Bioorganic & Medicinal Chemistry 17 (2009) 6872–6878

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of heteroaromatic tropeines and heterogeneous binding to glycine receptors

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ARTICLE INFO

Article history:
Received 30 April 2009
Revised 12 August 2009
Accepted 14 August 2009
Available online 20 August 2009

Keywords:
[^{3}H]Strychnine binding
(Nor)tropeines
Binding heterogeneity
5-HT$_3$ receptors

ABSTRACT

Heteroaromatic carboxylic esters of (nor)tropine were synthesized. Tropine esters displaced [^{3}H]strychnine binding to glycine receptors of rat spinal cord with low Hill slopes. Two-site displacement resulted in nanomolar $IC_{50,1}$ and micromolar $IC_{50,2}$ values, and $IC_{50,2}/IC_{50,1}$ ratios up to 615 depending on the heteroaromatic rings and *N*-methyl substitution. Nortropeines displayed high affinity and low heterogeneity. $IC_{50,1}$ and $IC_{50,2}$ values of tropeines did not correlate suggesting different binding modes/sites. Glycine potentiated only the nanomolar displacement reflecting positive allosteric interactions and potentiation of ionophore function. Affinities of three (nor)tropeines were different for glycine receptors but identical for 5-HT$_3$ receptors.

1. Introduction

Glycine receptors (GlyRs) belong to the pentameric ('Cys-loop') family of ligand-gated ion channels. Five GlyR subunits out of α1–α3 and β form channels selective for chloride ions. They mediate inhibitory neurotransmission in mammalian spinal cord. GlyRs have primary roles in processing motor and sensory signals, while GlyRs containing α3 subunits participate in inflammatory pain sensitization.[1] Therefore GlyRs might be targets of muscle relaxant, sedative, and analgesic agents. There is an increasing consensus that selective and positive allosteric modulation of GlyRs might be exploited pharmacologically,[1,2] similar to the facilitation of A-type γ-aminobutyric acid ($GABA_A$) receptors by benzodiazepines, barbiturates, and neurosteroids. Unfortunately however, no drugs exist which target GlyRs, while some GlyR subunit-selective agents affect other receptors as well. For example, some cannabinoids exert differential modulation of GlyR subunits α1–α3.[3] Due to structural similarities of GlyRs with other pentameric receptors of neurotransmitters (e.g., $GABA_A$, nicotinic acetylcholine, and 5-HT$_3$-type serotonin), their ligand selectivities overlap. For example, tropisetron and other 'setron'-type antagonists of 5-HT$_3$ receptors potentiate and inhibit the ionophore function of GlyRs in nano- and micromolar concentrations, respectively.[4]

Tropane alkaloids, occurring as esters of carboxylic acids and tropine or ψ-tropine are commonly found in plants of different families, such as in Solanaceae, Erythroxylaceae, and Convolvulaceae. Because of their valuable pharmacological properties, a large number of tropine esters (tropeines) have been synthesized. It is promising that the bidirectional modulating effects of tropeines have been found to be GlyR subunit-dependent.[5] Consensus structure of the pharmacologically desirable high-affinity potentiation has been associated with aromatic esters of tropine.[6] Selectivity has been shifted from 5-HT$_3$ receptors towards GlyRs via benzoic esters of tropine.[7] A substituted benzoic ester of nortropine, nor-*O*-zatosetron has displayed nanomolar affinity and preference for point-mutated recombinant human α1 GlyRs leading to hyperekplexia, an inherited neurological disease.[8] Tropisetron has deserved utmost interest because it potentiated α1 GlyR function in femtomolar concentrations.[9] This allosteric modulator might have the highest affinity identified for pentameric receptors but this is overshadowed by its sub-nanomolar antagonism on 5-HT$_3$ receptors. Also remarkably, the presence of the agonist glycine (10 μM) has led to heterogeneous (nanomolar and micromolar) displacement by tropisetron for [^{3}H]strychnine binding to spinal GlyRs with high heterogeneity ($IC_{50,2}/IC_{50,1}$ >100) versus homogeneous, micromolar displacement without glycine.[6] Here, we revealed displacing heterogeneity of tropeines which was intrinsic, in the absence of glycine. Since glycine potentiated only the high-affinity phase of displacement, this can be associated with the advantageous potentiation of GlyR function by tropeines. In contrast, micromolar displacement can be attributed to GlyR antagonism which blocks the chloride channels. These opposite potentiating and inhibitory effects can be separated and hopefully lead to GlyR-selective potentiating agents which lack the high affinity of 'setrons' (tropisetron, bemesetron) to 5-HT$_3$ receptors.

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doi:10.1016/j.bmc.2009.08.029

We explored the $IC_{50,2}/IC_{50,1}$ ratios of binding heterogeneity with the replacement of the indole ring of tropisetron by other heteroaromatic rings in order to find the structural requirements of increasing heterogeneity and selective potentiation of GlyRs.

2. Results and discussion

2.1. Chemistry

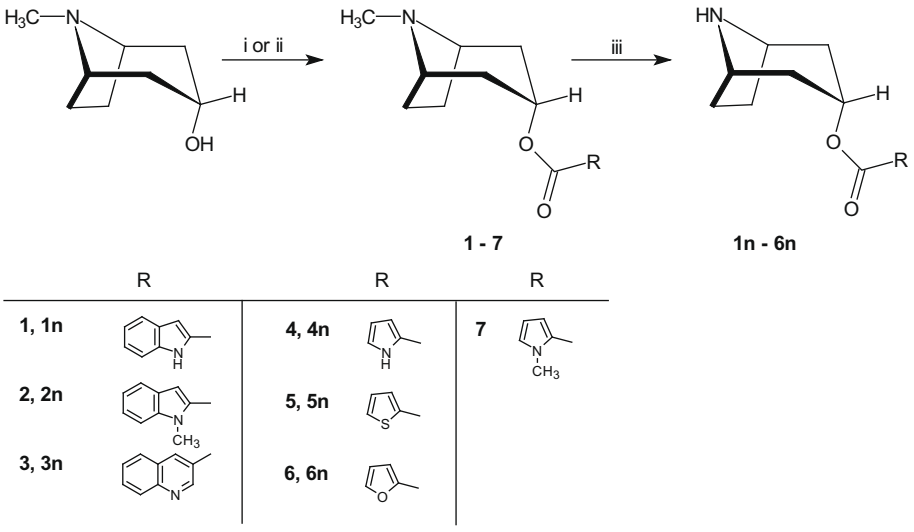
The esterification of tropine with substituted benzoic acid derivatives can be carried out, applying the usual acylation processes, in acceptable yields.[10–12] Preparation of the new heteroaromatic tropine esters however, was much more difficult. We tried several acylation methods but they gave the desired products generally in poor yields. To achieve better results, finally we chose the *trans*-esterification method for the synthesis of **1– 3**.[13,14] On the other hand, **4**–**7** were prepared using the acid chlorides, prepared from the sodium salts of the corresponding acids with oxalyl chloride (Scheme 1).

