that of Hibert in which he more exactly defined the spatial geometry of molecules in their active conformation.[16,17] This concern regarding steric hindrance appears to be confirmed by 4-quinazolinone **13**. This compound contains a larger 2-functionality and was found to be almost 2000-fold less potent than **11d**. This steric intolerance at the 2-position may also explain the lower potency of quinuclidine **12b** compared with tropane **11d**. In the corresponding benzamides, tropane **7** and quinuclidine **6** are of approximately equal potency.[15] The quinuclidine, therefore, probably has a lower degree of steric tolerance than the tropane.

The results presented for the benzotriazines support the hypothesis that, in the active conformation of the benzamides, the orientation of the benzamide is as expected, with the carbonyl oxygen pointing toward the 6-position and the NH hydrogen bonded to the *o*-methoxy group. This is the exact opposite to the orientation recently proposed by Schmidt and Peroutka, where simple structural overlap criteria were applied without consideration of conformational energies.[18] Indeed it is physically impossible for the benzotriazines to adopt the conformation proposed for the benzamides by these authors. In contrast, the relative restriction of freedom, with consequential structural definition, is wholly consistent with our own and Hibert's model with regard to the orientation of the carbonyl and basic side chain. The positional requirements for the aromatic ring are less clear. There is no possible overlap between the aromatic rings of the benzotriazines and the indolines related to **4**. The necessary requirement of both a 5-chloro and 4-amino group in the benzamides, and equivalent substitution in the benzotriazines, therefore suggests that these functionalities are complimenting the structural requirements met by the benzo-fused ring in the 6,5-bicyclic and carbazole classes of highly potent 5-HT_3 receptor antagonists.

Acknowledgment. The authors are indebted to D. N. Nelson for the receptor binding studies on compound **11d**.

Supplementary Material Available: Spectral and physical data for **8a–c, 11a–d, 12b, 13** (2 pages). Ordering information is given on any current masthead page.

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Frank D. King,* Steven Dabbs
Jose Bermudez, Gareth J. Sanger
SmithKline Beecham Pharmaceuticals
Coldharbour Road
The Pinnacles
Harlow, Essex
England

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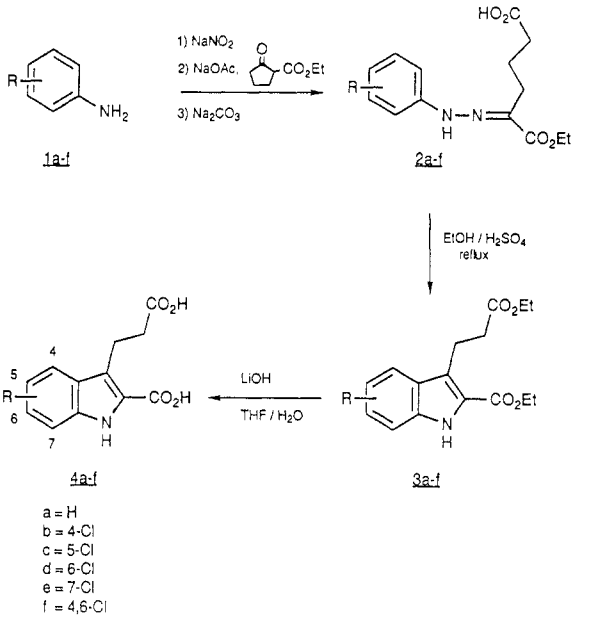
3-(2-Carboxyindol-3-yl)propionic Acid Derivatives: Antagonists of the Strychnine-Insensitive Glycine Receptor Associated with the *N*-Methyl-D-aspartate Receptor Complex

