Supporting Information

Methotrexate anchored carbon dots as theranostic probes: Digitonin conjugation enhances cellular uptake and cytotoxicity

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Conjugation of DG onto CDs.

PEG diamine passivated CDs were modified with adipic acid (AD) via EDC chemistry to get carboxyl terminated CDs. CDs (5 mL, 1mg/ mL) were mixed with 2 mL of AD (0.013 M, H_2O) and 2 mL of EDC (0.26 M, H_2O) after adjusting the pH to ~ 4.2. The reaction mixture was kept overnight at 4 °C and the resulting solution was purified by dialyzing against distilled water using a cellulose ester dialysis membrane [MWCO = 3500] for 2 days in order to remove any unreacted reactants. Further, DG was conjugated onto CDAD by DCC/DMAP reaction. CDAD (10 mL, 0.5 mg/mL) was stirred with 1 mL of DCC (0.015 M, DMSO) and 500 μL of DMAP (0.001 M, DMSO), for the activation of –COOH groups on CDAD. 1 mL of DG (0.013 M, DMSO/H_2O) was then added to the reaction mixture and stirred overnight at room temperature (28 ^{0}C). The solution was dialyzed against DMSO/H_2O mixture using a cellulose ester dialysis membrane [MWCO = 3500] for 2 days and the resulting solution (CDDG) was used for further studies.

Conjugation of MX onto CD to form CM

Methotrexate (MX) was conjugated onto CD using EDC chemistry via the formation of amide linkage between carboxylic acid group of MX and amine groups of CDs. 1 mL of MX solution (5 mg/mL) was added to CD (10 mL, 1mg/ mL) in presence of EDC (0.13 M). The pH of the solution was maintained at 4.2 and the reaction was kept overnight at 4ºC. The reaction mixture was then dialyzed against distilled water using cellulose ester dialysis membrane [MWCO = 3500].for 24 h to remove any unreacted reactants. The resulting solution (CM) was used for the studies.