Tropeines **1**–**6** were subjected to demethylation to obtain *nor* derivatives. Demethylation methods described in the literature for the synthesis of nortropane derivatives[15,16] are well developed processes. By use of ethyl chloroformate, demethylation takes place readily under the formation of methyl chloride and *N*-ethoxycarbonyl derivatives. However, the hydrolysis of the ethoxycarbonyl group requires vigorous reaction conditions and the ester group is also cleaved. To avoid the cleavage of the ester function 2,2,2-trichloroethyl chloroformate can be applied, because the removal of the trichloroethoxycarbonyl group from the nitrogen atom can be achieved with zinc in acetic acid solution. Demethylation and subsequent cleavage of the carbamate can be performed in one pot using α-chloroethyl chloroformate in refluxing chloroform in the presence of $NaHCO_3$, resulting in the heteroaromatic nortropine esters.[17] The mild reaction conditions and good yields prompted us to use this simple reaction for the synthesis of the *nor* derivatives **1n**, **2n**, **3n**, **4n**, **5n**, and **6n** (Scheme 1). It should be mentioned that no demethylation of the pyrrole nitrogen atom of **2** could be observed. Preparation of the demethylated derivative **7n** was unsuccessful in pure form, due to low conversions and formation of several byproducts. Compounds **8** and **8n** were prepared as described.[18]

2.2. Receptor binding

All compounds exhibited concentration-dependent, full displacement of specific [^{3}H]strychnine binding to spinal GlyRs (Figs. 1 and 2). Table 1 summarizes the parameters of fitting to the displacement data. Nortropeines (**1n**, **3n, 4n, 5n, 6n**, and **8n)** exerted homogenous displacement; their Hill slope values were not significantly different from unity (Table 1). In contrast, sigmoidal fitting for tropine esters resulted in Hill slopes significantly lower than unity. These displacements with shallow Hill slopes were fitted with two sites significantly better than with one site. Figure 1 illustrates this with **3** (continuous vs dotted lines). Figure 1 also demonstrates that displacement by nortropeine **3n** did not show a low-affinity phase while that of its N-methylated analogue **3** did. On the other hand, the high-affinity $IC_{50,1}$ values of **3** and **3n** were similar (Fig. 1). Moreover, the $IC_{50,1}$ values of tropeines were not significantly different from the IC_{50} of the respective *nor* analogues (**1**–**1n**, **2**–**2n**, **3**–**3n, 4**–**4n, 5**–**5n**, and **6**–**6n** pairs in Table 1). For most compounds in Table 1, the minor contribution of the high-affinity fraction ($fraction_1$) resulted in greater SEM values of the corresponding lg $IC_{50,1}$ values. In view of the distinct binding heterogeneity of heteroaromatic tropeines versus nortropeines observed here, we have re-evaluated now the displacement data for the 3α-(3′-pyridinecarbonyloxy)-(nor)tropane pair **8–8n** synthesized and evaluated earlier according to an allosteric model.[18] Table 1 shows that they fit in the general trend: nortropeine **8n** displayed homogeneous displacement while tropeine **8** with its shallow displacement was fitted with two sites significantly better. Overall, tropeines bind to different populations of GlyR sites in distinctive manner: with high-affinity (nanomolar) $IC_{50,1}$ and low-affinity (micromolar) $IC_{50,2}$ while nortropeines have almost identical, nanomolar affinities to these populations of binding sites, without distinction.

Native GlyRs of rat spinal cord have heterooligomeric α1β subunit composition.[2] Point-mutation of amino acid N102 in the agonist-binding interface cavity of recombinant α1(β) GlyRs has abolished inhibition but not potentiation by tropisetron suggesting the existence of discrete binding sites for femtomolar potentiation and micromolar inhibition.[9] This is strongly supported by distinct patterns of point mutations at the agonist-binding cavity of recombinant human α1 GlyRs expressed in Xenopus oocytes which abolished either nanomolar potentiation or micromolar



Scheme 1. Synthesis of tropine and nortropine esters. Reagents and conditions: (i) Method A: RCOOEt, Na, 120 °C; (ii) Method B: RCOCl, K_2CO_3, CH_3CN, reflux; (iii) $CH_3CH(Cl)$-OCOCl, $NaHCO_3$, $CHCl_3$ reflux.

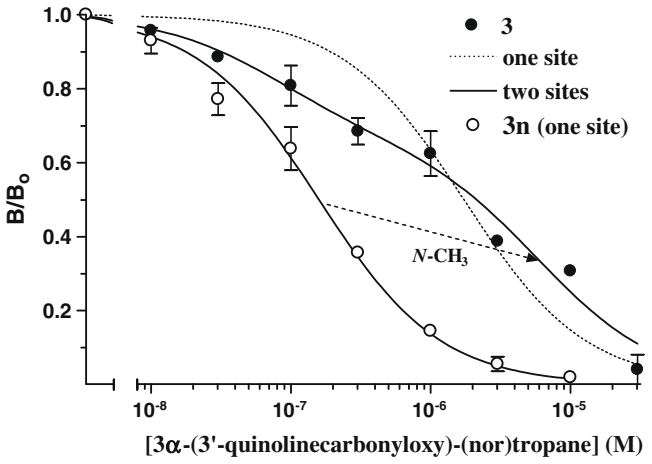


Figure 1. Concentration-dependent displacement of [^{3}H]strychnine binding to striatal GlyRs by a representative pair, tropeine **3** and nortropeine **3n**. Specific [^{3}H]strychnine binding in the presence (B) versus absence (control, B_o) of displacing tropeines. Data are mean ± SEM of four experiments. Displacement by **3n** (○) can be fitted with one site while that of the corresponding **3** (●) can be fitted to two sites (continuous curve) significantly better than to one site with Hill slopes of one (dotted curve). The dashed arrow shows that N-methylation of **3n** shifts the $IC_{50,2}$ value of **3**.

