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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Carbamate derivatives of felbamate as potential anticonvulsant agents

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Abstract Several monocarbamate compounds derived from felbamate were synthesized and 11 target compounds (***1***, ***4***, and ***6–14***) were initially evaluated in mice MES and PTZ models in our laboratory. Carbamate compounds with varying substituents on the oxygen (***1–4***) gave anticonvulsant activity with a wide range of ED_{50} in MES test from <20 mg/kg (***2***) to >300 mg/kg (***4***) and compounds with different groups on the nitrogen (***5–14***) also were quite active in the range of 15 mg/kg (***14***) to 170.5 mg/kg (***6***). This suggested that the spatial limitation in the MES model seemed flexible especially on the nitrogen end. All tested compounds showed some activity against mice scPTZ test, but none had the ED_{50} value <50 mg/kg. Ten selected compounds (***1*** and ***6–14***) for subsequent pharmacological evaluation in NIH all gave positive mice MES activity except ***8*** and ***9***, which were unexpectedly active in rats after further evaluations. Among the compounds, ***1***, ***8***, and ***9*** advanced to the quantitative study and ***1*** and ***9*** provided the highest PI values, 15 and 21, respectively, in the rat oral MES test.

Keywords Carbamate · Felbamate · Anticonvulsant

Introduction

Many carbamate compounds have demonstrated potential therapeutic uses. Meprobamate, an anxiolytic dicarbamate compound (Fig. 1) has been known to

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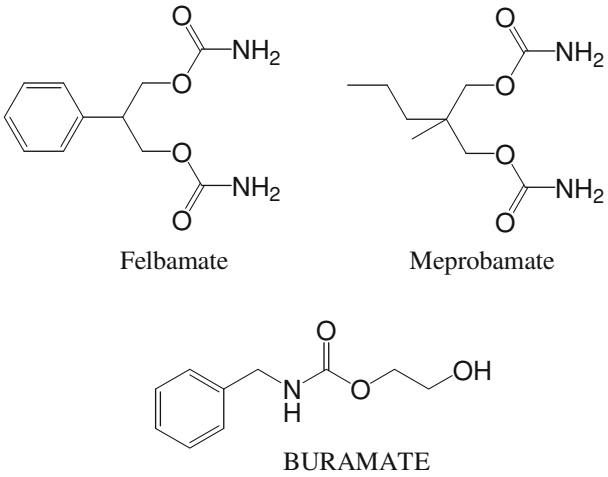


Fig. 1 Felbamate, meprobamate, and buramate

possess anticonvulsant activity, and the search for additional anticonvulsant carbamates continued through 1960 s. The duration of action of these carbamate-related compounds was typically very short, and this has led to their discontinuance as therapeutic agents (Murray and Kier, 1977). According to the SAR of those dicarbamates, derivatives with two alkyl groups attached at C-2 possess stronger muscle paralyzing action, whereas the presence of a phenyl group at the 2 position enhance anticonvulsant activity (Ludwing *et al.*, 1969). For example, felbamate, 2-phenyl-1,3-propanediol dicarbamate, despite its structural similarity to meprobamate, lacks anxiolytic properties and provides higher anticonvulsant activity in some cases (Ludwing *et al.*, 1969; Kupferberg, 1995). Felbamate was approved by the United States FDA in 1993 as a new anticonvulsant drug. Its clinical use has been rather limited subsequent to the finding of significant toxicological issues of felbamate, i.e., aplastic anemia and hepatotoxicity (Frey and Bartels, 1997). 3-Carbamoyl-2-phenyl propionaldehyde is a likely intermediate in the metabolism of felbamate to its principal metabolite, 3-carbamoyl-2-phenyl propionic acid, and the aldehyde intermediate would undergo a cascade of chemical reactions responsible for the toxic properties of the parent drug, felbamate (Ludwing *et al.*, 1969; Thompson *et al.*, 1996; Fig. 2). The aldehyde undergoes a β-elimination to α,β unsaturated aldehyde (2-phenylpropenal), which is known to be toxic or a reversible cyclization process forms a urethane compound as a reservoir. These intermediates have been identified as urinary metabolites in humans (Thompson *et al.*, 2000; Dieckhaus *et al.*, 2001) (see Scheme 1).

A felbamate analog that cannot undergo β-elimination by having a methyl substituent at the prochiral carbon was reported with ED_{50} of 102 mg/kg in the mice MES test by oral administration (Ludwing *et al.*, 1969). A compound with a fluorine substituent on the same carbon of felbamate structure also has been suggested (Dieckhaus and Macdonald, 2001), and successfully synthesized with supportive results in metabolic stability tests (Parker *et al.*, 2005). Besides the replacement of

