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

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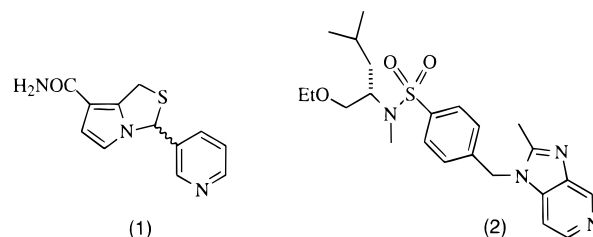Dominique Dormont,[‡] and Jean-Jacques Godfroid*,[§]

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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

Figure 1. **1**: RP 48740 (racemic) and RP 55778 [(+)-enantiomer]. **2**: BB-882.
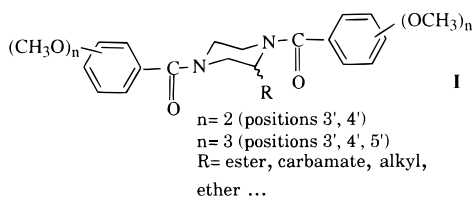
dementia and inflammatory syndrome.[4] Tissues such as brain are sites of HIV replication and may act as a reservoir for the virus. TNF-α up-regulates HIV-1 production in HIV-infected macrophages, and PAF, in turn, appears to increase TNF-α synthesis.[5−7] As a consequence, it is also of interest to 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

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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]
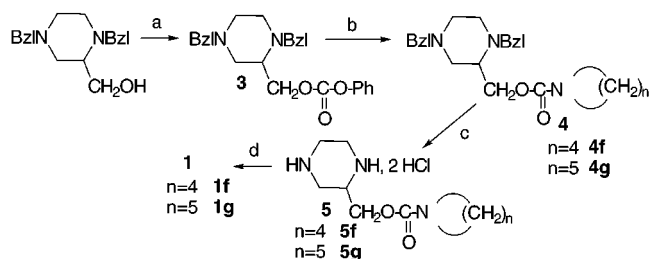
Consequently, the "cache-oreilles" effect was improved with the hypothesis of the multipolarized cylinder.[19] The influence of the hydrophobicity was also studied.[20] Moreover, we have shown that the PAF antagonistic effect was only weakly enantiospecific in this series.[17] Starting from these different 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.
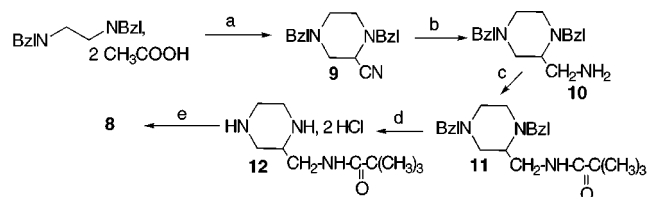
Chemistry

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$_3$N, CH_2Cl_2.

Scheme 2[a]

[a] Reagents: (a) BrCH$_2$CHBrCN, Et$_3$N, benzene, reflux; (b) Na, EtOH, reflux; (c) (CH$_3$)$_3$CCOCl, Et$_3$N, CH_2Cl_2; (d) H_2, Pd/C, HCl, EtOH; (e) 3,4,5-(MeO)$_3$PhCOCl, Et$_3$N, CH_2Cl_2.
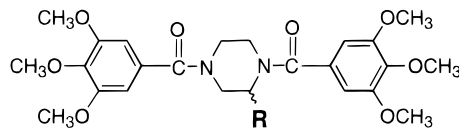
The amide **8** needed another process as described in Scheme 2. *N,N'*-Dibenzylethylenediamine was condensed with 2,3-dibromopropionitrile to form the 1,4-dibenzyl-2-cyanopiperazine **(9)**. The nitrile function was reduced into the amine **10** which was reacted with trimethylacetyl chloride to give the amide **11**. Debenzylation and "cache-oreilles" formation were conducted as for **1**.
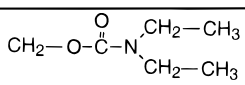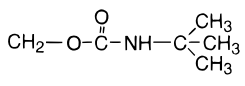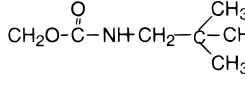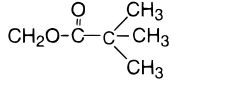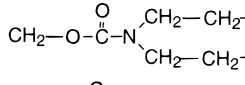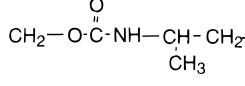
Results and Discussion

