Articles

Selective NR1/2B *N*-Methyl-D-aspartate Receptor Antagonists among Indole-2-carboxamides and Benzimidazole-2-carboxamides

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(4-Benzylpiperidine-1-yl)-(6-hydroxy-1*H*-indole-2-yl)-methanone (**6a**) derived from (*E*)-1-(4-benzylpiperidin-1-yl)-3-(4-hydroxy-phenyl)-propenone (**5**) was identified as a potent NR2B subunit-selective antagonist of the NMDA receptor. To establish the structure−activity relationship (SAR) and to attempt the improvement of the ADME properties of the lead, a series of compounds were prepared and tested. Several derivatives showed low nanomolar activity both in the binding and in the functional assay. In a formalin-induced hyperalgesia model in mice, **6a** and (4-benzylpiperidine-1-yl)-[5(6)-hydroxy-1*H*-benzimidazol-2-yl]-methanone (**60a**) were as active as besonprodil (**2**) after oral administration. A CoMSIA model was developed based on binding data of a series of indole- and benzimidazole-2-carboxamides.

Introduction

There is a high unmet medical need for new drugs in the therapy of neuropathic pain. The principal cause of this fact is that until recently little was known of the mechanisms underlying the various neuropathic pain conditions. The current armamentarium of pharmacotherapy for neuropathic pain includes tricyclic antidepressants, anticonvulsants, opioids, and unconventional agents like topical capsaicin. Suboptimal therapeutic efficacies and/or side effects are a serious limitation to the use of these medications.[1] Several compounds with novel mechanisms of action are being studied to address this problem. The already marketed gabapentin and its follow-up compound pregabalin bind with high affinity to the $\alpha_2\delta$ accessory protein of voltage-gated calcium channels widely distributed in the central nervous system. Noradrenaline and serotonin reuptake inhibitors, cannabinoid receptor agonists, and *N*-methyl-D-aspartate (NMDA) receptor antagonists are also the focus of current attention.[2] The NMDA receptor is heteromeric and its activity may be modulated by interaction at one of several different binding sites. One of the most promising targets of this receptor is the so-called ifenprodil binding site, situated on the NR2B receptor subtype.[3] Substantial effort has already been invested in finding potent, selective, and drug-like NR2B antagonists.[4] Moreover, a recent clinical trial with **1** (traxoprodil, (+) CP-101,606)[5] provided additional impetus by demonstrating the attenuation of neuropathic pain by NR2B subtype selective NMDA antagonists in humans.[6]

A substantial proportion of the known NR2B subtype selective NMDA antagonists are 1,4-disubstituted piperidines like **1**, **2** (besonprodil, CI-1041),[7] **3** (Ro-25−6981),[8] or **4**.[9] There are, however, examples of potent NR2B antagonists in which the piperidine nitrogen is not basic, for example, in compound **5**, indicating that the basic nitrogen is not a condition of activity[10] (Figure 1). Numerous endogenous ligands of important receptors in the CNS contain basic nitrogen, and basic nitrogen is an important feature of the pharmacophore of several agents acting on ion channels (e.g., hERG channels) as well. This may result in the recognition of basic nitrogen containing exogenous ligands by several of these receptors and ion channels and as a consequence in off-target activities. The elimination of potential off-target activities from lead structures is an important issue in the NR2B antagonist field, too.[11] To avoid or at least mitigate possible adverse side effects, the nonbasic cinnamide derivative **5** was selected as a chemical starting point of our research aimed at identifying novel, potent NR2B subtype-selective NMDA receptor antagonists for the treatment of neuropathic pain. The IC_{50} value of compound **5** for the inhibition of NMDA evoked increase of intracellular Ca^{2+} level in rat primary cortical cell culture was 131 nM, as measured in our laboratories. One of our first modifications of this structure was the incorporation of an NH group between the α carbon atom and the benzene ring of the cinnamic acid part of the molecule, resulting in a 6-hydroxyindole-2-carboxamide derivative, compound **6a**, with more than 6-fold higher potency (IC_{50}: 18 nM). Herein we elaborate the structure−activity relationship (SAR) around indole-2-carboxamide **6a**. Our investigations were aimed at clarifying the influence of the following features in general formula **A** on the biological activity: a, nature and position of the H-bond donor moiety **Q**; b, nature of **X**; c, position of the substituent of the piperidine ring; d, nature of spacer **Y**; e, the nature and position of **Z**; and f, possible substitution of the pyrrole ring in the indole for other carbo- or heterocycles (**V** and **W**). The goal of the lead optimization was to increase the potency and to improve the drug-likeness, that is, the ADME properties of the compounds. To achieve these goals, a series of analogues of compound **6a** were prepared and tested in vitro and some representative members of the series in vivo as well (Figure 2).

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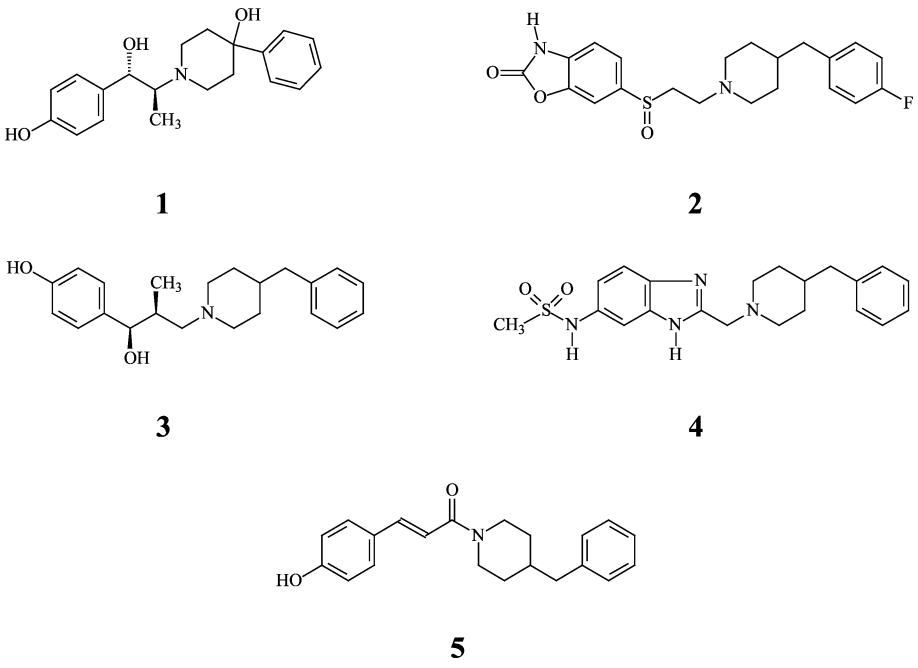


Figure 1. Structures of NR2B selective NMDA receptor antagonists.

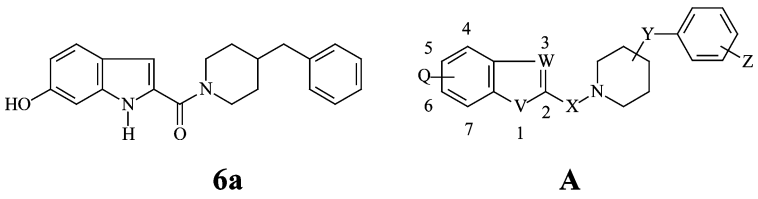
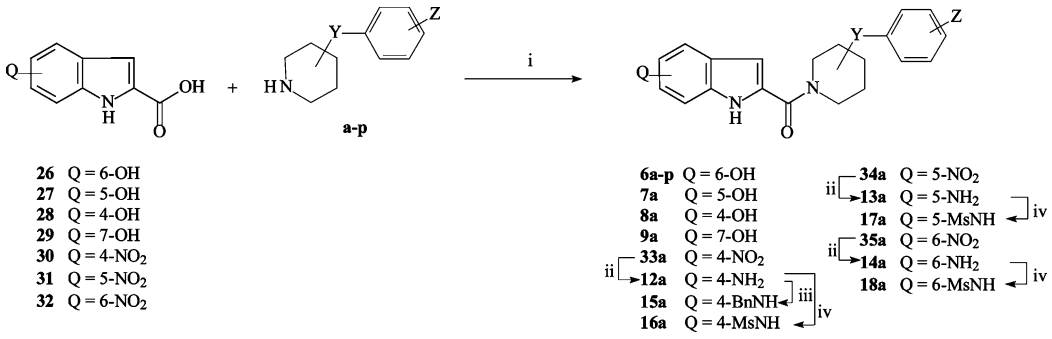


Figure 2. Lead compound (**6a**) and the general formula of the prepared analogues.

Scheme 1[a]



[a] Reagents and conditions: (i) HBTU, TEA, DMF, 0 °C−rt, 16 h; (ii) H_2, 10% Pd/C, MeOH, rt; (iii) PhCHO, $Na(AcO)_3BH_3$, AcOH, DCE, rt, 2 h; (iv) MsCl, TEA, $CHCl_3$, rt, 10 h.

Based on the data generated among indole- and benzimidazole-2-carboxamides, a CoMSIA model was constructed for the facilitation of the design of further, more potent compounds. Preliminary results of this work were published earlier.[12]

Chemistry

Compounds **6a**−**6p**, **7a**−**9a**, **19**−**22**, and **33a**−**35a** were prepared from appropriately substituted indole-2-carboxylic acids (**26**−**32**), which were coupled to piperidines (**a**−**p**) using a standard amide coupling reaction.[13] Nitroindolecarboxamides (**33a**−**35a**) were transformed via the corresponding amino derivatives (**12a**−**14a**) to benzylamino derivative **15a** or to methansulfonamides (**16a**−**18a**) with standard procedures (Scheme 1).

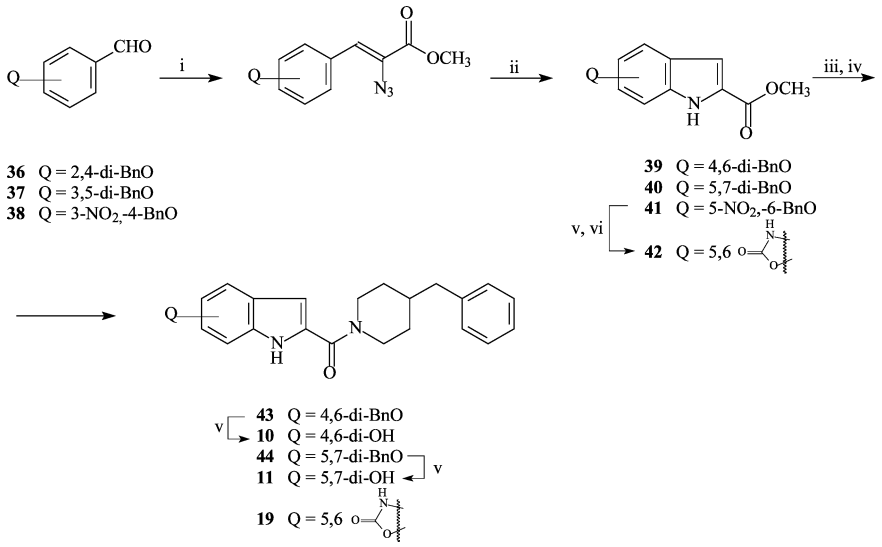
The preparation of some analogues (**43**, **44**, and **19**) was started with Hemetsberger−Knittel indole synthesis.[14] The dihydroxy derivatives (**10** and **11**) were prepared from the corresponding dibenzyloxy derivatives (**43** and **44**) by catalytic hydrogenolysis (Scheme 2).

A related series of heterotricyclic carboxamides (**20**−**22**) were synthesized according to the Fischer/Japp−Klingemann procedure[15] (Scheme 3).

Amidine **23** was prepared from 6-benzyloxy-1*H*-indole-2-carboxylic acid (**51**) via the corresponding nitrile (**52**) using a Pinner-type procedure[16] (Scheme 4).

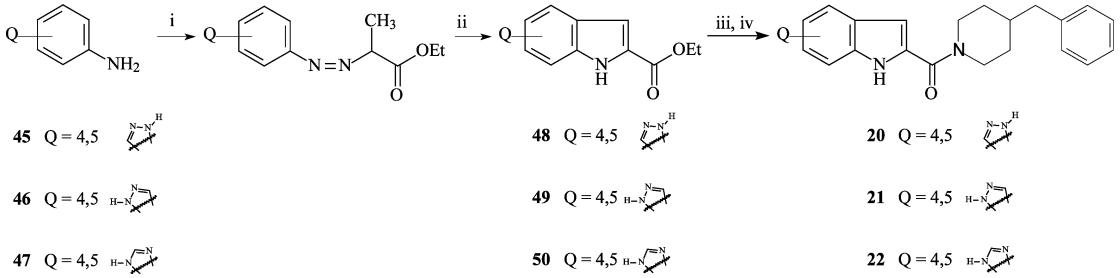
Compound **24** was prepared by reducing carboxamide **6a** with $LiAlH_4$ (Scheme 5).

