Nonpeptide HIV Protease Inhibitors. Differential Introduction of Alkylamino Groups into the Two Aryl Rings of Haloperidol

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In efforts to synthesize haloperidol analogues with improved properties as HIV protease inhibitors, methods were sought to introduce mono- and dialkylamino groups into the two aromatic rings of the parent structure. We report here that the reaction of haloperidol with alkylamines in the presence of a strong base ($NaNH_2$) regiospecifically introduces the alkylamino group into the chlorophenyl ring, whereas the same reaction in the presence of a weak base (K_2CO_3) results in exclusive replacement of the fluorine of the fluorophenyl ring. Different amine functions can be introduced at the two rings by sequential reactions in the presence of, respectively, a strong and a weak base. The reaction catalyzed by $NaNH_2$ involves, at least in part, benzyne formation, whereas the reaction catalyzed by K_2CO_3 involves direct nucleophilic addition to the aromatic ring. The regiospecificity of the reaction is due to conjugation of the fluorophenyl ring to a ketone group. The fluorophenyl ring is activated by the ketone towards nucleophilic aromatic substitution but is deactivated by the same function when it is converted by strong base to the enolate anion. Carbonyl conjugation of one of two haloaryl groups appears to be a general strategy for regiospecific introduction of alkylamino functions into complex aromatic molecules. The alkylamino derivatives actually prepared are comparable to haloperidol as inhibitors of the HIV-1 protease.

The proteases encoded by the HIV viral genome process the polyprotein precursors produced from the *gag* and *pol* genes into the proteins required for replication and assembly of the mature virus.[1] Inactivation of the HIV-1 protease (HIV-1PR) by mutation of the two catalytic aspartyl residues produces non-infectious virions.[2] The protease is therefore an attractive target for the design of anti-AIDS therapeutic agents, and many potent peptide inhibitors of the enzyme with K_I values in the nanomolar or subnanomolar range have been synthesized.[3] However, none of the inhibitors so far developed has proven to be of clinical utility because of the low bioavailability often associated with peptidic agents.[3] There is intense interest, therefore, in the development of nonpeptidic inhibitors of the HIV proteases, a task facilitated by the availability of high-resolution crystallographic structures for the enzyme.[4–6] Using a computer-based docking strategy, we identified haloperidol, a clinically used antipsychotic agent, as an inhibitor of HIV-1PR with $K_I = 100\ \mu M$.[7] The toxicity of haloperidol and the derivatives so far examined, however, is too high for the inhibitors to be of practical utility.[7,8] The toxicity of haloperidol is not well defined but it is not limited to its interaction with the D2 receptor because the *N*-oxide derivative, which does not bind to the receptor, is as toxic as haloperidol itself. To determine whether the toxicity of the molecule is due to membrane disruption caused by the presence of a polar group at one end and a lipophilic terminus at the other, we sought to synthesize derivatives with polar functionalities at both ends. These efforts have led to the development of a remarkable approach that makes possible the introduction of different alkylamino groups at the two ends of the molecule starting with the parent structure. The methodology developed for functionalization of haloperidol may be of practical utility in the functionalization of other complex structures containing two aryl rings.

Results and Discussion

The reaction of haloperidol with dialkylamines follows quite different pathways depending on the nature of the base used to promote the reaction. If the reaction is carried out with sodium amide as the base, the reaction occurs exclusively at the chlorophenyl ring to give either the *p*-(dialkylamino)-substituted haloperidol or a mixture of the *p*- and *m*-(dialkylamino)-substituted haloperidol derivatives (Table 1, entries 1–4). The *para*-substituted product, if formed, is always the minor product. On the other hand, if the reaction is carried out with a weak base such as potassium carbonate, the reaction occurs at the fluorophenyl ring and gives exclusively the *p*-(dialkylamino)-substituted haloperidol derivative. The same reaction is observed, albeit sometimes with lower yields, if the reaction is carried out without a base other than the secondary amine itself (Table 1, entries 6 and 8). The regiospecificity of the reaction is the same with alkylamines as with dialkylamines (Table 1). It is therefore possible, through the choice of base, to specifically substitute a mono- or dialkylamino group into either the chlorophenyl or fluorophenyl moiety of haloperidol.

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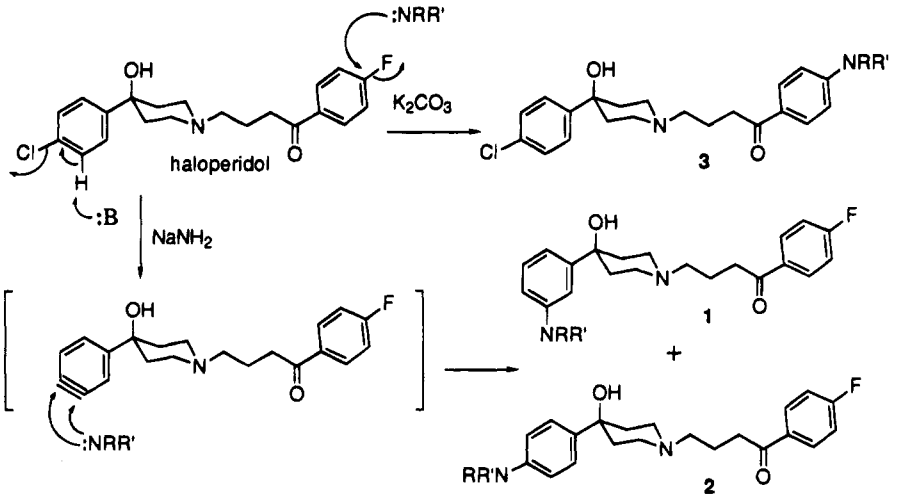
Table 1. Haloperidol Adducts Isolated under Different Reaction Conditions. The Structures of the Products Are Given in Scheme 1

