

**Epoxide functionalization on cholane side chains in the  
identification of G-protein coupled bile acid receptor (GPBAR1)  
selective agonists**

Simona De Marino, Adriana Carino, Dario Masullo, Claudia Finamore, Valentina Sepe,  
Silvia Marchianò, Francesco Saverio Di Leva, Vittorio Limongelli, Stefano Fiorucci, and  
Angela Zampella

**Supplementary Information**

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**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data ( $\text{CDCl}_3$ ) of steroidal nucleus for compounds **1**<sup>a</sup> (700 and 175 MHz) and **4**<sup>b</sup>(400 and 100 MHz)

Position	<b>1</b>		<b>4</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1	1.04, 1.78	35.4	0.97, 1.80	35.3
2	1.33, 1.67	30.2	1.33, 1.66	30.5
3	3.60 m	71.6	3.62 m	71.9
4	1.40, 1.87	29.1	1.50, 1.76	36.4
5	1.61	48.3	1.38	42.0
6	4.04 dt (12.0, 4.6 Hz)	68.0	1.26, 1.85	27.2
7	1.10, 1.64	34.9	1.09, 1.42	26.4
8	1.42	34.9	1.41	35.8
9	1.35	39.7	1.40	40.2
10	-	35.7	-	34.6
11	1.17, 1.38	20.7	1.25, 1.41	20.8
12	1.14, 1.96	39.8	1.17, 1.97	40.4
13	-	42.6	-	42.7
14	1.12	56.0	1.05	56.5
15	1.06, 1.57	24.2	1.04, 1.56	24.2
16	1.28, 1.87	28.1	1.26, 1.85	28.3
17	1.12	56.0	1.13	56.1
18	0.65 s	12.1	0.65 s	12.0
19	0.91 s	23.4	0.92 s	23.4

<sup>a</sup> The data reported for compound **1** are identical with those of compound **2**, **5** and **6**. <sup>b</sup> The data reported for compound **4** are identical with that of compound **3**.

**Table 2.**NMR data (CDCl<sub>3</sub>) of side chains for compounds **1**, **2**, **5** and **6**<sup>a</sup>

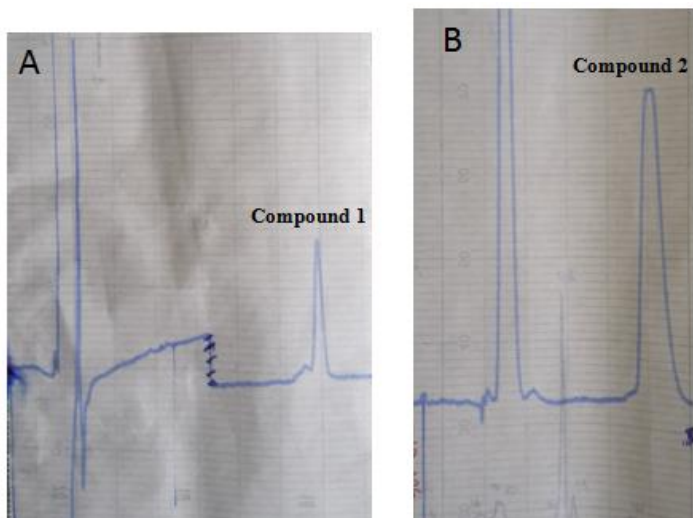
Position	<b>1</b>		<b>2</b>		<b>5</b>		<b>6</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}^{\text{b}}$	$\delta_{\text{C}}$
20	1.42	35.2	1.43	35.5	1.62	34.2	1.65	34.1
21	0.93 d (7.0 Hz)	18.6	0.93 d (6.7 Hz)	18.7	0.99 d (6.5 Hz)	18.9	1.04 (1.05) d	19.0
22	1.21, 1.47	32.3	1.18, 1.58	32.0	1.17, 1.72	34.8	1.63	38.8
23	1.40, 1.60	25.6	1.41, 1.58	29.3	2.74 t (6.1)	63.4	2.93 m	53.6
24	2.66 t (6.0 Hz)	64.7	2.88 m	52.9 (52.7)	-	58.7	2.80 m (2.72) 2.47 m (2.40)	48.1
25	-	58.3	2.47 m, 2.75 m	47.3 (47.1)	1.32 s	24.8		
26	1.30 s	24.9			1.25 s	19.2		
27	1.25 s	18.6						

<sup>a</sup> NMR data at 700 MHz (**1** and **2**), 500 MHz (**5**) and 400 MHz (**6**) respectively.