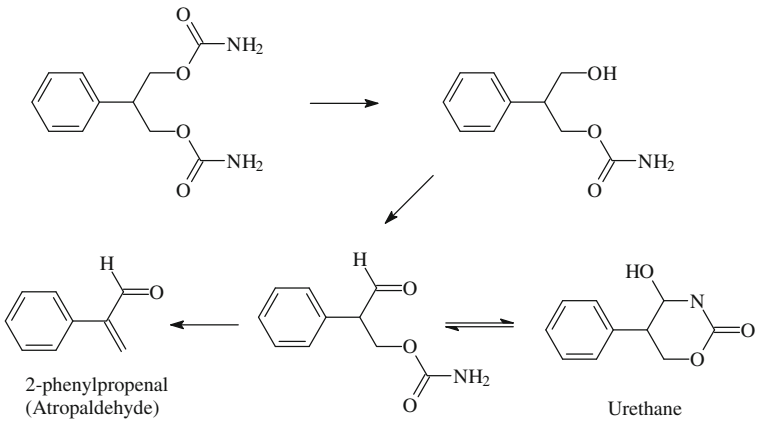
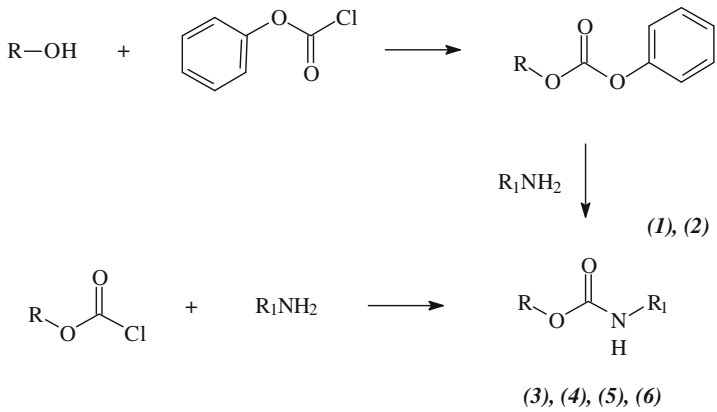


Fig. 2 Felbamate metabolic pathway and toxic species



Scheme 1 Synthesis of Carbamate derivatives

the hydrogen to avoid the formation of toxic metabolite, other methods may apply as well. The oxygen in one of the carbamate moieties may be replaced by another atom, such as nitrogen or carbon. Limited SAR information is available for monocarbamate analogs that lack the possibility of cascade metabolic pathway to form toxic species as with felbamate (Yamagami *et al.*, 1982; Tanaka *et al.*, 1985). N-methyl or N,N-dimethyl substituted benzyl carbamates were more potent than unsubstituted benzyl carbamate, whereas N,N-dimethyl substitution decreases anticonvulsant activity in phenylethyl or phenylpropyl carbamates (Tanaka *et al.*, 1985). Among those compounds, phenyl ethyl carbamate (Fig. 3) was the most potent. Interestingly, carbamates with a hydroxyl group in structure, e.g., the mono-decarbamylated alcohol of felbamate were found to be active in convulsion protection (Ludwing *et al.*, 1969). The hydroxylated carbamates are structurally similar to buramate (Fig. 1), a hypnotic and anesthetic/CNS depressant (Aurousseau, 1960; Hazard *et al.*, 1951).

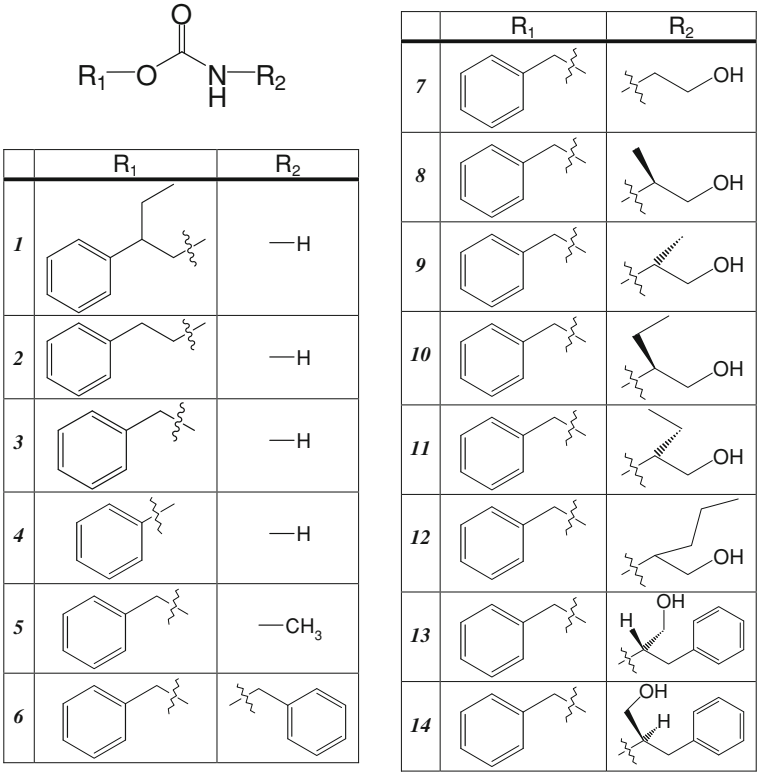
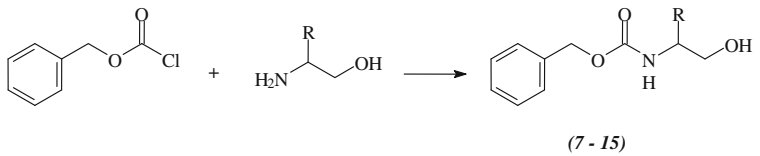


