Supporting Information

Quantitative Evaluation of the Relative Cell Permeability of Peptoids and Peptides

*Yong-Uk Kwon and Thomas Kodadek**

Department of Internal Medicine and Division of Translational Research, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390

thomas.kodadek@utsouthwestern.edu

Contents

Illustration of the design of the cell permeability assay

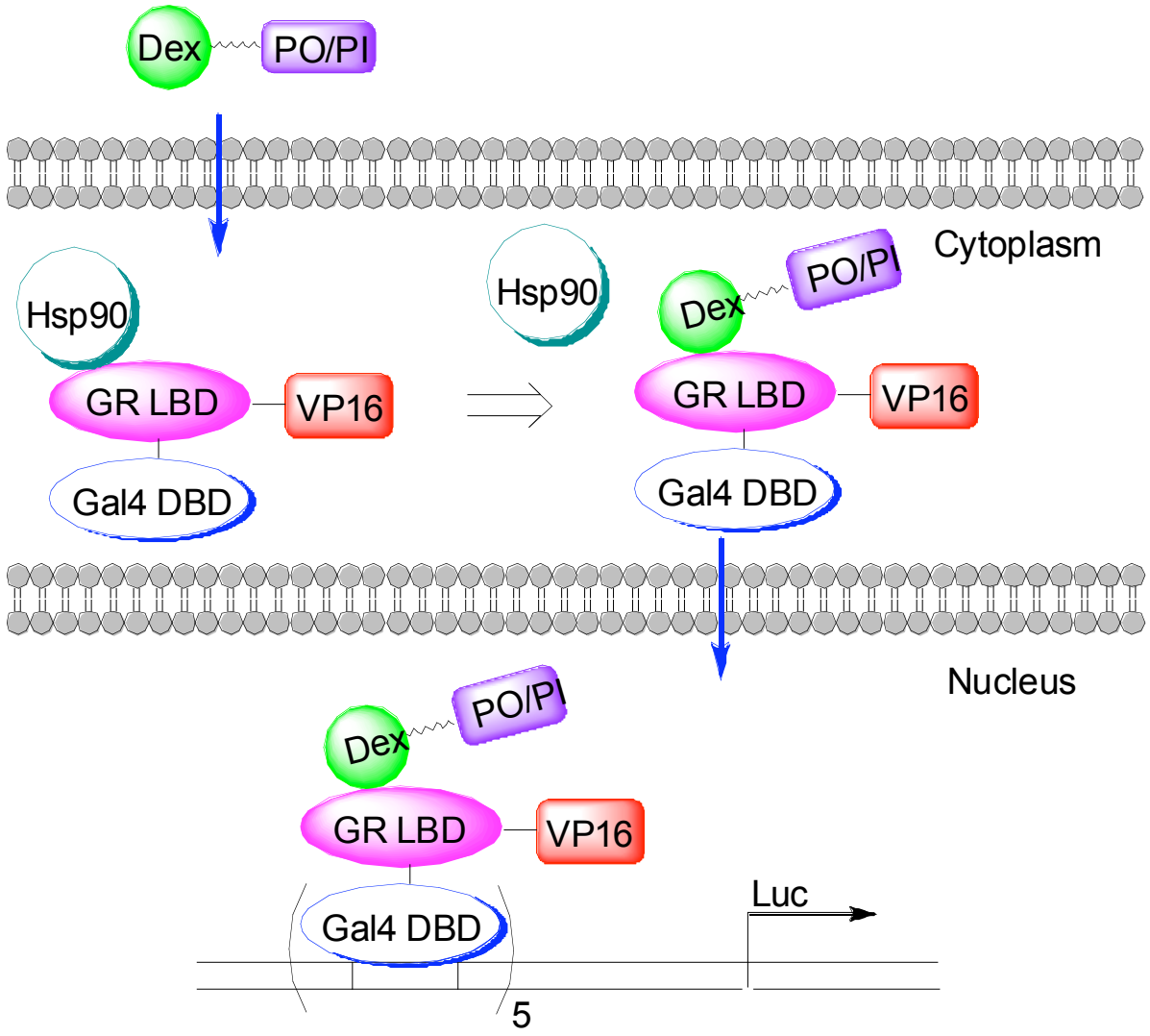


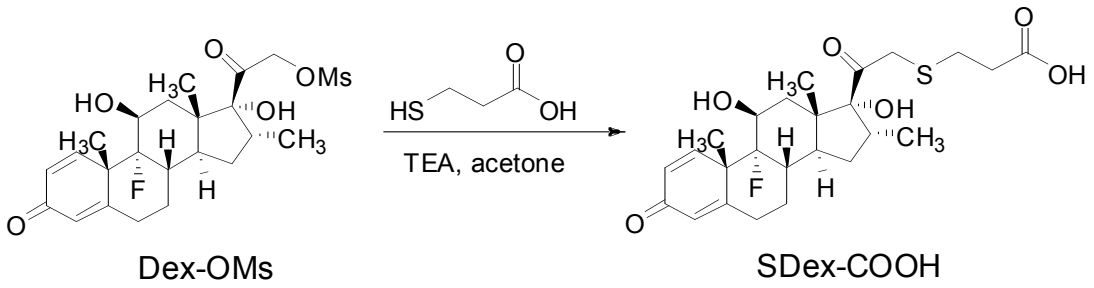
Figure S1. Schematic illustration of the design of the cell permeability assay for SDex-CO-PO/PI conjugates. See the text for details.

Synthesis of compounds

Materials and equipments. All commercial reagents were used as obtained without further purification. *O*-t-Butyl-2-amino ethanol was purchased from CSPS Pharmaceuticals. Polystyrene A RAM macrobeads (500-560 μm; capacity: 0.56 mmol/g) and Rink amide AM resin (200-400 mesh, capacity: 0.71 mmol/g) were obtained from Rapp Polymere and Novabiochem, respectively. NMR

