European Journal of Medicinal Chemistry 150 (2018) 195–205

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Anti-inflammatory hybrids of secondary amines and amide-sulfamide derivatives

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ARTICLE INFO

Article history:
Received 12 January 2018
Received in revised form
15 February 2018
Accepted 27 February 2018
Available online 2 March 2018

Keywords:
CXCR4
Hybrids
Amide-sulfamide
Inflammation
Inflammatory cell accumulation

ABSTRACT

The CXCR4/CXCL12 chemokine axis can chemotactically accumulate inflammatory cells to local tissues and regulate the release of inflammatory factors. Developing novel CXCR4 modulators may provide a desirable strategy to control the development of inflammation. A series of novel hybrids were designed by integrating the key pharmacophores of three CXCR4 modulators. The majority of compounds displayed potent CXCR4 binding affinity. Compound **7a** exhibited 1000-fold greater affinity than AMD3100 and significantly inhibited invasion of CXCR4-positive tumor cells. Additionally, compound **7a** blocked mice ear inflammation by 67% and suppressed the accumulation of inflammatory cells in an *in vivo* mouse ear edema evaluation. Western blot analyses revealed that **7a** inhibited the CXCR4/CXCL12-mediated phosphorylation of Akt and p44 in a dose-dependent manner. Moreover, compound **7a** had no observable cytotoxicity and displayed a favorable plasma stability in our preliminary pharmacokinetic study. These results confirmed that this is a feasible method to develop CXCR4 modulators for the regulation and reduction of inflammation.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most frequently administered drugs due to their anti-inflammatory, analgesic, and antipyretic properties [1]. Nonselective NSAIDs show unfavorable dose-dependent gastric mucosal injury side effects due to their inhibition of biosynthesis of necessary PGs by COX-1 in the stomach. Selective COX-2 inhibitors reduce the risk of gastric ulcers compared with nonselective NSAIDs, but they have been associated with an increased risk of cardiovascular side effects, including myocardial infarction, stroke, and hypertension [1-3].

The C-X-C chemokine receptor type 4 (CXCR4)/C-X-C chemokine ligand 12 (CXCL12) axis has been shown to be involved in various pathological conditions, including HIV infection, cancer and inflammation [4, 5]. Evidence has shown that the CXCR4/CXCL12 chemokine axis can chemotactically accumulate inflammatory cells (neutrophils, monocytes, and lymphocytes) to local tissues and regulate the release of inflammatory factors that cause inflammatory responses [6, 7]. Therefore, developing novel anti-inflammatory agents targeting CXCR4 may be a desirable strategy to avoid the side effects of NSAIDs.

As shown in Fig. 1, AMD3100 is the first small molecule CXCR4 antagonist to enter clinical trials for the treatment of HIV infection [8]. However, AMD3100 was not approved due to poor oral bioavailability and serious cardiotoxicity [9-11]. Taking AMD3100 as the lead compound, our research group discovered a promising bis-secondary amine drug candidate, Q-122, which is under Phase II clinical trials. Subsequent work mainly focused on modifying the two amino groups of the secondary amine structure to obtain compound RB-55 with better CXCR4 inhibitory activity, proving that the amide- sulfamide structure is a new, promising scaffold targeting CXCR4 [12].

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https://doi.org/10.1016/j.ejmech.2018.02.085
0223-5234/

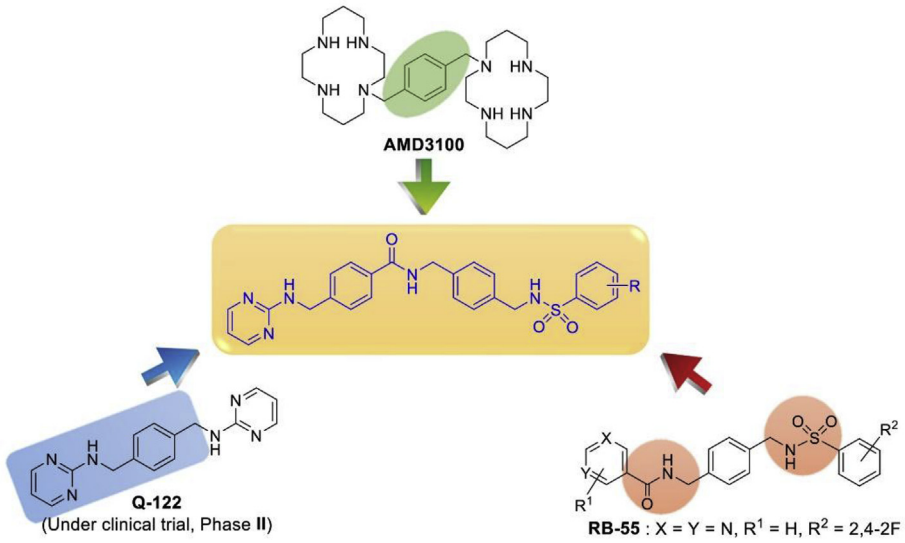

Fig. 1. Strategy for the discovery of novel amide-sulfamide anti-inflammatory agents.

