Synthesis and Radiolabeling of (*S*)-4-Amino-5-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide, the Active Enantiomer of [^{125}I]Iodozacopride, and Re-evaluation of Its 5-HT$_3$ Receptor Affinity

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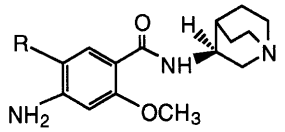
We report an improved synthesis of unlabeled (*S*)-iodozacopride, the radiolabeling of (*S*)-[^{125}I]iodozacopride *via* deschloro-(*S*)-zacopride, and a re-evaluation of its affinity for the 5-HT$_3$ receptor. Unlabeled (*S*)-iodozacopride was prepared in seven steps from 4-aminosalicylic acid *via* alkaline hydrolysis of its 4-acetamide derivative. Catalytic hydrogenation of (*S*)-iodozacopride gave deschloro-(*S*)-zacopride, identical to that obtained from (*S*)-3-amino-quinuclidine and 4-amino-2-methoxybenzoic acid *via* its corresponding 1-imidazole derivative. Radioiodination to produce (*S*)-[^{125}I]iodozacopride was accomplished by treatment of deschloro-(*S*)-zacopride with 5 mCi sodium ^{125}iodide and chloramine-T in hydrochloric acid. Purification of the reaction products using an HPLC system capable of detecting chlorinated side-products revealed a mixture of 2.1 mCi (1.3 nmol) (*S*)-[^{125}I]iodozacopride and (*S*)-zacopride (1.5 nmol). Saturation analysis of the binding of the purified (*S*)-[^{125}I]iodozacopride to whole rat brain homogenates gave an estimated K_D of 1.10 ± 0.07 nM. As anticipated, this is approximately half the K_D reported for binding of racemic [^{125}I]iodozacopride, and differs from the previously reported value by an order of magnitude. Analysis of the apparent binding affinity of a 1 : 1 mixture of (*S*)-[^{125}I]iodozacopride and (*S*)-zacopride suggests that the previous result may have been confounded by contamination of the product with unlabeled (*S*)-zacopride. Competition analysis of the displacement of (*S*)-[^{125}I]iodozacopride binding by unlabeled (*S*)-iodozacopride and (*S*)-zacopride gave K_i values of 0.95 and 0.21 nM, respectively.

Key words 5-HT$_3$ receptor affinity; (*S*)-iodozacopride; radioligand binding

The 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor antagonist zacopride[1,2] and its corresponding 5-iodo analogue, 4-amino-5-iodo-2-methoxy-*N*-(1-azabicyclo-[2.2.2]oct-3-yl)benzamide (iodozacopride),[3] have been used extensively to study 5-HT$_3$ receptors in the central nervous system (CNS) and periphery.[1-7] Autoradiographic and biochemical studies of CNS binding sites using tritium-labeled [^{3}H]zacopride have been limited, however, because of low 5-HT$_3$ receptor densities (*ca.* 1 fmol/mg tissue)[3,4] and moderate specific activity (maximum 87 Ci/mmol) of [^{3}H]zacopride. Although radiolabeled [^{125}I]iodozacopride has the potential advantage of high specific activity (maximum 2170 Ci/mmol) and a 60 d half-life that provides a convenient shelf-life for *in vitro* investigations,[3] the synthesis of unlabeled iodozacopride and its radioiodination[8,9] have certain shortcomings. Koscielniak *et al.*[8] synthesized iodozacopride hydrochloride from deschlorozacopride, using *N*-chlorosuccinimide in chloroform. This method requires preparative HPLC purification, and the synthesis of a precursor, deschlorozacopride, was not described. Ponchant *et al.*[9] found that the iodine atom could be introduced into methyl 4-amino-2-methoxybenzoate, which was subsequently converted to iodozacopride *via* its trimethylaluminum complex. Each of these steps requires column chromatography to remove impurities which is cumbersome and inefficient.

Radiolabeled racemic [^{125}I]iodozacopride was synthesized by Koscielniak *et al.*[8] and by Ponchant *et al.*[9] using *N*-[^{125}I]iodosuccinimide as the iodinating reagent. Synthesis of the precursor was not described and the compound was reportedly obtained from Delalande (Rueil Malmaison, France).[8,9] The estimated affinities (K_D) of (*RS*)-[^{125}I]iodozacopride for the 5-HT$_3$ receptor were 2.6 and 4.3 nM.[8,9] This is six to ten times lower than that of racemic [^{3}H]zacopride (0.50—0.76 nM),[1,2] and is consistent with the decrease in affinity seen in similar 5-HT$_3$ receptor ligands when an iodine atom is substituted for a chlorine atom in the aromatic 5-position.[10,11]

Only the (*S*)-enantiomers of these ligands have substantial antagonist activity at the 5-HT$_3$ receptor.[4,6,7] (*S*)-[^{125}I]Iodozacopride is commercially available from Amersham International and is used to characterize interactions at the 5-HT$_3$ receptor. However, data for the racemic compound,[3] rather than that of the *S*-form, are referred to in describing its pharmacological properties (Amersham Life Sciences 1996). Gehlert *et al.*[12] reported the synthesis of the active *S* enantiomer of [^{125}I]iodozacopride, using the precursor deschloro-(*S*)-zacopride, prepared by catalytic hydrogenation of (*S*)-zacopride. The isolation and purification of the precursor was not reported. This group characterized the binding affinity of their product using saturation analysis and displacement of binding with other known 5-HT$_3$ receptor ligands. Surprisingly, the reported K_D for their labeled compound was 0.19 nM,[12] which is fourteen to twenty-two times higher than that reported for the racemate,[8,9] and



