Bioorganic & Medicinal Chemistry Letters 21 (2011) 6485–6490

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of novel Cobactin-T based matrix metalloproteinase inhibitors via a ring closing metathesis strategy

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ARTICLE INFO

Article history:
Received 19 July 2011
Revised 12 August 2011
Accepted 15 August 2011
Available online 28 August 2011

Keywords:
Matrix metalloproteinases
Ring closing metathesis
MMP-2
MMP-9
Cobactin-T

ABSTRACT

The discovery of potent *N*-hydroxyl caprolactam matrix metalloproteinase (MMP) inhibitors (**6**) based on the natural product Cobactin-T (**2**) is described. The synthetic method, which utilizes the ring closing metathesis reaction, is compatible to provide complementary (*R*) and (*S*) enantiomers. These compounds tested against MMP-2 and 9, show that the *R* stereochemistry (i.e., **16**), which is opposite for that found in the natural product Cobactin-T is >1000-fold more active with IC_{50} values of 0.2–0.6 nM against both enzymes. The variation in the incorporation of the sulfonamide enzyme recognition element ($Ar_2XAr_1SO_2N(R^1)$, **6**), along with alterations in the RCM/double bond chemistry (R^2) provided a series of sub nanomolar MMP inhibitors. For example, compounds **16** and **34** were found to be the most potent with IC_{50} values against MMP-2 and MMP-9 found to be between 0.2 and 0.6 nM with **34** being the most potent compound discovered (MMP-2 IC_{50} = 0.39 nM and MMP-9 IC_{50} = 0.22 nM). Compounds **16** and **34** showed acceptable drug-like properties in vivo with compound **34** showing oral bioavailability.

Matrix metalloproteinases are a family of zinc containing metal enzymes responsible for degrading all types of extracellular matrix proteins, as well as processing bioactive molecules. These include the digestion of a large range of connective and soft tissues such as collagen and gelatin, the cleavage of cell surface receptors, and the release of apoptotic ligands. There are currently about 27 MMPs, which are classified by function and substrate specificity. Specific examples are: collagenases (MMP-1, 8, and 13); stromelysins (MMP-3, 10, and 11); and gelatinases (MMP-2 and 9). These enzymes have become the focus of therapeutic intervention in many diseases including osteoarthritis, cancer, macular degeneration, cardiovascular disease, and stroke.[1,2] The gelatinases play a role in regulating angiogenesis and arterial remodeling in vascular pathologies such as heart disease, tumor metathesis, and ischemic stroke. During ischemic stroke, both gelatinases are up regulated and involved in degradation of the extra-cellular matrix and blood brain barrier. Much of the brain damage is linked to this cerebral hemorrhaging as inflammatory cells are allowed to penetrate the brain tissue and cause further neurological injury, as well as edema. Initial results have shown that treatment with hydroxamic acid based broad spectrum MMP inhibitors such as BB-94 (**1**, Fig. 1) in animal models of ischemic stroke lead to diminished hemorrhaging and infarct.[2,3]

As part of a drug discovery program, we became interested in the role of the gelatin degrading matrix metalloproteinases 2

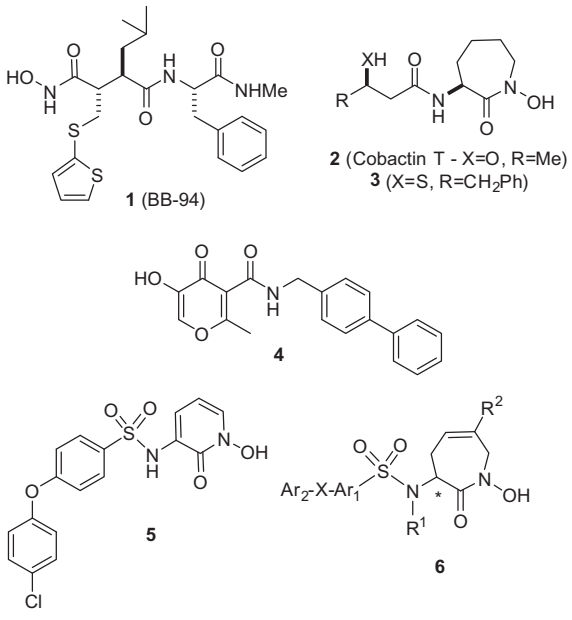

Figure 1. Structures of matrix metalloproteinase inhibitors.