entry	base	amine	T (°C)	medium	R, R′	product (yield, %)
1	$NaNH_2$	Bu_2NH	80–90	$HNBu_2$	R = R′ = Bu	**1a** (37)
2	$NaNH_2$	$BuNH_2$	78 (reflux)	H_2NBu	R = Bu, R′ = H	**1b** (58)
3	$NaNH_2$	Et_2NH	55 (reflux)	$HNEt_2$	R = R′ = Et	**1c** (61)
4	$NaNH_2$	$C_6H_{11}NH_2$	80–90	$C_6H_{11}NH_2$	R = C_6H_{11}, R′ = H	**1d** (41)
5	K_2CO_3	Bu_2NH	reflux	DMF	R = R′ = Bu	**3e** (42)
6	no base	Bu_2NH	reflux	$HNBu_2$	R = R′ = Bu	**3e** (8)
7	K_2CO_3	piperidine	reflux	DMF	R, R′ = C_5H_{11}	**3f** (89)
8	no base	piperidine	reflux	piperidine	R, R′ = C_5H_{11}	**3f** (89)
9	K_2CO_3	$C_6H_{11}NH_2$	reflux	DMF	R = C_6H_{11}, R′ = H	**3g** (92)
10	K_2CO_3	$Me_2N(CH_2)_2NH_2$	reflux	DMF	R = $Me_2N(CH_2)_2$, R′ = H	**3h** (92)
11	K_2CO_3	$Me_2N(CH_2)_2NHEt$	reflux	DMF	R = $Me_2N(CH_2)_2$, R′ = Et	**3i** (30)
12	K_2CO_3	$Me_2N(CH_2)_3NH_2$	reflux	DMF	R = $Me_2N(CH_2)_3$, R′ = H	**3j** (80)

Scheme 1



Formation of both the *para*- and *meta*-substituted products in the reactions supported by sodium amide rules out a simple aromatic substitution mechanism and strongly suggests that the reaction proceeds *via* a benzyne intermediate (Scheme 1). Benzyne formation involves deprotonation of the carbon adjacent to the halogen followed by elimination of the halide to give the benzyne intermediate that is trapped by the amine. Studies with deuterium-labeled substrates have shown that deprotonation *ortho* to the fluorine of a fluorophenyl ring is preferentially followed by reprotonation rather than halide elimination, resulting in much faster hydrogen exchange than benzyne formation.[9] Benzyne formation is thus expected to strongly favor the chlorophenyl ring in a direct competition between chlorophenyl and fluorophenyl rings, as observed here for haloperidol. No products indicative of benzyne formation at the fluorophenyl terminus were detected.

The regiochemistry of the addition of amines to benzyne depends largely on the electronic influence of the other substituents on the ring and on steric factors.[10] A mixture of *meta*- and *para*-substituted products is expected on electronic grounds when the inductive effect is relatively weak, as in the case of the *para*-carbon substituent in haloperidol. A mixture of *meta*- and *para*-substituted products is obtained, for example, in the reaction of *p*-methylchlorobenzene with sodium amide and ammonia.[9] Predominant or exclusive formation of the *meta*-substituted products thus indicates that steric and possibly other environmental factors control the alkylamine addition step of the reaction.

The most unexpected finding is that the sodium amide-catalyzed reaction of alkylamines and haloperidol does not result in substitution into the fluorophenyl ring, whereas reaction with weak bases results in exclusive, high-yield replacement of the fluorine by the alkylamino moiety. The absence of *meta*-substituted products from reactions with the fluorophenyl ring suggests that this reaction, in contrast to that with the chlorophenyl ring, is the result of a nucleophilic aromatic substitution that does not proceed via a benzyne mechanism. The key to this difference in reactivity of the two halogen-substituted phenyl rings is undoubtedly the carbonyl group conjugated to the fluorophenyl ring. The carbonyl group plays a straightforward role in favoring nucleophilic aromatic substitution of the fluorine, as it stabilizes the negative charge introduced by the addition of a nucleophile to the aromatic ring. The conjugated carbonyl group also provides a rationale for the fact that benzyne reactions are not observed at the fluorophenyl ring and for the surprising result that nucleophilic replacement of the fluorine is observed with weak but not strong bases. Deprotonation of the carbon adjacent to the carbonyl group by strong bases, a rapid reaction, produces the enolate anion which is negatively charged and thus decreases the reactivity of the fluorophenyl ring toward reactions that increase the electron density on the aromatic ring (Scheme 2). Enolate formation thus can suppress both nucleophilic aromatic substitution and benzyne formation. The use of weak bases, however, promotes deprotonation of either the amine or intermediate formed by amine addition to the aromatic ring but does not promote stable enolate formation. Weak bases thus catalyze the nucleophilic aromatic substitution reaction.

