Tricyclic Indole-2-carboxylic Acids: Highly in Vivo Active and Selective Antagonists for the Glycine Binding Site of the NMDA Receptor

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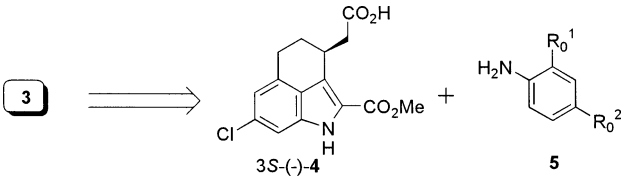
Received May 31, 2002

A series of tricyclic indole-2-carboxylic acid derivatives were synthesized and evaluated by the radioligand binding assay and the anticonvulsant effects in the mouse NMDA-induced seizure model. Among them, derivatives of 3*S*-(−)-**4** such as **3a**, **3f**, and **3g** which had certain zwitterionic anilides showed high affinity to the NMDA-glycine binding site. The absolute configuration of 3*S*-(−)-**4** was confirmed by X-ray crystallographic analysis. In particular, **3g** (SM-31900) was found to be a highly active glycine antagonist for both in vitro and in vivo assays (K_i = 1.0 ± 0.1 nM, ED_{50} = 2.3 mg/kg, iv) and also showed high selectivity for the glycine site. In addition, **3g** was soluble enough in aqueous media (>10 mg/mL at pH 7.4) to use for medications by intravenous injection.

Introduction

The *N*-methyl-D-aspartate (NMDA) receptor is a member of the glutamate receptor superfamily and plays important roles for neurotransmission in the central nervous system (CNS) and synaptic plasticity that underlies learning processes and memory.[1] The NMDA receptor is associated with a ligand gated calcium ion channel which is activated by cooperation of glutamate and glycine[2] and blocked by certain ion channel blockers[3] such as phencyclidine (PCP) and dizocilpine (MK-801). Overactivation of the NMDA receptor is, however, thought to be involved in neuronal cell death caused by not only hypoxic conditions such as stroke but also chronic neurodegenerative disorders such as Alzheimer's and Hungtington's diseases.[4] Therefore, antagonists, especially, acting on the glycine binding site of the NMDA receptor have been recognized to be potential therapeutic agents for such disorders.[1b,5] The glycine antagonists discovered initially, however, were found to show poor in vivo activities because the structural features were unfavorable for penetration of the blood−brain barrier.[6] Although much effort has been devoted to identifying in vivo active glycine antagonists by many laboratories, only a few compounds such as ACEA 1021,[7] L-701,324,[8] and ZD9379[9] have successfully showed in vivo activities so far.[10] GV150526A[11] was also reported to be in vivo active and entered clinical trials, although the trials were terminated at the phase III. We have synthesized a series of tricyclic quinoxalinediones **1** (Figure 1) as a new class of potent NMDA−glycine antagonists including SM-18400 (**1a**) which showed extremely high affinity to the glycine site (K_i = 0.4 nM) and exhibited neuroprotective properties in several animal models.[12,13] We have also synthesized a series of tricyclic azakynurenic acids **2** using a novel Stille type coupling reaction.[14] Moreover, we recently identified another potent tricyclic series based on indole-2-carboxylic acid, as exemplified by **3a** (K_i = 0.8 nM).[15] Herein, we report the detailed study on this new series, i.e., tricyclic indole-2-carboxylic acids **3**, in which we have further synthesized and evaluated the derivatives of **3a** and identified **3g** as a potent glycine antagonist in both in vitro and in vivo, as determined by the radioligand binding assay and the anticonvulsant effects in the mouse NMDA-induced seizure model.

Scheme 1. Synthetic strategy for tricyclic indole-2-carboxylic acid derivatives **3**.



Chemistry

Tricyclic indole-2-carboxylic acid derivatives (**3**) bearing various anilide groups can be prepared by condensation of tricyclic indole monocarboxylic acid 3*S*-(−)-**4** with the corresponding anilines **5** followed by deprotections (Scheme 1). Synthesis of common intermediate 3*S*-(−)-**4** was described in our previous report.[15] Since we succeeded in obtaining suitable crystals of (−)-**4** by recrystallization from 2-propanol, the absolute configuration of the (−)-isomer could be determined to be *S* by single X-ray crystallographic analysis as shown in Figure 2.

Aniline **5d** was prepared as shown in Scheme 2. Methyl 2-nitrophenylacetate (**6**), which was readily prepared from commercially available 2-nitrophenylacetic acid, was hydrogenated with 10% palladium on carbon in ethyl acetate to give **5d**. Aniline **5e** was synthesized according to Scheme 3. Mesylation of 4-nitrobenzyl alcohol (**7**) followed by treatment with potas-

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10.1021/jm020239l CCC: $25.00
Published on Web 01/24/2003

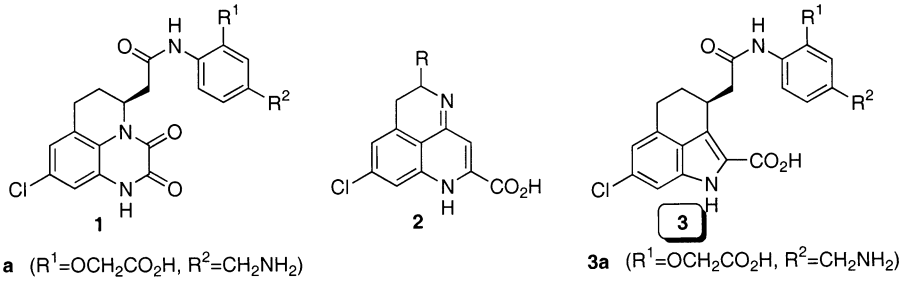


Figure 1. Tricyclic NMDA antagonists acting on the glycine site.

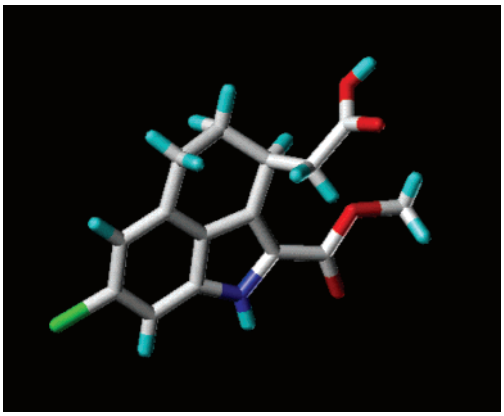
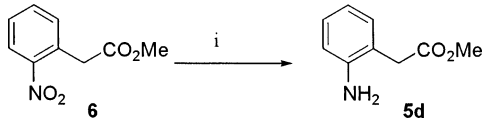


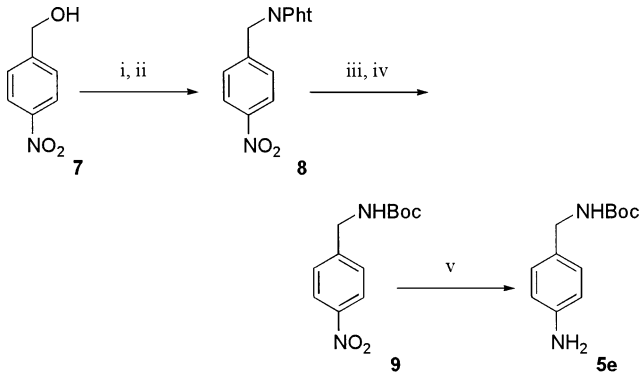
Figure 2. X-ray structure of 3*S*-(−)-**4**.

Scheme 2[a]



[a] Reagents and conditions: (i) H_2, 10% Pd/C, EtOAc, rt, quant.

Scheme 3[a]

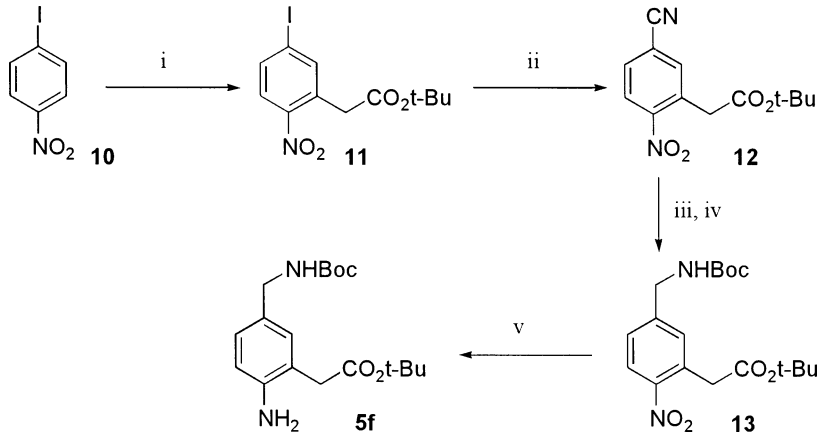


[a] Reagents and conditions: (i) MsCl, Et_3N, toluene, 0 °C; (ii) phthalimide-K, DMF, rt, 91% from **7**; (iii) $H_2NNH_2 \cdot H_2O$, $TsOH \cdot H_2O$, THF, reflux; (iv) Boc_2O, CH_2Cl_2, rt, 87% from **8**; (v) H_2, 10% Pd/C, EtOAc, rt, 99%.

