

**Supplementary Information for:**

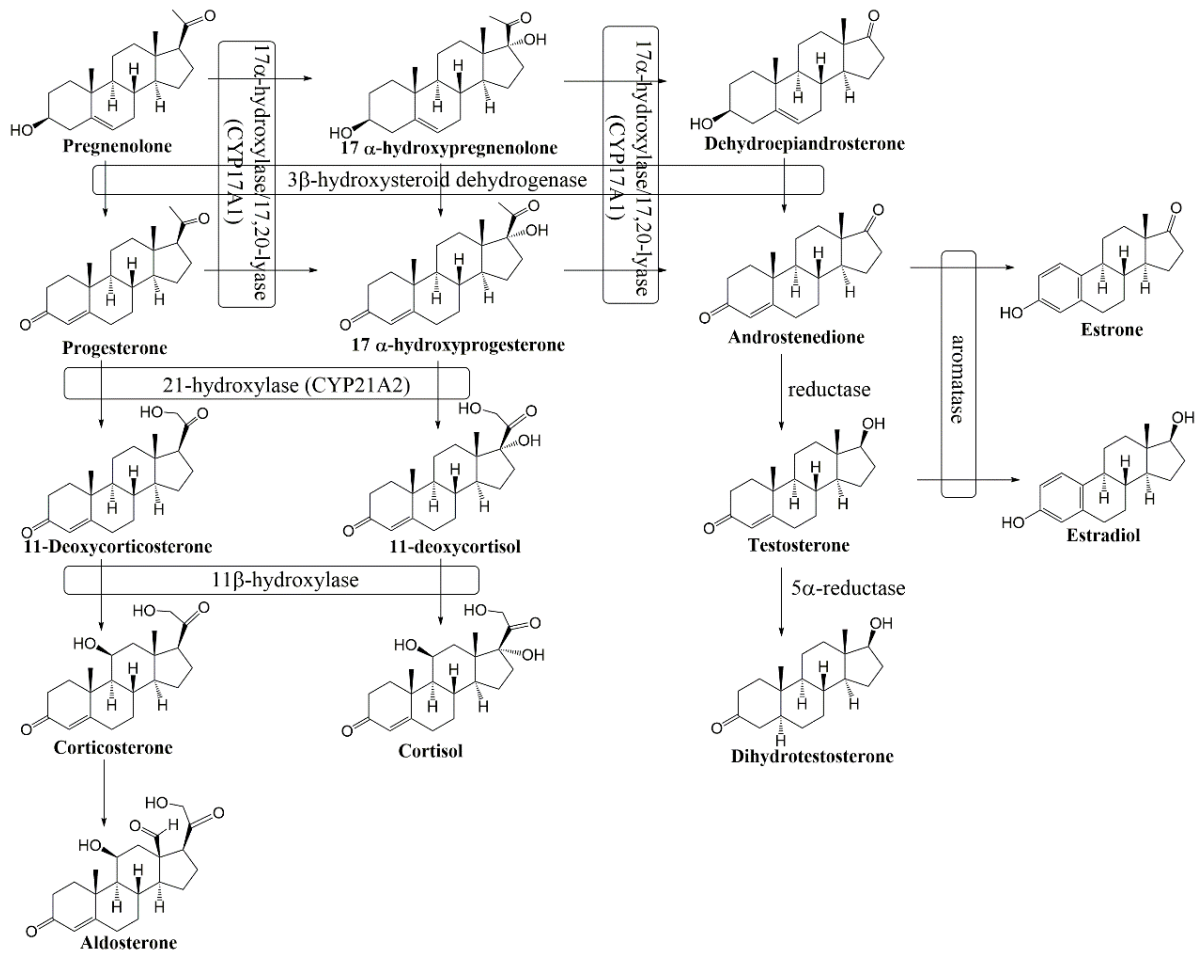
**Pregnenolonyl- $\alpha$ -Glucoside Exhibits Marked Anti-Cancer and CYP17A1 Enzymatic Inhibitory Activities**

