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Identification of potent and selective MMP-13 inhibitors

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Abstract—A potent, selective series of MMP-13 inhibitors has been derived from a weak (3.2 μM) inhibitor that did not bear a zinc chelator. Structure-based drug design strategies were employed to append a Zn-chelating group to one end of the molecule and functionality to enhance selectivity to the other. A compound from this series demonstrated rat oral bioavailability and efficacy in a bovine articular cartilage explant model.

MMP-13 (collagenase 3), a member of the matrix metalloproteinase family of zinc dependent enzymes, has been identified as an important target for the treatment of osteoarthritis (OA).[1,2] This enzyme is known to efficiently degrade type II collagen, the enzyme's preferred substrate and the main structural component in cartilage. Its expression has been shown to be upregulated in OA.[3,4] In addition, small molecule inhibitors of MMP-13 have been found to inhibit the degradation of type II collagen in articular cartilage explants.[5] The potential for orally bioavailable MMP-13 inhibitors to slow the progression of OA, for which there are at present only agents that provide symptomatic relief, has led to several clinical trials. Unfortunately, many broad spectrum MMP inhibitors have been found to have dose-limiting toxicity in the form of musculoskeletal side effects including joint stiffness and inflammation.[6,7] While the inhibition of specific MMPs such as MMP-1[8–10] or MMP-14[11,12] has been postulated to be responsible for musculoskeletal syndrome (MSS) the exact cause of this pathology is not yet clear.[13,14] Therefore, in an effort to reduce the likelihood of MSS in an effective therapeutic for OA, we sought a potent inhibitor of MMP-13 with a high degree of selectivity over other MMPs.

Our design of a selective, orally bioavailable MMP-13 inhibitor began with CL-82198, a high-throughput screening (HTS) hit that had an IC_{50} of 3.2 μM against MMP-13 and was selective over MMP-1 and MMP-9. We demonstrated earlier that a potent, selective, hydroxamate-containing inhibitor could be realized by using structure-based methods to hybridize the benzofuran carboxamide portion of CL-82198 directly to a hydroxamate head piece (see Scheme 1).[15] We now disclose the design, synthesis, and biological activity of a series of carboxylic acid inhibitors with a rigid linker to the benzofuran carboxamide P1′ terminus along with additional functionalities that impart selectivity over a broader range of MMPs.

The design of these rigid P1′ analogs was the result of a detailed, structure-based computational analysis,[16] where the replacement of the ubiquitous hydroxamic acid group as the chelator of the MMP-13 active site zinc with a carboxylate group was specifically explored. Since the carboxylate group is a less effective Zn-chelator[17] than the hydroxamate (note that the carboxylate analog of WAY-170523 is not active at 10 μM[18]), several features of the scaffold of WAY-170523 that were believed to contribute negatively to its free energy of binding needed to be optimized. These included entropic penalties resulting from the conformationally mobile $–O–CH_2–CH_2–$ linker, the positioning of the amide group, and minimal enthalpic interactions beyond the hydroxamate moiety. This analysis led us to utilize

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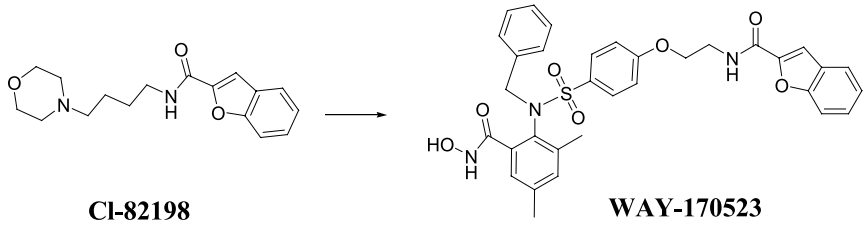


Scheme 1. Evolution of potent, selective inhibitor WAY-170523 from HTS hit CL-82198.

a scaffold exemplified by compound **1**, with a carboxylate zinc chelator and a sulfonamide group to H-bond to LEU185 and ALA186. To correctly position the benzofuran moiety of the P1′ group, a biphenyl P1′ linker was installed to rigidify the molecule, fill the hydrophobic S1′ tunnel, and to π-stack with HIS222. Most importantly, it places the terminal benzofuran carboxamide in a manner that retains the selectivity and binding features of CL-82198. An overlay illustrating these features is shown in Figure 1.

