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Pyrazole derived from (+)-3-carene; a novel potent, selective scaffold for sphingosine-1-phosphate ($S1P_1$) receptor agonists

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ABSTRACT

High throughput screening and hit to lead optimization led to the identification of 'carene' as a promising scaffold showing selective $S1P_1$ receptor agonism. In parallel to this work we have established a pharmacophore model for the $S1P_1$ receptor highlighting the minimal structural requirement necessary for potent receptor agonism.

FTY720 (fingolimod™) is a novel immunomodulator that is highly effective in animal models of transplantation and autoimmunity.[1] Once daily oral treatment with **FTY720** has been shown to be effective in preventing relapsing–remitting multiple sclerosis in a Phase II clinical trial and may represent a novel modality for immunomodulator therapy.[2]

Detailed mechanistic studies have shown that **FTY720** effectively inhibits the egress of T-cells and B-cells from lymph nodes, thereby reducing the number of activated cells that recirculate to peripheral inflammatory tissues.[3] More specifically, it was demonstrated that **FTY720** is acting via its phosphorylated form **FTY720-P**, which is a potent agonist on 4 out of the 5 known S1P receptors.[4] **FTY720-P** acts as a functional antagonist at the $S1P_1$ receptor inducing internalization of the receptor in lymphocytes resulting in blockage of S1P directed migration of the lymphocytes out of the lymph node (see Fig. 1).[5]

The understanding of the complex biology of the S1P receptors has been facilitated by the development of tool compounds with different selectivity and different pharmacokinetic profiles. Numerous groups have used and reported medicinal chemistry efforts using **SEW2871**[6] as a lead structure, leading to, for example, **AUY954** a selective $S1P_1$ agonist with similar efficacy to **FTY720** in rat heterotopic heart transplantation.[7]

High throughput screening of the Novartis Compound Library was carried out by recording compound mediated Ca^{2+} mobilization in CHO cells over-expressing the human $S1P_1$ receptor.[8] Primary hits were validated in a [^{35}S]-GTPγS binding assay using cell membranes prepared from the $hS1P_1$ CHO cells.[7] This led to the identification of **5** as an interesting $S1P_1$ receptor agonist scaffold. It is noteworthy that this pyrazole scaffold is derived from the natural monoterpene (+)-3-carene and has found application in the resolution of racemic *cis*-Permethric and *cis*-*Z*-cyhalothric acids.[9]

In the absence of ligand-bound X-ray co-crystal structures, conclusive information about the biologically active conformation of $S1P_1$ agonists is not available. A common solution to this problem is the use of homology models and point-mutation experiments to

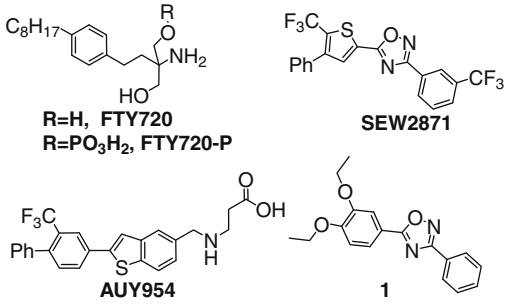

Figure 1. Representative $S1P_1$ agonists.

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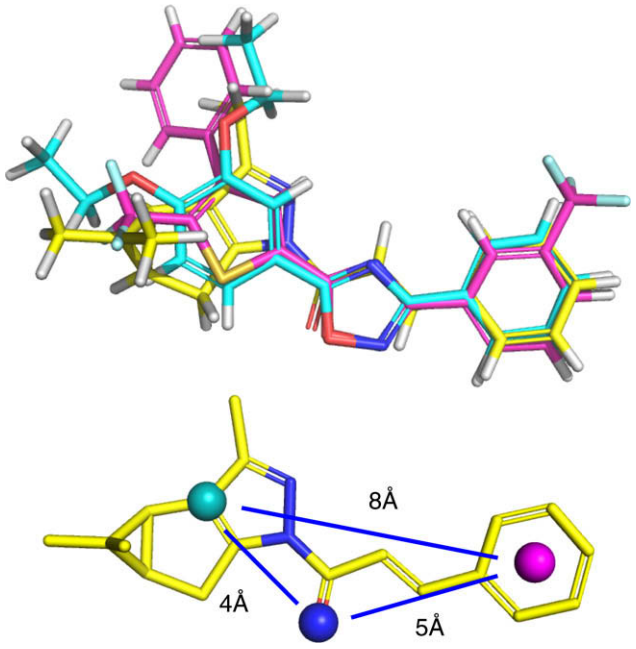


