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Synthesis of (nor)tropeine (di)esters and allosteric modulation of glycine receptor binding

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Abstract—(Hetero)aromatic mono- and diesters of tropine and nortropine were prepared. Modulation of [^{3}H]strychnine binding to glycine receptors of rat spinal cord was examined with a ternary allosteric model. The esters displaced [^{3}H]strychnine binding with nano- or micromolar potencies and strong negative cooperativity. Coplanarity and distance of the ester moieties of diesters affected the binding affinity being nanomolar for isophthaloyl-bistropane and nortropeines. Nortropisetron had the highest affinity ($K_A \sim 10$ nM). Two esters displayed negative cooperativity with glycine in displacement, while three esters of low-affinity and nortropisetron exerted positive cooperativity with glycine.

1. Introduction

Glycine is the major inhibitory neurotransmitter in spinal cord. Glycine receptors (GlyRs) function as pentameric anion channels belonging to the 'cysteine-loop' superfamily of ionotropic neurotransmitter receptors. Pharmacological fine-tuning of these receptors can be performed by allosteric agents: for example, *A*-type γ-aminobutyric acid receptors by 1,4-benzodiazepines and neurosteroids. GlyRs play predominant roles in the processing of motor and sensory signals, neuronal development, inflammatory pain sensitization, and in inherited neurological disorders such as hyperekplexia.[1] Although they are potential targets of muscle relaxant, sedative, and analgesic agents, they are still 'therapeutic orphans'.[2] Due to the structural homology of cysteine-loop receptors, especially of their binding cavities, 5-HT$_3$ type serotonin receptor antagonists such as tropisetron potentiate GlyR function.[3] Preliminary structure–activity analysis has concluded that tropeines (tropine esters) serve as a consensus structure of positive allosteric modulation of GlyRs: most potent 'Gly-positive' agents are aromatic esters of 3α-hydroxy-tropane (tropine).[4] Starting from 5-HT$_3$ receptor antagonists we have developed substituted benzoate esters of nortropine as high-affinity, GlyR-selective allosteric modulators.[5] Since tropisetron has shown GlyR subunit-selectivity,[6,7] tropeines might serve as a promising lead to develop selective modulators of GlyRs. Here, we extended the structure–activity analysis of tropeines to modify their alcohol as well as acid parts. Further, diesters were also examined because several advantages of twin drugs might be exploited in medicinal chemistry.[8] A ternary allosteric model was used which has been successfully applied for the binding of the antagonist [^{3}H]strychnine to GlyRs.[9] By this way we determined not only the binding affinity of allosteric modulators (K_A) but also the cooperativity factor β of their allosteric interactions with glycine which is a relevant property in glycinergic neurotransmission.

2. Results and discussion

2.1. Chemistry

Tropane alkaloids occur as esters of relatively simple carboxylic acids and tropine, or Ψ-tropine. A large number of different esters were also synthesized, owing to their pharmacological significance. Several acylation methods[10,11] are known for the preparation of tropeines and nortropeines, which can be applied for the synthesis of the compounds described in this paper.

Keywords: Glycine receptors; Tropeines; Ternary allosteric model; Glycine displacement.
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3α-Succinyl-bis-*N*-ethyl-nortropane (**2**) and 3α-terephthaloyl-bis-tropane (**4**) (Fig. 4) were prepared from *N*-ethyl-nortropine and tropine, respectively, with the corresponding dicarboxylic acid chloride according to a known procedure.[12]

Acylation of the hydroxyl group of tropine can also be performed by means of ethyl esters. For the synthesis of 3α-(3′-pyridinecarbonyloxy)-tropane (**5**)[13] the transesterification method was used. Nicotinic acid ethyl ester and tropine were heated at 120 °C in the presence of catalytic amount of sodium, under reduced pressure, whereby the alcohol produced was distilled off. 3α-(3′-Indole-3′-carbonyloxy)-tropane (tropisetron) was obtained according to the procedure described.[14] 3β-(9′-Xantenecarbonyloxy)-tropane was obtained in excellent yield from Ψ-tropine and from the acyl chloride in CH_2Cl_2 in the presence of TEA.

Several methods are known for the demethylation of tropane derivatives.[15,16] By use of ethyl chloroformate, demethylation takes place readily under the formation of methyl chloride and *N*-ethoxycarbonyl derivatives. However, the removal of the ethoxycarbonyl group requires vigorous reaction conditions, so that the ester function is also cleaved. Therefore, the use of 2,2,2-trichloroethyl chloroformate is much more appropriate for the demethylation of tropeines, and it was used for the preparation of **7**, **8**, and **9** (Scheme 1 and Fig. 5). The removal of the trichloroethoxycarbonyl group from the nitrogen atom can be achieved with zinc in acetic acid solution (Scheme 1). This reductive procedure does not affect the ester group, and nortropeines can be obtained in good yield.

