# Structure–Activity Study of 5-Substituted 1-Carbobenzoxy-2-iminohydantoins as Potential Anticonvulsant Agents

Zhong-Yue Sun, Chul-Hoon Kwon,* and John N. D. Wurpel

*Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, New York 11439*

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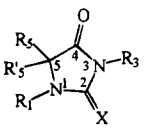
On the basis of our previous findings, a series of 5-substituted 2-iminohydantoins has been synthesized and tested for anticonvulsant activity to better understand the SAR of 2-iminohydantoins. Among the compounds tested, (*S*)-(+)-**1**-carbobenzoxy-2-iminohydantoin analogs with ethyl (**6**)-, *n*-propyl (**7a**)-, isopropyl (**8**)-, allyl (**9**)-, and *sec*-butyl (**11**)-substituted groups at the C5 of the iminohydantoin ring provided the best activities against the MES test with $ED_{50}$ values in the range of 52–74 mg/kg. All of the above compounds except **8** also showed activity against the scMET test with $ED_{50}$ values in the range of **141**–223 mg/kg. All significantly active compounds (**1**, **6**, **7a**, **8**, **9**, and **11**) possessed aliphatic hydrocarbon side chains of two- to three-carbon lengths at the C5 position. All of the compounds with no or minimal activity had either shorter or longer side chains. The compounds substituted at the C5 position by aryl groups, arylalkyl groups, or alkyl and arylalkyl groups containing heteroatoms also showed no activity against the MES and scMET tests. The results suggested that the C5 side chain with the correct stereochemistry in 2-iminohydantoins provides optimal anticonvulsant activity when the side chains are aliphatic hydrocarbons with a length, ignoring branching, of two to three carbons.

## Introduction

Since the introduction of hydantoins (Chart 1) as antiepileptic drugs,[1] numerous structural modifications of hydantoins have been made to optimize their activity and their structure–activity relationship (SAR) has been well established.[2–7] In general, at least one aromatic group such as a phenyl ring is essential for the anticonvulsant activity of the hydantoins. Maximal activity against the maximal electroshock seizure test (MES test) is achieved when two phenyl substituents are at the C5 position of the hydantoin ring, viz., phenytoin. An alkyl substitution at the N3 position can increase the activity against the subcutaneous pentylenetetrazole seizure test (scMET test); however, a bulky substituent at the N1 position diminishes the anticonvulsant activity. In contrast to the hydantoins, much less is known about the imino analogs, viz., 2-iminohydantoins (Chart 1). It was previously found[8] in our laboratory that the SAR of 2-iminohydantoin analogs appears to be quite different from that of hydantoins. First, compounds with mono- or diphenyl substitution at the C5 of the iminohydantoin ring did not have anticonvulsant activity. For example, 5,5-diphenyl-2-iminohydantoin, the closest analog of phenytoin, failed to show any significant activity. Methylation of this compound at the N3 position or carbobenzoxy (Z) substitution at the N1 position did not provide a compound with substantial activity. Second, (*S*)-5-isobutyl-2-iminohydantoin showed some activity, and the activity was greatly increased when the N1 position of this compound was substituted with a Z group, i.e. (*S*)-(+)-1-carbobenzoxy-5-isobutyl-2-iminohydantoin (**1**). The $ED_{50}$ values of **1** were 98.6 and 141.7 mg/kg in mice against the MES test and the scMET test, respectively. In addition, evaluation of the individual enantiomers of the chiral iminohydantoins showed that the anticonvulsant activity resided primarily in the compounds made from the corresponding L-amino acids. These results suggested that the side chains at the N1 and C5 positions of the 2-iminohydantoin ring play important roles in providing anticonvulsant activity.

**Chart I**




X = O Hydantoin
X = NH 2-Iminohydantoin

In this study, we have focused on the variation of the C5 side chain (R5 region) of the 2-iminohydantoin ring with a Z group attached at the N1 position. The compounds selected were 1-carbobenzoxy-2-iminohydantoin analogs with methyl (**4**)-, ethyl (**6**)-, *n*-propyl (**7a**)-, isopropyl (**8**)-, allyl (**9**)-, *n*-butyl (**10**)-, *sec*-butyl (**11**)-, 2-acetamido (**12**)-, 3-propanamido (**13**)-, 2-(methylthio)ethyl (**14**)-, and (indol-3-yl)methyl (**16**)-substituted groups at the C5 position with *S*-configuration, with *n*-propyl (**7b**) and (benzylthio)methyl (**15**) substituents at the C5 position with *R*-configuration, and with dimethyl groups (**5**) at the C5 position (Table 1).