Figure 2. Top: FieldTemplater alignment of the $S1P_1$ agonists **SEW2871** (magenta), **1** (cyan), and carene **5** (yellow). Bottom: A pharmacophore describing the common features of the ligands. The features are a planar hydrophobic group (cyan), an aromatic ring (magenta), and a hydrogen-bond acceptor (blue). The distances indicated are derived from these three structures.

rationalize observed SAR.[10] Another approach to obtaining approximate information to help understand details of the SAR landscape is to flexibly align several known agonists with consistent SAR. We performed such an alignment of **SEW2871**, **1**,[11] and carene analogue **5** using the FieldTemplater software.[12] This procedure results in a very good alignment, volume similarity of 0.75, and overall Cresset similarity of 0.66 (see Fig. 2).

The alignment shows that the central core of all three ligands have a similar shape and suggest a common pharmacophore, shown in Figure 2 below for carene **5**. This common pharmacophore, the minimal structural requirement for activity in these series, is defined by the following features: a planar hydrophobic (or aromatic) group, a hydrogen-bond acceptor, and an aromatic ring. This pharmacophore is consistent with the SAR reported here for the carenes, **SEW2871** and its derivatives, as well as the $S1P_1$ agonists discovered via HTS by Schürer et al., and finally the large series of $S1P_1$ agonists built around a series of five-membered aromatic heterocycles reported by Vachal et al.[6h] This minimal pharmacophore reflects the fact that a polar interaction with the $S1P_1$ receptor, (cf. **FTP-720-P** and **AUY954**) is not required for agonism. This has been demonstrated by Schürer et al.[11] and is further reinforced by the results presented below. In this model it is not necessary to invoke ion–dipole interactions via the trifluoromethyl group in order to explain the activity of **SEW2871**.

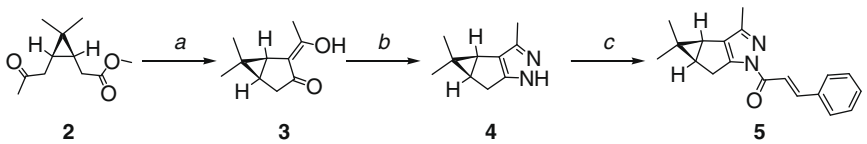
Following the protocol developed by Tkachev,[13] it is possible to prepare large quantities of **4** via the spontaneous cyclization of readily available keto ester **2** to afford bis-ketone **3**, which can be reacted with hydrazine to generate the tri-cyclic pyrazole core **4**. The nitrogen in position 1 of pyrazole **4** can be reacted with a variety of electrophiles such as activated carboxylic acids, sulfonyl chlorides, allyl or alkyl to yield analogues **5**–**19**. The acylation and alkylation reactions of **4** proceed with complete regioselectivity (see Scheme 1).

As described in our pharmacophore analysis, it was clear that carene analogues **5** represented a good starting point for hit to lead optimization as it met the minimal structural requirement for $S1P_1$ potency and offered a high level of rigidity. This rigidity is helpful for achieving high selectivity across the S1P receptor isoforms. Table 1 shows the importance of the H-bond acceptor and linker geometry of the carene analogues. Deletion of the key acceptor oxygen atom from the amide bond of **5** to yield amine **6**, results in a significant decrease in agonism across the S1P receptor panel as predicted by the pharmacophore model. Replacing the amide of **5** with a sulfonamide, **7**, leaves the key acceptor intact but substantially changes the shape of the ligand, resulting in a loss of activity. Reduction of rigidity via hydrogenation of the double bond of **5** to analogue **8** demonstrates that increasing the flexibility of the ligand has a detrimental effect on potency. Finally, analogues **9–11** highlight the importance of the appropriately positioned phenyl group and the overall ligand shape.