Fig. 3 Structures of carbamate compounds

In this report, a series of monocarbamates with or without hydroxyethyl group on the nitrogen were synthesized. They were (R,S)-2-phenylbutyl carbamate ***1***, phenyl ethyl carbamate ***2***, benzyl carbamate ***3***, phenyl carbamate ***4***, benzyl N-methyl carbamate ***5***, dibenzoyl carbamate ***6***, O-benzyl-N-(2-hydroxy ethyl) carbamate ***7***, (S)-O-benzyl-N-2-hydroxy-1-methyl ethyl carbamate ***8***, (R)-O-benzyl-N-2-hydroxy-1-methyl ethyl carbamate ***9***, and the racemic mixture ***15***, and (S)-O-benzyl-N-1-hydroxy methyl propyl carbamate ***10***, (R)-O-benzyl-N-1-hydroxy methyl propyl carbamate, ***11***, (R,S)-O-benzyl-N-(1-hydroxy methyl butyl) carbamate ***12***, (S)-O-benzyl-N-1-benzyl-2-hydroxy ethyl carbamate ***13***, (R)-O-benzyl-N-1-benzyl-2-hydroxy ethyl carbamate ***14***. The compounds were evaluated for their potential anticonvulsant activity in both MES and scPTZ animal-seizure models. On the basis of the preliminary pharmacological results, further studies were conducted for the chosen compounds in different models (see Scheme 2).

Chemistry

2-Phenyl butyl carbamate (***1***) was prepared via the nucleophilic substitution with the corresponding phenyl carbonate according to Wang and Merrifield (1969) and



Scheme 2 Synthesis of Hydroxy ethyl carbamate compounds

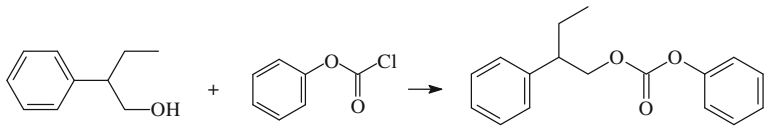


Fig. 4 Intermediate in the synthesis of (**1**)

Sieber and Iselin (1968). In short, 2-phenyl butyl alcohol was initially treated with phenyl chloroformate in basic organic solvent under low temperature (−5°C) to form its corresponding arylalkyl phenyl carbonates in excellent yield (Fig. 4). The carbonate then reacted with excess ammonia to produce this branched aryl alkyl carbamate. The other carbamate compounds (***3***, ***4***, ***5***, ***6***) and benzyl N-hydroxyl alkyl carbamates (***7–15***) were synthesized by following basically the same procedure by reacting benzyl chloroformate (for ***3***, ***5***, ***6***, ***7–15***) or phenyl chloroformate (for ***4***) with ammonia (for ***3***, ***4***), methylamine (for ***5***), benzylamine (for ***6***), or different hydroxyl alkyl amines.

Experimental section

Chemistry

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. Microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA. The following instruments were used: IR, Perkin-Elmer Model 281 or Perkin-Elmer FT-IR Spectrometer SPECTRUM 1000; ^{1}H NMR, Varian EM-360-L CW, 60 MHz (Me_4Si as internal standard) or Bruker-400 MHz (Me_4Si as internal standard); polarimeter, Perkin-Elmer Model 241. Silica gel GF plates (250 μm, 10 × 20 cm^2, Analtech) were used for thin-layer chromatography (TLC). All chemicals and solvents were reagent grade and were purchased from commercial vendors. Compounds **2**, **3**, **4**, and **5** were purchased from Sigma-Aldrich (St. Louis, MO) and were used directly for the anticonvulsant tests.

O-2-Phenyl-butyl-O′-phenyl carbonate

Phenyl chloroformate (5 ml, 0.04 mol) in 20 ml of CH_2Cl_2 was added drop wise into a solution of 5 g (0.033 mol) of (±)-β-ethyl-phenethyl alcohol and 4 ml

(0.05 mol) of pyridine in 80 ml of CH_2Cl_2 under ice bath temperature with stirring for 1 hour. After stirring overnight at room temperature, the mixture was poured into crushed ice and diluted with 100 ml of methylene chloride. The organic phase was separated, washed with water three times (3 × 250 ml), dried over anhydrous sodium sulfate, and then evaporated in vacuo at 30°C to give a thick, colorless, oily liquid. TLC $Rf = 0.73$ in CH_2Cl_2/Hexane (1:1). This was used for the subsequent step without further purification.

