Articles

Design, Synthesis, X-ray Analysis, and Dopamine Receptor-Modulating Activity of Mimics of the "C5" Hydrogen-Bonded Conformation in the Peptidomimetic 2-Oxo-3(R)-[(2(S)-pyrrolidinylcarbonyl)amino]-1-pyrrolidineacetamide

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3(R)-(7a(S)-Hexahydro-1-oxo-3,3-dimethyl-1*H*-pyrrolo[1,2-*c*]imidazol-2-yl)-2-oxo-1-pyrrolidineacetamide (2) and 3(R)-[1-(2,5-dioxopyrrolidino[3,4-*c*]piperazino)]-2-oxo-1-pyrrolidineacetamide (3) were designed and prepared as mimics of the "C5" hydrogen-bonded structure found in the crystal structure of 2-oxo-3(R)-[(2(S)-pyrrolidinylcarbonyl)amino]-1-pyrrolidineacetamide (1). Both compounds effectively restrict the ψ_1 torsional angle to very near the value found in the X-ray structure of 1 as seen in the X-ray crystallographic determination of 2 and methyl 3(R)-[1-(2,5-dioxopyrrolidino[3,4-*c*]piperazino)]-2-oxo-1-pyrrolidineacetate (11), a diketopiperazine intermediate in the synthesis of 3. These analogs were tested for their ability to enhance the binding of the dopamine D₂ receptor agonist *N*-propylnorapomorphine (NPA) in the absence and presence of 5'-guanylylimidodiphosphate (Gpp(NH)p). Both compounds enhanced [³H]-NPA binding in a dose-dependent manner by increasing both the binding affinity of the agonist and the number of high-affinity sites available for binding. Both 2 and 3 also attenuated the Gpp(NH)p-induced conversion of D₂ receptor high-affinity states to the low-affinity states.

Introduction

The γ -lactam compound 2-oxo-3(R)-[(2(S)-pyrrolidinylcarbonyl)amino]-1-pyrrolidineacetamide (1, Figure 1) was prepared previously in our laboratory as a conformationally constrained analog of the dopamine receptor-modulating peptide Pro-Leu-Gly-NH₂ (PLG).¹ This analog was found to be 1000 times more potent than PLG in enhancing the binding of the high-affinity agonist N-propylnorapomorphine (NPA) to dopamine D_2 receptors.² Although 1 was prepared as a mimic of the postulated type II β -turn bioactive conformation of PLG,¹ the X-ray crystal structure of 1 showed it to be semiextended at the C-terminus with the N-terminal prolyl residue existing in a "C5" conformation that is stabilized by a (peptide)NH-N(amino) intramolecular hydrogen bond.³ In an effort to determine whether the "C5" conformation is playing a role in the high biological activity of 1, two analogs of 1, imidazolidinone 2 and diketopiperazine 3, have been synthesized as potential mimics of this conformation. X-ray crystallography of 2 and 11, a late intermediate in the synthesis of 3, has been used to analyze these mimetics.

Syntheses

Compound 2 was prepared by the slow crystallization of 1^1 from a 50:50 mixture of methanol and acetone. As a crystalline solid, compound 2 is stable indefinitely. However, the hydrolytic stability of the imidazolidinone



Figure 1. Synthesized mimetics (2 and 3) of the "C5" hydrogen-bonded conformation of 1 as found in the X-ray crystal structure.

ring as found in compound 2 can vary depending upon the structure of the compound in which it is incorporated.^{4,5} Whereas some imidazolidinones have been prepared in aqueous buffer solutions, others are rapidly hydrolyzed under similar conditions. Thus, the potential usefulness of 2 as a stable "C5" mimic for biological assay was assessed. An HPLC assay was developed which measured the amount of 2 present in the buffer solution. Time course analysis of the concentration of 2 in the buffer solution revealed that decomposition of 2 to 1 occurred predominantly within the first hour and that only about 33% of 2 was intact after 12 h.

Compound **3** was prepared from *N*-(benzyloxycarbonyl)-L-proline *tert*-butyl ester⁶ and **6**.¹ The benzyloxycarbonyl group was removed from Cbz-Pro-O-*t*-Bu by hydrogenation, and the free amine obtained was coupled with benzyloxyacetyl chloride and Et₃N in CH₂Cl₂ to

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CH₂CO₂Me

CH2CO2Me

4 N HCI

dioxane

7 ö





give 4 as seen in Scheme 1. Removal of the *tert*-butyl ester to give 5 was done quantitatively by using TFA in CH₂Cl₂. Compound 6 was deprotected with HCl/ dioxane to give 7 which was coupled with 5 in 77% yield by using the water-soluble 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride in the presence of 1-hydroxybenzotriazole and Et₃N to give 8. Hydrogenolysis of the benzyl ether to alcohol 9 required 18 h to complete. Closure to give the diketopiperazine ring in 11 was carried out under Mitsonobu conditions,⁷ but went in poor yield. The major product formed during this reaction was 10, as a result of the nucleophilic addition of the diethyl azodicarboxylate anion to the hydroxyl carbon with the loss of the hydroxyl group as triphenylphosphine oxide. The composition of 10 was supported by MS, ¹H, and ¹³C NMR. The desired product could not be separated from 10 using normal low- or medium-pressure chromatography on silica gel. Separation of 11 from 10, however, was achieved using HPLC on a silica gel column with $CH_2Cl_2/MeOH$ (10:1) as the eluant. Compound 11 was converted to 3 with NH₃/MeOH. This product was isolated as a single peak via HPLC on silica gel.

X-ray Crystallography

The molecular structures of **2** and **11** with the atomic numbering schemes are shown in Figures 2 and 3, respectively.8 Compound 2 crystallizes with two molecules, 2A and 2B, in the asymmetric unit. Crystallographic data for 2 and 11 are summarized in Table 1.



Figure 2. ORTEP drawing of 2A and 2B with crystallographic numbering system. The non-hydrogen atoms are shown at the 50% probability level. The water molecule was omitted for clarity.

Figure 3. ORTEP drawing of 11 with crystallographic numbering system. The non-hydrogen atoms are shown at the 50% probability level.

