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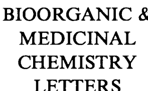

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Design and Synthesis of Novel Inhibitors of Gelatinase B

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Abstract—A new method was developed to identify nonpeptidic metalloproteinase inhibitors with novel zinc binding groups. Application of this method to matrix metalloproteinase-9 resulted in the identification of aminomethyl benzimidazole analogue **7a** with an IC_{50} = 13 μM. © 2002 Elsevier Science Ltd. All rights reserved.

Matrix metalloproteinases (MMPs) are a family of zinc-containing endopeptidases that are involved in the degradation and remodeling of connective tissues. The activities of MMPs under normal physiological conditions are modulated by endogenous inhibitors, such as the TIMPs (tissue inhibitor of metalloproteinases).[1] Disruption of this balance leads to overactivation of the MMPs, which causes excessive tissue degradation.[2] This has, in turn, been associated with a variety of disease states including arthritis,[3] tumor metastasis,[4] and periodontal disease.[5] Due to the clear therapeutic potential for inhibiting MMPs, the development of MMP inhibitors has been under intensive investigation.[6]

Virtually all MMP inhibitors have been designed upon a key zinc binding pharmacophore to which is appended additional, typically peptidomimetic functionality to provide enhanced affinity and selectivity. Hydroxamic acid is by far the most commonly used zinc binding pharmacophore and has provided the highest affinity MMP inhibitors. However, inhibitors incorporating the hydroxamic acid pharmacophore are often rapidly metabolized and show poor selectivity profiles across the MMPs.[7] Herein, we report a new method to identify completely nonpeptidic MMP inhibitors that incorporate novel zinc binding pharmacophores.

In the first step of the method a collection of low molecular weight compounds (< 150 daltons) containing potential zinc-binding functional groups are screened against a metalloprotease of interest. Assays are performed at high concentrations to identify even weakly inhibitory pharmacophores. In the second step analogues of the identified pharmacophores are assayed to provide SARs. In the final step, focused libraries based upon the preliminarys SARs are synthesized to identify more active compounds.

We have chosen gelatinase B (MMP-9) as a prototypical MMP to evaluate this method since it is one of the most highly expressed MMPs in and around a wide variety of tumors and has been implicated in tumor aggressiveness and increased metastatic potential.[8] A collection of potential zinc binders was assayed at 5 mM against murine gelatinase B.[9] The inhibitory activities of a representative set of these compounds are listed in Table 1. Acetohydroxamic acid, which was included in this study as a bench mark, is a very weak inhibitor (< 15% inhibition at 20 mM). This weak inhibitory activity is consistent with the 17 mM IC_{50} value of this compound against stromelysin (MMP-3) as reported by Fesik.[10] Among the compounds tested, aminomethyl benzimidazole showed particularly promising activity. MMP inhibitors have not previously been reported that incorporate an imidazole as the zinc-binding group. This is surprising considering that in MMPs three conserved active site histidine residues coordinate to zinc. The aminomethyl benzimidazole is also advantageous for further optimization because diversity can be introduced at multiple positions on this scaffold using efficient synthetic chemistry. Evaluation of a series of aminomethyl benzimidazole analogues indicated that substitution on the aromatic ring (Fig. 1) results in compounds with improved activity (data not shown). These results strongly suggested that aminomethyl benzimidazole binds to the enzyme active site rather than inhibiting simply by sequestering zinc, since substitution on the aromatic ring is distant from the metal-binding functionality. Furthermore, these results indicated that the preparation of focused libraries of

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Table 1. Percentage inhibition of potential zinc-binders

Compd	% Inhibition at 5 mM	Compd	% Inhibition at 5 mM[a]
O, NHOH	<5	N, NH_2	75±5
S, N, OH	80±5	N, NH_2	<5
O, O	76±5	H, N, N, NH_2	65±5
H, N, N, OH	26±5	NH_2, NH_2	10±5
CO_2H	<5	N, H, NH_2	<5
N, OH	<5	H_2N, HN, NH_2	<5
S, N, H, N, O	<5	H_2N, NH_2	<5

[a]Values are averages of three experiments.

Figure 1. Aminomethyl benzimidazole analogues.

aminomethyl benzimidazoles with diversity displayed on the aromatic ring should result in the identification of compounds with improved inhibitory activity.

The solid-phase synthesis sequence used to prepare focused aminomethyl benzimidazole libraries is depicted in Scheme 1. First, 5-nitro chloromethyl benzimidazole is loaded onto the DHP linker (**1**) using camphorsulfonic acid as an acid catalyst.[11,12] Nucleophilic substitution upon the support-bound chloromethylbenzimidazole **2** with phthalimide is followed by reduction of the aromatic nitro group to afford aniline **3**. Secondary amines **4**, amides **5** and ureas **6** are then readily prepared by reductive amination, acylation or coupling with isocyanates, respectively. Aminomethyl benzimidazoles **7**–**9** are obtained by deprotection of the phthalimide group with hydrazine at room temperature[13] followed by cleavage from support with 90/10 TFA/H_2O at 65 °C for 25 min.

