# Tropone Skeleton Enhances the Dispersion Stability of Nano-prodrugs



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# Tropone Skeleton Enhances the Dispersion Stability of Nano-Prodrugs

*Keita Tanita, Yoshitaka Koseki, Takaaki Kamishima, and Hitoshi Kasai**

Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai, Miyagi 980-8577, Japan

Keywords

Drug delivery system, Reprecipitation method, Tropone skeleton

Abstract

We synthesized hinokitiol-modified SN-38 prodrugs and fabricated their nano-prodrugs which are nanoparticles composed of only prodrug molecules by the reprecipitation method. These nano-prodrugs possessed high dispersion stability, and further research revealed that the tropone skeleton of hinokitiol is the key structure for the dispersion stability in our prodrug design. In addition, the nanoparticles of hinokitiol-modified SN-38 showed much higher cytotoxicity against cancer cells than irinotecan, the pharmaceutically available prodrug of SN-38.

Nano meter-sized drug (nanodrug) has been gaining many attentions as therapeutic agents [1]. Especially, in the field of cancer treatment, drugs which are ranged from 10–200 nm in size, would be able to avoid exclusion from the body, and to circulate in the blood for a longer time [2]. Furthermore, these nanodrugs easily reach tumor site through the gap about 200 nm in the blood vessels around tumor tissues. This passive targeting effect is known as enhanced permeability and retention (EPR) effect [2], and various types of nanodrugs have been reported. However, the conventional formulation strategies using nano carriers (e.g., liposome [3] and polymer micelle [4]) can lead to several problems, such as undesirable side effects caused by the carriers themselves or their metabolites [5].

With this background, our group has been developing nano-prodrugs (NPs) which are nanoparticles composed of only prodrug molecules by the reprecipitation method [6]–[8]. We selected 7-ethyl-10-hydroxycamptothecin (SN-38, Figure 1) as a component of NPs. SN-38 is known for its strong antitumor activity, but is poorly soluble in water. In fact, irinotecan (Figure 1), a water-soluble prodrug of SN-38, is currently used. In our previous study, we synthesized SN-38 dimer as a homodimeric prodrug, and their NPs showed much higher in vitro cytotoxicity compared to irinotecan due to improvement of the hydrophobicity [9]. Despite good anticancer effect, these nanoparticles were aggregated with in several days, and difficult to apply for in vivo application. Based on the above results, we developed substituent-modified SN-38 NPs [10, 11]. These NPs, which were prepared by changing the substituents, showed good dispersion stability, but their drug loading capacity was lower than that of homodimeric prodrugs. Therefore, we could not produce NPs that have both good dispersion stability and pharmacological efficacy.

In this study, we designed hinokitiol-modified SN-38 (SN-38×Hinoki, Figure 1). Hinokitiol, a natural monocyclic monoterpenoid, is known for its antibacterial activity [12] and some anticancer activities [13]. We synthesized SN-38×Hinoki as a heterodimeric prodrug and fabricated their NPs which possess high drug loading capacity of 86%.

SN-38×Hinoki was obtained 87% in yield by esterification of glutaric acid-conjugated SN-38 (SN-38×Glu) and hinokitiol under dehydration condensation conditions. When we fabricated SN-38×Hinoki NPs by the reprecipitation method, 0.1 mM water dispersion of SN-38×Hinoki NPs showed good dispersion stability and did not aggregate for more than one week. Then, the particle size and the dispersion stability profile of SN-38×Hinoki NPs were evaluated by scanning electron microscopy (SEM) and dynamic light scattering (DLS). The size distribution profile measured by DLS revealed that one week's particle size distribution was good (Figure 2a), and the zeta-potential of the NPs was −40 mV. SEM images revealed that SN-38×Hinoki NPs were approximately 200 nm in size (Figure 2b). Based on these results, we suggested that most SN-38×Hinoki NPs were within the range of appropriate particle sizes that exert EPR effect.

Furthermore, SN-38, SN-38×Hinoki NPs, and irinotecan were added to a cell culture medium of human hepatoma HepG2 cells, at a concentration range of 0.04–10 μM. SN-38×Hinoki NPs showed much higher cytotoxicity as compared to irinotecan, which is the pharmaceutically available prodrug of SN-38 (Figure 3). Our findings were consistent with those of previous study [14].

Despite SN-38×Hinoki NPs showed good stability as NPs, the interplay between dispersion stability and the key structure of prodrug design had not been well investigated. Therefore, we synthesized various types of substituents-conjugated SN-38 (Figure 4). All prodrugs were applied

to the reprecipitation method, and their structure-activity relationship between their dispersion stability and molecular structure of substituents was evaluated based on SEM image. As shown in Table 1, only NPs which possess the tropone skeleton (Figure 5) in the substituents showed good dispersion stability. In the case of entries 1–5, these NPs were aggregated immediately after fabrication. These results suggested that crystal growth derived from SN-38 was proceeded when substituents having low molecular weight were conjugated to SN-38. In other case (entries 6–9 and hinokitiol), we successfully fabricated nanoparticles. These prodrugs possessed a flexible linker part and a large steric hindrance part, and it suggested their molecular structure suppressed crystal growth. Furthermore, only prodrugs that had substituent tropone skeleton (entry 9 and hinokitiol) showed good dispersion stability even after 1 week. The tropone skeleton is a non-benzenoid aromatic with a unique resonance structure not found in other aromatics (i.e., partial positive charge on the carbon atom and a partial negative charge on the oxygen atom). We predicted that the partial charge derived from the tropone skeleton improved the dispersion stability of NPs (Figure 5). Based on the results, it was found that the tropone skeleton of the substituents contributed to the dispersion stability of nanodrugs.