Following the chemistry outlined in Scheme 2[19] the compounds in Table 1[20] were prepared, wherein the carboxylic acid was connected to the benzofuran via a biphenyl sulfonamide spacer, resulting in a series of rigid and potent inhibitors of MMP-13. These analogs were evaluated for selectivity for MMP-13 over MMP-14 and MMP-2. As MMP-2 is highly homologous to MMP-13 in and around the S1′ pocket, it was hoped that compounds that demonstrated selectivity over MMP-2 would also possess enhanced levels of selectivity over a wide variety of other MMPs.

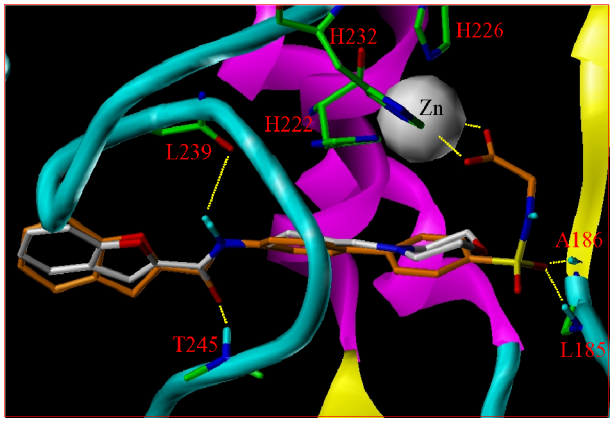
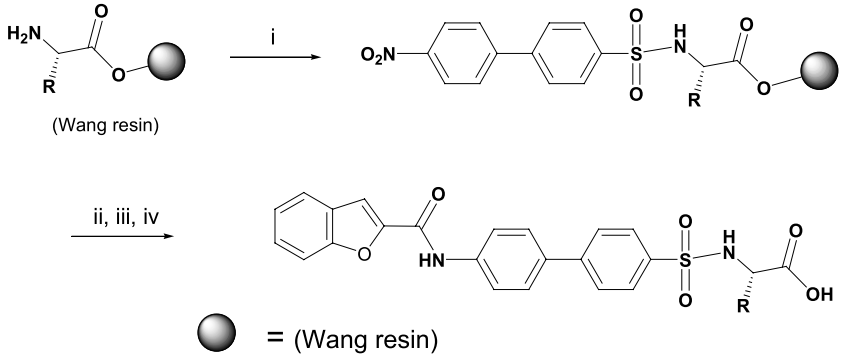


Figure 1. Overlay of CL-82198 and compound **1**.

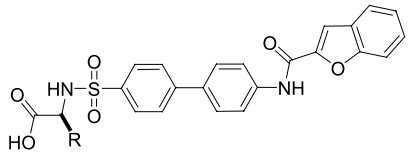
While the glycine-based compound **1** was reasonably potent, a 17-fold improvement in activity was obtained for compound **2**, with the isopropyl group derived from valine. The increased potency of compound **2** is attributed to both the restricted conformational flexibility of the amino acid portion of the molecule due to its bulky isopropyl group, and the increased burial, or shielding of the sulfonamide H-bond with ALA186. The rigidity and length of the P1′ moiety also provide selectivity for MMP-13 over MMP-1 ($IC_{50} > 16$ μM), MMP-9 ($IC_{50} = 1.1$ μM), and MMP-14 ($IC_{50} = 2.2$ μM) (Table 4). As can be seen in Table 1, MMP-13 activity is retained for both hydrophobic (compounds **2** and **4**) and hydrophilic (compounds **3**, **5**, and **6**) α-substituents. This is not surprising given the solvent exposed nature of this region.[21] Having identified compound **2** with excellent MMP-13 potency and promising selectivity, the activity of this analog in a bovine articular cartilage explant model,[22] and its pharmacokinetic properties were assessed. We were gratified to find that compound **2** demonstrated low clearance in rats (2 mL/min/kg at 2 mg/kg iv), reasonable half-life on both iv (3.7 h) and oral dosing (3 h at 5 mg/kg po), as well as bioavailability of 24%. It was also a potent inhibitor of cartilage degradation in the explant assay with an EC_{50} of 4 nM.

