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Highly Specific and Broadly Potent Inhibitors of Mammalian Secreted Phospholipases A_2

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Abstract

We report a series of inhibitors of secreted phospholipases A_2 (sPLA$_2$s) based on substituted indoles, 6,7-benzoindoles, and indolizines derived from LY315920, a well-known indole-based sPLA$_2$ inhibitor. Using the human group X sPLA$_2$ crystal structure, we prepared a highly potent and selective indole-based inhibitor of this enzyme. Also, we report human and mouse group IIA and IIE specific inhibitors and a substituted 6,7-benzoindole that inhibits nearly all human and mouse sPLA$_2$s in the low nanomolar range.

Introduction

Secreted phospholipases A_2 (sPLA$_2$s)[a] are a family of disulfide-rich, Ca^{2+}-dependent enzymes that hydrolyze the *sn*-2 position of glycero-phospholipids to release a fatty acid and a lysophospholipid.[1] The mouse genome encodes 10 sPLA$_2$s (groups IB, IIA, IIC, IID, IIE, IIF, III, V, X, XIIA), whereas the human genome encodes all of these except the group IIC enzyme, which occurs as a pseudogene.[2,3] More than a decade ago there was interest in human group IIA sPLA$_2$ (hGIIA) as an anti-inflammatory drug target because it was found at high concentrations in synovial fluid from arthritis patients,[4] although a clinical trial with an inhibitor against hGIIA failed to show efficacy in the treatment of rheumatoid arthritis.5 Interest in inhibitors of sPLA$_2$s has remained because of the possible involvement of these enzymes in inflammation. For example, studies with mGX- and mGV-deficient mice show that these sPLA$_2$s contribute to airway inflammation in a mouse model of allergic asthma.[6,7] Studies with macrophages from mGV-deficient mice show a partial reduction in eicosanoid production in response to agonists.[8]

Substituted indoles and indolizines first reported by workers at Lilly and Shionogi are the most potent sPLA$_2$ inhibitors and the ones with drug potential in terms of pharmacokinetic profiles. Compounds in this group include the indolizine Indoxam and the substituted indoles Me-Indoxam and **1** (LY315920; Figure 1).[9–12] The development of these compounds is an early example of structure-guided improvement of binding potency starting from a lead compound obtained through high-throughput screening[13] and making use of the X-ray structure of hGIIA.[14]

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Supporting Information Available: Details of synthetic methods, including NMR and MS data, for all other described compounds, HPLC traces showing purity of key target compounds, molecular modeling details, and *E. coli* membrane enzyme assay procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

[a]Abbreviations: hGIIA, human group IIA secreted phospholipase A_2 (likewise for other group names); mGIIA, mouse group IIA secreted phospholipase A_2 (likewise for other group names); sPLA$_2$, secreted phospholipase A_2.

With the availability of the full set of mouse and human recombinant $sPLA_2$s, it has been recently possible to explore the specificity of these compounds against all mammalian family members.[15–17] For example, Me-Indoxam inhibits hGIIA, mGIIA, mGIIC, hGIIE, mGIIE, hGV, and mGV $sPLA_2$s with low nanomolar potency, is less potent on hGIB, mGIB, hGX, and mGX, and inhibits hGIID, mGIID, hGXIIA, and mGXIIA only at micromolar concentrations.[15] Compound **1** potently inhibits hGIIA, mGIIA, hGIIE, mGIIE, hGX, and mGX enzymes and is less potent on the other mammalian sPLA2s.[17] In the current study we have taken a structure-guided approach using the X-ray structure of hGX[16,18] to obtain inhibitors in the class shown in Figure 1 that are highly specific for hGX. Along the way we also obtained a highly specific inhibitor that binds only to hGIIA, mGIIA, hGIIE, and mGIIE as well as a broadly potent inhibitor that shows strong inhibition against human and mouse GIB, GIIA, GIID, GIIE, GIIF, GV and GX $sPLA_2$s. These compounds may be useful in the study of the role of various mammalian $sPLA_2$s in cellular and whole animal responses.

Chemistry

Reported compounds were prepared using slightly modified routes.[9–12,17,19] The substituted indole and 6,7-benzoindole inhibitors were prepared using analogous routes starting from 2-carbomethoxy-4-methoxy-indole **4a** and 2-carbomethoxy-4-methoxy-6,7-benzoindole **4b**, respectively. However, because **4b** could not be purchased commercially, it was prepared from commercially available 3-methoxy-2-naphthalenemethanol **2a** (Scheme 1). 3-Methoxy-2-naphthalenemethanol (**2a**) was oxidized with PCC to form the aldehyde **2b**. The aldehyde was treated with methyl azidoacetate and sodium methoxide to form the azidocinamate **3**. Ring closure of **3** was achieved via the Hemetsberger reaction to give 2-carbomethoxy-4-methoxy-6,7-benzoindole **4b**.

Indole-based inhibitors **11c**, **11d**, **12a**, and **12b** were prepared by N-1 benzylation of commercially available **4a** using sodium hydride as the base to yield **5a** (Scheme 2). The methyl ester was saponified to form the 2-carboxylic acid indole **6a**. The 2-acetyl indole **7a** was formed by treatment of **6a** with methyllithium. Reduction of the ketone was carried out with $NaBH_4$ to yield **8a**. Deoxygenation of **8a** was achieved using a mixture of $NaBH_4$ and trifluoroacetic acid to give **9a**. The 2-isobutyl indole intermediate **9b** was prepared in a similar fashion as **9a** except isobutyllithium was used in place of methyllithium to form **7b** with subsequent transformations to give **9b**. Compounds **10a–d** were prepared by first deprotecting the 4-methoxy substituent on **9a** and **9b** using BBr_3 followed by addition of the appropriate alkyl bromoactetate or 2-bromo-*N*-(arylsulfonyl)acetamide with sodium hydride as the base. Addition of the oxalamide group to the indole was carried out by treating **10a–d** with oxalyl chloride followed by addition of ammonia gas to give compounds **11a–d**. Deprotection of the indole esters **11a** and **11b** was carried out with NaOH to give **12a** or with trifluoroacetic acid to yield **12b**.

