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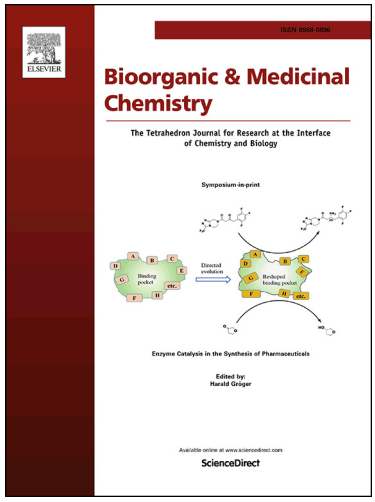

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Design, synthesis, and *in vitro* bioactivity evaluation of fluorine-containing analogues for sphingosine-1-phosphate 2 receptor

Zonghua Luo[a] , Hui Liu[a] , Robyn S. Klein[b,c,d] , and Zhude Tu[a, *]

[a] *Department of Radiology, Washington University School of Medicine, St. Louis, MO 63110, USA*
[b] *Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA*
[c] *Department of Neuroscience, Washington University School of Medicine, St. Louis, MO 63110, USA*
[d] *Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA*

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ABSTRACT

Twenty eight new aryloxybenzene analogues were synthesized and their *in vitro* binding potencies toward S1PR2 were determined using a [^{32}P]S1P competitive binding assay. Out of these new analogues, three compounds, **28c** (IC_{50} = 29.9 ± 3.9 nM), **28e** (IC_{50} = 14.6 ± 1.5 nM), and **28g** (IC_{50} = 38.5 ± 6.3 nM) exhibited high binding potency toward S1PR2 and high selectivity over the other four receptor subtypes (S1PR1, 3, 4, and 5; IC_{50} > 1000 nM). Each of the three potent compounds **28c**, **28e**, and **28g** contains a fluorine atom that will allow developing F-18 labeled PET radiotracers for imaging S1PR2.

1. Introduction

Sphingosine 1-phosphate (S1P) is an essential membrane-derived bioactive sphingolipid mediator which plays critical regulatory functions in the cardiovascular, nervous, and immune systems.[1] S1P is generated from the phosphorylation of sphingosine by sphingosine kinases (Sphk1 and 2) and presents at high levels in the blood and lymph.[2] S1P exerts its functions by interacting with a family of five G protein-coupled receptor subtypes, S1PR1, 2, 3, 4, and 5, originally named as EDG-1, 5, 3, 6, and 8.[3] Each subtype has its specific tissue expression and response to different biological process.[4, 5] Out of these five subtypes, S1PR1, 2, and 3 are extensive ubiquitously expressed in a variety of cell types and tissues; S1PR4 expression is primarily in hematopoietic and immune system cells; S1PR5 is primarily expressed in the spleen and central nervous system (CNS).[6, 7] Among these receptors, S1PR2 plays a pivotal role in mediating different cellular functions and pathologies, such as endothelial,[8] metabolic,[9, 10] muscle,[11] neuronal,[12, 13] and kidney functions.[14] Conjugated bile acid induced activation of the S1PR2 signaling pathway plays a critical role in obstructive cholestasis and invasive growth of esophageal adenocarcinoma (EAC) cells, which represent a novel therapeutic target for cholestatic liver diseases and EAC.[15, 16] During demyelination process in CNS diseases, S1PR2 regulates blood-brain barrier (BBB) function and an increased expression of S1PR2 is observed in disease-susceptible regions of both female SJL experimental allergic encephalomyelitis (EAE) mice and female multiple sclerosis (MS) patients with compared to their male counterparts.[17] In the kidney of diabetic rats and mesangial cells under high glucose condition, the highest S1PR2 mRNA expression was observed compared to other four S1P subtypes, S1PR1, 3, 4, and 5.[18] Together, S1PR2 protein may serve a promising biomarker for assessing the progress of diseases such as cholangiopathies, MS, and diabetic nephropathy. Positron emission tomography (PET) is a unique imaging modality and can provide a non-invasive method to quantitatively assess the target protein distribution *in vivo* in tissues. It was widely used in preclinical and clinical investigations. PET imaging with a suitable S1PR2 specific radiotracer could help advance our understanding of S1PR2 modulating functions in related diseases. Therefore, identification

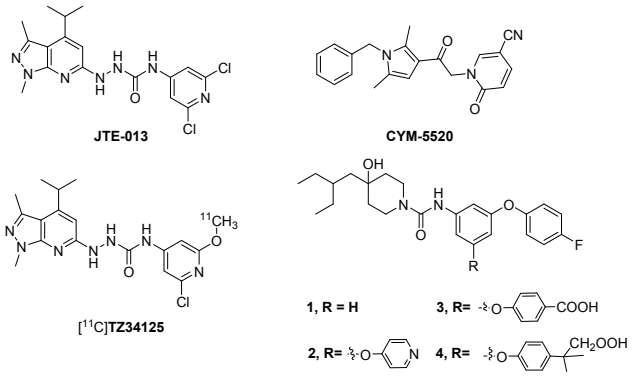


Figure 1. Structures of S1PR2 compounds

of S1PR2 specific ligands is imperative. Despite recognition of this potential, only a few compounds have been reported specifically for S1PR2 (**Figure 1**). JTE-013, a well-known antagonist for S1PR2, is widely used in preclinical studies[19]; CYM-5520 is an allosteric S1PR2 selective agonist, but it was not competitive with S1P in a [^{33}P]S1P competitive binding assay.[20] Takuya Seko's group recently, reported four potent S1PR2 compounds **1-4 (Figure 1)** that have high antagonist activity. Compounds **2**, **3**, and **4** showed improved metabolic stability compared to compound **1**.[21-23] Our group reported the radiosynthesis of [^{11}C]**TZ34125**, a JTE-013 analogue, and performed *in vivo* evaluation in SJL mice that confirmed the sexual dimorphism of S1PR2 expression in the cerebellum.[24] Nevertheless, no F-18 S1PR2 radiotracer was reported. To identify an F-18 PET radiotracer for imaging S1PR2 expression *in vitro* and *in vivo* for related diseases, herein we reported our recent efforts on the exploration of the new structural S1PR2 ligands.

After analyzing the structure of compound **1**, it was dissected into three fragments A, B, and C as shown in **Figure 2**. Our exploration of new S1PR2 analogues used three strategies. First, we focused on the optimization of fragment A: the proton at the 5-position of benzyl ring was replaced with different moieties including alkoxy heterocycle, trifluoromethyl, aryloxy, and arylthio groups. Second, we focused on the optimization of fragment B: different electronegativity functional groups were used to replace the fluorine at 4'-position of the aromatic ring. Third, we focused on the optimization of fragment C: the piperdin-4-ol was replaced with pyrrolidin-3-ol and azetidin-3-ol and different alkyl chains were used to replace the 2-(ethyl)butyl tail. The goal of our efforts was to identify S1PR2 ligands with high binding potency and high selectivity. The newly synthesized analogues were screened for their *in vitro* binding potency and selectivity using [^{32}P]S1P as the competitive radioligand in an assay with cell membranes that were enriched for the different receptor subtypes. Out of these new analogues, three new compounds were discovered having high S1PR2 binding potency (IC_{50} < 50 nM) and high selectivity for S1PR2. Consequently, the structure-activity relationship analysis of these new analogues was explored.

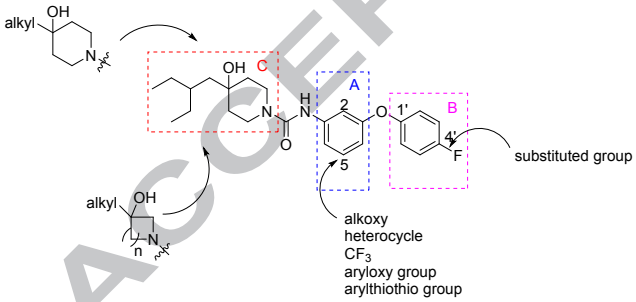
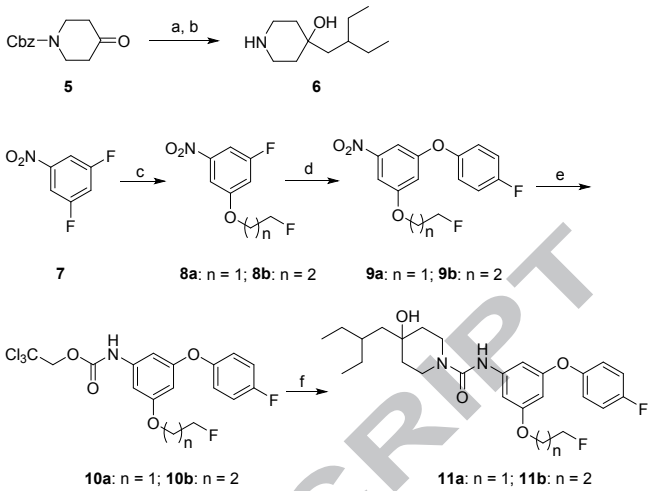


Figure 2. Design strategy of new S1PR2 analogues

2. Results and Discussion

2.1 Chemistry

Our exploration of new S1PR2 analogues was accomplished by following our abovementioned three strategies. Firstly, fluorine substituted alkoxy, 1-methyl-4-piperazine, 1*H*-pyrazole, and trifluoromethyl groups was used to replace the hydrogen atom at the 5-position of the aromatic ring in the fragment A to generate compounds **11a-b** and **17a-c**. Briefly, the synthesis of compounds **11a-b** were accomplished by following Scheme 1. Briefly, benzyl 4-oxopiperidine-1-carboxylate (**5**) was treated with 2-ethylbutylmagnesium bromide in the presence of

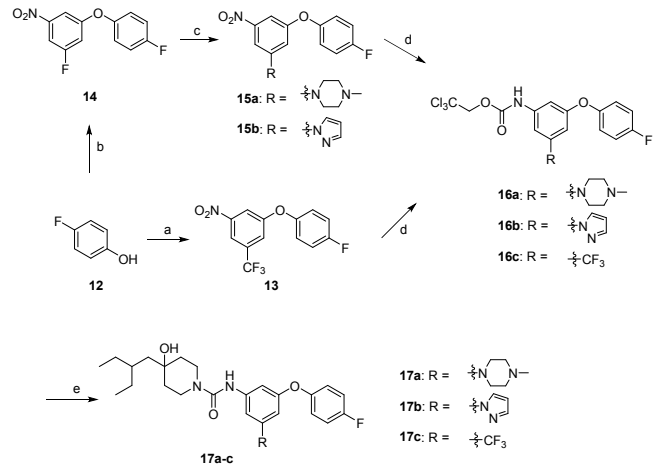


Scheme 1. Synthesis of **11a-b**. Reagents, conditions, and yields: (a) $LaCl_3$-2LiCl solution, 2-ethylbutylmagnesium bromide, 0 ºC - RT; (b) Pd/C, H_2, methanol, RT; 81%; (c) 2-fluorethanol or 3-fluoropyopan-1-ol, NaH, DMF, RT; 33-35%; (d) 4-fluorophenol, K_3PO_4, DMA, 100 ºC; 91-99%; (e) (i) Pd/C, H_2, ethyl acetate, RT; (ii) 2,2,2-trichloroethyl chloroformate, ethyl acetate, $NaHCO_3$, 0 ºC – RT; (f) **6**, DIPEA, DMA, 100 ºC; 97-98%.

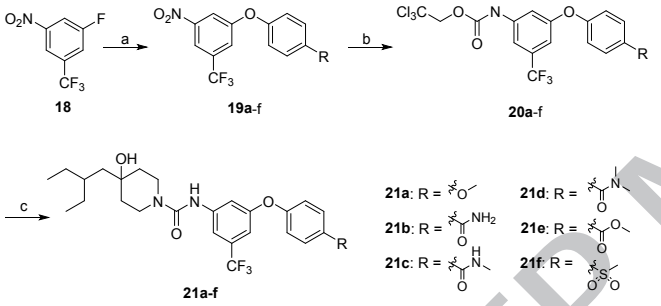
lanthanum trichloride-lithium chloride complex at 0 ºC to afford alcohol intermediate, following by removal of carboxybenzyl (Cbz) group through Pd-catalyzed hydrogenation in methanol to yield 4-(2- ethylbutyl)piperidin-4-ol (**6**). Meanwhile, commercially available 1,3-difluoro-5-nitrobenzene (**7**) was treated with 2-fluorethanol or 3-fluoro-1-propanol and sodium hydride (NaH) in *N*,*N*-dimethylformamide (DMF) to yield **8a-b**, which were subjected to a substitution reaction using 4-fluorophenol to afford aryloxy intermediates **9a-b** in the presence of K_3PO_4 at 100 ºC in *N*,*N*-dimethylacetamide (DMA). The Pd-catalyzed hydrogenation of **9a-b** gave anilines, followed by treating with 2,2,2-trichloroethyl chloroformate and $NaHCO_3$ gave intermediates **10a-b**. The subsequent coupling reaction of **10a-b** with 4-(2-ethylbutyl)piperidin-4-ol (**6**) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) at 100 ºC in DMA afforded target compound **11a-b**.

