Supporting Information

Glucuronidation of [6]-shogaol, [8]-shogaol and [10]-shogaol by human tissues and expressed UGT enzymes: identification of UGT2B7 as the major contributor

Liangliang He,[†a] Jinjin Xu,[†a] Qi Wang,[ab] Yezi Zhang,[a] Zifei Qin,*[ac] Yang Yu,[a,d] Zhengming Qian,[b] Zhihong Yao*[ad] and Xinsheng Yao[a,d]

[a]College of Pharmacy, Jinan University, Guangzhou 510632, P.R. China;

[b]Key Laboratory of State Administration of Traditional Chinese Medicine, Sunshine 瑞

[c]Department of Pharmacy, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, P. R. China;

[d]Guangdong Provincial Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, China;

[†]These authors contributed equally to this work.

*Correspondence authors.

Associate Prof. Zhihong Yao, Tel: (086) 20-85221767, Fax: (086) 20-85221559,

E-mail: yaozhihong_jnu@163.com; yaozhihong.jnu@gmail.com;

Dr. Zifei Qin, Tel: (086) 20-85221707, E-mail: qzf1989@163.com

Figure Caption

Fig. S1 Kinetic profiles for glucuronidation of shogaols by pooled human liver microsomes (HLM) and pooled human intestine microsomes (HIM). Kinetic profile for glucuronidation of [6]-shogaol by HLM (A). Kinetic profile for glucuronidation of [8]-shogaol by HLM (B). Kinetic profile for glucuronidation of [10]-shogaol by HLM (C). Kinetic profile for glucuronidation of [6]-shogaol by HIM (D). Kinetic profile for glucuronidation of [8]-shogaol by HIM (E). Kinetic profile for glucuronidation of [10]-shogaol by HIM (F).

Fig. S2 Kinetic profiles for glucuronidation of [6]-shogaol by expressed UDP-glucuronosyltransferase enzymes. Kinetic profile for glucuronidation of [6]-shogaol by expressed UGT1A1 (A). Kinetic profile for glucuronidation of [6]-shogaol by expressed UGT1A6 (B). Kinetic profile for glucuronidation of [6]-shogaol by expressed UGT1A10 (C). Kinetic profile for glucuronidation of [6]-shogaol by expressed UGT2B7 (D). Kinetic profile for glucuronidation of [6]-shogaol by expressed UGT2B15 (E).

Fig. S3 Kinetic profiles for glucuronidation of [8]-shogaol by expressed UDP-glucuronosyltransferase enzymes. Kinetic profile for glucuronidation of [8]-shogaol by expressed UGT1A1 (A). Kinetic profile for glucuronidation of [8]-shogaol by expressed UGT1A8 (B). Kinetic profile for glucuronidation of [8]-shogaol by expressed UGT1A10 (C). Kinetic profile for glucuronidation of [8]-shogaol by expressed UGT2B4 (D). Kinetic profile for glucuronidation of [8]-shogaol by expressed UGT2B7 (E).

Fig. S4 Kinetic profiles for glucuronidation of [10]-shogaol by expressed UDP-glucuronosyltransferase enzymes. Kinetic profile for glucuronidation of [10]-shogaol by expressed UGT1A7 (A). Kinetic profile for glucuronidation of [10]-shogaol by expressed UGT1A8 (B). Kinetic profile for glucuronidation of [10]-shogaol by expressed UGT1A10 (C). Kinetic profile for glucuronidation of [10]-shogaol by expressed UGT2B4 (D). Kinetic profile for glucuronidation of [10]-shogaol by expressed UGT2B7 (E).

Fig. S5 (a) Correlation analysis between [6]-shogaol 4'-*O*-glucuronidation and AZT glucuronidation in a bank of individual human liver microsomes (n = 9). (b) Correlation analysis between [8]-shogaol 4'-*O*-glucuronidation and AZT glucuronidation in a bank of individual human liver microsomes (n = 9). (c) Correlation analysis between [10]-shogaol 4'-*O*-glucuronidation and AZT glucuronidation in a bank of individual human liver microsomes (n = 9).

Fig. S6 The HRESIMS spectrum (S6-1) and 13C-NMR spectrum (S6-2) of reference standard of [6]-shogaol.

Fig. S7 The HRESIMS spectrum (S6-1) and 13C-NMR spectrum (S6-2) of reference standard of [6]-shogaol.

Fig. S8 The HRESIMS spectrum (S6-1) and 13C-NMR spectrum (S6-2) of reference standard of [6]-shogaol.

