Benzimidazoles as NMDA Glycine-Site Antagonists: Study on the Structural Requirements in 2-Position of the Ligand

Gerd Dannhardt* and Beate K. Kohl

Department of Pharmacy, Johannes Gutenberg University, D-55099 Mainz, Germany

Key Words: *NMDA-receptor; glycine antagonists; benzimidazoles; binding studies; structure-activity relationships*

Summary

A series of different substituted benzimidazole derivatives has been synthesized and evaluated for the ability to displace [^{3}H]MDL-105,519 to rat cortical membranes. Two benzimidazole-2-carboxylic acids **9 b** and **9 c,** in this substitution pattern not yet described as glycine antagonists, showed IC_{50} values of 0.89 µM (**9 b**) and 38.0 µM (**9 c**). Replacement of the carboxylate function in 2-position by a sulfonic acid moiety appreciably increased solubility, but decreased the affinity giving evidence for the strong need of the carboxylate group within the ligand. Further structure-activity studies using benzimidazol-2-one derivatives with an acetic acid moiety adjacent to a ring nitrogen revealed new insights into the importance of amide functionalities within the heterocycle for the affinity of antagonist glycine-site ligands.

Introduction

Antagonists acting on the strychnine-insensitive glycine site (glycine$_B$) of the NMDA (*N*-methyl-D-aspartate) receptor have long been considered as promising therapeutic agents for a huge number of acute and chronic neurodegenerative disorders [1]. Recent studies [2] on glycine$_B$ antagonists as potential therapeutics, however, indicate that the wide range of putative applications for this class of drugs might have to be restricted to a core of disorders including epilepsy (status epilepticus) and central ischaemic processes, which are of a special pharmaceutical interest considering the high morbidity and invalidity associated with these emergencies. A further field of interest, in which glycine-site antagonists were proved to be active is hyperalgesia suggesting that glycine antagonists could present a new analgesic class [3]. The advantages of glycine antagonists over other NMDA receptor antagonists include the lack of psychotomimetic side effects as well as the absence of vacuolisation in the cingulate cortex. However, glycine$_B$ antagonists are not totally free of side effects. The side effect profile previously observed includes sedation, ataxia, and myorelaxation [2].

A difficulty still facing medicinal chemists designing glycine antagonists is to attain the triad: affinity in vitro, potency in vivo, and satisfactory solubility in physiological media [1]. The glycine unit incorporated in many antagonistic ligands for the glycine site gives rise to an often decreased in vivo activity and unfavourable properties concerning the physicochemical profile, i.e. the hydrophilic-lipophilic balance and solubility. Replacement of the carboxylate function in 2-position by other acidic but solubility enhancing groups could therefore present a possibility to afford new compounds with altered bioavailability.

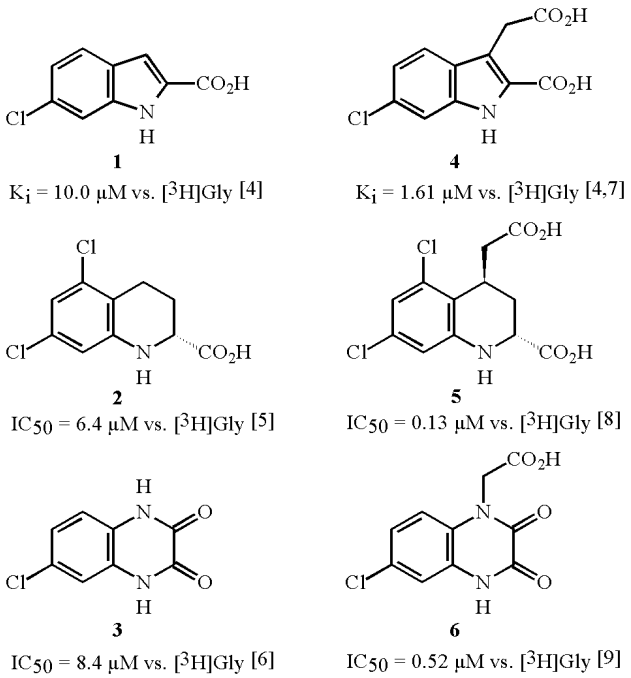

Figure 1

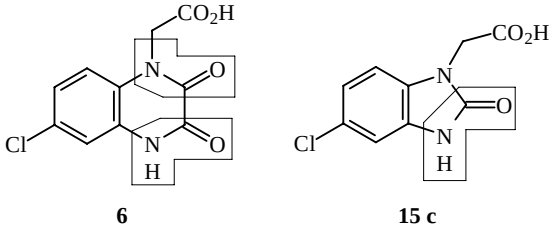

Figure 2

Generally, the carboxylate function plays a role of interest concerning the molecular architecture of the glycine binding site since it could be demonstrated for diverse heterocycles, i.e. indoles, tetrahydroquinolines, and quinoxaline-2,3-diones (Figure 1), that a carboxylate group introduced into the northern region of the ligand causes an increase in affinity giving evidence for a second acidic binding pocket in the receptor protein.

We therefore decided to evaluate a series of benzimidazoles bearing a sulfonic acid function instead of a carboxylate group in 2-position of the heterocycle in order to increase the solubility and to refine structure-activity relationships according to the 2-position.

