High-Affinity α-Aminobutyric Acid A/Benzodiazepine Ligands: Synthesis and Structure−Activity Relationship Studies of a New Series of Tetracyclic Imidazoquinoxalines†

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A series of tetracyclic imidazoquinoxaline analogs was developed which constrain the carbonyl group of the partial agonist 3-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)-5-[(dimethylamino)carbonyl]-4,5-dihydroimidazo[1,5-*a*]quinoxaline (**2**, U-91571) away from the benzene ring. These analogs orient the carbonyl group in the opposite direction of the previously reported full agonist 1-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)-12,12a-dihydroimidazo[1,5-*a*]pyrrolo[2,1-*c*]quinoxalin-10(11*H*)-one (**3**, U-89267). A number of approaches were utilized to form the "bottom" ring of this tetracyclic ring system including intramolecular cyclizations promoted by Lewis acids or base, as well as metal−carbenoid conditions. The size and substitution pattern of the additional ring was widely varied. Analogs within this series had high affinity for the benzodiazepine receptor on the α-aminobutyric acid A chloride ion channel complex. From TBPS shift and Cl^- current assays, the *in vitro* efficacy of compounds within this class ranged from antagonists to partial agonists with only **18a** identified as a full agonist. Additionally, several analogs were quite potent at antagonizing metrazole-induced seizures indicating possible anticonvulsant or anxiolytic activity. Unlike **3**, analogs in this series did not have high affinity for the diazepam insensitive $\alpha_6\beta_2\delta_2$ subtype. These results suggest that either constraining the carbonyl group away from the benzene ring or the greater planarity that results from the additional cyclic structure provides analogs with partial agonist properties and prevents effective interaction with the $\alpha_6\beta_2\delta_2$ subtype.

Introduction

The development of the benzodiazepine class of drugs for the treatment of a variety of neurological indications has proven to be an outstanding success story in the field of drug therapy. However, these compounds often produce undesirable side effects when used as anti-anxiety or hypnotic agents. These side effects include sedation, physical dependence, amnesia, muscle relaxation, and ethanol potentiation. The development of a benzodiazepine receptor-based anxiolytic agent devoid of these side effects would constitute a major advance in the field and has been the focus of significant research efforts.[1]

Benzodiazepines exert their influence by interacting with the benzodiazepine receptor (BzR) located on the α-aminobutyric acid A ($GABA_A$) chloride ion channel complex. Associated with the $GABA_A$ ion channel are a variety of recognition sites for small molecules, which can directly influence the ability of this channel to transport chloride ion across neuronal membranes. In addition to the benzodiazepine receptor, there exist binding sites for γ-aminobutyric acid (GABA), barbiturates, picrotoxin (and other convulsant agents), and neurosteroids.[2] When GABA, the major inhibitory neurotransmitter in the central nervous system (CNS), binds to its receptor, the flow of chloride ion through the channel is increased and the excitability of the neuron is reduced.[3] Of the many types of receptor−ligand interactions that influence this GABA-induced chloride flux, the benzodiazepine receptor and its ligands have been the most widely studied, with many structural classes discovered which span the entire efficacy spectrum. Full agonists potentiate the GABA-induced chloride flux to further decrease the excitability of the neuron and have found wide-spread use as anxiolytic, hypnotic, and anticonvulsant agents. In contrast, inverse agonists which decrease the flow of chloride ion are proconvulsant and anxiogenic in nature. Antagonists which have minimal or no effect on the chloride flux have neutral efficacy. Presumably, partial agonists lie within this efficacy continuum.[4] This is especially intriguing in that partial agonists may display anti-anxiety properties but, due to their lowered intrinsic efficacy, lack the undesirable side effects often associated with full agonists.[5]

The heterogeneity of the $GABA_A$ receptor population has been confirmed with molecular biology studies having identified several different receptor subtypes[6] which are composed of multiple subunits (α, β, γ, δ).[7] While classical benzodiazepines interact with most subtypes with nearly equal affinity,[8] several atypical BzR ligands are selective for the $\alpha_1\beta_2\delta_2$ subtype.[7a,8] In contrast, most benzodiazepine ligands do not interact with the diazepam insensitive $\alpha_6\beta_2\delta_2$ subtype located in cerebellar granule cells, whereas ligands such as Ro 15-4513 have high affinity for this subtype.[9a] The $\alpha_6\beta_2\delta_2$ subtype has been hypothesized to play a role in ethanol