The synthesis of **17a-c** were achieved by following Scheme 2. Commercially available 4-fluorophenol (**12**) reacted with 1-fluoro-3-nitro-5-(trifluoromethyl)benzene or 1,3-difluoro-5-nitrobenzene afforded **13** or **14**, respectively. Intermediate **14** was coupled with 1-methylpiperozine or 1*H*-pyrazole using K_2CO_3 as a base in dimethyl sulfoxide (DMSO) to afford **15a** or **15b**. After palladium-catalyzed reduction of **13** and **15a-b**, the resulting anilines were reacted with 2,2,2-trichloroethyl chloroformate to afford **16a-c**. The target compounds **17a-c** was prepared by reacting the carbamates **16a-c** with 4-(2-ethylbutyl)piperidin-4-ol (**6**) as described for **11a-b**.

Secondly, to explore the impact of the substitution group at 4'-position of the aromatic ring in fragment B, we retained the trifluoromethyl group because trifluoromethyl-containing compound **17c** showed moderate binding potency. Compounds **21a-f** were synthesized with different substitution groups at 4'-position as shown in Scheme 3. Briefly, commercially available 1- fluoro-3-nitro-5-(trifluoromethyl)benzene (**18**) reacted with differently substituted phenols in the presence of K_3PO_4 in DMA afforded **19a-f**. The palladium-catalyzed hydrogenation of **19a-f** gave anilines, followed by condensation with 2,2,2-trichloroethyl chloroformate to afford key intermediates **20a-f**. Coupling of intermediates **20a-f** with 4-(2-ethylbutyl)piperidin-4-ol (**6**) yielded the target compounds **21a-f**.

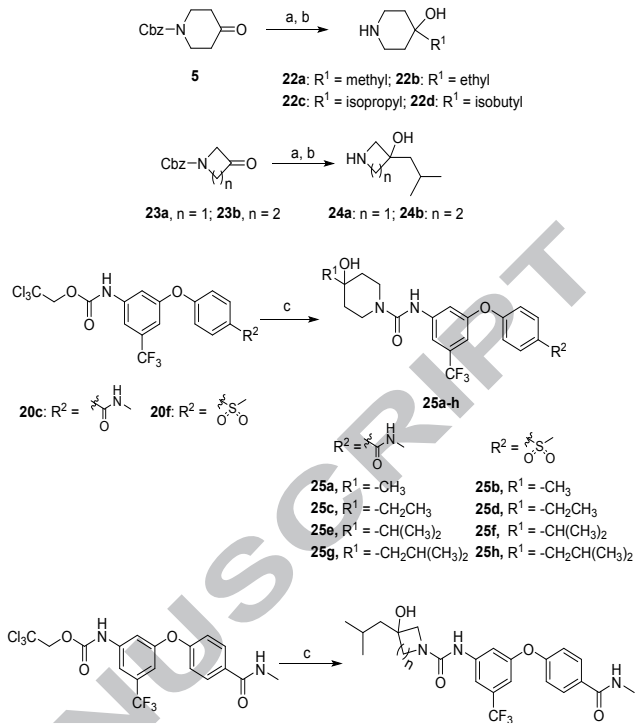


Scheme 2. Synthesis of **17a-c**. Reagents, conditions, and yields: (a) 1-fluoro-3-nitro-5-(trifluoromethyl)benzene, K_3PO_4, DMA, 100 ºC; 93%; (b) 1,3-difluoro-5-nitrobenzene, K_3PO_4, DMA, 100 ºC; 95%; (c) 1-methylpiperozine or 1*H*-pyrazole, K_2CO_3, DMSO, 65 ºC; 42-53%; (d) (i) Pd/C, H_2, ethyl acetate, RT; (ii) 2,2,2-trichloroethyl chloroformate, ethyl acetate, $NaHCO_3$, 0 ºC - RT; (e) **6**, DIPEA, DMA, 100 ºC ; 22-44%.



Scheme 3. Synthesis of target compounds **21a-f**. Reagents, conditions, and yields: (a) phenols, K_3PO_4, DMA, 100 ºC; 60-99%; (b) (i) Pd/C, H_2, ethyl acetate, RT; (ii) 2,2,2-trichloroethyl chloroformate, ethyl acetate, $NaHCO_3$, 0 ºC - RT; (c) **6**, DIPEA, DMA, 100 ºC; 39-81%.

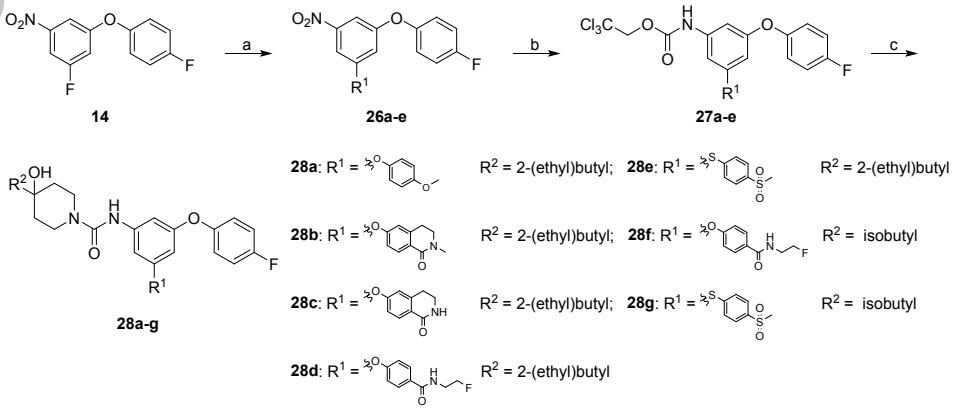
Thirdly, to further evaluate the impact of the side chain and *N*-containing heterocycle in fragment C, we changed 2-(ethyl)butyl side chain with different alkyl chains and replaced the piperdin-4-ol with pyrrolidin-3-ol and azetidin-3-ol. Compounds **25a-j** were synthesized by following Scheme 4. The amines (**22a-d** and **24a-b**) were prepared ahead using the same procedure as described for compound **6** with corresponding Grignard's agents and



Scheme 4. Synthesis of different amines **22a-d**, **24a-b**, and target compounds **25a-j**. Reagents, conditions, and yields: (a) $LaCl_3$-2LiCl solution, Grignard's agents, 0 ºC - RT; (b) Pd/C, H_2, methanol, RT; 92-98%; (c) amines (**22a-d** or **24a-b**), DIPEA, DMA, 100 ºC; 42-99%.

ketones. The subsequent coupling two carbamates **20c** and **20f** with corresponding amines (**22a-d** and **24a-b**) afforded compounds **25a-j**.

Finally, to further investigate the substituted group at the 5-position of the middle aromatic ring in fragment A, a series of substituted aryloxy group and arylthio group were used to replace the trifluoromethyl group. New analogues **28a-g** were synthesized by following Scheme 5. Briefly, compound **14** reacted with 4-methoxylphenol, 6-hydroxy-2-methyl-3,4-dihydroisoquinolin-1(2*H*)-one, 6-hydroxy-3,4-dihydroisoquinolin-1(2*H*)-one, *N*-(2-fluoroethyl)-4-hydroxybenzamide, or 4-(methylsulfonyl)benzene-thiol in the presence of K_3PO_4 gave compounds **26a-e**, followed by palladium-catalyzed hydrogenation afforded corresponding anilines. The resulting anilines were treated with 2,2,2-trichloroethyl chloroformate and $NaHCO_3$ to afford intermediates **27a-e**. The subsequent



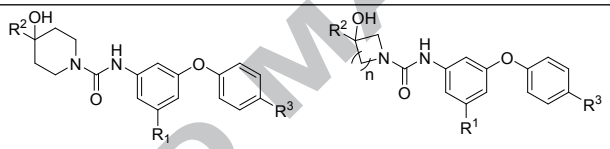
Scheme 5. Synthesis of **28a-g**. Reagents, conditions, and yields: (a) phenols or thiophenol, K_3PO_4, DMA, 100 ºC; 15-99%; (b) (i) Pd/C, H_2, ethyl acetate, RT; (ii) 2,2,2-trichloroethyl chloroformate, ethyl acetate,$NaHCO_3$, 0 ºC - RT; 90-99%; (c) **6** or **22d**, DIPEA, DMA, 100 ºC; 24-88%.

condensation reaction of **27a-e** with **6** or **22d** in the presence of DIPEA at 100 ºC in DMA yielded target compounds **28a-g**.

2.2 Biological binding studies

In vitro binding of these newly synthesized compounds **11a-b**, **17a-c**, **21a-f**, **25a–j**, and **28a–g** toward S1PR2 was determined by radioligand [^{32}P]S1P competitive cell membrane binding assay following our published protocol.[20] The results are shown in Table 1-2. The strategy of introducing alkoxy, heterocycle, or trifluoromethyl group in fragment A gave compounds **11a-b** and **17a-c**. The *in vitro* S1PR2 binding data showed that compound **17c**, with a trifluoromethyl group at the 5-position exhibit moderate binding activity with an IC_{50} value of 362.3 nM, which is comparable to the lead compound **1** having IC_{50} value of 310 nM for S1PR2. The fluorine-containing alkoxy compounds, **11a** and **11b** showed low binding activities with IC_{50} > 1000 nM. The substitution with another two *N*-containing heterocycles, 1-methyl-4-piperazine and 1*H*-pyrazole didn't improve the binding activity either; both **15a** and **15b** had IC_{50} > 1000 nM. Subsequently, our further exploration of new analogues focused on structural optimization of compound **17c**. From one side, as shown in Scheme 3, we first retained trifluoromethyl group at 5-position in the fragment A and checked the impact of various substituted groups at 4'-position in fragment B (**21a-f**). Our binding data indicated that mono-methyl carbamide ($CONHCH_3$) and methylsulfonyl (SO_2CH_3) group improved the binding activity, compound **21c** and **21f** had IC_{50} values of 278.5 and 270.3 nM, respectively. The other functional groups, like OCH_3, $CONH_2$, $CON(CH_3)_2$, and $COOCH_3$ in **21a**, **21b**, **21d**, and **21e** decreased the binding activity with IC_{50} >1000 nM. To investigate the impact of the side chain in the fragment C, compounds **25a-h** were synthesized and tested. We observed that the replacement of 2-(ethyl)butyl chain with methyl, ethyl, or isopropyl chains caused the loss of biological activity, compounds **25a-f** had IC_{50} > 1000 nM. Interestingly, we observed compound **25g** (IC_{50} = 359.3 nM) and **25h** (IC_{50} = 296.5 nM) with a isobutyl side chain resulted in comparable binding activity compared to the 2-(ethyl)butyl compounds **21c** and **21f**. It suggested that the size and the steric hindrance of the side chain lead to increase the S1PR2 binding activity. Another strategy to modify the fragment C was to change the piperidine heterocycle moiety. The results revealed that the compounds **25i** and **25j**, with pyrrolidine and azetidine heterocycles, respectively, showed IC_{50} values that were over 1000 nM, which indicated the piperidine heterocycle was the favorable heterocycle moiety. From the other side, compound **2**, having a 4-pyridineoxyl moiety showed potent binding activity with an IC_{50} value of 45 nM, we replaced the trifluoromethyl group at 5-position with different alkoxy moieties in fragment A and

Table 1 Structures and binding potencies (IC_{50} ± SD) of compounds **11a-b**, **17a-c**, **21a-f**, and **25a-j** toward S1PR2. [a]

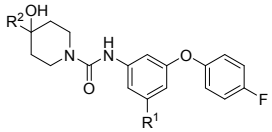


1, 11a-b, 17a-c, 21a-f, and 25a-h **25i-j**

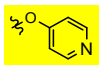
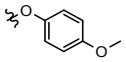
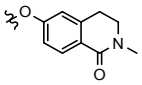
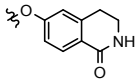
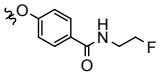
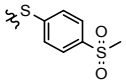
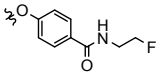
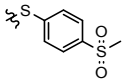
Compd.	n	R^1	R^2	R^3	S1PR2 IC_{50} (nM)
JTE-013	-	-	-	-	66.8 ± 7.8
1[b]	-	H	2-(ethyl)butyl	F	310
11a	-	OCH_2CH_2F	2-(ethyl)butyl	F	> 1000
11b	-	$O(CH_2)_3F$	2-(ethyl)butyl	F	> 1000
17a	-	1-methylpiperazine	2-(ethyl)butyl	F	> 1000
17b	-	1*H*-pyrazole	2-(ethyl)butyl	F	> 1000
17c	-	CF_3	2-(ethyl)butyl	F	362.3 ± 85.0
21a	-	CF_3	2-(ethyl)butyl	OCH_3	> 1000
21b	-	CF_3	2-(ethyl)butyl	$CONH_2$	> 1000
21c	-	CF_3	2-(ethyl)butyl	$CONHCH_3$	278.5 ± 27.2
21d	-	CF_3	2-(ethyl)butyl	$CON(CH_3)_2$	> 1000
21e	-	CF_3	2-(ethyl)butyl	$COOCH_3$	> 1000
21f	-	CF_3	2-(ethyl)butyl	SO_2CH_3	270.3 ± 72.0
25a	-	CF_3	methyl	$CONHCH_3$	> 1000
25b	-	CF_3	methyl	SO_2CH_3	> 1000
25c	-	CF_3	ethyl	$CONHCH_3$	> 1000
25d	-	CF_3	ethyl	SO_2CH_3	> 1000
25e	-	CF_3	isopropyl	$CONHCH_3$	> 1000
25f	-	CF_3	isopropyl	SO_2CH_3	> 1000
25g	-	CF_3	isobutyl	$CONHCH_3$	359.3 ± 50.1
25h	-	CF_3	isobutyl	SO_2CH_3	296.5 ± 32.3
25i	1	CF_3	isobutyl	$CONHCH_3$	> 1000
25j	2	CF_3	isobutyl	$CONHCH_3$	> 1000

