.4, 56.0, 18.9. ESI-MS (m/z): 390.0 $[M + H]^+$.

*2,5-Dichloro-N-(4-chloro-2-methylphenyl)-N-(prop-2-yn-1-yl)-benzenesulfonamide (**14**).* 2,5-Dichloro-*N*-(4-chloro-2-methylphenyl)-*N*-(prop-2-yn-1-yl)benzenesulfonamide (**14**) was prepared according to the general procedure as an oil, 51.2 mg in 61% yield. ^{1}H NMR (400 MHz, chloroform-d): δ 7.83 (d, J = 2.1 Hz, 1H), 7.52−7.43 (m, 2H), 7.25 (d, J = 2.5 Hz, 1H), 7.08 (dd, J = 8.5, 2.5 Hz, 1H), 6.96 (d, J = 8.5 Hz, 1H), 4.85−4.33 (m, 2H), 2.25 (d, J = 2.7 Hz, 4H). ^{13}C NMR (101 MHz, chloroform-d): δ 141.4, 138.8, 135.3, 134.8, 134.0, 133.3, 133.2, 132.3, 132.0, 131.6, 130.5, 127.0, 77.8, 74.4, 42.5, 18.1. ESI-MS (m/z): 388.0 $[M + H]^+$.

*2,5-Dichloro-N-(4-chloro-2-methylphenyl)-N-(cyclopropylmethyl)benzenesulfonamide (**15**).* 2,5-Dichloro-*N*-(4-chloro-2-methylphenyl)-*N*-(cyclopropylmethyl)benzenesulfonamide (**15**) was prepared according to the general procedure to afford 38.8 mg of colorless oil as the product, yield 95%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.78 (d, J = 2.3 Hz, 1H), 7.46 (d, J = 8.6 Hz, 1H), 7.42 (ddd, J = 8.5, 2.4, 1.0 Hz, 1H), 7.23 (d, J = 2.5 Hz, 1H), 7.05 (dd, J = 8.5, 2.5 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 3.69 (dd, J = 14.0, 7.5 Hz, 1H), 3.57 (dd, J = 14.1, 7.0 Hz, 1H), 2.27 (s, 3H), 0.91 (ddd, J = 17.2, 7.9, 3.9 Hz, 1H), 0.44 (q, J = 7.3, 6.7 Hz, 2H), 0.10 (ddd, J = 39.0, 9.3, 4.4 Hz, 2H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 141.5, 139.0, 135.5, 134.4, 133.5, 133.1, 133.0, 132.2, 131.7, 131.3, 130.1, 126.7, 58.2, 18.1, 10.4, 4.2, 4.0. ESI-MS m/z = 404.0 $[M + H]^+$.

*2,5-Dichloro-N-(4-chloro-2-methylphenyl)-N-(2-methoxyethyl)-benzenesulfonamide (**16**).* 2,5-Dichloro-*N*-(4-chloro-2-methylphenyl)-*N*-(2-methoxyethyl)benzenesulfonamide (**16**) was prepared according to the general procedure to afford 34.0 mg of colorless oil as the product, yield 82%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.82 (t, J = 1.8 Hz, 1H), 7.49−7.39 (m, 2H), 7.22 (t, J = 1.7 Hz, 1H), 7.06 (dd, J = 8.7, 2.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 3.94 (tq, J = 20.2, 8.4, 6.9 Hz, 2H), 3.40 (td, J = 5.7, 1.4 Hz, 2H), 3.27 (d, J = 1.4 Hz, 3H), 2.24 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 141.4, 140.5, 138.8, 135.2, 134.5, 133.5, 133.1, 132.2, 131.6, 131.4, 130.2, 126.8, 69.8, 58.6, 52.0, 17.9. ESI-MS m/z = 408.0 $[M + H]^+$.

*2,5-Dichloro-N-(4-chloro-2-methylphenyl)-N-(2-hydroxyethyl)-benzenesulfonamide (**17**).* 2,5-Dichloro-*N*-(4-chloro-2-methylphenyl)-*N*-(2-hydroxyethyl)benzenesulfonamide (**17**) was prepared according to the general procedure to afford 34.0 mg of colorless oil as the product, yield 59%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.81 (d, J = 2.3 Hz, 1H), 7.51−7.41 (m, 2H), 7.22 (d, J = 2.6 Hz, 1H), 7.10 (dd, J = 8.5, 2.5 Hz, 1H), 6.99 (d, J = 8.5 Hz, 1H), 4.52 (s, 1H), 3.92 (dt, J = 8.2, 5.6 Hz, 2H), 3.67 (q, J = 5.5 Hz, 2H), 2.21 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 140.9, 138.7, 135.0, 134.7, 133.7, 133.2, 133.1, 132.2, 132.0, 131.5, 130.1, 127.0, 60.1, 54.9, 17.9. ESI-MS m/z = 808.9 $[2M + Na]^+$.

*2,5-Dichloro-N-(4-chloro-2-methylphenyl)-N-(2-fluoroethyl)-benzenesulfonamide (**18**).* To a stirring solution of **17** in 1 mL of DCM cooled to 0 °C under nitrogen, Morph-Dast (48.8 μL, 0.40 mmol, 4.0 equiv) was added dropwise. The reaction mixture was

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warmed to room temperature and allowed to stir overnight until the starting material was consumed. Upon completion of the reaction, Morph-Dast was quenched slowly by saturated sodium bicarbonate solution. The reaction mixture was diluted with water and extracted with DCM. The combined organics were washed with brine, dried over sodium sulfate, and then concentrated. The crude product was purified by silica gel chromatography using 50% of DCM in hexane to afford 19.5 mg of colorless oil as the product, yield 49%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.78 (d, J = 2.2 Hz, 1H), 7.51−7.41 (m, 2H), 7.24 (d, J = 2.4 Hz, 1H), 7.07 (dd, J = 8.5, 2.4 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 4.58−4.38 (m, 2H), 4.18−3.99 (m, 2H), 2.25 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 141.3, 138.5, 134.94, 134.86, 133.8, 133.2, 133.1, 132.3, 131.61, 131.60, 130.2, 127.0, 80.97 (d, J = 173.1 Hz), 52.83 (d, J = 21.4 Hz), 17.91 (d, J = 1.7 Hz). ESI-MS m/z = 430.0 $[M + Cl]^-$.

*2,5-Dichloro-N-(4-chloro-2-methylphenyl)-N-(difluoromethyl)-benzenesulfonamide (**19**).* A well-stirred slurry of 2,5-dichloro-*N*-(4-chloro-2-methylphenyl)benzenesulfonamide (40.9 mg, 0.12 mmol, 1.0 equiv), potassium carbonate (51.3 mg, 0.35 mmol, 2.9 equiv), and sodium chlorodifluoroacetate (53.5 mg, 0.35 mmol, 2.9 equiv) in 2 mL of acetonitrile was heated to 60 °C for 72 h. The resulting slurry was then concentrated in vacuo, diluted with 2 mL of DCM and 2 mL of water. The organic phase was separated, dried, and concentrated in vacuo. The crude mixture was further purified by silica gel chromatography using DCM to afford 41.6 mg of white solid as the product, yield 89%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.75 (t, J = 1.4 Hz, 1H), 7.58−7.39 (m, 3H), 7.30 (d, J = 2.5 Hz, 1H), 7.06 (dd, J = 8.4, 2.5 Hz, 1H), 6.69 (dd, J = 8.5, 1.6 Hz, 1H), 2.23 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 143.1, 136.6, 136.6, 134.9, 133.5, 133.5, 133.4, 132.5, 131.8, 130.4, 127.3, 126.7, 111.3 (dd, J = 252.1, 246.9 Hz), 18.1 (d, J = 3.3 Hz). ESI-MS: compound did not ionize.

*2,5-Dichloro-N-(4-chloro-2-fluorophenyl)-N-ethylbenzenesulfonamide (**20**).* Step 1. 2,5-Dichloro-*N*-(4-chloro-2-fluorophenyl)-benzenesulfonamide was prepared according to the general procedure using 4-chloro-2-fluoroaniline (33.6 mg, 0.20 mmol, 1.0 equiv), yield 97%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.97 (d, J = 2.5 Hz, 1H), 7.51−7.41 (m, 3H), 7.22 (s, 1H), 7.07 (dt, J = 8.6, 1.8 Hz, 1H), 7.03 (dd, J = 9.9, 2.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 153.8 (d, J = 249.3 Hz), 137.3, 134.4, 133.4, 132.9, 131.6 (d, J = 9.6 Hz), 131.4, 130.0, 125.3 (d, J = 3.8 Hz), 124.1, 122.3 (d, J = 12.5 Hz), 116.6 (d, J = 22.8 Hz). ESI-MS m/z = 352.0 $[M-H]^+$.