Compounds 2 and 11 both mimic the proline conformation present in the parent γ -lactam crystal structure with respect to the torsion angle ψ_1 (Figure 1). Due to the "C5" hydrogen bond, this torsion angle is $-0.8(2)^{\circ}$ in 1. The values for this torsion angle in **2A**, **2B**, and **11** are 18.1(2)°, 20.2(2)°, and 31.2(2)°, respectively. Stereoviews of separate overlays of the N-terminal portions of **2A** and **11** with **1** are shown in Figure 4A,B, respectively.

Molecule 11 exists in an extended conformation analogous to that seen in the X-ray structure of 1. Both **2A** and **2B**, on the other hand, exist in a type II β -turn configuration. The ϕ,ψ angles ($\phi_2 = -42.2(3)^\circ, \psi_2 =$ 133.3(2)°, $\phi_3 = 89.1(3)^\circ$, $\psi_3 = -6.9(3)^\circ$) for **2B** deviate slightly from the ideal values ($\phi_2 = -60^\circ$, $\psi_2 = 120^\circ$, ϕ_3 = 80°, $\psi_3 = 0^\circ$) for a type II β -turn but are still within the defined values.⁹ The resultant $4 \rightarrow 1$ hydrogen bond has a N(1B)-O(3B) distance of 2.949(3) Å. Likewise,

Table 1. Crystal Data for Compounds 2 Hemihydrate and 11

parameter	2 hemihydrate	11		
empirical formula	C14H23N4O3.5	C14H19N3O5		
formula weight (amu)	303.36	309.32		
crystal habit	stout needle	triangular prism		
crystal	0.35 imes 0.10 imes	$0.20 \times 0.20 \times$		
dimensions (mm)	0.05	0.20×0.10		
crystal system	monoclinic	orthorhombic		
a (Å)	10.569 (2)	8.422(2)		
<i>b</i> (Å)	12.365 (2)	8.980 (2)		
c (Å)	12.578 (2)	19.265 (3)		
β (°)	113.80(2)			
$V(Å^3)$	1504 (1)	1457 (1)		
Z	4	4		
space group	P21 (No. 4)	P212121 (No. 19)		
calcd density (g/cm)	1.340	1.410		
F_{000}	652	656		
μ (Cu K α) (cm ⁻¹)	7.67	8.66		
no. of reflections				
total	4912	2516		
unique	4453	2202		
no. of refl $(I > 3.00\sigma(I))$	4124	1941		
$R_{\rm int}$	0.029	0.018		
absorption corrections	1.00, 0.93	1.00, 0.95		
(max., min.)				
extinction coefficient		$0.76092 imes 10^{-5}$		
no. of variables	405	200		
R	0.035	0.035		
$R_{ m w}$	0.042	0.042		
S	1.59	1.50		
max. shift/error	0.01	0.01		
largest excess in final	-0.23	0.24		
diff map (e ⁻ /Å ³)				

Α

Figure 4. Stereoviews of an overlay of **2A** with **1** (A) and the analogous view for **11** with **1** (B). The molecules were joined by fitting the atoms N3A, C7A, C8A, and N4A for **2A** and the analogous atoms for compounds **1** and **11**. The rms deviations for the respective overlays were 0.152 and 0.122. The γ -lactam portions of the molecules were excluded for clarity.

the ϕ, ψ angles ($\phi_2 = -43.6(3)^\circ$, $\psi_2 = 121.3(2)^\circ$, $\phi_3 = 80.5-(3)^\circ$, $\psi_3 = -0.6(3)^\circ$) for **2A** are very near ideal except for ϕ_2 . The $4 \rightarrow 1$ hydrogen bond is shorter in **2A** with a N(1A)-O(3A) distance of 2.836(3) Å.

Pharmacology

Table 2 describes the modulation of [³H]spiroperidol/ [³H]NPA competitive binding to D_2 receptors in the presence or absence of 100 μ M 5'-guanylylimidodiphosphate (Gpp(NH)p), a nonhydrolyzable analog of GTP. This competition experiment is illustrated for 3 in Figure 5. A similar competition curve was obtained for 2, but it is not depicted. Computer analysis of the competition data clearly shows that [3H]NPA discriminates between two affinity states of the receptor labeled by [3H]spiroperidol.10 The receptor high-affinity and low-affinity state ratio $(R_{\rm H}/R_{\rm L})$ for the untreated control membranes was 0.96 and 0.83, respectively, for experiments with 2 and 3, thus indicating that the populations of the two states of the receptor are approximately the same. However, the K_i values for [³H]NPA binding significantly differ for the high-affinity and low-affinity states. The K_i for the high-affinity state in the control experiments for 2 and 3 was 0.07 and 0.08 nM, respectively, whereas the K_i for the low-affinity state was 74 and 69 nM. Membranes treated with either 2 or 3 showed a 2–4-fold decrease in the K_i (0.03 and 0.02 nM, respectively) for the high-affinity state and a statistically insignificant decrease in the K_i for the low-affinity state of the receptor (61 and 56 nM, respectively). Furthermore, the ratio of high- to low-affinity sites $(R_{\rm H}/$ $R_{\rm L}$) in the 2- and 3-treated membranes increased significantly from 0.96 to 1.91 and from 0.83 to 1.97, respectively.

Inclusion of Gpp(NH)p in the assay causes a significant decrease in the number of high-affinity states of the receptor as observed in the control experiments for 2 and 3 where the ratio $R_{\rm H}/R_{\rm L}$ becomes 0.23 and 0.28, respectively. An increase in the K_i value of about 2-fold for the high-affinity state was also observed in the presence of Gpp(NH)p. When the membranes are treated with either 2 or 3, the conversion of high- to low-affinity states induced by Gpp(NH)p was less than that observed in the absence of these compounds. $R_{\rm H}$ / $R_{\rm L}$ ratios of 0.56 and 0.71 were obtained for 2 and 3, respectively, as compared to $R_{\rm H}/R_{\rm L}$ ratios of 0.23 and 0.28 in the control experiments. In these experiments, the high-affinity receptor state still experiences an approximate 2-4-fold decrease in the K_i for NPA. The K_i for the low-affinity state with Gpp(NH)p present and either 2 or 3 increases with borderline statistical significance.