(*S*)-iodozacopride (R = I)
deschloro-(*S*)-zacopride (R = H)
(*S*)-zacopride (R = Cl)

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approximately twice that of (*S*)-[^{3}H]zacopride (0.21—0.67 nM).[4,6,7,10,13] These latter results are remarkable in that unlabeled (*RS*)-iodozacopride (K_i 6.1 nM) was ten times less active than (*RS*)-zacopride (K_i 0.60 nM).[8] Thus, the true affinity of (*S*)-[^{125}I]iodozacopride for the 5-HT$_3$ receptor is open to question. Since the validity of the conclusions reached by those who employ (*S*)-[^{125}I]iodozacopride in research is dependent upon a precise estimate of the affinity of this ligand at the 5-HT$_3$ receptor, it is imperative that an accurate estimate of this affinity be determined.

We now report an efficient synthesis and chemical characterization of deschloro-(*S*)-zacopride and unlabeled (*S*)-iodozacopride, as well as improved radiolabeling of the active enantiomer of [^{125}I]iodozacopride. We have also repeated the radiolabeling experiment of Gehlert *et al.* using an HPLC system capable of separating chlorinated and iodinated side-products, and re-examined the affinity of (*S*)-[^{125}I]iodozacopride for the 5-HT$_3$ receptor in rat brain.

Synthesis

Dialkylation of 4-aminosalicylic acid with dimethyl sulfate in the presence of potassium hydroxide gave methyl 4-amino-2-methoxybenzoate, **1**, as described by Murakami *et al.* (Chart 1).[14] Subsequent hydrolysis of **1** to the corresponding acid, **2**, and transformation to the reactive imidazole derivative (not isolated), followed by coupling with (*S*)-3-aminoquinuclidine (3-AQN)[15] gave deschloro-(*S*)-zacopride. The low lipophilicity of deschloro-(*S*)-zacopride made its extraction with ether inefficient. Instead, the crude product was purified by elution with a 1 : 1 mixture of ethyl acetate and ethanol through a short silica column, or by extraction with chloroform.

(*S*)-Iodozacopride was prepared by alkaline hydrolysis of the corresponding 4-acetamide derivative obtained by a modification of the method of Koscielniak *et al.*[8] Thus, 4-aminosalicylic acid was converted to its methyl ester, **3**, by treatment with sulfuric acid in methanol according to Drain *et al.*[16] The salicylate, **3**, was reacted with acetyl chloride in pyridine to give the corresponding *N*-acetyl derivative, **4**.[16] Compound **4** was then iodinated in the 5-position by a method similar to that used for the synthesis of epidepride and (*S*)-5-iodo-2,3-dimethoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide (MIZAC).[10,17] Iodine chloride was generated *in situ* by addition of chloramine-T (sodium *N*-chloro-4-methylbenzenesulfonate) to sodium iodide in dimethylformamide (DMF).[17] The resulting methyl 4-acetamido-5-iodosalicylate, **5**, was alkylated with dimethyl sulfate to give ester **6**. Care was taken to avoid the use of excess alkylating reagent with a prolonged reaction time, which produces the undesired *N*-methylacetamide derivative as a byproduct. Hydrolysis of **6** in 0.5 M sodium hydroxide gave 4-acetamido-5-iodo-2-methoxybenzoic acid, **7**. Use of stronger base or prolonged heating caused unwanted hydrolysis of the

