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Article

Discovery of Novel Sphingosine-1-Phosphate-1 Receptor Agonists for the Treatment of Multiple Sclerosis

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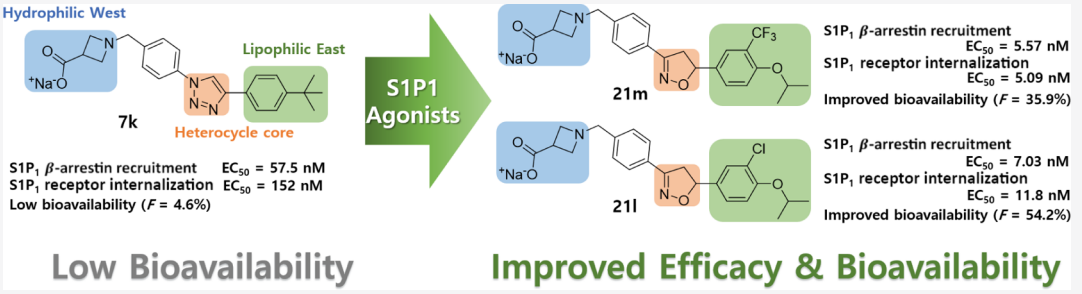

ABSTRACT: The sphingosine-1-phosphate-1 ($S1P_1$) receptor agonists have great potential for the treatment of multiple sclerosis (MS) because they can inhibit lymphocyte egress through receptor internalization. We designed and synthesized triazole and isoxazoline derivatives to discover a novel $S1P_1$ agonist for MS treatment. Of the two scaffolds, the isoxazoline derivative was determined to have excellent *in vitro* efficacy and drug-like properties. Among them, compound **21l** was found to have superior drug-like properties as well as excellent *in vitro* efficacies (EC_{50} = 7.03 nM in β-arrestin recruitment and EC_{50} = 11.8 nM in internalization). We also confirmed that **21l** effectively inhibited lymphocyte egress in the peripheral lymphocyte count test and significantly improved the clinical score in the experimental autoimmune encephalitis MS mouse model.

INTRODUCTION

Multiple sclerosis (MS) is a neuroinflammatory autoimmune disease of the central nervous system (CNS), characterized by demyelination, axonal loss, and paralysis.[1] MS is more commonly found in Caucasians, and 85% of the MS patients belong to the relapsing−remitting MS (RRMS), one of the four classified types of the disease.[2,3] Its pathogenesis is still unclear, but it has been hypothesized that autoreactive T cells migrate across the blood−brain barrier (BBB) and mediate the pathological responses to myelin antigens, resulting in demyelination and neurodegeneration.[4]

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid that mediates diverse biological responses, such as lymphocyte trafficking, cardiac function, inflammation, and vascular development through five related G-protein-coupled receptors (GPCRs), $S1P_{1-5}$ receptors.[5] Of the five receptors, the sphingosine-1-phosphate-1 ($S1P_1$) receptor is responsible for regulating the lymphocytes egress from the lymphoid tissue to the lymph.[6] This particular GPCR has been highlighted as a potential drug target because it has been found that the $S1P_1$ receptor is internalized and degraded by synthetic agonists, resulting in lymphocyte sequestration in the lymph node and immune suppression.[7,8] Thus, such functionally antagonistic $S1P_1$ receptor agonists are considered great therapeutic agents in autoimmune diseases, including MS.

Fingolimod (FTY720, Gilenya) is a representative $S1P_1$ receptor agonist and an immunosuppressant approved in 2010 to be dosed orally for the treatment of RRMS.[9] It is essentially a prodrug as it is readily phosphorylated into FTY720-phosphate (FTY720-P), the active pharmacological species, and displays a full agonistic activity toward S1P receptors *in vivo*.[10] This first-generation MS therapeutic drug is a nonselective agonist of $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$ receptors, which has been shown to have accompanying adverse side effects when orally dosed in clinical studies, such as bradycardia and declining pulmonary function.[11−14]

The off-target activity against the $S1P_3$ receptor was initially thought to be associated with a transient heart rate reduction based on rodent studies.[15,16] Therefore, to reduce these

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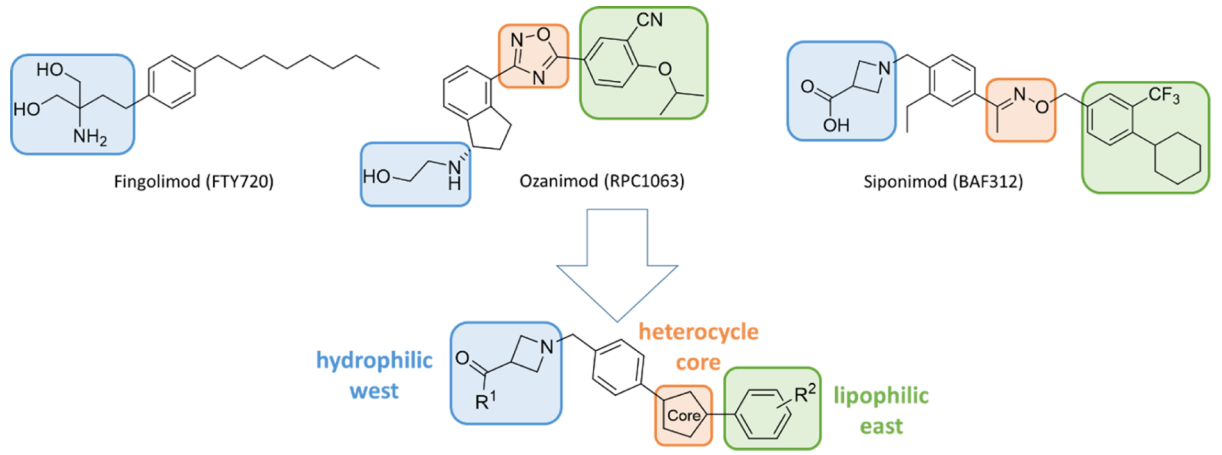
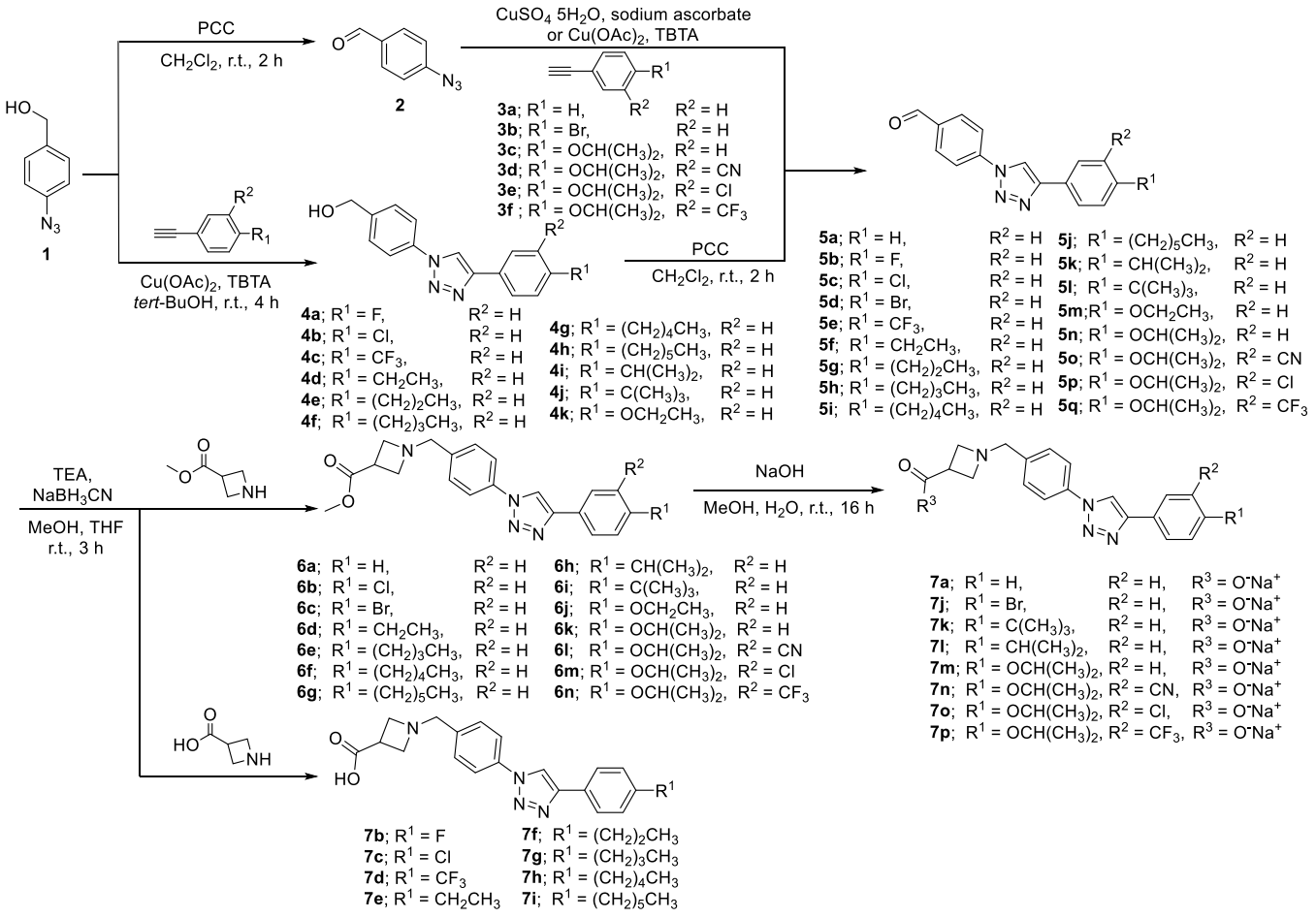


Figure 1. Structural analysis of FDA-approved $S1P_1$ receptor agonists and integration of various heterocyclic cores with the hydrophilic west and lipophilic east regions.

Scheme 1. Synthesis of Compounds 6 and 7



adversities, the selectivity against the $S1P_3$ receptor has been emphasized in developing the second-generation MS drug, such as siponimod (BAF312, Mayzent) and $S1P_3$-sparing $S1P_1$ full agonists.[17−22] Recently, ozanimod (RPC1063, Zeposia), a new selective $S1P_1$ agonist approved by the FDA as an oral treatment for RRMS in 2020, was also developed with the aim of excellent off-target selectivity for the $S1P_3$ receptor to minimize cardiac abnormalities.[23−25] However, recent clinical studies have shown that these rodent studies have not been translated to human studies and that some cardiovascular side effects are still observed with the $S1P_3$-sparing $S1P_1$ receptor agonists.[13,22]

The structures of FTY720, the first $S1P_1$ receptor agonist drug, had undergone structural modifications to improve potency, receptor selectivity, and drug-like properties. Its amino-alcohol group was considered a critical structural element in maintaining potency;[26] thus, hydrophilicity on the west region was retained in later developed agonists, BAF312

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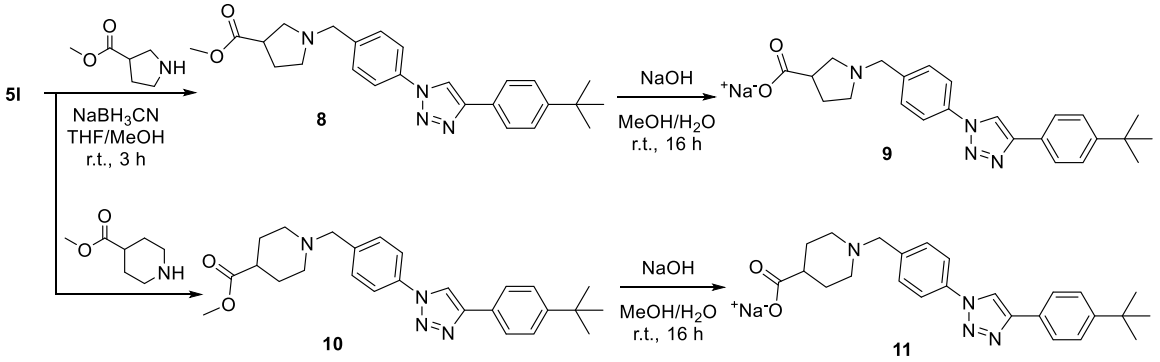
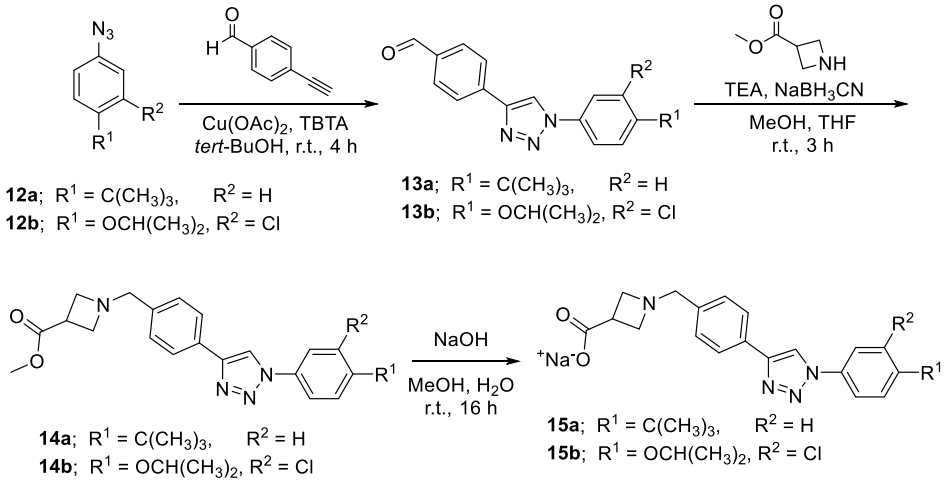
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 2. Synthesis of Compounds 9 and 11



Scheme 3. Synthesis of Compounds 14 and 15



and RPC1063 (Figure 1). Other structural elements of FTY720, such as the phenyl core and the long lipophilic tail, were also modified with other heterocyclic and aryl systems, respectively. Particularly, $S1P_1$ receptor agonists with oxadiazole and thiazole as heterocycle cores have been reported.[27−35] Hence, we have endeavored to develop and synthesize selective $S1P_1$ receptor agonists based on such a structural frame, composed of a heterocycle core with a hydrophilic west region and a lipophilic east region (Figure 1). We introduced triazole or isoxazoline rings as heterocycle cores, aryl groups with various functional groups in the lipophilic east region, and mainly azetidine carboxylic acid in the hydrophilic west region. The synthesized compounds were evaluated for their $S1P_1$ receptor agonistic activities, drug-like properties, and *in vivo* efficacies.

RESULTS AND DISCUSSION

Chemical Synthesis. The synthetic derivatives were divided into three parts: hydrophilic west, heterocycle core, and lipophilic east. Triazole and isoxazoline rings were introduced as the heterocycle core (Figure 1). First, we synthesized triazole derivatives (**6a−6n**, **7a−7p**, and **8−11**) following standard protocols (Schemes 1 and 2). The Sandmeyer reaction with the commercially available (4-aminophenyl)methanol yielded (4-azidophenyl)methanol (**1**), the oxidation of benzyl alcohol with pyridinium chlorochromate (PCC) yielded 4-azidobenzaldehyde (**2**), and the Cu(I)-catalyzed azide/alkyne-"click" chemistry of 4-azidobenzaldehyde (**2**) with phenylacetylene derivatives (**3a−3f**) yielded the corresponding triazole derivatives (**5a**, **5d**, and **5n−5q**). Alternatively, click chemistry of (4-azidophenyl)methanol (**1**) with phenylacetylene derivatives yielded the corresponding triazole derivatives (**4a−4k**). Subsequent oxidation of benzyl alcohol derivatives with PCC yielded the corresponding benzaldehydes (**5b**, **5c**, and **5e−5m**). Next, reductive amination of the aldehyde derivatives (**5**) with azetidine derivatives yielded the corresponding compounds (**6a−6n** and **7b−7i**). Last, hydrolysis of the methyl ester derivatives with sodium hydroxide yielded the final triazole compounds (**7a−7p**).

The triazole ring (**14a−15b**) was reversed following a standard protocol (Scheme 3). First, the click chemistry of azidobenzene derivatives (**12a** and **12b**) with 4-ethynylbenzaldehyde yielded the reversed triazole derivatives (**13a** and **13b**). Reductive amination of the reversed triazole aldehydes with methyl azetidine-3-carboxylate yielded the corresponding derivatives (**14a** and **14b**). Hydrolysis of the methyl ester derivatives with sodium hydroxide yielded the final compounds (**15a** and **15b**).

Next, various isoxazoline derivatives were synthesized by introducing an isoxazoline ring as a heterocycle core (Scheme 4). Starting from the reaction of the commercially available terephthalaldehyde with sodium borohydride, 4-(hydroxymethyl)benzaldehyde (**16**) was obtained. Then, the reaction of 4-(hydroxymethyl)benzaldehyde (**16**) with hydroxylamine hydrochloride resulted in 4-(hydroxymethyl)-benzaldehyde oxime (**17**). Cyclization of the oxime with the desired styrenes yielded the corresponding isoxazoline

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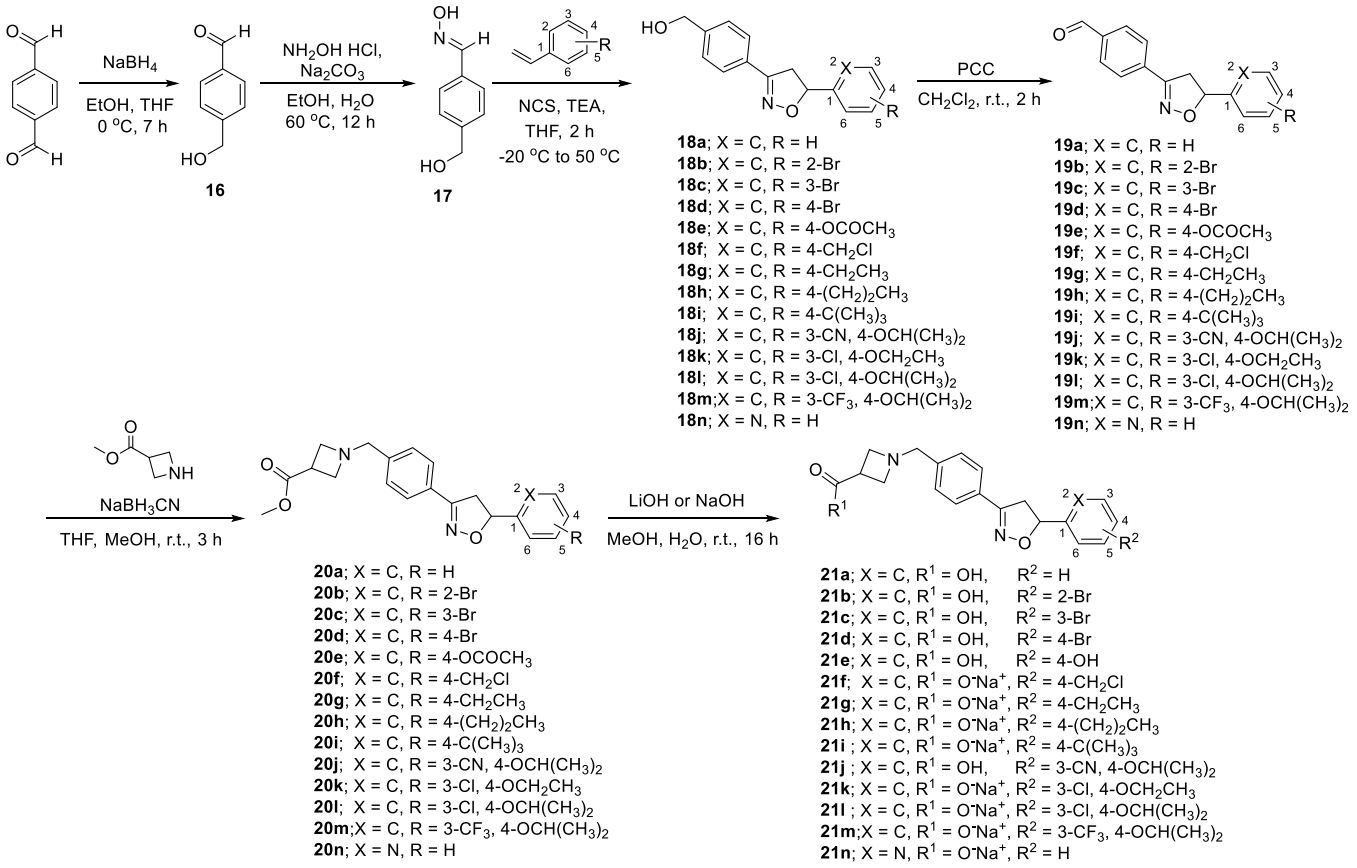
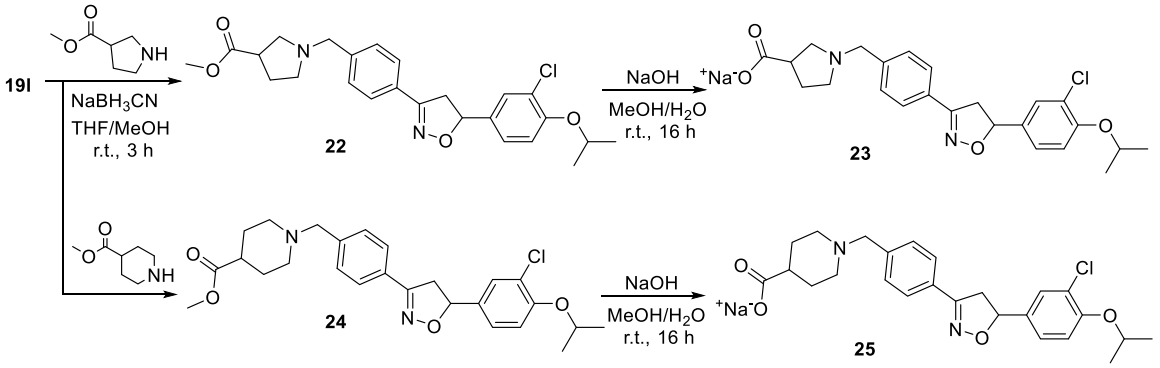
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 4. Synthesis of Compounds 20 and 21



Scheme 5. Synthesis of Compounds 22−25



derivatives (**18a−18n**). Meanwhile, oxidation of the benzyl alcohol derivatives with PCC yielded the corresponding benzaldehyde derivatives (**19a−19n**), and reductive amination of the aldehyde derivatives (**19**) with methyl azetidine-3-carboxylate yielded the corresponding compounds (**20a−20n**). Lastly, hydrolysis of the methyl ester derivatives with lithium hydroxide or sodium hydroxide yielded the final compounds (**21a−21n**).

We introduced pyrrolidine and piperidine rings instead of the azetidine ring to compare the difference in efficacy with the amine ring size (Schemes 2 and 5). Reductive amination of the aldehyde derivatives (**5l** and **19l**) with pyrrolidine and piperidine gave the corresponding derivatives (**8**, **10**, **22**, and **24**). Hydrolysis of the methyl ester derivatives with sodium hydroxide gave the final compounds (**9**, **11**, **23**, and **25**).

Evaluation of the Synthesized Compounds as $S1P_1$ Receptor Agonists. To evaluate the functionally antagonistic $S1P_1$ receptor agonist activities of the synthesized compounds, the compounds were assessed for their abilities to recruit β-arrestin to the $S1P_1$ receptor and internalize the $S1P_1$ receptor from the cell surface using commercially available *in vitro* assay systems.[36,37] The effects of three types of heterocyclic derivatives of triazole, reversed triazole, and isoxazoline were expressed as a value of the half-maximal effective concentration (EC_{50}) (Tables 1−4).

First, various derivatives containing a triazole ring as a heterocycle core were synthesized, and then, the *in vitro* efficacies were evaluated (Table 1). Compound **7k** with a *tert*-butyl group on the para position of a benzene ring in R^2 emerged as a lead compound with moderate nanomolar activities in β-arrestin recruitment and $S1P_1$ receptor internal-

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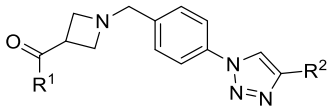
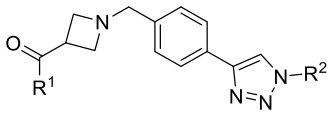
Table 1. Effects of Triazole Ring Derivatives on Ligand Binding to the GPCR



Compd.	R^1	R^2	$S1P_1$ β-arrestin[a] EC_{50} (nM)	$S1P_1$ Internalization[a] EC_{50} (nM)	$S1P_3$ β-arrestin[b] EC_{50} (nM)
FTY720-P[c]			1.67 ± 0.06	0.70 ± 0.07	97.7 ±1.22
6a	OCH_3		>1,000	>1,000	>10,000
7a	O^-Na^+		>1,000	>1,000	>10,000
7b	OH		>1,000	>1,000	>10,000
6b	OCH_3		>1,000	>1,000	>10,000
7c	OH		>1,000	>1,000	>10,000
6c	OCH_3		>1,000	>1,000	>10,000
7j	O^-Na^+		>1,000	>1,000	>10,000
7d	OH		867 ± 5.65	876 ± 8.96	>10,000
7e	OH		419 ± 8.59	378 ± 6.92	>10,000
7f	OH		19.1 ± 0.34	66.5 ± 0.35	>10,000
6e	OCH_3		72.6 ± 2.76	63.2 ± 1.13	>10,000
7g	OH		29.6 ± 0.55	36.4 ± 0.12	>10,000
6f	OCH_3		>1,000	813 ± 37.7	>10,000
7h	OH		196 ± 6.43	111 ± 1.15	>10,000
6g	OCH_3		>1,000	>1,000	>10,000
7i	OH		87.3 ± 1.67	385 ± 5.07	>10,000
7l	O^-Na^+		83.4 ± 9.08	160 ± 21.2	>10,000
6i	OCH_3		219 ± 2.33	313 ± 10.1	>10,000
7k	O^-Na^+		57.5 ± 4.25	152 ± 10.5	>10,000
6j	OCH_3		402 ± 14.5	>1,000	>10,000
6k	OCH_3		52.3 ± 1.62	111 ± 7.21	>10,000
7m	O^-Na^+		43.3 ± 1.44	24.6 ± 0.82	>10,000
6l	OCH_3		6.49 ± 0.07	11.1 ± 0.19	>10,000
7n	O^-Na^+		1.84 ± 0.03	3.16 ± 0.06	>10,000
6m	OCH_3		8.35 ± 0.12	25.0 ± 0.84	>10,000
7o	O^-Na^+		1.87 ± 0.06	2.59 ± 0.08	>10,000
6n	OCH_3		4.74 ± 0.18	4.06 ± 0.14	>10,000
7p	O^-Na^+		1.31 ± 0.04	0.84 ± 0.03	>10,000

[a]The activities of β-arrestin recruitment and $S1P_1$ receptor internalization were determined based on CHO-K1 EDG1 and HEK293 EDG1 cells, respectively, with the mean ± SEM of EC_{50} values shown. [b]The activity of β-arrestin recruitment to the $S1P_3$ receptor was determined based on CHO-K1 EDG3 cells, with the mean ± SEM of EC_{50} values shown. [c]FTY720-P: phosphate form of fingolimod, a well-known immunomodulating drug mostly used for the treatment of MS.

Table 2. Effects of Reversed Triazole Ring Derivatives on Ligand Binding to the GPCR



Compd.	R^1	R^2	$S1P_1$ β-arrestin[a] EC_{50} (nM)	$S1P_1$ Internalization[a] EC_{50} (nM)	$S1P_3$ β-arrestin[b] EC_{50} (nM)
14a	OCH_3		131 ± 1.49	209 ± 2.85	>10,000
15a	O^-Na^+		64.9 ± 2.42	106 ± 12.3	>10,000
14b	OCH_3		4.91 ± 0.04	13.8 ± 0.10	>10,000
15b	O^-Na^+		3.72 ± 0.05	2.25 ± 0.16	>10,000

[a]The activities of β-arrestin recruitment and $S1P_1$ receptor internalization were determined based on CHO-K1 EDG1 and HEK293 EDG1 cells, respectively, with the mean ± SEM of EC_{50} values shown. [b]The activity of β-arrestin recruitment to the $S1P_3$ receptor was determined based on CHO-K1 EDG3 cells, with the mean ± SEM of EC_{50} values shown.

ization (Table 1). The following para position modification with various lipophilic chains such as ethyl, propyl, butyl, pentyl, and hexyl chains revealed that the butyl group substituted into the R^2 benzene ring (**7g**) significantly increased the agonistic activity by 2- to 4-fold. We also introduced ethoxy and isopropoxy groups into the para position of the benzene ring on the lipophilic east side (**6j** and **6k**), which resulted in the isopropoxy group modification proving more potent than the other (**6k**). Next, an electron-withdrawing group (CN, Cl, and CF_3) was additionally introduced at the meta position of compound **7m** having an isopropoxy group. As a result, compounds **7n**, **7o**, and **7p** (**7n**: CN, **7o**: Cl, and **7p**: CF_3) showed significantly enhanced β-arrestin recruitment and $S1P_1$ receptor internalization efficacies compared to compound **7m** (Table 1).

