Bioorganic & Medicinal Chemistry 18 (2010) 3588–3600

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design of new potent and selective secretory phospholipase A_2 inhibitors. 6-Synthesis, structure–activity relationships and molecular modelling of 1-substituted-4-[4,5-dihydro-1,2,4-(4*H*)-oxadiazol-5-one-3-yl(methyl)]-functionalized aryl piperazin/one/dione derivatives

Nadia Meddad-Belhabich [a], Darina Aoun [a], Atimé Djimdé [a], Catherine Redeuilh [a], Georges Dive [b], France Massicot [c,†], François Chau [a], Françoise Heymans [a], Aazdine Lamouri [a,*]

[a] Laboratoire ITODYS, équipe Pharmacochimie Moléculaire, Université Paris Diderot, 15 rue de Baïf, 75205 Paris Cedex 13, France
[b] Centre d'Ingénierie des Protéines, Université de Liège, 13, Allée du 6 Août, Sart Tilman, 4000 Liège, Belgium
[c] Université Paris Descartes, UFR Pharmacie 4, avenue de l'Observatoire, 75006 Paris, France

ARTICLE INFO

Article history:
Received 5 November 2009
Accepted 20 March 2010
Available online 27 March 2010

Keywords:
Phospholipase A_2 inhibitors
Structure–activity relationships
Piperazin/one/dione

ABSTRACT

The group IIA human non-pancreatic secretory phospholipase A_2 (hnp-sPLA$_2$) is one of the enzymes implied in the inflammatory process. In the course of our work on inhibitors of this enzyme we investigated the influence of rigidity of the piperazine region on the biological activity. Several modifications were explored. Various linkers, such as amide, urea, carbamate, or alkoxyphenyl were inserted between the piperazine and the lipophilic chain. Also, modification of the piperazine core to incorporate carbonyl groups was studied. In an in vitro fluorimetric assay using the human GIIA (HPLA$_2$) and porcine pancreatic GIB enzymes, compound **60a** (Y = phenoxy, R = $C_{18}H_{37}$, Z = CH_2) had the optimal activity with an IC_{50} = 30 nM on HPLA$_2$. By means of molecular modelling we attempted to get informations towards comprehension of differences in activity.

1. Introduction

Phospholipases A_2 (PLA$_2$) family members are esterases that hydrolyze glycerophospholipids at the *sn*-2 position. The reaction products are lysophospholipids and free fatty acids.[1] Among them, arachidonic acid (AA) is metabolized by enzymes such as cyclo-oxygenases (Cox-1 and Cox-2) and lipo-oxygenases and lead to the formation of prostaglandins, prostacyclin, thromboxans and leukotrienes, with important roles in numerous physiological and pathophysiological processes. Moreover, lyso Platelet-Activating Factor (lyso-PAF) is acetylated by the PAF–acetyltransferase to yield PAF, another well known pro-inflammatory mediator. The superfamily of PLA$_2$ comprises a number of very different proteins that can be divided into five principal kinds of enzymes, the sPLA$_2$s, the cPLA$_2$s, the Ca^{2+}-independent PLA$_2$s (iPLA$_2$s), and the PAF acetylhydrolases (PAF–AH), and the lysosomal PLA$_2$s. Assignment of the enzymes to a certain group is based on the catalytic mechanism (His/Asp, Ser/Asp or Ser/ His/Asp hydrolase) as well as functional and structural features.[2]

The sPLA$_2$s are small secreted proteins (14–18 kDa) usually containing 5–8 disulfide bonds. This group of enzymes requires μM levels of Ca^{2+} for catalysis. Generally sPLA$_2$s show a high activity with anionic phospholipid (PL) but only the GV and GX PLA$_2$s also hydrolyze phosphatidylcholine (PC) vesicles.[3]

The sPLA$_2$s are found in plants,[4] insects,[5,6] molluscs,[7] reptiles[8] and mammals.[9] sPLA$_2$-IB is found in large amounts in the pancreas and its principal function is the digestion of dietary lipids.[10] This enzyme was identified and cloned in other tissues such as lung, spleen, kidney and ovary,[11,12] and it has been proposed to be involved in various physiological and pathophysiological responses such as cell proliferation,[13] cell contraction,[14,15] lipid mediators release,[16] acute lung injury[17] and endotoxic shock.[18]

The sPLA$_2$s appear to play a role in several inflammatory diseases. GIIA PLA$_2$ is present at high concentrations in synovial fluid from patients suffering from rheumatoid arthritis.[19] sPLA$_2$s are involved in adult respiratory stress syndrome (ARDS), inflammatory bowel disease, pancreatitis, sepsis[20] and atherosclerosis.[21–23] Mammalian sPLA$_2$s GIIA, GV, and GIID promote degranulation of mast cells,[24] possess potent anticoagulant activity,[25,26] bactericidal properties and particularly the human GIIA, GV, GX and GXII and the murine GIIA, GIID, GIIE and GV.[27,28] It is the GIIA that has physiologically significant bactericidal activity.[28]

* Corresponding author. Tel.: +33 (0)1 57 27 88 78; fax: +33 (0)1 57 27 72 63.
E-mail address: aazdine.lamouri@univ-paris-diderot.fr (A. Lamouri).
† Present address.

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.03.049

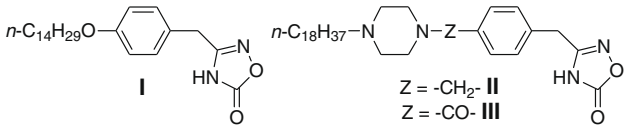


Figure 1.

The function of GIIA PLA_2 in tumorigenesis is a controversial issue. It is not clear if this enzyme serves as a tumour suppressor or tumour promotor.[29]

In a previous paper, starting from our lead compound **I**[30,31] (Fig. 1), we designed and synthesized piperazinic derivatives **II** and **III**[32] which were specific group II versus group I $sPLA_2$ inhibitors. 1-octadecyl-4-[4′-(4,5-dihydro-(4*H*)-1,2,4-oxadiazol-5-one-3-ylmethyl) benzyl] piperazine (**II**) and 1-octadecyl-4-[4′-(4,5-dihydro-(4*H*)-1,2,4-oxadiazol-5-one-3-yl methyl)benzoyl]piperazine (**III**) exhibited a micromolar range IC_{50} towards group IIA PLA_2, while inactive at 100 μM, the highest concentration used, on group I PLA_2 in an in vitro enzymatic assay. In addition, as compound **I** was active in the carrageenan-induced oedema model when ip administrated, but inactive per os because of its high lipophilicity (log *P* = 7), compound **III** was active either by ip or per os routes, probably for its ability to get protonated at physiological pH, leading to a better bioavailability.

In this last series, the activity appeared to be enhanced by rigidifying the piperazinic ring either by introduction of the carbonyl or branching directly the phenyl to the nitrogen as compared to a methylene linker between piperazine and phenyle.[32] Here we report a SAR study by examining the influence of structural modifications of **II** and **III** on inhibiting activity and selectivity towards GIIA versus GIB $sPLA_2$s. They included replacing either one nitrogen or both by different functions as amide, carbamate, urea and/or branching directly an alkyloxyphenyl group, keeping a long C_{10}–C_{18} alkyl chain. We also diminished flexibility of piperazine ring by branching one (or two) methyl group(s) on one (or two) carbon atom(s) of this cycle or replacing one (or two) methylene(s) by one (or two) carbonyl(s) leading to substituted piperazinones and piperazinediones. All of these derivatives were evaluated in our enzymatic assay for their inhibitory potency towards GIB and GIIA $sPLA_2$s. This SAR was completed by a molecular modelling study to explain the observed results.

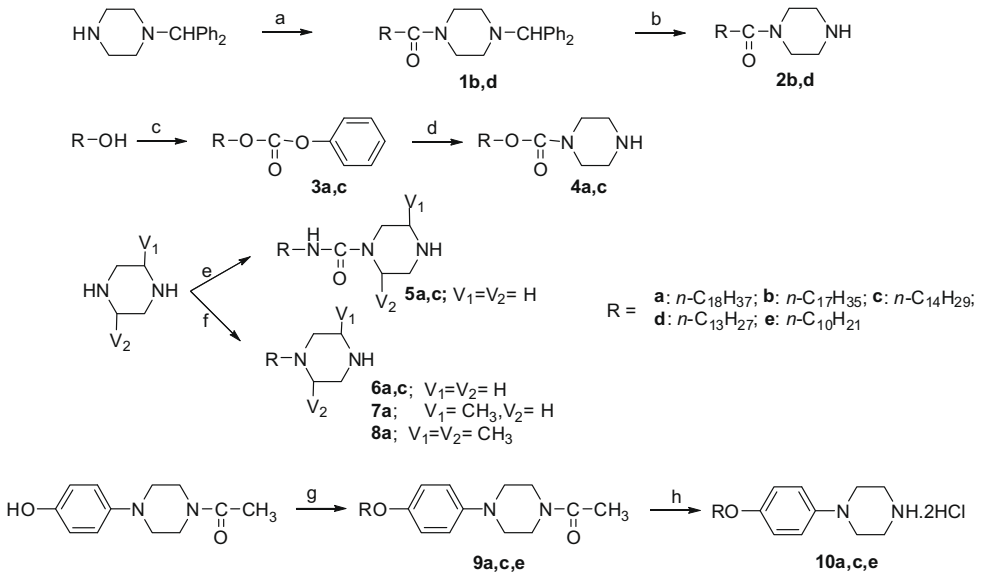
2. Chemistry

To ovoid the N,N′-disubstitution, N-monosubstituted piperazines were prepared as depicted in Scheme 1 either in two steps starting from diphenylmethylpiperazine[33] or by using an excess of piperazine.[32] Diphenylmethylpiperazine was treated with the appropriate acid chloride to give the 4-acyl-1-diphenylmethylpiperazines **1b**,**d** which were deprotected by hydrogenolysis into the 1-acylpiperazines **2b**,**d**. The carbamates **4a**,**c** were obtained after preparing first the carbonates **3a**,**c** by esterification of the corresponding primary alcohol by phenyl chloroformate followed by treatment with an excess of piperazine. In the same manner, introduction of the urea function was obtained by addition of an excess of piperazine to the chosen alkylisocyanate to give **5a**,**c**. As for alkylpiperazines **6a**,**c**, **7a** and **8a**, they were prepared as described by Boukli et al.[32] starting from the corresponding 1-alkylbromide and either piperazine or mono or dimethyl piperazines.

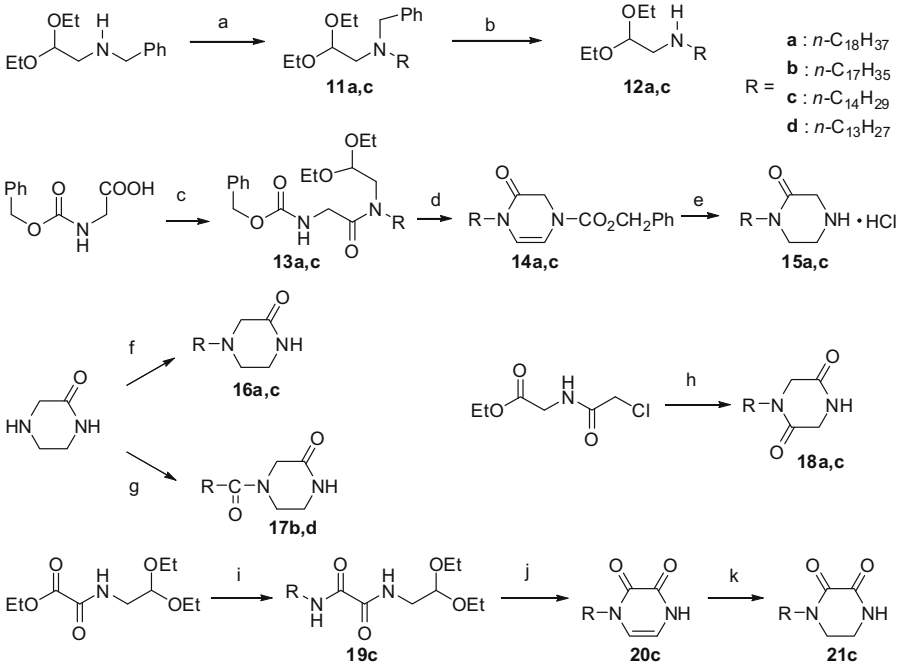
The introduction of an alkyloxyphenyl group was achieved as outlined in Scheme 1 by O-alkylation of the commercially available 1-acetyl-4-(4-hydroxyphenyl)piperazine. This one was condensed on the corresponding alkylbromide by means of its sodium salt obtained with sodium hydride, leading to **9a**,**c**,**e**. De-acetylation was performed using hydrogen chloride and afforded *N*-alkyloxyphenylpiperazines **10a**,**c**,**e** as hydrochlorides.

