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Design of new potent and selective secretory phospholipase A_2 inhibitors. Part 5: Synthesis and biological activity of 1-alkyl-4-[4,5-dihydro-1,2,4-[4*H*]-oxadiazol-5-one-3-ylmethylbenz-4′-yl(oyl)] piperazines

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Abstract—Among the different PLA_2s identified to date, the group IIA secretory PLA_2 ($sPLA_2$ GIIA) is implied in diverse pathological conditions. In this work we describe the synthesis, inhibitory activities, and structure–activity relationships (SAR) of a new class of substituted piperazine derivatives. The in vitro fluorimetric assay using two groups of enzymes, GIB and GIIA, revealed several compounds as highly potent inhibitors (IC_{50} = 0.1 μM). The in vivo activity assessed by ip or per os administration in a carrageenan-induced edema test in rats showed that two compounds proved to be as potent as indomethacin (10 mg/kg).

1. Introduction

The phospholipases A_2 (PLA_2) super family is a wide variety of enzymes that by definition hydrolyze the *sn*–2 ester bond of membrane glycerophospholipids, leading to a lysophospholipid and releasing a fatty acid. When the latter is arachidonic acid (AA),[1] it is further metabolized by cyclooxygenases (COX-1 and COX-2) or lipoxygenase (LOX) to give prostaglandins, thromboxans, and leukotrienes, well-known mediators of inflammation. When the lysophospholipid is the lyso-PAF, it can be converted by the acetyltransferase into Platelet-Activating Factor (PAF), another mediator of inflammatory process and tissue injury.[2] PLA_2 enzymes are classified into four types (12 groups): cytosolic calcium-dependent PLA_2 ($cPLA_2$), calcium-independent PLA_2 ($iPLA_2$), low molecular-weight secretory PLA_2 ($sPLA_2$), and PAF acetylhydrolase (PAF-AH).[3,4] Despite that all enzymes in each group share a number of characteristics, the calcium-dependent $sPLA_2$s which are worth attention demonstrate a diverse range of functions and properties. Some appear to be tissue-specific.[5] Group IIA, V, and X secretory PLA_2s ($sPLA_2$s) are recognized to be involved in AA release and subsequent eicosanoïd production under inflammatory conditions.[6] Particularly group IIA PLA_2 (GIIA PLA_2) was demonstrated to play an important pathophysiological role in various inflammatory diseases, such as arthritis,[7] septic shock,[8] adult respiratory distress syndrome,[9] multiple injuries,[10] diffuse peritonitis,[11,12] and acute pancreatitis.[13–15] In these human pathologies, highly significant increases in serum catalytic PLA_2 activity have been described which correlate well with the severity of the disease. Furthermore, $sPLA_2$s play a pivotal role in propagation and amplification of other inflammatory disorders. The observation that many tumors have elevated eicosanoïd levels[16,17] shows their central role in cancer development. Then the importance to find a drug able to neutralize the $sPLA_2$ involved is undeniable. Another advantage of PLA_2 inhibitors is the reduction of PAF levels. A multitude of structurally diverse compounds described in the literature[18–22] have been

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reported to be PLA_2 inhibitors in vitro and some have shown anti-inflammatory activity,[23] but the multiple forms of PLA_2 tend to complicate the elucidation of the cellular mechanisms that regulate AA release and the subsequent eicosanoïd production. We have demonstrated previously[24–26] that a good GIIA PLA_2 inhibitor must respect a number of parameters, among them: (i) an adequate length of chain[26] which allows a good interaction with the hydrophobic pocket of the active site; (ii) an aromatic ring able to induce a π–π stacking interaction; and (iii) an oxadiazolone heterocycle[24] whose role is capital by interacting with calcium in its anionic form and which seems largely responsible for the selectivity.[25] This might explain the high affinity and the good activity of compound **II** (Fig. 1), our first leader.[26]

In this new series (Scheme 4) we first introduce a piperazine ring in the alkyl chain of **II** and modulate chemically the piperazine nitrogen substituent which carries the oxadiazolone heterocycle in order to rigidify more or less the system. Furthermore, a possible protonation of the nitrogen atom(s) of the piperazinic cycle might be able to lower lipophilicity as compared to our inhibitors reported before[24–26] presaging a better biodisponibility. In fact, this series is a combination of previously described families of active but not selective inhibitors of GIB and GIIA $sPLA_2$s as **I**,[19] and active, selective but highly lipophilic inhibitor of GIIA PLA_2 as **II**[24–26] (Fig. 1).

All the synthesized compounds were evaluated as $sPLA_2$ inhibitors (GIB and GIIA) in an in vitro enzymatic test (see biological section). Some of them were then selected and an in vivo activity was assayed using the carrageenan-induced paw edema test in rats. We report here the synthesis and SAR study of this novel series and discuss with regard to the anti-PLA_2 and in vivo activities.

2. Chemistry

This new series of molecules displays piperazine dissymmetric derivatives with one of the two nitrogens substituted by an alkyl chain and the second carrying an oxadiazolone cycle through the benzyl(oyl) group. The key intermediates of the synthesis are the nitriles **2**–**4**, **6**, **10**, and **15** which were prepared using different methods as described below. Nearly all compounds shown in Table 1 were synthesized starting from the corresponding substituted piperazines **1a**–**e** (Scheme 1) prepared by direct monoalkylation of piperazine with the appropriate alkyl halide in very good yields. To avoid dialkylation, this reaction was carried out, by drop by drop addition of the alkyl halide to a large excess of piperazine.

The alkyl piperazines **1a**–**c** were then alkylated on *N*-4 position using either bromo-*p*-tolunitrile or 4-bromomethylphenylacetonitrile **7** leading to compounds **2a**–**c** and **3a**–**c**, respectively. The **1a**–**e** derivatives were also acylated either with 4-cyanobenzoyl chloride to give the intermediates **4a**–**c** or with 4-chloro (or bromo)methylbenzoyl chloride at low temperature to prevent a nucleophilic substitution at the 4-chloromethylene level leading to **5a**–**e**. This latter was then treated by sodium cyanide to give the nitriles **6a**–**e**.

4-Bromomethylphenylacetonitrile **7** which is not commercially available was prepared as outlined in Scheme 1, from 4-methylphenylacetonitrile using *N*-bromosuccinimide (NBS) and a catalytic amount of 2,2′-azobis-2-methylpropionitrile (AIBN). Due to the presence of two bromation sites in the molecule and the radicalar process of the reaction, this step was hardly controllable. The bad yield (35%) in the obtention of **7** is explained by the formation of two supplementary derivatives in nearly equivalent amounts: the 4-bromomethylphenylbromo acetonitrile (23%) and the 4-methylphenylbromoacetonitrile (25%). Nevertheless, the isolation of **7** was possible using distillation.

Compounds without a linker between piperazine and phenyl were prepared in five steps from diethanolamine (Scheme 2). N-Alkylation by an alkyl bromide according to Jerzy et al.[27] led to the derivatives **8a**–**c**. The hydroxyl groups were converted into chlorides **9a**–**c** using thionyl chloride according to Newman et al.[28] Cyclization into piperazines **10a**–**c** was then performed by action of the 4-aminophenylacetonitrile on the corresponding dichloride.

The introduction of two methylenes between phenyl and oxadiazolone moieties was performed through the preparation of the nitrile carboxylic acid derivative **14a** by the method of Brouard et al.[29] adapted to our use. Coupling **14a** with the alkyl piperazine **1a** gave the intermediate **15a** (Scheme 3). To introduce one more methylene group, the method above was unsuccessful. However, a solution was found by the preparation of the bromide derivative **11** from action of phosphore tribromide on 3-phenylpropan-1-ol (Scheme 3). Treatment of **11** with acetyl chloride under Friedel and Crafts conditions gave

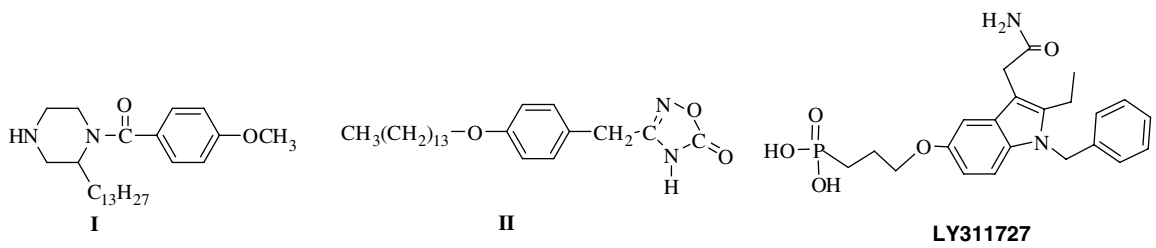
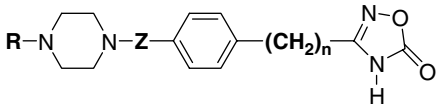