The key pharmacophores of compounds AMD3100, Q-122, and RB-55 were incorporated into a single structure and a series of long-chain hybrids were designed and synthesized in the following study. The anti-CXCR4 effect, *in vivo* anti-inflammatory activity, pharmacokinetic properties, and cytotoxicity were also systematically screened.

2. Results and discussion

2.1. Chemistry

The synthetic route chosen to synthesize the targeted compounds was outlined in Scheme 1. Compounds **2a-q** were synthesized by sulfonylation of the starting material 4-(Boc-aminomethyl) benzylamine (**1**) with the corresponding sulfonyl chlorides in dichloromethane (DCM). The protective group Boc was subsequently removed in the presence of trifluoroacetic acid (TFA) producing the benzylamine intermediates **3a-q**. Compound **5** was prepared by reductive amination of another starting material terephthaldicarboxaldehyde (**4**) with 2-amino-pyrimidine. The subsequent oxidation reaction of compound **5** with Jones reagent afforded the key intermediate **6**. The final compounds **7a-q** were synthesized by the acylation of intermediate **3a-q** with acid **6**.

2.2. Primary binding affinity screening

All of the prepared compounds were first screened with a binding affinity assay as described in our previous publications [5, 12-15]. The screening protocol is a competitive CXCR4 binding assay between biotinylated TN14003, a potent CXCR4 peptidic inhibitor, and the target compounds **7a-q** at concentrations of 1, 10, 100, and 1000 nM. The effective concentration (EC) is used to measure the affinity, which is defined as the lowest concentration at which a significant reduction in the rhodamine fluorescent color is observed as compared to control (Fig. 3, without CXCR4 modulators). Thus, this initial screening is a semi-quantitative, primary screening of the level of activity, which is different from IC_{50}.

The vast majority of compounds showed comparable or even better CXCR4 binding affinity than that of AMD3100 (Table 1 and Fig. 2). Compound **7b** displayed 100-fold, and compounds **7a** and **7i** exhibited 1000-fold more potent activity than AMD3100. In terms of the benzenesulfonyl side chain, receptor affinity was obviously increased with no substitution or substitution with an electron-donating group ($-CH_3$). Moreover, when substituted with electron-withdrawing groups (-F, $-NO_2$, $-CF_3$), binding affinity remained weak, as all the ECs were no less than 1000 nM. Only 3-Cl, 4-Cl and 3,4-difluoro-substituted derivatives showed favorable activity. More importantly, when a nitrogen atom, heterocyclic, or aromatic ring were introduced to the benzenesulfonyl moiety, the affinity weakened significantly.

2.3. Matrigel invasion assay

Activation of CXCR4 through its ligand CXCL12 mediates migration and invasion. Thus, a Matrigel invasion assay was performed to probe whether the selected compounds with better binding affinities to CXCR4 can also block CXCR4/CXCR12 mediated chemotaxis and invasion [13, 15]. The target compounds and cells were added to the upper chamber of a vessel and CXCL12 was added to the lower chamber as a chemoattractant in serum-free medium. Binding of CXCR4 at the cell surface with the selected compounds would block CXCL12 chemotaxis to the cells. Therefore, MDA-MB-231 human breast cancer cells at the top chamber treated with the compounds would be inhibited to migrate from the top chamber through the Matrigel-coated filter pores to the bottom of the filter. Inhibition of cell invasion with each tested compound was calculated by comparing results to the cell invasion without treatment. The results of Matrigel invasion are summarized in Fig. 3.

All selected compounds ($EC \leq 100$ nM) effectively inhibited tumor cell invasion. Compared to the negative control group, the number of cells invading through the chamber decreased significantly, exceeding 65% inhibitory activity (Fig. 4). The compounds' anti-invasive activities was improved when sulfonamide side chain was substituted. Compounds **7d** (4-CH_3) and **7i** (3-Cl) showed the most effective anti-invasive activity, reaching 87% and 88% inhibition, respectively, which is superior to the reference drug AMD3100 (55%). The above invasion results give eloquent proof that the amide-sulfamide structure is a potent scaffold to block CXCR4 function.