Synthesis and Biological Evaluation of 3-Heterocyclyl-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazines and Analogues as Subtype-Selective Inverse Agonists for the $GABA_A\alpha5$ Benzodiazepine Binding Site

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The identification of a novel series of 7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]-phthalazines as $GABA_A\alpha5$ inverse agonists, which have both binding and functional (efficacy) selectivity for the benzodiazepine binding site of α5- over α1-, α2-, and α3-containing $GABA_A$ receptor subtypes, is described. Binding selectivity was determined to a large part by the degree of planarity of the fused ring system whereas functional selectivity was dependent on the nature of the heterocycle at the 3-position of the triazolopyridazine ring. 3-Furan and 5-methylisoxazole were shown to be optimal for $GABA_A\alpha5$ functional selectvity. 3-(5-Methylisoxazol-3-yl)-6-(2-pyridyl)methyloxy-1,2,4-triazolo[3,4-*a*]phthalazine (**43**) was identified as a full inverse agonist at the $GABA_A\alpha5$ subtype with functional selectivity over the other $GABA_A$ receptor subtypes and good oral bioavailability.

Introduction

Inhibitory neurotransmission throughout the mammalian central nervous system is predominantly mediated through $GABA_A$ receptors.[1] These are ligand-gated ion channels that, in addition to binding γ-aminobutyric acid (GABA), are the site of action of a number of pharmacologically important allosteric modulators including barbiturates, neurosteroids, loreclezole, anaesthetics, ethanol, and benzodiazepines (BZs).[2] $GABA_A$ receptors open in response to the binding of GABA, resulting in chloride ion flux into the cell and hyperpolarization of the resting membrane potential, leading to inhibition of neuronal activity. The BZ binding site on the GABA receptor has historically received most attention, and binding of ligands at this site modulates the effects of GABA. Compounds that bind to the BZ site have a range of activities, from full agonist, e.g., diazepam and lorazepam (positive allosteric modulators) that increase the frequency of channel opening, resulting in hyperpolarization of the membrane potential and therefore reduced neuronal excitability, to inverse agonists, e.g., DMCM (negative allosteric modulators) that reduce the frequency of channel opening which causes a depolarization and therefore increased neuronal excitability. Antagonists such as Ro 15-1788 (flumazenil) bind to the BZ site and have no effect on chloride ion flux but will block the action of both agonists and inverse agonists.[3] Between the extremes of full agonism and full inverse agonism lies a spectrum of efficacies which includes partial agonists such as bretazenil and partial inverse agonists such as FG 7142. The behavioral effects of BZ site ligands are reflected in their efficacy. Full agonists are anxiolytic and sedative and also have cognition impairing[4,5] and anticonvulsant properties. Inverse agonists are anxiogenic and are either convulsant[6−8] in their own right or enhance the efficacy of a convulsant compound (proconvulsant). In animal tests of learning and memory, BZ receptor inverse agonists have the opposite effects to BZ receptor agonists and can enhance learning and memory.[9]

To date, 16 $GABA_A$ receptor subunits have been identified (α1−α6, β1−β3, γ1−γ3, δ, ϵ, θ, π) using molecular cloning techniques.[10] $GABA_A$ receptors that bind BZs are a pentameric assembly of proteins made up from at least one α subunit, one β subunit, and one γ subunit with current evidence suggesting a stoichiometry of two α subunits, two β subunits, and one γ subunit. Analysis of recombinant $GABA_A$ receptors has established that the benzodiazepine binding site occurs at the interface of the α and γ subunits. Consequently, the combination of α and γ subunits strongly influences both the affinity of benzodiazepine ligands for the receptor and the efficacy of benzodiazepine ligands for the enhancement of GABA-induced chloride ion flux.[11] Since the predominant γ subunit in the brain is the γ2, the pharmacology of native $GABA_A$ receptors is dictated by the α subunit present.[12] When transfected into cells containing α, β, and γ2 subunits, the α1 subunit confers a BZ I type pharmacology and the α2, α3, and α5 subunits confer a BZ II type pharmacology,[13,14] a $GABA_A$ receptor population accounting for approximately 75% of total brain $GABA_A$ receptors.[15] A third class of $GABA_A$ sites with low affinity for benzodiazepines contains either the α4 or α6 subunits, the so-called diazepam insensitive receptors; a difference which can be solely

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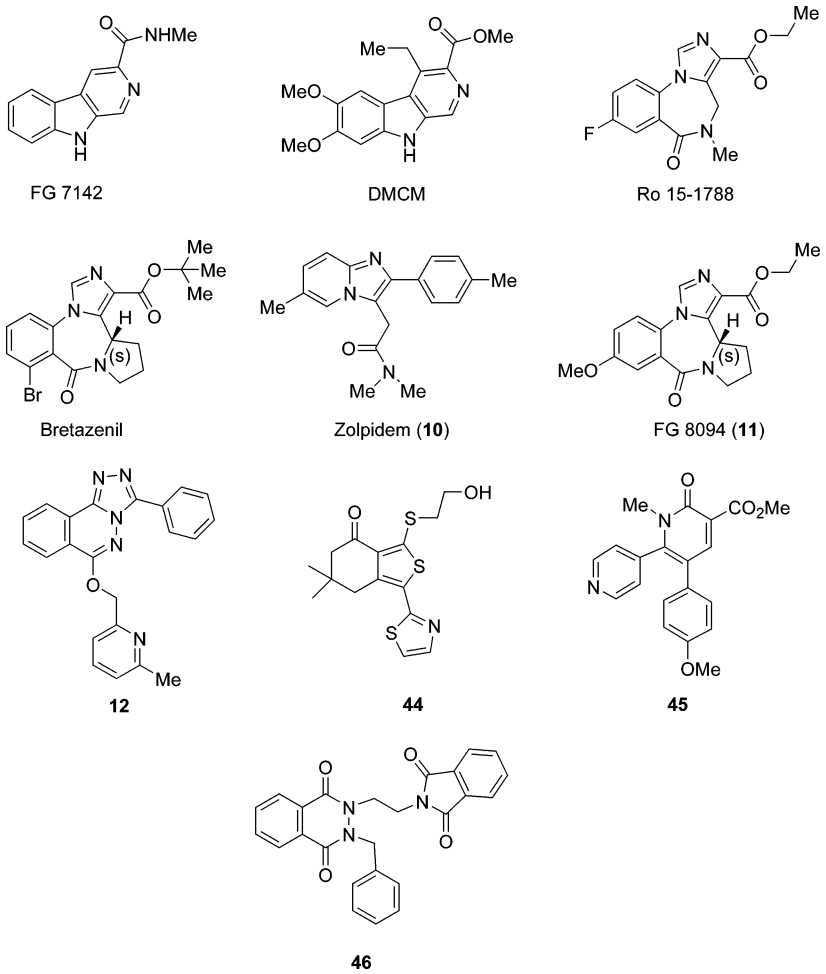
Chart 1. GABA$_A$ Benzodiazepine Binding Site Ligands

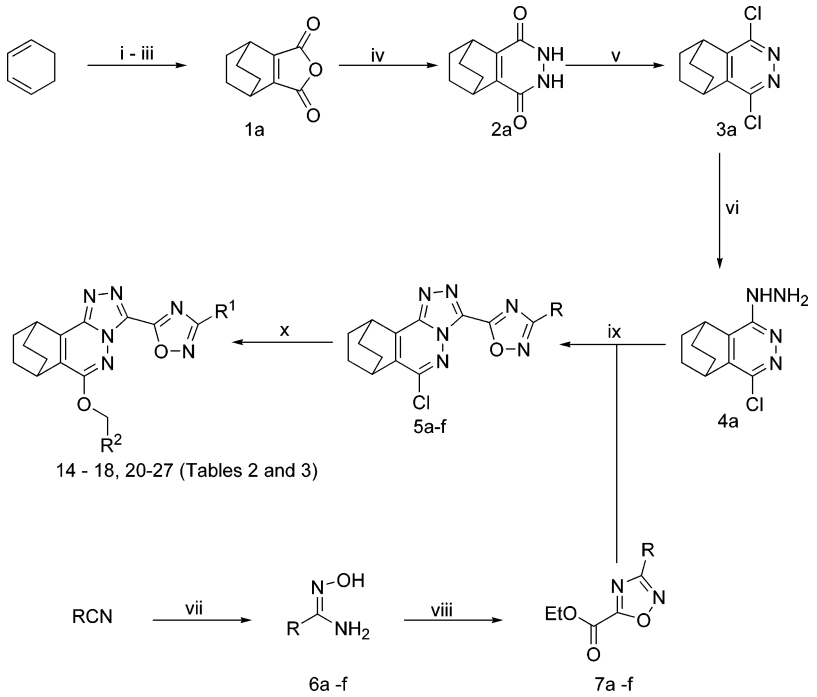


attributed to the presence of an arginine residue in α4 and α6 subunits which in α1, α2, α3, and α5 subunits is histidine.[16] Thus the major BZ-sensitive GABA$_A$ receptor subtypes in the mammalian brain are α1βxγ2, α2βxγ2, α3βxγ2, and α5βxγ2.

The distribution of the GABA$_A$ subtypes has been determined using a combination of in situ mRNA hybridization, quantitative immunoprecipitation, and immunohistochemistry.[12,17] The α1 subtypes are predominantly localized in the cerebellum and cortex, the α2 and the α3 are found mainly in the cortex and hippocampus, and the α5 subtype is predominantly in the hippocampus. The distinct neuroanatomical distributions of the different subunit-containing GABA$_A$ receptors were therefore consistent with different subtypes being associated with different physiological processes, and this has been examined recently using gene-targeting approaches. These results were obtained using knock-in mice in which individual GABA$_A$ receptor subunits were rendered diazepam-insensitive by introducing a His to Arg change into murine α subunit genes. It has been demonstrated that GABA$_A$ receptors containing an α1 subunit mediate the sedative/muscle relaxant effects of benzodiazepines, whereas those containing an α2 or α3 subunit mediate anxiolytic/anticonvulsant effects.[18−20] The role of GABA$_A$ receptors containing an α5 subunit has remained largely undefined. Although the α5 subtypes represent less than 5% of total brain GABA$_A$ receptors, they are primarily expressed in the dendritic fields of the hippocampus where they account for ∼20% of all GABA$_A$ receptors.[21,22] The localization of the α5 subtype to this region suggests an involvement in the physiological processes underlying learning and memory and it has been shown recently by targeted disruption of the α5 gene in mice that α5 containing GABA$_A$ receptors play a key role in cognitive processes.[23] In this study, α5-/- mice showed a significant improvement in a water maze model of hippocampal learning in comparison with the wild-type controls.

Nonselective benzodiazepine agonists such as diazepam and triazolam, which have equal affinity for the BZ sensitive GABA$_A$ subtypes, are used therapeutically as anxiolytics and anticonvulsants. However, they also impair learning and memory processes. The sedative agent Zolpidem, which has selectivity for the α1 subtype and very low affinity for the α5 subtype, was shown to induce significantly smaller deficits in a memory task compared to triazolam.[24] These data indirectly suggest that modulation of the α5 subtype may affect learning and memory processes. Since nonselective BZ inverse agonists are anxiogenic and either convulsant or proconvulsant, they cannot be used to treat human cognitive disorders. Work in our laboratory has focused on the identification of GABA$_A$α5 receptor inverse agonists which have subtype selectivity over the α1-, α2- and α3-containing GABA$_A$ subtypes. It was hypothesised that such compounds would have utility as cognition enhancers without the side-effects associated with nonselective GABA$_A$ modulation. Subtype selectivity may

Scheme 1[a]



[a] Reagents and conditions: (i) Acetylene dicarboxylate, THF, 60 °C; (ii) H_2, Pd on C, ethyl acetate; (iii) acetic anhydride, 100 °C; (iv) $NH_2NH_2.H_2O$, NaOAc, acetic acid, H_2O, reflux; (v) $POCl_3$, reflux; (vi) $NH_2NH_2.H_2O$, ethanol, reflux; (vii) $NH_2OH{\cdot}H_2O$, K_2CO_3, ethanol, reflux; (viii) EtO_2CCOCl, either pyridine/1,2-dichloroethane, 0 °C−80 °C or NaH/THF; (ix) dioxane, reflux; (x) RCH_2OH, NaH, DMF, + 25 °C then 65 °C for 15 h.

