Bioorganic & Medicinal Chemistry Letters 22 (2012) 1788–1792

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of CS-2100, a potent, orally active and $S1P_3$-sparing $S1P_1$ agonist

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ARTICLE INFO

Article history:
Received 29 October 2011
Revised 25 November 2011
Accepted 3 December 2011
Available online 2 January 2012

Keywords:
$S1P_1$
Agonist
CS-2100
Lymphocyte
HvGR

ABSTRACT

$S1P_3$-sparing $S1P_1$ agonists have attracted attention as a suppressant of autoimmunity with reduced side effects. Our synthetic efforts and extensive SAR studies led to the discovery of **10b** named CS-2100 with the EC_{50} value of 4.0 nM for human $S1P_1$ and over 5000-fold selectivity against $S1P_3$. The in vivo immunosuppressive efficacy was evaluated in rats on host versus graft reaction and the ID_{50} value was determined at 0.407 mg/kg. The docking studies of CS-2100 with the homology model of $S1P_1$ and $S1P_3$ showed that the ethyl group on the thiophene ring of CS-2100 was sterically hindered by Phe263 in $S1P_3$, not in the case of Leu276 in $S1P_1$. This observation gives an explanation for the excellent $S1P_3$-sparing characteristic of CS-2100.

Sphingosine-1-phosphate (S1P) (**1**, Fig. 1) is a bioactive sphingolipid that plays a role in a wide range of physiological processes such as cell differentiation, morphogenesis and motility, through its interaction with the five-membered S1P family ($S1P_1$–$S1P_5$) of G-protein coupled receptors (GPCRs).[1] Among these five receptors, in particular, $S1P_1$ modulators have recently been focused as a suppressant of autoimmunity by affecting lymphocyte trafficking, through the rapid progress of studies on FTY720 (fingolimod) (**2**).[2]

The systemic administration of FTY720 induces a dose–responsive lowering of circulating lymphocytes and its immunosuppressive actions have been reported to result from the active phosphate ester metabolite, FTY720-P (**3**)[3], which is an agonist of $S1P_{1,3,4,5}$ but not of $S1P_2$. From intense studies on both FTY720 and S1P receptors, it has also been revealed that the agonism of $S1P_1$ alone is sufficient to control lymphocyte recirculation.[4] On the other hand, $S1P_3$ is implicated in bradycardia as reported in rodents.[5] Recent studies suggested that only removal of the $S1P_3$ agonism is insufficient to exclude the cardiovascular side effect.[6a] However, a great deal of research efforts focused on the exploration of $S1P_3$-sparing $S1P_1$ agonists,[6] with an aim to reduce potential side effects but preserve the impressive efficacy as demonstrated in clinical trials of FTY720 for the treatment of transplant rejection[7] and remitting relapsing multiple sclerosis.[8]

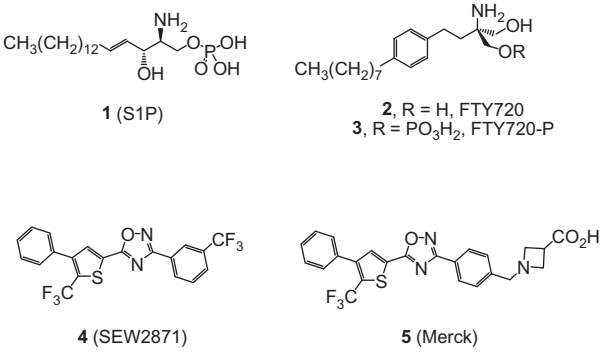

Figure 1. Structures of S1P, FTY720, FTY720-P, SEW2871 and one of the 1,2,4-oxadiazole derivatives reported by Merck.