Step 2. The title compound was prepared according to the general procedure to afford 38.1 mg of colorless oil as the product, yield 98%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.86 (t, J = 2.1 Hz, 1H), 7.49−7.39 (m, 2H), 7.29 (t, J = 8.3 Hz, 1H), 7.14 (d, J = 8.6 Hz, 1H), 7.08 (dd, J = 10.0, 2.1 Hz, 1H), 3.82 (q, J = 7.1 Hz, 2H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 159.7 (d, J = 256.3 Hz), 138.6, 135.6 (d, J = 9.9 Hz), 134.1 (d, J = 1.4 Hz), 133.7, 133.1, 132.9, 131.8, 130.6, 125.1 (d, J = 3.7 Hz), 123.6 (d, J = 12.1 Hz), 117.5 (d, J = 23.6 Hz), 46.8 (d, J = 2.5 Hz), 14.4. ESI-MS m/z = 382.0 $[M + H]^+$.

*2,5-Dichloro-N-(2,4-difluorophenyl)-N-ethylbenzenesulfonamide (**21**).* Step 1. 2,5-Dichloro-*N*-(2,4-difluorophenyl)benzenesulfonamide was prepared according to the general procedure using 2,4-difluoroaniline (244 mg, 1.0 mmol, 1.0 equiv) as the aniline, yield 98%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.92 (t, J = 1.5 Hz, 1H), 7.49 (td, J = 9.0, 5.7 Hz, 1H), 7.45 (d, J = 1.5 Hz, 2H), 7.15 (s, br, 1H), 6.83 (dddd, J = 9.2, 7.8, 2.9, 1.6 Hz, 1H), 6.75 (ddd, J = 10.7, 8.2, 2.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 160.5 (dd, J = 249.4, 11.2 Hz), 154.9 (dd, J = 249.1, 12.3 Hz), 137.5, 134.3, 133.4, 132.8, 131.3, 130.1, 126.0 (dd, J = 9.7, 1.7 Hz), 119.5 (dd, J = 12.9, 3.9 Hz), 112.0 (dd, J = 22.4, 3.9 Hz), 104.4 (dd, J = 26.8, 23.4 Hz). ESI-MS m/z = 336.0 $[M-H]^+$.

Step 2. The title compound was prepared according to the general procedure to afford 36.0 mg of colorless oil as the product, yield 99%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.84 (d, J = 2.2 Hz, 1H), 7.50−7.39 (m, 2H), 7.32 (q, J = 7.9, 7.3 Hz, 1H), 6.88 (t, J = 8.0 Hz, 1H), 6.79 (t, J = 9.5 Hz, 1H), 3.83 (q, J = 6.0, 4.9 Hz, 2H), 1.14 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 162.7 (dd, J = 252.1, 11.5 Hz), 160.2 (dd, J = 255.5, 12.5 Hz), 138.6, 134.4 (d, J = 10.0 Hz), 133.6, 133.1, 132.9, 131.8, 130.6, 121.1 (d, J = 16.2 Hz), 111.9 (dd, J = 22.3, 3.7 Hz), 105.0 (dd, J = 26.4, 24.0 Hz), 46.9 (d, J = 2.4 Hz), 14.4. ESI-MS m/z = 366.0 $[M + H]^+$.

*2,5-Dichloro-N-(2,4-dichloro-6-methylphenyl)-N-ethylbenzenesulfonamide (**22**).* Step 1. 2,5-Dichloro-*N*-(2,4-dichloro-6-methylphenyl)benzenesulfonamide was prepared according to the general procedure using 2,4-dichloro-6-methylaniline (177.1 mg, 1.0 mmol, 1.0 equiv) as the aniline. 4-(Dimethylamino)pyridine was added in the catalytic amount (3.40 mg, 0.024 mmol, 0.024 equiv), and the reaction mixture was heated to 60 °C for 9 days. The crude was further purified by silica gel chromatography using 100% of DCM in hexane to afford 246 mg of white solid as the product, yield 65%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.87 (t, J = 1.4 Hz, 1H), 7.48 (d, J = 1.4 Hz, 2H), 7.18 (ddd, J = 8.8, 2.5, 0.8 Hz, 2H), 6.73 (s, br, 1H), 2.44 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 142.9, 140.1, 134.3, 133.9, 133.8, 133.3, 132.6, 130.4, 130.2, 130.0, 129.7, 127.2, 19.8. ESI-MS m/z = 382.0 $[M-H]^+$.

Step 2. The title compound was prepared according to the general procedure to afford 39.1 mg of colorless oil as the product with quantitative yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.87 (d, J = 1.9 Hz, 1H), 7.43 (s, 1H), 7.21 (d, J = 4.9 Hz, 1H), 4.14 (dq, J = 14.4, 7.3 Hz, 1H), 3.73 (dq, J = 14.1, 7.1 Hz, 1H), 2.43 (s, 3H), 1.14 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 144.5, 140.2, 135.9, 134.7, 133.4, 133.1, 133.0, 132.8, 131.5, 130.6, 130.1, 128.0, 46.6, 20.0, 14.3. ESI-MS m/z = 434.2 $[M + Na]^+$.

*2,5-Dichloro-N-(4-chloro-2,6-difluorophenyl)-N-ethylbenzenesulfonamide (**23**).* Step 1. 2,5-Dichloro-*N*-(4-chloro-2,6-difluorophenyl)benzenesulfonamide was prepared according to the general procedure using 4-chloro-2,6-difluoroaniline (249 mg, 1.0 mmol, 1.0 equiv) as the aniline. 4-(Dimethylamino)pyridine was added in the catalytic amount (3.40 mg, 0.024 mmol, 0.024 equiv), and the reaction mixture was heated to 70 °C for 7 days. The crude was further purified by silica gel chromatography using 50% of DCM in hexane to afford 203 mg of white flake as the product, yield 54%. ^{1}H NMR ($CDCl_3$, 101 MHz): δ ppm 7.93 (t, J = 1.5 Hz, 1H), 7.56−7.46 (m, 2H), 7.01−6.91 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 158.7 (t, J = 255.7, 5.1 Hz), 138.6, 134.7 (t, J = 12.5 Hz), 134.1, 133.2, 132.7, 130.6, 130.2, 113.5−113.1 (m), 111.3 (t, J = 16.5 Hz). ESI-MS m/z = 370.0 $[M-H]^+$.

Step 2. The title compound was prepared according to the general procedure to afford 21.0 mg of colorless oil as the product, yield 98%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.93 (d, J = 2.1 Hz, 1H), 7.52−7.41 (m, 2H), 6.99 (d, J = 7.6 Hz, 2H), 3.78 (q, J = 7.2 Hz, 2H), 1.15 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 162.2 (d, J = 5.4 Hz), 159.7 (d, J = 5.4 Hz), 137.4 (d, J = 282.5 Hz), 136.0 (d, J = 25.6 Hz), 133.7, 133.2, 132.9, 131.5, 130.6, 113.5 (dd, J = 24.6, 3.4 Hz), 46.6, 14.1. ESI-MS m/z = 421.9 $[M + Na]^+$.

*Ethyl 2-(4-((2,5-Dichloro-N-ethylphenyl)sulfonamido)phenyl)-acetate (**24**).* Step 1. Ethyl 2-(4-((2,5-dichlorophenyl)sulfonamido)-phenyl)acetate was prepared according to the general procedure using 2,5-dichlorobenzenesulfonyl chloride (248 mg, 3.0 mmol, 1.0 equiv) as sulfonyl chloride and ethyl (4-aminophenyl)acetate (540.4 mg, 3.0 mmol, 1.0 equiv) as the aniline, yield 95%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.97 (t, J = 1.4 Hz, 1H), 7.42 (d, J = 1.4 Hz, 2H), 7.22 (s, br, 1H), 7.15 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 8.5 Hz, 2H), 4.12 (q, J = 7.2 Hz, 2H), 3.52 (s, 2H), 1.22 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 171.4, 137.6, 134.2, 134.0, 133.5, 132.6, 132.0, 131.7, 130.4, 129.5, 121.9, 61.0, 40.6, 14.1. ESI-MS m/z = 386.1 $[M-H]^+$.

Step 2. The title compound was prepared according to the general procedure to afford 809 mg of yellow oil as the product, yield 98%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.81 (d, J = 2.4 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.38 (dd, J = 8.5, 2.4 Hz, 1H), 7.21 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 4.13 (q, J = 7.1 Hz, 2H), 3.87 (q, J = 7.1 Hz, 2H), 3.56 (s, 2H), 1.22 (t, J = 7.1 Hz, 3H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 171.1, 138.5, 136.5, 134.4, 133.4, 132.9, 132.8, 132.2, 130.3, 130.2, 129.4, 61.0, 47.6, 40.9, 14.7, 14.1. ESI-MS m/z = 416.1 $[M + H]^+$.

