Article

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The effect of XlogP and Hansen solubility parameters on small molecule modified paclitaxel anti-cancer drug conjugates self-assembled into nanoparticles

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Abstract:

Small molecule modified anti-cancer drug conjugates (SMMDCs) can self-assemble into nanoparticles (NPs) as therapeutic NP platforms for cancer treatment. Here we demonstrate that the XlogP and Hansen solubility parameters of paclitaxel (PTX) SMMDCs is essential for SMMDCs self-assembling into NPs. The amorphous state of PTX SMMDCs will also affect SMMDCs self-assembling into NPs. However, the anti-tumor activity of these PTX SMMDCs NPs decreased along with their XlogP values, indicating that a suitable XlogP value for designing the SMMDCs is important for self-assembling into NPs and for possessing anti-tumor activity. For higher level XlogP SMMDCs, a degradable linker should be considered in the design of SMMDCs to overcome the problem of lower anti-tumor activity. It is preferable that the hydrophilic groups in the SMMDCs should be present on the surface of self-assembling NPs.

Keywords

Small molecule modified anti-cancer drug conjugates (SMMDCs); Paclitaxel (PTX); Self-assemble; Nanoparticles (NPs); XlogP; Hansen solubility parameters.

Introduction:

Several therapeutic nanoparticle (NP) platforms, such as liposomes, lipid NPs, albumin NPs and polymeric micelles, have been widely used as delivery vehicles for anti-cancer drugs.[1-6] However, the ratio of drug to carrier material in these NPs is relatively low (below 1:10 or less).

Although many polymer-drug conjugates have been reported to self-assemble forming nanomedicines by themselves, the drug occupancy rate of these polymer-drug conjugates is not very high since the molecular weight of the polymer is higher than that of the anti-cancer drug.[7, 8]

In order to increase the drug occupancy rate, small molecule modified anti-cancer drug conjugates (SMMDCs), which have a higher drug occupancy, have been synthesized. Interestingly, these SMMDCs can self-assemble into NPs in water and the anti-tumor activity of these SMMDCs was confirmed *in vitro* and *in vivo*. Therefore, many SMMDCs have been designed and prepared for self-assembling into NPs.[9-17] However, to some extent, the selection of small molecular weight agents and anti-cancer drugs for preparing SMMDCs has been seemingly random. The supporting principle for the selection of small molecular weight agents and anti-cancer drugs has been unclear. The intrinsic characteristic change of SMMDCs compared with that of small molecular weight agents and anti-cancer drugs has also been unclear.

It is well-known that many anti-cancer drugs have a high lipophilicity and low water-solubility and so we wanted to know whether the lipophilicity and solubility of the SMMDCs were changed compared with that of small molecular weight agents and anti-cancer drugs. Also, it is important to know whether these changes are key factors for SMMDCs self-assembling into NPs.

Here we selected paclitaxel as an anti-cancer drug to prepare a series of small molecule modified anti-cancer drug conjugates (SMMDCs) and used the XlogP (the theoretical n-octanol-water partition coefficient calculated by the XLOGP3 method, representing the lipophilicity) and Hansen solubility parameters (HSPs, representing the water-solubility) as significant factors to investigate the relationship between these parameters for self-assembly into NPs.

Results & Discussion

The effect of XlogP and Hansen solubility parameters on SA-PTX, OA-PTX, CLA-PTX and ALA-PTX self-assembled into NPs

Recently, it has been reported that oleic acid-S-PTX (OA-S-PTX) can self-assemble into NPs.[12] The reported mechanism may be due to the intermolecular π–π stacking facilitated by the unsaturated alkyl chains in oleic acid with the planar structures of PTX.[12] Considering the results of our previous research, we know that the conjugated linoleic acid-paclitaxel conjugate (CLA-PTX) can self-assemble into NPs in water.[18] Therefore, we considered whether the conjugated double bond in CLA was also essential for CLA-PTX self-assembling into NPs. So, we selected stearic acid (SA), oleic acid (OA) and alpha-linolenic acid (ALA) to synthesize the SA-PTX, OA-PTX and ALA-PTX, respectively. SA, OA, CLA and ALA are all straight-chain fatty acids with a chain length of 18 carbon atoms, but containing different unsaturated bonds (no unsaturated bond (SA), a double bond (OA), two double bonds (CLA) or three separate double bonds (ALA).

We investigated whether SA-PTX, OA-PTX or ALA-PTX could also self-assemble into NPs. Our results indicated that, like CLA-PTX, SA-PTX, OA-PTX and ALA-PTX all spontaneously formed NPs in water with a particle size in the range 80-170 nm, as shown in Fig. 1A, 1B and S1, indicating that the unsaturated bond contained in the fatty acids was not a key factor for fatty acid-PTX conjugates self-assembling into NPs.

We determined the retention time of SA-PTX, OA-PTX, CLA-PTX and ALA-PTX in order to determine the hydrophobicity of SA-PTX, OA-PTX, CLA-PTX and ALA-PTX compared with PTX. Our results indicated that the retention times of SA-PTX, OA-PTX, CLA-PTX and ALA-PTX were all longer than that of PTX (Fig. 1C), showing that the hydrophobicity of SA-PTX, OA-PTX, CLA-PTX and ALA-PTX was increased compared with that of PTX due to the introduction of fatty acid.

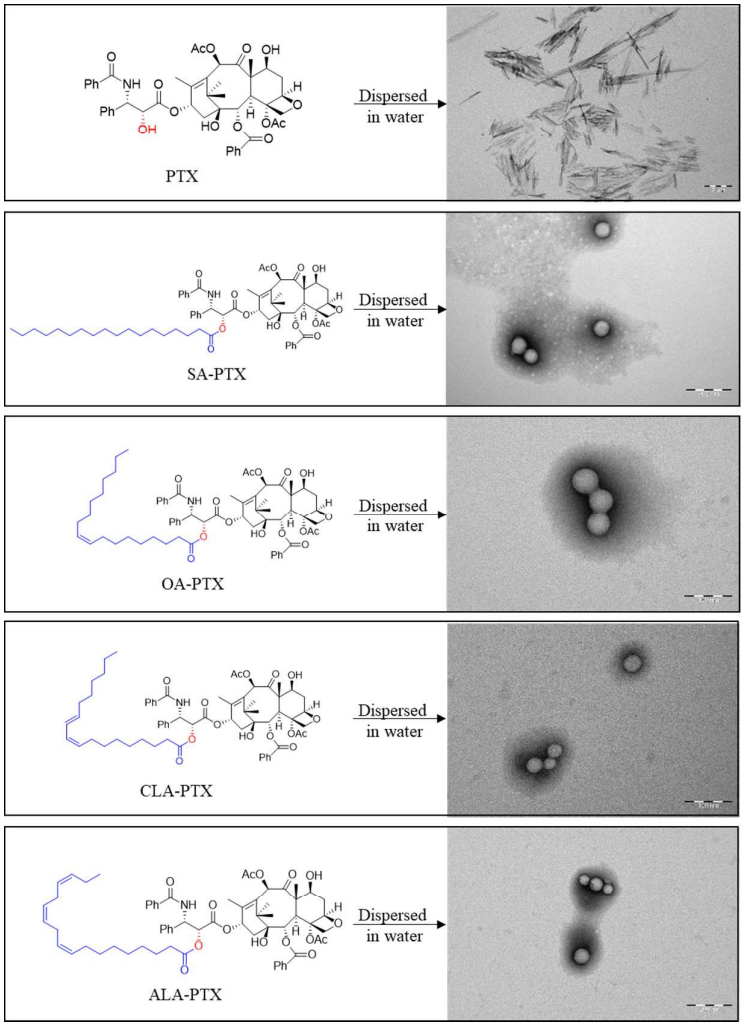
It is well known that the n-octanol-water partition coefficient is an important parameter for evaluating hydrophobicity and lipophilicity. Therefore, the theoretical

n-octanol-water partition coefficient (expressed by XlogP) of PTX, SA-PTX, OA-PTX, CLA-PTX and ALA-PTX was calculated using the XLOP3 method.[19] As shown in Fig. 1D, the calculated XlogP values of SA-PTX, OA-PTX, CLA-PTX and ALA-PTX were all significantly higher than that of PTX.

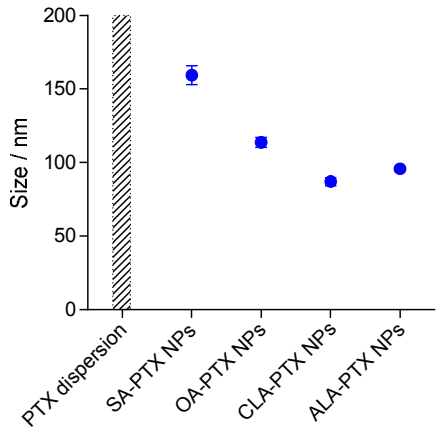
According to theoretical concepts,[20] the total cohesion can be divided into three individual components, which are the group contribution to the dispersion forces, the group contribution to the polar forces, and the group contribution to the hydrogen-bonding energy. The corresponding solubility parameters were divided into three parts: dispersion solubility parameters (δ_d), polar solubility parameters (δ_p) and hydrogen-bonding solubility parameters (δ_h). The total solubility parameter (d_t) is also called the three-dimensional solubility parameter. These four solubility parameters (also called Hansen solubility parameters) can be calculated by using the group contribution method (GCM) proposed by Beerbower.[21] In the present research, the Hansen solubility parameters (δ_d, δ_p, δ_h and δ_t) of PTX, SA-PTX, OA-PTX, CLA-PTX and ALA-PTX were calculated by using the GCM. As shown in Fig. 1E, the results indicated that the Hansen solubility parameters of SA-PTX, OA-PTX, CLA-PTX and ALA-PTX were all significantly lower than that of PTX.