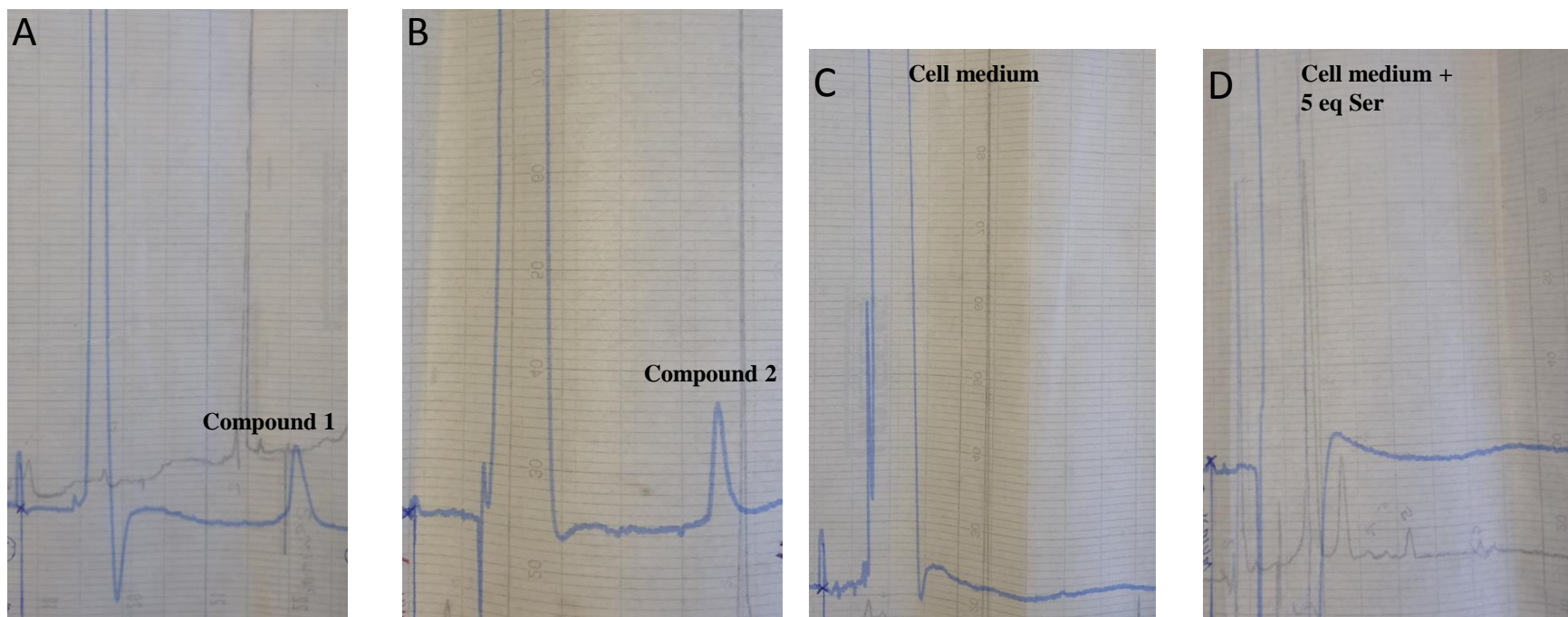
<sup>b</sup> Selected <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta_{\text{H}}$  4.00 (dt, J= 12.0, 4.5 Hz, H-6), 3.50 (m, H-3), 2.95 (m, H-23), 2.78 (2.70) (t, J = 4.5 Hz, H-24), 2.46 (2.38) (dd, J= 5.1, 2.7 Hz, H-24), 1.08 (1.07) (d, J=6.8 Hz, H<sub>3</sub>-21), 0.93 (s, H<sub>3</sub>-19), 0.71 (s, H<sub>3</sub>-18)