Preparation of the 6,7-benzoindole inhibitors **11g**, **11h**, **12e**, and **12f** was done using identical routes described for the substituted indole inhibitors (Scheme 2). Compounds **14a** and **14b,** N-methyl amides **15a** and **15b**, and all **11d** derivatives were prepared using analogous steps to those outlined in Scheme 2. All Indoxam derivatives (**15c** and **16a–c**) were prepared using similar techniques to those already described.[12]

Results and Discussion

Molecular Modeling

We recently reported that compound **1** was 30 times more potent than the 2-methyl indole against hGX.[17] We explored this gain in selectivity by docking indole compounds with larger 2-alkyl groups into the hGIIA and hGX $sPLA_2$ active sites of existing X-ray crystal

structures[13,16] using the FLO/QXP docking program.[20] An overlay of the hGIIA and hGX enzyme structures (rms C_α = 0.98 Å) revealed a region of extra space in the hGX active site not present in hGIIA. This difference in hydrophobic space results mostly from a change in one amino acid residue. hGIIA has an isoleucine whereas hGX has a valine in the active site region which is contacted by the 2-position substituent on the indole ring (Figure 2). Larger 2-alkyl substituents would clash with this portion of the hGIIA active site but not in the case of hGX. Our designs were supported by data from workers at Shionogi showing that 2-isobutyl indole and indole-like inhibitors selectively inhibited the hGX enzyme.[21] However, this report only included IC_{50} values for these compounds against hGIB, hGIIA, hGV, and hGX. As a group X specific inhibitor would be extremely useful, we wanted to test 2-isobutyl indole derivatives against all human and mouse $sPLA_2$ enzymes.

Also, in attempts to increase hydrophobicity of these compounds in order to make them more cell permeable, docking studies revealed that larger substituents such as arylsulfonamides or alkylsulfonamides could replace the carboxylic acid OH group on the indole scaffold. In our previous studies, addition of a methyl group to the 6-position on the indole scaffold did not affect inhibition potency against the various $sPLA_2$s tested.[17] Larger groups including a benzene ring fused to the 6,7-position of the indole scaffold were also docked into the active site without affecting key binding interactions.

In Vitro Inhibiton

Using a fluorometric $sPLA_2$ assay,[16] the substituted indoles, 6,7-benzoindoles, and indolizines were tested against the full panel of human and mouse $sPLA_2$ enzymes, with the exception of mGIIC (because humans contain a group IIC pseudogene) and mGXIIA, which has 94% sequence identity to hGXIIA.[15] All reported compounds in this study except **13a–i**, **14b**, and **15a–c** were tested against hGIII and hGXIIA $sPLA_2$ enzymes, and gave <50% inhibition for both enzymes at 1.6 μM concentrations. The active sites of GIII and GXIIA $sPLA_2$ are predicted to be significantly different than those of the other mammalian $sPLA_2$s, and this probably explains why the indole/indolizine set of inhibitors lack potency on GIII and GXIIA enzymes. IC_{50} values generated against hGIID were obtained using the [^{3}H]oleic acid-labeled *E. coli* membrane assay, which was preferred for this enzyme because of the higher sensitivity achieved over the fluorometric assay. Data in Table 1 show that **11d** and **12b** are highly selective for hGX over all other human and mouse $sPLA_2$s. Thus, the large isobutyl group is well tolerated only by hGX, which is consistent with modeling studies. Interestingly, these compounds lack potency against mGX despite the fact that hGX and mGX share 72% sequence identity. Structural alignment reveals that mGX does not contain a valine in the active site region that contacts the indole 2-position like hGX, but rather a leucine. This extra hydrophobic bulk sterically excludes the 2-isobutyl indoles from the mGX active site in similar fashion as with GIIA. Other $sPLA_2$s such as GIB, GIIE, and GV also have an isoleucine in this region like the GIIA enzyme. However, GIID and GIIF have a valine in this region like human GX, which supports the fact that the 2-isobutyl compounds **11h** and **12f** display somewhat increased potency against GIID and GIIF enzymes.

A small subset of **11d** derivatives were synthesized and tested against hGX $sPLA_2$ (Table 2). As initial docking studies predicted that the phenylsulfonamide group would extend out of the active site, it was surprising to see a 38-fold difference in inhibition for compounds **13b–d** when the phenyl ring was substituted with a chlorine at the *para*-, *meta*-, and *ortho*-positions (Table 2). Compounds **13d**, and **13f**, with substitutions at the *ortho*-position with a chloro- or methyl- group, resulted in higher inhibition potency over **11d** (Table 2). It is possible that the extra methyl or chlorine groups pack into a small pocket of the active site, which would increase the binding affinity. However, replacing the phenylsulfonamide on **11d** with a methylsulfonamide (**13h**) also increases potency against hGX (Table 2). Without a crystal

structure, it is difficult to conclude how this pheynlsulfonamide is contacting the enzyme active site.

The 6,7-benzoindole inhibitors display general potency against all tested human and mouse $sPLA_2$ enzymes (Table 3). Because the extra hydrophobic bulk is predicted not to make direct contact with the enzyme, the increased potency is likely due to increased partitioning of the inhibitor into the phospholipid substrate vesicles, which increases the ratio of X_I/K_I^* (X_I is the mole fraction of inhibitor in the interface and K_I^* is the interfacial dissociation constant).[22, 23] Of particular note is compound **12e** that inhibited human and mouse groups IB, IIA, IID, IIE, IIF, V, and X $sPLA_2$s with an IC_{50} of less than 350 nM (Table 3). We also sought structurally similar compounds that would be devoid of $sPLA_2$ binding activity because such compounds are useful as controls in cellular studies. The X-ray structure of an Indoxam analogue bound to hGIIA and Me-Indoxam bound to hGX show that the carboxyl group of the substituent at the 4-position of the indole directly coordinates to the active site Ca^{2+}.[16,24] We thus synthesized **14a** and **14b** with only a methoxy group at the 4-position to remove the interaction made between the inhibitor and Ca^{2+}. Surprisingly, while **14b** (Figure 3) gave an IC_{50} of 1000 nM against hGX (data not included in table), **14a** had an IC_{50} of 14 and 34 nM against human and mouse GIIA, respectively (Table 3). Compound **14a** was also potent against hGIIE and mGIIE, consistent with trends observed for other potent group IIA indole-based inhibitors. Poor inhibiting control compounds were successfully designed by introduction of an N-methyl group on the oxalamide of the indole scaffold to give compounds **15a–15c** (Figure 3). Analysis of the co-crystal structure containing Me-Indoxam in the hGX active site reveals that the introduced N-methyl group disrupts a key hydrogen bond with either a histidine or aspartate residue, while also introducing extra hydrophobic bulk into the active site.[16] All N-methyl oxalamide control compounds had IC_{50} values that were >30-fold higher than their parent compound (Figure 3).

The 2-isobutyl Indoxam derivative **16a** was synthesized and found to poorly inhibit $sPLA_2$ enzymatic activity (Table 4). Since Indoxam does not inhibit hGX in the low nanomolar range (Table 4), it is not surprising that **16a** fails to inhibit hGX. This result suggests that poor inhibition of hGX activity by Indoxam or it derivatives has more to do with the indolizine heterocyle and not the substituents present on the ring. Interestingly, the 8-oxopropanone derivative **16b** and the 8-methoxy derivative **16c** were selectively potent against hGIIA and hGIIE which was similar to the gain in selectivity displayed by **14a**. We also prepared **15c** (Figure 3), which did not significantly inhibit hGIIA at concentrations below 1600 nM.

