Bioorganic & Medicinal Chemistry Letters 19 (2009) 2315–2319

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and evaluation of arylalkoxy- and biarylalkoxy-phenylamide and phenylimidazoles as potent and selective sphingosine-1-phosphate receptor subtype-1 agonists

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ARTICLE INFO

Article history:
Received 10 December 2008
Revised 17 February 2009
Accepted 19 February 2009
Available online 23 February 2009

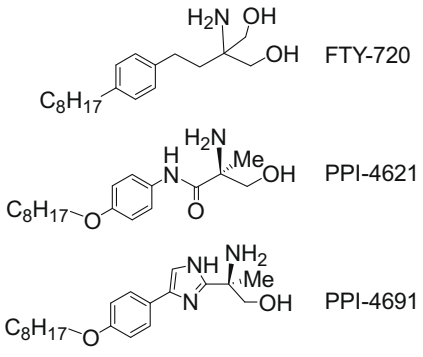
Keywords:
S1P
S1P1 agonist
EDG1
Lymphopenia
FTY-720
Sphingosine-1-phosphate
Arylalkoxy-phenylamide
Arylalkoxy-phenylimidazoles
Biarylalkoxy-phenylamide
Biarylalkoxyphenylimidazoles

ABSTRACT

In pursuit of potent and selective sphingosine-1-phosphate receptor agonists, we have utilized previously reported phenylamide and phenylimidazole scaffolds to explore extensive side-chain modifications to generate new molecular entities. A number of designed molecules demonstrate good selectivity and excellent in vitro and in vivo potency in both mouse and rat models. Oral administration of the lead molecule **11c** (PPI-4667) demonstrated potent and dose-responsive lymphopenia.

Sphingosine-1-phosphate (S1P) receptors, a class of G-protein coupled receptors (GPCRs), are important molecular receptors for drug discovery and development because of the significant role they play in a diversity of physiological and pathophysiological processes.[1] Activation or antagonism of members of this cluster of five receptors ($S1P_{1-5}$) with the natural ligand S1P can induce various effects on cardiovascular and immune system function and other yet poorly defined effects on additional physiological systems including pulmonary function.[1]

FTY-720 phosphate, a potent non-selective S1P receptor agonist, has profound immunomodulatory activity through alteration of lymphocyte trafficking.[2] This activity is due to activation of S1P receptor subtype 1 ($S1P_1$) signaling cascades. In contrast, agonists of S1P receptor subtype 3 ($S1P_3$) have been associated with negative chronotropic effects in preclinical studies which may translate into clinical side effects including bradycardia.[3] We recently reported two classes of S1P receptor agonists with excellent potency at the $S1P_1$ receptor with good oral activity (Scheme 1).[4] In order to retain the positive therapeutic properties while further reducing the potential for side effects of non-selective S1P receptor agonists like FTY-720, we have explored structural modifications to



Scheme 1.

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doi:10.1016/j.bmcl.2009.02.073

these scaffolds to further improve the agonist selectivity for S1P receptor subtype 1 over 3. In the design of potent sphingosine-1-phosphate receptor agonists in the phenylamide and phenylimidazole scaffolds, we reported two potent lead molecules, PPI-4621 and PPI-4691 (Scheme 1), with moderate selectivity for $S1P_1$ versus $S1P_3$ and significant in vivo activity in mouse.[4]

To further improve agonist selectivity for $S1P_1$ over $S1P_3$, we explored extensive tail modifications and developed a robust structural–activity relationship (SAR) in the phenylamide scaffold series (Fig. 1). The initial effort was focused on carbocycle and heterocycle insertion (Q) in the tail region of PPI-4621, where X can be an inserted linker, and developed an early SAR to access potent orally active molecules using in vivo lymphopenia as the biological endpoint.

One general approach for the synthesis of the desired agonists is described in Scheme 2. Nucleophilic substitution of the desired alcohol[5] on 1-fluoro-4-nitrobenzene (**1**, Z = F) in the presence of base afforded nitrobenzene **2**. An alternative approach to synthesis of nitrobenzene **2** was through Mitsunobu reaction of 4-nitrophenol (**1**, Z = OH) with the desired alcohol.

Hydrogenation of the nitro group followed by peptidic coupling of the aniline with (*S*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid using either *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC), 1-hydroxybenzotriazole (HOBt), and *N*,*N*-diisopropylethylamine (DIPEA) or *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and DIPEA afforded protected amino-alcohols **3**. An alternative approach to synthesis of **3a–3o** was through HATU coupling of (*S*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid with 4-aminophenol followed by copper(II)-promoted coupling of various arylboronic acids with the phenol to generate biaryl-ether desired products. Removal of the Boc protecting group with trifluoroacetic acid (TFA) afforded final compound **4**.

