Selective Inhibition of Matrix Metalloproteinase Isozymes and in Vivo Protection against Emphysema by Substituted γ-Keto Carboxylic Acids

Dawei Ma,*,† Yongwen Jiang,†,# Fangping Chen,‡,# Li-kun, Gong,‡ Ke Ding,† Yong Xu,† Renxiao Wang,† Aihua Ge,† Jin Ren,*,‡ Jingya Li,§ Jia Li,§ and Qizhuang Ye§

State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Lu, Shanghai 200032, China, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Lu, Shanghai 201203, China, and Chinese National Center for Drug Screening, Shanghai Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, 189 Guo-Shou-Jing Road, Shanghai 201203, China

Received November 1, 2005

Abstract: The synthesis and matrix metalloproteinase (MMP) inhibitory activity of a series of γ-keto carboxylic acids are described. Among nine MMP isozymes tested, compound **1j** displays selective inhibition of MMP-2, -9, and -12 with IC_{50} values between 0.20 and 1.51 μM, and in male golden Syrian hamsters, it shows protection against PPE-induced emphysema.

There is increasing evidence[1−3] that some matrix metalloproteinases, particularly macrophage elastase (MMP-12), play an important role in the progression of emphysema, a disease caused by prolonged exposure to particles and gases such as those in tobacco smoke.[4] The earliest direct observation for this hypothesis was reported by Hautamaki et al.[2] who found that wild-type mice possessing the metalloelastase gene (MMP-$12^{+/+}$) developed emphysema after chronic exposure to cigarette smoke, while mice lacking this gene (MMP-$12^{-/-}$) did not. Recently, it was proposed that MMP-12 mediates smoke-induced inflammation by releasing TNF-α from macrophages with subsequent endothelial activation, neutrophil influx, and proteolytic matrix breakdown caused by neutrophil-derived proteases.[4] Accordingly, it is expected that selective inhibitors for MMP-12 should serve as novel therapeutic agents for treatment of chronic obstructive pulmonary disease (COPD). However, to the best of our knowledge, there have appeared no reports regarding in vivo protection against emphysema by selective MMP-12 inhibitors although much effort has in recent years been directed to the development of selective MMP inhibitors.[5,6] In studies toward selective MMP inhibitors,[7] we have shown that some substituted γ-keto acids inhibit MMP-12 selectively and display promising in vivo protection against emphysema.

During the past decade, a variety of different structural classes of MMP inhibitors have been discovered using different methods including structure-based design and combinatorial chemistry.[5a] From these results, it can be seen that the requirements for a molecule to be an effective inhibitor of MMPs include a functional group, such as a carboxylic acid, hydroximic acid, or sulfhydryl, capable of chelating the active-site Zn(II) ion (zinc binding group, ZBG), at least one functional group that provides a hydrogen bond interaction with the enzyme backbone, and one or more side chains that undergo effective van der Waals interactions with subsites of the enzyme. Carboxylates, represented by compound **6**[8] (Figure 1), have been shown to be effective MMP inhibitors. In light of these studies, we have designed and synthesized a series of γ-keto carboxylic acids **1** in which the γ-oxo-benzenebutanoic acid skeleton was retained to maintain the necessary Zn(II)-binding group and substituents at either the 4-position of the benzene ring or the β-position of the carboxyl group were introduced to provide van der Waals interactions with the enzyme. It was hoped to identify some structurally novel compounds with higher inhibitory activity and selectivity.

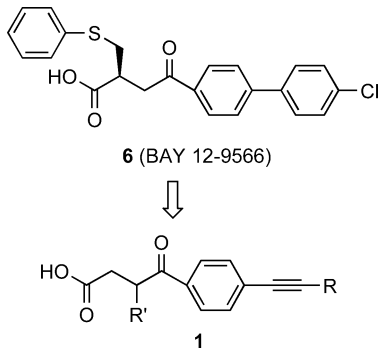

Figure 1. Design of a new class of γ-keto acids as selective MMP inhibitors.

