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A Monoselective Sphingosine-1-Phosphate Receptor-1 Agonist Prevents Allograft Rejection in a Stringent Rat Heart Transplantation Model

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Summary

FTY720 is an immunomodulator with demonstrated efficacy in a phase II trial of relapsing multiple sclerosis. FTY720-phosphate, the active metabolite generated upon phosphorylation in vivo, acts as a potent agonist on four of the five known sphingosine-1-phosphate ($S1P_1$) receptors. AUY954, an aminocarboxylate analog of FTY720, is a low nanomolar, monoselective agonist of the $S1P_1$ receptor. Due to its selectivity and pharmacokinetic profile, AUY954 is an excellent pharmacological probe of $S1P_1$-dependent phenomena. Oral administration of AUY954 induces a profound and reversible reduction of circulating lymphocytes and, in combination with RAD001 (Certican/Everolimus, an mTOR inhibitor), is capable of prolonging the survival of cardiac allografts in a stringent rat transplantation model. This demonstrates that a selective agonist of the $S1P_1$ receptor is sufficient to achieve efficacy in an animal model of transplantation.

Introduction

Sphingosine-1-phosphate (S1P) is an evolutionarily conserved bioactive sphingolipid. S1P is generated as a metabolite of ceramide and secreted into serum by mast cells, platelets, and monocytes. It has been implicated as a second messenger in cellular proliferation and survival and in protection against ceramide-mediated apoptosis. In addition, S1P regulates diverse physiological processes such as cell migration, angiogenesis, vascular maturation [1, 2], and immunity [3–6] by serving as a ligand to cell-surface G-protein-coupled S1P receptors [7, 8].

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FTY720 is a synthetic analog of the natural product myriocin that shares structural homology with the endogenous sphingolipid sphingosine. As a novel immunomodulatory agent, FTY720 exhibited promising activity in animal models of organ transplantation and autoimmunity [9, 10]. In combination with cyclosporin (CsA), FTY720 has proven to be efficacious in preventing kidney rejection in humans [11] and entered phase III clinical trials for transplant rejection [12]. However, further development for this indication was discontinued since FTY720 did not provide benefits over standard of care. FTY720 is now being developed for the treatment of multiple sclerosis and is entering a phase III trial after having reduced clinical relapse in a phase II study by greater than 50% relative to placebo in 6 months [13].

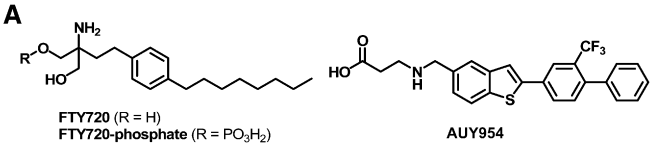
Upon phosphorylation in vivo primarily by sphingosine kinase 2 (SK2) [14], FTY720 acquires the ability to mimic sphingosine-1-phosphate (S1P) and acts as a potent agonist at four of the five known sphingosine-1-phosphate receptors, namely $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$ (Figure 1) [3, 15]. It has been convincingly demonstrated that FTY720-phosphate-mediated S1P receptor agonism causes inhibition of lymphocyte egress from thymus and lymph nodes, which is—at least in part—responsible for the immunomodulatory activity of FTY720 [16–18]. Recent reports demonstrate the requirement of the $S1P_1$ receptor for the desired pharmacodynamic response in vivo [19]. In addition, a transient and asymptomatic bradycardia has been reported as the primary clinical adverse effect of FTY720 [20], and $S1P_3$ has been demonstrated to regulate heart rate in rodents [21], apparently by activating G-protein-coupled inward-rectifying potassium (GIRK) channels in atrial myocytes [22]. We therefore sought to determine the minimal receptor profile required to provide full protection of organ transplants in vivo. To accomplish this, we prepared a series of novel amino carboxylate analogs and profiled their selectivity against the S1P receptors by using functional GTPγS binding assays. Here, we demonstrate that a compound with monoselectivity for the sphingosine-1-phosphate receptor-1 ($S1P_1$) is capable of potently reducing circulating lymphocytes and protecting rat heart allografts in vivo in combination therapy with a subtherapeutic dose of RAD001.

Results and Discussion

Design and Synthesis of AUY954: A $S1P_1$ Monoselective Agonist

FTY720 acts as a prodrug and is converted to an active aminophosphate metabolite through sphingosine-kinase-2-mediated phosphorylation in vivo. Due to potential complexities associated with prodrugs such as species differences between the kinase and phosphatase responsible for controlling drug levels, we and others [23] sought to prepare non-prodrug aminocarboxylate bioisoteres. Recently, a series of orally bioavailable tricyclic carboxylate analogs have also been reported as $S1P_1$ selective agonists [23]. These and earlier compound series developed by the Merck group [24]

A



B

(GTPγS binding assay, EC_{50}, nM)

compound	$hS1P_1$	$mS1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$
S1P	0.9 ± 0.7	1.4 ± 0.61	8.9 ± 2.3	0.16 ± 0.11	8.6 ± 3.8	11 ± 9
(S)-FTY720-P	0.3 ± 0.1	-	>10000	3.1 ± 0.45	0.6 ± 0.2	0.3 ± 0.2
AUY954	1.2 ± 0.6	0.9 ± 0.26	>10000	1210 ± 127	>1000	340 ± 80

Figure 1. Chemical Structures and Functional Activity of FTY720, FTY720-Phosphate, and AUY954 on S1P Receptors

(A) Chemical structures of FTY720, FTY720-phosphate, and AUY954.