The preparation of N-monosubstituted piperazinones (**15a**,**c**, **16a**,**c**, **17b**,**d**) and piperazinediones (**18a**,**c**, **21c**) was performed following synthetic strategies depicted in Scheme 2. *N*-(2,2-Diethoxyethyl)benzylamine was alkylated with the appropriate alkyl bromide leading to *N*-alkyl-*N*-(2,2-diethoxyethyl)benzylamines **11a**,**c**, followed by hydrogenolysis in the presence of catalytic palladium. Using reported procedures[34,35] with managed modifications, condensation of these *N*-alkyl-2,2-diethoxyethylamines **12a**,**c** with benzyloxycarbonylaminoacetic acid, in the presence of DCC and HOBt, allowed to obtain the corresponding carbamic acid benzyl esters **13a**,**c** which were cyclized into 1,4-disubstituted 3,4-dihydro-2*H*-pyrazin-2-ones **14a**,**c** in the presence of *p*-TsOH. Subsequent catalytic hydrogenolysis in acidic conditions led to 1-alkylpiperazin-2-ones **15a**,**c** as hydrochlorides.

Commercially available piperazin-2-one was treated with alkyl bromide[36] and acyl chloride to give 4-alkylpiperazin-2-ones **16a**,**c** and 4-acylpiperazin-2-ones **17b**,**d**, respectively.



Scheme 1. [a]Reagents and conditions: (a) RCOCl, Et_3N, dry toluene; (b) H_2/Pd/C, EtOH, 50 °C; (c) PhOCOCl, Et_3N, THF, 0 °C; (d) piperazine, CH_2Cl_2; (e) RNCO, CH_2Cl_2; (f) R-Br, THF/CH_2Cl_2; (g) R-X, NaH, DMF; (h) 12 N HCl, EtOH, reflux.

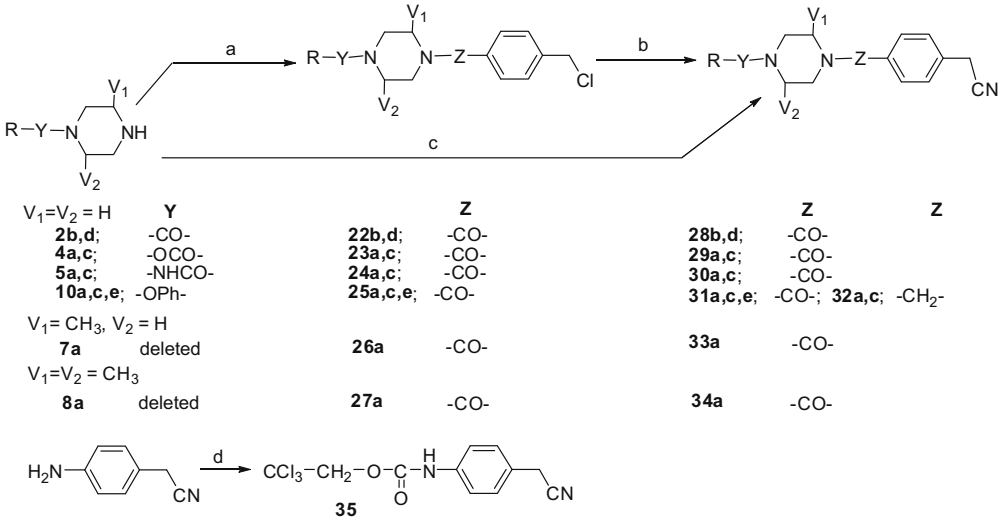


Scheme 2. [a]Reagents and conditions: (a) R-Br, K_2CO_3, Kl, CH_3CN, reflux; (b) H_2/Pd/C, EtOH, 40 °C; (c) **12a**,**d**, DCC, HOBT, Et_3N, CH_2Cl_2, reflux; (d) *p*-TsOH·H_2O, toluene, 60 °C; (e) H_2/Pd/C, EtOH, 12 N HCl; (f) R-Br, K_2CO_3, DMF; (g) RCOCl, Et_3N, CH_2Cl_2; (h) RNH_2, DMSO, 60 °C; (i) RNH_2, EtOH, reflux; (j) AcOH, 12 N HCl; (k) H_2/Pd/C, AcOET.

N-Chloroacetyl glycine ethyl ester was treated with the appropriate alkyl amine, then subsequent cyclization into 1-alkyl-piperazine-2,5-diones **18a**,**c** occured under heating in DMSO.[37] *N*-(2,2-Diethoxyethyl)oxamic acid ethyl ester was prepared from oxalic acid diethyl ester and 2,2-diethoxyethylamine according to a described procedure.[38] Treatment with tetradecylamine under reflux in ethanol then, cyclization of the so obtained *N*-(2,2-diethoxyethyl)-*N*′-tetradecyl oxalamide **19c** using concentrated hydrochloric acid,[39] led to 1-tetradecyl-1,4-dihydropyrazine-2,3-dione **20c** which was hydrogenated into 1-tetradecyl piperazine-2,3-dione **21c** in the presence of palladium.

The key intermediates to oxadiazolone formation are the nitrile function. Depending on the case, this was either achieved through preparation of chloride intermediates or required synthesis of activated reactants as outline in Scheme 3. Therefore chloride intermediates **22–27** were obtained by acylation of the monosubstituted **2b**,**d**, **4a**,**c**, **5a**,**c**, **10a**,**c**,**e**, disubstituted **7a** and trisubstituted **8a** piperazines using 4-chloromethylbenzoyl chloride at low temperature (0 °C) to avoid reaction on the chloromethyl moiety as described previously,[23] nucleophilic substitution of the chloride atom by sodium cyanide[40] providing the nitriles **28–31**, **33**, **34** (Z = –CO–). Action of 4-bromomethylphenylacetonitrile which was prepared according to the previously published procedure[23] on **10a**,**c** gave their corresponding cyanide **32a**,**c** derivatives. At last, activation of 4-aminophenylacetonitrile was performed using 2,2,2-trichloroethylchloroformate leading to the carbamate **35**.

In the piperazinone and piperazine dione series, the syntheses of the nitrile precursors of oxadiazolone moiety, substituted



Scheme 3. [a]Reagents and conditions: (a) *p*-ClCOPhCH_2Cl, Et_3N, CH_2Cl_2, 0 °C; (b) NaCN, DMSO, 80 °C; (c) *p*-BrCH_2PhCH_2CN, K_2CO_3, Kl, CH_3CN, reflux; (d) CCl_3CH_2OCOCl, Et_3N, CH_2Cl_2, 0 °C.

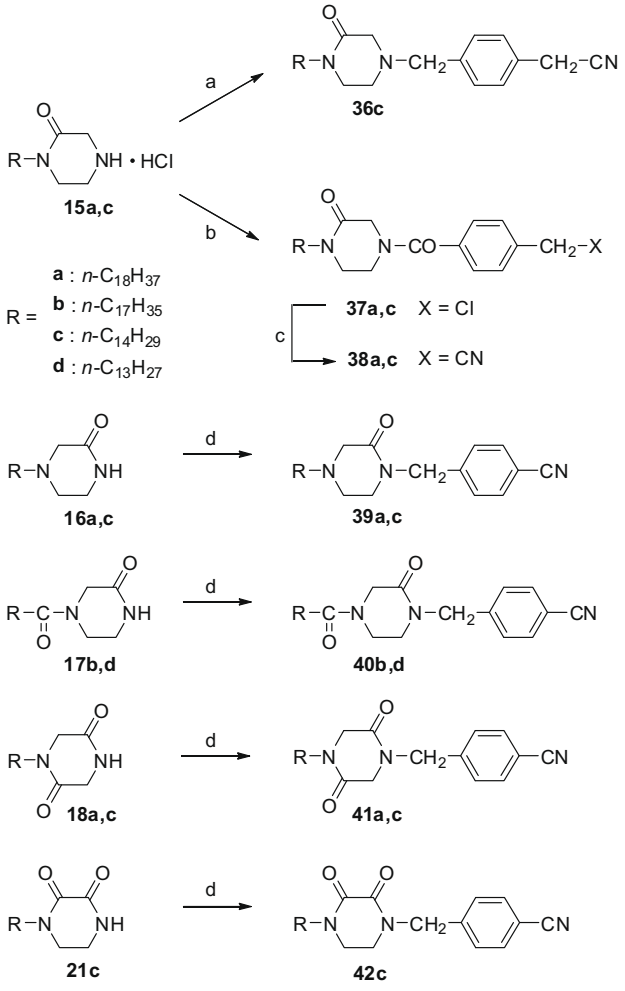
phenylacetonitrile **36c**, **38a**,**c** and benzonitrile **39a**,**c**, **40b**,**d**, **41a**,**c**, **42c** derivatives, are shown in Scheme 4.

Treatment of 1-tetradecylpiperazin-2-one hydrochloride **15c** with (4-bromomethyl) phenylacetonitrile in the presence of potassium iodide conducted to 4-(4-cyanomethylbenzyl)-1-tetradecylpiperazin-2-one **36c** in alkaline conditions and under reflux in acetonitrile.[41]

Using 4-chloromethylbenzoyl chloride, the 1-alkylpiperazin-2-one hydrochlorides **15a**,**c** were acylated. The chloro atom of the 1-alkyl-4-(4-chloromethylbenzoyl)piperazin-2-one intermediates **37a**,**c** was substituted by sodium cyanide in DMSO,[42] leading to 1-alkyl-4-(4-cyanomethylbenzoyl)piperazin-2-ones **38a**,**c**.

In a same manner, 1-alkylpiperazin-3-ones **16a**,**c**, 1-acylpiperazin-3-one **17b**,**d**, 1-alkylpiperazine-2,5-dione **18a**,**c** and 1-tetradecylpiperazine-2,3-dione **21c** were deprotonated by sodium hydride and alkylated[36] with (4-bromomethyl)benzonitrile to give 1-alkyl-4-(4-cyanobenzyl)piperazin-3-ones **39a**,**c**, 1-acyl-4-(4-cyanobenzyl)- piperazin-3-ones **40b**,**d**, 1-alkyl-4-(4-cyanobenzyl) piperazine-2,5-diones **41a**,**c** and 4-(4-cyanobenzyl)-1-tetradecylpiperazine-2,3-dione **42c**, respectively.

All cyano derivatives **28–36** and **38–42** were transformed into the amidoximes **43–56** by aqueous hydroxylamine.[43] The oxadiazolone derivatives **57–70** were then obtained after treatment with phenyl chloroformate according to Kohara et al.[44] adapted to our use, followed by cyclization in refluxing toluene of the crude carbonate intermediates (Scheme 5). Compounds **71** and **72** (Z = –CO–NH–) were obtained by reacting **64** with the mono substituted piperazines **5a**,**c** and **6a**,**c**, respectively.



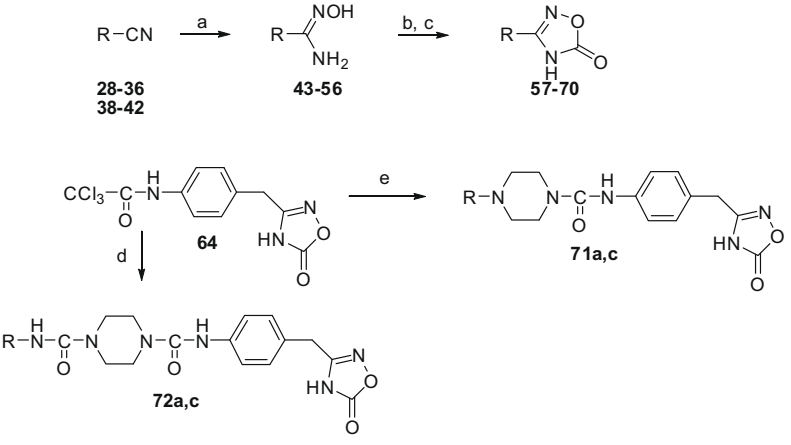
Scheme 4. [a]Reagents and conditions: (a) *p*-NCCH$_2$–Ph–CH$_2$Br, K_2CO_3, KI, CH_3CN, reflux; (b) *p*-ClCH$_2$–Ph–COCl, Et_3N, benzene; (c) NaCN, DMSO, 50 °C; (d) (i) NaH, THF, 0 °C; (ii) *p*-BrCH$_2$–Ph–CN, rt.

