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NOVEL INDOLACTAM-BASED INHIBITORS OF MATRIX METALLOPROTEINASES

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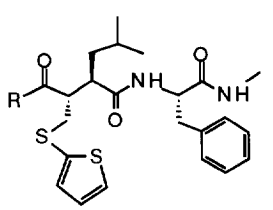
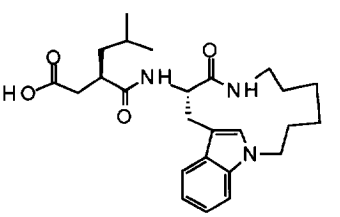
Abstract: *Potent collagenase inhibitors incorporating a novel indolactam macrocycle, which are accessible by the intramolecular alkylation of N-t-Boc-L-Trp-NH(CH_2)$_6$OTs under phase transfer conditions, show enhanced activity compared to their acyclic analogs.*

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases which include collagenases, stromelysins, and gelatinases. Collectively, these enzymes can degrade all the major components of the extracellular matrix, the breakdown of which occurs in both normal and pathological processes.[1,2] Collagenase and stromelysin-1 have been implicated in the loss of functioning cartilage observed in rheumatoid arthritis.[3,4] Endogenous tissue inhibitors of metalloproteinases, (TIMPs), regulate MMP proteolytic activities *in vivo* by forming a tight enzyme-inhibitor, non covalent complex (K_i < 1nM)[5] but are unable to prevent the release of proteoglycans from interleukin-1 stimulated cultured articular cartilage, presumably because of their large size and low pI.[6] Low molecular weight hydroxamate-based MMP inhibitors however, can suppress the degradation of proteoglycan within articular cartilage *in vitro*, an indication that synthetic inhibitors of requisite potency and pharmacokinetic properties may be of therapeutic value in rheumatoid arthritis and osteoarthritis.[6] Potencies in the nanomolar range can be achieved with peptidic inhibitors whose design is based on the sequence of the collagen cleavage site and a strong zinc binding group, usually hydroxamate.[7] Inhibitors with partial selectivity towards gelatinase[8] and stromelysin[9] have also been reported. Our objective was to develop inhibitors of MMPs with minimal peptidic character in order to achieve *in vivo* activity while exploring diverse zinc ligands. As illustrated for BB-94 and "BB-94-acid" with human fibroblast collagenase (HFC), carboxylate-based inhibitors are usually 10^2 - 10^4 less active than the corresponding hydroxamates.



BB-94 R = -NHOH K_i ~ 0.5 nM
"BB-94 acid" R = -OH K_i = 1500 nM



1b K_i = 25 nM

In this Letter we describe a series of conformationally constrained, peptidomimetic inhibitors, **1**, containing a novel

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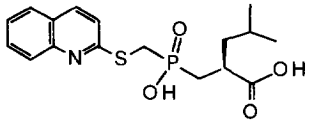
indolactam moiety as a key feature. These compounds possess superior potency to their acyclic counterparts as inhibitors of several MMPs. For example, the potency observed with the carboxylate **1b** versus collagenases is comparable to that of simple acyclic hydroxamates; $HONHCOCH_2CH(iBu)CO.Leu.Gly.OEt$[10] and $HONHCOCH_2CH(iBu)CO.Tyr(OMe).NHMe$[2] have IC_{50}s of 20 nM vs HFC.

The design of inhibitors **1** stems from the work of Johnson[10a,11] on macrocyclic lactam inhibitors generated by joining the side chain of the P_2' amino acid residue to the C-terminal amide nitrogen (nominally the P_3' nitrogen). Potency comparable to the parent was maintained provided that the ring size was sufficiently large (13 member) to allow the amide bond to assume a *trans* conformation. In the present work, the C-terminal amide nitrogen is linked to the indole nitrogen, which generates a more rigid structure by virtue of the N1, C3 substitution of the pyrrole ring.

The syntheses of indolactam based inhibitors **1**a-f (Table 1) require the preparation of fragments that contain a combined P_1' entity, a zinc binding ligand (**3-7**), and the indolactam fragment **10** (Scheme 1). Coupling the two fragments using HOBT/NMP/EDCI methodology followed by unmasking of the zinc binding group produced compounds **1**a-f.