*2-(4-((2,5-Dichloro-N-ethylphenyl)sulfonamido)phenyl)acetic Acid (**25**).* Lithium hydroxide monohydrate (25.6 mg, 1.0 mmol, 5.0

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equiv) was added to compound 23 (85.3 mg, 0.2 mmol, 1.0 equiv) in a mixed solvent of 1.2 mL of tetrahydrofuran, 0.8 mL of methanol, and 0.4 mL of distilled water. The mixture was stirred at room temperature overnight. The reaction was quenched by adding 1 M HCl after TLC showed complete disappearance of starting material. The crude material was concentrated in vacuo and further purified by silica gel chromatography using 5% of methanol in DCM to afford 71.5 mg of white solid as the product, yield 90%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.80 (d, J = 2.4 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.37 (dd, J = 8.5, 2.4 Hz, 1H), 7.21 (d, J = 8.4 Hz, 2H), 7.14 (d, J = 8.4 Hz, 2H), 3.86 (q, J = 7.1 Hz, 2H), 3.60 (s, 2H), 1.13 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 177.2, 138.5, 136.9, 133.5, 133.5, 133.0, 132.9, 132.2, 130.4, 130.4, 129.5, 47.6, 40.5, 14.7. ESI-MS m/z = 388.0 [M + H$^+$].

*2,5-Dichloro-N-ethyl-N-(4-(2-hydroxyethyl)phenyl)-benzenesulfonamide (**26**).* Ethyl 2-(4-((2,5-dichloro-*N*-ethylphenyl)-sulfonamido)phenyl)acetate (**24**, 160 mg, 0.4 mmol, 1.0 equiv) in 2.4 mL of tetrahydrofuran was treated dropwise with 2.0 M lithium aluminum hydride in tetrahydrofuran (0.2 mL, 0.4 mmol, 1.0 equiv). After stirring for 2 h at room temperature, the reaction mixture was carefully quenched by 17.3 μL of water, 17.3 μL of 15% NaOH, and then 52.0 μL of water. The mixture was stirred for 20 min and filtered through a plug of celite to remove the solids. The celite plug was rinsed repeatedly with ethyl acetate. The crude mixture was concentrated in vacuo and further purified by silica gel chromatography using 5% of acetone in DCM to afford 91.9 mg of white solid as the product, yield 42%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.80 (d, J = 2.4 Hz, 1H), 7.46−7.34 (m, 2H), 7.15 (d, J = 8.4 Hz, 2H), 7.10 (d, J = 8.5 Hz, 2H), 3.85 (q, J = 6.9 Hz, 2H), 3.82−3.76 (m, 2H), 2.81 (t, J = 6.6 Hz, 2H), 1.13 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 139.1, 138.5, 135.8, 133.4, 132.9, 132.9, 132.2, 130.3, 129.9, 129.5, 63.3, 47.6, 38.7, 14.7. ESI-MS m/z = 374.0 [M + H]$^+$.

*N-(4-(2-Aminoethyl)phenyl)-2,5-dichloro-N-ethylbenzenesulfonamide (**27**).* Step 1. To a mixture of alcohol **26** (37.2 mg, 0.10 mmol, 1.0 equiv) in 2.6 mL of tetrahydrofuran were added diethyl azodicarboxylate (38.8 mg, 0.20 mmol, 2.0 equiv), triphenylphosphine (54.7 mg, 0.20 mmol, 2.0 equiv), and diphenylphosphoryl azide (61.4 mg, 0.20 mmol, 2.0 equiv). The mixture was stirred at room temperature for 5 h. After removal of solvent, the residue was purified by silica gel chromatography using 50% DCM in hexane to afford 22.0 mg of *N*-(4-(2-azidoethyl)phenyl)-2,5-dichloro-*N*-ethylbenzenesulfonamide as a colorless oil, yield 55%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.81 (s, 1H), 7.48−7.34 (m, 2H), 7.15 (q, J = 8.5, 8.0 Hz, 4H), 3.88 (q, J = 7.1 Hz, 2H), 3.48 (t, J = 7.1 Hz, 2H), 2.86 (t, J = 7.2 Hz, 2H), 1.15 (td, J = 7.1, 1.5 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 138.5, 138.4, 136.2, 133.4, 132.9, 132.8, 132.3, 130.3, 129.7, 129.6, 52.1, 47.6, 34.9, 14.7. ESI-MS m/z = 399.0 [M + H]$^+$.

Step 2. To a solution of the azide from step 1 (8.40 mg, 0.020 mmol, 1.0 equiv) in 0.3 mL of methanol was added 10% Pd−C (0.6 mg). The resulting mixture was stirred for 4 h under a hydrogen atmosphere. The mixture was filtered through a pad of celite, and the filtrate was concentrated. The residue was purified by silica gel chromatography using 15% methanol in chloroform to afford 3.7 mg of colorless oil as the product, yield 47%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.81 (d, J = 2.4 Hz, 1H), 7.47−7.35 (m, 2H), 7.18−7.06 (m, 4H), 3.87 (q, J = 7.1 Hz, 2H), 2.94 (t, J = 6.9 Hz, 2H), 2.71 (t, J = 6.9 Hz, 2H), 1.15 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 140.2, 138.6, 135.6, 133.4, 132.9, 132.8, 132.2, 130.3, 129.7, 129.5, 47.6, 43.2, 39.5, 14.7. ESI-MS m/z = 373.1 [M + H]$^+$.

*N-(4-((2,5-Dichloro-N-ethylphenyl)sulfonamido)phenethyl)-acetamide (**28**).* Acetyl chloride (0.700 mg, 0.0088 mmol, 1.5 equiv) was added to a stirred solution of amine **27** (2.20 mg, 0.0059 mmol, 1.0 equiv) and solid sodium bicarbonate (2.20 mg, 0.0088 mmol, 1.5 equiv) in 0.75 mL of chloroform. The resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with DCM (1 mL) and water (2 mL) and stirred for 30 min. The aqueous and organic layers were partitioned, and the aqueous layer was extracted with DCM (2 × 1 mL). The combined organic layers were dried over sodium sulfate. The residue was purified by silica gel chromatography using 5% methanol in DCM to afford 1.2 mg of white solid as the product, yield 49%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.82 (d, J = 2.4 Hz, 1H), 7.49−7.36 (m, 2H), 7.12 (s, 4H), 5.42 (s, br, 1H), 3.87 (q, J = 7.1 Hz, 2H), 3.48 (q, J = 6.7 Hz, 2H), 2.79 (t, J = 7.1 Hz, 2H), 1.94 (s, 3H), 1.15 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 170.0, 139.2, 138.5, 136.0, 133.4, 132.9, 132.2, 130.4, 129.62, 129.60, 47.6, 40.4, 35.2, 23.3, 14.7. ESI-MS m/z = 415.1 [M + H]$^+$.

*2,5-Dichloro-N-ethyl-N-(4-(2-(4-methylpiperazin-1-yl)-2-oxoethyl)phenyl)benzenesulfonamide (**29**).* To a solution of acid **25** (9.50 mg, 0.025 mmol, 1.0 equiv) in 0.3 mL of dimethylformamide were added 1-methylpiperazine (2.80 mg, 0.028 mmol, 1.1 equiv), HATU (10.5 mg, 0.028 mmol, 1.1 equiv), and *N*,*N*-diisopropylethylamine (8.7 μL, 0.050 mmol, 2.0 equiv). The mixture was sealed in a LC−MS vial and stirred at room temperature for 2 h. The crude mixture was purified by silica gel chromatography using 5% methanol in chloroform to afford 6.7 mg of white solid as the product, yield 57%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.81 (d, J = 2.5 Hz, 1H), 7.48−7.36 (m, 2H), 7.16 (q, J = 8.5 Hz, 4H), 3.88 (q, J = 7.1 Hz, 2H), 3.69 (s, 2H), 3.64 (t, J = 5.1 Hz, 2H), 3.42 (t, J = 5.1 Hz, 2H), 2.35 (t, J = 5.1 Hz, 2H), 2.26 (s, 3H), 2.21 (t, J = 5.0 Hz, 2H), 1.15 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 168.9, 138.5, 136.3, 135.4, 133.4, 132.9, 132.9, 132.2, 130.4, 129.7, 129.6, 54.8, 54.5, 47.6, 45.9, 41.7, 40.3, 14.7. ESI-MS m/z = 470.1 [M + H]$^+$.