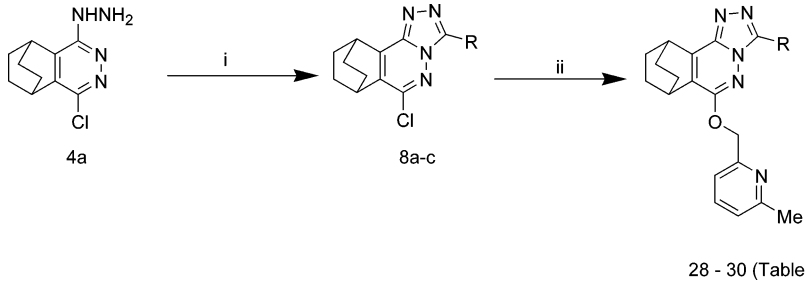
be achieved in two ways: (1) binding selectivity (higher affinity for the $GABA_A\alpha5$ subtype than for the other subtypes) or (2) functional selectivity (selective inverse agonism for the $GABA_A\alpha5$ receptor). There are a few reports in the literature of compounds with binding selectivity for the $GABA_A\alpha5$ receptor. Most notable are the imidazobenzodiazepines[25−28] and analogues of diazepam.[29] The imidazobenzodiazepine FG-8094 (L-655,-708, **11**) is the most widely reported $GABA_A\alpha5$ binding selective inverse agonist.[30,31] We have recently reported the identification of a novel series of 6,7-dihydro-2-benzothiophen-4(5*H*)-ones as $GABA_A\alpha5$ selective inverse agonists.[32] From this series, the 2-hydroxyethyl derivative **44** was shown to enhance cognitive performance in rats without showing the convulsant or proconvulsant activity that is associated with nonselective $GABA_A$ receptor inverse agonists.[33] We have also recently reported on the identification of 6-(4-pyridyl)-5-(4-methoxyphenyl)-3-carbomethoxy-1-methyl-1*H*-pyridin-2-one (**45**) as a $GABA_A\alpha2/\alpha3$ functionally selective inverse agonist.[34]

We reported in a preceding paper a series of 3-phenyl-6-(2-pyridyl)methyloxy-1,2,4-triazolo[3,4-*a*]phthalazines (e.g. **12**) as high affinity $GABA_A$ ligands which was optimized to give $GABA_A\alpha2/\alpha3$ agonists with subtype selectivity over $GABA_A\alpha1$ receptors.[35] In this paper, we describe the identification of a novel series of 7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazines as $GABA_A\alpha5$ inverse agonists which have binding selectivity over the other $GABA_A$ subtypes and the further extension of this work to give an orally bioavailable, functionally selective $GABA_A\alpha5$ receptor inverse agonist that enhances performance in rodent tests of learning and memory but is without anxiogenic and convulsant effects.

Synthetic Methods

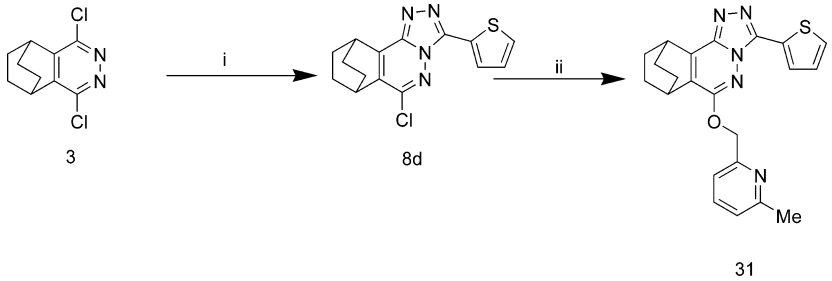
Synthesis of the 1,2,4-oxadiazole analogues (Tables 2 and 3) was perfomed as shown in Scheme 1. Hydrazine **4a**[36] was prepared in three steps and 56% overall yield from the known anhydride **1a**.[37] Refluxing **4a** in dioxane with the ethyl-(1,2,4-oxadiazol-5-yl)carboxylate (**7a**−**f**) resulted in cyclization of the intermediate carboxylic hydrazide to give the triazolopyridazines **5a**−**f**. Substitution changes on the oxymethylene group were achieved by reaction of **5a** with a range of carbinols (Table 2). Treatment of pyridine **15** with *m*-chloroperbenzoic acid gave the *N*-oxide **19** in 47% yield. A range of five- and six-ring heterocycles were explored at the 3-position of the triazolopyridazine ring (Table 4), and these were prepared according to Schemes 2−5. For the 3- and 2-furans (**28** and **29** respectively) and 3-thiophene (**30**), the carboxylic acid chlorides were commerically available so the triazolopyridazines were prepared by refluxing the acid chlorides with hydrazine **4a** in dioxane and triethylamine followed by reaction with 6-methyl-2-pyridylcarbinol (Scheme 2). The 2-thiophene derivative **31** was prepared from 2-thiophenecarboxylic hydrazide according to Scheme 3. Cyclization of the intermediate carboxylic hydrazide was accomplished by refluxing in xylene in the presence of triethylamine. Several compounds (**32**−**38**) were prepared from the corresponding heterocycle carboxylic acids (Schemes 4 and 5). For example, triazoles **35** and **36** were prepared by coupling hydrazine **4a** with 1,2,4-triazolecarboxylic acid, mediated with 1,1′-carbonyldiimidazole, followed by heating in xylene to give the triazolopyridazine **8j** (Scheme 5). This was methylated with methyl iodide, and the regioisomers **8k** and **8l** were separated by silica gel chromatography[38] then were reacted with 6-methyl-2-

Scheme 2[a]



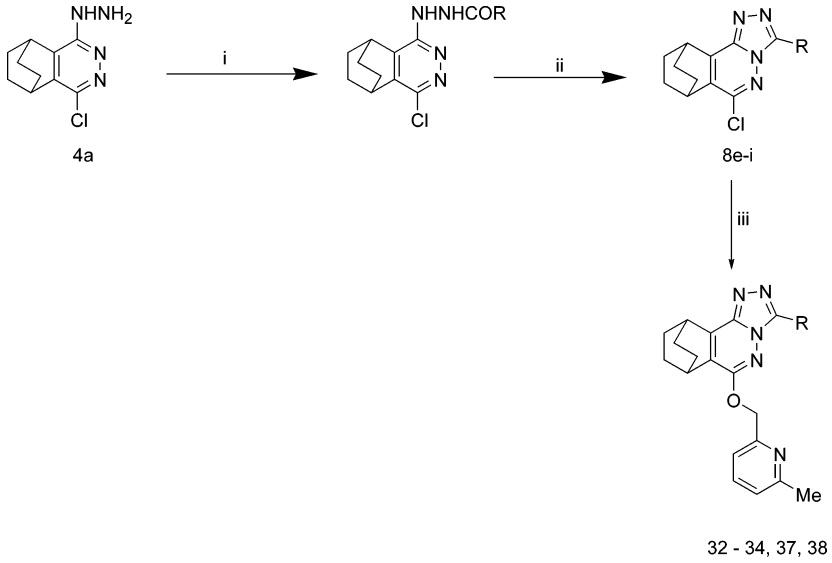
[a] Reagents and conditions: (i) RCOCl, dioxane, NEt_3 reflux; (ii) 6-methyl-2-pyridylcarbinol, NaH, DMF, +25 °C.

Scheme 3[a]



[a] Reagents and conditions: (i) 2-Thiophenecarboxylic hydrazide, xylene, NEt_3, reflux; (ii) 5-methyl-2-pyridylcarbinol, NaH, DMF, +25 °C.

Scheme 4[a]



[a] Reagents and conditions: (i) RCO_2H, 1,1′-carbonyldiimidazole, DMF; (ii) xylene, *p*-toulene sulfonic acid (cat.), reflux, 16 h; (iii) 6-methyl-2-pyridylcarbinol, NaH, DMF, +25 °C.

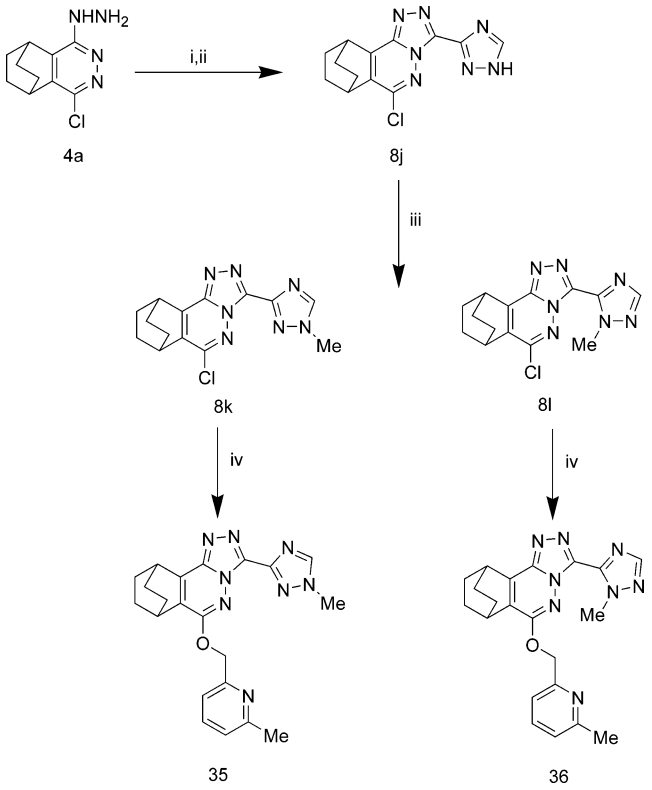
pyridylcarbinol to give **35** and **36**, respectively. The tetrahydro-methano-1,2,4-triazolophthalazine derivatives **39** and **40** and tetrahydro-1,2,4-triazolophthalazine **41** were prepared from 2-norbornene-2,3-dicarboxylic anhydride (**1b**) and tetrahydrophthalic anhydride (**1c**), respectively, according to Scheme 6. 5-Methylisoxazole-3-carboxylic acid was prepared by carefully refluxing acetonylacetone with nitric acid. 1,2,4-Triazolophthalazines **42** and **43** were similarly prepared from 1,4-dichlorophthalazine **3d** as shown in Scheme 7.[39]

Results and Discussion

The binding affinity of all compounds for cloned human α1β3γ2, α2β3γ2, α3β3γ2, and α5β3γ2 $GABA_A$ receptors was determined by displacement of [^{3}H]Ro15-1788 in L(tk$^-$) cells.[40] The in vitro efficacies of selected compounds were determined using two- electrode voltage clamp recording in *Xenopus laevis* oocytes, which transiently expressed α1β3γ2, α2β3γ2, α3β3γ2, and α5β3γ2 $GABA_A$ receptors, by measurement of the modulatory effect on the GABA EC_{20} ion current.[41] Additionally, the efficacy of selected compounds was determined using whole cell patch clamp recording from mammalian fibroblast L(tk$^-$) cells that stably expressed human $GABA_A$ receptors in the presence of a submaximal dose (EC_{20}) of GABA.[42]

The 1,2,4-triazolophthalazine **12** was developed from the 2,3-dihydrophthalazine-1,4-dione **46** (K_i: α1β3γ2 = 6.4 μM, α2β3γ2 =1.8 μM, α3β3γ2 = 1.4 μM, α5β3γ2 = 4.8 μM), which was identified from screening the

Scheme 5[a]

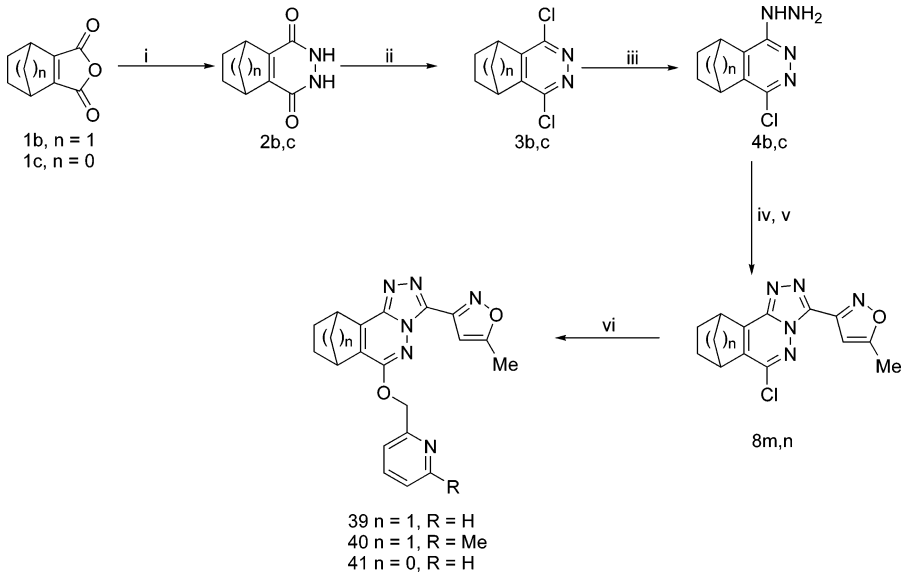


[a] Reagents and conditions: (i) 1,2,4-Triazolecarboxylic acid, 1, 1′-carbonyldiimidazole, THF; (ii) xylene, NEt_3.HCl, 140 °C, 16 h; (iii) Mel, K_2CO_3, DMF, silica gel chromatography eluting with CH_2Cl_2/MeOH (95:5), (iv) 6-methyl-2-pyridylcarbinol, NaH, DMF, +25 °C.

