Hydroxy-1,2,5-oxadiazolyl Moiety as Bioisoster of the Carboxy Function. Synthesis, Ionization Constants, and Pharmacological Characterization of γ-Aminobutyric Acid (GABA) Related Compounds

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Received December 28, 2005

Three 4-substituted 1,2,5-oxadiazol-3-ols containing aminoalkyl substituents (analogues and homologues of γ-aminobutyric acid (GABA)) were synthesized to investigate the hydroxy-1,2,5-oxadiazolyl moiety as a bioisoster for a carboxyl group at GABA receptors. The pK_a values of the target compounds were close to those of GABA. At $GABA_A$ receptors of cultured cerebral cortical neurons, weak agonist and partial agonist profiles were identified, demonstrating the 4-hydroxy-1,2,5-oxadiazol-3-yl unit to be a nonclassical carboxyl group bioisoster.

Introduction

Isosteric replacement of functional groups in a lead compound is a widely used approach to study receptor chemistry and to develop new drugs with optimized behavior.[1] When this replacement affords products with broadly similar biological properties, the groups are called bioisosters.[2,3] A number of clear bioisosteric relationships have been established for many functional groups, in particular for the carboxyl group, which successfully has been substituted by heterocycles such as tetrazole, 3-hydroxyisoxazole, 3-hydroxyisothiazole, 3-hydroxy-1,2,5-thiadiazole, and 3-cyclobutene-1,2-dione. These cyclic systems have been used extensively to design amino acid mimetics active at subtypes of central nervous system (CNS) receptors.[4−7] The 1,2,5-oxadiazole (furazan) system and its 2-oxide (furoxan) are heterocyclic rings whose pharmacochemistry some of us have been studying for many years.[8] The former is a classical isoster of the 1,2,5-thiadiazole ring. Similar to the hydroxy-substituted 1,2,5-thiadiazoles, the hydroxy-1,2,5-oxadiazoles are known to display marked acidic properties.[9] Consequently, the 4-hydroxy-1,2,5-oxadiazol-3-yl moiety could reasonably behave as the bioisoster of the carboxy function. In this first paper we report the results of a work devoted to obtain potential biomimetics of the γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in CNS.

In GABA neurotransmission, synaptically released GABA exerts its effects through activation of ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors. After unbinding from the receptor, GABA is taken up by GABA transporters of which four subtypes have been cloned (GAT1−4).[10] To pharmacologically characterize the $GABA_A$ receptors, a number of ligands bioisosterically derived from GABA, such as the selective agonists muscimol (**1**) and 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]-pyridine-3-ol (THIP, gaboxadol, **2**) and the antagonist gabazine (**3**), have been developed over the years[11] (Figure 1). THIP, which has a particular partial agonist profile at cloned $GABA_A$ receptors, is currently undergoing phase III clinical investigation for treatment of sleep disorders.[12] Whereas THIP shows very potent nonopioid analgesic effects and novel hypnotic effects in human clinical studies, it seems likely that partial $GABA_A$ agonists showing lower levels of efficacy such as 5-(4-piperidyl)-3-isoxazolol (4-PIOL, **4**)[13] may be of therapeutic interest in certain CNS disorders such as schizophrenia.[14] Information about the mechanism of receptor−ligand interactions resulting in partial agonism at the molecular level is still not available, thus making the design of new $GABA_A$ agonists with a range of different efficacies relevant.

