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Synthesis and biological evaluation of novel benzofuroxan-based pyrrolidine hydroxamates as matrix metalloproteinase inhibitors with nitric oxide releasing activity

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ABSTRACT

On the basis of the strategy of "multifunctional drugs", a series of novel matrix metalloproteinase inhibitors (MMPIs) containing benzofuroxan scaffold as a nitric oxide donor were designed, synthesized and evaluated. All synthesized compounds, especially **16a**, exhibited potent MMP-2,9 inhibitory activities, anti-proliferative activities and could produce high levels of NO in Hela cells. They were also evaluated for both of their anti-invasion and anti-angiogenesis effects. Furthermore, compared with **LY52**, **16a** demonstrated competitive antitumor activity *in vivo*. These hybrid NO-MMPIs might offer suitable scaffolds to develop valuable MMP inhibitors for the further discovery of novel anti-cancer drugs.

1. Introduction

The matrix metalloproteinases (MMPs) are a family of structurally and functionally related zinc-dependent endoproteases that are involved in the degradation and remodeling of the extracellular matrix (ECM).[1] MMPs have been linked to physiological and pathological processes such as cancer growth, arthritis, inflammation and cardiovascular.[2–4] Among all the identified MMP subtypes, MMP-2 (Gelatinases A) and MMP-9 (Gelatinases B), have been implicated in virtually all aspects of cancer progression (angiogenesis, invasion and metastasis), making them important targets for intervention.[5]

Nitric oxide (NO) is a key mediator that plays very important roles in mammalian physiology and pathophysiology. High levels of NO and its metabolic derivatives, the reactive nitrogen species (RNS) and reactive oxygen species (ROS), have profound effects on tumor cells proliferation, angiogenesis and metastasis by functional proteins.[6] Recently, there is some evidence that the production of NO by some synthesized NO-releasing compounds has shown potent cytotoxicity against human carcinoma cells *in vitro* and anti-metastasis activity *in vivo*.[7,8] Indeed, exogenous NO-donors, such as furoxans, have shown stable against acid and base, and it can produce high levels of NO, leading to potent cytotoxicity against broad types of cancer cells.

Previous studies have demonstrated that increased NO decreases MMP-2,9 expression and activity. The exact relationship between NO and MMP-2,9 is evidently complicated. Increased expression of MMP-2 and MMP-9 is responsive to various cytokines including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α).[9–11] High-output levels of nitric oxide (NO) is reported to interact with these signaling pathways by the inducible isoform of NO synthase (iNOS), thus MMP-inhibitory NO donor hybrid might be useful to downregulate MMP-2,9 expression and it could play a significant role for the pharmaceutical treatment in pathological situations.[12,13]

The current authors' and their colleagues have recently endeavored to identify pyrrolidine derivatives as effective MMP inhibitors, exemplified by **LY52** (Fig. 1).[14,15] Vast number of sulfonamide-based derivatives as potential MMP inhibitors including **AG 3340** and **RS 130,830** have also been reported (Fig. 1).[16,17] Accordingly, the introduction of the sulfonamide group and caffeoyl group into the MMP inhibitors enhanced their zinc-binding effect, and also enable it to plunge into the enzyme binding domain deeply. Inspired by the theory of 'multifunctional drugs', we thereof have designed and synthesized a series of novel NO-MMPIs containing benzofuroxan as the NO donor,[18] which is integrated at the C-4 position of the pyrrolidine scaffold. The 4-phenoxyphenylsulfonyl and 3,4-dimethoxyphenylacryloyl group are

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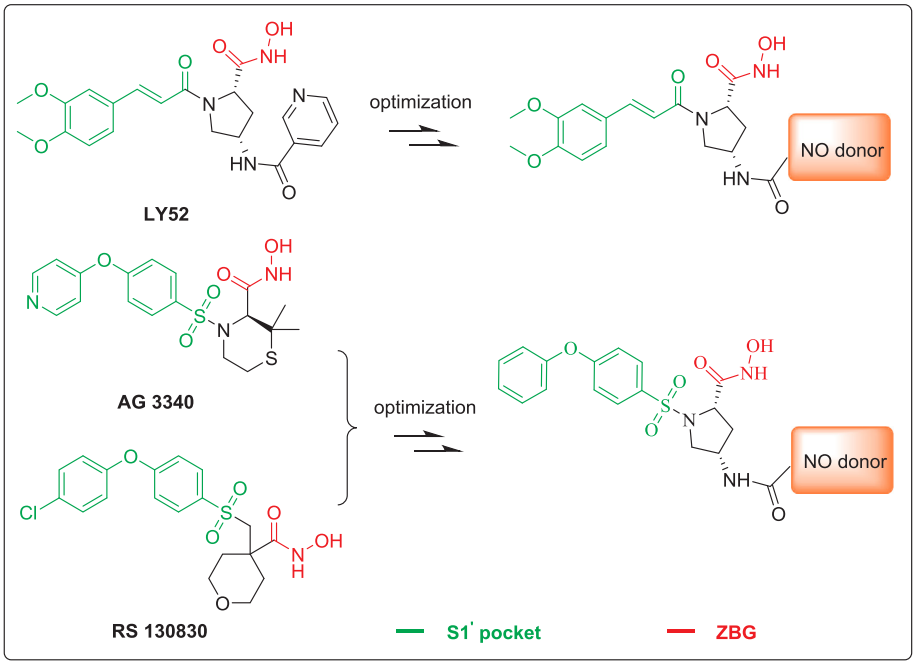


