Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 1783–1786

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Improved Gelatinase A Selectivity by Novel Zinc Binding Groups Containing Galardin Derivatives

Franck Augé,[a] William Hornebeck,[b] Martine Decarme[b] and Jean-Yves Laronze[a,*]

[a]*UMR 6013 'Isolement, Structure, Transformation et Synthèse de Produits Naturelles', Faculté de Pharmacie, IFR53 Biomolécules, Université de Reims Champagne-Ardenne, 51 Rue Cognacq Jay, 51096 Reims Cedex, France*

[b]*FRE 2534 CNRS, Faculté de Médecine, IFR53 Biomolécules, Université de Reims Champagne-Ardenne, 51 Rue Cognacq Jay, 51096 Reims Cedex, France*

Received 3 December 2002; revised 21 February 2003; accepted 21 February 2003

Abstract—The synthesis of several analogues of galardin, a MMP inhibitor, are presented with their in vitro inhibitory activity against MMP-1 and MMP-2. These compounds contain a distinct Zinc Binding Group (ZBG). Those having a 2-acylated-heterocycle as well as a 2-arylamide function do not exhibit a good inhibition/selectivity against the enzymes tested. On the contrary, those that are based on a hydrazide scaffold present potent selectivity for MMP-2 versus MMP-1.

Zn-metalloproteases have been, for a long time, targets for new drugs. The major example was the pioneering work of Ondetti[1] on inhibitors of angiotensin converting enzyme, which led to the first antihypertensive drug of this family (Captopril®, Fig. 1). Nearly all the zinc-containing metalloprotease inhibitors are designed around, at least, two pharmacophores, a Zinc Binding Group (ZBG) and a peptide-like group. These compounds behave as competitive inhibitors, providing that the latter group can fit into one or more substrate recognizing subsites of the target enzyme and that the ZBG binding mode can mimic one of the transition states occurring during the substrate hydrolysis.

Hydroxamic acid was soon found to be one of the most powerful ZBG. However the use of this ZBG in drugs led to extreme undesirable effects, due to the poor bioavailability of such function and especially to its high transition-metal binding ability. For example, bufexamac[2] (*p*.butoxybenzylhydroxamic acid, Parfenac®), a non-steroidal anti-inflammatory drug, typically used in mild skin disorders, sometimes exhibit undesirable effects by initiating multiform exudative erythema and was therefore withdrawn from the market in many countries. As a consequence, the hydroxamic acid function is no longer present in any major drug.

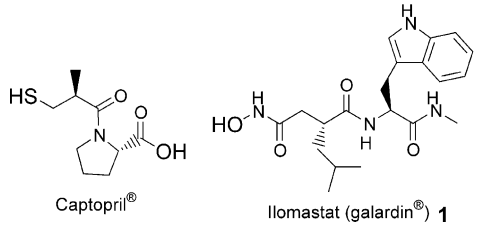

Figure 1. Examples of Zn-metalloprotease inhibitors containing a ZBG.

To avoid the use of hydroxamic acid in matrix metalloprotease (MMP) inhibitors, other ZBG containing compounds as carboxylate, phosphonate, thiol ... were synthesized. However, associated with this structural change, there is an important loss of activity compared to the hydroxamate counterpart.[3] In continuation of our work on MMP[4] and MMP inhibitors,[5] we have synthesized and evaluated the MMP inhibitory capacity of galardin[6] derivatives having novel ZBGs which theoretically might behave as potent chelating agents suggested by our previous calculations. Our challenge was to find a new organic group able to bind to the catalytic zinc ion of MMP with, not only a good affinity for this site, but also a good specificity, in the view to avoid undesirable effect, inherent in this type of inhibitor.[7]

*Corresponding author. Tel.: +33-326913589; fax : +33-326918025; e-mail: jy.laronze@univ-reims.fr

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doi:10.1016/S0960-894X(03)00214-2

Although clinical trials have proved MMP inhibitors to fail in last-stage metastatic cancers,[8] it must be kept in mind that they could be useful in the first stage of tumor progression, provided they display MMP selectivity, in order to preclude extreme side effects like musculoskeletal ones.