[a] IC_{50} values were determined by at least two independent experiments, each run was performed in duplicate. [b] reference[21]

Table 2 Structures and binding affinities (IC_{50} ± SD) of **28a-g** toward S1PRs.[a]



2 and 28a-g

Compd.	R^1	R^2	IC_{50} (nM)				
			S1PR1	S1PR2	S1PR3	S1PR4	S1PR5
JTE-013	-	-	> 1000	66.8 ± 7.8	> 1000	> 1000	> 1000
S1P[b]	-	-	1.4 ± 0.3	3.6 ± 0.5	0.4 ± 0.2	151 ± 82	3.1 ± 1.1
2[c]		2-(ethyl)butyl	> 10000	45	> 10000	> 10000	> 10000
28a		2-(ethyl)butyl	N.T.	188.5 ± 32.9	N.T.	N.T.	N.T.
28b		2-(ethyl)butyl	> 1000	73.3 ± 10.6	> 1000	> 1000	> 1000
28c		2-(ethyl)butyl	> 1000	29.9 ± 3.9	> 1000	> 1000	> 1000
28d		2-(ethyl)butyl	> 1000	66.7 ± 7.8	> 1000	> 1000	> 1000
28e		2-(ethyl)butyl	> 1000	14.6 ± 1.5	> 1000	> 1000	> 1000
28f		isobutyl	N.T.	194.5 ± 35.9	N.T.	N.T.	N.T.
28g		isobutyl	> 1000	38.5 ± 6.3	> 1000	> 1000	> 1000

[a] IC_{50} values were determined by at least two independent experiments, each run was performed in duplicate; for compounds with IC_{50} < 100 nM, at least three independent experiments were performed, each run was performed in duplicate; N.T. means not test; [b] reference[25]; [c] reference[21]

identified compounds **28a-g**. The piperdine heterocycle moiety with isobutyl or 2-(ethyl)butyl side chain was retained because these two side chains were discovered as favorable pharmacophores in current analogues. As shown in Table 2, the aryloxy moieties containing compounds exhibited improved S1PR2 binding activity compared to compound **17c**. Compound **28a** showed an IC_{50} value of 188.5 nM; compounds **28b** and **28c**, bearing a cyclization of carbamide group exhibited good S1PR2 binding potency that compound **28b** had an IC_{50} value of 73.3 nM and compound **28c** had an IC_{50} value of 29.9 nM; Compound **28d** possessing a fluoroethyl carbamide group had an IC_{50} value of 66.7 nM; compound **28e**, containing a 4-(methylsulfonyl)benzene-thiol ether moiety was the most potent S1PR2 compound with an IC_{50} value of 14.6 nM, which is more potent than the 4-pyridineoxyl lead compound **2** having IC_{50} value of 45 nM. Additionally, both of compounds **28f** (IC_{50} = 194.5 nM) and **28g** (IC_{50} = 38.5 nM) possessing an isobutyl side chain exhibited less potency for S1PR2 than the corresponding **28d** and **28e**, suggesting 2-(ethyl)butyl side chain is the most favorable moiety for S1PR2 binding activity. The representative competitive binding curves of compounds **JTE-013**, **28b**, **28c**, **28d**, **28e**, and **28g** toward S1PR2 were shown in Figure 3. Six different concentrations from 0.01 to 1000 nM were used to determine the binding potencies. As shown in the competitive binding curves, compound **28e** has the best inhibition effect at 1.0 nM compared to the other compounds and it gave the best binding potency toward S1PR2 with an average IC_{50} value of 14.6 nM.

From the *in vitro* binding data shown in Table 1 and Table 2, the following structure-activity relationship information was generated: a) when the 5-position substitution groups were introduced into the fragment A, the binding potency order of the substituents is aryloxy > CF_3 > alkoxyl and *N*-containing heterocycles, the aryloxy group play an important role in regulating the S1PR2 binding activity; b) when the 4'-position substitution groups were introduced into the fragment B, the stronger electronegativity of substituents offered the higher S1PR2 binding activity with the order as SO_2CH_3 > $CONHCH_3$ > F > $CONH_2$, $CON(CH_3)_2$, $COOCH_3$, OCH_3; c) when optimizing the fragment C, comparing with different group, the piperidine ring was the most favorable heterocycle and the steric hindrance of side chains resulted in increased S1PR2 binding potency with the order as 2-(ethyl)butyl > isobutyl > isopropyl, ethyl, and methyl. The structure-activity relationship information of this scaffold is valuable for guiding the future design of new S1PR2 compounds.

Because compounds **28b**, **28c**, **28d**, **28e**, and **28g** showed high S1PR2 binding potency with IC_{50} < 100 nM, their binding potencies toward the other S1P receptor subtypes, S1PR1, 3, 4, and 5 were also assessed to determine their *in vitro* binding selectivity for S1PR2. As shown in Table 2, all five compounds had no significant binding toward the other four subtypes

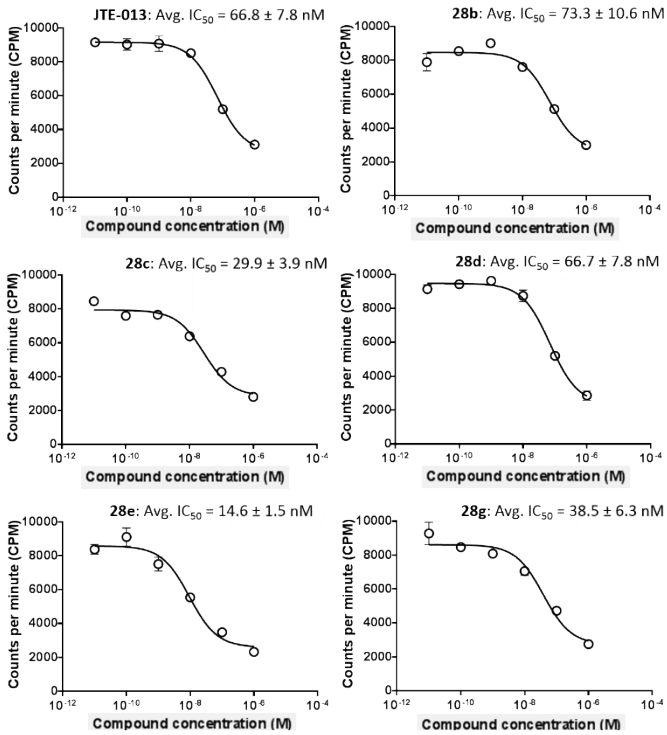


Figure 3. The representative competitive binding curves of compounds **JTE-013**, **28b**, **28c**, **28d**, **28e**, and **28g** toward S1PR2. The averaged IC_{50} values were obtained from three independent experiments.

S1PR1,3,4, and 5 (IC_{50} > 1000 nM), indicating that they are highly selective for S1PR2.

3. Conclusion

In summary, we successfully synthesized a series of ligands for S1PR2. The *in vitro* data suggested that compounds **28c**, **28e**, and **28g** exhibit high S1PR2 binding potencies with IC_{50} values of 29.9, 14.6, and 38.5 nM, respectively. They are more potent for S1PR2 than compound JTE-013 and lead compounds **1** and **2**. In addition, compounds **28c**, **28e**, and **28g** also displayed high selectivity over S1PR 1, 3, 4, and 5. The initial structure-activity relationship analysis indicates that aryloxy substitution at 5-position of fragment A plays a key role in retaining the high potency for S1PR2; the strong electronegativity substituent at 4'-position of fragment B is favorable to the S1PR2 binding; the piperidine ring with steric hindrance side chain in fragment C also provides S1PR2 favorable binding. Three S1PR2 potent compounds **28c**, **28e**, and **28g** contain fluorine atom that provides the position for introducing F-18 isotope to make the F-18 labeling counterparts. Further evaluation of the F-18 labeled radiotracer may lead to identify an F-18 PET radiotracer for imaging S1PR2 *in vivo*. Further characterizations of their *in vitro* and *in vivo* suitability to be PET radiotracers for assessing the S1PR2 expression in living animals are ongoing in our lab.

4. Experimental

4.1 Chemistry

Commercially available starting materials, reagents, and solvents were used as received. Unless otherwise indicated, all reactions were conducted in oven-dried glassware. In general, anhydrous reactions were performed under nitrogen. Reactions were monitored by thin-layer chromatography (TLC) carried out on pre-coated glass plates of silica gel (0.25 mm) 60 F_{254} from EMD Chemicals Inc. Visualization was accomplished with ultraviolet light (UV 254 nm), or by shaking the TLC plate in a sealed jar containing silica gel and iodine. Flash column chromatography was performed using 230–400 mesh silica gel purchased from Silicycle. All work-up and purification procedures were carried out with reagent grade solvents in the air. Yields refer to isolate yield unless otherwise stated. Melting points were determined on a MEL-TEMP 3.0 apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 400 MHz instrument. Chemical shifts are reported in parts per million (ppm) and are calibrated using residual undeuterated solvent as an internal reference ($CDCl_3$: δ 7.26 ppm; CD_3OD: δ 3.31 ppm; DMSO: δ 2.50 ppm). Data are reported as follows: chemical shift, multiplicity, coupling constants (Hz), and integration. High resolution positive ion mass (HRMS) analyses were conducted on a Bruker MaXis 4G Q-TOF mass spectrometer with electrospray ionization source.

*4.1.1 Synthesis of 4-(2-ethylbutyl)piperidin-4-ol (**6**)*

To a dried round two neck bottomed flask equipped with a magnetic stir bar were added 0.6 M $LaCl_3$-2LiCl in THF (12.5 mL, 7.5 mmol) under nitrogen, 0.25 M 2-ethylbutylmagnesium bromide in THF (30 mL, 7.5 mmol) was added slowly through syringe at 0 °C. After stirring at room temperature for 3 h, a solution of benzyl 4-oxopiperidine-1-carboxylate (**5**) (1.2 g, 5.0 mmol) in THF (5.0 mL) was added into the mixture. The reaction was stirred for another18 h until the reaction was completed as determined by TLC and then quenched with 25% acetic acid. The mixture was extracted with ethyl acetate, the ethyl acetate layer was washed with saturated brine and dried over anhydrous MgSO4. After filtration and concentration, the crude product was used directly for the next step without purification.

To a round-bottomed flask equipped with a magnetic stir bar were added above crude product, 10% Pd/C (0.2 g) and methanol (10.0 mL). The reaction was bubbled with hydrogen gas for 6 h at room temperature until the reaction was completed as determined by TLC and then filtered through celite. The filtrate was concentrated under reduced pressure to afford yellow oil product **6**. Yield: 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.18 (s, 1H), 2.98 (d, *J* = 33.4 Hz, 4H), 1.96 (s, 1H), 1.63 (d, *J* = 24.9 Hz, 4H), 1.46 – 1.30 (m, 7H), 0.86 (t, *J* = 7.1 Hz, 6H).

*4.1.2 General procedure to synthesize **8a-b**.*

A solution of 2-fluoroethanol or 3-fluoropyopan-1-ol (1.0 eq) in DMF (0.25 M) was stirred and cooled to 0 °C, then NaH (2.0 eq) was added. After stirring for 15 min, a solution of 1,3-difluoro-5-nitrobenzene (**7**) (1.0 eq) in DMF (0.5 M) was added to the mixture and stirred at room temperature for 12 h until the reaction was completed as determined by TLC. The reaction then was diluted with water and extracted with ethyl acetate. The organic layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was purified on a silica gel column, eluted with hexane/ethyl acetate to afford **8a-b**.

*4.1.2.1 1-Fluoro-3-(2-fluoroethoxy)-5-nitrobenzene (**8a**).*

Compound **8a** was eluted with hexane/ethyl acetate (10/1, V/V) as yellow oil. Yield: 33%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 7.55 (m, 2H), 6.99 (dt, J = 9.6, 2.3 Hz, 1H), 4.87 – 4.83 (m, 1H), 4.75 – 4.71 (m, 1H), 4.35 – 4.31 (m, 1H), 4.29 – 4.25 (m, 1H).

*4.1.2.2 1-Fluoro-3-(3-fluoropropoxy)-5-nitrobenzene (**8b**)*

Compound **8b** was eluted with hexane/ethyl acetate (10/1, V/V) as yellow oil. Yield: 35%. ^{1}H NMR (400 MHz, $CDCl_3$) δ

7.58 – 7.56 (m, 1H), 7.55 – 7.50 (m, 1H), 6.98 – 6.92 (m, 1H), 4.71 (t, J = 5.7 Hz, 1H), 4.59 (t, J = 5.7 Hz, 1H), 4.17 (t, J = 6.1 Hz, 2H), 2.29 – 2.14 (m, 2H).

