Journal of Medicinal Chemistry

Article

Identification of Tricyclic Agonists of Sphingosine-1-Phosphate Receptor 1 (S1P1) Employing Ligand-Based Drug Design

Hai-Yun Xiao, Scott H. Watterson, Charles M. Langevine, Anurag S. Srivastava, Soo S Ko, Yanlei Zhang, Robert Joseph Cherney, Weiwei Guo, John L Gilmore, James E Sheppeck, Dauh-Rurng Wu, Peng Li, Duraisamy Ramasamy, Pirama Nayagam Arunachalam, Arvind Mathur, Tracy L. Taylor, David J. Shuster, Kim W. McIntyre, Ding Ren Shen, Melissa Yarde, Mary Ellen Cvijic, Anthony M. Marino, Praveen V. Balimane, Zheng Yang, Dana M. Banas, Georgia Cornelius, Celia J. D´Arienzo, Bethanne M. Warrack, Lois D. Lehman-McKeeman, Luisa M. Salter-Cid, Jenny H. Xie, Joel C. Barrish, Percy H Carter, Alaric J. Dyckman, and T. G. Murali Dhar

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.6b01099 • Publication Date (Web): 11 Oct 2016

Just Accepted

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

	Lehman-McKeeman, Lois; Bristol-Myers Squibb Company, Investigative Toxicology (MS H24-03) Salter-Cid, Luisa; Bristol-Myers Squibb Pharmaceutical Research and Development, Discovery Biology Xie, Jenny; Bristol-Myers Squibb Pharmaceutical Research and Development, Discovery Biology Barrish, Joel; Achillion Pharmaceuticals Inc Carter, Percy; BMS, R&D Dyckman, Alaric; Bristol-Myers Squibb, Discovery Chemistry Dhar, T. G. Murali; Bristol-Myers Squibb, Discovery Chemistry

Identification of Tricyclic Agonists of Sphingosine-1-Phosphate Receptor 1 ($S1P_1$) Employing Ligand-Based Drug Design

*Hai-Yun Xiao[‡], Scott H. Watterson[‡], Charles M. Langevine[‡], Anurag S. Srivastava[‡], Soo S. Ko[‡], Yanlei Zhang[‡], Robert J. Cherney[‡], Wei-Wei Guo[‡], John L. Gilmore[‡], James E. Sheppeck II[‡], Dauh-Rurng Wu[‡], Peng Li[‡], Duraisamy Ramasamy[#], Piramanayagam Arunachalam[#], Arvind Mathur[‡], Tracy L. Taylor[‡], David J. Shuster[‡], Kim W. McIntyre[‡], Ding-Ren Shen[‡], Melissa Yarde[‡], Mary Ellen Cvijic[‡], Anthony M. Marino[‡], Praveen V. Balimane[‡], Zheng Yang[‡], Dana M. Banas[‡], Georgia Cornelius[‡], Celia J. Darienzo[‡], Bethanne M. Warrack[‡], Lois Lehman-McKeeman[‡], Luisa M. Salter-Cid[‡], Jenny Xie[‡], Joel C. Barrish[‡], Percy H. Carter[‡], Alaric J. Dyckman[‡] and T. G. Murali Dhar[‡]**

‡ Research and Development, Bristol-Myers Squibb Company, P.O. Box 4000, Princeton, New Jersey 08543-4000, United States

BMS Biocon Research Center, Bangalore, India

RECEIVED DATE

ABSTRACT: Fingolimod (**1**) is the first approved oral therapy for the treatment of relapsing remitting multiple sclerosis. While the phosphorylated metabolite of fingolimod was found to be a non-selective S1P receptor agonist, agonism specifically of $S1P_1$ is responsible for the peripheral blood lymphopenia believed to be key to its efficacy. Identification of modulators that maintain activity on $S1P_1$ while sparing activity on other S1P receptors could offer equivalent efficacy with reduced liabilities. We disclose in this paper a ligand based drug design approach that led to the discovery of a series of potent

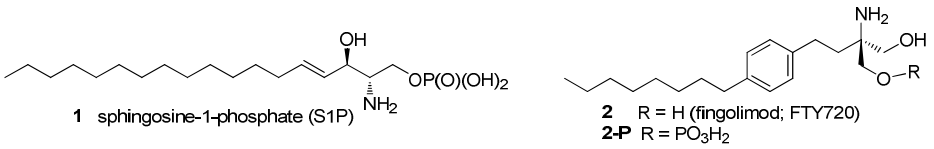
tricyclic agonists of $S1P_1$ with selectivity over $S1P_3$ and were efficacious in a pharmacodynamic model of suppression of circulating lymphocytes. Compound **10** had the desired pharmacokinetic (PK) and pharmacodynamic (PD) profile and demonstrated maximal efficacy when administered orally in a rat adjuvant arthritis model.

INTRODUCTION

The G-protein coupled receptor (GPCR) $S1P_1$ (sphingosine-1-phosphate-subtype 1) has emerged as an attractive drug target in recent years. Activation of $S1P_1$ by synthetic agonists, but not by activation with the endogenous ligand S1P, leads to sustained internalization and degradation of cell surface receptors.[1] This functional antagonism of the receptor results in immunosuppression through disruption of normal lymphocyte trafficking, with lymphocytes sequestered in lymph nodes and secondary lymphoid organs.[2] Extensive validation of this mechanism has been demonstrated across a range of pre-clinical models of chronic autoimmune disease.[3] The success of the non-selective S1P receptor agonist fingolimod (FTY720, **2**, Figure 1), approved by the FDA in 2010 for the treatment of relapsing remitting multiple sclerosis (RRMS), has spurred interest in the clinical evaluation of it and additional $S1P_1$ agonists for a growing list of disorders (Crohn's disease, ulcerative colitis, psoriasis, polymyositis, progressive MS, lupus).[4] Fingolimod is a pro-drug, requiring in vivo phosphorylation to form FTY720-P (**2-P**, Figure 1) which is a potent agonist of 4 of the 5 S1P receptors ($S1P_{1,3,4,5}$).[2] In addition to the pro-drug class of $S1P_1$ agonists, exemplified by fingolimod, direct-acting agonists that replace the amino-phosphonate group with moieties that mimic its interactions and thus do not require bioactivation have been reported by a number of research teams and are in clinical development.[5-13] The focus of research programs following fingolimod has primarily been aimed at identifying agonists with improved S1P family selectivity, specifically to avoid interaction with $S1P_3$, which was believed to be associated with clinical side effects such as the transient reduction in heart rate (bradycardia). In rodent studies, fingolimod was unable to elicit heart rate reductions in $S1P_3$ knock-out mice, whereas it did

induce bradycardia in wild type controls.[14] Furthermore, synthetic $S1P_1$-selective agonists that lacked activity on $S1P_3$ did not induce bradycardia in rodents. Surprisingly, in human trials multiple $S1P_3$-sparing $S1P_1$ agonists have now been shown to offer no substantial advantage over fingolimod in regards to bradycardia.[15] In humans, as opposed to non-clinical species, regulation of heart rate appears to have a significant $S1P_1$ related component.[15] Nevertheless, as $S1P_3$ does not contribute to the desired efficacy, a drive for more selective $S1P_1$ agonists has remained a viable approach for research programs, including our own.

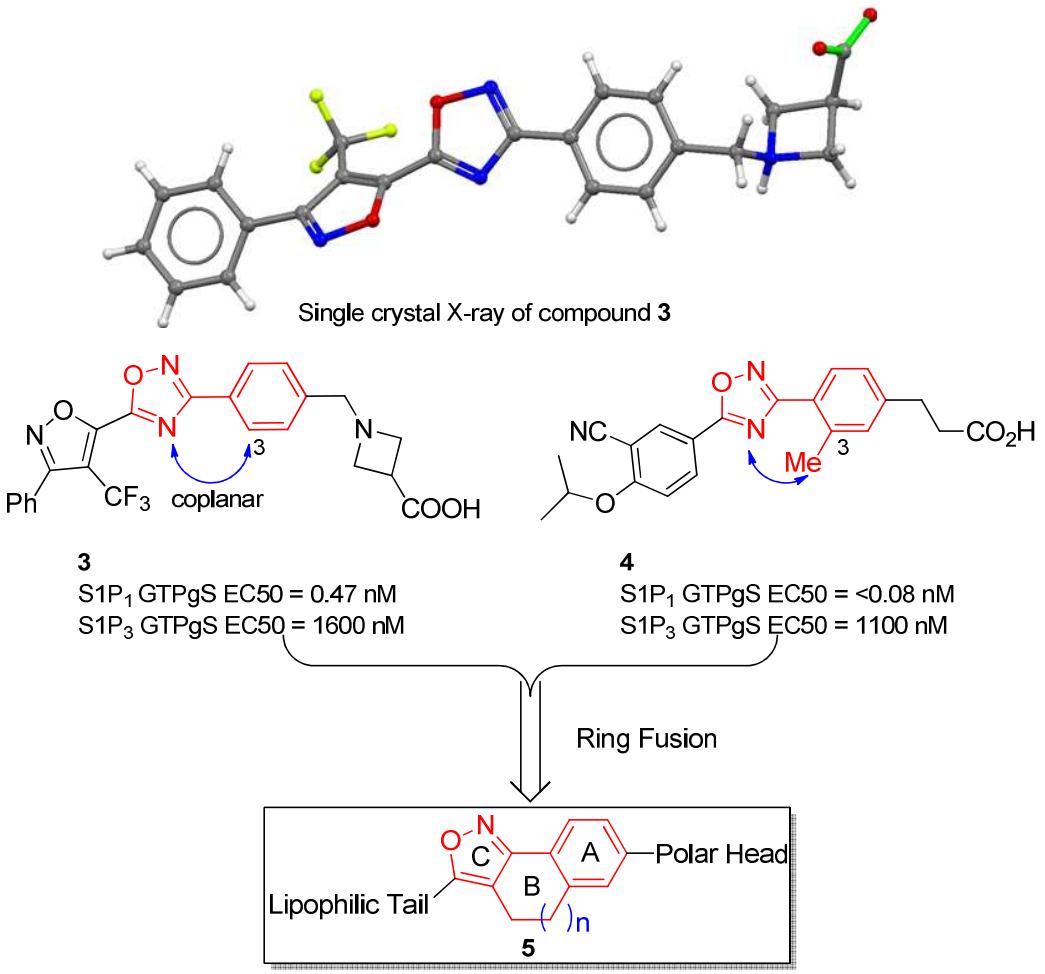
Figure 1. Structure of S1P, fingolimod (FTY720), and its phosphate



We recently disclosed the identification of compound **3** as a potent functional antagonist of $S1P_1$ with selectivity over $S1P_3$ (Figure 2).[16] The single crystal X-ray structure of **3** indicated that the phenyl ring and the 1,2,4-oxadiazole ring are coplanar in orientation (Figure 2). This suggested that ring fusion of the oxadiazole ring with the 3-position of the phenyl ring would maintain planarity of the scaffold thus leading to a novel series of tricyclic $S1P_1$ receptor modulators. However, in the absence of X-ray co-crystal structures of $S1P_1$ with functional antagonists, it was not clear if substituents at the 3-position of the phenyl ring would be tolerated by the receptor. Yan et al.[17] disclosed a series $S1P_1$ receptor modulators with a methyl substituent at the 3-position of the phenyl ring (**4**, Figure 2). These compounds were potent functional antagonists at $S1P_1$ with excellent selectivity over $S1P_3$, suggesting that substituents at the 3-postion were tolerated by the receptor. These data gave us the impetus to follow-up on our ligand based drug design hypothesis for the generation of novel $S1P_1$ modulators (Figure 2). As discussed below, this novel scaffold (**5**) allowed us to explore the structure-activity

relationship (SAR) of the polar head piece, phenyl ring (A), central ring (B), heterocyclic ring (C) and the lipophilic tail piece as outlined in Fig. 2.

Figure 2. Design of tricyclic modulators of $S1P_1$

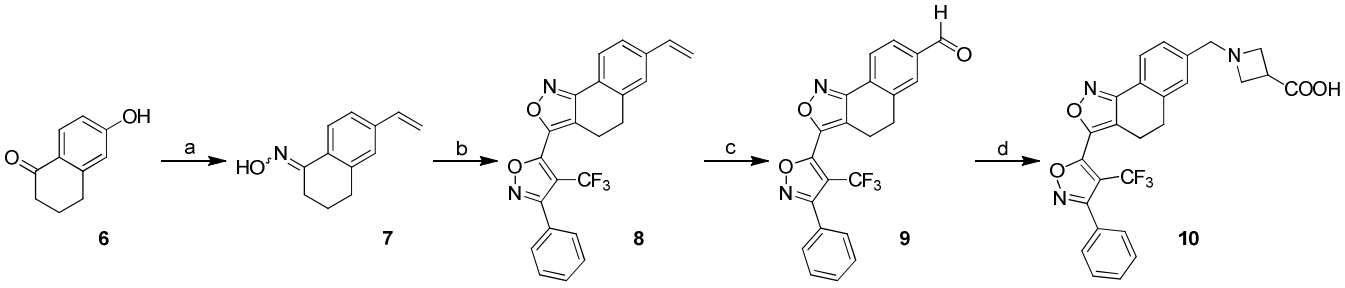


CHEMISTRY

Schemes 1-4 outline the synthesis of representative examples of compounds with variations to the phenyl ring, central ring, heterocyclic ring, the lipophilic tail piece and the polar head piece.[18] The key step in the synthesis of the bis-isoxazole **10** (Scheme 1) is the condensation of dilithiated 1-tetralone oxime with methyl 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylate.[19] For optimal yields, LiTMP was the base of choice to affect this condensation - LDA or *n*-BuLi gave poor yields. In the case of the tricyclic chroman (**24**), the tetralone oxime route led to intractable mixtures of products during the

condensation step. An alternative approach employing oxyamination of a β−diketone with hydroxylamine via an eneamine led to the desired product, albeit in low yield (Scheme 2, see transformation **18** to **19**).[20] The key step in the synthesis of the regioisomeric isoxazole tailpiece (**36**) was an intramolecular 1,3-dipolar cycloaddition of a nitrile oxide which was generated in situ from the oxime precursor **32** (Scheme 3).[21] The synthesis of the thiazole (**47**, Scheme 4) was accomplished in good yield via condensation of the acylamino ketone (**41**) with Lawesson's reagent.[22]

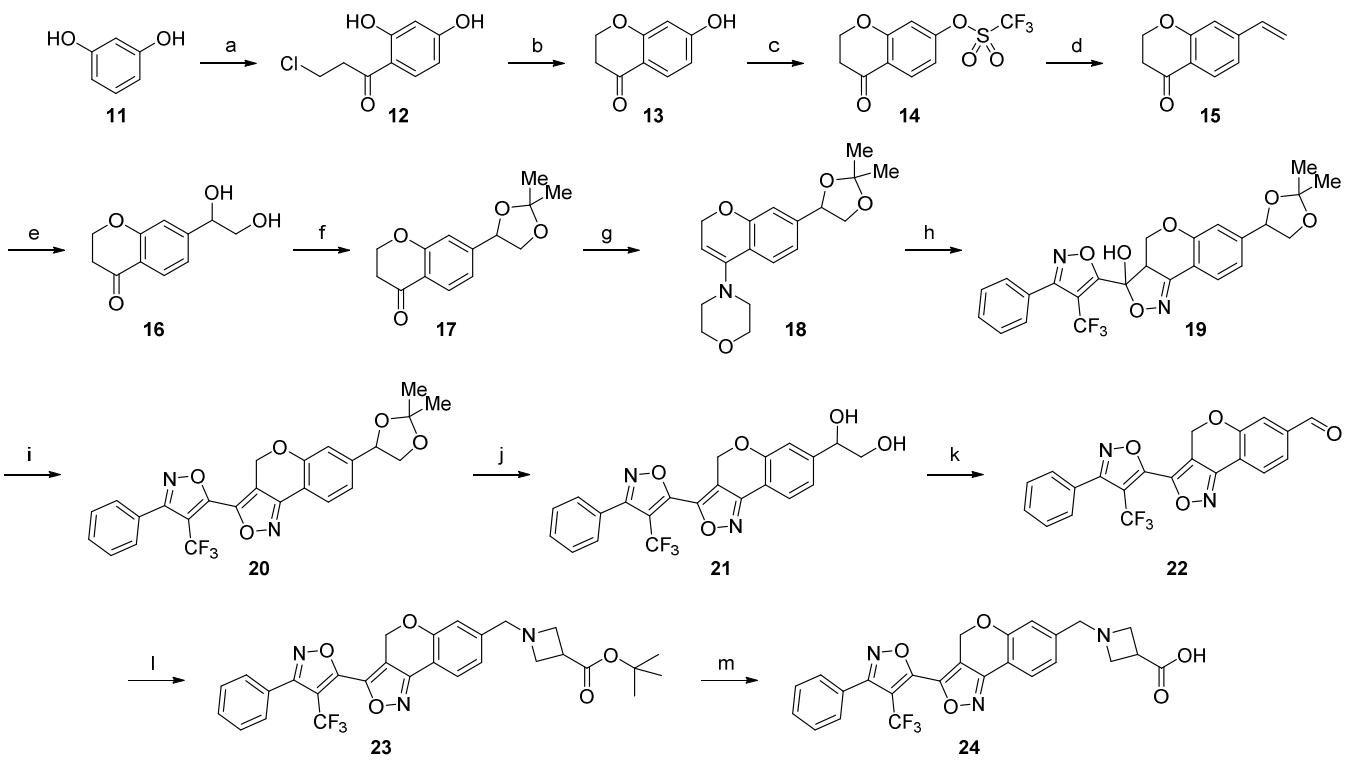
Scheme 1[a]. Synthesis of **10**



6 7 8 9 10

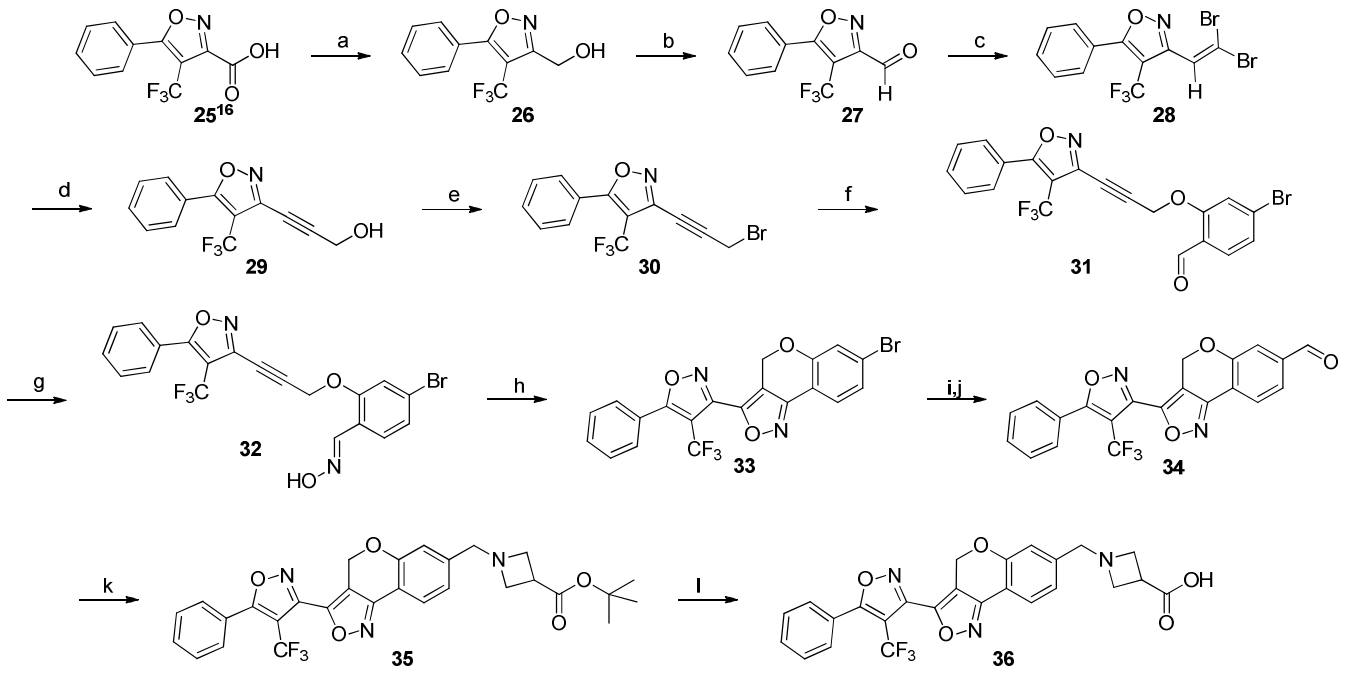
[a] Reagents and conditions: (a) (1) Tf_2O, Py, 0 °C to RT, 4 h, 94%; (2) tributyl(vinyl)stannane, LiCl, $Pd(PPh_3)_4$, dioxane, 100 °C, 14 h, 80%; (3) $NH_2OH.HCl$, NaOAc, MeOH, 80 °C, 1.5 h, 70%; (b) (1) TMS-Cl, DIPEA, THF, 0 °C; (2) LiTMP, THF, methyl 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylate[16], -78 °C to 0 °C; (3) $SOCl_2$, Py, PhMe, RT to 90 °C, 25% for 3 steps; (c) OsO_4, NMO, THF, overnight then $NaIO_4$, H_2O, 30 min, 100%; (d) (1) tert-butyl azetidine-3-carboxylate, $NaBH(OAc)_3$, $Ti(OiPr)_4$, MeOH, DCE, 2 h, 79%; (2) TFA, 90%