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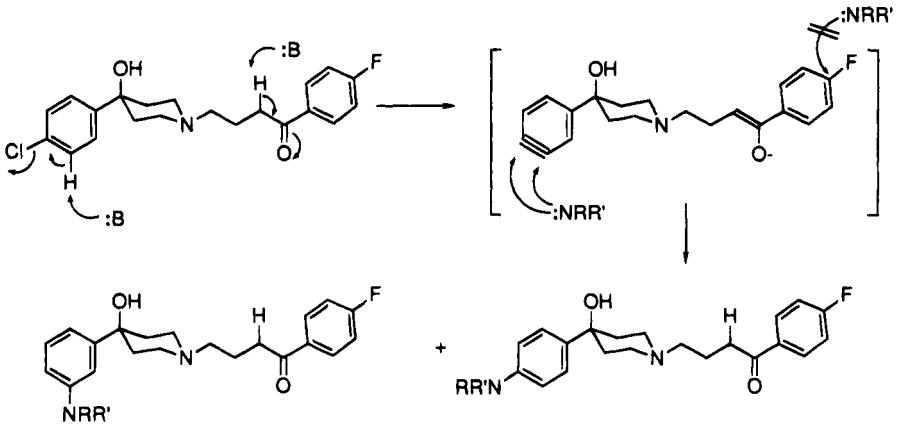
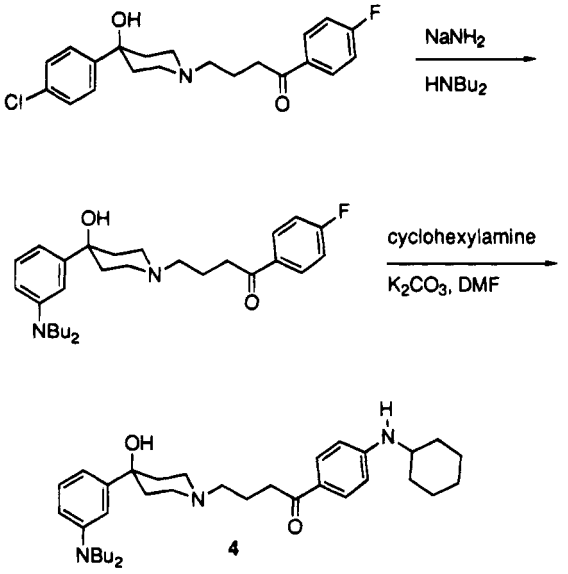
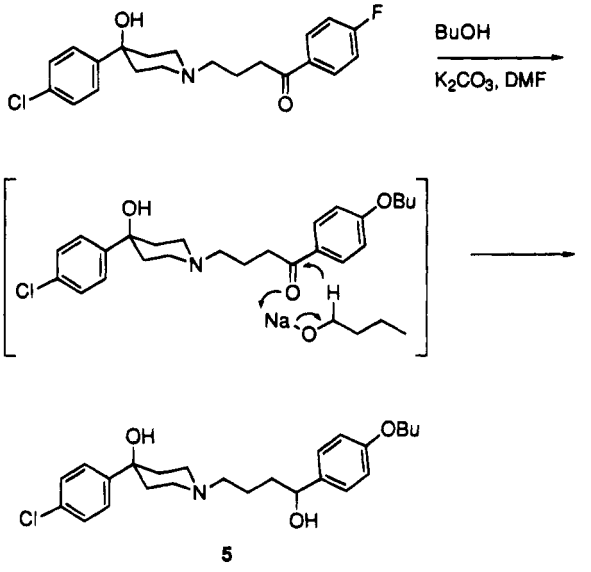
Scheme 2



Scheme 3



Scheme 4



If the above rationale for the regiospecificity of the reaction is correct, it should be possible, by sequential use of the two reaction conditions, to introduce alkylamino groups into both aryl rings. Indeed, reaction of haloperidol with dibutylamine in the presence of sodium amide, followed by reaction of the dibutylamino-substituted product thus obtained with cyclohexylamine and potassium carbonate, gives an 85% yield of the haloperidol derivative in which the chlorine has been replaced by a dibutylamino substituent and the fluorine by a cyclohexylamino group (Scheme 3).

The possibility of regiospecifically substituting alkoxy groups into haloperidol was similarly explored with butanol as the nucleophile and potassium carbonate as the base. This reaction, however, resulted not only in replacement of the fluorine by a butoxy group but also in reduction of the ketone to the alcohol by a Meerwein–Pondorf–Verley process (Scheme 4). Replacement of the fluorine by the alkoxy group, which replaces an electron-withdrawing by an electron-donating substituent, appears to promote the ketone reduction reaction because neither the fluorophenyl alcohol nor the alkoxy-substituted ketone was isolated.

Comparison of the alkylamino derivatives with haloperidol itself shows that the alkylamino functions only modestly alter HIV-1PR inhibitory activity. Replacement of the *p*-chloro group by a *p*- (**1a**) or *m*-(dibutylamino) (**2a**) function increases inhibitory activity up to 3-fold but replacement by a butylamino, diethylamino, or cyclohexylamino group does not improve the activity (Table 2). Replacement of the fluorine by a cyclohexylamino moiety has little effect but its replacement by other alkylamino functions decreases inhibitory activity by a factor of 2–3. Furthermore, replacement of the chloride by alkylamino groups does not decrease toxicity, although similar replacement of the fluorine decreases toxicity from 2- to 20-fold (Table 2). The compounds nevertheless remain more toxic than haloperidol (IC_{50} = 115 μM, LD_{50} = 500 μM).

Table 2. Inhibition of HIV-1PR and Cell Toxicity

compd	IC_{50}[a] (μM)	LD_{50} (μM)
1a	75	10
2a	35	10
1b	170	140
1c	>200	130
1d	115	10
2d	120	35
3e	>200	70
3f	>200	100
3g	95	25
3h	290	60
3c	355	60
3j	210	200
4	530	20
haloperidol	115	500

[a] The IC_{50} values are accurate to within ±10%.

