Eur. J. Med. Chem. 36 (2001) 809–828
© 2001 Éditions scientifiques et médicales Elsevier SAS. All rights reserved
PII: S0223-5234(01)01274-0/FLA

Original article

Structure–activity relationships in platelet-activating factor (PAF). 11-From PAF-antagonism to phospholipase A_2 inhibition: syntheses and structure–activity relationships in 1-arylsulfamido-2-alkylpiperazines

Carine Binisti[a], Léon Assogba[a], Estéra Touboul[a], Carine Mounier[b], Jack Huet[a], Jean-Edouard Ombetta[a], Chang Zhi Dong[a], Catherine Redeuilh[a], Françoise Heymans[a], Jean-Jacques Godfroid[a,*]

[a]Laboratoire de Pharmacochimie Moléculaire, Université Paris 7-Denis Diderot, 2 Place Jussieu, F-75251 Paris cedex 05, France
[b]Unité des Venins, Institut Pasteur, 25, rue du Docteur Roux, F-75724 Paris cedex 15, France

Received 30 April 2001; revised 13 August 2001; accepted 5 September 2001

Abstract – 1-Benzoyl-2-alkyl piperazines are strong inhibitors of Group I and II secreted PLA_2s. An improvement of their activity was obtained by replacing the amide function by a sulfamide and by introduction of electrodonor substituents on the *para* position of the benzenesulfonyl moiety. Neither the position on one of the carbon of the piperazine ring nor the absolute configuration of this carbon have an effect on the affinity for one or the other group of PLA_2, but the lipophilicity remains for these series an essential parameter. In addition structure–activity relationships allow new hypothesis on interaction of these piperazine derivatives with the catalytic site of PLA_2s. © 2001 Éditions scientifiques et médicales Elsevier SAS

phospholipase A_2 inhibitor / structure–activity relationships / piperazine

1. Introduction

Phospholipases A_2 (PLA_2s) catalyse the hydrolysis of glycerophospholipids at the *sn*-2 position and generate free fatty acids and lysophospholipids. This can provide, in some cases, substrates for the biosynthesis of prostaglandins, thromboxanes, leukotrienes and other oxygenated metabolites of arachidonic acid (i.e. eicosanoids), as well as Platelet Activating Factor (PAF), well known mediators of inflammatory processes and tissue injury. PLA_2s may also be involved in antibacterial defenses, cellular proliferation and apoptosis, as well as in some cancers [1]. To date the most widely studied PLA_2s are the low molecular weight (14 kDa), secreted PLA_2 ($sPLA_2$), and the high molecular weight (85 kDa), intracellular PLA_2 ($cPLA_2$) [2]. There are numerous other PLA_2 activities described in the literature that indicate that the PLA_2s may be a much more diverse group of enzymes that had been previously expected [2]. The secreted PLA_2s have been first divided into three main groups (I, II, III) according to similarities found in their primary sequences [3, 4]. With the recent cloning of several new $sPLA_2$s, this family of proteins is growing and presents now ten different groups [5–11]. Secreted non-pancreatic PLA_2 ($snpPLA_2$) was identified as an enzyme from Group II, 14 kDa, micromolar concentration calcium-dependent, which preferentially hydrolyses phospholipids in negatively charged membrane. The high molecular weight (85 kDa) cytosolic PLA_2 differs from the secreted PLA_2 by its specificity for 2-arachidonoyl phospholipids, its activation by submicromolar calcium concentration and by phosphorylation [12, 13]. $SnpPLA_2$ has been isolated from human synovial fluid as well as human cells from various sources [4, 14–17]. Currently its exact physiological role is not known. However, high concentrations of $snpPLA_2$ have been found in the

* Correspondence and reprints
E-mail address: godfroid@paris7.jussieu.fr (J.-J. Godfroid).

synovial fluid of patients with rheumatoid arthritis [18] and have been implicated in many other inflammatory diseases such as asthma, psoriasis [19], Crohn's disease, ulcerative colitis [20, 21], and septic shock [22]. Because of its implication in inflammatory processes, a potent and selective inhibitor of snpPLA$_2$ activity would be a useful drug for the treatment of a wide range of common clinical inflammatory disorders, and an accurate pharmacological tool for investigating the exact role of these proteins in such diseases. Various PLA$_2$ inhibitors have been described and discussed, including some active site-directed substrate and transition state analogs. Although these agents are potent inhibitors of PLA$_2$ activity, they are not likely to be cell permeant and their cytotoxicity reduces their interest for in vivo studies. Other molecules structurally different from substrate analogs have been reported to inhibit snpPLA$_2$ activity with good potency, but these compounds are not specific and also act on other enzymatic systems [23–26]. In 1995 and 1996, a series of indole derivatives was shown to specifically inhibit snpPLA$_2$ [27–30], opening new prospects for future in vivo analysis.

The three dimensional structure of human synovial fluid PLA$_2$, and of porcine and bovine pancreatic PLA$_2$ (Group I) have been determined by X-ray crystallographic and NMR studies both in their native form and in a complex with a transition state analogue [31–34]. These studies show that the structural features of the active site are similar and allow to postulate a catalytic mechanism for the hydrolysis of glycerophospholipids [35], as well as to design new secretory PLA$_2$ inhibitors [23, 36]. Hydrocarbon chain length is an important factor for the activity of potential inhibitors. Studies with phospholipid analogues demonstrated that ten carbons are required in the *sn*-2 acyl chain for optimum binding to cobra venom PLA$_2$ [37], whereas the optimal length for the *sn*-1 alkyl chain is four carbons in the case of porcine pancreatic PLA$_2$ [38].

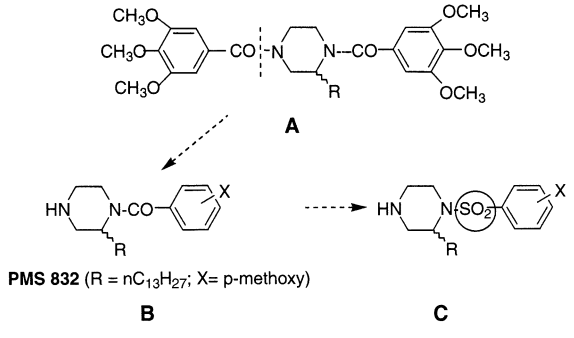


Figure 1. From PAF-antagonist **A** toPLA$_2$ inhibitors **B** [39] and this work **C**.

In previous reports we described novel structures that are good inhibitors of secreted PLA$_2$s [39, 40]. There, the piperazine derivatives of general structure **B** (*figure 1*) showed good inhibitory potency of PLA$_2$ activity from rabbit platelet lysate, and from bovine or porcine pancreatic PLA$_2$s, with IC$_{50}$ values in the micromolar range. Preliminary structure–activity relationships (SAR) analysis displayed that hydrocarbon chain length (R) was the main factor for the inhibitory potency, as well as the H on the secondary nitrogen (N$_4$) of the 2-alkyl piperazine derivatives **B**, which is presumed to be implicated in a H-bond with the imidazolyl moiety of the catalytically active His$_{48}$. Moreover, it was postulated that the carbonyl function could partly chelate a calcium ion [39].

In this work, we report a new series of derivatives of general structure **C** (*figure 1*). The carbonyl group was replaced by a sulfonyl moiety, which could have a stronger binding affinity for calcium ion than carbonyl moiety.

2. Chemistry

The main part of compounds shown in *tables I* and *II* was synthesised starting from the corresponding substituted piperazines prepared as described previously and the regioselective method we used for arylamide derivatives **B** in Binisti et al. [40], but adapted to sulfamide obtention. Alkenyl piperazines needed the preparation of 1-bromo-7-dodecenes *Z* (compound **6**) and *E* (compound **9**) in six steps as illustrated in *figure 2*.

Refluxing the two phases mixture of hexane-1,6-diol and aqueous hydrobromic acid in benzene afforded the 6-bromohexan-1-ol (**1**). After protection of the hydroxy function using 3,4-dihydro-2*H*-pyran, the protected compound **2** was condensed with the organolithium derivative of 1-hexyne to give the acetylenic compound **3**. Catalytic hydrogenation of **3** using 5% Pd-on-barium sulphate and quinoline [41] produced exclusively the *Z*-double bound (compound **4**). Deprotection of **4** using HCl gas in methanol gave the alcohol **5** which was converted into the bromo derivative **6** by treatment with phosphorus tribromide. Various methods are described for reduction of

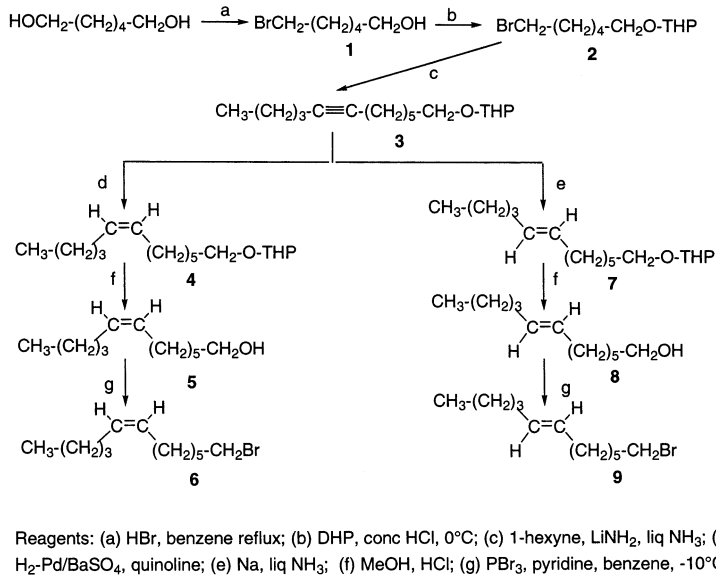


Figure 2. Synthesis of 1-bromo-7-dodecenes *Z* **6** and *E* **9**.

the triple bond to the *E*-double bond [42, 43]. In the case of compound **3**, only the Birch reduction [44] afforded the desired product **7**, which was converted into the bromide **9** following the same process as for **4**–**6**.

To prepare the non-commercially available arylsulfonyl chlorides, five synthetic pathways were developed depending on the substituent on the aromatic ring as described in *figure 3.*

Isopropylbenzene treated with H_2SO_4 gave the sul-

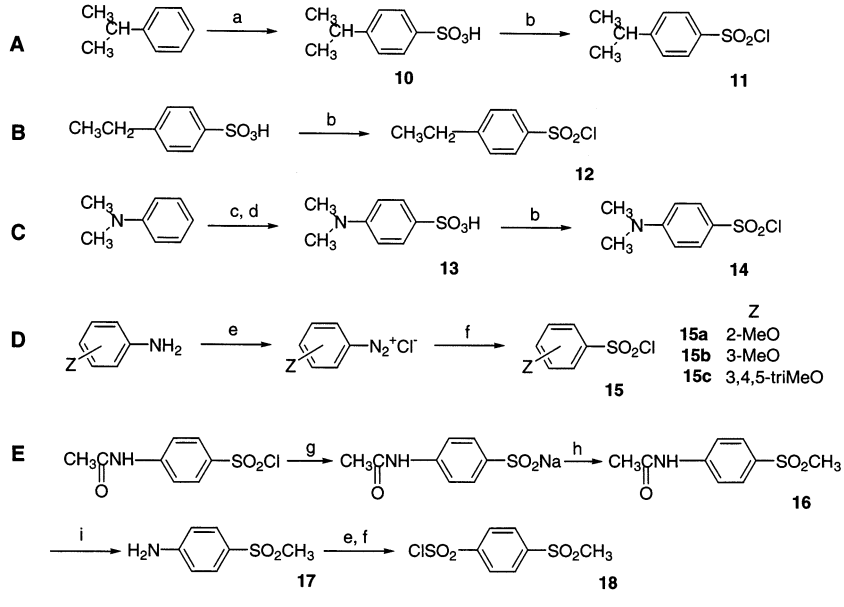
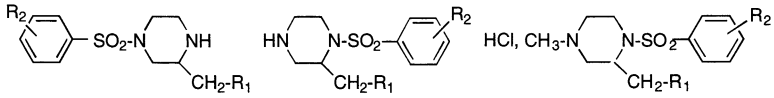


Figure 3. Preparation of non-commercially available arylsulfonyl chlorides.

Table I. Physical data for 1-arylsulfonyl-3-*n*-alkylpiperazines (**21**), 1-arylsulfonyl-2-*n*-alkylpiperazines (**24**), 1-*p*-tolylsulfonyl-2-*n*-octyl-4-methylpiperazine hydrochloride (**25**), and 1-*p*-chlorophenylsulfonyl-2-*n*-tridecyl-4-methylpiperazine (**26**).