According to these findings, we speculated that the increased XlogP and the decreased Hansen solubility parameters of SA-PTX, OA-PTX, CLA-PTX and ALA-PTX, owing to the introduction of a fatty acid, might be one of the key factors for SA-PTX, OA-PTX, CLA-PTX and ALA-PTX self-assembling into NPs. Considering the results of X-ray di□raction (Fig.1F), SA-PTX, OA-PTX, CLA-PTX and ALA-PTX, unlike PTX, were in an amorphous state. We believe that a compound with a higher lipophilicity and lower water solubility could easily precipitate in water. If its crystallinity decreased, then this compound would precipitate as spheres forming nanoparticles in water.

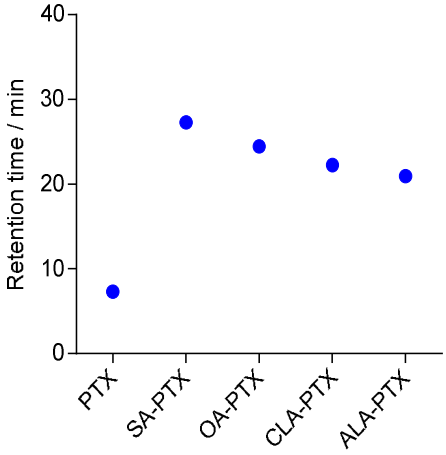
A


PTX
Dispersed in water
SA-PTX
Dispersed in water
OA-PTX
Dispersed in water
CLA-PTX
Dispersed in water
ALA-PTX
Dispersed in water

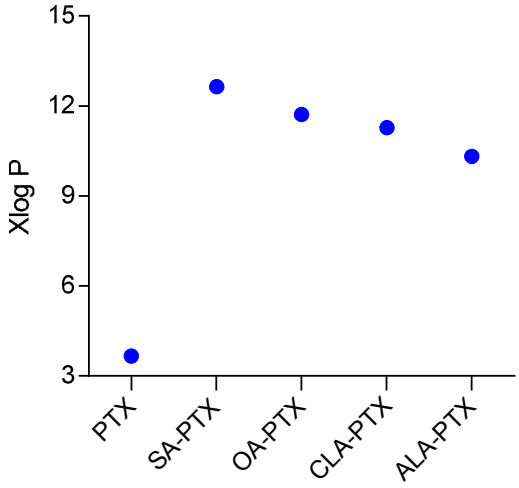
B


Size / nm
200
150
100
50
0
PTX dispersion
SA-PTX NPs
OA-PTX NPs
CLA-PTX NPs
ALA-PTX NPs

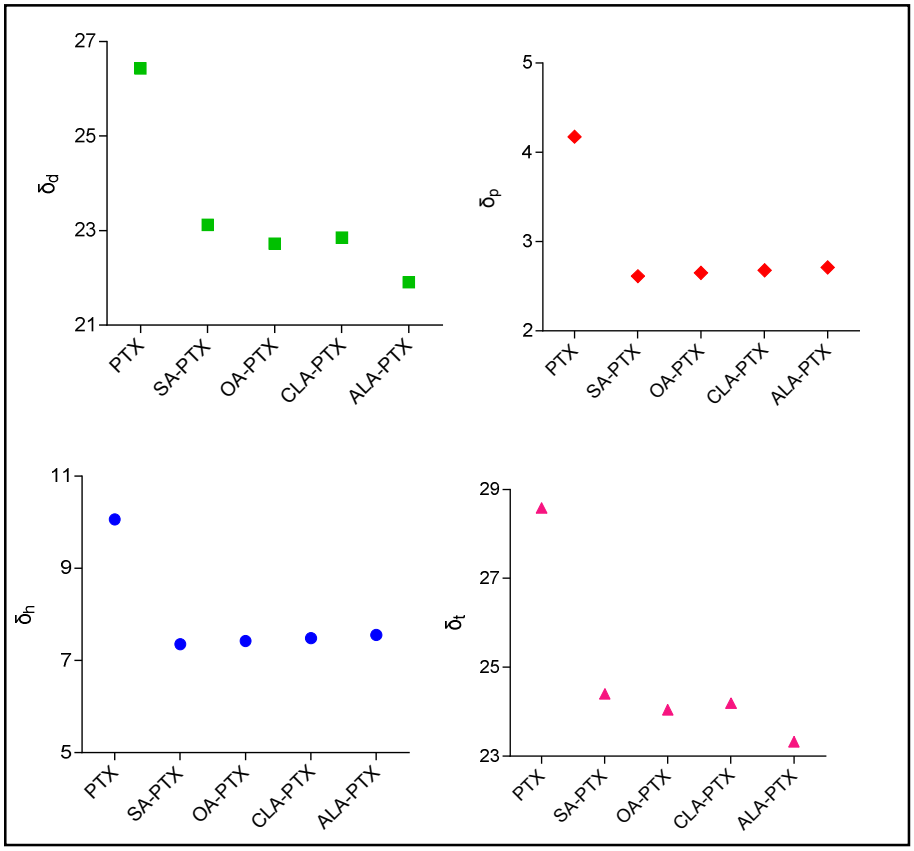
C


Retention time / min
40
30
20
10
0
PTX
SA-PTX
OA-PTX
CLA-PTX
ALA-PTX

D


Xlog P
15
12
9
6
3
PTX
SA-PTX
OA-PTX
CLA-PTX
ALA-PTX

E


δd
27
25
23
21
δp
5
4
3
2
δh
11
9
7
5
δt
29
27
25
23
PTX
SA-PTX
OA-PTX
CLA-PTX
ALA-PTX

F

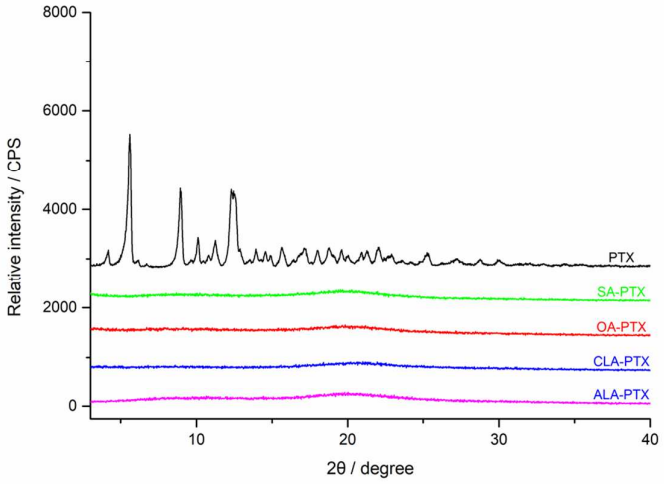


Fig.1. The effect of XlogP and Hansen solubility parameters on SA-PTX, OA-PTX, CLA-PTX and ALA-PTX self-assembling NPs.
A: The structures of PTX, SA-PTX, OA-PTX, CLA-PTX and ALA-PTX and TEM images of PTX, SA-PTX, OA-PTX, CLA-PTX and ALA-PTX NPs.
B: Particle size of PTX, SA-PTX, OA-PTX, CLA-PTX and ALA-PTX NPs.
C: The retention time of PTX, SA-PTX, OA-PTX, CLA-PTX and ALA-PTX determined by high performance liquid chromatography (HPLC).
D: Theoretical n-octanol-water partition coefficients (XlogP) of PTX, SA-PTX, OA-PTX, CLA-PTX and ALA-PTX calculated using XLOGP3.
E: Hansen solubility parameters (HSPs) of PTX, SA-PTX, OA-PTX, CLA-PTX and ALA-PTX according to Beerbower, including dispersion (δ_d), polar (δ_p) and hydrogen bonding (δ_h,) and total solubility parameter (δ_t).
F: The X-ray diffraction (XRD) spectra of PTX, SA-PTX, OA-PTX, CLA-PTX and ALA-PTX.