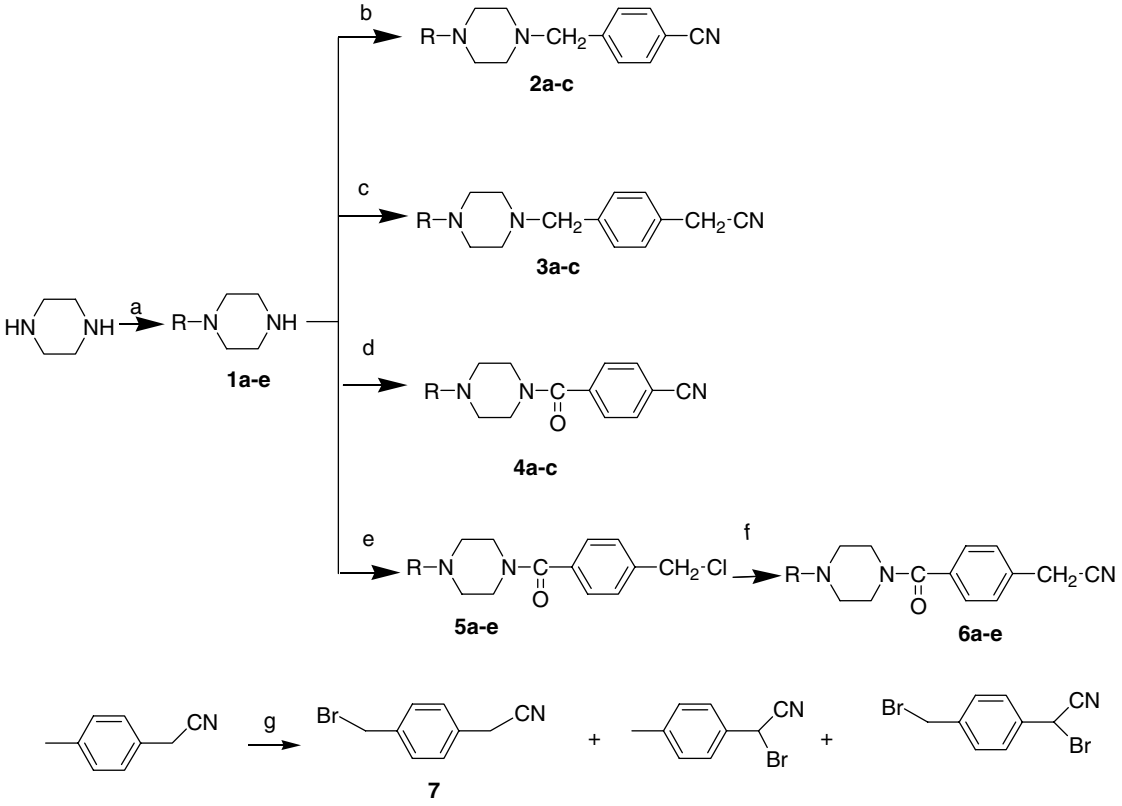
Figure 1. Synthetic PLA_2 inhibitors.

Table 1. Inhibition of the enzymatic activity of porcine pancreatic PLA_2 (GIB) $PPLA_2$ and human PLA_2 (GIIA) $HPLA_2$ by piperazine derivatives of PMS 1062, using the fluorimetric assay

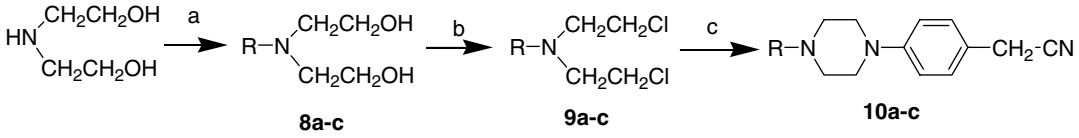


Compound	R	Z	*n*	Log,*P*[a]	IC_{50} (μM)	
					$HPLA_2$	$PPLA_2$
23a	$C_{14}H_{29}$	CH_2	0	5.301	>100	>100
23b	$C_{16}H_{33}$	CH_2	0	6.339	>100	>100
23c	$C_{18}H_{37}$	CH_2	0	7.370	5.08	>100
24a	$C_{14}H_{29}$	CH_2	1	5.593	>10	>100
24b	$C_{16}H_{33}$	CH_2	1	6.631	10	>100
24c	$C_{18}H_{37}$	CH_2	1	7.669	2.2	>100
25a	$C_{14}H_{29}$	CO	0	5.480	>100	>100
25b	$C_{16}H_{33}$	CO	0	6.526	>100	>100
25c	$C_{18}H_{37}$	CO	0	7.564	1.1	>100
26a	$C_{14}H_{29}$	CO	1	5.780	9	>100
26b	$C_{16}H_{33}$	CO	1	6.814	4	>100
26c	$C_{18}H_{37}$	CO	1	7.856	0.8	>100
26d	$C_{20}H_{41}$	CO	1	8.894	0.1	>100
26e	$C_{22}H_{45}$	CO	1	9.932	0.1	>100
27a	$C_{14}H_{29}$	Deleted	1	5.949	4.5	>100
27b	$C_{16}H_{33}$	Deleted	1	6.983	3.5	>100
27c	$C_{18}H_{37}$	Deleted	1	8.017	2.71	>100
28a	$C_{14}H_{29}$	CO	2	6.297	18.7	>100
29a	$C_{14}H_{29}$	CO	3	6.814	5.3	>100
II				7.08[b]	4	>100
LY311727					0.47	8

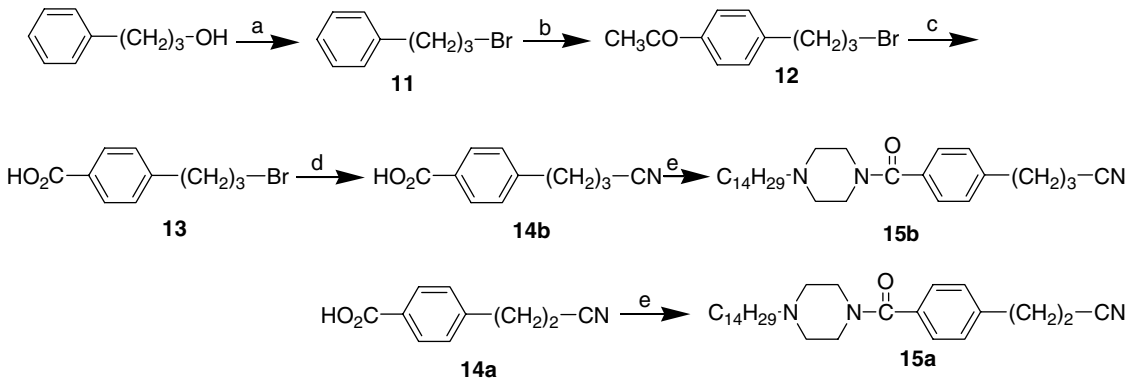
[a] Log *P* of the free base was calculated using the Rekker's hydrophobic fragmental constants method.[33]
[b] See Ref. 25.



Scheme 1. Reagents and conditions: (a) R–Br, THF, CH_2Cl_2, rt; (b) $BrCH_2pC_6H_4CN$, K_2CO_3, KI, CH_3CN, reflux; (c) **7**, K_2CO_3, KI, CH_3CN, reflux; (d) $ClCOpC_6H_4CN$, Et_3N, anhyd C_6H_6; (e) $ClCOpC_6H_4CH_2Cl$, Et_3N, C_6H_6, rt; (f) NaCN, DMSO, 60 °C; (g) NBS, AIBN, CCl_4, reflux.



Scheme 2. Reagents and conditions: (a) R–Br, K_2CO_3, KI, CH_3CN; (b) $SOCl_2$, $CHCl_3$, rt; (c) $H_2N_pC_6H_4CH_2CN$, KI, CH_3CN, reflux.



Scheme 3. Reagents: (a) PBr_3, CH_2Cl_2; (b) CH_3COCl, $AlCl_3$, CS_2; (c) NaOH, Br_2, H_2O, dioxane; (d) NaCN, DMF; (e) **1a**, DCC, Et_3N, CH_2Cl_2.

the ketone **12** which was transformed into the corresponding acid **13** by a bromoform reaction. Substitution of the bromide using sodium cyanide led to the cyano-acid **14b** which was then coupled with the alkyl piperazine to give the nitrile **15b**.

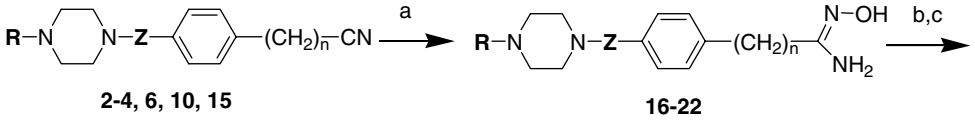
Treatment of all these nitrile derivatives as described previously,[24] that is, first by hydroxylamine to lead to the expected amidoximes **16**–**22**, second using phenylchloroformate, then heating the carbonate obtained in refluxing toluene for cyclization (Scheme 4), transformed them into the final oxadiazolones **23**–**29**.

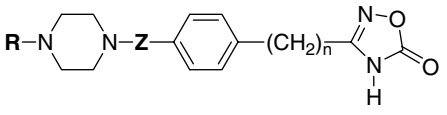
In order to improve the yields of different steps (synthesis of compounds **28a** and **29a**), we chose to follow another strategy consisting in condensation of alkylpiperazine **1a**–**e** with the oxadiazolylmethylbenzoic acid, prepared as previously reported by Kitamura et al.[30] in the presence of dicyclohexylcarbodiimide (DCC), a coupling agent largely used in peptide synthesis.

3. Results and discussion

In our previous publications leading to **II**,[24–26] our home reference, we showed on one hand the necessity of an optimal length of 14 carbons for hydrophobic interactions[26] with the sub-sites of the enzyme, an aromatic ring probably for the interactions π–π or cation–π and on the other hand, a heterocycle: the oxadiazolone, a polar hydrophilic head[24] which could interact with the calcium ion inside the active site and would be responsible for the selectivity. [25]

In this work, we introduce the piperazinic ring while keeping the same modifications as those studied for **II**,





	Z	n	
16	CH_2	0	**23**
17	CH_2	1	**24**
18	CO	0	**25**
19	CO	1	**26**
20	deleted	1	**27**
21	CO	2	**28**
22	CO	3	**29**

Scheme 4. Reagents and conditions: (a) NH_2OH, HCl, K_2CO_3, EtOH, reflux; (b) ClCOOPh, NEt_3, CH_2Cl_2; (c) toluene, reflux.

that is, number of methylenes between the oxadiazolyl and phenyl groups (compounds **23a**, **24a**, **28a**, and **29a**) and variation of the alkyl chain length between 14 and 22 carbon atoms. The piperazine ring, relatively flexible but supposed to be a little more rigid, due to the privileged chair conformations, than the alkyl chain in **II**, was also introduced to estimate the variation of the flexibility at this level on activity. For this reason, we rendered this cycle less flexible by substitution of the nitrogen carrying the oxadiazolyl bearing part by a carbonyl group (compounds **25a–c** and **26a–e**) or branching directly the phenyl group (compounds **27a–c**). Our principal aim was also to reduce the global lipophilicity rendered possible by nitrogen protonation at physiological pH, for a better biodisponibility compared to that of **II** which appeared inactive in vivo.

