Structure−Activity Relationships in Platelet-Activating Factor (PAF). 10. From PAF Antagonism to Inhibition of HIV-1 Replication

ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · JULY 2000

Impact Factor: 5.45 · DOI: 10.1021/jm9911276 · Source: PubMed

13 AUTHORS, INCLUDING:

Nawal Serradji
Paris Diderot University
20 PUBLICATIONS **217** CITATIONS
SEE PROFILE

Nathalie Dereuddre-Bosquet
Atomic Energy and Alternative Energies Com…
96 PUBLICATIONS **1,769** CITATIONS
SEE PROFILE

Aazdine Lamouri
Paris Diderot University
41 PUBLICATIONS **473** CITATIONS
SEE PROFILE

Changzhi Dong
Paris Diderot University
17 PUBLICATIONS **350** CITATIONS
SEE PROFILE

Structure−Activity Relationships in Platelet-Activating Factor (PAF). 10. From PAF Antagonism to Inhibition of HIV-1 Replication

Nawal Serradji,[§] Okkacha Bensaid,[§] Marc Martin,[‡] Erwan Kan,[§] Nathalie Dereuddre-Bosquet,[†,‡] Catherine Redeuilh,[§] Jack Huet,[§] Françoise Heymans,[§] Aazdine Lamouri,[§] Pascal Clayette,[†,‡] Chang Zhi Dong,[§] Dominique Dormont,[‡] and Jean-Jacques Godfroid*[,§]

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, 60-68 Avenue de la Division Leclerc, BP 6, 92265 Fontenay aux Roses Cedex, France; and SPI-BIO, 2 rue du Buisson aux Fraises, 91741 Massy, France

Received January 24, 2000

Excessive levels of PAF and cells of macrophage lineage appear to play an important role in neuronal cell injury, inflammatory syndrome, and HIV replication in CNS resulting in AIDS dementia complex (ADC). The beneficial effects of PAF receptor antagonists are evident and give rise to expected therapeutic strategies for neurotrauma. Piperazine derivatives bearing a "cache-oreilles" (ear-muff) electronic distribution are able to inhibit in vitro PAF effects and, thus, could be used in pathologies where this mediator is involved. Therefore, their potential anti-HIV activity was investigated, and we find that (i) these PAF antagonists are effectively active in HIV-infected monocyte-derived macrophages (MDM) but there is no correlation between both anti-HIV and anti-PAF activities; (ii) the presence of a carbamate function (compounds **1a**−**d**) is favorable to the antiviral activity; (iii) the lipophilicity of the substituent on the piperazinic cycle seems to be less important for the anti-PAF activity than for the antiviral one. Our leading compound, PMS 601 (compound **1a**), presents a dual activity with IC_{50} of 8 and 11 μM for anti-PAF and anti-HIV activity, respectively, without cytotoxic events at 1000 μM in MDM. Although its mode of action is not clearly defined, these data suggest that PMS 601, which displays no effect on acellular reverse transcriptase or protease tests, deserves further investigation in the treatment of HIV-1-associated dementia.

Introduction

HIV infection is associated with inflammatory syndrome and neurologic dysfunctions. These pathophysiological tissular events are both initiated byproductively HIV-infected macrophages and/or activated macrophages that release high levels of the proinflammatory cytokine tumor necrosis factor-α (TNF-α) and the phospholipid mediator platelet-activating factor (PAF).[1] In the central nervous system (CNS) of patients with HIV-associated dementia, the number of TNF-α mRNA-expressing macrophages is elevated compared to that of HIV-infected patients without dementia. Likewise, PAF is elevated in cerebrospinal fluid of patients presenting dementia and altered immune system.[2] Because direct infection of neurons by HIV is unlikely and because TNF-α and PAF are both neurotoxic, there is a consensus to consider that neuronal dysfunction and apoptosis are mediated by these soluble factors released by macrophages or apparented cells in CNS, microglia.[3] PAF is probably the key element in this phenomenon since TNF-α-mediated neuronal apoptosis can also be decreased either by PAF acetylhydrolase,[2] the main catabolic enzyme for PAF, or by a PAF receptor antagonist.[2] Blocking pathologic effects of PAF may therefore be a pivotal step in the treatment of HIV-associated dementia and inflammatory syndrome.[4] Tissues such as brain are sites of HIV replication and may act as a reservoir for the virus. TNF-α up-regulates HIV-1 production in HIV-infected macrophages, and PAF, in turn, appears to increase TNF-α synthesis.[5−7] As a consequence, it is also of interest to interfere with PAF-mediated signal transduction to see if it leads to diminished HIV replication in tissues. The relative efficacy in tissues and particularly in CNS of antiretrovirals administered today to HIV-infected patients[8] takes further interest of PAF antagonists as adjuvant therapy of HIV infection. According to Weissman et al.[9] and Le Naour et al.[10] PAF receptor antagonists, such as RP 55778 [(+)3-(3-pyridyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-7-carboxamide] (Rhône-Poulenc; Figure 1), the active (+)-enantiomer of the racemic compound RP 48740[11] and inhibiting TNF-α synthesis,[12] which has demonstrated in vitro anti-HIV effects in HIV-1-infected monocytic cell lines or monocyte-derived macrophages

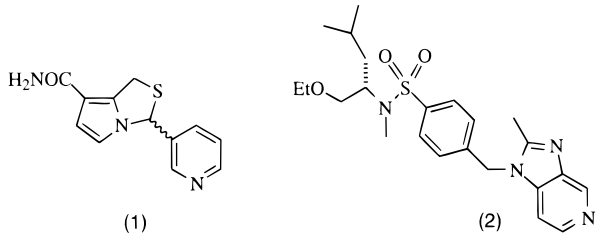


Figure 1. **1**: RP 48740 (racemic) and RP 55778 [(+)-enantiomer]. **2**: BB-882.

