ACS Medicinal Chemistry Letters

Letter

Design of selective sPLA2-X inhibitor (-)-2-{2-[carbamoyl-6-(trifluoromethoxy)-1H-indol-1-yl]pyridine-2-yl}propanoic acid

Fabrizio Giordanetto, Laurent Knerr, Peter Nordberg, Daniel Pettersen, Nidhal Selmi, Hans-Georg Beisel, Hanna de la Motte, Åsa Månsson, Mikael Dahlstrom, Ingemar Starke, Johan Broddefalk, Gabrielle Saarinen, Fredrik Klingegård, Eva Hurt-Camejo, Birgitta Rosengren, Johannes Wikström, Maria Wågberg, Johan Brengdahl, Mattias Rohman, Jenny Sandmark, Tomas Akerud, Robert G. Roth, Frank Jansen, and Marie Ahlqvist

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.7b00507 • Publication Date (Web): 23 Jun 2018

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Design of selective sPLA$_2$-X inhibitor (-)-2-{2-[carbamoyl-6-(trifluoromethoxy)-1H-indol-1-yl]pyridine-2-yl}propanoic acid

Fabrizio Giordanetto[a*,†] Laurent Knerr[a*], Peter Nordberg[a], Daniel Pettersen[a], Nidhal Selmi[a], Hans-Georg Beisel[a,#], Hannah de la Motte[a,¤], Åsa Månsson[a,Ø], Mikael Dahlström[a], Johan Broddefalk[a], Gabrielle Saarinen[a,§], Fredrik Klingegård[a,¶], Eva Hurt-Camejo[b], Birgitta Rosengren[c], Johannes Wikström[c], Maria Wågberg[c], Johan Brengdahl[d], Mattias Rohman[d], Jenny Sandmark[e], Tomas Åkerud[e], Robert G. Roth[e], Frank Jansen[f], Marie Ahlqvist[g]

[a]Medicinal Chemistry, [b]Translational Sciences, [c]Bioscience and [g]Drug Metabolism and Pharmacokinetics, Cardiovascular and Metabolic Diseases, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden.
[d]Reagents and Assay Development,[e]Structure and Biophysics, [f]Mechanistic Biology and Profiling, Discovery Sciences, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden.

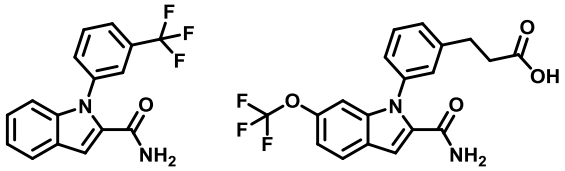
KEYWORDS. secreted phospholipase A2 type X; sPLA2-X; inhibitor; atherosclerosis, coronary artery disease, carotid ligation

ABSTRACT: A lead generation campaign identified indole-based sPLA$_2$-X inhibitors with a promising selectivity profile against other sPLA$_2$ isoforms. Further optimisation of sPLA$_2$ selectivity and metabolic stability resulted in the design of (-)-**17**, a novel, potent and selective sPLA$_2$-X inhibitor with an exquisite pharmacokinetic profile characterised by high absorption and low clearance, and low toxicological risk. (-)-**17** was tested in an ApoE$^{-/-}$ murine model of atherosclerosis to evaluate the effect of reversible, pharmacological sPLA$_2$-X inhibition on atherosclerosis development. Despite being well tolerated and achieving adequate systemic exposure of mechanistic relevance, (-)-**17** did not significantly affect circulating lipid and lipoprotein biomarkers, had no effect on coronary function or histological markers of atherosclerosis.

Based on our previously reported hit identification efforts,[1] the discovery and *in vivo* characterisation of (-)-2-{2-[carbamoyl-6-(trifluoromethoxy)-1H-indol-1-yl]pyridine-2-yl}propanoic acid, a novel sPLA$_2$-X inhibitor with significant selectivity over the other main sPLA$_2$ isoforms, is presented, alongside its lack of efficacy in the murine carotid artery ligation model of atherosclerosis.[2]

Preliminary structure-based evolution of the initial fragment hit **1** resulted in a lead series offering a bidentate coordination of the calcium ion present in the sPLA$_2$-X catalytic site.[1] One of the most promising compounds in the series yielded a greater than 250-fold sPLA$_2$-X potency improvement over the original fragment hit (**2**, Figure 1). However, in order to fulfil the project goal of evaluating the therapeutic potential of selective sPLA$_2$-X inhibition in a rodent model of atherosclerotic disease, further optimisation was required. Specifically, maximising selectivity towards sPLA$_2$-X was of interest, in order to minimise confounding effects originating from inhibition of alternative sPLA$_2$ isoforms. Furthermore, a significant improvement of the metabolic properties of **2** was needed to afford a pharmacokinetic profile compatible with prolonged *in vivo* dosing. Based on the existing data, we decided to target a 3-fold improvement in both sPLA$_2$-X selectivity and mouse unbound Clearance over **2** (Figure 1).