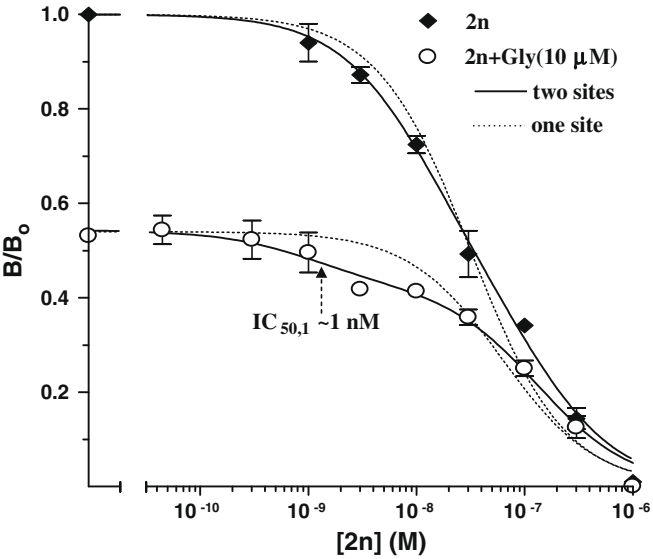


Figure 2. Displacement of [^{3}H]strychnine binding by **2n** in the absence (♦) and presence (○) of 10 μM glycine. Data are mean ± SEM of three to six experiments. The data can be fitted to two sites (continuous curve) significantly better than to one site (dotted curve). Glycine displaced the control [^{3}H]strychnine binding to ~53% and selectively shifted the high-affinity fraction to the left.

inhibition of glycine-elicited chloride currents by 3α-(3′-methoxy-benzoyloxy)-nortropane.[19] Tropisetron appeared to have different binding orientations in α/α versus α/β subunit interfaces of GlyRs.[9] Coexpression of the major subunit α1 with β has resulted in potentiation of agonist-elicited GlyR function by the heteroaromatic tropisetron two orders of magnitude stronger than inhibition.[5] Here, heteroaromatic tropeines displayed similarly heterogeneous displacing potencies on native α1β GlyR binding. Glycine potentiated selectively the high-affinity $IC_{50,1}$ of tropeines for [^{3}H]strychnine binding. All these findings support that positive cooperativity between tropeine binding of nanomolar affinity and agonist binding can be associated with potentiation of GlyR function. On the other hand, micromolar tropeine concentrations prevent agonist binding and lead to closed GlyR channels. $IC_{50,1}$ and $IC_{50,2}$ values of the tropeines did not correlate with each other ($r^2 = 0.02$, plot not shown). This supports the existence of different modes/sites of high- and low-affinity tropeine bindings. Thus, we postulate that tropeine binding with nanomolar affinity can be associated with the advantageous potentiation while micromolar binding elsewhere corresponds to inhibition of GlyRs. This is why we were interested in heterogeneous binding and selective potentiation.

The $IC_{50,2}/IC_{50,1}$ ratios of binding heterogeneity varied between 1 (that is, no apparent distinction for most nortropeines) and 615 (Table 1). We did observe slight heterogeneity of displacement for only one nortropeine, **2n** as illustrated in Figure 2. Fitting to two sites (continuous line) revealed small but statistically significant heterogeneity as compared to fitting to one site (dotted line). It resulted in a high-affinity fraction of about 54% and $IC_{50,2}/IC_{50,1} = 11$ for **2n** (Table 1). We also examined displacement by **2n** in the presence of a partially displacing concentration, 10 μM of the GlyR agonist glycine to confirm the involvement of GlyRs. Figure 2 shows that displacing heterogeneity was more substantial in the presence of glycine due to a leftward shift of only the high-affinity phase. This represents strong, selective potentiation of the high affinity resulting in $IC_{50,1}$ ~1 nM (Fig. 2) while the low affinity was not affected (Table 1). Glycine increased the $IC_{50,2}/IC_{50,1}$ ratio of **2n** from 11 to 124 (Table 1). It should be noted that not only tropisetron but also other 5-HT_3 receptor antagonists, bemesetron and zatosetron which are substituted benzoyloxy-tropanes, resulted in heterogeneous displacement of [^{3}H]strychnine binding in the presence of 10 μM glycine with the appearance of a high-affinity phase.[5] Therefore displacement data for several substituted benzoyloxy-(nor)tropanes studied previously[5,7] were re-evaluated. All these nortropeines and most tropeines displayed apparently homogeneous displacement with Hill slopes around unity. However, displacement by bemesetron, 3α-(3′,5′-dichlorobenzoyloxy)-tropane was fitted to two sites significantly better than to one site (data not shown). Moreover, 10 μM glycine selectively shifted the high-affinity phase to the left. Glycine increased the displacing heterogeneity from 18 to 292 but it did not affect the $IC_{50,2}$ value significantly (data not shown). Glycine also potentiated the displacement by 3α-(3′-indolecarbonyloxy)-nortropane (nortropisetron).[18] However, even though 10 μM glycine decreased the Hill slope of displacement by nortropisetron, two phases could not be significantly separated.[18] Since the 1′-methylated indole derivative **2** bound with high heterogeneity and tropeine **4** with a 2′-pyrrole ring displayed the greatest heterogeneity (Table 1), we merged them and synthesized the 1′-methylated pyrrole derivative **7**. Although the Hill slope of displacement by **7** was below unity (Table 1), two phases could not be significantly separated. If the high-affinity fraction is small, it might be undetectable. Thus, the $IC_{50,2}/IC_{50,1}$ ratio might be higher but it cannot be determined (see the arrow at **7** in Fig. 3). These previous and present findings can be interpreted now that glycine reveals/increases the heterogeneity of (nor)tropeine binding to GlyRs. The selective potentiating effects of glycine on the high-affinity fraction of displacement by (nor)tropeines indicate positive allosteric interactions with glycine binding. This interaction in vitro and the high binding affinity of about 1 nM for **2n** might be exploited pharmacologically in positive modulation of glycinergic neurotransmission.

Binding heterogeneity varied within three orders of magnitude depending on the structures of heteroaromatic rings and *N*-methyl substitution (Table 1). Heterogeneity was increased in two ways (see the arrows in the Graphical abstract): (1) Glycine further potentiated the high binding affinity without affecting the low-affinity component. (2) N-Methylation attenuated the low-affinity tropeine binding while it did not significantly affect the high affinity ones.

Table 1
Displacing potencies of (nor)tropeines for [^{3}H]strychnine binding to GlyRs of rat spinal cord and for [^{3}H]granisetron binding to 5-HT$_3$-type serotonin receptors of rat forebrain