During the past 15 years evidence has accumulated implicating the acidic amino acids, glutamic acid and aspartic acid, as excitatory neurotransmitters in the mammalian central nervous system. Several distinct glutamate receptor complexes have been identified and classified according to the relatively selective ligands *N*-methyl-D-aspartic acid (NMDA), kainic acid, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). From a potential therapeutic point of view, the NMDA receptor complex has attracted considerable interest. Overstimulation of this receptor has been implicated in the etiology of several neurodegenerative disorders[1] and may play a role in human epilepsy.[2] This particular receptor complex possesses several allosteric binding sites which can alter cellular responses to glutamic acid. In particular, glycine acting at a strychnine-insensitive binding site has been shown to facilitate the action of glutamic acid[3] and, more recently, has been suggested as a cotransmitter required for activation of the NMDA receptor.[4-8] The role of glycine has largely been deduced from the actions of quinoline,[5,6] quinoxaline,[7] and aminopyrrolidinone,[8] or indole[9] antagonists which possess varying degrees of selectivity for the glycine site. A potent, selective antagonist of this glycine binding site, therefore, may have potential clinical applications as an anticonvulsant or neuroprotective agent. In this communication we report that 3-(4,6-dichloro-2-carboxyindol-3-yl)-propionic acid (**4f**) and other indolepropionic acid derivatives represent a new class of selective NMDA antagonists acting at the strychnine-insensitive glycine binding site.

Scheme I



Indoles **4a–f** were synthesized in good yields by utilizing the Japp–Klingemann reaction.[10] In general, hydrazones **2a–f** were prepared by condensation of 2-(ethoxycarbonyl)cyclopentanone with the corresponding benzenediazonium salt, isolated, and cyclized without further purification under Fischer indole reaction conditions

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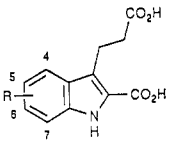
Table I

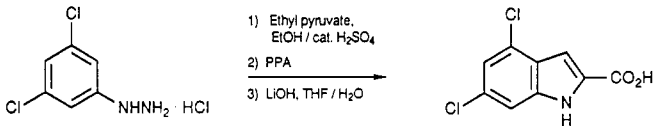


4a-f

compound[a]	R	mp,[b] °C	% yield (from aniline)	IC_{50}, μM [^{3}H]gly[c]	[^{3}H]CPP[d]	c-GMP[e]	CPP/gly
4a	H	184–6	40	30	34	ND	1
4b	4-Cl	255–6	14	6	59	ND	10
4c	5-Cl	249–50	54	14	25	ND	1.8
4d	6-Cl	234–6	8	2.7	283	ND	105
4e	7-Cl	255–6	14	90	>100	ND	>1
4f	4,6-Cl_2	273–80	40	0.14	358	5.0	2550
4,6-dichloroindole-2-carboxylic acid (**5**)		238–9	85[f]	6	>1000	ND	>150
indole-2-carboxylic acid (**6**)				106	4200	ND	40
kynurenic acid (**7**)				16	75	167	5
7-chlorokynurenic acid (**8**)				0.4	162	8	405
5,7-dichlorokynurenic acid (**9**)[g]				0.08	12	2.5	150

[a] Satisfactory elemental analysis (C, H, N) were obtained on compounds **4a–f** and **5**. [b] Recrystallized from EtOAc/hexane. [c] Competition assay against tritiated glycine for rat cortical and hippocampal membrane strychnine-insensitive glycine binding sites. [d] Competition assay against tritiated CPP for rat cortical and hippocampal membrane glutamate binding sites. [e] Inhibition of glutamate stimulated accumulation of cyclic GMP in neonatal rat cerebral slices. [f] Yield from 3,5-dichlorophenylhydrazine hydrochloride. [g] From ref 4. ND = not determined.

Scheme II



5

(Scheme I). The resulting diesters (**3a–f**) were purified by flash silica gel chromatography (EtOAc/hexane) followed by recrystallization. In the case of the isomeric pairs **3b** and **3d**, prepared in equimolar proportions from 3-chloroaniline, separation was most conveniently achieved at this step in the synthesis. The relatively deactivated dichloro derivative **2f** cyclized poorly when treated with ethanol/H_2SO_4 (25% yield, 24-h reflux); however, the resulting diethyl ester of **2f** could be cleanly converted to **3f** in high yield by treatment with *p*-toluenesulfonic acid (2 equiv) in refluxing toluene for 1 h. Saponification of **3a–f** was achieved with 3 equiv of LiOH in aqueous tetrahydrofuran, whereupon the desired diacids **4a–f** were obtained in good yield and purified by recrystallization (EtOAc/hexane). Finally, 4,6-dichloroindole-2-carboxylic acid (**5**) was prepared for comparison against the known indole-2-carboxylic acid (**6**) (Scheme II). Thus, the hydrazone prepared from commercially available 3,5-dichlorophenylhydrazine hydrochloride and ethyl pyruvate in ethanol containing concentrated H_2SO_4 was cyclized in high yield utilizing polyphosphoric acid at 95 °C. Compounds **3a–f**, **4a–f**, and **5** were all fully characterized by elemental analysis (C, H, N), 300-MHz NMR, IR, and MS.