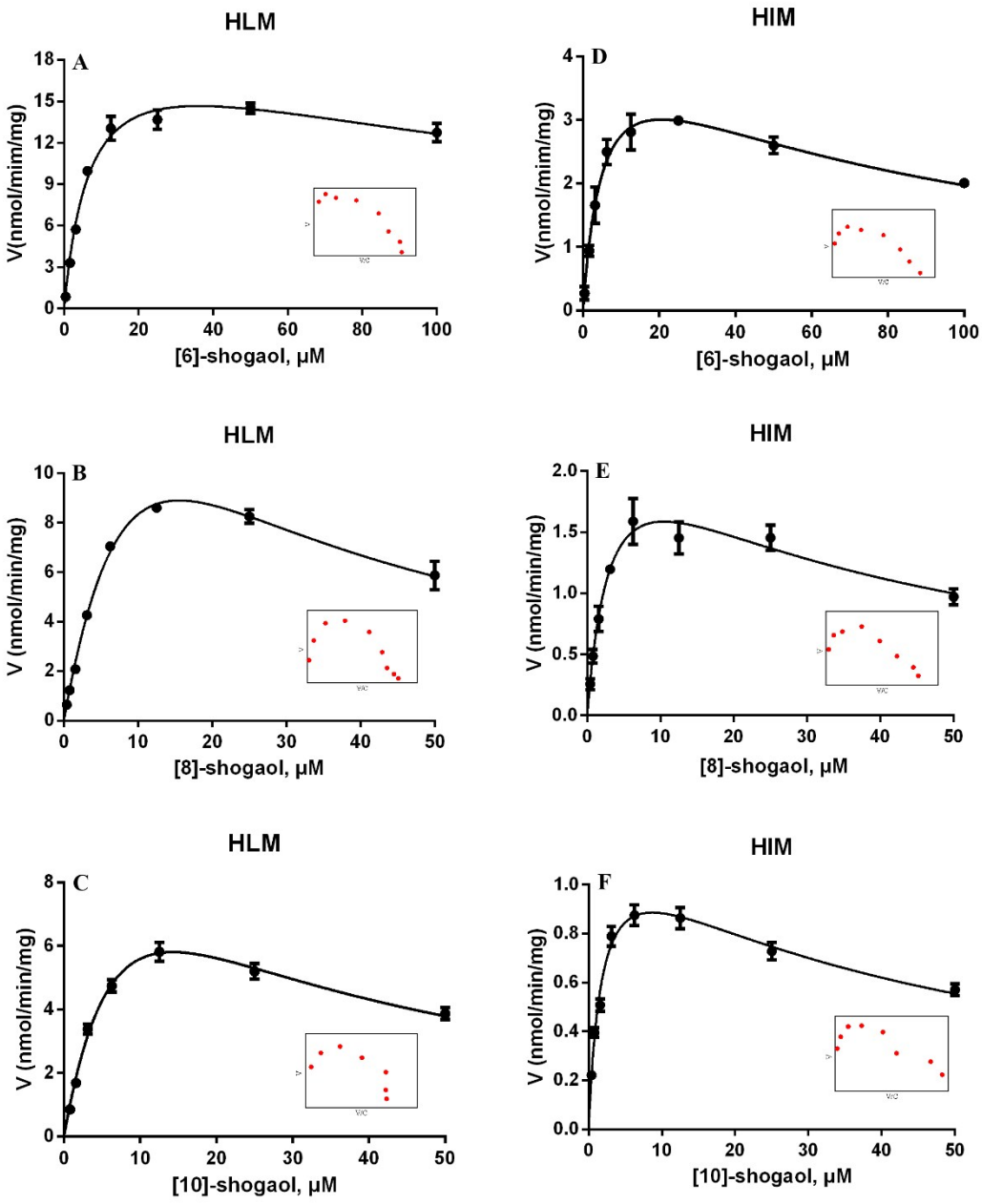

HLM
A
V(nmol/mim/mg)
[6]-shogaol, μM
HIM
D
V(nmol/mim/mg)
[6]-shogaol, μM
HLM
B
V (nmol/min/mg)
[8]-shogaol, μM
HIM
E
V (nmol/min/mg)
[8]-shogaol, μM
HLM
C
V (nmol/min/mg)
[10]-shogaol, μM
HIM
F
V (nmol/min/mg)
[10]-shogaol, μM

