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Discovery of (pyridin-4-yl)-2*H*-tetrazole as a novel scaffold to identify highly selective matrix metalloproteinase-13 inhibitors for the treatment of osteoarthritis

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ABSTRACT

Potent, highly selective and orally-bioavailable MMP-13 inhibitors have been identified based upon a (pyridin-4-yl)-2*H*-tetrazole scaffold. Co-crystal structure analysis revealed that the inhibitors bind at the S_1' active site pocket and are not ligands for the catalytic zinc atom. Compound **29b** demonstrated reduction of cartilage degradation biomarker (TIINE) levels associated with cartilage protection in a pre-clinical rat osteoarthritis model.

Osteoarthritis (OA) is a degenerative joint disease characterized by the erosion of articular cartilage leading to pain and reduced mobility. An estimated 26.9 million people in the US suffer from clinical osteoarthritis.[1] The primary treatment strategy for the majority of patients is the use of acetaminophen, NSAIDs, or to a lesser extent opioids to alleviate the symptomatic pain of the disease.[2] A significant unmet medical need exists for therapeutics that treat the underlying pathology of OA, namely the loss of articular cartilage. Matrix metalloproteinase-13 (MMP-13) is a zinc dependent protease responsible for cleavage of type II collagen, the major structural protein in articular cartilage. MMP-13 has been found to be expressed in the articular cartilage of OA patients, and transgenic mice over-expressing MMP-13 in articular cartilage demonstrate changes characteristic to human OA.[3] Consequently inhibition of MMP-13 activity in the joint is a compelling strategy to arrest joint destruction and halt the progression of disease in OA patients.

Several broad-spectrum metalloproteinase inhibitors have been investigated in human clinical trials for cancers and inflammatory diseases; however, a specific dose limiting toxicity termed muscular skeletal syndrome (MSS), characterized by a tendonitis-like stiffening of the joint, was a common observation.[4] The lack of this finding in the phenotype of MMP-13 null mice[5] as well as individuals possessing a missense mutation of the MMP-13 gene[6] suggests one or more metalloproteinases other than MMP-13 is the causative factor. Therefore, the discovery of highly selective MMP-13 inhibitors is a critical design criteria for new agents. The poor metalloproteinase selectivity profile of early drugs is partly the consequence of inhibitor design dependent on the use of a strong zinc metal chelator, usually a hydroxamic acid, to achieve tight enzyme binding.[4b] More recent strategies utilizing structure based optimization of the P1′–P3′ inhibitor binding elements in conjunction with weaker, monodentate zinc binding groups have afforded inhibitors which spare specific metalloproteinases among the 23 known enzymes, however, truly specific MMP-13 inhibitors have remained elusive to this approach.[7]

A remarkably different selectivity profile demonstrating the desired high specificity for MMP-13 was reported by Johnson for the quinazolinone **1** (MMP-13/CD[8] IC_{50} = 0.67 nM, IC_{50} >30 μM for nine MMPs tested), Figure 1.[9] Compound **1** was reported to significantly reduce cartilage lesion areas when dosed orally in a rabbit anterior

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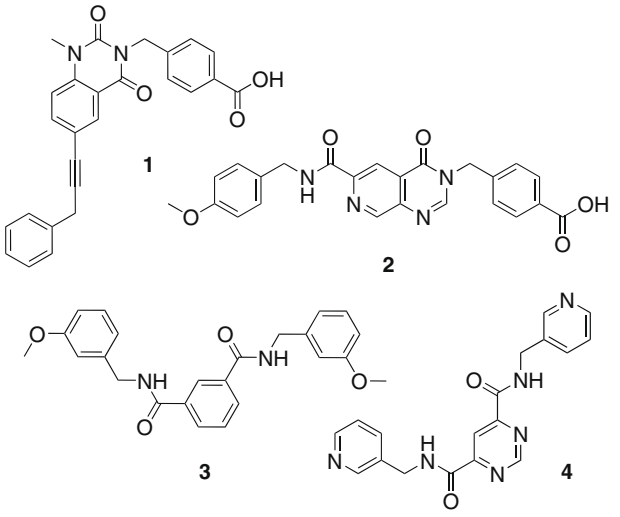
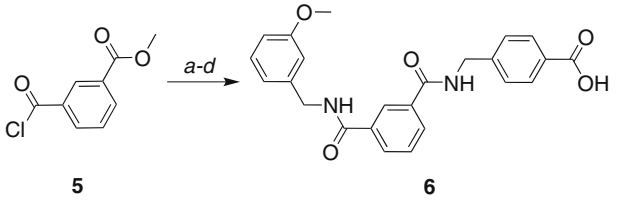


Figure 1. Reported non-zinc binding, highly selective MMP-13 inhibitors (**1**, **2**, and **4**) and monocyclic scaffold based MMP-13 inhibitor screening hit **3**.

cruciate ligament transection/partial meniscectomy model while not showing MMS-like fibroplasia in rats.[10] As opposed to classical MMP inhibitor design where binding is dominated by a zinc-inhibitor complex, a co-crystal structure of **1** with the catalytic domain of MMP-13 revealed a non-zinc binding mode relying solely on interactions between the inhibitor and the S_1' binding pocket. Further optimization of this bicyclic core afforded pyrido[3,4-*d*]pyrimidin-4-one **2** described by Li et al.[11]