2-Phenylbutyl carbamate (*1*)

Crude O-2-phenyl-butyl-O′-phenyl carbonate prepared above was dissolved in 40 ml DMF and then 17 ml of ammonia (2.0 M solution in Ethanol, 0.034 mol) was added to the solution at room temperature with stirring. Additional ammonia was added after 6 hours and every 12 hours until the starting carbonate compound disappeared as visualized on TLC plate. A total of 50 ml of ammonia (2.0 M solution in ethanol, 0.1 mol) was added. The mixture was evaporated in vacuo to the half of its volume, and then 150 ml of water was added slowly. After keeping at 0°C overnight, the product was filtered to give 5.97 g (92.3% yield) of white crystalline product. mp 104–106°C; TLC $Rf = 0.39$ in CH_2Cl_2; TLC $Rf = 0.13$ in CH_2Cl_2/Hexane (1:1); IR (SPECTRUM 1000, KBr, cm^{-1}) 1686 (C=O), 2899, 2970 (alkyl), 3027, 3062, 3085 (ArH), 3326, 3411 (NH); ^{1}H NMR ($CDCl_3$) δ 0.63–1.07 (t, $J = 7$ Hz, 3H, CH_3), 1.43–2.00 (fused m, 2H, CH_2), 2.60–3.10 (m, 1H, CH), 4.10–4.40 (d, $J = 7$ Hz, 2H, OCH_2), 4.40–4.90 (br, 2H, NH_2), 7.21 (s, 5H, ArH). Anal. ($C_{11}H_{15}NO_2$), C, H, N.

N,O-Dibenzyl carbamate (*6*)

Benzyl chloroformate (3.8 ml, 25 mmol) in 15 ml of CH_2Cl_2 was added gradually into a solution of benzylamine (2.73 ml, 25 mmol) and pyridine (3 ml, 37.5 mmol) in 25 ml of CH_2Cl_2 in 100-ml round-bottom flask at ice bath temperature with stirring for 0.5 hours. After stirring overnight at room temperature, the mixture was poured into crushed ice and diluted with 50 ml of CH_2Cl_2. The organic phase was separated, washed with water three times (3 × 200 ml), dried over anhydrous sodium sulfate, and then evaporated in vacuo to concentrated liquid. This liquid was diluted in 100 ml of ethyl acetate, washed with acidic water three times (3 × 200 ml, pH 1–2), dried over anhydrous sodium sulfate, and then evaporated in vacuo to half of its volume and crystallized at 0°C to give 2.58 g (42.9% yield) of white crystalline product. mp 60–61.5°C; TLC $Rf = 0.71$ in CH_2Cl_2; ^{1}H NMR ($CDCl_3$) δ 4.25–4.50 (d, $J = 6$ Hz, 2H, NCH_2), 5.10 (s, 2H, OCH_2), 7.23, 7.30 (2 s, 10H, ArH). Anal. ($C_{15}H_{15}NO_2$), C, H, N.

O-Benzyl-N-(2-hydroxy ethyl) carbamate (*7*)

Benzyl chloroformate (3 ml, 20 mmol) in 10 ml of THF was added into the mixture of ethanolamine (1.22 ml, 20 mmol) and pyridine (2.5 ml, 30 mmol) in 25 ml of THF at ice bath temperature. After stirring overnight at room temperature, 75 ml of

water and 50 ml of EtOAc were added into the reaction mixture in a separatory funnel. The organic phase was washed with water three times, with acidic water (pH 1) three times, then separated and dried over anhydrous sodium sulfate. Evaporation in vacuo gave a thick liquid. The liquid solidified when the liquid-containing, round-bottomed flask was placed on dry ice. The crude solid product was purified by preparative TLC to give 1.82 g (46.7% yield) of crystalline compound. mp 61–62°C; TLC $Rf = 0.31$ in CH_2Cl_2; TLC $Rf = 0.50$ in EtOAc/ CH_2Cl_2 (1:1); TLC $Rf = 0.70$ in EtOAc; ^{1}H NMR ($CDCl_3$) δ 3.15–3.50 (t, $J = 4.5$ Hz, 2H, OCH_2), 3.50–3.85 (m, 2H, NCH_2), 5.10 (s, 2H, OCH_2), 7.33 (s, 5H, ArH). Anal. ($C_{10}H_{13}NO_3$), C, H, N.

O-Benzyl-N-(1*S*)-2-hydroxy-1-methyl ethyl carbamate (*8*)

Benzyl chloroformate (2.0 ml, 13 mmol) in 10 ml of THF was added gradually into a solution of (S)-(+)-2-amino-1-propanol (1 g, 13 mmol) and pyridine (1.2 ml, 14.5 mmol) in 25 ml of THF in a 100-ml round-bottom flask at ice bath temperature with stirring. After stirring overnight at room temperature, 50 ml of 10% NaOH in water was added and the mixture was kept stirring for additional 0.5 hours. The mixture was then evaporated in vacuo, and the residue was diluted with CH_2Cl_2 (100 ml). The organic layer was separated and washed with acidic water (pH 1) three times. This organic layer was separated again and evaporated in vacuo to give a thick liquid. The liquid was then placed in a Petri dish and exposed to air overnight to give a crude solid. Further purification by preparative TLC (solvent system EtOAc: $CH_2Cl_2 = 1{:}9$) provided 555 mg (20.9% yield) of crystalline compound. mp 80–82°C; $[\alpha]_D^{23} = -4.6$ (c = 1.0, MeOH); TLC $Rf = 0.53$ in EtOAc/CH_2Cl_2 (1:2); ^{1}H NMR ($CDCl_3$) δ 1.03–1.43 (m, 3H, CH_3), 3.43–4.17 (fused m, 3H, OCH_2, NCH), 5.10 (s, 2H, OCH_2), 7.37 (s, 5H, ArH). Anal. ($C_{11}H_{15}NO_3$), C, H, N.