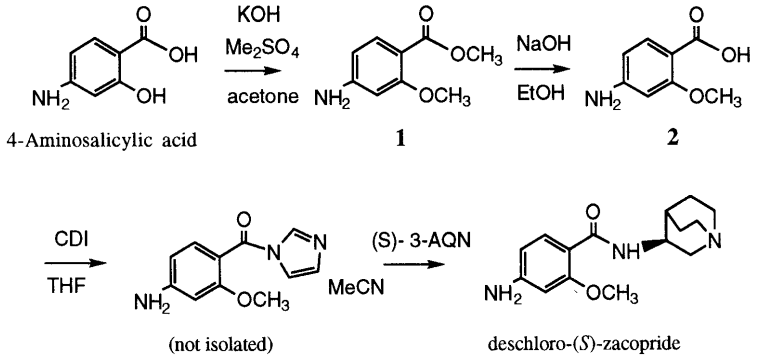

Chart 1

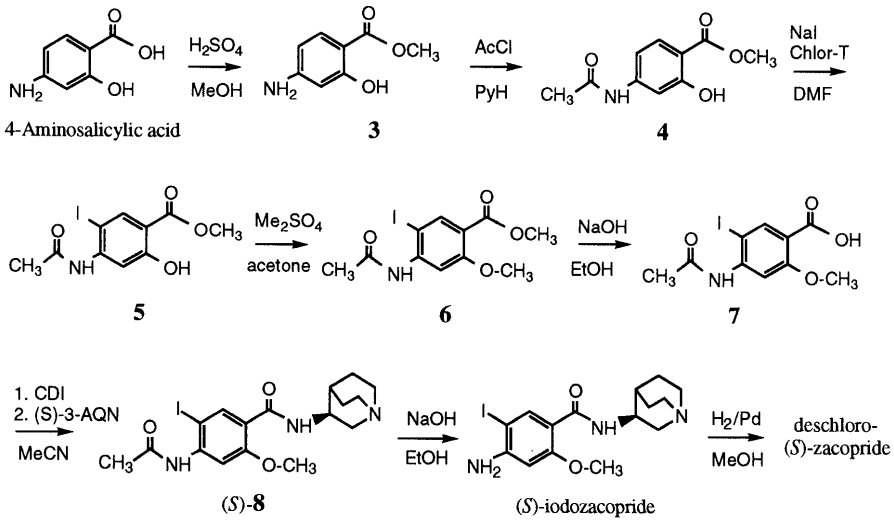

Chart 2

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acetyl group. The acid **7** was converted to the corresponding *N*-imidazolobenzamide (not isolated) and reacted with (*S*)-3-AQN to give (*S*)-N^4-acetyl-5-iodozacopride, (*S*)-**8**. Acid hydrolysis of (*S*)-**8** resulted in undesired partial deiodination as detected by NMR. In contrast, hydrolysis of the acetyl group of (*S*)-**8** with 5 M sodium hydroxide gave exclusively the desired (*S*)-iodozacopride. The UV spectrum of (*S*)-iodozacopride was identical to that reported for racemic iodozacopride, prepared from methyl 4-amino-5-iodo-2-methoxybenzoate.[9] Palladium-catalyzed hydrogenation of (*S*)-iodozacopride under acidic conditions gave deschloro-(*S*)-zacopride, identical to that above (Chart 1).

Results and Discussion

In contrast to the previously reported methods,[8,9] synthesis of (*S*)-iodozacopride *via* its 4-acetamide derivative, (*S*)-**8** can readily be scaled up without major changes in the purification conditions. In the radiolabeling of (*S*)-iodozacopride, Gehlert *et al.* reported successful use of chloramine-T as the oxidizing agent in neutral phosphate buffer.[12] In our hands, however, no radiolabeled product could be obtained using these conditions. Reacting 15 nmol deschloro-(*S*)-zacopride and 110 nmol chloramine-T in 50 μl 0.2 M Na_2HPO_4 buffer at pH 7.5 failed to produce (*S*)-[^{125}I]iodozacopride, but gave substantial amounts of (*S*)-zacopride. Ponchant *et al.*[9] also failed to prepare [^{125}I]iodozacopride with chloramine-T. Further, they reported only a 4% radiochemical yield in the reaction of deschloro-zacopride with [^{125}I]iodobeads in acetonitrile, and contamination from a radiolabeled byproduct that lacked affinity for the 5-HT_3 receptor.[9] Under acidic conditions, however, and using one tenth the amount of chloramine-T, 42% (2.1 mCi) (*S*)-[^{125}I]iodozacopride together with an approximately equal amount of (*S*)-zacopride was produced. These were easily separated from the starting material using cyano column HPLC; retention times for deschloro-(*S*)-zacopride and (*S*)-zacopride were 13 and 21 min, respectively, whereas that of (*S*)-[^{125}I]iodozacopride was 26 min (Fig. 1). The chemical yields of (*S*)-zacopride and (*S*)-[^{125}I]iodozacopride, relative to the amount of precursor, were 14% and 12%, respectively. An unidentified peak (10%) at 18 min was presumed to be the 3-chloro isomer of (*S*)-zacopride. A small amount (2%) of the corresponding 3-[^{125}I]iodo derivative of (*S*)-zacopride, (*S*)-4-amino-5-chloro-3-[^{125}I]-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide ([^{125}I]-TRIZAC),[18] was detected at 31 min (Fig. 1). Radiochemical purity of (*S*)-[^{125}I]iodozacopride was >98% with a specific activity of 1800 Ci/mmol.

Analysis of the binding of (*S*)-[^{125}I]iodozacopride to sites in rat brain homogenate, as described by Kilpatrick *et al.*,[19] using bemesetron to define nonspecific binding, identified a single binding site with a K_D of 1.10 $\pm$ 0.07 nM and a B_{max} of 0.68 $\pm$ 0.05 fmol/mg tissue (Fig. 2A, B). Inhibition of the binding of 0.3 nM (*S*)-[^{125}I]iodozacopride by (*S*)-iodozacopride, (*S*)-zacopride, and (*S*)-5-chloro-3-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide (DAIZAC),[11] using 1.10 nM as an estimate of K_D, gave K_i values of 0.95, 0.21, and 0.12 nM, respectively (Fig. 3). Inhibition of the binding of 0.1 nM [^{125}I]DAIZAC (K_D 0.15 nM)[11] by (*S*)-iodozacopride, (*S*)-zacopride, and DAIZAC, gave K_i values of 1.05, 0.24, and 0.19 nM, respectively (data not shown).