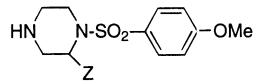


21 **24** **25,26**

Compound	R_1	R_2	m.p. (°C)	Recrystallisation solvents	Formula
21c	nC_7H_{15}	*p*-CH_3	54.7 [a]	hexane	$C_{19}H_{32}N_2O_2S{\cdot}HCl$
21d	nC_9H_{19}	*p*-CH_3	65	pentane	$C_{21}H_{36}N_2O_2S$
21e	$nC_{12}H_{25}$	*p*-CH_3	75.3	CH_2Cl_2–hexane	$C_{24}H_{42}N_2O_2S$
21f	$nC_{14}H_{29}$	*p*-CH_3	78.2 [a]	CH_2Cl_2–hexane	$C_{26}H_{47}N_2O_2S{\cdot}HCl{\cdot}1/2H_2O$
21g	$nC_{16}H_{33}$	*p*-CH_3	82.2 [a]	CH_2Cl_2–hexane	$C_{28}H_{51}N_2O_2S{\cdot}HCl{\cdot}1/2H_2O$
24a	nC_3H_7	*p*-CH_3	yellow oil	–	$C_{15}H_{24}N_2O_2S{\cdot}HCl$
24b	nC_5H_{11}	*p*-CH_3	yellow oil	–	$C_{17}H_{28}N_2O_2S{\cdot}HCl$
24c	nC_7H_{15}	*p*-CH_3	128.1 [a]	pentane	$C_{19}H_{32}N_2O_2S{\cdot}HCl$
24d	nC_9H_{19}	*p*-CH_3	102.5 [a]	ether–hexane	$C_{21}H_{36}N_2O_2S{\cdot}HCl$
24e	$nC_{12}H_{25}$	*p*-CH_3	125.1 [a]	ether–hexane	$C_{24}H_{42}N_2O_2S{\cdot}HCl$
24f	$nC_{14}H_{29}$	*p*-CH_3	135.6 [a]	ether–hexane	$C_{26}H_{46}N_2O_2S{\cdot}HCl$
24g	$nC_{16}H_{33}$	*p*-CH_3	140.2 [a]	ether–hexane	$C_{28}H_{50}N_2O_2S{\cdot}HCl$
24h	nC_7H_{15}	*p*-H	117.5 [a]	CH_2Cl_2–ether	$C_{18}H_{30}N_2O_2S{\cdot}HCl$
24i	nC_7H_{15}	*p*-SO_2CH_3	207.5 [a]	CH_2Cl_2–ether	$C_{19}H_{32}N_2O_4S_2{\cdot}HCl$
24j	nC_7H_{15}	*p*-Cl	182.3 [a]	ether	$C_{18}H_{29}ClN_2O_2S{\cdot}HCl$
24k	nC_7H_{15}	*p*-OCH_3	103.5 [a]	CH_2Cl_2–ether	$C_{19}H_{32}N_2O_3S{\cdot}HCl$
24l	nC_7H_{15}	*o*-OCH_3	170 [a]	CH_2Cl_2–hexane	$C_{19}H_{32}N_2O_3S{\cdot}HCl$
24m	nC_7H_{15}	*m*-OCH_3	89.6 [a]	CH_2Cl_2–hexane	$C_{19}H_{32}N_2O_3S{\cdot}HCl$
24n	nC_7H_{15}	3,4,5-triOCH_3	150.2 [a]	CH_2Cl_2–hexane	$C_{21}H_{36}N_2O_5S{\cdot}HCl$
24o	$nC_{12}H_{25}$	*p*-H	49.3	ether–hexane	$C_{23}H_{40}N_2O_2S$
24p	$nC_{12}H_{25}$	*p*-SO_2CH_3	74.4	ether–CH_2Cl_2–hexane	$C_{24}H_{42}N_2O_4S_2$
24q	$nC_{12}H_{25}$	*p*-Cl	162.6 [a]	acetone	$C_{23}H_{39}ClN_2O_2S{\cdot}HCl$
24r	$nC_{12}H_{25}$	*p*-OCH_3	65.4	hexane	$C_{24}H_{42}N_2O_3S$
24r	$(7Z)nC_{12}H_{23}$	*p*-OCH_3	yellow oil	–	$C_{24}H_{40}N_2O_3S$
24r	$(7E)nC_{12}H_{23}$	*p*-OCH_3	yellow oil	–	$C_{24}H_{40}N_2O_3S$
24s	$nC_{12}H_{25}$	*p*-Br	70.8	ether–hexane	$C_{23}H_{39}BrN_2O_2S$
24t	$nC_{12}H_{25}$	*p*-NH_2	85.1	pentane	$C_{23}H_{41}N_3O_2S{\cdot}1/2H_2O$
24u	$nC_{12}H_{25}$	*p*-$N(CH_3)_2$	65.8	ether–pet (35–60 °C)	$C_{25}H_{45}N_3O_2S$
24v	$nC_{12}H_{25}$	*p*-$NHCOCH_3$	81.6	ether–hexane	$C_{25}H_{43}N_3O_3S$
24w	$nC_{12}H_{25}$	*p*-$NHCO_2Et$	103	ether–hexane	$C_{26}H_{45}N_3O_4S$
24x	$nC_{12}H_{25}$	*p*-$NHCOC_6H_5$	137.5	CH_2Cl_2–hexane	$C_{30}H_{45}N_3O_3S$
24y	$nC_{12}H_{25}$	*p*-$CH(CH_3)_2$	63.9	hexane	$C_{26}H_{46}N_2O_2S$
24z	$nC_{12}H_{25}$	*p*-CH_2CH_3	39.5	hexane	$C_{25}H_{44}N_2O_2S$
25	nC_7H_{15}	*p*-CH_3	170.6 [a]	CH_2Cl_2–hexane	$C_{20}H_{34}N_2O_2S{\cdot}HCl$
26	$nC_{12}H_{25}$	*p*-Cl	150.9 [a]	ether–hexane	$C_{24}H_{41}ClN_2O_2S{\cdot}HCl$

[a] m.p. of the hydrochloride salt.

Table II. Physical data for compounds **30**, **34** and **38**.



Compound	*Z*	m.p. (°C)	Recrystallisation solvents	Formula
34	$CH_2OC_{14}H_{29}$	104.5 [a]	acetone–pet (35–60 °C)	$C_{26}H_{46}N_2O_4S{\cdot}HCl$
38	$CH_2OCOC_{13}H_{27}$	158.1	ether–hexane	$C_{26}H_{44}N_2O_5S$
30	$CO_2C_{14}H_{29}$	38.5 [a]	ether–pet (35–60 °C)	$C_{26}H_{44}N_2O_5S{\cdot}HCl$

[a] m.p. of the hydrochloride salt.

Table III. IR and ^{1}H-NMR data of compounds **24a–z**.

Compound	IR (film)	^{1}H-NMR
24a	3380 (NH), 1594 (C=C Ar)	($CDCl_3$) 0.78 (t, 3H); 1.3 (br s, 4H); 1.55 (m, 2H); 1.82 (s, 1H exch with D_2O); 2.40 (s, 3H); 2.70, 3, 3.60 (3m, 7H); 7.70 (d, 2H); 7.30 (d, 2H)
24b	3350 (NH), 1596 (C=C Ar)	($CDCl_3$) 0.80 (t, 3H); 1.2 (br s, 6H); 1.60 (m, 2H); 1.70 (s, 1H exch with D_2O); 2.35 (s, 3H); 2.30, 3, 3.60 (3m, 7H); 7.60 (d, 2H); 7.25 (d, 2H)
24c	3340 (NH), 1595 (C=C Ar)	($CDCl_3$) 0.79 (t, 3H); 1.25 (br s, 12H); 1.50 (m, 2H); 1.30 (s, 1H exch with D_2O); 2.34 (s, 3H); 2.7, 3, 3.6 (3m, 7H); 7.70 (d, 2H); 7.30 (d, 2H)
24d	3380 (NH), 1595 (C=C Ar)	($CDCl_3$) 0.79 (t, 3H); 1.25 (br s, 16H); 1.50 (m, 2H); 1.30 (s, 1H exch with D_2O); 2.35 (s, 3H); 2.7, 3, 3.6 (3m, 7H); 7.70 (d, 2H); 7.30 (d, 2H)
24e	3288 (NH), 1598 (C=C Ar)	($CDCl_3$) 0.83 (t, 3H); 1.20 (br s, 22H); 1.60 (m, 2H); 2.38 (s, 3H); 2.15, 3, 3.6 (3m, 7H); 7.58 (d, 2H); 7.28 (d, 2H)
24f	3300 (NH), 1598 (C=C Ar)	($CDCl_3$) 0.82 (t, 3H); 1.20 (br s, 26H); 1.55 (m, 2H); 1.90 (s, 1H exch with D_2O); 2.40 (s, 3H); 2.25, 3, 3.80 (3m, 7H); 7.65 (d, 2H); 7.25 (d, 2H)
24g	3360 (NH), 1595 (C=C Ar)	($CDCl_3$) 0.73 (t, 3H); 1.20 (br s, 30H); 1.50 (m, 2H); 1.35 (s, 1H exch with D_2O); 2.45 (s, 3H); 2.55, 3, 3.60 (3m, 7H); 7.80 (d, 2H); 7.40 (d, 2H)
24h	3387 (NH), 1587 (C=C Ar)	($CDCl_3$) 0.81 (t, 3H); 1.20 (br s, 12H); 1.55 (m, 2H); 1.52 (s, 1H exch with D_2O); 2.60, 3, 3.75 (3m, 7H); 7.80 (d, 2H); 7.40 (m, 3H)
24i	3340 (NH), 1590 (C=C Ar)	($CDCl_3$) 0.81 (t, 3H); 1.15 (br s, 12H); 1.50 (m, 2H); 1.49 (s, 1H exch with D_2O); 2.60, 3.25, 3.70 (3m, 7H); 3 (s, 3H); 8 (m, 4H)
24j	3350 (NH), 1580 (C=C Ar)	($CDCl_3$) 0.82 (t, 3H); 1.12 (br s, 12H); 1.70 (m, 2H); 1.50 (s, 1H exch with D_2O); 3.15, 3.90 (2m, 7H); 7.70 (d, 2H); 7.45 (d, 2H)
24k	3340 (NH), 1590 (C=C Ar)	($CDCl_3$) 0.81 (t, 3H); 1.13 (br s, 12H); 1.70 (m, 2H); 1.60 (s, 1H exch with D_2O); 3.10, 3.90 (2m, 7H); 3.80 (s, 3H); 7.70 (d, 2H); 6.80 (d, 2H)
24l	3388 (NH), 1591 (C=C Ar)	($CDCl_3$) 0.81 (t, 3H); 1.15 (br s, 12H); 1.50 (m, 2H); 1.60 (s, 1H exch with D_2O); 2.65, 3.15, 3.75 (3m, 7H); 3.80 (s, 3H); 7.85 (d, 1H); 7.45 (d, 1H); 6.90 (d, 2H)
24m	3432 (NH), 1596 (C=C Ar)	($CDCl_3$) 0.81 (t, 3H); 1.20 (br s, 12H); 1.55 (m, 2H); 1.60 (s, 1H exch with D_2O); 2.75, 3, 3.65 (3m, 7H); 3.80 (s, 3H); 7.30 (m, 3H); 7 (m, 1H)
24n	3410 (NH), 1586 (C=C Ar)	($CDCl_3$) 0.80 (t, 3H); 1.20 (br s, 12H); 1.55 (m, 2H); 1.60 (s, 1H exch with D_2O); 2.65, 3.15, 3.70 (3m, 7H); 3.85 (s, 9H); 7.00 (s, 2H)
24o	3325 (NH), 1585 (C=C Ar)	($CDCl_3$) 0.82 (t, 3H); 1.15 (br s, 22H); 1.55 (m, 2H); 1.55 (s, 1H exch with D_2O); 2.75, 3, 3.70 (3m, 7H); 7.75 (d, 2H); 7.45 (m, 3H)
24p	3321 (NH), 1583 (C=C Ar)	($CDCl_3$) 0.81 (t, 3H); 1.15 (br s, 22H); 1.55 (m, 2H); 1.58 (s, 1H exch with D_2O); 2.60, 3.25, 3.65 (3m, 7H); 3.00 (s, 3H); 8.00 (m, 4H)
24q	3372 (NH), 1586 (C=C Ar)	($CDCl_3$) 0.82 (t, 3H); 1.20 (br s, 22H); 1.65 (m, 2H); 1.60 (s, 1H exch with D_2O); 3.05, 3.80 (2m, 7H); 7.65 (d, 2H); 7.45 (d, 2H)
24r	3335 (NH), 1574–1596 (C=C Ar)	($CDCl_3$) 0.82 (t, 3H); 1.20 (br s, 22H); 1.55 (m, 2H); 1.45 (s, 1H exch with D_2O); 2.60, 3, 3.65 (3m, 7H); 3.8 (s, 3H); 7.70 (d, 2H); 6.85 (d, 2H)
24r′	3352 (NH), 1652 (C=C *cis*), 1579–1596 (C=C Ar)	($CDCl_3$) 0.83 (t, 3H); 1.24 (br s, 14H); 1.96 (m, 4H); 1.55 (m, 2H);1.50 (s, 1H exch with D_2O); 2.6 (m, 2H); 2.60, 3, 3.65 (3m, 7H); 3.80 (s, 3H); 5.30 (t, 2H); 7.70 (d, 2H); 6.80 (d, 2H)
24r″	3351 (NH), 1674 (C=C *trans*), 1579–1596 (C=C Ar)	($CDCl_3$) 0.81 (t, 3H); 1.16 (br s, 14H); 1.88 (m, 4H); 1.50 (m, 2H);1.55 (s, 1H exch with D_2O); 2.6 (m, 2H); 2.55, 3, 3.70 (3m, 7H); 3.75 (s, 3H); 5.31 (m, 2H); 7.65 (d, 2H); 6.80 (d, 2H)
24s	3337 (NH), 1574 (C=C Ar)	($CDCl_3$) 0.83 (t, 3H); 1.20 (br s, 22H); 1.60 (m, 2H); 1.50 (s, 1H exch with D_2O); 2.57, 3, 3.68 (3m, 7H); 7.60 (m, 4H)
24t	3434–3324 (NH, NH_2), 1601 (C=C Ar)	($CDCl_3$) 0.80 (t, 3H); 1.15 (br s, 22H); 1.50 (m, 2H); 1.50 (s, 3H exch with D_2O); 2.60, 3, 3.70 (3m, 7H); 7.50 (d, 2H); 6.55 (d, 2H)
24u	3330 (NH), 1580 (C=C Ar)	($CDCl_3$) 0.85 (t, 3H); 1.20 (br s, 22H); 1.55 (m, 2H); 1.45 (s, 1H exch with D_2O); 2.75, 3.60 (2m, 7H); 3 (s, 6H); 7.60 (d, 2H); 6.6 (d, 2H)
24v	3180–3300 (several bands, NH), 1690 (C=O), 1590–1612 (C=C Ar)	($CDCl_3$) 0.80 (t, 3H); 1.17 (br s, 22H); 1.55 (m, 2H); 1.60 (s, 1H exch with D_2O); 2.20 (s, 3H); 2.67, 3.10, 3.68 (3m, 7H); 7.65 (d, 2H); 7.75 (d, 2H); 7.45 (s, 1H exch with D_2O)
24w	3175–3435 (several bands, NH), 1730 (C=O), 1596–1617 (C=C Ar)	($CDCl_3$) 0.77 (t, 3H); 1.20 (br s, 22H); 1.50 (m, 2H); 1.25 (t, 3H); 1.50 (s, 1H exch with D_2O); 2.63, 3, 3.60 (3m, 7H); 4.15 (q, 2H); 6.75 (s, 1H exch with D_2O); 7.40 (d, 2H); 7.60 (d, 2H)
24x	3360 (NH), 1662 (C=O), 1593 (C=C Ar)	($CDCl_3$) 0.80 (t, 3H); 1.20 (br s, 22H); 1.55 (m, 2H); 1.25 (t, 3H); 1.45 (s, 1H exch with D_2O); 2.60, 3, 3.60 (3m, 7H); 4.15 (q, 2H); 6.75 (s, 1H exch with D_2O); 7.40 (d, 3H); 7.75 (d, 6H); 8.00 (s, 1H exch with D_2O)
24y	3356 (NH), 1598 (C=C Ar)	($CDCl_3$) 0.82 (t, 3H); 1.20 (br s, 22H); 1.50 (m, 2H); 1.30 (d, 6H); 1.45 (s, 1H exch with D_2O); 2.6 (m, 1H); 2.75, 3.60 (2m, 7H); 7.70 (d, 2H); 7.25 (d, 2H)
24z	3350 (NH), 1597 (C=C Ar)	($CDCl_3$) 0.9 (t, 3H); 1.25 (br s, 22H); 1.60 (m, 2H); 1.30 (t, 3H); 1.45 (s, 1H exch with D_2O); 2.6 (m, 2H); 2.80, 3.67 (2m, 7H); 7.70 (d, 2H); 7.30 (d, 2H)

