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Structure−Activity Relationships in Platelet-Activating Factor. 12. Synthesis and Biological Evaluation of Platelet-Activating Factor Antagonists with Anti-HIV-1 Activity

Nawal Serradji,[†] Marc Martin,[‡] Okkacha Bensaid,[†,§] Salvatore Cisternino,[∥] Christophe Rousselle,[∥] Nathalie Dereuddre-Bosquet,[‡,⊥] Jack Huet,[†] Catherine Redeuilh,[†] Aazdine Lamouri,[†] Chang-Zhi Dong,[†] Pascal Clayette,[‡,⊥] Jean-Michel Scherrmann,[∥] Dominique Dormont,[‡] and Françoise Heymans*[,†]

Unité de Recherche Pharmacochimie Moléculaire et Systèmes Membranaires, Laboratoire de Pharmacochimie Moléculaire, Université Paris 7—Denis Diderot, case 7066, 2 Place Jussieu, 75251 Paris Cedex 05, France; CEA, Service de Neurovirologie, DSV/DRM, CRSSA, EPHE, Université Paris-Sud Orsay, 18 route du Panorama, BP 6, 92265 Fontenay aux Roses, France; SPI-BIO c/o Service de Neurovirologie, CEA, Fontenay aux Roses Cedex, France; INSERM U26, Hôpital Fernand Widal, 200 rue du Faubourg Saint-Denis, 75475 Paris Cedex 10, France; and COSNA, Laboratoire de Chimie Organique, Substances Naturelles et Analyses, Faculté des Sciences, Université Aboubakr Belkaid, B.P 119, Tlemcen, Algérie

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The HIV-1 central nervous system infection leads to the onset of neurological impairments called AIDS dementia complex (ADC). PAF plays an important role in this pathology, as it is an HIV-1-induced neurotoxin produced by infected or activated macrophages and microglia, in the brain. We previously reported that PAF-antagonists bearing a trisubstituted piperazine presented in vitro anti-HIV-1 activity in human macrophages. To improve the pharmacological activities of our lead compound, **1a**, we modified its carbamate function and evaluated both its antiretroviral and anti-PAF activities. One carbamate derivative (**10c**) demonstrated a similar antiviral activity but a higher anti-PAF potency, whereas **4a**, with an ureide function, presents an increased antiviral activity and can be considered as a pure antiretroviral drug, as it does not present PAF-antagonism. Moreover, we measured the ability of **1a** to cross the blood−brain barrier, using the in situ mouse brain perfusion method and its plasmatic concentrations after iv and po administration. The transport parameter measured (K_{in}) proves that **1a** is able to cross this biological barrier, but a pharmacokinetic study reveals its weak bioavailability in rats.

Introduction

The infection of central nervous system (CNS) by the human immunodeficiency virus (HIV) occurs early after the systemic infection[1] and leads to the onset of neurological dysfunctions named acquired immune deficiency syndrome (AIDS) dementia complex (ADC), observed in 15−20% of patients.[2,3] Productive replication of HIV-1 in brain macrophages and microglia is a critical component of viral pathogenesis. However, how virus−macrophage interactions lead to neurological disease remains incompletely understood.[4] Neither, it is clear whether ADC results from the trafficking into the brain of infected or activated monocytes or direct infection of the brain by the virus. Nevertheless, there is a consensus to say that secretory products from HIV-1-infected macrophages are the likely source of neurotoxic activities. Indeed, after an antigenic stimulation in vitro or contact with neural cells in vivo, infected monocytes release high levels of the proinflammatory cytokine tumor necrosis factor-α (TNF-α) and the phospholipidic mediator platelet-activating factor (PAF).[5−7] HIV-1 production is up-regulated by TNF-α in infected macrophages, and PAF, in turn, appears to increase TNF-α synthesis in HIV-infected cells of monocytic lineage.[8,9] High levels of PAF are detected in cerebrospinal fluids (CSF) of patients with neurologic dysfunctions and correlated with the degree of neurological impairments. Moreover, when added at concentrations close to those found in CSF of HIV-infected patients, PAF produces a dose-dependent neurotoxicity, inhibited by NMDA uncompetitive antagonists MK-801 and memantine.[7] The neurotoxicity induced by HIV-1-infected macrophages can be prevented by PAF-acetylhydrolase (PAF-AH), the main enzyme responsible of PAF catabolism.[10]

Even if the virus plasma level falls below the limit of detection (20−500 HIV RNA copies/mL, depending on the assay used) and neurological disorders are markedly improved in 50−60% of the patients treated by highly active antiretroviral therapy (HAART), the reappearance of HIV in the blood of these patients suggests that this treatment may not eradicate the virus in certain reservoirs, such as the CNS, where it is rapidly replicated and delivered to the periphery. This hypothesis is supported by the weak penetration or the efflux of actual antiretroviral drugs into the brain.[11]

Taking together, these results suggest that PAF plays an important role in the HIV-1-induced neurologic dysfunctions and prompted us to evaluate, in our previous publication, the antiviral activity of PAF antagonists. This led us to the discovery of our lead compound, PMS 601 (compound **1a** previously described,[12] Figure 1), a trisubstituted piperazine that is

* To whom correspondence should be addressed. Tel: 33 1 4427 6050. Fax: 33 1 4427 5641. E-mail: heymans@ccr.jussieu.fr.
[†] Université Paris 7—Denis Diderot.
[‡] CEA.
[§] Université Aboubakr Belkaid.
[∥] INSERM U26.
[⊥] SPI-BIO.

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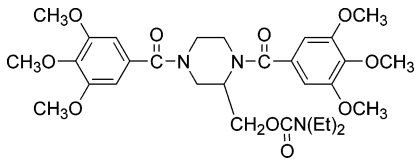


Figure 1. Compound **1a** (PMS 601).

a PAF antagonist (8 μM IC_{50}) and possesses anti-HIV-1 activity (11 μM IC_{50}). It has been shown that it is able to diminish both HIV-1 replication and the neurotoxic effects induced by the important production of PAF by the infected cells in brain (data not shown).[12,13] To improve the biological activities of **1a**, we modified the carbamate linkage to ureate and reversed carbamate and thionocarbamate. Different alkyl substituents of the above functions were used to maintain either the structure skeleton or lipophilicity. Some less lipophilic ester or monosubstituted carbamate derivatives than what have been published[12] are also described in this work to complete this structure−activity relationship study.

We verified whether **1a**, the most studied compound of the series, is able to cross the blood−brain barrier (BBB), using the in situ brain perfusion technique, and reach the virus in one of its main reservoirs and measured its pharmacokinetic parameters in male Sprague−Dawley rats.

Chemistry

By action of commercially available isocyanates on the amine **1**, reported by Serradji et al.,[12] urea derivatives **2a,b** were synthesized following method A in Scheme 1, while the carbamate **2c** was prepared by condensing isopropyl chloroformate with the amine **1**. The phenyl carbamate **7** and the ureide **6** were obtained from the condensation of phenyl chloroformate on the alcohol **5**, as previously described,[14] and the amine **1**, respectively. *N*,*N*-Disubstituted ureides **8a,b** were obtained by treatment of phenyl carbamate **6** (method B, Scheme 1) with the corresponding amines and **8c** by action of the appropriate amine on the phenyl carbonate **7**. The benzyl groups of **2a**−**c** and **8a**−**c** were removed by catalytic hydrogenolysis to afford the corresponding 2-substitued piperazines **3a**−**c** and **9a**−**c**, which, when treated with 3,4,5-trimethoxybenzoyl chloride, led to **4a**−**c** and **10a**−**c**.

As outlined in Scheme 2, the ester **12** was derived from the previously described compound **11** by debenzylation.[14] The piperazinic nitrogens were then substituted using 3,4,5-trimethoxybenzoyl chloride, and the ester **13** thus obtained was reduced into the corresponding alcohol **14** by sodium borohydride in MeOH. The thionocarbamoyl functions (**15a,b**) were introduced by condensation of the alcoholate of **14** with the commercial thiocarbamoyl chloride, while ester **15c** was synthesized by reacting **14** with propanoyl chloride.

Tritylation followed by acylation with 3,4,5-trimethoxybenzoyl chloride of **16**[14] afforded **18** through the intermediate **17**. An acid deprotection of **18** into **19** and condensation of the latter with syringic acid using DCC led to **20**, as described in Scheme 3. **[^{3}H]1a** (compound **21**, Scheme 3) was prepared by alkylation of compound **20** with commercial [^{3}H]methyl iodide (Amersham, France).

Results and Discussion

Evaluation of New 1a Derivatives. All the compounds were tested for their ability to inhibit PAF-induced platelet aggregation on one hand and for their activity in MDM infected with the reference macrophage-tropic HIV-1/Ba-L strain on the other hand,[12,13] and the activities are reported in Table 1.

In these experiments, **1a** demonstrated identical effects as previously described with an anti-HIV IC_{50} and an anti-PAF IC_{50} equal to 11 and 8 μM, respectively.[12]

The introduction of an ureide function as in **10a**, a direct analogue of **1a**, leads to the loss of both anti-PAF and anti-HIV activities, apparently due to the log *P* decrease ($f_W = -0.564$) or the presence of a new NH moiety. To distinguish which is the main factor responsible of this diminution, **10b**, an isolipophilic derivative of **1a**, was synthesized and tested. The result shows that this compound remains less active than our lead in both biological assays. Taken together with the result of **1d**, the carbamate analogue of **10b** previously described,[12] it seems clear that the new NH moiety induces a drastic influence on the anti-PAF activity, as already proposed in our previous publication for an amide analog,[12] and suggests that other factors than lipophilicity are involved. Indeed, we cannot exclude an intramolecular H-bond between the ureide linkage and the amide group or between the compound and its biological target. This could modify, especially in the first case, the "cache-oreilles" effect and prevent the molecule from interacting efficiently with the PAF−R.

