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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

Mark E. Schnute [a,*], Patrick M. O'Brien [b], Joe Nahra [b], Mark Morris [b], W. Howard Roark [b], Cathleen E. Hanau [a], Peter G. Ruminski [a], Jeffrey A. Scholten [a], Theresa R. Fletcher [a], Bruce C. Hamper [a], Jeffery N. Carroll [a], William C. Patt [b], Huey S. Shieh [a], Brandon Collins [a], Alexander G. Pavlovsky [b], Katherine E. Palmquist [a], Karl W. Aston [a], Jeffrey Hitchcock [a], Michael D. Rogers [a], Joseph McDonald [a], Adam R. Johnson [b], Grace E. Munie [a], Arthur J. Wittwer [a], Chiu-Fai Man [b], Steven L. Settle [a], Olga Nemirovskiy [a], Lillian E. Vickery [a], Arun Agawal [b], Richard D. Dyer [b], Teresa Sunyer [a]

[a] Global Research and Development, Pfizer Inc., 700 Chesterfield Parkway West, St. Louis, MO 63017, USA
[b] Global Research and Development, Pfizer Inc., 2800 Plymouth Road, Ann Arbor, MI 48105, USA

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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

* Corresponding author. Tel.: +1 636 247 3662; fax: +1 636 247 5400.
E-mail address: mark.e.schnute@pfizer.com (M.E. Schnute).

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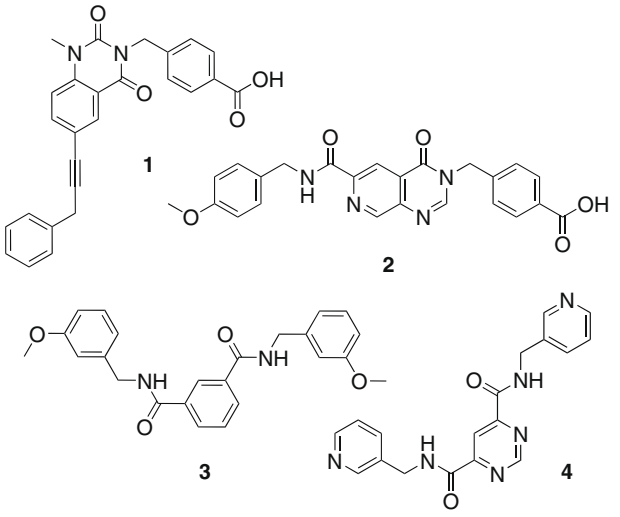
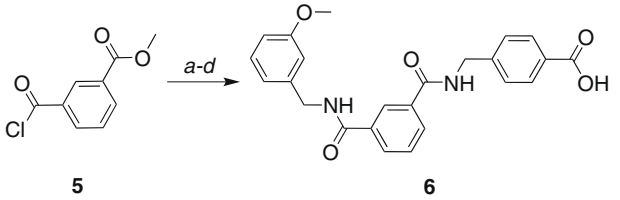


Figure 1. Reported non-zinc binding, highly selective MMP-13 inhibitors (**1**, **2**, and **4**) and monocyclic scaffold based MMP-13 inhibitor screening hit **3**.

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, $MeOH/H_2O$.

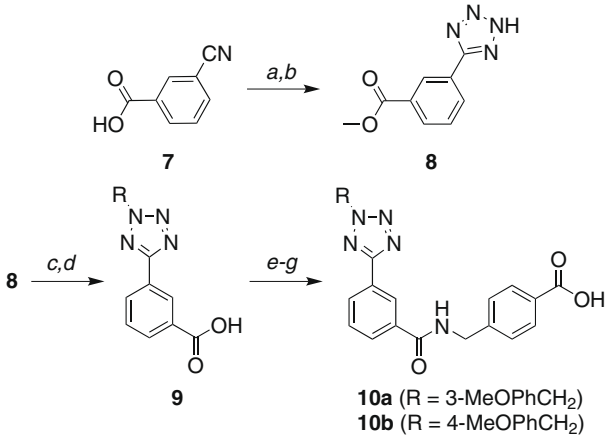
Table 1
MMP-13 inhibitory activity of amides **3**, **6**, and **10**