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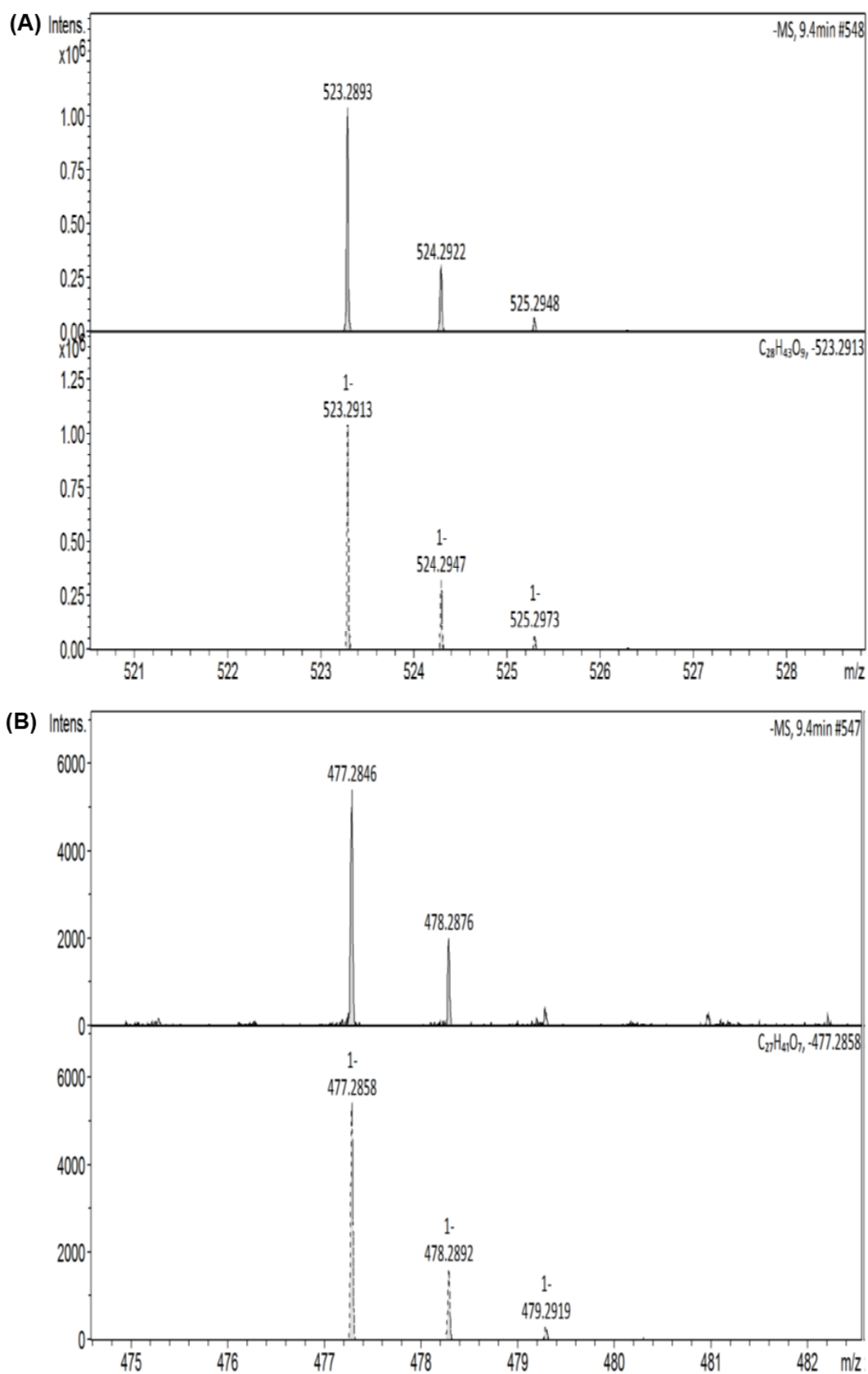
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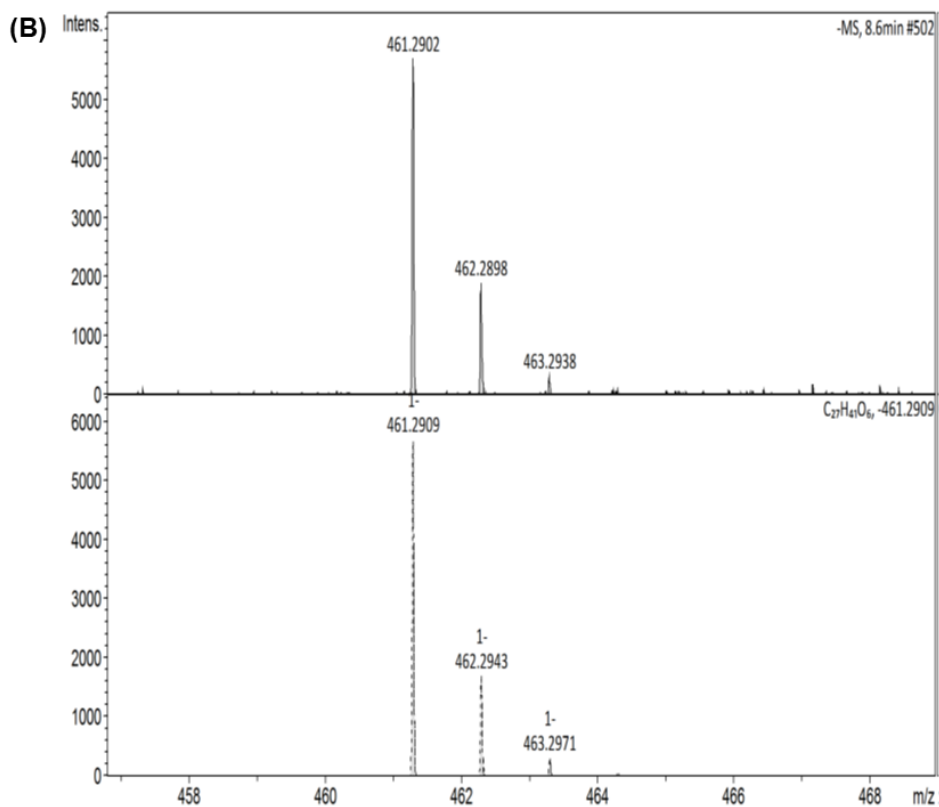
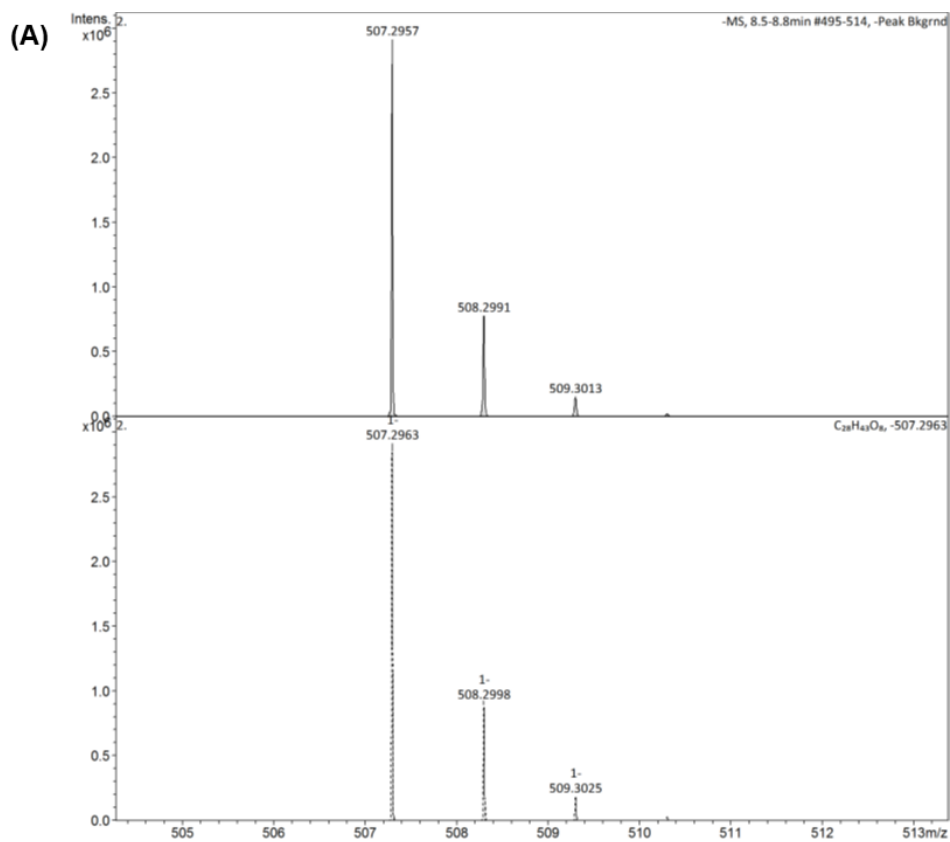
**Scheme S1.** Biosynthesis of steroid hormones from pregnenolone.



