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Newly acylated SN-38 homodimers: carrier-free nano-prodrugs for chemotherapy

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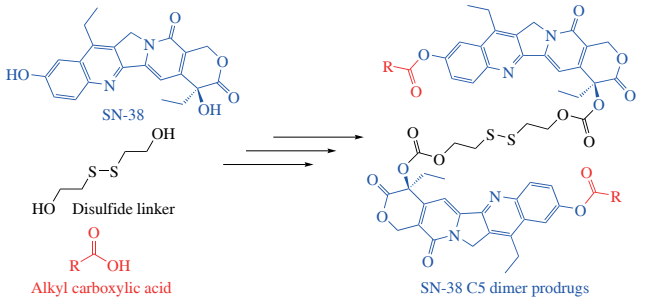
Homodimers based on 7-ethyl-10-hydroxycamptothecin (SN-38) containing $S_2[(CH_2)_2OC(O)]_2$ bridge and acyl substituents were synthesized and used for fabrication of nano-prodrugs. The latter demonstrated remarkable dispersion and stability over one month when stored at a temperature of 4 °C, with an ideal size of nanoparticles in the range of 80–130 nm that is required for EPR effect. The homodimer containing cyclopropylacetyl substituent chain exhibited significant anticancer efficacy against the HCT-116 and A-549 cell lines, with IC_{50} values having been 0.07 ± 0.01 and 0.29 ± 0.02 μM, respectively.



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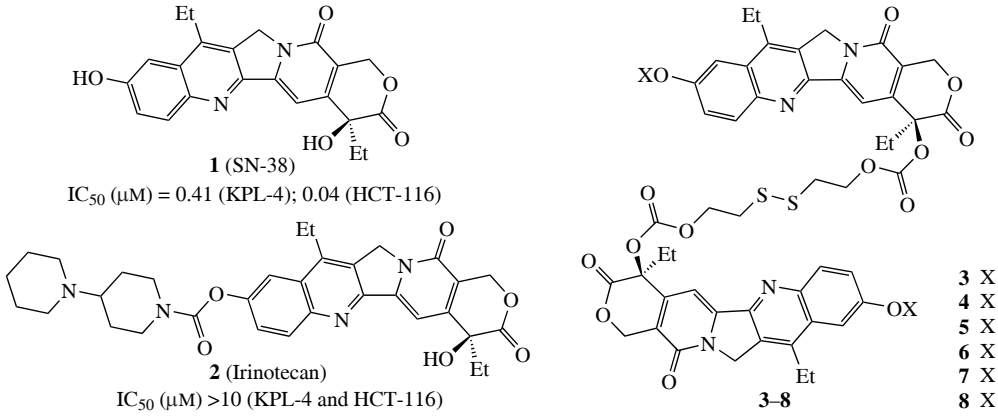
The application of nanotechnology for the administration of anticancer agents is widespread, primarily due to the various challenges linked to these agents. These challenges include poor solubility in water, insufficient cellular permeability, liver disposition, swift clearance from the bloodstream and kidneys, a limited therapeutic range, lack of selectivity for cancer cells, undesirable side effects, and rapid absorption by normal tissues.[1,2] Nanocarrier-based drug delivery system (DDS) has often been employed to enhance the therapeutic effectiveness of drug molecules. Generally, nanocarriers are composed of inorganic materials, including carbon-based, magnetic, silica-based, and gold-based nanomaterials, as well as organic molecules such as polymeric nanoparticles and liposomes.[3–5] Nanocarrier-based DDSs enhance the effectiveness of drug molecules; nonetheless, they are accompanied by several disadvantages. These drawbacks encompass intricate synthesis procedures, restricted drug loading capabilities, slow drug release profiles, possible bio-incompatibility of the carriers, non-specific systemic toxicity, and the potential for triggering immune responses, among other concerns.[6,7] In response to these limitations, the development of carrier-free nanodrugs has emerged. Our research group has reported reprecipitation method for the fabrication of nanodrugs that do not require the use of carriers, which demonstrate a remarkable efficiency in cellular uptake.[8–11] In this study, we fabricated nano-prodrugs (NPDs) utilizing the same reprecipitation technique.