Merck sample collection, as previously described.[35] Compound **12** has low nanomolar binding affinity for all the $GABA_A$ receptor subtypes and is essentially an antagonist at all subtypes in the *Xenopus oocytes* efficacy assay (Table 1). As well as a suitable starting point for the discovery of $GABA_A$ agonists with subtype selectivity for the $GABA_A\alpha2$ and α3 receptors, compound **12** was also considered to be a suitable starting point for a program to identify novel $GABA_A\alpha5$ selective inverse agonists with binding selectivity and/or functional (efficacy) selectivity. Structural modification of the triazolophthalazine ring identified the tetrahydro-(7,10-ethano)-1,2,4-triazolophthalazine **13** with high affinity for the $GABA_A\alpha5$ receptor and having up to 40-fold binding selectivity over the $GABA_A\alpha1$-containing subtype.[45] In the efficacy assay, **13** was shown to be a weak partial agonist at the $GABA_A\alpha5$ receptor and a partial agonist at the other $GABA_A$ subtypes. Since there is evidence that within a series of $GABA_A$ ligands, the efficacy can be modulated by modifying the hydrophilicity of the molecule[33,34] (i.e., increasing hydrophilicity generally lowers efficacy to give $GABA_A$ inverse agonists), we decided to explore heterocyclic replacements for the 3-phenyl group of triazolophthalazines **12** and **13** and to make modifications to the pyridine ring. Replacement of the phenyl ring of **13** with 3-methyl-1,2,4-oxadiazole gave **14** with low nanomolar affinity for $GABA_A\alpha5$ and 50-fold binding selectivity over the α1-containing subtype. Most importantly, oxadiazole **14** was shown to be a $GABA_A$ inverse agonist having greatest inverse efficacy for the α5 subtype in *Xenopus* oocytes. Indeed, **14** had greater inverse efficacy for the α5 subtype than the literature standard FG8094 (**11**). Unfortunately, **14** was shown to have poor oral bioavailability in rat (F_{oral} <1%), most likely due to rapid first pass metabolism.

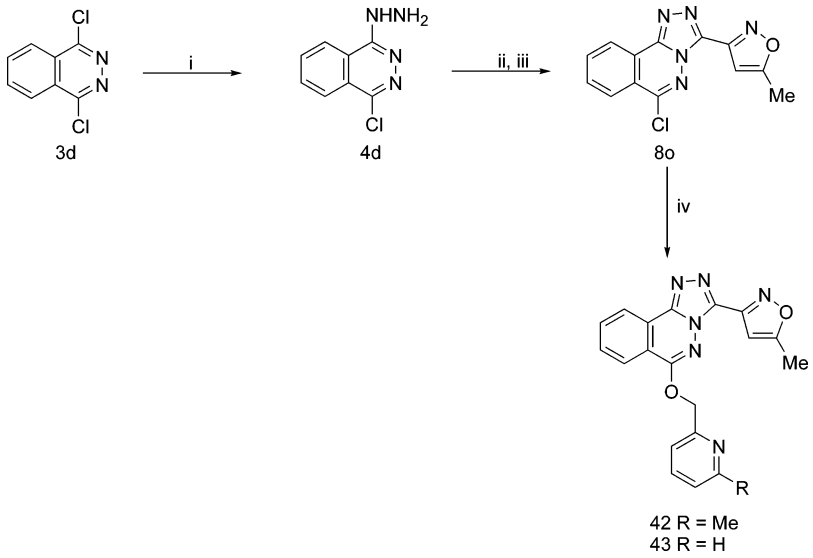
Changes to the pyridine and oxadiazole rings of **14** were explored with the aim of further improving $GABA_A\alpha5$ receptor binding affinity and selectivity and to improve oral bioavailability. The results of changes to the pyridine ring are shown in Table 2, and, in general, significant structural changes to the pyridine ring could be tolerated without loss in binding affinity for the $GABA_A\alpha5$ subtype. Both the desmethyl analogue **15** and 2,3-dimethyl analogue **16** gave a modest improvement in $GABA_A\alpha5$ binding affinity over **14**, and both compounds maintained inverse agonism. In terms

Scheme 6[a]



[a] Reagents and conditions: (i) $NH_2NH_2{\cdot}H_2O$, NaOAc, acetic acid, H_2O, reflux; (ii) $POCl_3$, reflux; (iii) $NH_2NH_2.H_2O$, ethanol, reflux; (iv) 5-methylisoxazole-3-carboxylic acid, 1,1′-carbonyldiimidazole, DMF; (v) xylene, NEt_3.HCl, reflux, 16h; (vi) 6-methyl-2-pyridylcarbinol or pyridine-2-methanol, NaH, DMF, +25 °C.

Scheme 7[a]



[a] Reagents and conditions: (i) $NH_2NH_2.H_2O$, ethanol, reflux; (ii) 5-methylisoxazole-3-carboxylic acid, bis(2-oxo-3-oxazolidinyl)phosphinic chloride, triethylamine, dichloromethane; (iii) xylene, NEt_3.HCl, reflux, 16 h; (iv) pyridine-2-methanol, NaH, DMF, +25 °C.

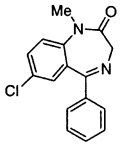
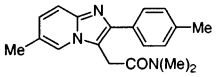
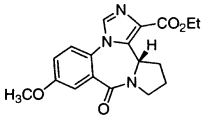
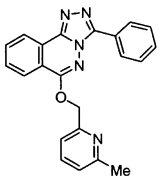
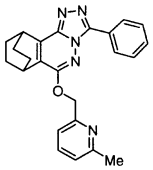
of binding selectivity, **16** was shown to be the most selective compound in the series having 124-fold selectivity for GABA$_A$α5 containing receptors over the GABA$_A$α1 subtype and up to 25-fold selectivity over the other GABA$_A$ subtypes. Neither the position of the nitrogen in the pyridine ring nor the presence of a basic nitrogen were shown to be crucial for binding affinity since both the 3-pyridyl derivative **18** and the *N*-oxide **19** maintained high binding affinity. Five-membered ring heterocycles were also well tolerated as demonstrated by the imidazole **17**, although with 5−10-fold reduced affinity relative to the pyridine analogues. We have previously shown in a related series of triazolophthalazines that the pyridine nitrogen is crucial for binding affinity since the corresponding phenyl analogues have 100-fold lower affinity.[35] We now show, however, that the pyridine ring can be replaced with substituted phenyl rings, e.g., 2-bromophenyl (**20**) and 2-cyanophenyl (**21**), without loss of affinity or efficacy. Moreover, **20** has good functional selectivity for the GABA$_A$α5 receptor subtype. Increasing the ring size of the heterocycle was also well tolerated as shown for quinoline **22**.

The results of exploring changes to the oxadiazole methyl group of **14** are shown in Table 3. Again, increasing the size of the substituent at this position was generally well tolerated. Both the ethyl oxadiazole **23** and the isopropyl analogue **24** maintained good binding selectivity for GABA$_A$α5 receptors. Phenyl substituent, e.g., **25** was less well tolerated, but affinity could be reinstated by the addition of nitrogen into the ring so that pyridines **26** and **27** have a comparable affinity to **14** but much better binding selectivity.

To explore SAR further, replacements for the 1,2,4-oxadiazole ring of **14** were sought and structural modifications to the triazolopyridazine ring were explored. Both the size of the heterocyclic ring and arrangement of heteroatoms in the heterocycle at the 3-position of the triazolopyridazine ring were shown to be important for binding affinity and efficacy (Table 4). 3-Furan was shown to be a good replacement for 1,2,4-oxadiazole so that **28** has comparable binding affinity and binding selectivity to **14** and improved functional selectivity for the α5 subtype in the *Xenopus* oocytes efficacy assay. The furan **28** is an inverse agonist at the α5 subtype only, with weak partial agonism at the α1 subtype and antagonism at both the α2 and α3 subtypes, and is thus functionally selective for GABA$_A$α5. The isomeric furan **29** and the two isomeric thiophenes **30** and **31** had either lower affinity or reduced inverse agonism relative to **28**. 5-Methylisoxazole **32** was also a good replacement for the oxadiazole ring and, like the 3-furan **28**, had excellent functional selectivity with inverse agonism for the α5 subtype only. In fact, 5-methylisoxazole was shown to be the optimal five-membered heterocycle for both binding and functional selectivity in the 7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine series with the 2-methyloxazole (**33**), thiazole (**34**), and triazole analogues **35** and **36** having reduced affinity. Of the six-membered heterocycles, pyrazine (**38**) was shown to have the best profile albeit with lower inverse agonism for α5-containing receptors relative to isoxazole **32**.

Assessment of the pharmacokinetics of **32** in rats showed it to have low oral bioavailability (F_{oral} 3%). Since **32** had the best selectivity profile, further structure activity studies were performed with this compound, exploring the effect of changes to the 7,8,9,10-tetrahydro-(7,10-ethano) group on both GABA$_A$ subtype selectivity, efficacy, and oral bioavailability (Table 5). The methylene bridgehead derivative **40**, profiled as a mixture of enantiomers, was shown to maintain both GABA$_A$α5 inverse agonism and binding selectivity over the other subtypes. Removal of the methylene bridge to give the fused tetrahydrophenyl derivative **41** resulted in an approximately 7-fold improvement in binding affinity for all the GABA$_A$ subtypes relative to the corresponding 7,8,9,10-tetrahydro-(7,10-methano) derivative **39**, but with some loss in both binding selectivity and functional selectivity over the GABA$_A$α3 containing subtype. Functional selectivity was regained by replacing the fused tetrahydrophenyl ring with a benzo fused ring to give the 1,2,4-triazolophthalazine **42**. Removal of the methyl group from the pyridine ring

Table 1. Binding Affinity and Efficacy of Standard Compounds at the $GABA_A$ Receptor Subtypes

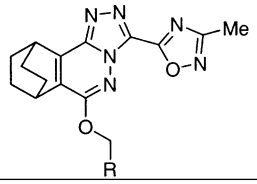
		K_i (nM) GABA-A$\alpha x\beta 3\gamma 2$ receptors[a] (%Efficacy[b])			
Compd.[c]	Structure	α5	α1	α2	α3
Diazepam (9)		11.2 (+73±9)	14.3 (+156±10)	26.2 (89±7)	23.7 (+211±19)
Zolpidem (10)		>10,000	52.1 (+133±31)	255.1 (187±23)	608.5 (142±25)
FG8094 (11)		0.45 (-12± 2)	48.5 (-12±2)	22.4 (-17±3)	24.5 (-3±3)
(12)		1.3 (+11.0±6)	1.9 (+0.7±3)	1.0 (-3.8±5)	0.33 (-8.3±3)
(13)		3.0 (+14±2)	122.2 (+53±12)	44.5 (+35±6)	23.7 (+44±8)

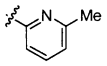
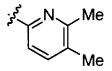
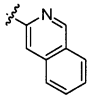
[a] K_i values for benzodiazepine sites on stably transfected $GABA_A$ receptors $\alpha x\beta 3\gamma 2$ ($x = 1$, 2, 3, or 5). Inhibition curves were carried out using receptors labeled with[^{3}H]Ro15−1788 at a concentration of around twice the K_d. K_i values were calculated according to the Cheng-Prussof equation. Data shown are mean values for 3−6 determinations. [b] % Changes in electrophysiological response at a maximal concentration of test compound (100 × K_i) in the presence of GABA (EC_{20} GABA concentrated) in *Xenopus* oocytes expressing $\alpha x\beta 3\gamma 2$. Data are expressed as the mean ± standard error of at least four separate oocytes. [c] All compounds were characterized by proton NMR and mass spectra and gave satisfactory elemental analyses.

of **42** gave a significant enhancement in inverse agonism for the $GABA_A\alpha 5$ subtype only, so that **43** is a high efficacy $GABA_A\alpha 5$ inverse agonist with essentially an antagonist profile at the other subtypes. The reduced binding selectivity observed for both the tetrahydrophenyl analogue **41** and the triazolophthalazines **42** and **43** compared with the bicyclic derivatives **32** and **40** suggests that the degree of planarity in this region of space is an important determinant for binding affinity. The more planar triazolophthalazines generally have higher affinity for all $GABA_A$ receptor subtypes, and in particular for $GABA_A$ α1, α2, and α3 subtypes, and are consequently less binding selective for the α5 subtype. Both the fused norbornene derivative **40** and the 1,2,4-triazolophthalazine **42** showed essentially no improvement in oral bioavailability over the bicyclo[2.2.2]octane-fused analogue **32**, with both compounds showing high plasma clearance in rat. However, removal of the methyl group from the pyridine ring of **42** gave a significant improvement in oral bioavailability, so that **43** has 41% oral bioavailability in rat. The improved oral bioavailability of **43** over **42** is due to reduced plasma clearance and suggests that the methyl group of the methylpyridine **42** may be a site of metabolism. Compound **43** is thus a high affinity, orally bioavailable, functionally selective $GABA_A\alpha 5$ inverse agonist and was therefore considered to be a useful tool for determining the role of $GABA_A\alpha 5$ receptors in memory and cognition.