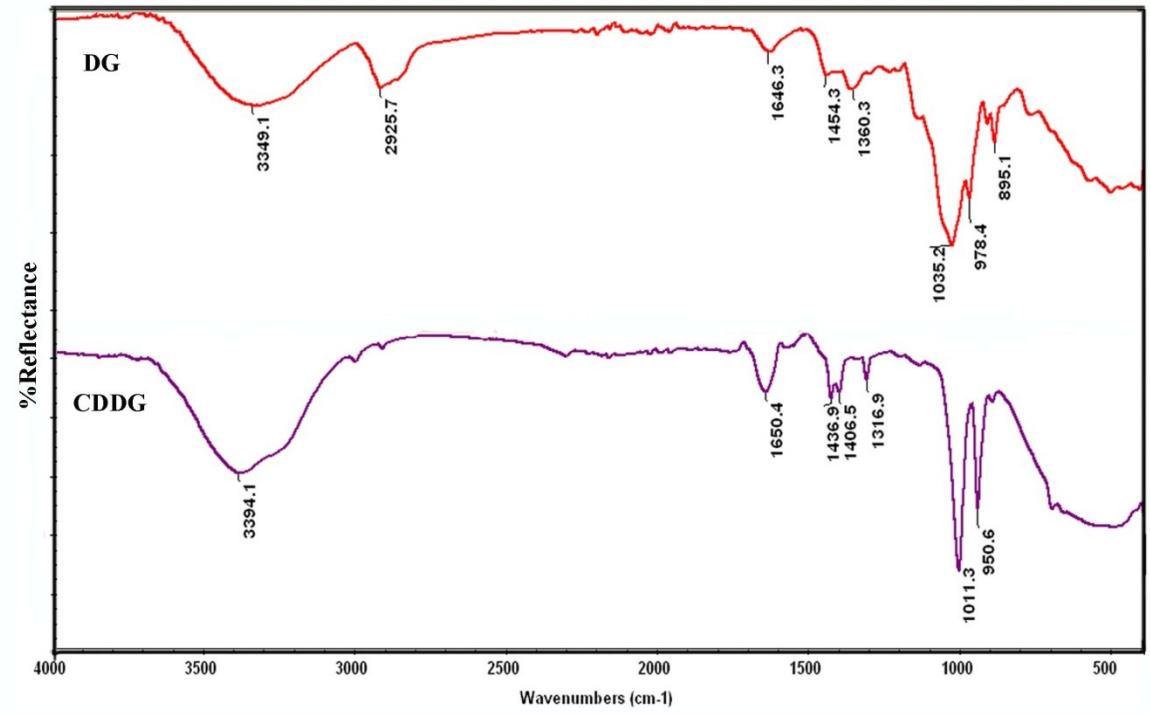

Figure S1. FTIR spectra of DG and CDDG

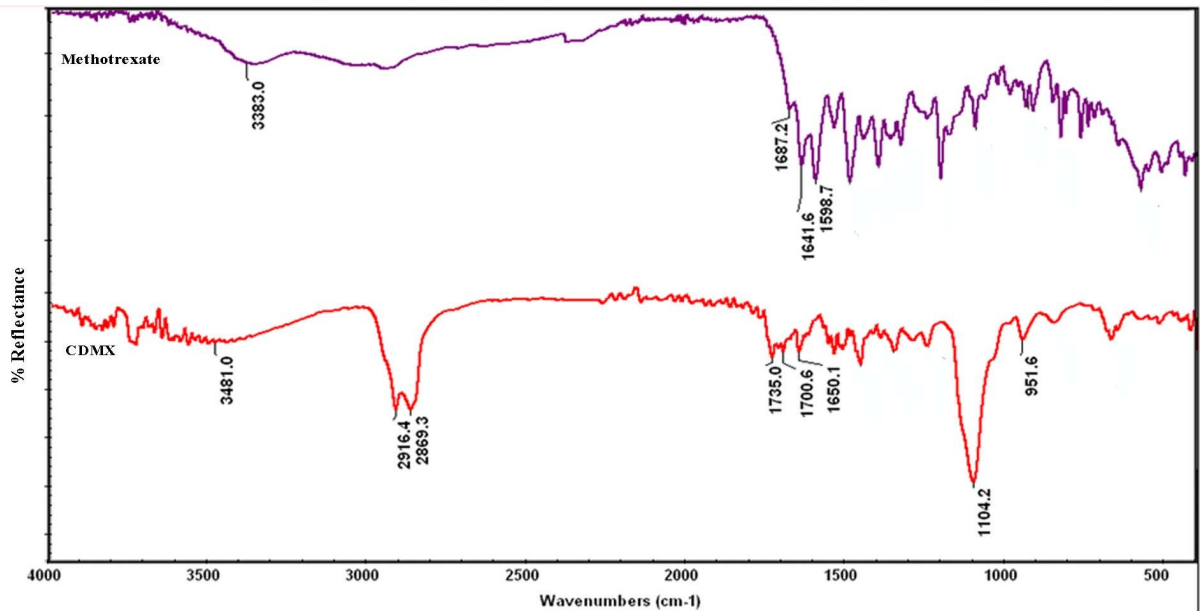

Figure S2. FTIR spectra of Methotrexate and CDMX

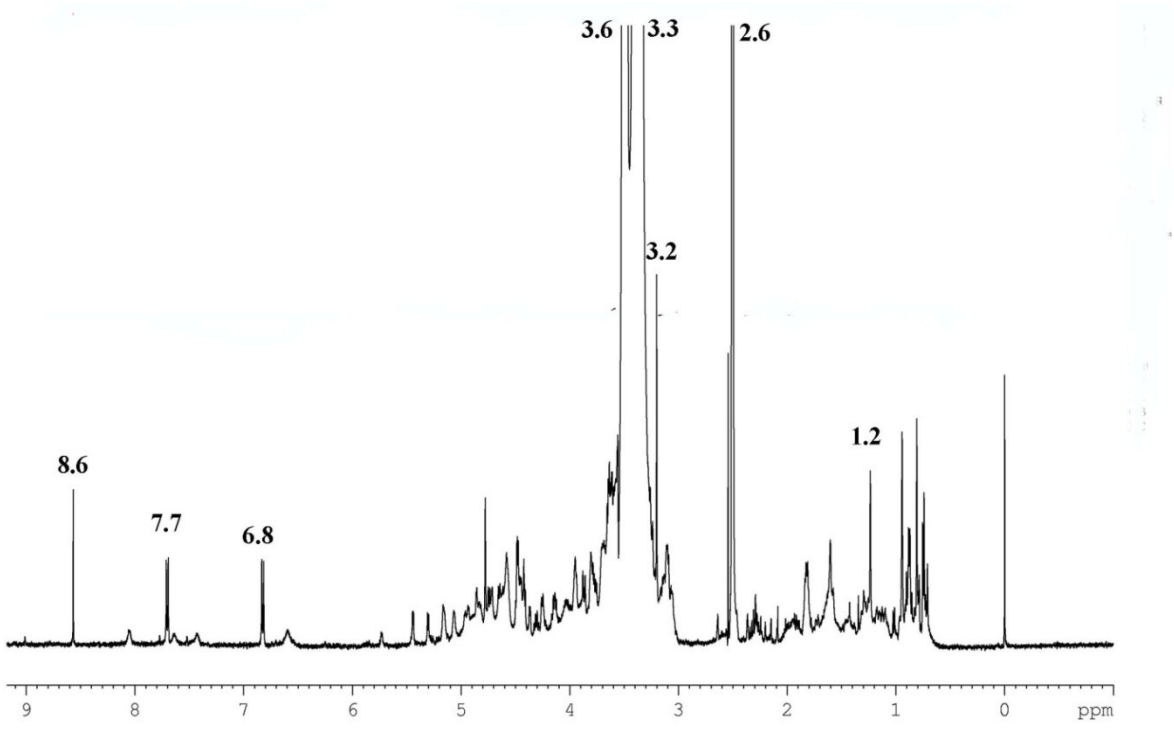

Figure S3. H[1]NMR spectrum of CDMX

