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Short communication

# Design, synthesis and biological evaluation of nitrogen-containing macrocyclic bisbibenzyl derivatives as potent anticancer agents by targeting the lysosome

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ABSTRACT

A series of novel nitrogen-containing macrocyclic bisbibenzyl derivatives was designed, synthesized, and evaluated for antiproliferative activity against three anthropic cancer cell lines. Among these novel molecules, the tri-*O*-alkylated compound **18a** displayed the most potent anticancer activity against the A549, MCF-7, and k562 cancer cell lines, with $IC_{50}$ values of 0.51, 0.23, and 0.19 μM, respectively, which were obviously superior to those of the parent compound riccardin D, and were 3–10-fold better than those of the clinical used drug ADR. The bis-Mannich derivative **11b** also exhibited significantly enhanced antiproliferative potency, with submicromolar $IC_{50}$ values. Structure-activity relationship analyses of these newly synthesized compounds were also performed. Mechanistic studies indicated that these compounds could target the lysosome to induce lysosomal membrane permeabilization, and could also induce cell death that displayed features characteristic of both apoptosis and necrosis.



## 1. Introduction

Lysosomes are acidic intracellular organelles that are involved in several cellular processes, including receptor degradation, autophagy, apoptosis, post-translational protein maturation, and the extracellular release of active enzymes [1–3]. They control the recycling of large amounts of cellular organelles and macromolecules through the actions of more than 50 acid hydrolytic enzymes. Interestingly, lysosomes in cancer cells are larger, less stable, more numerous, and exhibit greater cathepsin activity than those in normal cells [3,4]. Additionally, weakly basic drugs have been found to accumulate specifically in lysosomes, after which they can no longer easily diffuse out of the vesicles because they become protonated [5,6]. This accumulation of weakly basic drugs could lead to changes in osmolality within the lysosomes, which results in lysosomal swelling and rupture that leads to lysosomal membrane permeabilization (LMP) [7]. LMP usually causes lysosomal proteases to leak into the cytosol and trigger initiation of either the apoptotic or necrotic cell death pathways [8,9]. Therefore, the lysosome is a critical target for anticancer therapy [10,11], and agents that interact with lysosomes show promise for the development of novel and potent anticancer drugs.

Macrocyclic bisbibenzyls are a series of phenolic natural products that are mainly found in liverworts [12]. These natural products exhibit versatile biological activities, including antifungal, antibacterial, antiviral, anti-mitotic, antioxidant, cytotoxic, muscle-relaxing, LXR-modulating, and NOS-inhibiting activities [13–17]. Therefore, bisbibenzyls are of great interest to natural product researchers because of their potential applications as pharmacological agents. Riccardin D, a macrocyclic bisbibenzyl isolated from *Marchantia polymorpha* L., has been shown to exhibit robust anticancer activity [18,19]. In our previous study, we prepared two Mannich base derivatives of riccardin D—RDN-1 and RDN-2 (Fig. 1)—that exhibited markedly improved anticancer activity compared with the parent riccardin D. Interestingly, both molecules were found to accumulate in the lysosomes. Mechanistic

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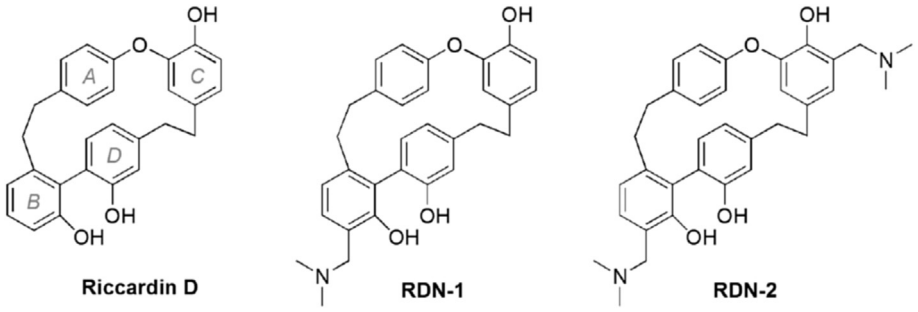


**Fig. 1.** The chemical structures of riccardin D, RDN-1 and RDN-2.

studies indicated that both compounds could induce LMP and cathepsin release from the lysosomes into the cytosol, and furthermore could induce cell death that displayed features characteristic to both apoptosis and necrosis. We then predicted that the introduction of basic groups to riccardin D might improve its anticancer activity by promoting lysosome targeting [20,21].