The in vitro efficacy profile of **43** at the BZ receptor site was further characterized using whole-cell patch-clamp recording from L(tk$^-$) cells in the presence of a submaximal GABA concentration (EC_{20}) (Table 6). The selective inverse agonism of **43** shown in *X. laevis* oocytes for $GABA_A\alpha 5$ receptors was mirrored in the whole cell patch clamp experiment with **43**, causing a concentration-dependent suppression of the current activated by an EC_{20} GABA concentration. The EC_{50} for **43** on the α5 subtype was 2.5 nM with a maximum inhibition of −45%, i.e., **43** shows greater inverse agonism than the imidazobenzodiazepine FG-8094 (**11**). At the other subtypes, **43** was shown to be either a low efficacy partial inverse agonist or an antagonist. In addition, **43** had much lower affinity for $GABA_A$ receptors containing either the α4 or α6 subunits (K_i 244 nM and 4410 nM, respectively), the so-called diazepam-insensitive $GABA_A$ receptor subtypes.[39] We recently reported on the behavioral profile of the 6,7-dihydro-2-benzothiophen-4(5*H*)-one **44** which was shown to enhance cognitive performance in rats in the delayed-matching-to-place (DMTP) version of the watermaze test at 0.3 mg/kg ip.[33] Triazolophthalazine **43** was also profiled in the DMTP watermaze test and showed a

Table 2. Binding Affinity and Efficacy of 3-Methyl-1,2,4-oxadiazoles at the GABA$_A$ Receptor Subtypes



		K_i (nM) GABA-Aαxβ3γ2 receptors[a] (%Efficacy[b])			
Compd.[c]	R	α5	α1	α2	α3
(14)		1.4 (-35±8)	75.7 (-15±1)	16.8 (-10±3)	11.0 (-21±5)
(15)		0.77 (-38±7)	28.2	8.50	3.9
(16)		0.39 (-25±8)	48.6 (+1±5)	9.8	8.6 (-21±0.8)
(17)		3.8 (-26±0.5)	158.6	35.9	19.4
(18)		0.93	26.8	5.7	4.2
(19)		3.9	74.3	15.4	14.7
(20)		1.0 (-42±3)	58.5 (-12±4)	16.5 (-18±3)	6.2 (+12±4)
(21)		0.44 (-45±5)	17.5	2.9	1.7
(22)		0.91	42.9	29.2	4.1

[a–c] Footnotes as for Table 1.

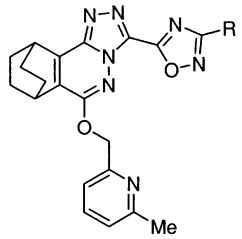
significant enhancement in performance at 1.0 mg/kg after oral dosing.[43] In addition, like **44**, **43** was shown to have a behaviorally benign profile in rodents with no convulsant and only weak proconvulsant effects.[44]

These data provide further support for the proposal that the different behavioral effects of benzodiazepines are mediated by different GABA$_A$ receptor subunits and that a compound acting specifically at the GABA$_A$α5 receptor subunit may be useful for the treatment of cognitive disorders, e.g., Alzheimer's disease, without the side-effects associated with nonselective GABA$_A$α5 receptor inverse agonists.

Conclusion

A novel series of high affinity GABA$_A$α5 inverse agonists having binding and/or functional selectivity over the other GABA$_A$ receptor subtypes was developed from a screening lead from the Merck sample collection. Incorporation of heterocycles into the 3-position of a series of 7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolophthalazines was shown to give GABA$_A$ receptor inverse agonists. The nature of the heterocycle was shown to be important for determining functional selectivity for the GABA$_A$α5 subtype and in particular a furan-3-yl or 5-methylisoxazol-3-yl group was found to be optimal for selectivity. Modifications of the 1,2,4-triazolophthalazine ring determined the requirements for binding selectivity and the 7,8,9,10-tetrahydro-(7,-10-ethano)-1,2,4-triazolophthalazine ring was shown to give excellent binding selectivity. Determinants for oral bioavailability were identified which led to the orally bioavailable GABA$_A$α5 receptor inverse agonist 3-(5-methylisoxazol-3-yl)-6-(2-pyridyl)methyloxy-1,2,4-triazolo[3,4-*a*]phthalazine (**43**). Clinical studies with compounds such as **43** may provide new therapies for Alzheimer's disease with the potential for having a greater therapeutic window and fewer side effects than to currently marketed drugs.

Table 3. Binding Affinity of 3-Substituted-1,2,4-oxadiazoles at the $GABA_A$ Receptor Subtypes. Changes to the Oxadiazole Substituent



		K_i (nM) GABA-A$\alpha x\beta 3\gamma 2$ receptors [a]			
Compd.[b]	R	α5	α1	α2	α3
(23)	Et	3.50	171.0	34.0	21.0
(24)		6.5	>1000	68.4	83.9
(25)		19.2	>1000	203.3	226.9
(26)		3.3	>1000	166.3	69.6
(27)		2.1	>1000	208.8	26.4

[a,b] Footnotes as for Table 1.

Experimental Section

Chemical Methods. General Procedures. All reactions were carried out under a positive pressure of nitrogen. Glassware for water-sensitive reactions were dried in an oven at 120 °C overnight. Melting points are reported uncorrected. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Brucker AM360 or AC250 spectrometers. Deuterated chloroform (99.8%D) or DMSO-d_6 (99.9%D) were used as solvents. Chemical shift values (δ), from Me_4Si as internal standard, are expressed in ppm and coupling constants (J) in hertz. Mass spectra were obtained with a VG Quattro spectrometer operating in electrospray positive ion mode. Anhydrous solvents were purchased from the Aldrich Chemical Co. Organic solutions were dried over Na_2SO_4 or $MgSO_4$. Flash chromatography was performed on silica gel Fluka Art. No. 60738. Thin-layer chromatography (TLC) was carried out on Merck 5 cm × 10 cm plates with silica gel 60 F_{254} as sorbant. Microanalyses were determined by Butterworth Laboratories, 54-56 Waldegrave Road, Teddington, UK. Commerically available starting materials were used as supplied.

4,5-Diazatricyclo[6.2.2.2,7]dodec-2(7)-ene-3,6-dione (2a). Bicyclo[2.2.2]oct-2-ene-2,3-dicarboxylic acid anhydride (**1a**)[37] (60.8 g, 0.342 mol) was dissolved in 50% aqueous acetic acid (1600 mL) with sodium acetate trihydrate (55.5 g, 1.2 mol equiv) and hydrazine hydrate (19.82 mL, 1.2 mol equiv). The reaction mixture was heated under reflux for 16 h then allowed to cool. The solid produced was collected by filtration and washed with water and diethyl ether before drying in a vacuum oven at 80 °C to give 59.3 g (90.4%) of **2a**: Mp 214−215 °C; δ_H (250 MHz, DMSO-d_6): 1.16 (4H, d, J 7.1), 1.69 (4H, d, J 7.1), 3.18 (2H, s), 11.31 (2H, br s, NH); m/z (ES^+) 193 ($M + H^+$).

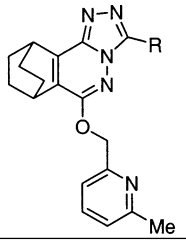
3,6-Dichloro-4,5-diazatricyclo[6.2.2.2,7]dodeca-2(7),3,5-triene (3a). 4,5-Diazatricyclo[6.2.2.*2,7*]dodec-2(7)-ene-3,6-dione (59.2 g, 0.308 mmol)) was dissolved in phosphorus oxychloride (300 mL) and heated under reflux for 14 h. The solvent was removed under vacuum and azeotroped 2× with toluene. The residue was dissolved in dichloromethane (200 mL) and stirred rapidly, and the solution was neutralized by the addition of solid and aqueous sodium hydrogen carbonate (cautiously). When effervescence had ceased, the organic layer was separated and the aqueous layer was extracted with dichloromethane (2 × 200 mL). The combined organic layers were dried ($MgSO_4$), filtered, and evaporated to give 59.5 g (84%) of **3a**: Mp > 370 °C; δ_H (250 MHz, $CDCl_3$): 1.39 (4 H, d, J 8.1), 1.92 (4 H, d, J 8.1), 3.47 (2 H, s); m/z (ES^+) 229 ($M + H^+$).

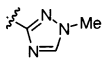
3-Chloro-6-hydrazino-4,5-diazatricyclo[6.2.2,7]dodeca-2(7),3,5-triene (4a). 3a (40.0 g, 0.175 mol) was added to a stirred solution of hydrazine monohydrate (56.8 g, 1.13 mol) in ethanol (600 mL) and the solution refluxed for 18 h. The mixture was cooled to room temperature and the solvent removed in vacuo. Water (150 mL) was added to the residue and the mixture acidified to pH 1−2 with 5 N hydrochloric acid. The aqueous was extracted with dichloromethane (×3) and then basified with powdered K_2CO_3 and extracted with dichloromethane (×3). The combined dichloromethane was dried ($MgSO_4$) and evaporated to give **4a** (29.0 g, 74%): Mp 136−140 °C; δ_H (250 MHz, $CDCl_3$): 1.27−1.45 (4H, m), 1.79−1.91 (4H, m), 3.05 (1H, s), 3.40 (1H, s), 3.94 (2H, br s), 6.18 (1H, br s); m/z (ES^+) 225 ($M + H^+$).

6-Chloro-3-(3-methyl-1,2,4-oxadiazol-5-yl)-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (5a). (a) Pyridine (32.7 mL, 0.404 mol) was added dropwise to a stirred suspension of acetamide oxime (10.0 g, 0.135 mol), in 1,2-dichloroethane (500 mL), at room temperature, under nitrogen. The mixture was cooled to 0 °C and ethyl oxalyl chloride (22.6 mL, 0.202 mol) added dropwise over 0.2 h. After stirring at 0 °C for 0.1 h, the mixture was warmed to room temperature for 0.3h and then to 80 °C and stirred for 2.1 h. The mixture was diluted with dichloromethane (200 mL) and washed with 2 N HCl (100 mL), water (2 × 100 mL), and brine (100 mL). The solution was dried ($MgSO_4$) and evaporated in vacuo to give ethyl [3-(methyl)-1,2,4-oxadiazol-5-yl)]carboxylate **(7a)** (20.05 g, 95%); NMR δ_H (250 MHz, $CDCl_3$): 1.46 (3H, t, J 7.2), 2.52 (3H, s), 4.55 (2H, q, J 7.2).

