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Synthesis of radioiodinated probes targeted toward matrix metalloproteinase-12

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ABSTRACT

Matrix metalloproteinase-12 (MMP-12, macrophage elastase) is a member of the MMP family that is responsible for the degradation of extracellular matrix, and is associated with the inflammatory process of chronic obstructive pulmonary disease (COPD). COPD, characterized by progressive and irreversible airflow obstruction, is recently a major cause of mortality and morbidity worldwide. Herein, to develop radioiodinated probes for the early diagnosis of COPD, we designed and synthesized novel MMP-12-targeted dibenzofuran compounds (**1**–**3**) with a variety of linker structures (carbamate, amide, and sulfonamide). In competitive enzyme activity assays, it was revealed that the linker structures significantly affected the inhibitory activity against and selectivity for MMP-12. Compound **1**, with carbamate linker, demonstrated potent MMP-12 inhibitory activity (IC_{50} = 8.5 nM) compared to compound **2**, with amide linker, and compound **3**, with sulfonamide linker. Using bromo-substituted carbamate **13** as a radioiodination precursor, [^{125}I]**1** was successfully prepared to high radiochemical purity (over 98%) and good specific radioactivity (4.1 GBq/μmol). These results suggest that radioiodinated compound **1** is potent as a novel MMP-12-targeted probe.

Matrix metalloproteinases (MMPs) are enzyme families of zinc-dependent endopeptidases, and 24 members of MMPs have been discovered in humans.[1–4] MMPs catalyze the remodeling of several extracellular matrix molecules, including collagen, proteoglycan, laminin, and elastin.[5,6] In addition, MMPs participate in cleaving cell surface proteins and mediate the processing of bioactive molecules.[1] Among these, MMP-12, known as a macrophage elastase, is secreted predominantly from alveolar macrophages.[7–9] MMP-12 is also associated with pulmonary inflammatory diseases, including chronic obstructive pulmonary disease (COPD).[10,11]The number of COPD patients is recently increasing, and could become the third leading global cause of death.[12,13] Since COPD is considered to be an irreversible disease,[14,15] the early detection of MMP-12 related to pulmonary inflammation diseases is important in order to determine appropriate therapeutic strategies to delay the disease progression,[16] thereby leading to improvement in prognosis and quality of life of patients.

For the treatment of COPD, Li et al. previously reported selective inhibitors of MMP-12 based on dibenzofuran scaffolds through structure-activity relationship (SAR) studies.[17] Although the shape and size of the specificity loops were similar between MMP-12 and MMP-13, the dibenzofuran derivatives with a carbamate linker revealed higher affinity for MMP-12 than for MMP-13 due to a change in the dihedral angle and a restriction in the rotation of the core structure associated with the S1′ pocket.[17] Of these derivatives, MMP-408 revealed potent inhibitory activity against and selectivity to MMP-12, and prevented lung inflammation induced by recombinant human MMP-12 in C57BL/6 mice. In accordance with these reports, we planned to develop a novel radioiodinated MMP-408 derivative **1** (Fig. 1) as an MMP-12 *in vivo* imaging probe. In addition, in order to investigate the linker effective for the interaction with the S1′ pocket of MMP-12,[17] we also designed compound **2** with an amide linker and compound **3** with a sulfonamide linker. Herein, we synthesized a series of MMP-12-targeted iodinated compounds (**1**–**3**) to diagnose early-stage COPD and evaluated their *in vitro* inhibitory activities against MMP-12 and MMP-13. Compound **1**, with potent inhibitory activity toward MMP-12, was labeled with radioiodine.