**Table 3.**NMR data (CDCl<sub>3</sub>) of side chains for compounds **3** and **4** (400 MHz, CDCl<sub>3</sub>)

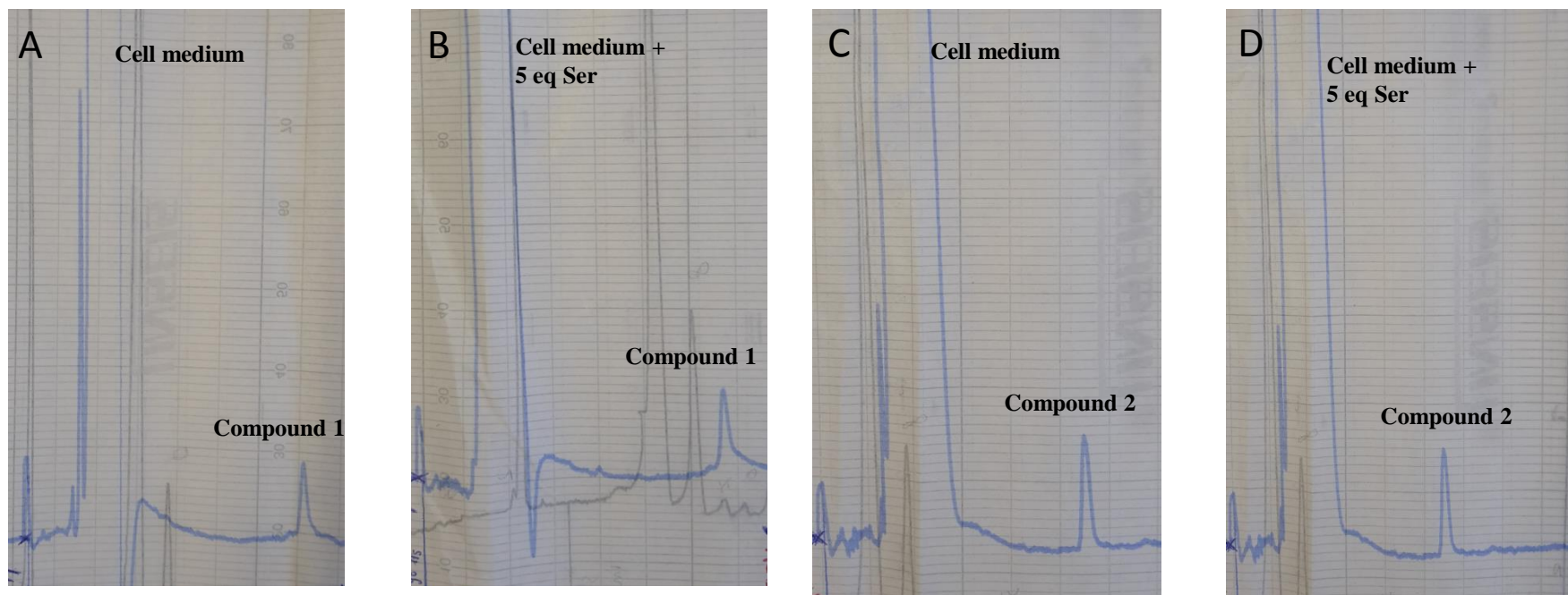
Position	<b>3</b>		<b>4</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
20	1.42	35.2	1.43	35.5
21	0.92 d (7.0 Hz)	18.5	0.93 d (6.7 Hz)	18.5
22	1.20, 1.47	32.2	1.19, 1.58	32.0
23	1.40, 1.60	25.6	1.41, 1.58	29.2
24	2.67 t (6.0 Hz)	64.7	2.87 m	52.9 (52.7)
25	-	58.3	2.46 m, 2.75 m	47.3 (47.1)
26	1.30 s	24.9		
27	1.26 s	18.6		



**Fig. S1.** Purification of compound **1** (Panel A,  $t_R = 15$  min) and compound **2** (Panel B,  $t_R = 11.2$  min) by HPLC, equipped with a differential refractometer, on Phenomenex Luna C18 (5  $\mu\text{m}$ ; 4.6 mm i.d. x 250 mm) with MeOH/H<sub>2</sub>O (85:15) as eluent (flow rate 1 mL/min).

