Structure–Activity Relationships of Strychnine Analogs at Glycine Receptors

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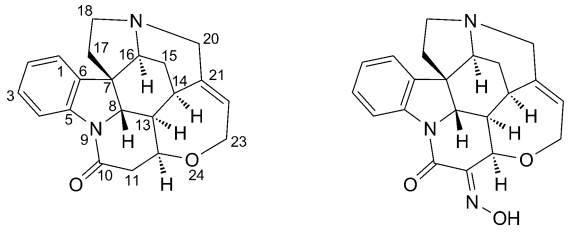
Nine strychnine derivatives including neostrychnine, strychnidine, isostrychnine, 21,22-dihydro-21-hydroxy-22-oxo-strychnine, and several hydrogenated analogs were synthesized, and their antagonistic activities at human $\alpha1$ and $\alpha1\beta$ glycine receptors were evaluated. Isostrychnine has shown the best pharmacological profile exhibiting an IC_{50} value of 1.6 μM at $\alpha1$ glycine receptors and 3.7-fold preference towards the $\alpha1$ subtype. SAR Analysis indicates that the lactam moiety and the C(21)=C(22) bond in strychnine are essential structural features for its high antagonistic potency at glycine receptors

Introduction. – Strychnine (**1**), the major alkaloid from the plant *Strychnos nux vomica*, exhibits pharmacological activity at several neurotransmitter receptors, including a number of ligand-gated ion channels. Its most pronounced pharmacological action is a strong antagonistic activity at glycine receptors (GlyRs), often referred to as 'strychnine-sensitive glycine receptors', which are anionic chloride channels linked to hyperpolarization and inhibition of neuronal firing [1–3]. Strychnine displays nanomolar K_i values at recombinant and native GlyRs in binding and functional assays [2][3].

Although dozens of simple structural analogs of strychnine are known, only a limited number of pharmacological studies has been reported to date, most of them on the convulsive effect and lethal dosis in animal experiments [4–7].

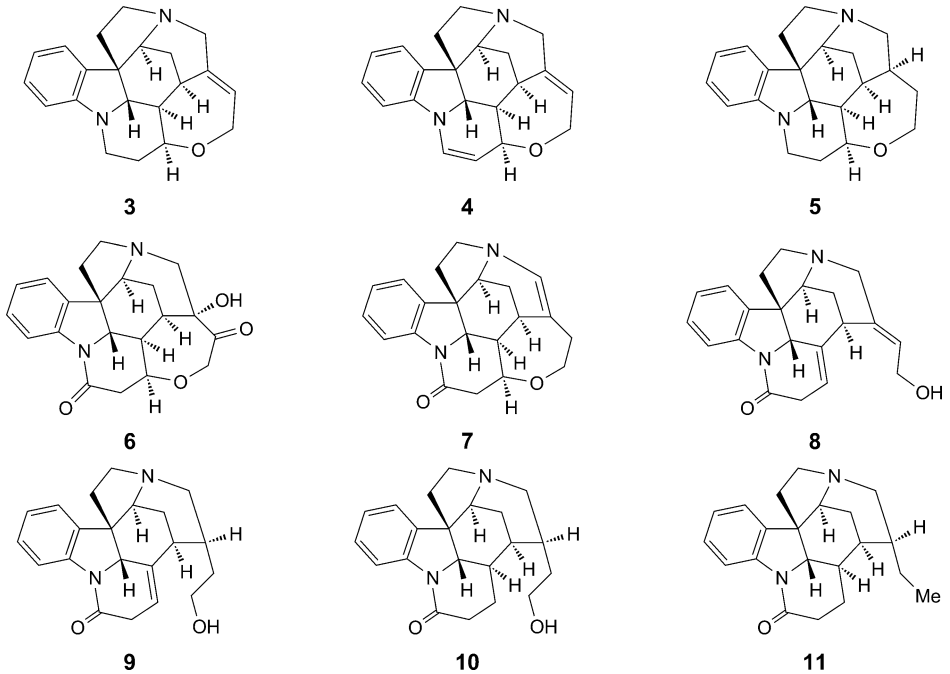
In the course of our studies on GlyR ligands, we have recently reported the functional properties of five tertiary and twelve quaternary strychnine analogs at $\alpha1$ and $\alpha1\beta$ GlyRs [8]. While all quaternary compounds were inactive, the tertiary analogs 2-nitrostrychnine, 21,22-dihydrostrychnine, *Wieland–Gumlich* aldehyde, and (*Z*)-11-isonitrososstrychnine displayed reduced antagonistic potencies at both GlyRs, when compared to strychnine. The only structure modification that did not impair the activity of the parent compound was an (*E*)-configurated hydroxyimino group at C(11) (compound **2**). However, none of the analogs exhibited pronounced selectivity towards $\alpha1$ or $\alpha1\beta$ receptors (*Fig. 1*).