Scheme 2[a]



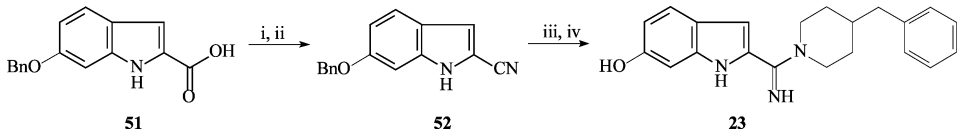
[a] Reagents and conditions: (i) methyl azidoacetate, NaOMe, MeOH, 0 °C, 4 h; (ii) heating in xylene; (iii) **38**, **39**: $KOSi(CH_3)_3$, THF, reflux, 1 h; **41**: KOH, EtOH, reflux; (iv) 4-benzylpiperidine, HBTU, TEA, DMF, 0 °C−rt, 16 h; (v) H_2, 10% Pd/C, MeOH, rt; (vi) ClCOCl, THF, TEA, rt.

Scheme 3[a]



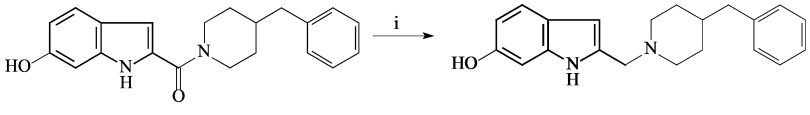
[a] Reagents and conditions: (i) (1) $NaNO_2$, H_2O, HCL, 0 °C; (2) KOH, NaOAc, EtOH, MeCOCH(Me)COOEt, 0 °C; (ii) PPA, 120 °C; (iii) KOH, EtOH, reflux; (iv) 4-benzylpiperidine, HBTU, TEA, DMF.

Scheme 4[a]



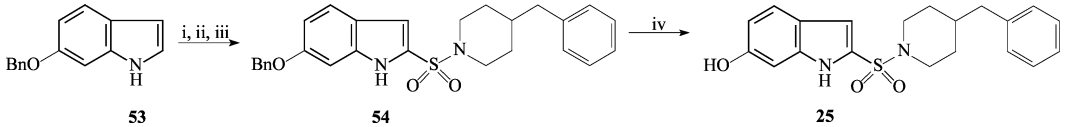
[a] Reagents and conditions: (i) (1) $SOCl_2$, $CHCl_3$, reflux, 1 h; (2) NH_3; (ii) $POCl_3$ $CHCl_3$, reflux, 2 h; (iii) HCl, MeOH, 5 h; (iv) 4-benzylpiperidine.

Scheme 5[a]



[a] Reagents and conditions: (i) $LiAlH_4$, THF, rt, 1 h.

Scheme 6[a]

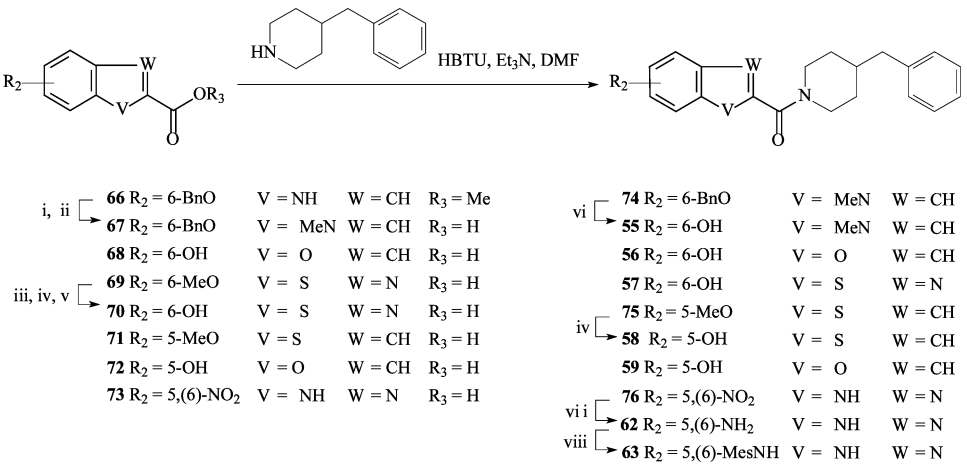


[a] Reagents and conditions: (i) *t*-Boc_2O, DMAP, DCM, rt, 2 h; (ii) (1) *n*-BuLi, SO_2, THF, −70 °C−rt; (2) NCS, DCM, 5 °C; (iii) 4-benzylpiperidine, rt, 24 h; (iv) H_2, 10% Pd/C, MeOH, rt.

The synthesis of sulfonamide **25** was started from 6-benzyloxy-1*H*-indole (**53**). Lithiation was followed by the formation of sulfonamide **54** in a one-pot procedure. Then hydrogenolysis resulted in compound **25** (Scheme 6).

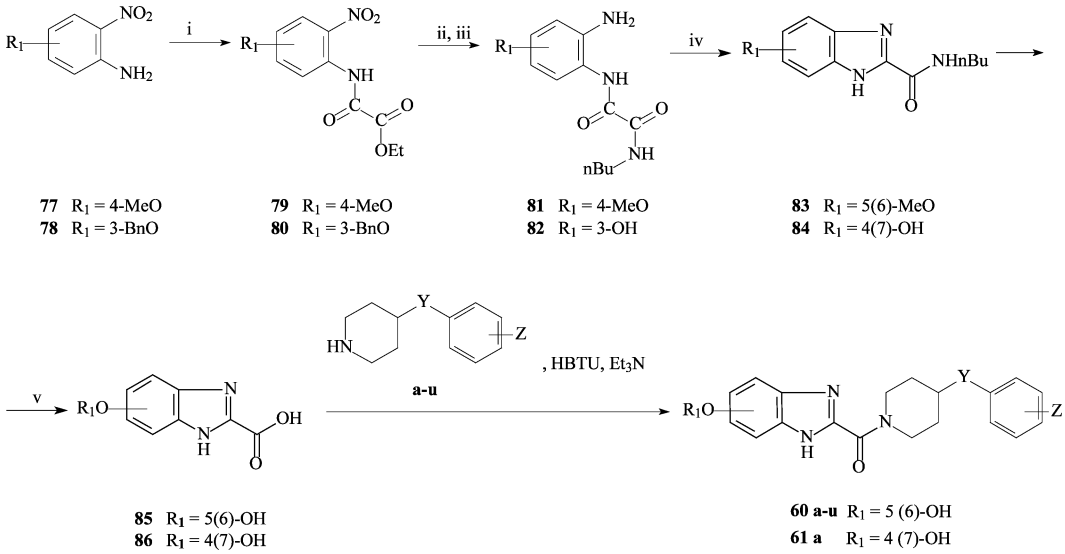
Piperidines used in the above syntheses were either commercially available or prepared by procedures known from the literature. 4-Phenoxypiperidines were prepared by reacting 4-chloropyridine with phenols, and then the obtained pyridine

Scheme 7[a]



[a] Reagents and conditions: (i) MeI, KO*t*-Bu, DMF, rt, 24 h; (ii) $KOSi(CH_3)_3$, THF, reflux, 1 h; (iii) $SOCl_2$, MeOH, 0 °C−rt, 10 h; (iv) BBr_3, DCM, −15 °C−rt, 10 h; (v) KOH, MeOH, reflux 1 h; (vi) 33% HBr−AcOH, 10 min; (vii) H_2, 10% Pd/C, MeOH, rt; (viii) MesCl, pyridine, DCM, rt, 5 h.

Scheme 8[a]



[a] Reagents and conditions: (i) ClCOCOOEt, Et_3N, CH_2Cl_2, 0 °C−rt; (ii) *n*-$BuNH_2$, toluene, rt, 10 h; (iii) H_2, 10% Pd/C, MeOH, rt; (iv) 240 °C, 10 min; (v) 48% HBr, reflux, 48 h.

derivatives were saturated.[17] 4-Benzyloxypiperidines were obtained by *O*-benzylation of *N*-Boc-4-piperidinol,[18] while 4-phenoxymethylpiperidines were prepared by alkylation of phenols with *N*-Boc-4-mesyloxymethylpiperidine.[19] For the preparation of 2-, 3-, and 4-(substituted benzyl)piperidines, a new and efficient method was developed.[20]

Compounds **74**, **56**, **57**, **75**, **59**, and **76** were prepared from the corresponding heterobicyclic carboxylic acids (**67**, **68**, **70**, **71**, **72**, and **73**) and 4-benzylpiperidine, using a standard amide coupling reaction.[21] Reagents were commercially available or prepared from commercially available starting materials with standard procedures. Some of the products were further reacted, like **74** and **75**, that were debenzylated and demethylated, respectively, to obtain the corresponding hydroxy derivatives **55** and **58**. From 5(6)-nitro-1*H*-benzimidazole-2-carboxylic acid amide **76**, the 5(6)-MsNH-derivative **63** was prepared via the 5(6)-amino-derivative **62** (Scheme 7).

The syntheses of 5(6)-hydroxy-1*H*-benzimidazole- and 4(7)-hydroxy-1*H*-benzimidazole-2-carboxylic acid (**85** and **86**) were performed analogously to the procedure described in the literature.[22] For acylation of piperidine derivatives **a**−**u**, HBTU coupling was applied, resulting in hydroxybenzimidazole-2-carboxamides **60a**−**u** and **61a** (Scheme 8).

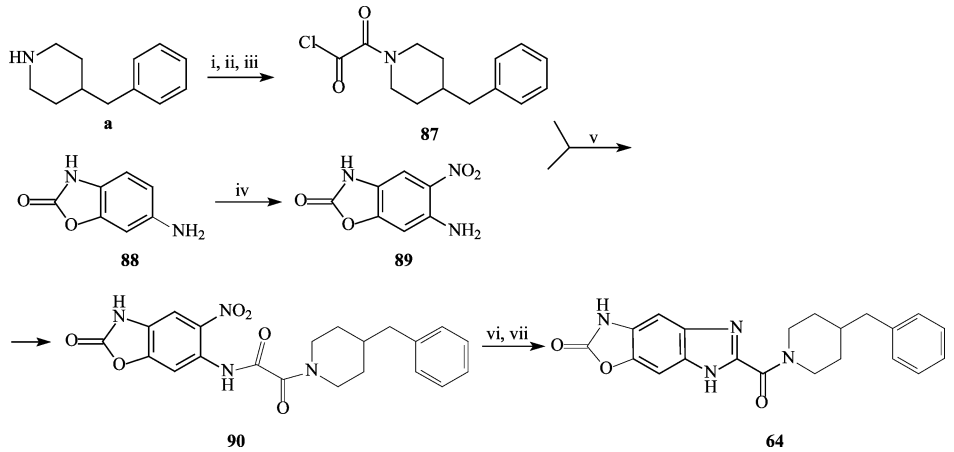
Heterotricyclic carboxamide **64** was prepared with a benzimidazole synthesis, similar to that depicted in Scheme 8, starting from aniline derivative **89** and acid chloride **87**, which in turn were prepared from 6-aminobenzoxazolinone (**88**) and 4-benzylpiperidine (**a**), respectively (Scheme 9).

The synthesis of heterotricyclic carboxamide **65** started from 5(6)-hydroxy-1*H*-benzimidazole-2-carboxylic acid. Nitration at position 4(7) and coupling with 4-benzylpiperidine resulted in carboxamide **92**, which was subsequently reduced to **93**. Ring closure with CDI led to the final product (Scheme 10).

Results and Discussion

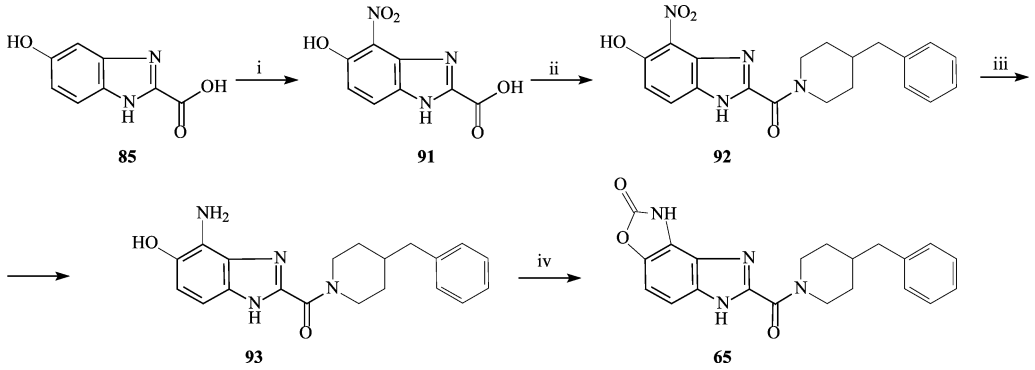
All compounds described were evaluated in a functional assay by a fluorimetric measurement of NMDA-evoked elevation of cytoplasmic calcium concentration ($[Ca^{2+}]_i$) in primary rat neocortical cell cultures.[23] Neurons in these cultures express NMDA receptors containing multiple types of NR2 subunits of which NR2B is the predominant one.[24] Compounds active in this test were then evaluated in an NR2B-selective binding assay using [^{3}H]-Ro-25-6981 as radioligand.[25] We found that the results obtained from the functional test were suitable for the establishment of SAR during lead-optimization, while acceptable QSAR models could only be obtained using binding

Scheme 9[a]



[a] Reagents and conditions: (i) ClCOCOOEt, DIPEA, DCM, 0 °C; (ii) (1) KOH, MeOH, rt; (2) HCl; (iii) $SOCl_2$, reflux, 2 h; (iv) $NaNO_3$, TFA, rt; (v) $CHCl_3$, TEA, rt; (vi) H_2, 10% Pd/C, MeOH, rt; (vii) 240 °C, 10 min.

