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Design and synthesis of 3-pyrrol-3-yl-3*H*-isobenzofuran-1-ones as inhibitors of human cytosolic phospholipase $A_2\alpha$

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Abstract

A series of 3-pyrrol-3-yl-3*H*-isobenzofuran-1-ones was synthesized and assessed for the ability to inhibit cytosolic phospholipase $A_2\alpha$ (cPLA$_2\alpha$). Several of these compounds were found to be active in both a cell based assay and an isolated enzyme assay. The most potent inhibitor was the thiazolidine-2,4-dione substituted derivative **35**. With IC_{50}-values of 0.7 μM and 7.3 μM in the cellular and isolated enzyme assay, respectively, it possesses similar inhibitory potency as the known cPLA$_2\alpha$ inhibitor arachidonyltrifluoromethyl ketone (AACOCF$_3$). Structure–activity relationship studies revealed that the evaluated isobenzofuran-1-ones seem to exert their cellular activities not only by a direct interaction with the enzyme but also by other as yet unknown mechanisms.

Keywords: *Cytosolic phospholipase $A_2\alpha$, inhibitors, isobenzofuran-1-one, structure–activity relationships*

Introduction

Cytosolic phospholipase $A_2\alpha$ (cPLA$_2\alpha$) is an esterase that selectively cleaves the *sn*-2 position of arachidonoyl-glycerophospholipids of biomembranes to generate free arachidonic acid and lysophospholipids [1]. Arachidonic acid in turn is metabolized to a variety of inflammatory mediators including prostaglandins and leukotrienes. Lysophospholipids with an alkyl ether moiety at the *sn*-1 position can be acetylated to platelet activating factor (PAF), another mediator of inflammation [2]. Thus, inhibition of cPLA$_2\alpha$ is considered as an attractive target for the design of new anti-inflammatory drugs [3–5].

First-generation cPLA$_2\alpha$ inhibitors were analogues of arachidonic acid with the COOH group replaced by $COCF_3$ (AACOCF$_3$, **1**) or $CH_2PO(OCH_3)F$ (MAFP) [3]. Inhibitors of cPLA$_2\alpha$ with very high *in vitro* potency reported later are thiazolidinediones from Shionogi, such as compound **2** (Figure 1) [6], and propan-2-ones from AstraZeneca [7].

We here describe the synthesis and biological evaluation of a series of 3-pyrrol-3-yl-3*H*-isobenzofuran-1-ones. Some of these compounds show inhibition of cPLA$_2\alpha$ in a cell free as well as in a cellular test system with IC_{50} values in the low micromolar range.

Material and methods

Chemical synthesis

Column chromatography was performed on silica gel 60, 230–400 mesh (= flash chromatography) or 70–230 mesh from Merck, Darmstadt, Germany. Preparative HPLC was performed on a RP18 column (Kromasil 100, 5 μm, 10 mm (I.D.) × 250 mm protected with an analogously filled guard column 10 mm (I.D.) × 50 mm, CS-chromatographie service, Langerwehe, Germany). Melting points were determined on a Büchi B-540 apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Mercury Plus 400 spectrometer (400 MHz). Mass

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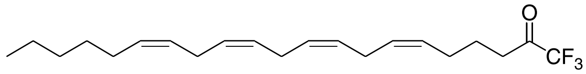

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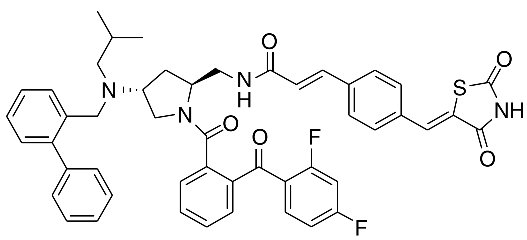

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Figure 1. Structures of known cPLA$_2\alpha$ inhibitors.

spectra were obtained on Finnigan GCQ and LCQ apparatuses applying electron beam ionization (EI) and electrospray ionisation (ESI), respectively. The purity of the target compounds was determined using two diverse HPLC systems with UV detection at 254 nm. The first one applied a reversed phase C18 column (Nucleosil 100 RP18, 10 μm, 4.0 mm (I.D.) × 250 mm, Macherey & Nagel, Düren, Germany) eluting the compounds isocratically with CH_3CN/H_2O containing 0.1% H_3PO_4 at a flow rate of 1 mL/min. In the second system separation was performed using a cyano phase (LiChrospher 100 CN, 10 μm, 4.0 mm (I.D.) × 250 mm, Merck, Darmstadt, Germany) under reversed phase conditions, eluting with CH_3CN/H_2O containing 0.1% H_3PO_4 at a flow rate of 0.8 mL/min. With exception of **17** and **30**, all target compounds showed purities greater than 95% in both systems. The purities evaluated for **17** and **30** were 91 and 93%, respectively, in each case. The reference inhibitor arachidonyltrifluoromethyl ketone ($AACOCF_3$) was purchased from Biomol, Hamburg, Germany.

2,5-Dimethyl-1-phenylpyrrole-3-carbaldehyde oxime (4). 2,5-Dimethyl-1-phenylpyrrole-3-carbaldehyde (**3**) (2.5 g, 12.5 mmol) was dissolved in methanol (50 mL) by heating. After addition of an aqueous solution (20 mL) of hydroxylamine-HCl (0.87 g, 12.5 mmol) and sodium carbonate (1.33 g, 12.5 mmol) the mixture was refluxed for 3 h. The precipitate formed was filtered off by suction and washed with cold methanol to yield **4** [8] (2.36 g, 88%); mp 196°C. ^{1}H-NMR (DMSO-d_6): δ 1.90 (s, 3H), 2.06 (s, 3H), 6.08 (s, 1H), 7.24–7.50 (m, 5H), 8.03 (s, 1H), 10.37 (s, 1H). ^{13}C-NMR (DMSO-d_6): δ 10.86, 12.64, 103.89, 113.06, 127.85, 127.94, 128.69, 128.78, 129.20, 137.27, 143.10.

(2,5-Dimethyl-1-phenylpyrrol-3-yl)methylamine (5). A solution of $TiCl_4$ (3.3 mL, 20 mmol) in 1,2-dimethoxyethane (20 mL) was treated under a nitrogen atmosphere at 0°C with $NaBH_4$ (2.27 g, 60 mmol). A suspension of **4** (3.0 g, 14 mmol) in 1,2-dimethoxyethane (10 mL) was added slowly and the resulting mixture was allowed to warm up to room temperature. After being stirred for 14 h at this temperature, water (40 mL) and 25% ammonium hydroxide (40 mL) were added with ice cooling. The precipitate formed was filtered off with suction. The filtrate was extracted with CH_2Cl_2 and the precipitate was washed with CH_2Cl_2. The CH_2Cl_2 phases were combined and the solvent distilled off. The residue was dissolved in dilute HCl and washed with diethyl ether. The aqueous phase was alkalized with dilute aqueous KOH-solution and extracted with diethyl ether. The organic layer was dried (Na_2SO_4) and concentrated to give **5** as an oil (1.90 g, 68%). ^{1}H-NMR ($CDCl_3$): δ 1.68 (s, broad, 2H), 1.99 (s, 3H), 2.02 (s, 3H), 3.69 (s, 2H), 5.94 (s, 1H), 7.18–7.47 (m, 5H). ^{13}C-NMR ($CDCl_3$): δ 10.92, 13.13, 38.52, 105.88, 121.16, 124.74, 127.67, 128.13, 128.36, 128.44, 129.04, 129.10, 138.96. MS (EI): m/z (%) = 200 (12) [M$^+$], 184 (100).

N-(2,5-Dimethyl-1-phenylpyrrol-3-ylmethyl)-2,2,2-trifluoroacetamide (6). A solution of **5** (750 mg, 3.7 mmol) in dry THF (10 mL) was treated under a nitrogen atmosphere at 0°C with ethyl trifluoroacetate (0.45 mL, 3.7 mmol). After being stirred at this temperature for 10 min, the solvent was distilled off and the residue was purified by chromatography on silica gel (hexane/ethyl acetate, 4:1) to give **6** as an oil (0.96 g, 87%). ^{1}H-NMR ($CDCl_3$): δ 1.98 (s, 3H), 2.01 (s, 3H), 4.37 (d, 2H, J = 5 Hz), 5.91 (s, 1H), 6.37 (s, broad, 1H), 7.19–7.47 (m, 5H). ^{13}C-NMR ($CDCl_3$): δ 10.78, 12.91, 36.78, 106.29, 112.90, 115.88 (q), 127.01, 128.02, 128.09, 128.84, 129.18, 138.28, 156.55 (q). MS (EI): m/z (%) = 522 (95) [M$^+$], 463 (100).