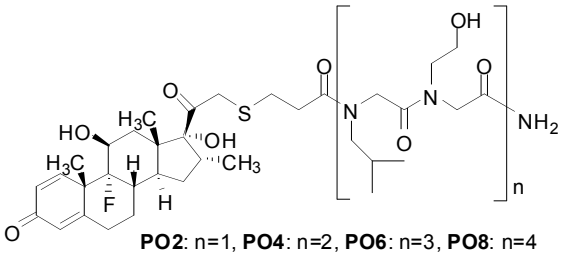
spectra were recorded on a Varian 300 MHz spectrometer. Preparative HPLC was performed on a Waters binary HPLC system with a C18 reverse-phase column with the gradient elution of water/acetonitrile with 0.1 % TFA. MS (MALDI-TOF) was performed on a Voyager-DE PRO biospectrometry workstation (Applied Biosystems) with α-hydroxy cinnamic acid as a matrix. HRMS (ESI) was measured on an ESI LTQ-FT mass spectrometer in Washington University. The synthesis of peptides was performed in a New Brunswick Scientific Innova 4000 incubator shaker. The synthesis of peptoids under microwave conditions was performed in a 1000 W Whirlpool microwave oven (model MT1130SG) with 10% power. Standard glass peptide synthesis vessels (Chemglass) were used for the synthesis in the incubator shaker and in the microwave oven.