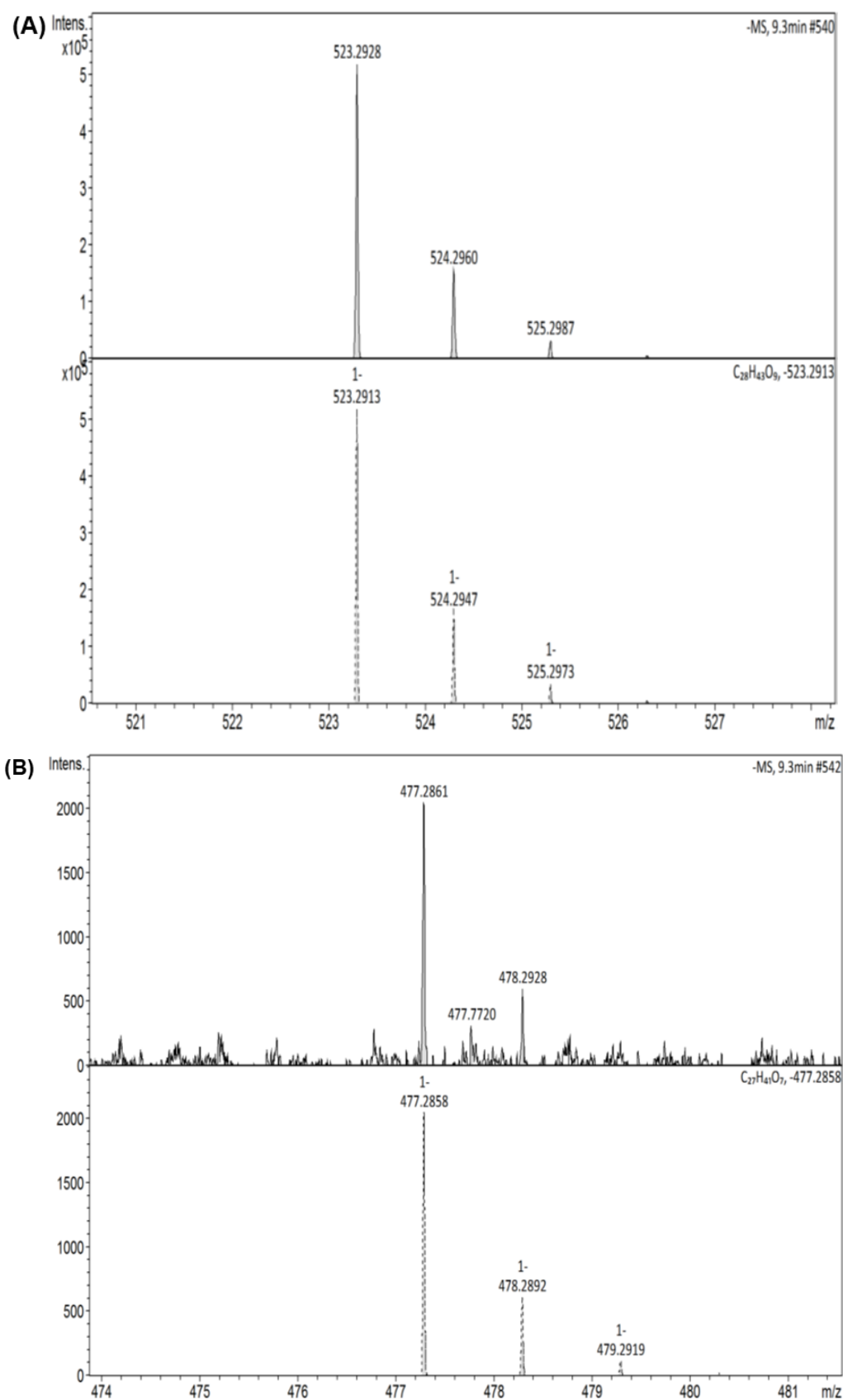
**Fig. S1** High resolution UPLC/ESI-MS of PREG- $\alpha$ -Glc. (A) Theoretical (Mw = 523.2913) and experimental (Mw = 523.2893) data of  $[M + HCOO - H]^-$ . (B) Theoretical (Mw = 477.2858) and experimental (Mw = 477.2838) data of  $[M - H]^-$ .