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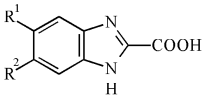
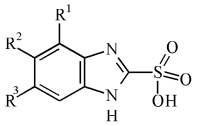
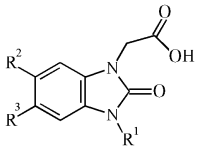
Table 1. Structures of benzimidazole-2-carboxylic acids and benzimidazole-2-sulfonic acids.

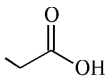
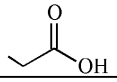




Cpd.	R^1	R^2	Cpd.	R^1	R^2	R^3
9 a	H	Cl	**11 a**	H	H	F
9 b	Cl	Cl	**11 b**	H	H	Cl
9 c	H	NO_2	**11 c**	Cl	H	Cl
			11 d	H	Cl	Cl
			11 e	H	H	Br
			11 f	Br	H	Br
			11g	H	H	CH_3
			11 h	H	CH_3	CH_3

Table 2. Structures of benzimidazolones.



Cpd.	R^1	R^2	R^3
15 a	H	H	H
15 b	H	H	Cl
15 c	H	Cl	H
18 a		H	H
18 b		H	Cl

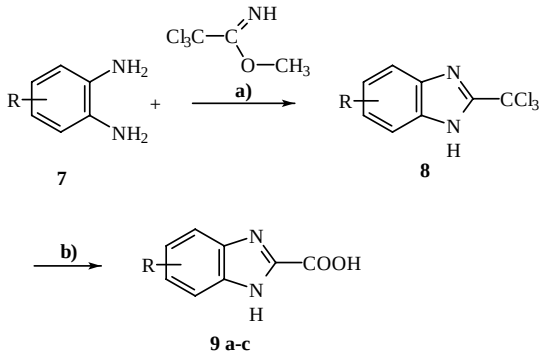
A further study based on a small series of benzimidazolones with the favourable carboxylate function in the northern region of the molecule should clarify the importance of an amide structure in 2-position of the heterocycle (compound **15 c**) relative to the bis-amide structure (compound **6**) as depicted in Figure 2.

Chemistry

The compounds, which were synthesized and evaluated for their ability to displace [^{3}H]MDL-105,519, are summarized in Tables 1 and 2. The physical data of compounds **9 a–c**, **11 a–h**, **15 a–c** and **18 a, b** are given in Table 3.

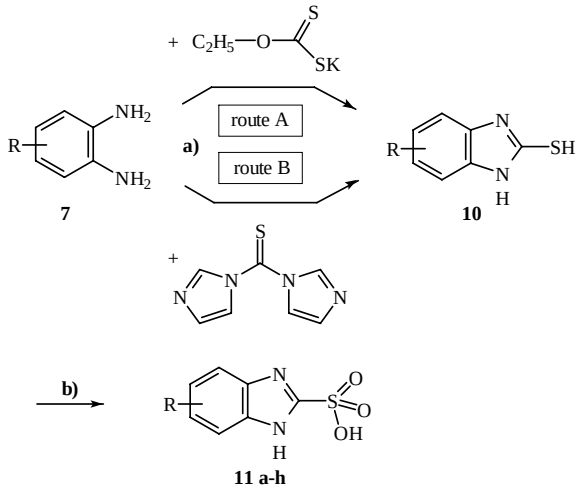
Synthesis of the benzimidazole-2-carboxylic acid derivatives **9 a–c** was carried out as depicted in Scheme 1. According to a procedure described by Louvet et al. [10] the *o*-phenylenediamine derivative **7** was reacted with trichloromethyl acetimidate in acetic acid to give the corresponding trichloromethylbenzimidazole derivative **8**, which was isolated and treated with a solution of sodium hydroxide to afford the carboxylic acids **9 a–c**.

The preparation of the benzimidazole-2-sulfonic acid derivatives **11 a–h** was performed according to Scheme 2. For the routine synthesis of the benzimidazole-2-thiol derivative **10** the appropriate *o*-phenylenediamine **7** was refluxed with



Scheme 1. a) CH_3COOH, 1 h, room temp. b) 1 N NaOH.

potassium ethyl xanthate in ethanol 95% (route A) [11]. As an alternative synthetic pathway to afford **10** under milder conditions suitable for readily oxidizing diamines, compound **7** was reacted with 1,1′-thiocarbonyldiimidazole in dry tetrahydrofuran as exemplified for the 5,6-dichloro-benzimidazole-2-thiol (route B). The thiol group of compound **10** was easily transferred into the sulfonic acid moiety by an oxidation procedure using H_2O_2 10% and sodium hydroxide under reflux conditions [12]. Purification from hot water yielded compounds **11 a–h**.



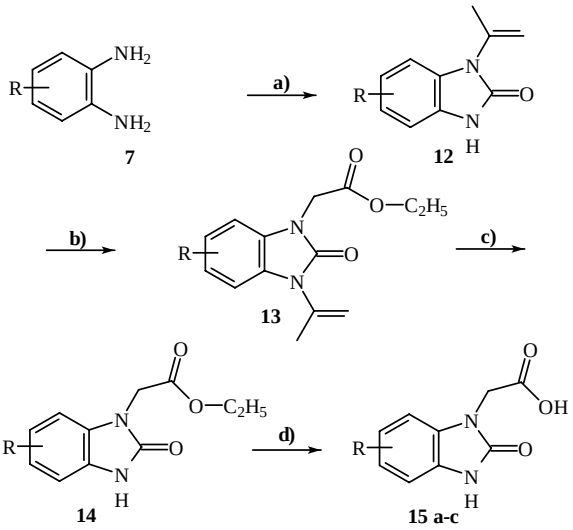
Scheme 2. a) route A: EtOH 95%, reflux, 3 h; route B: THF, 3 h, 0 °C and 12 h room temp. b) H_2O_2 10%, NaOH, reflux.