Scheme S1. Synthesis of SDex-COOH

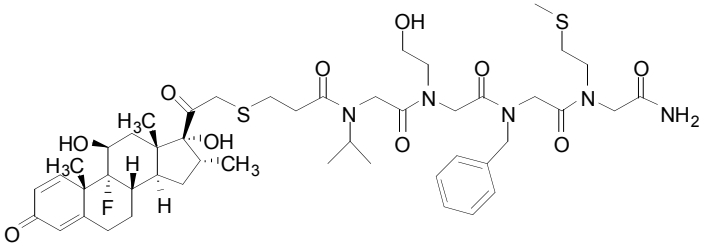


Dex-OMs SDex-COOH

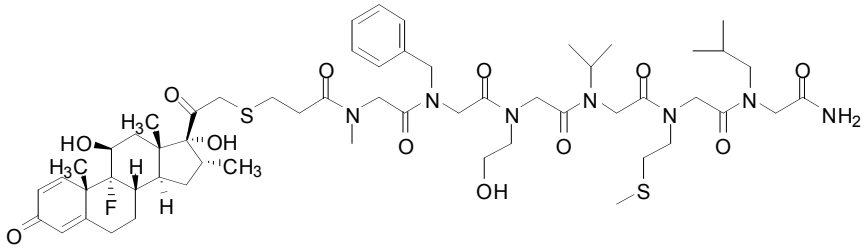
Dexamethasone-21-thiopropionic acid (SDex-COOH). To a solution of 3-mercaptopropionic acid (0.175 mL, 2 mmol) and triethylamine (0.56 mL, 4 mmol) in acetone was added dexamethasone-21-mesylate1 (471 mg, 1mmol) portionwise. After the resulting solution was stirred at rt overnight, it was poured into ice-cold water and acidified with 1N HCl to pH 2. The precipitate was filtered and dried in vacuo to give **SDex-COOH** (456 mg, 95%). 1H NMR (DMSO-d_6) δ 0.78 (d, J = 6.9 Hz, 3H), 0.88 (s, 3H), 1.06 (m, 1H), 1.34 (m, 1H), 1.48 (s, 3H), 1.51 (m, 1H), 1.62 (q, J = 11.8 Hz, 1H), 1,76 (m, 1H), 2.16-2.53 (m, 7H), 2.67-2.73 (m, 2H), 2.94 (m, 1H), 3.44 (d, J = 16.8 Hz, 1H), 3.85 (d, J = 16.8 Hz, 1H), 4.14 (m, 1H), 5.08 (s, 1H), 5.31 (d, J = 3.0 Hz, 1H), 6.01 (s, 1H), 6.22 (dd, J = 1.8, 10.2 Hz, 1H), 7.29 (d, J = 10.2 Hz, 1H); HRMS (ESI): m/z: calcd for $C_{25}H_{34}FO_6S$ 481.2060; found 481.2060 $[M + H]^+$.



PO2: n=1, **PO4**: n=2, **PO6**: n=3, **PO8**: n=4
SDex-CO-Peptoids



PO4-1: SDex-CO-N_{Val}-N_{Hse}-N_{Phe}-N_{Met}-NH_2

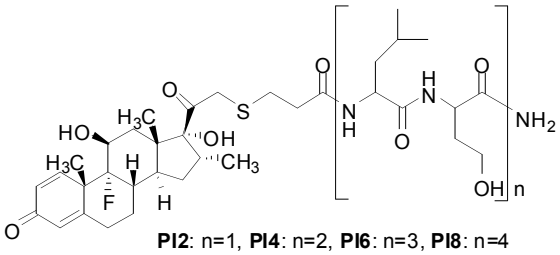


PO6-1: SDex-CO-N_{Ala}-N_{Phe}-N_{Hse}-N_{Val}-N_{Met}-N_{Leu}-NH_2

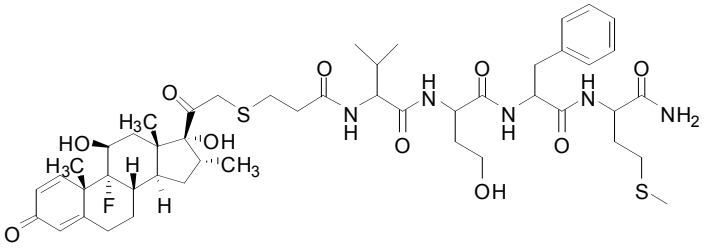
Figure S2. Chemical structures of SDex-CO-peptoid conjugates.

General procedure for the synthesis of SDex-CO-peptoid conjugates. The synthesis of peptoids was performed employing a microwave-assisted protocol.[2] Polystyrene A RAM macrobeads (30 μmol) in DMF were allowed to swell at room temperature for 1 h. After DMF was drained, the beads were incubated with 20 % piperidine for 30 min. The beads were thoroughly washed with DMF (8 × 3 mL) and then treated with 2 M bromoacetic acid (1.0 mL) and 3.2 M diisopropylcarbodiimide (DIC) (1.0 mL). The beads were thoroughly washed with DMF (8 × 3 mL) and then treated with the primary amines (1~2 M in DMF, 1.5 mL). Both acylation and displacement of bromide by the primary amines were performed twice for 15 s in a 1000W microwave oven set to deliver 10% power. The beads were shaken manually for 30 s between microwave pulses. The procedure was repeated. For attachment of SDex-COOH to the peptoid, a solution of SDex-COOH (2.5 eq.), HATU (2.5 eq.), HOAt (2.5 eq.), diisopropylethylamine (10 eq.) and 2,6-lutidine (7 eq.) in DMF (1.5 mL) was added to the beads. After

