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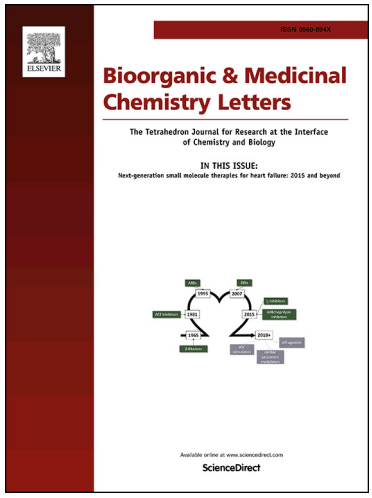

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Synthesis and Biological Activities Evaluation of Sanjuanolide and its Analogues

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[d]**Author Contributions:** These three authors contributed equally to this work.

Declarations of interest: none

Abbreviations: Ethyl acetate (EtOAc), Dichloromethane (DCM), Chloromethyl methyl ether (MOMCl), *p*-Toluenesulfonic acid (*p*-TsOH), Tetrahydrofuran (THF), 4-Dimethylaminopyridine (DMAP), Tetraphenylporphyin (TPP).

ABSTRACT

Sanjuanolide, psorachalcone A and its seven new analogues were synthesized via a combinatorial strategy by aldol reaction. In order to investigate the effect between electron density in π-conjugated systems and biological activities, several electron-withdrawing and electron-donating groups were introduced at C-4 and the phenolic hydroxyl groups of sanjuanolide. The two natural products and its seven new analogues were investigated for their inhibitory effects against five cancer cell lines. Moreover, the hydroxyisoprenyl group may be important to maintain the biological activities of sanjuanolide.

Keywords: Isoprenylated chalcone; Hydroxyisoprenyl group; Natural product; Analogues; Biological activity

Chalcones are abundant in seeds, fruit skin or peel, bark, and flowers of most edible plants. They have been considered derivatives of a three-carbon *α,β*-unsaturated carbonyl system (1,3-diphenyl-2-propen-1-one) composed of two phenolic rings. Many of the chalcones exhibit important pharmacological properties, such as anti-inflammatory,[1] anti-bacterial,[2] anti-cancer,[3] anti-oxidant,[4] analgesic,[5] and anti-pyretic effects.[6,7] Sanjuanolide, a new isoprenylated chalcone which contains an unique hydroxyisoprenyl group, was isolated from *G. hypoleuca. Dalea frutescens* by Shaffer *et al* in 2016.[8] Specifically, sanjuanolide showed slightly greater cytotoxic activities against PC-3 and DU 145 prostate cancer cell lines.

To our knowledge, the unique hydroxyisoprenyl group was rarely in natural products of chalcones. According to Literature search by reaxys, chalcones with hydroxyisoprenyl at A ring and three-carbon *α,β*-unsaturated carbonyl system have exhibited cytotoxic or antifungal activities mostly, typically including sanjuanolide (**1**), psorachalcone A (**2**),[9] xanthohumol D (**3**),[10] xanthoangelol D (**4**),[11] compound **5**,[12] paratocarpin E (**7**),[13] bartericin A (**8**),[14] angusticornin A (**6**) and angusticornin B (**9**)[15] (Figure 1).

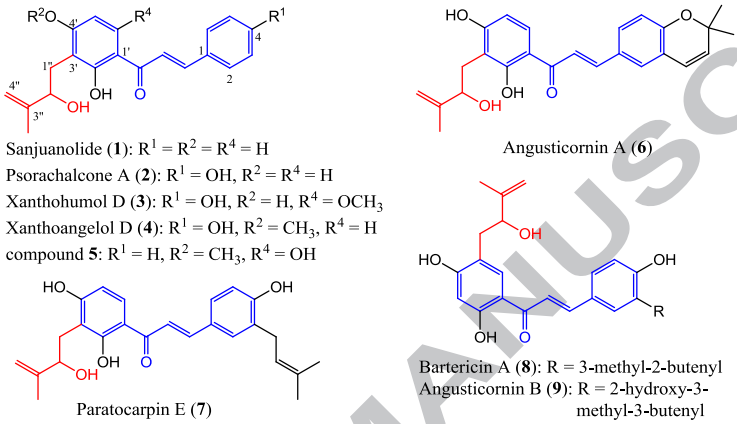
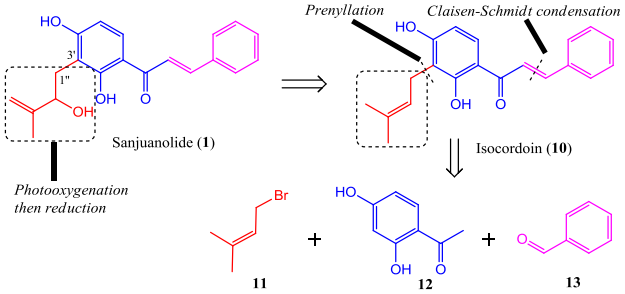