Compounds **15 a–c** were synthesized as shown in Scheme 3. The isopropenyl intermediate **12** was prepared by reacting the *o*-phenylenediamine derivative **7** with ethyl acetoacetate according to Rossi et al. [13] and Gómez-Parra et al. [14]. Compound **12** was converted into the ester derivative **13** following a standard procedure [15] using freshly prepared sodium ethoxide and ethyl bromoacetate. After acidic cleavage of the isopropenyl moiety with diluted sulfuric acid and saponification of the ethyl ester using 2 N sodium hydroxide, the carboxylic acids **15 a–c** were precipitated by acidification of the reaction mixture with concentrated hydrochloric acid.

Table 3. Physical data of the final compounds.

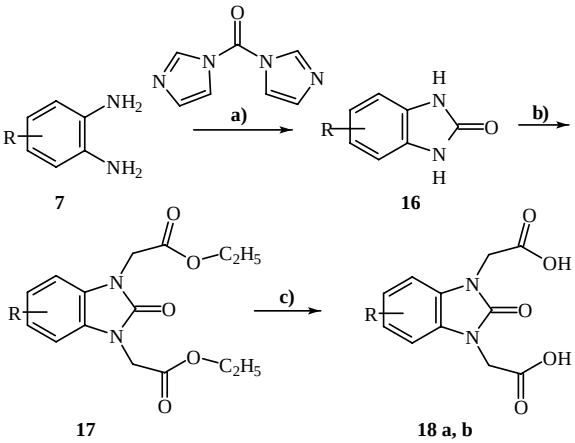
Cpd.	Mp. (°C)	IR (KBr)	^{1}H-NMR (200 MHz), δ (ppm), *J* (Hz)/ MS (EI, 70 eV): *m/z*
9 a	162	3500 s (NH), 2500 w (-COOH), 1680–1650 s (-C=N-; –C=O), 1600 s (arom.)	7.22 (d, 1H, *J* = 1.46, Ar-H), 7.34 (d, 1 H, *J* = 8.78, Ar-H), 7.66 (d, 1 H, *J* = 8.79, Ar-H), 8.28 (s, 1 H, NH) 198 ($M^{+\bullet}$, 0.1%), 154 (32%), 125 (13%)
9 b	220	3480 s (NH), 2500 w (-COOH), 1680–1650 s (-C=N-; C=O), 1600 s (arom.)	7.85 (s, 2 H, Ar-H) 190 [$(M - CO_2)^+$, 9%], 188 (56%), 186 (88%), 159 (10%)
9 c	192	3460 s (NH), 2500 w (-COOH), 1680-1650 s (-C=N-; C=O), 1600 s (arom.), 1530 s (N=O)	7.78 (d, 1 H, *J* = 2.93, Ar-H), 8.11 (d, 1 H, *J* = 9.20, Ar-H), 8.22 (s, 1 H, NH), 8.53 (d, 1 H, *J* = 9.20, Ar-H) 207 ($M^{+\bullet}$, 0,34%), 164 (9%), 163 (100%), 44 (43%)
11 a	>300	3530 s (-OH), 3480 s (NH), 1640 s (-C=N-), 1610 m (arom.), 1260 m (SO_2-OH), 1060 s (-SO_2-OH)	7.39 (d, 1 H, *J* = 1.96, Ar-H), 7.46 (d, 1 H, *J* = 8.3, Ar-H), 7.50 (d, 1 H, *J* = 8.3, Ar-H) 216 ($M^{+\bullet}$, 7%), 136 (100%), 109 (44%)
11 b	>300	3560 s (-OH), 3480 s (NH), 1640 s (-C=N-), 1250 s (SO_2-OH), 1080 s (-SO_2-OH)	7.54 (d, 1 H, *J* = 1.47, Ar-H), 7.63 (dd, 1 H, *J* = 7.32, Ar-H), 7.70 (d, 1 H, *J* = 7.32, Ar-H) MS (FD)[a]: 234 ($M^{+\bullet}$, 5%), 232 (4%)
11 c	>250	3590 s (-OH), 3460 s (NH), 1640 s (-C=N-), 1610 s (arom.), 1240 s (SO_2-OH), 1070 s (-SO_2-OH)	6.70 (s, 1 H, NH), 7.49 (m, 2 H, Ar-H) 267 ($M^{+\bullet}$, 62%), 234 (56%), 196 (62%), 174 (65%)
11 d	>300	3440 s (NH), 1670 s (-C=N-), 1610 s (arom.), 1270 s (SO_2-OH), 1070 s (-SO_2-OH)	7.9 (s, 2 H, Ar-H) MS (FD)[a]: 268 ($M^{+\bullet}$, 3%)
11 e	>250	3540 s (-OH), 3460 s (NH), 1640 s (-C=N-), 1610 s (arom.), 1270 s (SO_2-OH), 1060 s (-SO_2-OH)	7.54 – 7.73 (m, 3 H, Ar-H)) 186 [$(M - 91)^+$, 3%], 118 (100%), 93 (63%), 80 (13%), 79 (11%)
11 f	>300	3540 s (-OH), 3460 s (NH), 1640 s (-C=N-), 1600 s (arom.), 1270 s (SO_2-OH), 1070 s (C-Br)	7.63 (d, 1 H, *J* = 1.47, Ar-H), 7.69 (d, 1 H, *J* = 1.47, Ar-H) 355 ($M^{+\bullet}$, 2%), 353 (1%), 275 (20%), 77 (100%)
11g	>300	3460 s (NH), 2960 s (-CH_3), 1640 s (-C=N-), 1610 s (arom.), 1470 s (CH_3), 1260 s (-SO_2-OH), 1060 s (-SO_2-OH)	2.45 (s, 3 H, -CH_3), 7.48 (m, 3 H, Ar-H) 212 ($M^{+\bullet}$, 2%), 132 (100%), 131 (75%), 77 (14%)
11 h	>300	3460 s (NH), 2960 s (-CH_3), 1640 s (-C=N-), 1610 s (arom.), 1470 s (CH_3), 1260 s (-SO_2-OH), 1060 s (-SO_2-OH)	2.34 (s, 6 H, -CH_3), 7.46 (s, 2 H, Ar-H) 226 ($M^{+\bullet}$, 1%), 146 (100%), 131 (65%), 118 (8%)
15 a	>250	3400 s (NH), 3300 s (-COOH), 1700 s (-CO), 1680 s (NHCO), 1470 s (-CH_2-)	4.26 (s, 2 H, -CH_2), 6.97 (m, 4 H, Ar-H), 10.80 (s, 1 H, NH) 195 ($M^{+\bullet}$, 15%), 192 (100%), 148 (68%), 147 (79%), 119 (88%)
15 b	>250	3460 s (NH), 3000 m (-COOH), 1710–1690 s (CO), 1600 s (arom.), 1430 m (-CH_2-)	4.02 (s, 2 H, -CH_2), 6.89 (m, 3 H, Ar-H), 11.01 (s, 1 H, NH) 226 ($M^{+\bullet}$, 2%), 182 (3%), 167 (72%), 166 (24%), 92 (100%), 78 (50%)
15 c	>250	3400 s (NH), 3000 m (-COOH), 1700 s (-CO), 1680 (NHCO), 1480 s (-CH_2-)	4.02 (s, 2 H, -CH_2), 6.90 (m, 3 H, Ar-H), 11.01 (s, 1 H, NH) 229 ($M^{+\bullet}$, 3%), 228 (30%), 226 (88%), 182 (87%), 181 (100%), 153 (60%)
18 a	>250	3350 s (-COOH), 2980 m (-CH_2-), 1700 s (-CO), 1600 s (arom.), 1480 s (-CH_2-)	4.57 (s, 4 H, -CH_2), 7.06 (m, 4 H, Ar-H) 252 ($M^{+\bullet}$, 2%), 250 (100%), 205 (44%), 162 (39%), 133 (35%)
18 b	>240	3000 m (-COOH), 1700 s (-CO), 1600 m (arom.)	4.63 (s, 4 H, -CH_2), 7.09 (d, 1 H, *J* = 8.30, Ar-H), 7.20 (d, 1 H, *J* = 8.28, Ar-H), 7.28 (s, 1 H, Ar-H), 11.08 (s, 2 H, -COOH) 286 ($M^{+\bullet}$, 32%), 284 (100%), 239 (44%), 228 (30%), 181 (97%), 153 (50%)