*4.1.3 General procedure to synthesize **9a-b**.*

To a round-bottomed flask equipped with a magnetic stir bar was added **8a**–**b** (1.0 eq), 4-fluorophenol (1.2 eq), potassium phosphate (2.0 eq), and DMA (1.0 M). The reaction vessel was immersed in a 100 °C preheated oil bath for 12 h until the reaction was completed as determined by TLC. After cooling, the reaction was diluted with water and extracted with ethyl acetate. The organic layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was pure enough for the next step.

*4.1.3.1 1-(2-Fluoroethoxy)-3-(4-fluorophenoxy)-5-nitrobenzene (**9a**).*

Yellow oil, yield: 99%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (t, *J* = 2.1 Hz, 1H), 7.37 (t, *J* = 2.1 Hz, 1H), 7.14 – 6.99 (m, 4H), 6.83 (t, *J* = 2.3 Hz, 1H), 4.83 – 4.79 (m, 1H), 4.71 – 4.68 (m, 1H), 4.31 – 4.26 (m, 1H), 4.24 – 4.20 (m, 1H).

*4.1.3.2 1-(4-Fluorophenoxy)-3-(3-fluoropropoxy)-5-nitrobenzene (**9b**).*

Yellow oil, yield: 91%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (s, 1H), 7.34 (s, 1H), 7.14 – 6.98 (m, 4H), 6.80 (s, 1H), 4.70 (t, *J* = 5.9 Hz, 1H), 4.58 (t, *J* = 5.9 Hz, 1H), 4.19 – 4.11 (m, 2H), 2.25 – 2.14 (m, 2H).

*4.1.4 General procedure to synthesize **10a-b**.*

To a round-bottomed flask equipped with a magnetic stir bar were added **9a-b** (1.0 eq), 10% Pd/C, and ethyl acetate (0.05 M). The reaction was bubbled with hydrogen gas for 12 h at room temperature until the reaction was completed as determined by TLC. The mixture then was filtered through celite. To the filtrate was added $NaHCO_3$ (2.0 eq) followed by adding 2,2,2-trichloroethyl chloroformate (1.0 eq) slowly through syringe under nitrogen at 0 ºC. The reaction was warmed to room temperature and stirred for 3 h until the reaction was completed as determined by TLC. The mixture then was washed with water, saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was used directly for the next step without further purification.

*4.1.5 General procedure to synthesize **11a-b**.*

To a round-bottomed flask equipped with a magnetic stir bar were added **10a–b** (1.0 eq), **6** (1.2 eq), DIPEA (2.0 eq), and DMA. The reaction vessel was immersed in a 100 °C preheated oil bath for 12 h until the reaction was completed as determined by TLC. After cooling, the reaction mixture was diluted with water and extracted with ethyl acetate. The ethyl acetate layer was washed with 1 N HCl, saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was purified on a silica gel column, eluted with hexane/ethyl acetate to afford **11a-b**.

*4.1.5.1 4-(2-Ethylbutyl)-N-(3-(2-fluoroethoxy)-5-(4-fluorophenoxy)phenyl)-4-hydroxypiperidine-1-carboxamide (**11a**).*

Compound **11a** was eluted with hexane/ethyl acetate (2/3, V/V) as yellow oil. Yield: 97%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 – 6.74 (m, 5H), 6.57 (s, 1H), 6.51 (s, 1H), 6.21 (s, 1H), 4.78 – 4.72 (m, 1H), 4.67 – 4.60 (m, 1H), 4.22 – 4.16 (m, 1H), 4.15 – 4.09 (m, 1H), 3.83 – 3.72 (m, 2H), 3.35 – 3.22 (m, 2H), 1.64 – 1.57 (m, 4H), 1.42 – 1.31 (m, 8H), 0.85 (t, *J* = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.93 (d, *J* = 243.4 Hz), 159.90, 159.04, 154.32, 152.31 (d, *J* = 2.0 Hz), 141.31, 120.89 (d, *J* = 8.1 Hz), 116.25 (d, *J* = 24.2 Hz), 102.18, 100.41, 99.87, 81.74 (d, *J* = 171.7 Hz), 70.17, 67.23 (d, *J* = 21.2 Hz), 46.74, 40.59, 37.00, 35.37, 27.30, 10.81. HRMS (ESI) m/z $[M + H]^+$ calcd. for $C_{26}H_{35}F_2N_2O_4$ 477.2559, found 477.2550.

*4.1.5.2 4-(2-Ethylbutyl)-N-(3-(4-fluorophenoxy)-5-(3-fluoropropoxy)phenyl)-4-hydroxypiperidine-1-carboxamide (**11b**).*

Compound **11b** was eluted with hexane/ethyl acetate (1/1, V/V) as yellow oil. Yield: 98%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 – 6.95 (m, 4H), 6.92 – 6.89 (m, 1H), 6.47 (s, 1H), 6.36 (s, 1H), 6.19 (s, 1H), 4.66 (t, *J* = 5.8 Hz, 1H), 4.54 (t, *J* = 5.8 Hz, 1H), 4.04 (t, *J* = 6.1 Hz, 2H), 3.78 (d, *J* = 12.8 Hz, 2H), 3.33 – 3.24 (m, 2H), 2.18 – 2.06 (m, 2H), 1.63 – 1.58 (m, 4H), 1.41 – 1.34 (m, 7H), 1.08 (s, 1H), 0.85 (t, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.28, 158.93, 158.85 (d, *J* = 242.4 Hz), 154.47, 152.45 (d, *J* = 2.0 Hz), 141.37, 120.77 (d, *J* = 8.1 Hz), 116.21 (d, *J* = 23.2 Hz), 101.95, 100.60, 99.58, 80.65 (d, *J* = 164.6 Hz), 70.20, 63.63 (d, *J* = 6.1 Hz), 46.75, 40.44, 37.05, 35.35, 30.26 (d, *J* = 20.2 Hz), 27.29, 10.81. HRMS (ESI) m/z $[M + H]^+$ calcd. for $C_{27}H_{37}F_2N_2O_4$ 491.2716, found 491.2709.

*4.1.6 1-(4-Fluorophenoxy)-3-nitro-5-(trifluoromethyl)benzene (**13**).*

To a round-bottomed flask equipped with a magnetic stir bar was added 1-fluoro-3-nitro-5-(trifluoromethyl)benzene (2.5 g, 12 mmol), 4-fluorophenol (**12**) (1.1 g, 10 mmol), potassium phosphate (4.2 g, 20 mmol), and DMA (15 mL). The reaction vessel was immersed in a 100 °C preheated oil bath for 12 h until the reaction was completed as determined by TLC. After cooling, the reaction was diluted with water and extracted with ethyl acetate. The ethyl acetate layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was purified on a silica gel column, eluted with hexane/ethyl acetate (50/1, V/V) to afford **13**. Yield: 93%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.89 (t, *J* = 2.1 Hz, 1H), 7.52 (s, 1H), 7.21 – 7.12 (m, 2H), 7.12 – 7.05 (m, 2H).

*4.1.7 Synthesis of 1-fluoro-3-(4-fluorophenoxy)-5-nitrobenzene (**14**).*

To a round-bottomed flask equipped with a magnetic stir bar were added 1,3-difluoro-5-nitrobenzene (**7**) (8.0 g, 50 mmol), 4-fluorophenol (**12**) (6.2 g, 55 mmol), Cs_2CO_3 (17.9 g, 55 mmol), and DMA (80 mL). The reaction vessel was immersed in a 65 °C preheated oil bath for 12 h until the reaction was completed as determined by TLC. After cooling, the reaction was diluted with water and extracted with ethyl acetate. The ethyl acetate layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was pure enough for the next step. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 8.1 Hz, 1H), 7.54 (s, 1H), 7.13 – 7.03 (m, 4H), 6.96 (d, *J* = 9.2 Hz, 1H).

*4.1.8 General procedure to synthesize **15a-b**.*

To a round-bottomed flask equipped with a magnetic stir bar was added **14** (1.0 eq), 1-methylpiperazine or 1*H*-pyrazole (1.0 eq), K_2CO_3 (1.0 eq), and DMSO (2.0 M). The reaction vessel was immersed in a 65 °C preheated oil bath for 12 h until the reaction was completed as determined by TLC. After cooling, the reaction was diluted with water and extracted with ethyl acetate. The ethyl acetate layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude

product was purified on a silica gel column, eluted with hexane/ethyl acetate to afford **15a–b**.

*4.1.8.1 1-(3-(4-Fluorophenoxy)-5-nitrophenyl)-4-methylpiperazine (**15a**).*

Compound **15a** was eluted with hexane/ethyl acetate (1/5, V/V) as yellow oil. Yield: 42%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (s, 1H), 7.14 – 6.97 (m, 5H), 6.79 (s, 1H), 3.34 – 3.22 (m, 4H), 2.61 – 2.51 (m, 4H), 2.36 (s, 3H).

*4.1.8.2 1-(3-(4-Fluorophenoxy)-5-nitrophenyl)-1H-pyrazole (**15b**).*

Compound **15b** was eluted with hexane/ethyl acetate (5/1, V/V) as yellow semi-solid. Yield: 53%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.98 (d, J = 2.4 Hz, 1H), 7.73 (d, J = 12.4 Hz, 2H), 7.62 (s, 1H), 7.18 – 7.05 (m, 4H), 6.53 (s, 1H).

*4.1.9 General procedure to synthesize **16a-c**.*

To a round-bottomed flask equipped with a magnetic stir bar were added **13** or **15a–b** (1.0 eq), 10% Pd/C, and ethyl acetate (0.2 M). The reaction was bubbled with hydrogen gas for 12 h at room temperature until the reaction was completed as determined by TLC. The mixture then was filtered through celite. To the filtrate was added $NaHCO_3$ (2.0 eq) followed by adding 2,2,2-trichloroethyl chloroformate (1.0 eq) slowly through syringe under nitrogen at 0 ºC. The reaction was warmed to room temperature and stirred for 3 h until the reaction was completed as determined by TLC. Then, the mixture was washed with water, saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was used directly for the next step without further purification.

*4.1.10 General procedure to synthesize **17a-c**.*

To a round-bottomed flask equipped with a magnetic stir bar were added **16a–c** (1.0 eq), **6** (1.2 eq), DIPEA (2.0 eq) and DMA (0.5 M). The reaction vessel was immersed in a 100 °C preheated oil bath for 12 h until the reaction was completed as determined by TLC. After cooling, the reaction was diluted with water and extracted with ethyl acetate. The ethyl acetate layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was purified on a silica gel column to afford **17a–c**.

*4.1.10.1 4-(2-Ethylbutyl)-N-(3-(4-fluorophenoxy)-5-(4-methylpiperazin-1-yl)phenyl)-4-hydroxypiperidine-1-carboxamide (**17a**).*

Compound **17a** was eluted with ethyl acetate/methanol (5/1, V/V) as yellow oil. Yield: 20%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 – 6.91 (m, 5H), 6.45 (s, 1H), 6.32 (s, 1H), 6.21 (s, 1H), 3.80 – 3.71 (m, 2H), 3.31 – 3.20 (m, 2H), 3.16 (s, 4H), 2.50 (s, 4H), 2.31 (s, 3H), 1.65 – 1.56 (m, 4H), 1.40 – 1.25 (m, 8H), 0.83 (t, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.69, 158.68 (d, J = 242.4), 154.78, 153.02 (d, J = 2.02), 152.93, 141.33, 120.40 (d, J = 8.08), 116.19 (d, J = 23.2), 102.20, 100.93, 100.78, 70.22, 54.99, 48.58, 46.86, 46.11, 40.54, 37.14, 35.42, 27.37, 10.92. HRMS (ESI) m/z $[M + H]^+$ calcd. for $C_{29}H_{42}FN_4O_3$ 513.3235, found 513.3226.

*4.1.10.2 4-(2-Ethylbutyl)-N-(3-(4-fluorophenoxy)-5-(1H-pyrazol-1-yl)phenyl)-4-hydroxypiperidine-1-carboxamide (**17b**).*

Compound **17b** was eluted with hexane/ethyl acetate (2/1, V/V) as yellow semi-solid. Yield: 30%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.65 (s, 1H), 7.50 (s, 1H), 7.08 – 6.90 (m, 6H), 6.80 (s, 1H), 6.41 (s, 1H), 3.85 – 3.70 (m, 2H), 3.32 – 3.21 (m, 2H), 1.57 (s, 4H), 1.42 – 1.30 (m, 7H), 1.26 (s, 1H), 0.84 (t, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.17 (d, J = 243.4 Hz), 159.12, 154.56, 152.32 (d, J = 3.0 Hz), 141.87, 141.52, 141.21, 127.21, 121.03 (d, J = 8.8 Hz), 116.54 (d, J = 23.2 Hz), 107.82, 107.19, 105.13, 103.44, 70.31, 46.89, 40.58, 37.18, 35.47, 27.43, 10.96. HRMS (ESI) m/z $[M + H]^+$ calcd. for $C_{27}H_{34}FN_4O_3$ 481.2609, found 481.2603.