7-Ethyl-10-hydroxycamptothecin (SN-38) **1** is a potent inhibitor of DNA topoisomerase I, an enzyme essential for the unwinding of DNA helices and the relaxation of supercoiled DNA during the replication process.[12] Although SN-38 exhibits significant efficacy as an anticancer agent, its application in clinical settings is hindered by a range of chemical and pharmacological obstacles.[13] It cannot be formulated in any solution dosage form due to insolubility in water and other pharmaceutically acceptable solvents or oils. Irinotecan **2** (Figure 1), a water-soluble prodrug of SN-38, was developed to overcome the challenge of insolubility and approved by Food and Drug Administration (FDA) for second-line treatment in cases of progressive metastatic carcinoma of the colon or rectum.[14,15] In February 2024, FDA approved irinotecan liposome (Onivyde®) in combination with oxaliplatin, fluorouracil, and leucovorin, to be used as a first-line treatment for metastatic pancreatic adenocarcinoma.[16] However, the administration of this drug leads to various side effects due to unintended transformation into the active metabolite SN-38 by carboxylesterase enzymes, predominantly found in the liver.[17,18] Besides its restricted solubility, the presence of the α-OH group at the C^{20} chiral center of SN-38 promotes the hydrolysis of the lactone ring, resulting in its conversion to the carboxylate form at a physiological pH of 7.4, which has no therapeutic effect.[19] The incorporation of a linker to the α-OH group at the C^{20} chiral center of SN-38 leads to the prevention of lactone ring hydrolysis and the transformation into the drug's active form by carboxylesterase enzymes, because of steric hindrance. In our previous research, we synthesized prodrugs **3**–**8** which consisted of two molecules of SN-38 connected *via* a disulfide linker (see Figure 1). These prodrugs are stable toward hydrolysis by carboxylesterase, and selectively release SN-38 in environments with high concentrations of GSH such as cancer cells.[20] Among the prodrugs examined, compound **5** demonstrated significant antitumor efficacy, with no severe adverse effects reported. In this research, we introduced new prodrugs by modifying the acyl structure of **5** while maintaining a consistent carbon number, aiming to assess how steric hindrance of the side chain influences anticancer activity.



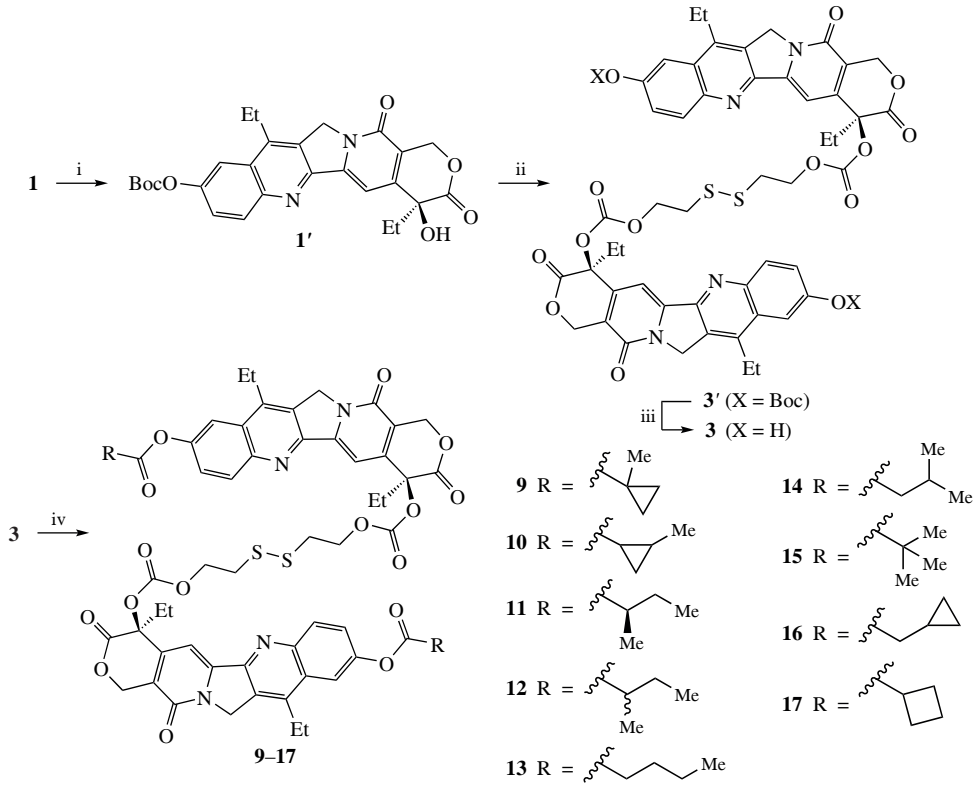
	IC$_{50}$ (μM)	
	KPL-4	HCT-116
3 X = H	0.37	0.18
4 X = MeC(O)	0.36	0.28
5 X = PrC(O)	0.41	0.06
6 X = *n*-C_5H_{11}C(O)	1.66	0.33
7 X = *n*-C_7H_{15}C(O)	9.67	0.68
8 X = *n*-C_9H_{19}C(O)	>10	0.96