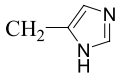
With compound **2** as a promising lead, the role of the benzofuran and the amide linker in providing potency and selectivity was examined. Following the chemistry shown in Scheme 3 the compounds in Table 2 were



Scheme 2. Reagents: (i) 4′-nitrobiphenyl-4-sulfonyl chloride, iPr$_2$NEt, CH_2Cl_2; (ii) $SnCl_2$, DMF; (iii) R′COCl, iPr$_2$NEt, CH_2Cl_2; (iv) TFA.

Table 1. Structure–activity relationships of different amino acid side chains



Compound	R	MMP-13[a]	MMP-2[a]	MMP-14[a]
1	H	22	47	13,900
2	Isopropyl	1.3	5.0	2,200
3	CH_2CONH_2	2.53	10	
4	Isobutyl	2.19	8.4	
5	CH_2OH	4.5	27.8	
6		8.1	37	

[a] IC_{50} (nM).

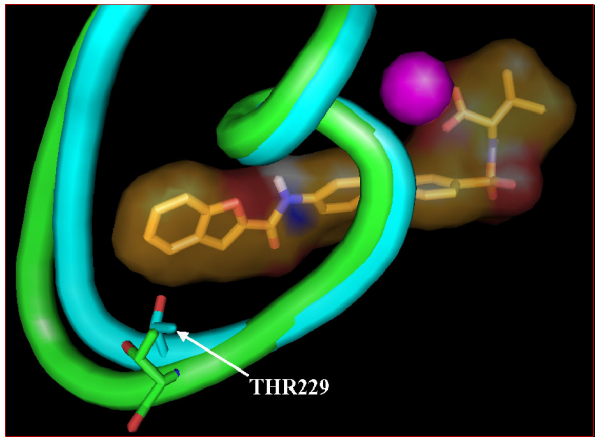
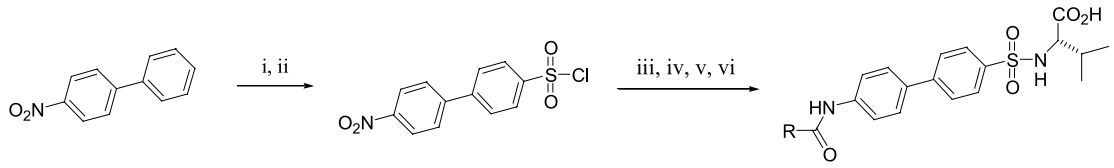


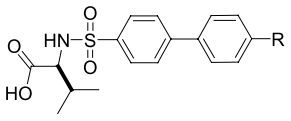
Figure 2. Overlay of protein crystal structure of compound **2**-MMP-13 (green) and MMP-2 (blue).

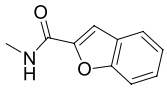
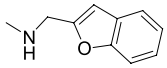
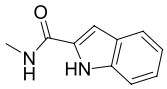
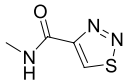
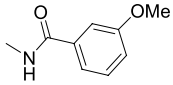


Scheme 3. Reagents and conditions: (i) $ClSO_3H$, $CHCl_3$, 60 °C; (ii) $SOCl_2$, reflux; (iii) L-valine *tert*-butyl ester hydrochloride, CH_2Cl_2, $NaHCO_3$; (iv) $SnCl_2$, DMF; (v) RCOCl, Hunigs base, CH_2Cl_2; (vi) TFA.

prepared. Both the amide linker and the benzofuran were found to be optimal. Thus, replacement of the amide linker with a methylene (compound **7**) resulted in a 3.8-fold loss of activity. Replacing the benzofuran with an indole resulted in a 37-fold loss of potency, and replacement of the benzofuran with monocyclic aryl or heterocyclic moieties resulted in compounds with a loss in potency of approximately 10-fold.

