Novel 1-Phenylcycloalkanecarboxylic Acid Derivatives as Potential Anticonvulsant Agents^{†,‡}

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A series of analogues based on the anticonvulsant carbetapentane (1, 2-[2-(diethylamino)ethoxy]ethyl 1-phenyl-1-cyclopentylcarboxylate) was prepared as potential novel anticonvulsant drugs. Structure-activity relationships of analogues in which the ester function and cyclopentane moieties were modified have been investigated by evaluating their ability to prevent seizures in the rat maximal electroshock test. These compounds (11, $ED_{50} = 16 \mu mol/kg$; 12, $ED_{50} = 86 \mu mol/kg$, and 23, $ED_{50} = 173 \mu mol/kg$) were effective anticonvulsants. Compound 11, an alkyl ether derivative of 1, was more potent than the parent compound ($ED_{50} = 48 \mu mol/kg$) and also showed a 2-fold increase in potency compared to that of the prototypic anticonvulsant drug diphenylhydantoin.

Epilepsy is a general term that describes a central nervous system disorder which can manifest itself in a variety of abnormalities in EEG activity and motor episodes. Different types of seizures, characterized by the clinical manifestation of the attacks and the pattern of the EEG, require specific pharmacotherapeutic drug treatments.¹ A large number of patients with epilepsy cannot be treated successfully with current antiepileptic drugs.² In addition there is a need for new anticonvulsants that are more effective than those that are currently used for intractable epilepsy, such as those with complex partial seizures. Also the acute and chronic toxicity of currently available anticonvulsants such as embryotoxicity and hepatotoxicity demands the development of novel drugs which may be devoid of these side effects.² Furthermore, the development of medications for nonepileptic seizures (i.e. trauma-, drug- or toxin-induced) which may possess a completely different pharmacotherapeutic profile needs to be addressed. Once again, novel agents may provide tools to study these types of seizures as well as potentially furnishing new treatment modalities.

Carbetapentane (1, 2-[2-(diethylamino)ethoxy]ethyl 1-phenyl-1-cyclopentylcarboxylate) and dextromethorphan (2, (+)-3-methoxy-17-methylmorphinan) are nonopioid cough-suppressant drugs that demonstrate anticonvulsant activity in a variety of experimental seizure models.³ The rat maximal electroshock (MES) test is a seizure model that identifies drugs that are effective in generalized tonic-clonic (grand mal and partial) seizures.² In this model, both 1 and 2 block convulsions and potentiate the anticonvulsant action of the prototypic antiepileptic drug, diphenylhydantoin (5,5-diphenyl-2,4-imidazolinedione).⁴

Carbetapentane and dextromethorphan bind with nanomolar potency to high- and low-affinity dextromethorphan binding sites in guinea pig brain.⁵⁻⁷ It has been speculated that the anticonvulsant activity of these drugs may be mediated through one or both of these sites.⁸

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Alternatively, others have suggested that the anticonvulsant action of 2 may be due to the interaction of this drug at the N-methyl-D-aspartate (NMDA) receptor, as a noncompetitive antagonist,⁹ although the results of other studies fail to fully support this mechanism.³ Unlike 2, compound 1 does not protect against the lethality induced by NMDA¹⁰ and it does not antagonize the effects of NMDA in electrophysiological studies.⁹ Therefore, these data suggest that the anticonvulsant action of 1 is due to a mechanism distinct from that of NMDA antagonism.¹⁰

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^a(a) MeOH/HCl; (b) LiAlH₄/THF; (c) SOCl₂/benzene; (d) NaH/DMF, 95-100 °C.

Carbetapentane was first introduced primarily as an antispasmodic agent and later was used as a cough-suppressant drug.¹¹ Some derivatives of 1 in which the cyclopentyl ring was replaced by cyclohexane, thiopyran, and pyran have been evaluated as antispasmodic agents.¹² More recently, compound 1 has been described as a potent anticonvulsant.⁴ In an effort to prepare potential new anticonvulsant agents, chemical modification of the carbetapentane structure was explored to identify those structural features necessary for optimal anticonvulsant activity.

Although in the carbetapentane molecule there are several possible sites of chemical modification, the work described herein is concerned only with the ester function and the cycloalkane moiety. The ester functionality may be susceptible to metabolic degradation, and therefore improved biochemical stability and subsequent increases in duration of action may be achieved by chemical modification. Thus the ester function was bioisosterically replaced with an amide, the carbonyl group was reduced to a methylene group, and compounds with either a secondary or tertiary amine were synthesized. The cyclopentyl ring was expanded to a six-membered ring, in addition to the modifications described above, to investigate whether the resulting increased lipophilicity of these agents would improve anticonvulsant potency. The prepared compounds were examined for their ability to protect against seizures in the rat MES test.