sium phthalimide in DMF gave phthalimide **8**. Deprotection of **8** with hydrazine monohydrate in THF in the presence of *p*-toluenesulfonic acid followed by treatment with di-*tert*-butyl dicarbonate (Boc_2O) afforded **9**, which was then hydrogenated as described above to give **5e**. Although synthesis of aniline **5f** was described in ref 16, we used a more convenient route as shown in Scheme 4. Commercially available 1-iodo-4-nitrobenzene (**10**) was treated with *tert*-butyl chloroacetate under the conditions of the vicarious nucleophilic substitution (VNS) reaction[17] to afford compound **11**. Palladium-catalyzed cyanation of **11** using zinc cyanide[18] gave compound **12** in practical yield. Selective reduction of **12** with a combination of borane−THF complex and trimethylborate in THF followed by treatment with Boc_2O afforded desired nitrobenzene **13**, which was then hydrogenated as described above to give **5f**. Anilines **5g** and **5h** were respectively synthesized according to the route shown in Scheme 5. At first, we used esters such as ethyl or *tert*-butyl ester as a protecting group of carboxylic acid at the side chain of the anilines. Such anilines, however, readily underwent undesired lactam-formation during storage or under coupling conditions with 3*S*-(−)-**4**. To solve this problem, we chose morpholinoamide as a protecting group that was stable enough under storage or the coupling conditions and was readily cleaved to the carboxylic acid by hydrolysis. A mixture of methyl (*S*)-(−)-lactate (**14g**) and morpholine was heated in the presence of a catalytic amount of sodium hydride and the resulting amide was converted into tosylate **15g**. The optical purity of **15g** was confirmed to be more than 99% ee after recrystallization, as determined by HPLC analysis on a chiral stationary column. 3-Hydroxy-4-nitrobenzoic acid (**16**) was reduced with a combination of borane−pyridine complex/boron trifluoride diethyl etherate/trimethyl borate in THF to give benzyl alcohol **17** in high yield.[19] This product was coupled with **15g** in the presence of potassium carbonate to yield ether **18g**. In this step, a S_N2 type reaction would take place with complete inversion at the stereogenic center because the chiral stationary phase HPLC analysis of crude **18g** showed an optical purity of more than 99% ee without loss of the ee from **15g**. The absolute configuration of **18g** must be *R*, as confirmed by a model reaction.[20] Similarly to the synthesis of **5e**, **18g** was converted into the desired aniline **5g** in five steps. The Boc-protected nitrobenzylamine **21g** was purified by recrystallization from toluene, and the chiral stationary phase HPLC analysis of this product showed 99.9% ee. The isomer **5h** was also prepared from methyl (*R*)-(+)-lactate (**14h**) as described above.

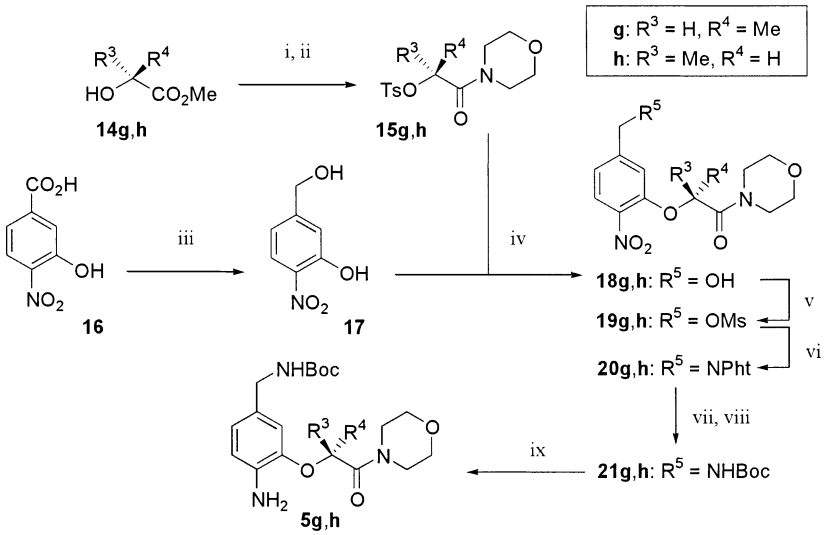
Preparation of compounds **3a**−**c** in Table 1 was described in the previous report.[15] Compounds **3d**−**h** in Table 1 were prepared according to the route outlined in Scheme 6. Condensation of 3*S*-(−)-**4** with aniline **5d** using *N*,*N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (Bop-Cl) in dichloromethane in the presence of triethylamine afforded anilide **22d**. Both methyl esters of **22d** were hydrolyzed simultaneously with aqueous sodium hydroxide in a mixed solvent of methanol and THF to give **3d**. In a similar way, **5f** was condensed with 3*S*-(−)-**4** to afford anilide **22f**, which was hydrolyzed as described above and then treated with hydrogen chloride in 1,4-dioxane to give **3f** as a hydrochloride salt.

Scheme 4[a]



[a] Reagents and conditions: (i) *tert*-butyl chloroacetate, *t*-BuOK, DMF, 0 °C, 51%; (ii) $Zn(CN)_2$, $(Ph_3P)_4Pd$, DMF, 80 °C, 84%; (iii) $(MeO)_3B$, BH_3·THF, THF, rt; (iv) Boc_2O, THF, rt, 50% from **12**; (v) H_2, 10% Pd/C, EtOAc, rt, quant.

Scheme 5[a]



[a] Reagents and conditions: (i) morpholine, NaH, 50 °C; (ii) NaH, TsCl, THF, 0 °C, 73% from **14g,h**, >99% ee; (iii) $(MeO)_3B$, BF_3·OEt_2, BH_3·pyridine, 1,2-dichloroethane, rt 94%; (iv) K_2CO_3, DMF 50 °C, >99% ee; (v) MsCl, Et_3N, CH_2Cl_2, 0 °C, 99% from **17**; (vi) phthalimide-K, DMF, rt, 67%; (vii) H_2NNH_2·H_2O, TsOH·H_2O, THF, reflux, 92%; (viii) Boc_2O, EtOAc, rt, 93%, 99.9% ee; (ix) H_2 10%, Pd/C, EtOAc, rt, quant.

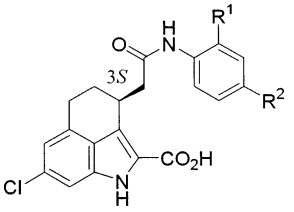
Condensation of 3*S*-(−)-**4** with **5e** was carried out with a combination of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (WSC) and 1-hydroxybenzotriazole (HOBt) in DMF to afford **22e**, which was then subjected to the deprotection sequence as described above to give **3e** as a hydrochloride salt. Treatment of 3*S*-(−)-**4** with oxalyl chloride in ethyl acetate in the presence of DMF gave the corresponding acid chloride which was then reacted with **5g** in the presence of triethylamine to afford anilide **22g**. Succesive deprotection of **22g** was performed at first with aqueous lithium hydroxide in a mixed solvent of methanol and THF and then treatment with hydrogen chloride in acetic acid to give **3g** as a hydrochloride salt. Under these conditions no chromatographic purifications were required through the entire sequence from starting materials, **16** and **14g**, to **3g**. The final product **3g** was also analyzed by HPLC and both its ee and de were estimated to be 99.9%. In a manner similar to **3g**, **3h** was prepared from aniline **5h**.

Biological Evaluations

The affinities of compounds **3a**−**h** and **1a** for the glycine site of the NMDA receptor were determined by the displacement of the tritium labeled selective glycine site antagonist [^{3}H] **1a** (K_d = 0.43 nM)[13e] from rat cortical membrane preparations. The respective IC_{50} values are listed in Table 1. The affinity of the selected compound **3g** for the glycine site was also evaluated by using [^{3}H] 5,7-dichlorokynurenic acid (DCKA),[21] [^{3}H] glycine,[22] as well as [^{3}H] **1a** (Table 2). The affinities of **3g** for the glutamate binding site of the NMDA receptor and other glutamate receptor subtypes such as 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA) and kainic acid (KA) receptors were evaluated using [^{3}H] glutamate,[23] [^{3}H] AMPA,[24] and [^{3}H] KA[25] as radiolabeled ligands, respectively (Table 2).