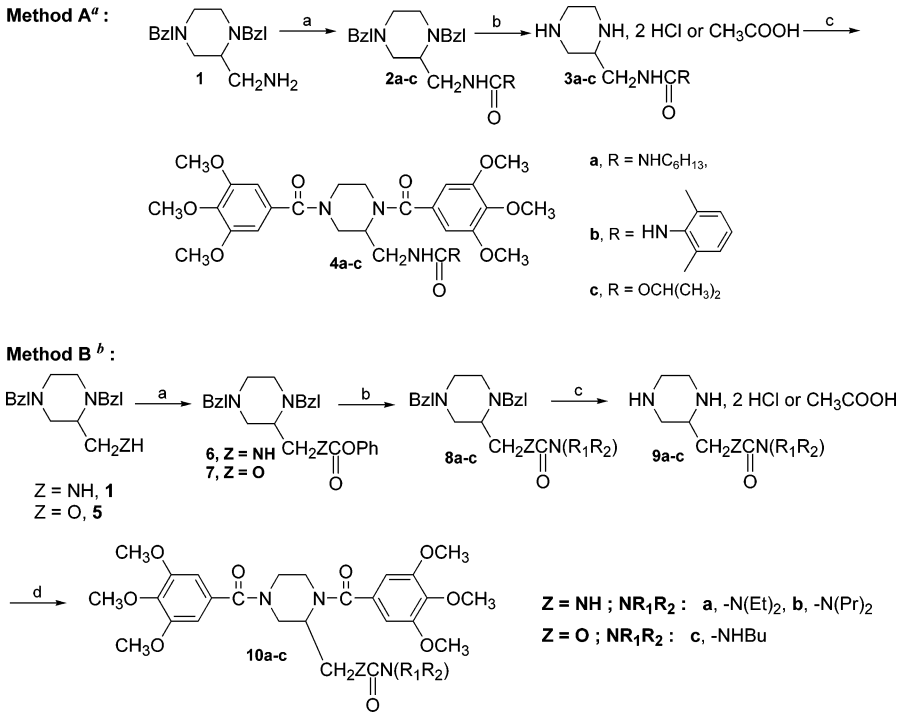
We have shown in our previous publication[12] that the replacement of the *N*,*N*-diethylamino moiety by a pyrrolidino or a piperidino group in the C-substituent of **1a** had no influence or increased its potency on the PAF-R, while its anti-HIV capacity was reduced. However, using the *N*-monoalkylamino group with a weak steric hindrance of the alkyl chain close to the carbonyl function decreased less the antiviral activity. This prompted us to synthesize compound **4a**, in which a linear hexyl was used and whose lipophilicity is similar to that of **10b**. It is worthwhile to note that this compound is not only much more active than **10b** but also 10-fold more potent than **1a** against HIV, although its anti-PAF property is not improved. This underlines the importance of the second NH for the antiviral activity in these isolipophilic compounds. The decrease of this activity for **4b** ($IC_{50} = 32$ μM) could be explained by the steric effect generated by the *o*-methyl substituents on the phenyl group.

However, compared to **1a**, only the anti-PAF activity, but not at all the antiviral potency, was improved when an *n*-butyl group was used, instead of two ethyls, in the carbamate moiety to preserve a similar global lipophilicity, as demonstrated by the biological data of **10c**.

Moreover, when the nitrogen and the oxygen atoms are inverted (compound **4c**), the anti-PAF activity is unchanged and the anti-HIV activity is weakly diminished. This suggests that the order of the atoms in the carbamate function would not be important to keep both activities.

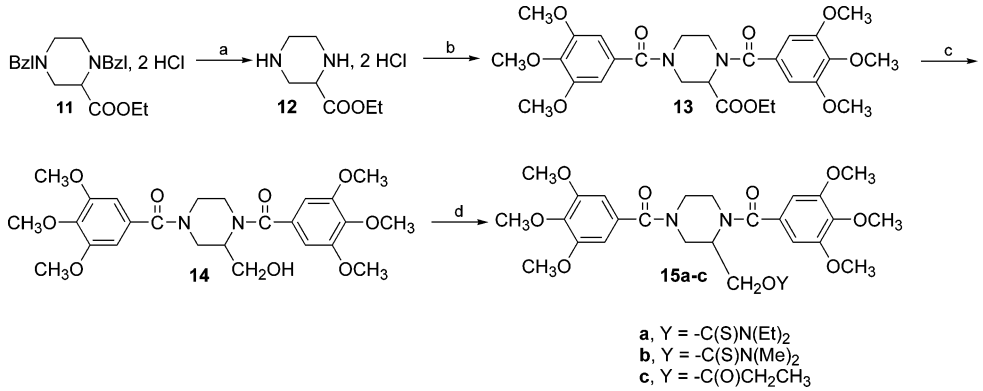
The ester **15c** ($f_W = 0.488$) was synthesized to complete our preliminary results, since the ester ana-

Scheme 1[a]



[a] Reagents: (a) O=C=N−R, Et_2O or RCOCl, Et_3N, toluene: (b) H_2, Pd/C (10%), EtOH/HCl or CH_3COOH, 40 °C; (c) 3,4,5-$(MeO)_3$PhCOCl, Et_3N, CH_2Cl_2. [b] Reagents: (a) PhOCOCl, Pyr, CH_2Cl_2, 0 °C; (b) $HN(R_1R_2)$, reflux; (c) H_2, Pd/C (10%), EtOH/HCl or CH_3COOH, heat; (d) 3,4,5-$(MeO)_3$PhCOCl, Et_3N, CH_2Cl_2.

Scheme 2[a]



[a] Reagents: (a) H_2, Pd/C, EtOH, heat; (b) 3,4,5-$(MeO)_3$PhCOCl, Et_3N, CH_2Cl_2; (c) $NaBH_4$, MeOH, 0 °C; (d) YCl, NaH, DMF, 70−80 °C or Et_3N, CH_2Cl_2.

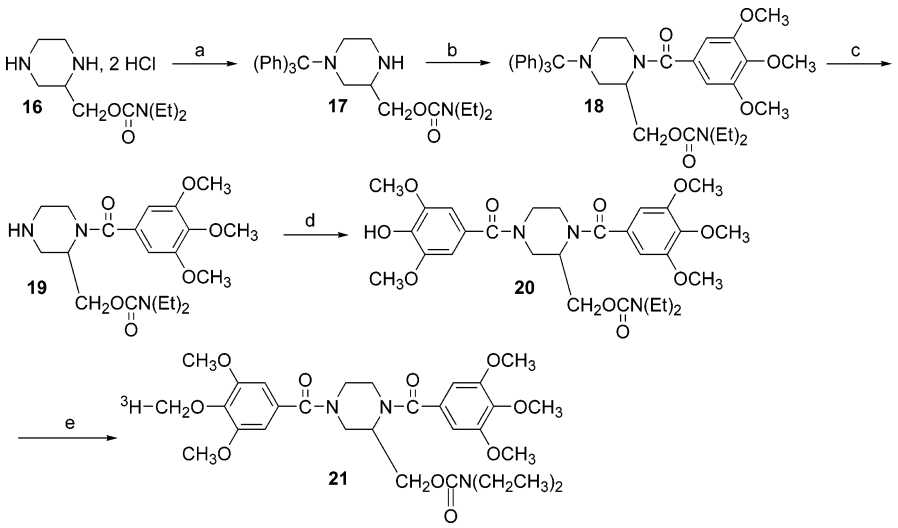
logue (W = $CH_2OCOCMe_3$) previously described[12] possesses a potent anti-PAF activity but did not present a good antiviral one. The result obtained with **15c** shows clearly that this lack should not be due to the high lipophilicity of pivalate (f_W = 1.526), as **15c** is isolipophilic to **1a** (f_W = 0.488), and that the ester bond is not favorable for the antiviral activity.

When the carbonyl function in the C-substituent of **1a** was changed to a thiocarbonyl as in **15a**, an increase of the lipophilicity was not associated with an augmentation of the anti-PAF activity as expected. In addition, with this modification, the molecule becomes highly toxic on MDM at 10 μM and is inactive at 1 μM. Thus, its antiviral activity could not be evaluated. The **1a** isolipophilic compound **15b** ($\times c4_W$ = 0.611) presents a higher anti-PAF potency but a moderate antiviral activity, showing that the introduction of a sulfur atom in this substituent does not improve both of them.

Blood−Brain Barrier Permeability of 1a. One of the main defects of HAART to eradicate the virus seems to be related to the weak penetration of these drugs into certain tissues, such as the CNS, which, in consequence, could be considered as a virus reservoir. New antiviral agents capable to cross the BBB efficiently represent potential candidates to struggle against HIV toward a complete eradication of the virus. This promped us to evaluate the intrinsic permeability of **1a** into the brain, the most studied compound of the series.[12] This evaluation was performed in mice using **[^{3}H]1a** and the in situ brain perfusion method.[15]

Cerebral vascular volume was preserved, and thus, the BBB integrity was maintained during the perfusion, as shown by the fact that no significant penetration was observed when radioactive [^{14}C]sucrose, a marker of brain vascular volume, was coperfused during brief periods.

Scheme 3[a]



[a] Reagents: (a) Ph_3CCl, Et_3N, CH_2Cl_2; (b) 3,4,5-$(MeO)_3C_6H_2COCl$, Et_3N, CH_2Cl_2; (c) HCl, MeOH; (d) 4-OH-3,5-$(MeO)_2C_6H_2COOH$, DCC, HOBT, CH_2Cl_2, reflux; (e) $[^3H]CH_2Cl$, K_2CO_3, CH_3CN/toluene.