Chemistry

The first two series of phenylcycloalkane carboxylic acid derivatives were prepared according to the methods outlined in Schemes I–III. In Scheme I, methyl esters 5 and 6 were obtained by classical methods, and LiAlH₄ reduction gave the desired alcohols 7 and 8. The replacement of the ester function of compound 1 with a methylenoxy group to give 11 and 12 was effected by alkylation of the alcohol with alkyl chloride 10. In an attempt to increase the yield of this reaction, different reaction temperatures were explored, but decomposition to another compound that was presumed to be a side chain elimination product resulted. Attempts to directly reduce the ester function of 1 using a mixture of sodium borohydride–boron tri-



9 + $Zn(N_3)_2$ 2Py + DIAD + Ph₃P

° (a) 95–100 °C; (b) Et₂NH·HCl, K_2CO_3/DMF , 110 °C; (c) 110–120 °C; (d) NH₂NH₂, 40% KOH; (e) H₂–10% Pd/C, 40 psi/EtOH.

Scheme III^a



 a (a) SOCl_/benzene; (b) pentene-stabilized CHCl_3, H_2O/NaH-CO_3; (c) $9/Et_3N/toluene;$ (d) Red-Al/toluene; (e) 37% form-aldehyde/NaBH_3CN.

fluoride etherate according to the method described by Pettit et al.¹³ was unsuccessful, resulting in a complex mixture of products from which the desired compound could not be identified or isolated.

The diamino side chain (20) needed for the preparation of the amide derivatives 21 and 22 was synthesized according to the method outlined in Scheme II. The first approach was the synthesis of compound 18, under Gabriel conditions,¹⁴ followed by hydrazinolysis. N-Substituted hydrazine intermediate 18 was synthesized by two methods. Although the yield obtained in both methods is not higher than 30%, the second method was preferred over the first because one step of synthesis was avoided. The hydrazinolysis of 18 was accomplished by refluxing in an ethanolic solution with hydrazine hydrate and the intermediate was decomposed under strong alkaline conditions using a 40% KOH solution according to the method described by Mosher.¹⁵ The second approach to compound

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Table I. Structures and Physical Properties of Carbetapentane Analogues



no.	n	X	salt	recrystn solvent	mp, °C	formula
11	1	-CH ₂ O-	oxalate	2-PrOH/ether	67 -69	C ₂₂ H ₃₅ NO ₆ ·1/2H ₂ O
12	2	$-CH_2O-$	oxalate	2-PrOH/ether	84-85	$C_{23}H_{37}NO_6$
21	1	-CONH-	fumarate	2-PrOH	118-120	$C_{24}H_{36}N_2O_6$
22	2	-CONH-	citrate	EtOHEtAcO	91-92	$C_{27}H_{42}N_2O_9$
23	2	-CO ₂ -	citrate	MeOH/ether	86-87	$C_{27}H_{41}NO_{10}$
24	1	-CH ₂ NH-	dioxalate	MeOH/EtOH	169-171	$C_{24}H_{38}N_2O_9$
25	2	-CH ₂ NH-	dioxalate	EtOH	123-124	$C_{25}H_{40}N_2O_9^{-1}/_2H_2O$
26	1	$-CH_2N(CH_3)-$	dioxalate	2-PrOH/ether	107-108	$C_{25}H_{40}N_2O_9$ ·1/2H2O

 Table II. Anticonvulsant Activity of Carbetapentane Analogues in the MES Test^a

	ED ₅₀ (95% CL ^b)		
compound	mg/kg, sc	$\mu mol/kg, sc$	
diphenylhydantoin	8 (5-14)	30 (18-49)	
1	25 (16-38)	48 (31-72)	
11	7 (4-12)	16 (9-31)	
12	37 (21-64)	86 (50-151)	
21	IAc		
22	IA		
23	93 (43-203)	173 (80-375)	
24	IA		
25	IA		
26	IA		

 ${}^{a}n = 10$ for all groups. ${}^{b}CL$, confidence limits. ^cInactive at doses up to 50 mg/kg.

20 was to catalytically reduce the azide derivative 19, which was obtained by treatment of the primary alcohol 9 with zinc azide, in the form of its more stable bis-pyridine complex, under Mitsunobu conditions.¹⁶ This last approach gave the best results, and improved isolation of the azide is currently being pursued to obtain better yields. Amide derivatives 21 and 22 were synthesized from acid chlorides 13 and 14 and amine 20 as depicted in Scheme III.¹⁷ Compound 23 was synthesized by reaction of acid chloride 14 with compound 9 in toluene-triethylamine. Reduction of amides 21 and 22 using Red-Al (Aldrich Chemical Co.) afforded the secondary amines 24 and 25, respectively. Compound 26 was obtained via a modification of the procedure of Borch and Hassid.¹⁸