Anticipating that the second objective would have been more stringent from a design point of view, due to its partial dependency on lipophilicity, it was decided to a) identify areas on the molecule where polarity could be introduced and b) incorporate molecular modifications in accordance with the available sPLA$_2$ structural framework and associated selectivity hypotheses. The aim was to balance the contrasting effects of reduced lipophilicity on sPLA$_2$-X inhibition and pharmacokinetics while monitoring sPLA$_2$ selectivity. Considering the ionized class and physicochemical nature of the compounds, and associated implications for DMPK properties,[3] we opted to target a ca. 0.5 logD$_{7.4}$ reduction from **2**.



1
sPLA$_2$-X IC$_{50}$: 6.9 μM

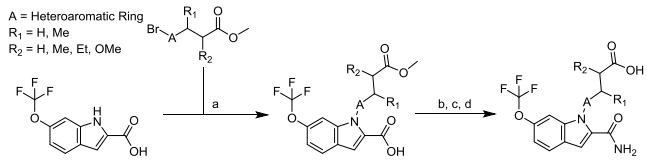
2
sPLA$_2$-X IC$_{50}$: 0.026 μM
sPLA$_2$-IIa/X IC$_{50}$ ratio: 12x
Mouse C57Bl/6 Cl$_u$: 1446 mL/min/kg
logD$_{7.4}$: 1.2

Figure 1. Initial fragment hit **1** and derived lead **2**.

Compounds **3**-**9** (Table 1) were designed to introduce polar elements in the region that, being furthest away from the bottom of the lipophilic binding pocket, appeared most amenable to modification, based on the X-ray of **2** bound to sPLA$_2$-X.[1] Synthesis started by preparation of the adequate heterocyclic halides, bearing a propanoate side chain in analogy to **2**, as

shown in Scheme 1. The halides so obtained were coupled under *N*-arylation/Ullmann conditions[4] with 6-trifluoromethoxy-indole-2-carboxylic acid to generate the corresponding *N*-heteroaryl-indoles in modest yields. Standard TBTU coupling produced the indole-2-carboxamides in good to quantitative yields. Finally hydrolysis of the corresponding esters yielded the carboxylic acid products **3-9** (See supporting information).

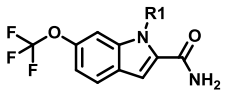
Scheme 1. General synthesis of 1-heteroaryl-1*H*-indole-2-carboxamide derivatives.[a]

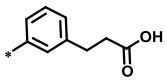
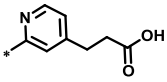
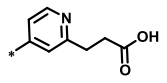
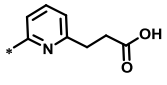
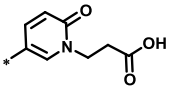
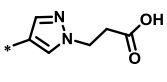
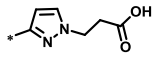
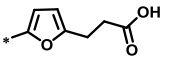


[a] Reagents and conditions : a) $Cu(OAc)_2$, DBU, DMSO, µw 110°C-180°C, 7 min – 24 h (15 – 25%) b) NH_4Cl, TBTU, NMM, DMF, rt. 1.5 – 16h (74-100%) c) Chiral chromatography d) LiOH, THF, MeOH, water, rt. 3 – 19h (45 – 83%)

Of the three pyridine regioisomers evaluated (**3**-**5**), the 2,6-disubstituted one (**5**) was the only one that maintained comparable potency to its phenyl counterpart. It is noteworthy that such modification had no effect on $sPLA_2$-X selectivity. Inhibition of HDL hydrolysis as mediated by $sPLA_2$-X, an important translational biomarker of lipoprotein modification, was also maintained. Introduction of marked polarity on the aryl moiety via a N-substituted pyridine-2-one (**6**) reduced potency by more than 100-fold. Decreased lipophilicity by installment of five-membered heterocyclic rings also diminished $sPLA_2$-X inhibition with pyrazoles (**7**, **8**) having a more pronounced effect than furane (**9**). Importantly, all the pyridine heterocycles had the sought effect of reducing metabolism, as shown in Table 1.

Table 1. $sPLA_2$ isoform potencies and human hepatocyte intrinsic clearance for compounds **2**-**9**.