The synthetic pathways to the target molecules **1** are shown in Scheme 1. The key intermediates **4** were obtained by two routes. One was direct esterification of **2**, a Friedel−Crafts reaction product of bromobenzene with succinic anhydride, to afford **4g**. The other is alkylation of aryl ketones **3** with ethyl bromoacetate mediated by LDA. The ketones **3a**−**c** and **3f** were prepared by a direct Friedel−Crafts reaction of bromobenzene with a suitable acyl chloride, while **3d** and **3e** were prepared by a Wittig reaction of a 2,4′-dibromoacetophenone-derived ylide with an aromatic aldehyde followed by hydrogenation. Sonogashira reaction of esters **4** with different 1-alkynes catalyzed by Pd/CuI gave the coupling products **5**. Upon treatment with aqueous NaOH in methanol, these esters were hydrolyzed to furnish the target compounds **1**.

The synthetic γ-keto carboxylic acids **1** were evaluated as MMP inhibitors using galardin as a standard as described in our previous paper.[7] As summarized in Table 1, when R was an aliphatic substituent, the resultant compounds **1a**−**d** showed weak inhibition of MMP-2 and MMP-12. Introduction of an alkyl group at the α-position of the ketone moiety slightly enhanced the potency (compare **1c** with **1d**). The potency was further increased by about 10-fold by switching the alkyl substituents of the alkyne moiety to aryl (compare **1d** to **1e**). Better selectivity for MMP-12 over MMP-2 was seen when R′ was a larger group (compare **1e** with **1f**−**h**). It is noteworthy that **1j** showed the highest potency toward MMP-12, indicating that replacing the phenyl group in **1g** with a 4-chlorophenyl group led to increased inhibitory activity. Although this compound had considerable potency for MMP-2 and MMP-9, it did not show activity against MMP-1, MMP-7, MMP-14,

* To whom correspondence should be addressed. For D. Ma: phone 86-21-54925130; fax 86-21-64166128; e-mail madw@mail.sioc.ac.cn. For J. Ren: phone 86-21-50806031; fax 86-21-50807088; e-mail: jren@mail.shcnc.ac.cn.
† Shanghai Institute of Organic Chemistry.
‡ Shanghai Institute of Meteria Medica.
§ Chinese National Center for Drug Screening.
# These authors contributed equally to this work.

10.1021/jm051101g CCC: $33.50
Published on Web 12/21/2005

Scheme 1

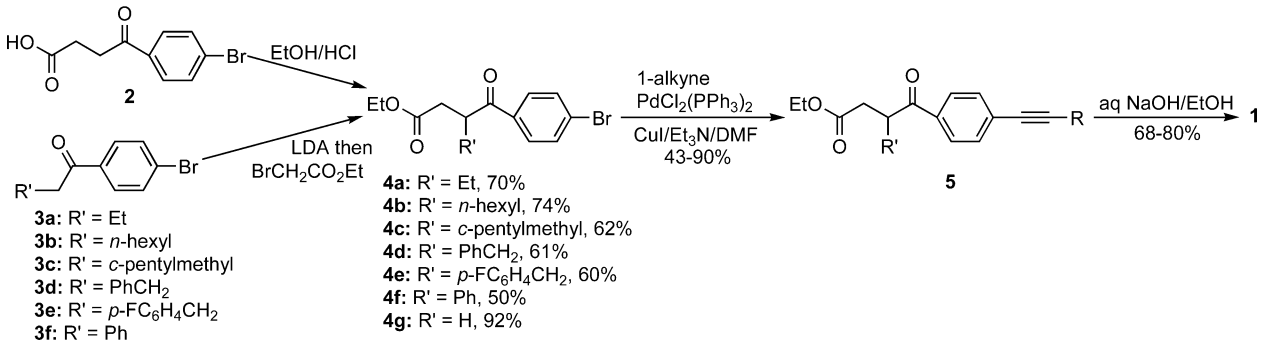

Table 1. IC_{50} Values for Inhibition of MMPs by Compounds **1** (μM)