The concentration-dependent effect of Gpp(NH)p induced reduction in high-affinity [3H]NPA binding to dopamine D₂ receptors was studied in the presence of increasing concentrations of either 2 or 3. The results for 3 are displayed in Figure 6 and show that in the control membranes a gradual decrease in [3H]NPA binding was observed up to 100 mM Gpp(NH)p. At this concentration of Gpp(NH)p, only 25% of the maximum high-affinity [³H]NPA binding was observed. However, in the membranes preincubated with 1, 10, and 100 nM 3, the inhibition curve significantly shifted to the right at all three concentrations, showing the attenuation of the affect of Gpp(NH)p. The concentration required to cause 50% inhibition of [³H]NPA binding significantly increased from control $(0.7 \,\mu\text{M})$ to that measured in the presence of 1 nM 3 (1.5 μ M). Analogous results were obtained for 2 (data not shown).

Similarly, the ability of 2 and 3 to reverse the Gpp-(NH)p-induced inhibition of high-affinity [³H]NPA binding was studied. The membranes were preincubated with 100 μ M Gpp(NH)p and then assayed for an increase in [³H]NPA binding with different concentrations of 2 or 3. Details shown in Figure 7 for 3 show

Table 2. Modulation of [3H]Spiroperidol/[3H]NPA Competition by 2 and 3^a

	binding parameters				
membrane	$K_{\rm H}({\rm nM})$	$K_{\rm L} ({\rm nM})$	$R_{ m H}$ (%)	$R_{\rm L}$ (%)	$R_{ m H}/R_{ m L}$
control					
-Gpp(NH)p	0.07 ± 0.008	74 ± 2	49 ± 2	51 ± 3	0.96 ± 0.06
+Gpp(NH)p	0.12 ± 0.010	50 ± 3	19 ± 2.5	81 ± 3.5	0.23 ± 0.07
pretreatment with 2					
-Gpp(NH)p	0.03 ± 0.005^{b}	61 ± 3	$65 \pm 3^{\circ}$	34 ± 2	1.91 ± 0.015^{b}
+Gpp(NH)p	0.07 ± 0.006^{b}	77 ± 4	$36\pm2.5^{\circ}$	64 ± 1.8	0.56 ± 0.013^{b}
control					
-Gpp(NH)p	0.08 ± 0.005	69 ± 3.5	46 ± 1	55 ± 1.8	0.83 ± 0.001
$+\dot{\mathbf{G}}\mathbf{pp}(\mathbf{NH})\mathbf{p}$	0.19 ± 0.020	50 ± 3	22 ± 0.8	78 ± 1.2	0.28 ± 0.002
pretreatment with 3					
-Gpp(NH)p	0.02 ± 0.003^{b}	56 ± 2.5	$65 \pm 4^{\circ}$	33 ± 3.8	1.97 ± 0.007^{b}
+Ĝpp(NH)p	0.05 ± 0.006^b	64 ± 3.5	$42 \pm 3^{\circ}$	59 ± 1.8	0.71 ± 0.003^b

^a Competition data as shown in Figure 5 were computer analyzed as described in the methods section. $K_{\rm H}$ and $K_{\rm L}$ represent the inhibitor constant ($K_{\rm i}$) of agonist calculated for high- and low-affinity components of [³H]spiroperidol binding, respectively. Percent $R_{\rm H}$ and percent $R_{\rm L}$, respectively, are percentage of sites in high- or low-affinity form for the agonist. Values are mean \pm SEM of four to five separate experiments carried out in triplicate. ^b p < 0.001 different from the respective control group. ^c p < 0.005 different from the respective control group.

Figure 5. Competition curves of [${}^{3}H$]spiroperidol/[${}^{3}H$]NPA in control as well as in membranes pretreated with **3** and the affect of Gpp(NH)p. \blacksquare , control; \blacktriangle , control and **3**; \checkmark , control and Gpp(NH)p plus **3**; \blacklozenge , control and Gpp(NH)p. The concentration of [${}^{3}H$]spiroperidol in the assay was 0.5 nM. Gpp(NH)p concentration was 100 μ M, and **3** was present at 1 nM. Computer analysis (cDATA analysis) of the curves indicated two sites, high- and low-affinity sites of the agonist binding with binding parameters as described in Table 2. Each value is an average of five separate experiments. The SEM varied by less than 10%.

there was no increase in $[{}^{3}H]NPA$ binding in Gpp(NH)ppretreated membranes at any concentration. However, when the membranes are simultaneously treated with Gpp(NH)p and 1, 10, or 100 nM **3**, a significant increase in the $[{}^{3}H]NPA$ binding was observed. This ability to antagonize Gpp(NH)p-induced reductions in $[{}^{3}H]NPA$ binding was also exhibited by **2** (data not shown).

Discussion

The ability of 1 to modulate high-affinity dopamine D_2 receptors in the CNS was reported previously.¹⁰ Although designed to encourage type II β -turn formation, 1 had an extended conformation in the X-ray structure with an unusual "C5" hydrogen-bonded conformation at the prolyl N-terminus.³ Since this interaction does not preclude turn formation in solution, we designed and analyzed 2 and 3 as ψ_1 torsional angle constraints to mimic this "C5" interaction.

X-ray analyses of **2** and **11** show that both of the designed ψ_1 constraints are good mimetics of the ψ_1

Figure 6. Effect of Gpp(NH)p on high-affinity [³H]NPA binding in control and 3-treated striatal membranes. \blacksquare , control membranes; \blacktriangle , membranes treated with 1-nM 3; \checkmark , membranes treated with 10 nM 3; \blacklozenge , membranes treated with 100 nM 3. The details of treatment with 3 and the assay conditions are described in the Experimental Section. The results are expressed as percentage of maximum specific binding obtained from control membranes in the absence of Gpp(NH)p. Each value is an average of four separate experiments. The SEM varied by less than 10%.

torsion angle observed for the prolyl residue in the structure of 1. Compound 3 failed to crystallize; however, the conformation of the diketopiperazine ring in 3 is not expected to deviate significantly from that in 11. These ψ_1 constraints do not interfere with β -turn formation, as indicated by the observed solid-state conformation of 2. Of the two molecules in the asymmetric unit, 2A forms a more ideal β -turn structure (ideal $\psi_2 = 120^{\circ})^9$ than 2B as evident in the values for their ψ_2 torsion angle ($\psi_2 = 121.3(2)^{\circ}$ and $133.3(2)^{\circ}$, respectively).

