2-Haloaporphines as Potent Dopamine Agonists

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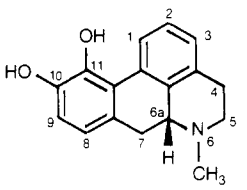
The synthesis of 2-amino- and 2-halo-substituted aporphines is described. The key step is the substitution of a hydroxy group in the 2-position with an amino group effected by a Smiles rearrangement reaction of the 2-methylpropanamide derivative **6**. The affinity of the new compounds for the dopamine D-2 receptor in the anterior pituitary gland was evaluated. 2-Fluoroapomorphine was the most potent compound, being 1.5 times more potent than (−)-apomorphine. The structure–activity relationships are discussed in relation to a previously proposed receptor model.

Since the discovery of useful dopamine (DA) agonist activity in hydroxylated aporphine alkaloids such as (*R*)-(−)-apomorphine (I),[1] continuing interest has developed in delineating the portions of the aporphine molecular structure responsible for dopaminergic properties and the interactions with DA receptors.[2,3] (*R*)-Apomorphine (APO) and its (*R*)-*N*-*n*-propyl analogue (NPA) have been reported to be the active enantiomer for DA agonist activity whereas the *S*-(+) enantiomer of APO[4] and NPA[5,6] have been reported to show antagonistic activity. The tetracyclic ring system of APO has served as an excellent model of the study of DA–receptor interactions, particularly since the catechol ring and the amino group analogous to those of DA are held in rigid conformation and multiple sites for chemical substitution are available in the aporphine system. We have thus used the aporphine ring system as a template for studying DA receptors.[2,3,5,6] In 1981 we reported that 2-hydroxyapomorphine (THA) derivatives were potent DA agonists in several biochemical and pharmacological test systems although the 2-OH group reduced binding affinity and pharmacological potency.[7,8] These and previous studies led to the suggestion that the 6a*R* configuration and the 10,11-hydroxy groups enable aporphine molecules to fit the presumptive DA receptor,[5] whereas an additional 2-hydroxy moiety interferes with intrinsic agonist activity. The consequence of replacing other substituents such as halogen or amino groups at the 2-position of apomorphine serves as the basis of the present study.



I

Chemistry

In exploration of methods for the preparation of 2-halo-substituted aporphines several approaches were considered. Nitration of apomorphines yield primarily 8-nitro-substituted apomorphines.[9] An attractive approach seemed to be the selective substitution of the 2-hydroxy group in trihydroxyapomorphine (THA) with an amino group, which could then easily be transformed to halogens by using the Sandmeyer reaction. Most of the methods for the transformation of a phenol derivative to the corresponding aniline use rather drastic reaction conditions and are limited in scope. Two general methods have been reported for this conversion. One was based on the thermal rearrangement of 4-(aryloxy)-2-phenylquinazolines,[10] and the other was a rearrangement of aryl diethyl phosphates by potassium/potassium amide in liquid ammonia.[11] It might be anticipated that these methods would not be applicable to the aporphines, the main risk being ring opening leading to formation of phenanthrene derivatives.[12] However a facile procedure for this conversion was based on a Smiles rearrangement reaction.[13] Generally, Smiles rearrangements have required aromatic ring systems activated to nucleophilic attack by electron-withdrawing substituents. However, by use of 2-(aryloxy)-2-methylpropanamides, e.g., **6** (Scheme I), and hexamethylphosphoric triamide (HMPA) as a solvent, activated anions were generated and the scope of the Smiles rearrangement was extended to include nonactivated and deactivated aromatic ring systems as reported by Bayles et al.[14] In this latter case the α-methyl groups were essential for the reaction to occur. We successfully adapted a modification of this reaction for the preparation of the key intermediate, the 2-amino aporphine derivative **8**. We have previously used a methylenedioxy function to protect the catechol grouping in

