acknowledged for analytical data. Dr. S. A. Dodson is acknowledged for his sourcing and obtaining a supply of the chiral 3-aminoquinuclidine. We would also like to thank Dr. M. N. Chang for his helpful comments and discussions.

Registry No. 1 (n = 1), 3041-17-6; 1 (n = 2), 73345-01-4; **2** (n = 1), 103386-91-0; **2** (n = 2), 112998-54-6; **3** (n = 1), 138572-42-6; **3** (n = 2), 138572-63-1; **4** (n = 1), 6169-78-4; **4** (n = 2), 51060-43-6; **5** (n = 1), 31457-17-7; **5** (n = 2), 138572-64-2; **6b**, 124794-82-7; **6c**, 138572-65-3; **6d**, 138572-66-4; **7**, 138572-43-7; **8**, 138572-44-8; **9**, 138572-45-9; **10**, 138572-46-0; 11, 124911-57-5; **12**, 138572-47-1; **13**, 124773-73-5; **13a**, 124773-72-4; **13b**, 124773-75-7; **14**, 138572-48-2; **15**, 4068-78-4; **15** (acid), 321-14-2; **16**, 138572-49-3; **17**, 138572-50-6; **18**, 138572-51-7; **19a**, 138572-52-8; **19b**, 138572-67-5; **19c**, 138572-68-6; **19d**, 138605-33-1; **20a**, 138572-53-9; **20b**, 138605-34-2; **20c**, 138572-69-7; **20d**, 138572-70-0; **21a**, 138572-54-0; **21b**, 138572-71-1; **21c**, 138572-72-2; **21d**, 138572-73-3; **22**, 138663-20-4; **23**, 5533-04-0; **24**, 138572-55-1; **24a**, 138572-74-4; **25**, 182-50-3; **25a**, 138572-75-5; **26**, 138572-56-2; (±)-**27**, 76883-48-2; (*S*)-**27**, 120570-05-0; (*R*)-**27**, 123536-15-2; (±)-**28**, 138572-57-3; (*S*)-**28**, 138663-21-5; (*R*)-**28**, 138663-22-6; **29**, 138572-58-4; **30**, 138572-59-5; **31**, 138572-60-8; **32**, 138572-61-9; **33**, 138572-62-0; CH_3OCH_2Cl, 107-30-2; NO_2BF_4, 13826-86-3; α-tetralone, 529-34-0; benzosuberone, 826-73-3; triphenylphosphine cyclohexyl bromide, 7333-51-9; 3-bromocyclohexene, 1521-51-3; 3-bromo-2-butanone, 814-75-5; 3-chloro-1-butene, 563-52-0; salicylaldehyde, 90-02-8; 2-methoxy-5-chlorobenzoic acid, 3438-16-2.

Development of High-Affinity 5-HT_3 Receptor Antagonists. 2. Two Novel Tricyclic Benzamides

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Rhône-Poulenc Rorer Central Research, 640 Allendale Road, King of Prussia, Pennsylvania 19406. *Received August 23, 1991*

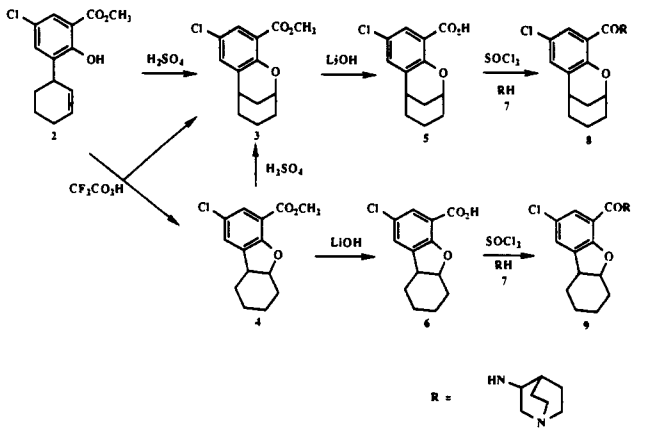
Two new classes of potent 5-HT_3 agents have been developed and examined as inhibitors of cytotoxic drug induced emesis in the ferret and dog. The absolute configuration of the most active molecules **10** and **18** have been determined by X-ray crystallography. These two compounds are more potent than known 5-HT_3 receptor antagonists both in vivo and in vitro in blocking 5-HT_3 receptor activation and preventing chemotherapeutic induced emesis. Compared with 5-HT_3 antagonists, such as GR 38032F, zacopride, BRL 43694, and ICS 205-930, compound **10** was more potent in (1) inhibiting binding to 5-HT_3 receptor binding sites in rat cortex (K_i = 0.17 nM), (2) blocking the von Bezold–Jarisch effect in the rat (lowest effective dose, 1 μg/kg iv), and (3) inhibiting 5-HT-induced contraction of guinea pig ileum (lowest effective concentration, 10^{-9} M). This novel agent was as effective given po as when given iv in reducing cisplatin-induced emetic episodes in the ferret (ED_{50} = 4 μg/kg iv or po). A 1 mg/kg po dose of **10** virtually abolished cisplatin-induced emesis for 10 h in the ferret. However, it was inactive against apomorphine or copper sulfate-induced vomiting. These data, coupled with receptor binding studies of ligands for D_2-dopamine, a_1, a_2, 5-HT_1, 5-HT_2, and muscarinic receptors demonstrate that **10** is a highly selective 5-HT_3 receptor antagonist with remarkable potency in vivo.

Introduction

An unfortunate side effect of chemotherapeutic treatment of cancer is nausea and vomiting which has stimulated efforts to discover an effective and safe antiemetic agent to block this side effect of neoplastic agents. 5-HT_3 antagonists have demonstrated potent antagonism of chemotherapy- or radiation-induced emesis in man.[1] Metoclopramide, which was originally thought to block emesis via antagonism of dopamine D_2 receptors, is the drug most often used to inhibit the emesis produced by such agents. It has been found to be a relatively weak 5-HT_3 antagonist,[2] requiring high dosage, and it suffers from the fact that it exerts D_2-dopamine receptor blocking side effects.[3] Recently several compounds exhibiting high affinity for 5-HT_3 receptor have been identified as potent antiemetic agents. Members of this class of compounds, which include ICS 205-930,[4] MLD 72222,[5] BRL 43674,[6] GR 38032F,[7] and aromatic thiazoles[8] have been shown effective clinically in the treatment of chemotherapy-induced emesis.[9] 5-HT_3 receptor binding sites have been indicated in the ferret area postrema and the nucleus tractus solitarius.[9] Injection of GR 38032F directly into this region of the ferret brain has completely prevented cisplatin-induced emesis.[10,11]

Previously,[12] we have described the initial structure–activity relationships of a series of bicyclic and tricyclic benzamides as 5-HT_3 receptor antagonists leading to a tricyclic benzamide (**1**) as the most potent 5-HT_3 receptor antagonist. In this paper we describe the synthesis and properties of two new classes of tricyclic benzamides that

Scheme I



are potent antagonists of chemotherapy-induced emesis in animals and exhibit high affinity for 5-HT_3 receptor

* To whom all correspondence should be sent.

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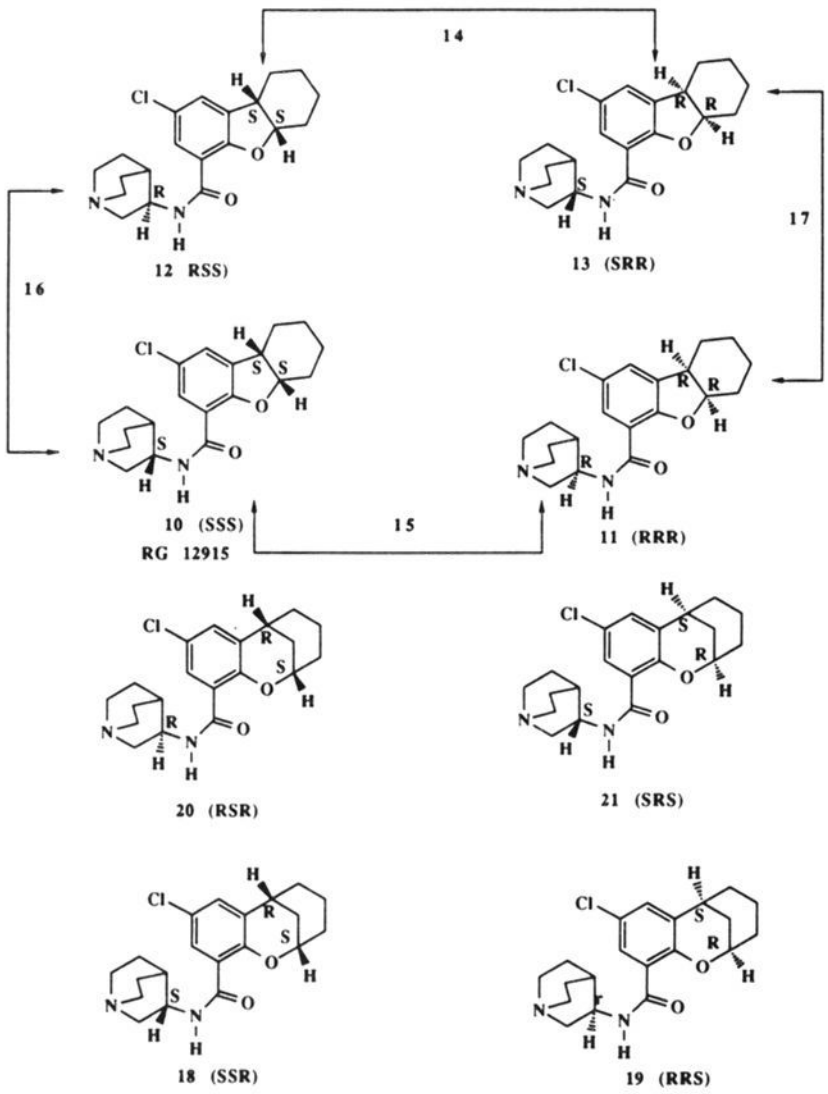
Scheme II



binding sites with negligible affinity for dopaminergic binding sites.