3. Results and discussion

All the synthesized compounds were evaluated as sPLA$_2$ inhibitors in in vitro enzymatic tests using either human PLA$_2$ GIIA (HPLA$_2$) or porcine pancreatic PLA$_2$ GIB (PPLA$_2$) (see biological section) and the results are reported in Tables 1 and 2. The log *P* values were calculated using the Rekker's hydrophobic fragmental constants method.[49]

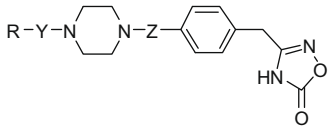
Our previous work[32] showed that introduction of a piperazine ring (compound **II**) in the structure of compound **I** did not affect neither the inhibitory effect against GIIA sPLA$_2$ with IC$_{50}$ of 2.2 and 3.4 μM, respectively, nor the selectivity GIIA/GIB (Table 1). In addition, it offered the ability of protonation at physiological pH then lowering the lipophilicity supposed to enhance biodistribution. It appeared also that rigidifying the piperazine ring by replacing Z = CH$_2$, compound **II**, by Z = CO, compound **III**, with IC$_{50}$ of 2.2 and 0.8 μM, respectively, permitted to reach the value of Lilly reference, **LY311727**[45] (IC$_{50}$ = 0.47 μM), measured in the same conditions as ours.

Starting with these results we undertook in one hand to rigidify more the piperazine ring from the outside by replacing its two nitrogens by different functions as amide, carbamate and urea or



Scheme 5. [a]Reagents and conditions: (a) NH_2OH 50% aq, EtOH, reflux; (b) ClCOOPh, Et_3N, CH_2Cl_2, 0 °C; (c) toluene, reflux; (d) **5a**,**c**, toluene, reflux; (e) **6a**,**c**, toluene, reflux.

Table 1
Inhibition of the enzymatic activity of human PLA_2 (GIIA) $HPLA_2$ and porcine pancreatic PLA_2 (GIB) $PPLA_2$ by piperazine derivatives of compound **I**, using the fluorimetric assay



Compd	R	Y	Z	Log P[a]	IC_{50}[b] (μM)	
					$HPLA_2$	$PPLA_2$
57b	n-$C_{17}H_{35}$	–CO–	–CO–	7.0	1.6	>75
57d	n-$C_{13}H_{27}$	–CO–	–CO–	4.9	2.7	>75
58a	n-$C_{18}H_{37}$	–O–CO–	–CO–	6.9	0.15	59.77
58c	n-$C_{14}H_{29}$	–O–CO–	–CO–	4.9	2.7	33
59a	n-$C_{18}H_{37}$	–NH–CO–	–CO–	6.9	0.33	24.54
59c	n-$C_{14}H_{29}$	–NH–CO–	–CO–	4.8	4.88	42.23
60a	n-$C_{18}H_{37}$	–O–C_6H_4–	–CH_2–	10	0.03	7.41
60c	n-$C_{14}H_{29}$	–O–C_6H_4–	–CH_2–	7.9	>10	>10
61a	n-$C_{18}H_{37}$	–O–C_6H_4–	–CO–	10.2	0.07	7.4
61c	n-$C_{14}H_{29}$	–O–C_6H_4–	–CO–	8.1	0.22	5.33
61e	n-$C_{10}H_{21}$	–O–C_6H_4–	–CO–	6.1	3.25	21.57
71a	n-$C_{18}H_{37}$	Deleted	–CO–NH–	7.3	0.05	>50
71c	n-$C_{14}H_{29}$	Deleted	–CO–NH–	5.2	2.04	>25
72a	n-$C_{18}H_{37}$	–NH–CO–	–CO–NH–	6.3	0.29	3.58
72c	n-$C_{14}H_{29}$	–NH–CO–	–CO–NH–	4.3	3.04	33
I[31]				7.08	3.4	>100
II[32]	n-$C_{18}H_{37}$	Deleted	–CH_2–	7.67	2.2	>100
III[32]	n-$C_{18}H_{37}$	Deleted	–CO–	7.85	0.8	>100
LY311727[45]					0.47	8

[a] Calculated using Rekker's hydrophobic fragmental constants.[49]
[b] Determined with three or more independent sample preparations using the fluorimetric binding assay and given as mean value with SD less than 20% of the mean value.

branching a phenyl group directly on one of the nitrogens (Table 1). In another hand, the piperazine ring was also rendered less flexible from the inside by substitution with one or two methyl groups or introducing carbonyl groups in the place of one or two methylenes (Table 2).

At the first glance, results reported in Tables 1 and 2 show that all the synthesized compounds are active on $HPLA_2$ with a very high selectivity for this group II enzyme versus GIB. Comparing the global lipophilicity of the molecules calculated using Rekker's hydrophobic fragmental constants,[49] it appears that the best results are obtained with log $P \geqslant 7$ as found for our initial leader compound **I** with the advantage that, in some of them, the amine function of the piperazine allows protonation, then lowering the calculated lipophilicity of the free base (compounds **60**, **61**, **62**, **63**, **65**, **67**, **68** and **71**) by about three log P units.

3.1. Rigidification outside the piperazine ring

Table 1 shows that replacing the first methylene group of the alkyl chain of **III** by a carbonyl as in **57b** divides the activity by two as shown by the IC_{50} of 0.8 and 1.6 μM, respectively. A better result was obtained with Y = OCO to reach a 10 times more active compound (**58a**, IC_{50} = 0.15 μM) and 5 times with Y = NHCO in **59a** (IC_{50} = 0.33 μM). It is relevant noting here that **57b**, **58a** and **59a** being isolipophilic, the difference cannot involve lipophilic interactions but appears as a consequence of the modified functional groups introduced. One could hypothesize that an unfavourable effect of the C=O in the amide **57b** could be compensated by the oxygen in the carbamate **58a** or the NH in the urea **59a** as the ability of a hydrogen bonding in the catalytic site.

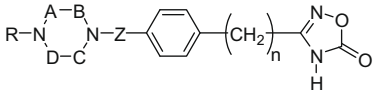
A very good result is also obtained by intercaling a phenoxy moiety between the nitrogen and the alkyl chain as shown by comparing **60a** and **II** (0.03 and 2.2 μM, respectively) in one hand, **61a** and **III** (0.07 and 0.8 μM, respectively) leading to at least a ten times gain in activity.

The same advantage is reached by replacing one of the amine functions of **II** by an ureide in compound **71a** (IC_{50} = 0.05 μM) or both, as in **72a** (IC_{50} = 0.29 μM).

3.2. Variation inside the piperazine

Table 2 shows that substituting one of the methylenes of the piperazine with a methyl group (compound **62a**) or two

Table 2
Inhibition of the enzymatic activity of human PLA_2 (GIIA) $HPLA_2$ and porcine pancreatic PLA_2 (GIB) $PPLA_2$ by piperazinone/piperazinedione derivatives of compound **I**, using the fluorimetric assay



Compd	R	A	B	C	D	Z	n	Log P[a]	IC_{50}[b] (μM)	
									$HPLA_2$	$PPLA_2$
62a	n-$C_{18}H_{37}$	CH_2	CH_2	$CHCH_3$	CH_2	CO	1	8.4	0,58	>100
63a	n-$C_{18}H_{37}$	$CHCH_3$	CH_2	$CHCH_3$	CH_2	CO	1	8.9	0.23	>100
65c	n-$C_{14}H_{29}$	CO	CH_2	CH_2	CH_2	CH_2	1	4.7	30	>85
66a	n-$C_{18}H_{37}$	CO	CH_2	CH_2	CH_2	CO	1	5.8	1.7	>100
66c	n-$C_{14}H_{29}$	CO	CH_2	CH_2	CH_2	CO	1	3.8	10	>100
67a	n-$C_{18}H_{37}$	CH_2	CO	CH_2	CH_2	CH_2	0	6.5	0.97	>50
67c	n-$C_{14}H_{29}$	CH_2	CO	CH_2	CH_2	CH_2	0	4.5	>25	>25
68b	n-$C_{17}H_{35}CO$	CH_2	CO	CH_2	CH_2	CH_2	0	5.3	26	>50
68d	n-$C_{13}H_{27}CO$	CH_2	CO	CH_2	CH_2	CH_2	0	3.3	>75	>75
69a	n-$C_{18}H_{37}$	CH_2	CO	CH_2	CO	CH_2	0	5.7	1.3	>100
69c	n-$C_{14}H_{29}$	CH_2	CO	CH_2	CO	CH_2	0	3.6	57	>100
70c	n-$C_{14}H_{29}$	CO	CO	CH_2	CH_2	CH_2	0	nd*	40.7	>75
III[32]	n-$C_{18}H_{37}$	CH_2	CH_2	CH_2	CH_2	CO	1	7.85	0.8	>100
IV[32]	n-$C_{18}H_{37}$	CH_2	CH_2	CH_2	CH_2	CH_2	0	7.37	5.08	>100
LY311727[45]									0.47	8

[a] Calculated using Rekker's hydrophobic fragmental constants.[49]
[b] Determined with three or more independent sample preparations using the fluorimetric binding assay and given as mean value with SD less than 20% of the mean value.
* Not determined.

(compound **63a**) has few effect on the activity, compounds **62a**, **63a** and **III** bearing IC_{50} of the same range, 0.58, 0.23 and 0.8 μM, respectively. In the other hand, replacing a methylene of the piperazine by a carbonyl beside the alkyl chain decreases the activity, compound **66a** (IC_{50} = 1.7 μM) being half time less active as **III** and this result could be compared to the lost of activity between **III** and **57b**. Introduction of a carbonyl in the piperazinic ring was attempted to obtain more or less flexible compounds but it was dependent on the success of the synthesis for this series. For example, we could not assess through our synthesis, compounds bearing carbonyls in the piperazine as in **67**–**70**, with Z = CO and n different from zero. Lacking with these two spacers is, with our experience,[30,31] very unfavourable to activity as shown by comparing the IC_{50} of **III** (Z = CO, n = 1, IC_{50} = 0.8 μM), **II** (Z = CH_2, n = 1, IC_{50} = 2.2 μM) and **IV** (Z = CH_2, n = 0, IC_{50} = 5.08 μM) in one hand, and those of **66c** (10 μM) and **65c** (30 μM) in the other hand. Nevertheless when the CO group is close to the oxadiazolone bearing moiety, compounds more or less active as **III** were obtained as shown by the IC_{50} of **67a** (0.97 μM) and **69a** (1.3 μM), then from four to five times more active than **IV**, in spite of their lower lipophilicity.

As it appeared that lipophilicity was not concerned in this series, we wondered if other interactions could be involved to explain these results either intramolecular or in relation with the active site. That is the reason why some of them were selected to be studied through molecular modelling.

First, the geometries of the molecules were optimized for all the degrees of freedom at the ab initio RHF level using the double ζ basis set 6-31G.[46] For several compounds, an additional conformational analysis was performed as for **61** and reoptimization of the local minima thus trapped. All the calculations were performed using the GAUSSIAN software.[47] For all the studied molecules, the alkyl appendage was limited to a propyl substituent.

Optimization of the geometries shows that most of the compounds (compounds **III**, **57**, **58**, **59**, **61**, **63** and **66**) with Z = CO and n = 1 have nearly the same profile as illustrated in Figure 2 (completed in Supplementary data) with the same 'up' orientation of the oxadiazolone ring with regard to the rest of the molecule. In **57**, **58** and **59**, the carbonyl on each site of the piperazine nitrogens fixes the rigid geometry due to the amide function. This feature also appears with one carbonyl in **III**, **61**, **63** and **66**. The conformational analysis of the **61** molecule allows locating four conformers with a very similar relative energy (see Supplementary data). In return, when Z = CH_2, n = 0 (compounds **IV**, **67**, **68**, **69** and **70**), the oxadiazolone moiety adopts a very different 'down' position which could explain the less activity of these molecules (Fig. 3). The ureas **71** and **72**, as for them, appear in a third type privileged conformation as a consequence of the lengthening between the piperazine and the phenyl rings, which seems favourable, as shown by their activity (Fig. 4).

The electrostatic potential was computed at the same level as the geometry optimization. The 3D envelope is drawn at −20 kcal/mol level. All the maps show a strong electrostatic potential at the level of the oxadiazolone and several others depending of the functional groups introduced to rigidify the piperazine ring. The characteristic potential appears in the amide region for Z = CO in **57**, **61**, **63** and **III** (Fig. 5). In compounds bearing another carbonyl in the Y region, outside (**57**, **58**, **59**) or inside (**66**) another important electrostatic potential appears when compared to **III** which could moderate the activity depending on other factors as hydrogen bonding abilities evoked previously (Fig. 6). It is to note that a similar electrostatic profile is shown in **69** with the two carbonyls inside the piperazine ring leading to comparable IC_{50}. In another hand, those two negative wells joint together in **70** (Fig. 7) could explain the poor activity of this last.