(B) EC_{50} in nanomolar (mean ± SEM) for the compounds against S1PRs in a γ-GTPS-binding assay.

demonstrated that the aminophosphate "head group" can be functionally mimicked by an amino carboxylate and is sufficient to achieve potent agonist activity. The discovery of the $S1P_1$ selective agonist SEW02871 [21] demonstrated that modifications made to the parts of the inhibitor that interact with the hydrophobic channel of the S1P receptor allow $S1P_1$ subtype selectivity to be achieved. SEW02871 achieves significant agonist activity (EC_{50} = 14 ± 8 nM) despite being incapable of forming ionic interactions with the receptor [25]. It is postulated that the trifluoromethyl groups contributes significantly to the binding affinity. AUY954 (Figure 1) was inspired by combining structural features derived from screening leads related to SEW02871 that were obtained from a high-throughput screen of the $S1P_1$ receptor. Although the trifluoromethyl biphenyl substitutent of AUY954 was identified by a systematic structure activity relationship (SAR) analysis, it can be retrospectively viewed as an isosteric replacement of the trifluoromethyl-thiophene-phenyl fragment of SEW02871. The potential for molecular mimicry between AUY954 and SEW02871 [25] can be visualized when these compounds are docked to a homology model of $S1P_1$ (Figure 2). Interestingly, an energetically preferred binding mode has the two compounds closely overlapped, especially in the trifluoromethyl region. AUY954 was finally obtained after installing different amino carboxylate "head groups" to a diverse assortment of benzo-fused heterocycles that possessed the trifluoromethyl-biphenyl pharmacophore (unpublished data) [26]. AUY954 exhibited monoselectivity for $S1P_1$ over all other S1P receptors ($S1P_{2–5}$) as assessed by GTPγS-binding assays (see section below). The synthesis of AUY954 was completed in seven steps (Figure 3). Thus, the boronic acid **2** was obtained from benzothiophene compound **1** upon treatment of lithium diisopropylamide and trimethyl borate. Its coupling counterpart (**5**) was prepared in two steps starting with Suzuki coupling of commercially available 3-trifluoromethyl-4-bromoaniline (**3**) with phenylboronic acid followed by a diazotization-bromination

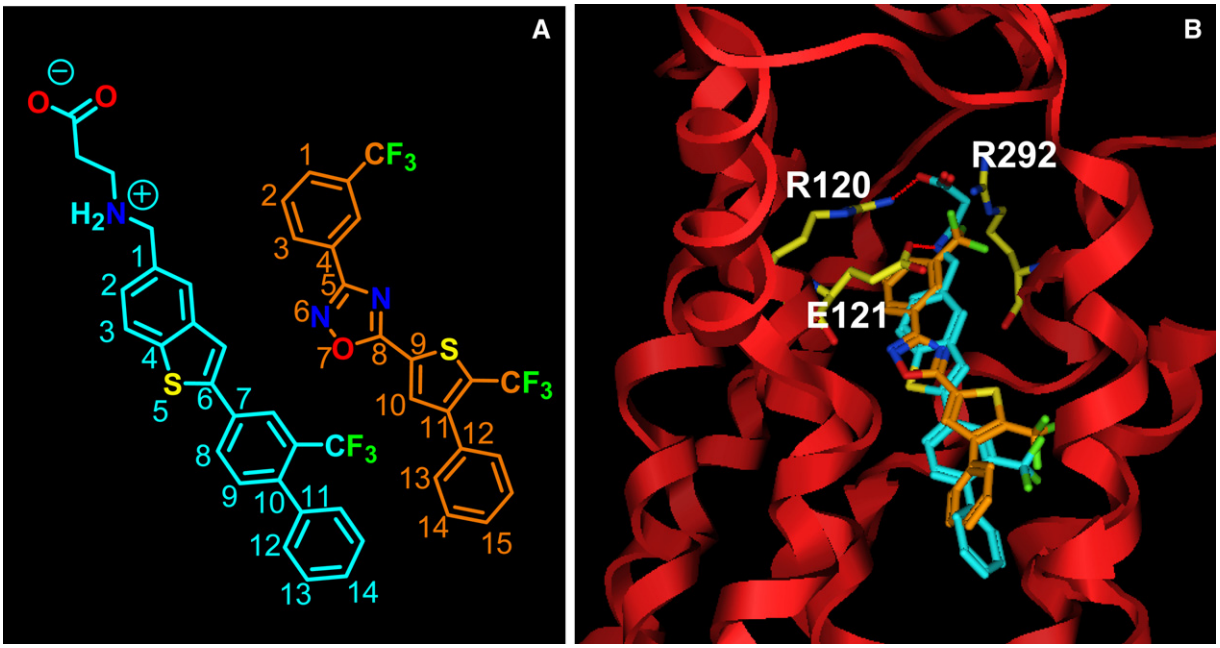


Figure 2. Comparative Binding Mode of AUY954 and SEW02871 Bound to Homology Model of $S1P_1$

AUY954 and SEW02871 are shown in cyan and brown, respectively (heteroatoms are colored red for oxygen, blue for nitrogen, green for fluorine, and yellow for sulfur), and $S1P_1$ in red ribbons. Possible ionic interactions are shown as dotted lines to Glu121 to amine and Arg120 to acid of AUY954.