Bioorganic & Medicinal Chemistry 21 (2013) 6447–6455

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis of amino-hydroxy-benzocycloheptenones as potent, selective, non-peptidic dinuclear zinc metalloaminopeptidase inhibitors

Mira Al-Lakkis-Wehbe, Bérénice Chaillou, Albert Defoin, Sébastien Albrecht *, Céline Tarnus *

Université de Haute Alsace, Laboratoire de Chimie Organique et Bioorganique, EA4466, ENSCMu, 3, rue Alfred Werner, F-68093 Mulhouse Cedex, France

ARTICLE INFO

Article history:
Received 6 May 2013
Revised 20 August 2013
Accepted 23 August 2013
Available online 4 September 2013

Keywords:
Racemic trisubstituted benzocycloheptane derivatives
Synthetic inhibitors
Selective inhibition
M1 aminopeptidases
M28 aminopeptidases

ABSTRACT

Racemic trisubstituted benzocycloheptanes were synthesized and evaluated for their ability to inhibit metalloaminopeptidase activities. A highly selective nanomolar inhibitor of a prototypical '*two zinc*' aminopeptidase from the M28 family was observed with these tridentate species, while bidentate analogs proved to be highly selective for the '*one zinc*' M1 family of enzymes. The selectivity profile of these new, low molecular weight structures may guide the design of specific, non-peptidic inhibitors of binuclear aminopeptidases.

1. Introduction

Metalloaminopeptidases (APs) remove amino acids from unblocked N-termini of peptides and proteins. This family of exopeptidases is widely distributed in nature, with representative members present in animal cells, plants, bacteria and fungi.[1,2] Although some APs are secreted, the vast majority are either cytosolic or membrane-bound enzymes.[3–5] They play pivotal roles in many biological processes such as pain, blood pressure regulation, immune cell response and cell migration in mammals and/or amino acid metabolism in parasites for examples.[6–9]

Most metalloaminopeptidases catalyse the same chemical reaction with the active site region related to that of thermolysin. Generally cleavage of the scissile peptide bond at the N-terminus of a peptide or a protein, is dependent upon the chemical structure and composition of amino-acid residues that flank the cleavage site ($P_1 * P'_1$).[10] They can be broadly classified into two sub-families on the basis of the number of their active site zincs. While some of these enzymes contain only one zinc, others possess a binuclear metal centre, usually referred to as a co-catalytic unit.[10] Since all these APs are zinc-dependent enzymes sharing a large substrate specificity, the development of specific inhibitors is undoubtedly a daunting task and is of utmost importance in order to gain a deeper understanding of the respective biological functions of APs and to assess their potential as drug targets. In the present study we focused on aminopeptidases that prefers hydrophobic residues in P1 position. Recent reviews illustrate the various concepts developed up to now and the lack of selectivity, when reported, observed in many cases.[11–15]

We have previously reported aminobenzoheptanone **1a** as a specific lead structure for the design of selective inhibitors of the 'one-zinc' M1 family of aminopeptidases, with an excellent Ligand Efficiency (LE = 0.63).[16] In this series of compounds, well-chosen substitutions with appropriate functional groups led to the most potent inhibitor of mammalian APN (CD13), **1b** (K_i = 60 pM).[17] The present study explored further modifications of chemotype **1a** in order to design specific inhibitors for binuclear aminopeptidases. We have considered trisubstituted benzocycloheptane derivatives as target structures based on bestatin **2** efficacy on 'two zinc' aminopeptidases.[18] Actually, bestatin **2**, usually used as a standard inhibitor of APN with a K_i value in the micromolar range, is a much more potent inhibitor of the binuclear aminopeptidases with nano- to subnanomolar affinities.[18] We have decided first of all to synthesize racemic aminohydroxyketones (*cis* and *trans*) **3a**–**b**, as well as the corresponding aminodiols **4**, **5** and the *cis*-aminohydroxyketone **6**. We have also investigated the preferred position for these functional groups, with the amino group moved to position 7 in the *cis*-aminodiol **7** and the *cis*-aminoketol **8**. The reported inhibitory activity of these new compounds on APN (EC 3.4.11.2) and *Aeromonas proteolytica* AP (EC 3.4.11.10), two

* Corresponding authors. Tel.: +33 3 89 33 68 59; fax: +33 3 89 33 68 60.
E-mail addresses: sebastien.albrecht@uha.fr (S. Albrecht), celine.tarnus@uha.fr (C. Tarnus).