Scheme 2[a]. Synthesis of **24**



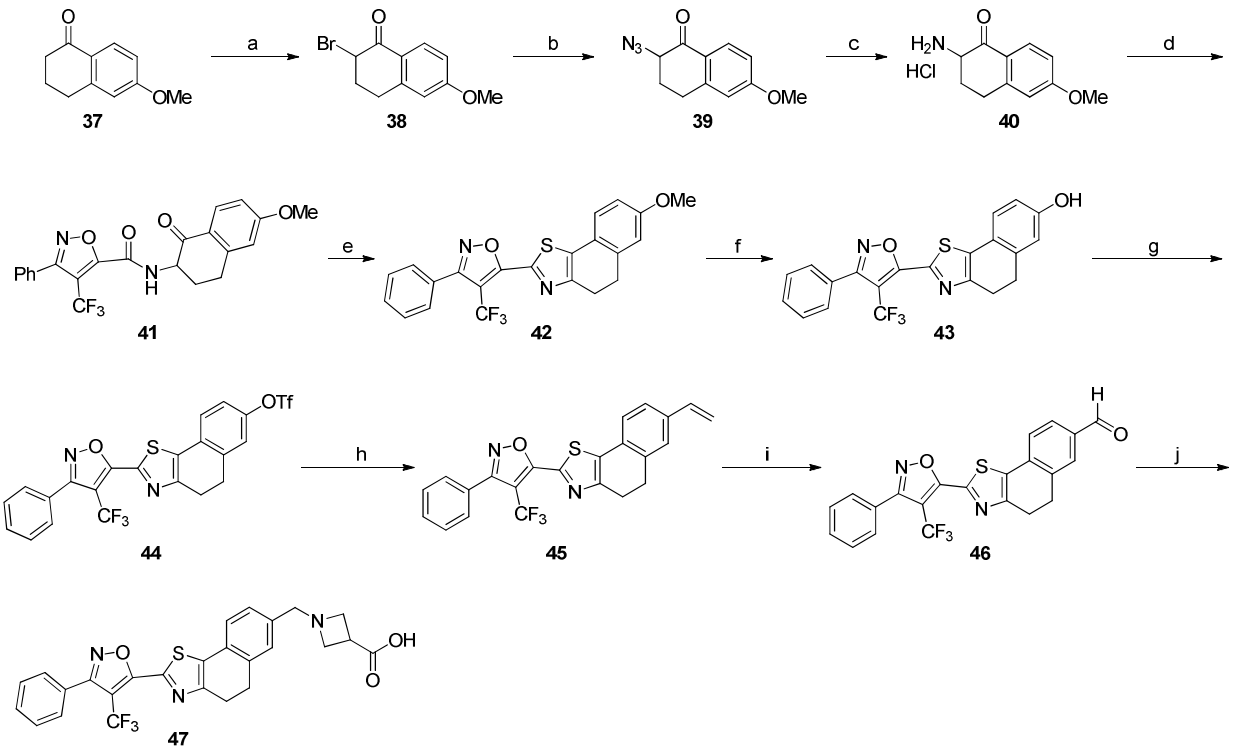
[a] Reagents and conditions: (a) 3-chloropropanoic acid, CF_3-SO_3H, 80 °C, 75%; (b) 2M aq. NaOH, 5 °C-RT; 6M aq. H_2SO_4, 81%; (c) $(CF_3$-$SO_2)_2O$, pyridine, 86%; (d) tributyl(vinyl)stannane, lithium chloride, $Pd(PhP)_4$, dioxane, 100 °C, 80%; (e) OsO_4, NMO, THF, 99%; (f) dimethoxypropane, (1R)-(-)-camphorsulfonic acid, 58%; (g) $TiCl_4$, morpholine, toluene, 89%; (h) 3-phenyl-4-(trifluoromethyl)isoxazole-5-carbonyl fluoride[16], Et_3N, CH_3CN: NH_2OH-HCl, NaOAc, H_2O, 45 °C, 17%; (i) $SOCl_2$, pyridine, toluene, 62%; (j) TFA, 100%; (k) $NaIO_4$, THF, H_2O; (l) tert-Butyl azetidine-3-carboxylate, acetic acid salt, AcOH, titantium(IV) isopropoxide, MeOH, CH_2Cl_2, 64%; (m) TFA; MeOH slurry crystallization, 99%.

Scheme 3[a]. Synthesis of **36**



[a] Reagents and conditions: (a) i-BuOCOCl, N-methylmorpholine, THF; $NaBH_4$, THF-MeOH, 63%; (b) Dess-Martin reagent, CH_2Cl_2, 99%%; (c) CBr_4, PPh_3, THF, 67%; (d) n-BuLi,THF; paraformaldehyde, 63%; (e) PBr_3, CH_2Cl_2, 40-60%; (f) 4-bromo-2-hydroxybenzaldehyde, K_2CO_3, DMF, >85%; (g) NH_2OH-HCl, Et_3N, MeOH, 97%; (h) NaOCl, Et_3N, CH_2Cl_2, 73%; (i) tributyl(vinyl)stannane, lithium chloride, $(PhP)_4Pd$, dioxane, 100 °C, 67%; (j) OsO_4, NMO, THF; $NaIO_4$, THF, H_2O, 95%; (k) tert-Butyl azetidine-3-carboxylate, acetic acid salt, AcOH, titantium(IV) isopropoxide, MeOH, CH_2Cl_2, 58%; (l) TFA; neutralization; MeOH; slurry crystallization, 65%.

Scheme 4[a]. Synthesis of isomeric tricyclic $S1P_1$ modulator **47**



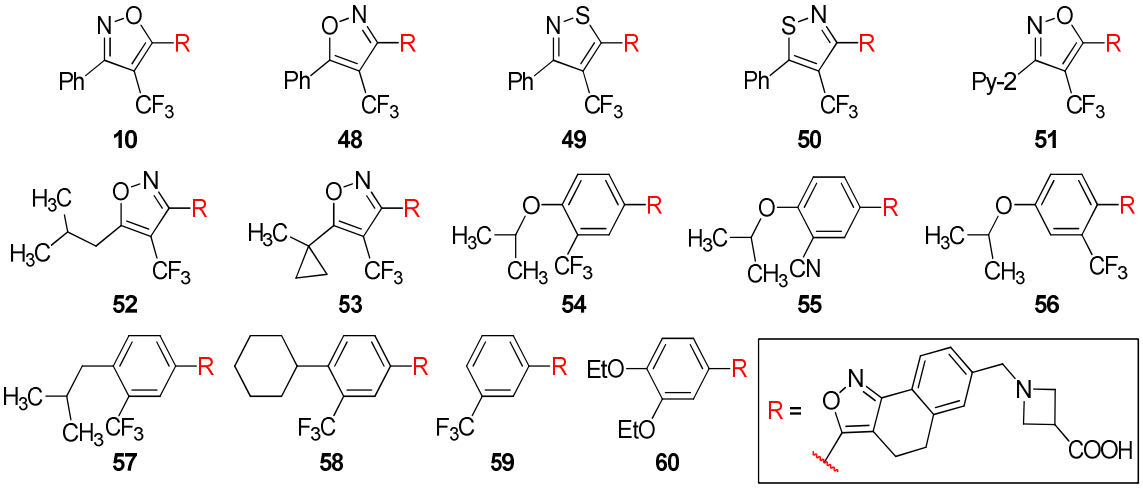
[a] Reagents and conditions: (a) NBS, pTsOH, 60 °C, 11 min; (b) NaN_3, acetone, water, 3h rt, 69% over two steps; (c) Pd/C, 2N HCl, MeOH, 50 psi H_2, rt 4h, 74%; (d) (1) 3-Phenyl-4-trifluoromethyl)isoxazole-5-carboxylic acid[16], oxalyl chloride, DMF, DCM, 0 °C; (2) DMAP, Hunig's base, DCM, rt overnight, 40%; (e) Lawesson's reagent, THF, 30 min, 120 °C μ-wave, 60%; (f) BBr_3, DCM, 6h, rt, 100%; (g) Triflic anhydride, pyridine, 0 °C, 89%; (h) Tributyl(vinyl)stannane, LiCl, $Pd(PPh_3)_4$, dioxane, 100 °C, overnight; (i) OsO_4, NMO, THF, 3h, rt then $NaIO_4$, H_2O, 90 min, 47% over two steps; (j) (1) Azetidine-3-carboxylic acid, AcOH, MeOH, 1,2-dichloroethane, 1h, 80 °C; (2) $NaCNBH_3$, rt, 1h, 25%

RESULTS AND DISCUSSION

All compounds were evaluated in a functional GTPγS assay for both human $S1P_1$ and $S1P_3$ receptor agonism. Compounds with good $S1P_1$ potency, and selectivity over $S1P_3$, and acceptable profiles in liability assays (metabolic stability, CYP inhibition, etc.) were initially evaluated in a rat pharmacokinetic/ pharmacodynamic (PK/ PD) model where rats were dosed orally with the compound. Blood was drawn at the 4 and 24 hour time points to measure both circulating lymphocytes relative to control and plasma concentration of each compound. Compounds which showed reduction in circulating lymphocytes at low doses were then evaluated in PK studies and then progressed to chronic

efficacy models such as the rat adjuvant arthritis model. The following tables summarize SAR related to the lipophilic tail piece (Table 1), central ring B (Table 2), heterocyclic ring C (Table 3), phenyl ring A (Table 4) as well as the PK/PD properties of these compounds.

Table 1. Lipophilic tail piece SAR.

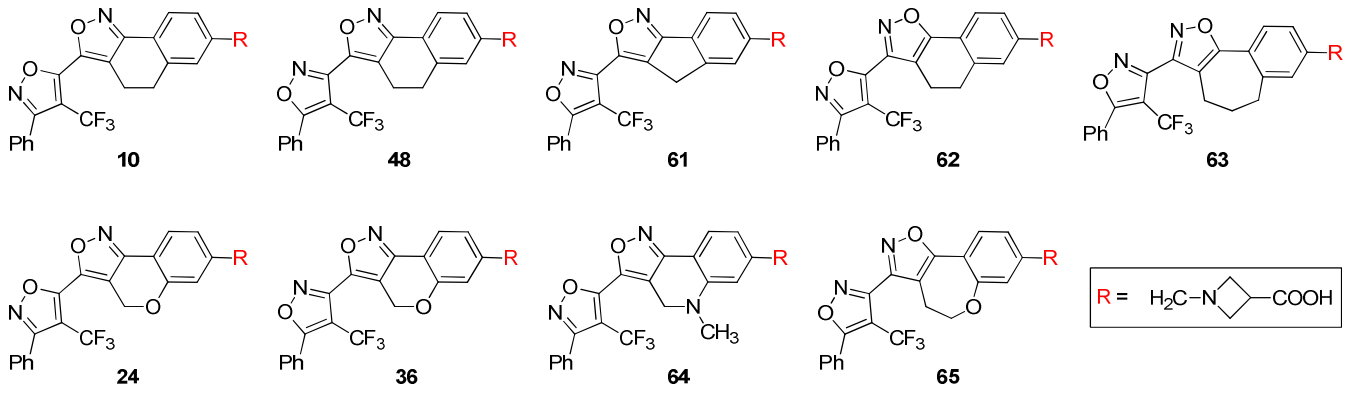


	In Vitro Activity			Rat Blood Lymphocyte Reduction Assay				
Cmpd	*h*S1P$_1$ GTPγS EC$_{50}$ nM[a]	*h*S1P$_3$ GTPγS EC$_{50}$ nM[a]	S1P$_3$/ S1P$_1$ Sel.	Reduction at 4 h (1.0 mpk)	Plasma Conc.at 4h (nM)	Reduction at 24 h (1.0 mpk)	Plasma Conc. at 24h (nM)	Conc. 4h/24h ratio
3	0.47±0.25	1,600±320	3,400x	78%	2,100	91%	700	3
10	0.59±0.19	4,400±330	7,500x	86% 83% (0.3)	120 55	85% 80% (0.3)	27 13	4 4
48	0.48±0.27	1,900 (n=2)	4,000x	83%	160	87%	43	4
49	0.58±0.16	4,600±2,900	7,900x	74%	190	69%	32	6
50	0.33 (n=2)	1,400±870	4,200x	82% (0.4)	150	85% (0.4)	26	6
51	1.0±0.86	1,300[b]	1,300x	82% (0.5)	120	69% (0.5)	1	120
52	1.5[b]	>3,100[b]	>2,100x	86%	520	80%	61	9
53	1.3[b]	1,300[b]	1,000x	85%	190	34%	4	48
54	1.5±1.5	26,000[b]	17,000x	57%	28	21%	5	6
55	4.6±2.6	>63,000±25,000	>14,000x	-5%	<LLQ	12%	<LLQ	
56	24[b]	11,000[b]	460x	ND	ND	ND	ND	
57	1.3±1.0	2,800[b]	2,200x	74%	110	76%	50	2
58	0.98±0.29	2,100 (n=2)	2,100x	35% (0.5)	68	32% (0.5)	17	4
59	310±32	>63,000±30,000	>200x	ND	ND	ND	ND	
60	1.6±1.4	14,000[b]	8,800x	ND	ND	ND	ND	

[a] EC$_{50}$ values are shown as mean values of at least three determinations; [b] EC$_{50}$ values are shown as a single determination; ND = Not Determined

As expected, based on our earlier effort[16] lipophilic groups are well tolerated in this region of the receptor leading to potent functional antagonists of $S1P_1$. Within this group however, both isomeric isoxazoles, (**10** and **48**) and isothiazoles (**49** and **50**) exhibited picomolar potencies for $S1P_1$ in the GTPγS assay with good selectivity against $S1P_3$. It is also interesting to note the SAR trends in the 5-membered heteroaryl vs. the phenyl analogs – for example, a CF_3 group on the 5-membered heteroaryl ring, which is adjacent to the carbon atom anchoring the tricyclic group (R, in Table 1), led to very potent functional antagonism at $S1P_1$. In the case of the phenyl analogs, however, ortho substitution of the CF_3 group to the R group led to a relative loss in potency at $S1P_1$ in the functional assay (compare compound **52**, **57**, and **54** with **56**). Potency is regained when the CF_3 group is moved to the meta position in relation to the R group (compare **56** and **54**). It is also important to note that lipophilic moieties at the para position of the phenyl ring are critical in imparting functional potency at $S1P_1$ – the data for **59** is consistent with this SAR. Although subtle SAR trends described above are difficult to explain from a functional potency perspective, it is quite possible that optimal occupancy of the lipophilic pocket and the dihedral angle between the R group and the lipophilic tail piece may be important factors contributing to the modest potency differences observed in this region of the molecule. Since a number of compounds in Table 1 showed excellent functional potency for $S1P_1$ while maintaining selectivity for $S1P_3$, they were advanced to the rat blood lymphocyte reduction (BLR) pharmacokinetic/ pharmacodynamic (PK/PD) model. Compounds with the isoxazole and isothiazole tail pieces showed sustained PD effects at 24 hours at low plasma concentrations – see for example, data for **50**, **48** and **10** in Table 1. Since we had a good understanding of the liability profile of the isoxazoles compared to the isothiazoles based on our earlier work,[16] the isoxazoles (**48** and **10**) were determined to be the optimized lipophilic tail pieces for exploring SAR of the rest of the tricyclic scaffold.

Table 2. SAR of the central ring, B.



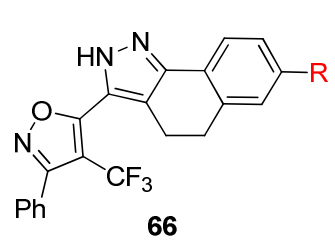
Cmpd	*In Vitro* Activity: *h*S1P$_1$ GTPγS EC$_{50}$ nM[a]	*In Vitro* Activity: *h*S1P$_3$ GTPγS EC$_{50}$ nM[a]	*In Vitro* Activity: S1P$_3$/S1P$_1$ Sel.	Rat Blood Lymphocyte Reduction Assay: Reduction at 4 h (1.0 mpk)	Rat Blood Lymphocyte Reduction Assay: Plasma Conc. at 4h (nM)	Rat Blood Lymphocyte Reduction Assay: Reduction at 24 h (1.0 mpk)	Rat Blood Lymphocyte Reduction Assay: Plasma Conc. at 24h (nM)	4h/24h ratio
10	0.59±0.19	4,400±330	7,500x	83% (0.3)	55	80% (0.3)	13	4
61	6.8±1.4	32,000[b]	4,700x	14%	21	3%	<LLQ	
62	1.3±0.37	4,400[b]	3,400x	81% 66% (0.3)	200 34	85% 79% (0.3)	46 8	4 4
63	31±23	12,000 (n=2)	390x	ND	ND	ND	ND	
24	0.25 (n=2)	>31,000±15,000	>120,000x	82% (0.5)	330	89% (0.5)	56	6
36	0.27±0.18	1,200±1,300	4400x	87% (0.5)	230	93% (0.5)	46	5
64	1.6±0.32	>63,000±31,000	>39,000x	ND	ND	ND	ND	
65	33[b]	1,600[b]	48x	ND	ND	ND	ND	

[a] EC$_{50}$ values are shown as mean values of at least three determinations; [b] EC$_{50}$ values are shown as a single determination; ND = Not Determined

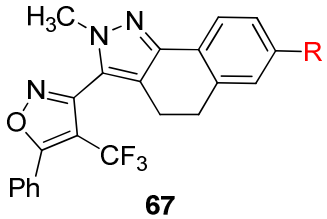
Table 2 summarizes SAR of the central ring B. Of the carbocyclic analogs examined, the six membered ring (**10, 48**) was optimal – the five membered analog (**61**) was approximately an order of magnitude less potent in the S1P$_1$ GTPγS assay. Comparing the six and seven membered analogs (**62** vs. **63**), the six membered analog was significantly more potent. This data was also consistent with the data for the seven membered oxepane analog **65**. The significant loss in potency for the seven membered analogs compared to the five and six membered compounds supports our initial hypothesis about the importance of maintaining planarity between the phenyl group (which anchors the polar

headpiece) and the central heterocyclic ring for optimal potency and selectivity for the $S1P_1$ receptor. Of the heterocyclic rings examined, the pyran analogs (**24** and **36**) were equipotent to the carbocyclic analogs (**10** and **48**) in the $S1P_1$ GTPγS assay. Since both **24** and **36** were about equipotent in the $S1P_1$ GTPγS assay, we tested them in the rat BLR assay for further progression. As outlined in Table 2, both analogs were efficacious in reducing lymphocyte count at very low doses and these reductions were similar to what was observed with the carbocyclic analog **10**. We decided to use the carbocyclic analog **10** for further analoging, since the synthetic feasibility of exploring SAR appeared to be easier with this compound compared to pyrans **24** and **36**.