*2,5-Dichloro-N-ethyl-N-(4-(2-(4-methylpiperazin-1-yl)-2-oxoethyl)phenyl)benzenesulfonamide (**29**).* To a solution of acid 25 (9.50 mg, 0.025 mmol, 1.0 equiv) in 0.3 mL of dimethylformamide were added 1-methylpiperazine (2.80 mg, 0.028 mmol, 1.1 equiv), HATU (10.5 mg, 0.028 mmol, 1.1 equiv), and *N*,*N*-diisopropylethylamine (8.7 μL, 0.050 mmol, 2.0 equiv). The mixture was sealed in a LC−MS vial and stirred at room temperature for 2 h. The crude was purified by silica gel chromatography using 5% methanol in chloroform to afford 6.7 mg of white solid as the product, yield 57%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.81 (d, J = 2.5 Hz, 1H), 7.48−7.36 (m, 2H), 7.16 (q, J = 8.5 Hz, 4H), 3.88 (q, J = 7.1 Hz, 2H), 3.69 (s, 2H), 3.64 (t, J = 5.1 Hz, 2H), 3.42 (t, J = 5.1 Hz, 2H), 2.35 (t, J = 5.1 Hz, 2H), 2.26 (s, 3H), 2.21 (t, J = 5.0 Hz, 2H), 1.15 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 168.9, 138.5, 136.3, 135.4, 133.4, 132.9, 132.9, 132.2, 130.4, 129.7, 129.6, 54.8, 54.5, 47.6, 45.9, 41.7, 40.3, 14.7. ESI-MS m/z = 470.1 [M + H]$^+$.

*N-(2,4-Difluorophenyl)-N-ethylbenzenesulfonamide (**30**).* *N*-(2,4-Difluorophenyl)-*N*-ethylbenzenesulfonamide (**30**) was prepared according to the general procedure using benzenesulfonyl chloride (17.7 mg, 0.10 mmol, 1.0 equiv) and *N*-ethyl-2,4-difluoroaniline (23.6 mg with 70% purity, 0.10 mmol, 1.0 equiv) to afford 29.6 mg of colorless oil as the product, yield 79%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.73−7.66 (m, 2H), 7.63−7.54 (m, 1H), 7.52−7.43 (m, 2H), 7.20 (td, J = 8.6, 6.0 Hz, 1H), 6.86 (dddd, J = 9.0, 7.8, 2.9, 1.4 Hz, 1H), 6.79 (ddd, J = 10.3, 8.4, 2.8 Hz, 1H), 3.60 (q, J = 7.2 Hz, 2H), 1.08 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 162.4 (dd, J = 251.4, 11.4 Hz), 160.2 (dd, J = 255.3, 12.6 Hz), 139.0, 133.6 (dd, J = 10.2, 2.4 Hz), 132.8, 128.9, 127.4, 122.1 (dd, J = 12.1, 4.1 Hz), 111.7 (dd, J = 22.2, 3.8 Hz), 105.0 (dd, J = 26.2, 24.2 Hz), 45.4 (d, J = 2.7 Hz), 14.1. ESI-MS m/z = 298.1 [M + H]$^+$.

*2-Chloro-N-(2,4-difluorophenyl)-N-ethylbenzenesulfonamide (**31**).* 2-Chloro-*N*-(2,4-difluorophenyl)-*N*-ethylbenzenesulfonamide (**31**) was prepared according to the general procedure using 2-chloro-benzenesulfonyl chloride (21.1 mg, 0.10 mmol, 1.0 equiv) and *N*-ethyl-2,4-difluoroaniline (23.6 mg with 70% purity, 0.10 mmol, 1.0 equiv) to afford 29.3 mg of colorless oil as the product, yield 77%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.84 (d, J = 8.0 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 7.32−7.24 (m, 2H), 6.83 (t, J = 8.3 Hz, 1H), 6.76 (td, J = 9.5, 8.4, 2.8 Hz, 1H), 3.84 (q, J = 7.2 Hz, 2H), 1.13 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 162.5 (dd, J = 251.6, 11.4 Hz), 160.3 (dd, J = 255.6, 12.6 Hz), 137.2, 134.4 (dd, J = 10.3, 2.2 Hz), 133.7, 132.3, 132.1 (d, J = 13.3 Hz), 126.7, 121.4 (dd, J = 12.3, 4.2 Hz), 111.7 (dd, J = 22.2, 3.8 Hz), 104.9 (dd, J = 26.2, 24.1 Hz), 46.6 (d, J = 2.5 Hz), 14.4. ESI-MS m/z = 332.0 [M + H]$^+$.

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*3-Chloro-N-(2,4-difluorophenyl)-N-ethylbenzenesulfonamide (**32**).* 3-Chloro-*N*-(2,4-difluorophenyl)-*N*-ethylbenzenesulfonamide (**32**) was prepared according to the general procedure using 3-chlorobenzenesulfonyl chloride (21.1 mg, 0.10 mmol, 1.0 equiv) and *N*-ethyl-2,4-difluoroaniline (23.6 mg with 70% purity, 0.10 mmol, 1.0 equiv) as the aniline to afford 25.9 mg of colorless oil as the product, yield 70%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.69 (t, J = 1.9 Hz, 1H), 7.58−7.52 (m, 2H), 7.41 (t, J = 7.9 Hz, 1H), 7.22 (td, J = 8.7, 6.0 Hz, 1H), 6.88 (dddd, J = 9.0, 7.7, 2.8, 1.4 Hz, 1H), 6.81 (ddd, J = 10.4, 8.4, 2.8 Hz, 1H), 3.60 (q, J = 7.2 Hz, 2H), 1.09 (td, J = 7.1, 0.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 162.6 (dd, J = 11.5 Hz), 160.0 (dd, J = 12.6 Hz), 140.8, 135.1, 133.7 (dd, J = 10.1, 2.3 Hz), 132.9, 130.2, 127.4, 125.4, 121.7 (dd, J = 12.1, 4.2 Hz), 111.9 (dd, J = 22.2, 3.8 Hz), 105.1 (dd, J = 26.2, 24.1 Hz), 45.7 (d, J = 2.7 Hz), 14.1. ESI-MS m/z = 332.0 $[M + H]^+$.

*2,4-Dichloro-N-(2,4-difluorophenyl)-N-ethylbenzenesulfonamide (**33**).* 2,4-Dichloro-*N*-(2,4-difluorophenyl)-*N*-ethylbenzenesulfonamide (**33**) was prepared according to the general procedure using 2,4-dichloro-benzenesulfonyl chloride (24.1 mg, 0.10 mmol, 1.0 equiv) and *N*-ethyl-2,4-difluoroaniline (28.3 mg with 70% purity, 0.10 mmol, 1.0 equiv) to afford 26.3 mg of colorless oil as the product, yield 73%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.76 (d, J = 8.6 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.31 (td, J = 8.7, 6.0 Hz, 1H), 7.25 (dd, J = 8.6, 2.1 Hz, 1H), 6.86 (dddd, J = 9.1, 7.8, 2.9, 1.5 Hz, 1H), 6.78 (ddd, J = 10.3, 8.4, 2.8 Hz, 1H), 3.83 (q, J = 7.1 Hz, 2H), 1.13 (td, J = 7.2, 0.7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 162.6 (dd, J = 252.1, 11.4 Hz), 160.2 (dd, J = 255.5, 12.6 Hz), 139.5, 135.9, 134.5 (dd, J = 10.1, 2.2 Hz), 133.3, 133.0, 131.8, 127.0, 121.1 (dd, J = 12.3, 4.1 Hz), 111.8 (dd, J = 22.2, 3.8 Hz), 105.0 (dd, J = 26.2, 24.1 Hz), 46.8 (d, J = 2.5 Hz), 14.4. ESI-MS m/z = 366.0 $[M + H]^+$.

*3,5-Dichloro-N-(2,4-difluorophenyl)-N-ethylbenzenesulfonamide (**34**).* 3,5-Dichloro-*N*-(2,4-difluorophenyl)-*N*-ethylbenzenesulfonamide (**34**) was prepared according to the general procedure using 3,5-dichloro-benzenesulfonyl chloride (27.3 mg, 0.10 mmol, 1.0 equiv) and *N*-ethyl-2,4-difluoroaniline (29.7 mg with 70% purity, 0.10 mmol, 1.0 equiv). The reaction mixture was heated to 70 °C for 3 days. The crude was further purified by silica gel chromatography using DCM to afford 27.6 mg of white solid as the product, yield 68%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.56 (s, 3H), 7.26 (td, J = 8.7, 5.9 Hz, 1H), 6.92 (dddd, J = 9.0, 7.7, 2.8, 1.4 Hz, 1H), 6.85 (ddd, J = 10.9, 8.4, 2.8 Hz, 1H), 3.61 (q, J = 7.2 Hz, 2H), 1.11 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 162.7 (dd, J = 252.4, 11.5 Hz), 159.9 (dd, J = 255.1, 12.7 Hz), 142.0, 136.0, 133.9 (dd, J = 10.2, 2.3 Hz), 132.7, 125.6, 121.4 (dd, J = 12.1, 4.1 Hz), 112.1 (dd, J = 22.2, 3.8 Hz), 105.2 (dd, J = 26.3, 24.1 Hz), 45.9 (d, J = 2.7 Hz), 14.1. ESI-MS m/z = 365.9 $[M + H]^+$.