	(Nor)tropeines	GlyR						5HT$_3$R
		One site		Two sites				IC$_{50}$ nM
		−lg IC$_{50}$	$-n_H$	Fraction$_1$	−lg IC$_{50,1}$	−lg IC$_{50,2}$	$\frac{IC_{50,2}}{IC_{50,1}}$	
1	3α-(2′-Indolecarbonyloxy)-tropane	5.33 ± 0.11	0.76 ± 0.06[a]	0.13 ± 0.05	7.22 ± 0.60	5.10 ± 0.09	133	
1n	3α-(2′-Indolecarbonyloxy)-nortropane	6.63 ± 0.08	0.92 ± 0.10				∼1[c]	32 ± 7
2	3α-(1′-Methyl-2′-Indolecarbonyloxy)-tropane	5.80 ± 0.05	0.68 ± 0.08[a]	0.15 ± 0.03	7.99 ± 0.41	5.62 ± 0.05	240	18 ± 1
2n	3α-(1′-Methyl-2′-Indolecarbonyloxy)-nortropane	7.47 ± 0.05	0.82 ± 0.05[a]	0.54 ± 0.23	7.94 ± 0.24	6.90 ± 0.29	11	25 ± 5
2n	—″— + 10 μM Gly	7.15 ± 0.07	0.73 ± 0.11[a]	0.22 ± 0.07[b]	8.97 ± 0.46	6.87 ± 0.12	124	
3	3α-(3′-Quinolinecarbonyloxy)-tropane	5.72 ± 0.11	0.61 ± 0.05[a]	0.32 ± 0.10	7.14 ± 0.37	5.26 ± 0.16	76	
3n	3α-(3′-Quinolinecarbonyloxy)-nortropane	6.78 ± 0.07	0.94 ± 0.07				∼1[c]	
4	3α-(2′-Pyrrolecarbonyloxy)-tropane	4.95 ± 0.05	0.63 ± 0.09[a]	0.16 ± 0.03	7.47 ± 0.34	4.68 ± 0.07	615	
4n	3α-(2′-Pyrrolecarbonyloxy)-nortropane	6.89 ± 0.04	0.91 ± 0.13				∼1[c]	
5	3α-(2′-Thiophenecarbonyloxy)-tropane	5.72 ± 0.07	0.71 ± 0.04[a]	0.23 ± 0.07	7.05 ± 0.31	5.37 ± 0.09	47	
5n	3α-(2′-Thiophenecarbonyloxy)-nortropane	7.38 ± 0.08	0.89 ± 0.11				∼1[c]	
6	3α-(2′-Furanecarbonyloxy)-tropane	5.25 ± 0.07	0.73 ± 0.05[a]	0.14 ± 0.03	7.30 ± 0.35	5.00 ± 0.06	200	
6n	3α-(2′-Furanecarbonyloxy)-nortropane	7.10 ± 0.04	1.07 ± 0.19				∼1[c]	
7	3α-(1′-Methyl-2′-Pyrrolecarbonyloxy)-tropane	5.12 ± 0.08	0.86 ± 0.09[a]				⩾1[c]	
8	3α-(3′-Pyridinecarbonyloxy)-tropane[d]	5.37 ± 0.05	0.74 ± 0.07[a]	0.19 ± 0.03	7.23 ± 0.26	4.64 ± 0.07	390	
8n	3α-(3′-Pyridinecarbonyloxy)-nortropane[d]	7.00 ± 0.03	1.20 ± 0.03				∼1[c]	

Fitting parameters are mean ± SEM of four experiments for sigmoidal fitting to one site, while fitting with two-site displacement was performed for the pooled data of four to six experiments.

[a] Significantly lower than unity in Student's *t* test (P <0.05) indicating binding heterogeneity. For these compounds fitting with two sites was significantly better (P <0.001) than with one site according to the *F* test.

[b] Ten-micromolar glycine reduced the binding to 0.54 ± 0.02. Fraction$_1$ = 0.22 corresponds to 40.7% of that.

[c] Significant heterogeneity of displacement was not observed.

[d] Binding data were taken from Ref. 18 and refitted.

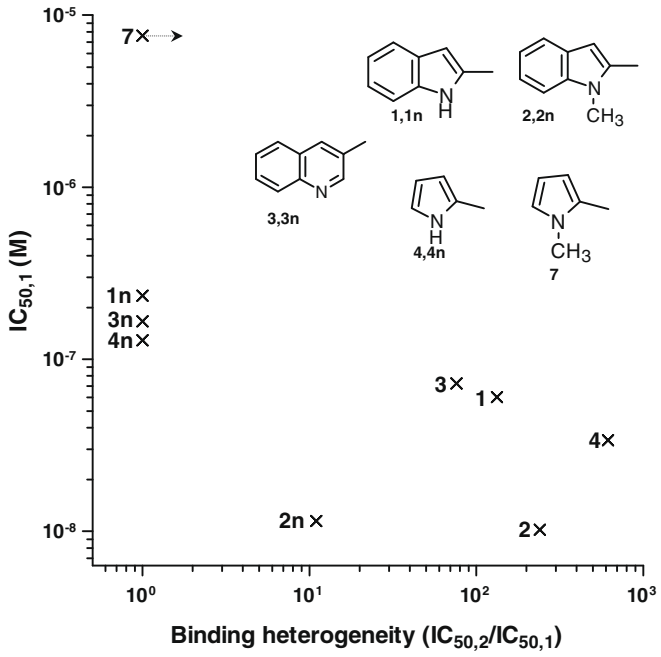


Figure 3. N-containing heterocyclic esters strongly affect the logarithmic plot of the displacing potency with high affinity (IC$_{50,1}$) of (nor)tropeines on [^{3}H]strychnine binding to GlyRs and binding heterogeneity (IC$_{50,2}$/IC$_{50,1}$). Data are taken from Table 1. The structures of R moieties are inserted.

Beside the distinctive effects of N-methylation of the nortropane ring, structure–activity relationships of systematically modified (hetero)aromatic rings can be also evaluated. Figure 3 shows that the binding properties can be affected significantly by the different position and stereo-electronic properties of the heteroaromatic N atom and its different interactions with GlyR moieties. This is supported by the following findings. (1) A shift of the carbonyloxy attachment between the aromatic and tropane rings from position 3′ to 2′ of the indole ring significantly affects the shape of the tropeine molecule. Consequently, the binding affinity of the 2′-derivative **1n** was much lower than that of the isomeric 3′-derivative nortropisetron with IC$_{50}$ = 17 nM.[18] (2) Binding heterogeneities of **2** with a 1′-methyl-2′-indole ring and of **4** with a 2′-pyrrole ring were great (Fig. 3), while no heterogeneity could be detected for **7** with a 1′-methyl-2′-pyrrole ring. This discrepancy indicates a crucial role of the N heteroatom in high-affinity binding, thus in heterogeneity. (3) N-methylation of the indole rings of **1** and **1n** increased the high affinity and binding heterogeneity of **2** and **2n** (Fig. 3). (4) Ring enlargement of 3′-indole into 3′-quinoline derivatives decreased the binding affinity of nortropeine **3n** while the binding of the 3′-quinoline tropeine **3** was much more heterogeneous. (5) The absence of the phenyl ring of 2′-indole derivative **1** also led to more potent and heterogeneous binding for the 2′-pyrrole derivative **4** (Fig. 3). On the other hand, the phenyl group in 3′-quinoline derivatives **3** and **3n** had no apparent effects in comparison with 3′-pyridine derivatives **8** and **8n**. The presence of the pyridine N atom in **8** did not affect the high binding affinity either, in comparison with the corresponding benzoyloxy-tropane,[7] but it introduced great heterogeneity in GlyR binding. The rank order of displacing potencies for the heteroatoms in (nor)tropeines was N > O > S. Overall, heteroatoms in the aromatic (thiophene, furane, pyridine, and pyrrole) rings resulted in great binding heterogeneity (**4**, **6**, and **8**).

