MedChemComm

Accepted Manuscript

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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

Characterization of a selective inhibitor for matrix metalloproteinase-8 (MMP-8)

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

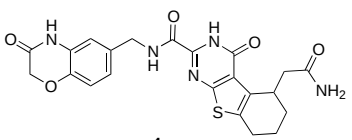
MMP-8 has been implicated in various diseases. Selective MMP-8 inhibitors are needed to ascertain the role of this enzyme. We synthesized two inhibitors reported previously as selective for MMP-8. Compound 1 selectively inhibited MMP-8 and MMP-13; compound 2 was a potent broad-spectrum inhibitor, notwithstanding that it is used as selective.

Matrix metalloproteinases (MMPs) constitute a family of 26 enzymes that perform important functions in restructuring the extracellular matrix environment.[1] The roles of these enzymes in the course of many physiological processes are well documented.[2] However, one or more of the MMPs might also be expressed in the course of various pathological events.[3-6] Indeed, the expression of MMPs in either physiological or pathological conditions is highly regulated. These enzymes are expressed as zymogens, which need to be activated by proteolysis.[7] The active enzymes are further regulated by their complex formation with tissue inhibitors of matrix metalloproteinases (TIMPs), an association that inhibits the activity of the enzymes. It is the uncomplexed activated MMP that can play a biological role in the living organism.

As it pertains to the pathological condition, these enzymes are worthy targets for inhibition by small-molecule inhibitors. However, broad-spectrum inhibition of MMPs, as explored in investigations of cancer, has not borne fruit. Aside from the multiplicity of these enzymes, the situation is complicated by the fact that some MMPs might play salutatory effects in the given disease, whereas others might play detrimental (pathological) roles. When multiples of these enzymes are expressed, the study becomes complicated.

We have used selective inhibitors of MMPs to great advantage in elucidation of the roles of specific MMPs in various diseases.[8-13] These selective agents behave as "surgical" tools that arrest the functions of specific enzymes at the precise time points in the progression of the disease. A comparable experimental control is not possible with the use of MMP-knockout animals, as the knockout organism will lack the given MMP from the moment of the fertilization of the egg. Moreover, gene ablation of a particular MMP might result in compensatory increases in other MMPs, which might exhibit some overlap in their activities. Hence, small-molecule inhibitors that exhibit high selectivity in inhibition of a targeted MMP are keenly needed for use in wild-type organisms.

MMP-8 has been implicated in inflammation,[14] cancer progression,[15] and wound repair.[16, 17] In this vein, we have had a need for a selective MMP-8 inhibitor for use in our investigations of these mechanisms. A search of the literature identified two inhibitors, compounds **1** and **2**, which have been billed and used as selective toward inhibition of MMP-8. We synthesized these compounds by a variation of methods that have been reported for the preparation of each, one in six steps and the other in four steps. The breadth of activity of these two inhibitors was



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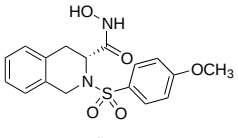

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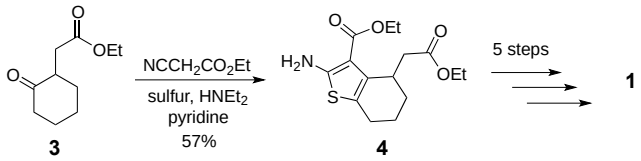
evaluated *in vitro* to explore the degree of selectivity of each in inhibition of representative MMPs in assessment of the usefulness of the inhibitors for *in vivo* work in animal models.

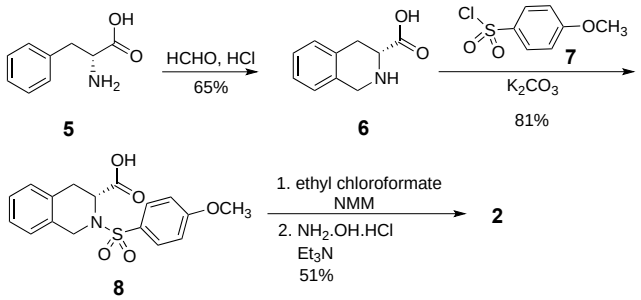
The synthesis of compound **1** has been reported using a linear sequence of seven steps from the commercially available ethyl 2-oxo-cyclohexylacetate (**3**) in an overall yield of 6%.[18] Our initial attempts to synthesize ethyl 2-amino-4-ethoxycarbonylmethyl-4,5,6,7-tetrahydrobenz[*b*]thiophene-3-carboxylate (**4**) followed the reported two-step sequence.[18] This process did not work well in our hands as the conditions were harsh and led to the formation of side products, which proved difficult to separate from the desired product. The yield hence was low. In order to overcome these difficulties, we devised the one-step transformation of **3** to **4**, which furnished the product in 57% yield. As depicted in Scheme 1, compound **3** was treated with ethyl cyanoacetate and sulfur in pyridine in the presence of diethylamine at room temperature. Compound **4** was obtained in 57% after column purification. With this intermediate in hand, the target molecule was then synthesized following the earlier procedure in five additional steps.[18] This synthesis was accomplished in six steps with an overall yield of 10%.