exch, exchange.

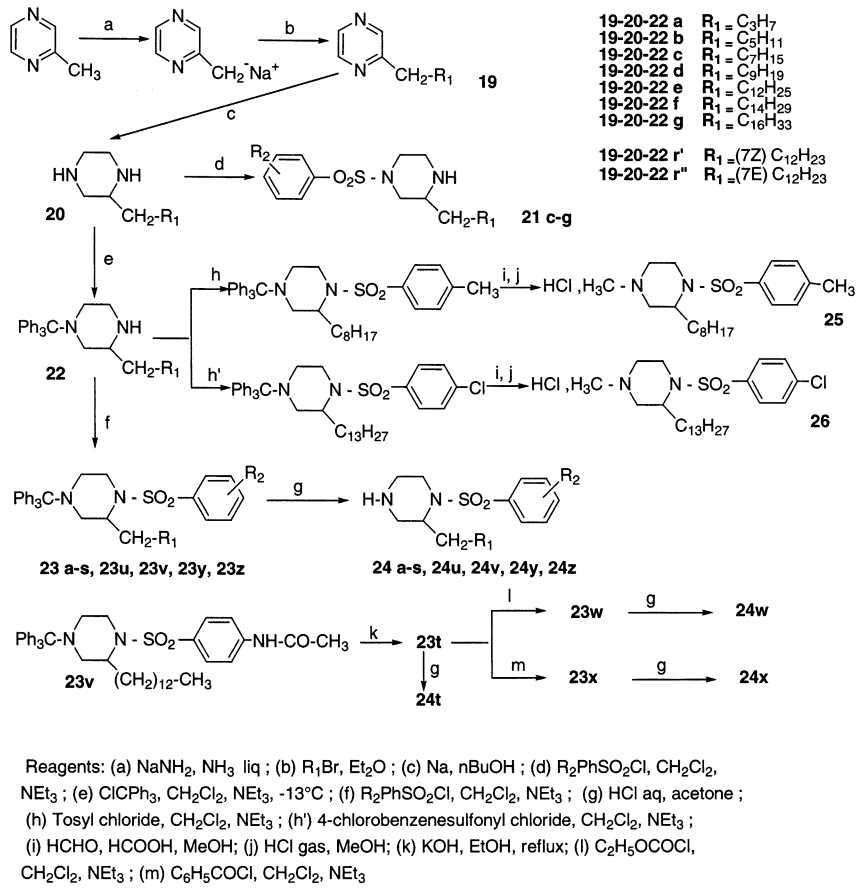


Figure 4. General synthesis of 1-arylsulfonyl-2-substituted piperazines.

fonic acid **10**, which was converted into the corresponding chloride **11** by means of phosphorus pentachloride [45]. 4-Ethyl benzenesulfonic acid treated in the same way gave the chloride **12**. Treatment of *N*,*N*-dimethylaniline with bistrimethylsilyl sulfate followed by hydrolysis of the silyl ester intermediate [46, 47] afforded the sulfonic acid **13**, which was transformed into its chloride as **10**. The 2-methoxy, 4-methoxy and 3,4,5-trimethoxybenzene sulfonyl chlorides were prepared from the corresponding anilines using the procedure described by Meervein et al. [48]. Treatment of these amines with

Table IV. IR and ^{1}H-NMR data of compounds **30**, **34** and **38**.

Compound	IR (film)	^{1}H-NMR
34	3369 (NH), 1578–591 (C=C Ar)	($CDCl_3$) 0.83 (t, 3H); 1.19 (br s, 22H); 1.4 (m, 2H); 1.50 (s, 1H exch with D_2O); 3.40 (t, 2H); 2.70, 3.50 (2m, 7H); 3.7 (d, 2H); 3.80 (s, 3H); 4.1 (m, 1H); 7.70 (d, 2H); 6.90 (d, 2H)
38	3320 (NH), 1738 (COO), 1597–622 (C=C Ar)	($CDCl_3$) 0.81 (t, 3H); 1.18 (br s, 20H); 1.50 (s, 1H exch with D_2O); 1.6 (m, 2H); 2.22 (t, 2H); 2.62, 3, 3.58 (3m, 7H); 3.80 (s, 3H); 4.4 (d, 2H); 7.70 (d, 2H); 6.90 (d, 2H)
30	3342 (NH), 1751 (COO), 1579–601 (C=C Ar)	($CDCl_3$) 0.81 (t, 3H); 1.20 (br s, 22H); 1.5 (m, 2H); 1.55 (s, 1H exch with D_2O); 2.22 (t, 2H); 2.80, 3.35 (2m, 7H); 3.80 (s, 3H); 4 (m, 2H); 4.1 (dd, 2H); 7.65 (d, 2H); 6.85 (d, 2H)

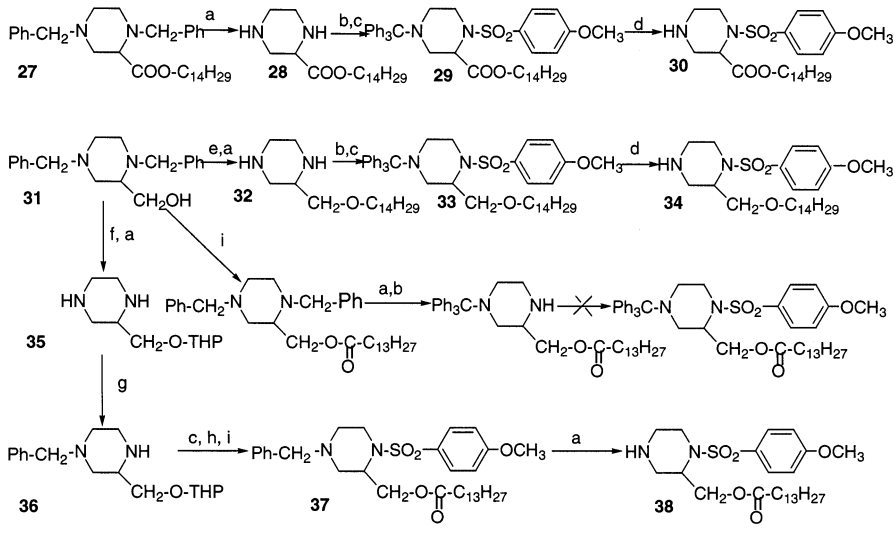
sodium nitrite and concentrated hydrochloric acid afforded the corresponding diazonium salts which were converted into the corresponding sulfonyl chlorides **15a**, **15b** and **15c** using cuprous chloride in anhydrous acetic acid saturated with SO_2. 4-Acetamidobenzenesulfonyl chloride treated with sodium sulfite and sodium hydrogencarbonate afforded the sulfinic acid sodium salt. Reaction of this salt with dimethylsulfate gave the ester **16** and the selective hydrolysis of the amide function led to the amine **17** which was converted into the sulfonyl chloride **18** using the same procedure as described for **15**.

The alkyl pyrazines **19a–g** and the corresponding piperazines **20a–g** were prepared as described by Tavet et al. [49]. The same method was applied for the synthesis of the alkenyl derivatives **19r′,r″** and **20r′,r″** (*figure 4*).

The preparation of substituted piperazines containing a functional group, ester (**28**) and ether (**32**), was achieved as described by Binisti et al. [39]. All the 1-arylsulfonyl-2-substituted (or 3-substituted) piperazines were prepared following the strategy described previously for the benzoyl derivatives **B** [39] and were obtained as outlined in *figures 4* and *5* except for compounds **24t**, **24w** and **24x** (*figure 4*) which were synthesised from 1-(4′-acetamidobenzenesulfonyl)-2-*n*-tridecyl-4-triphenylmethylpiperazine (**23v**). Selective hydrolysis of the amide bond with ethanolic KOH afforded **23t**, which was treated with ethyl chloroformate or benzoyl chloride to give **23w** and **23x**, respectively. Removal of the trityl protecting group gave **24t**, **24w** and **24x** (*table III*).

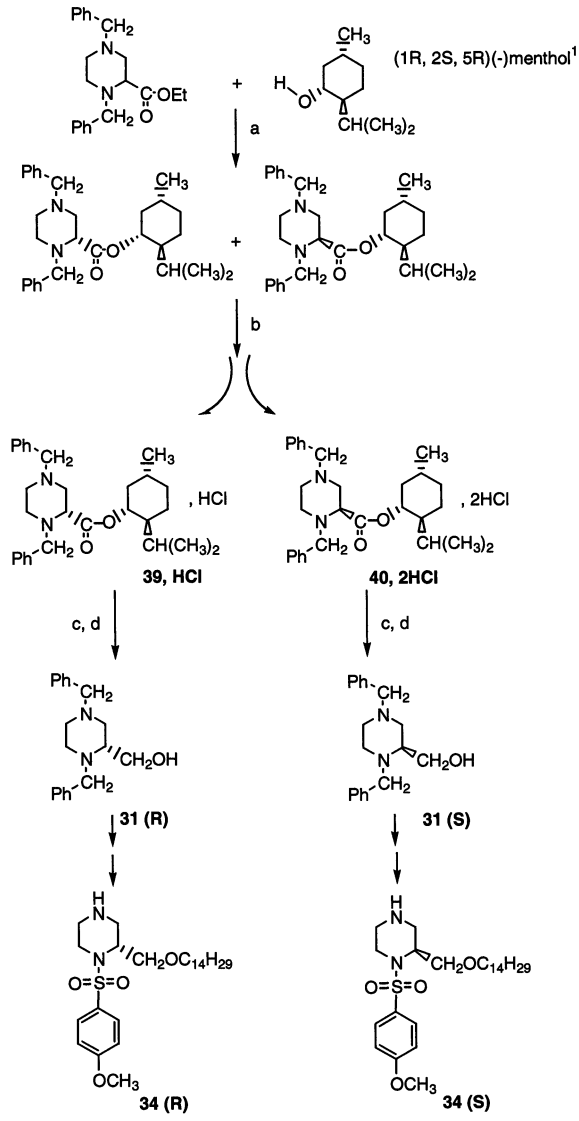
The two enantiomers of 1-(4′-methoxybenzenesulfonyl)-2-*n*-tetradecyloxymethylpiperazine (**34**) were prepared from the racemic ethyl 1,4-dibenzyl-2-piperazine carboxylate according to *figure 6*.