O-Benzyl-N-(1R)-2-hydroxy-1-methyl ethyl carbamate (*9*) was prepared from (R)-(–)-2-amino-1-propanol (1.0 g, 13 mmol) according to the procedure for ***8*** to give 564 mg (21.5% yield) of crystalline compound. mp 80–82°C; [α]D23 = +5.0 (c = 1.0, MeOH); TLC Rf = 0.53 in EtOAc/ CH2Cl2 (1:2); Anal. ($C_{11}H_{15}NO_3$), C, H, N.

O-Benzyl-N-(1S)-1-hydroxymethyl propyl carbamate (*10*) was prepared from (S)-(+)-2-amino-1-butanol (1.0 g, 11 mmol) according to the procedure for ***8*** to give 590 mg (24.1% yield) of crystalline compound. mp 64–66°C; $[\alpha]_D^{23} = -27.3$ (c = 1.0, MeOH); TLC $Rf = 0.56$ in EtOAc/ CH_2Cl_2 (1:2); Anal. ($C_{12}H_{17}NO_3$), C, H, N.

O-Benzyl-N-(1R)-1-hydroxymethyl propyl carbamate (*11*) was prepared from (R)-(–)-2-amino-1-butanol (1.0 g, 11 mmol) according to the procedure for ***8*** to give 741 mg (30.2% yield) of crystalline compound. mp 65–67°C; $[\alpha]_D^{23} = +26.5$ (c = 1.0, MeOH); TLC $Rf = 0.56$ in EtOAc/ CH_2Cl_2 (1:2); ^{1}H NMR [Bruker-400 MHz] ($CDCl_3$) δ 0.96–1.00 (t, $J = 7.2$ Hz, 3H, CH_3), 1.45–1.52 (m, 1H, CH_2), 1.56–1.64 (m, 1H, CH_2), 1.66 (s, exchangeable H), 2.22 (s, exchangeable H), 3.62 (fused s, 2H, OCH_2), 3.71 (fused s, 1H, NCH), 5.13 (s, 2H, OCH_2), 7.38 (s, 5H, ArH). Anal. ($C_{12}H_{17}NO_3$), C, H, N.

(R,S)-O-Benzyl-N-(1-Hydroxymethyl butyl) carbamate (*12*)

Benzyl chloroformate (1.5 ml, 9.7 mmol) in 10 ml of THF was added gradually into a solution of *dl*-2-amino-1-pentanol (1.0 g, 9.7 mmol) and pyridine (1.2 ml, 14.5 mmol) in 25 ml of THF in a 100 ml round bottom flask at ice bath temperature with stirring for 0.5 hours. After stirring overnight at room temperature, 50 ml of 10% NaOH aqueous solution was added and kept stirring for additional 5 hours. The mixture was concentrated *in vacuo*, and then EtOAc (50 ml) was added. The separated organic layer was washed with acidic water (pH 1) three times. The organic layer was evaporated in vacuo to give a thick liquid. The liquid solidified upon cooling to give 1.363 g of white solid. Recrystallization of the solid with CH_2Cl_2 and Hexane provided 1.01 g (44% yield) white crystalline compound. mp 71–72°C; TLC $Rf = 0.58$ in EtOAc/CH_2Cl_2 (1:2); ^{1}H NMR ($CDCl_3$) δ 0.70–1.17 (m, 3H, CH_3), 1.17–1.63 (fused m, 4H, CH_2CH_2), 3.46–3.80 (fused m, 3H, OCH_2, NCH), 5.07 (s, 2H, OCH_2), 7.30 (s, 5H, ArH). Anal. ($C_{13}H_{19}NO_3$), C, H, N.

O-Benzyl-N-(1S)-1-Benzyl-2-hydroxy ethyl carbamate (*13*) was prepared from (S)-(−)-2-amino-3-phenyl-1-propanol (1.0 g, 6.6 mmol) according to the procedure for ***8*** to give 1.50 g (80.0% yield) of crystalline compound. mp 91–93°C; $[\alpha]_D^{23} = -43.9$ (c = 1.0, MeOH); TLC Rf = 0.72 in EtOAc/ CH_2Cl_2 (1:2); Anal. ($C_{17}H_{19}NO_3$), C, H, N.

O-Benzyl-N-(1R)-1-Benzyl-2-hydroxy ethyl carbamate (*14*) was prepared from (R)-(+)-2-amino-3-phenyl-1-propanol (1.0 g, 6.6 mmol) according to the procedure for ***8*** to give 1.30 g (70% yield) of crystalline compound. mp 90.5–92.5°C; $[\alpha]_D^{23} = +42.8$ (c = 1.0, MeOH); TLC Rf = 0.72 in EtOAc/ CH_2Cl_2 (1:2); ^{1}H NMR ($CDCl_3$) δ 2.70–3.05 (m, 2H, $ArCH_2$), 3.45–3.75 (fused s, 2H, OCH_2), 3.75–4.17 (fused m, 1H, NCH), 5.10 (s, 2H, OCH_2), 7.27 (s, 5H, ArH), 7.37 (s, 5H, ArH). Anal. ($C_{17}H_{19}NO_3$), C, H, N.

