

Supplementary Materials for

Pathways for Dolutegravir Transformation from a Daily Oral to a Once-a-Year Parenteral Medicine

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DTG prodrugs chemical characterization

Proton (^1H)- and carbon (^{13}C)- NMR spectra for MDTG, M2DTG, M3DTG, and M4DTG were recorded on a Varian Unity/Inova-500 NB (500 MHz, Varian Medical Systems Inc., Palo Alto, CA)

MDTG ^1H NMR (500 MHz, CDCl_3): δ 10.20 (s, 1 H), 8.45 (s, 1 H), 7.35 (dd, $J = 15.0, 8.2$ Hz, 1 H), 6.83 (app. dd, $J = 19.1, 9.3$ Hz, 1 H), 5.26 (br. s, 1 H), 4.85–5.01 (m, 1 H), 4.62 (br. s, 1 H), 4.30 (app. d, $J = 12$ Hz, 2 H), 4.17 (dd, $J = 13.3, 5.9$ Hz, 1 H), 4.0 (app. d, $J = 6.3$ Hz, 1 H), 2.73 (t, $J = 7.6$ Hz, 2 H), 2.17 (td, $J = 14.5, 7.2$ Hz, 1 H), 1.80 (app. t, $J = 7.5$ Hz, 2 H), 1.51–1.61 (m, 2 H), 1.40–1.49 (m, 2 H), 1.36 (d, $J = 7.0$ Hz, 3 H), 1.26 (br. s, 20 H), 0.90 (t, $J = 6.6$ Hz, 3 H). ^{13}C NMR (125 MHz, CDCl_3): δ 171.9, 171.1, 163.3, 163.1, 161.6, 161.1, 159.6, 130.6, 130.5, 129.2, 121.3, 121.2, 119.5, 111.2, 111.1, 103.9, 103.7, 103.5, 76.1, 53.1, 36.5, 33.8, 31.9, 29.7, 29.6, 29.5,

29.4, 29.3, 29.2, 29.0, 24.4, 22.6, 15.8, 14.1. HRMS (ESI) m/z : $[M + H]^+$: calculated for $C_{34}H_{45}F_2N_3O_6$, 629.33 (100%), 630.33 (36.8%), 631.33 (3.9%); found, 630.30.

M2DTG: 1H NMR (500 MHz, $CDCl_3$): 10.18 (t, $J = 5.7$ Hz, 1H), 8.44 (s, 1H), 7.33 (dd, $J = 14.9$, 8.3 Hz, 1H), 6.75-6.85 (m, 2H), 5.23 (t, $J = 4.6$ Hz, 1H), 4.94 (t, $J = 6.1$ Hz, 1H), 4.60 (b, 2H), 4.29 (app. d, $J = 10.4$ Hz, 1H), 4.16 (dd, $J = 13.4$, 6.0 Hz 1H), 2.71 (t, $J = 7.7$ Hz, 2H), 1.78 (p, $J = 7.7$ Hz, 2H), 1.42 (p, $J = 7.3$ Hz, 2H), 1.34 (d, $J = 7.0$ Hz, 3H), 1.25 (b, 28H), 0.88 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.0, 171.1, 163.4, 163.3, 163.2, 161.8, 161.3, 159.8, 143.1, 130.7, 130.6, 130.5, 129.2, 121.3, 121.2, 119.6, 111.3, 111.1, 103.9, 103.8, 103.6, 76.2, 53.1, 36.6, 33.9, 31.9, 29.7, 29.6, 29.5, 29.3, 29.2, 29.1, 24.5, 22.7, 15.9, 14.1. HRMS (ESI) m/z : $[M + H]^+$: calculated for $C_{38}H_{54}F_2N_3O_6^+$, 685.39 (100%), 686.39 (41.1%), 687.40 (8.2%); found, 686.38.

M3DTG: 1H NMR (500 MHz, $CDCl_3$): 10.17 (t, $J = 5.7$ Hz, 1H), 8.42 (s, 1H), 7.33 (dd, $J = 14.9$, 8.4 Hz, 1H), 6.75-6.85 (m, 2H), 5.23 (t, $J = 4.8$ Hz, 1H), 4.95 (t, $J = 6.1$ Hz, 1H), 4.60 (b, 2H), 4.28 (d, $J = 12.2$ Hz, 1H), 4.15 (dd, $J = 13.4$, 6.0 Hz 1H), 3.98 (d, $J = 7.2$ Hz, 2H), 2.71 (t, $J = 7.7$ Hz, 2H), 2.18 (tt, $J = 14.5$, 7.2 Hz, 2H), 1.78 (p, $J = 7.6$ Hz, 2H), 1.42 (p, $J = 7.3$ Hz, 2H), 1.33 (d, $J = 7.0$ Hz, 3H), 1.25 (b, 36H), 0.88 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.0, 171.1, 163.4, 163.3, 163.2, 161.7, 161.2, 159.7, 143.2, 130.7, 130.6, 130.5, 129.2, 121.3, 121.2, 119.6, 111.3, 111.1, 103.9, 103.8, 103.6, 76.2, 53.1, 36.5, 33.9, 31.9, 29.7, 29.6, 29.5, 29.3, 29.2, 29.1, 24.5, 22.7, 15.9, 14.1. HRMS (ESI) m/z : $[M + H]^+$: calculated for $C_{42}H_{62}F_2N_3O_6^+$, 741.45 (100%), 742.46 (45.4%), 743.46 (7.4%); found, 742.40.

M4DTG: ^1H NMR (500 MHz, CDCl_3): 10.21 (t, $J = 5.7$ Hz, 2H), 8.50 (s, 2H), 7.33 (dd, $J = 14.9$, 8.3 Hz, 2H), 6.75-6.85 (m, 4H), 5.21 (t, $J = 3.9$ Hz, 2H), 4.92 (t, $J = 6.0$ Hz, 2H), 4.59 (b, 4H), 4.33 (dd, $J = 13.4$, 3.8 Hz, 2H), 4.18 (dd, $J = 12.7$, 4.8 Hz 2H), 3.96 (d, $J = 6.7$ Hz, 4H), 2.71 (t, $J = 7.7$ Hz, 4H), 2.17 (tt, $J = 14.5$, 7.2 Hz, 2H), 1.72-1.84 (m, 5H), 1.42 (p, $J = 7.2$ Hz, 4H), 1.32 (d, $J = 7.0$ Hz, 6H), 1.25 (b, 24H). ^{13}C NMR (125 MHz, CDCl_3): δ 172.0, 171.1, 163.4, 163.3, 163.2, 161.7, 161.2, 159.7, 143.2, 130.7, 130.6, 130.5, 129.2, 121.3, 121.2, 119.6, 111.3, 111.1, 103.9, 103.8, 103.6, 76.2, 53.1, 36.6, 33.7, 29.7, 29.6, 29.5, 29.3, 29.1, 24.5, 15.9. HRMS (ESI) m/z : $[\text{M} + \text{H}]^+$: calculated for $\text{C}_{58}\text{H}_{69}\text{F}_4\text{N}_6\text{O}_{12}^+$, 1116.48 (100%), 1117.49 (62.7%), 1118.49 (19.3%); found, 1117.45.

Thermal analyses

Differential scanning calorimetry (DSC) thermograms were obtained using a Discovery X3 Differential Scanning Calorimeter (TA Instruments, New Castle, DE, USA), while thermogravimetric analysis (TGA) was performed using a TGA 550 (TA Instruments, New Castle, DE, USA) with a thermal analysis operating system (TRIOS software, TA DSC 25 Instruments). For DSC, about 8 mg of sample was hermetically sealed in an aluminum pan (Tzero pans, TA Instruments), while an empty pan was used as a reference. The pans were heated from 30 - 300 °C at the rate of 10 °C/min in a nitrogen environment (flow rate 20 mL/min). TGA was performed from 30 - 300 °C at the rate of 10 °C/min in a nitrogen environment (flow rate 20 mL/min).