Pharmacology

In general, MES causes a generalized convulsion characterized by an initial tonic extension of the forelimbs, progressing immediately to tonic hindlimb extension followed by clonic jerking. The ability of the compounds to

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Figure 1. Anticonvulsant dose-response effect of 1 and compounds 11, 12 and 23. Percent protection refers to the percentage of rats (n = 10 per group) that were protected against MES-induced seizure (quantal data). Each dose-response curve represents the computer-derived regression line analyzed for linearity.¹⁹

block tonic hindlimb extension in the rat was recorded for each MES convulsion. We have previously shown that the shock parameters (see the Experimental Section) used in this study induce MES, and not threshold seizures.⁴

Discussion

The structures and physical properties of the prepared compounds are displayed in Table I. In Table II, protection of MES-induced seizures in the rat by compound 1 and the synthetic analogues described herein are shown. Three of the analogues were effective anticonvulsants, with compound 11 being more potent than the parent compound.

In this first series of compounds, structure-activity relationship trends can be summarized as follows: within like series, cycloalkane expansion results in decreased activity, e.g. 1 vs 23 and 11 vs 12, while replacement of the ester with an ether function results in increased activity, e.g. 1 vs 11 and 23 vs 12. Introduction of a nitrogen function (i.e. amide or amine) results in inactive compounds. Since replacement of the ester with an ether resulted in increased anticonvulsant potency, a time course study will allow us to compare the duration of the anticonvulsant effect of compound 11 with 1 as well as to determine the time of peak effect.

In Figure 1, the anticonvulsant dose-response effect of 1, and the synthetic analogues 11, 12, and 23, is shown. All four drugs produced a linear dose-response curve (1, r = 0.963; 11, r = 0.994; 12, r = 0.945; 23, r = 0.997) with dose-related increases in protection for MES-induced

convulsions. Statistical comparison¹⁹ of these functions indicated no deviation from parallelism.

In this first series of analogues, three of the prepared analogues (11, 12, and 23) protected rats against MESinduced seizures and represent new anticonvulsant agents. We are presently investigating the binding profile of these compounds at dextromethorphan, σ , and phencyclidine sites in an attempt to correlate receptor binding affinity with anticonvulsant activity. Pharmacological evaluation of these analogues in other seizure models is currently being pursued to further explore their anticonvulsant profile.

Experimental Methods

Synthesis. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Thin-layer chromatography (silica gel GF, Analtech, Delaware) was used to detect product homogeneity. Flash column chromatography (silica gel, grade 60, 230-400 mesh; Aldrich Chemical Company, Milwaukee, WI) was used for purification. The solvent system used for all chromatography was CHCl₃/MeOH/NH₄OH (90:10:1) unless otherwise specified. Drying refers to the use of Na₂SO₄. ¹H NMR spectra were obtained either on a Bruker AC 300 MHz or a Varian XL 300 MHz NMR spectrometer using tetramethylsilane as an internal standard. IR spectra were determined on a Nicolet Model 105 IR spectrometer using either KBr pellets or CHCl₃ cells. EIMS and CIMS (NH₃) were obtained on a Finnegan 1015 mass spectrometer. All compounds exhibited NMR, IR, and mass spectral data consistent with those of the structures assigned. Elemental analyses were performed by Spang Microanalytical Laboratory (Eagle Harbor, MI) and were within 0.4% of the theoretical values.

1-Phenyl-1-cyclohexanecarboxylic Acid (4). A modification of the procedure for hydrolysis of nitriles by Bannard et al.²⁰ was used to obtain compound 4. A solution of the commercially available (Lancaster Synthesis) 1-phenyl-1-cyclohexanecarbonitrile (20.0 g, 108 mmol) in 80 mL of 48% HBr was stirred at reflux for 4 days. The solution was basified with 10% NaOH (w/v) to pH 8-9 and washed with ether $(3 \times 25 \text{ mL})$. Acidification of the aqueous layer with 1 N HCl to pH 2-3 and extraction of the product with ether (4 × 25 mL) gave 15.9 g of 4 (72%), mp 118-121 °C (lit.²¹ mp 121 °C).

Methyl 1-Phenyl-1-cyclopentanecarboxylate (5). A solution of 3 (2.00 g, 10.51 mmol) in 30 mL of MeOH saturated with HCl was stirred at reflux for 2 h. The reaction mixture was allowed to cool and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with 2 N NaOH (3 × 25 mL). The organic layer was washed with water $(2 \times 25 \text{ mL})$ and dried. Evaporation of the solvent gave 1.89 g (88%) of 5 as a pale yellow liquid and was used in the next step without further purification: CIMS m/z 205 (M + 1).