Demethylation of scopolamine and the subsequent cleavage of the carbamate was performed using α-chloroethyl chloroformate[17] in refluxing dichloroethane and methanol, respectively, to get norscopolamine (**12**, Fig. 6).

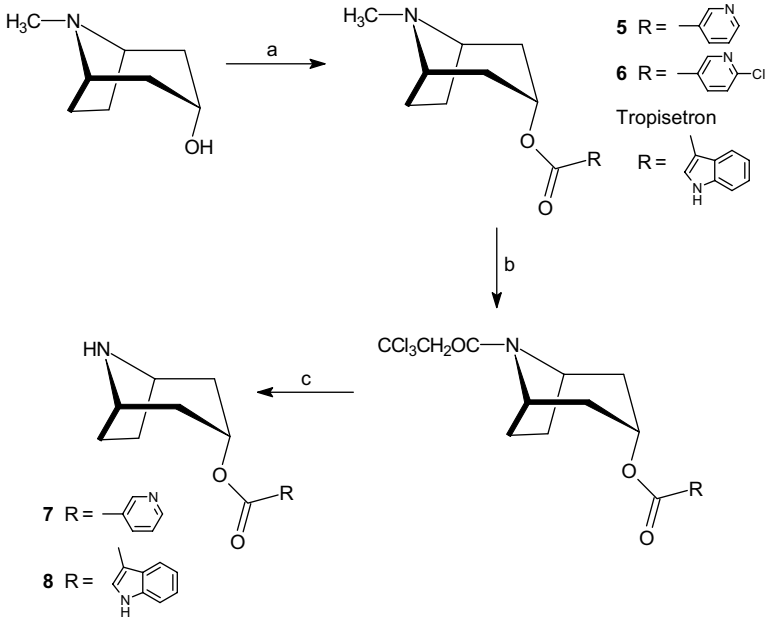
Compounds **1**, **3**, **6**, **10**, and **11** were donated by Professor László Gyermek (Harbor-UCLA Medical Center, Torrance, CA, USA).[12,18]

2.2. Binding to glycine receptors

Most esters resulted in concentration-dependent, full displacement of specific [^{3}H]strychnine binding as shown for compounds **3** and **8** in Figure 1. Since residual binding accompanying maximal displacement cannot be differentiated accurately from non-specific binding, the extent of strong negative cooperativity could not be exactly determined via the ternary allosteric model and Eq. 2 resulting in $\alpha \gg 10$ in Table 1.

Since tropeines proved to be most potent modulators of GlyRs as benzoate esters, we examined their diesters as twin agents. The 3α-isophthaloyl-bistropane (**3**) was much more potent than 3α-terephthaloyl-bistropane **4** (Table 1) or most of the substituted benzoyloxy-monotropanes[5] to show the importance of the distance and orientation of two coplanar ester moieties. The planar trans orientation of the diester 3α-fumaroyl-bistropane **1** resulted in fair potency (K_A = 7.5 μM) but the deconjugation of ester moieties (e.g., succinyl) rendered compound **2** completely inactive (Table 1). Extension of the tropane skeleton of 3α-fumaroyl-bistropane (**1**) into 3α-fumaroyl-bisgranatane (**11**) also resulted in a reduced binding affinity (from K_A = 7.5 to 20.8 μM in Table 1).

The acid parts of the esters were also modified. The heteroaromatic nicotinic ester 3α-(3′-pyridinecarbonyloxy)-tropane (**5**) resulted in K_A = 2.2 μM, while its 6′-chloro



Scheme 1. Synthesis of tropine and nortropine esters. Reagents and conditions: (a) for the preparation of compounds **5** (HBr) and **6**, tropine, RCOOEt, Na, 120 °C; (b) for compounds **7** (HCl) and **8** (HCl), $ClCOOCH_2CCl_3$, K_2CO_3, toluene, reflux; (c) Zn, CH_3COOH, water, room temperature.

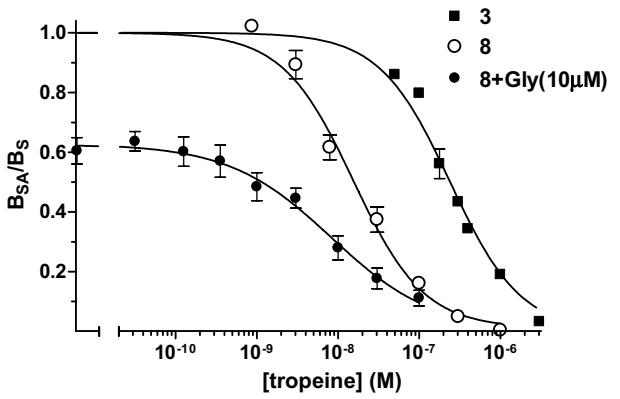


Figure 1. Concentration-dependent displacement of [^{3}H]strychnine binding by 3α-isophthaloyl-bistropane (**3**, ■), nortropisetron (**8**, ○) and the effect of 10 μM glycine on nortropisetron displacement (•). Points are means ± SEM of 3–4 experiments. Fitting to Eq. 2 resulted in K_A and α values of Table 1.

