Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Materials and general methods:

Chemicals: Fmoc-amino acids were obtained from GL Biochem (Shanghai). All the other Starting materials were obtained from *Alfa*. Commercially available reagents were used without further purification, unless noted otherwise. Nanopure water was used for all experiments. All other chemicals were reagent grade or better.

General methods: The synthesized compounds were characterized using ^{1}H NMR (Bruker ARX 300) using DMSO-d_6 as the solvent and ESI-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System. HPLC was conducted at LUMTECH HPLC (Germany) system using a C18 RP column with MeOH (0.1% of TFA) and water (0.1% of TFA) as the eluents, TEM samples were prepared as following: a copper coated with a thin layer of carbon layer was dipped into the hydrogel, and then it was kept in a desicator overnight. The dried sample was performed at the Tecnai G2 F20 system, operating at 200 kV, LC-MS was conducted at the LCMS-20AD (Shimadzu) system, and rheology was performed on an AR 1500ex (TA instrument) system using a parallel plates (40 mm) at the gap of 500 μm. ARES 1500ex can not control the strain when the mechanical strength (G' value) of the sample is low. For example, for a solution sample with low viscosity, though we set the strain value to 1%, the machine can not control the strain and sometimes the strain will be 100% which will destroy the gels. Therefore, the Hgel needs to be incubated for 1 hour before measurement.

Syntheses and characterizations:

The synthesis of Dex-FFFK(Taxol/HCPT)E-ss-EE is easy and straightforward. Fmoc-amino acids, Fmoc-succinated cystamine reported in our previous paper,[13] and succinated Dex were used for solid phase peptide synthesis to enable Dex-FFFKE-ss-EE to be purified by HPLC. Dex-FFFKE-ss-EE was then used to couple with N-hydroxysuccinimide (NHS), the active ester of HCPT/Taxol derivatives in high yields (>80% after HPLC purification). Such a simple synthetic pathway ensures large-scale preparation of the compounds for practical use.

Preparation of Dex-SA: 1.0 g (2.55 mmol) of Dexamethasone was dissolved in 30 mL of pyridine and 0.77 g (7.65 mmol) of succinic anhydride, 0.1 eq DMAP was then added. After being stirred at room temperature overnight, the mixture was evaporated under reduced pressure. The resulting residue was then treated with 40 mL of water. The mixture was stirred for 10 min and then centrifuged. The resulting precipitate was washed again with H_2O. White powder of the product was obtained in a yield of 86%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.27(d, J=10.11, 1H), 6.19-6.24 (q, 1H), 5.99 (m, 1H), 5.42 (d, 1H), 5.17

(s, 1H), 4.99-5.06 (m, 1H), 4.75-4.82 (m, 1H), 4.10-4.15 (m, 1H), 2.82-2.91 (m, 1H), 2.58-2.61 (m, 3H), 2.26-2.40 (m, 3H), 2.09-2.18 (m, 2H), 1.53-1.79 (m, 6H), 1.05-1.096 (m, 1H). MS: calc. M^+ = 492.2, obsvd. $(M+1)^+$ = 493.1.

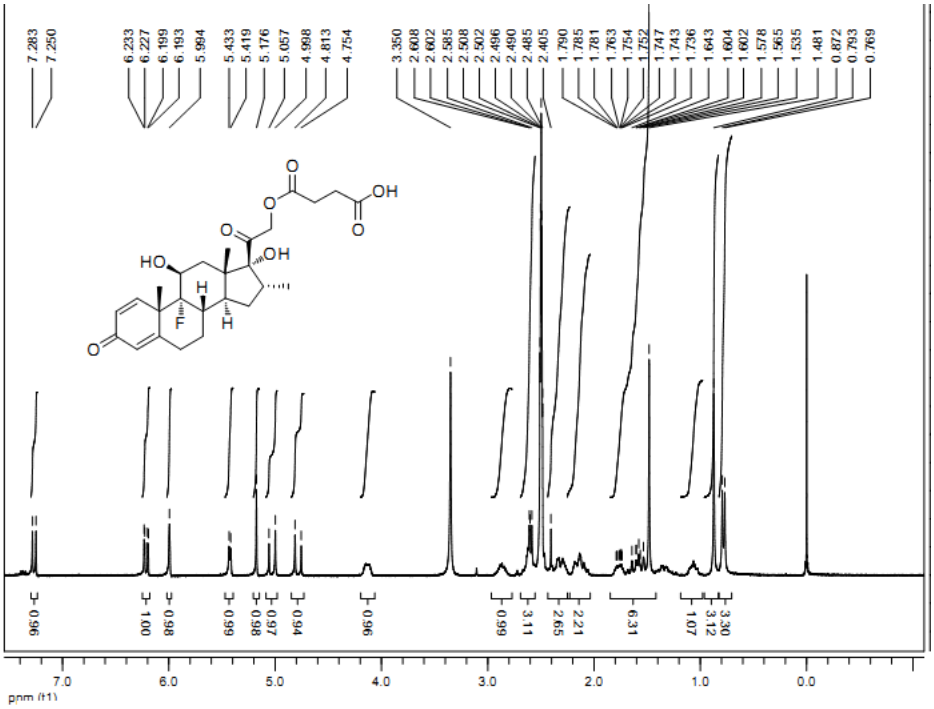
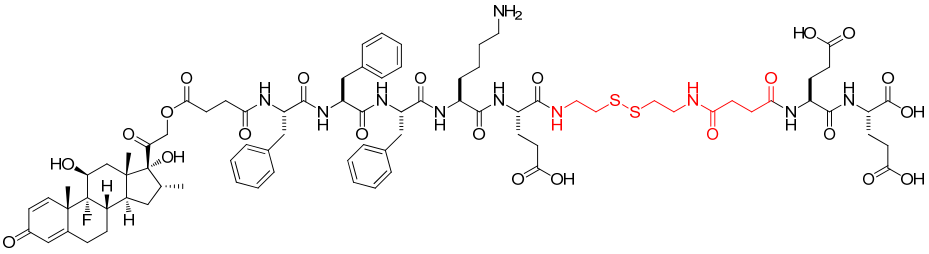


Figure S-1. ^{1}H NMR of Dex-SA

Peptide Synthesis: The peptide derivative was prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin, the corresponding N-Fmoc protected amino acids with side chains properly protected by a tert-butyl group, and Fmoc-succinated cystamine. The first amino acid was loaded on the resin at the C-terminal with the loading efficiency about 0.6 mmol/g. 20% piperidine in anhydrous N,N'-dimethylformamide (DMF) was used during deprotection of Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. At the final step, Dex-succ was used to attach on the peptide. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 mL per gram of resin), followed by five steps of washing using DCM for 2 min (5 mL per gram of resin). The peptide derivative was cleaved using 95% of trifluoroacetic acid with 2.5% of TMS and 2.5% of H_2O for 20 minutes. 20 mL per gram of resin of ice-cold diethylether was then added to cleavage reagent. The resulting precipitate was centrifuged for 10 min at room temperature at 10,000 rpm. Afterward the supernatant was decanted and the resulting solid was dissolved in DMSO for HPLC separation. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.02-8.17 (m, 9H), 7.15-7.31 (m, 15H), 6.21-6.25 (m, 1H), 6.01 (s, 1H), 5.40-5.42 (m, 1H), 5.16 (s, 1H), 4.98-5.03 (m, 1H), 4.72-4.77 (m, 1H), 4.56-4.59 (m, 1H),

4.45-4.49 (m, 3H), 4.24-4.29 (m, 4H), 4.13-4.15 (m, 2H), 2.95-3.06 (m, 3H), 2.84-2.88 (m, 3H), 2.72-2.76 (m, 6H), 2.62-2.68 (m, 3H), 2.41-2.44 (m, 2H), 2.22-2.34 (m, 12H), 2.12-2.19 (m, 3H), 1.88-1.93 (m, 3H), 1.73-1.81 (m, 3H), 1.61-1.68 (m, 3H), 1.48-1.56 (m, 7H), 1.30-1.34 (m, 3H), 1.03-1.09 (m, 1H), 0.87 (s, 3H), 0.78 (d, J=7.18, 3H). MS: calc. M^+ = 1682.7, obsvd. $(M+1)^+$ = 1683.6.