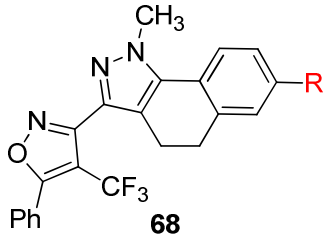
Table 3. SAR of the heterocyclic ring, C.



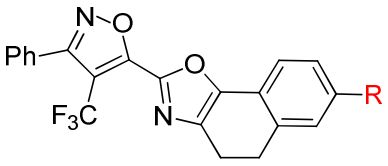
66



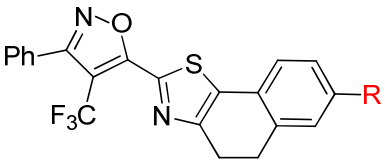
67



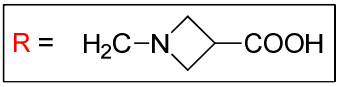
68



69



47



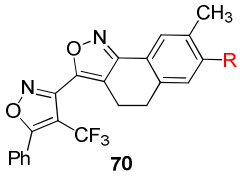
	In Vitro Activity			Rat Blood Lymphocyte Reduction Assay				
Cmpd	*h*$S1P_1$ GTPγS EC_{50} nM[a]	*h*$S1P_3$ GTPγS EC_{50} nM[a]	$S1P_3$/ $S1P_1$ Sel.	Reduction at 4 h (1.0 mpk)	Plasma Conc. at 4h (nM)	Reduction at 24 h (1.0 mpk)	Plasma Conc. at 24h (nM)	4h/24h ratio
10	0.59±0.19	4,400±330	7,500x	83% (0.3)	55	80% (0.3)	13	4
66	190±23	>63,000±30,000	>330x	ND	ND	ND	ND	
67	380±150	>63,000±30,000	>170x	ND	ND	ND	ND	
68	21±16	>63,000±35,000	>3,000x	ND	ND	ND	ND	
69	5.5±3.6	920[b]	170x	62%	110	2%	11	10
47	2.4 (n=2)	12,000[b]	5,000x	80%	230	85%	84	3

[a] EC_{50} values are shown as mean values of at least three determinations; [b] EC_{50} values are shown as a single determination; ND = Not Determined

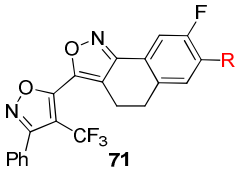
Table 3 summarizes the SAR of the heterocyclic ring C, (Fig. 1). It is clear from Table 3 that of the various heterocyclic rings examined, the [1,2-*c*]isoxazole (**10**) is the most potent in the $S1P_1$ GTPγS assay. It is of interest to note that the isomeric [2,1-*d*]oxazole (**69**) is an order of magnitude less potent vs. $S1P_1$ in the GTPγS assay compared to compound **10**. Additionally, in the rat BLR PK/PD assay compound **69** was significantly less efficacious in reducing lymphocyte counts through 24 hours compared to the isoxazole (**10**). On the contrary, the thiazole analog **47,** which is similar in potency to **69** in the $S1P_1$ GTPγS assay, was highly efficacious in reducing lymphocyte counts in the rat BLR assay. This is likely due to the higher plasma concentration of **47** compared to **69** at the 24 hour time point.

Although we did not do exhaustive SAR studies on the aryl ring (A, in Fig. 2), the limited SAR work suggested that the phenyl analog (**10**) provided the best balance of $S1P_1$ potency vs. selectivity for $S1P_3$ in the GTPγS assay (Table 4).

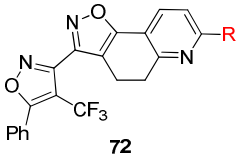
Table 4. SAR of Ring A.



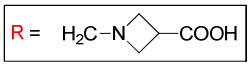
70



71



72

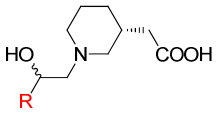


	In Vitro Activity			Rat Blood Lymphocyte Reduction Assay				
Cmpd	*h*$S1P_1$ GTPγS EC_{50} nM[a]	*h*$S1P_3$ GTPγS EC_{50} nM[a]	$S1P_3$/ $S1P_1$ Sel.	Reduction at 4 h (1.0 mpk)	Plasma Conc. at 4h (nM)	Reduction at 24 h (1.0 mpk)	Plasma Conc. at 24h (nM)	4h/24h ratio
10	0.59±0.19	4,400±330	7,500x	83% (0.3)	55	80% (0.3)	13	4
70	0.87[b]	1,200[b]	1,400x	85%	190	34%	4	48
71	1.6±0.32	15,000[b]	9,400x	74% (0.5)	320	79% (0.5)	46	7
72	17[b]	>63,000 (n=2)	>3,700x	76%	460	32%	47	10

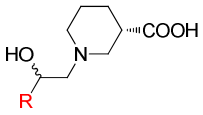
[a] EC_{50} values are shown as mean values of at least three determinations; [b] EC_{50} values are shown as a single determination; ND = Not Determined

Since we have shown that the azetidine carboxylic acid head piece can be replaced by an ethanolamine group,[23] we decided to explore this SAR along with other polar moieties in the tricylic chemotype (Table 5).

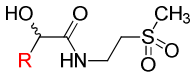
Table 5. SAR of polar head piece.



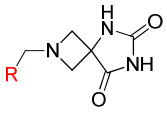
73 and **74**



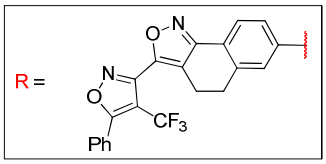
75 and **76**



77 and **78**



79



	In Vitro Activity			Rat Blood Lymphocyte Reduction Assay				
Cmpd	*h*S1P$_1$ GTPγS EC$_{50}$ nM[a]	*h*S1P$_3$ GTPγS EC$_{50}$ nM[a]	S1P$_3$/ S1P$_1$ Sel.	Reduction at 4 h (1.0 mpk)	Plasma Conc. at 4h (nM)	Reduction at 24 h (1.0 mpk)	Plasma Conc. at 24h (nM)	4h/24h ratio
10	0.59±0.19	4,400±330	7,500x	83% (0.3)	55	80% (0.3)	13	4
73	4.0±1.9	7,400[b]	1,900x	70%	190	72%	160	1
74	0.33±0.20	1,700 (n=2)	5,200x	81% 70% (0.3)	280 93	87% 78% (0.3)	77 32	4 3
75	0.21±0.045	13,000 (n=2)	62,000x	76%	140	76%	49	3
76	26±7.8	16,000[b]	620x	28%	140	20%	21	7
77	200±89	18,000[b]	90x	ND	ND	ND	ND	
78	1.6±0.58	6,200[b]	3,900x	50 (0.5)	190	43 (0.5)	26	
79	51±9.0	23,000[b]	450x	ND	ND	ND	ND	

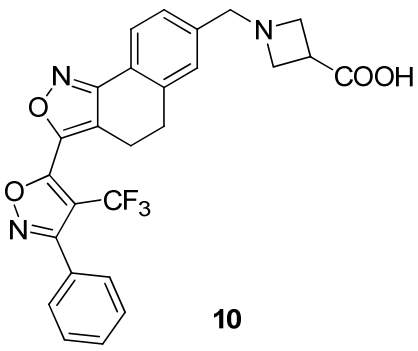
[a] EC$_{50}$ values are shown as mean values of at least three determinations; [b] EC$_{50}$ values are shown as a single determination; ND = Not Determined

As is evident from Table 5, ethanolamines such as **74** and **75** and α-hydroxy amides such as **78** showed potencies comparable to compound **10** in the S1P$_1$ GTPγS assay while showing good selectivity for S1P$_3$. It is interesting to note that chirality at the carbon anchoring the hydroxy group is important in determining potency and selectivity for S1P$_1$ in both the ethanolamine and the α-hydroxy amide series (the absolute configuration of the isomers **73-78** was not established in this series).

It is clear from the discussion above, that a number of compounds show excellent lymphopenia at low doses in the rat BLR assay. However, compound **10** remained one of the lead compounds in

terms of showing a sustained PD response at very low doses (Figure 3) with an ED_{50} of 0.05 mg/kg (EC_{50} = 2.5 ± 0.8 nM) in addition to displaying an excellent liability profile (Table 6). Moreover, the PK profile of the compound across species (Table 7) was very promising (low clearance, moderate to high bioavailability and volumes of distribution) and the compound was advanced to the rat adjuvant arthritis (AA) model, a preclinical model of rheumatoid arthritis. The rationale for testing compound **10** in a rat AA model is as follows. While the current market approval for Gilenya, an $S1P_1$ agonist, is for RRMS, $S1P_1$ agonists have been or are currently being clinically evaluated for a wide range of autoimmune disorders (psoriasis, ulcerative colitis, Crohn's disease, organ transplant, lupus). At the time of these investigations, arthritis was of primary interest for this program.

Figure 3. Rat lymphocyte reduction of compound **10**



10

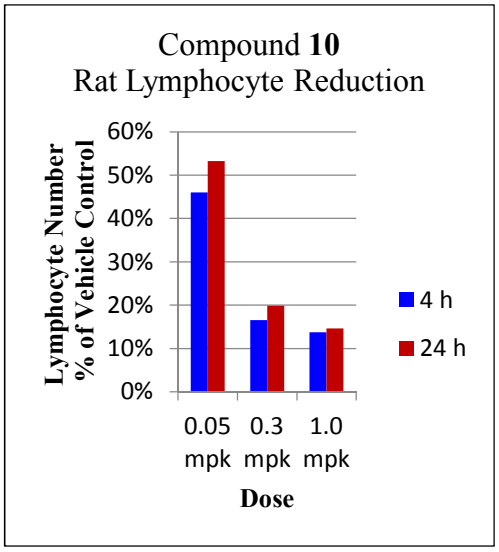


Table 6. Partial *in vitro* profiling data for compound **10**

Parameter	Result
Human $S1P_1$ binding (IC_{50})	0.38 nM
Human $S1P_1$ GTPγS EC_{50} (Ymax)	0.59 nM (95%)
Human $S1P_1$ cAMP EC_{50} (Ymax)	0.032 nM (93%)
Human $S1P_3/S1P_1$ selectivity	7,500
Human $S1P_4/S1P_1$ selectivity	2.4x
Human $S1P_5/S1P_1$ selectivity	2.4x
Rat $S1P_1$ GTPγS (EC_{50})	0.31 nM
Rat $S1P_3/S1P_1$ selectivity	6100x
Protein Binding (bound)	99.6% human 99.3% mouse 99.6% rat 99.5% dog 99.5% monkey
hERG (Patch Clamp)	IC_{50} = 2 μM
Na^+ (Patch Clamp)	12% @ 10 μM (1 and 4

Ca^{+} (Patch Clamp)	Hz)
PXR TransAct, EC_{50}	11% @ 10 μM
	>50 μM
CYP[a] inhibition (IC_{50})	>40 μM 1A2, 2B6, 2C9,
	2C19, 2D6, 3A4-BZR
	= 14 μM 2C8
PAMPA permeability	280 nm/s @ pH 7.7
	260 nm/s @ pH 5.5
Met Stab, $T_{1/2}$	>120 min (h, m, r, d, mk)

[a] CYP = cytochrome P450

Figure 4. Rat adjuvant arthritis study of compound **10**

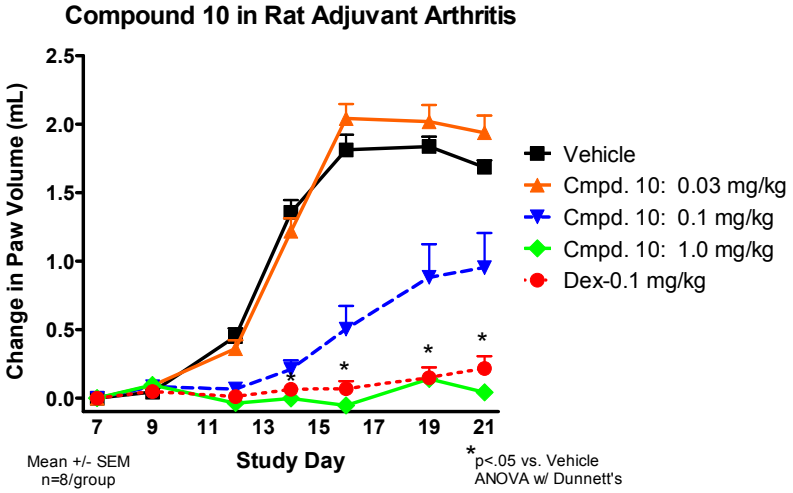


Table 7. Pharmacokinetic parameters for compound **10**

Parameter	Mouse[a]	Rat[a]	Cyno[a,b]	Dog[a]
po dose (mg/kg)	5	1	0.5	0.1
iv dose (mg/kg)	2	1	0.5	0.1
C_{max} (μM)	3.6	0.14	0.41	0.09
T_{max} (h)	3	4	4	8
AUC (μM*h) PO	30.3	1.5	3.4	2.3
$T_{1/2}$ (h)	15	7.9	18	23.1
CL (mL/min/kg)	1.7	8.5	2.4	1.4
V_{ss} (L/kg)	2.2	4.8	3.6	2.5
F_{po} (%)	80	43	80	95

[a] Average of three animals; [b] cynomologus monkey.

Arthritis was induced in male Lewis rats by a subcutaneous injection of complete Freund's adjuvant at the base of the tail. Compound **10** was dosed orally (PO), once a day (QD), for 21 days at 0.03, 0.1 and 1.0 mg/kg. Dexamethasone served as the positive control in the study and was dosed at 0.1 mg/kg PO/QD. Paw volumes (day 7 to day 21) were measured using water displacement

plethysmometry. As is evident from Figure 4, a dose of 0.03 mg/kg had little impact on disease development. However, a dose of 0.1 mg/kg provided >50% suppression of paw swelling, and the higher dose of 1mg/kg resulted in robust inhibition equivalent to the positive control dexamethasone. On the basis of the suppression of paw swelling observed in this study, the ED_{50} for compound **10** was determined to be 0.09 mg/kg (EC_{50} = 24 nM) which is comparable to other $S1P_1$ modulators disclosed in the literature.[16,23]

CONCLUSION

In summary, a series of tricyclic $S1P_1$ receptor agonists with selectivity over $S1P_3$ have been identified employing a ligand-based drug design approach. SAR in this series suggested that (a) a number of lipophilic tail pieces are tolerated (b) the azetidine carboxylic acid (**10**) and the hydroxy nipecotic acid (**74** and **75**) are optimal head pieces (c) six membered carbocylic and pyran rings are optimal central rings and (d) [1,2-*c*]isoxazole is the preferred heteroaromatic ring. Based on the extensive SAR carried out, compound **10** was identified as having the optimal properties in terms of potency for $S1P_1$, selectivity vs. $S1P_3$ with an excellent liability profile. This compound showed sustained pharmacodynamic effects in a rat blood lymphocyte reduction assay and efficacy in a rat adjuvant arthritis model, with an ED_{50} comparable to reported $S1P_1$ modulators. Based upon this favorable profile, compound **10** was further evaluated for suitability as a clinical development candidate.

Experimental

All commercially available chemicals and solvents were used without further purification. Reactions were performed under an atmosphere of nitrogen. All new compounds gave satisfactory ^{1}H NMR, LC/MS and/or HRMS, and mass spectrometry results. ^{1}H NMR spectra were obtained on a Bruker 400 MHz or a JEOL 500 MHz NMR spectrometer using the residual TMS signal of deuterated NMR solvent as internal reference. Electrospray ionization (ESI) mass spectra were obtained on a Waters ZQ single

quadrupole mass spectrometer. All MS measurements were made on open access systems. The purity of tested compounds determined by analytical HPLC was >95%. HPLC Methods are listed below:

Method A: A linear gradient using 5% acetonitrile, 95% water, and 0.05% TFA (Solvent A) and 95% acetonitrile, 5% water, and 0.05% TFA (Solvent B); *t* = 0 min., 10% B, *t* = 15 min., 100% B (20 min.) was employed on a SunFire C18 3.5u 3.0 x 150 mm column. Flow rate was 1.0 ml/min and UV detection was set to 220/254 nm. The LC column was maintained at ambient temperature.

Method B: A linear gradient using 5% acetonitrile, 95% water, and 0.05% TFA (Solvent A) and 95% acetonitrile, 5% water, and 0.05% TFA (Solvent B); *t* = 0 min., 10% B, *t* = 15 min., 100% B (20 min.) was employed on a XBridge Ph 3.5u 3.0 x 150 mm column. Flow rate was 1.0 ml/min and UV detection was set to 220/254 nm. The LC column was maintained at ambient temperature.

Method C. A linear gradient using 10% methanol, 90% water, and 0.2% H_3PO_4 (Solvent A) and 90% methanol, 10% water, and 0.2% H_3PO_4 (Solvent B); *t* = 0 min., 0% B, *t* = 4 min., 100% B (5 min.) was employed on a YMC S5 CombiScreen 4.6 x 50 mm column. Flow rate was 4.0 ml/min and UV detection was set to 220 or 254 nm. The LC column was maintained at ambient temperature.

Method D. A linear gradient using 10% methanol, 90% water, and 0.2% H_3PO_4 (Solvent A) and 90% methanol, 10% water, and 0.2% H_3PO_4 (Solvent B); *t* = 0 min., 0% B, *t* = 4 min., 100% B (5 min.) was employed on a Chromolith SpeedROD 4.6 x 50 mm column. Flow rate was 4.0 ml/min and UV detection was set to 220 nm. The LC column was maintained at ambient temperature.

6-Vinyl-3,4-dihydronaphthalen-1(2H)-one oxime (7). To a solution of 6-hydroxy-3,4-dihydronaphthalen-1(2H)-one (2 g, 12.33 mmol) in anhydrous pyridine (10 mL) was added trifluoromethanesulfonic anhydride (2.5 mL, 14.80 mmol) at 0 °C over a period of 5 min. The reaction mixture was allowed to come to room temperature and stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure. The resulting brownish red residue was partitioned between ether (60 mL) and water (30 mL). The ether layer was sequentially washed with 1N

hydrochloric acid (20 mL), sat. aq. $NaHCO_3$ (20 mL), brine (20 mL), dried over sodium sulfate, concentrated, and purified by silica gel column chromatography using hexane/ethyl acetate to yield 5-oxo-5,6,7,8-tetrahydronaphthalen-2-yl trifluoromethanesulfonate (3.4 g, 11.55 mmol, 94 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.14 (1 H, d, *J*=8.4 Hz), 7.17-7.24 (2 H, m), 3.02 (2 H, t, *J*=6.1 Hz), 2.69 (2 H, t, *J*=6.5 Hz), 2.15-2.22 (2 H, m).