3.1. In vitro activity

All the synthesized compounds were tested on group IIA versus IB enzymes. As shown in Table 1, all compounds **23–29** are, at the first glance, either inactive on the PLA_2 of the two groups (**23a**, **23b**, **25a–25b**) or, like the reference product **II**, only inhibitors of group II $sPLA_2$, thus with the required selectivity. Several of them, **26a–c**, are twice less (**26a**, $IC_{50} = 9\ \mu M$) to 40 times more active (**26d** and **26e**, $IC_{50} = 0.1\ \mu M$) than the reference **II** ($IC_{50} = 4\ \mu M$) and the more active **26d** and **26e** are as potent as Lilly's Laboratories, **LY311727**[21] a compound very structurally different from ours which was described in the literature as very potent and that we included in our assay for comparison, being then tested in the same conditions as ours.

In this study, we can verify that, in agreement with our previous results,[24] a methylene spacer between the phenyl ring and the oxadiazolone is favorable to inhibition: **23a** and **23b** as well as **25a** and **25b** (n = 0) are inactive while **26a**, **26b**, **27a**, and **27b** (n = 1) are as active as the reference **II**. Nevertheless, this can be compensated by an increase of the lipophilicity as shown for **23c** and **25c** which have curiously the same log *P* (close to 7 units) as our leader **II**. This improvement is necessarily due not only to the adequate lipophilic character, but it can be postulated that introduction of more flexibility between those cycles allows a better access of the oxadiazolone to the calcium binding site. But, no increase of the activity appears anymore with the addition of one or two methylenes to this linker (**28a** and **29a**).

Just introducing the piperazinic moiety in the alkyl chain of **II**, as shown in **24b** and **24c**, while keeping the same lipophilicity does not afford any improvement as the IC_{50}s are of the same order of magnitude. This is not surprising as; in this case, the sp^3 hybridization of the nitrogens is the same as, for the carbon atoms of the alkyl chain, leading to a rather high flexibility. On the other hand, as already described with non-selective inhibitors before,[19] compounds with Z = CO (**25c**, $IC_{50} = 1.1\ \mu M$, **26c**, $IC_{50} = 0.8\ \mu M$) are between two and three times more active than their analogs having $Z = CH_2$ (**23c**, $IC_{50} = 5.08\ \mu M$ and **24c**, $IC_{50} = 2.2\ \mu M$). Then it looks like if the partial rigidification of the piperazinic cycle, obtained when the nitrogen branched to the oxadiazolyl carrying moiety looses its sp^3 character to sp^2, was favorable to this activity. Good results are also obtained when Z is deleted and the phenyl directly branched to the nitrogen (**27a–c**), probably for the same reasons. Several hypothesis can explain this fact: (i) a beneficial change of the conformation due to the amide function in one case and the conjugation between the nitrogen doublet and the phenyl in the second, to adapt the most effective position in the catalytic site; (ii) a new electronic distribution with the presence of the carbonyl induced a negative electrostatic potential which can interact with a sub-site of the enzyme; (iii) a hydrogen bond formation between this same carbonyl and the catalytic site. Effectively, following the works of Poi et al.[31] which demonstrated the evidence and characterized a unique low barrier hydrogen bond (LBHB) between the imidazole ring of the histidine 48 and the oxygen of a transition state analog inhibitor, a conformational calculation study would certainly show whether the carbonyl group of the amidified piperazine is involved or not in a hydrogen bond with histidine 48 or another residue near the active site of the enzyme.

Concerning the lipophilicity, most of the active compounds of this series need a log *P* value for the free base (from 7.5 to 10 U) at least as high as reference **II** and in the homogenous series **26a–e**, by example, the activity increases as a function of this log *P* value. This is not surprising as the in vitro activity is measured directly on purified enzymes where the catalytic site is well known to be located at the end of a highly hydrophobic channel adapted to accommodate long alkyl chains of phospholipids.

3.2. In vivo activities

They were measured in two different models of inflammation, in one hand, in the classical carrageenan-induced edema test; compounds were administered ip or per os 1 h before the injection of carrageenan into the hind leg of the rats and the volume of edema was measured 0, 3 and 5 h after the injection of carrageenan. In the other hand, to evaluate the topical anti-inflammatory activity cutaneous inflammation was induced on the inner surface of the ear of anaesthetized mice by application of croton oil and the reduction of the edema by the selected compounds was measured after local application or per os administration.

In spite of its moderate in vitro activity, compound **26a** was selected by the pharmacologists, mainly for its low lipophilicity in this series, to research possible anti-inflammatory properties in a complementary pharmacological test. Compound **24a** was also selected for comparison because it has a similar lipophilicity to **26a** and as previous studies showed us some derivatives, in spite of no in vitro activity, sometimes presented surprisingly sometimes interesting effects in vivo. The two compounds administrated ip or po were evaluated on acute responses where mammalian group II $sPLA_2$ is supposed to be involved and compared to indomethacin, a well-known anti-inflammatory agent which acts at

Table 2. Local anti-inflammatory effect by topical administration of the pms compounds in the ear edema test induced by croton oil

Compound	Dose (mg/ear)/ (mmol)	Number of animals[a]	Edema inhibition (%)
24a	1/0.00212	56 (7)	51.45 ± 11.67
26a	1/0.00206	56 (7)	47.83 ± 12.34
Indomethacin	0.5/0.00139	72 (9)	43.11 ± 8.79

[a] The numbers in parentheses imply the number of performed experiments.

the COX level rather than inhibiting PLA_2. In the carrageenan-induced edema formation test, preliminary results by the intraperitoneal route show that the two compounds possessed an equivalent activity. Thus, at a 10-mg/kg dose, the inhibitions of edema by indomethacin and **24a** were 79% and 73%, respectively. By the oral route, **24a** had also a longer activity in time than that of indomethacin. In addition, 5 h after carrageenan injection, **26a** inhibited always 65% of the edema against 16% remaining effect for indomethacin, when the two products were administered orally at a dose of 10 mg/kg. In the same conditions the activity of **II** ($\log P_{cal} = 7$), *our home reference, disappeared completely. These results suggest that the high lipophilic character of* **II** could prevent this latter totally and the indomethacin ($\log P_{exp} = 4.27$) partially, from a good biodistribution. The protonation ability at physiological pH of our piperazinic derivatives leading to lower lipophilic compounds could explain the best per os activity. The same compounds were assayed for local anti-inflammatory effect by topical administration in the ear edema induced by croton oil and the data are reported in Table 2.

According to these results, **26a** and **24a** present equivalent activities. After administration of 1 mg/ear of these derivatives, 50% inhibition of the edema was observed compared to 40% obtained with indomethacin, 0.5 mg/ear.

However, in the oral edema experimental model induced by croton oil, compounds **24a** and **26a** were inactive and this suggests that oral administration of these compounds did not induce a systemic anti-inflammatory effect.

The difference in these in vivo results is probably due to the biodisponibility of the drug candidates in both inflammatory tissues.

4. Conclusions

As the result of this SAR investigation, we discovered more than twenty novel potent piperazinic derivatives that showed improved anti-PLA_2 activity with a high selectivity for GIIA versus GIB PLA_2s compared to the reference compound of Lilly **LY311727** and more active than our home reference **II**. In addition, two compounds **24a** and **26a** tested in vivo show interesting long-lasting activities in the carrageenan-induced edema formation as well after ip or per os administration. They also decreased significantly the inflammatory effect in the local edema experimental model induced by croton oil. They have a good potency to reduce the topical inflammatory effect due to the same agent, compared to indomethacin. Our synthetic work and the consecutive biological evaluation of the oxadiazolone–piperazine derivatives demonstrate the originality of this new class of compounds. Their potential therapeutic effect in the inflammatory process is promising. The exploration in the design of these new potent derivatives may allow a greater understanding of the structural parameters required for activity and may shed the light on the role of the oxadiazolone in the selectivity. It is possible that the effect of this oxadiazolone series on GIIA PLA_2 results from its interaction with a novel type of oxadiazolone-binding sub-site.

Further chemical modulations to obtain more active compounds and develop structure-activity relationships are required to verify our hypothesis on the possible contribution of the piperazine ring to the PLA_2 inhibitory effect. For this purpose, the synthesis of new compounds is in progress particularly with the aim to rigidify the piperazine ring as suggested by results in the present work. We are also investigating electronic and structural modifications using a molecular modeling approach to understand these requirements.

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 $60F_{254}$ (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 were uncorrected. The structure of all compounds was confirmed by IR and ^1H and ^{13}C NMR spectra. IR spectra were obtained in paraffin oil or KBr with an ATI Mattson Genesis Series FTIR spectrometer, and ^1H NMR or ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a BRUCKER 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; br, broad; m, multiplet. Elemental analyses were performed by 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.[32] using our reference II and using the reference control **LY311727** of Lilly company.[21] Enzymes tested were two secretory enzymes of human recombinant PLA_2 (group II) and a secretory enzyme of porcine pancreatic (group I) PLA_2. Palmitoyl-2-(10-pyrenyldecanoyl)-*sn*-glycero-3-phosphatidyl gly-cerolic 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.[24–26] The log *P* values were calculated using the Rekker's hydrophobic fragmental constants method.[33]

5.3. In vivo biological activity assay

5.3.1. Carrageenan-induced edema. In vivo activity of compounds was assessed by a classical carrageenan-induced edema test in rats, with indomethacin as a positive control. They were administered intraperitoneally or orally 1 h before the injection of carrageenan into the hind leg of the rats. The volume of edema was measured 0, 3, and 5 h after the injection of carrageenan. The doses used were 5, 10, and 20 mg/kg for the two products tested.

5.3.2. In vivo anti-inflammation. It concerns an assay for the in vivo anti-inflammatory activity of two compounds of those series, by the ear edema test as an acute inflammatory experimental model. Different samples of studied compounds were prepared.