Figure 1. Natural Chalcones with hydroxyisoprenyl group

However, there is no work has been done on the synthesis of these natural products. In addition, further study of sanjuanolide and its analogues by introduce electron-withdrawing and electron-donating groups at the two aromatic rings may provide new leads for drug discovery. Herein, the first total synthesis of sanjuanolide, psorachalcone A and its seven new analogues are described, and their inhibitory effects against PC-3, A375, PANC-1, A549 and MDA-MB-231 cell lines were investigated.

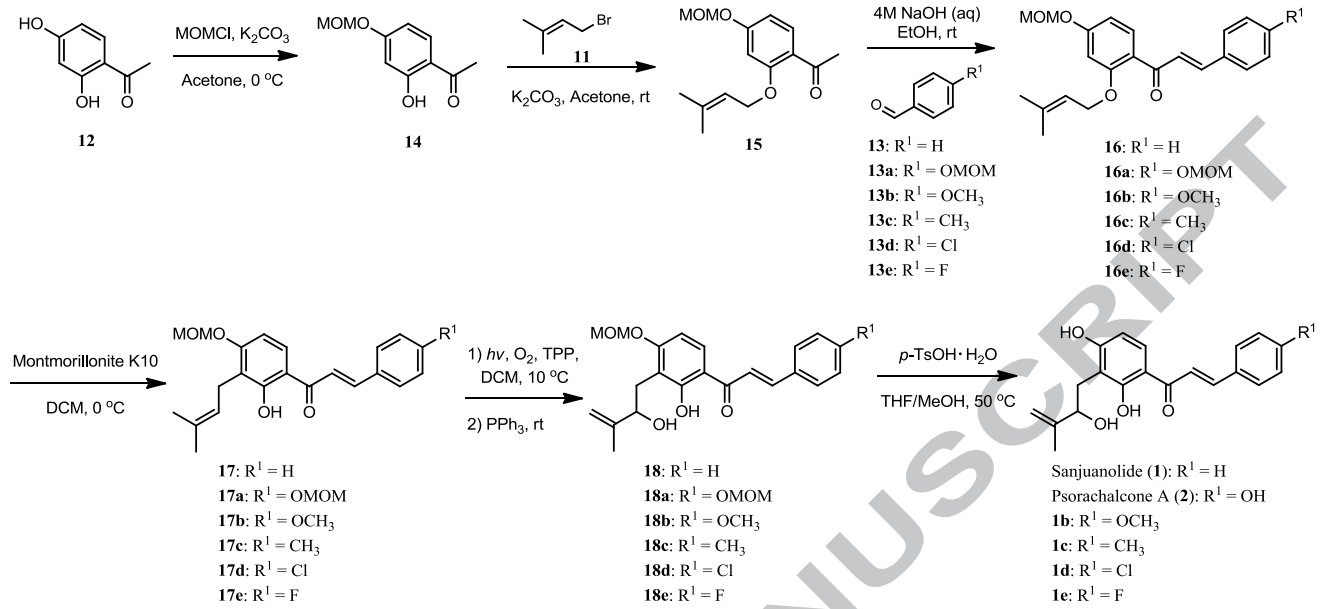
In consideration of the cytotoxic activity and typical structure, sanjuanolide was selected as the first synthetic target in our laboratory. According to Jean *et al*, photooxygenation of *ortho*-prenylphenols followed by a reduction at low temperature can yield *ortho*-(2-hydroxy-3-methylbut-3-enyl)phenols based on the Schenck reaction.[16] In this work, we intend to obtain the unique hydroxyisoprenyl groups from *ortho*-prenylphenols at the last two steps by retrosynthetic analysis of sanjuanolide (Scheme 1). Disconnection of isocordoin (**10**) through C3′–C1″ and C,C-double bond in *α,β*-unsaturated carbonyl system results in three commercialized raw materials 3,3-dimethylallyl bromide (**11**), 2,4-dihydroxyacetophenone (**12**) and benzaldehyde (**13**).