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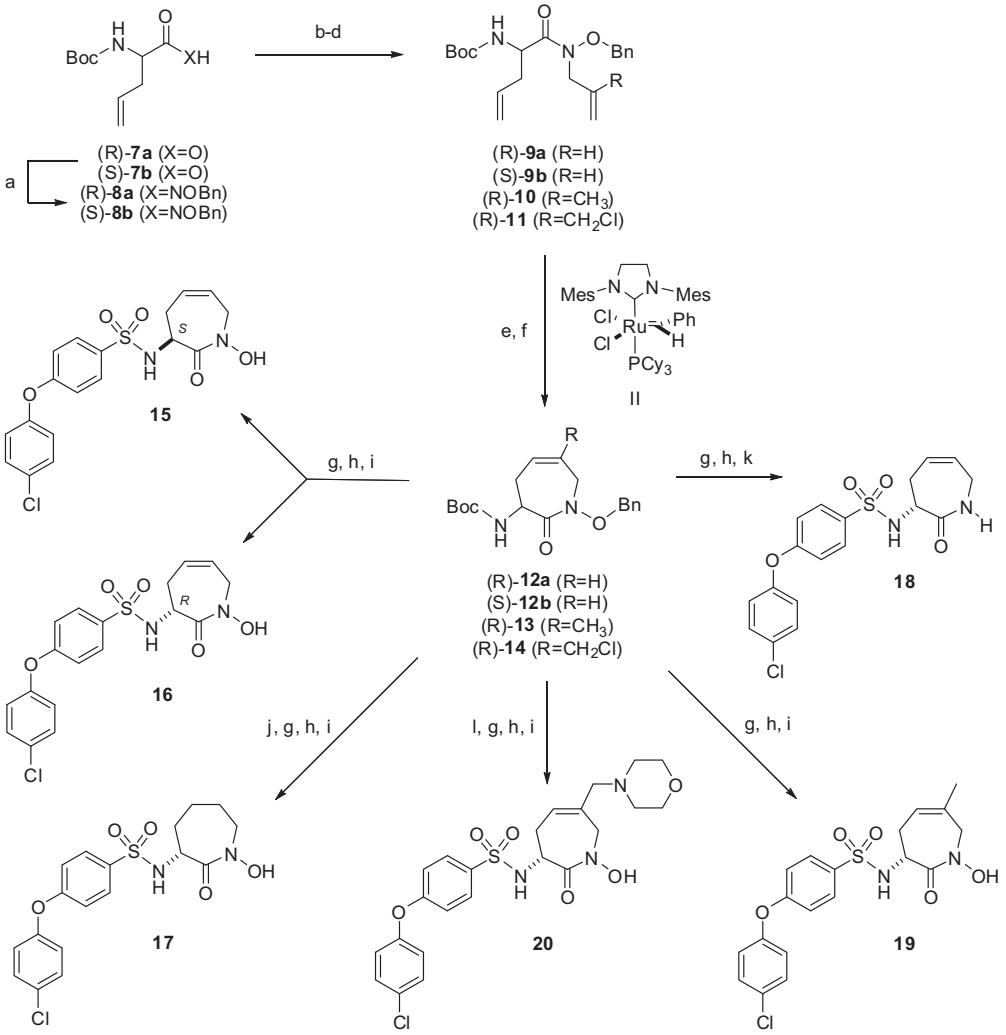
doi:10.1016/j.bmcl.2011.08.068