Structure–activity relationships of binding to GlyRs versus 5-HT$_3$ type serotonin receptors show substantial differences. This is demonstrated in Table 1 for compounds **1n**, **2**, and **2n** closely related to tropisetron. Their composite IC$_{50}$ values in the first data column of Table 1 varied within two orders of magnitude for GlyRs while their affinities to 5-HT$_3$ receptors were almost identical (last column). This means that (nor)tropeine binding to 5-HT$_3$ receptors is insensitive for *N*-methyl substituents of both indole and tropane rings. In contrast, N-methylation of **1n** 'created' binding heterogeneity for GlyRs differentially: in the nortropane ring via attenuating a low-affinity component for **1**, while in the indole ring via potentiating the high-affinity component for **2n**. Remarkably, the Gly-potentiated IC$_{50,1}$ ∼1 nM of **2n** was more potent than its IC$_{50}$ = 25 nM for 5-HT$_3$ receptors therefore it might lead to agents with promising selectivity for GlyRs.

3. Conclusions

Heteroaromatic esters of tropine discriminated between high- and low-affinity tropeine binding modes/sites on GlyRs. The ratios of high versus low binding affinities were increased (1) by glycine via further potentiating the high affinity and (2) by N-methylation of nortropeines via attenuating the low affinity. High-affinity (nanomolar) tropeine binding potentiated by glycine can serve as a lead to develop GlyR subunit-selective, positive allosteric modulators. It is promising that (i) binding heterogeneities up to 615 were observed depending on heteroaromatic (indole, quinoline, pyridine, pyrrole, furane, and thiophene) rings and *N*-methyl substitution; and (ii) structure–activity relationships of (nor)tropeines were different for GlyRs versus 5-HT_3 receptors. Consequently, **2n** displayed nanomolar affinity and preference for GlyRs versus 5-HT_3 receptors.

4. Experimental

4.1. Chemistry

The structures of the final products as free bases were confirmed by ^1H and ^{13}C NMR. Spectra were recorded on a Varian Unity 300 (300 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. Hydrochlorides or fumarates were prepared for receptor binding studies.

4.1.1. General procedure for the preparation of tropine esters

Method A: The ethyl ester of the carboxylic acid (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 over Na_2SO_4 and evaporated to give a thick, oily substance which was purified by column chromatography on silica gel using diethyl ether–isopropyl amine as eluent.

Method B: The potassium salt of the carboxylic acid (4.74 mmol) was suspended in CH_2Cl_2 (5 mL) and oxalyl chloride (0.62 mL, 7.1 mmol) was added dropwise to the mixture in an ice bath. After 30 min the reaction was refluxed for 3 h. Excess oxalyl chloride was removed under reduced pressure and the crude acid chloride was added to a solution of tropine (0.73 g, 5.21 mmol) and K_2CO_3 (1.31 g, 9.48 mmol) in acetonitrile (15 mL) at 0 °C. The mixture was stirred for 12 h at 0 °C. The solvent was removed in vacuo, the residue was quenched with water (10 mL) and then extracted with diethyl ether (2 × 8 mL). The organic layer was washed with saturated NaCl solution and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a yellow oil, which was purified by column chromatography on silica gel using diethyl ether–isopropyl amine as eluent.

4.1.1.1. 3α(2′-Indolecarbonyloxy)-tropane (1). *Method A:* White crystalline substance (1.28 g, 45%) mp >250 °C (HCl salt). ^1H NMR ($CDCl_3$) δ (ppm): 9.35 (br s, 1H, N–H), 7.71 (d, J = 8.1 Hz, 1H, H-7′), 7.44 (d, J = 8.3 Hz, 1H, H-4′), 7.33 (dd, J = 8.1 Hz, 7.1 Hz, 1H, H-6′), 7.19 (dd, J = 8.3 Hz, 7.1 Hz, 1H, H-5′), 7.05 (s, 1H, H-3′), 5.30 (t, J = 5.0 Hz, 1H, H-3), 3.22 (br s, 2H, H-1 and H-5), 2.34 (s, 3H, N–CH_3), 2.23–1.78 (m, 8H); ^{13}C NMR δ (ppm): 161.95 (C═O), 137.40 (C-8′), 128.30 (C-9′), 127.85 (C-2′), 125.75, (C-6′), 122.91 (C-4′), 121.24 (C-5′), 112.35 (C-7′), 108.57 (C-3′), 68.65 (C-3), 60.33 (C-1 and C-5), 40.79 (N–CH_3), 36.99 (C-2 and C-4), 26.16 (C-6 and C-7). Anal. Calcd for $C_{17}H_{20}N_2O_2$: C, 71.81; H, 7.09; N, 9.85. Found: C, 71.69; H, 6.95; N, 9.82.

4.1.1.2. 3α-(1′-Methyl-2′-indolecarbonyloxy)-tropane (2).

Method A: White crystalline substance (1.22 g, 41%) mp >250 °C (HCl salt). ^1H NMR ($CDCl_3$) δ (ppm): 7.70 (d, J = 8.0 Hz, 1H, H-7′), 7.41–7.33 (m, 2H, H-4′and H-6′), 7.25 (s, 1H, H-3′), 7.16 (dd, J = 8.2 Hz, 6.5 Hz, 1H, H-5′), 5.28 (t, J = = 5.1 Hz, 1H, H-3), 4.09 (s, 3H, N–CH_3-1′), 3.21 (br s, 2H, H-1 and H-5), 2.35 (s, 3H, N–CH_3), 2.23–1.84 (m, 8H); ^{13}C NMR δ (ppm): 161.91 (C═O), 140.09 (C-8′), 128.60 (C-9′), 126.21 (C-2′), 125.37, (C-6′), 122.88 (C-4′), 121.01 (C-5′), 110.64 (C-7′), 110.03 (C-3′), 67.81 (C-3), 60.33 (C-1 and C-5), 40.79 (N–CH_3), 36.99 (C-2 and C-4), 31.96 (N–CH_3-1′), 26.17 (C-6 and C-7). Anal. Calcd for $C_{18}H_{22}N_2O_2$: C, 72.46; H, 7.43; N, 9.39. Found: C, 72.55; H, 7.39; N, 9.08.