Table 2. Structure–activity relationships of different benzofuran mimetics



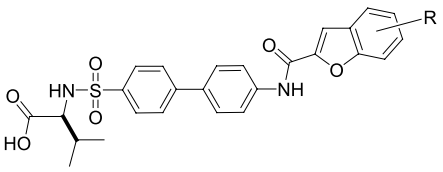
Compound	R	MMP-13[a]	MMP-2[a]	MMP-14[a]
2		1.3	5.0	2200
7		4.9	23.3	6600
8		35.4	19	5600
9		10.9	2.14	—
10		13.7	14	—

[a] IC_{50} (nM).

To improve the selectivity of compound **2** against MMP-2, the exact size, shape, and chemical environment of the S1′ pocket of MMP-2 compared with those of MMP-13 were studied[23] utilizing the protein crystal structure of compound **2** and MMP-13 (1ZTQ) and MMP-2 (1QIB[24,25]). As seen in Figure 2, the most striking difference between MMP-13 and MMP-2 is the size and conformation of the loop that give rise to the S1′ pocket. This difference is primarily due to the fact that the MMP-2 S1′ loop is two amino acids shorter in this vicinity; it is missing residues analogous to GLY248 and SER250 in MMP-13. This causes the loop to be displaced by 4 Å in the area around SER250 of MMP-13. This constriction of the MMP-2 S1′ loop results in the placement of THR229 in a manner that would clash with substituents at the benzofuran 4-position. Thus substitution at the 4-position of the benzofuran should be more easily accommodated by MMP-13, resulting in selectivity over MMP-2.

Having identified an approach to attain selectivity against MMP-2, and potentially other MMPs, the compounds shown in Table 3 were prepared utilizing the chemistry outlined in Scheme 3. As expected, substitution of the benzofuran with the 7-methoxy group did not provide selectivity for MMP-13 and in fact resulted in a reversal of selectivity. Substitution at the 5-position, with a variety of electron donating and withdrawing moieties, generally resulted in slightly increased potency,

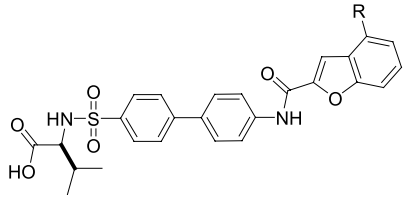
Table 3. Structure–activity relationships of different substituents on the benzofuran



Compound	R	MMP-13[a]	MMP-2[a]	Selectivity 2/13	MMP-14[a]
2	H	1.3	5.0	3.8	2,200
11	7-OMe	20.2	12.1	0.6	10,900
12	5-Cl	0.6	3.2	5.3	—
13	5-OMe	0.66	1.9	2.8	1.5
14	5-NO_2	0.7	2	2.8	—
15	5-NH_2	1.5	—	—	—
16	5-$NHCOCH_3$	0.98	4	4.1	—
17	5-$NHSO_2CH_3$	0.43	3.9	9.1	—
18	5-$NHSO_2Ph$	3.4	19	5.6	—
19	4-OMe	2.33	16	6.9	—
20	4-OBn	3.45	62	18	—
21	4-$OCH(CH_3)CO_2H$	14.9	1590	106.7	20,000

[a] IC_{50} (nM).

Table 4. Selectivity profiles for compounds **2** and **21**



Compound	R	MMP-13[a]	MMP-1[a]	MMP-2[a]	MMP-3[a]	MMP-7[a]	MMP-9[a]	MMP-14[a]
2	H	1.3	>16,000	5.0	50.5	19	1100	2,200
21	$OCH(CH_3)CO_2H$	14.9	—	1590	843	2500	5600	20,000

[a] IC_{50} (nM).

but did not affect selectivity significantly, again as expected from structural analysis of the enzymes. However, we were gratified that, as predicted, substitution at the benzofuran 4-position resulted in substantially greater selectivity. The 4-methoxy compound **19** was 6.9-fold selective over MMP-2, while the 4-benzyloxy compound **20** was 18-fold selective. Even more dramatic selectivity for MMP-13 over MMP-2, greater than 100-fold, could be obtained by the replacement of the 4-benzyloxy group with $-OCH(CH_3)CO_2H$ (compound **21**). As shown in Table 4, compound **21** also had increased selectivity for MMP-13 over MMP-3 and MMP-7, and MMP-14, as compared to **2**.