*2,6-Dichloro-N-(2,4-difluorophenyl)-N-ethylbenzenesulfonamide (**35**).* 2,6-Dichloro-*N*-(2,4-difluorophenyl)-*N*-ethylbenzenesulfonamide (**35**) was prepared according to the general procedure using 2,6-dichlorobenzenesulfonyl chloride (27.9 mg, 0.10 mmol, 1.0 equiv) and *N*-ethyl-2,4-difluoroaniline (24.5 mg with 70% purity, 0.10 mmol, 1.0 equiv) to afford 27.2 mg of white solid as the product, yield 65%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.45−7.33 (m, 3H), 7.33−7.23 (m, 1H), 6.87 (td, J = 9.1, 3.0 Hz, 1H), 6.77 (ddd, J = 10.9, 8.3, 2.9 Hz, 1H), 3.90 (q, J = 7.2 Hz, 2H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 162.7 (dd, J = 252.0, 11.5 Hz), 160.4 (dd, J = 255.5, 12.7 Hz), 136.1, 135.2, 134.7 (dd, J = 10.1, 2.2 Hz), 132.6, 131.5, 121.0 (dd, J = 12.3, 4.0 Hz), 111.8 (dd, J = 22.2, 3.8 Hz), 104.9 (dd, J = 26.3, 24.1 Hz), 46.6 (d, J = 2.4 Hz), 14.3. ESI-MS m/z = 366.0 $[M + H]^+$.

*3,4-Dichloro-N-(2,4-difluorophenyl)-N-ethylbenzenesulfonamide (**36**).* 3,4-Dichloro-*N*-(2,4-difluorophenyl)-*N*-ethylbenzenesulfonamide (**36**) was prepared according to the general procedure using 3,4-dichloro-benzenesulfonyl chloride (26.6 mg, 0.10 mmol, 1.0 equiv) and *N*-ethyl-2,4-difluoroaniline (28.0 mg with 70% purity, 0.10 mmol, 1.0 equiv). The reaction mixture was heated to 70 °C for 3 days. The crude was further purified by silica gel chromatography using DCM to afford 33.4 mg of white solid as the product, yield 84%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.79 (d, J = 2.0 Hz, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.50 (dd, J = 8.4, 2.1 Hz, 1H), 7.26 (td, J = 8.7, 5.9 Hz, 1H), 6.91 (t, J = 8.3 Hz, 1H), 6.83 (ddd, J = 10.8, 8.4, 2.8 Hz, 1H), 3.60 (q, J = 7.2 Hz, 2H), 1.10 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 162.6 (dd, J = 252.2, 11.5 Hz), 159.9 (dd, J = 255.1, 12.7 Hz), 139.0, 137.6, 133.8 (dd, J = 10.1, 2.3 Hz), 133.6, 131.0, 129.2, 126.4, 121.5 (dd, J = 12.0, 4.2 Hz), 112.0 (dd, J = 22.2, 3.7 Hz), 105.2 (dd, J = 26.3, 24.1 Hz), 45.8 (d, J = 2.8 Hz), 14.1. ESI-MS m/z = 366.0 $[M + H]^+$.

*2,3-Dichloro-N-(2,4-difluorophenyl)-N-ethylbenzenesulfonamide (**37**).* 2,3-Dichloro-*N*-(2,4-difluorophenyl)-*N*-ethylbenzenesulfonamide (**37**) was prepared according to the general procedure using 2,3-dichlorobenzenesulfonyl chloride (23.8 mg, 0.10 mmol, 1.0 equiv) and *N*-ethyl-2,4-difluoroaniline (22.0 mg with 70% purity, 0.10 mmol, 1.0 equiv) to afford 21.5 mg of colorless oil as the product, yield 61%. ^{1}H NMR ($CDCl_3$, 101 MHz): δ ppm 7.78 (dd, J = 8.0, 1.6 Hz, 1H), 7.64 (dd, J = 8.0, 1.6 Hz, 1H), 7.30 (td, J = 8.7, 6.0 Hz, 1H), 7.22 (t, J = 8.0 Hz, 1H), 6.85 (tdd, J = 9.0, 2.9, 1.4 Hz, 1H), 6.77 (ddd, J = 10.1, 8.4, 2.8 Hz, 1H), 3.84 (q, J = 7.1 Hz, 2H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 162.6 (dd, J = 252.1, 11.3 Hz), 160.2 (dd, J = 255.6, 12.5 Hz), 139.3, 135.7, 134.5, 134.4 (d, J = 8.0, 2.1 Hz), 130.8, 130.5, 126.9, 121.1 (dd, J = 12.4, 4.2 Hz), 111.8 (dd, J = 22.2, 3.8 Hz), 105.0 (dd, J = 26.2, 24.1 Hz), 46.9 (d, J = 2.1 Hz), 14.4. ESI-MS m/z = 366.0 $[M + H]^+$.

*2-Chloro-N-(2,4-difluorophenyl)-N-ethyl-5-fluorobenzenesulfonamide (**38**).* 2-Chloro-*N*-(2,4-difluorophenyl)-*N*-ethyl-5-fluorobenzenesulfonamide (**38**) was prepared according to the general procedure using 2-chloro-5-fluorobenzenesulfonyl chloride (24.4 mg, 0.10 mmol, 1.0 equiv) and *N*-ethyl-2,4-difluoroaniline (22.2 mg with 70% purity, 0.10 mmol, 1.0 equiv) to afford 30.6 mg of colorless oil as the product, yield 82%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.58 (dd, J = 8.2, 3.1 Hz, 1H), 7.50 (dd, J = 8.8, 4.7 Hz, 1H), 7.31 (td, J = 8.6, 6.1 Hz, 1H), 7.18 (ddd, J = 8.7, 7.3, 3.1 Hz, 1H), 6.87 (td, J = 8.4, 7.7, 2.9 Hz, 1H), 6.79 (ddd, J = 11.2, 8.5, 2.9 Hz, 1H), 3.85 (q, J = 7.2 Hz, 2H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 163.9 (d, J = 11.4 Hz), 161.5 (t, J = 11.8 Hz), 159.0 (t, J = 6.3 Hz), 138.9 (d, J = 6.5 Hz), 134.4 (dd, J = 10.2, 2.2 Hz), 133.4 (d, J = 7.5 Hz), 127.4 (d, J = 3.7 Hz), 121.1 (dd, J = 12.4, 4.1 Hz), 120.8 (d, J = 22.6 Hz), 119.3 (d, J = 26.3 Hz), 111.9 (dd, J = 22.3, 3.8 Hz), 105.0 (dd, J = 26.3, 24.1 Hz), 46.9 (d, J = 2.4 Hz), 14.4. ESI-MS m/z = 350.0 $[M + H]^+$.

*5-Chloro-N-(2,4-difluorophenyl)-N-ethyl-2-fluorobenzenesulfonamide (**39**).* 5-Chloro-*N*-(2,4-difluorophenyl)-*N*-ethyl-2-fluorobenzenesulfonamide (**39**) was prepared according to the general procedure using 5-chloro-2-fluorobenzenesulphonyl chloride (23.6 mg, 0.10 mmol, 1.0 equiv) and *N*-ethyl-2,4-difluoroaniline (22.8 mg with 70% purity, 0.10 mmol, 1.0 equiv) to afford 31.7 mg of colorless oil as the product, yield 88%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.68 (dd, J = 6.0, 2.7 Hz, 1H), 7.50 (dt, J = 8.9, 3.5 Hz, 1H), 7.31 (td, J = 8.7, 5.9 Hz, 1H), 7.17 (t, J = 9.1 Hz, 1H), 6.89 (td, J = 8.4, 7.8, 3.0 Hz, 1H), 6.80 (ddd, J = 10.9, 8.4, 2.8 Hz, 1H), 3.77 (q, J = 7.2 Hz, 2H), 1.13 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 162.7 (dd, J = 252.1, 11.6 Hz), 160.1 (dd, J = 254.5, 12.3 Hz), 157.5 (d, J = 256.5 Hz), 134.8 (d, J = 8.5 Hz), 134.4 (dd, J = 10.2, 2.3 Hz), 130.5, 129.5 (d, J = 3.7 Hz), 129.1 (d, J = 16.4 Hz), 121.1 (dd, J = 12.3, 4.2 Hz), 118.5 (d, J = 23.7 Hz), 112.0 (dd, J = 22.2, 3.7 Hz), 105.0 (dd, J = 26.4, 24.0 Hz), 46.2 (t, J = 3.1 Hz), 14.4. ESI-MS m/z = 350.0 $[M + H]^+$.