3-Chloro-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (7). A mixture of 2-(4-fluorobenzoyl)benzoic acid (5 g, 19 mmol) and thionyl chloride (5 mL) was heated with a catalytical amount of DMF under reflux for 1 h. The remaining thionyl chloride was distilled off under reduced pressure to yield **7** [16] (5.3 g, 98%) as solid. The crude product was used without further purification. It was stable under a nitrogen atmosphere at − 18°C for several weeks.

2,2,2-Trifluoro-N-{4-[1-(4-fluorophenyl)-3-oxo-1H-isobenzofuran-1-yl]-2,5-dimethyl-1-phenylpyrrol-3-ylmethyl}acetamide (8). A solution of $SnCl_4$ (0.43 mL, 3.7 mmol) in dry 1,2-dichloroethane (25 mL) was treated under a nitrogen atmosphere with **7** (813 mg, 3.1 mmol). After dissolution of **7**, compound **6**

(915 mg, 3.1 mmol) was added and the mixture was heated under reflux for 2 h. The reaction mixture was cooled, treated with ice-water and extracted with diethyl ether. The organic layer was dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by silica gel chromatography (hexane/ethyl acetate, 4:1) to give **8** (0.80 g, 50%) as solid; mp 173–174°C. ^{1}H-NMR ($CDCl_3$): δ 1.27 (s, 3H), 1.86 (s, 3H), 3.74 (dd, 1H, J = 5 Hz and 15 Hz), 3.99 (dd, 1H, J = 5 Hz and 15 Hz), 6.97 (t, 2H, J = 8 Hz), 7.02–7.09 (m, 3H), 7.33–7.42 (m, 5H), 7.45 (t, 1H, J = 8 Hz), 7.56 (t, 1H, J = 8 Hz), 7.85 (d, 1H, J = 8 Hz). ^{13}C-NMR ($CDCl_3$): δ 11.18, 13.22, 35.31, 90.46, 113.13, 115.89, 116.05 (q), 116.10, 116.67, 124.80, 125.05, 126.23, 127.54, 127.78, 127.87, 128.64, 128.66, 128.71, 129.46, 129.52, 129.61, 129.70, 134.19, 137.86, 138.23, 138.26, 152.52, 156.36 (q), 161.44, 163.91, 169.35. MS (EI): m/z (%) = 522 (5) [M^+], 301 (100).

3-(4-Aminomethyl-2,5-dimethyl-1-phenylpyrrol-3-yl)-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (9). To a solution of KOH (2.5 g) in water (7 mL) and methanol (10 mL) was added **8** (400 mg, 0.76 mmol). The mixture was stirred at room temperature for 30 min, diluted with water and extracted with diethyl ether and CH_2Cl_2. The organic phases were combined and the solvent distilled off. The residue was dissolved in dilute HCl and washed with diethyl ether. The aqueous phase was alkalized with dilute aqueous KOH-solution and extracted with diethyl ether. The organic layer was dried (Na_2SO_4) and concentrated to give **9** (257 mg, 78%) as solid; mp 193–195°C. ^{1}H-NMR (DMSO-d_6): δ 1.03 (s, 3H), 1.86 (s, 3H), 3.20 (d, 1H, J = 13 Hz), 3.28 (d, 1H, J = 13 Hz), 6.34 (d, 1H, 8 Hz), 6.95–7.16 (m, 8H), 7.34–7.58 (m, 4H). ^{13}C-NMR (DMSO-d_6): δ 10.48, 12.07, 37.10, 78.52, 113.50, 113.70, 121.48, 123.52, 123.83, 125.04, 125.74, 126.13, 126.24, 127.07, 127.99, 128.71, 130.52, 138.60, 143.72, 144.72, 144.74, 146.25, 159.21, 161.61, 173.04.

4-(2,4-Dioxothiazolidin-5-ylidenemethyl)-N-{4-[1-(4-fluorophenyl)-3-oxo-1H-isobenzofuran-1-yl]-2,5-dimethyl-1-phenylpyrrol-3-ylmethyl}benzamide (10). To a solution of **9** (250 mg, 0.59 mmol) in dry DMF (10 mL) was added 4-(2,4-dioxothiazolidin-5-ylidenemethyl)benzoic acid [9] (147 mg, 0.59 mmol). After addition of EDC-HCL (123 mg, 0.64 mmol) and 1-hydroxybenzotriazole (79 mg, 0.59 mmol), the mixture was stirred at room temperature for 1 h. Ethyl acetate was added and the organic solution was extracted twice with water and three times with brine. The organic layer was dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by silica gel chromatography ($CHCl_3$/methanol, 49:1) to give **10** (282 mg); 33 mg of the obtained solid were further purified by RP-HPLC applying acetonitrile/H_2O (73:27, v/v) as mobile phase. The eluates were concentrated under reduced pressure until most of the acetonitrile was distilled off. The remaining solvent was removed by freeze drying yielding pure **10** (26 mg). ^{1}H-NMR ($CDCl_3$): δ 1.35 (s, 3H), 2.01 (s, 3H), 3.80 (dd, 1H, J = 4 Hz and 15 Hz), 4.21 (dd, 1H, J = 6 Hz and 15 Hz), 6.89 (t, 1H, J = 8 Hz), 7.01–7.05 (m, 2H), 7.12–7.17 (m, 2H), 7.42–7.67 (m, 10H), 7.88 (s, 1H), 7.94 (d, 1H, J = 8 Hz), 8.01 (d, 2H, J = 8 Hz). ^{13}C-NMR ($CDCl_3$): δ 10.97, 13.05, 35.13, 90.67, 114.65, 115.56, 115.77, 116.31, 124.71, 124.82, 125.89, 127.21, 127.56, 127.64, 127.72, 128.34, 128.47, 128.93, 129.16, 129.21, 129.42, 130.00, 131.56, 133.97, 135.55, 135.69, 137.85, 137.93, 137.96, 152.54, 161.15, 163.61, 164.94, 167.05, 167.42, 169.44. MS (EI): m/z (%) = 657 (1) [M^+], 163 (100); MS (ESI-): m/z (%) = 656 (100) [$M^+ - 1$].

3-(2,5-Dimethyl-1-phenylpyrrol-3-yl)-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (11). Dry 1,2-dichloroethane (10 mL) was subsequently treated under a nitrogen atmosphere with $SnCl_4$ (0.18 mL, 1.55 mmol) and **7** (373 mg, 1.42 mmol). The mixture was stirred at room temperature for 10 min. Then 2,5-dimethyl-1-phenylpyrrole (250 mg, 1.35 mmol) was added and the resulting mixture heated at 50°C for 2 h. After cooling, water was added and the mixture was extracted twice with diethyl ether. The combined organic phases were washed with water, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (hexane/ethyl acetate, 4:1) to give **11** as solid (213 mg, 36%); mp 144–146°C. ^{1}H-NMR ($CDCl_3$): δ 1.60 (s, 3H), 1.85 (s, 3H), 5.49 (s, 1H), 6.92 (t, 2H, J = 9 Hz), 7.08–7.10 (m, 3H), 7.30–7.46 (m, 6H), 7.58 (t, 1H, J = 8 Hz), 7.83 (d, 1H, J = 8 Hz). ^{13}C-NMR ($CDCl_3$): δ 12.40, 12.98, 89.82, 107.20, 115.41, 115.62, 117.97, 123.86, 125.51, 125.98, 127.24, 127.95, 128.03, 128.37, 128.53, 128.98, 129.15, 129.42, 134.15, 138.32, 138.35, 138.42, 153.93, 161.33, 163.78, 170.72. MS (EI): m/z (%) = 397 (100) [M^+].