For MMP-12 selective inhibitors based on dibenzofuran scaffolds, SAR studies revealed that carboxylic acid was essential for zinc chelation, and the dibenzofuran structure was strongly

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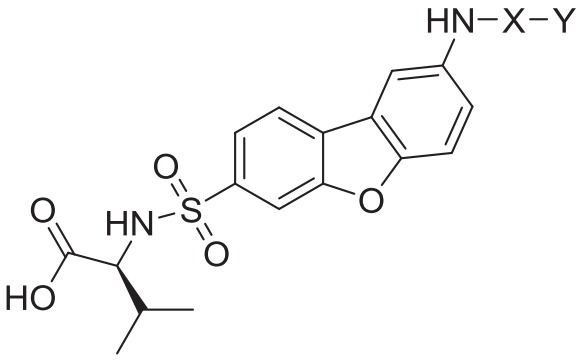


	X	Y
MMP408	CO	*O*-methyl
1	CO	*O*-(4-I)phenyl
2	CO	(4-I)phenyl
3	SO_2	(4-I)phenyl
4	CO	*O*-(4-F)phenyl

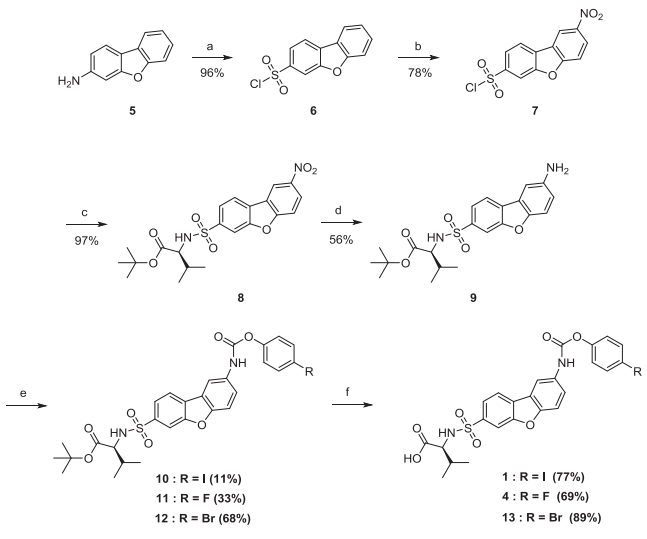
Fig. 1. Chemical structures of MMP-12-targeted compounds.

associated with the S1′ pocket;[17] however, substitution at the C7 or C8 position of the dibenzofuran structure was effective in affording MMP-12 selectivity over MMP-13.[17] Since fluorinated compound **4** also revealed MMP-12 selectivity over MMP-13,[17] we designed (*S*)-((8-(2-(4-iodophenyl)acetamido)dibenzo[*b*,*d*]furan-3-yl)sulfonyl)-L-valine (**1**) as the MMP-12-targeted iodinated compound (Fig. 1).

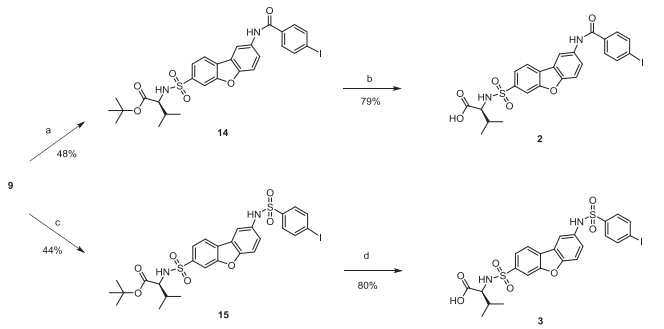
As previously reported,[17] the key intermediate **9** was prepared *via* a four-step reaction, as presented in Scheme 1. Dibenzofuran-3-sulfonyl chloride (**6**) was readily prepared from **5** with sulfur dioxide gas in the presence of CuCl. Nitration of **6** using trifluoroacetic acid and nitric acid produced the nitro derivative **7** with a yield of 78%. After the coupling reaction of **7** with L-valine *tert*-butyl ester, Pd/C catalyzed the hydrogenation generated intermediate **9** with a yield of 56%. Carbamate derivatives **10**–**12** were obtained through the reaction of intermediate **9** with different chloroformates. Target compound **1** was obtained through the deprotection of *tert*-butyl ester with trifluoroacetic acid, generated with a yield of 77%. Similarly, fluorinated compound **4** and brominated compound **13** were synthesized from **11** or **12** with yields of 69 and 89%, respectively. The synthesis of compound **2** with an amide linker and compound **3** with a sulfonamide linker is presented in Scheme 2. The condensation reaction of intermediate **9** with 4-iodobenzoyl chloride yielded derivative **14**, and the deprotection of *tert*-butyl ester of **14** with trifluoroacetic acid generated the desired compound **2** with a yield of 79%. The compound **3** was prepared by the reaction of intermediate **9** with 4-iodophenylsulfonyl chloride followed by treatment with trifluoroacetic acid.

