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Design and synthesis of pyrazolopyridine derivatives as sphingosine 1-phosphate receptor 2 ligands

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ABSTRACT

Eleven new sphingosine 1-phosphate receptor 2 (S1PR2) ligands were synthesized by modifying lead compound *N*-(2,6-dichloropyridin-4-yl)-2-(4-isopropyl-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-6-yl) hydrazine-1-carboxamide (JTE-013) and their binding affinities toward S1PRs were determined *in vitro* using [^{32}P]S1P and cell membranes expressing recombinant human S1PRs. Among these ligands, **35a** (IC_{50} = 29.1 ± 2.6 nM) and **35b** (IC_{50} = 56.5 ± 4.0 nM) exhibit binding potency toward S1PR2 comparable to JTE-013 (IC_{50} = 58.4 ± 7.4 nM) with good selectivity for S1PR2 over the other S1PRs (IC_{50} > 1000 nM). Further optimization of these analogues may identify additional and more potent and selective compounds targeting S1PR2.

Introduction

Sphingosine 1-phosphate (S1P) is a bioactive lysophospholipid that transmits signals through the family of G-protein coupled receptors S1PR1, 2, 3, 4, and 5, which were originally known as EDG-1, 3, 5, 6, and 8. Upon binding S1P, these five sphingosine 1-phosphate receptors (S1PRs) regulate diverse biological functions, from proliferation and survival to migration and secretion, across many cell types.[1–3] S1PRs are expressed in a wide variety of tissues, with each subtype exhibiting a different cell specificity. S1PR1, 2, and 3 are expressed ubiquitously, whereas S1PR4 is confined to lymphoid and hematopoietic tissues, and S1PR5 is primarily located in the white matter of the central nervous system (CNS) and spleen.[4,5]

Multiple sclerosis (MS) is an inflammatory disorder of the brain and spinal cord in which focal lymphocytic infiltration leads to damage of myelin and axons.[6] According to World Health Organization reports, the estimated number of people with MS increased from 2.1 million in 2008 to 2.3 million in 2013. MS significantly decreases life expectancy of patients by an average of 5–10 years.[7] The mechanisms that cause progression in MS are not well understood, thus major treatment strategies are aimed at improving neuronal function and limiting progression of disease after initial diagnosis.[8] The sphingolipid-like immunomodulator fingolimod (FTY720) was the first oral therapeutic agent for relapsing-remitting MS to be approved by the US Food and Drug Administration (FDA).[9] [γ-^{35}S]GTPγS binding assay showed that FTY720 is a potent agonist at four S1PRs (S1PR1, 3, 4, and 5), but inactive at S1PR2.[10] Several other S1PR1 agonists have also performed well in clinical trials with MS patients.[11] Robyn Klein's group recently demonstrated that S1PR2 plays an important role in regulating blood–brain barrier (BBB) function during inflammatory demyelination. An increased expression of S1PR2 is observed in disease-susceptible regions of both female SJL mice with experimental autoimmune encephalomyelitis (EAE) and female patients with MS compared with S1PR2 expression seen in their male counterparts.[12] Although therapeutics have focused on S1PR1 agonists, S1PR2 could be a potential biomarker for the prognosis and treatment of MS.

There is a need to discover potent and receptor sub-type selective ligands to better understand the biological role of these different receptors and identify therapeutic approaches for different pathological conditions.[11] Although many S1P receptor ligands have been reported, most are S1PR1 specific, and very few are specific for S1PR2 (Fig. 1). JTE-013, which was first reported in 2001, is one of the most widely-used potent and selective S1PR2 antagonist; it inhibits specific binding of radiolabeled S1P to cell membranes of Chinese hamster ovary (CHO) cells stably transfected with human or rat S1PR2.[13] Although CYM-5520 was reported as the first allosteric S1PR2 selective agonist, with an EC_{50} value of 0.48 μM, a [^{33}P]S1P competitive binding assay

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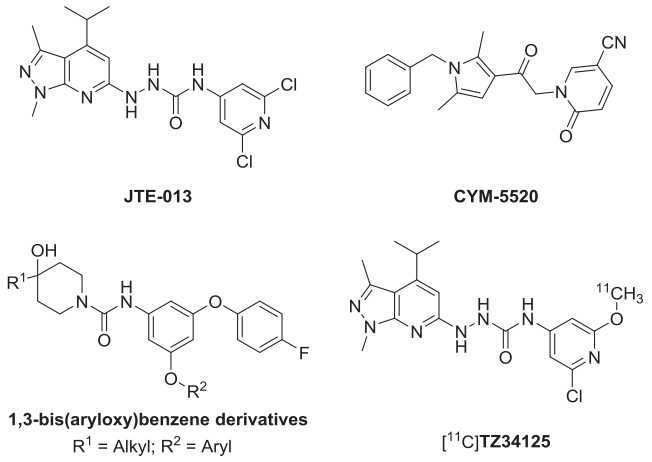

Fig. 1. Structures of S1PR2 ligands.

showed CYM-5520 was not competitive with S1P.[14] Recently, Takuya Seko's group identified several potent and selective S1PR2 antagonists in a novel series of 1,3-bis(aryloxy)benzene derivatives.[15–17] We reported a radiolabeled JTE-013 analogue, [^{11}C]**TZ34125** and its *in vivo* studies, suggesting the sexual dimorphism of S1PR2 expression in the cerebellum of cyclosporin pretreated SJL mice.[18] Here, we have continued our medicinal chemistry exploration of JTE-013 structural analogues. We divided the structure of JTE-013 into three fragments (A, B, and C) which were subsequently modified as shown in Fig. 2. Firstly, on one side fragment A was modified by reversing the pyrazole ring and adding one more carbonyl group to check the possibility of improving binding potency and stability, as reported;[19,20] on the other side, fragment A was modified by replacing the isopropyl group with a steric bioisotere group, trifluoromethyl to check its impact on the binding potency toward S1PR2.[21] Secondly, fragment B was modified by replacing the urea linker with heterocycle linker; this modification will reduce hydrogen bond donor (HBD) and cause reduction of molecular total polar surface area (tPSA) to improve the physicochemical property.[22] Thirdly, fragment C was modified by replacing the 2,6-dichloropyridine ring in JTE-013 with different fused heterocycles or substituted pyridines. Together, eleven new compounds were synthesized and their *in vitro* binding affinities were determined using [^{32}P]S1P and cell membranes expressing recombinant human S1PRs.