	R1	sPLA2-X, IIa, V IC50 (µM)[a]	sPLA2-X-HDL IC50 (µM)[a]	Hu HEP Clint (µL/min/10^-6)
2		0.026, 0.31, 2.2	0.31	9.33
3		0.11, 0.29, 0.72	0.64	<5
4		0.24, 1.2, 2.1	1.6	<5
5		0.029, 0.47, 2.3	0.25	<5
6		4.8, NA[b], NA[b]	ND[c]	ND[c]
7		>10, 4.5, 2.7	ND[c]	ND[c]
8		4.8, 4, NA[b]	ND[c]	ND[c]
9		0.43, 1.9, 5.9	ND[c]	ND[c]

[a]Results are mean of at least two experiments. Experimental errors within 20% of value. [b] Not active at highest tested concentration (10 µM). [c] Not determined.

Having successfully identified a polar element with neutral effect on potency and favorable impact on metabolism, optimisation of $sPLA_2$-X selectivity was in focus. Multiple sequence and crystallographic structure alignments of the three main $sPLA_2$ isoforms considered in this study indicated amino acid variation across the proteins in the area immediately surrounding the pyridine and carboxy linker.[1] Although the side chain differences were limited (I2-L29, L2-V29 and L2-I29, in $sPLA_2$-X, -IIa and -V, respectively), we hypothesized that these could react differently to ligand contacts and therefore drive selectivity between $sPLA_2$ isoforms, as previously reported.[5,6] Design of compounds **10**-**21** served to verify those assumptions, as shown in Table 2.

Compounds **10**-**21** were synthesized according to a similar synthetic scheme using the previously described Ullman coupling as a key step. Separation of the enantiomeric and diastereoisomeric pairs obtained was efficiently performed using preparative chiral HPLC (See supporting information).

Introduction of substituents at the alpha position respective to the carboxylic acid typically resulted in significant, stereoselective potency gains across $sPLA_2$ IIa and V isoforms and, as a result, reduced $sPLA_2$-X selectivity (cf. **10**, ***(ent)*-10** and **2**; **13**, ***(ent)*-13** and **3**; **14**, ***(ent)*-14** and **5**, Table 2), possibly due to the amino acid differences previously described. Here, methyl groups had the most profound effect, followed by methoxy and ethyl substituents (cf. **10** and **11**; **5** and **15**, Table 2). On the contrary, gem-dimethyl substitution at the same position was not tolerated (cf. **2**, **10** and **16**, Table 2). Intriguingly, when the substituents were installed on the beta position to the acid, improved selectivity between $sPLA_2$-X and, in an increasing order, $sPLA_2$-IIa and $sPLA_2$-V was apparent (cf. (*S*)-**12** and **2**; (-)-**17** and **5**, Table 2).

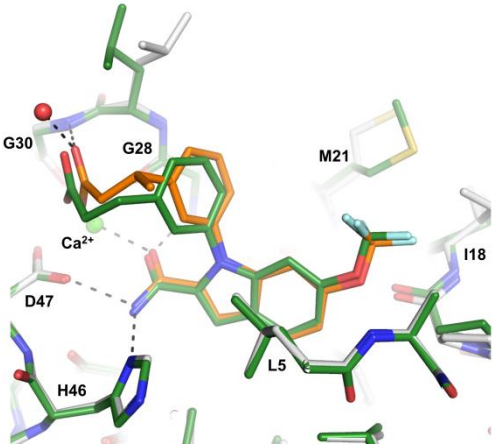


Figure 2. Overlay between the crystal structures of (*S*)-**12** (orange stick) and **2** (green stick) bound to $sPLA_2$-X (white and green stick, respectively) (Please see the supporting information for experimental details).

(*S*)-**12** and (-)-**17** emerged from this exploration as highly potent $sPLA_2$-X inhibitors (IC_{50}: 15 and 14 nM, $logD_{7.4}$: 1.3 and 0.9, respectively) of comparable selectivity over $sPLA_2$-IIa (60-