1 **2**

Fig. 1. *Structures of strychnine* (**1**) *and (*E*)-11-isonitrososstrychnine* (**2**)

To obtain more detailed SARs of the strychnine skeleton at GlyRs, nine tertiary strychnine derivatives (*Fig. 2*) were synthesized and pharmacologically evaluated in this study. The structure modifications involve the formal removal of the lactam O-atom (strychnidine (**3**)), introduction of a C(10)=C(11) bond into strychnidine (10,11-didehydrostrychnidine (**4**)), hydrogenation of the C(21)=C(22) bond in strychnidine (21,22-dihydrostrychnidine (**5**)), oxidation of the C(21)=C(22) bond (21,22-dihydro-21-hydroxy-22-oxostrychnine (**6**)), relocation of the C(21)=C(22) bond to C(20)=C(21) (neostrychnine (**7**)), opening of the tetrahydrooxepine ring (isostrychnine (**8**)), hydrogenation of the C(21)=C(22) bond of isostrychnine (21,22-dihydroisostrychnine (**9**)), hydrogenation of both C=C bonds of **8** (12,13,21,22-tetrahydroisostrychnine (**10**)), and removal of the OH group in **10** (23-deoxy-12,13,21,22-tetrahydroisostrychnine (**11**)).



3 **4** **5**

6 **7** **8**

9 **10** **11**

Fig. 2. *Structures of the strychnine analogs* **3**–**11** *studied in this work*

Results. – *Chemistry.* Strychnidine (**3**) and 10,11-didehydrostrychnidine (**4**) were synthesized by $LiAlH_4$ reduction of strychnine according to the procedure by *Swan* and *Wilcock* [9]. 21,22-Dihydrostrychnidine (**5**), previously reported to be accessible by $LiAlH_4$ reduction of 21,22-dihydrostrychnine [7], was synthesized by a more convenient approach using borane-THF as reducing agent. 21,22-Dihydro-21-hydroxy-22-oxostrychnine (**6**) was prepared by oxidation of strychnine with $KMnO_4$ in acetone [7]. Neostrychnine (**7**) was obtained according to a modified procedure by *Chakravarti* and *Robinson* [10] by heating strychnine in xylene with Pd/C 10% instead of the originally employed *Raney*-Ni. Isostrychnine (**8**) was prepared by heating strychnine in H_2O at 180° under microwave irradiation. 12,13,21,22-Tetrahydroisostrychnine (**10**) was obtained according to our previously reported procedure by hydrogenation of strychnine under microwave irradiation in H_2O at 180° and 60 bar H_2 pressure with PtO_2 as catalyst [11]. 21,22-Dihydroisostrychnine (**9**) and 23-deoxy-12,13,21,22-tetrahydroisostrychnine (**11**) were isolated as side-products of the latter reaction. Our alternative procedures for the synthesis of **5** and **7**–**11** are described in the *Exper. Part*.

Pharmacology. The functional properties of strychnine and the nine strychnine analogs as GlyR antagonists were characterized at the human $\alpha1$ and $\alpha1\beta$ receptor subtypes. The receptors were transiently expressed in tsA201 cells, and their abilities to inhibit the glycine-induced response in the *FLIPR*® membrane potential blue assay were determined. The IC_{50} values obtained for the compounds are compiled in the *Table*.