Methyl 1-Phenyl-1-cyclohexanecarboxylate (6). Compound 6 (1.91 g, 8.8 mmol, 89%) was prepared from 4 (2.00 g, 9.8 mmol) according to the procedure previously described for the synthesis of 5. This compound was homogeneous by TLC (ether/CH₂Cl₂, 2:1) and used in the next step without further purification: CIMS m/z 219 (M + 1).

1-Phenylcyclopentanemethanol (7). A solution of 5 (3.00 g, 14.71 mmol) in 10 mL of THF was added dropwise to a suspension of LiAlH₄ (1.25 g, 33.09 mmol) in 25 mL of dry THF. The reaction mixture was allowed to stir at reflux for 3 h. The excess LiAlH₄ was destroyed following the method described by Fieser and Fieser²² whereby the cooled reaction mixture was guenched

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by addition of 1.25 mL of water followed by addition of 1.25 mL of 15% NaOH (w/v) and by the final addition of 3.75 mL of water. The resulting aluminum salts were separated by filtration and washed with water $(5 \times 1 \text{ mL})$. The product was extracted with ether $(3 \times 25 \text{ mL})$ and dried; evaporation of the solvent gave 2.07 g (88%) of 7 as a white solid, mp 41-42 °C (lit.²³ mp 43-44 °C).

1-Phenylcyclohexanemethanol (8). Compound 8 was prepared (1.02 g, 5.43 mmol, 62%) from 6 (1.91 g, 8.76) using LiAlH₄ (0.75 g, 37.95 mmol) according to the procedure described for the synthesis of 7, with the exception that after destroying the excess hydride, separating the aluminum salts by filtration, and washing with water $(3 \times 4 \text{ mL})$, compound 8 precipitated out of solution. Filtration of the precipitate gave 8 as a white solid, mp 63-64 °C (lit.²³ mp 63-64 °C).

2-[2-(Dimethylamino)ethoxy]ethyl Chloride (10). A modification of the procedure described by Blicke and Biel²⁴ was used to obtain compound 10, beginning with the dropwise addition of SOCl₂ (17.02 mL, 233.42 mmol) to a solution of the commercially available 2-[2-(diethylamino)ethoxy]ethanol (9, Fluka) in benzene (100 mL). The reaction mixture was allowed to stir at reflux for 1.5 h, and the volatiles were removed under reduced pressure. The oily residue, which crystallized in ether, was nearly homogeneous by TLC and was used in the next step without further purification. The HCl salt obtained in this reaction was very hygroscopic. Attempts at purification of the free base via distillation resulted in decomposition: CIMS m/z 180 (M + 1), 182 (M + 3)

O-[2-[2-(Diethylamino)ethoxy]ethyl]-1-phenyl-1-cyclopentanemethanol (11). A solution of 7 (2.21 g, 12.56 mmol) in dry DMF (10 mL) was carefully added to NaH (1.00 g, 25.12 mmol, 60% suspension in mineral oil) previously washed with petroleum ether $(4 \times 5 \text{ mL})$, under an atmosphere of Ar, at 0 °C. After the addition was complete, the reaction mixture was allowed to stir at room temperature for 30 min. The HCl salt of 10 (13.56 g, 62.78 mmol) was dissolved in 10% NaOH (50 mL), and the free base was extracted with $CHCl_3$ (5 × 25 mL) and dried, and the solvent was evaporated under reduced pressure. The dark orange liquid residue was added dropwise to the reaction mixture. The addition funnel was washed with DMF (5 mL) and the reaction mixture was allowed to stir overnight at 95-100 °C. The reaction was carefully quenched with water (5 mL) and the product was extracted with ether $(2 \times 25 \text{ mL})$. The organic layer was washed with 1 N HCl $(3 \times 25 \text{ mL})$. The ether layer was dried and evaporated, affording unreacted starting material 7 (1.40 g, 7.95 mmol). The combined aqueous solution was basified to pH 9 with NH₄OH, extracted with $\tilde{C}HCl_3$ (3 × 25 mL), and dried. Removal of the solvent in vacuo afforded 11 as a pale yellow oil (0.74 g, 50% yield based on recovered starting material). The oxalate hemihydrate salt was obtained by dissolving the free base (0.74 g, 2.31 mmol) in a minimal volume of MeOH and adding it to a solution of oxalic acid (0.21 g, 2.31 mmol) in hot MeOH. The solvent was evaporated and the salt recrystallized from 2-PrOH/ether: mp 67-69 °C; ¹H NMR (D₂O) δ 1.22 (t, J = 7.3 Hz, 6 H), 1.68–1.72 (m, 4 H), 1.88–1.90 (m, 4 H), 3.10–3.21 (m, 6 H), 3.52–3.61 (m, 6 H), 3.66 (s, 2 H), 7.27–7.46 (m, 5 H); CIMS m/z319 (M + 1). Anal. (C₂₂H₃₅NO₆·¹/₂H₂O) C, H, N.