(R,S)-O-Benzyl- N-2-Hydroxy-1-methyl ethyl carbamate (*15*) was prepared by physically mixing ***8*** and ***9*** (1:1 ratio) thoroughly.

Pharmacological evaluations

Adult, male, Swiss-Webster mice (20–25 g, Taconic Farms, Germantown, NY) were used. The mice were housed in an environmentally controlled room (12-hour light/dark cycle, lights on 7:00 a.m.; 35–45% humidity; 20–30°C) with food and water available ad libitum, except when removed from cages for testing. Drugs used for antiepileptic testing were dissolved in normal saline or suspended in 30% PEG (polyethylene glycol) (Porter *et al.*, 1984). All test drugs were injected intraperitoneally in a volume of 10 ml/kg. Dose–response characteristics were determined from at least three groups of mice (4–8 mice per group) for each drug tested. The drugs were screened against 1) chemically induced seizures utilizing pentylenetetrazole as the convulsant (subcutaneous pentylenetetrazole seizure threshold test; *scMET test*), and 2) electrically induced seizures using a 50-mA current (maximal electroshock seizure test; *MES test*). All tests were performed at 0.5 hours after drug administration.

In the scMET test, pentylenetetrazole was administered subcutaneously in a loose fold of skin on the back of the neck in dose of 85 mg/kg (the convulsant dose, CD_{97}) (Porter *et al.*, 1984). The animals were observed for 30 minutes after injection of pentylenetetrazole, and absence of clonic spasms persisting for at least 5 seconds was considered that the compound can elevate the pentylenetetrazole-induced seizure threshold. In the MES test, corneal electrodes primed with a drop of electrolyte solution (0.9% sodium chloride) were applied to the eyes and an electrical stimulus (50 mA) was delivered for 0.2 seconds by an electroshock equipment (Electroshock Unit Model 11A, IITC, Landing, NJ). Abolition of the hind-leg tonic-extensor component (hind-leg tonic extension does not exceed a 90° angle to the plane of the body) was considered that the compound can prevent MES-induced seizure spread (Porter *et al.*, 1984).

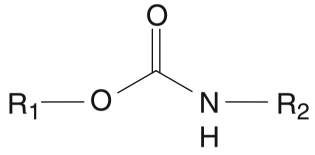
Further pharmacological evaluation data for selected compounds were provided by NIH-ADD program, i.e., mice ip for MES, scMET, and TOX; rat po for MES, scMET, and TOX; rat ip for MES, PTZ, TOX, and Flexible Format.

Results and discussion

2-Phenyl butyl carbamate (***1***) is a close structural analog of felbamate, but it has only one carbamate moiety and therefore cannot undergo β-elimination upon the expected metabolic decarbamylation. Its potential anticonvulsant activity was evaluated and compared with those of several other carbamates, including felbamate (Table 1). In general, all compounds showed some protection against seizures in animal models. In MES test, ***1*** was essentially as potent as felbamate, although not as potent as the unsubstituted phenylethyl carbamate ***2*** or benzyl carbamate ***3***. The N-methyl substituted ***5*** was just as potent as ***3***; however, the corresponding benzyl substituted compound ***6*** was less potent. Shortening the distance between the carbamate and phenyl ring by one carbon (compound ***4***) also resulted in reduction of potency. Among the compounds, ***1*** and ***6*** were selected and tested by the NIH-ADD program; ***6*** showed activity only at 300-mg/kg dose in MES test with no activity in PTZ test up to 300 mg/kg. On the other hand, ***1*** showed activity comparable to that produced in our laboratory. Calculated TD_{50} value from NIH was 114.3 mg/kg in mice rotorod test. Subsequent testing in rats by oral administration, ***1*** exhibited a potent activity with ED_{50} of 21.0 mg/kg in MES and ED_{50} of >40 mg/kg in PTZ tests. Furthermore, ***1*** did not show any toxicity up to 320 mg/kg in rats at the highest dose tested. This provided the PI (protection index) better than 15 for MES test and around 8 for PTZ test in rats. Compound ***1***, when tested in rats by intraperitoneal administration, exhibited a slightly better activity ($ED_{50} = 16.1$ mg/kg) in MES test but there was a substantially greater toxic profile by this route ($TD_{50} = 50.5$ mg/kg). Preliminary Hippocampal Kindling screen in rats (ip) was also performed for ***1***. At 100 mg/kg, ***1*** did not show activity at which felbamate is also inactive. Toxicity was however observed for ***1*** at 300 mg/kg and therefore testing could not be carried out.