Table. *Functional Properties of the Strychnine Analogs at the Human α1 and α1β GlyRs Transiently Expressed in tsA201 Cells in the* FLIPR®*Membrane Potential Blue Assay.* The EC_{50} values for glycine and the IC_{50} values for the analogs are given in μM with $pEC_{50} \pm$ S.E.M. and $pIC_{50} \pm$ S.E.M. values in brackets, respectively. In the antagonist experiments, EC_{50} concentrations of glycine as agonist were used. Data are the means of 3–4 individual experiments performed in duplicate.

	EC_{50} ($pEC_{50} \pm$ S.E.M.) [μM]	
	$\alpha1$	$\alpha1\beta$
Glycine	50 (4.30 ± 0.04)	35 (4.45 ± 0.04)
	IC_{50} ($pIC_{50} \pm$ S.E.M.) [μM]	
	$\alpha1$	$\alpha1\beta$
Strychnine (**1**)	0.16 (6.80 ± 0.08)	0.62 (6.21 ± 0.10)
Strychnidine (**3**)	~30 (~4.5)	~30 (~4.5)
10,11-Didehydrostrychnidine (**4**)	~50 (~4.3)	~100 (~4.0)
21,22-Dihydrostrychnidine (**5**)	~30 (~4.5)	~30 (~4.5)
21,22-Dihydro-21-hydroxy-22-oxostrychnine (**6**)	11 (4.96 ± 0.05)	~30 (~4.5)
Neostrychnine (**7**)	~30 (~4.5)	~100 (~4.0)
Isostrychnine (**8**)	1.6 (5.79 ± 0.12)	5.9 (5.23 ± 0.14)
21,22-Dihydroisostrychnine (**9**)	~30 (~4.5)	~30 (~4.5)
12,13,21,22-Tetrahydroisostrychnine (**10**)	~100 (~4.0)	~100 (~4.0)
23-Deoxy-12,13,21,22-tetrahydroisostrychnine (**11**)	~100 (~4.0)	~300 (~3.5)

Discussion. – Strychnine is the prototypical competitive antagonist of GlyRs displaying nanomolar affinities at recombinant and native receptors. However, it is not able to differentiate between the homomeric glycine receptor subtypes consisting exclusively of α subunits ($\alpha1-\alpha4$), and the heteromeric ones assembled from α and β subunits ($\alpha1\beta$, $\alpha2\beta$, $\alpha3\beta$, and $\alpha4\beta$). In the *FLIPR*® membrane potential blue assay, strychnine displayed only a slight four-fold preference for $\alpha1$ (IC_{50} 0.16 μM) over $\alpha1\beta$ receptors (IC_{50} 0.62 μM). In the search for potent competitive antagonists displaying selectivity towards the homomeric or heterodimeric GlyR subtypes, the functional properties of nine strychnine analogs obtained by simple structure modifications of the parent compound have been examined. The data are compiled in the *Table*. Although none of the derivatives was superior to strychnine, SAR enabled the identification of the structural elements that are essential for high antagonistic potency at $\alpha1$ and $\alpha1\beta$ receptors.