To evaluate the effects of different heterocycle substituents on $S1P_1$ receptor agonistic activities, reversed triazole ring derivatives were synthesized (Scheme 3 and Table 2). Overall, the introduction of a reversed triazole core gave results similar to the structure−activity relationship (SAR) pattern of the triazole core, as can be seen in Table 1. Compound **15a**, whose structure is comparable to that of compound **7k** except for a reversed triazole ring, exhibited similar agonistic effects to

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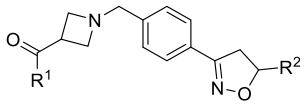
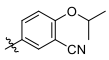
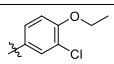
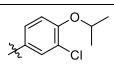
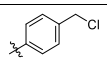
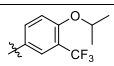
J. Med. Chem. XXXX, XXX, XXX−XXX

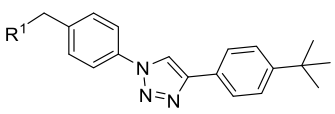
Table 3. Effects of Isoxazoline Ring Derivatives on Ligand Binding to the GPCR

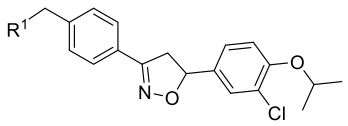


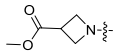
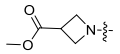
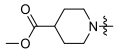
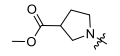
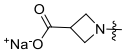
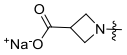
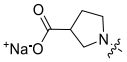
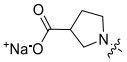
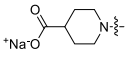
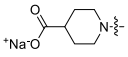
Compd.	R^1	R^2	$S1P_1$ β-arrestin[a] EC_{50} (nM)	$S1P_1$ Internalization[a] EC_{50} (nM)	$S1P_3$ β-arrestin[b] EC_{50} (nM)	Compd.	R^1	R^2	$S1P_1$ β-arrestin[a] EC_{50} (nM)	$S1P_1$ Internalization[a] EC_{50} (nM)	$S1P_3$ β-arrestin[b] EC_{50} (nM)
20a	OCH_3		>1,000	>1,000	>10,000	**21h**	O^-Na^+		25.9 ± 0.20	30.5 ± 1.23	>10,000
21a	OH		>1,000	>1,000	>10,000						>10,000
20b	OCH_3		>1,000	>1,000	>10,000	**20i**	OCH_3		>1,000	286 ± 4.78	>10,000
21b	OH		>1,000	>1,000	>10,000	**21i**	O^-Na^+		34.6 ± 0.27	112 ± 8.69	>10,000
20c	OCH_3		>1,000	>1,000	>10,000	**20j**	OCH_3		95.7 ± 3.96	346 ± 6.92	>10,000
21c	OH		>1,000	>1,000	>10,000	**21j**	OH		18.1 ± 0.14	34.6 ± 0.31	>10,000
20d	OCH_3		>1,000	>1,000	>10,000	**20k**	OCH_3		100 ± 0.50	122 ± 4.80	>10,000
21d	OH		567 ± 16.4	414 ± 5.80	>10,000	**21k**	O^-Na^+		20.4 ± 0.67	16.8 ±0.46	>10,000
21e	OH		>1,000	>1,000	>10,000	**20l**	OCH_3		23.1 ± 0.69	45.3 ± 3.24	>10,000
					>10,000	**21l**	O^-Na^+		7.03 ± 0.09	11.8 ± 0.28	>10,000
20f	OCH_3		>1,000	>1,000	>10,000	**20m**	OCH_3		86.6 ± 1.05	38.5 ± 0.81	>10,000
21f	O^-Na^+		>1,000	>1,000	>10,000	**21m**	O^-Na^+		5.57 ± 0.09	5.09 ± 0.08	>10,000
20g	OCH_3		>1,000	>1,000	>10,000	**20n**	OCH_3		392 ± 5.42	594 ± 14.9	>10,000
21g	O^-Na^+		542 ± 20.1	973 ± 24.3	>10,000	**21n**	O^-Na^+		346 ± 6.08	237 ± 6.13	>10,000

[a]The activities of β-arrestin recruitment and $S1P_1$ receptor internalization were determined based on CHO-K1 EDG1 and HEK293 EDG1 cells, respectively, with the mean ± SEM of EC_{50} values shown. [b]The activity of β-arrestin recruitment to the $S1P_3$ receptor was determined based on CHO-K1 EDG3 cells, with the mean ± SEM of EC_{50} values shown.

Table 4. Effects of Triazole and Isoxazoline Rings with Various Amine Ring Derivatives on Ligand Binding to the GPCR





Compd.	R^1	$S1P_1$ β-arrestin[a] EC_{50} (nM)	$S1P_1$ Internalization[a] EC_{50} (nM)	$S1P_3$ β-arrestin[b] EC_{50} (nM)	Compd.	R^1	$S1P_1$ β-arrestin[a] EC_{50} (nM)	$S1P_1$ Internalization[a] EC_{50} (nM)	$S1P_3$ β-arrestin[b] EC_{50} (nM)
6i		219 ± 2.33	313 ± 10.1	>10,000	**20l**		23.1 ± 0.69	45.3 ± 3.50	>10,000
10		>1,000	>1,000	>10,000	**22**		74.4 ± 1.93	146 ± 5.38	>10,000
7k		57.5 ± 4.25	152 ± 10.5	>10,000	**21l**		7.03 ± 0.09	11.8 ± 0.28	>10,000
9		279 ± 17.4	283 ± 3.88	>10,000	**23**		11.1 ± 0.13	14.3 ± 0.44	>10,000
11		>1,000	>1,000	>10,000	**25**		111 ± 2.74	80.6 ± 3.04	>10,000

[a]The activities of β-arrestin recruitment and $S1P_1$ receptor internalization were determined based on CHO-K1 EDG1 and HEK293 EDG1 cells, respectively, with the mean ± SEM of EC_{50} values shown. [b]The activity of β-arrestin recruitment to the $S1P_3$ receptor was determined based on CHO-K1 EDG3 cells, with the mean ± SEM of EC_{50} values shown.

compound **7k**. Similarly, the efficacies of compound **15b** were comparable to that of the corresponding compound **7o**.

Next, various derivatives were synthesized by replacing the triazole ring with an isoxazoline ring as a heterocycle core, and the *in vitro* efficacies were evaluated, which are shown in Table 3. Compound **21i**, an isoxazoline derivative with the same R^1 and R^2 substituents as that of the lead compound **7k**, displayed slightly improved potencies for both β-arrestin recruitment and $S1P_1$ receptor internalization (**7k**: EC_{50} = 57.5 nM and 152 nM vs **21i**: EC_{50} = 34.6 nM and 112 nM, respectively). Compounds with bromine (Br) groups on ortho, meta, and para positions were evaluated for their $S1P_1$ receptor agonist activities (**20b−d** and **21b−d**) to determine the effects of substituents in different positions on the R^2 benzene ring. In these three series of isoxazoline derivatives, only compound **21d** with a Br group in the para position showed some activity, highlighting the para substitution's importance. Similar to the substitution of triazole derivatives (**7n**, **7o**, and **7p**), ethoxy and isopropoxy groups were introduced at the para position of the R^2 benzene ring, and electron-withdrawing groups (CN, Cl,

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J. Med. Chem. XXXX, XXX, XXX−XXX

Table 5. CYP Inhibition and Microsomal Stability of the Synthesized Compounds

	CYP enzyme activities[a] (%)					microsomal stability[b] (%)			CYP enzyme activities[a] (%)					microsomal stability[b] (%)	
compd.	2C19	2D6	2C9	1A2	3A4	human	rodent[c]	compd.	2C19	2D6	2C9	1A2	3A4	human	rodent[c]
6a	68.3	86.1	91.3	82.3	>99	nd[d]	nd[d]	**10**	74.7	36.3	76.4	>99	36.3	14.6	8.90 (r)
6c	81.3	66.7	83.8	93.9	81.9	nd[d]	nd[d]	**11**	>99	92.2	>99	>99	>99	99.8	85.7 (m)
6e	54.6	13.8	49.9	>99	>99	15.9	nd[d]	**14a**	48.2	52.0	40.9	48.2	97.4	nd[d]	ndd
6f	71.4	21.6	54.3	98.3	98.2	13.4	nd[d]	**14b**	48.5	37.1	35.9	95.4	87.6	24.1	28.8 (m)
6g	65.0	54.8	65.2	95.6	>99	11.5	nd[d]	**15a**	>99	>99	98.3	75.5	>99	97.2	76.1 (m), 91.2 (r)
6i	88.1	76.4	82.6	72.4	96.9	18.9	nd[d]	**15b**	76.2	99.0	85.9	98.7	>99	98.5	>99 (m)
6k	82.3	66.1	86.0	95.1	92.7	nd[d]	nd[d]	**20a**	41.0	60.7	76.1	73.7	60.7	nd[d]	nd[d]
6l	23.0	69.2	38.4	>99	62.0	nd[d]	nd[d]	**20g**	38.8	36.5	64.2	82.9	95.0	nd[d]	nd[d]
6m	52.3	65.1	62.7	>99	>99	23.2	19.0 (m)	**20j**	55.3	83.6	55.9	71.0	77.1	nd[d]	nd[d]
6n	17.7	64.5	36.0	>99	77.7	40.1	26.2 (m)	**20k**	28.5	40.8	51.8	84.7	56.2	nd[d]	nd[d]
7a	92.2	>99	90.0	93.5	>99	nd[d]	nd[d]	**20l**	29.8	29.9	41.5	>99	52.6	25.3	36.4 (m)
7c	94.7	>99	90.3	72.0	>99	nd[d]	nd[d]	**20m**	22.6	75.2	49.8	88.4	64.1	22.6	29.0 (m)
7d	88.8	>99	95.6	97.9	83.0	nd[d]	nd[d]	**20n**	84.5	>99	90.6	89.6	82.6	nd[d]	nd[d]
7e	91.2	>99	>99	98.5	>99	>99	>99 (m), 94.9 (r)	**21a**	12.1	98.3	68.9	80.8	>99	nd[d]	nd[d]
7f	98.3	98.4	92.6	92.2	98.9	94.7	91.4 (m), 85.6 (r)	**21d**	65.2	93.5	96.4	99.4	84.5	nd[d]	nd[d]
7g	83.9	86.1	92.5	>99	>99	86.2	94.6 (m), 85.4 (r)	**21g**	>99	95.6	93.6	96.8	>99	nd[d]	nd[d]
7h	>99	>99	>99	84.2	>99	nd[d]	nd[d]	**21h**	89.1	>99	89.7	>99	>99	nd[d]	nd[d]
7i	>99	>99	>99	80.0	>99	nd[d]	nd[d]	**21i**	97.7	>99	>99	>99	>99	93.8	96.7 (m), 95.1 (r)
7j	99.0	96.5	89.4	99.2	96.1	nd[d]	nd[d]	**21j**	89.2	>99	>99	91.6	>99	90.7	>99 (m)
7k	>99	89.6	93.1	>99	91	95.1	87.6 (m), 85.2 (r)	**21k**	70.5	88.3	80.2	90.3	69.6	nd[d]	nd[d]
7l	89.7	98.5	>99	>99	>99	>99	83.7 (m), 90.0 (r)	**21l**	74.8	75.3	80.6	>99	76.6	81.2	>99 (m)
7m	>99	>99	>99	>99	>99	nd[d]	nd[d]	**21m**	79.7	>99	88.1	>99	>99	>99	90.4 (m)
7n	86.5	>99	97.8	>99	>99	91.4	86.9 (m)	**21n**	87.9	>99	>99	>99	>99	nd[d]	nd[d]
7o	97.1	99.6	>99	>99	>99	95.9	92.8 (m)	**22**	27.5	19.9	10.9	90.9	46.3	28.4	27.1 (m)
7p	61.5	>99	86.8	>99	>99	89.0	>99 (m)	**23**	83.0	77.5	80.1	>99	>99	62.1	71.8 (m)
9	>99	>99	>99	97.9	92.8	88.1	35.0 (m), 62.3 (r)	**25**	69.3	>99	70.0	>99	>99	73.5	84.5 (m)

[a]A CYP inhibition assay was performed using a P450-Glo assay system (Promega); the results are given as % of control (vehicle) activity. [b]*In vitro* microsomal stability of the synthesized compound; % remaining was determined after 30 min incubation with human or mouse microsomes. The % of the parent compound remaining was calculated by comparing the peak areas. [c]*In vitro* microsomal stability in mouse microsomes (m) and rat microsomes (r) are indicated in parentheses. [d]nd, not determined.

and CF_3) were introduced at the meta position (**20j−m** and **21j−m**). Corresponding with the results in Table 1, compounds with an ethoxy group were less potent than the compounds with an isopropoxy group (**20l** > **20k** and **21l** > **21k**). Compounds **21l** and **21m** with an isopropoxy group in the para position and a Cl or CF_3 group in the meta position exerted the most potency in a single-digit nanomolar range (**21l**: EC_{50} = 7.03 nM and 11.8 nM; **21m**: EC_{50} = 5.57 nM and 5.09 nM for β-arrestin recruitment and $S1P_1$ receptor internalization, respectively).

Insertions of larger-sized amine rings, such as pyrrolidine and piperidine, into compounds **7k** and **21l** were executed for the additional exploration of the SAR of triazole and isoxazoline heterocycle derivatives (Table 4). Triazole and isoxazoline ring derivatives with a pyrrolidine ring (**9** and **23**) showed a slight decrease in activities compared to the derivatives with azetidine rings (**7k** and **21l**). In addition, the derivatives substituted with a piperidine ring (**11** and **25**) caused a significant decrease in the activity, indicating that the activity decreased as the size of the ring increased. Overall, the carboxylic acid derivatives were relatively superior to the methyl ester derivatives at the R^1 position, regardless of the heterocycle core type, amine ring size, and functional group of R^2. For example, compared to that of compound **20l** containing a methyl ester in R^1, the efficacy of compound **21l** hydrolyzed with sodium salt was improved by more than 3 and 4 times in β-arrestin recruitment and $S1P_1$ receptor internalization, respectively (Table 3). Investigation on the $S1P_3/S1P_1$ receptor selectivity of the synthesized compounds also revealed that none of the compounds had significant activity against the $S1P_3$ receptor (Tables 1−4). Strikingly, **21l** demonstrated >1000-fold selectivity against the $S1P_3$ receptor while maintaining a high potency against the $S1P_1$ receptor as mentioned before.

***In Vitro* Drug-like Properties of the Synthesized Compounds.** The synthesized compounds' drug-like properties were assessed through cytochrome P450 (CYP) inhibition and microsomal stability tests described in our previous study.[38] The potential drug−drug interactions were tested by examining the compounds' inhibitory effects on CYP enzymes, consisting of subtypes 2C19, 2D6, 2C9, 1A2, and 3A4. Results were displayed as the remaining percentage of CYP activity after the treatment with 10 μM of each compound (Table 5). Overall, most compounds with a methyl ester moiety as the R^1

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J. Med. Chem. XXXX, XXX, XXX−XXX

substituent showed unfavorable inhibitions of CYP 2C19, 2D6, and 2C9, inhibiting more than 50% of the activities. Compounds with a sodium salt moiety did not inhibit more than 50% of the five CYP enzymes at 10 μM except **21a**.

The microsomal stability of the synthesized compound was determined by the percentage of the remaining parent compound after a 30 min incubation with human or rodent microsomes (Table 5). Similar to the CYP inhibition results, compounds with a methyl ester group on R^1 showed unfavorable microsomal stabilities in both humans and rodents. Neither the incorporation of various nitrogen heterocycles nor the differing sizes of amine rings produced an apparent trend in the compounds' microsomal stabilities. Based on the results of the analysis of the *in vitro* activity and drug-like properties, six potent compounds (**7k**, **7p**, **15a**, **21i**, **21l**, and **21m**) were selected for further pharmacokinetic (PK) studies.

PK Studies for the Selected Compounds. The six selected compounds were subjected to PK studies in rats, and the dose used for each compound was 1 or 10 mg/kg for intravenous injection (i.v.) or oral administration (p.o.), respectively (Table 6). **21i**, with an isoxazoline ring as a heterocycle core, showed the best PK profiles (F = 44.9%, AUC = 5187 ng*h/mL, and C_{max} = 1071 ng/mL) (Table 6) based on comparing triazole, reversed triazole, and isoxazoline compounds containing the same R^1 and R^2 substituents (**7k**, **15a**, and **21i**, respectively). A similar trend was observed with triazole and isoxazoline derivatives (**7p** and **21m**) having the same 4-isopropoxy-3-(trifluoromethyl)phenyl group at the R^2 position. **21m** with the isoxazoline heterocycle moiety showed better oral bioavailability (F = 35.9 *vs* 6.8%) and C_{max} value (876.0 *vs* 72.0 ng/mL) and a higher AUC value (2702.8 *vs* 258.4 ng*h/mL).

The PK profile of **21l** was further evaluated to compare the effects of R^2 modification on the isoxazoline ring. It was found that **21l**, with Cl and an isopropoxy group on the R^2 benzene moiety, displayed a more favorable PK profile than **21m**, with CF_3 and an isopropoxy group on R^2. Furthermore, **21l** had the highest oral bioavailability (F = 54.2%) among the selected compounds, with an exceptional AUC value (5044.9 ± 1061.0 ng*h/mL) and a significantly higher C_{max} value (1661.1 ± 916.6 ng/mL) (Table 6). PK characteristics of **21l** were also explored in male beagle dogs. The dose of the compound used was 2 or 10 mg/kg for i.v. or p.o., respectively. In conjunction with the favorable PK profile of **21l** in rats, a PK study of **21l** in dogs showed comparable PK parameters, with good oral bioavailability (F = 31.8%),and high AUC values (23,109.9 ± 7752.2 ng*h/mL) and C_{max} values (3979.4 ± 483.5 ng/mL) (Table 6).

To evaluate the potential for the heart rate effects in the clinic, **21l** and **21m** were treated with cultured cardiomyocytes derived from human-induced pluripotent stem cells (hiPSCs) to measure the electrophysiological signals using a micro-electrode array (MEA).[39] Parameters analyzed from these extracellular field potential (FP) recordings are considered similar to the heart rate, QT interval, and QRS amplitude on an electrocardiogram. Delayed and altered repolarization is defined as >20% FPDcF change[40] and is recognized as a surrogate indicator of preclinical arrhythmia risk. While hERG channel blockers such as E4031 exhibit extended FP duration (FPDcF) (>20%) at low concentrations (0.01 μM), **21l** and **21m** showed no difference compared to the reference at both

Table 6. PK Parameters of the Selected Compounds

		compd.									
		7k	**7p**	**15a**	**21i**	**21l**	**21m**	**21l**			
		rat	rat	rat	rat	rat	rat	dog			
intravenous[a]	$T_{1/2}$ (h)	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	2.4 ± 0.1	1.4 ± 0.3	1.2 ± 0.1	5.70 ± 1.2			
	$AUC_{0-\infty}$ (ng*h/mL)	186.4 ± 31.7	377.6 ± 39.9	370.8 ± 16.9	1155.5 ± 120.6	931.3 ± 95.7	752.4 ± 106.4	14,830.8 ± 5475.4			
	CL (mL/min/kg)	91.1 ± 18.1	43.9 ± 4.6	44.7 ± 2.2	13.1 ± 1.5	17.6 ± 2.0	22.3 ± 2.9	149.9 ± 62.5			
	V_{ss} (L/kg)	5.4 ± 1.1	2.9 ± 0.7	2.6 ± 0.4	2.6 ± 0.2	1.7 ± 0.2	1.7 ± 0.2	828.7 ± 134.2			
oral[a]	C_{max} (ng/mL)	19.8 ± 6.1	72.0 ± 31.1	23.9 ± 5.1	1071.2 ± 302.7	1661.1 ± 916.6	876.0 ± 347.3	3979.4 ± 483.5			
	T_{max} (h)	4.5 ± 1.0	0.6 ± 0.3	1.4 ± 1.7	2.3 ± 1.3	0.9 ± 0.8	0.8 ± 0.3	1.3 ± 0.5			
	$T_{1/2}$ (h)	1.2 ± 0.2	1.7 ± 0.7	1.4 ± 0.3	3.1 ± 0.1	1.4 ± 0.2	1.9 ± 0.2	4.9 ± 0.6			
	$AUC_{0-\infty}$ (ng*h/mL)	85.2 ± 15.4	258.4 ± 109.1	93.4 ± 20.0	5187.6 ± 1097.6	5044.9 ± 1061	2702.8 ± 735.1	23,109.9 ± 7752.2			
	F (%)	4.6	6.8	2.5	44.9	54.2	35.9	31.8			

[a]Rats (n = 4) were dosed with 1 mg/kg for i.v. and 10 mg/kg for p.o. Dogs (n = 3) were dosed with 2 mg/kg for i.v. and 10 mg/kg for p.o. Parameters were calculated from composite mean plasma concentration−time data. Data are expressed as the mean ± S.D. (%).

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J. Med. Chem. XXXX, XXX, XXX−XXX

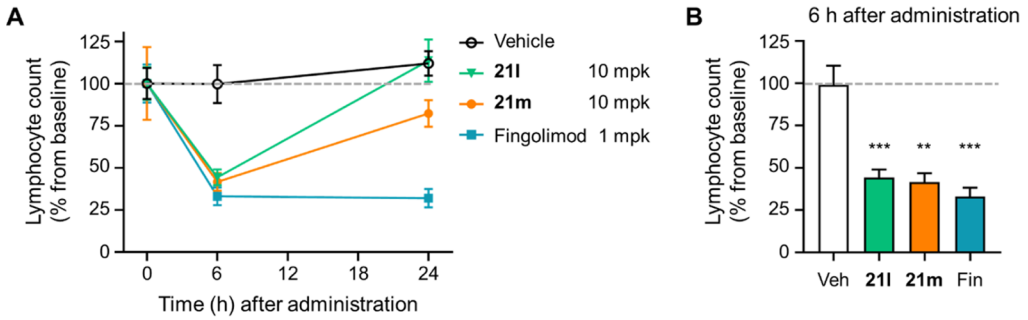

Figure 2. Reduction of the blood lymphocyte count by the treatment of **21l** and **21m** in rats. Rats were orally administered with the vehicle (n = 4), **21l** (10 mg/kg, n = 4), **21m** (10 mg/kg, n = 3), or positive control fingolimod (1 mg/kg, n = 4). (A) Blood lymphocyte counts were measured before (0 h) and after the administration (6 and 24 h). Blood lymphocyte count in each group before the administration (0 h) was considered as the baseline (100%). Data are presented as mean ± SEM. (B) Percentage of the blood lymphocyte count 6 h after the administration. The gray dotted line represents the baseline. $^{**}p < 0.01$ and $^{***}p < 0.001$, compared to vehicle-treated rats (one-way ANOVA with Dunnett's test). Data are presented as mean ± SEM. Veh; vehicle and Fin; fingolimod.

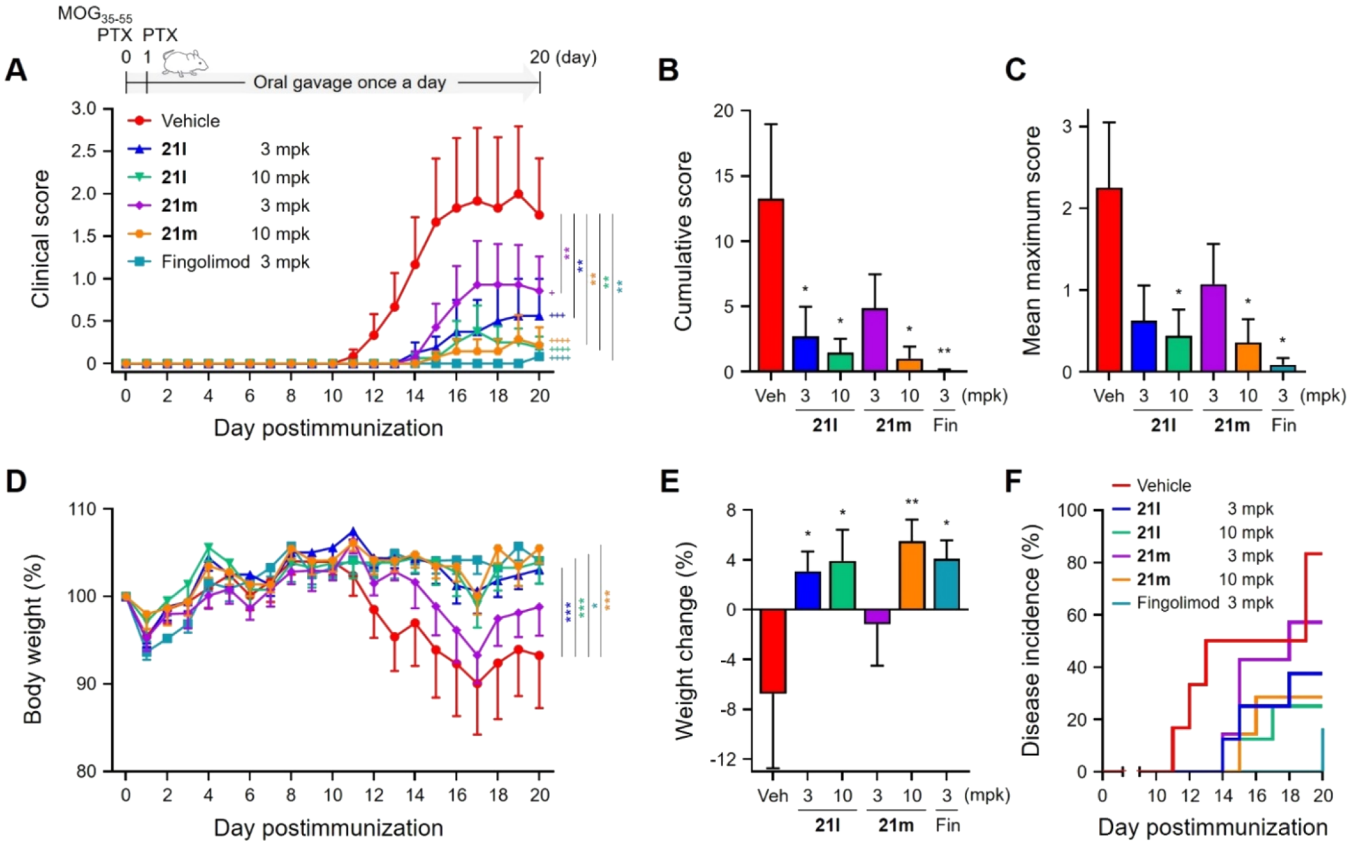

Figure 3. *In vivo* efficacy of **21l** and **21m** in EAE mice. (A) Top: schematic representation of the experimental procedure. EAE mice were daily treated with the vehicle (p.o., n = 6), **21l** (3 or 10 mg/kg, p.o., n = 8), **21m** (3 or 10 mg/kg, p.o., n = 7), or positive control fingolimod (3 mg/kg, p.o., n = 6). MOG, myelin oligodendrocyte glycoprotein and PTX, pertussis toxin. Bottom: mean clinical EAE scores. $^{**}p < 0.01$, compared with vehicle-treated mice (repeated measurements of one-way ANOVA with Dunnett's test). $^{+}p < 0.05$, $^{+++}p < 0.001$, and $^{++++}p < 0.0001$, compared with vehicle-treated mice (two-way ANOVA with Dunnett's test on day 20). (B,C) Cumulative scores (B) defined as the sum of the clinical score for each mouse and mean maximum scores (C) during the course of EAE. $^{*}p < 0.05$ and $^{**}p < 0.01$, compared with vehicle-treated mice (one-way ANOVA with Dunnett's test). Veh; vehicle and Fin; fingolimod. (D) Normalized daily weight changes in EAE mice. $^{*}p < 0.05$ and $^{***}p < 0.001$, compared with vehicle-treated mice (repeated measurements of one-way ANOVA with Dunnett's test). (E) Body weight changes on day 20. $^{*}p < 0.05$ and $^{**}p < 0.01$, compared with vehicle-treated mice (one-way ANOVA with Fisher's LSD). (F) Disease incidence rate monitored daily. Data are presented as mean ± SEM.

concentrations (1 and 0.1 μM), and no arrhythmia was observed (Figure S1 in the Supporting Information).

Therefore, compounds **21l** and **21m**, which are potent $S1P_1$ receptor agonists and have excellent drug-like properties, were selected for further *in vivo* studies.

***In Vivo* Reduction of the Peripheral Blood Lymphocyte Count by the Treatment of Compounds 21l and 21m in Rats.** Functionally antagonistic $S1P_1$ receptor agonists, such as fingolimod, inhibit $S1P_1$-mediated lymphocyte egress from the lymphoid tissue, leading to peripheral

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J. Med. Chem. XXXX, XXX, XXX−XXX

lymphopenia. Such agonist-induced lymphopenia contributes to the therapeutic effects against autoimmune diseases, such as MS.[7,41] Therefore, we examined the effectivity of the compounds to induce lymphopenia in the blood through a peripheral lymphocyte count (PLC) assay (Figure 2A). Blood samples were collected after the oral administration of test compounds. Consequently, 6 h after dosing the rates with **21l**, **21m** (10 mg/kg, respectively), and fingolimod (1 mg/kg), the blood lymphocyte counts for all samples decreased significantly compared to those before the administration of the test compounds (reduced from the baseline levels to 44.3, 41.6, and 33.1%, respectively; Figure 2B). As a result of a single dose administration, the number of peripheral lymphocytes in rats treated with fingolimod continued to decrease 24 h after the administration, whereas the blood lymphocyte counts in rats treated with compounds **21l** or **21m** returned to almost the baseline levels, suggesting that the cardiac toxicity of FTY720 caused by its prolonged potency on lymphocyte reduction could be overcome (Figure 2A).[23] Collectively, these results suggest that the administration of **21l** and **21m** can sufficiently inhibit the lymphocyte egress from the lymphoid tissue to the peripheral blood and that lymphopenia can be recovered within 24 h.