4.1.1.3. 3α-(3′-Quinolinecarbonyloxy)-tropane (3). *Method A:* White crystalline substance (1.51 g, 51%) mp >250 °C (HCl salt). ^1H NMR ($CDCl_3$) δ (ppm): 9.46 (d, J = 1.7 Hz, 1H, H-2′), 8.84 (d, J = 1.7 Hz, 1H, H-4′), 8.18 (d, J = 8.5 Hz, 1H, H-8′), 7.97 (d, J = 8.2 Hz, 1H, H-5′), 7.86 (dd, J = 8.5 Hz, 6.9 Hz, 1H, H-7′), 7.65 (dd, J = = 8.2 Hz, 6.9 Hz, 1H, H-6′), 5.37 (t, J = 5.2 Hz, 1H, H-3), 3.21 (br s, 2H, H-1 and H-5), 2.33 (s, 3H, N–CH_3), 2.29–1.89 (m, 8H); ^{13}C NMR δ (ppm): 165.05 (C═O), 150.23 (C-2′), 150.15 (C-9′), 139.10 (C-4′), 132.25 (C-7′), 129.82, (C-8′), 129.51 (C-10′), 127.86 (C-6′), 127.27 (C-5′), 124.02 (C-3′), 69.33 (C-3), 60.15 (C-1 and C-5), 40.88 (N–CH_3), 37.16 (C-2 and C-4), 26.24 (C-6 and C-7). Anal. Calcd for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45. Found: C, 73.14; H, 6.70; N, 9.33.

4.1.1.4. 3α-(2′-Pyrrolecarbonyloxy)-tropane (4). *Method B:* White crystalline substance (0.54 g, 49%) mp >250 °C (fumarate salt). ^1H NMR ($CDCl_3$) δ (ppm): 9.56 (br s, 1H, N–H) 6.97 (dd, J = 2.5 Hz, 1.3 Hz, 1H, H-5′), 6.88 (dd, J = 3.6 Hz, 1.3 Hz, 1H, H-3′), 6.26 (dd, J = 3.6 Hz, 2.5 Hz, 1H, H-4′), 5.19 (t, J = 5.0 Hz, 1H, H-3), 3.14 (br s, 2H, H-1 and H-5), 2.30 (s, 3H, N–CH_3), 2.24–1.78 (m, 8H); ^{13}C NMR δ (ppm): 160.91, (C═O), 123.73 (C-5′), 123.13 (C-2′), 114.99 (C-3′), 110.51 (C-4′), 67.65 (C-3), 60.06 (C-1 and C-5), 40.63 (N–CH_3), 36.91 (C-2 and C-4), 25.97 (C-6 and C-7). Anal. Calcd for $C_{13}H_{18}N_2O_2$: C, 66.64; H, 7.74; N, 11.96. Found: C, 66.44; H, 7.72; N, 12.21.

4.1.1.5. 3α-(2′-Thiophenecarbonyloxy)-tropane (5). *Method B:* White crystalline substance (0.65 g, 55%) mp >250 °C (HCl salt). ^1H NMR ($CDCl_3$) δ (ppm): 7.77 (dd, J = 3.7 Hz, 1.2 Hz, 1H, H-3′), 7.53 (dd, J = 5.0 Hz, 1.2 Hz, 1H, H-5′), 7.12–7.07 (m, 1H, H-4′), 5.19 (t, J = 5.2 Hz, 1H, H-3), 3.12 (br s, 2H, H-1 and H-5), 2.28 (s, 3H, N–CH_3), 2.23–1.78 (m, 8H); ^{13}C NMR δ (ppm): 161.60 (C═O), 134.82 (C-2′), 133.28 (C-3′), 132.32 (C-5′), 128.00, (C-4′), 68.64 (C-3), 60.01 (C-1 and C-5), 40.67 (N–CH_3), 36.88 (C-2 and C-4), 25.97 (C-6 and C-7). Anal. Calcd for $C_{13}H_{17}NO_2S$: C, 62.12; H, 6.82; N, 5.57. Found: C, 62.00; H, 6.95; N, 5.60.

4.1.1.6. 3α-(2′-Furanecarbonyloxy)-tropane (6). *Method B:* White crystalline substance (0.58 g, 52%) mp >250 °C (HCl salt). ^1H NMR ($CDCl_3$) δ (ppm): 7.55 (dd, J = 1.7 Hz, 0.8 Hz, 1H, H-5′), 7.09 (dd, J = 3.5 Hz, 0.8 Hz, 1H, H-3′), 6.47 (dd, J = 3.5 Hz, 1.7 Hz, 1H, H-4′), 5.19 (t, J = 5.2 Hz, 1H, H-3), 3.10 (br s, 2H, H-1 and H-5), 2.26 (s, 3H, N–CH_3), 2.22–1.78 (m, 8H); ^{13}C NMR δ (ppm): 158.24, (C═O), 146.49 (C-5′), 145.47 (C-2′), 117.54 (C-3′), 111.92 (C-4′), 68.43 (C-3), 60.36 (C-1 and C-5), 40.64 (N–CH_3), 36.82 (C-2 and C-4), 26.86 (C-6 and C-7). Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.05; H, 7.39; N, 6.08.

4.1.1.7. 3α-(1′-Methyl-2′-pyrrolecarbonyloxy)-tropane (7).

Method B: White crystalline substance (0.49 g, 42%) mp >250 °C (fumarate salt). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.85 (dd, J = 2.7 Hz, 1.4 Hz, 1H, H-5′), 6.73 (dd, J = 3.6 Hz, 1.4 Hz, 1H, H-3′), 6.07 (dd, J = 3.6 Hz, 2.7 Hz, 1H, H-4′), 5.23 (t, J = 4.7 Hz, 1H, H-3), 3.87 (s, 3H, CH_3-1′) 3.20 (br s, 2H, H-1 and H-5), 2.35 (s, 3H, N–CH_3), 2.22–1.80 (m, 8H); ^{13}C NMR δ (ppm): 160.08, (C=O), 130.26 (C-5′), 122.30 (C-2′), 117.53 (C-3′), 108.19 (C-4′), 64.41 (C-3), 60.94 (C-1 and C-5), 40.79 (N–CH_3), 39.96 (N–CH_3-1′), 36.93 (C-2 and C-4), 25.10 (C-6 and C-7). Anal. Calcd for $C_{14}H_{20}N_2O_2$: C, 67.71; H, 8.12; N, 11.28. Found: C, 67.52; H, 8.02; N, 11.31.

4.1.2. General procedure for the demethylation of tropeines

To a stirred mixture of the tropine ester (2 mmol) and $NaHCO_3$ (2.70 g, 32 mmol) in anhydrous $CHCl_3$ (25 mL), α-chloroethyl chloroformate (1.14 g, 0.86 mL, 8 mmol) was slowly added in nitrogen atmosphere at 40 °C. The temperature was then increased to 60 °C and the mixture stirred for 3 h. After cooling down the solvent was evaporated under reduced pressure. Methanol (5 mL) was added to the residue and refluxed for 2 h. The solution was then evaporated, saturated K_2CO_3 solution (10 mL) was added, and it was extracted with CH_2Cl_2 (3 × 5 mL). The solution was dried over Na_2SO_4 and evaporated to give a yellow oil, which was purified by column chromatography on silica gel using diethyl ether–isopropyl amine as eluent.