Scheme S-1. Chemical structure of Dex-FFFKE-ss-EE

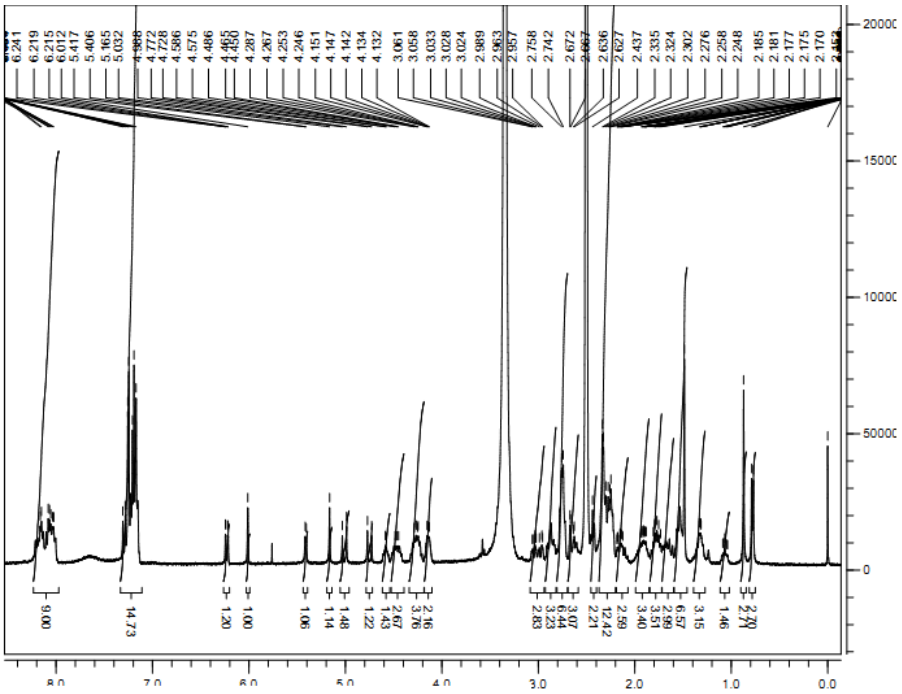


Figure S-2. ^{1}H NMR of Dex-FFFKE-ss-EE

Preparation of Taxol-SA: 0.50 g (0.59 mmol) of paclitaxel was dissolved in 12 mL of pyridine and 0.90 g (7.6 mmol) of succinic anhydride was added. After being stirred 3 hrs at room temperature, the mixture was evaporated under reduced pressure, then treated with 20 mL of water, stirred for 20 min, and filtered. The precipitate was dissolved in acetone, water was then slowly added. 0.52 g of fine crystals were collected.[1, 2] (Yield = 92.0%)

Preparation of HCPT-GA: a solution of 0.3g 10-hydroxycamptothecin (HCPT) and 0.282g Glutaric anhydride in 30 mL of pyridine was stirred for 48 hrs at room temperature. The reaction mixture was evaporated to dryness in vacuo. 0.01 M HCl (20 mL) was then added. The mixture was centrifuged and

the resulting precipitate was washed with HCl again. Yellow powder of the product was obtained in a yield of 94%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.23 (d, J=9.23, 1H), 7.94-7.95 (m, 1H), 7.69-7.72 (m, 1H), 7.36 (s, 1H), 6.57 (s, 1H), 5.45 (s, 2H), 5.31 (s, 2H), 2.73-2.76 (t, 2H), 2.39-2.43 (t, 2H), 1.86-1.94 (m, 4H), 0.88-0.92 (m, 3H). MS: calc. M^+ = 478.1, obsvd. $(M+1)^+$ = 479.2.

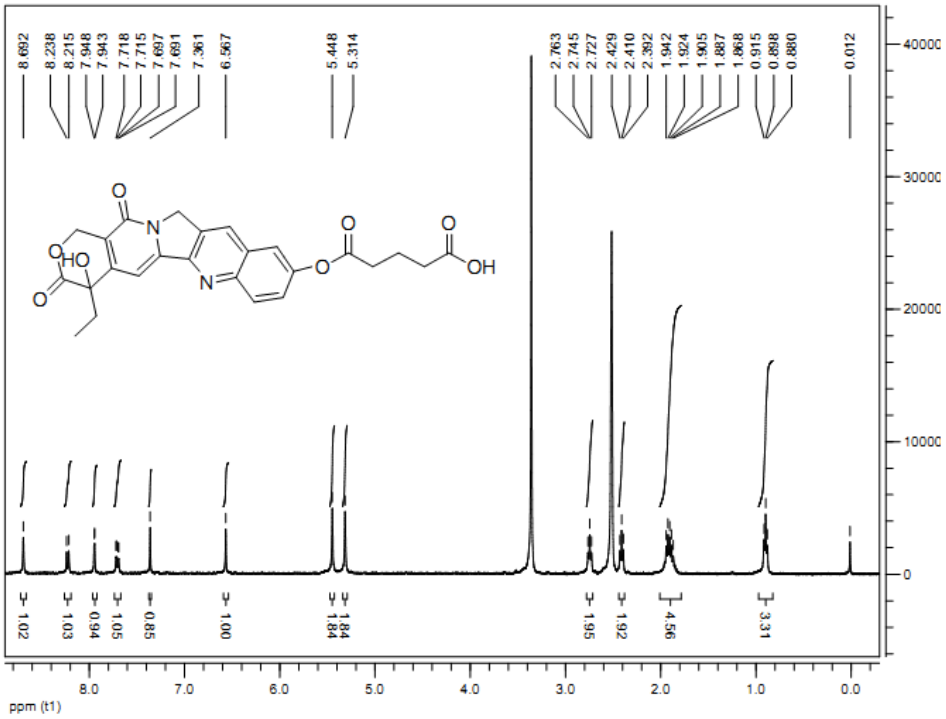
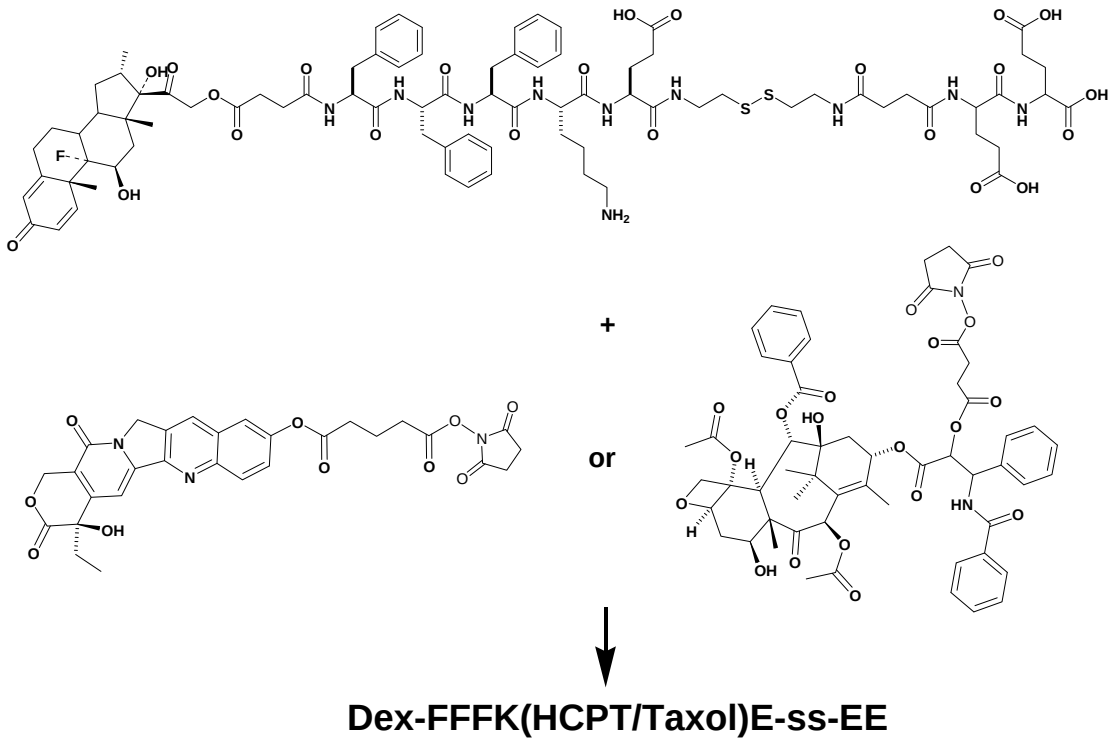


Figure S-3. ^{1}H NMR of HCPT-GA

Precursors of gelators were synthesized according to Scheme S-2.