The blood transport coefficient of **[^{3}H]1a** (K_{in} = 1.02 ± 0.05 μL/s/g), i.e., its brain uptake, obtained is not only higher than that of an hydrophilic compound such as urea, one of the least permeable compounds of the BBB ($K_{in} \approx 0.1$ μL/s/g), but also than that of morphine (K_{in} = 0.26 ± 0.04 μL/s/g), which is a well-known neuroactive drug.[16] This experiment enables us to compare the capacities of **1a** vs morphine to cross the BBB, but it does not establish its antiviral activity within the brain. Indeed, morphine acts through its receptor and **1a**'s mode of action is still unknown. This means that **1a** has an intrinsic capacity to cross the biological barrier and, consequently, is susceptible to inhibit HIV-1 replication and antagonize PAF activity in the brain, without changing BBB integrity. However, the brain extraction coefficient of **1a** (2.5%), obtained from the ratio ($K_{in\ 1a}$/$K_{in\ diazepam}$) × 100, shows clearly that **1a** uptake from the blood to the CNS is not complete, since diazepam, a lipophilic compound which freely crosses the BBB and thus was used to estimate the flow rate, has a blood transport coefficient of 42.5 μL/s/g.[16] However, this evaluation was performed on an intact biological membrane, and the BBB changes during invasion of the CNS by HIV-1 were not taken into account. Indeed, inflammatory and toxic molecules secreted by monocytes and microglia as well as viral proteins such as gp120, Tat, and Nef seem to be involved in the modifications of the functional integrity of tight junctions of brain endothelium.[17,18] On the other hand, the multidrug resistance pump P-glycoprotein (P-gp) has been identified as an important determinant of drug permeability across the BBB.[16] Thus, the potential influence of this protein on the penetration of **1a** through the BBB is also to be evaluated.

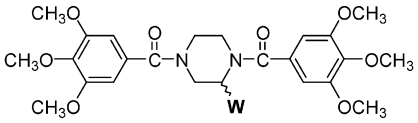
Pharmacokinetic Study of 1a. The objective of this investigation was to determine the pharmacokinetic profiles of **1a** after its single administration by intravenous and oral routes to male Sprague−Dawley rats. The bioavailability was also calculated. The main pharmacokinetic parameters (calculated for a dose of 1 mg/kg) are summarized in Table 2. To appreciate the anti-HIV efficacy of maximal **1a** concentrations found in plasma of rats, the micromolar values were calculated using the molecular weight of **1a** (MW = 603 g/mol). Using this calculation, we showed that the plasma concentrations of **1a** are equal to 141 ± 46 and 26 ± 6 μM after the iv and per os administrations, respectively. These calculations are between IC_{50} (20 ± 15 μM) and IC_{70} (43 ± 23 μM), and IC_{70} and IC_{90} (189 ± 109 μM) obtained in experiments performed with MDM infected with different macrophage-tropic strains (data not shown). It is probable that these concentrations are insufficient to enable a significant in vivo anti-HIV activity.

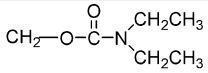
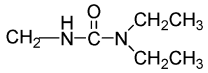
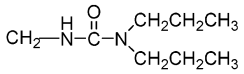
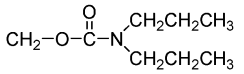
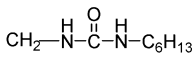
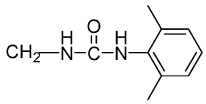
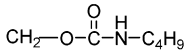
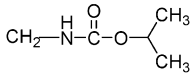
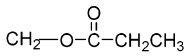
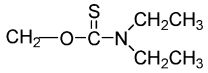
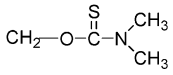
It has been reported very recently that BMS-378806, a trisubstituted piperazine derivative, is able to interfere with CD4−gp120 interactions.[19] It exhibits not only a good HIV-1 inhibitory profile with high specificity in a great number of different virus strains but also interesting pharmaceutical and pharmacokinetic properties, without crossing the BBB to any appreciable extent,[20] in three animal models. It is evident that this compound could be considered as a new HIV-1 attachment inhibitor. The important structural similarities of this series with **1a** do not lead to a similar mode of action, as we know that this compound is not an HIV-1 entry inhibitor in the cells (data not shown).

Conclusion

On the basis of our previous work, we have demonstrated in this study that using a *n*-butyl instead of diethyl moiety on the carbamate function only increases the anti-PAF activity. However, when the carbamate function is replaced by an ureide one and a linear chain is incorporated to maintain the lipophilicity, the antiviral activity is highly enhanced. This opens new perspectives in the development of original antiviral agents. Moreover, **1a**'s K_{in} and log $P_{octanol/water}$ (log $P_{octanol/water}$ = 0.8 vs log $P_{octanol/saline}$ = 1.02 ± 0.02 for **1a** and [^{3}H]AZT,[21] respectively), its ability to increase AZT activity in infected MDM, and its lack of toxicity in rats after oral administration (LD_{50} > 2000 mg/kg without mortality at this dose level, unpublished data) justify its use in the search for new derivatives with increased antiviral activity and bioavailability.

Table 1. Influence of Substituent W on in Vitro Anti-HIV and Anti-PAF Activities



compd	W	f_W [a]	Anti-HIV [b] IC$_{50}$ μM	Anti-PAF [c] IC$_{50}$ μM	CC$_{50}$ [d] μM
1a [e]		0.564	11	8	>1000
10a		-0.564	40	>10	>100
10b		0.474	>10	>10	>100
1d [e]		1.602	66±13 [f]	0.37	> 100
4a		0.763	1	>10	>100
4b		0.345	32	>10	>100
10c		0.763	10	0.67	>100
4c		0.334	25	5	>100
15c		0.488	>100	0.158	>100
15a		1.649	ND [g]	>5	>1
15b		0.611	76% [f]	1.6	>100

[a] Lipophilic contribution of W calculated from Rekker et al.[23] [b] Antiviral activity determined with HIV-1/Ba-L-infected monocyte-derived macrophages, from a single measurement (see the Experimental Section). [c] Inhibition of PAF-induced platelet aggregation using platelet-rich plasma (PRP) of New Zealand rabbits calculated from a dose−response curve as described in the Experimental Section (n = 5, mean ± 10%). [d] Fifty percent cytotoxicity concentration evaluated by neutral red staining. [e] See Serradji et al.[12] [f] Percent inhibition of HIV-1 replication at 100 μM. [g] Not determined because highly toxic at 10 μM.

Table 2. Pharmacokinetic Parameters for 1a in Rats[a,b]

	after iv administration	after po administration
C_{max} (ng/mL)	861 ± 338	33 ± 12.1
$t_{1/2}$ (h)	2.14 ± 0.17	2.29 ± 0.40
AUC (ng·h/mL)[c]	1828 ± 716	177 ± 106
bioavailability (%)	-	10.9 ± 1.1

[a] Three animals were included in this study and received each 100 mg/kg by iv route and 500 mg/kg by oral route. [b] All the results are normalized for an administration dose of 1 mg/kg of body weight in order to compare both routes of administration. [c] Area under curve.

Experimental Section

Chemistry. General Methods. 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 with silica gel 60 (70−230 mesh ASTM, Merck). All melting points were determined on a digital melting point apparatus (Electrothermal) and are uncorrected. The structures of all compounds were confirmed by IR and ^{1}H NMR spectra. IR spectra were obtained in paraffin oil with a ATI Mattson Genesis Series FTIR spectrometer, and ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or in 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 designated as follows: s, singlet, d, doublet, dd, doublet doublet, t, triplet, br s, broad singlet, m, multiplet, q, quadruplet, sex, sextuplet, sep, septuplet. 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.

1,4-Dibenzyl-2-(hexylaminocarbonylaminomethyl)-piperazine (2a). A solution of hexyl isocyanate (1.73 g, 13.56 mmol) in ether (50 mL) was added dropwise to a solution of the amine **1** (4 g, 13.56 mmol) in ether (50 mL). The solution was stirred for 2 h at room temperature, the solvent was eliminated in a vacuum, and the residue was chromatographed on silica gel column using $MeOH/CH_2Cl_2$ (3:97, v/v) as eluent to yield **2a** (5.26 g, 92%) as a wax: R_f 0.15 ($MeOH/CH_2Cl_2$, 5:95, v/v); IR (ν cm^{-1}) 3356 (NH), 3061 (ArH), 1629 (C=O amide), 1573 (ArC=C); ^1H NMR δ 7.19−7.06 (m, 10H, ArH), 5.31 (br s, 1H, NH), 4.99 (t, 1H, J = 5.5 Hz, NH), 3.92 (d, 1H, J = 13 Hz, C*H*-Ph), 3.50−2.81 (m, 4H, CH_2NC=ONCH_2), 3.38 (d, 1H, J = 13 Hz, C*H*-Ph), 3.32 (d, 1H, J = 13 Hz, C*H*-Ph), 3.14 (d, 1H, J = 13 Hz, C*H*-Ph), 2.55 (m, 4H, piperazine), 2.13 (m, 3H, piperazine), 1.37 and 1.19 (m, 8H, $(CH_2)_4$), 0.77 (t, 3H, J = 6.4 Hz, CH_3); ^{13}C NMR δ 158.68 (C=O), 138.13, 137.59, 128.91, 128.83, 128.10, 126.95, 126.89 (ArC=C), 62.81, 57.54, 55.78, 52.12, 50.03 (CH_2 benzyl and piperazine), 58.42 (CH piperazine), 40.45, 39.67, 31.41, 30.11, 26.46, 22.43 (CH_2), 13.89 (CH_3).