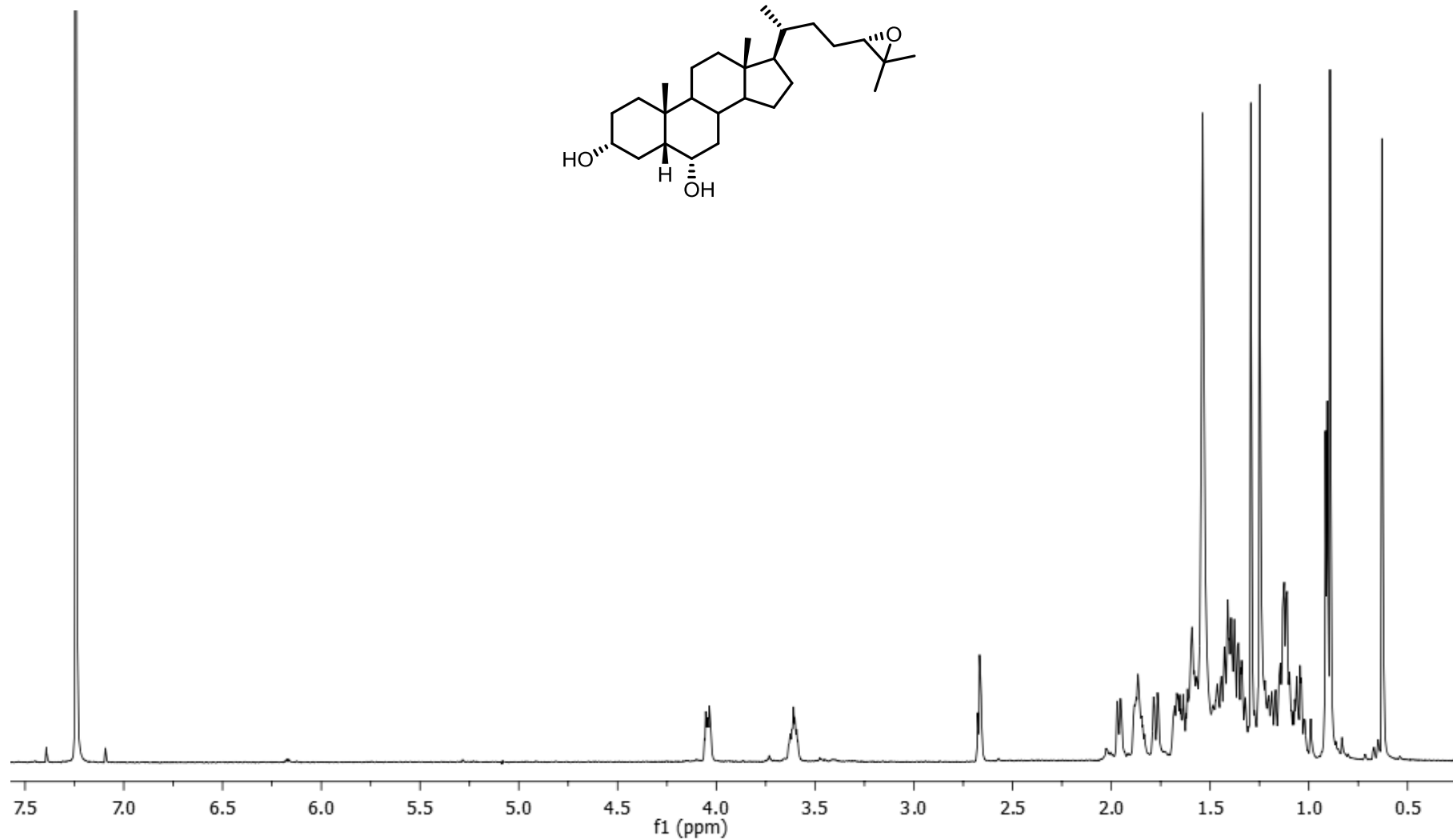
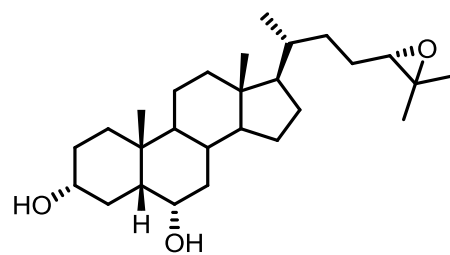


