Selective non-zinc binding MMP-2 inhibitors: Novel benzamide Ilomastat analogs with Anti-tumor Metastasis

Jiao Song, Peng Peng, Jun Chang, Ming-Ming Liu, Jian-Ming Yu, Lu Zhou, Xun Sun

PII: S0960-894X(16)30284-0
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.03.064
Reference: BMCL 23707

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 16 February 2016
Revised Date: 14 March 2016
Accepted Date: 16 March 2016

Please cite this article as: Song, J., Peng, P., Chang, J., Liu, M-M., Yu, J-M., Zhou, L., Sun, X., Selective non-zinc binding MMP-2 inhibitors: Novel benzamide Ilomastat analogs with Anti-tumor Metastasis, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.03.064

Selective non-zinc binding MMP-2 inhibitors: Novel benzamide Ilomastat analogs with Anti-tumor Metastasis

Jiao Song[a], Peng Peng[a], Jun Chang[a], Ming-Ming Liu[b], Jian-Ming Yu[a], Lu Zhou[b,*], Xun Sun[a,c,*]

[a] *Department of Natural Products Chemistry, School of Pharmacy, Fudan University, 826 Zhangheng Road, Shanghai 201203, China*

[b] *Department of Medicinal Chemistry, School of Pharmacy, Fudan University, 826 Zhangheng Road, Shanghai 201203, China*

[c] *Shanghai Key Lab of Clinical Geriatric Medicine, 221 West Yanan Road, Shanghai 200040, China*

*To whom correspondence should be addressed. Tel.: +86-21-51980003; Fax: +86-21-51980003.

*E-mail addres*s: zhoulu@fudan.edu.cn (L. Zhou); sunxunf@shmu.edu.cn (X. Sun)

Abstract

Novel Ilomastat analogs with substituted benzamide groups, instead of hydroximic acid groups, were designed, synthesized and evaluated against MMP-2 and MMP-9. Among these analogs, the most potent compound **10a** exhibited potent inhibitory activity against MMP-2 with IC_{50} value of 0.19 nM, which is 5 times more potent than that of Ilomastat (IC_{50} = 0.94 nM). Importantly, **10a** exhibited more than 8300 fold selectivity for MMP-2 vs MMP-9 (IC_{50} = 1.58 μM). Molecular docking studies showed that **10a** bond to the catalytic active pocket of MMP-2 by a non-zinc-chelating mechanism which was different from that of Ilomastat. Furthermore, the invasion assay showed that **10a** was effective in reducing HEY cells invasion at 84.6% in 50 μM concentration. For **10a**, the pharmacokinetic properties had been improved and especially the more desirable $t_{1/2z}$ was achieved compared with these of the lead compound Ilomastat.

***Keywords**:* MMP-2 and MMP-9, Ilomastat analogs, benzamide groups, enzyme inhibition, non-zinc binding, HEY cell invasion

Cancer is a key health issue across the world, causing substantial patient morbidity and mortality. World Cancer Report in 2014 reported that the death toll was 2.2 million in China, accounting for a quarter of global cancer deaths.[1] Cancer survival rate has been significantly improved over the years by early diagnosis and cancer growth inhibition.[2] However, as long as cancer metastasis occurs, it will be highly incurable and fatal which accounts for about 90% of cancer deaths.[3,4] Until recently, limited success has been achieved on prevention and inhibition of cancer metastasis.[2] Therefore, developing drugs to control cancer metastasis is still an urgent need to improve the clinical cancer survival rate.

Overexpression of matrix metalloproteinase (MMP) plays an important role in the context of tumor invasion and metastasis.[5,6] Extracellular matrix (ECM) is the most important physiological barrier for the metastasis of tumor cells.[7] Overexpression of MMP in tumor cells can destroy ECM, which could promote the invasion of cancer cells.[8] Thus, MMP inhibitors have been attractive anti-cancer targets. Among the MMP family, MMP-2 and MMP-9 were identified to be more critical in the invasion of tumor cells cross basement membranes.[9,10] Though they bind to a common substrate, MMP-2 has been selected as a promising target for anti-cancer drugs, whereas MMP-9 is regarded as a non-safe target for these drugs.[9,11] So far, many MMP-2 inhibitors have been developed as anti-cancer agents. However, owing to the low selectivity for MMP-2, most of these compounds were unsuccessful in clinical trials. Thus, searching for novel potent MMP-2 compounds with high selectivity is an important objective to overcome clinical cancer metastasis, such as lung, ovarian, breast and other malignant tumors.