*4.1.10.3 4-(2-Ethylbutyl)-N-(3-(4-fluorophenoxy)-5-(trifluoromethyl)phenyl)-4-hydroxypiperidine-1-carboxamide (**17c**).*

Compound **17c** was eluted with hexane/ethyl acetate (2/1, V/V) as yellow solid. Yield: 44%, M.P. 108 – 111 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, J = 21.6 Hz, 2H), 7.04 – 6.84 (m, 5H), 6.75 (s, 1H), 3.84 – 3.67 (m, 2H), 3.32 - 3.12 (m, 2H), 1.81 (s, 1H), 1.62 – 1.44 (m, 4H), 1.37 – 1.21 (m, 7H), 0.90 – 0.72 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.18 (d, J = 244.4 Hz), 158.54, 154.26, 151.73, 141.51, 132.12 (d, J = 32.3 Hz), 123.55 (d, J = 273.7 Hz), 121.04 (d, J = 8.1 Hz), 116.53 (d, J = 23.2 Hz), 112.04, 110.77, 108.63, 70.14, 46.73, 40.43, 37.01, 35.32, 27.27, 10.78. HRMS (ESI) m/z $[M + Na]^+$ calcd. for $C_{25}H_{30}F_4N_2NaO_3$ 505.2085, found 505.2078.

*4.1.11 General procedure to synthesize **19a-f**.*

To a round-bottomed flask equipped with a magnetic stir bar was added 1-fluoro-3-nitro-5-(trifluoromethyl)benzene (**18**) (1.0 eq), 4-substituted phenol (1.0 eq), potassium phosphate (2 eq), and DMA (2.0 M). The reaction vessel was immersed in a 100 °C preheated oil bath for 12 h until the reaction was completed as determined by TLC. After cooling, the reaction was diluted with water and extracted with ethyl acetate. The organic layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was purified on a silica gel column, eluted with hexane/ethyl acetate to afford **19a–f**.

*4.1.11.1 1-(4-Methoxyphenoxy)-3-nitro-5-(trifluoromethyl)benzene (**19a**).*

Coupling of 1-fluoro-3-nitro-5-(trifluoromethyl)benzene (**18**) (1.0 g, 4.8 mmol) with 4-methoxyphenol (0.5 g, 4.0 mmol) yielded **19a** (1.4 g, 99%), eluted with hexane/ethyl acetate (20/1, V/V). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.86 (s, 1H), 7.50 (s, 1H), 7.10 - 6.92 (m, 5H), 3.85 (s, 3H).

*4.1.11.2 4-(3-Nitro-5-(trifluoromethyl)phenoxy)benzamide (**19b**).*

Coupling of 1-fluoro-3-nitro-5-(trifluoromethyl)benzene (**18**) (1.1 g, 5.0 mmol) with 4-hydroxybenzamide (0.7 g, 5.0 mmol) yielded **19b** (1.6 g, 99%), eluted with hexane/ethyl acetate (1/2, V/V). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.93 (s, 1H), 7.85 (d, J = 8.9 Hz, 2H), 7.53 (s, 1H), 7.08 (d, J = 8.8 Hz, 2H).

*4.1.11.3 N-methyl-4-(3-nitro-5-(trifluoromethyl)phenoxy)benzamide (**19c**).*

Coupling of 1-fluoro-3-nitro-5-(trifluoromethyl)benzene (**18**) (1.8 g, 8.7 mmol) with 4-hydroxy-*N*-methylbenzamide (1.1 g, 7.3 mmol) yielded **19c** (2.5 g, 99%), eluted with hexane/ethyl acetate (1/2, V/V). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.97 (s, 1H), 7.86 (d, J = 8.8 Hz, 2H), 7.58 (s, 1H), 7.13 (d, J = 8.9 Hz, 2H), 6.13 (s, 1H), 3.04 (d, J = 4.9 Hz, 3H).

*4.1.11.4 N, N-dimethyl-4-(3-nitro-5-(trifluoromethyl)phenoxy)benzamide (**19d**).*

Coupling of 1-fluoro-3-nitro-5-(trifluoromethyl)benzene (**18**) (1.1 g, 5.0 mmol) with 4-hydroxy-*N*,*N*-dimethylbenzamide (0.8 g, 5.0 mmol) yielded **19d** (1.8 g, 99%), eluted with hexane/ethyl acetate (1/1, V/V). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H),

7.98 (s, 1H), 7.60 – 7.51 (m, 3H), 7.15 – 7.08 (m, 2H), 3.09 (s, 6H).

*4.1.11.5 Methyl 4-(3-nitro-5-(trifluoromethyl)phenoxy)benzoate (**19e**).*

Coupling of 1-fluoro-3-nitro-5-(trifluoromethyl)benzene (**18**) (1.1 g, 5.0 mmol) with methyl- 4-hydroxybenzoate (0.8 g, 5.0 mmol) yielded **19e** (1.0 g, 60%), eluted with hexane/ethyl acetate (10/1, V/V). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.13 (d, *J* = 8.2 Hz, 2H), 8.00 (s, 1H), 7.60 (s, 1H), 7.12 (d, *J* = 8.9 Hz, 2H), 3.94 (s, 3H).

*4.1.11.6 1-(4-(Methylsulfonyl)phenoxy)-3-nitro-5-(trifluoromethyl)benzene (**19f**).*

Coupling of 1-fluoro-3-nitro-5-(trifluoromethyl)benzene (**18**) (2.5 g, 12.0 mmol) with 4-(methylsulfonyl)phenol (1.7 g, 10.0 mmol) yielded **19f** (2.8 g, 78%), eluted with hexane/ethyl acetate (3/2, V/V). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 – 8.30 (m, 1H), 8.07 – 8.01 (m, 3H), 7.67 – 7.63 (m, 1H), 7.26 – 7.20 (m, 2H), 3.11 (s, 3H).

*4.1.12 General procedure to synthesize **20a-f**.*

To a round-bottomed flask equipped with a magnetic stir bar were added **19a-f** (1.0 eq), 10% Pd/C and ethyl acetate (0.2 M). The reaction was bubbled with hydrogen gas for 12 h at room temperature until the reaction was completed as determined by TLC. The mixture then was filtered through celite. To the filtrate was added $NaHCO_3$ (2.0 eq) followed by adding 2,2,2-trichloroethyl chloroformate (1.0 eq) slowly through syringe under nitrogen at 0 ºC. The reaction was warmed to room temperature and stirred for 3 h until the reaction was completed as determined by TLC. Then, the mixture was washed with water, saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was used directly for the next step without further purification.

*4.1.13 General procedure to synthesize **21a-f**.*

To a round-bottomed flask equipped with a magnetic stir bar were added **20a-f** (1.0 eq), **6** (1.2 eq), DIPEA (2.0 eq), and DMA (1.0 M). The reaction vessel was immersed in a 100 °C preheated oil bath for 12 h until the reaction was completed as determined by TLC. After cooling, the reaction was diluted with water and extracted with ethyl acetate. The ethyl acetate layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was purified on a silica gel column to afford **21a–f**.

*4.1.13.1 4-(2-Ethylbutyl)-4-hydroxy-N-(3-(4-methoxyphenoxy)-5-(trifluoromethyl)phenyl)piperidine-1-carboxamide (**21a**).*

Coupling of **20a** (229 mg, 0.5 mmol) with **6** (86 mg, 0.6 mmol) in the presence of DIPEA afforded **21a** (200 mg, 81%), eluted with hexane/ethyl acetate (1/1, V/V). White solid, M.P. 79 – 81 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (s, 1H), 7.15 (s, 1H), 6.99 (d, *J* = 9.0 Hz, 2H), 6.90 (d, *J* = 9.0 Hz, 2H), 6.82 (s, 1H), 6.46 (s, 1H), 3.90 – 3.83 (m, 2H), 3.81 (s, 3H), 3.32 – 3.22 (m, 2H), 1.65 – 1.58 (m, 5H), 1.35 – 1.18 (m, 6H), 1.04 (s, 1H), 0.93 (d, *J* = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.35, 156.33, 154.18, 149.02, 141.31, 132.07 (d, *J* = 33.3 Hz), 123.60 (d, *J* = 266.6 Hz), 121.13, 115.01, 111.32, 110.19, 108.23, 71.48, 55.57, 45.60, 40.34, 38.02, 33.75, 28.06, 11.34. HRMS (ESI) m/z $[M + Na]^+$ calcd. for $C_{26}H_{33}F_3N_2NaO_4$ 517.2290, found 517.2299.

*4.1.13.2 N-(3-(4-Carbamoylphenoxy)-5-(trifluoromethyl)phenyl)-4-(2-ethylbutyl)-4-hydroxypiperidine-1-carboxamide (**21b**).*

Coupling of **20b** (236 mg, 0.5 mmol) with **6** (95 mg, 0.6 mmol) in the presence of DIPEA afforded **21b** (100 mg, 39%), eluted with ethyl acetate. White solid, M.P. 98 – 101 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.84 (s, 1H), 7.94 (d, *J* = 8.3 Hz, 3H), 7.76 (s, 1H), 7.49 (s, 1H), 7.34 (s, 1H), 7.11 (d, *J* = 8.2 Hz, 2H), 6.92 (s, 1H), 3.85 – 3.70 (m, 2H), 3.14 (t, *J* = 11.6 Hz, 2H), 1.55 – 1.40 (m, 5H), 1.36 – 1.22 (m, 7H), 0.90 (d, *J* = 6.3 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 167.05, 158.32, 156.82, 154.08, 143.62, 130.52 (d, *J* = 32.3 Hz), 129.92, 129.83, 123.81 (d, *J* = 273.7 Hz), 118.38, 111.99, 110.55, 107.92, 68.38, 45.25, 42.06, 38.87, 34.95, 28.75, 11.30. HRMS (ESI) m/z $[M + Na]^+$ calcd. for $C_{26}H_{32}F_3N_3NaO_4$ 530.2243, found 530.2251.

*4.1.13.3 4-(2-Ethylbutyl)-4-hydroxy-N-(3-(4-(methylcarbamoyl)-phenoxy)-5 (trifluoromethyl)phenyl)piperidine-1-carboxamide (**21c**).*

Coupling of **20c** (243 mg, 0.5 mmol) with **6** (112 mg, 0.6 mmol) in the presence of DIPEA afforded **21c** (117 mg, 45%), eluted with hexane/ethyl acetate (1/7, V/V). White solid, M.P. 98 – 100 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.84 (s, 1H), 8.41 (d, *J* = 4.4 Hz, 1H), 7.89 (d, *J* = 8.6 Hz, 2H), 7.76 (s, 1H), 7.50 (s, 1H), 7.12 (d, *J* = 8.6 Hz, 2H), 6.91 (s, 1H), 3.79 (d, *J* = 13.1 Hz, 2H), 3.13 (t, *J* = 11.4 Hz, 2H), 2.78 (d, *J* = 4.4 Hz, 3H), 1.52 – 1.33 (m, 5H), 1.32 – 1.21 (m, 7H), 0.80 (t, *J* = 7.3 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 166.22, 158.58, 157.24, 154.48, 144.05, 130.94 (d, *J* = 31.3 Hz), 130.58, 129.77, 124.24 (d, *J* = 270.7 Hz), 118.85, 112.44, 110.97, 108.35, 68.87, 46.53, 37.13, 35.06, 31.09, 27.18, 26.67, 11.15. HRMS (ESI) m/z $[M + Na]^+$ calcd. for $C_{27}H_{34}F_3N_3NaO_4$ 544.2394, found 544.2391.

*4.1.13.4 N-(3-(4-(Dimethylcarbamoyl)phenoxy)-5-(trifluoromethyl)phenyl)-4-(2-ethylbutyl)-4-hydroxypiperidine-1-carboxamide (**21d**).*

Coupling of **20d** (250 mg, 0.5 mmol) with **6** (95 mg, 0.6 mmol) in the presence of DIPEA afforded **21d** (180 mg, 67%), eluted with hexane/ethyl acetate (1/10, V/V). White solid, M.P. 82 – 84 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.56 (s, 1H), 7.33 (d, *J* = 6.0 Hz, 2H), 7.13 (s, 1H), 6.97 (d, *J* = 6.1 Hz, 2H), 6.89 (s, 1H), 3.90 – 3.65 (m, 2H), 3.30 – 2.86 (m, 8H), 1.56 – 1.44 (m, 6H), 1.40 – 1.30 (m, 5H), 1.25 (s, 1H), 0.93 (d, *J* = 5.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.43, 157.69, 157.31, 154.67, 142.48, 132.35 (d, *J* = 33.3 Hz), 131.41, 129.12, 123.80 (d, *J* = 273.7 Hz), 118.95, 112.28, 111.52, 109.57, 70.14, 45.07, 42.54, 37.27, 35.01, 34.62, 28.38, 11.62. HRMS (ESI) m/z $[M + Na]^+$ calcd. for $C_{28}H_{36}F_3N_3NaO_4$ 558.2556, found 558.2661.

*4.1.13.5 Methyl 4-(3-(4-(2-ethylbutyl)-4-hydroxypiperidine-1-carboxamido)-5-(trifluoromethyl)phenoxy)benzoate (**21e**).*

Coupling of **20e** (487 mg, 1.0 mmol) with **6** (189 mg, 1.2 mmol) in the presence of DIPEA afforded **21e** (280 mg, 54%), eluted with hexane/ethyl acetate (1/1, V/V). White solid, M.P. 80 – 82 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.3 Hz, 2H), 7.42 (s, 1H), 7.00 (d, *J* = 8.3 Hz, 2H), 6.93 (s, 2H), 6.31 (s, 1H), 3.93 – 3.83 (m, 2H), 3.30 – 3.18 (m, 2H), 2.98 (s, 3H), 1.60 – 1.54 (m, 5H), 1.30 – 1.24 (m, 6H), 1.10 (s, 1H), 0.92 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.51, 160.64, 156.63, 154.08, 141.72, 132.49 (d, *J* = 33.3 Hz), 131.79, 125.27, 123.44 (d, *J* = 272.7 Hz), 117.92, 113.65, 111.88, 110.46, 70.13, 52.07, 45.97, 42.47, 37.10, 34.83, 27.28, 11.02. HRMS (ESI) m/z $[M + Na]^+$ calcd. for $C_{27}H_{33}F_3N_2NaO_5$ 545.2239, found 545.2230.