and 45-fold, respectively) and sPLA$_2$-V (360- and 390-fold, respectively). The two derivatives also inhibited the sPLA$_2$-X-mediated lipolytic effect on HDL with (-)-**17** being the most potent (IC_{50}: 42 and 110 nM, respectively). When their pharmacokinetic profile was evaluated in mice, (-)-**17** demonstrated superior properties, as indicated by a lower unbound clearance (Table 2). This is probably the result of its reduced lipophilicity (0.4 $logD_{7.4}$ units difference compared to (*S*)-**12**) and its combined impact on both metabolic stability and plasma protein binding. Importantly, the more polar character of (-)-**17** did not deteriorate its absorption profile, as indicated by adequate passive diffusion (Caco-2 P_{app}: 5.8×10^{-6} cm/sec) and oral bioavailability in mice (*F*: 95%). The absolute structure was assumed to be (*S*), as from molecular modelling based on the X-ray of (*S*)-**12** (Figure 2) and the comparable separation of sPLA$_2$-X activity in the corresponding enantiomeric pairs (*S*)-**12**-(*R*)-**12** and (-)-**17**-(+)-**17** (Table 3). The binding mode of (*S*)-**12,** closely resembled that of **2**, except for a slight upward shift of its phenyl ring (Figure 2). The additional methyl group at the benzylic position establishes van der Waals contacts with Y50 and K61. Further, the introduction of the methyl group slightly alters the conformation of the I2 side chain and subsequently alters its packing against K61. This suggests that the observed isoform selectivity results might stem from the slightly smaller space in the area around the pyridine, primarily caused by the I2 to L2 substitution. It is also likely that sequence differences in other parts of the active site, i.e. L5, V9 and L98 (F/L, I/I and F/L in IIa/V, respectively), may alter the binding mode of the entire ligand,[1] thus contributing to the effect. Furthermore, (-)-**17** maintained adequate potency for the mouse sPLA$_2$-X homolog (IC_{50}: 75 nM). Based on these promising preliminary characteristics, (-)-**17** was evaluated further to identify potential shortcomings. The compound showed no inhibition of ion channels implicated in cardiovascular function (hERG, $Na_v1.5$, IK_s, $Ca_v1.2$, $K_v4.3$) at the highest tested concentration (33 µM). (-)-**17** did not display any significant binding in a selectivity panel consisting of >100 different proteins when tested at 10 µM. No CYP450 isoforms were inhibited by (-)-**17** when dosed at up to 20 µM. In line with previously reported derivatives from the same series and lipophilicity range, (-)-**17** possessed adequate solubility and did not form any reactive metabolites (Table 3).

Owing to its favorable *in vitro-vivo* profile as well as its selective sPLA$_2$-X action, (-)-**17** was deemed suitable for long-term efficacy studies using the mouse carotid artery flow cessation model, modified as previously described[2]. Here, pharmacokinetic-pharmacodynamic (PK-PD) modelling based on the *in vitro* inhibitory potency (sPLA$_2$-X-mediated HDL hydrolysis IC_{50} and IC_{90}: 42 and 362 nM; mouse sPLA$_2$-X IC_{50} and IC_{90}: 75 and 820 nM), mouse plasma protein binding (F_u: 5%) and the measured PK profile (Table 3) predicted that once daily oral doses for (-)-**17** of 75 and 150 µmol/kg would allow for 24 hours coverage of IC_{50} and IC_{90}, respectively. This PK-PD hypothesis enabled testing a dynamic range of sPLA$_2$-X inhibition and its relevance to several pharmacological effects: a) modification of circulating lipoproteins and lipids, b) improvement of vascular function and c) reduction of atherosclerosis. The results from a three weeks administration of (-)-**17** to $ApoE^{-/-}$ mice that underwent carotid artery ligation are summarised in Figure 3 (See supplementary information for experimental details and additional results).

Table 3. *In vitro/vivo* profile[a] of (-)-**17**

Solubility (µM)	100
Caco-2 P_{app} (10^{-6} cm/s), Efflux Ratio	5.8, 0.4
Human Hepatocytes $T_{1/2}$ (min)	>173
hERG, $Na_v1.5$, IKs, $K_v4.3$ $Ca_v3.2$, $Ca_v1.2$ IC_{50} (µM)	>33
Reactive Metabolite Formation	No (0.0)
Cytochrome P450 IC_{50} (µM)	>20
Pharmacokinetics	C57Bl/6J Mouse
Plasma protein binding F_u (%)	5
Dose iv/po (µmol/kg)	10/50
CL_u (mL/min/kg)	306
F (%)	95
V_{ss} (L/kg)	0.52

[a] Please see the supporting information for experimental details.

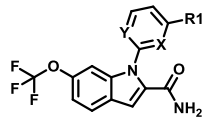
Treatment with (-)-**17** was overall well tolerated with no significant changes in the body weight of the animals and their liver enzyme levels. Histopathological analysis of vital organs did not reveal any significant findings. Importantly, plasma concentrations of (-)-**17** confirmed the PK-PD predictions with the 75 and 150 µmol/kg doses resulting in free compound exposures well in excess (>7-fold) of the *in vitro* sPLA$_2$-X HDL IC_{50} and IC_{90}, respectively, both after one week treatment and at the end of the study. When compared to the control group receiving only western diet, dosing of (-)-**17** did not significantly alter the plasma levels of triglycerides, free fatty acids and cholesterol (including esterified cholesterol, free cholesterol or any of its lipoprotein-associated fractions), despite their relative reductions with the 150 µmol/kg dose. Unfortunately, similar non-statistically significant trends were observed when the same analysis focused on liver, aorta and heart biosamples (data not shown). Coronary flow reserve (CFR) analysis of the left coronary artery using Doppler ultrasound-based echocardiography[7] did not reveal any differences between control and treatment groups. Furthermore, no significant changes in heart rate were observed upon treatment with (-)-**17**. Histological analysis of the left common carotid artery after ligation surgery[8] served to assess the effect of (-)-**17** on the development of atherosclerosis. Here, no significant reduction of artery wall thickness or increase in the overall lumen thickness was apparent compared to the untreated animal group. Additionally, administration of (-)-**17** did not result in a significant decrease of neointima and media formation, as highlighted in Figure 3.

