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Design, Synthesis and Biological Evaluation of 3-Amino-3-phenylpropionamide Derivatives as Novel μ Opioid Receptor Ligands

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Abstract—3-Amino-3-phenylpropionamide derivatives were produced as small molecule mimics of the cyclic octapeptide octreotide from readily available imine **1**. The compounds exhibit high affinity for the μ opioid receptor.

The human opioid receptors μ, δ, and κ have been classified as members of the G protein coupled receptor family.[1–3] The opioid receptors are abundantly present in the CNS and the periphery and mediate significant physiologic events that range from the perception of pain, mood and pleasure, to respiratory depression and regulation of the GI and immune systems.[4] The design and synthesis of potent and selective molecular probes that will define the biological significance of each receptor are highly active research areas in contemporary medicinal chemistry (for some leading examples, see refs 5–8). As part of a program that aimed at providing selective molecular probes for the μ receptor and its subtypes we sought to design and synthesize small molecule mimics of the cyclic octapeptide octreotide (SMS-201, 995), a selective μ ligand. The targeted molecules are represented by the 3-amino-3-phenylpropionamide derivatives shown in Figure 1.

Results and Discussion

Design

The cyclic octapeptide octreotide, D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol (SMS-201, 995), has been shown to exhibit affinity for the μ opioid receptor in addition to its somatostatin activity and good selectivity relative to the δ receptor.[9] More recently, interesting conformationally restricted cyclic peptides have been discovered as analogues of SMS-201, 995 with good affinity for the μ opiate receptor and reduced affinity for somatostatin.[10,11] In order to produce small molecule mimics of these cyclic peptides, our design efforts focused on the SAR and spatial relationship of the two phenylalanine residues in SMS-201, 995. In a similar approach, small molecule mimics of the δ selective cyclic peptide [(2S, 3R)-TMT1]DPDPE were designed by appropriately placing the aromatic pharmacophores of [(2S, 3R)-TMT1]DPDPE on a piperazine scaffold.[12]

The X-ray structure of SMS-201, 995 contains three molecules in the asymmetric unit cell and measurement of the distance between the centroids of the phenylalanine aromatic rings for each molecule gives 12.170 ± 0.11 Å. This conformation can be described as relatively 'extended'.[13] While this peptide is conformationally constrained, a more 'compact' conformation is also possible and supported by NMR studies.[14] A compact conformation can be achieved by modification of χ_1 of Phe-3 to be 60°, which decreases the distance between the two phenylalanine aromatic rings to 9.335 ± 0.40 Å. These distances provide us with a framework for the selection of the 3-amino-3-phenylpropionamide as a scaffold with the desired aromatic ring-hydrophobic group spatial relationship. For each 3-amino-3-phenylpropionamide the initial conformations were generated by carrying out a Monte Carlo conformational search with MacroModel v5.5 employing the MMFF force field with the GB/SA continuum solvation model.[15] In each Monte

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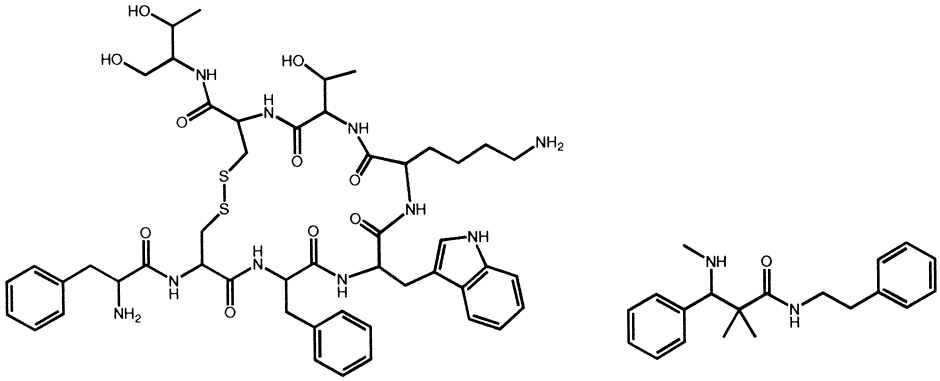


Figure 1. 2-Dimensional extended representations of octreotide (left) and a 3-amino-3-phenylpropionamide derivative (right).

