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Bioorganic & Medicinal Chemistry Letters 13 (2003) 2843–2846

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

MMPs Inhibitors: New Succinylhydroxamates with Selective Inhibition of MMP-2 over MMP-3

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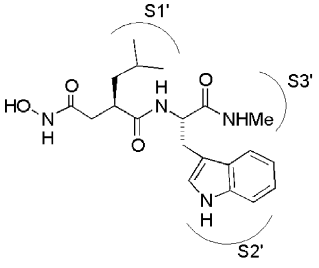
Received 11 December 2002; revised 30 April 2003; accepted 11 June 2003

Abstract—Some ilomastat analogues featuring an isobutylidene group or a 2-substituted indole nucleus were synthesized to evaluate their inhibitory activities against gelatinase A and stromelysin-1. Potent MMP-2 inhibition and good selectivity for that enzyme have been observed for compounds **1a**, **2** and **22**.

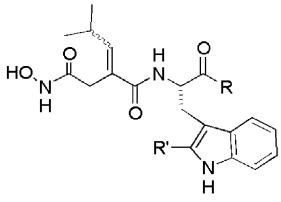
In humans, the MMP family comprises at least 22 members which can hydrolyze virtually any component of the extracellular matrix.[1] Their involvement in all stages of cancer progression is now well documented and intensive efforts have been made to design MMP inhibitors as cancer therapeutical agents. However, data from clinical trials have revealed undesirable side effects which can be partly attributed to the broad spectrum MMP inhibitory activity of those drugs.[2]

Gelatinase A (MMP-2) plays an active role in both tumor growth by favoring tumor angiogenesis and matrix invasion of cancer cells as melanoma cells. Its activation is catalyzed mainly by MT_1 and MT_2 MMP at the cell plasma membrane; on the contrary, other MMPs are processed through proteolytic cascades where stromelysin-1 (MMP-3) occupies a central position. In previous investigations,[3] we observed that long chain unsaturated fatty acids, but not their saturated counterparts, displayed MMP inhibitory activity. Besides, those compounds were found to exhibit higher inhibitory activity towards MMP-2 as compared to MMP-3[4] although both MMPs were described to contain deep S1′ hydrophobic pockets. We thus anticipated that introduction of an unsaturation at that site could bring specificity to MMP inhibitors.

We therefore prepared ilomastat (galardin®) analogues featuring an isobutylidene group at S1′ (**1**) and some modifications at S2′ and S3′ subsites (**2** and **3**) in order to increase their overall hydrophobicity.



Ilomastat (GM 6001)



1 R = NHMe, R' = H
2 R = OEt, R' = H
3 R = NHMe, R' = $(CH_2)_2NMe_2$

Chemistry

The access to the targeted molecules required the preparation of alkylidenesuccinic monoesters (Scheme 1). Our first attempts, using as the most direct approach, the Stobbe condensation between diethylsuccinate and isobutyraldehyde led to the expected half-ester with low yield, as frequently observed.[5] So we turned to the Wittig reaction, utilizing the process developed by Mc Combie to prepare arylidenesuccinates.[6] The phosphorane generated in situ by reaction between methyl maleate **4** and tri-*n*-butyl phosphine was reacted with isobutyraldehyde to yield the dimethyl alkylidene succinate **5** as a 9:1 *E*/*Z* mixture.[7] We also applied the

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0960-894X/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00590-0

Emmons–Wadsworth–Horner reaction,[8] following a procedure described by Dhokte et al.[9] The phosphonate **6** was condensed with isobutyraldehyde to yield the diethyl alkylidene succinate **7** as a 1:2 *E*/*Z* mixture. Both diesters **5** and **7** were then hydrolyzed and the resulting diacids were dehydrated, thus leading to the anhydrides **8a** (single *E*-isomer) and **8b** (*E*:*Z* mixture), respectively. Finally, refluxing **8a** in benzyl alcohol provided the monoester **9a** as the single *E*-isomer while **8b** treated by methanol or benzyl alcohol gave **9ba** and **9bb** as *E*/*Z* mixtures.

The dehydro analogues of ilomastat **1a** and **1b** were then prepared from succinates **9a** and **9ba** and (L)-tryptophan methylamide **10**[10] via a classical pathway[11] (Scheme 2). Coupling of **10** with monoester **9a** mediated by DCC gave the succinyltryptophanamide **12a**. After deprotection of the benzyl ester, the free acid **13a** was converted in two steps[12] into the pure *E*-hydroxamic acid **1a**.[13]

Alternatively, tryptophanamide **12b** obtained from **10** and methyl monoester **9ba** led to the *E*/*Z* 1:1 mixture **1b**.