Figure 1 Structures of SN-38 **1**, Irinotecan **2** and SN-38 prodrugs **3**–**8** with IC$_{50}$ values against KPL-4 and HCT-116 cells.[20]

Dimers **9**–**17** (Scheme 1) were designed based on *in vitro* anticancer activity of dimers **3**–**8** in our previous work. These prodrugs demonstrated resistance to hydrolysis; however, they selectively released SN-38 when the disulfide bond was cleaved by glutathione present in higher concentrations in cancer cells.[20] Compound **5** demonstrated a potency nearly equivalent to that of SN-38 when tested against KPL-4 and HCT-116 cell lines, and aggregation was not observed in the dispersion solution over an extended duration. Although compounds **3** and **4** also showed better potency than **5** against KPL-4 cell, the nanoparticles of these prodrugs aggregated immediately in the dispersion solution.[20] Therefore, we have selected dimers **9**–**17** for further investigation due to their five-carbon side chain, which is in proximity to compound **5** that possesses a four-carbon side chain. This selection facilitates increased diversity in the side chain while maintaining a uniform carbon count.

Synthesis of dimers **9**–**17** (see Scheme 1) involved Boc-protected at the phenolic group derivative **1′**. Two molecules of **1′** were linked with bis-carbonate disulfide linker by treating them with 2,2′-dithiodiethanol in the presence of triphosgene and DMAP in dichloromethane to get dimer **3′** *via* the *in situ* generation of chlorate intermediate.[21] Removal of Boc protection afforded bis-phenol **3** which was finally doubly acylated. The structures of synthesized compounds were confirmed by spectral data, *i.e*. ESI-HRMS, ^{1}H NMR, ^{13}C NMR, *etc*.

Prodrugs **9**–**17** were fabricated into nanoparticles using reprecipitation method.[8] The nano-prodrugs exhibited excellent dispersion and stability within the dispersion solution, as confirmed by dynamic light scattering (DLS) analysis. Furthermore, there were no significant alterations observed in the size or dispersion of the nanoparticles stored at 4 °C for an extended duration of one month. DLS analysis also revealed that the fabricated nanoparticles fall within the optimal size range (80–130 nm) necessary for the enhanced permeability and retention (EPR) effect [Figure 2(*a*)]. The size of the nanoparticles was also evaluated through the analysis of SEM images [Figure 2(*b*)] and the results were consistent with the findings from the DLS analysis.



Scheme 1 *Reagents and conditions*: i, $(Boc)_2O$, pyridine, CH_2Cl_2, room temperature, 3 h, 97%; ii, (*a*) triphosgene, DMAP, $S_2(CH_2CH_2OH)_2$, CH_2Cl_2, 0 → 20 °C, 1 h; (*b*) triphosgene, $S_2(CH_2CH_2OH)_2$, CH_2Cl_2, room temperature, 2 h, 71%; iii, CF_3CO_2H (10%), CH_2Cl_2, room temperature, 3 h, 99%; iv, RCOOH, EDC, DMAP, room temperature, 3 h, 58–71%.