*4.1.13.6 4-(2-Ethylbutyl)-4-hydroxy-N-(3-(4-(methylsulfonyl)phenoxy)-5-(trifluoromethyl)phenyl)piperidine-1-carboxamide (**21f**).*

Coupling of **20f** (253 mg, 0.5 mmol) with **6e** (112 mg, 0.6 mmol) in the presence of DIPEA afforded **21f** (160 mg, 59%), eluted with hexane/ethyl acetate (1/2, V/V). White solid, M.P. 86 – 88 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.9 Hz, 2H), 7.47 – 7.36 (m, 2H), 7.22 – 7.16 (m, 1H), 7.01 (d, *J* = 7.9 Hz, 2H), 6.85 (s, 1H), 3.83 – 3.65 (m, 2H), 3.25 – 3.10 (m, 2H), 2.98 (s, 3H), 1.56 – 1.37 (m, 5H), 1.36 – 1.19 (m, 7H), 0.76 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.52, 155.62, 154.22, 142.24, 134.54, 132.48 (d, *J* = 32.3 Hz), 129.73, 123.39 (d, *J* = 273.7 Hz), 118.23, 114.22, 112.59, 110.56, 70.07, 46.71, 44.71, 40.42, 37.00, 35.29, 27.25, 10.79. HRMS (ESI) m/z $[M + Na]^+$ calcd. for $C_{26}H_{33}F_3N_2NaO_5S$ 565.1954, found 565.1944.

*4.1.14 General procedure to synthesize **22a-d** and **24a-b**.*

To a dried round two neck bottomed flask equipped with a magnetic stir bar were added 0.6 M $LaCl_3$-2LiCl in THF (1.5 eq) under nitrogen, Grignard reagents in THF (1.5 eq) was added slowly through syringe at 0 ºC. After stirring at room temperature for 3 h, a solution of ketones (**5** or **23a-b**) (1.0 eq) in THF (1.0 M) was added into the mixture. The reaction was stirred for another18 h until the reaction was completed as determined by TLC and then quenched with 25% acetic acid. The mixture was extracted with ethyl acetate, the ethyl acetate layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was used directly for the next step without purification.

To a round-bottomed flask equipped with a magnetic stir bar were added above crude product, 10% Pd/C and methanol (0.5 M). The reaction was bubbled with hydrogen gas for 6 h at room temperature until the reaction was completed as determined by TLC and then filtered through celite. The filtrate was concentrated under reduced pressure to afford yellow oil product **22a-d** and **24a-b**.

*4.1.14.1 4-Methylpiperidin-4-ol (**22a**).*

Yield: 93%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 (s, 1H), 3.03 – 2.87 (m, 2H), 2.48 – 2.34 (m, 2H), 1.68 – 1.55 (m, 4H), 1.26 (s, 3H).

*4.1.14.2 4-Ethylpiperidin-4-ol (**22b**).*

Yield: 98%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (s, 1H), 3.00 – 2.86 (m, 2H), 2.44 – 2.23 (m, 2H), 1.72 – 1.39 (m, 7H), 0.95 – 0.80 (m, 3H).

*4.1.14.3 4-Isopropylpiperidin-4-ol (**22c**).*

Yield: 97%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.06 – 2.84 (m, 4H), 1.67 – 1.51 (m, 5H), 0.90 (d, *J* = 6.9 Hz, 6H).

*4.1.14.4 4-Isobutylpiperidin-4-ol (**22d**).*

Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.44 (s, 1H), 3.38 – 3.17 (m, 4H), 1.88 – 1.76 (m, 4H), 1.48 – 1.40 (m, 2H), 1.24 – 1.17 (m, 2H), 0.95 (d, *J* = 6.1 Hz, 6H).

*4.1.14.5 3-Isobutylazetidin-3-ol (**24a**).*

Yield: 93%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.95 – 3.87 (m, 2H), 3.75 – 3.65 (m, 2H), 1.96 – 1.82 (m, 1H), 1.75 – 1.58 (m, 2H), 0.91 (d, *J* = 5.8 Hz, 6H).

*4.1.14.6 3-Isobutylpyrrolidin-3-ol (**24b**).*

Yield: 92%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.75 – 3.41 (m, 4H), 2.12 – 1.78 (m, 4H), 1.71 – 1.49 (m, 3H), 1.02 – 0.91 (m, 6H).

*4.1.15 General procedure to synthesize **25a-j**.*

To a round-bottomed flask equipped with a magnetic stir bar were added **20c** or **20f** (1.0 eq), **22a-d** or **24a-b** (1.2 eq), DIPEA (2.0 eq), and DMA (1.0 M). The reaction vessel was immersed in a 100 ºC preheated oil bath for 12 h until the reaction was completed as determined by TLC. After cooling, the reaction was diluted with water and extracted with ethyl acetate. The ethyl acetate layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was purified on a silica gel column to afford **25a–j**.

*4.1.15.1 4-Hydroxy-4-methyl-N-(3-(4-(methylcarbamoyl)phenoxy)-5-(trifluoromethyl)phenyl)piperidine-1-carboxamide (**25a**).*

Coupling of **20c** (243 mg, 0.5 mmol) with **22a** (69 mg, 0.6 mmol) in the presence of DIPEA afforded **25a** (160 mg, 62%), eluted with hexane/ethyl acetate (1/5, V/V). White solid, M.P. 103 – 106 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.8 Hz, 2H), 7.50 (s, 1H), 7.40 (s, 1H), 7.27 (s, 1H), 7.12 (d, *J* = 8.7 Hz, 2H), 6.98 (s, 1H), 6.61 (s, 1H), 3.79 – 3.72 (m, 2H), 3.42 – 3.32 (m, 2H), 3.06 (s, 3H), 1.67 – 1.61 (m, 4H), 1.31 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.80, 158.15, 156.81, 154.07, 143.60, 130.51 (d, *J* = 32.3 Hz), 130.16, 129.33, 123.79 (d, *J* = 273.7 Hz), 118.42, 112.05, 110.59, 107.92, 66.02, 40.34, 38.21, 29.70, 26.23. HRMS (ESI) m/z $[M + Na]^+$ calcd. for $C_{22}H_{25}F_3N_3O_4$ 452.1792, found 452.1787.

*4.1.15.2 4-Hydroxy-4-methyl-N-(3-(4-(methylsulfonyl)phenoxy)-5-(trifluoromethyl)phenyl)piperidine-1-carboxamide (**25b**).*

Coupling of **20f** (253 mg, 0.5 mmol) with **22a** (69 mg, 0.6 mmol) in the presence of DIPEA afforded 2**5b** (160 mg, 68%), eluted with hexane/ethyl acetate (1/2, V/V). White solid, M.P. 94 – 96 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 7.4 Hz, 2H), 7.42 (s, 1H), 7.03 – 6.91 (m, 4H), 6.32 (s, 1H), 3.84 – 3.69 (m, 2H), 3.41 – 3.25 (m, 2H), 2.98 (s, 3H), 1.63 – 1.56 (m, 5H), 1.28 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.50, 155.71, 154.15, 142.11, 134.66, 132.59 (d, *J* = 32.3 Hz), 129.74, 123.37 (d, *J* = 273.7 Hz), 118.26, 114.14, 112.54, 110.71, 67.66, 44.67, 40.71, 38.25, 30.18. HRMS (ESI) m/z $[M + H]^+$ calcd. for $C_{21}H_{24}F_3N_2O_5S$ 473.1353, found 473.1343.

*4.1.15.3 4-Ethyl-4-hydroxy-N-(3-(4-(methylcarbamoyl)phenoxy)-5-(trifluoromethyl)phenyl)piperidine-1-carboxamide (**25c**).*

Coupling of **20c** (243 mg, 0.5 mmol) with **22b** (78 mg, 0.6 mmol) in the presence of DIPEA afforded **25c** (160 mg, 69%), eluted with ethyl acetate. White solid, M.P. 104 – 107 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.5 Hz, 2H), 7.42 (s, 1H), 7.28 (s, 1H), 7.01 (d, *J* = 8.4 Hz, 2H), 6.94 (s, 1H), 6.84 (s, 1H), 6.24 (s, 1H), 3.86 – 3.76 (m, 2H), 3.35 – 3.24 (m, 2H), 2.99 (d, *J* = 4.8 Hz, 3H), 1.61 – 1.53 (m, 6H), 1.12 (s, 1H), 0.93 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.79, 158.14, 156.81, 154.06, 143.61, 130.50 (d, *J* = 32.3 Hz), 130.16, 129.33, 123.79 (d, *J* = 273.7 Hz), 118.42, 112.04, 110.61, 107.89, 67.83, 40.05, 35.85, 34.78, 26.22, 7.14. HRMS (ESI) m/z $[M + H]^+$ calcd. for $C_{23}H_{27}F_3N_3O_4$ 466.1948, found 466.1940.

*4.1.15.4 4-Ethyl-4-hydroxy-N-(3-(4-(methylsulfonyl)phenoxy)-5-(trifluoromethyl)phenyl)piperidine-1-carboxamide (**25d**).*

Coupling of **20f** (253 mg, 0.5 mmol) with **22b** (78 mg, 0.6 mmol) in the presence of DIPEA afforded **25d** (240 mg, 99%),

eluted with hexane/ethyl acetate (1/2, V/V). White solid, M.P. 89 – 91 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.7 Hz, 2H), 7.50 (s, 1H), 7.41 (s, 1H), 7.12 (d, *J* = 8.7 Hz, 2H), 6.97 (s, 1H), 6.66 (s, 1H), 3.86 – 3.75 (m, 2H), 3.38 – 3.27 (m, 2H), 3.06 (s, 3H), 1.65 – 1.54 (m, 6H), 1.11 (s, 1H), 0.94 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.51, 155.68, 154.16, 142.17, 134.64, 132.56 (d, *J* = 33.3 Hz), 129.73, 123.38 (d, *J* = 273.7 Hz), 118.23, 114.15, 112.56, 110.68, 110.67, 44.67, 40.45, 36.04, 35.52, 6.96. HRMS (ESI) m/z $[M + H]^+$ calcd. for $C_{22}H_{26}F_3N_2O_5S$ 487.1509, found 487.1502.

*4.1.15.5 4-Hydroxy-4-isopropyl-N-(3-(4-(methylcarbamoyl)phenoxy)-5-(trifluoromethyl)phenyl)piperidine-1-carboxamide (**25e**).*

Coupling of **20c** (243 mg, 0.5 mmol) with **22c** (86 mg, 0.6 mmol) in the presence of DIPEA afforded **25e** (108 mg, 44%), eluted with ethyl acetate. White solid, M.P. 100 – 102 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.5 Hz, 2H), 7.40 (d, *J* = 11.8 Hz, 2H), 7.26 (s, 1H), 7.02 (d, *J* = 8.5 Hz, 2H), 6.95 (s, 1H), 6.52 (s, 1H), 3.91 (s, 3H), 3.83 – 3.76 (m, 2H), 3.38 – 3.28 (m, 2H), 1.90 – 1.80 (m, 1H), 1.65 – 1.62 (m, 2H), 1.45 – 1.41 (m, 2H), 1.06 (s, 1H), 0.98 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.00, 158.89, 157.12, 154.51, 141.94, 132.28 (d, *J* = 33.3 Hz), 130.07, 128.89, 123.53 (d, *J* = 374.7 Hz), 118.55, 112.60, 111.58, 109.88, 71.42, 40.25, 38.00, 33.77, 26.75, 16.33. HRMS (ESI) m/z $[M + H]^+$ calcd. for $C_{24}H_{29}F_3N_3O_4$ 480.2105, found 480.2097.

*4.1.15.6 4-Hydroxy-4-isopropyl-N-(3-(4-(methylsulfonyl)phenoxy)-5-(trifluoromethyl)phenyl)piperidine-1-carboxamide (**25f**).*

Coupling of **20f** (253 mg, 0.5 mmol) with **22c** (86 mg, 0.6 mmol) in the presence of DIPEA afforded **25f** (200 mg, 80%), eluted with hexane/ethyl acetate (1/2, V/V). White solid, M.P. 92 – 104 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.8 Hz, 2H), 7.50 (s, 1H), 7.41 (s, 1H), 7.12 (d, *J* = 8.8 Hz, 2H), 6.97 (s, 1H), 6.63 (s, 1H), 3.91 – 3.84 (m, 2H), 3.32 – 3.23 (m, 2H), 3.06 (s, 3H), 1.66 – 1.58 (m, 5H), 1.06 (s, 1H), 0.93 (d, *J* = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.50, 155.71, 154.08, 142.14, 134.68, 132.59 (d, *J* = 33.3 Hz), 129.74, 123.38 (d, *J* = 271.7 Hz), 118.23, 114.12, 112.48, 110.69, 71.43, 44.67, 40.34, 38.02, 33.75, 16.36. HRMS (ESI) m/z $[M + H]^+$ calcd. for $C_{23}H_{28}F_3N_2O_5S$ 501.1659, found 501.1666.