In this letter, we described our preliminary results on new non-hydroxamate MMP antagonists, with selectivity towards MMP-2 versus MMP-1. Three types of potential ZBGs have been devised and are shown in Figure 2: 1–4 ligands where X could be a hard or soft heteroatom (family **A**), 1–5 ligands (family **B**), hydrazides (family **C**), compared to hydroxamate (compound **D**). All these ligands are mimics of one transition state encountered in the MMP hydrolysis mechanism pathway, according to Lovejoy et al.[9]

Chemistry

Potential *A*-type ligands: 2-acylated heteroaromatic compounds (**2**) (Fig. 3).

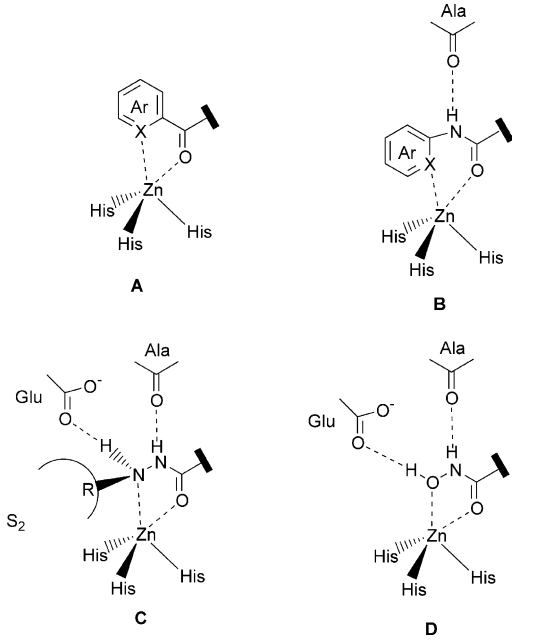


Figure 2. Possible binding mode of the different ZBG described in this paper.

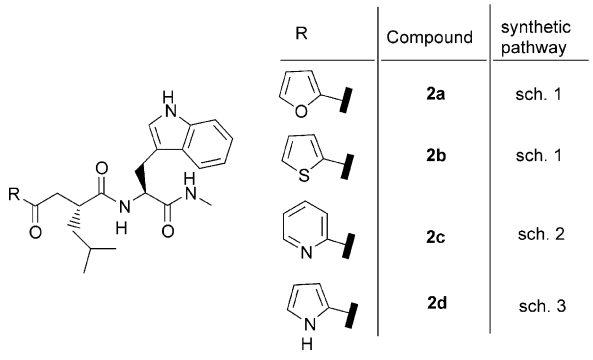
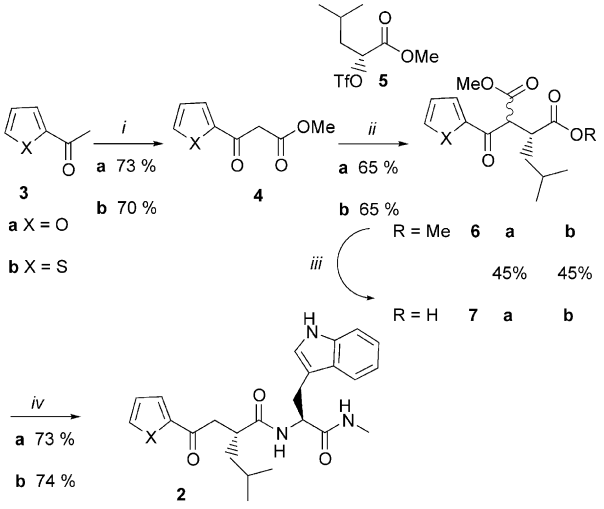


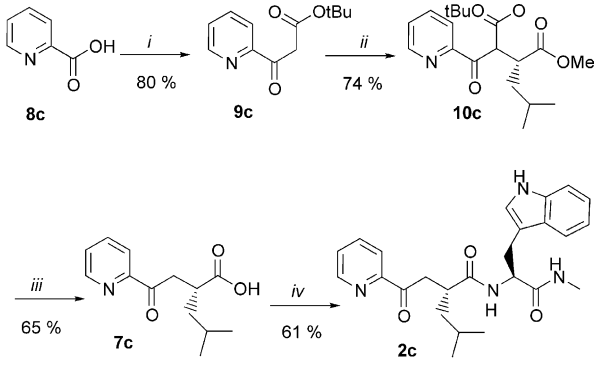
Figure 3. Compounds belonging to family **A**.