To a solution of 5-oxo-5,6,7,8-tetrahydronaphthalen-2-yl-trifluoromethanesulfonate (3.4 g, 11.55 mmol) in anhydrous dioxane was sequentially added tributyl(vinyl)stannane (3.73 mL, 12.71 mmol), lithium chloride (1.470 g, 34.7 mmol), and tetrakis(triphenylphosphine)palladium(0) (1.335 g, 1.155 mmol). The reaction mixture was purged with nitrogen gas for 5 min. and heated at 100 °C for 14 h. The reaction mixture was cooled to room temperature and filtered. The yellow residue was washed with ethyl acetate (3 x 20 ml). The filtrate was concentrated under reduced pressure. To the red oil was added ether (50 mL) and the contents were stirred at room temperature for 15 min. The contents were filtered and the resulting brick red residue was washed with ether (3 x 20 mL). The filtrate was concentrated under reduced pressure and the resulting red oil was purified by silica gel column chromatography using hexane/ethyl acetate to yield 6-vinyl-3,4-dihydronaphthalen-1(2H)-one (1.6 g, 9.29 mmol, 80 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.00 (d, *J*=8.14 Hz, 1 H), 7.36 (dd, *J*=8.14, 1.10 Hz, 1 H), 7.26 (s, 1 H), 6.72 (dd, *J*=17.61, 11.00 Hz, 1 H), 5.87 (dd, *J*=17.61, 0.66 Hz, 1 H), 5.37-5.41 (m, 1 H), 2.96 (t, *J*=6.05 Hz, 2 H), 2.63-2.67 (m, 2 H), 2.10-2.17 (m, 2 H).

To 6-vinyl-3,4-dihydronaphthalen-1(2H)-one (1.6 g, 9.29 mmol) in methanol (10 mL) was sequentially added hydroxylamine hydrochloride (0.775 g, 11.15 mmol) and sodium acetate (0.915 g, 11.15 mmol). The reaction mixture was heated at 80 °C (oil bath temp.) for 1.5 h. The reaction mixture was concentrated under reduced pressure and to the residue was added water (30 mL). The contents were triturated and filtered. The solid material was washed with water (2 x 20 mL) and dried overnight to yield 6-vinyl-3,4-dihydronaphthalen-1(2H)-one oxime (1.2 g, 6.41 mmol, 69.0 % yield). ^{1}H NMR

(400 MHz, $CDCl_3$) δ ppm 8.23 (br. s., 1 H), 7.85 (d, *J*=8.14 Hz, 1 H), 7.28 (s, 1 H), 7.18 (s, 1 H), 6.69 (dd, *J*=17.61, 10.78 Hz, 1 H), 5.78 (dd, *J*=17.61, 0.88 Hz, 1 H), 5.23-5.32 (m, 1 H), 2.71-2.87 (m, 4 H), 1.81-1.94 (m, 2 H).

3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-7-vinyl-4,5-dihydronaphtho[1,2-c]isoxazole (**8**). Preparation of LiTMP: To a stirred solution of 2,2,6,6-tetramethylpiperidine (10.88 mL, 64.5 mmol) in anhydrous THF (25 mL) was added n-BuLi (25.8 mL, 64.5 mmol) (2.5M in hexanes) dropwise at 0 °C under nitrogen. The pale yellow solution was then stirred at the same temperature for 20 min.

To a stirred solution of 6-vinyl-3,4-dihydronaphthalen-1(2H)-one oxime (**7**, 4.03 g, 21.50 mmol) and diisopropylethylamine (6.83 mL, 39.1 mmol) in anhydrous THF (50 mL) was added TMS-Cl (2.75 mL, 21.50 mmol) dropwise at 0 °C under nitrogen. The mixture was stirred at 0 °C for 30 min. The previously prepared LiTMP solution was added dropwise at -78 °C under nitrogen. After the mixture was stirred at the same temperature for 30 min, a solution of methyl 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylate[16] (5.3 g, 19.54 mmol) in anhydrous THF (10 mL) was added dropwise at -78 °C. The solution was stirred at -78 °C for 30 min and then warmed to 0 °C over 30 min. The reaction was quenched with saturated aqueous ammonium chloride solution (25 mL) and water (20 mL). EtOAc (50 mL) was added, contents stirred at room temperature for 30 min and filtered through a pad of celite. The filtrate was separated. The aqueous layer was extracted with EtOAc (2 x 25 mL). The combined organic solutions were washed with brine (50 mL), dried over sodium sulfate and concentrated to give a liquid. Flash chromatography purification (330g silica gel column, 15-30% ethyl acetate in hexanes) afforded 3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-7-vinyl-3,3a,4,5-tetrahydronaphtho[1,2-c]isoxazol-3-ol (5 g, 11.73 mmol, 60.0 % yield). The compound had an HPLC retention time = 3.74 min (condition C); LCMS (ESI) *m/z* Calcd for $C_{23}H_{18}F_3N_2O_3$ $[M + H]^+$ 427.13. Found: 426.9.

The 3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-7-vinyl-3,3a,4,5-tetrahydronaphtho[1,2-c]isoxazol-3-ol (5 g, 11.73 mmol) was mixed with thionyl chloride (1.712 mL, 23.45 mmol) and anhydrous toluene (80 mL). Pyridine (0.190 mL, 2.345 mmol) was then added dropwise. The mixture was stirred under nitrogen at room temperature for 30 min and 90 °C for 15 min. The mixture was concentrated under reduced pressure. The residue was mixed with saturated aqueous sodium bicarbonate solution (50 mL) and extracted with dichloromethane (100 mL, 2 x 50 mL). The combined dichloromethane extracts were dried over sodium sulfate, filtered through a pad of silica gel and concentrated under reduced pressure. Flash chromatography purification (220g silica gel column, 25->60% dichloromethane in hexanes) and trituration with methanol gave 3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-7-vinyl-4,5-dihydronaphtho[1,2-c]isoxazole (1.96 g, 4.80 mmol, 40.9 % yield). The compound had an HPLC retention time = 4.28 min (condition C); LCMS (ESI) *m/z* Calcd for $C_{23}H_{16}F_3N_2O_2$ $[M + H]^+$ 409.12. Found: 408.9.

3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho [1,2-c]isoxazole-7-carbaldehyde (9). To a stirred mixture of 3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-7-vinyl-4,5-dihydronaphtho[1,2-c]isoxazole (1.96 g, 4.80 mmol) and THF (20 mL) were added NMO (50% in water, 1.493 mL, 7.20 mmol) and osmium tetroxide (4% in water, 1.173 mL, 0.192 mmol) at room temperature. The mixture was vigorously stirred at room temperature overnight. A solution of sodium periodate (1.540 g, 7.20 mmol) in water (10 mL) was added. The mixture was stirred at room temperature under nitrogen for 30 min. Water (20 mL) was then added. The solid was filtered, washed with water (2 x 5 mL) and ethanol (2 mL) and dried to give 3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[1,2-c]isoxazole-7-carbaldehyde (1.92 g, 4.68 mmol, 98%). The compound had an HPLC retention time = 3.95 min (condition C); LCMS (ESI) *m/z* Calcd for $C_{22}H_{14}F_3N_2O_3$ $[M + H]^+$ 411.10. Found: 410.9.

1-((3-(3-Phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (10). To a stirred solution of 3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[1,2-c]isoxazole-7-carbaldehyde (2.1 g, 5.12 mmol), tert-butyl azetidine-3-carboxylate, AcOH (1.668 g, 7.68 mmol), and acetic acid (0.586 mL, 10.24 mmol) in anhydrous MeOH (10 mL) and anhydrous 1,2-dichloroethane (30 mL) was added titanium(IV) isopropoxide (3.05 mL, 10.24 mmol) dropwise at room temperature under nitrogen. The solution was stirred at room temperature for 1 hr before sodium triacetoxyborohydride (4.34 g, 20.47 mmol) was added in 10 portions over 3.5 hr. The mixture was stirred at room temperature for 2 hr. Saturated sodium bicarbonate (70 mL) was added slowly to make the reaction mixture basic. Celite was added, and the contents were stirred at room temperature for 30 min and then filtered through a pad of celite. The aqueous filtrate was separated and extracted with dichloromethane (3 x 20 mL). The combined organic solutions were dried (sodium sulfate) and concentrated. Flash chromatography purification (120g silica gel column, 25->70% ethyl acetate in hexanes) afforded tert-butyl 1-((3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl) azetidine-3-carboxylate (2.24 g, 4.06 mmol, 79 % yield). The compound had an HPLC retention time = 3.40 min (condition C); LCMS (ESI) *m/z* Calcd for $C_{30}H_{29}F_3N_3O_4$ $[M + H]^+$ 552.21. Found: 552.1.

To a stirred solution of tert-butyl 1-((3-(3-phenyl-4-(trifluoromethyl) isoxazol-5-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylate (2.24 g, 4.06 mmol) in dichloromethane (4 mL) was added TFA (4 mL) slowly at room temperature. The reaction mixture was stirred at room temperature for 2 hr. The mixture was concentrated to remove dichloromethane and TFA (6 mL) was added. The mixture was stirred at room temperature till the starting material disappeared. Dichloroethane (6 mL) was added and the reaction mixture was concentrated under reduced pressure. The residue was mixed with water (10 mL), made basic (pH ~8) with 1 N aqueous NaOH and acidified with 1N aqueous HCl to pH ~5. The mixture was sonicated for 30 min. The solid

was filtered and washed with water (4 x 10 mL). The residue was mixed with methanol (40 mL), sonicated for 30 min, filtered, washed with methanol (3 x 10 mL), and dried to give 1-((3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl) azetidine-3-carboxylic acid. HPLC t_r = 7.62 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{26}H_{21}F_3N_3O_4$ $[M + H]^+$ 496.15. Found: 496.0; ^{1}H NMR (500 MHz, *DMSO*) δ ppm 7.85 (1 H, d, *J*=7.90 Hz), 7.64 - 7.68 (2 H, m), 7.57 - 7.65 (3 H, m), 7.37 (1 H, s), 7.33 (1 H, d), 3.60 (2 H, s), 3.42 (2 H, br. s.), 3.23 (3 H, br.s.), 3.10 (2 H, d, *J*=6.41 Hz), 3.05 (2 H, d, *J*=6.41 Hz).

3-chloro-1-(2,4-dihydroxyphenyl)propan-1-one (12). To a stirred mixture of resorcinol (10 g, 91 mmol) and 3-chloropropanoic acid (9.95 g, 92 mmol) was added trifluoromethanesulfonic acid (50 g, 333 mmol) in one portion. The solution was heated at 80 °C for 30 min, cooled to room temperature over 15 min, and poured into dichloromethane (200 mL). The resulting solution was slowly poured into water (200 mL), and the layers were separated. The aqueous layer was extracted with dichloromethane (2 x 100 mL). The combined organic solutions were dried over anhydrous sodium sulfate, and filtered. Concentration under reduced pressure afforded 3-chloro-1-(2,4-dihydroxyphenyl)propan-1-one (13.62 g, 67.9 mmol, 74.8 % yield) as an orange semi-solid. The compound was used without any further purification. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 12.39 (1H, s), 7.56 (1H, d, *J*=8.58 Hz), 7.19 (1H, s), 6.17 - 6.49 (1H, m), 5.23 (1H, s), 3.78 - 4.01 (2H, m), and 3.34 (2H, t, *J*=6.71 Hz).

7-hydroxychroman-4-one (13). To a stirred 2M solution of sodium hydroxide (500 mL, 1000 mmol) at 5 ^{o}C was added 3-chloro-1-(2,4-dihydroxyphenyl)propan-1-one (13.6 g, 67.9 mmol) in one portion. The solution was warmed to room temperature over 2h, re-cooled to 5 ^{o}C, and the pH was adjusted to ~2 with 6M aqueous sulfuric acid (~50 mL). The orange precipitate formed was collected by vacuum filtration and dried under reduced pressure. The filtrate was extracted with ethyl acetate (3 x 100 mL), washed with brine, and dried over anhydrous sodium sulfate. Concentration under reduced pressure gave an orange solid. The two recovered solids were combined to give 7-hydroxychroman-4-

one (9.0 g, 54.8 mmol, 81% yield). LCMS (ESI) *m/z* Calcd for $C_9H_8O_3$ $[M + H]^+$ 165.1. Found: 165.0. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 7.77 (1H, d, *J*=8.58 Hz), 6.45 (1H, dd, *J*=8.69, 2.31 Hz), 6.33 (1H, d, *J*=2.42 Hz), 5.62 (1H, br. s.), 4.33 - 4.66 (2H, m), and 2.58 - 2.90 (2H, m).

4-oxochroman-7-yl trifluoromethanesulfonate (14). To a solution of 7-hydroxychroman-4-one (2.024 g, 12.3 mmol) in pyridine (10 mL) at 0 °C was added trifluoromethanesulfonic anhydride (2.500 mL, 14.8 mmol) over 5 min. The ice-bath was removed, and the reaction mixture was stirred at room temperature for 2.5 h. Pyridine was removed under reduced pressure, and the residue was diluted with ether (70 mL) and washed with water (30 mL). The organic layer was collected, and the aqueous layer was back-extracted with ether (25 mL). The combined organic layers were washed with 1N aqueous hydrochloric acid, 1N aqueous sodium hydroxide, brine, and dried over anhydrous sodium sulfate. Ether was removed under reduced pressure, and the crude product was purified by silica gel flash chromatography using a mixture of ethyl acetate in hexane (5% - 15% - 20%) to afford 4-oxochroman-7-yl trifluoromethanesulfonate (3.13 g, 10.6 mmol, 86% yield) as a yellow oil. HPLC t_r = 2.40 min. (Method D). LCMS (ESI) *m/z* Calcd for $C_{10}H_7F_3O_5S$ $[M + H]^+$ 297.0. Found: 297.2.

7-vinylchroman-4-one (15). To a solution of 4-oxochroman-7-yl trifluoromethanesulfonate (2.00 g, 6.75 mmol) in dioxane (10 mL) in a sealed tube was added sequentially tributyl(vinyl)stannane (2.18 mL, 7.43 mmol) and lithium chloride (0.859 g, 20.3 mmol). The mixture was degassed under reduced pressure and charged with nitrogen (2x). To the mixture was added tetrakis(triphenylphosphine)palladium(0) (0.780 g, 0.675 mmol), and the contents stirred under a strong stream of nitrogen for 5 min. The reaction mixture was sealed, immersed in an oil bath at 100 °C, and stirred overnight. The reaction mixture was cooled to room temperature and filtered under reduced pressure. The solid was washed with ethyl acetate (4 x 50 mL), and the filtrate was concentrated. The crude product mixture was diluted with ether (~100 mL), sonicated for several minutes, and filtered (the cake was rinsed with ether). The filtrate was concentrated under reduced pressure, and the residue was

purified by silica gel flash chromatography using a mixture of ethyl acetate in hexane (5% - 12% - 15%) to give 7-vinylchroman-4-one (0.938 g, 5.38 mmol, 80 % yield) as a yellow oil. HPLC t_r = 1.93 min. (Method D). LCMS (ESI) *m/z* Calcd for $C_{11}H_{10}O_2$ $[M + H]^+$ 175.1. Found: 174.9. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 7.87 (d, *J*=8.14 Hz, 1H), 7.09 (dd, *J*=8.25, 1.43 Hz, 1H), 6.98 (d, *J*=1.54 Hz, 1H), 6.69 (dd, *J*=17.61, 11.00 Hz, 1H), 5.88 (d, *J*=17.61 Hz, 1H), 5.42 (d, *J*=10.78 Hz, 1H), 4.52 - 4.58 (m, 2H), and 2.78 - 2.85 (m, 2H).

7-(1,2-dihydroxyethyl)chroman-4-one (16). To a mixture of 7-vinylchroman-4-one (0.801 g, 4.60 mmol) and a 50% solution of NMO in water (0.953 mL, 4.60 mmol) in tetrahydrofuran (11 mL) at room temperature was added a 4% aqueous solution of osmium tetroxide in water (1.44 mL, 0.184 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane (200 mL) and washed with water (40 mL). The organic layer was collected and was washed with brine (40 mL). The combined aqueous layers were extracted with dichloromethane (200 mL), and the combined organic layers were dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded 7-(1,2-dihydroxyethyl)chroman-4-one (0.950 g, 4.56 mmol, 99% yield) as a dark yellow oil. HPLC t_r = 0.587 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{11}H_{12}O_4$ $[M + H]^+$ 209.1. Found: 209.0.

7-(2,2-dimethyl-1,3-dioxolan-4-yl)chroman-4-one (17). To a mixture of 7-(1,2-dihydroxyethyl)chroman-4-one (0.950 g, 4.56 mmol) and 2,2-dimethoxypropane (1.68 mL, 13.7 mmol) in acetone (20 mL) at room temperature was added (1R)-(-)-camphorsulfonic acid (0.212 g, 0.913 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate and concentrated under reduced pressure. The aqueous residue was diluted with ethyl acetate, washed with a saturated aqueous solution of sodium bicarbonate, and brine. The organic layer was collected, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate and

concentrated to give the crude product as a dark oil, which was purified by flash silica gel chromatography using a 20% mixture of ethyl acetate and hexane to afford 7-(2,2-dimethyl-1,3-dioxolan-4-yl)chroman-4-one (0.657 g, 2.65 mmol, 58% yield) as a clear, colorless oil. HPLC t_r = 2.14 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{14}H_{16}O_4$ $[M + H]^+$ 249.1. Found: 248.9.

4-(7-(2,2-dimethyl-1,3-dioxolan-4-yl)-2H-chromen-4-yl)morpholine (18). To a mixture of 7-(2,2-dimethyl-1,3-dioxolan-4-yl)chroman-4-one (0.657 g, 2.65 mmol) and morpholine (1.15 mL, 13.2 mmol) in toluene (12 mL) at 0 °C was added a 1.0 M solution of titanium(IV) chloride in toluene (1.46 mL, 1.46 mmol) dropwise. The ice-bath was removed, and the orange, heterogeneous reaction mixture was stirred at room temperature overnight. The heterogeneous, orange reaction mixture was filtered through a pad of Celite and rinsed with toluene (3 x 20 mL). The pale yellow filtrate was concentrated under reduced pressure to give 4-(7-(2,2-dimethyl-1,3-dioxolan-4-yl)-2H-chromen-4-yl)morpholine (0.751 g, 2.37 mmol, 89% yield) as a yellow oil.

7-(2,2-dimethyl-1,3-dioxolan-4-yl)-3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-3a,4-dihydro-3H-chromeno[4,3-c]isoxazol-3-ol (19). To a homogeneous solution of 4-(7-(2,2-dimethyl-1,3-dioxolan-4-yl)-2H-chromen-4-yl)morpholine (0.266 g, 0.838 mmol) and triethylamine (0.232 mL, 1.68 mmol) in anhydrous acetonitrile (3.0 mL) at 0°C was added a solution of 3-phenyl-4-(trifluoromethyl)isoxazole-5-carbonyl fluoride[16] (0.217 g, 0.838 mmol) in acetonitrile (1.0 mL). The reaction mixture was stirred at 0 °C for 30 min. and then at room temperature for 60 min. A homogeneous solution of hydroxylamine hydrochloride (0.233 g, 3.35 mmol) and sodium acetate (0.275 g, 3.35 mmol) in water (0.544 mL, 30.2 mmol) was added, and the reaction mixture was heated at 45 °C for 60 min. The reaction mixture was diluted with dichloromethane, washed with water, and dried over anhydrous sodium sulfate. Concentration under reduced pressure followed by purification by flash silica gel chromatography using a mixture of ethyl acetate in hexane (5% - 12% - 20%) afforded 240 mg of the product mixture. The product was further purified by reverse phase preparative HPLC (2 x 120 mg)

to give, after neutralization, 7-(2,2-dimethyl-1,3-dioxolan-4-yl)-3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-3a,4-dihydro-3H-chromeno[4,3-c]isoxazol-3-ol (0.070 g, 0.139 mmol, 17% yield). HPLC t_r = 3.08 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{25}H_{21}F_3N_2O_6$ $[M + H]^+$ 503.1. Found: 502.9.