The in vivo activities of compounds **3a**, **3c**−**h**, and **1a** were measured in the mouse NMDA-induced seizure model.[12a,13b,26] A test compound was intravenously administered at a given dose (usually 3 mg/kg, iv) into each of 10 mice, 20 min prior to the intracerebroven-

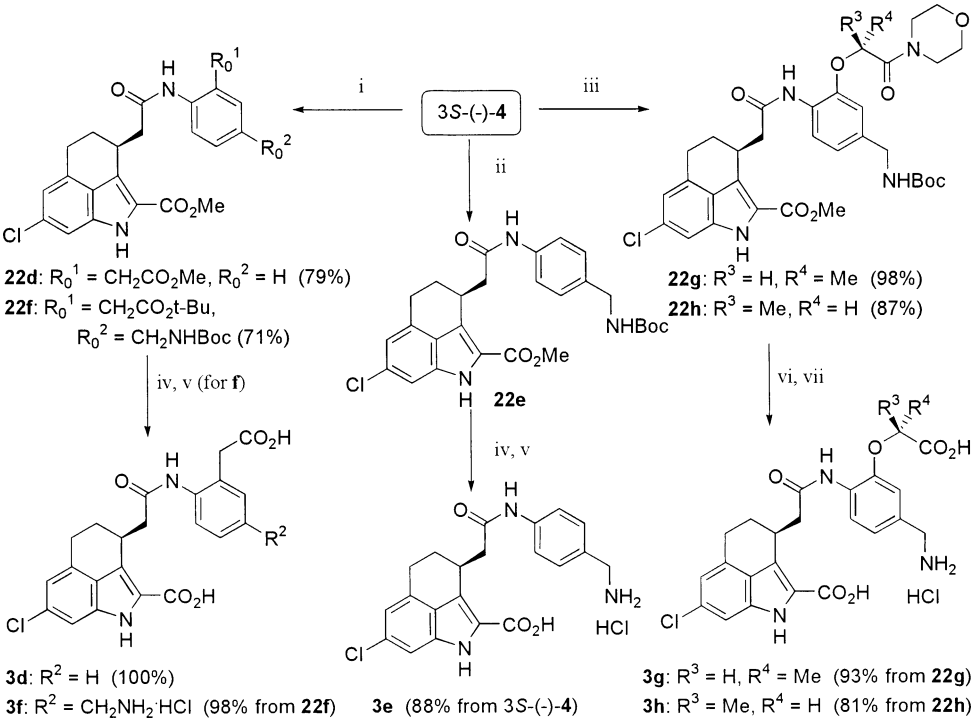
Table 1. Binding Affinity and Anticonvulsant Activity of Tricyclic Indole-2-carboxylic Acids **3a**−**h**



				mice NMDA-induced seizure model[b]	
compd	R^1	R^2	IC_{50} (nM)[a] vs [^{3}H] SM-18400	number protected/number tested (at 3 mg/kg, iv)	ED_{50} (mg/kg)
3a	OCH_2CO_2H	CH_2NH_2	1.2	2/10	5.0 (2.0−12.5)[c]
3b[d]	OCH_2CO_2H	CH_2NH_2	33	nt[e]	
3c	H	H	19	3/10	nd[f]
3d	CH_2CO_2H	H	7.6	3/10	nd[f]
3e	H	CH_2NH_2	18	3/10	nd[f]
3f	CH_2CO_2H	CH_2NH_2	1.5 ± 0.3	5/10	1.8 (0.63−5.1)[c]
3g	(1′*R*)-OC*H(CH_3)CO_2H	CH_2NH_2	2.7 ± 0.3	5/10	2.3 (0.71−5.1)[c]
3h	(1′*S*)-OC*H(CH_3)CO_2H	CH_2NH_2	29.0 ± 0.5	4/10	3.7 (2.5−10.0)[c]
1a	SM-18400		1.0 ± 0.1	10/10	0.41 (0.14−0.68)[c]

[a] The values of **3a**−**e** were determined from two independent experiments carried out in triplicate. The values of **3f**−**h**, and **1a** represent the mean ± SEM from three separate assays carried out in triplicate. See ref 13e for detail assay procedures. [b] See ref 12a, 13b, 26, 28 and see the text for detail assay procedures. [c] The values in parentheses show 95% confidence limits. [d] Compound **3b** was C-3 epimer of **3a** and prepared from 3*R*-(+)-**4**. See ref 15. [e] This compound was not tested in vivo. [f] The values of ED_{50} for these compounds were not determined.

Scheme 6[a]



[a] Reagents and conditions: (i) **5d** or **5f**, BopCl, Et_3N, CH_2Cl_2, rt; (ii) **5e**, WSC, HOBt, DMF, rt; (iii) $(COCl)_2$, DMF, EtOAc, rt, then **5g** or **5h**, Et_3N, EtOAc, rt; (iv) aq 2 N NaOH, THF, MeOH, rt; (v) 2 N HCl, 1,4-dioxane, rt; (vi) aq 2 N LiOH, THF, MeOH, rt; (vii) 0.5 N HCl, AcOH, rt.

tricular (icv) administration of NMDA (5 nmol). Under the conditions without pretreatment with the test compound, almost all of mice (9 to 10 of 10) exhibit tonic seizures. The number of mice which did not exhibit tonic seizures after icv administration of NMDA was counted as considered to be protected. For the compounds **3a**, **3f**−**h**, and **1a**, the ED_{50} values were determined by experiments at the doses of 1, 3, and 10 mg/kg under the conditions described above. These results (the number of protection at the given dose and/or the ED_{50} value) are summarized in Table 1.

Results and Discussion

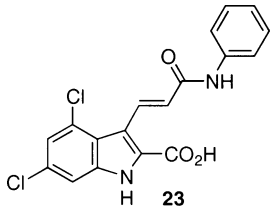
As revealed in the in vitro binding assays using [^{3}H] **1a** in Table 1 and [^{3}H] DCKA as in the previous report,[15] 3*S*-(−)-isomer **3a** (IC_{50} = 1.2 nM) was more active than its C-3 epimer **3b** (IC_{50} = 33 nM), and this result was consistent with our previous observation in the series

Table 2. Selectivity of **3g**: Affinities for Glycine Binding Site, Glutamate Binding Site of NMDA Receptor and Other Glutamate Receptor Subtypes[a]

NMDA−glycine site: K_i (nM)		NMDA binding site: K_i (nM)	non-NMDA receptor: K_i (nM)	
vs [^{3}H] DCKA[b]	vs [^{3}H] glycine[c]	vs [^{3}H] glutamate[d]	vs [^{3}H] AMPA[e]	vs [^{3}H] KA[f]
1.0 ± 0.1	11 ± 2	>10000	>10000	>10000

[a] See ref 28. [b] DCKA: 5,7-dichlorokynurenic acid. See ref 21. [c] See ref 22. [d] See ref 23. [e] AMPA: 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid. See ref 24. [f] KA: kainic acid. See ref 25.

of tricyclic quinoxalinediones.[12a,d,13a] In the previous studies, we optimized tricyclic quinoxalinediones for both in vitro and in vivo activity to obtain zwitterionic compound **1a**, which had an *o*-carboxymethoxy *p*-aminomethylanilide at the side chain.[13a] As expected, compound **3a** (IC_{50} = 1.2 nM, K_i = 0.8 nM[15]) which had the same anilide as **1a** showed affinity as high as **1a** (IC_{50} = 1.0 nM, K_i = 0.4 nM[13a,15]). Unfortunately, in the NMDA-induced seizure model, **3a** (2/10 at 3 mg/kg, ED_{50} = 5.0 mg/kg), however, showed reduced activity compared to **1a** (10/10 at 3 mg/kg, ED_{50} = 0.41 mg/kg). To identify more in vivo active compounds in this new series, we set a criterion that the anticonvulsant activity of the test compound should reach 50% protection at the dose of 3 mg/kg, iv (i.e. 5/10 at 3 mg/kg). Tricyclic indole-2-carboxylic acid derivatives having various anilide groups at the C-3 side chain were prepared and evaluated to seek a compound beyond the criterion. Selected examples are listed in Table 1. Among them, derivatives whose anilide parts were not zwitterionic such as **3c** (unsubstituted anilide, IC_{50} = 19 nM, 3/10 at 3 mg/kg), **3d** (*o*-carboxymethylanilide, IC_{50} = 7.6 nM, 3/10 at 3 mg/kg), and **3e** (*p*-aminomethylanilide, IC_{50} = 18 nM, 3/10 at 3 mg/kg) showed only moderate activity in both in vitro and in vivo. However, compound **3f** (IC_{50} = 1.5 nM, 5/10 at 3 mg/kg, ED_{50} = 1.8 mg/kg) which had a zwitterionic *o*-carboxymethyl- *p*-aminomethyanilide showed not only a high affinity comparable to **3a**, but excellent in vivo activity which could reach our criterion. It was noteworthy that a subtle structural difference between **3a** and **3f** affected their in vivo activities, i.e. where the former possessed an *o*-phenoxyacetic acid moiety {calculated pK_a = 3.08, calculated log *D* (pH 7.4) = −0.97}, whereas the latter had an *o*-phenylacetic acid moiety {calculated pK_a = 4.02, calculated log *D* (pH 7.4) = −0.79}. We speculated that the increased acidity and the existence of a hydrophilic oxygen atom in the phenoxyacetic acid moiety of **3a** might result in the reduced in vivo activity compared to **3f**. Thus, it occurred to us to introduce an electron-donating and lipophilic methyl group into the α position of the carboxyl group of **3a**. One isomer of such compounds, **3g** (IC_{50} = 2.7 nM, 5/10 at 3 mg/kg, ED_{50} = 2.3 mg/kg) retained affinity in in vitro and, indeed, significantly improved the in vivo activity compared to **3a**. The absolute configuration of the second asymmetric center of this compound was *R*, whereas the other epimer **3h** (IC_{50} = 29 nM, 4/10 at 3 mg/kg, ED_{50} = 3.7 mg/kg) showed an at least 11-fold reduced affinity relative to **3g**. Interestingly, **3h** which had 24-fold lower affinity than **3a** was even more potent than **3a** in in vivo. The calculated pK_a and log *D* (pH 7.4) for both **3g** and **3h** were 3.13 and −0.62, respectively. The increase of log *D* compared to **3a** might mainly contribute to improvement of the in vivo activity. It was also noteworthy that the activity of another indole-2-carboxylate-based glycine antagonist, GV-150526A (**23**, Figure 3)[11] was much weaker than that of our series such as **3a**−**h** in our seizure model.[27]



23

Figure 3. Indole-2-carboxylate-based glycine antagonist, GV-150526A.