**Fig. S2** High resolution UPLC/ESI-MS of PREG- $\alpha$ -2DG. (A) Theoretical (Mw = 507.2963) and experimental (Mw = 507.2957) data of  $[M + HCOO - H]^-$ . (B) Theoretical (Mw = 461.3093) and experimental (Mw = 461.2902) data of  $[M - H]^-$ .

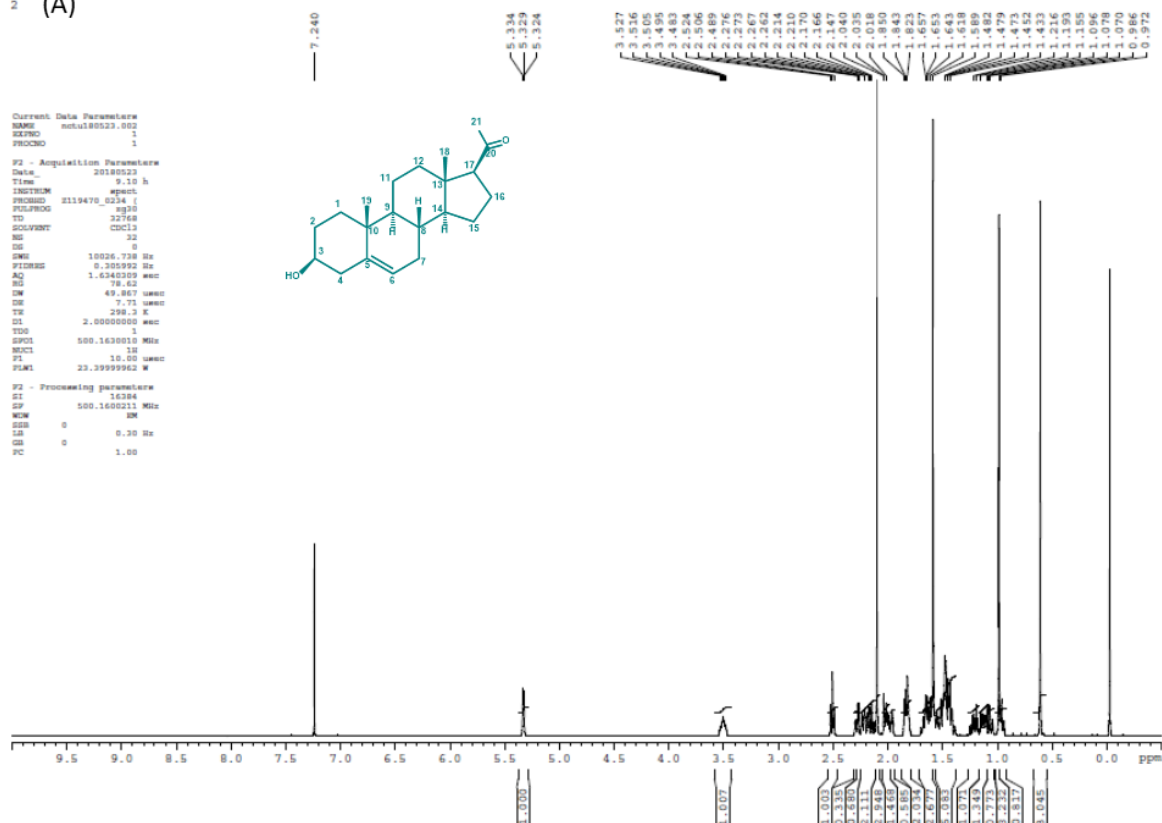


**Fig. S3** High resolution UPLC/ESI-MS of PREG- $\beta$ -Glc. (A) Theoretical (Mw = 523.2913) and experimental (Mw = 523.2928) data of  $[M + HCOO - H]^-$ . (B) Theoretical (Mw = 477.2858) and experimental (Mw = 477.3027) data of  $[M - H]^-$ .