(MMP-2) and 9 (MMP-9) in ischemic stroke to block soft tissue degradation. Historically, there are many types of inhibitors that have been discovered and most are proposed to bind to the zinc metal in the active site of the enzyme, while both non-zinc binding and zinc mechanism based inhibitors have shown a more limited interest.[4,5] Recently, there have been significant developments involving development of heterocyclic MMP inhibitors where the zinc binding group (ZBG) is buried in the heterocycle ring system.[5] These include ring systems such as hydroxy-pyrones (**4**) and pyridinones (**5**) termed HOPO's (Fig. 1).[6,7] These ring systems have been studied in MMP inhibitor development with success due to their known ability to chelate a range of metal ions including zinc. We envisioned utilizing the lysine derived *N*-hydroxyl caprolactam portion of Cobactin-T (**2**) as a zinc binding element for matrix metalloproteinases (**6**, Fig. 1). Synthetic studies on Cobactin-T have resulted in its total synthesis, as well as analogs of the mycobactins.[8,9] The amino-*N*-hydroxy caprolactam has also been exploited in the design of inhibitors of other zinc metal chelating metallo enzymes (**3**).[10] The naturally occurring *S*-isomer of the saturated lactam was reported previously by Miller, and in our enantiomeric synthesis of Cobactin-T utilizing the ring closing metathesis (RCM) reaction.[11] Placing an aryl sulfonamide on the nitrogen is a well known approach to getting proper enzyme recognition in all types of MMP inhibitors including carboxylic acid, hydroxamic acid, and heterocyclic compounds.[4,5] Occupancy of the S1′ pocket with a hydrophobic ligand should determine selectivity preferring deep S1′ pocket (MMP-2, 9, 13) over shallow S1′ pocket (MMP-1, 7) enzymes.[12] With this design criteria in mind, our approach was to construct the ring in an expedient manner using the ring closing metathesis (RCM) reaction from readily available allyl glycine based amino acid building blocks. This effort focuses on access to an enantiomeric route to explore the influence of stereochemistry on the biological properties of the molecules. Furthermore, to date none of the studies have provided complementary enantiomeric isomers, which could prove extremely useful in understanding biological activity and structure activity relationships.

The medicinal chemistry approach starts by assembling the caprolactam ring system using the ring closing metathesis reaction. The synthetic route begins from chiral Boc protected allyl glycines (**7a**/**7b**, Scheme 1). First, Boc-glycine coupling with *O*-benzyl-hydroxyl amine was accomplished to give **8a**/**8b**. Use of normal coupling conditions (i.e., DCC, EDC) resulted in racemization of the alpha carbon and loss of chirality. This was avoided by employing the more reactive coupling reagents HATU and HOAt giving greater than 96% enantioneric purity determined by chiral HPLC analysis. Next, alkylation with allyl bromide proceeded smoothly and in high yield to give diallyl compounds **9a**/**9b**. Reaction of these species with Grubb's second generation ruthenium catalyst **II** gave the