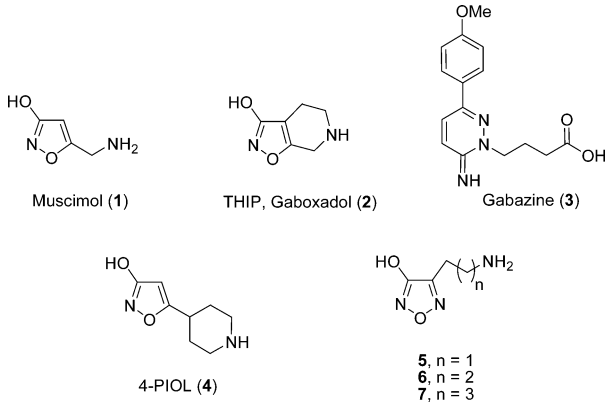

Figure 1. Structures of the $GABA_A$ receptor agonists muscimol (**1**), THIP (**2**), and 4-PIOL (**4**), the $GABA_A$ antagonist gabazine (**3**), and the new 1,2,5-oxadiazol-4-ols (**5**−**7**).

In this paper we report the synthesis, the ionization constants, and the pharmacological characterization at GABA receptors and GABA transporters of new analogues and homologues of GABA, **5**−**7** (Figure 1), in which the carboxyl group has been replaced by a 4-hydroxy-1,2,5-oxadiazol-3-yl moiety.

Results and Discussion

Chemistry. The synthetic pathway for preparing the final products **6** and **7** is depicted in Scheme 1. The common starting material was the 3,4-diphenylsulfonyl-1,2,5-oxadiazole **8**. By

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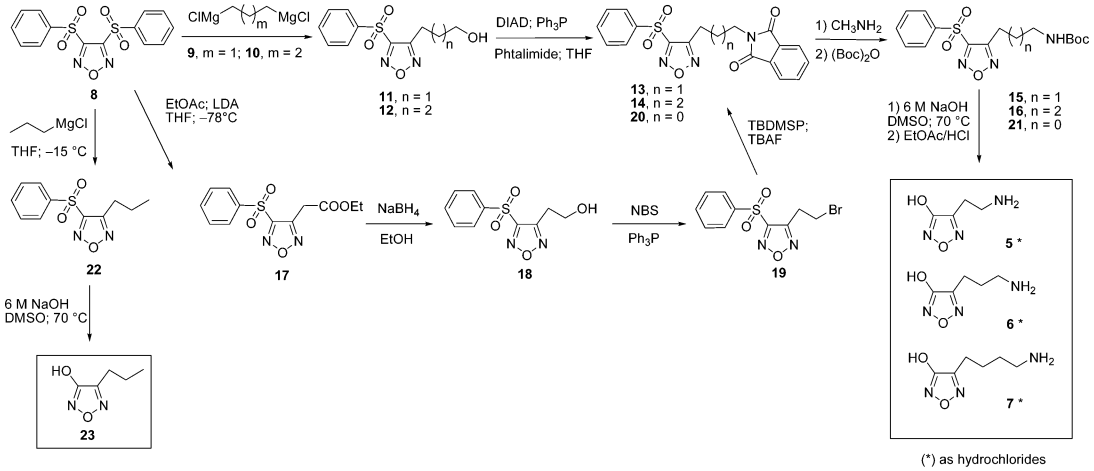
10.1021/jm051288b CCC: $33.50
Published on Web 06/21/2006

Scheme 1. Synthetic Strategy for the Preparation of the GABA-Related Compounds **5**−**7** and the Reference Compound **23**