compound	MMP-1	MMP-2	MMP-7	MMP-9	MMP-12	MMP-14	MMP-15	MMP-16	MMP-26
1a (R = CH_2OH, R′ = H)	*a*	15.37	*a*	*a*	24.69	*a*	*a*	*a*	*a*
1b (R = *n*-C_8H_{17}, R′ = H)	*a*	3.07	*a*	*a*	12.48	*a*	*a*	*a*	*a*
1c (R = CH_2OBn, R′ = H)	*a*	23.10	*a*	*a*	25.74	*a*	*a*	*a*	N.A.
1d (R = CH_2OBn, R′ = Et)	*a*	5.74	*a*	*a*	7.56	*a*	*a*	*a*	*a*
1e (R = Ph, R′ = Et)	*a*	0.44	*a*	3.60	0.48	30.60	13.94	32.04	18.07
1f (R = Ph, R′ = *n*-C_6H_{13})		1.66		5.41	0.45				
1g (R = Ph, R′ = *c*-pentylmethyl)		1.12		3.39	0.32				
1h (R = Ph, R′ = Bn)		2.11		9.29	0.60				
1i (R = Ph, R′ = 4-$FC_6H_4CH_2$)		3.43		10.03	1.14				
1j (R = 4-ClC_6H_4, R′ = *n*-C_6H_{13})	*a*	0.55	*a*	1.51	0.20	*a*	*a*	*a*	*a*
galardin	0.006	0.007		0.015	0.013	0.023	0.006	0.008	0.017

[a] No activity up to 50 μM.

MMP-15, MMP-16, and MMP-26 and could be considered as a relatively selective MMP inhibitor.

To rationalize the structure−activity relationship of our compounds, we have analyzed the three-dimensional structures of MMPs that are available in the Protein Data Bank.[9] Regarding the formation and the shape of their S1′ pockets, MMPs can be roughly clustered into three groups. As illustrated in Figure 2, the S1′ pockets on MMP-1 and MMP-7 are short. In contrast, the S1′ pockets on MMP-2, MMP-9, and MMP-12 are relatively long and wide. The characteristics of the S1′ pockets on MMP-14 and MMP-16 are more elusive since they could be very restricted (as on MMP-16) or a bit more open (as on MMP-14). In fact, MMP-14, MMP-15, and MMP-16 share a high sequence homology and form a subfamily of membrane-type matrix metalloproteinases (MT-MMPs). The orientation of one flexible glutamine residue, which locates at the middle of the S1′ pocket, largely determines the openness of this pocket. But in general, the S1′ pockets on MMP-14 and MMP-16 can be considered to be quite distinctive from their counterparts on MMP-2, MMP-9, and MMP-12. So, if there is a large alkynyl group at the 4-position of the phenyl ring (Table 1), such a compound can only be well-accommodated by MMP-2, MMP-9, and MMP-12. This may explain the high selectivity of this series of compounds to MMP-2, MMP-9, and MMP-12 over other subtypes. In addition, we found in our molecular docking results that the alkynyl groups at the 4-position on the phenyl ring can extend into the bottom of the S1′ pocket, forming favorable hydrophobic contacts with residues Ala216, Val217, Ala234, Val235, Met236, Phe237, Pro238, Tyr240, Tyr242, Val243, and Phe248 (as numbered on MMP-12, PDB entry 1JK3). This may explain the difference in inhibitory activity exhibited by different R groups. The most potent compound in this series, that is, compound **1j**, has a chlorine atom at the very end of the R group (Table 1). Our molecular docking results suggested that its potency may be due to the extra polar interaction formed between this chlorine atom and one polar residue at the bottom of S1′ pocket (Lys241, Thr227, and Arg424 in MMP-12, MMP-2, and MMP-9, respectively).