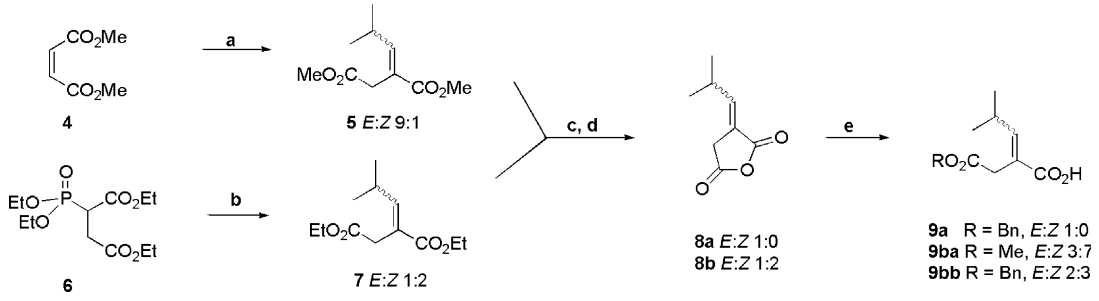
A similar sequence of reactions gave access to the *E*-ethyl carboxylate **2** from ethyl tryptophanate **11** and **9bb** via **14**.

With the compounds **1a**, **1b**, **2** in hand we were able to evaluate the influence of three parameters: (i) the presence of a double bond at P1′, (ii) the effect of its geometry and (iii) the importance of an amide function at the C-terminal part of the molecule.

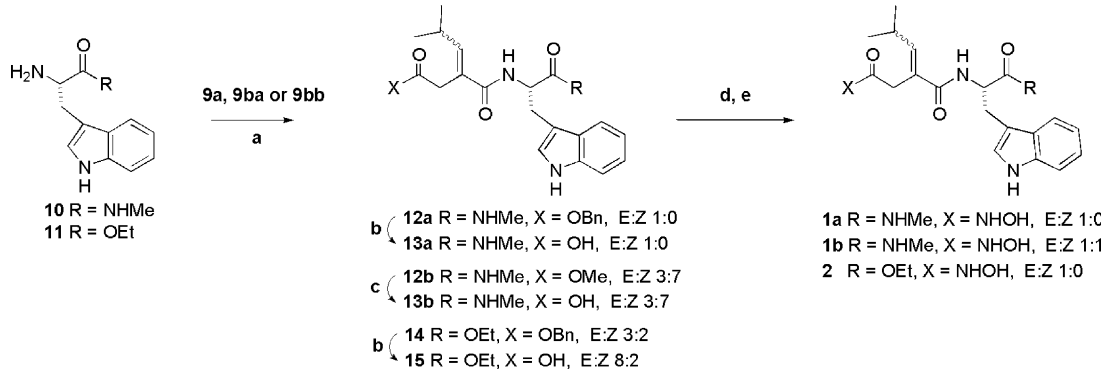
Substituted indole dehydro derivatives such as **3** were our next target. To this end we applied a method reported in a preceding paper:[14] the pseudopeptide backbone was generated through the reaction of a succinyl aminomalonate with a quaternarized gramine[15] and the hydroxamate was introduced at the beginning of the reaction sequence (Scheme 3).[16] Thus, the 2-(succinylamino)malonate **16** was prepared by reaction of **9bb** with diethyl aminomalonate. Unfortunately, hydrogenolysis of the benzylic ester was accompanied by hydrogenation of the double bond and led to the racemic carboxylic acid **17**. However, the synthesis was continued, at aim to obtain substituted indole derivatives of ilomastat. Thus the benzyl hydroxamate **18** derived from **17** was condensed with N_b-methyl-tetrahydro-β-carboline methyliodide **19**, affording the indole derivative **20**. Hydrolysis-decarboxylation of **20** followed by amidation gave the methyl amide **21** which was converted into the hydroxamic acid **22**.[17] This compound, even as a mixture of four diastereomers was biologically assayed.