*N-(2,4-Difluorophenyl)-N-ethyl-2,5-difluorobenzenesulfonamide (**40**).* *N*-(2,4-Difluorophenyl)-*N*-ethyl-2,5-difluorobenzenesulfonamide (**40**) was prepared according to the general procedure using 2,5-difluorobenzenesulfonyl chloride (27.5 mg, 0.10 mmol, 1.0 equiv) and *N*-ethyl-2,4-difluoroaniline (24.7 mg with 70% purity, 0.10 mmol, 1.0 equiv) to afford 32.8 mg of colorless oil as the product, yield 76%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.40 (ddd, J = 8.2, 5.2, 3.2 Hz, 1H), 7.30 (td, J = 8.7, 5.9 Hz, 1H), 7.21 (dtd, J = 18.0, 9.1, 4.9 Hz, 2H), 6.89 (td, J = 8.4, 7.6, 2.9 Hz, 1H), 6.80 (ddd, J = 10.8, 8.4, 2.6 Hz, 1H), 3.78 (q, J = 7.2 Hz, 2H), 1.13 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 162.7 (dd, J = 252.1, 11.5 Hz), 160.1 (dd, J = 255.0, 12.7 Hz), 156.29 (t, J = 2.6 Hz), 156.26 (dd, J = 497.8, 2.8 Hz), 134.3 (dd, J = 10.2, 2.3 Hz), 128.9 (dd, J = 17.5, 6.9 Hz), 121.6 (dd, J = 24.0, 8.6 Hz), 121.2 (dd, J = 12.4, 4.1 Hz), 118.5 (dd, J =

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24.8, 7.9 Hz), 117.6 (d, J = 26.9 Hz), 111.9 (dd, J = 22.3, 3.7 Hz), 105.0 (dd, J = 26.3, 24.0 Hz), 46.2 (t, J = 3.1 Hz), 14.4. ESI-MS m/z = 334.0 $[M + H]^+$.

*2,5-Dichloro-N-ethyl-N-(4-methylpyridin-3-yl)-benzenesulfonamide (**41**).* Step 1. To a scintillation vial equipped with a stir bar and Teflon-lined lid containing 3-amino-4-methylpyridine (441.9 mg, 4.1 mmol), *N*-methylimidazole (0.36 mL, 4.5 mmol), and DCM (14 mL) was slowly added 2,5-dichlorobenzenesulfonyl chloride (1.0 g, 4.1 mmol), and the reaction was stirred overnight. The reaction was condensed to dryness and purified by automated flash chromatography in 100% DCM to give 261.5 mg of 2,5-dichloro-*N*-(4-methylpyridin-3-yl)-benzenesulfonamide in 20% yield. ^{1}H NMR (400 MHz, chloroform-*d*): δ 8.32 (d, J = 4.9 Hz, 1H), 8.15 (s, 1H), 7.92 (d, J = 2.1 Hz, 1H), 7.55−7.47 (m, 2H), 7.15 (d, J = 4.9 Hz, 1H), 6.91 (s, 1H), 2.38 (s, 3H). ESI-MS (m/z): 317.0 $[M + H]^+$.

Step 2. Potassium carbonate (34.7 mg, 0.25 mmol), followed by iodoethane (34.4 mL, 0.25 mmol), was added to a solution of the sulfonamide from step 1 (44.6 mg, 0.14 mmol) in acetone (1.4 mL). The reaction was stirred at 60 °C overnight, then condensed to dryness and purified by automated flash chromatography in 100% DCM to give 28.5 mg of the title compound in 59% yield. ^{1}H NMR (400 MHz, chloroform-*d*): δ 8.49 (d, J = 1.7 Hz, 1H), 8.19 (d, J = 2.4 Hz, 1H), 7.53 (dd, J = 5.9, 1.7 Hz, 1H), 7.34−7.28 (m, 2H), 7.25 (dd, J = 8.4, 2.5 Hz, 1H), 4.26 (q, J = 7.4 Hz, 2H), 2.36 (s, 3H), 1.55 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, chloroform-*d*): δ 150.0, 144.0, 132.6, 132.3, 131.2, 130.5, 130.1, 129.9, 128.3, 127.0, 56.6, 19.6, 16.7. ESI-MS (m/z): 345.0 $[M + H]^+$.

*2,5-Dichloro-N-ethyl-N-(5-methylpyrazin-2-yl)-benzenesulfonamide (**42**).* Step 1. 2,5-Dichloro-*N*-(5-methylpyrazin-2-yl)benzenesulfonamide was synthesized according to the general procedure using 5-methylpyrazin-2-amine (12.2 mg, 0.10 mmol, 1.0 equiv) to afford 28.6 mg of white solid as the product, yield 89%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.31 (s, 1H), 8.15 (d, J = 2.4 Hz, 1H), 8.03 (s, 1H), 7.41 (dd, J = 8.6, 2.5 Hz, 1H), 7.34 (d, J = 8.5 Hz, 1H), 2.39 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 148.6, 144.9, 141.4, 138.1, 134.2, 133.6, 133.3, 132.9, 131.9, 130.1, 20.4. ESI-MS m/z = 317.9 $[M + H]^+$.

Step 2. The title compound was prepared according to the general procedure using the sulfonamide from step 1 (28.6 mg, 0.10 mmol, 1.0 equiv) and 1-bromoethane. The reaction was heated at 70 °C for 5 days. The crude was further purified by a silica gel plug and eluted by DCM to afford 16.0 mg of white solid as the product, yield 51%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.67 (d, J = 1.4 Hz, 1H), 8.17 (d, J = 1.4 Hz, 1H), 8.07 (d, J = 2.4 Hz, 1H), 7.43 (dd, J = 8.5, 2.5 Hz, 1H), 7.37 (d, J = 8.5 Hz, 1H), 3.98 (q, J = 7.0 Hz, 2H), 2.54 (s, 3H), 1.19 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 150.9, 145.8, 141.9, 141.3, 138.2, 133.8, 133.2, 133.2, 131.7, 130.4, 43.8, 20.9, 14.3. ESI-MS m/z = 346.0 $[M + H]^+$.

*2,5-Dichloro-N-ethyl-N-(2-methylpyrimidin-5-yl)-benzenesulfonamide (**43**).* Step 1. 2,5-Dichloro-*N*-(2-methylpyrimidin-5-yl)benzenesulfonamide was synthesized according to the general procedure using 2-methylpyrimidin-5-amine (11.6 mg, 0.10 mmol, 1.0 equiv) to afford 29.3 mg of white solid as the product, yield 87%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.42 (s, 2H), 7.97 (d, J = 2.0 Hz, 1H), 7.45−7.34 (m, 2H), 2.58 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 164.3, 158.9, 149.5, 137.5, 134.4, 133.6, 133.0, 131.5, 129.8, 110.0, 24.9. ESI-MS m/z = 317.9 $[M + H]^+$, $C_{11}H_9Cl_2N_3O_2S$ requires 317.0.

Step 2. The title compound was prepared according to the general procedure using the sulfonamide from step 1 (29.3 mg, 0.10 mmol, 1.0 equiv) to afford 30.7 mg of yellow solid as the product, yield 96%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.48 (s, 2H), 7.89 (s, 1H), 7.45 (d, J = 1.5 Hz, 2H), 3.84 (q, J = 7.2 Hz, 2H), 2.72 (s, 3H), 1.17 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 178.3, 167.6, 157.1, 137.7, 134.2, 133.4, 133.2, 132.2, 130.8, 130.2, 47.5, 25.7, 14.5. ESI-MS m/z = 346.0 $[M + H]^+$.

*2,5-Dichloro-N-ethyl-N-(6-methylpyridazin-3-yl)-benzenesulfonamide (**44**).* Step 1. 2,5-Dichloro-*N*-(6-methylpyridazin-3-yl)benzenesulfonamide was synthesized according to the general procedure using 2-methylpyrimidin-5-amine (12.6 mg, 0.10 mmol, 1.0 equiv) to afford 15.5 mg of white solid, yield 46%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.05 (d, J = 1.6 Hz, 1H), 7.57 (d, J = 9.5 Hz, 1H), 7.32−7.25 (m, 3H), 2.33 (s, 3H). ESI-MS m/z = 317.9 $[M + H]^+$.

