DOCTORAL THESIS

Structural Modification, Anticancer Activity Evaluation and Structure-Activity Relationship Studies Based on Natural Ent-Kaurane Diterpenoids

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ABSTRACT

Background: The healing power of plants has long been recognized in ethnomedicines such as Traditional Chinese Medicine (TCM) or Ayurvedic medicine. Up to date, medicinal plants are still serving as vital sources for drug discovery due to their containing abundant structurally diversified secondary metabolites. As an important class of plant metabolites, *ent*-kaurene diterpenes are widely distributed in the vascular and non-vascular terrestrial plants such as liverworts and the plants in the families of Lamiaceae, Compositae, Pteridaceae and Euphorbiaceae. Over the past decades, this class of natural products have received considerable attention owing to their broad spectrum of bioactivities ranging from antitumor, antibacterial, antiviral and anti-inflammatory activities. Structurally, compounds with a 6/6/6/5-fused tetracyclic ring system are the most common class among the discovered *ent*-kaurane diterpenoids. They possess multiple functional groups in their carbon skeletons. An *α*, *β*-unsaturated conjugate system in D-ring is constantly found in the *ent*-kauranes that to own antitumor activities. However, most natural *ent*-kaurenes possess moderate anticancer potency with observed toxicity in the biological systems, which hampers their further development as clinical drugs. Optimization of these *ent*-kaurane scaffolds based on structure-activity relationship (SAR) is considered as an effective strategy to address these problems. In the recent years, modification of *ent*-kaurane diterpenoids has resulted in numerous *ent*-kaurane derivatives with enhanced anticancer activities. As part of our effort, we carried out structure modification of some of our previously isolated *ent*-kauranes from *Isodon* medicinal plants, and observed anticancer activity enhancement. We thus believe the effective modifications of *ent*-kaurane diterpenoids could produce novel small molecule of entities with enhanced bioactivity potency and low toxicity for further development of *ent*-kauranes as anticancer drug candidates.

Objectives: 1) To elucidate the SAR of 5F as a representative *ent*-kaurane diterpenoid with the purpose of providing an effective modification approach that can

optimize its drug profiles and be applied on other *ent*-kaurane diterpenoids; 2) To construct a library of *ent*-kaurane derivatives using different natural *ent*-kaurane scaffolds based on their *α*, *β*-unsaturated ketone moieties; 3) To evaluate the *in vitro* and *in vivo* anticancer activities of the synthetic *ent*-kaurane analogues; 4) To prepare a pair of biotin-conjugated probes based on a natural *ent*-kaurane diterpenoid and its corresponding derivative, aiming to elucidate the underlying anticancer mechanism by identifying the direct binding target(s) of *ent*-kauranes *via* pull down assay.

Methods: To achieve the above objectives, 5F, an *ent*-kaurane diterpenoid isolated from *Pteris semipinnata,* was selected as a model compound for structure modification by considering its containing three important functional groups (carboxylic group, hydroxyl group and *α*, *β*-unsaturated ketone) on rings A, C, and D that can also be found in other *ent*-kauranes. Six series of 5F derivatives were designed and synthesized based on these functional groups. Two other *ent*-kaurane diterpenoids (henryin A and oridonin) have also been selected as scaffolds for the structural modification on the *α*, *β*-unsaturated ketone in the D-ring, resulting in the synthesis of additional series of *ent*-kaurane analogues. All the synthesized *ent*-kaurane analogues were tested for their *in vitro* bioactivities in various human cancer cell lines. One derivative was selected to be assessed for its *in vivo* antitumor efficacy in a xenograft mouse model. Further, we prepared a pair of biotin-conjugated probes based on 5F and its D-ring modified derivative, which will be used in the subsequent pull-down assay to identify the anticancer targets of *ent*-kauranes.

Results and Discussion: To investigate the meaningful SAR of 5F, six groups of novel 5F analogues were designed by the substitution with different types of small chemical entities on its 19-carboxylic group, 11-hydroxyl group and *α*, *β*-unsaturated ketone moiety, which yielded 68 derivatives of 5F. Our previous studies have found that the Michael addition miliusane-derivatives associated with the *α*, *β*-unsaturated ketone could still retain their anticancer activity. As far as we know, the *α*, *β*-unsaturated keto moiety might act as a Michael addition acceptor to react with universal nucleophiles (e.g. SH-enzymes or SH-coenzymes) in the biological systems,

which could result in unwanted side effects in humans when they are developed as drugs. We thus hypothesized that if the *α*, *β*-unsaturated ketone system in *ent*-kauranes is disrupted by substitution of some functional groups, the anticancer potency of the *ent*-kauranes will remain while their toxicity will be significantly reduced. By adding different substituents on the *α*, *β*-unsaturated ketone moiety, we synthesized 67 *ent*-kaurane derivatives based on 5F, henryin A and oridonin. The chemical structures of all the derivatives were confirmed by the MS and NMR data. The growth inhibitory effects of the newly generated *ent*-kaurane analogues were determined by SRB assay using the human cancer cell lines HCT116 (colorectal), MCF-7 (breast), A549 (lung), A375 (melanoma), and MIA PaCa-2 (pancreatic). The assay result showed that the esterification of 19-COOH and the oxidation of 11-OH could be beneficial to the anticancer efficacy, whereas the substitution of 11-OH was observed with reduced activity. The anti-proliferative efficacy of D-ring modified *ent*-kaurane derivatives in groups 1b-3b was divergent. Derivatives from groups 1b and 2b with anilines associated with *α*, *β*-unsaturated ketone showed comparable or even greater efficacy than their parent *ent*-kaurane diterpenoids. However, an opposite trend was observed for the derivatives in groups 1b-3b with various substituents other than anilines. Among all the series of *ent*-kaurane derivatives, the most potent compound **S2b** with *α*, *β*-unsaturated keto moiety showed an IC_{50} value of 0.232 μM, being 27-fold more effective than 5F against HCT116 cells. Compound **S8p** represented the most potent Michael adduct that possessed nearly 18 times more potent activity than 5F against MCF-7 cells with an IC_{50} value of 0.761 μM. Moreover, compound **S12i**, a Michael addition derivative of henryin A, was selected for *in vivo* antitumor evaluation. After 21 days of treatment, **S12i** significantly suppressed the tumor volume and the tumor weight by 54.5% at the doseage of 40 mg/kg/per 2 days, which was even more potent than the clinically used drug 5-FU. Notably, one mouse died in henryin A-treated group, while the body weight of mice treated with **S12i** exhibited no significant change.

Conclusion: A total of 135 *ent*-kaurane derivatives have been synthesized using

three naturally isolated *ent*-kaurane diterpenoids (5F, henryin A and oridonin) as the starting materials, 67 of which were obtained from the modification of the *α*, *β*-unsaturated keto moiety *via* hetero-Michael addition reaction. All the synthetic derivatives were assessed for their anticancer activities on different kinds of human cancer cell lines. SAR analysis of the synthetic *ent*-kaurane diterpenoids suggested that the 19-carboxylic group modification was essential for efficacy enhancement, and the *ent*-kaurane-based Michael adducts could be promising anticancer lead compounds for drug development.