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Original article

Discovery of oxazole and triazole derivatives as potent and selective $S1P_1$ agonists through pharmacophore-guided design

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ABSTRACT

We have discovered a series of triazole/oxazole-containing 2-substituted 2-aminopropane-1,3-diol derivatives as potent and selective $S1P_1$ agonists (prodrugs) based on pharmacophore-guided rational design. Most compounds showed high affinity and selectivity for $S1P_1$ receptor. Compounds **19b**, **19d** and **19p** displayed clear dose responsiveness in the lymphocyte reduction model when administered orally at doses of 0.3, 1.0, 3.0 mg/kg with reduced effect on heart rate. These three compounds were also identified to have favorable pharmacokinetic properties.

1. Introduction

Sphingosine-1-phosphate (S1P, **1**, Fig. 1), a metabolite of sphingomyelin, is a bioactive lysophospholipid that regulates an array of physiological processes including angiogenesis, endothelial barrier enhancement, airway and blood vessel constriction, alveolar epithelial barrier disruption, lymphocyte trafficking, heart rate modulation, neurite extension, and bone homeostasis [1]. S1P affects these functions through interacting with a class A family of G-protein coupled receptors named $S1P_{1-5}$ receptors [2,3]. In recent years, the role of $S1P_1$ receptor in autoimmune diseases such as multiple sclerosis (MS) and lupus erythematosus has become the focus of intense research [4,5], partly inspired by the therapeutic development of FTY720 (fingolimod, **2**), which was approved by the FDA as the first orally active drug for the treatment of relapsing-remitting MS (RRMS) in 2010 [6,7]. FTY720 was identified as a prodrug, phosphorylated *in vivo* by sphingosine kinase 2 (SPHK2) to the active monophosphate ester (FTY720-P, **3**), which can activate four of five $S1P_1$ receptors ($S1P_{1,3-5}$) at nanomolar level [8,9]. FTY720-P was shown to elicit its immunosuppressive effect through activation of $S1P_1$ receptor, which leads to sequestration of lymphocytes in secondary lymphoid organs, preventing them from trafficking to lymphoid tissues and blocking the egress of mature thymocytes from the thymus [10].

On the other hand, the activation of $S1P_3$ receptor is thought to be responsible for the cardiovascular side effect, since treatment with FTY720 resulted in bradycardia in normal but not in $S1P_3$ knockout mice [11,12]. However, the intensive study of BAF312 (**4**), a dual $S1P_{1,5}$ agonist sparing $S1P_3$ activity, suggested species-dependent bradycardia and a dominant role of $S1P_1$ in mediating heart rate in humans via activation of the G protein-coupled inwardly-rectifying potassium (GIRK/IKAch) channel in cardiomyocytes [13–15]. Since there are other side-effects associated with $S1P_3$ agonism such as macular edema and decreased pulmonary function, numerous research groups still focus on discovering $S1P_3$-sparing $S1P_1$ agonists to improve the safety profile [16–19].

Pharmacophore modeling has been identified as a well-behaved approach to explore common chemical characteristics among a considerable number of structures with great diversity [20]. In this

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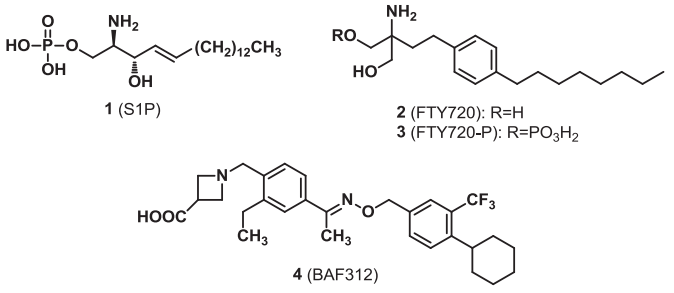

Fig. 1. Structures of S1P, FTY720, FTY720-P and BAF312.

study, a highly predictive 3D QSAR pharmacophore model based on a chemically diverse set of $S1P_1$ agonists was successfully developed, which could guide us to design desired molecules. On the basis of the best hypothesis, a series of 2-substituted 2-aminopropane-1,3-diol derivatives containing oxazole and triazole scaffold were designed and synthesized. In addition, the pharmacological effects such as $S1P_1$ and $S1P_3$ agonistic activity, peripheral blood lymphocyte lowering effects, influence on heart rate and pharmacokinetics (PK) profiles were also evaluated. Three compounds (**19b**, **19d** and **19p**) were found to have good *in vitro* and *in vivo* activities as well as favorable PK profiles, which had the potential for further development.

2. Results and discussion

2.1. Pharmacophore-guided molecular design

For the pharmacophore modeling studies, a set of 60 compounds was collected from the literature, which was divided into a training set of 36 compounds and a test set of 24 compounds [21]. The pharmacophore hypotheses were computed using HypoGen module implemented in Discovery Studio 3.0 and the top 10 hypotheses were generated (Table 1). The first hypothesis (Hypo1, Fig. 2) was the best pharmacophore hypothesis, characterized by the highest cost difference 61.601, lowest root mean square error 0.467 and the best correlation coefficient 0.975. The fixed cost, pharmacophore cost and null cost were 133.284, 137.367 and 198.968 respectively. The difference between null cost and fixed cost was large indicating that hypo1 has greater than 90% probability of correlating the data. An appropriate configuration cost value (11.069) was also obtained. Meanwhile, the predictive power and statistical significance of Hypo1 were validated using test set prediction and the Fischer randomization test [22]. The results demonstrated that we had successfully developed a reliable pharmacophore model with high predictivity.