## Chemistry

The 5-substituted 1-carbobenzoxy-2-iminohydantoins (Table 1) were synthesized from commercially available *N*-carbobenzoxyamino acids by the method previously reported.[8] For compounds **6**, **7b**, and **9**, the *N*-carbobenzoxyamino acids were prepared by acylation of the corresponding amino acids with benzyloxycarbonyl chloride. All compounds were made from the corresponding L-amino acids except **7b**, which was made from the corresponding D-amino acid, and **5**. Briefly, *N*-(Z-protected) amino acids were allowed to react initially with dicyclohexylcarbodiimide (DCC) and then with *N*-hydroxysuccinimide to form the activated succinimide esters. The succinimide esters were then coupled with

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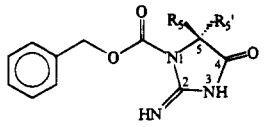
**Table 1.** Physical Properties of 5-Substituted 1-Carbobenzoxy-2-iminohydantoins[a]



| compd | $R_5$ | $R_5'$ | config | yield (%) | $R_f$[b] TLC | $[\alpha]^{23}_D$[c] (deg) | mp (°C) dec | formula | anal.[b] |
|---|---|---|---|---|---|---|---|---|---|
| **4** | $CH_3$ | H | *S* | 60 | 0.58 | +18.0 | 191–2 | $C_{12}H_{13}N_3O_3$ | C, H, N |
| **5** | $CH_3$ | $CH_3$ | | 62 | 0.64 | | 192–3 | $C_{13}H_{15}N_3O_3$ | C, H, N |
| **6** | $C_2H_5$ | H | *S* | 50[e] | 0.63 | +11.5 | 169–70 | $C_{13}H_{15}N_3O_3$ | C, H, N |
| **7a** | *n*-$C_3H_7$ | H | *S* | 65 | 0.64 | +15.3 | 173–4 | $C_{14}H_{17}N_3O_3$ | C, H, N |
| **7b** | H | *n*-$C_3H_7$ | *R* | 41[e] | 0.64 | −15.5 | 177–8 | $C_{14}H_{17}N_3O_3$ | C, H, N |
| **8** | *i*-$C_3H_7$ | H | *S* | 31 | 0.64 | +8.4 | 157–8 | $C_{14}H_{17}N_3O_3$ | C, H, N |
| **9** | $CH_2$=$CHCH_2$ | H | *S* | 40[e] | 0.64 | +15.8 | 152–3 | $C_{14}H_{15}N_3O_3$ | C, H, N |
| **10** | *n*-$C_4H_9$ | H | *S* | 53 | 0.65 | +26.5 | 177–8 | $C_{15}H_{19}N_3O_3$ | C, H, N |
| **11** | *sec*-$C_4H_9$ | H | *S* | 63 | 0.65 | +10.2 | 171–2 | $C_{15}H_{19}N_3O_3$ | C, H, N |
| **12** | $H_2NCOCH_2$ | H | *S* | 0 | | | | | |
| **13** | $H_2NCOC_2H_4$ | H | *S* | 53 | 0.13 | +16.0 | 188–9 | $C_{14}H_{16}N_4O_4$ | C, H, N |
| **14** | $CH_3SC_2H_4$ | H | *S* | 43 | 0.64 | +32.6 | 154–5 | $C_{14}H_{17}N_3O_3S$ | C, H, N, S |
| **15** | $PhCH_2SCH_2$ | H | *R* | 23 | 0.71 | +70.9 | 140–1 | $C_{19}H_{19}N_3O_3S$ | C, H, N, S |
| **16** | (indol-3-yl)$CH_2$ | H | *S* | 47 | 0.58 | +77.9 | 182–3 | $C_{20}H_{18}N_4O_3$ | C, H, N |

[a] The infrared and $^1H$ NMR spectra were consistent with the assigned structures. [b] $R_f$ values were determined on silica gel GF plates (250 μm, Analtech) using EtOAc/EtOH (9:1) as the eluent. [c] Specific optical rotation [α] values were measured in methanol at a concentration of 1% (g/mL), except compound **4** which was determined at 0.3% concentration. [d] All compounds gave satisfactory C, H, N, and S (when applied) analyses (±0.4%). [e] Based on the use of crude *N*-Z-amino acid precursors.

excess cyanamide in the presence of sodium hydroxide to produce the corresponding *N*-(Z-protected) aminoacylcyanamides. The acylcyanamides, which are usually unstable, undergo intramolecular cyclization spontaneously or by heat treatment in water to form the corresponding 5-substituted 1-Z-2-iminohydantoins. Weakly acidic conditions at room temperature were usually sufficient to catalyze the cyclization. Stronger conditions such as refluxing in MeOH/$H_2O$ or EtOAc at pH 3–4 were however required for the preparation of **8** and **9**.

Attempts to synthesize compound **12** failed. Its intermediate, the activated *N*-Z-asparagine succinimide ester, appeared to have been formed in the reaction, as indicated by TLC. The activated ester, however, did not undergo the coupling reaction with cyanamide but was hydrolyzed back to the starting material *N*-Z-asparagine as the reaction temperature was raised in an attempt to facilitate the coupling. Compound **13**, a one-carbon higher homolog of **12**, was however successfully synthesized. The reasons for the failure of the synthesis of **12** remain unclear.

