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Structure–activity relationship studies on 1-(5-carboxyindol-1-yl)-propan-2-one inhibitors of human cytosolic phospholipase $A_2\alpha$: Variation of the activated ketone moiety

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ABSTRACT

Indole-5-carboxylic acids with 3-aryloxy-2-oxopropyl residues in position 1 have been found to be potent inhibitors of human cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$). In course of structure–activity relationship studies, we investigated the effect of the substitution of the electrophilic ketone group in the middle part of the molecule by other polar residues, such as hydroxyimino, azido, acyloxy, acylamino, urea and carbamate, on enzyme inhibition. With an IC_{50} of 1.7 μM against $cPLA_2\alpha$ from human platelets, the 4-fluorophenylcarbamate derivative **23f** was the most active of the compounds tested.

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.[1,2] 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. Although several more phospholipases $A_2\alpha$ are present in the mammalian organism, the pre-eminence of $cPLA_2\alpha$ for lipid mediator generation was demonstrated especially by studies with $cPLA_2\alpha$ deficient mice. These animals, which display a reduced eicosanoid production, are resistant to disease in a variety of models of inflammation.[3,4] Therefore, $cPLA_2\alpha$ is considered as a target for the treatment of inflammatory diseases.[4–8]

First-generation $cPLA_2\alpha$ inhibitors were analogues of arachidonic acid with the COOH group replaced by $COCF_3$ (arachidonyl trifluoromethyl ketone, $AACOCF_3$, **1**) or $CH_2PO(OCH_3)F$ (methyl arachidonoyl fluorophosphonate, MAFP)[5] (Fig. 1). While these compounds possessed only a medium inhibitory potency, inhibitors of $cPLA_2\alpha$ discovered later on, such as thiazolidinediones from Shionogi,[9,10] benzhydrylindoles from Wyeth,[11] and propan-2-ones from AstraZeneca like ARC-70484XX (**2**)[12] showed a high in vitro activity.

In our group we have found the indole-5-carboxylic acid derivative **3**, structurally related to **2**, to be a potent inhibitor of $cPLA_2\alpha$.[13] During structure–activity relationship studies we have already varied the heterocyclic part as well as the octylphenoxy moiety of this lead compound.[14–18] Here, we report the effect of a modification of the activated ketone group of **3** positioned in the middle part of the molecule on inhibitory potency against $cPLA_2\alpha$.

The hydroxylamine-substituted carboxylic acid derivatives **7** and **8** were prepared by the route outlined in Scheme 1. The synthesis started from allyl 1-oxiranylmethylindole-5-carboxylate (**3**),[17] which was reacted with 4-octylphenol in presence of

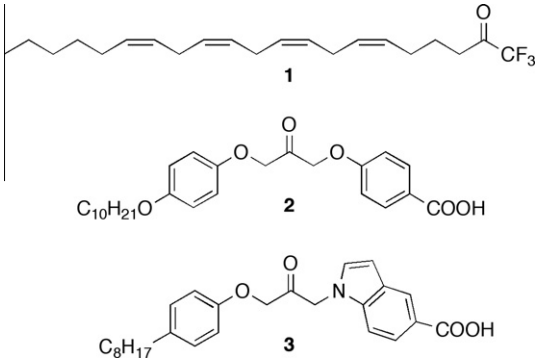

Figure 1. Described inhibitors of $cPLA_2\alpha$.