As seen in Table 2, the pharmacological effects of 2and 3 on D_2 dopamine receptors are of the same magnitude; furthermore, these effects are analogous to those seen previously with $1.^2$ However, the instability of 2 in the buffer solution used for the pharmacological testing precludes distinguishing whether the observed activity for this compound is due to the intact compound or to the release of 1 from 2. The synthesis of 3, which

Figure 7. Effect of 3 on [³H]NPA binding to striatal membranes pretreated with 100 μ M Gpp(NH)p for 2 h at 4 °C (open bars). The membranes were washed and assayed for [³H]NPA binding in the presence of indicated concentrations of 3. Striped bars depict membranes that were incubated simultaneously with 100 μ M Gpp(NH)_p and the indicated concentrations of 3 for 2 h at 4 °C before being assayed for [³H]NPA binding. The results are expressed as percentage of maximum specific binding obtained from membranes preincubated with assay buffer without Gpp(NH)p. Each value is an average (\pm SEM) of five separate experiments. p < 0.05.

has hydrolytic stability under physiological conditions, allowed for accurate biological analysis of the ψ_1 constraint.

Pharmacological analysis of **3** shows an increase in the ratio of high-affinity to low-affinity states of the dopamine D_2 receptor with a concurrent decrease in the K_i for the high-affinity state. This pattern is also true in the presence of Gpp(NH)p; however, the percentage of the high-affinity state is lower and the K_i is higher than in the experiments without Gpp(NH)p. Although **3** is unable to reverse the affect of Gpp(NH)p pretreatment on membranes, it protects the percentage of highaffinity states of the receptor if treatment is concurrent with Gpp(NH)p.

Molecule **3** restricts the rotation of the prolyl ring approximate to the plane of the β -turn backbone and suggests that the bioactive conformation of proline is very similar to that mimicked by this constraint. These results along with the previously reported dopamine receptor-modulating activity of a highly rigid spiro bicyclic peptidomimetic¹¹ suggest that the biologically active conformation of PLG is a type II β -turn with the proline residue in or near the plane of the backbone of this turn. The other possible important requirement for activity is the presence of lipophilic groups extending perpendicular to the β -turn. Efforts to test this topographical hypothesis are underway.

Experimental Section

General Aspects. All apparatus were oven-dried and cooled in a dessicator. THF and CH_2Cl_2 were distilled from Na/benzophenone and CaH_2 , respectively. Thin-layer chromatography was performed on Analtech 250 μ m silica gel HLF Uniplates that were visualized by UV, I₂, ninhydrin spray (amines), and 2,6-dichlorophenol indophenol spray (acids). Chromatographic purification on silica gel (Merck; grade 60, 240-400 mesh, 60 Å) was done by flash or gravity methods, and preparative HPLC was done with a Waters Associates 25 × 100 mm PrepPak cartridge. Optical rotations were measured on a Rudolph Research Autopol III polarimeter at the 589 nm Na D-line. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. ¹H and ¹³C NMR spectra were measured in

 $CDCl_3$ at 300 and 75.5 MHz, respectively, with $CDCl_3$ as the internal reference for ¹H (δ 7.26) and ¹³C (δ 77.06). J values are in hertz. In compounds where rotamers are present about the amide bond, ¹H and ¹³C resonances for both rotamers are listed.

3(R)-(7a(S)-Hexahydro-1-oxo-3,3-dimethyl-1H-pyrrolo-[1,2-c]imidazol-2-yl)-2-oxo-1-pyrrolidineacetamide (2). 2-Oxo-3(R)-[(2(S)-pyrrolidinylcarbonyl)amino]-1-pyrrolidineacetamide (1)¹ (0.20 g, 3.68 mmol) was dissolved in 1 mL of MeOH. One milliliter of HPLC grade acetone was added, and the solution was allowed to evaporate to dryness, producing large, blocky crystals of 2 quantitatively: mp 188-191 ° C; $[\alpha]_{\rm D} = +49.0^{\circ} (c \ 0.71, \text{MeOH}); \text{TLC } R_f (\text{CH}_2\text{Cl}_2/\text{MeOH}, 10:1) =$ 0.44; ¹H NMR (CDCl₃) δ 7.70 (s, 1 H, NH), 5.49 (s, 1 H, NH), 4.60 (d, J = 17.1 Hz, 1 H, CH₂CON), 3.89–3.92 (m, 1 H, α -CH), 3.75-3.78 (m, 1 H, 3-CH), 3.37 (d, J = 15.6 Hz, 1 H, CH₂-CON), 3.65-3.73 (m, 1 H, 5-CH), 3.37-3.44 (m, 1 H, 5-CH), $2.94-2.99 \text{ (m, 1 H, } \delta\text{-CH)}, 2.39-2.51 \text{ (m, 2 H, 4-CH and } \delta\text{-CH)},$ 2.09-2.26 (m, 2 H, 4-CH and β-CH), 1.65-1.96 (m, 3 H, β-CH and y-CH₂), 1.51 and 1.45 (2s, 6 H, (C(CH₃)₂); ¹³C NMR (CDCl₃) δ 176.7, 171.1, 170.9 (CO), 80.1 (C(CH_3)_2), 63.4 (Pro α -C), 52.5 (NCH₂CON), 49.3 (3-C), 47.5 (Pro δ-C), 46.0 (5-C), 28.5 (Pro β-C), 26.0 (4-C), 25.9 (Pro γ-C), 25.0, 23.1 (C(CH₃)₂); FAB MS m/z 295 [M + H]⁺. Anal. (C₁₄H₂₂N₄O₃·0.5 H₂O) C, H, N.