4.1.2.1. 3α-(2′-Indolecarbonyloxy)-nortropane (1n). White crystalline substance (0.39 g, 72%) mp >250 °C (HCl salt). ^{1}H NMR ($CDCl_3$) δ (ppm): 9.39 (br s, 1H, H-1′), 7.63 (d, J = 8.0 Hz, 1H, H-7′), 7.36 (d, J = 8.3 Hz, 1H, H-4′), 7.25 (dd, J = 8.0 Hz, 7.0 Hz, 1H, H-6′), 7.11 (s, 1H, H-3′), 7.06 (dd, J = 8.3 Hz, 7.0 Hz, 1H, H-5′), 5.28 (t, J = = 4.7 Hz, 1H, H-3), 3.53 (br s, 2H, H-1 and H-5), 2.26–1.81 (m, 8H); ^{13}C NMR δ (ppm): 162.00 (C=O), 137.40 (C-8′), 128.33 (C-9′), 127.84 (C-2′), 125.70, (C-6′), 122.90 (C-4′), 121.20 (C-5′), 112.32 (C-7′), 108.58 (C-3′), 69.46 (C-3), 53.73 (C-1 and C-5), 37.90 (C-2 and C-4), 29.89 (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, 70.87; H, 6.56; N, 10.02.

4.1.2.2. 3α-(1′-Methyl-2′-indolecarbonyloxy)-nortropane (2n). White crystalline substance (0.36 g, 63%) mp >250 °C (HCl salt). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.70 (d, J = 8.0 Hz, 1H, H-7′), 7.41–7.32 (m, 2H, H-4′and H-6′), 7.26 (s, 1H, H-3′), 7.16 (dd, J = 8.3 Hz, 6.5 Hz, 1H, H-5′), 5.31 (t, J = 5.1 Hz, 1H, H-3), 4.09 (s, 3H, N–CH_3-1′), 3.58 (br s, 2H, H-1 and H-5), 2.27–1.88 (m, 8H); ^{13}C NMR δ (ppm): 161.94 (C=O), 140.06 (C-8′), 128.59 (C-9′), 126.21 (C-2′), 125.35, (C-6′), 122.87 (C-4′), 121.00 (C-5′), 110.61 (C-7′), 110.05 (C-3′), 67.61 (C-3), 53.74 (C-1 and C-5), 37.90 (C-2 and C-4), 31.93 (N–CH_3-1′), 29.83 (C-6 and C-7). Anal. Calcd for $C_{17}H_{20}N_2O_2$: C, 71.81; H, 7.09; N, 9.85. Found: C, 72.00; H, 6.89; N, 9.88.

4.1.2.3. 3α-(3′-Quinolinecarbonyloxy)-nortropane (3n). White crystalline substance (0.42 g, 75%) mp >250 °C (HCl salt). ^{1}H NMR ($CDCl_3$) δ (ppm): 9.47 (d, J = 1.7 Hz, 1H, H-2′), 8.84 (d, J = 1.7 Hz, 1H, H-4′), 8.18 (d, J = 8.5 Hz, 1H, H-8′), 7.97 (d, J = 8.1 Hz, 1H, H-5′), 7.86 (dd, J = 8.5 Hz, 6.9 Hz, 1H, H-7′), 7.66 (dd, J = 8.1 Hz, 6.9 Hz, 1H, H-6′), 5.43 (t, J = 4.7 Hz, 1H, H-3), 3.61 (br s, 2H, H-1 and H-5), 2.25–1.90 (m, 8H); ^{13}C NMR δ (ppm): 165.05 (C=O), 150.23 (C-2′), 150.20 (C-9′), 139.10 (C-4′), 132.27 (C-7′), 129.86, (C-8′), 129.53 (C-10′), 127.89 (C-6′), 127.28 (C-5′), 124.01 (C-3′), 70.04 (C-3), 53.73 (C-1 and C-5), 37.97 (C-2 and C-4), 29.96 (C-6 and C-7). Anal. Calcd for $C_{17}H_{18}N_2O_2$: C, 72.32; H, 6.43; N, 9.92. Found: C, 72.08; H, 6.55; N, 9.98.

4.1.2.4. 3α-(2′-Pyrrolecarbonyloxy)-nortropane (4n). White crystalline substance (0.37 g, 85%) mp >250 °C (fumarate salt). ^{1}H NMR ($CDCl_3$) δ (ppm): 9.54 (br s, 1H, H-1′) 6.95 (dd, J = 2.4 Hz, 1.3 Hz, 1H, H-5′), 6.89 (dd, J = 3.5 Hz, 1.3 Hz, 1H, H-3′), 6.22 (dd, J = 3.5 Hz, 2.4 Hz, 1H, H-4′), 5.25 (t, J = 5.1 Hz, 1H, H-3), 3.56 (br s, 2H, H-1 and H-5), 2.20–1.83 (m, 8H); ^{13}C NMR δ (ppm): 160.96, (C=O), 123.65 (C-5′), 123.24 (C-2′), 115.05 (C-3′), 110.54 (C-4′), 68.29 (C-3), 53.59 (C-1 and C-5), 37.67 (C-2 and C-4), 25.53 (C-6 and C-7). Anal. Calcd for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72. Found: C, 65.21; H, 7.08; N, 12.99.

4.1.2.5. 3α-(2′-Thiophenecarbonyloxy)-nortropane (5n). White crystalline substance (0.37 g, 79%) mp >250 °C (HCl salt). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.79 (dd, J = 3.8 Hz, 1.2 Hz, 1H, H-3′), 7.55 (dd, J = 4.9 Hz, 1.2 Hz, 1H, H-5′), 7.14–7.07 (m, 1H, H-4′), 5.27 (t, J = 4.9 Hz, 1H, H-3), 3.54 (br s, 2H, H-1 and H-5), 2.23–1.70 (m, 8H). ^{13}C NMR δ (ppm): 159.99 (C=O), 133.12 (C-2′), 131.66 (C-3′), 130.69 (C-5′), 126.35 (C-4′), 67.69 (C-3), 51.90 (C-1 and C-5), 36.12 (C-2 and C-4), 28.01 (C-6 and C-7). Anal. Calcd for $C_{12}H_{15}NO_2S$: C, 60.73; H, 6.37; N, 5.90. Found: C, 60.64; H, 6.06; N, 5.99.

4.1.2.6. 3α-(2′-Furanecarbonyloxy)-nortropane (6n). White crystalline substance (0.36 g, 81%) mp >250 °C (HCl salt). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.59 (dd, J= 1.6 Hz, J = 0.7, 1H, H-5′), 7.13 (dd, J = 3.5, J = 0.7 Hz, 1H, H-3′), 6.51 (dd, J = 3.5 Hz, 1.6 Hz, 1H, H-4′), 5.29 (t, J = 4.7 Hz, 1H, H-3), 3.55 (br s, 2H, H-1 and H-5), 2.26–1.85 (m, 8H). ^{13}C NMR δ (ppm):158.28 (C=O), 146.51 (C-5′), 145.50 (C-2′), 117.57 (C-3′), 111.93 (C-4′), 69.14 (C-3), 53.61 (C-1 and C-5), 37.70 (C-2 and C-4), 29.56 (C-6 and C-7). Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 64.92; H, 6.89; N, 6.02.