## Results and Discussion

All compounds were first evaluated in our laboratory for their ability to protect against both MES and scMET tests in mice (Table 2). The pharmacological testing data of previously reported[8] compounds (*S*)-(+)-1-carbobenzoxy-5-isobutyl-2-iminohydantoin (**1**), 1-carbobenzoxy-2-iminohydantoin (**2**), and (*S*)-(+)-1-carbobenzoxy-5-benzyl-2-iminohydantoin (**3**) are also listed in the table for SAR comparisons. Compounds derived from L-amino acids (usually having the *S*-configuration) with small to medium size aliphatic hydrocarbon substituents at the C5 position, i.e., ethyl (**6**) *n*-propyl (**7a**), isopropyl (**8**), allyl (**9**), *sec*-butyl (**11**), and isobutyl (**1**) groups, showed substantial anticonvulsant activity against the MES test. All of the above compounds except **8** also exhibited significant activity against the scMET test. Compound **4**, which has a 5-methyl substituent, however showed lower activity against the MES test and

**Table 2.** Anticonvulsant Testing Data ($ED_{50}$) in Mice

| compd | optical config | $ED_{50}$[a] scMET[b] | $ED_{50}$[a] MES[c] |
|---|---|---|---|
| **1** | *S*-(+) | 141[d] (88.7–214) | 98.6[d] (67.0–141) |
| **2** | | NA[d] | + |
| **3** | *S*-(+) | >1000[d] | NA |
| **4** | *S*-(+) | NA | 171 (104–279)[e] |
| **5** | | NA | 228 (91.8–567) |
| **6** | *S*-(+) | 223 (118–422) | 69.2 (54.0–88.6) |
| **7a** | *S*-(+) | 141 (124–163)[f] | 53.3 (33.8–84.1) |
| **7b** | *R*-(−) | 374 (74.0–1893) | NA |
| **8** | *S*-(+) | + | 51.9 (29.5–91.2) |
| **9** | *S*-(+) | 193 (130–289) | 53.3 (44.2–64.4) |
| **10** | *S*-(+) | + | >1000 |
| **11** | *S*-(+) | 176 (86.2–360) | 74.4 (58.9–94.0) |
| **13** | *S*-(+) | NA | NA |
| **14** | *S*-(+) | NA | NA |
| **15** | *R*-(+) | NA | NA |
| **16** | *S*-(+) | + | NA |

[a] $ED_{50}$ values are in mg/kg of test drug delivered intraperitoneally and measured at 0.5 h. The time of peak effect was not determined. NA = not active up to 300 mg/kg. + denotes activity at 300 mg/kg, but $ED_{50}$ can not be estimated. [b] Subcutaneous pentylenetetrazole seizure test. [c] Maximal electroshock seizure test. [d] Previously tested.[8] [e] Numbers in parentheses are 95% confidence intervals. [f] Taken from the Epilepsy Branch, NIH.

no activity against the scMET test. Compound **10**, which has a 5-*n*-butyl substituent, had no activity against both the MES and scMET tests. Although compounds **1**, **10**, and **11** differ only by having different isomeric butyl groups at the C5 position, **1** (with 5-isobutyl) and **11** (with 5-*sec*-butyl) exhibited substantial anticonvulsant activity, while **10** (with 5-*n*-butyl) did not provide any significant activity. Compounds substituted at the C5 position by arylalkyl groups (**3** or **16**), alkyl or arylalkyl groups containing heteroatoms (**13**–**15**), or H (**2**) showed no activity against both the MES and scMET tests. The activity of 5,5-dimethyl-substituted compound **5** was comparable to that of the monomethyl-substituted **4**. The *R* isomer of 5-*n*-propyl-1-carbobenzoxy-2-iminohydantoin (**7b**), synthesized from D-norvaline, failed to show any significant activity against the MES test and provided a low activity against the scMET test as compared to its *S* isomer (**7a**), prepared from L-norvaline.

**Table 3.** Phase II Quantitative Evaluation[a]

| | $ED_{50}$ | | $TD_{50}$ | PI[b] | |
|---|---|---|---|---|---|
| | MES | scMET | | MES | scMET |
| 1[c] | 98.6 (67.0–141.4)[d] | 140.7 (88.7–214.4) | 349.5 (252.9–400) | 3.5 | 2.5 |
| **6** | 99.5 (83.5–113) | <500 | <500 | | |
| **7a** | 80.2 (49.5–108.7) | 140.7 (123.7–162.5) | 188.2 (155.2–237.6) | 2.3 | 1.3 |
| phenyltoin[e] | 9.5 | NA[f] | 65.5 | 6.9 | |

[a] $ED_{50}$ and $TD_{50}$ (rotorod ataxia test, neurotoxicity) values are in mg/kg delivered ip and measured at time of peak effect or peak deficit. [b] PI = protective index ($TD_{50}/ED_{50}$). [c] Previously reported.[8] [d] Numbers in parentheses are 95% confidence intervals. [e] Reference 9. [f] NA = not active.

On the basis of our preliminary data, compounds **6**, **7a**, **8**, **9**, and **11** were also independently evaluated by the Antiepileptic Drug Development (ADD) program, Epilepsy Branch, Neurological and Communicative Disorders and Stroke, NIH. The qualitative phase I studies involved three tests: maximal electroshock seizure, subcutaneous pentylenetetrazol seizure, and neurologic toxicity (rotorod ataxia test). All of the tested compounds showed activity at doses ≤300 mg/kg against both the MES and scMET tests, similar to results from our laboratory. In the neurologic toxicity test, results varied with the C5 substituent. For ethyl (**6**), no toxicity was observed up to 300 mg/kg. For *n*-propyl (**7a**) and allyl (**9**), toxicity was observed at 300 mg/kg. Compounds with isopropyl (**8**) and *sec*-butyl (**11**) were toxic at 100 mg/kg.