O-[2-[2-(Diethylamino)ethoxy]ethyl]-1-phenyl-1-cyclohexanemethanol (12). Compound 12 was prepared (1.00 g, 3.0 mmol, 39%) from 8 (1.45 g, 7.63 mmol) according to the procedure described for the synthesis of 11 and the product was purified by flash column chromatography. The oxalate salt was obtained by dissolving the free base (0.34 g, 1.01 mmol) in a minimal volume of hot MeOH and adding it to a solution of 0.09 g of oxalic acid (1.01 mmol) in hot MeOH. The solvent was evaporated and the salt recrystallized from 2-PrOH/ether: mp 84-85 °C; ¹H NMR $(D_2O) \delta 1.24$ (t, J = 7.3 Hz, 6 H), 1.32–1.66 (m, 8 H), 2.08–2.13 (m, 2 H), 3.12-3.23 (m, 6 H), 3.45-3.62 (m, 8 H), 7.29-7.53 (m, 5 H); CIMS m/z 334 (M + 1). Anal. (C₂₃H₃₇NO₆) C, H, N.

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1-Phenylcycloalkanecarboxylic Acid Derivatives

2-Chloroethyl 2-Phthalimidoethyl Ether (17). Following a modification of the procedure described by Cretcher et al.,²⁵ a mixture of potassium phthalimide (15, 3.16 g, 17.05 mmol), 2-chloroethyl ether (16, 3 mL, 25.6 mmol), and two drops of triethylamine was allowed to heat without stirring at 110–120 °C for 4 h. The volatiles were removed under reduced pressure. The mixture was dissolved in CHCl₃ and compound 17 was separated from the crude reaction mixture by flash column chromatography and eluted with CHCl₃. Evaporation of the solvent gave 2.30 g (53%) of 17, mp 70–71 °C (lit.²⁵ mp 69 °C).

[2-(2-Phthalimidoethoxy)ethyl]diethylamine (18). Compound 18 was obtained by two different methods.

Method A. A mixture of 17 (5.00 g, 19.71 mmol), diethylamine hydrochloride (6.48 g, 59.13 mmol), and K₂CO₃ (10.90 g, 78.84 mmol) in 25 mL of DMF was allowed to stir at 110 °C for 18 h. The solution was filtered through a pad of silica gel and the mixture was first eluted with ether (100 mL). The product was eluted with CHCl₃/MeOH/NH₄OH (90:10:1). Evaporation of the solvent gave 1.52 g (27%) of 18 which crystallized from ether: mp 132-134 °C; ¹H NMR (CDCl₃) δ 1.34 (t, J = 7.3 Hz, 6 H), 3.10-3.18 (m, 6 H), 3.73 (t, J = 5.3 Hz, 2 H), 3.90 (t, J = 5.3 Hz, 2 H), 3.97 (t, J = 4.7 Hz, 2 H), 7.76 (dd, J = 5.5 Hz, 3.1, 2 H), 7.87 (dd, J= 5.5 Hz, 3.1, 2 H); CIMS m/z 291 (M + 1).

Method B. A mixture of 15 (10.00 g, 53.98 mmol), 10 (16.63 g, 92.90 mmol, free base), and triethylamine (1 mL) was allowed to heat without stirring at 95–100 °C for 16 h. The crude reaction mixture was dissolved in CHCl₃, and 18 was purified by flash column chromatography to give 2.45 g (16%) of 18, which was identical to the product described in method A.

2-[2-(Diethylamino)ethoxy]ethylamine (20). Compound 20 was synthesized by two different methods.

Method A. A modification of the procedure described by Clinton et al.²⁶ was used whereby a solution of 18 (1.61 g, 5.55 mmol) in 20 mL of EtOH was allowed to stir at reflux with 64% hydrazine hydrate (0.55 mL, 11.10 mmol) for 2 h. The volatiles were removed, and the solid residue was dissolved in 50 mL of 40% KOH (w/v). The product was extracted with ether (5 × 25 mL) and dried. Evaporation of the solvent gave 0.73 g of 20 (82%) as a yellow oil. The dioxalate salt was prepared by dissolving the free base (0.05 g, 0.31 mmol) in a minimal volume of MeOH and adding it to a solution of 0.06 g of oxalic acid (0.62 mmol) in hot MeOH. The solvent was evaporated and the salt recrystallized from MeOH/2-PrOH: mp 123-125 °C; ¹H NMR (CD₃OD) δ 1.21 (t, J = 6.8 Hz, 6 H), 3.05 (t, J = 5.0, 2 H), 3.15–3.28 (m, 6 H), 3.64 (t, J = 5.0, 2 H), 3.73 (t, J = 5.0, 2 H); CIMS m/z 161 (M + 1).