(b) **7a** (0.987 g, 6.32 mmol) was added to a solution of **4a** (1.18 g, 5.3 mmol) in anhydrous dioxane (28 mL) and the reaction mixture stirred at reflux for 3 days. The solvent was

Table 4. Binding Affinity and Efficacy of 3-Heterocycyl-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-a]phthalazines at the GABA$_A$ Receptor Subtypes



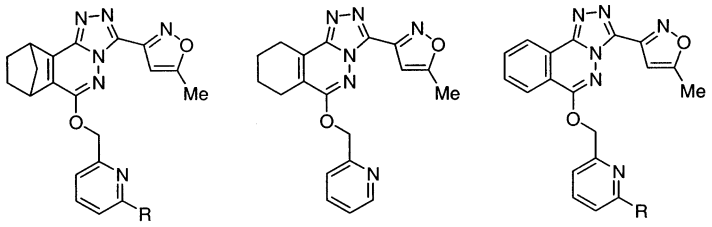
		K_i (nM) GABA-Aαxβ3γ2 receptors[a] (%Efficacy[b])			
Compd.[c]	R	α5	α1	α2	α3
(28)		3.1 (-24±4)	250 (+27±5)	63 (-5±2)	40 (-4 ±6)
(29)		13.0	1,000	275	115.7
(30)		3.7 (+7±1)	270	110	48
(31)		4.0	115	66	22
(32)		4.6 (-29±4)	280 (+21±7)	86 (+14±2)	41 (-1±3)
(33)		6.8	280	57	46
(34)		58	>1,000	>1,000	>1,000
(35)		213.7	>1,000	>1,000	>1,000
(36)		253	>1,000	>1,000	>1,000
(37)		15.3	518	227.3	104
(38)		1.8 (-9±3)	84.0 (+12±7)	28.4 (+8±3)	10.6 (+19±6)

[a–c] Footnotes as for Table 1.

removed under vacuum, and the residue was partitioned between dichloromethane (120 mL) and water (30 mL). The dichloromethane was separated, dried ($MgSO_4$), and evaporated in vacuo and the residue chromatographed on silica gel eluting with 85:15 CH_2Cl_2−ethyl acetate to give **5a** (0.32 g, 19%); δ_H (360 MHz, $CDCl_3$): 1.42−1.57 (4H, m), 1.95−2.08 (4H, m), 2.62 (3H, s), 3.64 (1H, s), 4.13 (1H, s); *m/z* (ES^+) 317 ($M + H^+$).

3-(3-Methyl-1,2,4-oxadiazol-5-yl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (14). To a solution of 6-methyl-2-pyridylcarbinol hydrochloride (0.275 g, 1.72 mmol), in DMF (10 mL), was added NaH (0.138 g of 60% dispersion in oil, 3.45 mmol), and the mixture was stirred at room temperature for 0.25 h. **5a** (0.227 g, 0.72 mmol) was added and the mixture stirred at room temperature for 2 h and then at 60 °C for 15 h. The solvent was removed under vacuum and the residue partitioned between water (20 mL) and ethyl acetate (100 mL). The aqueous was extracted further with ethyl acetate (×2), and the combined organic layers were dried ($MgSO_4$) and evaporated. The residue was triturated with ethyl acetate to give **14** (0.16 g, 56%); Mp 191−193 °C; δ_H (360 MHz, $CDCl_3$): 1.38−1.56 (4H, m), 1.86−2.03 (4H, m), 2.60 (3H, s), 2.60 (3H, s), 3.62 (1H, s), 4.02 (1H, s), 5.62 (2H, s), 7.14 (1H, d, *J* 7.7), 7.49 (1H, d, *J* 7.7), 7.65 (1H, dd, *J* 7.7 and 7.7); *m/z* (ES^+) 404 ($M + H^+$); Anal. ($C_{21}H_{21}N_7O_2 \cdot 0.2H_2O$): C, H, N.

Table 5. Binding Affinity and Efficacy of 5-Methylisoxazoles at the $GABA_A$ Receptor Subtypes



39 R = H
40 R = Me

41

42 R = Me
43 R = H

	K_i (nM) GABA-A$\alpha x\beta 3\gamma 2$ receptors[a] (%Efficacy[b])			
Compd.[c]	α5	α1	α2	α3
(39)	5.5 (-13±1)	57.7 -	19.7 -	17.6 (+0.6±4)
(40)	8.9 (-24±2)	147.3	38.4	33.5 (+11±4)
(41)	0.75 (-23±4)	4.2 (+0.4±4)	3.5 (-6±2)	1.6 (-15±3)
(42)	0.79 (-15±8)	0.88 (+15±5)	1.6 (+12±3)	1.3 (+3±3)
(43)	0.80 (-46±2)	1.4 (-2±1)	2.7 (+15±4)	1.4 (-4±3)

[a–c] Footnotes as for Table 1.

Table 6. Efficacy of **11** and **43** in L(tk−) Cells Expressing Human Recombinant $GABA_A$ Receptor Subtypes

	efficacy of $GABA_A$ $\alpha x\beta 3\gamma 2$ receptors (%)[a]			
compd[c]	α_5	α_1	α_2	α_3
(**11**)	−21 ± 3	−18 ± 3	−26 ± 2	−11 ± 4
(**43**)	−45 ± 3	−14 ± 4	−7 ± 3	−17 ± 5

[a] Maximum modulation of the current produced relative to a submaximal (EC_{20}) GABA response measured using whole cell patch clamp recording. Values are the arithmetic mean ± SEM from at least five individually fitted concentration−response curves. Each point represents data from a minimum of five cells.

Compounds **15**, **16**, **18**, and **20**−**22** were prepared from **5a** and the appropriate carbinol using the general procedure given for the preparation of **14**:

3-(3-Methyl-1,2,4-oxadiazol-5-yl)-6-(2-pyridyl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (15). δ_H (360 MHz, $CDCl_3$): 1.40−1.53 (4H, m), 1.89−2.00 (4H, m), 2.60 (3H, s), 3.61 (1H, s), 4.02 (1H, s), 5.66 (2H, s), 7.29 (1H, m), 7.72 (1H, t, *J* 7.8), 7.77 (1H, dd, *J* 7.6 and 6.1), 8.64 (1H, m); *m*/*z* (ES^+) 390 ($M + H^+$); Anal. ($C_{20}H_{19}N_7O_2 \cdot 0.1H_2O$): C, H, N.

6-(5,6-Dimethylpyridin-2-yl)methyloxy-3-(3-methyl-1,2,4-oxadiazol-5-yl)-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (16). Compound **16** was prepared from **5a** and 2-hydroxymethyl-5,6-dimethylpyridine (WO 93/21158) using the general procedure: Mp 139−141 °C; δ_H (250 MHz, $CDCl_3$): 1.38−1.60 (4H, m), 1.86−2.00 (4H, m), 2.31 (3H, s), 2.55 (3H, s), 2.61 (3H, s), 3.60 (1H, br s), 4.01 (1H, br s), 5.61 (2H, s), 7.48 (2H, s); *m*/*z* (ES^+) 418 ($M + H^+$).

3-(3-Methyl-1,2,4-oxadiazol-5-yl)-6-(3-pyridyl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (18). Yield 43%. Mp 167.5−168 °C; δ_H (360 MHz, $CDCl_3$): 1.36−1.52 (4H, m), 1.89−2.00 (4H, m), 2.63 (3H, s), 3.53 (1H, br s), 4.01 (1H, br s), 5.59 (2H, s), 7.37 (1H, dd, *J* 7.6 and 5.0), 8.02 (1H, br d, *J* 7.9), 8.62 (1H, d, *J* 5.0), 8.90 (1H, s); *m*/*z* (ES^+) 390 ($M + H^+$); Anal. ($C_{20}H_{19}N_7O_2$): C, H, N.

6-(2-Bromophenyl)methyloxy-3-(3-methyl-1,2,4-oxadiazol-5-yl)-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo-[3,4-*a*]phthalazine (20). Mp 217−218 °C; δ_H (360 MHz, $CDCl_3$): 1.38−1.53 (4H, m), 1.88−1.99 (4H, m), 2.61 (3H, s), 3.57 (1H, br s),4.01 (1H, br s), 5.63 (2H, s), 7.25 (1H, dt, *J* 1.8 and 7.6), 7.35 (1H, m), 7.64 (1H, dd, *J* 1.1 and 7.9), 7.74 (1H, dd, *J* 1.4 and 7.6); *m*/*z* (ES^+) 467 ($M + H^+$).

6-[2-(Cyano)phenyl]methyloxy-3-(3-methyl-1,2,4-oxadiazol-5-yl)-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo-[3,4-*a*]phthalazine (21). Mp 197−198 °C; δ_H (360 MHz, $CDCl_3$): 1.42−1.53 (4H, m), 1.90−1.99 (4H, m), 2.62 (3H, s), 3.60 (1H, br s), 4.01 (1H, br s), 5.73 (2H, s), 7.49 (1H, t, *J* 7.6), 7.64 (1H, dt, *J* 1.1 and 7.6) 7.76 (1H, d, *J* 7.6), 7.87 (1H, d, *J* 7.6); *m*/*z* (ES^+) 414 ($M + H^+$); Anal. ($C_{22}H_{19}N_7O_2$): C, H, N.

3-(3-Methyl-1,2,4-oxadiazol-5-yl)-6-(2-quinolino)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]-phthalazine (22). Mp 216−218 °C; δ_H (360 MHz, $CDCl_3$): 1.43−1.55 (4H, m), 1.92−2.02 (4H, m), 2.59 (3H, s), 3.65 (1H, br s), 4.03 (1H, br s), 5.84 (2H, s), 7.58 (1H, t, *J* 7.6), 7.73−7.86 (3H, m), 8.11 (1H, d, *J* 8.6), 8.24 (1H, d, *J* 8.3); *m*/*z* (ES^+) 440 ($M + H^+$); Anal. ($C_{24}H_{21}N_7O_2 \cdot 0.3H_2O$): C, H, N.

6-(2-Imidazolyl)methyloxy-3-(3-methyl-1,2,4-oxadiazol-5-yl)-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (17). (a) Sodium borohydride (0.42 g, 11 mmol) was added to a stirred solution of 1-{[2-(trimethylsilyl)ethoxy]-methyl}-1*H*-imidazole-2-carboxaldehyde (7.45 g, 33 mmol) (prepared as described in *J. Org. Chem.* **1986**, 51, 1891) in methanol (30 mL) at 0 °C. The solution was stirred at 0 °C for 0.67 h, brine (15 mL) added, and the mixture stirred at room temperature for 0.25h. The methanol was evaporated in vacuo and the aqueous solution washed with ethyl acetate (3 × 50 mL). The combined organic layers were dried (Na_2SO_4) and evaporated in vacuo to yield an oil which crystallized at 0 °C. The solid was washed and recrystallized from hexane to give 2-(hydroxymethyl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-imidazole (1.99 g, 26%); δ_H (250 MHz, $CDCl_3$): 0.00 (9H, s), 0.93 (2H, t, *J* 8.2), 3.54 (2H, t, *J* 8.2), 4.73 (2H, s), 4.77 (1H, br

s), 5.39 (2H, s), 6.94 (1H, d, *J* 1.4), 7.00 (1H, d, *J* 1.4); *m/z* (ES^+) 229 ($M + H^+$).

(b) 2-(Hydroxymethyl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-imidazole was reacted with the chlorophthalazine **5a** using the general procedure given for compound **14** to give 3-(3-methyl-1,2,4-oxadiazol-5-yl)-6-[2-{1-[2-(trimethylsilyl)ethoxy]methyl}-imidazolyl]methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine; (360 MHz, $CDCl_3$): δ_H 0.00 (9H, s, $SiMe_3$), 0.91 (2H, t, *J* 8.1), 1.40−1.56 (4H, m), 1.89−2.02 (4H, m), 2.62 (3H, s), 3.53−3.58 (3H, m), 4.05 (1H, br s), 5.50 (2H, s), 5.72 (2H, s), 7.14 (1H, d, *J* 1.4), 7.16 (1H, d, *J* 1.4); *m/z* (ES^+) 509 ($M + H^+$).