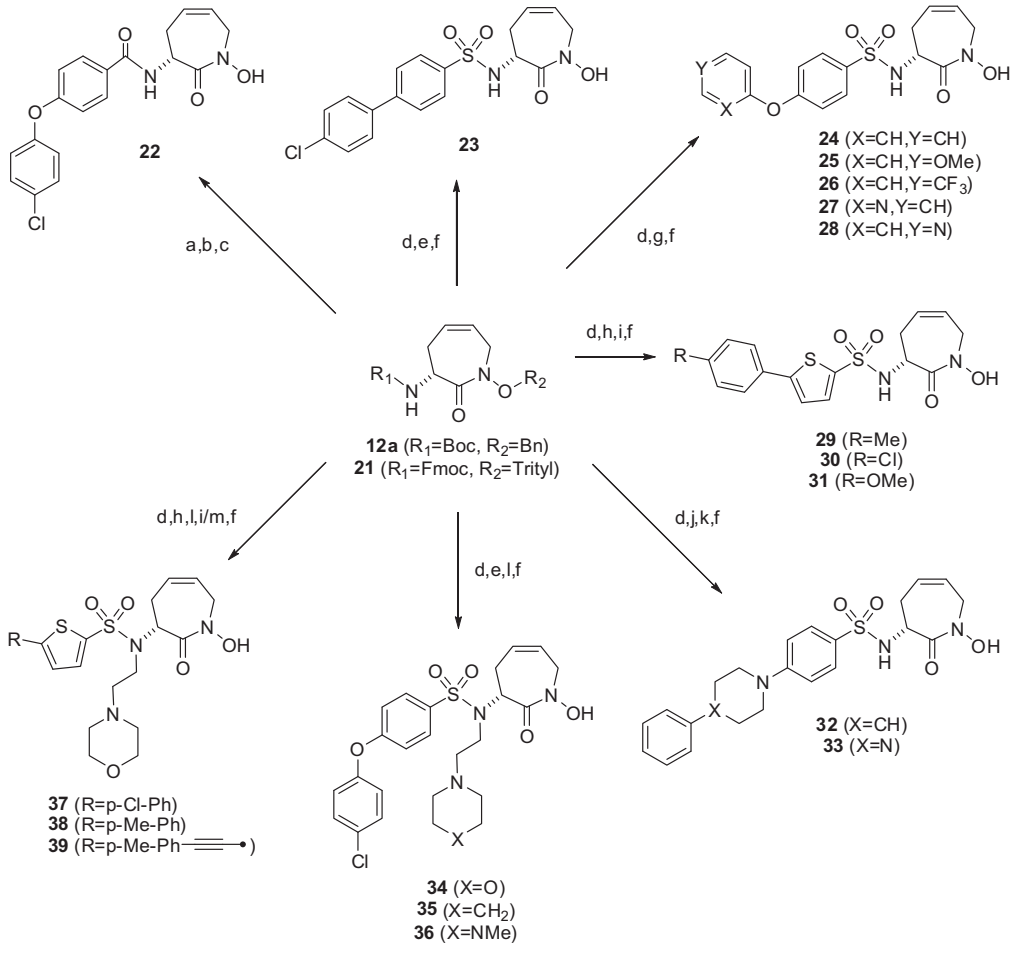


Scheme 1. Reagents and conditions: (a) $BnONH_2$, HATU, HOAt, *N*-methylmorphine, DCM; (b) for **9**: Cs_2CO_3, DMF, allyl bromide, rt; (c) for **10**: 2-methyl-allyl chloride, Cs_2CO_3, DMF, rt; (d) for **11**: 2-chloromethyl-allyl chloride, Cs_2CO_3, DMF, 60 °C; (e) for **12**: **II**, DCM, rt; (f) for **13** and **14**: **II**, 1,2-DCE, microwave, 150 °C; (g) TFA, DCM, rt; (h) 4-(4-ClPh)$OPhSO_2Cl$, pyridine, DCM; (i) $MeSO_3H$, rt; (j) $[CODIrPCy_3Pyr]PF_6$, THF, MeOH, H_2; (k) SmI_2, THF, rt; (l) morpholine, CH_3CN, 60 °C.

corresponding lactams **12a**/**b**.[13] At this point, the 4-(4-chlorophenoxy) phenyl sulfonamide group could be attached by first deprotecting the Boc group and then reacting the resulting primary amine with 4-(4-chloro-phenoxy)-phenyl sulfonyl chloride. Removal of the oxygen benzyl group using methane sulfonic acid gave the resulting (*S*) and (*R*) sulfonamido *N*-hydroxy caprolactams **15** and **16**. Next, we synthesized the saturated derivative. First, we reduced the double bond of lactam **12a** by using a heterogeneous iridium catalyst. This was followed by the Boc group deprotection, sulfonamide formation and benzyl ether deprotection sequence to provide the saturated compound **17**. Variation of the substituents on the double bond of the ring could be provided through alteration of the RCM reaction sequence, and two examples were explored. Thus, both a methyl and a morpholino-methyl substituent were installed by the following reactions: alkylation with either 2-methyl-allyl chloride (**8a**–**10**) or 2-chloromethyl-allyl chloride (**8a**–**11**); microwave promoted RCM (**10**/**11**–**13**/**14**),[14] morpholine addition (for **14**); and sulfonamide attachment followed by *O*-benzyl deprotection (**19** and **20**). Finally, to round out the heterocycle core modifications, reduction of the nitrogen oxygen bond was performed to provide lactam **18** to test the influence of the system without the zinc binding group.

Modifications in the appending nitrogen correspond to changes in the P1′ binding element. As such we focused on changes on the nitrogen atom and aromatic portions (Scheme 2). The amide version of **16** (compound **22**) was synthesized from the previously reported FMOC-Trityl protected lactam (**21**) by FMOC group removal, followed by amide formation, and trityl group deprotection.[11] All others consisted of sulfonamide modifactions made from the (*R*)-Boc-benzyl lactam **12a**. The first of these consisted of removing the biphenyl ether linkage (**23**), followed by biaryl ether expansion (**24**–**28**). These compounds were synthesized by the sequence of Boc group deprotection, sulfonamide formation with the corresponding commercially available sulfonyl chloride, and *O*-benzyl deprotection. Alternatively, 5-arylthiophene containing P1′ groups were installed by sufonylation with 5-bromothiophenesulfonyl chloride, followed by Suzuki coupling with 4 substituted aryl boronic acids, and benzyl group removal (**29**–**31**). Further extension
included the basic amine linkers replacing the aryl oxygen atom (**32**, **33**) synthesized by a 4-fluoro-phenyl sulfonamide and amine displacement sequence. Finally, sulfonamide N alkylation was accomplished by alkylation of the corresponding *N*-sulfonamido-*O*-benzyl lactam of **16** with *N*-chloroethyl heterocycles, followed by *O*-benzyl group deprotection (**34**–**36**) and also on the 5-arylthiophene series through the previously described chemistries (**37**–**39**) for the synthesis of **29**–**31** and **34**–**36**.

