Supporting Information

A Biopolymer-based and Inflammation-responsive Nanodrug for Rheumatoid Arthritis Treatment via Inhibiting JAK-STAT and JNK Signaling Pathways

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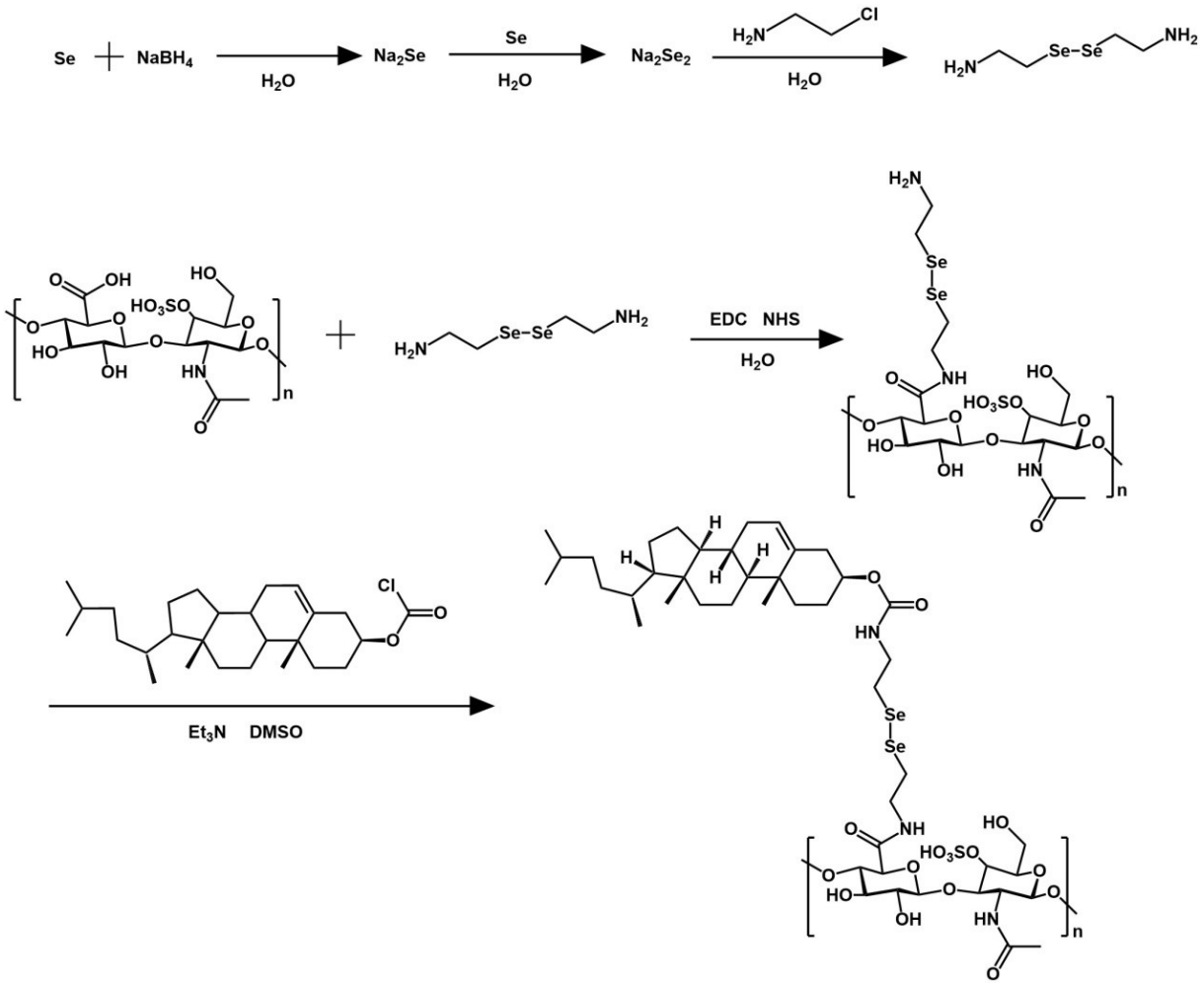
Experimental section

Instrumentation: ^{1}H NMR and ^{77}Se NMR spectra were recorded on a Bruker Advance 600 MHz NMR spectrometer. High-resolution mass spectrometry was performed on an AB Sciex Triple TOF 5600+ mass spectrometer. UV-vis spectra were recorded on a Hitachi U-3010 UV-vis spectrophotometer. Cells imaging were obtained on an Olympus IX 73 fluorescence microscope. The particle size and distribution were determined using a Malvern Zetasizer Nano laser dynamic scattering spectrometer at a fixed angle of 90° at 25 °C. Transmission electronic microscopy (TEM) experiments were performed by dropping a drop of the solution onto ultra-thin carbon film and the morphology of the particles was observed on a JEM-1400 TEM transmission electron microscope. Fluorescent imaging was performed on an AMI small animal fluorescence imaging system (Spectral Instruments Imaging Co.).

Cell culture: RAW264.7 cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin & streptomycin at 37 °C, 5% CO_2. L929 cells were cultured in DMEM medium supplemented with 10% FBS and 1% penicillin & streptomycin at 37 °C, 5% CO_2.



Scheme S1. Synthetic route for diselenide linker and CSSeSeChol.