action on this product of the Normant reagents **9** and **10**[15] in THF solution, alcohols **11** and **12** were obtained. These substances were treated with a mixture of triphenylphosphine (Ph_3P) and diisopropyl azodicarboxylate (DIAD) in the presence of phthalimide (Mitsunobu reaction) to give the related phthalimido derivatives **13** and **14**. These products were converted into analogues that are the Boc-protected derivatives **15** and **16**, which were purified by flash chromatography. These intermediates were transformed into the final hydrochlorides of **6** and **7** by treatment with NaOH in DMSO and then with HCl in ethyl acetate (EtOAc). For the synthesis of the strict GABA analogue **5**, a different synthetic procedure was explored (Scheme 1). The furazan **8** was treated in THF with EtOAc in the presence of lithium diisopropylamide (LDA) at low temperature to give the acetate **17**. The product results from the nucleophilic displacement of one of the two phenylsulfonyl groups of **8** under the action of the carbanion generated by action of LDA on EtOAc. Reduction of **17** with $NaBH_4$ in ethanol afforded the related alcohol **18** that was transformed into the corresponding bromide **19**, for action of Ph_3P and *N*-bromosuccinimide (NBS). The reaction of **19** with *tert*-butyldimethylsilylphthalimide (TBDMSP) in the presence of tetrabutylammonium fluoride (TBAF) produced the expected phthalimido derivative **20**. This product was transformed into the final GABA analogue **5**, through the intermediate **21**, following the same procedure used to prepare **6** and **7** from **13** and **14**, respectively. The 4-propyl-1,2,5-oxadiazol-3-ol (**23**), used as a reference in the ionization constant studies, was prepared as reported in Scheme 1. The action of propylmagnesium chloride on **8** gave the intermediate 1,2,5-oxadiazole derivative **22** that, by action of NaOH in DMSO solution, afforded the expected final compound **23**.

Ionization Constants. The pK_a ionization constants for the three final compounds **5**−**7** and for the reference compound **23** were obtained by potentiometric titration using a GLpKa apparatus. The pK_a values are in Table 1 with those of GABA.[17] Except for **23**, all of the products show two dissociation constants in the ranges 3.12−3.56 and 9.28−10.22 in accordance with their chemical structure. The pK_a obtained for **23** clearly indicates that the lowest pK_a of each product is related to the dissociation of the acidic −OH function, while the highest is related to the presence of the basic $-NH_2$ function. Although the observed ionization constants of **5**−**7** are slightly lower than the corresponding constants of GABA, the three compounds (similar to GABA) exist predominantly as zwitterions at physiological pH.

Table 1. pK_a Ionization Constants

compd	pK_{a1}	pK_{a2}
23[a]	3.77	
5[b]	3.12	9.28
6[b]	3.37	9.87
7[b]	3.56	10.22
GABA	4.04	10.71

[a] Titrations were performed using methanol as cosolvent (10−40 wt %). pK_a value was determined by extrapolation to zero content of cosolvent according to the Yasuda−Shedlovsky procedure.[16] SD = 0.03. [b] Determined by aqueous titration. SD ≤ 0.02.

For neutral amino acidic compounds such as GABA and **5**−**7** the distribution across of the blood−brain barrier is expected to be closely related to the I/U ratio of the compounds (the ratio between the concentration of the ionized and the un-ionized molecules), which is a function of the difference between the individual compound's two pK_a values. On the basis of the ionization constants (Table 1), the I/U ratio of GABA, which does not cross the blood−brain barrier, is estimated to be 0.8×10^6. The I/U ratios of **5**−**7** are at least as high as that of GABA, and these compounds are consequently not expected to cross the blood−brain barrier. Therefore, only an in vitro pharmacological characterization has been carried out.

In Vitro Pharmacology. Compounds **5**−**7** were characterized in receptor binding studies using rat brain membrane preparations and electrophysiologically at $GABA_A$ receptors. Furthermore, the compounds were evaluated in GABA uptake studies using cultured neurons and astrocytes and cloned GABA transporters from mice (mGAT1−4) expressed in HEK-293 cells.

The affinities of the new compounds for $GABA_A$ and $GABA_B$ receptor sites were determined using [^{3}H]muscimol and [^{3}H]GABA in the presence of the selective $GABA_A$ receptor agonist isoguvacine,[18] respectively (Table 2). In [^{3}H]muscimol binding, **5** and **6** showed affinities more than a 100-fold lower than those of GABA and THIP (**3**) but in the same range as that of 4-PIOL (**4**), whereas the IC_{50} of **7** was found to be greater than 100 μM. In the $GABA_B$ binding assay, **5** was the most potent displacer of [^{3}H]GABA binding ($IC_{50} = 2.0$ μM) whereas **6** was 10-fold weaker and **7** appeared to be inactive. At 1 mM, none of the new compounds, **5**−**7**, were able to inhibit GABA uptake in assays using neurons, astrocytes, or HEK293 cells expressing the cloned transporters mGAT1−4.