1,4-Dibenzyl-2-((2,6-dimethylphenyl)aminocarbonyl-aminomethyl)piperazine (2b). The process was the same as described for **2a** but using 2,6-dimethylphenyl isocyanate to yield **2b** (4.25 g, 71%) as a wax: R_f 0.65 ($MeOH/CH_2Cl_2$, 5:95, v/v); IR (ν cm^{-1}) 3337 (NH), 3023 (ArH), 1639 (C=O amide), 1563 (ArC=C); ^1H NMR δ 7.24−7.08 (m, 10H, ArH), 7.07−7.02 (m, 1H, NH−C_6H_3), 7.03−6.63 (m, 2H, NH−C_6H_3), 6.62 (br s, 1H, NH), 5.80 (d, 1H, J = 13 Hz, CH−Ph), 5.03 (br s, 1H, NH), 3.44−3.25 (m, 4H, CH_2−Ph, CH_2NHC=O), 2.96 (d, 1H, J = 13 Hz, CH−Ph), 2.90−2.30 (m, 4H, piperazine), 2.20 (s, 6H, CH_3), 2.08−1.90 (m, 2H, piperazine), 1.90−1.70 (m, 1H, piperazine); ^{13}C NMR δ 157.89 (C=O), 138.18, 137.49, 137.18, 134.17, 128.53, 128.32, 128.08, 127.49, 126.93, 126.69 (ArC=C), 62.88, 57.32, 55.43, 52.08, 50.19 (CH_2 benzyl and piperazine), 58.34 (CH piperazine), 39.49 (CH_2), 18.34 (CH_3).

1,4-Dibenzyl-2-(isopropyloxycarbonylaminomethyl)-piperazine (2c). A solution of isopropyl chloroformate (1 M in toluene, 44 mL) was added dropwise to a solution of **1** (10 g, 33.90 mmol) and Et_3N (25 mL) in toluene (100 mL). The solution was stirred at room temperature for 2 h, diluted with Et_2O (100 mL), washed with a saturated $NaHCO_3$ solution and water, dried over $MgSO_4$, and filtered, and the solvent was eliminated under reduced pressure to yield **2c** (8.65 g, 67%) as a wax: R_f 0.4 ($MeOH/CH_2Cl_2$, 5:95, v/v); IR (ν cm^{-1}) 1712 (C=O amide), 1602 (ArC=C); ^1H NMR δ 7.61 (br s, 4H, ArH), 7.34 (br s, 6H, ArH), 6.50 (br s, 1H, NH), 4.70−2.60 (m, 13H, CH_2−Ph, CH_2NC=O, piperazine), 3.41 (m, 1H, J = 7 Hz, OCH), 1.14 (br s, 6H, CH_3).

2-(Hexylaminocarbonylaminomethyl)piperazine, Diacetate (3a). To a solution of the ureate **2a** (6 g, 14.21 mmol) in glacial acetic acid (70 mL) was added 100 mg of Pd/C (10%), and this mixture was warmed at 40 °C and stirred under hydrogen atmosphere. After the disappearance of the starting material (shown by TLC), the suspension was filtered, the solvents were evaporated, and the residue was crystallized in acetic acid/ether to give **3a** (4.3 g, 83.5%) of as crystals: R_f 0.08 ($NH_4OH/MeOH/CH_2Cl_2$, 2:20:80, v/v/v); mp 119.8 °C; IR (ν cm^{-1}) 1732 (C=O acetic acid), 1646 (C=O ureate); ^1H NMR (DMSO-d_6) δ 7.48 (br s, 4H, NH_2^+), 6.38 (br s, 1H, NHC=O), 6.23 (br s, 1H, NHC=O), 3.40−2.30 (m, 11H, piperazine, CH_2NC=O), 1.89 (s, 6H, CH_3COO^-), 1.70−1.10 (m, 8H, $(CH_2)_4$), 0.94 (br s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 173.81 (C=O acetic acid), 158.38 (C=O ureate), 53.72 (CH piperazine), 46.36, 43.04 (CH_2 piperazine), 41.79, 39.38, 31.13, 30.01, 26.15, 22.16 (CH_2 ureate), 22.92 (CH_3 acetic acid), 13.95 (CH_3).

2-((2,6-Dimethylphenyl)aminocarbonylaminomethyl)-piperazine, Diacetate (3b). This compound was obtained from **2b** (4.24 g, 19.61 mmol) and glacial acetic acid (20 mL), following the process used for compound **3a**, as white crystals (3 g, 81%): R_f 0.14 ($NH_4OH/MeOH/CH_2Cl_2$, 2:20:80, v/v/v); mp 149 °C; IR (ν cm^{-1}) 1733 (C=O acetate), 1644 (C=O ureate), 1569 (ArC=C); ^1H NMR (DMSO-d_6) δ 7.91 (br s, 4H, NH_2^+), 7.00 (br s, 3H, ArH), 6.70 (br s, 1H, NHC=O), 3.40−2.30 (m, 9H, CH_2NHC=O, CH_2 piperazine), 2.15 (s, 6H, CH_3), 1.82 (s, 6H, CH_3 acetate); ^{13}C NMR (DMSO-d_6) δ 173.64 (C=O acetic acid), 156.33 (C=O ureate), 136.15, 135.59, 127.61, 125.63 (ArC=C), 53.68 (CH piperazine), 46.42, 43.16, 42.94 (CH_2 piperaine), 41.91 (CH_2 ureate), 22.69 (CH_3 acetic acid), 18.29 (CH_3).

2-(Isopropyloxycarbonylaminomethyl)piperazine, Dihydrochloride (3c). This compound was prepared from **2c** using the same process as for **3a** but with 12 N HCl instead of CH_3COOH, as a solid (4.2 g, 87%): R_f 0.60 ($NH_4OH/MeOH/CH_2Cl_2$, 2:20:80, v/v/v); mp 237 °C; IR (ν cm^{-1}) 1712 (C=O); ^1H NMR (DMSO-d_6) δ 10.01 (br s, 4H, NH_2^+), 4.72 (t, 1H, J = 6 Hz, NHC=O), 4.00−2.60 (m, 9H, CH_2N, CH_2 piperazine), 1.14 (d, 6H, J = 5.6 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 156.28 (C=O), 67.54 (OCH), 51.67 (CH piperazine), 42.08 (CH_2NH), 22.03 (CH_3).

2-(Hexylaminocarbonylaminomethyl)-1,4-bis(3,4,5-trimethoxybenzoyl)piperazine (4a). To a solution of **3a** (1.76 g, 4.86 mmol) and Et_3N (4.05 mL, 29.16 mmol) in CH_2Cl_2 (50 mL) was added dropwise a solution of 3,4,5-trimethoxybenzoyl chloride (2.8 g, 12.15 mmol) in CH_2Cl_2. The solution was stirred at room temperature overnight and EtOH (5 mL) was added. The organic layer was washed with saturated $NaHCO_3$ solution and water, dried over $MgSO_4$, and filtered, and the solvent was evaporated. The residue was chromatographed using a silica gel column with $MeOH/CH_2Cl_2$ (1:99, v/v) as eluent to give, after a crystallization in Et_2O/MeOH, **4a** (1.04 g, 46%) as white crystals: R_f 0.3 ($MeOH/CH_2Cl_2$, 5:95, v/v); mp 94.8 °C; IR (ν cm^{-1}) 3374 (NH), 1631 (C=O ureate), 1621 (C=O amide), 1584 (ArC=C); ^1H RMN δ 6.61 (s, 2H, ArH), 6.59 (s, 2H, ArH), 5.31 (br s, 1H, NH), 5.10−3.90 (m, 5H, NH, CH_2 and CH piperazine), 3.90−3.70 (m, 18H, CH_3O), 3.70−2.60 (m, 7H, $CH_2NCONCH_2$, CH_2 piperazine), 1.50−0.95 (m, 8H, $(CH_2)_4$), 0.77 (t, 3H, J = 6.4 Hz, CH_3); ^{13}C NMR δ 171.43, 170.84 (C=O amide), 158.43, 153.38, 153.28, 138.48, 130.09, 104.49 (ArC=C), 60.86, 60.79, 56.29, 56.21 (CH_3O), 40.50, 39.05, 31.40, 30.15, 26.45, 22.46 (CH_2 ureate), 13.91 (CH_3). Anal. ($C_{32}H_{46}N_4O_9 \cdot 0.75H_2O$) C, H, N.

2-((2,6-Dimethylphenyl)aminocarbonylaminomethyl)-1,4-bis(3,4,5-trimethoxybenzoyl)piperazine (4b). This compound was prepared from **3b**, using the same process as for **4a**, as a solid (890 mg, 17%): R_f 0.3 ($MeOH/CH_2Cl_2$, 5:95, v/v); mp 153.3 °C; IR (ν cm^{-1}) 3374 and 3235 (NH), 1651 (C=O ureate), 1624 (C=O amide), 1586 (ArC=C); ^1H RMN δ 7.10−6.80 (m, 3H, *m*- and *p*-ArH), 6.58 and 6.56 (s, 4H, *o*-ArH), 5.50−4.90 (m, 6H, NH, CH_2 piperazine), 4.85−3.75 (m, 12H, CH_3O), 3.75−2.60 (m, 10H, CH_2O, CH_2NHC=O, CH_2 piperazine), 2.35−1.60 (m, 7H, CH_3, NH); ^{13}C NMR δ 171.24, 170.79 (C=O amide), 156.84 (C=O ureate), 153.37, 153.15, 139.49, 139.40, 136.47, 134.03, 130.00, 128.36, 104.67, 104.43 (ArC=C), 60.85, 60.77, 56.29, 55.95 (CH_3O), 39.11 (CH_2 ureate), 18.17 (CH_3). Anal. ($C_{34}H_{42}N_4O_9 \cdot 0.75H_2O$) C, H, N.