Fig. S1

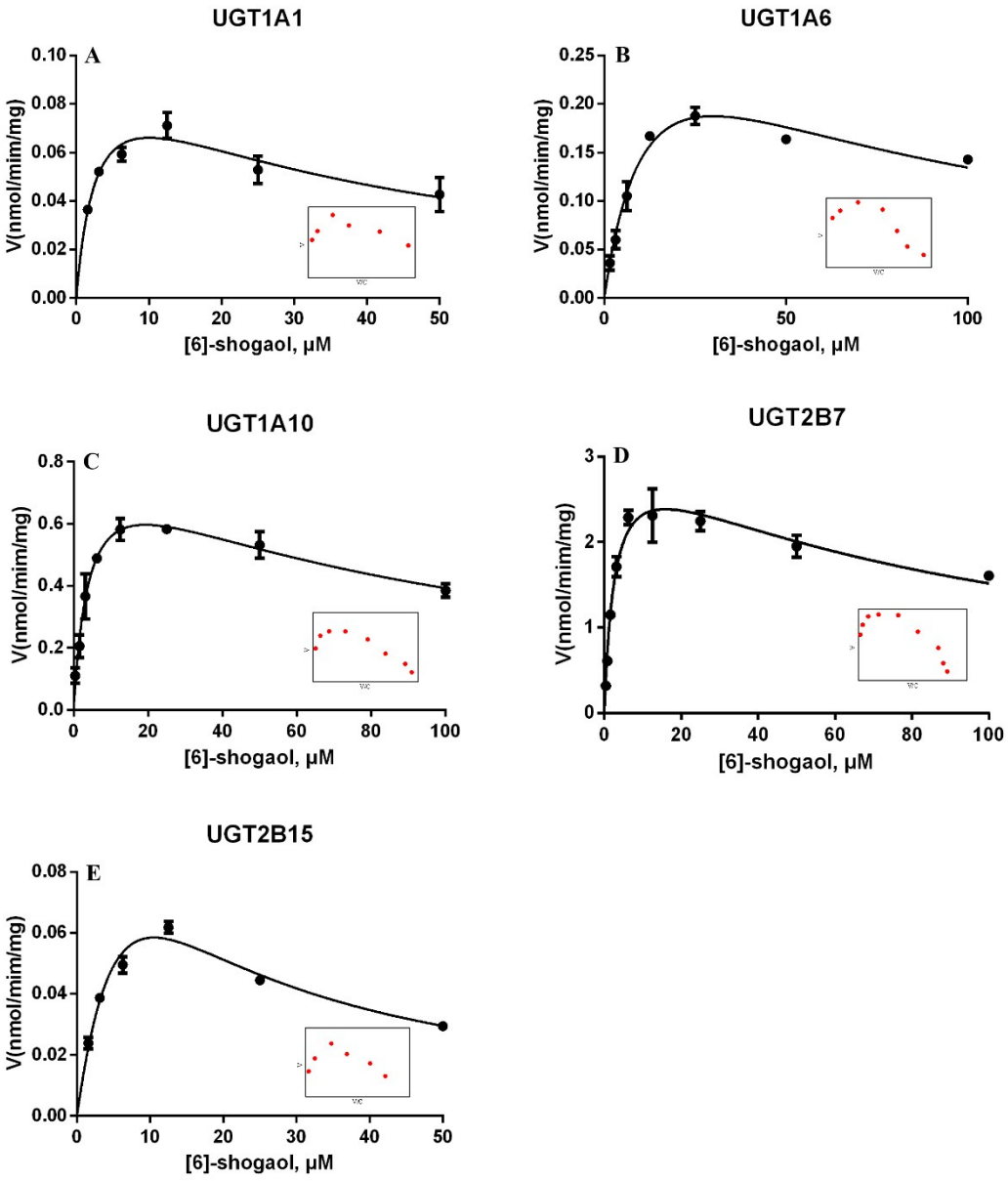

UGT1A1
A
V(nmol/mim/mg)
[6]-shogaol, μM
UGT1A6
B
V(nmol/mim/mg)
[6]-shogaol, μM
UGT1A10
C
V(nmol/mim/mg)
[6]-shogaol, μM
UGT2B7
D
V(nmol/mim/mg)
[6]-shogaol, μM
UGT2B15
E
V(nmol/mim/mg)
[6]-shogaol, μM