We selected **3g** (SM-31900) and evaluated further for the biological activities and found that **3g** had excellent neuroprotective effects in neuronal cells[28] and in several animal models.[29−31] In particular, **3g** was more potent than **3f** in a permanent suture middle cerebral artery occlusion (MCAo) model using normotensive rats.[29] The test compound was administered intravenously as a bolus injection immediately after induction of the occlusion followed by a continuous infusion for 24 h. The percentage of cortical infarct volume reduction using **3g** and **3f** were 84% (*statistically significant*) and 30% (*not significant*) at the same dosage of 20 mg/kg bolus + 20 mg/kg/h continuous infusion, respectively. For comparison, **1a** also significantly reduced the infarct volume by 50% at a dosage of 5 mg/kg bolus + 5 mg/kg/h continuous infusion in the same test. Furthermore, **3g** was also neuroprotective in the permanent electrocoagulation MCAo model using spontaneously hypertensive rats (SHRs).[30] In this test, **3g** was administered 30 min after the occlusion and still demonstrated significant infarct reduction of 40% at a dosage of 1.5 mg/kg bolus + 6 mg/kg/h continuous infusion for 24 h. As shown in Table 2, **3g** showed high selectivity for the glycine site (K_i = 1.0 ± 0.1 nM vs [^{3}H] DCKA, K_i = 11 ± 2 nM vs [^{3}H] glycine) compared to the glutamate site (K_i >10 μM vs [^{3}H] glutamate) and other subtypes such as AMPA and KA receptors (K_i >10 μM vs [^{3}H] AMPA, K_i >10 μM vs [^{3}H] KA).[28] In addition, **3g** had no affinities for more than 160 other receptors, ion channels, and enzymes at 10μM. Although we had already introduced another highly potent candidate **1a**, new candidate **3g** turned out to be superior to **1a** in terms of pharmacokinetics and solubility in aqueous media. Pharmacokinetic studies for both compounds were carried out in rats at the same dosage (10 mg/kg, iv) and showed that **3g** had more favorable pharmacokinetic parameters than **1a** {clearance (mL/min/kg), plasma half-life (min) for **3g**: 4.5, 83; for **1a**: 39, 38, respectively}. The brain access of **3g** was also confirmed {the area under the concentration−time curve from time zero to time infinity ($AUC_{0-\infty}$) in whole brain homogenates for **3g**: 10 μg·min/g}, which was better than that of **1a** {the AUC value for the observed time period (AUC_{0-2h}) in whole brain homogenates for

1a: 3.3 μg·min/g}. Solubility was a very important character for intravenous administration that was needed for the treatment of acute neurodegenerative diseases such as stroke. Thus, it was also noteworthy that **3g** was highly soluble (>10 mg/mL) in aqueous media at pH 7.4. In contrast, the solubility of **1a** was only 1.9 mg/mL at pH 7.4, though **1a** could be more soluble in alkaline conditions.

Conclusion

We have successfully identified tricyclic indole-2-carboxylic acids as a new class of potent NMDA−glycine antagonists. Particularly, **3g** was the most promising one in terms of its neuroprotective effects, high selectivity for the glycine site, and high solubility in aqueous media. Further pharmacological and toxicological evaluations of **3g** are in progress.

Experimental Section

General Notes. Melting points were measured on either a Thomas-Hoover or a Yanaco melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a JEOL GX-270 or JEOL JNM-LA300 spectrometers using tetramethylsilane as an internal standard. LC mass spectra were obtained on a PE SCIEX API 150EX spectrometer. Elemental analyses, low-resolution mass spectra, and high-resolution mass spectra were obtained from Sumitomo Analytical Center, Inc. Thin-layer chromatography and flash column chromatography was performed on silica gel glass-backed plates (5719, Merck & Co.) and silica gel 60 (230−400 or 70−230 mesh, Merck & Co.), respectively. Optical rotations were measured on a JASCO DIP-370. Optical purity was determined by HPLC using Hitachi L 6000 pump and L 4000 UV detector or Shimadzu LC-10A pump and SPD-10A UV detector with chiral columns (CHIRALCEL OJ, Daicel; SUMICHIRAL OA-2500, Sumitomo Analytical Center).

***N*-((2*S*)-2-Hydroxypropanoyl)morpholine.** To a mixture of methyl (*S*)-(−)-lactate (**14g**, 50.0 g, 0.480 mol) and morpholine (46.0 mL, 0.528 mol) with stirring at 0 °C was added slowly portionwise 60% sodium hydride (1.92 g, 0.0480 mol), and the resulting mixture was stirred at 50 °C for 3 h. Excess morpholine was removed by azeotropic evaporation with toluene to give the title compound (81.4 g) as crude material. This product was used in the following reaction without further purification: MS (EI) *m*/*z* 159 (M^+); HRMS (EI) calcd for $C_7H_{13}NO_3$ 159.0895 found 159.0815.

***N*-((2*S*)-2-*p*-Toluensulfonyloxypropanoyl)morpholine (15g).** To a suspension of 60% sodium hydride (20.2 g, 0.504 mol) in THF (400 mL) with stirring at 0 °C was added dropwise a solution of *N*-((2*S*)-2-hydroxypropanoyl)morpholine (81.4 g) obtained above in THF (400 mL), and the mixture was warmed gradually and stirred at 50 °C for 30 min. After the mixture was cooled to 0 °C, a solution of *p*-toluenesulfonyl chloride (110 g, 0.576 mol) in THF (400 mL) was added dropwise followed by stirring for 2 h at 0−5 °C. The reaction mixture was diluted with water, acidified with aqueous 1 N HCl to pH 1, and extracted with ethyl acetate. The organic layer was washed with water and brine successively, dried over sodium sulfate, and concentrated to afford the crude product. To the residue were added diethyl ether (100 mL) and hexane (30 mL). The precipitated crystals were collected by filtration, washed with diethyl ether, and dried in vacuo to give **15g** (110 g, >99% ee, 73% yield from methyl (*S*)-(−)-lactate). The enantiomeric excess was determined by using HPLC on a CHIRALCEL OJ with 1:5 ethanol/hexane as an eluent at a flow rate of 1.0 mL/min (UV detection, 254 nm). The (−)-enantiomer, **15g**, eluted at the retention time of 22.9 min, and the (+)-enantiomer, **15h**, eluted at the retention time of 21.6 min: mp 77−78 °C; $[\alpha]^{25}_D = -26.6°$ (*c* 1.0, MeOH); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.47 (d, 3H, J = 6.6 Hz), 2.46 (s, 3H), 3.42 (m, 1H), 3.57 (m, 3H), 3.63 (m, 4H), 5.27 (q, 1H, J = 6.6 Hz), 7.35 (d, 2H, J = 8.3 Hz), 7.81 (d, 2H, J = 8.3 Hz); MS *m*/*z* 313 (M^+); HRMS (EI) calcd for $C_{14}H_{19}NO_5S$ 313.0984 found 313.0975.

5-Hydroxymethyl-2-nitrophenol (17). To a solution of 3-hydroxy-4-nitrobenzoic acid (**16**, 50.0 g, 0.273 mol) in 1,2-dichloroethane (1.0 L) were added trimethyl borate (45.4 g, 0.437 mol) and boron trifluoride diethyl etherate (62.0 g, 0.437 mol) followed by dropwise addition of borane−pyridine complex (38.1 g, 0.410 mol). After stirring at room temperature for 4 h, methanol (100 mL) was added dropwise at 0 °C. The mixture was concentrated, and toluene was added to the residue. The resulting mixture was extracted with aqueous 1 N NaOH three times, and the combined aqueous layers were acidified with aqueous 12 N HCl to pH 1 and extracted with ethyl acetate twice. The organic layers were combined, washed with water and brine successively, dried over magnesium sulfate, and concentrated in vacuo to give the title compound (43.3 g, 94%): mp 97−98 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.94 (bs, 1H), 4.77 (s, 2H), 6.96 (dd, 1H, J = 8.6, 1.7 Hz), 7.17 (d, 1H, J = 1.7 Hz), 8.09 (d, 1H, J = 8.6 Hz), 10.65 (s, 1H); MS (EI) *m*/*z* 169 (M^+); Anal. ($C_7H_7NO_4$): C, H, N.