Cytotoxicity assays

Following treatment with free drug and nanoparticles, cell vitality of MDMs was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as detailed in our previous works([29](#), [30](#)). The treatments comprised of NDTG or NM2DTG from 1.95 – 1,000 μ M or 2-fold dilutions of DTG or M2DTG from 0.20 - 100 μ M for 24 h. Untreated and vehicle-treated cells were used as controls. For each group, quadruplicate samples were used. The absorbance was measured at 490 nm on a Molecular Devices SpectraMax M3 plate reader with SoftMax Pro 6.2 software (Sunnyvale, CA, USA). Absorbance was compared to that of untreated control cells to determine cell vitality.

UPLC-TUV quantification of DTG and prodrugs

A Waters ACQUITY UPLC H-Class system with tunable ultraviolet/visible (TUV) detector and Empower 3 software measured drug concentrations. DTG, MDTG, M2DTG, and M3DTG samples were separated on a Phenomenex Kinetex 5 μ m C18 column (150 $\times$ 4.6 mm) (Torrance, CA, USA). DTG was detected at 254 nm, using isocratic elution with a mobile phase consisting of 65% 50 mM KH₂PO₄, pH 3.2/35% ACN at a flow rate of 1.0 mL/min ([29](#)). MDTG, M2DTG, and M3DTG were detected at 230 nm, using isocratic elution with mobile phases consisting of 90% ACN/10% water, 95% ACN/5% water, or 98% ACN/2% water ratio at a flow rate of 1.0 mL/min. M4DTG samples were separated on a Waters XBridge 3.5 μ m C8 column (150 $\times$ 3.0 mm) (Milford, MA, USA). M4DTG was detected at 230 nm, using isocratic elution

with a mobile phase consisting of 80% ACN/20% water ratio and 0.5 mL/min flow rate. Drug content was determined relative to peak areas of drug standards (0.05–50 µg/mL) in methanol.

Quantitation of DTG and M2DTG by UPLC-MS/MS

DTG and M2DTG were quantitated in mouse, rat, and rhesus macaque plasma and tissues by UPLC-MS/MS using a Waters ACQUITY H-class UPLC (Waters, Milford, MA, USA) connected to a Xevo TQ-S micro mass spectrometer and analyzed using MassLynx V4.1 software (Milford, MA, USA). All solvents for sample processing and UPLC-MS/MS analysis were LC-MS-grade (Fisher). For rodent plasma and blood samples, 25 µL of sample was added into 1 mL ACN spiked with 10 µL internal standard (IS). Samples were vortexed and centrifuged at $17,000 \times g$ for 10 minutes at 4 °C. The supernatants were collected and dried using a SpeedVac and reconstituted in 100 µL 20% water/80% methanol; 10 µL was injected for DTG, and M2DTG UPLC–MS/MS analyses. Standard curves were prepared in blank mouse or rhesus macaque plasma/blood in the range of 0.2-1000 ng/mL for respective analytes. For rodent tissue drug quantitation, 3-200 mg of sample was homogenized in 4-29 volumes of 0.1% v/v formic acid and 2.5 mM ammonium formate containing 90% methanol. 280 µL of Optima grade methanol containing 0.1% formic acid and 2.5 mM ammonium formate was added to 100 µL of rodent tissue homogenate, followed by addition of 80% methanol (10 µL), and IS (10 µL), followed by vortexing for 3 minutes and centrifugation at $20,000 \times g$ for 15 minutes. d3-Dolutegravir (d3-DTG), myristoylated darunavir (MDRV) and stearylatoled darunavir (SDRV), at a final concentration of 40, 20 and 20 ng/mL, respectively, were used as ISs for DTG and M2DTG analyses respectively. Finally, 85 µl of

supernatant was mixed with 15 μ L Optima grade water, and 10 μ L was injected for DTG, and M2DTG UPLC–MS/MS analyses. Standards were prepared similarly using blank tissue homogenates with 10 μ L of spiking solution (DTG/M2DTG, 5-5,000 ng/mL in 80% MeOH containing 0.1% formic acid and 2.5 mM ammonium formate). For DTG quantitation, chromatographic separation of 10 μ L DTG sample was performed on a Waters ACQUITY UPLC BEH Shield RP18 column (1.7 μ m, 2.1 mm x 100 mm) using a 10-minute gradient of mobile phase A (7.5 mM ammonium formate in water, adjusted to pH 3 using formic acid) and mobile phase B (100% ACN) at a flow rate of 0.25 mL/minute. For the first 3.5 minutes, the mobile phase composition was 35% B and was increased to 95% B in 0.5 minute and held constant for 1.5 minutes. Mobile phase B was then reset to 35% in 0.5 minute, and the column was equilibrated for 1 minute before the next injection. Chromatographic separation for M2DTG quantitation was achieved on a Waters ACQUITY UPLC BEH Shield RP18 column (1.7 μ m, 2.1 mm $\times$ 30 mm) using an 8-minute gradient of mobile phase A (7.5 mM ammonium formate in water, adjusted to pH 3 using formic acid) and mobile phase B (100% methanol) at a flow rate of 0.28 mL/minute. For M2DTG, the initial mobile phase composition was 85% B for the first 5 minutes, and increased to 95% B in 0.25 minute, held constant for 1 minute, reset to 85% in 0.25 minute and the column was equilibrated for 1 minute before the next injection. DTG and M2DTG were detected at a cone voltage of 10 V and 8 V, respectively, and a collision energy of 25 V and 30 V, and 50 V, 44 V, 14V, respectively. Multiple reaction monitoring (MRM) transitions used for DTG, M2DTG, d3-DTG, MDRV, and SDRV were 420.07>127.04, 420.07>277.12, 420.07>295.12, 686.38>126.96, 686.38>277.02, 686.38>420.07, 758.64> 602.55, 814.457>658.403, respectively. Spectra were

analyzed and quantified by MassLynx software version 4.1. All quantitations were determined using analyte peak area to internal standard peak area ratios.

