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SYNTHESIS OF CONFORMATIONALLY CONSTRAINED POTENTIAL INHIBITORS OF MAMMALIAN METALLOPROTEINASES

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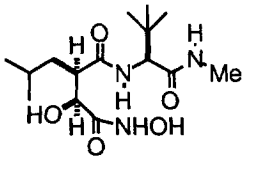
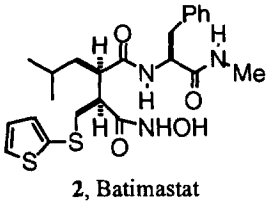
Abstract: The synthesis of carbocyclic molecules containing functional groups capable of interacting with appropriate binding sites in certain metalloproteinases is described. Preliminary biological evaluation reveals modest inhibitory activity for a thioether analog in the trisubstituted cyclopropane series.

Members of a family of Zn^{+2}-containing matrix metalloproteinases are thought to play a central role in the degradation of all of the major protein constituents in the extracellular matrix.[1] These enzymes are exemplified by the gelatinases (MMP-2 and 9), stromelysins (MMP-3,10, and 11) and collagenases (MMP-1, 8, and 13). An overactivity of these enzymes is implicated in various pathological conditions, such as arthritis and tumor metastasis. Considerable effort has recently focused on the synthesis of potential inhibitors,[2] particularly with the knowledge of X-ray crystal structure data of enzyme-bound inhibitors.[3] An important structural class of inhibitors includes succinamide hydroxamic acids as exemplified by Marimastat (**1**)[4] and Batimastat[5] (**2**, Figure 1), the former being an orally bioavailable drug presently undergoing clinical trials. Due to the involvement of several metalloproteinases in a given catalytic event, delineation of the roles of individual members is difficult although inhibitors with partial selectivities toward gelatinase and stromelysin have been reported.[6] A general mode of binding of these inhibitors implicates specific interactions with Zn^{+2}, which occupies an anchoring position for the hydroxamic acid part as revealed in X-ray structures of enzyme-inhibitor complexes.[3] Other binding interactions are also of interest as illustrated in Figure 1.

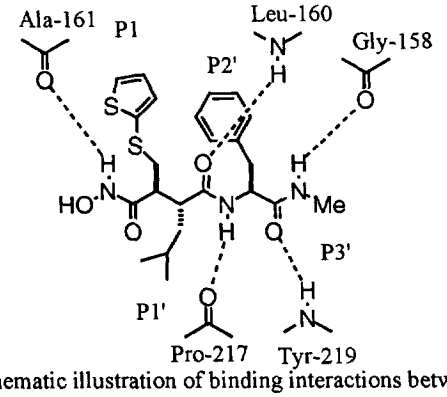


1, Marimastat



2, Batimastat

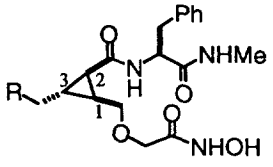
Figure 1



Schematic illustration of binding interactions between Batimastat (**2**) and Human Neutrophil Collagenase redrawn from ref. 3(c).

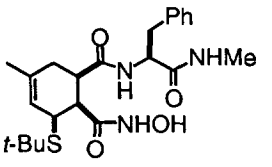
In this Letter, we report the synthesis and preliminary biological data of hydroxamic acid analogs which are constrained in the P1' region with the objective of probing the spatial and functional dispositions in prototypical inhibitors. The target structures shown in Figure 2 consist of the enantiopure cyclopropane motifs **3** and **4** and diastereomeric mixtures of the cyclohexene motifs **5a** and **5b**.[7] To the best of our knowledge, there are relatively few examples of conformationally constrained or "cyclic" variants of the P1' region in the literature.[8]

Figure 2



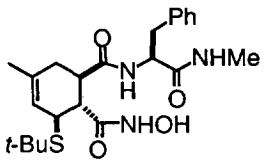
(1*R*,2*S*,3*S*)-isomer

3, R = OBn
4, R = SPh



(see ref 7)

