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

Silvia N. Calderon,§,∥ Amy Hauck Newman,*,§,∥ and Frank C. Tortella⊥

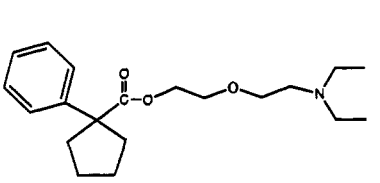
Department of Applied Biochemistry, and Neuropharmacology Branch, Walter Reed Army Institute of Research, Washington, DC, 20307-5100. *Received March 14, 1991*

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 μmol/kg; 12, ED_{50} = 86 μmol/kg, and 23, ED_{50} = 173 μmol/kg) were effective anticonvulsants. Compound 11, an alkyl ether derivative of 1, was more potent than the parent compound (ED_{50} = 48 μmol/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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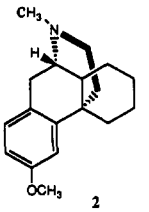

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Alternatively, others have suggested that the anticonvulsant action of 2 may be due to the interaction of this drug at the *N*-methyl-D-aspartate (NMDA) receptor, as a noncompetitive antagonist,[9] although the results of other studies fail to fully support this mechanism.[3] Unlike 2, compound 1 does not protect against the lethality induced by NMDA[10] 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]

† Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NIH publication 85-23. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4-3), AR 360-5.

‡ Portions of this paper were presented at the 22nd Medicinal Chemistry Symposium, July 1990, and the 20th Annual Meeting of the Society for Neurosciences, October 1990.

§ Department of Applied Biochemistry.

∥ Current address: Drug Development Group, Psychobiology Laboratory, NIDA Addiction Research Center, P.O. Box 5180, Baltimore, MD 21224.

⊥ Neuropharmacology Branch.

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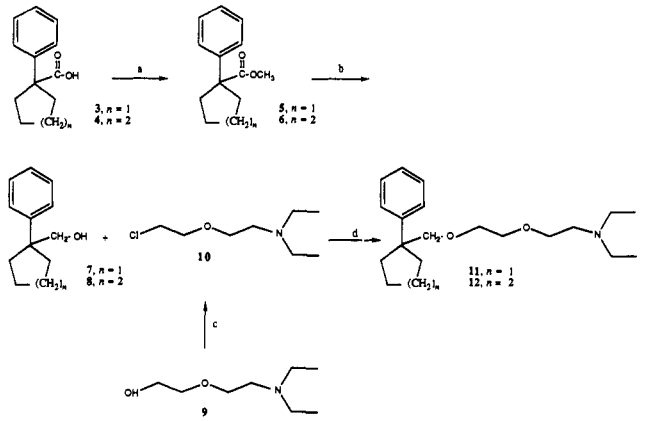
Scheme I[a]



[a] (a) MeOH/HCl; (b) $LiAlH_4$/THF; (c) $SOCl_2$/benzene; (d) NaH/DMF, 95–100 °C.

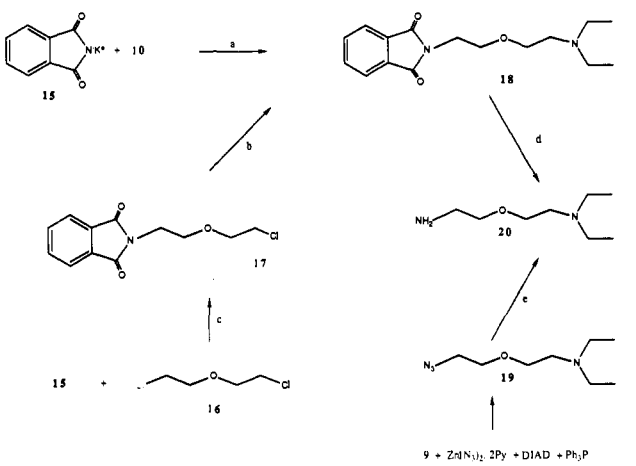
Carbetapentane was first introduced primarily as an antispasmodic agent and later was used as a cough-suppressant drug.[11] Some derivatives of **1** in which the cyclopentyl ring was replaced by cyclohexane, thiopyran, and pyran have been evaluated as antispasmodic agents.[12] More recently, compound **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

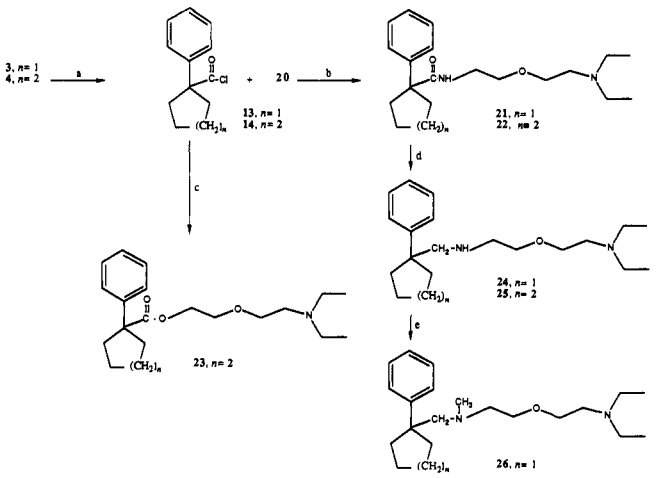
The first two series of phenylcycloalkane carboxylic acid derivatives were prepared according to the methods outlined in Schemes I–III. In Scheme I, methyl esters **5** and **6** were obtained by classical methods, and $LiAlH_4$ reduction gave the desired alcohols **7** and **8**. The replacement of 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 trifluoride 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.