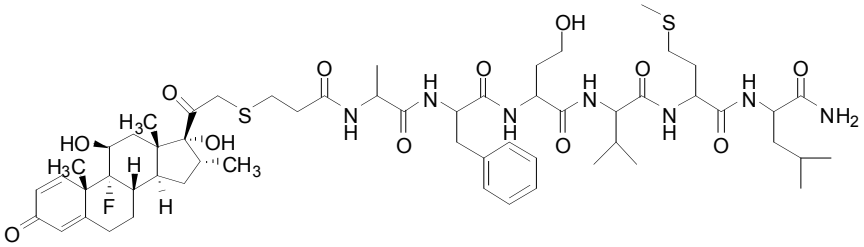
being incubated overnight at rt, the beads were thoroughly washed with DMF (8 × 3 mL) and dichloromethane (5 × 3 mL). Each SDex-CO-peptoid conjugate was released from the resin with concomitant removal of protecting groups by treating the beads with 3 mL of 95% TFA, 2.5% water, and 2.5% triisopropylsilane for 2 h. The suspension was filtered and the filtrate was concentrated by blowing nitrogen over the solution. The desired product was purified by preparative HPLC and lyophilized to dryness. The structures of SDex-CO-peptoid conjugates were confirmed by MALDI-TOF analysis (Table S1).



PI2: n=1, **PI4**: n=2, **PI6**: n=3, **PI8**: n=4
SDex-CO-Peptides



PI4-1: SDex-CO-Val-Hse-Phe-Met-NH_2



PI6-1: SDex-CO-Ala-Phe-Hse-Val-Met-Leu-NH_2

Figure S3. Chemical structures of SDex-CO-peptide conjugates.

General procedure for the synthesis of SDex-CO-peptide conjugates. The synthesis of peptides was performed using conventional Fmoc chemistry and peptide coupling method. Rink amide AM resins (30 μmol) in DMF were allowed to swell at room temperature for 1 h. After DMF was drained, the beads were incubated with 20 % piperidine for 30 min. The beads were thoroughly washed with

DMF (8 × 3 mL) and then treated with a solution of Fmoc-protected amino acid (3 eq.), HBTU (3 eq.) and HOBt (3 eq.) in 5% diisopropylethylamine/DMF for 1.5 h. The Fmoc deprotection and amino acid coupling steps were repeated. The attachment of SDex-COOH, cleavage of SDex-CO-peptide conjugate and final purification were carried out in the same manner as in the synthesis of SDex-CO-peptoid conjugates. The structures of SDex-CO-peptide conjugates were also confirmed by MALDI-TOF analysis (Table S1).

Table S1. Summary of data from MALDI-TOF and HPLC of SDex-CO-peptoid/peptide conjugates.

Compounds	MALDI-TOF [(M + Na)$^+$]			HPLC retention time (min)
	Chemical formula	MS (calculated)	MS (found)	
PO2	$C_{35}H_{52}FN_3NaO_8S$	716.3	716.3	29.5[a]
PO4	$C_{45}H_{70}FN_5NaO_{11}S$	930.4	930.3	30.4[a]
PO6	$C_{55}H_{88}FN_7NaO_{14}S$	1144.6	1144.6	30.7[a]
PO8	$C_{65}H_{106}FN_9NaO_{17}S$	1358.7	1358.8	31.0[a]
PI2	$C_{35}H_{52}FN_3NaO_8S$	716.3	716.3	29.1[a]
PI4	$C_{45}H_{70}FN_5NaO_{11}S$	930.4	930.5	30.1[a]
PI6	$C_{55}H_{88}FN_7NaO_{14}S$	1144.6	1144.7	30.3[a]
PI8	$C_{65}H_{106}FN_9NaO_{17}S$	1358.7	1358.7	31.7[a]
PO4-1	$C_{48}H_{68}FN_5NaO_{10}S_2$	980.4	980.6	27.5[b]
PO6-1	$C_{57}H_{84}FN_7NaO_{12}S_2$	1164.6	1164.6	28.8[b]
PI4-1	$C_{48}H_{68}FN_5NaO_{10}S_2$	980.4	980.5	27.1[b]
PI6-1	$C_{57}H_{84}FN_7NaO_{12}S_2$	1164.6	1164.4	29.1[b]

[a] 90% H_2O/acetonitrile with 0.1 TFA for 5min and then a gradient from 90% H_2O/acetonitrile to 10 % H_2O/acetonitrile with 0.1% TFA for 40 min. [b] A gradient from 90% H_2O/acetonitirile to 10 % H_2O/acetonitrile for 40 min.