7-(2,2-dimethyl-1,3-dioxolan-4-yl)-3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazole (20). *(This reaction was run twice.)* To a stirred suspension of 7-(2,2-dimethyl-1,3-dioxolan-4-yl)-3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-3a,4-dihydro-3H-chromeno[4,3-c]isoxazol-3-ol (0.035 g, 0.070 mmol) in anhydrous toluene (1.0 mL) at room temperature was added pyridine (0.012 mL, 0.153 mmol) followed by thionyl chloride (8.64 µl, 0.118 mmol). The reaction mixture was stirred at room temperature for 15 min. and then at 80 °C for 15 min. The reaction mixture was cooled to room temperature and concentrated. The solid residue was diluted with dichloromethane, washed with a saturated aqueous solution of sodium bicarbonate, and dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded a yellow film. The combined crude products were triturated with methanol with sonication. The solid was collected to provide 7-(2,2-dimethyl-1,3-dioxolan-4-yl)-3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazole (0.042 g, 0.087 mmol, 62% yield). HPLC t_r = 3.63 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{25}H_{19}F_3N_2O_5$ $[M + H]^+$ 485.1. Found: 484.9.

1-(3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazol-7-yl)ethane-1,2-diol (21). A solution of 7-(2,2-dimethyl-1,3-dioxolan-4-yl)-3-(3-phenyl-4-(trifluoromethyl)-isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazole (0.042 g, 0.087 mmol) in trifluoroacetic acid (1.00 ml, 13.0 mmol) was left standing at room temperature for 45 min. The trifluoroacetic acid was removed under reduced pressure, and the residue was diluted with dichloromethane, washed with a saturated aqueous solution of sodium bicarbonate, and dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded 1-(3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazol-7-

yl)ethane-1,2-diol (0.039 g, 0.088 mmol, 100% yield). HPLC t_r = 3.00 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{22}H_{15}F_3N_2O_5$ $[M + H]^+$ 445.1. Found: 444.9.

3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazole-7-carbaldehyde (22). To a homogeneous solution of 1-(3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazol-7-yl)ethane-1,2-diol (0.039 g, 0.088 mmol) in a mixture of tetrahydrofuran (1.0 mL) and water (0.065 mL) at room temperature was added sodium periodate (0.028 g, 0.132 mmol). The reaction mixture was stirred for 30 min. The solvent was removed under reduced pressure, and the residue was diluted with dichloromethane, washed with water, and dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded 36 mg of a ~1:1 mixture of 3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazole-7-carbaldehyde and 7-(2,2-dimethyl-1,3-dioxolan-4-yl)-3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazole as a yellow oil. The product was used in the next step without any further purification. HPLC t_r = 3.35 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{21}H_{11}F_3N_2O_4$ $[M + H]^+$ 413.1. Found: 412.9.

***tert*-butyl 1-((3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazol-7-yl)methyl)azetidine-3-carboxylate (23)**. To the mixture of 3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazole-7-carbaldehyde (0.036 g, 0.087 mmol) and 7-(2,2-dimethyl-1,3-dioxolan-4-yl)-3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazole from the previous reaction in methanol (0.333 mL) and dichloromethane (1.0 mL) at room temperature was added the *tert*-butyl azetidine-3-carboxylate, acetic acid salt (0.028 g, 0.131 mmol), acetic acid (10.00 µl, 0.175 mmol), and titanium(IV) isopropoxide (0.051 mL, 0.175 mmol) dropwise. The resulting homogeneous reaction mixture was stirred for 60 min. To the reaction mixture was added sodium triacetoxyborohydride (0.056 g, 0.262 mmol) in one portion, and the reaction mixture was stirred at room temperature for 30 min. The reaction was quenched with a saturated aqueous solution of sodium bicarbonate until the pH was slightly basic (~5 mL). The resulting emulsion was extracted with

dichloromethane (3x). and the organic layers were combined, dried over anhydrous sodium sulfate, and concentrated to give a pale yellow solid. The crude product was purified by flash silica gel chromatography using a mixture of ethyl acetate in hexane (20% - 35% - 50%) to afford *tert*-butyl 1-((3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazol-7-yl)methyl)azetidine-3-carboxylate (0.031g, 0.056 mmol, 64% yield). HPLC t_r = 3.01 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{29}H_{26}F_3N_3O_5$ $[M + H]^+$ 554.1. Found: 553.9.

1-((3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (24). A mixture of *tert*-butyl 1-((3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazol-7-yl)methyl)azetidine-3-carboxylate (0.031 g, 0.056 mmol) and trifluoroacetic acid (2.58 ml, 33.5 mmol) was left standing at room temperature for 45 min. The trifluoroacetic acid was removed under reduced pressure, and the residue was suspended in water, the pH was adjusted to 5 with sonication, and the resulting suspension was stirred for 1 h. The pH remained at 5, so the solid was collected by vacuum filtration, washed with water, and dried under reduced pressure to give a quantitative yield of the product. The compound was suspended in methanol and sonicated for 15 min. The product was collected by vacuum filtration, washed with methanol, and dried under reduced pressure to provide 1-((3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4H-chromeno[4,3-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (0.029 g, 0.058 mmol, 100% yield). HPLC t_r = 7.51 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{25}H_{18}F_3N_3O_5$ $[M + H]^+$ 498.1. Found: 498.0. ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm 7.79 (d, *J*=7.77 Hz, 1H), 7.59 - 7.69 (m, 5H), 7.09 (d, *J*=7.77 Hz, 1H), 7.04 (s, 1H), 5.61 (s, 2H), 3.58 (s, 2H), and 3.20 - 3.46 (m, 5H).

(5-Phenyl-4-(trifluoromethyl)isoxazol-3-yl)methanol (26). To a solution of 5-phenyl-4-(trifluoromethyl)isoxazole-3-carboxylic acid (6.0 g, 23.33 mmol) in tetrahydrofuran (40 mL) at 0°C was added N-methylmorpholine (3.85 mL, 35.0 mmol) followed by isobutyl chloroformate (4.60 mL, 35.0 mmol) via syringe over 5 min. The resulting suspension was stirred at 0°C for 15 min. The suspension

was then added to a suspension of sodium borohydride (1.589 g, 42.0 mmol) in tetrahydrofuran (80 mL) and methanol (24 mL) at -78°C via a 10 mL pipette. During the addition, frothing was observed. The reaction mixture was stirred for 3 h. The reaction mixture was slowly warmed to ~-20°C and quenched with a 1:9 mixture of acetic acid in water (40 mL). The reaction mixture was then stirred at room temperature for 60 min. The solvent was removed under reduced pressure, and the residue was diluted with ethyl acetate (240 mL), washed with water (40 mL), washed with a saturated aqueous solution of sodium bicarbonate (2 x 40 mL), and washed with brine (40 mL). The organic layer was collected, the aqueous layers were back-extracted with ethyl acetate (200 mL), and the combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash silica gel chromatography using a mixture of ethyl acetate in hexane (5% - 12% - 20%) afforded (5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)methanol (3.22 g, 13.24 mmol, 56.8 % yield) as a clear, colorless oil. HPLC t_r = 1.93 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{11}H_8F_3NO_2$ $[M + H]^+$ 244.2. Found: 244.0.

5-Phenyl-4-(trifluoromethyl)isoxazole-3-carbaldehyde (27). To a mixture of (5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)methanol (3.19 g, 13.12 mmol) in Dichloromethane (40 mL) at 0°C was added Dess-Martin Periodinane (6.12 g, 14.43 mmol). The reaction mixture was stirred at 0°C for 10 min. and then at room temperature for 1.5 h. By HPLC, the reaction was complete. A 1:1 mixture of saturated aqueous sodium thiosulfate and saturated aqueous bicarbonate (75 mL) was added slowly. The organic layer was collected, and the aqueous layer was extracted with dichloromethane (2 x 100 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered through a pad of Celite, and concentrated under reduce pressure to give a quantitative yield of 5-phenyl-4-(trifluoromethyl)isoxazole-3-carbaldehyde as a clear, colorless oil. HPLC t_r = 2.05 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{11}H_6F_3NO_2$ $[M + H]^+$ 242.0. Found: 242.0**.**

3-(2,2-dibromovinyl)-5-phenyl-4-(trifluoromethyl)isoxazole (28). To a solution of carbontetrabromide (12.24 g, 36.9 mmol) in dichloromethane (10 mL) at 0°C was added a solution of triphenylphosphine (4.84 g, 18.5 mmol) in dichloromethane (10 mL), and the resulting mixture was stirred at 0°C for 5 min. A solution of 5-phenyl-4-(trifluoromethyl)isoxazole-3-carbaldehyde (**27**, 1.59 g, 4.62 mmol) in dichloromethane (10 mL) was added via syringe, and the reaction mixture was stirred at 0 °C for 20 min. The reaction mixture was diluted with dichloromethane and washed with water. A white precipitate formed was removed by vacuum filtrating. The filtrate was washed with a saturated aqueous solution of sodium bicarbonate, washed with a saturated aqueous solution of ammonium chloride, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by flash silica gel chromatography using a mixture of ethyl acetate in hexane (1% - 3%) to give 3-(2,2-dibromovinyl)-5-phenyl-4-(trifluoromethyl)isoxazole (1.21 g, 3.05 mmol, 66.0 % yield). HPLC t_r = 3.10 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{12}H_6Br_2F_3NO$ $[M + H]^+$ 395.9. Found: 395.9 and 397.9. ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm 7.43 (s, 1H), 7.52 - 7.56 (m, 2H), 7.56 - 7.61 (m, 1H), and 7.74 (d, *J*=7.49 Hz, 2H).

3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)prop-2-yn-1-ol (29). To a solution of 3-(2,2-dibromovinyl)-5-phenyl-4-(trifluoromethyl)isoxazole (0.496 g, 1.19 mmol) in tetrahydrofuran (6.0 mL) at -78°C was slowly added a 2.5 M solution of butyl lithium in hexane (1.05 mL, 2.61 mmol). The reaction mixture was stirred at -78°C for 25 min. and then warmed to room temperature. Once at room temperature, paraformaldehyde (0.713 g, 23.74 mmol) was added, and the reaction mixture was stirred for 5 min. The mixture was diluted with ether, washed with water (2x), and washed with brine. The combined aqueous layers were extracted with ether, and the combined organics layers were dried over anhydrous sodium sulfate and concentrated. The residue was purified by flash silica gel chromatography using a mixture of ethyl acetate in hexane (1% - 5% - 20%) to give 3-(5-phenyl-4-

(trifluoromethyl)isoxazol-3-yl)prop-2-yn-1-ol (0.199 g, 0.745 mmol, 63% yield). HPLC t_r = 2.39 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{13}H_8F_3NO_2$ $[M + H]^+$ 268.1. Found: 268.2.

3-(3-bromoprop-1-ynyl)-5-phenyl-4-(trifluoromethyl)isoxazole (30). To a solution of 3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)prop-2-yn-1-ol (0.465 g, 1.740 mmol) in dichloromethane (9.0 mL) at 0°C was added a 1.0 M solution of tribromophosphine in dichloromethane (2.09 mL, 2.09 mmol). After 5 min., the ice-bath was removed, and the reaction mixture was stirred at room temperature overnight. Additional tribromophosphine (1.0 M in dichloromethane) (2.09 mL, 2.09 mmol) was added, and the reaction mixture was stirred at room temperature for 4.5 h. Additional tribromophosphine (1.0 M in dichloromethane) (2.09 mL, 2.09 mmol) was added, and the reaction mixture was stirred at room temperature over the weekend. The reaction mixture was diluted with dichloromethane, washed with a saturated aqueous solution of sodium bicarbonate, and dried over anhydrous sodium sulfate. Concentration under reduced pressure provided the crude product, which was loaded with a minimum amount of dichloromethane on a fritted funnel containing a layer of Celite topped with a layer of silica gel. The plug column was eluted with a mixture of ethyl acetate and hexane (1% - 3%) to give 3-(3-bromoprop-1-ynyl)-5-phenyl-4-(trifluoromethyl)isoxazole (0.219 g, 0.663 mmol, 38% yield) as a clear, colorless oil. HPLC t_r = 2.95 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{13}H_7BrF_3NO$ $[M + H]^+$ 329.9. Found: 329.9 and 331.9.

4-bromo-2-(3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)prop-2-ynyloxy)benzaldehyde (31). To a mixture of 3-(3-bromoprop-1-ynyl)-5-phenyl-4-(trifluoromethyl)isoxazole (0.219 g, 0.663 mmol) and 4-bromo-2-hydroxybenzaldehyde (0.133 g, 0.663 mmol) in dimethylformamide (2.0 mL) at room temperature was added potassium carbonate (0.115 g, 0.829 mmol). The reaction mixture was stirred at room temperature for 3 h, diluted with ethyl acetate (75 mL), washed with a 10% aqueous solution of lithium chloride (2 x 25 mL), and washed with brine (25 mL). The organic layer was collected, and the combined aqueous phases were extracted with ethyl acetate (2 x 25 mL). The

combined organic layers were dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded a tan solid which was triturated with methanol with sonication to give 4-bromo-2-(3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)prop-2-ynyloxy)benzaldehyde (0.223 g, 0.495 mmol, 75% yield). HPLC t_r = 3.28 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{20}H_{11}BrF_3NO_3$ $[M + H]^+$ 450.0. Found: 449.9 and 451.92. ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm 5.12 (s, 2H), 7.28 (d, *J*=8.32 Hz, 1H), 7.34 (d, *J*=1.66 Hz, 1H), 7.51 - 7.55 (m, 2H), 7.56 - 7.61 (m, 1H), 7.72 (d, *J*=7.21 Hz, 2H), 7.75 (d, *J*=8.32 Hz, 1H), and 10.44 (s, 1H).

(E)-4-bromo-2-(3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)prop-2-ynyloxy)benzaldehyde oxime (32). A mixture of 4-bromo-2-(3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)prop-2-ynyloxy)benzaldehyde (0.050 g, 0.111 mmol), hydroxylamine hydrochloride (9.26 mg, 0.133 mmol), and sodium acetate (0.018 g, 0.222 mmol) in methanol (2.0 mL) was heated at reflux for 30 min. The solvent was removed under reduce pressure, and the residue was diluted with dichloromethane, washed with water, and dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded (E)-4-bromo-2-(3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)prop-2-ynyloxy)benzaldehyde oxime (0.050 g, 0.107 mmol, 97 % yield). HPLC t_r = 3.26 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{20}H_{12}BrF_3N_2O_3$ $[M + H]^+$ 465.0. Found: 464.8 and 466.8.

7-bromo-3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4H-chromeno[4,3-c]isoxazole (33). To a mixture of sodium hypochlorite (23 mL), triethylamine (0.021 mL, 0.155 mmol), and dichloromethane (1.0 mL) at 0°C was added dropwise a solution of (E)-4-bromo-2-(3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)prop-2-ynyloxy)benzaldehyde oxime (0.036 g, 0.077 mmol) in dichloromethane (3 mL). The reaction mixture was stirred at 0°C for 30 min. and then at room temperature for 1.5 h. The reaction mixture was diluted with dichloromethane, and the organic layer was collected. The aqueous layer was extracted with dichloromethane (2x), and the combined organic layers were washed with brine and dried over anhydrous sodium sulfate. Concentration under reduced

pressure followed by trituration with methanol with sonication afforded 7-bromo-3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4H-chromeno[4,3-c]isoxazole (0.026 g, 0.056 mmol, 73% yield). HPLC t_r = 3.91 min. (Method C); LCMS (ESI) *m/z* Calcd for $C_{20}H_{10}BrF_3N_2O_3$ $[M + H]^+$ 463.0. Found: 462.9 and 464.9. ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm 7.78 (d, *J*=8.05 Hz, 1H), 7.73 (d, *J*=7.49 Hz, 2H), 7.60 - 7.65 (m, 1H), 7.55 - 7.59 (m, 2H), 7.23 - 7.27 (m, 2H), and 5.49 (s, 2H).

3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4H-chromeno[4,3-c]isoxazole-7-carbaldehyde (34). To a heterogeneous solution of 7-bromo-3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4H-chromeno[4,3-c]isoxazole (0.105 g, 0.227 mmol) in dioxane (2.0 mL) in a sealed tube was added sequentially tributyl(vinyl)stannane (0.073 mL, 0.249 mmol) and lithium chloride (0.029 g, 0.680 mmol). The mixture was degassed under reduced pressure and charged with nitrogen (3x). To the mixture was added tetrakis(triphenylphosphine)palladium(0) (0.026 g, 0.023 mmol), and the mixture was stirred under a strong stream of nitrogen for 5 min. The reaction mixture was sealed, immersed in an oil bath at 100 °C, and stirred overnight. The reaction mixture was concentrated under reduced pressure, and the residue was diluted with dichloromethane (10 mL), filtered through a pad of Celite, and rinsed with dichloromethane (10 mL). The filtrate was concentrated, and the residue was purified by flash silica gel chromatography using a 5% mixture of ethyl acetate in hexane to give 3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-7-vinyl-4H-chromeno[4,3-c]isoxazole (0.062 g, 0.151 mmol, 67% yield). HPLC t_r = 3.84 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{22}H_{13}F_3N_2O_3$ $[M + H]^+$ 411.1. Found: 411.0.

To a mixture of 3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-7-vinyl-4H-chromeno[4,3-c]isoxazole (0.060 g, 0.146 mmol) and a 50% aqueous solution of NMO (0.030 mL, 0.146 mmol) at room temperature was added a 4% aqueous solution of osmium tetroxide (0.046 mL, 5.85 μmol). The reaction mixture was stirred at room temperature for 5 h. The intermediate diol had an HPLC ret. time = 3.09 min and an $[M + H]^+$ = 445. Sodium periodate (0.047 g, 0.219 mmol) was added to the

homogeneous mixture followed by water (0.10 mL), and the reaction was stirred for 60 min. at room temperature. Concentration under reduced pressure afforded a tan solid residue that was then diluted with ethyl acetate (40 mL), washed with the water (10 mL), and washed with brine (10 mL). The organic layer was collected, and the aqueous layer was washed with ethyl acetate (40 mL). The combined organic layers were dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded 3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4H-chromeno[4,3-c]isoxazole-7-carbaldehyde (0.057 g, 0.138 mmol, 95% yield). HPLC t_r = 3.49 min. (Method D).

***tert*-butyl 1-((3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4H-chromeno[4,3-c]isoxazol-7-yl)methyl)azetidine-3-carboxylate (35)**. To a mixture of 3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4H-chromeno[4,3-c]isoxazole-7-carbaldehyde (0.057 g, 0.138 mmol), *tert*-butyl azetidine-3-carboxylate, acetic acid salt (0.045 g, 0.207 mmol), and acetic acid (0.016 mL, 0.276 mmol) in methanol (3.0 mL) and dichloroethane (0.900 mL) at room temperature was added titanium(IV) isopropoxide (0.081 mL, 0.276 mmol) dropwise. The resulting homogeneous reaction mixture was stirred for 90 min. To the reaction mixture was added sodium triacetoxyborohydride (0.088 g, 0.415 mmol) in one portion, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate until the pH was slightly basic (~5 mL). The resulting emulsion was extracted with dichloromethane (3x). The organic layers were combined, dried over anhydrous sodium sulfate, and concentrated to give a pale yellow solid. The crude product was purified by flash silica gel chromatography using a mixture of ethyl acetate in hexane (20% - 35% - 50%) to afford *tert*-butyl 1-((3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4H-chromeno[4,3-c]isoxazol-7-yl)methyl)azetidine-3-carboxylate (0.044 g, 0.079 mmol, 58% yield). HPLC t_r = 3.06 min. (Method D); LCMS (ESI) *m/z* Calcd for $C_{29}H_{26}F_3N_3O_5$ $[M + H]^+$ 554.2. Found: 554.2.