We suggest that the results of Gehlert *et al.* are the consequence of contamination of the radiolabeled product, (*S*)-[^{125}I]iodozacopride, with the chlorinated by-

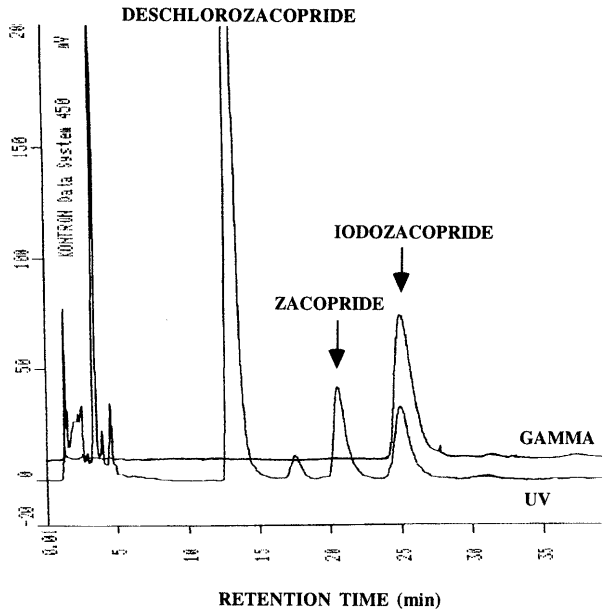

Fig. 1. Chromatogram of [^{125}I]Iodination of Deschloro-(*S*)-zacopride Using a Reverse-phase Cyano Column in 44% MeCN in 20 mM Phosphate Buffer as the Mobile Phase

Flow rate 1.5 ml/min. The upper trace shows the gamma radiation and the lower trace shows the UV absorption at 235 nm.

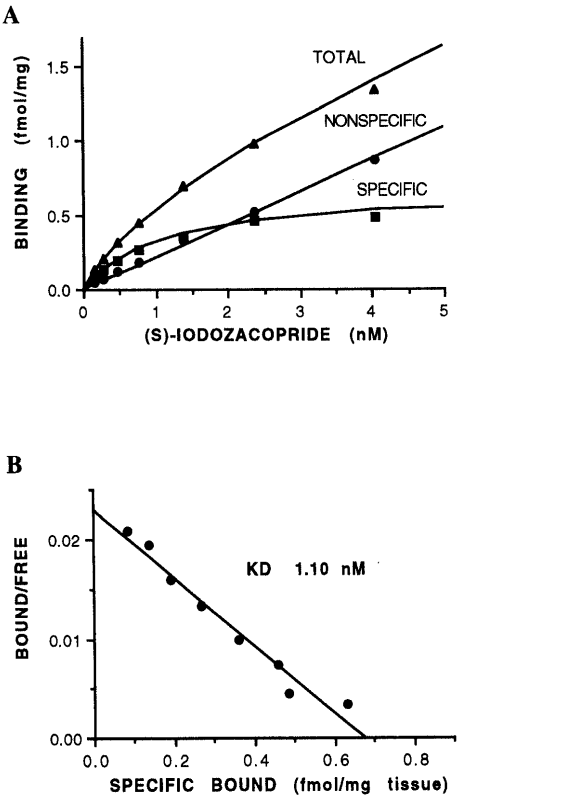

Fig. 2. Saturation (A) and Scatchard Analysis (B) of (*S*)-[^{125}I]Iodozacopride Binding to Whole Rat Brain Homogenates (15 mg/0.4 ml)

Incubation was performed for 1 h at 20 °C. Nonspecific binding was defined by co-incubation with 4 μM bemesetron.

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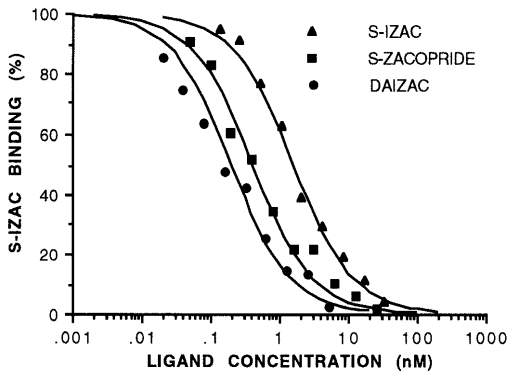

Fig. 3. Displacement of (*S*)-[^{125}I]Iodozacopride (0.3 nM) Binding in Rat Brain by (*S*)-Iodozacopride (S-IZAC), (*S*)-Zacopride, and DAIZAC

Nonspecific binding was defined by co-incubation with 10 μM (*S*)-iodozacopride.

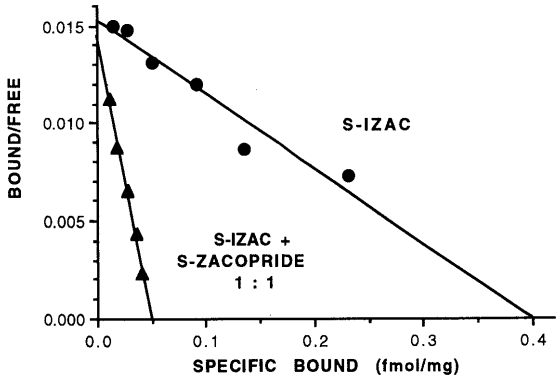

Fig. 4. Scatchard Analysis of (*S*)-[^{125}I]Iodozacopride Binding in the Absence and Presence of an Equimolar Amount of (*S*)-Zacopride

The analysis was performed without accounting for the presence of (*S*)-zacopride.