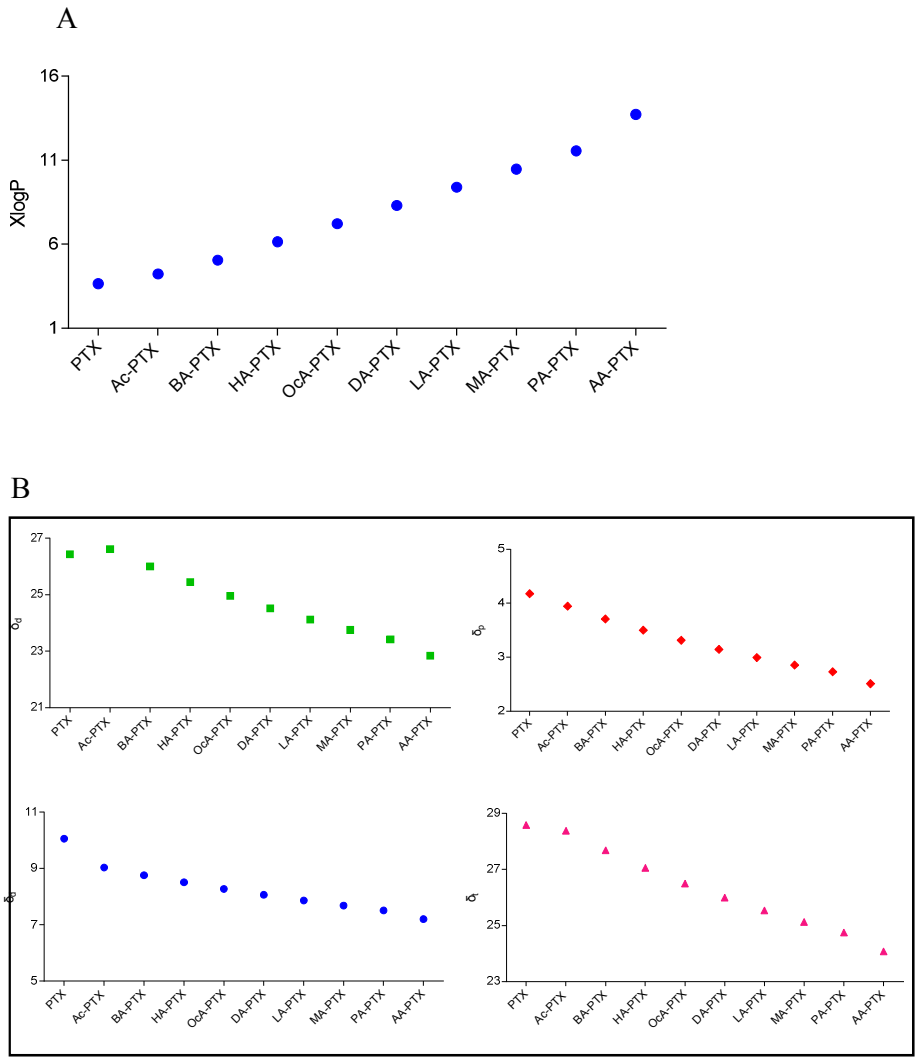
The effect of XlogP and Hansen solubility parameters on straight-chain fatty acid-PTX self-assembled into NPs

In order to confirm the importance of XlogP and Hansen solubility parameters for self-assembling NPs, we selected a series of straight-chain fatty acids (acetic acid (Ac, 2C), butyric acid (BA, 4C), hexanoic acid (HA, 6C), octanoic acid (OcA, 8C), decanoic acid (DA, 10C), lauric acid (LA, 12C), myristic acid (MA, 14C), palmitic acid (PA, 16C), and arachidic acid (AA, 20C)) and then we designed and synthesized a series of straight-chain fatty acid-PTX conjugates (Ac-PTX, BA-PTX, HA-PTX, OcA-PTX, DA-PTX, LA-PTX, MA-PTX, PA-PTX and AA-PTX).

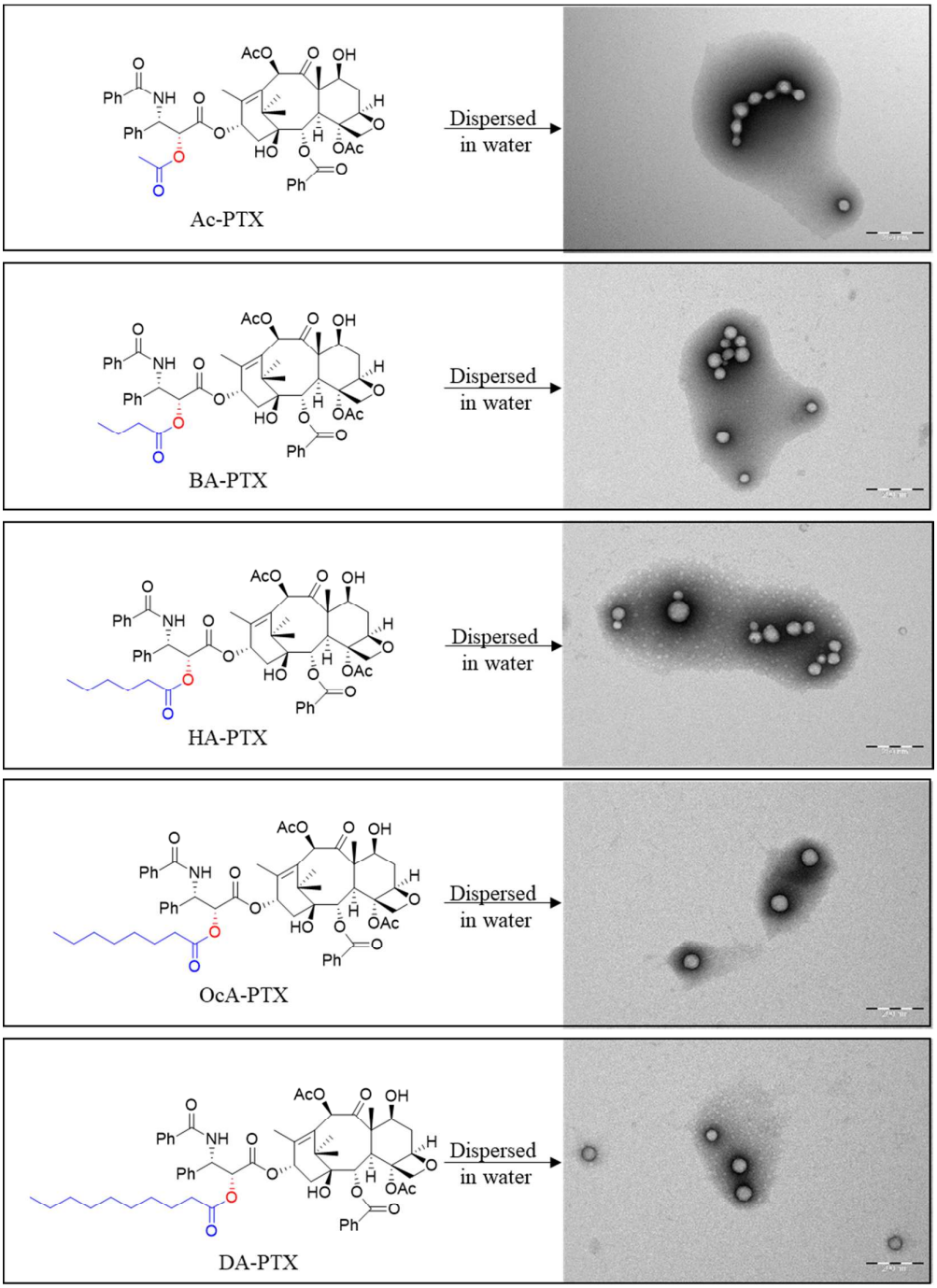
The calculated XlogP values of these fatty acid-PTX conjugates was all higher than that of PTX and increased with the chain length of the fatty acids (Fig.2A). Their Hansen solubility parameters were lower than that of PTX and decreased with the

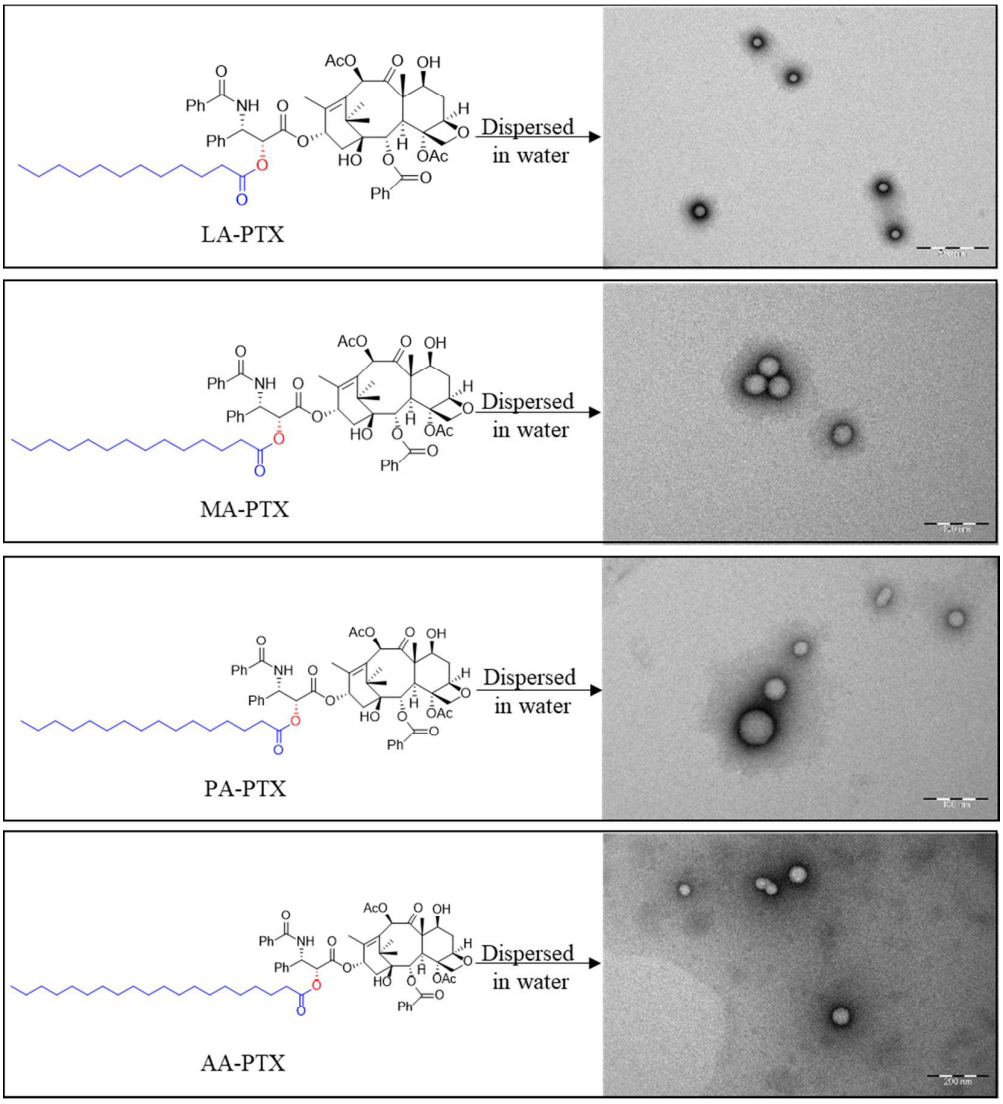
chain length of the fatty acids (Fig.2B). The self-assemble forming NPs of these fatty acid-PTX conjugates were investigated. As shown in Fig.2C, all these fatty acid-PTX conjugates spontaneously formed NPs in water with a particle size in the range 90-160 nm (Fig.2D and S1), indicating that the increased XlogP and the decreased Hansen solubility parameters might be key factors for straight-chain fatty acid-PTX conjugates self-assembling into NPs.

