

## Cobalt (III) Complexes as Novel Matrix Metalloproteinase-9 Inhibitors

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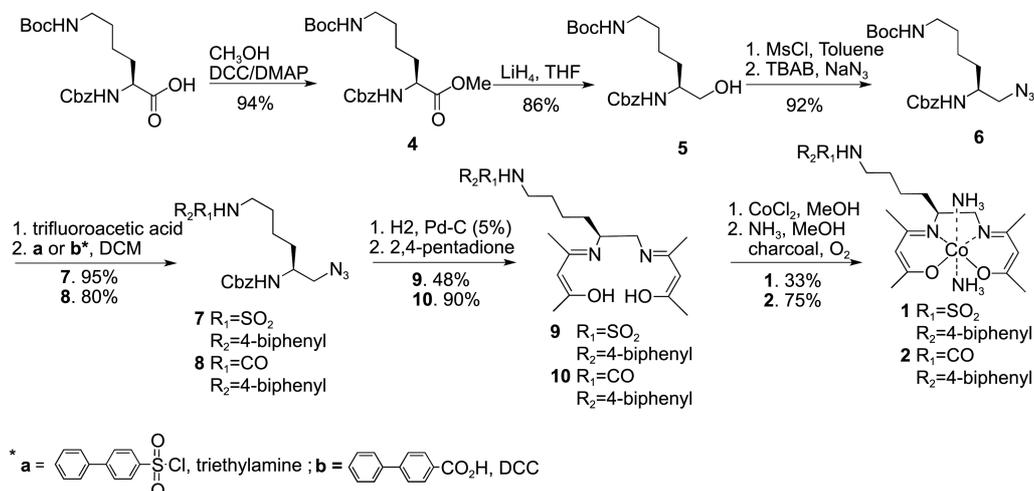
Matrix metalloproteinases (MMPs) are a family of zinc binding endopeptidases that play crucial roles in various physiological processes and diseases such as embryogenic growth, angiogenesis, arthritis, skin ulceration, liver fibrosis and tumor metastasis.<sup>1,2</sup> Because of their implications in a wide range of diseases, MMPs are considered as intriguing drug targets. The majority of MMP inhibitors are organic small molecules containing a hydroxamate functionality for the zinc binding group. This hydroxamate group binds to a zinc(II) center in a bidentate fashion and creates a distorted trigonal bipyramidal geometry.<sup>3</sup> Although the hydroxamate group is the most effective zinc binding moiety reported, it has shown two major limitations in clinical trials: low bio-availability and lack of specificity.<sup>4,5</sup> Because the hydroxamate group is prone to rapid metabolism and has a high binding affinity towards various transition metals, many efforts have been made to develop novel zinc binding groups, including reverse hydroxamate, phosphate, and pyridinone. However, the *in vitro* activity of these newly developed inhibitors are not as high as the hydroxamate derivatives.<sup>6,7</sup>

It was previously reported that cobalt(III) acacen complexes could interact with histidine residues of proteins and model peptides.<sup>8-11</sup> The cobalt(III) complexes bind histidine residues in active sites and on enzyme surfaces in a random fashion. Spectroscopic and chromatographic data suggested that the complexes bind to a histidine residue by axial ligand substitution. When an appropriate targeting group is attached, the cobalt(III) complexes can selectively inhibit the histidine-

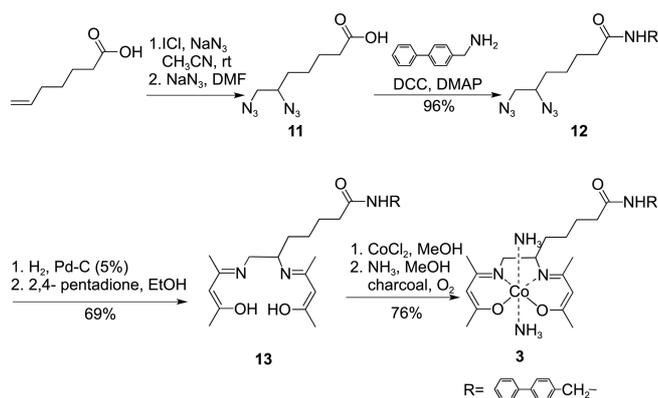
containing enzymes such as thermolysin,<sup>8</sup> human  $\alpha$ -thrombin,<sup>9</sup> and carbonic anhydrase.<sup>9</sup> Since histidine is the most commonly found residue in the active sites of zinc enzymes and zinc-binding proteins,<sup>12</sup> it has been demonstrated that the cobalt(III) complexes can interact with zinc finger proteins, such as HIV-1 nucleocapsid protein NCp7 and human zinc finger transcription factor *Sp1*.<sup>11</sup> It was also reported that a Co(III) complex with an oligonucleotide targeting Snail family zinc finger transcription factor resulted in a selective inactivation of a transcriptional activity implicated in embryonic development and breast cancer.<sup>13</sup>