As shown in Scheme 1, the synthesis of compound **7** was achieved by starting with compound **1**, which was synthesized as reported.[19] The pyridine *N*-oxide **2** was obtained using *meta*-chloroperoxybenzoic acid (*m*CPBA) to perform an *N*-oxidation reaction. The cyano group was introduced on the pyridine ring under trimethylsilyl cyanide (TMS-CN) to give cyanide intermediate **3**, which was then converted to amide **4** by hydration in the presence of 1 M NaOH and H_2O_2. The intermediate compound **6** was generated by reacting commercially available 2-chloro-6-methoxyisonicotinic acid **5** with diphenylphosphoryl azide. The final compound **7** was accomplished by a two-step one-pot reaction: the azide **6** was refluxed in toluene and converted to an isocyanate, which was followed by treating with 7-isopropyl-1,3-dimethyl-1*H*-pyrazolo-[4,3-*b*]pyridine-5 carbetamide **4** to afford compound **7**.

To generate trifluoromethyl substituted compounds **13a–d**, Scheme 2 was followed. Starting with 3-methyl-1*H*-pyrazol-5-amine **8a** or 1,3-dimethyl-1*H*-pyrazol-5-amine **8b**, cyclization was accomplished by reacting with ethyl 4,4,4-trifluoro-acetoacetate in propionic acid at 140 °C to give hydroxyheter-oarenes **9a** or **9b**, respectively. Bromination of compounds **9a** or **9b** using $POBr_3$ afforded bromoheteroarenes **10a** or **10b** in high yields, which were subjected to react with CuCN to afford cyanide intermediates **11a** or **11b**. After hydration, the amide intermediates **12a** and **12b** were obtained. Compounds **13a–d** were prepared using a two-step one-pot reaction procedure similar to the procedure of making compound **7**.

To investigate the impact of replacing the urea linker (fragment B) with a heterocycle linker, compounds **22** and **23** were synthesized by following Scheme 3. The commercially available 1,3-dimethyl-1*H*-pyrazol-5-amine **8b** was cyclized with ethyl isobutyrylacetate to afford 4-isopropyl-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-6-ol **14**. Under similar conditions as Scheme 2, the key cyanide intermediate 4-isopropyl-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-e-6-carbonitrile **16** was obtained. Next, the cyanide **16** was treated with hydroxylamine hydrochloride to afford *N*-hydroxy-4-isopropyl-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-6-

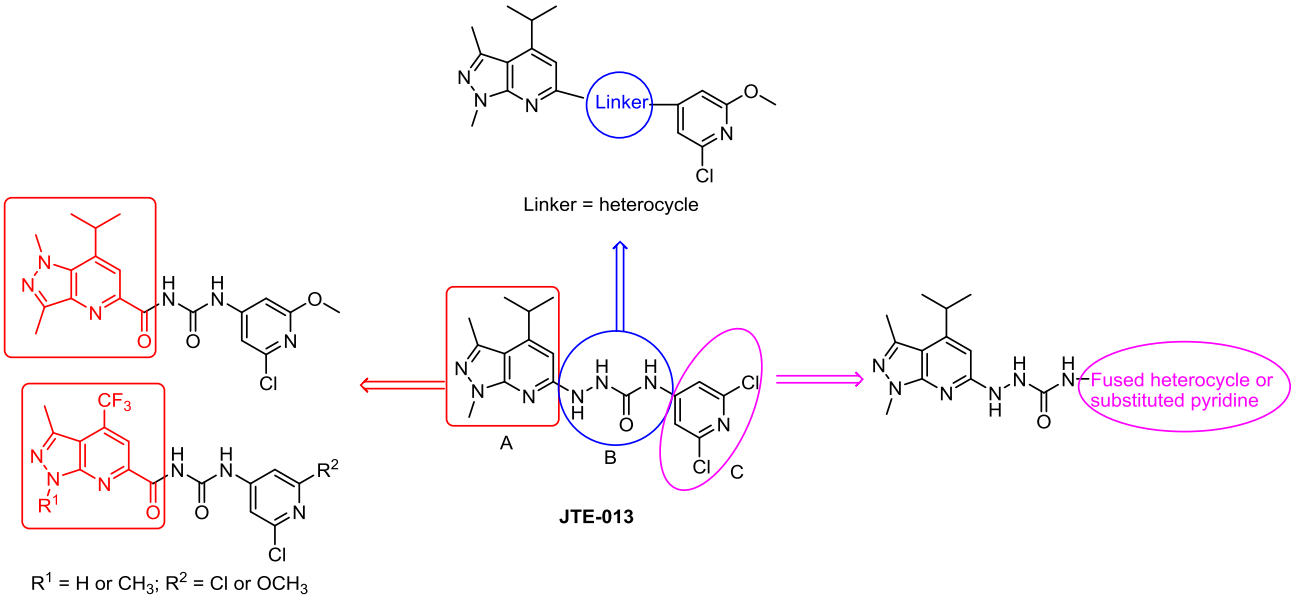

Fig. 2. Design strategy.