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THE ASYMMETRIC SYNTHESIS AND *IN VITRO* CHARACTERIZATION OF SUCCINYL MERCAPTOALCOHOL AND MERCAPTOKETONE INHIBITORS OF MATRIX METALLOPROTEINASES

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Abstract: A series of succinyl based mercaptoketones and diastereomeric mercaptoalcohols were prepared and evaluated *in vitro* as inhibitors of the matrix metalloproteinases collagenase-1 (MMP-1), stromelysin (MMP-3), and gelatinase-B (MMP-9).

The matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes that are involved in the remodeling and degradation of extracellular matrix proteins.[1-4] The MMPs play important roles in mediating many normal physiological activities including tissue growth, tissue remodeling, wound healing, and multiple processes involved in reproduction.[5-10] Because of the destructive potential of these enzymes, their activity and expression is tightly controlled by a variety of mechanisms.

The aberrant regulation of MMP production has been implicated in pathological tissue breakdown in numerous diseases processes, most notably arthritis and tumor metastasis.[1-4,11-14] More recently, increased levels of MMPs have been associated with different aspects of cardiovascular disease including smooth muscle cell migration in restenosis, and plaque formation and rupture in atherosclerosis.[15-17] Small molecule inhibitors of MMPs therefore hold great promise for the treatment of a wide variety of conditions, some of which are at present intractable. The potential utility of MMP inhibitors as therapeutic agents has further expanded with the recent recognition that tumor necrosis factor-α (TNF–α) converting enzyme (TACE), an enzyme responsible for the generation of soluble TNF-α, belongs to a family of enzymes related to the MMPs[18,19] and is inhibited by some small molecule MMP inhibitors (MMPIs).[20-22]

Approaches to identify inhibitors of MMP activity at all levels of their regulation have been undertaken, but the most widely pursued to date has been the design of molecules that bind to the catalytic site of the enzymes.[23] Peptidomimetics or pseudopeptides that incorporate a zinc binding group and P1 and/or P1' side chains that interact with the enzyme subsites are the most common class of MMP inhibitors, although sulfonamide based inhibitors[24] have been reported. The vast majority of these MMP inhibitors utilize a hydroxamic acid to chelate the active-site zinc atom,[23] while carboxylic acids,[25] thiols[26] and phosphinates and phosphonates[27] have been studied less thoroughly.

The search for novel zinc chelators has been driven by a desire to avoid potential complications presented by hydroxamic acid based inhibitors, including possible metabolic and pharmacokinetic problems which may present liabilities for a chronically administered therapeutic agent. To that end we have investigated

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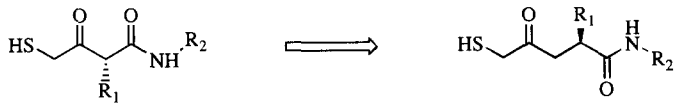
a novel series of malonyl mercaptoketones, **1**, [28] and now describe the synthesis and SAR of the related succinyl mercaptoketone class of MMP inhibitors, **2**.