Herein, we have reported the identification of a potent and selective series of MMP-13 inhibitors based on the integration of key components of the HTS hit CL-82198 and further analysis and design to obtain selectivity against the highly homologous MMP-2. While others have reported α-amino acid derived biphenyl sulfonamides as MMP-13 inhibitors,[20–23] this series attains excellent selectivity against MMP-2 as well as MMP-7, MMP-9, and MMP-14. Further investigation of this approach to selectivity will be reported in subsequent publications.

Acknowledgments

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16. Small molecule building and minimizations were performed in Sybyl (Tripos Inc) with the MMFF94s forcefield. Differences in the size, shape, and chemical environment of the MMP active sites were identified with the assistance of GRID (Molecular Discovery, Ltd.). Simulations of solvent-based electrostatics were accomplished with Zap_Bind (OpenEye Scientific Software), which employs a Poisson–Boltzmann continuum electrostatics method.
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18. Results not published.
19. All new compounds gave satisfactory ^{1}H NMR, MS in accord with their assigned structure.
20. (a) The inhibitory ability of small molecules was tested in a continuous fluorescent assay designed for each enzyme in which the substrate is a synthetic peptide containing a fluorescent group (7-methoxycoumarin or 2-aminobenzoyl) that is quenched by energy transfer to 2,4-dinitrophenyl. Enzymes were either prepared at Wyeth Research (MMP-1, -12, -13) or purchased from Calbiochem (San Diego, CA) (MMP-3, -7, -9), R&D Systems (Minneapolis, MN) (MMP-2), Chemicon International (Temecula, CA) (MMP-14), or Biomol (Plymouth Meeting, PA) (MMP-8). MMP-2 and MMP-14 were activated with AMPA. Enzymatic assay conditions for MMP-1, -2, -7, -9, -13, and -14 have been previously described in Ref. 20c and identical assay conditions were used for MMP-3 and -12, except MMP-3 used the substrate Mca-PKPLAL-Dpa-AR-NH2 (Bachem, King of Prussia, PA). Measurements were performed in fluorimeter plate readers at the excitation and emission wavelengths of the fluorophore. Note that while IC_{50} values were reported, the assays were established in such a way as to keep the IC_{50} and K_i within 2-fold of each other by manipulating the relationship between a given MMPs substrate concentration and its K_m as dictated by the Cheng–Prusoff equation (see Ref. 20b). Note also that the mode of inhibition is competitive as illustrated by the crystal structure of compound **2** and MMP-13.(b) Cheng, Y.-C.; Prusoff, W. *Biochem. Pharmacol.* **1973**, *22*, 3099; (c) Zhang, Y.; Xu, J.; Levin, J.; Hegen, M.; Li, G.; Robertshaw, H.; Brennan, F.; Cummons, T.; Clarke, D.; Vansell, N.; Nickerson-Nutter, C.; Barone, D.; Mohler, K.; Black, R.; Skotnicki, J.; Gibbons, J.; Feldmann, M.; Frost, P.; Larson, G.; Lin, L.-L. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 348.
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22. (a) Bovine articular cartilage explants were harvested from carpal joints up to 4 h after death. The explants were cultured in Dulbecco's modified Eagle's medium (DMEM) in the presence or absence of 5 ng IL-1/mL to induce synthesis and activation of proteolytic enzymes including MMP-13. Various concentrations of small molecule inhibitors were added to the culture wells. Culture medium was collected after 3, 7, 10, 14, and 17 days in culture and replaced with identical cytokine and inhibitor concentrations. After day 17, cartilage explants were collected and total collagen content in media and remaining in the explant were quantitated by biochemical analysis of hydroxyproline content using a modification of previously described in Refs. (b) and (c). Percent inhibition of collagen degradation by a small molecule inhibitor was calculated as percent reduction in collagen release over the 17-day experiment compared to collagen release with IL-1 alone and no inhibitor; (b) Bergman, I.; Loxley, R. *Anal. Chem.* **1963**, *35*, 1961; (c) Brown, S. W.; Worsfold, M.; Sharp, C. *BioTechniques* **2001**, *30*, 38.
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