Chemistry

The two novel benzamides **8** and **9** were synthesized as illustrated in Scheme I. Intramolecular cyclization of the salicylate **2** with trifluoroacetic acid provided a 1:3 mixture of **3** and **4**, respectively, which was separated by column chromatography.[13] However, intramolecular cyclization with sulfuric acid gave exclusively **3**. Sulfuric acid treatment of **4** induced complete isomerization to **3**. Basic hydrolysis of the mixture of **3** and **4** obtained from intramolecular cyclization with trifluoroacetic acid gave an insoluble solid which was identified as the sodium salt of acid **5**, while the mother liquor contained the soluble salt of acid **6**.

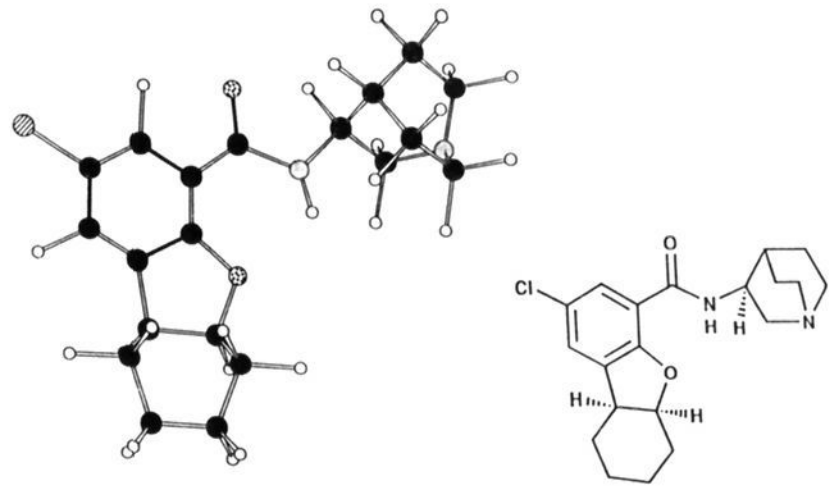


Figure 1. Compound **10**.

Structure determination of acid **6** was made by X-ray crystallography. Two different ring-fusion conformations were observed for this molecule in which the cyclohexane–benzofuran ring fusion were cis in both molecules. In molecule one, the five-membered ring joins the cyclohexane ring with oxygen axial and carbon equatorial, whereas, in molecule two, oxygen is equatorial and carbon is axial, revealing a mixture of enantiomers. The two enantiomers were resolved by classical resolution methods using α-methylbenzylamine as the resolving base, giving (−)-**6** and (+)-**6**.

Coupling of the isolated racemic **5** and racemic **6** with racemic 3-aminoquinuclidine (**7**) gave diastereomeric mixtures **8** and **9**, respectively. One important goal was to isolate all optical isomers of **8** and **9** and to ascertain their individual biological activities. Examination of the structure of *cis*-**9** reveals that because of the stereocenters at position 5a and 9a of the fused furan rings and position 3 of the quinuclidine the four possible optical isomers can have the stereochemical relationship *SSS*, *RRR*, *SRR*, and *RSS*[14] (Scheme II).

These four individual isomers were obtained by three different methods: (a) Separation of the four stereoisomers by chiral HPLC; using a semipreparative column, we were able to separate all four isomers of **9** and obtain 500 mg of each isomer **10**–**13** (Scheme II); (b) resolution of the four

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(13) This data is in accord with the reported cyclization of 2-(2′-cyclohexenyl)phenols to 1,2,3,4,4a,9b-hexahydrodibenzofuran and 3,4,5,6-tetrahydro-2,6-methano-2*H*-1-benzoxocin by (a) acid catalysis or photolysis (Frater, G. Y.; Schmid, H. *Helv. Chim. Acta* **1967**, *50*, 255–262.), (b) mercuric acetate (Hosokawa, T.; Miyagi, S.; Murahashi, S.; Sonoda, A.; Matsuura, Y.; Tanimoto, S.; Kakudo, M. Intramolecular Phenoxymercuration of 2-Allylphenols. Regioselectivity and Stereochemistry. *J. Org. Chem.* **1978**, *43*, 719–724.), and (c) phenylselenium chloride (Clive, D. L. J.; Chittatu, G.; Curtis, N. J.; Kiel, W. A.; Wong, C. K. Cyclofunctionalization of Ortho-Alkenyl Phenols: A New Method for Introducing the Benzeneseleno-Group. *J. Chem. Soc., Chem. Commun.* **1977**, *20*, 725–727.).

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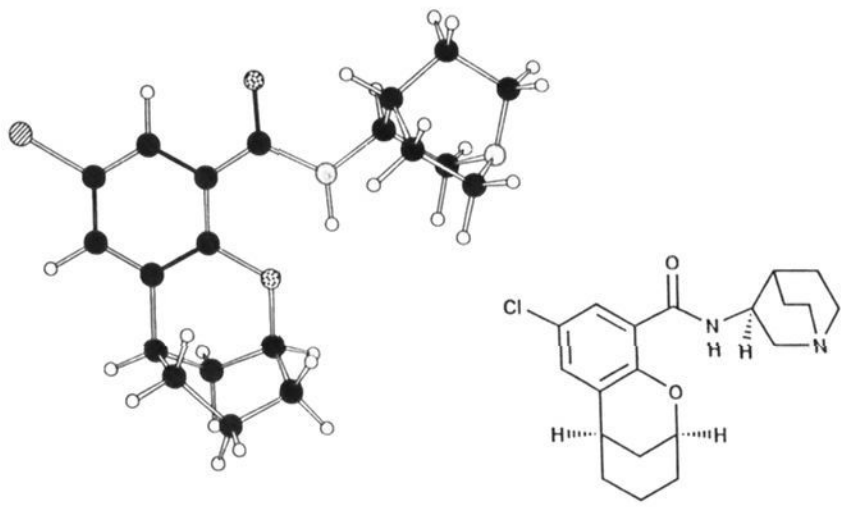


Figure 2. Compound 18.

stereoisomers by classical means; and (c) resolution of the *cis*-acids and the aminoquinuclidine followed by coupling of the resolved acids and the resolved amines.

Classical resolution of **9** with (1*S*)-(+)-10-camphorsulfonic acid gave the diastereomeric mixture **14** which by HPLC was shown to be a mixture of **12** and **13**. The X-ray data on this compound showed that the cationic portions of the two salts were enantiomeric with each other and had the absolute stereochemistry of *RSS* or *SRR*. Similarly, resolution of **9** with (1*R*)-(−)-10-camphorsulfonic acid gave the diastereomeric mixture **15** which by HPLC was a mixture of **10** and **11**. By default, the diastereomeric mixture **15** must also be a racemate of the two enantiomers **10** and **11**.

Coupling of the isolated (−)-**6** or (+)-**6** with racemic 3-aminoquinuclidine gave two new compounds, **16** and **17**, respectively, each being a diastereomeric mixture of **10** and **12** and **11** and **13**, respectively.

Coupling of (−)-**7** with (−)-**6** gave a single compound, which, by HPLC, was shown to be **10**, the most active stereoisomer of the *cis*-**9** compound. X-ray crystallographic data for this compound gave the absolute stereochemistry of **10** as *SSS*[15] (Figure 1). With this information, we were then able to assign the absolute stereochemistry to all of the four isomers of **9** as shown in Scheme II.

Examination of the structure of **8** shows that because of the stereocenters at position 5a, 9a of the fused furan ring and position 3 of the 3-aminoquinuclidine, the four enantiomers can have the stereochemical relationship *SSR*, *RRS*, *SRS*, and *RSR*, as shown in Scheme II.

The resolution of acid **5** was accomplished using chiral 4-nitro-α-methylbenzylamine to give (−)-**5** and (+)-**5**. X-ray crystallography data for (+)-**5** showed that the molecule was a *cis*-[3.3.1]pyrano fused ring compound with the absolute stereochemistry *SR*. The absolute stereochemistry of (−)-**5** must therefore be *RS*.