Biological Evaluation

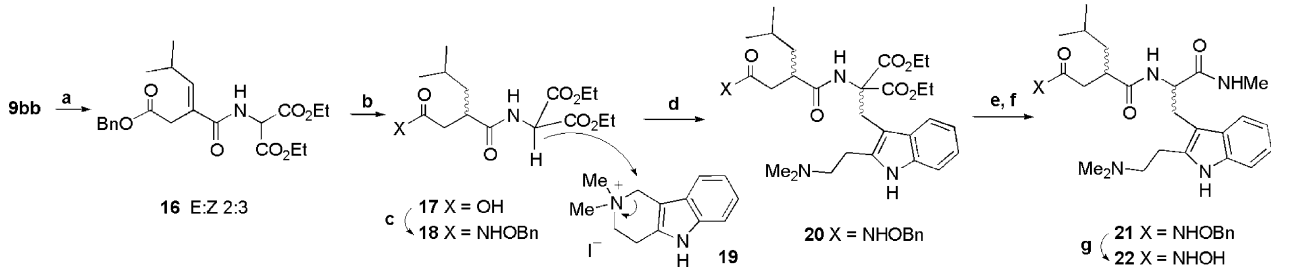
Compounds **1a**, **1b**, **2** and **22** were tested in vitro for inhibition of MMP-2 and MMP-3 and compared to ilomastat.[18] The results are shown in Table 1.



Scheme 1. Reagents and conditions: (a) isobutyraldehyde, $(n\text{-Bu})_3P$, THF, 25 °C, 96 h, 56%; (b) isobutyraldehyde, NaH, Et_2O, 25 °C, 3 h, 100%; (c) NaOH, H_2O, 25 °C, 72 h, 75%; (d) AcCl, reflux, 3 h, 100%; (e) ROH, reflux, 55–61%.



Scheme 2. Reagents and conditions: (a) DCC, CH_2Cl_2, 25 °C, 16 h, 49–61%; (b) H_2, Pd/C, EtOH, 25 °C, 3–4 h, 100%; (c) LiOH·H_2O, THF/H_2O (3:1), 25 °C, 3 h, 96%; (d) NH_2OBn·HCl, Et_3N, DCC, HOBt, CH_2Cl_2, 25 °C, 16 h, 77–100%; (e) H_2, Pd/C, EtOH, 25 °C, 5.5 h, 90–100%.



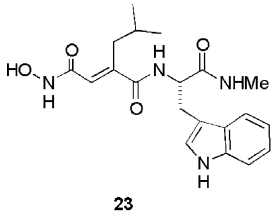
Scheme 3. Reagents and conditions: (a) diethyl aminomalonate, DCC, CH_2Cl_2, 25 °C, 2 h, 57%; (b) H_2, Pd/C, EtOH, 25 °C, 4.5 h, 79%; (c) $NH_2OBn{\cdot}HCl$, Et_3N, DCC, HOBt, CH_2Cl_2, 25 °C, 20 h, 84%; (d) NaH, THF, reflux, 16 h, 83%; (e) $LiOH{\cdot}H_2O$, THF/H_2O (3:1), 25 °C, 72 h, then HCl→pH 4, reflux, 16 h, 69%; (f) $MeNH_2{\cdot}H_2O$, EtOH, 25 °C, 24 h, 59%; (g) H_2, Pd/C, EtOH, 25 °C, 4.5 h, 98%.

Table 1. Inhibition data

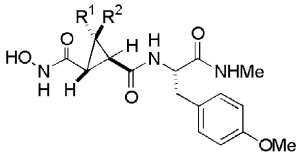
Compd	MMP-2[a]	MMP-3[b]	Selectivity[c]
Ilomastat[b]	0.104	0.191	2
1a	1.3	179	138
1b	19.6	910	46
2	5.97	2340	392
12b	NI[d]	NI[d]	—
22	5.73	665	116

[a]IC_{50} (nm): values are means of three experiments.
[b]Data generated in our laboratories.
[c]Selectivity: MMP-3; IC_{50}; MMP-2 IC_{5O}.
[d]NI: no inhibition.

The dehydroderivative **1a** was less potent as compared to ilomastat against MMP-2 (10-fold higher IC_{50}) and MMP-3 (1000-fold higher IC_{50}). A similar important decrease of potency was reported for the 'dehydroilomastat' **23** (~300-fold higher MMP-1 K_i).[19]



23



24a R^1 = i-Pr, R^2 = H
MMP-9 IC_{5O} 50μM
24b R^1 = H, R^2 = i-Pr, no activity at concentrations up to 100μM

However, introduction of an insaturation at S1′ subsite provided about a 100-fold greater selectivity for MMP-2 inhibition over MMP-3, that is a 70-fold increase as compared to ilomastat.

Potency and selectivity depend on the geometry of the double bond: the equimolar *E*/*Z* mixture **1b** displayed substancially lower inhibitory potency than the pure *E*-isomer.