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

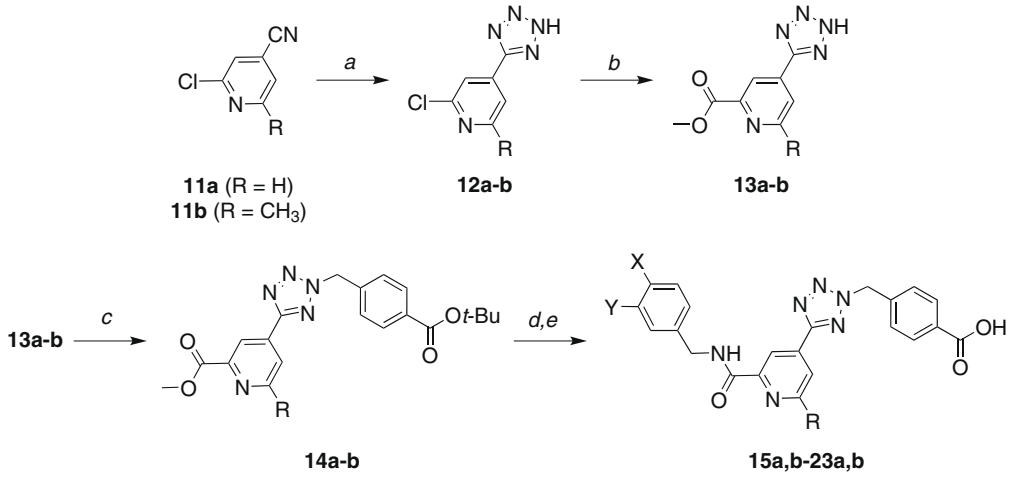
[a] Permeability × 10^{-6} cm/s (nd = not determined).

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.



Scheme 3. Synthesis of (pyridin-4-yl)-2*H*-tetrazole amides **15**. Reagents and conditions: (a) NaN_3, $Et_3N{\cdot}HCl$, toluene, reflux; (b) $Pd(OAc)_2$, DPPF, Et_3N, MeOH, CO (120 psi), 100 °C; (c) *tert*-butyl 4-(bromomethyl)benzoate, Et_3N, CH_3CN, 50 °C; (d) benzylamines, trimethyl aluminum, THF; (e) HCl/methanol or trifluoroacetic acid.

cyanopyridine **11a** to afford tetrazole **12a** which was then subjected to palladium mediated carbonylation to provide ester **13a**, Scheme 3. Alkylation of the tetrazole with *tert*-butyl 4-(bromomethyl)benzoate occurred with high regioselectivity at the 2-position to provide **14a**. Subsequent amide formation with a substituted benzylamine and ester hydrolysis provided (pyridin-4-yl)-2*H*-tetrazoles of the formula **15a**–**23a**. The 2-methylpyridine analogs **15b**–**23b** (R = Me) were also prepared through this route in which the cyanopyridine **11b** was prepared as described in Scheme 4 from pyridinyl carboxylic acid **24**. As shown in Table 2, MMP-13 inhibitory activity was maintained for the pyridinyl series with small groups at either the *meta* or *para*-positions of the benzylamide being well tolerated. Importantly, methyl group substitution on the pyridine ring as in **16b** afforded approximately a 10-fold improvement in MMP-13 potency. The most potent analog identified was the 3-methoxybenzylamide (**20b**, X = H, Y = OMe) (IC_{50} = 0.18 nM). Further increasing the size of the R substituent to *iso*-propyl, however, was detrimental to potency (IC_{50} = 1.4 μM). Compounds **15–20b** showed good CACO2 cell permeability which translated to good oral bioavailability in the rat for many analogs including the 3-methoxybenzyl analog **20b** (*F* = 59%).

Crystal structures of compounds **10b** and **17a** bound to the catalytic domain of MMP-13 are depicted in Figure 2. Consistent with previously reported binding conformations of compounds **1** and **4**, both compounds were found to bind deeply into the S_1' pocket without making contacts to the catalytic zinc atom. Notably, the position of the tetrazole ring and amide are transposed with respect to the central ring between the two structures. The tetrazole ring is proximal to the zinc atom in **10b** while distal in **17a**. In both cases the benzoic acid moiety was oriented deep into the S_1' pocket protruding under the S_1' specificity loop. Since both the tetrazole ring and amide are capable of hydrogen-bonding with Thr-245,

Table 2
MMP-13 inhibitory activity of amides **15**–**23**

Compds	X	Y	MMP-13/FL K_i[a] (nM)		CACO2[b]
			a, R = H	**b**, R = CH_3	R = CH_3
15	H	H		22.3	5.7
16	F	H	65.3	5.3	10.1
17	H	F	75.9(±21.1)	28.6(±0.35)	10.2
18	F	F	43.4	3.6	2.7
19	OCH_3	H	36.7	4.4	2.8
20	H	OCH_3	2.1(±0.22)	0.18(±0.03)	9.7
21	Cl	H	227		
22	H	Cl	18.1		
23	H	CF_3	16.5	2.5	nd

[a] Values are means of at least two experiments when standard error measurement is given in parentheses.
[b] Permeability × 10^{-6} cm/s (nd = not determined).