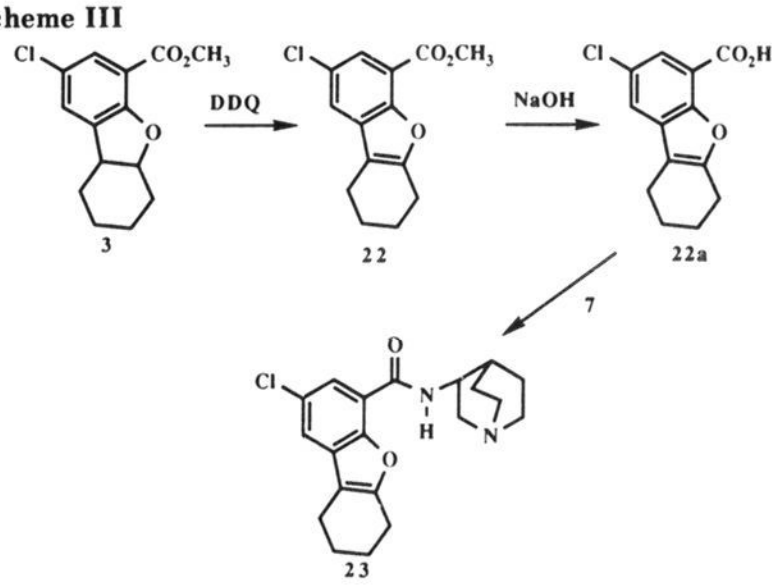
Coupling of each of the resolved acids with the resolved 3-aminoquinuclidine gave four separate stereoisomers **18**–**21**, evidenced by HPLC.

Now that the absolute stereochemistry for the (−)-**5** and (+)-**5** and (−)-**7** and (+)-**7** is known, the absolute stereochemical structures for the four isomers of **8** (**18**–**21**) are obvious, as shown in Scheme II.

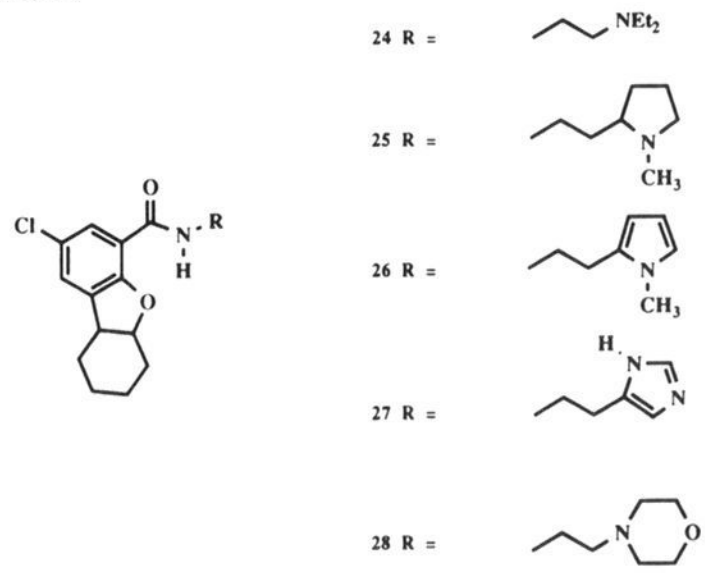
To further complete the proof of the absolute stereochemistry of these isomers, X-ray crystallographic data of the compound obtained from the coupling of acid (*SR*)-(+)-**6** and amine (*S*)-(−)-**7** showed the product to have the *SSR* configuration. This compound (**18**) was the most potent among the four isomers (Figure 2).

(15) This data is in support of the previous assumption of the absolute configuration of (−)-**7** and (+)-**7** as *S*(−) and *R*(+), respectively. Dorme, N.; Renand, A.; Langlois, M. (Delalande S. A.) EP0280-603, 1988.

Scheme III



Scheme IV



DDQ oxidation of ester **3** followed by basic hydrolysis gave acid **22a**, which on coupling with the racemic 3-aminoquinuclidine (**7**) produced **23**, as shown in Scheme III.

To investigate the effect of the aminoquinuclidine on the potency of **10**, compounds **24**–**28** were synthesized as usual by the reaction of **6** with appropriate amines as shown in Scheme IV.

Biological Results

Antiemetic activity of the two potent molecules **8** and **9** given po dose against cisplatin-induced emesis in the ferret[16] indicated the superiority of isomer **9** over **8**, as shown in Table I. Testing each individual isomer of **8** and **9** in this assay resulted in the identification of one isomer in each parent molecule, **10** and **18**, respectively, having the greater potency, with **10** having a slight edge over **18**. The enantiomers of each of these two compounds, **19** and **11**, respectively, were found to have the least activity; while each of the remaining pair of enantiomers, **20** and **21**, and **12** and **13**, respectively, were about equal but with moderate potency. Oxidation of the parent molecule **8** to **23** resulted in loss of two stereocenters and in diminished antiemetic potency.

A comparison of the oral antiemetic activity (Table I) with the ability to displace [^{3}H]quipazine binding from rat cortical tissue[17] (Table II) indicates a good correlation

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Table I. Antiemetic Profile against Cisplatin-Induced Emesis in the Ferret

	intravenously[a]					orally[b]	
compd[a]	% reduction in emetic episodes: ED_{50},[b] μg/kg	% increase in emetic latency period: ED_{100},[d] μg/kg	no. protected/ no. tested	% reduction in emetic episodes	% increase in latency to first emetic episode	% reduction in emetic episodes: (ED_{50},[c] μg/kg	% increase in emetic latency period: ED_{100}, μg/kg
8						79	22
9	6	11				5	11
10	4	7				4	5
11						84	>3000
12						56	107
13						41	258
18	9	6				7	10
19						>1000	>1000
20						85	408
21						61	290
23						33	272
24			0/5	2.2	6.5		
25			0/3	-38.1	-0.9		
26			0/4	51.7	1.0		
27			0/4	44.4	27.6		
28			2/4	48.9	110.9		
metaclopramide	1450	2100				6170	>5000
BRL 43694	226	82				119	117
GR 38032F	8	15				280	31

[a] Administered 30 minutes prior to cisplatin. [b] Administered 60 min prior to cisplatin. [c] Determined from 3–10 animals per dose at least three per compound. [d] ED_{100} = dose required to increase emetic latency 100% over control period. Range of mean emetic episodes in control treated animals: iv = 8.6–15.6; po = 6.9–8.4. Mean range of emetic latency period: iv = 50–60 minutes; po = 55–81 min.

Table II. Antagonism of [^{3}H]GR 65630 Binding by Various Agents

compd	n[a]	K_i ± SE	compd	n[a]	K_i ± SE
8	1	1.07 ± 0.57	**24**	1	>100
9	2	0.74 ± 0.14	**25**	1	>100
10	7	0.17 ± 0.02	**26**	1	>100
11	3	8.77 ± 1.82	**27**	1	29.6 ± 5.7
12	3	2.05 ± 0.12	**28**	1	>100
13	2	2.85 ± 1.16	BRL 43694	3	1.72 ± 0.03
18	1	0.30 ± 0.14	GR 38032F	3	6.16 ± 2.1
19	1	3.42 ± 0.84	ICS 205-930	5	2.1 ± 0.50
20	1	1.96 ± 0.55	MDI 72222	3	21.12 ± 8.6
21	2	0.69 ± 0.23	zacopride	3	1.51 ± 0.36

[a] n = number of experiments. On each experiment compounds were tested in six-point competition experiments with triplicate replication.

between antiemetic activity and binding affinity for the 5-HT_3 receptor for each individual isomer.

An important and unique aspect of the antiemetic profile of **10** and **18** is their potency and efficacy following oral administration against cisplatin-induced emesis in the ferret. Given po **10** and **18** had markedly lower ED_{50} values (0.004 and 0.007 mg/kg), respectively, than BRL 43694 (0.119 mg/kg), GR 38032F (0.28 mg/kg), and metoclopramide (6.17 mg/kg) in attenuating the emetic response to cisplatin in the ferret (Table I). After iv administration, **10** also had the lowest ED_{50} value (0.004 mg/kg) followed in ascending order by GR 38032F (0.008 mg/kg), BRL 43694 (0.226 mg/kg), and metoclopramide (1.95 mg/kg) (Table I).

As shown in Table III, **10** dose-dependently attenuated both the incidence and frequency of emetic episodes induced by cisplatin in the ferret. At doses of 0.03–1.0 mg/kg po, this agent reduced the number of emetic episodes by 90–100% and completely prevented emesis in 70–100% of the ferrets tested.

A comparison of the po/iv ratio of ED_{50} values further suggests that **10** (1.0) is more bioavailable than metoclopramide (4.3) and GR 38032F (35.0), but less than BRL 43694 (0.53).

Compound **10** exhibits a considerable duration of action against cisplatin-induced emesis in the ferret. Following pretreatment with a 1 mg/kg dose po, it was almost completely protective for up to 10 h. The estimated half-life for a 0.03 mg/kg dose po is 4.7 h based on pretreatment time.