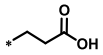
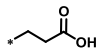
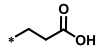
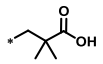
When compared to the vehicle group or positive treatment precedents using alternative modalities in the same in house disease model (e.g., 25 µmol/kg fluvastatin qd, as previously reported[9]), it was clear that, whilst being well tolerated, (-)-**17** failed to significantly affect any of the study endpoints. Lipoproteins and lipid biomarkers were postulated to be the most direct proof of concept endpoints due to the published lipid modifying and pro-inflammatory properties of sPLA$_2$-X.[10-12] The mechanistic hypothesis we followed implied that, because of marked improvements in lipid/lipoprotein metabolism and homeostasis, as mediated by sPLA$_2$-X inhibition, a significant reduction in the progression of atherosclerosis and therefore an overall improved coronary function would result. The data obtained with (-)-**17** would not seem to support such hypothesis. Potential causes for the observed lack of efficacy could include an underappreciated difference between *in vitro* and *in vivo* sPLA$_2$-X inhibition efficiency, requiring much higher compound exposure than the one sampled, although the latter was purposely designed with a

higher margin to mitigate this risk. It's possible that the beneficial effects of $sPLA_2$-X inhibition on atherosclerosis would require much longer treatment times given the pharmacodynamic coupling between lipid/lipoprotein homeostasis and atherosclerosis development in the chosen $ApoE^{-/-}$ carotid ligation model.[13] Alternatively, pharmacological inhibition of $sPLA_2$-X might not have direct enough relevance to a complex, multifactorial disease such as atherosclerosis in the $ApoE^{-/-}$ model employed here. Interestingly, a recent study has shown no association between genetic variants of PLA2G10, encoding $sPLA_2$-X, and coronary heart disease risk traits and outcome,[14] as opposed to PLA2G2A, encoding $sPLA_2$-IIa.[15]

In summary, started from a fragment-derived chemical series of $sPLA_2$-X inhibitors, optimisation of the $sPLA_2$ isoform selectivity and pharmacokinetic profile led to the design of (-)-2-{2-[carbamoyl-6-(trifluoromethoxy)-1H-indol-1-yl]pyridine-2-yl}propanoic acid, (-)-**17**, a novel, potent and selective $sPLA_2$-X inhibitor. Three weeks oral administration of (-)-**17** to carotid artery-ligated $ApoE^{-/-}$ mice fed on a western diet failed to reduce development of atherosclerosis, questioning the therapeutic validity of selective pharmacological $sPLA_2$-X inhibition.[16-18] Considering recent clinical failures in cardiovascular diseases treatment with the broad spectrum $sPLA_2$ inhibitor varespladib and lipoprotein-associated PLA_2 ($Lp\text{-}PLA_2$) inhibitor darapladib,[19] new insights into the causative association between these enzymes and atherosclerosis are required to progress novel, effective therapies.

Table 2. $sPLA_2$ potency and *in vivo* mouse unbound clearance for compound **10** – **29**



	X, Y	R1	$sPLA_2$-X IC_{50} (μM)[a]	$sPLA_2$-IIa IC_{50} (μM)[a]	$sPLA_2$-V IC_{50} (μM)[a]	$sPLA_2$-X-HDL IC_{50} (μM)[a]	C57Bl/6J Cl_u (mL/min/kg)[b]
2	CH, CH		0.026	0.31	2.2	0.31	1446
10	CH, CH		0.032	0.057	0.51	0.35	ND[c]
(***ent*)-10**[e]			0.1	0.81	>10	2.8	ND[c]
11	CH, CH		0.039	0.14	2.1	0.14	ND[c]
(***ent*)-11**[e]			0.17	2.7	NA[d]	ND[c]	ND[c]
(*S*)-**12**	CH, CH		0.015	0.82	5.1	0.11	1025
(*R*)-**12**			0.2	3.9	8.6	ND[c]	ND[c]
3	CH, N		0.11	0.29	0.72	0.64	ND[c]
13	CH, N		0.14	0.044	0.18	0.83	ND[c]
(***ent*)-13**[e]			0.36	0.34	1.6	3.4	ND[c]
5	N, CH		0.029	0.47	2.3	0.25	ND[c]
14	N, CH		0.065	0.092	0.51	0.25	ND[c]
(***ent*)-14**[e]			0.57	1.7	>10	ND	ND[c]
15	N, CH		0.17	0.17	1.9	0.33	ND[c]
(***ent*)-15**[e]			0.94	0.84	>10	ND[c]	ND[c]
16	N, CH		0.32	3.1	>10	ND[c]	ND[c]
(-)-**17**	N; CH		0.014	0.63	5.5	0.042	306
(+)-**17**			0.5	3.8	NA[d]	ND[c]	ND[c]