Scheme II[a]



[a] (a) 95–100 °C; (b) Et_2NH·HCl, K_2CO_3/DMF, 110 °C; (c) 110–120 °C; (d) NH_2NH_2, 40% KOH; (e) H_2–10% Pd/C, 40 psi/EtOH.

Scheme III[a]



[a] (a) $SOCl_2$/benzene; (b) pentene-stabilized $CHCl_3$, H_2O/$NaHCO_3$; (c) **9**/Et_3N/toluene; (d) Red-Al/toluene; (e) 37% formaldehyde/$NaBH_3CN$.

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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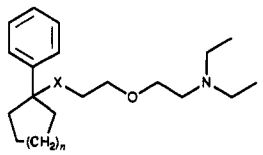
Table I. Structures and Physical Properties of Carbetapentane Analogues



no.	n	X	salt	recrystn solvent	mp, °C	formula
11	1	$-CH_2O-$	oxalate	2-PrOH/ether	67–69	$C_{22}H_{35}NO_6 \cdot {}^1/_2H_2O$
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	$-CH_2NH-$	dioxalate	MeOH/EtOH	169–171	$C_{24}H_{38}N_2O_9$
25	2	$-CH_2NH-$	dioxalate	EtOH	123–124	$C_{25}H_{40}N_2O_9 \cdot {}^1/_2H_2O$
26	1	$-CH_2N(CH_3)-$	dioxalate	2-PrOH/ether	107–108	$C_{25}H_{40}N_2O_9 \cdot {}^1/_2H_2O$

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

compound	ED_{50} (95% CL[b]) mg/kg, sc	ED_{50} (95% CL[b]) μmol/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	IA[c]	
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. [c] Inactive at doses up to 50 mg/kg.

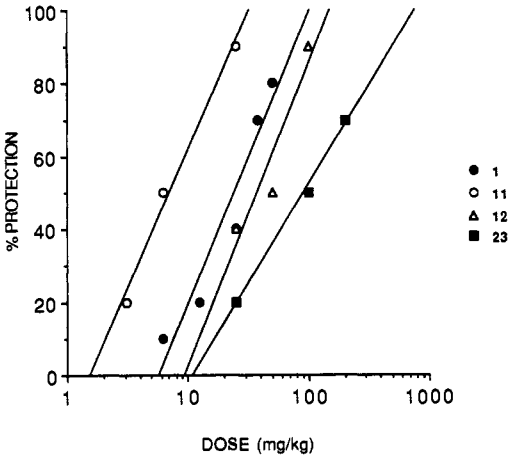


Figure 1. Anticonvulsant dose–response effect of 1 and compounds 11, 12 and 23. Percent protection refers to the percentage of rats (n = 10 per group) that were protected against MES-induced seizure (quantal data). Each dose–response curve represents the computer-derived regression line analyzed for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

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 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

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convulsions. Statistical comparison[19] 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 MES-induced 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 $CHCl_3$/MeOH/NH_4OH (90:10:1) unless otherwise specified. Drying refers to the use of Na_2SO_4. ^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 $CHCl_3$ cells. EIMS and CIMS (NH_3) 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 × 25 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 × 25 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/CH_2Cl_2, 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 $LiAlH_4$ (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 $LiAlH_4$ was destroyed following the method described by Fieser and Fieser[22] whereby the cooled reaction mixture was quenched 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 × 1 mL). The product was extracted with ether (3 × 25 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 $LiAlH_4$ (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 × 4 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 Biel[24] was used to obtain compound 10, beginning with the dropwise addition of $SOCl_2$ (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 × 5 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 × 25 mL). The organic layer was washed with 1 N HCl (3 × 25 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 NH_4OH, extracted with $CHCl_3$ (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 (D_2O) 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/z 319 (M + 1). Anal. ($C_{22}H_{35}NO_6 \cdot {}^1/_2H_2O$) 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) δ 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. ($C_{23}H_{37}NO_6$) C, H, N.