* To whom correspondence should be addressed. Tel: 33 1 4427 6050. Fax: 33 1 4427 5641. E-mail: godfroid@paris7.jussieu.fr.
[§] Université Paris 7 - Denis Diderot.
[‡] CEA.
[†] SPI-BIO.

10.1021/jm9911276 CCC: $19.00
Published on Web 05/12/2000

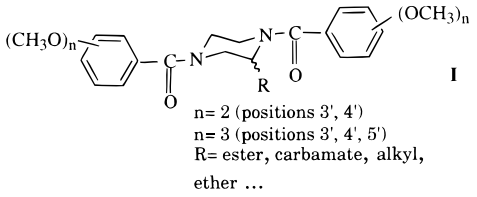


Figure 2. Piperazine analogues previously studied.[15]

(MDM), may represent potentially useful compounds in the treatment of HIV infection. The relatively potent inhibitory effect of RP 55778 (ED_{50} = 50 μM) on HIV-1-DAS replication, a primary macrophage-tropic HIV isolate, might permit its use as an adjuvant in AIDS therapy.[10] Moreover, a recent report showed that Lexipafant (BB-882; Figure 1), a PAF antagonist reducing organ failure and suppressing some aspects of the inflammatory response in acute pancreatitis,[13] is in phase I−II clinical trials in the treatment of AIDS dementia.[14]

At the end of the 1980s, we showed that 1,4-di(3,4,5-trimethoxybenzoyl)piperazine derivatives were able to inhibit in vitro PAF effects on platelet aggregation and could, as a consequence, potentially be used in pathologies where this lipid mediator is involved.[15] These compounds induce "cache-oreilles" effects, i.e., two strong electronegative wells (isocontoured at −10 kcal/mol) located at 180° to each other and at a relative constant distance (10−12 Å) according to the "Dive−Godfroid" receptor model.[16] This hypothesis was elaborated starting from electronic and geometric characteristics of published miscellaneous synthetic and natural PAF antagonists. Taking these features in account, very simple compounds[15] and more than 40 molecules of general formula **I** (Figure 2) were synthesized.[17,18]

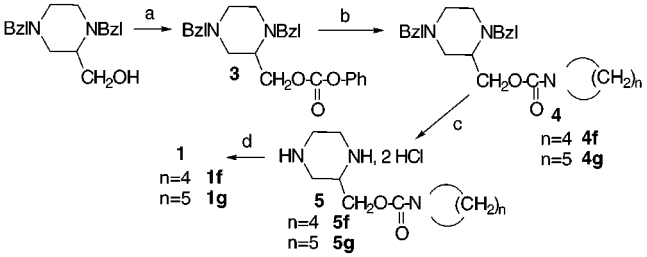
Consequently, the "cache-oreilles" effect was improved with the hypothesis of the multipolarized cylinder.[19] The influence of the hydrophobicity was also studied.[20] Moreover, we have shown that the PAF antagonistic effect was only weakly enantiospecific in this series.[17] Starting from these different hypotheses, we have also worked for a long time on the structural modifications leading to the discovery of dual biological activities on the same molecule: for instance, PAF antagonism and acetylcholinesterase[21] or PLA_2 inhibition.[22]

Taking into account the role of macrophages in HIV infection and inflammatory processes in CNS, we have investigated, in the present study, the putative anti-HIV effects of several PAF receptor antagonists in cultures of MDM infected by the reference macrophage tropic HIV-1/Ba-L strain in vitro.

Chemistry

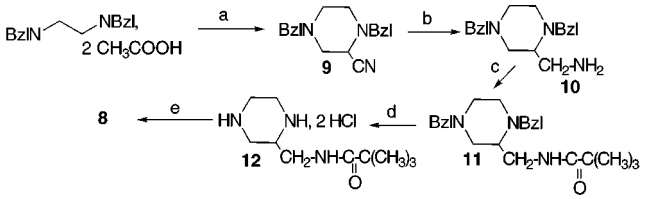
The carbamates **1a**−**e** and the ester **2** were prepared according to Lamouri et al.[17] Compounds **1f**−**g** were synthesized by the same manner as shown in Scheme 1. 1,4-Dibenzyl-2-hydroxymethylpiperazine was converted into the phenyl carbonate derivative **3**. Treatment with the appropriate amine gave the carbamate **4** which was debenzylated into the corresponding 2-substituted piperazine **5**. Introduction of the "cache-oreilles" was performed using 3,4,5-trimethoxybenzoyl chloride. Unsubstituted or alkyl-substituted derivatives **6a**−**d** and the alcohol **7** were prepared as described in Tavet et al.[18]

Scheme 1[a]



[a] Reagents: (a) PhOCOCl, pyr, CH_2Cl_2, 0 °C; (b) cyclic amine, reflux; (c) H_2, Pd/C, EtOH; (d) 3,4,5-$(MeO)_3$PhCOCl, Et_3N, CH_2Cl_2.

Scheme 2[a]



[a] Reagents: (a) $BrCH_2CHBrCN$, Et_3N, benzene, reflux; (b) Na, EtOH, reflux; (c) $(CH_3)_3CCOCl$, Et_3N, CH_2Cl_2; (d) H_2, Pd/C, HCl, EtOH; (e) 3,4,5-$(MeO)_3$PhCOCl, Et_3N, CH_2Cl_2.