product, (*S*)-zacopride. In fact, Ponchant *et al.* have recommended against the use of chloramine-T as an oxidation agent because of the large amount of zacopride resulting from chlorination in the aromatic 5-position.[9] The undetected presence of a compound with affinity for the receptor to which the radioligand binds produces the same effect as carrier-added dilution, *i.e.* it causes the effective specific radioactivity to be lower than anticipated. The error in the estimated K_D and B_{max} is further magnified when the contaminating agent is more potent than the radioligand.[20] To demonstrate that unintended contamination with (*S*)-zacopride would artifactually enhance the apparent receptor affinity of (*S*)-[^{125}I]iodozacopride, saturation binding was repeated in the presence of (*S*)-zacopride in a 1:1 ratio at each concentration of (*S*)-[^{125}I]iodozacopride, and re-analyzed, without accounting for the presence of (*S*)-zacopride. The apparent values of the K_D and B_{max} of (*S*)-[^{125}I]iodozacopride became 0.14 ± 0.02 nM and 0.06 ± 0.01 fmol/mg tissue, respectively (Fig. 4), similar to the results of Gehlert *et al.*[12] Such contamination could also explain why, in the hands of Gehlert *et al.*, several 5-HT$_3$ agonists and antagonists had affinities 6—10 times higher[12] than those reported by other investigators.[10,19,21]

In summary, unlabeled (*S*)-iodozacopride and its corresponding des-iodo precursor were prepared from 4-aminosalicylic acid. (*S*)-[^{125}I]Iodozacopride was prepared by treatment of deschloro-(*S*)-zacopride with sodium [^{125}I]iodide and chloramine-T in hydrochloric acid to give (*S*)-[^{125}I]iodozacopride with high specific activity. Formation of chlorinated by-products, *e.g.* (*S*)-zacopride, was documented by HPLC using conditions which completely separate the 5-chloro and 5-iodo derivatives. Our results with the purified product show that (*S*)-[^{125}I]iodozacopride binds to the 5-HT$_3$ receptor with a K_D of 1.10 nM, consistent with the K_i of 1.05 nM obtained from displacement of the selective 5-HT$_3$ receptor antagonist [^{125}I]DAIZAC[11] from binding sites in rat brain. Thus, the affinity of (*S*)-iodozacopride for the 5-HT$_3$ receptor is twice that of racemic iodozacopride and 6 times less than that of (*S*)-zacopride.

Experimental

Melting points were determined in open capillary tubes on a Haake/Buchler apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker NB 300 MHz spectrometer in $CDCl_3$ with Me_4Si as internal chemical shift standard. Rotatory powers at the sodium D line were measured in a 1-dm sample tube with a Rudolph Autopol III polarimeter. Combustion analyses were performed by Atlantic Microlabs, Norcross GA, and gave elemental compositions within $\pm 0.4\%$ of the calculated values unless otherwise noted. (*S*)-3-AQN[15] was liberated from the dihydrochloride salt (Aldrich SAF) by dissolving in two equivalents of NaOH and removal of the water by repeated azeotropic evaporation of anhydrous ethanol.[10] 4-Aminosalicylic acid was obtained from ACROS (Fisher Scientific). (*S*)-Zacopride was prepared from 4-amino-5-chloro-2-methoxybenzoic acid (Aldrich) and (*S*)-3-AQN *via* the corresponding 1-imidazolo derivative as described for the racemic compound.[22] Desamino-(*S*)-3-iodozacopride, (*S*)-5-chloro-3-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide (DAIZAC), was prepared from 5-chlorosalicylic acid as reported.[11]

(*S*)-4-Amino-5-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide, (*S*)-Iodozacopride (*S*)-4-Acetamido-5-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide ((*S*)-**8**; 0.22 g, 0.5 mmol) was dissolved in EtOH (20 ml) and 5 M NaOH (5 ml) was added. The mixture was heated to 80 °C for 2 h. The solvent was evaporated and the residue was extracted with ether (2 × 25 ml). Purification on a SiO_2 column in EtOAc–EtOH–NH_4OH (60 : 40 : 1) gave 0.21 g (*S*)-iodozacopride as an oil. Rotation: $[\alpha]_D^{20}$ −25° (c=0.40, EtOH). ^{1}H-NMR ($CDCl_3$) δ ppm 8.42 (s, 1, C-6 H), 7.97 (br d, 1, NH), 6.30 (s, 1, C-3 H), 4.61 (br, 2, NH_2), 4.11 (m, 1, C-3′ H), 3.90 (s, 3, OCH_3), 3.40 (dd, 1, C-2′ H), 2.86 (m, 4, C-6′, C-7′ H), 2.57 (dd, 1, C-2′ H), 1.98 (q, 1, C-4′ H), 1.5—1.7 (m, 4, C-5′, C-8′ H). Lit.[8] NMR (CD_3OD) C-6 H: δ 8.08 ppm. Lit.[9] NMR (D_2O) C-6 H: δ 8.18 ppm. UV (MeOH): λ_{max} (ε) 307 (9800), 275 (13700), 220 (25000), λ_{min} 293 (7900), 253 (5700).