Scheme S-2. Synthetic route for precursors of gelators

Preparation of Dex-FFFK(Taxol)E-ss-EE: 60 mg of Taxol-SA (0.06 mmol) was dissolved in 10 mL of dichloromethane. 1.1 equiv. (7.6 mg, 0.066 mmol) of N-Hydroxysuccinimide, 14.8 mg (0.07 mmol) of N,N'-dicyclohexylcarbodiimide, and catalytic amount of 4-dimethylamiopryidine were added. After being stirred 3hrs at room temperature, the solution was filtered to remove precipitation, dried and evaporated under reduced pressure to yield a white power. The white powder was used for the next step without further purification. The white powder obtained in last step was dissolved in 5 mL N,N-Dimethylformamide, 121 mg (0.07 mmol) of Dex-FFFKE-ss-EE was then added with 50 μL of N-Ethyldiisopropylamine. After being stirred overnight, HPLC was used for purification to yield the title product in a yield of 86%. ^{1}H NMR (400MHz, DMSO-d6) δ 8.12-8.19 (m, 4H), 8.02-8.09 (m, 4H), 7.97-7.99 (m, 3H), 7.86 (d, J=7.13, 1H), 7.72-7.76 (m, 1H), 7.64-7.67 (m, 2H), 7.54-7.58 (m, 1H), 7.48-7.50 (m, 2H), 7.44-7.47 (m, 4H), 7.28-7.31 (m, 1H), 7.24-7.26 (m, 4H), 7.16-7.20 (m, 12H), 6.30 (s, 1H), 6.21-6.24 (m, 1H), 6.01 (s, 1H), 5.80-5.85 (m, 1H), 5.51-5.55 (m, 1H), 5.40-5.42 (m, 2H), 5.33-5.35 (m, 1H), 5.17 (s, 1H), 4.99-5.04 (m, 1H), 4.90-4.95 (m, 2H), 4.73-4.78 (m, 1H), 4.64 (s, 1H), 4.59-4.61 (m, 1H), 4.44-4.50 (m, 2H), 4.23-4.28 (m, 3H), 4.09-4.17 (m, 4H), 3.98-4.03 (m, 2H), 3.55-3.58 (m, 2H), 3.17 (s, 1H), 2.82-3.07 (m, 8H), 2.72-2.78 (m, 6H), 2.59-2.66 (m, 5H), 2.41-2.44 (m, 3H), 2.29-2.39 (m, 13H), 2.23-2.25 (m, 7H), 2.10 (s, 3H), 1.88-1.99 (m, 4H), 1.72-1.83 (m, 7H), 1.57-1.74 (m, 5H), 1.49-1.50 (m, 8H), 1.32-1.36 (m, 3H), 1.24-1.28 (m, 2H), 0.99-1.10 (m, 8H), 0.88 (s, 3H), 0.78 (d, J=7.10, 3H). HR-MS: calc. M^{+} = 2619.03, obsvd. $(M+1)^{+}$ = 2620.0383.

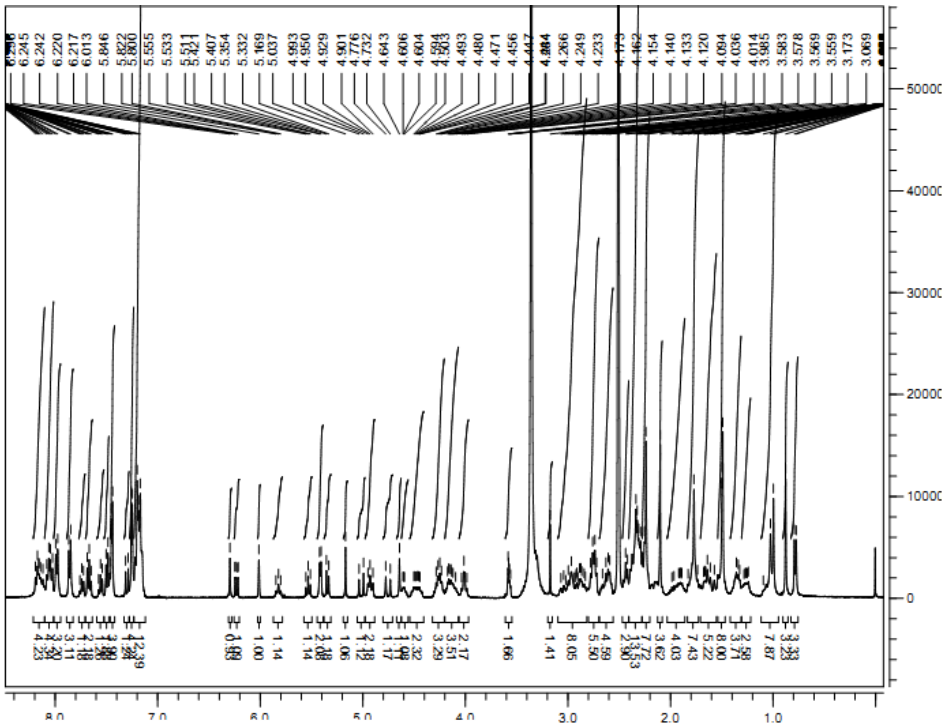


Figure S-4. ^{1}H NMR of Dex-FFFK(Taxol)E-ss-EE

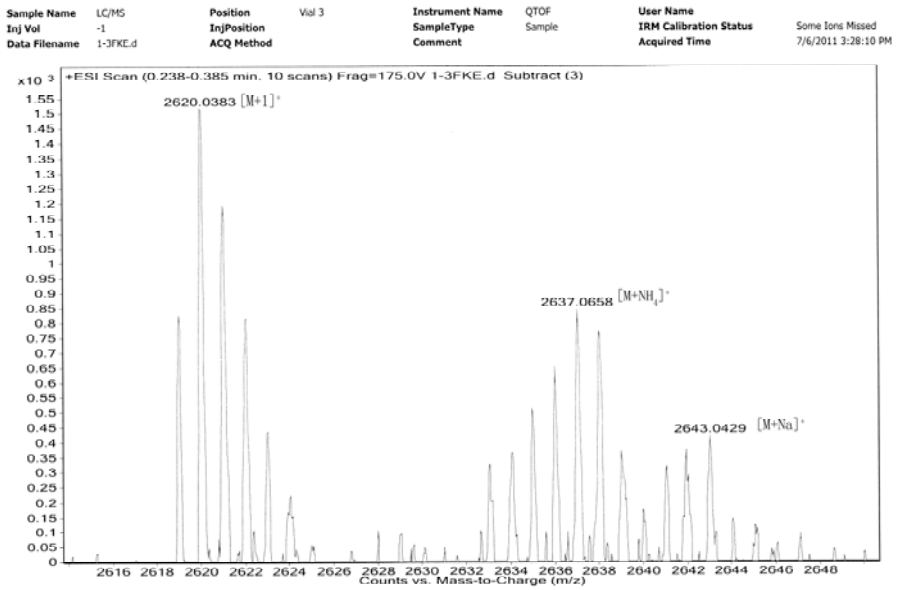


Figure S-5. HR-MS of Dex-FFFK(Taxol)E-ss-EE

Preparation of Dex-FFFK(HCPT)E-ss-EE: the same procedure for preparation of **Dex-FFFK(taxol)E-ss-EE** was used. The yield is 80%. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.02-8.23 (m, 9H), 7.82-7.92 (m, 2H), 7.67-7.69 (m, 1H), 7.09-7.35 (m, 15H), 6.55 (s, 1H), 6.21-6.24 (m, 1H), 6.01 (s, 1H), 5.40-5.44 (m, 3H), 5.30 (s, 2H), 5.16 (s, 1H), 4.98-5.03 (m, 1H), 4.72-4.77 (m, 1H), 4.58-4.61 (m, 1H), 4.43-4.50 (m, 2H), 4.13-4.30 (m, 6H), 3.29-3.31 (m, 2H), 2.97-3.06 (m, 4H), 2.83-2.91 (m, 3H), 2.56-2.78 (m, 9H), 2.23-2.43 (m, 14H), 2.09-2.16 (m, 2H), 1.85-1.94 (m, 5H), 1.23-1.79 (m, 15H), 1.03-1.08 (m, 1H), 0.86-0.90 (m, 4H), 0.76-0.78 (m, 2H). MS: calc. M^{+} = 2142.8, obsvd. $((M+2)/2)^{+}$ = 1073.0.