Fig. 1. Design strategy of the target compounds.

introduced into the *cis*-γ-amino-L-proline scaffold occupying the deep S1′ pocket, resulting effective enzyme interaction.[19–21] The hydroxamate was known as an effective ZBG to chelate the active site of catalytic zinc ions.[22] In the present study, we hereby described the synthesis and relevant biological evaluation of all the compounds.

2. Chemistry

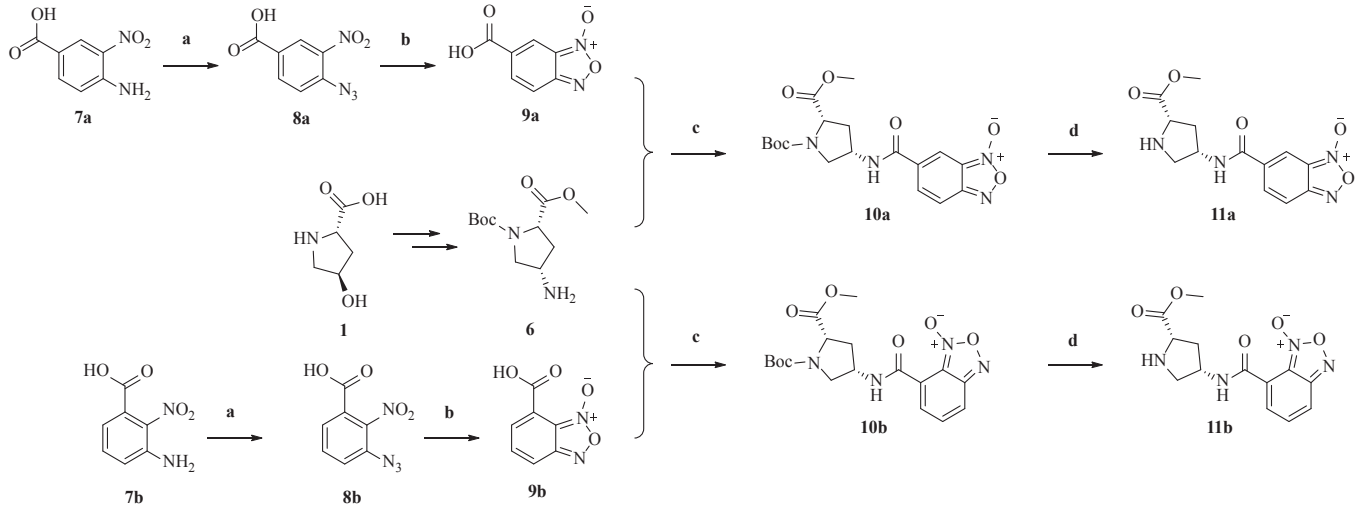
The routes of intermediate compounds **11a** and **11b** were efficiently synthesized following the procedures as illustrated in Scheme 1. The key intermediate (2*S*,4*S*)-1-*tert*-butyl-2-methyl-4-aminopyrrolidine-1,2-dicarboxylate (**6**) was prepared by esterification, acylation, sulfonation, SN_2 nucleophilic substitution, and Staudinger reaction.[23] The intermediate 5(6)-carboxybenzo[c][1,2,5]oxadiazole 1-oxide (**9a**) was prepared starting from the 4-amino-3-nitrobenzoic acid (**7a**) by diazo-reaction with sodium azide to get compound **8a**, then refluxed in toluene. The whole preparation processes of target compounds **9b** were similar as that of **9a**. The target compounds **16a** and **16b** were prepared *via* the synthetic route showed in Scheme 2. The intermediate compound **11a** and **11b** were treated with 4-Phenoxybenzene-1-sulfonyl chloride (**12**) to get compounds **14a** and **14b**, which be treated with NH_2OK in anhydrous methanol to yield the target compounds **16a** and **16b**.

The target compounds **17a** and **17b** were prepared *via* the synthetic route showed in Scheme 2. The intermediate compound **11a** and **11b** were treated with (*E*)-3-(3,4-Dimethoxyphenyl)-2-propenoic acid (**13**) to get compounds **15a** and **15b**, which be treated with NH_2OK in anhydrous methanol to yield the target compounds **17a** and **17b**.

3. Results and discussion

3.1. MMP-2 and MMP-9 inhibition assay

The newly synthesized benzofuroxan pyrrolidine derivatives were assayed for their inhibitory activities toward MMP-2 and MMP-9, and



Scheme 1. Synthesis route of intermediate **11a** and **11b**. Reagents and conditions: (a) 33% $NaNO_2$, 35% HCl, NaN_3, 84%; (b) toluene, 80%; (c) IBCF, THF, 76%; (d) TFA, DCM, 92%.