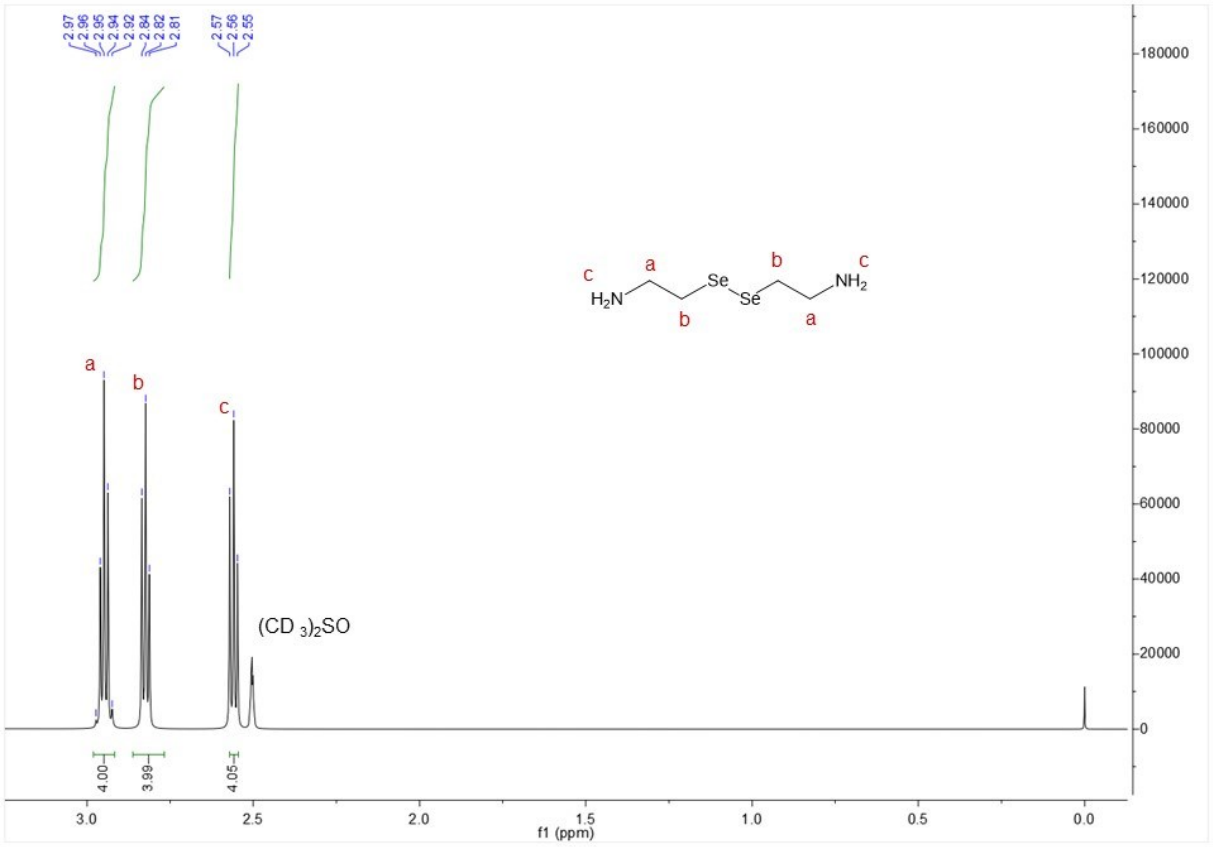


Figure S1. ^{1}H NMR spectrum for 2,2'-diselanediylbis(ethan-1-amine) (diselenide linker) (in DMSO-d6).

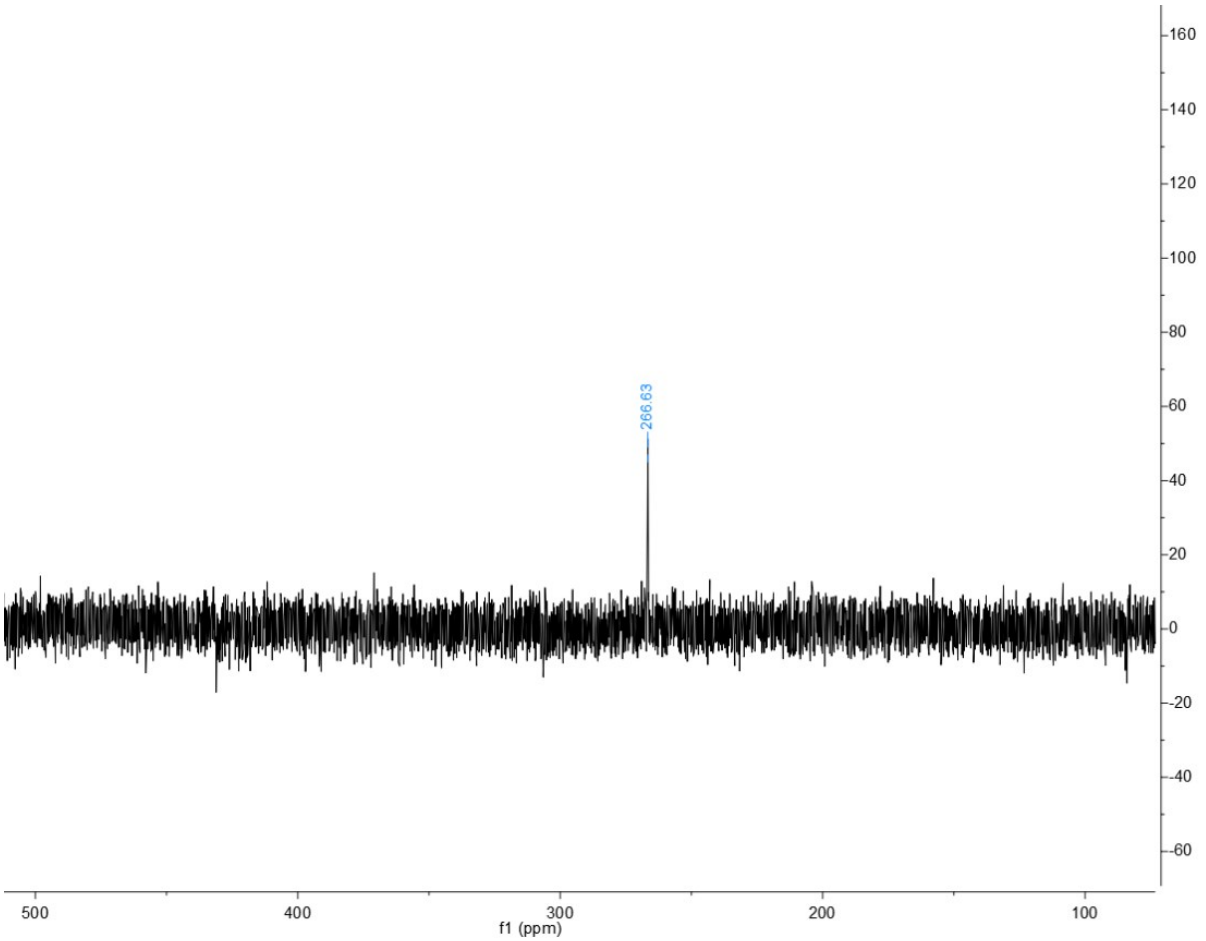


Figure S2. ^{77}Se NMR spectrum for 2,2'-diselanediylbis(ethan-1-amine) in DMSO-d6.

