

## Supplementary data

### **Simplified YM-26734 Inhibitors of Secreted Phospholipase A<sub>2</sub> Group IIA**

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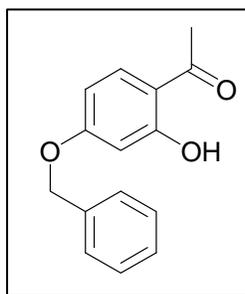
#### Table of Contents

1. Synthesis of YM-26734 and Derivatives	S2-S7
2. HPLC purification of YM-26734	S8-S9
3. <i>In vitro</i> Inhibition Assay	S10
4. Molecular Modeling	S11
5. References	S11

## Synthesis

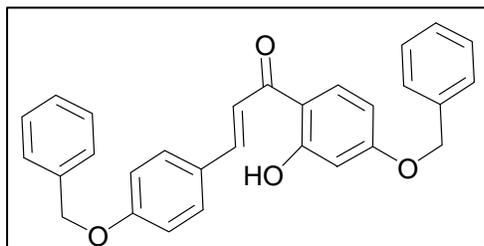
All reagents were purchased from commercial sources and used directly unless otherwise stated. Unless otherwise noted, reactions were performed under an atmosphere of dry nitrogen in oven dried glassware. Reactions were monitored for completeness by thin layer chromatography (TLC) using Merck 60F<sub>254</sub> silica plates and column chromatography was done with 60 Å silica gel purchased from Silicycle. <sup>1</sup>H NMR spectra were recorded on dilute solutions in CDCl<sub>3</sub>, CD<sub>3</sub>OD, CD<sub>3</sub>CN or DMSO-d<sub>6</sub>. NMR spectra were obtained on a Bruker AC-300 (300 MHz) and mass spectral data was obtained using a Bruker Daltonics Esquire electrospray-ion trap mass spectrometer (Bruker Esquire LC00066). Preparative reverse phase HPLC was performed on an automated Varian Prep Star system using a YMC S5 ODS column (20x100 mm, Waters Inc.). Unless otherwise noted, the following program was used to purify all final compounds tested for sPLA<sub>2</sub> inhibition: 0-60 minutes 50% CH<sub>3</sub>CN/50% H<sub>2</sub>O to 100% CH<sub>3</sub>CN, 60-90 minutes 100% CH<sub>3</sub>CN.

### YM-26734 and Derivatives



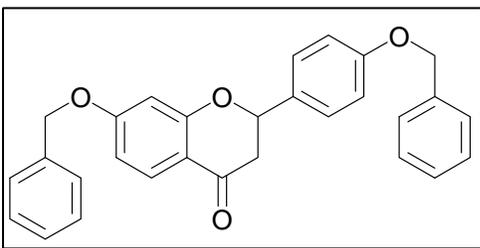
**1-(4-(benzyloxy)-2-hydroxyphenyl)ethanone (4).** Commercially available 2,4 dihydroxyacetophenone (**3**) ( 1.5g, 9.8 mmol) and K<sub>2</sub>CO<sub>3</sub> (1.63 g, 11.8 mmol) were added to 20 ml of dry CH<sub>3</sub>CN and heated to reflux for 1 hour. The reaction mixture was cooled to room temperature and Benzyl bromide (1.4mL, 11.8 mmol), dissolved in 10mL dry CH<sub>3</sub>CN, was added dropwise to the reaction mixture. After dropwise addition, the reaction mixture was refluxed for 2 hours and then cooled and poured onto 30 ml CH<sub>2</sub>Cl<sub>2</sub>. This mixture was washed with 3 x 20 ml H<sub>2</sub>O. The organic layer was dried over MgSO<sub>4</sub>, filtered, and the solvent was removed by rotary evaporation to

afford a white solid that was purified by column chromatography on silica gel (5% EtOAc/95% Hexanes) to afford **4** as a pale white solid (2.04 g, 86% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 2.56 (s, 3H), 5.09 (s, 2H), 6.53 (m, 2H), 7.33-7.41 (m, 5H), 7.64 (d, J = 7.8 Hz, 1H).



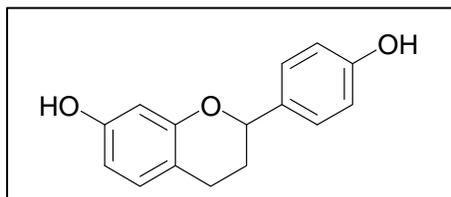
**1-(4-(benzyloxy)-2-hydroxyphenyl)-3-(4-(benzyloxy)phenyl)prop-2-en-1-one (6).** The benzyl protected acetophenone **4** (2.04 g, 8.42 mmol) and 4-benzyloxybenzaldehyde **5** (1.79g, 8.42 mmol) were suspended in 25 mL MeOH and heated to 50°C with stirring to help dissolve both starting materials. 25 mL of 40% KOH was then added and the reaction mixture was heated to reflux for 24 hours. Heat was removed and the

reaction mixture was poured onto 40 ml EtOAc and 40 mL H<sub>2</sub>O. The layers were separated and the aqueous layer was extracted with 3 x 20 mL EtOAc. The organic layers were combined, dried over MgSO<sub>4</sub>, filtered, and the solvent was removed by rotary evaporation. The crude yellow solid was purified by column chromatography on silica gel (10% EtOAc/90% Hexanes) to give **6** as a flaky yellow solid (2.58 g, 70% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 5.11 (d, J = 2.7 Hz, 4H), 6.53 (m, 2H), 7.02 (d, J = 8.7 Hz, 2H), 7.34-7.48 (m, 11H), 7.61 (d, J = 8.7 Hz, 2H), 7.82-7.89 (m, 2H).



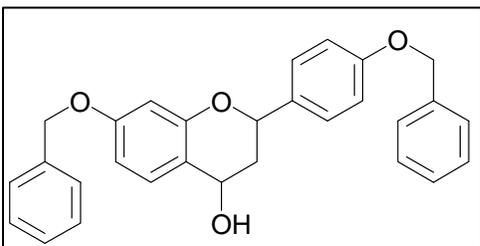
**7-(benzyloxy)-2-(4-(benzyloxy)phenyl)-2,3-dihydrochromen-4-one (7).** Compound **6** (155 mg, 0.24mmol) was added to 95 mL 10% H<sub>2</sub>SO<sub>4</sub> in MeOH and relaxed for 16 hrs. The reaction mixture was cooled to room temperature and poured onto 40mL EtOAc and 100mL saturated NaHCO<sub>3</sub>. The layers were separated and the aqueous layer was extracted with

2 x 30 mL EtOAc. The organic layers were combined, dried over MgSO<sub>4</sub>, filtered, and the solvent was removed by rotary evaporation. The crude light yellow solid was purified by column chromatography over silica gel (20% EtOAc/80% Hexanes) to afford **7** as a white solid (46 mg, 45% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 2.79 (dd, J = 16.8 Hz, J = 3.0 Hz, 1H), 3.05 (dd, J = 16.8 Hz, J = 13.2 Hz, 1H), 5.11 (d, J = 3.0 Hz, 4H), 5.41 (dd, J = 13.2 Hz, J = 2.7 Hz, 1H), 6.55 (d, J = 2.4 Hz, 1H), 6.69 (d, J = 8.8 Hz, 1H), 7.02 (d, J = 8.7 Hz, 2H), 7.34-7.48 (m, 11H), 7.88 (d, J = 8.7 Hz, 1H).



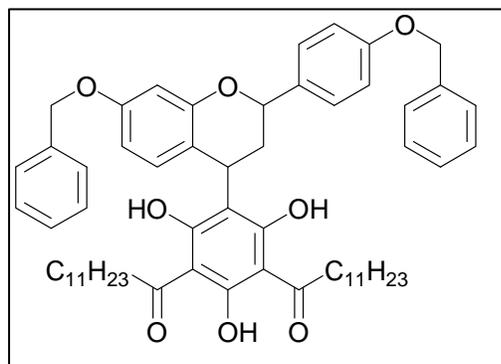
**7-4'-dihydroxyflavan.** Pd(OH)<sub>2</sub>-C (75 mg) was added to **7** in 7 ml 1:1 MeOH/THF and stirred under 1 atm H<sub>2</sub> for 2 hrs. The solvent was removed by rotary evaporation and the crude material was re-dissolved in 2mL 80% EtOAc/20% Hexanes and purified by column chromatography over silica gel to give a white solid

(26.8mg, 60% yield). A portion of this material was purified by HPLC using the following program: 0-5 minutes 25% MeOH/75% H<sub>2</sub>O, 5-30 minutes 25% MeOH/75% H<sub>2</sub>O to 100% MeOH, 30-35 minutes 100% MeOH. The solvent was removed from the fractions containing product by Speed-vac to afford a white solid (8.16 mg). <sup>1</sup>H NMR (300 MHz, MeOD) δ 1.93-2.16 (m, 2H), 2.64-2.72 (ddd, 1H), 2.82-2.94 (m, 1H), 4.92 (dd, J = 9.6 Hz, J = 2.1 Hz, 1H), 6.26 (d, J = 2.4 Hz, 1H), 6.32 (dd, J = 8.1 Hz, J = 2.4 Hz, 1H), 6.79 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.4 Hz 1H), 7.24 (d, J = 8.7 Hz, 2H). MS (ESI) 241 (MS-1).



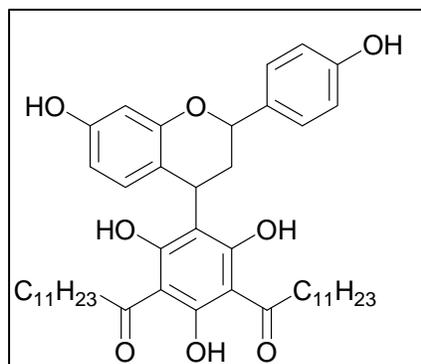
**7-(benzyloxy)-2-(4-(benzyloxy)phenyl)-3,4-dihydro-2H-chromen-4-ol (8).** Compound **7** (78.4 mg, 0.179 mmol) was dissolved in 7.5 ml of 1:1 MeOH/1,4 Dioxane and stirred under N<sub>2</sub> at 0°C. NaBH<sub>4</sub> (34 mg, 0.898 mmol) was added to the reaction mixture and it was stirred at 0°C for 4 hours. The reaction mixture was poured onto 20 mL EtOAc and washed with 3 x 15 ml ice cold 0.5 N HCl. The EtOAc layer was dried over

MgSO<sub>4</sub>, filtered, and the solvent was removed by rotary evaporation to afford **8** as a white solid (75.7mg, 96% yield). Product easily decomposes to give a bright pink color in mild to strong acidic conditions. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 2.04-2.24 (m, 1H), 2.44-2.51 (m, 1H), 4.99-5.12 (m, 6H), 6.50 (s, 1H), 6.64 (d, J = 8.7 Hz, 1H), 6.99, (d, J = 8.7 Hz, 2H), 7.31-7.45 (m, 13H).



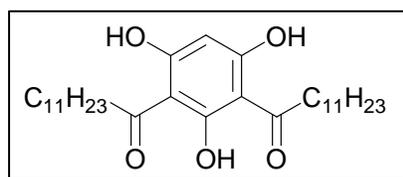
**7-(benzyloxy)-2-(4-(benzyloxy)phenyl)-4-(2,4,6-trihydroxy-3,5-(didodecanoyl)-phenyl)chroman (10).** Compound **9** (123 mg, 0.250 mmol) was suspended in 2.5 ml 4N HCl in Dioxane and stirred to dissolve most of the starting material. This mixture was added to compound **8** (73.5 mg, 0.167 mmol) in a 10 ml round bottom flask and stirred at room temperature for 4 hours. The reaction mixture was then cooled on ice and the pH of the reaction was made neutral by addition of 1.5N NaOH. The reaction mixture was then poured onto 15 ml H<sub>2</sub>O and 15ml EtOAc in a separatory funnel.

The layers were separated and the aqueous layer was washed 3 x 15 ml EtOAc. The organic layers were combined and dried over MgSO<sub>4</sub>, filtered, and the solvent was removed by rotary evaporation to afford a brown solid that was further purified by column chromatography over silica gel (95% Hexanes, 5% EtOAc) to afford **10** as a white solid (16.5mg, 11% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.85-0.922 (m, 6H), 1.25 (s, 32H), 1.58-1.69 (4H), 2.04-2.34 (m, 2H), 2.92 (t, J = 7.5 Hz, 2H), 3.14(t, J = 7.5 Hz, 2H), 4.64 (t, 0.23H), 5.04-5.22 (m, 6H), 6.12 (bs, 1H), 6.59-6.71 (m, 2H), 6.95-7.08 (m, 3H), 7.27-7.38 (m, 12H). MS (ESI) 910 (M-1).



**7-(hydroxy)-2-(4-(hydroxy)phenyl)-4-(2,4,6-trihydroxy-3,5-(didodecanoyl)-phenyl)chroman (YM-26734 or 1).** Compound **10** (6.8mg, 0.007 mmol) was dissolved in 2ml of 1:1 THF: MeOH. 5mg of 20% Pd(OH)<sub>2</sub>/C was added to the reaction mixture and it was stirred under 1 atm H<sub>2</sub> for 1.5 hours. The reaction mixture was then filtered over celite to remove the Pd(OH)<sub>2</sub>/C. The solvent was removed by rotary evaporation and the crude solid was purified by column chromatography over silica gel (30% EtOAc, 70% Hexanes) to afford **1** as a clear oil (2.6 mg, 47% yield). <sup>1</sup>H NMR (300

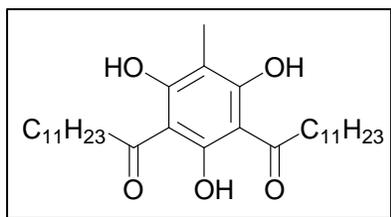
MHz, CDCl<sub>3</sub>) δ 0.88 (t, J = 6.9 Hz, 6H), 1.25 (s, 32H), 1.58-1.69 (4H), 2.07-2.39 (m, 2H), 2.92 (t, J = 7.2 Hz, 2H), 3.14(t, J = 7.5 Hz, 2H), 4.64 (t, 0.29H), 4.76-4.82 (m, 2H), 5.04-5.15 (m, 1.6H), 5.21 (m, 0.31H), 6.09 (bs, 1H), 6.43-6.57 (m, 2H), 6.80-6.87 (m, 2H), 6.97-7.04 (m, 1H), 7.34 (d, J = 8.4 Hz, 2H). MS (ESI) 729 (M-1).



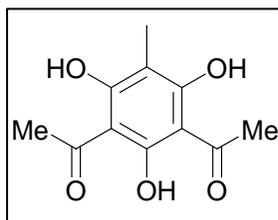
**2,4-(didodecanoyl)-1,3,5-trihydroxybenzene (9).**

Commercially available phloroglucinol (**11a**) (785mg, 6.22mmol) and lauric anhydride (5g, 13.07 mmol) were mixed together in 15ml BF<sub>3</sub>·OEt<sub>2</sub> and stirred overnight at 100°C. The reaction mixture was then poured onto 12.5g of NaOAc in 50ml H<sub>2</sub>O. The layers were separated and the aqueous layer was extracted with 3 x 25 ml ether. The ether layers were combined and washed with 3 x 25 mL NaHCO<sub>3</sub> and then 1 x 20 ml H<sub>2</sub>O. The ether layer was dried over MgSO<sub>4</sub>, filtered, and the solvent was removed by rotary evaporation to afford a brown oil that was further purified by column chromatography over silica gel (80% Hexanes, 20% EtOAc) to afford **9** as a

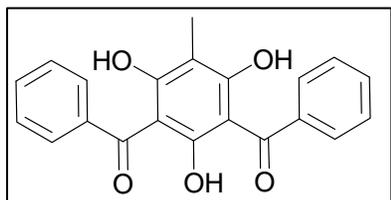
white/yellow solid (1.16g, 38% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.88 (t,  $J = 6.9$  Hz, 6H), 1.25 (s, 32H), 1.68 (q,  $J = 7.2$  Hz, 4H), 3.07 (t,  $J = 7.5$  Hz, 4H), 5.76 (s, 1H).



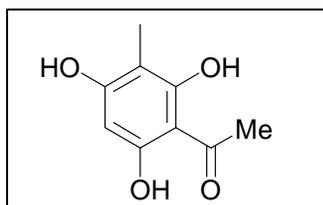
**3,5-(didodecanoyl)-2,4,6-trihydroxytoluene (17b).** This compound was prepared by a method analogous to **2,4-(didodecanoyl)-1,3,5-trihydroxybenzene** using commercially available 2,4,6-trihydroxytoluene and lauric anhydride.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.88 (t,  $J = 6.9$  Hz, 6H), 1.25 (s, 32H), 1.68 (q,  $J = 7.2$  Hz, 4H), 2.06 (s, 3H), 3.10 (t,  $J = 7.2$  Hz, 4H). MS (ESI) 503 (M-1).



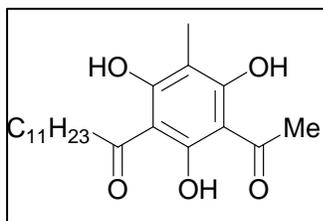
**3,5-(diacetyl)-2,4,6-trihydroxytoluene (17c).** This compound was prepared by a method analogous to **2,4-(didodecanoyl)-1,3,5-trihydroxybenzene** using commercially available 2,4,6-trihydroxytoluene and acetic anhydride. HPLC purification method was as follows: 0-5 minutes, 30% ACN/70%  $\text{H}_2\text{O}$ ; 5-35 minutes, 30% ACN/70%  $\text{H}_2\text{O}$ –100% ACN.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  2.09 (s, 3H), 2.74 (s, 6H).



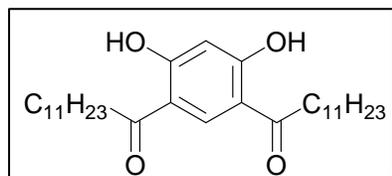
**3,5-(dibenzoyl)-2,4,6-trihydroxytoluene (17d).** This compound was prepared by a method analogous to **2,4-(didodecanoyl)-1,3,5-trihydroxybenzene** using commercially available 2,4,6-trihydroxytoluene and benzoic anhydride. HPLC purification method was as follows: 0-5 minutes, 30% ACN/70%  $\text{H}_2\text{O}$ ; 5-35 minutes, 30% ACN/70%  $\text{H}_2\text{O}$ –100% ACN.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  2.08 (s, 3H), 7.50 (m, 4H), 7.59 (m, 2H), 7.66 (d,  $J = 6.9$  Hz, 4H), 10.39 (s, 2H). MS (ESI) 347 (M-1).



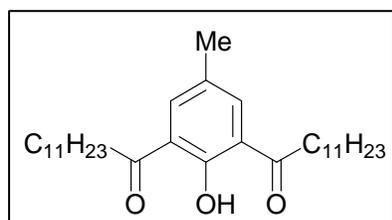
**3-acetyl-2,4,6-trihydroxytoluene (17e).** This compound was prepared by a method analogous to **2,4-(didodecanoyl)-1,3,5-trihydroxybenzene** using commercially available 2,4,6-trihydroxytoluene and acetic anhydride.  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{CN}$ )  $\delta$  1.93 (s, 3H), 2.60 (s, 3H), 5.96 (s, 1H), 7.53 (s, 1H), 8.18 (s, 1H). MS (ESI) 181 (M-1).



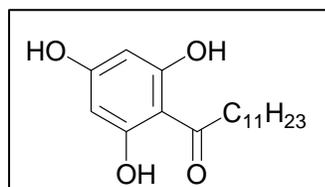
**3-acetyl-5-dodecanoyl-2,4,6-trihydroxytoluene (17f).** Compound **17e** was acylated with lauric acid chloride using a similar procedure to the preparation of **2-(dodecanoyl)-1,3,5-trihydroxybenzene**. HPLC purification method was as follows: 0-5 minutes, 30% ACN/70%  $\text{H}_2\text{O}$ ; 5-35 minutes, 30% ACN/70%  $\text{H}_2\text{O}$ –100% ACN, 35-50 minutes, 100% ACN.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.90 (t,  $J = 6.6$  Hz, 3H), 1.25-1.40 (s, 16H), 1.70 (m, 2H), 2.09 (s, 3H), 2.74 (s, 3H), 3.25 (t,  $J = 7.5$  Hz, 2H). MS (ESI) 363 (M-1).



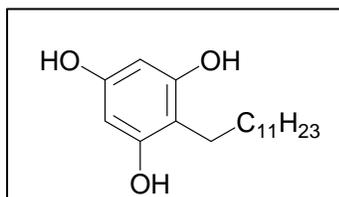
**2,4-(didodecanoyl)-1,5-dihydroxybenzene (12b).** Lauric acid (1g, 5mmol) was heated to 60°C in a round bottom flask. ZnCl<sub>2</sub> (1.36 g, 10mmol) was added to the heated lauric acid and the reaction suspension was heated for 30 minutes at 110°C. Commercially available 1,3-dihydroxybenzene (**11b**) (550mg, 5mmol) and lauric acid (3g, 15 mmol) were mixed together and added to the heated suspension of lauric and ZnCl<sub>2</sub>. This mixture was heated to 160°C and stirred at this temperature for 24 hours. This mixture was then suspended in 10mL of 10% EtOAc/90% Hexanes and purified by column chromatography over silica gel (90% Hexanes/10% EtOAc) to afford a light brown solid (253mg, 11% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.89 (t, J = 6.9 Hz, 6H), 1.25 (s, 32H), 1.78 (q, J = 7.2 Hz, 4H), 2.96 (t, J = 7.5Hz, 4H), 6.45 (s, 1H), 8.28 (s, 1H). MS (ESI) 473 (M-1).



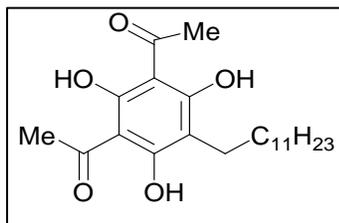
**2,4-(didodecanoyl)-1-hydroxy-2-methylbenzene (18b).** Approximately 2g (10mmol) of lauric acid chloride was prepared as described in the preparation of compound **13**. This was dissolved in 1mL nitrobenzene and added dropwise to a solution of p-cresol (410mg, 3.8mmol) and AlCl<sub>3</sub> (2.5g, 18.8mmol) in 4mL nitrobenzene at 0° C. The reaction mixture was raised to room temperature and stirred for one hour, and then the temperature was increased to 40° C and stirred for another hour. The temperature was then raised to 60° C and refluxed for an additional 5 hours. The flask was put on ice and 12mL of 6N HCl was added over several minutes, interspersed with the addition of 10mL nitrobenzene. The mixture was transferred to a separatory funnel, and the organic layer was collected. Nitrobenzene was distilled off by vacuum distillation at 100° C and 15 torr for one hour. Compound was purified on a 40g silica column using an automated Combiflash Rf machine (flow rate: 40mL/minute, 0-10 minutes, 100% hexanes, 10-25 minutes, 100% hexanes – 95% hexanes/5% EtOAc). Purified by HPLC under general method listed at top, but compound did not elute. Compound was then eluted off column using 100% MeOH to give pure **18b** as a clear oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.90 (t, J = 6.9 Hz, 6H), 1.25-1.4 (m, 32H), 1.7-1.8 (m, 4H), 2.35 (s, 3H), 3.05 (t, J = 7.5Hz, 4H), 7.76 (s, 2H). MS (ESI) 473 (M+1).



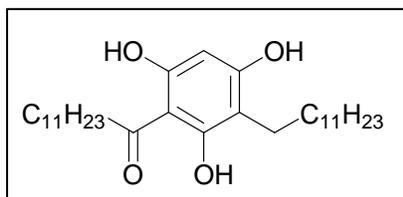
**2-(dodecanoyl)-1,3,5-trihydroxybenzene (13).** Lauric acid (2g, 10mmol) was dissolved in 5ml SOCl<sub>2</sub> and refluxed for 2 hours. The thionyl chloride solvent was removed by rotary evaporation. The lauric acid chloride product was added to a suspension of trihydroxybenzene (1.04g, 8.2mmol) and AlCl<sub>3</sub> (2.44g, 18.2 mmoles) in 20 ml dry CH<sub>2</sub>Cl<sub>2</sub>. After 3 hours stirring, the reaction mixture was poured onto 50ml H<sub>2</sub>O in a separatory funnel and extracted with 3 x 30 ml EtOAc. The organic layers were combined, dried over MgSO<sub>4</sub>, filtered, and the solvent was removed by rotary evaporation to afford a yellow oil that was further purified by column chromatography over silica gel (70% Hexanes, 30% EtOAc) to afford a white/yellow solid (1.12g, 44% yield). <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-DMSO) δ 0.88 (t, J = 6.9 Hz, 3H), 1.25 (s, 16H), 1.56 (m, 2H), 2.96 (t, J = 6.9 Hz, 2H), 5.76 (s, 2H), 10.35 (s, 1H).



**2-(decanoyl)-1,3,5-trihydroxybenzene (14).** Compound **13** (500mg, 1.62mmol) was added to a mixture containing 10ml conc. HCl, 5ml toluene, 5ml EtOH, and ZnHg amalgam (10g, prepared from 10g Zinc dust, 1g HgCl mixed in 5ml concentrated HCl and 15 ml H<sub>2</sub>O). This suspension was refluxed for 24 hrs with 1.5 ml additions of concentrated HCl every 8 hours. The reaction mixture was cooled to room temperature and the organic phase was separated from the reaction mixture. The organic phase was then poured onto 20ml brine and extracted with 3 x 20ml ether. The ether layers were combined, dried over MgSO<sub>4</sub>, filtered, and the solvent was removed by rotary evaporation. The crude mixture was purified by column chromatography over silica gel (70% Hexanes, 30% EtOAc) to afford **14** as a white solid that readily decomposes to a yellow/brown color when exposed to air in dry form. (274mg, 57% yield). <sup>1</sup>H NMR (300 MHz, MeOD) δ 0.89 (t, J = 6.9 Hz, 3H), 1.25 (s, 16H), 1.46 (m, 2H), 2.48 (t, J = 7.5 Hz, 2H), 5.84 (s, 2H). MS (ESI) 293 (M-1).



**2,4-(diacetyl)-6-(dodecanyl)-1,3,5-trihydroxybenzene (16).** This compound was prepared from **2-(decanoyl)-1,3,5-trihydroxybenzene** using a method analogous to the preparation of **3,5-(diacetyl)-2,4,6-trihydroxytoluene**. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.90 (t, J = 6.9 Hz, 3H), 1.25 (s, 16H), 1.46 (m, 2H), 2.56 (t, J = 7.7 Hz, 2H), 2.74 (s, 6H). MS (ESI) 377 (M-1).



**2-(dodecanoyl)-4-(dodecanoyl)-1,3,5-trihydroxybenzene (15).** This compound was prepared by a method analogous to the preparation of **2-(dodecanoyl)-1,3,5-trihydroxybenzene**. <sup>1</sup>H NMR (300 MHz, MeOD) δ 0.91 (t, J = 6.9 Hz, 6H), 1.25 (s, 32H), 1.46 (m, 2H), 1.67 (m, 2H), 2.49 (t, J = 7.5 Hz, 2H), 3.04 (t, J = 7.8Hz, 2H), 5.89 (s, 1H). MS (ESI) 477 (M+1).

## **Separation of YM-26734 Stereoisomers**

**Separation of Diastereomers:** 3mg of YM26734 was dissolved in 800ul DMF and purified by HPLC using the following program: 0-5 minutes (60% CH<sub>3</sub>CN/40% H<sub>2</sub>O), 5-50 minutes (60% CH<sub>3</sub>CN/40% H<sub>2</sub>O-100% CH<sub>3</sub>CN), 50-100 minutes (100% CH<sub>3</sub>CN). One of the diastereomers was separated from the shoulder of the other diastereomer peak. The diastereomers were labeled “cis” and “trans” based on the assignments made from the patent for this compound.<sup>1</sup>

*cis*-YM-26734 (+ +/- -): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.88 (t, J = 6.9 Hz, 6H), 1.25 (s, 32H), 1.58-1.69 (4H), 2.07-2.31 (m, 2H), 2.89-2.96 (m, 4H), 3.14(t, J = 7.5 Hz, 2H), 4.79 (s, 1H), 4.87 (s, 1H), 5.04-5.15 (m, 2H), 6.09 (bs, 1H), 6.43-6.57 (m, 2H), 6.85 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 8.7 Hz, 1H), 7.34 (d, J = 8.4 Hz, 2H). MS (ESI) 729 (M-1).

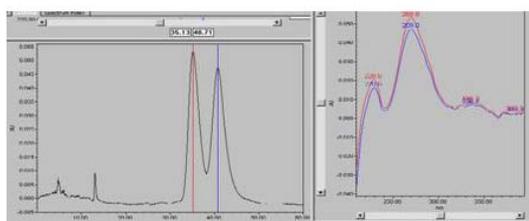
*trans*-YM-26734 (+ -/- +): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.88 (t, J = 6.9 Hz, 6H), 1.25 (s, 32H), 1.58-1.69 (4H), 2.01 (m, 1H), 2.28-2.39 (m, 2H), 2.91 (m, 2H), 3.12(m, 2H), 4.61 (t, 1H), 4.71 (bs, 1H), 4.84 (bs, 1H), 5.21 (m, 1H), 6.47 (d, J = 8.1 Hz, 1H), 6.57 (s, 1H), 6.81 (d, J = 8.4 Hz, 2H), 7.02 (d, J = 8.1 Hz, 1H), 7.24 (d, J = 8.4 Hz, 2H). MS (ESI) 729 (M-1).

**Separation of Enantiomers (+ +, - -, + -, - +):** Separation of enantiomers was performed by HPLC using a Daicel Chirex column consisting of N-(3,5-dimethylphenyl)-acetamide attached to cellulose-coated silica as the stationary phase (ODH0CE-FB025). The column was equilibrated with 95% Hexane/5% Isopropanol and the enantiomers were eluted isocratically at 1 ml/min. Elution of each enantiomer was monitored by UV fluorescence at 280 nm. Peaks corresponding to the enantiomers were collected, and the solvent was removed under N<sub>2</sub>. Multiple HPLC runs were performed to generate enough pure enantiomer for quantification and *in vitro* studies. Quantification of each enantiomer was performed using NMR with 0.1uL spike of DMSO and a pulse delay of 17.5 seconds. See below graphs for elution time of each enantiomer. The *cis*- and *trans*- enantiomers were arbitrarily labeled (+ +) and (- -) and (+ -) and (- -) respectively. Determination of absolute stereochemistry was not performed.

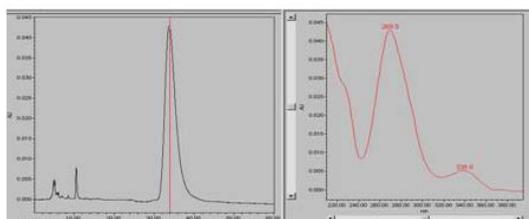
The HPLC and UV traces below show final purity of the diastereomers, *cis*-enanatiomers and *trans*-enanatiomers of YM-26734 used for *in vitro* studies.

## Separation of cis-YM26734

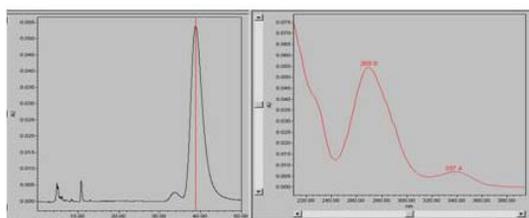
### Separation of Enantiomers (OD-H Analytical Column)



Racemic Mixture



(+ +)



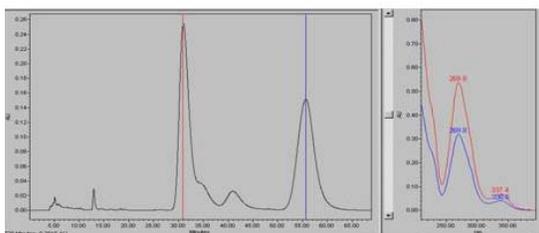
(- -)

≈97.6% Pure

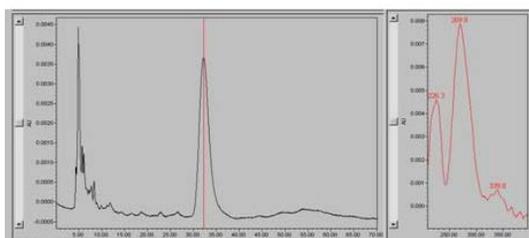
≈ 95% ee

## Separation of trans-YM26734

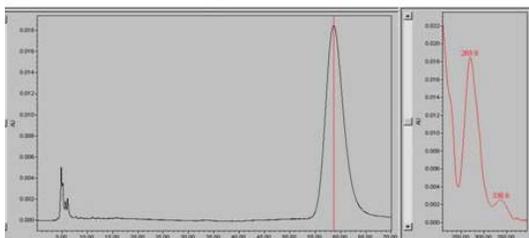
### Separation of YM2 Enantiomers (OD-H Analytical Column)



Racemic Mixture



(+ -)



(- +)

## ***In vitro* Fluorometric Assay of sPLA<sub>2</sub> Inhibition<sup>2</sup>**

**Preparation of pyrPG vesicle stock solution.** 1mg of 1-hexadecanoyl-2-(10-pyrenedecanoyl)-*sn*-glycero-3-phosphoglycerol (pyr PG) (prepared in house, but can also be purchased from Molecular Probes, Cat. No. H3809) was dissolved in 1mL toluene: ethanol (1:1 solution) (isopropanol can be used in place of ethanol) and vortexed and sonicated repeatedly while also warming tube with hands to aide solubility of pyrPG (assuming that all of the pyrPG goes into solution, the concentration of this Stock Solution should be about 1.168mM (FW=856.09g/mol for pyrPG)). This stock solution is stored in the -20C.

**Preparation of pyrPG working solution.** 300uL of the pyrPG stock solution is taken and added to a 5mL glass vial. The solvent is removed by Speed-vac and dissolved in 1mL 100% EtOH. This solution is vortexed and sonicated repeatedly while warming in hands to dissolve pyrPG. After sonication and vortexing for 5-10 minutes, the entire sample is transferred to an eppendorf tube and centrifuged at 13,000-14,000 rpm for 3 minutes to remove insoluble salts and particulates. The liquid layer is transferred to a clean vial and the Absorbance at 342 nm is measured. This is done by adding 10uL of the pyrPG solution to 500uL 100% EtOH in a quartz cuvette and measuring the absorbance at 342nm (100% EtOH is used as the blank). The extinction coefficient for pryPG at 342nm is  $40,000\text{cm}^{-1}\text{M}^{-1}$ . The final concentration of pyrPG should be between 100-200 uM pyrPG and the pyrPG concentration should not be below 100 uM (this would require the addition of more pyrPG into the assay, and consequently more EtOH which affects the specific activity of the sPLA<sub>2</sub>). When not in use, store at -20C.

**Assay Procedure:** To seven wells of a 96-well microtiter plate was added 100  $\mu\text{L}$  of solution A (27  $\mu\text{M}$  bovine serum albumin, 50 mM KCl, 1 mM  $\text{CaCl}_2$ , 50 mM Tris-HCl, pH 8.0) followed by the desired concentration of inhibitor (1  $\mu\text{L}$  in DMSO from serial-diluted stock solutions) or 1  $\mu\text{L}$  of DMSO for control reactions. To the first well was added an additional 100 uL of solution A as a negative control. Solution B was prepared by mixing the appropriate amount of sPLA<sub>2</sub>, depending on the specific activity, to Solution A. This solution was prepared immediately prior to each set of assays, to avoid loss of enzymatic activity due to sticking to the walls of the container. Solution B was delivered in 100  $\mu\text{L}$  portions to all seven wells except the first well. Quickly after the addition of Solution B, the assay was initiated by the addition of 100  $\mu\text{L}$  of Solution C (4.2 uM 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphoglycerol (pyrPG, Molecular Probes) vesicles in assay buffer (50 mM KCl, 1 mM  $\text{CaCl}_2$ , 50 mM Tris-HCl, pH 8.0)) to all seven wells. The fluorescence (excitation = 342 nm, emission = 395 nm) was read with a microtiter plate spectrophotometer (Fluorocount, Packard Instruments). Control reactions without enzyme or inhibitor were run with each assay of seven wells and the percent inhibition calculated from the initial slopes of fluorescence versus time. The amount of enzyme used per well are as follows: hGIIA, 6 ng; hGV, 12.5 ng; hGX, 17.7 ng; mGIIA, 3.4 ng; mGV, 12.7 ng; mGX, 10.7 ng; rGIIA, 3.8 ng. All of the recombinant mouse and human sPLA<sub>2</sub>s were prepared as described.<sup>3</sup> Recombinant rat group IIA sPLA<sub>2</sub> was obtained as a gift from M.Janssen (University of Utrecht, The Netherlands).<sup>4</sup>

## Molecular Docking of YM-26734

Docking was performed using the program FLO99<sup>5</sup>. YM-26734 was overlaid onto Indole 8 in the active site of hGIIA using the crystal structure reported by Schevitz and co-workers<sup>6</sup> (PDB: 1DB4). The inhibitor was manually positioned into the active site such that one of the dodecanoyl oxygens and the *para*-hydroxy group are directed towards the Ca<sup>2+</sup> ion in the active site. The other dodecanoyl oxygen and its vicinal hydroxyl group were oriented towards a neighboring lysine residue. The dodecanoyl chains were replaced by propanoyl chains to simplify inhibitor posing. Figure 2 was created using PyMol (DeLano, W.L. The PyMOL Molecular Graphics System (2002) DeLano Scientific, San Carlos, CA, USA. <http://www.pymol.org>).

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