To elucidate the *in vitro* affinity for MMP-12 and MMP-13, we performed competitive enzyme activity assays by reacting the synthesized fluorogenic peptide substrates with recombinant human MMP-12 and MMP-13. The IC_{50} values of compounds **1**–**3** were evaluated using compound **4** as a positive control (Table 1). Iodinated compound **1** revealed a higher potent inhibitory activity against MMP-12 (IC_{50} = 8.5 nM) than for MMP-13. The IC_{50} value of **1** was almost similar to that of fluorinated compound **4**, indicating that replacing the fluorine atom with an iodine atom at the para position of the phenyl carbamate moiety did not affect the inhibitory activities against MMP-12 and MMP-13. Replacing the carbamate linker with an amide or sulfonamide linker (**2** and **3**) resulted in the reduction of the inhibitory activities against MMP-12 and MMP-13. Compound **2**, with the amide linker, demonstrated selectivity toward MMP-12; however, the inhibitory activity of **2** for MMP-12 was about 60 times lower compared to that of **1**. Compound **3**, with the sulfonamide linker, revealed less selectivity and lower inhibitory activity against both MMP-12 and MMP-13. These results suggested that compound **1** could be a useful probe for MMP-12. Radiosynthesis was then performed using compound **1**.

The radioiodinated compound [^{125}I]**1** was prepared as presented in Scheme 3. Due to its relatively long half-life and low energy gamma-rays, ^{125}I was selected as a radioiodine in this study. The [^{125}I]**10** was radiosynthesized by copper-catalyzed halogen exchange reaction[18] of brominated precursor **12** with sodium [^{125}I]iodide followed by direct heating at 130 °C for 30 min. Since compound **13** (the esterolysis product of **12**) also revealed MMP-12 inhibitory activity (IC_{50} = 11 nM) similar to compound **1** (Table 1), it was necessary to completely separate [^{125}I]**10** and **12** after radioiodination. As presented in Fig. 2, the radio-HPLC analysis of the crude mixture indicated that the radioactive peak derived from the desired [^{125}I]**10** was detected at 15 min, which was

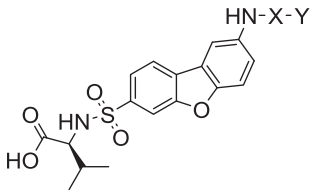


Scheme 1. Synthesis of **1**, **4**, and **13**. Reagents and conditions: (a) glacial acetic acid, cHCl, $NaNO_2$ in H_2O, SO_2 (gas), CuCl $2H_2O$, rt, (b) HNO_3, TFA, rt, (c) L-valine *t*-butyl ester, *N*,*N*-diisopropylethylamine, rt, (d) H_2, Pd/C, (e) DMAP, 4-iodophenyl chloroformate or 4-bromophenyl chloroformate or 4-fluorophenyl chloroformate, rt, (f) TFA, CH_2Cl_2.



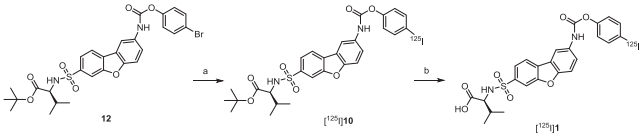
Scheme 2. Synthesis of **2** and **3**. Reagents and conditions: (a) trimethylamine, 4-iodobenzoyl chloride, (b) TFA, CH_2Cl_2, (c) trimethylamine, 4-iodophenylsulfonyl chloride, (d) TFA, CH_2Cl_2.

Table 1
In vitro affinity of MMP-12-targeted compounds for MMP-12 and MMP-13.



Compound	X	Y	IC_{50}	
			MMP-12	MMP-13
1	CO	*O*-(4-I)phenyl	8.5 ± 5.7	69 ± 11
2	CO	(4-I)phenyl	502 ± 169	>2000
3	SO_2	(4-I)phenyl	297 ± 70	403 ± 98
4	CO	*O*-(4-F)phenyl	6.2 ± 4.0	65 ± 2.2
13	CO	*O*-(4-Br)phenyl	11 ± 6.0	51 ± 9.9

Data represent mean ± SD (n = 3).