In this work, a series of the cobalt(III) complexes are prepared as novel MMP inhibitors. Since cobalt(III) complexes have high affinity towards histidine residues and zinc binding proteins, they can be engineered to disrupt the zinc binding active site of MMP. Furthermore, the cobalt(III) complexes may be able to provide better solubility and higher binding affinity than the known organic inhibitors. To test this hypothesis, a series of cobalt(III) complexes were designed and synthesized. For active site directing groups, biphenyl sulfonate and biphenyl amide group were chosen based on the structures of well known MMP inhibitors.<sup>14</sup> *In vitro* activity of these complexes was evaluated for MMP-9 (gelatinase B), because MMP-9 is one of the most highly expressed MMPs in tumors and has been implicated in tumor aggressiveness.<sup>5</sup>

The syntheses of cobalt(III) complexes are shown in Schemes 1-2. The synthesis of **1** and **2** begins with the methylation of commercially available Cbz-Lys(Boc)-OH as



**Scheme 1.** Synthesis of cobalt(III) complexes **1** and **2**.



**Scheme 2.** Synthesis of cobalt(III) complex **3**.

shown in Scheme 1. Compound **4** was reduced by lithium borohydride to give **5** in an 86% yield. Compound **5** was mesylated and then refluxed with sodium azide and tetrabutyl ammonium bromide in toluene to give **6**. Subsequent deprotection of the Boc group followed by coupling with 4-biphenyl sulfonyl chloride or 4-biphenyl carboxylic acid using a peptide coupling reagent produced **7** and **8** respectively. These compounds were reduced and deprotected by hydrogenation on palladium carbon to obtain the diamine compounds. Overnight stirring of the diamine compounds with 2,4-pentanedione in ethanol afforded ligands **9** and **10**. The acacen ligands were metalated using cobalt(II) chloride, and oxidized in methanolic ammonia solution with oxygen and charcoal to give the final products **1** and **2**.

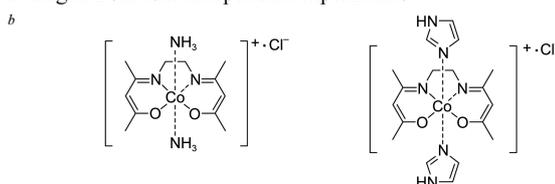
To investigate a potential distance effect of the spacer between the cobalt(III) complex and the biphenyl group, compound **3** was designed and synthesized as described in Scheme 2. The synthesis of **3** started with 6-heptenoic acid. Addition of iodine azide, prepared *in situ* from sodium azide and iodine monochloride,<sup>15</sup> followed by subsequent azide addition produced **11** in a 87% yield. Compound **11** was coupled with 4-biphenyl methylamine (**16**) using a peptide coupling agent to give **12** in a 96% yield. Compound **12** was reduced by hydrogenation to afford the diamine intermediate, and the diamine was reacted with 2,4-pentanedione to provide the acacen ligand **13**. Final product **3** was prepared from **13** by following the same procedure for the metalation of **1** and **2**.

*In vitro* activity of compound **1-3** for MMP-9 was evaluated using a fluorescent substrate assay (Table 1). For comparison, two unmodified cobalt(III) complexes with no targeting moiety were tested as a control group. The structures of these molecules are also shown in Table 1. The results indicate that all cobalt(III) complexes, including the control group, are effective as MMP-9 inhibitors. The substitutionally inert imidazole complex [Co(acacen)(Im)<sub>2</sub>]<sup>8,11</sup> differs from the synthesized compounds by more than an order of magnitude in IC<sub>50</sub>, which indicates that the cobalt(III) complex can efficiently inhibit MMP-9 by disrupting the zinc binding active site *via* axial ligand exchange. The imidazole complex showed a poor but some degree of inhibition (IC<sub>50</sub> = 47 μM); this is likely due to a weak

**Table 1.** Inhibition of MMP-9 by cobalt(III) complexes

Compound	IC <sub>50</sub> (μM) <sup>a</sup>
<b>1</b>	2.6 ± 0.51
<b>2</b>	0.83 ± 0.03
<b>3</b>	1.3 ± 0.16
[Co(acacen)(NH <sub>3</sub> ) <sub>2</sub> ] <sup>b</sup> Cl <sup>b</sup>	6.9 ± 1.3
[Co(acacen)(Im) <sub>2</sub> ] <sup>b</sup> Cl <sup>b</sup>	47 ± 5.5

<sup>a</sup>IC<sub>50</sub> values were determined at six different concentrations and averaged from four independent experiments.



interaction between one of the imidazole ligands and the active site zinc ion.