Compounds **1**, **6**, **7a**, **8**, and 11 were also evaluated by the ADD Program, NIH, for their oral activity in the MES test in the rat at a dose of 50 mg/kg in phase IVa screening. All compounds showed oral activity in this qualitative screen except **1**.

Among the five compounds (**6**, **7a**, **8**, **9**, and **11**), **6** and **7a** showed both significant anticonvulsant activity and low rotorod ataxia toxicity and were advanced to phase II trials by the ADD Program, NIH, for quantification of their anticonvulsant activity and neurotoxicity in mice by determining the median effective dose ($ED_{50}$) and median toxic dose ($TD_{50}$) (Table 3). It should be noted that the MES $ED_{50}$ values of **6** and **7a** determined by our laboratory (Table 2) are 69.2 (54.0–88.6) and 53.3 mg/kg (33.8–84.1), respectively, with 95% confidence intervals shown in parentheses, and these values are slightly lower but comparable to the results shown in Table 3.

In summary, among the compounds synthesized and screened in this research, (*S*)-(+)-1-carbobenzoxy-2-iminohydantoin analogs with 5-substituted ethyl (**6**), propyl (**7a**), isopropyl (**8**), allyl (**9**), and *sec*-butyl (**11**) groups show substantial anticonvulsant activity against the MES test with $ED_{50}$ values in the range of 52–74 mg/kg. All of the compounds except **8** also have activity against the scMET test with $ED_{50}$ values in the range of 141–223 mg/kg. Ignoring branching, all of the significantly active compounds have an aliphatic hydrocarbon moiety with two- to three-carbon chain lengths at the C5 position of the 2-iminohydantoin ring. The activity is significantly decreased when the side chain length is more than three carbons or less than two carbons. Upon the basis of our previous studies[8] and the results for the enantiomers of **7**, the correct stereochemistry of the C5 substituent is also important. The results suggest that the side chain length at the C5 position with the correct stereochemistry in the 1-carbobenzoxy-2-iminohydantoin ring plays an important role in providing anticonvulsant activity.

## Experimental Section

**Chemistry.** Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA. The instruments used are as follows: IR, Perkin-Elmer Model 281; $^1$H NMR, Varian EM-360L CW, 60 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 purchased from commercial vendors.

**(*S*)-(+)-1-Carbobenzoxy-5-methyl-2-iminohydantoin (4).** A mixture of *N*-carbobenzoxy-L-alanine (5.00 g, 22.4 mmol), 1,3-dicyclohexylcarbodiimide (4.67 g, 22.4 mmol), and *N*-hydroxysuccinimide (2.66 g, 22.4 mmol) was stirred in 190 mL of THF at ice bath temperature for 3.5 h. The reaction mixture was filtered, and the filtrate was added dropwise to a solution of cyanamide (2.83 g, 67.2 mmol) in 165 mL of alkaline water [26.9 mL of 10% (g/v) NaOH, 67.2 mmol] at ice bath temperature. After stirring overnight at room temperature, the mixture was concentrated *in vacuo* at 40 °C to remove THF and was then filtered. The aqueous filtrate (pH 10) was further basified with 10% NaOH to pH 12 and extracted with methylene chloride (3 × 120 mL). The aqueous layer was separated, acidified to pH 2 with 10% HCl, and extracted with ethyl acetate (4 × 100 mL). The EtOAc extract was dried over anhydrous sodium sulfate and evaporated *in vacuo* to give a thick yellow liquid. Addition of $H_2O$ to this liquid resulted in formation of a solid product, which was purified by recrystallization with hot ethanol to give 3.33 g (59.6% yield) of white crystalline **4**: mp 191–192 °C dec; $[\alpha]^{23}_D$ = +18.0° (*c* = 0.3, MeOH); TLC $R_f$ = 0.58 in EtOAc/EtOH (9:1); IR (Nujol, cm$^{-1}$) 3400 (NH), 3060 ($C_6H_5$), 1750, 1720, 1710 (C=O), 1640 (C=N); $^1$H NMR [$CDCl_3$/DMSO-$d_6$ (1:1)] δ 9.00 (br, 1H, NH), 7.90 (br, 1H, NH), 7.32 (s, 5H, ArH), 5.27 (s, 2H, $OCH_2$), 3.90–4.32 (fused q, 1H, CH), 1.25–1.50 (d, 3H, $CH_3$). Anal. ($C_{12}H_{13}N_3O_3$) C, H, N.