Compounds **2a**[10] and **2b** have been synthesized following the method of Hoffman et al.,[11] starting from arylic ketones **3a** and **3b** (Scheme 1). As known procedures[12] to prepare **4** from **3** were ineffective, we adapted the Krapcho's method[13] for our purpose. The coupling of the known chiral triflate[14] **5** gave β-dicarbonyl compounds **6**, further hydrolyzed and decarboxylated into acids **7** with acceptable yield, without any racemization of the surviving chiral center. Derivatives **7** were coupled with *N*-methyltryptophanamide already synthesized in our laboratory.[5b]

The above method could not be used for the pyridine derivative **2c**, because the intermediate **6c** gave only very small amounts of targeted acid **7c** due to the hydroxide induced the cleavage of the β-dicarbonyl system in this case. Finally, **7c** was prepared following Scheme 2 via *tert*-butylester **9c**. Amidation by *N*-methyltryptophamide did not proceed with DCC-HOBt as well as with other more or less classical coupling reagent usual in peptide syntheses. Fortunately the reaction worked conveniently with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-methylmorpholinium chloride (DMTMM) developed by Kunishima et al.[15]



Scheme 1. Synthetic route to prepare compounds **2a** and **2b**. (i) NaH, $CO(OMe)_2$; (ii) NaH, **5**, THF; (iii) LiOH, H_2O, 0 °C then PhH reflux; (iv) *N*-methyltryptophanamide, DCC-HOBt, CH_2Cl_2.



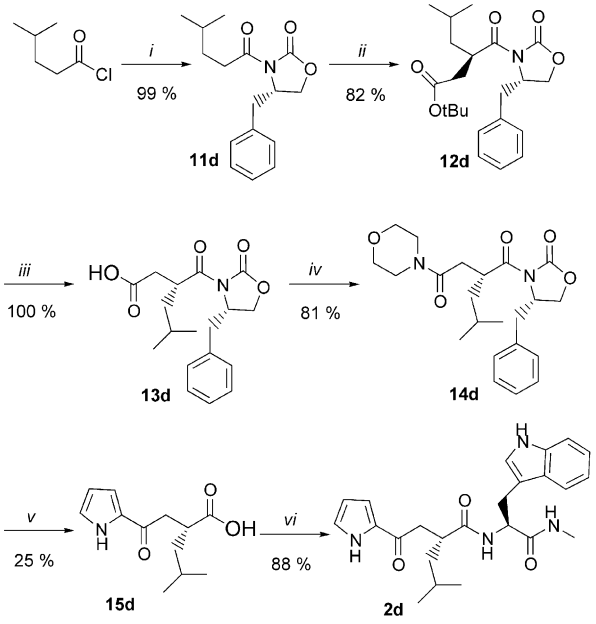
Scheme 2. Synthetic route to prepare the compound **2c**. (i) $COlm_2$, LDA-THF −78 °C, $MeCO_2tBu$; (ii) NaH, THF, **5**; (iii) 1M TFA in CH_2Cl_2 then LiOH; (iv) *N*-methyltryptophanamide, DMTMM, MeOH.

The synthesis of pyrrole derivative **2d** cannot be carried out by the above methods, since it needs protection of the pyrrole nitrogen by a benzyl group which we were unable to remove at the last stage of the synthesis. So, in view of that, pyrrole was introduced in the latter stages of the synthesis, thus avoiding its protection (Scheme 3).