The retention time and X-ray diffraction of these fatty acid-PTX conjugates were also determined. The retention times of these fatty acid-PTX conjugates were all longer than that of PTX and increased with the chain length of the fatty acid (Fig.2E). All these fatty acid-PTX conjugates were in an amorphous state (Fig.2F).

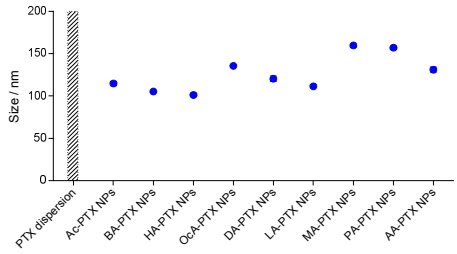


C

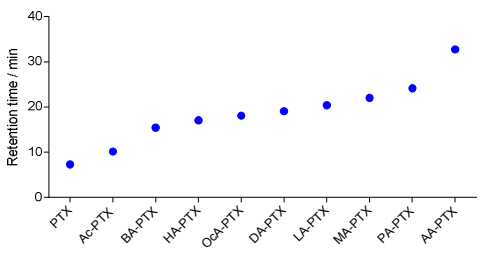

Ac-PTX
Dispersed in water
BA-PTX
Dispersed in water
HA-PTX
Dispersed in water
OcA-PTX
Dispersed in water
DA-PTX
Dispersed in water


LA-PTX
Dispersed in water
MA-PTX
Dispersed in water
PA-PTX
Dispersed in water
AA-PTX
Dispersed in water

D


Size / nm
PTX dispersion
Ac-PTX NPs
BA-PTX NPs
HA-PTX NPs
OcA-PTX NPs
DA-PTX NPs
LA-PTX NPs
MA-PTX NPs
PA-PTX NPs
AA-PTX NPs

E


Retention time / min
PTX
Ac-PTX
BA-PTX
HA-PTX
OcA-PTX
DA-PTX
LA-PTX
MA-PTX
PA-PTX
AA-PTX

F

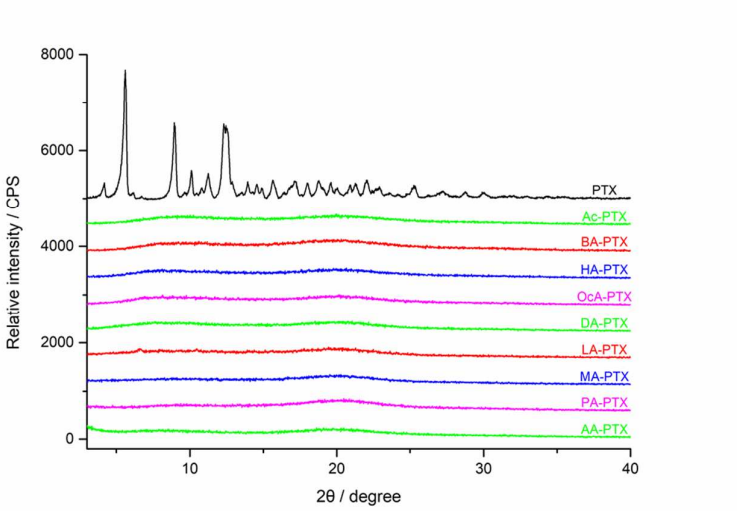


Fig.2. The effect of XlogP and Hansen solubility parameters on straight-chain fatty acid-PTX self-assembling NPs

A: Theoretical n-octanol-water partition coefficients (XlogP) of PTX and straight-chain fatty acid-PTX calculated by XLOGP3.

B: Hansen solubility parameters (HSPs) of PTX and straight-chain fatty acid-PTX according to Beerbower, including the dispersion (δ_d), polar (δ_p) and hydrogen bonding (δ_h) and total solubility parameter (δ_t).

C: The structures of PTX and straight-chain fatty acid-PTX and TEM images of PTX, and straight-chain fatty acid-PTX NPs.

D: Particle size of PTX and straight-chain fatty acid-PTX NPs.

E: The retention time of PTX and straight-chain fatty acid-PTX determined by high performance liquid chromatography (HPLC).

F: The X-ray diffraction (XRD) spectra of PTX and straight-chain fatty acid-PTX.

The effect of XlogP and Hansen solubility parameters on B-G-PTX, BeA-PTX and SaA-PTX self-assembled into NPs

In order to further confirm the importance of the XlogP and Hansen solubility parameters for self-assembling NPs, we designed and synthesized the N-tert-butoxycarbonyl glycine-paclitaxel conjugate (B-G-PTX), in which the N-(tert-butoxycarbonyl) glycine had a branched structure and heteroatom-containing small molecule, the benzoic acid-paclitaxel conjugate (BeA-PTX), in which the benzoic acid was the simplest aromatic acid, and the salicylic acid-paclitaxel conjugate (SaA-PTX).

The calculated XlogP values of B-G-PTX, BeA-PTX and SaA-PTX was all higher than that of PTX (Fig.3A). Most of the Hansen solubility parameters B-G-PTX, BeA-PTX and SaA-PTX were lower than that of PTX (Fig.3B). We found that

B-G-PTX, BeA-PTX and SaA-PTX spontaneously formed NPs in water with a particle size in the range of 100-130 nm (Fig.3C, 3D and S1). These results indicated that the increased XlogP and decreased Hansen solubility parameters might be also key factors for non-straight-chain fatty acid-PTX conjugates self-assembling into NPs.

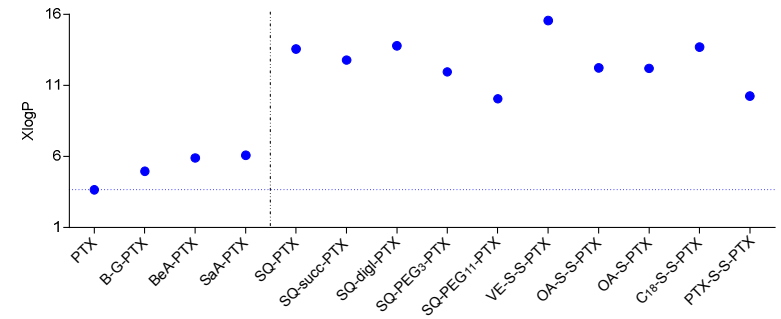
As shown in Fig.3E, the retention times of B-G-PTX, BeA-PTX and SaA-PTX were all longer than that of PTX. In addition, B-G-PTX, BeA-PTX and SaA-PTX were all in an amorphous state (Fig.3F).

The effect of XlogP and Hansen solubility parameters on reported PTX SMMDCs self-assembled into NPs

It has been reported that several PTX SMMDCs can self-assemble into NPs, and these PTX SMMDCs include SQ-PTX,[9] SQ-succ-PTX,[9] SQ-digl-PTX,[9] SQ-PEG_3-PTX,[9] SQ-PEG_{11}-PTX,[9] VE-S-S-PTX,[10] OA-S-S-PTX,[11] OA-S-PTX,[12] C_{18}-S-S-PTX[13] and PTX-S-S-PTX.[14] The XlogP and Hansen solubility parameters of these PTX SMMDCs were calculated. As shown in Fig.3A and 3B, all the XlogP values were increased compared with PTX and all the Hansen solubility parameters were decreased compared with PTX.