Method B. A modification of the procedure described by Viaud and Rollin¹⁶ was followed. A zinc azide-bispyridine complex (5.52 g, 18 mmol) was prepared and suspended in a solution of 2-[2-(diethylamino)ethoxy]ethanol (9, 4.12 mL, 24 mmol) and triphenylphosphine (12.6 g, 48 mmol) in 100 mL of anhydrous toluene. To this stirred solution was added diisopropyl azodicarboxylate (9.44 mL, 48 mmol) dropwise. The reaction mixture was allowed to stir at room temperature for 2 h. The solvent was evaporated under vacuum and a precipitate of triphenylphosphine oxide was separated by filtration. Distillation of the crude mixture under reduced pressure (1 mm) resulted in collection of the main fraction (19) at 25 °C: IR 2080 cm⁻¹; CIMS m/z 187 (M + 1). A solution of the clear distillate in 100 mL of EtOH was catalytically reduced by hydrogenation in a Parr hydrogenator (40 psi) with 10% Pd/C (500 mg), at room temperature for 48 h. Evaporation of the solvent gave 3.66 g (48%) of an oily residue that was homogeneous by TLC (CHCl₃/MeOH/NH₄OH 70:30:1). The dioxalate salt was prepared by dissolving the free base (0.70 g, 4.38 mmol) in a minimal volume of MeOH and adding it to a solution of 0.79 g oxalic acid (8.75 mmol) in hot MeOH. The solvent was evaporated and the salt recrystallized from MeOH/2-PrOH. The product obtained was identical to the compound described in method A.

N-[2-[2-(Diethylamino)ethoxy]ethyl]-1-phenyl-1-cyclopentanecarboxamide (21). A solution of 1-phenyl-1-cyclopentanecarboxylic acid (3, 0.99 g, 5.18 mmol) in 2.60 mL of SOCl₂ was allowed to stir at reflux for 3 h under Ar. The volatiles were removed under reduced pressure, and the acid chloride (13) obtained was dissolved in 10 mL of pentene-stabilized CHCl₃. The resulting solution was added dropwise to a mechanically stirred solution of amine 20 (0.63 g, 3.94 mmol) and NaHCO₃ (0.66 g, 7.88 mmol) in 15 mL of water. The biphasic reaction mixture was allowed to stir at room temperature for 1 h. The CHCl₃ layer was separated and evaporated. The residue was purified by flash column chromatography to give 0.93 g (71%) of 21 as an oily residue that was homogeneous by TLC. The fumarate salt was prepared by dissolving the free base (100 mg, 0.30 mmol) in a minimal volume of hot MeOH and adding it to a solution of 0.35 g of fumaric acid (0.30 mmol) in hot MeOH. The solvent was evaporated and the salt was recrystallized from 2-PrOH: mp 118–120 °C; ¹H NMR (D₂O) δ 1.15 (t, J = 7.2 Hz, 6 H), 1.58–1.63 (m, 4 H), 1.82–1.92 (m, 2 H), 2.33–2.42 (m, 2 H), 3.01–3.09 (m, 4 H, 3.19-3.23 (m, 4 H), 3.37 (t, J = 5.3 Hz, 2 H), 3.52 (t, J = 4.9Hz, 2 H), 7.11–7.29 (m, 5 H); CIMS m/z 332 (M + 1). Anal. $(C_{24}H_{36}N_2O_6)$ C, H, N.

N-[2-[2-(Diethylamino)ethoxy]ethyl]-1-phenyl-1-cyclohexanecarboxamide (22). Compound 22 was prepared (0.72 g, 2.08 mmol, 45%) from 14 (1.34 g, 6.01 mmol) according to the procedure described for the synthesis of 21. The citrate salt was prepared by dissolving the free base (0.29 g, 0.84 mmol) in a minimal volume of hot EtOH and adding it to a solution of 0.16 g of citric acid (0.84 mmol) in hot EtOH. The solvent was evaporated and the salt was recrystallized from EtOH/ethyl acetate: mp 91-92 °C; ¹H NMR (D₂O) δ 1.15 (t, J = 7.2 Hz, 6 H), 1.43-1.57 (m, 6 H), 1.71-1.80 (m, 2 H), 2.26-2.31 (m, 2 H), 3.55 (t, J = 4.9 Hz, 2 H), 7.13-7.35 (m, 5 H) CIMS m/z 347 (M + 1). Anal. (C₂₇H₄₂N₂O₉) C, H, N.