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.08.044

prototypes of the one and two-zinc aminopeptidases, respectively, showed that the trisubstituted analogue **6**, proved to be an interesting lead chemotype for selective inhibition of the two zinc families of aminopeptidases. It is proposed to behave as a new tridentate zinc binding motif (Scheme 1).

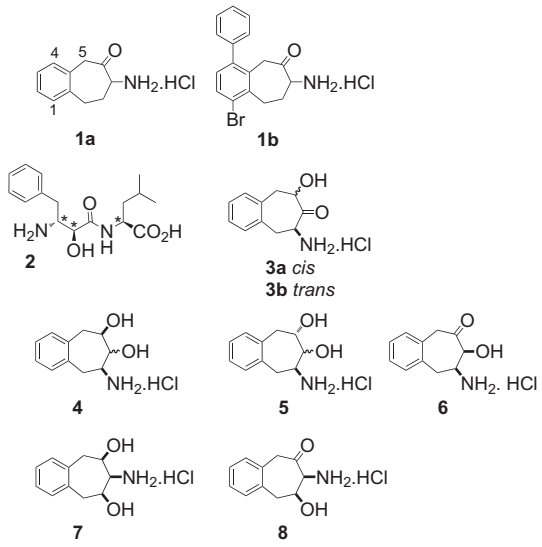
2. Chemistry

The known benzoheptanone **9**[19–21] was the starting material for the synthesis of the target structures and its functionalization was performed according to described procedures.[16] For the first series, the amine function was introduced by substitution of a bromine atom with azide, whereas a reductive amination of ketone allowed the access to the second series.

2.1. First series, amino group in position 6 or 8

The *cis*-bromosilyloxy-ketone **13** was a key intermediate for the synthesis of the first series (Scheme 2, analogues **3a** and **3b**). It was obtained from ketone **9** according to the silyloxylation method[16] which led to α-silyloxy-ketone **11**. This method consisted in silylation of the enol ether with silyl triflate and triethylamine[22] into **10** and then oxidation-rearrangement with *m*-CPBA.[23] In this case, the *tert*-butyldimethylsilyl group was preferred due to its better stability during the following steps. Formation of the trimethylsilylenol ether **12** was achieved by the same procedure. The enol ether **12** was brominated with NBS into the *cis*-bromosilyloxyketone **13** in quantitative yield from ketone **11**.[24] Bromine substitution with sodium azide in DMF[25] occurred with isomerization and partial elimination to give a 60:22:18 mixture of the *cis* and *trans* azidoketones **14a** and **14b**, and the known silyloxyenone **15**[16], respectively.

Isomers **14a** and **14b** were isolated and the catalytic hydrogenation of the azide function over palladium in ethanol in presence of 1 N aqueous hydrochloride acid led to the aminohydroxyketones **3a** and **3b**, as stable crystalline hydrochlorides in ca. 50% yield. For the *cis*-isomer **14a**, the reduction in acidic medium produced a spontaneous desilylation leading to the amine **3a**. By contrast, the *trans*-isomer **14b** gave a partially O-silylated compound which was totally deprotected into **3b** by further action of hydrogen chloride in dry ether. The aminohydroxyketones **3a** and **3b** were in these conditions obtained from the ketone **9** in ca. 16% and 4% yield, respectively. The *cis* and *trans* configuration of the amines **3a** and **3b** was deduced from the NMR data using the theoretical calculation for the benzocycloheptane conformation (see Section 5.9).[16,17,26,27]



Scheme 1. Chemical structures of racemic trisubstituted derivatives **3–8** and enantiopure bestatin **2**.