substitution decreased the potency of 3α-(6′-chloro-3′-pyridinecarbonyloxy)-tropane (**6**) to K_A = 5.7 μM (Table 1). N-Demethylation in 3α-(3′-pyridinecarbonyloxy)-nortropane (**7**) strongly increased the affinity to K_A = 82 nM (Table 1) as observed previously for tropine benzoates.[5]

Indolic tropeines are also active on GlyRs.[3,4] Tropisetron is particularly promising since its femtomolar concentrations potentiate α_1 GlyRs[19] and it has shown subunit-selectivity for both potentiation and inhibition of GlyRs.[6,7] Therefore, N-demethylated tropisetron (**8**) was prepared. Nortropisetron **8** was the most potent displacer with K_A = 17 nM, more than 100 times stronger than tropisetron at GlyRs.[4] A tricyclic xantene ester was also prepared and examined. The binding site tolerated 3β-(9′-xantenecarbonyloxy)-nortropane **9** fairly (K_A = 3.1 μM in Table 1). The disadvantages of 3β orientation of the ester group[5] and non-planarity of its two phenyl rings were compensated by the advantageous N-demethylation. 3α-(Hydroxydiphenylacetoxy)-*N*-allyl-nortropane (**10**) with its non-conjugated phenyl rings was quite inactive (Table 1). The separation of the phenyl ring from the carbonyloxy moiety by a hydroxyalkyl group in atropine and the annellation of an epoxide ring to the tropane ring of atropine made scopolamine inactive on GlyRs.[4] However, N-demethylation restored the affinity of norscopolamine (**12**) to K_A = 8 μM (Table 1).

The cooperativity factor β of the allosters with glycine in displacement has been found to correlate well with the modulation of ionophore activity of GlyRs: allosteric agents can be distinguished which have positive (β < 1), negative (β > 1), and neutral (β ∼ 1) cooperativities with glycine.[9] Therefore, concentration-dependent displacement of [^{3}H]strychnine binding by glycine was examined simultaneously in the absence and presence of the tropeines at low occupancy of their binding sites. Figure 2 compares the distinctive effects of positive versus negative allosteric agents. Representative compounds were isophthaloyl-bistropane **3** and nortropisetron **8** in Figure 2. Negative modulators (β > 1) resulted in displacement curves of glycine merging into control (Fig. 2A), while positive modulators (β < 1) shifted the curve downwards (Fig. 2B).

The presence of 10 μM glycine has led to the appearance of a high-affinity (nanomolar), but minor displacement phase of [^{3}H]strychnine binding by tropisetron.[4] Therefore, nortropisetron displacement was also examined in the presence of 10 μM glycine which resulted in displacement to about 60% (Fig. 1). However, Figure 1 shows that no distinct high-affinity phase could be observed for nortropisetron, although glycine significantly decreased the slope value of displacement to $n = 0.75 \pm 0.04$ as well as the IC_{50} value to 9.3 ± 2.6 nM nortropisetron (mean ± SEM of three experiments). Thus, nortropisetron displays one of the highest affinities reported for GlyR ligands. The decreased slope and increased potency can be reconciled with displacement heterogeneity due to the appearance

Table 1. Binding affinity and cooperativity factors of tropane derivatives with [^{3}H]strychnine (α) and glycine (β) for GlyRs of rat spinal cord

Compounds	K_A (μM)	α[a]	β
1. 3α-Fumaroyl-bistropane	7.5 ± 2.4	2.6 ± 0.4	ND[b]
2. 3α-Succinyl-bis-*N*-ethyl-nortropane	≫100		
3. 3α-Isophthaloyl-bistropane (50 nM)	0.197 ± 0.013	≫10	3.4 ± 0.7
4. 3α-Terephthaloyl-bistropane (2.5 μM)	24.6 ± 3.0	≫10	0.14 ± 0.01
5. 3α-(3′-Pyridinecarbonyloxy)-tropane	2.2 ± 0.1	6.9 ± 1.5	ND[b]
6. 3α-(6′-Chloro-3′-pyridinecarbonyloxy)-tropane (1 μM)	5.7 ± 0.2	≫10	0.45 ± 0.08
7. 3α-(3′-Pyridinecarbonyloxy)-nortropane (10 nM)	0.082 ± 0.019	≫10	2.2 ± 1.1
8. 3α-(3′-Indolecarbonyloxy)-nortropane[c] (5 nM)	0.017 ± 0.003	≫10	0.73 ± 0.09
9. 3β-(9′-Xantenecarbonyloxy)-nortropane (0.3 μM)	3.1 ± 0.5	≫10	0.26 ± 0.05
10. 3α-(Hydroxydiphenylacetoxy)-*N*-allyl-nortropane	∼52		
11. 3α-Fumaroyl-bisgranatane (6 μM)	20.8 ± 2.5	≫10	1.2 ± 0.7
12. Norscopolamine[d]	8.0 ± 0.6	≫10	ND[b]

Data are means ± SEM fitted to binding data of three to six experiments. Fitting to Eq. 2 resulted in K_A and α values, while β values were determined via Eq. 1. K_L values were 18.9 ± 8.6 μM (mean ± SEM of 10 experiments).