Conclusions

The synthetic studies described in this paper indicate that regiospecific substitution of alkylamine groups into either of two halophenyl rings can be achieved if one of the rings is conjugated to a carbonyl group. The difference in reactivity can be used, in fact, to regiospecifically introduce different alkylamino groups into the two rings. This methodology has been exploited here to produce haloperidol derivatives of interest as potential inhibitors of HIV-1PR, but should be generally applicable to molecules with two halophenyl moieties. The biological results suggest that the amphiphilic properties of haloperidol are not a critical determinant of its cellular toxicity.

Experimental Section

General Procedures. Commercial reagents were used without further purification. Melting points were determined by the capillary method and are uncorrected. Column chromatography was performed on silica gel (230–400 mesh). All the reactions were worked up by cooling the reaction mixture to room temperature, pouring it into EtOAc (200 mL), and washing the resulting mixture with water (3 × 100 mL) and dilute HCl (50 mL). The organic layer was then separated, washed with water (100 mL) and brine (20 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (9:1 CH_2Cl_2/MeOH).

4-[4-[3-(Dibutylamino)phenyl]-4-hydroxypiperidino]-4′-fluorobutyrophenone (1a). A mixture of *n*-Bu_2NH (25 mL) and $NaNH_2$ (780 mg, 20 mmol) was heated at 80 °C for 30 min. Haloperidol (1.16 g, 3 mmol) was added slowly to the warm mixture, and the resulting mixture was heated at 80 °C for another 3 h. Workup and purification gave a yellow solid (535 mg, 37%): mp 96–97 °C; IR (Nujol, cm^{-1}) 3580 (OH), 1685 (C=O), 1610 (C=C), 1590 (C=C), 1450 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 7.98 (dd, J = 8.6, 5.46 Hz, 2H), 7.1 (m, 3H), 6.81 (s, 1H), 6.68 (d, J = 7.63 Hz, 1H), 6.5 (dd, J = 8.12, 1.8 Hz, 1H), 3.24 (t, J = 7.4 Hz, 4H), 3.0 (t, J = 6.9 Hz, 2H), 2.9 (brd, J = 11.2 Hz, 2H), 2.6 (m, 4H), 2.2 (dd, J = 13.4, 3.8 Hz, 2H), 2.0 (m, 2H), 1.77 (d, J = 13.3 Hz, 2H), 1.54 (m, 4H), 1.33 (q, J = 7.4 Hz, 4H), and 0.91 ppm (t, J = 7.3 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) 13.9, 20.3, 20.6, 29.4, 35.9, 37.2, 49.3, 50.7, 57.3, 70.6, 107.7, 110.5, 111.2, 115.4, 115.7, 129.1, 130.7, 133.2, 148.2, 148.4, 165.0, 167.3, and 197.8 ppm; LRMS (CI), m/z 469 (MH^+), 451 ($MH^+ - H_2O$), 287, 123 (C_7H_4FO); HRMS (EI) calcd for $C_{29}H_{41}FN_2O_2$ 468.3152, found 468.3159. The regioisomer **4-[4-[4-(Dibutylamino)phenyl]-4-hydroxypiperidino]-4′-fluorobutyrophenone (2a)** was also isolated as a dark yellow solid (200 mg, 14%): mp 74–75 °C; IR (Nujol, cm^{-1}) 3579 (OH), 1680 (C=O), 1530 (C=C), 1480 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 8.0 (m, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.12 (m, 2H), 6.6 (d, J = 8.4 Hz, 2H), 3.24 (t, J = 7.2 Hz, 4H), 3.02 (m, 2H), 2.88 (brd, J = 10.49 Hz, 2H), 2.59 (m, 4H), 2.08 (m, 4H), 1.77 (brd, J = 13.05 Hz, 2H), 1.55 (m, 4H), 1.34 (q, J = 7.1 Hz, 4H), and 0.95 ppm (t, J = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) 13.9, 20.2, 21.1,29.3, 36.1, 37.6, 49.4, 50.6, 57.5, 70.2, 111.3, 115.4, 115.7, 125.4, 130.5, 130.7, 134, 147.2, 165, 167.2, and 198.2 ppm; LRMS (CI) m/z 469 (MH^+), 451 ($MH^+ - H_2O$), 407 ($MH^+ - H_2O - C_3H_7$), 285, 158, 123 (C_7H_4FO); HRMS (EI) calcd for $C_{29}H_{41}FN_2O_2$, 468.3152, found 468.3140.

4-[4-[3-(Butylamino)phenyl]-4-hydroxypiperidino]-4′-fluorobutyrophenone (1b). A mixture of Et_2NH (25 mL) and $NaNH_2$ (780 mg, 20 mmol) was heated at reflux for 2 h (turned dark brown). Haloperidol (1.16 g, 3 mmol) was added slowly to the warm mixture, and the resulting mixture was heated at reflux overnight. Workup and purification gave a yellow solid (717 mg, 58%): mp 94–96 °C; IR (Nujol, cm^{-1}) 3570 (OH), 1685 (C=O), 1620 (C=C), 1485 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 8.0 (m, 2H), 7.12 (m, 3H), 6.75 (s, 1H), 6.72 (d, J = 8 Hz, 1H), 6.48 (d, J = 8.5 Hz, 1H), 3.5 (brs, 1H), 3.09 (t, J = 7 Hz, 2H), 3.0 (t, J = 7 Hz, 2H), 2.83 (d, J = 10.9 Hz, 2H), 2.5 (m, 4H), 1.97–2.16 (m, 4H), 1.7 (d, J = 12.7 Hz, 2H), 1.57 (m, 2H), 1.49 (q, J = 7.55 Hz, 2H), and 0.95 ppm (t, J = 7.25 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) 198.5, 168, 150, 148.5, 130.7, 130.6, 129.2, 115.7, 115.4, 113.2, 111.1, 109.5, 71, 57.7, 49.4, 43.7, 37.9, 36.2, 31.6, 21.4, 20.3, and 13.9 ppm; LRMS (CI) m/z 413 (MH^+), 395 ($MH^+ - H_2O$), 274 ($MH^+ - C_7H_4FO - CH_3$), 229, 123 (C_7H_4FO); HRMS (EI) calcd for $C_{25}H_{33}FN_2O_2$ 412.2526, found 412.2526.