The amide **8** needed another process as described in Scheme 2. *N*,*N*′-Dibenzylethylenediamine was condensed with 2,3-dibromopropionitrile to form the 1,4-dibenzyl-2-cyanopiperazine **(9)**. The nitrile function was reduced into the amine **10** which was reacted with trimethylacetyl chloride to give the amide **11**. Debenzylation and "cache-oreilles" formation were conducted as for **1**.

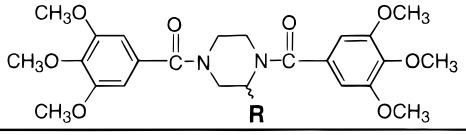
Results and Discussion

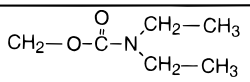
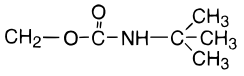
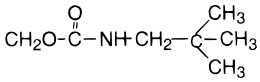
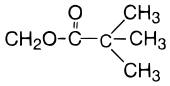
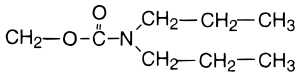
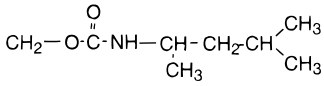
All compounds, some of them known as PAF receptor antagonists,[18] were tested for their ability to inhibit PAF-induced platelet aggregation on the one hand and for their activity in MDM infected with the reference macrophage-tropic HIV-1/Ba-L strain on the other hand.

First Screening (Table 1). In the first set, known[18,19] and new molecules possessing anti-PAF activities with IC_{50} from 0.1 to 8 μM were tested against HIV. They are listed in Table 1 by increasing lipophilicity in order to evaluate the importance of this parameter in the activities. The percentage of inhibition of HIV-1 replication at a single dose of 100 μM reported in Table 1 shows that (i) these PAF antagonists are effectively active in HIV-infected MDM but there is no correlation between anti-HIV and anti-PAF activities [**1a** is the leading compound in anti-HIV-1 activity (95% inhibition) and the weakest potent PAF antagonist in this series; the best PAF antagonist, the ester **2**, produces very moderate (25%) inhibition of HIV-1 replication]; (ii) the *N*-substituted carbamate group (compounds **1a**−**d**) seems to present the highest potency against HIV-1 replication (from 52% to 95% at 100 μM); (iii) the lipophilicity seems to be less important for the anti-PAF activity than for the anti-HIV activity (compound **1e** vs **1a**).

As no toxicity was observed in this series (except for compound **6a**) at 100 μM and as the best compound, **1a**, is the least hydrophobic, we decided to synthesize and test molecules with f_R (lipophilic contribution of R, calculated from Rekker's method[23]) ranging between f_R(**1a**) ± 1.5.

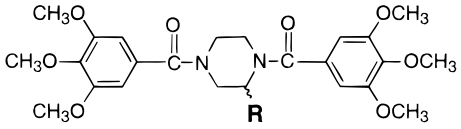
Table 1. Lipophilicity Influence on in Vitro Anti-HIV and Anti-PAF Activities of the First Set of Molecules

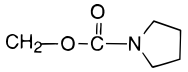
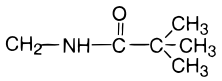
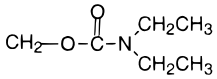
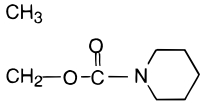


compd	R	f_R[a]	MR[b]	Anti-HIV[c] % inhibition	Anti-PAF[d] IC_{50} μM	CC_{50}[e] μM
1a		0.564	34.47	95 ±0	8	>1000
1b		0.853	34.52	52 ±26	0.13	>100
1c		1.372	39.17	74 ±4	1	>100
2		1.526	31.09	25 ±21	0.1	>100
1d		1.602	43.79	66 ±13	0.37	>100
1e		1.891	43.80	-2 ±12	0.28	>100
6a	$(CH_2)_9$-CH_3	5.372	47.5	40 ±10[f]	0.24	>50

[a] Lipophilic contribution due to R, calculated from Rekker et al. [b] Molecular refractivity due to R, calculated from Hansch et al. [c] Percent (%) inhibition at 100 μM dose of HIV-1 replication using monocyte-derived macrophages infected by the HIV-1/Ba-L strain (see Experimental Section, all the values are the mean ± SD). [d] Inhibition of PAF-induced platelet aggregation using PRP of New Zealand rabbits calculated from dose−response curve as described in the Experimental Section ($n = 5$, mean ± 10%). [e] Fifty percent (50%) cytotoxicity concentration evaluated by neutral red staining. [f] Measured at 10 μM dose, toxic for higher doses.

Table 2. Biological Data of the Second Set of Molecules



compd	R	f_R[a]	MR[b]	Anti-HIV[c] IC_{50} μM	Anti-PAF[d] IC_{50} μM	CC_{50}[e] μM
7	CH_2OH	-0.951	7.19	>100	1.27	>100
6b	H	0.182	1.03	>100	1.78	>100
1f		0.2	33.66	>100	10	>100
8		0.331	33.55	>100	>100	>100
1a		0.564	34.47	11±7	8	>1000
6c	CH_3	0.701	5.65	66*	0.56	>100
1g		0.719	38.31	95*	0.08	>100
6d	CH_2CH_3	1.22	10.30	230*	0.46	>100
RP 55778				50[f]	16[g]	

[a,b,d,e] See Table 1. [c] Antiviral activity measured toward HIV-1/Ba-L-infected monocyte-derived macrophages. [f] Inhibition of HIV-1-DAS replication.[10] [g] IC_{50} of the racemic compound, RP 48740.[11] *Single measurement.