Concentration determination of SDex-COOH conjugates. SDex-COOH conjugates were dissolved in DMSO. The concentration of each compound was determined by the distinct absorbance of

dexamethasone at 242 nm using an extinction coefficient of $1.2 * 10^4$ $M^{-1}cm^{-1}$.

In vitro competition GR binding assays

The GR binding affinity of SDex-COOH conjugates was determined using the glucocorticoid receptor Competitor Assay Kit (Invitrogen).[3] SDex-COOH conjugates were mixed with 1 nM fluormone and 4 nM recombinant human glucocorticoid receptor in 100 μL of the buffer and incubated in the dark at 25 °C for 1.5 h. Fluorescence polarization was measured on a Panvera Beacon 2000 fluorometer (Invitrogen). The concentration of a SDex-COOH conjugate that results in a half-maximum decrease in the polarization value is defined as the IC_{50} of the compound, which is a measure of the relative binding affinity of the test compound for glucocorticoid receptor. The binding data were fit to the equation [$P = (1-C/(IC_{50} + C)) * (P_{max} - P_{min}) + P_{min}$] where C is the concentration of the test compound, P is the polarization of the sample, P_{max} is the polarization value observed when no competitor is added to the glucocorticoid receptor–fluormone complex, P_{min} is the polarization value that represents maximum competition and was measured when 1 μM dexamethasone was used as the glucocorticoid receptor competitor.

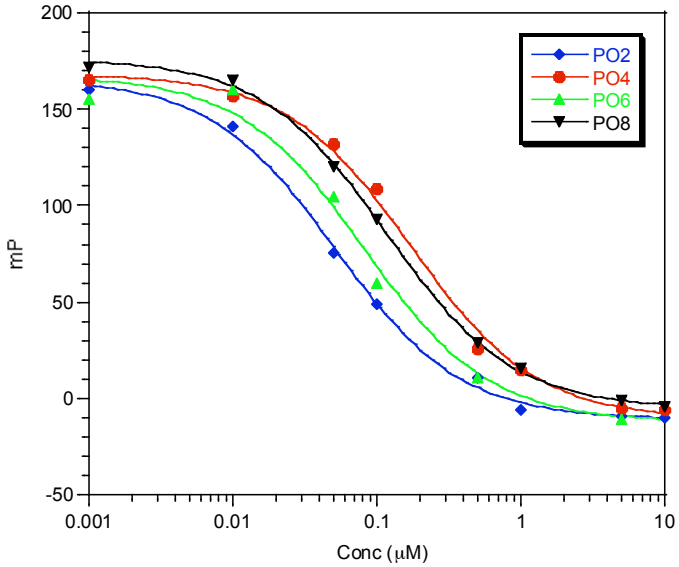


Figure S4. Fluorescence polarization assay for *in vitro* GR binding affinity of **PO2**, **PO4**, **PO6** and **PO8**.

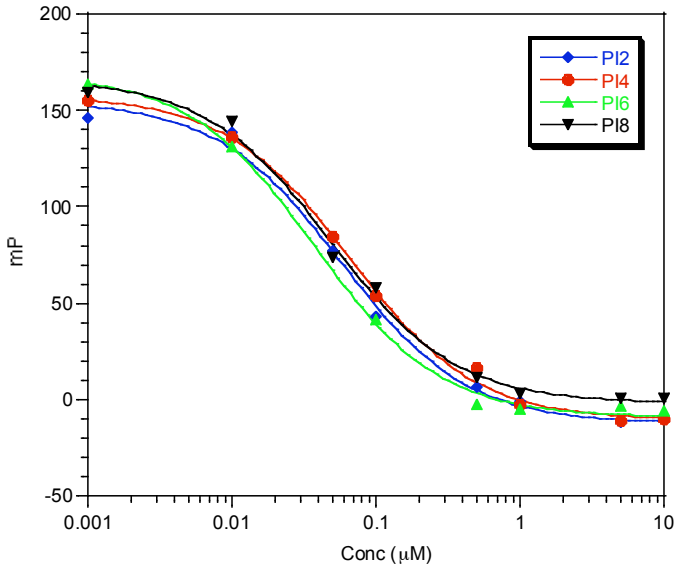


Figure S5. Fluorescence polarization assay for *in vitro* GR binding affinity of **PI2**, **PI4**, **PI6** and **PI8**.