**1-Carbobenzoxy-5,5-dimethyl-2-iminohydantoin (5)** was prepared from *N*-carbobenzoxymethylalanine (5.00 g, 21.1 mmol) according to the procedure for **4**. After acidifying the aqueous layer (separated from the methylene chloride extraction) to pH 2, some cyclized solid product was spontaneously formed and filtered. The filtrate was further extracted with EtOAc to give a thick yellow liquid which was solidified by addition of water. These two portions of crude product were identical as indicated by TLC. They were combined and recrystallized with hot ethanol to give 4.50 g (61.8% yield) of white crystalline **5**: mp 192–193 °C dec; TLC $R_f$ = 0.64 in EtOAc/EtOH (9:1); IR (Nujol, cm$^{-1}$) 3410 (NH), 3050 ($C_6H_5$), 1740, 1720, 1700 (C=O), 1655 (C=N); $^1$H NMR ($CDCl_3$) δ 7.86 (br, 1H, NH), 7.37 (s, 5H, ArH), 5.27 (s, 2H, $OCH_2$), 1.48 (s, 6H, $CH_3$). Anal. ($C_{13}H_{15}N_3O_3$) C, H, N.

***N*-Carbobenzoxy-L-2-amino-*n*-butyric Acid.** Benzyloxycarbonyl chloride (8.30 g, 48.5 mmol) in 40 mL of THF was added to a solution of L-2-amino-*n*-butyric acid (5.00 g, 48.5

mmol) in 160 mL of alkaline THF/$H_2O$ (1:1) solution (48.5 mmol of NaOH) at ice bath temperature. The reaction mixture was stirred at room temperature overnight, and the pH was adjusted to pH 8–9 with 30% NaOH. The mixture was concentrated to half of its volume *in vacuo*. The resulting aqueous solution (pH 9) was further basified to pH 12 with 10% NaOH and extracted with methylene chloride (3 × 100 mL). The aqueous layer was separated, acidified to pH 2 with 10% HCl, and extracted with EtOAc (3 × 100 mL). The separated organic layer was dried over anhydrous sodium sulfate, and the filtrate was evaporated *in vacuo* to give 7.00 g (60.9% yield) of the crude product as a yellow solid. This solid was used to synthesize **6** without further purification; $[\alpha]^{23}_D = -14.4°$ ($c$ = 1.0, MeOH); TLC $R_f$ = 0.72 in EtOAc/AcOH (99:1); $^1$H NMR ($CDCl_3$) $\delta$ 9.10 (br, 1H, NH), 7.50 (s, 5H, ArH), 5.20 (s, 2H, $OCH_2$), 4.13–4.60 (m, 1H, CH), 1.60–2.20 (m, 2H, $CH_2$), 0.83–1.06 (fused t, 3H, $CH_3$).

**(*S*)-(+)-1-Carbobenzoxy-5-ethyl-2-iminohydantoin (6)** was prepared from the crude *N*-carbobenzoxy-L-2-amino-*n*-butyric acid (5.70 g, 24.0 mmol) according to the procedure for 4 to give a thick yellow liquid. This liquid was heated at 50–60 °C for 30 min in MeOH/$H_2O$ (1:1) solution. The crude solid product was obtained after evaporation of the solvent and recrystallized from hot EtOH to give 3.10 g (49.4% yield) of white crystalline **6**: mp 169–170 °C dec; $[\alpha]^{23}_D = +11.5°$ ($c$ = 1.0, MeOH); TLC $R_f$ = 0.63 in EtOAc/EtOH (9:1); IR (Nujol, $cm^{-1}$) 3400 (NH), 3040 ($C_6H_5$), 1750, 1725, 1710 (C=O), 1670 (C=N); $^1$H NMR ($CDCl_3$) $\delta$ 8.0 (br, 1H, NH), 7.35 (s, 5H, ArH), 5.26 (s, 2H, $OCH_2$), 4.20–4.40 (fused t, 1H, CH), 1.80–2.26 (m, 2H, $CH_2$), 0.60–0.96 (fused t, 3H, $CH_3$). Anal. ($C_{13}H_{15}N_3O_3$) C, H, N.

**(*S*)-(+)-1-Carbobenzoxy-5-*n*-propyl-2-iminohydantoin (7a)** was prepared from *N*-carbobenzoxy-L-norvaline (15.00 g, 59.7 mmol) according to the procedure for **4** to give a thick yellow liquid. This liquid was heated with water at 60 °C for 30 min to give a solid product. The crude product was crystallized from hot EtOH to give 10.60 g (64.5% yield) of white crystalline product: mp 173–174 °C dec; $[\alpha]^{23}_D = +15.3°$ ($c$ = 1.0, MeOH); TLC $R_f$ = 0.64 in EtOAc/EtOH (9:1); IR (Nujol, $cm^{-1}$) 3410 (NH), 3040 ($C_6H_5$), 1720, 1715, 1710 (C=O), 1675 (C=N); $^1$H NMR ($CDCl_3$) $\delta$ 7.96 (br, 1H, NH), 7.36 (s, 5H, ArH), 5.27 (s, 2H, $OCH_2$), 4.18–4.38 (fused t, 1H, CH), 1.80–2.10 (fused q, 2H, $CH_2$), 1.00–1.60 (m, 2H, $CH_2$), 0.75–1.00 (fused d, 3H, $CH_3$). Anal. ($C_{14}H_{17}N_3O_3$) C, H, N.

