Supplementary data

Norsampsone E, an unprecedented decarbonyl polycyclic polyprenylated acylphloroglucinols with homoadamantyl core from Hypericum sampsonii

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UV spectrum of Norsampsone E (1) in CH$_3$OH.

IR (KBr disc) spectrum of Norsampsone E (1).

HR-ESI-MS spectrum of Norsampsone E (1).
$^1$H NMR (AV-400, 400 MHz) spectrum of Norsampsone E (1) in CDCl$_3$


$^{13}$C NMR spectrum (AV-400, 100 MHz) of Norsampsone E (1) in CDCl$_3$
HSQC spectrum (AV-400) of Norsampsone E (1) in CDCl$_3$

$^1$H–$^1$H COSY spectrum (AV-400) of Norsampsone E (1) in CDCl$_3$
HMBC spectrum (AV-400) of Norsampsone E (1) in CDCl$_3$

NOESY spectrum (AV-400) of Norsampsone E (1) in CDCl$_3$
UV spectrum of hypersampsone X (2) in CH$_3$OH.

IR (KBr disc) spectrum of hypersampsone X (2).

HR-ESI-MS spectrum of hypersampsone X (2).
$^1$H NMR (AV-600, 600 MHz) spectrum of hypersampsone X (2) in CDCl$_3$

$^{13}$C NMR spectrum (AV-600, 150 MHz) of hypersampsone X (2) in CDCl$_3$
HSQC spectrum (AV-600) of hypersampsone X (2) in CDCl$_3$

$^1$H–$^1$H COSY spectrum (AV-600) of hypersampsone X (2) in CDCl$_3$
HMBC spectrum (AV-600) of hypersampsone X (2) in CDCl$_3$

NOESY spectrum (AV-600) of hypersampsone X (2) in CDCl$_3$
Bioassays

Biacore assay

**RXRa-LBD protein purified.** The 0.79 kb DNA fragment corresponding to the ligand-binding domain (LBD) of human RXRa (genes 592-1386) was excised from MCF-7 cells and sub-cloned into pET-15b between the BamHI and NheI restriction site. Transformed E. coli BL21(DE3) were grown at 37°C in LB medium until OD$_{600}$ = 0.6-0.8. Protein expression was initiated by 0.4 mM IPTG, and this procedure sustained at 18 °C for 16 h. Following centrifugation, resuspension and sonication processes, the combined protein was purified by Ni$^{2+}$-NTA agarose column at low temperatures.

**SPR assay.** The measurements were performed on the Biacore T200 (Biacore GE) at 25 ºC in a running buffer comprising PBS (pH 7.5), 150 mM NaCl, 10 mM MgCl$_2$ and 0.1% P20. CM5 chips (Biacore GE) were first treated with EDC-NHS mixture at a flow rate of 10 ml/min. Purified RXRa-LBD protein was immobilized on the sensor chip by the standard amine coupling protocol with resonance unit around 8000 RU. All test samples were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions and further diluted in PBS. Gradient concentrations of each compound (100 μM, 50 μM, 25 μM, 12.5 μM, 6.25 μM, 3.125 μM) were injected through flow cells immobilized with purified RXRa-LBD protein. The chip is being exposed to ligand solution during 0–120 s, and the ligand is dissociated from the chip by running buffer from 120 to 420 s. The K$_D$ values were calculated from the experimental curve with Biacore T200 evaluation software package. The formation of surface-bound complexes was analyzed according to the interaction type of A+B↔AB.

![Figure 1. SPR results of compound 1-2 binding to RXRa-LBD.](image-url)
RXRα transcriptional activity assay

**Cell Culture.** The human renal epithelial cells (293T) (ATCC) were cultured in 37 °C in DMEM (Hyclone) containing 10% fetal bovine serum (FBS, Hyclone) for 24 h.

**Experimental Methods.** The previous dual-luciferase reporter gene assay with some modification was used in the present study. In brief, approximately $1.5 \times 10^4$ cells/well were seeded in 96-well plates. The two target plasmids, 20 ng pBind RXRα LBD (provided by Dr. Xiao-kun Zhang from the Burnham Institute for Medical Research, Cancer Center, La Jolla, CA, USA.) and 50 ng PG5 LUC (provided by Dr. Xiao-kun Zhang from the Burnham Institute for Medical Research, Cancer Center, La Jolla, CA, USA.), were transfected by Liposome 2000 (Invitrogen) in the cell. After 24 h, the cells were exposed to the test compound for 12 h. Then the cells were rinsed with PBS and lysed by buffered solution (1 × PLB) on the oscillating platform for 15 minutes. According to the introduction of the Dual-Luciferase Reporter Assay System (promega), the activities of Firefly luciferase (FL) and Rellina luciferase (RL) were checked.

$$\text{Relative luciferase activity (\%) = FL / RL} \times 100\%$$

![Figure 2. Effects of compounds 1-2 (5, 10, and 20μM) on the transcriptional activities of RXRα](image)

**Reference**
