

## DOCTORAL THESIS

### Discovery of COX-2 selective inhibitors from saussurea laniceps using an enzyme-anchored nanomagnetic ligand fishing platform

Chen, Qilei

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## Abstract

Serious cardiovascular side effects are reported from synthetic cyclooxygenase-2 (COX-2) selective nonsteroidal anti-inflammatory drugs, the most common medication for rheumatoid arthritis (RA) and osteoarthritis (OA). Natural products from herbal medicine are inspirational source of safe and effective remedy due to its distinguished chemical diversity. Nanomagnetic ligand fishing using enzyme-anchored-magnetic nanoparticles (MNPs) is an advanced selective bioseparation strategy based on macromolecular target–ligand binding, which can screen enzyme inhibitors from complex mixtures. “Snow lotus” herbs have been clinically applied as safe and effective treatment for arthritis throughout centuries in Asia. Some major chemicals from the herbs have been found with anti-COX-2 activities. It is therefore hypothesized that novel and safe COX-2 selective inhibitors can be separated from a most representative snow lotus herb via ligand fishing using COX-2-functionalized MNPs (COX-2–MNPs), and that the efficacy and safety of the screened COX-2 ligands can be verified by subsequent evaluation.

*Saussurea laniceps* Hand.-Mazz. (SL), *S. medusa* Maxim. (SM) and *S. involucrata* (Kar. et Kir.) Sch.Bip. (SI) are three authenticated sources of “snow lotus” herbs. An ultra-high performance liquid chromatography hyphenated with diode array detector and quadrupole time of flight-mass spectrometry (UPLC-DAD-QTOF-MS) method was developed to analyze 49 herbal samples for species analysis and overall quality evaluation. With 25 simultaneously identified constituents, of which 12 were quantified, the chemical determination, four-dimensional principle component analysis (4D-PCA), and orthogonal hierarchical cluster analysis (2D-HCA) showed a distinctive bioactive component profile of SL from the other two species, and explained the therapeutic potency of SL. As a result, SL has been chosen as a model herb to screen for novel and safe COX-2 selective inhibitors.

With systematic uniform experimental designs and statistical modeling, COX-2–MNPs with high magnetic moments and outstanding enzyme activity have been synthesized. Four COX-2-selective compounds, namely, chlorogenic acid, syringin, umbelliferone, and scopoletin, were separated from the herbal extract using fine-tuned fishing protocol and were identified by UPLC-DAD-QTOF-MS. All the four ligands were proved with evidently lower *in vitro* and *in vivo* cardiotoxicity than celecoxib, a known selective COX-2 inhibitor. Some of them exerted potent anti-inflammatory activities on cells, and their optimum combination ratios were investigated. Among the ligands, scopoletin showed most evident therapeutic potential in rats with adjuvant-induced arthritis and anterior cruciate ligament

transection (ACLT)-induced OA, respectively, by alleviating clinical statuses, immune responses, and joint pathological features. An equal mixture of scopoletin and syringin brought possible synergistic remedial effects on rat OA. Molecular docking results explained the structure-specific enzyme-binding affinities of the ligands; the ligands' inhibition on COX-2 may involve direct interaction as well as upstream signaling pathways.

In conclusion, promising candidates of COX-2 selective inhibitors, *e.g.* scopoletin, have been screened and validated on a nanomagnetic ligand fishing platform using COX-2-MNPs from the extract of SL, a most representative snow lotus herb with distinctive chemical composition and outstanding therapeutic efficacies. The quality evaluation strategy of snow lotus herbs combining chemical determination and multidimensional chemometric analysis can be applied in other multi-original herbal medicines. The nanomagnetic ligand fishing platform of compound bio-separation and multi-model bio-evaluation should be equally valuable for uncovering other therapeutic chemicals in different natural sources.

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