***In Vivo* Efficacy of Compounds 21l and 21m in a Mouse Experimental Autoimmune Encephalomyelitis Model.** Compounds **21l** and **21m** were evaluated in a myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$-induced experimental autoimmune encephalitis (EAE) mouse model, the most used preclinical model for MS with accompanying pathological features of human MS, such as paralysis, CNS inflammation, BBB disruption, or demyelination.[42] EAE was induced by immunization with MOG_{35-55}/complete Freund's adjuvant (CFA), followed by two intraperitoneal injections of pertussis toxin (PTX) in C57BL/6 mice. MOG_{35-55}-immunized mice were prophylactically dosed with **21l**, **21m**, or positive control fingolimod (3 or 10 mg/kg/day, p.o.) once daily from the day of immunization (day 0, before the disease onset) up to day 20 (Figure 3A). We observed significantly lower daily mean clinical scores in the EAE mice treated with **21l** or **21m** than those in the EAE mice treated with the vehicle, indicating that both compounds dose-dependently suppress disease progression (Figure 3A). In addition, **21l**- and **21m**-treated mice showed a significant reduction in the disease severity with an attenuated cumulative score (Figure 3B) and mean maximum score (Figure 3C). During EAE progression, disease severity is reflected in weight loss due to excessive inflammatory reactions and decreased food intake following paralysis. The weight loss observed in vehicle-treated EAE mice was remarkably recovered in **21l**- or **21m**-treated mice (Figure 3D,E). We found out that **21l** or **21m** treatments delayed the disease onset and reduced the disease incidence in a dose-dependent manner. Furthermore, we found that **21l** was slightly superior to **21m** (Figure 3F). Taken together, **21l** effectively ameliorated the disease progression and overall severity in EAE mice, showing favorable drug-like properties.

CONCLUSIONS

A series of functionally antagonistic compounds against the $S1P_1$ receptor were synthesized following the discovery of a novel triazole core scaffold. Based on the lead compound, **7k**, further optimization was executed, yielding the improved compounds **21l** and **21m** with an isoxazoline ring as a heterocycle core. Such modifications led to better therapeutic potencies in $S1P_1$ receptor internalization and β-arrestin recruitment, highly favorable properties in CYP inhibition and microsomal stability assays, and superior PK profiles. Furthermore, **21l** and **21m** were confirmed to show *in vitro* selectivities against $S1P_3$. The *in vivo* examination of the two compounds through a PLC assay has demonstrated that compounds **21l** and **21m** significantly decreased the blood lymphocyte counts in rats. Treatment of EAE mice with **21l** effectively ameliorated the disease progression and MS's severity, suggesting that **21l** is a novel $S1P_1$ receptor agonist for MS treatment.

EXPERIMENTAL SECTION

General Methods. All chemicals, reagents, and solvents were obtained from commercially available sources as reagent grade products and were used without further purification. Yields reported are for purified products and were not optimized. Synthesized compounds were analyzed by thin-layer chromatography (TLC), ^1H and ^{13}C nuclear magnetic resonance (NMR), melting point (MP), high-resolution mass spectrometry (HRMS), and high-performance liquid chromatography (HPLC) analyses. The reactions were monitored using analytical TLC plates (Merck, Cat no. 1.05715) and analyzed by ultraviolet light at 254 and 280 nm. The reactions were purified by medium-pressure liquid chromatography (Biotage, Isolera one). MPs were measured in open capillary tubes using OptiMelt melting point equipment (Stanford Research Systems, Inc.). The NMR spectra were recorded at 400 MHz (^1H)/100 MHz (^{13}C) or 300 MHz (^1H)/75 MHz (^{13}C) using Bruker spectrometers. Chemical shifts (δ) were reported in parts per million downfield from tetramethylsilane. HPLC analysis was performed using a Waters E2695 system equipped with a YMC-Triart C18/S-5 μm/12 nm/Lot no. 17452 (150 mm × 4.6 mm diameter). The HPLC data were recorded using the following parameters: DW (0.1% AcOH)/acetonitrile. Method A: 10/90 → 100/0 in 15 min, +5 min isocratic, and a flow rate of 0.5−1.0 mL/min; method B: 30/70 → 100/0 in 15 min, +5 min isocratic, and a flow rate of 1.0 mL/min; and λ = 254 and 280 nm. All compounds were >95% pure. HRMS was performed with electrospray ionization (ESI) on a Q-Exactive (Thermo Fisher Scientific) instrument.

General Procedure for Intermediate Compounds 2, 5b−5c, 5e−5m, and 19 (Method A). To a mixture of alcohol derivatives (**1**, **4a−4k**, and **18**) (1.0 equiv) in anhydrous dichloromethane (CH_2Cl_2) ($[C] \sim 0.1$ M) was added PCC (3.0 equiv). The resulting suspension was stirred at room temperature (1−2 h). The reaction mixture was evaporated in vacuo. The obtained residue was purified by column chromatography on SiO_2. The detailed methods and data for each intermediate compound are described in the Supporting Information.

General Procedure for Intermediate Compounds 4, 5a, 5d, 5n−5q, and 13 (Method B). (a) To a mixture of phenyl azide derivatives (**1**, **3b**, and **12**) (1.0 equiv) in *tert*-butanol ($[C] \sim 0.1$ M) were added acetylene derivatives (1.2 equiv), copper (II) acetate (0.4 M), and tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (0.1 equiv), and the resulting mixture was stirred at room temperature (4 h). The reaction mixture was diluted with distilled water and extracted with ethyl acetate. The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated in vacuo. The obtained residue was purified by column chromatography on SiO_2. (b) To a mixture of phenyl azide derivatives (**3a** and **3c−3f**) (1.0 equiv) in tetrahydrofuran (THF) and distilled water in a ratio of 4:1 ($[C] \sim 0.1$ M) were added acetylene derivatives (1.2 equiv), sodium ascorbate (0.05 equiv), and copper(II) sulfate pentahydrate (0.01 equiv), and the mixture was stirred at room temperature (4 h). The reaction mixture was diluted with distilled water and extracted with ethyl acetate. The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated in vacuo. The obtained residue was purified by column chromatography on SiO_2. The detailed methods and data for each intermediate compound are described in the Supporting Information.

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J. Med. Chem. XXXX, XXX, XXX−XXX

General Procedure for Intermediate Compounds 3c−3f (Method C). To a mixture of the desired phenol derivatives (1.0 equiv) in *N*,*N*-dimethylformamide ([*C*] ∼ 0.1 M) were added 2-bromopropane (1.3 equiv) and potassium carbonate (5.0 equiv), and the resulting mixture was stirred at 60 °C (12 h). The reaction mixture was diluted with distilled water and extracted with ethyl acetate. The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated in vacuo. The resulting residue was purified by column chromatography on SiO_2 to give the desired isopropoxybenzene derivatives. The detailed methods and data for each intermediate compound are described in the Supporting Information.

General Procedure for Final Compounds 6, 7b−7i, 8, 10, 14, 20, 22, and 24 (Method D). To a mixture of benzaldehyde derivatives (**5**, **13**, and **19**) (1.0 equiv) in anhydrous MeOH and THF in a ratio of 1:1 ([*C*] ∼ 0.1 M) was added a mixture of amine derivatives (1.5 equiv) with triethylamine (3.0 equiv). The resulting suspension was stirred at room temperature (0.5 h). Then, sodium cyanoborohydride (4.0 equiv) was added and stirred at room temperature (2−3 h). The reaction mixture was evaporated in vacuo. The product residue was washed with ethyl acetate and distilled water. The combined organic layer was dried with anhydrous Na_2SO_4. The product mixture was filtered and evaporated in vacuo. The obtained residue was purified by column chromatography on SiO_2.

General Procedure for Final Compounds 21a−21e and 21j (Method E). To a mixture of methyl ester derivatives (**20a−20e** and **20j**) (1.0 equiv) in MeOH ([*C*] ∼ 0.1 M) was added aqueous lithium hydroxide (2.0 equiv) dropwise at 4 °C. The resulting suspension was stirred at room temperature (24 h). After 24 h, to the reaction mixture was added 3 M HCl dropwise (pH ∼ 3), and the temperature was increased to 25 °C. The product mixture was extracted with CH_2Cl_2. The combined organic layer was dried with anhydrous Na_2SO_4. The product mixture was filtered and evaporated in vacuo.

General Procedure for Final Compounds 7a, 7j−7p, 9, 11, 15, 21, 23, and 25 (Method F). To a mixture of methyl ester or carboxylic acid derivatives (**6a**, **6c**, **6i−6n**, **8**, **10**, **14**, **20**, **22**, and **24**) (1.0 equiv) in MeOH ([*C*] ∼ 0.1 M) was added NaOH (1.05 equiv) in distilled water ([*C*] ∼ 0.1 M), and the resulting suspension was stirred at room temperature (16 h). The reaction mixture was evaporated in vacuo, and the obtained residue was dissolved in cold MeOH and filtered off. The combined MeOH is then removed in vacuo to yield a white salt.

General Procedure for Intermediate Compounds 18a−18n (Method G). To a mixture of commercially available terephthalaldehyde (5.00 g, 37.3 mmol) in EtOH and THF (50 mL, a ratio of 2:3) was added $NaBH_4$ (0.35 g, 9.3 mmol) at low temperature. The resulting suspension was stirred at the same temperature (7 h). The reaction mixture was quenched by adjusting the pH to 5−6 with an aqueous 2 M HCl solution. The resulting solution was extracted with ethyl acetate (3×), and the combined organic layer was dried with anhydrous Na_2SO_4. The resulting solution was evaporated in vacuo and was purified by column chromatography to give **16**. To a mixture of **16** (0.30 g, 2.2 mmol) in EtOH (3 mL) were added an aqueous hydroxylamine (0.62 g, 8.8 mmol) solution and sodium carbonate (0.94 g, 8.8 mmol). The resulting suspension was refluxed at 60 °C (12 h). The product mixture was neutralized by aqueous 1 N HCl solution and extracted with ethyl acetate. The combined organic layer was dried with anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography to give **17**. To a mixture of 4-[(hydroxyimino)-methyl] phenyl methanol **17** (1.0 equiv) in anhydrous THF ([*C*] ∼ 0.1 M) was added *N*-chlorosuccinimide (2.0 equiv), and the resulting mixture was stirred at −20 °C (1 h). The reaction mixture was refluxed at 60 °C (0.5 h). To the reaction mixture were slowly added trimethylamine (3.0 equiv) and styrene derivatives (1.5 equiv) at 50 °C, and the resulting suspension was stirred at the same temperature (2 h). The reaction mixture was diluted with cold distilled water and extracted with CH_2Cl_2. The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated in vacuo. The obtained residue was purified by column chromatography on SiO_2. The detailed methods and data for each intermediate compound are described in the Supporting Information.

*Preparation of Methyl 1-(4-(4-Phenyl-1H-1,2,3-triazol-1-yl)-benzyl)azetidine-3-carboxylate (**6a**).* Using method D, **5a** (0.06 g, 0.2 mmol), methyl azetidine-3-carboxylate hydrochloride (0.05 g, 0.3 mmol), triethylamine (0.1 mL, 0.7 mmol), and sodium cyanoborohydride (0.03 g, 0.4 mmol) gave **6a** as an ivory solid (0.04 g, 47%); R_f = 0.14 (*n*-hexane/EtOAc 1/5); MP: 148−150 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.17 (s, triazole ring-**H**), 7.90 (d, *J* = 7.36 Hz, 2 Ar**H**), 7.73 (d, *J* = 8.3 Hz, 2 Ar**H**), 7.44−7.47 (m, 4 Ar**H**), 7.36 (t, *J* = 7.3 Hz, 1 Ar**H**), 3.72 (s, COOC**H**$_3$), 3.68 (s, NC**H**$_2$), 3.52−3.58 (m, 2 azetidine ring-**H**), 3.33−3.38 (m, 3 azetidine ring-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.48 (**C**(O)), 148.38 (triazole ring-**C**), 138.70, 136.10, 130.29, 129.71, 128.92, 128.41, 125.86, 120.54, 117.56 (Ar**C**), 62.67 (N**C**H$_2$), 56.89 (azetidine ring-**C**), 52.00 (COO**C**H$_3$), 33.96 (azetidine ring-**C**); HPLC purity: 3.4 min, 98.1%; HRMS (M + H)$^+$ (ESI$^+$): 349.1662 [M + H]$^+$ (calcd for $C_{20}H_{20}N_4O_2H^+$, 349.1665).

*Preparation of Methyl 1-(4-(4-(4-Chlorophenyl)-1H-1,2,3-triazol-1-yl) benzyl)azetidine-3-carboxylate (**6b**).* Using method D, **5c** (0.28 g, 1.0 mmol), methyl azetidine-3-carboxylate hydrochloride (0.17 g, 1.1 mmol), triethylamine (0.41 mL, 3.0 mmol), and sodium cyanoborohydride (0.06 g, 1.0 mmol) gave **6b** as a white solid (0.16 g, 41%); R_f = 0.50 (EtOAc/MeOH 19/1); MP: 144−146 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.33 (s, triazole ring-**H**), 7.96−7.98 (m, 2 Ar**H**), 7.88 (d, *J* = 8.6 Hz, 2 Ar**H**), 7.58−7.60 (m, 2 Ar**H**), 7.52 (d, *J* = 8.5 Hz, 2 Ar**H**), 3.64 (s, NC**H**$_2$, COOC**H**$_3$), 3.37−3.47 (m, 2 azetidine ring-**H**), 3.30−3.36 (m, 1 azetidine ring-**H**), 3.24−3.27 (m, 2 azetidine ring-**H**); ^{13}C NMR (CD_3OD, 75 MHz): δ 173.42 (**C**(O)), 147.09 (triazole ring-**C**), 136.21, 133.90, 129.96, 128.78, 126.88, 120.42, 120.15, 119.11 (Ar**C**), 61.66 (N**C**H$_2$), 56.14 (azetidine ring-**C**), 51.17 (COO**C**H$_3$), 33.53 (azetidine ring-**C**); HPLC purity: 4.7 min, 97.2%; HRMS (M + H)$^+$ (ESI$^+$): 383.1275 [M + H]$^+$ (calcd for $C_{20}H_{19}ClN_4O_2H^+$, 383.1275).

*Preparation of Methyl 1-(4-(4-(4-Bromophenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**6c**).* Using method D, **5d** (0.27 g, 0.83 mmol), methyl azetidine-3-carboxylate hydrochloride (0.19 g, 1.2 mmol), triethylamine (0.35 mL, 2.5 mmol), and sodium cyanoborohydride (0.10 g, 1.7 mmol) gave **6c** as a white solid (0.17 g, 49%); R_f = 0.12 (*n*-hexane/EtOAc 1/1); MP: 188.5−190.5 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.33 (s, 1 triazole ring-**H**), 7.87−7.91 (m, 4 Ar**H**), 7.71 (d, *J* = 8.5 Hz, 2 Ar**H**), 7.52 (d, *J* = 8.3 Hz, 2 Ar**H**), 3.64−3.66 (m, NC**H**$_2$, COOC**H**$_3$), 3.47 (br, 2 azetidine ring-**H**), 3.35−3.37 (m, 1 azetidine ring-**H**), 3.24−3.29 (m, 2 azetidine ring-**H**); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 173.58 (**C**(O)), 146.68 (triazole ring-**C**), 139.44, 132.47, 130.09, 130.03, 127.76, 121.77, 120.47, 120.43 (Ar**C**), 62.12 (N**C**H$_2$), 56.70 (azetidine ring-**C**), 52.15 (COO**C**H$_3$), 33.75 (azetidine ring-**C**); HPLC purity: 4.6 min, 98.8%; HRMS (M + H)$^+$ (ESI$^+$): 427.0770 [M + H]$^+$ (calcd for $C_{20}H_{19}BrN_4O_2H^+$, 427.0770).

*Preparation of Methyl 1-(4-(4-(4-Ethylphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**6d**).* Using method D, **5f** (1.00 g, 3.6 mmol), methyl azetidine-3-carboxylate hydrochloride (0.60 g, 4.0 mmol), triethylamine (1.51 mL, 10.8 mmol), and sodium cyanoborohydride (0.23 g, 3.6 mmol) gave **6d** as a white solid (0.23 g, 17%); R_f = 0.14 (*n*-hexane/EtOAc 1/1); MP: 146−148 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.23 (s, triazole ring-**H**), 7.83−7.91 (m, 4 Ar**H**), 7.48−7.53 (m, 2 Ar**H**), 7.31−7.37 (m, 2 Ar**H**), 3.64 (s, COOC**H**$_3$), 3.62−3.64 (m, NC**H**$_2$), 3.41−3.48 (m, 2 azetidine ring-**H**), 3.29−3.39 (m, 1 azetidine ring-**H**), 3.22−3.28 (m, 2 azetidine ring-**H**), 2.66 (q, *J* = 15.1 Hz, C**H**$_2$CH$_3$), 1.22 (t, *J* = 7.6 Hz, CH$_2$C**H**$_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 173.56 (**C**(O)), 147.83 (triazole ring-**C**), 144.40, 139.13, 135.97, 130.07, 128.81, 128.19, 125.83, 120.33, 119.56 (Ar**C**), 62.05 (N**C**H$_2$), 56.65 (azetidine ring-**C**), 52.13 (COO**C**H$_3$), 33.73 (azetidine ring-**C**), 28.42 (**C**H$_2$CH$_3$), 15.92 (CH$_2$**C**H$_3$).

*Preparation of Methyl 1-(4-(4-(4-Butylphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**6e**).* Using method D, **5h** (0.67 g, 2.2 mmol), methyl azetidine-3-carboxylate hydrochloride (0.37 g, 2.4 mmol), triethylamine (0.92 mL, 6.6 mmol), and sodium

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J. Med. Chem. XXXX, XXX, XXX−XXX

cyanoborohydride (0.55 g, 8.8 mmol) gave **6e** as a white solid (0.60 g, 68%); R_f = 0.45 (EtOAc/MeOH 19/1); MP: 147−149 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 9.97 (s, triazole ring-**H**), 8.64−8.70 (m, 4 Ar**H**), 8.29 (d, J = 8.5 Hz, 2 Ar**H**), 8.08 (d, J = 8.1 Hz, 2 Ar**H**), 4.44−4.45 (m, COOC**H**$_3$, NC**H**$_2$), 4.24−4.28 (m, 2 azetidine ring-**H**), 4.06−4.16 (m, 3 azetidine ring-**H**), 3.43 (t, J = 7.6 Hz, alkyl chain-C**H**$_2$), 2.36−2.44 (m, alkyl chain-C**H**$_2$), 2.10−2.19 (m, alkyl chain-C**H**$_2$), 1.73 (t, J = 7.3 Hz, alkyl chain-C**H**$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.49 (**C**(O)), 148.51, 143.37, 138.58, 136.17, 129.71, 128.98, 127.66, 125.79, 120.51, 117.22 (Ar**C**, triazole ring-**C**), 62.67 (N**C**H$_2$), 56.89 (azetidine ring-**C**), 52.02 (COO**C**H$_3$), 35.48 (alkyl chain-**C**H$_2$), 33.97 (alkyl chain-**C**H$_2$), 33.55 (azetidine ring-**C**), 22.36 (alkyl chain-**C**H$_2$), 13.97 (alkyl chain-**C**H$_3$); HPLC purity: 6.3 min, 98.7%; HRMS (M + H)$^+$ (ESI$^+$): 405.2291 [M + H]$^+$ (calcd for $C_{24}H_{28}N_4O_2H^+$, 405.2291).

*Preparation of Methyl 1-(4-(4-(4-Pentylphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**6f**).* Using method D, **5i** (0.67 g, 2.1 mmol), methyl azetidine-3-carboxylate hydrochloride (0.35 g, 2.3 mmol), triethylamine (0.88 mL, 6.3 mmol), and sodium cyanoborohydride (0.07 g, 1.1 mmol) gave **6f** as a white solid (0.44 g, 50%); R_f = 0.48 (EtOAc/MeOH 19/1); MP: 130−132 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.22 (s, triazole ring-**H**), 7.83−7.89 (m, 4 Ar**H**), 7.50 (d, J = 8.3 Hz, 2 Ar**H**), 7.32 (d, J = 8.0 Hz, 2 Ar**H**), 3.64−3.68 (m, COOC**H**$_3$, NC**H**$_2$), 3.43−3.46 (m, 2 azetidine ring-**H**), 3.30−3.36 (m, 1 azetidine ring-**H**), 3.23−3.26 (m, 2 azetidine ring-**H**), 2.62 (t, J = 7.6 Hz, alkyl chain-C**H**$_2$), 1.57−1.65 (m, alkyl chain-C**H**$_2$), 1.29−1.36 (m, alkyl chain-C**H**$_2$C**H**$_2$), 0.88 (t, J = 6.8 Hz, alkyl chain-C**H**$_3$); ^{13}C NMR (CD_3OD, 75 MHz): δ 173.42 (**C**(O)), 148.31, 143.34, 138.07, 136.22, 129.85, 128.64, 127.39, 125.38, 120.02, 118.42 (Ar**C**, triazole ring-**C**), 61.81 (N**C**H$_2$), 56.16 (azetidine ring-**C**), 51.12 (COO**C**H$_3$), 35.25 (alkyl chain-**C**H$_2$), 33.55 (azetidine ring-**C**), 31.19 (alkyl chain-**C**H$_2$), 30.89 (alkyl chain-**C**H$_2$), 22.17 (alkyl chain-**C**H$_2$), 12.96 (alkyl chain-**C**H$_3$); HPLC purity: 12.0 min, 97.2%; HRMS (M + H)$^+$ (ESI$^+$): 419.2447 [M + H]$^+$ (calcd for $C_{25}H_{30}N_4O_2H^+$, 419.2447).

*Preparation of Methyl 1-(4-(4-(4-Hexylphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**6g**).* Using method D, **5j** (0.75 g, 2.3 mmol), methyl azetidine-3-carboxylate hydrochloride (0.38 g, 2.5 mmol), triethylamine (0.94 mL, 6.8 mmol), and sodium cyanoborohydride (0.07 g, 1.1 mmol) gave **6g** as a white solid (0.36 g, 37%); R_f = 0.48 (EtOAc/MeOH 19/1); MP: 129−131 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.22 (s, triazole ring-**H**), 7.87 (dd, J = 8.0, 15.9 Hz, 4 Ar**H**), 7.51 (d, J = 8.2 Hz, 2 Ar**H**), 7.32 (d, J = 7.8 Hz, 2 Ar**H**), 3.64−3.66 (m, COOC**H**$_3$, NC**H**$_2$), 3.43−4.47 (m, 2 azetidine ring-**H**), 3.30−3.38 (m, 1 azetidine ring-**H**), 3.24−3.27 (m, 2 azetidine ring-**H**), 2.63 (t, J = 7.6 Hz, alkyl chain-C**H**$_2$), 1.59−1.62 (m, alkyl chain-C**H**$_2$), 1.24−1.30 (m, alkyl chain-C**H**$_2$C**H**$_2$C**H**$_2$), 0.85−0.88 (m, alkyl chain-C**H**$_3$); ^{13}C NMR (CD_3OD, 75 MHz): δ 173.41 (**C**(O)), 148.32, 143.34, 138.04, 136.23, 129.87, 128.65, 127.39, 125.38, 120.04, 118.43 (Ar**C**, triazole ring-**C**), 61.79 (N**C**H$_2$), 56.16 (azetidine ring-**C**), 51.12 (COO**C**H$_3$), 35.29 (alkyl chain-**C**H$_2$), 33.54 (azetidine ring-**C**), 31.46 (alkyl chain-**C**H$_2$), 31.17 (alkyl chain-**C**H$_2$), 28.61 (alkyl chain-**C**H$_2$), 22.27 (alkyl chain-**C**H$_2$), 12.99 (alkyl chain-**C**H$_3$); HPLC purity: 7.6 min, 95.7%; HRMS (M + H)$^+$ (ESI$^+$): 433.2605 [M + H]$^+$ (calcd for $C_{26}H_{32}N_4O_2H^+$, 433.2604).

*Preparation of Methyl 1-(4-(4-(4-Isopropylphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**6h**).* Using method D, **5k** (0.55 g, 1.9 mmol), methyl azetidine-3-carboxylate hydrochloride (0.31 g, 2.1 mmol), triethylamine (0.79 mL, 5.7 mmol), and sodium cyanoborohydride (0.12 g, 1.9 mmol) gave **6h** as a white solid (0.23 g, 31%); R_f = 0.33 (*n*-hexane/EtOAc 1/1); MP: 150−152 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.23 (s, triazole ring-**H**), 7.84−7.92 (m, 4 Ar**H**), 7.48−7.53 (m, 2 Ar**H**), 7.35−7.40 (m, 2 Ar**H**), 3.64 (s, COOC**H**$_3$), 3.62−3.64 (m, NC**H**$_2$), 3.42−3.48 (m, 2 azetidine ring-**H**), 3.30−3.39 (m, 1 azetidine ring-**H**), 3.23−3.28 (m, 2 azetidine ring-**H**), 2.90−2.99 (m, C**H**(CH$_3$)$_2$), 1.25 (d, J = 6.9 Hz, CH(C**H**$_3$)$_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 173.57 (**C**(O)), 148.99, 147.80 (triazole ring-**C**), 139.22, 135.97, 130.05, 128.37, 127.35, 125.85, 120.31, 119.59 (Ar**C**), 62.12 (N**C**H$_2$), 56.68 (azetidine ring-**C**), 52.13 (COO**C**H$_3$), 33.72 (azetidine ring-**C**), 24.25 (CH(**C**H$_3$)$_2$).

*Preparation of Methyl 1-(4-(4-(4-(tert-Butyl)phenyl)-1H-1,2,3-triazol-yl) benzyl)azetidine-3-carboxylate (**6i**).* Using method D, **5l** (0.46 g, 1.5 mmol), methyl azetidine-3-carboxylate hydrochloride (0.34 g, 2.3 mmol), triethylamine (1.26 mL, 9.1 mmol), and sodium cyanoborohydride (0.10 g, 1.0 mmol) gave **6i** as a white solid (0.25 g, 63%); R_f = 0.35 (EtOAc/MeOH 9/1); MP: 151−153 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.24 (s, triazole ring-**H**), 7.86−7.91 (m, 4 Ar**H**), 7.49−7.54 (m, 4 Ar**H**), 3.64−3.83 (m, COOC**H**$_3$, NC**H**$_2$), 3.43−3.47 (m, 2 azetidine ring-**H**), 3.31−3.38 (m, 1 azetidine ring-**H**), 3.24−3.27 (m, 2 azetidine ring-**H**), 1.33 (s, C(C**H**$_3$)$_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 173.56 (**C**(O)), 151.22, 147.73, 139.21, 135.97, 130.03, 127.99, 126.18, 125.59, 120.31, 119.62 (Ar**C**, triazole ring-**C**), 62.12 (N**C**H$_2$), 56.68 (azetidine ring-**C**), 52.12 (COO**C**H$_3$), 34.88 (**C**H(CH$_3$)$_3$), 33.74 (azetidine ring-**C**), 31.53 (CH(**C**H$_3$)$_3$); HPLC purity: 5.8 min, 99.6%; HRMS (M + H)$^+$ (ESI$^+$): 405.2292 [M + H]$^+$ (calcd for $C_{24}H_{28}N_4O_2H^+$, 405.2291).

*Preparation of Methyl 1-(4-(4-(4-Ethoxyphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**6j**).* Using method D, **5m** (0.16 g, 0.6 mmol), methyl azetidine-3-carboxylate hydrochloride (0.09 g, 0.6 mmol), triethylamine (0.23 mL, 1.7 mmol), and sodium cyanoborohydride (0.04 g, 0.6 mmol) gave **6j** as a white solid (0.17 g, 78%); R_f = 0.50 (EtOAc/MeOH 19/1); MP: 132−134 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.16 (s, triazole ring-**H**), 7.84−7.89 (m, 4**H**), 7.50 (d, J = 8.6 Hz, 2**H**), 7.04−7.06 (m, 2**H**), 4.08 (q, J = 7.0 Hz, OC**H**$_2$CH$_3$), 3.62−3.63 (m, COOC**H**$_3$, NC**H**$_2$), 3.43−3.47 (m, 2 azetidine ring-**H**), 3.31−3.37 (m, 1 azetidine ring-**H**), 3.23−3.27 (m, 2 azetidine ring-**H**), 1.36 (t, J = 7.0 Hz, OCH$_2$C**H**$_3$); ^{13}C NMR (CD_3OD, 75 MHz): δ 173.41 (**C**(O)), 162.33, 159.43, 137.93, 136.29, 129.89, 126.77, 122.37, 120.04, 117.88, 114.56 (Ar**C**, triazole ring-**C**), 63.21 (N**C**H$_2$), 61.74 (O**C**H$_2$CH$_3$), 56.15 (azetidine ring-**C**), 51.13 (COO**C**H$_3$), 33.53 (azetidine ring-**C**), 13.72 (OCH$_2$**C**H$_3$); HPLC purity: 4.0 min, 99.1%; HRMS (M + H)$^+$ (ESI$^+$): 393.1926 [M + H]$^+$ (calcd for $C_{22}H_{24}N_4O_3H^+$, 393.1927).

