Guanidino N-Substituted and N,N-Disubstituted Derivatives of the κ-Opioid Antagonist GNTI

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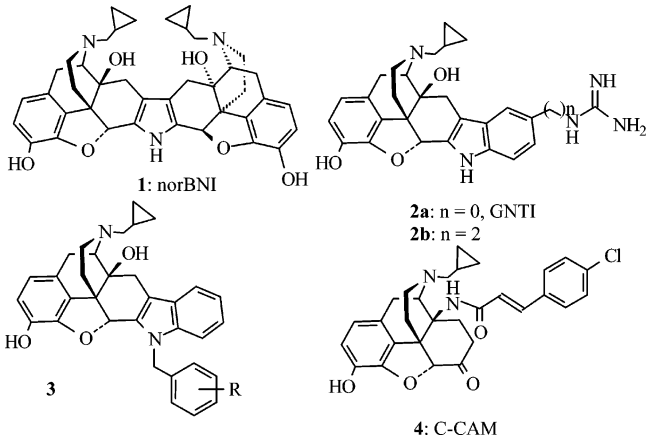
Derivatives of the highly selective κ-opioid receptor antagonist GNTI (**2a**) have been prepared. Binding and functional studies conducted on cloned human opioid receptors expressed in Chinese hamster ovarian (CHO) cells suggested that adding a benzyl or a substituted benzyl group to the guanidino moiety led, in general, to a retention of high κ-affinity and antagonist potency. Disubstitution of the guanidino moiety led to reduced κ-selectivity.

Introduction

One of the principal goals for medicinal chemists within the field of drug abuse, and in particular opioid abuse, has been the development of selective antagonists for each of the opioid receptors (μ, κ, and δ). The availability of such agents greatly facilitates the study of the physiological function of these receptors. Though the role of opioids in the treatment of pain has long been recognized, it has become increasingly apparent that they have a significant role to play in a wider range of clinical situations and thus there is a continuing need for the development of selective ligands, both as tools for basic research and as leads for potential pharmacotherapies. Of the three opioid receptor types, the μ-receptor has been the most thoroughly investigated due to its involvement in the treatment of pain and opiate abuse. It is, however, becoming increasingly apparent that both the κ- and δ-receptors represent viable molecular targets for a number of indications. Of particular interest to ourselves are reports on the role of κ-opioid agonists in cocaine abuse, and in particular, the findings that κ-agonists can block many of cocaine's behavioral effects.[1−6] Thus κ-agonists may be of use in the development of a treatment for cocaine abuse.

κ-Antagonists have also been studied in the preclinical setting with reports of their utility in improving recovery after traumatic brain injury in rats,[7] for determining the underlying mechanisms that cause the motor fluctuations that develop during the treatment of Parkinson's disease,[8] and as antidepressants in the forced swim test in rats.[9] In feeding studies, administration of the κ-antagonist norBNI (**1**) significantly reduced deprivation intake and suppressed other forms of food intake in rats[10] and has also been shown to attenuate drinking in genetically polydipsic mice.[11]

At present, norBNI, discovered by Portoghese and coworkers, is the κ-antagonist of choice.[12−14] Extensive investigation of the structural requirements for κ-antagonist selectivity has led to GNTI (**2a**), a simplified



structure based on the indolomorphinan naltrindole.[15,16] Initial reports suggest that **2a** has comparable, or better, κ-antagonist selectivity than **1**[15,16] and shares with **1** an extended duration of action.[17] We were interested in the possibilities that the guanidine group provided for further structural elaboration. Introduction of a lipophilic group into the side chain of μ- and δ-opioid antagonists can have a profound effect on the selectivity, efficacy, and reversibility of the ligands. For example, introduction of arylalkyl groups to the 14-position in a series of δ-antagonists related to naltrindole resulted in a μ-agonist/low-efficacy δ-partial agonist profile.[18] In addition, the indole *N*-benzyl derivative of naltrindole, BNTI (**3**, R = H),[19,20] has no means of forming a covalent bond to the receptor and yet is reported to have an in vivo profile very similar to that of the isothiocyanate-containing BNTII (**3**, R = p-NCS), with antagonist effects lasting up to 5 days. A similar effect has been observed with the well-known μ-receptor irreversible antagonist C-CAM (**4**).[21−23] The available evidence does not suggest that **4** forms a covalent bond with the receptor, yet it displays a pharmacological profile consistent with nonsurmountable binding to the receptor. Presumably, in these cases noncompetitive binding is a result of extremely tight binding involving the benzyl and cinnamoyl groups. If such an effect were replicated in the GNTI (**2a**) series at the κ-receptor, it would result in the first nonsurmountable κ-antagonist based on a