For **60** and the compounds where Z = CH_2 (**IV**, **65**), the nitrogen character becomes sp^3 and their lone pairs generate their own isocontour depending on the geometry.

4. Conclusions

We have shown that in the oxadiazolone series of PLA_2 GIIA inhibitors, rigidifying the molecules by means of a piperazine ring whose flexibility and conformation can be modulated through different functional groups could enhance the activity. The best compounds here were those bearing a phenoxy group directly attached to the nitrogen of the piperazine. The advantage is to keep the protonation ability of the nitrogen, useful for bioavailability. The introduction of carbonyls not only suppressed this protonation, but also

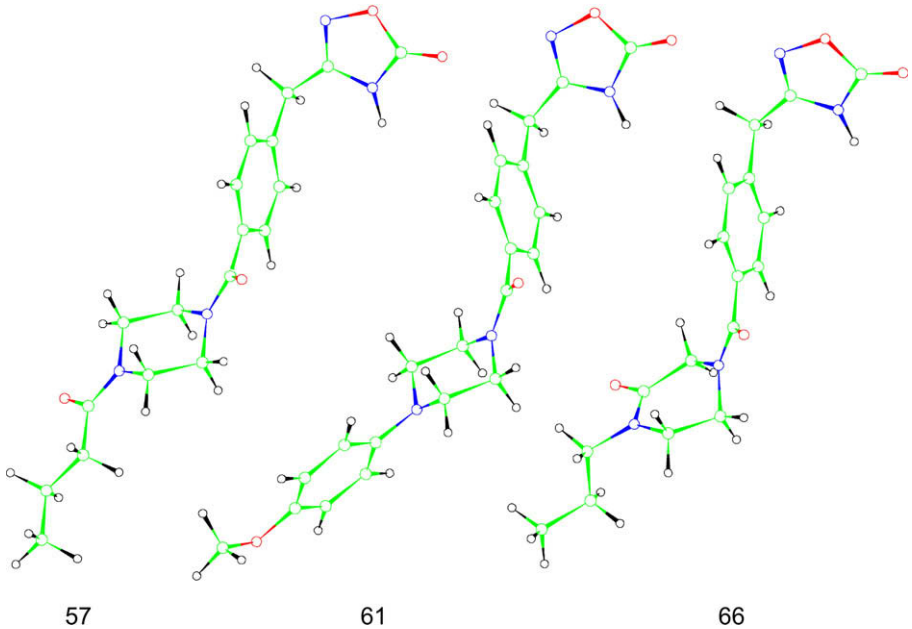


Figure 2.

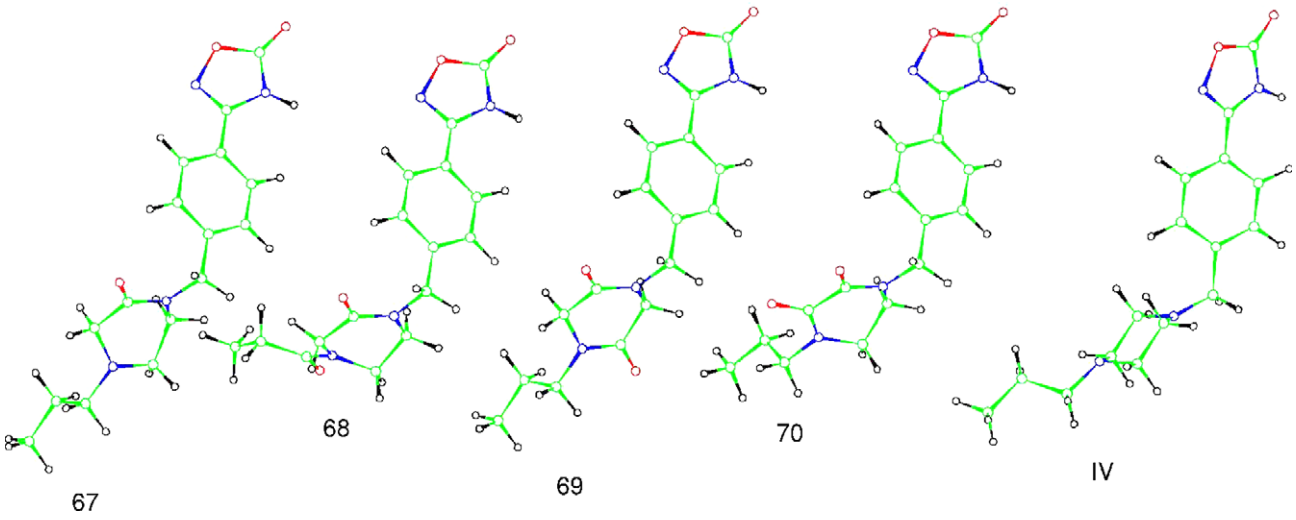


Figure 3.

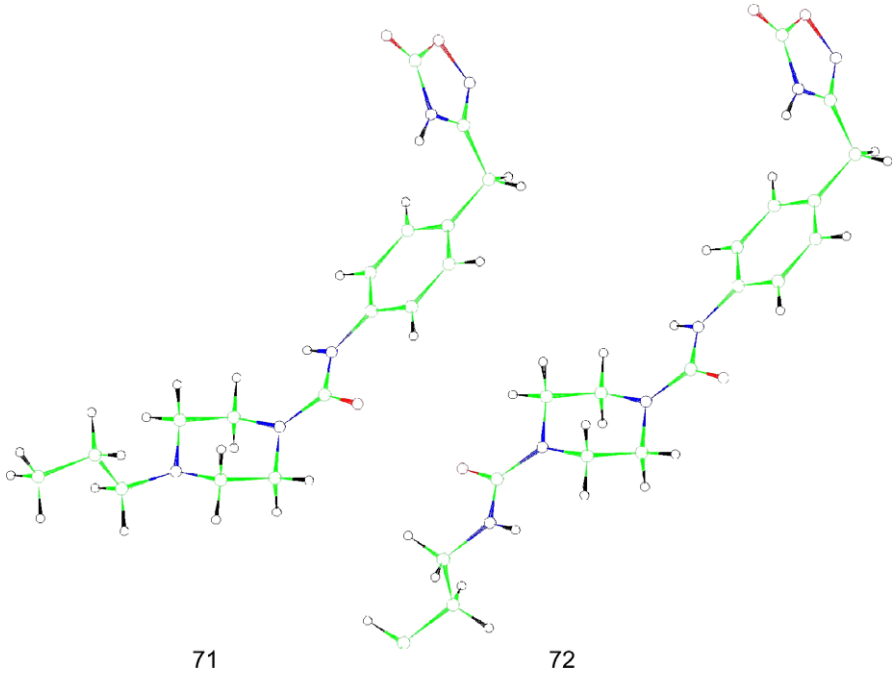


Figure 4.

generated an electronic character which seems unfavourable to the inhibitory effect.

5. Experimental

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 60F254 (layer thickness 0.2 mm) from Merck. Column chromatography purification was carried out on silica gel 60 (70–230 mesh ASTM, Merck). All melting points were determined on a digital melting point apparatus (electrothermal) and are uncorrected. The structure of all compounds was confirmed by IR and NMR spectra. IR spectra were obtained in paraffin oil with an ATI Mattson Genesis Series FTIR spectrometer; ^{1}H and ^{13}C NMR spectra were recorded at 200 MHz and 50 MHz, respectively, in $CDCl_3$ or otherwise specified, on a BRUKER AC 200 spectrometer using hexamethyldisiloxane (HMDS) as an internal standard. Chemical shifts are given in ppm and peak multiplicities are described as follows: s, singlet, ls, large singlet, d, doublet, t, triplet, 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.

5.2. In vitro biological activity assay

PLA_2 activity was evaluated by the method of Radvanyi et al.[48] Enzymes used were two secretory enzymes: human recombinant PLA_2 of group II, $HPLA_2$ (GIIA) and porcine pancreatic PLA_2 of group I, $PPLA_2$ (GIB). Palmitoyl-2-(10-pyrenyldecanoyl)-*sn*-glycero-3-

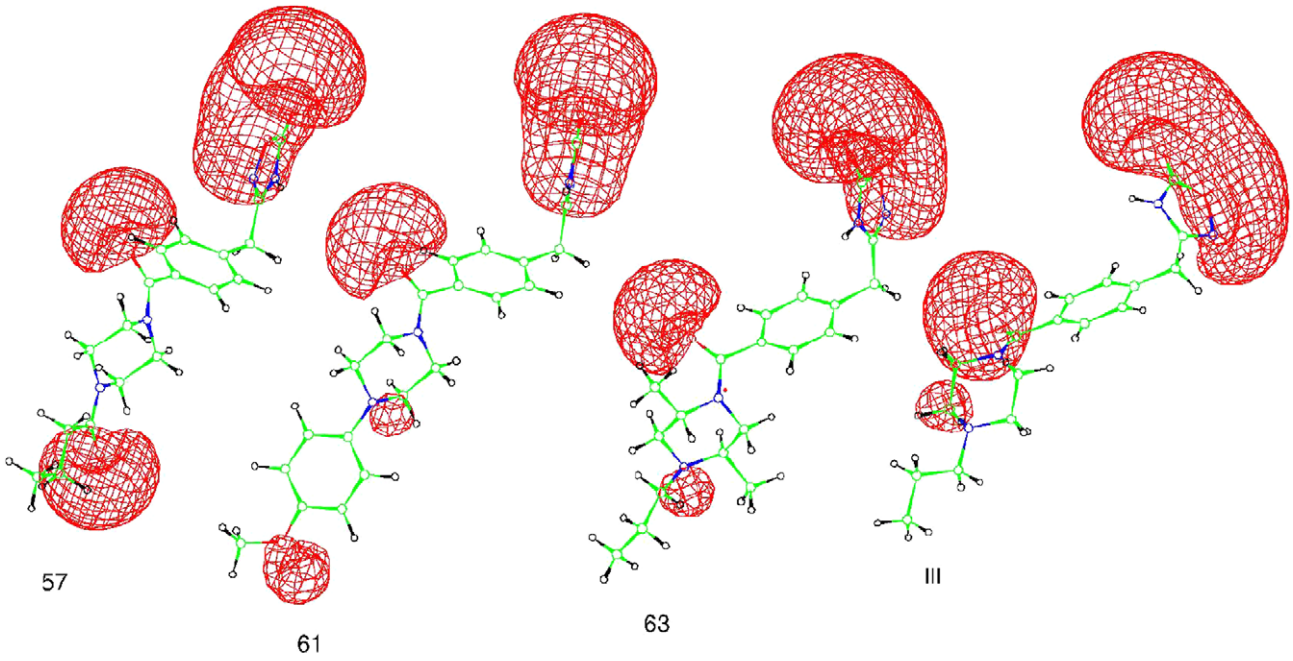


Figure 5.

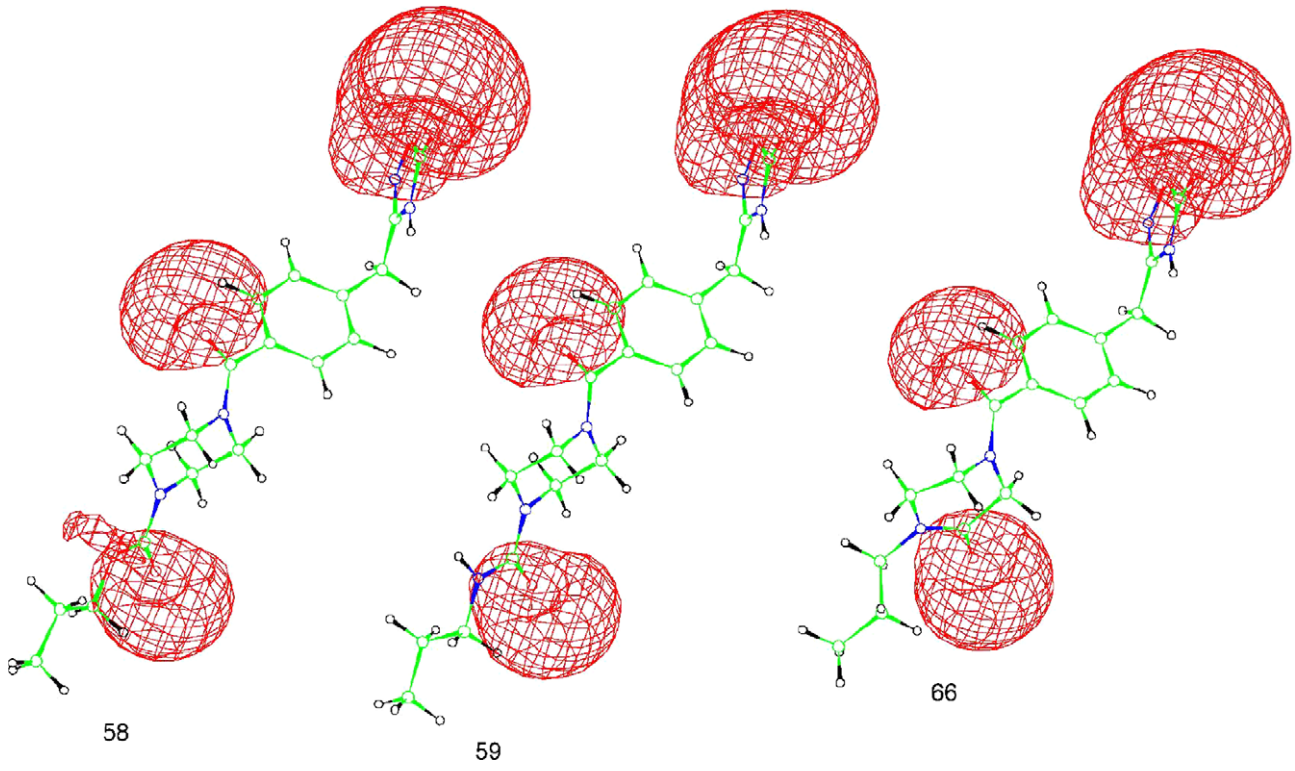


Figure 6.