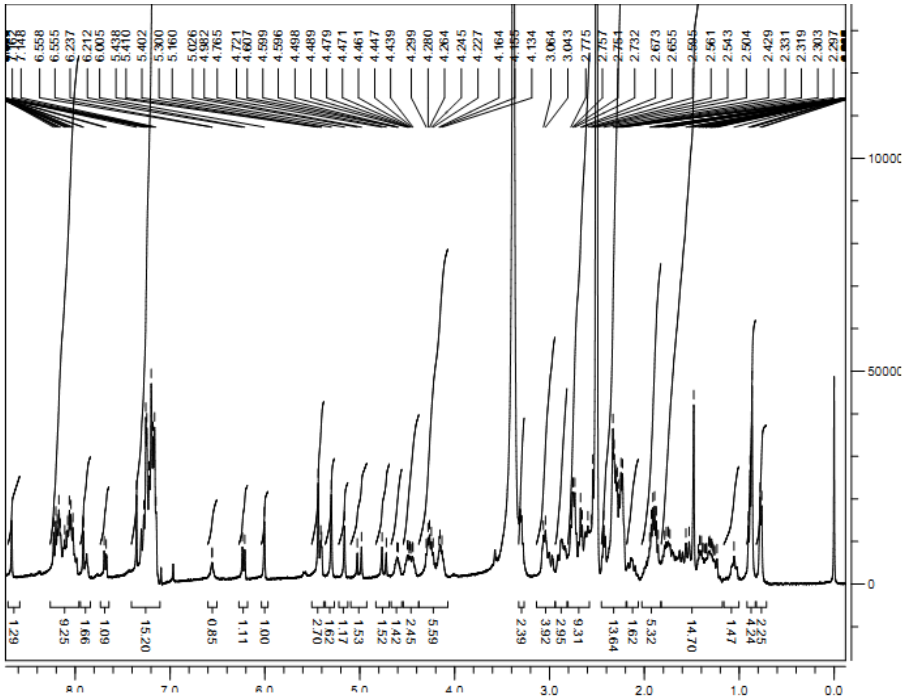


Figure S-6. ^{1}H NMR of Dex-FFFK(HCPT)E-ss-EE

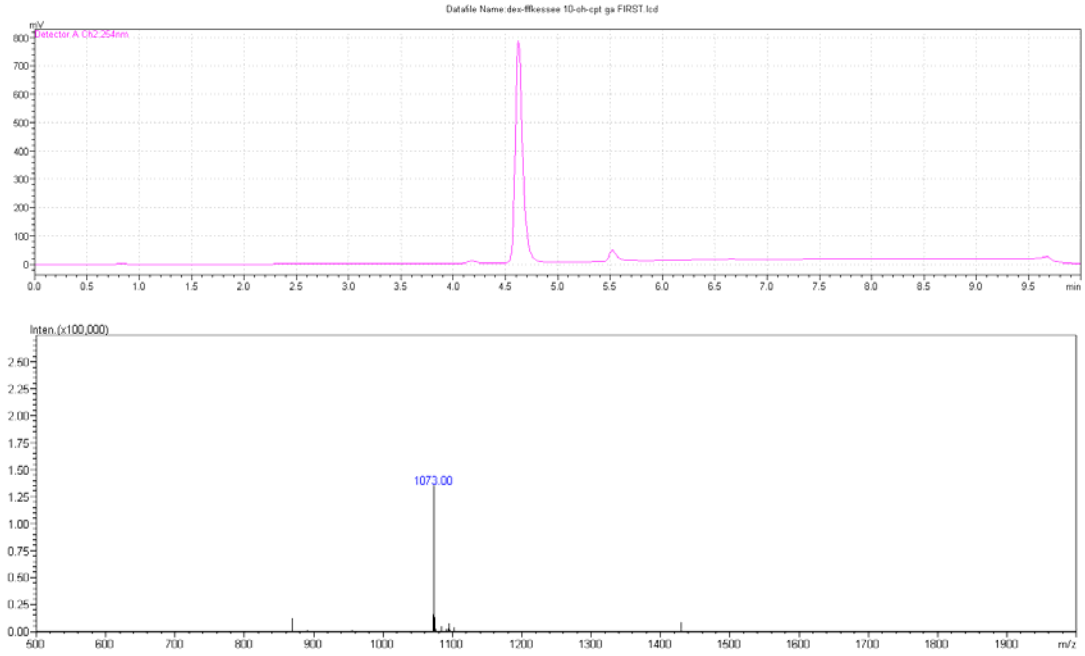


Figure S-7. LCMS spectrum of Dex-FFFK(HCPT)E-ss-EE

Formation of gels: 5 mg of Dex-FFFK(taxol/HCPT)E-ss-EE was dissolved in 0.49 mL of PBS buffer solution (pH = 7.4). 1.0 equiv. of DTT or 2.0 equiv. of GSH in 10 μL of PBS buffer (pH = 7.4, adjusted by Na_2CO_3) was then added to the above solution. Gel was formed after the solution being kept at room temperature (22-25 ^{0}C) for less than 5 minutes and about 2 hours for Tgel and Hgel, respectively.

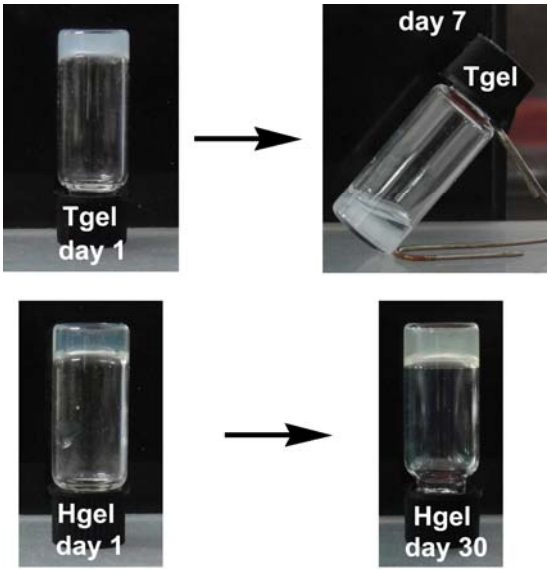


Figure S-8. Optical images of Tgel and Hgel at different times at room temperature (22-25 ^{0}C)

Table S-1. Stability of hydrogels at 37 ^{0}C

	Tgel	Tgel:Hgel = 3:1	Tgel:Hgel = 2:2	Tgel:Hgel = 1:3	Hgel
without BSA	~2 days	~4 days	~7 days	>2 weeks	>2 weeks
with BSA (50%)	~7 days	>2 weeks	>2 weeks	>2 weeks	>2 weeks

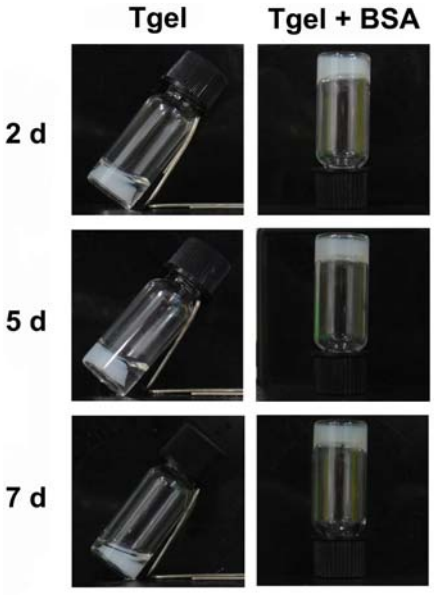


Figure S-9. Optical images of Tgel with or without BSA (50% wt to Dex-FFFK(Taxol)E-ss-EE) at different times and at 37 ^{0}C

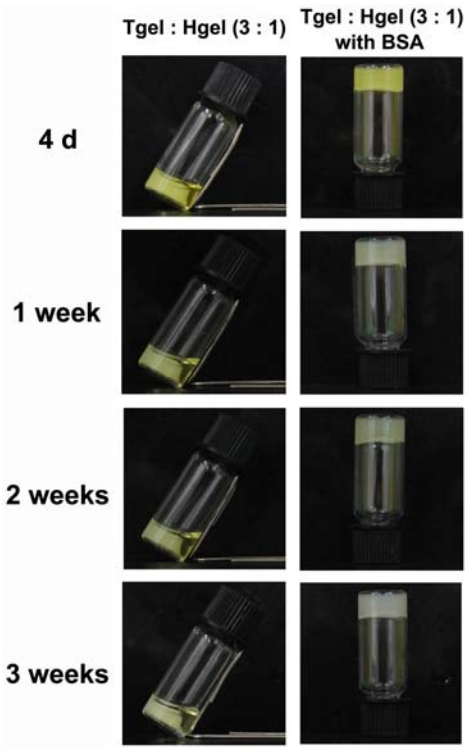


Figure S-10. Optical images of mixed gels (Tgel : Hgel = 3 : 1) with or without BSA (50% wt to precursors of gelators) at different times and at 37 ^{0}C

