Bioorganic & Medicinal Chemistry Letters 22 (2012) 2794–2797

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Indole-propionic acid derivatives as potent, $S1P_3$-sparing and EAE efficacious sphingosine-1-phosphate 1 ($S1P_1$) receptor agonists

Qinghua Meng, Baowei Zhao, Qiongfeng Xu, Xuesong Xu, Guanghui Deng, Chengyong Li, Linbo Luan, Feng Ren, Hailong Wang, Heng Xu, Yan Xu, Haibo Zhang, Jia-Ning Xiang, John D. Elliott, Taylor B. Guo, Yonggang Zhao, Wei Zhang, Hongtao Lu, Xichen Lin *

Research and Development, GlaxoSmithKline Pharmaceuticals, 898 Halei Road, Zhangjiang Hi-tech Park, Pudong, Shanghai 201023, China

ARTICLE INFO

Article history:
Received 10 December 2011
Revised 8 February 2012
Accepted 24 February 2012
Available online 3 March 2012

Keywords:
Sphingosine-1-phosphate
Agonist
Lymphopenia
Experimental autoimmune encephalomyelitis (EAE)
SAR

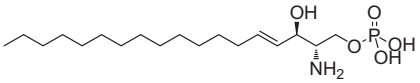
ABSTRACT

Novel indole-propionic acid derivatives were developed as sphingosine-1-phosphate (S1P) receptor agonists through a systematic SAR study. The optimized and $S1P_3$ selective $S1P_1$ agonist **9f** induced peripheral blood lymphocyte reduction in vivo and has an excellent efficacy in mouse experimental autoimmune encephalomyelitis (EAE).

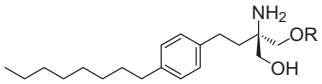
Sphingonsine-1-phosphate[1] (S1P) induces a range of cellular responses by its interaction with the S1P family of G-protein coupled receptors (GPCRs). A total of five S1P receptors are known: $S1P_1$–$S1P_5$. The in vivo immunosuppressive efficacy of a non-selective S1P receptor agonist has been evidenced by the numerous preclinical and clinical studies of FTY720 (fingolimod, Fig. 1), which has been recently approved for the treatment of remitting relapsing multiple sclerosis (RRMS) by FDA.[2] Recent studies have demonstrated that S1P receptor agonists affect lymphocyte trafficking and an interaction with $S1P_1$ receptors on lymphocytes drives the observed pharmacodynamics.[3] On the other hand, fingolimod's $S1P_3$ activity has been linked to mediate vascular side effects seen in clinical trials.[4] Hence, there is a need for $S1P_1$ receptor agonist compounds with selectivity over $S1P_3$ which is expected to show a reduced tendency to induce bradycardia and hypertension.[4] The understanding of FTY720 unique mode of action has triggered intensive effort towards the discovery of $S1P_1$ agonists with increased degree of selectivity versus $S1P_3$.[5] Herein, we report our efforts to identify orally active $S1P_1$, selective over $S1P_3$.

We recently identified 3-[4-(5-aryl-1, 2, 4-oxadiazol-3-yl)-1*H*-indol-1-yl]propanoic acid series (**4**) (Fig. 2) are potent $S1P_1$ receptor agonists without $S1P_3$ activity.[6] In order to fully understand the SAR, we synthesized different substituent patterns of 1,3,4-oxadiazole and propanoic acids at the indole ring (**5**). As showed in Scheme 1, treatment of the nitrile (**5a**) with hydroxylamine provided *N*-hydroxylamidine (**5b**), which was coupled with various benzoic acids followed by dehydration at elevated temperature to afford the oxadiazole esters (**5c**). The esters were hydrolyzed to afford the desired acids (**5**). The indole derivatives and benzoic acids used to prepare the new analogs were synthesized based on literature precedents.[7]

A direct comparison of different substitution patterns of the indole showed that pEC_{50} of the agonists varied depending on the orientations of the indole in Tango assay[8] (Table 1). The selectivity over $S1P_3$ is generally good as determined by $S1P_3$ Ca^{2+} mobilization in $hS1P_3$ assay.[9] In this set, **6c** showed the best $S1P_1$ potency and $S1P_3$ selectivity, therefore, was selected for further exploration.



1, sphingosine-1-phosphate, S1P



2, R = H, FTY720
3, R = $PO(OH)_2$, FTY720 phosphate

Figure 1. Structure S1P (**1**), FTY720 (**2**), FTY720 phosphate (**3**).