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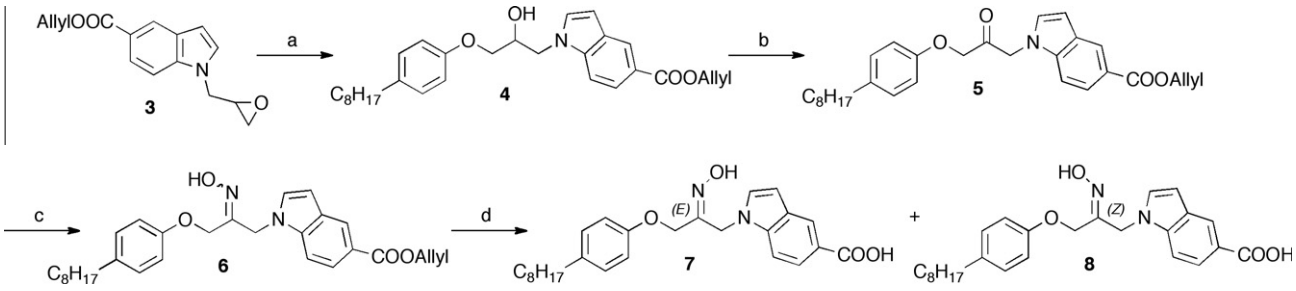
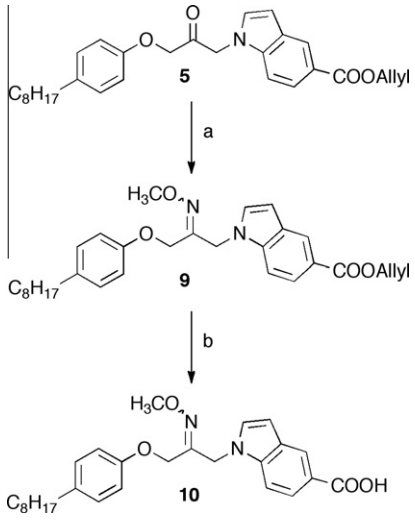
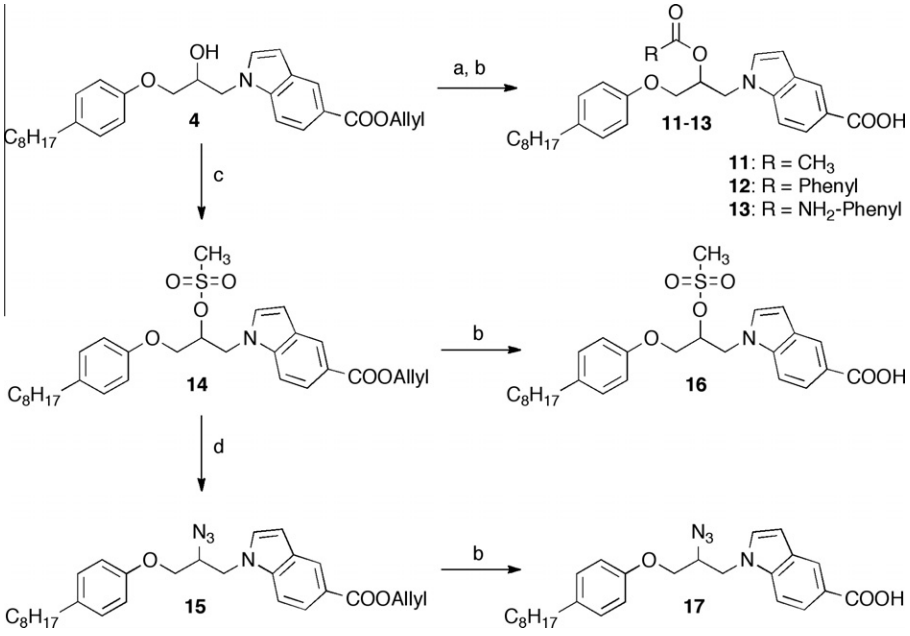


Scheme 1. Reagents and conditions: (a) 4-Octylphenol, 4-dimethylaminopyridine, 120 °C, 40 min, 56%; (b) Dess–Martin reagent, CH_2Cl_2, room temp, 4 h, 86%; (c) hydroxylamine hydrochloride, pyridine, reflux, 4 h, 96%; (d) $Pd(PPh_3)_4$, CH_3COOH, THF, room temp, 24 h, **7**: 22%; **8**: 36%.



Scheme 2. Reagents and conditions: (a) Methoxylamine hydrochloride, pyridine, reflux, 4 h, 88%; (b) $Pd(PPh_3)_4$, CH_3COOH, THF, room temp, 24 h, 83%.