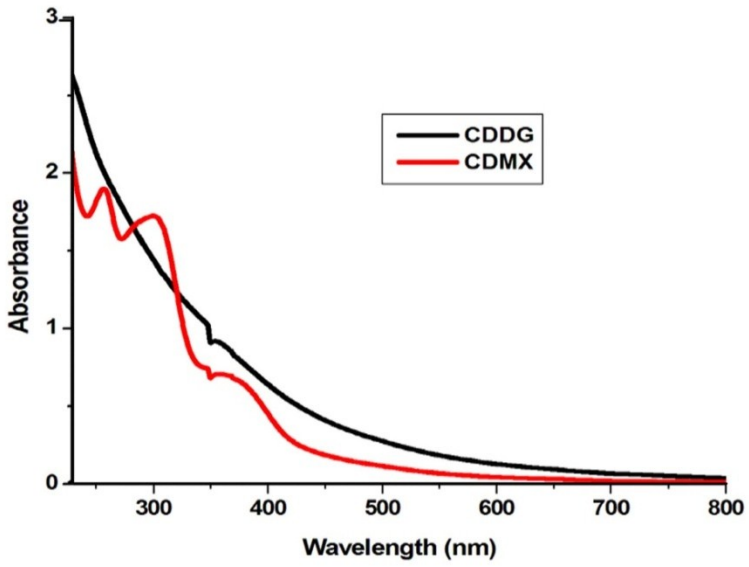

Figure S4. UV-Visible absorption spectra of CDDG and CDMX

Table S1. Sizes of materials obtained from DLS

Sample	Size (nm)
CD	10 ± 3.0
CDDG	147 ± 8.0

Table S2. Zeta potential by DLS

Sample	Zeta potential (mV)
CD	-20.90
CDDG	-4.99
CDMX	0.572