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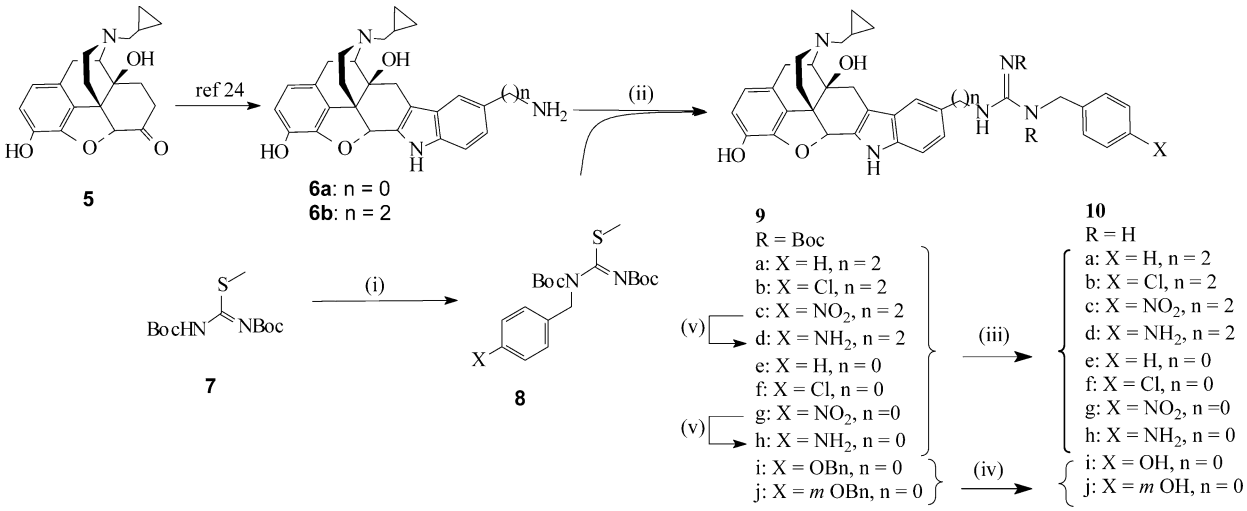
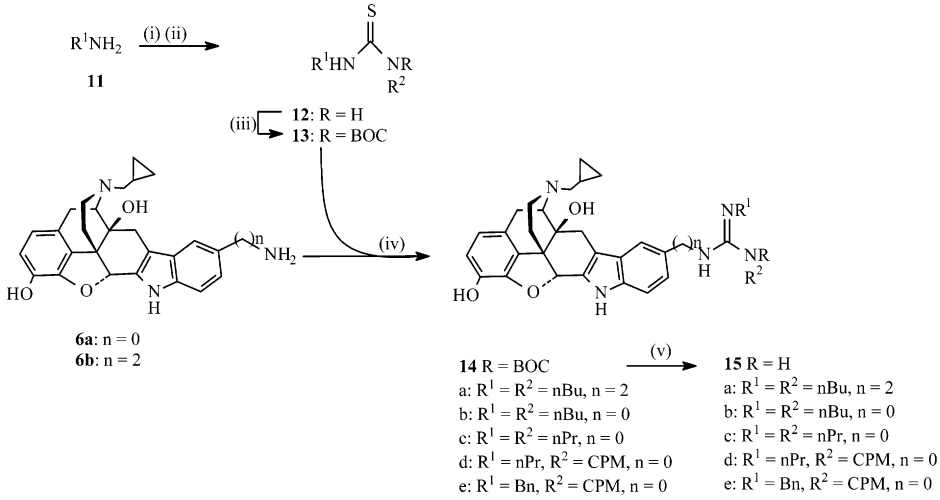
Scheme 1[a]



[a] (i) NaH, X-$C_6H_4CH_2Br$, DMF; (ii) $HgCl_2$, NEt_3, DMF; (iii) TFA/CH_2Cl_2; (iv) HCl/MeOH; (v) Raney Ni/cyclohexene.

Scheme 2[a]



[a] (i) Cl_2CS, $CaCO_3$, H_2O; (ii) RNH_2, acetone; (iii) NaH, $(BOC)_2O$, THF; (iv) $HgCl_2$, NEt_3, DMF; (v) TFA, CH_2Cl_2.

κ-antagonist, and not κ-agonist, structure. For these reasons benzyl-substituted analogues of **2** were targeted along with a small number of disubstituted aliphatic analogues to extend the SAR.

Chemistry

The synthesis of guanidines **2a** and **2b** has been previously reported by Stevens et al.[24] They were obtained by mercury-assisted condensation of amines **6a**,**b** with bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea. To access the related *N*-benzyl guanidines it was decided to utilize *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*-benzyl-2-methylthiopseudourea and substituted benzyl analogues (**8**), in an analogous reaction. The guanidinylating agents (**8**) were prepared by reaction of the appropriately substituted benzyl bromide with 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (**7**) in the presence of sodium hydride. Coupling with amine **6b**, to give **9a**−**c**, was carried out at 50 °C in the presence of mercuric chloride, with the less reactive aniline **6a** requiring extended reaction time at 60 °C to yield **9e**−**g**. Removal of the *tert*-butoxycarbonyl protecting group was accomplished using standard conditions of trifluoroacetic acid to give the products (**10a**−**c**,**e**−**g**) as their trifluoroacetic acid salts. The nitro analogues **9c**,**g** were also converted to their amino counterparts **9d**,**h** by Raney Ni catalyzed transfer hydrogenation in 50% MeOH/cyclohexene before removal of the protecting groups with trifluoroacetic acid to yield **10d**,**h**.

The *p*- and *m*-hydroxy analogues (**10i**,**j**) were prepared in similar fashion (Scheme 1), the appropriate guanidinylating agents being synthesized from the *m*- and *p*-benzyloxybenzyl bromides reported previously.[25,26] Simultaneous removal of the *tert*-butoxycarbonyl and benzyl protecting groups of **10i** and **10j** was found to proceed most efficiently in HCl/MeOH to give **10i** and **10j** as their hydrochloride salts.

Disubstituted thioureas **15** were prepared by coupling of **6a** and **6b** with symmetric and unsymmetric *N*,*N*′-disubstituted thioureas (**13**), themselves prepared from the appropriate amines and thiophosgene (Scheme 2). The coupling would not take place directly with thioureas (**12**), in agreement with literature precedent,[27] and required the presence of a *tert*-butoxycarbonyl group on one of the nitrogens. Interestingly, in the same report[27] it is suggested that the thiourea group should have one proton on each of the nitrogens in order for the coupling reaction to be successful. This does not

Table 1. Antagonist Potency in [^{35}S]GTPγS Assays Performed in Cloned Human Opioid Receptors[a]

