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Aza-THIP and Related Analogues of THIP as $GABA_C$ Antagonists

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Abstract—The potency of a series of eight compounds structurally related with 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP), a potent $GABA_A$ partial agonist exhibiting $GABA_C$ ρ_1 antagonist effect ($K_i = 25$ μM), was determined electrophysiologically using homomeric human $GABA_C$ ρ_1 receptors expressed in *Xenopus* oocytes. Protolytic properties (pK_a values for the acidic bioisosteric groups) and the presence of steric bulk in the molecules appear to be structural parameters of importance for blockade of the $GABA_C$ ρ_1 receptor. Within this series of moderately potent $GABA_C$ antagonists, only 4,5,6,7-tetrahydropyrazolo[5,4-*c*]pyridin-3-ol (Aza-THIP) does not interact detectably with $GABA_A$ receptors, and Aza-THIP has the potential of being a useful tool for molecular and behavioural pharmacological studies.

Introduction

4-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system (CNS), inhibits neuronal activity through two classes of GABA receptors, ionotropic and metabotropic GABA receptors. Based on molecular biology and pharmacology, the ionotropic GABA receptors can be devided into two groups, $GABA_A$ and $GABA_C$ receptors.[1,2] The ionotropic GABA receptors are ligand gated ion channels conducting chloride. $GABA_A$ receptors are hetero-oligomeric receptors being assembled of different subunits and are believed to have a pentameric structure made up of five subunits.[3] $GABA_C$ receptors, however, form homooligomeric receptors from the ρ subunits, although some evidence for heterooligomeric $GABA_C$ receptors exists.[1,2] To date, several subunits have been identified that make up the $GABA_A$ receptors including α_{1-6}, β_{1-4}, γ_{1-4}, δ, ε, π, while the $GABA_C$ receptors are built only by ρ_{1-3}.[1,2]

The GABA system is believed to be involved in several neurological and psychiatric disorders, and in particular the $GABA_C$ receptors appear to be involved in a number of inherited diseases of the eye.[2,4,5] These aspects have focused interest on the $GABA_C$ receptor as a novel therapeutic target making the search for ligands as experimental tools and potential drugs important. In addition to the reference antagonist ligand for the $GABA_C$ receptors, (1,2,3,6-tetrahydropyridine-4-yl) methylphosphinic acid (TPMPA),[6] the $GABA_A$ partial agonist 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP)[7,8] (Fig. 1) has been shown to be a moderately potent competitive $GABA_C$ antagonist.[9]

In this paper we describe the pharmacology of eight compounds structurally related to THIP (Fig. 1) at functional $GABA_C$ ρ_1 receptors expressed in *Xenopus* oocytes to elucidate the structural determinants for blockade of the $GABA_C$ receptors. A new and improved synthetic route was developed for the synthesis of 4,5,6,7-tetrahydroisothiazolo[5,4-*c*]pyridin-3-ol (Thio-THIP) (Scheme 1) making it possible to extend the pharmacological characterization of the series of THIP analogues.

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Results

Briefly, isonicotinamide was alkylated with benzylbromide giving a pyridinium salt (**1**) followed by reduction with sodium borohydride to provide **2** in good yield (Scheme 1). After reduction the benzyl group was replaced by the methoxycarbonyl protecting group (**3**). Sulphur was introduced by a Michael addition of thioacetic acid giving thioester **4** in good yield. Compound **4** was hydrolized to the corresponding thiol, oxidized to the disulphide which was then cyclized using sulphuryl chloride, resulting in protected Thio-THIP (**5**). Compound **5** was deprotected with 33% hydrogen bromide in glacial acetic acid giving Thio-THIP in 85% yield.