Considering the XlogP values of these reported PTX SMMDCs, we found that the XlogP was increased 1.75- to 3.25-fold compared with PTX (Fig. 3G). For our synthetic PTX SMMDCs, such as Ac-PTX, BA-PTX, HA-PTX, B-G-PTX, BeA-PTX and SaA-PTX, we found that the increases in their XlogP were all below 1.0-fold compared with PTX (Fig. 3G). In addition, the self-assembling NPs of SaA-PTX and B-G-PTX were found to become unstable if kept for two days. Although the increased XlogP values of SaA-PTX and B-G-PTX were not the lowest among Ac-PTX, BA-PTX, HA-PTX, B-G-PTX, BeA-PTX and SaA-PTX, we found that the Hansen solubility parameters of SaA-PTX and B-G-PTX were more similar to that of PTX, especially the calculated δ_p and δ_h values which were only decreased about 9.6% and 3.8% (SaA-PTX), 2.6% and 8.6% (B-G-PTX) (Fig. 3H), respectively.

A


XlogP
16
11
6
1
PTX
B-G-PTX
BeA-PTX
SaA-PTX
SQ-PTX
SQ-succ-PTX
SQ-digl-PTX
SQ-PEG3-PTX
SQ-PEG11-PTX
VE-S-S-PTX
OA-S-S-PTX
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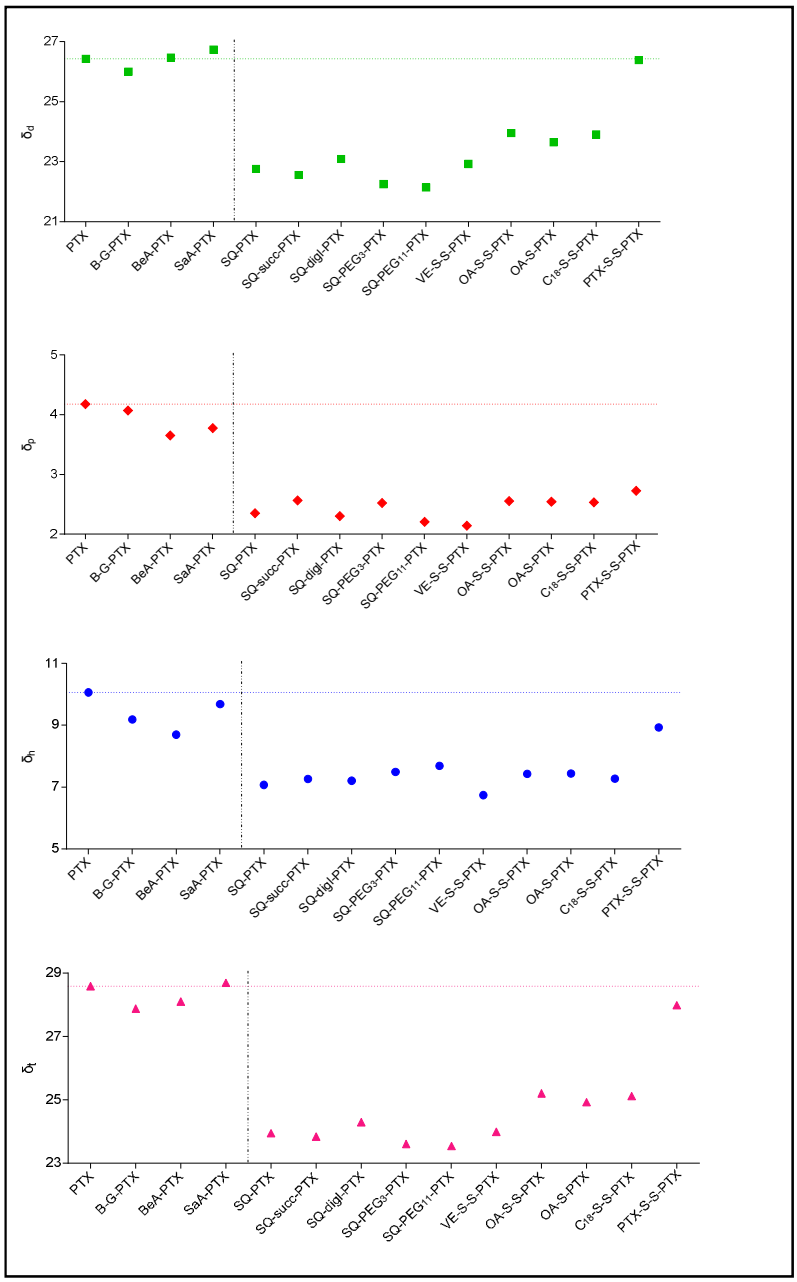
C18-S-S-PTX
PTX-S-S-PTX

B


δd
δp
δh
δt
PTX
B-G-PTX
BeA-PTX
SaA-PTX
SQ-PTX
SQ-succ-PTX
SQ-digl-PTX
SQ-PEG3-PTX
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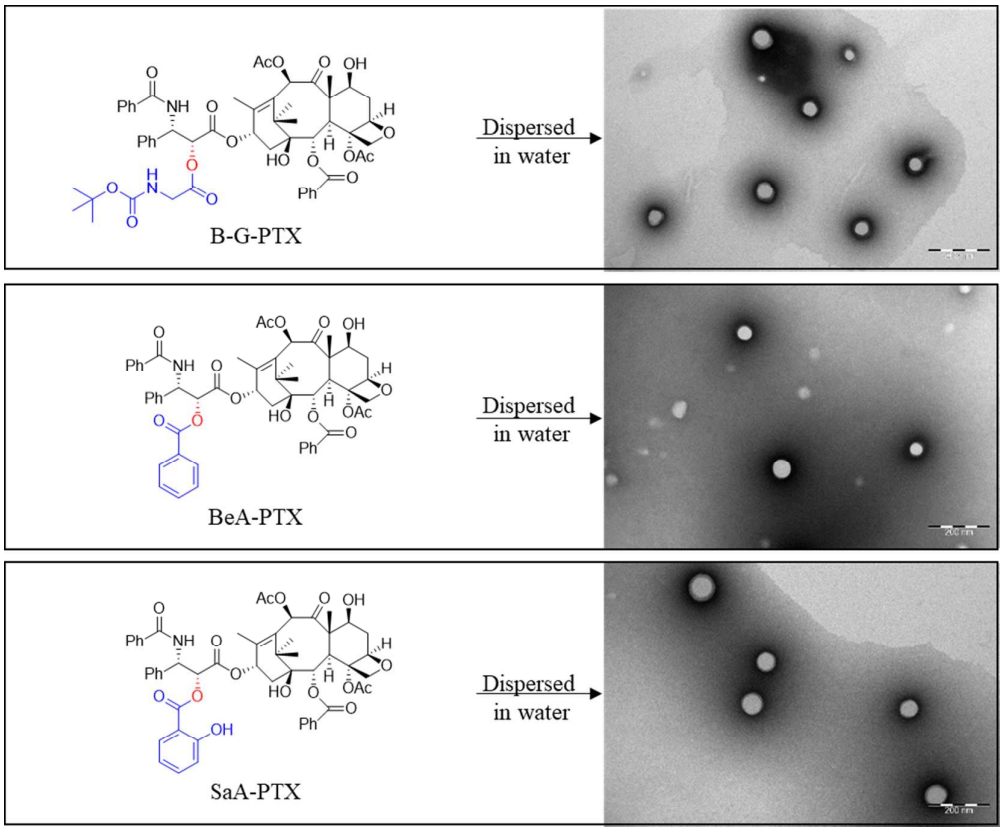
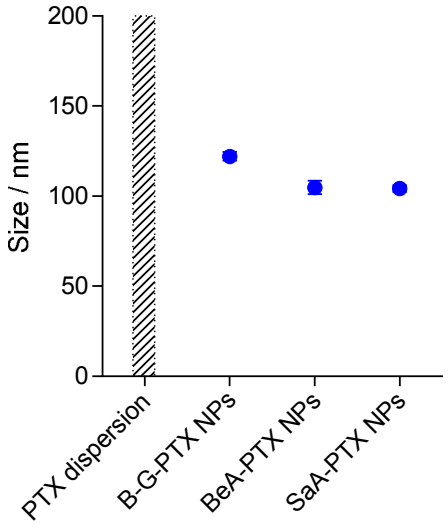
OA-S-PTX
C18-S-S-PTX
PTX-S-S-PTX