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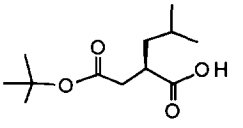

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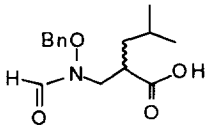

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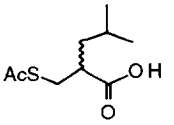

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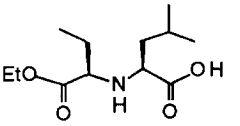

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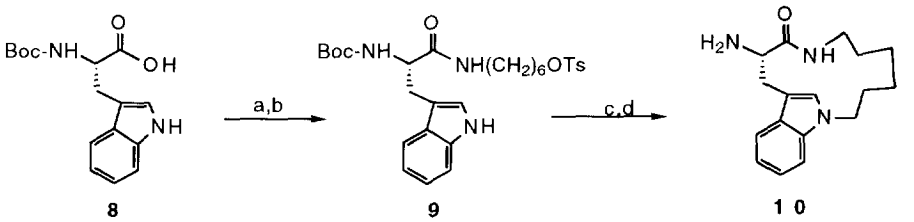
The preparation of **10** involves the intramolecular alkylation of the precursor tosylate **9,** prepared from alcohol **8** with tosyl chloride/pyridine at 0 °C. The key cyclization step occurs in about 40% yield with 3-4 equivalents of NaH in THF. The N-Boc precursor of indolactam **10** had $[\alpha]_D$ = -68.8° (c 0.5, $CHCl_3$), mp 222-223 °C. Several byproducts are formed under these cyclization conditions resulting from competing amide and carbamate alkylations and amide acylation by the Boc group. A practical and higher yielding method involves the use of phase transfer conditions such as $Bn(Et)_3N^+Cl^-$/KOH/CH_2Cl_2 or $(Bu)_4N^+HSO_4^-$/NaOH/benzene[12], typically generating product **10** in unoptimized yields of 50-77%, when carried out on 5g-10g scale. The indole ring in **10** and **1b** imposes a major anisotropic effect on the -$(CH_2)_6$- alkyl resonances, with upfield shifts to δ 0.5 and -0.5 ppm, ($C_{10/11}$-H) due to the near orthogonal disposition of the indole relative to the macrolactam ring. A *trans* amide geometry prevails and an nOe is observed between indole C_5-H and Cα-H (and between indole C_2-H and macrocyclic C_4-H_a/C_8-H_a), indicative of an *anti* relationship between Cα-H and indole C_2-H (*insert*).[13]

The quinoyl-2-thiomethylphosphinate fragment **3** was prepared in a chiral form as shown in Scheme 2. The mesylate

of intermediate **11**,[14] prepared in quantitative yield, was reacted with the sodium salt of 2-quinoline thiol in DMF initially at 0 °C and subsequently at room temperature overnight to yield **12** in 66% yield. Base hydrolysis provided the corresponding diacid which was resolved with (-)-cinchonidine. The undesired diastereomer salt precipitates out of solution. Processing of the mother liquor yielded desired product with ca. 90% optical purity. Fragment (R)-**3** thus obtained had $[\alpha]_D$ = -8.70° (c 4.94, CH_3OH). The undesired (S)-**3** isomer had $[\alpha]_D$ = +10.68° (c 4.87, CH_3OH). Coupling of (R)-**3** with indolactam amine **10** using HOBT/NMP/EDCI or CDI yielded **1a**.

The assembly of inhibitors **1b,c** required intermediate **4**, available through the alkylation of (4S)-N-(4-methylpentanoyl)-4-benzyl-2-oxazolidinone with tert-butyl bromoacetate, followed by basic peroxide hydrolysis.[15] Coupling of the resulting tert-butyl (R)-2-isobutyl succinic acid **4** with amine **10**, provided inhibitor **1b** after TFA treatment. Further coupling of **1b** with O-benzyl hydroxylamine followed by careful hydrogenolysis to avoid over-reduction of the hydroxamate moiety to the parent amide, gave **1c**.



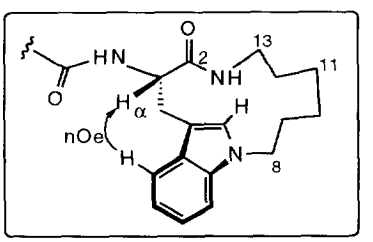
Scheme 1:

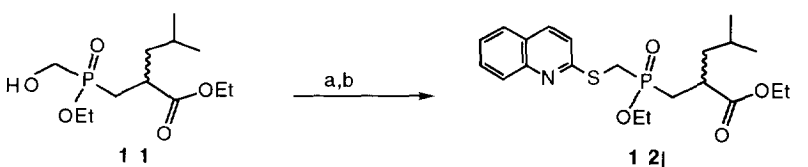
a) $HN(CH_2)_6OH$/EDCI/DMAP/DMF (97%)