				K_e (nM) ± SEM				
compd	*n*	R1	R2	*μ*-CHO membranes DAMGO	*δ*-CHO membranes DPDPE	*κ*-CHO membranes U69,593	*μ*/*κ*	*δ*/*κ*
2b	2	H	H	1.25 ± 0.12	0.88 ± 0.15	0.40 ± 0.06	3	2
10a	2	H	benzyl	2.94 ± 0.31	1.36 ± 0.10	0.13 ± 0.01	23	10
10b	2	H	*p*-chlorobenzyl	2.61 ± 0.41	1.48 ± 0.14	0.23 ± 0.02	11	6
10c	2	H	*p*-nitrobenzyl	2.20 ± 0.60	1.34 ± 0.24	0.17 ± 0.01	13	8
10d	2	H	*p*-aminobenzyl	1.57 ± 0.13	0.95 ± 0.14	0.25 ± 0.03	6	4
10e	0	H	benzyl	1.41 ± 0.17	4.09 ± 0.63	0.06 ± 0.01	24	68
10f	0	H	*p*-chlorobenzyl	5.24 ± 1.13	7.67 ± 1.36	0.14 ± 0.01	37	55
10g	0	H	*p*-nitrobenzyl	3.71 ± 0.59	16.66 ± 1.30	0.18 ± 0.01	21	93
10h	0	H	*p*-aminobenzyl	1.22 ± 0.06	10.80 ± 0.80	0.09 ± 0.01	14	120
10i	0	H	*p*-hydroxybenzyl	12.66 ± 0.84	18.31 ± 0.95	0.10 ± 0.01	127	183
10j	0	H	*m*-hydroxybenzyl	4.28 ± 0.52	4.35 ± 0.22	0.13 ± 0.02	33	33
15a	2	butyl	butyl	4.62 ± 0.59	1.05 ± 0.08	0.39 ± 0.03	12	3
15b	0	butyl	butyl	5.66 ± 0.39	5.24 ± 0.67	0.44 ± 0.03	13	12
15c	0	propyl	propyl	4.59 ± 0.80	2.43 ± 0.31	0.26 ± 0.05	18	9
15d	0	propyl	cyclopropylmethyl	3.26 ± 0.32	6.31 ± 0.34	0.08 ± 0.01	41	79
15e	0	benzyl	cyclopropylmethyl	2.75 ± 0.24	3.28 ± 0.37	0.17 ± 0.05	16	19
1	norBNI			18.9 ± 1.8	4.42 ± 0.38	0.04 ± 0.004	484	113
2a[b]	GNTI	H	H	3.23	15.49	0.04	81	389

[a] Values are means from five or six experiments. [b] Converted from pA_2 values from ref 16.

Table 2. Binding Affinities to Cloned Human Opioid Receptors Transfected into Chinese Hamster Ovary (CHO) Cells[a]

				K_i (nM) ± SEM				
compd	*n*	R1	R2	*μ* [^{3}H]-DAMGO	*δ* [^{3}H]Cl-DPDPE	*κ* [^{3}H]U69,593	*μ*/*κ*	*δ*/*κ*
2b	2	H	H	5.69 ± 1.28	4.93 ± 1.28	0.49 ± 0.00	12	10
10a	2	H	benzyl	3.54 ± 0.25	7.24 ± 0.86	1.42 ± 0.17	2	5
10b	2	H	*p*-chlorobenzyl	7.74 ± 1.98	19.18 ± 0.17	2.41 ± 0.22	3	8
10c	2	H	*p*-nitrobenzyl	9.21 ± 3.73	11.70 ± 0.28	2.14 ± 0.34	4	5
10d	2	H	*p*-aminobenzyl	7.78 ± 2.71	5.05 ± 0.23	0.95 ± 0.04	8	5
10e	0	H	benzyl	10.47 ± 1.87	26.81 ± 6.47	0.86 ± 0.20	12	31
10f	0	H	*p*-chlorobenzyl	29.78 ± 0.50	85.05 ± 5.06	0.66 ± 0.05	45	129
10g	0	H	*p*-nitrobenzyl	26.28 ± 3.82	95.82 ± 3.49	1.61 ± 0.45	16	60
10h	0	H	*p*-aminobenzyl	7.89 ± 2.45	23.91 ± 5.69	0.63 ± 0.10	13	38
10i	0	H	*p*-hydroxybenzyl	14.67 ± 3.65	41.86 ± 0.93	3.26 ± 0.12	4.5	13
10j	0	H	*m*-hydroxybenzyl	14.16 ± 4.11	17.58 ± 0.29	2.74 ± 0.74	5	6
15a	2	butyl	butyl	7.89 ± 0.57	6.73 ± 1.80	4.80 ± 0.02	1.5	1.5
15b	0	butyl	butyl	18.27 ± 0.34	15.04 ± 3.26	6.96 ± 0.85	3	2
15c	0	propyl	propyl	9.83 ± 0.09	8.52 ± 1.99	2.72 ± 0.39	4	3
15d	0	propyl	cyclopropylmethyl	22.44 ± 7.27	15.48 ± 3.47	2.38 ± 0.37	9	6.5
15e	0	benzyl	cyclopropylmethyl	17.73 ± 0.30	18.06 ± 1.72	3.91 ± 0.60	4.5	5
1	norBNI			21.0 ± 5.0	5.7 ± 0.9	0.20 ± 0.05	105	28
2a[b]	GNTI	H	H	36.9 ± 2.3	70.0 ± 0.3	0.18 ± 0.10	205	389

[a] Data are the average from two experiments, each carried out in triplicate. [b] Data from ref 16.

appear to be the case in the current work and may argue against formation of the previously proposed carbodiimide intermediate in these instances.[27]

Results and Discussion

Opioid agonist and antagonist activity was determined using the [^{35}S]GTPγS assay in cloned human opioid receptors transfected into Chinese hamster ovary (CHO) cells (Table 1).[28,29] None of the compounds stimulated [^{35}S]GTPγS binding for any type of opioid receptor but were found to be antagonists of the selective agonists DAMGO (*μ*), Cl-DPDPE (*δ*), and U69,593 (*κ*) (Table 1). The ligands were also evaluated in competition binding assays in CHO cells transfected with cloned human opioid receptors (Table 2).[29] The displaced radioligands were [^{3}H]DAMGO (*μ*), [^{3}H]Cl-DPDPE (*δ*), and [^{3}H]U69,593 (*κ*).