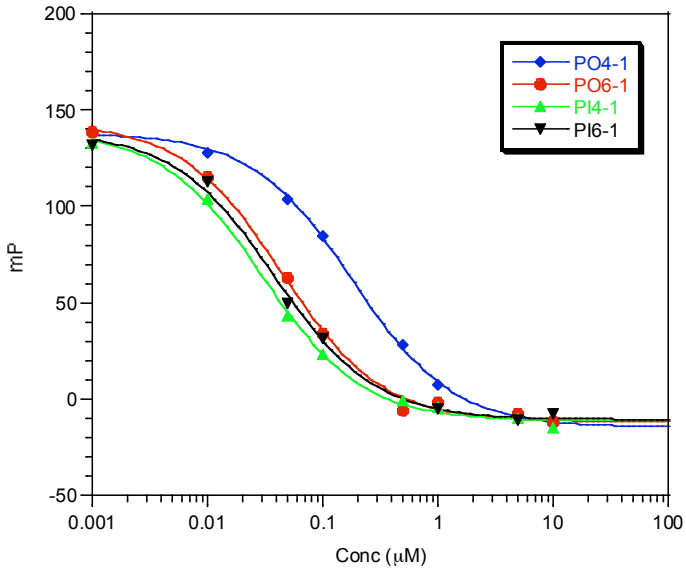


Figure S6. Fluorescence polarization assay for *in vitro* GR binding affinity of **PO4-1**, **PO6-1**, **PI4-1** and **PI6-1**.

Cell culture, DNA transfection and luciferase assays using HeLa cells

HeLa cells (~2.0 * 10^4 cells/well) were split in 96-well plates and maintained at 37 °C under 5% CO_2 in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (vol/vol) fetal calf serum. After 1 day, cells were transfected in OPTI-MEM I medium by the Lipofectamine Plus method (Invitrogen) with 0.101 □g total DNAs including 0.05 □g of Gal4DBD-GRLBD-VP16, 0.05 □g of pG5B reporter plasmid and 1 ng of *Renilla reniformis* luciferase plasmid (pRL-SV40) which were prepared as previously mentioned.[3] After DNA transfection, cells were maintained at 37 °C under 5% CO_2 for 3h. Cells in DMEM were treated with SDex-COOH conjugates dissolved in DMSO at various concentrations. Luciferase assays were conducted 40 h after treatment of each compound with a dual-luciferase reporter assay system (Promega). The luminescence was measured on a Sirius luminometer (Berthold detection systems). The value obtained was normalized with respect to the *Renilla reniformis* luciferase luminescence. The result for the control cells to which no ligand was added was treated as 1, the baseline for the induction. Other results were normalized based on this baseline and finally presented

as fold of inductions. The concentration of SDex-COOH conjugate that results in a half-maximum increase of the induction level is defined as EC_{50}. The maximum induction level is defined as IND_{max}. The induction data were fit to the equation [$I = (C/(EC_{50} + C)) * (IND_{max} - 1) + 1$] where I is the fold of induction, C is the concentration of the test compound.

Figure S7. Dose-dependent induction of luciferase expression of **PO2**, **PO4**, **PO6** and **PO8**.