To assess the antiemetic efficacy of **10** in a second species, it was evaluated for the prevention of cisplatin-induced emesis in the dog.[19] Pretreatment with a 1 mg/kg po dose of **10** significantly reduced the mean number of emetic episodes from 16.5 (vehicle treated) to 0.14 and also increased the emetic latency period from 102 (vehicle treated) to 280 min. In this study, 6/7 (86%) of the dogs tested were completely protected from vomiting.

In the ferret, **10** (1 mg/kg po) also completely prevented the emesis associated with combined administration of the cytotoxic agents cyclophosphamide and doxorubicin,[6] while vehicle-treated ferrets had an average of 13.5 emetic episodes. Therefore, the antiemetic efficacy of **10** is not limited solely to the chemotherapeutic drug cisplatin.

Administration of metoclopramide (1 mg/kg po) prevented apomorphine (D_2-dopamine receptor) mediated emesis in 3/4 (75%) dogs. In contrast, **10** (1 mg/kg po) failed to protect any (0/4) of the dogs tested in this assay from vomiting.[19] Similarly, **10** failed to displace [^{3}H]-spiroperidol at D_2 binding sites in rat cortical membranes. The K_i value for metoclopramide in the [^{3}H]spiroperidol binding assay was 233 nM, while **10** at 10 000 nM failed to displace 50% of the ligand for the D_2 binding site.[20]

In contrast, **10** potently competes for [^{3}H]GR 65630 (a ligand for the 5-HT_3 binding site) binding in rat cortical

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Table III. Oral Antiemetic Profile of **10** against Cisplatin-Induced Emesis in the Ferret

compd[a] (mg/kg)	no. protection/no. tested	% protected	mean % reduction in emetic episodes	mean % increase in emetic latency
0.5% methylcellulose	0/22	0		
RG 12915				
(0.003)	1/4	25	24	53*
(0.010)	5/12	42	57*	105**
(0.030)	4/4	100	100*	195**,[b]
(0.100)	6/6	100	100**	195**,[b]
(1.000)	7/10	70	90**	171**

[a] Administered 60 min prior to cisplatin. Mean number of emetic in 0.5% methylcellulose group = 7.6. *p <0.05 or **p < 0.01 vs 0.5% methylcellulose control. [b] When animals did not vomit, 240 min (i.e. duration of the study) was used to calculate the increase in emetic latency.

tissue with a K_i value of 0.17 nM.[17] The K_i value for **10** was 10, 36, 12, and 124 times lower than those derived from BRL 43694, GR 38032F, ICS 205-930, and MDL 72222, respectively (Table II). This result in the binding assay, as well as the blockade of both the von Bezold–Jarisch effect in the rat[21,22] and serotonin-induced contraction of the guinea pig ileum,[23] suggests that **10** is one of the most potent 5-HT$_3$ antagonist yet studied.

Compound **10** significantly reduced the von Bezold–Jarisch effect at 1.0 μg/kg iv (lowest effective dose) as compared to ICS 205-930 (9 μg/kg iv), BRL 43694 (9 μg/kg iv), GR 38032F (27 μg/kg iv), and MDL 72222 (>200 μg/kg iv). This data again suggests a potent 5-HT$_3$ receptor blocking action for **10**. Compound **10** was very weak in displacing ligands for the 5-HT$_1$ (IC_{50} = 100 μM), 5-HT$_2$ (IC_{50} = 2 μM),[21] and muscarinic cholinergic (IC_{50} = 7 μM)[22] binding sites.

Compound **10** produces dose-related and potent antagonism of 5-HT-induced contraction of the guinea pig ileum (lowest effective concentration, 10^{-9} μM). It is also a potent antagonist of the actions of the specific 5-HT$_3$ agonist 2-methylserotonin in this preparation.[23]

In contrast to BRL 24924, cisapride, or zacopride, **10** at 0.125–1 mg/kg po did not enhance gastric emptying of a liquid meal in the dog.[24] This suggests that there is no direct relationship between the inhibition of 5-HT$_3$ receptor interactions and enhancement of gastric emptying.[25]

In Tables I and II, a comparison of the 5-HT$_3$ receptor binding and cisplatin-induced emesis antagonist activity of compound **9** and its tetrahydro analogue **23** and its amine side-chain analogues **24–28** have been made. Compound **9** is superior in both binding and antiemetic activities compared to the other derivatives.

5-HT$_3$ Receptor Site Model

The structural requirements reported for 5-HT$_3$ receptor binding consist of an aromatic ring, a hydrogen-bond acceptor, and either a hydrogen-bond donor or a positively charged center.[26–28] In the present series of compounds these correspond to the phenyl, carbonyl, and amino functionalities (the latter is presumably protonated under physiological conditions). To further explore binding interactions, a computer modeling study of the receptor site was carried out using BRL 43694, GR 38032F, ICS 205930, **10**, and **12** (the *SSS* and *RSS* isomers of **10**, respectively) as ligands, and SYBYL version 5.2 software for computation.

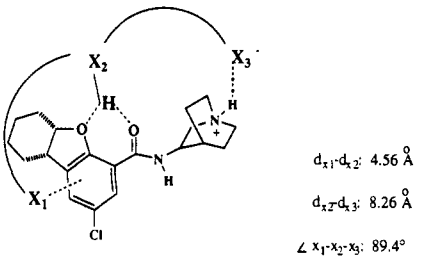

Figure 3. 5-HT$_3$ receptor site model/RG 12915.

Three-dimensional structures of the ligands were either determined by X-ray crystallographic analysis or built from premodeled structural fragments and then minimized using MAXIMIN2. Following protonation, conformational profiles were generated by varying the dihedral angles of all rotatable bonds through a 360° range in increments of 10° or less. For each conformer steric energy, dihedral angle values, and selected interatomic distances were reported. By comparing distance data, a spatial configuration of the aryl, carbonyl, and amino groups accessible to all ligands was identified. One or more conformers containing this pharmacophore were selected for each ligand and minimized, with the lowest-energy minima being retained for further study.

To define receptor site geometry, the minima were aligned by superimposing their aryl and amide functionalities; charges were then added using the Pullman method. A hydroxy group and carboxyl anion (from water and formic acid, respectively), representing the hydrogen-bond donor and acceptor sites in the receptor, were placed approximately 2 Å away from the corresponding sites in the aligned ligands and were connected by a flexible dummy bond. An explicit aryl binding site was not used; its position could be determined from that of the ligand aromatic rings. Constants were defined for maintaining aryl ring alignment and proper hydrogen-bonding geometry. The MULTIFIT option was then used to minimize total system energy, allowing both intramolecular and intermolecular geometries to vary. The resulting receptor site model is shown in Figure 3 (X_1, X_2, and X_3 denote average

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aryl ring position and receptor H-bond donor and receptor sites, respectively).[29] For all ligands the bound conformation either coincided with or was close in energy to a local or global minimum.

Compounds **10** and **12**, which differ only in stereochemistry of the quinucliding ring, show identical behavior toward site points X_1 and X_2. The amine hydrogen in **10** is directed toward X_3, optimally positioned for hydrogen bonding. The change in ring stereochemistry forces the same hydrogen in **12** away from X_3, resulting in weaker electrostatic interaction. The loss of hydrogen bonding may explain the reduced 5-HT_3 binding affinity shown by *R* vs *S* quinuclidine derivatives.

A rotational study of the N-quinucliding bond in **10** shows one well-defined minimum which is adopted by both **10** and **12** when receptor-bound; steric factors otherwise limit rotational freedom. Two minima are found for the aryl–carbonyl bond, with the phenyl and carbonyl groups coplanar and the dibenzofuran and carbonyl oxygens in a cisoid or transoid relationship. The combination of rotational and stereochemical factors essentially locks **10** with a conformation ideal for binding. The reduced binding shown by analogues **24–28**, where the quinuclidine is replaced by at least partially acyclic moieties, may result from increased molecular flexibility. The cisoid conformer of **10** is 2.5 kcal/mol higher in energy than the transoid one; however, the additional binding interaction to X_2 may overcome this difference.

Experimental Section

All melting points were determined on a Thomas-Hoover Unimelt capillary melting point apparatus using open capillaries and are uncorrected. Analytical results are indicated by the elements' symbols and are within ±0.4% of the theoretical values. ^{1}H NMR spectra were recorded on a Varian EM-390 instrument using tetramethylsilane as an internal standard and are consistent with the assigned structures. Separation by chiral HPLC was undertaken using a Chiracel OD (Diacel Supplier) column with hexane/ethanol as the mobile phase. This gave good separation of all optical isomers. The HPLC percentage is recorded as the area percent.