1-((3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4H-chromeno[4,3-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (36). A mixture of *tert*-butyl 1-((3-(5-phenyl-4-

(trifluoromethyl)isoxazol-3-yl)-4H-chromeno[4,3-c]isoxazol-7-yl)methyl)azetidine-3-carboxylate (0.044 g, 0.079 mmol) and trifluoroacetic acid (3.00 mL, 39.0 mmol) was left standing at room temperature for 60 min. The trifluoroacetic acid was removed under reduced pressure, and the residue was suspended in water, the pH was adjusted to ~5 using 1N NaOH with sonication, and the resulting suspension was stirred overnight. The pH remained at 5, so the solid was collected by vacuum filtration, washed with water, and dried under reduced pressure to give a quantitative yield of the product. The compound was suspended in methanol and sonicated for 15 min. and then stirred overnight. The product was collected by vacuum filtration, washed with methanol, and dried under reduced pressure to provide 1-((3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4H-chromeno[4,3-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (0.026 g, 0.052 mmol, 65% yield). HPLC t_r = 8.92 min. (Method B); LCMS (ESI) *m/z* Calcd for $C_{25}H_{18}F_3N_3O_5$ $[M + H]^+$ 498.1. Found: 498.1. ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm 7.78 (dd, *J*=7.21, 5.83 Hz, 3H), 7.70 - 7.75 (m, 1H), 7.64 - 7.70 (m, 2H), 7.08 (d, *J*=7.77 Hz, 1H), 7.03 (s, 1H), 5.51 (s, 2H), 3.58 (s, 2 H), 3.42 (br. s., 2H), and 3.22 (br. s., 3H).

2-bromo-6-methoxy-3,4-dihydronaphthalen-1(2H)-one (38). 6-methoxy-3,4-dihydronaphthalen-1(2H)-one (1 g, 5.67 mmol), NBS (1.010 g, 5.67 mmol) and pTsOH (0.108 g, 0.567 mmol) were ground in a porcelain mortar and the mixture was heated at 60 °C for 11 mins in a scintillation vial with stirring. The reaction was carried out in 4 separate vials with same scale (total 4x1 g of 6-methoxy-3,4-dihydronaphthalen-1(2H)-one). After cooling the contents of the 4 vials were combined and dissolved in 80 mL of dichloromethane. The solution was washed in sequence with saturated sodium bicarbonate (2x), water and then dried over magnesium sulfate. The reaction mixture was concentrated under reduced pressure to yield 2-bromo-6-methoxy-3,4-dihydronaphthalen-1(2H)-one as an oily residue. The crude product was used for next reaction without purification. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm 8.07 (d, *J*=8.8 Hz, 1H), 6.88 (dd, *J*=8.8, 2.6 Hz, 1H), 6.73 (d, *J*=2.4 Hz, 1H),

4.70 (t, *J*=4.2 Hz, 1H), 3.88 (s, 3H), 3.35 - 3.25 (m, 1H), 2.87 (dt, *J*=16.9, 4.4 Hz, 1H), 2.53 - 2.41 (m, 2H)

2-azido-6-methoxy-3,4-dihydronaphthalen-1(2H)-one (39). To a solution of 2-bromo-6-methoxy-3,4-dihydronaphthalen-1(2H)-one (5.71 g, 22.4 mmol) in acetone (40 mL) was added a solution of sodium azide (2.184 g, 33.6 mmol) in water (10 mL) dropwise, and the mixture was stirred at room temp for 3 hrs. The reaction mixture was diluted with ethyl acetate and washed with water twice and then brine. The combined aqueous layers were back extracted with ethyl acetate. The organic layers were combined and dried over sodium sulfate and evaporated to yield an oily residue. The crude product was purified by flash chromatography (120 g silica gel cartridge, eluting with 5:95 followed by 1:9 and 2:8 EtOAc-hexane). Crystallization from ethyl acetate – hexanes afforded a total of 3.37 g of 2-azido-6-methoxy-3,4-dihydronaphthalen-1(2H)-one (69.3% yield over two steps). ^{1}H NMR (400MHz, $CDCl_3$) δ ppm 8.04 (d, *J*=8.8 Hz, 1H), 6.86 (dd, *J*=8.7, 2.5 Hz, 1H), 6.70 (d, *J*=2.4 Hz, 1H), 4.19 (dd, *J*=11.8, 4.7 Hz, 1H), 3.87 (s, 3H), 3.03 (dd, *J*=7.8, 4.5 Hz, 2H), 2.34 (dq, *J*=13.2, 4.6 Hz, 1H), 2.16 - 2.05 (m, 1H)

2-Amino-6-methoxy-3,4-dihydronaphthalen-1(2H)-one, HCl (40). A mixture of 2-azido-6-methoxy-3,4-dihydronaphthalen-1(2H)-one (4.88 g, 22.47 mmol), 2.0 M HCl in water (33.7 mL, 67.4 mmol) and MeOH (125 mL) under nitrogen was charged with 10% Pd/C (50% wet) and stirred under a 50 psi hydrogen atmosphere for 4h. The reaction mixture was then filtered through a plug of celite using methanol. The reaction mixture was concentrated. The residue was suspended in MeOH and treated with EtOAc and the resulting solids were collected by filtration and dried *in vacuo*. 2-Amino-6-methoxy-3,4-dihydronaphthalen-1(2H)-one, HCl (3.8 g, 74.3% yield) was isolated. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.99 (1 H, d, *J*=8.80 Hz), 6.95 (1 H, dd, *J*=8.80, 2.64 Hz), 6.89 (1 H, d, *J*=2.42 Hz), 4.22 (1 H, dd, *J*=13.98, 4.95 Hz), 3.89 (3 H, s), 3.20 - 3.29 (1 H, m), 3.12 (1 H, ddd, *J*=17.17, 4.62, 2.64 Hz), 2.46 - 2.53 (1 H, m, *J*=12.30, 4.75, 4.75, 2.64 Hz), 2.17 (1 H, dddd, *J*=13.64, 12.87, 12.65, 4.62 Hz)

N-(6-methoxy-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)-3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxamide (41). To a solution of 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylic acid[16] (1407 mg, 5.47 mmol) in dichloromethane (12 mL) at 0 °C was added oxalyl chloride (0.878 mL, 10.03 mmol) followed by DMF (0.018 mL, 0.228 mmol), and the cooling bath was removed and the mixture was stirred for 25 min. The reaction mixture was evaporated and the residue was azeotroped with anhydrous ethylene chloride (3x). The residue was dissolved in 10 mL of dichloromethane and was added to a stirred solution of 2-amino-6-methoxy-3,4-dihydronaphthalen-1(2H)-one hydrochloride (1038 mg, 4.56 mmol) and dimethyl aminopyridine (55.7 mg, 0.456 mmol) in 5 mL of dichloromethane by a syringe. Then Hunig's Base (2.389 mL, 13.68 mmol) was added, and the mixture was stirred overnight. The reaction mixture was diluted with ethyl acetate and then washed with saturated sodium bicarbonate followed by brine. It was dried over anhydrous magnesium sulfate, concentrated under reduced pressure and purified by flash chromatography (80g redisep cartridge was presaturated with 10%EtOAc-Hexanes. The crude was loaded as a solution in dichloromethane. The column was eluted with 10% EtOAc-Hex.). N-(6-methoxy-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)-3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxamide (0.78 g, 1.812 mmol, 39.8 % yield) was obtained. LCMS (ESI) *m/z* Calcd for $C_{22}H_{17}F_3N_2O_4$ $[M + H]^+$ 431.11. Found: 431.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.06 (1 H, d, *J*=8.80 Hz), 7.82 (1 H, d, *J*=4.40 Hz), 7.59 - 7.64 (2 H, m), 7.49 - 7.57 (2 H, m), 6.90 (1 H, dd, *J*=8.80, 2.42 Hz), 6.75 (1 H, d, *J*=2.20 Hz), 4.77 (1 H, dt, *J*=13.42, 4.84 Hz), 3.90 (3 H, s), 3.30 (1 H, ddd, *J*=17.28, 13.09, 4.62 Hz), 3.05 (1 H, ddd, *J*=17.22, 4.24, 2.31 Hz), 2.95 - 3.02 (2 H, m)

5-(7-methoxy-4,5-dihydronaphtho[2,1-d]thiazol-2-yl)-3-phenyl-4-(trifluoromethyl)isoxazole (42). A mixture of the N-(6-methoxy-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)-3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxamide (133 mg, 0.309 mmol) and Lawesson's Reagent (187 mg, 0.464 mmol) in THF (3.0 ml) was heated for 30 min at 120 °C in a microwave. The resulting bright

yellow mixture was combined with 14 mg of crude from an earlier run, concentrated *in vacuo* and purified by flash chromatography (with 10% followed by 20% ethyl acetate-hexanes). 5-(7-methoxy-4,5-dihydronaphtho[2,1-d]thiazol-2-yl)-3-phenyl-4-(trifluoromethyl)isoxazole (0.088 g, 0.205 mmol, 60.3 % yield) was obtained. LCMS (ESI) *m/z* Calcd for $C_{22}H_{15}F_3N_2O_2S$ $[M + H]^+$ 429.08. Found: 429.2. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.66 (1 H, s), 7.65 (1 H, d, *J*=1.54 Hz), 7.49 - 7.56 (3 H, m), 7.35 (1 H, d, *J*=8.36 Hz), 6.86 (1 H, d, *J*=2.42 Hz), 6.83 (1 H, dd, *J*=8.36, 2.64 Hz), 3.87 (3 H, s), 3.16 - 3.21 (2 H, m), 3.09 - 3.15 (2 H, m)

2-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]thiazol-7-ol (43). To a solution of 5-(7-methoxy-4,5-dihydronaphtho[2,1-d]thiazol-2-yl)-3-phenyl-4-(trifluoromethyl)isoxazole (0.088 g, 0.205 mmol) in dichloromethane (4 mL) was added 1M boron tribromide (1.027 mL, 1.027 mmol) in dichloromethane dropwise, and the mixture was stirred at room temperature for 6h. The reaction was quenched by the slow addition of saturated ammonium chloride and the product was extracted with ethyl acetate and washed with brine. The combined aq. layers were back extracted with ethyl acetate once and the combined organic layer was dried over sodium sulfate. Evaporation of the solvent gave an oily residue. It was purified by flash chromatography (40 g redisep cartridge eluting with 2:8 followed by 3:7 ethyl acetate-hexanes). 2-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]thiazol-7-ol (0.085 g, 0.205 mmol, 100 % yield) was obtained. LCMS (ESI) *m/z* Calcd for $C_{21}H_{13}F_3N_2O_2S$ $[M + H]^+$ 415.06. Found: 415.16. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.65 (1 H, s), 7.63 (1 H, d, *J*=1.54 Hz), 7.47 - 7.57 (3 H, m), 7.29 (1 H, d, *J*=8.14 Hz), 6.78 (1 H, d, *J*=2.42 Hz), 6.75 (1 H, dd, *J*=8.14, 2.64 Hz), 3.14 - 3.21 (2 H, m), 3.05 - 3.12 (2 H, m)

2-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl trifluoromethanesulfonate (44). To a solution of 2-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]thiazol-7-ol (0.085g, 0.205 mmol) in pyridine (5.0 mL) at 0 °C was added

trifluoromethanesulfonic anhydride (0.042 mL, 0.246 mmol). After 30 min, the cooling bath was removed. After 2h the reaction mixture was concentrated under reduced pressure. The residue was partitioned between ether and 1N HCl. The organic layer was washed with saturated sodium bicarbonate, brine, dried over anhyd. sodium sulfate and concentrated to yield a tan solid which were washed with hexanes. The mother liquor was purified by silica gel flash chromatography (with 10% ethyl acetate-hexanes). The desired product was combined with the solid obtained earlier. 2-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl trifluoromethanesulfonate (0.1 g, 0.183 mmol, 89 % yield) was obtained. LCMS (ESI) *m/z* Calcd for $C_{22}H_{12}F_6N_2O_4S_2$ $[M + H]^+$ 547.01. Found: 546.84. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.64 - 7.68 (2 H, m, 7.50 - 7.59 (3 H, m), 7.47 (1 H, d, *J*=8.36 Hz), 7.24 (1 H, d, *J*=2.42 Hz), 7.21 (1 H, dd, *J*=8.14, 2.42 Hz), 3.23 - 3.28 (2 H, m), 3.17 - 3.23 (2 H, m)

3-phenyl-4-(trifluoromethyl)-5-(7-vinyl-4,5-dihydronaphtho[2,1-d]thiazol-2-yl)isoxazole (45). A mixture of 2-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl trifluoromethanesulfonate (0.1 g, 0.183 mmol), tributyl(vinyl)tin (0.059 mL, 0.201 mmol) and lithium chloride (0.023 g, 0.549 mmol) in dioxane (2.5 mL) were added to a microwave reaction vessel. To this was then added the tetrakis(triphenylphosphine)palladium(0) (0.021 g, 0.018 mmol) and argon was bubbled through the reaction mixture for 3 min. The vessel was capped and the reddish brown mixture was immersed in a 100 °C oil bath. After overnight stirring, the reaction mixture was cooled and diluted with ethyl acetate. It was filtered through celite. The filter cake was washed with ethyl acetate (3 times). The ethyl acetate layers were combined, concentrated under reduced pressure and the residue stirred in diethyl ether for 30 min. It was then filtered through celite and washed with diethyl ether. The ether layer was evaporated and the residue was purified by flash silica gel chromatography. The crude product was used as such for the subsequent step without further purification. LCMS (ESI) *m/z* Calcd for $C_{23}H_{15}F_3N_2OS$ $[M + H]^+$ 425.09. Found: 424.96.

2-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]thiazole-7-carbaldehyde (46). To a solution of 3-phenyl-4-(trifluoromethyl)-5-(7-vinyl-4,5-dihydronaphtho[2,1-d]thiazol-2-yl)isoxazole (0.066 g, 0.155 mmol) in THF (1.0 ml) at rt. was added a solution of NMO (0.040 g, 0.342 mmol) in water (1 ml) followed by addition of osmium tetroxide (2.5% in butanol (0.32 ml, 3.11 μmol). The reaction was stirred at room temperature for 3h when the diol formation was found to be over as per LC/MS ((M+H) = 458.96). To the reaction was then added the sodium periodate (0.050 g, 0.233 mmol) in 1ml of water. Stirring was continued for 90 min. and the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over magnesium sulfate, concentrated and purified by flash silica gel chromatography. 2-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]thiazole-7-carbaldehyde (31 mg, 0.073 mmol, 46.8 % yield over two steps) was obtained. LCMS (ESI) *m/z* Calcd for $C_{22}H_{13}F_3N_2O_2S$ $[M + H]^+$ 427.06. Found: 426.95. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 10.02 (1 H, s), 7.83 (1 H, s), 7.81 (1 H, dd, *J*=7.92, 1.54 Hz), 7.64 - 7.68 (2 H, m), 7.50 - 7.59 (4 H, m), 3.23 - 3.29 (4 H, m)

1-((2-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)methyl)azetidine-3-carboxylic acid, TFA (47). To a stirring solution of the 2-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]thiazole-7-carbaldehyde (0.031 g, 0.073 mmol) and azetidine-3-carboxylic acid (8.82 mg, 0.087 mmol) in methanol (1.5 mL) and 1,2-dichloroethane (1.5 mL) was added at room temperature about 3-4 drops of acetic acid. The reaction mixture was immersed in an oil bath heated at 80 °C for 1h. It was then cooled to room temperature and treated with sodium cyanoborohydride (5.48 mg, 0.087 mmol). The reaction mixture was stirred at room temperature for 1h, concentrated and purified by reverse phase prep. HPLC (HPLC conditions: Phenomenex Luna C18 5 micron column (250 x 30mm); 25-100% CH_3CN/water (0.1% TFA); 25 minute gradient; 30 mL/min.). 1-((2-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]thiazol-7-yl)methyl)azetidine-3-carboxylic acid, TFA (12 mg, 0.018 mmol, 25.3

% yield) was obtained. HPLC t_r = 9.084 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{26}H_{20}F_3N_3O_3S$ $[M + H]^+$ 512.12. Found: 511.97. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.53 - 7.66 (6 H, m), 7.46 (1 H, s), 7.42 (1 H, dd, *J*=7.81, 1.65 Hz), 4.43 (2 H, s), 4.29 - 4.41 (4 H, m), 3.65 - 3.75 (1 H, m), 3.16 - 3.25 (4 H, m)

1-((3-(5-Phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (48). This compound was prepared starting from methyl 5-phenyl-4-(trifluoromethyl)isoxazole-3-carboxylate[16] using the sequence outlined in Scheme 1. HPLC t_r = 7.70 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{26}H_{21}F_3N_3O_4$ $[M + H]^+$ 496.15. Found: 496.2. ^{1}H NMR (400 MHz, CD_3OD+$CDCl_3$) δ ppm 8.06 (1 H, d, *J*=7.9 Hz), 7.77 (2 H, d, *J*=7.3 Hz), 7.59-7.71 (3 H, m), 7.51 (1 H, s), 7.48 (1 H, d, *J*=7.7 Hz), 4.42 (2 H, s), 4.25-4.34 (4 H, m), 3.55-3.65 (1 H, m), 3.14 (4 H, s).

1-((3-(3-phenyl-4-(trifluoromethyl)isothiazol-5-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (49).This compound was prepared starting from methyl 3-phenyl-4-(trifluoromethyl)isothiazole-5-carboxylate[18a] using the sequence outlined in Scheme 1. HPLC t_r = 9.16 min. (Method B); LCMS (ESI) *m/z* Calcd for $C_{26}H_{21}F_3N_3O_3S$ $[M + H]^+$ 512.13. Found: 512.0. ^{1}H NMR (500 MHz, *DMSO-d*$_6$) δ ppm 7.84 (1 H, d, *J*=7.8 Hz), 7.60 - 7.65 (2 H, m), 7.53 - 7.58 (3 H, m), 7.35 (1 H, s), 7.32 (1 H, d, *J*=7.8 Hz), 3.59 (2 H, s), 3.42 (2 H, s), 3.16 - 3.25 (3 H, m), 3.02 (2 H, t, *J*=6.9 Hz), 2.93 (2 H, t, *J*=6.9 Hz).

1-((3-(5-phenyl-4-(trifluoromethyl)isothiazol-3-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (TFA salt) (50). This compound was prepared starting from methyl 5-phenyl-4-(trifluoromethyl)isothiazole-3-carboxylate[18a] using the sequence outlined in Scheme 1. HPLC 93.6%; t_r = 9.05 min. (Method B); LCMS (ESI) *m/z* Calcd for $C_{26}H_{21}F_3N_3O_3S$ $[M + H]^+$ 512.13. Found: 512.1. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.05 (1 H, d, *J*=7.9 Hz), 7.55 - 7.63 (5 H,

m), 7.53 (1 H, s), 7.49 (1 H, dd, *J*=7.9, 1.8 Hz), 4.47 (2 H, s), 4.32 - 4.43 (4 H, m), 3.67 - 3.78 (1 H, m), 3.11 (4 H, s).

1-((3-(3-(Pyridin-2-yl)-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (51). HPLC t_r = 6.89 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{25}H_{19}F_3N_4O_4$ $[M + H]^+$ 497.1. Found: 497.0. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 3.07 (dd, *J*=12.54, 5.50 Hz, 3H), 3.42 (br. s., 2H) 3.60 (s, 2H), 7.33 (d, *J*=7.92 Hz, 1H), 7.37 (s, 1H), 7.65 (dd, *J*=6.71, 4.95 Hz, 1H), 7.85 (d, *J*=7.92 Hz, 1H), 7.95 (d, *J*=7.70 Hz, 1H), 8.07 (td, *J*=7.65, 1.43 Hz, 1H), and 8.81 (d, *J*=4.40 Hz, 1H).

1-((3-(5-Isobutyl-4-(trifluoromethyl)isoxazol-3-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (52). HPLC t_r = 3.36 min. (Method C); LCMS (ESI) *m/z* Calcd for $C_{24}H_{24}F_3N_3O_4$ $[M + H]^+$ 476.2. Found: 476.3. ^{1}H-NMR (400 MHz, CD_3OD) δ ppm 7.95 (d, *J*=7.96 Hz, 1H), 7.43 (dd, 2H), 7.39 (d, *J*=7.92 Hz, 1H), 4.37 (s, 2H), 4.14-4.30 (m, 4H), 3.56-3.77 (m, 1H), 2.92-3.11 (m, 4H), 2.87 (d, *J*=7.37 Hz, 2H), 2.07 (m, 1H), 0.94 (s, 6H).