Conclusion

A series of indole- and indolizine-based compounds were synthesized and tested against the full set of human and mouse $sPLA_2$ enzymes. Compound **11d** was found to be selectively potent against hGX over all other human and mouse $sPLA_2$ enzymes. Derivatives of **11d**, such as **13h**, were also found to bind with higher affinity to the hGX enzyme active site and may help in further studies of hGX $sPLA_2$ function. An inhibitor selective for mouse and human GIIA and GIIE $sPLA_2$ (**14a**) as well as selective human GIIA and GIIE inhibitors (**16b** and **16c**) were also identified from this group of compounds. Compound **12e** is potent against human and mouse groups IB, IIA, IID, IIE, IIF, V, and X and is the most generally potent $sPLA_2$ inhibitor reported to date. It is also the first reported potent inhibitor of groups IID and IIF $sPLA_2$s. The inhibitors we describe may be useful in probing the roles of $sPLA_2$s in inflammatory diseases such as asthma and arthritis.

Experimental Section

Enzyme Inhibition Assays

For compounds with IC_{50}s <1600 nM in the fluorometric assay or <1300 nM in the *E. coli* membrane assay, inhibitor concentrations were varied with five different concentrations used to determine IC_{50} values. All IC_{50} values were obtained by nonlinear regression curve-fitting of percent inhibition versus log [inhibitor] using the Kaleidagraph software.

Fluorometric Assay

Microtiter plate assay of $sPLA_2$s using pyrene-labeled phosphatidylglycerol as the substrate was performed as described previously[16] with the exception that seven wells were used per assay instead of eight.

E. coli Membrane Assay

IC_{50} values calculated for hGIID were done using a modified procedure from that reported previously.[25] See Supporting Information for details.

Synthesis

All reagents were purchased from Sigma-Aldrich and used directly unless otherwise stated. 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 $60F_{254}$ silica plates, and column chromatography was done with 60 Å silica gel purchased from Silicycle. ^{1}H NMR spectra were recorded on dilute solutions in $CDCl_3$, CD_3OD, or DMSO-d_6. NMR spectra were obtained on a Bruker AC-300 (300 MHz) and electrospray ionization mass spectra were acquired on a Bruker Esquire LC00066 for all compounds. Preparative reverse phase HPLC was performed on an automated Varian Prep Star system using a YMC S5 ODS column (20 × 100 mm, Waters Inc.).

Representative Procedure for Synthesis of Substituted 6,7-Benzoindole Inhibitors (Compound 12e): Preparation of 1-Benzyl-2-carbomethoxy-4-methoxy-6,7-benzoindole (5b)

Compound **4b** (synthesis described in Supporting Information; 800 mg, 3.14 mmol) was dissolved in 10 mL dry DMF and stirred at 0 °C and sodium hydride (140 mg, 5.5 mmol) was added. After stirring for five minutes at 0 °C, benzylbromide (820 uL, 6.90 mmol) was added and the reaction was stirred for 30 min at room temperature. The reaction mixture was poured onto 20 mL of H_2O and 20 mL of EtOAc in a separatory funnel. The layers were separated and the organic layer was washed with 3 × 10 mL H_2O and the combined aqueous layer was back-extracted with 1 × 20 mL EtOAc. The combined organic layer was dried over $MgSO_4$ and filtered, and the solvent was removed by rotary evaporation. The crude white solid was purified by column chromatography on silica gel (20% EtOAc/80% hexanes) to give a white solid (820 mg, 75% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85 (s, 3H), 4.06 (s, 3H), 6.34 (bs, 2H), 6.77 (s, 1H), 7.09 (d, J = 7.2 Hz, 2H), 7.16–7.31 (m, 4H), 7.37 (t, J = 7.2 Hz, 1H), 7.68 (s, 1H), 7.78 (d, J = 8.1 Hz, 1H), 8.06 (d, J = 8.4 Hz, 1H).

Preparation of 1-Benzyl-2-carboxylic acid-4-methoxy-6,7-benzoindole (6b)

Compound **5b** (485 mg, 1.41 mmol) was suspended in 15 mL of 30% KOH/MeOH/THF (2:1:1) and refluxed for 2.0 h (all the solid dissolved during reflux). After refluxing, the reaction was cooled on ice and the pH was made acidic using 2 N HCl, causing the product to precipitate. The white solid was collected by vacuum filtration and washed with 1 × 10 mL of cold water and 2 × 10 mL of cold hexanes to give a white solid (400 mg, 86% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.02 (s, 3H), 6.41 (bs, 2H), 6.98 (m, 3H), 7.20–7.26 (m, 2H), 7.32 (t, J = 7.5 Hz, 2H), 7.39 (t, J = 8.1 Hz, 1H), 7.49 (s, 1H), 7.86 (d, J = 7.5 Hz, 1H), 8.12 (d, J = 8.4 Hz, 1H).

Preparation of 1-Benzyl-2-acetyl-4-methoxy-6,7-benzoindole (7c)

Compound **6b** (920 mg, 1.12 mmol) was dissolved in 40 mL of dry THF to which 6.6 mL of 1.25 M MeLi in diethyl ether was added dropwise and stirred at room temperature for 2.5 h. Saturated NH_4Cl (8 mL) was added followed by the addition of 2 N HCl until the mixture had an acidic pH. The reaction mixture was then poured onto 30 mL of EtOAc and 30 mL of H_2O in a separatory funnel. The layers were separated and the water phase was washed with 2 × 20 mL of EtOAc. The organic layers were combined, dried over $MgSO_4$ and filtered, and the solvent was removed by rotary evaporation. The crude white solid was triturated in 15 mL of 1:1 EtOAc/hexanes and separated from the solvent by vacuum filtration. The white solid collected via vacuum filtration was washed with 2 × 10 mL of 1:1 EtOAc/hexanes giving **6b**. Additional product could be purified from the combined filtrate and washings by removing the solvent and repeating the trituration step described above followed by chromatography on silica gel (20% EtOAc/80% hexanes) of the filtrate and washings combined together from the second trituration step. The purified product was combined to afford a white solid (366 mg, 40% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.63 (s, 3H), 4.08 (s, 3H), 6.35 (bs, 2H), 6.77 (s, 1H), 7.07 (d, *J* = 6.9 Hz, 2H), 7.20–7.31 (m, 4H), 7.39 (t, *J* = 8.1 Hz, 1H), 7.67 (s, 1H), 7.78 (d, *J* = 8.1 Hz, 1H), 8.09 (d, *J* = 8.7 Hz, 1H).