Ilomastat (**1**), a zinc-binding inhibitor, is in phase III clinical trials (Fig. 1).[12] Its hydroxamic acid specifically forms a bidentate complex with the active site zinc.[13] Ilomastat was one of the most potent MMP inhibitors and had a good biological activity to many diseases, such as tumor, sudden liver failure and postoperative corneal reparation.[14] However, Ilomastat was a non-selective broad spectrum inhibitor,[15,16] since the hydroxamic acid could interact at other subsites containing zinc ion and/or some other oxidative states of metals, such as iron (III).[15,17-19] In addition, it also had some other drawbacks, such as poor oral bioavailability (administration via injection).[19-21]

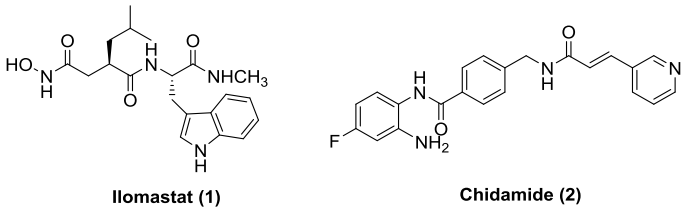


Figure 1. Chemical structures of Ilomastat (**1**) and Chidamide (**2**)

To improve the poor selectivity of Ilomastat, many research groups had endeavored to explore and develop other alternative zinc-binding group replacements, such as carboxylate, hydrazide, thiol, phosphonic acid and other heterocycles.[22-24] Unfortunately, these modified analogs without the hydroxamic acid feature usually had lower inhibitory activity for MMP-2 compared to their hydroxamate counterparts.[22] Until recently, only a very few success has been achieved on finding compounds which could efficiently and selectively inhibit MMP-2. Herein, we found a series of Ilomastat analogs with hydroxamate group replaced by benzamide group. Interestingly, one resulted analog, **10a**, exhibited very potent MMP-2 inhibitory activity (IC_{50} = 0.19 nM) and had more than 8300-fold selectivity over MMP-9.

The rationale to design these compounds was based on the idea to hybrid the functional groups from both Ilomastat (**1**) and Chidamide (**2**) (Fig. 2). Chidamide was a histone deacetylase (HDAC) inhibitor, which has a good subtype selectivity and anti-cancer activity.[25] It was approved in the global market in 2015 for the treatment of recurrent and refractory peripheral T-cell lymphoma (PTCL). HDACs and MMPs are both zinc-dependent enzymes.[23] Recent years, several classes of HDAC inhibitors had been found to have potent and specific anti-cancer activities in preclinical and clinical studies.[26] Initially, HDACs inhibitors were hydroxamic acid derivatives, whose hydroxamate group binds to zinc ion in an active site (Fig. 2), such as trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA). These were also non-selective broad spectrum inhibitors. Later, new HDACs inhibitors, exampled by Chidamide, were developed with subtype selectivity, whereas the benzamide group was used as a surrogate of the hydroxamate group.[27] Worth to mention, some HDACs inhibitors could interact with MMPs and had effective anti-metastatic activity. Lu *et al*.[28] reported that TSA, which suppresses MCF-7 breast carcinoma cell invasion and up-regulates TET1 expression, could promote the expression of tissue inhibitors of metalloproteinase 2/3 (TIMP 2/3) and inhibit transcriptional activity of MMP-2 and MMP-9 matrix metalloproteinase. Molecular docking studies showed that Chidamide binds to the catalytic active pocket of HDAC by a similar zinc-chelating mechanism

to TSA; however, the benzamide group not only chelates with catalytic zinc ion, but also can form new π–π stacking interactions and hydrogen bonds significantly different from TSA.[27] This could partially explain the higher selectivity of Chidamide than TSA.