Indoles **4a–f** were evaluated for their ability to compete for the strychnine-insensitive binding of tritiated glycine in assays using rat cortical and hippocampal membranes as described in the literature.[11] The IC_{50} values presented in Table I represent the concentration of compound required to reduce glycine binding by 50%. Similar experiments were performed with labeled 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP)[11] in order to evaluate the affinity of these compounds for the glutamate recognitions site on the NMDA receptor. In the case of **4f**, these experiments were extended to include [^{3}H]kainic acid[12] and [^{3}H]AMPA.[13] The most potent compound (**4f**) was identified as an antagonist by its ability to inhibit NMDA-stimulated accumulation of cyclic GMP[11] (see Table I).

The results of these binding studies (Table I) reveal that 3-(2-carboxyindol-3-yl)propionic acid derivatives represent a new class of glycine antagonists and illustrate several important structural features. The unsubstituted lead compound **4a** (IC_{50} = 30 μM) compared favorably with kynurenic acid (**7**) (IC_{50} = 16 μM), a known, nonselective antagonist, and was about 3-fold more potent than indole-2-carboxylic acid (**6**) (IC_{50} = 106 μM). The effect of chlorine substitution into the benzene ring of the indole nucleus produced substantial changes in binding potency, an effect which has been previously recognized in a series of kynurenic acid analogues.[6,14] Incorporation of chlorine into either the 4-, 5-, or 6-position (**4b–d**) resulted in enhanced binding, the 4- and 6-chloro derivatives being the most potent (5- and 10-fold more potent than **4a**, respectively). 7-Chloroindole analogue **4e**, however, had less affinity for the receptor. Incorporation of chlorine atoms into both the 4- and 6-positions (**4f**) afforded one of the most potent glycine antagonists known to date with an IC_{50} value of 0.14 μM, considerably more potent than would be predicted from the activities of the monochloro analogues **4b** and **4d**. This compound is over 200-fold more potent than the parent compound **4a** and 50-fold more active than 4,6-dichloroindole-2-carboxylic acid (**5**), which lacks the propionic acid side chain. Furthermore, it is essentially equipotent with 5,7-dichlorokynurenic acid (**9**).[4]

Introduction of chlorine atoms into these molecules, especially at the 6-position, has a second important consequence. Whereas 3-(2-carboxyindol-3-yl)propionic acid (**4a**) is equipotent at both the glycine and glutamate binding sites of the receptor complex, the 6-chloro (**4d**) and 4,6-dichloro (**4f**) analogues are not only more potent at the glycine site but have significantly less affinity for the

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glutamate site (Table I). In fact, **4f** is 2550-fold selective for the glycine site on the basis of these ligand-binding studies. Although we have not extensively studied the structure–activity relationships of this series of compounds at the other excitatory amino acid binding site, we did establish that **4f** is highly selective toward the glycine site compared to both the kainate (IC_{50} = 418 μM) and AMPA (IC_{50} = 273 μM) sites, respectively. In retrospect, the affinity of **4a** for the glutamate binding site can be rationalized on the basis of the structural relationship with the amino dicarboxylic acids, glutamic acid and aspartic acid.