The synthesis of compound **2** has been given only as a scheme in the literature and the detailed procedures were not provided.[19] Based on the reported synthetic route,[19] we devised a slight

modification of it to arrive at the target molecule. As outlined in Scheme 2, the synthesis started from (*R*)-phenylalanine (**5**). Compound **6**, which was prepared according to the literature,[20] was used for the next step directly to afford compound **8** by the literature method.[21] With **8** in hand, the initial attempts to prepare compound **2** followed a two-step procedure involving activation of the acid by thionyl chloride and subsequent treatment with hydroxylamine hydrochloride. However, TLC analysis indicated the presence of a complex mixture from which it was difficult to purify the desired product. We resorted to a different method. Compound **8** was used to form a mixed anhydride by the reaction with ethyl chloroformate in the presence of NMM, which was allowed to react with hydroxylamine hydrochloride using triethylamine as base to afford the final product in good yield. In summary, this four-step sequence furnished the desired compound **2** with an overall yield of 27%.



Scheme 1. Synthetic scheme for the preparation of compound **1**.



Scheme 2. Synthetic scheme for the preparation of compound **2**.

Compound **1** was a selective competitive inhibitor of MMP-8 and MMP-13, with K_i values of 25 ± 2 nM and 0.54 ± 0.30 nM, respectively (Table 1). Inhibition of MMP-1, -2, -3, -7, -9, and MMP-14 was poor, with dissociation constants (K_i) in the micromolar range and at least 120-fold greater than that of MMP-8. The K_i values for compound **1** had not been reported previously; however the reported IC_{50} values were 7.4 nM for MMP-8, <25 nM for MMP-13, >2500 nM for MMP-3, and >10000 nM for MMP-1, -2, -7, -9, and -14.[18]

On the other hand, compound **2** strongly inhibited all the tested MMPs in a competitive manner, with K_i values in the nanomolar range for MMP-1, -3, and -7 and in the picomolar range for MMP-2, -8, -9, -13, and -14 (Table 1), for which it is as a tight-binding inhibitor. The enzyme kinetics for compound **2** have not been reported previously according to a search of the literature, except for that of MMP-8 for which an IC_{50} value of 4 nM was indicated.[19] We have since learned that compound **2** is being marketed by several commercial entities as a selective MMP-8 inhibitor. According to our results, although this compound is exceptionally potent, it is not selective for any MMP.

Table 1. Kinetic Parameters for Inhibition of MMPs

MMP	Compound 1	Compound 2
	K_i (nM)	K_i (nM)
MMP-1*	24000 ± 3000	6.8 ± 0.3
MMP-2	3000 ± 200	0.10 ± 0.01
MMP-3*	17000 ± 2000	1.2 ± 0.3
MMP-7	53000 ± 6000	135 ± 26
MMP-8*	25 ± 2	0.6 ± 0.1
MMP-9*	76000 ± 8000	0.37 ± 0.02
MMP-13	0.53 ± 0.30	0.023 ± 0.008
MMP-14*	45000 ± 4000	0.22 ± 0.04

*Catalytic domain

Conclusions

Of the two compounds reported as selective MMP-8 inhibitors, only compound **1** exhibits selectivity in inhibition of MMP-8 and MMP-13. This compound is not commercially available. However, compound **2** is currently commercially available and is marketed as a selective MMP-8 inhibitor. Compound **2** has been used in several studies to ascertain the role of MMP-8 in the pathology of diseases. Using this inhibitor, MMP-8 was indicated to play a role in bacterial meningitis by preventing detachment of infected human brain microvascular endothelial cells and restoring blood-brain barrier permeability.[22] Compound **2** was evaluated in mice with experimental autoimmune encephalomyelitis, an animal model for multiple sclerosis, and found to reduce the severity of the disease.[23] The use of inhibitor **2** was instrumental in implicating MMP-8 in fibrocyte migration.[24] Compound **2** was found to decrease ventilator-induced lung injury in mice,[25] which led to the conclusion that MMP-8 promotes acute inflammation after ventilator-induced lung injury. In light of our results indicating the broad spectrum of activity for inhibition of MMPs by compound **2**, we regretfully conclude that the mechanistic role of MMP-8 in the pathology of these diseases might not have been established.

Notes and references

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† Electronic Supplementary Information (ESI) available: syntheses of compounds **1** and **2** and enzyme inhibition. See DOI: 10.1039/b000000x/

1 A. Name, B. Name and C. Name, *Journal Title*, 2000, **35**, 3523; A. Name, B. Name and C. Name, *Journal Title*, 2000, **35**, 3523.

1. A. Page-McCaw, A. J. Ewald and Z. Werb, *Nat Rev Mol Cell Biol*, 2007, **8**, 221-233.
2. T. H. Vu and Z. Werb, *Genes Dev*, 2000, **14**, 2123-2133.
3. E. Dejonckheere, R. E. Vandenbroucke and C. Libert, *Drug Discov Today*, 2011, **16**, 762-778.
4. C. Gialeli, A. D. Theocharis and N. K. Karamanos, *FEBS J*, 2011, **278**, 16-27.
5. W. C. Parks, C. L. Wilson and Y. S. Lopez-Boado, *Nat Rev Immunol*, 2004, **4**, 617-629.
6. V. W. Yong, S. M. Agrawal and D. P. Stirling, *Neurotherapeutics*, 2007, **4**, 580-589.
7. R. Visse and H. Nagase, *Circ Res*, 2003, **92**, 827-839.