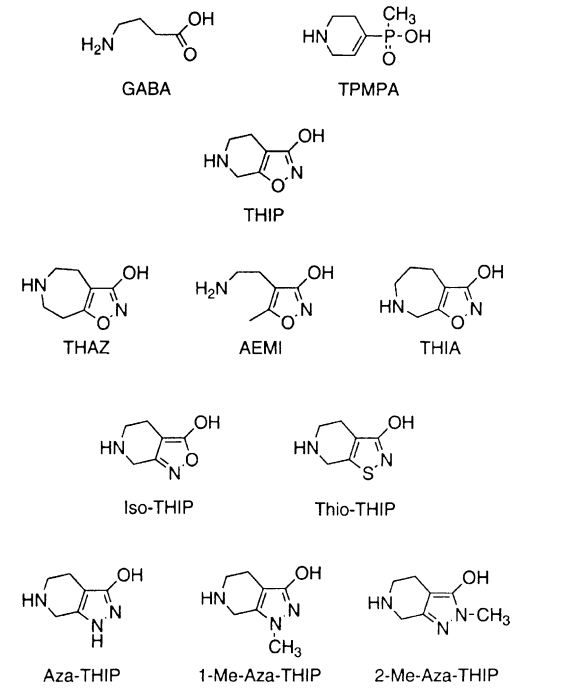
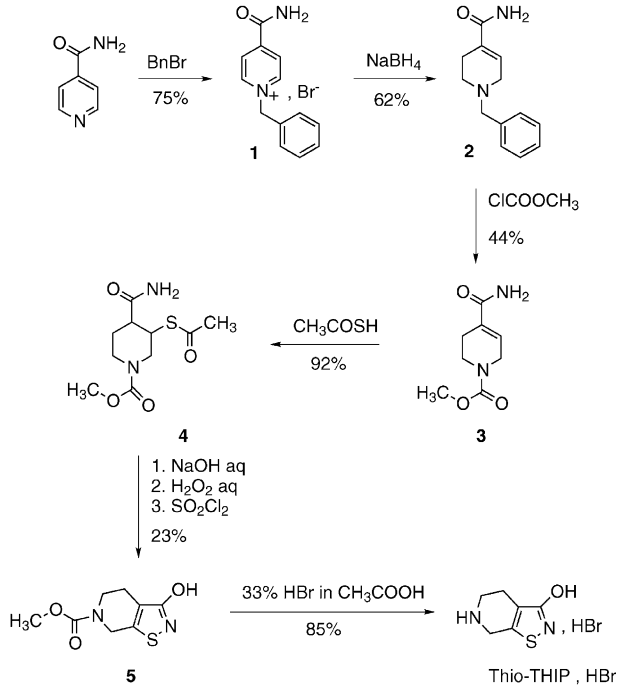


Figure 1. Structures of GABA and a number of heterocyclic analogues of GABA characterized as $GABA_C$ antagonists in the present study.



Scheme 1.

The overall yield based on commercially available isonicotinamide was markedly improved and involved fewer steps as compared with the previously described synthesis of Thio-THIP.[10]

The activities of the analogues of THIP, including the synthesized Thio-THIP, on functional $GABA_C$ ρ_1 receptors expressed in *Xenopus* oocytes and their affinities for $GABA_A$ receptors in rat brain membrane preparations using either [^{3}H]muscimol or [^{3}H]GABA as the radioligand, are summarized in Table 1. The compounds under study were shown to be either inactive or competitive antagonists at functional ρ_1 receptors expressed in *Xenopus* oocytes.

In this study the reference compound TPMPA was shown to be a potent ρ_1 receptor antagonist with a K_i value of 3.2 μM, whereas THIP was markedly weaker as an antagonist at ρ_1 receptors ($K_i = 25$ μM). Thio-THIP, Aza-THIP and Iso-THIP showed activity at $GABA_C$ receptors ranging from weak to potent antagonism. Iso-THIP was a potent antagonist with a K_i value of 7.9 μM only some three times weaker than TPMPA, whereas Thio-THIP was a weak antagonist ($K_i = 91$ μM). Aza-THIP was approximately equipotent with THIP having a K_i of 31 μM. The shift of the GABA concentration–response curve by Aza-THIP is shown in Figure 2. Both Iso-THIP and Thio-THIP show affinity for the $GABA_A$ receptors, whereas Aza-THIP shows no detectable $GABA_A$ receptor affinity (Table 1), hence Aza-THIP was shown to be a selective $GABA_C$ antagonist. The inhibition of a 3 μM GABA response by 300 μM Aza-THIP is shown in Figure 3. The two methylated Aza-THIP analogues showed no activity at $GABA_C$ ρ_1 receptors and no affinity for $GABA_A$ receptors (Table 1).