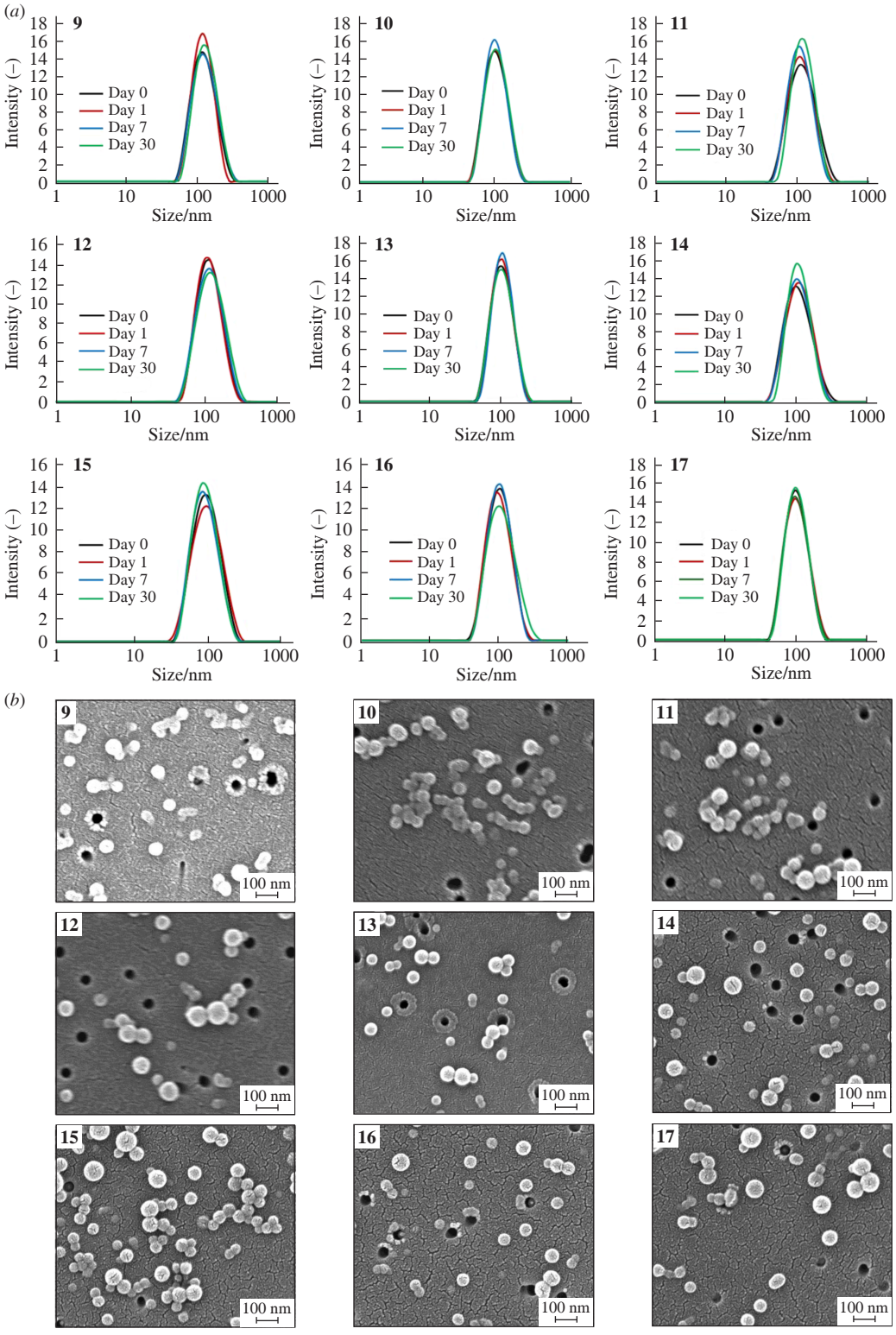


Figure 2 (*a*) Dynamic light scattering (DLS) analysis and (*b*) SEM images of fabricated nano-prodrugs **9**–**17**.