* Corresponding author. Tel.: +86 21 6159 0718; fax: +86 21 6159 0730.
E-mail address: Xichen.2.Lin@gsk.com (X. Lin).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.02.083

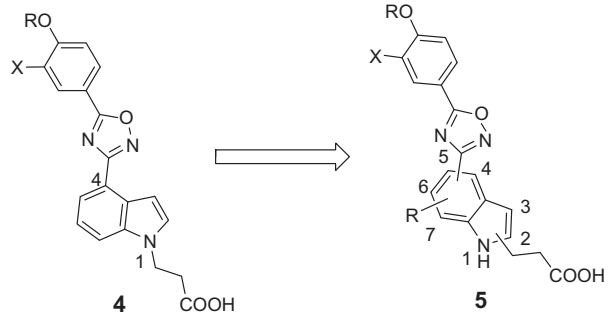
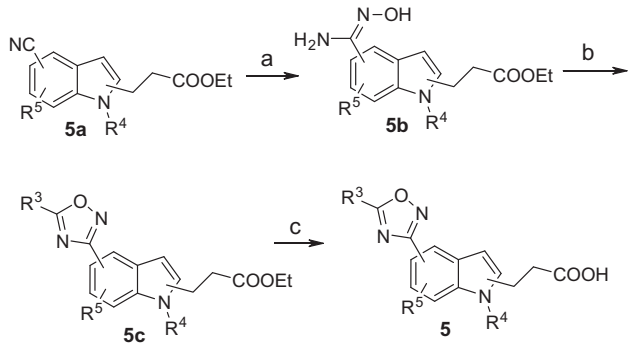
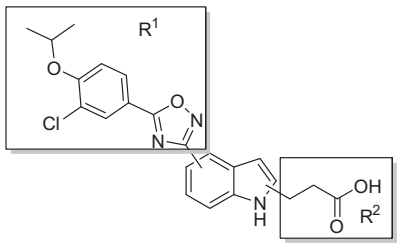


Figure 2. General structures of 3-[4-(5-Aryl-1,2,4-oxadiazol-3-yl)-1*H*-indol-1-yl]propanoic acid (**4**) series and the new scaffold (**5**).



Scheme 1. Synthesis of indole propanoic acids. Reagent: (a) $NH_2OH{\cdot}HCl$, $NaHCO_3$, EtOH; (b) R_3COOH, EDC, HOBt, TBAF, THF; (c) NaOH, MeOH, H_2O and then HCl.

Table 1
$S1P_1$ and $S1P_3$ pEC_{50} values for compounds (**6a**–**6e**)



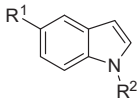
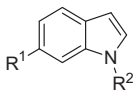
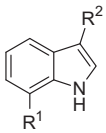
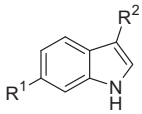
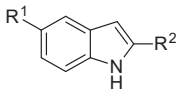
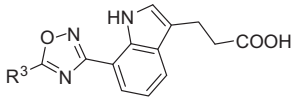
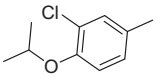
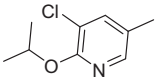
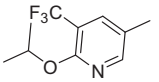
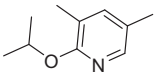
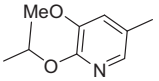
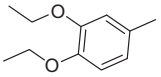
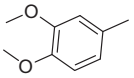
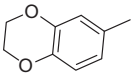
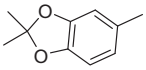
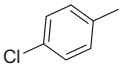
Compound	Structure	$S1P_1$ pEC_{50}	$S1P_3$ pEC_{50}
6a		8.6	<5.0
6b		6.4	<5.0
6c		10.9	<5.0
6d		9.6	<5.0
6e		9.7	<5.0

Table 2
$S1P_1$ and $S1P_3$ pEC_{50} values for compounds (**6c** and **7a**–**7k**)



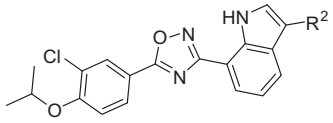
Compound	R^3	$S1P_1$ pEC_{50}	$S1P_3$ pEC_{50}
6c		10.9	<5.0
7a		9.4	<5.0
7b		9.6	<5.0
7c		8.8	<5.0
7d		7.0	<5.0
7e		8.5	<5.0
7f		5.9	<5.0
7g		<5.5	<5.0
7h		6.4	<5.0
7i		5.5	<5.0
7j		<5	<5.0
7k		<5	<5.0