Transesterification of ethyl ester to menthyl ester was carried out using (1*R*, 2*S*, 5*R*)-menthol according to Aebischer et al. [50]. The hydrochloric salts of both diastereoisomers **39** and **40** were separated by crystallisation. Then the free bases were regenerated in usual conditions and the ester function was reduced into alcohol (**31** (**R**) and **31** (**S**)). The optical purity of these alcohols was checked by ^{1}H-NMR using the chiral shift reagent tris-[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium. In the absence of chiral chelate, the ^{1}H-NMR spectrum of the racemic alcohol **31** shows two dd (3.60 and 4.06 ppm) attributed to the protons H'_2 and two doublets (3.47 and 3.98 ppm) for the protons H'_1. In the presence of the chiral chelate, the protons H'_2 formed two unre-



Reagents: (a) H_2-Pd/C; (b) Ph_3CCl, CH_2Cl_2, NEt_3, -13°C; (c) 4-methoxybenzene sulfonyl chloride, CH_2Cl_2, NEt_3 ; (d) HCl, acetone; (e) NaH, tetradecyl bromide, DMF; (f) DHP, APTS; (g) benzyl chloride, K_2CO_3, KI, acetone; (h) MeOH, HCl; (i) n-tetradecanoyl chloride, pyridine, toluene

Figure 5. Preparation of substituted piperazines containing a functional group.



Reagents :[1]1R, 2S, 5R-5-methyl-2-(1-methylethyl)cyclohexanol; (a) NaH, dry toluene; (b) EtOH, aq HCl; (c) $LiAlH_4$, dry ether; (d) 5M aq NaOH

Figure 6. Preparation of enantiomers of 1-(4′-methoxybenzenesulfonyl)-2-*n*-tetradecyloxymethylpiperazine (**34**).

solved signals (4.55 and 5.15 ppm) and the proton H'_1 two dd (3.75 and 4.30 ppm), each doublet of dd representing 50% of the mixture (win NMR programme Brüker). In the case of resolved alcohol **31** (**S**), the ^{1}H-NMR spectrum shows only two doublets for the proton H'_1 (3.85 and 4.50 ppm) and in consequence compound **31** (**S**) is optically pure (±5%, precision of NMR). The alcohols **31** (**S**) and (**R**) were treated separately as the racemic alcohol **31** to give the two enantiomers **34** (**S**) and **34** (**R**) (*table IV*).

3. Biological results and discussion

All the final products were tested for their inhibitory effects on the enzymatic activity of two types

of PLA_2: (i) a Group I PLA_2 from bovine pancreas (Bp PLA_2); and (ii) a Group II PLA_2 from a rabbit platelet lysate (Rpl PLA_2).

3.1. Specificity

The various compounds inhibited the bovine pancreatic PLA_2 and the rabbit platelet lysate PLA_2 at similar levels, thus, showing no specificity towards Group I or II PLA_2s (*table V*). The slight discrepancy between both enzymes can only be attributed to the difference between a purified enzyme (bovine pancreatic) and a non-purified one (platelet lysate).

3.2. Position isomers (series **21** and **24**)

As shown in *tables VI* and *VII*, compounds differing by the position of the tosyl group in position 4 (series **21**) or 1 (series **24**) show slightly different inhibitory activities. The best compound **24e** is twice more potent than the corresponding **21e** on bovine pancreatic PLA_2. That is why we decided to develop the series **24**, where the hydrophobic appendix is in position 2 on the piperazine cycle.

3.3. Sulfamide function

In our previous publication [39], compounds were substituted on nitrogen of the piperazine ring through an amide function. **PMS 832** (see *figure 1*) was selected from this study and investigated further [40]. The sulfamide **24r**, analogous to **PMS 832** is twice more potent on the two enzymes with IC_{50} of 1.9 and 3.3 against 3.9 and 7.2, respectively.

As shown later, this improvement of activity should be related to a better chelation with calcium ion. This is reinforced by the fact that an electron donor group on *para* position such as methoxy (**24r**) or dimethylamino (**24n**) enhances the activity, probably by increasing the chelation of the O=S=O to the calcium ion. However, an electron-donating substituent on *ortho* position decreases the activity. Such substituent on this position, leads to steric hindrance, and therefore could modify the planeity of O=S=O group versus the phenyl ring.

3.4. Hydrophobicity of the tail

The lipophilic character Σf was evaluated using the hydrophobic fragmental constant f of Rekker et al. [51]. As in the case of amide compounds **B** (*figure 1*) [39], the inhibitory activity of the sulfamide series **C** depends on the length of the alkyl chain, with an optimum for 13 carbons as shown for compounds **21e** (*tables VI*) and **24e** (*table VII*). This optimum is the same for both types of enzymes.

3.5. Effect of the nature of the hydrophobic tail

Table VIII shows that introducing a functional group at the level of the alkyl chain leads to a dramatic loss of activity, particularly for ester groups (compounds **30** and **38**). This feature can be the consequence of a reduced flexibility. By contrast, an ether function does not modify the activity: compound **34**, isolipophilic with compound **24r** shows the same activity with IC_{50} of 2.1 and 2.9 (for **34**) close to 1.9 and 3.3 (for **24r**) on both enzymes, respectively.

3.6. Substitution on N_4

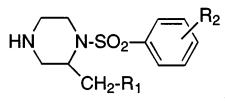
As shown in *table V*, blocking the nitrogen in position 4 by a methyl group (compounds **25** and **26**) suppressed the inhibitory potency, in spite of the fact that the global lipophilicity of these compounds are very similar (the difference is only 0.23 $\log P$ unit) to that of the corresponding compounds **24c** and **24q** not substituted on this nitrogen. This feature illustrates the necessity for these compounds to possess a free NH, which might establish an hydrogen bond with the site of action of the enzyme, probably the imidazole core of His_{48}, essential amino-acid involved in the catalytic process.

3.7. Substitution on the phenyl ring

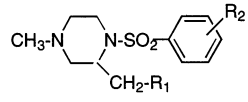
The substituents on the phenyl group (*table IX*) were selected according to cluster analysis described by Hansch et al. [52, 53] for their physicochemical properties: lipophilicity (Σf), steric (Es) and electronic (σp) effects. In this selection, the correlation matrix shows the orthogonality of the parameters (*table X*), and allows quantitative structure–activity relationship calculations (*table XI*).

Moreover, *table V* shows that the biological responses do not vary in a large range except with low or high lipophilic appendices on position 2. Surprisingly the initial and rational choice of substituents R_2 on the benzene ring gives the same results, except with a lipophilic and bulky substituent (suddenly inactive). In spite of these features we have performed a complete SAR analysis.

Table V. Biological data for 1-arylsulfonyl-2-*n*-alkylpiperazines (**24**) and methyl derivatives **25** and **26**.



24

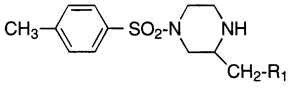


25, 26

Compound	R_1	R_2	Bp [a]PLA_2 IC_{50} (μM)	Rpl [b]PLA_2 IC_{50} (μM)
24a	nC_3H_7	*p*-CH_3	>100	>100
24b	nC_5H_{11}	*p*-CH_3	100	100
24c	nC_7H_{15}	*p*-CH_3	17.3	26.8
24d	nC_9H_{19}	*p*-CH_3	8.8	11.7
24e	$nC_{12}H_{25}$	*p*-CH_3	2.45	4.5
24f	$nC_{14}H_{29}$	*p*-CH_3	9	20
24g	$nC_{16}H_{33}$	*p*-CH_3	80	100
24h	nC_7H_{15}	*p*-H	19.3	40
24i	nC_7H_{15}	*p*-SO_2CH_3	51.25	60
24j	nC_7H_{15}	*p*-Cl	16.7	17.8
24k	nC_7H_{15}	*p*-OCH_3	10	18.9
24l	nC_7H_{15}	*o*-OCH_3	37.7	40
24m	nC_7H_{15}	*m*-OCH_3	17	19.6
24n	nC_7H_{15}	3,4,5-triOCH_3	33.8	39.2
24o	$nC_{12}H_{25}$	*p*-H	8	8.6
24p	$nC_{12}H_{25}$	*p*-SO_2CH_3	3	4.6
24q	$nC_{12}H_{25}$	*p*-Cl	7.5	7.2
24r	$nC_{12}H_{25}$	*p*-OCH_3	1.9	3.3
24r′	$(7Z)nC_{12}H_{23}$	*p*-OCH_3	3.8	5.5
24r″	$(7E)nC_{12}H_{23}$	*p*-OCH_3	2.3	3.5
24s	$nC_{12}H_{25}$	*p*-Br	5.4	5.3
24t	$nC_{12}H_{25}$	*p*-NH_2	2	3.5
24u	$nC_{12}H_{25}$	*p*-$N(CH_3)_2$	1.4	1.7
24v	$nC_{12}H_{25}$	*p*-$NHCOCH_3$	2.2	3.5
24w	$nC_{12}H_{25}$	*p*-$NHCO_2Et$	2.2	3.6
24x	$nC_{12}H_{25}$	*p*-$NHCOC_6H_5$	2.5	4
24y	$nC_{12}H_{25}$	*p*-$CH(CH_3)_2$	100	100
24z	$nC_{12}H_{25}$	*p*-CH_2CH_3	100	100
25	nC_7H_{15}	*p*-CH_3	>100	>100
26	$nC_{12}H_{25}$	*p*-Cl	>100	>100

[a] Bovine pancreatic PLA_2.
[b] Rabbit platelet lysate PLA_2.

Table VI. Biological data for 1-*p*-tolylsulfonyl-3-*n*-alkylpiperazines (**21**).



21

Compound	R_1	Bp [a]PLA_2 IC_{50} (μM)
21c	nC_7H_{15}	>100
21d	nC_9H_{19}	12.1
21e	$nC_{12}H_{25}$	5.5
21f	$nC_{14}H_{29}$	6.8
21g	$nC_{16}H_{33}$	>100

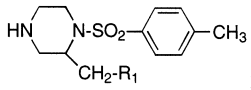
[a] Bovine pancreatic PLA_2.

Eq. (1) in *table XI* obtained from data in *table IX* shows that the hydrophobic effect of the substituent on phenyl ring plays prevalent role in *anti*-PLA_2 activity.

3.8. Enantiospecificity

PLA_2s are enantiospecific and hydrolyse solely phospholipids of the (*R*) series. Our inhibitors possess a chiral centre, located at the carbon atom of the piperazine ring carrying the hydrophobic tail. The resolution of the racemate was then performed on the ester derivative **34** (see Section 2). *Table VIII* shows that both enantiomers **34** (**R**) and **34** (**S**) present a close inhibitory activity contrary to the substrate analogs.

Table VII. Biological data for 1-*p*-tolylsulfonyl-2-*n*-alkylpiperazines (**24**).



24

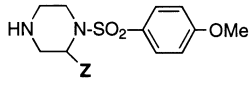
Compound	R_1	$\Sigma f\ R_1$ [a]	Bp [b]PLA_2 IC_{50} (μM)	Rpl [c]PLA_2 IC_{50} (μM)
24a	nC_3H_7	1.739	>100	>100
24b	nC_5H_{11}	2.777	100	100
24c	nC_7H_{15}	3.815	17.3	26.8
24d	nC_9H_{19}	4.853	8.8	11.7
24e	$nC_{12}H_{25}$	6.410	2.45	4.5
24f	$nC_{14}H_{29}$	7.448	9	20
24g	$nC_{16}H_{33}$	8.486	80	100

[a] Calculated from Ref. [51].
[b] Bovine pancreatic PLA_2.
[c] Rabbit platelet lysate PLA_2.

3.9. Global hydrophobicity

The relation between the inhibitory effect and the global hydrophobicity follows a bilinear type correlation according to Kubinyi [54, 55]. That attests the importance of the lipophilic character in the interaction between the inhibitor and the enzyme (see Eq. (4) obtained from *table XII* data).

Table VIII. Biological data for compounds **24r**, **30**, **38** and racemic **34**, **34** (**R**) and **34** (**S**).