[a]Results are mean of at least two experiments. Experimental errors within 20% of value. [b]Unbound clearance (Cl_u) is defined as Cl/F_u where F_u is the compound's fraction that is unbound in plasma. Clearance is calculated from noncompartmental analysis concentrations in fasted C57Bl/6J male mice (i.v./p.o. N=2). [c]Not determined. [d]Not active at highest tested concentration (10 μM). [e]Enantiomers that were not characterized further are labelled using the (*ent*)-prefix.

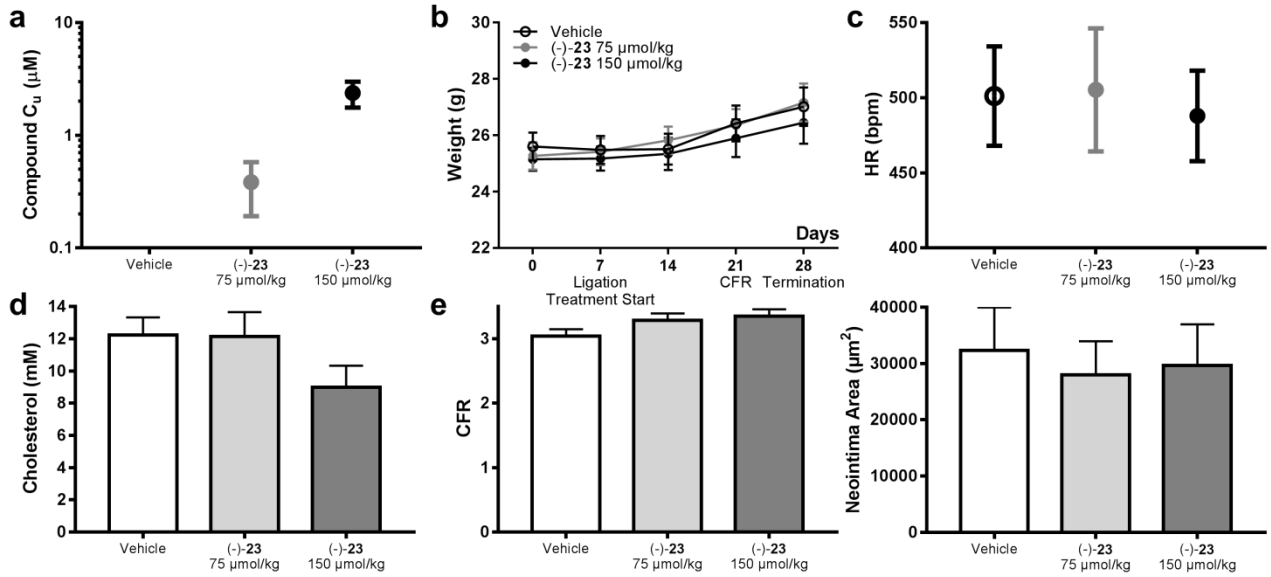


Figure 3. Effect of **(-)-17** in an ApoE$^{-/-}$ murine carotid artery ligation model of atherosclerosis (N=16/group). a) Plasma unbound concentrations of **(-)-17** at study termination. ApoE-/- mice body weight (b), heart rate (c) and total cholesterol (d). Coronary flow reserve (CFR) analysis (e) and left carotid artery neointima area (f) (Please see the supporting information for experimental details).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the

Experimental details, synthesis, assay protocols and X-ray crystallographic statistics (PDF)

AUTHOR INFORMATIONS

Correponding authors

*(FG) Tel.: +1 (212)4780822 . Email : fabrizio.giordanetto@DEShawResearch.com

*(LK) Tel.: +46 317065788. Email : laurent.knerr@astrazenenca.com

Present adresses :

†F.G: D.E. Shaw Research, 120W 45th Street, New York, New York 10036, United States

#H-G.B.: Medivir AB, Huddinge, Sweden

¤H.d.l.M.: RISE Research Institutes of Sweden Division Bioeconomy, Gothenburg, Sweden.

ØÅ.M.: Alfa Laval Lund AB, Lund, Sweden

§G.S.: SCA Hygiene products AB, Stockholm, Sweden.

¶F.K.: SciLifeLab, Drug Discovery & Development Platform, Solna, Sweden.

Notes

The authors declare no competing financial interests

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of the Structure Analysis and Separation Science groups of AstraZeneca Gothenburg.