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doi:10.1016/j.bmcl.2011.12.019

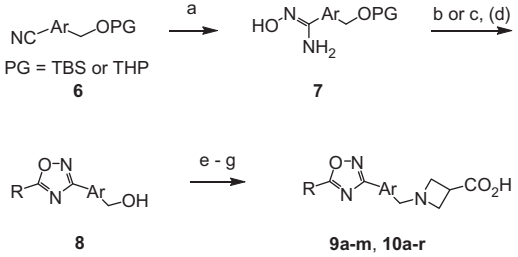
In 2004, Scripps and Norvartis reported SEW2871 (**4**),[5b,9] a non phosphate type $S1P_1$ selective agonist, which has attracted attention as the second stream of $S1P_1$ agonists. On the other hand, Merck also disclosed a series of non phosphate type $S1P_1$ agonists, representatively, compound **5** in their precedent patent.[10] Encouraged by our success in the discovery of a phosphate type $S1P_1$ agonist, CS-0777 as a promising clinical candidate,[11] we continued to investigate new $S1P_1$ agonists and initiated the SAR studies based on the structure of compound **5**. Herein, we describe the discovery process for a potent $S1P_3$-sparing $S1P_1$ agonist, CS-2100 and report its SAR study with consideration of the homology model based on the X-ray crystal structure of bovine rhodopsin.

Based on the structure of **5**, we planned to (1) convert the inner benzene ring to other aromatics and (2) transform the left-hand side 4-phenyl-5-trifluoromethyl thiophene structure to procure a patentable basal structure.

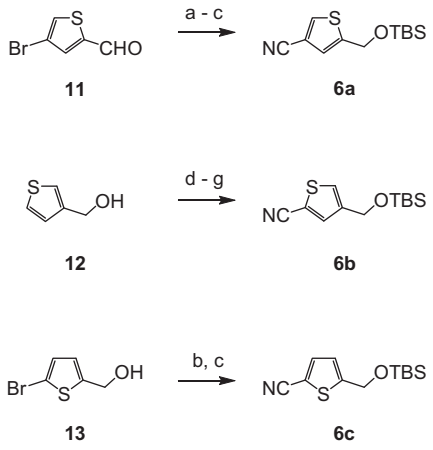
The general synthetic method is outlined in Scheme 1. Aromatic nitriles **6** were reacted with aqueous hydroxyamine to give amidoximes **7**. Subsequent 1,2,4-oxadiazole formation[12] provided a series of 1,2,4-oxadiazoles **8** with simultaneous deprotection of the TBS group except for the THP-protected one. As for THP-deprotection, it was conducted after 1,2,4-oxadiazole formation. Bromination or chlorination of **8** and the succedent substitution by methyl azetidine-3-carboxylate, followed by hydrolysis provided **9a**–**m** and **10a**–**r**.

Regarding the preparation of aromatic nitriles **6**, the synthetic routes are summarized in Scheme 2. **6a** was prepared from aldehyde **11** by reduction, TBS-protection and following cyanidation with copper cyanide. **6b** was obtained from alcohol **12** by TBS-protection, formylation and nitrile formation through dehydration of the oxime. **6c** was provided by the similar method for preparation of **6a**.

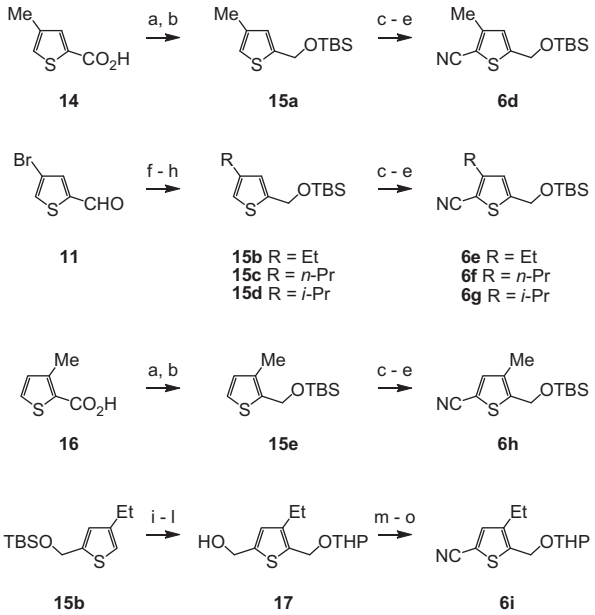
For the purpose of structural optimization, our synthetic effort was extended to various alkylated thiophenes. The synthetic routes are summarized in Scheme 3. Starting from carboxylic acid **14**, reduction and TBS-protection provided **15a**. Subsequent formylation, oxime formation and dehydration gave **6d**. Regarding **6e–g**, aldehyde **11** was converted to **15b–d**, respectively by reduction, TBS-protection and a Ni-catalyzed cross coupling reaction with the corresponding Grignard reagents. As for **15d**, isopropenyl magnesium bromide was used for a cross-coupling reaction and then the olefin was hydrogenated because the use of isopropyl magnesium bromide resulted in a mixture of *n*-propylated and isopropylated compounds. The transformation of **15b–d** to **6e–g** was performed by the same method as the conversion from **15a** into **6d**. Compound **6h** was prepared from carboxylic acid **16** in the same manner as preparation of **6d**. **6i**, which is the only THP-protected thiophene, was synthesized via the intermediate **17** prepared from **15b** in four steps including formylation, reduction,