***N*-Carbobenzoxy-D-norvaline.** Benzyloxycarbonyl chloride (14.60 g, 85.4 mmol) in 30 mL of THF was added to a solution of D-norvaline (10.00 g, 85.4 mmol) in alkaline THF/$H_2O$ (1:1) solution (85.4 mmol of NaOH) at ice bath temperature. The reaction was allowed to proceed for 3 h at this temperature, another equivalent of benzyloxycarbonyl chloride and NaOH (in 20 mL of water) was added, and the reaction mixture was stirred at room temperature overnight. The pH value of the reaction mixture was maintained at pH 8–9 during the course of the reaction by adjustment with 30% NaOH. The mixture was concentrated to half of its volume *in vacuo*, and the resulting aqueous solution (pH 9) was basified to pH 12 with 10% NaOH and extracted with methylene chloride (3 × 100 mL). The aqueous layer was separated, acidified to pH 2.5, and extracted with EtOAc (3 × 100 mL). The separated organic layer was dried over anhydrous sodium sulfate and evaporated *in vacuo* to give 18.20 g (84.9% yield) of the crude product as a yellow solid. This was used to synthesize compound **7b** without further purification: TLC $R_f$ = 0.75 in EtOAc/AcOH (99:1); $^1$H NMR ($CDCl_3$) $\delta$ 9.70 (br, 1H, NH), 17.33 (s, 5H, ArH), 5.30 (s, 2H, $OCH_2$), 4.10–4.80 (fused t, 1H, CH), 1.17–2.25 (m, 4H, $CH_2$), 0.70–1.17 (fused t, 3H, $CH_3$).

**(R)-(−)-1-Carbobenzoxy-5-*n*-propyl-2-iminohydantoin (7b)** was prepared from the crude *N*-carbobenzoxy-D-norvaline (10.00 g, 39.8 mmol) according to the procedure for **7a** to give 4.52 g (41.3% yield) of white crystalline product: mp 177–178 °C dec; $[\alpha]^{23}_D = -15.5°$ ($c$ = 1.0, MeOH); TLC $R_f$ = 0.64 in EtOAc/EtOH (9:1); IR (Nujol, $cm^{-1}$) 3400 (NH), 3040 ($C_6H_5$), 1750, 1720, 1710 (C=O), 1670 (C=N); $^1$H NMR ($CDCl_3$) $\delta$ 8.00 (br, 1H, NH), 7.66 (s, 5H, ArH), 5.28 (s, 2H, $OCH_2$), 4.06–4.73 (fused t, 1H, CH), 1.63–2.13 (fused q, 2H, $CH_2$), 0.90–1.63 (m, 2H, $CH_2$), 0.60–0.90 (fused d, 3H, $CH_3$). Anal. ($C_{14}H_{17}N_3O_3$) C, H, N.

**(*S*)-(+)-1-Carbobenzoxy-5-isopropyl-2-iminohydantoin (8)** was prepared from *N*-carbobenzoxy-L-valine (5.00 g, 19.9 mmol) according to the procedure for 4 to give a thick yellow liquid. This liquid was dissolved in 800 mL of MeOH/$H_2O$ (1:1) solution, adjusted to pH 3.5 with 10% HCl, and refluxed for 3 h. The solid product was obtained after evaporation of methanol to one-fourth of its volume *in vacuo*, and this was recrystallized from hot ethanol to give 1.70 g (31.0% yield) of white crystalline **8**: mp 157–158 °C dec; $[\alpha]^{23}_D = +8.4°$ ($c$ = 1.0, MeOH); TLC $R_f$ = 0.64 in EtOAc/EtOH (9:1); IR (Nujol, $cm^{-1}$) 3430, 3400 (NH), 3030 ($C_6H_5$), 1750, 1720, 1705 (C=O), 1660 (C=N); $^1$H NMR ($CDCl_3$) $\delta$ 8.20 (br, 1H, NH), 7.32 (s, 5H, ArH), 5.25 (s, 2H, $OCH_2$), 4.10–4.15 (fused d, 1H, CH), 2.20–2.70 (m, 1H, CH), 10.77–1.20 (m, 6H, $CH_3$). Anal. ($C_{14}H_{17}N_3O_3$) C, H, N.

***N*-Carbobenzoxy-L-2-amino-4-pentenoic acid** was prepared from L-2-amino-4-pentenoic acid (3.00 g, 26.1 mmol) accordng to the procedure for *N*-carbobenzoxy-D-norvaline, except 1.5 equiv of benzyloxycarbonyl chloride and sodium hydroxide were used in the reaction. One equivalent of each was added at the beginning of the reaction, and the remaining half-equivalent was added 3 h after the first addition. The crude product (6.40 g, 98.6% yield) was obtained as a yellow solid. This was used to synthesize compound **9** without further purification: $[\alpha]^{23}_D = +10.3°$ ($c$ = 1.0, MeOH); TLC $R_f$ = 0.67 in EtOAc/AcOH (99:1); $^1$H NMR ($CDCl_3$) $\delta$ 8.86 (br, 1H, NH), 7.36 (s, 5H, ArH), 4.90–5.50 (m, 5H, $OCH_2$, $CH{=}CH_2$), 4.35–4.65 (m, 1H, CH), 2.45–2.80 (fused t, 2H, $CH_2$).