[a] Most of the esters resulted in full displacement of specific binding not distinguishable from non-specific binding (see Fig. 1) therefore their α values could not be exactly determined (α ≫ 10).

[b] ND, not determined.

[c] Nortropisetron.

[d] 6β,7β-Epoxy-1αH,5αH-nortropan-3α-ol (−)tropate.

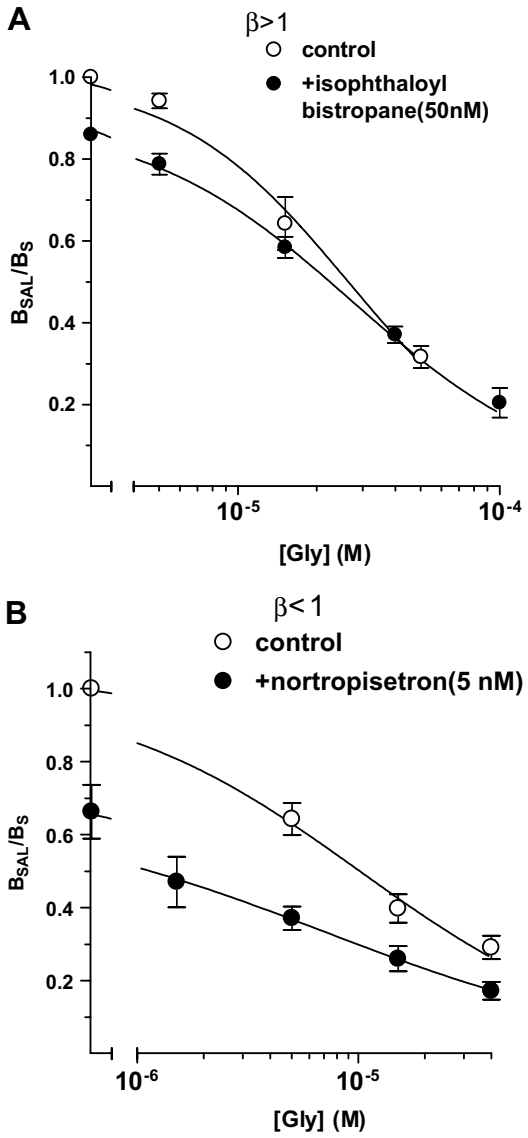


Figure 2. Displacement of [^{3}H]strychnine binding by glycine in the absence (control, ○) and presence of 50 nM 3α-isophthaloyl-bistropane (A) and 5 nM nortropisetron (B) representing negative (A, $\beta > 1$) and positive (B, $\beta < 1$) cooperativities, respectively, with glycine. Ratios of [^{3}H]strychnine binding in the presence of glycine and the tropeines (B_{SAL}) over control (B_S). Data are means ± SEM of three experiments. Fitting to Eq. 1 resulted in the β values of Table 1.

of a minor high-affinity phase. We can conclude that glycine 'creates' a minor component of 'superhigh'-affinity displacement by nortropisetron but it cannot be separated from the major nanomolar phase. Consequently, low nanomolar concentrations of nortropisetron show mutual positive binding cooperativity with glycine which is promising for the development of high-affinity, subunit-selective positive modulators of GlyRs.

Tropeines **3** and **8** with high, nanomolar affinity displayed negative cooperativity with glycine, while the ones with low, micromolar affinity (**4**, **6**, and **9**) showed positive cooperativity (Table 1). Figure 3 demonstrates a good correlation ($r^2 = 0.89$) between the logarithms of K_A and β values of mono and bis (nor)tropeines. A similar correlation was found for a structurally homogeneous group of tropine benzoates.[5] In contrast, the bisgranatane ester **11** with about neutral cooperativity (Table 1) did not fit in the correlation of Figure 3. This confirms the preference for tropeine structures in the allosteric modulation of GlyRs. Nortropisetron did not fit in the correlation either, possibly because of its indole-specific, high-affinity, positive allosteric interactions with glycine.