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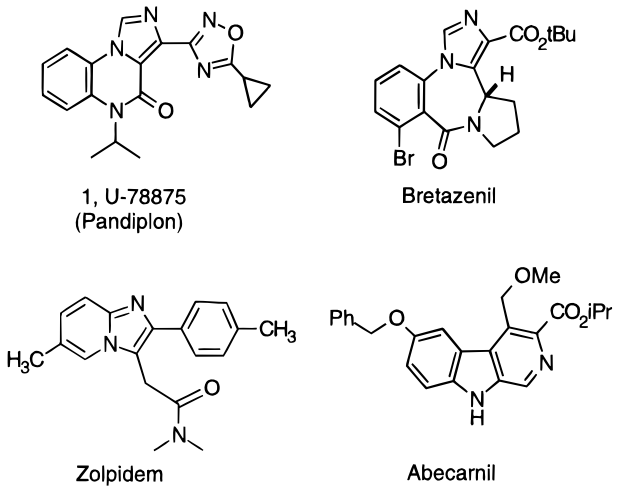


Figure 1.

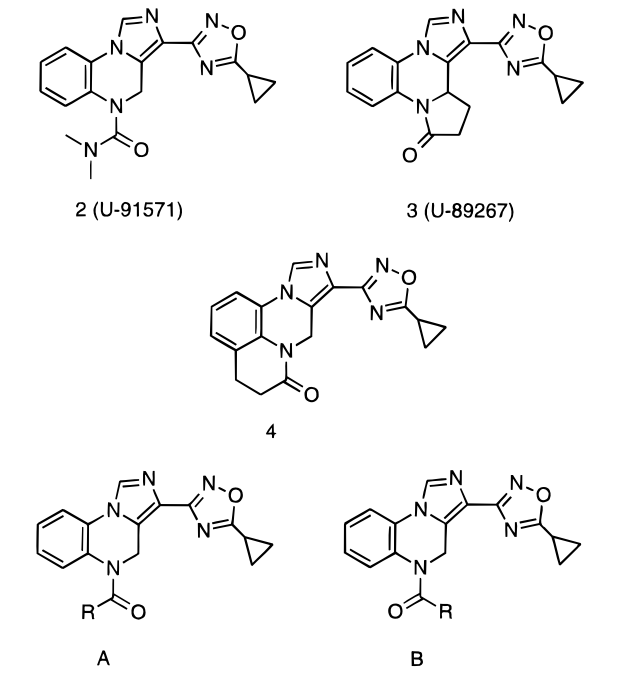


Figure 2.

potentiation,[9a] although this has recently been disputed.[9b] Efficacy differences for various BzR ligands between these subtypes have also been noted.[10] The discovery of selective ligands for the various GABA$_A$ subtypes, and the elucidation of their function, remains an important goal in this area.