Compound	Z	$\Sigma f\ Z$ [a]	Bp [b]PLA_2 IC_{50} (μM)	Pl [c]PLA_2 IC_{50} (μM)
24r	$nC_{13}H_{27}$	6.929	1.9	3.3
30	$CO_2C_{14}H_{29}$	6.545	>100	>100
38	$CH_2OCOC_{13}H_{27}$	6.775	>100	>100
34	$CH_2OC_{14}H_{29}$	6.950	2.6	2.47
34 (**R**)	$CH_2OC_{14}H_{29}$	6.950	3	2.76
34 (**S**)	$CH_2OC_{14}H_{29}$	6.950	2.1	2.9

[a] Calculated from Ref. [51].
[b] Bovine pancreatic PLA_2.
[c] Platelet lysate PLA_2.

$$\log(1/IC50) = \underset{n=21}{0.568 \pm (0.070)} \log P \underset{r^2=0.802}{} - \underset{s=0.31}{2.749 \pm (0.351)}[\log(1e^{-8}P+1)] \underset{F=11.12}{} + \underset{\alpha<0.05}{1.864 \pm (0.429)} \log(1/IC_{50}) = 0.568 \quad (4)$$

4. Conclusion

Starting from a series of compounds highly potent as PAF-antagonists **A** (*figure 1*), we have modified in two steps the pharmacophore and obtained activities on PLA_2 in the micromolar range. As for the amides series **B** [39], the sulfamide series **C** is not specific on Group II PLA_2. As described for substrate analogues [37, 38], the global hydrophobicity of the inhibitors is essential, assuming that the interaction occurs in a large hydrophobic pocket. According to the literature results on the active site structure, the hydrophobic well interacting with both substitute tails is large. This last feature is in accordance with the non-enantiospecificity regarding the carbon bearing the hydrophobic

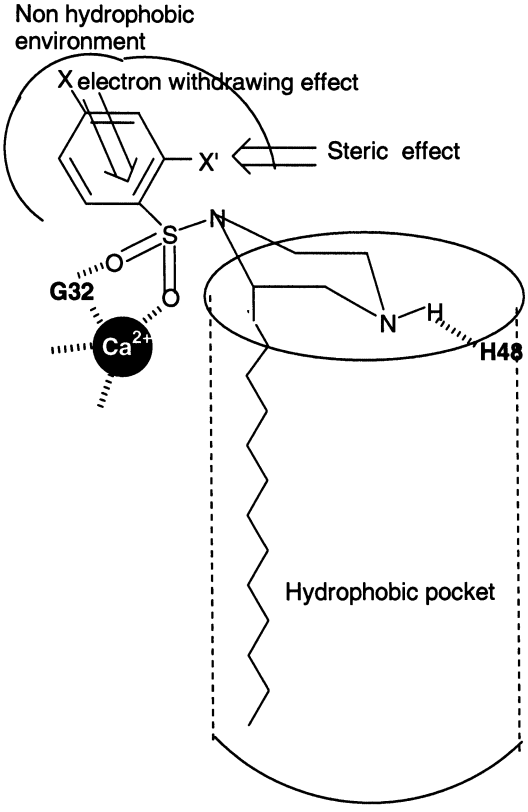
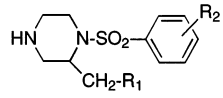


Figure 7. Expected interactions between 1-arylsulfamido-2-alkylpiperazines and the PLA_2 catalytic site.

Table IX. Biological data and physicochemical parameters for 1-arylsulfonyl-2-*n*-alkylpiperazines (**24**).



24

Compound	R_1	R_2	$\Sigma f\ R_2$ [a]	σp	Es	Bp [b]PLA_2 IC_{50} (μM)	Log $1/IC_{50}$
24e	$nC_{12}H_{25}$	*p*-CH_3	0.701	−0.17	−1.24	2.45	5.6108
24p	$nC_{12}H_{25}$	*p*-SO_2CH_3	−1.163	0.72	–	3	5.5229
24q	$nC_{12}H_{25}$	*p*-Cl	0.924	0.23	−0.97	7.5	5.1249
24r	$nC_{12}H_{25}$	*p*-OCH_3	0.262	−0.27	−0.55	1.9	5.7212
24s	$nC_{12}H_{25}$	*p*-Br	1.116	0.23	−1.16	5.4	5.2676
24t	$nC_{12}H_{25}$	*p*-NH_2	−1.23	−0.66	−0.61	2	5.6990
24u	$nC_{12}H_{25}$	*p*-$N(CH_3)_2$	−0.842	−0.83	–	1.4	5.8539
24v	$nC_{12}H_{25}$	*p*-$NHCOCH_3$	−0.589	0.00	–	2.2	5.6576
24w	$nC_{12}H_{25}$	*p*-NHCOOEt	0.452	−0.15	–	2.2	5.6576
24x	$nC_{12}H_{25}$	*p*-$NHCOC_6H_5$	0.55	−0.19	–	2.5	5.6021
24y	$nC_{12}H_{25}$	*p*-$CH_2(CH_3)_2$	1.739	−0.15	−1.71	100	4.0000
24z	$nC_{12}H_{25}$	*p*-CH_2CH_3	1.220	−0.15	−1.31	100	4.0000

[a] Calculated from Ref. [51].
[b] Bovine pancreatic PLA_2.

Table X. Correlation matrix.

	Σf	σp	Es
Σf	1	–	–
σp	0.025	1	–
Es	0.235	0.002	1

tail and its position on the cycle. These remarks confirm our previous results [39] on amide series **B**. The sulfamide group induces a better inhibitory effect than the amide one (two-fold for compound **24r** compared to **PMS 832** (see *figure 1*)). In the other hand, *para*-substituents with donor effect increase the activity, may be by enhancing the electronic distribution on the sulfamide group allowing a better chelation with the calcium ion. That is confirmed by steric effect in *ortho* substitution (comparison of compound **24k** with **24l**). It seems that this interaction area requires hydrophobic and steric constraints (*figure 7*). These features seem to confirm that these inhibitors act at the catalytic site. Nevertheless a thorough study at the biochemical level has to be performed in order to evaluate these hypotheses.

5. Experimental protocols

5.1. Chemistry

All materials were obtained from commercial suppliers and used without further purification. Thin-layer chromatography was performed on TLC plastic sheets of silica gel $60F_{254}$ (layer thickness 0.2 mm) from Merck.

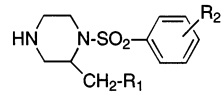
Table XI. Equations relating to lipophilic, electronic and steric effects on the aromatic ring.

$$\underset{n=12}{\log(1/\mathrm{IC50})} = \underset{r^2=0.811}{-0.467 \pm (0.117)}\Sigma f \underset{s=0.309}{-0.337 \pm (0.098)}\Sigma f \underset{F=9.730}{+5.849 \pm (0.141)} \quad 0.01<\alpha<0.05 \tag{1}$$

$$\underset{n=12}{\log(1/\mathrm{IC50})} = \underset{r^2=0.284}{-0.094 \pm (0.047)}\sigma p \underset{s=0.5696}{+5.925 \pm (0.350)} \quad F=3.976 \quad 0.025<\alpha<0.05 \tag{2}$$

$$\underset{n=7}{\log(1/\mathrm{IC50})} = \underset{r^2=0.626}{0.549 \pm (0.263)}\mathrm{Es} \underset{s=0.508}{+5.471 \pm (0.301)} \quad F=8.353 \quad 0.05<\alpha<0.1 \tag{3}$$

Table XII. Global hydrophobicity and biological data for 1-arylsulfonyl-2-*n*-alkylpiperazines **24**.



24

Compound	R_1	R_2	Log *P*	Bp [a]PLA_2 IC_{50} (μM)	Log $1/IC_{50}$
24b	nC_5H_{11}	*p*-CH_3	4.481	100	4.0000
24c	nC_7H_{15}	*p*-CH_3	5.519	17.3	4.7619
24d	nC_9H_{19}	*p*-CH_3	6.557	8.8	5.0555
24e	$nC_{12}H_{25}$	*p*-CH_3	8.114	2.45	5.6108
24h	nC_7H_{15}	*p*-H	4.000	19.3	4.7144
24I	nC_7H_{15}	*p*-SO_2CH_3	3.649	51.25	4.2903
24j	nC_7H_{15}	*p*-Cl	5.742	16.7	4.7773
24k	nC_7H_{15}	*p*-OCH_3	5.080	10	5.0000
24l	nC_7H_{15}	*o*-OCH_3	5.080	37.7	4.4237
24m	nC_7H_{15}	*m*-OCH_3	5.080	17	4.7696
24n	nC_7H_{15}	3,4,5-triOCH_3	5.240	33.8	4.4711
24o	$nC_{12}H_{25}$	*p*-H	7.595	8	5.0969
24p	$nC_{12}H_{25}$	*p*-SO_2CH_3	6.244	3	5.5229
24q	$nC_{12}H_{25}$	*p*-Cl	8.337	7.5	5.1249
24r	$nC_{12}H_{25}$	*p*-OCH_3	7.675	1.9	5.7212
24s	$nC_{12}H_{25}$	*p*-Br	8.529	5.4	5.2676
24t	$nC_{12}H_{25}$	*p*-NH_2	6.571	2	5.6990
24u	$nC_{12}H_{25}$	*p*-$N(CH_3)_2$	7.886	1.4	5.8539
24v	$nC_{12}H_{25}$	*p*-$NHCOCH_3$	6.824	2.2	5.6576
24w	$nC_{12}H_{25}$	*p*-$NHCO_2Et$	7.865	2.2	5.6576
24x	$nC_{12}H_{25}$	*p*-$NHCOC_6H_5$	8.252	2.5	5.6021

[a] Bovine pancreatic PLA_2.

Column chromatography purification was carried out on silica gel 60 (70–230 mesh ASTM, Merck). All m.p. were determined on a digital m.p. apparatus (Electrothermal) and are uncorrected. The structures of all compounds were confirmed by IR and ^{1}H-NMR spectra. IR spectra were obtained in paraffin oil with a ATI Mattson Genesis Series FTIR spectrometer, and ^{1}H-NMR spectra were recorded in $CDCl_3$ on a BRUCKER AC 200 spectrometer using hexamethyldisiloxane (HMDS) as an internal standard. Chemical shifts are given in ppm and peak multiplicities are designated as follows: s, singlet, d, doublet, t, triplet, br, broad, m, multiplet. Elemental analyses were obtained from the 'Service Régional de Microanalyse', Université Pierre et Marie Curie, Paris, France and were within ±0.4% of theoretical values.

*5.1.1. 6-Bromohexan-1-ol (**1**)*

A mixture of hexane-1,6-diol (238 g, 2 mol) and HBr (48% aq. solution, 250 mL) in 500 mL C_6H_6 was refluxed for 72 h. Upon cooling, the reaction mixture was decanted and the aq. phase extracted with ether (3×100 mL) and $CHCl_3$ (100 mL). The combined organic layers were concentrated and the residue was dissolved in ether (200 mL), washed with saturated $NaHCO_3$, then with water until neutral pH. The organic phase was dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by high vacuum distillation to yield 257 g (70%) of **1** as a colourless oil: $E_{0.05} = 70$ °C; IR (film) ν 3340_{OH} cm^{-1}; ^{1}H-NMR δ 1.35–1.95 (m, 8H, CH_2), 3.35–3.70 (m, 4H, CH_2O and CH_2Br), 2.4 (s, 1H, D_2O exchange, OH).

*5.1.2. 6-Bromo-1-(tetrahydropyran-2′-yloxy)hexane (**2**)*

To a stirred solution of **1** (92 g, 510 mmol) in dry ether (100 mL) at 0 °C were added dropwise 55.1 mL (600 mmol) of dihydropyran containing concentrated (37%) HCl in a catalytic amount (80 μL). The mixture was stirred for 2 h at 0 °C, then 12 h at room temperature (r.t.). The reaction mixture was diluted with ether, washed with saturated aq. $NaHCO_3$, then with water until neutral pH. The organic layer was dried over

$MgSO_4$, filtered, and evaporated to dryness. The crude product was purified by high vacuum distillation to afford 120 g (89%) of **2** as a colourless liquid: $E_{0.05} =$ 100–102 °C; ^{1}H-NMR δ 1.4, 1.8 (2m, 14H, CH_2), 3.35 (t, 2H, CH_2Br), 3.4, 3.8 (2m, 4H, CH_2O), 4.5 (t, 1H, CH).

*5.1.3. 1-(Tetrahydropyran-2′-yloxy)-7-dodecyne (**3**)*

In a 1 L three-necked round bottom flask, equipped with a magnetic stirring bar, a dry ice condenser and a dropping funnel, a suspension of 14 g (610 mmol) of lithium amide in 400 mL liquid NH_3 was maintained at −60 °C in an acetone bath. 1-Hexyne (35 mL, 300 mmol) was added dropwise and the mixture was stirred for 2 h at ammoniac refluxing temperature. Then a solution of 60 g (226 mmol) of **2** in 200 mL anhydrous THF was added dropwise over a 20 min period and the mixture was stirred in the same conditions for an additional 2 h. The solution was allowed to warm to r.t. and stirred overnight under a fume-cupboard. The LiBr salts were filtered and washed with ether. The filtrate was concentrated and the residue taken up in ether was washed with water until neutral pH. The organic phase was dried over $MgSO_4$, filtered and the solvent evaporated in vacuo. The crude product was purified by high vacuum distillation to yield 51 g (85%) of **3** as a colourless oil: $E_{0.05} = 154$ °C; ^{1}H-NMR δ 0.84 (t, 3H, CH_3), 1.4, 1.8 (2m, 18H, CH_2), 2.2 (t, 4H, CH_2–C≡C), 3.4, 3.8 (2m, 4H, CH_2O), 4.5 (t, 1H, CH).