phosphatidyl glycerolic acid was used as a fluorescent substrate in the fluorometric assay which was performed with a Perkin–Elmer LS50 luminescence spectrometer in a unit dosage polystyrene cell having a size of 1 cm. The concentration of the fluorescent substrate was determined by UV Unicam spectrometry in a quartz cell. The experimental protocol was the same as described in our previous publications.[30–32]

5.3. 4-Diphenylmethyl-1-octadecanoylpiperazine (1b)

A solution of *N*-diphenylmethyl piperazine (20 g, 79 mmol) in Et_3N (12 mL, 120 mmol) and dry toluene (200 mL) was cooled in an ice bath. Octadecanoyl chloride (26.4 g, 100 mmol) solubilized in dry toluene (150 mL) was added dropwise and the mixture was stirred at 0 °C for 1 h. The organic layer was washed with a

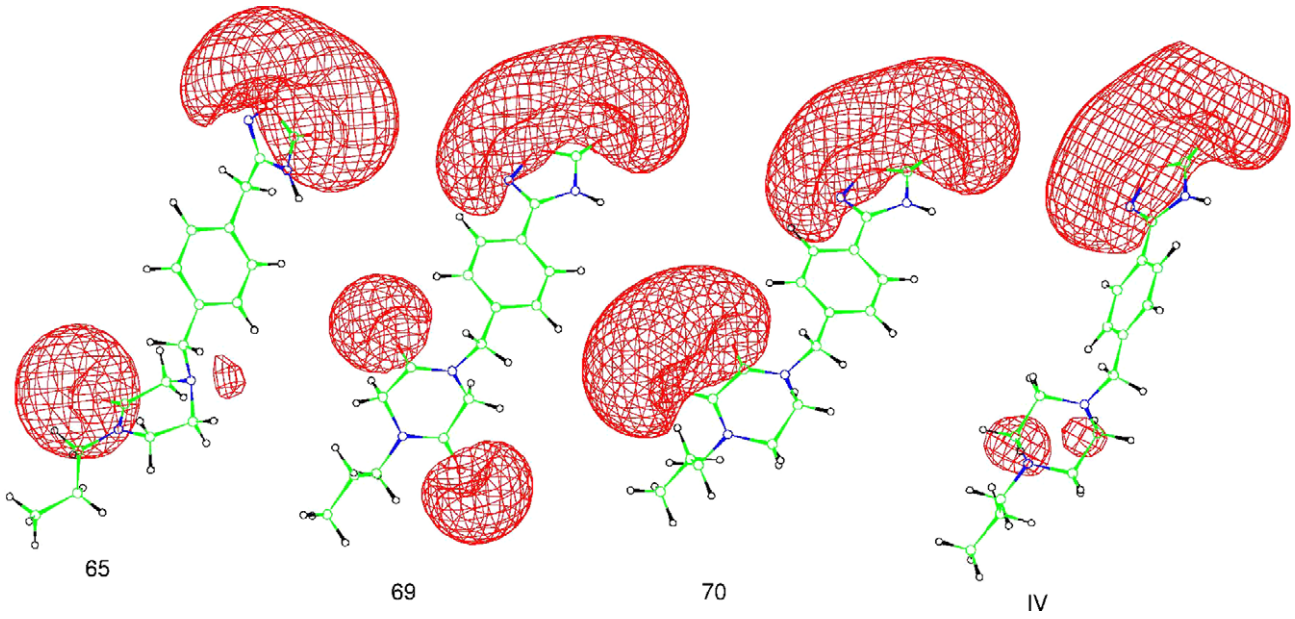


Figure 7.

saturated Na_2CO_3 solution and twice with water. After drying ($MgSO_4$), filtration and evaporation, the residue was chromatographed on a silica gel column using CH_2Cl_2 as eluent to yield 36.85 g (90%) of **1b** as a yellow oil. R_f 0.4 (CH_2Cl_2/MeOH, 95:5, v/v); IR cm^{-1}: 1647 (C=O), 1600 ($C=C_{ar}$); ^1H NMR: δ 6.98–7.25 (m, 10H), 4.05 (s, 1H), 3.44 (t, 2H, J = 4.7 Hz), 3.27 (t, 2H, J = 4.9 Hz), 2.19 (t, 4H, J = 4.9 Hz), 2.09 (t, 2H, J = 8 Hz), 1.38 (t, 2H, J = 6.8 Hz), 1.08 (ls, 28H), 0.71 (t, 3H, J = 6.4 Hz).

5.4. 1-Octadecanoylpiperazine (2b)

To a solution of the amide **1b** (24 g, 69 mmol) in EtOH (150 mL) was added 100 mg Pd–C, and this mixture was warmed (50 °C) with stirring under hydrogen atmosphere. After disappearance of the starting material (12 h) as shown by TLC, the suspension was filtered and the catalyst washed several times with EtOH. The solvents were evaporated and the residue chromatographed on a silica gel column using CH_2Cl_2 as eluent to yield 10 g (72.5%) of **2b** as a yellow powder: mp 98 °C; R_f 0.25 (CH_2Cl_2/MeOH, 90:10, v/v); IR cm^{-1}: 3380 (N–H), 1647 (C=O); ^1H NMR (CD_3OD): δ 3.65 (t, 4H, J = 4.7 Hz), 3.24 (ls, 1H), 3.01 (ls, 4H), 2.34 (t, 2H, J = 7.6 Hz), 1.52 (t, 2H, J = 6.7 Hz), 1.22 (ls, 28H), 0.85 (t, 3H, J = 5.8 Hz).

5.5. Octadecylphenylcarbonate (3a)

A solution of 1-octadecanol (10 g, 37 mmol) in Et_3N (6.25 mL, 44 mmol) and freshly distilled THF (80 mL) was cooled in an ice bath. A solution of phenyl chloroformate (5.63 mL, 44 mmol) in THF (30 mL) was added dropwise and the mixture was stirred at 0 °C for 1 h. The solvent was evaporated and the residue, taken up in CH_2Cl_2, was washed with a saturated Na_2CO_3 solution and twice with water. After drying ($MgSO_4$), filtration and evaporation, the residue was chromatographed on a silica gel column using CH_2Cl_2 as eluent to yield 9.42 g (66%) of carbonate **3a** as an oil: R_f 0.52 (CH_2Cl_2/MeOH, 98:2, v/v); IR cm^{-1}: 1753 (C=O), 1110 (C–O); ^1H NMR: δ 7.35–7.08 (m, 5H), 4.17 (t, 2H, J = 6.6 Hz), 1.69–1.64 (m, 2H), 1.19 (ls, 30H), 0.81 (t, 3H, J = 6.6 Hz).

5.6. *N*-Octadecyloxycarbonylpiperazine (4a)

A mixture of carbonate **3a** (9.42 g, 24 mmol) and piperazine (20.77 g, 241 mmol) in 100 mL THF/CH_2Cl_2 (3:1) was stirred at room temperature for 1 h. The solvents were evaporated; the residue was taken up in CH_2Cl_2 and washed twice with water. The organic phase was then dried over $MgSO_4$, filtered and concentrated in vacuo. The product was crystallized in acetone to yield 4.5 g (76%) of **4a** as white crystals: mp 54 °C; R_f 0.30 (CH_2Cl_2/MeOH, 90:10, v/v); IR cm^{-1}: 3425 (NH), 1709 (CO), 1198 (C–O); ^1H NMR: δ 4.00 (t, 2H, J = 6.6 Hz), 3.37 (t, 4H, J = 5.2 Hz), 2.75 (t, 4H, J = 4.9 Hz), 1.93 (s, 1H), 1.55 (t, 2H, J = 6.8 Hz), 1.19 (ls, 30H), 0.81 (t, 3H, J = 6.7 Hz).

5.7. *N*-Octadecylaminocarbonylpiperazine (5a)

A mixture of octadecyl isocyanate (4.42 g, 15 mmol) and piperazine (13 g, 151 mmol) in CH_2Cl_2 (70 mL) was stirred at room temperature for 1 h. The solution was washed twice with water. The organic phase was then dried over $MgSO_4$, filtered and concentrated to yield 5.21 g (91%) of **5a** as white crystals: mp 72 °C; R_f 0.46 (CH_2Cl_2/MeOH/NH_4OH, 80:20:2, v/v/v); IR cm^{-1}: 3364 (NH), 1620 (C=O); ^1H NMR: δ 4.68 (t, 1H, J = 5 Hz), 3.26 (t, 4H, J = 5.2 Hz), 3.14 (q, 2H, J = 7 Hz), 2.77 (t, 4H, J = 4.8 Hz), 1.73 (s, 1H), 1.42 (t, 2H, J = 6.4 Hz), 1.19 (ls, 30H), 0.81 (t, 3H, J = 6.2 Hz).

5.8. 3-Methyl-1-octadecylpiperazine (7a)

A mixture of 45 g (0.45 mol) of commercially available 2-methylpiperazine and 15 g (45 mmol) of octadecylbromide in 250 mL of THF/CH_2Cl_2 (50:50, v/v) was stirred at room temperature for 3 h. After evaporation of the solvents, the residue was taken up in CH_2Cl_2 and washed twice with water. The organic layer was then dried over $MgSO_4$, filtered and concentrated to afford 12.7 g (80%) of **7a** as a white solid: mp 48.5 °C. R_f 0.12 (CH_2Cl_2/MeOH, 80:20, v/v); IR cm^{-1}: 3440 (N–H); ^1H NMR: δ 3.00–2.2 (m, 9H), 1.50–1.3 (m, 2H), 1.20 (1s, 30H), 0.98 (d, 3H, J = 6 Hz), 0.81 (t, 3H, J = 6.6 Hz).

5.9. 1-Acetyl-4-(4-octadecyloxyphenyl)piperazine (9a)

To a solution of 1-acetyl-4-(4-hydroxyphenyl)piperazine (2.36 g, 0.059 mol) in DMF (100 mL) was added dropwise a suspension of NaH (60% dispersion in mineral oil, 10.8 g, 0.049 mol) in DMF (30 mL). The mixture was stirred at room temperature for

30 min and 1-bromooctadecane (13.8 g, 0.049 mol) in DMF (50 mL) was added dropwise. The resulting mixture was warmed (60 °C) with stirring for 2 h. After cooling, water (100 mL) was added and the precipitated crystals were collected, washed with water and dried to provide **9a** (15.57 g, 95%) which was used in the next step without purification: mp 117 °C; R_f 0.40 (CH_2Cl_2/MeOH, 95:5, v/v); IR cm^{-1}: 1621 (C═O), 1597 (C═C_{ar}); ^1H NMR: δ 6.85 (q, 4H, J = 9.1 Hz), 3.89 (t, 2H, J = 6.5 Hz), 3.73 (t, 2H, J = 4.6 Hz), 3.60 (t, 2H, J = 4.7 Hz), 3.01 (d, 4H, J = 4.3 Hz), 2.12 (s, 3H), 1.80–1.7 (m, 2H), 1.19 (ls, 30H), 0.81 (t, 3H, J = 6.1 Hz).