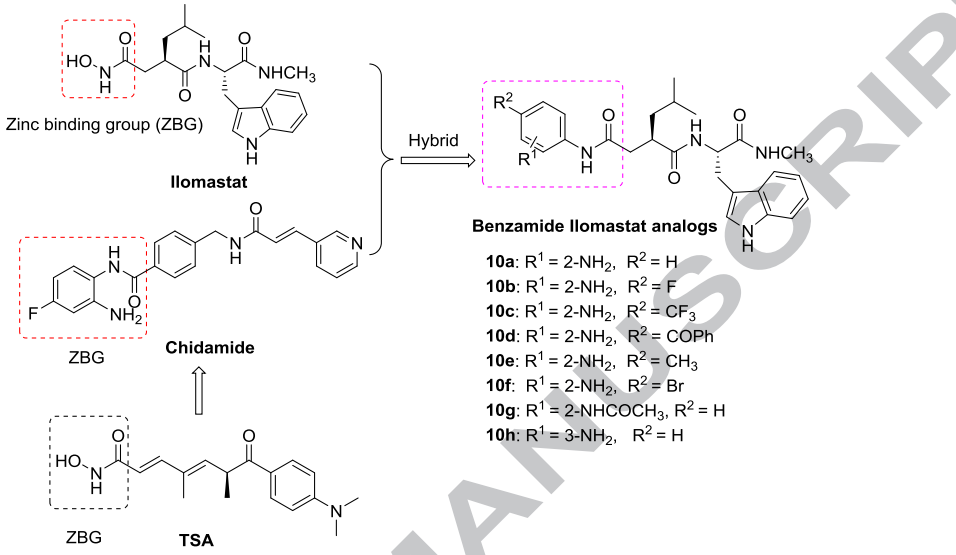
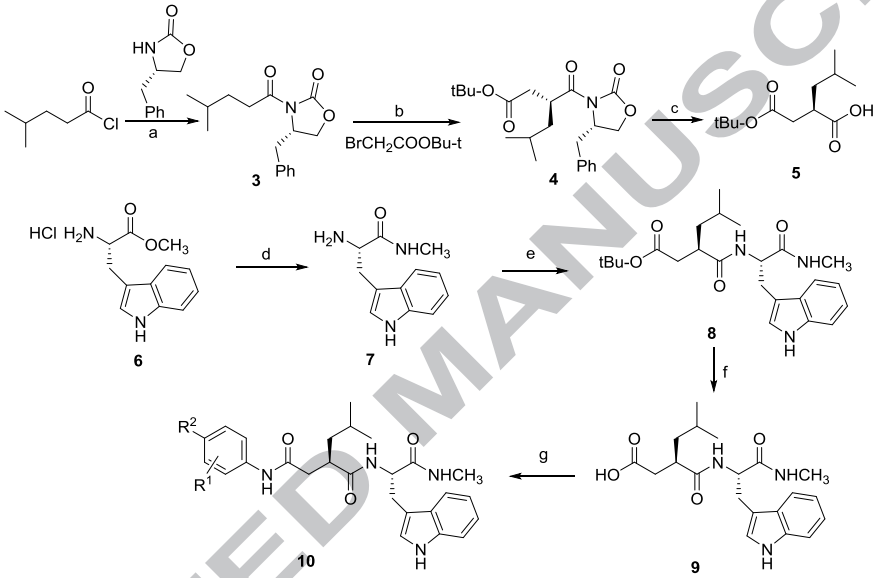


Figure 2. Design strategy for benzamide Ilomastat analogs

Thus, we used the same strategy as that for Chidamide to incorporate a benzamide group into Ilomastat (hydroxamate replacement) to design a series of novel compounds which may have good activity, higher selectivity, and improved oral bioavailability. Based on previous structure-activity relationship (SAR) study of Chidamide, para-substituents on benzamide ring could significant affect the HDACs inhibitory activity.[29] This information could help our SAR study to rationally design a small focused library. Thus, eight new benzamide compounds were designed and synthesized (Fig. 2). These analogs were evaluated for their inhibitory activity against both MMP-2 and MMP-9. Meanwhile, their binding modes with MMP-2 and MMP-9 were also explored.

The syntheses of the target compounds **10a-h** were accomplished as indicated in Scheme 1, followed the reference method[12] with some minor modifications. Commercially available 4-methylvaleryl chloride was used as the starting material, which coupled with a chiral auxiliary (*S*)-4-benzyl-2-oxazolidinone to provide compound **3** in 90% yield. Compound **3** was then treated with K-HMDS and *tert*-butyl bromoacetate to obtain **4** in a moderate yield. The auxiliary of **4** was removed by using hydrogen peroxide and lithium hydroxide to afford in **5** in high yield. The intermediate **5** was coupled with **7** to give **8** in 40% yield, which was

hydrolyzed under TFA condition to give carboxylic acid **9** in 70% yield. The last step for amide formation to form **10** was turned out to be problematic when CDI was used as the coupling reagent.[30] After many attempts, analogs **10a-h** could be synthesized in 20-50% yields by using EDCI hydrochloride salt as a coupling reagent. The reaction for this step was relatively messy. The desired products were isolated and their structures were characterized by ^{1}H NMR, ^{13}C NMR and high resolution mass spectrum. In addition, spectra of these compounds were also further compared with the patterns of same moiety presented in intermediates or products reported.[30,31]

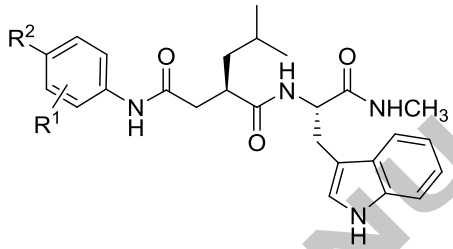


Scheme 1. General synthetic scheme for analogs **10a-h**. Regents and conditions: a) n-BuLi (2.5 M, 1.0 equiv), anhydrous THF, -78°C→-45°C→RT, nitrogen; b) K-HMDS (1M, 1.2 equiv), anhydrous THF, -78°C→RT, nitrogen, overnight; c) H_2O_2 (30%, 4.0 equiv), LiOH (0.8 M, 1.6 equiv), $NaNO_2$ (4.0 equiv), THF/H_2O (v/v = 4:1), 0°C; d) CH_3NH_2/CH_3OH (30%, 10.0 equiv), RT, overnight; e) **5** (1.0 equiv), EDCI (1.2 equiv), DMAP (0.5 equiv), anhydrous THF, RT, overnight; f) TFA/DCM (v/v = 2:1), anisole (0.5 mL), RT, 0.5 h; g) EDCI (1.5 equiv), DMAP (0.5 equiv), aniline, anhydrous DCM, nitrogen, RT, overnight.