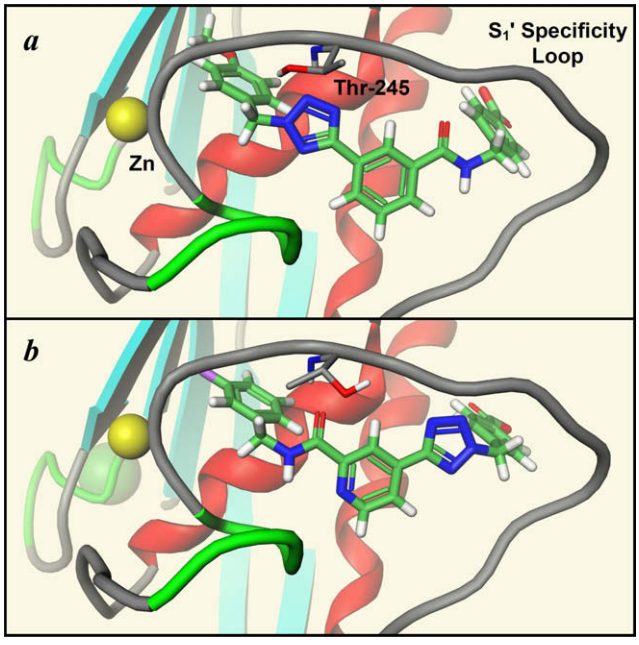
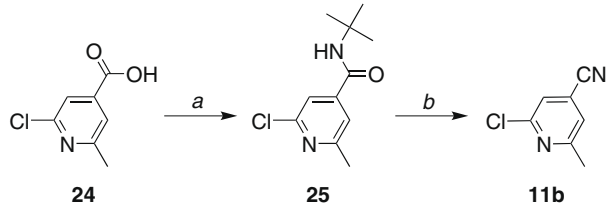
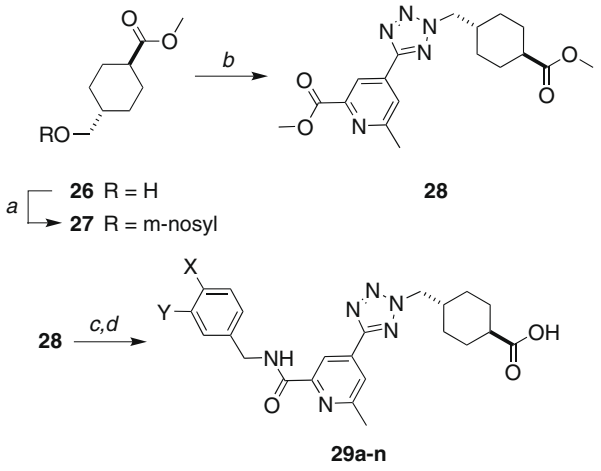


Figure 2. Co-crystal structures of MMP-13 catalytic domain and MMP-13 inhibitor (a) compound **10b**, PDB 3KEC and (b) compound **17a**, PDB 3KEJ.



Scheme 4. Synthesis of 2-chloro-6-methylisonicotinonitrile **11b**. Reagents and conditions: (a) oxalyl chloride, CH_2Cl_2; *t*-ButylNH_2; (b) $POCl_3$, toluene, reflux, 15 h.



Scheme 5. Synthesis of (pyridin-4-yl)-2*H*-tetrazole amides **29a–n**. Reagents and conditions: (a) 3-nitrobenzenesulfonyl chloride, Et_3N, CH_2Cl_2; (b) **13b**, *i*-Pr_2EtN, CH_3CN; (c) benzylamines, DMF, 60 °C, 3 days; (d) LiOH, THF/H_2O.

