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Design, synthesis and biological evaluation of glutamic acid derivatives as anti-oxidant and anti-inflammatory agents

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ABSTRACT

A series of glutamic acid derivatives was synthesized and evaluated for their antioxidant activity and stability. We found several potent and stable glutamic acid derivatives. Among them, compound **12b** exhibited good *in vitro* activity, chemical stability and cytotoxicity. A prototype compound **12b** showed an anti-inflammatory effect in LPS-stimulated RAW 264.7 cell lines and in a zebrafish model.

Reactive oxygen species (ROS) are produced in living organisms either due to normal cell metabolisms *in situ* or due to environmental factors. When ROS (free radicals) such as superoxide anions, hydroxyl radicals, hydrogen peroxide, and hypochlorous acid cannot be effectively eliminated, their accumulation in the body generates oxidative stress. This can cause chronic and degenerative illnesses such as inflammation, cardiovascular diseases, cancer, aging-related disorders, metabolic disorders, and atherosclerosis.[1] ROS attack unsaturated fatty acids resulting in membrane lipid peroxidation, reduced membrane fluidity, loss of enzymes and receptor activities and damage to membrane proteins, ultimately leading to cell inactivation.[2] Despite the existence of natural defense mechanisms, excessive production of ROS over the lifetime of a cell causes progressive oxidative damage and ultimately cell death.[3]

Many antioxidant compounds are extracted from plants and tested as the main ingredients of cosmetics. Ramalin, isolated from the Antarctic lichen, *Ramalina terebrata* (Ramalinaceae) has antioxidant and anti-inflammatory activities.[4–6] It has also been shown *in vivo* to inhibit melanogenesis.[7]

Despite these properties, the use of Ramalin is limited because it is unstable, and spontaneously decomposes at room temperature.[8] To overcome its instability, a method for stabilizing Ramalin using vitamin C has been reported, however, the instability associated with the parent structure unsolved. This situation prompted us to structurally modify Ramalin to improve its stability as well as its potency. In the present study, we report the synthesis, structure activity relationships (SAR) and biological evaluation of glutamic acid derivatives as anti-oxidant and anti-inflammatory agents (see Fig. 1).

The Ramalin structure can be divided based on three functional units such as an ortho hydroxy phenyl group, hydrazine group and L-glutamic acid as shown in Fig. 2. We tried to modify and optimize Ramalin to identify more stable and potent derivatives.

The synthesis of glutamic acid derivatives **4**(**a–l**), **6a**, **8a**, and **12a,b** is depicted in Scheme 1. A commercially available aniline derivative **1**(**a–l**) was reacted with sodium nitrite under acidic conditions to obtain the phenyl hydrazine hydrochloride **2**(**a–l**). Compound **2**(**a–l**) reacted with 1-benzyl N-carbobenzoxy-L-glutamate **13** and O-(benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate to give Cbz protected glutamic acid derivatives **3**(**a–l**). Benzyloxy carbamate and benzyl groups were deprotected with hydrogen gas and palladium on charcoal to obtain **4**(**a–l**). 2-Hydroxy aniline **1a** coupled with 1-benzyl N-carbobenzoxy-L-glutamate **13** followed by hydrogenation to give **6a**.

(2-(Benzyloxy)phenyl)hydrazine **2a** was coupled with 5-(benzyloxy)-2-(((benzyloxy)carbonyl)amino)-5-oxopentanoic acid **14**

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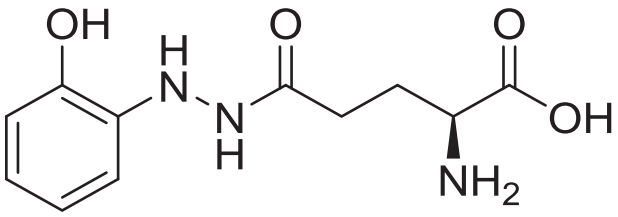


Fig. 1. Structure of Ramalin.

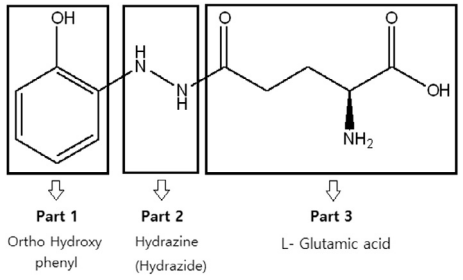


Fig. 2. Structure of Ramalin divided into three functional units.