Scheme 10[a]



[a] Reagents and conditions: (i) $NaNO_3$, TFA, rt; (ii) 4-benzylpiperidine, HBTU, TEA, DMF; (iii) H_2, 10% Pd/C, THF, rt; (iv) 1,1′-carbonyldiimidazole, THF, rt.

Table 1. In Vitro Data for Reference Compounds

	NMDA-evoked $\Delta[Ca^{2+}]_i$[a] $IC_{50} \pm$ S.E.M. (nM)	*n*	[^{3}H]Ro-25,6981 binding $IC_{50} \pm$ S.E.M. (nM)	*n*
1	41 ± 5	7	7 ± 1	6
2	6.6 ± 1.2	3	4 ± 1	3
3	159 ± 29	3	6 ± 1	3
4	8.6 ± 1.7	10	3.5 ± 1.1	3
5	131 ± 10	2	196 ± 43	3

[a] NMDA-evoked changes of intracellular Ca^{2+}.

data. IC_{50} values of the reference compounds **1**−**5** measured in the above assays are shown in Table 1.

The results in Table 2 show that the 5- and 4-hydroxy analogues of compound **6a** (**7a** and **8a**) had comparable activities, while the 7-hydroxy analogue (**9a**) showed inferior activity. This indicated that there is probably more than one H-bond acceptor moiety in the corresponding area of the receptor. Further evidence for this assumption came from the observation that the 4,6-dihydroxy analogue (**10**) had enhanced activity compared to the monohydroxy derivatives. This compound was actually found to be equipotent with besonprodil (**2**), as can be seen in Table 1. The reduced potency of compound **9a** (over **6a**−**8a**) and **11** (over **10**) is likely to reflect poor interaction of the 7-hydroxyl with the receptor, possibly because of its probable intramolecular hydrogen bonding with the indole NH group. Among the amino analogues (**12a**−**14a**), only the 4-amino derivative (**12a**) showed high activity, while among the methansulfonamido isomers (**16a**−**18a**), the 6-MsNH derivative was the most active. Due to the anticipated high metabolism of the phenol moiety[26] and the mutagenetic liability of aniline derivatives,[27] our objective was to identify phenol and aniline replacements that might improve the drug-likeness of our compounds. Analogues bearing a condensed heterocycle on the indole part (**19**−**22**) showed acceptable to good activites, proving that this mode of formation of an H-bond donor moiety in this area was a viable option.

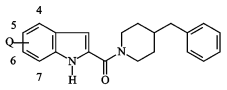
Replacing the carboxamide functionality in compound **6a** by carboxamidine (**23**) resulted in a somewhat more active analogue, whereas compounds with CH_2 or SO_2 in place of CO (**24** and **25**, respectively) almost completely lost their activity (Table 3).

The effect of varying the position of the benzyl group on the piperidine ring was investigated on 4-methoxybenzyl derivatives **6b**−**6d**. In this instance, substitution at either position 3 (**6c**) or position 2 (**6d**) substantially decreased potency (Table 4).

Another sensitive region of compound **6a** is the spacer (Y) between position 4 of the piperidine and the terminal benzene ring. Both elimination (**6e**) and elongation (**6f**) of the methylene group resulted in less-active compounds. A similar reduction in potency was found when CH_2 was substituted for CO, CHOH, or NH (**6j**−**6l**). Somewhat surprisingly, the activities of the benzyloxypiperidine and the phenoxymethylpiperidine derivatives (**6h** and **6i**) are closer to that of compound **6a** than to that of the geometrically more similar phenylethylpiperidine derivative (**6f**). This is apparently a novel example for the Friedman's paradox[28] (Table 5).

As far as the substitution pattern of the terminal benzene was concerned, it was found after having prepared and tested a small

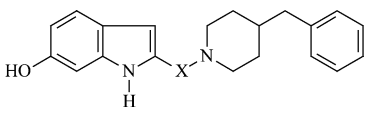
Table 2. In Vitro Data for Compounds with Various Q-s in Formula **A**



	Q	NMDA-evoked $\Delta[Ca^{2+}]_i$[a] IC_{50} ± S.E.M. (nM)	n	[^{3}H]Ro-25,6981 binding IC_{50} ± S.E.M. (nM)	n
6a	6-OH	18 ± 4	13	12±2	4
7a	5-OH	25 ± 8	3	31±10	3
8a	4-OH	16 ± 2	8	18±5	4
9a	7-OH	165 ± 30	4	380±141	3
10	4,6-di-OH	6.5 ± 0.7	3	6±1	3
11	5,7-di-OH	31 ± 3	3	106±19	3
12a	4-NH_2	4.3 ± 0.9	7	12	2
13a	5-NH_2	596 ± 113	4	N.D.	-
14a	6-NH_2	627 ± 102	3	N.D.	-
15a	4-Bn-NH	47 ± 10	7	191	1
16a	4-MsNH	119 ± 25	3	215	1
17a	5-MsNH	631 ± 114	4	1545	1
18a	6-MsNH	30 ± 5	6	9	2
19	5,6- 	17 ± 4	7	23 ± 6	3
20	4,5- 	58 ± 11	5	64	2
21	4,5- 	20 ± 5	4	5 ± 2	3
22	4,5- 	50 ± 11	3	6	2

[a] NMDA-evoked changes of intracellular Ca^{2+}.

Table 3. In Vitro Data for Compounds with Various X-s in Formula **A**

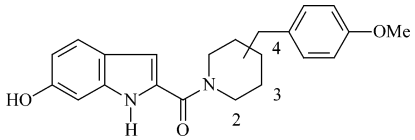


	X	NMDA-evoked $\Delta[Ca^{2+}]_i$[a] IC_{50} ± S.E.M. (nM)	*n*	[^{3}H]Ro-25,6981 binding IC_{50} (nM)	*n*
23	C═NH	8.5 ± 1.7	5	10	2
24	CH_2	1158 ± 231	3	N.D.	
25	SO_2	>8000	1	N.D.	

[a] NMDA-evoked changes of intracellular Ca^{2+}.

series of derivatives that substituents in all positions on the benzene ring of the benzylpiperidine part of compound **A** decreased the activity (**6m**−**6p**; Table 6).

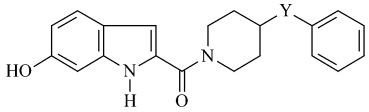
Table 4. Influence on the In Vitro Data of the Position of the Benzyl Substituent on the Piperidine Ring in Formula **A**



	subst.	NMDA-evoked $\Delta[Ca^{2+}]_i$[a] IC_{50} ± S.E.M. (nM)	*n*	[^{3}H]Ro-25,6981 binding IC_{50} (nM)	*n*
6b	4-(4-MeO)Bn	80 ± 14	3	17	2
6c	3-(4-MeO)Bn	2206 ± 406	7	N.D.	
6d	2-(4-MeO)Bn	>8000 ± 2	2	N.D.	

[a] NMDA-evoked changes of intracellular Ca^{2+}.

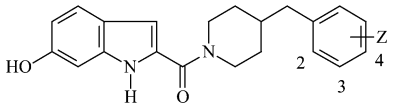
Table 5. In Vitro Data for Compounds with Various Y in Formula **A**



	Y	NMDA-evoked $\Delta[Ca^{2+}]_i$[a] IC_{50} ± S.E.M. (nM)	*n*	[^{3}H]Ro-25,6981 binding IC_{50} ± S.E.M. (nM)	*n*
6e		>8000 ± 2	2	N.D.	
6f	CH_2CH_2	875 ± 187	5	359 ± 93	3
6g	O	107 ± 27	3	89	2
6h	OCH_2	16 ± 3	9	47 ± 4	3
6i	CH_2O	36 ± 6	7	19 ± 2	3
6j	CO	744 ± 131	4	N.D.	
6k	CH_2OH	111 ± 23	4	60	2
6l	NH	348 ± 75	6	1084	2

[a] NMDA-evoked changes of intracellular Ca^{2+}.

Table 6. In Vitro Data for Compounds with Various Z in Formula **A**



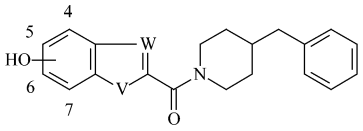
	Z	NMDA-evoked $\Delta[Ca^{2+}]_i$[a] IC_{50} ± S.E.M. (nM)	*n*	[^{3}H]Ro-25,6981 binding IC_{50} ± S.E.M. (nM)	*n*
6m	4-F	90 ± 15	6	12 ± 3	3
6n	4-Me	208 ± 34	3	28	2
6o	2-MeO	249 ± 55	3	N.D.	
6p	3-F	38 ± 8	6	31	1

[a] NMDA-evoked changes of intracellular Ca^{2+}.

Then we wanted to assess the importance of the NH group in compound **6a**. The N−Me analogue (**55**) was inactive. Similarly, the corresponding benzofuran and benzothiazole derivatives **56** and **57** had very weak activity. The 5-hydroxybenzo(*b*)thiophene-2-carboxamide (**58**) and the 5-hydroxy isomer of benzofuran **56** (**59**) were also inactive. These results indicated that the NH in compound **6a** has a critical role in the interaction with the receptor. This observation was supported by the fact that benzimidazole-2-carboxamide **60a** showed very good activity. The additional N in the molecule resulted in a 9-fold increase in potency compared to **6a**, making this compound one of the most potent NR2B selective NMDA antagonists reported (Table 7).

After having identified **60a** as an exceptionally potent NR2B selective NMDA antagonist the optimization of this compound started. A selection of the derivatives prepared and tested is shown in Table 8. The 4(7)-hydroxy isomer of **60a** (**61a**) was practically equipotent with the lead. Substitution of the OH for NH_2 resulted in a less-active analogue (**62**). The corresponding

Table 7. In Vitro Data for Compounds with Various V-s and W-s in Formula **B**



	subst.	V	W	NMDA-evoked $\Delta[Ca^{2+}]_i$[a] $IC_{50} \pm$ S.E.M. (nM)	*n*	[^{3}H]Ro-25,6981 binding IC_{50} (nM)	*n*
55	6-OH	NMe	CH	5773 ± 958	4	N.D.	
56	6-OH	O	CH	6920 ± 1280	4	>10 000	1
57	6-OH	S	N	2597 ± 369	3	N.D.	
58	5-OH	S	CH	5613 ± 1066	5	>10 000	1
59	5-OH	O	CH	1901 ± 295	5	>10 000	1
60a	5(6)-OH	NH	N	2.2 ± 0.4	7	4	2

[a] NMDA-evoked changes of intracellular Ca^{2+}.

mesylamino derivative **63**, however, was slightly more active than **60a**. Substitution the OH in **60a** for NH as part of an oxazolinone ring attached to the benzimidazole (**64** and **65**) led to less-active compounds. Interestingly, modifications of Y and Z (**60m**−**60u**) did not influence significantly the activity of the compounds.

Selectivity toward NR2A subunit containing NMDA receptors was tested by the same functional assay using cells expressing recombinant NR1/NR2A receptors, and none of the compounds exhibited significant activity up to 15 μM concentration. Compound **60a** was selected for further examination of selectivity toward other NR2 subunit containing NMDA receptors. Compound **60a**, up to 10 μM concentration, did not inhibit (inh. < 20%) NMDA-evoked current in NR1/NR2A and NR1/NR2D transfected cells measured by standard patch clamp technique.[29]

Reference compounds **1**−**3**, **6a**, and **60a** were assayed in the mouse formalin test adapted from Hunskaar et al.[30] The first phase (0−10 min) of the typical biphasic pain response is regarded as acute nociception, while the second phase, lasting generally from 10 to 60 min, represents persistent pain. NMDA antagonists are known potent inhibitors of this late phase of formalin response that represents persistent pain.[31] Therefore, we measured pain-related licking behavior at the peak of the second phase of the typical biphasic pain response. ED_{50} values after oral administration were >20 mg/kg, 2.4 mg/kg, 1.7 mg/kg, and 1.6 mg/kg for compounds **1**, **2**, **6a**, and **60a**, respectively. Compound **3** was found inactive p.o., although it had an ED_{50} value of 5.1 mg/kg after i.p. administration.