[a] For FD-mass spectra see Experimental Part



Scheme 3. a) ethyl acetoacetate, xylene, KOH b) NaOEt, ethyl bromoacetate c) diluted H_2SO_4 d) 2 N NaOH, room. temp.

The *N,N′*-disubstituted derivatives **18 a** and **18 b** were yielded as outlined in Scheme 4. For this purpose the *o*-phenylenediamine derivative **7** was treated with *N,N′*-carbonyldiimidazole to afford compound **16**, which was reacted with potassium hydroxide in acetone followed by slow addition of ethyl bromoacetate to obtain compound **17** [16]. Saponification of **17** with 2 N sodium hydroxide followed by acidification with concentrated hydrochloric acid gave the crude bis-carboxylic acids (**18 a, b**), which were recrystallized from hot ethanol.



Scheme 4. a) THF, 72 h, room. temp. b) KOH, acetone, 30 min reflux, ethyl bromoactate, 1 h reflux c) 2 N NaOH, 72 h room. temp.

Pharmacology

The binding affinities of compounds **9a–c, 11 a–h, 15 a–c** and **18a, b** were determined by measuring the ability to displace [^{3}H]-MDL-105,519 to rat cortical membranes according to the method of Siegel et al. [17]. Each value is an average of triplicates. Tissue preparation was performed according to Foster and Wong [18] using the brains of male Sprague-Dawley rats (250–260 g body weight). The photometric protein determination method by Hartfree [19] was used to quantify the final amount of protein (250–500 µg/ml) in the membrane preparation. The IC_{50} value of compound **9 a** [6] is included for purpose of comparison.