Table 2 summarizes our efforts to further improve $S1P_1$ receptor agonism while retaining overall S1P receptor selectivity. We kept the hydrophobic and the H-bond acceptor linker moieties constant and studied the effect of phenyl group modification on the S1P receptors panel. Within the carene series, the phenyl group tolerates a range of different substitution patterns ranging from 3,4-dimethoxy **12**, 2-chloro **13**, to more polar substituents such as trifluoromethyl **14**, sulfonamide **15, 16**, or various heterocycles **17–19.** Introduction of a trifluoromethyl substituent on the carene phenyl group **14**, mimicking the trifluoromethyl-phenyl group of **SEW2871**, does not lead to substantial change in $S1P_1$ agonism, though it does increase activity on $S1P_5$. Increasing polarity in the *meta* position by replacing the phenyl ring with a pyridine or adding polar substituents increases agonism on both $S1P_1$ and $S1P_5$. Moving the polar substituent to the *para* position, such as sulfonamide **16**, substantially decreases agonism on $S1P_1$ and $S1P_5$. The polar head groups in *meta* could be involved in interactions with Arg120 or Glu121[10c] an interaction which is lost when the substituent is moved to *para*. The 4-pyridine **17**, on the other hand shows increased agonism across the panel.

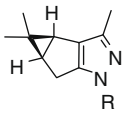
The variety of polar substituents tolerated in the carene series offer a handle to tune ADME properties such as solubility by increasing PSA or introducing charged groups. In conclusion we have optimized carene series via phenyl group modification yielding analogue **19:** a subnanomolar $S1P_1$ agonist with micromolar activity on $S1P_3$ and 250-fold selectivity over $S1P_4$ and $S1P_5$.

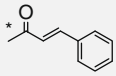
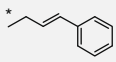
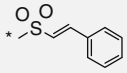
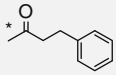
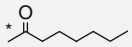
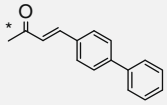
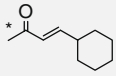
In vitro potent and selective $S1P_1$ agonists **17** and **19** were further evaluated in an in vivo PK/PD model, where lymphocyte count is measured in Lewis rat blood at different time points. Both after 3 mg/kg po and 0.3 mg/kg iv application no significant reduction in lymphocyte count was observed correlating with a lack of blood exposure. Further studies revealed that the compounds were unstable at pH >7 with a $t_{1/2}$ of approximately 3 h for analogues **17**. Characterization of the decomposition product revealed that the amide bond was cleaved to yield amine **4** and the correspond-



Scheme 1. General synthetic route for the preparation of acyl carene. Reagents and conditions: (a) NaOMe–MeOH, reflux, 15 min, (60–80%); (b) $NH_2NH_2{\cdot}H_2O$, ethylene glycol, reflux, 6 h (70–85%); (c) EDCI, HOBt, CH_2Cl_2, rt, 16 h (82%).

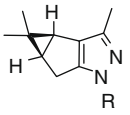
Table 1
Structure–activity relationship of H-bond acceptor linker on S1P receptor panel

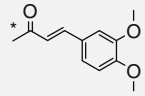
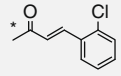
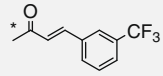
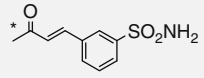
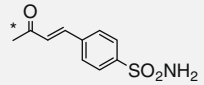
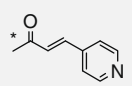
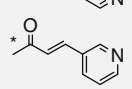
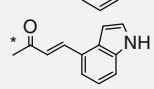


Compds	R	EC_{50}* hS1P$_1$	EC_{50}* hS1P$_3$	EC_{50}* hS1P$_4$	EC_{50}* hS1P$_5$
5		0.034 (82)	>1 (94)	>10 (−2)	1.76 (90)
6		>10 (45)	>10 (14)	>10 (10)	>10 (10)
7		>10 (7)	>10 (−2)	>10 (−19)	>10 (−1)
8		0.086 (55)	>10 (38)	>10 (0)	>10 (26)
9		>10 (25)	>10 (44)	>10 (−6)	>10 (2)
10		0.28 (76)	>10 (8)	>10 (1)	>10 (22)
11		>10 (18)	>10 (3)	>10 (15)	>10 (29)

* EC_{50} obtained in [^{35}S] GTPγS binding assays[7] are reported in μM. Value in bracket represents the E_{max}, % inhibition at 10 μM compared to S1P. Values reported are the mean of two experiments.