Molecular Modeling

In total, 59 indole- and benzimidazole-carboxamide derivatives were used for this CoMSIA[32] study, for which IC_{50} values were determined in an in vitro binding assay.[23]

The molecules were clustered by their UNITY fingerprints, resulting in 12 clusters. From each cluster, one molecule was selected for external validation of the QSAR model (test set), with the condition to cover the biological activity range. The remaining 47 molecules were used as a training set for the 3D-QSAR analysis. The structures of compounds employed in this study and their measured, as well as calculated, biological activities (converted to $-\log IC_{50}$) can be found in the Supporting Information.

The structural energy minimization was performed using the standard Tripos Force Field and Gasteiger−Hückel charges with conjugate gradient method and a 0.05 kcal/mol energy gradient convergence criterion. Conformational analyses were carried out by random search with an energy cutoff of 10 kcal/mol.

The alignment of the molecules is a crucial step in the grid-based 3D-QSAR techniques. The molecules employed in our study were superimposed based on the pharmacophoric points of the pharmacophore model built with the help of DISCO technique.[33] The resulting model (see Figure 3) consists of three hydrophobic centers (H1−3), a donor atom (DA1) and the corresponding acceptor site (AS1), and an acceptor atom (AA1) and the corresponding donor site (DS1) as common to all compounds involved in the model development. Additional donor site (DS2) and acceptor sites (AS2, AS3) exist in some subset of the compounds studied, improving their biological activity. The molecules were aligned by least-square fitting to the common pharmacophoric points.

The superimposed molecules were placed in a rectangular regular grid with 2 Å spacing extending at least 4 Å beyond each molecule in all directions. Molecular similarity indices were used as descriptors (independent variables) in the 3D-QSAR analyses. Steric, electrostatic, and hydrophobic as well as hydrogen-bond donor and acceptor features were considered in this CoMSIA study. Similarity indices were calculated at the grid points between each compound and a probe atom with radius of 1 Å and charge, hydrophobic, and hydrogen bond properties of +1. The default setting of the attenuation factor was applied ($\alpha = 0.3$).

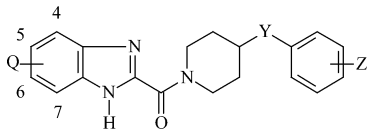
Regression analysis of the resulting field matrix was performed by partial least-square (PLS) method. The optimal number of components was chosen by the leave-one-out (LOO) cross-validation technique on the basis of the highest q^2 value. A minimum sigma standard deviation threshold (column filtering) of 2.0 was used. A statistically significant CoMSIA model was derived from the training set of 47 compounds. Good cross-validated r^2 (0.547) and conventional r^2 (0.972) values were obtained, that is, however, a necessary but not sufficient condition for a model to be truly predictive. Cross-validated r^2 (i.e., q^2) is considered usually as a measure of the predictivity of the method, although that is often not the case.[34] External validation is an absolute requirement for a predictive QSAR model. The biological activities of the 12 test set molecules were predicted with the above-described CoMSIA model, with a predictive r^2 of 0.666. The experimental and calculated activity values (expressed as $-\log IC_{50}$) for the training and test set are presented in the Supporting Information and graphically depicted in Figure 4. The predicted values do not differ significantly from the measured ones in that the deviations are well within one logarithmic unit.

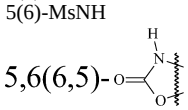
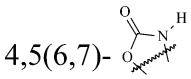
The 3D-QSAR study carried out using CoMSIA has led to the identification of those regions important for interactions between ligand and receptor and provided an insight into the structural variations that enabled us to design further compounds with high activity. CoMSIA contour maps are shown in Figure 5.

Conclusion

The conformational constraint of cinnamide **5** via the incorporation of an NH group resulted in indole-2-carboxamide **6a**, having an additional H-bond donor moiety compared to **5**. It was found that these structural changes caused a significant increase both in potency and in affinity. During the lead optimization, it was found that the majority of numerous structural modifications resulted in less-active compounds. The most significant exception was the insertion of a further atom into the indole skeleton, yielding **60a**. The benzimidazole-2-

Table 8. In Vitro Data for Benzimidazole-2-carboxamides



	Q	Y	Z	NMDA-evoked $\Delta[Ca^{2+}]_i$[a] $IC_{50} \pm$ S.E.M. (nM)	*n*	[^{3}H]Ro-25,6981 binding $IC_{50} \pm$ S.E.M. (nM)	*n*
61a	4(7)-OH	CH_2	H	3.0 ± 0.4	4	10 ± 4	3
62	5(6)-NH_2	CH_2	H	40 ± 10	3	143	2
63	5(6)-MsNH	CH_2	H	1.3 ± 0.2	4	3	2
64	5,6(6,5)- 	CH_2	H	193 ± 42	11	N.D.	
65	4,5(6,7)- 	CH_2	H	328 ± 70	3	N.D.	
60m	5(6)-OH	CH_2	4-F	1.2 ± 0.3	5	4	2
60q	5(6)-OH	CH_2	3-Me	2.4 ± 0.5	6	5	2
60r	5(6)-OH	CH_2	3-MeO	2.5 ± 0.6	3	3	1
60s	5(6)-OH	O	4-Me	1.1 ± 0.2	4	3	2
60t	5(6)-OH	O	4-Cl	1.7 ± 0.4	6	14	2
60u	5(6)-OH	O	4-F	2.9 ± 0.6	3	6	2

[a] NMDA-evoked changes of intracellular Ca^{2+}.

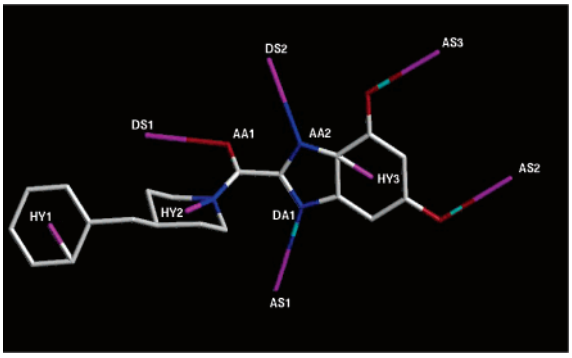


Figure 3. Pharmacophore model. AA, acceptor atom; DS, donor site; DA, donor atom; AS, acceptor site; HY, hydrophobic center.

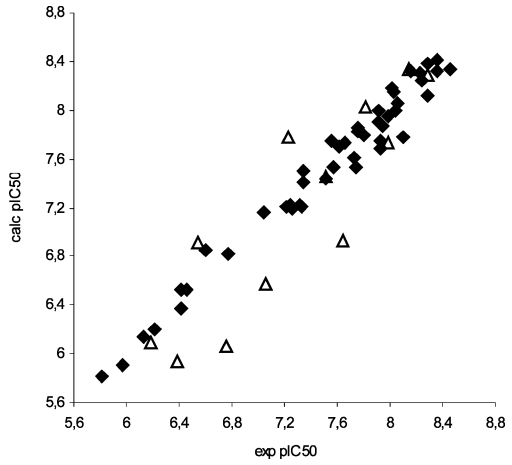


Figure 4. Calculated vs experimental biological activity of training ◆ and test △ set molecules.

carboxamide analogues obtained in this way were more potent derivatives than the corresponding indoles. In the case of the one of most active compounds (**60a**), the NR2B versus NR2A, NR2D subtype selectivities were determined. Compounds **6a** and **60a** had antinociceptive effect in the mouse formalin test after oral administration, and these effects were comparable to that of the reference compound besonprodil (**2**). On the basis of the affinities of a selection of the indole- and benzimidazole-2-carboxamides measured in the [^{3}H]-Ro-25,6981 binding assay, a CoMSIA model was developed to facilitate the design of further active analogues.

Experimental Section

General. All reagents and solvents were of commercial quality and used without further purification, unless indicated otherwise. ^{1}H NMR spectra were obtained on a Varian Unity Inova 300 or a Varian Unity Inova 500 spectrometer. Chemical shifts are reported in parts per million relative to TMS as internal standard. High-resolution MS measurements (EI, 70 eV) were carried out on a Finnigan MAT 95XP mass spectrometer; perfluorotributyl amine was used as a reference compound. All prepared compounds (end products and isolated intermediates) were characterized by high performance liquid chromatography coupled to a mass selective detector (LC/MS) using HP 1100 Binary Gradient chromatography system with Microplate Sampler (Agilent, Waldbronn), controlled by ChemStation software. HP diode array detector was used to acquire UV spectra at 225 and 240 nm. All experiments were performed using HP MSD (Agilent, Waldbronn) single quadruple spectrometer equipped with an electrospray ionization source to determine the structure. Melting points were determined in open glass capillaries using a Büchi 535 melting point apparatus and are uncorrected. Column chromatography was performed on Kieselgel 60 (Merck).

Measurement of $[Ca^{2+}]_i$. Primary cultures of cortical neurones were initiated from neocortices of 17-day old Wistar rat embryos. The cortical tissue was cut into small pieces, washed, and trypsinised (0.25% trypsin, 4 min). The resulting suspension was filtered through a 70 μm mesh and centrifuged at 125 *g* for 5 min. The cell pellet was resuspended in DMEM[a] containing 10% foetal bovine serum and antibiotics (0.25 μg/mL amphotericin B, 100 U/mL penicillin G, 100 μg/mL streptomycin). Cells were plated in standard 96-well microplates previously coated with poly-D-lysine at a concentration of 2×10^5 to 4×10^5 cells/cm^2. Cultures were kept at 37 °C in a humidified atmosphere of 95% air/5% CO_2. After 3 days in culture, half of the culture medium was replaced with

[a] Abbreviations: DMEM, Dulbecco's modified eagle medium; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; EGTA, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; TRIS−HCl, tris(hydroxymethyl)aminomethane hydrochloride.

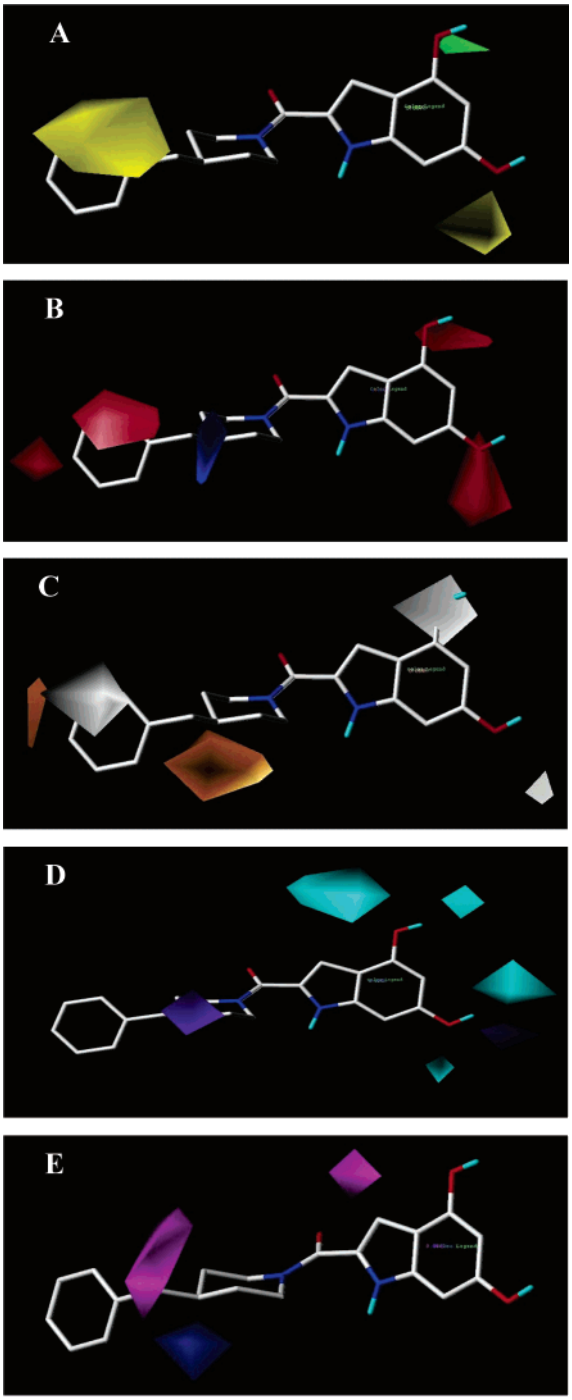


Figure 5. CoMSIA contour maps. A, steric field (green region, steric bulk enhances activity; yellow region, sterick bulk detracts from activity); B, electrostatic field (blue region, positive charge favored; red region, positive charge unfavored); C, hydrophobic field (orange region, hydrophobic groups favored; white region, hydrophobic groups unfavored); D, hydrogen donor field (cyan region, hydrogen donor favored; purple region, hydrogen donor unfavored); E, hydrogen acceptor field (magenta region, hydrogen acceptor favored; blue region, hydrogen acceptor unfavored).

fresh medium (DMEM containing 10% bovine serum and antibiotics) containing 50 μM 5-fluoro-5′-deoxyuridine to stop the proliferation of glial elements. On the fifth day, half of the medium was replaced with serum-free (N2) medium (DMEM supplemented with 5 μg/mL insulin, 0.1 mg/mL transferrin, 5 μg/mL Na-selenite, 0.02 μg/mL progesterone, 0.1 mM putrescine, 25 mM D-glucose, 0.25 μg/mL amphotericin B, 100 U/mL penicillin G, 100 μg/mL streptomycin). The cultures were used for the $[Ca^{2+}]_i$ measurements after 3−10 days in vitro.