Each of the compounds was found to be a potent *κ*-antagonist with varying selectivity over the *μ*- and *δ*-receptors (Table 1). *κ*-Antagonist potency was consistent throughout the series with little variation between compounds (K_e = 0.06−0.44 nM). Those ligands lacking the ethylene spacer group were consistently more selective for the *κ*-receptor than those with the spacer group **10e**−**h** vs **10a**−**d** and **15a** vs **15b**. This effect was particularly prominent for *κ*/*δ*-selectivity but less so for *κ*/*μ*. This appears to confirm earlier reports suggesting that in the majority of cases a spacer group was not beneficial for *κ*-selectivity.[24] In neither series (*n* = 0, 2) was there any evidence of consistent effects of the substituents on the benzyl ring, with the unsubstituted, *p*-amino, *p*-nitro, *p*-chloro, and *m*-hydroxy all having similar *κ*-antagonist potency and selectivity. The one compound that stood out was **10i**, having a *p*-hydroxy substituent. While **10i** retained antagonist potency at *κ*-receptors, it was less potent than the other ligands at *δ*- and *μ*-receptors, leading to increased *κ*-selectivity (183-fold over *δ* and 127-fold over *μ*). These results are intriguing as the *p*-hydroxy group of **10i** can exactly overlay the second phenolic group of norBNI (**1**) and thus may be interacting unfavorably with the same site/residues on the *μ*- and *δ*-receptors. While GNTI (**2a**) was

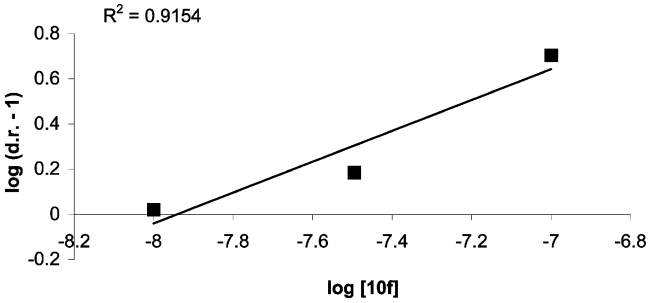


Figure 1. Schild plot of the κ-antagonist activity of **10f**.

not evaluated in this current study, data from the same source and using the same assays as in the present study have been reported previously. Thus, some comparison can be made between **2a** and the current series of compounds with reasonable confidence. **10i** appears similar to both **1** and **2a** in these assays. Of the three, **1** is the most selective for κ/μ, but the least selective for κ/δ. **2a** is the opposite, with the greatest selectivity over δ and the least over μ, while **10i** displays a balance, with good selectivity over both δ and μ receptors.

The dialkylguanidines (**15a**−**e**) were again selective antagonists for the κ-receptor, but in general with somewhat reduced selectivity as compared to the benzyl guanidines (Table 1). The most direct comparison is between **10e**, having a benzyl group, and **15e**, having benzyl and cyclopropylmethyl groups. **10e** showed higher selectivity, particularly with respect to κ/δ. That **15d** also displayed higher selectivity than **15e** suggests that the increase in steric bulk on having both cyclopropylmethyl and benzyl groups is detrimental for selectivity. This is a result of slightly reduced κ-antagonist potency and slightly increased μ- and δ-antagonist potencies for **15e** compared to **15d**.

In the binding assays, all of the ligands bound with highest affinity at the κ receptor (Table 2) with Hill slopes approximating unity, indicating competitive binding. Selectivity varied considerably, but with the broad trends comparable to those noted in the functional assay. Thus, benzyl guanidines **10a**−**d** had substantially lower κ-selectivity than their analogues **10e**−**h**; this was particularly evident for κ/δ selectivity. There again appears to be no consistent SAR relating to the ring substituent within this series. Whereas in the functional assays **10i** was most selective, in the binding assays it was the *p*-chloro analogue **10f**. GNTI (**2a**) and its benzyl analogues ($n = 0$) each had higher selectivity for κ/δ than κ/μ, whereas, as found in the functional assays, norBNI (**1**) was more selective for κ/μ than κ/δ.

The difference between the disubstituted guanidines (**15a**−**e**) and the monosubstituted benzyl guanidines was even more pronounced in the binding assays than in the functional assays, with **15a**−**e** displaying little or no κ-selectivity against the other two opioid receptors.

In the [^{35}S]GTPγS assays, while the ligands caused a shift in the agonist dose−response curves, there was no indication of flattening of the dose−response curve that would be expected if the compounds were acting in a nonsurmountable manner. However, in C6(δ) and CHO(κ), but not C6(μ) cells, the compounds tended to display wash-resistant binding that could be indicative of pseudo-irreversibility (data not shown). As norBNI (**1**) also displayed similar binding characteristics in these cell lines, it was felt that further studies in the mouse vas deferens (mvd) would provide a more accurate assessment of their reversibility. To this end, two compounds, **10b** and **10f**, were evaluated for κ-antagonist activity in the mvd. Both compounds produced surmountable antagonism and parallel shifts of the dose−response curve for the selective κ-agonist E2078.[30] For **10f** the antagonism was studied in more detail, and the effects were found to be dose-dependent. A Schild plot of the antagonist activity of **10f** indicates a linear relationship with a slope approximating unity (0.7), again indicating competitive binding characteristics (Figure 1). It was noted that the K_e of 20 nM obtained in this mvd assay is considerably higher than the value suggested by the binding and GTPγS data.

Conclusions

The addition of benzyl and substituted-benzyl groups to the guanidino moiety of GNTI (**2a**) results in ligands that retain high affinity and selectivity for the κ-opioid receptor. Disubstituted guanidines displayed reduced selectivity compared to their monosubstituted counterparts, primarily as a result of lower κ-affinity. That the benzyl group of these ligands may interact with the same site on the receptor as the second phenolic group of norBNI (**1**) seemed to be confirmed by the substantial selectivity displayed by the *p*-hydroxy analogue (**10i**) in the functional assays. The discrepancy between wash-resistant binding in the C6(δ) and CHO(κ) cell lines, yet fully reversible binding in the mvd, may suggest that wash-resistance is not a suitable definition of irreversibility.