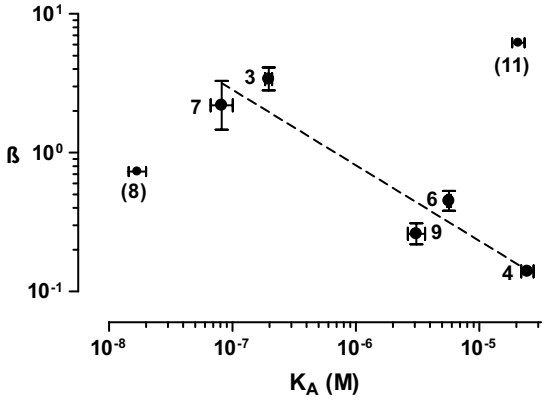


Figure 3. Correlation between the logarithms of binding affinity (K_A) and cooperativity factors with glycine (β) of tropine esters for [^{3}H]strychnine binding to rat spinal GlyRs. Data are logarithms of the means ± SEM in Table 1. Correlation coefficient: $r^2 = 0.89$. Nortropisetron and 3α-fumaroyl-bisgranatane in parentheses (**8** and **11**) did not fit in the correlation and were excluded.

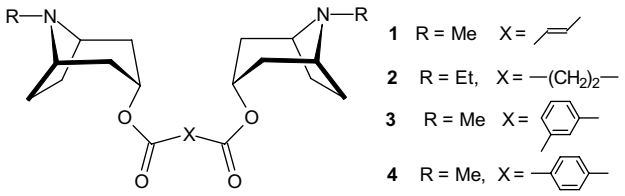


Figure 4. The structures of dicarboxylic acid bis-tropine esters **1**, **2**, **3**, **4** (HCl).

It should be noted that nortropeines including nortropisetron have recently shown nanomolar displacing potencies on [^{3}H]strychnine binding to homomeric GlyRs containing α_1, the major human subunit, transiently expressed in human embryonic kidney 293 cells[20] and these potencies have been amplified by the hyperekplexia mutation R271L of α_1 subunits. Consequently, displacement of [^{3}H]strychnine binding offers an in vitro method to evaluate agents against neurological disorders associated with inherited mutations of GlyRs.[20]

3. Conclusions

This study extended the structure–activity analysis of tropine esters and allosteric modulation of GlyR binding with the modification of acid and alcohol parts. The tropeine binding site tolerated tricyclic carboxylate esters and twin tropeines as well. Not only nortropeines but also properly oriented bistropine esters with conju-

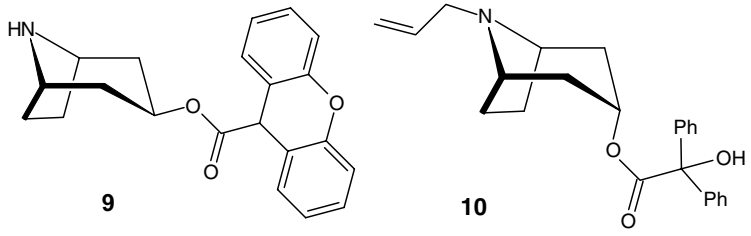


Figure 5. The structures of 3β-(9′-xantenecarbonyloxy)-nortropane (**9**) and 3α-(hydroxydiphenylacetoxy)-*N*-allyl-nortropane (**10**).

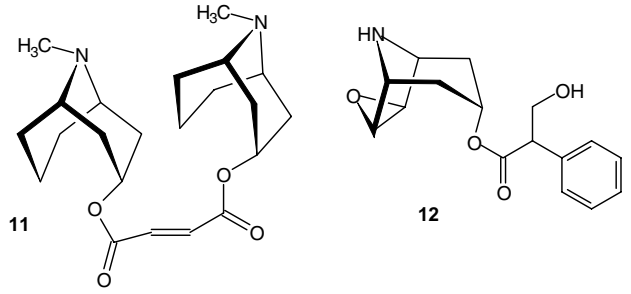


Figure 6. The structures of 3α-fumaroyl-bisgranatane (**11**) and norscopolamine (**12**).

gated aromatic dicarboxylic acids in meta position showed nanomolar displacing potencies. The highest affinity of nortropisetron with $K_A \sim 10$ nM is a promising lead to develop GlyR subunit-selective agents.

4. Experimental

4.1. Chemistry

The structures of the final products as free bases were confirmed by ^{1}H and ^{13}C NMR. Spectra were recorded on a Bruker Avance 250 (250 MHz) spectrometer, in $CDCl_3$ solutions. Chemical shifts (δ) are expressed in ppm relative to the internal standard TMS. Infrared spectra were obtained with a Perkin-Elmer 1605 FT-IR spectrometer. The purity of the compounds was controlled with microanalysis, which was carried out on a Heraeus Micro Rapid CHN. Melting points were determined on a Büchi SMP 20 apparatus.

4.1.1. General procedure for the preparation of the free bases. Powdered K_2CO_3 (138 mg, 1 mmol) was added to a solution of the salt (0.5 mmol) in water (2 mL) and the mixture was extracted with CH_2Cl_2 (2 × 3 mL). The combined organic phases were washed with water (4 mL), dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to yield the free base, which was used for NMR and microanalysis.