The testing of these compounds against the enzymes of interest (MMP-2 and 9) was concurrent with the synthesis efforts. We found some very encouraging and exciting results (Table 1). First, the (*S*)-analog **15**, which contains the stereochemistry found in the Cobactin-T (**2**) containing natural products, showed modest



Scheme 2. Reagents and conditions: (a) Piperidine, DMF; (b) 4-(4-ClPhO)PhCOCl, *i*Pr$_2$NEt, DCM; (c) Amberlyst 15 ion exchange resin, DCM, MeOH; (d) TFA, DCM; (e) 4-(4-ClPh)PhSO$_2$Cl, Pyr, DCM; (f) MeSO$_3$H; (g) 4-(Ar)OPhSO$_2$Cl, Pyr, DCM; (h) 5-Br-thiophene-SO$_2$Cl, Pyr, DCM; (i) 4-ArB(OH)$_2$, Pd(OAc)$_2$, DMF, microwave, 150 °C; (j) 4-F-PhSO$_2$Cl, Pyr, DCM; (k) 4-Ph-piperdine or 4-Ph-piperazine, DMSO; (l) Na$_2$CO$_3$, DMF, *N*-(ClCH$_2$CH$_2$)-morpholine, *N*-(ClCH$_2$CH$_2$)-piperidine, or *N*-(ClCH$_2$CH$_2$)-*N*′-Me-piperazine; (m) 4-Me-PhCCH, Pd(PPh3)$_4$, CuCl$_2$, NEt$_3$, DMF.

Table 1
IC_{50} Values for cobactin based mmp inhibitors (**15**–**39**)

Compound	MMP		Enzyme	IC_{50}[a] (nM)	
	MMP-2	MMP-9	MMP-1	MMP-3	MMP-13
15	1599 ± 36	1117 ± 18			
16	0.57 ± 0.04	0.58 ± 0.03	>1000	86	0.4
17	3.7	5.9			
18	>1000	>1000			
19	2	1.3			
20	0.53 ± 0.01	0.46 ± 0.02	>1000	39	0.2
22	2319	5091			
23	85	1506			
24	1	2			
25	2	1			
26	2	2			
27	15	23			
28	3	12			
29	4	9			
30	11	16			
31	3.9	11	>1000	493	3
32	>1000	>1000			
33	>1000	>1000			
34	0.39 ± 0.01	0.22 ± 0.03	>1000	90	0.7
35	0.55	0.21			
36	0.49	0.2			
37	16	17			
38	7	11			
39	2.4	0.8			

[a] Values are means of two to three separate experiments. Each dose response was ran in duplicate.

activity against both enzymes in the single digit micromolar range. However, when testing the (*R*)-enantiomer **16** potencies were found to be below 1 nM in both MMP-2 and 9 assays. The large difference in activity regarding stereochemistry has been observed in sulfonamide phosphate MMP inhibitors but not carboxylic acid sulfonamides.[5,15] However stereo chemical preference is an unknown in the case of Cobactin-T containing compounds, and represents a novel development. Noteworthy is the compound without the double bond (**17**) has about 10-fold less activity than **16**. Furthermore, compound **16** is greater than 100-fold more potent than activity reported for compound **4** suggesting the azepenone scaffold (**6**) from the ring closing metathesis is the most potent heterocycle system reported to date.[7] The importance of the zinc binding properties of this system were confirmed since the normal lactam **18** which lacks the hydroxyl amine functionality had no activity in our assays.