Table 4. Binding studies of benzimidazole-2-carboxylic acids **9 a–c**.

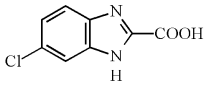
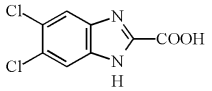
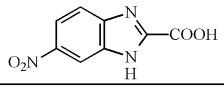
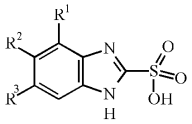
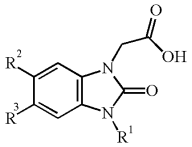
Cpd.	Structure	IC_{50} [µM] vs. [^{3}H]MDL-105,519
		Reference [6] and internal standard compound
9 a		1.86
9 b		0.89
9 c		38.0

Table 5. Binding studies of benzimidazole-2-sulfonic acids **11 a–h.**



Cpd.	IC_{50} [µM] vs. [^{3}H]MDL-105,519	Cpd.	IC_{50} [µM] vs. [^{3}H]MDL-105,519
11 a	399	**11 e**	347
11 b	445	**11 f**	319
11 c	304	**11 g**	401
11 d	223	**11 h**	328

Table 6. Binding studies of benzimidazolones **15 a–c**.



Cpd.	IC_{50} [µM] vs. [^{3}H]MDL-105,519	Cpd.	IC_{50} [µM] vs. [^{3}H]MDL-105,519
15 a	>1000	**18 a**	no displacement
15 b	>1000	**18 b**	no displacement
15 c	>1000		

Results and Discussion

All the final compounds synthesized were evaluated for their ability to displace [^{3}H]MDL-105,519 to rat cortical membranes. The results are summarized in Tables 4–6.

The benzimidazole-2-carboxylic acids **9 a–c** generally show considerable binding affinities in the range of 0.89 to 38.0 (IC_{50} values [µM] vs. [^{3}H]MDL-105,519). Compared to

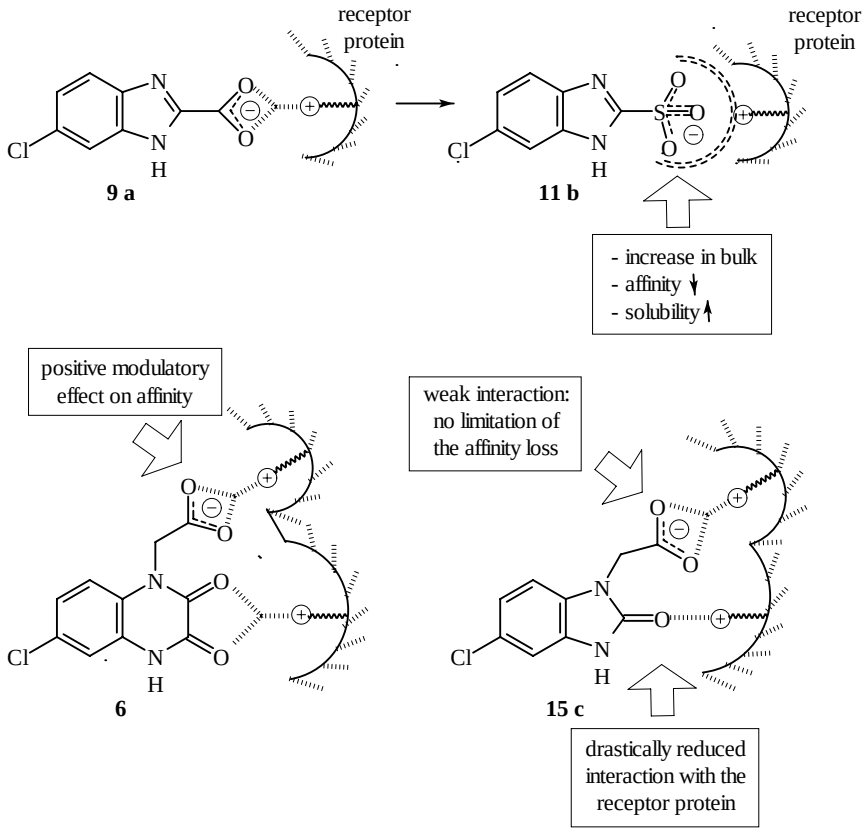


Figure 3

the standard compound **9 a**, the dichlorosubstituted analogue **9 b** might be considered as equipotent, whereas the nitro compound **9 c** leads to a 20-fold loss in receptor affinity, a fact also observed in other antagonists for the glycine site.

The displacement studies for the benzimidazole-2-sulfonic acids **11 a–h** indicate in a very drastic manner that carboxyl-replacement by the solubility enhancing sulfonic acid moiety is not tolerated to gain affine agents in this class of compounds. Compounds **11 a–h** were of excellent solubility even under aqueous conditions but the 240–250 fold loss in affinity as demonstrated for compounds **11 b** and **11 d** compared to their carboxylic acid analogues **9 a** and **9 b** makes them tools for structure-activity studies which clearly show the tightrope walk between in vitro potency and lack of potency. A possible reason for the decreased affinity in the benzimidazole-2-sulfonic acid series might be the increased bulk of the tetrahedral sulfonic acid group relative to the carboxylic group predominantly orientated into the plane. Thus an optimal fitting of the sulfonic acid function into the acidic binding pocket might be impeded, leading to a markedly reduced physical contact (coulombic interactions) of the ligand with the receptor protein. Findings by former groups [6], indicating that glycine-antagonist potency might be altered by varying substitution patterns of the benzene ring fused to the heterocycle, led us to investigate the effects of halogen and methyl substituents to the 4-, 5-, and 6-positions in the sulfonic acid series. In contrast to other classes of glycine antagonists, no appreciable modifications in binding affinity as a result of changes in substitution could be observed.