THAZ, a very weak glycine receptor antagonist,[11] was a moderately potent antagonist ($K_i = 23$ μM) at ρ_1 receptors, whereas the monocyclic analogue, AEMI, of THAZ was shown to be a weak antagonist ($K_i = 142$ μM). In contrary to AEMI, THAZ showed some affinity for $GABA_A$ receptors. THIA, an isomer of THAZ and also a glycine receptor antagonist,[11] shows no affinity for the $GABA_A$ receptors and showed no activity at $GABA_C$ ρ_1 receptors.

Discussion

THIP is a conformationally restricted analogue of GABA, which adopts a well-defined conformation. Of the eight compounds tested in this study, Iso-THIP, Aza-THIP and Thio-THIP are structurally and conformationally closely related to THIP, adopting essentially rigid conformations. All of these compounds are

Table 1. Effects of TPMPA, THIP and a number of THIP analogues at $GABA_C$ ρ_1 receptors and on $GABA_A$ receptor binding[a]

Compd	PK_a values	Human ρ_1 receptors K_i (μM) (pK_i±SEM)	$GABA_A$ receptor binding K_i (μM) (pK_i±SEM)
TPMPA	—	3.2 (5.49±0.11)	39 (4.40±0.01)
THIP	4.4; 8.5[b]	25 (4.60±0.06)	0.16 (6.79±0.03)
THAZ	4.8; 9.2[c]	23 (4.63±0.04)	16 (4.78±0.04)
AEMI	5.1; 10.4[d]	142 (3.87±0.07)	>100
THIA	4.6; 8.6[b]	NA[e]	>100
Iso-THIP	3.0, 9.1[f]	7.9 (5.10±0.06)	90 (4.05±0.03)
Thio-THIP	6.1; 8.5[c]	91 (4.04±0.03)	52 (4.29±0.04)
Aza-THIP	6.3; 9.9[f]	31 (4.51±0.02)	>100
1-Me-Aza-THIP	7.1; 9.2[f]	NA[e]	>100
2-Me-Aza-THIP	5.8; 9.8[f]	NA[e]	>100

K_i values are calculated from pK_i values found in brackets. The pK_i values represent mean ±SEM of at least three experiments.
[a]Standard [^{3}H]muscimol binding on rat brain synaptic membranes determined using a method described previously.[18]
[b]Data from Krogsgaard-Larsen (1977).[17]
[c]Data from Krogsgaard-Larsen et al. (1983).[10]
[d]Data from Hjeds and Krogsgaard-Larsen (1976).[16]
[e]NA for no activity at 300 μM with or without 1 μM of GABA.
[f]Data from Krogsgaard-Larsen and Roldskov-Christiansen (1979).[14]

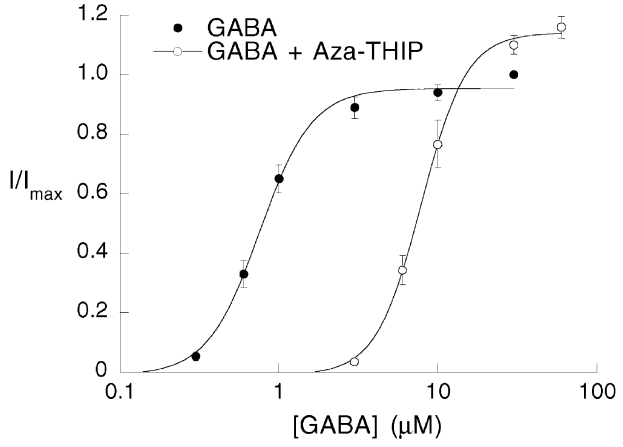


Figure 2. Concentration–response curves for GABA and GABA in the presence of 300 μM Aza-THIP in *Xenopus* oocytes injected with ρ_1. Data points are mean values±standard errors from three individual oocytes. Curves were fitted using the equation described in ref 16.