Second Screening (Table 2). Here are reported the IC_{50} in both activities. The best compound is still **1a**. Surprisingly, similar compounds such as **1f**,**g** show very poor inhibition of HIV-1 replication. It is difficult to attribute this feature to the difference of f_R which is very small, −0.36 and +0.15, respectively, but probably due to a steric effect induced by the cyclic structures. Small alkyl derivatives such as **6c**,**d** (R = Me or Et; Table 2)

are unexpectedly less potent than analogue **6a** of the first set of compounds. Introducing more hydrophilic substituents as in alcohol **7** as well as replacing the carbamate function by an amide in compound **8** led to totally inactive compounds. Moreover, no correlation appears between both activities and the molecular refractivity (MR), calculated from Hansch's method[24] and traducing the size and the electronic effects of R.

Consequently, compound **1a** became our reference molecule as PMS 601 with an IC_{50} of 11 μM against HIV, with the absence of cytotoxic events at 100−1000 μM, as shown after microscopical observation of the cells and neutral red staining, and was selected for further investigations. Structure−activity relationships are in progress: for instance, the role of enantiospecificity in inhibition of HIV-1 replication (negligible for PAF antagonism[17]) is under investigation. Its mode of action is up to now unknown. Nevertheless, preliminary results show that PMS 601 (compound **1a**) presents antiviral activity in other cell systems and interferes with both early and late steps in the HIV biological cycle[25] but inhibits, in acellular tests, neither the reverse transcriptase (RT) nor the HIV protease activity (as performed in refs 31 and 26, respectively).

Conclusion

In this paper, we have shown that one molecule, PMS 601 (compound **1a**), possesses both anti-HIV-1 and anti-PAF potencies in the micromolar range. As compared with RP 55778, PMS 601 is more potent (11 μM vs 50 μM[10]) and does not antagonize AZT.[25] As for Lexipafant (published without reported anti-HIV activity), PMS 601 may be a good candidate for preclinical trials in the treament of HIV infection.

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 on silica gel 60 (70−230 mesh ASTM, Merck). All melting points were determined on a digital melting point apparatus (Electrothermal) and are uncorrected. The structures of all compounds were confirmed by IR and ^{1}H NMR spectra. IR spectra were obtained in paraffin oil with a ATI Mattson Genesis Series FTIR spectrometer, and ^{1}H NMR spectra were recorded in $CDCl_3$ on a BRUCKER AC 200 spectrometer using hexamethyldisiloxane (HMDS) as an internal standard. Chemical shifts are given in ppm and peak multiplicities are designated as follows: s, singlet; d, doublet; t, triplet; br s, broad singlet; m, multiplet. Elemental analyses were obtained from the "Service régional de microanalyse" (Université Pierre et Marie Curie), Paris, France, and were within ±0.4% of theoretical values.

1,4-Dibenzyl-2-(phenoxycarbonyloxymethyl)piperazine (3). A solution of 1,4-dibenzyl-2-(hydroxymethyl)piperazine (14 g, 47.3 mmol) in Et_3N (14.2 mL, 192.8 mmol) and CH_2Cl_2 (95 mL) was cooled in an ice bath. A solution of phenyl chlorocarbonate (11.1 g, 71 mmol) in CH_2Cl_2 (95 mL) was added dropwise and the mixture was stirred at 0 °C for 1 h and at room temperature for 48 h. The solution was washed with a saturated $NaHCO_3$ solution and with water until neutral pH. After drying ($MgSO_4$), filtration and evaporation, the residue was chromatographed on a silica gel column using CH_2Cl_2 as eluent to yield 8 g (40.6%) of carbonate **3** as a viscous white product: R_f 0.52 ($CHCl_3$/MeOH, 98:2, v/v); IR (ν cm^{-1}) 3383 (NH), 2955 (ArCH), 1746 (C=O), 1603 (C=C); ^{1}H NMR δ 7.2 (m, 15H, ArH), 4.3 (m, 2H, CH_2OCO), 3.8 (d, 1H, J = 12 Hz, CH−Ph), 3.4 (d, 1H, J = 12 Hz, CH−Ph), 3.3 (s, 2H, CH_2−Ph), 2.9−2 (m, 7H, piperazine CH and CH_2).

1,4-Dibenzyl-2-(pyrrolidinocarbonyloxymethyl)-piperazine (4). A solution of carbonate **3** (3.12 g, 7.5 mmol) in pyrrolidine (9.4 mL, 112.8 mmol) was refluxed for 60 h. After cooling and evaporation of the excess of amine, the residue was taken up in CH_2Cl_2 and washed with water. After drying ($MgSO_4$), filtration and evaporation, a chromatography on a silica gel column using 0.5% MeOH in CH_2Cl_2 as eluent yielded 1.48 g (50.2%) of **4** as an oil: R_f 0.38 ($CHCl_3$/MeOH, 95:5, v/v); IR (ν cm^{-1}) 3030 (ArCH), 1761 (C=O), 1593 (ArC=C); ^{1}H NMR δ 7.2 (m, 10H, ArH), 4 (m, 2H, CH_2OCO), 3.8 (d, 1H, CH−Ph), 3.4 (d, 1H, CH−Ph), 3.3 (s, 2H, CH_2−Ph), 2.9−2 (m, 7H, piperazine CH and CH_2) and pyrrolidine CH_2N), 1.8 (large s, 4H, pyrrolidine CH_2).