*5.1.4. (7Z)-1-(Tetrahydropyran-2′-yloxy)-7-dodecene (**4**)*

A stirred and warmed (40 °C) solution of 51 g of **3** (197 mmol) and 2 mL quinoline in 200 mL *n*-hexane was hydrogenated with 5.1 g of 5% Pd on $BaSO_4$ under ca. 20 psi of hydrogen for 24 h. The reaction was monitored by NMR following the disappearance of the signal at 2.2 ppm (CH_2–C≡C) while a signal at 5.2 ppm (CH=CH) was appearing, afterwhat the catalyst was filtered through Celite, and the filtrate was concentrated. The residue **4** was used immediately without further purification.

*5.1.5. (7Z)-1-Hydroxy-7-dodecene (**5**)*

The crude **4** was dissolved in 75 mL absolute MeOH and added to a solution of 75 mL absolute MeOH saturated with anhydrous HCl gas. The mixture was stirred for several min, then left 3 h at 4 °C. Evaporation of the solvent left a residue which was partitioned between H_2O (100 mL) and Et_2O (100 mL). The organic layer was washed with aq. saturated $NaHCO_3$, then H_2O, dried, and concentrated in vacuo. High vacuum distillation of the crude residue gave 32.5 g (92%) of **5** as a colourless viscous liquid: $E_{0.1} = 80$ °C; ^{1}H-NMR δ 0.82 (t, 3H, CH_3), 1.3 (m, 12H, CH_2), 2.05 (m, 4H, CH_2–C=C), 2.25 (s, 1H, D_2O exchange, OH), 3.50 (t, 2H, CH_2O), 5.25 (t, 2H, CH=CH).

*5.1.6. (7Z)-1-Bromo-7-dodecene (**6**)*

To a stirred solution of PBr_3 (6.1 mL, 65 mmol) in anhydrous C_6H_6 (12.5 mL) were added dropwise 2.8 mL (34 mmol) of anhydrous pyridine. The mixture was stirred at r.t. for 15 min, then chilled at −10 °C and 30 g (164 mmol) of **5**, 1 mL (12.5 mmol) of anhydrous pyridine in 12.5 mL dry C_6H_6 were added dropwise. The mixture was stirred at r.t. for an additional 48 h. Then it was diluted with 200 mL ether and washed successively with a solution of $NaHCO_3$ (5.5 g in 100 mL H_2O), H_2O until pH 7, and 200 mL HCl 1 N. The organic layer was washed with H_2O several time, dried over $MgSO_4$ and the solvent was evaporated. The crude product was distilled under high vacuum to afford 20 g (50%) of compound **6** as a colourless liquid: $E_{0.1} =$ 88 °C; ^{1}H-NMR δ 0.83 (t, 3H, CH_3), 1.3 (m, 12H, CH_2), 1.91 (m, 4H, CH_2–C=C), 3.35 (t, 2H, CH_2Br), 5.3 (t, 2H, CH=CH).

*5.1.7. (7E)-1-(Tetrahydropyran-2′-yloxy)-7-dodecene (**7**)*

In a 1 L three-necked round bottom flask, equipped with a magnetic stirring bar, a dry ice condenser and a dropping funnel, and maintained at −60 °C in an acetone bath, 250 mL of ammoniac gas were condensed. Na (12 g, 520 mmol) was added in small portions. The mixture was stirred at ammoniac refluxing temperature and 38 g (150 mmol) of **3** in 150 mL anhydrous ether were added dropwise over a 20 min period, afterwhat it was stirred in the same conditions for an additional 2 h. The solution was allowed to warm to r.t. and was stirred overnight under a fume-cupboard. Then it was diluted with ether (200 mL) and poured over ice. The organic layer was separated and washed with water until pH 7, dried ($MgSO_4$) and concentrated. The crude product was purified by high vacuum distillation to give 30.4 g (yield 72%) of **7** as a colourless liquid: $E_{0.1} = 134$ °C; ^{1}H-NMR δ 0.82 (t, 3H, CH_3), 1.4, 1.8 (2m, 22H, CH_2), 1.95 (m, 4H, CH_2–C=C), 3.4, 3.8 (2m, 4H, CH_2O), 4.5 (t, 1H, CH), 5.35 (m, 2H, CH=CH).

*5.1.8. (7E)-7-Dodecen-1-ol (**8**)*

This compound was prepared using the procedure described above for **5** to give **8** in a 91% yield: $E_{0.1} =$

102–104 °C; ^{1}H-NMR δ 0.80 (t, 3H, CH_3), 1.3 (m, 12H, CH_2), 2.05 (m, 4H, CH_2–C=C), 3.45 (t, 2H, CH_2O), 5.30 (m, 2H, CH=CH).

*5.1.9. (7E)-1-Bromo-7-dodecene (**9**)*

This compound was prepared following the procedure described for **6** and gave **9** in a 47% yield: $E_{0.05} = 90$ °C; ^{1}H-NMR δ 0.83 (t, 3H, CH_3), 1.35 (m, 12H, CH_2), 1.95 (m, 4H, CH_2–C=C), 3.40 (t, 2H, CH_2Br), 5.3 (m, 2H, CH=CH).

*5.1.10. p-Isopropylbenzenesulfonic acid (**10**)*

A mixture of cumene (14 mL, 0.1 mol) and concentrated H_2SO_4 (2.7 mL, 0.05 mol) was stirred at 160 °C for 3 h with a dean stark trap. At the end of the reaction, the excess cumene was removed under vacuum ($E_{15} = 30$ °C). The crude product was diluted with $CHCl_3$ and washed with water. The aqueous layer was concentrated to yield *p*-isopropylbenzene sulfonic acid (dihydrate) as white crystals. The crystals were placed in a 250 mL round bottom flask equipped with a distilling column, and then steam distillated with CCl_4 (50 mL) to remove the remaining H_2O, yielding 16.5 g (82.5%) of anhydrous *p*-isopropylbenzenesulfonic acid (**10**).

*5.1.11. p-Isopropylbenzenesulfonyl chloride (**11**)*

To an ice cooled solution of phosphorus pentachloride (17 g, 79 mmol) in dry CH_2Cl_2 (200 mL) was added portionwise 14 g (72.5 mmol) of **10**. The reaction mixture was allowed to stir for 3 h at 0 °C. The solvent was removed under vacuum, and the residue was dissolved in ether, washed with water, dried ($MgSO_4$), filtered and concentrated in vacuo. The crude compound was chromatographed on silica gel using 5% ether in petroleum spirit (35–60 °C) as the eluent to yield 9.1 g (60%) of pure **11** as a greenish oil: IR ν $1580_{ArC=C}$ cm^{-1}; ^{1}H-NMR δ 1.25 (d, 6H, CH_3), 3 (m, 1H, CH), 7.4, 7.95 (2d, 4H, ArH).

*5.1.12. p-Ethylbenzenesulfonyl chloride (**12**)*

p-Ethylbenzenesulfonic acid (5 g, 26.88 mmol) was treated with PCl_5 as described above yielding 3.5 g (64%) of **12** as a greenish oil after purification by column chromatography on silica gel eluted with 5% ether in petroleum spirit (35–60 °C): IR (film) ν $1575_{ArC=C}$ cm^{-1}; ^{1}H-NMR δ 1.25 (t, 3H, CH_3), 2.8 (q, 2H, CH_2), 7.4, 7.9 (2d, 4H, ArH).

*5.1.13. p-N,N-Dimethylaminobenzenesulfonyl chloride (**14**)*

Bistrimethylsilyl sulfate (5 g, 20 mmol) and *N*,*N*-dimethylaniline (2.5 g, 20 mmol) were placed in a 250 mL round bottom flask equipped with a distilling column. The mixture was stirred at 170 °C for 4 h. The white pasty solid obtained was washed with ether to remove the remaining *N*,*N*-dimethylaniline and dissolved in 20 mL H_2O. The aqueous solution was concentrated and the residue dried under high vacuum to yield 4 g (96%) of anhydrous *p*-*N*,*N*-dimethylaminobenzenesulfonic acid (**13**) as a white solid which was treated with PCl_5 in an analogous manner to **11** to give, after recrystallisation from C_6H_{14}, 4 g (92%) of pure **14** as greenish crystals: m.p. 63 °C; IR ν $1580_{ArC=C}$ cm^{-1}; ^{1}H-NMR δ 3 (d, 6H, CH_3), 6.65, 7.80 (2d, 4H, ArH).

*5.1.14. o-Methoxybenzenesulfonyl chloride (**15a**)*

o-Methoxybenzenesulfonyl chloride (**15a**) was prepared as described for **15b** below and purified by high vacuum distillation (yield 22%): $E_{0.1} = 110$ °C; IR ν $1600_{ArC=C}$ cm^{-1}; ^{1}H-NMR δ 3.9 (s, 3H, CH_3), 7.2, 7.5 (2m, 4H, ArH).

*5.1.15. m-Methoxybenzenesulfonyl chloride (**15b**)*

To a cooled (−10 °C) and stirred solution of *m*-methoxyaniline in concentrated (37%) HCl (60 mL) and glacial acetic acid (18 mL) was added dropwise a solution of NaN_3 (12 g, 174 mmol) in 20 mL H_2O, in a rate to keep the temperature below 5 °C. After the addition, the mixture was allowed to stir for an additional 45 min at −10 °C to form the diazonium salt.

In a 1 L round bottom flask containing 250 mL glacial AcOH, SO_2 gas was bubbled through a diffusion apparatus until saturation of the solution. Then 4 g of $CuCl_2$ was added to the colourless solution, which became greenish–yellow. SO_2 gas was allowed to bubble for an additional 30–45 min until the solution became blue–green showing that the $CuCl_2$ was dissolved. The mixture was chilled in an ice bath at +10 °C and the diazonium salt previously formed was added portionwise in a rate to keep the temperature below 30 °C, and then stirred for 1 h at r.t. Then 300 mL water and 200 mL ether were added. The aqueous phase was extracted with ether until the organic layer became colourless. The combined organic layers were washed with saturated aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated in vacuo. The crude product (26 g) was purified twice by high vacuum distillation to afford 6 g (18%) of pure **15b** as a

yellow oil: $E_{0.1} = 116$ °C; IR ν $1600_{ArC=C}$ cm^{-1}; ^{1}H-NMR δ 3.8 (s, 3H, CH_3), 7.0, 7.5 (2m, 4H, ArH).

*5.1.16. 3,4,5-Trimethoxybenzenesulfonyl chloride (**15c**)*

3,4,5-Trimethoxybenzenesulfonyl chloride (**15c**) was prepared as described above and purified by column chromatography eluted with $CHCl_3$ (yield 24%): IR ν $1590_{ArC=C}$ cm^{-1}; ^{1}H-NMR δ 3.9 (s, 9H, CH_3), 7.4 (s, 2H, ArH).

*5.1.17. Methyl 4-acetamidobenzenesulfone (**16**)*

To a stirred and warmed (80 °C) solution of Na_2SO_3 (24 g, 187 mmol) and $NaHCO_3$ (16.5 g, 196 mmol) in 100 mL H_2O was added portionwise 4-acetamidobenzenesulfonyl chloride (23 g, 98 mmol). The mixture was stirred at 90 °C for 3 h, then filtered, and the filtrate concentrated under vacuum. The residue was recrystallised from water (50 mL) to yield 22 g (100%) of sodium 4-acetamidobenzenesulfinate.

To a stirred solution of sodium 4-acetamidobenzenesulfinate (22 g, 99.6 mmol), $NaHCO_3$ (16 g, 190 mmol) and dimethylsulfate (15 mL) in water (6 mL), were added dropwise 30 mL H_2O. The mixture was stirred and refluxed for 20 h. After cooling, water (30 mL) was added and the insoluble material filtered and dried to give **16** (8.5 g, 40%) as white crystals: m.p. 182–183 °C; IR ν 3351_{NH}, 1686_{NCO}, $1588_{ArC=C}$ cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.10 (s, 3H, $NCOCH_3$), 3.10 (s, 3H, SO_2CH_3), 7.85 (s, 4H, ArH), 10.4 (br s, 1H, D_2O exchange, NH).

*5.1.18. Methyl 4-aminobenzenesulfone (**17**)*

A mixture of **16** (8.5 g, 40 mmol) and KOH (2.3 g, 40 mmol) in absolute EtOH (100 mL) was refluxed for 3 h. The reaction was monitored by TLC. After the starting material had disappeared, the solvent was removed under vacuum. The residue was triturated with water and filtered to yield 5.85 g (75%) of **17**, which was used in the next step without purification.

*5.1.19. 4-Methylsulfinylbenzenesulfonyl chloride (**18**)*

4-Methylsulfinylbenzenesulfonyl chloride (**18**) was prepared as described for **15b** but using CH_2Cl_2 for the extraction. A white powder was obtained after removing of the solvent and was used (in excess) without purification for the synthesis of **24p**.

Representative examples of the reactions presented in *figure 4* are given below. 2-Alkylpyrazines **19a–g** and 2-alkylpiperazines **20a–g** were prepared as described by Tavet et al. [49].