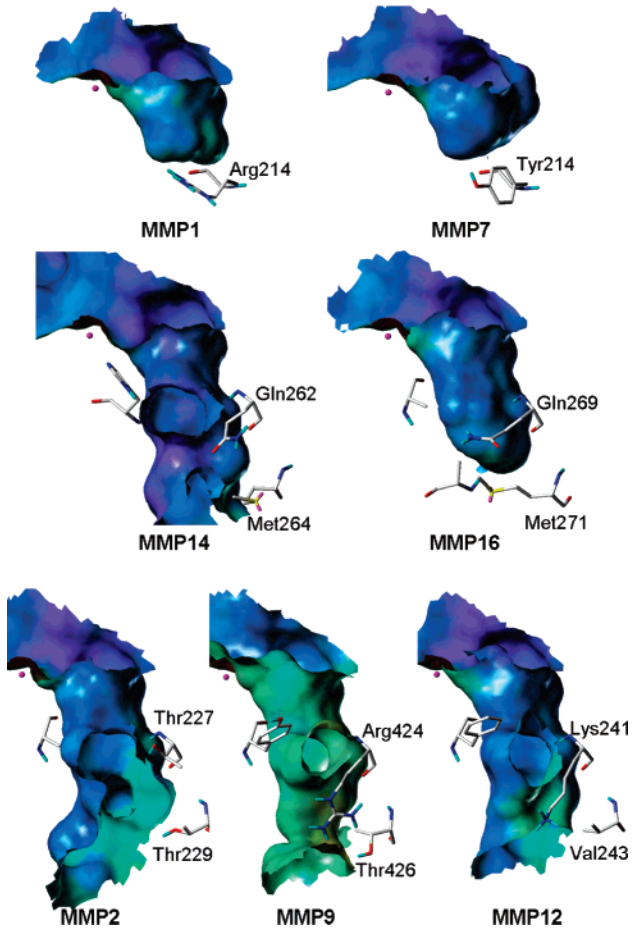

Figure 2. S1′ pocket of MMPs generated by the MOLCAD program encoded in Sybyl 6.8.[8] Key residues determining the size and the shape of the binding pocket are labeled. The structures of MMPs were retrieved from the RCSB Protein Data Bank.[9] The PDB entries used for making these figures are 1HFC (MMP-1), 1QIB (MMP-2), 1MMQ (MMP-7), 1GKC (MMP-9), 1JK3 (MMP-12), 1BUV (MMP-14), and 1RM8 (MMP-16).

Table 2. Effect of **1j** on Wet Weight of Left Lung, V_L, LV and RVHR in Hamster

group	*n*	weight of left lung (g)	V_L (mL)	LV (mL/g)	RVHR
saline	5	0.188 ± 0.011	1.45 ± 0.78	1.20 ± 0.48	233.31 ± 25.39
PPE	5	0.240 ± 0.030^{b}	2.28 ± 0.21^{b}	2.39 ± 0.45^{b}	302.49 ± 10.73^{b}
1j/PPE	5	$0.203 \pm 0.018^{a,c}$	$1.96 \pm 0.57^{b,c}$	$1.82 \pm 0.38^{a,c}$	$255.00 \pm 22.73^{a,c}$
1j/saline	5	0.196 ± 0.014^{c}	1.07 ± 0.74^{c}	1.14 ± 0.50^{c}	237.10 ± 30.22^{c}

^{a} $p < 0.05$. ^{b} $p < 0.01$ vs Saline group. ^{c} $p < 0.01$ vs PPE groups.