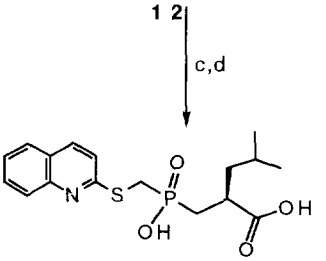
b) TsCl/pyr/0° (76%)

c) NaH/THF/0° (~40%) or
KOH/ $Bn(Et)_3N^+Cl^-/CH_2Cl_2/H_2O$/RT (50-77%)

d) 50%TFA/CH_2Cl_2; OH^- (100%)



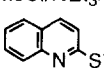




(R)-**3**

Scheme 2:

a) $MsCl/NEt_3$/-20°->RT/3h

b)  /DMF/0°->RT/18h

c) NaOH/THF/MeOH/RT/18h

d) (-)-cinchonidine/EtOH

Inhibitors **1**d,e were obtained by initially carrying out a Michael addition of O-benzyl hydroxylamine or thiol acetic acid respectively to 2-methylene-4-methylpentanoic acid. Coupling of the resulting acids with **10** followed by basic ammonia gave **1**e, whereas **1**d required a subsequent formylation and hydrogenolysis step. The amino carboxylate inhibitor **1**f was generated by first coupling benzyl leucinate with the triflate of (S)-ethyl lactate followed by hydrogenolysis, and then coupling with amine **10**. Base hydrolysis of the ethyl ester intermediate gave **1**f.[16]

All of the indole-lactam based inhibitors display excellent activity versus HFC and HNC (human neutrophil collagenase) and have increased binding to most MMPs by at least ten fold relative to the acyclic hexyl amide analogs **2**. The lactam component is tolerated least well by matrilysin and stromelysin, paralleling the comparative affinities for tryptophan at P_2'.[17] For all the enzymes investigated, the phosphinate **1**a, with the novel quinoylthiomethyl group targeted for the P_1-P_2 region of the active site, shows significant enhancements (18-80 fold) in potency over the acyclic variant. The enhancements are less marked for the carboxylate **1**b, but are still significant (4-12 fold). The most potent inhibitor, **1**c, which exhibits uniformly good activity in the subnanomolar to nanomolar range, is more potent versus HFC than its linear version **2**c by at least an order of magnitude. For **1**c, this incremental potency gain for the cyclic C-terminus is not seen with the other enzymes tested. We speculate that the difference in incremental potency between the phosphinate and the hydroxamate pairs may be the result of a more favourable π-stacking interaction between a backbone amide carbonyl (Gly_{179} in HFC,[18] Asn_{179} in matrilysin[19]) and the indole moiety in the phosphinate case.[20] Significant positional differences between bound inhibitors **1**b, **1**c, and a sulfodiimine analog (0.5 Å mean variation in the position of polar atoms) are observed in matrilysin complexes.[19] Such displacements extend to the indole moiety and likely affect the differential binding observed.

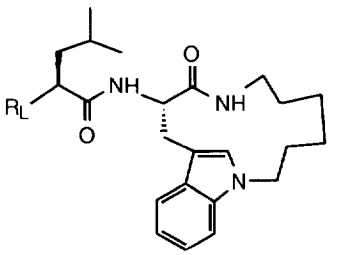
It is notable that the activity of carboxylate **1**b versus HFC/HNC (K_i = 14-25 nM) falls in a range that makes it the most potent carboxylate inhibitor reported to date, and an attractive candidate for further study. Similarly, the amino-carboxylate **1**f, possesses activity greater by an order of magnitude than that reported for other members of this class.[21] The formylhydroxylamine **1**d (reverse hydroxamate), is a potent inhibitor with a K_i of ~0.5 nM (single isomer). As expected, a concomitant loss in activity relative to hydroxamate **1**c is observed.[22] The activity of the simple mercaptan **1**e (K_i = 2.3 nM) is comparable to β-mercaptocarboxylate dipeptide inhibitors from the SKB group,[7d] designed to coordinate to the zinc ion in a bidentate manner.

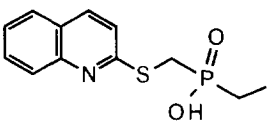
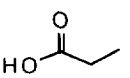
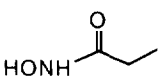
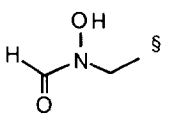
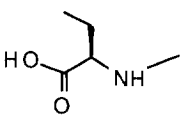
The marked potency and selectivity of indolactam-based inhibitors towards collagenolytic enzymes appears to result from the constraining influence of the ring, imposing a conformation very close to the preferred bound conformation, and the preference for isobutyl binding in S_1'. The presumed greater entropic advantage is even more apparent in the 12-membered lactam.[23] Studies are in progress to determine the pharmacokinetic properties of these inhibitors and their *in vivo* activity in models of cartilage degradation and arthritis.