A set of designed molecules are reported in Table 1. These amino-alcohols were orally administered to determine their in vivo activity by measuring redistribution of circulating lymphocytes in the mouse 6 h after administration of the compounds. When the tail was modified to a phenyl group or phenyl group substituted at positions 4- and/or 3- with an electron conductive halogen, an electron withdrawing group, or an electron rich alkyl or alkoxyl, no lymphopenia was observed (compounds **4a**–**4e** and **4g**–**4o**). However, the 4-biphenyl tail provided significant lymphopenia. When the X was changed from O to CH_2CH_2O little or no change in absolute lymphocyte count was observed regardless of the substitution on the phenyl group (Q) or when a naphthyl group was utilized (compounds **4p**–**4v**). Change of the R-Q group to a 4-biaryl system provided good to excellent activity especially when R

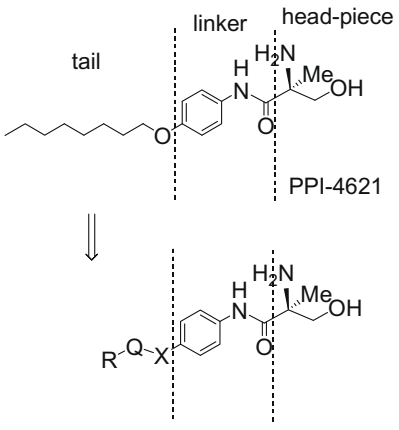
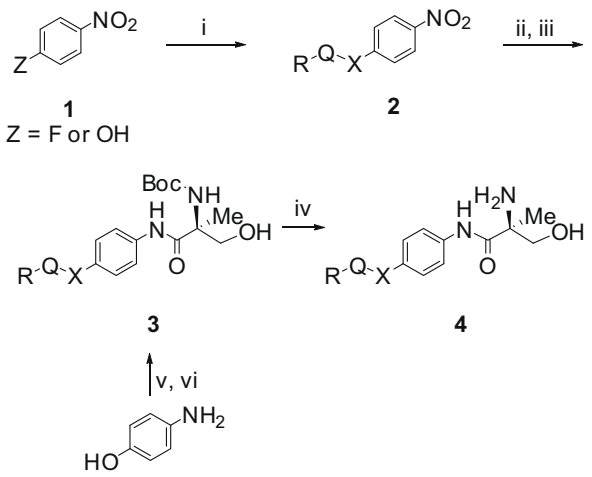


Figure 1. General approach for tail modification in PPI-4621.



Scheme 2. Reagents and conditions: (i) alcohol, NaH or KO^{t}Bu, THF, 60–70 °C, or alcohol, PPh_3, diethylazodicarboxylate, CH_2Cl_2; (ii) H_2, 10% Pd/C, MeOH or N_2H_4, 10% Pd/C, EtOH, 80 °C; (iii) (*S*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid, EDC and HOBt or HATU, DIPEA, DMF or CH_2Cl_2; (iv) TFA, CH_2Cl_2; (v) (*S*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid, HATU, DIPEA, DMF; (vi) $ArB(OH)_2$, $Cu(OAc)_2$, molecular sieves, pyridine, CH_2Cl_2.

was unsubstituted phenyl or contained small-sized substitutions regardless of the electronic character (**4w**–**4ae**). When 3-biphenyl was used in place of 4-biphenyl the compound lost activity (**4af**). Heteroaromatic substitution at R was also well tolerated (**4ag**–**4ak**). When the X group was modified to C4 and C5 ethers, moderate to excellent in vivo activity was observed with R-Q being a simple aryl or cyclohexyl group.

In another series of tail modifications, we chose to explore the invention of a substituted phenyl system without the ether connection. The compounds were synthesized as described in Scheme 3. Suzuki cross-coupling of the boronic acid **6** with 4-bromoaniline **5** provided 4-biphenylamino **7**.[6] 4-Biphenylamino **7** was then coupled with (*R*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid treated with TFA to generate the final compound **9**. The in vivo biological activity is reported in Table 2. When R was a *para*-substituted Me or Et little or no reduction in circulating lymphocyte count was observed (**9a** and **9b**). *meta*-Substitution with an alkyloxy group gave a similar result to **9a** and **9b** regardless of the nature of the alkyl group. The 3,4-methylenedioxy group (**9g**) demonstrated the greatest lymphopenia, 40%, in this series. Therefore, regardless of the R substitution on the phenyl group, low to moderate lymphopenia was induced by this series of molecules.

In order to determine the agonist binding activity for both $S1P_1$ and $S1P_3$, we selected several amino-alcohols based on the absolute in vivo lymphocyte reduction and synthesized the corresponding phosphates. These were synthesized through two different approaches as reported in Scheme 4. In the first approach, the reaction of free amine or Boc-protected amino-alcohol with excess diethyl chlorophosphate in the presence of triethylamine afforded phospho-triester **10**. The phopho-triester **10** was then treated with excess bromotrimethylsilane to give the final phosphate **11** after preparative HPLC purification. An alternative approach for phosphate synthesis was through synthesis of the intermediate di-*tert*-butyl phosphate using di-*tert*-butyl diisopropylphosphoramidite and 1*H*-tetrazole followed by oxidation to phosphate ester and removal of the *tert*-butyl groups by a method analogous to that reported by Clemens and co- workers.[7] S1P and synthetic phosphate agonist binding activities were measured using a [^{33}P]S1P receptor binding assay according to an earlier report (Table 3).[4] In the 4-biphenyl system when X was O, the agonist showed low