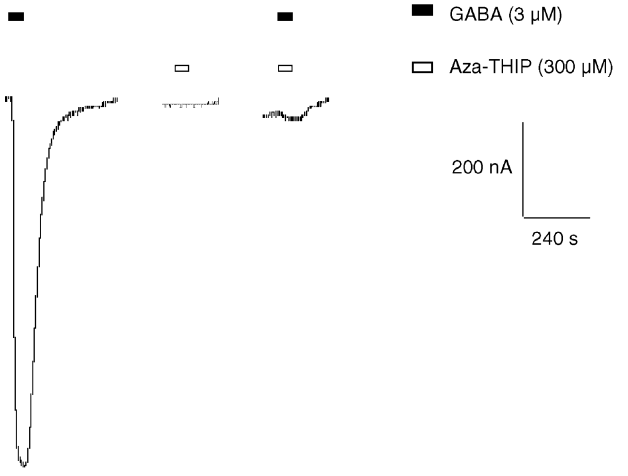


Figure 3. At homooligomeric ρ_1 receptors expressed in *Xenopus* oocytes, GABA (3 μM) (duration indicated by filled bar) activates the receptor while Aza-THIP (300 μM) does not activate the receptor (duration indicated by unfilled bar). However, when Aza-THIP (300 μM) is co-applied with GABA (3 μM), the GABA response is reduced.

antagonists at the $GABA_C$ ρ_1 receptor and most likely bind to the receptor in a manner similar to that of THIP.

Within this series of $GABA_C$ antagonists, Iso-THIP is the most potent being four times more potent than THIP. Aza-THIP was approximately equipotent with THIP at the $GABA_C$ ρ_1 receptor and shows no affinity for the $GABA_A$ receptor (K_i>100 μM). This indicates that Aza-THIP is selective for $GABA_C$ ρ_1 receptors. Thio - THIP was less potent at $GABA_C$ ρ_1 receptors, being three times weaker than THIP. The lower activity of Thio-THIP may be due to the introduction of the sulphur atom in the 3-isothiazolol ring representing additional steric bulk as compared with oxygen in the 3-isoxazolol ring of THIP, as elucidated crystallographically,[12] and to the lower acidic character of the 3-isothiazolol ring (pK_a 6.1) as compared to the 3-isoxazolol ring of THIP (pK_a 4.4) (Table 1). This proposed effect of steric bulk is in agreement with the pharmacophore model proposed by Chebib et al.,[13] where the binding site at $GABA_C$ receptors is envisaged to be narrow. This implies that larger substituents may interact unfavorably with the binding site. Furthermore, the different pK_a values of THIP and Thio-THIP suggest, that a larger fraction of the molecules of the latter compound at physiological pH contains a nonionized 3-isothiazolol group, assumed to be pharmacologically inactive.

Neither of the two methylated analogues of Aza-THIP show activity at $GABA_C$ ρ_1 receptors, and the lack of activity of 1-Me-Aza-THIP and 2-Me-Aza-THIP may be explained by steric interaction and by low acidic character of the 3-pyrazolol nuclei of these two compounds (pK_a 7.1 and 5.8, respectively) (Table 1). In these two compounds, the methyl group may protrude into an unfavorable area in the receptor binding pocket

in agreement with the receptor model proposed by Chebib et al.[13]

The homologue of THIP, THAZ, contains a larger seven membered ring and is therefore a more flexible molecule. THAZ is a moderately potent antagonist ($K_i = 23$ μM) at $GABA_C$ ρ_1 receptors. Being equipotent with THIP at this receptor, THAZ may be able to attain a conformation similar to that of THIP in order to fit into the $GABA_C$ binding pocket. In contrast, AEMI, which is an open ring analogue of THAZ, is markedly more flexible, and a conformation of AEMI comparable with those of THIP and THAZ is unlikely to be energetically favourable. Additionally, the methyl group in the 5-position of the 3-isoxazolol ring of AEMI may protrude into an area of steric hindrance causing AEMI to be some seven times weaker than THAZ (Table 1).

THIA, an isomer of THAZ (Fig. 1) shows no activity at $GABA_C$ ρ_1 receptors and no affinity for the $GABA_A$ receptors. THIA may not be able to adopt a conformation where the amino group is in the right position to interact with the receptor.

Conclusion

TPMPA has so far been the most useful antagonist for studying $GABA_C$ receptors. However, Aza-THIP, which has now been shown to be a specific competitive $GABA_C$ antagonist, may be a supplement to TPMPA as a reference $GABA_C$ compound. TPMPA shows strong antagonist activity at $GABA_C$ receptors and, in contrast to Aza-THIP, weak activity at both $GABA_A$ (Table 1) and metabotropic $GABA_B$ receptors.[6]

Using a series of compounds related to THIP, we have shown that only minor structural changes affect their potency as $GABA_C$ antagonists. The acidic character of the 3-isoxazolol ring of Iso-THIP (pK_a 3.0) is more pronounced than that of the 3-isoxazolol ring of THIP (pK_a 4.4) (Table 1), suggesting that this difference in potency may be explained by a stronger electrostatic interaction of Iso-THIP than of THIP with the receptor. The structural and protolytic similarity between THIP and Aza-THIP suggests that the latter compound, like THIP, is capable of penetrating the blood-brain-barrier. Thus, although Aza-THIP is only moderately potent as a $GABA_C$ antagonist, it may be a useful compound for behavioural pharmacological studies.