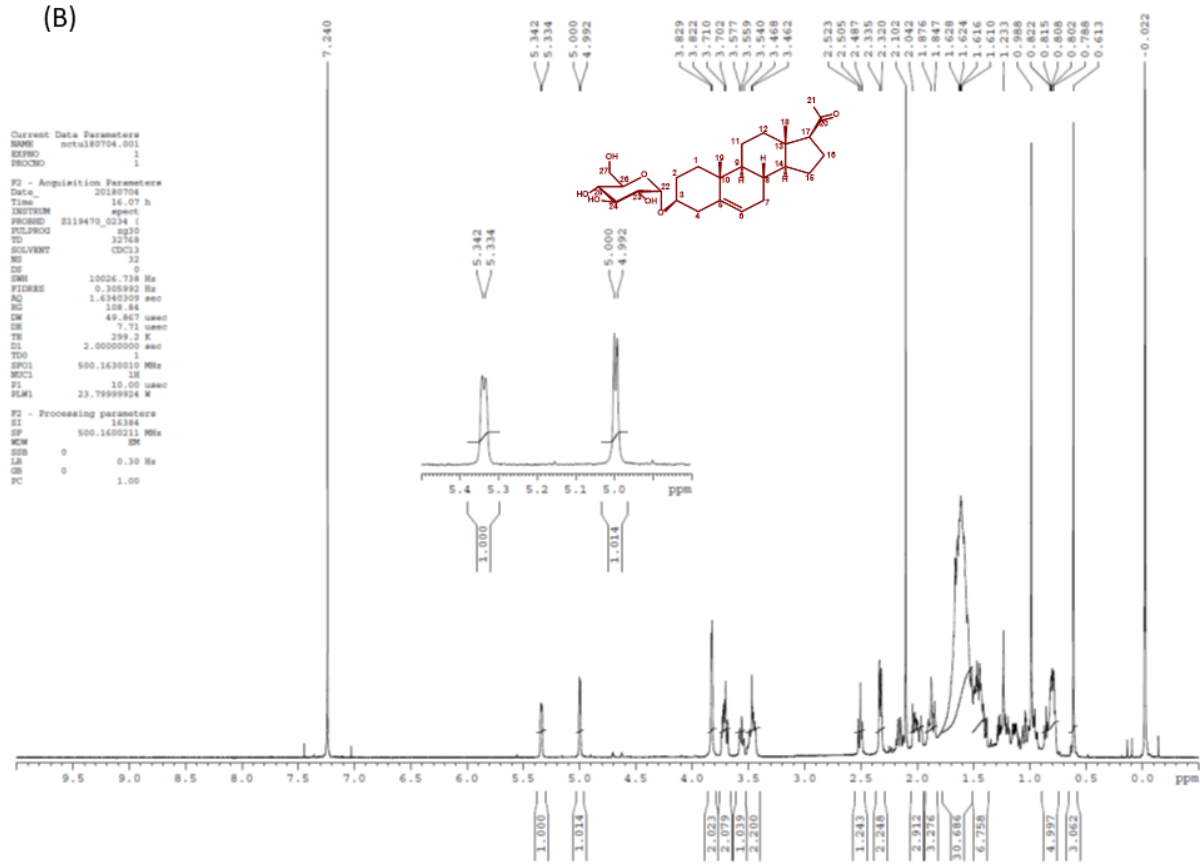


**Fig. S4** NMR of (A)  $^1\text{H}$ -PREG, (B)  $^1\text{H}$ -PREG- $\alpha$ -Glc, and (C)  $^{13}\text{C}$ -PREG- $\alpha$ -Glc.

2 (A)



(B)



(c)

exp7 CARBON

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SAMPLE          PRESATURATION
date    Dec 21 2018  satmode      n
solvent  odcl3      wet          n
file    /home/peng/vn-          SPECIAL
mrays/data/Wu-preg-          temp    25.0
-a-glucoside-C.fid          gain    30
ACQUISITION          spin    not used
sw      44642.9      hat      0.008
at      1.468      pw90    14.000
mp      131072     alfa    10.000
fb      17000
be      8          il      n