*5.1.20. 1-p-Tolylsulfonyl-3-n-tridecylpiperazine (**21e**)*

To a stirred and cooled (−13 °C) solution of 2-*n*-tridecylpiperazine dihydrochloride salt **20e** (1 g, 3.8 mmol) and triethylamine (2 mL, 3 equiv.) in dry CH_2Cl_2 (50 mL) was added dropwise *p*-toluenesulfonyl chloride (0.86 g, 1 equiv.) in dry CH_2Cl_2 (50 mL). The resulting solution was stirred overnight at −13 °C. The mixture was allowed to warm to r.t., and was washed with a $NaHCO_3$ solution (1 g, 100 mL H_2O). The organic layer was washed with water, dried ($MgSO_4$) and the solvent was evaporated under vacuum. The crude product was chromatographed on a silica gel column eluted first with 1% MeOH in CH_2Cl_2 followed by 5% MeOH in $CHCl_3$. The fractions showing a single spot (TLC) were pooled and evaporated to dryness. The residue was then recrystallised from a mixture of ether–pentane (5:95, v/v) to give 1.3 g (82%) of **21e** as a white powder: IR ν 3340_{NH}, $1605_{C=C}$ cm^{-1}; ^{1}H-NMR δ 0.83 (t, 3H, CH_3), 1.2 (br s, 22H, $(CH_2)_{11}$), 1.6 (m, 2H, CH_2–piperazine), 2.38 (s, 3H, CH_3–Ar), 2–2.3, 2.9–3.15, 3.4–3.8 (3 m, 7H, piperazine H), 7.28, 7.58 (2d, 4H, ArH), 1.6 (s, 1H, D_2O exchange, NH).

A similar procedure starting from the corresponding 2-alkylpiperazines **20** gave compounds **21c**, **21d**, **21f**, **21g**. All showed the similar IR and ^{1}H-NMR spectra as for **21e**, except for the integration of the signal at 1.2 ppm.

*5.1.21. 1-Triphenylmethyl-3-n-tridecylpiperazine (**22e**)*

To a cooled (−13 °C) solution of 2-*n*-tridecylpiperazine dihydrochloride salt **22e** (5 g, 19 mmol) and triethylamine (8 mL, 57 mmol) in dry CH_2Cl_2 (100 mL) was added dropwise a solution of triphenylmethyl chloride (5.3 g, 19 mmol) in dry CH_2Cl_2 (100 mL). The mixture was stirred overnight at −13 °C, and then allowed to warm to r.t. $NaHCO_3$ (1 g) in 100 mL water was added, and the mixture was stirred for 10 min. The organic layer was decanted and washed with water until neutral pH. The aqueous phases were extracted with ether (2×100 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and subjected to column chromatography on silica gel using first CH_2Cl_2, then 5% MeOH in $CHCl_3$ as eluents. This afforded 9 g of a yellow oil which was triturated with cold C_6H_{14} to give 7 g (73%) of pure **22e** as white crystals: m.p. 81–82 °C; IR ν 3340_{NH}, $1596_{ArC=C}$ cm^{-1}; ^{1}H-NMR δ 0.81 (t, 3H, CH_3), 1.15 (br s, 24H, $(CH_2)_{12}$), 3, 1.4 (2m, 7H, piperazine H), 1.3 (s, 1H, D_2O exchange, NH), 7.4, 7.15 (2m, 15H, ArH).

*5.1.22. General procedure for the synthesis of 1-(arylsulfonyl)-2-n-alkypiperazines (**24**)*

The following procedure was used to prepare the 1-(arylsulfonyl)-2-*n*-alkyl piperazines, except for compounds **24t**, **24w**, **24x** of which synthesis is presented in details.

To a solution of **22** (4 mmol) and triethylamine (4 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise a solution of 4 mmol of the corresponding benzenesulfonyl chloride in dry CH_2Cl_2 (50 mL). The mixture was stirred for 3 h at r.t. and treated as described for compound **21e**. The crude product was dissolved in C_2H_4O (30 mL) and added to a solution of 30 mL C_2H_4O and 2 mL concentrated HCl (37%). The mixture was stirred several minutes, then left 3 h without stirring at r.t. The solvent was removed under vacuum and the residue was dissolved in CH_2Cl_2, washed with aq. saturated $NaHCO_3$ (100 mL) and water (3×100 mL). The aqueous layers were extracted with ether. The combined organic layers were dried over $MgSO_4$, filtered and the solvents removed in vacuo. Chromatography over silica gel eluted with CH_2Cl_2, then 5% MeOH in CH_2Cl_2 afforded the corresponding 1-(arylsulfonyl)-2-*n*-alkylpiperazine (**24a**–**s**, **24u**, **24v**, **24y**–**z**, see *tables I* and *III* for details).

*5.1.23. 1-(p-Aminobenzenesulfonyl)-2-n-tridecyl-4-triphenylmethylpiperazine (**23t**)*

A mixture of **23v** (19 g, 26.8 mmol) and KOH (26.8 mmol) in absolute EtOH (180 mL) was heated under reflux for 48 h. The reaction was monitored by TLC. After the starting material had disappeared, the solvent was removed under vacuum. The crude product was dissolved in CH_2Cl_2 (100 mL) and washed with water. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure affording 17.8 g (100%) of **23t**: m.p. 128.7 °C; IR ν 3380_{NH_2}, $1592_{ArC=C}$ cm^{-1}; ^{1}H-NMR δ 0.81 (t, 3H, CH_3), 1.1 (br s, 22H, $(CH_2)_{11}$), 1.6 (m, 2H, CH_2–piperazine), 2.4–2.8, 2.9–3.15, 3.4–3.75 (3m, 7H, piperazine H), 4 (s, 2H, D_2O exchange, NH_2), 6.6, 7.3 (2m, 19H, ArH).

*5.1.24. 1-(p-Aminobenzenesulfonyl)-2-n-tridecylpiperazine (**24t**)*

Compond **23t** (2 g, 3 mmol) was dissolved in C_2H_4O (30 mL) and added to 30 mL C_2H_4O containing 2 mL concentrated (37%) HCl. The mixture was stirred for several min then left 3 h at r.t. Solvent was removed in vacuo, the residue dissolved in CH_2Cl_2, and washed with aq. saturated $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$) and concentrated to give 1.4 g of crude product. Silica gel column chromatography starting with 3% MeOH in $CHCl_3$ then with 10% MeOH in $CHCl_3$ as eluents gave 1.1 g of the desired product as a thick oil. The oil was triturated with pentane to give 1 g (83%) of **24t**: m.p. 85.1 °C (see *tables I* and *III* for details).

*5.1.25. 1-(p-Ethylcarbamoylbenzenesulfonyl)-2-n-tridecylpiperazine (**24w**)*

To a solution of **23t** (4 g, 6 mmol) and triethylamine (0.83 mL, 6 mmol) in dry toluene (100 mL) was added dropwise at r.t. a solution of ethylchloroformate (1.04 g, 9 mmol) in dry toluene (100 mL). The mixture was stirred for 3 h at 80 °C. The toluene was removed under reduced pressure; the residue was taken up in CH_2Cl_2 (100 mL) and washed with $NaHCO_3$ (2.5 g, 100 mL H_2O). The organic layer was washed with H_2O, dried over $MgSO_4$, filtered and evaporated to yield the crude 1-(*p*-ethylcarbamoylbenzenesulfonyl)-2-*n*-tridecyl-4-triphenylmethyl piperazine **23w** which was then detritylated according to the procedure used for **23t**. Compound **24w** (400 mg, 13.5%) was obtained after purification by column chromatography on silica gel eluted first with $CHCl_3$, then with 2% MeOH in $CHCl_3$ and recrystallisation from ether–pentane (5:95, v/v): m.p. 103 °C (see *tables I* and *III* for details).

*5.1.26. 1-(p-N-Benzoylaminobenzenesulfonyl)-2-n-tridecylpiperazine (**24x**)*

To a solution of **23t** (4 g, 6 mmol) and triethylamine (0.83 mL, 6 mmol) in dry CH_2Cl_2 (100 mL) was added dropwise at r.t. a solution of benzoylchloride (0.84 g, 6 mmol) in dry CH_2Cl_2 (100 mL). The mixture was stirred for 3 h at r.t., then the reaction was quenched with 2 g $NaHCO_3$ in 100 mL of H_2O. The organic layer was washed with H_2O, dried over $MgSO_4$, filtered and evaporated to yield the crude 1-(*p*-*N*-benzoylaminobenzenesulfonyl)-2-*n*-tridecyl-4-triphenylmethyl piperazine **23x**, which was detritylated in the same manner as for **23t**. Column chromatography on silica gel (CH_2Cl_2, then 2% MeOH in $CHCl_3$) afforded 3 g of an oily product which was triturated with CH_2Cl_2–C_6H_{14} (5:95, v/v) to give 2.7 g (85.4%) of **24x** as white crystals: m.p. 137.5 °C (see *tables I* and *III* for details).

*5.1.27. 1-p-Tolylsulfonyl-2-n-octyl-4-methylpiperazine hydrochloride (**25**)*

A solution of **22c** (1 g, 2.9 mmol) in 5 mL of absolute MeOH, formaldehyde (0.3 mL, 8.15 mmol), and formic

acid (0.3 mL, 8 mmol) was refluxed for 20 h. The MeOH was evaporated under vacuo and the residue was diluted with ether (100 mL), washed with saturated $NaHCO_3$ (100 mL) and H_2O. The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The free amine was dissolved in anhydrous EtOH (20 mL) and HCl gas was bubbled through the solution until saturation. EtOH was evaporated under vacuo and the residue crystallised from ether to afford **25** as the hydrochloride salt (1 g, 88%): m.p. 170.6 °C (see *tables I* and *III* for details).

The above procedure was used to prepare 1-*p*-chlorobenzenesulfonyl-2-*n*-tridecyl-4-methylpiperazine-(**26**) (90%) crystallised from ether as the hydrochloride salt: m.p. 150.9 °C (see *tables I* and *III* for details).

*5.1.28. (8′Z)-2-(8′-Tridecen-1′-yl)-pyrazine (**19r′**)*

Using the procedure described by Tavet et al. [49], **6** was condensed with 2-methylpyrazine, affording after purification by high vacuum distillation 16 g (76%) of **19r′** as a yellowish oil: $E_{0.05} = 127$ °C; ^{1}H-NMR δ 0.83 (t, 3H, CH_3), 1.35 (m, 14H, CH_2), 1.91 (m, 4H, CH_2–C=C), 2.7 (t, 2H, CH_2–pyrazine), 5.3 (t, 2H, CH=CH), 8.4 (m, 3H, Ar H).

*5.1.29. (8′E)-2-(8′-Tridecen-1′-yl)-pyrazine (**19r″**)*

Compound **19r″** was obtained in 76% using the same process as for **19r′**: $E_{0.05} = 120$–130 °C; ^{1}H-NMR δ 5.35–5.15 (m, 2H, CH=CH).

*5.1.30. (8′Z)-2-(8′-Tridecen-1′-yl)-piperazine (**20r′**)*

This compound was prepared according to the method described in Tavet et al. [49]. The crude product was purified by crystallisation of the diacetate salt from C_2H_4O–EtOH. Usual treatment of this salt afforded pure **20r′** (76%): ^{1}H-NMR (CD_3OD) δ 0.83 (t, 3H, CH_3), 1.30 (m, 16H, CH_2), 1.9 (m, 4H, CH_2–C=C), 2.1–2.4, 2.5–3.1, 3.8–4 (3 m, 7H, piperazine H), 5.35 (t, 2H, CH=CH).

*5.1.31. (8′E)-2-(8′-Tridecen-1′-yl)-piperazine (**20r″**)*

Compound **20r″** was obtained in 77% yield using the same process as for **20r′**.

*5.1.32. (8′Z)-1-(p-Methoxybenzenesulfonyl)-2-(8′-tridecen-1′-yl)-piperazine (**24r′**)*

This compound was prepared following the procedure described for **22e** and the general procedure for **24**. Chromatography on silica gel (CH_2Cl_2–MeOH, 99:1, v/v) afforded **24r′** as a yellowish oil.

*5.1.33. (8′E)-1-(p-Methoxybenzenesulfonyl)-2-(8′-tridecen-1′-yl)-piperazine (**24r″**)*

Compound **9** was converted to **24r″** via the same processes as described above for **24r′**. 1-*p*-methoxybenzenesulfonyl-2-*n*-tetradecyloxycarbonylpiperazine (**30**), 1-*p*-methoxybenzenesulfonyl-2-*n*-tetradecyloxymethylpiperazine (**34**) and 1-*p*-methoxybenzenesulfonyl-2-*n*-tetradecanoyloxymethylpiperazine (**38**), were synthesised from the corresponding substituted piperazines **28**, **32** and **35** prepared according to published procedure [39] adapted to sulfamide formation (see *tables II* and *IV* for details).

5.1.34. Resolution of 1,4-dibenzyl-2-piperazinemethanol via crystallisation of the menthyl ester [50]

*5.1.34.1. (R)-1,4-Dibenzyl-2-hydroxymethylpiperazine **31 (R)***

To a cooled (0 °C) suspension of $LiAlH_4$ (1.1 g, 29 mmol) in 30 mL dry ether was added dropwise a solution of **39** (13 g, 29 mmol) in 100 mL dry ether. The mixture was allowed to stir at 0 °C for 24 h. The excess hydride was quenched by the dropwise addition of 5 M NaOH. The mixture was filtered and the solvent evaporated. The residue was taken up in ether (150 mL) and washed with 2 N aq. HCl (150 mL). The aqueous layer was neutralised with Na_2CO_3, extracted with ether and dried over Na_2SO_4. Ether was removed under vacuo and the residue was triturated with C_6H_{14} affording 7.8 g (91%) of **31** (**R**) as white crystals: m.p. 71.8 °C; $[\alpha]_{589}$ + 31.6° ($c = 2.6$, $CHCl_3$).