The strong effect of minor changes in the eastern region of the ligand on the binding affinity could be further demonstrated by the benzimidazolone derivatives **15 a–c**. Compound **15 c** showed an extremely poor affinity (IC_{50} > 1000 μM vs. [^{3}H]MDL-105,519) compared to the quinoxaline-2,3-dione derivative **6** (IC_{50} = 0.52 μM vs. [^{3}H]Gly) evaluated by Novonordisk [9]. This result indicates the importance of a second amide moiety within the heterocycle, which dramatically enhances the binding affinity. The glycine unit in the northern region of **15 c** (Figure 3) had obviously not the ability to compensate the lack of the additional amide moiety within the heterocycle. This finding accumulates the evidence already suggested in Figure 1, that the glycine unit in the northern region of the quinoxalinedione derivative **6** positively modulates the affinity to the receptor protein however contributing to the interaction with the glycine binding site to an all in all smaller extent.

The inability of compounds **18 a** and **18 b** to displace [^{3}H]MDL-105,519 is in line with the common pharmacophore model for glycine antagonists suggesting a H-bond donor group in the southern position of the ligand. A summary of the structure-activity relationships outlined above is illustrated in Figure 3.

Conclusions

The binding studies indicated in a very clear manner, that structural elements often giving rise to unfavourable physical properties of the whole molecule as for example the carboxylate group or the bis-amide moiety, are in the case of glycine$_B$ antagonists exactly the molecular parts, which strongly interact with the receptor protein and mainly contribute to the binding affinity. The research interest leading to a further development of glycine$_B$ antagonists concerns both the evaluation of structures suitable as diagnostic tools and the design of therapeutically interesting compounds. The discrepancy between solubility, affinity, and of course bioavailability to the CNS forces medicinal chemists to search for completely new leads and further strategies to optimize drug delivery in a specific manner (i.e. Brain Specific Chemical Delivery Systems (CDS) [20]).

Acknowledgements

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Experimental Part

Chemistry

Melting points were measured on a Büchi apparatus (Dr. Tottoli) and are uncorrected. IR spectra (KBr) were recorded on a Beckman IR Model 4220 spectrophotometer. ^{1}H NMR spectra were obtained on a Bruker AC-200 (200 MHz) and were consistent with proposed structures. Chemical shifts are described in parts per million. Tetramethylsilane was used as internal standard. Coupling constants (*J*) are reported in hertz. Mass spectra (electron impact) were obtained on a Varian MAT 311 A spectrometer. FD-mass spectra were performed on a Finnigan MAT 95 FD-spectrometer (5 kV ionisation energy). For the compounds recrystallized from water, elemental analyses were not be obtained due to the strong tendency of these compounds to incorporate the solvent. Thin-layer chromatography (TLC) was carried out with E. Merck silica gel 60 F_{254} plates. Yields are reported in per cent from their theoretically calculated value.

1,1′-Thiocarbonyldiimidazole and 1,1′-carbonyldiimidazole were purchased from Fluka. All other chemicals were obtained from Aldrich Chemical Co. (Steinheim, Germany). For column chromatography silica gel (230–240 mesh ASTM) from Merck was used.

Benzimidazole-2-carboxylic Acids ***9 a–c***

The synthesis of compounds **9 a–c** was performed as described by Louvet [10] for 4,6-dichlorobenzimidazole-2-carboxylic acid.

Benzimidazole-2-sulfonic Acids ***11 a–h***

Route A: 75 mmol of the corresponding *o*-phenylenediamine derivative and 82 mmol potassium ethyl xanthate were dissolved in a mixture of 75 ml ethanol 95% and 11 ml water and heated under reflux conditions for 3 h [11]. After adding 3 g of active charcoal the mixture was heated for another 10 min, filtered, and diluted with 75 ml of tap water (60–70 °C). The product was precipitated by the addition of diluted acetic acid (6.25 ml glacial acetic acid in 12.5 ml water). Purification of the crude product was achieved by recrystallization from ethanol 95% (yields 40–60%).