3-(2,5-Dimethyl-1-phenylpyrrol-3-yl)-3-methyl-3H-isobenzofuran-1-one (12). A mixture of 2,5-dimethyl-1-phenylpyrrole (1.0 g, 5.84 mmol) and 2-acetylbenzoic acid (0.96 g, 5.84 mmol) was heated at 120–140°C for 2 h. After being cooled, the reaction mixture was purified by silica gel chromatography (hexane/ethyl acetate, 9:1) to give **12** as solid (370 mg, 20%); mp 153°C. ^{1}H-NMR ($CDCl_3$): 1.69 (s, 3H), 1.96 (s, 3H), 2.01 (s, 3H), 5.95 (s, 1H), 7.20–7.25 (s, 2H), 7.40–7.44 (m, 3H), 7.48–7.52 (m, 2H), 7.63–7.67 (m, 1H), 7.88–7.90 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.10, 13.03, 28.41, 86.95, 105.63, 117.64, 122.49, 125.69, 126.09, 126.99, 127.65, 128.32, 128.63, 128.89, 129.37, 134.17, 138.43, 155.22, 170.60. MS (EI): m/z (%) = 317 (51) [M^+], 302 (100).

3-(2,5-Dimethyl-1-phenylpyrrol-3-yl)-3H-isobenzofuran-1-one (13). A solution of 2,5-dimethyl-1-phenylpyrrole (300 mg, 1.75 mmol) and 2-formylbenzoic acid (263 mg, 1.75 mmol) in toluene (10 mL) was refluxed under a nitrogen atmosphere for 2 h. The solvent was distilled off and the residue was purified by silica gel chromatography (hexane/ethyl acetate, 9:1) followed by preparative RP-HPLC (acetonitrile/water, 715:285). Recrystallization from ethanol/H_2O gave **13** as solid (45 mg, 8%); mp 120–121°C. ^{1}H-NMR ($CDCl_3$): 1.92 (s, 3H), 2.09 (s, 3H), 5.48 (s, 1H), 6.50 (s, 1H), 7.21–7.23 (m, 2H), 7.43–7.51 (m, 4H), 7.55 (t, 1H, J = 7 Hz), 7.66–7.70 (m, 1H), 7.95 (d, 1H, J = 8 Hz). ^{13}C-NMR ($CDCl_3$): δ 11.16, 12.96, 78.44, 105.24, 114.01, 123.39, 125.54, 127.05, 128.39, 128.48, 129.12, 129.48, 129.58, 134.02, 138.48, 150.27, 171.05. MS (EI): m/z (%) = 303 (100) [M^+], 244 (63).

3-(4-Fluorophenyl)-3-(1,2,5-trimethylpyrrol-3-yl)-3H-isobenzofuran-1-one (14). Under a nitrogen atmosphere $AlCl_3$ (573 mg, 4.3 mmol) and **7** (985 mg, 3.7 mmol) were added to 1,2-dichloroethane (20 mL) at 0°C. Then the mixture was treated dropwise with 1,2,5-trimethylpyrrole (0.5 mL, 3.7 mmol) ensuring that the temperature did not exceed 20°C. After being stirred at room temperature for 1 h, the reaction mixture was treated with ice water and extracted with diethyl ether. The organic phase was dried (K_2CO_3) and the solvent evaporated. The residue was purified by silica gel chromatography (hexane/ethyl acetate, 4:1) to give **14** as solid (750 mg, 60%); mp 158–160°C. ^{1}H-NMR ($CDCl_3$): δ 1.88 (s, 3H), 2.13 (s, 3H), 3.35 (s, 3H), 5.45 (s, 1H), 6.95–7.00 (m, 2H), 7.38–7.51 (m, 4H), 7.61–7.65 (m, 1H), 7.88–7.90 (m, 1H). MS (EI): m/z (%) = 335 (66) [M^+], 276 (100).

3-(2,5-Dimethylpyrrol-3-yl)-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (15). 2,5-Dimethylpyrrole (1.1 mL, 10 mmol) was reacted with **7** (2.8 g, 10 mmol) and $SnCl_4$ (1.5 mL, 12 mmol) in 1,2-dichloroethane (30 mL) in a similar manner as described for the synthesis of **11**. The product was recrystallized from ethanol to yield **15** (1.9 g, 57%); mp 185–186°C. ^{1}H-NMR ($CDCl_3$): δ 1.87 (s, 3H), 2.15 (s, 3H), 5.43 (s, 1H), 6.98 (t, 2H, J = 9 Hz), 7.39–7.49 (m, 4H), 7.63 (t, 1H, J = 8 Hz), 7.68 (s, br, 1H), 7.88 (d, 1H, J = 8 Hz). MS (EI): m/z (%) = 321 (66) [M^+], 262 (100).

Compounds **16–23**, **26**, **28**, **30–33** and **35** were synthesized by reacting the appropriate substituted 2,5-dimethylpyrroles with 3-chloro-3-(4-fluorophenyl)-3*H*-isobenzofuran-1-one (7) in a similar manner as described for the synthesis of **11**. The 1-aryl-2,5-dimethylpyrroles needed for the synthesis of these compounds were obtained by refluxing benzylamine, benzhydrylamine and substituted anilines, respectively, with hexane-2,5-dione and a catalytic amount *p*-toluenesulfonic acid in toluene at a water separator for 2 h followed by purification with silica gel chromatography.

The carboxylic acid derivative **24** was obtained by saponification of its ethyl ester **23** with aqueous KOH in ethanol. The amide **25** was prepared by refluxing the carbonitrile **22** with solid KOH in *tert*-butanol. The aniline derivatives **27** and **29** were synthesized by hydrolyzing their acetates **26** and **28**, respectively, with half concentrated HCl in methanol or ethanol. The dihydroxy-derivative **34** was obtained by cleaving the methylether of the corresponding 3-hydroxy-4-methoxy compound **33** with 2-bromobenzo[1,3,2]dioxaborol in dry CH_2Cl_2.

3-(1-Benzyl-2,5-dimethylpyrrol-3-yl)-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (16). Mp 141°C. ^{1}H-NMR ($CDCl_3$): δ 1.83 (s, 3H), 2.07 (s, 3H), 4.99 (s, 2H), 5.56 (s, 1H), 6.86 (d, 2H, J = 7 Hz), 6.96–7.01 (m, 2H), 7.23 (t, 1H, J = 8 Hz), 7.28–7.32 (m, 2H), 7.39–7.44 (m, 2H), 7.48–7.53 (m, 2H), 7.63–7.67 (m, 1H), 7.90–7.92 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 11.88, 12.64, 47.19, 89.85, 107.12, 115.36, 115.57, 117.78, 123.80, 125.38, 125.67, 125.90, 126.43, 127.40, 127.74, 127.83, 128.20, 128.98, 129.06, 134.05, 137.73, 138.41, 138.44, 153.79, 161.13, 163.58, 170.70. MS (EI): m/z (%) = 411 (100) [M^+].

3-(1-Benzhydryl-2,5-dimethylpyrrol-3-yl)-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (17). Mp 84°C. ^{1}H-NMR ($CDCl_3$): δ 1.68 (s, 3H), 1.79 (s, 3H), 5.53 (s, 1H), 6.72 (s, 1H), 6.93–6.99 (m, 2H), 7.06–7.09 (m, 4H), 7.28–7-37 (m, 8H), 7.44–7.46 (m, 1H), 7.48–7.52 (m, 1H), 7.61–7.65 (m, 1H), 7.90–7.92 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 13.28, 14.49, 62.52, 89.79, 108.34, 115.38, 115.59, 118.02, 123.91, 125.46, 125.97, 127.51, 127.72, 127.81, 128.45, 128.59, 128.69, 128.76, 129.08, 129.60, 134.05, 139.04, 139.22, 153.96, 163.71, 170.98. MS (EI): m/z (%) = 487 (8) [M^+], 167 (100).

3-(1-Biphenyl-4-yl-2,5-dimethylpyrrol-3-yl)-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (18). Mp 91°C. ^{1}H-NMR ($CDCl_3$): δ 1.73 (s, 3H), 1.99 (s, 3H), 5.60 (s, 1H), 6.99–7.05 (m, 2H), 7.25 (d, 2H, J = 9 Hz), 7.38 (t, 1H, J = 7 Hz), 7.44–7.56 (m, 6H), 7.61–7.69 (m, 5H), 7.92–7.94 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.49, 13.08, 89.83, 107.34, 115.43, 115.64, 118.12, 123.88, 125.53, 126.00, 127.36, 127.97, 127.99, 128.05, 128.39, 128.82, 129.05, 129.15, 129.18, 134.18, 137.52, 138.31, 138.34, 140.21, 141.28, 153.93, 161.35, 163.81, 170.73. MS (EI): m/z (%) = 473 (68) [M^+], 414 (100).