4.1.2. 3α-Succinyl-bis-*N*-ethyl-nortropane (2). To a solution of *N*-ethyl-nortropine (1.55 g, 10 mmol) in CH_2Cl_2 (5 mL) is added dropwise succinyl chloride (0.775 g, 5 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for 1 h. The solvent was then removed in vacuum, and to the oily residue water (10 mL) was added. The solution was made alkaline with powdered K_2CO_3, and then it was extracted with CH_2Cl_2 (2× 5 mL). The combined organic phases were washed with H_2O (4 mL), dried over $MgSO_4$, and evaporated under reduced pressure to yield the free base, which was crystallized from diisopropyl ether (1.51 g, 38%), mp: 104 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 5.02 (t, $J = 5.2$ Hz, 2H, H-3), 3.21 (br s, 4H, H-1 and H-5), 2.61 (s, 4H), 2.39 (q, $J = 7.2$ Hz, 4H, CH_2–CH_3), 2.14 (t, $J = 4.2$ Hz, 2H, CH_2–CH_2), 2.07 (t, $J = 4.2$ Hz, 2H, CH_2–CH_2), 1.93 (br s, 8H), 1.05–1.71 (m, 4H), 1.08 (t, $J = 7.2$ Hz, 6H, CH_2–CH_3); ^{13}C NMR δ (ppm): 171.85 (C═O), 68.32 (C-3), 57.54 (C-1 and C-5), 46.07 (CH_2–CH_2), 36.56 (C-2 and C-4), 29.96 (CH_2–CH_3), 26.33 (C-6 and C-7), 14.13 (CH_2–CH_3). Anal. Calcd for $C_{22}H_{36}N_2O_4$: C, 67.32; H, 9.24; N, 7.14. Found: C, 67.01; H, 9.29; N, 6.99.

4.1.3. 3α-Terephthaloyl-bistropane (4). To a solution of tropine (1.41 g, 10 mmol) in CH_2Cl_2 (5 mL) is added dropwise terephthaloyl chloride (1.02 g, 5 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for 1 h. The solvent was then removed in vacuum, and to the oily residue acetone (5 mL) was added. The precipitate was filtered to yield white crystalline substance (1.15 g, 56%) mp >240 °C (HCl salt). ^{1}H NMR ($CDCl_3$) δ (ppm): 8.03 (s, 4H, H-Ar), 5.20 (t, $J = 4.9$ Hz, 2H, H-3), 3.12 (br s, 4H, H-1 and H-5), 2.90 (s, 6H, N–CH_3), 1.61–2.25 (m, 16H); ^{13}C NMR δ (ppm): 165.23 (C═O), 134.75 (C-Ar), 129.71 (C-Ar) 68.95 (C-3), 59.99 (C-1 and C-5), 40.68 (N–CH_3), 36.86 (C-2 and C-4), 26.08 (C-6 and C-7). Anal. Calcd for $C_{24}H_{32}N_2O_4$: C, 69.88; H, 7.82; N, 6.79. Found: C, 69.55; H, 7.65; N, 6.97.

4.1.4. 3α-(3′-Pyridinecarbonyloxy)-tropane (5). Nicotinic acid ethyl ester (1.52 g, 10 mmol), tropine (1.55 g, 11 mmol), and sodium (0.05 g 1.15 mmol) were heated at 120 °C for 24 h at 15 mm in vacuum, whereby ethanol produced was distilled off. Methanol was added to the mixture, and it was stirred for 10 min. The solvent was then removed, water was added to the residue, and it was extracted with diethyl ether. The ethereal solution was dried and evaporated to give thick yellow oil (1.7 g, 69%). It was dissolved in acetone and acidified with cc. HBr solution to afford white crystals (1.85 g), recrystallized from ethanol, mp >240 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 9.23 (d, $J = 1.3$ Hz, 1H, H-2′), 8.81 (dd, $J = 4.7$ Hz, $J = 1.3$ Hz, 1H, H-4′), 8.32 (d, $J = 7.9$ Hz, 1H, H-6′), 7.44 (dd, $J = 4.7$ Hz, 7.9 Hz, 1H, H-5′), 5.31 (t, $J = 5.1$ Hz, 1H, H-3), 3.20 (br s, 2H, H-1 and H-5), 2.33 (s, 3H, N–CH_3), 1.84–2.17 (m,

8H); ^{13}C NMR δ (ppm): 164.79 (C=O), 153.65 (C-6′), 151.00 (C-2′), 137.27 (C-4′), 126.88 (C-3′), 123.71 (C-5′), 69.06 (C-3), 60.06 (N–CH_3), 40.80 (C-1 and C-5), 37.00 (C-2 and C-4), 26.07 (C-6 and C-7). Anal. Calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.39; H, 7.15; N, 11.60.