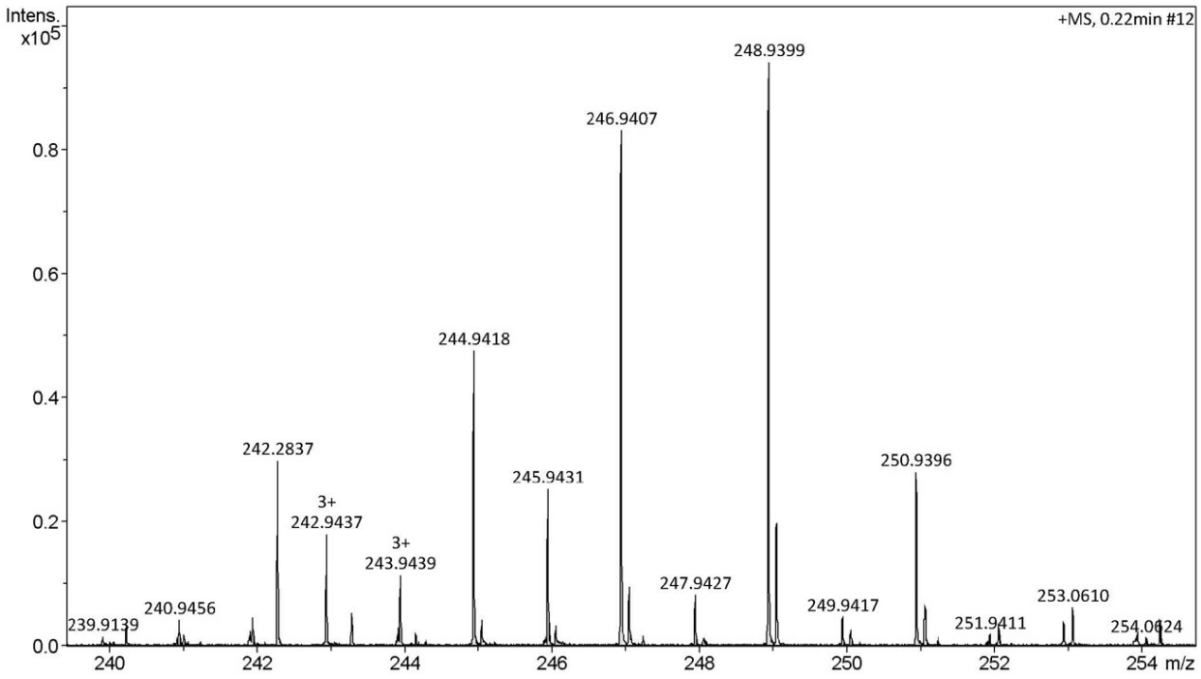


Figure S3. High resolution mass spectrum (HR-MS) for 2,2'-diselanediylbis(ethan-1-amine). m/z: $[M+H]^+$ 248.9399.
(The isotopic peaks are due to the contribution from isotopes of the elements)