Fig. S2

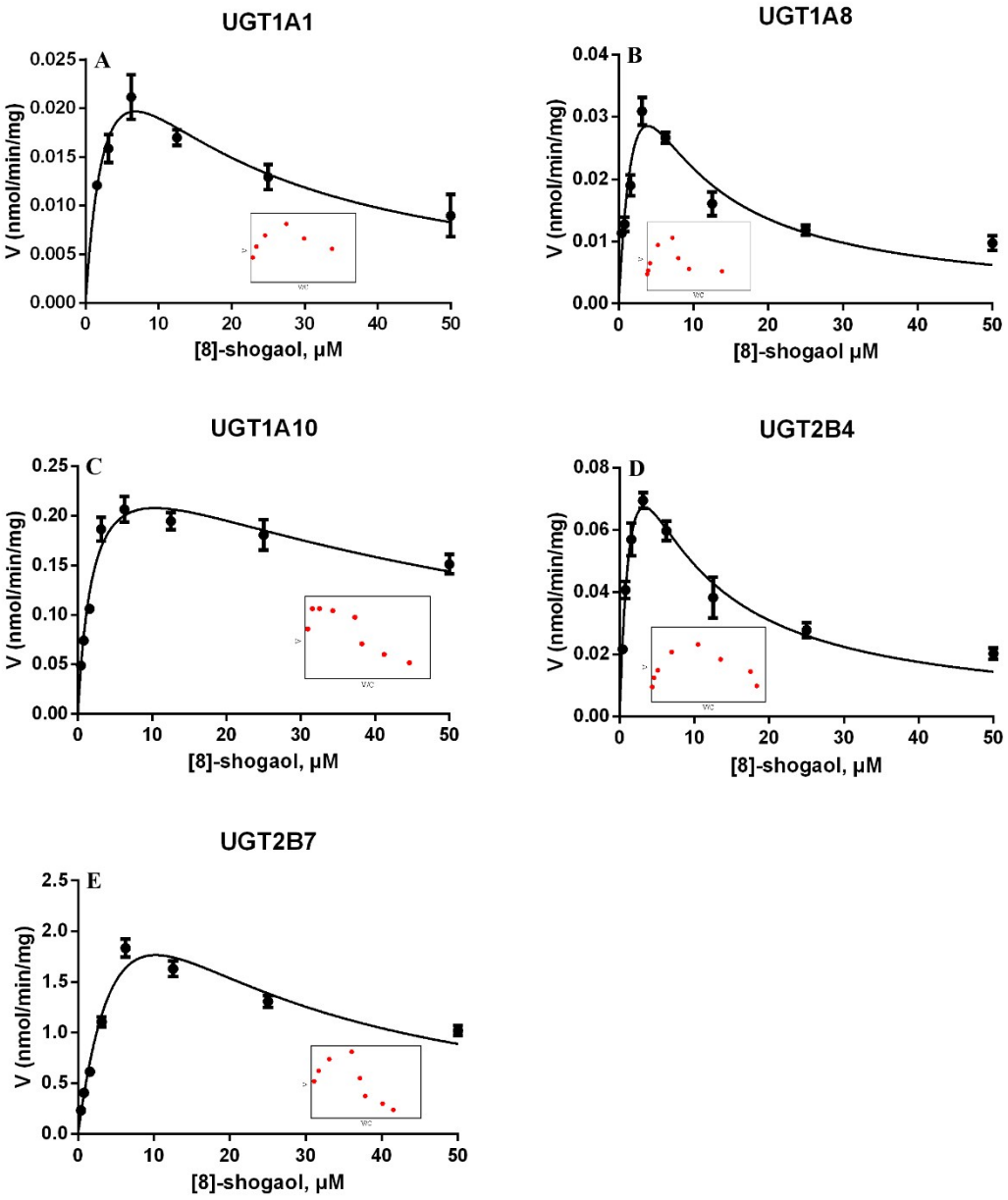

UGT1A1
A
V (nmol/min/mg)
[8]-shogaol, μM
UGT1A8
B
V (nmol/min/mg)
[8]-shogaol μM
UGT1A10
C
V (nmol/min/mg)
[8]-shogaol, μM
UGT2B4
D
V (nmol/min/mg)
[8]-shogaol, μM
UGT2B7
E
V (nmol/min/mg)
[8]-shogaol, μM