Carlo search, 2000 possible conformers were generated. Full geometry optimizations were then carried out with Gaussian98 (6-31G(d) basis set) on the 10 lowest energy conformations for each compound within a 5 kcal/mol window.[16] The pharmacophoric elements of each compound (aromatic ring, basic nitrogen, and hydrophobic group) were then aligned and evaluated against the compact and extended conformations of SMS-201,995. The overlap of SMS-201, 995 (green) with a 3-amino-3-phenylpropionamide derivative (gold) as shown in Figure 2 illustrates our design strategy.

Synthesis

A convergent approach was designed for the preparation of the desired opiates (see Scheme 1). Thus, the precursor β-lactam **3** was obtained in high yields after treatment of imine **1** with titanium tetrachloride and addition of the commercially available ketene acetal **2** followed by tandem cyclization of an intermediate amino ester.[17] The lactam **3** serves as an internal nitrogen-protecting group and sets the stage for ring opening reactions. Thus, the desired propionamides **4** were produced in high yields after addition of lithium amides of primary amines to the lactam **3**. The methyl ethers **4d–i** were deprotected in high yields with boron tribromide in dichloromethane to produce the phenols **5**. The tertiary amines **6** were formed by reductive alkylation under standard conditions.

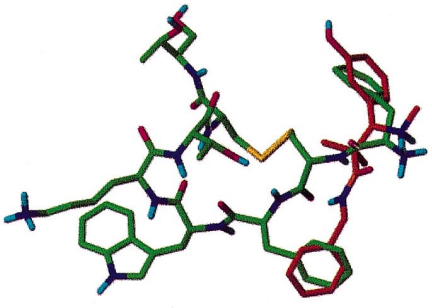
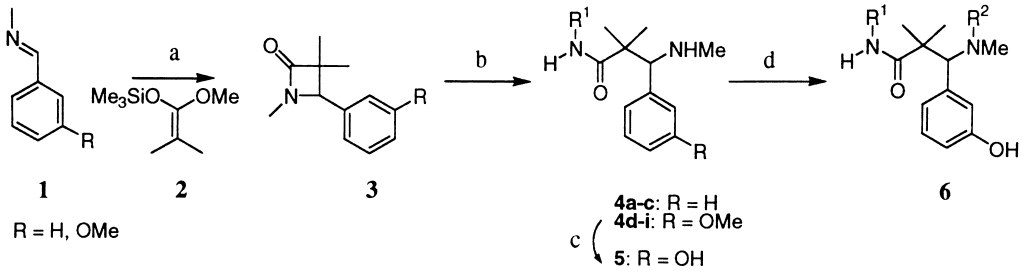


Figure 2. Overlap of SMS-201, 995 with a 3-amino-3-phenylpropionamide derivative.

The in vitro binding affinities for compounds **4**, **5** and **6** were determined.[19] The binding assays were performed according to standard protocols. For the δ receptor, radioligand studies were carried out on CHO cells expressing the human δ opioid receptor. Cells were collected and homogenized (50 mM Tris HCl, pH 7.4 and 1 mM EGTA) centrifuged, the pellet washed once and resuspended in buffer at 0.8 mg/mL and frozen (−80 °C). Binding to the δ receptor was initiated with the addition of cell membranes to 96 well plates containing 0.5 nM [^{3}H] naltrindole (Tocris: specific activity 50 Ci/mmol) and incubated for 90 min at 37 °C. Non-specific binding was estimated using 1 μM cold naltrindole.