4-dimethylaminopyridine. Oxidation of the resulting alcohol intermediate **4** to the ketone **5** was carried out with Dess–Martin periodinane reagent. Reaction with hydroxylamine hydrochloride in pyridine led to a mixture of the *E*- and *Z*-isomers of oxime derivative **6**. Cleavage of the allyl ester of **6** was achieved by Pd(0)-catalyzed allyl transfer. Obtained oxime isomers **7** and **8** were separated by reversed phase HPLC. Since NOE-experiments were not successful, the stereochemistry of the two compounds was assigned on the basis of their ^{13}C NMR spectra. It has been shown that in the ^{13}C NMR spectra the resonances of the carbonyl carbon and both α-carbons of a ketone all shift upfield on oxime formation, with the effect for the carbon in *syn*-position to the hydroxy group of the oxime being greater than for the *anti* carbon.[19–22] In the spectrum of compound **7** the signals of the carbons standing in α-position to the oxime moiety appear at 66 ppm (phenoxy-C) and 40 ppm (indolyl-C), respectively, while the chemical shifts of the corresponding signals of **8** are 61 ppm and 46 ppm. Because in the spectrum of compound **7** the indolyl-C signal is shifted more upfield than in the spectrum of **8** (40 vs 46 ppm), this carbon stands in *syn*-position to the oxime hydroxy group of **7**. Vice versa, the resonance of the phenoxy-C of **8** is seen at lower ppm values than in case of compound **7** (61 vs 66 ppm), showing that the hydroxy moiety of **8** is *syn* to the phenoxy-C. The assignment of the signals of the phenoxy-C and indolyl-C in the ^{13}C NMR spectra of **7** and **8** was made with the help of NOE- and $^{1}H/^{13}C$-correlation spectra (see Supplementary data).

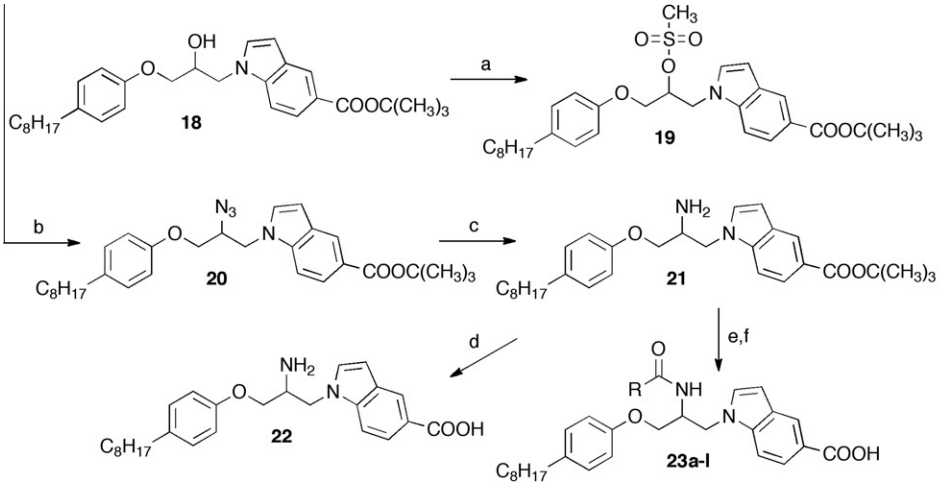


Scheme 3. Reagents and conditions: (a) **11**: acetyl chloride, pyridine, CH_2Cl_2, 0 °C, 6 h, 90%; **12**: benzoyl chloride, pyridine, CH_2Cl_2, 0 °C, 6 h, 99%; **13**: phenylisocyanate, triethylamine, THF, reflux, 4 h, 66%; (b) $Pd(PPh_3)_4$, CH_3COOH, THF, room temp, 5–24 h, 25–94%; (c) methanesulfonyl chloride, pyridine, room temp, 4 h, 91%; (d) trimethylsilyl azide, tetrabutylammonium fluoride, THF, reflux, 4 h, 90%.

The methyloxime-substituted indole-5-carboxylic acid **10** was synthesized by reaction of allyl ester derivative **5** with methoxylamine hydrochloride followed by Pd(0) catalyzed ester hydrolysis (Scheme 2). The target compound **10** was afforded as a 1:1 mixture of the *E*- and *Z*-isomers.

The test substances with acetoxy-, benzoyloxy-, phenylcarbamoyloxy- and methanesulfonyloxy-substituents at the central propyl part of the molecule (**11**–**13** and **16**) were prepared by reaction of **4** with acetyl chloride, benzoyl chloride, phenyl isocyanate and methanesulfonyl chloride, respectively, in presence of an amine base and subsequent ester cleavage by palladium catalysis (Scheme 3).