By attaching a biphenyl group as an active site directing moiety, the potency of each compound increased 3 to 6-fold. Although phenylsulfonamide groups are considered to be an efficient pharmacophore for many zinc enzymes,<sup>16</sup> the biphenylsulfonamide derivative of the cobalt(III) complex **1** (IC<sub>50</sub> = 2.6 μM) was not as effective as its amide counterpart **2** (IC<sub>50</sub> = 0.83 μM). When the spacer length was increased, IC<sub>50</sub> value of **3** (1.3 μM) became greater than IC<sub>50</sub> of **2**. These results indicate that the shorter length of the spacer between the cobalt(III) complex and the biphenyl group might be more beneficial to the efficiency of enzyme inhibition.

In summary, we have synthesized a series of novel MMP-9 inhibitors containing cobalt(III) complexes. The synthesized cobalt(III) complexes are effective as enzyme inhibitors and the attachment of a biphenyl group enhanced the efficiency of enzyme inhibition up to 6-fold. When compared to the reported non-hydroxamate MMP inhibitors,<sup>14</sup> the synthesized complexes showed comparable *in vitro* potency. The enzyme assay showed that the cobalt(III) complex can disrupt the zinc binding active site of MMP-9 and is proposed to work *via* a ligand exchange mechanism. Since histidine residues are essential for the catalytic activity of a large percentage of enzymes and zinc finger proteins, these cobalt(III) complexes can serve as a prototype inhibitor towards various zinc containing enzymes and proteins.

## Experimental Section

**General Methods.** Unless otherwise noted, materials and solvents were purchased from commercial suppliers and used without further purification. All organic reactions were performed under an atmosphere of N<sub>2</sub> in oven-dried glassware unless otherwise stated. Thin-layer chromatography was performed on Merck 60F 254 silica gel plates. Visualization of the developed chromatogram was performed by CAM stain and platinum stain. Flash chromatography was carried out using Fisher Grade 60 Å 230-400 mesh silica gel and Sigma-Aldrich Brockmann I standard grade 58 Å 150 mesh aluminum oxide. Organic extracts were dried over

MgSO<sub>4</sub> and were concentrated using a Büchi rotary evaporator under reduced pressure. NMR spectra were obtained on a Varian Inova spectrometer at 500 MHz and a Varian Mercury spectrometer at 400 MHz. NMR chemical shifts are reported in ppm and referenced to residual protonated solvent. Mass spectrometry samples were analyzed in MeOH using electrospray (ESI) ionization using a Varian 1200L Quadrupole MS system.

**Synthesis and Characterization.** Compounds **1-3** were synthesized by following the pathway described in Scheme 1, 2. Detailed synthetic procedures and spectroscopic data are available in the supporting information.

**Enzyme Activity Assay.** MMP-9 (recombinant human gelatinase B expressed in mammalian cells) activity was measured using a 96-well microplate fluorescent assay kit purchased from Calbiochem, following the procedure provided with the kit. Active form of MMP-9 was purchased from Calbiochem. Experiments were performed using a Gemini EM Fluorescence/Chemi-luminescence Plate reader and Corning Costar black 96-well plates. 2 mM stock solution of each inhibitor was prepared by dissolving in DMSO and further diluted ( $\times 50$ ) into the assay buffer provided with the kit. MMP-9 (1  $\mu\text{g}/\text{mL}$ ) was incubated with 6 different concentrations of each inhibitor for 30 min at room temperature. After addition of the substrate solution to each well, the plate was incubated at 37 °C for 2 h. Upon cleavage of the fluorogenic substrate<sup>17</sup> (0.15 mM), fluorescence intensity ( $\lambda_{\text{ex}} = 320$  nm,  $\lambda_{\text{em}} = 405$  nm) of each well was measured. The IC<sub>50</sub> values were calculated from control reactions without the inhibitor. The mean IC<sub>50</sub> was obtained from four independent measurements. For reference, the known MMP-9 inhibitor, (2*R*)-[(4-biphenylsulfonyl)-amino]-*N*-hydroxy-3-phenylpropion- amide was tested. The observed IC<sub>50</sub> value is 0.031  $\mu\text{M}$ , and the reported value is

0.030  $\mu\text{M}$ .<sup>18</sup>

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