N-[(Benzyloxy)acetyl]-L-proline tert-Butyl Ester (4). Z-Pro-O-t-Bu⁶ (3.25 g, 10.6 mmol) was dissolved in 20 mL of distilled MeOH, and 300 mg of 10% Pd/C was added under a blanket of Ar. The mixture was hydrogenated at 40 psi for 1 h. The solution was filtered through filter paper followed by filtration through a 0.45 μ m PTFE filter disk. Concentration was done under vacuum below 25 °C to give the free amine as a clear oil. Residual solvents were azeotroped from dry CH2- Cl_2 twice, and the resulting oil was kept under vacuum for 1 h prior to its use. The resulting oil was dissolved in CH₂Cl₂ (20 mL) and cooled to -20 °C. Triethylamine (1.56 mL, 11.2 mmol) was added slowly with stirring. Benzyloxyacetyl chloride (1.76 mL, 11.2 mmol) in 10 mL CH_2Cl_2 was added over 5 min. This was stirred for 1 h at -20 °C and then at room temperature for an additional 1 h. Fifty milliliters of CH₂Cl₂ was added, and this was partitioned with 20 mL of 10% citric acid. The organic fraction was washed with 20 mL each of 1 M NaHCO₃, H_2O , and saturated NaCl. The extract was dried (MgSO₄) and concentrated to give a white foam. Crystallization from Et₂O/petroleum ether (60-70 °C) gave 2.90 g (85% yield) of a white solid: mp 81–83 °C; $[\alpha]_D = -71.4^\circ$ (c 1.0, MeOH); TLC R_f (CH₂Cl₂/MeOH, 20:1) = 0.78; both ¹H and ¹³C NMR spectra indicated the presence of rotamers about the amide bond; ¹H NMR (CDCl₃) δ 7.28-7.38 (m, 5 H, Ph H's), 4.56-4.67 (2d, 2 H, PhCH₂), 4.41 (m, 1 H, α -CH), 3.95-4.14 (m, 2 H, OCH₂CO), 3.47-3.65 (m, 2 H, δ-CH), 1.82-2.19 (m, 4 H, β - and γ -CH₂), 1.39 and 1.46 (2s, 9 H, (C(CH₃)₃); ¹³C NMR (CDCl₃) & 172.0 (NCO), 169.0 (COO), 137.8, 129.1, 129.1, 128.8, 128.8, 128.6, 128.5 (Ph C), 82.6, 82.0 (C(CH₃)₃), 73.9, $73.6\,(PhCH_2),\,70.4,\,70.0\,(OCH_2CO),\,60.4,\,60.1\,(\alpha\text{-}C),\,47.5,\,46.9$ (δ-C), 32.2, 29.4 (β-C), 28.6, 28.5 (C(CH₃)₃), 25.5, 22.5 (γ-C); FAB MS m/z 320 [M + H]⁺. Anal. (C₁₈H₂₅NO₄) C, H, N.

N-[(Benzyloxy)acetyl]-L-proline (5). Compound 4 (2.5 g, 7.83 mmol) was dissolved in CH_2Cl_2 (15 mL), and this was stirred under Ar. Trifluoroacetic acid (10 mL, 130 mmol) was added slowly. After 2 h, the solution was concentrated to yield a clear oil. Azeotropic removal of the excess TFA was done using $H_2O/MeOH(2\times)$ followed by an anhydrous toluene and two dry CH_2Cl_2 treatments. Storage in vacuo gave a colorless oil in quantitative yield (2.06 g, 100%): $[\alpha]_D = -33.6^\circ$ (c 1.0, MeOH); TLC R_f (CH₂Cl₂/MeOH, 20:1) = 0.30; both ¹H and ¹³C NMR spectra indicated the presence of rotamers about the amide bond; ¹H NMR (CDCl₃) & 9.58, 9.45 (2s, 1 H, COOH), $7.30{-}7.34~(m,~5$ H, Ph H's), $4.55{-}4.60~(m,~1$ H, $\alpha{-}CH),~4.52,$ 4.60 (2s, 2 H, PhCH₂), 4.19, 4.22 (2s, 2 H, OCH₂CO), 3.47-3.61 (m, 2 H, δ -CH₂), 2.00–2.26 (m, 4 H, β - and γ -CH₂); ¹³C NMR (CDCl₃) & 176.6 (COOH), 171.5 (NCO), 136.9, 136.7, 129.3, 129.2, 129.0, 129.0, 128.9, 128.9 (Ph C), 74.3, 74.2 $(PhCH_2)$, 69.9, 68.6 (OCH_2CO) , 60.1, 60.0 $(\alpha$ -C), 48.4, 47.4 $(\delta$ -C), 31.9, 29.1 (β -C), 25.3, 22.2 (γ -C); FAB MS m/z 264 [M + $H]^+$. Anal. (C₁₄ $H_{17}NO_4$) C, H, N.

Methyl 3(R)-[[[1-[(Benzyloxy)acetyl]-2(S)-pyrrolidin-

yl]carbonyl]amino]-2-oxo-1-pyrrolidineacetate (8). Methyl 3(R)-[N-(tert-butoxycarbonyl)amino]-2-oxo-1-pyrrolidineacetate⁴ (6; 2.13 g, 7.83 mmol) was deprotected by adding 40 mmol of a 4 N HCl in dioxane solution to the solid under a stream of Ar. The solid dissolved quickly, and as the deprotection occurred, a white solid, 7, precipitated from solution. After 1h, the solution was concentrated followed by azeotropic removal of residual dioxane and hydrochloric acid with freshly distilled CH_2Cl_2 (2×). Compound 7 was obtained as a white foam, and this was stored under vacuum until needed. N-[(Benzyloxy)acetyl]-L-proline (5; 2.06 g, 7.83 mmol) was dissolved in 40 mL of dry CH₂Cl₂. To this stirred solution under Ar was added hydroxybenzotriazole (1.06 g, 7.83 mmol). Compound 7 in CH_2Cl_2 was added dropwise. The suspension was cooled to -20 °C before adding Et₃N (1.09 mL, 7.83 mmol). This provided a clear solution into which 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1.50 g, 7.83 mmol) was added all at once. The dry ice bath was removed, and the solution was stirred under Ar for 2 days. An additional 40 mL of CH₂Cl₂ was added, and this was partitioned with 25 mL of a 10% citric acid solution. The precipitated HOBt was removed by filtration. The organic layer was separated and washed with 25 mL each of 1 M NaHCO₃, H₂O, and saturated NaCl. Concentration of the organic layer under vacuum gave a pinkish oil which was gravity chromatographed on silica gel (Merck; 200-400 mesh, 60 Å) using $CH_2Cl_2/MeOH$ (20:1) as the eluant. The desired fractions were combined and concentrated to give 2.5 g (77%) of a white foam: $[\alpha]_D = -61.0^{\circ} (c$ 1.95, MeOH); TLC R_f (CH₂Cl₂/MeOH, 20:1) = 0.18; ¹H NMR $(\text{CDCl}_3) \delta$ 7.45 (d, J = 3 Hz, 1 H, CONH), 7.26–7.29 (m, 5 H, Ph H's), 4.58 (s, 2 H, PhCH₂), 4.34-4.51 (m, 2 H, Pro α-CH and 3-CH), 3.81-4.18 (m, 2 H, CH₂CO₂Me), 4.09 (s, 2 H, OCH₂-CO), 3.65 (s, 3 H, OCH₃), 3.24-3.47 (m, 4 H, Pro δ -CH₂ and 5-CH₂), 2.45–2.60 (m, 1 H, 4-CH), 1.84–2.32 (m, 5 H, Pro β -and γ -CH₂ and 4-CH); ¹³C NMR (CDCl₃) δ 172.6, 171.6, 169.2, 168.8 (CO), 137.4, 128.5, 128.2, 127.9 (Ph C), 73.2 (PhCH₂), 69.0 (OCH₂CO), 60.1 (Pro α-C), 52.3 (OCH₃), 51.0 (3-C), 46.4 (CH_2CO_2Me) , 44.7 (Pro δ -C), 44.5 (5-C), 27.4 (Pro β -C), 27.0 (4-C), 22.0 (Pro γ -C); FAB MS m/z 418 [M + H]⁺. Anal. $(C_{21}H_{27}N_3O_6 CH_2Cl_2) C, H, N.$