It is known that emphysema can be induced in hamsters by the intratracheal instillation of porcine pancreatic elastase (PPE).[10] This model was therefore used to examine the in vivo activity of our selective MMP-12 inhibitor **1j**. Forty male golden Syrian hamsters were divided into four groups, which were treated with saline, PPE (400 IU/kg), PPE/**1j** (400 IU/kg and 5 mg/(kg·day)) and saline/**1j** (5 mg/(kg·day)), respectively (**1j** was injected intraperitoneally for 28 days after saline or PPE was instilled intratracheally). After 28 days, the hamsters were killed and their lungs were examined by measuring the wet weight of left lung, lung volumes (V_L), relative lung volumes (LV, LV = V_L/body weight $\times 10^2$), and right ventricular hypertrophy (RVH, RVH = right ventricle/(left ventricle + interventricular septum) $\times 10^3$). As indicated in Table 2, all these parameters increased significantly for the PPE-treated group in comparison with the parameters from the saline-treated control hamsters. This is consistent with those results reported previously and clearly shows emphysema to have been produced in PPE-treated hamsters. This trend was inhibited greatly by **1j**, as evidenced by the lower wet weight of left lung, lung volumes, relative lung volumes, and right ventricular hypertrophy observed in PPE/**1j**-treated hamsters when compared with PPE-treated hamsters. It should be noted that the data from **1j**/saline treated hamsters are close to those from saline-treated control hamsters. Furthermore, morphometric results in the PPE/**1j**-treated group imply that **1j** tends to protect the integrity of the alveolar septal walls and protect elastic fibers from cleavage. Histologic data in the hamster revealed that **1j** significantly protects against lung hemorrhage on the first day after PPE is injected intratracheally. From these results, it is concluded that **1j** provides in vivo protection against emphysema.

In conclusion, we have found that some γ-keto carboxylic acids are relatively selective inhibiters of MMP-12. The most potent compound in this series also displayed promising in vivo protection against PPE-induced emphysema in male golden Syrian hamsters. This result provides additional evidence for involvement of MMP-12 in development of emphysema and should stimulate further exploration of MMP-12 as a target for treatment of emphysema.

Acknowledgment. The authors are grateful to the Chinese Academy of Sciences, National Natural Science Foundation of China (Grants 20321202 and 20132030), and Science and Technology Commission of Shanghai Municipality (Grants 02JC14032 and 05DZ19334) for their financial support.

Supporting Information Available: Experimental procedure for the preparation of compounds **1** and pharmacological studies of the compounds **1j**. This material is available free of charge via the Internet at http://pubs.acs.org/.

References

(1) For a review, see: Belvisi, M. G.; Bottomley, K. M. The Role of Matrix Metallopropeinases (MMPs) in the Pathophysiology of Chronic Obstructive Pulmonary Diease (COPD): a Therapeutic Role for Inhibitors of MMPs. *Inflammation Res*. **2003**, *52*, 95−100.

(2) Hautamaki, R. D.; Kobayashi, D. M.; Senior, R. M.; Shapiro, S. D. Requirement for Macrophage Elastase for Cigarette Smoke-Induced Emphysema in Mice. *Science* **1997**, *277*, 2002−2004.

(3) For a review, see: Buhl, R.; Farmer, S. G. Current and Future Pharmacologic Therapy of Exacerbations in Chronic Obstructive Pulmonary Disease and Asthma. *Proc. Am. Thorac. Soc*. **2004**, *1*, 136−142.

(4) Churg, A.; Wang, R. D.; Tai, H.; Wang, X.; Xie, C.; Dai, J.; Shapiro, S. D.; Wright, J. L. Macrophage Metalloelastase Mediates Acute Cigarette Smoke-Induced Inflammation via Tumor Necrosis Factor-a Release. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 1083−1089.