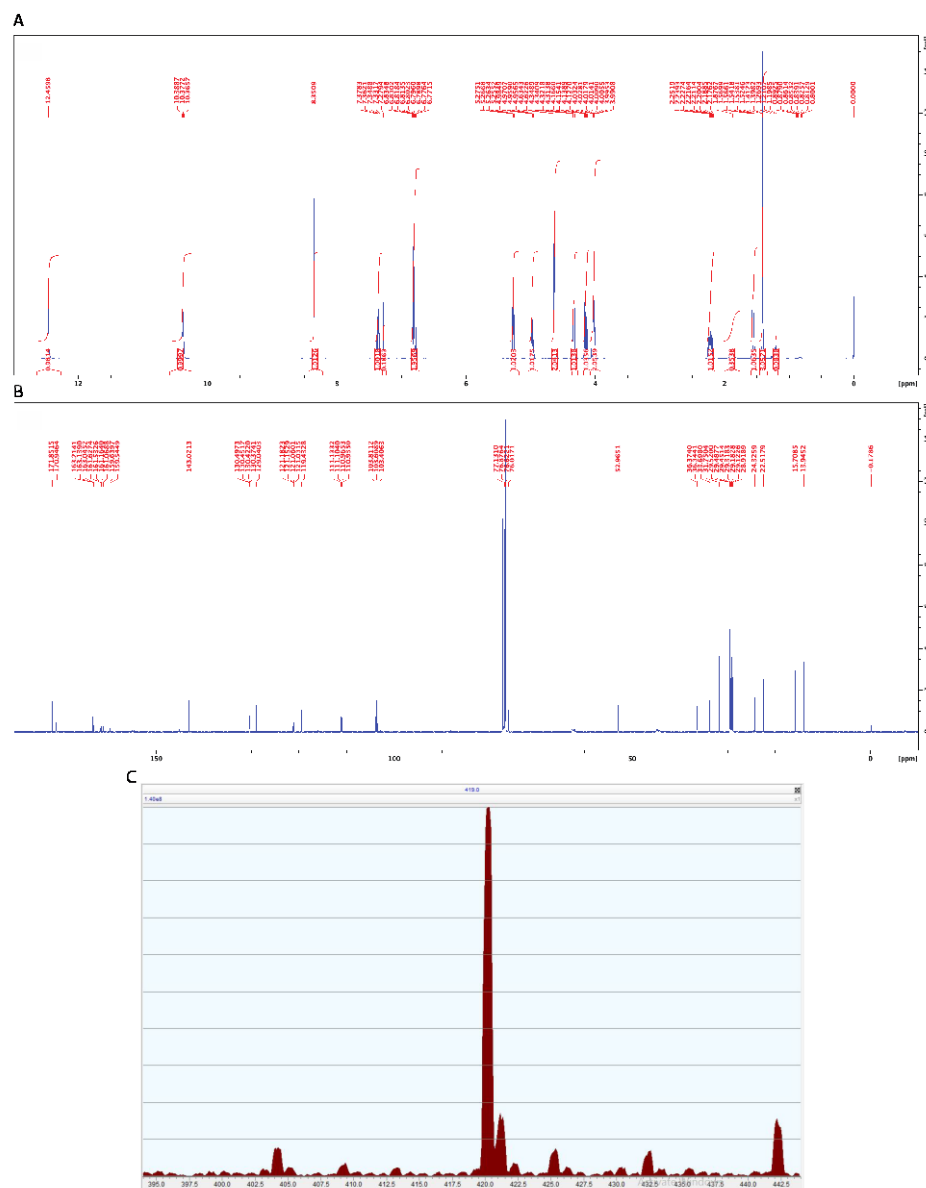
Carboxylesterase quantification

ELISA kits for the quantification of specific carboxylesterases for Rat Carboxylesterase 1 (CES1; catalog no. MBS452470) and Rat Cocaine esterase (CES2; catalog no. MBS7216469) were purchased from MyBioSource (San Diego, CA, USA) and used per manufacturer instructions. Male SD rats (SASCO) were euthanized using isoflurane, exsanguinated, and subjected to whole body perfusion. Tissues, including liver, kidney, muscle, spleen, lymph nodes, and gut were collected and homogenized according to protocols established previously ([29](#), [30](#)) in 5 volumes of ice-cold PBS. Rat plasma was used without homogenization or dilution. Heat-inactivation was achieved by incubation at 60 °C for a minimum of 20 min. All the kit components and the samples were brought to room temperature before use and processed as per the protocols provided with the kits. The plates were analyzed at 450 nm on a Molecular Devices SpectraMax M3 plate reader with SoftMax Pro 6.2 software (Sunnyvale, CA, USA). CES concentrations were calculated relative to the absorbance of the standard curve after normalizing to blanks.

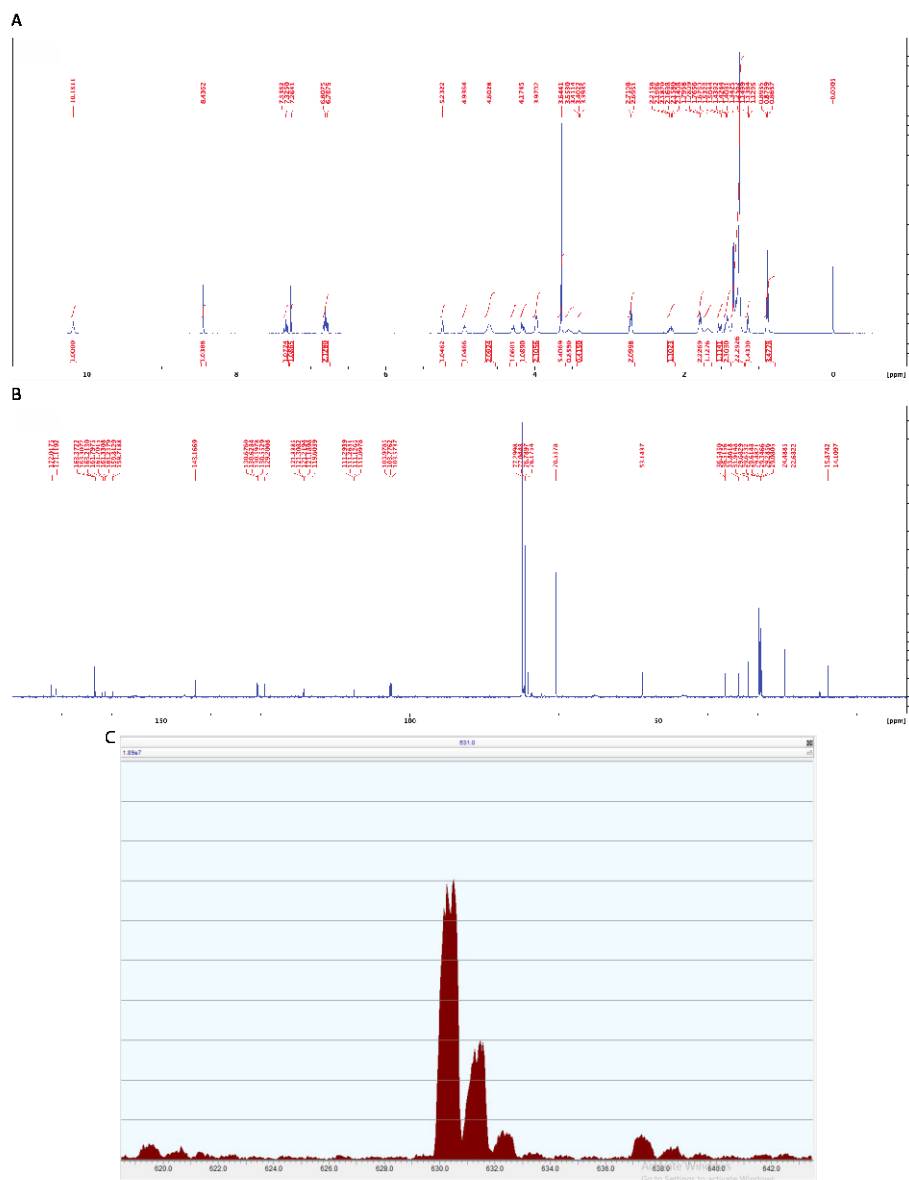
Molecular docking

A series of induced fit molecular docking simulations (Maestro Suite 2020-2, Schrödinger, New York City, NY, USA) were conducted with Carboxylesterase 1. The three-dimensional crystal structures of CES1 (PDB ID: 1YA8) protein were retrieved from the Research Collaboratory for