As shown in (Tables 2 and 4) -isopropoxyl substituted aromatic rings are crucial for the in vitro potency (**6c** and **7a**–**7c**). Replacements of the isopropoxyl group with other alkoxyl groups (**7e**–**7h**) or aliphatic rings (**7j**–**7k**) yielded much less activity. The 4-isopropoxyl substituted phenyl ring (**6c**) is more potent than its pyridine analogs (**7a**). Compounds with electron withdrawing groups such as –Cl, $–CF_3$, showed higher potency than that with electron donating group such as –Me, –OMe. Based on these data, 3-chloro-4-isopropoxyl phenyl group was chosen as the moiety for further investigation.

Among all carboxylic acids with various distances, the 3-indolepropionic acid **6c** was the most potent $S1P_1$ agonist (Table 3). We also evaluated carboxylic acid bioisosteres including the primary, secondary amides and tetrazoles which provided similar potency.

As the final step, we focused on various substituents on the indole moiety for further SAR study. Methylated indole ring **9a** was found to be optimal to enhance $S1P_1$ potency. Depending on the electron properties and position, other substituents on the indole ring significantly affected the $S1P_1$ pEC_{50}. Electron withdrawing groups are favorable over electron donating groups (**9f** vs **9e**). 4- or 5-F substituted molecules gave much improved $S1P_1$ potency than 6-F substituted one (**9f, 9g** vs **9h**).

Table 3
$S1P_1$ and $S1P_3$ pEC_{50} values for compounds (**7c** and **8a–8g**)



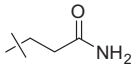
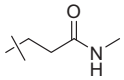
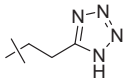
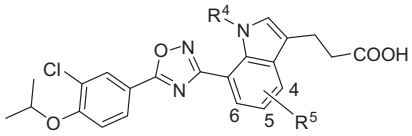
Compound	R^2	$S1P_1$ pEC_{50}	$S1P_3$ pEC_{50}
8a	–COOH	8.3	<5.0
8b	$-CH_2COOH$	7.4	<5.0
6c	$-(CH_2)_2COOH$	10.9	<5.0
8c	$-(CH_2)_3COOH$	9.4	<5.0
8d	$-(CH_2)_4COOH$	10.3	<5.0
8e		10.5	<5.0
8f		10.4	<5.0
8g		10.1	<5.0

Table 4
$S1P_1$ and $S1P_3$ pEC_{50} values for compounds (**9a–9h**)



Compound	R^4	R^5	$S1P_1$ pEC_{50}	$S1P_3$ pEC_{50}
9a	–Me	/	11.2	<5
9b	–Et	/	9.5	<5
9c	–Pr	/	6.7	<5
9d	–*i*Pr	/	7.7	<5
9e	–Me	4-OMe	8.2	<5
9f	–Me	4-F	>11	<5
9g	–Me	5-F	>11	<5
9h	–Me	6-F	7.6	<5

Table 5
In vivo profile of **9f**

Assay	**9f**	
Lymphopenia[a]	21%@4 h 114@24 h	
Brain/Blood ratio[b]	0.35@1 h 0.48@2 h 0.48@4 h	
Mouse PK[c]	CL_b (mL/min/kg) V_{ss} (L/kg) C_{max} (ng/mL) $T_{1/2}$ (h) *F*, po (%)	3.22 0.84 1700 3.83 100

[a] Effect of a single po dose (1 mg/kg) on circulating lymphocyte counts in naïve B6 mice over 24 h. Presented as % cells relative to time 0 normalized against vehicle.
[b] The brain and blood concentration was analyzed after a single ip dose (2 mg/kg).
[c] Male C57BL/6 mice, 1/2 mg/kg (iv/po).

With these data, **9f** was tested in an in vivo peripheral lymphocyte reduction assay.[10] Following a single administration at an oral dose of 1 mg/kg, **9f** led to 21% peripheral lymphocyte reduction in mice at 4 h. Next we measured the time course for **9f** lymphopenia profile and whether the effects could be reversed upon time. The lymphocyte count normalized at 24 h after the single dose. Compared to fingolimod,[11] **9f** showed much less severe and more transient lymphopenia effect (Table 5). Given the promising lymphopenia data, we further evaluated **9f** in CNS, PK and EAE study. Mice CNS penetration assays demonstrated that **9f** was able to enter the CNS with a brain to blood ratio of 0.48. Pharmacokinetics analysis of **9f** in mice revealed a low systemic clearance, low volume of distribution and high C_{max}. Compound **9f** also had good oral bioavailability (100%) and a moderate terminal half-life (3.83 h) (Table 5). On the basis of its $S1P_1$ receptor agonist potency, selectivity and pharmacokinetic profiles, **9f** was evaluated in a mouse experimental autoimmune encephalomyelitis (EAE) model with a head-to-head comparation with fingolimod.[12] As shown in Figure 3, compound **9f** showed a comparable in vivo efficacy to fingolimod.