(c) A stirred solution of the preceding product (355 mg, 0.698 mmol) in 5 N HCl (14 mL) was heated at 40 °C for 2.3 h. Ethanol was added and the mixture evaporated in vacuo. The residue was azeotroped with ethanol (×2), partitioned between dichloromethane and water, and basified with saturated potassium carbonate solution. The organic layer was separated and the aqueous phase re-extracted with dichloromethane (×1). The combined organic extracts were washed with water (×1), dried ($MgSO_4$), and evaporated in vacuo, and the residue was recrystallized from ethyl acetate−hexane to give **17** (0.19 g, 72%); Mp 187 °C; δ_H (360 MHz, $CDCl_3$): 1.36−1.50 (4H, m), 1.88−2.00 (4H, m), 2.66 (3H, s), 3.56 (1H, br s), 4.01 (1H, br s), 5.58 (2H, br s), 7.10 (2H, s); *m/z* (ES^+) 379 ($M + H^+$); Anal. ($C_{18}H_{18}N_8O_2 \cdot 0.05H_2O$): C, H, N.

3-(3-Methyl-1,2,4-oxadiazol-5-yl)-6-(2-pyridyl-*N*-oxide)-methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo-[3,4-*a*]phthalazine (19). *m*-Chloroperbenzoic acid (62 mg of a 55% purity sample, 0.20 mmol) was added to a stirred solution of **15** (43 mg, 0.11 mmol) in dichloromethane (3 mL) at room temperature. After stirring for 17 h, the mixture was diluted with dichloromethane, washed with saturated potassium carbonate solution (×2) and water (×1), dried ($MgSO_4$), and evaporated in vacuo. The residue was chromatographed on silica gel, eluting with 95:15 CH_2Cl_2−methanol, and then recrystallized from ethyl acetate/hexane to give **19** (21 mg, 47%); Mp 225−227 °C; δ_H (360 MHz, $CDCl_3$): 1.42−1.54 (4H, m), 1.92−2.02 (4H, m), 2.60 (3H, s), 3.62 (1H, br s), 4.03 (1H, br s), 5.85 (2H, s), 7.31−7.33 (2H, m), 7.86 (1H, m), 8.33 (1H, m); *m/z* (ES^+) 406 ($M + H^+$); Anal. ($C_{20}H_{19}N_7O_3 \cdot H_2O$): C, H, N.

Compounds **23**−**25** were prepared from hydrazine **4a** and the appropriate 1,2,4-oxadiazole carboxylic ethyl ester **7b**−**d** using the general procedures given for the preparation of **14**:

3-(3-Ethyl-1,2,4-oxadiazol-5-yl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (23). Mp 169−171 °C; δ_H (360 MHz, $CDCl_3$): 1.40−1.54 (7H, m), 1.90−2.00 (4H, m,), 2.60 (3H, s), 2.97 (2H, q, *J* 7.6), 3.61 (1H, br s), 4.01 (1H, br s), 5.62 (2H, s), 7.14 (1H, d, *J* 7.9), 7.49 (1H, d, *J* 7.6), 7.64 (1H,t, *J* 7.6); *m/z* (ES^+) 418 ($M + H^+$); Anal.($C_{22}H_{23}N_7O_2$): C, H, N.

3-(3-Isopropyl-1,2,4-oxadiazol-5-yl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (24). Mp 185−187 °C; δ_H (360 MHz, $CDCl_3$): 1.40−1.52 (10H, m), 1.88−2.00 (4H, m), 2.60 (3H, s), 3.30 (1H, septet, *J* 6.8), 3.61 (1H, br s), 4.01 (1H, br s), 5.62 (2H, s), 7.14 (1H, d, *J* 7.6), 7.50 (1H, d, *J* 7.6), 7.63 (1H, t, *J* 7.8); *m/z* (ES^+) 432 ($M + H^+$); Anal. ($C_{23}H_{25}N_7O_2$): C, H, N.

3-(3-Phenyl-1,2,4-oxadiazol-5-yl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (25). δ_H (360 MHz, $CDCl_3$): 1.36−1.62 (4H, m), 1.87−2.08 (4H, m), 2.60 (3H, s), 3.61 (1H, br s), 4.04 (1H, br s), 5.64 (2H, s), 7.14 (1H, d, *J* 7.6), 7.48−7.66 (5H, m), 8.24−8.32 (2H, m); *m/z* (ES^+) 466 ($M + H^+$).

6-(6-Methylpyridin-2-yl)methyloxy-3-[3-(pyridin-3-yl)-1,2,4-oxadiazol-5-yl]-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (26). (a) General Procedure for the Synthesis of 6b−**f**: **3-Pyridylcarboxamide Oxime (6e).** A solution of 3-cyanopyridine (10.0 g, 96 mmol) in ethanol (100 mL) was added to a stirred slurry of hydroxylamine hydrochloride (13.3 g, 192 mmol) and potassium carbonate (15.9 g, 120 mmol) in ethanol (150 mL). The mixture was heated at reflux for 16 h and then cooled to room temperature and filtered through a pad of Celite. The solution was evaporated in vacuo to give **6e** as a brown oil and as a mixture of geometric isomers: δ_H (360 MHz, DMSO-d_6): 5.97 (2H, br s), 7.40 (0.55H, m), 7.50 (0.45H, m), 8.02 (0.5H, m), 8.20 (0.5H, m), 8.56 (0.55H, m), 8.70 (0.45H, m), 8.86 (0.55H, m), 9.04 (0.45H, m), 9.77 (1H, br s).

(b) General procedure for the Synthesis of 7b−**f**: **Ethyl [3-(Pyridin-3-yl)-1,2,4-oxadiazol-5-yl]carboxylate (7e).** A mixture of **6e** (3.0 g, 22 mmol) and 4 Å molecular sieves in THF (120 mL) was stirred vigorously at room temperature under nitrogen. Sodium hydride (0.96 g of a 60% dispersion in oil, 24 mmol) was added, the mixture stirred for 0.1 h, and then ethyl oxalyl chloride (3.0 g, 22 mmol) added. The mixture was heated at reflux for 1 h, cooled to room temperature, and filtered through Celite. The solvent was evaporated in vacuo and the residue dissolved in CH_2Cl_2, washed with water (×2), dried ($MgSO_4$), and evaporated in vacuo. The residue was chromatographed on silica gel, eluting with ethyl acetate−hexane 50:50 to give **7e** (1.30 g, 28%), δ_H (360 MHz, $CDCl_3$): 1.51 (3H, t, *J* 7.0), 4.59 (2H, q, *J* 7.1), 7.47 (1H, m,), 8.43 (1H, m), 8.78 (1H, m), 9.39 (1H, m).

(c) 6-(6-Methylpyridin-2-yl)methyloxy-3-[3-(pyridin-3-yl)-1,2,4-oxadiazol-5-yl]-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (26). Prepared from the preceding ethyl ester **7e** and hydrazine **4a** using the general procedure described for **14**: Mp 202−204 °C; δ_H (250 MHz, $CDCl_3$): 1.42−1.58 (4H, m), 1.90−2.08 (4H, m), 2.61 (3H, s), 3.64 (1H, br s), 4.05 (1H, br s), 5.67 (2H, s), 7.16 (1H, d, *J* 7.5), 7.49−7.52 (2H, m), 7.65 (1H, t, *J* 7.6), 8.56 (1H, m), 8.80 (1H, m), 9.51 (1H, br s); *m/z* (ES^+) 467 ($M + H^+$); Anal. ($C_{25}H_{22}N_8O_2 \cdot 1.2H_2O \cdot 1.5HCl$): C, H, N.

6-(6-Methylpyridin-2-yl)methyloxy-3-[3-(pyridin-4-yl)-1,2,4-oxadiazol-5-yl]-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (27). Prepared from ethyl ester **7f** and **4a** using the general procedure: Mp 272−274 °C; δ_H (250 MHz, $CDCl_3$): 1.42−1.58 (4H, m), 1.90−2.05 (4H, m), 2.61 (3H, s), 3.64 (1H, br s), 4.05 (1H, br s), 5.67 (2H, s), 7.16 (1H, d, *J* 7.8), 7.49 (1H, d, *J* 7.8), 7.64 (1H, d, *J* 7.7), 8.13−8.16 (2H, m), 8.84−8.86 (2H, m); *m/z* (ES^+) 467 ($M + H^+$); Anal. ($C_{25}H_{22}N_8O_2$): C, H, N.

General Procedure for the Preparation of 8a−**c. 6-Chloro-3-(3-furyl)-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (8a).** To a solution of **4a** (1.0 g, 4.45 mmol), in xylene (20 mL), were added triethylamine (0.67 g, 6.7 mmol) and 3-furoyl chloride (0.58 g, 4.4 mmol). The mixture was stirred at room temperature for 1 h and then heated at reflux for 16h. The solution was cooled to room temperature, the solvent evaporated under reduced pressure, and the residue partitioned between CH_2Cl_2 (150 mL) and water (30 mL). The aqueous phase was separated and extracted further with CH_2Cl_2 (×2). The combined extracts were dried (Na_2SO_4) and evaporated, and the residue was chromatographed on silica gel eluting with ethyl acetate to afford **8a** (0.75 g, 56%); δ_H (250 MHz, $CDCl_3$): 1.41−1.55 (4H, m, 2), 1.90−2.05 (4H, m, 2), 3.57 (1H, s), 4.04 (1H, s), 7.30−7.31 (1H, m), 7.59−7.60 (1H, m), 8.61 (1H, s); *m/z* (ES^+) 301 ($M + H^+$).

Compounds **28**−**30** were prepared from **8a**−**c**, respectively, and 6-methyl-2-pyridylcarbinol as described for **14**:

3-(3-Furyl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (28). Mp 205−206 °C; δ_H (250 MHz, $CDCl_3$): 1.38−1.56 (4H, m), 1.84−2.02 (4H, m), 2.63 (3H, s), 3.58 (1H, s), 3.96 (1H, s), 5.58 (2H, s), 7.14 (1H, d, *J* 7.6), 7.26 (1H, s), 7.31 (1H, d, *J* 7.7), 7.55−7.56 (1H, m), 7.64 (1H, dd, *J* 7.7 and 7.6), 8.50 (1H, s); *m/z* (ES^+) 388 ($M + H^+$); Anal. ($C_{22}H_{21}N_5O_2 \cdot 0.25\ H_2O$): C, H, N.

3-(2-Furyl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (29). δ_H (360 MHz, $CDCl_3$): 1.40−1.56 (4H, m), 1.86−2.00 (4H, m), 2.62 (3H, s), 3.56 (1H, br s), 3.96 (1H, br s), 5.58 (2H, s), 6.58−6.62 (1H, m) 7.12 (1H, d, *J* 7.6), 7.32 (1H, d, *J* 7.6), 7.43 (1H, d, *J* 1.8), 7.60−7.68 (2H, m); *m/z* (ES^+) 388 ($M + H^+$); Anal. ($C_{22}H_{21}N_5O_2$): C, H, N.

3-(3-Thiophenyl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (30). δ_H (250 MHz, $CDCl_3$): 1.39−1.57 (4H, m), 1.86−2.00 (4H, m), 2.63 (3H, s), 3.57 (1H, s), 3.96 (1H, s), 5.58 (2H, s), 7.14 (1H, d, *J* 7.6), 7.26 (1H, s), 7.30 (1H, d, *J* 7.7), 7.55−7.56 (1H, m), 7.63 (1H, dd, *J* 7.7 and 7.6), 8.40 (1H, s); *m*/*z* (ES^+) 404 ($M + H^+$); Anal. ($C_{22}H_{21}N_5OS$): C, H, N.

3-(2-Thiophenyl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo-[3,4-*a*]phthalazine (31). To a solution of **3** (2.0 g, 87.3 mmol), in xylene (50 mL), was added triethylamine (0.88 g, 87.1 mmol) and 2-thiophene carboxylic hydrazide (1.24 g, 87.3 mmol). The mixture was stirred at room temperature for 1 h and then heated at reflux for 3 days. The solution was cooled to room temperature and filtered and the solvent evaporated under reduced pressure. The residue was chromatographed on silica gel eluting with CH_2Cl_2−MeOH 96:4 to afford **8d** (0.17 g, 6.2%); *m*/*z* (ES^+) 317 ($M + H^+$). To a solution of 6-methyl-2-pyridylcarbinol (0.10 g, 0.80 mmol), in DMF (10 mL), was added NaH (0.045 g of 60% dispersion in oil, 1.1 mmol), and the mixture was stirred at room temperature for 0.25 h. **8d** (0.17 g, 0.54 mmol) was added and the mixture stirred at room temperature for 2 h. The reaction mixture was poured into water, and the resultant solid product was filtered off and recrystallized from ethyl acetate to give **31** (0.05 g, 23%); δ_H (250 MHz, $CDCl_3$): 1.40−1.56 (4H, m), 1.86−2.03 (4H, m), 2.64 (3H, s), 3.58 (1H, s), 3.98 (1H, s), 5.64 (2H, s), 7.12−7.24 (2H, m), 7.37 (1H, d, *J* 7.7), 7.48 (1H, dd, *J* 6.5 and 1.5); 7.68 (1H, m); 8.19 (1H, dd, *J* 5.5 and 1.5); *m*/*z* (ES^+) 404 ($M + H^+$); Anal. ($C_{22}H_{21}N_5OS \cdot 0.25H_2O$): C, H, N.