After inducing edema on one ear of mice by the application of croton oil, the samples of each compound as described above were dissolved in 80% chloroform. The resulting solution was applied on the ear at a dose of 1 mg of each compound per ear. The other ear was applied only with the solvent, that is, 80% chloroform. At the end of 5 h of the initiation of the experiment, the ear tissue at the level of edema was collected by a gimlet from the skin, and the tissue collected was compared with that collected from the control ear by a gimlet, to calculate the percent inhibition.

5.3.3. Systemic anti-inflammatory effect. The samples of the compounds (Table 2) were suspended in CMS, and administered orally at a ratio of 80 mg of each compound per mouse. At 1 h after the initiation of the experiment, edema was induced by the application of croton oil. At 5 h after the application of croton oil, the tissues where edema had been developed were collected by a gimlet, and compared with the tissue collected from a control part, to calculate the percent inhibition. To calculate the statistical significance, the results obtained in each of the control group and the tested group were evaluated by Student's *t*-test.

5.4. 1-Alkylpiperazines (1a–e)

One example is given for 1-octadecylpiperazine **1c**. To a solution of piperazine (13 g, 0.15 mol) in a mixture of THF/CH_2Cl_2 (3:1) (100 mL) was added 1-bromooctadecane (5 g, 15 mmol) and the solution was stirred at room temperature for 1 h. The solvents were evaporated and the residue solubilized in CH_2Cl_2 was then washed twice with water. The organic layer was dried ($MgSO_4$), filtered, and evaporated. Recrystallization in acetone led to **1c** (4.5 g, 89%) as white crystals: mp 61.5 °C; IR (KBr, ν cm^{-1}): 3440 (N—H); ^1H NMR ($CDCl_3$) δ: 6–8 (1s, 1H, NH), 2.85 and 2.33 (2t, 8H, J = 4.88 and 4.50 Hz, H of piperazine), 2.21 (t, 2H, J = 7.56 Hz, CH_2—N), 1.40 (m, 2H, CH_2—CH_2—N), 1.20 (1s, 30H, CH_2), 0.80 (t, 3H, J = 6.6 Hz, CH_3).

5.5. 1-(4-Cyanobenzyl)-4-tetradecylpiperazine (2a)

A vigorously stirred suspension of 1-tetradecylpiperazine **1a** (6 g, 21 mmol), 4-cyanobenzyl bromide (4.93 g, 25 mmol), K_2CO_3 (9 g, 65 mmol), and KI (0.5 g) in CH_3CN (200 mL) was heated to reflux until TLC analysis showed the reaction to be complete. The mixture was filtered, and K_2CO_3 washed several times with CH_2Cl_2. The solvents were evaporated under vacuum, the resulting residue was diluted with CH_2Cl_2 (150 mL) and washed with water until neutral pH. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The product was recrystallized in acetone to provide **2a** (7.4 g, 88%) as white crystals: mp 49.1 °C, IR (KBr, ν cm^{-1}): 2248 (CN), 1607 (C=C_{ar}); ^1H NMR ($CDCl_3$) δ: 7.52 and 7.37 (2d, 4H, J = 8.06 Hz, Ar H), 3.49 (s, 2H, Ph—CH_2—N), 2.44 (ls, 8H, piperazine H), 2.35 (t, 2H, J = 7.64 Hz, CH_2—N), 1.65–1.42 (m, 2H, CH_2—CH_2—N), 1.18 (1s, 22H, CH_2), 0.80 (t, 3H, J = 6.66 Hz, CH_3). Compounds **2b** (89%) and **2c** (74%), mp 42 °C, were prepared using the same process.

5.6. 1-(4-Cyanomethylbenzyl)-4-tetradecylpiperazine (3a)

5.6.1. 4-Bromomethylphenylacetonitrile (7). In a 1-L Erlenmeyer flask, 4-methylphenyl acetonitrile (25 g, 0.19 mol) was dissolved in CCl_4 (300 mL). To the solution, NBS (41 g, 0.23 mol) freshly crystallized in acetic acid prior to use and 2,2′-Azobis(2-methylpropionitrile) (AIBN) 0.5 g were added. After refluxing for 3 h, the solution was cooled and washed three times with water. The organic phase was dried over $MgSO_4$, filtered and evaporated in vacuum. Distillation of the residue under reduced pressure (1 mm Hg) allowed successive recovery of three fractions at 95, 110, and 140 °C. The last one (T_{eb} = 140 °C) corresponding to the desired compound was crystallized from diethyl ether at −18 °C, to produce **7** (14 g, 35%) as white crystals: mp 63 °C; IR (KBr, ν cm^{-1}): 2224 (CN), 1594 (C=C_{ar}); ^1H NMR ($CDCl_3$) δ: 7.34 and 7.24 (2d, 4H, J = 8.28 Hz, Ar H), 4.41 (s, 2H, CH_2—Br), 3.68 (s, 2H, CH_2—CN).

5.6.2. 1-Tetradecylpiperazine **1a** (7 g, 24 mmol), 4-bromomethylphenylacetonitrile (6 g, 28 mmol), K_2CO_3 (9.93 g, 71 mmol), and KI (0.5 g) were mixed in acetonitrile (200 mL) and refluxed for 6 h. The suspension was then filtered, and the solid was washed several times with CH_2Cl_2. The solvents were evaporated, and the residue taken up in CH_2Cl_2 (150 mL) was washed with water until neutral pH. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel eluted by MeOH/CH_2Cl_2 (1:99, v/v) as eluent, to afford the title nitrile (8.2 g, 83%) as a brown oil: IR (film, ν cm^{-1}): 2248 (CN), 1607 (C=C_{ar}); ^1H NMR ($CDCl_3$) δ: 7.25 and 7.18 (2d, 4H, J = 8.23 Hz, Ar H),

3.63 (s, 2H, CH_2—CN), 3.42 (s, 2H, Ph—CH_2—N), 2.40 (ls, 8H, piperazine H), 2.27 (t, 2H, J = 7.64 Hz, CH_2—N), 1.55–1.4 (m, 2H, CH_2—CH_2—N), 1.17 (ls, 22H, CH_2), 0.80 (t, 3H, J = 6.7 Hz, CH_3).

5.7. 1-(4-Cyanobenzoyl)-4-tetradecylpiperazine (4a)

Tetradecylpiperazine **1a** (6.5 g, 23 mmol) and Et_3N (4.79 mL, 34 mmol) were dissolved in dry benzene (100 mL) and the mixture was stirred at 0 °C. 4-Cyanobenzoyl chloride (4.95 g, 29 mmol) solubilized in benzene (20 mL) was then added dropwise. After 2 h stirring at room temperature, the solvent was evaporated; the residue was taken up in CH_2Cl_2, washed with an alkaline solution and with water until neutrality. The organic phase was dried over $MgSO_4$, filtered and concentrated in vacuum. The product was then purified by chromatography on a silica gel column with CH_2Cl_2 as eluent, to afford **4a** as a brownish oil (6 g, 63%): IR (film, ν cm^{-1}): 2251 (CN), 1620 (NC—O), 1607 (C=C_{ar}); ^{1}H NMR ($CDCl_3$) δ: 7.63 and 7.42 (2d, 4H, J = 8.48 Hz, Ar H), 3.72 and 3.30 (2ls, 4H, CH_2—N—C—O of piperazine), 2.44 (ls, 4H, H of piperazine), 2.29 (t, 2H, J = 7.9 Hz, CH_2—N), 1.55–1.35 (m, 2H, CH_2—CH_2—N), 1.18 (ls, 22H, CH_2), 0.81 (t, 3H, J = 6.7 Hz, CH_3). Compounds **4b** (71%) and **4c** (76%) were obtained in the same manner from **1b** and **1c**, respectively.

5.8. 1-(4-Cyanomethylbenzoyl)-4-octadecylpiperazine (6c)

5.8.1. 1-(4′-Bromomethylbenzoyl)-4-octadecylpiperazine (5c). Octadecylpiperazine **1c** (4.4 g, 13 mmol) and Et_3N (2.7 mL, 19 mmol) were dissolved in anhydrous benzene (100 mL). The mixture was stirred at 0 °C, and 4-bromomethylbenzoyl chloride (3.04 g, 13 mmol) was added dropwise. After stirring for 2 h at room temperature, the solvent was evaporated and the resulting residue was diluted in CH_2Cl_2. It was then washed with alkaline solution, and several times with water until neutralization. The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude product was purified by chromatography on silica gel eluted with CH_2Cl_2 and yielded 5 g (72%) of pure **5c** as an oil: IR (film, ν cm^{-1}): 1624 (NC—O), 1607 (C=C_{ar}); ^{1}H NMR ($CDCl_3$) δ: 7.38 and 7.31 (2d, 4H, J = 3 Hz, Ar H), 4.42 (s, 2H, CH_2—Br), 3.72 and 3.38 (2ls, 4H, CH_2—N—C—O of piperazine), 2.41 (ls, 4H, H of piperazine), 2.29 (t, 2H, J = 8 Hz, CH_2—N), 1.55–1.35 (m, 2H, CH_2—CH_2—N), 1.18 (ls, 30H, CH_2), 0.81 (t, 3H, J = 6.75 Hz, CH_3).

5.8.2. The bromide derivative **5c** (5.35 g, 10 mmol) prepared in the step above was dissolved in dimethylsulfoxide (70 mL). The solution was stirred at 0 °C and sodium cyanide (1.96 g, 40 mmol) was added by portions. The mixture was then heated at 80 °C for 1 h. The reaction medium was diluted with CH_2Cl_2 and water. The organic phase was washed several times with water, dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by chromatography on a silica gel column, with CH_2Cl_2 as eluent. This yielded the title nitrile **6c** (3 g, 61%) as a thick honey-colored oil: IR (film, ν cm^{-1}): 2251 (CN), 1620 (NC—O), 1607 (C=C_{ar}); ^{1}H NMR ($CDCl_3$) δ: 7.36 and 7.30 (2d, 4H, J = 8.50 Hz, Ar H), 3.71 (s, 2H, CH_2—CN), 3.72 and 3.38 (2ls, 4H, CH_2—N—C—O of piperazine), 2.37 (ls, 4H, H of piperazine), 2.29 (t, 2H, J = 7.65 Hz, CH_2—N), 1.41–1.35 (m, 2H, CH_2—CH_2—N), 1.18 (ls, 30H, CH_2), 0.81 (t, 3H, J = 6.6 Hz, CH_3).