Cells grown in standard 96-well microplates were loaded with a fluorescent Ca^{2+}-sensitive dye, fluo-4/AM (2−2.5 μM), for 60−90 min. To stop dye loading, cells were washed twice with extracellular medium (145 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 10 mM HEPES, 20 mM glucose, 10 μM glycine, pH = 7.4). After washing, the test compounds (diluted in extracellular medium from a DMSO stock solution, final DMSO concentration was <0.1%) were added to the cells. Measurements $[Ca^{2+}]_i$ were carried out with a plate reader fluorimeter (Fluoroskan Ascent, Labsystems); after recording baseline fluorescence (5 min), elevation of $[Ca^{2+}]_i$ was induced by application of 40 μM NMDA, and the fluorescence was recorded for an additional 5 min. Conversion of raw fluorescence data to calcium concentration values was performed using the equation

$$[Ca^{2+}]_i = K_d \frac{F - F_{min}}{F_{max} - F}$$

where K_d is the in vitro dissociation constant of fluo-4, F_{max} and F_{min} are the maximal and minimal fluorescence (determined in the presence of 5 μM ionomycin and 20 mM EGTA, respectively). In some of the experiments this calibration was not performed and raw fluorescence data was used for analysis. All treatments on a single plate were measured in multiple wells, and the average value of all wells of a treatment was used for analysis. Inhibitory potency of a compound at a single concentration point was expressed as percent inhibition of the control NMDA response. Sigmoidal concentration−inhibition curves were fitted to the data derived from at least three independent experiments using GraphPad Prism software. IC_{50} values were determined as the concentration that produced half of the maximal inhibition.

NR2B Receptor Binding. Rat forebrain was homogenized in 50 volumes of ice-cold 50 mM TRIS−HCl buffer at pH 7.4 containing 10 mM EDTA. The homogenate was centrifuged at 48 000 *g* for 10 min at 4 °C. The pellet was suspended in the same buffer, and the homogenate was incubated at 37 °C for 10 min and centrifuged at 48 000 *g* for 10 min at 4 °C. The pellet was resuspended in the same buffer and frozen at −70 °C until use.

For the binding assay, membrane was thawed at 37 °C and centrifuged at 48 000 *g* for 10 min at 4 °C, and the pellet was washed two times in a 5 mM TRIS−HCl buffer (pH 7.4). The final pellet was suspended in 5 mM TRIS−HCl buffer at pH 7.4 and was used at a final concentration of 1 mg protein/mL. An amount equal to 0.45 mL membrane suspension was incubated with 4 nM [^{3}H]Ro-25,6981 in the presence or absence of different concentrations of compounds for 2 h at room temperature. The final incubation volume was 500 μL. Nonspecific binding, assessed in the presence of Ro-25,6981 (10 μM), was 5−15% of the total binding. The assay was stopped by filtration on Whatman GF/B glass fiber filter. The filters were washed three times with 5 mL ice-cold 5 mM TRIS−HCl buffer, and radioactivity of the filters was counted by liquid scintillation spectrometry in 2 mL HISafe scintillation cocktail using a LKB-Wallac 1409 liquid scintillation counter.

All dilutions were made by 5% DMSO from 10 mM stock solutions prepared in DMSO.

The ligand displacement by the compounds was determined using a minimum of seven concentrations The specific radioligand binding is defined as the difference between total binding and the nonspecific binding determined in the presence of an excess of unlabeled ligand. Results are expressed as a percent of inhibition of specific binding obtained in the presence of compounds. IC_{50} values (i.e., concentration of compound giving 50% inhibition of specific binding) were calculated from concentration−displacement curves by sigmoidal fitting using Origin 6.0 software (Microcal).

Mouse Formalin Test. Male NMRI mice (20−25 g) from Charles River (Isaszeg, Hungary) were used for tests. Prior to the oral treatments, solid food was withdrawn for 14−16 h, but mice had free access to 20% glucose solution.

An amount equal to 20 μL of 1% formalin in 0.9% saline was injected subcutaneously into the dorsal surface of the right hindpaw.

Test drugs or the vehicle (5% Tween 80) were orally administered 15 min before the formalin injection. Time spent by licking or biting of the injected paw was then registered 20−25 min post-formalin.

Increasing doses of drugs were administered and compared to a parallel vehicle treated group (min 5 mice/dose group).

ED_{50} values were calculated by Boltzmann's sigmoidal curve fitting, using Microgal Origin 6.0 software. Licking time data were statistically analyzed by Mann−Whitney U test.

Molecular Modeling. All calculations were performed using the Sybyl 6.8 software package from Tripos.[35]

Chemistry. (4-Benzylpiperidine-1-yl)-(6-hydroxy-1*H*-indole-2-yl)-methanone (6a). A mixture of 6-hydroxy-indole-2-carboxylic acid (5.0 g, 28.2 mmol), triethylamine (4.4 mL, 31.6 mmol), 4-benzylpiperidine (5.0 g, 28.5 mmol), and HBTU (12.0 g, 31.6 mmol) in DMF (50 mL) was stirred at room temperature for 6 h. The reaction mixture was concentrated, purified by column chromatography (4:1 toluene/methanol), and recrystallized from ethanol to give **6a** as a white solid (6.75 g, 71%): mp 214−215 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 11.08 (d, J = 1.5 Hz, 1H), 9.12 (s, 1H), 7.36 (d, J = 8.5 Hz, 1H), 7.32−7.25 (m, 2H), 7.23−7.15 (m, 3H), 6.79−6.75 (m, 1H), 6.63−6.60 (m, 1H), 6.57 (dd, J = 8.5, 2.4 Hz, 1H), 4.49−4.38 (m, 2H), 3.03−2.82 (m, 2H), 2.55 (d, J = 7 Hz, 2H), 1.92−1.74 (m, 1H), 1.72−1.59 (m, 2H), 1.27−1.07 (m, 2H) ppm.

(4-Benzylpiperidine-1-yl)-(4,6-dihydroxy-1*H*-indole-2-yl)-methanone (10). Step 1: Methyl 4,6-Dibenzyloxy-1*H*-indole-2-carboxylate (39). To a solution of sodium methoxide (1.4 g, 60.8 mmol sodium) in methanol (45 mL), a mixture of 2,4-dibenzyloxybenzaldehyde (**36**; 4.75 g, 14.9 mmol), methyl azidoacetate (6.9 g, 60.0 mmol), and methanol (20 mL) were added dropwise, at 0 °C, under an Ar atmosphere. The mixture was stirred at 0 °C for 4 h, then diluted with water (300 mL), and extracted with chloroform. The organic layer was washed with water, dried over Na_2SO_4, and concentrated, and the residue was crystallized with ethanol to give methyl (*Z*)-2-azido-3-(2,4-dibenzyloxy)-acrylate (2.2 g, 38%): mp 94−95 °C. This material was added in small portions to a stirred solution of boiling xylene (50 mL of 2.2 g, 5.7 mmol). After completion of the addition, the reaction mixture was refluxed until the gas formation (about 0.5 h), then cooled to room temperature. The precipitated product was filtered off and recrystallized from isopropanol to give **39** (1.3 g, 63.7%): mp 174 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 11.74 (s, 1H), 7.55−7.25 (m, 10H), 7.08 (s, 1H), 6.61−6.55 (m, 1H), 6.41 (d, J = 1.8 Hz, 1H), 5.22 (s, 2H), 5.10 (s, 2H), 3.83 (s, 3H) ppm.

Step 2: (4-Benzylpiperidine-1-yl)-(4,6-dibenzyloxy-1*H*-indole-2-yl)-methanone (43). A mixture of **39** (1.25 g, 3.5 mmol), potassium trimethylsilanolate (0.5 g, 3.9 mmol), and THF (20 mL) was refluxed for 2 h. The reaction mixture was cooled, poured into water (100 mL), and acidified with HCl. The precipitated product was filtered off, washed with water, and dried to give 4,6-dibenzyloxy-1*H*-indole-2-carboxylic acid (1.0 g 83.2%): mp 180 °C.

A mixture of this material (1.0 g, 2.68 mmol), triethylamine (0.41 mL, 2.85 mmol), 4-benzylpiperidine (0.5 g, 2.85 mmol), and HBTU (1.1 g, 2.9 mmol) in DMF (20 mL) was stirred at room temperature for 6 h. The reaction mixture was concentrated and purified by column chromatography (4:1 toluene/methanol) to give **43** as a white solid (0.75 g, 52%): ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 11.35 (d, J = 2.0 Hz, 1H), 7.56−7.08 (m, 15H), 6.66−6.62 (m, 1H), 6.60−6.57 (m, 1H), 6.37 (d, J = 1.8 Hz, 1H), 5.21 (s, 2H), 5.07 (s, 2H), 4.50−4.36 (m, 2H), 3.08−2.80 (m, 2H), 2.54 (d, J = 7.0 Hz, 2H), 1.94−1.72 (m, 1H), 1.72−1.55 (m, 2H), 1.30−1.04 (m, 2H) ppm.

Step 3: (4-Benzylpiperidine-1-yl)-(4,6-dihydroxy-1*H*-indole-2-yl)-methanone (10). To a solution of **43** (0.75 g, 1.4 mmol) in methanol (20 mL), 10% Pd/C catalyst (0.05 g) was added and hydrogenated for 2 h. The catalyst was filtered off, the filtrate was concentrated, and the residue was recrystallized from isopropanol to give (**10**; 0.3 g, 60.7%): mp 245−246 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 10.92 (d, J = 2.0 Hz, 1H), 9.43 (s, 1H), 8.91 (s, 1H), 7.35−7.24 (m, 2H), 7.24−7.13 (m, 3H), 6.69−6.65 (m, 1H), 6.28−6.23 (m, 1H), 5.97 (d, J = 1.9 Hz, 1H), 4.53−4.36 (m, 2H), 3.03−2.82 (m, 2H), 2.55 (d, J = 7.2 Hz, 2H), 1.94−1.74 (m, 1H), 1.73−1.58 (m, 2H), 1.28−1.09 (m, 2H) ppm.

(4-Amino-1*H*-indol-2-yl)-(4-benzyl-piperidin-1-yl)-methanone (12a). Step 1: (4-Benzyl-piperidin-1-yl)-(4-nitro-1*H*-indol-2-yl)-methanone (33a). A mixture of 4-nitroindole-2-carboxylic acid (2.68 g, 13.0 mmol), triethylamine (1.5 mL, 10.8 mmol), 4-benzylpiperidine (1.88 g, 10.8 mmol), and HBTU (4.1 g, 10.8 mmol) in DMF (130 mL) was stirred at room temperature for 6 h. The reaction mixture was concentrated and purified by column chromatography (chloroform) and crystallized with diethyl ether to give **33a** as a yellow solid (3.1 g, 79%): mp 139−142 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10−1.32 (m, 2H), 1.62−1.78 (m, 2H), 1.78−1.97 (m, 1H), 2.57 (d, J = 7.2 Hz, 2H), 3.02 (br m, 2H), 4.38 (br m, 2H), 7.14−7.34 (m, 6H), 7.41 (t, J = 8.1 Hz, 1H), 7.90 (dt, J = 8.1, 0.9 Hz, 1H), 8.11 (dd, J = 8.1, 0.9 Hz, 1H), 12.43 (br s, 1H).