2-((1*R*)-1-Morpholinocarbonylethoxy)-4-hydroxymethylnitrobenzene (18g). Potassium carbonate (52.7 g, 0.381 mol) was added to a solution of **17** (43.0 g, 0.254 mol) and **15g** (83.6 g, 0.267 mol) in DMF (150 mL), and the mixture was stirred at 50 °C for 6 h. Water was added, and the resulting mixture was extracted with dichloromethane twice. The organic layers were combined and washed with aqueous 5% potassium carbonate solution, aqueous 1 N HCl, and water successively, dried over magnesium sulfate, and concentrated in vacuo to give **18g** (93.2 g, >99% ee). This product was used in the following reaction without further purification. The enantiomeric excess was estimated by using HPLC on a CHIRALCEL OJ with 1:1 2-propanol/hexane as an eluent at a flow rate of 0.4 mL/min (UV detection, 254 nm). The (*R*)-enantiomer, **18g**, eluted at the retention time of 17.7 min, and the (*S*)-enantiomer, **18h**, eluted at the retention time of 25.7 min. The crude product can be purified by silica gel column chromatography with 1:1 chloroform/ethyl acetate to give the analytically pure sample: $[\alpha]^{25}_D = -87.3°$ (*c* 0.25, MeOH); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.67 (d, 3H, J = 6.9 Hz), 3.51 (m, 3H), 3.68 (m, 4H), 3.87 (m, 1H), 4.15 (t, 1H, J = 5.6 Hz), 4.72 (d, 2H, J = 5.6 Hz), 5.09 (q, 1H, J = 6.9 Hz), 7.02 (dd, 1H, J = 8.3, 1.6 Hz), 7.11 (d, 1H, J = 1.6 Hz), 7.80 (d, 1H, J = 8.3 Hz); MS (FAB) *m*/*z* 311 (MH^+); HRMS (FAB) calcd for $C_{14}H_{19}N_2O_6$ (MH^+) 311.1243 found 311.1238.

2-((1*R*)-1-Morpholinocarbonylethoxy)-4-methanesulfonyloxymethylnitrobenzene (19g). Methanesulfonyl chloride (36.1 g, 0.315 mol) was added dropwise slowly to a solution of crude **18g** (93.0 g) and triethylamine (50.1 mL, 0.360 mol) in dichloromethane (500 mL) with stirring at 0 °C. After stirring at 0 °C for 40 min, aqueous 1 N HCl (300 mL) was added, and the organic layer was separated, washed with water, dried over magnesium sulfate, and concentrated in vacuo to give the title compound (97.0 g, 99% from **17**). This product was used in the following reaction without further purification: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.70 (d, 3H, J = 6.9 Hz), 3.09 (s, 3H), 3.48 (m, 3H), 3.69 (m, 4H), 3.92 (m, 1H), 5.08 (q, 1H, J = 6.9 Hz), 5.24 (s, 2H), 7.09 (dd, 1H, J = 8.2, 1.7 Hz), 7.11 (d, 1H, J = 1.7 Hz), 7.85 (d, 1H, J = 8.2 Hz); LC-MS (ESI) *m*/*z* 389 (MH^+).

2-((1*R*)-1-Morpholinocarbonylethoxy)-4-phthalimidomethylnitrobenzene (20g). To a solution of **19g** (96.7 g, 0.249 mol) in DMF (800 mL) was added potassium phthalimide (50.8 g, 0.274 mol), and the mixture was stirred at room temperature for 1.5 h. Water was added, and the resulting mixture was extracted with 2:1 ethyl acetate/toluene twice. The organic layers were combined, washed with water (three times) and brine successively, dried over magnesium sulfate, and concentrated to give the crude product (108 g). To the residue thus obtained were added diethyl ether (400 mL) and toluene (50 mL). The precipitated crystals were collected by filtration, washed with diethyl ether and dried in vacuo to give **20g** (73.6 g, 67%): mp 124−125 °C; $[\alpha]^{25}_D = -85.1°$ (*c* 0.22,

MeOH); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.65 (d, 3H, J = 6.9 Hz), 3.49−3.31 (m, 3H), 3.71−3.51 (m, 4H), 3.88 (m, 1H), 4.81 (d, 1H, J = 15.2 Hz), 4.88 (d, 1H, J = 15.2 Hz), 5.02 (q, 1H, J = 6.9 Hz), 7.08 (dd, 1H, J = 7.3, 1.7 Hz), 7.10 (d, 1H, J = 1.7 Hz), 7.75 (m, 2H), 7.80 (d, 1H, J = 7.3 Hz), 7.87 (m, 2H); MS (FAB) m/z 440 (MH^+); HRMS (FAB) calcd for $C_{22}H_{22}N_3O_7$ (MH^+) 440.1458 found 440.1462.

2-((1*R*)-1-Morpholinocarbonylethoxy)-4-aminomethylnitrobenzene. To a solution of **20g** (73.4 g, 0.167 mol) in THF (800 mL) were added hydrazine monohydrate (32.4 mL, 0.668 mol) and *p*-toluenesulfonic acid monohydrate (3.18 g, 0.0167 mol). The mixture was heated under reflux for 6 h. The resulting mixture was cooled to room temperature, basified with aqueous 5% potassium carbonate solution to pH 10, and extracted with dichloromethane three times. The organic layers were combined, washed with water, dried over magnesium sulfate, and concentrated in vacuo to give the title compound (47.3 g, 92%): mp 116−117 °C; $[\alpha]^{25}_D$ = −57.8° (*c* 0.19, MeOH); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.50 (bs, 2H), 1.69 (d, 3H, J = 6.9 Hz), 3.56−3.38 (m, 3H), 3.69 (m, 4H), 3.93 (s, 2H), 3.96 (m, 1H), 5.09 (q, 1H, J = 6.9 Hz), 7.05 (d, 1H, J = 8.3 Hz), 7.13 (s, 1H), 7.83 (d, 1H, J = 8.3 Hz); MS (FAB) m/z 310 (MH^+); HRMS (FAB) calcd for $C_{14}H_{20}N_3O_5$ (MH^+) 310.1403 found 310.1426.

2-((1*R*)-1-Morpholinocarbonylethoxy)-4-*tert*-butoxycarbonylaminomethylnitrobenzene (21g). Di-*tert*-butyl dicarbonate (36.6 g, 0.167 mol) was added to a solution of 2-((1*R*)-1-morpholinocarbonylethoxy)-4-aminomethylnitrobenzene (47.1 g, 0.152 mol) in ethyl acetate (800 mL), and the mixture was stirred at room temperature for 1 h. After the solvent was removed in vacuo, the residue was dissolved in hot toluene (400 mL). The solution thus obtained was allowed to cool to room temperature, then further cooled to 0 °C and stirred for 2 h. The precipitated crystals were collected by filtration, washed with toluene, and dried at 50 °C in vacuo to give **21g** (58.1 g, 93%, 99.9% ee). The enantiomeric excess was determined by using HPLC on a CHIRALCEL OJ with 1:1 2-propanol/hexane as an eluent at a flow rate of 0.4 mL/min (UV detection, 254 nm). The (*R*)-enantiomer, **21g**, eluted at the retention time of 18.4 min, and the (*S*)-enantiomer, **21h**, eluted at the retention time of 14.8 min: mp 147−148 °C; $[\alpha]^{25}_D$ = −58.7° (*c* 1.0, MeOH); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.68 (d, 3H, J = 6.9 Hz), 3.55−3.37 (m, 3H), 3.69 (m, 4H), 3.91 (m, 1H), 4.32 (d, 2H, J = 6.4 Hz), 5.06 (q, 1H, J = 6.9 Hz), 5.07 (m, 1H), 7.00 (m, 2H), 7.82 (d, 1H, J = 8.6 Hz); MS (FAB) m/z 410 (MH^+); HRMS (FAB) calcd for $C_{19}H_{28}N_3O_7$ (MH^+) 410.1927 found 410.1923.

2-((1*R*)-1-Morpholinocarbonylethoxy)-4-*tert*-butoxycarbonylaminomethylaniline (5g). Compound **21g** (20.0 g, 48.9 mmol) in ethyl acetate (500 mL) was hydrogenated over 10% palladium on carbon (including 50% of water, 5.0 g) at room temperature under an atmospheric pressure of hydrogen for 3 h. The reaction mixture was dried over magnesium sulfate and filtered through a Celite pad, and the filtrate was concentrated in vacuo to give the title compound (18.6 g, 100%): $[\alpha]^{D}_{25}$ = −11.3° (*c* 0.40, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.60 (d, 3H, J = 6.6 Hz), 3.63−3.52 (m, 8H), 3.89 (bs, 2H), 4.14 (d, 2H, J = 7.3 Hz), 4.80 (bs, 1H), 4.98 (q, 1H, J = 6.6 Hz), 6.66 (d, 1H, J = 7.6 Hz), 6.70 (s, 1H), 6.73 (d, 1H, J = 7.6 Hz); LC-MS (ESI) m/z 380 (MH^+); MS (FAB) m/z 379 (M^+); HRMS (FAB) calcd for $C_{19}H_{29}N_3O_5$ (M^+) 379.2107 found 379.2109.