The preparation of the corresponding aminodiols **4a**,**b** and **5a**,**b** is presented in Scheme 3 from the azido-ketones **14a** and **14b**, respectively. The reduction of **14a** and **14b** was carried out in acidic medium, with sodium cyanoborohydride in the presence of acetic acid to avoid any basic isomerization (otherwise observed). The *cis*-isomer **14a** led to a 60:40 diastereoisomeric mixture of **16a** and **16b**, the all-*cis* compound **16a** being preponderant. The *trans*-isomer **14b** gave the silylated alcohols **17a** and **17b** as a 80:20 diastereoisomeric mixture, the major one being the *cis*-azidoalcohol **17a**. O-deprotection by action of dry hydrogen chloride in ether provided the crystallized diol mixture **18a** and **18b**, which was directly oxidized by Dess–Martin periodinane (DMP) to give, after recrystallization, the pure ketone **19a** in 60% yield from the *trans*-azide **14b**. The good regioselectivity of the oxidation might be due to a preferential oxidation on the less hindered C-6 hydroxyl function.

The catalytic hydrogenolysis of the azide function either of the silyloxyl mixtures **16a** and **16b**, or the diol mixture **18a** and **18b** was performed over palladium in ethanol in the presence of hydrochloric acid. In the case of **16a**,**b** a spontaneous O-deprotection occurred to furnish a 60:40 mixture of the corresponding amines hydrochlorides **4a** and **4b** in 65% yield from the azido-ketone **14a**. The diols **18a**,**b** were hydrogenolyzed directly to a 93:7 mixture of the amines hydrochlorides **5a** and **5b** in 49% yield from the azido-ketone **14b**.

Finally, the hydroxyketone **19a** was hydrogenolyzed over Adams's catalyst into the *cis*-hydroxyaminoketone **6** in 10% yield after recrystallization. This poor yield might be due to the low temperature stability of compound **6**. Indeed, thermal degradation occured at 60 °C and may be responsible for the difficulties encountered in obtaining and purifying compound **6**. However, it was only characterized by ^{1}H and ^{13}C NMR. In ^{1}H NMR spectrum in CD_3OD compound **6** appeared only as a hemiketal suggesting a fast hydration of the ketone.[16,27]

The target amino-diols **4a**,**b**, as well as **5a**,**b** and the aminohydroxyketone **6** were thus synthesized in ca. 16%, 7% and 0.9% overall yield from the ketone **9**, respectively.

2.2. Second series: amine function in position 7

The starting compound in this second series was the unsaturated aminoalcohol **20** as a 80:20 *cis*–*trans* mixture. The synthesis of this intermediate was already described in a precedent work.[16]

The second hydroxyl group was introduced by regioselective hydride reduction of the major *cis*-epoxide **21** which was obtained from **20** by reaction with *m*-CPBA. Its *cis*-configuration corresponded to the standard regioselectivity observed for the epoxidation of α-hydroxylated double bond. Its reduction by the diisobutylaluminium hydride (DIBAL)[28] at low temperature occurred selectively at the benzylic position into the diol **22** in good yield.

N-deprotection of this diol with dry hydrogen chloride in ether/dioxane gave the all *cis*-amino-diol **7** as hydrochloride salt in 23% overall yield from the ketone **9**.

The amido-diol **22** was smoothly oxidized with Dess–Martin periodinane (DMP, 1.3 equiv) in order to react only one hydroxyl function.[29] The corresponding hydroxy-ketone **23** was isolated in good yield and its acidic N-deprotection provided the *cis*-hydroamino-ketone **8** in 56% yield from the diol **22** or 20% overall yield from the ketone **9** (Scheme 4).