Table 3
MMP-13 inhibitory activity of amides **29a–n**

Compds	X	Y	MMP-13/FL K_i[a] (nM)
29a	H	H	31.3
29b	F	H	4.4(±0.15)
29c	OCH_3	H	4.2(±0.6)
29d	H	F	22.1(±2.2)
29e	H	CN	6.7
29f	H	OH	1.5
29g	H	OCH_3	0.16(±0.04)
29h	H	$OCH(CH_3)_2$	1.5
29i	F	F	7.5
29j	F	OH	1.4
29k	F	OCH_3	0.30(±0.01)
29l	F	Cl	0.26(±0.02)
29m	F	CF_3	0.62
29n	F	CH_3	0.22(±0.1)

[a] Values are means of at least two experiments when standard error measurement is given in parentheses.

the reversal of orientation appears to be governed by the preferential alignment of the carboxylic acid towards the solvent exposed region of the pocket.

In order to increase hydrophobic contacts in the tunnel region beneath the S_1' specificity loop, the benzoic acid was replaced with a trans-cyclohexyl carboxylic acid residue, Scheme 5. Alkylation of tetrazole **13b** with *m*-nosylate **27** obtained from alcohol **26** afforded preferentially (>30:1) 2-substituted tetrazole ester **28**. Amide formation followed by ester hydrolysis provided the desired carboxylic acids **29a–n**. MMP-13 inhibitory activity was found to be comparable to the phenyl analogs, Table 3. Stereochemistry of the ring substitution was found to be important since the cis-isomer of compound **29b**, prepared by an analogous route, was found to be significantly less active (K_i = 59.2 nM) than the trans-isomer. Substitution at the *meta*-position of the benzylamide was well tolerated as was fluorine substitution at the *para*-position. Compound **29b** demonstrated potent inhibition of full length MMP-13 (K_i = 4.4 nM) and exhibited exquisite selectivity (K_i >25 μM) against a set of 13 MMP enzymes,[17] TACE, ADAMTS-4, and ADAMTS-5. Compound **29b** also inhibited cytokine stimulated type II collagen degradation from human articular cartilage[18] in a dose dependent manner (IC_{50} = 2 μM). Excellent oral bioavailability was also observed in the rat (F = 100%, 1 mg/kg dose) with low clearance (14 mL/min/kg, $T1/2_{Eff}$ = 2.8 h).

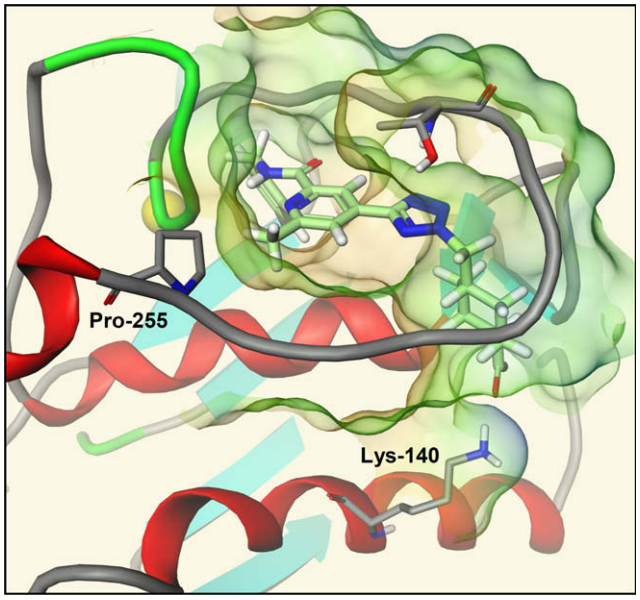


Figure 3. Co-crystal structures of MMP-13 catalytic domain and MMP-13 inhibitor compound **29b** (PDB 3KEK).

The co-crystal structure of compound **29b** and the catalytic domain of MMP-13 showed **29b** in a similar conformation to that found for **17a** with the tetrazole ring distal to the zinc atom, Figure 3. The cyclohexane ring protrudes beneath the S_1' specificity loop with the terminal carboxylic acid contacting Lys-140. The methyl substituent on the pyridine ring which significantly improved MMP-13 inhibition was found to fill a hydrophobic region in the southern portion of the S_1' pocket. The methyl group buttresses against Pro-255 further enforcing the orientation of the tetrazole substituent through the tunnel region beneath the specificity loop.