The inhibitory activities of synthesized compounds against MMP-2 and MMP-9 were evaluated by using a fluorimetric assay[16] and the results were illustrated in Table 1. Ilomastat was also tested as a positive control. Overall, four compounds (**10a-b**, **10f** and **10h**) displayed a certain extent inhibitory activity toward MMP-2. Among them, 2-amino-phenyl derivative **10a** showed the most potent inhibitory activity (IC_{50} = 0.19 nM) against MMP-2, which was 5 times more potent than that of Ilomastat (IC_{50} = 0.94 nM). In addition, **10a** exhibited high selectivity to MMP-2, which was more than 8300 fold over MMP-9. When the 2-amine group (analog **10a**) was switched to

3-position (analog **10h**) or acylation of 2-amino group (analog **10g**), both compounds lost potency against MMP-2 (6- and 110-fold decreased respectively) compared with **10a**, though increased undesired potency for MMP-9. Retaining R^1 group as the amino group and modifying R^2 group by introducing electron-donating or electron-withdrawing group, all resulted analogs **10b-f** displayed much lower inhibitory activity against MMP-2. Except **10a**, compounds **10b-h** also exhibited low selectivity for MMP-2 vs MMP-9.

Table 1. MMP-2 and MMP-9 inhibitory activity of **10a-h**



compound	R^1	R^2	MMP-2[a]	MMP-9[a]	Selectivity[b]
10a	2-NH_2	H	0.19	1579.01	8310
10b	2-NH_2	F	2.20	7.75	3.52
10c	2-NH_2	CF_3	$>10^4$	$>10^4$	-
10d	2-NH_2	COPh	$>10^4$	$>10^4$	-
10e	2-NH_2	CH_3	$>10^4$	7297.04	-
10f	2-NH_2	Br	21.80	27.32	1.25
10g	2-$NHCOCH_3$	H	$>10^4$	155.19	-
10h	3-NH_2	H	2.05	13.52	6.59
Ilomastat			0.94	0.55	0.58

a: Inhibition data are expressed as IC_{50} (nM).

b: relative selectivity[15]: MMP-9 (IC_{50}) / MMP-2 (IC_{50})

-: no obvious inhibition;

Ilomastat: positive control

Molecular docking studies were carried out to explore the binding modes of **10a** with MMP-2 and MMP-9. Initial protein structures of MMP-2 and MMP-9 were taken from structural models deposited in the Protein Data Bank (PDB) with the entry code 1HOV and 4H3X, respectively. It was found that **10a** binds to both MMP-2 and MMP-9 in non-zinc binding modes. For MMP-2, the benzamide group of **10a** interacts with the residues of the specific loop, presenting van der Waals interactions

with residues Tyr 142, Leu 116, Thr 143, Leu 137, Ala 139, Ile 141, and Ala 136 sitting at the bottom of the S1' sub-site,[32] while the 2-amino group can form hydrogen bonds with Ala 136 and Ile 141 (Fig. 3A and 3B). For MMP-9, due to the limited pocket size, the benzamide group locates at the entrance of the S1' hydrophobic pocket and presents weak van der Waals interactions with residues Pro 421, Met 422 and Tyr 423 (Fig. 4A and 4B). Since S1' pockets in MMP-2 and MMP-9 have different shapes and sizes,[33] only MMP-2 has enough space to accommodate the benzamide group. In addition, the indole group of **10a** is localized in the S2 cavity of MMP-2, and the phenyl ring can form a π–π interaction with His 124 and weak van der Waals interactions with residues Ala 84, His 85, and Ala 86.[15] For MMP-9, the indole ring of **10a** deviates from S2 cavity, which results only weak van der Waals interactions with residues Ala 189, His 190, and Ala 191. These may explain why **10a** had a high selectivity against MMP-2 vs MMP-9. Meanwhile, we measured the lipid-water partition coefficient (log P) for both **10a** and Ilomastat. The log P of **10a** was 1.91, which was higher than Ilomastat (log P = 1.04). This could also potentially contribute the feasibility of **10a** to bind with hydrophobic active pockets of MMP-2 than Ilomastat.