Methyl 5-Chloro-3-(3′-cyclohexenyl)salicylate (2). A slurry of methyl 5-chloro-3-(3′-cyclohexenyloxy)salicylate (603.6 g, 2.26 mol) and cesium carbonate (33.5 g, 0.1 mol) was heated, under a nitrogen blanket, to 155–175 °C for about 4 h. On completion of the reaction the mixture was cooled to 80 °C and toluene (500 mL) added. The mixture was stirred for 1 h, and the solids were removed by filtration. The filter cake was washed with toluene (2 × 65 mL). The filtrates were combined and concentrated to dryness to give 557.3 g (92%) of **4** as a brown oil. The oil was crystallized from denatured ethanol (550 mL). The solid was filtered, washed with cold denatured ethanol, and dried under house vacuum at 40 °C overnight to give 429.6 g (71.2%) of **4** as a tan solid: mp 59–61 °C; ^{1}H NMR ($CDCl_3$) δ 11.03 (s, 1 H), 7.62 (d, 1 H), 7.30 (d, 1 H), 5.96 (m, 1 H), 5.94 (m, 1 H), 3.92 (s, 3 H), 3.84 (m, 1 H), 1.34–2.2 (m, 6 H); HPLC 99.79%.

8-Chloro-3,4,5,6-tetrahydro-2,6-methano-2*H*-1-benzoxocin-10-carboxylic Acid (5). To concentrated sulfuric acid (519 mL) was added in portions Claisen ester **2** (345 g, 1.3 mol), and the temperature was kept below 50 °C. After stirring at room temperature for 1 h the mixture was poured onto water (4.2 L), followed by neutralization with 50% aqueous sodium hydroxide (843 mL). This basic mixture was extracted with toluene (2.5 L), the toluene dried with sodium sulfate, filtered, and evaporated to dryness to give 351.6 g of methyl 8-chloro-3,4,5,6-tetrahydro-2,6-methano-2*H*-1-benzoxocin-10-carboxylate (**3**) as an oil.

Ester **3** (351.6 g) was added to a solution of lithium hydroxide monohydrate (131.0 g, 3.12 mol) in water (2.6 L) and the mixture heated to reflux for 1.5 h, resulting in the formation of a heavy precipitate. The reaction mixture was cooled to room temperature and ethyl acetate (1.7 L) was added, followed by acidification with concentrated hydrochloric acid (276 mL).

The ethyl acetate was removed and the acidic aqueous layer was further extracted with ethyl acetate (500 mL). The ethyl acetate extracts were combined, dried with sodium sulfate, filtered, and evaporated to dryness to give 327.7 g (100%) of acid **5** as an off-white solid.

Recrystallization from acetonitrile gave 253.6 g of acid **5**: mp 110–20 °C; ^{1}H NMR ($CDCl_3$) δ 10.94 (s, 1 H), 7.98 (d, 1 H), 7.22 (d, 1 H), 4.98 (s, 1 H), 3.10 (s, 1 H), 1.2–2.3 (m, 8 H); achiral HPLC 99.53%.

2-Chloro-*cis*-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxylic Acid (6). A mixture of **2** (508.6 g, 1.91 mol) and trifluoroacetic acid (1850.0 g, 16.23 mol) was heated to reflux. On completion of the reaction, the mixture was cooled to 35 °C and evaporated to dryness; then methanol (250 mL) was added to the warm mixture, followed by 1 N aqueous lithium hydroxide (3500.0 mL), and the mixture heated to reflux. When no more starting material remained, the mixture was cooled to −5 °C. The precipitated solids were filtered and washed with 1 N aqueous lithium hydroxide (1000 mL) and then ethyl acetate (1000 mL). The solid was treated with ethyl acetate (3000 mL) and deionized water (1000 mL) and acidified with 10% aqueous hydrochloric acid to a pH of 1–2. The layers were separated and the ethyl acetate concentrated to dryness to give 239.0 g (43.9% based on **2** used) of **6** as an off-white solid: mp 143–4 °C; ^{1}H NMR ($CDCl_3$) δ 10.84 (s, 1 H), 7.76 (d, 1 H), 7.28 (d, 1 H), 4.94 (m, 1 H), 3.3 (m, 1 H), 1.2–2.2 (m, 8 H); HPLC 99.69%; chiral HPLC shows 49.89%:48.22% of a mixture of enantiomers. X-ray analysis confirmed the structure of the molecule as a combination of two enantiomers.

2-Chloro-(5a*S*,9a*S*)-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxylic Acid [(5a*S*,9a*S*)-6]. To a stirred suspension of (*S*)-4-nitro-α-methylbenzylamine hydrochloride (300.0 g, 1.48 mol) was added 50% aqueous sodium hydroxide (120.36 g). The mixture was extracted with methylene chloride (550 mL), the methylene chloride dried with sodium sulfate, filtered, and concentrated to dryness to give 255.5 g (103.9%) of the free base as an oil which was used directly. To a stirred solution of the above oil (197.1 g, 1.19 mol) in methanol (3560 mL) was added **6** (300.0 g, 1.19 mol). The mixture was stirred at room temperature for 10 min and then heated to reflux to give a clear solution. On cooling to room temperature the solids were filtered, washed with methanol (300 mL), and dried in vacuo at 60 °C to give 152.0 g (30.6% of total) of (5a*S*,9a*S*)-6-(*S*)-4-nitro-α-methylbenzylamine salt as a beige solid.

A stirred suspension of the above salt (152.0 g, 0.36 mol) in methanol (1.5 L) was heated to reflux. The suspension was filtered hot and the solid air-dried to give 48.1 g (31.6%) of a beige solid (A) (chiral HPLC showed 91% purity).

The methanolic filtrate was heated to reflux to give a clear solution. On cooling to room temperature the solid was filtered, washed with methanol (100 mL), and dried in vacuo to give 51.0 g (33.6%) of a solid (B) (chiral HPLC showed 96.4% purity).

The methanolic filtrate was concentrated to dryness, combined with solid A (48.1 g) and refluxed with methanol (1.0 L). Further methanol was added to complete dissolution of the solid at reflux. On cooling to room temperature the solid was filtered, washed with methanol (200 mL), and dried in vacuo to give 44.5 g (29.3%) of solid C (chiral HPLC showed 97.5% purity).

Solids B and C were combined (95.5 g) and converted to the free acid as follows.

To a mixture of 1 N aqueous hydrochloric acid (300 mL) and methylene chloride (300 mL) was added 2-chloro-(5a*S*,9a*S*)-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxylic acid [(5a*S*,9a*S*)-6] (*S*)-4-nitro-α-methylbenzylamine salt (solids B and

(29) Position X_1 represents the average position of a ligand aryl ring when the ligand is bound to the receptor. It was determined by averaging the positions of the aryl ring centroids, for the compounds used in receptor site generation, when each was bound to the receptor site model. Positions X_2 and X_3 represent the positions of the oxygen atom of the hydroxyl probe and the midpoint of the oxygen atoms of the carbonyl probe, when these probes occupy the postulated hydrogen-bond-donor and -acceptor sites, respectively, of the receptor site model. The distances/angles between these points were used in lieu of their Cartesian coordinates in order to present the geometry of the receptor site model.

C) (95.5 g, 0.38 mol). The organic layer was removed and the aqueous acid was extracted with methylene chloride (100 mL). The methylene chloride extracts were combined, dried with sodium sulfate, filtered, and concentrated in vacuo to give 58.3 g (101.2%) of (5a*S*,9a*S*)-**6** as a white solid. A mixture of the acid (5a*S*,9a*S*)-**6** (58.3 g, 0.233 mol) and acetonitrile (85.0 mL) was heated to reflux to give a clear, light yellow solution. On cooling, the precipitated solid is filtered, washed with acetonitrile (100 mL), and dried in vacuo at 60 °C to give 46.2 g (79.2%) of (5a*S*,9a*S*)-**6** as a white solid: mp 150–4 °C; $[\alpha]^{RT}_D$ = −34.0° (c = 0.05, MeOH); ^{1}H NMR ($CDCl_3$) δ 11.60 (s, 1 H), 7.76 (d, 1 H), 7.3 (d, 1 H), 4.9 (m, 1 H), 3.28 (m, 1 H), 1.2–2.2 (m, 8 H); chiral HPLC 99.9%; achiral HPLC 99.79%. The structure of (5a*S*,9a*S*)-**6** was confirmed by X-ray analysis.

***N*-(1-Azabicyclo[2.2.2]octan-3(*S*)-yl)-2-chloro-(5a*S*,9a*S*)-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxamide Hydrochloride (10).** To a solution of **9** (139.5 g, 0.552 mol) in chloroform (500 mL) preheated to about 40 °C was added, under a nitrogen blanket, thionyl chloride (84.9 g, 0.713 mol) over a period of 45 min. On completion of the reaction, excess thionyl chloride and solvent were removed under vacuum. The oil was stored under nitrogen until the 3-aminoquinuclidine free base was prepared.

