Supporting Information

A Benzyl Ether-Linked Glucuronide Derivative of 10-Hydroxycamptothecin Designed for Selective Camptothecin-Based Anticancer Therapy

Yu-Ling Leu,*[,†,‖] Chien-Shu Chen,[‡,‖] Yih-Jang Wu,[‡] and Ji-Wang Chern*[,‡]

Department of Pharmacy, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan, and School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan

Table of Contents:

Elemental Analysis Data

Compound	Formula		C%	H%	N%
9	$C_{20}H_{21}NO_{13}$	Calcd	49.69	4.38	2.90
		Found	49.63	4.37	2.73
10	$C_{20}H_{23}NO_{13}$	Calcd	49.49	4.78	2.89
		Found	49.43	4.72	2.75
11	$C_{21}H_{25}NO_{15}S$	Calcd	44.76	4.47	2.49
		Found	44.83	4.42	2.36
12	$C_{40}H_{37}N_3O_{17}\cdot H_2O$	Calcd	56.54	4.63	4.95
		Found	56.65	4.32	4.86
13	$C_{34}H_{31}N_3O_{14}\cdot CH_3OH$	Calcd	56.99	4.78	5.70
		Found	57.19	4.97	5.21
7	$C_{33}H_{29}N_3O_{14}\cdot 10H_2O$	Calcd	45.47	5.67	4.82
		Found	45.34	4.82	4.64

HPLC Analysis of Compound 7

(A) Conditions

Sample: compound **7** (0.1 mg/mL)

Column: LiChroCART RP-18, 250 x 4 mm, 5 µm particle size

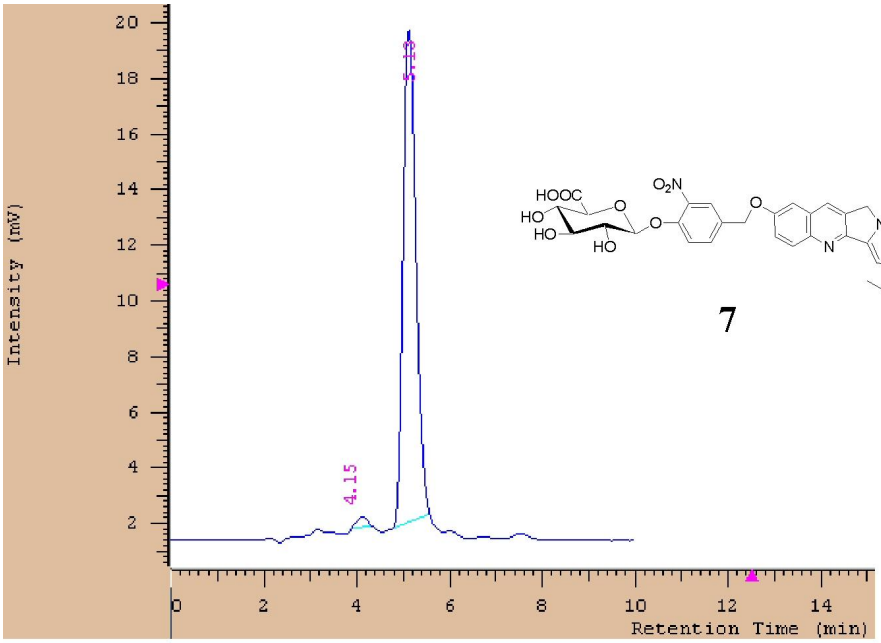
Mobile phase: 60% MeOH and 0.1% phosphoric acid solution

Flow rate: 1 mL/min

UV-detector: Hitachi L-4000 (254 nm)

(B) Purity of compound **7** by HPLC: 97.4% (retention time 5.13 min)

