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Synthesis and biological evaluation of the codrug of Leonurine and Aspirin as cardioprotective agents

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ABSTRACT

The novel codrugs of Leonurine and Aspirin, compounds **545** and **503** have been synthesized and evaluated on their cardioprotective effects. Preliminary pharmacological studies showed that both compounds **545** and **503** were able to increase cell viability of hypoxia-induced H9c2 cells, and compound **545** exhibited at least ten fold potency than **503** and their parent drugs (Leonurine and Aspirin). Further mechanisms studies indicated that the cardioprotective effect of **545** due to its (1) anti-oxidative ability by increasing SOD and CAT enzymes activity and decreasing MDA content and LDH leakage rate, (2) anti-apoptosis activity by regulating apoptosis-associated proteins expression during hypoxia, (3) anti-inflammatory effect by suppression of pro-inflammatory mediators. All of these results demonstrate that compound **545** as a new class of Leonurine analogue could be a drug candidate in our further drug development studies.

The concept of codrug or mutual prodrug refers to two or more compounds with similar or different therapeutic effects bonded via a covalent chemical linkage. It has to be hydrolyzed to provide two (or more) different drugs in cells or organs. So the ester group and amide group are the prefer linkage in the codrug designing. As it well known that codrugs could elicit synergistic action or help the parent drugs to target specific site/organ/cells respectively. Therefore, the drug design basing on codrug strategy has become an efficient approach for drug optimization.[1]

Because of the high mortality, acute myocardial ischemia (AMI), has been one of the most serious diseases threatening human health. Sustained ischemia causes several types of damage to cardiac tissues. The causes of AMI are complicated and varied. As we know, apoptosis, oxidative stress and inflammation are tightly related with the development and progression of AMI. Therefore, preservation of cardiac tissues and cells from the deleterious effects of apoptosis, oxidative stress and inflammation would be an effective approach to treat AMI.

Leonurine (**LEO**) is an effective ingredient derived from *Leonurus artemisia* which has long been used in Chinese traditional medicine. The pharmaceutical studies on **LEO** show that it has cardioprotective effects both in vitro and in vivo due to its anti-oxidation and anti-apoptosis properties.[6–9] Moreover, the animal studies of **LEO** display cardioprotective effects on myocardial ischemia (MI) disease in rats after intraperitoneal injection.[10] However, several issues, such as low content in natural resource, relatively short half-life and moderate biological activity,[11,12] have blocked the progress on the therapeutic development of **LEO**. To improve pharmacological efficacy of **LEO**, structure modification might be a practicable way to gain more potent drug candidates.

Aspirin (ASA), one of the nonsteroidal anti-inflammatory drugs (NSAIDs), is the most versatile pharmaceutical agent. It is used most commonly in the treatment of pain, fever, and inflammation. It also has an antiplatelet effect. So long-term use of Aspirin at low doses could help prevent heart attacks, strokes, and blood clot formation in people at high risk of developing blood clots. Otherwise, some evidences show that Aspirin may be effective at preventing certain types of cancer, particularly colorectal cancer. Due to its significant and versatile pharmaceutical effects, Aspirin has become a good choice for developing new drugs by the strategy of codrug designing.[2–5] Several codrugs of Aspirin have been reported and even used in clinics, such as Benorilate.

As inspired by the studies of codrugs, we were intrigued to assume whether combining **LEO** with another cardioprotective compound such as Aspirin to form new compound will have a dual mode of cardioprotective action and be able to improve cardioprotective efficiency of the precursor. Based on these considerations, compound **545** and **503**, two codrugs of **LEO** and Aspirin as well as **LEO** and salicylic acid, have been designed and synthesized.