Methyl 3(R)-[[[1-(Hydroxyacetyl)-2(S)-pyrrolidinyl]carbonyl]amino]-2-oxo-1-pyrrolidineacetate (9). Compound 8 (2.1 g, 5.03 mmol) was dissolved in distilled MeOH (5 mL) and added to a solution of 10% Pd/C (200 mg) in 5 mL of MeOH under Ar. Hydrogenation was done at 40 psi for 18 h. Gravity filtration through filter paper followed by passage of the solution through a $0.45 \,\mu m$ PTFE filter disk gave a clear solution. Concentration under vacuum produced 1.31 g (80%) of a white foam: $[\alpha]_{D} = -60.0^{\circ} (c \ 0.70, \text{ MeOH}); \text{ TLC } R_{f} (\text{CH}_{2} - 60.0^{\circ})$ $Cl_2/MeOH$, 20:1) = 0.15; ¹H NMR (CDCl₃) δ 7.33 (d, 1 H, CONH), 4.64 (d, 1 H, Pro a-CH), 4.42-4.45 (m, 1 H, 3-CH), $4.17 (d, 2 H, COCH_2O), 4.17 (d, J = 5.7 Hz, 1 H, CH_2CO_2Me),$ $3.99 (d, J = 5.7 Hz, 1 H, CH_2CO_2Me), 3.74 (s, 3 H, OCH_3),$ 3.28-3.54 (m, 4 H, Pro δ -CH₂ and 5-CH₂), 2.60-2.69 (m, 1 H, 4-CH), 1.89–2.42 (m, 5 H, Pro β - and γ -CH₂ and 4-CH); ¹³C NMR (CDCl₃) & 173.4, 172.7, 171.9, 169.4 (CO), 61.4 (COCH₂-OH), 60.9 (Pro a-C), 53.0 (OCH₃), 51.9 (3-C), 46.0 (CH₂CO₂-Me), 45.5 (Pro δ-C), 45.1 (5-C), 28.3 (Pro β-C), 27.9 (4-C), 25.3 (Pro γ -C); FAB MS m/z 328 [M + H]⁺. Anal. (C₁₄H₂₁N₃O₆) C, H, N.

Methyl 3(*R*)-[1-(2,5-Dioxopyrrolidino[3,4-c]piperazino)]-2-oxo-1-pyrrolidineacetate (11). Compound 9 (0.59 g, 1.80 mmol) was dissolved in freshly distilled THF (50 mL). The solution was stirred under Ar while triphenylphosphine (0.50 g, 1.89 mmol) was added all at once. This was cooled to -78 °C and a solution of diethyl azodicarboxylate (312 μ L, 1.98 mmol) in 20 mL of THF was added dropwise. The resulting solution was stirred for 15 min before warming to -20 °C over 2 h. It was stirred at -20 °C for an additional hour and then warmed to room temperature where it was kept for 24 h. Concentration under vacuum gave an oil which was chromatographed under medium-pressure on a silica gel column using EtOAc/*i*-PrOH (25:1) as the eluant. An oil (140 mg) was collected which contained the desired product contaminated with 10. [Spectral characteristics for 10: ¹H NMR (CDCl₃) δ 7.59 (bs, 2 H, NH), 4.62–4.65 (m, 1 H, Pro α -CH), 4.24–4.51 $(m, 3 H, 3-CH and NCH_2CONH_2), 4.14-4.22 (m, 4 H, CH_2O_2C),$ 3.96-4.02 (m, 2 H, NCH₂CON), 3.74 (s, 3 H, CO₂CH₃), 3.37- $3.57 (m, 4 H, Pro \delta$ -CH₂ and 5-CH₂), 2.56-2.64 (m, 1 H, 4-CH), 2.37-2.45 (m, 1 H, Pro β -CH), 1.88-2.18 (m, 4 H, Pro β -CH and γ -CH₂ and 4-CH), 1.26 (t, J = 2.5 Hz, 6 H, CH₂CH₃); ¹³C NMR (CDCl₃) δ 172.8, 171.6, 168.8, 167.9 (CO ester and amide), 156.7, 155.6 (CO carbamate), 62.8 (COCH₂OH), 61.7 (Pro a-C), 60.1 (CH₂CH₃), 52.2 (OCH₃), 51.9 (3-C), 46.5 (CH₂-CO₂Me), 44.7 (Pro δ -C), 44.4 (5-C), 28.5 (Pro β -C), 27.9 (4-C), 24.8 (Pro γ-C), 14.4 (CH₃CH₂); FAB MS m/z 486 [M + H]⁺.] The desired product was purified via HPLC using CH₂Cl₂/ MeOH (20:1) on a silica gel preparatory column (Waters RCM, 25×100 mm). A single peak corresponding to 4.6 mg was obtained. The retention time was 29 min with a flow rate of 3.0 mL/min. Concentration under vacuum gave a colorless oil which crystallized from EtOAc/hexane to give blocky crystals: $[\alpha]_D = -54.8^\circ$ (c 0.46, MeOH); TLC R_f (EtOAc/i-PrOH, 25:1) = 0.11; ¹H NMR (CDCl₃) δ 4.77 (t, J = 9.6 Hz, 1 H, Pro α -CH), 4.25 (d, J = 17.7 Hz, 1 H, NCH₂CON), 4.20 (d, J =15.9 Hz, 1 H, CH_2CO_2Me), 4.13 (t, J = 7.5 Hz, 1 H, 3-CH), $3.95 (d, J = 17.7 Hz, 1 H, NCH_2CON), 3.80 (d, J = 15.9 Hz, 1$ H, CH₂CO₂Me), 3.75 (s, 3 H, OCH₃), 3.43-3.80 (m, 4 H, Pro δ-CH₂ and 5-CH₂), 2.32-2.47 (m, 1 H, 4-CH), 1.85-2.24 (m, 5 H, Pro β - and γ -CH₂ and 4-CH); ¹³C NMR (CDCl₃) δ 171.4, 169.3, 168.7, 163.4 (CO), 67.7 (Pro α -C), 61.9 (NCH₂CON), 57.2 (NCH₂COO), 53.1 (OCH₃), 51.4 (3-C), 45.9 (Pro δ-C), 45.2 (5-C), 29.4 (Pro β -C), 23.3 (4-C), 23.2 (Pro γ -C); high-resolution FAB MS m/z 310.1401 (C₁₄H₁₉N₃O₅ + H⁺ requires 310.1403).