Experimental

Chemistry

The following compounds were synthesized by published procedures: 4,5,6,7-tetrahydropyrazolo[5,4-*c*]pyridin-3-ol (Aza-THIP),[14] 1-methyl-4,5,6,7-tetrahydropyrazolo[5,4-*c*]pyridin-3-ol (1-Me-Aza-THIP),[14] 2-methyl-4,5,6,7-tetrahydropyrazolo[5,4-*c*]pyridin-3-ol (2-Me-Aza-THIP),[14] 4,5,6,7 - tetrahydroisoxazolo[3,4 - *c*]pyridin - 3 - ol (Iso-THIP),[14] 5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*d*]azepin-3-ol (THAZ),[15] 4-(2-aminoethyl)-5-methylisoxazol-3-ol (AEMI),[16] THIP[17] and 5,6,7,8 - tetrahydro - 4*H* - isoxazolo[5,4-*c*]azepin-3-ol (THIA).[17]

Melting points were determined in open capillary tubes and are corrected. Elemental analyses were performed at Analytical Research Department, H. Lundbeck A/S, Denmark, or by Mr. J. Theiner, Department of Physical Chemistry, University of Vienna, Austria, and are within ±0.4% of the calculated values unless otherwise stated. Nuclear Magnetic Resonance Spectra (NMR) were recorded on a 300 MHz Varian Gemini spectrometer.

4-Aminocarbonyl-1-benzylpyridiniumbromide (1). To a solution of isonicotinamide (10.0 g, 81.9 mmoles) in ethanol (250 mL) was added benzyl bromide (29.2 mL, 245.6 mmoles) and the reaction mixture was refluxed (90° C) for 4 h. After cooling, diethylether (250 mL) was added, and the reaction mixture was then placed at 4° C overnight for crystallization to provide crude **1** as white crystals (19.3 g, 80%). An analytical sample was recrystallized (methanol): mp 243.5–246.0° C (decomposed); ^{1}H NMR (D_2O) δ 8.93 (2H, d, $J = 6.6$ Hz), 8.19 (2H, d, $J = 6.6$ Hz), 7.35 (5H, s), 5.73 (2H, s), 4.65 (3H, s); ^{13}C NMR (D_2O) δ 166.62, 148.65, 145.47, 132.15, 130.11, 129.62, 129.30, 126.45, 66.33. Analysis ($C_{13}H_{13}BrN_2O$) C, H, Br, N.

1-Benzyl-1,2,3,6-tetrahydropyridine-4-carboxylic acid amide (2). A solution of crude **1** (19.3 g, ca. 65.8 mmoles) in methanol (350 mL) was cooled to 0° C. Sodium borohydride (2.62 g, 69.1 mmoles) was added over 3 h and the mixture was then allowed to stir at 0° C for 30 min and at 25° C for 16 h. The mixture was evaporated in vacuo and the residue was extracted with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and evaporated in vacuo to give crude **2** (8.87 g, 62%). An analytical sample was dissolved in 2 M hydrochloric acid, evaporated in vacuo and crystallized (methanol) to provide analytically pure hydrochloride of **2**: mp 236.0–238.0° C (decomposed); ^{1}H NMR (D_2O) δ 7.34 (5H, m), 6.38–6.35 (1H, m), 4.23 (2H, s), 3.74–3.67 (2H, m), 3.50 and 3.07 (1H, two broad signals), 2.50–2.43 (2H, m); ^{13}C NMR (D_2O) δ 170.50, 131.25, 130.25, 130.13, 129.38, 128,75, 126.00, 59.00, 49.38, 48.00, 21.25. Analysis ($C_{13}H_{17}ClN_2O$) C, H, Cl, N.