*5.1.35. (S)-1,4-Dibenzyl-2-hydroxymethylpiperazine **31 (S)***

This compound was prepared according to the above procedure from **40**, to give **31** (**S**) in an 88% yield: m.p. 73 °C; $[\alpha]_{589}$ − 30.8° ($c = 2.44$, $CHCl_3$).

Compounds **34** (**R**) and **34** (**S**) were synthesised according to published procedure [50]. **34** (**R**): m.p. 75.3 °C; $[\alpha]_{589}$ + 33.3° ($c = 2.6$, $CHCl_3$) and **34** (**S**): m.p. 74.8 °C; $[\alpha]_{589}$ − 33.4° ($c = 2.6$, $CHCl_3$).

5.2. Biology

5.2.1. Materials

Bovine pancreatic PLA_2 and fatty-acid free bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Rabbit platelets were prepared as described by Mounier et al. [56]. The fluorescent substrate for PLA_2 assay, 1-palmitoyl-2-(10-pyrenylde-

canoyl)-*sn*-glycero-3-monomethyl phosphatidic acid (10-pyrene PA), was obtained from Interchim (Montluçon, France).

5.2.2. PLA_2 assay

PLA_2 activity was evaluated by the method of Radvanyi et al. [57] using the fluorescent phospholipid analogue 10-pyrene PA as the substrate. Bovine pancreatic PLA_2 or PLA_2 from rabbit platelet lysate were used to test the potency of various inhibitors. We have shown the specificity of this assay for detecting secretory PLA_2, since the cytosolic PLA_2 was not active on substrates with a pyrene group at the *sn*-2 position [58]. In a total volume of 1 mL, the standard reaction medium contained: 50 mM Tris–HCl (pH 7.5), 100 mM NaCl, 1 mM EGTA, 2 μM substrate, fatty-acid free BSA solution in water (15 μM or 0.1%) and 5 nM of pancreatic PLA_2 or 10 μL of platelet lysate (0.4 μg of proteins). The fluorescence ($\lambda_{ex} = 342$ nm and $\lambda_{em} = 388$ nm) of the enzymatic reaction medium (blank) was recorded for 1 min with a spectrofluorimeter SFM 25 (Kontron Instruments) equipped with a Xenon lamp. The reaction was then initiated by addition of $CaCl_2$ (10 mM, final concentration). The increase in fluorescence was continuously recorded for 2 min and PLA_2 activity was calculated as described by Radvanyi et al. [57]. When used, the inhibitor was added to the reaction medium after introduction of BSA. The activity was expressed in micromoles of fluorescent 10-pyrene PA hydrolyzed per min and per mg of bovine pancreatic PLA_2, or in nmole of fluorescent 10-pyrene PA hydrolyzed per min per 10^9 cells, in the case of rabbit platelet lysate. The standard error of the mean of three independent experiments was less than 10%. This allowed the determination of the IC_{50} values (concentration of inhibitors producing 50% inhibition) of each compound.

References

[1] Lambeau G., Lazdunski M., Trends Pharmacol. Sci. 20 (1999) 162–170.

[2] Dennis E.A., J. Biol. Chem. 269 (1994) 13057–13060.

[3] Heinrikson R.L., Krueger E.T., Keim P.S., J. Biol. Chem. 252 (1977) 4913–4921.

[4] Waite M., The Phospholipases in Handbook of Lipid Research, Plenum Press, New York, 1987, pp. 1–332.

[5] Valentin E., Koduri R.S., Scimeca J.-C., Carle G., Gelb M.H., Lazdunski M., Lambeau G., J. Biol. Chem. 274 (1999) 19152–19160.

[6] Valentin E., Ghomashchi F., Gelb M.H., Lazdunski M., Lambeau G., J. Biol. Chem. 274 (1999) 31195–31202.

[7] Ishizaki J., Suzuki N., Higashino K., Yokota Y., Ono T., Kawamoto K., Fujii N., Arita H., Hanasaki K., J. Biol. Chem. 274 (1999) 24973–24979.

[8] Valentin E., Lambeau G., Biochim. Biophys. Acta 1488 (2000) 59–70.

[9] Suzuki N., Ishizaki J., Yokota Y., Higashino K., Ono T., Ikeda M., Fujii N., Arita H., Hanasaki K., J. Biol. Chem. 275 (2000) 5785–5793.

[10] Valentin E., Ghomashchi F., Gelb M.H., Lazdunski M., Lambeau G., J. Biol. Chem. 275 (2000) 7492–7496.

[11] Gelb M.H., Valentin E., Ghomashchi F., Lazdunski M., Lambeau G., J. Biol. Chem. 275 (2000) 39823–39826.

[12] Clark J.D., Milona N., Knopf J.L., Proc. Natl. Acad. Sci. USA 87 (1990) 7708–7712.

[13] Clark J.D., Lin L.L., Kriz R.W., Ramesha C.S., Sultzman L.A., Lin A.Y., Milona N., Knopf J.L., Cell 65 (1991) 1043–1051.

[14] Van Den Bosch H., Aarsman A.J., Verkleij H.M., New Trends, Lipid Mediators Res, Karger Editions, Basel, 1989, pp. 257–261.

[15] Rosenthal M.D., Gordon M.N., Buescher E.S., Slusser J.H., Harris L.K., Franson R.C., Biochem. Biophys. Res. Commun. 208 (1995) 650–656.

[16] Aarsman A.J., De Jong J.G.N., Arnoldussen E., Neys F.W., Van Wassenaar P.D., Van Den Bosch H., J. Biol. Chem. 264 (1989) 10008–10014.

[17] Minami T., Tojo H., Shinomura Y., Matsusawa Y., Okamoto M., Biochim. Biophys. Acta 1170 (1993) 125–130.

[18] Bomalaski J.S., Clark M.A., Arthritis Rheum. 36 (1993) 190–198.

[19] Forster S., Ilderton E., Norris J.F.B., Summerly R., Yardley H.J., Br. J. Dermatol. 112 (1985) 135–147.

[20] Minami T., Tojo H., Shinomura Y., Matsuzawa Y., Okamoto M., Gut 35 (1994) 1593–1598.

[21] Nevalainen T.J., Grönroos J.M., Kallajoki M., Lab. Invest. 72 (1995) 201–208.

[22] Vadas P., Pruzanski W., Trends Shock Res. Circ. Shock 39 (1993) 160.

[23] Beaton H.G., Bennion C., Connoly S., Cook A.R., Gensmantel N.P., Hallam C., Hardy K., Hitchin B., Jackson C.G., Robinson D.H., J. Med. Chem. 37 (1994) 557–560.

[24] Burke J.R., Gregor K.R., Tramposch K.M., J. Biol. Chem. 270 (1995) 274–280.

[25] LeMahieu R.A., Carson M., Han R.J., Madison V.S., Hope W.C., Chen T., Morgan D.W., Hendrickson H.S., J. Med. Chem. 36 (1993) 3029–3031.

[26] Oinuma H., Takamura T., Hasegawa T., Nomoto K.I., Naitoh T., Daiku Y., Hamano S., Kakisawa H., Minami N., J. Med. Chem. 34 (1991) 2260–2267.

[27] Schewitz R.W., Bach N.J., Carlson D.G., Chirgadze N.Y., Clawson D.K., Dillard R.D., Draheim S.E., Hartley L.W., Jones N.D., Mihelic E.D., Olkowski J.L., Snyder D.W., Sommers C., Wery J., Nat. Struct. Biol. 2 (1995) 458–465.

[28] Dillard R.D., Bach N.J., Draheim S.E., Berry D.R., Carlson D.G., Chirgadze N.Y., Clawson D.K., Hartley L.W., Johnson L.M., Jones N.D., McKinney E.R., Mihelich E.D., Olkowski J.L., Schewitz R.W., Smith A.C., Snyder D.W., Sommers C.D., Wery J.P., J. Med. Chem. 39 (1996) 5137–5158.

[29] Dillard R.D., Bach N.J., Draheim S.E., Berry D.R., Carlson D.G., Chirgadze N.Y., Clawson D.K., Hartley L.W., Johnson L.M., Jones N.D., McKinney E.R., Mihelich E.D., Olkowski J.L., Schewitz R.W., Smith A.C., Snyder D.W., Sommers C.D., Wery J.P., J. Med. Chem. 39 (1996) 5119–5136.

[30] Draheim S.E., Bach N.J., Dillard R.D., Berry D.R., Carlson D.G., Chirgadze N.Y., Clawson D.K., Hartley L.W., Johnson L.M., Jones N.D., McKinney E.R., Mihelich E.D., Olkowski J.L., Schewitz R.W., Smith A.C., Snyder D.W., Sommers C.D., Wery J.P., J. Med. Chem. 39 (1996) 5159–5175.

[31] Thunnissen M.M.G.M., Eiso A.B., Kalk K.H., Drenth J., Dijkstra B.W., Kuipers O.P., Dijkman R., De Haas G.H., Verheij H.M., Nature 347 (1990) 689–691.

[32] Slotboom A.J., Verheij H.M., De Haas G.H., in: Phospholipids, Ansell G.B. (Ed.), Amsterdam, 1982, pp. 359–434.

[33] Scott D.L., White S.P., Browning J.L., Rosa J.J., Gelb M.H., Sigler P.B., Science 254 (1991) 1007–1010.

[34] Wery J.P., Schewitz R.W., Clawson D.K., Bobbitt J.L., Dow E.R., Gamboa G., Goodson J.T., Hermann R.B., Kramer R.M., McClure D.B., Mihelic E.D., Putnam J.E., Sharp J.D., Stark D.H., Teater C., Warrick M.W., Jones N.D., Nature 352 (1991) 79–82.

[35] Scott D.L., White S.P., Otwinowski Z., Yuan W., Gelb M.H., Sigler P.B., Science 250 (1990) 1541–1546.

[36] Pisabarro T., Ortiz A.R., Palomer A., Cabré F., Garcia L., Wade R.C., Gago F., Mauleon D., Carganico G., J. Med. Chem. 37 (1994) 337–341.

[37] Yu L., Dennis E.A., J. Am. Chem. Soc. 114 (1992) 8757–8763.

[38] Van den Berg L., Verheij H.M., Dijkman R., De Haas G.H., Biochim. Biophys. Acta 1124 (1992) 66–70.

[39] Binisti C., Touboul E., Mounier C., Heymans F., Bon C., Godfroid J.J., J. Lipid Mediat. Cell Signal. 15 (1996) 125–144.

[40] Binisti C., Mounier C., Touboul E., Heymans F., Bon C., Godfroid J.J., J. Lipid Mediat. Cell Signal. 16 (1997) 171–187.

[41] Lindlar H., Helv. Chim. Acta 35 (1952) 446.

[42] Magoon E.F., Slaugh L.H., Tetrahedron 23 (1967) 4509–4515.

[43] Evans D.A., Nelson J.V., J. Am. Chem. Soc. 102 (1980) 774–782.

[44] Henne A.L., Greenly K.W., J. Am. Chem. Soc. 65 (1943) 2020–2021.

[45] Kazuhiko A., Tsuyoshi K., Satoru U., Akihiko M., Yoshifumi I., Fumihiro H., Yoshito Y., Eiji I., Takashi Y., J. Med. Chem. 36 (1993) 1356–1363.

[46] Bourgeois P., Duffaut N., Bull. Soc. Chim. II 3–4 (1980) 195–199.

[47] Voronkov M.G., Roman V.K., Maletina E.A., Synthesis (1982) 277–280.

[48] Meerwein H., Dittmar G., Gollner R., Hafner K., Mensch F., Steinfort O., Chem. Ber. 90 (1957) 841–852.

[49] Tavet F., Lamouri A., Heymans F., Dive G., Touboul E., Blavet N., Godfroid J.J., J. Lipid Mediat. Cell Signal. 15 (1996) 145–159.

[50] Aebischer B., Frey P., Haerter H.P., Herrling P.L., Mueller W., Olverman H.J., Watkins J.C., Helv. Chim. Acta 72 (1989) 1043–1051.

[51] Rekker R.F., Eur. J. Med. Chem. Chim. Ther. 14 (1979) 479–488.

[52] Hansch C., Leo A., Unger S.H., Kim K.H., Nikaitani D., Lien, Eur. J. Med. Chem. 16 (1973) 1207–1216.

[53] Hansch C., Unger S.H., J. Med. Chem. 16 (1973) 1218–1222.

[54] Kubinyi H., Arzheim-Forsch. Drug Res. 26 (1976) 1991–1997.

[55] Kubinyi H., J. Med. Chem. 20 (1977) 625–629.

[56] Mounier C., Hatmi M., Faili A., Bon C., Vargaftig B.B., J. Pharm. Exp. Ther. 264 (1993) 1460–1467.

[57] Radvanyi F., Jordan L., Russo-Marie F., Bon C., Anal. Biochem. 117 (1989) 103–109.

[58] Mounier C., Faili H., Vargaftig B.B., Bon C., Hatmi M., Eur. J. Biochem. 216 (1993) 169–175.