Compounds **1a**,**b** can be considered as conformationaly constrained analogues of ilomastat. The double bond confers to the *E*-isomer **1a** a conformation close to that of the biological active conformation of ilomastat while the *Z*-isobutylidene group cannot be easily accommodated in the S1′ pocket. This observation is consistent with the work of Martin and colleagues[20] on other constrained inhibitors such as the cyclopropane derivatives **24a** and **24b**.

Replacement of the methylamide residue at P3′ by an ethoxycarbonyl group in compound **3** caused about a 5-fold decrease in potency against MMP-2 but a significant enhancement of selectivity.

Similarly, Castelhano and coworkers[21] have reported that replacement of the P2′–P3′ amide bond of ilomastat by imidazole maintained potency against MMPs and induced selectivity for MMP-1 and MMP-8 over MMP-3.

On the other hand, a selectivity for MMP-3 over MMP-2 may be obtained by replacement of the methyl group of methylamide by a benzhydryl[22] or α-methylbenzyl[23] group.

The substituted indole derivative **22** showed lower inhibitory activities, but a selectivity for MMP-2 was also observed. This result suggests that selectivity against MMP-2 over MMP-3 can also be modulated by modification of the P2′ group. Work is in progress to prepare the single isomer with the 'right' configuration at the two stereogenic centers.

To exclude the possibility that binding of compounds to exosites distinct from the MMP active site might be responsible for the observed effect, compound **12b** bearing a methoxy group in the hydroxamate moiety was evaluated for its MMP inhibitory capacity.[4] It proved to be uneffective on both MMP-2 and MMP-3.

Although **1a**, **2** and **22** are not as effective as ilomastat, their selectivity for MMP-2 makes these derivatives interesting for more detailed SAR studies.

Acknowledgements

We thank the 'Ministère de l'Education Nationale, de la Recherche et de la Technologie' for financial support, the 'ville de Reims' for a PhD fellowship (V.M.), P. Sigaut (MS), and C. Petermann (NMR) for spectroscopic recordings. Fruitful discussions with Professor J. Lévy have been appreciated.

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13. **1a**: ^{1}H NMR (DMSO-d_6) δ 10.82 (s, 1H), 10.57 (brs, 1H), 8.87 (s, 1H), 8.14 (d, 1H, $J=8$ Hz), 7.92 (m, 1H), 7.62 (d, 1H, $J=7.7$ Hz), 7.34 (d, 1H, $J=7.7$ Hz), 7.15 (s, 1H), 7.07 (t, 1H, $J=7.7$ Hz), 6.99 (t, 1H, $J=7.7$ Hz), 6.04 (d, 1H, $J=9.8$ Hz), 4.55–4.40 (m, 1H), 3.30–2.92 (m, 4H), 2.70–2.50 (m, 1H), 2.61 (d, 3H, $J=4.4$ Hz), 0.98 (d, 3H, $J=6.5$ Hz), 0.93 (d, 3H, $J=6.5$ Hz).
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16. Attempts at condensation of the succinylamino-malonate **16** with **19** were fruitless.
17. **22**: *R*R*:R*S** 1:1 mixture; ^{1}H NMR (DMSO-d_6) δ 10.65 (s, 2H), 10.48 (s, 1H), 10.48 (s, 1H), 10.38 (brs, 1H), 8.12 (d, 1H, $J=8.1$ Hz), 7.92 (d, 1H, $J=8.1$ Hz), 7.80 (m, 1H), 7.68 (m, 1H), 7.51 (d, 1H, $J=7.7$ Hz), 7.47 (d, 1H, $J=7.7$ Hz), 7.20 (d, 2H, $J=7.8$ Hz), 6.98 (t, 2H, $J=7.1$ Hz), 6.90 (t, 2H, $J=7.1$ Hz), 4.52 (m, 1H), 4.40 (m, 1H), 2.60 (s, 3H), 2.50 (s, 3H), 2.24 (s, 12H), 0.80 (d, 3H, $J=6.1$ Hz), 0.75 (d, 3H, $J=6.1$ Hz), 0.45 (d, 6H, $J=4.7$ Hz).
18. Assays of inhibition activity were conducted using the fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (Bachem), as previously described.[4] For those studies, APMA-activated recombinant proenzyme MMP-2 (Calbiochem PF 03710) was used; since either APMA or enzyme treatment of rec pro MMP-3 (Calbiochem) led to irreproducible extent of the enzyme activation, assays were performed with truncated rec MMP-3 lacking PEX and Pro domains instead (Calbiochem 444217). Nevertheless, the extent of inhibition was similar using either deleted PEX or total length.
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