The free base of 3(*S*)-aminoquinuclidine was prepared from 112 g (0.56 mol) of the dihydrochloride salt[19] and dissolved in toluene (700 mL). To this solution, under nitrogen, was added dropwise the 2-chloro-(5a*S*,9a*S*)-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carbonyl chloride of (5a*S*,9a*S*)-**6** (139.5 g, 0.55 mol) at 25 °C. The temperature was maintained at 40 °C. On completion of the reaction, toluene (300 mL) was added and a solution of 50% aqueous sodium hydroxide (45 g) in methanol (800 mL) was added. Further methanol (500 mL) was added to break up the solids. The mixture was extracted with water (2000 mL). The aqueous phase was removed, diluted with water (2000 mL), and extracted with toluene (2 × 1000 mL). The toluene extracts were combined, washed with water to a pH of 6, dried with magnesium sulfate, and filtered, and the toluene was removed under vacuum to give 171 g (86%) of **10** as a glassy solid. The glassy solid was dissolved in diethyl ether (150 mL) and a saturated etherial solution of hydrogen chloride was added in portions. Further hydrogen chloride was bubbled through the slurry to ensure complete salt formation. The solids were collected by vacuum filtration, washed with ether (3 × 100 mL), and dried at 60 °C in vacuo to give 175.7 g (80%) of **10**: mp 256 °C; $[\alpha]_D$ = −1.4° (c = 49 mg/mL, H_2O); ^{1}H NMR ($CDCl_3$) δ 8.1 (d, 1 H), 7.75 (d, 1 H), 7.22 (d, 1 H), 5.0 (m, 1 H), 4.56 (s, 1 H), 3.77 (m, 1 H), 3.4–3.5 (m, 1 H), 2.38 (s, 1 H), 1.2–2.4 (m, H); chiral HPLC 99.08%; achiral HPLC 99.59%. The structure of **10** was determined by X-ray analysis.

X-ray Structure Determination of 10. The X-ray structure determination was performed with a Enraf-Nonius CAD-4 diffractometer and the TEXSAN program system (TEXSAN, 1987) software on a Digital Equipment Corp. MicroVax II computer. The crystal was orthorhombic, space group *P*2,2,2, with cell constants *a* = 7.4530 (4) Å, *b* = 9.5633 (5) Å, and *c* = 27.831 (3) Å.

Conversion of Acid 6 to 5. A mixture of racemic acid **6** (300 mg, 1.19 mmol) and concentrated sulfuric acid (25 mL) was stirred at room temperature for 1 h. The solution turned dark amber as compound **6** dissolved. The mixture was cooled in an ice bath and water (50 mL) was added in portions at 25 °C. The cloudy mixture was extracted with toluene (2 × 50 mL); the toluene was dried with sodium sulfate, filtered, and evaporated to dryness to give a glassy solid. The NMR showed the mixture to be racemic acid **5**: ^{1}H NMR ($CDCl_3$) δ 10.2 (s, 1 H), 7.2 (d, 1 H), 7.2 (d, 1 H), 4.9 (m, 1 H), 3.3 (m, 1 H), 1.2–2.4 (m, 8 H).

***N*-(1-Azabicyclo[2.2.2]oct-3-yl)-2-chloro-*cis*-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxamide (9).** To a suspension of racemic acid **6** (10.0 g, 39.6 mmol) in chloroform (15.0 mL) at about 38 °C, under nitrogen, was added thionyl chloride (9.5 g, 79.8 mmol) in chloroform (10.0 ml) over a period of 30 min. On completion of the reaction, all the solvents were removed under vacuum to give an oil.

The free base of racemic 3-aminoquinuclidine (**7**) was prepared by adding a 1.0 M methanolic sodium hydroxide solution (110 mL) to a slurry of racemic 3-aminoquinuclidine dihydrochloride (10.0 g, 50 mmol) in absolute ethanol (10.0 mL). The ensuing precipitate was removed and the solution evaporated to dryness. The solid was dissolved in benzene and the insolubles filtered. The benzene was evaporated to dryness to give a voluminous white foam which on exposure to air became a sticky gum. The free base (5.58 g, 44 mmol) was dissolved in chloroform (10.0 mL) and added dropwise to the acid chloride, made previously, dissolved in chloroform (10.0 mL).

On completion of the reaction the mixture was treated with 0.5 N sodium hydroxide in methanol; the chloroform was separated, washed with water to a pH of 7, dried with magnesium sulfate, filtered, and evaporated to dryness to give 13.5 g (95% yield) of **9**, as a sticky solid. Chiral HPLC showed it to be a combination of four stereoisomers (**10**–**13**): ^{1}H NMR ($CDCl_3$) δ 7.99 (d, 1 H), 7.87 (d, 1 H), 7.19 (d, 1 H), 4.89 (m, 1 H), 4.15 (m, 1 H), 3.40 (m, 1 H), 3.26 (s, 1 H), 2.5–3.0 (m, 6 H), 2.1–1.3 (m, 12 H); HPLC 97.4%; chiral HPLC 24.5%:23.9%:25.7%:22.3%.

***N*-(1-Azabicyclo[2.2.2]octan-3-yl)-8-chloro-2,6-methano-3,4,5,6-tetrahydro-2*H*-1-benzoxocin-10-carboxamide (8).** The procedure was identical to that described for the preparation of **9** but starting with racemic acid **5** (4.9 g, 19.4 mmol) and coupling with racemic 3-aminoquinuclidine to give 4 g of the title compound: ^{1}H NMR ($CDCl_3$) δ 11.27 (2, 1 H), 8.47 (m, 1 H), 7.85 (t, 1 H), 7.09 (t, 1 H), 5.37 (s, 1 H), 4.95 (d, 1 H), 4.50 (m, 1 H), 3.90–3.2 (m, 8 H), 3.03 (s, 1 H), 2.39 (s, 1 H), 1.2–2.2 (m, 9 H); chiral HPLC 24.7%:24.9%:24.7%:24.7%.

2-Chloro-(5a*R*,9a*R*)-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxylic Acid [(5a*R*,9a*R*)-6]. This stereoisomer was prepared in the same manner as for (5a*S*,9a*S*)-**6**, but starting with racemic acid **6** and performing the resolution with (*R*)-4-nitro-α-methylbenzylamine hydrochloride, to give the resolved salt, which was converted to the resolved (5a*R*,9a*R*)-**6** free acid, with 1 N hydrochloric acid: mp 148 °C; $[\alpha]_D$ = +32.8° (c = 0.05, MeOH); ^{1}H NMR ($CDCl_3$) δ 9.85 (s, 1 H), 7.77 (d, 1 H), 7.29 (d, 1 H), 4.93 (m, 1 H), 3.3 (m, 1 H), 1.4–2.2 (m, 8 H); chiral HPLC 99.53%.

***N*-[1-Azabicyclo[2.2.2]octan-3(*S*)-yl]-2-chloro-(5a*R*,9a*R*)-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxamide Hydrochloride (13).** This stereoisomer was prepared in the same manner as for isomer **10**, starting with 15 g (0.059 mol) of (5a*R*,9a*R*)-**6** and 16.0 g (0.08 mol) of 3(*S*)-aminoquinuclidine dihydrochloride, to give 21 g (90%) of compound **13**: mp ~256 °C; ^{1}H NMR ($CDCl_3$) δ 7.95 (d, 1 H), 7.82 (d, 1 H), 7.26 (d, 1 H), 4.93 (m, 1 H), 4.49 (m, 1 H), 3.77 (m, 1 H), 3.1–3.6 (m, 6 H), 2.99 (s, 1 H), 2.45 (m, 1 H), 1.3–2.3 (m, 12 H); HPLC 99.5%; chiral HPLC 98.7%.

***N*-[1-Azabicyclo[2.2.2]octan-3(*R*)-yl]-2-chloro-(5a*S*,9a*S*)-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxamide Hydrochloride (12).** The procedure was identical to that described for the preparation of **10** but starting with (5a*S*,9a*S*)-**6** (15 g, 0.059 mol) and coupling with 3(*R*)-aminoquinuclidine to give 21 g of the title compound: mp ~257 °C; ^{1}H NMR ($CDCl_3$) δ 12.10 (d, 1 H), 7.97 (d, 1 H), 7.80 (d, 1 H), 7.24 (d, 1 H), 4.93 (m, 1 H), 4.49 (m, 1 H), 3.79 (m, 1 H), 3.2–3.6 (m, 6 H), 3.12 (s, 1 H), 2.43 (m, 1 H), 1.3–2.3 (m, 12 H); HPLC 99.6%; chiral HPLC 98.7%.

***N*-[1-Azabicyclo[2.2.2]octan-3(*R*)-yl]-2-chloro-(5a*R*,9a*R*)-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxamide Hydrochloride (11).** The procedure was identical to that described for the preparation of **10**, but starting with (5a*R*,9a*R*)-**6** (15 g, 0.059 mol) and coupling with 3(*R*)-aminoquinuclidine to give 23 g of the title compound: mp ~256 °C; ^{1}H NMR ($CDCl_3$) δ 11.87 (d, 1 H), 8.07 (d, 1 H), 7.76 (d, 1 H), 7.23 (d, 1 H), 4.99 (m, 1 H), 4.55 (m, 1 H), 3.77 (m, 1 H), 3.1–3.5 (m, 6 H), 2.39 (s, 1 H), 1.3–2.3 (m, 12 H); HPLC 92.5%; chiral HPLC 97.1%.