4-[4-[3-(Diethylamino)phenyl]-4-hydroxypiperidino]-4′-fluorobutyrophenone (1c). A mixture of Et_2NH (25 mL) and $NaNH_2$ (780 mg, 20 mmol) was heated at reflux for 2 h. Haloperidol (1.16 g, 3 mmol) was then added slowly, and the resulting mixture was heated at reflux for another 20 h. Workup and purification gave a yellow solid (755 mg, 61%): mp 67–68 °C; IR (CH_2Cl_2, cm^{-1}) 3599 (OH), 1697 (C=O), 1598 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 8.0 (m, 2H), 7.13 (m, 3H), 6.84 (s, 1H), 6.7 (d, J = 7.54 Hz, 1H), 6.56 (dd, J = 8.2, 3.24 Hz, 1H), 3.36 (q, J = 6.8 Hz, 4H), 2.97 (t, J = 7 Hz, 2H), 2.82 (brd, J = 10.7 Hz, 2H), 2.52 (m, 4H), 2.1 (td, J = 12.4, 3.5 Hz, 2H), 2.0 (t, J = 7.05 Hz, 2H), 1.71 (brd, J = 12.8 Hz, 2H), and 1.13 ppm (t, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) 198.1, 167.1, 163.7, 149.2, 147.6, 130.6, 130.4, 128.9, 115.5, 115.2, 111.4, 110.3, 107.9, 70.9, 57.6, 49.3, 44.1, 37.8, 36.1, 21.3, and 12.4 ppm; LRMS (EI) m/z 412 (M^+), 394 ($M^+ - H_2O$), 379 ($M^+ - H_2O - CH_3$), 261 ($M^+ - C_9H_8FO$), 245 (261 − CH_3), 165 ($C_{10}H_{10}FO$), 123 (C_7H_4FO); HRMS (EI) calcd for $C_{25}H_{33}FN_2O_2$ 412.2526, found 412.2526.

4-[4-[3-(Cyclohexylamino)phenyl]-4-hydroxypiperidino]-4′-fluorobutyrophenone (1d). A mixture of cyclohexylamine (25 mL) and $NaNH_2$ (780 mg, 20 mmol) was heated at 80–90 °C for 2 h (turned dark brown). Haloperidol (1.16 g, 3 mmol) was then added slowly, and the resulting mixture was heated at 80–90 °C for another 8 h. Workup and purification gave a brown solid (540 mg, 41%): mp 104–106 °C; IR (CH_2Cl_2, cm^{-1}) 3599 (OH), 1678 (C=O), 1604 (C=C), 1511 (C=C), 1437 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 8.0 (m, 2H), 7.12 (m, 3H), 6.7 (d, J = 6.7 Hz, 2H), 6.46 (d, J = 7.48 Hz, 1H), 3.25 (m, 1H), 3.01 (t, J = 6.8 Hz, 2H), 2.55 (brd, J = 11.2 Hz, 2H), 2.58 (m, 4H), 1.9–2.2 (m, 6H), 1.6–1.8 (m, 4H), and 1.08–1.4 ppm (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) 198.7, 150, 148.5, 132, 130.5, 129.5, 115.5, 115.6, 113, 111, 109, 70.9, 57.6, 51.6, 49.4, 37.7, 36.1, 33.4, 25.9, 24.9, and 21.2 ppm; LRMS (CI) m/z 439 (MH^+), 421 ($MH^+ - H_2O$), 391, 255, 123 (C_7H_4FO); HRMS (EI) calcd for $C_{27}H_{35}FN_2O_2$ 438.2713, found 438.2669. The regioisomer **4-[4-[4-(Cyclohexylamino)-phenyl]-4-hydroxypiperidino]-4′-fluorobutyrophenone (2d)** was also obtained as a yellow solid (200 mg, 15%): mp 131–132 °C; IR (CH_2Cl_2, cm^{-1}) 3586 (OH), 1702 (C=O), 1598 (C=C), 1530 (C=C), 1431 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 8.01 (m, 2H), 7.23 (d, J = 8.24 Hz, 2H), 7.12 (d, J = 8.48 Hz, 2H), 6.55 (d, J = 8.27 Hz, 2H), 3.21 (m, 1H), 2.95 (m, 2H), 2.73 (m, 2H), 2.48 (m, 4H), 1.9–2.1 (m, 6H), 1.6–1.8 (m, 4H), and 1.1–1.4 ppm (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) 198, 168, 149, 137, 130.7, 130.6, 125.5, 115.7, 115.4, 112.7, 70, 57.7, 51.6, 49.5, 38, 36.2, 33.4, 25.8, 24.9, and 21.5 ppm; LRMS (CI), m/z 439 (MH^+), 421 ($MH^+ - H_2O$), 395, 255, 165 ($C_{10}H_{10}FO$), 123 (C_7H_4FO); HRMS (EI) calcd for $C_{27}H_{33}FN_2O$ ($M - H_2O$) 420.2576, found 420.2596.