Strychnidine **3** and didehydrostrychnidine **4** are formally obtained from the parent compound by removal of the lactam O-atom and additional introduction of a C(10)=C(11) bond into the former lactam ring, respectively. Compounds **3** and **4** displayed *ca.* 200-fold and *ca.* 300-fold lower antagonistic potencies at $\alpha1$ receptors, and *ca.* 50-fold and *ca.* 150-fold lower activities at $\alpha1\beta$ receptors, respectively, when compared to strychnine. Saturation of the C(21)=C(22) bond of **3** maintained the low inhibitory activity at both $\alpha1$ and $\alpha1\beta$. The resulting dihydrostrychnidine **5** exhibited the same IC_{50} values as strychnidine at both subtypes (IC_{50} *ca.* 30 μM). The findings indicated that the lactam moiety of strychnine was an essential structural feature for the antagonistic activity at GlyRs. Relocation of the C(21)=C(22) bond of strychnine to C(20)=C(21) to give neostrychnine **7** was also detrimental for the inhibitory action at both GlyRs ($\alpha1$: IC_{50} ~30 μM; $\alpha1\beta$: IC_{50} *ca.* 100 μM). Moreover, oxidation of the C(21)=C(22) bond to a 21-hydroxy-22-oxo moiety to give compound **6** also resulted in significantly decreased antagonistic potency at $\alpha1$ (IC_{50} 11 μM) and $\alpha1\beta$ (IC_{50} *ca.* 30 μM), revealing the C(19)=C(20) bond as another essential structural motif for the action at GlyRs. The latter conclusion is supported by the finding that hydrogenation of strychnine to the 21,22-dihydro analog was previously reported to cause 30-fold and 10-fold decreased binding for $\alpha1$ and $\alpha1\beta$, respectively [8]. In accordance with these findings, **3** and 21,22-dihydrostrychnine are known to possess considerably lower convulsive and lethal effects than strychnine in animal experiments [6][7]

Isostrychnine (**8**) is a ring-opened analog of the parent compound in which both structural features important for the antagonistic action at GlyRs, *i.e.*, the lactam ring and the C(21)=C(22) bond, are still present. Indeed, isostrychnine is the most potent analog in the series displaying IC_{50} values of 1.6 and 5.9 μM at $\alpha1$ and $\alpha1\beta$, respectively. Although ten times less potent than strychnine, it maintains the three-fold preference towards the $\alpha1$ subtype. As expected, the saturated analogs lacking the C(21)=C(22) bond, **9** ($\alpha1$: IC_{50} *ca.* 30 μM; $\alpha1\beta$: IC_{50} *ca.* 30 μM), and **10** ($\alpha1$: IC_{50} *ca.* 100 μM; $\alpha1\beta$: IC_{50} *ca.* 100 μM) exhibited considerably reduced antagonistic potencies. Interestingly, the formal removal of the OH group from **10** to give **11** ($\alpha1$: IC_{50} *ca.* 100 μM; $\alpha1\beta$: IC_{50} *ca.* 300 μM) had no influence on the inhibitory activity.

In summary, the lactam group and the C(21)=C(22) bond of strychnine could be identified as essential structural features required for strong antagonistic activity at glycine $\alpha1$ and $\alpha1\beta$ receptors. The absence of one of these structural elements or

changes of their relative spatial orientation result in reduced antagonistic potency. The findings are important for the future design of potent strychnine derived GlyR antagonists.

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Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2, 0.063–0.200 mm; *Merck*). M.p.: cap. melting-point apparatus (*Gallenkamp*, *Sanyo*); uncorrected. ^{1}H- and ^{13}C-NMR spectra: *Bruker AV-400* spectrometer; δ in ppm rel. to $CDCl_3$ as internal standard, J in Hz. EI-MS: *Finnigan MAT 90*; in *m/z*.

