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Boronic acid-containing aminopyridine- and aminopyrimidinecarboxamide CXCR1/2 antagonists: Optimization of aqueous solubility and oral bioavailability

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ABSTRACT

The chemokine receptors CXCR1 and CXCR2 are important pharmaceutical targets due to their key roles in inflammatory diseases and cancer progression. We have previously identified 2-[5-(4-fluoro-phenylcarbamoyl)-pyridin-2-ylsulfanylmethyl]-phenylboronic acid (SX-517) and 6-(2-boronic acid-5-trifluoromethoxy-benzylsulfanyl)-*N*-(4-fluoro-phenyl)-nicotinamide (SX-576) as potent non-competitive boronic acid-containing CXCR1/2 antagonists. Herein we report the synthesis and evaluation of aminopyridine and aminopyrimidine analogs of SX-517 and SX-576, identifying (2-{(benzyl)[(5-boronic acid-2-pyridyl)methyl]amino}-5-pyrimidinyl)(4-fluorophenylamino)formaldehyde as a potent chemokine antagonist with improved aqueous solubility and oral bioavailability.

The chemokine receptors CXCR1 and CXCR2 are major targets of drug development in the pharmaceutical industry[1,2] due to their key role in neutrophil chemotaxis and its association with inflammation.[3] The endogenous ligands for these G-protein coupled receptors (GPCRs) include the CXCR2-specific growth-related oncogene α (GRO α, or CXCL1) and interleukin-8 (IL8, or CXCL8), which binds to both receptors.[4] Experiments with animals lacking CXCR1 and/or CXCR2 activity have demonstrated that their inhibition could be beneficial in the treatment of several diseases, including asthma,[5] chronic obstructive pulmonary disease (COPD),[6] and inflammatory bowel disease[7]), cancer (melanoma,[8] pancreatic,[9,10] and colon[11,12] cancer), Alzheimer's disease,[13] and traumatic brain injury.[14] Several previously reported CXCR1 and/or CXCR2 inhibitors representing the key structural classes are summarized in Figure 1. The diaryl urea CXCR2 antagonist SB656933 was evaluated in clinical trials for the treatment of COPD[15,16] and cystic fibrosis,[17] and GlaxoSmithKline has recently advanced into the clinic a new structurally-similar inhibitor danirixin **1**,[18] with trials currently recruiting patients for COPD and respiratory syncytial virus infections. The CXCR2 antagonist navarixin (SCH527123, **2**)[19] from Merck utilized a cyclic urea bioisostere 3,4-diaminocyclobut-3-ene-1,2-dione instead of the diaryl urea to generate a potent inhibitor that was evaluated in clinical trials for the treatment of COPD,[20,21] asthma,[22] and psoriasis. Their Phase 2 trial demonstrated safety and efficacy in moderate to severe COPD patients.[21] AstraZeneca's pyrimidine-based CXCR1/2 inhibitors AZD8309,[23] AZD5069 **3**,[24] and AZD4721 (structure undisclosed) have been clinically evaluated to treat COPD[25,26] and asthma.[2] Dompé's ketoprofen derivative reparixin **4**, an inhibitor of CXCL8 receptor CXCR1 and CXCR2 activation,[27,28] is being explored for the reduction of post-surgical inflammation after transplantation surgeries.[2] Novartis[29,30] and Pfizer[23,31] also have active CXCR2 programs.

We have previously reported a novel class of dual allosteric CXCR1/2 antagonists that utilize an aromatic boronic acid moiety on a nicotinamide core.[32,33] Our first-generation inhibitor **5** (SX-517) exhibited anti-inflammatory activity in vivo, but its preclinical development was halted due to its metabolic instability. A focused SAR effort to increase metabolic stability led to the discovery of our second-generation inhibitor **6** (SX-576), which was less susceptible to oxidation of the boronic acid.[32] Modeling of the

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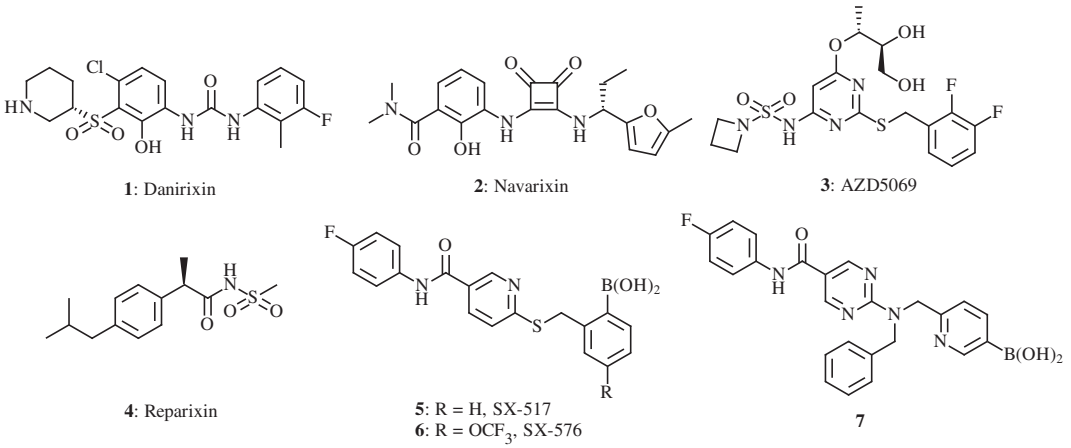
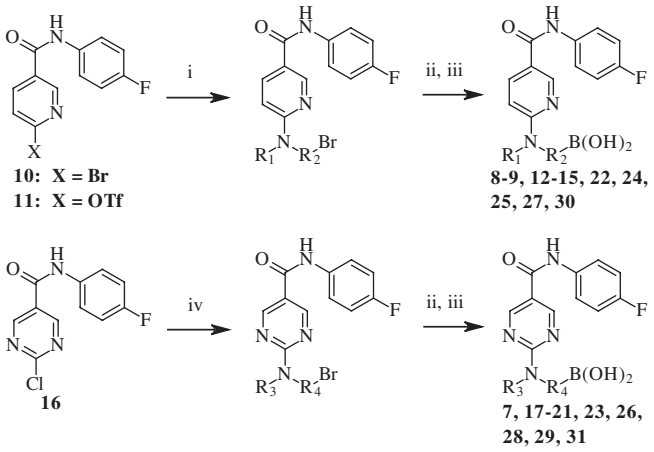