5.10. 1-(4-Octadecyloxyphenyl)piperazine dihydrochloride (10a)

A mixture of **9a** (15 g, 33 mmol) in EtOH (150 mL) and 12 N HCl (30 mL) was warmed to reflux for 3 h. The solvent was evaporated under reduced pressure to give a viscous solid. The taking up in MeOH (100 mL) made precipitate crystals which were collected and dried to provide 14 g (85%) of **10a**: mp 220 °C; R_f 0.15 (CH_2Cl_2/MeOH, 90:10, v/v); IR cm^{-1}: 1595 (C═C_{ar}), 1146 (C–O); ^1H NMR: δ 9.96 (ls, 1H), 7.69 (d, 2H, J = 8.7 Hz), 6.87 (d, 2H, J = 8.7 Hz), 3.98 (ls, 8H), 3.83 (t, 2H, J = 6.5 Hz), 1.68–1.6 (m, 2H), 1.14 (ls, 30H), 0.81 (t, 3H, J = 6.1 Hz).

5.11. *N*-(2,2-Diethoxyethyl)-*N*-tetradecylbenzylamine (11c)

To a mixture of *N*-(2,2-diethoxyethyl)benzylamine (51.6 g, 0.23 mol), potassium carbonate (63.9 g, 0.46 mol) and potassium iodide (1 g, 6.02 mmol) in acetonitrile (700 mL) was added 1-bromotetradecane (64 g, 0.23 mol) and the reaction mixture was refluxed for 12 h. After cooling to room temperature, the mixture was filtered and evaporated under reduced pressure. The crude residue was taken up into CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, then filtered and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (99.5:0.5, v/v) as eluent to obtain **11c** as a colorless oil (88.5 g, 91%): R_f 0.46 (CH_2Cl_2/MeOH, 97:3, v/v); IR cm^{-1}: 1603 (C═C_{ar}), 1118, 1065 (CH_2O); ^1H NMR: δ 7.30–7.13 (m, 5H), 4.47 (t, 1H, J = 5.16 Hz), 3.56 (s, 2H), 3.62–3.35 (m, 4H), 2.55 (d, 2H, J = 5.17 Hz), 2.41 (t, 2H, J = 7.23 Hz), 1.39 (t, 2H, J = 6.87 Hz), 1.19–1.07 (m, 28H), 0.81 (t, 3H, J = 6.37 Hz).

5.12. *N*-(2,2-Diethoxyethyl)tetradecylamine (12c)

To a solution of **11c** (88 g, 0.2 mol) in ethanol (300 mL) was added 10% Pd–C (0.5 g). The suspension was shaken vigorously under H_2 (50 PSI) and heated at 40 °C for 48 h. After cooling to room temperature and filtration through a glass frit funnel covered with a layer of Celite 545, the solvent was removed under reduced pressure and the resulting residue was purified by column chromatography on silica gel using CH_2Cl_2 to afford **12c** as a colorless oil (59 g, 90%): R_f 0.3 (CH_2Cl_2/MeOH, 95:5, v/v); IR cm^{-1}: 3391 (NH), 1125, 1069 (CH_2O); ^1H NMR: δ 4.59 (t, 1H, J = 5.55 Hz), 3.57 (m, 4H), 2.63 (d, 2H, J = 5.57 Hz), 2.58 (t, 2H, J = 7.25 Hz), 2.32 (s, 1H), 1.45 (m, 2H), 1.15 (m, 28H), 0.81 (t, 3H, J = 6.40 Hz).

5.13. *N*-Tetradecyl carbamic acid benzyl ester (13c)

A mixture of **12c** (53.8 g, 0.16 mol), *N*-carbobenzyloxyglycine (33.1 g, 0.16 mol), triethylamine (46 mL, 0.3 mol) and 1-hydroxybenzotriazole (26 g, 0.19 mol) were dissolved in dichloromethane (120 mL). The solution was stirred for 10 min then *N,N′*-dicyclohexylcarbodiimide (37 g, 0.18 mol) was added and the reaction mixture was heated to reflux for 2 h. After cooling to room temperature, the mixture was filtered. The organic layer was washed with water and brine, subsequently, then dried over $MgSO_4$, and concentrated in vacuo. The crude residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (99:1, v/v) as eluent to obtain **13c** as a colorless oil (82 g, 96.5%): R_f 0.43 (CH_2Cl_2/MeOH, 98:2, v/v); IR cm^{-1}: 1720 (OC═O), 1649 (NC═O); ^1H NMR: δ 7.17 (m, 5H), 5.85 (ls, 1H), 5.01 (s, 2H), 4.54 (t, 1H, J = 5.26 Hz), 4.02–3.92 (m, 2H), 3.65–3.48 (m, 2H), 3.20 (m, 6H), 1.44 (m, 2H), 1.17 (m, 22H), 1.09 (t, 6H, J = 6.98 Hz), 0.79 (t, 3H, J = 6.20 Hz).

5.14. 3,4-Dihydro-1-tetradecyl-2*H*-pyrazin-2-one (14c)

To a solution of compound **13c** (23 g, 47 mmol) in toluene (250 mL) was added a catalytic amount of *p*-TsOH (936 mg, 4.7 mmol). After heating to 60 °C for 3 h, the solution was allowed to cool to room temperature and washed with water. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography using CH_2Cl_2 to obtain **14c** as a yellow oil (15 g, 75%): R_f 0.61 (CH_2Cl_2/MeOH, 98:2, v/v); IR cm^{-1}: 1700 (OC═O), 1669 (C═O, C═C); ^1H NMR: δ 7.25 (m, 5H), 6.29 (dd, 1H, J = 21.58 Hz, J = 5.98 Hz), 5.42 (dd, 1H, J = 18.78 Hz, J = 6.01 Hz), 5.12 (s, 2H), 4.59 (s, 2H), 3.39 (t, 2H, J = 7.24 Hz), 1.33 (m, 2H), 1.18 (m, 22H), 0.81 (t, 3H, J = 6.32 Hz).

5.15. 1-Tetradecylpiperazin-2-one hydrochloride (15c)

To a solution of **14c** (10.5 g, 24.6 mmol) in ethanol (100 mL) were added 10% Pd–C (1 g) and concentrated hydrochloric acid (5 mL). The suspension was shaken vigorously under H_2 (50 PSI) and heated to 40 °C for 6 h. After cooling to room temperature and filtration through a glass frit funnel covered with a layer of Celite 545, the solvent was evaporated in vacuo and the resulting residue was purified by crystallization in ether to afford **15c** as a yellow powder (7 g, 86%): mp 161,6 °C (decomposed); R_f 0.24 (CH_2Cl_2/MeOH, 90:10, v/v); IR cm^{-1}: 3451 (NH_2), 1655 (C═O); ^1H NMR: δ 10.30–9.45 (ls, 1H), 8.90–7.70 (ls, 1H), 4.18 (m, 2H), 3.93 (m, 2H), 3.63 (m, 2H), 3.30 (m, 2H), 1.46 (m, 2H), 1.19 (m, 22H), 0.81 (t, 3H, J = 6.35 Hz).

5.16. 1-Tetradecylpiperazin-3-one (16c)

To a mixture of piperazin-2-one (1.9 g, 19 mmol) and potassium carbonate (5.25 g, 38 mmol) in DMF (20 mL) was added dropwise a solution of 1-bromotetradecane (5.28 g, 19 mmol) in DMF (20 mL). After stirring for 24 h at room temperature, water (50 mL) was added and the mixture was extracted with ether. The organic layer was washed with brine, dried over $MgSO_4$, then filtered and concentrated in vacuo. The crude residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH, 98:2, v/v, to give **16c** as a white powder (4.7 g, 83%): mp 87 °C; R_f 0.48 (CH_2Cl_2/MeOH, 90:10, v/v); IR cm^{-1}: 3193 (NH), 1667(C═O); ^1H NMR: δ 6.32 (ls, 1H), 3.32 (m, 2H), 3.06 (s, 2H), 2.57 (t, 2H, J = 5.43 Hz), 2.34 (t, 2H, J = 7.35 Hz), 1.42 (m, 2H), 1.19 (m, 22H), 0.81 (t, 3H, J = 6.46 Hz).

5.17. 1-Tetradecanoylpiperazin-3-one (17d)

A solution of piperazin-2-one (1 g, 10 mmol) and triethylamine (1.7 mL, 12 mmol) in dichloromethane (80 mL) was stirred for 10 min at room temperature then cooled to 0 °C and tetradecanoyl chloride (3 mL, 11 mmol) was added. The reaction mixture was stirred for 12 h at room temperature, then washed with a saturated solution of $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified by crystallization in acetone to afford **17d** as a white powder (2.8 g, 90%): mp 77 °C; R_f 0.26

(CH_2Cl_2/MeOH, 93:7, v/v); IR cm^{-1}: 3204 (NH), 1670 (C=O), 1655 (C=O); ^1H NMR: δ 7.03–6.71 (ls, 1H), 4.18–4.07 (s, 2H), 3.75–3.61 (t, 2H, J = 5.20 Hz), 3.33 (t, 2H, J = 2.59 Hz), 2.25 (t, 2H, J = 7.40 Hz), 1.57 (t, 2H, J = 7.40 Hz), 1.19 (m, 20H), 0.81 (t, 3H, J = 6.39 Hz).

5.18. 1-Tetradecylpiperazine-2,5-dione (18c)

A solution of *N*-chloroacetylglycine ethyl ester (1 g, 5.57 mmol) and 1-tetradecylamine (4.74 g, 22.3 mmol) in DMSO (58 mL) was heated at 60 °C for 48 h. After cooling to room temperature, water (130 mL) was added and the mixture was extracted three times with ether. The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH, 98:2, v/v, to obtain **18c** as a white powder (1.4 g, 81%): mp 147 °C; R_f 0.46 (CH_2Cl_2/MeOH, 90:10, v/v); IR cm^{-1}: 3260 (NH), 1677 (C=O), 1650 (NC=O); ^1H NMR: δ 6.93 (ls, 1H), 3.94 (s, 2H), 3.88 (s, 2H), 3.32 (t, 2H, J = 7.44 Hz), 1.49 (m, 2H), 1.19 (m, 22H), 0.81 (t, 3H, J = 6.44 Hz).

5.19. *N*-(2,2-Diethoxyethyl)-*N*′-tetradecyloxamide (19c)

A solution of *N*-(2,2-diethoxyethyl)oxamate ethyl ester (16.33 g, 70 mmol) and 1-tetradecylamine (14.9 g, 70 mmol) in ethanol (250 mL) was stirred under argon atmosphere and heated to reflux for 6 h. After cooling to room temperature, the mixture was filtered and the resulting solid was purified by crystallization in ethanol to afford **19c** as a yellow powder (21.5 g, 77%): mp 87.5–89 °C; R_f 0.55 (CH_2Cl_2/MeOH, 97:3, v/v); IR cm^{-1}: 3302 (NH), 1688, 1650 (C=O), 1133, 1073 (C–O); ^1H NMR: δ 7.65 (t, 1H, J = 6.20 Hz), 7.55 (t, 1H, J = 4.80 Hz), 4.47 (t, 1H, J = 5.40 Hz), 3.73–3.55 (m, 2H), 3.52–3.35 (m, 4H), 3.24 (q, 2H, J = 6.75 Hz), 1.48 (t, 2H, J = 6.81 Hz), 1.15 (m, 28H), 0.81 (t, 3H, J = 6.40 Hz).

5.20. 1,4-Dihydro-1-tetradecylpyrazine-2,3-dione (20c)

Compound **19c** (21 g, 52.5 mmol) was dissolved in a mixture of acetic acid (250 mL) and hydrochloric acid 12 N (0.55 mL). The solution was heated to reflux for 30 min, and after cooling to room temperature, evaporated in vacuo. The resulting residue was purified by crystallization in CH_2Cl_2/MeOH to obtain **20c** as a white powder (15 g, 93%): mp 122 °C (decomposed); R_f 0.25 (CH_2Cl_2/MeOH, 97:3, v/v); IR cm^{-1}: 3225 (NH), 1665, 1640 (C=O), 1593 (C=C); ^1H NMR: δ 6.5 (d, 1H, J = 5.72 Hz), 6.15 (d, 1H, J = 5.8 Hz), 3.73 (t, 2H, J = 7.29 Hz), 1.64 (m, 2H), 1.18 (m, 22H), 0.80 (t, 3H, J = 6.88 Hz).

5.21. 1-Tetradecylpiperazine-2,3-dione (21c)

To a suspension of **20c** (6 g, 19.5 mmol) in ethyl acetate (300 mL) was added 10% Pd–C (1 g). The mixture was shaken vigorously under H_2 (50 PSI) and heated to 40–60 °C for 48 h. After cooling to room temperature and filtration through a glass frit funnel covered with a layer of Celite 545, the solvent was evaporated in vacuo and the resulting yellow solid **21c** was used without further purification: mp 101 °C (decomposed); R_f 0.25 (CH_2Cl_2/MeOH, 93:7, v/v); IR cm^{-1}: 3239 (NH), 1698, 1671 (C=O); ^1H NMR: δ 8.17 (ls, 1H), 3.42 (m, 6H), 1.51 (m, 2H), 1.18–1.04 (m, 22H), 0.81 (t, 3H, J = 6.52 Hz).