General Procedure for the Synthesis of 32−38: 3-(5-Methylisoxazol-3-yl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (32). (a) A mixture of acetonylacetone (10 g, 88 mmol) and nitric acid (sp. gr. 1.42)/water (2:3) (50 mL) was cautiously brought to reflux under a stream of nitrogen and boiled for 1 h. The solution was cooled to room temperature and aged overnight. The resultant solid was collected by filtration, washed with chilled water (2 × 7 mL) and hexane, and dried in vacuo to give 5-methylisoxazole-3-carboxylic acid (4.4 g, 40%), δ_H (250 MHz, $CDCl_3$) 2.50 (3H, d, *J* 0.8), 6.41 (1H, d, *J* 0.8).

(b) 1,1′-Carbonyldiimidazole (2.0 g, 12.6 mmol) was added to a stirred solution of 5-methylisoxazole-3-carboxylic acid in DMF (50 mL). The solution was stirred for 0.5 h before adding **4a** (2.63 g, 11.7 mmol). After 1 h at room temperature, the solution was poured into water, and the resultant precipitate was filtered, washed with water (30 mL) and hexane (100 mL), and dried in vacuo to give the ketohydrazine (3.1 g, 79%). A solution of the ketohydrazine (1.0 g, 3.0 mmol) and triethylamine hydrochloride (0.2 g, 1.45 mmol), in xylene (30 mL), was heated at reflux for 16 h. The solution was cooled to room temperature and the solvent removed in vacuo. The residue was chromatographed on silica gel, eluting with ethyl acetate, to give **8e** (0.60 g, 63%): Mp 186−188 °C; δ_H (250 MHz, $CDCl_3$) 1.22−1.54 (4H, m), 1.94−2.06 (4H, m), 2.58 (3H, s), 3.60 (1H, s), 4.08 (1H, s), 6.90 (1H, s); *m*/*z* (ES^+) 316 ($M + H^+$).

(c) To a solution of 6-methyl-2-pyridylcarbinol (0.47 g, 3.8 mmol), in DMF (50 mL), was added sodium hydride (0.15 g of a 60% dispersion in oil, 3.8 mmol), and the mixture was stirred at room temperature for 0.25 h. After this time, **8e** (1.0 g, 3.2 mmol) was added and the reaction mixture stirred at 55 °C for 16 h. The solvent was removed under vacuum and the residue partitioned between ethyl acetate and water. The aqueous was separated and extracted further with ethyl acetate (×3). The combined extracts were dried (Na_2SO_4/$MgSO_4$) and evaporated, and the residue was chromatographed on silica gel eluting with ethyl acetate to give **32** (0.35 g, 28%): Mp 223−225 °C; δ_H (250 MHz $CDCl_3$) 1.38−1.54 (4H, m), 1.84−1.98 (4H, m), 2.58 (3H, s), 2.60 (3H, s), 3.58 (1H, s), 3.98 (1H, s), 5.59 (2H, s), 6.85 (1H, s), 7.14 (1H, d, *J* 7.7), 7.44 (1H, d, *J* 7.6), 7.64 (1H, dd, *J* 7.7 and 7.6); *m*/*z* (ES^+) 403 ($M + H^+$). Anal. ($C_{22}H_{22}N_6O_2 \cdot 0.3H_2O$): C, H, N.

3-(2-Methyloxazol-4-yl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]-phthalazine (33). (a) Triethylamine (8.2 mL, 59 mmol) was added to a stirred mixture of L-serine ethyl ester hydrochloride (5.00 g, 29.5 mmol) and ethyl acetimidate hydrochloride (3.64 g, 29.5 mmol) in dichloromethane (150 mL) at 0 °C under nitrogen. The mixture was stirred at 0 °C for 1.25 h and at room temperature for 19 h and then partitioned between dichloromethane and water. The organic layer was separated, washed with water (×3), dried ($MgSO_4$), and evaporated in vacuo and the residue chromatographed on silica gel, eluting with diethyl ether, to give ethyl (2-methyl-2-oxazolin-4-yl)-carboxylate (2.68 g, 37%), δ_H (360 MHz, $CDCl_3$) 1.31 (3H, t, *J* 7.0), 2.04 (3H, s), 4.16−4.51 (4H, m), 4.70 (1H, m); *m*/*z* (ES^+) 158 ($M + H^+$).

(b) A stirred mixture ethyl(2-methyl-2-oxazolin-4-yl)carboxylate (2.18 g, 13.9 mmol) and *N*-bromosuccinimide (3.70 g, 20.8 mmol) in dichloromethane (90 mL) at −15 °C under nitrogen was irradiated for 7 h. A second portion of *N*-bromosuccinimide (0.45 g, 2.5 mmol) was added and the mixture irradiated for a further 4 h. The reaction mixture was filtered and the filtrate diluted with dichloromethane, washed with water (×3), dried ($MgSO_4$), and evaporated in vacuo. The residue was chromatographed on silica gel, eluting with diethyl ether−hexane 70:30 to give ethyl (2-methyloxazol-4-yl)carboxylate (0.95 g, 44%), δ_H (250 MHz, $CDCl_3$) 1.38 (3H, t, *J* 7.3), 2.52 (3H, s), 4.39 (2H, *J* 7.3), 8.13 (1H, s); *m*/*z* (ES^+) 156 ($M + H^+$).

(c) A solution of sodium hydroxide (0.97 g, 24 mmol) in water (10 mL) was added to a stirred solution of ethyl (2-methyloxazol-4-yl)carboxylate (0.94 g, 6.1 mmol) in methanol (6 mL). After 1.25 h at room temperature, the methanol was evaporated in vacuo and the aqueous solution cooled to 0−5 °C and acidified to pH 1 with 5 N HCl. Ethanol was added, the solvents were evaporated in vacuo, and the residue was azeotroped with ethanol. The resulting solid was mixed with ethanol, filtered, and evaporated in vacuo. The process was repeated to give 2-methyloxazole-4-carboxylic acid (0.776 g, 100%), δ_H (360 MHz, DMSO-d_6) 2.44 (3H, s), 8.60 (1H, s); *m*/*z* (ES^+) 128 ($M + H^+$).

(d) Compound **33** was prepared from 2-methyloxazole-4-carboxylic acid and **4a** using the general procedure: Mp 195−197 °C; δ_H (360 MHz, $CDCl_3$): 1.42−1.54 (4H, m), 1.90−1.98 (4H, m), 2.59 (3H, s), 2.64 (3H, s), 3.58 (1H, br s), 4.00 (1H, br s), 5.60 (2H, s), 7.15 (1H, d, *J* 7.6), 7.32 (1H, d, *J* 7.6), 7.65 (1H, dd, *J* 7.6 and 7.6), 8.56 (1H, s); *m*/*z* (ES^+) 403 ($M + H^+$); Anal. ($C_{22}H_{22}N_6O_2$): C, H, N.

3-(2-Methylthiazol-4-yl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (34). δ_H (360 MHz, $CDCl_3$): 1.40−1.52 (4H, m), 1.88−2.02 (4H, m), 2.64 (3H, s), 2.86 (3H, s), 3.60 (1H, br s), 4.03 (1H, br s), 5.63 (2H, s), 7.14 (1H, d, *J* 7.6), 7.24 (1H, d, *J* 7.6), 7.66 (1H, dd, *J* 7.6 and 7.6), 8.38 (1H, s); *m*/*z* (ES^+) 419 ($M + H^+$); Anal. ($C_{22}H_{22}N_6SO$): C, H, N.

3-(2-Methyltriazol-5-yl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (35). δ_H (360 MHz, $CDCl_3$) 1.40−1.54 (4H, m), 1.86−1.96 (4H, m), 2.60 (3H, s), 3.59 (1H, s), 3.99 (1H, s), 4.10 (3H, s), 5.59 (2H, s), 7.13 (1H, d, *J* 7.6), 7.46 (1H, d, *J* 7.7), 7.63 (1H, dd, *J* 7.6 and 7.7), 8.25 (1H, s); *m*/*z* (ES^+) 403 ($M + H^+$); Anal. ($C_{21}H_{22}N_8O$): C, H, N.

3-(1-Methyltriazol-5-yl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (36). δ_H (250 MHz, $CDCl_3$) 1.36−1.54 (4H, m), 1.86−1.96 (4H, m), 2.58 (3H, s), 3.59 (1H, s), 3.97 (1H, s), 4.30 (3H, s), 5.56 (2H, s), 7.13 (1H, d, *J* 7.6), 7.46 (1H, d, *J* 7.6), 7.61 (1H, dd, *J* 7.6 and 7.6), 8.15 (1H, s); *m*/*z* (ES^+) 403 ($M + H^+$); Anal. ($C_{21}H_{22}N_8O$): C, H, N.

3-(2-Pyridyl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,-10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (37). δ_H (360 MHz, $CDCl_3$) 1.42−1.55 (4H, m), 1.89−2.00 (4H, m), 2.60 (3H, s), 3.58 (1H, s), 4.02 (1H, s), 5.57 (2H, s), 7.13 (1H, d, *J* 7.6), 7.37 (1H, d, *J* 7.8), 7.39−7.41 (1H, m), 7.63 (1H, dd, *J* 7.6 and 7.8), 7.85−7.90 (1H, m), 8.38 (1H, d, *J* 8.0),

8.85−8.87 (1H, m); *m/z* (ES$^+$) 399 (M + H$^+$); Anal. ($C_{23}H_{22}N_6O$): C, H, N.

3-(2-Pyrazinyl)-6-(6-methylpyridin-2-yl)methyloxy-7,8,9,-10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazine (38): Mp 204−206 °C; δ_H (360 MHz, $CDCl_3$) 1.40−1.56 (4H, m), 1.87−2.02 (4H, m), 2.61 (3H, s), 3.60 (1H, s), 4.03 (1H, s), 5.57 (2H, s), 7.14 (1H, d, *J* 7.7), 7.35 (1H, d, *J* 7.7), 7.64 (1H, dd, *J* 7.7 and 7.7), 8.68 (1H, d, *J* 1.5), 8.82 (1H, dd, *J* 1.3 and 1.5), 9.65 (1H, d, *J* 1.3); *m/z* (ES$^+$) 400 (M + H$^+$); Anal. ($C_{22}H_{21}N_7O$): C, H, N.

(±)3-(5-Methylisoxazol-3-yl)-6-(2-pyridyl)methyloxy-7,8,9,10-tetrahydro-(7,10-methano)-1,2,4-triazolo[3,4-*a*]-phthalazine (39). (a) (±)-3-Chloro-6-hydrazino-4.5-diaza-tricyclo[6.2.1.2,7]undeca-2(7),3,5-triene (4b). Prepared from 2-norbornene-2,3-dicarboxylic anhydride using the general procedure described for **4a**: δ_H (250 MHz, $CDCl_3$) 1.10−1.24 (2H, m), 1.58 (1H, m), 1.80 (1H, m), 1.96−2.10 (2H, m), 3.49 (1H, d, *J* 1.8), 3.55 (1H, d, *J* = 1.6).

(b) **39** was prepared from **4b** and 5-methylisoxazole-3-carboxylic acid using the procedures described for **32**: δ_H (360 MHz, $CDCl_3$) 1.22−1.34 (2H, m), 1.69 (1H, d, *J* 9.2), 1.95 (1H, d, *J* 9.2), 2.05−2.16 (2H, m), 2.58 (3H, s), 3.76 (1H, br s), 4.13 (1H, br s), 5.62 (1H, d, *J* 13.1), 5.66 (1H, d, *J* 13.1), 6.81 (1H, d, *J* 0.7), 7.30 (1H, t, *J* 6.2), 7.72 (1H, d, *J* 7.6), 7.78 (1H, m), 8.66 (1H, m); *m/z* (ES$^+$) 375 (M + H$^+$); Anal. ($C_{20}H_{18}N_6O_2 \cdot 0.45\ H_2O$): C, H, N.