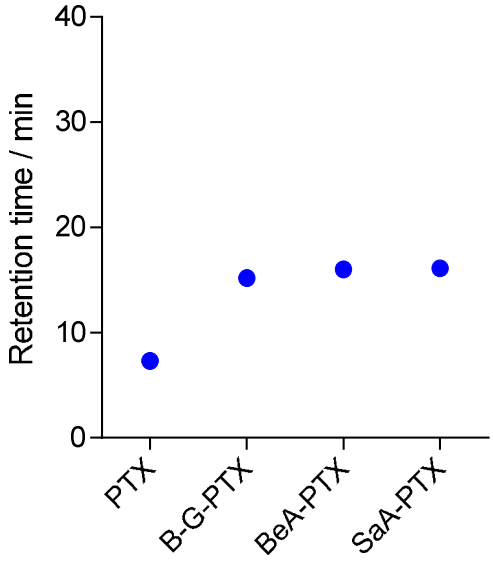
C


B-G-PTX
Dispersed in water
BeA-PTX
Dispersed in water
SaA-PTX
Dispersed in water

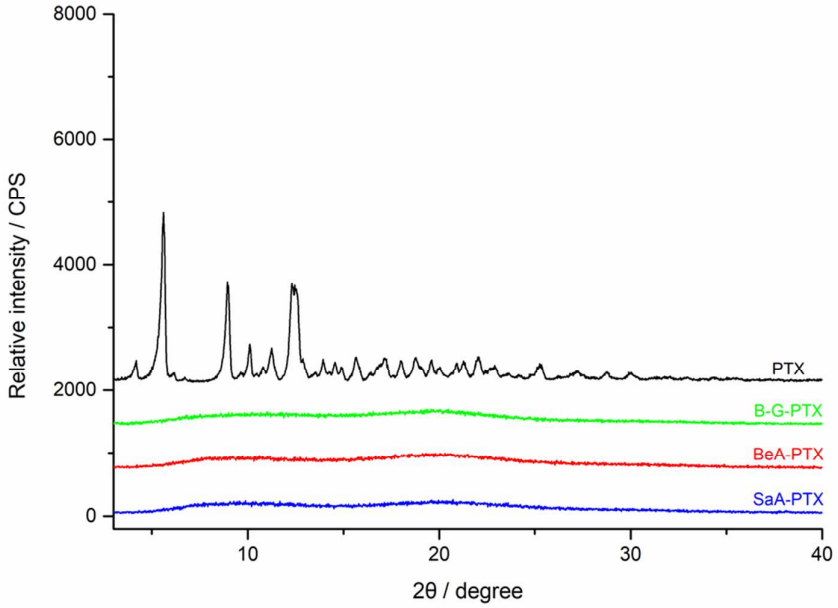
D


Size / nm
200
150
100
50
0
PTX dispersion
B-G-PTX NPs
BeA-PTX NPs
SaA-PTX NPs

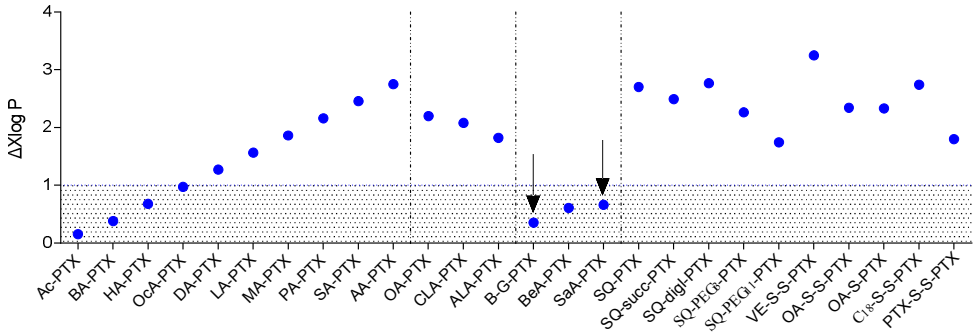
E


Retention time / min
40
30
20
10
0
PTX
B-G-PTX
BeA-PTX
SaA-PTX

F


8000
6000
4000
2000
0
Relative intensity / CPS
10
20
30
40
2θ / degree
PTX
B-G-PTX
BeA-PTX
SaA-PTX

G


ΔXlog P
4
3
2
1
0
Ac-PTX
BA-PTX
HA-PTX
OcA-PTX
DA-PTX
LA-PTX
MA-PTX
PA-PTX
SA-PTX
AA-PTX
OA-PTX
CLA-PTX
ALA-PTX
B-G-PTX
BeA-PTX
SaA-PTX
SQ-PTX
SQ-succ-PTX
SQ-digl-PTX
SQ-PEG6-PTX
SQ-PEG11-PTX
VE-S-S-PTX
OA-S-S-PTX
OA-S-PTX
C18-S-S-PTX
PTX-S-S-PTX

H

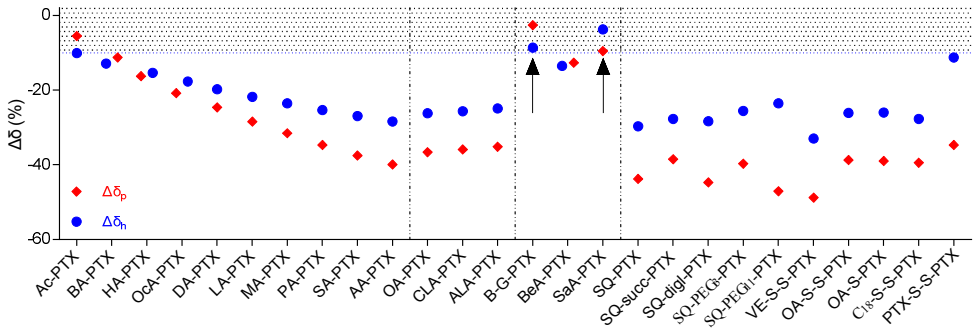

Δδ (%)
0
-20
-40
-60
Δδp
Δδh
Ac-PTX
BA-PTX
HA-PTX
OcA-PTX
DA-PTX
LA-PTX
MA-PTX
PA-PTX
SA-PTX
AA-PTX
OA-PTX
CLA-PTX
ALA-PTX
B-G-PTX
BeA-PTX
SaA-PTX
SQ-PTX
SQ-succ-PTX
SQ-digl-PTX
SQ-PEG6-PTX
SQ-PEG11-PTX
VE-S-S-PTX
OA-S-S-PTX
OA-S-PTX
C18-S-S-PTX
PTX-S-S-PTX

Fig.3. The effect of XlogP and Hansen solubility parameters on B-G-PTX, BeA-PTX and SaA-PTX self-assembling NPs and reported PTX SMMDC NPs
A: Theoretical n-octanol-water partition coefficients (XlogP) of PTX and B-G-PTX, BeA-PTX, SaA-PTX and reported PTX SMMDCs calculated by XLOGP3.
B: Hansen solubility parameters (HSPs) of PTX and B-G-PTX, BeA-PTX, SaA-PTX and PTX SMMDCs according to Beerbower, including dispersion (δ_d), polar (δ_p) and hydrogen bonding (δ_h) and total solubility parameter (δ_t).
C: The structures of PTX and B-G-PTX, BeA-PTX and SaA-PTX and TEM images of PTX and B-G-PTX, BeA-PTX and SaA-PTX NPs.
D: Particle size of PTX and B-G-PTX, BeA-PTX and SaA-PTX NPs.
E: The retention time of PTX and B-G-PTX, BeA-PTX and SaA-PTX determined by high performance liquid chromatography (HPLC).
F: The X-ray diffraction (XRD) spectra of PTX and B-G-PTX, BeA-PTX and SaA-PTX.
G: The ΔXlogP values of our synthetic PTX SMMDCs and reported PTX SMMDCs (ΔXlogP=(XlogP value of PTX SMMDCs - XlogP value of PTX)/ XlogP value of PTX).
H: The ΔHansen solubility parameters values of our synthetic PTX SMMDCs and reported PTX SMMDCs (ΔHansen solubility parameters=(Hansen solubility parameters value of PTX-Hansen solubility parameters value of PTX SMMDCs)*100%/ Hansen solubility parameters value of PTX).

Considering the results of the XlogP and Hansen solubility parameters of our synthetic PTX SMMDCs and reported PTX SMMDCs, we suggested that for designing self-assembling PTX SMMDC NPs, the XlogP value of PTX SMMDCs should increase more than 1.0-fold compared with that of the parent drug PTX, otherwise, their Hansen solubility parameters of δ_p and δ_h, especially the δ_h value, would decrease more than 10% compared with the parent drug PTX.

The effect of XlogP and Hansen solubility parameters on SMMDCs self-assembled into NPs

The lipophilicity of compounds can be correlated with the pharmacodynamic drug property. Increasing lipophilicity will reduce solubility[22] and, so, if a compound has a higher lipophilicity, it will tend to precipitate in water. In addition, the crystallinity of organic compounds is influenced by strong hydrogen bonding and the dipole force between molecules. Compounds exhibiting strong hydrogen bonding and a dipole force tend to form one-dimensional nanostructures, while compounds with weak hydrogen bonding and a weak dipole force readily form nanoparticles.[23] Here, we selected XlogP as the parameter for evaluating the compound lipophilicity and the polar solubility (δ_p) and hydrogen bonding solubility (δ_h) as parameters for evaluating the compound crystallinity.

Considering the XlogP results of our synthetic PTX SMMDCs (16 compounds) and

reported PTX SMMDCs (10 compounds), we suggested that for designing self-assembling SMMDC NPs, the XlogP value of SMMDCs should increase more than 1.0-fold compared with that of the parent drug, otherwise the Hansen solubility of the polar solubility parameter (δ_p) and hydrogen bond solubility (δ_h), especially the δ_h value, should decrease more than 10% compared with that of the parent drug. The amorphous state of SMMDCs also had an impact on SMMDCs self-assembling into NPs.