Compound **29b** was evaluated in vivo for its ability to inhibit type II collagen neoepitope (TIINE),[19] a biomarker of cartilage degradation, and its ability to prevent cartilage lesions in a preclinical rat medial meniscus tear (MMT) model of osteoarthritis.[20] Compound **29b** was dosed orally to MMT operated rats 10 days post surgery, when cartilage lesions were forming and TIINE was elevated in tissue and synovial fluid. The effect of compound **29b** on synovial fluid TIINE was evaluated four hours after dosing, Figure 4.[21] A dose dependent inhibition of TIINE levels was observed affording 55% inhibition at a dose of 30 mg/kg. Exposure levels of **29b** in the synovial fluid lavage were 0.05, 0.36, 3.89 μM (n = 4) for the 3, 10 and 30 mg/kg dose groups, respectively. In order to evaluate the ability of the MMP-13 inhibitor to prevent cartilage lesions, compound **29b** was dosed orally to MMT operated rats for 28 days (50 mg/kg, bid) post surgery.[22] A statistically significant

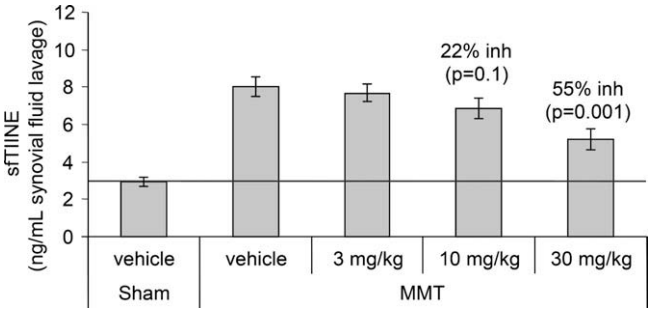


Figure 4. TIINE in synovial fluid of rat knees 10 days post-operative MMT and 4 h post dose of **29b** (n = 8).

21% (SEM ± 6%, $p = 0.02$) reduction in the medial tibial cartilage degradation score was observed upon histological evaluation of the knee joints compared to vehicle control animals.

Hit to lead optimization of phenyl dicarboxamide **3** has identified highly selective and orally-bioavailable MMP-13 inhibitors based upon a (pyridin-4-yl)-2*H*-tetrazole scaffold. The inhibitors bind deeply within the MMP-13 S_1' pocket and do not interact with the catalytic zinc atom as determined by co-crystal structure analysis. Compound **29b** upon oral dosing was found to inhibit production of type II collagen neoepitope (TIINE), a biomarker of cartilage degradation, and afforded cartilage protection in a preclinical rat osteoarthritis model. These findings further support the role of MMP-13 in the progression of osteoarthritis disease and suggests new templates for the design of pharmacologically active agents to treat the disease.

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14. MMP-13/FL activity and MMP selectivity assays were performed using a fluorogenic substrate, Mca-Arg-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-Glu-Arg-NH_2 except in the case of MMP-3/CD where the assay employed Mca-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH_2 as the substrate: Tortorella, M. D.; Tomasselli, A. G.; Mathis, K. J.; Schnute, M. E.; Woodard, S. S.; Munie, G.; Williams, J. M.; Caspers, N.; Wittwer, A. J.; Malfait, A.-M.; Shieh, H.-S. *J. Biol. Chem.* **2009**, *284*, 24185.
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17. Compound **29b** was evaluated in enzyme assays utilizing full length MMP-1, MMP-2, MMP-9, and MMP-12 or catalytic domain MMP-3, MMP-7, MMP-8, MMP-14, MMP-15, MMP-16, MMP-24, MMP-25, and MMP-26.
18. Articular cartilage degradation assay was performed in OA human cartilage explant cultures as described in Ref. 23. Cytokine cocktail contained 0.1 ng/mL IL-1β + 50 ng/mL oncostatin M, and type II collagen degradation was quantified using a TIINE assay in the explant culture conditioned media as described in Ref. 19a.
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21. TIINE and drug levels were measured in the synovial fluid lavage obtained by injecting 100 μl of saline into the rat knee and withdrawing fluid from the synovial space following repeated flexion of the joint (approximately 20-fold dilution). Rat MMP-13/FL $K_i = 2.4$ nM, rat plasma protein binding = 95%.
22. Rat MMT model was performed as described in Ref. 20b. Male Sprague Dawley rats (12 animals per group) were dosed with vehicle or compound **29b** beginning one day after surgery and then for a period of 28 days. Plasma concentrations of **29b** ($n = 3$ animals) fluctuated from 41 to 289 μM, but the mean was very similar throughout the dosing period when evaluated at day 21: 123, 263, 114, 274, 90 and 196 μM ($t = 0.5$, 1, 2, 4, 8 and 12 h of dosing). Cartilage lesions were scored by a certified pathologist at Bolder BioPath (Boulder, CO). The Pfizer institutional animal care and use committee reviewed and approved the animal use in these studies. The animal care and use program is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.
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