The functional properties of **5**−**7** at $GABA_A$ receptors in primary cultures of cerebral cortical neurons were investigated using standard patch-clamp techniques. Because of extensive

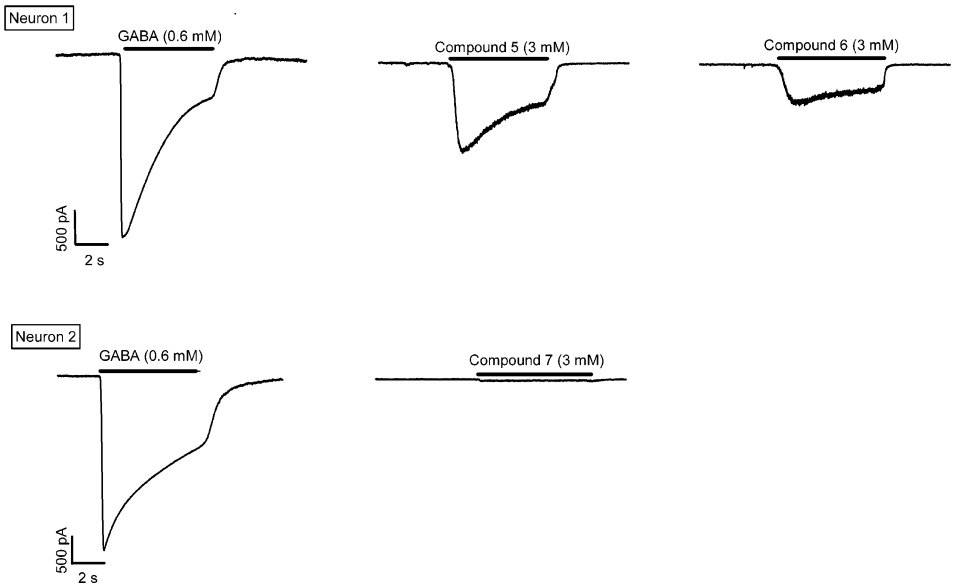


Figure 2. Representative current traces from two different neurons comparing the responses evoked by 3 mM of each of the compounds **5**−**7** with a close to maximum reponse of GABA (0.6 mM). Each compound was applied for approximately 5 s as shown by the thick bar above each trace. The neurons have been chosen so that the amplitudes of the GABA responses are nearly identical, thereby making the traces for **5**−**7** directly comparable.

Table 2. Affinities for GABA Receptor Sites[a]

compd	[^{3}H]muscimol binding K_i (μM)[b]	[^{3}H]GABA binding IC_{50} (μM)[b]
GABA	0.049 (0.043, 0.056)	0.013 (0.011, 0.014)
3 (THIP)	0.16[c]	nd[e]
4 (4-PIOL)	9.1 (8.2, 10)[d]	>100[d]
5	13 (11, 16)	2.0 (1.5, 2.6)
6	27 (25, 30)	25 (19, 33)
7	>100	>100

[a] Standard receptor binding on rat brain synaptic membranes, $n = 3$. [b] Mean and SEM were calculated assuming a normal distribution of the logarithm of the IC_{50} and K_i values. Hence, numbers in parentheses (min, max) indicate the antilog of the log of the mean ± SEM of IC_{50} and K_i. [c] Data from ref 19. [d] Data from ref 4. [e] nd: not determined.