Methyl (3*S*)-7-Chloro-3-(2-((1*R*)-1-morpholinocarbonylethoxy)-4-*tert*-butoxycarbonylaminomethylphenyl)-aminocarbonylmethyl-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylate (22g). Oxalyl chloride (16.9 g, 133 mmol) was added dropwise slowly to a suspension of methyl (3*S*)-(−)-7-chloro-3-carboxymethyl-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylate (3*S*-(−)-**4**, 39.0 g, 127 mmol) in ethyl acetate (390 mL) and DMF (0.196 mL) with stirring at 0 °C, and then the mixture was stirred at room temperature for 2 h. The solvent and the excess oxalyl chloride were removed in vacuo, and the residue was dissolved in ethyl acetate (260 mL) to give a solution of the acid chloride of 3*S*-(−)-**4** in ethyl acetate. To a solution of **5g** (57.7 g, 152 mmol) in ethyl acetate (880 mL) was added triethylamine (26.5 mL, 190 mmol) followed by dropwise addition of the solution obtained above of the acid chloride of 3*S*-(−)-**4** in ethyl acetate with stirring at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for a further 1 h. The reaction mixture was acidified with aqueous 5% $KHSO_4$ and extracted with ethyl acetate. The organic layer was washed with water, saturated aqueous $NaHCO_3$, water, and brine successively, dried over magnesium sulfate, and concentrated in vacuo to give crude product (88.5 g). The crude product thus obtained was dissolved in hot toluene (450 mL). The resulting solution was cooled gradually and stirred at room temperature. The precipitated crystals were collected by filtration, washed with toluene, and dried in vacuo at 40 °C to give the title compound (83.0 g, 98%): mp 189−190 °C, dec; $[\alpha]^{25}_D$ = −68.4° (*c* 0.23, MeOH); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.57 (d, 3H, J = 6.6 Hz), 2.04 (m, 1H), 2.29 (m, 1H), 2.59 (dd, 1H, J = 14.0, 10.1 Hz), 2.84 (m, 2H), 3.12 (m, 1H), 3.43 (m, 2H), 3.59 (m, 6H), 3.83 (s, 3H), 4.03 (m, 1H), 4.23 (m, 2H), 4.88 (m, 1H), 4.95 (q, 1H, J = 6.6 Hz), 6.86 (m, 2H), 6.93 (d, 1H, J = 8.3 Hz), 7.18 (s, 1H), 8.36 (d, 1H, J = 8.3 Hz), 8.82 (s, 1H), 9.02 (s, 1H); MS (FAB) m/z 669 (MH^+); HRMS (FAB) calcd for $C_{34}H_{42}N_4O_8Cl$ (MH^+) 669.2691 found 669.2724.

Methyl (3*S*)-7-Chloro-3-(2-((1*R*)-1-carboxyethoxy)-4-*tert*-butoxycarbonylaminomethylphenyl)aminocarbonylmethyl-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylate. To a solution of **22g** (86.4 g, 129 mmol) in a mixed solvent of THF (440 mL) and methanol (440 mL) was added aqueous 2 N LiOH (440 mL, 880 mmol), and the mixture was stirred at room temperature for 16 h. The organic solvents were removed in vacuo, and the resulting aqueous layer was washed with toluene (three times), acidified with aqueous 13% $KHSO_4$ to pH 2 and extracted with 3:1 ethyl acetate/THF three times. The organic layers were combined, washed with water and brine successively, treated with activated charcoal and magnesium sulfate, and filtered. The filtrate was concentrated in vacuo to give crude product (73.1 g). The crude product (72.0 g) was suspended in acetonitrile (360 mL), heated under reflux, cooled gradually, and stirred at room temperature for 1 h. The crystals formed were collected by filtration, washed with ice-cooled acetonitrile, and dried in vacuo to give the title compound (70.3 g, 94%): mp 228−229 °C; $[\alpha]^{25}_D$ = −61.6° (*c* 0.22, MeOH); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.56 (d, 3H, J = 6.6 Hz), 1.85 (m, 1H), 2.13 (m, 1H), 2.81−2.62 (m, 3H), 3.10 (m, 1H), 3.87 (m, 1H), 4.04 (m, 2H), 4.75 (q, 1H, J = 6.6 Hz), 6.84 (m, 3H), 7.15 (s, 1H), 7.31 (m, 1H), 7.89 (d, 1H, J = 8.3 Hz), 9.07 (s, 1H), 11.42 (s, 1H), 12.99 (bs, 2H); MS (FAB) m/z 586 (MH^+); HRMS (FAB) calcd for $C_{29}H_{33}N_3O_8Cl$ (MH^+) 586.1956 found 586.1956.

(3*S*)-7-Chloro-3-(2-((1*R*)-1-carboxyethoxy)-4-aminomethylphenyl)aminocarbonylmethyl-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylic Acid Hydrochloride (3g). To a solution of methyl (3*S*)-7-chloro-3-(2-((1*R*)-1-carboxyethoxy)-4-*tert*-butoxycarbonylaminomethylphenyl)aminocarbonylmethyl-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylate (70.0 g, 119 mmol) in acetic acid (280 mL) was added 1 N HCl in acetic acid (240 mL, 240 mmol) at room temperature, and the mixture was stirred at the same temperature for 2 h. Toluene (1.04 L) was added and stirring was continued for 30 min. The precipitated solid was collected by filtration, washed with toluene, and dried in vacuo to give the title compound (61.2 g, 99%) as an amorphous solid. The enantiomeric excess was determined to be 99.9% ee by chiral HPLC. Chiral HPLC conditions: column, SUMICHIRAL OA-2500; mobile phase, acetonitrile/5 mM aqueous 1-heptanesulfonic acid, sodium salt (adjusted to pH 2.5 with H_3PO_4) (27:73); flow rate: 1.0 mL/min; UV detection, 240 nm; t_R for (−)-enantiomer **3g**, 26.3 min; (+)-enantiomer, 30.5 min. The diastereomeric excess and the purity were determined to be 99.9% de and 98.8 area %, respectively, by reverse phase HPLC. HPLC conditions: column, SUMIPAX ODS A-212; mobile phase, acetonitrile/5 mM aqueous 1-heptanesulfonic acid, sodium salt (adjusted to pH 2.5 with H_3PO_4) (33: 66 for 20 min, then gradient

to 95:5 over 20 min); flow rate: 1.0 mL/min; UV detection, 240 nm; t_R for **3g** and its enantiomer, 16.2 min; diastereomers (**3h** and its enantiomer), 18.0 min. Although the material obtained above was pure enough to use for pharmacological tests, **3g** could also be recrystallized from 1:10 water/2-propanol to give white crystals as monohydrate: mp 210−214 °C dec (for amorphous), 231−237 °C, dec (for crystals); $[\alpha]^{25}_D = -68.7$ (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56 (d, 3H, J = 6.6 Hz), 1.87 (m, 1H), 2.10 (m, 1H), 2.60−2.80 (m, 3H), 3.08 (m, 1H), 3.89 (m, 1H), 3.93 (s, 2H), 4.84 (q, 1H, J = 6.6 Hz), 6.83 (s, 1H), 7.06 (d, 1H, J = 8.1 Hz), 7.15 (s, 1H), 7.19 (s, 1H), 8.02 (d, 1H, J = 8.1 Hz), 8.34 (bs, 3H), 9.22 (s, 1H), 11.45 (s, 1H); Anal. ($C_{24}H_{25}N_3O_6Cl_2 \cdot H_2O$): C, H, N (for crystals).

2-((1*S*)-1-Morpholinocarbonylethoxy)-4-*tert*-butoxycarbonylaminomethylaniline (5h). The title compound was prepared from methyl (*R*)-(+)-lactate by the procedure similar to that described above in the preparation of **5g**: $[\alpha]^D_{25}$ = +11.2° (*c* 0.40, $CHCl_3$); The other spectral properties of the title compound were identical with those of **5g**.

(3*S*)-7-Chloro-3-(2-((1*S*)-1-carboxyethoxy)-4-aminomethylphenyl)aminocarbonylmethyl-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylic Acid Hydrochloride (3h). The title compound was prepared from 3*S*-(−)-**4** and **5h** by the procedure similar to that described above in synthesis of **3g**: mp 204−206 °C, dec; $[\alpha]^{25}_D = -34.0°$ (*c* 0.22, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.57 (d, 3H, J = 6.8 Hz), 1.84 (m, 1H), 2.07 (m, 1H), 2.67−2.81 (m, 3H), 3.10 (m, 1H), 3.86 (m, 1H), 3.93 (s, 2H), 4.81 (q, 1H, J = 6.4 Hz), 6.84 (s, 1H), 7.05 (d, 1H, J = 8.2 Hz), 7.15 (s, 1H), 7.17 (s, 1H), 7.99 (d, 1H, J = 8.2 Hz), 8.32 (bs, 3H), 9.31 (s, 1H), 11.45 (s, 1H); Anal. ($C_{24}H_{27}N_3O_7Cl_2 \cdot {}^6/_5H_2O$): C, H, N.

Methyl (2-Aminophenyl)acetate (5d). A procedure similar to that described in synthesis of **5g** from **21g** was carried out with methyl (2-nitrophenyl)acetate (**6**, 174 mg, 0.893 mmol) to give the title compound (148 mg, 100%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.57 (s, 2H), 3.69 (s, 3H), 4.06 (bs, 2H), 6.70−6.78 (m, 2H), 7.07−7.13 (m, 2H); MS (FAB) *m*/*z* 165 (M^+); HRMS (FAB) calcd for $C_9H_{11}NO_2$ (M^+) 165.0790 found 165.0781.