Route B: 14 mmol 1,1′-Thiocarbonyldiimidazole and 14 mmol 4,5-dichloro-*o*-phenylenediamine were dissolved in 500 ml dry THF (freshly distilled over K^0/benzophenone) each and added dropwise into 100 ml of cooled and dry THF under an atmosphere of nitrogen. The reaction mixture was stirred for 3 h at 0 °C followed by a 12 h stirring procedure at room temp., evaporated under reduced pressure and the resulting residue recrystallized from ethanol 95% using charcoal (yield 43%).

o-Phenylenediamine derivatives

o-Phenylenediamine derivatives, which were not commercially available comprise the following compounds:

– 3,5-dichloro-*o*-phenylenediamine
– 3,5-dibromo-*o*-phenylenediamine
– 4-bromo-*o*-phenylenediamine

The first two *o*-phenylenediamine derivatives were synthesized from the commercially available mono-nitro compounds using the following procedure: The appropriate mono-nitro compound (5 g) and powdered tin (15 g) were added to 80 ml hydrochloric acid (18%) and boiled under reflux for 1 h. After this time, the colour of the mixture had changed from dark yellow to pale yellow, indicating a successful reduction. Undissolved tin was filtered off, the resulting clear solution was diluted with 50 ml water and extracted with diethylether (2 × 50 ml). The aqueous layer was now poured into an excess of diluted sodium hydroxide and the resulting primary amine extracted with diethylether (3 × 100 ml). The organic layers were combined, dried over magnesium sulfate, filtered, and evaporated under reduced pressure. After a short cooling procedure, the oily residue turned into a white solid (yield 60%).

4-Bromo-*o*-phenylenediamine was synthesized from 4-bromo-2-nitroaniline, which had to be prepared according to Gardner [21] and Philipps [22]. 4-Bromo-2-nitro-aniline was reduced according to the above described procedure.

Benzimidazole-2-sulfonic Acids ***11 a–h***

11 mmol of the corresponding benzimidazole-2-thiol was dissolved in diluted sodium hydroxide, an excess of H_2O_2 10% added and the mixture stirred under reflux conditions [12]. After the onset of a very vigorous reaction, reflux was continued for another 10 min. Cooling of the resulting solution and acidification with concentrated hydrochloric acid to pH 1–2 caused precipitation of the product. Further purification of the crude product was achieved by recrystallization from hot water. The crystals obtained in the last step were dried in vacuo (yield 90%).

Benzimidazolones **15 a–c**

Compounds **15 a–c** were prepared from their corresponding 1-isopropenyl-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-one derivatives. These were synthesized from the *o*-phenylenediamine derivatives with ethyl acetoacetate according to Rossi et al. [13] and Gómez-Parra et al. [14]. The resulting compounds were reacted with freshly prepared sodium ethoxide and ethyl bromoacetate following a standard procedure [15]. Cleavage of the isopropenyl moiety with diluted H_2SO_4 and saponification of the ethyl ester with 2 N sodium hydroxide afforded **15 a–c** (yields 45–50%).

1,3-Bis-Substituted Benzimidazole-2-ones **18 a,b**

Compound **18 a** was prepared according to Clark [23] and Kloetzel [16]. For the synthesis of **18 b** 5-chloro-2,3-dihydro-1*H*-benzo[*d*]imidazole-2-one was synthesized as follows:

80 mmol 4-chloro-*o*-phenylenediamine and 80 mmol 1,1′-carbonyldiimidazole were dissolved in 400 ml dry tetrahydrofuran each and added dropwise to 150 ml dry tetrahydrofuran. After the addition of both educts the reaction mixture was stirred for 72 h at room temperature. The formation of a grey precipitate indicated a successful reaction. Finally the precipitate was collected by filtration and purified by chromatography on silica gel (eluent: CH_2Cl_2/MeOH 9:1). 5-Chloro-2,3-dihydro-1*H*-benzo[*d*]imidazole-2-one was reacted to yield **18 b** as described for **18 a** (yield 60%).

Pharmacology

All centrifugation steps for the tissue preparation were performed on a Beckman Optima^{TM} LE-80 K centrifuge using a Ti 60 rotor. D (+)-Sucrose and tris(hydroxymethyl)aminomethane were purchased from Fluka. Stock solutions were made using DMSO (Aldrich) and Tris-buffer. Serial dilutions

were made with Ampuwa® (Fresenius). [^{3}H]-MDL-105,519 (74.0 Ci/mmol) (Amersham) was generously provided by Hoechst Marion Roussel. Incubations were terminated using the Hoefer® FH 225 V Ten-Place Filter Manifold and collection box from Pharmacia Biotech. Glass fibre filters (GF 52) were obtained from Schleicher & Schuell. Scintillation liquid (Ultima Gold™) was purchased from Packard. Radioactivity was determined using a Wallac 1409 scintillation counter and MultiCalc™ Routine (Laboratory Data Management Program V 2.6).

Tissue Preparation

Tissue preparation was performed according to the method described by Foster and Wong[18]. Male Sprague-Dawley rats (200–260 g) were decapitated and their brains removed rapidly. The cortex was dissected and homogenised in 15 volumes of ice-cold 0.32 M sucrose using a glass Teflon homogeniser (potter-Elvehjem). The homogenate was centrifuged at 4200 rpm for 8 min. The pellet was discarded and the supernatant centrifuged at 16000 rpm for 30 min. The resulting pellet was resuspended in 15 ml of ice-cold Ampuwa® and centrifuged at 10000 rpm for 30 min. Then the supernatant and the isolated buffy coat were centrifuged at 30000 rpm for 30 min. The pellet was now resuspended in 15 ml 5 mM Tris-buffer (pH 7.4; 4 °C) and centrifuged at 30000 rpm for 30 min. The last step was repeated 6 times. Resuspension of the pellet in 5 ml 5 mM Tris-buffer (pH 7.4) resulted the final preparation, which was frozen rapidly at –80 °C. On the day of assay the membranes were thawed and washed with 15 ml 5 mM Tris-buffer and centrifuged at 30000 rpm for 30 min. The membrane preparation used for the displacement studies was suspended in assay buffer (50 mM Tris-buffer; pH 8.0; 4 °C).