2-[2-(Diethylamino)ethoxy]ethyl 1-Phenyl-1-cyclohexanecarboxylate (23). A solution of 4 (1.02 g, 5 mmol) and 2.5 mL of SOCl₂ in 25 mL of toluene was allowed to stir at reflux for 2 h under Ar. The solvent was evaporated under reduced pressure and the acid chloride (14) was dissolved in 20 mL of toluene. A solution of 9 (0.85 mL, 5 mmol) and triethylamine (0.75 mL) was added dropwise to the acid chloride solution. The mixture was allowed to stir at reflux for 3 h and then stand at room temperature overnight. The triethylamine hydrochloride formed in the reaction was separated by filtration and the filtrate was washed with toluene $(3 \times 1 \text{ mL})$. The solvent was evaporated and the residue was dissolved in 25 mL of 20% NH4OH. The product was extracted with $CHCl_3$ (3 × 25 mL) and the combined organic fraction was washed with water $(2 \times 25 \text{ mL})$ and dried. The solvent was evaporated, affording 23 as a pale yellow oil (1.72 g, 99%). The citrate salt was prepared by dissolving the free base (0.50 g, 1.44 mmol) in a minimal volume of hot MeOH and adding it to a solution of 0.28 g of citric acid (1.44 mmol) in hot MeOH. Addition of anhydrous ether resulted in the crystalline citrate salt, which was recrystallized from MeOH/ether: mp 86-87 °C; ¹H NMR (D₂O) δ 1.20 (t, J = 7.2 Hz, 6 H), 1.41–1.85 (m, 8 H), 2.38-2.41 (m, 2 H), 3.07-3.14 (m, 6 H), 3.57-3.70 (m, 4 H), 4.28 (t, J = 4 Hz, 2 H), 7.33-7.50 (m, 5 H); CIMS m/z 348 (M + 1).Anal. $(C_{27}H_{41}NO_{10})$ C, H, N.

N-[2-[2-(Diethylamino)ethoxy]ethyl]-1-phenyl-1-cyclopentanemethylamine (24). A solution of 21 (1.00 g, 3.01 mmol) in 15 mL of toluene was added dropwise over a solution of Red-Al (Aldrich Chemical Co., 2.95 mL, 9.94 mmol) in 15 mL of toluene. The mixture was allowed to stir at reflux overnight. The excess hydride was carefully quenched with water and the product was extracted with ether $(4 \times 25 \text{ mL})$. The organic layer was washed with 1 N HCl (4×25 mL) and the combined aqueous solution was basified to pH 9 with NH₄OH, extracted with CHCl₃ (4 \times 25 mL), and dried. The solvent was evaporated, affording 24 as a pale yellow oil (0.70 g 74%). The dioxalate salt was prepared by dissolving the free base (0.22 g, 0.65 mmol) in a minimal volume of hot MeOH and adding it to a solution of 0.12 g oxalic acid (1.3 mmol) in hot MeOH. The solvent was evaporated and the salt recrystallized from MeOH/EtOH: mp 169–171 °C; ¹H NMR (D₂O) δ 1.22 (t, J = 7.3 Hz, 6 H), 1.66–1.76 (m, 4 H), 1.88–1.94 (m, 2 H), 2.08-2.13 (m, 2 H), 3.10-3.19 (m, 8 H), 3.37 (s, 2 H),

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3.65–3.71 (m, 4 H), 7.37–7.50 (m, 5 H); CIMS m/z 319 (M + 1). Anal. (C₂₄H₃₈N₂O₉) C, H, N.

N-[2-[2-(Diethylamino)ethoxy]ethyl]-1-phenyl-1-cyclohexanemethylamine (25). Compound 25 was prepared (0.23 g, 0.69 mmol, 51%) from 22 (470 mg, 1.36 mmol) according to the procedure described for the synthesis of 24. The dioxalate hydrate salt was generated by dissolving the free base (0.23 g, 0.69 mmol) in a minimal volume of hot EtOH and adding it to a solution of 0.13 g oxalic acid (1.38 mmol) in hot EtOH. Addition of ether resulted in a white crystalline salt. Recrystallization from EtOH gave the pure oxalate salt of 25: mp 123-124 °C; ¹H NMR (D₂O) δ 1.23 (t, J = 7.3 Hz, 6 H), 1.31-1.72 (m, 8 H), 2.21-2.26 (m, 2 H), 3.12-3.23 (m, 8 H), 3.27 (s, 2 H), 3.65-3.70 (m, 4 H), 7.41-7.57 (m, 5 H); CIMS m/z 333 (M + 1). Anal. (C₂₅H₄₀N₂O₈, ¹/₂H₂O) C, H, N.