Methyl 7-Chloro-(3*S*)-3-(2-{[2-(2-methoxy-2-oxoethyl)-phenyl]amino}-2-oxoethyl)-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylate (22d). To a solution of methyl (3*S*)-(−)-7-chloro-3-carboxymethyl-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylate (3*S*-(−)-**4**, 36.5 mg, 0.125 mmol), **5d** (20.8 mg, 0.126 mmol), and triethylamine (53 μL, 0.38 mmol) in dichloromethane (1.5 mL) was added *N*,*N*-bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (35.3 mg, 0.139 mmol) followed by stirring at room temperature for 16 h. The reaction mixture was acidified with aqueous 5% $KHSO_4$ and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography with 6:1 chloroform/ethyl acetate to give the title compound (45.2 mg, 79%): mp 215−218 °C; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.79−1.94 (m, 1H), 2.07−2.15 (m, 1H), 2.55−2.66 (m, 2H), 2.78−2.87 (m, 1H), 3.01−3.14 (m, 1H), 3.60 (s, 3H), 3.72 (s, 2H), 3.85 (s, 3H), 3.86 (m, 1H), 6.89 (s, 1H), 7.14−7.19 (m, 2H), 7.25−7.29 (m, 2H), 7.41 (d, 1H, J = 7.6 Hz), 9.36 (s, 1H), 11.61 (s, 1H); LC-MS (ESI) *m*/*z* 455 (MH^+); MS (FAB) *m*/*z* 455 (MH^+); HRMS (FAB) calcd for $C_{24}H_{24}N_2O_5Cl$ (MH^+) 455.1374 found 455.1372.

(3*S*)-3-(2-{[2-(Carboxymethyl)phenyl]amino}-2-oxoethyl)-7-chloro-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylic Acid (3d). To a solution of **22d** (37.4 mg, 0.0822 mmol) in 1,2-dimethoxyethane (3.0 mL) was added aqueous 1 N NaOH (1.50 mL, 1.50 mmol), and the mixture was stirred at room temperature for 4 h. The reaction mixture was acidified with aqueous 5% $KHSO_4$ and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo to give the title compound (35.1 mg, 100%): mp 211−213 °C, dec; $[\alpha]^{25}_D = -87.2°$ (*c* 0.03, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.78−1.91 (m, 1H), 2.04−2.13 (m, 1H), 2.55−2.70 (m, 2H), 2.74−2.85 (m, 1H), 3.01−3.15 (m, 1H), 3.62 (s, 2H), 3.87 (m, 1H), 6.86 (s, 1H), 7.12−7.17 (m, 2H), 7.23−7.28 (m, 2H), 7.45 (d, 1H, J = 7.9 Hz), 9.38 (s, 1H), 11.44 (s, 1H), 12.52 (bs, 2H); MS (FAB) *m*/*z* 427 (MH^+); HRMS (FAB) calcd for $C_{22}H_{20}N_2O_5Cl$ (MH^+) 427.1061 found 427.1047; Anal. ($C_{22}H_{19}N_2O_5Cl \cdot {}^1/_5H_2O$): C, H, N.

2-(4-Nitrobenzyl)-1*H*-isoindole-1,3(2*H*)-dione (8). A procedure similar to that described in synthesis of **20g** from **18g** was carried out with 4-nitrobenzyl alcohol (**7**, 2.24 g, 14.6 mmol) to give 3.76 g of the title compound (91% from 4-nitrobenzyl alcohol): mp 171−174 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.93 (s, 2H), 7.59 (d, 2H, J = 8.6 Hz), 7.73−7.78 (m, 2H), 7.84−7.91 (m, 2H), 8.18 (d, 2H, J = 8.6 Hz); LC-MS (ESI) *m*/*z* 283 (MH^+); MS (FAB) *m*/*z* 283 (MH^+); HRMS (FAB) calcd for $C_{15}H_{11}N_2O_4$ (MH^+) 283.0719 found 283.0732.

***tert*-Butyl 4-Nitrobenzylcarbamate (9).** A procedure similar to that described in synthesis of **21g** from **20g** was carried out with **8** (772 mg, 2.74 mmol) to give 600 mg of the title compound (87% from **8**): mp 105−108 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.46 (s, 9H), 4.41 (d, 2H, J = 5.9 Hz), 5.00 (bm, 1H), 7.44 (d, 2H, J = 8.6 Hz), 8.19 (d, 2H, J = 8.6 Hz); LC-MS (ESI) *m*/*z* 253 (MH^+); MS (FAB) *m*/*z* 253 (MH^+); HRMS (FAB) calcd for $C_{12}H_{17}N_2O_4$ (MH^+) 253.1188 found 253.1181.

***tert*-Butyl 4-Aminobenzylcarbamate (5e).** A procedure similar to that described in synthesis of **5g** from **21g** was carried out with **9** (134 mg, 0.533 mmol) to give the title compound (118 mg, 99%): mp 60−64 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.45 (s, 9H), 3.64 (bs, 2H), 4.18 (d, 2H, J = 5.6 Hz), 4.71 (bm, 1H), 6.64 (d, 2H, J = 8.6 Hz), 7.07 (d, 2H, J = 8.6 Hz); LC-MS (ESI) *m*/*z* 223 (MH^+); MS (FAB) *m*/*z* 223 (MH^+); HRMS (FAB) calcd for $C_{12}H_{19}N_2O_2$ (MH^+) 223.1447 found 223.1461.

Methyl (3*S*)-3-{2-[(4-{[(*tert*-Butoxycarbonyl)amino]-methyl}phenyl)amino]-2-oxoethyl}-7-chloro-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylate (22e). To a solution of 3*S*-(−)-**4** (50.0 g, 0.162 mmol) and **5e** (40.0 mg, 0.179 mmol) in DMF (1.0 mL) were added 1-hydroxybenzotriazole (30.0 mg, 0.195 mmol) and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (37 mg, 0.195 mmol), and the mixture was stirred at room temperature for 20 h. The resulting mixture was acidified with aqueous 5% $KHSO_4$, and extracted with 1:1 toluene/ethyl acetate. The organic layer was washed successively with water, aqueous 5% $NaHCO_3$, and brine, dried over magnesium sulfate, and concentrated in vacuo to give the title compound (100 mg) as a crude material. This product was used in the following reaction without further purification: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.05 (m, 1H), 2.30 (m, 1H), 2.52 (dd, 1H, J = 13.7, 9.2 Hz), 2.71 (dd, 1H, J = 13.7, 4.3 Hz), 2.88 (m, 1H), 3.01 (m, 1H), 3.90 (s, 3H), 3.95 (m, 1H), 4.27 (d, 2H, J = 5.3 Hz), 4.81 (bm, 1H), 6.91 (s, 1H), 7.20 (s, 1H), 7.24 (d, 2H, J = 8.4 Hz), 7.50 (d, 2H, J = 8.4 Hz), 7.79 (s, 1H), 8.60 (s, 1H); LC-MS (ESI) *m*/*z* 512 (MH^+); MS (FAB) *m*/*z* 512 (MH^+); HRMS (FAB) calcd for $C_{27}H_{31}N_3O_5Cl$ (MH^+) 512.1952 found 512.1961.

(3*S*)-3-{2-[(4-{[(*tert*-Butoxycarbonyl)amino]methyl}-phenyl)amino]-2-oxoethyl}-7-chloro-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylic Acid. To a solution of **22e** (100 mg) obtained above in a mixed solvent of THF (2.0 mL) and methanol (2.0 mL) was added aqueous 2 N NaOH (2.0 mL, 4.0 mmol), and the mixture was stirred at room temperature for 21 h. The reaction mixture was acidified with aqueous 5% $KHSO_4$, and the precipitated crystals were collected by filtration and dried in vacuo at 40 °C to give the title compound (72.0 mg, 89% from 3*S*-(−)-**4**): mp 213−218 °C, dec; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.39 (s, 9H), 1.85 (m, 1H), 2.04 (m, 1H), 2.49 (m, 1H), 2.67 (dd, 1H, J = 14.5, 3.6 Hz), 2.79 (m, 1H), 3.05 (m, 1H), 3.90 (m, 1H), 4.03 (d, 2H, J = 6.4 Hz), 6.84 (s, 1H), 7.16 (d, 2H, J = 8.4 Hz), 7.33 (t, 1H, J = 6.4 Hz), 7.52 (d, 2H, J = 8.4 Hz), 9.86 (s, 1H), 11.41 (s, 1H), 12.91 (bs, 1H); LC-MS (ESI) *m*/*z* 498 (MH^+); MS (FAB) *m*/*z* 498 (MH^+); HRMS (FAB) calcd for $C_{26}H_{29}N_3O_5Cl$ (MH^+) 498.1796 found 498.1807.