(C) HPLC trace for compound **7**

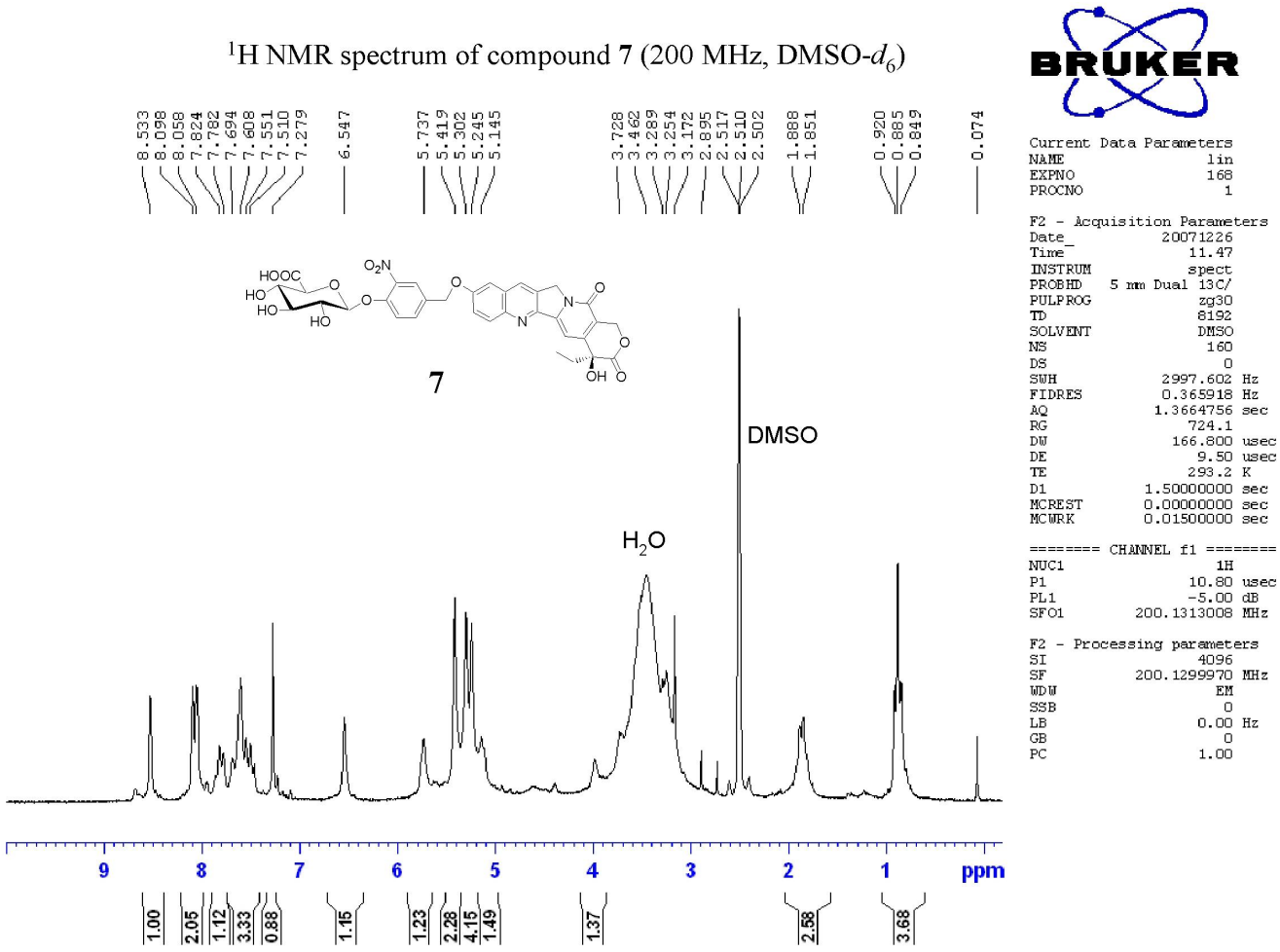


Peak Quantitation: AREA
Calculation Method: AREA%

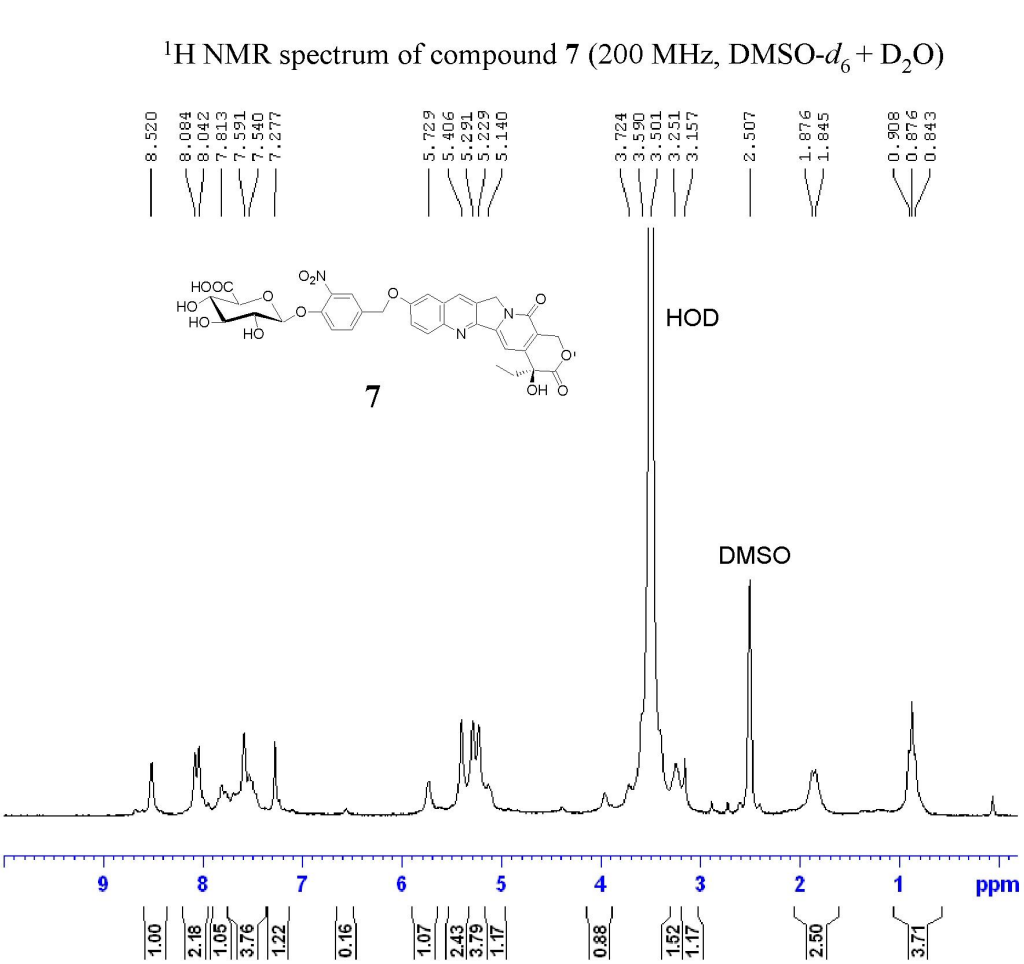
No.	RT	Area	Conc 1	BC
1	3.18	1617	0.474	BB
2	4.15	7106	2.084	BB
3	5.13	332271	97.441	BB
		340994	100.000	

^{1}H NMR Spectra of Compound 7

(A) ^{1}H NMR (200 MHz, DMSO-d_6)



(B) ^{1}H NMR (200 MHz, DMSO-d_6 + D_2O)



```
Current Data Parameters
NAME                  lin
EXPNO                 171
PROCNO                  1

F2 - Acquisition Parameters
Date_            20071226
Time                16.18
INSTRUM             spect
PROBHD    5 mm Dual 13C/
PULPROG             zg30
TD                  8192
SOLVENT             DMSO
NS                   128
DS                     0
SWH             2997.602 Hz
FIDRES          0.365918 Hz
AQ              1.3664756 sec
RG                   512
DW                166.800 usec
DE                  9.50 usec
TE                 293.2 K
D1              1.50000000 sec
MCREST          0.00000000 sec
MCWRK           0.01500000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 10.80 usec
PL1                -5.00 dB
SFO1          200.1313008 MHz

F2 - Processing parameters
SI                  4096
SF            200.1299977 MHz
WDW                   EM
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  1.00
```