**(*S*)-(+)-Carbobenzoxy-5-allyl-2-iminohydantoin (9)** was prepared from crude *N*-carbobenzoxy-L-2-amino-4-pentenoic acid (6.40 g, 25.7 mmol) according to the procedure for 4. Concentrated hydrochloric acid (1 mL) was added to the EtOAc extract which had been dried over anhydrous sodium sulfate. This solution was refluxed for 0.5 h and concentrated. The remaining thick yellow liquid was dissolved in 800 mL of MeOH/$H_2O$ (1:1) solution, which was allowed to stand for 3 days. The solid product was obtained after evaporation of the methanol, and this was recrystallized from hot EtOH/$H_2O$ to give 2.80 g (39.9% yield) of white crystalline **9**: mp 152–153 °C dec; $[\alpha]^{23}_D = +15.8°$ ($c$ = 1.0, MeOH); TLC $R_f$ = 0.64 in EtOAc/EtOH (9:1); IR (Nujol, $cm^{-1}$) 3410, 3380 (NH), 3040 ($C_6H_5$), 1750, 1720, 1710 (C=O), 1670 (C=N); $^1$H NMR ($CDCl_3$) $\delta$ 7.96 (br, 1H, NH), 7.40 (s, 5H, ArH), 5.30 (s, 2H, $OCH_2$), 4.60–5.70 (m, 3H, $CH{=}CH_2$), 2.60–2.80 (fused t, 2H, $CH_2$). Anal. ($C_{14}H_{15}N_3O_3$) C, H, N.

**(*S*)-(+)-1-Carbobenzoxy-5-*n*-butyl-2-iminohydantoin (10)** was prepared from *N*-carbobenzoxy-L-norleucine (5.00 g, 18.9 mmol) according to the procedure for **7a** to give a solid product. This crude product was recrystallized with EtOH/EtOAc to give 2.90 g (53.2% yield) of white crystalline product: mp 177–178 °C dec; $[\alpha]^{23}_D = +26.5°$ ($c$ = 1.0, MeOH); TLD $R_f$ = 0.65 in EtOAc/EtOH (9:1); IR (Nujol, $cm^{-1}$) 3400 (NH), 3030 ($C_6H_5$), 1750, 1720, 1700 (C=O), 1655 (C=N); $^1$H NMR ($CDCl_3$/DMSO-$d_6$) $\delta$ 7.93 (br, 1H, NH), 7.38 (s, 5H, ArH), 5.28 (s, 2H, $OCH_2$), 4.15–4.30 (fused t, 1H, CH), 1.77–2.10 (m, 2H, $CH_2$), 1.00–1.35 (m, 4H, $CH_2$), 0.70–1.00 (fused d, 3H, $CH_3$). Anal. ($C_{15}H_{19}N_3O_3$) C, H, N.

**(*S*)-(+)-1-Carbobenzoxy-5-*sec*-butyl-2-iminohydantoin (11)** was prepared from *N*-carbobenzoxy-L-isoleucine (4.50 g, 17.0 mmol) according to the procedure for **7a** to give 3.07 g (62.6% yield) of white crystalline product: mp 171–172 °C dec; $[\alpha]^{23}_D = +10.2°$ ($c$ = 1.0, MeOH); TLC $R_f$ = 0.65 in EtOAc/EtOH (9:1); IR (Nujol, $cm^{-1}$) 3390, 3190 (NH), 3040 ($C_6H_5$), 1745, 1710 (C=O), 1660 (C=N); $^1$H NMR ($CDCl_3$) $\delta$ 7.90 (br, 1H, NH), 7.36 (s, 5H, ArH), 5.26 (s, 2H, $OCH_2$), 4.20–4.23 (d, 1H, CH), 2.30–2.36 (m, 3H, CH and $CH_2$), 0.73–1.00 (m, 6H, $CH_3$). Anal. ($C_{15}H_{19}N_3O_3$) C, H, N.

**(*S*)-(+)-1-Carbobenzoxy-5-(3-propanamido)-2-iminohydantoin (13)** was prepared from *N*-carbobenzoxy-L-glutamine (5.00 g, 18.0 mmol) according to the procedure for **5** to give 2.85 g (52.5%) of white crystalline product **13**: mp 188–189 °C dec; $[\alpha]^{23}_D = +16.0°$ ($c$ = 1.0, MeOH); TLC $R_f$ = 0.13 in EtOAc/EtOH (9:1); IR (Nujol, $cm^{-1}$) 3380 (NH), 3080 ($C_6H_5$), 1700 (C=O), 1665 (C=N); $^1$H NMR [$CDCl_3$/DMSO-$d_6$ (1:1)] $\delta$

9.06 (br, 1H, NH), 7.96 (br, 1H, NH), 7.38 (s, 5H, ArH), 6.60 and 7.20 (br, 2H, $CONH_2$), 5.60 (s, 2H, $OCH_2$), 4.20–4.40 (fused t, 1H, CH), 1.85–2.52 (m, 4H, $CH_2$). Anal. ($C_{14}H_{16}N_4O_4$) C, H, N.