2-(Pyrrolidinocarbonyloxymethyl)piperazine, Hydrochloride (5). To a solution of the carbamate **4** (1.48 g, 3.7 mmol) in EtOH (40 mL) and 12 N HCl (1 mL) was added 100 mg Pd/C (10%), and this solution was warmed (40 °C) with stirring under hydrogen atmosphere. After disappearing of the starting material (3 h) as shown by TLC, the suspension was filtered and the catalyst washed several times with EtOH and H_2O. The solvents were evaporated to give 1.04 g (98.3%) of **4** which was used in the next step without purification: R_f 0.4 ($CHCl_3$/MeOH/NH_4OH, 80:20:2, v/v/v).

2-(Pyrrolidinocarbonyloxymethyl)-1,4-di(3,4,5-trimethoxybenzoyl)piperazine (1f). To a solution of diamine hydrochloride **5** (1.04 g, 3.63 mmol) in CH_2Cl_2 (50 mL) in the presence of Et_3N (3 mL, 41 mmol) was added dropwise 3,4,5-trimethoxybenzoyl chloride (2.33 g, 10.1 mmol) in CH_2Cl_2 (30 mL). After stirring overnight at room temperature, EtOH (2 mL) was added and the solution was washed twice with water, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed on a silica gel column using 2% MeOH in CH_2Cl_2 as eluent and crystallized in a mixture of MeOH/ether/hexane to yield 0.59 g (29%) of **1f**: mp 145.1 °C; R_f 0.43 ($CHCl_3$/MeOH, 95:5, v/v); IR (ν cm^{-1}) 1694 (C=O carbamate), 1622 (C=O amide), 1603 (C=C); ^{1}H NMR δ 6.6 (s, 4H, ArH), 5.6−4 (m, 5H, CH_2OCO, piperazine CH_2 and CH), 3.8 (s, 18H, CH_3O), 3.6−2.3 (m, 8H, piperazine and pyrrolidine CH_2NC=O), 1.8 (s, 4H, pyrrolidine CH_2).

1,4-Dibenzyl-2-cyanopiperazine (9). A mixture of *N,N′*-dibenzylethylenediamine diacetate (79 g, 221 mmol) and Et_3N (123 mL, 884 mmol) in benzene (500 mL) was warmed until dissolution. Then 2,3-dibromopropionitrile (38.54 g, 300 mmol) in benzene (150 mL) was added dropwise. After 3 h of reflux with stirring and filtration, the solid was washed with ether and the filtrate was washed with saturated $NaHCO_3$ (100 mL) and twice with water. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified on a silica gel column using ether/petroleum ether (10:90, v/v) as eluent to yield 45 g (98.2%) of **9** as crystals: mp 61.7 °C; R_f 0.33 (ether/petroleum ether, 30:70, v/v); IR (ν cm^{-1}) 3030 (ArCH), 1622, (C=O amide) 2224 (CN), 1602 (ArC=C); ^{1}H NMR δ 7.2 (m, 10H, ArH), 3.7 (m, 4H, CH_2Ph), 3.7−3.3 (m, 7H, piperazine CH_2 and CH).

2-Aminomethyl-1,4-dibenzylpiperazine (10). A mixture of nitrile **9** (24.7 g, 85 mmol) in absolute EtOH (200 mL) was warmed to reflux. Sodium (7.85 g, 341.4 mmol) was added portionwise through the condenser. After stirring overnight at room temperature, the sodium ethoxide was neutralized by a stoichiometric quantity of acetic acid (19.5 mL) in ether (60 mL). The salts were filtered and washed with ether and the filtrate was concentrated in vacuo. The residue was taken up with ether, washed with water, dried ($MgSO_4$), filtered and evaporated yielding 16.1 g (64%) of **10** as a wax: R_f 0.08 (CH_2Cl_2/MeOH, 90:10, v/v); IR (ν cm^{-1}) 3333 (NH_2), 3027 (ArCH), 1602 (ArC=C); ^{1}H NMR δ 7.2 (m, 10H, ArH), 4 (s, 2H, NH_2), 3.7 (m, 4H, CH_2Ph), 3.5−2.2 (m, 9H, CH_2N and CHN).

1,4-Dibenzyl-2-(2,2-dimethylpropionylaminomethyl)-piperazine (11). To a solution of **10** (3.86 g, 13.1 mmol) in CH_2Cl_2 (100 mL) in the presence of Et_3N (5.5 mL, 39.3 mmol) was added dropwise 2,2-dimethylpropanoyl chloride (2.63 mL, 19.6 mmol) in CH_2Cl_2 (30 mL). After 1 h stirring at room

temperature, the mixture was refluxed for 4 h. After adding EtOH (2 mL), the solution was washed twice with water, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was chromatographed on a silica gel column using MeOH/CH_2Cl_2, 0.5:99.5, v/v as eluent to yield 2.44 g (49%) of the amide **11**: R_f 0.36 (CH_2Cl_2/MeOH, 95:5, v/v); IR (ν cm^{-1}) 3383 (NH), 2955 (ArCH), 1746 (C═O), 1603 (ArC═C); ^{1}H NMR δ 7.2 (m, 10H, ArH), 6.4 (s, 1H, NH), 3.8 (m, 2H, CH_2NC═O), 3.3 (m, 5H, CH_2Ph and piperazine CH), 2.7−2.2 (m, 6H, piperazine CH_2), 1.2 (m, 9H, CH_3).

2-(2,2-Dimethylpropionylaminomethyl)piperazine, Hydrochloride (12). To a solution of amide **11** (2.44 g, 6.43 mmol) in EtOH (40 mL) and 12 N HCl (1 mL) was added 100 mg Pd/C, and this mixture was warmed (40 °C) with stirring under hydrogen atmosphere. After disappearance of the starting material (3 h) as shown by TLC, the suspension was filtered and the catalyst washed several times with EtOH and H_2O. The solvents were evaporated and the residue crystallized in MeOH/ether to give 1.2 g (68.6%) of **12**: mp 278.6 °C; R_f 0.13 ($CHCl_3$/MeOH/NH_4OH, 80:20:2, v/v/v).