Preparation of 1-(1-Benzyl-4-methoxy-1*H*-6,7-benzoindol-2-yl)-ethanol (8c)

Compound **7c** (366 mg, 1.11 mmol) was dissolved in 30 mL of 75% EtOH/25% THF, and $NaBH_4$ (100 mg, 3.33 mmol) was added to the mixture and stirred at room temperature for 16 h. The reaction mixture was then poured onto 30 mL EtOAc and 30 mL H_2O in a separatory funnel. The layers were separated and the water phase was washed with 2 × 20 mL of EtOAc. The organic layers were combined and washed with 2 × 20 mL of H_2O and 1 × 20 mL of satd NaCl. The organic layer was dried over $MgSO_4$ and filtered, and the solvent was removed by rotary evaporation to give **8c** as a white solid that was used without further purification (355 mg, 96% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.72 (d, *J* = 6.3 Hz 3H), 4.08 (s, 3H), 4.99 (m, 1H), 5.96 (d, *J* = 20.7 Hz, 1H), 6.09 (d, *J* = 20.7 Hz, 1H), 6.81 (s, 1H), 6.85 (s, 1H), 7.05 (d, *J* = 6.9 Hz, 2H), 7.15 (t, *J* = 6.9 Hz, 1H), 7.20–7.31 (m, 4H), 7.79 (d, *J* = 8.1 Hz, 1H), 7.93 (d, *J* = 8.7 Hz, 1H).

Preparation of 1-Benzyl-2-ethyl-4-methoxy-1*H*-6,7-benzoindole (9c)

Compound **8c** (420 mg, 1.26 mmol) was dissolved in 20 mL of dry CH_2Cl_2 and added dropwise to a mixture of 14 mL of 99% trifluoroacetic acid (TFA) and $NaBH_4$ (243 mg, 6.3 mmol) at 20 °C (prepare TFA/$NaBH_4$ mixture in an ice bath by careful dropwise addition of TFA to $NaBH_4$ and let stir until $NaBH_4$ fully dissolves before raising temperature). The reaction mixture was stirred at room temperature for 30 min and then poured onto 30 mL of satd $NaHCO_3$ and 30 mL of CH_2Cl_2 in a separatory funnel. Once bubbling ceased, the layers were separated and the water phase was washed with 2 × 20 mL of CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$ and filtered, and the solvent was removed by rotary evaporation. The crude material was purified by column chromatography on silica gel (20% EtOAc/80% hexanes) to afford a white solid (335 mg, 84% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (t, *J* = 7.5 Hz 3H), 2.75 (q, *J* = 7.5 Hz 2H), 4.07 (s, 3H), 5.77 (s, 2H), 6.56 (s, 1H), 6.80 (s, 1H), 7.05 (d, *J* = 6.9 Hz, 2H), 7.13 (t, *J* = 6.9 Hz, 1H), 7.20–7.31 (m, 4H), 7.79 (d, *J* = 8.1 Hz, 1H), 7.93 (d, *J* = 8.7 Hz, 1H).

Preparation of Methyl 2-(1-Benzyl-2-ethyl-1*H*-6,7-benzoindol-4-yloxy)acetate (10e)

Compound **9c** (80 mg, 0.25 mmol) was dissolved in 8 mL of dry CH_2Cl_2 and stirred at 0 °C. BBr_3 (1.0 M in CH_2Cl_2; 635 μL, 0.635 mmol) was added in portions over 5 min to the reaction mixture at 0 °C, and the reaction mixture was stirred for 3 h at room temperature or until product formation was complete as indicated by TLC. H_2O (8 mL) was added to the reaction mixture

to quench excess BBr_3, and the reaction mixture was poured onto 20 mL of CH_2Cl_2 and 20 mL of H_2O in a separatory funnel. The layers were separated and the water phase was washed with 2 × 20 mL of CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$ and filtered, and the solvent was removed by rotary evaporation to give the 4-hydroxy-6,7-benzoindole intermediate, which is unstable and decomposes giving a purple color upon exposure to air. After drying in vacuo for 30 min, this compound was then immediately dissolved in 4 mL of DMF and stirred in an ice bath. Sodium hydride (10.3 mg, 0.41 mmol) was added to the reaction mixture and stirred 5 min at 0 °C, with subsequent addition of methyl bromoacetate (40 μL, 0.456 mmol). The reaction was stirred at room temperature for 30 min. Additional portions of sodium hydride were added at 0 °C until the reaction was shown to be complete by TLC. The reaction mixture was then poured onto 20 mL of H_2O and 20 mL of EtOAc in a separatory funnel. The layers were separated and the organic layer was washed with 4 × 10 mL of H_2O, and the combined aqueous layer was back-extracted with 1 × 20 mL of EtOAc. The combined organic layers were dried over $MgSO_4$ and filtered, and the solvent was removed by rotary evaporation. The crude material was purified by column chromatography on silica gel (20% EtOAc/80% hexanes) to afford a white solid (23 mg, 24% yield over two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (t, *J* = 7.5 Hz 3H), 2.75 (q, *J* = 7.5 Hz 2H), 3.85 (s, 3H), 4.90 (s, 2H), 5.76 (s, 2H), 6.69 (s, 1H), 6.76 (s, 1H), 7.05 (d, *J* = 6.9 Hz, 2H), 7.15 (t, *J* = 6.9 Hz, 1H), 7.20–7.31 (m, 4H), 7.75 (d, *J* = 8.1 Hz, 1H), 7.95 (d, *J* = 8.4 Hz, 1H).

Preparation of Methyl-2-(3-(2-amino-2-oxoacetyl)-1-benzyl-2-ethyl-1*H*-6,7-benzoindol-4-yloxy)acetate (11e)

Compound **10e** (22.4 mg, 0.06 mmol) was dissolved in 7 mL of dry CH_2Cl_2 and added dropwise to 14 mL of dry CH_2Cl_2 containing oxalyl chloride (26 μL, 0.30 mmol) at room temperature. The reaction mixture was stirred overnight at room temperature. Ammonia gas was then bubbled into the reaction mixture for five minutes. The reaction mixture was then poured into a separatory funnel containing 20 mL of 2 N HCl. The layers were separated and the aqueous layer was extracted with 2 × 10 mL of CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$ and filtered, and the solvent was removed by rotary evaporation. The crude mixture was purified by column chromatography over silica gel (70% EtOAc/30% hexanes) to give a white yellow solid (10.9 mg, 41% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, *J* = 7.5 Hz 3H), 2.94 (q, *J* = 7.5 Hz 2H), 3.81 (s, 3H), 4.88 (s, 2H), 5.42 (bs, 1H), 5.81 (s, 2H), 6.72 (bs, 1H), 6.81 (s, 1H), 7.10 (d, *J* = 6.9 Hz, 2H), 7.17 (t, *J* = 6.9 Hz, 1H), 7.20–7.31 (m, 4H), 7.74 (d, *J* = 7.8 Hz, 1H), 7.92 (d, *J* = 8.4 Hz, 1H). MS (ESI, pos. ion) *m*/*z*: 467 ($M + Na^+$).