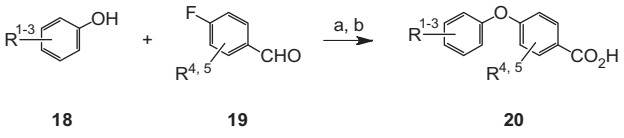
Scheme 1. Reagents and conditions: (a) aq. NH_2OH, EtOH (52–98%); (b) RCOCl, Et_3N, CH_2Cl_2, then TBAF, THF (65–98%); (c) RCO_2H, WSC–HCl or DCC, HOBt, CH_3CN, then TBAF, THF (40-94%); (d) PPTS, EtOH, 60 °C (92%) for THP deprotection; (e) CBr_4, PPh_3, CH_2Cl_2 or $SOCl_2$, cat. DMF, toluene; (f) Methyl azetidine-3-carboxylate-HCl or Ethyl azetidine-3-carboxylate-HCl, *i*-Pr_2NEt, CH_3CN (44–93%, two steps). (g) 1 N aq. NaOH or LiOH, then AcOH or oxalic acid (**10c**, **10g**, **10h** and **10i** were solidified as oxalates.), (39–95%).



Scheme 2. Reagents and conditions: (a) $NaBH_4$, MeOH (quant.); (b) TBSCl, imidazole, DMF (quant.); (c) CuCN, DMF (58% for **6a**, 56% for **6c**); (d) TBSCl, imidazole, DMF (86%). (e) *n*-BuLi, THF, then DMF (54%); (f) NH_2OH–HCl, Et_3N, CH_2Cl_2–MeOH; (g) DCC, toluene, 90 °C (95%, two steps).



Scheme 3. Reagents and conditions: (a) BH_3, THF; (b) TBSCl, imidazole, DMF (67% for **15a**, 88% for **15e**, two steps); (c) *n*-BuLi, DMF, THF (47–89%); (d) NH_2OH–HCl, Et_3N, CH_2Cl_2–MeOH; (e) DCC, toluene, 90 °C (69–100%, two steps); (f) $NaBH_4$, MeOH; (g) TBSCl, imidazole, DMF (quant. two steps); (h) EtMgBr, $NiCl_2$(dppp), THF (95%) for **15b**. *n*-PrMgBr, $NiCl_2$(dppp), Et_2O (72%) for **15c**. Isopropenyl magnesium bromide, $NiCl_2$(dppp), Et_2O (82%), then H_2, $RhCl(PPh_3)_3$, benzene (86%) for **15d;** (i) *n*-BuLi, DMF, THF (86%); (j) $NaBH_4$, MeOH (94%); (k) 3,4-dihydro-2*H*-pyrane, p-TsOH; (l) TBAF, THF (78%, two steps); (m) PDC, MS4A, CH_2Cl_2 (87%); (n) NH_2OH–HCl, Et_3N, CH_2Cl_2; (o) DCC, toluene, 90 °C (95%, two steps)



Scheme 4. Reagents and conditions: (a) K_2CO_3, DMF, 100 °C; (b) $NaClO_2$, KH_2PO_4, 2-methyl-2-butene, THF-*t*-BuOH–H_2O (33%-quant., two steps)

THP-protection and TBS-deprotection. Subsequent oxidation by PDC and following nitrile formation through dehydration of the oxime provided **6i**.