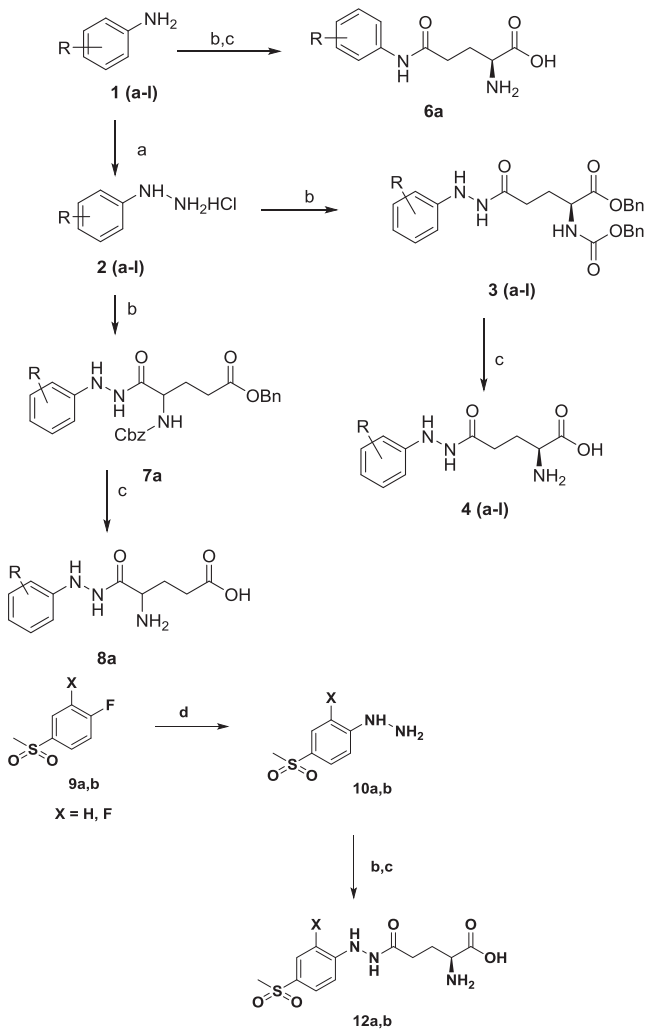
to afford **7a** followed by hydrogenation to give **8a**. 1-Fluoro-4-(methylsulfonyl)benzene **9a** and 1,2-difluoro-4-(methylsulfonyl) benzene **9b** were treated with hydrazine monohydrate afforded the hydrazine hydrochlorides **10a,b**. Subsequent condensation with 1-benzyl N-carbobenzoxy-L-glutamate **13** followed by hydrogenation was carried out to obtain **12a,b**.

The *in vitro* antioxidant activity of the synthesized glutamic acid derivatives was evaluated using a DPPH assay. The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol.[9] This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to a colorless ethanol solution. The DPPH assay is an easy and rapid method to evaluate antioxidants by spectrophotometry. Thus it allows assessment of multiple products at a time. Sixteen different types of glutamic acid derivatives were screened by using the DPPH assay. Ascorbic acid was used as a reference compound.

Initially, the SARs of glutamic acid derivatives were investigated and the results are summarized in Table 1. Ramalin exhibited antioxidant activity with an IC_{50} value of 8.67 μg/ml, which is 2-fold higher than that of ascorbic acid. Replacement of hydrazine in Ramalin with an amide (**6a**) caused loss of *in vitro* activity. In addition, opposite glutamic acid (**8a**) and protected glutamic acid derivatives (**3a**) showed decreased antioxidant activities. On the basis of these data, we retained the hydrazine and glutamic acid moieties and further optimized the hydroxyphenyl moiety.

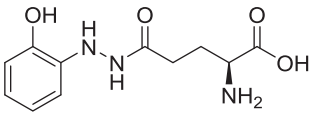
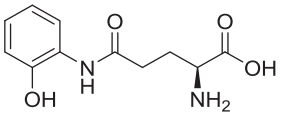
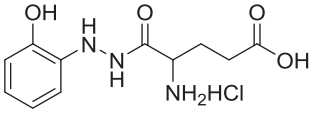
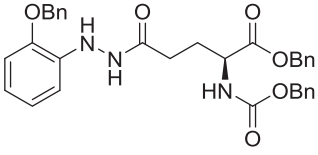
Diverse mono-substituted phenylhydrazine glutamic acid derivatives were synthesized and evaluated for their activity. As can be seen in Table 2, the o-methyl derivative (**4b**) showed better *in vitro* activity than Ramalin (**4a**) with an IC_{50} value of 6.24 μg/ml. The o-ethyl derivative (**4c**) exhibited decreased activity. Although several meta substituted compounds were synthesized and evaluated, however the activities were not effective than Ramalin. Para methyl (**4h**) and methylsulfonyl (**12a**) derivatives were found to possess single digit IC_{50} value of 9.99 and 9.57 μg/ml, respectively. Based on these data, we further optimized the phenylhydrazine moiety with more substituents.

Several disubstituted phenylhydrazine glutamic acid derivatives were synthesized and evaluated. As shown in Table 3, 2,4-di-substituted glutamic acid derivative **4j** showed better *in vitro* activity than 3,5-di-substituted **4l**. Therefore, more 2,5-di-sub-



Scheme 1. Reagents and conditions: (a) 1) Sodium nitrite, 6 M HCl, H_2O, 0 °C; 2) Tin chloride, 6 M HCl, 0 °C, 75% (over two steps); (b) 1-Benzyl N-carbobenzoxy-l-glutamate **13** or 5-(benzyloxy)-2-(((benzyloxy)carbonyl)-amino)-5-oxopentanoic acid **14**, o-(benzotriazol-1-yl)-N,N,N′,N′-tetramethy-luroniumtetrafluoroborate, DIPEA, DMF, room temperature, 85%; (c) H_2, Pd/C, methanol, room temperature, 80–85%; (d) Hydrazine monohydrate, ethanol, reflux, 70%.

Table 1
Anti-oxidant activity of Ramalin derivatives.

compound	Structure	IC_{50} (μg/ml)
4a(Ramalin)		8.67
6a		162.42
8a		37.19
3a		44.98
Ascorbic acid		17.38