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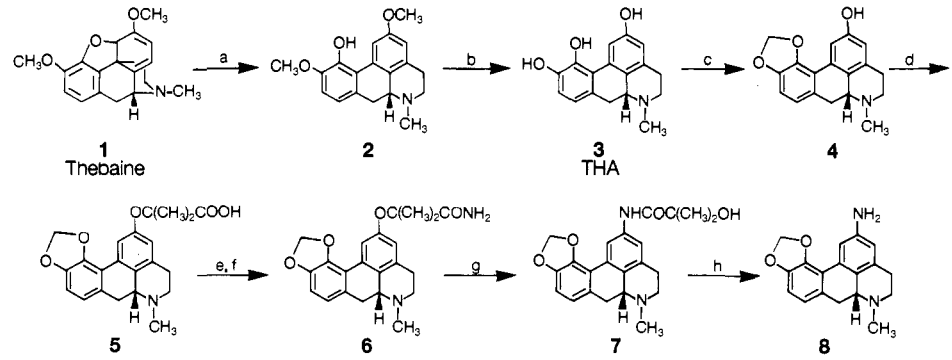
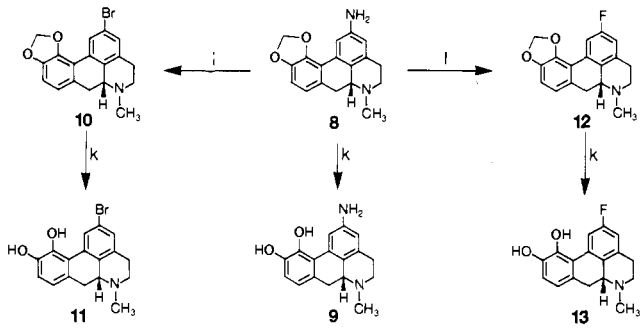
Scheme I[a]



[a] Reagents: (a) CH_3SO_3H; (b) HBr; (c) CH_2Br_2, DMSO; (d) $(CH_3)_2CO$, $CHCl_3$, NaOH; (e) $SOCl_2$; (f) NH_3; (g) NaH, HMPA; (h) HCl.

Scheme II[a]



[a] Reagents: (i) $NaNO_2$, CuBr, Δ; (k) BBr_3, CH_2Cl_2; (l) $NaNO_2$, HPF_6, Δ.

aporphines[15] for the further manipulation of the 2-hydroxy group in THA (**3**).

The synthetic route is shown in Scheme I. The readily available opioid thebaine (**1**) was rearranged in methanesulfonic acid, which has been found to be superior to hydrochloric acid used previously.[16] The product, 2,10-dimethoxy-11-hydroxyaporphine (**2**) was demethylated with refluxing hydrogen bromide to the trihydroxyaporphine (**3**). The catechol grouping was protected for further transformation as a methylenedioxy group by using methylene dibromide in dimethyl sulfoxide. The 2-hydroxy methylenedioxy compound **4** was treated with sodium hydroxide and chloroform in acetone to give the 2-methylpropanoic acid derivative (**5**). This acid was converted to the corresponding amide (**6**) via the acid chloride. The Smiles rearrangement reaction of the propionamide (**6**) was effected with sodium hydride in HMPA with retention of the configuration at the 6a carbon. The rearranged product (**7**) was not isolated but was subjected to acid hydrolysis to give the 2-aminoaporphine (**8**), which was characterized as a mono(hydrochloride) salt.

The further conversion of the protected 2-amino derivative (**8**) to the 2-bromo compound (**10**) and the 2-fluoro compound (**12**) is depicted in Scheme II. The methylenedioxy-protected halogen derivatives were prepared by using the Sandmeyer reaction with cuprous bromide for the synthesis of the bromo compound (**10**). The fluoro compound was obtained by the Schiemann reaction through the thermal decomposition of the diazonium hexafluorophosphate salt. The target catechol derivatives **9**, **11**, and **13** were synthesized by demethylenation with boron tribromide in methylene chloride.

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Table I. Affinities and Proportions of High- and Low-Affinity States of the Dopamine Receptor of the Porcine Anterior Pituitary As Recognized by Aporphines

compd	R	K_D^{high}, nM	K_D^{low}, nM	D-2^{high}, %	D-2^{low}, %
9	NH_2	19.9	3300	38	62
11	Br	6.75	728	49	51
13	F	0.43	94.3	32	68
(−)-apomorphine	H	0.66	127	50	50