Preparation of 2-(3-(2-Amino-2-oxoacetyl)-1-benzyl-2-ethyl-1*H*-6,7-benzoindol-4-yloxy) acetic Acid (12e)

Compound **11e** (10.9 mg, 0.024 mmol) was dissolved in 5 mL of MeOH/THF (5:1) with 0.5 mL of 1.5 M NaOH added to the reaction mixture and stirred for 2.5 h at room temperature. The reaction mixture was then poured onto 20 mL of 2 N HCl and 20 mL of CH_2Cl_2 in a separatory funnel. The layers were separated and the aqueous layer was extracted with 2 × 10 mL of CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and filtered, and the solvent was removed by rotary evaporation to yield **12e** quantitatively. A portion of **12e** was purified by HPLC using the following program (eluting solvents each contained 0.08% TFA): 0–5 min 30% MeOH/70% H_2O, 5–30 min 30% MeOH/70% H_2O–70% MeOH/30% H_2O, 30–32 min 70% MeOH/30% H_2O–100% MeOH, 32–35 min 100% MeOH. The product eluted at 24.5 min and the solvent was removed by Speed-Vac to afford a white/yellow solid (4.9 mg). ^{1}H NMR (300 MHz, MeOD) δ 1.26 (t, *J* = 7.2 Hz 3H), 3.04 (q, *J* = 7.5 Hz 2H), 4.93 (s, 2H), 5.99 (s, 2H), 6.96 (s, 1H), 7.15–7.23 (m, 3H), 7.27–7.39 (m, 4H), 7.83 (d, *J* = 6.9 Hz, 1H), 8.07 (d, *J* = 8.4 Hz, 1H). MS (ESI, pos. ion) *m*/*z*: 431 (M+).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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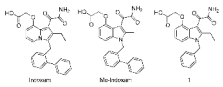


Figure 1.
Substituted indole and indolizine $sPLA_2$ inhibitors.

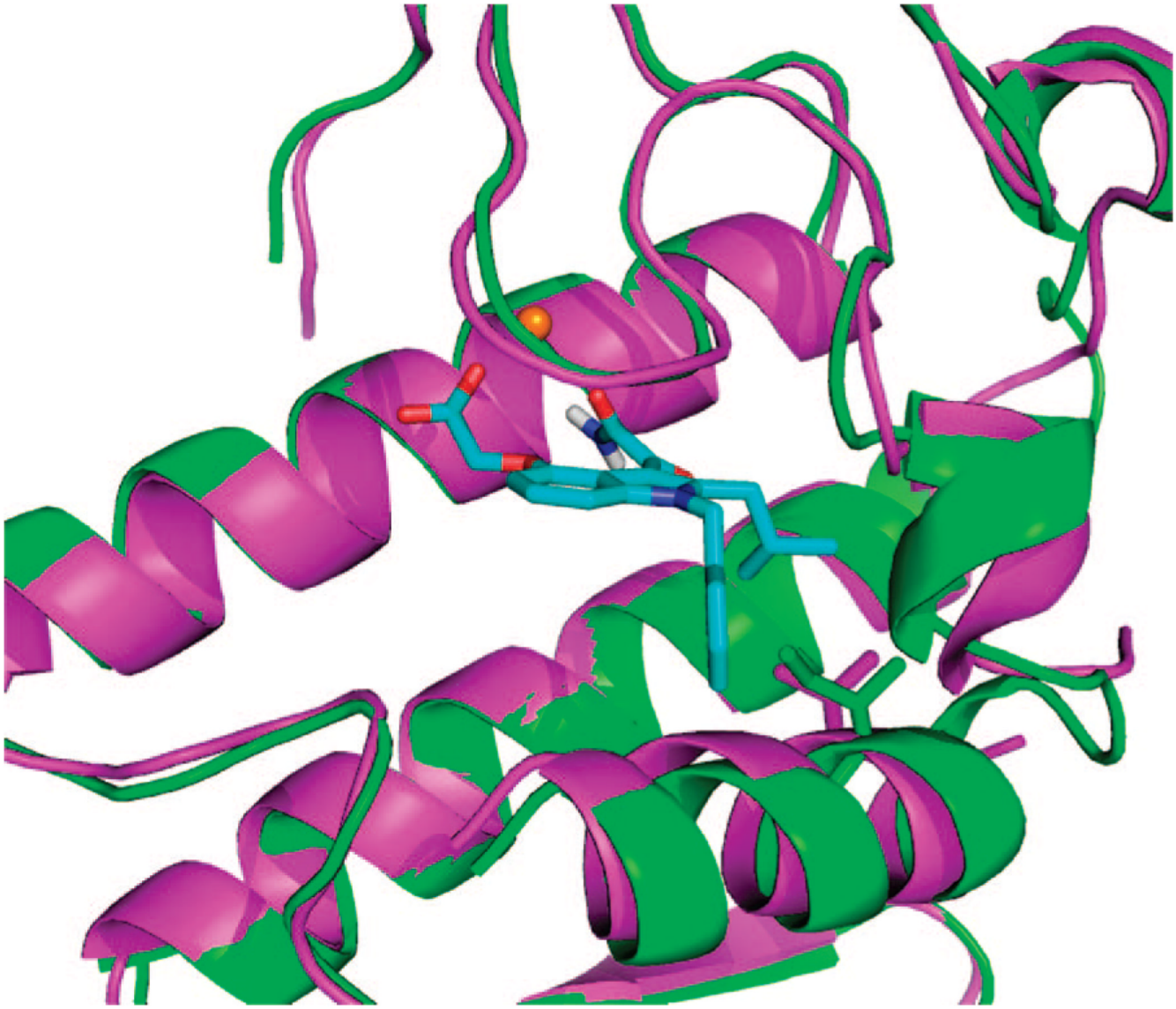


Figure 2.
An overlay of hGIIA (green) and hGX (magenta) with **12b** docked into the active site. The isoleucine of hGIIA, but not the valine of hGX, provides extra hydrophobic bulk near the 2-isobutyl group on the indole ring and presumably excludes the 2-isobutyl indole from the active site.