**Fig. S2. A-B)** HPLC traces of standard solution prepared diluting 1  $\mu\text{L}$  of a 60  $\mu\text{M}$  solution in DMSO of the compound **1** and **2**, respectively, with 499  $\mu\text{L}$  of MeOH/H<sub>2</sub>O (85:15); **C)** HPLC trace of cell medium alone (D-MEM additioned with 10% FBS); **D)** HPLC trace of cell medium additioned with 5 eq. Ser. HPLC was performed on Phenomenex Luna C18 (5  $\mu\text{m}$ ; 4.6 mm i.d. x 250 mm) with MeOH/H<sub>2</sub>O (85:15) as eluent (flow rate 1 mL/min).

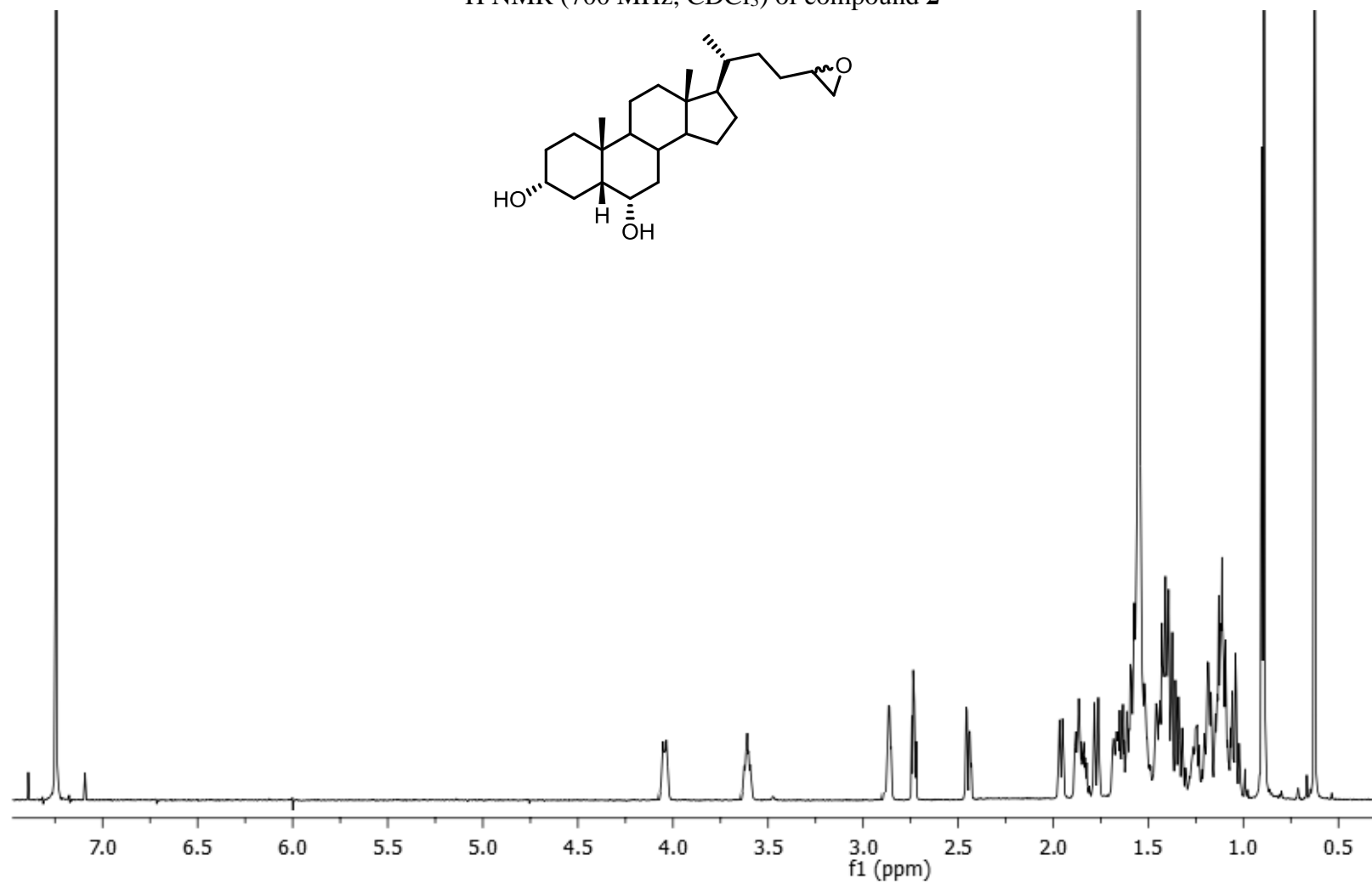