3(R)-[1-(2,5-Dioxopyrrolidino[3,4-c]piperazino)]-2-oxo-1-pyrrolidineacetamide (3). Compound 11 (4.6 mg) was dissolved in 0.5 mL of HPLC grade MeOH and cooled to -20°C. To this stirred solution was slowly added an equal portion of MeOH saturated with NH₃. The resulting solution was capped tightly and stirred at this temperature for 4 h. Concentration in vacuo gave a clear oil which resisted crystallization attempts. High-pressure liquid chromatography on a C18 reverse phase analytical column (Waters RCM, 8×100 mm) using neat MeOH (flow rate at 1.0 mL/min) allowed the isolation of a single peak at 4.0 min which corresponded to the desired product: 3.4 mg (78% yield); $[\alpha]_D = -3.5^\circ$ (c 0.34, MeOH); TLĈ R_f (CH₂Cl₂/MeOH 10:1) = 0.12; ¹H NMR (CDCl₃) δ 7.29 (bs, 1 H, NH₂), 5.49 (bs, 1 H, NH₂), 4.54 (t, J = 15.9 Hz, 1 H, Pro α -H), 4.52 (d, J = 15.9 Hz, 1 H, NCH₂CON), 4.17 (t, J = 7.5 Hz, 1 H, 3-CH), 3.90 (d, J = 15.9 Hz, 1 H, NCH₂CON), $3.88 (d, J = 17.7 Hz, 1 H, CH_2CONH_2), 3.45 (d, J = 17.7 Hz,$ 1 H, CH₂CONH₂), 3.39-3.93 (m, 4 H, Pro δ-CH₂ and 5-CH₂), 2.45-2.57 (m, 1 H, 4-CH), 2.20-2.39 (m, 2 H, Pro β -CH and 4-CH), 1.90–2.11 (m, 3 H, Pro β -CH and γ -CH₂); ¹³C NMR (CDCl₃) δ 171.0, 169.3, 169.1, 163.3 (CO), 60.7 (Pro α-C), 59.8 $(COCH_2N)$, 55.7 (CH_2CONH_2) , 54.1 (3-C), 47.4 (Pro δ -C), 45.8 (5-C), 29.3 (Pro β -C), 23.7 (4-C), 23.3 (Pro γ -C); high-resolution FAB MS m/z 295.1397 (C₁₃H₁₈N₄O₄ + H⁺ requires 295.1406).

HPLC Assay. A 100 nM concentration of 2 in a 50 mM Tris buffer solution at pH 7.4 was prepared. A 50 μ L aliquot of this solution at various intervals was diluted with 500 μ L of a 10:90 CH₃CN/MeOH solution. Water was removed with molecular sieves, and the solution was evaporated under a stream of dry N₂. This procedure stops the hydrolytic decomposition of compound 2. The residue was dissolved in 50 μ L of a 10:90 CH₃CN/MeOH solution, and 20 μ L was injected onto the HPLC. The method was standardized by comparing the integrations for a 100 nM concentration of 2—in a 10:90 CH₃-CN/MeOH solution—both with and without this preparation.

X-ray Diffraction. Colorless crystals of 2 hemihydrate were grown from acetone/methanol. Colorless crystals of 11 were grown from ethyl acetate/hexane. All measurements were made on an Enraf-Nonius CAD-4 diffractometer with graphite monochromated Cu K α radiation ($\lambda = 1.54178$ Å) using the $\omega - 2\theta$ scan mode at 173(1) K by using a Molecular Structure Corporation low-temperature device. Intensities were corrected for Lorentz and polarization effects. Equivalent reflections were merged, and absorption effects were corrected using the ψ -scan method.¹² The structures were solved by direct methods using SHELXS86¹³ and refined using the TEXSAN structure analysis package.¹⁴ All non-hydrogen atoms were refined anisotropically. Hydrogens bonded to carbon atoms were placed in calculated positions (0.95 \AA) and not refined. Hydrogens bonded to heteroatoms (2 hemihydrate) were located in difference Fourier maps, and their positional parameters were refined. Crystal data are given in Table 1.

Membrane Preparation. The bovine striatal membranes were prepared essentially as described in previous reports.¹⁵ The tissues were homogenized in 10 volumes of 0.25 M sucrose in a Potter-Elvehjem homogenizer and centrifuged at 1000g for 15 min. The supernatant was then centrifuged for 1 h at 10000g. The resulting pellet was resuspended in 50 mM Tris-HCl, 1 mM EDTA buffer (pH 7.4) and stored at -80 °C in small aliquots. On the day of use, the membrane preparations were thawed and diluted with Tris-HCl buffer containing 1 mM EDTA, 5 mM MgCl₂, 0.1 mM dithiothreitiol, 0.1 mM phenylmethanesulfonyl fluoride, 0.1 mM benzamide, and 1 mg/mL soybean trypsin inhibitor before using for the ligand-binding assay.