dl      3.500     in      n
nt      15000     dp      y
ot      15000     hs      nn
TRANSMITTER          PROCESSING
tn      C13       lb      3.00
sfrq    175.970  fn      262144
tof     2679.2   DISPLAY
tpwr    59      ep      -879.8
pw      7.000   wp      39588.7
DECOUPLER          rfl    2960.5
dn      H1      rfp    0
dof     0      rp    13.2
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decwave  w      PLOT
dpcr    39     wc    200
dmf     10582  sc    0
va      690051
th
ai      ph      2
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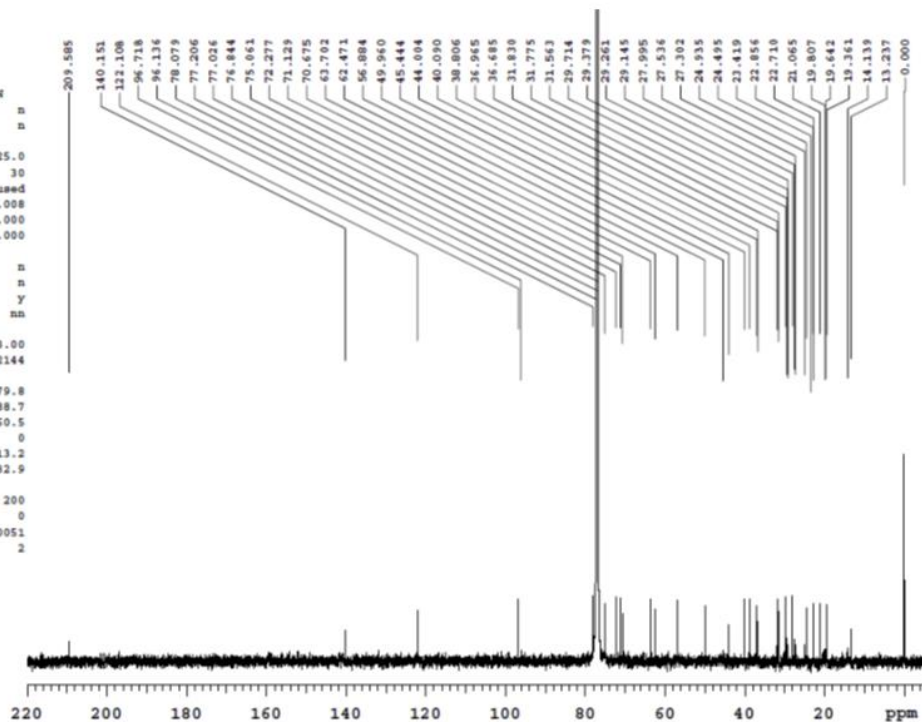
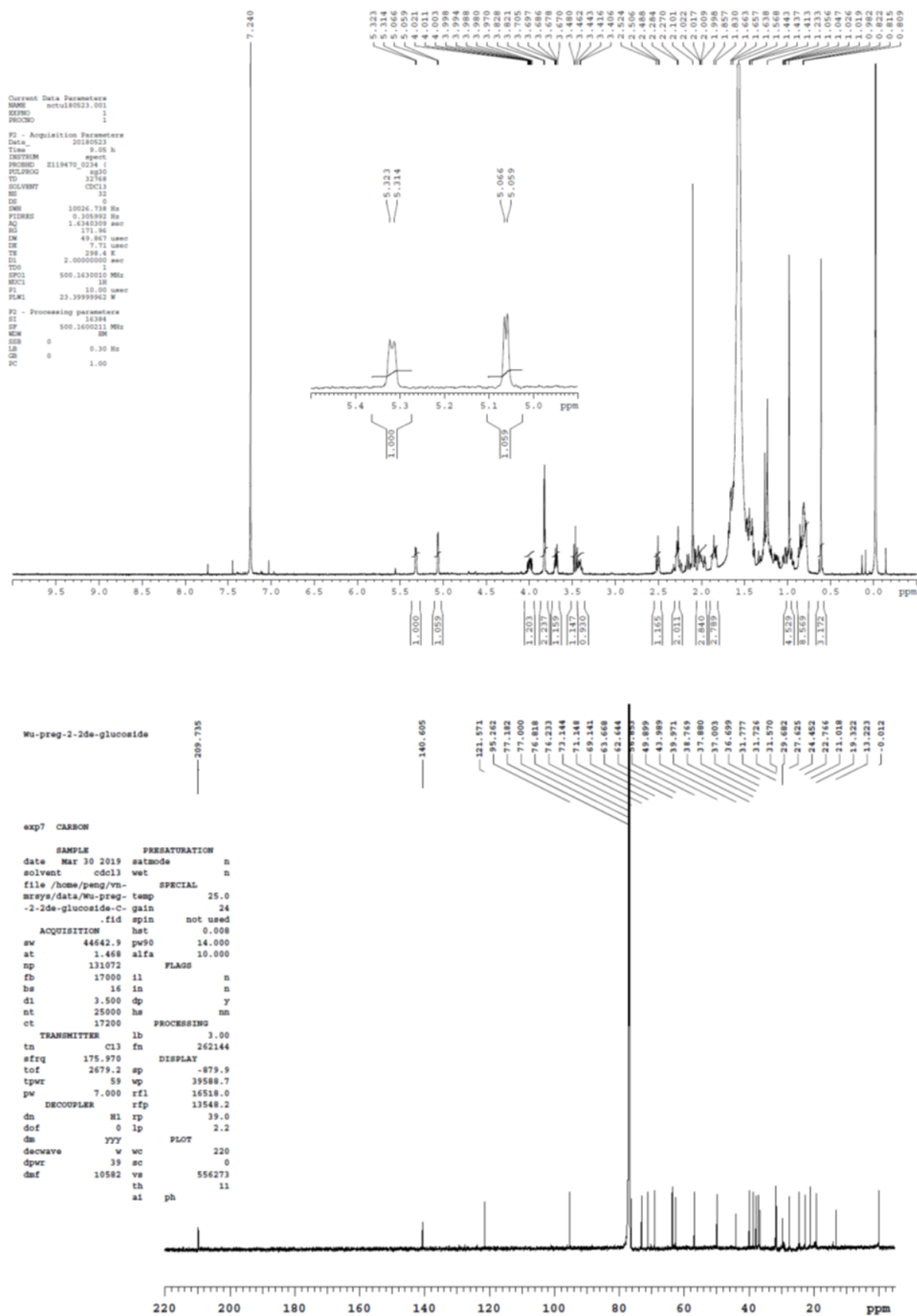