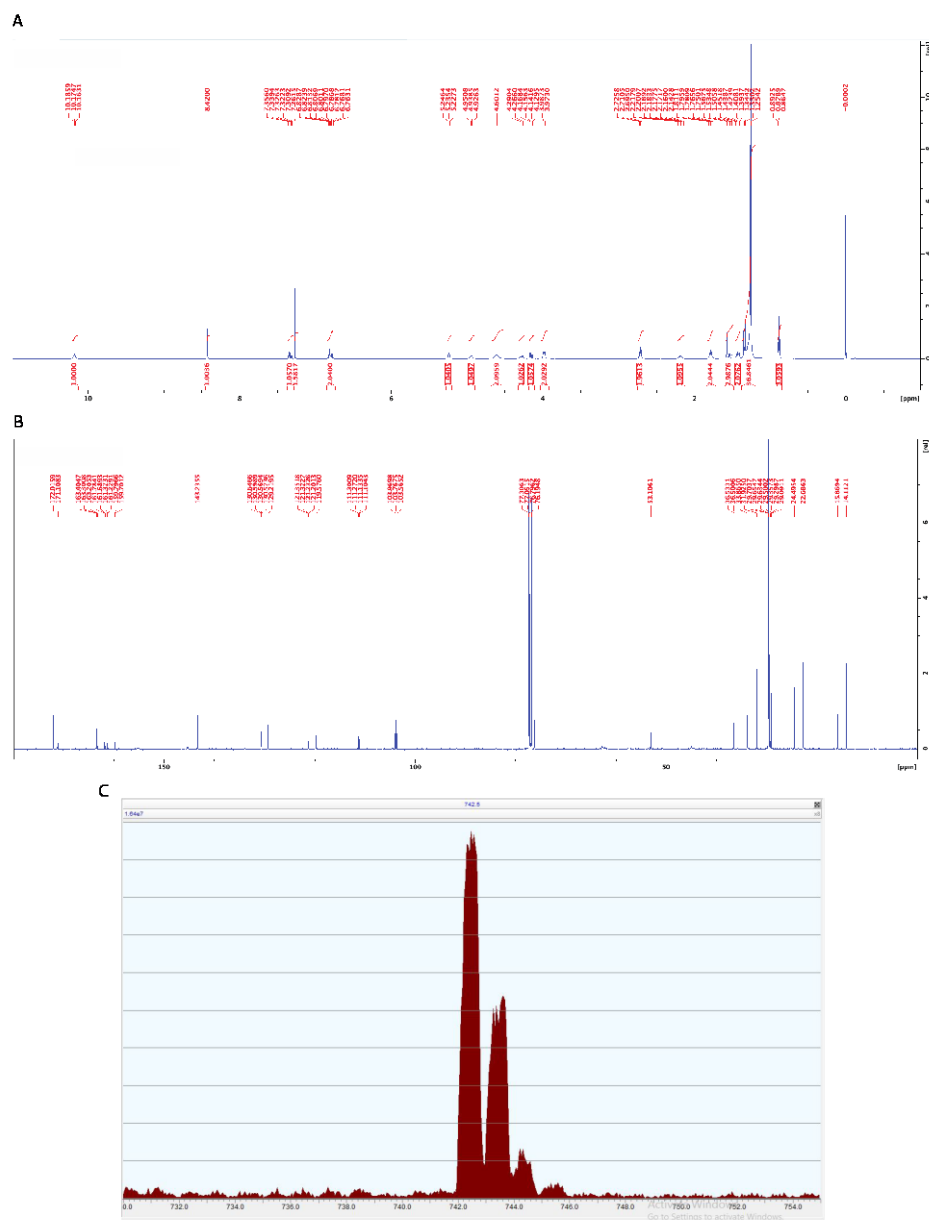
Structural Bioinformatics Protein Data Bank (RCSB PDB) database. Structure optimization was performed using the Protein Preparation Wizard (Epik, Impact, and Prime). Prodrug structures were prepared prior to docking using the LigPrep application for the conversion of structures from 2-dimensional to 3-dimensional, correction of improper bond distances, bond orders, generation of ionization states, and energy minimization processes. The prepared protein and ligand structures were then used for Induced Fit Docking (IFD). Flexible protein-ligand docking was performed using the Induced Fit Docking application, which combines Grid-based Ligand Docking with Energetics (GLIDE) and Prime refinement modules. The ligand 2-methylbutanoic acid in the crystal 1YA8 binding site complex was used to define the binding site. It was then used as the centroid of the workplace, which represents protein putative functional sites. SiteMap module was used to highlight regions within the binding site suitable for occupancy by hydrophobic groups or by ligand hydrogen-bond donors, acceptors, or metal-binding.



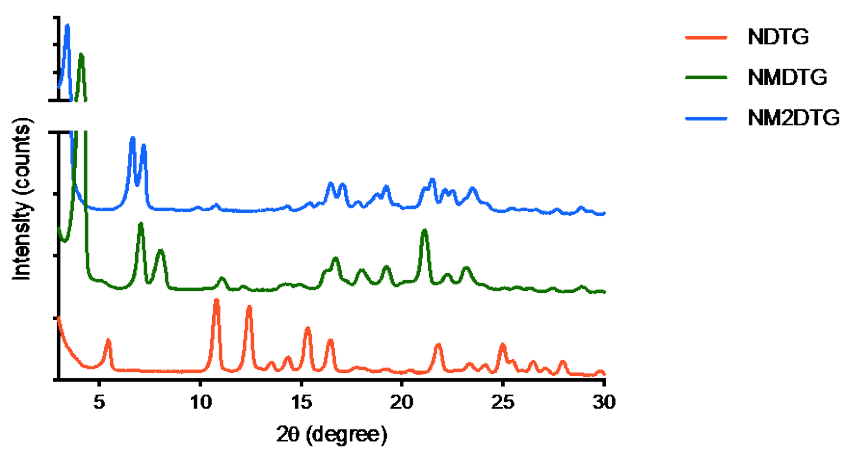
Supplementary figure 1. Structural characterization of DTG. (A) ^1H and (B) ^{13}C NMR of DTG. (C) ESI infusion of DTG generated a signal at 419.904 m/z. (A-C) Experiments were repeated five independent times with equivalent results.



Supplementary figure 2. Structural characterization of MDTG. (A) ^1H and (B) ^{13}C NMR of MDTG. (C) ESI infusion of MDTG generated a strong signal at 630.3 m/z. (A-C) Experiments were repeated five independent times with equivalent results.

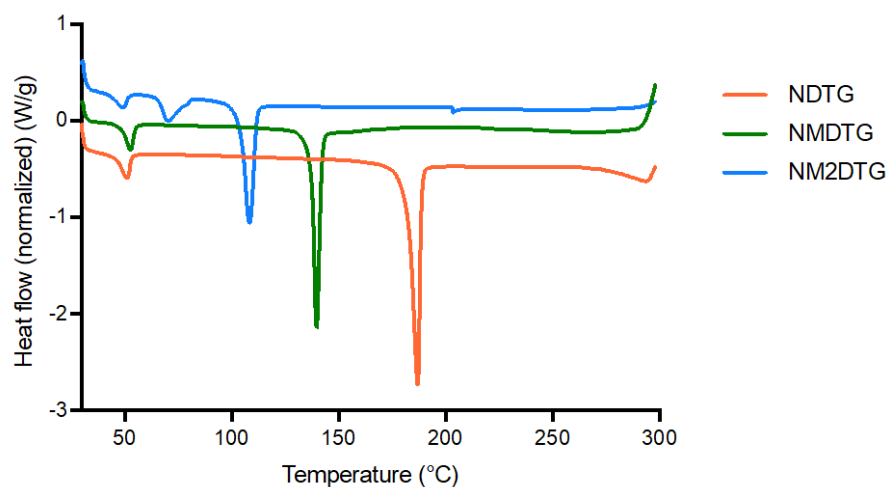


Supplementary figure 4. Structural characterization of M3DTG. (A) ¹H and (B) ¹³C NMR of M3DTG. (C) ESI infusion of M3DTG generated a strong signal at 742.4 m/z. (A-C) Experiments were repeated five independent times with equivalent results.

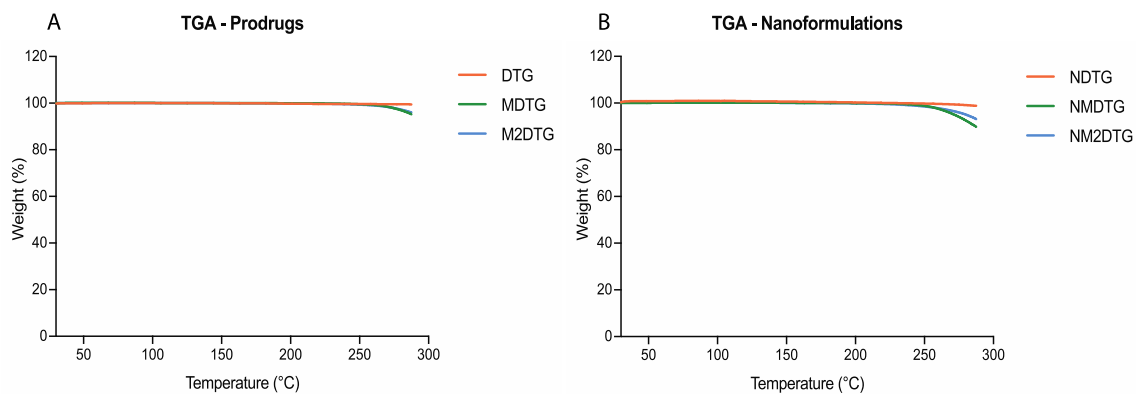


Supplementary figure 6. X-ray diffraction (XRD) of nanoformulated DTG and prodrugs.