Figure 1. Chemokine antagonists.

binding of **6** with CXCR2, constructed from the recently solved structure of CXCR1,[34] revealed that this class of antagonists has a different binding model than previously described for other classes of compounds. However, the poor aqueous solubility of **6** led us to examine whether heteroatom replacement of the sulfur could improve its oral bioavailability. Herein, we describe our focused SAR studies that led to the identification of a third-generation compound **7**, which exhibited improved aqueous solubility and oral bioavailability while maintaining activity in an animal model of pulmonary inflammation.

Starting from our first- (**5**) and second- (**6**) generation inhibitors, we synthesized compounds that maintained a boronic acid in the 2-position of the phenyl ring, replacing the sulfur in **5** with secondary (**8**) and tertiary (**9**) amines, utilizing either a bromonicotinamide[35] or triflatenicotinamide[36] intermediate (**10**, **11**), depending on the reactivity of the amine. Generally, primary and *N*-methyl secondary amines were coupled with the bromonicotinamide intermediate, and more sterically-hindered secondary amines were coupled with the triflatenicotinamide intermediate. The general synthesis of the inhibitors is shown in Scheme 1. Shifting the position of the boronic acid to the 3- (**12**, **13**) and 4-positions (**14**, **15**) was also explored. The corresponding pyrimidine compounds were also prepared from a chloropyrimidinamide[37] intermediate (**16**), with the boronic acid in the 2- (**17**), 3- (**18**, **19**), and 4-positions (**20**, **21**). The corresponding pyrimidine analog to **8** could not be prepared using our standard synthetic methods, because the palladium-catalyzed conversion of the halogen to the boronic acid pinacol ester instead led to reduction of the halogen to an unsubstituted benzyl ring. Alternate routes to the desired product also proved unsuccessful.

The compounds were screened for their ability to inhibit calcium flux in a previously described cell-based assay.[33,38] Briefly, the synthesized compounds were incubated for 30 min with rat basophilic leukemia (RBL) cells stably transfected with either CXCR1 or CXCR2. After the addition of IL8, the release of intracellular calcium was measured via FLUO-4AM detection in a fluorescent microplate reader. The activity of the compounds against CXCR1 and CXCR2 is summarized in Table 1. In contrast to what was observed with our sulfur-containing compounds, the 2-position for the boronic acid was not well tolerated. Instead, the 4-position appeared to yield the most potent compounds, such as **21**. The lack of activity for the 2-position compounds was likely due to neighboring group interactions between the boronic acid and the amine.[39] Due to these observations, the 4-boronic acid, 2-pyridinyl analogs (**22**, **23**) were prepared and found to maintain inhibitory activity at CXCR2. These compounds also exhibited improved



Scheme 1. Reagents and conditions: (i) primary or secondary amine, DIPEA, anhyd DMF or NMP, 120–160 °C, 16–72 h; (ii) $PdCl_2$(dppf), bis(pinacolato)diboron, potassium acetate, anhyd. DMF, 80 °C, 3–6 h; (iii) KHF_2, then TMS–Cl/H_2O or HCl/dioxane or aq formic acid; (iv) primary or secondary amine, triethylamine, anhyd NMP, rt, 16–72 h.

aqueous solubility, making them good scaffolds for further substitution.

The addition of a phenyl ring (**24**, **7**) at the tertiary amine significantly improved potency of the inhibitors, in contrast to the addition of another pyridine ring (**25**, **26**). Additions of a tetrahydropyran ring (**27**), carboxylic acid (**28**), or amine (**29**) in efforts to further improve aqueous solubility, yielded inactive compounds. However, the addition of a furan ring (**30**, **31**) yielded compounds with similar potency to the phenyl compounds. The third generation inhibitors are summarized in Table 2. Interestingly, aminopyrimidine-based **7** was significantly more potent than its aminopyridine analog **24**, while the aminopyridine-based **30** was significantly more potent than its aminopyrimidine analog **31**. It was somewhat surprising that changing a single atom between the paired compounds made such a difference in their activity. Since they were the most potent in the cell-based assays, **7**[40] and **30**[41] were selected for further evaluation.

Compared with **6**, **7** and **30** were more potent inhibitors of IL8-mediated calcium flux in RBL cells stably transfected with CXCR1 or CXCR2, but they were not as potent at inhibiting calcium flux in isolated neutrophils, and **7** (185 ± 52 nM) was more potent than **30** (321 ± 96 nM). Due to its higher activity, **7** was selected for further testing.