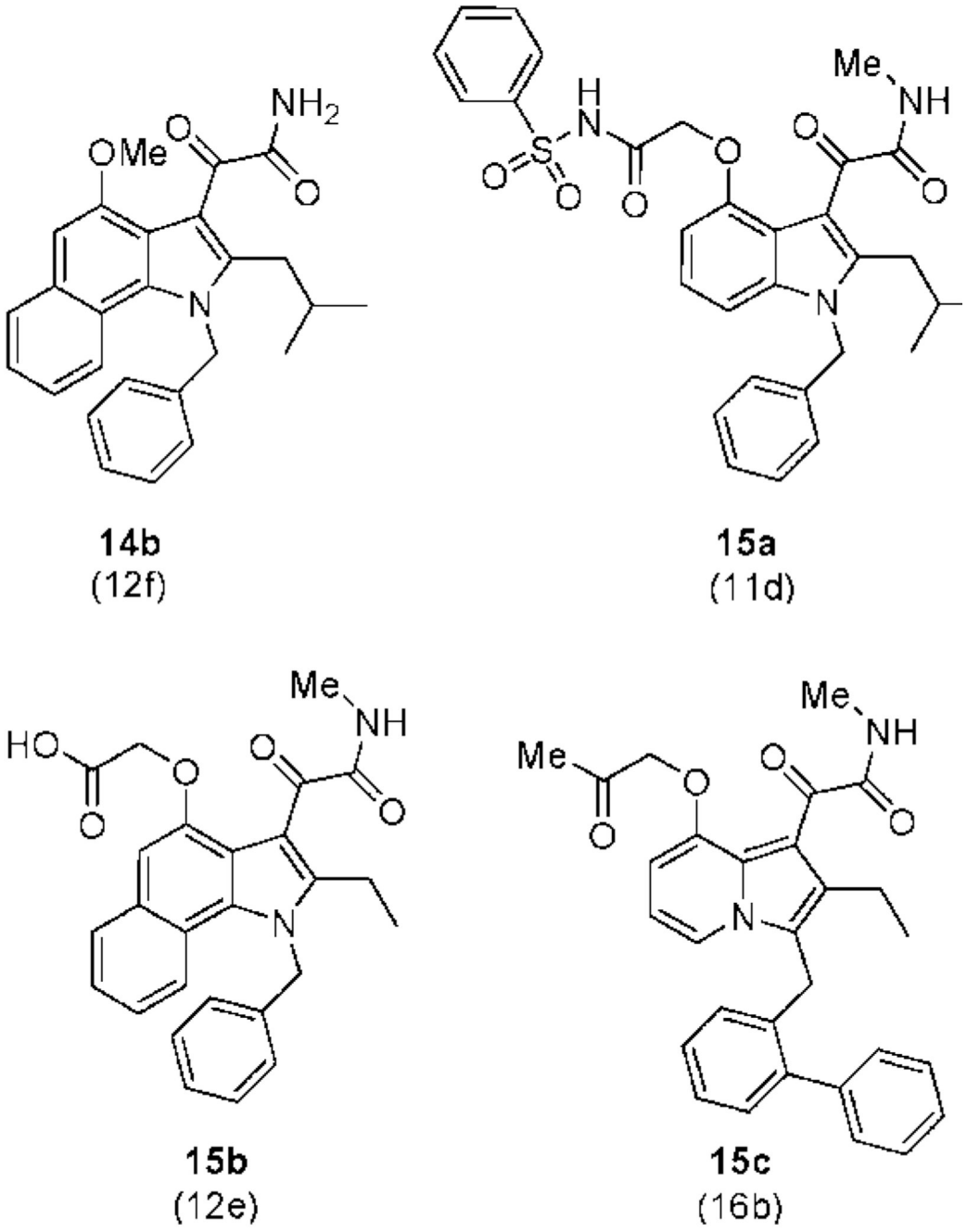
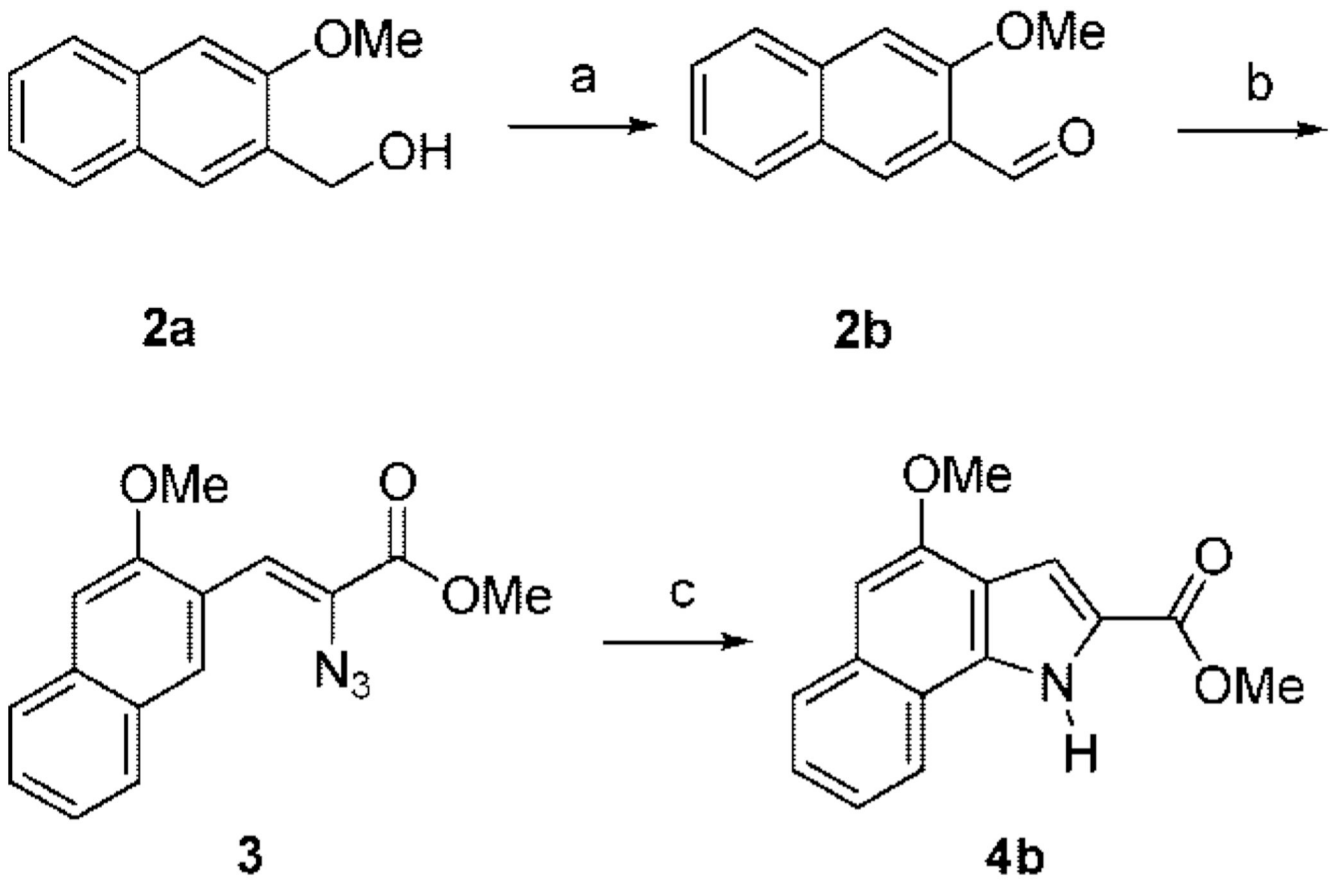


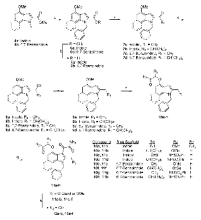
Figure 3.

Control compounds designed by removing the functionality from the 4-postion (**14b**) or by methylating the oxalamide (**15a–c**). Control compounds are >30-fold less potent than their parent compound (listed below the compound in parenthesis) when tested against hGX (**14b** and **15a**), human and mouse GIIA, GV, and GX (**15b**), or human GIIA (**15c**).