2-(2,2-Dimethylpropionylaminomethyl)-1,4-di(3,4,5-trimethoxybenzoyl)piperazine (8). To a solution of diamine hydrochloride **12** (1.2 g, 4.4 mmol) in Et_3N (3 mL, 41 mmol) and CH_2Cl_2 (50 mL) was added dropwise 3,4,5-trimethoxybenzoyl chloride (2.33 g, 10.1 mmol) in CH_2Cl_2 (30 mL). After stirring overnight at room temperature, EtOH (2 mL) was added and the solution was washed twice with water, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed on a silica gel column using 2% MeOH in CH_2Cl_2 as eluent to yield 0.9 g (34.6%) of **9**: mp 146.5 °C; R_f 0.29 ($CHCl_3$/MeOH, 95:5, v/v); IR (ν cm^{-1}) 3391 (NH), 1740 (C═O amide), 1608 (ArC═O), 1584 (ArC═C); ^{1}H NMR δ 6.6 (s, 4H, ArH), 6.4 (s, 1H, NH), 4.5 (m, 4H, CH_2NC═O), 3.7 (s, 18H, CH_3O), 3.6 (m, 2H, CH_2N), 3.3 (m, 3H, CH_2 and CHNC═O), 1.1 (m, 9H, CH_3).

Biological Methods. 1. Platelet Aggregation. The inhibition of platelet aggregation was determined using platelet-rich plasma (PRP) of New Zealand rabbits by the method of Cazenave et al.[27] Blood samples were collected from 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 (number of determinations: 5−6).

2. Monocyte-Derived Macrophage Isolation. Human peripheral blood mononuclear cells (PBMC) were obtained from healthy HIV-, HCV-, HBV-seronegative blood donor by Ficoll-Hypaque density gradient centrifugation (MSL 2000, Eurobio, Les Ulis, France). Monocytes were isolated from PBMC by countercurrent centrifugal elutriation as previously described with an enrichment degree ≥95%.[28,29] Freshly isolated human monocytes were resuspended in medium A: RPMI 1640 medium (Roche Diagnostics, Meylan, France) supplemented with 10% heat-inactivated (56 °C for 30 min) fetal calf serum (FCS) (Roche Diagnostics), 2 mM L-glutamine (Roche Diagnostics), and 1% tri-antibiotic mixture (penicillin, streptomycin, neomycin, PSN; Life Technologies, Grand Island, NY). Cells were placed at 1 million cells/well of 48-well plates (Becton Dickinson Labware, Lincoln Park, NJ) and maintained 7 days in humidified 5% CO_2 atmosphere to allow their differentiation in MDM.

3. Drugs. Drugs were solubilized in RPMI 1640 or dimethyl sulfoxide (DMSO, Sigma) and conserved at −80 °C. Dilutions were performed in cell culture medium.

4. Virus. MDM were infected with the reference macrophage-tropic HIV-1/Ba-L strain.[30] In the Service de Neurovirologie, this virus was amplified in vitro using human phytohemagglutinin (PHA)-P-activated umbilical blood mononuclear cells (UBMC). The viral stock was ultracentrifugated to eliminate soluble factors such as cytokines, resuspended in RPMI 1640 and titrated using PHA-P-activated PBMC. Fifty percent (50%) tissue culture infectious doses ($TCID_{50}$) were calculated using Kärber's formula.[31]

5. Antiviral Assay. One million MDMs were pretreated 1 h in the presence of different concentrations of compounds and infected with 10 000 $TCID_{50}$ (0.01 moi, multiplicity of infection) of the HIV-1/Ba-L strain. After 24 h, MDM were washed once to eliminate excess virus and fed with fresh medium A. Twice a week, supernatants were removed and stored at −20 °C in order to measure viral replication by RT activity dosage. Cell culture medium and drugs were then renewed, and cells were microscopically observed to assess possible drug-induced cytotoxicity. Moreover, at the end of the culture, cytotoxicity was confirmed using neutral red assay and, for PMS 601, by testing until 1 mM dose.

6. Dosage of Viral Replication. HIV replication was assessed by the dosage of RT activity in cell culture supernatants, as described elsewhere.[32]

7. Data Analysis. All experiments were repeated with cells isolated from a second blood donor. Results were expressed as the mean of RT activity ± standard deviation (SD). Fifty percent (50%) inhibition concentrations (IC_{50}) were calculated using cumulative RT activity and microcomputer software (J. and T. C. Chou, Biosoft, Cambridge, U.K.). Fifty percent (50%) cytotoxic concentrations (CC_{50}) were determined using the same microcomputer software and DO values (neutral red staining assay).

Acknowledgment. The authors thank the Centre de Transfusion Sanguine des Armées (CTSA, Clamart, France), the Service de Cytaphérèse de l'Hôpital Saint-Louis (Paris, France), and the Maternité de Sainte-Félicité (Paris, France) as well as N. Boggetto and Prof. M. Reboud (Institut Jacques Monod, CNRS-Universités Paris 6 et 7, Paris, France) for the antiprotease assay. This work was supported by the Scientific Council of the Université Paris 7 - Denis Diderot (Paris, France), the Agence Nationale de Recherches sur le SIDA (ANRS, Paris, France), the Fondation pour la Recherche Médicale-Sidaction (Paris, France), the Institut de Formation Supérieure Biomédicale (IFSBM, Villejuif, France), the Association Claude Bernard (ARC, Paris, France), the Association Naturalia et Biologia (NEB, Paris, France), and the Association pour la Recherche en Neurovirologie (ARN, Griselles, France).