Step 2: (4-Amino-1*H*-indol-2-yl)-(4-benzyl-piperidin-1-yl)-methanone (12a). To a solution of **33a** (2.7 g, 7.5 mmol) in methanol (110 mL), 10% Pd/C catalyst (0.5 g) was added and hydrogenated for 3 h. The catalyst was filtered off, the filtrate was concentrated, and the residue was crystallized with diethyl ether to give **12a** (1.96 g, 78.4%): mp 165−168 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10−1.30 (m, 2H), 1.60−1.74 (m, 2H), 1.75−1.95 (m, 1H), 2.56 (d, J = 6.9 Hz, 2H), 2.94 (br m, 2H), 4.47 (d, J = 13.5 Hz, 2H), 5.33 (s, 2H), 6.13 (dd, J = 7.5, 0.6 Hz, 1H), 6.60 (d, J = 8.1 Hz, 1H), 6.84 (dd, J = 8.1, 7.5 Hz, 1H), 7.14−7.33 (m, 5H), 11.08 (s, 1H).

(4-Benzylamino-1*H*-indol-2-yl)-(4-benzyl-piperidin-1-yl)-methanone (15a). To a solution of **12a** (0.33 g, 1.0 mmol), benzaldehyde (0.1 g, 1.0 mmol), and acetic acid (0.12 mL, 2.0 mmol) in 1,2-dichloroethane (10 mL) sodium triacetoxyborohydride (0.32 g, 1.5 mmol) was added below 10 °C, and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into 8% $NaHCO_3$ (50 mL) and extracted with chloroform. The organic layer was dried over Na_2SO_4 and concentrated, and the residue was dissolved in EtOAc and HCl in EtOAc (2.5 M, 1 mL) was added. The precipitated HCl salt was filtered off and washed with diethyl ether to give **15a** (0.3 g, 65.2%): mp 136−145 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10−1.30 (m, 2H), 1.60−1.75 (m, 2H), 1.75−1.97 (m, 1H), 2.57 (d, J = 7.2 Hz, 2H), 2.95 (br m, 2H), 4.40 (d, J = 12.6 Hz, 2H), 4.52 (s, 2H), 6.67 (br s, 1H), 7.00−7.46 (m, 14H), 11.63 (s, 1H).

***N*-[2-(4-Benzyl-piperidine-1-carbonyl)-1*H*-indol-4yl]-methanesulfonamide (16a).** To a stirred solution of **12a** (0.33 g, 1.0 mmol) and triethylamine (0.21 mL, 1.5 mmol) in chloroform (10 mL), dropwise a solution of methanesulfonyl chloride (0.1 mL, 1.2 mmol) in chloroform (5 mL) was added below 10 °C, and the mixture was stirred at room temperature for 10 h. The reaction mixture was poured into water (30 mL) and extracted with chloroform. The organic layer was dried over Na_2SO_4 and concentrated, and the residue was treated with diethyl ether and the crystals were filtered to give **16a** (0.25 g, 61%): mp 228−230 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.12−1.32 (m, 2H), 1.62−1.76 (m, 2H), 1.76−1.98 (m, 1H), 2.58 (d, J = 7.2 Hz, 2H), 2.94 (br m, 2H), 2.97 (s, 3H), 4.46 (d, J = 13.2 Hz, 2H), 7.01 (dd, J = 7.5, 0.9 Hz, 1H), 7.08−7.33 (m, 8H), 9.60 (s, 1H), 11.60 (s, 1H).

(4-Benzylpiperidin-1-yl)-(1,6-dihydro-pirrolo[2,3-*g*]indazol-7-yl)-methanone (20). Step 1: Ethyl 1,6-Dihydro-pyrrolo[2,3-*g*]indazole-7-carboxylate (48). To a stirred mixture of 6-amino-indazol (**45**; 6.66 g, 50.0 mmol), water (40 mL), and HCl (25 mL), a solution of sodium nitrite (3.5 g, 50.7 mmol) in water (10 mL) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 0.5 h. Then the so obtained solution was added to a mixture of water (86 mL), KOH (5.0 g, 267 mmol), sodium acetate (15.0 g, 183 mmol), ethanol (60 mL), and ethyl 2-methyl-acetoacetate (8.0 mL, 50.9 mmol, purity 90%). The reaction mixture was stirred at 0 °C for 1 h. The precipitated product was filtered off, washed with water, and dried to give ethyl 2-[(1*H*-indazol-6-yl)-hydrazono]-propionate (8.16 g, 66%): mp 210−211 °C. A mixture of an aliquot of this material (4.0 g, 16.25 mmol) and polyphosphoric acid (20

g) was slowly warmed to 120 °C and kept at this temperature for 0.5 h. The reaction mixture was cooled to room temperature and water (30 mL) and HCl (15 mL) were added. The so obtained mixture was extracted with ethyl acetate, dried over Na_2SO_4, and concentrated to give **48** (1.6 g, 43%): mp 120−121 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (t, J = 7.2 Hz, 3H), 4.36 (q, J = 7.2 Hz, 2H), 7.25 (dd, J = 8.7, 0.9 Hz, 1H), 7.42 (dd, J = 0.9, 0.6 Hz, 1H), 7.58 (d, J = 8.7 Hz, 1H), 8.10 (s, 1H), 12.30 (br s, 1H).

Step 2: (4-Benzylpiperidin-1-yl)-(1,6-dihydro-pirrolo[2,3-*g*]-indazol-7-yl)-methanone (20). A stirred mixture of **48** (1.6 g, 7.0 mmol), ethanol (160 mL), water (10 mL), and KOH (1 g, 17.8 mmol) was refluxed for 5 h. The mixture was concentrated, and the residue was dissolved in water and acidified with 1 N HCl solution. The precipitated crystals were filtered off and washed with water to give 1,6-dihydro-pyrrolo[2,3-*g*]indazole-7-carboxylic acid (1.1 g, 78%): mp 270−275 °C. A mixture of an aliquot of this material (0.38 g, 1.9 mmol), triethylamine (0.3 mL, 2.1 mmol), 4-benzylpiperidine (0.35 mL, 2.2 mmol), and HBTU (0.76 g, 2.0 mmol) in DMF (10 mL) was stirred at room temperature for 6 h. The reaction mixture was concentrated, purified by column gel chromatography (95:5 chloroform/methanol), and crystallized with diethyl ether to give **20** as a white solid (0.25 g, 37%): mp 209−210 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10−1.32 (m, 2H), 1.64−1.77 (m, 2H), 1.79−2.00 (m, 1H), 2.58 (d, J = 7.2 Hz, 2H), 3.00 (br m, 2H), 4.49 (d, J = 13.2 Hz, 2H), 7.00 (dd, J = 2.4, 0.9 Hz, 1H), 7.16−7.34 (m, 6H), 7.47 (d, J = 9.0 Hz, 1H), 7.99 (d, J = 1.5 Hz, 1H), 11.90 (s, 1H), 13.19 (s, 1H). HRMS ($C_{22}H_{22}N_4O$) calcd, 358.1788; found, 358.1784.

2-[(4-Benzyl-piperidin-1-yl)-imino-methyl]-1*H*-indol-6-ol (23). Step 1: 6-Benzyloxy-1*H*-indole-2-carbonitrile (52). To a suspension of 6-benzyloxy-1*H*-indole-2-carboxylic acid (**51**; 3.0 g, 11.2 mmol) in chloroform (60 mL), thionyl chloride (3.0 mL, 41.1 mmol) and DMF (1 drop) were added at ambient temperature, and the mixture was refluxed for 1 h. The reaction mixture was poured into a mixture of 25% ammonia solution (20 mL) and ice. After evaporation of the chloroform, the precipitated product was filtered off and washed with water to yield 2.72 g (90.6%) of the 6-benzyloxy-1*H*-indole-2-carboxylic acid amide: mp 186 °C. A mixture of this material (2.72 g, 10.2 mmol) and $POCl_3$ (2.72 mL, 29.1 mmol) in chloroform (20 mL) was refluxed for 2 h. The reaction mixture was poured into ice. The organic layer was separated and concentrated in vacuo. The residue was purified by column chromatography (4:1 hexane/ethyl acetate) to give **52** as a white solid (1.63 g, 64.2%): mp 123−124 °C; ^{1}H NMR (500 MHz, DMSO-d_6, 30 °C) δ 12.15 (s, 1 H), 7.56 (d, J = 7.8 Hz, 1 H), 7.50−7.45 (m, 2 H), 7.43−7.37 (m, 2 H), 7.36−7.31 (m, 1 H), 7.27 (s, 1 H), 6.98 (d, J = 2.0 Hz, 1 H), 6.89 (dd, J = 7.8, 2.0 Hz, 1 H), 5.16 (s, 2 H) ppm.

Step 2: 2-[(4-Benzyl-piperidin-1-yl)-imino-methyl]-1*H*-indol-6-ol (23). A mixture of **52** (1.0 g, 4.0 mmol) and saturated HCl solution in MeOH (20 mL, 52%) was stirred at room temperature for 5 h. The reaction mixture was concentrated in vacuo. The residue was dissolved in MeOH (20 mL). To this solution was added 4-benzyl-piperidin (5.0 g, 28.0 mmol), and the mixture was stirred at the same temperature for 24 h. The reaction mixture was concentrated and purified by column chromatography (3:1 $CHCl_3$/MeOH) and crystallized with diethyl ether to give the HCl salt of **23** as a white solid (61 mg): mp 219 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 11.90 (vbr s, 1H), 7.36 (d, J = 8.6 Hz, 1H), 7.33−7.24 (m, 2H), 7.23−7.14 (m, 3H), 6.78−6.73 (m, 1H), 6.66−6.62 (m, 1H), 6.59 (dd, J = 8.6, 2.1 Hz, 1H), 4.09−3.90 (m, 2H), 3.01−2.85 (m, 2H), 2.55 (d, J = 7.1 Hz, 2H), 1.91−1.71 (m, 1H), 1.69−1.55 (m, 2H), 1.40−1.20 (m, 2H) ppm.

2-(4-Benzyl-piperidin-1-ylmethyl)-1*H*-indol-6-ol (24). To a solution of **6a** (1.0 g, 3.0 mmol) in THF (20 mL), $LiAlH_4$ (0.2 g, 5.2 mmol) was added at room temperature under an Ar atmosphere. The mixture was stirred for 1 h and decomposed with ethanol (5 mL). The reaction mixture was concentrated and purified by column chromatography (4:1 toluene/methanol) and crystallized with ethanol to give **24** as a white solid (0.15 g, 15.6%): mp 180 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 10.48 (d, J = 1 Hz, 1H), 8.73 (s, 1H), 7.33−7.21 (m, 2H), 7.20−7.09 (m, 4H), 6.70−6.67 (m, 1H), 6.45 (dd, J = 8.4, 2.4 Hz, 1H), 6.08−6.03 (m, 1H), 3.46 (s, 2H), 2.88−2.71 (m, 2H), 2.49 (d, J = 7.0 Hz, 2H), 1.95−1.78 (m, 2H), 1.60−1.38 (m, 3H), 1.30−1.09 (m, 2H) ppm.

2-(4-Benzyl-piperidin-1-sulfonyl)-1*H*-indol-6-ol (25). Step 1: 6-Benzyloxy-2-(4-Benzyl-piperidin-1-sulfonyl)-1*H*-indol (54). A mixture of 6-benzyloxy-1*H*-indole (3.51 g, 15.7 mmol), *t*-Boc_2O (3.68 g, 16.9 mmol), and DMAP (0.18 g, 1.4 mmol) in dichloromethane (50 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated and purified by column chromatography (4:1 hexane/ethyl acetate) to give 6-benzyloxy-indole-1-carboxylic acid *tert*-butyl ester as a white solid (5.03 g, 99.0%): mp 105 °C. Under an Ar atmosphere to a solution of the above *t*-Boc intermediate (1.0 g, 3.1 mmol) in dry THF (10 mL), 2.5 M *n*-butyllithium in hexane (1.25 mL, 3.12 mmol) was added dropwise at −70 °C. After an additional 0.5 h, dry SO_2 gas was introduced over the solution surface for 15 min to give a copious precipitate. This suspension was allowed to warm to room temperature over 2 h and diluted with hexane (30 mL), and the white precipitate was collected by filtration to give lithium sulfinate salt. The salt was suspended in dichloromethane (10 mL) and cooled to 5 °C, and *N*-chlorosuccinimide (0.45 g, 3.37 mmol) was added portionwise. After 2 h, the mixture was filtered and the solvent was evaporated to give a brown oil. This crude sulfonyl chloride was dissolved in THF (10 mL), and 4-benzyl-piperidin (1.1 g, 6.1 mmol) was added. The reaction mixture was stirred for 24 h, concentrated, and purified by column chromatography (4:1 toluene/MeOH) to give **54** as a white solid (0.25 g, 17.5%): ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 11.95 (s, 1 H), 7.56 (d, J = 8.7 Hz, 1H), 7.50−7.06 (m, 10H), 6.98−6.94 (m, 1H), 6.91−6.84 (m, 2H), 5.14 (s, 2H), 3.70−3.58 (m, 2H), 2.45 (d, J = 7.0 Hz, 2H), 2.35−2.20 (m, 2H), 1.70−1.40 (m, 3H), 1.32−1.10 (m, 2H) ppm.