**Fig. S3.** A) HPLC trace of compound **1** in cell medium (D-MEM with 10% FBS); B) HPLC trace of compound **1** in cell medium/5 eq. Ser; C) HPLC trace of compound **2** in cell medium; D) HPLC trace of compound **2** in cell medium/5 eq. Ser. HPLC was performed on Phenomenex Luna C18 (5  $\mu$ m; 4.6 mm i.d. x 250 mm) with MeOH/H<sub>2</sub>O (85:15) as eluent (flow rate 1 mL/min).

$^1\text{H}$  NMR (700 MHz,  $\text{CDCl}_3$ ) of compound **1**

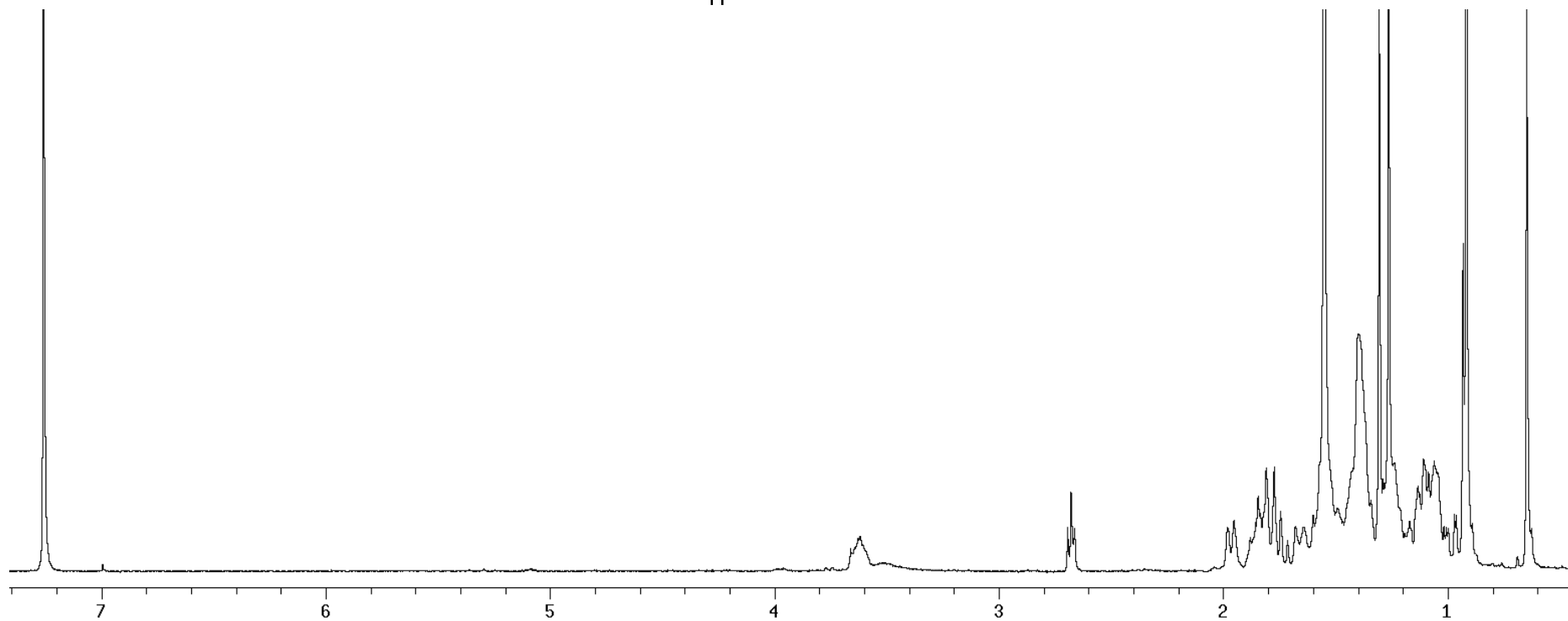
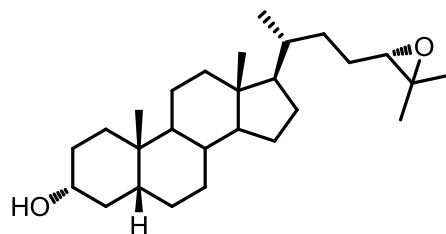