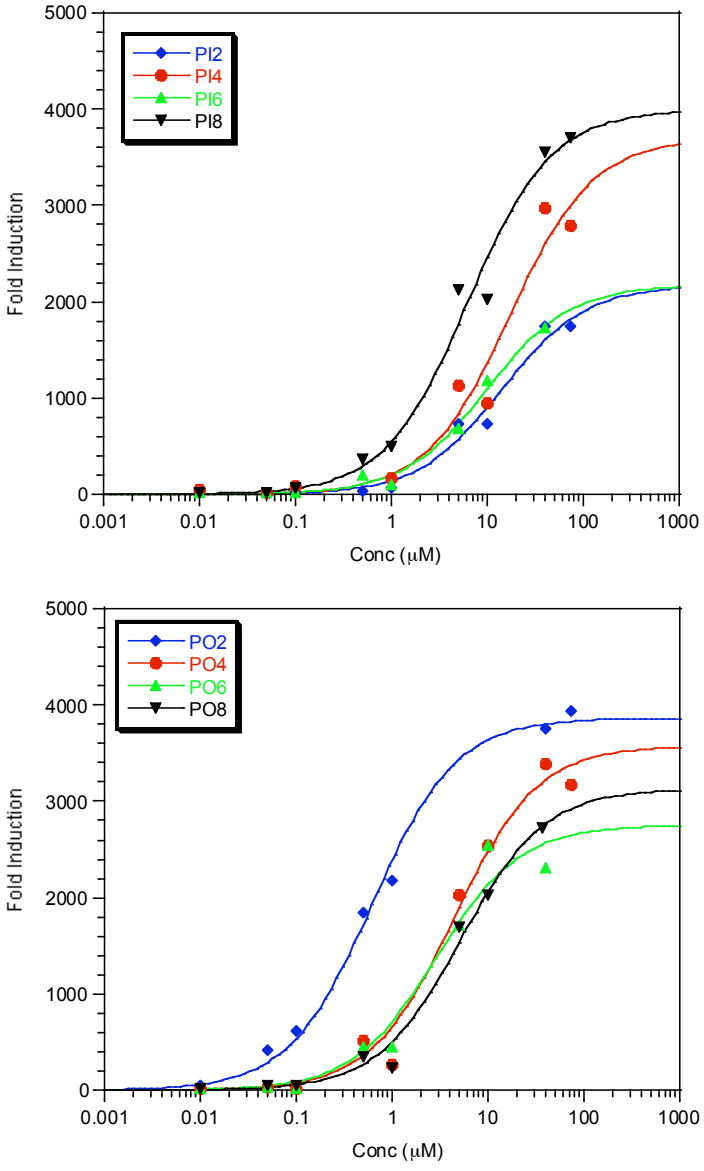


Figure S8. Dose-dependent induction of luciferase expression of **PI2**, **PI4**, **PI6** and **PI8**.

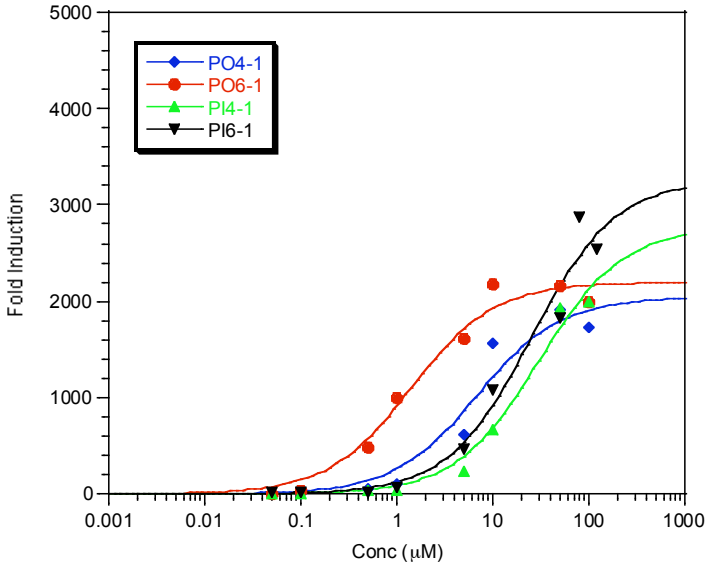


Figure S9. Dose-dependent induction of luciferase expression of **PO4-1**, **PO6-1**, **PI4-1** and **PI6-1**.

Cell culture, DNA transfection and luciferase assays using COS7 and MCF7 cells

Luciferase assays were similarly repeated using COS7 and MCF7 cells (~2.0 * 10^4 cells/well) which were transfected in OPTI-MEM I medium by the Lipofectamine Plus method (Invitrogen) with 20.5 ng total DNAs, including 10 ng of Gal4DBD-GRLBD-VP16, 10 ng of pG5B reporter plasmid and 0.5 ng of *Renilla reniformis* luciferase plasmid (pRL-SV40).[3]