*4.1.15.7 4-Hydroxy-4-isobutyl-N-(3-(4-(methylcarbamoyl)phenoxy)-5-(trifluoromethyl)phenyl)piperidine-1-carboxamide (**25g**).*

Coupling of **20c** (243 mg, 0.5 mmol) with **22d** (94 mg, 0.6 mmol) in the presence of DIPEA afforded **25g** (159 mg, 64%), eluted with hexane/ethyl acetate (1/7, V/V). White solid, M.P. 190 – 192 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.80 (s, 1H), 8.38 (s, 1H), 7.86 (d, *J* = 7.5 Hz, 2H), 7.72 (s, 1H), 7.47 (s, 1H), 7.09 (d, *J* = 7.4 Hz, 2H), 6.88 (s, 1H), 3.85 – 3.65 (m, 2H), 3.20 – 3.00 (m, 2H), 2.75 (s, 3H), 2.47 (s, 1H), 1.85 – 1.70 (m, 1H), 1.52 – 1.21 (m, 6H), 0.87 (d, *J* = 5.5 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 166.21, 158.57, 157.24, 154.50, 147.82, 144.04, 140.77, 131.09, 130.58, 129.77, 118.86, 112.45, 109.69 (d, *J* = 267.7 Hz), 68.79, 51.67, 40.48, 37.29, 26.67, 25.37, 23.17. HRMS (ESI) m/z $[M + Na]^+$ calcd. for $C_{25}H_{30}F_3N_3NaO_4$ 516.2081, found 516.2069.

*4.1.15.8 4-Hydroxy-4-isobutyl-N-(3-(4-(methylsulfonyl)phenoxy)-5-(trifluoromethyl)phenyl)piperidine-1-carboxamide (**25h**).*

Coupling of **20f** (253 mg, 0.5 mmol) with **22d** (94 mg, 0.6 mmol) in the presence of DIPEA afforded **25h** (210 mg, 82%), eluted with hexane/ethyl acetate (1/2, V/V). White solid, M.P. 87 – 90 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 2H), 7.50 (s, 1H), 7.42 (s, 1H), 7.12 (s, 2H), 6.97 (s, 1H), 6.72 (s, 1H), 3.87 – 3.72 (m, 2H), 3.41 – 3.24 (m, 2H), 3.06 (s, 3H), 1.94 – 1.70 (m, 1H), 1.71 – 1.40 (m, 7H), 1.07 – 0.90 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.52, 155.64, 154.20, 142.21, 134.58, 132.51 (d, *J* = 32.3 Hz), 129.73, 123.38 (d, *J* = 275.7 Hz), 118.22, 114.17, 112.55, 110.60, 70.03, 51.89, 44.67, 40.42, 37.05, 24.82, 23.24. HRMS (ESI) m/z $[M + Na]^+$ calcd. for $C_{24}H_{29}F_3N_2NaO_5S$ 537.1641, found 537.1641.

*4.1.15.9 3-Hydroxy-3-isobutyl-N-(3-(4-(methylcarbamoyl)phenoxy)-5-(trifluoromethyl)phenyl)azetidine-1-carboxamide (**25i**).*

Coupling of **20c** (243 mg, 0.5 mmol) with **24a** (78 mg, 0.6 mmol) in the presence of DIPEA afforded **25i** (110 mg, 47%), eluted with hexane/ethyl acetate (1/5, V/V). White solid, M.P. 107 – 109 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.80 (s, 1H), 8.42 (s, 1H), 7.90 (d, *J* = 7.4 Hz, 2H), 7.77 (s, 1H), 7.52 (s, 1H), 7.12 (d, *J* = 7.5 Hz, 2H), 6.92 (s, 1H), 3.95 – 3.65 (m, 4H), 2.78 (s, 3H), 1.91 – 1.77 (m, 1H), 1.65 – 1.45 (m, 2H), 1.22 (s, 1H), 0.88 (d, *J* = 5.3 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 166.22, 158.46, 157.45, 156.37, 143.47, 131.11 (d, *J* = 32.3 Hz), 130.68, 129.79, 124.19 (d, *J* = 273.7 Hz), 118.98, 111.63, 110.24, 108.36, 69.11, 63.86, 47.30, 26.67, 24.52, 23.50. HRMS (ESI) m/z $[M + H]^+$ calcd. for $C_{23}H_{27}F_3N_3O_4$ 466.1948, found 466.1939.

*4.1.15.10 3-Hydroxy-3-isobutyl-N-(3-(4-(methylcarbamoyl)phenoxy)-5-(trifluoromethyl)phenyl)pyrrolidine-1-carboxamide (**25j**).*

Coupling of **20c** (243 mg, 0.5 mmol) with **24b** (86 mg, 0.6 mmol) in the presence of DIPEA afforded **25j** (100 mg, 42%), eluted with hexane/ethyl acetate (1/5, V/V). White solid, M.P. 113 – 116 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.48 (d, *J* = 45.8 Hz, 2H), 8.11 – 7.43 (m, 4H), 7.13 (s, 2H), 6.93 (s, 1H), 3.55 – 3.40 (m, 2H), 2.90 – 2.67 (m, 2H), 2.10 – 1.68 (m, 3H), 1.60 – 1.37 (m, 2H), 1.24 (s, 1H), 0.93 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 165.81, 158.17, 156.81, 153.43, 143.45, 130.52 (d, *J* = 32.3 Hz), 130.15, 129.35, 123.82 (d, *J* = 271.7 Hz), 118.41, 111.94, 110.49, 107.89, 58.16, 47.16, 45.37, 44.48, 26.25, 24.37, 24.18, 24.13. HRMS (ESI) m/z $[M + H]^+$ calcd. for $C_{24}H_{29}F_3N_3O_4$ 480.2105, found 480.2098.

*4.1.16 General procedure to synthesize **26a-e**.*

To a round-bottomed flask equipped with a magnetic stir bar was added **14** (1.0 eq), corresponding phenols (1.0 eq), K_3PO_4 (2.0 eq) and DMA (1.0 M). The reaction vessel was immersed in a 100 °C preheated oil bath for 12 h until the reaction was completed as determined by TLC. After cooling, the reaction was diluted with water and extracted with ethyl acetate. The organic layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was purified on a silica gel column, eluted with hexane/ethyl acetate to afford **26a–e**.

*4.1.16.1 1-(4-Fluorophenoxy)-3-(4-methoxyphenoxy)-5-nitrobenzene (**26a**).*

Compound **26a** was eluted with hexane/ethyl acetate (10/1, V/V). Yield: 93%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (dd, *J* = 4.8, 2.1 Hz, 2H), 7.12 – 6.96 (m, 6H), 6.96 – 6.89 (m, 2H), 6.88 – 6.84 (m, 1H), 3.80 (s, 3H).

*4.1.16.2 6-(3-(4-Fluorophenoxy)-5-nitrophenoxy)-2-methyl-3,4-dihydroisoquinolin-1(2H)-one (**26b**).*

Compound **26b** was pure enough for next step. Yield: 99%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 8.6 Hz, 1H), 7.49 (d, *J* = 12.3 Hz, 1H), 7.24 (s, 1H), 7.17 – 6.90 (m, 6H), 6.83 (s, 1H), 3.59 (t, *J* = 6.6 Hz, 2H), 3.16 (s, 3H), 3.00 (t, *J* = 6.6 Hz, 2H).

*4.1.16.3 6-(3-(4-Fluorophenoxy)-5-nitrophenoxy)-3,4-dihydroisoquinolin-1(2H)-one (**26c**).*

Compound **26c** was pure enough for next step. Yield: 40%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.74 – 7.35 (m, 2H), 7.19 – 6.78 (m, 6H), 6.65 (s, 1H), 3.74 – 3.47 (m, 2H), 3.11 – 2.84 (m, 2H).

*4.1.16.4 N-(2-Fluoroethyl)-4-(3-(4-fluorophenoxy)-5-nitrophenoxy)benzamide (**26d**).*

Compound **26d** was eluted with hexane/ethyl acetate (3/2, V/V). Yield: 31%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.7 Hz, 2H), 7.51 – 7.45 (m, 2H), 7.14 – 7.10 (m, 4H), 7.08 – 7.04 (m, 2H), 6.95 (t, *J* = 2.2 Hz, 1H), 6.48 (s, 1H), 4.68 (t, *J* = 4.7 Hz, 1H), 4.56 (t, *J* = 4.7 Hz, 1H), 3.83 (dd, *J* = 10.2, 5.1 Hz, 1H), 3.76 (dd, *J* = 10.2, 5.1 Hz, 1H).

*4.1.16.5 (3-(4-Fluorophenoxy)-5-nitrophenyl)(4-(methylsulfonyl-)phenyl)sulfane (**26e**).*

Compound **26e** was eluted with hexane/ethyl acetate (2/1, V/V). Yield: 15%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 – 7.88 (m, 3H), 7.65 (s, 1H), 7.47 (d, *J* = 8.3 Hz, 2H), 7.28 (s, 1H), 7.16 – 7.02 (m, 5H), 3.08 (s, 3H).

*4.1.17 General procedure to synthesize **27a-e**.*

To a round-bottomed flask equipped with a magnetic stir bar were added **26a–e** (1.0 eq), 10% Pd/C and ethyl acetate. The reaction was bubbled with hydrogen gas for 16 h at room temperature until the reaction was completed as determined by TLC. The mixture then was filtered through celite. To the filtrate was added $NaHCO_3$ (2.0 eq) followed by adding 2,2,2-trichloroethyl chloroformate (1.0 eq) slowly through syringe under nitrogen at 0 ºC. The reaction was warmed to room temperature and stirred for 3 h until the reaction was completed as determined by TLC. Then, the mixture was washed with water, saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was directly used for next step without purification.

*4.1.17.1 2,2,2-Trichloroethyl (3-(4-fluorophenoxy)-5-(4-methoxyphenoxy)phenyl)carbamate (**27a**).*

Yield: 96%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 – 6.97 (m, 6H), 6.90 – 6.87 (m, 2H), 6.79 (s, 1H), 6.76 (s, 1H), 6.67 (s, 1H), 6.32 (t, *J* = 2.2 Hz, 1H), 4.76 (s, 2H), 3.80 (s, 3H).

*4.1.17.2 2,2,2-Trichloroethyl (3-(4-fluorophenoxy)-5-((2-methyl-1-oxo-1,2,3,4-tetrahydroisoquinolin-6-yl)oxy)phenyl)carbamate (**27b**).*

Yield: 96%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.5 Hz, 1H), 7.24 (s, 1H), 7.14 – 6.96 (m, 4H), 6.96 – 6.82 (m, 3H), 6.81 – 6.75 (m, 1H), 6.39 (s, 1H), 4.77 (s, 2H), 3.55 (t, *J* = 6.6 Hz, 2H), 3.14 (s, 3H), 2.96 (t, *J* = 6.5 Hz, 2H).

*4.1.17.3 2,2,2-Trichloroethyl (3-(4-fluorophenoxy)-5-((1-oxo-1,2,3,4-tetrahydroisoquinolin-6-yl)oxy)phenyl)carbamate (**27c**).*

Yield: 95%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 – 7.99 (m, 1H), 7.16 (s, 1H), 7.09 – 7.00 (m, 4H), 6.96 – 6.91 (m, 1H), 6.91 – 6.86 (m, 2H), 6.84 – 6.79 (m, 1H), 6.41 (s, 1H), 6.13 (s, 1H), 4.78 (s, 2H), 3.60 – 3.51 (m, 2H), 2.96 (t, *J* = 6.1 Hz, 2H).

*4.1.17.4 2,2,2-Trichloroethyl (3-(4-((2-fluoroethyl)carbamoyl)phenoxy)-5-(4-fluorophenoxy)phenyl)carbamate (**27d**).*

Yield: 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.7 Hz, 2H), 7.10 – 6.97 (m, 7H), 6.88 (s, 1H), 6.83 (s, 1H), 6.45 (s, 1H), 6.39 (t, *J* = 2.1 Hz, 1H), 4.77 (s, 2H), 4.66 (t, *J* = 4.7 Hz, 1H), 4.54 (t, *J* = 4.7 Hz, 1H), 3.81 (dd, *J* = 10.3, 5.0 Hz, 1H), 3.74 (dd, *J* = 10.2, 5.1 Hz, 1H).

*4.1.17.5 2,2,2-Trichloroethyl (3-(4-fluorophenoxy)-5-((4-(methylsulfonyl)phenyl)thio)phenyl)carbamate (**27e**).*

Yield: 99%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.5 Hz, 2H), 7.36 (d, *J* = 8.6 Hz, 2H), 7.12 (s, 1H), 7.08 – 6.98 (m, 6H), 6.90 (s, 1H), 4.79 (s, 2H), 3.04 (s, 3H).