(*S*)-4-Amino-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide, Deschloro-(*S*)-zacopride a) From Substituted Benzoic Acid: 4-Amino-2-methoxybenzoic acid (**2**; 1.67 g, 10 mmol) was dissolved in MeCN (20 ml) and 1,1′-carbonyldiimidazole (CDI) (1.62 g, 10 mmol) was added and the mixture was stirred at 20 °C for 1 h. (*S*)-3-AQN (1.5 g of free base,[10] 12 mmol) was added and the mixture was heated to reflux for 16 h. The solvent was evaporated. The residue was treated with 1 M NaOH (20 ml) and extracted with ether (50 ml) to remove impurities. Extraction of the aqueous layer with $CHCl_3$ (2 × 25 ml), drying (Na_2SO_4) and evaporation of the solvent gave 1.92 g (70%) deschloro-(*S*)-zacopride as a viscous oil. Crystallization from EtOAc (50 ml) gave 0.96 g in the first crop and 0.32 g after reducing the volume of the mother liquors to 20 ml, mp 187—189 °C. Rotation: $[\alpha]_D^{20}$ −24° (c=0.24, EtOH). ^{1}H-NMR ($CDCl_3$) δ ppm 8.09 (br d, 1, NH), 7.98 (d, 1, J=8.5 Hz, C-6 H), 6.33 (dd, 1, J=8.6, 2.0 Hz, C-5 H), 6.21 (d, 1, J=2.0 Hz, C-3 H), 4.17 (m, 1, C-3′ H), 4.12 (br, 2, NH_2), 3.91 (s, 3, OCH_3), 3.41 (dd, 1, C-2′ H), 2.85 (m, 4H), 2.09 (dd, 1, C-2′ H), 2.01 (q, 1, C-4′ H), 1.72 (m, 3 H), 1.54 (m, 1H). Lit.[8] NMR (CD_3OD) C-6 H: δ 7.64 ppm. *Anal.* Calcd for $C_{15}H_{21}N_3O_2$: C, 65.43; H, 7.69; N, 15.26; O, 11.62. Found: C, 64.54; H, 7.57; N, 15.04.

b) From (*S*)-Iodozacopride: (*S*)-4-Amino-5-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide (0.2 g, 0.5 mmol) was dissolved in MeOH (30 ml) and 12 M HCl (0.05 ml), followed by 10% Pd on carbon (0.05 g). Hydrogenation with H_2 (11 ml) at 1 atm for 12 h, followed by

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filtration and evaporation of the solvent gave 172 mg deschloro(*S*)-zacopride·HCl as crystalline residue. ^{1}H-NMR ($CDCl_3$) δ ppm 8.01 (br d, 1, NH), 7.94 (d, 1, J=8.5 Hz, C-6 H), 6.35 (dd, 1, J=8.6, 2.0 Hz, C-5 H), 6.22 (d, 1, J=2.0 Hz, C-3 H), 4.50 (m, 1, C-3′ H), 4.10 (br, 2, NH_2), 3.95 (s, 3, OCH_3), 3.70 (dd, 1, C-2′ H), 3.2—3.4 (m, 4 H), 3.13 (dd, 1, C-2′ H), 2.45 (q, 1, C-4′ H), 2.80 (m, 3H), 1.92 (m, 1H).

Methyl 4-Amino-2-methoxybenzoate (1) 4-Aminosalicylic acid (10.0 g, 65 mmol) was dissolved in acetone (200 ml). Pellets of KOH (9.2 g, 143 mmol) were added followed by dropwise addition of dimethyl sulfate (19.5 g, 155 mmol) at 25 °C. Stirring was continued at room temperature for 2.5 h. The solvent was evaporated and the residue was diluted with water (150 ml). Extraction with EtOAc (2 × 150 ml), washing with 5% $NaHCO_3$ (2 × 25 ml), drying (Na_2SO_4), and evaporation of the solvent to 70 ml gave 5.92 g (50%) **1**, mp 153—155 °C. Lit.[14] mp 155—156 °C. ^{1}H-NMR ($CDCl_3$) δ ppm 7.73 (d, 1, J=8.4 Hz, C-6 H), 6.25 (dd, 1, J=8.5, 2.1 Hz, C-5 H), 6.22 (d, 1, J=2.1 Hz, C-3 H), 4.21 (br, 2, NH_2), 3.85 (s, 3, OCH_3), 3.83 (s, 3, OCH_3).

4-Amino-2-methoxybenzoic Acid (2) Methyl 4-amino-2-methoxybenzoate (**1**; 5.0 g, 28 mmol) was dissolved in EtOH (50 ml) and 2 N NaOH (15 ml, 30 mmol) was added. The mixture was heated to reflux for 40 min and the solvent was removed by evaporation. The crystalline residue was dissolved in hot water (150 ml), filtered, and neutralized to pH 3 by addition of 12 M HCl. Cooling gave 3.0 g (67%) **2** after recrystallization from MeOH (15 ml). mp 147—149 °C. Lit.[14] mp 149—150 °C. ^{1}H-NMR ($CDCl_3$) δ ppm 7.94 (d, 1, J=8.5 Hz, C-6 H), 6.36 (dd, 1, J=8.6, 2.0 Hz, C-5 H), 6.24 (d, 1, J=2.0 Hz, C-3 H), 4.32 (br, 2, NH_2), 4.08 (s, 3, OCH_3).