2-(Isopropyloxycarbonylaminomethyl)-1,4-bis(3,4,5-trimethoxybenzoyl)piperazine (4c). This compound was prepared from **3c**, using the same process as for **4a**, as a solid (3 g, 70%): R_f 0.3 ($MeOH/CH_2Cl_2$, 5:95, v/v); mp 138 °C; IR (ν cm^{-1}) 3368 (NH), 2976 (ArCH), 1717 (C=O carbamate), 1622 (C=O amide), 1585 (ArC=C); ^1H NMR δ 6.60 (s, 2H, ArH), 6.58 (s, 2H, ArH), 5.00 (br s, 1H, NH), 4.80 (sep, 1H, J = 6.2 Hz, OCH), 4.60−3.90 (m, 3H, CH_2NHC=O, piperazine), 3.81, 3.79 and 3.70 (s, 18H, OCH_3), 3.70−2.70 (m, 6H, piperazine), 1.15 (d, 6H, J = 6.2 Hz, CH_3); ^{13}C NMR δ 170.97, 170.67 (C=O amide), 156.22 (C=O carbamate), 153.25, 139.30, 139.18, 130.25, 129.81, 104.21 (ArC=C), 67.94 (OCH), 60.56, 56.03, 55.96 (CH_3O), 39.31 (CH_2), 21.94, 21.84 (CH_3). Anal. ($C_{29}H_{39}N_3O_{10} \cdot 0.5H_2O$) C, H, N.

1,4-Dibenzyl-2-(phenoxycarbonylaminomethyl)piperazine (6). To a cooled solution, in an ice bath, of **1** (1.85 g, 6.27 mmol) and pyridine (1 mL, 12.54 mmol) in CH_2Cl_2 (20 mL) was added dropwise a solution of phenylchloroformate (1.2 mL, 9.40 mmol) in CH_2Cl_2 (5 mL). The solution was stirred at 0 °C for 1 h and for 3 h at room temperature. It was then washed with a saturated $NaHCO_3$ solution and water, dried ($MgSO_4$), and filtered, and the solvent was evaporated in a vacuum. The residue was chromatographed using a silica gel

column with MeOH/CH_2Cl_2 (1:99, v/v) as eluent to give **6** (2.26 g, 86.8%) as a wax: R_f 0.55 (MeOH/CH_2Cl_2, 5:95, v/v); IR (ν cm^{-1}) 3338 (NH), 1730 (C=O amide), 1594 (ArC=C); ^{1}H NMR (60 MHz) δ 7.20 (br s, 15H, ArH), 5.83 (br s, 1H, NH), 4.00−3.00 (m, 6H, CH_2−NH, CH_2−Ph), 2.83−2.18 (m, 7H, CH_2 and CH piperazine).

1,4-Dibenzyl-2-(phenoxycarbonyloxymethyl)piperazine (7). This compound was prepared from **5**, using the same process as for **6**, as a wax (1.97 g, 88%); R_f 0.31 (MeOH/CH_2Cl_2, 5:95, v/v); IR (ν cm^{-1}) 3062 (ArCH), 1758 (C=O), 1599 (ArC=C); ^{1}H NMR δ 7.27−7.00 (m, 15H, ArH), 4.42−4.34 (m, 2H, CH_2O), 3.85 (d, 1H, J = 13 Hz, CH−Ph), 3.44−3.29 (m, 2H, CH_2−Ph), 3.32 (d, 1H, J = 13 Hz, CH−Ph), 2.81−2.79 (m, 1H, C*H*-CH_2O), 2.66−2.50 (m, 2H, piperazine), 2.37−2.22 (m, 4H, piperazine); ^{13}C NMR δ 153.55 (C=O), 138.72, 138.04, 129.37, 128.91, 128.65, 128.18, 127.00, 126.91, 125.93, 120.96 (ArC=C), 67.34, 62.84, 58.55, 55.34, 52.61, 49.48 (CH_2 benzyl and piperazine), 58.08 (CH piperazine).

1,4-Dibenzyl-2-(*N*,*N*-diethylaminocarbonylaminomethyl)piperazine (8a). A solution of **6** (3.2 g, 7.71 mmol) in Et_2NH (10 mL) was refluxed for 20 h. After elimination of the excess of amine under reduced pressure, the residue was taken up with CH_2Cl_2, washed with 1 N NaOH and water, dried ($MgSO_4$), and filtered, and the solvent was eliminated in a vacuum. A chromatography on a silica gel column using MeOH/CH_2Cl_2 (2:98, v/v) as eluent gave **8a** (1.84 g, 61%) as a wax: R_f 0.29 (MeOH/CH_2Cl_2, 5:95, v/v); IR (ν cm^{-1}) 3366 (NH), 1636 (C=O ureate), 1589 (ArC=C); ^{1}H NMR δ 7.19 (br s, 10H, ArH), 5.01 (br s, 1H, NH), 3.94 (d, 1H, J = 13.6 Hz, CH−Ph), 3.42 (d, 1H, J = 13 Hz, CH−Ph), 3.33 (d, 1H, J = 13 Hz, CH−Ph), 3.24 (d, 1H, J = 13.6 Hz, CH−Ph), 3.19 (m, 2H, CH_2NH), 3.13 (q, 4H, J = 7 Hz, N(C$H_2$$CH_3$)$_2$), 2.69 (m, 4H, piperazine), 2.19 (m, 3H, piperazine), 1.03 (t, 6H, J = 7 Hz, N(CH_2CH_3)$_2$).

1,4-Dibenzyl-2-(*N*,*N*-dipropylaminocarbonylaminomethyl)piperazine (8b). This compound was prepared, using the same process as for **8a**, as a wax (1.97 g, 88%): R_f 0.31 (MeOH/CH_2Cl_2, 5:95, v/v); IR (ν cm^{-1}) 3379 (NH), 1636 (C=O ureate), 1590 (ArC=C); ^{1}H NMR δ 7.18 (br s, 10H, ArH), 5.05 (t, 1H, J = 4 Hz, NH), 3.95 (d, 1H, J = 13.5 Hz, CH−Ph), 3.41 (d, 1H, J = 13.1 Hz, CH−Ph), 3.32 (d, 1H, J = 13.1 Hz, CH−Ph), 3.02 (m, 6H, CH_2NH, N(C$H_2$$CH_2CH_3$)$_2$), 2.80−2.35 (m, 4H, piperazine), 2.35−1.90 (m, 3H, piperazine), 1.46 (m, 4H, J = 7.4 Hz, N(CH_2C$H_2$$CH_3$)$_2$), 0.80 (t, 6H, J = 7.4 Hz, N(CH_2CH_2CH_3)$_2$); ^{13}C NMR δ 157.57 (C=O), 138.41, 137.49, 128.93, 128.35, 128.12, 127.99, 126.88, 126.78 (ArC=C), 62.84, 57.26, 55.69, 52.34, 50.33 (CH_2 benyl and piperazine), 58,21 (CH piperazine), 48.87, 40.06, 21.61 (CH_2 ureate), 11.18 (CH_3).

1,4-Dibenzyl-2-(*N*-butylaminocarbonyloxymethyl)piperazine (8c). This compound was prepared from **7** (5 g, 12 mmol), using the same process as for **8a**, as a wax (4 g, 84%): R_f 0.24 (MeOH/CH_2Cl_2, 3:97, v/v); IR (ν cm^{-1}) 3339 (NH), 1712 (C=O amide), 1602 (ArC=C); ^{1}H NMR δ 7.17 (m, 10H, ArH), 4.68 (br s, 1H, NH), 4.25 (dd, 1H, J = 11.3 and 3.8 Hz, CHOC=O), 4.20 (dd, 1H, J = 11.3 and 4.5 Hz, CH−Ph), 3.92 (d, 1H, J = 13.5 Hz, CH−Ph), 3.06 (m, 2H, NH−CH_2), 2.80−1.90 (m, 7H, piperazine), 1.29 (m, 4H, NH−CH_2(CH_2)$_2$−CH_3), 0.84 (t, 3H, J = 7 Hz, CH_3); ^{13}C NMR δ 156.31 (C=O), 138.62, 138.03, 128.88, 128.04, 126.76 (ArC=C), 63.63, 62.81, 58.34, 55.70, 52.66, 49.93 (CH_2 benzyl and piperazine), 19.74 (CH_2), 13.61 (CH_3).

2-(*N*,*N*-Diethylaminocarbonylaminomethyl)piperazine, Dihydrochloride (9a). This compound was prepared as described for compounds **3** from **8a** (1.48 g, 4.67 mmol) and purified by crystallization in MeOH/Et_2O, as crystals (1.34 g, 35%): R_f 0.1 (NH_4OH/MeOH/CH_2Cl_2, 2:20:80, v/v/v); mp 103 °C; IR (ν cm^{-1}) 1617 (C=O); ^{1}H NMR (DMSO-d_6) δ 9.95 (br s, 4H, NH_2^+), 6.71 (br s, 1H, NH), 3.90−2.80 (m, 13H, CH_2NH, N(C$H_2$$CH_3$)$_2$, piperazine), 1.01 (t, 6H, J = 6.7 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 156.95 (C=O), 52.22 (CH piperazine), 42.29, 40.33, 39.92 (CH_2 piperazine), 39.30 (CH_2), 13.87 (CH_3).