The fabricated nano-prodrugs **9**–**17** were evaluated for *in vitro* anticancer activity in the range of 0.04–10 μM (based on SN-38 **1** concentration) against colon (HCT-116), breast (MCF-7) and lung (A-549) cancer cells (Table 1) following the method used in previous paper.[20,22] Additionally, the nano-prodrugs were tested for cytotoxic effects on normal human dermal fibroblasts neonatal foreskin cells (NHDF-Neo) to determine the specificity of the prodrugs. Prodrug **16** containing cyclopropyl acetyl side chain demonstrated anticancer activity against HCT-116 and A-549 cells with IC_{50} values of 0.07 ± 0.01 and 0.29 ± 0.02 μM, respectively, which were comparable to those of parent drug SN-38 **1** (IC_{50} values of 0.04 ± 0.01 and 0.24 ± 0.01 μM, respectively). The therapeutic indices determined for HCT-116 and A-549 cells were 63.72 ± 2.15 and 16.39 ± 1.93, respectively, representing the highest values among the fabricated nano-prodrugs. Nano-prodrug **16** also showed highest potency against breast cancer among the fabricated nano-prodrugs with IC_{50} value of 1.76 ± 0.06 μM. All the nano-prodrugs **9**–**17** and SN-38 **1** demonstrated greater efficacy against HCT-116, MCF-7, and A-549 cell lines in comparison to irinotecan. This improved effectiveness relates to the hydrophilic properties of irinotecan, which result in a reduced affinity for cell membranes.

Table 1 *In vitro* anticancer activity of SN-38 C5 dimers.

Dimer	CC_{50}/μM[a]	IC_{50}/μM[b]			TI[c]		
	NHDF-Neo	HCT-116	MCF-7	A-549	HCT-116	MCF-7	A-549
9	8.62±0.51	0.37±0.09	5.41±1.09	0.82±0.17	24.91±1.55	1.38±0.11	12.51±1.52
10	6.78±0.82	0.34±0.05	5.51±1.21	1.46±0.17	20.72±1.37	1.05±0.10	5.36±0.96
11	7.36±0.16	0.27±0.07	6.19±1.04	0.75±0.19	30.63±1.52	1.06±0.11	11.49±1.41
12	3.13±0.06	0.18±0.03	4.52±0.80	0.33±0.06	18.17±1.53	0.83±0.12	10.05±1.12
13	3.89±0.66	0.20±0.03	5.12±0.69	0.81±0.15	19.93±1.76	0.68±0.12	4.93±0.85
14	5.19±0.48	0.20±0.05	6.44±0.60	0.73±0.14	27.60±1.78	0.77±0.04	8.03±1.06
15	8.38±0.23	0.26±0.04	4.86±0.23	1.3±0.19	33.39±1.08	1.67±0.05	6.67±1.21
16	4.77±0.83	0.07±0.01	1.76±0.06	0.29±0.02	63.72±2.15	2.67±0.48	16.39±1.93
17	3.69±0.40	0.20±0.08	3.01±0.31	0.6±0.23	21.74±1.97	1.14±0.11	6.93±1.40
SN-38 (**1**)	0.66±0.12	0.04±0.01	0.73±0.08	0.24±0.01	16.50±1.31	0.82±0.05	2.80±0.86
Irinotecan (**2**)	>10	>10	>10	>10	–	–	–

[a] CC_{50}: 50% cytotoxic concentration against normal cells (concentrations of SN-38 **1** were detected). [b] IC_{50}: 50% inhibitory concentration against cancer cells. [c] Therapeutic index (TI): CC_{50}/IC_{50}.

In summary, nine new dimer prodrugs based on SN-38 have been synthesized and fabricated as nano-prodrugs by reprecipitation method, and the resulting nanoparticles fall within an ideal size range of 80–130 nm, required for EPR effect. Prodrug **16** exhibited significant anticancer activity as compared with SN-38 against HCT-116 and A-549 with IC_{50} values of 0.07±0.01 and 0.29±0.02 μM, respectively. This research has presented a new approach to the design of prodrug molecules that integrate SN-38 homodimers with diverse side chains, with the objective of creating more potent and clinically applicable anticancer agents.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7664.

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