Methyl 4-Aminosalicylate (3) 4-Aminosalicylic acid (10.5 g, 0.07 mol) was dissolved in MeOH (400 ml). This was followed by dropwise addition of 18 M sulfuric acid (7 ml, 0.12 mol) to a warm solution and heating to refluxing temperature for 36 h. Evaporation of the solvent, addition of ice-water (300 ml), partial neutralization with 10 M NaOH (13 ml), and filtration gave 11.6 g (53%) precipitated **3**, mp 119 °C. Lit.[16] mp 119—121 °C.

Methyl 4-Acetamidosalicylate (4) Methyl 4-aminosalicylate (**3**; 10.5 g, 63 mmol) was dissolved in pyridine (75 ml) and a solution of acetyl chloride (5.3 g, 68 mmol) in $CHCl_3$ (25 ml) was slowly added at 0 °C. The mixture was stirred for 2 h at 20 °C. The solvent was evaporated and the residue was diluted with water (300 ml) and the precipitate was filtered. Recrystallization from EtOAc (75 ml) gave 7.72 g (58%) **4**. mp 146—148 °C. Lit.[16] mp 150 °C.

Methyl 4-Acetamido-5-iodosalicylate (5) Methyl 4-acetamidosalicylate (**4**; 8.4 g, 40 mmol) was dissolved in a suspension of NaI (6.5 g, 43 mmol) and DMF (85 ml), and chloramine-T (9.6 g, 42 mmol) was added in portions at 10 °C. After 1 h the mixture was diluted with 400 ml water containing sodium metabisulfite (0.4 g), acidified with 12 M HCl, and the product was extracted with ether (2 × 100 ml). Drying (Na_2SO_4) and evaporation gave 3.89 g (29%) **5**, after recrystallization from EtOAc (50 ml). mp 163—165 °C. ^{1}H-NMR ($CDCl_3$) δ ppm 11.73 (s, 1, OH), 8.20 (s, 1, C-6 H), 8.08 (s, 1), 7.56 (br, 1, C-3 H), 3.93 (s, 3, OCH_3), 2.26 (s, 3, $COCH_3$). *Anal.* Calcd for $C_{10}H_{10}INO_4$: C, 35.84; H, 3.01; I, 37.87; N, 4.18; O, 19.10. Found: C, 35.74; H, 3.01; I, 37.97; N, 4.10.

Examination of the mother liquors by NMR revealed the presence of 50% of the positional isomer methyl 4-acetamido-3-iodosalicylate. ^{1}H-NMR ($CDCl_3$) δ ppm 11.89 (s, 1, OH), 7.97 (d, 1, J=8.9 Hz, C-6 H), 7.83 (br, 1, NH), 7.81 (d, 1, J=8.9 Hz, C-5 H), 3.95 (s, 3, OCH_3), 2.28 (s, 3, $COCH_3$). Both the 5- and 3-iodo compounds showed one unseparable TLC spot on silica-gel in hexane–EtOAc (1 : 1).

Methyl 4-Acetamido-5-iodo-2-methoxybenzoate (6) Methyl 4-acetamido-5-iodosalicylate (**5**; 4.8 g, 14 mmol) was mixed with K_2CO_3 (4.8 g, 35 mmol) and dimethyl sulfate (2.0 g, 16 mmol) in acetone (100 ml) and heated to reflux for 16 h. Filtration and evaporation of the solvent, followed by extraction with ether (2 × 100 ml), drying (Na_2SO_4), evaporation, and recrystallization from EtOAc (30 ml), gave 4.18 g (86%) **6**, mp 179—181 °C. ^{1}H-NMR ($CDCl_3$) δ ppm 8.26 (s, 1, C-6 H), 8.22 (s, 1, C-3 H), 7.62 (br, 1, NH), 3.92 (s, 3, OCH_3), 3.87 (s, 3, OCH_3), 2.28 (s, 3, $COCH_3$). *Anal.* Calcd for $C_{11}H_{12}INO_4$: C, 37.84; H, 3.46; I, 36.35; N, 4.01; O, 18.33. Found: C, 37.92; H, 3.52; I, 36.24; N, 3.95.

4-Acetamido-5-iodo-2-methoxybenzoic Acid (7) Methyl 4-acetamido-5-iodo-2-methoxybenzoate (**6**; 3.48 g, 10 mmol) was dissolved in EtOH (34 ml) and 0.5 N NaOH (14 ml, 7 mmol) was added. Heating to 75 °C for 45 min, addition of water (100 ml), acidifying with HCl, and extraction with ether (2 × 50 ml), drying (Na_2SO_4) evaporation, and recrystallization from EtOAc (45 ml), gave 2.81 g (84%) **7**, mp 201—203 °C. ^{1}H-NMR ($CDCl_3$) δ ppm 8.54 (s, 1, C-6 H), 8.39 (s, 1, C-3 H), 7.73 (br, 1, NH), 4.09 (s, 3, OCH_3), 2.31 (s, 3, $COCH_3$). *Anal.* Calcd for $C_{11}H_{12}INO_4$: C, 35.84; H, 3.01; I, 37.87; N, 4.18; O, 19.10. Found: C, 34.93; H, 3.19; I; 36.70; N, 4.06.