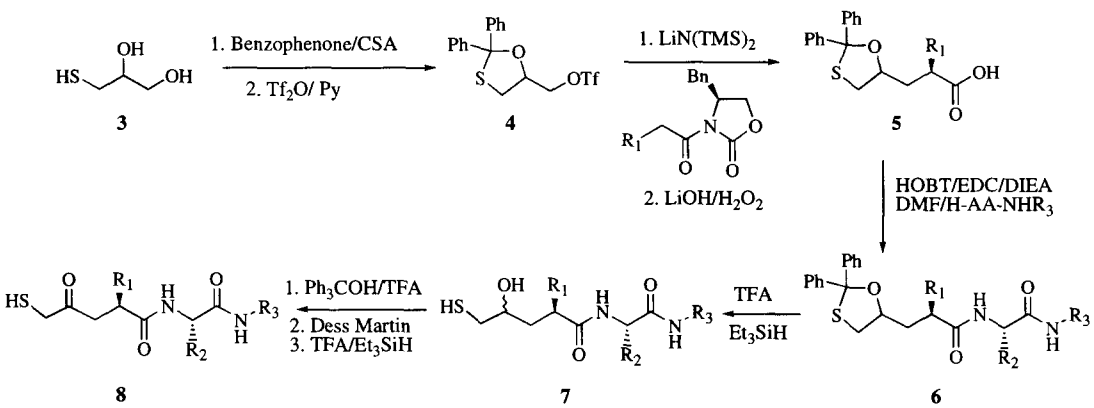


1 **2**

Chemistry

The first compounds prepared in this series were the dipeptide mercaptoalcohol **7** (R_1 = *n*-heptyl, R_2 = *i*-Bu, R_3 = Leu(N-Et)) and the corresponding mercaptoketone **8** (R_1 = *n*-heptyl, R_2 = *i*-Bu, R_3 = Leu(N-Et), Scheme 1). The most efficient route to inhibitors of this type, shown in Scheme 1, produces the desired mercaptoketones in 5 steps from the benzophenone hemithioketal of 3-mercapto-1,2-propanediol. However, this route provides the mercaptoalcohols as mixtures of diastereomers which must be separated chromatographically. The *in vitro* potency of **7** (Table 1) versus MMP-3 and MMP-9 thus demanded that we develop a practical synthetic route, incorporating differentially protected thiol and alcohol groups, that would allow access to either diastereomerically pure mercaptoalcohol, as well as the corresponding mercaptoketone.