References

(1) Walh, L. M.; Corcoran, M. L.; Pyle, S. W.; Arthur, L. O.; Harel-Bellan, A.; Farrar, W. L. Human Immunodeficiency Virus Glycoprotein (gp 120) Induction of Monocyte Arachidonic Acid Metabolites and Interleukines. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 621−625.

(2) Perry, S. W.; Hamilton, J. A.; Tjoelker, L. W.; Dbaibo, G.; Dzenko, K. A.; Epstein, L. G.; Hannun, Y.; Whittaker, J. S.; Dewhurst, S.; Gelbard, H. A. Platelet-Activating Factor Receptor Activation, an Initiator Step in HIV-1 Neuropathogenesis. *J. Biol. Chem.* **1998**, *273*, 17660−17664.

(3) Kolson, D. L.; Lavi, E.; Gonzalez-Scarano, F. The Effects of Human Immunodeficiency Virus in the Central Nervous System. *Adv. Virus Res.* **1998**, *50*, 1−47.

(4) Gelbard, H. A.; Nottet, H. S.; Swindells, S.; Jett, M.; Dzenko, K. A.; Genis, P.; White, R.; Wang, L.; Choi, Y. B.; Zhang, D.; Lipton, S. A.; Tourtellote, W. W.; Epstein, L. G.; Gendelman, H. E. Platelet-Activating Factor: a Candidate Human Immunodeficiency Virus Type 1-Induced Neurotoxin. *J. Virol.* **1994**, *68*, 4628−4635.

(5) Dagenais, P.; Thivierge, M.; Parent, J.-L.; Stankova, J.; Rola-Pleszczynski, M. Augmented Expression of Platelet-Activating Factor Receptor Gene by TNF-alpha through Transcriptional Activation in Human Monocytes. *J. Leukocyte Biol.* **1997**, *61*, 106−112.

(6) Rola-Pleszczynski, M. Priming of Human Monocytes with PAF Augments their Production of Tumor Necrosis Factor. *J. Lipid Mediators* **1990**, *2*, S77−S82.