5.9. 1-(4-Cyanomethylphenyl)-4-octadecylpiperazine (10c)

5.9.1. *N*-Octadecyldiethanolamine (8c). This derivative was prepared according to Jerzy et al.,[27] starting from diethanolamine (10 g, 95 mmol), octadecyl bromide (37.96 g, 0.114 mol), K_2CO_3 (39.33 g, 0.285 mol) and KI (0.5 g) in CH_3CN (200 mL) to afford **8c**, after crystallization from acetone, as 33 g (quantitative) of white crystals: mp 49 °C; IR (KBr, ν cm^{-1}): 3310 (O—H); ^{1}H NMR ($CDCl_3$) δ: 3.53 (t, 4H, J = 5.43 Hz, CH_2—O), 3.27 (1s, 2H, OH), 2.57 (t, 4H, J = 5.43 Hz, N—CH_2—C—O), 2.44 (t, 2H, J = 7.06 Hz, CH_2—N), 1.41–1.34 (m, 2H, CH_2—CH_2—N), 1.18 (ls, 30H, CH_2), 0.80 (t, 3H, J = 5.85 Hz, CH_3).

5.9.2. *N*,*N*′-Di(chloroethyl)octadecylamine (9c). In this step we adapted to our use the method of Newman et al.[28] The diol **8c** (13 g, 36 mmol) was dissolved in $CHCl_3$ (100 mL) and cooled to 0 °C, and, thionyl chloride (7.95 mL, 0.109 mmol) was added dropwise. After completion of the addition, the reaction mixture was refluxed for 3 h. The solvent and excess of thionyl chloride were evaporated and the residue taken up in CH_2Cl_2 was washed with a Na_2CO_3 saturated aqueous solution and several times with water until neutralization. The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by chromatography on silica gel eluted by ether/petroleum ether (5:95, v/v) and yielded the chloride derivative **9c** (10 g, 70%) as an oil: IR (film, ν cm^{-1}): no hydroxyle; ^{1}H NMR ($CDCl_3$) δ: 3.38 (t, 4H, J = 5.43 Hz, CH_2—Cl), 2.75 (t, 4H, J = 7.30 Hz, N—CH_2—CH_2—Cl), 2.43 (t, 2H, J = 6.67 Hz, CH_2—N), 1.40–1.36 (m, 2H, CH_2—CH_2—N), 1.16 (ls, 30H, CH_2), 0.80 (t, 3H, J = 5.95 Hz, CH_3).

5.9.3. Compound **9c** (3 g, 7.6 mmol), 4-aminophenylacetonitrile (2 g, 15 mmol), and KI (0.2 g) in CH_3CN (100 mL) were mixed and the suspension was stirred under reflux for 16 h. The solvent was then evaporated and the residue was diluted in CH_2Cl_2, washed with alkaline solution and then several times with water. The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum. The resulting residue was crystallized from acetone to produce the title substituted **10c** piperazine (2.66 g, 77%) as white crystals: mp 94 °C; IR (KBr, ν cm^{-1}): 2252 (CN), 1607 (C=C_{ar}); ^{1}H NMR ($CDCl_3$) δ: 7.17 and 6.84 (2d, 4H, J = 8.61 Hz, Ar H), 3.62 (s, 2H, CH_2—CN), 3.61 and 3.22 (2ls, 8H, H of piperazine), 2.92 (t, 2H, J = 8.32 Hz, CH_2—N), 1.88–1.82 (m, 2H, CH_2—CH_2—N), 1.19 (ls, 30H, CH_2), 0.81 (t, 3H, J = 5.95 Hz, CH_3).

5.10. 4-(3-Cyanopropyl)benzoic acid (14b)

5.10.1. 1-Bromo-3-phenylpropane (11). To a solution of 3-phenylpropan-1-ol (10 g, 73 mmol) in anhydrous

CH_2Cl_2 (150 mL), a solution of PBr_3 1M in CH_2Cl_2 (36 mmol, 50 mL) was added dropwise. The reaction mixture was stirred at room temperature for 1 h. After washing several times with water, the organic phase was dried and evaporated. The residue was purified by chromatography on silica gel eluted with ether/petroleum ether (5:95, v/v) to give the bromide derivative **11** (11.6 g, 80%) as a viscous liquid: IR (film, ν cm^{-1}): 1605 (C=C_{ar}); ^1H NMR ($CDCl_3$) δ: 7.25–7.06 (m, 5H, Ar H), 3.29 (t, 2H, J = 6.59 Hz, CH_2Br), 2.68 (t, 2H, J = 7.34 Hz, Ph$CH_2CH_2CH_2Br$), 2.14–1.99 (m, 2H, $CH_2CH_2CH_2Br$).

5.10.2. 4-(3-Bromopropyl)acetophenone (12). The bromide derivative **11** (25 g, 125 mmol) solubilized in acetyl chloride (20 mL) was added dropwise at 0 °C to a solution of aluminum trichloride (17.5 g, 88 mmol) and acetyl chloride (50 mL) in CS_2 (100 mL). The mixture was stirred at room temperature for 2 h. Excess of acetyl chloride and CS_2 were removed by evaporation under reduced pressure. The residue was diluted in CH_2Cl_2, washed successively with alkaline solution, several times with water, dried over $MgSO_4$ and concentrated under vacuum, to yield the title substituted acetophenone **12** (23.8 g, 79%) as a yellow liquid: $bp_{3\ mm\ Hg}$ 140–145 °C; R_f 0.25 (ether/petroleum ether, 50:50, v/v); IR (film, ν cm^{-1}): 1670 (C—O), 1605 (C=C_{ar}); ^1H NMR ($CDCl_3$) δ: 7.79 (ls, 2H, Ar H), 7.23 (ls, 2H, Ar H), 3.26 (t, 2H, J = 6.49 Hz, CH_2Br), 2.7 (t, 2H, J = 6.26 Hz, Ph$CH_2CH_2CH_2Br$), 2.48 (s, 3H, CH_3), 2.08–1.9 (m, 2H, $CH_2CH_2CH_2Br$).

5.10.3. 4-(3-Bromopropyl)benzoic acid (13). To a NaOH (33 g, 82.5 mmol) solution in water (200 mL), Br_2 (50 mL) and dioxane (100 mL) were successively added dropwise. The mixture was cooled to 0 °C and **12** (22 g, 91.2 mmol) was added dropwise. Stirring was maintained at room temperature until the brown color of bromide disappeared (1 h). The mixture was then carefully acidified with an aqueous solution of 12 N HCl (20 mL). The precipitate was filtered, washed several times with water, and dried to afford **26** (18 g, 85%) as a yellow solid: mp 120 °C; IR (KBr, ν cm^{-1}): 3340 (OH), 1700 (C—O); ^1H NMR ($CDCl_3$) δ: 7.9 (d, 2H, J = 8.16 Hz, Ar H), 7.2 (d, 2H, J = 8.3 Hz, Ar H), 3.3 (t, 2H, J = 6.47 Hz, CH_2Br), 2.78 (t, 2H, J = 7.7 Hz, Ph $CH_2CH_2CH_2Br$), 2.18–2.04 (m, 2H, $CH_2CH_2CH_2Br$).

5.10.4. The bromide derivative **13** (18 g, 77.5 mmol) prepared above was converted into the nitrile **14b** by the same procedure as described for the preparation of **6c** and yielded **14b** (11 g, 75%) with a viscous appearance. IR (film, ν cm^{-1}): 3345 (OH), 1700 (C—O), 2253 (CN); ^1H NMR ($CDCl_3$) δ: 7.9 (d, 2H, J = 8.09 Hz, Ar H), 7.2 (d, 2H, J = 8.09 Hz, Ar H), 2.8 (t, 2H, J = 7.44 Hz, CH_2CN), 2.28 (t, 2H, J = 6.99 Hz, Ph$CH_2CH_2CH_2CN$), 2.02–1.91 (m, 2H, $CH_2CH_2CH_2CN$).

5.11. 4-(2-Cyanoethyl)benzoic acid (14a)

This intermediate was prepared according to the method of Brouard et al.[29] starting from 4-(2-chloro-2-cyanoethyl)benzoic acid (10 g, 47 mmol), glacial acetic acid (250 mL), zinc powder (1.56 g, 23 mmol) and gave compound **14a** (5.68 g, 68%) as a white solid: mp 165 °C; IR (KBr, ν cm^{-1}): 1700 (C—O), 2252 (CN); ^1H NMR ($CDCl_3$) δ: 7.9 (d, 2H, J = 8.17 Hz, Ar H), 7.25 (d, 2H, J = 8.13 Hz, Ar H), 2.95 (t, 2H, J = 7.22 Hz, CH_2CN), 2.6 (t, 2H, J = 7.36 Hz, CH_2CH_2CN).

5.12. 1-[4-*N*-(Hydroxyamidino)benzyl]-4-tetradecylpiperazine (16a)

To a suspension of K_2CO_3 (8 g, 57 mmol) and NH_2OH, HCl (3.2 g, 46 mmol) in refluxing absolute EtOH (150 mL), was added dropwise the nitrile **2a** (3.7 g, 9.2 mmol) dissolved in absolute EtOH (50 mL) and the mixture was refluxed for 6 h. Salts were filtered and washed several times with CH_2Cl_2. The filtrate was concentrated under reduced pressure and the crude product was taken up in CH_2Cl_2. The organic phase was washed with water until neutrality, dried over $MgSO_4$, then filtered. After evaporation of the solvent, the product was crystallized in acetone to lead to the amidoxime **16a** (2.7 g, 69%) as white crystals: mp 104.7 °C, IR (KBr, ν cm^{-1}): 3490 (O—H), 3374 (NH_2), 1655 (C—N), 1607 (C—C_{ar}); ^1H NMR ($CDCl_3$) δ: 7.50 and 7.28 (2d, 4H, J = 8 Hz, Ar H), 4.78 (s, 2H, NH_2), 3.46 (s, 2H, Ph—CH_2—N), 2.43 (ls, 8H, piperazine H), 2.25 (t, 2H, J = 8 Hz, CH_2—N), 1.5–1.35 (m, 2H, CH_2—CH_2—N), 1.18 (1s, 22H, CH_2), 0.80 (t, 3H, J = 7 Hz, CH_3). Compounds **16b** and **16c** were obtained following the same process with approximately the same yield.