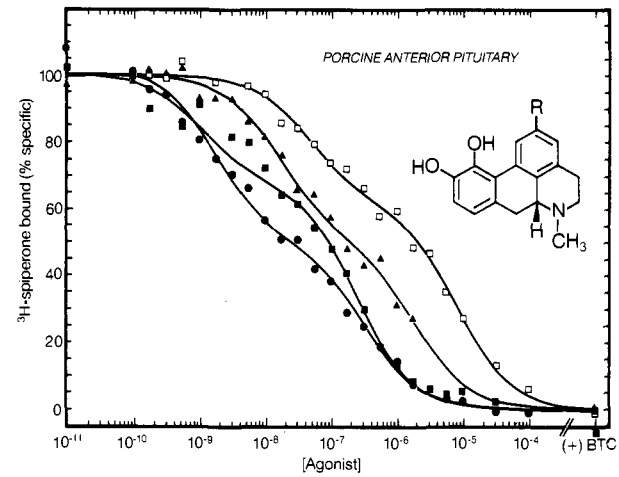

Figure 1. Competition curves of aporphines for the binding of [^{3}H]spiperone. The symbols denote the following: (□) compound **9**, R = NH_2; (▲) compound 11, R = Br; (■) compound **13**, R = F; (●) (−)-apomorphine.

Results and Discussion

Pharmacological and biochemical evidence indicate that DA receptors can be divided into two categories, D-1 and D-2 receptors.[17] The D-1 receptors are positively coupled to adenylate cyclase, whereas the D-2 receptors are uncoupled or negatively coupled to adenylate cyclase.[18] Moreover, each dopamine receptor can exist in two states, high- and low-affinity states defined by the affinity for the agonist dopamine.[19] It has been suggested that the D-2 high-affinity state is the functional state in the anterior pituitary gland.[20]

The new aporphine compounds were tested for their ability to displace [^{3}H]spiperone in porcine anterior pi-

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tuitary gland. The results are summarized in Table I, showing the affinity and proportions of the high- and low-affinity states of the D-2 receptor. Also, the competition curves for the 2-substituted aporphines for the binding of [^{3}H]spiperone are shown in Figure 1.

By substituting the hydrogen atom in the 2-position of apomorphine with an amino group, the affinity for the D-2 receptor decreases about 30-fold. However, introduction of halogen atoms increases the affinity compared to the amino compound, the 2-bromo derivative being 10-fold less potent than apomorphine. In the 2-fluoro compound the potency is restored to the level of apomorphine, and this compound is about 1.5 times more active than apomorphine itself. About the same potency ratios were found for the low-affinity state.

On the basis of structure–activity relationship studies for many different aporphines and related DA agonists a hypothetical receptor model has been proposed.[5] The main features are the M_1 and M_2 binding sites capable of interacting with the hydroxyl groups on the agonist molecule, an M_3 site that interacts with an OH substituent in the 2-position of ring A, and an electronegative site B that interacts with the protonated nitrogen atom on the agonist. Substituents on the nitrogen atom, e.g., a propyl group, could fit in a lipophilic cavity on the receptor. Also, steric factors on the recognition site have been proposed to account for the reduced potencies of O-methylated aporphines and the enantioselectivity exerted by the aporphines.

It has been shown previously that introducing a hydroxyl group in the 2-position of the aporphines leads to a reduced affinity for the D-2 receptor measured as the ability to displace [^{3}H]ADTN and [^{3}H]apomorphine from calf caudate membranes.[3] For example, (–)-2,10,11-trihydroxy-*N*-*n*-propylnorapomorphine [(–)-TNPA] has an IC_{50} value of 5.5 nM in the [^{3}H]ADTN binding assay compared to (–)-10,11-dihydroxy-*N*-*n*-propylnorapomorphine [(–)-NPA] with an IC_{50} = 2.0 nM. The corresponding values for (–)-THA is 1.1 nM and 0.66 for apomorphine in the displacement of [^{3}H]spiperone (cf. reference 21 and Table I). Substituting the 2-OH group in THA with an amino group decreases the affinity for the D-2 receptor even further, i.e., about 20-fold.

A number of explanations could account for the substituent effects observed at C-2. There may be a π stacking interaction between the receptor and the A-ring of the aporphines. This interaction could be reinforced by the small, electron-withdrawing fluoro group and disrupted by hydroxyl, amino, or bromo substituents through either steric or electronic factors. Alternatively, there may be a hydrophobic pocket in the receptor into which the polar hydroxy and amino groups are not readily incorporated (the bromine group being excluded because of its size).[22] 2-Fluoroapomorphine (**13**) is more potent than apomorphine and is one of the most active aporphines prepared so far. Further work is in progress to further elucidate the effect of substituents in the 2-position of the aporphine ring and to further enhance the biological effects of such aporphines.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. TLC was performed with precoated silica gel 13181 foils (Eastman-Kodak, Rochester, NY) to follow the reactions and check the purity of the products. The spots were visualized by staining with iodine vapors. NMR (Varian T-60) and mass spectra (12-90-G Nuclide) were consistent with the assigned structures. Optical rotations were obtained on a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by Atlantic Microlaboratories, Atlanta, GA, and by Analytische Laboratorium, Elbach, West Germany. Analytical results indicated by elemental symbols were within ±0.4% of the theoretical values.