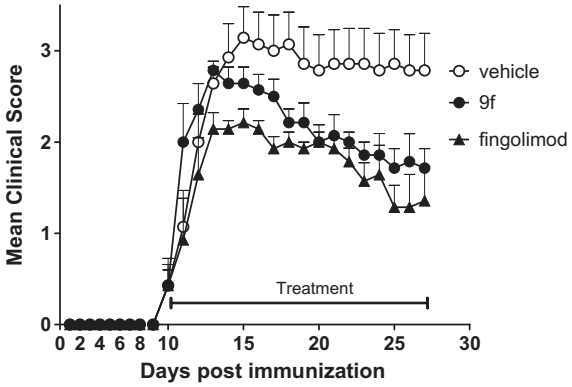


Figure 3. EAE results of compound **9f**.

In conclusion, we conducted a systematic SAR analysis and identified potent and selective $S1P_1$ receptor agonists. Within the set of indole compounds, **9f** showed excellent PK profile and similar efficacy to fingolimod. Details of further evaluation of **9f** in other preclinical studies and safety evaluation will be reported in due course.

Acknowledgments

We thank Drs. Eric Y. Yang, Hong Lu, Zongping Zhang, and Jiansong Yang for help for comments and suggestions.

References and notes

1. For recent reviews on S1P receptors, see: (a) Cusack, K. P.; Stoffel, R. H. *Curr. Opin. Drug. Discov. Devel.* **2010**, *13*, 481; (b) Verzijl, D.; Peters, S. L. M.; Alewijnse, A. E. *Mol. Cells* **2010**, *29*, 99; (c) Rosen, H.; Gonzalez-Cabrera, P. J.; Sanna, M. G.; Brown, S. *Annu. Rev. Biochem.* **2009**, *78*, 743; (d) Brinkmann, V. *Pharmacol. Ther.* **2007**, *115*, 84; (e) Meyer-zu-Heringdorf, D.; Jakobs, K. H. *Biochim. Biophys. Acta* **2006**, *1768*, 923; (f) Gardell, S. E.; Dubi, A. E.; Chun, J. *Trends Mol. Med.* **2006**, *12*, 65.
2. Brinkmann, V.; Billich, A.; Baumruker, T.; Heining, P.; Schmouder, R.; Francis, G.; Aradhye, S.; Burtin, P. *Nature Rev. Drug Discov.* **2010**, *9*, 883.
3. (a) Matloubian, M.; Lo, C. G.; Cinamon, G.; Lesneski, M. J.; Xu, Y.; Brinkmann, V.; Allende, M. L.; Proia, R. L.; Cyster, J. G. *Nature* **2004**, *427*, 355; (b) Allende, M. L.; Dreier, J. L.; Mandala, S.; Proia, R. L. *J. Biol. Chem.* **2004**, *279*, 15396.
4. (a) Forrest, M.; Sun, S.-Y.; Hajdu, R.; Bergstrom, J.; Card, D.; Doherty, G.; Hale, J.; Keohane, C.; Meyers, C.; Milligan, J.; Mills, S.; Nomura, N.; Rosen, H.; Rosenbach, M.; Shei, G.-J.; Singer, I. I.; Tian, M.; West, S.; White, V.; Xie, J.; Proia, R. L.; Mandala, S. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 758; (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.
5. (a) Buzard, D. J.; Thatte, J.; Lerner, M.; Edwards, J.; Jones, R. M. *Expert Opin. Ther. Patents* **2008**, *18*, 1141; (b) Crosignani, S.; Bombrun, A.; Covini, D.; Maio, M.; Marin, D.; Quattropani, A.; Swinnen, D.; Simpson, D.; Sauer, W.; Francon, B.; Martin, T.; Cambet, Y.; Nichols, A.; Martinou, I.; Burgat-Charvillon, F.; Rivron, D.; Donini, C.; Schott, O.; Eligert, V.; Novo-Perez, L.; Vitte, P.-A.; Arrighi, J.-F. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1516; (c) Nishi, T.; Miyazaki, S.; Takemoto, T.; Suzuki, K.; Iio, Y.; Nakajima, K.; Ohnuki, T.; Kawase, Y.; Nara, F.; Inaba, S.; Izumi,