5.13. 1-[4-(*N*-Hydroxyamidinomethyl)benzyl]-4-tetradecylpiperazine (17a)

This intermediate was obtained in the same conditions as **16a** using K_2CO_3 (13.08 g, 94 mmol), NH_2OH, HCl (5.48 g, 78 mmol) in absolute ethanol (150 mL), and the nitrile **3a** (6.5 g, 15 mmol). After evaporation of the solvent, the residue was crystallized from acetone to give the title amidoxime **17a** (4.62 g, 65%) as white crystals: mp 74 °C; IR (KBr, ν cm^{-1}): 3490 (O—H), 3374 (NH_2), 1655 (C—N), 1607 (C=C_{ar}); ^1H NMR ($CDCl_3$) δ: 7.20 and 7.15 (2d, 4H, J = 6 Hz, Ar H), 4.38 (s, 2H, NH_2), 3.43 (s, 2H, CH_2—C—N), 3.35 (s, 2H, Ph—CH_2—N), 2.41 (m, 8H, piperazine H), 2.25 (t, 2H, J = 8 Hz, CH_2—N), 1.55–1.36 (m, 2H, CH_2—CH_2—N), 1.18 (ls, 22H, CH_2), 0.80 (t, 3H, J = 6.13 Hz, CH_3).

5.14. 1-[4-(*N*-Hydroxyamidino)benzoyl]-4-tetradecylpiperazine (18a)

1-[4-(*N*-Hydroxyamidino)benzoyl]-4-tetradecylpiperazine (**18a**) was prepared in the same conditions as for **16a**, from **4a** (6 g, 14 mmol), to give **18a** (4 g, 64%) as white crystals (acetone): mp 143.6 °C; IR (KBr, ν cm^{-1}): 3486 (O—H), 3373 (NH_2), 1657 (NC—O), 1625 (C—N), 1582 (C=C_{ar}); ^1H NMR ($CDCl_3$) δ: 7.58 and 7.34 (2d, 4H, J = 8 Hz, Ar H), 4.43 (s, 2H, NH_2), 3.73 and 3.40 (2m, 4H, CH_2—N—C—O of piperazine), 2.55 (m, 4H, H of piperazine), 2.29 (t, 2H, J = 6.64 Hz, CH_2—N), 1.39 (m, 2H, CH_2—CH_2—N), 1.18 (ls, 30H, CH_2), 0.81 (t, 3H, J = 6.03 Hz, CH_3). Starting from nitriles **4b** and **4c** compounds: **18b** (34%), mp 130 °C and **18c** (23%), mp 130.2 °C, were obtained by the same manner.

5.15. 1-[4-(*N*-Hydroxyamidinomethyl)benzoyl]-4-octadecylpiperazine (19c)

The same procedure was used as described for **12a** starting from **6c** (6 g, 12 mmol), K_2CO_3 (10.26 g, 74 mmol) and NH_2OH, HCl (4.30 g, 61 mmol). The crude product was crystallized from acetone to give 4 g (67%) of the title oxime **19c** as white crystals: mp 105.2 °C; IR (KBr, ν cm^{-1}): 3486 (O—H), 3373 (NH_2), 1657 (NC—O), 1625 (C—N), 1582 (C=C_{ar}); ^{1}H NMR ($CDCl_3$) δ: 7.30 and 7.24 (2d, 4H, J = 8 Hz, Ar H), 4.44 (s, 2H, NH_2), 3.42 (s, 2H, CH_2—C—N), 3.73 and 3.40 (2ls, 4H, CH_2—N—C—O of piperazine), 2.37 (ls, 4H, H of piperazine), 2.29 (t, 2H, J = 8 Hz, CH_2—N), 1.50–1.35 (m, 2H, CH_2—CH_2—N), 1.18 (ls, 30H, CH_2), 0.81 (t, 3H, J = 7 Hz, CH_3).

5.16. 1-[4-(*N*-Hydroxyamidinomethyl)phenyl]-4-octadecylpiperazine (20c)

The same procedure as described for **16a** was used to prepare **20c** starting from NH_2OH, HCl (1.52 g, 21 mmol), K_2CO_3 (3.64 g, 26 mmol) and the nitrile **10c** (2 g) prepared above. The crude product was purified by chromatography on a silica gel column with CH_2Cl_2 as eluent, and the resulting oil was crystallized from acetone to afford the title amidoxime **20c** (600 mg, 28%) as white crystals: mp 110.1 °C; IR (KBr, ν cm^{-1}): 3489 (O—H), 3375 (NH_2), 1655 (C—N), 1607 (C—C_{ar}); ^{1}H NMR ($CDCl_3$) δ: 7.08 and 6.80 (2d, 4H, J = 8.60 Hz, Ar H), 4.36 (s, 2H, NH_2), 3.44 (s, 2H, CH_2—C—N), 3.14 and 2.56 (2ls, 8H, H of piperazine), 2.34 (t, 2H, J = 7.34 Hz, CH_2—N), 1.52–1.41 (m, 2H, CH_2—CH_2—N), 1.19 (ls, 30H, CH_2), 0.81 (t, 3H, J = 7 Hz, CH_3).

5.17. 1-[4-(4,5-Dihydro-1,2,4-[4*H*]-5-oxo-oxadiazol-3-yl)benzyl]-4-tetradecylpiperazine (23a)

This synthesis was carried out in two steps as described elsewhere.[24] In a 100-mL round-bottomed flask, **16a** (1 g, 2.3 mmol) and Et_3N (279 mg, 2.75 mmol) were dissolved in dry CH_2Cl_2 (40 mL). The solution was stirred at 0 °C for 1 h and phenyl chloroformate (431 mg, 2.75 mmol) was added. After stirring at 0 °C 1 h more, the solution was washed successively with saturated aqueous Na_2CO_3 and three times with water, dried over $MgSO_4$, filtered, and then concentrated in vacuum. The carbonate intermediate obtained was taken up in anhydrous toluene (40 mL), and heated to reflux for 12 h. The toluene was evaporated under reduced pressure, and the resulting residue was purified by chromatography on a silica gel column using CH_2Cl_2/MeOH (98:2, v/v) as eluent and crystallization in acetone, to produce the title compound **23a** (717 mg, 26%) as white crystals: mp 118.7 °C; IR (KBr, ν cm^{-1}): 1732 (NC—O), 1688 (C—N), 1599 (C=C_{ar}); ^{1}H NMR ($CDCl_3$) δ: 8.77 (ls, 1H, NH), 7.75 and 7.26 (2d, 4H, J = 8 Hz, Ar H), 3.51 (s, 2H, Ph—CH_2—N), 2.9 and 2.65 (2ls, 8H, piperazine H), 2.5 (t, 2H, J = 7.64 Hz, CH_2—N), 1.7–1.45 (m, 2H, CH_2—CH_2—N), 1.18 (1s, 22H, CH_2), 0.80 (t, 3H, J = 6 Hz, CH_3).

^{13}C NMR ($CDCl_3$) δ: 169.09 (C—O), 163.50 (C—N), 139.84 (Cq_{ar}—CH_2—C—N), 129.39 (CH_{ar}), 126.56 (CH_{ar}), 126.31 (NCH_2Cq_{ar}), 61.88 (N—CH_2—Ph), 57.84 (CH_2N), 52.43 (CH_2N—CH_2—Ph), 50.79 (CH_2NC_{13}), 31.88 (CH_2CH_2N), 29.30 (CH_2), 27.03 (CH_2), 24.88 (CH_2), 22.65 (CH_2), 14.09 (CH_3).

Anal. Calcd for $C_{27}H_{44}N_4O_2/0.5H_2O$ (465): C, 69.67; H, 9.67; N, 12.04. Found: C, 69.70; H, 9.89; N, 11.87.

Final compounds **23b** (63%), mp 120.6 °C, and **23c** (37%), mp 109 °C, were prepared by the same reaction starting from the corresponding amidoximes **16b** and **16c**, respectively.

Compound **23b**, Anal. Calcd for $C_{29}H_{48}N_4O_2$ (484): C, 71.90; H, 9.91; N, 11.57. Found: C, 71.85; H, 10.09; N, 11.47.

Compound **23c**, Anal. Calcd for $C_{31}H_{52}N_4O_2/0.5H_2O$ (521): C, 71.40; H, 10.17; N, 10.34. Found: C, 71.10; H, 10.21; N, 10.06.

5.18. 1-[4-(4,5-Dihydro-1,2,4-[4*H*]-5-oxo-oxadiazol-3-ylmethyl)benzyl]-4-tetradecylpiperazine (24a)

1-[4-(4,5-Dihydro-1,2,4-[4*H*]-5-oxo-oxadiazol-3-ylmethyl)-benzyl]-4-tetradecylpiperazine (**24a**) was obtained in 26% yield as described for **23a** from the amidoxime **17a** (1.8 g, 4 mmol) and Et_3N (0.66 mL, 4 mmol), while a mixture of acetone and ether was used for crystallization: mp 98 °C; IR (KBr, ν cm^{-1}): 1732 (NC—O), 1688 (C—N), 1599 (C=C_{ar}); ^{1}H NMR ($CDCl_3$) δ: 8–12 (ls, 1H, NH), 7.27 and 7.18 (2d, 4H, J = 8.07 and 9.12 Hz, Ar H), 3.67 (s, 2H, CH_2—C—N), 3.35 (s, 2H, Ph—CH_2—N), 2.56 and 2.34 (2m, 8H, piperazine H), 2.46 (t, 2H, J = 7.64 Hz, CH_2—N), 1.47 (m, 2H, CH_2—C—N), 1.18 (ls, 22H, CH_2), 0.80 (t, 3H, J = 6.13 Hz, CH_3).