Resolution of 9 with (1*S*)-(+)-10-Camphorsulfonic Acid. Compound **9** (11.24 g, 31.1 mmol) was dissolved in 95% ethanol (70 mL) and a solution of (1*S*)-(+)-10-camphorsulfonic acid (7.24 g, 28.4 mmol) in 95% ethanol (10.0 mL) was added. To this reaction mixture was added diethyl ether (515 mL) and the mixture was left in a refrigerator for 12 h. The reaction mixture was filtered and the solids air-dried to give 7.07 g of material which by HPLC was shown to be 86% of the racemic pair **12** and **13**. Three recrystallizations from ethanol gave 2.3 g of material, which by HPLC was 94% of the racemate (**14**): ^{1}H NMR ($CDCl_3$) δ 12.22 (d, 1 H), 7.94 (d, 1 H), 7.81 (d, 1 H), 7.24 (d, 1 H), 4.93 (m, 1 H),

4.50 (m, 1 H), 3.76 (m, 1 H), 3.1–3.5 (m, 6 H), 2.43 (m, 1 H), 1.3–2.3 (m, 12 H); chiral HPLC 47.2%:46.4%.

Resolution of 9 with (1*R*)-(−)-10-Camphorsulfonic Acid. The mother liquors from the reaction of **9** with (1*S*)-(+)-10-camphorsulfonic acid were evaporated to dryness, and the material was converted to free base **9**. This material was treated with (1*R*)-(−)-10-camphorsulfonic acid in the same manner as for the (1*S*)-diastereoisomer, to give 77% by HPLC of the racemates **10** and **11**. Recrystallization from toluene/ether gave 2.5 g of racemate **15**, a combination of **10** and **11**: ^{1}H NMR ($CDCl_3$) δ 12.10 (d, 1 H), 8.03 (d, 1 H), 7.79 (d, 1 H), 7.23 (d, 1 H), 4.98 (m, 1 H), 4.54 (m, 1 H), 3.74 (m, 1 H), 3.1–3.5 (m, 6 H), 2.35 (m, 1 H), 1.3–2.3 (m, 12 H); chiral HPLC 46.86%:42.57%.

8-Chloro-3,4,5,6-tetrahydro-2(*S*),6(*R*)-methano-2*H*-1-benzoxocin-10-carboxylic Acid [(2*S*,6*R*)-5]. (*S*)-4-Nitro-α-methylbenzylamine hydrochloride (139.3 g, 0.69 mol) was added to 50% aqueous sodium hydroxide (59 mL) and the mixture extracted with methylene chloride (2 × 700 mL). The methylene chloride was separated, dried with sodium sulfate, filtered, and evaporated to dryness to give the free base of **4** as an oil, which was dissolved in methanol (1.0 L).

Racemic acid **5** (173.7 g, 0.69 mol) was added to the above methanolic solution and stirred to give a homogeneous solution. Within 0.5 h a white precipitate formed. The solvent was removed under reduced pressure and the crude salt mixture was suspended in ethyl acetate (700 mL) and methanol (500 mL) and heated to reflux to give a clear solution. On cooling to room temperature the solids were filtered and washed with ethyl acetate. The solids were recrystallized from refluxing ethyl acetate/methanol (400 mL/250 mL) to give 27.37 g of (2*S*,6*R*)-**5** salt as a white solid. ($[\alpha]_D$ = +27.61° (c = 5.0, MeOH).

The resolved salt of (2*S*,6*R*)-**5** was partitioned between methylene chloride (400 mL) and aqueous 1 N hydrochloric acid. The layers were separated, and the aqueous layer was reextracted with methylene chloride (200 mL). The combined organic layers were dried with sodium sulfate, filtered, and concentrated to dryness to give an oil, which on trituration with ether gave 17.49 g of the resolved acid (2*S*,6*R*)-**5**: mp 114–9 °C; ^{1}H NMR ($CDCl_3$) δ 10.79 (s, 1 H), 7.95 (d, 1 H), 7.23 (d, 1 H), 4.98 (s, 1 H), 3.11 (s, 1 H), 1.2–2.3 (m, 8 H); chiral HPLC 100%; $[\alpha]_D$ = +26.4° (c = 5, MeOH).

8-Chloro-3,4,5,6-tetrahydro-2(*R*),6(*S*)-methano-2*H*-1-benzoxocin-10-carboxylic Acid [(2*R*,6*S*)-5]. By a similar procedure, resolution of racemic acid **5** with (*R*)-4-nitro-α-methylbenzylamine gave the second enantiomer (2*R*,6*S*)-**5**: mp 114–9 °C; ^{1}H NMR ($CDCl_3$) δ 10.58 (d, 1 H), 7.94 (d, 1 H), 7.23 (d, 1 H), 4.99 (s, 1 H), 3.11 (s, 1 H), 1.6–2.2 (m, 8 H); chiral HPLC 99.6%; $[\alpha]^{RT}_D$ = −25.6° (c = 5, MeOH).

***N*-[1-Azabicyclo[2.2.2]octan-3(*S*)-yl]-8-chloro-2(*S*),6-(*R*)-methano-3,4,5,6-tetrahydro-2*H*-1-benzoxocin-10-carboxamide (18).** The free base of 3(*S*)-aminoquinuclidine dihydrochloride (5.0 g, 0.025 mol) was prepared as described previously and dissolved in toluene (60 mL). To this was added the acid chloride prepared from (2*S*,6*R*)-**5** (5.0 g, 0.02 mol) in toluene (20 mL). The usual workup gave 6.0 g (84%) of **18** as a glassy solid: $[\alpha]^{RT}_D$ = +12.83° (c = 5, MeOH); mp 115 °C; ^{1}H NMR ($CDCl_3$) δ 8.31 (d, 1 H), 7.91 (d, 1 H), 6.99 (d, 1 H), 4.75 (s, 1 H), 4.05 (m, 1 H), 3.31 (m, 1 H), 2.4–3.1 (m, 6 H), 1.2–2.2 (m, 12 H); MS *m*/*z* = 360; achiral HPLC 98.77%; chiral HPLC 97.88%. The structure of **18** was determined by X-ray analysis.

X-ray Structure Determination of 18. The X-ray structure determination was performed with a Enraf-Nonius CAD-4 diffractometer and TEXSAN program system (TEXSAN, 1987) software on a Digital Equipment Corp. MicroVax II computer. The crystals were monoclinic, space group $P2_1$, with cell constants *a* = 12.712 (3) Å, *b* = 12.833 (2) Å, and *c* = 13.692 (2) Å.

***N*-[1-Azabicyclo[2.2.2]octan-3(*S*)-yl]-8-chloro-2(*R*),6-(*S*)-methano-3,4,5,6-tetrahydro-2*H*-1-benzoxocin-10-carboxamide (21).** By the same procedure as above, condensation of **7** (5 g, 25.1 mmol), 3(*S*)-aminoquinuclidine, and the acid chloride prepared from (2*R*,2*S*)-**5** (5 g, 19.8 mmol) gave 4.8 g (68% yield) of **21**: ^{1}H NMR ($CDCl_3$) δ 8.38 (d, 1 H), 8.01 (d, 1 H), 7.09 (d, 1 H), 4.82 (s, 1 H), 4.12 (m, 1 H), 3.42 (m, 1 H), 3.05 (s, 1 H), 2.95–2.55 (m, 6 H), 1.3–2.2 (m, 12 H); chiral HPLC 92.5%.

***N*-[1-Azabicyclo[2.2.2]octan-3(*R*)-yl]-8-chloro-2(*S*),6-(*R*)-methano-3,4,5,6-tetrahydro-2*H*-1-benzoxocin-10-carboxamide (20).** By the same procedure as above, condensation of **7** (6 g, 30.1 mmol), 3(*R*)-aminoquinuclidine, and the acid chloride prepared from 6 g (23.8 mmol) of acid (2*S*,6*R*)-**5** gave 5 g (59% yield) of **20**: ^{1}H NMR ($CDCl_3$) δ 8.39 (d, 1 H), 8.01 (d, 1 H), 7.05 (d, 1 H), 4.82 (s, 1 H), 4.13 (m, 1 H), 3.42 (m, 1 H), 3.05 (s, 1 H), 2.95–2.55 (m, 6 H), 1.3–2.2 (m, 12 H); HPLC 92.8%; chiral HPLC 88.2%.

***N*-[1-Azabicyclo[2.2.2]octan-3(*R*)-yl]-2-chloro-2(*R*),6-(*S*)-methano-3,4,5,6-tetrahydro-2*H*-1-benzoxocin-10-carboxamide (19).** By the same procedure as above, condensation of **7** (5 g, 25.1 mmol), 3(*R*)-aminoquinuclidine, and the acid chloride prepared from 5 g (19.8 mmol) of acid (2R,6S)-**5** gave 4.7 g (66% yield) of **19**: ^{1}H NMR ($CDCl_3$) δ 8.39 (d, 1 H), 8.01 (d, 1 H), 7.09 (d, 1 H), 4.83 (s, 1 H), 4.16 (m, 1 H), 3.40 (m, 1 H), 3.05 (s, 1 H), 2.95–2.55 (m, 6 H), 1.3–2.2 (m, 12 H); chiral HPLC 95.7%.