Table 2
Structure–activity relationship of head group on S1P receptor



Compds	R	EC_{50}* hS1P$_1$	EC_{50}* hS1P$_3$	EC_{50}* hS1P$_4$	EC_{50}* hS1P$_5$
12		0.025 (89)	>10 (46)	>10 (30)	0.90 (80)
13		0.016 (89)	>1 (69)	>10 (−19)	>10 (42)
14		0.026 (65)	>10 (46)	>10 (17)	0.325 (56)
15		0.011 (92)	1.35 (84)	0.116 (21)	0.082 (76)
16		0.119 (68)	>1 (68)	>10 (−22)	>10 (16)
17		0.007 (92)	0.345 (83)	0.10 (32)	0.13 (93)
18		0.017 (90)	>1 (61)	0.10 (30)	0.345 (87)
19		0.002 (90)	0.995 (84)	0.560 (28)	0.505 (130)

* EC_{50} obtained in [^{35}S] GTPγS binding assays[7] are reported in μM. Value in bracket represents the E_{max}, % inhibition at 10 μM compared to S1P. Values reported are the mean of two experiments.

ing carboxylic acid. It is hypothesized that the acyl carene is acting as an activated carboxylic acid when subjected to various nucleophiles and that amine **4** is acting as a leaving group.

In summary we have discovered and optimized pyrazoles derived from (+)-3-carene, as a novel class of $S1P_1$ agonists with excellent selectivity against all the other S1P isoforms. During the course of this work, we have established a common pharmacophore with existing S1P agonists and defined the minimal structural requirements needed for $S1P_1$ agonism across chemical series.