^{13}C NMR ($CDCl_3$) δ: 167.51 (C—O), 163.26 (C—N), 135.80 (Cq_{ar}—CH_2—C—N), 134.39 (Cq_{ar}), 129.41 (CH_{ar}), 128.84 (CH_{ar}), 61.68 (N—CH_2—Ph), 57.79 (CH_2N), 52.15 (CH_2N—CH_2—Ph), 50.80 (CH_2NC_{13}), 32.40(CH_2C—N), 31.89 (CH_2CH_2N), 29.53 (CH_2), 27.21 (CH_2), 25.08 (CH_2), 22.66 (CH_2), 14.08 (CH_3).

Anal. Calcd for $C_{28}H_{46}N_4O_2$ (470): C, 71.48; H, 9.78; N, 11.91. Found: C, 71.71; H, 10.06; N, 11.68.

Final compounds **24b** (20%), mp 99 °C, and **24c** (21%), mp 101 °C, were prepared by the same reaction starting from the corresponding amidoximes **17b** and **17c**, respectively.

Compound **24b**, Anal. Calcd for $C_{30}H_{50}N_4O_2/1.5H_2O$ (525): C, 68.57; H, 10.09; N, 10.06. Found: C, 68.50; H, 9.97; N, 10.00.

Compound **24c**, Anal. Calcd for $C_{32}H_{54}N_4O_2$ /0.5 H_2O (535): C, 71.77; H, 10.28; N, 10.46. Found: C, 71.45; H, 10.16; N, 10.18.

5.19. 1-[4-(4,5-Dihydro-1,2,4-[4*H*]-5-oxo-oxadiazol-3-yl) benzoyl]-4-tetradecylpiperazine (25a)

This compound was obtained under the same conditions as for **23a** from **18a** (4 g, 9 mmol). The residue was purified by chromatography on a silica gel column eluted

with CH_2Cl_2/MeOH (98:2, v/v), then crystallization in acetone, leading to **25a** (2.5 g, 59%) as white crystals: mp 114.8 °C; IR (KBr, ν cm^{-1}): 1780 (OC—O), 1734 (C—N), 1640 (NC—O), 1607 (C=C_{ar}); ^1H NMR ($CDCl_3$) δ: 8–12 (most, 1H, NH), 7.16 (s, 4H, Ar H), 3.77 and 3.36 (2m, 4H, CH_2—N—C—O of piperazine), 2.52 (m, 4H, H of piperazine), 2.35 (t, 2H, J = 5.86 Hz, CH_2—N), 1.43 (m, 2H, CH_2—CH_2—N), 1.18 (ls, 30H, CH_2), 0.81 (t, 3H, J = 6.21 Hz, CH_3).

^{13}C NMR ($CDCl_3$) δ: 169.26 (C—O), 162.88 (C—O), 158.36 (C—N), 138.17 (CH_{ar}—C—N), 127.36 (CH_{ar}), 126.71 (CH_{ar}), 125.69 (CO—Cq_{ar}), 58.37 (CH_2N), 53.09 (CH_2NCO), 52.55 (CH_2NC_{13}), 31.85 (CH_2CH_2N), 29.43 (CH_2), 27.31 (CH_2), 26.32 (CH_2), 22.63 (CH_2), 14.08 (CH_3).

Anal. Calcd for $C_{27}H_{41}N_4O_2$/1H_2O/1HCl (524): C, 61.83; H, 8.58; N, 10.68. Found: C, 62.10; H, 8.64; N, 10.50.

Compounds **25b** (52%), mp 111.4 °C, and **25c** (20%), mp 113.6 °C, were obtained using the same procedure from the corresponding amidoximes **18b** and **18c**.

Compound **25b**, Anal. Calcd for $C_{29}H_{46}N_4O_3$ (498): C, 69.87; H, 9.23; N, 11.24. Found: C, 70.03; H, 9.27; N, 11.06.

Compound **25c**, Anal. Calcd for $C_{31}H_{50}N_4O_3$ (526): C, 68.38; H, 9.55; N, 10.29. Found: C, 68.54; H, 9.40; N, 10.05.

5.20. 1-[4′-(4,5-dihydro-1,2,4[4*H*]-5-oxo-oxadiazol-3-ylmethyl)benzoyl]-4-octadecylpiper azine (26c)

The same procedure as described for **23a** was followed starting from the amidoxime **19c** (1.3 g, 2.53 mmol), NEt_3 (0.45 mL, 3.28 mmol) and phenyl chloroformate (0.4 mL, 3.03 mmol). The product was crystallized from acetone to give 500 mg (37%) of the final compound **26c** as white crystals: mp 121 °C; IR (KBr, ν cm^{-1}): 1780 (OC—O), 1734 (C—N), 1640 (NC—O), 1607 (C=C_{ar}); ^1H NMR ($CDCl_3$) δ: 8–12 (most, 1H, NH), 7.16 (s, 4H, Ar H), 3.79 (s, 2H, CH_2—C—N), 3.77 and 3.36 (2m, 4H, CH_2—N—C—O of piperazine), 2.52 (m, 4H, H of piperazine), 2.35 (t, 2H, J = 5.86 Hz, CH_2—N), 1.43 (m, 2H, CH_2—C—N), 1.18 (ls, 30H, CH_2), 0. 81 (t, 3H, J = 6.21 Hz, CH_3).

^{13}C NMR ($CDCl_3$) δ: 170.11 (C—O), 163.81 (C—O), 160.43 (C—N), 135.76 (CO—Cq_{ar}), 133.33 (Cq_{ar}), 129.00 (CH_{ar}), 127.36 (CH_{ar}), 58.40 (CH_2N), 53.19 (CH_2NCO), 52.54 (CH_2NC_{13}), 31.79 (CH_2C—N), 31.68 (CH_2CH_2N), 29.47 (CH_2), 27.36 (CH_2), 26.33 (CH_2), 22.68 (CH_2), 14.11 (CH_3).

Anal. Calcd for $C_{32}H_{52}N_4O_3$/1.5H_2O (540): C, 69.94; H, 9.65; N, 10.20. Found: C, 70.09; H, 9.80; N, 9.90.

Final compounds **26a** (41%), mp 120.7 °C, **26b** (35%), mp 120.2 °C, **26d** (40%), mp 123.7 °C, and **26e** (44%), mp 121.3 °C, were prepared by the same reaction starting from the corresponding amidoximes **19a**, **19b**, **19d**, and **19e**, respectively.

Compound **26a**, Anal. Calcd for $C_{28}H_{44}N_4O_3$ (484): C, 69.42; H, 9.09; N, 11.57. Found: C, 69.26; H, 9.29; N, 11.58.

Compound **26b**, Anal. Calcd for $C_{30}H_{56}N_4O_3$ (508): C, 70.86; H, 11.02; N, 11.02. Found: C, 70.69; H, 11.20; N, 10.93.

Compound **26d**, Anal. Calcd for $C_{34}H_{56}N_4O_3$/1H_2O (586): C, 69.62; H, 9.89; N, 9.55. Found: C, 69.44; H, 9.80; N, 9.46.

Compound **26e**, Anal. Calcd for $C_{36}H_{60}N_4O_3$ (596): C, 72.48; H, 10.06; N, 9.39. Found: C, 72.38; H, 10.21; N, 9.20.

5.21. 1-[4-(4,5-Dihydro-1,2,4[4*H*]-5-oxo-oxadiazol-3-ylmethyl)phenyl]-4-octadecylpiperazine (27c)

The same procedure as described for **23a** was used starting from the amidoxime **20c** (600 mg, 1.2 mmol), NEt_3 (0.22 mL, 1.6 mmol), and phenyl chloroformate (0.2 mL, 1.6 mmol). The crude product was crystallized from acetone to obtain the final compound **27c** (210 mg, 33%) as white crystals: mp 147.3 °C; IR (KBr, ν cm^{-1}): 1740 (OC—O), 1716 (C—N), 1607 (C=C_{ar}) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.33 (s, 1H, NH), 7.12 and 6.74 (2d, 4H, J = 8.62 and 8.60 Hz, Ar H), 3.71 (s, 2H, CH_2—C—N), 3.14 and 2.56 (2ls, 8H, H of piperazine), 2.34 (t, 2H, J = 7.34 Hz, CH_2—N), 1.47 (m, 2H, CH_2—C—N), 1.19 (ls, 30H, CH_2), 0.81 (t, 3H, J = 6.05 Hz, CH_3).

^{13}C NMR ($CDCl_3$) δ: 163.71 (C—O), 160.53 (C—N), 134.76 (NCq_{ar}), 132.33 (Cq_{ar}), 129.32 (CH_{ar}), 127.34 (CH_{ar}), 62.52 (CH_2NPh), 61.19 (CH_2NC_{13}), 58.54 (CH_2N), 31.80 (Ph—CH_2), 31.70 (CH_2CH_2N), 29.50–26.39 (CH_2), 27.39 (CH_2), 26.42 (CH_2), 22.65 (CH_2), 14.06 (CH_3).

Anal. Calcd for $C_{31}H_{52}N_4O_2$/1H_2O (530): C, 70.20; H, 10.18; N, 10.56. Found: C, 70.60; H, 10.41; N, 10.48.

Compounds **27a** (42%), mp 123.4 °C, and **27b** (35%), mp 132.6 °C, were obtained using the same procedure from the corresponding amidoximes **20a** and **20b**.

Compound **27a**, Anal. Calcd for $C_{27}H_{44}N_4O_2$ (456): C, 71.05; H, 9.64; N, 12.28. Found: C, 71.31; H, 9.70; N, 12.00.