5a



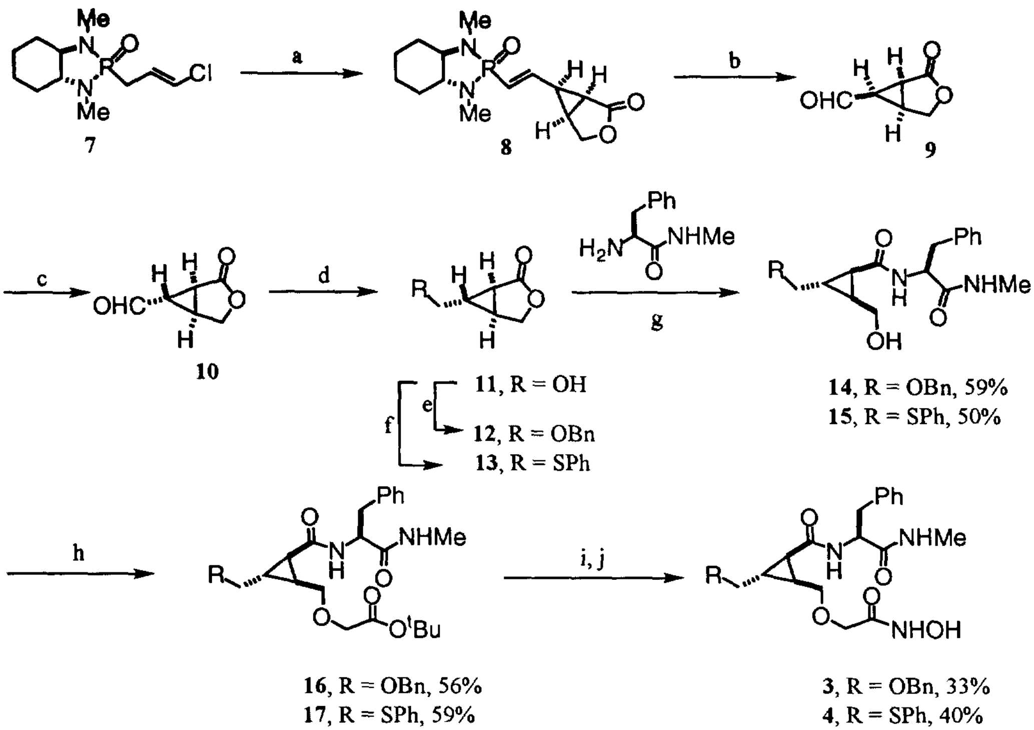
(see ref 7)

5b

Design considerations: We considered the synthesis of prototypical cyclic structures that incorporated functionalities capable of interacting with potential binding sites on collagenases. In the absence of the recently published X-ray data,[3] we initially sought to maintain the pseudo succinate backbone with an *erythro* relationship[9] of the hydroxamic acid and amide appendages as exemplified by the cyclopropane and cyclohexene analogs **3**, **4**, and **5a** shown in Figure 2. Although these motifs did not exhibit optimal fits with the extended bioactive conformation of enzyme-bound Batimastat after the information became available in print,[3] they were nevertheless considered as test cases with the potential for improvement by stereochemical and functional group adjustments following their biological evaluation. A thioether substituent has often been found to be beneficial in the succinamide hydroxamic acid series despite some evidence that it may not act as a Zn^{+2} chelator. For example, succinamide inhibitors with 4-(*t*-butylphenyl)thiomethyl or thiol-substituents displayed comparable potencies.[10] Furthermore, X-ray crystallographic studies of complexes of succinamide inhibitors[6] have shown a marked preference for extended conformations of the inhibitors with the P3' subsite directed away from the enzyme and on the opposite side of the inhibitor backbone to the P1' specificity pocket. However, a recent report from Dupont Merck scientists[11] would suggest that not all structural classes bind in an identical fashion. For example, a compound, predicted by modeling to be of suboptimal activity, was a potent inhibitor of collagenase and was shown to induce a conformational change in the enzyme structure.[11] Thus, a new structural class can reveal hitherto unknown modes of binding, hence create new structural motifs for further refinement.

Synthesis: The synthesis of the cyclopropane analogs **3** and **4** took advantage of a highly stereoselective method for the preparation of chiral, non-racemic compounds in the same series.[12] Thus, treatment of the readily accessible chloroallyl phosphonamide **7** with *n*-BuLi followed by trapping with (5*H*)-furanone afforded the desired cyclopropane **8** (80%, >98:2), with the large phosphonamide substituent being *endo* relative to the lactone ring (Scheme 1). Ozonolysis of **8** was followed by equilibration of aldehyde **9** to the more stable *exo* aldehyde **10**.

Scheme 1



(a) i. *n*-BuLi, THF, -78 °C, 5 min; ii. 2(5*H*)-furanone, THF, -78 °C, 1 h, 80%; (b) Ozone, CH_2Cl_2, -78 °C then DMS, -78 °C to rt, 1 h, 39%; (c) DBU, toluene, rt, 48h, 63%; (d) $NaBH_4$, MeOH, 0 °C, 10 min, 98%; (e) NaH, BnBr, Bu_4NI (cat.), DMF, 0 °C, 4 h, 69%; (f) PhSSPh, Bu_3P, THF, rt, 48h, 67%; (g) Amine, 2-hydroxypyridine, toluene, reflux, 48 h; (h) NaH, *tert*-butyl bromoacetate, Bu_4NI (cat.), DMF, 0 °C to rt, 14 h; (i) TFA, CH_2Cl_2, 0 °C to rt, 4 h; (j) $H_2NOH.HCl$, BOP, $^iPr_2NEt$, MeCN, rt, 16 h.