Scheme 1a.

[a]Reagents and conditions: (a) PCC, NaAcetate in CH_2Cl_2; (b) methyl azidoacetate, NaOMe in THF; (c) xylene or toluene, reflux.

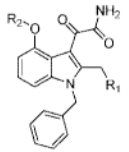


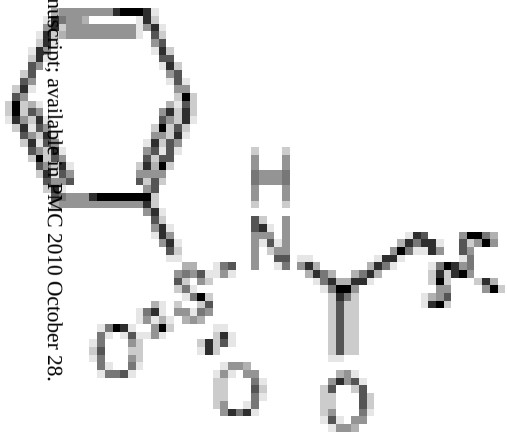
Scheme 2a.

[a]Reagents and conditions: (a) NaH, BnBr in DMF; (b) 30% KOH/MeOH/THF (2:1:1), reflux; (c) MeLi or isopropyllithium in THF; (d) $NaBH_4$ in EtOH/THF; (e) $NaBH_4$, TFA in CH_2Cl_2; (f) (1) BBr_3 in CH_2Cl_2, (2) NaH R_2COCHR_3Br in DMF; (g) (1) oxalyl chloride in CH_2Cl_2, (2) NH_3(g); (h) 1.5 M NaOH in MeOH/THF or 20% TFA in CH_2Cl_2.

Table 1

st Human and Mouse sPLA2s[a]



	IC$_{50}$(nM)													
R2	**hGIB**	**mGIB**	**hGIIA**	**mGIIA**	**hGIID[c]**	**mGIID**	**hGIIE**	**mGIIE**	**hGIIF**	**mGIIF**	**hGV**	**mGV**	**hGX**	**mGX**
	750±150	140±75	125±20	70±20	60±10	430±90	50±20	75±20	130±30	>1600	500±50	750±100	75±10	75±10
	>1600	950±80	250±20	60±10	170±30	890±45	7±1	12±2	1100±60	>1600	100±20	60±7	15±3	30±7

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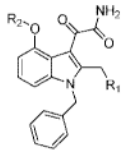
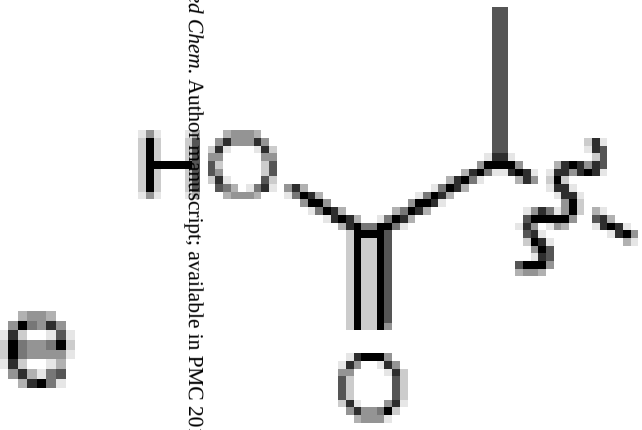
R2	IC$_{50}$(nM)											
	hGIB	mGIB	hGIIA	mGIIA	hGIID[c]	mGIID	hGIIE	mGIIE	hGIIF	mGIIF	hGV	mGV	hGX	mGX
	>1600	>1600	>1600	>1600	700±230	>1600	>1600	>1600	>1600	>1600	>1600	>1600	21±7	>1600



R2	IC$_{50}$(nM)													
	hGIB	**mGIB**	**hGIIA**	**mGIIA**	**hGIID**[c]	**mGIID**	**hGIIE**	**mGIIE**	**hGIIF**	**mGIIF**	**hGV**	**mGV**	**hGX**	**mGX**
	80±20	100±30	170±40	60±10	120±40	>1600	9±4	140±50	90±30	470±190	44±9	57±20	22±2	20±5
	>1600	580±10	>1600	>1600	500±90	>1600	1300±18	>1600	550±100	>1600	>1600	>1600	50±16	>1600

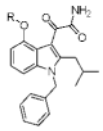
lysis with standard deviations. Each compound was screened at 1660 nM and reported as >1600 nM if the inhibition was <50%.

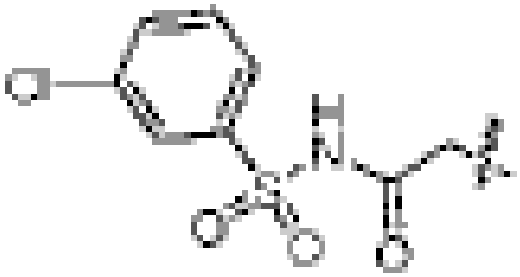
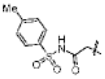
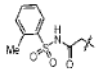
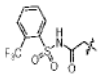
t of this compound against hGV and mGV gave 110 ± 30 and 160 ± 20 nM, respectively.

nd was screened at 1330 nM and reported as >1300 nM if the inhibition was <50%.

Table 2

IC_{50} Values of **11d** Derivatives against hGX sPLA2[a]

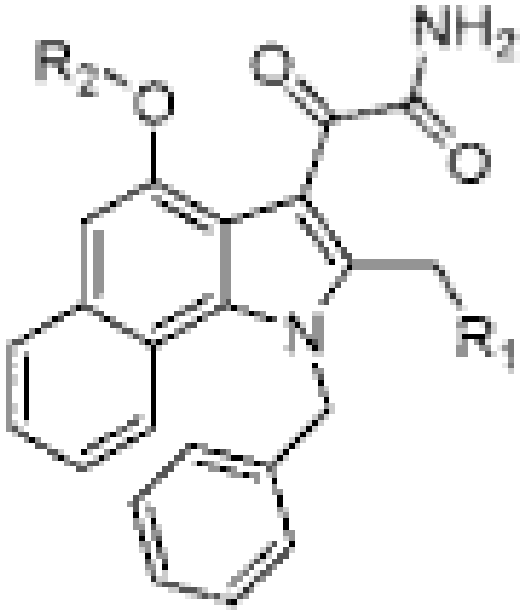


		hGX
Comp	**R**	**IC_{50} (nM)**
13a		80±10
13b		540±60
13c		140±30
13d		14±3
13e		320±30
13f		11±1
13g		70±20
13h		7±2
13i		30±10

[a] IC_{50} values are reported as the mean of duplicate or triplicate analysis with standard deviations.