Binding Assays. The receptor binding assays were performed using 150-200 mg membrane proteins, [3H]spiroperidol (0.2 nM), and $[^{3}\text{H}]$ NPA (0.05 nM) in a total volume of 1.0 mL of buffer. The nonspecific binding was determined in parallel assays in the presence of 1.0 mM(+)-butaclamol. Fifty nanomolar ketanserin was included in the [3H]spiroperidolbinding assays to occlude the presence of serotonergic sites. Incubation of membranes with ligands was carried out for 1 h at 22 °C. At the end of the incubation, the bound and free ligands were separated by rapid filtration on Whatman GF/B filters. The filters were washed with 3×5 mL of Tris EDTA buffer and the radioactivity was determined in a Beckman scintillation counter (Model 1780). For the competition experiments, [3H]spiroperidol and varying concentrations of [3H]NPA were added to the reaction mixture in the absence and presence of 100 μ M Gpp(NH)p. The effect of 2 and 3 on radioligand-binding assays was followed by directly incubating the ligands, compounds, and Gpp(NH)p as well as by preincubating the membranes with Gpp(NH)p. For preincubation experiments, the membranes (0.5 mg/mL) were incubated with 100 mM Gpp(NH)p for 2 h at 4 $^{\circ}\rm{C}$ and then centrifuged at 30000g for 20 min. The pellet was washed and resuspended in the assay buffer.

Data Analysis. The binding data were analyzed as previously described.¹⁶ In brief, curves were analyzed using weighted nonlinear curve-fitting programs obtained from EMF Software, Knoxville TN. Data were subjected to analysis for either one site or multiple binding sites together with statistical analysis comparing "goodness of fit" between one- or twoaffinity state models. A two-site model was selected only if a statistically significant improvement of the fit data was obtained over a one-site model. The IC₅₀ values obtained from the competition curves were converted to K_i values using the Cheng and Prusoff equation.¹⁷

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Supplementary Material Available: Tables of positional parameters, bond distances, and bond angles for 2 and 11 (5 pages). Ordering information is given on any current masthead page.

References

- Yu, K.-L.; Rajakumar, G.; Srivastava, L. K.; Mishra, R. K.; Johnson, R. L. Dopamine Receptor Modulation by Conformationally Constrained Analogues of Pro-Leu-Gly-NH₂. J. Med. Chem. 1988, 31, 1430-1436.
- (2) Mishra, R. K.; Śrivastava, L. K.; Johnson, R. L. Modulation of High-Affinity CNS Dopamine D₂ Receptor by L-Pro-L-Leu-Glycinamide (PLG) Analogue 3(R)-(N-L-Prolylamino)-2-oxo-1pyrrolidineacetamide. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 1990, 14, 821-827.
- (3) Valle, G.; Crisma, M.; Toniolo, C.; Yu, K.-L.; Johnson, R. L. Crystal-State Structural Analysis of Two γ-Lactam-Restricted Analogs of Pro-Leu-Gly-NH₂. Int. J. Peptide Protein Res. 1989, 33, 181-190.
- (4) Rasmussen, G. J.; Bundgaard, H. Prodrugs of Peptides. 15. 4-Imidazolidinone Prodrug Derivatives of Enkephalins to Prevent Aminopeptidase-Catalyzed Metabolism in Plasma and Absorptive Mucosae. Int. J. Pharm. 1991, 76, 113-122.
- (5) Bundgaard, H. Design of Prodrugs: Bioreversible Derivatives for Various Functional Groups and Chemical Entities. In *Design* of *Prodrugs*; Bundgaard, H., Ed.; Elsevier Biomedical Press: Amsterdam, 1985; pp 61-63.
- (6) Guttmann, St. Synthese de la Val⁵-D-Phe⁸-angiotensine-I et Nouvelle Synthese de la Val⁵-angiotensine-I. (Synthesis of Val⁵-D-Phe⁸-Angiotensin I and Novel Synthesis of Val⁵-Angiotensin I.) Helv. Chim. Acta **1961**, 44, 721-744.
- (7) Genin, M. J.; Ojala, W. H.; Gleason, W. B.; Johnson, R. L. Synthesis and Crystal Structure of a Peptidomimetic Containing the (R)-4,4-Spiro Lactam Type-II β -Turn Mimic. J. Org. Chem. **1993**, 58, 2334-2336.
- (8) The authors have deposited atomic coordinates for these structures with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, upon request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Rd., Cambridge CB2 1EZ, U.K.
- (9) Rose, G. D.; Gierasch, L. M.; Smith, J. A. Turns in Peptides and Proteins. Adv. Protein Chem. 1985, 37, 1-109.
- (10) Srivastava, L. K.; Bajwa, S. B.; Johnson, R. L.; Mishra, R. K. Interaction of L-Prolyl-L-Leucyl Glycinamide with Dopamine D₂ Receptor: Evidence for Modulation of Agonist Affinity States in Bovine Striatal Membranes. J. Neurochem. 1988, 50, 960-967.
- (11) Genin, M. J.; Mishra, R. K.; Johnson, R. L. Dopamine Receptor Modulation by a Highly Rigid Spiro Bicyclic Peptidomimetic of Pro-Leu-Gly-NH₂. J. Med. Chem. **1993**, 36, 3481-3483.
- (12) North, A. C. T.; Phillips, D. C.; Mathews, F. S. A Semi-empirical Method of Absorption Correction. Acta Crystallogr. 1968, 24A, 351-359.
- (13) Sheldrick, G. M. SHELXS86. Program for the Solution of Crystal Structures; University of Gottingen: Germany, 1986.
- (14) TEXSAN-TEXRAY Structure Analysis Package, Molecular Structure Corporation, 3200 Research Forest Dr., The Woodlands, TX 77381; 1985.
- (15) Kazmi, S. M. I.; Ramwami, J.; Srivistava, L. K.; Rajakumar, G.; Ross, G. M.; Cullen, M.; Mishra, R. K. Characterization of High-Affinity Dopamine D₂ Receptors and Modulation of Affinity States by Guanine Nucleotides in Cholate-Solubilized Bovine Striatal Preparations. J. Neurochem. 1986, 47, 1493-1502.
- (16) Kazmi, S. M. I.; Mishra, R. K. Comparative Pharmacological Properties and Functional Coupling of μ and δ Opioid Receptor Sites in Human Neuroblastoma SH-SY5Y Cells. Mol. Pharmacol. 1987, 32, 109-118.
 (17) Cheng, Y. C.; Prusoff, W. H. Relationship Between the Inhibition
- (17) Cheng, Y. C.; Prusoff, W. H. Relationship Between the Inhibition Constant (K_I) and the Concentration of Inhibitor Which Causes 50 Per Cent Inhibition (I₅₀) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.