3-[1-(4-Chlorophenyl)-2,5-dimethylpyrrol-3-yl]-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (19). Mp 190°C. ^{1}H-NMR ($CDCl_3$): δ 1.67 (s, 3H), 1.92 (s, 3H), 5.58 (s, 1H), 6.98–7.03 (m, 2H), 7.12 (d, 2H, J = 9 Hz), 7.42–7.47 (m, 4H), 7.50–7.54 (m, 1H), 7.65–7.69 (m, 1H), 7.91–7.93 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.37, 12.95, 89.64, 107.55, 115.45, 115.67, 118.45, 123.82, 125.46, 126.02, 127.22, 127.91, 127.99, 128.86, 129.22, 129.72, 129.82, 134.21, 134.37, 136.92, 138.21, 153.81, 161.35, 163.81, 170.64. MS (EI): m/z (%) = 433 (26), 431 (73) [M^+], 372 (100).

3-[2,5-Dimethyl-1-(4-methylphenyl)pyrrol-3-yl]-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (20). Mp 191°C. ^{1}H-NMR ($CDCl_3$): δ 1.67 (s, 3H), 1.93 (s, 3H), 2.40 (s, 3H), 5.57 (s, 1H), 6.98–7.03 (m, 2H), 7.05 (d, 2H, J = 8 Hz), 7.24 (d, 2H, J = 8 Hz), 7.45–7.50 (m, 2H), 7.52–7.54 (m, 1H), 7.65–7.69 (m, 1H), 7.91–7.93 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.38, 12.97, 21.38, 89.89, 107.03, 115.39, 115.61, 117.78, 123.88, 125.52, 125.95, 127.30, 127.96, 128.04, 128.24, 129.05, 129.14, 130.04, 134.15, 135.78, 138.28, 138.36, 138.39, 153.98, 161.32, 163.78, 170.75. MS (EI): m/z (%) = 411 (59) [M^+], 352 (100).

3-[2,5-Dimethyl-1-(4-methoxyphenyl)pyrrol-3-yl]-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (21). Mp 188°C. ^{1}H-NMR ($CDCl_3$): δ 1.67 (s, 3H), 1.92 (s, 3H), 3.85 (s, 3H), 5.56 (s, 1H), 6.94–7.03 (m, 4H), 7.09 (d, 2H, J = 8 Hz), 7.45–7.54 (m, 4H), 7.64–7.69 (m, 1H), 7.91–7.92 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.51, 13.10, 55.74, 89.85, 106.81, 114.44, 115.29, 115.50, 117.54, 123.76, 125.35, 125.81, 127.37, 127.81, 127.89, 129.01, 129.09, 129.35, 130.97, 134.04, 138.19, 153.75, 159.17, 161.08, 163.53, 170.52. MS (EI): m/z (%) = 427 (13) [M^+], 368 (100).

3-[1-(4-Cyanophenyl)-2,5-dimethylpyrrol-3-yl]-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (22). Mp 83°C. ^{1}H-NMR ($CDCl_3$): δ 1.70 (s, 3H), 1.94 (s, 3H), 5.63 (s, 1H), 6.98–7.03 (m, 2H), 7.31 (d, 2H, J = 9 Hz), 7.42–7.47 (m, 2H), 7.50–7.54 (m, 2H), 7.65–7.69 (m, 1H), 7.77 (d, 2H, J = 9 Hz), 7.90–7.92 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.68, 13.24, 89.38, 108.40, 112.33, 115.49, 115.70, 118.18, 119.34, 123.72, 125.27, 125.99, 126.98, 127.78, 127.86, 128.48, 129.26, 129.34, 133.39, 134.24, 137.85, 137.88, 142.29, 153.46, 161.21, 163.66, 170.35. MS (EI): m/z (%) = 422 (68) [M^+], 363 (100).

Ethyl 4-{3-[1-(4-fluorphenyl)-3-oxo-1,3-dihydroisobenzofuran-1-yl]-2,5-dimethylpyrrol-1-yl}benzoate (23). Mp 61°C. ^{1}H-NMR ($CDCl_3$): δ 1.41 (t, 3H, J = 7 Hz), 1.70 (s, 3H), 1.94 (s, 3H), 4.40 (q, 2H, J = 7 Hz), 5.60 (s, 1H), 6.98–7.04 (m, 2H), 7.26 (d, 2H, J = 8 Hz), 7.44–7.48 (m, 2H), 7.48–7.54 (m, 2H), 7.66–7.70 (m, 1H), 7.92 (m, 1H), 8.17 (d, 2H, J = 8 Hz). ^{13}C-NMR ($CDCl_3$): δ 12.61, 13.19, 14.73, 61.61, 89.57, 107.83, 115.39, 115.61, 118.66, 123.73, 125.31, 125.91, 126.99, 127.79, 127.87, 128.37, 128.60, 129.13, 130.34, 130.70, 134.12, 137.99, 138.02, 142.19, 153.59, 161.15, 163.60, 165.79, 170.41. MS (EI): m/z (%) = 469 (27) [M^+], 410 (100).

4-{3-[1-(4-Fluorphenyl)-3-oxo-1,3-dihydroisobenzofuran-1-yl]-2,5-dimethylpyrrol-1-yl}benzoic acid (24). A mixture of **23** (560 mg, 1.19 mmol), ethanol (10 mL) and 10% aqueous KOH (6 mL) was heated under reflux for 5 min, cooled, acidified with dilute H_3PO_4 and extracted with diethyl ether. The organic phase was dried (Na_2SO_4) and the solvent evaporated. The residue was purified by silica gel chromatography eluting with diethyl ether to give **24** as solid (464 mg, 88%); mp 144°C. ^{1}H-NMR ($CDCl_3$): δ 1.71 (s, 3H), 1.96 (s, 3H), 5.62 (s, 1H), 6.98–7.04 (m, 2H), 7.29 (d, 2H, J = 9 Hz), 7.44–7.48 (m, 2H), 7.51–7.54 (m, 2H), 7.66–7.70 (m, 1H), 7.91–7.93 (m, 1H), 8.20 (d, 2H, J = 9 Hz). ^{13}C-NMR ($CDCl_3$): δ 12.68, 13.25, 89.62, 108.03, 115.45, 115.66, 118.86, 123.74, 125.31, 125.98, 127.03, 127.81, 127.89, 128.58, 128.61, 129.15, 129.19, 131.41, 134.19, 137.94, 137.97, 143.12, 153.59, 161.19, 163.64, 170.53, 170.93. MS (EI): m/z (%) = 441 (9) [M^+], 382 (100).

4-{3-[1-(4-Fluorophenyl)-3-oxo-1,3-dihydroisobenzofuran-1-yl]-2,5-dimethylpyrrol-1-yl}benzamide (25). A mixture of **22** (350 mg, 0.83 mmol), powdered KOH (85%) (210 mg, 3.3 mmol) and *tert*-butanol (15 mL) was heated under reflux for 10 min. The reaction mixture was cooled, diluted with water, acidified with dilute HCl and extracted with diethyl ether. The organic phase was washed twice with water, dried (Na_2SO_4) and concentrated to give **25** as solid (338 mg, 92%); mp 121°C. ^{1}H-NMR ($CDCl_3$): δ 1.68 (s, 3H), 1.93 (s, 3H), 5.59 (s, 1H), 6.28

(s, broad, 2H), 6.98–7.03 (m, 2H), 7.25 (d, 2H, J = 8 Hz), 7.42–7.47 (m, 2H), 7.50–7.54 (m, 2H), 7.65–7.69 (m, 1H), 7.89–7.93 (m, 3H). ^{13}C-NMR ($CDCl_3$): δ 12.64, 13.21, 89.67, 107.85, 115.44, 115.65, 118.66, 123.75, 125.27, 125.94, 127.06, 127.79, 127.87, 128.63, 128.66, 129.19, 133.15, 134.20, 137.93, 137.96, 141.54, 153.61, 161.18, 163.63, 168.84, 170.55. MS (EI): m/z (%) = 440 (100) [M^+].