Molecular Modeling Studies

1. Preparation of Protein and Ligands. The 2.6 Å resolution X-ray crystal structure of human *β*-D-glucuronidase (PDB code 1BHG,[1] the Protein Data Bank: http://www.rcsb.org/pdb) was retrieved for docking studies. The B-chain of protein and ligand molecules were removed from the crystal structure. To the remaining protein structure were added hydrogen atoms, and then the protein atoms were assigned with Kollman All-atom partial charges. The 3-D structures of the substrate molecules were built and optimized by energy minimization using the Tripos force field in the software package SYBYL 7.1 (Tripos, Inc., St. Louis, MO). The partial atomic charges were calculated using the Gasteiger-Marsili method.[2]

2. Building a Model of *β*-Glucuronidase in Complex with the Substrate *p*-Nitrophenyl *β*-Glucuronide. To build a protein model whose active site exists in the probable substrate-bound conformation, the known substrate *p*-nitrophenyl *β*-glucuronide was first docked into the active site of *β*-glucuronidase using the docking program GOLD 2.0.[3] The docking site was defined as all atoms within 12 Å of a specified central point (x,y,z coordinates: 89.37, 89.18, 90.61). For each of the 50 independent genetic algorithm (GA) runs, a maximum number of 100000 GA operations were performed on a single population of 100 individuals. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, respectively. Default cutoff values of 2.5 Å for hydrogen bonds and 4.0 Å for van der Waals distance were used. The GoldScore scoring function was employed. From the docking results, a high-scoring docked pose of *p*-nitrophenyl *β*-glucuronide having the glucuronyl moiety oriented properly toward the key residues Glu451 and Glu540 involved in the enzyme catalysis reaction[4] was chosen to represent its most probable binding mode. The obtained docking model was further subjected to energy minimization for refinement. Prior to energy minimization, the Glu451 residue in the active site was assigned a protonated form that was consistent with its role in acting as a proton donor during catalysis. In addition, a structural subset was specified as the ligand and the amino acid residues within a 10 Å radius of the ligand. The complex structure was then refined through a two-step minimization, allowing (i) only the ligand and the side chains of

the subset to relax and then (ii) only all the atoms of the subset to relax. The energy minimization calculations were performed in SYBYL 7.1 using the Tripos force field, the Powell method, a distance-dependent dielectric constant of 1*r*, and a nonbonded cutoff of 8 Å. The minimization was terminated when the energy gradient convergence criterion of 0.01 kcal/(mol·Å) was reached. Figure S1 shows the refined docking model of *p*-nitrophenyl *β*-glucuronide with *β*-glucuronidase.

3. Prediction of the Complex Structures of 7 and 6 with *β*-Glucuronidase. The modeled complex structure of *β*-glucuronidase with the substrate *p*-nitrophenyl *β*-glucuronide was used for the prediction of a favorable binding mode of **7** or **6** to *β*-glucuronidase by docking and then geometry optimization as described above, with the exception of the defined docking region around the central point (x,y,z coordinates: 79.2, 86.5, 94.1) within a radius of 18 Å. The predicted binding models of **7** and **6** with *β*-glucuronidase (Figures S2 and S3, respectively) were further scored using the programs AutoDock,[5] SCORE,[6] and LigScore[7] to estimate the binding affinity of the substrate. In the present study, all computer simulations were performed on the Silicon Graphics Origin 3800 system and an Octane workstation.