Experimental Section

Column chromatography was performed under gravity, over silica gel 60 (35−70 μm) purchased from Merck. Preparative TLC was performed on plates made with Kieselgel 60 $PF_{254+366}$ for preparative TLC, obtained from Merck. The thickness of the silica layer was approximately 1 mm. Analytical TLC was performed using aluminum-backed plates coated with Kieselgel 60 F_{254}, from Merck. The chromatograms were visualized using either UV light (UVGL-58, short wavelength), ninhydrin (acidic), or potassium permanganate (basic). Melting points were carried out using a Reichert-Jung Thermo Galen Kopfler block or a Gallenkamp MFB-595 melting point apparatus and are uncorrected. High- and low-resolution fast atom bombardment (FAB) mass spectra were recorded on a Fisons VG AutoSpec Q instrument, with a matrix of *m*-nitrobenzyl alcohol. High- and low-resolution electron impact (EI) mass spectra were recorded using EI ionization at 70 eV, on a VG AutoSpec instrument, equipped with a Fisons autosampler. ^{1}H NMR and ^{13}C NMR spectra were recorded using either JEOL 270 (operating at 270 MHz for ^{1}H and 67.8 MHz for ^{13}C), JEOL Lambda 300 (operating at 300 MHz for ^{1}H and 75.4 MHz for ^{13}C), or JEOL EX 400 (operating at 400 MHz for ^{1}H and 100.5 MHz for ^{13}C) spectrometers. Chemical shifts (δ) are measured in ppm. Spectra were referenced internally using the residual solvent resonance. Coupling constants (J) are expressed in hertz, and the multiplicities are abbreviated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). Only diagnostic peaks have been quoted for proton NMR. Microanalysis was performed with a Perkin-Elmer 240C analyzer. Analytical RP-HPLC was performed with a Beckman System Gold 125 solvent module, equipped with a Beckman System Gold 166 detector ($\lambda = 254$ nm). The column stationary phase was Beckman ultrasphere ODS, 5 μm (15 cm × 4.6 mm). A mobile phase of [MeOH/0.3% NH_4CO_3 (80:20)] was used at a flow rate of 1 mL/min. Infrared spectroscopy was performed

on either a Perkin-Elmer 782 Instrument or a Perkin-Elmer RX 1 FT-IR Instrument. Anhydrous THF, DMF, DCM, and MeOH were purchased from Aldrich. HPLC solvent grade chloroform and MeOH were purchased from Merck. All other solvents used were GPR grade, purchased from Merck or Fisher Scientific. Chemicals were purchased from Aldrich, Fluka, Lancaster, and Acros chemical companies.

Details are given for representative examples **10f** and **15d**. Full experimental details for all compounds are provided as Supporting Information.