4-[4-(4-Chlorophenyl)-4-hydroxypiperidino]-4′-(dibutylamino)butyrophenone (3e). To a solution of haloperidol (1.16 g, 3 mmol) in dry DMF (30 mL) were added K_2CO_3 (1.7 g, 12 mmol) and *n*-Bu_2NH (2 mL), and the reaction mixture was heated at reflux for 3 days. Workup and purification gave a pale yellow solid (610 mg, 42%): mp 124–126 °C; IR (CH_2Cl_2, cm^{-1}) 3580 (OH), 1660 (C=O), 1598 (C=C), 1530 (C=C), 1418 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 7.85 (d, J = 8.9 Hz, 2H), 7.45 (d, J = 8.5 Hz, 2H), 7.3 (d, J = 8.5 Hz, 2H), 6.59 (d, J = 8.9 Hz, 2H), 3.32 (t, J = 7.8 Hz, 4H), 2.94 (m, 4H), 2.64 (m, 4H), 2.22 (m, 2H), 2.04 (m, 2H), 1.74 (brd, J = 13.2 Hz, 2H), 1.59 (m, 4H), 1.38 (q, J = 7.2 Hz, 4H), and 0.96 ppm (t, J = 7.2 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) 197.5, 151.5, 146.5, 132.6, 130.5, 128.2, 126.1, 123.8, 110.2, 70.3, 57.7, 50.6, 49.1, 37.5, 35, 29.2, 21.2, 20.2, and 13.9 ppm; LRMS (EI) m/z 484 (M^+), 466 ($M^+ - H_2O$), 274 ($C_{18}H_{24}NO$), 204 ($C_{14}H_{22}N$); HRMS (EI) calcd for $C_{29}H_{41}ClN_2O_2$ 484.2857, found 484.2850.

4-[4-(4-Chlorophenyl)-4-hydroxypiperidino]-4′-piperidinobutyrophenone (3f). To a solution of haloperidol (1.16 g, 3 mmol) in dry DMF (30 mL) were added K_2CO_3 (1.7 g, 12 mmol) and piperidine (2 mL), and the mixture was heated at reflux for 3 h. Workup and purification gave a white solid (1.18 g, 89%): mp 157–159 °C; IR (CH_2Cl_2, cm^{-1}) 3598 (OH), 1668 (C=O), 1594 (C=C), 1430 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 7.88 (d, J = 8.95 Hz, 2H), 7.42 (d, J = 8.5 Hz, 2H), 7.31 (d, J = 8.5 Hz, 2H), 6.86 (d, J = 8.95 Hz, 2H), 3.4 (m, 2H), 2.9 (m, 4H), 2.55 (m, 4H), 2.13 (m, 2H), 2.0 (m, 2H), and 1.6–1.8 ppm (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz) 198, 154.4, 146.7, 132.8, 130.2, 128.4, 126.3, 126.1, 113.3, 70.9, 58, 49.3, 48.6, 38.1, 35.5, 25.4, 24.4, and 21.8 ppm; LRMS (EI) m/z 440 (M^+), 422 ($M^+ - H_2O$), 188 ($C_{12}H_{14}NO$); HRMS (EI) calcd for $C_{26}H_{33}ClN_2O_2$ 440.2231, found 440.2211.

4-[4-(4-Chlorophenyl)-4-hydroxypiperidino]-4′-(cyclohexylamino)butyrophenone (3g). To a solution of haloperidol (1.16 g, 3 mmol) in dry DMF (30 mL) were added K_2CO_3 (1.7 g, 12 mmol) and cyclohexylamine (2 mL), and the reaction mixture was heated at reflux for 5 h. Workup and purification gave a white solid (1.255 g, 92%): mp 135–136 °C; IR (CH_2Cl_2, cm^{-1}) 3605 (OH), 1660 (C=O), 1604 (C=C), 1524 (C=C), 1486 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 7.75 (d, J = 8.61 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H), 7.24 (d, J = 8.41 Hz, 2H), 6.48 (d, J = 8.6 Hz, 2H), 4.25 (d, J = 7.7 Hz, 1H), 3.30 (m, 1H), 2.82–3.0 (m, 6H), 2.57 (m, 4H), 2.14 (m, 2H), 2.0 (m, 4H), 1.70 (m, 4H), 1.34 (t, J = 12.2 Hz, 2H), and 1.18 ppm (t, J = 11.15 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) 197.7, 151.3, 146.7, 132.5, 130.6, 130.2, 128.2, 126.1, 125.4, 111.4, 110.5, 70.4, 57.7, 51.1, 49.1, 37.5, 35.2, 33, 25.6, 24.7, and 21.3 ppm; LRMS (EI) m/z 454 (M^+), 436 ($M^+ - H_2O$), 244 ($C_{16}H_{22}NO$), 202 ($C_{13}H_{16}NO$); HRMS (EI) calcd for $C_{27}H_{35}ClN_2O_2$ 454.2387, found 454.2387.