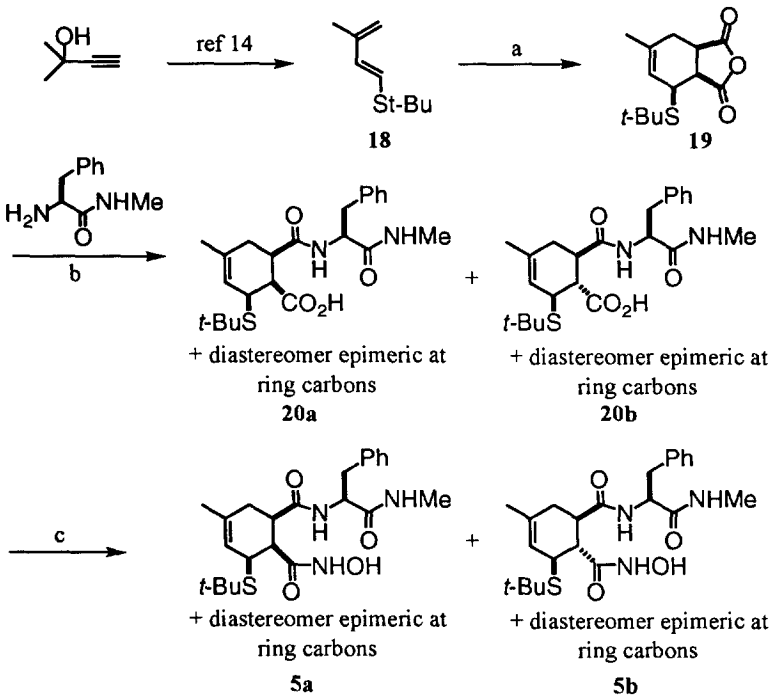
Reduction of the latter proceeded smoothly to yield alcohol **11** in good overall yield. Conversion to the benzyl ether **12**, and opening the lactone with L-Phe-NHMe was most conveniently performed in the presence of 2-hydroxypyridine as a catalyst[13] to afford alcohol **14**. Treatment of **14** with NaH followed by *t*-butyl bromoacetate yielded *t*-butyl ester **16**, which was further manipulated to afford the target hydroxamic acid **3**. Unfortunately, facile cyclization to the imide precluded the synthesis of the hydroxamic acid analog derived from the oxidation of **14** to the corresponding carboxylic acid, hence the option for the extended analog **16**.

The synthesis of **4** followed the same route to intermediate **11**, which was converted to its phenylthio derivative **13**, and the latter was further manipulated to the intended target following a sequence similar to one described above (Scheme 1).

The cyclohexenyl hydroxamic acid derivatives **5a** and **5b** were obtained as mixtures of diastereomers via a Diels-Alder condensation of 1-*t*-butylthio-3-methyl-1,3-butadiene **18**[14] and maleic anhydride (Scheme 2). Under thermal conditions, the racemic all-*cis* adduct **19** was obtained in 64% yield. Condensation of **19** with chiral, non-racemic L-Phe-NHMe led to a mixture of four diastereomers expressed as **20a** and **20b** (Scheme 2) in

approximately equal amounts resulting from a regioselective opening at the carbonyl group remote from the bulky thio substituent.

Scheme 2



(a) Toluene, reflux, 64% (b) lutidine, CH_2Cl_2, 0 °C → rt, quant (c) $NH_2OH.HCl$, EDC, HOBt, DMF, 77%

Two of these diastereomers **20b** consisted of the *trans-trans* products due to epimerization. Previous studies with *meso* anhydrides and L-amino acid esters had demonstrated that one isomer could be selectively obtained in such reactions.[15] In view of the difficulty in the separation of these isomers into individual compounds by flash chromatography, recrystallizations or conversion to various derivatives, they were transformed into the corresponding mixtures of hydroxamic acids **5a** and **5b**, and subjected to enzymatic tests as such.

Results and Discussion: Compounds **3**, **4**, the mixtures of diastereomers containing the hydroxamic acid isomers represented as **5a** and **5b**, as well as the corresponding reduced cyclohexyl analogs (not shown) were evaluated against gelatinases A and B and against L1210 cell lines.[16] All except **4** were found to be inactive (IC_{50} >1000 μM). In spite of the modest level of activity of compound **4** (IC_{50} = 32 μM vs gelatinase A and 36 μM vs gelatinase B), it is of interest that the replacement of sulfur with oxygen (as in compound **3**) resulted in a drastic loss of potency. The inclusion of elements of conformational constraints in the design of potential enzyme inhibitors or receptor antagonists has been shown to have dramatically beneficial effects on the biological activity of the given system.[17] Clearly, the cyclohexenyl-derived constrained analogs **5a** and **5b** and their saturated

analogs are not suitable fits for the active site in gelatinases A and B. Knowledge of the bioactive conformation of Batimastat through enzyme-bound crystal structural data[3] will allow the refinement of the constrained structures in the P1' and other areas of productive binding for the design of potent analogs. Results pertaining to these new prototypes as well as other analogs in this intriguing area of metalloproteinase inhibition are under active study and will be reported in due course.

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