Radioligand Binding-Studies

Stock solutions (10 mM) were made in Tris-buffer/dimethyl sulfoxide (1:1). To improve solubility of the compounds bearing carboxylic acid moieties, an equimolar quantity of diluted sodium hydroxide was added. Serial dilutions of the stock solutions were made in Ampuwa®.

Incubations were performed according to the methods modified from Siegel et al. [17]. Membranes were suspended and incubated in 50 mM Tris-buffer (pH 8.0; 4 °C) for 60 min at 4 °C with a fixed [^{3}H]-MDL-105,519 concentration of 2 nM. Non-specific binding was defined by the addition of unlabeled glycine at 1 mM. Incubations were terminated by a filtration procedure. The samples, all in duplicate for each concentration, were rinsed two times with 2.5 ml ice-cold assay buffer over glass fibre filters (GF 52) from Schleicher & Schuell under constant vacuum. Following rapid separation, the filters were placed in 5 ml Ultima Gold™ scintillation liquid, each. Radioactivity retained on the filters was determined with a scintillation counter.

References

[1] G. Dannhardt, B. K. Kohl, *Curr. Med. Chem.* **1998**, *5*, 253–263.

[2] W. Danysz, C. G. Parsons, M. Karcz-Kubicha, A. Schwaier, P. Popik, K. Wedzony, J. Lazarewicz, G. Quack, *Amino Acids* **1998**, *14*, 235–239.

[3] M. Quartaroli, C. Carignani, G. Dal-Forno, M. Mugnaini, A. Ugolini, R. Arban, L. Bettelini, G. Maraia, F. Belardetti, A. Reggiani, D. G. Trist, E. Ratti, R. Di Fabio, M. Corsi, *J. Pharmacol. Exp. Ther.* **1999**, *290*, 158–169.

[4] N. M. Gray, M. S. Dappen, B. K. Cheng, A. A. Cordi, J. P. Biesterfeldt, W. F. Hood, J. B. Monahan, *J. Med. Chem.* **1991**, *34*, 1283–1292.

[5] P. D. Leeson, R. W. Carling, K. W. Moore, A. M. Moseley, J. D. Smith, G. Stevenson, T. Chan, R. Baker, A. C. Foster, S. Grimwood, J. A. Kemp, G. R. Marshall, K. Hoogsteen, *J. Med. Chem.* **1992**, *35*, 1954–1968.

[6] P. D. Leeson, R. Baker, R. W. Carling, N. R. Curtis, K. W. Moore, B. J. Williams, A. C. Foster, A. E. Donald, J. A. Kemp, G. R. Marshall, *J. Med. Chem.* **1991**, *34*, 1243–1252.

[7] P. D. Leeson, L. L. Iversen, *J. Med. Chem.* **1994**, *37*, 4053–4067.

[8] R. W. Carling, P. D. Leeson, A. M. Moseley, J. D. Smith, K. Saywell, M. D. Tricklebank, J. A. Kemp, G. R. Marshall, A. C. Foster, S. Grimwood, *Bioorg. Med. Chem. Lett.* **1993**, *3*, 65–70.

[9] A. S. Jørgensen, C. E. Stidsen, P. Faarup, F. C. Grønvald (Novo Nordisk). WO 91/13878. **1991** [Chem. Abstr. **1991** *115*. 280059 u].

[10] P. Louvet, G. Lallement, *Eur. J. Med. Chem.* **1993**, *28*, 71–75.

[11] J. A. van Allan, B. D. Deacon, *Org. Synth.* **1950**, *30*, 56–57.

[12] W. Knobloch, K. Rintelen, *Arch. Pharm.* **1958**, *63*, 180–184.

[13] A. Rossi, A. Hunger, J. Kebrle, K. Hoffmann, *Helv. Chim. Acta* **1960**, *163*, 1298–1313.

[14] V. Gómez-Parra, M. Jiménez, F. Sánchez, T. Torres, *Liebigs Ann. Chem.* **1989**, 539–544.

[15] J. Davoll, D. H. Laney, *J. Chem. Soc.* **1960**, 314–318.

[16] I. J. Pachter, M. C. Kloetzel, *J. Amer. Chem Soc.* **1952**, *74*, 1321–1322.

[17] B. W. Siegel, B. M. Baron, B. L. Harrison, R. S. Gross, C. Hawes, P. Towers, *J. Pharmacol. Exp. Ther.* **1996**, *279*, 62–68.

[18] A. C. Foster, E. H. F. Wong, *Br. J. Pharmacol.* **1987**, *91*, 403–409.

[19] E. F. Hartfree, *Anal. Biochem.* **1972**, *48*, 422–427.

[20] E. Pop, *Curr. Med. Chem.* **1997**, *4*, 279–294.

[21] D. Gardner, D. R. P. 620825, *Friedlaender* **1933**, *22*, 529–530.

[22] M. A. Philipps, *Soc.* **1930**, 2400–2401.

[23] R. L. Clark, A. A. Pessolano, *J. Amer. Chem. Soc.* **1958**, *80*, 1657–1667.

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