In terms of structure–activity relationships, there are several important aspects which can be demonstrated from this work. Chlorine atom substitution in the 4- and 6-positions of the indole ring, but not the 5- and 7-positions, plays an important role in determining antagonist potency. Preliminary molecular modeling studies indicate that a combination of dipole effects and subtle pH changes due to chlorine substitution appear to correlate with antagonist activity.[15] The enhancing effect of the propionic acid side chain is an important discovery and suggests that there is another pocket in the receptor whose occupancy can lead to even more potent antagonists. This speculation is consistent with other reports from our group.[14] We are currently exploring the nature of this secondary binding site in an extensive structure–activity study. In conclusion, we have discovered a new series of potent antagonists of the strychnine-insensitive glycine binding site associated with the NMDA receptor complex. These compounds should allow for a more detailed understanding of the important features which define the pharmacophore for this receptor.[16]

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(16) In vivo data indicate that **4f** has anticonvulsant activity against quinolinic acid induced seizures in mice (ED_{50} = 2 μg icv).

Francesco G. Salituro,* Boyd L. Harrison
Bruce M. Baron, Philip L. Nyce
Kenneth T. Stewart, Ian A. McDonald
Merrell Dow Research Institute
Cincinnati Center
2110 E. Galbraith Road
Cincinnati, Ohio 45215
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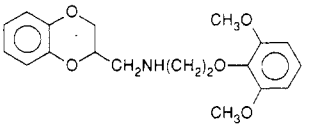
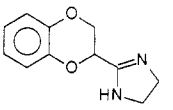
3-Phenyl Analogues of 2-[[[2-(2,6-Dimethoxyphenoxy)ethyl]amino]-methyl]-1,4-benzodioxan (WB 4101) as Highly Selective α_1-Adrenoreceptor Antagonists[1]

A benzodioxan nucleus bearing an appropriate substituent at position 2 can discriminate markedly among α-adrenoreceptor subtypes. In fact, 1 (WB 4101)[2] and 2 (idazoxan, RX 781094),[3] both carrying a 1,4-benzodioxan-2-yl moiety as a basic feature but having a different 2-substituent, are highly selective for α_1- and α_2-adrenoreceptors, respectively. A variety of 1 and 2 analogues have been studied involving a modification of the dehydrodioxane ring. Compounds in which one of the two oxygen atoms has been replaced by a methylene,[4-6] carbonyl,[4] or sulfur[4,5,7,8] and those in which the ring size has been altered to give furan,[6,9] indole,[4] and naphthalene[4,5] derivatives were investigated. All these structural modifications have shown that (a) the oxygens at positions 1 and 4 of the benzodioxan moiety play a different role in receptor binding and (b) replacement of the dehydrodioxane ring by other systems gives rise to a drop in affinity toward α_1-adrenoreceptors.[4] None of these manipulations performed on the structure of 1 has led to a significant improvement of affinity or selectivity for α-adrenoreceptors.



1 (WB 4101)



2 (Idazoxan)

Since 1 is a very potent α_1-adrenoreceptor antagonist an improvement of its affinity would not represent a major achievement unless there is also a concomitant increase in selectivity. The objectives of this study were to improve the selectivity toward α_1-adrenoreceptors by modifying the dehydrodioxane ring of 1. The starting point was the observation that replacement of a hydrogen at position 2 or 3 of **2** with a substituent such as a methyl can dramatically alter the drug–receptor interaction.[10] In fact, the 3-methyl analogue of **2** turned out to be a very weak α-antagonist compared to both **2** and its 2-methyl analogue.[10] Thus, it appears that the 3-position in 1,4-benzodioxan-bearing compounds might be crucial for the affinity toward α_2-adrenoreceptors. We thought that the introduction of a substituent such as a phenyl ring at position 3 of **1** could decrease the affinity for α_2-adrenoreceptors while leaving hopefully unaffected that for α_1-adrenoreceptors, thus giving rise to an improvement of the α_1-selectivity. To this end, we describe here the synthesis and the pharmacological profile in the isolated rat vas deferens of isomers **4** and **5**. Moreover, compound **6** was included in this study to verify whether the insertion of a phenyl ring in the structure of **3**, which displays a biological profile close to that of 1,[4] causes an effect on α-adrenoreceptor blocking activity similar to that observed

(1) Part 3 of a series entitled: Structure-Activity Relationships in 1,4-Benzodioxan-Related Compounds. For Parts 1 and 2, see, respectively, ref 4 and Giardinà, D.; Gulini, U.; Piloni, M. G.; Melchiorre, C. *Farmaco Ed. Sci.*, in press.
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