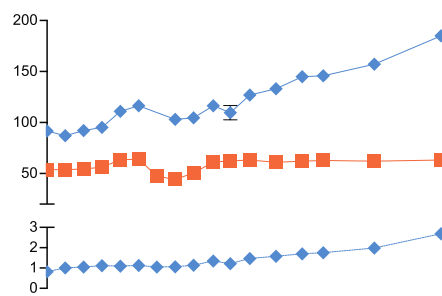
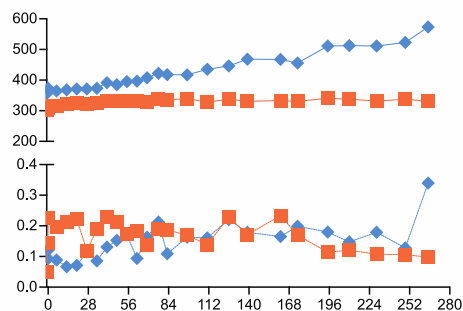
Overlay of powder X-ray diffraction (XRD) patterns of NDTG, NMDTG, and NM2DTG at $2\theta = 2-70$ at $1^\circ/\text{min}$.



Supplementary figure 7. Differential scanning calorimetry of DTG prodrug nanoformulations. Overlay of differential scanning calorimetry (DSC) thermograms of lyophilized NDTG, NMDTG, and NM2DTG nanoformulations at a ramp of 10 °C/min from 30-300 °C.

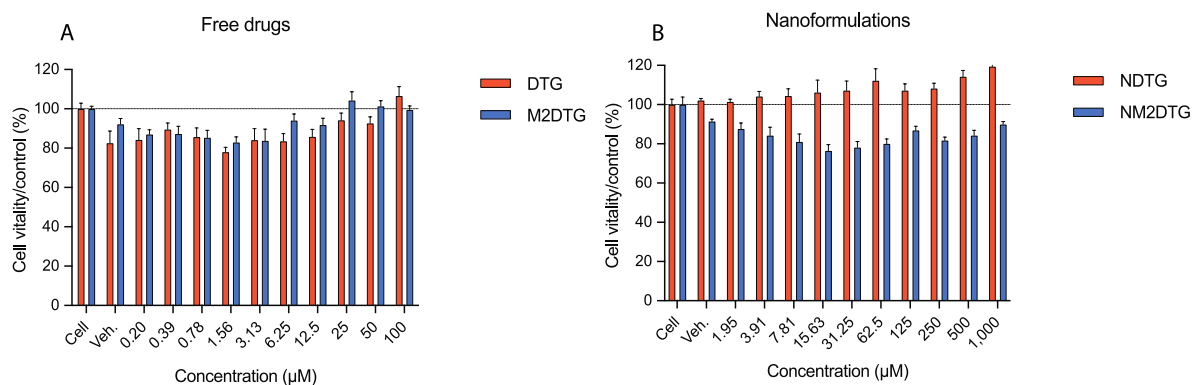


Supplementary figure 8. Thermogravimetric analyses (TGA) of DTG, prodrugs, and nanoformulations. (A) Overlay of TGA thermograms of DTG, MDTG, and M2DTG at a ramp of 10 °C/min from 30-300 °C. (B) Overlay of TGA thermograms of lyophilized NDTG, NMDTG, and NM2DTG nanoformulations at a ramp of 10° C/min from 30-300°C.

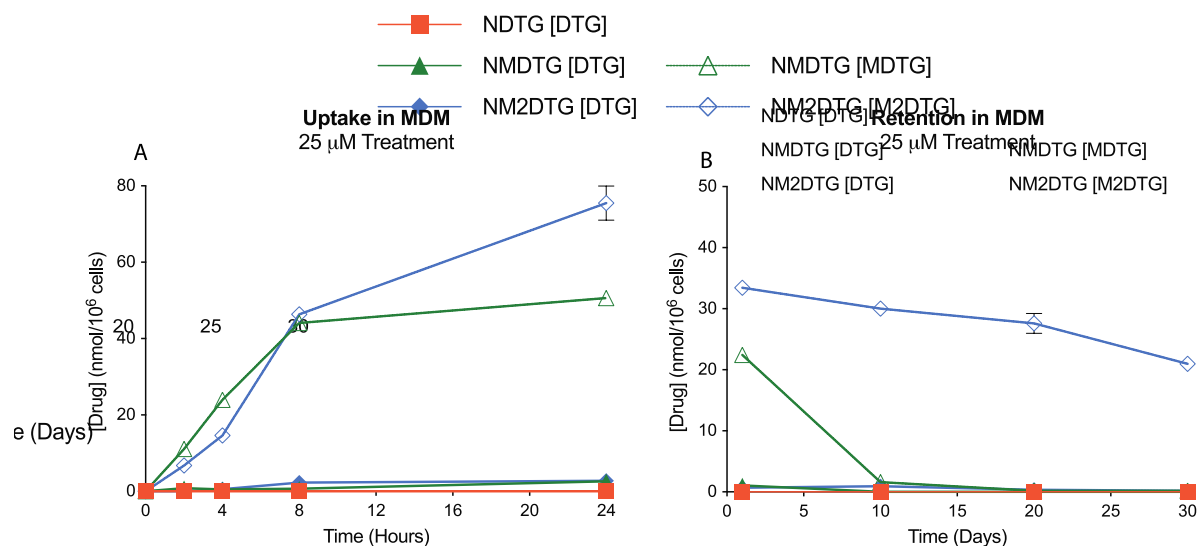


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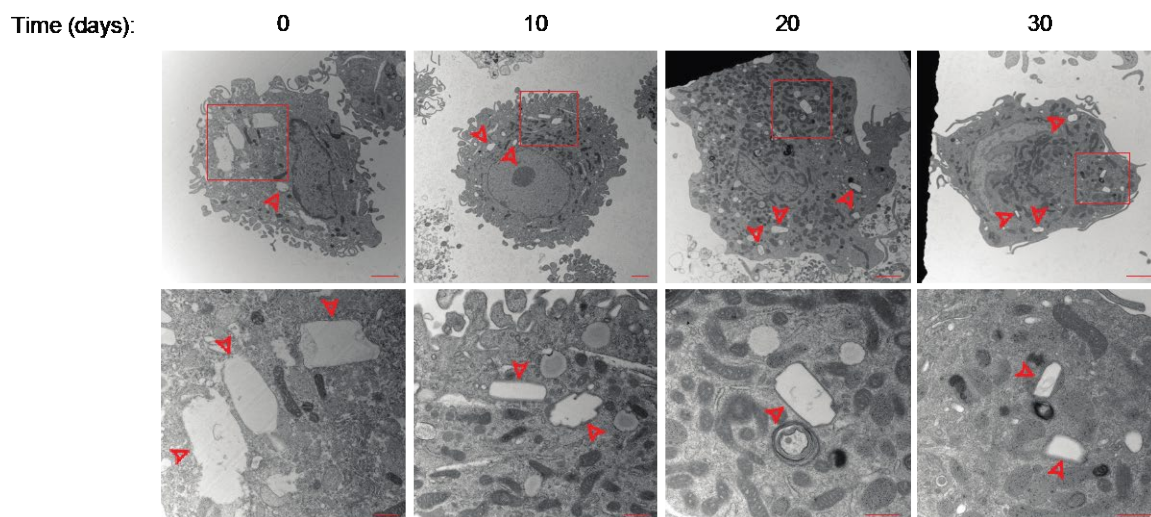
Supplementary figure 9. Nanoformulation stability. (A) Hydrodynamic diameter (size), polydispersity index (PDI), and (B) zeta potential of NDTG and NM2DTG formulations at 4 °C were evaluated over 265 days, as determined by dynamic light scattering (DLS). (C) Drug concentration stability of NDTG and NM2DTG within the nanoformulation. Results are expressed as the mean $\pm$ SEM for N = 3.



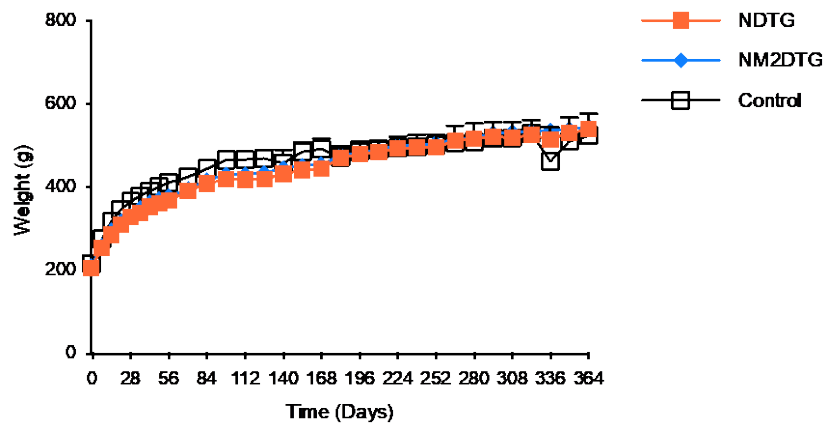
Supplementary figure 10. Cytotoxicity assay. Cell vitality was assessed in MDM by MTT assay after 24 h treatment with (A) DTG or M2DTG over a range of concentrations (0.2 - 100 µM); or (B) NDTG or NM2DTG nanoformulations over a range of concentrations (1.95 - 1000 µM). Results were normalized to untreated control cells.