Step 2: 2-(4-Benzyl-piperidin-1-sulfonyl)-1*H*-indol-6-ol (25). To a solution of **54** (0.2 g, 0.43 mmol) in methanol (20 mL), 10% Pd/C catalyst (0.2 g) was added and hydrogenated for 6 h. The catalyst was filtered off, the filtrate was concentrated, and the residue was purified by column chromatography (4:1 toluene/aceton) and crystallized with hexane to give **25** (20 mg): mp 92 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 11.60 (s, 1H), 9.38 (s, 1H), 7.44 (d, J = 8.7 Hz, 1H), 7.28−7.20 (m, 2H), 7.19−7.07 (m, 3H), 6.84−6.81 (m, 1H), 6.80−6.76 (m, 1H), 6.65 (dd, J = 8.7, 2.2 Hz, 1H), 3.68−3.58 (m, 2H), 2.64 (d, J = 7.0 Hz, 2H), 2.34−2.20 (m, 2H), 1.68−1.40 (m, 3H), 1.30−1.10 (m, 2H) ppm.

(4-Benzylpiperidin-1-yl)-(6-hydroxy-1-methyl-1*H*-indol-2-yl)-methanone (55). Step 1: 6-Benzyloxy-1-methyl-1*H*-indole-2-carboxylic Acid (67). Under an Ar atmosphere to a solution of 6-benzyloxy-1*H*-indole-2-carboxylic acid methyl ester (**66**; 1.0 g, 3.5 mmol) in DMF (10 mL), KO*t*-Bu (1.0 g, 8.9 mmol) and iodomethane (0.5 mL, 8.0 mmol) were added at room temperature. The mixture was stirred at this temperature for 24 h and then diluted with water (100 mL). The precipitated crystals were filtered off, washed with water, and recrystallized from isopropanol to give 6-benzyloxy-1-methyl-1*H*-indole-2-carboxylic acid methyl ester (0.85 g, 81%): mp 80−81 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 7.56 (d, J = 8.7 Hz, 1H), 7.53−7.47 (m, 2H), 7.45−7.31 (m, 3H), 7.22−7.16 (m, 2H), 6.87 (dd, J = 8.7, 2.4 Hz, 1H), 5.19 (s, 2H), 5.07 (s, 2H), 3.98 (s, 3H), 3.83 (s, 3H) ppm. A mixture of 6-benzyloxy-1-methyl-1*H*-indole-2-carboxylic acid methyl ester (7.22 g, 24.4 mmol), potassium trimethylsilanolate (4.33 g, 33.7 mmol), and THF (140 mL) was refluxed for 1 h. The reaction mixture was cooled, poured into water (100 mL), and acidified with HCl. The precipitated product was filtered off, washed with water, and dried to give **67** (4.43 g, 64.4%): mp 210−212 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 13.50−11.50 (vbr s, 1H), 7.56 (d, J = 8.7 Hz, 1H), 7.53−7.47 (m, 2H), 7.45−7.30 (m, 3H), 7.20−7.16 (m, 2H), 6.85 (dd, J = 8.7, 2.4 Hz, 1H), 5.19 (s, 2H), 3.98 (s, 3H) ppm.

Step 2: (4-Benzylpiperidin-1-yl)-(6-hydroxy-1-methyl-1*H*-indol-2-yl)-methanone (55). A mixture of **67** (0.8 g, 2.8 mmol), triethylamine (0.4 mL, 2.8 mmol), 4-benzylpiperidine (0.6 g, 3.4 mmol), and HBTU (1.1 g, 2.9 mmol) in DMF (20 mL) was stirred

at room temperature for 6 h. The reaction mixture was concentrated and purified by column chromatography (4:1 toluene/methanol) to give **74** as a white solid (0.5 g, 40%). A mixture of **74** (0.5 g, 1.1 mmol) and 33% HBr solution in AcOH (5 mL) was stirred at room temperature for 10 min. The reaction mixture was diluted with diethyl ether. The precipitated crystals were filtered off, purified by column chromatography (4:1 toluene/MeOH), and crystallized with isopropanol to give **55** as a white solid (0.18 g, 45%): mp 180 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 9.22 (s, 1H), 7.36 (d, J = 8.7 Hz, 1H), 7.32−7.24 (m, 2H), 7.23−7.14 (m, 3H), 6.76−6.73 (m, 1H), 6.63 (dd, J = 8.7, 2.1 Hz, 1H), 6.48 (d, J = 1 Hz, 1H), 4.38−4.16 (m, 2H), 3.61 (s, 3H), 3.04−2.76 (m, 2H), 2.55 (d, J = 7.2 Hz, 2H), 1.91−1.72 (m, 1H), 1.70−1.54 (m, 2H), 1.27−1.08 (m, 2H) ppm.

(4-Benzylpiperidin-1-yl)-(6-hydroxybenzofuran-2-yl)-methanone (56). A mixture of 6-hydroxybenzofuran-2-carboxylic acid (**68**; 0.36 g, 2.0 mmol), triethylamine (0.3 mL, 2.1 mmol), 4-benzylpiperidine (0.35 g, 2.0 mmol), and HBTU (0.76 g, 2.0 mmol) in DMF (10 mL) was stirred at room temperature for 6 h. The reaction mixture was concentrated, and the residue was purified by column chromatography (4:1 toluene/aceton) and crystallized with methanol to give **56** (0.37 g, 55%): mp 183−186 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.08−1.30 (m, 2H), 1.59−1.74 (m, 2H), 1.74−1.94 (m, 1H), 2.55 (d, J = 7.2 Hz, 2H), 2.94 (br m, 2H), 4.22−4.42 (m, 2H), 6.81 (dd, J = 8.5, 2.1 Hz, 1H), 6.92−6.96 (m, 1H), 7.13−7.34 (m, 6H), 7.49 (dd, J = 8.5, 0.5 Hz, 1H), 9.79 (br s, 1H).

(4-Benzylpiperidin-1-yl)-(6-hydroxybenzothiazol-2-yl)-methanone (57). Step 1: 6-Hydroxybenzothiazole-2-carboxylic Acid (70). To a stirred solution of 6-methoxybenzothiazole-2-carboxylic acid (**69**; 8.3 g, 40.0 mmol) in methanol (80 mL), thionyl chloride (4.8 mL, 65.8 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature for 10 h. The precipitated crystals were filtered off and washed with methanol to give methyl 6-methoxybenzothiazole-2-carboxylate (6.0 g, 67%): mp 130−135 °C. To a stirred solution of methyl 6-methoxybenzothiazole-2-carboxylate (6.0 g, 27 mmol) in dichloromethane (60 mL), a solution of BBr_3 (2.6 mL, 27.5 mmol) in dichloromethane (10 mL) was added dropwise at −15 °C. The reaction mixture was stirred at room temperature for 10 h, poured into ice water, and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (2:1 hexane/EtOAc) to give methyl 6-hydroxybenzothiazole-2-carboxylate (2.0 g, 36%): mp 200−206 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.95 (s, 3H), 7.12 (dd, J = 9.0, 2.7 Hz, 1H), 7.49 (dd, J = 2.7, 0.6 Hz, 1H), 8.01 (dd, J = 9.0, 0.6 Hz, 1H), 10.24 (br s, 1H).

A stirred mixture of methyl 6-hydroxybenzothiazole-2-carboxylate (2.0 g, 9.6 mmol), ethanol (100 mL), and KOH (1.1 g, 16.9 mmol) was refluxed for 1 h. The reaction mixture was cooled to 10 °C and the precipitated crystals were filtered off, washed with ethanol, dissolved in water, and acidified with 20% aqueous sulfuric acid. The precipitated crystals were filtered off and washed with water to give **70** (1.85 g, 99%): mp 128−132 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.10 (dd, J = 9.0, 2.4 Hz, 1H), 7.46 (d, J = 2.4 Hz, 1H), 8.00 (d, J = 9.0 Hz, 1H), 10.20 (br s, 1H).

Step 2: (4-Benzylpiperidin-1-yl)-(6-hydroxybenzothiazol-2-yl)-methanone (57). Compound **57** was prepared from **70** and 4-benzylpiperidine according to the procedure described above for **56**: mp 68−70 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.08−1.36 (m, 2H), 1.59−1.98 (m, 3H), 2.55 (d, J = 7.1 Hz, 2H), 2.83 (t, J = 12.2 Hz, 1H), 3.19 (J = 12.3 Hz, 1H), 4.45 (d, J = 12.3 Hz, 1H), 5.21 (d, J = 12.5 Hz, 1H), 7.04 (dd, J = 8.9, 2.5 Hz, 1H), 7.13−7.34 (m, 5H), 7.42 (d, J = 2.5 Hz, 1H), 7.91 (d, J = 8.9 Hz, 1H), 10.07 (br s, 1H).

(4-Benzylpiperidin-1-yl)-(5-hydroxybenzothiophen-2-yl)-methanone (58). Step 1: 1-(4-Benzylpiperidin-1-yl)-1-(5-methoxybenzothiophen-2-yl)-methanone (75). Compound 75 was prepared from 71 and 4-benzylpiperidine according to the procedure described above for 56: mp 128−130 °C (diethyl ether).

Step 2: (4-Benzylpiperidin-1-yl)-(5-hydroxybenzothiophen-2-yl)-methanone (58). To a solution of **75** (0.68 g, 1.9 mmol) in dichloromethane (10 mL), BBr_3 solution in dichloromethane (4 mL, 1 M) was added dropwise at −20 °C. The reaction mixture was stirred at room temperature for 10 h and concentrated. The residue was stirred with 20 mL of 5% aqueous $NaHCO_3$ solution. The precipitated crystals were filtered off and washed with water to give **58** (0.63 g, 94%): mp 162 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10−1.30 (m, 2H), 1.59−1.73 (m, 2H), 1.74−1.92 (m, 1H), 2.55 (d, J = 7.2 Hz, 2H), 2.94 (br m, 2H), 4.25 (br m, 2H), 6.94 (dd, J = 8.7, 2.4 Hz, 1H), 7.14−7.33 (m, 6H), 7.49 (d, J = 0.6 Hz, 1H), 7.75 (d, J = 8.7 Hz, 1H), 9.50 (br s, 1H).

(4-Benzylpiperidin-1-yl)-[5(6)-hydroxy-1*H*-benzimidazol-2-yl]-methanone (60a). Step 1: *N*-(4-Methoxy-2-nitro-phenyl)-oxalamic Acid Ethyl Ester (79). To a solution of **77** (33.6 g, 0.2 mol) and Et_3N (30.8 mL, 0.22 mol) in dichloromethane (1000 mL), ethyl chlorooxoacetate (23.4 mL, 0.21 mol) was added dropwise below 5 °C. The reaction mixture was stirred at room temperature for 24 h, washed with water, dried over Na_2SO_4, and concentrated. The residue was stirred with *n*-hexane (500 mL) and filtered to give **79** (55.1 g, 96.2%): mp 136 °C; ^{1}H NMR (500 MHz, DMSO-d_6, 30 °C) δ 11.12 (s, 1H), 7.89 (d, J = 9.1 Hz, 1H), 7.59 (d, J = 3.0 Hz, 1H), 7.39 (dd, J = 9.1, 3.0 Hz, 1H), 4.33 (q, J = 7.2 Hz, 2H), 3.86 (s, 3H), 1.33 (t, J = 7.2 Hz, 3H) ppm.

Step 2: *N*-(2-Amino-4-methoxy-phenyl)-*N*'-butyl-oxalamide (81). To a suspension of **79** (44.0 g, 164 mmol) in toluene (330 mL), *n*-butylamine (16.8 mL, 170 mmol) was added at room temperature. The reaction mixture was stirred for 10 h and concentrated, and the residue was crystallized with diethyl ether. The precipitated product was filtered off, washed with diethyl ether, and dried to give *N*-butyl-*N*'-(4-methoxy-2-nitro-phenyl)-oxalamide (45.3 g, 93.3%): mp 127−128 °C; ^{1}H NMR (500 MHz, DMSO-d_6, 30 °C) δ 10.25 (vbr s, 1H), 9.06 (t, J = 6.2 Hz, 1H), 8.22 (d, J = 9.1 Hz, 1H), 7.61 (d, J = 3.1 Hz, 1H), 7.39 (dd, J = 9.1, 3.1 Hz, 1H), 3.86 (s, 3H), 3.28−3.20 (m, 2H), 1.56−1.49 (m, 2H), 1.37−1.27 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H) ppm.