*Preparation of Methyl 1-(4-(4-(4-Isopropoxyphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**6k**).* Using method D, **5n** (0.06 g, 0.2 mmol), methyl azetidine-3-carboxylate hydrochloride (0.05 g, 0.3 mmol), triethylamine (0.09 mL, 0.6 mmol), and sodium cyanoborohydride (0.02 g, 0.4 mmol) gave **6k** as an ivory solid (0.05 g, 56%); R_f = 0.16 (*n*-hexane/EtOAc 1/5); MP: 129−131 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.07 (s, triazole ring-**H**), 7.80 (d, J = 8.8 Hz, 2 Ar**H**), 7.72 (d, J = 8.4 Hz, 2 Ar**H**), 7.45 (d, J = 8.3 Hz, 2 Ar**H**), 6.96 (d, J = 8.7 Hz, 2 Ar**H**), 4.61 (sept, J = 6.0 Hz, OC**H**(CH$_3$)$_2$), 3.73 (s, COOC**H**$_3$), 3.69 (s, NC**H**$_2$), 3.58 (br, 2 azetidine ring-**H**), 3.36−3.40 (m, 3 azetidine ring-**H**), 1.36 (d, J = 6.0 Hz, OCH(C**H**$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.42 (**C**(O)), 158.18, 148.33, 138.29, 136.23, 129.74, 127.19, 122.69, 120.48, 116.67, 116.20 (Ar**C**, triazole ring-**C**), 69.99 (O**C**H(CH$_3$)$_2$), 62.56 (N**C**H$_2$), 56.82 (azetidine ring-**C**), 52.03 (COO**C**H$_3$), 33.91 (azetidine ring-**C**), 22.05 (OCH(**C**H$_3$)$_2$); HPLC purity: 5.0 min, 99.6%; HRMS (M + H)$^+$ (ESI$^+$): 407.2082 [M + H]$^+$ (calcd for $C_{23}H_{26}N_4O_3H^+$, 407.2083).

*Preparation of Methyl 1-(4-(4-(3-Cyano-4-isopropoxyphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**6l**).* Using method D, **5o** (0.19 g, 0.5 mmol), methyl azetidine-3-carboxylate hydrochloride (0.12 g, 0.8 mmol), triethylamine (0.23 mL, 1.7 mmol), and sodium cyanoborohydride (0.07 g, 1.1 mmol) gave **6l** as an ivory solid (0.1 g, 41%); R_f = 0.16 (*n*-hexane/EtOAc 1/5); MP: 140−142 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.13 (s, triazole ring-**H**), 8.10 (d, J = 2.2 Hz, 1 Ar**H**), 8.08 (d, J = 2.2 Hz, 1 Ar**H**), 8.01 (d, J = 2.2 Hz, 1 Ar**H**), 7.71 (d, J = 8.4 Hz, 2 Ar**H**), 7.46 (d, J = 8.4 Hz, 2 Ar**H**), 7.05 (d, J = 8.8 Hz, 1 Ar**H**), 4.70 (sept, J = 6.0 Hz, OC**H**(CH$_3$)$_2$), 3.71 (s, COOC**H**$_3$), 3.68 (s, NC**H**$_2$), 3.54−3.56 (m, 2 azetidine ring-**H**), 3.33−3.38 (m, 3 azetidine ring-**H**), 1.42 (d, J = 6.0 Hz, OCH(C**H**$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.47 (**C**(O)), 159.84, 146.37 (triazole ring-**C**), 138.95, 135.90, 131.57, 131.07, 129.76, 123.25, 120.53, 117.27, 116.29, 114.15, 103.53 (Ar**C**), 72.19 (O**C**H(CH$_2$)$_3$), 62.64 (N**C**H$_2$), 56.90 (azetidine ring-**C**), 52.02 (COO**C**H$_3$), 33.95 (azetidine ring-**C**), 21.87 (OCH(**C**H$_3$)$_2$); HPLC

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J. Med. Chem. XXXX, XXX, XXX−XXX

purity: 4.9 min, 97.9%; HRMS (M + H)$^+$ (ESI$^+$): 432.2038 [M + H]$^+$ (calcd for $C_{24}H_{25}N_5O_3H^+$, 432.2036).

*Preparation of Methyl 1-(4-(4-(3-Chloro-4-isopropoxyphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**6m**).* Using method D, **5p** (0.050 g, 0.15 mmol), methyl azetidine-3-carboxylate hydrochloride (0.033 g, 0.22 mmol), sodium cyanoborohydride (0.018 g, 0.29 mmol), and triethylamine (0.06 mL, 0.44 mmol) gave **6m** as a clear oil (0.031 g, 61%); R_f = 0.07 (*n*-hexane/EtOAc 1/1); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.25 (s, 1 triazole ring-**H**), 7.96 (s, 1 Ar**H**), 7.84−7.87 (m, 3 Ar**H**), 7.50 (d, J = 8.0 Hz, 2 Ar**H**), 7.31 (d, J = 8.8 Hz, 1 Ar**H**), 4.71−4.77 (m, OC**H**(CH$_3$)$_2$), 3.62−3.63 (m, COOC**H$_3$**, NC**H$_2$**), 3.44 (t, J = 6.7 Hz, 2 azetidine ring-**H**), 3.32−3.35 (m, 1 azetidine ring-**H**), 3.22−3.26 (m, 2 azetidine ring-**H**), 1.32 (d, J = 6.0 Hz, OCH(C**H$_3$**)$_2$); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.99 (**C**(O)), 152.72, 145.89 (triazole ring-**C**), 138.69, 135.34, 129.51, 126.72, 125.01, 123.66, 122.84, 119.69, 119.09, 115.95 (Ar**C**), 71.19 (O**C**H(CH$_3$)$_2$), 61.52 (N**C**H$_2$), 56.11 (azetidine ring-**C**), 51.57 (COO**C**H$_3$), 33.16 (azetidine ring-**C**), 21.69 (OCH(**C**H$_3$)$_2$); HPLC purity: 4.7 min, 98.2%; HRMS (M + H)$^+$ (ESI$^+$): 441.1696 [M + H]$^+$ (calcd for $C_{23}H_{25}ClN_4O_3H^+$, 441.1693).

*Preparation of Methyl 1-(4-(4-(4-Isopropoxy-3-(trifluoromethyl)-phenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**6n**).* Using method D, **5q** (0.13 g, 0.3 mmol), methyl azetidine-3-carboxylate hydrochloride (0.07 g, 0.5 mmol), triethylamine (0.14 mL, 1.0 mmol), and sodium cyanoborohydride (0.04 g, 0.7 mmol) gave **6n** as an ivory solid (0.08 g, 48%); R_f = 0.19 (*n*-hexane/EtOAc 1/5); MP: 98−100 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.14 (s, triazole ring-**H**), 8.02−8.06 (m, 2 Ar**H**), 7.72 (d, J = 8.4 Hz, 2 Ar**H**), 7.45 (d, J = 8.4 Hz, 2 Ar**H**), 7.09 (d, J = 8.8 Hz, 1 Ar**H**), 4.70 (sept, J = 6.0 Hz, OC**H**(CH$_3$)$_2$), 3.71 (s, COOC**H$_3$**), 3.68 (s, NC**H$_2$**), 3.53−3.57 (m, 2 azetidine ring-**H**), 3.33−3.38 (m, 3 azetidine ring-**H**), 1.39 (d, J = 6.0 Hz, OCH(C**H$_3$**)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.49 (**C**(O)), 156.12, 147.17 (triazole ring-**C**), 138.81, 135.95, 130.40, 129.69 (Ar**C**), 124.78 (q, J_{C-F} = 7.3 Hz), 123.52 (q, J_{C-F} = 270 Hz), 122.21 (Ar**C**), 120.44, 120.23 (q, J_{C-F} = 30.5 Hz), 117.17, 114.70 (Ar**C**), 71.50 (O**C**H(CH$_3$)$_2$), 62.63 (N**C**H$_2$), 56.87 (azetidine ring-**C**), 51.98 (COO**C**H$_3$), 33.95 (azetidine ring-**C**), 21.84 (OCH-(**C**H$_3$)$_2$); HPLC purity: 6.5 min, 97.6%; HRMS (M + H)$^+$ (ESI$^+$): 475.1959 [M + H]$^+$ (calcd for $C_{24}H_{25}F_3N_4O_3H^+$, 475.1957).

*Preparation of Sodium 1-(4-(4-Phenyl-1H-1,2,3-triazol-1-yl)-benzyl)azetidine-3-carboxylate (**7a**).* Using method F, **6a** (0.03 g, 0.1 mmol) and NaOH (4.3 mg, 0.1 mmol) gave **7a** as a white solid (0.02 g, 67%); MP: 282−284 °C (decomp.); ^{1}H NMR (CD_3OD, 400 MHz): δ 8.92 (s, triazole ring-**H**), 7.93 (d, J = 8.5 Hz, 2 Ar**H**), 7.89 (d, J = 8.5 Hz, 2 Ar**H**), 7.55 (d, J = 8.5 Hz, 2 Ar**H**), 7.46−7.50 (m, 2 Ar**H**), 7.37−7.39 (m, 1 Ar**H**), 3.73 (s, NC**H$_2$**), 3.56−3.60 (m, 2 azetidine ring-**H**), 3.36−3.41 (m, 2 azetidine ring-**H**), 3.19−3.28 (m, 1 azetidine ring-**H**); ^{13}C NMR (CD_3OD, 100 MHz): δ 179.53 (**C**(O)), 148.19, 138.49, 136.13, 130.04, 129.97, 128.62, 128.18, 125.41, 120.04, 118.86 (Ar**C**, triazole ring-**C**), 62.09 (N**C**H$_2$), 57.68 (azetidine ring-**C**), 36.54 (azetidine ring-**C**); HPLC purity: 8.0 min, 99.3%; HRMS (M + H)$^+$ (ESI$^+$): 335.1506 [M + H]$^+$ (calcd for $C_{19}H_{18}N_4O_2H^+$, 335.1508).

*Preparation of 1-(4-(4-(4-Fluorophenyl)-1H-1,2,3-triazol-1-yl)-benzyl)azetidine-3-carboxylic Acid (**7b**).* Using method D, **5b** (0.36 g, 1.4 mmol), 3-azetidine carboxylic acid (0.15 g, 1.4 mmol), and sodium cyanoborohydride (0.04 g, 0.7 mmol) gave **7b** as a white solid (0.27 g, 57%); R_f = 0.18 (CH_2Cl_2/MeOH 8/2); MP: 182−184 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.90 (s, triazole ring-**H**), 7.92−7.99 (m, 2 Ar**H**), 7.86−7.90 (m, 2 Ar**H**), 7.53−7.56 (m, 2 Ar**H**), 7.19−7.25 (m, 2 Ar**H**), 3.73 (s, NC**H$_2$**), 3.56−3.60 (m, 2 azetidine ring-**H**), 3.36−3.42 (m, 2 azetidine ring-**H**), 3.21−3.28 (m, 1 azetidine ring-**H**); ^{13}C NMR (CD_3OD, 75 MHz): δ 179.54 (**C**(O)), 162.87 (d, J_{C-F} = 244.8 Hz), 147.26 (triazole ring-**C**), 138.53, 136.08, 129.96, 127.40 (d, J_{C-F} = 8.2 Hz), 126.52, 120.02, 118.74 (Ar**C**), 115.43 (d, J_{C-F} = 21.9 Hz), 62.09 (N**C**H$_2$), 57.68 (azetidine ring-**C**), 36.54 (azetidine ring-**C**); HPLC purity: 3.5 min, 97.1%; HRMS (M + H)$^+$ (ESI$^+$): 353.1413 [M + H]$^+$ (calcd for $C_{19}H_{17}FN_4O_2H^+$, 353.1414).

*Preparation of 1-(4-(4-(4-Chlorophenyl)-1H-1,2,3-triazol-1-yl)-benzyl)azetidine-3-carboxylic Acid (**7c**).* Using method D, **5c** (0.20 g, 0.7 mmol), 3-azetidine carboxylic acid (0.07 g, 0.7 mmol), and sodium cyanoborohydride (0.02 g, 0.4 mmol) gave **7c** as a white solid (0.13 g, 51%); R_f = 0.20 (CH_2Cl_2/MeOH 8/2); MP: 208−210 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.33 (s, triazole ring-**H**), 7.95−7.98 (m, 2 Ar**H**), 7.87−7.91 (m, 2 Ar**H**), 7.55−7.59 (m, 2 Ar**H**), 7.50−7.52 (m, 2 Ar**H**), 3.64 (s, NC**H$_2$**), 3.39−3.46 (m, 2 azetidine ring-**H**), 3.21−3.26 (m, 3 azetidine ring-**H**); ^{13}C NMR (CD_3OD, 75 MHz): δ 179.57 (**C**(O)), 147.03 (triazole ring-**C**), 138.51, 136.04, 133.83, 129.96, 128.85, 128.76, 126.86, 120.02, 119.08 (Ar**C**), 62.06 (N**C**H$_2$), 57.68 (azetidine ring-**C**), 49.69 (O**C**H$_3$), 36.52 (azetidine ring-**C**); HPLC purity: 4.5 min, 98.8%; HRMS (M + H)$^+$ (ESI$^+$): 369.1120 [M + H]$^+$ (calcd for $C_{19}H_{17}ClN_4O_2H^+$, 369.1118).

*Preparation of 1-(4-(4-(4-Trifluoromethylphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylic Acid (**7d**).* Using method D, **5e** (0.05 g, 0.2 mmol), 3-azetidine carboxylic acid (0.02 g, 0.2 mmol), and sodium cyanoborohydride (0.01 g, 0.1 mmol) gave **7d** as a white solid (0.06 g, 99%); R_f = 0.15 (CH_2Cl_2/MeOH 8/2); MP: 192−194 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.47 (s, triazole ring-**H**), 8.16 (d, J = 8.1 Hz, 2 Ar**H**), 7.88−7.91 (m, 4 Ar**H**), 7.52 (d, J = 8.4 Hz, 2 Ar**H**), 3.64 (s, NC**H$_2$**), 3.40−3.43 (m, 2 azetidine ring-**H**), 3.23−3.33 (m, 3 azetidine ring-**H**); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.67 (**C**(O)), 146.32 (triazole ring-**C**), 139.56, 135.78, 134.72, 130.11, 128.79 (q, J_{C-F} = 31.7 Hz), 128.15, 126.48 (q, J_{C-F} = 3.6 Hz), 126.32, 124.7 (q, J_{C-F} = 271.3 Hz), 121.37, 120.49 (Ar**C**), 62.11 (N**C**H$_2$), 56.84 (azetidine ring-**C**), 34.00 (azetidine ring-**C**); HPLC purity: 5.1 min, 99.3%; HRMS (M + H)$^+$ (ESI$^+$): 403.1384 [M + H]$^+$ (calcd for $C_{20}H_{17}F_3N_4O_2H^+$, 403.1382).

*Preparation of 1-(4-(4-(4-Ethylphenyl)-1H-1,2,3-triazol-1-yl)-benzyl)azetidine-3-carboxylic Acid (**7e**).* Using method D, **5f** (0.30 g, 1.1 mmol), 3-azetidine carboxylic acid (0.12 g, 1.1 mmol), and sodium cyanoborohydride (0.03 g, 0.5 mmol) gave **7e** as a white solid (0.21 g, 55%); R_f = 0.20 (CH_2Cl_2/MeOH 8/2); MP: 193−195 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.23 (s, triazole ring-**H**), 7.82−7.90 (m, 4 Ar**H**), 7.48−7.53 (m, 2 Ar**H**), 7.31−7.36 (m, 2 Ar**H**), 3.63 (s, NC**H$_2$**), 3.16−3.42 (m, 5 azetidine ring-**H**), 2.65 (q, J = 7.6 Hz, C**H$_2$**CH$_3$), 1.22 (t, J = 7.6 Hz, CH$_2$C**H$_3$**); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.88 (**C**(O)), 147.80, 144.37, 139.28, 135.96, 130.03, 128.78, 128.22, 125.81, 120.29, 119.55 (Ar**C**, triazole ring-**C**), 62.17 (N**C**H$_2$), 55.31 (azetidine ring-**C**), 34.33 (azetidine ring-**C**), 28.42 (**C**H$_2$CH$_3$), 14.50 (CH$_2$**C**H$_3$); HPLC purity: 3.7 min, 95.2%; HRMS (M + H)$^+$ (ESI$^+$): 363.1820 [M + H]$^+$ (calcd for $C_{21}H_{22}N_4O_2H^+$, 363.1821).

*Preparation of 1-(4-(4-(4-Propylphenyl)-1H-1,2,3-triazol-1-yl)-benzyl)azetidine-3-carboxylic Acid (**7f**).* Using method D, **5g** (0.30 g, 1.0 mmol), 3-azetidine carboxylic acid (0.11 g, 1.1 mmol), and sodium cyanoborohydride (0.03 g, 0.5 mmol) gave **7f** as a white solid (0.21 g, 54%); R_f = 0.20 (CH_2Cl_2/MeOH 8/2); MP: 190−192 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.22 (s, triazole ring-**H**), 7.83−7.89 (m, 4 Ar**H**), 7.47−7.54 (m, 2 Ar**H**), 7.28−7.35 (m, 2 Ar**H**), 3.63 (s, NC**H$_2$**), 3.42−3.46 (m, 2 azetidine ring-**H**), 3.21−3.37 (m, 3 azetidine ring-**H**), 2.57−2.62 (m, alkyl chain-C**H$_2$**), 1.61−1.69 (m, alkyl chain-C**H$_2$**), 0.93 (t, J = 7.3 Hz, alkyl chain-C**H$_3$**); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 174.72 (**C**(O)), 147.81, 142.75, 139.32, 135.95, 130.04, 129.39, 128.25, 125.75, 120.31, 119.60 (Ar**C**, triazole ring-**C**), 62.19 (N**C**H$_2$), 56.89 (azetidine ring-**C**), 37.47 (alkyl chain-**C**H$_2$), 34.09 (azetidine ring-**C**), 24.43 (alkyl chain-**C**H$_2$), 14.08 (alkyl chain-**C**H$_3$); HPLC purity: 5.5 min, 100%; HRMS (M + H)$^+$ (ESI$^+$): 377.1976 [M + H]$^+$ (calcd for $C_{22}H_{24}N_4O_2H^+$, 377.1978).

*Preparation of 1-(4-(4-(4-Butylphenyl)-1H-1,2,3-triazol-1-yl)-benzyl)azetidine-3-carboxylic Acid (**7g**).* Using method D, **5h** (0.07 g, 0.2 mmol), 3-azetidine carboxylic acid (0.02 g, 0.2 mmol), and sodium cyanoborohydride (0.008 g, 0.1 mmol) gave **7g** as a white solid (0.05 g, 51%); R_f = 0.20 (CH_2Cl_2/MeOH 8/2); MP: 157−159 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.38−12.47 (br, COO**H**), 9.36 (s, triazole ring-**H**), 8.03 (d, J = 8.37 Hz, 2 Ar**H**), 7.79−7.87 (m, 4 Ar**H**), 7.32 (d, J = 8.1 Hz, 2 Ar**H**), 4.41 (s, NC**H$_2$**), 4.07−4.09 (m, 4 azetidine ring-**H**), 3.62−3.65 (m, 1 azetidine ring-**H**), 2.60−2.65 (m, alkyl chain-C**H$_2$**), 1.54−1.64 (m, alkyl chain-C**H$_2$**), 1.29−1.39 (m,

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J. Med. Chem. XXXX, XXX, XXX−XXX

alkyl chain-CH_2), 0.91 (t, J = 7.3 Hz, alkyl chain-CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.42 (**C**(O)), 147.81, 142.95, 138.54, 136.10, 130.23, 129.32, 128.19, 125.77, 120.32, 119.58 (Ar**C**, triazole ring-**C**), 61.63 (NCH_2), 56.68 (azetidine ring-**C**), 35.04 (alkyl chain-CH_2), 33.86 (azetidine ring-**C**), 33.45 (alkyl chain-CH_2), 22.18 (alkyl chain-CH_2), 14.22 (alkyl chain-CH_3); HPLC purity: 6.3 min, 95.4%; HRMS (M + H)$^+$ (ESI$^+$): 391.2127 [M + H]$^+$ (calcd for $C_{23}H_{26}N_4O_2H^+$, 391.2134).

*Preparation of 1-(4-(4-(4-Pentylphenyl)-1H-1,2,3-triazol-1-yl)-benzyl)azetidine-3-carboxylic Acid (**7h**).* Using method D, **5i** (0.14 g, 0.4 mmol), 3-azetidine carboxylic acid (0.05 g, 0.5 mmol), and sodium cyanoborohydride (0.01 g, 0.2 mmol) gave **7h** as a white solid (0.10 g, 55%); R_f = 0.22 (CH_2Cl_2/MeOH 8/2); MP: 157−159 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.31 (s, triazole ring-**H**), 8.03 (d, J = 8.5 Hz, 2 Ar**H**), 7.85 (d, J = 8.1 Hz, 2 Ar**H**), 7.77 (d, J = 8.5 Hz, 2Ar**H**), 7.33 (d, J = 8.1 Hz, 2 Ar**H**), 4.42 (s, NCH_2), 4.08−4.17 (m, 4 azetidine ring-**H**), 3.58−3.66 (m, 1 azetidine ring-**H**), 2.60−2.64 (m, alkyl chain-CH_2), 1.57−1.65 (m, alkyl chain-CH_2), 1.31−1.35 (m, alkyl chain-CH_2CH_2), 0.88 (t, J = 7.3 Hz, alkyl chain-CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.70 (**C**(O)), 147.81, 142.98, 139.26, 135.96, 130.05, 129.33, 128.21, 125.76, 120.30, 119.58 (Ar**C**, triazole ring-**C**), 62.16 (NCH_2), 56.85 (azetidine ring-**C**), 35.35 (alkyl chain-CH_2), 34.03 (azetidine ring-**C**), 31.35 (alkyl chain-CH_2), 30.99 (alkyl chain-CH_2), 22.42 (alkyl chain-CH_2), 14.39 (alkyl chain-CH_3); HPLC purity: 7.1 min, 96.8%; HRMS (M + H)$^+$ (ESI$^+$): 405.2292 [M + H]$^+$ (calcd for $C_{24}H_{28}N_4O_2H^+$, 405.2291).

*Preparation of 1-(4-(4-(4-Hexylphenyl)-1H-1,2,3-triazol-1-yl)-benzyl)azetidine-3-carboxylic Acid (**7i**).* Using method D, **5j** (0.14 g, 0.4 mmol), 3-azetidine carboxylic acid (0.04 g, 0.4 mmol), and sodium cyanoborohydride (0.01 g, 0.2 mmol) gave **7i** as a white solid (0.09 g, 50%); R_f = 0.22 (CH_2Cl_2/MeOH 8/2); MP: 242−244 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.50−13.00 (br, COO**H**), 9.31 (s, triazole ring-**H**), 8.02 (d, J = 8.6 Hz, 2 Ar**H**), 7.85 (d, J = 8.1 Hz, 2 Ar**H**), 7.76−7.87 (d, J = 8.5 Hz, 2 Ar**H**), 7.32 (d, J = 8.2 Hz, 2 Ar**H**), 4.39 (s, NCH_2), 4.07−4.13 (m, 4 azetidine ring-**H**), 3.59−3.63 (m, 1 azetidine ring-**H**), 2.60−2.64 (m, alkyl chain-CH_2), 1.56−1.61 (m, alkyl chain-CH_2), 1.23−1.33 (m, alkyl chain-$CH_2CH_2CH_2$), 0.88 (t, J = 7.3 Hz, alkyl chain-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.34 (**C**(O)), 147.99, 143.12, 137.44, 132.06, 129.38, 128.07, 125.80, 120.56, 119.70 (Ar**C**, triazole ring-**C**), 62.16 (NCH_2), 55.10 (azetidine ring-**C**), 35.40 (alkyl chain-CH_2), 32.66 (azetidine ring-**C**), 31.57 (alkyl chain-CH_2), 31.28 (alkyl chain-CH_2), 28.78 (alkyl chain-CH_2), 22.53 (alkyl chain-CH_2), 14.42 (alkyl chain-CH_3); HPLC purity: 8.9 min, 98.6%; HRMS (M + H)$^+$ (ESI$^+$): 419.2449 [M + H]$^+$ (calcd for $C_{25}H_{30}N_4O_2H^+$, 419.2447).

*Preparation of Sodium 1-(4-(4-(4-Bromophenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**7j**).* Using method F, **6c** (0.13 g, 0.31 mmol), NaOH (0.013 g, 0.31 mmol), and THF gave **7j** as a white solid (0.047 g, 35%); MP: 252.5−254.5 °C (decomp.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.33 (s, 1 triazole ring-**H**), 7.89 (d, J = 8.3 Hz, 2 Ar**H**), 7.84 (d, J = 8.3 Hz, 2 Ar**H**), 7.70 (d, J = 8.4 Hz, 2Ar**H**), 7.48 (d, J = 8.4 Hz, 2 Ar**H**), 3.55 (s, NCH_2), 3.26 (t, J = 7.4 Hz, 3 azetidine ring-**H**), 3.09 (t, J = 7.1 Hz, 2 azetidine ring-**H**); ^{13}C NMR (CD_3OD, 100 MHz): δ 173.59 (**C**(O)), 146.68 (triazole ring-**C**), 139.44, 132.47, 130.09, 130.03, 127.76, 121.77, 120.47, 120.43 (Ar**C**), 62.12 (NCH_2), 56.70, 52.15, 33.75 (azetidine ring-**C**); HPLC purity: 4.8 min, 98.4%; HRMS (M + H)$^+$ (ESI$^+$): 413.0614 [M + H]$^+$ (calcd for $C_{19}H_{17}BrN_4O_2H^+$, 413.0613).

*Preparation of Sodium 1-(4-(4-(4-(tert-Butyl)phenyl)-1H-1,2,3-triazol-1-yl) benzyl)azetidine-3-carboxylate (**7k**).* Using method F, **6i** (0.03 g, 0.08 mmol) and NaOH (0.005 g, 1.2 mmol) gave **7k** as a white solid (0.02 g, 55%); R_f = 0.05 (*n*-hexane/EtOAc 1/1); MP: 279−281 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.22 (s, triazole ring-**H**), 7.85−7.87 (m, 4 Ar**H**), 7.47−7.53 (m, 4 Ar**H**), 3.56 (NCH_2), 3.26−3.30 (m, 2 azetidine ring-**H**), 3.08−3.11 (m, 2 azetidine ring-**H**), 2.74−2.78 (m, 1 azetidine ring-**H**), 1.32 (s, $C(CH_3)_3$); ^{13}C NMR (CD_3OD, 100 MHz): δ 179.56 (**C**(O)), 151.46, 148.20 (triazole ring-**C**), 138.45, 136.14, 129.95, 127.15, 125.50, 125.20, 120.01, 118.54 (Ar**C**), 62.11 (NCH_2), 57.68 (azetidine ring-**C**), 36.54 (azetidine ring-**C**), 34.14 (**C**$(CH_3)_3$), 30.30 (C(**C**$H_3)_3$); HPLC purity: 6.0 min, 99.0%; HRMS (M + H)$^+$ (ESI$^+$): 391.2127 [M + H]$^+$ (calcd for $C_{23}H_{26}N_4O_2H^+$, 391.2134).

*Preparation of Sodium 1-(4-(4-(4-Isopropylphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**7l**).* Using method F, **6h** (0.09 g, 0.2 mmol) and NaOH (0.005 g, 0.2 mmol) gave **7l** as a white solid (0.07 g, 75%); R_f = 0.26 (CH_2Cl_2/MeOH 8/2); MP: 270−272 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.22 (s, triazole ring-**H**), 7.84−7.89 (m, 4 Ar**H**), 7.49−7.57 (m, 2 Ar**H**), 7.37 (d, J = 8.2 Hz, 2 Ar**H**), 3.62 (s, NCH_2), 3.35−3.46 (m, 2 azetidine ring-**H**), 3.13−3.24 (m, 3 azetidine ring-**H**), 2.88−2.98 (m, C**H**$(CH_3)_2$), 1.23 (d, J = 6.9 Hz, m, $CH(CH_3)_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.92 (**C**(O)), 147.79 (triazole ring-**C**), 139.38, 135.93, 130.04, 128.37, 127.35, 125.84, 120.29, 119.60 (Ar**C**), 57.05, 34.38 (azetidine ring-**C**), 33.72 (**C**$H(CH_3)_2$), 24.26 ($CH(CH_3)_2$); HPLC purity: 6.7 min, 98.5%; HRMS (M + H)$^+$ (ESI$^+$): 377.1979 [M + H]$^+$ (calcd for $C_{22}H_{24}N_4O_2H^+$, 377.1978).