4-[4-(4-Chlorophenyl)-4-hydroxypiperidino]-4′-[(*N*,*N*-dimethyl-*N*′-ethyl)amino]butyrophenone (3i). To a solution of haloperidol (1.16 g, 3 mmol) in dry DMF (20 mL) were added K_2CO_3 (1.7 g, 12 mmol) and *N*,*N*-dimethylethylenediamine (3 mL), and the reaction mixture was heated at reflux for 24 h. Workup and purification gave a yellow solid (430 mg, 30%): mp 54–56 °C; IR (CH_2Cl_2, cm^{-1}) 3603 (OH), 1668 (C=O), 1606 (C=C), 1537 (C=C), 1532 (C=C), 1430 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 7.82 (d, J = 8.68 Hz, 2H), 7.39 (d, J = 8.28 Hz, 2H), 7.25 (d, J = 8.28 Hz, 2H), 6.60 (d, J = 8.70 Hz, 2H), 3.43 (m, 4H), 2.83 (m, 4H), 2.46 (m, 6H), 2.24 (s, 6H), 1.8–2.1 (m, 4H), 1.66 (brd, J = 12.7 Hz, 2H), and 1.17 ppm (t, J = 6.85 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) 12.2, 21.9, 35.4, 38.1, 45.2, 45.7, 48.5, 49.2, 56.2, 57.9, 70.6, 110.1, 124.6, 126.1, 128.1, 130.5, 132.4, 147.1, 151, and 197.9 ppm; LRMS (CI) m/z 472 (MH^+), 456 ($M^+ - CH_3$), 438 ($M^+ - CH_3 - H_2O$), 426, 367, 289, 261 ($M^+ - C_{11}H_{10}ClNO$), 190 ($C_{12}H_{16}NO$), 176 ($C_{11}H_{14}NO$); HRMS (EI) calcd for $C_{27}H_{38}ClN_3O_2$ 471.2653, found 471.2649.

4-[4-(4-Chlorophenyl)-4-hydroxypiperidino]-4′-[[2-(dimethylamino)ethyl]amino]butyrophenone (3h). To a solution of haloperidol (1.16 g, 3 mmol) in dry DMF (30 mL) were added K_2CO_3 (1.7 g, 12 mmol) and *N*,*N*-dimethylethylenediamine (3 mL), and the reaction mixture was heated at reflux for 5 h. Workup and purification gave a white solid (1.25 g, 92%): mp 107–109 °C; IR (CH_2Cl_2, cm^{-1}) 3604 (OH), 1691 (C=O), 1606 (C=C), 1526 (C=C), 1425 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 7.80 (d, J = 8.57 Hz, 2H), 7.39 (d, J = 8.55 Hz, 2H), 7.25 (d, J = 8.51 Hz, 2H), 6.54 (d, J = 8.64 Hz, 2H), 4.93 (m, 1H), 3.18 (m, 2H), 2.8 (m, 4H), 2.4–2.55 (m, 6H), 2.21 (s, 3H), 2.18 (s, 3H), 1.9 (m, 2H), and 1.7 ppm (brd, J = 12.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) 21.9, 35.3, 38.1, 40.2, 45, 49.2, 57.5, 70.6, 111.3, 126.1, 128.2, 130.5, 132.5, 147, 152.2, 161.2, and 198 ppm; LRMS (CI) m/z 444 (MH^+), 371 ($M^+ - C_4H_{10}N$), 301, 284, 267 ($M^+ - C_{11}H_{16}NO$), 232 ($M^+ - C_{11}H_{16}ClNO$); HRMS (EI) calcd for $C_{25}H_{34}ClN_3O_2$, 443.2340; found, 443.2351.

4-[4-(4-Chlorophenyl)-4-hydroxypiperidino]-4′-[[(3-(dimethylamino)propyl]amino]butyrophenone (3j). To a solution of haloperidol (1.16 g, 3 mmol) in dry DMF (30 mL) were added K_2CO_3 (1.7 g, 12 mmol) and 3-(dimethylamino)-propylamine (3 mL), and the reaction mixture was heated at reflux for 3 h. Workup and purification gave a white solid (1.12 g, 80%): mp 137–139 °C; IR (CH_2Cl_2, cm^{-1}) 3593 (OH), 1668 (C=O), 1600 (C=C), 1532 (C=C), 1430 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 7.81 (d, J = 8.31 Hz, 2H), 7.40 (d, J = 8.53 Hz, 2H), 7.27 (d, J = 8.39 Hz, 2H), 6.52 (d, J = 8.13 Hz, 2H), 5.30 (s, 1H), 3.24 (m, 2H), 3.9 (t, J = 7 Hz, 2H), 2.8 (brd, J = 10.6 Hz, 2H), 2.35–2.5 (m, 6H), 2.22 (s, 6H), 1.9–2.1 (m, 4H), and 1.6–1.8 ppm (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) 22.2, 26.3, 35.5, 38.4, 42.7, 45.5, 49.3, 58.1, 58.2, 71, 111.2, 126, 126.1, 128.3, 130.5, 147, 152.5, and 198.1 ppm; LRMS (CI) m/z 458 (MH^+), 442 ($M^+ - CH_3$), 258 ($MH^+ - C_{12}H_{17}N_2O + H^+$), 247, 212; HRMS (EI) calcd for $C_{26}H_{36}ClN_3O_2$ 457.2496, found 457.2477.