2-(*N*,*N*-Dipropylaminocarbonylaminomethyl)piperazine, Diacetate (9b). This compound was prepared as described for compounds **3** from **8b** (1.92 g, 4.55 mmol) and purified by crystallization in acetic acid/H_2O, as crystals (870 mg, 53%): R_f 0.33 (NH_4OH/MeOH/CH_2Cl_2, 2:20:80, v/v/v); mp 134 °C; IR (ν cm^{-1}) 1633 (C=O); ^{1}H NMR (DMSO-d_6) δ 6.32 (br s, 1H, NHC=O), 5.14 (br s, 4H, NH_2^+), 3.10−2.10 (m, 13H, CH_2NH, N(C$H_2$$CH_2CH_3$)$_2$, piperazine), 1.81 (s, 6H, CH_3COO-), 1.43m (4H, J = 7.2 Hz, N(CH_2C$H_2$$CH_3$)$_2$), 0.80 (t, 6H, J = 7.2 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 157.24 (C=O), 53.89 (CH piperazine), 47.81, 47.02, 43.42, 42.67, 39.51, 21.31 (CH_2 piperazine and ureate), 11.15 (CH_3).

2-(*N*-Butylaminocarbonyloxymethyl)piperazine, Dihydrochloride (9c). This compound was prepared as described for compounds **3**, from **8c** (4 g, 10.05 mmol), as crystals (2.4 g, 82%): R_f 0.14 (NH_4OH/MeOH/CH_2Cl_2, 2:20:80, v/v/v); mp 188 °C; IR (ν cm^{-1}) 1760 (C=O amide); ^{1}H NMR (DMSO-d_6) δ 10.12 (br s, 4H, NH_2^+), 7.26 (br s, 1H, NHC=O), 4.22 (m, 2H, CH_2OCO), 4.00−2.80 (m, 9H, CH_2−NH, piperazine), 1.30 (m, 4H, $(CH_2)_2$), 0.84 (t, 3H, J = 13 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 155.28 (C=O), 63.80 (CH_2O), 50.79 (CH piperazine), 41.33, 40.07 (CH_2 piperazine), 39.32, 31.38, 19.42 (CH_2 carbamate), 13.67 (CH_3).

2-(*N*,*N*-Diethylaminocarbonylaminomethyl)-1,4-bis-(3,4,5-trimethoxybenzoyl)piperazine (10a). This compound was prepared as described for compounds **4**, from **9a**, as crystals (800 mg, 81%): R_f 0.18 (MeOH/CH_2Cl_2, 5:95, v/v); mp 148 °C; IR (ν cm^{-1}) 3389 (NH), 1634 (C=O ureate), 1619 (C=O amide), 1584 (ArC=C); ^{1}H NMR δ 6.61 (br s, 4H, ArH), 4.95 (br s, 1H, NH), 4.14 (m, 3H, piperazine), 3.82 and 3.79 (2s, 18H, CH_3O), 3.60−2.60 (m, 10H, CH_2NH, N(C$H_2$$CH_3$)$_2$, piperazine), 1.03 (t, 6H, J = 7 Hz, CH_3); ^{13}C NMR δ 171.43, 170.95 (C=O), 157.04 (C=O ureate), 153.39, 153.29, 139.48, 130.28, 130.06, 104.41 (ArC=C), 60.81, 56.31, 56.22 (CH_3O), 49.81, 47.10, 43.45, 39.51, 41.11 (CH_2), 13.78 (CH_3). Anal. ($C_{30}H_{42}N_4O_9$·0.75H_2O) C, H, N.

2-(*N*,*N*-Dipropylaminocarbonylaminomethyl)-1,4-bis-(3,4,5-trimethoxybenzoyl)piperazine (10b). This compound was prepared as described for compounds **4**, from **9b**, as crystals (500 mg, 61%): R_f 0.50 (MeOH/CH_2Cl_2, 5:95, v/v); mp 78.2 °C; IR (ν cm^{-1}) 3395 (NH), 1636 (C=O ureate), 1617 (C=O amide), 1584 (ArC=C); ^{1}H NMR δ 6.60 and 6.58 (s, 4H, ArH), 4.88 (br s, 1H, NH), 4.50−3.95 (m, 3H, piperazine), 3.82 and 3.79 (2s, 18H, CH_3O), 3.60−3.30 (m, 10H, CH_2NH, N(C$H_2$$CH_2CH_3$)$_2$, piperazine), 1.47 (m, 4H, J = 7.2 Hz, N(CH_2C$H_2$$CH_3$)$_2$), 0.81 (t, 6H, J = 7.2 Hz, CH_3); ^{13}C NMR δ 171.43, 170.95 (C=O amide), 157.43 (C=O ureate), 153.39, 153.29, 139.51, 130.31, 130.07, 104.47 (ArC=C), 60.83, 56.31, 56.23 (CH_3O), 49.82, 43.40, 39.75, 49.01, 21.70 (CH_2), 11.27 (CH_3). Anal. ($C_{32}H_{46}N_4O_9$·H_2O) C, H, N.

2-(*N*-Butylaminocarbonyloxymethyl)-1,4-bis(3,4,5-trimethoxybenzoyl)piperazine (10c). This compound was prepared as described for compounds **4**, from **9c**, as crystals (610 mg, 29%): R_f 0.36 (MeOH/CH_2Cl_2, 5:95, v/v); mp 101.4 °C; IR (ν cm^{-1}) 3390 (NH), 1717 (C=O carbamate), 1620 (C=O amide), 1586 (ArC=C); ^{1}H NMR δ 6.58 and 6.57 (s, 4H, ArH), 4.74 (br s, 1H, NH), 4.60−3.90 (m, 5H, CH_2O, piperazine), 3.80−3.79 (s, 18H, CH_3O), 3.60−2.60 (m, 6H, CH_2NH, piperazine), 1.28 (m, 4H, $NHCH_2$(CH_2)$_2$$CH_3$), 0.82 (t, 3H, J = 7 Hz, CH_3); ^{13}C NMR δ 170.83 (C=O amide), 155.65 (C=O carbamate), 153.27, 139.43, 130.16, 130.01, 104.33 (ArC=C), 60.89 (CH_2O), 60.74, 56.17 (CH_3O), 40.71, 31.76, 19.69 (CH_2), 13.51 (CH_3). Anal. ($C_{30}H_{41}N_3O_{10}$·0.5H_2O) C, H, N.

2-Ethyloxycarbonylpiperazine, Dihydrochloride (12). This compound was prepared from **11** (21 g, 62.13 mmol) as described for compound **3a** but using 12 N HCl instead of glacial acetic acid, as a solid (14 g, 97%): R_f 0.39 (NH_4OH/MeOH/CH_2Cl_2, 2:20:80, v/v/v); mp 199 °C; IR (ν cm^{-1}) 1756 (C=O); ^{1}H NMR (DMSO-d_6) δ 9.74 (br s, 4H, NH_2^+), 4.56 (m, 1H, piperazine), 4.11 (m, 2H, CH_2OC=O), 2.80−2.60 (m, 6H, piperazine), 1.20 (br s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 156.03 (C=O), 62.73 (CH_2O), 51.70 (CH piperazine), 13.86 (CH_3).

2-Ethyloxycarbonyl-1,4-bis(3,4,5-trimethoxybenzoyl)-piperazine (13). This compound was prepared using the same process as for **4a**, from **12**, as a solid (13.1 g, 39.6%): R_f 0.4 (MeOH/CH_2Cl_2, 3:97, v/v); mp 122 °C; IR (ν cm^{-1}) 1735 (C=O ester), 1635 (C=O amide), 1586 (ArC=C); ^{1}H NMR δ

6.59 (s, 4H, ArH), 5.40−4.30 (m, 3H, piperazine), 4.15 (m, 2H, CH_2OC=O), 3.81 and 3.79 (2s, 18H, OCH_3), 3.60−2.60 (m, 4H, piperazine), 1.19 (t, 3H, J = 7 Hz, OCH_2CH_3); ^{13}C NMR δ 171.13, 170.54 (C=O amide), 169.08 (C=O ester), 153.32, 153.14, 139.51, 129.81, 104.52, 104.13 (ArC=C), 61.86 (CH_2O), 60.72, 56.12 (CH_3O), 52.32 (CH piperazine), 44.95 (CH_2 piperazine), 13.91 (CH_3).

2-Hydroxymethyl-1,4-bis(3,4,5-trimethoxybenzoyl)piperazine (14). $NaBH_4$ (7 g, 183.1 mmol) was added portionwise to a cooled (ice bath) solution of **13** (10 g, 18.31 mmol) in MeOH (100 mL). The solution was stirred for 1 h at 0 °C and overnight at room temperature. The solvent was then evaporated and the residue taken up in CH_2Cl_2. The solution was washed several times with water, dried over $MgSO_4$, and filtered, and the solvent was eliminated under reduced pressure. The compound was crystallized in MeOH/Et_2O, and 6.50 g (70%) of white crystals was obtained: R_f 0.45 (MeOH/CH_2Cl_2, 10:90, v/v); mp 139 °C; IR (ν cm^{-1}) 3370 (OH), 1636 (C=O), 1581 (ArC=C); ^{1}H NMR δ 6.59 (s, 4H, ArH), 5.25−3.90 (m, 4H, CH_2O, OH, piperazine), 3.80 and 3.79 (s, 18H, CH_3O), 3.70−2.70 (m, 6H, piperazine); ^{13}C NMR δ 171.51 (C=O), 153.23, 139.60, 139.24, 130.23, 129.47, 104.52, 104.14 (ArC=C), 60.73, 56.11 (CH_3O), 59.16 (CH_2OH).