21,22-Dihydrostrychnidine (**5**). Borane/THF soln. (1M; 8 ml) was added dropwise to a stirred soln. of *21,22-dihydrostrychnine* (500 mg, 1.54 mmol) in dry THF (100 ml) at r.t. The mixture was heated under reflux for 7 h, allowed to cool, and 2M aq. HCl (4 ml) was added dropwise under ice cooling. After heating for 30 min under reflux, the mixture was basified under ice cooling with 25% aq. NH_3 and extracted with CH_2Cl_2. The combined org. layers were washed with H_2O (3×15 ml), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by CC (SiO_2; $CHCl_3$/MeOH/25% aq. NH_3 100:10:1) to yield **5** (80 mg, 16%). White solid M.p. 216–218° ([7]: 221–224° [12]: 212–214° [12]) ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.08 (*ddd*, J=7.6, 7.6, 0.8, H–C(2)); 7.01 (*dd*, J=7.4, 0.8, H–C(4)); 6.69 (*ddd*, J=7.6, 7.4, 0.9, H–C(3)); 6.43 (*d*, J=7.6, H–C(1)); 3.97 (*ddd*, J=13.0, 3.9, 3.9, H_a–C(23)); 3.84 (*d*, J=11.7, H–C(8)); 3.72 (*ddd*, J=6.2, 6.2, 3.6, H–C(12)); 3.58–3.45 (*m*, H_b–C(23), H_a–C(20)); 3.42 (*t*, J=3.0, H–C(16)); 3.14 (*ddd*, J=12.1, 10.0, 8.1, H_a–C(18)); 3.07–2.91 (*m*, H_a–C(10), H_b–C(20)); 2.85 (J=12.1, 8.5, 3.5, H_b–C(18)); 2.54 (J=13.6, 8.5, 8.1, H_a–C(17)); 2.40–2.29 (*m*, H_b–C(10), H–C(14)); 2.10–1.93 (*m*, H_b–C(17), H_a–C(15), H_a–C(11), H_a–C(22), H–C(21)); 1.89–1.68 (*m*, H_b–C(11), H_b–C(7), H–C(13)); 1.65 (*ddd*, J=14.0, 3.7, 3.7, H_b–C(22)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 151.2 (C(5)); 134.3 (C(6)); 128.0 (C(2)); 122.0 (C(4)); 118.3 (C(3)); 107.8 (C(1)); 78.9 (C(12)); 68.7 (C(8)); 68.5 (C(23)); 62.0 (C(16)); 58.2 (C(10)); 54.1 (C(18)); 51.9 (C(7)); 47.0 (C(13)); 41.6 (C(20)); 41.4 (C(17)); 35.3 (C(14)); 34.6 (C(22)); 31.4 (C(15)); 31.2 (C(21)); 28.7 (C(11). EI-MS: 323.1 (27), 322.1 (100, M^+), 180.1 (20).

Neostrychnine (=*20,21-Didehydro-21,22-dihydrostrychnidin-10-one*; **7**). *Strychnine* (**1**; 300 mg, 0.90 mmol) and Pd/C 10% (200 mg) were heated under reflux in xylene (50 ml) for 3 h. Pd/C was filtered off through a pad of *Celite®545* and washed with xylene. The filtrates were combined, the solvent was removed *in vacuo*, and the residue was purified by CC (SiO_2; $CHCl_3$/MeOH/25% aq. NH_3 100:10:1) to give **7** (65 mg, 22 %). White solid. M.p. 216–218° ([10]: 228°). ^{1}H-NMR: in accordance with those reported in [13]. ^{13}C-NMR ($CDCl_3$, 100 MHz): 170.4 (C=O); 141.6 (C(5)); 135.7 (C(20)); 134.4 (C(6)); 128.4 (C(1)); 124.5 (C(2)); 122.0 (C(3)); 116.1 (C(4)); 112.3 (C(21)); 77.5 (C(12)); 67.8 (C(23)); 65.2 (C(8)); 59.7 (C(16)); 53.9 (C(13)); 53.6 (C(7)); 52.6 (C(18)); 46.8 (C(17)); 41.5 (C(11)); 34.5 (C(22)); 28.6 (C(14)); 27.4 (C(15)). EI-MS: 335.1 (23), 334.1 (100, M^+), 277 (22), 167 (26), 149 (47).

Isostrychnine (=*(3a*R,*11b*S,*12*S,*14*E*)-14-(2-Hydroxyethylidene)-2,3,10,12,13,13a-hexahydro-9*H,*11b*H-*1,12-ethanopyrido[1,2,3-*lm*]pyrrolo[2,3-*d*]carbazol-9-one*; **8**). Strychnine (**1**; 1 g, 3.00 mmol) was suspended in H_2O (180 ml), and the mixture was heated for 1 h at 180° under microwave irradiation (heating rate, 5 min from 25° to 180°). H_2O was evaporated, and the residue was subjected to CC (SiO_2; $CHCl_3$/MeOH/25% aq. NH_3 100:10:1) to give 175 mg (17%) of a 3.4:1 mixture of **8** and its C(11)=C(12) bond isomer as indicated by ^{1}H-NMR. The latter was further purified by CC (SiO_2; CH_2Cl_2/MeOH 100:15) to give **8** (75 mg, 7%). White solid. M.p. 218–220°([14]: 214–215, [15]: 223–224°. ^{1}H- and ^{13}C-NMR: in accordance with those reported in [16]. EI-MS: 335.1 (19), 334.1 (78, M^+), 316.1 (100), 303.1 (81), 220.1 (43), 167.1 (46).