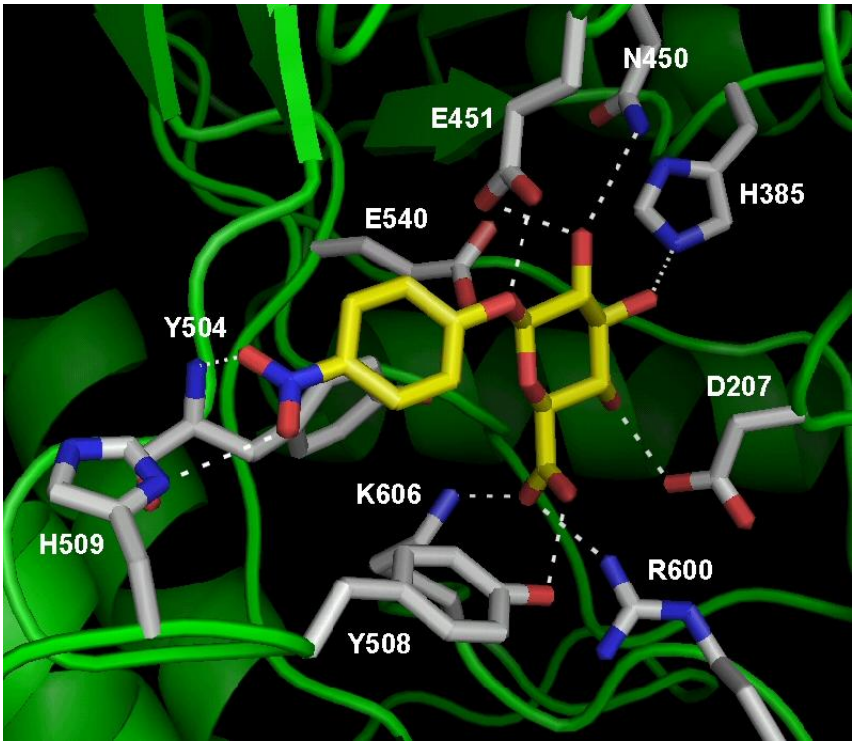


Figure S1. Predicted binding model of *p*-nitrophenyl *β*-glucuronide in complex with human *β*-glucuronidase. The dashed lines indicate hydrogen-bonding interactions.

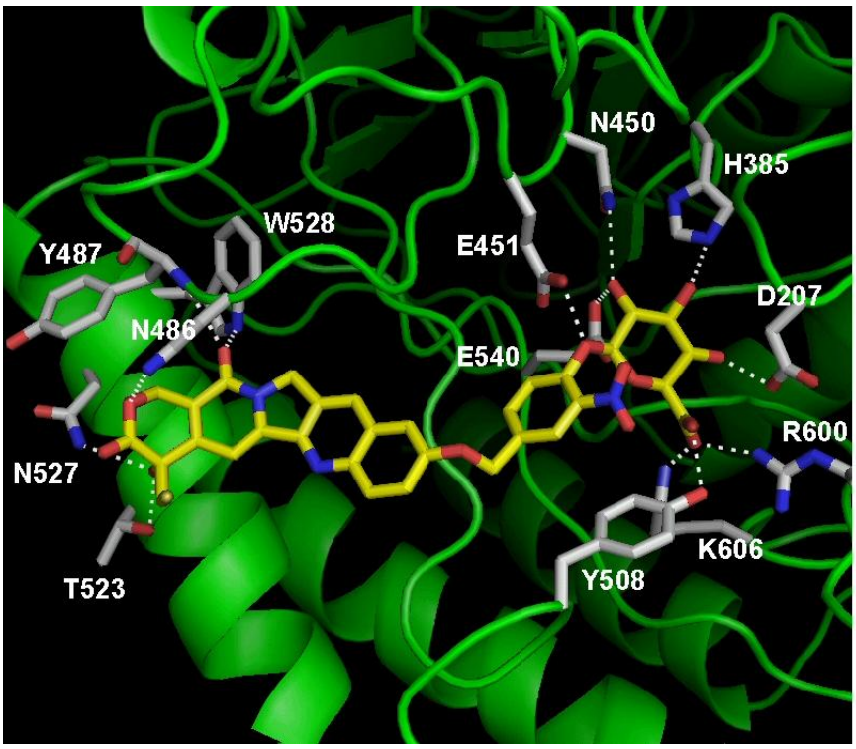


Figure S2. Predicted binding model of **7** in complex with human *β*-glucuronidase. The dashed lines indicate hydrogen-bonding interactions.