Hypo1 consisted of spatial arrangement of five chemical features: one positive ionizable (PI), one negative ionizable (NI) and three hydrophobic (HY) features (Fig. 2). According to the known SAR, the structure of $S1P_1$ agonists can be divided into two parts: one is the "polar head group", consisting of a phosphate group (or carboxyl group), and an amino group, which can form salt bridge interaction with Arg120 and Glu121 in $S1P_1$ receptor; the other is the lipophilic chain which can form Van der Waals and π–π stacking interactions in a hydrophobic cavity of $S1P_1$ receptor. Our previous research revealed that structural rigidity of the lipophilic chain was required to increase $S1P_1$ agonistic potency and subtype selectivity [23]. Through analyzing the shape and composition of Hypo1, it was found that PI and NI features represented the "polar head group" and three linear aligned HY features represented the structural rigid lipophilic chain, which demonstrated that Hypo1 truly revealed the key characteristics of $S1P_1$ agonists.

On the basis of Hypo1, we designed a series of compounds containing the amino phosphate "polar head group" which mapped to the PI and NI features and the lipophilic chain with increased rigidity composed of aromatic rings which mapped to HY features (Fig. 3). A set of 1,2,3-triazole-containing 2-aminopropane-1,3-diol derivatives (prodrugs) were efficiently synthesized through Cu(I)-catalyzed 1,3-dipolar alkyne-azide cycloaddition (CuAAC), a key reaction in click chemistry which was widely used in drug design [24,25]. Triazole ring located in the middle of the lipophilic chain and served as a linker between two phenyl rings to enable lipophilic chain map well to HY features. Replacement of the triazole ring with oxazole ring provided another series of compounds which can also map Hypo1 well. For instance, compound **12a** and **20a** both had a high fitvalue (a measure of how well the ligand fits the pharmacophore) when mapped to Hypo1.

2.2. Chemistry

The preparation of 1,2,3-triazole-containing derivatives **11a**–**p** and their phosphates **12a**–**p** is shown in Scheme 1. Reacting 4-bromophenethyl bromide (**5**) with diethyl acetamidomalonate in the presence of sodium ethoxide produced **6**. Reduction of **6** with $NaBH_4/K_2HPO_4$ buffer gave diol **7**. Pd-catalyzed Sonogashira reaction was performed between **7** and trimethylsilylacetylene in the presence of $PdCl_2(PPh_3)_2$, CuI, PPh_3 and Et_3N to give **8** [26]. Treatment of **8** with K_2CO_3 provided terminal alkyne **9**. Anilines were converted to the corresponding phenyl azides using $TMSN_3$/*t*-BuONO and then reacted with **9** through a Cu(I)-catalyzed Huisgen azide–alkyne cycloaddition (CuAAC) using $CuSO_4$ and sodium ascorbate to afford triazoles **10a**–**j** [27]. In addition, CuAAC reaction of **9** with benzyl or aliphatic azides which were prepared from benzyl or aliphatic bromide with NaN_3 was carried out to furnish **10k**–**p** [28]. Hydrolysis of **10a**–**p** with NaOH followed by

Table 1
Results of pharmacophore hypotheses generated using training set for $S1P_1$ agonists.

Hypothesis	Total cost	Δcost[a]	RMS	Correlation	Features[b]
1	137.367	61.601	0.467	0.975	PI, NI, HY, HY, HY
2	138.383	60.585	0.520	0.968	PI, NI, HY, HY, HY
3	146.746	52.222	0.819	0.920	PI, NI, HY, HY
4	146.814	52.154	0.847	0.913	PI, NI, HY, HY
5	146.826	52.142	0.855	0.912	PI, NI, HY, HY
6	148.89	50.078	0.927	0.895	PI, NI, HY, HY, HY
7	149.097	49.871	0.920	0.897	PI, NI, HY, HY
8	149.488	49.48	0.903	0.901	PI, NI, HY, HY
9	150.466	48.502	0.948	0.890	PI, NI, HY, HY, HY
10	150.704	48.264	0.975	0.883	PI, NI, HY, HY, HY

[a] Cost difference = null cost-total cost. Null cost = 198.968. Fixed cost = 133.284. Configuration cost = 11.0694. All cost units are in bits.
[b] PI, positive ionizable; NI, negative ionizable; HY, hydrophobic.

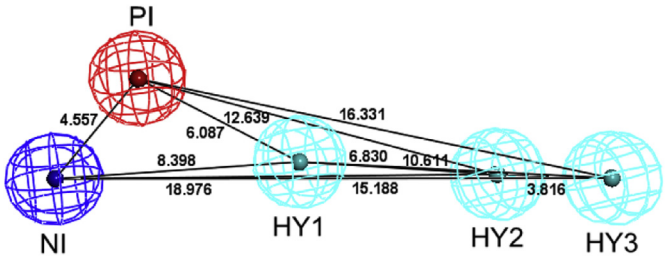

Fig. 2. Top scoring HypoGen pharmacophore Hypo1. The hypothesis features are color coded as follows: positive ionizable (PI), red; negative ionizable (NI), blue; hydrophobic (HY), light-blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)