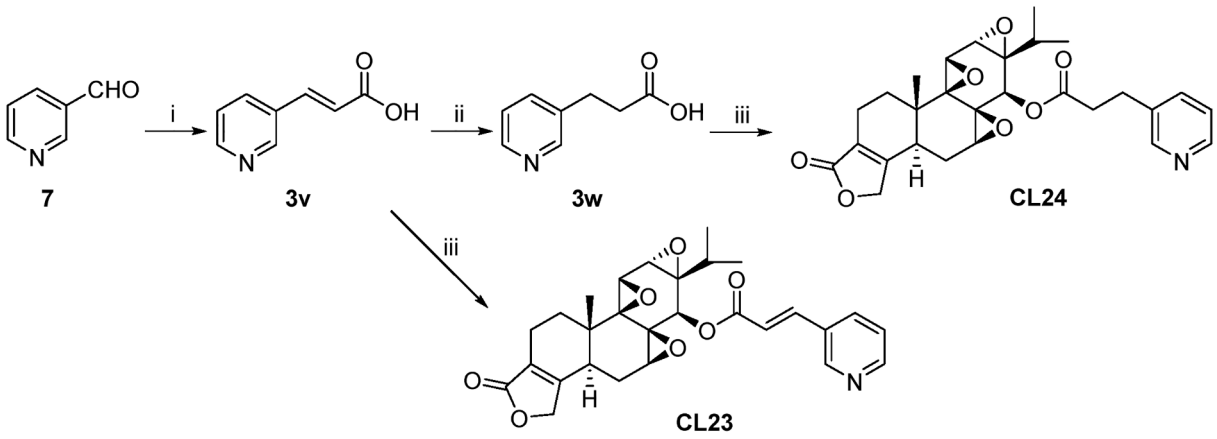

Scheme 4. Synthesis of triptolide derivatives **CL23-24**: (i) malonic acid, pyridine, aniline, toluene, reflux; (ii) H_2, Pd/C, rt; (iii) triptolide, EDCI, DMAP, CH_2Cl_2, ice bath to rt.

Table 1
In vitro cytotoxic activity of triptolide derivatives in HepG2 cells.

compound	IC_{50}[a] (μM)	compound	IC_{50} (μM)	compound	IC_{50} (μM)
Triptolide	0.468 ± 0.022	**CL9**	0.591 ± 0.054	**CL17**	0.783 ± 0.037
CL1	0.654 ± 0.026	**CL10**	0.564 ± 0.049	**CL18**	1.715 ± 0.066
CL2	0.686 ± 0.030	**CL11**	0.439 ± 0.036	**CL19**	0.578 ± 0.017
CL3	0.689 ± 0.048	**CL12**	0.838 ± 0.029	**CL20**	0.355 ± 0.075
CL4	0.663 ± 0.080	**CL13**	0.381 ± 0.045	**CL21**	1.021 ± 0.044
CL5	0.413 ± 0.032	**CL14**	0.591 ± 0.062	**CL22**	0.818 ± 0.023
CL6	0.454 ± 0.055	**CL15**	0.696 ± 0.068	**CL23**	0.538 ± 0.020
CL7	0.899 ± 0.030	**CL16**	0.620 ± 0.024	**CL24**	0.839 ± 0.040
CL8	0.695 ± 0.044	–	–	–	–

[a] IC_{50}: The drug concentration required for 50% inhibition of cell proliferation, while the maximum concentration used here was 4 μM, and the results are presented as average values.

derivatives (**CL1-24**) on HepG2 cells was next detected, and represented by the IC_{50} values,[29] which were summarized in Table 1.

The results revealed that by introduction of cinnamic acid and its analogues, these triptolide esters can still retain the cytotoxicity, albeit slightly reduced. Among them, the phosphate groups substituted analogue **CL20** showed the highest potency and was slightly more active than parent natural triptolide, with the lowest IC_{50} value (IC_{50} = 0.355 μM). A preliminary SAR analysis of these test compounds indicated that the conjugated double bond of cinnamic acid moiety was important for antitumor activity (**CL23** vs. **CL24**). The cytotoxic activity was found to reduce when a substituent was introduced at the para position of the benzene ring, either electron donating or electron withdrawing groups (see **CL1** and **CL5-12**). Inversely, the substitution with fluorine at the ortho position of the phenyl ring led to a slight increase (see **CL13**). As the number of substituents on the phenyl ring increased, the antitumor activity would decrease (see **CL1-5** and **CL13-14**). In addition, the replacement of benzene ring with pyridine ring resulted in a slight loss of cytotoxic activity (**CL5** vs. **CL23**).

In summary, starting from the commercially available benzaldehyde analogues, a practical synthesis of novel cinnamic acid triptolide ester derivatives and their biological evaluation of CCK-8 determination have been accomplished. The compound **CL20** has shown the most potent antitumor activity, although its underlying molecular mechanism remains to be clarified. Given that the majority of target compounds in this study exhibited significant inhibition in the HepG2 cells, more comprehensive SAR studies and further pharmacological studies would be warranted.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2022.128760.

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