12,13,21,22-Tetrahydroisostrychnine (=*(3a*R,*11a*R,*11b*S,*12*S*)-14-(2-Hydroxyethyl)-2,3,10,11,11a,12,13,13a-octahydro-9*H,*11b*H-*1,12-ethanopyrido[1,2,3-*lm*]pyrrolo[2,3-*d*]carbazol-9-one*; **10**). Strychnine (**1**; 6.00 mmol, 2.00 g) was dissolved in 200 ml of 10% aq. AcOH, 0.2 g of PtO_2 was added, and the

mixture was hydrogenated in a high pressure microwave reactor [11] at 270° and 60 bar H_2 pressure for 4.5 h (heating rate, 10 min 25° to 270°). After cooling to 25°, the catalyst was filtered off, and the solvent was evaporated. The residue was treated with 150 ml of 2.0M NaOH, extracted with CH_2Cl_2 (3 × 120 ml), and dried ($MgSO_4$). The solvent was evaporated, and the residue was purified by CC (SiO_2; (R_f ($CHCl_3$/MeOH/25% aq. NH_3 100:10:1) 0.10) to give **10** (1.05 g, 52%). Colorless foam. $[\alpha]_D^{23} = -23.0°$ ($c = 2.0$, $CHCl_3$) ([17]: $[\alpha]_D^{23} = -24.5$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.11 (*d*, *J* = 7.9, H–C(4)); 7.25–7.15 (*m*, H–C(1), H–C(3)); 7.10 (*t*, *J* = 7.4, H–C(2)); 4.06 (*d*, *J* = 5.5, H–C(8)); 3.68 (*t*, *J* = 6.3, C(23)); 3.09 (*td*, *J* = 11.9, 7.2, H_a–C(11)); 2.99 (*dd*, *J* = 11.9, 6.9, H_b–C(11)); 2.92 (*dd*, *J* = 10.7, 3.6, H_a–C(20)); 2.88 (*m*, H–C(16)); 2.49 (*m*, C(18)); 2.27–2.43 (*m*, C(12), H_b–C(21)); 2.07–2.18 (*m*, H_a–C(17), H–C(13)); 1.95 (*m*, H–C(21)); 1.65–1.80 (*m*, H–C(14), CH_2(15), H_b–C(17)); 1.53 (*m*, H_a–C(22)); 1.43 (*m*, H_b–C(22)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 169.2 (C=O); 141.1 (C(5)); 138.8 (C(6)); 127.9 (C(3)); 125.1 (C(2)); 122.0 (C(1)); 117.7 (C(4)); 67.5 (C(8)); 67.0 (C(16)); 60.2 (C(23)); 55.3 (C(22)); 52.8 (C(7)); 50.4 (C(11)); 37.8 (C(12)); 37.7 (C(21)); 34.7 (C(22)); 32.1 (C(14)); 29.7 (C(18)); 28.7 (C(17)); 26.8 (C(13)); 26.6 (C(15)).