4.1.5. General procedure for the demethylation of tropeines. Tropeines (5 mmol) were dissolved in toluene (10 mL) and a catalytic amount of K_2CO_3 was added. The solution was boiled and trichloroethyl chloroformate[5,16] (7.5 mmol) in toluene (5 mL) was added dropwise in 20 min while stirring. The mixture was refluxed for additional 2 h, cooled, and extracted twice with 10% acetic acid solution. The organic phase was separated, dried over Na_2SO_4, and evaporated. The oily material was crystallized from diisopropyl ether and used without further purification. Yield: 85–90%.

4.1.6. General procedure for the removal of the trichlorocarboethoxy group. *N*-Trichlorocarboethoxy-nortropeine[5] (4.5 mmol) dissolved in acetic acid (47 mL) was added dropwise to a suspension of zinc dust (1.46 g, 22 mmol) in water (5 mL) at room temperature. After stirring for 2 h, most of the acetic acid was removed in vacuum, and the residue was cooled to 10 °C, and made alkaline by adding an aqueous solution of 15% of NaOH and 5% of potassium–sodium tartrate. The solution was then saturated with K_2CO_3 and extracted three times with CH_2Cl_2. The organic phases were combined, the solution was dried over Na_2SO_4 and evaporated to give oily materials that could be either crystallized, or were converted into their hydrochloride salts. Yield: 50–61%.

4.1.6.1. 3α-(3′-Pyridinecarbonyloxy)-nortropane (7). White crystal (0. 52 g, 50%); mp > 240 °C (HCl salt); ^{1}H NMR ($CDCl_3$) δ (ppm): 9.16 (d, J = 1.4 Hz, 1H, H-2′), 8.72 (dd, J = 4.8 Hz, J = 1.4 Hz, 1H, H-4′), 8.22 (d, J = 7.9 Hz, 1H, H-6′), 7.35 (dd, J = 4.8 Hz, 7.9 Hz, 1H, H-5′), 5.30 (t, J = 4.8 Hz, 1H, H-3), 3.53 (br s, 2H, H-1 and H-5), 1.82–2.41 (m, 8H); ^{13}C NMR δ (ppm): 163.56 (C=O), 152.38 (C-6′), 149.75 (C-2′), 135.98 (C-4′), 125.60 (C-3′), 122.41 (C-5′), 68.48 (C-3), 52.27 (C-1 and C-5), 36.38 (C-2 and C-4), 28.42 (C-6 and C-7). Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found: C, 66.98; H, 6.85; N, 12.02.

4.1.6.2. 3α-(3′-Indolecarbonyloxy)-nortropane (8). Tropisetron was demethylated and transformed to the *nor* derivative according to the general procedure described above. Its hydrochloride salt was recrystallized from ethanol; (0.74 g, 61%); mp >240 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 11.17 (br s, 1H, N*H*), 7.66–7.81 (m, 1H, H-Ar), 7.50 (s, 1H, H-Ar), 7.11 (m, 1H, H-Ar), 6.78–6.86 (m, 2H, H-Ar), 4.90 (t, J = 4.7 Hz, 1H, H-3), 3.19 (2H, br s, H-1, H-5), 1.51–1.86 (m, 8H); ^{13}C NMR δ (ppm): 164.55 (C=O), 136.79 (C-8′), 131.69 (C-9′), 128.1 (C-2′), 126.20 (C-5′), 122.62 (C-4′), 121.49 (C-6′), 120.81 (C-7′), 112.35 (C-3′), 66.95 (C-3), 53.28 (C-1 and C-5), 37.49 (C-2 and C-4), 29.36 (C-6 and C-7). Anal. Calcd for $C_{16}H_{18}N_2O_2$: C, 71.09; H, 6.71; N, 10.36. Found: C, 70.83; H, 6.68; N, 10.40.

4.1.7. 3β-(9′-Xantenecarbonyloxy)-nortropane (9). Ψ-Tropine (1.41 g, 0.01 mol) and TEA (1.1 g, 11 mmol) were dissolved in CH_2Cl_2 (15 mL), and 9-xantenecarboxylic acid chloride (2.69 g, 11 mmol) was added dropwise, at room temperature in CH_2Cl_2 (10 mL). The solution was then stirred for 2 h and extracted with saturated $NaHCO_3$ solution. The organic phase was separated, dried with Na_2SO_4, and evaporated at reduced pressure. The Ψ-tropine ester was demethylated and the carbamate was cleaved according to the general procedure to yield white crystalline substance (2.85 g, 85%), mp 141 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 6.92–7.21 (m, 8H, H-Ar), 5.30 (s, 1H, H-9′), 4.66–4.95 (m, 1H, H-3), 3.57 (br s, 2H, H-1 and H-5), 1.79–1,41 (m, 8H); ^{13}C NMR δ (ppm): 171.83 (C=O), 151.63, 129.48, 129.18, 123.65, 118.55, 117.37 (aromatics), 68.15 (C-3), 54.40 (C-1 and C-5), 37.02 (C-2 and C-4), 28.31 (C-6 and C-7). Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.31; N, 4.18. Found: C, 75.07; H, 6.18; N, 4.19.