ABBREVIATIONS USED

sPLA$_2$, secreted phospholipase A$_2$; ApoE, Apolipoprotein E; HEP, hepatocytes; PK-PD, pharmacokinetics-pharmacodynamics; CFR, coronary flow reserve; Lp-PLA$_2$, lipoprotein-associated phospholipase A$_2$.

References:

1. Knerr, L.; Giordanetto, F.; Nordberg, P.; Pettersen, D.; Selmi, N.; Beisel, H.-G.; de la Motte, H.; Olsson, T.; Perkins, T. D. J.; Herslöf, M.; Månsson, Å.; Dahlström, M.; Broddefalk, J.; Saarinen, G.; Klingegård, F.; Hurt-Camejo, E.; Rosengren, B.; Brengdahl, J.; Janssen, J.; Rohman, M.; Sandmark, J.; Hallberg, K.; Åkerud, T.; Roth, R. G.; Ahlqvist, M. Discovery of a series of indole-2 carboxamides as selective secreted phospholipase A$_2$ type X (sPLA$_2$-X) inhibitors – submitted.

2. Kumar, A.; Lindner, V. Remodeling With Neointima Formation in the Mouse Carotid Artery After Cessation of

Blood Flow. *Arteriosclerosis, Thrombosis, and Vascular Biology* **1997**, *17*, 2238–2244.

3. Charifson, P.S.; Walters, W.P. Acidic and Basic Drugs in Medicinal Chemistry: A Perspective. *J. Med. Chem.* **2014**, *57*, 9701–9717.

4. Huang, H.; Yan, X.; Zhu, W.; Liu, H.; Jiang, H.; Chen, K. Efficient copper-promoted N-arylations of aryl halides with amines. *J. Comb. Chem.* **2008,** *10*, 617–619.

5. Giordanetto, F.; Pettersen, D.; Starke, I.; Nordberg, P.; Dahlström, M.; Knerr, L.; Selmi, N.; Rosengren, B.; Larsson, L.-O.; Sandmark, J. et al. Discovery of AZD2716: A Novel Secreted Phospholipase A2 (sPLA2) Inhibitor for the Treatment of Coronary Artery Disease. *ACS Med. Chem. Lett.* **2016**, *7*, 884–889.

6. Mouchlis, V.D.; Magrioti, V.; Barbayianni, E.; Cermak, N.; Oslund, R.C.; Mavromoustakos, T.M.; Gelb, M.H.; Kokotos, G. Inhibition of secreted phospholipases A2 by 2-oxoamides based on α-amino acids: Synthesis, in vitro evaluation and molecular docking calculations. *Bioorg. Med. Chem.* **2011**, *19*, 735–743.

7. Westergren, H.U.; Grönros, J.; Heinonen, S.E.; Miliotis, T.; Jennbacken, K.; Sabirsh, A.; Ericsson, A.; Jönsson-Rylander, A.C.; Svedlund, S.; Gan, L.M. Impaired Coronary and Renal Vascular Function in Spontaneously Type 2 Diabetic Leptin-Deficient Mice. *PLOS one* **2015**, *10*, e0130648.

8. De Wilde, D.; Trachet, B.; De Meyer, G.R.; Segers, P. Shear Stress Metrics and Their Relation to Atherosclerosis: An In Vivo Follow-up Study in Atherosclerotic Mice. *Ann Biomed. Eng*. **2016**, *44*, 2327-2338.

9. Nakamura, K.; Sasaki, T.; Cheng, X.W.; Iguchi, A.; Sato, K.; Kuzuya, M. Statin prevents plaque disruption in apoE-knockout mouse model through pleiotropic effect on acute inflammation. *Atherosclerosis* 2009, *206*, 355–361.

10. Singer A. G., Ghomashchi F., Le Calvez C., Bollinger J., Bezzine S., Rouault M., Sadilek, M.; Nguyen, E.; Lazdunski, M.; Lambeau, G.; Gelb, M.H. Interfacial kinetic and binding properties of the complete set of human and mouse groups I, II, V, X, and XII secreted phospholipases A2. *J. Biol. Chem.* **2002**, *277*, 48535–48549.

11. Hanasaki K., Yamada K., Yamamoto S., Ishimoto Y., Saiga A., Ono T., Ikeda, M.; Notoya, M.; Kamitani, S.; Arita, H. Potent modification of low density lipoprotein by group X secretory phospholipase A2 is linked to macrophage foam cell formation. *J. Biol. Chem.* **2002**, *277*, 29116–29124.

12. Curfs D.M., Ghesquiere S.A., Vergouwe M.N., van der Made I., Gijbels M.J., Greaves D.R., Verbeek, J.S.; Hofker, M.H.; de Winther, M.P. Macrophage secretory phospholipase A2 group X enhances anti-inflammatory responses, promotes lipid accumulation, and contributes to aberrant lung pathology. *J. Biol. Chem.* **2008**, *283*, 21640–21648.