(7) Howard, A. D.; Erickson, K. L. The Induction and Augmentation of Macrophage Tumoricidal Responses by Platelet-Activating Factor. *Cell. Immunol.* **1995**, *164*, 105−112.
(8) Enting, H. E.; Hoetelmans, R. M. W.; Lange, J. M. A.; Burger, D. M.; Beijnen, J. H.; Portegies, P. Antiretroviral Drugs and the Central Nervous System. *AIDS* **1998**, *12*, 1941−1955.
(9) Weissman, D.; Poli, G.; Bousseau, A.; Fauci, A. S. A Platelet-Activating Factor Antagonist, RP 55778, Inhibits Cytokine-Dependent Induction of Human Immunodeficiency Virus Expression in Chronically Infected Promonocytic Cells. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2537−2541.
(10) Le Naour, R.; Clayette, P.; Henin, Y.; Mabondzo, A.; Raoul, H.; Bousseau, A.; Dormont, D. Infection of Human Macrophages with an Endogenous Tumor Necrosis Factor-Alpha (TNF-alpha)-Independent Human Immunodeficiency Virus Type-1 Isolate is Unresponsive to the TNF-alpha Synthesis Inhibitor RP 55778. *J. Gen. Virol.* **1994**, *75*, 1379−1388.
(11) Sedivy, P.; Caillard, C. G.; Floch, A.; Folliard, F.; Mondot, S.; Robaut, C.; Terlain, B. 48740 RP, a Specific PAF-Acether Antagonist. *Prostaglandins* **1985**, *30*, 688.
(12) Floch, A.; Bousseau, A.; Hetier, E.; Floch, F.; Bost, P.-E.; Cavero, I. RP 55778, a PAF Receptor Antagonist, Prevents and Reverses LPS-Induced Hemoconcentration and TNF Release. *J. Lipid Mediators* **1989**, *1*, 349−360.
(13) Johnson, C. D. Platelet-Activating Factor and Platelet-Activating Factor Antagonists in Acute Pancreatitis. *Dig. Surg.* **1999**, *16*, 93−101.
(14) Schifitto, G.; Sacktor, N.; Marder, K.; McDermott, M. P.; McArthur, J. C.; Kieburtz, K.; Small, S.; Epstein, L. G. Randomized Trial of the Platelet-Activating Factor Antagonist Lexipafant in HIV-Associated Cognitive Impairment. Neurological AIDS Research Consortium. *Neurology* **1999**, *53*, 391−396.
(15) Lamotte-Brasseur, J.; Heymans, F.; Dive, G.; Lamouri, A.; Redeuilh, C.; Hosford, D.; Braquet, P.; Godfroid, J.-J. PAF-Receptor and the "Cache-Oreilles" Effect. Simple PAF Antagonists. *Lipids* **1991**, *26*, 1167−1171.
(16) Dive, G.; Godfroid, J.-J.; Lamotte-Brasseur, J.; Batt, J.-P.; Heymans, F.; Dupond, L.; Braquet, P. PAF-Receptor. 1. "Cache-Oreilles" Effects of Selected High-Potency Platelet-Activating Factor (PAF) Antagonists. *J. Lipid Mediators* **1989**, *1*, 201−215.
(17) Lamouri, A.; Heymans, F.; Tavet, F.; Dive, G.; Batt, J.-P.; Blavet, N.; Braquet, P.; Godfroid, J.-J. Design and Modelling of New Platelet-Activating Factor Antagonists. 1. Synthesis and Biological Activity of 1,4-Bis(3′,4′,5′-trimethoxybenzoyl)-2-[[(substitued carbonyl and carbamoyl)oxy]methyl]piperazines. *J. Med. Chem.* **1993**, *36*, 990−1000.
(18) Tavet, F.; Lamouri, A.; Heymans, F.; Dive, G.; Touboul, E.; Blavet, N.; Godfroid, J.-J. Design and Modelling of New Platelet-Activating Factor Antagonists. 2[1]. Synthesis and Biological Activity of 1,4-Bis(3′,4′,5′-trimethoxybenzoyl)-2-alkyl and 2-alkoxymethylpiperazines. *J. Lipid Mediators Cell Signalling* **1997**, *15*, 145−159.
(19) Batt, J.-P.; Lamouri, A.; Tavet, F.; Heymans, F.; Dive, G.; Godfroid, J.-J. New Hypothesis on the Conformation of the PAF-Receptor from Studies on the Geometry of Selected Platelet-Activating Factor-Antagonists. *J. Lipid Mediators* **1991**, *4*, 343−346.
(20) Heymans, F.; Dive, G.; Lamouri, A.; Bellahsene, T.; Touboul, E.; Huet, J.; Tavet, F.; Redeuilh, C.; Godfroid, J.-J. Design and Modelling of New Platelet-Activating Factor Antagonists. 3[1]. Relative Importance of Hydrophobicity and Electronic Distribution in Piperazinic Series. *J. Lipid Mediators Cell Signalling* **1997**, *15*, 161−173.
(21) Le Texier, L.; Favre, E.; Ronzani, N.; Massicot, F.; Blavet, N.; Pirotzky, E.; Godfroid, J.-J. Structure−Activity Relationships in Platelet-Activating Factor (PAF). 8. Tetrahydrofuran Derivatives as Dual PAF Antagonists and Acetylcholinesterase Inhibitors: Anti-Acetylcholinesterase Activity and Comparative SAR. *J. Lipid Mediators Cell Signalling* **1996**, *13*, 207−222.
(22) Binisti, C.; Touboul, E.; Mounier, C.; Heymans, F.; Bon, C.; Godfroid, J.-J. Structure−Activity Relationships in Platelet-Activating Factor. 9. From PAF-Antagonism to PLA_2 Inhibition. *J. Lipid Mediators Cell Signalling* **1996**, *15*, 125−144.
(23) Rekker, R. F.; De Kort, H. M. The Hydrophobic Fragmental Constant; an Extension to a 1000 Data Point Set. *Eur. J. Med. Chem.* **1979**, *14*, 479−488.
(24) Hansch, C.; Leo, A.; Hunger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. "Aromatic" Substituent Constants for Structure−Activity Correlations. *J. Med. Chem.* **1973**, *16*, 1207−1216.
(25) Martin, M.; Serradji, N.; Dereuddre-Bosquet, N.; Le Pavec, G.; Lamouri, A.; Heymans, F.; Godfroid, J. J.; Clayette, P.; Dormont, D. Anti-HIV-1 and Antiinflammatory Properties of a New PAF Antagonist, PMS 601. *Antimicrob. Agents Chemother.*, submitted.
(26) Bouras, A.; Boggetto, N.; Benatalah, Z.; de Rosny, E.; Sicsic, S.; Reboud-Ravaux, M. Design, Synthesis, and Evaluation of Conformationally Constrained Tongs, New Inhibitors of HIV-1 Protease Dimerization. *J. Med. Chem.* **1999**, *42*, 957−962.
(27) Cazenave, J. P.; Benveniste, J.; Mustard, J. F. Aggregation of Rabbit Platelets by Platelet-Activating Factor is Independent of the Release Reaction and the Arachidonate Pathway and Inhibited by Membrane-Active Drugs. *Lab. Invest.* **1979**, *41*, 275−285.
(28) Dereuddre-Bosquet, N.; Clayette, P.; Martin, M.; Benveniste, O.; Fretier, P.; Jaccard, P.; Vaslin, B.; Lebeaut, A.; Dormont, D. Lack of IL-10 Expression in Monocyte-Derived Macrophages in Response to in vitro Infection by HIV-1 Isolates. *AIDS Res. Hum. Retroviruses* **1997**, *13*, 9961−966.
(29) Fidgor, C. G.; Leemans, J. M. M.; Bont, W. S.; Vries, J. E. Theory and Practice of Centrifugal Elutriation (CE). Factors Influencing the Separation of Human Blood Cells. *Cell. Biophys.* **1983**, *5*, 105−118.
(30) Gartner, S.; Markovitz, P.; Markovitz, D. M.; Kaplan, M. H.; Gallo, R. C.; Popovic, M. The Role of Mononuclear Phagocytes in HTLV−III/LAV Infection. *Science* **1986**, *233*, 215−219.
(31) Kärber, G. Beitrag Zur Kollektiven Behandlung Pharmakologischer Reihenversuche. *Arch. Exp. Pathol. Pharmakol.* **1931**, *162*, 956−959.
(32) Rey, F.; Spire, B.; Dormont, D.; Barré-Sinoussi, F.; Montagnier, L.; Chermann, J. C. Characterization of RNA Dependent DNA Polymerase of a New Human T-Lymphotropic Retrovirus (Lymphadenopathy Associated Virus). *Biochem. Biophys. Res. Commun.* **1984**, *121*, 126−133.

JM9911276