*Preparation of Sodium 1-(4-(4-(4-Isopropoxyphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**7m**).* Using method F, **6k** (0.04 g, 0.1 mmol) and NaOH (4.3 mg, 0.1 mmol) gave **7m** as a white solid (0.02 g, 55%); MP: 279−281 °C (decomp.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.14 (s, triazole ring-**H**), 7.83−7.86 (m, 4 Ar**H**), 7.48 (d, J = 8.3 Hz, 2 Ar**H**), 7.03 (d, J = 8.7 Hz, 2 Ar**H**), 4.65−4.71 (m, O**C**$H(CH_3)_2$), 3.55 (s, NCH_2), 3.26−3.27 (m, 2 azetidine ring-**H**), 3.07−3.11 (m, 2 azetidine ring-**H**), 2.71−2.78 (m, 1 azetidine ring-**H**), 1.30 (d, J = 6.0 Hz, $OCH(CH_3)_2$); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 179.53 (**C**(O)), 158.30, 148.19 (triazole ring-**C**), 138.39, 136.17, 129.95, 126.80, 122.32, 119.97, 117.91, 115.87 (Ar**C**), 69.64 (O**C**$H(CH_2)_3$), 62.10 (NCH_2), 57.68 (azetidine ring-**C**), 36.54 (azetidine ring-**C**), 20.93 ($OCH(CH_2)_3$); HPLC purity: 4.7 min, 100%; HRMS (M + H)$^+$ (ESI$^+$): 393.1926 [M + H]$^+$ (calcd for $C_{22}H_{24}N_4O_3H^+$, 393.1927).

*Preparation of Sodium 1-(4-(4-(3-Cyano-4-isopropoxyphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**7n**).* Using method F, **6l** (0.08 g, 0.2 mmol) and NaOH (8.2 mg, 0.2 mmol) gave **7n** as a yellow solid (0.07 g, 85%); MP: 272−274 °C (decomp.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.29 (s, triazole ring-**H**), 8.18−8.22 (m, 2 Ar**H**), 7.83 (d, J = 8.8 Hz, 2 Ar**H**), 7.50 (d, J = 8.4 Hz, 1 Ar**H**), 7.44 (d, J = 8.8 Hz, 1 Ar**H**), 4.87 (sept, J = 6.0 Hz, O**C**$H(CH_3)_2$), 3.56 (s, NCH_2), 3.27−3.31 (m, 2 azetidine ring-**H**), 3.09−3.12 (m, 2 azetidine ring-**H**), 2.67−2.81 (m, 1 azetidine ring-**H**), 1.36 (d, J = 6.0 Hz, $OCH(CH_3)_2$); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 176.22 (**C**(O)), 159.51, 145.88 (triazole ring-**C**), 140.55, 135.60, 132.07, 130.79, 129.97, 123.82, 120.23, 120.06, 116.74, 115.47, 102.43 (Ar**C**), 72.16 (O**C**$H(CH_3)_2$), 62.88 (NCH_2), 58.94 (azetidine ring-**C**), 37.47 (azetidine ring-**C**), 22.11 ($OCH(CH_3)_2$); HPLC purity: 4.8 min, 98.1%; HRMS (M + H)$^+$ (ESI$^+$): 418.1879 [M + H]$^+$ (calcd for $C_{23}H_{23}N_5O_3H^+$, 418.1879).

*Preparation of Sodium 1-(4-(4-(3-Chloro-4-isopropoxyphenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**7o**).* Using method F, **6m** (0.031 g, 0.070 mmol) and NaOH (0.003 g, 0.07 mmol) gave **7o** as a yellow solid (0.032 g, 100%); MP: 238−240 °C (decomp); ^{1}H NMR (CD_3OD, 400 MHz): δ 8.82 (s, 1 triazole ring-**H**), 7.91 (s, 1 Ar**H**), 7.84 (d, J = 8.5 Hz, 2 Ar**H**), 7.75−7.77 (m, 1 Ar**H**), 7.51 (d, J = 8.5 Hz, 2 Ar**H**), 7.14 (d, J = 8.7 Hz, 1 Ar**H**), 4.65−4.71 (m, O**C**$H(CH_3)_2$), 3.72 (s, NCH_2), 3.55−3.59 (m, 2 azetidine ring-**H**), 3.31−3.35 (m, 2 azetidine ring-**H**), 3.20−3.24 (m, 1 azetidine ring-**H**), 1.36 (d, J = 6.0 Hz, $OCH(CH_3)_2$); ^{13}C NMR (CD_3OD, 100 MHz): δ 179.44 (**C**(O)), 153.68, 146.93 (triazole ring-**C**), 138.25, 136.12, 130.01, 127.22, 124.95, 124.13, 123.58, 119.99, 118.43, 115.67 (Ar**C**), 71.71 (O**C**$H(CH_3)_2$), 61.96 (NCH_2), 57.65, 36.49 (azetidine ring-**C**), 20.92 ($OCH(CH_3)_2$); HPLC purity: 9.2 min, 98.9%; HRMS (M + H)$^+$ (ESI$^+$): 427.1538 [M + H]$^+$ (calcd for $C_{22}H_{23}ClN_4O_3H^+$, 427.1537).

*Preparation of Sodium 1-(4-(4-(4-Isopropoxy-3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-1-yl)benzyl)azetidine-3-carboxylate (**7p**).* Using method F, **6n** (0.05 g, 0.1 mmol) and NaOH (4.7 mg, 0.1 mmol) gave **7p** as a yellow solid (0.03 g, 64%); MP: 280−282 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.34 (s, triazole ring-**H**), 8.14−8.16 (m, 2 Ar**H**), 7.86 (d, J = 8.4 Hz, 2 Ar**H**), 7.44−7.50 (m, 3 Ar**H**), 4.86 (sept, J = 6.0 Hz, O**C**$H(CH_3)_2$), 3.57 (s,

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J. Med. Chem. XXXX, XXX, XXX−XXX

NCH_2), 3.28−3.33 (m, 2 azetidine ring-H), 3.11−3.15 (m, 2 azetidine ring-H), 2.50−2.84 (m, 1 azetidine ring-H), 1.32 (d, J = 5.6 Hz, $OCH(CH_3)_2$); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 176.90 (C(O)), 155.82, 146.43 (triazole ring-C), 140.38, 135.67, 131.18, 129.92, 124.10 (q, J_{C-F} = 5.2 Hz), 122.88, 122.76, 120.18, 119.82 (ArC), 118.96 (q, J_{C-F} = 27.6 Hz), 116.12 (ArC), 71.51 ($OCH(CH_3)_2$), 62.86 (NCH_2), 58.86 (azetidine ring-C), 37.32 (azetidine ring-C), 22.13 ($OCH(CH_3)_2$); HPLC purity: 5.2 min, 98.6%; HRMS $(M + H)^+$ (ESI^+): 461.1799 $[M + H]^+$ (calcd for $C_{23}H_{23}F_3N_4O_3H^+$, 461.1801).

*Preparation of Methyl 1-(4-(4-(4-(tert-Butyl)phenyl)-1H-1,2,3-triazol-1-yl) benzyl)pyrrolidine-3-carboxylate (**8**).* Using method D, **5l** (0.50 g, 1.6 mmol), methyl pyrrolidine-3-carboxylate hydrochloride (0.41 g, 2.5 mmol), trimethylamine (0.69 mL, 4.9 mmol), and sodium cyanoborohydride (0.05 g, 0.8 mmol) gave **8** as a white solid (0.62 g, 90%); R_f = 0.30 (*n*-hexane/EtOAc 1/1); MP: 162−164 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.23 (s, triazole ring-H), 7.87 (t, J = 7.9 Hz, 4 ArH), 7.64 (d, J = 8.3 Hz, 2 ArH), 7.38 (d, J = 7.8 Hz, 2 ArH), 3.64 (s, $COOCH_3$), 3.56−3.60 (m, NCH_2), 3.39−3.46 (m, 2 pyrrolidine ring-H), 3.28−3.38 (m, 1 pyrrolidine ring-H), 3.16−3.26 (m, 2 pyrrolidine ring-H), 1.34 (s, $C(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 175.08 (C(O)), 151.23, 147.74 (triazole ring-C), 140.12, 136.01, 130.34, 127.97, 126.18, 125.60, 120.29, 119.62 (ArC), 58.62 (NCH_2), 55.36 (pyrrolidine ring-C), 53.59 (pyrrolidine ring-C), 52.14 ($COOCH_3$), 41.66 (pyrrolidine ring-C), 34.88 ($C(CH_3)_3$), 31.53 ($C(CH_3)_3$), 27.62 (pyrrolidine ring-C).

*Preparation of Sodium 1-(4-(4-(4-(tert-Butyl)phenyl)-1H-1,2,3-triazol-1-yl)benzyl)pyrrolidine-3-carboxylate (**9**).* Using method F, **8** (0.15 g, 0.4 mmol) and NaOH (0.01 g, 0.4 mmol) gave **9** as a white solid (0.02 g, 25%); R_f = 0.10 (CH_2Cl_2/MeOH 17/3); MP: 150−152 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.23 (s, COOH), 7.85−7.90 (m, 4 ArH), 7.50−7.55 (m, 4 ArH), 3.61−3.70 (m, NCH_2), 3.29−3.30 (m, 1 pyrrolidine ring-H), 2.62−2.76 (m, 2 pyrrolidine ring-H), 2.50−2.58 (m, 2 pyrrolidine ring-H), 1.93−2.02 (m, 2 pyrrolidine ring-H), 1.32 (s, $C(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 179.31 (C(O)), 151.25, 147.73 (triazole ring-C), 140.67, 135.75, 130.31, 127.91, 126.17, 125.63, 120.20, 119.68 (ArC), 59.52 (NCH_2), 58.32 (pyrrolidine ring-C), 54.36 (pyrrolidine ring-C), 45.24 (pyrrolidine ring-C), 34.85 ($C(CH_3)_3$), 31.51 ($C(CH_3)_3$), 28.78 (pyrrolidine ring-C); HPLC purity: 5.7 min, 97.4%; HRMS $(M + H)^+$ (ESI^+): 405.2293 $[M + H]^+$ (calcd for $C_{24}H_{28}N_4O_2H^+$, 405.2291).

*Preparation of Methyl 1-(4-(4-(4-(tert-Butyl)phenyl)-1H-1,2,3-triazol-1-yl)benzyl)piperidine-4-carboxylate (**10**).* Using method D, **5l** (0.25 g, 0.8 mmol), methyl piperidine-4-carboxylate hydrochloride (0.16 g, 0.9 mmol), triethylamine (0.34 mL, 2.5 mmol), and sodium cyanoborohydride (0.21 g, 3.3 mmol) gave **10** as a white solid (0.17 g, 49%); R_f = 0.30 (*n*-hexane/EtOAc 1/1); MP: 198−200 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.30 (s, triazole ring-H), 8.03−0.06 (m, 2 ArH), 7.86−7.88 (m, 2 ArH), 7.67−7.70 (m, 2 ArH), 7.51−7.54 (m, 2 ArH), 3.60−3.61 (m, $COOCH_3$, NCH_2), 2.98−3.06 (m, 1 piperidine ring-H), 2.32−2.58 (m, 3 piperidine ring-H), 2.11−2.21 (m, 1 piperidine ring-H), 1.80−1.89 (m, 2 piperidine ring-H), 1.44−1.77 (m, 2 piperidine ring-H), 1.33 (s, $C(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.94 (C(O)), 151.33, 147.89 (triazole ring-C), 137.20, 134.44, 129.59, 127.86, 126.21, 125.63, 120.72, 119.72, 116.16 (ArC), 60.95 (NCH_2), 51.92 (piperidine ring-C), 51.28 ($COOCH_3$), 31.53 (piperidine ring-C), 28.36 ($C(CH_3)_3$), 28.00 ($C(CH_3)_3$); HPLC purity: 13.9 min, 95.6%; HRMS $(M + H)^+$ (ESI^+): 433.2605 $[M + H]^+$ (calcd for $C_{26}H_{32}N_4O_2H^+$, 433.2604).

*Preparation of Sodium 1-(4-(4-(4-(tert-Butyl)phenyl)-1H-1,2,3-triazol-1-yl)benzyl)piperidine-4-carboxylate (**11**).* Using method F, **10** (0.10 g, 0.2 mmol) and NaOH (0.005 g, 0.2 mmol) gave **11** as a white solid (0.06 g, 57%); R_f = 0.18 (CH_2Cl_2/MeOH 8/2); MP: 182−184 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.96 (s, triazole ring-H), 8.06 (d, J = 8.4 Hz, 2 ArH), 7.87 (d, J = 8.4 Hz, 2 ArH), 7.74 (d, J = 8.5 Hz, 2 ArH), 7.55 (d, J = 8.4 Hz, 2 ArH), 4.24 (s, NCH_2), 3.33−3.38 (m, 2 piperidine ring-H), 2.93−2.95 (m, 2 piperidine ring-H), 2.53−2.56 (m, 1 piperidine ring-H), 2.11−2.14 (m, 2 piperidine ring-H), 1.92−1.95 (m, 2 piperidine ring-H), 1.38 (s, $C(CH_3)_3$); ^{13}C NMR (CD_3OD, 75 MHz): δ 164.84 (C(O)), 151.83, 148.45 (triazole ring-C), 137.75, 132.19, 127.15, 125.56, 125.21, 120.34, 118.48 (ArC), 60.09 (NCH_2), 51.77 (piperidine ring-C), 34.17 (piperidine ring-C), 30.27 ($C(CH_3)_3$), 26.18 ($C(CH_3)_3$); HPLC purity: 5.4 min, 99.0%; HRMS $(M + H)^+$ (ESI^+): 419.2448 $[M + H]^+$ (calcd for $C_{25}H_{30}N_4O_2H^+$, 419.2447).

*Preparation of Methyl 1-(4-(1-(4-(tert-Butyl)phenyl)-1H-1,2,3-triazol-4-yl)benzyl)azetidine-3-carboxylate (**14a**).* Using method D, **13a** (0.15 g, 0.5 mmol), methyl azetidine-3-carboxylate hydrochloride (0.07 g, 0.6 mmol), triethylamine (0.20 mL, 1.5 mmol), and sodium cyanoborohydride (0.03 g, 0.5 mmol) gave **14a** as a white solid (0.18 g, 93%); R_f = 0.20 (*n*-hexane/EtOAc 1/1); MP: 151−153 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.22 (s, triazole ring-H), 7.84−7.92 (m, 4 ArH), 7.64 (d, J = 8.4 Hz, 2 ArH), 7.38 (d, J = 7.9 Hz, 2 ArH), 3.63 (s, $COOCH_3$), 3.58 (s, NCH_2), 3.38−3.50 (m, 2 azetidine ring-H), 3.25−3.35 (m, 1 azetidine ring-H), 3.15−3.25 (m, 2 azetidine ring-H), 1.34 (s, $C(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 173.59 (C(O)), 151.83, 147.58 (triazole ring-C), 138.56, 134.81, 129.47, 129.31, 127.11, 125.72, 120.17, 119.83 (ArC), 62.61 (NCH_2), 56.67 (azetidine ring-C), 52.11 ($COOCH_3$), 34.99 (azetidine ring-C), 33.75 ($C(CH_3)_3$), 31.48 ($C(CH_3)_3$); HPLC purity: 5.8 min, 98.2%; HRMS $(M + H)^+$ (ESI^+): 405.2293 $[M + H]^+$ (calcd for $C_{24}H_{28}N_4O_2H^+$, 405.2291).

*Preparation of Methyl 1-(4-(1-(3-Chloro-4-isopropoxyphenyl)-1H-1,2,3-triazol-4-yl)benzyl)azetidine-3-carboxylate (**14b**).* Using method D, **13b** (0.12 g, 0.35 mmol), methyl azetidine-3-carboxylate hydrochloride (0.80 g, 0.53 mmol), triethylamine (0.15 mL, 1.1 mmol), and sodium cyanoborohydride (0.044 g, 0.70 mmol) gave **14b** as yellow oil (0.085 g, 55%); R_f = 0.12 (*n*-hexane/EtOAc 1/1); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.23 (s, 1 triazole ring-H), 8.05 (d, J = 2.6 Hz, 1 ArH), 7.84−7.88 (m, 3 ArH), 7.37−7.44 (m, 3 ArH), 4.80 (sept, J = 6.0 Hz, $OCH(CH_3)_2$), 3.63 (s, $COOCH_3$), 3.58 (s, NCH_2), 3.43 (t, J = 6.8 Hz, 2 azetidine ring-H), 3.28−3.31 (m, 1 azetidine ring-H), 3.23 (t, J = 6.7 Hz, 2 azetidine ring-H), 1.34 (d, J = 6.0 Hz, $OCH(CH_3)_2$); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 173.6 (C(O)), 153.5, 147.6 (triazole ring-C), 138.6, 130.5, 129.4, 129.4, 125.7, 123.6, 122.3, 120.5, 120.0, 116.6 (ArC), 72.2 ($OCH(CH_3)_2$), 62.6 (NCH_2), 56.7 (azetidine ring-C), 52.1 ($COOCH_3$), 33.8 (azetidine ring-C), 22.2 ($OCH(CH_3)_2$); HPLC purity: 5.2 min, 97.5%; HRMS $(M + H)^+$ (ESI^+): 441.1695 $[M + H]^+$ (calcd for $C_{23}H_{25}ClN_4O_3H^+$, 441.1693).

Preparation of Sodium 1-(4-(1-(4-(tert-Butyl)phenyl)-1H-1,2,3-triazol-4-yl) benzyl)azetidine-3-carboxylate (15a). Using method E, **14a** (0.10 g, 0.5 mmol) and lithium hydroxide (0.09 g, 7.2 mmol) gave a salt-free acid as a white solid (0.02 g, 25%); R_f = 0.26 (CH_2Cl_2/MeOH 8/2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.26 (s, triazole ring-H), 7.83−7.93 (m, 4 ArH), 7.64 (d, J = 8.4 Hz, 2 ArH), 7.39 (d, J = 7.8 Hz, 2 ArH), 3.60 (s, NCH_2), 3.41 (d, J = 6.4 Hz, 2 azetidine ring-H), 3.20−3.22 (m, 3 azetidine ring-H), 1.34 (s, $C(CH_3)_3$). Using method F, the salt-free acid (0.03 g, 0.08 mmol) and NaOH (0.005 g, 1.2 mmol) gave **15a** as a white solid (0.02 g, 55%); R_f = 0.05 (*n*-hexane/EtOAc 1/1); MP: 250−252 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.25 (s, triazole ring-H), 7.86−7.88 (m, 4 ArH), 7.64 (d, J = 8.0 Hz, 2 ArH), 7.36 (d, J = 7.6 Hz, 2 ArH), 3.52 (S, NCH_2), 3.28−3.32 (m, 2 azetidine ring-H), 3.10−3.14 (m, 2 azetidine ring-H), 2.79−2.83 (m, 1 azetidine ring-H), 1.34 (C-$(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 177.29 (C(O)), 162.33, 151.79, 147.68 (triazole ring-C), 139.61, 134.83, 129.17, 127.09, 125.63, 120.15, 119.76 (ArC), 63.34 (NCH_2), 58.82 (azetidine ring-C), 37.26 (azetidine ring-C), 34.98 ($C(CH_3)_3$), 31.48 ($C(CH_3)_3$); HPLC purity: 7.1 min, 95.1%; HRMS $(M + H)^+$ (ESI^+): 391.2127 $[M + H]^+$ (calcd for $C_{23}H_{26}N_4O_2H^+$, 391.2134).

Preparation of Sodium 1-(4-(1-(3-Chloro-4-isopropoxyphenyl)-1H-1,2,3-triazol-4-yl)benzyl)azetidine-3-carboxylate (15b). Using method F, **14b** (0.09 g, 0.20 mmol) and NaOH (0.009 g, 0.21 mmol) gave **15b** as a white solid (0.090 g, 100%); MP: 218.5−219.5 °C (decomp.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.23 (s, 1 triazole ring-H), 8.05 (d, J = 2.7 Hz, 1 ArH), 7.85−7.89 (m, 1 ArH), 7.83 (d, J = 8.2 Hz, 2 ArH), 7.42 (d, J = 9.2 Hz, 1 ArH), 7.36 (d, J = 8.2 Hz, 2 ArH), 4.80 (sept, J = 6.0 Hz, $OCH(CH_3)_2$), 3.51 (s, NCH_2), 3.26 (t, J = 7.5 Hz, 2 azetidine ring-H), 3.08 (t, J = 7.3 Hz, 2 azetidine ring-H),

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J. Med. Chem. XXXX, XXX, XXX−XXX

2.74 (t, J = 7.8 Hz, 1 azetidine ring-**H**), 1.34 (d, J = 6.0 Hz, OCH(CH_3)$_2$); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 176.9 (**C**(O)), 153.5, 147.7 (triazole ring-**C**), 139.7, 130.5, 129.2, 129.1, 125.6, 123.6, 122.2, 120.4, 119.9, 116.6 (Ar**C**), 72.2 (O**C**H(CH_3)$_2$), 63.3 (NCH_2), 58.8 (azetidine ring-**C**), 37.3 (azetidine ring-**C**), 22.2 (OCH(CH_3)$_2$); HPLC purity: 5.5 min, 99.2%; HRMS (M + H)$^+$ (ESI$^+$): 427.1537 [M + H]$^+$ (calcd for $C_{22}H_{23}ClN_4O_3H^+$, 427.1537).

*Preparation of Methyl 1-(4-(5-Phenyl-4,5-dihydroisoxazol-3-yl)-benzyl)azetidine-3-carboxylate (**20a**).* Using method D, **19a** (0.38 g, 1.5 mmol), methyl azetidine-3-carboxylate hydrochloride (0.35 g, 2.3 mmol), triethylamine (0.64 mL, 4.6 mmol), and sodium cyanoborohydride (0.19 g, 3.0 mmol) gave **20a** as clear oil (0.031 g, 57%); R_f = 0.23 (*n*-hexane/EtOAc 1/5); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.65 (d, J = 8.2 Hz, 2 Ar**H**), 7.28−7.41 (m, 7 Ar**H**), 5.70−5.75 (m, 1 isoxazoline ring-**H**), 3.75−3.80 (m, 1 isoxazoline ring-**H**), 3.73 (s, COOC**H**$_3$), 3.69 (s, NC**H**$_2$), 3.64 (br, 2 azetidine ring-**H**), 3.30−3.36 (m, 3 azetidine ring-**H**, 1 isoxazoline ring-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.49 (**C**(O)), 155.94 (isoxazoline ring-**C**), 140.97, 139.96, 128.79, 128.77, 128.37, 128.22, 126.84, 125.88 (Ar**C**), 82.53 (isoxazoline ring-**C**), 62.99 (NCH_2), 56.86 (azetidine ring-**C**), 51.99 (COOCH_3), 43.21 (isoxazoline ring-**C**), 33.96 (azetidine ring-**C**); HPLC purity: 3.3 min, 96.0%; HRMS (M + H)$^+$ (ESI$^+$): 351.1707 [M + H]$^+$ (calcd for $C_{21}H_{22}N_2O_3H^+$, 351.1709).

*Preparation of Methyl 1-(4-(5-(2-Bromophenyl)-4,5-dihydroisox-azol-3-yl)benzyl)azetidine-3-carboxylate (**20b**).* Using method D, **19b** (0.19 g, 0.5 mmol), methyl azetidine-3-carboxylate hydrochloride (0.13 g, 0.8 mmol), triethylamine (0.24 mL, 1.7 mmol), and sodium cyanoborohydride (0.07 g, 1.1 mmol) gave **20b** as clear oil (0.070 g, 28%); R_f = 0.33 (*n*-hexane/EtOAc 1/5); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.64 (d, J = 8.4 Hz, 2 Ar**H**), 7.55−7.59 (m, 2 Ar**H**), 7.29−7.35 (m, 3 Ar**H**), 7.15−7.20 (m, 1 Ar**H**), 5.96−6.01 (m, 1 isoxazoline ring-**H**), 3.93−4.00 (m, 1 isoxazoline ring-**H**), 3.72 (s, COOC**H**$_3$), 3.71 (s, NC**H**$_2$), 3.52−3.64 (m, 2 azetidine ring-**H**), 3.33−3.36 (m, 3 azetidine ring-**H**), 3.17−3.23 (m, 1 isoxazoline ring-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.48 (**C**(O)), 155.84 (isoxazoline ring-**C**), 140.65, 140.17, 132.74, 129.36, 128.75, 128.09, 127.83, 127.43, 126.88, 120.84 (Ar**C**), 81.42 (isoxazoline ring-**C**), 63.00 (NCH_2), 56.86 (azetidine ring-**C**), 51.97 (COOCH_3), 42.98 (isoxazoline ring-**C**), 33.96 (azetidine ring-**C**); HPLC purity: 5.09 min, 97.9%; HRMS (M + H)$^+$ (ESI$^+$): 429.0817 [M + H]$^+$ (calcd for $C_{21}H_{21}BrN_2O_3H^+$, 429.0814).

*Preparation of Methyl 1-(4-(5-(3-Bromophenyl)-4,5-dihydroisox-azol-3-yl)benzyl)azetidine-3-carboxylate (**20c**).* Using method D, **19c** (0.55 g, 1.6 mmol), methyl azetidine-3-carboxylate hydrochloride (0.38 g, 2.5 mmol), triethylamine (0.69 mL, 5.0 mmol), and sodium cyanoborohydride (0.21 g, 3.3 mmol) gave **20c** as a clear oil (0.25 g, 35%); R_f = 0.22 (*n*-hexane/EtOAc 1/5); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.62 (d, J = 8.4 Hz, 2 Ar**H**), 7.53 (s, 1 Ar**H**), 7.42 (d, J = 7.6 Hz, 1 Ar**H**), 7.29−7.32 (m, 3 Ar**H**), 7.20−7.24 (m, 1 Ar**H**), 5.65−5.69 (m, 1 isoxazoline ring-**H**), 3.73−3.80 (m, 1 isoxazoline ring-**H**), 3.70 (s, COOC**H**$_3$), 3.62 (s, NC**H**$_2$), 3.50−3.55 (m, 2 azetidine ring-**H**), 3.25−3.35 (m, 3 azetidine ring-**H**, 1 isoxazoline ring-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.47 (**C**(O)), 155.87 (isoxazoline ring-**C**), 143.39, 140.23, 131.23, 130.37, 128.85, 128.78, 128.00, 126.86, 124.43, 122.80 (Ar**C**), 81.50 (isoxazoline ring-**C**), 62.97 (NCH_2), 56.86 (azetidine ring-**C**), 51.97 (COOCH_3), 43.27 (isoxazoline ring-**C**), 33.96 (azetidine ring-**C**); HPLC purity: 4.7 min, 98.2%; HRMS (M + H)$^+$ (ESI$^+$): 429.0816 [M + H]$^+$ (calcd for $C_{21}H_{21}BrN_2O_3H^+$, 429.0814).

*Preparation of Methyl 1-(4-(5-(4-Bromophenyl)-4,5-dihydroisox-azol-3-yl)benzyl)azetidine-3-carboxylate (**20d**).* Using method D, **19d** (0.9 g, 2.7 mmol), methyl azetidine-3-carboxylate hydrochloride (0.61 g, 4.0 mmol), triethylamine (1.1 mL, 8.1 mmol), and sodium cyanoborohydride (0.34 g, 5.4 mmol) gave **20d** as a clear oil (0.18 g, 16%); R_f = 0.22 (*n*-hexane/EtOAc 1/5); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.64 (d, J = 8.4 Hz, 2 Ar**H**), 7.49−7.53 (m, 2 Ar**H**), 7.36 (d, J = 10.0 Hz, 2 Ar**H**), 7.29−7.32 (m, 2 Ar**H**), 5.67−5.72 (m, 1 isoxazoline ring-**H**), 3.77−3.82 (m, 1 isoxazoline ring-**H**), 3.75 (s, COOC**H**$_3$), 3.68 (s, NC**H**$_2$), 3.52−3.65 (m, 2 azetidine ring-**H**), 3.26−3.39 (m, 3 azetidine ring-**H**, 1 isoxazoline ring-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.52 (**C**(O)), 155.91 (isoxazoline ring-**C**), 140.19, 140.05, 131.87, 128.80, 128.08, 127.56, 126.85, 122.11 (Ar**C**), 81.75 (isoxazoline ring-**C**), 62.99 (NCH_2), 56.87 (azetidine ring-**C**), 52.00 (COOCH_3), 43.22 (isoxazoline ring-**C**), 33.96 (azetidine ring-**C**); HPLC purity: 9.1 min, 98.3%; HRMS (M + H)$^+$ (ESI$^+$): 429.0817 [M + H]$^+$ (calcd for $C_{21}H_{21}BrN_2O_3H^+$, 429.0814).

*Preparation of Methyl 1-(4-(5-(4-Acetoxyphenyl)-4,5-dihydroi-soxazol-3-yl)benzyl)azetidine-3-carboxylate (**20e**).* Using method D, **19e** (0.58 g, 1.9 mmol), methyl azetidine-3-carboxylate hydrochloride (0.43 g, 2.8 mmol), triethylamine (0.80 mL, 5.6 mmol), and sodium cyanoborohydride (0.24 g, 3.8 mmol) gave **20e** as a white sticky solid (0.15 g, 20%); R_f = 0.30 (*n*-hexane/EtOAc 1/1); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.66 (d, J = 8.0 Hz, 2 Ar**H**), 7.43 (d, J = 8.4 Hz, 2 Ar**H**), 7.35 (d, J = 8.1 Hz, 2 Ar**H**), 7.15 (d, J = 8.4 Hz, 2 Ar**H**), 5.71−5.76 (m, 1 isoxazoline ring-**H**), 3.82−3.89 (m, 1 isoxazoline ring-**H**), 3.60−3.62 (m, COOC**H**$_3$, NC**H**$_2$), 3.37−3.44 (m, 2 azetidine ring-**H**, 1 isoxazoline ring-**H**), 3.25 (br s, 3 azetidine ring-**H**), 2.27 (s, OCOC**H**$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.51 (**C**(O)), 169.43 (**C**(O)), 155.93 (isoxazoline ring-**C**), 150.47, 140.10, 138.57, 128.80, 128.23, 127.03, 126.85, 121.92 (Ar**C**), 81.93 (isoxazoline ring-**C**), 63.02 (NCH_2), 56.88 (azetidine ring-**C**), 51.99 (COOCH_3), 43.24 (isoxazoline ring-**C**), 33.97 (azetidine ring-**C**) 21.12 (OCOCH_3).