Fig. S3

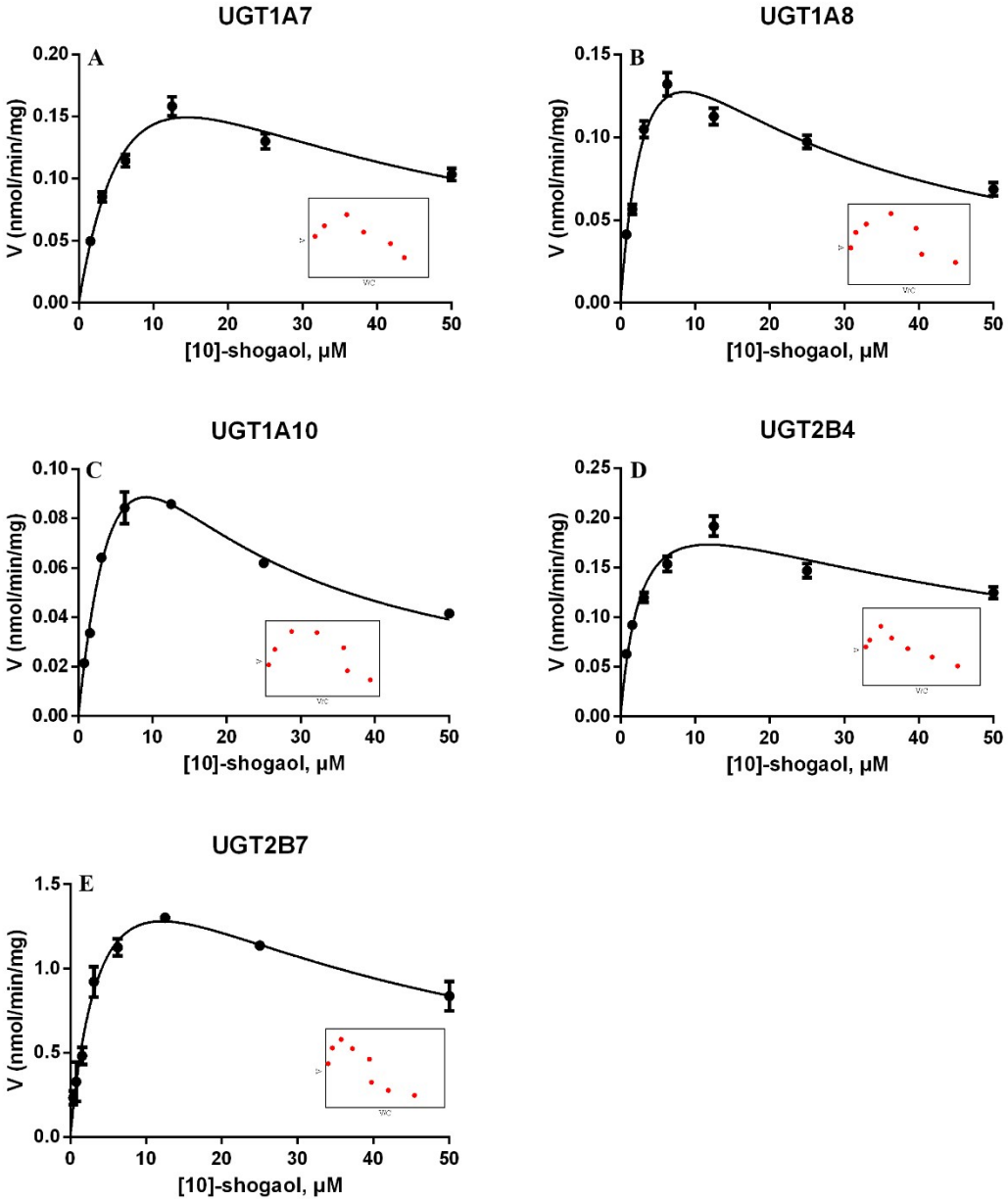

UGT1A7
A
V (nmol/min/mg)
[10]-shogaol, μM
UGT1A8
B
V (nmol/min/mg)
[10]-shogaol, μM
UGT1A10
C
V (nmol/min/mg)
[10]-shogaol, μM
UGT2B4
D
V (nmol/min/mg)
[10]-shogaol, μM
UGT2B7
E
V (nmol/min/mg)
[10]-shogaol, μM