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References and notes

1. (a) Fujita, T.; Hirose, R.; Yoneta, M.; Sasaki, S.; Inoue, K.; Kiuchi, M.; Hirase, S.; Chiba, K.; Sakamoto, H.; Arita, M. *J. Med. Chem.* **1996**, *39*, 4451; (b) Chiba, K.; Yanagawa, Y.; Masubuchi, Y.; Kataoka, H.; Kawaguchi, T.; Ohtsuki, M.; Hoshino, Y. *J. Immunol.* **1998**, *160*, 5037.
2. O'Connor, P.; Comi, G.; Montalban, X.; Antel, J.; Radue, E. W.; de Vera, A.; Pohlmann, H.; Kappos, L. *Neurology* **2009**, *72*, 73.
3. (a) Brinkmann, V.; Cyster, J. G.; Hla, T. *Am. J. Transplant.* **2004**, *4*, 1019; (b) Brinkmann, V.; Lynch, K. R. *Curr. Opin. Immunol.* **2002**, *14*, 569.
4. For recent reviews on S1P receptor, see: (a) Cyster, J. G. *Annu. Rev. Immunol.* **2005**, *23*, 127; (b) Rosen, H.; Goetzl, E. J. *Nat. Rev. Immunol.* **2005**, *5*, 560; (c) Brinkmann, V. *Pharmacol. Ther.* **2007**, *115*, 84; (d) Cooke, N. G.; Zécri, F. Z. *Annu. Rep. Med. Chem.* **2007**, *42*, 245; (e) Marsolais, D.; Rosen, H. *Nat. Rev. Drug Disc.* **2009**, *8*, 297.
5. (a) Matloubian, M.; Charles, C. G.; Cinamon, G.; Lesneski, M. J.; Xu, Y.; Brinkmann, V.; Allende, M. L.; Maria, L.; Proia, R. L.; Cyster, J. G. *Nature* **2004**, *427*, 355; (b) Schwab, S. R.; Pereira, J. P.; Matloubian, M.; Xu, Y.; Huang, Y.; Cyster, J. G. *Science* **2005**, *309*, 1735.
6. For SEW2871 related publication, see: (a) Hale, J. J.; Lynch, C. L.; Neway, W.; Mils, 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. *J. Med. Chem.* **2004**, *47*, 6662; (b) Sanna, M. G.; Liao, J.; Jo, E.; Alfonso, C.; Ahn, M.-Y.; Peterson, M. S.; Webb, B.; Lefebvre, S.; Chun, J.; Gray, N.; Rosen, H. *J. Biol. Chem.* **2004**, *279*, 13839; (c) Anari, M. R.; Creighton, M. D.; Ngui, J. S.; Tschrret-Guth, R. A.; Teffera, Y.; Doss, G. A.; Tang, W.; Yu, N. X.; Ciccotto, S. L.; Hobra, D. F.; Coleman, J. B.; Vincent, S. H.; Evans, D. C. *Drug Metab. Dispos.* **2006**, *34*, 1367; (d) Li, Z.; Chen, W.; Hale, J. J.; Lynch, C. L.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M. J.; Milligan, J. A.; Shei, G.-J.; Chrebet, G.; Parent, S. A.; Bergstrom, J.; Card, D.; Forrest, M.; Quackenbush, E. J.; Wickham, L. A.; Vargas, H.; Evans, R. M.; Rosen, H.; Mandala, S. *J. Med. Chem.* **2005**, *48*, 6169; (e) Colandrea, V. J.; Legiec, I. E.; Huo, P.; Yan, L.; Hale, J. J.; Mills, S. G.; Bergstrom, J.; Card, D.; Chebret, G.; Hajdu, R.; Keohane, C. A.; Milligan, J. A.; Rosenbach, M. J.; Shei, G.-J.; Mandala, S. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2905; (f) Yan, L.; Budhu, R.; Huo, P.; Lynch, C. L.; Hale, J. J.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M. J.; Milligan, J. A.; Shei, G.-J.; Chrebet, G.; Bergstom, J.; Card, D.; Mandala, S. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3564; (g) 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. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3679; (h) Vachal, P.; Toth, L.; Hale, J. J.; Yan, L.; Mills, S. G.; Chrebet, G.; Keohane, C. A.; Hajdu, R.; Milligan, J. A.; Rosenbach, M. J.; Mandala, S. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3684; (i) Yan, L.; Huo, P.; 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.; Mandala, S. M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 828.
7. Pan, S.; Mi, Y.; Pally, C.; Beerli, C.; Chen, A.; Guerini, D.; Hinterding, K.; Nuesslein-Hildesheim, B.; Tuntland, T.; Lefebvre, S.; Liu, Y.; Gao, W.; Chu, A.; Brinkmann, V.; Bruns, C.; Streiff, M.; Cannet, C.; Cooke, N.; Gray, N. *Chem. Biol.* **2006**, *13*, 1227.
8. Ullrich, T.; Ghobrial, M.; Peters, C.; Billich, A.; Guerini, D.; Nussbaumer, P. *Chem. Med. Chem.* **2008**, *3*, 356.
9. Popov, S. A.; Tkachev, A. V. *Tetrahedron: Asymmetry* **1995**, *6*, 1013.
10. (a) Deng, Q.; Clemas, J. A.; Chrebet, G.; Fischer, P.; Hale, J. J.; Li, Z.; Mills, S. G.; Bergstrom, J.; Mandala, S.; Mosley, R.; Parent, S. A. *Mol. Pharmacol.* **2007**, *71*, 724; (b) Fujiwara, Y.; Osborne, D. A.; Walker, M. D.; Wang, D.-A.; Bautista, D. A.; Liliom, K.; Van Brocklyn, J. R.; Parrill, A. L.; Tigyi, G. *J. Biol. Chem.* **2007**, *282*, 2374; (c) Jo, E.; Sanna, M. G.; Gonzalez-Cabrera, P. J.; Thangada, S.; Tigyi, G.; Osborne, D. A.; Hla, T.; Parrill, A. L.; Rosen, H. *Chem. Biol.* **2005**, *12*, 703.
11. Schürer, S. C.; Brown, S. J.; Gonzalez-Cabrera, P. J.; Schaeffer, M.-T.; Chapman, J.; Jo, E.; Chase, P.; Spicer, T.; Hodder, P.; Rosen, H. *Chem. Biol.* **2008**, *3*, 486.
12. FieldTemplater v2.1.0. http://www.cresset-bmd.com.
13. Popov, S. A.; Denisov, A. Y.; Gatilov, Y. V.; Bagryanskaya, I. Y.; Tkachev, A. V. *Tetrahedron: Asymmetry* **1994**, *5*, 479.