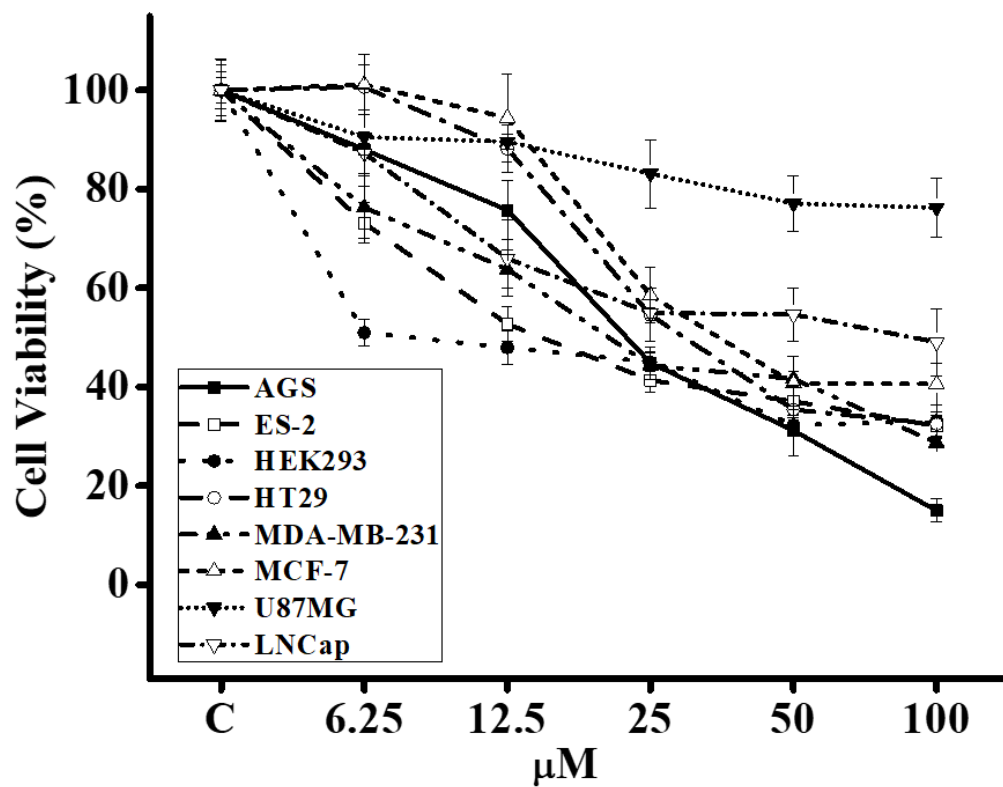
Fig. S5 <sup>1</sup>H and <sup>13</sup>C-NMR of PREG- $\alpha$ -2DG.