$^1\text{H}$  NMR (700 MHz,  $\text{CDCl}_3$ ) of compound **2**

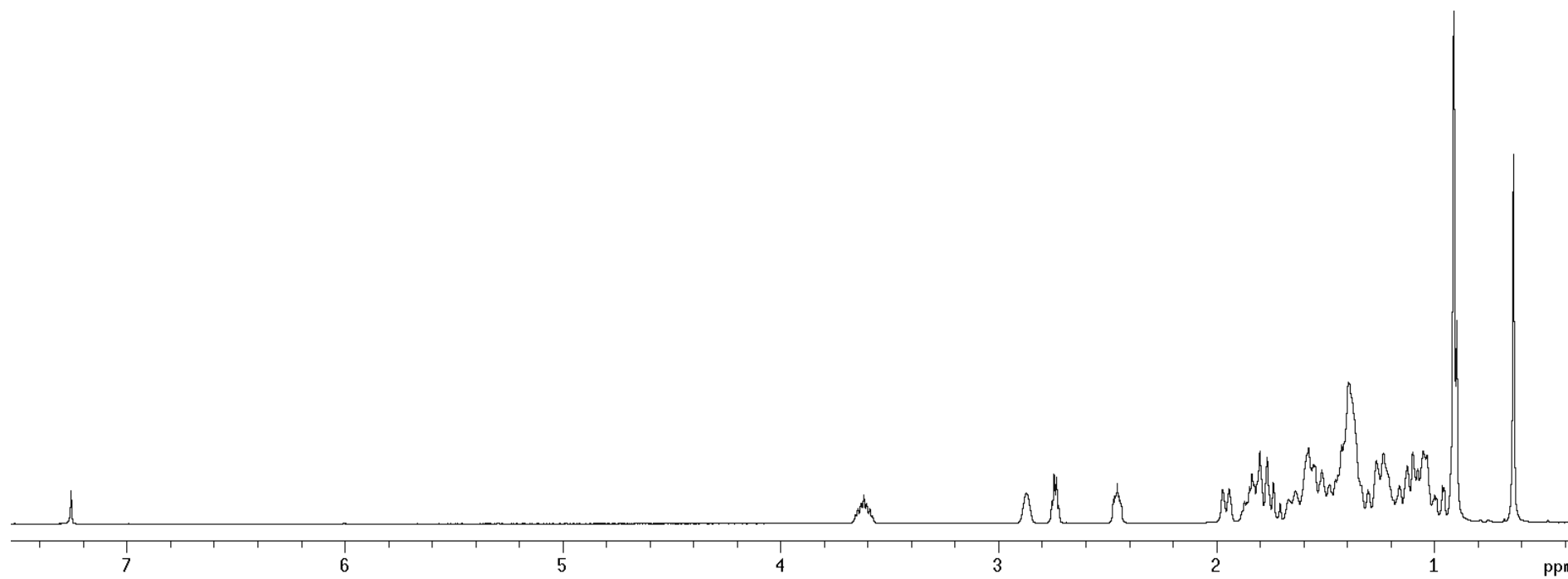
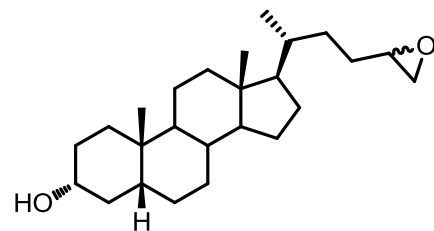