(*S*)-4-Acetamido-5-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-benzamide ((*S*)-8) 4-Acetamido-5-iodo-2-methoxybenzoic acid (**7**; 0.52 g, 1.5 mmol) was converted to the *N*-imidazolo derivative (not isolated) by reaction with CDI (0.25 g, 1.5 mmol) in dry THF (15 ml) at 20 °C for 1 h, followed by (*S*)-3-AQN (0.25 g, 2.0 mmol) and the mixture was heated to reflux for 5 h. The solvent was evaporated. Purification on a SiO_2 column in EtOAc–EtOH–NH_4OH (50 : 50 : 1) gave (*S*)-**8** as light yellow crystals, mp 196 °C (dec.). Rotation: $[\alpha]_D^{20}$ −18° (c=0.40, EtOH). ^{1}H-NMR ($CDCl_3$) δ ppm 8.56 (s, 1, C-6 H), 8.27 (s, 1, C-3 H), 8.19 (br, 1, NH), 7.68 (br, 1, NH), 4.13 (m, 1, C-3′ H), 4.00 (s, 3, OCH_3), 3.42 (dd, 1, C-2′ H), 2.86 (m, 4 H), 2.59 (dd, 1, C-2′ H), 2.28 (s, 3, $COCH_3$), 2.01 (q, 1, C-4′ H), 1.65 (m, 4 H). *Anal.* Calcd for $C_{17}H_{22}IN_3O_3$: C, 46.06; H, 5.00; I, 28.63; N, 9.48; O, 10.83. Found: C, 46.20; H, 5.07; I; 28.74; N, 9.45.

Radiolabeling with I-125 a) Method According to Gehlert *et al.*: A 1 mM solution of deschloro-(*S*)-zacopride (30 μl, 30 nmol) in 0.2 M Na_3PO_4 was added to 1 mCi Na ^{125}I (0.4 nmol), followed by a 20 mM aqueous solution of chloramine-T (7 μl, 210 nmol). No radiolabeled product was obtained unless the labeling reaction was conducted with an excess HCl. After 3 h at 20 °C, the reaction mixture was injected into a reverse phase HPLC column (Dynamax Cyano, 60 Å) and eluted with 44% MeCN–20 mM Na_2HPO_4 buffer at pH 6.7. The radioactive peak at 23 min (flow rate 1.5 ml/min) was collected to give 0.24 mCi (*S*)-[^{125}I]iodozacopride. Retention times for (*S*)-zacopride and deschloro-(*S*)-zacopride were 17 and 13 min, respectively. Estimated yields of (*S*)-zacopride and (*S*)-[^{125}I]iodozacopride relative to deschloro-(*S*)-zacopride were 18% and 0.5%, respectively.

b) Conditions for Improved Radiosynthesis: A 3 mM solution of deschloro-(*S*)-zacopride (10 μl, 30 nmol) in 20 mM Na_2HPO_4 was added to 5 mCi Na ^{125}I (2.3 nmol), followed by a 1.3 mM aqueous solution of chloramine-T (7 μl, 10 nmol) and 0.2 M HCl (10 μl). After 3 h at 20 °C, the reaction mixture was purified by HPLC as described above. The radioactive peak at 23 min (flow rate 1.5 ml/min) was collected to give 2.09 mCi (*S*)-[^{125}I]iodozacopride. The relative amounts of zacopride and (*S*)-[^{125}I]iodozacopride were 46% and 54%, respectively, according to their UV peaks. Comparison of a 0.1 mM solution of unlabeled iodozacopride (20 μl, 2 nmol) gave a specific activity of 1800 Ci/mmol. Radiochemical yield was 43% and radiochemical purity >98%.

5-HT$_3$ Receptor Binding Male Harlan-Sprague-Dawley rats (200—250 g) were sacrificed, their brains removed and dissected on an ice-cold porcelain dish. The cerebellum was removed and the brain homogenized (1 g/13 ml) using a Brinkman Polytron (15 s at 12700 rpm) in a buffer at pH 7.4 containing 50 mM HEPES, 2.4 mM $MgCl_2$, and 5 mM $CaCl_2$.[10,19] The homogenate was centrifuged at 10000 × ***g*** for 15 min at +4 °C. The membrane pellet was resuspended in the same volume of buffer, centrifuged a second time, and resuspended in fresh buffer. For saturation analysis, the tissue was incubated at +20 °C for 1 h with 0.10—4.3 nM of (*S*)-[^{125}I]iodozacopride which had been diluted (1 + 9) with unlabeled (*S*)-iodozacopride. For competition studies, the tissue was incubated with 0.02—80 nM concentrations of the displacing ligands and either 0.3 nM of (*S*)-[^{125}I]iodozacopride (K_D 1.1 nM) or 0.1 nM of [^{125}I]-DAIZAC (K_D 0.15 nM).[11] Specific 5-HT-3 receptor binding was determined by co-incubation with 4 μM bemesetron (MDL-72222).[19] Bound and free (*S*)-[^{125}I]iodozacopride or [^{125}I]DAIZAC were separated by filtration through Whatman GF/B filters presoaked in 0.3% polyethylenimine, using a Brandel model M-24R cell harvester. The filters were rinsed three times for 10 s with ice-cold buffer and placed in plastic tubes. Gamma spectrometry was performed with an ICN Isomedic Model 4/600 HE instrument with an efficiency of 80%. Data from binding experiments were analyzed by LUNDON software. Errors in the calculated affinities and receptor densities represent the standard error of the linear fit.

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