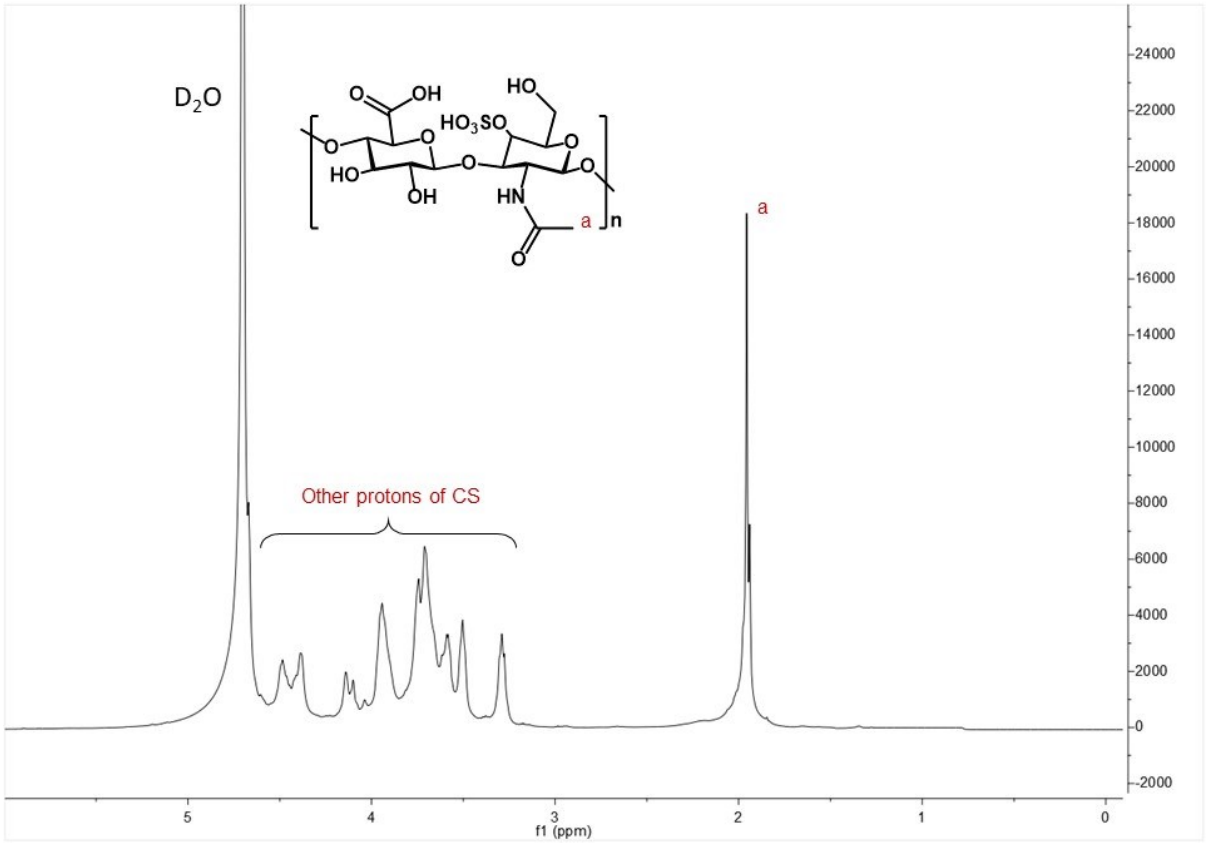


Figure S4. ^{1}H NMR spectrum for CS in D_2O.

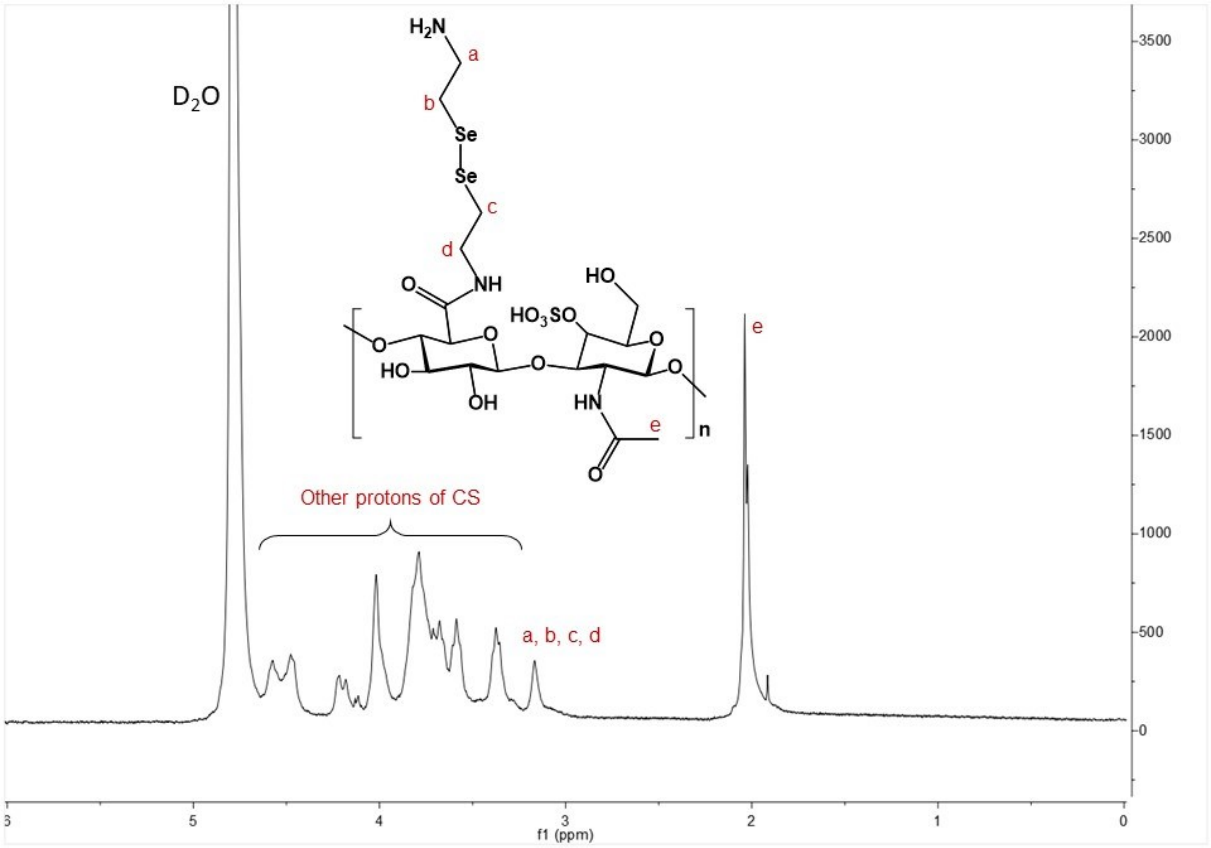


Figure S5. ^{1}H NMR spectrum for CS-SeSe in D_2O.
Note: After being incorporated into side chains of CS polymer, the protons in diselenide linker exhibit a single and relatively broad ^{1}H NMR peak at around 3.2 ppm in D_2O, which is similar to that reported by a previous literature (Li et al., *J. Am. Chem. Soc.* 2018, 140, 4164.)