1,3-Bis-*tert*-butoxycarbonyl-1-(4′-chlorobenzyl)-2-methyl-2-thiopseudourea (8b). To 1,3-bis-*tert*-butoxycarbonyl-2-methyl-2-thiopseudourea (**7**) (2 g, 6.90 mmol) in dry DMF (20 mL) in an ice-bath was added NaH (60% in oil, 0.334 g, 8.36 mmol), and the mixture stirred for 1 h. 4-Chlorobenzyl bromide (1.56 g, 7.60 mmol) was added, and the mixture stirred at room temperature for a further 12 h. Water (30 mL) was added and the mixture extracted with ethyl acetate (3×). The combined organic layers were washed with brine (2×), dried (Na_2SO_4), and concentrated to give crude **8b**. Column chromatography [hexane/EtOAc (9:2)] provided pure **8b** (2.21 g, 5.33 mmol, 77%): R_f = 0.42 [hexane/EtOAc (9:2)];^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 [s, 9H, C(CH_3)$_3$], 1.47 [s, 9H, C(CH_3)$_3$], 2.24 (s, 3H, SCH_3), 4.68 (s, 2H, CH_2), and 7.24 (s, 4H, Ar*H*); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 16.0 (S*C*H$_3$), 28.3 [C(*C*H$_3$)$_3$], 28.4 [C(*C*H$_3$)$_3$], 52.0 (*C*H$_2$), 82.0 [*C*(CH$_3$)$_3$], 83.1 [*C*(CH$_3$)$_3$], 128.7 (Ar), 129.4 (Ar), 133.4 (quaternary Ar), 136.0 (quaternary Ar), 151.9 (*C*═O), 157.9 (*C*═O), and 162.7 (*C*═N); FAB$^+$MS *m*/*z* 415 [(M + 1)$^+$, 30%] and 259 (65); HRMS (FAB) *m*/*z* 415.1439 (M + 1)$^+$, $C_{19}H_{28}N_2O_4SCl$ requires 415.1458.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5′-bis-*tert*-butoxycarbonyl-(*N*′-4-chlorobenzyl)guanidinyl-3,14-dihydroxyindolo[2′,3′:6,7]morphinan (9f). 1,3-Bis-*tert*-butoxycarbonyl-1-(4′-chlorobenzyl-2-methyl-2-thiopseudourea (**8b**) (0.243 g, 0.59 mmol), 5′-amino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2′,3′:6,7]morphinan (**6a**) (0.126 g, 0.29 mmol), $HgCl_2$ (0.079 g, 0.29 mmol), triethylamine (0.059 g, 0.082 mL, 0.59 mmol), and DMF (10 mL) were stirred at 50 °C for 24 h. The solution was subsequently filtered and sodium bicarbonate (30 mL) added. The solution was extracted with ethyl acetate and the organic layer washed successively with water and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the crude product mixture, which was purified by column chromatography−gradient elution (CH_2Cl_2) until unreacted **8b** had been removed then [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)], affording 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5′-bis-*tert*-butoxycarbonyl-(*N*′-4-chlorobenzyl)guanidinyl-3,14-dihydroxyindolo[2′,3′:6,7]morphinan (0.103 g, 0.13 mmol, 44%): R_f = 0.47 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.16−0.27 [m, 2H, NCH$_2$CH(C*H*HC*H*H)], 0.56−0.68 [m, 2H, NCH$_2$CH(CH*H*CH*H*)], 0.88−1.00 [m, 1H, NCH$_2$C*H*(CH$_2$CH$_2$)], 1.37 [s, 9H, C(CH_3)$_3$], 1.43 [s, 9H, C(CH_3)$_3$], 5.60 [s, 1H, C(5)*H*], 6.55 [d, *J* = 8.2 Hz, 1H, C(1)*H*], 6.59 [d, *J* = 8.2 Hz, 1H, C(2)*H*], 6.70 (s, 1H, C(4′)*H*], 6.78 [d, *J* = 8.6 Hz, 1H, C(6′)*H*], 7.21 [d, *J* = 8.6 Hz, 1H, C(7′)*H*], and 7.25−7.48 [m, 4H, Ar*H*]; ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 3.4 [NCH$_2$CH-(*C*H$_2$CH$_2$)], 3.9 [NCH$_2$CH(CH$_2$*C*H$_2$)], 9.2 [NCH$_2$*C*H(CH$_2$CH$_2$)], 22.9 [*C*(10)], 27.2 [C(*C*H$_3$)$_3$], 27.6 [*C*(15)], 27.7 [C(*C*H$_3$)$_3$], 28.4 [*C*(8)], 43.5 [*C*(16)], 47.6 (*C*H$_2$Ph), 47.9 [quaternary *C*(13)], 59.1 [*C*(18)], 61.8 [*C*(9)], 72.3 [quaternary *C*(14)], 79.7 [*C*(CH$_3$)$_3$], 82.6 [*C*(CH$_3$)$_3$], 84.3 [*C*(5)], 110.2 (Ar), 111.2 (Ar), 112.0 (Ar), 116.6 (Ar), 118.1 (Ar), 118.4 (Ar), 124.2 (Ar), 126.3 (Ar), 127.8 (Ar), 128.2 (Ar), 129.9 (Ar), 130.1 (Ar), 130.2 (Ar), 130.4 (Ar), 132.8 (Ar), 135.1 (Ar), 135.3 (Ar), 139.0 (Ar), 142.6 (C═O) and 162.7 (*C*═N); FAB$^+$MS *m*/*z* 796 [(M + 1)$^+$, 100%], 696 (20), and 596 (30); HRMS (FAB) *m*/*z* 796.3463 (M + 1)$^+$, $C_{44}H_{51}N_5O_7$Cl requires 796.3477.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5′-(*N*′-4-chlorobenzyl)guanidinyl-3,14-dihydroxyindolo-[2′,3′:6,7]morphinan (10f). 17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5′-bis-*tert*-butoxycarbonyl-(*N*′-4-chlorobenzyl)guanidinyl-3,14-dihydroxyindolo[2′,3′:6,7]morphinan (**9f**) (0.100 g, 0.13 mmol) was dissolved in dichloromethane (4 mL) and allowed to stir for 10 min at 0 °C. Trifluoroacetic acid (2 mL) was added and the solution allowed to warm to room temperature. Stirring was continued for 12 h, after which the solution was concentrated under reduced pressure. Washing the resultant oil with diethyl ether afforded a precipitate that could be isolated by vacuum filtration. Further purification was achieved by recrystallization (methanol/diethyl ether) to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5′-(*N*′-4-chlorobenzyl)guanidinyl-3,14-dihydroxyindolo[2′,3′:6,7]morphinan (**10f**) as the bistrifluoroacetic acid salt (0.077 g, 0.09 mmol, 74%): mp 186−189 °C; R_f = 0.13 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3630−2475 (br, bonded OH and NH) and 1678 (br, C═N, NH and NH$_2$); ^{1}H NMR (400 MHz, CD_3OD) δ 0.48−0.59 [m, 2H, NCH$_2$CH(C*H*HC*H*H)], 0.73−0.91 [m, 2H, NCH$_2$CH(CH*H*CH*H*)], 1.09−1.22 [m, 1H, NCH$_2$C*H*(CH$_2$CH$_2$)], 5.71 [s, 1H, C(5)*H*], 6.64 [d, *J* = 8.2 Hz, 1H, C(1)*H*], 6.68 [d, *J* = 8.2 Hz, 1H, C(2)*H*], 6.98−7.02 (m, 1H, Ar*H*), and 7.28−7.46 [m, 6H, Ar*H*]; ^{13}C NMR (100.5 MHz, CD_3OD) δ 3.5 [NCH$_2$CH-(*C*H$_2$CH$_2$)], 6.3 [NCH$_2$CH(CH$_2$*C*H$_2$)], 6.9 [NCH$_2$*C*H(CH$_2$CH$_2$)], 25.1 [*C*(10)], 29.7 [*C*(15)], 30.2 [*C*(8)], 45.3 [*C*(16)], 47.5 [quaternary *C*(13)], 48.0 (*C*H$_2$Ph), 58.9 [*C*(18)], 63.6 [*C*(9)], 73.5 [quaternary *C*(14)], 84.8 [*C*(5)], 109.9 (Ar), 113.7 (Ar), 118.0 (Ar), 119.2 (Ar), 120.5 (Ar), 122.2 (Ar), 122.4 (Ar), 126.6 (Ar), 128.3 (Ar), 129.6 (Ar), 129.7 (Ar), 130.1 (Ar), 132.3 (Ar), 134.4 (Ar), 136.4 (Ar), 138.0 (Ar), 141.8 (Ar), 144.5 (Ar), and 157.5 (*C*═NH); FAB$^+$MS *m*/*z* 596 [(M + 1)$^+$, 100%]; HRMS (FAB) *m*/*z* 596.2416 (M + 1)$^+$, $C_{34}H_{35}N_5O_3Cl$ requires 596.2428. Anal. ($C_{34}H_{34}N_5O_3Cl$·2TFA·3H$_2$O) C, H, N.