desensitization of $GABA_A$ receptors in receptor binding assays, resulting in an increased apparent affinity,[20] a marked discrepancy between affinity in [^{3}H]muscimol binding and the agonist activity is normally seen for agonists and highly efficacious partial agonists, whereas for antagonists and low efficacious partial agonists, the affinity and the potency are in good agreement. On the basis of this, it was decided to include **7** in these studies despite the low affinity, primarily to look for possible antagonist or partial agonist activities. Figure 2 shows representative current traces from **5**−**7** obtained from two different neurons and suggests that the compounds possess agonist properties at the $GABA_A$ receptor. The currents were evoked by high concentrations of each compound, i.e., 3 mM, and compared to a close to maximal response of GABA (600 μM). The average peak currents induced by the compounds relative to GABA were 40% ± 9% ($N = 7$) for **5**, 14% ± 2.6% ($N = 7$) for **6**, and 0.8% ± 0.17% ($N = 3$) for **7**.

To further elucidate whether the currents elicited by **5**−**7** were $GABA_A$ receptor mediated, antagonist experiments with gabazine were conducted. Cells were pretreated with gabazine (100 μM) for 15 s followed by application of a mixture of gabazine (100 μM) and **5**, **6**, or **7** (3 mM). Gabazine blocked the responses elicited by **5**, **6**, or **7** nearly completely (**5**, 99.5% ± 0.5%, $N = 7$; **6**, 100%, $N = 7$; **7**, 97% ± 3%, $N = 4$). The close to complete inhibition by the specific $GABA_A$ antagonist demonstrates that the currents evoked by each of **5**−**7** are $GABA_A$ receptor mediated.

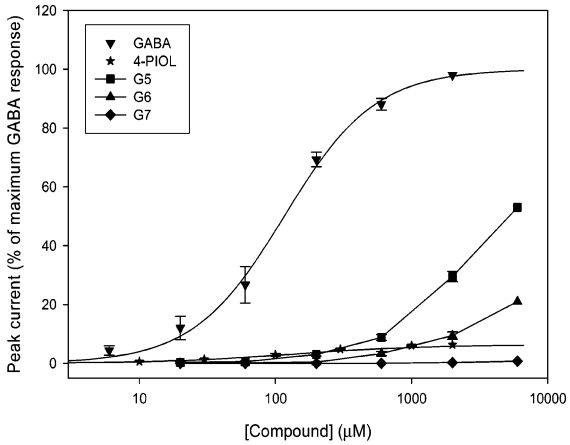


Figure 3. Concentration response curves for **5**−**7**. Currents are normalized to maximum GABA response (refer to Supporting Information for details). Data are presented as the mean ± SE from seven to nine neurons for **5**−**7**. For GABA and 4-PIOL the solid lines represent the best fit to the Hill-type function shown in the Supporting Information.

The agonist profiles of **5**−**7** were further characterized in concentration−response experiments (Figure 3) in which concentration−response curves for GABA and 4-PIOL based on previously reported data[21] have been included for comparison. Although exact estimates for potencies and efficacies of **5**−**7** cannot be extracted because maximum responses have not been reached, **5** and **6** clearly possess efficacies greater than that of the partial agonist 4-PIOL ($E_{max} \approx 5\%$ [21]) in cortical neurons, whereas 4-PIOL is more efficacious than **7**. In terms of potencies, all three compounds **5**−**7** turned out to be less potent than 4-PIOL ($EC_{50} = 139$ μM [21]).

To investigate whether **5**−**7** possess partial agonist properties, the interactions between the full $GABA_A$ receptor agonist isoguvacine at 20 μM (corresponding to E_{20}) and **5**−**7** were studied (Figure 4). In this type of experiment, a partial agonist with a low efficacy is expected to competitively displace a full agonist from the receptor, and at increasing concentration of partial agonist the combined response will approach the maximum response of the partial agonist alone. Compounds **5**