1-((3-(5-(1-Methylcyclopropyl)-4-(trifluoromethyl)isoxazol-3-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (53). HPLC t_r = 3.25 min. (Method C); LCMS (ESI) *m/z* Calcd for $C_{24}H_{22}F_3N_3O_4$ $[M + H]^+$ 474.2. Found: 474.2. 1H-NMR (500mhz, CD_3OD) δ ppm 7.94 (d, J = 7.63 Hz, 1H), 7.40 (s, 1H), 7.10-7.44 (m, 1H), 4.36 (s, 2H), 4.10-4.32 (m, 4H), 3.50-3.80 (m, 1H), 2.68-3.13 (m, 4H), 1.39 (s, 3H), 1.00-1.23 (m, 2H), 0.63-1.02 (m, 2H).

1-((3-(4-Isopropoxy-3-(trifluoromethyl)phenyl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (hydrochloric acid salt) (54). HPLC t_r = 8.05 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{26}H_{26}F_3N_2O_4$ $[M + H]^+$ 487.18. Found: 487.1; ^{1}H NMR (400MHz, CD_3OD) δ 8.04 - 7.93 (m, 3H), 7.58 - 7.45 (m, 2H), 7.39 (d, *J*=8.8 Hz, 1H), 4.51 - 4.31 (m, 7H), 3.78 - 3.68 (m, 1H), 3.15 - 3.04 (m, 4H), 1.40 (d, *J*=6.2 Hz, 6H)

1-((3-(3-Cyano-4-isopropoxyphenyl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (55). HPLC t_r = 2.69 min (Method C); LCMS (ESI) *m/z* Calcd for $C_{26}H_{26}N_3O_4$ $[M + H]^+$ 444.19. Found: 444.2; ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.02-8.07 (2 H, m), 7.97 (1 H, d, *J*=7.9 Hz), 7.51 (1 H, s), 7.46 (1 H, dd, *J*=7.8, 1.2 Hz), 7.38 (1 H, d), 4.38 (2 H, s), 4.13-4.23 (5 H, m), 3.40-3.50 (1 H, m), 3.04-3.13 (4 H, m), 1.44 (6 H, d, *J*=5.9 Hz).

1-((3-(4-Isopropoxy-2-(trifluoromethyl)phenyl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (TFA) (56). HPLC t_r = 3.01 min (Method C); LCMS (ESI) *m/z* Calcd for $C_{26}H_{26}F_3N_2O_4$ $[M + H]^+$ 487.18. Found: 487.2; ^{1}H NMR (500 MHz, CD_3OD) δ ppm 8.01 (1 H, d, *J*=7.8 Hz), 7.55 (1 H, d, *J*=8.6 Hz), 7.50 (1 H, s), 7.48 (1 H, dd, *J*=8.0, 1.7 Hz), 7.36 (1 H, d, *J*=2.5 Hz), 7.31 (1 H, dd, *J*=8.6, 2.5 Hz), 4.79 (1 H, spt, *J*=6.1 Hz), 4.46 (2 H, s), 4.32-4.41 (4 H, m), 3.71 (1 H, quin, *J*=8.3 Hz), 3.04 (2 H, t, *J*=7.1 Hz), 2.77 (2 H, t, *J*=7.2 Hz), 1.39 (6 H, d, *J*=6.1 Hz).

1-((3-(4-Isobutyl-3-(trifluoromethyl)phenyl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (TFA) (57). HPLC t_r = 3.55 min. (Method C); LCMS (ESI) *m/z* Calcd for $C_{27}H_{28}F_3N_2O_3$ $[M + H]^+$ 485.21. Found: 485.4. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.83-7.94 (m, 3 H) 7.50 (d, *J*=8.32 Hz, 1 H) 7.38 (s, 1 H) 7.34 (dd, *J*=8.05, 1.66 Hz, 1 H) 4.33 (s, 2 H) 4.18-4.28 (m, 3 H) 3.58 (qd, *J*=8.37, 8.18 Hz, 1 H) 3.15 (1H, hidden under CD_3OD peak) 2.96-3.01 (m, 4 H) 2.62 (d, *J*=6.94 Hz, 2 H) 1.85-1.94 (m, 1 H) 0.84 (d, *J*=6.66 Hz, 6 H).

1-((3-(4-cyclohexyl-3-(trifluoromethyl)phenyl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (TFA) (58). HPLC t_r = 9.63 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{29}H_{30}F_3N_2O_3$ $[M + H]^+$ 511.22. Found: 511.2; ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.99 - 8.05 (3 H, m), 7.78 (1 H, d, *J*=8.6 Hz), 7.51 (1 H, d, *J*=1.1 Hz), 7.47 (1 H, dd, *J*=7.9, 1.8 Hz), 4.46 (2 H, s), 4.33 - 4.38 (4 H, m), 3.63 - 3.74 (1 H, m), 3.09 - 3.15 (4 H, m), 3.00 (1 H, t, *J*=11.7 Hz), 1.77 - 1.96 (5 H, m), 1.54 - 1.67 (2 H, m, *J*=12.0, 12.0, 11.9, 2.1 Hz), 1.35 - 1.53 (3 H, m).

1-((3-(3-(Trifluoromethyl)phenyl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (TFA) (59). HPLC t_r = 3.45 min (Method C); LCMS (ESI) *m/z* Calcd for $C_{23}H_{20}F_3N_2O_3$ $[M + H]^+$ 429.14. Found: 429.1; ^{1}H NMR (400MHz, CD_3OD) δ 8.30 - 8.24 (m, 2H), 8.20 (d, *J*=7.9 Hz, 1H), 8.03 - 7.94 (m, 2H), 7.71 (d, *J*=1.3 Hz, 1H), 7.67 (dd, *J*=7.8, 1.7 Hz, 1H), 4.65 (s, 2H), 4.61 - 4.50 (m, 4H), 3.95 - 3.85 (m, 1H), 3.50 (dt, *J*=3.3, 1.7 Hz, 2H), two protons under MeOH peak.

1-((3-(3,4-diethoxyphenyl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (60). HPLC t_r = 7.93 min. (Method B); LCMS (ESI) *m/z* Calcd for $C_{26}H_{29}N_2O_5$ $[M + H]^+$ 449.21. Found: 449; ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.96 (1 H, d, *J*=7.8 Hz), 7.48 (1 H, s), 7.44 (1 H, dd, *J*=7.9, 1.5 Hz), 7.32 - 7.38 (2 H, m), 7.10 (1 H, d, *J*=8.0 Hz), 4.36 (2 H, s), 4.12 - 4.21 (8 H, m), 3.37 - 3.46 (1 H, m), 3.01 - 3.12 (4 H, m), 1.42 - 1.49 (6 H, m).

1-((3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4H-indeno[1,2-c]isoxazol-6-yl)methyl)azetidine-3-carboxylic acid (TFA) (61). HPLC t_r = 7.72 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{25}H_{19}F_3N_3O_4$ $[M + H]^+$ 482.13. Found: 482.4. ^{1}H - NMR (400 MHz, CD_3OD) δ ppm 8.04 (1 H, d, *J*=7.9 Hz), 7.75 - 7.81 (3 H, m), 7.59 - 7.73 (4 H, m), 4.54 (2 H, s), 4.33 - 4.44 (4 H, m), 4.10 (2 H, s), 3.72 (1 H, quin, *J*=8.3 Hz).

1-((3-(3-Phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (62). HPLC t_r = 7.92 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{26}H_{21}F_3N_3O_4$ $[M + H]^+$ 496.15. Found: 496.0. ^{1}H NMR (500MHz, DMSO-d_6) δ 7.72 - 7.66 (m, 3H), 7.65 - 7.58 (m, 3H), 7.35 (s, 1H), 7.31 (d, *J*=7.5 Hz, 1H), 3.59 (br. s., 2H), 3.42 (br. s., 2H), 3.23 (br. s., 2H), 3.17 (s, 1H), 3.14 - 3.08 (m, 3H), 3.01 - 2.95 (m, 2H)

1-((3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-5,6-dihydro-4H-benzo[3,4]cyclohepta[1,2-d]isoxazol-8-yl)methyl)azetidine-3-carboxylic acid (63). HPLC t_r = 8.04 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{27}H_{23}F_3N_3O_4$ $[M + H]^+$ 510.16. Found: 510.2.

1-((5-Methyl-3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydroisoxazolo[4,3-c]quinolin-7-yl)methyl)azetidine-3-carboxylic acid (64). HPLC t_r = 3.47 min. (Method C); LCMS (ESI) *m/z* Calcd for $C_{26}H_{21}F_3N_4O_4$ $[M + H]^+$ 511.2. Found: 511.3. 1H-NMR (400 MHz, CD_3OD) δ ppm 8.09 (d, *J=7.92 Hz*, 1H), 7.78 (d, *J=7.26*, 2H), 7.32-7.77 (m, 5H), 4.44 (s, 2H), 3.47 (s, 2H), 3.09-3.21 (m, 4H), 2.78-3.01 (m, 4H).

1-((3-(5-Phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4,5-dihydrobenzo[6,7]oxepino[4,5-d]isoxazol-8-yl)methyl)azetidine-3-carboxylic acid (65). HPLC t_r = 8.00 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{26}H_{20}F_3N_3O_5$ $[M + H]^+$ 512.1. Found: 512.5. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 7.95 (d, *J*=7.92 Hz, 1H), 7.80 (d, *J*=7.26 Hz, 2H), 7.64 - 7.76 (m, 3H), 7.17 (dd, *J*=8.25, 1.43 Hz, 1H), 7.05 (d, *J*=1.10 Hz, 1H), 4.37 (t, *J*=4.84 Hz, 2H), 3.58 (s, 2H), 3.16 - 3.46 (m, 5H), 3.14 (t, *J*=4.95 Hz, 2H).

1-((3-(3-Phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydro-2H-benzo[g]indazol-7-yl)methyl)azetidine-3-carboxylic acid (HCl) (66). HPLC t_r = 2.89 min (Method C); LCMS (ESI) *m/z* Calcd for $C_{26}H_{22}F_3N_4O_3$ $[M + H]^+$ 495.16. Found: 495.1.

1-((2-methyl-3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4,5-dihydro-2H-benzo[g]indazol-7-yl)methyl)azetidine-3-carboxylic acid, TFA (67). HPLC t_r = 2.96 min. (Method C); LCMS (ESI) *m/z* Calcd for $C_{27}H_{24}F_3N_4O_3$ $[M + H]^+$ 509.18. Found: 509.2 ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.90 (1 H, d, *J*=8.1 Hz), 7.82 - 7.87 (2 H, m), 7.61 - 7.72 (3 H, m), 7.40 (2 H, dd, *J*=4.1, 2.3 Hz), 4.41 (2 H, s), 4.30 - 4.38 (4 H, m), 3.91 (3 H, s), 3.71 (1 H, quin, *J*=8.4 Hz), 3.02 (2 H, t, *J*=7.3 Hz), 2.76 (2 H, t, *J*=7.3 Hz).

1-((1-methyl-3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4,5-dihydro-1H-benzo[g]indazol-7-yl)methyl)azetidine-3-carboxylic acid, TFA (68). LCMS (ESI) *m/z* Calcd for $C_{27}H_{24}F_3N_4O_3$ [M +

H]$^+$ 509.18. Found: 509.2 ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.35-7.85 (m, 8 H) 4.3-4.6 (hidden under broad CD_3OD peak, 9H) 3.7-3.9 (m, 1 H) 2.85-3.2 (m, 4 H).

1-((2-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[2,1-d]oxazol-7-yl)methyl)azetidine-3-carboxylic acid, TFA (69). HPLC t_r = 7.54 min. (Method B); LCMS (ESI) *m/z* Calcd for $C_{26}H_{20}F_3N_3O_4$ [M + H]$^+$ 496.14. Found: 496.06. ^{1}H NMR (400MHz, CD_3OD) δ ppm 7.67 - 7.62 (m, 3H), 7.61 - 7.53 (m, 3H), 7.45 (d, *J*=4.6 Hz, 2H), 4.40 (s, 2H), 4.32 - 4.27 (m, 4H), 3.65 - 3.55 (m, 1H), 3.29 - 3.23 (m, 2H), 3.08 - 3.03 (m, 2H)

1-((8-Methyl-3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)azetidine-3-carboxylic acid (70). HPLC t_r = 3.60 min. (Method C); LCMS (ESI) *m/z* Calcd for $C_{27}H_{22}F_3N_3O_4$ [M + H]$^+$ 510.2. Found: 510.2. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.80 (d, J=7.26 Hz, 2H), 7.58-7.75 (m, 3H), 7.44 (s, 1H), 4.43 (br. s., 2H), 4.21 (br. s, 4H), 3.40-3.63 (m, 1H), 3.10 (s, 4H), 2.51 (s, 3H).

1-((8-Fluoro-3-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)azetidine-3-carboxylic acid (71). HPLC t_r = 3.54 min. (Method C). LCMS (ESI) *m/z* Calcd for $C_{26}H_{20}F_4N_3O_4$ [M + H]$^+$ 514.14. Found: 514.07. ^{1}H-NMR (400 MHz, CD_3OD) δ ppm 7.70 - 7.93 (1H, m), 7.37 - 7.81 (6H, m), 4.51 (2H, s), 4.31 (4H, br. s.), 2.92 - 3.27 (4H, m), and 2.31 (1H, s).

1-((3-(5-Phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4,5-dihydroisoxazolo[5,4-f]quinolin-7-yl)methyl)azetidine-3-carboxylic acid (72). HPLC t_r = 6.89 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{25}H_{19}F_3N_4O_4$ [M + H]$^+$ 497.1. Found: 497.2. ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm 8.06 (d, *J*=8.05 Hz, 1H), 7.80 (d, *J*=7.21 Hz, 2H), 7.70 - 7.74 (m, 1H), 7.64 - 7.69 (m, 2H), 7.36 (d, *J*=8.05 Hz, 1H), 5.75 (s, 2H), 3.70 (s, 2H), 3.49 (t, *J*=7.63 Hz, 2H), 3.16 - 3.34 (m, 5H), 3.05 - 3.10 (m, 2H).

2-((3R)-1-(2-Hydroxy-2-(3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)ethyl)piperidin-3-yl)acetic acid.HCl (73 and **74).**

Chiral separation of **73** and **74** was accomplished at the ester stage (ChiralCel OJ-H 25 X 3cm , 5μm; 40 ^{0}C; 140mL/min; CO2/ MEOH+0.1%DEA = 85/15; 256 nm). Peak 1 (t_r = 7.7 min, ester of **74**) and peak 2 (t_r = 8.8 min, ester of **73**). Peaks 1 and 2 were hydrolyzed individually to give the corresponding acids **74** and **73**.

73. HPLC t_r = 7.80 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{30}H_{29}F_3N_3O_5$ $[M + H]^+$ 568.21. Found: 568.1; ^{1}H NMR (400MHz, DMSO-d_6) δ 12.34 (br. s., 1H), 9.89 (br. s., 1H), 7.94 (d, *J*=7.9 Hz, 1H), 7.80 (d, *J*=7.3 Hz, 2H), 7.76 - 7.63 (m, 3H), 7.53 (s, 1H), 7.49 (d, *J*=8.1 Hz, 1H), 6.35 (br. s., 1H), 5.22 (br. s., 1H), 3.65 (dd, *J*=19.7, 12.2 Hz, 2H), 3.39 (br. s., 3H), 3.29 - 3.20 (m, 2H), 2.97 - 2.84 (m, 1H), 2.81 - 2.67 (m, 1H), 2.45 - 2.18 (m, 3H), 1.94 - 1.75 (m, 3H), 1.43 - 1.04 (m, 2H)

74. HPLC t_r = 7.84 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{30}H_{29}F_3N_3O_5$ $[M + H]^+$ 568.21. Found: 568.1; ^{1}H NMR (400MHz, DMSO-d_6) δ 12.35 (br. s., 1H), 9.84 (br. s., 1H), 7.94 (d, *J*=7.9 Hz, 1H), 7.80 (d, *J*=7.3 Hz, 2H), 7.76 - 7.64 (m, 3H), 7.53 (s, 1H), 7.50 (d, *J*=8.1 Hz, 1H), 6.39 (br. s., 1H), 5.22 (br. s., 1H), 3.68 (d, *J*=10.8 Hz, 1H), 3.58 (d, *J*=11.0 Hz, 1H), 3.43 - 3.18 (m, 5H), 2.96 - 2.70 (m, 2H), 2.39 - 2.19 (m, 3H), 1.99 - 1.71 (m, 3H), 1.34 - 1.09 (m, 2H)

(3S)-1-(2-Hydroxy-2-(3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)ethyl)piperidine-3-carboxylic acid. HCl (75 and **76)**.

Chiral separation of **75** and **76** was accomplished at the ester stage (ChiralCel OJ-H 25 X 3cm , 5μm; 40 ^{0}C; 150mL/min; CO2/ MEOH+0.1%DEA = 75/25; 250 nm). Peak 1 (t_r = 3.4 min, ester of **75**) and Peak 2 (t_r = 4.5 min, ester of **76**). Peaks 1 and 2 were hydrolyzed individually to give the corresponding acids **75** and **76**.

75. HPLC t_r = 3.15 min. (condition C); LCMS (ESI) *m/z* Calcd for $C_{29}H_{27}F_3N_3O_5$ $[M + H]^+$ 554.19. Found: 554.1. ^{1}H NMR (400MHz, DMSO-d_6) δ 7.95 (d, *J*=7.9 Hz, 1H), 7.80 (d, *J*=7.0 Hz, 2H), 7.76 - 7.64 (m, 3H), 7.54 (s, 1H), 7.51 (d, *J*=8.1 Hz, 1H), 6.38 (br. s., 1H), 5.23 (br. s., 1H), 3.83 (br. s.,

1H), 3.56 (br. s., 1H), 3.32 (br. s., 4H), 3.06 - 2.80 (m, 3H), 2.05 (d, *J*=12.3 Hz, 1H), 2.00 - 1.72 (m, 3H), 1.48 (d, *J*=10.6 Hz, 1H)

76. HPLC t_r = 3.14 min. (condition C); LCMS (ESI) *m/z* Calcd for $C_{29}H_{27}F_3N_3O_5$ $[M + H]^+$ 554.19. Found: 554.0. ^{1}H NMR (400MHz, DMSO-d_6) δ 7.95 (d, *J*=7.9 Hz, 1H), 7.80 (d, *J*=7.3 Hz, 2H), 7.76 - 7.64 (m, 3H), 7.52 (s, 1H), 7.49 (d, *J*=7.9 Hz, 1H), 6.39 (br. s., 1H), 5.26 (br. s., 1H), 3.80 (br. s., 1H), 3.67 (d, *J*=10.8 Hz, 1H), 3.56 - 3.36 (m, 1H), 3.35 - 3.14 (m, 3H), 3.04 - 2.88 (m, 3H), 2.06 (d, *J*=11.7 Hz, 1H), 2.00 - 1.74 (m, 3H), 1.56 - 1.39 (m, 1H)

2-hydroxy-N-(2-(methylsulfonyl)ethyl)-2-(3-(5-phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)acetamide (77 and **78)**

Chiral separation of **77** and **78** was accomplished at the hydroxy acid stage (Chiralcel OJ-H 5 x 25 cm, column temperature 20 °C, isocratic elution with mobile phase 35% acetonitrile + 0.1 TFA in CO_2, 280 mL/min, 250 nm). Peak 1 (t_r = 7.26 min, acid of **77**) and peak 2 (t_r = 9.88 min, acid of **78**). Peaks 1 and 2 were coupled individually with 2-(methylsulfonyl)ethanamine using HATU to yield **77** and **78**.