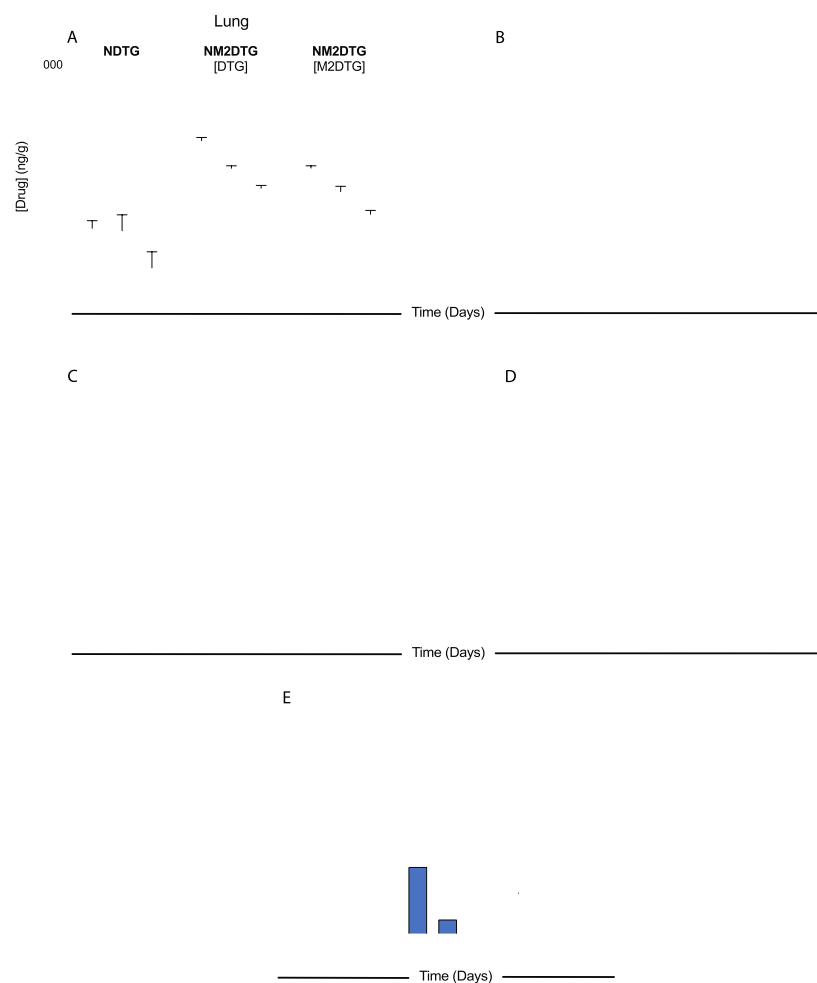
Supplementary figure 11. Pharmacological characterization of NM2DTG in human monocyte-derived macrophages. (A) Drug uptake in MDM was measured over a 24-h period after treatment with drug concentration of 5 μ M. (B) Drug retention in MDM was measured over a 30-day observation period after treatment with drug concentration of 5 μ M for 8 h. Results are expressed as the mean $\pm$ SEM for N = 3 replicates.



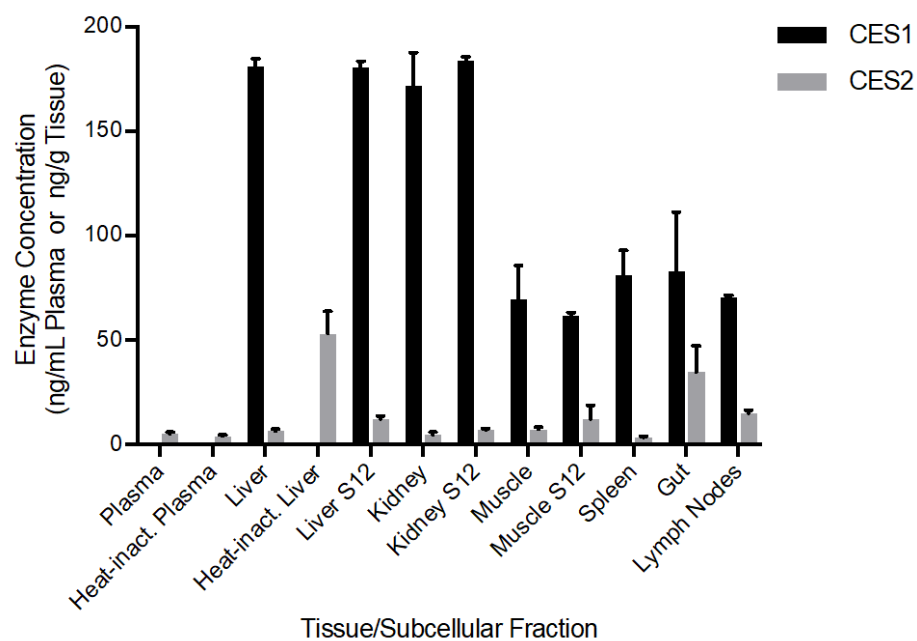
Supplementary figure 12. Transmission electron microscopy (TEM) of MDM treated with NM2DTG *in vitro*. TEM images of MDM after treatment with 25 μ M NM2DTG for 8 h. Cells were collected immediately, 10, 20, or 30 days after treatment. Red arrowheads indicate nanocrystals retained in the cytoplasm. Scale bars - 2 μ m top, 500 nm bottom.



Supplementary figure 13. Toxicity of NM2DTG in male SD rats. Weight of male SD rats administered a single IM dose of NDTG or NM2DTG at 45 mg DTG-eq./kg in the caudal thigh up to day 364.

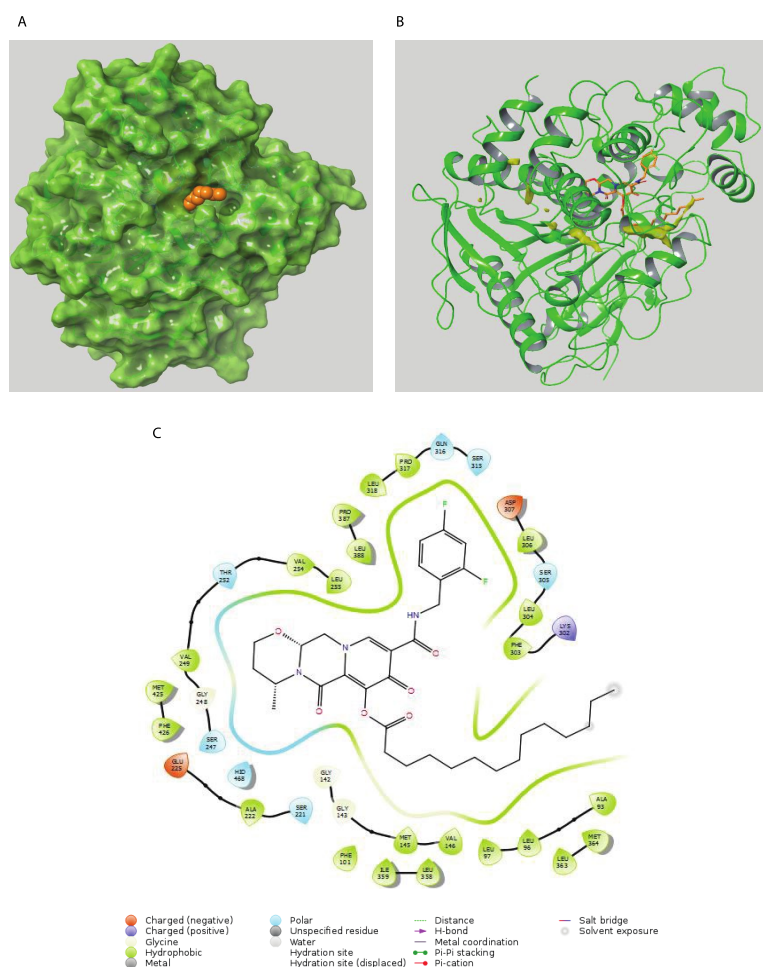


Supplementary figure 14. Biodistribution of NM2DTG in male SD rats. Male SD rats were administered with a single 45 mg DTG-eq./kg IM dose of NDTG or NM2DTG in the caudal thigh. Tissue biodistribution was assessed on days 57, 175, and 364. Parent drug (DTG) and prodrug M2DTG) levels were determined in (A) lung, (B) gut, (C) kidney, (D) rectum, and (E) brain. All drug levels were quantified by UPLC-MS/MS. Results are expressed as mean $\pm$ SEM.