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.
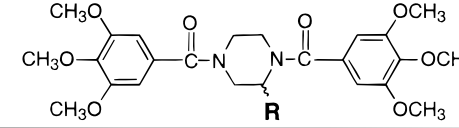
First Screening (Table 1). In the first set, known[18,19] and new molecules possessing anti-PAF activities with IC_{50} from 0.1 to 8 μM were tested against HIV. They are listed in Table 1 by increasing lipophilicity in order to evaluate the importance of this parameter in the activities. The percentage 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**).
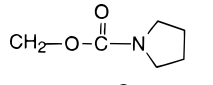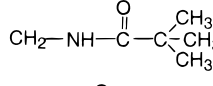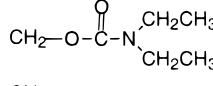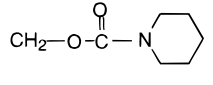
As no toxicity was observed in this series (except for compound **6a**) at 100 μM and as the best compound, **1a**, is the least hydrophobic, we decided to synthesize and test molecules with f_R (lipophilic contribution of R, calculated from Rekker's method[23]) ranging between f_R(**1a**) ± 1.5.

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	CH$_2$—O—C(=O)—N(CH$_2$—CH$_3$)$_2$	0.564	34.47	95 ±0	8	>1000
1b	CH$_2$—O—C(=O)—NH—C(CH$_3$)$_3$	0.853	34.52	52 ±26	0.13	>100
1c	CH$_2$O—C(=O)—NH—CH$_2$—C(CH$_3$)$_3$	1.372	39.17	74 ±4	1	>100
2	CH$_2$O—C(=O)—C(CH$_3$)$_3$	1.526	31.09	25 ±21	0.1	>100
1d	CH$_2$—O—C(=O)—N(CH$_2$—CH$_2$—CH$_3$)$_2$	1.602	43.79	66 ±13	0.37	>100
1e	CH$_2$—O—C(=O)—NH—CH(CH$_3$)-CH$_2$CH(CH$_3$)$_2$	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$_2$OH	-0.951	7.19	>100	1.27	>100
6b	H	0.182	1.03	>100	1.78	>100
1f	CH$_2$—O—C(=O)—N(pyrrolidine)	0.2	33.66	>100	10	>100
8	CH$_2$—NH—C(=O)—C(CH$_3$)$_3$	0.331	33.55	>100	>100	>100
1a	CH$_2$—O—C(=O)—N(CH$_2$CH$_3$)$_2$	0.564	34.47	11±7	8	>1000
6c	CH$_3$	0.701	5.65	66*	0.56	>100
1g	CH$_2$—O—C(=O)—N(piperidine)	0.719	38.31	95*	0.08	>100
6d	CH$_2$CH$_3$	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_2-Cl_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$_2$Cl$_2$, 0.5:99.5, v/v as eluent to yield 2.44 g (49%) of the amide **11**: R_f 0.36 (CH$_2$Cl$_2$/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$_2$NC=O), 3.3 (m, 5H, CH$_2$Ph 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$_2$O. 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$_4$OH, 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$_3$N (3 mL, 41 mmol) and CH$_2$Cl$_2$ (50 mL) was added dropwise 3,4,5-trimethoxybenzoyl chloride (2.33 g, 10.1 mmol) in CH$_2$Cl$_2$ (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$_2$-Cl$_2$ 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$_2$NC=O), 3.7 (s, 18H, CH$_3$O), 3.6 (m, 2H, CH$_2$N), 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 phytohe-magglutinin (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).

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