Evan's Chiral oxazolidine **11d** was alkylated to the *tert*-butylester **12d** with complete control of the chirality of the new center. The cleavage of the *tert*-butyl group gave the acid **13d**, which was further transformed into morpholinamide **14d**. The Vilsmeier–Haack arylation and oxazolidine removal was performed in the same experiment, unfortunately in low yield. Finally **15d** was coupled to *N*-methyltryptophanamide with DMTMM.

Potential *B*-type ligands: arylamides **16** (Fig. 4).

They were prepared along Scheme 4: Evan's chiral oxazolidine **12d** was cleaved to the acid ester **17**, which was coupled with *N*-methyltryptophanamide in the presence of DMTMM. The *tert*-butyl group was removed by TFA, in refluxing methylene chloride, giving acid **18**. Arylamide **16c** was smoothly obtained from **18** and



Scheme 3. Synthetic route for the compound **2d**. (i) ref 16: (ii) $BrCH_2CO_2tBu$, HMDS −78 °C; (iii) TFA 1M CH_2Cl_2; (iv) DCC-HOBt, morpholine; (v) 1/$POCl_2$, 2/pyrrole, 3/LiOH, H_2O_2; (vi) *N*-methyltryptophanamide, DMTMM.

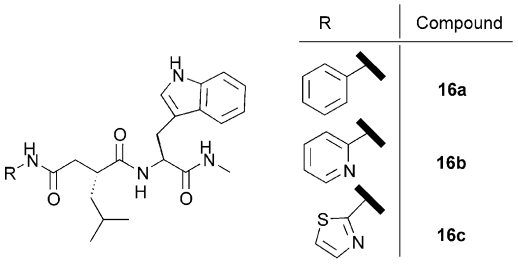
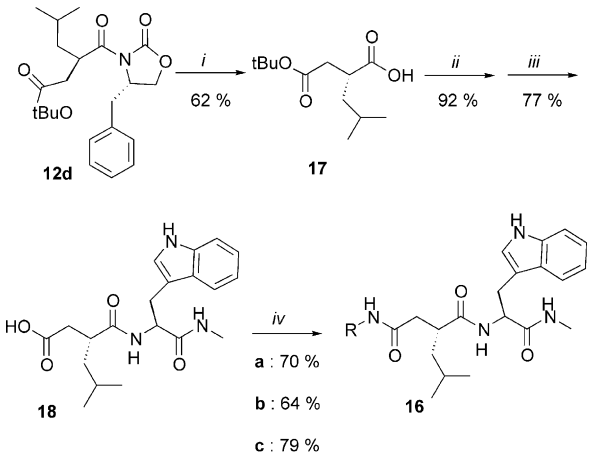


Figure 4. Compounds belonging to family B.



Scheme 4. Synthetic route to prepare compounds **16a**, **16b**, **16c**. (i) LiOH, H_2O_2 in THF/H_2O, 0 °C; (ii) *N*-methyltryptophanamide, DMTMM, THF; (iii) 1M TFA in CH_2Cl_2, reflux; (iv) DMTMM + conditions (see text).

2-aminothiazine in THF which was not the case for weekly basic aniline and 2-aminopyridine, which are not able to deprotonate acid **18** into its carboxylate counterpart. Adding *N*-methylmorpholine (NMM) overcame this problem, giving **16a** and **16b** in acceptable yields.

Potential *C*-type ligands: hydrazides **19** (Fig. 5).

Hydrazides **19b** and **19c** were prepared in low yield from acid **18**, via DCC-HOBt coupling of methylhydrazine and phenylhydrazine, respectively.

Hydrazine itself did not cleanly react with **18** in these conditions, but **19a** was obtained in 45% yield from **18**, using the DMTMM/NMM system in THF. The same approach allowed smooth preparation of sulfonylhydrazide **19d** in 67% yield (DMTMM/NMM in THF). For the best of our knowledge, DMTMM-assisted synthesis of hydrazides has, until now, not been documented.