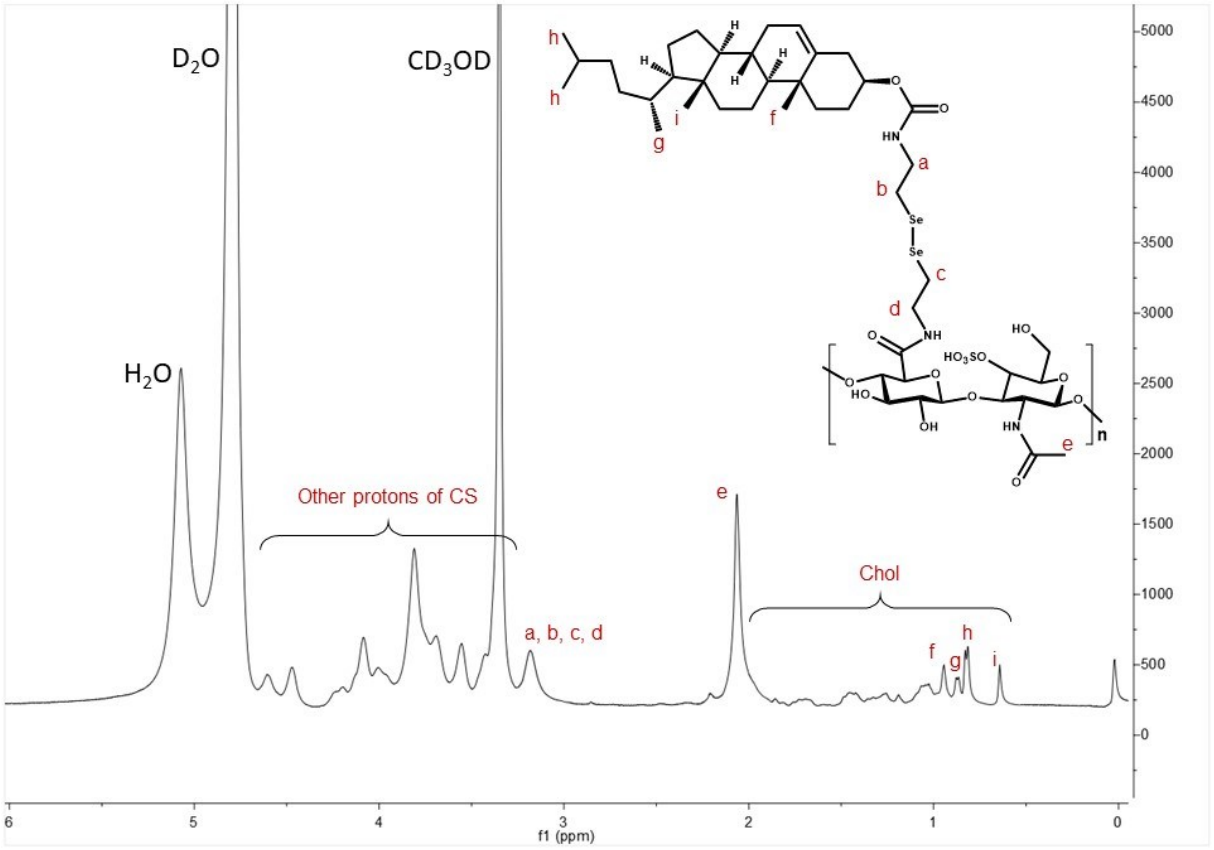


Figure S6. ^{1}H NMR spectrum of CSSeSeChol in D_2O/CD_3OD (volume 1:1).

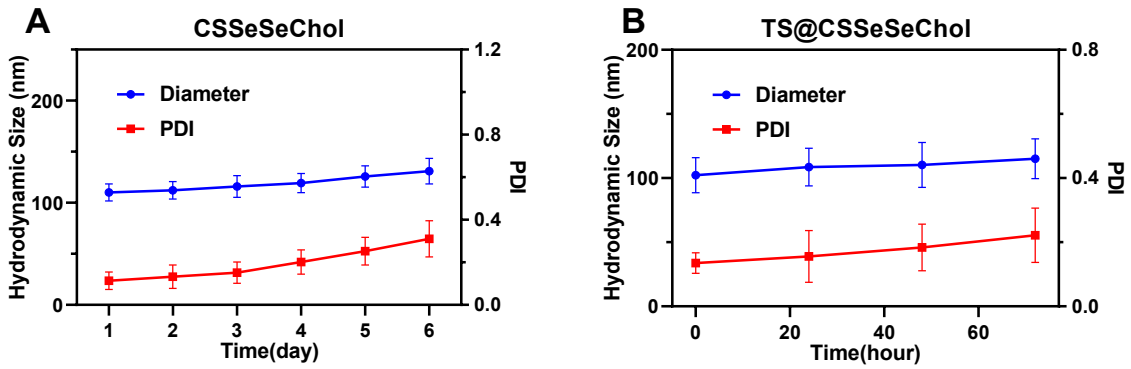


Figure S7. (A) Change in hydrodynamic diameters and polydisperse index as determined by DLS for CSSeSeChol nanoparticles (1 mg/mL) stored in PBS (pH 7.4) at 37 °C from day one to day six. (B) Change in hydrodynamic diameters and polydisperse index as determined by DLS for TS@CSSeSeChol nanoparticles (1 mg/mL) incubated in pH 7.4 PBS (with 10% fetal bovine serum) at 37 °C for varied time.

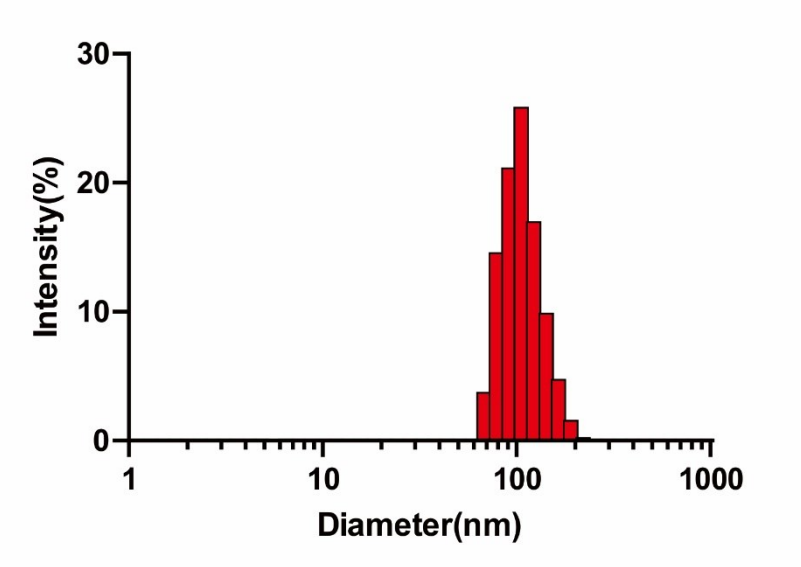


Figure S8. Size distributions of the TS@CSSeSeChol in PBS (pH 7.4) at 25 °C.