Bemesetron (MDL-72,222) (**10**) was purchased from RBI (Natick, MA).

4.2. Biology: membrane preparation and binding studies

4.2.1. GlyRs

Crude synaptic membranes were prepared from spinal cords of male Wistar rats as described.[20] The binding assay was performed in one ml membrane suspensions in 50 mM Tris citrate (pH 7.4) plus 200 mM KSCN with 2 nM [^{3}H]strychnine (10 μCi/mmol, Dupont-NEN). Samples were incubated at 0 °C for 40 min. Duplicate aliquots were filtered on Whatman GF/B filters under vacuum and washed by 3 × 3-mL ice cold buffer. Radioactivity of the filters was measured by scintillation spectrometry. Nonspecific binding was determined in the presence of 2 mM glycine. Nonlinear regression (GraphPad Prism Version 2.0, San Diego, CA) and sigmoidal fitting were used to determine the (nor)tropeine concentration to elicit 50% displacement (IC_{50}) of specific [^{3}H]strychnine binding and the Hill slopes of displacement (n_H) according to Eq. 1:

$$B/B_o = 1/(1 + 10\exp[(X - \lg\ IC_{50})n_H]) \quad (1)$$

where X is the logarithm of (nor)tropeine concentration, while B and B_o are specific binding of [^{3}H]strychnine in the presence and absence of displacers, respectively. Fitting to two phases of displacement was also performed to determine $IC_{50,1}$ and $IC_{50,2}$ values according to Eq. 2:

$$B/B_o = \text{fraction}_1/(1 + 10\exp(X - \lg\ IC_{50,1})) + (1 - \text{fraction}_1)/(1 + 10\exp(X - \lg IC_{50,2})) \quad (2)$$

where fraction_1 is the high-affinity phase. Another constant fraction represented displacement by 10 μM glycine in Eq. 2. Fitting to Eq. 2 was disregarded when it was not significantly better than sigmoidal fitting via Eq. 1 according to the F test.

4.2.2. 5-HT$_3$ type serotonin receptors

Crude membranes were prepared from forebrains (cerebral cortex + hippocampus) of male Wistar rats as described.[21] Briefly,

forebrains were homogenized in 10 mM HEPES containing 140 mM NaCl (pH 7.5), centrifuged at 30,000*g* for 20 min, washed by homogenization and centrifugation and frozen. Before the binding assay, the suspensions were thawed and centrifuged at 10,000*g* for 10 min. For 5-HT_3 receptor binding, membrane suspensions in 10 mM HEPES buffer containing 140 mM NaCl (pH 7.5) were incubated with 0.2 nM [^{3}H]granisetron (82 Ci/mmol, Du Pont-NEN) at 0 °C for 2.5 h. Nonspecific binding was determined in the presence of 10 μM granisetron. Duplicate aliquots were filtered on Whatman GF/B filters pre-soaked in 0.25% polyethyleneimine under vacuum. Fitting to Eq. 1 where $n_H = 1$ was used to determine the IC_{50} values of tropeines.

Acknowledgments

This work was supported by a Grant K 62203 of the Hungarian Science Research Fund OTKA. Technical assistance is acknowledged to Mrs. Ilona Kawka and Mrs. Valéria Székely-Marjay.

References and notes

1. Zeilhofer, H. U.; Zeilhofer, U. B. *Neurosci. Lett.* **2008**, *437*, 170.
2. Laube, B.; Maksay, G.; Schemm, R.; Betz, H. *Trends Pharmacol. Sci.* **2002**, *23*, 519.
3. Yang, Z.; Aubrey, K. R.; Alroy, I.; Harvey, R. J.; Vandenberg, R. J.; Lynch, J. W. *Biochem. Pharmacol.* **2008**, *76*, 1014.
4. Chesnoy-Marchais, D. *Br. J. Pharmacol.* **1996**, *118*, 2115.
5. Supplisson, S.; Chesnoy-Marchais, D. *Mol. Pharmacol.* **2000**, *58*, 763.
6. Maksay, G. *Neuropharmacology* **1998**, *37*, 1633.
7. Maksay, G.; Nemes, P.; Bíró, T. *J. Med. Chem.* **2004**, *47*, 6384.
8. Maksay, G.; Bíró, T.; Laube, B.; Nemes, P. *Neurochem. Int.* **2008**, *52*, 235.
9. Yang, Z.; Ney, A.; Cromer, B. A.; Ng, H. L.; Parker, M. W.; Lynch, J. W. *J. Neurochem.* **2007**, *100*, 758.
10. Langlois, M.; Soulier, J. L.; Yang, D.; Bremont, B.; Florac, C.; Rampillon, V.; Giudice, A. *Eur. J. Med. Chem.* **1993**, *28*, 869.
11. Wallace, J. L.; Kidd, M. R.; Cauthen, S. E.; Woodyard, J. D. *J. Heterocycl. Chem.* **1978**, *15*, 315.
12. Gyermek, L.; Lee, C.; Cho, Y.-M. U.S. Patent 6,376,510; *Chem. Abstr.* **2002**, *136,* 340861.
13. Zeile, K.; Adickes, F.; Wick, H. U.S. Patent 2,824,106; *Chem. Abstr.* **1958**, *52,* 7365b.
14. Donatsch, P.; Engel, G.; Hugi, B.; Richardson, B. P.; Stadler, H. R.; Stadler, B. M.; Stadler, S. A.; Breuleux, G. U.S. Patent 4,789,673, *Chem. Abstr.* **1988**, *114,* 81598.
15. Kraiss, G.; Nádor, K. *Tetrahedron Lett.* **1971**, *1*, 57.
16. Wallace, J. L.; Kidd, M. R.; Cauthen, S. E.; Woodyard, J. D. *J. Pharm. Sci.* **1980**, *69*, 1357.
17. Olofson, F. A.; Martz, J. T.; Senet, J.-P.; Piteau, M.; Malfroot, T. *J. Org. Chem.* **1984**, *49*, 2081.
18. Maksay, G.; Nemes, P.; Vincze, Z.; Bíró, T. *Bioorg. Med. Chem.* **2008**, *16*, 2086.
19. Maksay, G.; Laube, B.; Schemm, R.; Grudzinska, J.; Drwal, M.; Betz, H. *J. Neurochem.* **2009**, *109*, 1725.
20. Maksay, G.; Bíró, T. *Neuropharmacology* **2002**, *43*, 1087.
21. Maksay, G.; Bíró, T.; Bugovics, Gy. *Eur. J. Pharmacol.* **2005**, *514*, 17.