(5) For a review, see: (a) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. Design and Therapeutic Application of Matrix Metalloproteinase Inhibitors. *Chem. Rev*. **1999**, *99*, 2735−2776. For some recent examples of the development of selective MMP inhibiors, see: (b) Li, J.; Rush, T. S.; Li, W.; DeVincentis, D.; Du, X.; Hu, Y.; Thomason, J. R.; Xiang, J. S.; Skotnicki, J. S.; Tam, S.; Cunningham, K. M.; Chockalingam, P. S.; Morris, E. A.; Levin, J. I. Synthesis and SAR of Highly Selective MMP-13 Inhibitors. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 4961−4966. (c) Dublanchet, A.-C.; Ducrot, P.; Andrianjara, C.; O'Gara, M.; Morales, R.; Compère, D.; Denis, A.; Blais, S.; Cluzeau, P.; Courté, K.; Hamon, J.; Moreau, F.; Prunet, M.-L.; Tertre, A. Structure-Based Design and Synthesis of Novel Non-Zinc Chelating MMP-12 Inhibitors. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 3787−3790. (d) Kim, S.-H.; Pudzianowski, A. T.; Leavitt, K. J.; Barbosa, J.; McDonnell, P. A.; Metzler, W. J.; Rankin, B. M.; Liu, R.; Vaccaro, W.; Pitts, W. Structure-Based Design of Potent and Selective Inhibitors of Collagenase-3 (MMP-13). *Bioorg. Med. Chem. Lett*. **2005**, *15*, 1101−1106. (e) Matziari, M.; Beau, F.; Cuniasse, P.; Dive, V.; Yiotakis, A. Evaluation of P1′-Diversified Phosphinic Peptides Leads to the Development of Highly Selective Inhibitors of MMP-11. *J. Med. Chem*. **2004**, *47*, 325−336. (f) Pikul, S.; Ohler, N. E.; Ciszewski, G.; Laufersweiler, M. C.; Almstead, N. G.; De, B.; Natchus, M. G.; Hsieh, L. C.; Janusz, M. J.; Peng, S. X.; Branch, T. M.; King, S. L.; Taiwo, Y. O.; Mieling, G. E. *J. Med. Chem*. **2001**, *44*, 2499−2502.

(6) For a report regarding protection against emphysema in a smoke-induced emphysema model by a broad-spectrum MMP inhibitor, see: Selman, M.; Cisneros-Lira, J.; Gaxiola, M.; Ramírez, R.; Kudlacz, E. M.; Mitchell, P. G.; Pardo, A. *Chest* **2003**, *123*, 1633−1641.

(7) Ma, D.; Wu, W.; Yang, G.; Li, J.-Y.; Li, J. Ye, Q. Tetrahydroisoquinoline Based Sulfonamide Hydroxamates as Potent Matrix Metalloproteinase. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 47−50.

(8) Hibner, B.; Card, A.; Flynn, C.; Casazza, A. M.; Tarabpletti, G.; Rieppi, M.; Giavazzi, R. BAY 12-9566, a novel, biphenyl matrix metalloproteinase inhibitor, demonstrates an-invasive and anti-angiogenic properties. *Proc. Am. Assoc. Cancer Res.* **1979**, *39*, Abstract 2063.

(9) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J.; Meyer, E. E. J.; Brice, M. D. et al. The Protein Data Bank: A Computer Based Archival File for Macromolecular Structures. *J. Mol. Biol.* **1977**, *112*, 535−542.

(10) (a) Lucey, E. C.; Goldstein, R. H.; Stone, P. J.; Snider, G. L. Remodeling of Alveolar Walls after Elastase Treatment of Hamsters. *Am. J. Respir. Crit. Care Med.* **1998**, *158*, 555−564. (b) Ofulue, A. F.; Ko, M.; Abboud, R. T. Time course of neutrophil and macrophage elastinolytic activities in cigarette smoke-induced emphysema. *Am. J. Physiol*. **1998**, *275* (*Lung Cell. Mol. Physiol. 19*), L1134−L1144. (c) Avelar, E.; Jalili, T.; Dong, L.; Arvizo, J.; Hu, P.; Litwin, S. E.; Mattson, J. P. PKC translocation and ERKI/2 activation in compensated right ventricular hypertrophy secondary to chronic emphysema. *BMC Physiol.* **2005**, *5*, No 6.

JM051101G