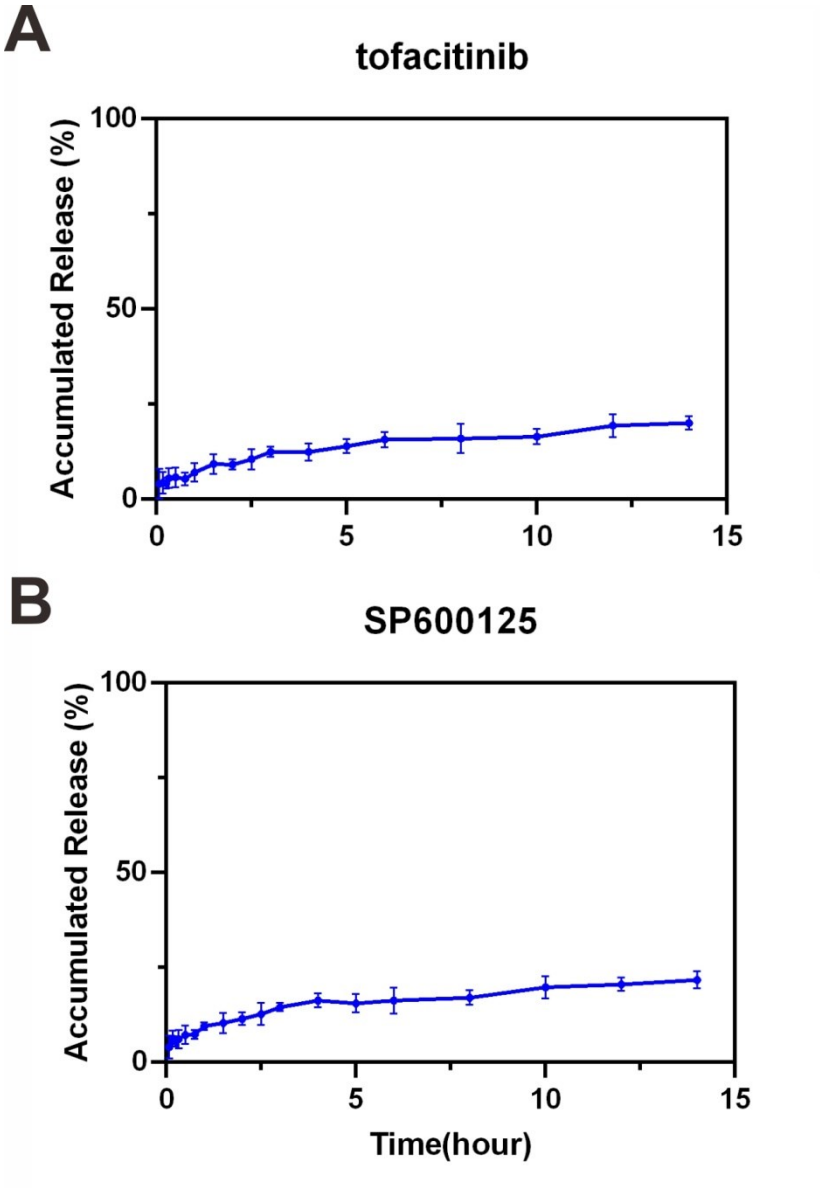


Figure S9. In vitro release profiles of tofacitinib (A) and SP600125 (B) from TS@CSSeSeChol in PBS (pH 7.4, with 10% FBS).

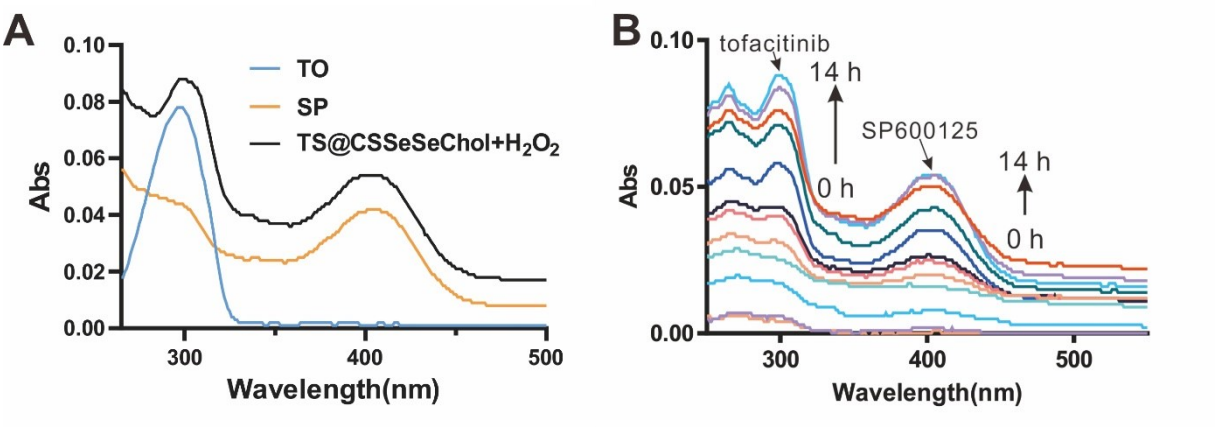


Figure S10. (A) The absorption spectra of free tofacitinib and free SP600125 in DMSO and the absorption spectrum of TS@CSSeSeChol treated with 100 μM H_2O_2 for 14 h in PBS (pH 7.4, containing 10% DMSO). (B) Absorption spectra for the supernatant of the nanodrug TS@CSSeSeChol upon treatment with 100 μM H_2O_2 for varied time in pH 7.4 PBS (containing 10% DMSO) at 25 °C and after dialysis.