Scheme 1. Retrosynthetic Analysis of Sanjuanolide (1)

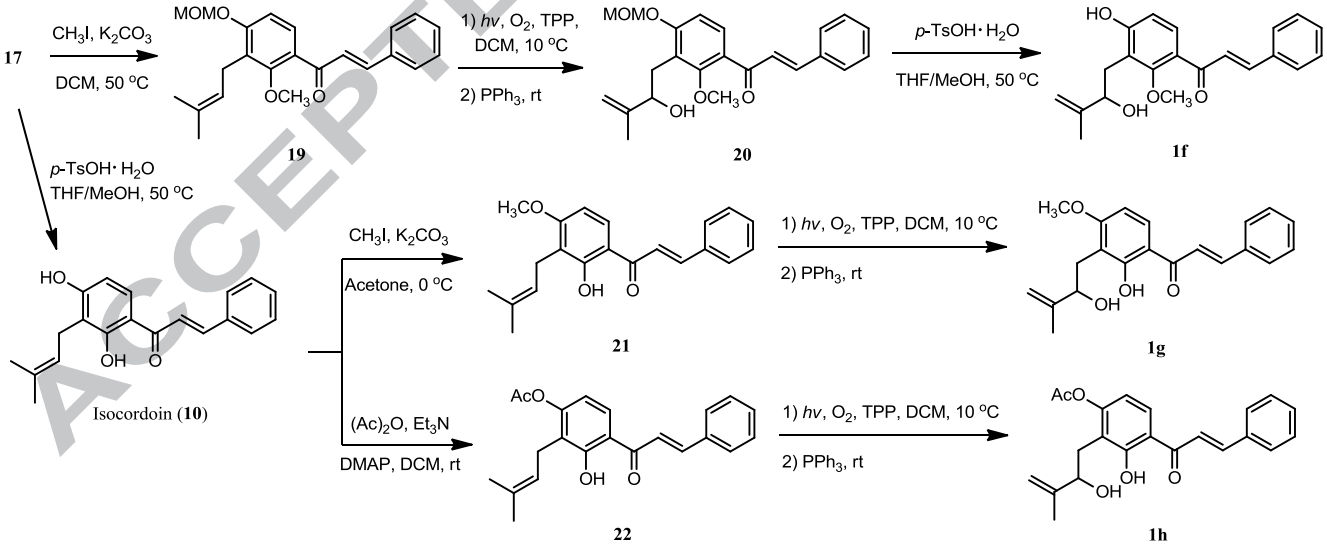
As described by Jain *et al*,[17] prenylation of 2,4-dihydroxyacetophenone with 2-methylbut-3-en-2-ol in the presence of Lewis acidic medium, was adopted in our laboratory at the beginning. However, by the limit of low yields and high challenge in the process of purification (Low solubility of the two monoprenylated prenylated derivatives in eluent), we used MOM (methoxymethyl) to pro-

tect the C4′-hydroxy group of **12** selectively in 92% yield (Scheme 2). Then the MOM-protected compound **14** was prenylated with bromide **11** to afford **15** (90% yield), which was further reacted with benzaldehyde in the presence of 4 M NaOH to afford chalcone **16** by Claisen-Schmidt condensation (85% yield).[18]



Scheme 2. Synthesis of sanjuanolide, psorachalcone A and analogues 1b–e.

Chalcone **16** was subjected to a montmorillonite K10-promoted sigmatropic rearrangement to afford the *ortho*-prenylated chalcone **17** in 35% yield (along with 25% *para*-prenylated chalcone as a by-product).[19] With the intermediate **17** in hand, photooxidation was taken in the presence of tetraphenylporphyin (TPP) as a photosensitizer. Moreover, we chose the temperature at 10 °C (compare to –30 °C reported by Jean *et al*)[16] to ensure **17** could be converted completely. Then PPh_3 was added to the reaction mixture, and the resulting solution was allowed to warm to room temperature naturally (55% yield). Finally, deprotection of **18** using *p*-TsOH at 50 °C in MeOH/THF afforded synthetic sanjuanolide (**1**), which was obtained as a yellow powder (88% yield).