Fig. S4

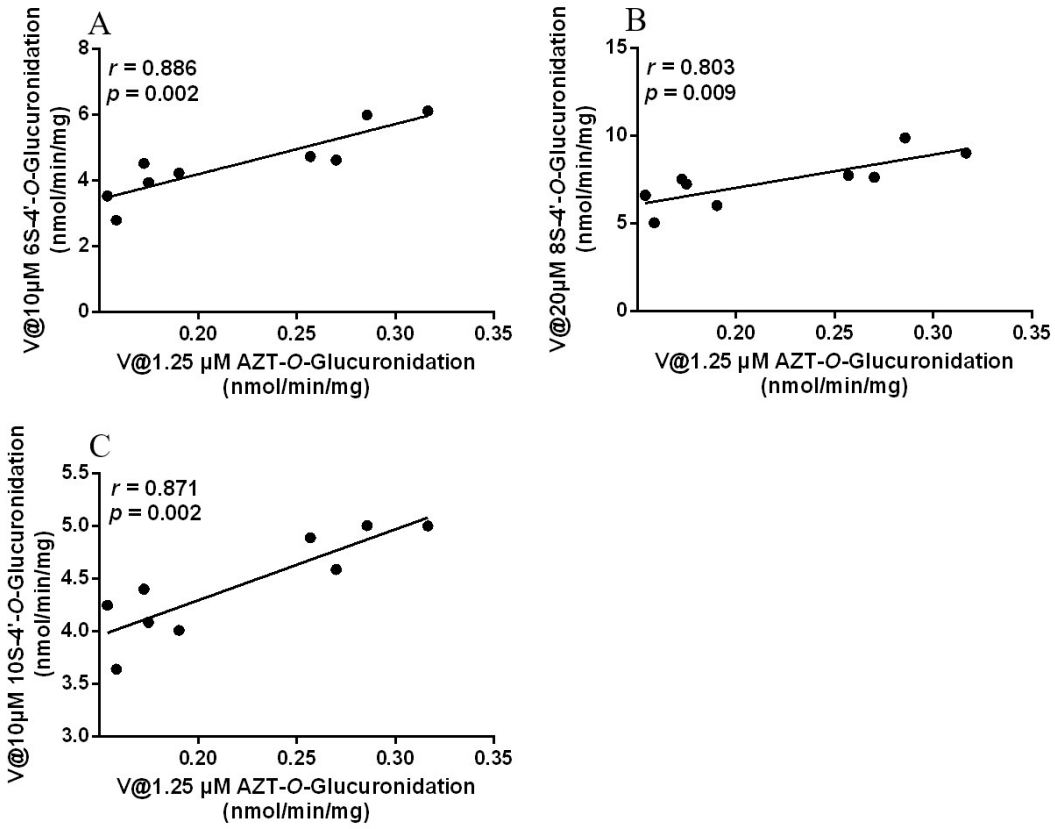

A
r = 0.886
p = 0.002
V@10μM 6S-4'-O-Glucuronidation (nmol/min/mg)
V@1.25 μM AZT-O-Glucuronidation (nmol/min/mg)
B
r = 0.803
p = 0.009
V@20μM 8S-4'-O-Glucuronidation (nmol/min/mg)
V@1.25 μM AZT-O-Glucuronidation (nmol/min/mg)
C
r = 0.871
p = 0.002
V@10μM 10S-4'-O-Glucuronidation (nmol/min/mg)
V@1.25 μM AZT-O-Glucuronidation (nmol/min/mg)

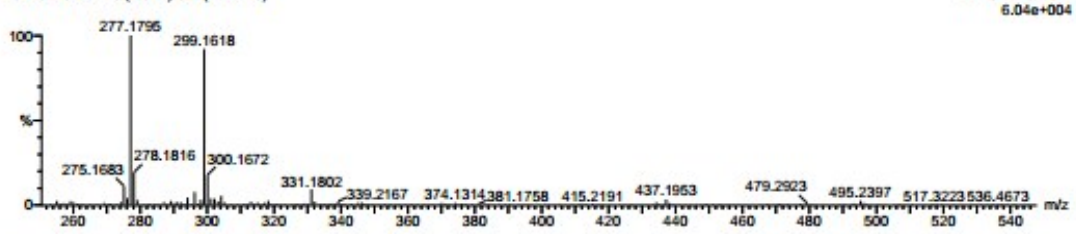
Fig. S5

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
86 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-100 O: 0-50 23Na: 0-1
G3A1
20170710-16 225 (1.817) Cm (221:228)

1: TOF MS ES+
6.04e+004



Minimum:				-1.5				
Maximum:		10.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
277.1795	277.1804	-0.9	-3.2	5.5	11.5	n/a	n/a	C17 H25 O3