According to the sequence in Scheme 1, a spatially separate library of 176 aminomethyl benzimidazoles was prepared in Robins plates using 88 aldehydes, 66 carboxylic acids and 22 isocyanates. Twenty randomly selected compounds were evaluated by LC–MS and NMR. The average overall yield for the six-step sequence was 30% and the average purity was greater than 75%.

The library was screened against gelatinase B at 100 μM. Inhibitory activities for six of the most potent compounds were determined after compound resynthesis and purification (Table 2). Hydrophobic side chains are clearly preferred at R_1, with the 2-benzyloxybenzyl substituent providing the most potent compound (**7a**).[14]

Scheme 1. Reagents and conditions: (a) 5-nitro chloromethyl benzimidazole (5 equiv relative to the resin), CSA (0.5 equiv relative to the resin), THF/dichloroethane (1:1), 70 °C, overnight; (b) phthalimide (6 equiv), K_2CO_3 (4 equiv), KI (3 equiv), DMF, 70 °C, overnight; (c) 1 M $SnCl_2{\cdot}2H_2O$ (30 equiv), NMP, rt, 4 h; (d) R^1CHO (10 equiv), $NaCNBH_3$ (20 equiv), HOAc (1%), DMA, rt, overnight; (e) R^1CO_2H (10 equiv), HATU (10 equiv), *i*Pr_2NEt (10 equiv), DMF, rt, overnight; (f) R^1NCO (10 equiv), DMF, rt, overnight; (g) 60% NH_2NH_2 in DMF, rt, 4 h; (h) 90% TFA/H_2O (v/v), 65 °C, 25 min.

Table 2. Inhibition data of aminomethyl benzimidazole analogues identified from the library against gelatinase

7a-c, 8a-c

Compd	R^1	IC_{50} ($\mu M^{a,b}$)	Compd	R^1	IC_{50} ($\mu M^{a,b}$)
7a		13±3	**8a**		46±6
7b		56±8	**8b**		85±10
7c		92±10	**8c**		75±10

[a]IC_{50} values were determined at seven different concentrations using fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 with [S] = 10 μM and K_m = 9.3 μM.
[b]IC_{50} values are averages from three experiments.

To identify the key structural elements responsible for inhibition, a series of analogues of **7a** were synthesized and tested (Table 3). Reducing the size of the R_1 substituent results in a dramatic drop in activity (**7d** and **7e**), highlighting the importance of an extended hydrophobic group at R_1. As illustrated for compounds **7f**–**7g**, the inhibitory activity is not significantly affected as long as an extended hydrophobic group is maintained at R_1.

Table 3. Inhibition data of analogues with R^1 and R^2 modifications

Compd	R_1	R_2	IC_{50} (μM)[a,b]
7a		H	13±3
7d	H	H	> 500
7e		H	195±10
7f		H	51±5
7g		H	48±5
7h		CH_3CO	83±5

[a]See Table 2 legend.
[b]See Table 2 legend.

The roles of the imidazole nitrogen and the terminal amino group were also determined (Table 4). As shown for compound **10**, replacing one of the nitrogens in the benzimidazole with carbon led to a 4-fold loss of activity. As observed for compounds **10**–**13**, removal, replacement with a hydroxyl group or acetylation of the terminal amino group each results in greater than 10-fold loss of activity. Clearly, the terminal amino group provides an important contribution to binding activity.

Finally, in order to characterize the physicochemical properties of lead compound **7a** both the aqueous solubility and CMC (critical micelle concentration) were determined. The aqueous solubility of **7a** is greater than 4 mM and the CMC in water is greater than 100 μM, indicating that **7a** is well behaved at inhibitory concentrations.

Table 4. Inhibition data of analogues with R^3 and R^4 modifications

Compd	R_3	R_4	IC_{50} (μM)[a,b]
7a	N	NH_2	13±3
10	CH	NH_2	72±8
11	N	H	> 300
12	N	OH	> 100
13	N	NHAc	> 100

[a]See Table 2 legend.
[b]See Table 2 legend.

In conclusion, we have reported a new method for the discovery of MMP inhibitors that has resulted in the identification of a completely nonpeptidic gelatinase B inhibitor **7a** ($IC_{50} = 13$ μM). The novel aminomethyl benzimidazole core structure has multiple potential sites for modification providing a number of opportunities for further optimization.

Acknowledgements

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14. (**7a**) ^{1}H NMR (300 MHz, chloroform-*d*) 3.98 (s, 2H), 4.36 (s, 2H), 4.39 (s, 2H), 5.10 (s, 2H), 6.52–6.68 (m, 2H), 6.83–6.95 (m, 2H), 7.17–7.43 (m, 8H); ^{13}C NMR (75.4 MHz, chloroform-*d*) 157.2, 154.2, 152.3, 145.4, 137.4, 133.8, 131.3, 129.5, 129.0, 128.7, 128.3, 128.2, 127.6, 121.2, 116.7, 111.8, 112.1, 109.2, 70.5, 44.9, 40.4; HRMS (ES) calcd for $C_{22}H_{23}N_4O$ (M+1): 359.1872. Found: 359.1871. Anal. calcd for $C_{22}H_{25}Cl_3N_4O$: C, 56.48; H, 5.39; N, 11.98. Found: C, 56.67; H, 5.71; N, 11.80.