77. HPLC t_r = 9.25 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{26}H_{23}F_3N_3O_6S$ $[M + H]^+$ 562.13. Found: 562. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.93 (1 H, d, *J*=7.92 Hz), 7.77 (2 H, d, *J*=7.48 Hz), 7.59 - 7.72 (3 H, m), 7.49 - 7.57 (2 H, m), 5.09 (1 H, s), 3.73 (2 H, q, *J*=6.38 Hz), 3.34 (2 H, q, *J*=6.38 Hz), 3.08 (4 H, s), 2.97 (3 H, s)

78. HPLC t_r = 9.23 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{26}H_{23}F_3N_3O_6S$ $[M + H]^+$ 562.13. Found: 562. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.94 (1 H, d, *J*=7.92 Hz), 7.78 (2 H, d, *J*=7.48 Hz), 7.59 - 7.71 (3 H, m), 7.49 - 7.56 (2 H, m), 5.09 (1 H, s), 3.73 (2 H, q, *J*=6.24 Hz), 3.34 (2 H, q, *J*=6.24 Hz), 3.08 (4 H, s), 2.97 (3 H, s)

2-((3-(5-Phenyl-4-(trifluoromethyl)isoxazol-3-yl)-4,5-dihydronaphtho[1,2-c]isoxazol-7-yl)methyl)-2,5,7-triazaspiro[3.4]octane-6,8-dione (79). HPLC t_r = 7.37 min. (Method A); LCMS (ESI) *m/z* Calcd for $C_{27}H_{21}F_3N_5O_4$ $[M + H]^+$ 536.15. Found: 536.3; ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.92 (1 H, d, *J*=7.9 Hz), 7.77 (2 H, d, *J*=7.3 Hz), 7.58-7.71 (3 H, m), 7.40 (1 H, s), 7.37 (1 H, d, *J*=7.7 Hz), 3.78 (2 H, s), 3.75 (2 H, d, *J*=8.6 Hz), 3.48 (2 H, d, *J*=8.8 Hz), 3.08 (4 H, s).

Receptor [35S] GTPγS Binding Assays

Compounds were loaded in a 384 Falcon v-bottom plate (0.5 μl/well in a 3-fold dilution). Membranes prepared from $S1P_1$/CHO cells or EDG3-Ga15-bla HEK293T cells were added to the compound plate (40 μl/well, final protein 3 μg/well) with multidrop liquid handler (Thermo Scientific, Waltham, MA). [^{35}S]GTP (1250 Ci/mmol, Perkin Elmer) was diluted in assay buffer: 20 mM HEPES, pH7.5, 10 mM $MgCl_2$, 150 mM NaCl, 1 mM EGTA, 1 mM DTT, 10 μM GDP, 0.1% fatty acid free BSA, and 10 μg/ml Saponin to 0.4 nM. 40 μl of the [^{35}S] GTP solution was added to the compound plate with a final concentration of 0.2 nM. The reaction was kept at room temperature for 45 min. At the end of incubation, all the mixtures in the compound plate were transferred to a 384 well FB filter plates via GPCR robot system. The filter plate was washed with water 4 times by using the modified manifold Embla plate washer and dried at 60°C for 45 min. 30 μl of MicroScint 20 scintillation fluid was added to each well for counting at Packard TopCount. EC_{50} is defined as the agonist concentration that corresponds to 50% of the Ymax (maximal response) obtained for each individual compound tested.

All procedures involving animals were reviewed and approved by the Institutional Animal Care Use Committee.

Blood Lymphocyte Reduction Assay (BLR) in Rat:

Lewis rats were dosed orally with test article (as a solution or suspension in the vehicle) or vehicle alone (polyethylene glycol 300, "PEG300"). Blood was drawn at 4hr and 24h by retro-orbital bleeding under

isoflurane anesthesia. Blood lymphocyte counts were determined on an ADVIA 120 Hematology Analyzer (Siemens Healthcare Diagnostics). The results were expressed as a reduction in the number of circulating lymphocytes as compared to the vehicle treated group at the 4 hr and 24 hr measurement. The results represent the average results of all animals within each treatment group (n = 3-4).

Rat Adjuvant Induced Arthritis Assay (AA)

Male Lewis rats (200-225 g; Harlan, n=8 treatment group) were immunized at the base of the tail with 100 μl of 10 mg/ml freshly ground *Mycobacterium butyricum* (Difco Laboratories) in incomplete Freund's adjuvant (Sigma). Animals were dosed once daily with the test article (as a solution or suspension in the vehicle) or vehicle alone (PEG300) starting from the day of immunization. The volumes of their hind paws were measured in a water displacement plethysmometer (Ugo Basile, Italy). The baseline paw measurements were taken before onset of the disease (day 7). The paw measurements were then taken three times a week until the end of the study on day 21.

ASSOCIATED CONTENT

Supporting Information. Single crystal X-ray structure of compounds **3** and **10** along with the parameters like crystal system, space group, unit cell parameters, temperature of data collection etc. This information is available free of charge on the ACS Publications website at DOI:

Author Information

Corresponding Author

* T. G. Murali Dhar: (Phone) 609-252-4158,

murali.dhar@bms.com

Author Contributions

The manuscript contains contributions from all authors.

Notes

The authors declare no competing financial interests.

Abbreviations: S1P, Sphingosine-1-phosphate; S1P1-5, Sphingosine-1-phosphate receptors 1-5; GTPγS, Guanosine-5'-O-[gamma-thio]triphosphate; SAR, structure-activity relationship.

REFERENCES

1. (a) Matloubian, M.; Lo,G. G.; Cinamon, G.; Lesneski, M. J.; Xu, Y.; Brinkman, 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*, 355-360. (b) 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. Investigating the molecular mechanisms through which FTY720-P causes persistent S1P1 receptor internalization. *Br. J. Pharmacol*. **2014**, *171*, 4797-4807.
2. (a) Chun J, Hartung H. Mechanism of action of oral fingolimod (FTY720) in multiple sclerosis. *Clinical neuropharmacology* **2010**, *33*, 91-101. (b) Mandala, S.; Hajdu, R.; Bergstorm, J.; Quackenbush, E.; Xie, J.; Milligan, J.; Thornton, R.; Shei, G-J.; Card, D.; Keohane, CA.; Rosenbach, M.; Hale, J.; Lynch, C. L.; Rupprecht, K.; Parsons, W.; Rosen, H. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science* **2002**, *296*, 346-349. (c) 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.
3. Gonzalez-Cabrera, P. J.; Brown, S.; Studer, S. M.; Rosen, H. S1P signaling: new therapies and opportunities. *F1000Prime Reports* **2014**, *6* (doi:10.12703/P6-109).
4. 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.
5. 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-benyl-3-carboxyazetidine sphingosine-1-phosphate-1 receptor agonists. *J. Med. Chem*. **2004**, *47*, 6662-6665.

6. Dyckman, A. J. Recent Advances in the Discovery and Development of Sphingosine-1-Phosphate-1 Receptor Agonists. *Ann. Rep. Med. Chem*. **2012**, *47*, 195-207.
7. (a) 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 ($S1P_3$)-sparing $S1P_1$ agonists active at low oral doses. *J. Med. Chem*. **2016**, *59*, 1003−1020. (b) Buzard, D. J.; Schrader, O.; Zhu, X.; Lehmann, J.; Johnson, B.; Kasem, M.; Kim, S.-H.; Kawasaki, A.; Lopez, L.; Moody, J.; Han, S.; Gao, Y.; Edwards, J.; Barden, J.; Thatte, J.; Gatlin, J.; Jones, R. M. Design and synthesis of new trycyclic indoles as potent modulators of the S1P1 receptor. *Bioorg. Med. Chem. Lett*. **2015**, *25*, 659-663. (c) Buzard, D. J.; Lopez, L.; Moody, J.; Kawasaki, A.; Schrader, T. O.; Kasem, M.; Johnson, B.; Zhu, X.; Thoresen, L.; Kim, S. H.; Gharbaoui, T.; Sengupta, D.; Calvano, L.; Krishnan, A.; Gao, Y.; Semple, G.; Edwards, J.; Barden, J.; Morgan, M.; Usmani, K.; Chen, C.; Sadeque, A.; Chen, W.; Christopher, R. J.; Thatte, J.; Fu, L.; Solomon, M.; Whelan, K.; Al-Shamma, H.; Gatlin, J.; Gaidarov, I.; Anthony, T.; Le, M.; Unett, D. J.; Stirn, S.; Blackburn, A.; Behan, D. P.; Jones, R. M. (7-Benzyloxy-2,3-dihydro-1H-pyrrolo[1,2-a]indol-1-yl)acetic acids as S1P1 functional agonists. *ACS Med. Chem. Lett*. **2014**, *5*, 1334-1339. (d) Skidmore, J.; Heer, J., Jhonson, 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 receptor agonists: effects of acidic, basic, and zwitterionic chemotypes

on pharmacokinetic and pharmacodynamics profiles. *J. Med. Chem*. **2014**, *57*, 10424-10442. (e) Bolli, M. H.; Abele, S.; Birker, M.; Brave, R.; Bur, D.; de Kanter, R.; Kohl, C.; Grimont, J.; Hess, P.; Lescop, C.; Mathys, B.; Muller, C.; Nayler, O.; Rey, M.; Scherz, M.; Schmidt, G.; Seifert, J.; Steiner, B.; Velker, J.; Weller, T. Novel S1P1 receptor agonists – Part 3: From thiophenes to pyridines. *J. Med. Chem*. **2014**, *57*, 110-130. (f) Bolli, M. H.; Velker, J.; Muller, C.; Mathys, B.; Birker, M.; Bravo, R.; Bur, D.; de Kanter, R.; Hess, P.; Kohl, C.; Lehmann, D.; Meyer, S.; Nayler, O.; Rey, M; Scherz, M.; Steiner, B. Novel S1P1 receptor agonists – Part 2: From bicylco[3.1.0]hexane-fused thiophenes to isobutyl substituted thiophenes. *J. Med. Chem*. **2014**, *57*, 78-97. (g) Tian, Y.; Jin, J.; Wang, X.; Hu, J.; Xiao, Q.; Zhou, W.; Chen, X.; Yin, D. Discovery of oxazole and triazole derivatives as potent and selective S1P1 agonists through pharmacophore-guided design. *Eur. J. Med. Chem*. **2014**, *85*, 1-15. (h) Horan, J. C.; Sanyal, S.; Choi, Y.; Hill-Drzewi, M.; Patnaude, L.; Anderson, S.; Fogal, S.; Mao, C.; Cook, B. N.; Gueneva-Boucheva, K.; Fisher, M. B.; Hickey, E.; Pack, E.; Bannen, L. C.; Chan, D. S.; Mac, M. B.; Ng, S. M.; Wang, Y.; Xu, W.; Modis, L. K.; Lemieux, R. M. Piperazinyl-oxadiazoles as selective sphingosine-1-phosphate receptor agonists. *Bioorg. Med. Chem. Lett*. **2014**, *24*, 4807-4811.

8. Bolli, M. H.; Abele, S.; Binkert, C.; Bravo, R.; Buchmann, S.; Bur, D.; Gatfield, J.; Hess, P.; Kohl, C.; Mangold, C.; Mathys, B.; Menyhart, K.; Müller, C.; Nayler, O.; Scherz, M.; Schmidt, G.; Sippel, V.; Steiner, B.; Strasser, D.; Treiber, A.; Weller, T. 2-Imino-thiazolidin- 4-one derivatives as potent, orally active S1P1 receptor agonists. *J.Med. Chem*. **2010**, *53*, 4198−4211.
9. Pan, S.; Gray, N.; Gao, W.; Mi, Y.; Fan, Y.; Wang, X.; Tuntland, T.; Che, J.; Lefebvre, S.; Chen, Y.; Chu, A.; Hinterding, K.; Gardin, A.; End, P.; Heining, P.; Bruns, C.; Cooke, N. G.; Nuesslein-Hildesheim, B. Discovery of BAF312 (siponimod), a potent and selective S1P receptor modulator. *ACS Med. Chem. Lett*. **2013**, *4*, 333−337.

10. Bar-Or, A.; Zipp, F.; Scaramozza, M.; Vollmer, T.; Due, B.; Thangavelu, K.; Fischer, T.; Selmaj, K. Effect of Ceralifimod (ONO-4641), a Sphingosine-1-Phosphate Receptor-1 and -5 Agonist, on Magnetic Resonance Imaging Outcomes in Patients with Multiple Sclerosis: Interim Results from the Extension of the DreaMS Study. *Neurology* **2014**, *82*, suppl. 3.161.
11. Olson, A.; Hartung, J.; Timony, G.; Peach, R.; Boehm, M.; Rosen, H.; Smith, H.; Pan, C.; Brooks, J.; Gujrathu, S. Safety and Tolerability of Orally Administered RPC1063, a Novel S1P1 Receptor Modulator, in Healthy Adult Volunteers, Results of a Phase 1 Study. *Neurology* **2013**, *80* (meeting abstracts 1): P01.178.
12. Buzard, D. J.; Kim, S. H.; Lopez, L.; Kawasaki, A.; Zhu, X.; Moody, J.; Thoresen, T.; Calderon, I.; Ullman, B.; Han, S.; Lehmann, J.; Gharbaoui, T.; Sengupta, D.; Calvano, L.; Montalban, A. G.; Ma, Y-A.; Sage, C.; Gao, Y.; Semple, G.; Edwards, J.; Barden, J.; Morgan, M.; Chen, W.; Usmani, K.; Chen, C.; Sadeque, A.; Christopher, R. J.; Thatte, J.; Fu, L.; Solomon, M.; Mills, D.; Whelan, K.; Al-Shamma, H.; Gatlin, J.; Le, M.; Gaidarov, I.; Anthony, T.; Unett, D. J.; Blackburn, A.; Rueter, J.; Stirn, S.; Behan, D. P.; Jones, R. P. Discovery of APD334: Design of a clinical stage functional antagonist of the sphingosine-1-phosphate-1 receptor. *ACS Med. Chem. Lett*., **2014**, *5*, 1313-1317.
13. Xu, J.; Gray, F.; Henderson, A.; Hicks, K.; Yang, J.; Thompson, P.; Oliver, J. Safety, pharmacokinetics, pharmacodynamics, and bioavailability of GSK2018682, a sphingosine-1-phosphate receptor modulator, in healthy volunteers. *Clinical Pharmacology in Drug Development* **2014**, *3*, 170–178.
14. Forrest, M.; Sun, S-Y.; 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.

15. Gergely, P.; Nuesslein-Hildesheim, B.; Guerini, D.; Brinkmann, V.; Traebert, M.; Bruns, C.; Pan, S.; Gray, N. S.; Hinterding, K.; Cooke, N. G.; Groenewegen, A.; Vitaliti, A.; Sing, T.; Luttringer, O.; Yang, J.; Gardin, A.; Wang, N.; Crumb, W. J. Jr.; Saltzman, M.; Rosenberg, M.; Wallstrom, E. The selective sphingosine 1-phosphate receptor modulator BAF312 redirects lymphocyte distribution and has species-specific effects on heart rate. *Br. J. Pharm*. **2012**, *167*, 1035-1047.

16. Watterson, S. H.; Guo, J.; Spergel, S. H.; Langevine, C. M.; Moquin, R. V.; Shen, D. R.; Yarde, M.; Cvijic, M. E.; Banas, D.; Liu, R.; Suchard, S. J.; Gillooly, K.; Taylor, T.; Rex-Rabe, S.; Shuster, D. J.; McIntyre, K. W.; Cornelius, G.; D'Arienzo, C.; Marino, A.; Balimane, P.; Warrack, B.; Salter-Cid, L.; McKinnon, M.; Barrish, J. C.; Carter, P. H.; Pitts, W. J.; Xie, J.; Dyckman, A. J. Potent and selective agonists of sphingosine 1-phosphate 1 (S1P1): Discovery and SAR of a novel isoxazole based series. *J. Med. Chem*. **2016**, *59*, 2820-2840.

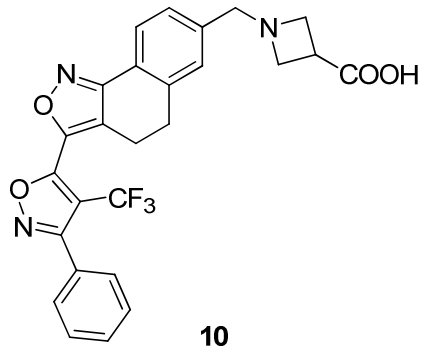
17. Yan, L.; Huo, P.; Doherty, G.; Toth, L.; Hale, J. J.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M. J.; Milligan, J. A.; Shei, G-J.; Chrebet, G.; Bergstrom, J.; Card, D.; Quackenbush, E.; Wickham, A.; Mandala, S. M. Discovery of 3-arylpropionic acids as potent agonists of sphingosine-1-phosphate receptor-1 (S1P1) with high selectivity against all other known S1P receptor subtypes. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 3679-3683.

18. (a) Dhar, T. G. M.; Xiao, H-Y.; Watterson, S. H.; Ko, S.; Dyckman, A. J.; Langevine, C. M.; Das, J.; Cherney, R. J. Patent application WO2011/059784. **2011**. (b) Xiao, H-X.; Watterson, S. H.; Langevine, C. M.; Srivastava, A. S.; Ko, S. S.; Gilmore, J. L.; Sheppeck, J. E.; Zhang, Y.; Cherney, R. J.; Li. L.; Wu, D-R.; Ramasamy, D.; Arunachalam, P.; Mathur, A.; Taylor, T. L.; Shuster, D. J.; McIntyre, K. W.; Chen, D-R.; Yarde, M.; Cvijic, M. E.; Marino, A. M.; Balimane, P. V.; Yang, Z.; Banas, D. M.; Liu, R.; Cornelius, G.; Darienzo, C. J.; Lehman-McKeeman, L.; Salter-Cid, L.; Xie, J.; Barrish, J. C.; Carter, P. H.; Dyckman, A. J.; Dhar, T. G. M. Novel tricyclic modulators of sphingosine-1-phosphate receptor ($S1P_1$). *Abstracts of Papers*, 244th

National Meeting & Exposition of the American Chemical Society, Indianapolis, IN, U.S., September 8-12, 2013; American Chemical Society: Washington, DC, 2013; MEDI-66.

19. Beam, C. F.; Schady, D. A.; Rose, K. L.; Kelley, W. Jr.; Rakkhit, R.; Hornsby, C. D.; Studer-Martinez, S. L. *Synth. Commun*. **2000**, *30*, 3391-3404.
20. Ohigashi, A.; Kanda, A.; Tsuboi, H.; Hashimoto, N. *Org. Process Res. Dev*. **2005**, *9*, 179-184.
21. Liaskopoulos, T.; Skoulika, S.; Tsoungas, P. G.; Varvounis, G. *Synthesis*, **2008**, 711-718.
22. Nishio, T.; Ori, M. *Helv. Chim. Acta*. **2001**, *84*, 2347-2354.
23. Gilmore, J. L.; Sheppeck, J. E.; Watterson, S. H.; Haque, L.; Mukhopadhyay, P.; Tebben, A. J.; Galella, M. A.; Shen, D. R.; Yarde, M.; Cvijic, M. E.; Borowski, V.; Gillooly, K. M.; Taylor, T.; McIntyre, K. W.; Warrack, B.; Levesque, P. C.; Li, J. P.; Cornelius, G.; D'Arienzo, C. J.; Marino, A. M.; Balimane, P. V.; Salter-Cid, L.; Barrish, J. C.; Pitts, W. J.; Carter, P. H.; Xie, J.; Dyckman, A. J. Discovery and structure activity relationship (SAR) of a series of ethanolamine-based direct-acting agonists of sphingosine-1-phosphate (S1P1). *J. Med. Chem*. **2016**, 59, 6248-6264.

Table of Contents Graphic



10

***h*S1P$_1$ GTPγS EC$_{50}$ = 0.59 nM**

***h*S1P$_3$ GTPγS EC$_{50}$ = 4,400 nM**