Methyl 2-Chloro-6,7,8,9-tetrahydrodibenzofuran-4-carboxylate (22). To a solution of racemic **5** (85 mg, 0.32 mmol) in 1,4-dioxane (3 mL) was added DDQ (80 mg, 0.35 mmol) in a single portion at room temperature. The resulting solution was heated at reflux overnight (18 h). After cooling, the reaction mixture was diluted with ether and washed with 1.5 N sodium hydroxide, until the organic layer was colorless, and then with brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated. Flash chromatography (5% ethyl acetate/hexanes) gave the desired compound **22**: ^{1}H NMR ($CDCl_3$) δ 7.85 (d, *J* = 4.3 Hz, 1 H), 7.52 (d, *J* = 4.3 Hz, 1 H), 4.00 (s, 3 H), 2.78 (m, 2 H), 2.68 (m, 2 H), 1.95 (m, 4 H).

2-Chloro-6,7,8,9-tetrahydrodibenzofuran-4-carboxylic Acid (22a). To a solution of the ester (500 mg, 1.9 mmol) in methanol (5 mL) was added 1 N aqueous sodium hydroxide (1.0 mL) in a single portion at room temperature. The resulting solution was heated at reflux for 3 h. After cooling, methanol was removed in vacuo and the residue taken up in water and acidified to pH <2 with 1 N hydrochloric acid. The white precipitate was filtered and dried under vacuum.

***N*-(1-Azabicyclo[2.2.2]octan-3-yl)-2-chloro-6,7,8,9-tetrahydrodibenzofuran-4-carboxamide (23).** To a suspension of carboxylic acid **22a** (300 mg, 1.2 mmol) in chloroform (3 mL) was added triethylamine (0.34 mL, 2.4 mmol) in a single portion at room temperature. The resulting solution was cooled to 0 °C and ethyl chloroformate (0.14 mL, 1.44 mmol) was added dropwise via a syringe. Stirring was continued for 1.5 h at 0 °C and a solution of racemic 3-aminoquinuclidine dihydrochloride (1.2 g, 6 mmol) in saturated aqueous potassium carbonate (3 mL) was added in a single portion at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred at room temperature overnight (16 h). The reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated. Flash chromatography (10% methanol/chloroform) provided the desired product **23** in a very low yield as a white crystalline solid: mp 196–8 °C.

2-Chloro-*cis*-5a,6,7,8,9,9a-hexahydro-*N*-[2-(1-methyl-2-pyrrolidinyl)ethyl]dibenzofuran-4-carboxamide (25). Ethyl chloroformate (0.56 g, 0.0052 mol) was added to an ice-cold solution of 2-chloro-*cis*-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxylic acid (1.3 g, 0.0051 mol) in chloroform (15 mL) containing triethylamine (0.77 g, 0.0076 mol) and the mixture stirred at 0 °C for 1 h; then 2-(2-aminoethyl)-1-methylpyrrolidine (0.7 g, 0.0052 mol) was added and the mixture stirred overnight while it was allowed to come to room temperature. After a water wash, the chloroform solution was dried with magnesium sulfate, filtered, and evaporated to dryness to give 2.3 g of the product as an oil. The material was purified by dry column chromatography (10% methanol/chloroform) and 0.9 g (49%) of the product was obtained as a light amber oil: ^{1}H NMR ($CDCl_3$) δ 1.3–3.7 (m, 2 H), 4.8 (q, 1 H), 7.2 (d, 1 H), 7.9 (d, 1 H).

2-Chloro-*cis*-5a,6,7,8,9,9a-hexahydro-*N*-[2-(1-methyl-2-pyrrolyl)ethyl]dibenzofuran-4-carboxamide (26). Ethyl chloroformate (0.56 g, 0.0052 mol) was added to an ice-cold solution of 2-chloro-*cis*-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxylic acid (1.3 g, 0.0051 mol) in chloroform (15 mL) containing triethylamine (0.77 g, 0.0076 mol) and the mixture stirred at 0 °C for 1 h then 2-(2-aminoethyl)-1-methylpyrrole (0.7 g, 0.0052 mol) was added and the mixture stirred overnight while it was

allowed to come to room temperature. After a water wash, the chloroform solution was dried with magnesium sulfate, filtered, and evaporated to dryness to give 2.3 g of the product as an oil. The material was purified by dry column chromatography (10% methanol/chloroform) and 1.3 g of the product was obtained as a light amber oil which crystallized at room temperature. After trituration with ethyl ether, 0.8 g (44%) of the product was obtained as a cream-colored solid: mp 104-5 °C.

***N*-[2-(4-Morpholinyl)ethyl]-2-chloro-*cis*-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxamide (28).** To a stirred mixture of acid **5** (2.0 g, 8 mmol) and triethylamine (1.22 g, 12 mmol) in chloroform (12.0 mL) at 0 °C was first added ethyl chloroformate (0.95 g, 9 mmol), and then after 2 h of stirring, a solution of 4-(2-aminoethyl)morpholine (1.14 g, 9 mmol) in chloroform (3.0 mL) was added and stirring was continued overnight. It was then diluted with chloroform (100 mL), washed with water, dried over anhydrous magnesium sulfate, and evaporated to dryness to give 3.30 g of an oily product which was purified on a silica gel column (60% ethyl acetate/hexanes) to give 2.2 g (80%) of a white solid (**28**): mp 99 °C; MS m/z = 364 (M^+).

***N*-[2-(4-Imidazolyl)ethyl]-2-chloro-*cis*-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxamide (27).** By the same procedure as above, 2.0 g (8 mmol) of acid **5** and 0.99 g (9 mmol) of 4-(2-aminoethyl)imidazole gave 3.12 g of an oily mixture which was purified on a silica gel column (8% methanol/chloroform) to give 1.19 g (43%) of **27** as a white solid: mp 209-10 °C; MS m/z = 345 (M^+).

***N*-[2-(*N'*,*N'*-Diethylamino)ethyl]-2-chloro-*cis*-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxamide (24).** By the same procedure as above, 1.0 g (0.004 mol) of racemic acid **5** and 0.6 g (0.005 mol) of *N'*,*N'*-diethylenediamine gave an oily product which was taken up in ether, washed with 1 N sodium hydroxide and then water, dried over magnesium sulfate, filtered, and evaporated to dryness to give an oily product which was chromatographed over silica gel column (1:1 ethyl acetate/hexanes) to give a solid product (**24**) (0.84 g): mp 96-8 °C; MS m/z = 350 (M^+); ^{1}H NMR ($CDCl_3$) δ 8.15 (s, 1 H), 7.88 (m, 1 H), 7.17 (m, 1 H), 4.81 (m, 1 H), 3.52 (m, 2 H), 3.22 (m, 1 H), 2.57 (m, 6 H), 1.68 (m, 8 H), 1.03 (m, 6 H).

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Synthesis and in Vitro Biological Profile of All Four Isomers of the Potent Muscarinic Agonist 3-(3-Methyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane

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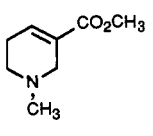
The four stereoisomers of the muscarinic agonist **7** have been synthesized from enantiomerically pure *exo*-azanorbornane esters (**13a,b**). The esters were obtained in optically active form by separation of the carboxamide diastereomers **12a,b**, formed from the borane complex of *exo*-azanorbornane-3-carboxylate **10** and a chiral amine auxiliary. Using the known chirality of (*R*)-α-methylbenzylamine, an X-ray analysis was accomplished on **12a** in order to determine the absolute configuration of the azanorbornane C4 chiral center. Each of the chiral esters **13a,b** was separately transformed into the oxadiazoles with concomitant epimerization at C3 of the azanorbornane ring to afford the thermodynamic equilibrium mixture of isomers. Chromatographic separation followed by analysis of each isomer by NMR and GC allowed the absolute stereochemistry of all four isomers of **7** to be confirmed. Full biological evaluation in biochemical and pharmacological assays revealed that the 3*R*,4*R* isomer was the most active on receptor binding studies and the most potent on the pharmacological preparations, showing a 50-fold increase in potency at the M_2 and M_3 sites compared to M_1.

The finding that specific cholinergic deficits present in brain tissues of patients diagnosed as having Alzheimer's disease[1] has led to the hypothesis that an enhancement of cholinergic neurotransmission may alleviate the deficits in memory and cognition. The clinical trials of a number of directly acting muscarinic agonists such as arecoline (**1**),[2] RS86 (**2**),[3] and pilocarpine (**3**)[4] have proved disappointing.



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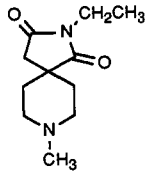

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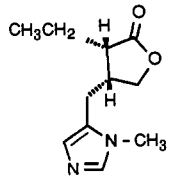

3

We have previously suggested[5] that these results may be understood in terms of the low cortical efficacy of these ligands. Within the cerebral cortex the postsynaptic pirenzepine-sensitive muscarinic receptors (coupled to phosphatidylinositol turnover) lack an effective receptor reserve, and therefore partial agonists such as **1**, **2**, and **3** produce a small maximal response relative to a full agonist such as carbachol (**4**).[6,7]

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