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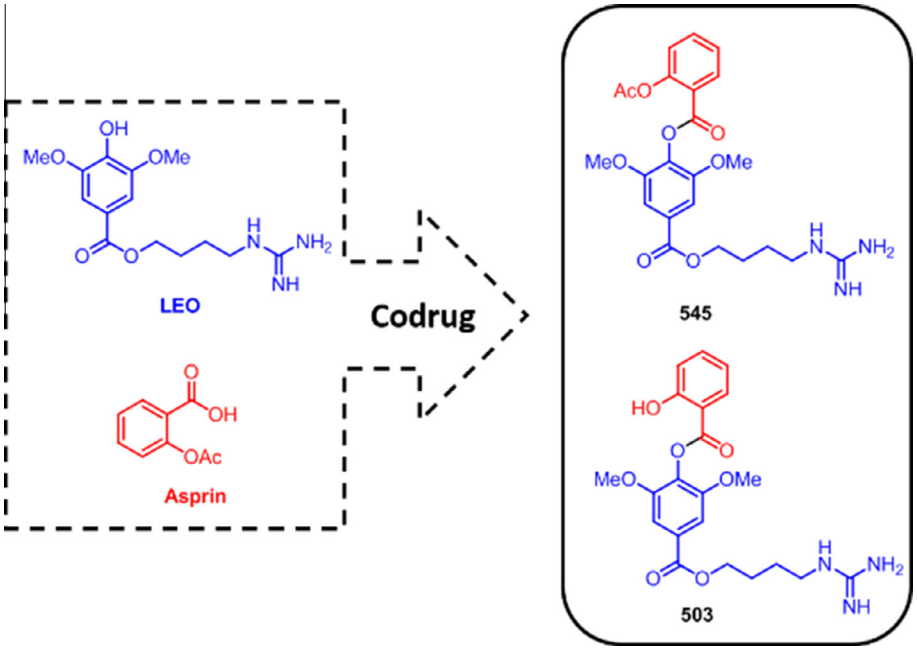
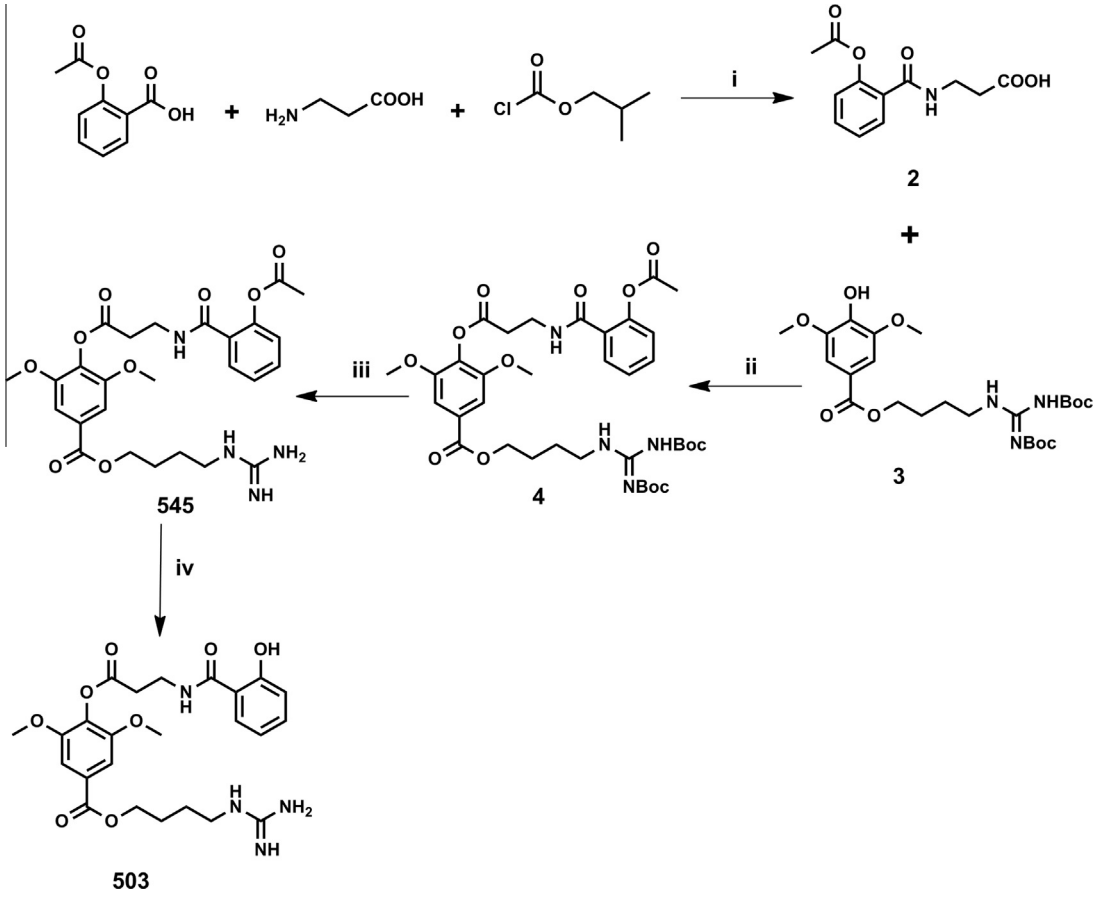


Figure 1. Chemical structure of codrugs and their parent drugs.



Scheme 1. The synthetic route of Leonurine-(β-alanine)-Aspirin conjugates (**545** and **503**). Conditions: (i) TEA, THF, −5 °C, 3 h, (ii) DPTs/DIC, CH_2Cl_2, rt, 12 h (iii) TFA, CH_2Cl_2, rt, 2 h, (iv) hydrochloric acid–methanol solutions, CH_2Cl_2, rt, 2 h.

The preliminary studies indicated both compounds exhibited cardioprotective effects, and compound **545** even possessed higher biological activities than its parent drugs (at less 10-fold than its parent drugs, **LEO** and Aspirin). These results encouraged us to further explore the pharmaceutical mechanisms of compound **545**. Therefore, the effects of compound **545** on anti-oxidant, anti-apoptosis and anti-inflammatory were evaluated. The results suggest compound **545** is able to prevent cellular apoptosis by up-regulating the expression of Bcl-2 and down-regulating Bax even under a lower concentration (10-fold less than its parent drugs, **LEO** and

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