N-(4-{3-[1-(4-Fluorophenyl)-3-oxo-1,3-dihydroisobenzofuran-1-yl]-2,5-dimethylpyrrol-1-yl}phenyl) acetamide (26). Mp 240°C. ^{1}H-NMR ($CDCl_3$): δ 1.63 (s, 3H), 1.90 (s, 3H), 2.23 (s, 3H), 5.55 (s, 1H), 6.97–7.03 (m, 2H), 7.04–7.13 (m, 2H), 7.42–7.47 (m, 2H), 7.50–7.54 (m, 4H), 7.66–7.70 (m, 1H), 7.76 (s, broad, 1H), 7.90–7.92 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.56, 13.16, 24.98, 90.15, 107.10, 115.42, 115.64, 117.67, 120.37, 123.85, 125.22, 125.87, 127.35, 127.78, 127.87, 128.82, 128.96, 129.16, 133.77, 134.27, 137.99, 138.02, 138.21, 153.82, 161.16, 163.62, 168.82, 170.96. MS (EI): m/z (%) = 454 (5) [M^+], 227 (100).

3-[1-(4-Aminophenyl)-2,5-dimethylpyrrol-3-yl]-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (27). A solution of **26** (250 mg, 0.55 mmol) in methanol (5 mL) was adjusted to pH 1 with dilute HCl. After heating under reflux for 6 h, the reaction mixture was cooled, neutralized with 25% aqueous KOH solution and extracted twice with diethyl ether. The combined organic phases were washed with water, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (hexane/ethyl acetate, 2:3) to give **27** as solid (64 mg, 28%); mp 213°C. ^{1}H-NMR ($CDCl_3$): δ 1.66 (s, 3H), 1.92 (s, 3H), 3.82 (s, 2H), 5.53 (s, 1H), 6.70 (d, 2H, J = 9 Hz), 6.92 (d, 2H, J = 9 Hz), 6.97–7.02 (m, 2H), 7.44–7.52 (m, 4H), 7.64–7.68 (m, 1H), 7.90–7.91 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.33, 12.92, 89.80, 106.37, 115.09, 115.30, 117.04, 123.59, 125.19, 125.61, 127.25, 127.64, 127.72, 128.70, 128.81, 128.97, 129.04, 133.83, 138.06, 138.09, 146.15, 153.63, 160.90, 163.34, 170.42. MS (EI): m/z (%) = 412 (14) [M^+], 353 (100).

N-(4-{3-[1-(4-Fluorophenyl)-3-oxo-1,3-dihydroisobenzofuran-1-yl]-2,5-dimethylpyrrol-1-yl}phenyl)-N-methylacetamide (28). Mp 73°C. ^{1}H-NMR ($CDCl_3$): δ 1.70 (s, 3H), 1.95 (s, 3H), 2.00 (s, 3H), 3.34 (s, 3H), 5.59 (s, 1H), 6.98–7.03 (m, 2H), 7.22–7.32 (m, 4H), 7.49–7.54 (m, 2H), 7.65–7.69 (m, 1H), 7.90–7.92 (m, 1H). MS (EI): m/z (%) = 468 (100) [M^+].

3-[2,5-Dimethyl-1-(4-methylaminophenyl)pyrrol-3-yl]-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (29). A solution of **28** (180 mg, 0.38 mmol) in a small amount of ethanol was treated with half concentrated HCl (5 mL) and heated under reflux for 4 h. The reaction mixture was poured into ice-water, alkalized with aqueous KOH and extracted three times with diethyl ether. The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (hexane/ethyl acetate, 7:3) to give **29** as solid (83 mg, 51%); mp 210°C. ^{1}H-NMR ($CDCl_3$): δ 1.66 (s, 3H), 1.93 (s, 3H), 2.87 (s, 3H), 3.90 (s, broad, 1H), 5.53 (s, 1H), 6.62 (d, 2H, J = 9 Hz), 6.95–7.02 (m, 4H), 7.44–7.52 (m, 4H), 7.63–7.67 (m, 1H), 7.89–7.91 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.60, 13.20, 31.09, 102.93, 106.53, 112.45, 115.33, 115.55, 117.19, 123.85, 125.49, 125.88, 127.60, 127.76, 127.92, 128.00, 129.04, 129.15, 129.44, 134.05, 149.01, 153.93, 163.60, 167.50, 169.47. MS (EI): m/z (%) = 426 (100) [M^+].

3-[1-(4-Dimethylaminophenyl)-2,5-dimethylpyrrol-3-yl]-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (30). Mp 74°C. ^{1}H-NMR ($CDCl_3$): δ 1.70 (s, 3H), 1.95 (s, 3H), 3.00 (s, 6H), 5.58 (s, 1H), 6.73 (d, 2H, J = 9 Hz), 6.98–7.05 (m, 4H), 7.48–7.56 (m, 4H), 7.65–7.69 (m, 1H), 7.91–7.92 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.48, 13.08, 40.68, 89.97, 106.43, 112.20, 115.19, 115.41, 117.05, 123.75, 125.32, 125.69, 126.81, 127.52, 127.79, 127.88, 128.76, 128.92, 129.27, 133.96, 149.88, 153.80, 161.00, 163.44, 170.52. MS (EI): m/z (%) = 440 (27) [M^+], 213 (100).

3-(4-Fluorophenyl)-3-[1-(4-hydroxyphenyl)-2,5-dimethylpyrrol-3-yl]-3H-isobenzofuran-1-one (31). Mp 238°C. ^{1}H-NMR ($CDCl_3$): δ 1.61 (s, 3H), 1.91 (s, 3H), 5.54 (s, 1H), 6.92–7.02 (m, 7H), 7.42–7.46 (m, 2H), 7.51–7.55 (m, 2H), 7.67–7.71 (m, 1H), 7.92–7.94 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.49, 13.11, 90.82, 106.71, 115.43, 115.64, 116.22, 117.08, 123.84, 125.09, 125.99, 127.48, 127.78, 127.86, 129.21, 129.26, 130.43, 134.43, 137.82, 137.85, 153.96, 156.26, 161.18, 163.63, 171.75. MS (EI): m/z (%) = 413 (100) [M^+].

3-(4-Fluorophenyl)-3-[1-(3-hydroxyphenyl)-2,5-dimethylpyrrol-3-yl]-3H-isobenzofuran-1-one (32). Mp 217°C. ^{1}H-NMR ($CDCl_3$): δ 1.66 (s, 3H), 1.93 (s, 3H), 5.53 (s, 1H), 6.52 (s, broad, 1H), 6.68–6.75 (m, 2H), 6.92–7.01 (m, 3H), 7.25–7.29 (m, 1H), 7.41–7.44 (m, 2H), 7.50–7.53 (m, 2H), 7.66–7.70 (m, 1H), 7.90–7.93 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.54, 13.15, 107.05, 115.41, 115.62, 115.74, 117.44, 120.41, 123.85, 125.17, 125.95, 127.21, 127.85, 127.93, 128.99, 129.21, 130.12, 134.36, 137.87, 137.90, 139.32, 153.91, 156.85, 161.18, 163.63. MS (EI): m/z (%) = 413 (36) [M^+], 354 (100).

3-(4-Fluorophenyl)-3-[1-(3-hydroxy-4-methoxyphenyl)-2,5-dimethylpyrrol-3-yl]-3H-isobenzofuran-1-one (33). Mp 88°C. ^{1}H-NMR ($CDCl_3$): δ 1.67 (s, 3H), 1.93 (s, 3H), 3.94 (s, 3H), 5.53 (s, 1H), 5.76 (s, broad, 1H), 6.66 (dd, 1H, J = 3 Hz and 8 Hz), 6.75 (d, 1H, J = 3 Hz), 6.88 (d, 1H, J = 8 Hz), 6.97–7.03 (m, 2H), 7.44–7.53 (m, 4H), 7.64–7.68 (m, 1H), 7.91–7.92 (m, 1H). ^{13}C-NMR ($CDCl_3$): δ 12.52, 13.10, 56.37, 89.91, 106.80, 110.58, 114.85, 115.32, 115.54, 117.52, 120.10, 123.80, 125.40, 125.86, 127.35, 127.86, 127.94, 129.04, 131.67, 134.05, 138.24, 145.91, 146.41, 153.80, 161.13, 163.58, 170.60. MS (EI): m/z (%) = 443 (8) [M^+], 261 (100).