2-Hydroxy-10,11-(methylenedioxy)apomorphine (4). Finely ground NaOH (4.8 g, 0.12 mol) was added to a solution of 2,10,11-trihydroxyapomorphine hydrobromide (**3**) (14.5 g, 0.04 mol), prepared from thebaine (1) as previously described,[16] in 500 mL of dry DMSO at ambient temperature under N_2. Stirring was continued for 1 h. Methylene dibromide (9.1 g, 0.052 mol) was added, and the mixture was heated at 80 °C for 4 h. After cooling, the solution was poured onto ice water and extracted with AcOEt. Drying ($MgSO_4$) and evaporation of the organic solvent under reduced pressure gave 9.6 g of an oil. The title compound was isolated by flash chromatography (SiO_2 0.04–0.66 mm, 600 g) and 5% MeOH in CH_2Cl_2 as eluent. Recrystallization of the product from AcOEt afforded 7.2 (61%) of the desired compound **4**, mp 193–195 °C dec. Anal. ($C_{18}H_{17}NO_3$) C, H, N.

2-[(Aminocarbonyl)dimethylmethoxy]-10,11-(methylenedioxy)aporphine (6). The MDO-phenol **4** (2.95 g, 10 mmol) was suspended in 50 mL of dry acetone at room temperature under nitrogen. Pulverized NaOH (2.04 g, 66 mmol) was added in two portions, and the reaction mixture was heated to reflux. Chloroform (1.78 g, 15 mmol) was added dropwise, and refluxing was continued for 4 h. After cooling, 5 mL of water was added, the solution was concentrated in vacuo, and the aqueous solution was cooled and acidified with concentrated HCl. The solvent was evaporated and the residue dried azeotropically by evaporation of EtOH and then $CHCl_3$. The acid hydrochloride (4.9 g crude yield) was used directly in the next step.

The crude propionic acid derivative **5** (4.0 g) was dissolved in 50 mL of $SOCl_2$ and stirred at room temperature overnight. The excess of thionyl chloride was removed under reduced pressure. The crude acid chloride was dissolved in 20 mL of dry THF and added dropwise with stirring to an ice-cooled concentrated ammonia solution. After the addition, the solution was allowed to reach room temperature, and stirring was continued for an additional 2 h. The solution was concentrated in vacuo, and the aqueous solution was extracted with AcOEt. The combined extracts were dried ($MgSO_4$) and evaporated, yielding 4.2 g of dark brown oil. The amide **6** was isolated by flash chromatography on a silica column (150 g, 0.040–0.063 mm) with 5% MeOH in CH_2Cl_2 as eluent to yield 2.3 g (60%) of a light brown oil that crystallized on standing. Recrystallization from benzene gave 1.9 g of **6**: mp 195–197 °C; MS (EI, 70 eV) *m/z* (rel intensity) 380 (M, 32%), 379 (21%), 295 (22%), 294 (100%), 293 (13%), 252 (12%), 236 (11%). Anal. ($C_{22}H_{24}N_2O_4$) C, H, N.

2-Amino-10,11-(methylenedioxy)aporphine Hydrochloride (8). The amide **6** (3.0 g, 7.8 mmol) was dissolved in 50 mL of dry HMPA and treated with NaH (50% in oil, 0.42 g) under N_2 at room temperature. The solution was heated at 100 °C with stirring for 1 h. The reaction mixture was cooled, poured into ice water, and extracted with AcOEt. The combined extract was washed with water and dried ($MgSO_4$). Removal of the solvent gave 3.6 of crude hydroxy amide **7**.