2-Hydroxyethyl benzylcarbamate ***7*** is structurally identical to buramate (benzyl 2-hydroxyethylcarbamate) except the order of carbamate is reversed. Compound ***7***

Table 1 Pharmacological results for carbamates



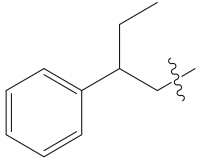
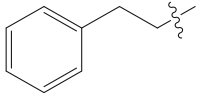
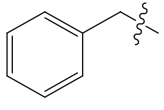
Compounds	R_1	R_2	SJU mice (ip) (mg/kg)	NIH mice (ip) (mg/kg)	NIH rats (po) (mg/kg)
(1)		–H	MES 71.7 (24.4–210.6) PTZ 123.6 (37.1–412.3)	MES ++ [3/3] PTZ + – MES 72.7 (66.1–75.9) PTZ 87.6 (72.5–106.5) – TD_{50} = 114 (93.2–140.9)	MES [4/4][a] – MES 21 (14.1–30.7) PTZ > 40 – TD_{50} > 320
(2)		–H	MES 17.7[(1)] PTZ –		
(3)		–H	MES 53.6[(1)] MES 30.6 (17.3–54.1) PTZ 80.2 (28.9–222.6)		

Table 1 continued

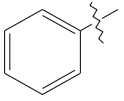
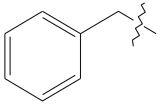
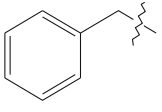
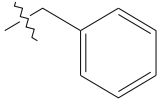
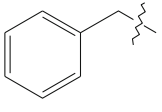
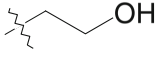
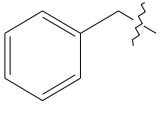
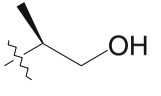
Compounds	R_1	R_2	SJU mice (ip) (mg/kg)	NIH mice (ip) (mg/kg)	NIH rats (po) (mg/kg)
(4)		–H	MES + PTZ 101.3 (31.3–327.4)		
(5)		$–CH_3$	MES 40.5[(1)] PTZ –		
(6)			MES 170.5 (104.1–279.2) PTZ 114.7 (44.2–297.6)	MES + PTZ –	
(7)			MES 25.2 (9.6–66.) PTZ 170.5 (104.1–279.2)	MES + PTZ –	
(8) S-isomer			MES 54.7 (11.4–263.1) PTZ 258.3 (52.9–1260.7)	MES – PTZ –	MES [4/4][a] – MES 67.6 (34.0 to 105.6) PTZ > 125 – TD_{50} > 360

Table 1 continued

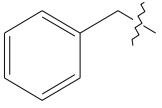
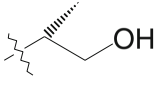
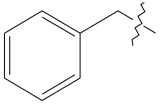
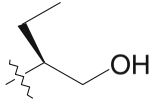
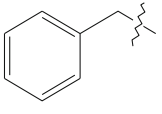
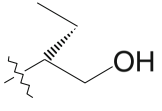
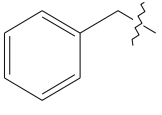
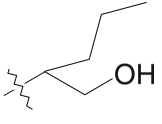
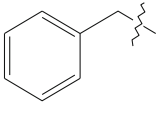
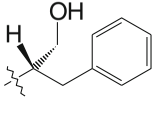
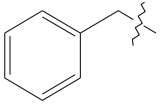
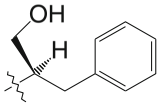
Compounds	R_1	R_2	SJU mice (ip) (mg/kg)	NIH mice (ip) (mg/kg)	NIH rats (po) (mg/kg)
(9) R-isomer			MES 60.1 (23.5–154.2) PTZ 226 (178.6–286.0)	MES − PTZ −	MES [2/4][a] – MES 23.9 (13.8–36.7) PTZ > 250 – TD_{50} > 500
(10) S-isomer			MES 122.6 (53–283.4) PTZ 110.7 (44.6–274.7)	MES + PTZ −	MES [3/4][a]
(11) R-isomer			MES 80.5 (32.6–198.4) PTZ 143.6 (56.3–366.5)	MES + PTZ −	MES [1/4][a]
(12)			MES 51.8 (22.8–117.6) PTZ 280.6 (104.1 to 279.2)	MES + PTZ +	
(13) S-isomer			MES 33.5 (13.6–82.4) PTZ ?	MES + PTZ −	

Table 1 continued

Compounds	R_1	R_2	SJU mice (ip) (mg/kg)	NIH mice (ip) (mg/kg)	NIH rats (po) (mg/kg)
(14) R-isomer			MES 14.9 (5.4–40.7) PTZ ?	MES + PTZ −	
	Felbamate		MES 52 (po)[2], 81[3], 35[4] PTZ 548[3], not dose dependent[4]	MES 35.5 (28.6–40.7) PTZ 126 (72.8 to 192)	MES 25.3 (19.1–30.5) PTZ > 250 – TD_{50} > 500
	Meprobamate		MES 165 (po)[2], 127[4] PTZ 66[4]		

Ranges in parentheses indicate 95% confidence intervals

[a] Rat MES test by oral route, dose = 30 mg/kg [No. of animal affected/number of animal being tested]

"++" Denotes activity at dose of 100 mg/kg

"+" Denotes activity at dose of 300 mg/kg

"?" Denotes some activity but not dose-dependent, therefore, ED_{50} cannot be determined