Compound **27b**, Anal. Calcd for $C_{29}H_{48}N_4O_2$ (484): C, 71.90; H, 9.91; N, 11.57. Found: C, 72.00; H, 9.72; N, 11.50.

5.22. 1-[4-(4,5-Dihydro-1,2,4[4*H*]-5-oxo-oxadiazol-3-ylpropyl)benzoyl]-4-tetradecylpiperazine (29a)

A mixture of 4-(3-cyanopropyl)benzoic acid **14b** (2.5 g, 13.2 mmol) and 4-tetradecylpiperazine **1a** (3.75 g, 13.2 mmol) in 50 mL CH_2Cl_2 was cooled to 0 °C. Dicyclohexylcarbodiimide (DCC) (2.7 g, 13.2 mmol) was added and the mixture was stirred overnight warming to room temperature. The solution was washed with saturated $NaHCO_3$ (2× 50 mL), 10% HCl (2× 50 mL) and water. The organic layer was then dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting residue was purified by chromatography on a silica gel column

using MeOH/CH_2Cl_2 (5:95, v/v) as eluent and yielded the final compound **29a** (3.95 g, 60%) as an oil: IR (film, ν cm^{-1}): 3422 (NH), 1774 (OCON), 1730 (C—N), 1613 (C=C_{ar}); ^1H NMR ($CDCl_3$) δ: 8.41 (ls, 1H, NH), 7.2 (d, 2H, J = 7.89 Hz, ArH), 7.05 (d, 2H, J = 7.92 Hz, ArH), 3.73 (ls, 2H, $CONCH_2$), 3.43 (ls, 2H, $CONCH_2$), 2.58 (t, 2H, J = 7.2 Hz, $CONCH_2$), 2.58 (t, 2H, J = 7.30 Hz, CH_2C—N), 2.54–2.35 (m, 8H, $2C_{14}NCH_2$, NCH_2C_{13}, $CH_2CH_2CH_2C$—N), 1.89–1.82 (m, 2H, $CH_2CH_2CH_2C$—N), 1.42 (ls, 2H, $NCH_2CH_2C_{12}$), 1.4–1.1 (m, 22H, CH_2), 0.81 (t, 3H, J = 6.76 Hz, CH_3).

^{13}C NMR ($CDCl_3$) δ: 170.53 (C—O), 161.17 (C—O), 159.71 (C—N), 142.84 (OCq_{ar}), 132.85 (Cq_{ar}), 128.57 (CH_{ar}), 127.01 (CH_{ar}), 58.31 (NCH_2C_{13}), 52 (NCH_2), 48 (NCH_2), 34.4 (CH_2), 31.75 (CH_2C—N), 29.50–26.39 (CH_2), 22.41 (CH_2), 22.52 (CH_2), 13.97 (CH_3).

Anal. Calcd for $C_{30}H_{48}N_4O_3$/0.25 AcOEt (534): C, 69.66; H, 9.36; N, 10.48. Found: C, 69.33; H, 9.43; N, 10.54.

5.23. 1-[4-(4,5-Dihydro-1,2,4[4*H*]-5-oxo-oxadiazol-3-ylethyl)benzoyl]-4-tetradecylpiperazine (28a)

Compound **28a** was obtained as a yellow powder in 60% yield following the same procedure as used for the synthesis of **29a** from the corresponding cyanoacid **14a**. Mp 97 °C; IR (KBr, ν cm^{-1}): 1700 (C—O), 2252 (CN); ^1H NMR ($CDCl_3$) δ 9.33 (ls, 1H, NH), 7.2 (d, 2H, J = 8 Hz, ArH), 7.01 (d, 2H, J = 8.03 Hz, ArH), 3.75 (ls, 2H, $CONCH_2$), 3.39 (ls, 2H, $CONCH_2$) 2.82 (t, 2H, J = 8.2 Hz, CH_2C—N), 2.54 (t, 2H, J = 7.19 Hz, CH_2CH_2C—N), 2.36–2.28 (m, 6H, $2C_{14}NCH_2$, NCH_2C_{13}), 1.42 (ls, 2H, $NCH_2CH_2C_{12}$), 1.3–1.1 (m, 22H, CH_2), 0.81 (t, 3H, J = 6.76 Hz, CH_3).

^{13}C NMR ($CDCl_3$) δ 170.41 (C—O), 160.81 (C—O), 158.9 (C—N), 141.54 (OCq_{ar}), 133.42 (Cq_{ar}), 128.55 (CH_{ar}), 127.1 (CH_{ar}), 58.36 (NCH_2C_{13}), 53 (NCH_2), 50 (NCH_2), 31.81 (CH_2), 31.06 (CH_2C—N), 29.56–26.52 (CH_2), 22.58 (CH_2), 14.02 (CH_3).

Anal. Calcd for $C_{29}H_{46}N_4O_3$/0.25 AcOEt (520): C, 69.13; H, 9.22; N, 11.06. Found: C, 69.30; H, 9.52; N, 11.06.

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References and notes

1. Murakami, M.; Kudo, I. *J. Biochem. (Tokyo)* **2002**, *131*, 285.
2. Harris, S. G.; Padilla, J.; Koumas, L.; Ray, D.; Phipps, R. P. *Trends Immunol.* **2002**, *23*, 144.
3. Murakami, M.; Kudo, I. *Adv. Immunol.* **2001**, *77*, 163.
4. Valentin, E.; Lambeau, G. *Biochim. Biophys. Acta.* **2000**, *1488*, 59.
5. Laye, J. P.; Gill, J. H. *Therapeutic Focus*, DDT, 2003; Vol. 8, No. 15, p 710.
6. Balsinde, J.; Dennis, E. A. *J. Biol. Chem.* **1996**, *271*, 6758.
7. Bomalaski, J. S.; Clark, M. A. *Arthritis Rheum.* **1993**, *36*, 190.
8. Vadas, P. *Biochim. Biophys. Acta* **1997**, *1346*, 193.
9. Baur, M.; Schmid, O.; Landauer, B. *Klin. Wochenschr.* **1989**, *67*, 196.
10. Uhl, W.; Büchler, M.; Nevalainen, T. J.; Deller, A.; Beger, H. G. *J. Trauma* **1990**, *30*, 1285.
11. Büchler, M.; Deller, A.; Malfertheiner, P.; Kleine, H. O.; Uhl, W. *Klein. Wochenschr.* **1989**, *67*, 217.
12. Uhl, W.; Beger, H. G.; Hoffman, G.; Hanisch, E.; Schild, A.; Waydhas, C.; Entholzner, E.; Muller Kellermann, W.; Vogeser, M. *J. Am. Coll. Surg.* **1995**, *180*, 323.
13. Büchler, M.; Malfertheiner, P.; Schädlich, H.; Nevalainen, T. J.; Fress, H.; Berger, H. G. *Gastroenterology* **1989**, *97*, 1521.
14. Bird, N. C.; Goodman, A. J.; Johnson, A. G. *Br. J. Surg.* **1989**, *76*, 731.
15. Kortesuo, P. T.; Nevalainen, T. J.; Büchler, M.; Uhl, W. *Eur. J. Clin. Chem. Clin. Biochem.* **1992**, *30*, 263.
16. Marks, F.; Muller-Decker, K.; Furstenberger, G. *Toxicology* **2000**, *153*, 11.
17. Tapiero, H.; Ba, G. N.; Couvreur, P.; Tew, K. D. *Biomed. Pharmacother.* **2002**, *56*, 215.
18. Uhl, W.; Schrag, H.; Schmitter, J. N.; Aufenanger, J. *Br. J. Surg.* **1998**, *85*, 618.
19. Binisti, C.; Assogba, L.; Touboul, E.; Mounier, C.; Huet, J.; Ombetta, J. E.; Dong, C.-Z.; Redeuilh, C.; Heymans, F.; Godfroid, J.-J. *Eur. J. Med. Chem.* **2001**, *36*, 809.
20. 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*, 5137.
21. 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.
22. Maguire, A. R.; Plunkett, S. J.; Papot, S.; Clynes, M.; O'Connor, R.; Touley, S. *Bioorg. Med. Chem.* **2001**, *9*, 745.
23. Glaser, K. B. *Adv. Pharmacol.* **1995**, *32*, 31.
24. Assogba, L.; Ahamada-Himidi, A.; Meddad-Bel Habich, N.; Aoun, D.; Boukli, L.; Massicot, F.; Mounier, C.; Huet, J.; Lamouri, A.; Ombetta, J.-E.; Godfroid, J.-J.; Dong, C.-Z.; Heymans, F. *Eur. J. Med. Chem.* **2005**, *40*, 850.
25. 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.
26. Plocki, S.; Aoun, D.; Ahamada-Himidi, A.; Tavarès-Camarinha, 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**, 2747.
27. Jerzy, R.; Pietraseiwick, M.; Duszynska, B.; Cegla, M. T. *J. Med. Chem.* **1992**, *35*, 2369.
28. Newman, A. H.; Kline, R. H.; Allen, A. C.; Izenwasser, S.; Clifford, G.; Katz, J. L. *J. Med. Chem.* **1995**, *38*, 3933.
29. Brouard, C; Dupont, B.; Leroy, J. M.; Sito, J. P. *Ger. Offen.* 1975, Deutsches Patent No. GWXXBX DE, 243809, 19750220.
30. Kitamura, S.; Fukushi, H.; Miyawaki, T.; Kawamura, M.; Konishi, N.; Terashita, Z.; Naka, T. *J. Med. Chem.* **2001**, *44*, 2438.
31. Poi, M. J.; Tomaszewski, J. W.; Dunlap, C. Y. C. A.; Andersen, N. H.; Gelb, M. H.; Tsai, M.-D. *J. Mol. Biol.* **2003**, *329*, 997.
32. Radvanyi, F.; Jordan, L.; Russo-Marie, F.; Bon, C. *Anal. Biochem.* **1989**, *117*, 103.
33. Rekker, R. F.; De Kort, H. M. *Eur. J. Med. Chem.* **1979**, *14*, 479.