Based on the findings described above, a series of riccardin D derivatives with weakly basic groups, especially groups that contained nitrogen atoms, was designed. Our modification strategy for riccardin D is shown in Fig. 2. We first modified the Mannich side chains of RDN-1 and RDN-2, and changed the methylamino groups to either ethylamino, pyrrolidinyl, piperidinyl, morpholino, thiomorpholino, 4-hydroxypiperidinyl, 4-(4-methoxyphenyl)piperazinyl, 4-methylpiperazinyl, bis((dibutylamino)methyl) amino, or bis(2-methoxyethyl)amino groups to evaluate how the Mannich side chains influence anticancer activities. Second, we directly introduced a nitrogen atom into the arene ring of riccardin D and prepared some derivatives based on the structure of aniline to determine the effect of these substitutions on the cytotoxic activity of riccardin D. Finally, we focused on the modification of phenolic groups of riccardin D by introducing O-alkylation of different nitrogen-containing electrophiles, and the resulting mono- and tri-*O*-alkylated derivatives were obtained for structure-activity relationship (SAR) studies.

Herein, we describe the synthesis of a series of novel nitrogen-containing riccardin D derivatives and the evaluation of these compounds for antiproliferative activities using the MTT assay. The anticancer mechanisms of two representative derivatives were also investigated using flow cytometry and confocal microscopy. Together, these findings indicated that these novel weakly basic derivatives of macrocyclic bisbibenzyl represent a novel class of potent anticancer agents that target lysosomes.

## 2. Results and discussion

### 2.1. Chemistry

Riccardin D was prepared in 11 steps with a satisfactory yield following a procedure reported previously [22], and was used as a staring material for further structural modifications. The general procedure is outlined in Scheme 1. Mannich reactions of riccardin D were carried out using different secondary amines [23]. The reaction of riccardin D, amine, and formaldehyde at a molar ratio of 1:1.5:1.5 in ethanol yielded the mono-Mannich derivatives **10a**–**10m** as the main product at a moderate yield. The position of the Mannich side chain was then determined based on a NOE spectrum. As shown in Fig. 3, correlations between $H_2$-13′ and H-5′ were determined for representative compound **10l** (Figs. S25 and S26), which indicated that mono-Mannich derivatives have Mannich side-chains on the 4′-position of arene *B*. The substitution pattern in the mono-Mannich derivatives was consistent with that of RDN-1, which might occur because of the stereo-hindrance effect was reduced (compared with 2-position on arene *D*) and a higher electron density (compared with 13-position on arene *C*) was present at the 4′-position of arene *B*. When the quantity of the secondary amine and formaldehyde were increased to 3 and 3 equivalents, respectively, the bis-Mannich derivatives **11a**–**11m** were obtained as the main products. Basic groups were introduced at the 4′-position on arene *B* and 13-position on arene *C*, which was confirmed by the NOE spectrum of the representative derivative **11l**. As shown in Fig. 3, correlations of $H_2$-13′ with H-5′, and $H_2$-17′ with H-14 were determined (Figs. S53 and S54, respectively).

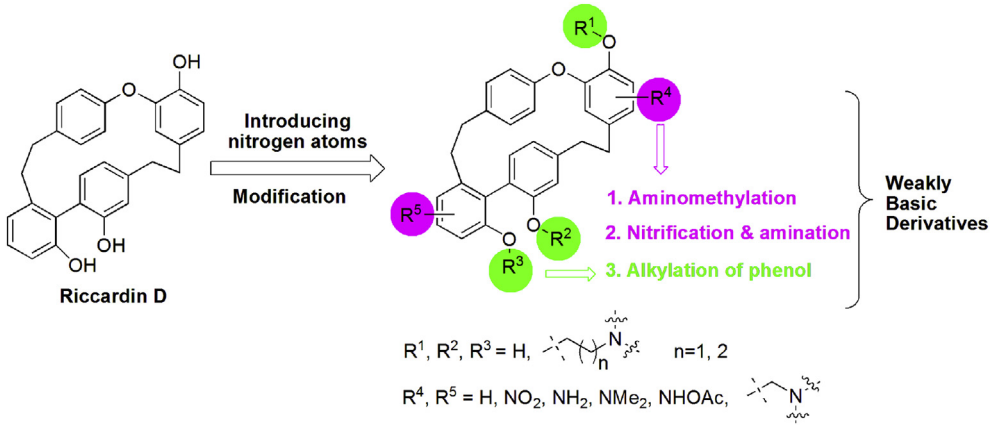


**Fig. 2.** Modification strategy for riccardin D derivatives.