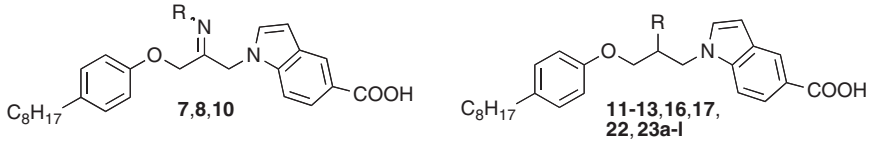
For the synthesis of the 2-azidopropyl-substituted indole-5-carboxylic acid **17**, first the methanesulfonyloxy group of compound **14** was converted to an azide with trimethylsilyl azide and tetrabutylammonium fluoride in THF (Scheme 3). Then the



Compound	R
23a	CH_3
23b	Phenyl
23c	NHPhenyl
23d	OCH_3
23e	OPhenyl
23f	O(4-F-Phenyl)
23g	O(4-Cl-Phenyl)
23h	O(4-OCH_3-Phenyl)
23i	O(3-F-Phenyl)
23j	O(2-F-Phenyl)
23k	O(2-Cl-Phenyl)
23l	O(2-OCH_3-Phenyl)

Scheme 4. Reagents and conditions: (a) Methanesulfonyl chloride, pyridine, room temp, 4 h, 85%; (b) trimethylsilyl azide, tetrabutylammonium fluoride, THF, reflux, 3 h, 69%; (c) H_2, Pd/C, methanol, THF, room temp, 2 h, 99%; (d) trifluoroacetic acid, CH_2Cl_2, room temp, 18 h, 95%; (e) **23a**,**b**: acyl chloride, pyridine, CH_2Cl_2, 0 °C, 2.5–5 h, 82–84%; **23c**: phenylisocyanate, triethylamine, THF, reflux, 3.5 h, 23%; **23d**,**e**,**g**: substituted chloroformate, ethyl(diisopropyl)amine, CH_2Cl_2, DMF, room temp, 16–18 h, 57–86%; **23f**: 4-fluorophenyl chloroformate, triethylamine, 4-dimethylaminopyridine, CH_2Cl_2, DMF, room temp, 14 d, 12%; **23h**–**l**: substituted chloroformate, ethyl(diisopropyl)amine, THF, room temp, 0.5–1 h, 78–95%; (f) trifluoroacetic acid, CH_2Cl_2, room temp, 2–24 h, 20–97%.

Table 1
Inhibition of cPLA$_2\alpha$-activity



Compd	R	Inhibition of cPLA2α IC_{50}[a] (μM)
7	OH (*E*)	>10 (42%[b])
8	OH (*Z*)	>10 (32%[b])
10	OCH_3 (*E*/*Z*)	>10 (22%[b])
11	$OCOCH_3$	n.a.[c]
12	OCOPhenyl	>10 (18%[b])
13	OCONHPhenyl	n.a.[c]
16	OSO_2CH_3	>10 (16%[b])
17	N_3	4.3
22	NH_2	>10 (37%[b])
23a	$NHCOCH_3$	n.a.[c]
23b	NHCOPhenyl	n.a.[c]
23c	NHCONHPhenyl	n.a.[c]
23d	$NHCOOCH_3$	>10 (21%[b])
23e	NHCOOPhenyl	4.6
23f	NHCOO(4-F–Phenyl)	1.7
23g	NHCOO(4-Cl–Phenyl)	>10 (28%[b])
23h	NHCOO(4-OCH_3–Phenyl)	>10 (23%[b])
23i	NHCOO(3-F–Phenyl)	5.1
23j	NHCOO(2-F–Phenyl)	n.a.[c]
23k	NHCOO(2-Cl–Phenyl)	n.a.[c]
23l	NHCOO(2-OCH_3–Phenyl)	n.a.[c]
1 ($AACOCF_3$)		2.3
3		0.035

[a] Values are the means of at least two independent determinations; errors are within ±20%.
[b] Inhibition at 10 μM.
[c] n.a.: not active at 10 μM.

allyl ester moiety of obtained intermediate **15** was hydrolyzed as described above.