Step 2. The title compound was prepared according to the general procedure using the sulfonamide from step 1 (15.5 mg, 0.050 mmol, 1.0 equiv) and 1-bromoethane to afford 13.0 mg of yellow solid as the product, yield 77%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.28−8.16 (m, 2H), 7.38 (m, 2H), 7.25 (d, J = 9.5 Hz, 1H), 4.33 (q, J = 7.2 Hz, 2H), 2.42 (s, 3H), 1.34 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 153.7, 149.0, 142.2, 132.8, 132.6, 132.29, 132.28, 130.4, 129.5, 127.0, 51.3, 20.9, 13.2. ESI-MS m/z = 346.0 $[M + H]^+$.

*2,5-Dichloro-N-ethyl-N-(6-methylpyridin-3-yl)-benzenesulfonamide (**45**).* Step 1. 2,5-Dichloro-*N*-(6-methylpyridin-3-yl)benzenesulfonamide was synthesized according to the general procedure using 5-amino-2-methylpyridin (11.1 mg, 0.10 mmol, 1.0 equiv) e to afford 26.2 mg of white solid as the product, yield 86%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.10 (d, J = 2.7 Hz, 1H), 7.93 (d, J = 1.8 Hz, 1H), 7.48 (dd, J = 8.4, 2.7 Hz, 1H), 7.40−7.36 (m, 2H), 7.02 (d, J = 8.3 Hz, 1H), 2.40 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 155.1, 141.7, 137.6, 134.0, 133.3, 132.8, 131.5, 130.7, 129.9, 129.8, 123.9, 23.3. ESI-MS m/z = 317.0 $[M + H]^+$.

Step 2. The title compound was prepared according to the general procedure using the sulfonamide from step 1 (26.2 mg, 0.10 mmol, 1.0 equiv) and 1-bromoethane to afford 26.1 mg of colorless oil as the product, yield 92%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.23 (d, J = 2.6 Hz, 1H), 7.83 (d, J = 2.3 Hz, 1H), 7.48 (dd, J = 8.3, 2.6 Hz, 1H), 7.44 (d, J = 8.6 Hz, 1H), 7.40 (dd, J = 8.4, 2.3 Hz, 1H), 7.13 (d, J = 8.3 Hz, 1H), 3.87 (q, J = 7.1 Hz, 2H), 2.52 (s, 3H), 1.15 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 158.5, 149.5, 138.1, 137.5, 133.8, 133.2, 133.0, 132.2, 131.9, 130.3, 123.6, 47.7, 24.1, 14.6. ESI-MS m/z = 345.0 $[M + H]^+$.

*2,5-Dichloro-N-(5-chloropyridin-2-yl)-N-ethylbenzenesulfonamide (**46**).* Step 1. 2,5-Dichloro-*N*-(5-chloropyridin-2-yl)-benzenesulfonamide was synthesized according to the general procedure using 2-amino-5-chloropyridine (14.0 mg, 0.10 mmol, 1.0 equiv) to afford 16.0 mg of white solid as the product, yield 47%. ^{1}H NMR (d_4-MeOH, 400 MHz): δ ppm 8.17 (d, J = 2.6 Hz, 1H), 8.05 (d, J = 2.5 Hz, 1H), 7.66 (dd, J = 8.8, 2.7 Hz, 1H), 7.56 (dd, J = 8.5, 2.5 Hz, 1H), 7.49 (d, J = 8.6 Hz, 1H), 7.02 (d, J = 8.8 Hz, 1H). ESI-MS m/z = 336.9 $[M + H]^+$.

Step 2. The title compound was prepared according to the general procedure using the sulfonamide from step 1 (16.0 mg, 0.050 mmol, 1.0 equiv) and 1-bromoethane to afford 7.5 mg of colorless oil as the product, yield 43%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.27 (d, J = 2.6 Hz, 1H), 8.07 (d, J = 2.4 Hz, 1H), 7.64 (dd, J = 8.7, 2.6 Hz, 1H), 7.45 (d, J = 8.7 Hz, 1H), 7.42 (dd, J = 8.5, 2.4 Hz, 1H), 7.37 (d, J = 8.5 Hz, 1H), 4.00 (q, J = 7.0 Hz, 2H), 1.19 (t, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 149.8, 146.9, 138.4, 137.5, 133.7, 133.2, 133.1, 131.7, 130.5, 129.1, 121.4, 43.8, 14.4. ESI-MS m/z = 364.9 $[M + H]^+$.

*2,5-Dichloro-N-(3-chloropyridin-4-yl)-N-ethylbenzenesulfonamide (**47**).* Step 1. 2,5-Dichloro-*N*-(3-chloropyridin-4-yl)-benzenesulfonamide was synthesized according to the general procedure using 2 4-amino-3-chloropyridine (13.6 mg, 0.10 mmol, 1.0 equiv) to afford 24.1 mg of white solid as the product, yield 71%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.14 (s, 1H), 8.04 (d, J = 2.6 Hz, 1H), 7.36 (d, J = 1.5 Hz, 1H), 7.28 (q, J = 7.9, 7.3 Hz, 2H), 6.75 (s, 1H). ESI-MS m/z = 336.9 $[M + H]^+$.

Step 2. The title compound was prepared according to the general procedure using the sulfonamide from step 1 (11.4 mg, 0.050 mmol, 1.0 equiv) and 1-bromoethane to afford 5.9 mg of white solid as the product, yield 48%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.13 (d, J = 2.1 Hz, 1H), 7.85 (s, 1H), 7.54 (s, 2H), 7.30 (d, J = 3.2 Hz, 2H), 3.99 (q, J = 7.3 Hz, 2H), 1.43 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 158.5, 141.7, 138.3, 138.1, 132.6, 132.4, 132.2, 130.7, 129.5, 125.0, 113.9, 53.3, 16.0. ESI-MS m/z = 365.0 $[M + H]^+$.

*2,5-Dichloro-N-ethyl-N-(3-fluoropyridin-4-yl)-benzenesulfonamide (**48**).* Step 1. 2,5-Dichloro-*N*-(3-fluoropyridin-4-yl)benzenesulfonamide was synthesized according to the general

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procedure using 4-amino-3-fluoropyridine (12.6 mg, 0.10 mmol, 1.0 equiv) to afford 16.6 mg of white solid as the product, yield 46%. ^{1}H NMR (d_4-MeOH, 400 MHz): δ ppm 8.84 (t, J = 7.8 Hz, 1H), 8.19 (d, J = 5.0 Hz, 1H), 8.15 (d, J = 2.2 Hz, 1H), 7.94 (d, J = 6.7 Hz, 1H), 7.56 (d, J = 7.3 Hz, 1H), 7.46 (s, 1H). ESI-MS m/z = 320.9 [M + H]$^+$.

Step 2. The title compound was prepared according to the general procedure using the sulfonamide from step 1 (8.3 mg, 0.050 mmol, 1.0 equiv) and 1-bromoethane to afford 7.6 mg of colorless oil as the product, yield 84%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.14 (d, J = 2.0 Hz, 1H), 7.74 (dd, J = 5.8, 2.0 Hz, 1H), 7.65 (t, J = 7.7 Hz, 1H), 7.55 (dd, J = 7.4, 2.0 Hz, 1H), 7.32 (d, J = 2.3 Hz, 2H), 4.05 (q, J = 7.3 Hz, 2H), 1.47 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 153.1, 151.9 (d, J = 258.5 Hz), 141.7, 137.4 (d, J = 1.4 Hz), 132.6, 132.6, 132.3, 130.8, 129.6, 126.9 (d, J = 36.5 Hz), 115.1 (d, J = 5.7 Hz), 53.5, 16.0. ESI-MS m/z = 349.0 [M + H]$^+$.

*5-Chloro-1-((2,5-dichlorophenyl)sulfonyl)indoline (**49**).* 5-Chloro-1-((2,5-dichlorophenyl)sulfonyl)indoline (**49**) was synthesized according to the general procedure using 5-chloro-2,3-dihydro-(1*H*)-indole (30.7 mg, 0.20 mmol, 1.0 equiv). The reaction mixture was heated to 70 °C for 5 days. The crude was further purified by silica gel chromatography using 66% of DCM in hexane to afford 70.6 mg of white solid as the product, yield 98.5%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.13 (d, J = 2.8 Hz, 1H), 7.48−7.35 (m, 2H), 7.18 (d, J = 8.6 Hz, 1H), 7.12−7.01 (m, 2H), 4.20 (td, J = 8.3, 3.9 Hz, 2H), 3.08 (t, J = 8.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 139.9, 137.8, 134.0, 133.3, 133.1, 133.1, 132.0, 130.5, 128.7, 127.5, 125.5, 114.8, 50.7, 27.8. ESI-MS m/z = 362.0 [M + H]$^+$.