Step 3: A mixture of *N*-butyl-*N*'-(4-methoxy-2-nitro-phenyl)-oxalamide (27.0 g, 91 mmol) and Pd/C (7.3 g, 5%) catalyst in methanol (1200 mL) was hydrogenated for 3 h. Acetone (600 mL) was added to the reaction mixture. The catalyst was filtered off and washed with acetone, the filtrate was concentrated, and the residue was crystallized with diethyl ether to give **81** (21.8 g, 90.1%): mp 180−181 °C; ^{1}H NMR (500 MHz, DMSO-d_6, 30 °C) δ 9.72 (s, 1H), 8.81 (t, J = 6.1 Hz, 1H), 7.13 (d, J = 8.6 Hz, 1H), 6.38 (d, J = 2.8 Hz, 1H), 6.20 (dd, J = 8.6, 2.8 Hz, 1H), 4.94 (br s, 2H), 3.68 (s, 3H), 3.24−3.16 (m, 2H), 1.54−1.45 (m, 2H), 1.35−1.24 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H) ppm.

Step 4: 5(6)-Methoxy-1*H*-benzimidazole-2-carboxylic Acid Butylamide (83). Compound **81** (41.0 g, 154 mmol) was stirred at 240 °C for 10 min under N_2. The mixture was cooled to room temperature, diethyl ether (300 mL) was added, and the mixture was stirred for 1 h. The precipitated product was filtered off, washed with diethyl ether, and dried to give **83** (26.5 g, 69.5%): mp 125−126 °C; ^{1}H NMR (500 MHz, DMSO-d_6, 50 °C) δ 12.41 (vbr s, 1H), 8.65 (t, J = 6.0 Hz, 1H), 7.55 (br s, 1H), 7.08 (br s, 1H), 6.94 (dd, J = 8.8, 1.6 Hz, 1H), 3.82 (s, 3H), 3.39−3.30 (m, 2H), 1.62−1.52 (m, 2H), 1.40−1.30 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H) ppm.

Step 5: 5(6)-Hydroxy-1*H*-benzimidazole-2-carboxylic Acid (85). A mixture of **83** (26.0 g, 105 mmol) and 48% aqueous hydrobromic acid (780 mL) was refluxed for 12 h. The mixture was cooled to room temperature, and the precipitated product was filtered off, washed with water until pH neutral, and dried to give **85** (14.3 g, 76.2%): mp 206−207 °C; ^{1}H NMR (300 MHz, D_2O + NaOD, 30 °C) δ 7.38 (m, 1H), 6.70 (m, 2H) ppm.

Step 6: (4-Benzylpiperidin-1-yl)-[5(6)-hydroxy-1*H*-benzimidazol-2-yl]-methanone (60a). Compound **60a** was prepared from **85** and 4-benzylpiperidine according to the procedure described above for **56**: mp 186 °C (toluene); ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 12.61 (s, 1H), 9.40 (s, 1H), 7.50 (d, J = 8.9 Hz, 1H), 7.36−7.24 (m, 2H), 7.23−7.14 (m, 3H), 6.84 (d, J = 2.4 Hz, 1H), 6.74 (dd, J = 8.9, 2.4 Hz, 1H), 5.68−5.50 (m, 1H), 4.61−4.43 (m,

1H), 3.23−3.07 (m, 1H), 2.85−2.70 (m, 1H), 2.55 (d, $J = 7.0$ Hz, 2H), 1.98−1.61 (m, 3H), 1.36−1.03 (m, 2H) ppm.

6-(4-Benzylpiperidin-1-carbonyl)-3,5-dihydro-imidazo[4′,5′:4,5]benzo[1,2-*d*]-oxazol-2-one (64). Step 1: 6-Amino-5-nitro-3*H*-benzoxazol-2-one (89). To a solution of 6-amino-3*H*-benzoxazol-2-one (**88**; 2.0 g, 13.3 mmol) in trifluoroacetic acid (20 mL), sodium nitrate (1.2 g, 14.1 mmol) was added below 20 °C. The reaction mixture was stirred at room temperature overnight and then concentrated. The residue was purified by column chromatography (4:1 toluene/MeOH) to give **89** (2.50 g, 96.0%): mp 198 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 11.64 (br s, 1H), 7.54 (s, 2H), 7.50 (s, 1H), 6.85 (s, 1H) ppm.

Step 2: (4-Benzylpiperidin-1-yl)-oxoacetyl Chloride (87). To a solution of 4-benzylpiperidine (10.0 g 57 mmol) and *N*-ethyldiisopropylamine (10 mL, 57.4 mmol) in dichloromethane (100 mL), ethyl oxalyl chloride (7.05 mL, 63.1 mmol) was added dropwise at 0 °C. The mixture was stirred at this temperature for 30 min. The organic phase was washed with water, dried on Na_2SO_4, and concentrated to give (4-benzylpiperidin-1-yl)-oxoacetic acid ethyl ester (15.5 g, 99%) as a brown oil. A mixture of (4-benzylpiperidin-1-yl)-oxoacetic acid ethyl ester (15.5 g, 56 mmol) and KOH (5.0 g, 75.9 mmol) in methanol (250 mL) was stirred at room temperature for 6 h. The reaction mixture was concentrated, and the residue was taken up in water and acidified with 1 N HCl. The precipitated product was filtered off, washed with water, and dried to give (4-benzylpiperidin-1-yl)-oxoacetic acid (11.95 g, 85%): mp 115 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91−1.23 (m, 2H), 1.51−1.92 (m, 3H), 2.53 (d, $J = 7.1$ Hz, 2H), 2.64 (td, $J = 12.7$, 3.0 Hz, 2H), 3.05 (td, $J = 12.4$, 2.7 Hz, 2H), 3.46−3.58 (m, 2H), 4.12−4.27 (m, 2H), 7.12−7.35 (m, 5H), 13.96 (br s, 1H). A mixture of (4-benzylpiperidin-1-yl)-oxoacetic acid (26.2 g, 106 mmol) and thionyl chloride (50 mL) was refluxed for 2 h. The mixture was cooled and concentrated to give **87** (28.0 g, 99.5%) as an oil.

Step 3: 2-(4-Benzylpiperidin-1-yl)-*N*-(5-nitro-2-oxo-2,3-dihydro-benzoxazol-6-yl)-2-oxo-acetamide (90). To a solution of **89** (3.23 g, 16.56 mmol) and triethylamine (2.58 mL, 18.5 mmol) in chloroform (100 mL), **87** (5.25 g, 19.75 mmol) in chloroform (20 mL) was added dropwise at 20 °C. The reaction mixture was stirred at room temperature for 2 h. The organic phase was washed with water, dried on Na_2SO_4, and concentrated. The residue was purified by column chromatography (4:1 toluene/MeOH) to give **90** (3.3 g, 47.0%) as an amorphous solid foam: ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 12.16 (br s, 1H), 11.15 (s, 1H), 7.73−7.69 (m, 2H), 7.34−7.25 (m, 2H), 7.24−7.14 (m, 3H), 4.41−4.19 (m, 1H), 4.15−3.97 (m, 1H), 3.16−2.99 (m, 1H), 2.80−2.64 (m, 1H), 2.55 (d, $J = 7.1$ Hz, 2H), 1.95−1.75 (m, 1H), 1.75−1.53 (m, 2H), 1.33−1.02 (m, 2H) ppm.

Step 4: 6-(4-Benzylpiperidin-1-carbonyl)-3,5-dihydro-imidazo[4′,5′:4,5]benzo-[1,2-*d*]oxazol-2-one (64). A mixture of **90** (3.3 g, 7.7 mmol) and Pd/C (0.3 g, 10%) catalyst in methanol (30 mL) was hydrogenated for 8 h. The catalyst was filtered off, and the filtrate was concentrated. The residue was purified by column chromatography (2:1 toluene/acetone) to give *N*-(5-amino-2-oxo-2,3-dihydro-benzoxazol-6-yl)-2-(4-benzylpiperidin-1-yl)-2-oxo-acetamide (1.06 g, 34.5%): mp 296 °C. An aliquot of this material (1.0 g, 2.5 mmol) was heated to 240 °C for 10 min and then cooled. The obtained mixture was purified by column chromatography (1:1 toluene/acetone) to give **64** (0.16 g, 17%): mp >290 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 13.00 (vbr s, 1H), 11.70 (vbr s, 1H), 7.53 (br s, 1H), 7.40−7.04 (m, 6H), 5.68−5.43 (m, 1H), 4.65−4.42 (m, 1H), 3.28−3.09 (m, 1H), 2.87−2.71 (m, 1H), 2.55 (d, $J = 7.0$ Hz, 2H), 1.97−1.77 (m, 1H), 1.77−1.60 (m, 2H), 1.37−1.05 (m, 2H) ppm.

7-(4-Benzylpiperidin-1-carbonyl)-1,6-dihydro-3-oxa-1,6,8-triaza-as-indacen-2-one (65). Step 1: 5(6)-Hydroxy-4(7-)nitro-1*H*-benzimidazole-2-carboxylic Acid (91). To a solution of **85** (2.0 g, 11.2 mmol) in trifluoroacetic acid (25 mL), sodium nitrate (1.0 g, 11.7 mmol) was added in several portions below 20 °C. The mixture was stirred at 20 °C for 2 h. The reaction mixture was poured into ice water, and the precipitated product was filtered off, washed with water, and dried to give **91** (1.7 g, 68%): mp 218 °C.

Step 2: (4-Benzylpiperidin-1-yl)-(5(6)-hydroxy-4(7)-nitro-1*H*-benzimidazol-2-yl)methanone (92). Compound **92** was prepared from **91** and 4-benzylpiperidine according to the procedure described above for **56**: mp 102 °C (diethyl ether); ^{1}H NMR (500 MHz, DMSO-d_6, 50 °C) δ 12.68 (s, 1H), 7.84 (br s, 1H), 7.32−7.24 (m, 3H), 7.22−7.13 (m, 2H), 7.08−6.91 (m, 1H), 5.40−4.30 (m, 2H), 3.24−3.06 (m, 1H), 2.83−2.72 (m, 1H), 2.56 (d, $J = 7.1$ Hz, 2H), 1.94−1.81 (m, 1H), 1.79−1.56 (m, 2H), 1.33−1.12 (m, 2H) ppm.

Step 3: (4(7)-Amino-5(6)-hydroxy-1*H*-benzimidazol-2-yl)-(4-benzylpiperidin-1-yl)methanone (93). A mixture of **92** (1.0 g, 2.6 mmol) and Pd/C (0.4 g, 10%) catalyst in methanol (30 mL) was hydrogenated for 2 h. The catalyst was filtered off, and the filtrate was concentrated. The residue was treated with diethyl ether, and the crystalline product was filtered, washed with diethyl ether, and dried to give **93** (0.5 g, 54%): mp 108 °C; ^{1}H NMR (500 MHz, DMSO-d_6, 30 °C) δ 12.43 (br s, 1H), 8.72 (br s, 1H), 7.32−7.25 (m, 3H), 7.22−7.15 (m, 2H), 6.81 (d, $J = 8.6$ Hz, 1H), 6.75 (d, $J = 8.6$ Hz, 1H), 5.79−5.67 (m, 1H), 4.75 (s, 2H), 4.65−4.44 (m, 1H), 3.22−3.10 (m, 1H), 2.83−2.71 (m, 1H), 2.55 (d, $J = 7.1$ Hz, 2H), 1.94−1.76 (m, 1H), 1.76−1.61 (m, 2H), 1.31−1.08 (m, 2H) ppm.

Step 4: 7-(4-Benzylpiperidin-1-carbonyl)-1,6-dihydro-3-oxa-1,6,8-triaza-as-indacen-2-one (65). To a solution of **93** (0.45 g, 1.28 mmol) in THF (5 mL), 1,1′-carbonyldiimidazole (0.2 g, 1.37 mmol) was added at 20 °C. The reaction mixture was stirred at room temperature for 2 h and then concentrated. The residue was purified by column chromatography (4:1 toluene/MeOH) and crystallized with isopropanol to give **65** (0.45 g, 93.5%): mp >270 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 13.30 (br s, 1H), 12.22 (br s, 1H), 7.40−7.15 (m, 7H), 5.40−5.25 (m, 1H), 4.60−4.46 (m, 1H), 3.28−3.14 (m, 1H), 2.89−2.75 (m, 1H), 2.56 (d, $J = 7.0$ Hz, 2H), 1.98−1.60 (m, 3H), 1.35−1.10 (m, 2H) ppm.

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Supporting Information Available: Experimental details and data for compounds **6b**−**6p**, **7**, **8**, **9**, **11**, **13a**−**14a**, **17a**, **18a**, **19**, **21**, **22**, **59**, **60m**−**60u**, **61a**, **62**, and **63**. HPLC and HRMS analysis of the final compounds. Experimental and calculated biological activities (expressed as $-\log IC_{50}$) of compounds used in the CoMSIA study. Structures of molecules in the CoMSIA study. This material is available free of charge via the Internet at http://pubs.acs.org.

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