4-[4-[3-(Dibutylamino)phenyl]-4-hydroxypiperidino]-4′-(cyclohexylamino)butyrophenone (4). To a solution of 4-[4-[3-(dibutylamino)phenyl]-4-hydroxypiperidino]-4′-fluorobutyrophenone (35 mg, 0.075 mmol) in dry DMF (10 mL) were added K_2CO_3 (123 g, 0.9 mmol) and cyclohexylamine (1 mL), and the reaction mixture was heated at reflux for 20 h. Workup and purification gave a yellow solid (34 mg, 84%): mp 150–151 °C; IR (CH_2Cl_2, cm^{-1}) 3589 (OH), 1668 (C=O), 1623 (C=C), 1521 (C=C), 1424 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 7.8 (d, J = 8.7 Hz, 1H), 7.17 (t, J = 8 Hz, 1H), 6.81 (s, 1H), 6.70 (d, J = 7.7 Hz, 1H), 6.54 (m, 3H), 4.17 (d, J = 7.7 Hz, 1H), 3.0–3.3 (m, 7H), 2.7–3.0 (m, 4H), 2.5 (t, 2H), 2.0–2.2 (m, 4H), 1.7–1.9 (m, 6H), 1.5 (q, J = 7.9 Hz, 4H), 1.1–1.45 (m, 10H), and 0.92 ppm (t, J = 7.27 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14, 20.3, 24.8, 25.7, 29.4, 33, 34.9, 36.6, 49.2, 50.7, 51.2, 57.4, 70.4, 107.6, 110.8, 111.1, 111.6, 126.5, 129.3, 130.6, 147.8, 148.3, 151.4, and 198 ppm; LRMS (EI) m/z 547 (M^+), 529 ($M^+ - H_2O$), 287, 273, 244 ($C_{16}H_{22}NO$), 217 ($C_{14}H_{19}NO$), 120 (C_7H_6NO); HRMS (EI) calcd for $C_{35}H_{53}N_3O_2$ 547.4157, found 547.4137.

1-(*n*-Butoxyphenyl)-4-[4-(4-chlorophenyl)-4-hydroxypiperidino]-*n*-butyl Alcohol (5). $NaNH_2$ was slowly added to *n*-butanol (50 mL) (fizzing, turned yellow). Haloperidol (1.6 g, 3 mmol) was then added, and the resulting mixture was heated at reflux for 1 h. Workup and purification gave a white solid (0.95 g, 75%): mp 128–129 °C; IR (CH_2Cl_2, cm^{-1}) 3576 (OH), 1617 (C=C), 1515 (C=C), 1430 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) 7.41 (d, J = 8.5 Hz, 2H), 7.25 (m, 4H), 6.82 (d, J = 8.45 Hz, 2H), 4.58 (m, 1H), 3.93 (t, J = 6.5 Hz, 2H), 2.98 (brd, J = 10.45 Hz, 1H), 2.81 (brd, J = 9.88 Hz, 1H), 2.4–2.6 (m, 4H), 2.1–2.3 (m, 2H), 1.65–1.95 (m, 8H), 1.46 (q, J = 7.4 Hz, 2H), and 0.96 ppm (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) 13.8, 19.2, 23.7, 31.3, 37.5, 39.5, 48.5, 49.8, 58.6, 67.7, 70.6, 73.2, 114.2, 126.2, 126.7, 128.3, 132.8, 137.5, 146.3, and 158 ppm; LRMS (EI) m/z 431 (M^+), 379 ($M^+ - OH - Cl$), 226, 193 ($C_{11}H_{12}ClN$), 165 ($C_{10}H_{13}O_2$); HRMS (EI) calcd for $C_{25}H_{34}ClNO_4$ 431.2227, found 431.2219.

***In Vitro* Assay of HIV-1PR Inhibition.** HIV-1PR was assayed against the decapeptide Ala-Thr-Leu-Asn-Phe-Pro-Ile-Ser-Pro-Trp as previously described.[11] Conversion of the decapeptide to the two pentapeptides was quantified by HPLC[7] and compared with product standard curves. IC_{50} determinations were carried out at pH 5.5. Stock solutions of the inhibitors (2–10 mM) were prepared in DMSO. Compounds were added to assay solutions and the final DMSO concentration adjusted to 5% (vol/vol). Base line values were determined from enzymatic reactions containing 5% DMSO in the absence of inhibitor. HIV-1PR (70 nM) was preincubated with the respective inhibitor for 1 min at 25 °C before initiating the reaction by addition of the decapeptide substrate (250 μM final concentration). The extent of inhibition is independent of the incubation time, indicating reversible binding.

Cell Toxicity Assay. A stable cell line (COSA6) has been established that produces all of the HIV-1 HXB2 proteins with the exception of envelope gp 160 (5F). Cytotoxicity of the inhibitors on this cell line was measured using the MTT cell viability assay.[12] The tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is cleaved by dehydrogenases in active mitochondria of living cells to yield a change in color from yellow to purple. To obtain the LD_{50} values (concentration that kills 50% of the cells), the concentration at which the absorbance at 570 nm was half of that

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for the untreated cells was determined. Solutions of inhibitor were prepared in DME H-21 containing penicillin (100 U/mL), streptomycin sulfate (100 μg/mL), xanthine (0.25 mg/mL), hypoxanthine (14 μg/mL), mycophenolic acid (25 μg/mL), and 10% fetal bovine serum. Compounds were tested in duplicate, in serial dilutions generally ranging from 5 to 500 μM concentrations in the presence of 1% DMSO. Cells were incubated with the inhibitor solution for 4 h at 37 °C.

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Supplementary Material Available: ^{1}H and ^{13}C NMR spectra of **1a–d**, **2a**, **d**, **3e–j**, **4**, and **5** (28 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.