***N*-Propyl-*N*′-cyclopropylmethylthiourea (12d).** Calcium carbonate (1.97 g, 19.67 mmol) was dissolved in H_2O (2 mL) and added to a stirred solution of propylamine (1.16 g, 19.67 mmol) in $CHCl_3$ (30 mL). Thiophosgene (4.52 g, 3 mL, 39.34 mmol) was added and the solution stirred at room temperature for 24 h. The aqueous layer was washed with H_2O and concentrated to give propylisothiocyanate; R_f = 0.72 [EtOAc/hexane (1:1)]. Propylisothiocyanate (0.44 g, 4.38 mmol) was then dissolved in acetone (15 mL) and added dropwise to aminomethylcyclopropane (0.31 g, 4.38 mmol) in acetone (15 mL). The solution was refluxed gently for 3 h, concentrated, and purified by column chromatography [hexane/EtOAc (1:1)], providing the desired *N*-propyl-*N*′-cyclopropylmethylthiourea (0.42 g, 2.44 mmol, 56%): R_f = 0.38 [EtOAc/hexane (1:1)]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.21−0.30 [m, 2H, NCH$_2$-CH(C*H*HC*H*H)], 0.52−0.62 [m, 2H, NCH$_2$CH(CH*H*CH*H*)], 0.97 (t, *J* = 7.3 Hz, 3H, CH_3), 1.02−1.13 [m, 1H, NCH$_2$C*H*(CH$_2$CH$_2$)], 1.64 [q, *J* = 7.3 Hz, 2H, CH_2CH$_3$), 3.30−3.41 [m, 4H, 2 × NCH_2], and 6.24 [br s, 2H, 2 × N*H*]; ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 3.5 [NCH$_2$CH(*C*H$_2$CH$_2$)] and [NCH$_2$CH(CH$_2$*C*H$_2$)], 10.0 [NCH$_2$*C*H(CH$_2$CH$_2$)], 11.3 (*C*H$_3$), 22.1 (*C*H$_2$CH$_3$), 45.9 (N*C*H$_2$), 49.4 (N*C*H$_2$CH), and 181.0 (*C*═S); FAB$^+$MS *m*/*z* 173 [(M + 1)$^+$, 100%]; HRMS (FAB) *m*/*z* 173.1116 (M + 1)$^+$, $C_8H_{17}N_2S$ requires 173.1112.

***N*-*tert*-Butoxycarbonyl-*N*-propyl-*N*′-cyclopropylmethylthiourea (13d).** *N*-Propyl-*N*′-cyclopropylmethylthiourea (**12d**) (0.41 g, 2.38 mmol) was added to NaH (0.19 g, 60% in oil, 4.75 mmol) in THF (40 mL) at 0 °C and stirred for 10 min. (*tert*-Butoxycarbonyl)$_2$O (0.60 g, 2.73 mmol) was added and the mixture stirred at room temperature for 12 h before addition of 10% NaOH and stirring for 20 min. The organic layer was then separated and the aqueous layer further extracted with EtOAc. The combined organic layers were concentrated and subsequently treated with hexane, which caused unreacted starting material to precipitate out. The solid was removed by filtration and the filtrate subsequently purified by column chromotography [EtOAc/hexane (1:3)], yielding the *N*-*tert*-butoxycarbonyl-*N*-propyl,*N*′-cyclopropylmethylthiourea (0.30 g, 1.09 mmol, 46%): R_f = 0.66 [EtOAc/hexane (1:3)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.22−0.49 [m, 4H, NCH$_2$CH(C*H*HC*H*H) and NCH$_2$CH(CH*H*CH*H*)], 0.51−0.57 [m, 1H, NCH$_2$C*H*(CH$_2$-CH$_2$)], 0.88 (t, *J* = 7.4 Hz, 3H, CH_3), 1.51 [s, 9H, C(CH_3)$_3$], 1.65 [q, *J* = 7.4 Hz, 2H, CH_2CH$_3$], 3.39−3.60 (m, 2H, NHCH_2), 4.16−4.30 (m, 2H, CONCH_2), and 10.86 (br s, 1H, N*H*); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 4.0 [NCH$_2$CH(*C*H$_2$CH$_2$)], 4.1 [NCH$_2$CH(CH$_2$*C*H$_2$)], 11.0 [NCH$_2$*C*H(CH$_2$CH$_2$)], 11.7 (*C*H$_3$),