Acknowledgment. We are indebted to Dr. Steve Bender of Agouron Pharmaceuticals, Inc. for providing samples of BB-94 and "BB-94 acid" and Dr. H. Van Wart for generous gifts of HNC and HNG. We thank Valerie Robinson for NMR studies of **1**a and Dr. John Nestor for a helpful critique of the manupscript.

Table 1: Indolactam-Based Inhibitors of Matrix Metalloproteinases

(1a-f)



Cpd	R_L	Ki (nM) HFC	HNC	HNG	Matrilysin	HFS
1a		5 (2, pH6.5)	4	14	78	806
2a§	"	432	102	300	1420	6575
1b		25 (5, pH6.5)	14	115	850	4500
2b	"	171	163	472	>620	nd
1c		0.1	0.4	0.2	3	9
2c	"	3	0.5	0.3	5	nd
1d§		1.0	nd	2.0	18	15
1e		2.3	nd	nd	114	>>800
1f		20	nd	nd	3225	25000

HFC, human fibroblast collagenase; HNC human neutrophil collagenase; Gel, human neutrophil gellatinase; HFS, human fibroblast stromelysin. Enzyme activities were measured using fluorometric assays,[24] the standard deviation of all Ki values are less than twenty percent.

2a,b,c Tryptophan hexylamide acyclic analog of **1a,b,c**; nd: not determined; §two stereoisomers.

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12. Dr. Melwyn A. Abreo and Chuck Agree of Agouron Pharmaceuticals, Inc., have carried out the cyclization using $(Bu)_4N^+HSO_4^-$/NaOH/benzene in 77% yield.
13. **1b**: ^1H NMR ($CDCl_3$) δ 7.93 (d, 1H, J=8.24 Hz, NH), 7.84 (d, 1H, J=7.63 Hz, $C_{5,\,ind}$-H), 7.29 (d, 1H, J=7.63 Hz, $C_{8,\,ind}$-H), 7.20 (t, 1H, $C_{7,\,ind}$-H), 7.11 (t, 1H, $C_{6,\,ind}$-H), 6.68 (s, 1H, $C_{2,\,ind}$-H), 5.3 (dd, 1H, J=3.97,7.93, NH), 4.82 (m , 1H, C_{α}-H), 4.25 (m, 1H, C_{8a}-H), 3.73 (m, 1H, C_{8b}-H), 3.56 (m, 1H, C_{13a}-H), 2.96 (m, 1H, CHC(O)NH-), 2.80 (dd, 1H, J=16,17, 9.04 Hz, -CH_2C(O)-), 2.50 (dd, 1H, J=16.17, 3.97 Hz, -CH2C(O)-), 2.36 (m, 1H, C_{13b}-H), 1-1.9 (m, 6H), 0.56 (m, 1H), -0.43 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 175.41 (s, C(O)NH), 175.27 (s, C(O)NH),171.91 (s, COOH),136.03 (s), 127.18 (s), 126.97 (d, C_2-ind), 121.83 (d, C_7-ind), 119.40 (d,d $C_{5,6}$-ind), 109.74 (d, C_8-ind), 109.05 (s), 54.43 ($C\alpha$), 47.46 (t, C_8), 41.55(t, CH_2-CH), 40.67 (d, CH-C(O)-), 39.19 (t, C_{13}), 37.54 (t, -CH_2-C(O)-), 29.90 (t, C_4), 27.91, 27.43, 26.15, 24.44 (4t, $C_{9\text{-}12}$), 25.82 (d, $CH(CH_3)_2$), 22.88, 22.20 ($CH(CH_3)_2$). FAB MS m/z 442 $(M + H)^+$, 424 $(M\text{-}H_2O)^+$.
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20. The Tyrosine cyclic analog of **1c** (C-terminal amide nitrogen joined to the phenol group by a six-methylene linker) is equipotent to the acyclic counterpart.
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22. As determined in a dipeptidic series, the acetyl analog is relatively inactive; see also ref 10a.
23. The activity of the 12-membered analog of **1b**, prepared in low yield by following the steps in Scheme 1 but substituting with 5-amino-1-pentanol, is about 2-3 times higher than **1b**.
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