5.22. 1-(4-Chloromethylbenzoyl)-4-octadecanoylpiperazine (22b)

A mixture of 1-octa decanoylpiperazine **2b** (3.28 g, 9.3 mmol) and Et_3N (1.41 mL, 14 mmol) in CH_2Cl_2 (50 mL) was stirred at 0 °C for 10 min. A solution of 4-(chloromethyl)benzoyl chloride (2.11 g, 11.16 mmol) in CH_2Cl_2 (25 mL) was added dropwise and the mixture was stirred at room temperature for 2 h. The solution was washed with a saturated Na_2CO_3 solution and twice with water. After drying ($MgSO_4$), filtration and evaporation, the residue was chromatographed on a silica gel column using CH_2Cl_2 as eluent to yield 3.47 g (79%) of **22b** as white crystals: mp 53 °C; R_f 0.26 (CH_2Cl_2/MeOH, 95:5, v/v); IR cm^{-1}: 1662 ($C=O_{al}$), 1630 ($C=O_{ar}$), 1600 ($C=C_{ar}$); ^1H NMR: δ 7.35 (q, 4H, J = 8.1 Hz), 4.57 (s, 2H), 3.8–3.3 (m, 8H), 2.31 (t, 2H, J = 7.3 Hz), 1.7–1.45 (m, 2H), 1.22 (ls, 28H), 0.85 (t, 3H, J = 6.1 Hz).

5.23. 1-(4-Cyanomethylbenzoyl)-4-octadecanoylpiperazine (28b)

To a solution of **22b** (3 g, 6 mmol) in dimethylsulfoxide (30 mL) was added NaCN (1.16 g, 24 mmol) and the mixture was warmed (80 °C) for 2 h. After adding CH_2Cl_2 (30 mL), the solution was washed twice with water, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was chromatographed on a silica gel column using MeOH/CH_2Cl_2 (99:1, v/v) as eluent to yield 1.9 g (64%) of the nitrile **28b**: mp 104 °C; R_f 0.36 (CH_2Cl_2/MeOH, 90:10, v/v); IR cm^{-1}: 2251 (CN), 1675 ($C=O_{al}$), 1625 ($C=O_{ar}$), 1600 ($C=C_{ar}$); ^1H NMR: δ 7.36 (q, 4H, J = 9.4 Hz), 3.80 (s, 2H), 3.49–3.58 (m, 8H), 2.3 (t, 2H, J = 7.1 Hz), 1.56–1.62 (m, 2H), 1.21 (ls, 28H), 0.83 (t, 3H, J = 6.4 Hz).

5.24. 1-(4-Cyanomethylbenzyl)-4-(4-octadecyloxyphenyl) piperazine (32a)

A mixture of **10a** (3.28 g, 7 mmol), 4-bromomethylphenylacetonitrile[23] (1.52 g, 7 mmol), K_2CO_3 (2.5 g, 18.2 mmol) and KI (0.5 g) in acetonitrile (200 mL) was heated to reflux for 6 h. The suspension was filtered and K_2CO_3 washed several times with CH_2Cl_2. The solvents were evaporated under vacuo and the residue taken up in CH_2Cl_2 (150 mL) was washed with water until neutral pH. After drying ($MgSO_4$), filtration and evaporation, a crystallization in acetone gave 2.60 g (66%) of **32a** as white crystals: mp 100 °C; R_f 0.6 (CH_2Cl_2/MeOH, 98:5, v/v); IR cm^{-1}: 2251 (CN), 1257 (C–O), 1600 ($C=C_{ar}$); ^1H NMR: δ 7.33 (q, 4H, J = 8.6 Hz), 6.86 (q, 4H, J = 9.1 Hz), 3.89 (t, 2H, J = 6.52 Hz), 3.73 (s, 2H), 3.56 (s, 2H), 3.09 (t, 4H, J = 5 Hz) 2.60 (t, 4H, J = 5.1 Hz), 1.74 (q, 2H, J = 7 Hz), 1.25 (ls, 30H), 0.88 (t, 3H, J = 6.2 Hz).

5.25. 4-(2,2,2-Trichloroethoxycarbonylamino) phenylacetonitrile (35)

A solution of 4-aminophenylacetonitrile (10 g, 75 mmol) in Et_3N (16 mL, 0.11 mol) and CH_2Cl_2 (100 mL) was cooled in an ice bath. A solution of 2,2,2-trichloroethyl chloroformate (19.23 g, 90 mmol) in CH_2Cl_2 (30 mL) was added dropwise and the mixture was stirred at 0 °C for 1 h. The solution was washed with a saturated Na_2CO_3 solution and twice with water. After drying ($MgSO_4$), filtration and evaporation, the residue was crystallized in acetone/pentane (10:90, v/v) to give 13.56 g (86%) of **35** as white crystals: mp 140 °C; R_f 0.46 (CH_2Cl_2/MeOH, 99:1, v/v); IR cm^{-1}: 3306 (NH), 2256 (CN), 1642 (C=O), 1607 ($C=C_{ar}$); ^1H NMR (DMSO-d_6): δ 10.20 (s, 1H), 7.4 (q, 4H, J = 8.4 Hz), 4.93 (s, 2H), 3.95 (s, 2H).

5.26. 4-(4-Cyanomethylbenzyl)-1-tetradecylpiperazin-2-one (36c)

To a mixture of 1-tetradecylpiperazin-2-one hydrochloride **15c** (2.95 g, 8.8 mmol), K_2CO_3 (2.6 g, 17.6 mmol) and KI (0.5 g) in acetonitrile (100 mL) was added 4-bromomethylphenylacetonitrile[23] (2.2 g, 10 mmol). The reaction mixture was heated to reflux for 4 h, then filtered and the solvent was removed in vacuo. The

residue was taken up in CH_2Cl_2 and washed with a saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (99:1, v/v) as eluent to give **36c** as a yellow oil (3.6 g, 95%); R_f 0.48 (CH_2Cl_2/MeOH, 95:5, v/v); IR cm^{-1}: 2249 (CN), 1647 (CO), 1505 (C=C); ^1H NMR: δ 7.19 (m, 4H), 3.67 (s, 2H), 3.47 (s, 2H), 3.26 (m, 4H), 3.07 (s, 2H), 2.58 (t, 2H, J = 5.39 Hz), 1.47 (m, 2H), 1.18 (m, 22H), 0.81 (t, 3H, J = 6.40 Hz).

5.27. 4-(4-Chloromethylbenzoyl)-1-tetradecylpiperazin-2-one (37c)

A solution of 1-tetradecylpiperazin-2-one hydrochloride **15c**, hydrochloride (6 g, 18 mmol) and triethylamine (7.6 mL, 54 mmol) in benzene (140 mL) was cooled to 0 °C and a solution of 4-chloromethylbenzoyl chloride (4.1 g, 21.6 mmol) in benzene (20 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The solvent was removed in vacuo. The remaining residue was taken up in CH_2Cl_2 and washed with a saturated Na_2CO_3 solution then with water. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (99:1, v/v) to afford **37c** as a yellow powder (4.6 g, 57%): mp 60.5 °C; R_f 0.28 (CH_2Cl_2/MeOH, 97:3, v/v); IR cm^{-1}: 1645 (CO), 1575 (C=C); ^1H NMR: δ 7.42–7.33 (m, 4H), 4.53 (s, 2H), 4.15 (ls, 2H), 3.81 (m, 2H), 3.33 (m, 4H), 1.49 (m, 2H), 1.18 (m, 22H), 0.81 (t, 3H, J = 6.39 Hz).

5.28. 1-(4-Cyanomethylbenzoyl)-1-tetradecylpiperazin-2-one (38c)

The intermediate **37c** (4.3 g, 9.6 mmol) was dissolved in DMSO (67 mL) and the solution was cooled to 0 °C. Sodium cyanide (1.87 g, 38.3 mmol) was added in small portions and the reaction mixture was heated to 50–55 °C for 2 h. After cooling to room temperature, water was added and the solution was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (99:1, v/v) as eluent to yield **38c** as a brown oil (3.7 g, 88%): R_f 0.22 (CH_2Cl_2/MeOH, 97:3, v/v); IR cm^{-1}: 2251 (CN), 1649 (CO), 1575 (C=C); ^1H NMR: δ 7.43–7.32 (m, 4H), 4.12 (ls, 2H), 3.88 (m, 2H), 3.74 (s, 2H), 3.33 (m, 4H), 1.49 (m, 2H), 1.19 (m, 22H), 0.81 (t, 3H, J = 6.39 Hz).

5.29. 1-(4-Cyanobenzyl)-4-tetradecylpiperazin-2-one (39c)

Sodium hydride (60% dispersion in oil, 560 mg, 14 mmol) was added to a stirred solution of 1-tetradecylpiperazin-3-one **16c** (3.47 g, 11.68 mmol) in freshly distilled THF (50 mL). The mixture was stirred for 1 h at room temperature under argon atmosphere and a solution of *p*-cyanobenzyle bromide (2.97 g, 15.2 mmol) in THF (10 mL) was added dropwise at 0 °C. After stirring for 30 min, the reaction mixture was allowed to warm to room temperature and stirred for 18 h. MeOH (2 mL) was added and the solvent was removed in vacuo. The resulting residue was taken up in CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (99:1, v/v) to afford **39c** as a white powder (4.3 g, 90%): mp 59.5 °C; R_f 0.26 (CH_2Cl_2/MeOH, 97:3, v/v); IR cm^{-1}: 2230 (CN), 1638 (CO), 1596 (C=C); ^1H NMR: δ 7.55 (d, 2H, J = 8.18 Hz), 7.30 (d, 2H, J = 8.19 Hz), 4.57 (s, 2H), 3.19 (t, 2H, J = 5.52 Hz), 3.16 (s, 2H), 2.59 (t, 2H, J = 5.39 Hz), 2.32 (t, 2H, J = 7.30 Hz), 1.41 (m, 2H), 1.19 (m, 22H), 0.81 (t, 3H, J = 6.33 Hz).

5.30. 1-(4-*N*-Hydroxyamidinomethylbenzoyl)-4-octadecanoylpiperazine (43b)

To a solution of **28b** (2 g, 4 mmol) in ethanol (50 mL) was added NH_2OH (50% w/w solution in water, 0.47 mL, 16 mmol). After refluxing at 80 °C for 5 h, concentration to dryness afforded 2.15 g (97%) of amidoxime **43b** as a yellow solid in sufficient purity for use in the next step without purification: mp 128 °C; R_f 0.44 (CH_2Cl_2/MeOH, 90:10, v/v); IR cm^{-1}: 3370 (OH), 3488 (NH), 1656 (C=O_{al}), 1634 (C=O_{ar}), 1610 (C=N); ^1H NMR: δ 7.32 (s, 4H), 4.55 (s, 2H), 3.58–3.48 (m, 8H), 3.45 (s, 2H), 2.31 (t, 2H, J = 7.3 Hz), 1.45–1.65 (m, 2H), 1.23 (ls, 28H), 0.82 (t, 3H, J = 6.67 Hz).

5.31. 1-[4-(4,5-Dihydro-1,2,4-(4*H*)-5-oxo-oxadiazol-3-ylmethyl) benzoyl]-4-octadecanoyl piperazine (57b)

Phenyl chloroformate (0.75 g, 4 mmol) was added dropwise to an ice-cooled mixture of amidoxime **43b** (2.15 g, 4 mmol) and Et_3N (0.68 g, 4.8 mmol) in dry THF (30 mL). The resulting mixture was stirred at 0 °C for 1 h, the solvent was evaporated under reduced pressure and the residue, taken up in CH_2Cl_2, was washed with a saturated Na_2CO_3 solution and twice with water. After drying ($MgSO_4$), filtration and evaporation, the residue was dissolved in toluene and heated under reflux for 4 h. The reaction mixture was concentrated in vacuo and the residue was chromatographed on a silica gel column using CH_2Cl_2/MeOH (98:2, v/v) as eluent to yield 1.5 g (68%) of **57b** as white crystals: mp 94 °C; R_f 0.51 (CH_2Cl_2/MeOH, 90:10, v/v); IR cm^{-1}: 3460 (N–H), 1780 (OCON), 1665 (C=O_{al}), 1636 (C=O_{ar}), 1610 (C=N); ^1H NMR: δ 11.20 (s, 1H), 7.25 (s, 4H), 3.79 (s, 2H), 3.75–3.30 (m, 8H), 2.3 (t, 2H, J = 4 Hz), 1.7–1.45 (m, 2H), 1.20 (ls, 28H), 0.82 (t, 3H, J = 5.9 Hz); ^{13}C NMR : δ 172.24, 170.23, 160.00, 157.69, 135, 134.03, 129.03, 127.48, 45.30, 41.33, 33.29, 31.73, 31.10, 29.51, 29.17, 25.11, 22.50, 13.95. Anal. ($C_{32}H_{50}N_4O_4$) C, H, N.