3-[1-(3,4-Dihydroxyphenyl)-2,5-dimethylpyrrol-3-yl]-3-(4-fluorophenyl)-3H-isobenzofuran-1-one (34). To a solution of 2-bromobenzo[1,3,2]dioxaborol (560 mg, 2.82 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise under a nitrogen atmosphere at −70°C a solution of **33** (250 mg, 0.56 mmol) in dry CH_2Cl_2 (5 mL). The reaction mixture was allowed to warm up to room temperature and held there for 24 h. After addition of water, the organic phase was separated and the aqueous phase was extracted exhaustively with diethyl ether. The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (hexane/ethyl acetate/formic acid, 6:4:0.05). The product fractions were washed with dilute $NaHCO_3$ solution and water. After drying over Na_2SO_4, the solvent was evaporated to yield **34** as solid (123 mg, 51%); mp 116°C. ^{1}H-NMR (DMSO-d_6): δ 1.53 (s, 3H), 1.93 (s, 3H), 5.43 (s, 1H), 6.44–6.47 (m, 1H), 6.51 (s, 1H), 6.78 (d, 1H, J = 8 Hz), 7.15–7.20 (m, 2H), 7.42–7.45 (m, 2H), 7.58–7.62 (m, 1H), 7.68–7.70 (m, 1H), 7.76–7.80 (m, 1H), 7.85–7.87 (m, 1H). 9.27 (s, broad, 2H). ^{13}C-NMR (DMSO-d_6): δ 12.10, 12.72, 89.08, 106.33, 115.24, 115.35, 115.59, 115.68, 116.93, 118.83, 119.28, 124.04, 124.15, 125.24, 126.75, 127.42, 127.71, 127.79, 128.70, 129.51, 134.79, 138.20, 145.32, 145.50, 153.13, 160.32, 162.61, 169.33. MS (EI): m/z (%) = 429 (95) [M^+], 370 (100).

5-(4-{3-[1-(4-Fluorophenyl)-3-oxo-1,3-dihydroisobenzofuran-1-yl]-2,5-dimethyl-pyrrol-1-yl}benzylidene) thiazolidine-2,4-dione (35). ^{1}H-NMR (DMSO-d_6): δ 1.61 (s, 3H), 1.92 (s, 3H), 5.57 (s, 1H), 7.16–7.21 (m, 2H), 7.42–7.48 (m, 4H), 7.60–7.64 (m, 1H), 7.70–7.73 (m, 3H), 7.78–7.82 (m, 1H), 7.85 (s, 1H), 7.89–7.90 (m, 1H), 12.68 (s, 1H). ^{13}C-NMR (DMSO-d_6): δ 12.05, 12.62, 88.58, 107.27, 115.20, 115.41, 117.91, 123.84, 123.91, 124.34, 125.09, 126.57, 127.03, 127.55, 127.63, 128.77, 129.37, 130.37, 130.74, 132.57, 134.65, 137.74, 137.77, 138.46, 152.76, 160.16, 162.59, 167.05, 167.42, 169.01. MS (EI): m/z (%) = 524 (63) [M^+], 465 (100).

Biological assays

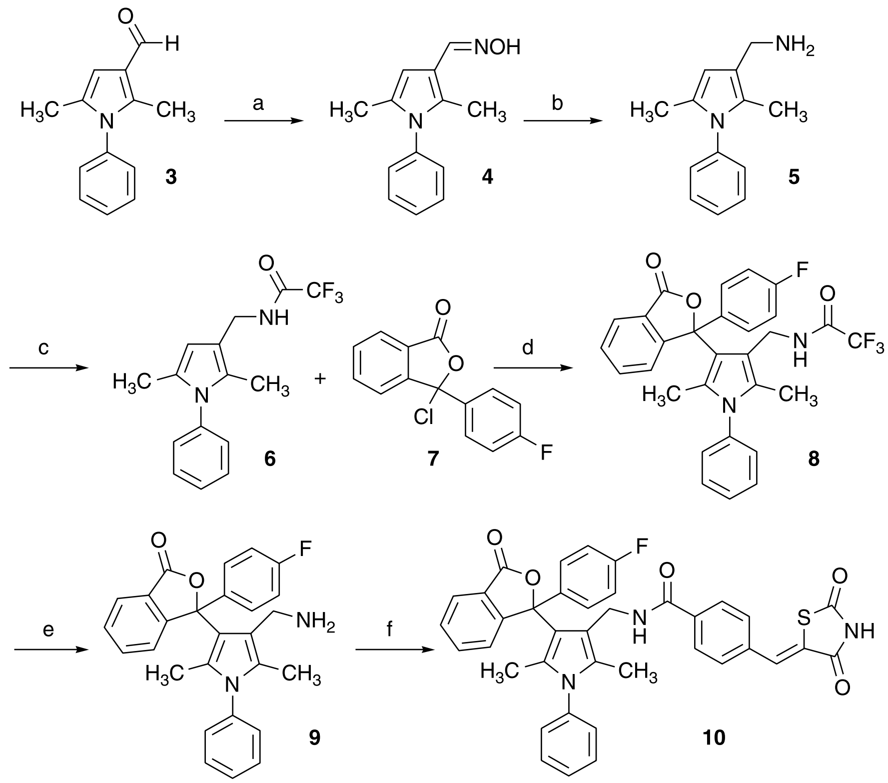
Assay with the isolated enzyme. The ability of test compounds to inhibit cPLA$_2$α isolated from human platelets was performed as previously described [10]. Briefly, sonicated covesicles consisting of 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (0.2 mM) and 1,2-dioleoyl-*sn*-glycerol (0.1 mM) were used as substrate. Enzyme reaction was terminated by addition of a mixture of acetonitrile, methanol and 0.1 M aqueous EDTA-Na_2 solution, which contained 4-undecyloxybenzoic acid as internal standard and nordihydroguaiaretic acid as oxygen scavenger. cPLA$_2$α activity was determined by measuring the arachidonic acid released by the enzyme with reversed phase HPLC and UV-detection at 200 nm after cleaning up the samples by solid phase extraction.

Cell assay. The ability of compounds to inhibit cPLA$_2$α activity in intact cells was determined by measuring the calcium ionophore A23187-induced arachidonic acid release from human platelets with HPLC/UV-detection according to a method previously described [12]. Deviating from this procedure, for HPLC-separation of arachidonic acid a RP18 multospher 100 column, 3 μm, 3.0 mm (I.D.) × 125 mm, with a RP18 multospher 100 guard column, 5 μm, 3.0 mm (I.D.) × 20 mm (CS-chromatographie service, Langerwehe, Germany) was applied. The mobile phase consisted of acetonitrile/10 mM $(NH_4)_2HPO_4$ buffer adjusted to pH 7.4 with *ortho*-phosphoric acid (50:50, v/v). The flow rate was 0.33 mL/min and the injected sample volume was 300 μL. The detection wavelength was 200 nm applying a Waters 2487 UV-detector. After each run the column was washed with 0.6 mL methanol. 3-(4-Decyloxyphenyl)propanoic acid was applied as internal standard.