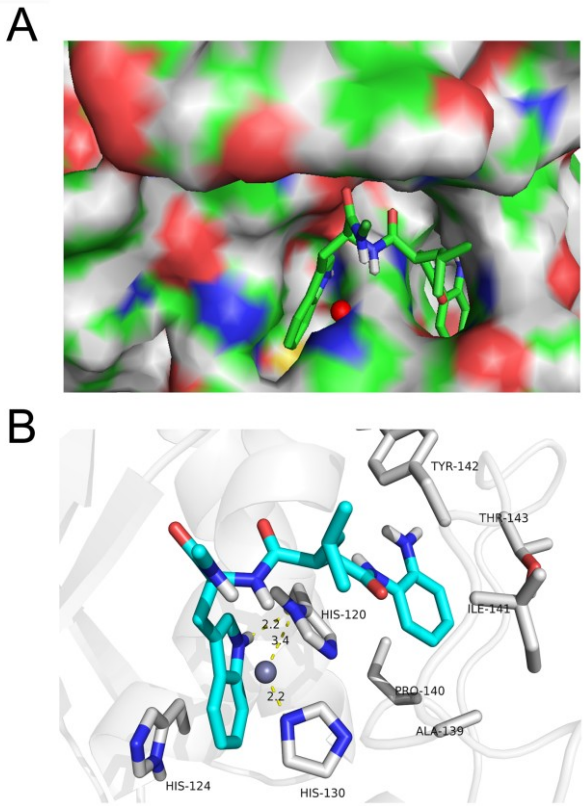


Figure 3. The surface map and interactions between **10a** and MMP-2. A) The surface map of

interactions between **10a** and MMP-2; B) the interactions between **10a** and residues of active sites of MMP-2.

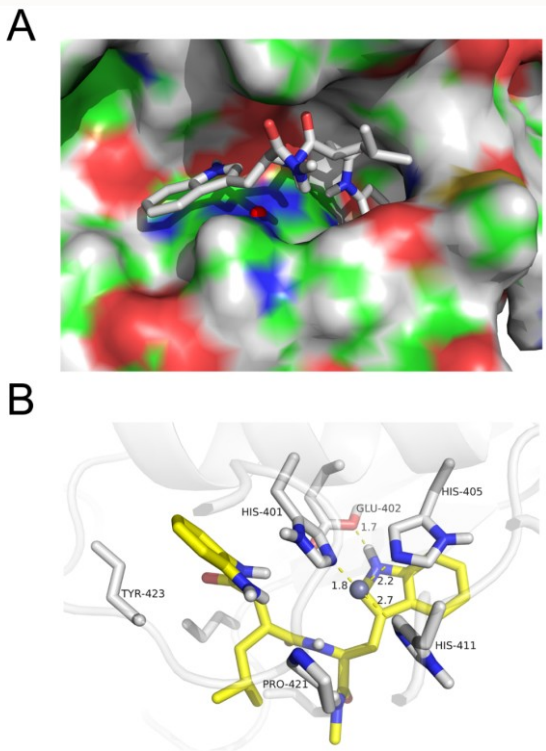


Figure 4. The surface map and interactions between **10a** and MMP-9. **A**) The surface map of interactions between **10a** and MMP-9; **B**) The interactions between **10a** and residues of active sites of MMP-9.

The anti-invasion effect of **10a** on human ovarian cancer cell line HEY was evaluated using matrigel based Transwell invasion assay. First, the cytotoxicity of **10a** was determined on HEY cells using MTT assay. As shown in Figure S1 (see supporting information), cell viability was not significantly affected by treatment with **10a** at 6.25-100 μM compared with the control (DMSO). However, solid particles were observed by microscope in the cell culture medium at the concentration of 100 μM, indicating the saturation of **10a** in culture at this concentration. Therefore, non-cytotoxic concentrations (12.5, 25 and 50 μM) of **10a** were applied in the subsequent Transwell invasion assay. It showed that the number of cells invading matrigel membrane and migrating from the upper to the lower chamber was dose-dependently reduced, after the treatment with **10a** (Fig. 5A). At concentration of 50 μM, 84.6% reduction of cellular invasive and migratory activity was achieved, as shown in Fig. 5B. This clearly demonstrated that **10a** possessed effective activity against cellular invasion and migration.