2-(*N*,*N*-Diethylthionocarbamoyloxymethyl)-1,4-bis(3,4,5-trimethoxybenzoyl)piperazine (15a). To a suspension of NaH (60% in mineral oil, 100 mg, 2.4 mmol) in DMF (5 mL) was added dropwise a solution of the alcohol **14** (1 g, 2 mmol) in DMF (10 mL). When the alcoholate was formed, a solution of *N*,*N*-diethylthiocarbamoyle chloride (370 mg, 2.4 mmol) in DMF (10 mL) was added dropwise to the former suspension. The mixture was heated at the end of the addition to 70−80 °C and stirred for 23 h. Then saturated aqueous NaCl (50 mL) was added, the reaction mixture was extracted with EtOAc (3 × 50 mL) and dried over $MgSO_4$, and the solvent was removed in a vacuum. The residue was chromatographed using a silica gel column with MeOH/CH_2Cl_2 (1:99, v/v) as eluent and crystallized in MeOH/EtOH to yield the title compound as yellow crystals (800 mg, 65%): R_f 0.43 (MeOH/CH_2Cl_2, 5:95, v/v); mp 175.3 °C; IR (ν cm^{-1}) 3045 (ArH), 1614 (C=O), 1582 (ArC=C); ^{1}H NMR δ 6.60 (s, 2H, ArH), 6.59 (s, 2H, ArH), 5.40−4.60 and 4.60−4.00 (m, 5H, CH_2OC=S, piperazine), 3.82, 3.80 and 3.79 (3s, 18H, CH_3O), 3.75−3.10 (m, 4H, S=CN(CH_2CH_3)$_2$, piperazine), 3.41 (q, 2H, J = 7 Hz, S=CN(CH_2CH_3)$_2$), 1.14 and 0.98 (t, 6H, J = 7 Hz, CH_3); ^{13}C NMR δ 186.30 (C=S), 170.84, 170.71 (C=O), 153.41, 153.28, 138.48, 139.30, 130.12, 104.43, 104.05 (ArC=C), 66.53 (CH_2O), 60.78, 56.29 (CH_3O), 47.86, 43.31 (CH_2), 13.20, 11.80 (CH_3). Anal. ($C_{30}H_{41}N_3O_9S\cdot0.5H_2O$) C, H, N.

2-(*N*,*N*-Dimethylthionocarbamoyloxymethyl)-1,4-bis-(3,4,5-trimethoxybenzoyl)piperazine (15b). Compound **15b** (1.2 g, 51%) was prepared as a solid from the alcohol **14** and *N*,*N*-dimethylthiocarbamoyle chloride as described for compound **15a**: R_f 0.24 (MeOH/CH_2Cl_2, 3:97, v/v); mp 171.3 °C; IR (ν cm^{-1}) 1623 (C=O), 1582 (ArC=C); ^{1}H NMR δ 6.59 and 6.57 (s, 4H, ArH), 5.40−3.90 (m, 5H, CH_2OC=S, piperazine), 3.82, 3.80 and 3.79 (3s, 18H, CH_3O), 3.70−3.35 (m, 2H, piperazine), 3.25 (s, 3H, CH_3), 3.20−2.30 (m, 5H, piperazine); ^{13}C NMR δ 186.88 (C=S), 170.65 (C=O), 153.23, 139.32, 139.10, 130.00, 104.15, 103.89 (ArC=C), 66.38 (CH_2O), 60.64, 56.18, 56.14 (CH_3O), 42.67, 37.53 (CH_3). Anal. ($C_{28}H_{37}N_3O_9S$) C, H, N.

2-(Ethylcarbonyloxymethyl)-1,4-bis(3,4,5-trimethoxybenzoyl)piperazine (15c). To a solution of the alcohol **14** (500 mg, 1 mmol) and Et_3N (140 μL, 2 mmol) in CH_2Cl_2 (10 mL) was added dropwise propanoyl chloride (208 μL, 4 mmol) in CH_2Cl_2 (5 mL). After stirring overnight at room temperature, the solution was washed with water, dried over $MgSO_4$, and concentrated in a vacuum. Purification on a silica gel column using CH_2Cl_2 as eluent and crystallization in MeOH/ether yielded **15c** (460 mg, 82%) as crystals: R_f 0.39 (MeOH/CH_2Cl_2, 5:95, v/v); mp 139.6 °C; IR (ν cm^{-1}) 2954 (ArCH), 1746 (C=O ester), 1630 (C=O amide), 1586 (ArC=C); ^{1}H NMR δ 6.55 (s, 4H, ArH), 5.20−3.95 (m, 5H, CH_2O, piperazine), 3.80 (br s, 18H, CH_3O), 3.50−2.60 (m, 4H, piperazine), 2.16 (m, 2H, CH_2CH_3), 1.00 (t, 3H, J = 7 Hz, CH_2CH_3); ^{13}C NMR δ.173.73 (C=O ester), 170.69 (C=O), 153.25, 139.41, 130.04, 129.86, 104.17 (ArC=C), 59.94 (CH_2O), 60.68, 56.07 (CH_3O), 27.10 (CH_2), 8.69 (CH_3). Anal. ($C_{28}H_{36}N_2O_{10}$) C, H, N.

3-(*N*,*N*-Diethylaminocarbonyloxymethyl)-1-(triphenylmethyl)piperazine (17). To a solution of **16** (6.26 g, 21.73 mmol) and Et_3N (12 mL, 86.92 mmol) in CH_2Cl_2 (100 mL) was added dropwise a solution of triphenylmethyl chloride (6 g, 21.73 mmol) in CH_2Cl_2 (100 mL). The solution was stirred for 6 h and was then washed with saturated $NaHCO_3$ solution and water until neutral pH, dried ($MgSO_4$), and filtered, and the solvent was eliminated in a vacuum. The residue (9.9 g, 99.7%) was used for next steps without any further purification: R_f 0.093 (MeOH/CH_2Cl_2, 3:97, v/v); IR (ν cm^{-1}) 3320 (NH), 3045 (ArCH), 1695 (C=O carbamate), 1595 (ArC=C); ^{1}H NMR δ 7.40 (m, 6H, ArH), 7.10 (m, 9H, ArH), 3.86 (m, 2H, CH_2O), 3.50−2.45 (m, 8H, N(CH_2CH_3)$_2$), 2.00−1.10 (m, 4H, piperazine), 0.97 (m, 6H, N(CH_2CH_3)$_2$); ^{13}C NMR δ 155.46 (C=O), 129.26, 127.40, 125.91 (ArC=C), 66.85 (CH_2O), 54.68 (CH piperazine), 51.50, 48.78, 45.75 (CH_2 piperazine), 41.67, 41.21 (NCH_2CH_3), 13.93, 13.45 (NCH$_2CH_3$)

2-(*N*,*N*-Diethylaminocarbonyloxymethyl)-1-(3,4,5-trimethoxybenzoyl)-4-(triphenylmethyl)piperazine (18). To a stirred solution of **17** (7.5 g, 16.45 mmol) and Et_3N (7 mL, 49.35 mmol) in CH_2Cl_2 (60 mL) was added dropwise a solution of 3,4,5-trimethoxybenzoyl chloride (4.44 g, 19.25 mmol) in CH_2Cl_2 (50 mL). The mixture was stirred overnight at room temperature and then washed with a saturated $NaHCO_3$ solution and water. The organic layer was dried over $MgSO_4$, filtered, and concentrated in a vacuum. Purification on a silica gel column using CH_2Cl_2 as eluent yielded **18** (9.52 g, 89%) as a wax: R_f 0.33 (MeOH/CH_2Cl_2, 3:97, v/v); IR (ν cm^{-1}) 1701 (C=O carbamate), 1616 (C=O amide), 1507 (ArC=C); ^{1}H NMR δ 7.38 and 7.19 (m, 15H, ArH), 6.49 (s, 2H, ArCH), 4.99 and 4.41 (m, 2H, piperazine), 3.83 (m, 2H, CH_2O), 3.74 and 3.73 (s, 9H, CH_3O), 3.59 (q, 2H, J = 7 Hz, NCH_2CH_3), 3.60−2.60 (m, 6H, N(CH_2CH_3)$_2$, piperazine), 1.79 (m, 1H, piperazine), 1.09 (m, 6H, CH_3); ^{13}C NMR 169.97 (C=O amide), 155.78 (C=O carbamate), 152.96, 138.96, 130.86, 129.13, 127.53, 126.20, 104.31 (ArC=C), 62.83 (CH_2O), 60.66, 56.02 (CH_3O), 49.25 (CH piperazine), 48.97, 48.00, 44.93 (CH_2 piperazine), 41.69, 41.12 (NCH_2CH_3), 13.90, 13.43 (NCH$_2CH_3$).