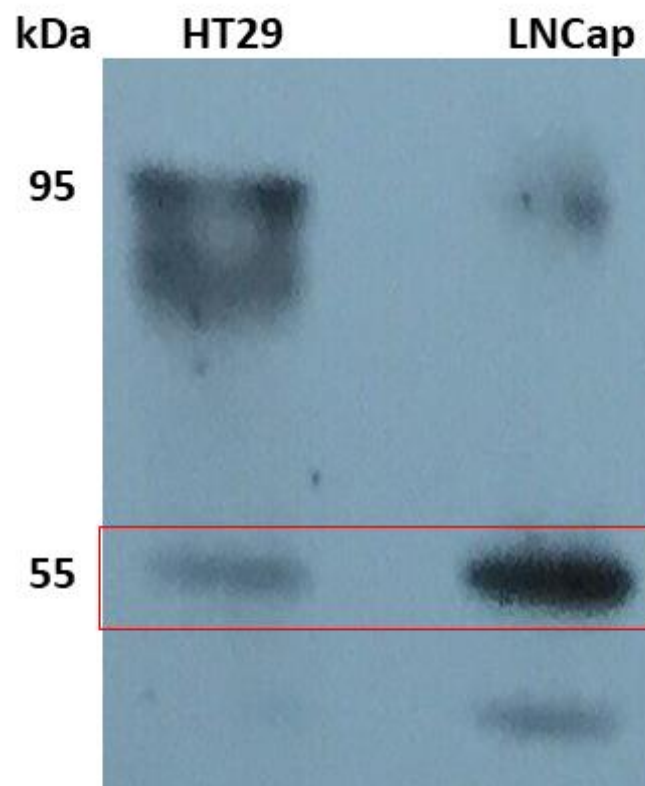




**Fig. S7** Dose-dependent cell viability assay of abiraterone on various cell types. AGS (■), ES-2 (□), HEK293 (●), HT-29 (○), MDA-MB-231 (▲), MCF-7 (△), U87MG (▼), and LNCap (▽).



**Fig. S8.** Western blot analysis of CYP17A1 protein expression within HT29 and LNCap cells, respectively.



**Table S1.** IC<sub>50</sub> values of cytotoxic activity of PREG (1), PREG- $\alpha$ -Glc (2), PREG- $\alpha$ -2-DG (3), and PREG- $\beta$ -Glc (4) on various cancer cell types.

Cell Name	PREG, 1 ( $\mu$ M)	PREG- $\alpha$ -Glu, 2 ( $\mu$ M)	PREG- $\alpha$ -2DG, 3 ( $\mu$ M)	PREG- $\beta$ -Glu, 4 ( $\mu$ M)
HEK293	>200	>200	>200	>200
AGS	>200	36.5 $\pm$ 8.0	>200	>200
ES-2	179.5 $\pm$ 25.1	50.9 $\pm$ 3.0	65.8 $\pm$ 10.7	>200
HT-29	91.9 $\pm$ 19.0	23.5 $\pm$ 9.9	>200	>200
LNCap	>200	115.1 $\pm$ 0.4	149.0 $\pm$ 6.8	>200
MCF-7	>200	90.6 $\pm$ 7.5	52.8 $\pm$ 4.3	>200
MDA-MB-231	>200	54.3 $\pm$ 5.6	>200	>200
U87MG	>200	140.2 $\pm$ 9.7	81.9 $\pm$ 5.0	>200