Fig. S6-1

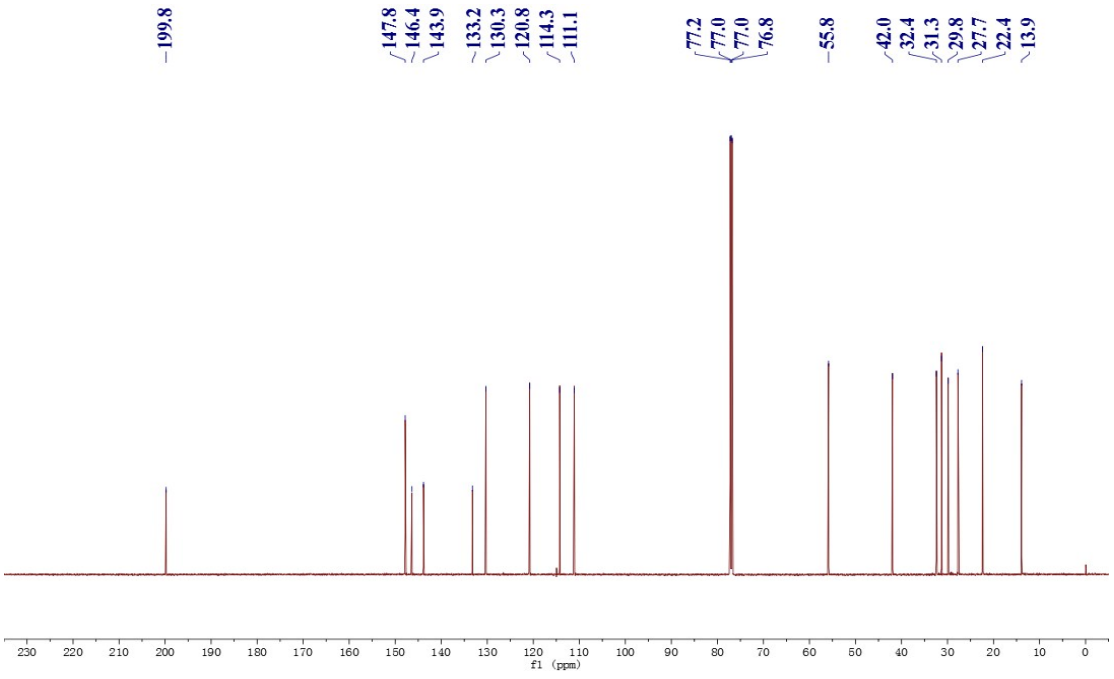


Fig. S6-2

Fig. S6

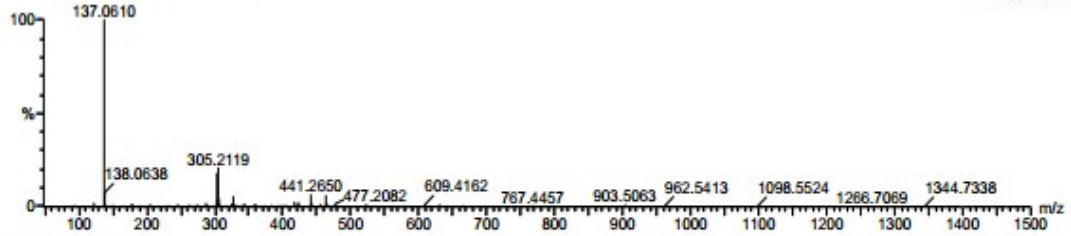
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
101 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 O: 0-50 Na: 0-1

G7F3
2017110618 255 (2.053) Cm (252:256)

1: TOF MS ES+
8.99e+005



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
305.2119	305.2117	0.2	0.7	5.5	28.7	n/a	n/a	C19 H29 O3