"−" Denotes no activity up to 300 mg/kg

(1) Tanaka *et al.* (1985); (2) Ludwing *et al.* (1969); (3) Kupferberg (1995); (4) Frey and Bartels (1997)

also is related to 3-hydroxy-2-phenylpropyl carbamate, the mono-decarbamylated alcohol metabolite of felbamate, which was reported to be active with the ED_{50} of 154 mg/kg in MES test in mice by oral administration (Ludwing *et al.*, 1969). Several 2-substituted benzyl 1-hydroxy-2-ylcarbamates (***7–15***) were synthesized and tested for their potential anticonvulsant activity profiles. The unsubstituted benzyl 1-hydroxy-2-ylcarbamate ***7*** exhibited a potent activity with ED_{50} of 25.2 mg/kg in MES test and 170.5 mg/kg in PTZ test. 2-Methyl substituted individual stereoisomers ***8*** and ***9*** were less potent than ***7*** with no difference in activity between the isomers. 2-Ethyl isomers ***10*** and ***11*** were even less potent with some differences in activity between the isomers. The racemic mixture of 2-propyl substituted isomer ***12*** was roughly equipotent to ***8*** and ***9***. On the other hand, 2-benzyl isomers ***13*** and ***14*** showed significant activity with ED_{50} of 33.5 mg/kg in MES test for the S isomer ***13*** and 14.9 mg/kg for the R isomer ***14***. Both isomers gave some protection at different dose in mice PTZ tests, but activity was not dose-dependant, therefore, ED_{50} values could not be calculated. The 2-substituted benzyl 1-hydroxy-2-ylcarbamates (***7–15***) were subsequently tested by the NIH-ADD program. All compounds showed activity at 300 mg/kg in MES test with the exception of ***8*** and ***9***, even though both were potently active in MES test in mice by intraperitoneal administration in our lab. In PTZ test, only ***12*** showed activity at the dose of 300 mg/kg. Although ***8*** and ***9*** were inactive at up to 300 mg/kg dose in both MES and PTZ tests, they showed noticeable toxicity (data not shown). The individual stereo isomers ***8***, ***9***, ***10***, and ***11*** were tested further in rats by oral administration; interestingly, ***10*** provided 3/4 protections, but ***11*** protected only 1/4 in MES test at 30 mg/kg. Compound ***8*** protected 4/4 and ***9*** protected 2/4. Subsequent quantitative testing showed the R isomer ***9*** was more potent with the ED_{50} of 23.9 mg/kg in MES with the TD_{50} of >500 mg/kg, thus providing the PI >20. Toxicity and preliminary Hippocampal Kindling screen in rats by intraperitoneal administration were performed for ***9***. At 100 mg/kg, ***9*** did not show activity, and toxicity was observed at 300 mg/kg, therefore, testing could not be done. Furthermore, Flexible Format in rats (ip) was evaluated for ***8*** and ***9***. R isomer ***9*** provided substantial activity at 30 minutes, whereas the activity of S isomer ***8*** disappeared at earlier stage at 10 minutes (Table 2). This may explain the difference in the ED_{50} values in rat MES oral activity between the two isomers (see Table 3).

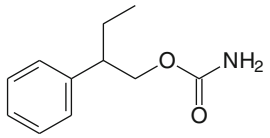
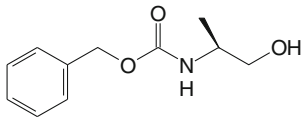
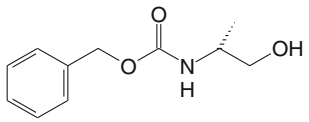
In summary, 11 of the listed 14 target compounds (***1***, ***4***, and ***6–14***) in Table 1 were evaluated in mice MES and PTZ models in our laboratory and literature data were cited for the remaining 3 (***2***, ***3***, and ***5***). Carbamate compounds with substituents on the oxygen in different sizes and lengths (***1–4***) all gave some anticonvulsant activity with a wide range of ED_{50} in MES test from <20 mg/kg (***2***) to >300 mg/kg (***4***). Compounds with different groups on the nitrogen (***5–14***) also were quite active in the range of 15 mg/kg (***14***) to 170.5 mg/kg (***6***). This suggested the spatial limitation seemed flexible especially on the nitrogen end. All tested compounds showed some activity against mice scPTZ test, but none had the ED_{50} value <50 mg/kg. Selected ten compounds (***1*** and ***6–14***) were subsequently submitted to NIH for further pharmacological evaluation. They all gave positive mice MES activity except ***8*** and ***9***, which were unexpectedly active in rats after further evaluations. Among the compounds, ***1***, ***8***, and ***9*** advanced to the quantitative study

Table 2 NIH results of Flexible Format—Rats IP

		5 min	10 min	15 min	30 min	60 min
S isomer ***8***	MES	3/4	1/4	0/4	0/4	1/4
(20 mg/kg)	TOX	0/4	0/4	0/4	0/4	0/4
R isomer ***9***	MES	1/4	0/4	1/4	2/4	0/4
(20 mg/kg)	TOX	0/4	0/4	0/4	0/4	0/4

No. of animals affected / no. of animals being tested

Table 3 ED_{50}, TD_{50}, and PI comparison of potential compounds (NIH rats PO)

#	Structure	ED_{50} (mg/kg)	TD_{50} (mg/kg)	PI
1		MES 20.98 PTZ > 40	>320	MES > 15.3 PTZ (8)
8		MES 67.58 PTZ > 125	>360	MES > 5.3 PTZ (2.88)
9		MES 23.85 PTZ > 250	>500	MES > 21 PTZ (2)

and ***1*** and ***9*** provided the highest PI values 15 and 21, respectively, in the rat oral MES test.

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