**(*S*)-(+)-1-Carbobenzoxy-5-[2-(methylthio)ethyl]-2-iminohydantoin** (14) was prepared from *N*-carbobenzoxy-L-methionine (5.00 g, 17.7 mmol) according to the procedure for 4 to give a solid product. This crude product was recrystallized from EtOH/EtOAc to give 2.34 g (43.1% yield) of white crystalline product 14: mp 154–155 °C dec; $[\alpha]^{23}_D = +32.6°$ ($c$ = 1.0, MeOH); TLC $R_f$ = 0.64 in EtOAc/EtOH (9:1); IR (Nujol, $cm^{-1}$) 3400 (NH), 3050 ($C_6H_5$), 1750, 1720, 1710 (C=O), 1670 (C=N); $^1H$ NMR [$CDCl_3$/DMSO-$d_6$ (1:1)] $\delta$ 9.10 (br, 1H, NH), 7.86 (br, 1H, NH), 7.38 (s, 5H, ArH), 5.30 (s, 2H, $OCH_2$), 4.26–4.40 (fused t, 1H, CH), 2.20–2.40 (m, 4H, $CH_2$), 1.90 (s, 3H, $SCH_3$). Anal. ($C_{14}H_{17}N_3O_3S$) C, H, N, S.

**(*R*)-(+)-1-Carbobenzoxy-5-[(benzylthio)methyl]-2-iminohydantoin (15)** was prepared from *N*-carbobenzoxy-L-[(*S*)-benzyl]cysteine (5.00 g, 14.5 mmol) according to the procedure for 4 to give 1.25 g (23.4% yield) of white crystalline product 15: mp 140.5–141.5 °C dec; $[\alpha]^{23}_D = +70.9°$ ($c$ = 1.0, MeOH); TLC $R_f$ = 0.71 in EtOAc/EtOH (9:1); IR (Nujol, $cm^{-1}$) 3410 (NH), 3040 ($C_6H_5$), 1755, 1720 (C=O), 1670 (C=N); $^1H$ NMR ($CDCl_3$) $\delta$ 7.90 (br, 1H, NH), 7.05–7.50 (m, 10H, ArH), 5.18 (s, 2H, $OCH_2$), 4.28–4.48 (t, 1H, CH), 3.60 (s, 2H, $SCH_2$), 3.03–3.10 (fused d, 2H, $CH_2$). Anal. ($C_{19}H_{19}N_3O_3S$) C, H, N, S.

**(*S*)-(+)-1-Carbobenzoxy-5-[(indol-3-yl)methyl]-2-iminohydantoin** (16) was prepared from *N*-carbobenzoxy-L-tryptophan (6.80 g, 20.0 mmol) according to the procedure for 4 to give a thick yellow liquid. This liquid was first purified by flash chromatography.[10] Silica gel (40 $\mu$m, Baker) was packed on a column with EtOAc under a positive nitrogen pressure. The crude sample dissolved in EtOAc (25%) was loaded onto the column, and the column was eluted with EtOAc/EtOH (95:5) at a flow rate of 1.5 in./min under a positive nitrogen pressure. The fractions containing the desired product were combined and evaporated *in vacuo* to give a pure powder. This powder was recrystallized from hot ethanol to give 3.20 g (47.0% yield) of white crystalline 16: mp 182–183 °C dec; $[\alpha]^{23}_D = +77.9°$ ($c$ = 1.0, MeOH); TLC $R_f$ = 0.58 in EtOAc/EtOH (9:1); IR (Nujol, $cm^{-1}$) 3440, 3420 (NH), 3060 ($C_6H_5$), 1740, 1700 (C=O), 1640 (C=N); $^1H$ NMR [$CDCl_3$/DMSO-$d_6$ (1:1)] $\delta$ 10.40 (br, 1H, NH of indole), 8.70 (br, 1H, NH), 6.62–7.48 (m, 10H, ArH), 5.32 (s, 2H, $OCH_2$), 4.32–4.52 (fused t, 1H, CH), 3.36–3.48 (fused d, 2H, $CH_2$). Anal. ($C_{20}H_{18}N_4O_3$) C, H, N.

**Pharmacology.** Adult, male Swiss Webster mice (20–25 g, Taconic Farms, Germantown, NY) were used. The mice were housed in an environmentally controlled room (12 h light/dark cycle, lights on 07:00 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 1% carboxymethyl cellulose (CMC). 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/group) for each drug tested. The drugs were screened against (1) chemically induced seizures utilizing pentylenetetrazole as the convulsant (subcutaneous pentylenetetrazole seizure threshold test) and (2) electrically induced seizures using a 50 mA current (maximal electroshock seizure test. All tests were performed at 0.5 h after drug administration.

In the scMET test, pentylenetetrazole was administered subcutaneously in a loose fold of skin on the back of the neck in a dose of 85 mg/kg (the convulsant dose, CD97[9]). The animals were observed for 30 min after injection of pentylenetetrazole, and absence of clonic spasms persisting for at least 5 s was considered the end point for elevation of the pentylenetetrazole-induced seizure threshold by active compounds. 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 s by electroshock equipment (Electroshock Unit Model 11 A, 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 the end point for a compound's prevention of MES-induced seizure spread.[9]

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