Results and discussion

Chemistry

3-Pyrrol-3-yl-3*H*-isobenzofuran-1-one derivative **10** was synthesized by the route outlined in Scheme 1. 2,5-Dimethyl-1-phenylpyrrole-3-carbaldehyde (**3**) was reacted with hydroxylamine-HCl in the presence of Na_2CO_3 to give oxime **4** [8]. Amine **5** was afforded by reduction of the oxime functionality of **4** by $TiCl_4$/$NaBH_4$. Acetylation of **5** with ethyl trifluoroacetate led to the amide **6**. Treatment of this compound with **7** in the presence of $SnCl_4$ in a



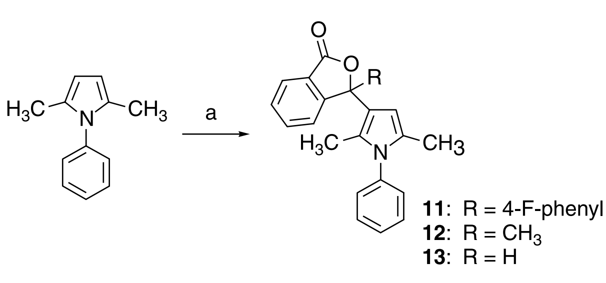
Scheme 1. (a) Hydroxylamine-HCl, Na_2CO_3, methanol, reflux; (b) $TiCl_4$, $NaBH_4$, 1,2-dimethoxyethane, room temp.; (c) ethyl trifluoroacetate, THF, 0°C; (d) **7**, $SnCl_4$, 1,2-dichlororethane, reflux; (e) KOH, water, methanol, room temperature; (f) 4-(2,4-dioxothiazolidin-5-ylidenemethyl)benzoic acid, EDC, 1-hydroxybenzotriazole, DMF, room temp.

Friedel-Crafts like reaction gave the intermediate **8**. Compound **7** necessary for this reaction was synthesized from 2-(4-fluorobenzoyl)benzoic acid and thionyl chloride. Cleavage of the trifluoroacetyl group of **8** by KOH provided the 3-pyrrol-3-yl-3*H*-isobenzofuran-1-one **9**, which was converted to the target compound **10** by reaction with 4-(2,4-dioxothiazolidin-5-ylidenemethyl)benzoic acid [9].

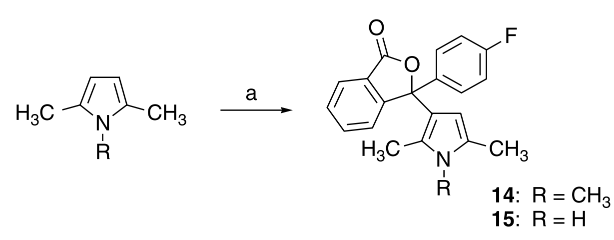
The analogous isobenzofuran-1-one **11** lacking the thiazolidine-containing side chain at the pyrrole scaffold was obtained by reaction of 2,5-dimethyl-1-phenylpyrrole with **7** applying $SnCl_4$ as catalyst (Scheme 2). Compound **12** possessing a methyl group instead of a 4-fluorophenyl substituent at position 3 of the isobenzofuran-1-one system was synthesized by heating 2,5-dimethyl-1-phenylpyrrole with 2-acetylbenzoic acid at 120–140°C. Reaction of 2,5-dimethyl-1-phenylpyrrole with 2-formylbenzoic acid led to the analogous isobenzofuran-1-one with only a pyrrole substituent at position 3.

The dimethyl- and trimethylpyrrolyl-substituted 3-(4-fluorophenyl)isobenzofuran-1-ones **14** and **15** were prepared by reaction of 2,5-dimethylpyrrole and 1,2,5-trimethylpyrrole with **7**, applying $AlCl_3$ and $SnCl_4$, respectively, as catalyst (Scheme 3).

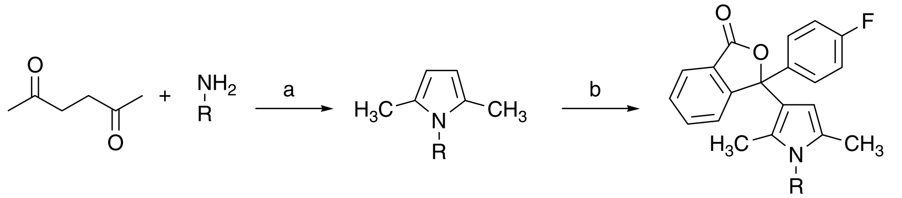
Derivatives of **11** bearing substituents in the phenyl ring of the pyrrole nucleus (**16–23,26,28,30–33,35**) were prepared as shown in Scheme 4. Reaction of substituted aniline derivatives with hexane-2,5-dione resulted in the formation of phenyl substituted 2,5-dimethyl-1-phenylpyrroles, which were converted to the target compounds by reaction with **7** in the



Scheme 2. (a) **11**: **7**, $SnCl_4$, 1,2-dichlororethane, 50°C; **12**: 2-acetylbenzoic acid, 120–140°C; **13**: 2-formylbenzoic acid, toluene, reflux.



Scheme 3. (a) **14**: **7**, $AlCl_3$, 1,2-dichlororethane, room temp.; **15**: **7**, $SnCl_4$, 1,2-dichlororethane, 50°C.



16-23, **26**, **28**, **30-33**, **35**

Compd.	R
16	benzyl
17	benzhydryl
18	4-phenylphenyl
19	4-Cl-phenyl
20	4-CH_3-phenyl
21	4-OCH_3-phenyl
22	4-CN-phenyl
23	4-$COOC_2H_5$-phenyl
26	4-$NHCOCH_3$-phenyl
28	4-NCH_3COCH_3-phenyl
30	4-$N(CH_3)_2$-phenyl
31	4-OH-phenyl
32	3-OH-phenyl
33	3-OH-4-OCH_3-phenyl
35	2,4-dioxothiazolidin-5-ylidenemethyl

Scheme 4. (a) *p*-Toluenesulfonic acid, toluene, reflux; (b) **7**, $SnCl_4$, 1,2-dichlororethane, 50°C.

presence of $SnCl_4$. The ethyl ester **23** was cleaved with aqueous KOH in ethanol to yield the carboxylic acid derivative **24**. Refluxing carbonitrile **22** with solid KOH in *tert*-butanol led to the amide **25**. The aniline derivatives **27** and **29** were synthesized by hydrolyzing their acetates **26** and **28**, respectively, with half concentrated HCl in methanol or ethanol. The dihydroxy-derivative **34** was obtained by cleaving the methylether of the corresponding 3-hydroxy-4-methoxy compound **33** with 2-bromobenzo[1,3,2]-dioxaborol in dry CH_2Cl_2.

Biological evaluation

All newly synthesized isobenzofuran-1-one derivatives were evaluated in an assay applying cPLA$_2\alpha$ isolated from human platelets [10]. Sonicated covesicles consisting of 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine and 1,2-dioleoyl-*sn*-glycerol were used as enzyme substrate. A possible problem of assays using such an aggregated form of phospholipids is that a test compound could inhibit the enzyme not by binding to its active site but merely by altering the substrate assembly and hence causing the enzyme to desorb from the lipid–water-interface. To exclude this way of action, the mole fraction of inhibitor in the interface has to be kept low [11]. Thus, the highest concentration of test compounds evaluated was 33 μM, while the concentration of the vesicle forming lipids was 300 μM. The enzyme activity was determined by measuring the enzyme product arachidonic acid formed after an incubation time of 60 minutes with HPLC and UV-detection at 200 nm.

Several of the active compounds were also tested in cellular situation. In this assay cPLA$_2\alpha$ of intact human platelets was stimulated with calcium ionophore A23187. cPLA$_2\alpha$-catalysed liberation of arachidonic acid from membrane phospholipids was measured with HPLC and UV-detection at 200 nm [12]. To avoid metabolism of arachidonic acid via cyclooxygenase-1 and 12-lipoxygenase pathways, the dual cyclooxygenase/12-lipoxygenase inhibitor 5,8,11,14-eicosatetraynoic acid (ETYA) was added to the platelets in these experiments.

Since lysis of the platelets by a test compound may falsely indicate enzyme inhibition, we also determined the cell lytic potency of each compound by turbidimetry [13]. In these experiments it was found that none of the compounds showed cell lytic properties at concentrations near its IC_{50} against cPLA$_2\alpha$.

Structure–activity relationships

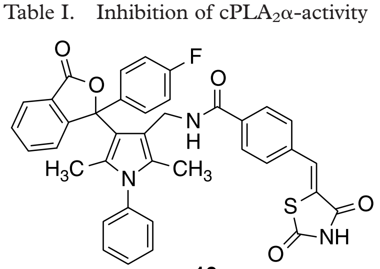
Important pharmacophoric groups of the potent Shionogi cPLA$_2\alpha$ inhibitors such as **2** (Figure 1) are the *o*-(benzoyl)benzoyl-moiety and the thiazolidinedione part of the molecules. In our effort to develop new inhibitors of cPLA$_2\alpha$, we wanted to synthesize compounds, in which these residues are attached to a 1-phenyl-substituted pyrrole.