Scheme 1

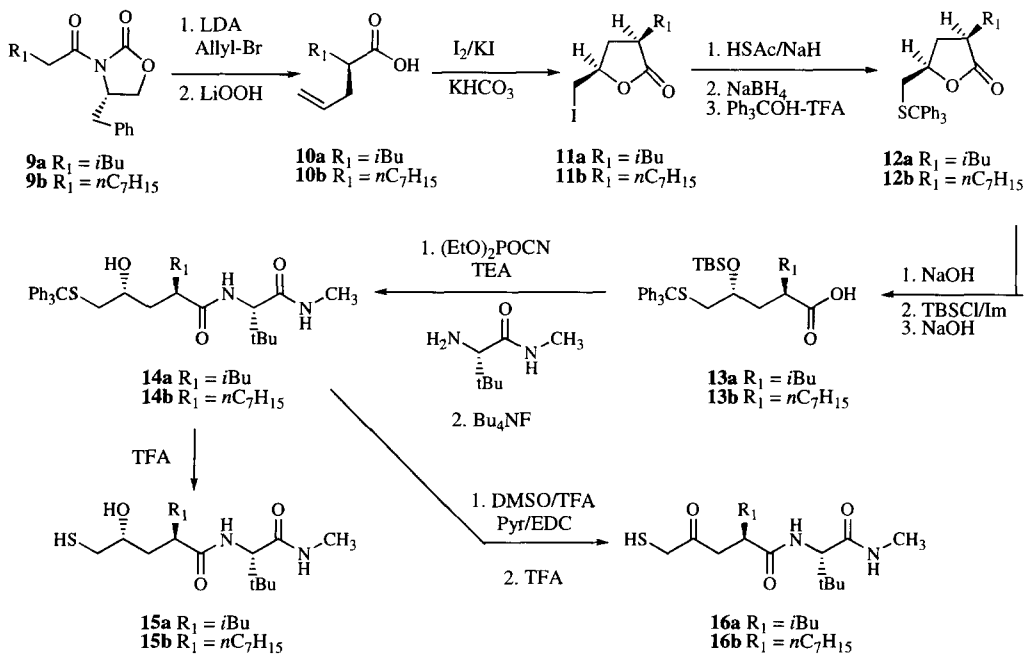


8 **7** **6**

Thus, the γ,Δ-unsaturated carboxylic acids **10a** and **10b** are prepared in 78% and 73% yield respectively, via Evans' alkylation of the appropriately substituted oxazolidinones, **9**, with allyl bromide followed by cleavage of the chiral auxiliary with lithium hydroperoxide (Scheme 2).[29,30] Iodolactonization of **10a,b** with I_2/KI/$KHCO_3$ then provides the *cis*-substituted iodide **11a** in 63% isolated yield (cis:trans, 1.9:1) and **11b** in 71% isolated yield (cis:trans, 2.4:1) after column chromatography.[31] The thiol functionality is next introduced from the iodides **11** via displacement of the iodide with AcSH/NaH/THF followed by reductive cleavage of the thioacetate and reprotection as the trityl sulfides **12a** (48%) and **12b** (57%). Basic hydrolysis of the lactone then provides the hydroxy-acid which is bis-silylated and selectively deprotected to give carboxylic acids **13a** (90%) and **13b** (79%), in which the hydroxyl and thiol moieties are differentially protected. Peptide coupling of acids **13a** and **13b** with t-butyl glycine-N-methylamide and subsequent desilylation provides **14a** (53%) and **14b** (62%). Detritylation of the sulfides with TFA/Et_3SiH then furnishes the desired mercaptoalcohols **15a** and **15b** in 16% and 40% yield, respectively. The poor yields obtained for this final

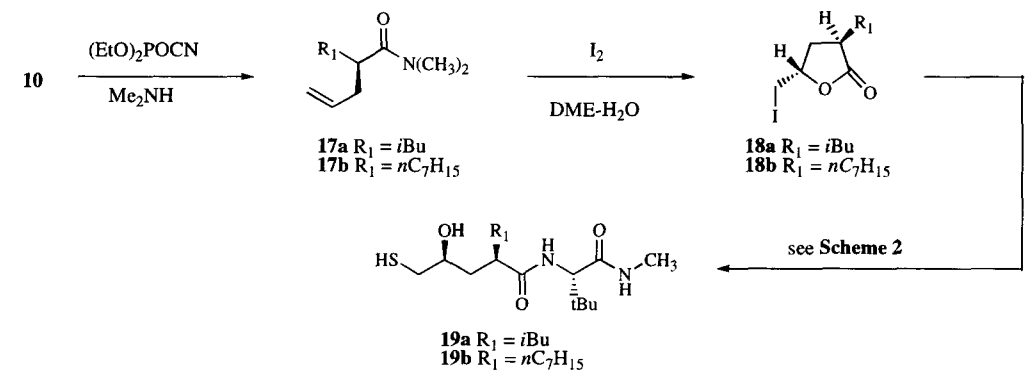
deprotection were due to facile lactonization of **15** under the reaction conditions to produce t-butyl glycine-N-methylamide and the free thiols analogous to lactones **12**. The corresponding mercaptoketones, **16**, are accessed via oxidation of alcohols **14a** and **14b** and subsequent detritylation to give **16a** (32%) and **16b** (42%).

Scheme 2



The epimeric mercaptoalcohols, **19a** and **19b**, are also readily available from carboxylic acids **10** (Scheme 3). Thus, conversion of the acids into the corresponding dimethyl carboxamides, **17**, followed by reaction with iodine in DME/H_2O provides the *trans*-iodolactones (trans:cis, 6:1) **18a** (62%) and **18b** (63%).[32] Conversion of these *trans*-iodolactones into mercaptoalcohols **19a** and **19b** then proceeds as in Scheme 2 in 14% and 19% overall yields, respectively.

Scheme 3

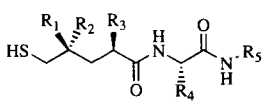


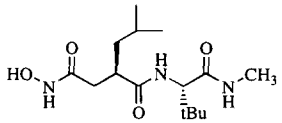
Biology

In order to assess the dependence of inhibitory potency on the size of the P1' substituent and the stereochemistry of the hydroxyl group, each of the mercaptoalcohols and mercaptoketones was tested *in vitro*[33] versus MMP-1 (collagenase-1), MMP-3 (stromelysin-1), and MMP-9 (gelatinase-B), enzymes implicated in the pathology of osteoarthritis and rheumatoid arthritis.[1-4,11-14] MMP-9 has also been implicated in the pathology of tumor metastasis.[13,14] Several features of the resulting SAR, shown in Table 1, are notable.