*4.1.18 General procedure to synthesize **28a-g**.*

To a round-bottomed flask equipped with a magnetic stir bar were added **27a-e** (1.0 eq), **6** or **22d** (1.2 eq), DIPEA (2.0 eq) and DMA. The reaction vessel was immersed in a 100 °C preheated oil bath for 12 h until the reaction was completed as determined by TLC. After cooling, the reaction was diluted with water and extracted with ethyl acetate. The ethyl acetate layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was purified on a silica gel column, eluted with hexane/ethyl acetate to afford **28a–g**.

*4.1.18.1 4-(2-Ethylbutyl)-N-(3-(4-fluorophenoxy)-5-(4-methoxyphenoxy)phenyl)-4-hydroxypiperidine-1-carboxamide (**28a**).*

Compound **28a** was eluted with hexane/ethyl acetate (1/1, V/V) as white solid. Yield: 52%, M.P. 79 – 80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (tt, *J* = 10.2, 3.0 Hz, 6H), 6.88 – 6.83 (m, 2H), 6.75 (t, *J* = 2.0 Hz, 1H), 6.64 (t, *J* = 2.0 Hz, 1H), 6.57 (s, 1H), 6.25 (t, *J* = 2.2 Hz, 1H), 3.79 (s, 3H), 3.72 (d, *J* = 12.9 Hz, 2H), 3.31 – 3.21 (m, 2H), 1.59 (dd, *J* = 8.8, 3.8 Hz, 4H), 1.36 (dd, *J* = 15.0, 8.8 Hz, 7H), 0.84 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (101 MHz, CDCl3) δ 160.07, 159.12, 158.91 (d, *J* = 243.4), 156.09, 154.05, 152.28 (d, *J* = 3.0), 149.38, 141.13, 121.07, 120.76 (d, *J* = 8.1), 116.26 (d, *J* = 23.2), 114.84, 103.44, 103.22, 102.33, 70.12, 55.60, 46.71, 40.74, 36.90, 35.36, 27.30, 10.82. HRMS (ESI) m/z $[M + H]^+$ calcd. for $C_{31}H_{38}FN_2O_5$ 537.2759, found 537.2751.

*4.1.18.2 4-(2-Ethylbutyl)-N-(3-(4-fluorophenoxy)-5-((2-methyl-1-oxo-1,2,3,4-tetrahydroisoquinolin-6-yl)oxy)phenyl)-4-hydroxypiperidine-1-carboxamide (**28b**).*

Compound **28b** was eluted with hexane/ethyl acetate (1/3, V/V) as white solid. Yield: 24%, M.P. 80 – 82 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.4 Hz, 1H), 7.07 – 6.95 (m, 3H), 6.94 – 6.84 (m, 2H), 6.81 (s, 1H), 6.75 (s, 1H), 6.57 (s, 1H), 6.32 (s, 1H), 3.85 – 3.71 (m, 2H), 3.53 (t, *J* = 6.5 Hz, 2H), 3.34 – 3.20 (m, 2H), 3.12 (s, 3H), 2.94 (t, *J* = 6.5 Hz, 2H), 1.62 – 1.52 (m, 4H), 1.42 – 1.30 (m, 7H), 1.16 (s, 1H), 0.84 (t, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.49, 159.90, 159.14, 158.92 (d, *J* = 243.4 Hz), 157.12, 154.47, 152.17 (d, *J* = 2.0 Hz), 142.20, 140.19, 130.13, 124.12, 120.82 (d, *J* = 8.1 Hz), 116.48, 116.28 (d, *J* = 23.2 Hz), 116.00, 105.62, 105.11, 103.78, 70.06, 47.98, 46.73, 40.38, 37.05, 35.28, 35.04, 27.91, 27.26, 10.80. HRMS (ESI) m/z $[M + Na]^+$ calcd. for $C_{34}H_{40}FN_3NaO_5$ 612.2844, found 612.2846.

*4.1.18.3 4-(2-Ethylbutyl)-N-(3-(4-fluorophenoxy)-5-((1-oxo-1,2,3,4-tetrahydroisoquinolin-6-yl)oxy)phenyl)-4-hydroxypiperidine-1-carboxamide (**28c**).*

Compound **28c** was eluted with ethyl acetate as white solid. Yield: 26%, M.P. 81 – 83 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.5 Hz, 1H), 7.07 – 6.96 (m, 3H), 6.96 – 6.89 (m, 1H), 6.88 – 6.77 (m, 2H), 6.56 (s, 1H), 6.33 (s, 1H), 5.95 (s, 1H), 3.85 – 3.71 (m, 2H), 3.61 – 3.48 (m, 2H), 3.34 – 3.18 (m, 2H), 2.99 –

2.87 (m, 2H), 1.65 – 1.52 (m, 5H), 1.44 – 1.31 (m, 7H), 1.15 (s, 1H), 0.84 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.00, 160.41, 159.24, 158.97 (d, *J* = 243.4 Hz), 157.03, 154.45, 152.13 (d, *J* = 2.0 Hz), 142.15, 141.14, 130.07, 123.60, 120.88 (d, *J* = 8.1 Hz), 116.52, 116.32 (d, *J* = 23.2 Hz), 116.30, 105.61, 105.10, 103.90, 70.13, 46.72, 40.41, 40.09, 37.06, 35.32, 28.43, 27.27, 10.80. HRMS (ESI) m/z [M + Na]$^+$ calcd. for $C_{33}H_{38}FN_3NaO_5$ 598.2688, found 598.2680.

*4.1.18.4 4-(2-Ethylbutyl)-N-(3-(4-((2-fluoroethyl)carbamoyl)phenoxy)-5-(4-fluorophenoxy)phenyl)-4-hydroxypiperidine-1-carboxamide (**28d**).*

Compound **28d** was eluted with hexane/ethyl acetate (7/3, V/V) as white solid. Yield: 88%, M.P. 79 – 82 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 7.6 Hz, 2H), 7.14 – 6.93 (m, 6H), 6.90 (s, 1H), 6.86 – 6.76 (m, 3H), 6.31 (s, 1H), 4.68 – 4.46 (m, 2H), 3.84 – 3.62 (m, 4H), 3.26 – 3.12 (m, 2H), 1.58 – 1.44 (m, 4H), 1.38 – 1.22 (m, 8H), 0.88 – 0.78 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ 166.16, 159.35, 158.84 (d, *J* = 241.39 Hz), 158.94, 157.41, 154.60, 152.46, 144.02, 129.81, 129.59, 121.50 (d, *J* = 8.1 Hz), 118.39, 117.00 (d, *J* = 24.2 Hz), 104.51, 104.03, 102.45, 82.60 (d, *J* = 166.7 Hz), 68.88, 46.52, 40.38, 37.18, 35.07, 27.18, 11.15. HRMS (ESI) m/z [M + Na]$^+$ calcd. for $C_{33}H_{39}F_2N_3NaO_5$ 618.2750, found 618.2727.

*4.1.18.5 4-(2-Ethylbutyl)-N-(3-(4-fluorophenoxy)-5-((4-(methylsulfonyl)phenyl)thio)phenyl)-4-hydroxypiperidine-1-carboxamide (**28e**).*

Compound **28e** was eluted with hexane/ethyl acetate (2/3, V/V) as white solid. Yield: 27%, M.P. 78 – 80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 8.2 Hz, 2H), 7.21 (s, 1H), 7.14 (s, 1H), 7.05 – 6.93 (m, 4H), 6.72 (s, 1H), 6.59 (s, 1H), 3.85 – 3.70 (m, 2H), 3.24 – 3.15 (m, 2H), 3.02 (s, 3H), 1.65 – 1.53 (m, 4H), 1.44 – 1.28 (m, 7H), 1.14 (s, 1H), 0.84 (t, *J* = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.06 (d, *J* = 243.4 Hz), 159.00, 154.25, 151.94 (d, *J* = 2.0 Hz), 145.85, 142.15, 137.33, 132.65, 127.90, 127.79, 120.92 (d, *J* = 9.1 Hz), 119.08, 116.86, 116.45 (d, *J* = 24.2 Hz), 110.06, 70.11, 46.72, 44.50, 40.39, 37.03, 35.31, 27.28, 10.82. HRMS (ESI) m/z [M + Na]$^+$ calcd. for $C_{31}H_{37}FN_2NaO_5S_2$ 623.2020, found 623.2018.

*4.1.18.6 N-(3-(4-((2-fluoroethyl)carbamoyl)phenoxy)-5-(4-fluorophenoxy)phenyl)-4-hydroxy-4-isobutylpiperidine-1-carboxamide (**28f**).*

Compound **28f** was eluted with hexane/ethyl acetate (1/2, V/V) as white solid. Yield: 70%, M.P. 81 – 83 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.2 Hz, 2H), 7.06 – 6.90 (m, 7H), 6.86 – 6.75 (m, 3H), 6.31 (s, 1H), 4.68 – 4.46 (m, 1H), 4.55 – 4.46 (m, 1H), 3.82 – 3.60 (m, 4H), 3.19 (t, *J* = 11.5 Hz, 2H), 1.87 – 1.74 (m, 1H), 1.60 – 1.46 (m, 4H), 1.39 – 1.28 (m, 3H), 0.93 (d, *J* = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.44, 159.72, 159.28, 158.99 (d, *J* = 243.4 Hz), 157.35, 154.55, 152.04 (d, *J* = 2.0 Hz), 142.05, 128.97, 128.89, 120.96 (d, *J* = 8.1 Hz), 118.10, 116.34 (d, *J* = 23.2 Hz), 105.01, 104.83, 103.55, 82.54 (d, *J* = 167.7 Hz), 70.03, 51.86, 40.54, 40.31, 37.06, 24.84, 23.22. HRMS (ESI) m/z [M + Na]$^+$ calcd. for $C_{31}H_{35}F_2N_3NaO_5$ 590.2437, found 590.2430.

*4.1.18.7 N-(3-(4-Fluorophenoxy)-5-((4-(methylsulfonyl)phenyl)-thio)phenyl)-4-hydroxy-4-isobutylpiperidine-1-carboxamide (**28g**).*

Compound **28g** was eluted with hexane/ethyl acetate (2/3, V/V) as white solid. Yield: 35%, M.P. 79 – 81 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 5.6 Hz, 2H), 7.41 – 7.15 (m, 4H), 7.12 – 6.95 (m, 4H), 6.88 (s, 1H), 6.75 (s, 1H), 3.90 – 3.68 (m, 2H), 3.40 – 3.18 (m, 2H), 3.04 (s, 3H), 1.95 – 1.75 (m, 1H), 1.70 – 1.50 (m, 4H), 1.46 – 1.28 (m, 3H), 0.99 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.10 (d, *J* = 244.4 Hz), 159.07, 154.15, 151.93 (d, *J* = 3.0 Hz), 145.75, 141.99, 137.46, 132.85, 127.99, 127.83, 120.95 (d, *J* = 8.1 Hz), 118.96, 116.95, 116.48 (d, *J* = 23.2 Hz), 109.95, 70.13, 51.94, 44.53, 40.43, 37.10, 24.84, 23.27. HRMS (ESI) m/z [M + Na]$^+$ calcd. for $C_{29}H_{33}FN_2NaO_5S_2$ 595.1707, found 595.1693.

4.2 Sphingosine-1-phosphate receptors binding assay

[^{32}P]S1P was freshly prepared by following our previously published protocol[26] and dissolved in DMSO, which was further diluted to 0.3-0.6 nM with assay buffer (50 mM HEPES-Na, pH 7.5, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% fatty acid-free BSA). The test compounds were dissolved in DMSO and diluted into six different concentrations (0.03, 0.3, 3.0, 30, 300, and 3000 nM) with assay buffer. The commercial cell membranes expressing recombinant human S1PRs were diluted with assay buffer to make a 20-40 μg/mL of solution. To a 96-well plate was added 50 μL of cell membranes, 50 μL of test compounds, and 50 μL of [^{32}P]S1P. Each well has a final volume of 150 μL containing 0.1-0.2 nM of [^{32}P]S1P, 1-2 μg of membrane protein (S1PRs), and different concentrations (0.01-1000 nM) of test compounds. The plate was incubated for 60 min at room temperature with shaking and terminated by collecting the membranes onto 96-well glass fiber (GF/B) filtration plates (Millipore, Billerica, MA). Each filter was washed with 200 μL of assay buffer for a total of five washes. The filter bound radionuclide was measured by a Beckman LS 3801 scintillation counter using Cherenkov counting. The IC_{50} values were fitted from GraphPad Prism 6 using one site Nonlinear Regression.

Abbreviations

S1P, sphingosine 1-phosphate; S1PR, sphingosine 1-phosphate receptor; CNS, central nervous system; HTS, high throughput screening; BBB, blood-brain barrier; PET, positron emission tomography; DMF, *N*,*N*-dimethylformamide; DMA, *N*,*N*-dimethylacetamide; DIPEA, *N*,*N*-diisopropylethylamine; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran; RT, room temperature; TLC, thin-layer chromatography

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Graphical Abstract

Design, synthesis, and in vitro bioactivity evaluation of fluorine-containing analogues for sphingosine-1-phosphate 2 receptor

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Zonghua Luo[a] , Hui Liu[a] , Robyn S. Klein[b,c,d] , and Zhude Tu[a*]

[a] *Department of Radiology, Washington University School of Medicine, St. Louis, MO 63110, USA*

[b] *Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA*

[c] *Department of Neuroscience, Washington University School of Medicine, St. Louis, MO 63110, USA*

[d] *Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA*