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Simplified YM-26734 inhibitors of secreted phospholipase A_2 group IIA

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ABSTRACT

Simplified analogs of YM-26734, a known inhibitor of secreted phospholipase A_2 (sPLA$_2$) group IIA, were synthesized and found to also display potent inhibition at low nanomolar concentrations. Analogs were based on the didodecanoylphloroglucinol portion of YM-26734 which contains the predicted active site calcium binding group.

Secreted phospholipases A_2 (sPLA$_2$s) are a family of interfacial enzymes that catalyze the *sn*-2 esterolysis of glycerophospholipids.[1] Products of sPLA$_2$ enzymatic activity include arachidonic acid, a well-known precursor of pro-inflammatory eicosanoids.[1] Currently, there are ten known mammalian sPLA$_2$s, (groups IB, IIA, IIC (pseudo gene in humans), IID, IIE, IIF, III, V, X, XIIA) all of which contain a conserved active site that consists of a Ca^{2+} binding loop and an Asp-His catalytic dyad.[2,3] Over the past two decades there has been an increasing body of research implicating the involvement of sPLA$_2$s, especially groups IIA (GIIA), V, and X, in different inflammatory processes including arthritis,[4] asthma,[5] atherosclerosis,[6] and sepsis.[7] The involvement of one or more sPLA$_2$s in these diseases and other processes highlights a critical need to understand their biological functions.

One powerful approach towards elucidating sPLA$_2$ function is through the use of small molecule inhibitors. A number of different academic and industrial laboratories have explored small molecule active site inhibitors of sPLA$_2$.[8] Some of the most potent inhibitors, displaying low nanomolar potency, include the functionalized indole[9] and pyrazole[10] scaffolds from Eli Lilly, D-tyrosine analogs reported by Hansford and co-workers,[11] and the natural product analog YM-26734 (compound **1**, Fig. 1) from Yamanouchi Pharmaceuticals[12] (all reviewed in Ref. 1). With a number of potent inhibitor scaffolds available, our focus has turned towards the optimization of these inhibitors to increase cell permeability and/or selectivity among the sPLA$_2$ groups.

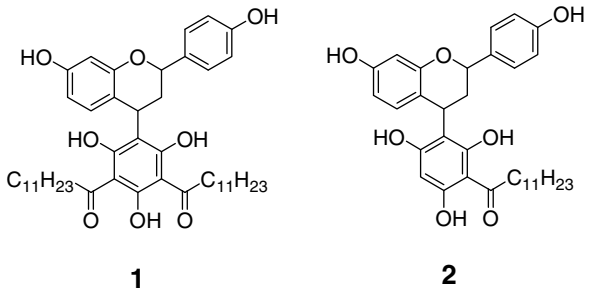

Figure 1. Potent GIIA sPLA$_2$ inhibitor YM-26734 (compound **1**) derived from YM-26567-1 (compound **2**). Data to assign chirality have not been published.

The natural product, YM-26567-1 (compound **2**, Fig. 1) isolated by Yamanouchi Pharmaceuticals from the fruit of *Horsfieldia amygdaline* was identified as a micromolar inhibitor of rabbit platelet sPLA$_2$.[13] Further development of this compound led to **1**, which had increased potency against rabbit platelet sPLA$_2$ with an IC_{50} of 85 nM.[12] Here, we further explore **1** as an inhibitor against mammalian sPLA$_2$ enzymes and elucidate its active site binding mode. This process led us to simplified analogs of **1** that displayed nearly identical inhibition potency against GIIA sPLA$_2$.

Compound **1** was prepared using a modified version of a previously reported procedure (Scheme 1).[14] Commercially available **3** was benzyl protected under basic conditions to yield **4** and subsequently refluxed with commercially available **5** in KOH to form the chalcone **6**. Compound **6** was refluxed in H_2SO_4/MeOH to form the

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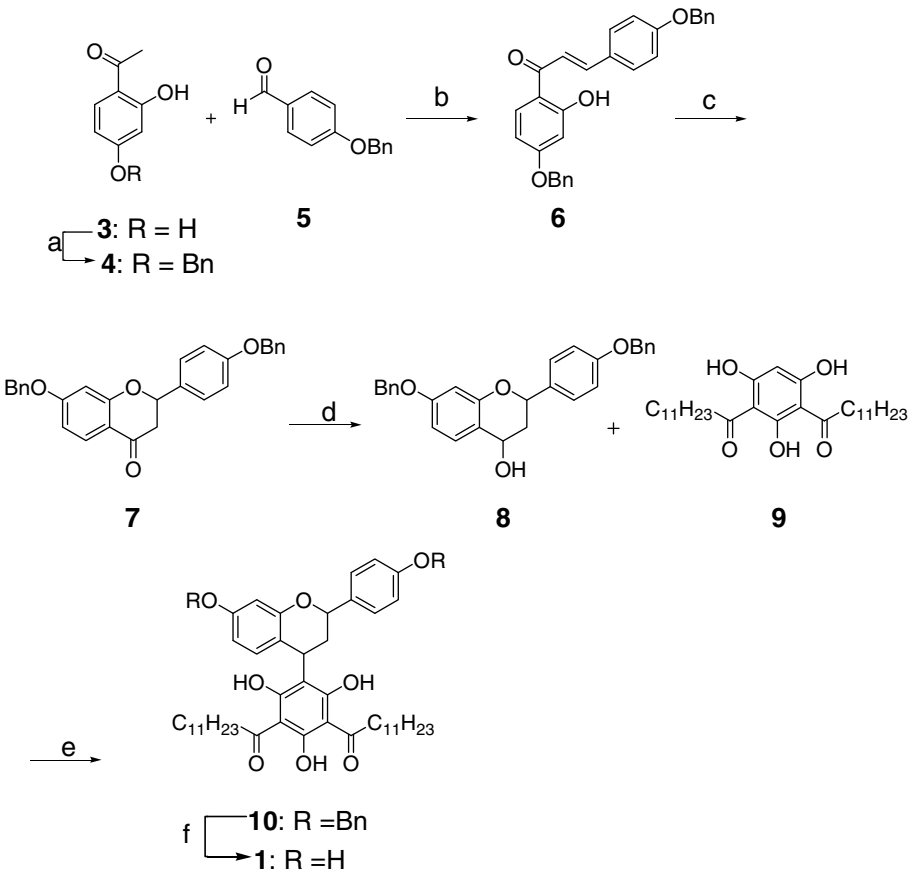
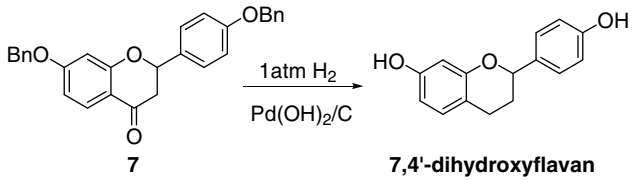


Scheme 1. Reagents: (a) BnBr, K_2CO_3; (b) 40% KOH; (c) 10% H_2SO_4/MeOH; (d) $NaBH_4$, MeOH; (e) 4 N HCl/dioxane; (f) 1 atm H_2, $Pd(OH)_2$/C.

flavanone **7** which was reduced with $NaBH_4$ to yield **8.** Compound **8** was condensed with **9** in HCl/dioxane to give **10**. Deprotection of **10** using $Pd(OH)_2$/C under H_2 afforded **1** as a mixture of four stereoisomers. Diastereomers were separated by HPLC using a reverse phase C18 column, and the enantiomers were isolated using a Daicel Chirex column (see Supplementary data). In addition, we prepared 7,4′-dihydroxyflavan from **7** under reducing conditions in H_2 and $Pd(OH)_2$/C (Scheme 2).

Di-acylation of phloroglucinol **11a** and resorcinol **11b** was done in either dodecanoic anhydride and $BF_3{\cdot}OEt_2$ or dodecanoic acid and $ZnCl_2$ to give **9** and **12b**, respectively (Scheme 3). Formation of **15** was done by monoacylating **11a** in dodecanoic acid chloride and $AlCl_3$ followed by complete reduction of the acyl group under Wolf Kishner conditions to give **14**. Monoacylation and di-acetylation of **14** were performed using dodecanoic acid chloride and $AlCl_3$ and acetic anhydride and $BF_3{\cdot}OEt_2$ to yield **15** and **16,** respectively. Compounds **17b–d**, **f**, and **18b** (Scheme 4) were prepared using similar chemistry as shown in Scheme 3.

Initially we tested **1** as a four-isomer mixture against human, mouse and rat GIIA, and human and mouse GV and GX $sPLA_2$ enzymes (Table 1) (see Supplementary data for all assay details).

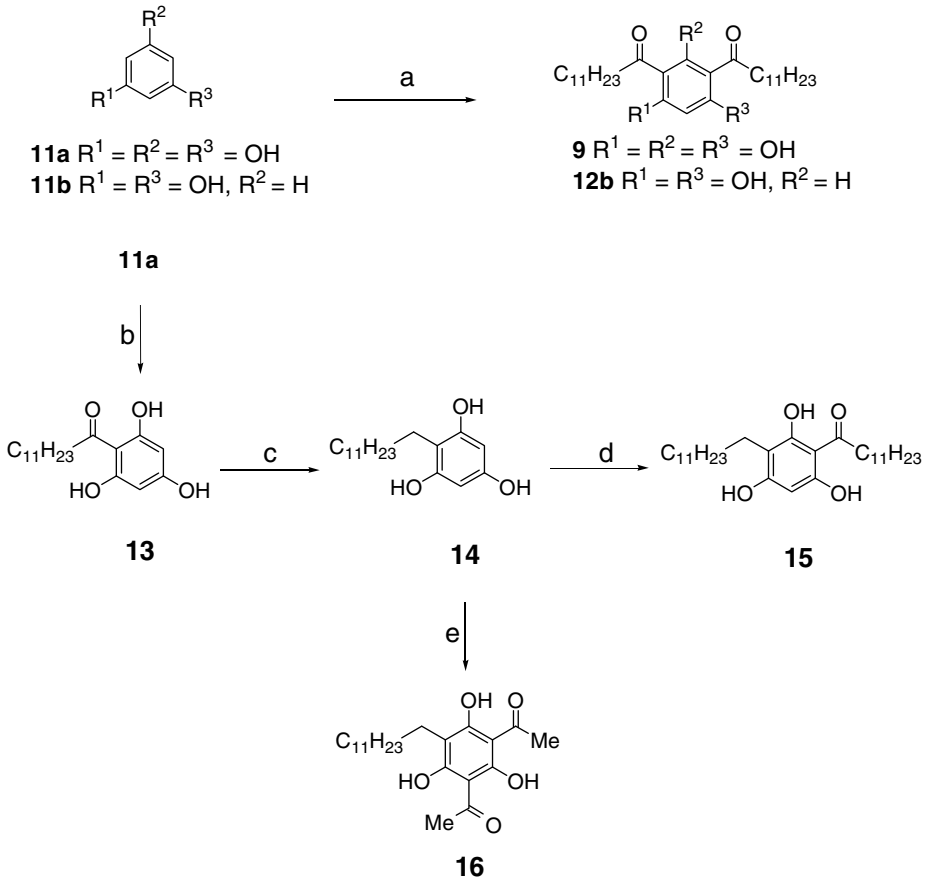


Scheme 2. Preparation of 7,4′-dihydroxyflavan.

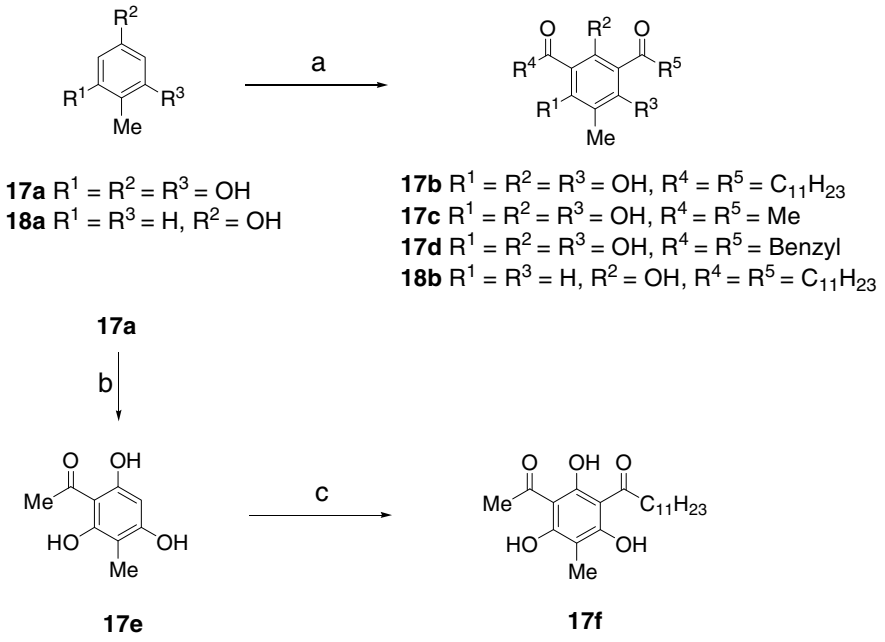
We found that **1** inhibited all GIIA enzymes and human GV at low nanomolar concentrations, displayed moderately potent inhibition against mouse GV and showed no inhibition of human and mouse GX at low micromolar concentrations. These results are consistent with the 85 nM IC_{50} value previously reported for rabbit GIIA $sPLA_2$.[12] However, Hamaguchi and co-workers recently reported IC_{50} values of 1 μM and 0.2 μM for **1** against GIIA and GX, respectively (the authors did not disclose whether this was human or mouse $sPLA_2$).[15] These discrepancies in potency are probably due to the differences in substrate and assay conditions used to obtain IC_{50} values.

In order to assess if one stereoisomer is more potent over the others, we isolated all four stereoisomers of **1** and tested them individually against rat GIIA $sPLA_2$ (Table 2). Interestingly, all four isomers of **1** had IC_{50}s between 60 and 120 nM. We found this surprising because one would expect the dramatic structural diversity between the four isomers to result in different binding affinities.

Intrigued by this result, we decided to model the binding of **1** in the active site of human GIIA $sPLA_2$. We manually positioned **1** into the GIIA active site by overlaying it onto Indole 8 from the co-crystal structure reported by Schevitz and co-workers (PDB code: 1DB4).[16] Compound **1** was oriented into the active site by creating a binding interaction between the oxygen from one of the dodecanoyl chains and the *para*-hydroxy group with the active site calcium ion involved in stabilizing the transition state (Fig. 2). We chose this binding pose to mimic the acetylacetonate bidentate ligands that coordinate to metals including calcium.[17] This positioned the other dodecanoyl oxygen and its vicinal hydroxyl group to contact a neighboring lysine residue (Fig. 2). In this orientation, the dodecanoyl alkyl chains are able to fill the active site as they extend out into the solvent.



Scheme 3. Reagents: (a) dodecanoic anhydride, $BF_3{\cdot}Et_2O$ or dodecanoic acid, $ZnCl_2$; (b) $C_{11}H_{23}COCl$, $AlCl_3$; (c) ZnHg, HCl; (d) $C_{11}H_{23}COCl$, $AlCl_3$; (e) acetic anhydride, $BF_3{\cdot}Et_2O$.



Scheme 4. Reagents: (a) alkyl or benzyl anhydride, $BF_3{\cdot}Et_2O$ or $C_{11}H_{23}COCl$, $AlCl_3$; (b) acetic anhydride, $BF_3{\cdot}Et_2O$; (c) $C_{11}H_{23}COCl$, $AlCl_3$.

Since the large structural variations of the four isomers of **1** are all located on the flavanoid moiety which is predicted to extend out of the active site, the binding affinity would not change among the isomers of **1**. The IC_{50} values of the four stereoisomers in Table 2 support this binding orientation.

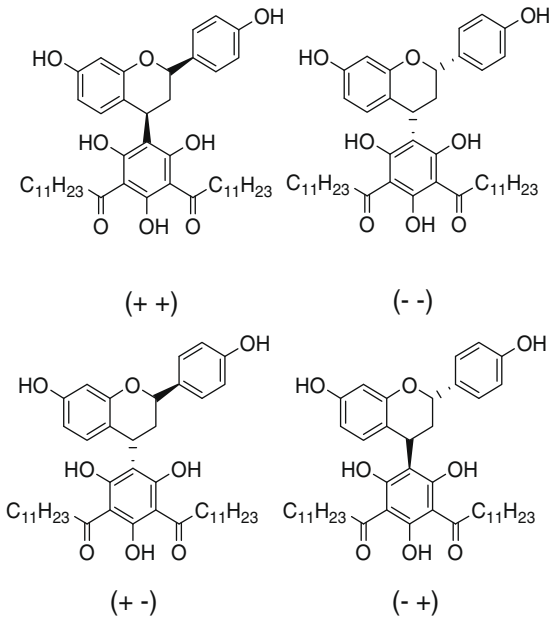
Didodecanoylphloroglucinol (**9**) and 7,4′-dihydroxyflavan were prepared as two separate moieties and tested against human, mouse and rat GIIA $sPLA_2$s. 7,4′-Dihydroxyflavan displayed IC_{50}s of >3300 nM against human, mouse and rat GIIA $sPLA_2$s (data not shown), whereas **9** inhibited mouse and rat GIIA with low nanomo-

Table 1
Inhibition of compound **1** against $sPLA_2$s

$sPLA_2$	IC_{50} (nM)
hGIIA	80 ± 20
mGIIA	30 ± 5
rGIIA	120 ± 5
hGV	110 ± 20
mGV	520 ± 140
hGX	>1600
mGX	>1600

IC_{50} values are reported as the mean of triplicate analysis with standard deviations.

Table 2
Inhibition of compound **1** stereoisomers[a] against rat GIIA $sPLA_2$s



Compound **1** stereoisomer	IC_{50} (nM)
+ +	60 ± 10
– –	70 ± 10
+ –	110 ± 10
– +	120 ± 30

IC_{50} values are reported as the mean of duplicate analysis with standard deviations.
[a] Absolute stereochemistry was not determined.

lar potency. Interestingly, we found that **9** does not inhibit human GIIA and GV $sPLA_2$s with the same level of potency as **1** for reasons we do not fully understand. Notwithstanding the lack of potency of **9** on human GIIA and GV $sPLA_2$s, this result is consistent with patent work previously reported by Yamanouchi Pharmaceuticals.[18] They reported that **9** and other diacylphloroglucinol derivatives inhibit rabbit platelet $sPLA_2$ with IC_{50}'s somewhere in the range of 0.010–30 μM. Our work shows that **9** and **17b**, a methyl derivative of **9**, inhibit mouse and rat GIIA with low nanomolar potency (Table 3).

To investigate whether or not the carbonyl oxygens or hydroxyl groups are important for $sPLA_2$ inhibition, we synthesized **12b**, **15**, and **18b**. The inhibition potency for these three compounds was >1600 nM against human and mouse GIIA, GV, and GX $sPLA_2$s (data not shown). This supports our predicted binding mode where the

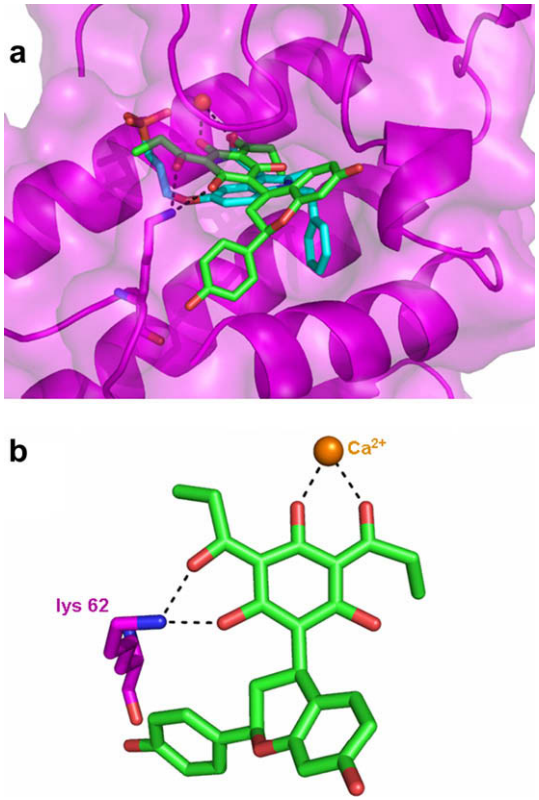


Figure 2. (a) Compound **1** (green) overlaid onto Indole 8 (light blue) in the human GIIA $sPLA_2$ active site (PDB code: 1DB4). (b) The predicted interactions of compound **1** made with the active site Ca^{2+} and lys 62 models the acetylacetonate bidentate ligand (the dodecanoyl chains were shortened to propanoyl chains to simplify docking).

Table 3
Inhibition of compounds **9** and **17b** against mammalian $sPLA_2$s

	IC_{50} (nM)						
Inh.	hGIIA	mGIIA	rGIIA	hGV	mGV	hGX	mGX
9	≈1600	110 ± 40	90 ± 30	≈1600	≈1600	>1600	>1600
17b	690 ± 130	60 ± 10	80 ± 20	530 ± 100	≈1600	>1600	>1600

Compounds were screened in duplicate at 1660 nM and reported as either ≈1600 nM or >1600 nM if the inhibition was ⩽50%. IC_{50} values below 1600 nM are reported as the mean of triplicate analysis using four to five inhibitor concentrations per analysis with standard deviations.

hydroxyl and the carbonyl groups are most likely involved in coordinating the calcium ion and neighboring lysine residue.

We also prepared **16**, **17c**, **17d**, and **17f**. All four of these compounds exhibited IC_{50} values of >1600 nM against human and mouse GIIA, GV, and GX $sPLA_2$s. The loss of extra hydrophobicity most likely lowers the amount of inhibitor partitioned onto the vesicle surface which lowers the amount of inhibitor that the enzyme 'sees' for inhibition. The fact that **16** does not give potent inhibition suggests that the dodecanoyl groups also play an important role in binding the active site of the enzyme.

In summary, we have tested a class of inhibitors derived from YM-26734 (compound **1**) against GIIA, GV, and GX $sPLA_2$s. The predicted binding pose of compound **1** in the active site of human GIIA $sPLA_2$ led to compounds **9** and **17b**. These two compounds inhibited mouse and rat GIIA $sPLA_2$ with similar potency observed for compound **1**. This class of hydrophobic inhibitors may be useful for mouse and rat cellular studies of $sPLA_2$ function where cell-permeable inhibitors with low nanomolar inhibition are needed.

Acknowledgments

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Supplementary data

Synthesis of all compounds, enantiomeric separation of compound **1**, and enzyme assay details are available as Supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.09.041.

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