13. Ait-Oufella, H.; Herbin, O.; Lahoute, C.; Coatrieux, C.; Loyer, X.; Joffre, J.; Laurans, L.; Ramkhelawon, B.; Blanc-Brude, O.; Karabina, S.; Girard, C.A.; Payré, C.; Yamamoto, K.; Binder, C.J.; Murakami, M.; Tedgui, A.; Lambeau, G.; Mallat, Z. Group X secreted phospholipase A2 limits the development of atherosclerosis in LDL receptor–null mice. *Arterioscler. Thromb. Vasc. Biol*. **2013**, *33*, 466-473.

14. Guardiola, M.; Exeter, H.J.; Perret, C.; Folkersen, L.; Van't Hooft, F.; Eriksson, P.; Franco-Cereceda, A.; Paulsson-Berne, G.; Palmen, J.; Li, K. et al. PLA2G10 Gene Variants, sPLA2 Activity, and Coronary Heart Disease Risk. *Circ. Cardiovasc. Genet.* **2015**, *8*, 356–362.

15. Kugiyama, K.; Ota, Y.; Takazo, K.; Moriyama, Y.; Kawano, H.; Miyao, Y.; Sakamoto, T.; Soejima, H.; Ogawa, H.; Doi, H.; Sugiyama, S.; Yasue, H. Circulating levels of secretory type II phospholipase A(2) predict coronary events in patients with coronary artery disease. *Circulation*, **1999**, *100*, 1280–1284.

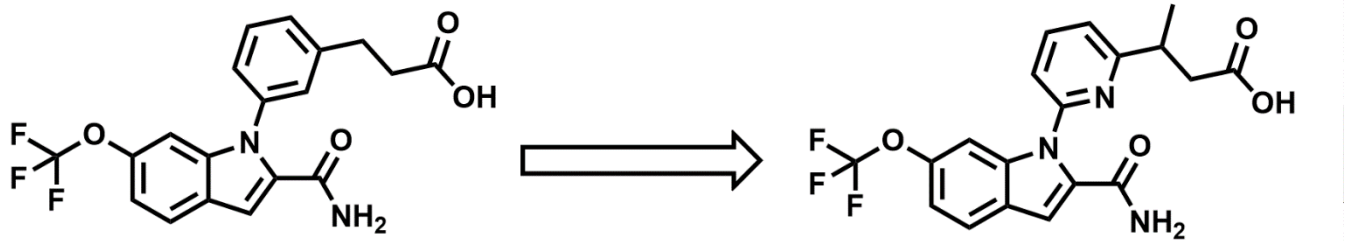
16. Watanabe, K.; Fujioka, D.; Saito, Y.; Nakamura, T.; Obata, J.E.; Kawabata, K.; Watanabe, Y.; Mishina, H.; Tamaru, S.; Hanasaki, K.; Kugiyama, K. Group X secretory PLA2 in neutrophils plays a pathogenic role in abdominal aortic aneurysms in mice. *Am. J. Physiol. Heart. Circ. Physiol.*, **2012**, *302*, H95-104.

17. Schewe, M.; Franken, P.F.; Sacchetti, A.; Schmitt, M.; Joosten, R.; Böttcher, R.; van Royen, M.E.; Jeammet, L.; Payré, C.; Scott, P.M.; Webb, N.R.; Gelb, M.; Cormier, R.T.; Lambeau, G.; Fodde, R. Secreted phospholipases A2 are intestinal stem cell niche factors with distinct roles in homeostasis, inflammation, and cancer. *Cell Stem Cell*. **2016**, *19*, 38-51.

18. Hallstrand, T.S.; Lai, Y.; Hooper, K.A.; Oslund, R.C.; Altemeier, W.A.; Matute-Bello, G.; Gelb, M.H. Endogenous secreted phospholipase A2 group X regulates cysteinyl leukotrienes synthesis by human eosinophils. J. Allergy Clin. Immunol. **2016**, *137*, 268-277.

19. Kokotou, M.G.; Limnios, D.; Nikolaou, A.; Psarra, A.; and Kokotos, G. Inhibitors of phospholipase A2 and their therapeutic potential: an update on patents (2012-2016). *Expert Opin. Ther. Pat.* **2017**, *27*, 217–225.

SYNOPSIS TOC



2

$sPLA_2$-X HDL IC_{50}: 310 nM

Mouse C57Bl/6 Cl_u: 1446 mL/min/kg

(-)-17

$sPLA_2$-X HDL IC_{50}: 42 nM

Mouse C57Bl/6 Cl_u: 306 mL/min/kg

No effect on $ApoE^{-/-}$ atherosclerosis model