Table 3

ibitors against Human and Mouse sPLA2s[a]



	IC$_{50}$ (nm)													
R2	**hGIB**	**mGIB**	**hGIIA**	**mGIIA**	**hGIID**[b]	**mGIID**	**hGIIE**	**mGIIE**	**hGIIF**	**mGIIF**	**hGV**	**mGV**	**hGX**	**mGX**
	1300±290	1000±40	100±20	90±20	35±10	1000±590	16±1	48±9	550±120	>1600	140±6	70±10	90±20	35±5

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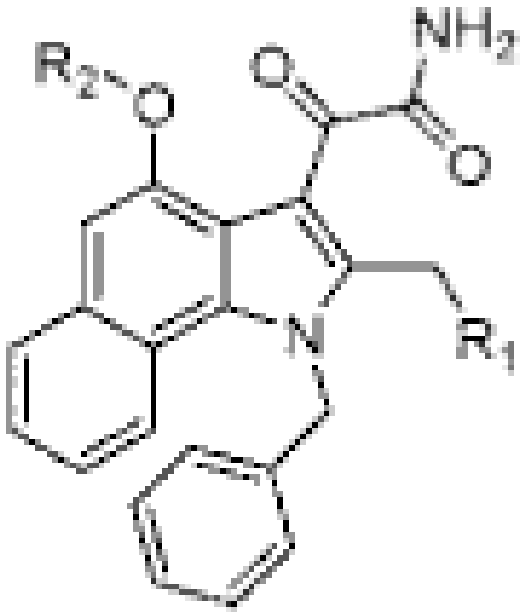

	IC$_{50}$ (nm)													
R2	**hGIB**	**mGIB**	**hGIIA**	**mGIIA**	**hGIID**[b]	**mGIID**	**hGIIE**	**mGIIE**	**hGIIF**	**mGIIF**	**hGV**	**mGV**	**hGX**	**mGX**
	>1600	920±180	>1600	>1600	270±150	530±30	840±290	>1600	290±50	450±120	>1600	>1600	30±4	>1600

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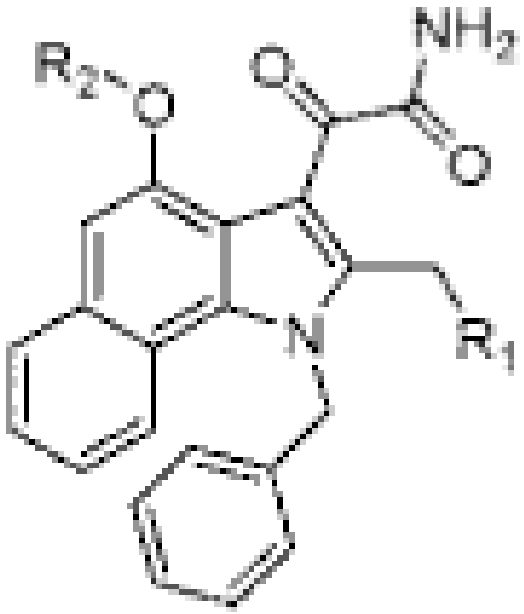
R2	IC_{50} (nm)												
	hGIB	mGIB	hGIIA	mGIIA	hGIID[b]	mGIID	hGIIE	mGIIE	hGIIF	mGIIF	hGV	mGV	hGX	mGX
	84±3	160±40	40±2	30±1	7±3	320±5	7±2	18±2	50±3	170±33	35±7	20±1	20±3	6±1

R2	IC_{50} (nm)												
	hGIB	mGIB	hGIIA	mGIIA	hGIID[b]	mGIID	hGIIE	mGIIE	hGIIF	mGIIF	hGV	mGV	hGX	mGX
	1400±40	290±20	>1600	>1600	80±20	640±190	260±10	1500±170	90±10	130±20	>1600	>1600	10±2	>1600



	IC$_{50}$ (nm)													
R2	**hGIB**	**mGIB**	**hGIIA**	**mGIIA**	**hGIID**[b]	**mGIID**	**hGIIE**	**mGIIE**	**hGIIF**	**mGIIF**	**hGV**	**mGV**	**hGX**	**mGX**
Me	810±80	1600±30	14±2	34±1	240±4	>1600	20±6	150±4	>1600	>1600	>1600	>1600	1500±270	>1600

sis with standard deviations. Each compound was screened at 1660 nM and reported as >1600 nM if the inhibition was <50%.

d was screened at 1330 nM and reported as >1300 nM if the inhibition was <50%.

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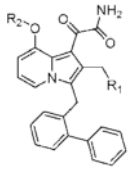
Table 4

inst Human and Mouse sPLA2s[a]



	IC_{50} (nm)													
R2	**hGIB**	**mGIB**	**hGIIA**	**mGIIA**	**hGIID**[b]	**mGIID**	**hGIIE**	**mGIIE**	**hGIIF**	**mGIIF**	**hGV**	**mGV**	**hGX**	**mGX**
	700±30	1000±60	60±10	150±40	>1300	>1600	10±2	35±15	>1600	>1600	100±5	170±10	>1600	900±300
	>1600	>1600	>1600	>1600	>1300	>1600	>1600	>1600	970±50	1100±200	>1600	>1600	>1600	>1600

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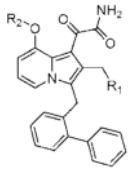
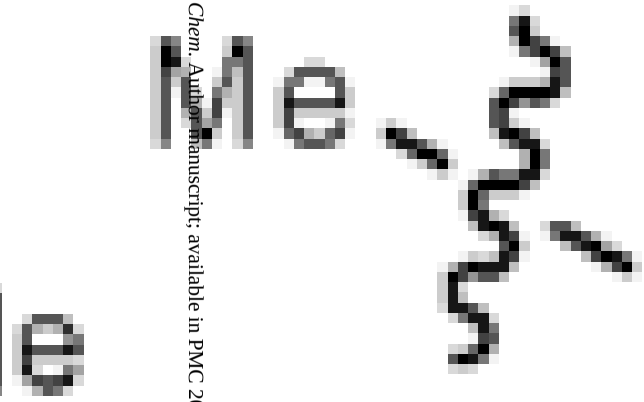
R2	IC_{50} (nm)												
	hGIB	mGIB	hGIIA	mGIIA	hGIID[b]	mGIID	hGIIE	mGIIE	hGIIF	mGIIF	hGV	mGV	hGX	mGX
	>1600	1200±110	30±10	>1600	>1300	>1600	90±15	410±20	>1600	>1600	>1600	>1600	>1600	>1600



	IC$_{50}$ (nm)													
R2	hGIB	mGIB	hGIIA	mGIIA	hGIID[b]	mGIID	hGIIE	mGIIE	hGIIF	mGIIF	hGV	mGV	hGX	mGX
	>1600	320±20	35±2	>1600	>1300	>1600	50±10	230±110	>1600	>1600	>1600	>1600	>1600	>1600

sis with standard deviations. Each compound was screened at 1660 nM and reported as >1600 nM if the inhibition was <50%.

d was screened at 1330 nM and reported as >1300 nM if the inhibition was <50%.

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