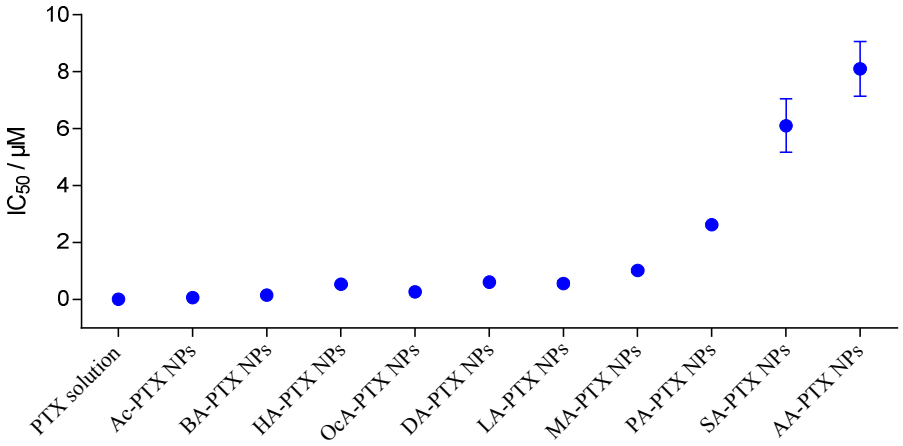
The effect of XlogP on the *in vitro* anti-tumor activity of SMMDCs self-assembled into NPs

Lipophilicity is also a very commonly used parameter in Quantitative Structure-Activity Relationship (QSAR) analysis for predicting the biological activity of a compound. Here, we examined the effect of the Xlogp of PTX SMMDCs on the anti-tumor activity of these PTX SMMDC NPs. The *in vitro* anti-tumor activity of our synthetic PTX SMMDC NPs was investigated in MCF-7 cells and MCF-7/A cells. We selected the IC_{50} value as a parameter which was defined as the drug concentration which inhibited the tumor cell growth by 50%. A higher IC_{50} value means lower *in vitro* anti-tumor activity. As shown in Fig. 4A and 4B, the IC_{50} value of PTX SMMDC NPs was increased along with the XlogP values (Fig. 4A vs Fig. 2A, Fig. 4B vs Fig. 2A), indicating that the *in vitro* anti-tumor activity of SMMDC NPs was reduced along with the increased XlogP values. For designing SMMDCs with potential ability to self-assemble into NPs, their XlogP should increase at a relatively higher level compared with that of the parent drug, however, the *in vitro* anti-tumor activity of these SMMDCs NPs decreased along with the XlogP, indicating that a suitable XlogP value for designing SMMDCs is important for self-assembling into NPs and for possessing anti-tumor activity.

In addition, we also investigated the *in vitro* anti-tumor activity of these PTX SMMDCs in MCF-7 cells. As shown in Supporting Information, the IC_{50} value of PTX SMMDCs was increased along with the XlogP values (Fig. S2), similar with that of PTX SMMDC NPs.

Considering to the reported PTX SMMDCs, their XlogP values were relative high, however, some PTX SMMDCs contained disulfide bonds as a linker, such as VE-S-S-PTX,[10] OA-S-S-PTX,[11] OA-S-PTX,[12] C_{18}-S-S-PTX[13] and PTX-S-S-PTX.[14] These disulfide bonds could respond to the tumor microenvironment to release the parent drug PTX. For SQ-PTX,[9] SQ-succ-PTX,[9] SQ-digl-PTX,[9] SQ-PEG_3-PTX[9] and SQ-PEG_{11}-PTX,[9] the *in vitro* anti-tumor activity of SQ-succ-PTX and SQ-digl-PTX was relatively higher than that of the others being more susceptible to hydrolysis to form PTX. The *in vitro* anti-tumor activity of SQ-PTX, with a higher XlogP value, was less potent than free PTX. Therefore, for a higher XlogP, the degradable linker should be considered in the design of SMMDCs for overcoming the problem of lower anti-tumor activity.

A



B

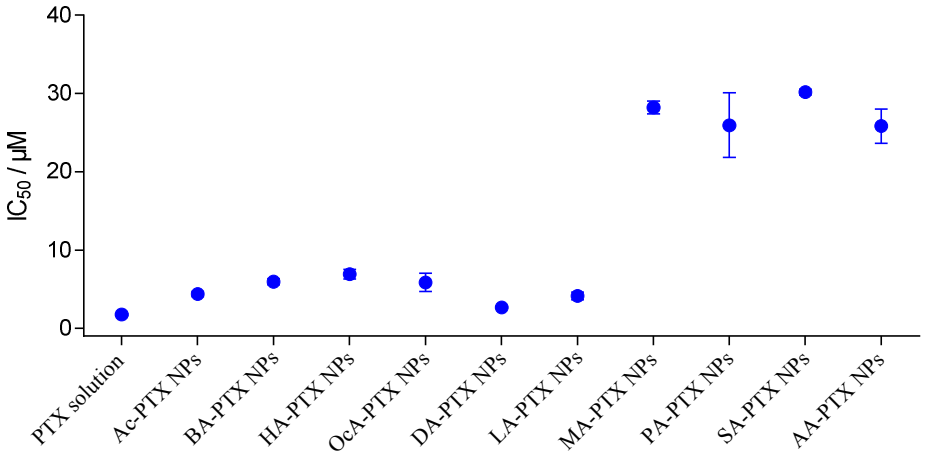


Fig.4. The effect of XlogP on the *in vitro* anti-tumor activity of PTX SMMDCs self-assembling NPs.
The *in vitro* anti-tumor activity of our synthetic PTX SMMDC NPs was investigated in MCF-7 cells (**A**) and MCF-7/A cells (**B**).

Probable structure of SMMDCs self-assembling NPs

It is well known that amphiphilic block copolymers are spontaneously formed when polymer micelles composed of a hydrophilic shell and a lipophilic core are dispersed in water. For our synthetic self-assembling PTX SMMDCs, the small molecular weight compounds and PTX were all hydrophobic compounds, however, the hydrophilic groups, such as hydroxyl, is still present in PTX. The hydrophobic groups preferred to come together due to hydrophobic forces, while the hydrophilic groups tended to extend into the water, resulting in the formation of a hydrated layer. Therefore, the hydrophobic groups of small molecular weight compounds preferentially exist in the inner region of self-assembling NPs, while, the anti-tumor drug, containing hydrophilic group, preferentially are present on the surface of self-assembling NPs (Fig. 5). In order to confirm this hypothesis, we investigated the zeta potential of our synthetic PTX SMMDC self-assembling NPs. As shown in Fig. 6, the zeta potentials of PTX NPs were all negative. Also, a negative zeta potential has also been reported in PTX SMMDC self-assembling NPs, such as SQ-PTX NP (-36.88 mV),[9] SQ-succ-SQ NP (-44.33 mV),[9] SQ-digl-PTX NP (-38.03 mV),[9] SQ-PEG_3-PTX NP (-41.42 mV),[9] SQ-PEG_{11}-PTX NP (-40.88 mV),[9] VE-S-S-PTX NP

(−29.2 mV),[10] C_{18}-S-S-PTX NP (-26.2 ± 0.64 mV)[13] and PTX-S-S-PTX NP (−33.9 ± 0.777 mV).[14] While, a positive zeta potential has been reported irinotecan (Ir) SMMDC self-assembling NPs (Cb-Ir NP, +3.4 mV).[17] We suggested that there was a hydroxyl group in PTX, while, there was a piperidyl group in Ir. These results confirmed that hydrophilic groups are preferentially located on the surface of self-assembling NPs.

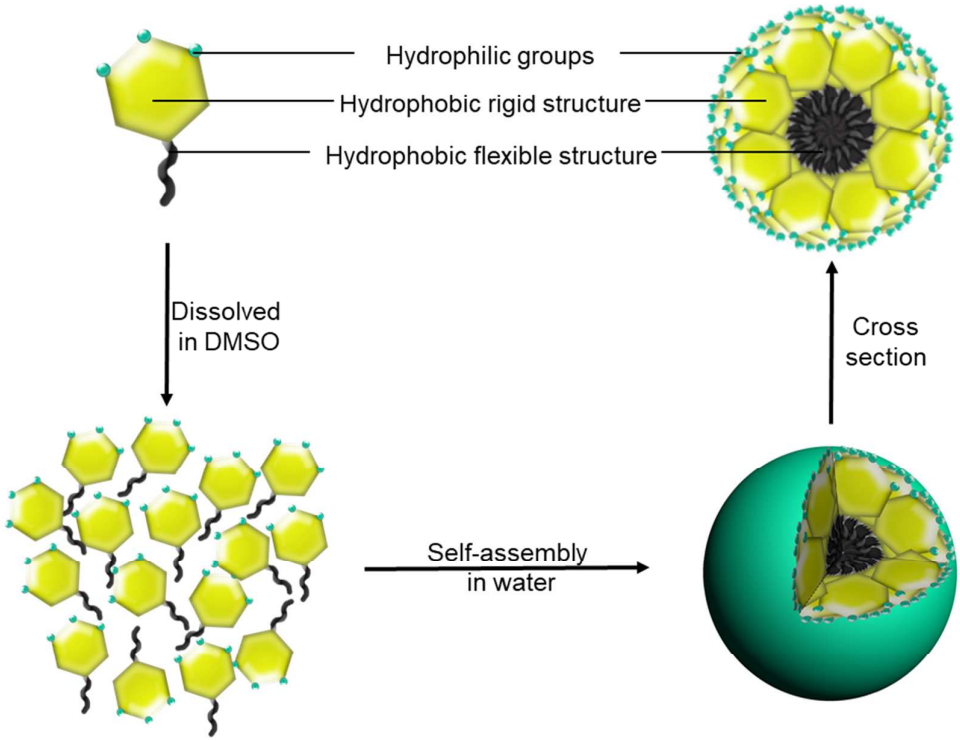


Fig.5. The schematic diagram of SMMDC self-assembling into NPs
The hydrophobic groups in small molecular weight agents preferred to gather together due to hydrophobic forces, while the hydrophilic groups in the anti-tumor drug tended to stretch into the water, resulting in the formation of a hydrated layer owing to hydration. Therefore, the hydrophobic group of small molecular weight agents preferred to exist in the inner part of self-assembling NPs, while, the anti-tumor drug, containing hydrophilic groups, preferred to be located on the surface of self-assembling NPs.