This crude amide (3.0 g) was dissolved in 50 mL of 5 M HCl solution and heated at 100 °C for 1 h. After evaporation of the solvent, the residue (2.9 g) was treated with 5 mL of EtOH and stirred for 1 h at room temperature. AcOEt (5 mL) was added to the suspension, and the precipitate was collected by filtration and recrystallized from 95% EtOH to give 1.7 g of the amine mono(hydrochloride) **8**: mp 252–253 °C dec; $[\alpha]^{25}_D$ = –53.2° (*c* = 0.44, MeOH); MS *m/z* (rel. intensity) 294 (M, 58%), 293 (100%), 292 (13%), 251 (12%), 235 (11%). Anal. ($C_{18}H_{19}ClN_2O_2$) C, H, Cl, N.

2-Bromo-10,11-(methylenedioxy)aporphine (10). To a solution of the amine hydrochloride **8** (750 mg, 2.3 mmol) in 3 mL of 48% aqueous HBr solution at 0 °C was added dropwise with stirring a solution of $NaNO_2$ (157 mg, 2.3 mmol) in 0.1 mL of H_2O. The reaction mixture was stirred for 1 h at 0 °C. Cuprous bromide dissolved in 0.5 mL of 48% HBr was added, and the solution was heated at 80 °C for $^1/_2$ h. After cooling, 3 mL of water was added and the reaction mixture was made alkaline with concentrated

(22) We acknowledge with thanks this suggestion by one of the reviewers of the manuscript.

ammonia. The precipitate was filtered off, washed with water, and dried. The product was triturated with AcOEt, and the insoluble material was collected by filtration. The solvent was removed under reduced pressure to give 580 mg of product. TLC analysis of the product (CH_2Cl_2–MeOH 9:1) showed it to consist of two major components with R_f values 0.74 and 0.63. These were separated by chromatography on a SiO_2 column (30 g, 0.040–0.060 mm) and eluted with AcOEt. The compound with the higher R_f value is a dibrominated compound as indicated by mass spectrometry. The compound with the lower R_f value is the desired monobromo derivatives (**10**): yield 295 mg (36%); mp 163–164 °C; MS m/z (rel. intensity) 359/357 (M, 65/75%), 358/356 (100/92%), 278 (41%), 235 (39%), 176 (24%), 110 (33%). Anal. ($C_{18}H_{16}BrNO_2$) C, H, Br, N.

2-Fluoro-10,11-(methylenedioxy)aporphine (12). A solution of the 2-aminoaporphine hydrochloride **8** (130 mg, 0.4 mmol) in 2 mL of water was treated with a solution of $NaNO_2$ (35 mg, 0.5 mmol) in 0.1 mL of water dropwise with stirring at 0 °C. After $^1/_2$ h 75% HPF_6 (130 mg, 0.62 mmol) was added. The suspension was diluted with 1 mL of water, and stirring was continued for 1 h at 0 °C. The reaction mixture was filtered, and the precipitate was washed with MeOH–Et_2O (1:10) and dried over P_2O_5 in vacuo. The dry diazonium salt was decomposed portionwise by heating at 170 °C for 10 min. After cooling, a saturated Na_2CO_3 solution was added, and the mixture was extracted with AcOEt and dried ($MgSO_4$). Evaporation of the solvent afforded 95 mg of crude material. The fluoro compound was isolated by chromatography on a SiO_2 column (10 g) with AcOEt as eluent: yield 65 mg (55%); mp 185–187 °C. Anal. ($C_{18}H_{16}FNO_2$) C, H, F, N.

General Method for the Demethylenation Reaction 2-Aminoapomorphine Hydrobromide (9). To a solution of the MDO-amine **8** (15.0 mg, 0.5 mmol) in 5 mL of CH_2Cl_2 was added dropwise with stirring 1 mL of 1 M BBr_3 in CH_2Cl_2 (1 mmol) under nitrogen at ambient temperature. Stirring was continued overnight. Methanol (1 mL) was added, and the solvent was evaporated in vacuo. The resulting product was recrystallized from methanol–ether, affording 110 mg (63% of the 2-aminoapomorphine hydrobromide: mp 216–218 °C; MS m/z (rel. intensity) 282 (M, 57%), 281 (100%), 280 (24%). Anal. ($C_{17}H_{19}BrN_2O_2$) C, H, Br, N.

2-Bromoapomorphine Hydrobromide (11). This compound was prepared from the corresponding MDO derivative (**10**) (70 mg, 0.2 mmol) as described from the amino compound above: yield 45 mg (54%); mp >300 °C dec; MS m/z (rel. intensity) 347/345 (M, 65/85%), 346/344 (100/97%), 266 (73%), 223 (41%), 110 (35%). Anal. $C_{17}H_{17}Br_2NO_2$) C, H, Br, N.