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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 $CHCl_3$ and compound **17** was separated from the crude reaction mixture by flash column chromatography and eluted with $CHCl_3$. 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 K_2CO_3 (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 $CHCl_3$/MeOH/NH_4OH (90:10:1). Evaporation of the solvent gave 1.52 g (27%) of **18** which crystallized from ether: mp 132–134 °C; ^1H NMR ($CDCl_3$) 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 $CHCl_3$, 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 (CD_3OD) 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 Rollin[16] 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 ($CHCl_3$/MeOH/NH_4OH 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 $SOCl_2$ 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 $CHCl_3$. The resulting solution was added dropwise to a mechanically stirred solution of amine **20** (0.63 g, 3.94 mmol) and $NaHCO_3$ (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 $CHCl_3$ 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 (D_2O) 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 = 4.9 Hz, 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 (D_2O) 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), 3.03–3.11 (m, 6 H), 3.23–3.30 (m, 2 H), 3.42 (t, J = 5.3 Hz, 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. ($C_{27}H_{42}N_2O_9$) 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 $SOCl_2$ 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 × 1 mL). The solvent was evaporated and the residue was dissolved in 25 mL of 20% NH_4OH. The product was extracted with $CHCl_3$ (3 × 25 mL) and the combined organic fraction was washed with water (2 × 25 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 (D_2O) 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 × 25 mL). The organic layer was washed with 1 N HCl (4 × 25 mL) and the combined aqueous solution was basified to pH 9 with NH_4OH, extracted with $CHCl_3$ (4 × 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 (D_2O) 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. ($C_{24}H_{38}N_2O_9$) 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; ^{1}H NMR (D_2O) 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. ($C_{25}H_{40}N_2O_9 \cdot {}^1/_2H_2O$) C, H, N.

***N*-[2-[2-(Diethylamino)ethoxy]ethyl]-*N*-methyl-1-phenyl-1-cyclopentanemethylamine (26).** A modification of the procedure for N-methylation of amines by Borch and Hassid[18] was used to obtain compound **26**. To a solution of **24** (0.32 g, 1 mmol) in CH_3CN was added 37% formaldehyde (0.24 mL, 3 mmol), followed by $NaBH_3CN$ (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 $CHCl_3$ 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; ^{1}H NMR (CD_3OD) 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. ($C_{25}H_{40}N_2O_9 \cdot {}^1/_2H_2O$) 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 dextromethorphan.[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.

Acknowledgment. S.N.C. was supported by a National Research Council Award, sponsored by LTC Marvin Oleshansky, Walter Reed Army Institute of Research. We gratefully acknowledge Dr. Jeffrey Witkin for helpful discussion in preparing this manuscript. We thank Mr. Wesley White for obtaining the NMR spectra and Mr. Noel Whittaker for obtaining the mass spectra. We also thank SSG Lydia Robles and SSG Eddy Echevarria for their technical assistance in running the MES test.

Registry No. **3**, 77-55-4; **4**, 1135-67-7; **5**, 4535-96-0; **6**, 17380-78-8; **7**, 59115-90-1; **8**, 68692-77-3; **9**, 140-82-9; **10**, 79611-69-1; **10**·HCl, 114456-81-4; **11**, 136089-15-1; **11**·oxalate, 136089-25-3; **12**, 136089-16-2; **12**·oxalate, 136115-32-7; **13**, 17380-62-0; **14**, 2890-42-8; **15**, 1074-82-4; **16**, 111-44-4; **17**, 58290-51-0; **18**, 136089-17-3; **19**, 136089-18-4; **20**, 136089-19-5; **20**·2oxalate, 136089-26-4; **21**, 136089-20-8; **21**·fumarate, 136089-27-5; **22**, 136089-21-9; **22**·citrate, 136089-28-6; **23**, 13877-96-8; **23**·citrate, 136089-29-7; **24**, 136089-22-0; **24**·2oxalate, 136089-30-0; **25**, 136089-23-1; **25**·2oxalate, 136089-31-1; **26**, 136089-24-2; **26**·2oxalate, 136089-32-2; 1-phenyl-1-cyclohexanecarbonitrile, 2201-23-2; diethylamine hydrochloride, 660-68-4.

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

David J. Triggle,*,† Yong Who Kwon,† Philip Abraham,‡ J. Bruce Pitner,‡ S. Wayne Mascarella,‡ and F. I. Carroll*,‡

Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, North Carolina 27709, and Department of Biochemical Pharmacology, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14260.
Received October 29, 1990

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 pA_2 values for antagonism of methacholine-induced tension responses in guinea pig ileum. Binding affinities were measured by competition against [^{3}H]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 [^{3}H]QNB or [^{3}H]NMS and [^{3}H]oxotremorine-M ([^{3}H]Oxo-M). Nine muscarinic antagonists, including azaprophen, did not discriminate significantly between the subtypes of muscarinic receptors. K_I 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_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 (1*R*,3*R*,5*S*)-6-[1(*R*)-phenylethyl]-6-azabicyclo[3.2.1]octan-3α-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

† State University of New York at Buffalo.
‡ Research Triangle Institute.

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