21,22-Dihydroisostrychnine (=*(3a*R,*11b*S,*12*S*)-14-(2-Hydroxyethyl)-2,3,10,12,13,13a-hexahydro-9*H,*11b*H-*1,12-ethanopyrido[1,2,3*-lm*]pyrrolo[2,3*-d*]carbazol-9-one*; **9**). Compound **9** (100 mg, 5%) was obtained as a minor fraction during the purification of **10** by CC (SiO_2; R_f ($CHCl_3$/MeOH/25% aq. NH_3 100:10:1) 0.15). White solid. M.p. 243–245° ([17]: 244–246°). ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.10 (*d*, *J* = 7.7, H–C(4)); 7.24–7.15 (*m*, H–C(1), H–C(3)); 7.07 (*td*, *J* = 7.5, 1.0, H–C(2)); 5.75 (*m*, H–C(12)); 4.25 (*t*, *J* = 2.9, H–C(8)); 3.71 (*td*, *J* = 6.4, 2.0, C(23)); 3.39 (*t*, *J* = 3.0, H–C(16)); 3.24–2.90 (*m*, H_a–C(11), H_a–C(18), H_b–C(21), H_a–C(20), H_b–C(11)); 2.53 (*m*, H–C(14)); 2.45 (*m*, H_a–C(7)); 2.30 (*ddd*, *J* = 13.9, 9.0, 1.5, H_b–C(17)); 2.14–2.03 (*m*, H_a–C(15), H–C(21)); 1.79 (*t*, *J* = 12.1, H_b–C(20)); 1.59–1.48 (*m*, H_a–C(22), H_b–C(15)); 1.35 (*dddd*, *J* = 13.5, 6.7, 6.7, 6.7, H_b–C(22). ^{13}C-NMR ($CDCl_3$, 100 MHz): 168.7 (C=O); 141.2 (C(5)); 141.1 (C(13)); 136.3 (C(6)); 128.2 (C(3)); 124.4 (C(2)); 122.4 (C(12)); 122.3 (C(1)); 114.4 (C(4)); 70.8 (C(8)); 65.4 (C(16)); 60.0 (C(23)); 54.6 (C(11)); 53.7 (C(20)); 51.7 (C(7)); 48.7 (C(17)); 37.3 (C(18)); 36.7 (C(14)); 36.1 (C(21)); 35.4 (C(22)); 27.4 (C(15)). EI-MS: 337.1 (23), 336.1 (78, M^+), 316.1 (30), 305.1 (33), 291.1 (42), 220.1 (45), 167.1 (49), 149.0 (69).

23-Deoxy-12,13,21,22-tetrahydroisostrychnine (=*(3a*R,*11a*R,*11b*S,*12*S*)-14-Ethyl-2,3,10,11,11a,12,13,13a-octahydro-9*H,*11b*H-*1,12-ethanopyrido[1,2,3*-lm*]pyrrolo[2,3*-d*]carbazol-9-one*; **11**). Compound **11** (75 mg, 4%) was obtained as a minor fraction during the purification of **10** by CC (SiO_2; R_f ($CHCl_3$/MeOH/25% aq. NH_3 100:10:1) 0.49). White foam. M.p. 125–130° ([18]: 174–176°). ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.10 (*d*, *J* = 7.8, H–C(4)); 7.23–7.15 (*m*, H–C(1), H–C(3)); 7.09 (*td*, *J* = 7.4, 1.0, H–C(2)); 4.04 (*d*, *J* = 5.5, H–C(8)); 3.09 (*m*, H_a–C(11)); 2.99 (*m*, H_b–C(11)); 2.92–2.86 (*m*, H_a–C(20), H–C(16)); 2.53–2.47 (*m*, CH_2(18)); 2.40–2.26 (*m*, CH_2(12), H_b–C(20)); 2.16–2.06 (*m*, H_a–C(17), H–C(14)); 1.75–1.61 (*m*, CH_2(15), H–C(13), H_b–C(17), H–C(21)); 1.31–1.17 (*m*, CH_2(22)); 0.93 (*t*, *J* = 7.4, Me(23)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 168.2 (C=O); 140.2 (C(5)); 138.0 (C(6)); 126.8 (C(23)); 124.0 (C(2)); 121.0 (C(1)); 116.7 (C(4)); 66.6 (C(8)); 66.2 (C(16)); 54.3 (C(11)); 51.9 (C(7)); 49.3 (C(20)); 41.6 (C(13)); 36.8 (C(12)); 30.2 (C(21)); 28.7 (C(18)); 27.6 (C(15)); 22.7 (C(17)); 22.5 (C(14)); 23.3 (C(22)); 10.8 (Me(23)). EI-MS: 323.1 (10), 322.1 (44, M^+), 316.1 (30), 167.0 (36), 149.0 (100), 124.1 (65).