As part of the original high-throughput screening which led to the eventual discovery of compound **1**, phenyl dicarboxamide **3** was also identified, Figure 1. Compound **3** was a modest inhibitor of MMP-13/CD (IC_{50} = 1.35 μM) and demonstrated high selectivity against a range of MMPs (IC_{50} >100 μM, MMP-1, 2, 3, 7, 9, 14).[12] Concurrent to this work, Wendt and co-workers reported a similar pyrimidine dicarboxamide **4** that demonstrated a comparable activity and selectivity profile.[13] The discovery of compound **3** suggested that the rigid fused bicyclic core structures of compounds **1** and **2** were not required for MMP-13 potency. This offered the opportunity to merge activity-based learnings from these series with a simplified core ring template that could provide improved physiochemical properties. Herein, we describe the optimization of the monocyclic hit **3** to provide highly potent, selective, and orally-bioavailable MMP-13 inhibitors that demonstrate cartilage protection in preclinical OA animal models.

The introduction of the benzoic acid motif of lead compound **1** had previously been shown to improve MMP-13 potency in hit to lead efforts.[9] Therefore, initial efforts to optimize compound **3** were to evaluate the translation of these findings in this series by replacing one of the 3-methoxybenzyl substituents to afford compound **6**, Scheme 1. This modification afforded a significant



Scheme 1. Synthesis of phenyl dicarboxamide **6**. Reagents and conditions: (a) 3-methoxybenzylamine, Et_3N, CH_2Cl_2; (b) NaOH, $MeOH/H_2O$; (c) methyl 4-(aminomethyl)benzoate, Et_3N, HOBt, EDAC·HCl, CH_2Cl_2; (d) NaOH, $MeOH/H_2O$.

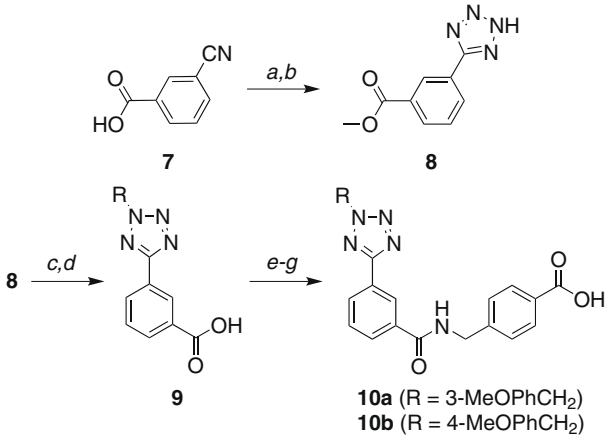
Table 1
MMP-13 inhibitory activity of amides **3**, **6**, and **10**

Compds	MMP-13/CD IC_{50} (nM)	MMP-13/FL IC_{50} (nM)	Solubility (μg/mL)	CACO2[a]
3	1350	nd	nd	nd
6	77	1700	60	0
10a	38	530	<3	0
10b	58	130	<3	0

[a] Permeability × 10^{-6} cm/s (nd = not determined).

improvement in MMP-13 inhibitory activity (IC_{50} = 77 nM) compared to **3** while retaining the exquisite selectivity profile, Table 1. Unfortunately, CACO2 cell permeability of **6** and subsequently oral bioavailability were found to be very poor. To address these findings, a strategy to replace one of the amides with an isosteric ring was pursued and specifically tetrazoles **10** were prepared, Scheme 2. Sodium azide addition to 3-cyanobenzoic acid (**7**) followed by esterification afforded ester **8**. Alkylation of **8** with representative benzylchlorides provided predominately (10:1) the N2-regioisomer **9**. Subsequent amide formation and ester hydrolysis provided analogs **10a** and **b**. Comparable MMP-13 potency to the parent amide **6** was observed, but oral bioavailability remained low (*F* = 6%, **10b**) consistent with the continued low CACO2 cell permeability, Table 1. On the other hand, compound **10b** was the first compound of this series to demonstrate good potency against full length MMP-13 (IC_{50} = 130 nM) as measured in a type II collagen cleavage assay.[9] Since we felt inhibition of the full length construct to be critical, subsequent biological evaluation utilized an enzyme assay based on MMP-13/FL cleavage of a fluorogenic peptide.[14]

Potential replacements for the central phenyl ring were next considered. The promising MMP-13 potency reported for pyrido[3,4-*d*]pyrimidin-4-one **2**[11] suggested that incorporation of nitrogen into the ring adjacent to the amide could be tolerated. Recent reports have suggested that engaging secondary amides in intramolecular hydrogen bonds is a potential strategy to improve the permeability of poorly orally-bioavailable compounds.[15] In this case, we envisioned the pyridine nitrogen atom as the putative hydrogen bond acceptor.[16] Due to the pseudosymmetric nature of the series, alignment of **10b** with **2** now places the benzoic acid functionality as a substituent on the tetrazole ring. Synthesis of the desired pyridine analogs proceeded by sodium azide addition to



Scheme 2. Synthesis of (phenyl)-2*H*-tetrazole amides **10**. Reagents and conditions: (a) NaN_3, Et_3N·HCl, toluene, reflux; (b) HCl, methanol; (c) 3- or 4-methoxybenzylchloride, Et_3N, CH_3CN, reflux; (d) LiOH, THF/H_2O; (e) oxalyl chloride, CH_2Cl_2, 3 h; (f) methyl 4-(aminomethyl)benzoate, Et_3N, CH_2Cl_2; (g) LiOH, THF/H_2O.