*Preparation of Methyl 1-(4-(5-(4-(Chloromethyl)phenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate (**20f**).* Using method D, **19f** (0.42 g, 1.4 mmol), methyl azetidine-3-carboxylate hydrochloride (0.32 g, 2.1 mmol), triethylamine (0.59 mL, 4.2 mmol), and sodium cyanoborohydride (0.17 g, 2.8 mmol) gave **20f** as a clear oil (0.13 g, 23%); R_f = 0.15 (*n*-hexane/EtOAc 1/5); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.64 (d, J = 8.4 Hz, 2 Ar**H**), 7.37−7.41 (m, 4 Ar**H**), 7.33 (d, J = 8.0 Hz, 2 Ar**H**), 5.71−5.75 (m, 1 isoxazoline ring-**H**), 4.58 (s, ArC**H**$_2$Cl), 3.75−3.81 (m, 1 isoxazoline ring-**H**), 3.73 (s, COOC**H**$_3$), 3.65 (s, NC**H**$_2$), 3.53−3.56 (m, 2 azetidine ring-**H**), 3.28−3.36 (m, 2 azetidine ring-**H**, 1 isoxazoline ring-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.49 (**C**(O)), 155.94 (isoxazoline ring-**C**), 141.28, 140.10, 137.47, 129.02, 128.79, 128.20, 126.84, 126.26 (Ar**C**), 82.06 (isoxazoline ring-**C**), 62.97 (NCH_2), 56.86 (azetidine ring-**C**), 51.99 (COOCH_3), 45.82 (ArCH_2Cl), 43.21 (isoxazoline ring-**C**), 33.96 (azetidine ring-**C**); HPLC purity: 10.1 min, 96.9%; HRMS (M + H)$^+$ (ESI$^+$): 399.1480 [M + H]$^+$ (calcd for $C_{22}H_{23}ClN_2O_3H^+$, 399.1475).

*Preparation of Methyl 1-(4-(5-(4-Ethylphenyl)-4,5-dihydroisox-azol-3-yl)benzyl)azetidine-3-carboxylate (**20g**).* Using method D, **19g** (0.066 g, 0.24 mmol), methyl azetidine-3-carboxylate hydrochloride (0.054 g, 0.35 mmol), triethylamine (0.10 mL, 0.71 mmol), and sodium cyanoborohydride (0.030 g, 0.47 mmol) gave **20g** as a white solid (0.042 g, 46%); R_f = 0.12 (*n*-hexane/EtOAc 1/1); MP: 61−62 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.63 (d, J = 8.2 Hz, 2 Ar**H**), 7.29−7.32 (m, 4 Ar**H**), 7.19 (d, J = 8.0 Hz, 2 Ar**H**), 5.69 (dd, J = 4.2, 10.8 Hz, 1 isoxazoline ring-**H**), 3.72−3.76 (m, 1 isoxazoline ring-**H**), 3.70 (s, COOC**H**$_3$), 3.62 (s, NC**H**$_2$), 3.50−3.55 (m, 2 azetidine ring-**H**), 3.29−3.35 (m, 3 azetidine ring-**H**, 1 isoxazoline ring-**H**), 2.64 (q, J = 7.6 Hz, C**H**$_2$CH$_3$), 1.22 (t, J = 7.6 Hz, CH$_2$C**H**$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.52 (**C**(O)), 155.98 (isoxazoline ring-**C**), 144.43, 139.96, 138.11, 128.76, 128.47, 128.24, 126.82, 125.98 (Ar**C**), 82.57 (isoxazoline ring-**C**), 63.05 (NCH_2), 56.89 (azetidine ring-**C**), 51.98 (COOCH_3), 43.08 (isoxazoline ring-**C**), 33.99 (azetidine ring-**C**), 28.58 (**C**H$_2$CH$_3$), 15.57 (CH$_2$**C**H$_3$); HPLC purity: 4.5 min, 98.4%; HRMS (M + H)$^+$ (ESI$^+$): 379.2021 [M + H]$^+$ (calcd for $C_{23}H_{26}N_2O_3H^+$, 379.2022).

*Preparation of Methyl 1-(4-(5-(4-Propylphenyl)-4,5-dihydroisox-azol-3-yl)azetidine-3-carboxylate) (**20h**).* Using method D, **19h** (0.10 g, 0.34 mmol), methyl azetidine-3-carboxylate hydrochloride (0.078 g, 0.51 mmol), triethylamine (0.14 mL, 1.0 mmol), and sodium cyanoborohydride (0.043 g, 0.68 mmol) gave **20h** as a white sticky solid (0.048 g, 36%); R_f = 0.17 (*n*-hexane/EtOAc 1/1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.63 (d, J = 8.1 Hz, 2 Ar**H**), 7.30 (t, J = 8.1 Hz, 4 Ar**H**), 7.17 (d, J = 8.0 Hz, 2 Ar**H**), 5.68 (dd, J = 8.5, 10.8 Hz, 1 isoxazoline ring-**H**), 3.72−3.76 (m, 1 isoxazoline ring-**H**), 3.70 (s, COOC**H**$_3$), 3.63 (s, NC**H**$_2$), 3.53 (s, 2 azetidine ring-**H**), 3.31−

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J. Med. Chem. XXXX, XXX, XXX−XXX

3.35 (m, 2 azetidine ring-**H**, 1 isoxazoline ring-**H**), 2.57 (t, J = 7.4 Hz, alkyl chain-C**H**$_2$), 1.57−1.67 (m, alkyl chain-C**H**$_2$), 0.92 (t, J = 7.3 Hz, alkyl chain-C**H**$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.5 (**C**(O)), 156.0 (isoxazoline ring-**C**), 139.9, 138.1, 128.8, 128.7, 128.5, 126.8, 125.9 (Ar**C**), 82.6 (isoxazoline ring-**C**), 63.0 (N**C**H$_2$), 56.9 (azetidine ring-**C**), 51.9 (COO**C**H$_3$), 43.1 (isoxazoline ring-**C**), 37.7 (alkyl chain-**C**H$_2$), 33.9 (azetidine ring-**C**), 24.5 (alkyl chain-**C**H$_2$), 13.8 (alkyl chain-**C**H$_3$).

*Preparation of Methyl 1-(4-(5-(4-(tert-Butyl)phenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate (**20i**).* Using method D, **19i** (0.91 g, 3.0 mmol), methyl azetidine-3-carboxylate hydrochloride (0.49 g, 3.3 mmol), triethylamine (1.24 mL, 8.9 mmol), and sodium cyanoborohydride (0.74 g, 11.8 mmol) gave **20i** as a white solid (0.54 g, 45%); R_f = 0.26 (*n*-hexane/EtOAc 1/1); MP: 142−144 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.64 (d, J = 7.9 Hz, 2 Ar**H**), 7.39 (d, J = 8.2 Hz, 2 Ar**H**), 7.32 (dd, J = 3.4, 8.3 Hz, 4 Ar**H**), 5.71 (dd, J = 8.3, 10.9 Hz, 1 isoxazoline ring-**H**), 3.68−3.79 (m, 1 isoxazoline ring-**H**, COOC**H**$_3$), 3.63 (s, NC**H**$_2$), 3.57−3.60 (m, 1 azetidine ring-**H**, 1 isoxazoline ring-**H**), 3.39−3.42 (m, 2 azetidine ring-**H**), 3.21−3.36 (m, 2 azetidine ring-**H**), 1.28 (s, C(C**H**$_3$)$_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 173.33 (**C**(O)), 162.33, 156.74, 151.04, 138.32, 132.53, 129.58, 127.14, 126.39, 125.81 (Ar**C**, isoxazoline ring-**C**), 82.31 (isoxazoline ring-**C**), 56.52 (azetidine ring-**C**), 52.19 (COO**C**H$_3$), 49.06 (azetidine ring-**C**), 42.45 (isoxazoline ring-**C**), 34.74 (**C**(CH$_3$)$_3$), 33.61 (azetidine ring-**C**), 31.55 (C(**C**H$_3$)$_3$); HPLC purity: 9.8 min, 95.5%; HRMS (M + H)$^+$ (ESI$^+$): 407.2336 [M + H]$^+$ (calcd for $C_{25}H_{30}N_2O_3H^+$, 407.2335).

*Preparation of Methyl 1-(4-(5-(3-Cyano-4-isopropoxyphenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate (**20j**).* Using method D, **19j** (0.07 g, 0.2 mmol), methyl azetidine-3-carboxylate hydrochloride (0.04 g, 0.3 mmol), triethylamine (0.08 mL, 0.6 mmol), and sodium cyanoborohydride (0.02 g, 0.4 mmol) gave **20j** as a clear oil (0.60 mg, 66%); R_f = 0.31 (*n*-hexane/EtOAc 1/5); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.63 (d, J = 8.2 Hz, 2 Ar**H**), 7.52−7.56 (m, 2 Ar**H**), 7.33 (d, J = 8.2 Hz, 2 Ar**H**), 6.96 (d, J = 8.7 Hz, 1 Ar**H**), 5.65−5.70 (m, 1 isoxazoline ring-**H**), 4.65 (sept, J = 6.0 Hz, OC**H**(CH$_3$)$_2$), 3.70−3.81 (m, 1 isoxazoline ring-**H**), 3.65 (s, COOC**H**$_3$), 3.54 (s, NC**H**$_2$), 3.31−3.37 (m, 2 azetidine ring-**H**), 3.24−3.30 (m, 3 azetidine ring-**H**, 1 isoxazoline ring-**H**), 1.41 (d, J = 6.0 Hz, OCH(C**H**$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.48 (**C**(O)), 159.77, 155.99 (isoxazoline ring-**C**), 140.32, 133.33, 131.87, 131.40, 128.81, 127.93, 126.85, 116.32, 114.04 (Ar**C**), 103.12, 81.09 (isoxazoline ring-**C**), 72.08 (O**C**H(CH$_3$)$_2$), 62.97 (N**C**H$_2$), 56.86 (azetidine ring-**C**), 51.97 (COO**C**H$_3$), 43.02 (isoxazoline ring-**C**), 33.95 (azetidine ring-**C**), 21.79 (OCH(**C**H$_3$)$_2$); HPLC purity: 4.3 min, 98.0%; HRMS (M + H)$^+$ (ESI$^+$): 434.2080 [M + H]$^+$ (calcd for $C_{25}H_{27}N_3O_4H^+$, 434.2080).

*Preparation of Methyl 1-(4-(5-(3-Chloro-4-ethoxyphenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate (**20k**).* Using method D, **19k** (0.13 g, 0.41 mmol), methyl azetidine-3-carboxylate hydrochloride (0.092 g, 0.61 mmol), triethylamine (0.17 mL, 1.2 mmol), and sodium cyanoborohydride (0.051 g, 0.81 mmol) gave **20k** as a white solid (0.15 g, 83%); R_f = 0.18 (*n*-hexane/EtOAc 1/1); MP: 96−97 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.62 (d, J = 8.2 Hz, 2 Ar**H**), 7.38 (d, J = 2.1 Hz, 1 Ar**H**), 7.31 (d, J = 8.2 Hz, 2 Ar**H**), 7.21 (dd, J = 2.1, 8.4 Hz, 1 Ar**H**), 6.89 (d, J = 8.5 Hz, 1 Ar**H**), 5.63 (dd, J = 8.2, 10.8 Hz, 1 isoxazoline ring-**H**), 4.09 (q, J = 6.9 Hz, OC**H**$_2$CH$_3$), 3.68−3.75 (m, 1 isoxazoline ring-**H**), 3.69 (s, COOC**H**$_3$), 3.62 (s, NC**H**$_2$), 3.50−3.53 (m, 2 azetidine ring-**H**), 3.24−3.34 (m, 3 azetidine ring-**H**, 1 isoxazoline ring-**H**), 1.45 (t, J = 7.0 Hz, OCH$_2$C**H**$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.51 (**C**(O)), 155.96 (isoxazoline ring-**C**), 154.37, 140.09, 133.82, 128.79, 127.93, 126.84, 125.35, 123.17, 113.39 (Ar**C**), 81.67 (isoxazoline ring-**C**), 64.86 (N**C**H$_2$), 63.02 (O**C**H$_2$CH$_3$), 56.87 (azetidine ring-**C**), 51.98 (COO**C**H$_3$), 43.05 (isoxazoline ring-**C**), 33.96 (azetidine ring-**C**), 14.66 (OCH$_2$**C**H$_3$); HPLC purity: 4.7 min, 98.5%; HRMS (M + H)$^+$ (ESI$^+$): 429.1581 [M + H]$^+$ (calcd for $C_{23}H_{25}ClN_2O_4H^+$, 429.1581).

*Preparation of Methyl 1-(4-(5-(3-Chloro-4-isopropoxyphenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate (**20l**).* Using method D, **19l** (0.35 g, 1.0 mmol), methyl azetidine-3-carboxylate hydrochloride (0.23 g, 1.5 mmol), triethylamine (0.40 mL, 3.0 mmol), and sodium cyanoborohydride (0.13 g, 2.0 mmol) gave **20l** as a yellow oil (0.20 g, 44%); R_f = 0.19 (*n*-hexane/EtOAc 1/1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.61 (d, J = 8.1 Hz, 2 Ar**H**), 7.37 (d, J = 2.0 Hz, 1 Ar**H**), 7.30 (d, J = 8.0 Hz, 2 Ar**H**), 7.19 (dd, J = 2.0, 8.4 Hz, 1 Ar**H**), 6.91 (d, J = 8.4 Hz, 1 Ar**H**), 5.61 (dd, J = 8.2, 10.8 Hz, 1 isoxazoline ring-**H**), 4.51−4.57 (m, OC**H**(CH$_3$)$_2$), 3.72 (dd, J = 10.9, 16.7 Hz, 1 isoxazoline ring-**H**), 3.69 (s, COOC**H**$_3$), 3.63 (s, NC**H**$_2$), 3.52−3.54 (m, 2 azetidine ring-**H**), 3.26−3.36 (m, 1 isoxazoline ring-**H**, 3 azetidine ring-**H**), 1.35 (d, J = 6.0 Hz, OCH(C**H**$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.49 (**C**(O)), 156.01 (isoxazoline ring-**C**), 153.54, 140.09, 134.06, 128.78, 128.21, 128.05, 126.82, 125.27, 124.45, 115.97 (Ar**C**), 81.66 (isoxazoline ring-**C**), 72.19 (O**C**H(CH$_3$)$_2$), 62.98 (N**C**H$_2$), 56.85 (azetidine ring-**C**), 51.96 (COO**C**H$_3$), 42.98 (isoxazoline ring-**C**), 33.95 (azetidine ring-**C**), 21.99 (OCH(**C**H$_3$)$_2$); HPLC purity: 5.3 min, 97.5%; HRMS (M + H)$^+$ (ESI$^+$): 443.1740 [M + H]$^+$ (calcd for $C_{24}H_{27}ClN_2O_4H^+$, 443.1738).

*Preparation of Methyl 1-(4-(5-(4-Isopropoxy-3-(trifluoromethyl)-phenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate (**20m**).* Using method D, **19m** (0.30 g, 0.79 mmol), methyl azetidine-3-carboxylate hydrochloride (0.18 g, 1.19 mmol), triethylamine (0.33 mL, 2.37 mmol), and sodium cyanoborohydride (0.10 g, 1.58 mmol) gave **20m** as a yellow oil (0.13 g, 35%); R_f = 0.19 (*n*-hexane/EtOAc 1/1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.62 (d, J = 8.0 Hz, 2Ar**H**), 7.54 (s, 1 Ar**H**), 7.48 (d, J = 8.5 Hz, 1 Ar**H**), 7.31 (d, J = 8.0 Hz, 2 Ar**H**), 6.98 (d, J = 8.6 Hz, 1 Ar**H**), 5.68 (dd, J = 8.7, 10.5 Hz, 1 isoxazoline ring-**H**), 4.60−4.63 (m, OC**H**(CH$_3$)$_2$), 3.74−3.77 (m, 1 isoxazoline ring-**H**), 3.73 (s, COOC**H**$_3$), 3.69 (s, NC**H**$_2$), 3.61−3.63 (m, 2 azetidine ring-**H**), 3.25−3.32 (m, 1 isoxazoline ring-**H**, 3 azetidine ring-**H**), 1.33 (d, J = 6.0 Hz, OCH(C**H**$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.49 (**C**(O)), 156.02 (isoxazoline ring-**C**), 140.19, 132.11, 130.69, 128.79, 128.16, 126.83 (Ar**C**), 125.05 (q, J_{C-F} = 4.9 Hz), 123.48 (q, J_{C-F} = 270.8 Hz), 120.02 (q, J_{C-F} = 30.5 Hz), 114.57 (Ar**C**), 81.75 (isoxazoline ring-**C**), 71.42 (O**C**H(CH$_3$)$_2$), 63.00 (N**C**H$_2$), 56.87 (azetidine ring-**C**), 51.95 (COO**C**H$_3$), 43.04 (isoxazoline ring-**C**), 33.96 (azetidine ring-**C**), 21.79 (OCH(**C**H$_3$)$_2$); HPLC purity: 5.1 min, 99.4%; HRMS (M + H)$^+$ (ESI$^+$): 477.2006 [M + H]$^+$ (calcd for $C_{25}H_{27}F_3N_2O_4H^+$, 477.2001).

*Preparation of Methyl 1-(4-(5-(Pyridin-2-yl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate (**20n**).* Using method D, **19n** (0.09 g, 0.3 mmol), methyl azetidine-3-carboxylate hydrochloride (0.08 g, 0.5 mmol), triethylamine (0.16 mL, 1.1 mmol), and sodium cyanoborohydride (0.04 g, 0.7 mmol) gave **20n** as a clear oil (0.06 g, 46%); R_f = 0.11 (*n*-hexane/EtOAc 1/5); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.57 (d, J = 4.1 Hz, 1 Ar**H**), 7.70 (td, J = 1.7, 7.6 Hz, 1 Ar**H**), 7.64 (d, J = 8.2 Hz, 2 Ar**H**), 7.56 (d, J = 7.8 Hz, 1 Ar**H**), 7.31 (d, J = 8.2 Hz, 2 Ar**H**), 7.20−7.23, (m, 1 Ar**H**), 5.81−5.85 (m, 1 isoxazoline ring-**H**), 3.78−3.85 (m, 1 isoxazoline ring-**H**), 3.66−3.74 (m, COOC**H**$_3$, 1 isoxazoline ring-**H**), 3.62 (s, NC**H**$_2$), 3.49−3.55 (m, 2 azetidine ring-**H**), 3.30−3.36 (m, 3 azetidine ring-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.45 (**C**(O)), 159.93, 156.31, 149.36, 140.00, 136.96, 128.74, 128.16, 126.93, 122.91, 120.57 (Ar**C**, isoxazoline ring-**C**), 82.40 (isoxazoline ring-**C**), 62.95 (N**C**H$_2$), 56.81 (azetidine ring-**C**), 51.96 (COO**C**H$_3$), 41.48 (isoxazoline ring-**C**), 33.92 (azetidine ring-**C**); HPLC purity: 5.1 min, 98.8%; HRMS (M + H)$^+$ (ESI$^+$): 352.1661 [M + H]$^+$ (calcd for $C_{20}H_{21}N_3O_3H^+$, 352.1661).

*Preparation of 1-(4-(5-Phenyl-4,5-dihydroisoxazol-3-yl)benzyl)-azetidine-3-carboxylic Acid (**21a**).* Using method E, **20a** (0.23 g, 0.7 mmol) and LiOH (0.033 g, 1.3 mmol) gave **21a** as a white solid (0.23 g, 97%); MP: 143−145 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.62 (br, COO**H**), 7.75 (d, J = 8.2 Hz, 2 Ar**H**), 7.65 (d, J = 8.2 Hz, 2 Ar**H**), 7.36−7.40 (m, 4 Ar**H**), 7.31−7.35 (m, 1 Ar**H**), 5.73−5.77 (m, 1 isoxazoline ring-**H**), 4.40 (s, NC**H**$_2$), 4.04−4.12 (m, 4 azetidine ring **H**), 3.85−3.92 (m, 1 isoxazoline ring-**H**), 3.63−3.67 (m, 1 azetidine ring-**H**), 3.40−3.44 (m, 1 isoxazoline ring-**H**); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 171.96 (**C**(O)), 156.62 (isoxazoline ring-**C**), 141.26, 133.09, 131.02, 130.39, 129.12, 128.63, 127.47, 126.67 (Ar**C**), 82.73 (isoxazoline ring-**C**), 56.74 (N**C**H$_2$), 54.58 (azetidine ring-**C**), 42.48

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J. Med. Chem. XXXX, XXX, XXX−XXX

(isoxazoline ring-**C**), 32.51 (azetidine ring-**C**); HPLC purity: 3.6 min, 96.8%; HRMS (M + H)$^+$ (ESI$^+$): 337.1551 [M + H]$^+$ (calcd for $C_{20}H_{21}N_3O_3H^+$, 337.1552).

*Preparation of 1-(4-(5-(2-Bromophenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylic Acid (**21b**).* Using method E, **20b** (26 mg, 0.06 mmol) and LiOH (0.003 g, 0.1 mmol) gave **21b** as a white solid (0.015 g, 60%); MP: 183−185 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.78 (d, J = 8.0 Hz, 2 Ar**H**), 7.69 (d, J = 8.0 Hz, 1 Ar**H**), 7.58 (d, J = 8.4 Hz, 2 Ar**H**), 7.45−7.46 (m, 2 Ar**H**), 7.28−7.32 (m, 1 Ar**H**), 5.93−5.98 (m, 1 isoxazoline ring-**H**), 4.40 (s, NC**H**$_2$), 4.12−4.14 (m, 4 azetidine ring-**H**), 4.01−4.05 (m, 1 isoxazoline ring-**H**), 3.59−3.64 (m, 1 azetidine ring-**H**), 3.34−3.37 (m, 1 isoxazoline ring-**H**); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 181.85, 177.21, 156.43 (isoxazoline ring-**C**), 140.43, 133.36, 130.99, 130.50, 130.21, 128.64, 127.69, 127.53, 121.38 (Ar**C**), 86.90 (isoxazoline ring-**C**), 81.74, 56.97 (N**C**H$_2$), 55.04 (azetidine ring-**C**), 42.34 (isoxazoline ring-**C**), 32.52 (azetidine ring-**C**); HPLC purity: 4.6 min, 99.3%; HRMS (M + H)$^+$ (ESI$^+$): 415.0659 [M + H]$^+$ (calcd for $C_{20}H_{19}BrN_2O_3H^+$, 415.0657).

*Preparation of 1-(4-(5-(3-Bromophenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylic Acid (**21c**).* Using method E, **20c** (0.08 g, 0.1 mmol) and LiOH (0.009 g, 0.3 mmol) gave **21c** as a white solid (0.07 g, 96%); MP: 116−118 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.75 (d, J = 8.0 Hz, 2 Ar**H**), 7.66 (d, J = 8.0 Hz, 2 Ar**H**), 7.59 (s, 1 Ar**H**), 7.54 (d, J = 8.8 Hz, 1 Ar**H**), 7.34−7.42 (m, 2 Ar**H**), 5.76−5.81 (m, 1 isoxazoline ring-**H**), 4.42 (s, NC**H**$_2$), 4.08−4.12 (m, 5 azetidine ring-**H**), 3.86−3.93 (m, 1 isoxazoline ring-**H**), 3.43−3.49 (m, 1 isoxazoline ring-**H**); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 171.03 (**C**(O)), 156.72 (isoxazoline ring-**C**), 144.11, 139.56, 132.05, 131.40, 131.03, 130.19, 129.31, 127.55, 125.66, 122.31 (Ar**C**), 81.71 (isoxazoline ring-**C**), 54.43 (N**C**H$_2$), 52.75 (azetidine ring-**C**), 42.54 (isoxazoline ring-**C**), 32.28 (azetidine ring-**C**); HPLC purity: 5.1 min, 98.6%; HRMS (M + H)$^+$ (ESI$^+$): 415.0662 [M + H]$^+$ (calcd for $C_{20}H_{19}BrN_2O_3H^+$, 415.0657).

*Preparation of 1-(4-(5-(4-Bromophenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylic Acid (**21d**).* Using method E, **20d** (0.12 g, 0.2 mmol) and LiOH (0.014 g, 0.5 mmol) gave **21d** as a white solid (0.07 g, 58%); MP: 152−254 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.58−7.63 (m, 4 Ar**H**), 7.32−7.37 (m, 4 Ar**H**), 5.69−5.74 (m, 1 isoxazoline ring-**H**), 3.81−3.96 (m, 1 isoxazoline ring-**H**), 3.50 (s, NC**H**$_2$), 3.22−3.26 (m, 2 azetidine ring-**H**, 1 isoxazoline ring-**H**), 3.04−3.07 (m, 2 azetidine ring-**H**), 2.74−2.78 (m, 1 azetidine ring-**H**); ^{13}C NMR (CD$_3$OD, 100 MHz): δ 178.90 (**C**(O)), 156.47, 140.34, 131.50, 129.45, 129.08, 127.64, 126.81, 121.59 (Ar**C**, isoxazoline ring-**C**), 81.97, 57.12, 42.37, 35.83, 22.75; HPLC purity: 4.8 min, 99.1%; HRMS (M + H)$^+$ (ESI$^+$): 415.0659 [M + H]$^+$ (calcd for $C_{20}H_{19}BrN_2O_3H^+$, 415.0657).

*Preparation of 1-(4-(5-(4-Hydroxyphenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylic Acid (**21e**).* Using method E, **20e** (0.050 g, 0.12 mmol) and LiOH (0.006 g, 0.25 mmol) gave **21e** as a white solid (0.032 g, 66%); MP: 184−185 °C (decomp.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.66 (s, COO**H**), 7.66 (d, J = 8.2 Hz, 2 Ar**H**), 7.41 (d, J = 8.0 Hz, 2 Ar**H**), 7.19 (d, J = 8.6 Hz, 2 Ar**H**), 6.78 (d, J = 8.5 Hz, 2 Ar**H**), 5.60 (t, J = 10.4 Hz, 1 isoxazoline ring-**H**), 3.73−3.80 (m, 3**H**), 3.55 (br, NC**H**$_2$), 3.40 (br, 1**H**), 3.30−3.37 (m, 3**H**); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 158.00, 156.79, 131.03, 129.59, 129.15, 128.65, 128.24, 127.12, 115.79 (Ar**C**, isoxazoline ring-**C**), 82.75 (isoxazoline ring-**C**), 56.29 (N**C**H$_2$), 49.74 (azetidine ring-**C**), 42.09 (isoxazoline ring-**C**), 33.69 (azetidine ring-**C**); HPLC purity: 1.9 min, 98.6%; HRMS (M + H)$^+$ (ESI$^+$): 353.1500 [M + H]$^+$ (calcd for $C_{20}H_{20}N_2O_4H^+$, 353.1501).

*Preparation of Sodium 1-(4-(5-(4-(Chloromethyl)phenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate (**21f**).* Using method F, **20f** (0.11 g, 0.2 mmol) and NaOH (0.013 g, 0.5 mmol) gave **21f** as a white solid (0.05 g, 45%); MP: 137−139 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.63 (d, J = 8.2 Hz, 2 Ar**H**), 7.39−7.47 (m, 4 Ar**H**), 7.32 (d, J = 8.2 Hz, 2 Ar**H**), 5.70−5.75 (m, 1 isoxazoline ring-**H**), 4.77 (s, C**H**$_2$Cl), 3.83−3.90 (1 isoxazoline ring-**H**), 3.49 (s, NC**H**$_2$), 3.40−3.42 (m, 1 isoxazoline ring-**H**), 3.17−3.25 (m, 2 azetidine ring-**H**), 3.02−3.06 (m, 2 azetidine ring-**H**), 2.67−2.73 (m, 1 azetidine ring-**H**); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.43 (**C**(O)), 156.75 (isoxazoline ring-**C**), 141.47, 140.02, 139.01, 129.48, 129.36, 128.48, 127.04, 126.63 (Ar**C**), 82.32 (isoxazoline ring-**C**), 57.76 (N**C**H$_2$), 53.12, 52.18, 46.75, 43.86, 42.51; HPLC purity: 5.6 min, 98.1%; HRMS (M + H)$^+$ (ESI$^+$): 385.1320 [M + H]$^+$ (calcd for $C_{21}H_{21}ClN_2O_3H^+$, 385.1319).