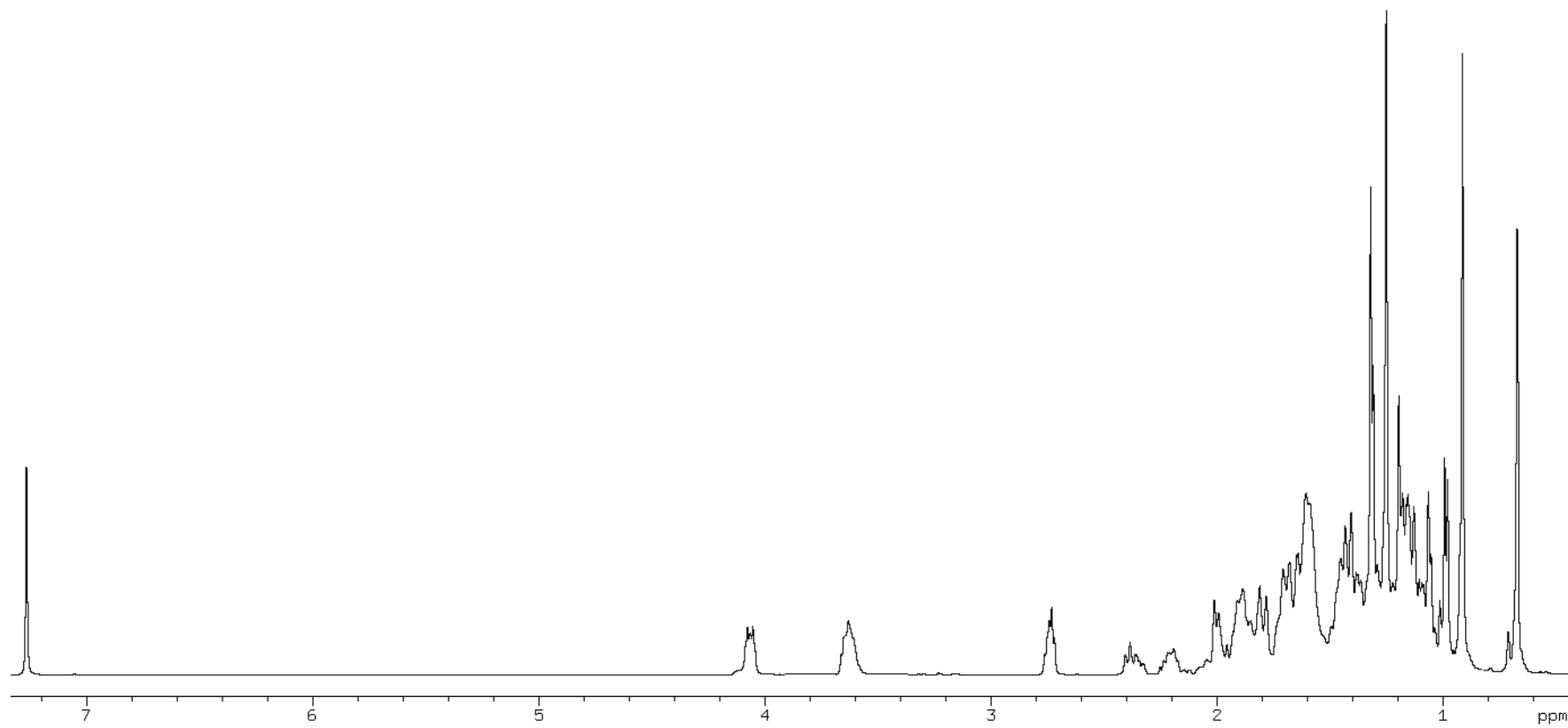
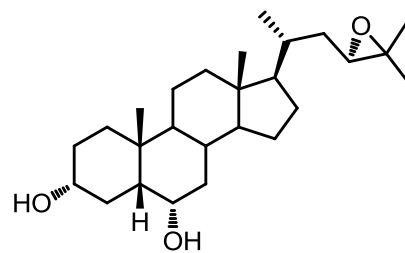
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) of compound **3**



$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) of compound **4**



$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) of compound **5**



$^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ) of compound **6**