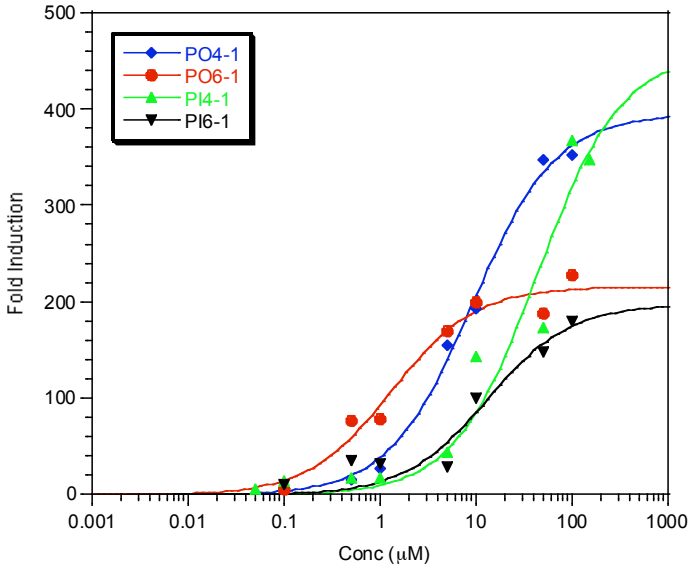


Figure S10. Dose-dependent induction of luciferase expression of **PO4-1**, **PO6-1**, **PI4-1** and **PI6-1** using COS7 cells.

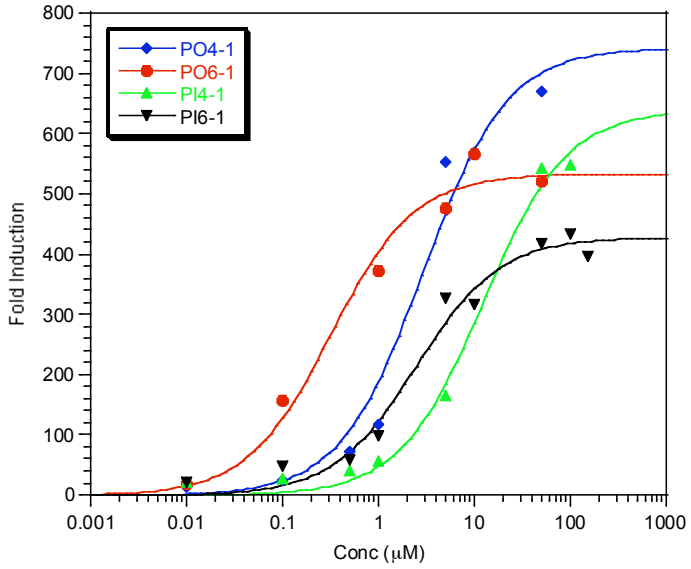


Figure S11. Dose-dependent induction of luciferase expression of **PO4-1**, **PO6-1**, **PI4-1** and **PI6-1** using MCF7 cells.

Table S2. Summary of parameters from *in vitro* GR binding affinity and luciferase assays using COS7 and MCF7 cells.

Compounds	*In vitro* GR binding affinity, IC_{50} (nM)	COS7 cells		MCF7 cells	
		Luciferase induction, EC_{50} (μM)	Relative permeability factor	Luciferase induction, EC_{50} (μM)	Relative permeability factor
PO4-1	182 ±12	9.1 ± 1.1	29.1	2.9 ± 0.8	25.7
PO6-1	44 ± 4	1.3 ± 0.3	11.6	0.32 ± 0.09	9.0
PI4-1	30 ± 2	43.7 ± 23.2		12.3 ± 2.4	
PI6-1	38 ± 4	13.0 ± 5.5		2.5 ± 0.5	

References

(1) Simons, S. S., Jr.; Pons, M.; Johnson, D. F. *J. Org. Chem.* **1980**, *45*, 3084-3088.

(2) (a) Olivos, H. J.; Alluri, P. G.; Reddy, M. M.; Salony, D.; Kodadek, T. *Org. Lett.* **2002**, *4*, 4057-4059. (b) Alluri, P. G.; Reddy, M. M.; Bachhawat-Sikder, K.; Olivos, H. J.; Kodadek, T. *J. Am. Chem. Soc.* **2003**, *125*, 13995-14004.

(3) Yu, P.; Liu, B.; Kodadek, T. *Nat. Biotechnol.* **2005**, *23*, 746-751.