8. R. D. Bonfil, A. Sabbota, S. Nabha, M. M. Bernardo, Z. Dong, H. Meng, H. Yamamoto, S. R. Chinni, I. T. Lim, M. Chang, L. C. Filetti, S. Mobashery, M. L. Cher and R. Fridman, *Int J Cancer*, 2006, **118**, 2721-2726.
9. J. Cui, S. Chen, C. Zhang, F. Meng, W. Wu, R. Hu, O. Hadass, T. Lehmidi, G. J. Blair, M. Lee, M. Chang, S. Mobashery, G. Y. Sun and Z. Gu, *Mol Neurodegen*, 2012, **7**, doi:10.1186/1750-1326-1187-1121.
10. Z. Gu, J. Cui, S. Brown, R. Fridman, S. Mobashery, A. Y. Strongin and S. A. Lipton, *J Neurosci*, 2005, **25**, 6401-6408.
11. D. H. Kim, C. Lilliehook, B. Roides, Z. Chen, M. Chang, S. Mobashery and S. A. Goldman, *J Neurosci*, 2008, **28**, 208-216.
12. A. Kruger, M. J. Arlt, M. Gerg, C. Kopitz, M. M. Bernardo, M. Chang, S. Mobashery and R. Fridman, *Cancer Res*, 2005, **65**, 3523-3526.
13. R. Ota, C. Kurihara, T. L. Tsou, W. L. Young, Y. Yeghiazarians, M. Chang, S. Mobashery, A. Sakamoto and T. Hashimoto, *J Cereb Blood Flow Metab*, 2009, **29**, 1547-1558.
14. P. Van Lint and C. Libert, *Cytokine Growth Factor Rev*, 2006, **17**, 217-223.
15. A. Gutierrez-Fernandez, A. Fueyo, A. R. Folgueras, C. Garabaya, C. J. Pennington, S. Pilgrim, D. R. Edwards, D. L. Holliday, J. L. Jones, P. N. Span, F. C. Sweep, X. S. Puente and C. Lopez-Otin, *Cancer Res*, 2008, **68**, 2755-2763.
16. E. Pirila, J. T. Korpi, T. Korkiamaki, T. Jahkola, A. Gutierrez-Fernandez, C. Lopez-Otin, U. Saarialho-Kere, T. Salo and T. Sorsa, *Wound Repair Regen*, 2007, **15**, 47-57.
17. M. Gooyit, Z. Peng, W. R. Wolter, H. Pi, D. Ding, D. Hesek, M. Lee, B. Boggess, M. M. Champion, M. A. Suckow, S. Mobashery and M. Chang, *ACS Chem Biol*, 2013.
18. G. Pochetti, R. Montanari, C. Gege, C. Chevrier, A. G. Taveras and F. Mazza, *J Med Chem*, 2009, **52**, 1040-1049.
19. H. Matter, M. Schudok, W. Schwab, W. Thorwart, D. Barbier, G. Billen, B. Haase, B. Neises, K. Weithmann and T. Wollmann, *Bioorg Med Chem*, 2002, **10**, 3529-3544.
20. A. P. Kozikowski, D. Ma, Y. P. Pang, P. Shum, V. Likic, P. K. Mishra, S. Macura, A. Basu, J. S. Lazo and R. G. Ball, *J Am Chem Soc*, 1993, **115**, 3957-3965.
21. H. Matter, M. Knauf, W. Schwab and E. F. Paulus, *J Am Chem Soc*, 1998, **120**, 11512-11513.
22. A. Schubert-Unkmeir, C. Konrad, H. Slanina, F. Czapek, S. Hebling and M. Frosch, *PLoS Pathog*, 2010, **6**, e1000874.
23. A. R. Folgueras, A. Fueyo, O. Garcia-Suarez, J. Cox, A. Astudillo, P. Tortorella, C. Campestre, A. Gutierrez-Fernandez, M. Fanjul-Fernandez, C. J. Pennington, D. R. Edwards, C. M. Overall and C. Lopez-Otin, *J Biol Chem*, 2008, **283**, 9465-9474.
24. C. Garcia-de-Alba, C. Becerril, V. Ruiz, Y. Gonzalez, S. Reyes, J. Garcia-Alvarez, M. Selman and A. Pardo, *Am J Respir Crit Care Med*, 2010, **182**, 1144-1152.
25. G. M. Albaiceta, A. Gutierrez-Fernandez, E. Garcia-Prieto, X. S. Puente, D. Parra, A. Astudillo, C. Campestre, S. Cabrera, A. Gonzalez-Lopez, A. Fueyo, F. Taboada and C. Lopez-Otin, *Am J Respir Cell Mol Biol*, 2010, **43**, 555-563.

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