4.1.8. Norscopolamine (12). To a solution of scopolamine (6β,7β-epoxy-1αH,5αH-tropan-3α-ol (−)tropate) (0.31 g, 1.0 mmol) in 1,2-dichloroethane (5 mL), α-chloroethyl chloroformate (0.17 g, 1.2 mmol) and TEA (0.12 g, 1.2 mmol) were added. The solution was refluxed for 10 h and then it was evaporated under reduced pressure. To the residue methanol (5 mL) was added and refluxed for 2 h. The solution was then evaporated, saturated K_2CO_3 solution was added, and it was extracted with CH_2Cl_2. The solution was dried over Na_2SO_4 and evaporated to give a yellow oil, which was crystallized from diisopropyl ether to give white crystalline substance (0.20 g, 69%), mp 95 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 7.22–7.36 (m, 5H, H-Ar), 5.00 (t, J = 4.9 Hz, 1H, H-3), 4.14–4.15 (m, 1H, H-2′), 3.71–3.82 (m, 2H, C*H*$_2$–OH), 3.26 (d, J = 2.9 Hz, 1H, H-6), 3.17 (br s, 1H, H-5), 3.02 (br s, 1H, H-1), 2.12 (d, J = 2.9 Hz, 1H, H-7), 1.95–2.13 (m, 2H), 1.67 (br d, J = 15.2 Hz, 1H), 1.39 (br d, J = 15.2 Hz, 1H). ^{13}C NMR δ (ppm): 171.99 (C=O), 136.23, 129.32, 128.42, 128.29 (aromatics), 67.14 (C-3), 63.96 (*C*H$_2$–OH), 54.83 (C-6), 54.26 (C-7), 53.79 (C-2′), 52.12 (C-1), 52.00 (C-5), 31.55 (C-2), 31.13 (C-4). Anal. Calcd for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.28; H, 6.71; N, 4.72.

4.2. Biology: membrane preparation and binding studies

Crude synaptic membranes were prepared from male Wistar rats as described.[9] Briefly, spinal cords homogenized in 5 mM Tris–HCl buffer (pH 7.4) were incubated on ice for 20 min and centrifuged at 30,000*g* for 20 min. The pellets were resuspended in 50 mM Tris–HCl buffer (pH 7.4), centrifuged similarly four times, and frozen. The thawed membranes were centrifuged in 50 mM Tris–HCl buffer containing 200 mM KSCN (pH 7.4) at 10,000*g* for 10 min before binding assays.

The binding assay was performed in 1 mL membrane suspensions in 50 mM Tris–HCl plus 200 mM KSCN with 3 nM [^{3}H]strychnine (10 μCi/mmol, Dupont-NEN). Samples were incubated at 0 °C for 35 min. Duplicate aliquots were filtered on Whatman GF/B filters under vacuum and washed by 3× 3-mL ice-cold buf-

fer. Radioactivity of the filters was measured by scintillation spectrometry. Non-specific binding was determined in the presence of 2 mM glycine.

4.3. Data analysis

Non-linear regression programs NLREG (PH Sherrod, Nashville, TN) and GraphPad Prism Version 4.0 (San Diego, CA) were used for fitting. The ternary allosteric model of GlyR binding was described previously.[9] Briefly, it contains three dissociation constants (K_S, K_L, and K_A) for the binding of the antagonist [^{3}H]strychnine (S), the agonist glycine (L), and the allosteric agents (A) as well as the cooperativity factors of A with [^{3}H]strychnine (α) and with glycine (β). Eq. 1[21] expresses the ratio of specific [^{3}H]strychnine binding in the presence of three ligands (B_{SAL}) over control (B_S, in the presence of [^{3}H]strychnine):

$$\frac{B_{SAL}}{B_S} = \frac{[S] + K_S}{[S] + K_S \times \frac{[L](K_A+[A]/\beta)+K_L(K_A+[A])}{K_L(K_A+[A]/\alpha)}} \quad (1)$$

In the absence of agonists Eq. 1 is simplified to:

$$\frac{B_{SA}}{B_S} = \frac{[S] + K_S}{[S] + \frac{K_S(K_A+[A])}{K_A+\frac{[A]}{\alpha}}} \quad (2)$$

K_A and α values were determined via Eq. 2. Then concentration-dependent displacement by glycine (control, in the absence of A) resulted in the determination of K_L via Eq. 1. Parallel displacement studies in the absence and presence of A at low occupancy (between 0.1 and 0.4 K_A) enabled us to determine β values via Eq. 1.

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