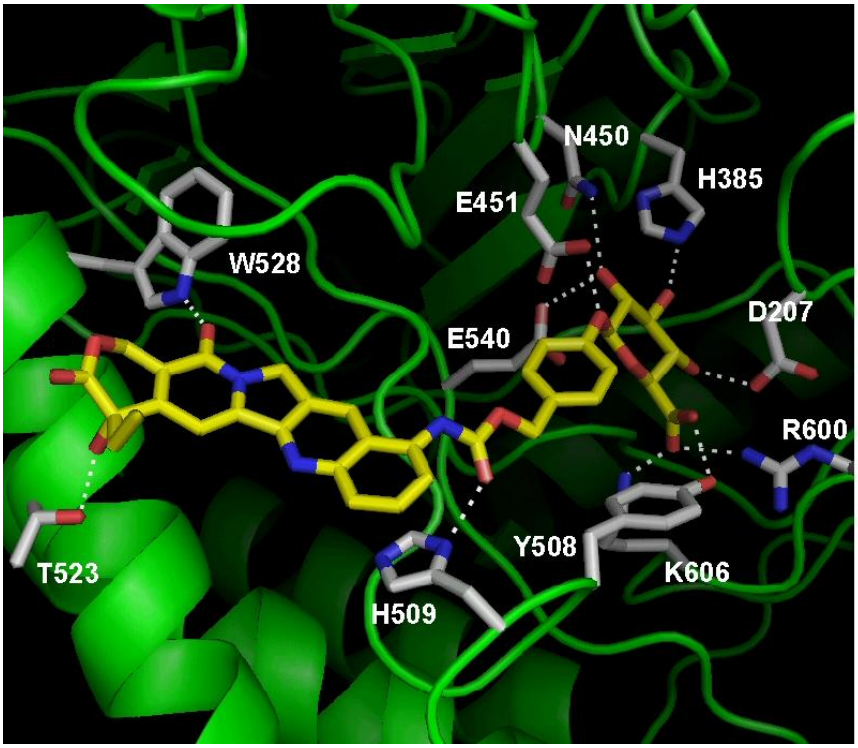


Figure S3. Predicted binding model of **6** in complex with human *β*-glucuronidase. The dashed lines indicate hydrogen-bonding interactions.

References

(1) Jain, S.; Drendel, W. B.; Chen, Z. W.; Mathews, F. S.; Sly, W. S.; Grubb, J. H. Structure of human β-glucuronidase reveals candidate lysosomal targeting and active-site motifs. *Nat. Struct. Biol.* **1996**, *3*, 375–381.

(2) Gasteiger, J.; Marsili, M. Iterative partial equalization of orbital electronegativity–a rapid access to atomic charges. *Tetrahedron* **1980**, *36*, 3219–3228.

(3) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol*. **1997**, *267*, 727–748.

(4) Islam, M. R.; Tomatsu, S.; Shah, G. N.; Grubb, J. H.; Jain, S.; Sly, W. S. Active site residues of human β-glucuronidase. Evidence for Glu^{540} as the nucleophile and Glu^{451} as the acid-base residue. *J. Biol. Chem.* **1999**, *274*, 23451–23455.

(5) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. *J. Comput. Chem.* **1998**, *19*, 1639–1662.

(6) Wang, R.; Liu, L.; Lai, L.; Tang, Y. SCORE: A new empirical method for estimating the binding affinity of a protein-ligand complex. *J. Mol. Model.* **1998**, *4*, 379–394.

(7) Venkatachalam, C. M.; Jiang, X.; Oldfield, T.; Waldman, M. LigandFit: A novel method for the shape-directed rapid docking of ligands to protein active sites. *J. Mol. Graph. Model.* **2002**, *21*, 289–307.