Scheme 3. Synthesis of [^{125}I]**1**. Reagents and conditions: (a) [^{125}I]NaI, $CuSO_4$ $5H_2O$, $(NH_4)_2SO_4$, (b) TFA, CH_2Cl_2.

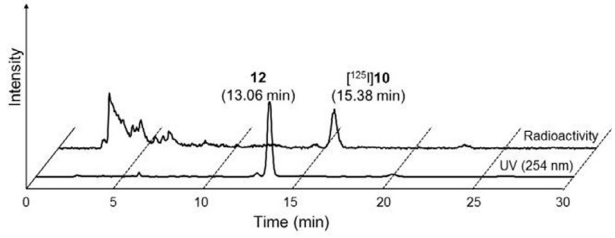


Fig. 2. HPLC analysis of [^{125}I]**10** coinjected with precursor compound **12**. HPLC conditions: the column was a COSMOSIL Cholester 4.6 mm × 250 mm, flow rate was 1.0 mL/min, UV excitation at 254 nm, mobile phase systems were CH_3CN (0.1% TFA): H_2O (0.1% TFA) = 75:25.

different from the bromo-substituted carbamates precursor **12** (13 min); however, the radiochemical yield of [^{125}I]**10** was only 2% (Table 2, Entry 1). Therefore, the reaction condition of Cu catalyzed

Table 2
Br/I halogen exchange reaction of compound **12**.

Entry	$Na^{125}I$ (MBq)	Temperature (°C)	Time (min)	Radiochemical Yield (%)
1	2.9	130	30	2
2	3.0	130	60	4
3	3.8	130	90	5
4	3.4	140	30	6
5	3.5	140	60	13
6	3.7	140	90	9
7	2.5	150	30	3
8	2.4	150	60	7
9	2.5	150	90	7

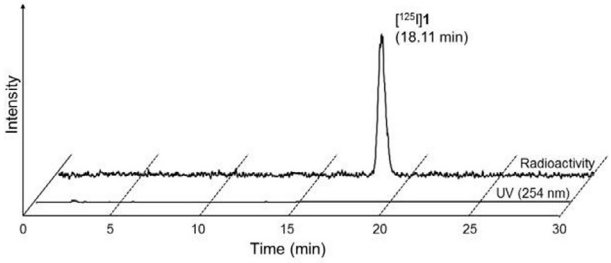


Fig. 3. HPLC analysis of [^{125}I]**1**. HPLC conditions: the column was a COSMOSIL Cholester 4.6 mm × 250 mm, flow rate was 1.0 mL/min, UV excitation at 254 nm, mobile phase systems were CH_3CN (0.1% TFA): H_2O (0.1% TFA) = 60:40.

halogen exchange reaction was optimized to improve the radiochemical yield of [^{125}I]**10**. As presented in Table 2, Entry 5, the radiochemical yield of [^{125}I]**10** was improved to 13% when the reaction was conducted at 140 °C for 60 min. Radioiodinated compound [^{125}I]**1** was obtained by the deprotection of the *tert*-butyl ester of [^{125}I]**10** with trifluoroacetic acid, with a yield of 91%. The radiochemical purity and specific activity of [^{125}I]**1** were over 98% and 4.1 GBq/μmol, respectively (Fig. 3).

We successfully designed and synthesized a series of MMP-12-targeted compounds (**1**–**3**) based on the dibenzofuran scaffold. In this study, we identified the linker structures that significantly affected the inhibitory activity against and selectivity for MMP-12. Of the new compounds, compound **1** with a carbamate linker demonstrated potent inhibitory activity against MMP-12. Copper-catalyzed halogen exchange reaction of brominated precursor **12** with [^{125}I]NaI yielded [^{125}I]**1** at high radiochemical purity (over 98%) and good specific radioactivity (4.1 GBq/μmol). These data indicate that radioiodinated compound **1** would be a potent MMP-12-targeted probe for *in vivo* imaging.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2017.11.027.

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