T.; Yuita, H.; Oshima, K.; Doi, H.; Inoue, R.; Tomisato, W.; Kagari, T.; Shimozato, T. *ACS Med. Chem. Lett.* **2011**, *2*, 368; (d) 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.; Mɇuller, C.; Nayler, O.; Scherz, M.; Schmidt, G.; Sippel, V.; Steiner, B.; Strasser, D.; Treiber, A.; Weller, T. *J. Med. Chem.* **2010**, *53*, 4198; (e) Cee, V. J.; Frohn, M.; Lanman, B. A.; Golden, J.; Muller, K.; Neira, S.; Pickrell, A.; Arnett, H.; Buys, J.; Gore, A.; Fiorino, M.; Horner, M.; Itano, A.; Lee, M. R.; McElvain, M.; Middleton, S.; Schrag, M.; Rivenzon-Segal, D.; Vargas, H. M.; Xu, H.; Xu, Y.; Zang, X.; Siu, J.; Wong, M.; Burli, R. W. *ACS Med. Chem. Lett.* **2011**, *2*, 107; (f) Demont, E. H.; Andrews, B. I.; Bit, R. A.; Campbell, C. A.; Cooke, J. W. B.; Deeks, N.; Desai, S.; Dowell, S. J.; Gaskin, P.; Gray, J. R. J.; Haynes, A.; Holmes, D. S.; Kumar, U.; Morse, M. A.; Osborne, G. J.; Panchal, T.; Patel, B.; Perboni, A.; Taylor, S.; Watson, R.; Witherington, J.; Willis, R. *ACS Med Chem. Lett.* **2011**, *2*, 444; (g) Buzard, D.; Han, S.; Thoresen, L.; Moody, J.; Lopez, L.; Kawasaki, A.; Schrader, T.; Sage, C.; Gao, Y.; Edwards, J.; Barden, J.; Thatte, J.; Fu, L.; Solomon, M.; Liu, L.; Al-Shamma, H.; Gatlin, J.; Le, M.; Xing, C.; Espinola, S.; Jones, R. M. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6013; (h) Kurata, H.; Kusumi, K.; Otsuki, K.; Suzuki, R.; Kurono, M.; Takada, Y.; Shioya, H.; Komiya, T.; Mizuno, H.; Ono, T.; Hagiya, H.; Minami, M.; Nakade, S.; Habashita, H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3885.

6. Ahmed, M.; Myatt, J.; Norton, D.; Rivers, D. A. PCT Int. Appl. 2008, WO 2008074821 A1.
7. (a) Sundberg, R. J.; The Chemistry of Indoles Academic Press: New York, 1970; (b) Gribble, G. W. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1045. and references therein.; (c) Schlosser, M.; Ginanneschi, A.; Lerous, F. *Eur. J. Org. Chem.* **2006**, 2956.
8. S1P1 Tango assay was conducted according to manufacturer's protocol (Catalog K1520, Invitrogen, Carlsbad, CA).
9. S1P3 GeneBLAzer assay was conducted according to manufacturer's protocol (Catalog K1713, Invitrogen, Carlsbad, CA).
10. (a) Mandala, S.; Hajdu, R.; Bergstrom, J.; Quackenbush, E.; Xie, J.; Milligan, J.; Thornton, R.; Shei, G. J.; Card, D.; Keohane, C., et al *Science* **2002**, *296*, 346; (b) Sanna, M. G.; Liao, J.; Jo, E.; Alfonso, C.; Ahn, M. Y.; Peterson, M. S.; Webb, B.; Lefebvre, S.; Chun, J.; Gray, N., et al *J. Biol. Chem.* **2004**, *279*, 434.
11. Papadopoulos, D.; Rundle, J.; Patel, R.; Marshall, I.; Stretton, J.; Eaton, R.; Richardson, J. C.; Gonzalez, M. I.; Philpott, K. L.; Reynolds, R. *J. Neurosci. Res.* **2010**, *88*, 346.
12. Peiris, M.; Monteith, G. R.; Roberts-Thomson, S. J.; Cabot, P. J. *J. Neurosci. Methods* **2007**, *163*, 245.