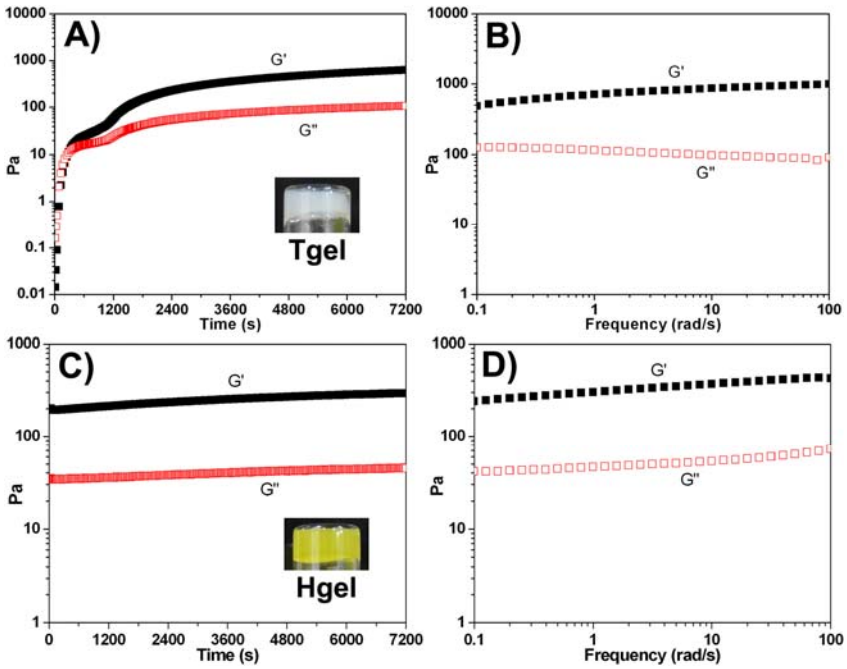


Fig. S-11. Rheological measurements at 37^{0}C with the mode of dynamic time sweep at the frequency of 2 rad/s and the strain of 1% for the PBS solutions containing 1.0% wt of A) Dex-FFFK(Taxol)E-ss-EE and C) Dex-FFFK(HCPT)E-ss-EE (incubated for one hour before measurement) with 1.0 equiv. of DTT and dynamic frequency sweep at the strain of 1% for the resulting gels: B) Tgel and D) Hgel

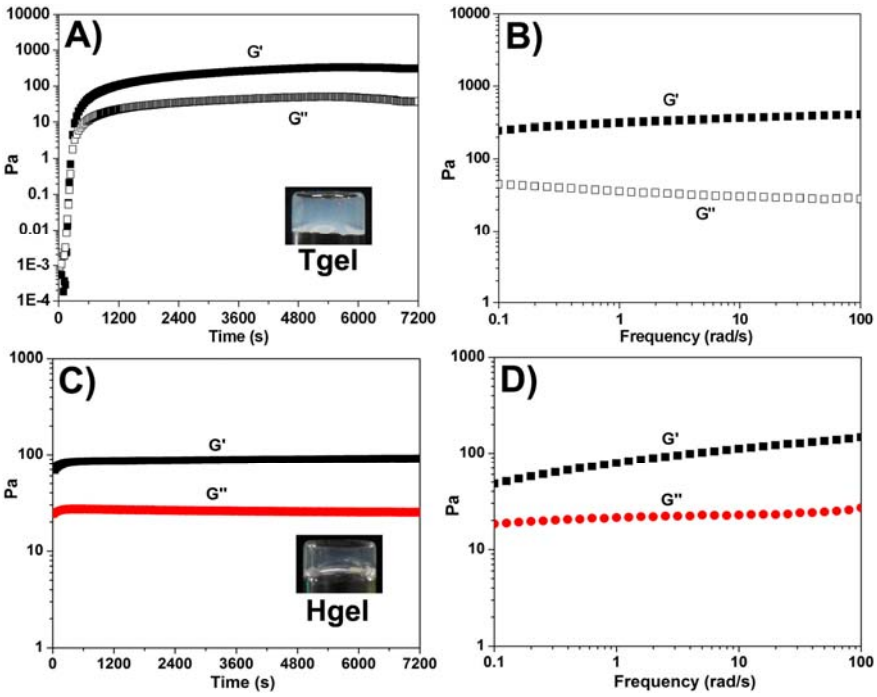


Figure S-12. Rheological measurements at room temperature with the mode of dynamic time sweep at the frequency of 2 rad/s and the strain of 1% for the PBS solutions containing 1.0 wt% of A) Dex-FFFK(Taxol)E-ss-EE and C) Dex-FFFK(HCPT)E-ss-EE (incubated for 2 hours before measurement) with 1.0 equiv. of DTT and dynamic frequency sweep at the strain of 1% for the resulting gels: B) Tgel and D) Hgel