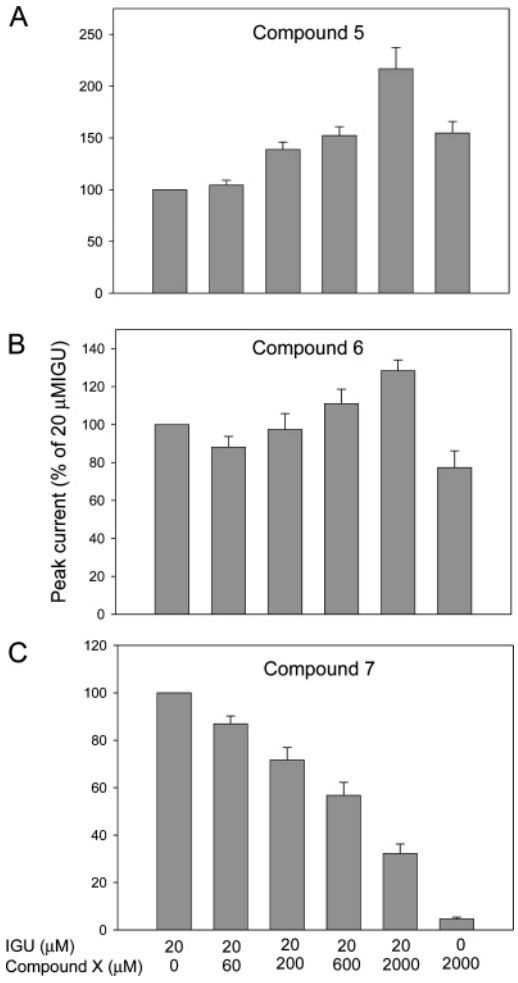


Figure 4. Concentration−interaction plots for **5**, **6**, and **7** in panels A, B, and C, respectively. Currents are normalized to the current induced by 20 μM isoguvacine (IGU). Data are presented as the mean ± SE from nine neurons for each compound. The concentrations are given below panel C. Note the different *y*-axis scale for each compound.

and **6** concentration-dependently increased the currents elicited by isoguvacine (parts A and B of Figure 4). This increased response could be explained by a modulating effect mediated by binding of **5** or **6** to distinct (allosteric) binding sites. However, on the basis of the results shown in Figure 3 and the sensitivity to gabazine, it is possible that **5** and **6** are $GABA_A$ receptor partial agonists with maximum responses larger than E_{20} of isoguvacine and GABA. The results obtained from the experiments with **7** were more clear (Figure 4C). This compound concentration-dependently inhibited the isoguvacine-induced currents (IC_{50} = 740 μM estimated by linear regression), demonstrating that **7** is a partial $GABA_A$ receptor agonist mainly possessing antagonist properties.

Conclusion

In the present study we have shown the 4-hydroxy-1,2,5-oxadiazol-3-yl moiety, when incorporated in GABA-related compounds, to have protolytical properties slightly more acidic than those of the carboxyl group of GABA. The GABA analogues and homologues **5**−**7** all behaved as agonists with lower potencies than those of GABA and 4-PIOL and displayed a range of efficacy levels in the rank order **7** < 4-PIOL < **6** < **5** < GABA. Compound **7** behaved as a true low-efficacy partial agonist and inhibited the currents induced by isoguvacine. A similar conclusion could not be disclosed for **5** or **6**, which may be binding to distinct sites from the GABA binding site. The results demonstrate that the 4-hydroxy-1,2,5-oxadiazol-3-yl unit can be used as a nonclassical carboxyl group bioisoster. The use of 4-hydroxy-1,2,5-oxadiazol-3-yl in other receptor systems in the design of new research tools with unique pharmacological properties and potential drug candidates will be exploited further.