*Preparation of Sodium 1-(4-(5-(4-Ethylphenyl)-4,5-dihydroisoxazol-3-yl)azetidine-3-carboxylate) (**21g**).* Using method F, **20g** (0.054 g, 0.14 mmol) and NaOH (0.006 g, 0.15 mmol) gave **21g** as a white solid (0.057 g, 99%); MP: 219−220 °C; ^{1}H NMR (CD$_3$OD, 400 MHz): δ 7.67 (d, J = 8.2 Hz, 2 Ar**H**), 7.37 (d, J = 8.2 Hz, 2 Ar**H**), 7.30 (d, J = 8.1 Hz, 2 Ar**H**), 7.21 (d, J = 8.0 Hz, 2 Ar**H**), 5.68 (dd, J = 8.8, 10.7 Hz, 1 isoxazoline ring-**H**), 3.82 (dd, J = 10.8, 17.0 Hz, 1 isoxazoline ring-**H**), 3.66 (s, NC**H**$_2$), 3.53 (t, J = 8.0 Hz, 2 azetidine ring-**H**), 3.32−3.37 (m, 2 azetidine ring-**H**, 1 isoxazoline ring-**H**), 3.18−3.24 (m, 1 azetidine ring-**H**), 2.63 (q, J = 7.6 Hz, alkyl chain-C**H**$_2$), 1.82 (t, J = 7.6 Hz, alkyl chain-C**H**$_3$); ^{13}C NMR (CD$_3$OD, 100 MHz): δ 179.57 (**C**(O)), 156.75 (isoxazoline ring-**C**), 144.32, 139.64, 139.64, 138.11, 128.97, 127.82, 126.54, 125.81 (Ar**C**), 82.71 (isoxazoline ring-**C**), 62.45 (N**C**H$_2$), 57.69 (azetidine ring-**C**), 42.28 (isoxazoline ring-**C**), 36.54 (azetidine ring-**C**), 28.16 (**C**H$_2$CH$_3$), 14.75 (CH$_2$**C**H$_3$); HPLC purity: 5.0 min, 97.8%; HRMS (M + H)$^+$ (ESI$^+$): 365.1864 [M + H]$^+$ (calcd for $C_{22}H_{24}N_2O_3H^+$, 365.1865).

*Preparation of Sodium 1-(4-(5-(4-Propylphenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate (**21h**).* Using method F, **20h** (0.048 g, 0.12 mmol) and NaOH (0.005 g, 0.13 mmol) gave **21h** as a white solid (0.054 g, 112%); MP: 213−214 °C; ^{1}H NMR (CD$_3$OD, 400 MHz): δ 7.67 (d, J = 8.3 Hz, 2 Ar**H**), 7.37 (d, J = 8.3 Hz, 2 Ar**H**), 7.29 (d, J = 8.1 Hz, 2 Ar**H**), 7.19 (d, J = 8.1 Hz, 2 Ar**H**), 5.67 (dd, J = 8.8, 10.7 Hz, 1 isoxazoline ring-**H**), 3.82 (dd, J = 10.8, 17.0 Hz, 1 isoxazoline ring-**H**), 3.65 (s, NC**H**$_2$), 3.53 (t, J = 8.1 Hz, 2 azetidine ring-**H**), 3.32−3.37 (m, 2 azetidine ring-**H**, 1 isoxazoline ring-**H**), 3.18−3.22 (m, 1 azetidine ring-**H**), 2.58 (t, J = 7.8 Hz, alkyl chain-C**H**$_2$), 1.59−1.65 (m, alkyl chain-C**H**$_2$), 0.92 (t, J = 7.3 Hz, alkyl chain-C**H**$_3$); ^{13}C NMR (CD$_3$OD, 100 MHz): δ 179.55 (**C**(O)), 156.77 (isoxazoline ring-**C**), 142.65, 139.66, 138.16, 128.98, 128.46, 126.54, 125.73 (Ar**C**), 82.74 (isoxazoline ring-**C**), 62.47 (N**C**H$_2$) 57.67 (azetidine ring-**C**), 42.38 (isoxazoline ring-**C**), 37.32 (alkyl chain-C**H**$_2$), 36.54 (azetidine ring-**C**), 24.31 (alkyl chain-C**H**$_2$), 12.67 (alkyl chain-C**H**$_3$); HPLC purity: 5.8 min, 99.3%; HRMS (M + H)$^+$ (ESI$^+$): 379.2021 [M + H]$^+$ (calcd for $C_{23}H_{26}N_2O_3H^+$, 379.2022).

*Preparation of Sodium 1-(4-(5-(4-(tert-Butyl)phenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate (**21i**).* Using method F, **20i** (0.10 g, 0.3 mmol) and LiOH (0.09 g, 3.7 mmol) gave the salt-free acid as a white solid (0.10 g, 99%); R_f = 0.18 (CH$_2$Cl$_2$/MeOH 4/1). Using method F, the salt-free acid (2.45 g, 6.2 mmol) and NaOH (0.25 g, 6.2 mmol) gave **21i** as a white solid (2.48 g, 96%); R_f = 0.10 (CH$_2$Cl$_2$/MeOH 4/1); MP: 134−136 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.63 (d, J = 8.2 Hz, 2 Ar**H**), 7.41 (d, J = 8.3 Hz, 2 Ar**H**), 7.30−7.33 (m, 4 Ar**H**), 5.65−5.69 (m, 1 isoxazoline ring-**H**), 3.78−3.85 (m, 1 isoxazoline ring-**H**), 3.50 (s, NC**H**$_2$), 3.36−3.40 (m, 1**H**), 3.25 (t, J = 7.6 Hz, 2**H**), 3.06 (t, J = 7.0 Hz, 2**H**), 2.74 (t, J = 7.7 Hz, 1**H**), 1.27 (s, C(C**H**$_3$)$_3$); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 176.87 (**C**(O)), 156.79 (isoxazoline ring-**C**), 151.01, 141.80, 138.36, 128.97, 128.16, 126.98, 126.43, 125.82 (Ar**C**), 82.12 (isoxazoline ring-**C**), 63.26 (N**C**H$_2$), 58.85, 37.35, 34.76, 31.57 (C(**C**H$_3$)$_3$); HPLC purity: 6.0 min, 99.3%; HRMS (M + H)$^+$ (ESI$^+$): 393.2176 [M + H]$^+$ (calcd for $C_{24}H_{28}N_2O_3H^+$, 393.2178).

*Preparation of 1-(4-(5-(3-Cyano-4-isopropoxyphenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylic Acid (**21j**).* Using method E, **20j** (0.19 g, 0.4 mmol) and LiOH (0.021 g, 0.8 mmol) gave **21j** as a white solid (0.12 g, 64%); MP: 216−218 °C (decomp.); ^{1}H NMR (CD$_3$OD, 400 MHz): δ 7.84 (d, J = 8.3 Hz, 2 Ar**H**), 7.64−7.67 (m, 2 Ar**H**), 7.56 (d, J = 8.3 Hz, 2 Ar**H**), 7.21−7.23 (m, 1 Ar**H**), 5.74−5.79 (m, 1 isoxazoline ring-**H**), 4.76−4.91 (m, OC**H**(CH$_3$)$_2$), 4.47 (s, NC**H**$_2$), 4.28−4.36 (m, 4 azetidine ring-**H**), 3.85−3.92 (m, 1 isoxazoline ring-**H**), 3.61−3.69 (m, 1 azetidine ring-**H**), 3.32−3.44 (m, 1 isoxazoline ring-**H**), 1.40 (d, J = 6.0 Hz, OCH(C**H**$_3$)$_2$); ^{13}C NMR (CD$_3$OD, 100 MHz): δ 173.04 (**C**(O)), 159.77, 156.27,

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J. Med. Chem. XXXX, XXX, XXX−XXX

133.44, 132.29, 131.73, 131.15, 130.89, 130.20, 127.38, 115.84, 114.13 (Ar**C**, isoxazoline ring-**C**), 102.23, 81.64 (isoxazoline ring-**C**), 71.85 (O**C**H(CH_3)$_2$), 57.65 (N**C**H$_2$), 55.92 (azetidine ring-**C**), 41.91 (isoxazoline ring-**C**), 32.99 (azetidine ring-**C**), 20.67 (OCH(**C**H$_3$)$_2$); HPLC purity: 8.9 min, 96.0%; HRMS (M + H)$^+$ (ESI$^+$): 420.1924 [M + H]$^+$ (calcd for $C_{24}H_{25}N_3O_4H^+$, 420.1923).

*Preparation of Sodium (1-(4-(5-(3-Chloro-4-ethoxyphenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate) (**21k**).* Using method F, **20k** (0.10 g, 0.24 mmol) and NaOH (0.01 g, 1.1 mmol) gave **21k** as a yellow solid (0.090 g, 86%); MP: 256−257 °C (decomp.); ^{1}H NMR (CD_3OD, 400 MHz): δ 7.67 (d, J = 8.2 Hz, 2 Ar**H**), 7.37−7.40 (m, 3 Ar**H**), 7.28 (dd, J = 2.1, 8.5 Hz, 1 Ar**H**), 7.05 (d, J = 8.5 Hz, 1 Ar**H**), 5.65 (dd, J = 8.6, 10.7 Hz, 1 isoxazoline ring-**H**), 4.11 (q, J = 6.9 Hz, O**C**H$_2$CH$_3$), 3.83 (dd, J = 10.8, 18.2 Hz, 1 isoxazoline ring-**H**), 3.67 (s, N**C**H$_2$), 3.55 (t, J = 8.0 Hz, 2 azetidine ring-**H**), 3.34−3.38 (m, 2 azetidine ring-**H**, 1 isoxazoline ring-**H**), 3.19−3.25 (m, 1 azetidine ring-**H**), 1.42 (t, J = 6.9 Hz, OCH$_2$C**H**$_3$); ^{13}C NMR (CD_3OD, 100 MHz): δ 179.50 (**C**(O)), 157.55, 154.16, 133.82, 129.46, 128.15, 127.54, 126.93, 125.76, 122.41, 113.82 (Ar**C**, isoxazoline ring-**C**), 81.79 (isoxazoline ring-**C**), 64.99 (N**C**H$_2$), 61.45 (O**C**H$_2$CH$_3$), 57.27 (azetidine ring-**C**), 42.35 (isoxazoline ring-**C**), 36.09 (azetidine ring-**C**), 13.74 (OCH$_2$**C**H$_3$); HPLC purity: 5.1 min, 95.9%; HRMS (M + H)$^+$ (ESI$^+$): 415.1424 [M + H]$^+$ (calcd for $C_{22}H_{23}ClN_2O_4H^+$, 415.1425).

*Preparation of Sodium 1-(4-(5-(3-Chloro-4-isopropoxyphenyl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate (**21l**).* Using method F, **20l** (0.13 g, 0.29 mmol) and NaOH (0.012 g, 0.30 mmol) gave **21l** as a yellow solid (0.12 g, 98%); MP: 193.5−194.5 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 7.69 (d, J = 8.1 Hz, 2 Ar**H**), 7.38−7.42 (m, 3 Ar**H**), 7.28 (dd, J = 2.1, 8.4 Hz, 1 Ar**H**), 7.07 (d, J = 8.5 Hz, 1 Ar**H**), 5.66 (dd, J = 8.5, 10.6 Hz, 1 isoxazoline ring-**H**), 4.61−4.89 (m, O**C**H(CH_3)$_2$), 3.84 (dd, J = 10.8, 17.0 Hz, 1 isoxazoline ring-**H**), 3.67 (s, N**C**H$_2$), 3.54 (t, J = 8.0 Hz, 2 azetidine ring-**H**), 3.32−3.40 (m, 1 isoxazoline ring-**H**, 2 azetidine ring-**H**), 3.20−3.26 (m, 1 azetidine ring-**H**), 1.35 (d, J = 6.0 Hz, OCH(C**H**$_3$)$_2$); ^{13}C NMR (CD_3OD, 100 MHz): δ 179.60 (**C**(O)), 156.79 (isoxazoline ring-**C**), 153.42, 139.76, 134.15, 128.98, 128.29, 127.75, 126.57, 125.33, 123.78, 115.65 (Ar**C**), 81.77 (isoxazoline ring-**C**), 71.68 (O**C**H(CH_3)$_2$), 62.48 (N**C**H$_2$), 57.69 (azetidine ring-**C**), 42.29 (isoxazoline ring-**C**), 36.54 (azetidine ring-**C**), 20.91 (OCH(**C**H$_3$)$_2$); HPLC purity: 5.7 min, 98.1%; HRMS (M + H)$^+$ (ESI$^+$): 429.1582 [M + H]$^+$ (calcd for $C_{23}H_{25}ClN_2O_4H^+$, 429.1581).

*Preparation of Sodium 1-(4-(5-(4-Isopropoxy-3-(trifluoromethyl)phenyl)-4,5-dihydroisoxazol-3-yl)benzyl)-azetidine-3-carboxylate (**21m**).* Using method F, **20m** (0.95 g, 0.20 mmol) and NaOH (0.008 g, 0.21 mmol) gave **21m** as a yellow solid (0.09 g, 92%); MP: 216−218 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 7.71 (d, J = 8.1 Hz, 2 Ar**H**), 7.55−7.57 (m, 2 Ar**H**), 7.43 (d, J = 8.1 Hz, 2 Ar**H**), 7.18 (d, J = 8.3 Hz, 1 Ar**H**), 5.72 (dd, J = 8.7, 10.6 Hz, 1 isoxazoline ring-**H**), 4.74 (sept, J = 6.0 Hz, O**C**H(CH_3)$_2$), 3.93 (s, N**C**H$_2$), 3.37−3.88 (m, 1 isoxazoline ring-**H**, 2 azetidine ring-**H**), 3.63−3.68 (m, 2 azetidine ring-**H**), 3.24−3.38 (m, 1 isoxazoline ring-**H**, 1 azetidine ring-**H**), 1.32 (d, J = 6.0 Hz, OCH(C**H**$_3$)$_2$); ^{13}C NMR (CD_3OD, 100 MHz): δ 178.16 (**C**(O)), 156.63, 155.95, 136.92, 132.37, 130.95, 129.44, 129.19 (Ar**C**, isoxazoline ring-**C**), 124.55 (q, J_{C-F} = 5.3 Hz), 123.65 (q, J_{C-F} = 270.0 Hz), 119.76, 119.45, 119.15, 118.85 (q, J_{C-F} = 30.3 Hz), 114.55 (Ar**C**), 81.93 (isoxazoline ring-**C**), 71.03 (O**C**H(CH_3)$_2$), 60.55 (N**C**H$_2$), 57.24, 42.22, 35.80, 20.74 (OCH(**C**H$_3$)$_2$); HPLC purity: 3.8 min, 100%; HRMS (M + H)$^+$ (ESI$^+$): 463.1848 [M + H]$^+$ (calcd for $C_{24}H_{25}F_3N_2O_4H^+$, 463.1845).

*Preparation of Sodium 1-(4-(5-(Pyridin-2-yl)-4,5-dihydroisoxazol-3-yl)benzyl)azetidine-3-carboxylate (**21n**).* Using method F, **20n** (0.04 g, 0.1 mmol) and NaOH (0.0054 g, 0.2 mmol) gave **21n** as a yellow solid (0.02 g, 53%); MP: 131−133 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.90 (d, J = 5.6 Hz, 1 Ar**H**), 8.69−8.73 (m, 1 Ar**H**), 8.23 (d, J = 8.0 Hz, 1 Ar**H**), 8.10 (d, J = 6.8 Hz, 1 Ar**H**), 7.84 (d, J = 8.0 Hz, 2 Ar**H**), 7.66−7.71, (m, 2 Ar**H**), 6.29−6.34 (m, 1 isoxazoline ring-**H**), 4.20−4.61 (m, 7**H**), 3.37−3.89 (m, 2**H**), 3.32−3.33 (m, 1**H**); ^{13}C NMR (CD_3OD, 100 MHz): δ 171.98, 171.47, 156.73, 154.70, 147.33, 141.82, 132.25 (d, J = 21.8 Hz), 130.55 (d, J = 8.2 Hz), 129.73, 127.73, 126.62, 124.85 (Ar**C**, isoxazoline ring-**C**), 78.14 (isoxazoline ring-**C**), 57.26 (d, J = 22.8 Hz, N**C**H$_2$), 55.27 (d, J = 25.6 Hz, azetidine ring-**C**), 41.89 (isoxazoline ring-**C**), 32.26 (azetidine ring-**C**); HPLC purity: 5.1 min, 95.3%; HRMS (M + H)$^+$ (ESI$^+$): 338.1503 [M + H]$^+$ (calcd for $C_{19}H_{19}N_3O_3H^+$, 338.1505).

*Preparation of Methyl 1-(4-(5-(3-(Chloro-4-isopropoxyphenyl)-4,5-dihydroisoxazol-3-yl)benzyl)pyrrolidine-3-carboxylate) (**22**).* Using method D, **19l** (0.43 g, 1.2 mmol), methyl pyrrolidine-3-carboxylate hydrochloride (0.41 g, 2.5 mmol), triethylamine (0.52 mL, 3.7 mmol), and sodium cyanoborohydride (0.16 g, 2.5 mmol) gave **22** as a yellow oil (0.23 g, 53%); R_f = 0.56 (*n*-hexane/EtOAc 1/1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.63 (d, J = 8.1 Hz, 2 Ar**H**), 7.35−7.39 (m, 3 Ar**H**), 7.21 (dd, J = 2.1, 8.4 Hz, 1 Ar**H**), 6.92 (d, J = 8.5 Hz, 1 Ar**H**), 5.63 (dd, J = 8.2, 10.8 Hz, 1 isoxazoline ring-**H**), 4.52−4.55 (m, O**C**H(CH_3)$_2$), 3.73 (dd, J = 10.9, 16.6 Hz, 1 isoxazoline ring-**H**), 3.67 (s, O**C**H$_3$), 3.65 (d, J = 4.0 Hz, N**C**H$_2$), 3.30 (dd, J = 8.2, 16.6 Hz, 1 isoxazoling ring-**H**), 3.01−3.05 (m, 1 pyrrolidine ring-**H**), 2.87 (t, J = 9.0 Hz, 1 pyrrolidine ring-**H**), 2.64−2.68 (m, 2 pyrrolidine ring-**H**), 2.54 (q, J = 7.6 Hz, 1 pyrrolidine ring-**H**), 2.08−2.12 (m, 2 pyrrolidine ring-**H**), 1.36 (d, J = 6.0 Hz, OCH(C**H**$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 175.43 (**C**(O)), 156.01, 153.59, 141.27, 134.11, 129.09, 128.14, 128.09, 126.77, 125.24, 124.56, 116.02 (Ar**C**, isoxazoline ring-**C**), 81.66 (isoxazoline ring-**C**), 72.25 (O**C**H(CH_3)$_2$), 59.67 (N**C**H$_2$), 56.63, 53.75 (pyrrolidine ring-**C**), 51.92 (O**C**H$_3$), 43.07 (pyrrolidine ring-**C**), 41.97 (isoxazoline ring-**C**), 27.69 (pyrrolidine ring-**C**), 22.01 (OCH(**C**H$_3$)$_2$); HPLC purity: 5.3 min, 98.7%; HRMS (M + H)$^+$ (ESI$^+$): 457.1895 [M + H]$^+$ (calcd for $C_{25}H_{29}ClN_2O_4H^+$, 457.1894).

*Preparation of Sodium 1-(4-(5-(3-Chloro-4-isopropoxyphenyl)-4,5-dihydroisoxazol-3-yl)benzyl)pyrrolidine-3-carboxylate (**23**).* Using method F, **22** (0.23 g, 0.50 mmol) and NaOH (0.021 g, 0.52 mmol) gave **23** as a yellow solid (0.28 g, quantitative yield); MP: 184−185 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 7.66 (d, J = 8.0 Hz, 2 Ar**H**), 7.38−7.42 (m, 3 Ar**H**), 7.25 (dd, J = 1.5, 8.4 Hz, 1 Ar**H**), 7.04 (d, J = 8.5 Hz, 1 Ar**H**), 5.63 (t, J = 10.2 Hz, 1 isoxazoline ring-**H**), 5.61−5.65 (m, O**C**H(CH_3)$_2$), 3.80 (dd, J = 10.8, 17.0 Hz, 1 isoxazoline ring-**H**), 3.61−3.69 (m, N**C**H$_2$), 3.31−3.36 (m, 1 isoxazoline ring-**H**), 2.89−2.99 (m, 2 pyrrolidine ring-**H**), 2.72−2.77 (m, 1 pyrrolidine ring-**H**), 2.61 (t, J = 8.0 Hz, 1 pyrrolidine ring-**H**), 2.49 (q, J = 8.2 Hz, 1 pyrrolidine ring-**H**), 2.00−2.09 (m, 2 pyrrolidine ring-**H**), 1.31 (d, J = 6.0 Hz, OCH(C**H**$_3$)$_2$); ^{13}C NMR (CD_3OD, 100 MHz): δ 181.64 (**C**(O)), 156.81, 153.42, 140.79, 134.16, 129.43, 128.19, 127.75, 126.48, 125.35, 123.78, 115.66 (Ar**C**, isoxazoline ring-**C**), 81.75 (isoxazoline ring-**C**), 71.69 (O**C**H(CH_3)$_2$), 59.73 (N**C**H$_2$), 57.75, 53.78, 45.15 (pyrrolidine ring-**C**), 42.32 (isoxazoline ring-**C**), 28.26 (pyrrolidine ring-**C**), 20.93 (OCH-(**C**H$_3$)$_2$); HPLC purity: 5.3 min, 98.7%; HRMS (M + H)$^+$ (ESI$^+$): 443.1741 [M + H]$^+$ (calcd for $C_{24}H_{27}ClN_2O_4H^+$, 443.1738).

*Preparation of Methyl 1-(4-(5-(3-Chloro-4-isopropoxyphenyl)-4,5-dihydroisoxazol-3-yl)benzyl)piperidine-4-carboxylate (**24**).* Using method D, **19l** (0.26 g, 0.77 mmol), methyl piperidine-4-carboxylate hydrochloride (0.22 g, 1.5 mmol), triethylamine (0.32 mL, 2.3 mmol), and sodium cyanoborohydride (0.097 g, 1.5 mmol) gave **24** as a clear oil (0.020 g, 6%); R_f = 0.30 (*n*-hexane/EtOAc 1/1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.63 (d, J = 8.2 Hz, 2 Ar**H**), 7.35−7.39 (m, 3 Ar**H**), 7.21 (dd, J = 2.1, 8.5 Hz, 1 Ar**H**), 6.92 (d, J = 8.5 Hz, 1 Ar**H**), 5.63 (dd, J = 8.1, 10.8 Hz, 1 isoxazoline ring-**H**), 4.57−4.51 (m, O**C**H(CH_3)$_2$), 3.73 (dd, J = 10.8, 16.6 Hz, 1 isoxazoline ring-**H**), 3.67 (s, COOC**H**$_3$), 3.50 (s, N**C**H$_2$), 3.30 (dd, J = 8.1, 16.6 Hz, 1 isoxazoline ring-**H**), 2.81−2.84 (m, 2 piperidine ring-**H**), 2.26−2.33 (m, 1 piperidine ring-**H**), 2.01−2.06 (m, 2 piperidine ring-**H**), 1.85−1.86 (m, 2 piperidine ring-**H**), 1.74−1.81 (m, 2 piperidine ring-**H**), 1.36 (d, J = 6.0 Hz, OCH(C**H**$_3$)$_2$).

*Preparation of Sodium 1-(4-(5-(3-Chloro-4-isopropoxyphenyl)-4,5-dihydroisoxazol-3-yl)benzyl)piperidine-4-carboxylate (**25**).* Using method F, **24** (0.02 g, 0.042 mmol) and NaOH (0.002 g, 0.045 mmol) gave **25** as a white solid (0.02 g, 99%); MP: 171−173 °C (decomp.); ^{1}H NMR (CD_3OD, 400 MHz): δ 7.69 (d, J = 8.2 Hz, 2 Ar**H**), 7.40−7.44 (m, 3 Ar**H**), 7.28 (dd, J = 2.1, 8.4 Hz, 1 Ar**H**), 7.08 (d, J = 8.5 Hz, 1 Ar**H**), 5.66 (dd, J = 8.6, 10.6 Hz, 1 isoxazoline

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J. Med. Chem. XXXX, XXX, XXX−XXX

ring-**H**), 4.60−4.67 (m, OC**H**$(CH_3)_2$), 3.84 (dd, J = 10.8, 17.0 Hz, 1 isoxazoline ring-**H**), 3.61 (s, NC$\mathbf{H_2}$), 3.35−3.40 (m, 1 isoxazoline ring-**H**), 2.92 (d, J = 11.6 Hz, 2 piperidine ring-**H**), 2.10−2.20 (m, 3 piperidine ring-**H**), 1.86 (s, 2 piperidine ring-**H**), 1.73−1.80 (m, 2 piperidine ring-**H**), 1.33 (d, J = 6.0 Hz, OCH(C$\mathbf{H_3}$)$_2$); ^{13}C NMR (CD_3OD, 100 MHz): δ 207.53 (**C**(O)), 156.81 (isoxazoline ring-**C**), 153.57, 141.36, 134.28, 129.94, 127.75, 126.45, 125.29, 123.79, 115.65, 113.63 (Ar**C**), 81.81 (isoxazoline ring-**C**), 71.67 (O**C**H-$(CH_3)_2$), 62.15 (N$\mathbf{C}H_2$), 53.05 (piperidine ring-**C**), 42.29 (isoxazoline ring-**C**), 28.62 (piperidine ring-**C**), 20.86 (OCH($\mathbf{C}H_3$)$_2$); HPLC purity: 4.9 min, 98.3%; HRMS (M + H)$^+$ (ESI$^+$): 457.1896 [M + H]$^+$ (calcd for $C_{25}H_{29}ClN_2O_4H^+$, 457.1894).

Cell Culture. PathHunter Chinese hamster ovary (CHO)-K1 endothelial differentiation gene 1 (EDG1) β-arrestin cells (93-0207C2; DiscoverX) were cultured in Dulbecco's modified Eagle medium (DMEM)/F12 (Biowest) supplemented with 10% (v/v) fetal bovine serum (Biowest), 2.05 mM L-glutamine (Gibco), 100 U/mL penicillin−streptomycin (Gibco), 300 μg/mL hygromycin B (Invitrogen), and 800 μg/mL Geneticin (G418) sulfate (Santa Cruz Biotechnology). PathHunter EDG1 total GPCR internalization human embryonic kidney (HEK) 293 cells (93-0784C1; DiscoverX) were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (Biowest), 100 U/mL penicillin−streptomycin (Gibco), 0.25 μg/mL puromycin (InvivoGen), and 200 μg/mL hygromycin B (Invitrogen). For the S1P receptor calcium flux assay, HEK293/Gα_{15} cells (HTSHEK-5L; Millipore) with stable expressed $S1P_1$ and $S1P_3$ receptors were cultured in DMEM/F12 (Biowest) supplemented with 10% (v/v) fetal bovine serum (Biowest), 2.05 mM L-glutamine (Gibco), nonessential amino acids (Gibco), 100 U/mL penicillin−streptomycin (Gibco), 1 μg/mL puromycin (Gibco), 200 μg/mL hygromycin B (Invitrogen), and 200 μg/mL Geneticin (G418) sulfate (Santa Cruz Biotechnology). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

$S1P_1$ Receptor β-Arrestin Recruitment Assay. The activity of β-arrestin recruitment following the $S1P_1$ receptor activation of each synthesized compound was evaluated based on the PathHunter CHO-K1 EDG1 β-arrestin cell line (93-0207C2; DiscoverX). The CHO-K1 EDG1 cells were engineered to coexpress two fragments of β-galactosidase at the $S1P_1$ receptor and β-arrestin, respectively. Interaction of the $S1P_1$ receptor with β-arrestin induces the formation of active β-galactosidase, and the β-arrestin recruitment activity is proportional to the subsequent chemiluminescent signal. The CHO-K1 EDG1 cells (1×10^4 cells/well) in cell plating 28 Reagent (DiscoverX) were seeded in 96-well white plates and incubated overnight at 37 °C. The test compound prepared in cell plating 28 Reagent (DiscoverX) was added to the wells and incubated for 90 min at 37 °C. After 1 h reaction with 50 μL of the detection reagent (PathHunter detection kit, 93-0001L; DiscoverX) at room temperature (20−23 °C) in the dark, the chemiluminescence signals were detected at all wavelengths with 1000 s integration time using a microplate reader (SpectraMax*i*3; Molecular Devices).

$S1P_1$ Receptor Internalization Assay. The ability to internalize the $S1P_1$ receptor of synthesized compounds was evaluated based on the PathHunter EDG1 total GPCR internalization HEK293 cell line (93-0784C1; DiscoverX). The internalization of the $S1P_1$ receptor in the endosome induces the complementation of two β-galactosidase fragments, expressed at the $S1P_1$ receptor or endosome. This activity increases the β-galactosidase activity detectable by chemiluminescence signal measurement. The HEK293 EDG1 cells (1×10^4 cells/well) in the cell plating 28 reagent (DiscoverX) were seeded in 96-well white plates and treated with the test compound prepared in the cell plating 28 reagent (DiscoverX) for 3 h at 37 °C. 50 μL of the detection reagent (PathHunter Detection Kit, 93-0001L; DiscoverX) was added to the wells and incubated for 1 h at room temperature in the dark. The luminescence was measured at all wavelengths using a microplate reader (SpectraMax*i*3; Molecular Devices).