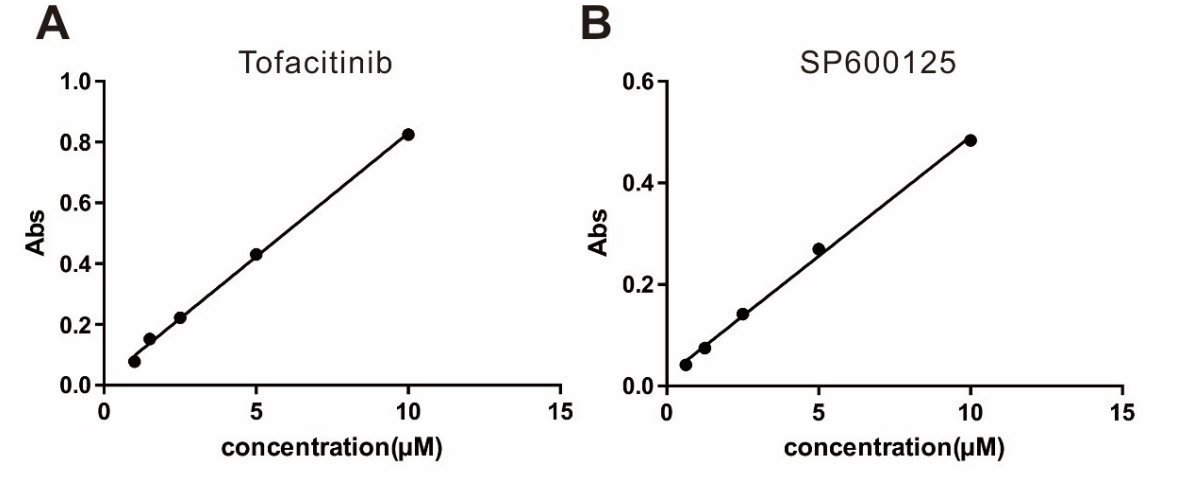


Figure S11. (A) The calibration curve (absorbance vs. concentration) of tofacitinib at 300 nm. (B) The calibration curve of SP600125 at 405 nm. The curves were used to determine the release profiles.

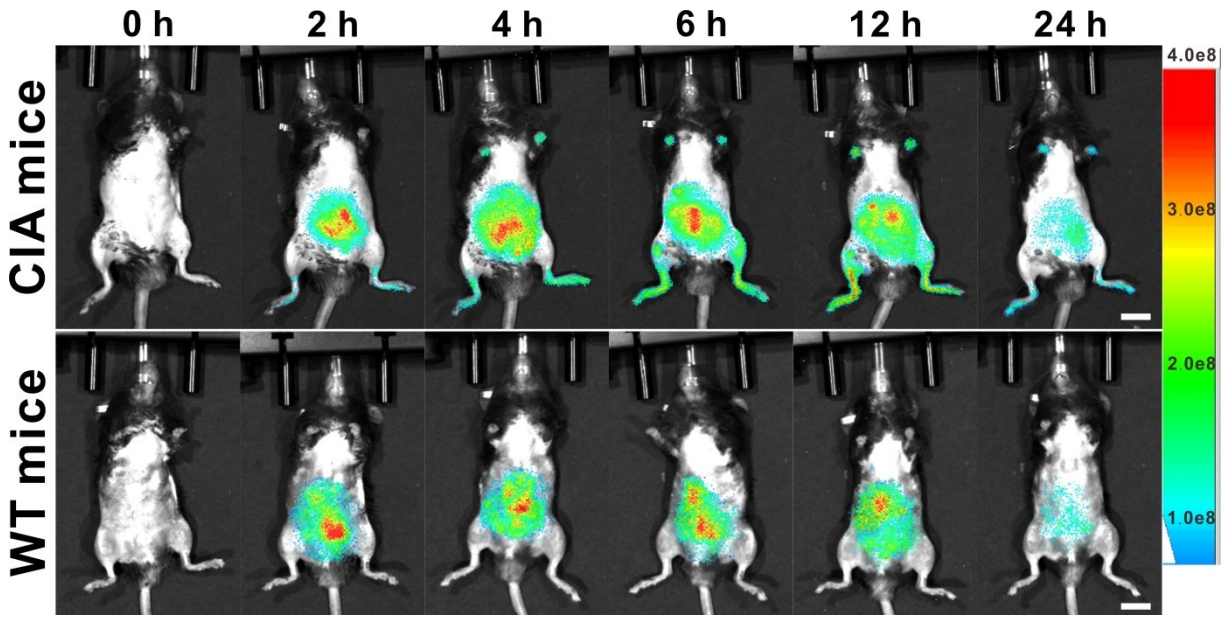


Figure S12. Whole-body fluorescence images for WT and CIA mice after intraperitoneal injection of dye-doped nanodrug at varied time periods. Scale bar: 1.30 cm. The trunks of the mice were shoved before imaging.

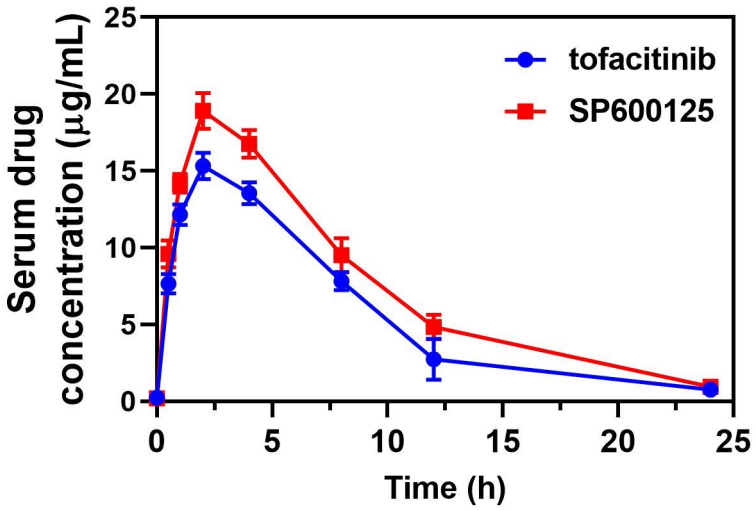


Figure S13. Pharmacokinetic profiles of tofacitinib and SP600125 in the bloodstream of CIA mice after i.p injection of TS@CSSeSeChol.