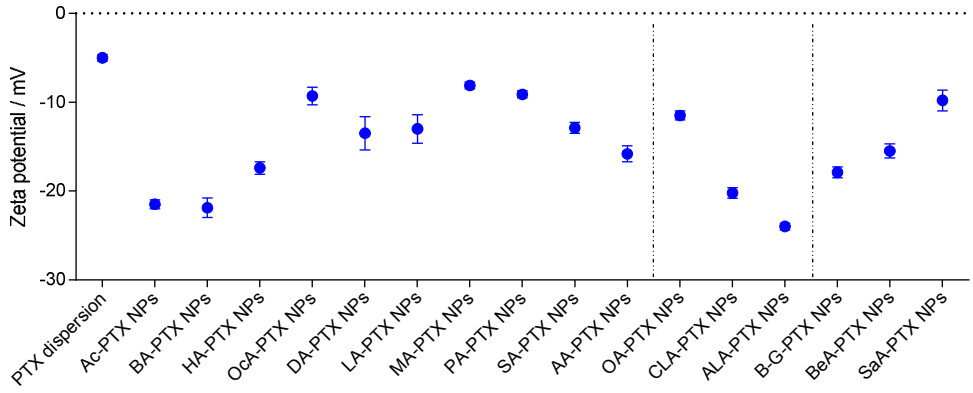


Fig.6. The zeta potential of our synthetic PTX SMMDC NPs.

Conclusion

We suggest that for designing self-assembling SMMDC NPs, the XlogP value of the SMMDCs should increase more than 1.0-fold compared with that of the parent drug, otherwise, their Hansen solubility of the polar solubility parameter (δ_p) and hydrogen bond solubility (δ_h), especially the δ_h value, should decrease more than 10% compared with the parent drug. The amorphous state of SMMDCs should also have an effect for SMMDCs self-assembling into NPs. However, the anti-tumor activity of these SMMDCs NPs decreased along with the XlogP, indicating that a suitable XlogP value for designing SMMDCs is important for self-assembling into NPs and for exhibiting anti-tumor activity. For higher level XlogP SMMDCs, a degradable linker should be considered in the design SMMDCs to overcome the problem of lower anti-tumor activity. The hydrophilic groups in the SMMDCs, should preferentially be located on the surface of self-assembling NPs.

Experimental Procedures

Materials

Paclitaxel (PTX) was obtained from Ouhe Co., Ltd. (Beijing, China). Other materials are shown in Supporting Information.

Synthesis of PTX SMMDCs

The detailed processes for PTX SMMDCs are shown in Supporting Information. In brief, PTX SMMDCs were synthesized through a direct esterifying reaction on the C2'-hydroxyl of PTX catalyzed by DCC/DMAP.[24]

Characterization of SMMDCs

Crystallinity The crystallinity of all samples was examined by powder X-ray diffraction (XRD) using a D/MAX 2000 rotating anode X-ray diffractometer (Rigaku Co., Japan) equipped with a Cu-Kα X-ray source (λ = 1.541 nm, 40 kV /100 mA),[18] as shown in Supporting Information.

Polarity The polarity of all samples was evaluated by high performance liquid chromatography (HPLC), involving an LC-20AT liquid chromatograph (SHIMADZU, Japan) and SPD-M20A diode array detector (SHIMADZU, Japan), as shown in Supporting Information.

Theoretical partition coefficient: XlogP value

The theoretical partition coefficient of each compound was calculated using the XLOGP3 developed by the Wang group.[19] Three-dimensional structural models of each compound were constructed with the ChemBio3D Ultra 2010 software. All final models were saved in Tripos Mol2 format as input data for the calculation.

Hansen solubility parameters

Based on the concept of dividing the total cohesive energy into individual components, the solubility parameters are constituted of three parts: dispersion (δ_d), polar (δ_p) and hydrogen bonding (δ_h).[20] The total solubility parameter (δ_t), also called the three-dimensional solubility parameter, can be defined as follows:

$$\delta_t=(\delta_d^2+\delta_p^2+\delta_h^2)^{\frac{1}{2}}$$

Only requiring knowledge of the compound's chemical structure, the group contribution method (GCM) is a commonly used theoretical method to calculate the HSPs. In this method, the cohesive energy of a molecule is considered to be an additive property and is the sum of the contributions from individual groups contained in the molecule. Using the GCM proposed by Beerbower,[21] the disperse solubility parameter (δ_d), polar solubility parameter (δ_p) and hydrogen bonding solubility parameter (δ_h) and the total solubility parameter (δ_t) were calculated as follows:

$$\delta_d = \frac{\sum_i F_{d_i}}{\sum_i V_i}$$

$$\delta_p = \frac{(\sum_i F_{p_i})^{\frac{1}{2}}}{\sum_i V_i}$$

$$\delta_h = \frac{\sum_i F_{h_i}}{(\sum_i V_i)^{\frac{1}{2}}}$$

where i is the structural group within the molecule, F_{d_i} is the group contribution to the dispersion forces, F_{p_i} is the group contribution to the polar forces, F_{h_i} is the group contribution to the hydrogen-bonding energy, and V_i is the group contribution to the molar volume of drug compounds or conjugates. The detail calculation is shown in Supporting Information.

Preparation and characterization of self-assembled NPs

All the self-assembled NPs in the present study were prepared by nano-precipitation, as shown in our previous report.[18] The particle size, particle size distribution and zeta potential were measured by dynamic light scattering (DLS). A transmission electron microscope (TEM) (JEM-1400Plus, JEOL Ltd. Tokyo, Japan) was used to examine the morphology of the NPs.

***In vitro* cytotoxicity studies**

The cytotoxicity of self-assembling NPs was evaluated against MCF-7 cells and MCF-7/ADR cells using a sulfonyl rhodamine B (SRB) colorimetric assay, as shown in our previous report.[18, 25]

Associated Content

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Additional details on materials, synthesis and characterization of PTX SMMDDCs, *in vitro* cytotoxicity studies, structural characteristics of PTX and synthetic PTX SMMDCs, calculation of HSPs for PTX according to Beerbower, XlogP and HSPs of PTX and PTX SMMDCs, DLS of PTX SMMDCs self-assembled nanoparticles, *in vitro* anti-tumor activity of PTX SMMDCs solution (PDF).

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Conflict of Interest statement:

The authors declare no competing financial interest.

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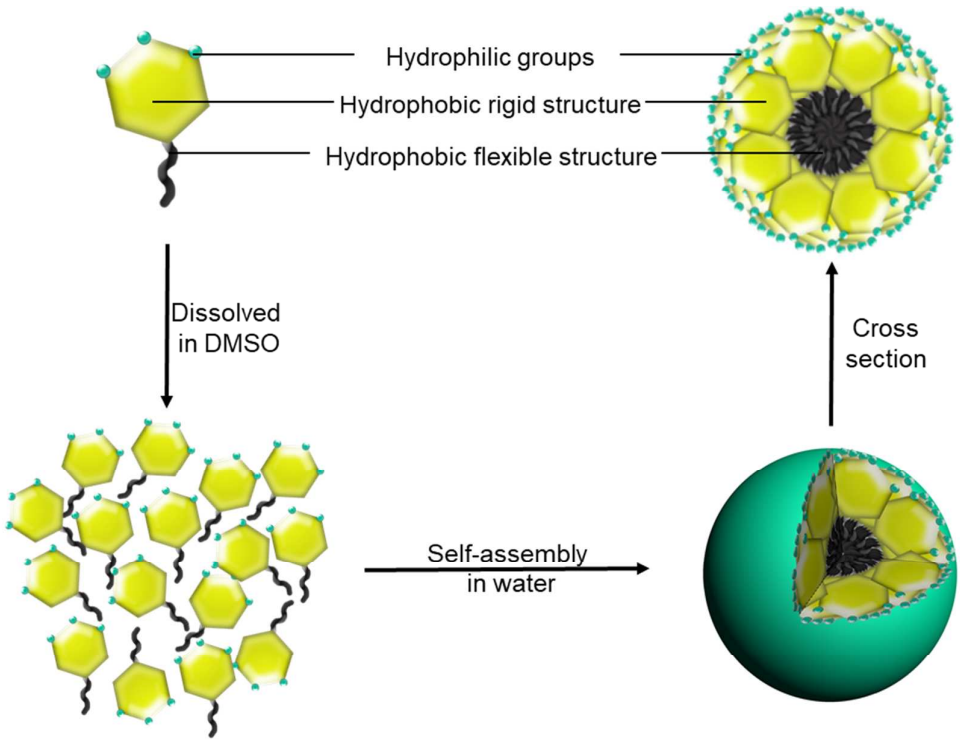
TOC graphic


Hydrophilic groups
Hydrophobic rigid structure
Hydrophobic flexible structure
Dissolved in DMSO
Cross section
Self-assembly in water