4-Aminocarbonyl-1,2,3,6-tetrahydropyridine-1-carboxylic acid methyl ester (3). Methyl chloroformate (6.13 mL, 79.7 mmoles) was added through a condenser to crude **2** (8.62 g, ca. 39.9 mmoles). The mixture was stirred for 7 h, evaporated in vacuo and extracted continuously with CH_2Cl_2 for 23 h. The CH_2Cl_2 phase was dried ($MgSO_4$), evaporated in vacuo and the residue recrystallized (ethanol–diethylether) to give **3** (3.22 g, 44%). An analytical sample was recrystallized (ethyl acetate): mp 128.0–130.5° C; ^{1}H NMR ($CDCl_3$) δ 6.60 (1H, s), 6.07 (2H, broad s), 4.19–4.04 (2H, m), 3.73 (3H, s), 3.65–3.49 (2H, m), 2.47–2.31 (2H, m); ^{13}C NMR ($CDCl_3$) δ 169.00, 156.60, 131.50, 130.50, 52.85, 43.90, 40.00, 24.00. Analysis ($C_8H_{12}N_2O_3$) C, H, N.

3-Acetylthio-4-aminocarbonylpiperidine-1-carboxylic acid methyl ester (4). Thioacetic acid (1.11 mL, 15.5 mmoles) was added to crude **3** (2.9 g, ca. 15.5 mmoles). Ethyl acetate (6 mL) was added and the mixture was stirred at 25° C for 20 h. Additional thioacetic acid (0.4 mL, 5.61 mmoles) was added and the mixture was stirred for another 72 h. The reaction mixture was evaporated in vacuo and recrystallized (ethyl acetate) affording **4** (2.92 g, 72%). An analytical sample was further recrystallized (ethyl acetate): mp 130.0–131.0° C; ^{1}H NMR ($CDCl_3$) δ 6.20–5.80 (2H, m), 4.22–3.98 (3H, m), 3.74–3.68 (3H, m), 3.43–3.30 (1H, m), 3.12–3.00 (1H, m), 2.84–2.76 (1H, m), 2.35 (3H, s), 2.00–1.88 (1H, m), 1.80–1.63 (1H, m); ^{13}C NMR ($CDCl_3$) δ 195.5, 174.0, 156.2, 53.0, 48.9, 44.9, 42.0, 31.0, 25.8. Analysis ($C_{10}H_{16}N_2O_4S$) C, H, N, S.

3-Hydroxy-4,5,6,7-tetrahydroisothiazolo[5,4-c]pyridine-6-caboxylic acid methylester (5). A solution of sodium hydroxide (1.08 g, 26.9 mmoles) in water (9 mL) was added to crude **4** (3.5 g, ca. 13.5 mmoles). After 1 h of stirring the reaction mixture was acidified to pH 5 with sulphuric acid (4 M) and extracted with CH_2Cl_2. The combined CH_2Cl_2 phases were dried ($MgSO_4$) and evaporated in vacuo. The residue (2.55 g) was dissolved in water (10 mL), and H_2O_2 (33%) (0.57 mL) was added dropwise at 45° C. After stirring for 4 h at 45° C the reaction mixture was cooled and evaporated in vacuo to give 2.55 g of crude product. Part of this residue (1.89 g) was suspended in $ClCH_2CH_2Cl$ (22 mL), and SO_2Cl_2 (1.06 mL, 13.1 mmoles) was added dropwise during 25 min. After stirring at room temperature for 24 h the reaction mixture was filtered. The crystals were washed with $ClCH_2CH_2Cl$ and recrystallized (ethanol) affording **5** (0.67 g, 23%): mp 179.5–181.5° C; ^{1}H NMR ($CDCl_3$) δ 9.08 (1H, broad s), 4.72–4.65 (2H, m), 3.77 (3H, s), 3.74–3.68 (2H, m), 2.52–2.62 (2H, m); ^{13}C NMR ($CDCl_3$) δ 159.2, 156.0, 153.6, 119.6, 53.2, 42.8, 41.0, 22.6. Analysis ($C_8H_{10}N_2O_3S$) C, H, N, S.