Further testing in MMP-2 and 9 provided interesting SAR trends (Table 1). Replacing the sulfonamide with a normal amide (**22**) resulted in greater than 1000-fold drop off in activity. The activity observed here is similar to other heterocycle amides such as **3**, and suggests these other scaffolds may be optimized by replacing the amide to a sulfonamide.[5,6] The biaryl ether oxygen was needed for the potent activity with the biphenyl series and a large drop off in activity ($\geqslant$100-fold) was seen between **16** and the biaryl compound **23**, although this compound had 10–20-fold selectivity towards MMP-2. The inclusion of other substituent patterns than the chloride at the para position (**24**–**26**) and a pyridine ring replacing the phenyl ring (**27**, **28**) in the biaryl ether assembly resulted in slightly less active (10–50-fold) compounds. The 5-aryl-thiophene compounds (**29**–**31**) were almost as potent and a modest drop off in activity was observed (about 10-fold) with similar activity to the aryl-pyridyl ether compounds (**27**, **28**). The replacement of the aryl oxygen with piperidine and piperazine rings (**32** and **33**) resulted in complete loss of activity. Alkylations on the sulfonamide nitrogen (**34–36**) and substitutions on the azepinone ring double bond (**19, 20**) slightly increased (**20**, **34**–**36**) or decreased (**19**) the potency compared to **16**. Although not as potent as the biaryl ether series, it is worth noting the best 5-aryl-thiophene compound discovered was the one with an alkyne linker and morpholine ethyl (**39**) which had sub nanmolar potency against MMP-9. Another interesting trend is that the heteroalkyl sulfonamides (**34**–**36** and **39**) seemed to have a 2–3-fold selectivity towards MMP-9 versus MMP-2. Overall, the *p*-chlorobiarylether and heterocycle (e.g., morpholine) substituted compounds **16**, **20** and **34**–**36** were the most potent with sub nanomolar IC_{50} values.

To determine interfamily MMP selectivity, we tested select compounds against three other MMP's (1, 3, and 13; Table 1). All four compounds (**16**, **20**, **31**, and **34**) showed the following trends. As it has been hypothesized that MMP-1 activity is associated with the side effect of musculo-skeletal syndrome (MSS) that is observed in the clinic, having MMP-1 inhibitory activity would be undesirable.[16] Therefore, it was significant that all were selective against MMP-1, showing no activity up to 1000-fold higher than their efficacy against MMP's 2 and 9. All had approximately 100-fold selectivity against MMP-3. None had selectivity against MMP-13, showing comparable values for all three enzymes (MMP 2, 9, and 13). This selectivity pattern is not completely surprising since MMP-2, 9, and 13 are all deep S1′ pocket enzymes while both MMP-1 and 3 have shallow S1′ pockets. As such, the selectivity is primarily due to the choice of the biaryl ether group on the sulfonamide.[12]

To get a better understanding of how these compounds might be fitting into the enzyme active site, a homology model of MMP-9 was built. Next, compounds **20** and **34** were docked into the active site containing the zinc metal (Fig. 2). We found that the best docking mode for these compounds was a pose in which the biaryl ether sulfonamide group fits into the deep S1′ pocket on the enzyme. The bent shape of this ligand is supported by the loss in activity observed upon removal of the biaryl oxygen (**23**) as well as replacement by thiophenes (**29–31**). In addition to the *N*-hydroxyl-lactam portion binding to the zinc atom, the model also picks up hydrogen bonding between the sulfonamide N–H and the amide carbonyl C–O of Pro-421. The other interaction