(±)3-(5-Methylisoxazol-3-yl)-6-(6-methylpyridin-2-yl)-methyloxy-7,8,9,10-tetrahydro-(7,10-methano)-1,2,4-triazolo[3,4-*a*]phthalazine (40). Prepared from **4b** using the general procedures: δ_H (360 MHz, $CDCl_3$) 1.22−1.33 (2H, m), 1.69 (1H, br d, *J* 9.2), 1.95 (1H, br d, *J* 9.2), 2.05−2.18 (2H, m), 2.57 (3H, s), 2.60 (3H, s), 3.76 (1H, br s), 4.13 (1H, br s), 5.58 (2H, s), 6.83 (1H, d, *J* 0.8), 7.14 (1H, d, *J* 7.6), 7.45 (1H, d, *J* 7.8), 7.64 (1H, dd, J 7.6, 7.8); *m/z* (ES$^+$) 389 (M + H$^+$).

3-(5-Methylisoxazol-3-yl)-6-(2-pyridyl)methyloxy-7,8,9,-10-tetrahydro-1,2,4-triazolo[3,4-*a*]phthalazine (41). Prepared from tetrahydrophthalic anhydride (**1c**) and 5-methyl-isoxazole-3-carboxylic acid using the general procedures: δ_H (360 MHz, $CDCl_3$) 1.90−1.98 (4H, m), 2.57 (3H, s), 2.74 (2H, br s), 3.14 (2H, br s), 5.61 (2H, s), 6.80 (1H, s), 7.26−7.31 (1H, m) 7.67 (1H, d, J 7.7), 7.77 (1H, dd, *J* 7.6,7.7), 8.64 (1H, m); *m/z* (ES$^+$) 363 (M + H$^+$). Anal. ($C_{19}H_{18}N_6O_2$): C, H, N.

3-(5-Methylisoxazol-3-yl)-6-(2-pyridyl)methyloxy-1,2,4-triazolo[3,4-*a*]phthalazine (43). (a) 1-Chloro-4-hydrazi-nophthalazine (4d). 1,4-Dichlorophthalazine (20.0 g, 0.100 mol) was added to a boiling solution of hydrazine monohydrate (37.3 mL, 0.765 mol) in ethanol (500 mL) and the mixture heated at reflux for 0.5 h. The mixture was cooled to room temperature and the solid collected by filtration and washed with ether. The material was taken with *n*-butanol and ammonia solution (sp. gr. 0.91) and heated until the solid dissolved. The organic layer was separated and evaporated in vacuo and the residue azeotroped with xylene (×2) and dried in vacuo to give **4d** (11.5 g, 59%), δ_H (250 MHz, DMSO-d_6) 7.84−8.04 (3H, m), 8.20 (1H, m); *m/z* (ES$^+$) 195 (M + H$^+$).

(b) 6-Chloro-3-(5-methylisoxazol-3-yl)-1,2,4-triazolo-[3,4-*a*]phthalazine (8o). 5-Methylisoxazole-3-carboxylic acid (5.24 g, 41.3 mmol), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (10.5 g, 41.2 mmol), and triethylamine (11.5 mL, 82.5 mmol) were added successively to a stirred suspension of **4d** (8.00 g, 41.2 mmol) in dichloromethane (1 L) at 0 °C under nitrogen. The mixture was stirred at 0 °C for 2h and at room-temperature overnight. The solvent was evaporated in vacuo, the residue was triturated with water, and the solid was filtered off, washed with hexane, and dried in vacuo. The solid (ketohydrazine) was added to a solution of triethylamine hydrochloride (2.2 g, 20% w/w) in xylene (500 mL) and was heated at reflux for 3 h. The mixture was cooled to room temperature and the solvent evaporated in vacuo. The residue was dissolved in dichloromethane, washed with water (× 2), dried ($MgSO_4$), and evaporated in vacuo and the solid recrystallized (dichloromethane/hexane) to give **8o** (6.8 g, 58%), δ_H (360 MHz, $CDCl_3$) 2.59 (3H, s), 6.90 (1H, s), 7.95 (1H, m), 8.07 (1H, m), 8.34 (1H, m), 8.78 (1H, s); *m/z* (ES$^+$) 286 (M + H$^+$).

(c) Sodium hydride (244 mg of a 60% dispersion in oil, 6.10 mmol) was added to a stirred solution of 2-pyridylcarbinol (470 mg, 4.27 mmol) in DMF (60 mL) at room temperature under nitrogen and the mixture stirred for 0.25 h. After this time, **8o** (1.160 g, 4.07 mmol) was added and the mixture stirred for 2 h. The solvent was removed in vacuo and the residue dissolved in dichloromethane, washed with water (×2), dried ($MgSO_4$), and evaporated in vacuo. Flash chromatography on silica gel eluting with methanol/dichloromethane 3:97 followed by recrystallization (dichloromethane/hexane) gave **43** (640 mg, 44%): mp 234−236 °C; δ_H (360 MHz, $CDCl_3$) 2.59 (3H, d, *J* 0.8), 5.77 (2H, s), 6.82 (1H, d, *J* 0.8), 7.30 (1H, m), 7.74−7.85 (3H, m), 7.95 (1H, m), 8.33 (1H, d, *J* 7.8), 8.64−8.72 (2H, m); *m/z* (ES$^+$) 359 (M + H$^+$); Anal. ($C_{19}H_{14}N_6O_2$ $0.1H_2O$): C, H, N.

3-(5-Methylisoxazol-3-yl)-6-(6-methylpyridin-2-yl)methyloxy-1,2,4-triazolo[3,4-*a*]phthalazine (42). Prepared from **8o** and 6-methyl-2-pyridylcarbinol using the procedure described for **43**: δ_H (360 MHz, $CDCl_3$) 2.59 (3H, d, *J* 0.8), 2.61 (3H, s), 5.73 (2H, s), 6.86 (1H, d, *J* 0.8 Hz), 7.16 (1H, d, *J* 7.6), 7.53 (1H, d, *J* 7.5), 7.66 (1H, dd, *J* 7.5, 7.6), 7.83 (1H, m), 7.97 (1H, t, *J* 8.2), 8.33 (1H, d, *J* 7.7), 8.70 (1H, d, *J* 7.7); *m/z* (ES$^+$) 373 (M + H$^+$); Anal. ($C_{20}H_{16}N_6O_2 \cdot 1.15\ H_2O$): C, H, N.

Biological Methods. Radioligand Binding Studies. L(tk−) cells expressing human recombinant GABA$_A$ receptors containing β3 and γ2s subunits in combination with various α subunits were harvested and binding performed as described elsewhere.[40] The displacement of [^{3}H]Ro15-1788 binding was measured in GABA$_A$ receptors containing either an α1, α2, α3, or α5 subunit and from the IC_{50} the K_i was calculated assuming respective KD values of [^{3}H]Ro 15-1788 binding of 0.92, 1.05, 0.58, and 0.45 nM at the α1, α2, α3, or α5 subtypes. Nonspecific binding was defined by the inclusion of 10 μM flunitrazepam for the α1, α2, α3, and α5 subtypes. The percentage inhibition of [^{3}H]Ro 15-1788 binding, the IC_{50} and the K_i values were calculated using ActivityBase (IDBS).

Electrophysiology. Voltage Clamp in *X. laevis* Oocytes. Adult female *X. laevis* were anaesthetised by immersion in a 0.4% solution of 3-aminobenzoic acid ethyl ester for 30−45 min (or until unresponsive). Ovary tissue was removed via a small abdominal incision, and Stage V and Stage VI oocytes were isolated with fine forceps. After mild collagenase treatment to remove follicle cells (Type IA, 0.5 mg mL^{-1}, for 8 min), the oocyte nuclei were directly injected with 10−20 nL of injection buffer (88 mM NaCl, 1 mM KCl, 15 mM HEPES, at pH 7, filtered through nitrocellulose) or sterile water containing different combinations of human GABA$_A$ subunit cDNAs (20 ng μL^{-1}) engineered into the expression vector pCDM8 or pcDNAI/Amp. Following incubation for 24−72 h, oocytes were placed in a 50 μL bath and perfused at 4−6 mL min^{-1} with modified Barth's medium (MBS) consisting of 88 mM NaCl, 1 mM KCl, 10 mM HEPES, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.91 mM $CaCl_2$, 2.4 mM $NaHCO_3$, at pH 7.5. Cells were impaled with two 1−3 MΩ electrodes containing 2 M KCl and voltage-clamped between −40 and −7 0mV.

The initial maximal response to GABA was defined by applying 3 mM GABA to the cell. Using this value, lower concentrations of GABA were titrated until a response approximately equal to 20% of the maximum (EC_{20}) was found (an EC_{20} dose was required to allow a maximum window of modulation of the GABA current due to the test compound and to minimize desensitization of the GABA current). This concentration was repeatedly applied until a constant value was maintained. Compounds were preapplied to the cell for 30 s prior to coapplication of the compound and the EC_{20} GABA concentration, and modulation of the GABA EC_{20} response expressed as a percentage increase of the control current. In all experiments drugs were applied in the perfusate until the peak of the response was observed. Noncumulative concentration−response curves were constructed allowing at least 3 min between each agonist application. Curves were fitted using a nonlinear square-fitting program to the equation $f(x) = B_{MAX}/[1 + (EC_{50}/x)]^n$ where x is the drug concentration, EC_{50} is the

concentration of drug eliciting a half-maximal response, and n is the Hill coefficient.

Whole Cell Patch-Clamp of Ltk⁻ Cells Stably Transfected with Human $GABA_A$ Receptors. Experiments were performed on Ltk⁻ cells expressing human cDNA combinations $\alpha1\beta3\ \gamma2$s, $\alpha2\beta3\ \gamma2$s, $\alpha3\beta3\ \gamma2$s, and $\alpha5\beta3\gamma2$s. Glass cover-slips containing the cells in a monolayer culture were transferred to a Perspex chamber on the stage of a Nikon Diaphot inverted microscope. Cells were continuously perfused with a solution containing 124 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1.25 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM D-glucose, at pH 7.2, and observed using phase-contrast optics. Patch-pipets were pulled with an approximate tip diameter of 2 μm and a resistance of 4 MΩ with borosilicate glass and filled with 130 mM CsCl, 10 mM HEPES, 10 mM EGTA, 3 mM Mg^+-ATP, pH adjusted to 7.3 with CsOH. Cells were patch-clamped in whole-cell mode using an Axopatch-200B patch-clamp amplifier. Drug solutions were applied with a double-barreled pipet assembly, controlled by a stepping motor attached to a Prior manipulator, enabling rapid equilibration around the cell. Increasing GABA concentrations were applied for 5 s pulses with a 30 s interval between applications. Curves were fitted using a non-linear square-fitting program to the equation $f(x) = B_{MAX}/[1 + (EC_{50}/x)^n]$ where x is the drug concentration, EC_{50} is the concentration of drug eliciting a half-maximal response, and n is the Hill coefficient.

Pharmacokinetic Methods. Six male SD rats, which had been surgically cannulated at the jugular vein, were deprived of food overnight and then given the test compound either intravenously (bolus injection in to tail vein) or orally ($n = 3$ per dose route). Serial blood samples (approximately 400 μL) were taken from the jugular vein cannula at time points up to 8 h. After each sample, an equivalent volume of heparinized saline (10 units/ml) was injected into the rat via the cannula. Plasma samples, prepared by centrifugation of whole blood, were frozen at −20 °C until analysis. Concentrations of comppound in plasma were determined by a LC-MS/MS assay. In brief, aliquots (100 μL) of plasma samples were spiked with 10 μL of DMSO (compensate for spiking of standards) and acetonitrile (200 μL) containing an internal standard (1 μg/mL). The tubes were capped and vortexed prior to centrifugation to separate the precipitated proteins. The supernatant was then analyzed by HPLC-MS/MS. Calibration curves (weighted $1/x$) were linear in the range 2.4 to 2000 ng/mL (typical r^2 values >0.995) with a limit of quantification (LOQ) of 2.4 ng/mL.

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Supporting Information Available: Microanalytic data for compounds in Tables 2−5. This material is available free of charge via the Internet at http://pubs.acs.org.

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