5.32. 1-[4-(4,5-Dihydro-1,2,4-(4*H*)-5-oxo-oxadiazol-3-ylmethyl)phenylaminocarbonyl]-4-octadecylpiperazine (71a)

A mixture of **64** (1.08 g, 3 mmol) and 1-octadecylpiperazine **6a**[23] (1 g, 3 mmol) in acetonitrile (20 mL) was heated to reflux for 5 h. A white precipitate appeared and the hot suspension was filtered to yield 1 g of **71a** (60%) as a beige powder: mp 160 °C; R_f 0.32 (CH_2Cl_2/MeOH, 90:10, v/v); IR cm^{-1}: 3356 (NH), 1788 (OCON), 1630 (C=O), 1597 (C=C_{ar}); ^1H NMR: δ 8.86 (s, 1H), 7.31 (q, 4H, J = 8.4 Hz), 3.79 (s, 2H), 3.33 (ls, 8H), 2.98 (t, 2H, J = 7.9 Hz), 1.7–1.55 (m, 2H), 1.23 (ls, 30H), 0.85 (t, 3H, J = 6.55 Hz); ^{13}C NMR: δ 159.76, 159.20, 154.55, 139.36, 128.82, 127.11, 119.85, 55.66, 50.72, 31.26, 30.00, 29.01, 28.79, 28.53, 26.11, 22.06, 13.93. Anal. ($C_{32}H_{53}N_5O_3$) C, H, N.

5.33. 1-[4-(4,5-Dihydro-1,2,4-(4*H*)-5-oxo-oxadiazol-3-ylmethyl) phenylaminocarbonyl]-4-octadecylaminocarbonylpiperazine (72a)

Compound **72a** was obtained in 56% yield following the same procedure as for **71a** but using **5a**: mp 198 °C; R_f 0.44 (CH_2Cl_2/MeOH, 90:10, v/v); IR cm^{-1}: 3365 (NH), 1787 (OCON), 1632 (C=O), 1599 (C=C_{ar}); ^1H NMR (DMSO-d_6): δ 8.55 (s, 1H), 7.42 (d, 2H, J = 8.2 Hz), 7.15 (d, 2H, J = 8.4 Hz), 6.5 (ls, 1H) 3.77 (s, 2H), 3.35 (ls, 8H), 2.99 (t, 2H, J = 5.72 Hz), 1.5–1.3 (m, 2H), 1.23 (m, 30H), 0.84 (t, 3H, J = 6.51 Hz); ^{13}C NMR (DMSO-d_6): δ 160.10, 159.42, 157.36, 154.94, 139.64, 128.77, 126.89, 119.82, 43.54, 43.26, 31.29, 30.10, 29.80, 29.02, 28.70, 26.43, 22.09, 13.95. Anal. ($C_{33}H_{54}N_6O_4$) C, H, N.

Acknowledgements

This work has been supported by the PAI contract P6/19. G.D. is senior research associate of the F.R.S and thanks the F.R.S. for the financial support of the high performance computing systems installed at Liège and Louvain-la-Neuve.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.03.049.

References and notes

1. Murakami, M.; Kudo, I. *J. Biochem.* **2002**, *131*, 285.
2. Schaloske, R. H.; Dennis, E. A. *Biochim. Biophys. Acta* **2006**, *1761*, 1246.
3. 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. *J. Biol. Chem.* **2002**, *277*, 48535.
4. Lee, H. Y.; Bahn, S. C.; Shin, J. S.; Hwang, I.; Back, K.; Doelling, J. H.; Beungtae Ryu, S. *Prog. Lipid Res.* **2005**, *44*, 52.
5. Kuchler, K.; Gmachl, M.; Sippl, M. J.; Kreil, G. *Eur. J. Biochem.* **1989**, *184*, 249.
6. Ryu, Y.; Oh, Y.; Yoon, J.; Cho, W.; Baek, K. *Biochim. Biophys. Acta* **2003**, *1628*, 206.
7. McIntosh, J. M.; Ghomashchi, F.; Gelb, M. H.; Dooley, D. J.; Stoehr, S. J.; Giordani, A. B.; Naisbitt, S. R.; Olivera, B. M. *J. Biol. Chem.* **1995**, *270*, 3518.
8. Manjunatha Kini, R. *Toxicon* **2003**, *42*, 827.
9. Valentin, E.; Lambeau, G. *Biochim. Biophys. Acta* **2000**, *1488*, 59.
10. De Haas, G. H.; Postema, N. M.; Nieuwenhuizen, W.; Van Deenen, M. *Biochim. Biophys. Acta* **1968**, *159*, 118.
11. Murakami, M.; Nakatani, Y.; Atsumi, G.; Inoue, K.; Kudo, I. *Crit. Rev. Immunol.* **1997**, *17*, 225.
12. Seilhamer, J. J.; Randall, T. L.; Yamanaka, M.; Johnson, L. K. *DNA* **1986**, *5*, 519.
13. Arita, H.; Hanasaki, K.; Nakano, T.; Oka, S.; Teraoka, H.; Matsumoto, K. *J. Biol. Chem.* **1991**, *266*, 19139.
14. Nakajima, M.; Hanasaki, K.; Ueda, M.; Arita, H. *FEBS Lett.* **1992**, *309*, 261.
15. Sommers, C. D.; Bobbitt, J. L.; Bemis, K. G.; Snyder, D. W. *Eur. J. Pharmacol.* **1992**, *216*, 87.
16. Kishino, J.; Ohara, O.; Nomura, K.; Kramer, R. M.; Arita, H. *J. Biol. Chem.* **1994**, *269*, 5092.
17. Rae, D.; Beechey-Newman, N.; Burditt, L.; Sumar, N.; Hermon-Taylor, J. *Scand. J. Gastroenterol. Suppl.* **1996**, *219*, 24.
18. Hanasaki, K.; Yakota, Y.; Ishizaki, J.; Itoh, T.; Arita, H. *J. Biol. Chem.* **1997**, *272*, 32792.
19. Seilhamer, J.; Pruzanski, W.; Vadas, P.; Plant, S.; Miller, J.; Kloss, J.; Johnson, L. *J. Biol. Chem.* **1989**, *264*, 5335.
20. Nevalainen, T. J.; Haapamaki, M. M.; Gronroos, J. M. *Biochim. Biophys. Acta* **2000**, *1488*, 83.
21. Pruzanski, W.; Lambeau, L.; Lazdunsky, M.; Cho, W.; Kopilov, J.; Kuksis, A. *Biochim. Biophys. Acta* **2005**, *1736*, 38.
22. Boyanovsky, B. B.; Van der Westhuyzen, D. R.; Webb, N. R. *J. Biol. Chem.* **2005**, *280*, 32746.
23. Wooton-Kee, C. R.; Boyanovsky, B. B.; Nasser, M. S.; de Villiers, W. J.; Webb, N. R. *Arterioscler. Thromb. Vasc. Biol.* **2004**, *24*, 762.
24. Enomoto, A.; Murakami, M.; Valentin, E.; Lambeau, G.; Gelb, M. H.; Kudo, I. *J. Immunol.* **2000**, *165*, 4007.
25. Kini, R. M. *Toxicon* **2005**, *45*, 1147.
26. Mounier, C. M.; Bon, C.; Kini, R. M. *Haemostasis* **2001**, *31*, 279.
27. Koduri, R. S.; Gronroos, J. O.; Laine, V. J.; Le Calvez, C.; Lambeau, G.; Nevalainen, T. J.; Gelb, M. H. *J. Biol. Chem.* **2002**, *277*, 5849.
28. Beers, S. A.; Buckland, A. G.; Koduri, R. S.; Cho, W.; Gelb, M. H.; Wilton, D. C. *J. Biol. Chem.* **2002**, *277*, 1788.
29. Laye, J. P.; Gill, J. H. *Drug Discovery Today* **2003**, *8*, 710.
30. Assogba, L.; Ahamada-Himidi, A.; Meddad-Belhabich, N.; Aoun, D.; Boukli, L.; Massicot, F.; Mounier, C.; Huet, J.; Lamouri, A.; Ombetta, J.-E.; Godfroid, J.; Dong, C.-Z.; Heymans, F. *Eur. J. Med. Chem.* **2005**, *40*, 850.
31. (a) Dong, C.-Z.; Ahamada-Himidi, A.; Plocki, S.; Aoun, D.; Touaibia, M.; Meddad-Bel Habich, N.; Huet, J.; Redeuilh, C.; Ombetta, J.-E.; Godfroid, J.-J.; Massicot, F.; Heymans, F. *Bioorg. Med. Chem.* **2005**, *13*, 1989; (b) Plocki, S.; Aoun, D.; Ahamada-Himidi, A.; Tavarès-Camar-inha, F.; Dong, C.-Z.; Massicot, F.; Huet, J.; Adolphe-Pierre, S.; Chau, F.; Godfroid, J.-J.; Gresh, N.; Ombetta, J.-E.; Heymans, F. *Eur. J. Org. Chem.* **2005**, *13*, 2747.
32. Boukli, L.; Touaibia, M.; Meddad-Belhabich, N.; Djimdé, A.; Park, C. H.; Kim, J.-J.; Yoon, J.-H.; Lamouri, A.; Heymans, F. *Bioorg. Med. Chem.* **2008**, *16*, 1242.
33. Ohtaka, H.; Kanazawa, T.; Ito, K.; Tsukamoto, G. *Chem. Pharm. Bull.* **1987**, *35*, 3270.
34. Dimaio, J.; Belleau, B. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1687.
35. Kitamura, S.; Fukushi, H.; Miyawaki, T.; Kawamura, M.; Konishi, N.; Terashita, Z.-I.; Naka, T. *J. Med. Chem.* **2001**, *44*, 2438.
36. Choi, S.-W.; Elmaleh, D. R.; Hanson, R. N.; Fischman, A. J. *J. Med. Chem.* **1999**, *42*, 3647.
37. Soukara, S.; Wünsch, B. *Synthesis* **1999**, *10*, 1739.
38. Bellouard, F.; Chuburu, F.; Kervarec, N.; Toupet, L.; Triki, S.; Le Mest, Y.; Handel, H. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3499.
39. Cheeseman, G. W. H.; Freestone, A. J.; Godwin, R. A.; Hough, T. L. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1888.
40. Adams, R.; Thal, A. F. *Org. Synth. Coll.* (second edition) **1941**, *1*, 107.
41. Jerzy, R.; Pietraseiwick, M.; Duszynska, B.; Cegla, M. T. *J. Med. Chem.* **1992**, *35*, 2369.
42. WU, C.; Decker, E. R.; Blok, N.; Bui, H.; Chen, Q.; Raju, B.; Bourgoyne, A.; Knowles, V.; Biediger, R.; Market, R.; Lin, S.; Dupré, B.; Kogan, T.; Holland, G.; Brock, T.; Dixon, R. *J. Med. Chem.* **1999**, *42*, 4485.
43. Choong, I. C.; Lew, W.; Lee, D.; Pham, P.; Burdett, M. T.; Lam, J. W.; Wiesmann, C.; Luong, T. N.; Fahr, B.; DeLano, W. L.; McDowell, R. S.; Allen, D. A.; Erlanson, D. A.; Gordon, E. M.; O'Brien, T. *J. Med. Chem.* **2002**, *45*, 5005.
44. Kohara, Y.; Kubo, K.; Imamiya, E.; Wada, T.; Inada, Y.; Naka, T. *J. Med. Chem.* **1996**, *39*, 5228.
45. Dillard, R. D.; Bach, N. J.; Draheim, S. E.; Berry, D. M.; 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.; Schevitz, R. W.; Smith, A. C.; Snyder, D. W.; Sommers, C.; Wery, J. P. *J. Med. Chem.* **1996**, *39*, 5119.
46. Ditchfield, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724.
47. GAUSSIAN 03, Revision C.02, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A. Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J.,M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A.; Gaussian, Inc., Wallingford CT, 2004.
48. Radvanyi, F.; Jordan, L.; Russo-Marie, F.; Bon, C. *Anal. Biochem.* **1989**, *117*, 103.
49. Rekker, R. F.; De Kort, H. M. *Eur. J. Med. Chem. Chim. Ther.* **1979**, *14*, 479.