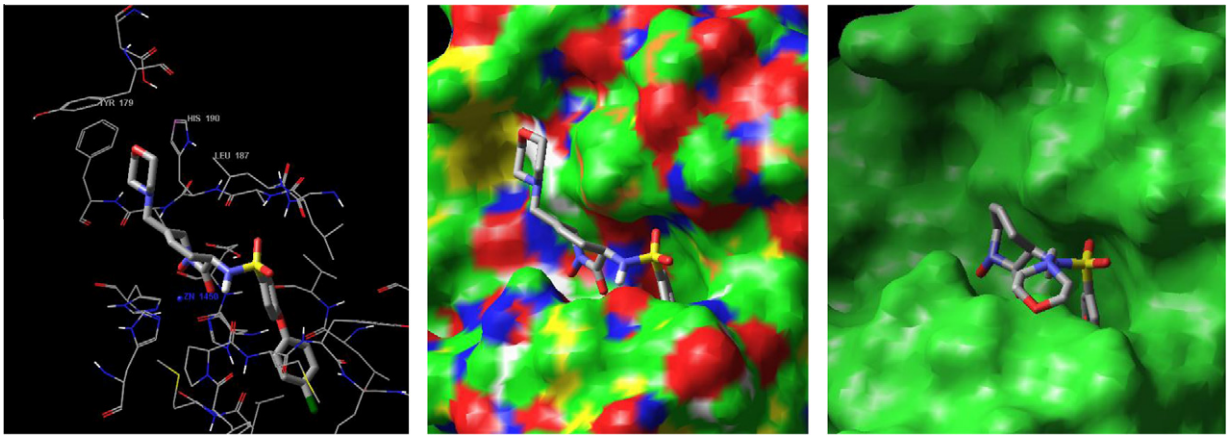


Figure 2. Pictures of compounds **20** and **34** docked in a homology model of MMP-9.

Table 2
Pharmacokinetic parameters for compounds **16** and **34**

Compound[a] (dose-mg/kg)	C_{max} (μM)	$T_{1/2}$ (h)	AUC (ng-h/mL)	V_{ss} (L/kg)	CL (mL/ min/kg)
16 (iv-0.5)	4.6	14	1226	3.3	7
34 (iv-0.5)	4.4	2	1831	1.4	19
34 (po-10)	38.3	1	—	—	—

[a] Iv experiments performed in rats, po experiment performed in mice.

identified is between the sulfonamide S–O group and the amide N–H of Leu-188. It is likely that this later interaction is more significant since it would prevail for all the compounds, especially in the case of N-alkylated derivatives **32** thru **34** as no sulfonamide N–H bond donor exists for these compounds. Furthermore, alteration of the sulfonamide to an amide (**22**) results in significant loss of activity. Lastly, compound **20** is an interesting example in that the model predicts the morpholine ring to occupy the S1 pocket of the enzyme. Although this might be a significant finding, there is no increase in potency observed (**16** vs **20**).

Next, we became interested in exploring the drug-like features of these compounds, specifically the pharmacokinetic and other important ADME parameters. Compounds **16** and **34** were evaluated in pharmacokinetic experiments in rats and mice (Table 2). Intravenous administration provided exposure data, with compound **16** showing a half-life of 14 h, while compound **34** had a shorter half-life of 2 h. Compound **34** was evaluated in an oral pharmacokinetic experiment in mice with strong evidence of good exposure (38 μM for a 10 mg/kg dose, Table 2). Some of the earlier developed MMP inhibitors, especially hydroxamic acids, suffer from lower exposure due to metabolism related to N–O bond reduction and glucaronidation. These results show that these compounds may have better metabolic stability imparted by introduction of the caprolactam ring system onto the hydroxamic acid functionality. Other experiments provided further evidence of favorable drug-like features.[17]

In summary, we have provided a series of novel MMP inhibitors based on the natural product series Cobactin-T (**2**). The incorporation of the double bond in the ring adds a series of benefits spanning from ease of synthesis via the RCM reaction to increased potency against the biologic target. The access to both optical isomers in this case elucidated a high degree of stereo recognition with the MMP enzymes, and also showed that the more active configuration was that opposite to the natural product itself. The ensuing SAR study performed showed an activity pattern resulting in a series of extremely potent enzyme inhibitors dependent mainly on the S1′ pocket recognition element contained on the sulfonamide. The highest activity resulted from the biaryl ether series. The other modifications provided potent inhibitors but with about equal activity to the initial discovery. Testing against other MMP's showed high to low selectivity and was dependent upon the classification (deep vs shallow S1′ pocket enzymes). Docking studies provided some rationale to understanding the SAR and were consistent with the perceived modes of binding. Finally, pharmacokinetic experiments showed these compounds to be highly bio available via several routes of administration.

Supplementary data

Supplementary data (experimental procedures and details are provided for the MMP assays used and also for the synthesis of compounds **8**–**20**, **23**–**29** and **34**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.068.

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17. Experiments with in vitro ADME-Tox assays for **16** and **34** are as follows: hERG binding: <50% @ 10 μM; CYP450 inhibition (3A4, 2D6) <50% @ 1 μM; AMES II = non-mutageneic; CEREP 50 targets: <50% @ 10 μM. This program was discontinued due to business reasons.