22.3 (CH_2CH_3), 28.4 [C(CH_3)$_3$], 51.1 (HNCH_2), 57.2 (CONCH_2), 83.3 [C(CH_3)$_3$], 151.7 (C=O), and 183.0 (C=S); FAB$^+$MS m/z 273 [(M + 1)$^+$, 25%] and 217 (85); HRMS (FAB) m/z 273.1644 (M + 1)$^+$, $C_{13}H_{25}N_2O_2S$ requires 273.1637.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5′-*tert*-butoxycarbonyl-(*N*′-propyl-*N*″-cyclopropylmethyl)guanidinyl-3,14-dihydroxyindolo[2′,3′:6,7]morphinan (14d). *N*-*tert*-Butoxycarbonyl-*N*-propyl-*N*′-cyclopropylmethylthiourea (**13d**) (0.152 g, 0.56 mmol), 5′-amino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2′,3′:6,7]morphinan (**6a**) (0.120 g, 0.28 mmol), $HgCl_2$ (0.152 g, 0.56 mmol), triethylamine (0.028 g, 0.040 mL, 0.28 mmol), and DMF (10 mL) were added together at 5°C under N_2 before being stirred at 50 °C for 48 h. The solution was subsequently filtered and sodium bicarbonate (30 mL) added. The solution was extracted with ethyl acetate and then washed successively with water and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the crude product mixture, which was purified by column chromatography−gradient elution (CH_2Cl_2) until unreacted **13d** had been removed and then [CH_3OH/CH_2Cl_2/NH_4OH (10:89:1)], affording 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5′-*tert*-butoxycarbonyl-(*N*′-propyl-*N*″-cyclopropylmethyl)guanidinyl-3,14-dihydroxyindolo[2′,3′:6,7]-morphinan (0.062 g, 0.09 mmol, 33%): R_f = 0.61 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.05−0.10 [m, 2H, NCH$_2$CH(CHHCHH)], 0.11−0.20 [m, 2H, NCH$_2$CH-(CHHCHH)], 0.35−0.44 [m, 2H, NCH$_2$CH(CHHCHH)],0.46−0.58 [m, 2H, NCH$_2$CH(CHHCHH)], 0.73−0.83 [m, 1H, NCH$_2$-CH(CH$_2$CH$_2$)], 0.84−0.98 [m, 1H, NCH$_2$CH(CH$_2$CH$_2$)], 1.47 [s, 9H, C(CH_3)$_3$], 5.60 [s, 1H, C(5)H], and 6.38−7.10 (m, 5H, ArH); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 3.3 [NCH$_2$CH(CH_2CH$_2$)], 3.4 [NCH$_2$CH(CH_2CH$_2$)], 3.8 [NCH$_2$CH(CH$_2$CH_2)], 3.9 [NCH$_2$CH-(CH$_2$CH_2)], 9.4 [NCH$_2$CH(CH$_2$CH$_2$)], 10.5 [NCH$_2$CH(CH$_2$CH$_2$)], 11.4 (CH_3), 21.8 (CH_2), 23.1 [C(10)], 28.3 [C(CH_3)$_3$], 28.8 (CH_2), 31.4 (CH_2), 43.6 (CH_2), 47.9 [quaternary C(13)], 59.5 [C(18)], 62.4 [C(9)], 72.7 [quaternary C(14)], 81.0 [quaternary C(CH$_3$)$_3$], 85.0 [C(5)], 110.7 (Ar), 111.7 (Ar), 117.5 (Ar), 117.9 (Ar), 118.5 (Ar), 124.5 (Ar), 127.3 (Ar), 129.6 (Ar), 130.6 (Ar), 134.2 (Ar), 139.6 (Ar), 143.4 (Ar), 153.9 (Ar), and 154.1 (C=N); FAB$^+$MS m/z 668 [(M + 1)$^+$, 100%] and 568 (30); HRMS (FAB) m/z 668.3819 (M + 1)$^+$, $C_{39}H_{50}N_5O_5$ requires 668.3812.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5′-(*N*′-propyl-*N*″-cyclopropylmethylthiourea)guanidinyl-3,14-dihydroxyindolo[2′,3′:6,7]morphinan (15d). 17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5′-*tert*-butoxycarbonyl-(*N*′-propyl-*N*″-cyclopropylmethyl)guanidinyl-3,14-dihydroxyindolo[2′,3′:6,7]morphinan (**14d**) (0.046 g, 0.07 mmol) was treated as described for **10f**, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5′-(*N*′-propyl-*N*″-cyclopropylmethyl)-guanidinyl-3,14-dihydroxyindolo[2′,3′:6,7]morphinan (**15d**) as the bistrifluoroacetic acid salt (0.039 g, 0.07 mmol, 99%): mp 177−178 °C; R_f = 0.24 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3700−2620 (br, bonded OH and NH) and 1678 (br, C=N, NH and NH$_2$); ^{1}H NMR (400 MHz, CD_3OD) δ 0.12−0.21 [m, 2H, NCH$_2$CH(CHHCHH)], 5.65 [s, 1H, C(5)H], 6.56 [d, J = 8.2 Hz, 1H, C(1)H], 6.59 [d, J = 8.2 Hz, 1H, C(2)H], 6.93 [dd, J_1 = 8.6 Hz, J_2 = 1.9 Hz, 1H, C(6′)H], 7.25 [d, J = 1.9 Hz, 1H, C(4′)H], and 7.38 [d, J = 8.6 Hz, 1H, C(7′)H]; ^{13}C NMR (100.5 MHz, CD_3OD) δ 3.7 [NCH$_2$CH(CH_2CH$_2$)], 4.2 [NCH$_2$CH(CH_2CH$_2$)], 6.5 [NCH$_2$CH(CH$_2$CH_2)], 7.1 [NCH$_2$CH-(CH$_2$CH$_2$)], 11.5 [NCH$_2$CH(CH$_2$CH$_2$)], 11.7 (CH_3), 23.6 [C(10)], 25.3 (CH_2), 30.0 (CH_2), 30.5 (CH_2), 44.6 [C(16)], 47.6 (CH_2), 47.9 [quaternary C(13)], 59.2 [C(18)], 63.9 [C(9)], 73.9 [quaternary C(14)], 85.2 [C(5)], 110.3 (Ar), 114.2 (Ar), 118.6 (Ar), 119.7 (Ar), 120.9 (Ar), 122.8 (Ar), 122.9 (Ar), 127.4 (Ar), 129.0 (Ar), 130.6 (Ar), 132.9 (Ar), 138.5 (Ar), 142.4 (Ar), 145.1 (Ar), and 156.6 (C=N); FAB$^+$MS m/z 568 [(M + 1)$^+$, 100%]; HRMS (FAB) m/z 568.3275 (M + 1)$^+$, $C_{34}H_{42}N_5O_3$ requires 568.3288. Anal. ($C_{34}H_{41}N_5O_3$·2TFA·2H_2O) C, H, N.

Biological Methods. The methods utilized have been reported previously.[29]

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Supporting Information Available: Spectra and experimental procedures for all compounds. This material is available free of charge via the Internet at https://pubs.acs.org.

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