New Songorine Derivatives as Novel G Protein-Coupled Receptor Antagonists: Syntheses and Preliminary Bioactivities Assay

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Abstract In our early screening process, songorine isolated from *Aconitum brachypodum* diels possessed prominent activity of inhibiting class B G protein-coupled receptors (GPCRs). For further study, a series of ester and carbonyl derivatives were synthesized and their potential inhibitory activities on class B GPCRs were also assayed by the Double Antibody Sandwich ELISA (DAS-ELISA) *in vitro*. Moreover, the structure-activity relationships (SARs) of songorine derivatives were discussed in detail. In this paper, the story behind this study was described.

Keywords songorine derivatives, G protein-coupled receptors, inhibition, synthesis

G protein-coupled receptors (GPCRs) are the largest family of membrane receptors such as epinephrine receptors and opioid receptors. They have seven transmembrane structures and abundant endogenous ligands, including neurotransmitters, polypeptides and hormones. GPCRs combine chemicals around cells, and activate a series of signaling pathways causing state changes in cells to regulate the personal growth, metabolism, endocrine, nerve conduction, and other important life activities.^[1] They are also one of the main targets for drugs, widely used in many major diseases including cancer, cardiovascular disease, diabetes, etc. So far, there are more than 800 GPCRs in human body, however, only 50 have been developed into drug targets, which can be divided into agonists and antagonists.^[2] Therefore, GPCRs are considered to possess broad application prospects in new therapeutic drugs.

Class B G protein-coupled receptors, also known as secretory G protein-coupled receptors, are peptide receptors binding to physiologically important peptide hormones to transmit downstream cell signals. Structurally, the Class B GPCRs are composed of an extracellular N-terminal domain (ECD) of 120–160 residues and seven transmembrane domains (TMD) of 310–420 residues.^[3] Class B GPCRs are critical drug targets for the treatment of a variety of human diseases, for instance, type II diabetes, obesity, cardiovascular diseases and mental diseases.

Songorine (Figure 1), a C22 diterpenoid alkaloid isolated from *AAconitum. brachypodum*, has various biological

activities, including anti-arrhythmia, anti-anxiety, analgesia, anti-inflammatory, anti-infection and promoting tissue regeneration.^[4–8] Our recent studies indicated that songorine (IC₅₀ = 3.21 nM) had an inhibitory effect on Class B GPCRs *in vitro* utilizing Double Antibody Sandwich ELISA (DAS-ELISA), a rapid and sensitive method completed by combining antigens with antibodies,^[9,10] which is suitable for preliminary screening.

Based on previous screening results, nineteen songorine derivatives, mainly ester and carbonyl derivatives, were synthesized. Furthermore, their inhibitory activities against Class B GPCRs were preliminarily investigated using similar methods mentioned above. During the experiment, four concentration gradients of 10 ng/mL, 1 ng/mL, 0.1 µg/mL, and 10 µg/mL were set and the corresponding inhibitory rates were calculated, which were eventually converted to IC₅₀ for our discussion of their activity and SARs.

According to the experimental data, we disclosed that most derivatives had a good inhibitory effect on Class B GPCRs *in vitro*. From the perspective of derivatives' structures, the ester structure in the derivatives was more important than the carbonyl group, and the inhibitory activities of most short-chain esters were more prominent than that of carbonyl derivatives. SARs of songorine derivatives in Class B GPCRs antagonism were summarized in Figure 2. By the way, the structures of the derivatives with better inhibitory activities were displayed in Figure 3, including IC₅₀ values.

Nowadays, the mainstream hypoglycemic drugs include insulin-secreting agents (glibenclamide, repaglinide, etc.), metformin, and α-glucosidase inhibitors (acarbose, etc.). Unfortunately, these drugs only direct at symptom, and there is still a lack of drugs for the etiological treatment of type II diabetes. Songorine and its derivatives are novel class B GPCRs antagonists connecting closely to metabolic diseases and have never been reported. Due to the particularity of Class B GPCRs, small molecule drugs targeting these kinds of receptors are not available. Therefore, these molecules are potential to develop as pharmaceuticals for type II diabetes, which is urgent and significant. However, diverse songorine derivatives and their

Figure 1 The chemical structure of songorine.

Figure 2 The SARs of songorine derivatives in GPCRs inhibitory activity.

Figure 3 The chemical structures and IC_{50} values of the most active songorine derivatives.

mechanisms of these effects need to be further explored in the future.

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