N-[2-[2-(Diethylamino)ethoxy]ethyl]-N-methyl-1phenyl-1-cyclopentanemethylamine (26). A modification of the procedure for N-methylation of amines by Borch and Hassid¹⁸ was used to obtain compound 26. To a solution of 24 (0.32 g, 1 mmol) in CH₃CN was added 37% formaldehyde (0.24 mL, 3 mmol), followed by NaBH₈CN (0.10 g, 1.6 mmol) at 0 °C. The reaction mixture was allowed to stir at room temperature for 1 h and then was neutralized to pH 6-7 by dropwise addition of glacial acetic acid. After stirring for 45 min, the volatiles were removed in vacuo, and 4 mL of 2 N KOH was added. Extraction with $CHCl_3$ (3 × 100 mL) was followed by washing the combined CHCl₃ fractions with 10 mL of 0.5 N KOH, drying, and evaporation of solvent in vacuo to give 0.31 g (92%) of 26 as the crude free base. The dioxalate salt was obtained by dissolving the free base (0.24 g, 0.71 mmol) in a minimal volume of hot MeOH and adding it to a solution of 0.13 g of oxalic acid (1.42 mmol) in MeOH. The solvent was evaporated and the salt was recrystallized from 2-PrOH/ether: mp 107-108 °C; ¹H NMR (CD₃OD) δ 1.29 (t, J = 7.2 Hz, 6 H), 1.62-1.68 (m, 2 H), 1.78-1.83 (m, 2 H),1.90-1.97 (m, 2 H), 2.19-2.27 (m, 2 H), 2.62 (s, 3 H), 3.11-3.21 (m, 4 H), 3.24-3.29 (m, 4 H), 3.64 (s, 2 H), 3.68-3.75 (m, 4 H), 7.28–7.51 (m, 5 H); CIMS m/z 333 (M + 1). Anal. (C₂₅H₄₀N₂O₉·1/₂H₂O) C, H, N.

Anticonvulsant Protocol. Male Sprague-Dawley rats (200-250 g; Zivic-Miller Laboratories), n = 10 per group, were randomly assigned as control of drug-treated animals. Both groups were subjected to a single transauricular maximal electroshock (MES, 2 s at 60 Hz and 50 mA) convulsion delivered through miniature alligator clips attached to the pinna of each ear. All compounds were administered subcutaneously (sc) and tested at 30 min, the time of peak anticonvulsant activity for dextrome-thorphan.⁴ All drugs were freshly prepared using appropriate dilutions in normal saline. Injection volumes were 1-2 mL/kg. Control groups received an appropriate vehicle injection.

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Synthesis, Molecular Modeling Studies, and Muscarinic Receptor Activity of Azaprophen Analogues

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Synthesis, radioligand binding, and pharmacologic activities of a series of muscarinic receptor ligands including and related to azaprophen (6-methyl-6-azabicyclo[3.2.1]octan- 3α -ol 2,2-diphenylpropionate, 1) have been measured to determine activity and selectivity for muscarinic receptor subtypes. Pharmacologic affinities of antagonists were determined as pA₂ values for antagonism of methacholine-induced tension responses in guinea pig ileum. Binding affinities were measured by competition against [³H]QNB binding in guinea pig ileum, rat heart and brain, and m_1 - or m_3 -transfected Chinese hamster ovary (CHO) cells. The efficacies of muscarinic agonists in brain were determined by the ratio of binding affinities against [³H]QNB or [³H]NMS and [³H]oxotremorine-M ([³H]Oxo-M). Nine muscarinic antagonists, including azaprophen, did not discriminate significantly between the subtypes of muscarinic receptors. K_1 values for receptor binding for azaprophen (1) were between 8.81×10^{-11} and 4.72×10^{-10} M in ileum, heart, brain, and m_1 - or m_3 -transfected CHO cells. The α - and β -benzilate esters 5 and 6 are as potent as azaprophen, and diphenylacetate esters 3 and 4 and N-(6)-benzyl α -isomer 7 are less potent than azaprophen. Significant stereoselectivity was exhibited with (+)-azaprophen being approximately 200 times more potent than the (-)-enantiomers and the 3β -ol isomer 2 being ca. 50 times less potent than azaprophen in all systems. A molecular modeling-molecular mechanics study was conducted to account for the difference. Putative muscarinic agonists (analogues and isomers of 6-methyl-6-azabicyclo[3.2.1]octan-3-ol acetate) did not discriminate muscarinic receptor subtypes with $K_{\rm I}$ values between 2.77 × 10⁻⁶ and 4.33 × 10⁻⁵ M without significant stereoselectivity in the systems examined. The most active analogue was (1R,3R,5S)-6-[1(R)-phenylethyl]-6-azabicyclo[3.2.1]octan-3 α -ol acetate. However, efficacies of these putative agonists were in general very low.

Introduction

A major contributing cause to the cognitive deficit in Alzheimer's disease is a selective degeneration of cholinergic neurons projecting into cortical and hippocampal regions.^{1,2} The origin of this deficit is not established, but

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