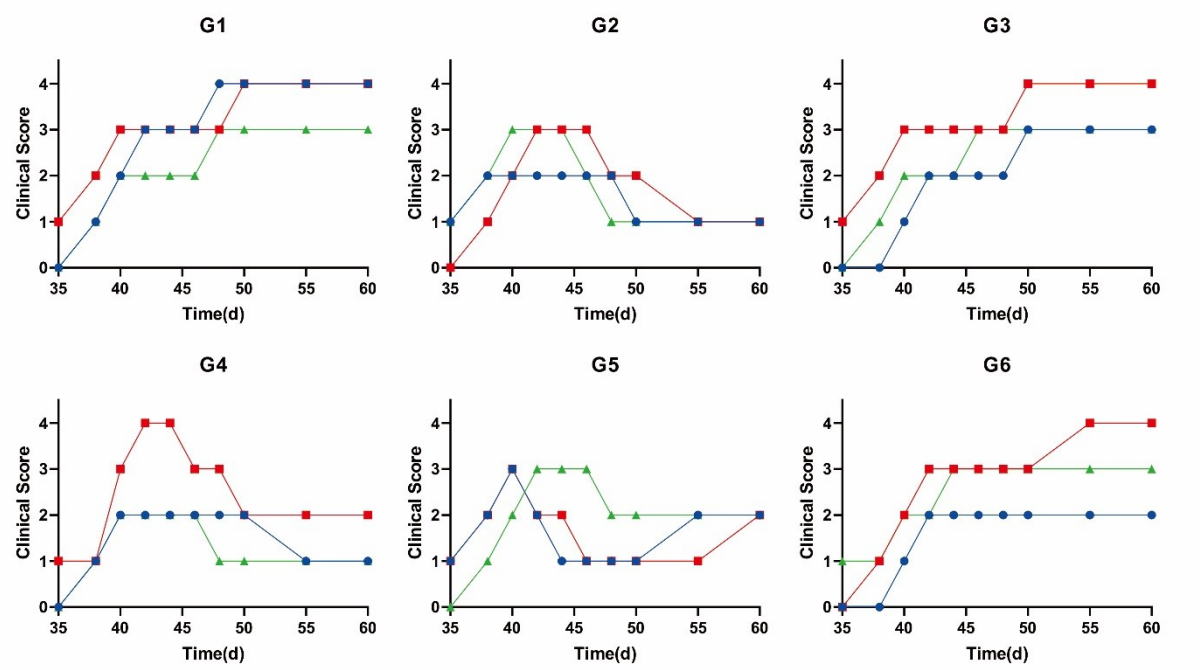


Figure S14. Clinical scores reflecting the severity of arthritis at right hindpaw of each mouse in different groups from day 35 to day 60.

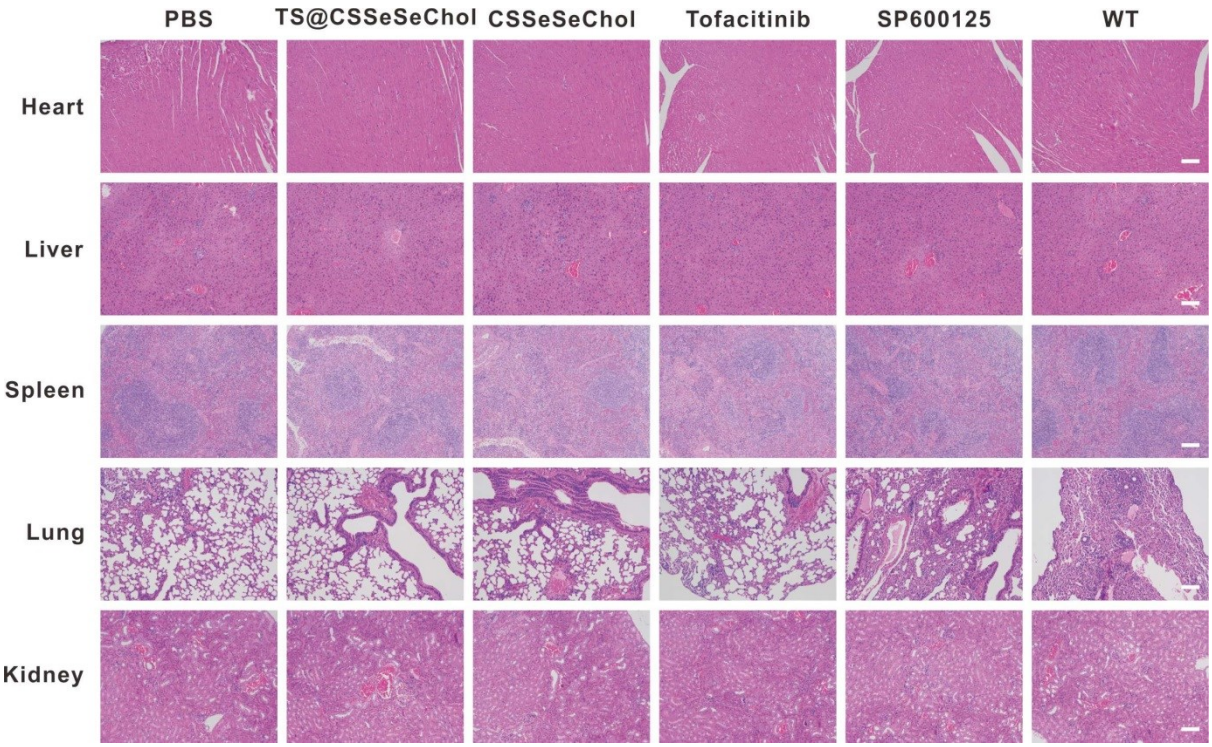


Figure S15. H&E staining of major organs for WT and CIA mice after treatment with various formulations. Scale bar: 200 μm.