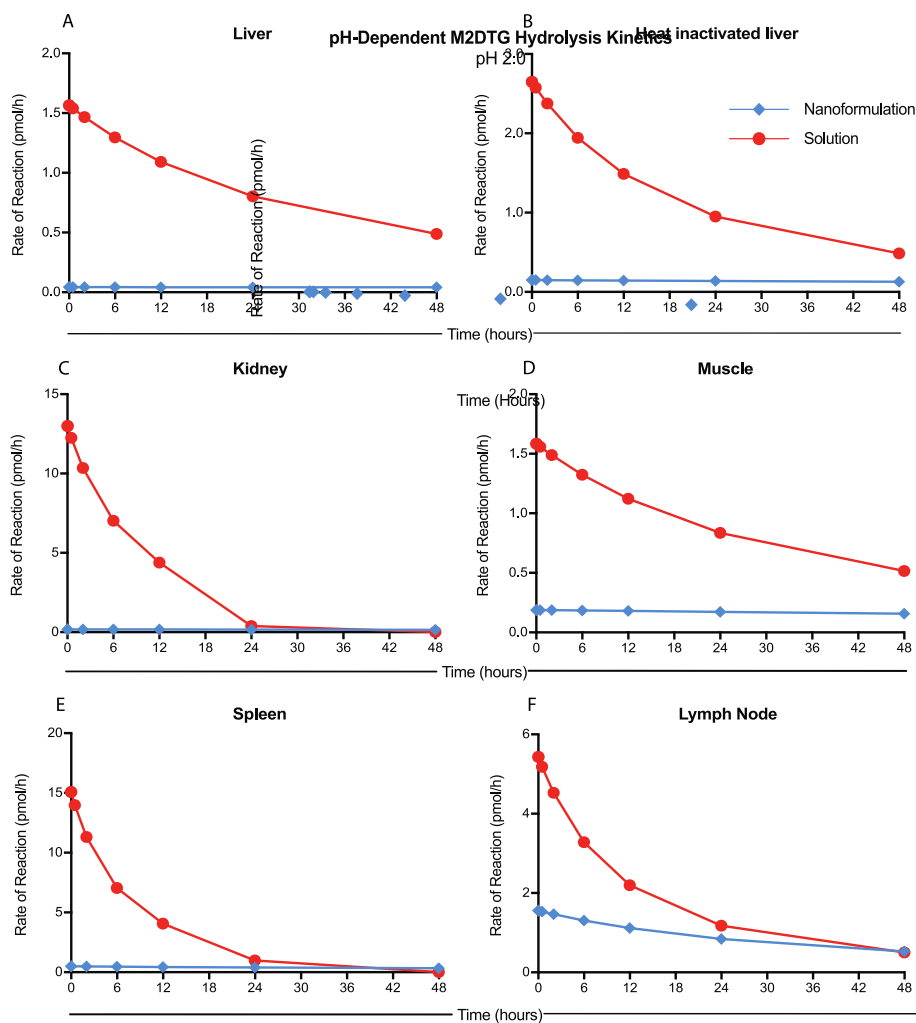


Supplementary figure 15. Carboxylesterase (CES) levels in rat tissue homogenates.

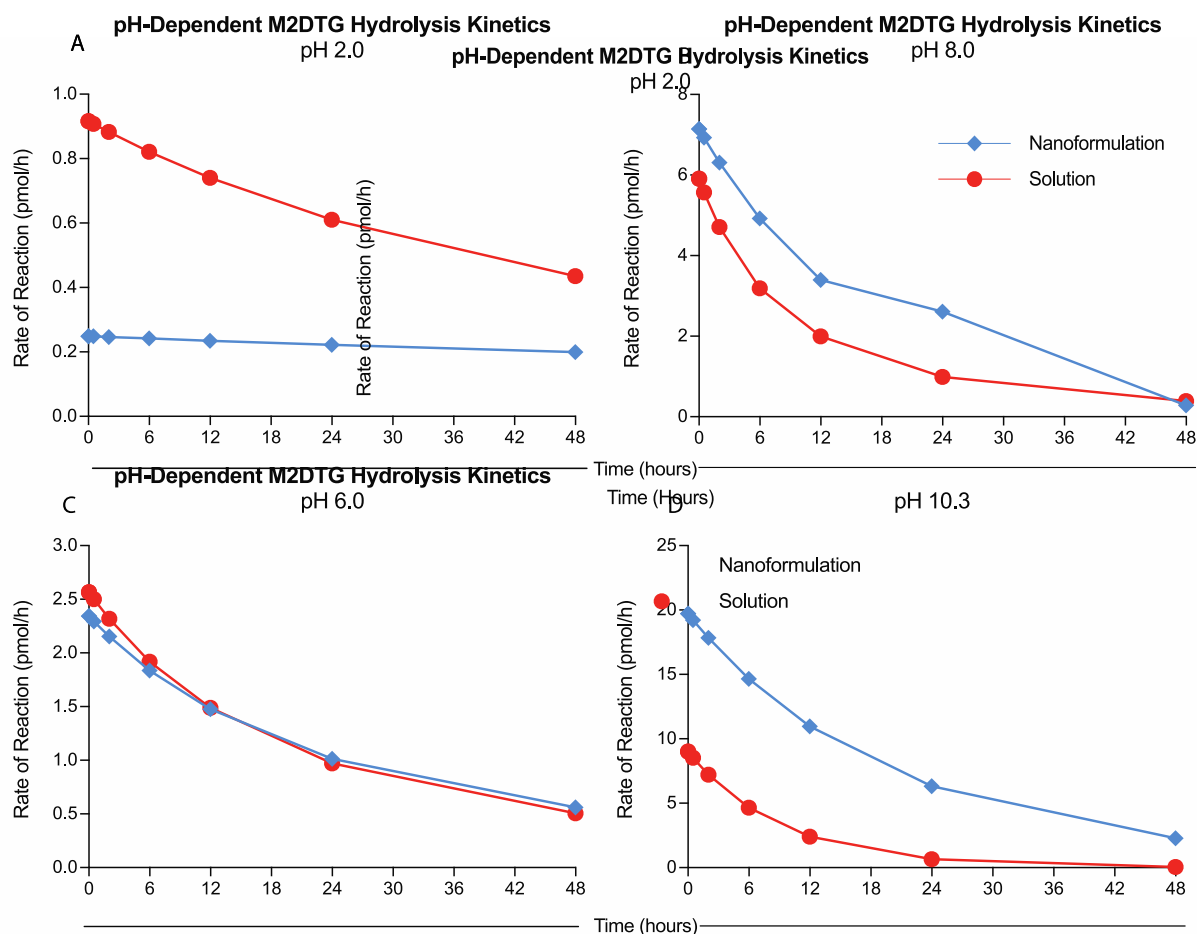
Quantification of CES isoform, CES1 and CES2, concentrations in various rat plasma, tissue homogenates, and subcellular fractions by ELISA.