FLIPR® *Membrane Potential Blue Assay.* The functional characterization of the strychnine analogs were performed by the *FLIPR*® membrane potential blue assay (*Molecular Devices*) [3]. TsA-201 Cells transiently transfected with cDNAs encoding for $\alpha 1$ or $\alpha 1\beta$ GlyRs were split into poly-D-lysine-coated black 96-wells plates with clear bottom (*BD Biosciences*, Bedford, MA). Later (16–24 h), the medium was aspirated, and the cells were washed with 100 µl of *Krebs* buffer (140 mM NaCl/4.7 mM KCl/2.5 mM $CaCl_2$/1.2 mM $MgCl_2$/11 mM *HEPES*/10 mM D-glucose; pH 7.4). *Krebs* buffer (50 µl) was added to the wells (in the antagonist experiments, various concentrations of the antagonist were dissolved in the buffer), and then an additional 50 µl of *Krebs* buffer supplemented with the FMP assay dye (1 mg/ml) was added to each well. Then, the plate was incubated at 37° in a humidified 5% CO_2 incubator for 30 min and assayed in a *NOVOstar*® plate reader (*BMG Labtechnologies*, DE-Offenburg), measuring emission (in fluorescence units (FU)) at 560 nm caused by excitation at 530 nm before and up to 1 min after addition of 33 µl of agonist soln. The experiments were performed in duplicate at least three times for each compound at each receptor using an EC_{50} concentration of glycine as agonist.

REFERENCES

[1] J. W. Lynch, *Physiol. Rev.* **2004**, *84*, 1051.
[2] B. Laube, G. Maksay, R. Schemm, H. Betz, *Trends Pharmacol. Sci.* **2002**, *23*, 519.
[3] A. A. Jensen, U. Kristiansen, *Biochem. Pharmacol.* **2004**, *67*, 1789.
[4] P. Karrer, C. H. Eugster, P. Waser, *Helv. Chim. Acta* **1949**, *32*, 2381.
[5] L. Szabo, L. Weimann, *Acta Pharm. Hung.* **1965**, *35*, 26.
[6] L. Szabo, L. Weimann, O. Clauder, *Acta Pharm. Hung.* **1968**, *38*, 84.
[7] G. M. Iskander, L. Bohlin, *Acta Pharm. Suec.* **1978**, *15*, 431.
[8] A. A. Jensen, P. Gharagozloo, N. J. M. Birsdall, D. P. Zlotos, *Eur. J. Pharmacol.* **2006**, *539*, 27.
[9] G. A. Swan, J. D. Wilcock, *J. Chem. Soc., Perkin Trans. 1* **1972**, 1068.
[10] R. N. Chakravarti, R. Robinson, *J. Chem. Soc.* **1947**, 78.
[11] E. Heller, A. Gutmann, G. Hiltensperger, W. Lautenschläger, M. J. Lohse, C. Schad, T. Schirmeister, F. Seufert, D. P. Zlotos, U. Holzgrabe, *G.I.T Laboratory Journal* **2013**, *1–2*, 32.
[12] A. E. Oxford, W. H. Perkin, R. Robinson, *J. Chem. Soc.* **1927**, 2389.
[13] J. D. Luther III, J. Valentini, J. C. Carter, *J. Magn. Reson.* **1974**, *15*, 132.
[14] A. Pictet, A. Bacovescu, *Chem. Ber.* **1905**, *38*, 2787.
[15] H. Leuchs, Nietschke, *Chem. Ber.* **1922**, 3171.
[16] M. Mori, M. Nakanishi, J. Kajishima, Y. Sato, *J. Am. Chem. Soc.* **2003**, *125*, 9801.
[17] H. Leuchs, H. Schulte, *Chem. Ber.* **1942**, *75B*, 1522.
[18] H. Leuchs, H. Schulte, *Chem. Ber.* **1942**, *75B*, 573.

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