2-(*N*,*N*-Diethylaminocarbonyloxymethyl)-1-(3,4,5-trimethoxybenzoyl)piperazine (19). To a solution of **18** (6.5 g, 9.98 mmol) in MeOH (100 mL) was added dropwise 12 N HCl (6 mL). The solution was stirred for 10 min at room temperature and the solvent was removed in a vacuum. The residue was then taken up with CH_2Cl_2, washed with a saturated $NaHCO_3$ solution and water, dried ($MgSO_4$), filtered, and concentrated. A rapid purification on a silica gel column using MeOH/CH_2Cl_2 as eluent (3:97, v/v) yielded **19** (3.8 g, 93%) as a wax: R_f 0.11 (MeOH/CH_2Cl_2, 3:97, v/v); IR (ν cm^{-1}) 3330 (NH), 1693 (C=O carbamate), 1632 (C=O amide), 1584 (ArC=C); ^{1}H NMR δ 6.55 (s, 2H, ArH), 4.90−4.40 (m, 2H, piperazine), 4.29 (m, 2H, CH_2O), 3.79 and 3.77 (s, 9H, CH_3O), 3.40−2.50 (m, 9H, N(CH_2CH_3)$_2$, piperazine), 1.37 (br s, 1H, NH), 1.02 (t, 6H, J = 7 Hz, CH_3); ^{13}C NMR δ 170.44 (C=O amide), 155.36 (C=O carbamate), 153.07, 138.95, 130.94, 104.08 (ArC=C), 61.44 (CH_2O), 60.62, 56.10 (CH_3O), 45.86 (CH piperazine), 41.67, 41.07 (NCH_2CH_3), 13.88, 13.25 (NCH$_2CH_3$).

2-(N,*N*-Diethylaminocarbonyloxymethyl)-4-(4-hydroxy-3,5-dimethoxybenzoyl)-1-(3,4,5-trimethoxybenzoyl)piperazine (20). A mixture of **19** (3 g, 7.33 mmol), syringic acid (1.5 g, 8.06 mmol), DCC (1.55 g, 8.06 mmol), and HOBT (1.2 g, 8.06 mmol) in CH_2Cl_2 (25 mL) was refluxed overnight. The solution was then filtered; washed with 1 N HCl, water, a saturated $NaHCO_3$ solution, and water; and dried over $MgSO_4$. After the evaporation of the solvent, purification on a silica gel column using MeOH/CH_2Cl_2 as eluent (1:99, v/v) and crystallization in MeOH/Et_2O yielded **20** (3.67 g, 84.5%) as crystals: R_f 0.21 (MeOH/CH_2Cl_2, 5:95, v/v); mp 129.3 °C; IR (ν cm^{-1}) 3373 (OH), 1686 (C=O carbamate), 1617 (C=O amide), 1586 (ArC=C); ^{1}H NMR δ 6.61 (s, 2H, ArH), 6.57 (s, 2H, ArH), 5.69 (s, 1H, OH), 5.3−3.9 (m, 5H, CH_2O and piperazine), 3.80

(s, 15H, CH_3O), 3.6−2.4 (m, 8H, NCH_2CH_3 and piperazine), 1.4−0.65 (m, 6H, CH_3); ^{13}C NMR δ.171.06, 170.62 (C=O amide), 155.05 (C=O amide), 153.26, 146.91, 139.46, 136.58, 130.21, 125.35, 104.42, 104.25 (ArC=C), 60.89 (CH_2O), 60.76, 56.38, 56.15 (CH_3O), 41.72, 41.01 (NCH_2CH_3), 13.80, 13.29 (NCH_2CH_3). Anal. ($C_{29}H_{39}N_3O_{10}\cdot 1.5H_2O$) C, H, N.

^{3}H-Labeling. To a solution of **20** (1.31×10^{-4} M, 1.1 equiv) and K_2CO_3 in toluene (1 mL) was added dropwise a solution of [^{3}H]CH_2I (10 mCi/mL, 1 mL, Amersham) in toluene. After the mixture was stirred at room temperature for 24 h and the addition of a few milliliters of CH_3CN, the resulting solution was stirred again for 30 h. Two compounds appeared after UV-detection on a TLC plate: the precursor **20** (R_f 0.21 in MeOH/CH_2Cl_2, 5:95, v/v) and the final compound **[^{3}H]1a** (compound **21**, R_f 0.48 in MeOH/CH_2Cl_2, 5:95, v/v). The solvent and traces of [^{3}H]CH_2I were then removed with a stream of N_2, and the residue taken up with CH_2Cl_2 was chromatographed using a TLC plate (MeOH/CH_2Cl_2, 3:97, v/v as eluent). Autoradiography (BIOMAX MR, Kodak) was then performed (3-h exposure in a cassette), the radioactive band corresponding to **1a** ($R_f \pm 0.05$) was cut, and the silica was recovered and extracted several times with MeOH/CH_2Cl_2 (10:90, v/v) to yield 10 μCi of **[^{3}H]1a**.

Biological Methods. Platelet Aggregation. The inhibition of platelet aggregation was conducted according to the published procedures.[12] Briefly, it was determined using platelet-rich plasma (PRP) of New Zealand rabbits by the method of Cazenave et al.[22] Blood samples were collected from the auricular artery into a citrate buffer (3.8%, pH 7.4), and PRP was obtained by centrifugation for 15 min at 1200 rpm. The antagonists were solubilized in EtOH at concentrations from 10^{-2} to 10^{-7} M and added to the incubated and stirred PRP for 2 min before PAF (2.5 nM) challenge. Platelet aggregation induced by PAF in the presence of the antagonists was monitored by continuous recording of light transmission in a dual-channel recorder (Cronolog Coultronics Apparatus) and was compared to a control aggregation induced by PAF alone. The drug concentration required to produce 50% inhibition (IC_{50}) was calculated from dose−response curves (five or six determinations).

Antiviral Assay. Antiviral assays and data analysis were conducted according to published procedures.[12,13] HIV-1 replication was assessed in cell culture supernatants by quantifying reverse transcriptase (RT), using the RetroSys kit (Innovagen).

Mouse Brain Perfusion Technique. Materials. [^{14}C]-sucrose (565 mCi/mmol) was purchased from Amersham Pharmacia Biotech (Orsay, France). Heparin sodium was obtained from Sanofi & Synthelabo (Gentilly, France). All other chemicals were commercial products of analytical grade.

Animals. Adult male OF-1 mice (30−40 g, 6−8 weeks old) were obtained from Iffa Credo (L'arbesle, France). Animals were housed in a room with a controlled environment (22 ± 3 °C; 55 ± 10% relative humidity) and maintained under a 12 h dark−light cycle (light from 6:00 a.m. to 6:00 p.m.). Animals had access to food and tap water ad libitum. All animals procedures complied with the Principles of Laboratory Animal Care (NIH publication # 85−23, revised 1985).

Surgical Procedures and Transport Studies. Mice were anaesthetized by intraperitoneal injection of xylazine (Bayer, Puteaux, France; 8 mg/kg) and ketamine (Parke Davis, Courbevoie, France; 140 mg/kg). Blood−brain transport of the compounds was measured in mice using the in situ brain perfusion technique recently described.[15] Briefly, the right common carotid was catheterized with polyethylene tubing (0.30 mm i.d. × 0.70 mm o.d., Biotrol Diagnostic, Chennevières-les-Louvre, France) that was filled with heparin (25 U/mL) and mounted on a 26 gauge needle. Before insertion of the catheter, the common carotid artery was ligated caudally. The external carotid was ligated rostral to the occipital artery at the level of the bifurcation of the common carotid artery. During surgery, body temperature was maintained from 37 to 38 °C using a rectal thermistor connected to a temperature monitor. The syringe containing the perfusion fluid was placed in an infusion pump (Harvard pump PHD 2000, Harvard Apparatus, Holliston, MA) and connected to the catheter. Before perfusion, the thorax of the animal was opened, the heart cut, and perfusion immediately started with a flow rate of 2.5 mL/min. The perfusion fluid consisted of bicarbonate-buffered physiological saline (mM): 128 NaCl, 24 $NaHCO_3$, 4.2 KCl, 2.4 NaH_2PO_4, 1.5 $CaCl_2$, 0.9 $MgCl_2$, and 9 D-glucose. The solution was gassed with 95% O_2 and 5% CO_2 for pH control (7.4) and warmed to 37 °C in a water bath. Tracers were added to the perfusate at a concentration of 0.3−0.4 μCi/mL. Perfusion was terminated by decapitation after 60 s. The brain was removed from the skull and dissected on ice. The right cerebral hemisphere was placed in tared vials and weighed. Aliquots of the perfusion fluid were also collected and weighed to determine tracer concentrations in the perfusate. Samples were digested in 1 mL of Solvable (Packard, Rungis, France) at 50 °C and mixed with 9 mL of Ultima gold XR (Packard). Dual label counting was performed simultaneously in a Packard Tri-Carb model 1900 TR (Packard).

Calculation of Blood−Brain Transport Parameters. The brain uptake was expressed as a blood−brain transfer coefficient K_{in} (μL/s/g) and was calculated from

$$K_{in} = V_{brain}/T \quad (1)$$

where T is the perfusion time (s).

The apparent volume of the distribution (V_{brain}) was calculated from the amount of radioactivity in the right brain hemisphere using the following equation

$$V_{brain} = X_{brain}/C_{perf} \quad (2)$$

where X_{brain} (dpm/g) is the calculated amount of **[^{3}H]1a** in the right cerebral hemisphere and C_{perf} (dpm/μL) is the [^{3}H]tracer concentration in the perfusion fluid.

Brain tissue radioactivity was corrected for vascular contamination (V_{vasc}) with the following equation

$$X_{brain} = X_{tot} - V_{vasc}C_{perf} \quad (3)$$

where X_{tot} (dpm/g) is the total quantity of tracer measured in the tissue sample (vascular + extravascular).

Brain vascular volume (V_{vasc}; μL/g) was estimated from the tissue distribution of [^{14}C]sucrose, which is known to diffuse very slowly across the BBB, using the following equation

$$V_{vasc} = X^*/C_{perf}^* \quad (4)$$

where X^* (dpm/g) is the amount of sucrose measured in the right brain hemisphere and C_{perf}^* (dpm/μL) is the concentration of the labeled sucrose in the perfusion fluid.

Data are presented as mean ± SD for five animals.

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