*6-Chloro-1-((2,5-dichlorophenyl)sulfonyl)-1,2,3,4-tetrahydroquinoline (**50**).* 6-Chloro-1-((2,5-dichlorophenyl)sulfonyl)-1,2,3,4-tetrahydroquinoline (**50**) was synthesized according to the general procedure using 6-chloro-1,2,3,4-tetrahydroquinoline (33.6 mg, 0.20 mmol, 1.0 equiv). The reaction mixture was heated to 60 °C for 7 days to afford 23.2 mg of white solid as the product, yield 31%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.12 (d, J = 2.5 Hz, 1H), 7.46 (dd, J = 8.5, 2.5 Hz, 1H), 7.41 (d, J = 8.5 Hz, 1H), 7.34 (d, J = 9.6 Hz, 1H), 7.07 (s, br, 2H), 3.85 (t, J = 6.0 Hz, 2H), 2.72 (t, J = 6.7 Hz, 2H), 1.89 (p, J = 6.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 139.3, 135.4, 133.9, 133.3, 133.3, 131.8, 131.5, 130.5, 129.9, 129.0, 126.5, 124.0, 46.5, 26.7, 22.2. ESI-MS m/z = 376.0 [M + H]$^+$.

*2,5-Dichloro-N-(4-chloro-2-methylphenyl)benzamide (**51**).* BOP-Cl (bis(2-oxo-3-oxazolidinyl)phosphinic chloride, 196 mg, 0.75 mmol, 1.5 equiv) was added to a stirred solution of 2,5-dichlorobenzoic acid (99.0 mg, 0.50 mmol, 1.0 equiv) in 5 mL of THF and i-Pr$_2$NEt (129 mg, 1.0 mmol, 2.0 equiv). The mixture was stirred for 1 h at room temperature. 4-Chloro-2-methylaniline (86.0 mg, 0.55 mmol, 1.1 equiv) was added and allowed to react for overnight at 50 °C under nitrogen. After completion, the crude mixture was poured onto ice. The precipitation was collected by filtration and washed by water to afford 157 mg of off-white solid, yield 91%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.70−7.63 (m, 2H), 7.35 (s, br, 2H), 7.17 (d, J = 8.6 Hz, 2H), 2.26 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 164.3, 136.5, 133.4, 133.3, 133.1, 131.4, 131.4, 130.4, 129.6, 128.9, 126.5, 125.8, 125.7, 17.8. ESI-MS m/z = 314.0 [M + H]$^+$.

*N-(2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)-2,5-dichloro-N-(4-chloro-2-methylphenyl)benzenesulfonamide (**52**).* *N*-(2-(3-(But-3-yn-1-yl)-3*H*-diazirin-3-yl)ethyl)-2,5-dichloro-*N*-(4-chloro-2-methylphenyl)benzenesulfonamide (**52**) was synthesized according to the general procedure, alkylating with 3-(but-3-yn-1-yl)-3-(2-iodoethyl)-3*H*-diazirine (6.9 mg, 0.024 mmol, 1.2 equiv) in the second step.[31] The crude mixture was further purified by a silica gel column and eluted by 20% of ethyl acetate in hexane to afford 6.3 mg of off-white solid as the product, yield 73%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.75 (t, J = 2.2 Hz, 1H), 7.51−7.40 (m, 2H), 7.22 (s, 1H), 7.07 (dt, J = 8.6, 2.3 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 3.65 (t, J = 9.2 Hz, 2H), 2.17 (s, 3H), 2.03−1.91 (m, 3H), 1.69 (dt, J = 16.4, 7.3 Hz, 1H), 1.61 (t, J = 7.2 Hz, 2H), 1.56 (s, 1H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 140.9, 138.5, 134.8, 134.5, 133.8, 133.3, 133.2, 132.2, 131.6, 131.5, 130.2, 127.0, 82.4, 69.5, 47.6, 32.7, 31.9, 26.1, 18.0, 13.2. ESI-MS m/z = 503.9 [M + Na]$^+$.

*2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)ethyl 2-(4-((2,5-Dichloro-N-ethylphenyl)sulfonamido)phenyl)acetate (**53**).* To a solution of acid **24** (7.8 mg, 0.020 mmol, 1.0 equiv) in 0.1 mL of dimethylformamide were added potassium carbonate (5.3 mg, 0.040 mmol, 2.0 equiv) and 3-(but-3-yn-1-yl)-3-(2-iodoethyl)-3*H*-diazirine (5.5 mg, 0.022 mmol, 1.1 equiv).[31] The reaction mixture was stirred at room temperature overnight. The crude mixture was purified by a silica gel plug and eluted by 50% of ethyl acetate in hexane to afford 8.7 mg of off-white solid as the product, yield 85%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.82 (t, J = 2.0 Hz, 1H), 7.44 (d, J = 8.6 Hz, 1H), 7.39 (dt, J = 8.5, 2.0 Hz, 1H), 7.25 (d, J = 8.6 Hz, 2H), 7.16 (d, J = 8.4 Hz, 2H), 3.99 (t, J = 6.3 Hz, 2H), 3.88 (q, J = 7.1 Hz, 2H), 3.62 (s, 2H), 2.03−1.94 (m, 3H), 1.72 (t, J = 6.3 Hz, 2H), 1.61 (t, J = 7.5 Hz, 2H), 1.15 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 170.8, 138.5, 136.6, 134.0, 133.5, 132.9, 132.9, 132.2, 130.3, 129.5, 82.5, 69.4, 59.7, 47.6, 40.8, 32.2, 32.1, 26.2, 14.7, 13.2. ESI-MS m/z = 507.9 [M + H]$^+$.

*N-(3-Azidophenyl)-2,5-dichloro-N-(prop-2-yn-1-yl)-benzenesulfonamide (**54**).* *N*-(3-Azidophenyl)-2,5-dichloro-*N*-(prop-2-yn-1-yl)benzenesulfonamide (**54**) was synthesized according to the general procedure to afford 15.8 mg of off-white solid as the product, yield 82%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.91 (dd, J = 2.0, 1.0 Hz, 1H), 7.46 (d, J = 1.9 Hz, 2H), 7.30 (t, J = 8.0 Hz, 1H), 7.05 (ddd, J = 8.0, 2.1, 0.9 Hz, 1H), 7.00 (ddd, J = 8.1, 2.3, 0.9 Hz, 1H), 6.96 (t, J = 2.1 Hz, 1H), 4.61 (d, J = 2.5 Hz, 2H), 2.29 (t, J = 2.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 141.2, 139.4, 138.1, 134.0, 133.2, 133.0, 132.3, 130.5, 130.5, 125.1, 119.7, 119.3, 77.9, 74.2, 42.2. ESI-MS m/z = 380.9 [M + Na]$^+$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00304.

NMR spectra for new compounds (PDF)
Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

A/PR/8/34, Influenza A/Puerto Rico/8/1934; A/WSN/33, Influenza A/Wisconsin/1933; ATCC, American Type Culture Collection; BSA, bovine serum albumin; CC_{50}, 50% cytotoxicity concentration; cDNA, complementary DNA; cmpd, compound; cRNPs, complementary ribonucleoproteins; DCM, dichloromethane; DMSO, dimethyl sulfoxide; EMEM, Eagle's minimum essential medium; ESI, electrospray ionization; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; h, hours; HA, hemagglutinin; HPLC, high-performance liquid chromatography; IC_{50}, 50% inhibitory concentration; LC−MS, liquid chromatography−mass spectrometry; M1, matrix protein 1; M2, matrix protein 2; MDCK cells, Madin−Darby Canine Kidney; min, minutes; MOI, multiplicity of infection; mRNA, messenger RNA; NMR, nuclear magnetic resonance; NP, nucleoprotein; NS, non-structural protein; NS1, non-structural protein 1; NS2, non-structural protein 2; PB1, polymerase basic subunit 1; PB2, polymerase basic subunit 2; PBS, phosphate buffered saline; PFA, paraformaldehyde; PFU, plaque forming units; poly(A) RNA, polyadenylated RNA; ppm, parts per million; qPCR, quantitative real-time PCR; RBC, red blood cells; RNA-FISH, RNA fluorescent in situ hybridization; RNAseq, RNA sequencing; smRNA-FISH, single molecule RNA fluorescent in situ hybridization; SSC buffer, saline-sodium citrate buffer; UV, ultraviolet; vRNPs, viral ribonucleoproteins

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