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# Selective non-zinc binding MMP-2 inhibitors: Novel benzamide Ilomastat analogs with anti-tumor metastasis

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ABSTRACT

Novel Ilomastat analogs with substituted benzamide groups, instead of hydroxamic 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 versus 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.



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]

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

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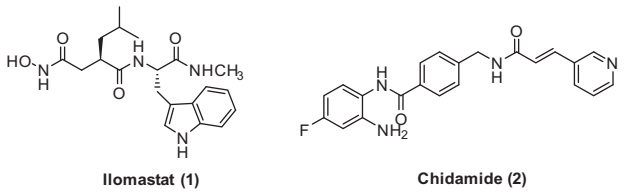


**Figure 1.** Chemical structures of Ilomastat (**1**) and Chidamide (**2**).

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.

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]

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

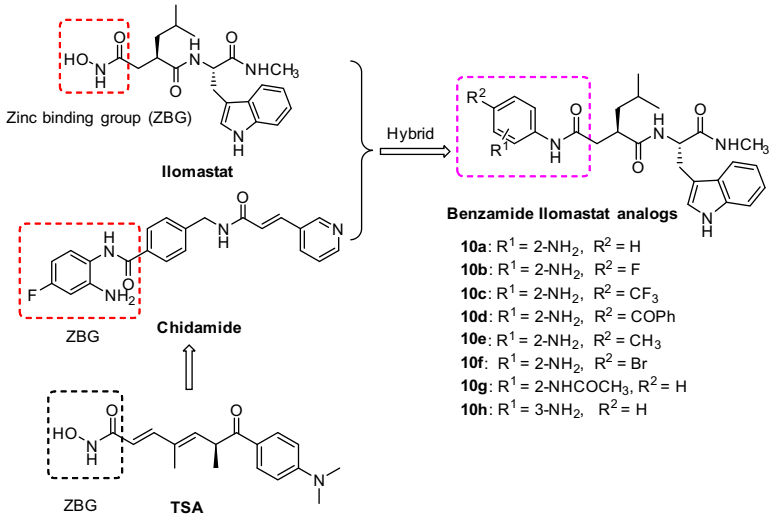


**Figure 2.** Design strategy for benzamide Ilomastat analogs.