4,5,6,7-Tetrahydroisothiazolo[5,4-c]pyridin-3-ol hydrobromide (Thio-THIP, HBr). To **5** (0.47 g, 2.19 mmoles) was added 33% hydrobromide in acetic acid (47 mL) and the mixture was stirred for 48 h. Evaporation and re-evaporation twice with toluene in vacuo gave crude Thio-THIP, HBr. Heating the residue in methanol (15 mL) afforded the product as white crystals after filtration (0.44 g, 85%): mp 221.0–222.0° C (decomposed); ^{1}H NMR (D_2O) δ 4.46–4.43 (2H, m), 3.52 (2H, t, J 6.3 and 6.0 Hz), 2.72–2.66 (2H, m); ^{13}C NMR (D_2O) δ 169.50, 146.50, 118.20, 41.30, 40.60, 18.95. Analysis ($C_6H_9BrN_2OS$) C, H, N.

Electrophysiological recording

Xenopus laevis was anaesthesized with 0.17% ethyl 3-aminobenzoate and a lobe of the ovaries was carefully removed. The lobe of the ovary was placed in oocyte releasing buffer 2 (OR2) (82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 5 mM HEPES, pH 7.5) with 2 mg/mL Collagenase A (Boehringer Mannheim) for 2 h. Defolliculated oocytes were then rinsed with frog Ringer solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.5) supplemented with 2.5 mM pyruvate, 0.5 mM theophylline and 50 μg/mL gentamycin. Stage V–VI oocytes were collected.

Human ρ_1 cDNA in pcDNA (Invitrogen, San Diego, CA, USA) was provided by Dr. George Uhl (National Institute for Drug Abuse, Baltimore, MD, USA). After linearization of the plasmid containing ρ_1 cDNA with restriction enzyme Xba 1, cRNA was synthesized using the 'Mmessage Mmachine' kit from Ambion (Austin, TX, USA). ρ_1 cRNA (10 ng/50 nL) was injected into defolliculated stage V–VI *Xenopus* oocytes and the oocytes were then incubated at 16° C on an orbital shaker for 3 to 8 days prior to recording.

Receptor activity was measured by two electrode voltage clamp recording using a Geneclamp 500 amplifier (Axon Instruments, Foster City, CA, USA), a MacLab 2e recorder (AD Instruments, Sydney, NSW, Australia) and Chart version 3.5 program. Oocytes were voltage clamped at −60 mV and continuously superfused with frog Ringer solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2 \cdot 2H_2O$, 1.8 mM $CaCl_2 \cdot 2H_2O$, 5 mM HEPES). For receptor activation measurements, the indicated concentrations of drug were added to the buffer solution.

Antagonist activity was measured as a pK_i or an IC_{50} value. The pK_i value was determined on the basis of a GABA concentration-response curve as a control followed by a GABA concentration-response curve in the presence of a fixed antagonist concentration on the same oocyte ($n \geq 3$). The IC_{50} value was determined by measuring the activation of the receptor by GABA in the presence of different antagonist concentrations. The GABA concentration was chosen as the concentration which produces 50% of max response, e.g., EC_{50}, based on a standard GABA concentration–response curve for each oocyte prior to the inhibition curve, thereby using a fixed relative response level of GABA.

Analysis of kinetic data

Current (I) as a function of GABA concentration was fitted by least squares to $I = I_{max}[GABA]^nH/(EC_{50}^nH + [GABA]^nH)$, where I_{max} is the maximum current, EC_{50} is the effective concentration that activates 50% of the maximum current and n_H is the Hill coefficient. The EC_{50} and the n_H were determined by fitting data from individual oocytes using Kaleidagraph 3.0 (1993). The pK_i value was calculated from $pK_i = \log(DR-1) - \log[Antagonist]$, where DR is the dose-ratio ($EC_{50}(GABA + antagonist)/EC_{50}(GABA)$).

The IC_{50} was determined by fitting data from individual oocytes using Kaleidagraph 3.0 (1993). Current as a function of antagonist concentration was fitted by least squares to $I = I_{max} - (I_{max}[Antagonist]^n/(IC_{50}^nH + [Antagonist]^n)$, where IC_{50} is the effective concentration of antagonist that inhibits 50% of the maximum current, I_{max} is maximum response, n is the slope of the inhibition curve and n_H is the Hill coefficient. The pK_i value

can be estimated from the IC_{50} by using $K_i = IC_{50}/(1 + ([GABA]/EC_{50})^n H))$, where [GABA] is the concentration of GABA used to determine the IC_{50}, EC_{50} is the effective concentration that activates 50% of the maximum current and n_H is the Hill coefficient of the GABA standard concentration-response curve. pK_i values are expressed as mean ± SEM ($n \geq 3$).

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