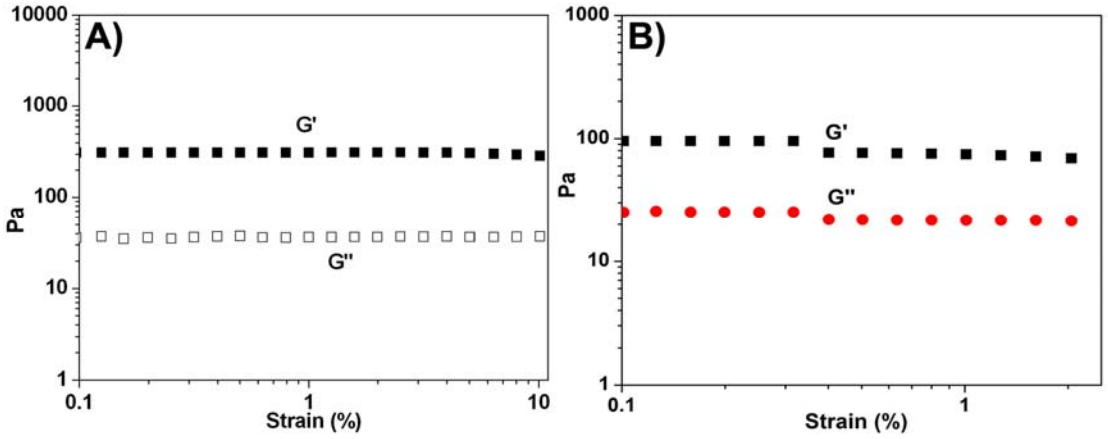


Figure S-13. Dynamic strain sweep of A) Tgel and B) Hgel at room temperature

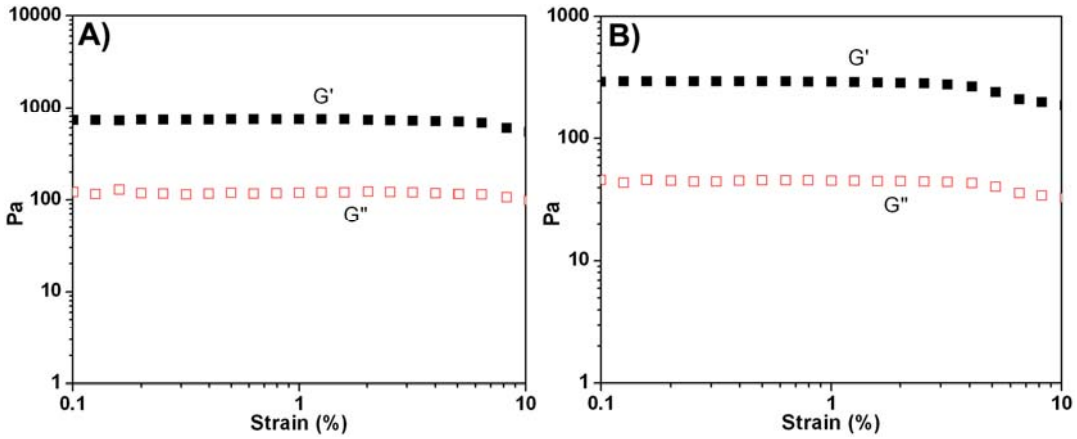


Figure S-14. Dynamic strain sweep of A) Tgel and B) Hgel at 37 ^{0}C

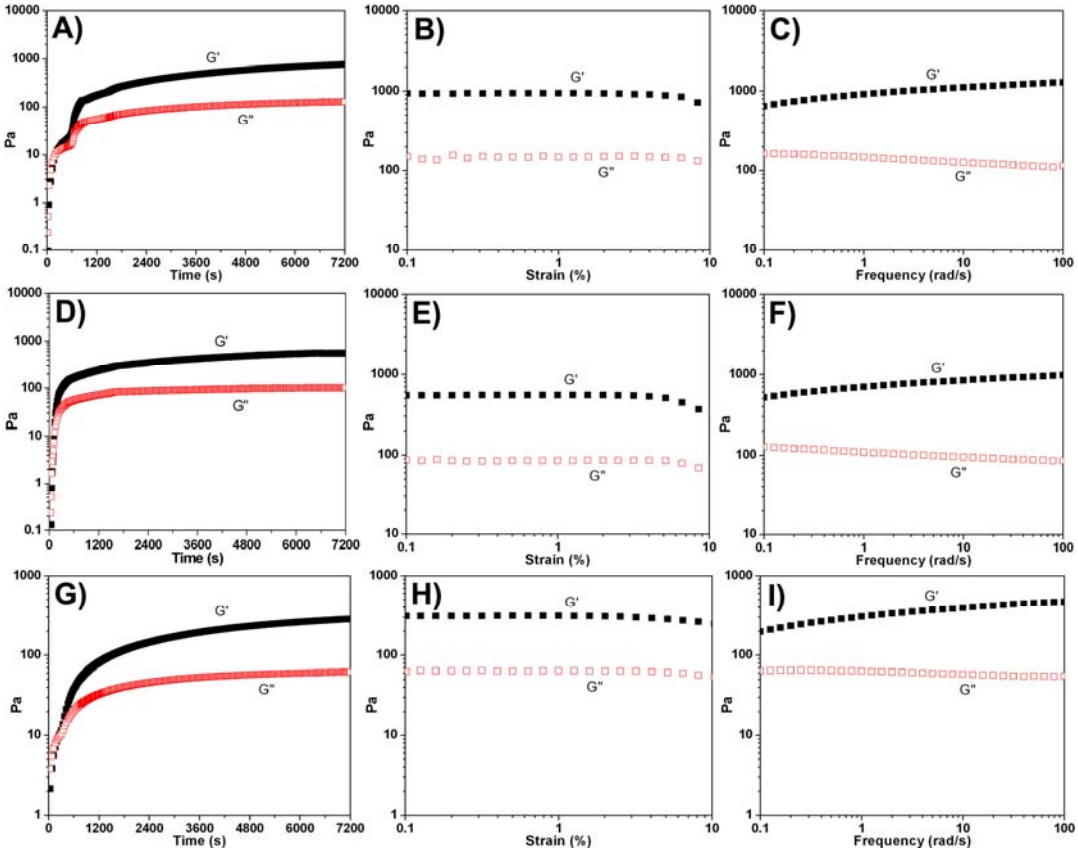


Figure S-15. Rheological measurements of mixed gels at 37 ^{0}C with the mode of dynamic time sweep at the frequency of 2 rad/s and the strain of 1% (A, D and G), with the mode of dynamic strain sweep at the frequency of 2 rad/s (B, E and H) and with the mode of dynamic frequency sweep at the strain of 1% (C, F and I), Tgel : Hgel = 3 : 1 (A, B and C), Tgel : Hgel = 2 : 2 (D, E and F) and Tgel : Hgel = 1 : 3 (G, H and I)

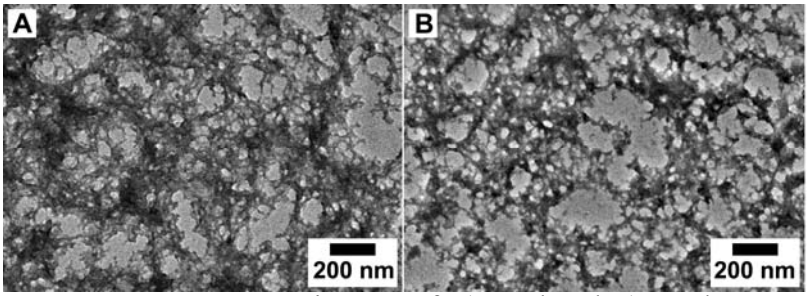


Fig. S-16. TEM images of A) Tgel and B) Hgel

Release profile of anti-cancer drugs: 0.4 mL of gels (1.0 wt%, 4 hours after the addition of DTT) was treated with 0.4 mL of fresh PBS buffer solutions (pH = 7.4). 0.4 mL of the upper buffer solution was taken out and used to run LC-MS at each time. The areas of peaks in LC-MS spectra were used to determine the percentage of Taxol or HCPT released from their corresponding gels. The experiment was conducted in 4 parallel experiments. The experiments were conducted at both room temperature (22-25 ^{0}C) and 37 ^{0}C.

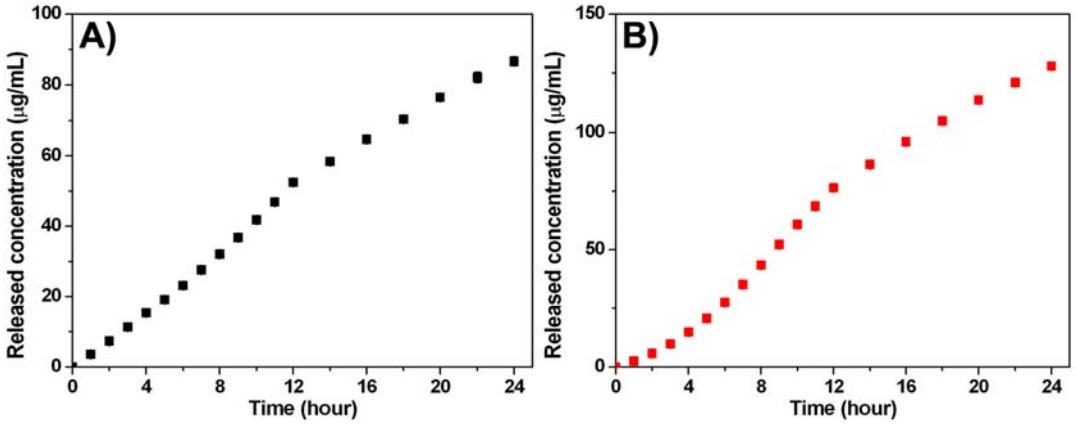


Figure S-17. Accumulative release profile of Taxol and HCPT from A) Tgel and B) Hgel, respectively, in 100 mM PBS buffers (pH = 7.4) and at room temperature

Description of Figure S-17 and S-18:

We only observed original drug molecules of Dex, Taxol, and HCPT releasing from the gels due to the hydrolysis of ester bond. There were no drug molecules conjugated with peptides released to upper PBS buffer solutions, suggesting the existence of extensive hydrogen bonds between peptides that could prevent peptide conjugates releasing to the upper solutions. The Tgel and Hgel released Taxol and HCPT at rates of about 3.61 μg/mL (0.11%) and about 5.33 μg/mL (0.31%) per hour in the first 24 hours, respectively. These preliminary results demonstrated the sustained release of original anti-cancer drugs from hydrogels. After the release experiments, we dissolved the gels and used them to run LC-MS. There were nearly equal molar percentage of the gelator (Dex-FFFK(Taxol)E) and dimer of it in Tgel. For Hgel,

the gelator of Dex-FFFK(HCPT)E and its dimer with a molar ratio of 1:3 were the two major components. The different components (precursor, gelator, and dimer of gelator) in both gels were probably due to the different gelation kinetics, as shown in Figure S-11A and 11C.

Table S-2. Original data of amounts of Taxol released from Tgel at room temperature

T = 22-25 Ⅲ	T_{gel}			
Time (hour)	Concentration (μg/mL)	error	Percentage (%)	error
1	3.64043	0.13414	0.11165	0.00411
2	7.39139	0.32414	0.22669	0.00994
3	11.37972	0.38621	0.34901	0.01184
4	15.38631	0.39822	0.47189	0.01221
5	19.09195	0.08945	0.58554	0.00274
6	23.18691	0.18773	0.71113	0.00576
7	27.63074	0.55251	0.84742	0.01695
8	32.10229	0.11207	0.98456	0.00344
9	36.82164	0.10808	1.1293	0.00331
10	41.78945	0.19292	1.28166	0.00592
11	46.97082	0.24316	1.44057	0.00746
12	52.49782	0.81933	1.61008	0.02513
14	58.42228	0.16382	1.79178	0.00502
16	64.71975	0.29193	1.98492	0.00895
18	70.47792	0.31584	2.16152	0.00969
20	76.39292	0.17542	2.34293	0.00538
22	82.04577	1.50055	2.5163	0.04602
24	86.60079	1.25781	2.656	0.03858

calculated from 4 parallel experiments

Table S-3. Original data of amounts of HCPT released from Hgel at room temperature

T = 22-25 Ⅲ	H_{gel}			
Time (hour)	Concentration (μg/mL)	error	Percentage (%)	error
1	2.55939	0.06069	0.15077	0.00358
2	5.81377	0.20232	0.34248	0.01192
3	9.88704	0.22381	0.58243	0.01318
4	14.91383	0.083	0.87855	0.00489
5	20.68041	0.07432	1.21825	0.00438
6	27.52255	0.40589	1.62131	0.02391
7	35.08124	0.20249	2.06658	0.01193
8	43.39312	0.20309	2.55622	0.01196
9	52.11038	0.14674	3.06974	0.00864
10	60.70084	0.3639	3.57579	0.02144
11	68.71582	0.24079	4.04794	0.01418
12	76.531	0.35643	4.50832	0.021
14	86.39394	0.45795	5.08933	0.02698
16	96.0362	0.07131	5.65734	0.0042