The pyrrole residue was chosen as central scaffold in these investigations, because structural variations of this activated heterocycle can be easily performed. During the synthesis of the concipated substances it became evident that under the reaction conditions applied the *o*-(benzoyl)benzoyl-residue rearranges into a 3*H*-isobenzofuran-1-one [14–16]. Biological evaluation of the 1-phenylpyrrole **10** with 3*H*-isobenzofuran-1-one and thiazolidienedione bearing substituents in position 3 and 4 showed that **10** possess some inhibitory potency against cPLA$_2\alpha$. Its IC$_{50}$-value in the isolated enzyme assay was 26 µM. In cellular situation **10** inhibited cPLA$_2\alpha$-mediated arachidonic acid release with an IC$_{50}$ of 3.1 µM. For comparison, the known cPLA$_2\alpha$-inhibitor arachidonyltrifluoromethyl ketone (**1**) showed IC$_{50}$-values of 2.3 µM and 3.3 µM in these assays (Table I).

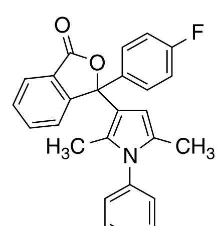
Since omission of the thiazolidinedione part of **10** led to a derivative with similar inhibition values (Table I), this structurally simpler compound (**11**) was taken as lead in our further inhibitor development. First, we substituted the 4-fluorophenyl residue of **11** by methyl and hydrogen. In contrast to **11**, which reduced cPLA$_2\alpha$ activity at 33 µM to 40%, the obtained isobenzofuran-1-ones **12** and **13** showed no enzyme inhibition at this concentration (Table II). Replacement of the 1-phenyl substituent of the pyrrole ring system of **11** by methyl (**14**) or hydrogen (**15**) did not change the inhibitory properties significantly. However, introduction of a benzyl moiety at the pyrrole nitrogen (**16**) caused a decrease of inhibitory potency, while a benzhydryl substituent (**17**), which is also present in several known cPLA$_2\alpha$ inhibitors [17], even led to inactivity at 33 µM.

Next, a variety of substituents were introduced into 4-position of the phenyl residue attached to the pyrrole ring of **11**. All compounds synthesized were tested in the isolated enzyme assay at 33 µM. Only compounds with H-donor substituents such as

Table I. Inhibition of cPLA$_2\alpha$-activity



10

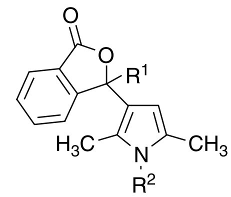


11

Compound	Vesicle assay with the isolated enzyme IC$_{50}$ (µM)[a]	Cellular assay with intact human platelets IC$_{50}$ (µM)[a]
10	26	3.1
11	>33[b]	8.0
1 (AACOCF$_3$)	2.3	3.3

[a]Values are the means of at least two independent determinations; errors are within ± 20%. [b]Inhibition of enzyme activity: 40% at 33 µM.

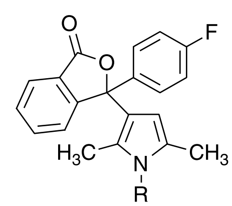
Table II. Inhibition of cPLA$_2\alpha$-activity



Compound	R[1]	R[2]	Vesicle assay with the isolated enzyme Inhibition at 33 µM (%)[a]
11	4-F-phenyl	Phenyl	40
12	CH$_3$	Phenyl	n.a.[b]
13	H	Phenyl	n.a.[b]
14	4-F-phenyl	CH$_3$	36
15	4-F-phenyl	H	35
16	4-F-phenyl	Benzyl	18
17	4-F-phenyl	Benzhydryl	n.a.[b]

[a]Values are the means of at least two independent determinations. [b]n.a.: not active at 33 µM.

Table III. Inhibition of cPLA$_2\alpha$-activity



Compound	R	Vesicle assay with the isolated enzyme Inhibition at 33 µM (%)[a]
11	Phenyl	40
18	4-phenylphenyl	n.a.
19	4-Cl-phenyl	n.a.
20	4-CH$_3$-phenyl	n.a.
21	4-OCH$_3$-phenyl	n.a.
22	4-CN-phenyl	n.a.
24	4-COOH-phenyl	51
25	4-CONH$_2$-phenyl	42
26	4-NHCOCH$_3$-phenyl	35
27	4-NH$_2$-phenyl	55
29	4-NHCH$_3$-phenyl	36
30	4-N(CH$_3$)$_2$-phenyl	n.a.
31	4-OH-phenyl	60
32	3-OH-phenyl	30
34	3,4-diOH-phenyl	20
35	2,4-dioxothiazolidin-5-ylidenemethyl	76

[a]Values are the means of at least two independent determinations. [b]n.a.: not active at 33 µM.

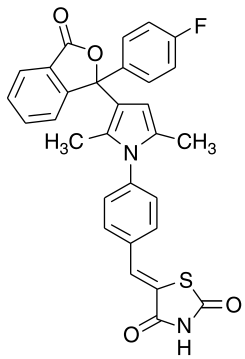


Figure 2. Structure of **35**.

COOH, $CONH_2$, OH or NH_2 in *para*-position showed some activity at this concentration (Table III).

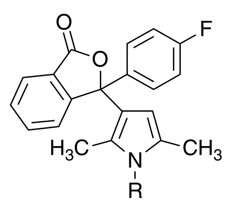
The most active of these derivatives was the 2,4-dioxothiazolidin-5-ylidenemethyl-substitued compound **35** (Figure 2) showing 76% inhibition at 33 μM (IC_{50}: 7.3 μM). Interestingly, the 3-hydroxy- and the 3,4-dihydroxyphenyl substituted pyrroles **32** and **34** were less active at 33 μM against the isolated enzyme than the 4-hydroxyphenyl derivative **31**.

The active 3-pyrrol-3-ylisobenzofuran-1-ones with 4-hydroxyphenyl-, 4-aminophenyl and 4-(2,4-dioxothiazolidin-5-ylidenemethylphenyl)-residues (**27**,**31**,**35**) were also investigated in the cellular system. Here, the IC_{50} of hydroxy- and amino-substituted derivatives **27** and **31** was 2.8 μM each. The thiazolidinedione **35** even revealed an IC_{50} of 0.7 μM in this assay (Table IV).

The evaluation of 3-hydroxy- and 3,4-dihydroxyphenyl-substituted compounds **32** and **34** in cellular situation led to an unexpected result. Although they were less active against the isolated enzyme, here their activity was even higher than that of the 4-hydroxy-derivative **27** (2.0 μM and 1.3 μM, respectively, vs. 2.8 μM). Taken together, the observed inhibition of cellular arachidonic acid release by the 3-pyrrol-3-ylisobenzofuran-1-ones investigated seems to be caused not only by a direct inhibition of $cPLA_2\alpha$. Other mechanisms may contribute to the reduction of cellular arachidonic acid liberation. Similar results have been evaluated for several indole-2-carboxylic acid derivatives before [18].

In conclusion, we have found some 3-pyrrol-3-ylisobenzofuran-1-ones to be $cPLA_2\alpha$ inhibitors. Since these isobenzofuranes show higher $cPLA_2\alpha$ inhibitory potency in cellular situation than against the isolated enzyme, it seems to be possible that they exert their cellular activities not only by a direct interaction with the enzyme but also by other as yet unknown mechanisms.

Table IV. Inhibition of $cPLA_2\alpha$-activity



Compound	R	Vesicle assay with the isolated enzyme IC_{50} (μM)[a]	Cellular assay with intact human platelets IC_{50} (μM)[a]
11	Phenyl	> 33[b]	8.0
27	4-NH_2-phenyl	27	2.8
31	4-OH-phenyl	22	2.8
32	3-OH-phenyl	> 33[c]	2.0
34	3,4-diOH-phenyl	> 33[d]	1.3
35	2,4-dioxothiazolidin-5-ylidenemethyl	7.3	0.7

[a]Values are the means of at least two independent determinations; errors are within ± 20%. [b]Inhibition of enzyme activity: 40% at 33 μM. [c]Inhibition of enzyme activity: 30% at 33 μM. [d]Inhibition of enzyme activity: 20% at 33 μM.

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