Experimental Section

General Procedure for the Synthesis of the Target Compounds 5−7. A solution of appropriate Boc-protected derivatives **15**, **16**, and **21** (2.83 mmol) and 5 M NaOH (5 mL, 25 mmol) in DMSO (8 mL) was heated at 70 °C for 2 h. The mixture was cooled at room temperature and then diluted with water (8 mL) and washed with ethyl ether (10 mL). The pH of the solution was adjusted to 3.6 by adding 6 M HCl, obtaining a milky mixture. The mixture was extracted with diethyl ether (4 × 15 mL), maintaining the pH of the aqueous layer constant by adding 6 M HCl. The collected organic layers were washed with water (8 mL), dried, and concentrated under reduced pressure, obtaining a white solid crude material. The crude product was dissolved in dry EtOAc (4 mL), and the resulting solution was mixed with a solution of HCl in dry EtOAc (1.3 M, 10 mL). The white precipitate was filtered and washed with dry EtOAc (2 mL) to afford the title compounds as hydrochlorides.

4-(2-Aminoethyl)-1,2,5-oxadiazol-3-ol Hydrochloride (5). Yield 54%; mp 161−164 °C. ^{1}H NMR (D_2O) δ 3.00/3.33 (2H, t, J = 6.8 Hz; $-CH_2NH_2$)/(2H, t, J = 6.8 Hz, furazanCH_2-). ^{13}C NMR (D_2O) δ 20.7, 39.8, 145.7, 163.1. Anal. ($C_4H_7N_3O_2$·HCl) C, H, N.

4-(3-Aminopropyl)-1,2,5-oxadiazol-3-ol Hydrochloride (6). Yield 73%; mp 177−179 °C. ^{1}H NMR (D_2O) δ 2.00 (2H, m, J = 7.6 Hz, $-CH_2CH_2CH_2-$), 2.70 (2H, t, J = 7.4 Hz, furazanCH_2-), 2.99 (2H, t, J = 7.7 Hz, $-CH_2NH_2$). ^{13}C NMR (D_2O) δ 19.4, 24.1, 40.0, 148.0, 163.0. Anal. ($C_5H_9N_3O_2$·HCl) C, H, N.

4-(4-Aminobutyl)-1,2,5-oxadiazol-3-ol Hydrochloride (7). Yield 68%; mp 176−178 °C. ^{1}H NMR (D_2O) δ 1.5−1.7 (4H, m, $-CH_2CH_2CH_2CH_2-$), 2.58 (2H, t, J = 7.0 Hz, furazanCH_2), 2.86 (2H, t, J = 7.0 Hz, $-CH_2NH_2$). ^{13}C NMR (D_2O) δ 21.5, 23.1, 26.4, 39.4, 148.8, 163.1. Anal. ($C_6H_{11}N_3O_2$·HCl) C, H, N.

4-Propyl-1,2,5-oxadiazol-3-ol (23). A 5 M NaOH (5 mL, 25 mmol) sample was added to a stirred solution of 22 (1 g, 3.98 mmol) in DMSO (8 mL). The mixture was heated at 70 °C for 1 h and then allowed to reach room temperature. The reaction mixture was diluted with water (8 mL), washed with ether (3 × 10 mL), and acidified to pH 3.6 by adding 6 M HCl. The milky mixture was extracted with ether (2 × 10 mL). The combined organic layers were washed with water (8 mL), dried, and evaporated in vacuo to give the title compound as a pale-yellow prismatic solid. Yield 92%; mp 34.8−35.5 °C. MS (CI) *m*/*z* 129 $(M + 1)^+$. ^{1}H NMR ($CDCl_3$) δ 1.02 (3H, t, J = 7.4 Hz, $-CH_2CH_3$), 1.79 (2H, se, J = 7.4 Hz, $-CH_2CH_3$), 2.70 (2H, t, J = 7.4 Hz, furazanCH_2). ^{13}C NMR ($CDCl_3$) δ 13.6, 20.1, 24.2, 148.2, 162.7. Anal. ($C_5H_8N_2O_2$) C, H, N.

Acknowledgment. This work was supported by MIUR (Grant COFIN 2003) and the Danish MRC (Grant 22-01-0291 to U.K.).

Supporting Information Available: Synthetic procedures for the preparation of the intermediates, experimental details used for receptor binding assays, uptake assays, in vitro electrophysiology, table of elemental analysis results of intermediates and final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM051288B