In conclusion, we succeeded in the fabrication of SN-38×Hinoki nano-prodrugs and found that hinokitiol is involved in improving the pharmacological activity and the dispersion stability of the NPs. The drug loading capacity of general nano drugs is 10% or lesser, whereas SN-38×Hinoki NPs possess a higher drug loading capacity of 86%. SN-38×Hinoki NPs showed much higher pharmaceutical efficacy than irinotecan, which is the pharmaceutically available prodrug of SN-38. We conclude that the tropone skeleton of hinokitiol can be used to fabricate NPs without losing the efficacy of pharmacological compounds and that it is a promising template for the development of novel drug design.

Acknowledgments

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Figure 1.

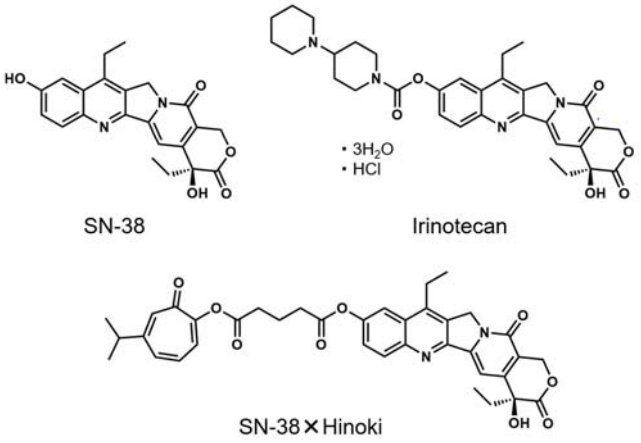


**Figure 1.** Chemical structure of SN-38 derivatives and irinotecan.

Figure 2.

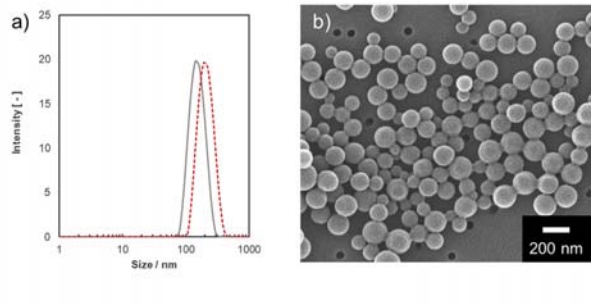


**Figure 2.** a) Size distribution profile of SN-38×Hinoki NPs. (Solid line: right after fabrication, dashed line: after 7 days). b) SEM image of SN-38×Hinoki NPs.

Figure 3.

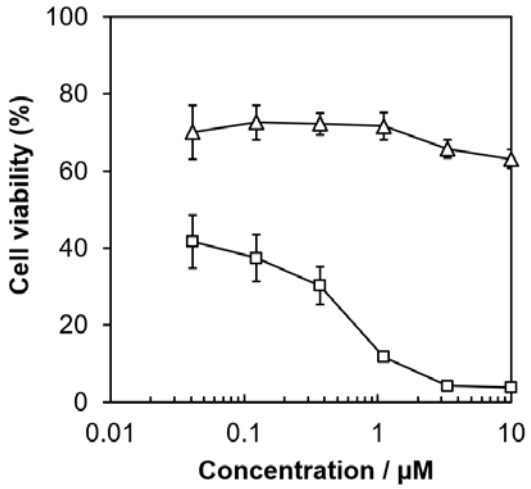


**Figure 3**. *In vitro* cytotoxicity of SN-38×Hinoki NPs (□) and irinotecan solution (△) in HepG2 cells. These results are indicated as mean ± standard deviation (n = 3).

Figure 4.

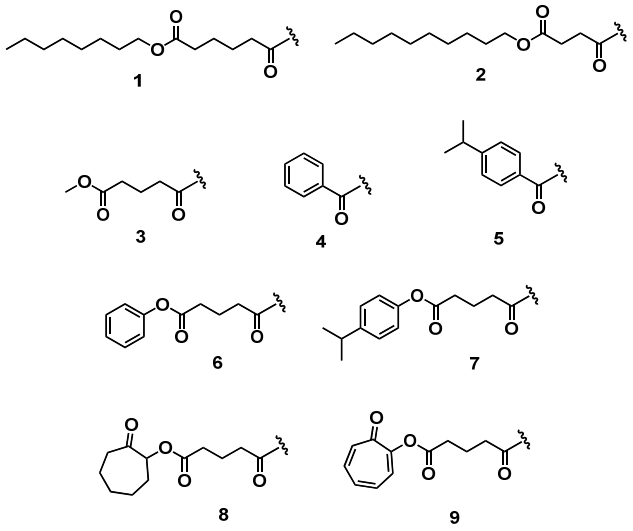


**Figure 4**. Chemical structure of substituents. The omitted chemical structure is SN-38.

Figure 5.

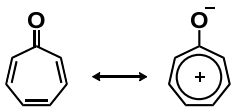


**Figure 5**. Unique resonance structure of tropone skeleton.

Table 1.

**Table 1.** Structure-activity relationship between substituents skeleton and dispersion stability of nanoparticles
Nano particle state ( - : aggregation, + : agglomerate or recrystallisation within several days, ++ : good dispersion )
Other line ( - : none, + : existence)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Hinoki |
|---|---|---|---|---|---|---|---|---|---|---|
| Nanoparticle state | - | - | - | - | - | + | + | + | ++ | ++ |
| Flexible linker | + | + | + | - | - | + | + | + | + | + |
| Aromatic group | - | - | - | + | + | + | + | - | + | + |
| Tropone skeleton | - | - | - | - | - | - | - | - | + | + |