Scheme 4 outlines the chemical approach used for the preparation of the amine-, amide-, urea- and carbamate-derivatives **22** and **23a–l**. The reaction sequence started from the *tert*-butyl indole-5-carboxylate **18**.[23] The hydroxy group of this compound was converted to an azide on the route described above for the synthesis of corresponding allyl ester derivative **15** (Scheme 3). Catalytic hydrogenation of the azide group of obtained compound **20** with Pd on charcoal led to the amine-substituted compound **21**. Reaction of this intermediate with acyl halogenides, phenylisocyanate and chloroformates, respectively, in presence of an amine base followed by hydrolysis of the *tert*-butyl ester with trifluoroacetic acid gave the desired target compounds **23a–l**. The unsubstituted amine **22** was afforded directly from **21** after ester cleavage. Because the syntheses of the target compounds **11**–**13**, **16**, **17**, **22** and **23a–l** were not performed under asymmetric conditions, all these substances were afforded as racemates (see Supplementary data).

Structure–activity relationship studies on the lead compound **3** have revealed that the carboxylic acid moiety in position 5 of the indole scaffold, the lipophilic residue bound to the phenoxy group of the molecule, and especially the activated ketone in the central part of the molecule are important for the high activity of the compound. On binding to cPLA$_2\alpha$, the electrophilic ketone moiety of **3** is supposed to form covalent binding interactions with a serine of the active site of the enzyme.[12,13] Metabolic reduction of this ketone group to a secondary alcohol leads to a loss of activity.[24] Experiments with rat liver microsomes have shown that such a reduction does not occur to a high extent as far as the molecule bears a long lipophilic residue.[17,18] However, when this lipophilic area of the molecule is reduced in order to make the compound more drug like, the extent of metabolic keto reduction increases significantly. Therefore, we investigated now, whether the metabolically unstable activated ketone group can be replaced by other polar groups, which are also able to interact with the serine of the active site either by dipol–dipol interactions and formation of H-bonds or by a covalent binding mode.

Evaluation of the inhibitory activity against cPLA$_2\alpha$[25,26] showed that the exchange of the ketone group by an oxime resulted in a drastic drop of activity. While the lead **3** inhibited the enzyme with an IC$_{50}$ of 0.035 μM, the IC$_{50}$s of both oxime isomers **7** (*E*) and **8** (*Z*) lay above 10 μM (Table 1). The *E*/*Z*-mixture of the methyloxime **10** also inhibited the enzyme to less than 50% at 10 μM. At the same concentration level the acetoxy-, benzoyloxy-, phenylcarbamoyloxy-, and methanesulfonyloxy-derivatives **11**, **12**, **13** and **16** showed no or only a marginal activity.

The first compound obtained in this series with an IC$_{50}$ less than 10 μM was the azide **17**. Its inhibitory potency lay in the same order of magnitude as that of the reference AACOCF$_3$ (**1**). Conversion of the azide group to an amine led to a decrease of activity. Compound **22** only showed an inhibition of 37% at 10 μM.

Next several derivatives of this amine were synthesized and tested. Acetylation (**23a**), benzoylation (**23b**) and conversion into a phenyl urea (**23c**) resulted in a loss of activity at 10 μM. In contrast, the *O*-methyl carbamate **23d** was slightly active at this concentration, and with an IC$_{50}$ of 4.6 μM the *O*-phenyl carbamate **23e** even possessed a considerable inhibitory potency. Like activated ketone compounds, phenyl carbamates are known to act as serine traps.[27,28] They can form covalent bonds via the nucleophilic attack of the hydroxyl of a serine residue of a protein, which leads to the displacement of the leaving group phenol. Finally, this leaving group was modified by introduction of fluoro-, chloro- and methoxy-substituents. A fluoro atom in position 4 of the phenyl ring increased activity about two- to threefold (IC$_{50}$ of **23f**: 1.7 μM), while the introduction of a lipophilic 4-chloro- (**23g**) as well as a polar 4-methoxy-substituent (**23h**) reduced activity. Switching the fluoro atom from 4 into 3 or 2 position of the phenyl residue was less favorable. While the 3-substituted derivative **23i** still was as active as the unsubstituted compound **23e**, the 2-fluoro derivative **23j** had no activity any more at 10 μM. Just so the 2-chloro- and 2-methoxy compounds **23k** and **23l** were inactive.

Taken together, all derivatives synthesized were less active than the lead **3**. However, with the carbamates **23e** and **23f** interesting new leads have been found. Compounds with such a structural element have not been described as cPLA$_2\alpha$ inhibitors yet. In further studies structural modifications of the indole as well as the octylphenyl part of the molecules will be performed in order to increase activity of the inhibitors.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.085.

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