Fig. S7-1

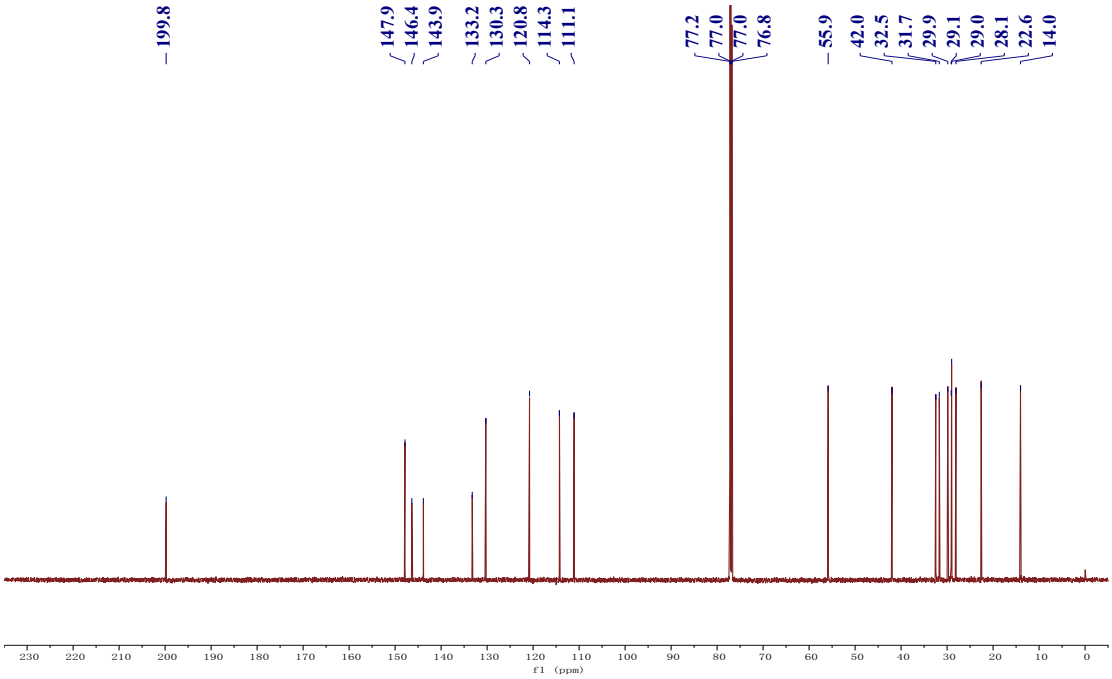


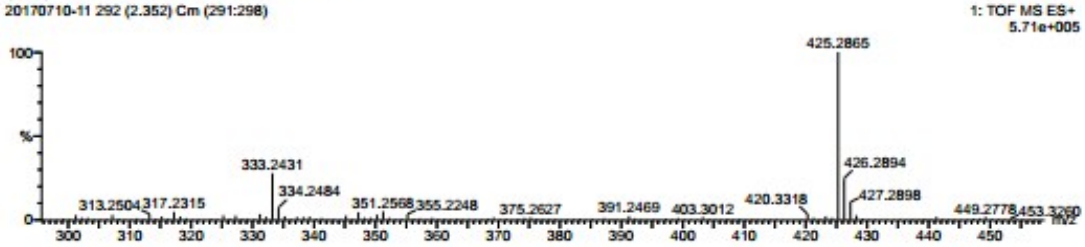
Fig. S7-2

Fig. S7

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
116 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-100 O: 0-50 23Na: 0-1
G11B5
20170710-11 292 (2.352) Cm (291:298)



Minimum:				-1.5				
Maximum:		10.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
333.2431	333.2430	0.1	0.3	5.5	24.3	n/a	n/a	C21 H33 O3

Fig. S8-1

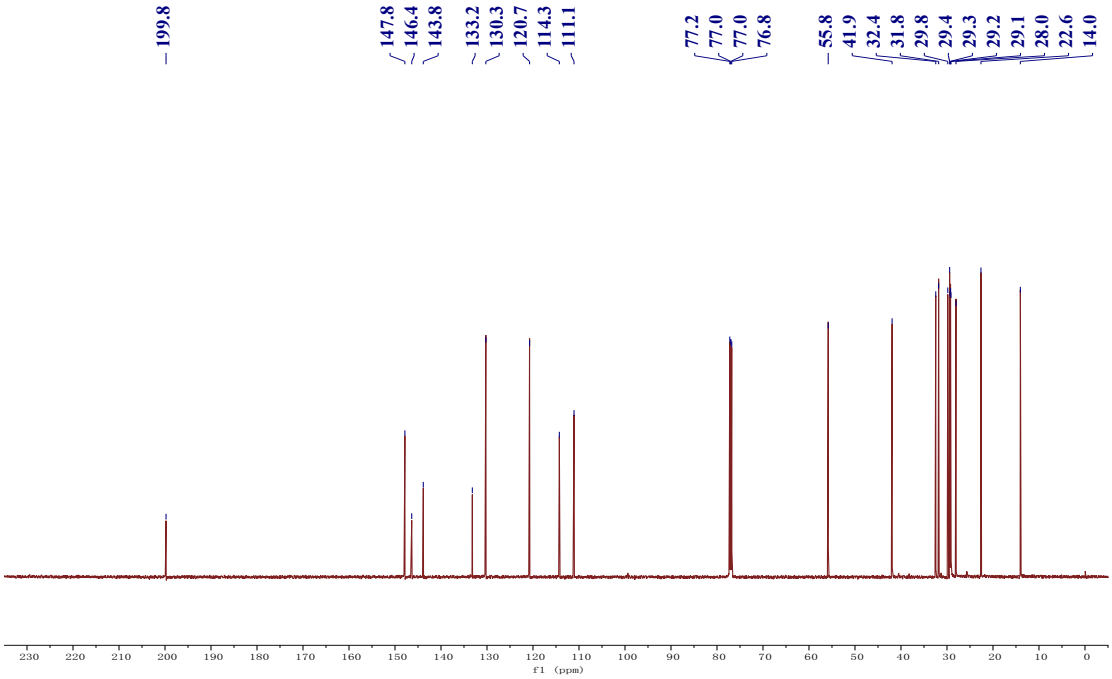


Fig. S8-2

Fig. S8