Scheme 3. Synthesis of analogues 1f–h.

Under the same strategy, psorachalcone A (**2**) and **1b**–**e** were prepared from the corresponding aldehyde **13a**–**e** using combinatorial approaches. Similarly, **1f** was synthesized starting from intermediate **17** through 3 steps (Scheme 3). Deprotection of the intermediate **17** using *p*-TsOH lead to isocordoin (**10**), which was further reacted with iodomethane or acetic anhydride separately to afford

prenylated chalcones **21** and **22**. And then, after the photooxidation step, analogues **1g** and **1h** were synthesized from **21** and **22** respectively. Additionally, photooxygenation of **10** followed by a reduction can also afford sanjuanolide in 46% yield.

The analytic data of synthetic sanjuanolide were compared with that of the natural sanjuanolide reported in the literatures,[8] including ^{1}H and ^{13}C NMR data (Table S1 in the Supporting Information). In addition, as described by Shaffer *et al*,[8] the configuration of the C-2″ stereocenter in **1** was converted to a virtual (~1:1) racemic mixture after several weeks of freezer storage. In this work, ^{1}H and ^{13}C NMR data also showed no discernible changes in these compounds according to our observations, and the samples were investigated as racemic mixtures by chiral HPLC.

The antiproliferative effects of sanjuanolide, psorachalcone A, isocordoin and the seven new analogues were evaluated in five cancer cells, using MTT assays as described in the *in vitro* screening protocol.[20] The ability of these compounds to inhibit the growth of cancer cells was summarized in Table 1. **1c–g** exhibited obvious activity against PC-3 and A375 cell lines. Especially, **1d** exhibited 3.4-fold and 3.0-fold greater potencies against the PC-3 and A375 cells compared to **1** respectively. On the contrary, isoprenylated chalcone **10** and 4-hydroxyl-substituted sanjuanolide (**2**, psorachalcone A) exhibited lower potencies as compared to **1** in PC-3 and A375 cells. In addition, **1f** exhibited moderate levels of activity against PANC-1 and MDA-MB-231 cell lines.

Table 1. Effects of sanjuanolide and its analogues on proliferation of cancer cell lines.

Compounds	IC_{50} (μM)[1]				
	PC-3	A375	PANC-1	A549	MDA-MB-231
1	17.5	13.1	> 25[2]	> 25	> 25
2	> 25	> 25	> 25	> 25	> 25
1b	> 25	> 25	> 25	> 25	> 25
1c	16.7	23.5	> 25	> 25	> 25
1d	5.1	4.3	22.5	> 25	23.4
1e	11.4	9.5	24.5	> 25	> 25
1f	13.5	9.2	13.8	> 25	12.1
1g	16.6	18.1	> 25	> 25	> 25
1h	20.4	17.7	> 25	> 25	> 25
10	> 25	23.2	> 25	> 25	> 25

[1] Cells were exposed to compounds for 48 h. All values are the mean of three independent experiments. [2] If a specific compound is given a value >25, it indicates that a specific IC_{50} cannot be calculated from the data points collected, meaning "no effect".

In summary, total synthesis of sanjuanolide, psorachalcone A and its seven new analogues were achieved from commercially available materials in acceptable yields through 6 or 7 steps (12% overall yield for sanjuanolide in 6 steps). Furthermore, this combinatorial approach can also be used for the preparation of chalcones with hydroxyisoprenyl group mentioned above. The compounds were subsequently evaluated for their anti-cancer activities. This preliminary structure-activity relationship study reveals that introducing an electron-withdrawing halogen atom (Cl or F) at C4 position or replace the C2′-hydroxy group with methoxy group may have a positive effect on their biological activities. The relationship between the hydroxyisoprenyl group and biological activities of chalcones still needs to be further investigated.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at xxx

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1. First syntheses of two new chalcones with hydroxyisoprenyl group were achieved
2. Preliminary structure-activity relationship study of sanjuanolide was investigated
3. The "hydroxyisoprenyl" group is shown to correlate with anti-tumor activities