Biology

Preliminary assay was performed by gelatin zymography on compounds **2**, **16**, **19** and also on galardin **1** and its carboxylic analogue **18** as comparison models. All compounds were incorporated into the incubation buffer at concentration from 10^{-4} to 10^{-7} mol L^{-1}. We used conditioned medium from HT 1080 fibrosarcome cells as a source of gelatinases (MMP-2 and MMP-9). Compounds **2a**, **2b**, **16a**, **16c**, **19b**, **19c** proved to be non inhibitory in such an assay and, thus, were not considered for the determination of IC_{50} using synthetic

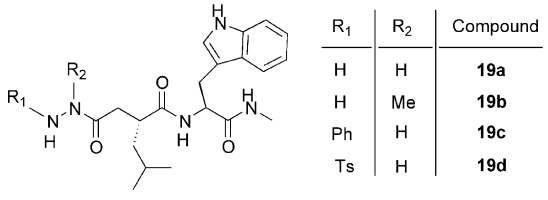


Figure 5. Compounds belonging to family **C**.

Table 1. Analytical data and biological activities on the most active compounds, compared to our model **18** and galardin

Compd	Mp °C	$[\alpha]_D^{21}$ (solvent)[a]	UV (λ_{max}, nm)	IC_{50} MMP-1 (nM)	IC_{50} MMP-2 (nM)
2c	79–80	−8.6 (CH_2Cl_2)	222,270,290	730	180
2d	109–110	+ 20.9 (CH_2Cl_2)	202,246,287	860	540
16b	87–88	−28.5 (MeOH)	205,223,278,291	60	50
19a	187–188	+ 7.2 (DMSO)	205,222,275,283,291	50	550
19d	192–193	−8.3 (MeOH)	204,222,280,292	$>10^4$	30
18				6	20
Galaradin				3	0.4

[a]c = 10 mg.mL^{-1}.

substrates. Inhibition of recMMP-1 and recMMP-2 (VWR Calbiochem) by galardin derivatives was determined using DNP-Pro-β-cyclohexylAla-Gly-Cys(Mes)-His-Ala-Lys(*N*-methylaminobenzoyl)-NH_2 (VWR Calbio-chem) and MCA-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2 (Bachem), respectively, according to previous published procedures.[5a,5c]

Experiments were performed at 37 °C, in a 50 mM Tris/HCl, 150 mM NaCl and 5 mM $CaCl_2$, pH 7.5 buffer, under steady state conditions, and IC_{50} values are reported in Table 1.

Discussion

Clearly **A**-type potential ZBG are not effective as MMP-inhibitors with the exception of compound **2c**. This is in spite of our calculation predicting a high stabilization of the complex $Zn^{2+}(His)_3$ by 2-acylpyridine **2d**. This lack of activity cannot be solely attributed to the bulkiness of the pyridine ring compared to the hydroxamate group, but rather to the greater conformational rigidity of these aromatic derivatives versus the galardin that hampers the access of inhibitor to its target (unpublished molecular mechanic results).

In the **B** family of ligands, **16b** showed increased activity, comparatively to its **A** counterpart, **2c**. This could be rationalized as being due to the extra hydrogen bond made with the carboxylate of the Glu residue involved in the hydrolysis mechanism (Fig. 2).

Much more interesting is the sulfonylhydrazide **19d** nearly 20 times more active than the non-sulfonylated derivative **19a**, with a high selectivity toward MMP-2 versus MMP-1. Two factors can contribute to this enhancement: the first one is the increased acidity of the N–H, close to the SO_2 electron-withdrawing group which enhances H-bonding to the Glu residue (Fig. 2). Such substitution also increases the partial negative charge on this nitrogen strengthening the bonding to the Zn ion.

Work is in progress to assign a more precise role to the arylsulfonylhydrazide group to help enhance its MMP-2 affinity and selectivity. A structure activity study is also in progress to optimize this compound by modifying the terminal *N*-methyltryptophanamide group by unnatural tryptophane derivatives.[17]

Acknowledgements

The authors thank the Association pour la Recherche sur le Cancer (ARC) for its financial support No. 9488.

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