Within the mono-peptide series (R_5 = Me) the size of the P1' substituent has little effect on binding to MMP-1 as the isobutyl derivatives **15a**, **16a**, and **19a** each have roughly the same inhibitory potencies as their corresponding *n*-heptyl analogs **15b**, **16b**, and **19b**. However, the dipeptide analogs, **7** and **8**, bearing the lengthy *n*-heptyl chain at the P1' site (R_3) are essentially inactive against MMP-1. Inhibition of MMP-3 and MMP-9 is strongly dependent on the chain length of the P1' moiety. In MMP-3 the shorter isobutyl side chain of compounds **16a** and **19a** causes a 50-fold diminution in potency relative to the analogous *n*-heptyl derivatives, **16b** and **19b**, and the P1' heptyl moiety is tolerated in dipeptide analogs **7** and **8**. The *n*-heptyl derivatives **15b**, **16b** and **19b** are also over 20-fold more active versus MMP-9 than isobutyl compounds **15a**, **16a** and **19a**, and much more potent than the dipeptide analogs. This predilection of MMP-3 and MMP-9 for the larger P1' *n*-heptyl group results in interesting selectivity profiles for the compounds in Table 1. Thus, the isobutyl analogs **15a**, **16a** and **19a** are each at least 15 times more potent against MMP-1 and MMP-9 than against MMP-3. Also, the *n*-heptyl analogs **16b** and **19b** are at least 25-fold more selective than the corresponding isobutyl analogs for MMP-9 over MMP-1. The most selective inhibitors are dipeptides **7** and **8**, which are over 100-fold more potent versus MMP-3 and MMP-9 than against MMP-1.

Table 1: *In Vitro* Activity of Succinyl Mercaptoalcohols and Mercaptoketones.



Compound	R_1	R_2	R_3	R_4	R_5	IC_{50} (nM) MMP-1	MMP-3	MMP-9
7	OH, H		*n*-C_7H_{15}	*i*-Bu	Leu(N-Et)	>4000	39	24
15a	H	OH	*i*-Bu	*t*-Bu	Me	46	3700	250
15b	H	OH	*n*-C_7H_{15}	*t*-Bu	Me	140	430	12
8	O		*n*-C_7H_{15}	*i*-Bu	Leu(N-Et)	>10000	36	20
16a	O		*i*-Bu	*t*-Bu	Me	11	480	8
16b	O		*n*-C_7H_{15}	*t*-Bu	Me	10	8	0.14
19a	OH	H	*i*-Bu	*t*-Bu	Me	11	470	8
19b	OH	H	*n*-C_7H_{15}	*t*-Bu	Me	5	9	0.14
20						5	470	12

The effect of the hydroxyl group stereochemistry on inhibitory potency was the same for all three enzymes studied. Compounds **19a** and **19b**, in which the hydroxyl and P1' groups are *syn*, are more than five times more active than the related *anti*- analogs **15a** and **15b**, and equivalent to the mercaptoketones **16a** and **16b**, respectively. Although the binding mode of these compounds to the MMPs is not known, this result is consistent with the mercaptoalcohols and mercaptoketones acting as bidentate zinc ligands, as has been proposed for other thiol MMP inhibitors,[26b,e] thus requiring the sulfhydryl and hydroxyl or ketone groups to be coplanar. The *syn* and *anti* mercaptoalcohols would then be forced to have the P1', P2', and P3' moieties oriented in dramatically different directions, resulting in the observed potency differences.

A direct comparison of the thiols **8**, **16a**, and **16b** with the analogous compounds of the malonyl class, described in the previous paper, reveals that there is no difference in activity between the two series. Finally, a comparison of the IC_{50}'s for compounds **16b** and **19b** with values for compound **20** (Ro31-9790[34]) in the same assays[33] demonstrates that the appropriately substituted mercaptoalcohols and mercaptoketones can provide analogs similar in potency to the corresponding hydroxamic acid-based inhibitors versus MMP-1 and may have improved potency versus MMP-3 and MMP-9. The *in vivo* activity of thiols **15a**, **15b**, **16a**, **16b**, **19a** and **19b** will be reported in due course.

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