18	104.92542	0.29345	6.18099	0.01729
20	113.55203	0.3323	6.68917	0.01958
22	121.01056	0.33702	7.12854	0.01985
24	127.88055	0.05069	7.53324	0.00299

calculated from 4 parallel experiments

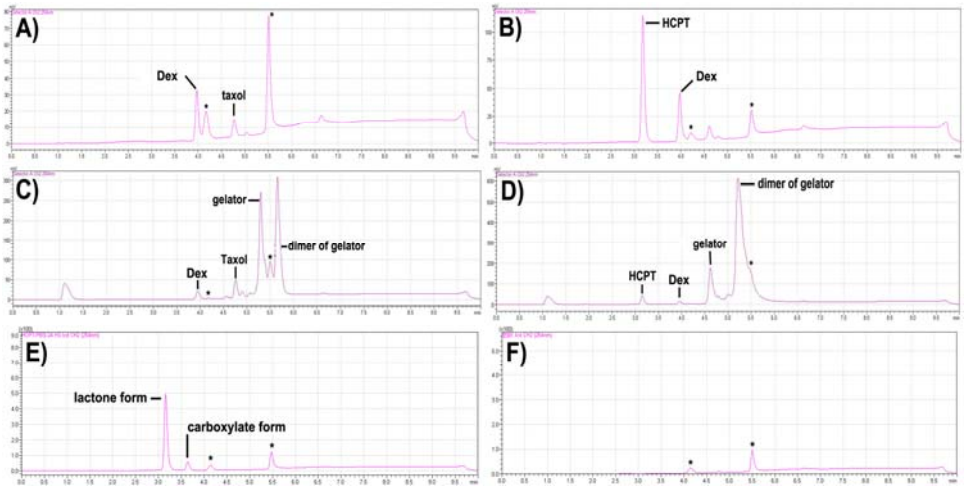


Figure S-18 HPLC traces of A) a representative PBS buffer in release profile on top of Tgel, B) a representative PBS buffer in release profile on top of Hgel, C) Tgel after release profile, D) Hgel after release profile, E) HCPT after being incubated in PBS buffer (pH = 7.4) for 24 hours, and F) blank to indicate the system peaks (by stars)

Table S-4. Original data of amounts of Taxol released from Tgel at 37 ^{0}C

T = 37 Ⅲ	T_{gel}			
Time (hour)	Concentration (μg/mL)	error	Percentage (%)	error
1	2.23441	0.31909	0.06853	0.00979
2	4.96642	0.27531	0.15232	0.00844
3	11.92094	0.49523	0.36561	0.01519
4	16.4431	1.15866	0.5043	0.03554
5	22.33497	1.09001	0.685	0.03343
6	24.957	0.70303	0.76542	0.02156
7	29.59698	2.13716	0.90772	0.06555
8	34.26288	3.3437	1.05082	0.10255
9	37.47801	3.36823	1.14943	0.1033
10	42.78435	4.49243	1.31217	0.13778
11	46.2575	3.65098	1.41869	0.11197
12	49.80827	4.22518	1.52759	0.12958
14	53.56349	5.19698	1.64276	0.15939
16	56.9129	5.23632	1.74549	0.1606

18	60.89713	5.37087	1.86768	0.16472
20	64.67783	5.3556	1.98363	0.16425
22	69.0811	4.25555	2.11868	0.13052
24	72.36934	4.33167	2.21953	0.13285

calculated from 4 parallel experiments

Table S-5. Original data of amounts of Taxol released from mixed gel of Tgel : Hgel = 3 : 1 at 37 ^{0}C

T = 37 Ⅲ	T_{gel} : H_{gel} = 3 : 1			
Time (hour)	Concentration (μg/mL)	error	Percentage (%)	error
1	1.32191	0.24707	0.0516	0.00964
2	2.64451	0.36099	0.10322	0.01409
3	4.35396	0.36774	0.16994	0.01435
4	6.31269	0.35749	0.2464	0.01395
5	7.35992	0.36137	0.28727	0.0141
6	11.70553	0.78252	0.45689	0.03054
7	14.84394	1.88409	0.57939	0.07354
8	17.78556	1.16982	0.69421	0.04566
9	20.40459	0.24936	0.79643	0.00973
10	22.65772	1.69682	0.88438	0.06623
11	24.7713	3.64893	0.96687	0.14243
12	26.07617	4.50798	1.01781	0.17596
14	28.26084	4.05023	1.10308	0.15809
16	29.88438	3.93129	1.16645	0.15345
18	32.51036	5.59002	1.26894	0.21819
20	34.36641	5.46752	1.34139	0.21341
22	35.96734	5.38315	1.40388	0.21011
24	38.45123	5.50012	1.50083	0.21468

calculated from 4 parallel experiments

Table S-6. Original data of amounts of Taxol released from mixed gel of Tgel : Hgel = 2 : 2 at 37 ^{0}C

T = 37 Ⅲ	T_{gel} : H_{gel} = 2 : 2			
Time (hour)	Concentration (μg/mL)	error	Percentage (%)	error
1	0.62984	0.58857	0.03512	0.03282
2	1.43506	1.0042	0.08002	0.05599
3	2.76495	1.68998	0.15417	0.09423
4	4.62266	2.54626	0.25776	0.14198
5	7.39152	1.48109	0.41215	0.08259
6	9.45213	3.08341	0.52705	0.17193
7	13.97818	2.45605	0.77942	0.13695

8	18.3997	1.30379	1.02597	0.0727
9	23.30551	1.54661	1.29952	0.08624
10	24.50251	2.28228	1.36626	0.12726
11	26.33977	5.0376	1.46871	0.2809
12	27.74697	4.98641	1.54717	0.27804
14	28.32112	5.48421	1.57919	0.3058
16	30.65807	5.02856	1.70949	0.28039
18	31.59808	5.6851	1.76191	0.317
20	33.02555	6.60529	1.84151	0.36831
22	34.09374	6.97273	1.90107	0.3888
24	35.10414	7.63444	1.95741	0.4257

calculated from 4 parallel experiments

Table S-7. Original data of amounts of Taxol released from mixed gel of Tgel : Hgel = 1 : 3 at 37 ^{0}C

T = 37 Ⅲ	T_{gel} : H_{gel} = 1 : 3			
Time (hour)	Concentration (μg/mL)	error	Percentage (%)	error
1	3.34787	0.59166	0.35638	0.06298
2	6.69575	1.18333	0.71277	0.12597
3	10.28923	2.19521	1.0953	0.23368
4	13.14818	2.59247	1.39964	0.27597
5	15.06281	2.22504	1.60345	0.23686
6	17.85007	4.05684	1.90016	0.43185
7	20.57821	3.45167	2.19057	0.36743
8	25.00849	6.67358	2.66218	0.71041
9	26.14994	6.70271	2.78369	0.71351
10	27.66909	7.45728	2.9454	0.79383
11	28.69875	8.57447	3.05501	0.91276
12	30.33004	8.06904	3.22866	0.85896
14	37.29262	8.32934	3.96983	0.88667
16	38.14102	9.12079	4.06015	0.97092
18	41.05926	11.71626	4.3708	1.24721
20	44.84698	12.62636	4.774	1.34409
22	46.99473	12.53223	5.00263	1.33407
24	49.28205	10.67773	5.24612	1.13665

calculated from 4 parallel experiments

Table S-8. Original data of amounts of HCPT released from Hgel at 37 ^{0}C

T = 37 Ⅲ	H_{gel}			
Time (hour)	Concentration (μg/mL)	error	Percentage	error

			(%)	
1	159.55775	1.99509	9.39929	0.11753
2	275.55902	8.77752	16.23274	0.51707
3	366.56698	3.66709	21.59388	0.21602
4	426.13876	2.80524	25.10316	0.16525
5	464.85797	7.12935	27.38405	0.41998
6	490.7525	9.68388	28.90945	0.57046
7	508.66356	10.96424	29.96456	0.64589
8	522.36382	11.91126	30.77162	0.70167
9	533.49881	12.64768	31.42757	0.74505
10	543.53608	12.45667	32.01885	0.7338
11	552.27586	12.32845	32.53369	0.72625
12	560.40946	12.50892	33.01283	0.73688
14	570.1835	12.67368	33.58861	0.74659
16	580.62334	13.50401	34.2036	0.7955
18	590.21742	14.31938	34.76877	0.84353
20	597.79694	14.7495	35.21527	0.86887
22	603.66925	15.26872	35.5612	0.89946
24	608.66094	15.61569	35.85525	0.9199

calculated from 4 parallel experiments

Table S-9. Original data of amounts of HCPT released from mixed gel of Tgel : Hgel = 3 : 1 at 37 ^{0}C