Rat brain tissue was utilized for the μ receptor assay. Thus, rat brains were homogenized (50 mM Tris HCl pH 7.4) and a crude membrane fraction was prepared and kept on ice. Receptor binding was initiated with the



Scheme 1. Preparation of 3-amino-3-phenylpropionamide derivatives.[18] (a) $TiCl_4$, CH_2Cl_2, rt., 10 h; 88%; (b) *n*-BuLi, R^1NH_2, THF, 0 °C; 15 min then add 3; 85–98% yield; (c) BBr_3, CH_2Cl_2, −78 °C to rt; 80–95% yield; (d) R^xCHO, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt; 10 h; 65–95% yield.

addition of rat brain membranes to 96-well plates containing 1.5 nM [^{3}H]DAMGO (NEN; specific activity 55 Ci/mmol) and incubated for 90 min at 22 °C. Non-specific binding was determined using 1 μM naltrexone. For the κ binding assay membranes (~5 mg/mL protein) prepared from HEK 293 cells transfected with the human κ opioid receptor were used. Receptor binding assays were initiated by the addition of cell membranes to 96-well plates containing 2 nM [^{3}H]U −69,593 (NEN; specific activity 40 Ci/mmol) and incubated for 1 h at 25 °C. All assays were terminated by rapid filtration using a Skatron harvester onto Whatman GF/C (δ assay), Wallac Filtermat B (μ assay) or Wallac Filtermat B (κ assay) and counted in a Beta Plate reader (Wallac).

Results of the biological evaluation of compounds **4**, **5** and **6** are included in Table 1. Biological data are reported in nM. Values reported are the arithmetic mean ± SEM in parenthesis of a minimum of three independent receptor binding assays, except when noted.

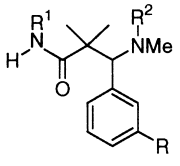
Compound **4a** with a phenethyl amide group represents a substrate with aromatic ring-hydrophobic group distance of 11.3 ± 0.2 Å. This value corresponds more closely to the phenylalanine aromatic group distance suggested by the X-ray structure of octreotide. Compound **4a** exhibits high affinity for the μ receptor, excellent selectivity over the δ receptor and modest affinity for the κ receptor. Compound **4b**, with a shorter benzyl amide group exhibits reduced affinity for the μ receptor and similar selectivity profile. The aromatic ring-hydrophobic group distance for **4b** was computed to be 9.9 Å, in line with a more compact conformation suggested by the NMR studies. Interestingly, compound **4c** with a non-aromatic hydrophobic amide substitution such as the cyclohexylmethyl shows binding affinity for the μ receptor similar to the benzamide **4b** and increased affinity for the κ receptor.

Introduction of a classic opioid-binding moiety, the 3-hydroxy substituent, to the benzylamino aromatic group (entries **5a**–**f**) delivers compounds with improved binding affinity for the μ receptor. Selectivity over the δ receptor is generally maintained with the introduction of the 3-hydroxy substituent and high binding affinity for the κ receptor is generally observed. Biological results for the methyl ether precursors **4d**–**i** are not included in Table 1 as the compounds generally show reduced affinity for the receptors (20–50-fold).

Substrates with amide substitutions such as the phenethyl in **5b** and 1-naphthylmethyl in **5d** exhibit the highest μ receptor binding affinities. Compound **5c**, with the larger phenylpropyl amide group, exhibits modestly lower affinity for the μ receptor and high affinity for the κ receptor. Compound **5e** with the hydrophobic cyclohexylmethyl amide group shows high binding affinity for the μ and κ receptors and good selectivity over the δ receptor. Compound **5f** with a smaller hydrophobic amide group, such as the cyclopropyl methyl group, has significantly reduced affinity for the μ receptor and modest affinity for the κ receptor. Generally, conversion of the secondary amines to tertiary amines (entries **6a**–**b**) via reductive alkylation with small aliphatic aldehydes delivered compounds with reduced binding affinity for the μ receptor.