2-Fluoroapomorphine Hydrobromide (13). The title compound was synthesized from the MDO derivative (**12**) (60 mg, 0.2 mmol) as described for the amino compound above: yield 42 mg (57%); mp >300 °C dec; MS m/z (rel. intensity) 285 (M, 67%), 284 (1005), 266 (13%), 242 (22%), 238 (13%). Anal. ($C_{17}H_{17}BrFNO_2$) C, H, Br, F, N.

Receptor Binding Assay. The dissociation constants (K_D values) for various agonist congeners at the high- and low-affinity states of the dopamine D-2 receptor were obtained by using [^{3}H]spiperone as follows.[23,24]

Pig anterior pituitaries (Bocknek Organic Material, Rexdale, Ontario, Canada) were stored at −70 °C. After thawing, the pituitary tissue was dissected free of neurointermediate lobe and attached hypophyseal stalk. The tissues were minced and homogenized (Brinkmann Polytron, 25 s, setting 7, full power being 10) in 20 volumes of buffer. The buffer contained 50 mM Tris-HCl (pH 7.4 at 20 °C), 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2 \cdot 6H_2O$, 1 mM EDTA, and 12 M nialamide. NaCl was omitted in order to permit the [^{3}H]spiperone to bind to the high-affinity state of D-2; it is known that 100 mM NaCl assists in converting high-affinity D-2 receptors ($D\text{-}2^{high}$) into $D\text{-}2^{low}$ receptors which have low affinity for dopamine.[23,24] The pituitary homogenate was passed through cheesecloth and centrifuged at 480g for 5 min at 0 °C. The supernatant was centrifuged at 49500g for 30 min at 0 °C, and the pellet was resuspended in buffer. This suspension was rehomogenized by Polytron for 10 s, preincubated for 10 min at 37 °C, and then put on ice for 45 min. The binding of [^{3}H]-spiperone (22–30 Ci/mmol; New England Nuclear, Boston, MA) to the homogenate was done at a final concentration of 200 pM in buffer containing 0.1% ascorbic acid. The incubation was started by adding 100 L of homogenate into tubes containing the test drug and [^{3}H]spiperone; the final volume was 5 mL (4 mg of original wet tissue/final mL). The tubes were incubated for 75 min at 20 °C.

The suspensions were then filtered (12 tubes simultaneously) by a cell harvester (Skatron, Lier, Norway) using two glass fiber filter mats stapled together (Skatron no. 7031, Sterling, VA) and a vacuum of 400–500 mmHg. The filter mat was rinsed for 15 s with 7 mL of 500 mM Tris-HCl (pH 7.4 at 20 °C). The filter circles were placed in liquid scintillation minivials along with 4 mL of scintillation fluid (Beckman Ready Solv EP). After 12 h of shaking (100 rpm at 4 °C), the vials were monitored for tritium in a refrigerated Packard 460C liquid scintillation spectrometer at 35% efficiency. Specific binding of [^{3}H]spiperone was defined as that binding which was inhibited by the presence of 1 M (+)-butaclamol (Research Biochemicals, Inc., Natick, MA). The K_D of [^{3}H]spiperone was 130 pM in the absence of NaCl and 64 pM in the presence of 100 mM NaCl. The competition data were analyzed by using the LIGAND program.[25] The program provided two statistical criteria to judge whether a two-site fit was better than a one-site fit or whether a three-site fit was better than a two-site fit.

Acknowledgment. This work is supported in part by USPHS Grants NS-15439 (to J.L.N) and BRSG SO 7 RR 0530-05 and by grants from the Medical Research Council (to D.G. and P.S.). Permission to complete the chemistry part of this work at Astra Alab AB is gratefully acknowledged. We also wish to thank Mallinckrodt Inc. for the generous gift of the thebaine alkaloid.

Registry No. **3**·HBr, 82331-73-5; **4**, 88247-26-1; **5**·HCl, 119771-33-4; **6**, 119771-34-5; **7**, 119771-35-6; **8**·HCl, 119771-36-7; **9**, 119771-32-3; **9**·HBr, 119771-37-8; **10**, 119771-38-9; **11**, 105013-79-4; **11**·HBr, 99307-14-9; **12**, 119771-39-0; **13**, 119771-41-4; **13**·HBr, 119771-40-3.

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