T = 37 Ⅲ	T_{gel} : H_{gel} = 3 : 1			
Time (hour)	Concentration (μg/mL)	error	Percentage (%)	error
1	77.92871	2.76278	21.40899	0.75901
2	128.57565	2.32656	35.32298	0.63917
3	160.9704	2.43606	44.22264	0.66925
4	180.74868	2.31367	49.65623	0.63562
5	192.83736	2.30637	52.9773	0.63362
6	200.6798	2.32558	55.13181	0.6389
7	205.33562	2.33259	56.41088	0.64082
8	208.2595	2.22144	57.21415	0.61029
9	210.01601	2.30042	57.69671	0.63198
10	211.08642	2.28355	57.99077	0.62735
11	211.73808	2.27177	58.1698	0.62411
12	212.03605	2.2692	58.25166	0.62341
14	212.296	2.32353	58.32308	0.63833
16	212.41359	2.25696	58.35538	0.62004
18	212.51404	2.242	58.38298	0.61593
20	212.51677	2.23822	58.38373	0.6149

22	212.51677	2.23822	58.38373	0.6149
24	212.51677	2.23822	58.38373	0.6149

calculated from 4 parallel experiments

Table S-10. Original data of amounts of HCPT released from mixed gel of Tgel : Hgel = 2 : 2 at 37 ^{0}C

T = 37 Ⅲ	T_{gel} : H_{gel} = 2 : 2			
Time (hour)	Concentration (μg/mL)	error	Percentage (%)	error
1	146.62364	2.54429	19.18153	0.33285
2	250.50102	4.49635	32.77093	0.58822
3	317.77242	5.3926	41.57148	0.70547
4	359.12928	5.84899	46.98185	0.76517
5	384.13458	5.998	50.25308	0.78467
6	399.60707	6.22953	52.27722	0.81496
7	408.68727	6.53494	53.46511	0.85491
8	414.36228	6.61792	54.20752	0.86577
9	417.79674	6.65959	54.65682	0.87122
10	419.76076	6.74311	54.91376	0.88214
11	421.03346	6.8292	55.08025	0.89341
12	421.84122	6.77385	55.18593	0.88617
14	422.33487	6.77762	55.25051	0.88666
16	422.73995	6.80685	55.3035	0.89048
18	422.99924	6.80183	55.33742	0.88983
20	423.1784	6.85308	55.36086	0.89653
22	423.24837	6.84149	55.37001	0.89501
24	423.29952	6.8375	55.3767	0.89449

calculated from 4 parallel experiments

Table S-11. Original data of amounts of HCPT released from mixed gel of Tgel : Hgel = 1 : 3 at 37 ^{0}C

T = 37 Ⅲ	T_{gel} : H_{gel} = 1 : 3			
Time (hour)	Concentration (μg/mL)	error	Percentage (%)	error
1	153.7697	18.74566	12.73189	1.55211
2	278.59432	18.74566	23.06718	1.55211
3	362.64589	12.60553	30.02652	1.04372
4	417.03465	10.70191	34.52982	0.8861
5	452.9317	11.85966	37.50204	0.98196
6	477.30895	14.51867	39.52044	1.20212
7	497.79429	24.23723	41.2166	2.0068
8	510.76771	30.07151	42.29078	2.48987
9	519.26547	33.96743	42.99438	2.81245

10	525.55868	37.55624	43.51545	3.1096
11	530.47014	40.82169	43.92211	3.37997
12	534.34811	43.63452	44.2432	3.61287
14	537.97263	46.29825	44.5433	3.83342
16	541.17946	48.66538	44.80882	4.02942
18	543.84996	50.45405	45.02994	4.17752
20	545.07198	50.439	45.13112	4.17627
22	546.69782	51.43077	45.26574	4.25839
24	548.1328	52.11974	45.38455	4.31543

calculated from 4 parallel experiments

Determination of IC_{50} values on HepG2 cells: The HepG2 cells were seeded in a 96-well plate with the density of 3,000 cells per-well (total medium volume of 75 μL). 24 hours post seeding, the solutions with a serial of concentrations (5 concentrations) of different compounds in 25 μL of medium were added to each well (five wells for each concentration). Cells without the treatment of the compounds were used as the control. The MTT assays were performed after an extra culture time of 48 hours. All compounds were removed and 100 μL fresh medium was added for each well, 20 μL of MTT solution (5 mg/mL) was added and incubated for 4 hours in 37 Ⅲ. Pipette out the spent media, formazon crystals at the bottom of each well were dissolved in 150 μL DMSO. After 15 minutes at room temperature, absorbance at wavelength of 490 nm was tested using a microplate reader (BIO-RAD, iMark^{TM}). The experiment was repeated for 3 times. IC_{50} values for the inhibition of cell viability were calculated from pharmacological inhibitory response curves using software Prism 5.0.

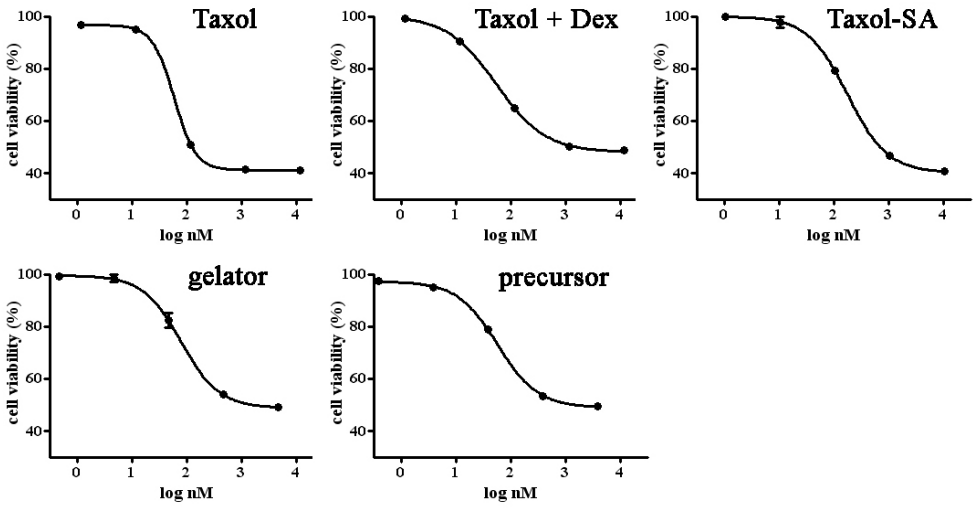


Figure S-19. Representative congress curve of cell inhibition of taxol-based compounds

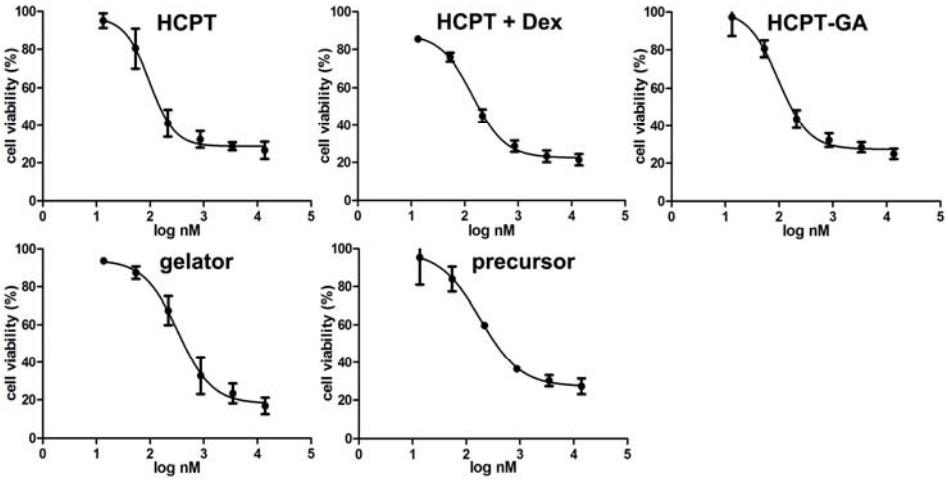


Figure S-20. Representative congress curve of cell inhibition of HCPT-based compounds

Reference:

(1) Deutsch, H. M.; Glinski, J. A.; Hernandez, M.; Haugwitz, R. D.; Narayanan, V. L.; Suffness, M.; Zalkow, L. H. In *J. Med. Chem.* 1989; Vol. 32, p 788.

(2) Gao, Y.; Kuang, Y.; Guo, Z.-F.; Guo, Z.; Krauss, I. J.; Xu, B. *J. Am. Chem. Soc.* **2009**, *131*, 13576.