In conclusion, the design and synthesis of small molecule mimics of the cyclic octapeptide SMS-201, 995 have been described herein. The designed propionamide derivatives were produced from readily available imines. In vitro biological evaluation revealed compounds with high affinity for the μ opioid receptor and excellent selectivity over the δ receptor, consistent with the data reported for SMS-201, 995 and its analogues. Selectivity over the κ receptor is generally low for substrates within this class of compounds. Receptor selectivity optimization, determination of the functional in vitro characteristics and behavioral in vivo properties of compounds within this group constitute the subject of our current investigation. Results will be reported in due course.

Table 1. Binding affinities (nM) for compounds **4**, **5** and **6** at μ, δ, and κ receptors



Compound	R1	R2	R	μ	δ	κ
4a	$PhCH_2CH_2$	H	H	13 (20–8.9)	>10,000	49[a] (50–48)
4b	$PhCH_2$	H	H	95 (139–65)	>10,000	467 (754–290)
4c	*c*-C_6H_{11}–CH_2	H	H	85 (110–66)	>10,000	1.2 (1.5–0.90)
5a	$PhCH_2$	H	OH	8.7 (11–6.6)	>10,000	5.0 (9.0–2.7)
5b	$PhCH_2CH_2$	H	OH	1.4 (1.7–1.2)	176 (320–97)	6.3 (13–3.1)
5c	$PhCH_2CH_2CH_2$	H	OH	12 (26–5.2)	>10,000	3.6 (7.3–1.8)
5d	1-naphthyl-CH_2	H	OH	1.4 (1.6–1.2)	163 (199–134)	1.3 (3.1–0.55)
5e	*c*-C_6H_{11}-CH_2	H	OH	4.4 (5.8–3.3)	881 (972–711)	5.7 (14–2.3)
5f	*c*-C_3H_5-CH_2	H	OH	364 (408–324)	781 (1000–522)	75 (118–47)
6a	$PhCH_2CH_2$	*c*-C_3H_5–CH_2	OH	28 (31–24)	710 (996–501)	2.9 (5.9–1.4)
6b	$PhCH_2CH_2$	*n*-C_4H_9	OH	26 (30–21)	>10,000	75 (198–28)

[a]Value represents the average of two experiments.

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18. All compounds are racemic and gave satisfactory spectral data. For example, see: 4-(3-methoxy-phenyl)-1,3,3-trimethyl-azetidin-2-one (**3**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (ap t, 1H), 6.84 (dd, J = 8.1, 2.1 Hz, 1H), 6.73 (d, J = 7.5 Hz, 1H), 4.25 (s, 1H), 3.80 (s, 3H), 2.85 (s, 3H), 1.40 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 159.9, 137.9, 129.8, 118.9, 113.0, 112.5, 112.4, 68.6, 56.5, 55.2, 27.2, 22.5, 17.7; MS (M + 1) 220.0. HRMS calcd for $C_{13}H_{17}NO_2$ + H 239.1495, found 239.1513. 3-(3-Hydroxy-phenyl)-2,2-dimethyl-3-methylamino-*N*-phenethyl-propionamide (**5b**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (br, 1H), 7.29–7.04 (comp, 6H), 6.79–6.74 (m, 1H), 6.72–6.70 (m, 1H), 6.64 (d, J = 7.7 Hz, 1H), 3.59–3.48 (comp, 2H), 3.43 (s, 1H), 2.79 (t, J = 6.8 Hz, 2H), 2.07 (s, 3H), 1.06 (s, 3H), 0.99 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.1, 156.6, 140.8, 139.1, 129.1, 128.1, 128.6, 126.4, 119.8, 115.9, 114.7, 71.0, 45.7, 40.6, 35.4, 34.7, 24.9, 21.0; MS (M + 1) 327.3. HRMS calcd for $C_{20}H_{26}N_2O_2$ + H 307.0930, found 307.0930.
19. Naloxone was used as a standard in the binding assays: μK_i = 1.4 nM, δK_i = 41 nM, κK_i = 1.2 nM.