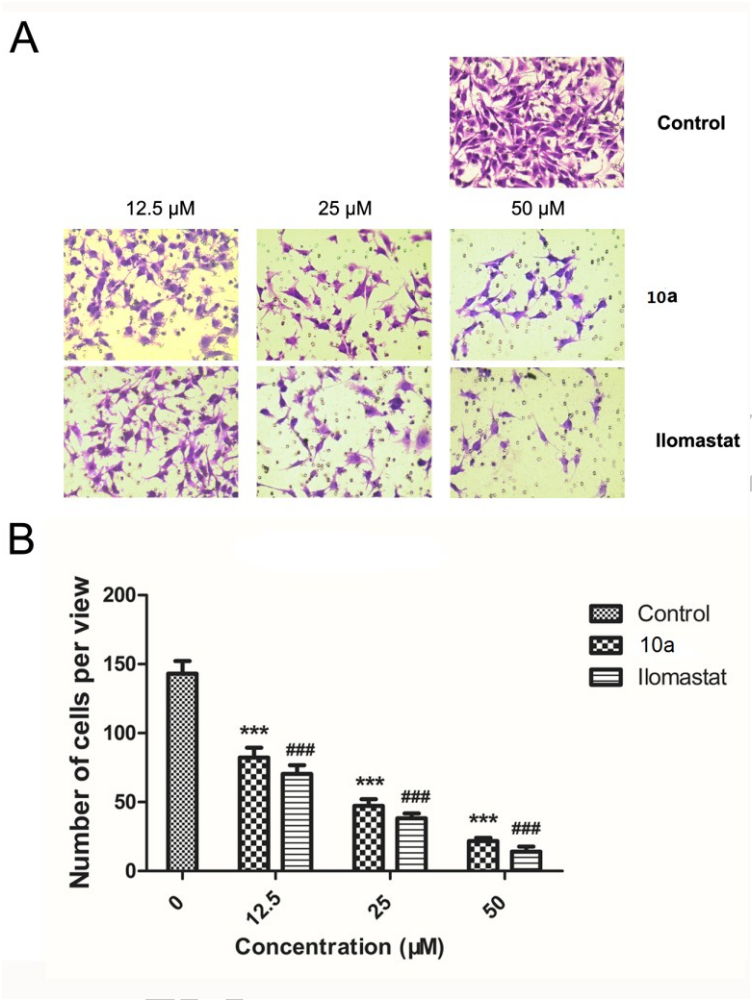


Figure 5. The effects of **10a** and Ilomastat on HEY cells invasion. A) The photomicrographs of control, **10a** and Ilomastat. After 24h of incubation with 12.5, 25, 50 μM of **10a** and Ilomastat, the numbers of cells per view were determined by crystal violet staining. B) The effects of HEY cells invasion with **10a** and Ilomastat. Different effects of **10a** and Ilomastat on invasion of HEY cells were evaluated using t test; $^{***}P < 0.001$ **10a** compared with Control and $^{###}P < 0.001$ Ilomastat compared with Control; No significant difference with the same concentration of **10a** and Ilomastat.

Furthermore, compound **10a** was dosed to Sprague-Dawley rat intravenously (10 mg/kg) and orally (10 mg/kg) for pharmacokinetic (PK) study. In general, PK properties of **10a** were better than that of Ilomastat (Table 2). After intravenous administration, $AUC_{0\text{-}24h}$ of **10a** was 3068.05 μg*h/L and slightly better than Ilomastat (2508.38 μg*h/L). Compound **10a** also showed lower clearance (3.13 L/h/kg) than that of Ilomastat (3.99 L/h/kg). As a consequence, the more desirable $t_{1/2z}$ of **10a** (7.88 h) was achieved compared with that of Ilomastat (0.25 h). After oral (P.O.) administration, compound **10a** showed a good $t_{1/2z}$ (5.64 h), thought its

clearance was too long. However, the absorption of Ilomastat was unable to be detected even at the lowest detection concentration. In addition, the oral bioavailability was slightly improved (%*F* of **10a** and Ilomastat was 4 and 0, respectively) at 10 mg/kg. Thus, overall PK profiles of **10a** have been improved to a certain extent compared with Ilomastat.

Table 2. Pharmacokinetic parameters of **10a** and Ilomastat after intravenous and oral administration at a dose of 10 mg/kg in Sprague-Dawley rats

Pharmacokinetic parameters	administration route			
	Intravenous (IV)		Oral (PO)	
	10a	Ilomastat	**10a**	Ilomastat
$AUC_{0\text{-}24h}$ (μg*h /L)	3068.05	2508.38	36.18	\
$AUC_{0\text{-}\infty}$ (μg*h /L)	3209.16	2509.32	140.44	\
V_z (L/kg)	1.43	35.76	579.41	\
$t_{1/2z}$ (h)	7.88	0.25	5.64	\
CL_z (L/h/kg)	3.13	3.99	71.20	\

$AUC_{0\rightarrow 24h}$, area under the concentration vs. time curve from 0 to 24h; $AUC_{0\rightarrow\infty}$, area under the concentration vs. Time curve from 0 to infinity; $t_{1/2z}$, terminal half-life; Clz, clearance; \: cannot be detected.