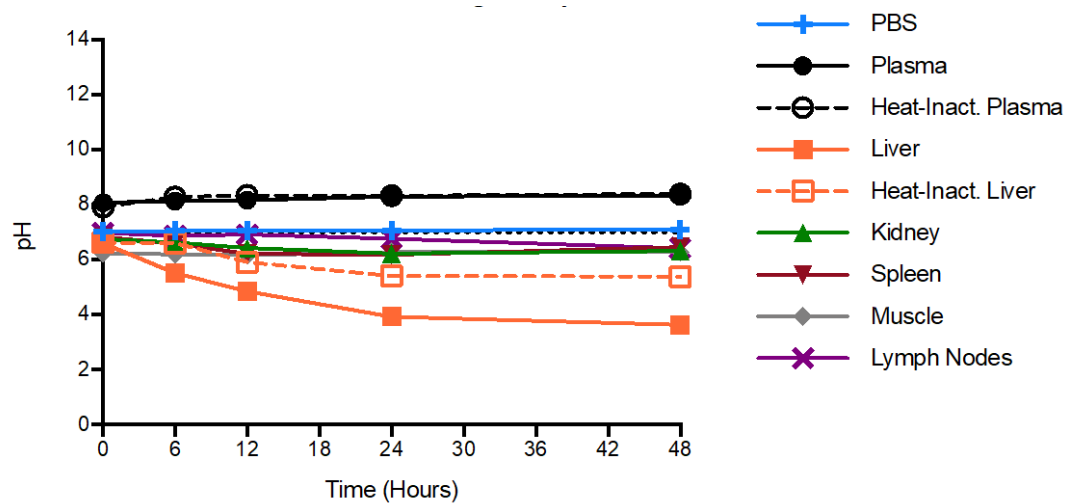
Supplementary figure 16. Molecular docking. (A) Three-dimensional crystal structure of carboxylesterase-1 (CES1; PDB ID: 1YA8) protein. (B) Docking solution of MDTG docked to CES1 (1YA8); MDTG is orange, the hydrophobic patch is yellow, and the protein ribbon is green. (C) Two-dimensional ligand interaction maps of CES1 with MDTG. The interaction of M2DTG with the enzyme could not be mapped due to meager affinity.



Supplementary figure 17. Reaction rate kinetics of the cleavage of M2DTG and NM2DTG in rat tissue homogenates. Rate of reaction for cleavage of M2DTG solution (dissolved in 1% (v/v) methanol) and NM2DTG nanoformulation in (A) liver, (B) heat-inactivated liver, (C) kidney, (D) muscle, (E) spleen, and (F) lymph node.



Supplementary figure 18. Reaction rate kinetics of the hydrolysis of M2DTG and NM2DTG in various pH buffers. Rate of reaction for hydrolysis of M2DTG solution (dissolved in 1% (v/v) methanol) and NM2DTG nanoformulation in buffers of (A) pH 2.0, (B) pH 6.0, (C) pH 8.0, and (D) pH 10.3.



Supplementary figure 19. Changes in rat plasma and tissue homogenate pH over time. The pH of rat plasma and various tissue homogenates was measured after preparation at 0, 6, 12, 24, and 48 h with incubation at 37°C and mechanical agitation. Tissues were homogenized in 5 volumes of ice-cold PBS using a TissueLyser.

Supplementary Table 1. Rat serum chemistry analysis

Serum Chemistry	Control			Day 3			Day 57			Day 175			Day 364		
				NM2DTG			NDTG			NM2DTG			NDTG		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
Albumin (g/dL)	4.2	0.3	4	4.0	0.0	3	4.8	0.2	3	5.2	0.1	3	4.6	0.2	4
Alkaline phosphatase (U/L)	226	24	4	405*	29	3	146	21	3	105	12	3	282	33	4
Alanine aminotransferase (U/L)	58	6	4	73	6	3	32	2	3	29	2	3	61	5	4
Amylase (U/L)	952	23	4	724*	21	3	792	23	3	688**	41	3	897	16	4
Total bilirubin (mg/dL)	0.3	0.0	4	0.2	0.0	3	0.2	0.0	3	0.2	0.0	3	0.2	0.0	4
Blood Urea Nitrogen (mg/dL)	22	1	4	18	1	3	23	1	3	26	1	3	22	1	4
Calcium (mg/dL)	11.7	0.1	4	12.3	0.1	3	9.3	0.4	3	10.0	0.4	3	11.8	0.2	4
Phosphorus (mg/dL)	6.8	0.3	4	11.8**	0.2	3	6.7	0.1	3	6.6	0.8	3	7.6	0.3	4
Creatinine (mg/dL)	0.4	0.1	4	0.4	0.1	3	0.3	0.1	3	0.6	0.0	3	0.4	0.1	4
Glucose (mg/dL)	145	10	4	185	6	3	162	16	3	155	84	3	166	15	4
Sodium (mmol/L)	145	3	4	144	1	3	141	2	3	143	1	3	143	1	4
Potassium (mmol/L)	8.0	0.3	4	6.0*	0.2	3	6.6	0.6	3	6.8	0.5	3	7.9	0.2	4
Total protein (g/dL)	6.9	0.2	4	5.8	0.1	3	6.5	0.3	3	6.8	0.1	3	7.0	0.2	4
Globulin (g/dL)	2.7	0.2	4	1.9	0.1	3	1.8	0.0	3	1.7	0.0	3	2.4	0.1	4

Two-way ANOVA with Geisser-Greenhouse correction and Dunnett's multiple comparisons test (Control vs. Treatment) *P < 0.05, **P < 0.01.

Supplementary Table 2. Mouse PK

PK Parameters	NDTG	NMDTG	NM2DTG	NM3DTG	NM4DTG
λ_z (1/day)	0.1991	0.0177	0.0041	0.0167	0.0304
$t_{1/2}$ (day)	3.48	39.09	167.90	41.51	22.77
AUC _{last} (day*ng/mL)	66561.10	53958.12	59294.17	2596.60	46454.75
AUC _{0-inf} (day*ng/mL)	66562.71	54012.26	76152.92	4183.62	46469.20
AUC % Extrapolation	0.0024	0.1002	22.14	37.93	0.0311
V _z /F (L/kg)	3.40	46.99	143.13	644.16	31.81
CL/F (L/day/kg)	0.68	0.83	0.59	10.76	0.97
MRT _{0-inf}	7.31	34.57	248.60	54.85	52.35

Supplementary Table 3. Rat PK analyses

PK Parameters	NDTG	NM2DTG
λ_z (1/day)	0.1529	0.0064
$t_{1/2}$ (day)	4.53	108.76
AUC _{last} (day*ng/mL)	90740.00	78012.16
AUC _{0-inf} (day*ng/mL)	90746.55	87112.41
AUC % Extrapolation	0.0072	10.45
V_z/F (L/kg)	3.24	82.72
CL/F (L/day/kg)	0.50	0.53
MRT _{0-inf}	5.74	147.36

Supplementary table 4. Rhesus Macaque PK

Rhesus macaque PK analyses (Dose 1)			
PK Parameters	T006	T017	R036
Rs _q adjusted	0.82	0.73	0.73
λ_z (1/day)	0.0088	0.0074	0.0080
$t_{1/2}$ (day)	78.49	93.82	86.32
AUC _{last} (day*ng/mL)	22379.87	23474.00	28804.68
AUC _{0-inf} (day*ng/mL)	26909.12	29429.79	35280.53
AUC % Extrapolation	16.83	20.24	18.36
V _z /F (L/kg)	189.36	206.97	158.84
CL/F (L/day/kg)	1.67	1.53	1.28
MRT _{0-inf}	116.02	126.68	117.47

Rhesus macaque PK analysis (Dose 2)			
PK Parameters	T006	T017	R036
Rsq adjusted	0.97	0.86	0.94
λ_z (1/day)	0.0091	0.0068	0.0106
$t_{1/2}$ (day)	76.01	101.82	65.20
AUC _{last} (day*ng/mL)	24909.05	27322.57	31610.31
AUC _{0-inf} (day*ng/mL)	29734.03	35548.98	37066.38
AUC % Extrapolation	16.23	23.14	14.72
V _z /F (L/kg)	165.96	185.95	114.20
CL/F (L/day/kg)	1.51	1.27	1.21
MRT _{0-inf}	102.26	141.76	60.20