CYP Inhibition Assay. According to the previously described method, the compounds' inhibitory activity against the CYP enzyme was evaluated using P450-Glo assays (Promega).[38] Briefly, the test compound in dimethylsulfoxide (DMSO) was mixed with the CYP enzyme and substrate in potassium phosphate buffer (pH 7.4) on a 96-well white plate and incubated for 10 min at room temperature. A reduced nicotinamide adenine dinucleotide phosphate (NADPH)-regenerating mixture containing substrate $NADP^+$, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase in potassium phosphate buffer was added to the wells and incubated at 37 °C for the optimized time according to the CYP subtype (10 min for 1A2 and 3A4, 20 min for 2C19, and 30 min for 2C9 and 2D6) to initiate the enzyme reaction. After the reaction with the luciferin detection reagent for 20 min at room temperature in the dark, the luminescence signals were recorded using a microplate reader (SpectraMax*i*3; Molecular Devices). All experiments were validated using a well-known inhibitor of each CYP subtype as a positive control: lansoprazole for 2C19, quinidine for 2D6, sulfaphenazole for 2C9, α-naphthoflavone for 1A2, and ketoconazole for 3A4.

Microsomal Stability Test. The microsomal stability of the synthesized compounds was evaluated based on the previously described method.[38] Briefly, the test compound (1 μM) was preincubated with human or mouse microsomes (0.5 mg/mL) in 0.1 M potassium phosphate buffer (pH 7.4) for 5 min at 37 °C, followed by incubation with the NADPH regeneration system for 30 min at 37 °C. Then, acetonitrile containing chlorpropamide was added to stop the reaction. The supernatant was collected after centrifugation at 14,000 rpm for 5 min at 4 °C and injected into an LC−MS/MS system for analysis. LC−MS/MS analysis was performed using a Shimadzu Nexera XR system and TSQ vantage (Thermo) with a Kinetex C18 column (2.1 × 100 mm, 2.6 μm particle size; Phenomenex). The mobile phase consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Xcalibur 1.6.1 was utilized to analyze the obtained result, and the amount of the remaining compound was calculated by comparing the peak area.

Measurement of the Peripheral Blood Lymphocyte Count. Male Wistar rats (5 wk, 160−180 g) were purchased from Orient Bio Inc. (Seongnam, South Korea). All test compounds were dissolved in 5% DMSO and 10% Kolliphor HS 15 (Sigma-Aldrich) in distilled water and orally administered. Blood samples were obtained from the lateral tail vein of the rat under anesthesia (4% isoflurane) at different time points and were drawn into a K2-EDTA-coated tube. The blood lymphocyte count was measured by using an automatic blood cell counter (Horiba).

EAE Mouse Model and Treatment. All animal studies were performed in accordance with the directives of the Animal Care and Use Committee of the Institutional Animal Care and Use Committee of KIST (Seoul, South Korea). Female C57BL/6 mice (10 weeks, 19−22 g) were purchased from Samtako (Seoul, South Korea) and housed in a temperature- and humidity-controlled animal facility (22 ± 1 °C, 12 h light−dark cycle).

EAE induction was performed using a Hooke Kit MOG_{35-55}/CFA Emulsion PTX (EK-2110; Hooke Laboratories) according to the manufacturer's instructions. Mice were immunized subcutaneously at two lower back sites (0.1 mL per each site) with the MOG_{35-55} peptide emulsified in CFA containing heat-killed *Mycobacterium tuberculosis* H37 Ra. PTX (150 ng) in phosphate-buffered saline was injected intraperitoneally on days 0 and 1 postimmunization. All test compounds (**21l**, **21m**, and fingolimod) were dissolved in 2.5% DMSO and 5% Kolliphor HS 15 (Sigma-Aldrich) in distilled water and administered by oral gavage once daily from day 0 to the end of the study at day 20. The EAE clinical score and body weight were recorded daily. The clinical scoring system was as follows: 0, no clinical signs; 0.5, limp tail-tip; 1, limp tail; 1.5, partial paralysis of one hind limb or a waddling gait; 2.0, partial paralysis of both hind limbs, dragging of one hind limb, or head tilt without tail paralysis; 2.5, dragging of both hind limbs or a strong head tilt that cause the mouse to fall over; 3.0, hind limbs paddling or complete paralysis of one hind leg; 3.5, complete paralysis of both hind limbs; 4.0, failure to return within 10 s if the body is overturned, hip rotation, or sunken hip; 4.5, partial paralysis of front limbs; and 5.0, death.

MEA Electrophysiology Studies in hiPSC-Derived Cardiomyocytes. For assessing the pro-arrhythmic potential of drug candidates, MEA electrophysiology studies were performed by

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J. Med. Chem. XXXX, XXX, XXX−XXX

NEXEL (South Korea), a member of the HESI comprehensive *in vitro* proarrhythmia assay (CiPA) working group. hiPSCs used NEXEL's proprietary Cardiosight-S, which consisted of a high-purity cardiomyocyte population derived from human iPSCs using the proprietary differentiation method. hiPSC-derived cardiomyocytes were cultured onto MEA plates (CytoView MEA 48/M768-tMEA-48w; Axion Biosystems). After 7 days of culture on MEA plates, the cells formed a spontaneously beating monolayer over the recording electrodes embedded in each well. At least 4 h prior to the experiment, the media inside the wells were completely changed with 300 μL of prewarmed media and equilibrated in a cell culture incubator at 37 °C/5% CO_2 before starting the experiments. The 48-well plate was transferred to the Axion MEA equipped with a CO_2 and temperature stage incubator (Axion Maestro MEA, Axion Biosystems). After checking that the wells were beating in a synchronous manner, the baseline was recorded for 5 min from each well. Test compounds were treated with a single dose (1 and 0.1 μM) per well by 10% medium change while maintaining a concentration of 0.1% DMSO as a negative control. Electrical activity was recorded for 5 min following 1 h exposure to test compounds or 0.01 μM E4031 as the positive control. Analysis software specific to each instrument was used to provide four primary endpoints from the cardiac FPs recorded: (1) spike amplitude, (2) FP duration (FPD), (3) beat period (BP), and (4) arrhythmia occurrence. FPD measurements were rate corrected using the Fridericia correction (FPDcF); subsequently, the percent change between the drug-treated and baseline condition was calculated for each well.

Statistical Analysis. Data are presented as mean ± SEM. For the β-arrestin recruitment assay and $S1P_1$ receptor internalization assay, the EC_{50} value was calculated from the dose−response curve using SigmaPlot 13.0 (Systat software). Significance was determined *via* one-way ANOVA with Dunnett's test, one-way ANOVA with Fisher's LSD, repeated measures one-way ANOVA with Dunnett's test, or two-way ANOVA with Dunnett's test using GraphPad Prism 7 (GraphPad software). $p < 0.05$ was considered significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c01979.

Synthetic methods and data for intermediates **1**, **2**, **3**, **4**, **5**, **6**, **8**, **12**, **13**, **16**, **17**, **18**, **19**, **20**, **24**, **26**, **27**, **28**, **29**, **30**, **31**, **32**, **33**, and **34**; additional details of the methods used; and ^1H and ^{13}C NMR spectra, HPLC analysis, and HR-MS data of final compounds **6**, **7**, **9**, **10**, **11**, **14**, **15**, **20**, **21**, **22**, **23**, and **25** (PDF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CFA, complete Freund's adjuvant; CHO, Chinese hamster ovary; DMEM, Dulbecco's modified Eagle medium; EAE, experimental autoimmune encephalitis; EDG1, endothelial differentiation gene 1; GPCR, G-protein coupled receptor; HEK, human embryonic kidney; MOG, myelin oligodendrocyte glycoprotein; MPLC, medium-pressure liquid chromatography; MS, multiple sclerosis; PLC, peripheral lymphocyte count; PTX, pertussis toxin; RRMS, relapsing−remitting multiple sclerosis; S1P, sphingosine-1-phosphate; $S1P_1$, sphingosine-1-phosphate-1 receptor; $S1P_3$, sphingosine-1-phosphate-3 receptor; $S1P_4$, sphingosine-1-phosphate-4 receptor; $S1P_5$, sphingosine-1-phosphate-5 receptor

■ REFERENCES

(1) Wu, L.; Li, N.; Zhang, M.; Xue, S.-L.; Cassady, K.; Lin, Q.; Riggs, A. D.; Zeng, D. MHC-mismatched Mixed Chimerism Augments Thymic Regulatory T-cell Production and Prevents Relapse of EAE in Mice. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 15994.

(2) O'Gorman, C.; Lucas, R.; Taylor, B. Environmental Risk Factors for Multiple Sclerosis: A Review with a Focus on Molecular Mechanisms. *Int. J. Mol. Sci.* **2012**, *13*, 11718−11752.

(3) Ghasemi, N.; Razavi, S.; Nikzad, E. Multiple Sclerosis: Pathogenesis, Symptoms, Diagnoses and Cell-Based Therapy. *Cell J.* **2017**, *19*, 1−10.

(4) Fletcher, J. M.; Lalor, S. J.; Sweeney, C. M.; Tubridy, N.; Mills, K. H. G. T cells in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis. *Clin. Exp. Immunol.* **2010**, *162*, 1−11.

(5) Obinata, H.; Hla, T. Sphingosine 1-Phosphate in Coagulation and Inflammation. *Semin. Immunopathol.* **2012**, *34*, 73−91.

(6) Sykes, D. A.; Riddy, D. M.; Stamp, C.; Bradley, M. E.; McGuiness, N.; Sattikar, A.; Guerini, D.; Rodrigues, I.; Glaenzel, A.; Dowling, M. R.; Mullershausen, F.; Charlton, S. J. Investigating the Molecular Mechanisms through which FTY720-P Causes Persistent S1P(1) Receptor Internalization. *Br. J. Pharmacol.* **2014**, *171*, 4797−4807.

(7) Cyster, J. G.; Schwab, S. R. Sphingosine-1-Phosphate and Lymphocyte Egress from Lymphoid Organs. *Annu. Rev. Immunol.* **2012**, *30*, 69−94.

(8) Pappu, R.; Schwab, S. R.; Cornelissen, I.; Pereira, J. P.; Regard, J. B.; Xu, Y.; Camerer, E.; Zheng, Y.-W.; Huang, Y.; Cyster, J. G.; Coughlin, S. R. Promotion of Lymphocyte Egress into Blood and Lymph by Distinct Sources of Sphingosine-1-Phosphate. *Science* **2007**, *316*, 295−298.

(9) Kunkel, G. T.; Maceyka, M.; Milstien, S.; Spiegel, S. Targeting the Sphingosine-1-Phosphate Axis in Cancer, Inflammation and Beyond. *Nat. Rev. Drug Discovery* **2013**, *12*, 688−702.

(10) Dhar, T. G. M.; Xiao, H.-Y.; Xie, J.; Lehman-McKeeman, L. D.; Wu, D.-R.; Dabros, M.; Yang, X.; Taylor, T. L.; Zhou, X. D.; Heimrich, E. M.; Thomas, R.; McIntyre, K. W.; Warrack, B.; Shi, H.; Levesque, P. C.; Zhu, J. L.; Hennan, J.; Balimane, P.; Yang, Z.; Marino, A. M.; Cornelius, G.; D'Arienzo, C. J.; Mathur, A.; Shen, D. R.; Cvijic, M. E.; Salter-Cid, L.; Barrish, J. C.; Carter, P. H.; Dyckman, A. J. Identification and Preclinical Pharmacology of BMS-986104: A Differentiated S1P(1) Receptor Modulator in Clinical Trials. *ACS Med. Chem. Lett.* **2016**, *7*, 283−288.

(11) Brinkmann, V.; Davis, M. D.; Heise, C. E.; Albert, R.; Cottens, S.; Hof, R.; Bruns, C.; Prieschl, E.; Baumruker, T.; Hiestand, P.; Foster, C. A.; Zollinger, M.; Lynch, K. R. The Immune Modulator FTY720 Targets Sphingosine 1-Phosphate Receptors. *J. Biol. Chem.* **2002**, *277*, 21453−21457.

(12) Matloubian, M.; Lo, C. G.; Cinamon, G.; Lesneski, M. J.; Xu, Y.; Brinkmann, V.; Allende, M. L.; Proia, R. L.; Cyster, J. G. Lymphocyte Egress from Thymus and Peripheral Lymphoid Organs is Dependent on S1P Receptor 1. *Nature* **2004**, *427*, 355.

(13) Camm, J.; Hla, T.; Bakshi, R.; Brinkmann, V. Cardiac and Vascular Effects of Fingolimod: Mechanistic Basis and Clinical Implications. *Am. Heart J.* **2014**, *168*, 632−644.

(14) Kappos, L.; Antel, J.; Comi, G.; Montalban, X.; Radue, E. W.; de Vera, A.; Pohlmann, H.; O'Connor, P. Oral Fingolimod (FTY720) in Relapsing Multiple Sclerosis: 24-month Results of the Phase II Study. *Mult. Scler.* **2006**, *12*, S101.

(15) Forrest, M.; Sun, S.-Y.; Hajdu, R.; Bergstrom, J.; Card, D.; Doherty, G.; Hale, J.; Keohane, C.; Meyers, C.; Milligan, J.; Mills, S.; Nomura, N.; Rosen, H.; Rosenbach, M.; Shei, G.-J.; Singer, I. I.; Tian, M.; West, S.; White, V.; Xie, J.; Proia, R. L.; Mandala, S. Immune Cell Regulation and Cardiovascular Effects of Sphingosine 1-Phosphate Receptor Agonists in Rodents are Mediated via Distinct Receptor Subtypes. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 758−768.

(16) Sanna, M. G.; Liao, J.; Jo, E.; Alfonso, C.; Ahn, M.-Y.; Peterson, M. S.; Webb, B.; Lefebvre, S.; Chun, J.; Gray, N.; Rosen, H. Sphingosine 1-Phosphate (S1P) Receptor Subtypes S1P1 and S1P3, respectively, Regulate Lymphocyte Recirculation and Heart Rate. *J. Biol. Chem.* **2004**, *279*, 13839−13848.

(17) Gergely, P.; Nuesslein-Hildesheim, B.; Guerini, D.; Brinkmann, V.; Traebert, M.; Bruns, C.; Pan, S.; Gray, N.; Hinterding, K.; Cooke, N.; Groenewegen, A.; Vitaliti, A.; Sing, T.; Luttringer, O.; Yang, J.; Gardin, A.; Wang, N.; Crumb, W.; Saltzman, M.; Rosenberg, M.; Wallström, E. The Selective Sphingosine 1-Phosphate Receptor Modulator BAF312 Redirects Lymphocyte Distribution and Has Species-specific Effects on Heart Rate. *Br. J. Pharmacol.* **2012**, *167*, 1035−1047.

(18) Gilmore, J. L.; Xiao, H.-Y.; Dhar, T. G. M.; Yang, M. G.; Xiao, Z.; Xie, J.; Lehman-McKeeman, L. D.; Gong, L.; Sun, H.; Lecureux, L.; Chen, C.; Wu, D.-R.; Dabros, M.; Yang, X.; Taylor, T. L.; Zhou, X. D.; Heimrich, E. M.; Thomas, R.; McIntyre, K. W.; Borowski, V.; Warrack, B. M.; Li, Y.; Shi, H.; Levesque, P. C.; Yang, Z.; Marino, A.

M.; Cornelius, G.; D'Arienzo, C. J.; Mathur, A.; Rampulla, R.; Gupta, A.; Pragalathan, B.; Shen, D. R.; Cvijic, M. E.; Salter-Cid, L. M.; Carter, P. H.; Dyckman, A. J. Identification and Preclinical Pharmacology of ((1R,3S)-1-Amino-3-((S)-6-(2-methoxyphenethyl)-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopentyl)methanol (BMS-986166): A Differentiated Sphingosine-1-phosphate Receptor 1 (S1P1) Modulator Advanced into Clinical Trials. *J. Med. Chem.* **2019**, *62*, 2265−2285.

(19) Xiao, Z.; Yang, M. G.; Dhar, T. G. M.; Xiao, H.-Y.; Gilmore, J. L.; Marcoux, D.; McIntyre, K. W.; Taylor, T. L.; Shi, H.; Levesque, P. C.; Marino, A. M.; Cornelius, G.; Mathur, A.; Shen, D. R.; Cvijic, M. E.; Lehman-McKeeman, L. D.; Sun, H.; Xie, J. H.; Carter, P. H.; Dyckman, A. J. Aryl Ether-Derived Sphingosine-1-Phosphate Receptor (S1P1) Modulators: Optimization of the PK, PD, and Safety Profiles. *ACS Med. Chem. Lett.* **2020**, *11*, 1766−1772.

(20) Demont, E. H.; Bailey, J. M.; Bit, R. A.; Brown, J. A.; Campbell, C. A.; Deeks, N.; Dowell, S. J.; Eldred, C.; Gaskin, P.; Gray, J. R. J.; Haynes, A.; Hirst, D. J.; Holmes, D. S.; Kumar, U.; Morse, M. A.; Osborne, G. J.; Renaux, J. F.; Seal, G. A. L.; Smethurst, C. A.; Taylor, S.; Watson, R.; Willis, R.; Witherington, J. Discovery of Tetrahydropyrazolopyridine as Sphingosine 1-Phosphate Receptor 3 (S1P3)-Sparing S1P1 Agonists Active at Low Oral Doses. *J. Med. Chem.* **2016**, *59*, 1003−1020.

(21) Harrington, P. E.; Croghan, M. D.; Fotsch, C.; Frohn, M.; Lanman, B. A.; Pennington, L. D.; Pickrell, A. J.; Reed, A. B.; Sham, K. K. C.; Tasker, A.; Arnett, H. A.; Fiorino, M.; Lee, M. R.; McElvain, M.; Morrison, H. G.; Xu, H.; Xu, Y.; Zhang, X.; Wong, M.; Cee, V. J. Optimization of a Potent, Orally Active S1P1 Agonist Containing a Quinolinone Core. *ACS Med. Chem. Lett.* **2012**, *3*, 74−78.

(22) Demont, E. H.; Arpino, S.; Bit, R. A.; Campbell, C. A.; Deeks, N.; Desai, S.; Dowell, S. J.; Gaskin, P.; Gray, J. R. J.; Harrison, L. A.; Haynes, A.; Heightman, T. D.; Holmes, D. S.; Humphreys, P. G.; Kumar, U.; Morse, M. A.; Osborne, G. J.; Panchal, T.; Philpott, K. L.; Taylor, S.; Watson, R.; Willis, R.; Witherington, J. Discovery of a Brain-Penetrant S1P3-Sparing Direct Agonist of the S1P1 and S1P5 Receptors Efficacious at Low Oral Dose. *J. Med. Chem.* **2011**, *54*, 6724−6733.

(23) Scott, F. L.; Clemons, B.; Brooks, J.; Brahmachary, E.; Powell, R.; Dedman, H.; Desale, H. G.; Timony, G. A.; Martinborough, E.; Rosen, H.; Roberts, E.; Boehm, M. F.; Peach, R. J. Ozanimod (RPC1063) is a Potent Sphingosine-1-Phosphate Receptor-1 (S1P(1)) and Receptor-5 (S1P(5)) Agonist with Autoimmune Disease-Modifying Activity. *Br. J. Pharmacol.* **2016**, *173*, 1778−1792.

(24) Tran, J. Q.; Hartung, J. P.; Olson, A. D.; Mendzelevski, B.; Timony, G. A.; Boehm, M. F.; Peach, R. J.; Gujrathi, S.; Frohna, P. A. Cardiac Safety of Ozanimod, a Novel Sphingosine-1-Phosphate Receptor Modulator: Results of a Thorough QT/QTc Study. *Clin. Pharmacol. Drug Dev.* **2018**, *7*, 263−276.

(25) Lassiter, G.; Melancon, C.; Rooney, T.; Murat, A.-M.; Kaye, J. S.; Kaye, A. M.; Kaye, R. J.; Cornett, E. M.; Kaye, A. D.; Shah, R. J.; Viswanath, O.; Urits, I. Ozanimod to Treat Relapsing Forms of Multiple Sclerosis: A Comprehensive Review of Disease, Drug Efficacy and Side Effects. *Neurol. Int.* **2020**, *12*, 89−108.

(26) Dyckman, A. J. Modulators of Sphingosine-1-phosphate Pathway Biology: Recent Advances of Sphingosine-1-phosphate Receptor 1 ($S1P_1$) Agonists and Future Perspectives. *J. Med. Chem.* **2017**, *60*, 5267−5289.

(27) Lescop, C.; Müller, C.; Mathys, B.; Birker, M.; de Kanter, R.; Kohl, C.; Hess, P.; Nayler, O.; Rey, M.; Sieber, P.; Steiner, B.; Weller, T.; Bolli, M. H. Novel S1P1 receptor agonists − Part 4: Alkylaminomethyl substituted aryl head groups. *Eur. J. Med. Chem.* **2016**, *116*, 222−238.

(28) Bolli, M. H.; Lescop, C.; Birker, M.; de Kanter, R.; Hess, P.; Kohl, C.; Nayler, O.; Rey, M.; Sieber, P.; Velker, J.; Weller, T.; Steiner, B. Novel S1P1 receptor agonists − Part 5: From amino-to alkoxy-pyridines. *Eur. J. Med. Chem.* **2016**, *115*, 326−341.

(29) Bolli, M. H.; Abele, S.; Birker, M.; Bravo, R.; Bur, D.; de Kanter, R.; Kohl, C.; Grimont, J.; Hess, P.; Lescop, C.; Mathys, B.; Müller, C.; Nayler, O.; Rey, M.; Scherz, M.; Schmidt, G.; Seifert, J.; Steiner, B.; Velker, J.; Weller, T. Novel $S1P_1$ Receptor Agonists − Part 3: From Thiophenes to Pyridines. *J. Med. Chem.* **2014**, *57*, 110−130.

(30) Xiao, H.-Y.; Watterson, S. H.; Langevine, C. M.; Srivastava, A. S.; Ko, S. S.; Zhang, Y.; Cherney, R. J.; Guo, W.-W.; Gilmore, J. L.; Sheppeck, J. E.; Wu, D.-R.; Li, P.; Ramasamy, D.; Arunachalam, P.; Mathur, A.; Taylor, T. L.; Shuster, D. J.; McIntyre, K. W.; Shen, D.-R.; Yarde, M.; Cvijic, M. E.; Marino, A. M.; Balimane, P. V.; Yang, Z.; Banas, D. M.; Cornelius, G.; D'Arienzo, C. J.; Warrack, B. M.; Lehman-McKeeman, L.; Salter-Cid, L. M.; Xie, J.; Barrish, J. C.; Carter, P. H.; Dyckman, A. J.; Dhar, T. G. M. Identification of Tricyclic Agonists of Sphingosine-1-phosphate Receptor 1 ($S1P_1$) Employing Ligand-Based Drug Design. *J. Med. Chem.* **2016**, *59*, 9837−9854.

(31) Ren, F.; Deng, G.; Wang, H.; Luan, L.; Meng, Q.; Xu, Q.; Xu, H.; Xu, X.; Zhang, H.; Zhao, B.; Li, C.; Guo, T. B.; Yang, J.; Zhang, W.; Zhao, Y.; Jia, Q.; Lu, H.; Xiang, J.-N.; Elliott, J. D.; Lin, X. Discovery of Novel 1,2,4-Thiadiazole Derivatives as Potent, Orally Active Agonists of Sphingosine 1-Phosphate Receptor Subtype 1 ($S1P_1$). *J. Med. Chem.* **2012**, *55*, 4286−4296.

(32) Nakamura, T.; Asano, M.; Sekiguchi, Y.; Mizuno, Y.; Tamaki, K.; Nara, F.; Kawase, Y.; Yabe, Y.; Nakai, D.; Kamiyama, E.; Urasaki-Kaneno, Y.; Shimozato, T.; Doi-Komuro, H.; Kagari, T.; Tomisato, W.; Inoue, R.; Nagasaki, M.; Yuita, H.; Oguchi-Oshima, K.; Kaneko, R.; Nishi, T. Synthesis and evaluation of CS-2100, a potent, orally active and S1P3- sparing S1P1 agonist. *Eur. J. Med. Chem.* **2012**, *51*, 92−98.

(33) Skidmore, J.; Heer, J.; Johnson, C. N.; Norton, D.; Redshaw, S.; Sweeting, J.; Hurst, D.; Cridland, A.; Vesey, D.; Wall, I.; Ahmed, M.; Rivers, D.; Myatt, J.; Giblin, G.; Philpott, K.; Kumar, U.; Stevens, A.; Bit, R. A.; Haynes, A.; Taylor, S.; Watson, R.; Witherington, J.; Demont, E.; Heightman, T. D. Optimization of Sphingosine-1-phosphate-1 Receptor Agonists: Effects of Acidic, Basic, and Zwitterionic Chemotypes on Pharmacokinetic and Pharmacodynamic Profiles. *J. Med. Chem.* **2014**, *57*, 10424−10442.

(34) Hale, J. J.; Lynch, C. L.; Neway, W.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M. J.; Milligan, J. A.; Shei, G.-J.; Parent, S. A.; Chrebet, G.; Bergstrom, J.; Card, D.; Ferrer, M.; Hodder, P.; Strulovici, B.; Rosen, H.; Mandala, S. A Rational Utilization of High-Throughput Screening Affords Selective, Orally Bioavailable 1-Benzyl-3-carboxyazetidine Sphingosine-1-phosphate-1 Receptor Agonists. *J. Med. Chem.* **2004**, *47*, 6662−6665.

(35) Li, Z.; Chen, W.; Hale, J. J.; Lynch, C. L.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M. J.; Milligan, J. A.; Shei, G.-J.; Chrebet, G.; Parent, S. A.; Bergstrom, J.; Card, D.; Forrest, M.; Quackenbush, E. J.; Wickham, L. A.; Vargas, H.; Evans, R. M.; Rosen, H.; Mandala, S. Discovery of Potent 3,5-Diphenyl-1,2,4-oxadiazole Sphingosine-1-phosphate-1 ($S1P_1$) Receptor Agonists with Exceptional Selectivity against $S1P_2$ and $S1P_3$. *J. Med. Chem.* **2005**, *48*, 6169−6173.

(36) Zhao, X.; Jones, A.; Olson, K. R.; Peng, K.; Wehrman, T.; Park, A.; Mallari, R.; Nebalasca, D.; Young, S. W.; Xiao, S.-H. A Homogeneous Enzyme Fragment Complementation-Based Beta-Arrestin Translocation Assay for High-Throughput Screening of G-Protein-Coupled Receptors. *J. Biomol. Screen* **2008**, *13*, 737−747.

(37) Hammer, M. M.; Wehrman, T. S.; Blau, H. M. A Novel Enzyme Complementation-Based Assay for Monitoring G-Protein-Coupled Receptor Internalization. *FASEB J.* **2007**, *21*, 3827−3834.

(38) Choi, J. W.; Kim, S.; Park, J.-H.; Kim, H. J.; Shin, S. J.; Kim, J. W.; Woo, S. Y.; Lee, C.; Han, S. M.; Lee, J.; Pae, A. N.; Han, G.; Park, K. D. Optimization of Vinyl Sulfone Derivatives as Potent Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) Activators for Parkinson's Disease Therapy. *J. Med. Chem.* **2019**, *62*, 811−830.

(39) Millard, D.; Dang, Q.; Shi, H.; Zhang, X.; Strock, C.; Kraushaar, U.; Zeng, H.; Levesque, P.; Lu, H.-R.; Guillon, J.-M.; Wu, J. C.; Li, Y.; Luerman, G.; Anson, B.; Guo, L.; Clements, M.; Abassi, Y. A.; Ross, J.; Pierson, J.; Gintant, G. Cross-Site Reliability of Human Induced Pluripotent stem cell-derived Cardiomyocyte Based Safety Assays Using Microelectrode Arrays: Results from a Blinded CiPA Pilot Study. *Toxicol. Sci.* **2018**, *164*, 550−562.

https://doi.org/10.1021/acs.jmedchem.1c01979
J. Med. Chem. XXXX, XXX, XXX−XXX

(40) Gintant, G.; Kaushik, E. P.; Feaster, T.; Stoelzle-Feix, S.; Kanda, Y.; Osada, T.; Smith, G.; Czysz, K.; Kettenhofen, R.; Lu, H. R.; Cai, B.; Shi, H.; Herron, T. J.; Dang, Q.; Burton, F.; Pang, L.; Traebert, M.; Abassi, Y.; Pierson, J. B.; Blinova, K. Repolarization Studies using Human Stem Cell-derived Cardiomyocytes: Validation studies and best practice recommendations. *Regul. Toxicol. Pharmacol.* **2020**, *117*, 104756.
(41) Marsolais, D.; Rosen, H. Chemical Modulators of Sphingosine-1-Phosphate Receptors as Barrier-Oriented Therapeutic Molecules. *Nat. Rev. Drug Discovery* **2009**, *8*, 297−307.
(42) Constantinescu, C. S.; Farooqi, N.; O'Brien, K.; Gran, B. Experimental Autoimmune Encephalomyelitis (EAE) as a Model for Multiple Sclerosis (MS). *Br. J. Pharmacol.* **2011**, *164*, 1079−1106.

https://doi.org/10.1021/acs.jmedchem.1c01979
J. Med. Chem. XXXX, XXX, XXX−XXX