In summary, we designed and synthesized a series of novel benzamide Ilomastat analogs as a small focused library to explore their inhibitory activity for MMP-2 and MMP-9. Among them, compound **10a** exhibited very potent inhibitory activity against MMP-2, which was 5 times more potent than that of Ilomastat. More importantly, **10a** showed high selectivity to MMP-2, which was more than 8300-fold over MMP-9. Molecular docking studies indicated that **10a** binds to the catalytic active pocket of MMP-2 by a non-zinc-chelating mechanism. In addition, the invasion assay indicated that **10a** had a good anti-invasion effect, reducing HEY cell invasion at 84.6% in 50 μM concentration. The pharmacokinetic properties of **10a** also have been improved and especially the $t_{1/2z}$ of **10a** was much longer than that of Ilomastat. Further optimization of **10a** to improve the oral bioavailability is ongoing and will be reported in due course.

Acknowledgements

The work was supported by grants from the National Natural Science Foundation of China (No: 81373276), Shanghai Municipal Committee of Science and Technology

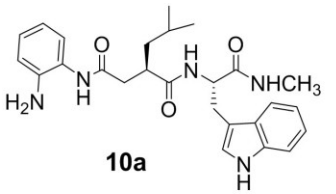
(No: 14XD1400300, 13431900101), and National Science and Technology Major Project of China (No. 2013ZX09J13103-02C).

References and notes

1. Siegel, R.; Ma, J.; Zou, Z.; Jemal, A. *CA Cancer J. Clin.* **2014,** *64*, 9.
2. Guan, X. *Acta Pharm. Sin. B.* **2015,** *5*, 402.
3. Wells, A.; Grahovac, J.; Wheeler, S.; Ma, B.; Lauffenburger, D. *Trends Pharmacol. Sci.* **2013,** *34*, 283.
4. Seyfried, T. N.; Huysentruyt, L. C. *Crit. Rev. Oncog.* **2013,** *18*, 43.
5. Shay, G.; Lynch, C. C.; Fingleton, B. *Matrix Biol.* **2015,** *44-46*, 200.
6. Bonnans, C.; Chou, J.; Werb, Z. *Nat. Rev. Mol. Cell. Biol.* **2014,** *15*, 786.
7. Wan, L.; Pantel, K.; Kang, Y. *Nat. Med.* **2013,** *19*, 1450.
8. Brown, G. T.; Murray, G. I. *J. Pathol.* **2015,** *237*, 273.
9. Overall, C. M.; Kleifeld, O. *Nat. Rev. Cancer* **2006,** *6*, 227.
10. Ruokolainen, H.; Paakko, P.; Turpeenniemi-Hujanen, T. *Clin. Cancer Res.* **2004,** *10*, 3110.
11. Higashi, S.; Hirose, T.; Takeuchi, T.; Miyazaki, K. *J. Biol. Chem.* **2013,** *288*, 9066.
12. Li, J.; Han, H.; Liu, K. l. *Chin. New Drugs J.* **2007,** *16*, 300.
13. Gupta, S. P.; Patil, V. M. *Exs.* **2012,** *103*, 35.
14. Hori, T.; Uemoto, S.; Walden, L. B.; Chen, F.; Baine, A. M.; Hata, T.; Kogure, T.; Nguyen, J. H. *Hepatol Res.* **2014,** *44*, 651.
15. Ledour, G.; Moroy, G.; Rouffet, M.; Bourguet, E.; Guillaume, D.; Decarme, M.; Elmourabit, H.; Auge, F.; Alix, A. J.; Laronze, J. Y.; Bellon, G.; Hornebeck, W.; Sapi, J. *Bioorg. Med. Chem.* **2008,** *16*, 8745.
16. Levy, D. E.; Lapierre, F.; Liang, W.; Ye, W.; Lange, C. W.; Li, X.; Grobelny, D.; Casabonne, M.; Tyrrell, D.; Holme, K.; Nadzan, A.; Galardy, R. E. *J. Med. Chem.* **1998,** *41*, 199.
17. Nicolotti, O.; Catto, M.; Giangreco, I.; Barletta, M.; Leonetti, F.; Stefanachi, A.; Pisani, L.; Cellamare, S.; Tortorella, P.; Loiodice, F.; Carotti, A. *Eur. J. Med. Chem.* **2012,** *58*, 368.
18. Moroy, G.; Denhez, C.; El Mourabit, H.; Toribio, A.; Dassonville, A.; Decarme, M.; Renault, J. H.; Mirand, C.; Bellon, G.; Sapi, J.; Alix, A. J.; Hornebeck, W.; Bourguet, E. *Bioorg. Med. Chem.* **2007,** *15*, 4753.