(3*S*)-3-(2-{[4-(Aminomethyl)phenyl]amino}-2-oxoethyl)-7-chloro-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylic Acid Hydrochloride (3e). To a suspension of 3-{2-[(4-{[(*tert*-butoxycarbonyl)amino]methyl}phenyl)amino]-2-oxoethyl}-7-chloro-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylic acid (65.0

mg, 0.130 mmol) in a mixture of 1,4-dioxane (3.0 mL) and acetic acid (2.0 mL) was added 4 N HCl in 1,4-dioxane (4.0 mL, 16 mmoL). The mixture was stirred at room temperature for 3 h and concentrated. The residual solid was rinsed with diethyl ether, collected by filtration, and dried in vacuo at 40 °C to give **3e** (56.0 mg, 99% yield): mp 206−214 °C, dec; $[\alpha]^{25}_D$ = −56.0° (*c* 0.07, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.86 (m, 1H), 2.04 (m, 1H), 2.51 (dd, 1H, *J* = 14.5, 11.2 Hz), 2.68 (dd, 1H, *J* = 14.5, 3.6 Hz), 2.79 (m, 1H), 3.07 (m, 1H), 3.89 (m, 1H), 3.96 (s, 2H,), 6.83 (s, 1H), 7.16 (s, 1H), 7.39 (d, 2H, *J* = 8.4 Hz), 7.64 (d, 2H, *J* = 8.4 Hz), 8.23 (bs, 3H), 10.05 (s, 1H), 11.43 (s, 1H), 12.89 (bs, 1H); MS (FAB) *m/z* 398 (MH^+ − HCl); Anal. ($C_{21}H_{21}N_3O_3Cl_2 \cdot {}^3/_2H_2O$): C, H, N.

***tert*-Butyl (5-Iodo-2-nitrophenyl)acetate (11).** To a suspension of potassium *tert*-butoxide (56.3 g, 0.508 mol) in DMF (1.00 L) with stirring at 0 °C was added dropwise a solution of 1-iodo-4-nitrobenzene (**10**, 50.0 g, 0.201 mol) and *tert*-butyl chloroacetate (33.3 g, 0.221 mol) in DMF (500 mL), and the mixture was stirred at 0 °C for 6 h. The resulting mixture was acidified with aqueous 5% $KHSO_4$ and extracted with 1:1 toluene/ethyl acetate. The organic layer was washed successively with water and brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography with 50:1 and then 40:1 hexane/ethyl acetate to give the title compound (37.2 g, 51%): mp 89−94 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.44 (s, 9H), 3.89 (s, 2H), 7.72 (s, 1H), 7.81 (s-like, 2H); LC-MS (ESI) *m/z* 364 (MH^+), 290 (M^+ − *t*-BuO); MS (FAB) *m/z* 364 (MH^+); HRMS (FAB) calcd for $C_{12}H_{15}NO_4I$ (MH^+) 364.0045 found 364.0040.

***tert*-Butyl (5-Cyano-2-nitrophenyl)acetate (12).** To a solution of **11** (36.2 g, 99.7 mmol) in DMF (350 mL) were added 60% zinc cyanide (11.7 g, 59.8 mmol) and tetrakis(triphenylphosphine)palladium(0) (6.91 g, 5.98 mmol), and the mixture was stirred at 80 °C for 3 h. After cooling to room temperature, water was added and the resulting mixture was extracted with 1:1 toluene/ethyl acetate. The organic layer was washed successively with water and brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography with 20:1 to 5:1 hexane/ethyl acetate to give the title compound (22.0 g, 84%): mp 88−90 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.44 (s, 9H), 3.98 (s, 2H), 7.67 (d, 1H, *J* = 1.7 Hz), 7.78 (dd, 1H, *J* = 8.2, 1.7 Hz), 8.17 (d, 1H, *J* = 8.2 Hz); LC-MS (ESI) *m/z* 263 (MH^+); MS (FAB) *m/z* 263 (MH^+); HRMS (FAB) calcd for $C_{13}H_{15}N_2O_4$ (MH^+) 263.1032 found 263.1016.

***tert*-Butyl (5-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-nitrophenyl)acetate (13).** To a solution of **12** (8.0 g, 30.5 mmol) and trimethyl borate (30.0 mL, 268 mmol) in THF (160 mL) was added dropwise 1.0 M borane−THF complex in THF solution (46.0 mL, 46.0 mmol), and the mixture was stirred at room temperature for 5 h. Methanol (50 mL) was added dropwise, and the resulting mixture was concentrated in vacuo. The residue was dissolved in THF (100 mL), to the solution was added di-*tert*-butyl dicarbonate (8.4 mL, 36.6 mmol), and the mixture was stirred at room temperature for 15 h. After the solvent was removed in vacuo, the residue was purified by silica gel column chromatography with 5:1 hexane/ethyl acetate to give the title compound (5.60 g, 50%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.46 (s, 9H), 3.93 (s, 2H), 4.37 (d, 2H, *J* = 7.3 Hz), 5.04 (bs, 1H), 7.23 (s, 1H), 7.35 (d, 1H, *J* = 8.6 Hz), 8.08 (d, 1H, *J* = 8.6 Hz).

***tert*-Butyl (2-Amino-5-{[(*tert*-butoxycarbonyl)amino]methyl}phenyl)acetate (5f).** A procedure similar to that described in synthesis of **5g** from **21g** was carried out with **13** (5.20 g, 14.2 mmol) to give the title compound (4.78 g, 100%): mp 77−80 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.45 (s, 9H), 3.44 (s, 2H), 4.05 (bs, 2H), 4.17 (d, 2H, *J* = 5.6 Hz), 4.70 (bs, 1H), 6.66 (d, 1H, *J* = 8.3 Hz), 6.98 (s, 1H), 7.00 (d, 1H, *J* = 8.3 Hz); LC-MS (ESI) *m/z* 337 (MH^+); MS (FAB) *m/z* 336 (M^+); HRMS (FAB) calcd for $C_{18}H_{28}N_2O_4$ (M^+) 336.2049 found 336.2029.

Methyl (3*S*)-3-(2-{[4-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-(2-*tert*-butoxy-2-oxoethyl)phenyl]amino}-2-oxoethyl)-7-chloro-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylate (22f). A procedure similar to that described in synthesis of **22d** was carried out with **5f** (3.94 g, 11.7 mmol) and 3*S*-(−)-**4** (3.00 g, 9.75 mmol) to give the title compound (4.32 g, 71%): mp 156−161 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.39 (s, 9H), 1.46 (s, 9H), 2.08 (m, 1H), 2.33 (m, 1H), 2.55 (dd, 1H, *J* = 13.9, 10.4 Hz), 2.85 (m, 2H), 3.10 (m, 1H), 3.47 (s, 2H), 3.93 (s, 3H), 4.04 (m, 1H), 4.26 (d, 2H, *J* = 5.6 Hz), 4.80 (bm, 1H), 6.90 (s, 1H), 7.12 (s, 1H), 7.19−7.23 (m, 2H), 7.90 (d, 1H, *J* = 8.3 Hz), 8.67 (s, 1H), 8.93 (s, 1H); LC-MS (ESI) *m/z* 626 (MH^+); MS (FAB) *m/z* 626 (MH^+); HRMS (FAB) calcd for $C_{33}H_{41}N_3O_7Cl$ (MH^+) 626.2633 found 626.2629.

(3*S*)-3-(2-{[4-(Aminomethyl)-2-(carboxymethyl)phenyl]amino}-2-oxoethyl)-7-chloro-1,3,4,5-tetrahydrobenz[*cd*]indole-2-carboxylic Acid Hydrochloride (3f). A procedure similar to that described in synthesis of **3e** from **22e** was carried out with **22f** (4.32 g. 6.90 mmol) to give the title compound (3.04 g, 98%): mp 217−218 °C, dec; $[\alpha]^{25}_D$ = −78.6° (*c* 0.21, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.82 (m, 1H), 2.09 (md, 1H, *J* = 12.9 Hz), 2.60 (m, 2H), 2.80 (md, 1H, *J* = 17.2 Hz), 3.11 (mt, 1H, *J* = 14.5 Hz), 3.60 (d, 1H, *J* = 16.5 Hz), 3.68 (d, 1H, *J* = 16.5 Hz), 3.89 (m, 1H), 3.97 (bs, 2H), 6.83 (s, 1H), 7.16 (s, 1H), 7.35 (s, 1H), 7.37 (d, 1H, *J* = 8.3 Hz), 7.52 (d, 1H, *J* = 8.3 Hz), 8.35 (bs, 3H), 9.49 (s, 1H), 11.44 (s, 1H); Anal. ($C_{23}H_{23}N_3O_5Cl_2 \cdot {}^6/_5H_2O$): C, H, N.

Acknowledgment. We are grateful to Prof. Y. Yoneda of Kanazawa University for ligand binding assays. We also wish to thank Messrs. K. Ohtani and H. Yasuda for pharmacological assays ([^{3}H] SM-18400 binding and rat MCAo model). We appreciate Mr. Y. Saito for pharmacokinetic study and Dr. K. Yanagi for discussion concerning X-ray crystallography. We acknowledge Drs. H. Kishimoto, A. Dan, W. E. Hume, Messrs. T. Toyoda, N. Sawada, T. Umezome, T. Tokunaga, and K. Shimago for their valuable discussions and contributions to the synthetic study. We thank Ms. Y. Saida for her technical assistance in biological evaluations. We also appreciate Dr. M. Nakamura for his helpful discussion of the pharmacology.

Supporting Information Available: X-ray crystallographic analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM020239L