19. Overall, C. M.; Kleifeld, O. *Br. J. Cancer* **2006,** *94*, 941.
20. Shi, Z. H.; Li, N. G.; Shi, Q. P.; Tang, H.; Tang, Y. P.; Li, W.; Yin, L.; Yang, J. P.; Duan, J. A. *Bioorg. Med. Chem. Lett.* **2013,** *23*, 1206.
21. Hodgson, J. *Nat. Biotech.* **1995,** *13*, 554.
22. Augé, F.; Hornebeck, W.; Decarme, M.; Laronze, J. Y. *Bioorg. Med. Chem. Lett.* **2003,** *13*, 1783.
23. Jacobsen, F. E.; Lewis, J. A.; Cohen, S. M. *Chem. Med. Chem.* **2007,** *2*, 152.
24. Li, N. G.; Tang, Y. P.; Duan, J. A.; Shi, Z. H. *Expert Opin. Ther. Pat.* **2014,** *24*, 1039.
25. Zhou, Y.; Pan, D. S.; Shan, S.; Zhu, J. Z.; Zhang, K.; Yue, X. P.; Nie, L. P.; Wan, J.; Lu, X. P.; Zhang, W.; Ning, Z. Q. *Biomed. Pharmacother.* **2014,** *68*, 483.
26. Moradei, O. M.; Mallais, T. C.; Frechette, S.; Paquin, I.; Tessier, P. E.; Leit, S. M.; Fournel, M.; Bonfils, C.; Trachy-Bourget, M. C.; Liu, J.; Yan, T. P.; Lu, A. H.; Rahil, J.; Wang, J.; Lefebvre, S.; Li, Z.; Vaisburg, A. F.; Besterman, J. M. *J. Med. Chem.* **2007,** *50*, 5543.
27. Pan, D.; Yang, Q. J.; Fu, X.; Shan, S.; Zhu, J. Z.; Zhang, K.; Li, Z. B.; Ning, Z. Q.; Lu, X. P. *Med. Chem. Commun.* **2014,** *5*, 1789.
28. Lu, H. G.; Zhan, W.; Yan, L.; Qin, R. Y.; Yan, Y. P.; Yang, Z. J.; Liu, G. C.; Li, G. Q.; Wang, H. F.; Li, X. L.; Li, Z.; Gao, L.; Chen, G. Q. *Mol. Med. Rep.* **2014,** *10*, 2595.
29. Jiao, J.; Hu, J. q.; Fang, H. *Chin. New Drugs J.* **2010,** *19*, 386.
30. Lu, X. P.; Li, Z. B.; Xu, X. K. *PCT Int. Appl.* **2015,** WO 2015149435 A1.
31. Kaiya, T.; Fujiwara, T.; Kohda, K. *Chem. Res. Toxicol.* **2000,** *13*, 993.
32. Feng, Y.; Likos, J. J.; Zhu, L.; Woodward, H.; Munie, G.; McDonald, J. J.; Stevens, A. M.; Howard, C. P.; De Crescenzo, G. A.; Welsch, D.; Shieh, H. S.; Stallings, W. C. *Biochim. Biophys. Acta* **2002,** *1598*, 10.
33. Rowsell, S.; Hawtin, P.; Minshull, C. A.; Jepson, H.; Brockbank, S. M. V.; Barratt, D. G.; Slater, A. M.; McPheat, W. L.; Waterson, D.; Henney, A. M.; Pauptit, R. A. *J. Mol. Biol.* **2002,** *319*, 173.

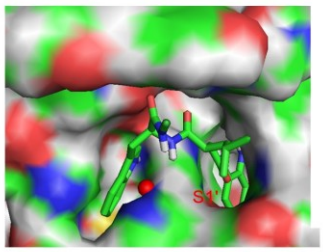
Graphic Abstract



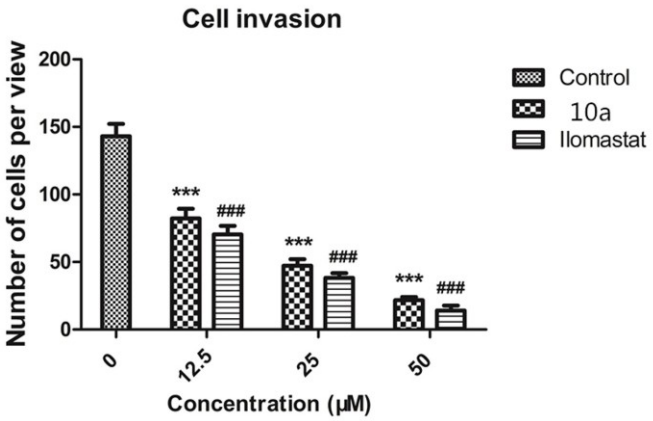
10a

MMP-2: IC_{50} = 0.19 nM

MMP-9: IC_{50} = 1.58 μM



10a interacts with MMP-2



Effect of reducing HEY cells invasion