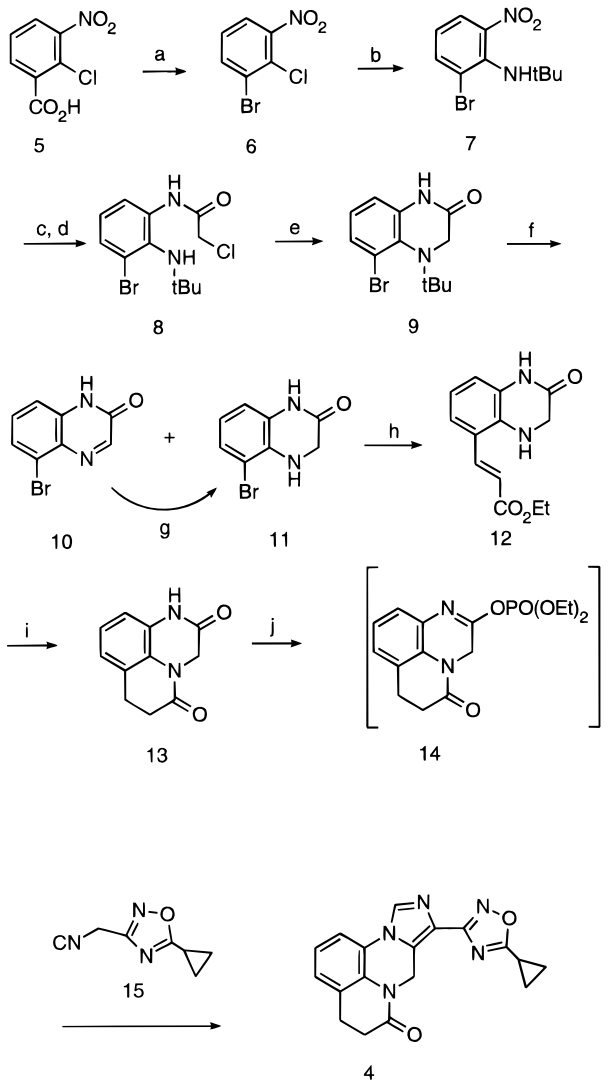
The search for viable partial agonists or subtype selective ligands has led to the development of a variety of compounds representing diverse structural types including imidazoquinoxalines, benzodiazepines, imidazopyridines, and β-carbolines (Figure 1). In an effort to identify replacement candidates for the partial agonist **1** (U-78875),[11] which was removed from clinical trials due to liver enzyme induction, a variety of analogs were prepared and evaluated. One class of compounds studied (Figure 2) consisted of imidazo[1,5-*a*]quinoxaline amides, carbamates, thiocarbamates, and ureas, of which **2** (U-91571) is representative.[12] Analogs within this series had varying efficacy; however, like **2**, most were partial agonists. Another related class of compounds that was explored involved a series of tetracycles as represented by **3** (U-89267), in which the carbonyl group was constrained to point toward the arene ring by incorporating a C(4)−N(5) tether (imidazo[1,5-*a*]-quinoxaline numbering, i.e., **2**).[13] Interestingly, derivatives from this subseries were full agonists by *in vitro* measurement (TBPS shift ratio) and were extremely potent in *in vivo* assays such as the metrazole antagonism assay. Furthermore, compounds such as **3** had unusually high affinity (13 nM) for the $\alpha_6\beta_2\delta_2$ subtype, whereas most derivatives from the "uncyclized" series (e.g., **2**) did not bind to this subtype.[12−14]

Molecular modeling studies of **2** indicated that there are two preferred conformations, depending on the force field used.[12] As depicted in Figure 2 the urea carbonyl group can point away from the aryl ring (A) or toward the aryl ring (B) as in **3**. Given the contrasting $\alpha_6\beta_2\delta_2$ selectivity and pharmacological activity between these two series (i.e., **2** and **3**), we were intrigued about what role the carbonyl orientation plays on affinity, efficacy, and subtype specificity and whether constraining it to a similar conformation as depicted for **2** would provide for partial agonist properties. In this study we have constrained the carbonyl group in the opposite direction of **3** as demonstrated by imidazoquinoxaline **4**. Both the constituency and the size of the bottom ring were varied to determine the effect on efficacy and binding. With one exception, these constrained analogs were found to be partial agonists by both *in vitro* assays (TBPS shift and Cl^- current) with only **18a** acting as a full agonist by chloride current assay. In addition, several analogs were extremely effective *in vivo* in a metrazole antagonism assay. Herein, we report the synthetic details of this investigation and the results from the biological testing of these tetracyclic-based partial agonist analogs.

Chemistry

The synthesis of the unsubstituted tetracyclic lactam analog **4** is presented in Scheme 1. A modified Hunsdiecker reaction[15] was utilized to convert 2-chloro-3-nitrobenzoic acid (**5**) to the known bromide **6** in high yield.[16] Addition of *tert*-butylamine to **6** provided nitroaromatic **7**, which was reduced with aqueous titanium trichloride and acylated directly with chloroacetyl chloride to provide amide **8**. Sodium iodide-induced cyclization of amide **8** gave quinoxalinone **9**. Treatment of **9** with trifluoroacetic acid (TFA) gave a mixture of the desired deprotected quinoxalinone **11** as well as a small amount (<5%) of the corresponding imine **10**. Complete conversion of imine **10** to **11** was realized when the crude reaction mixture was treated with sodium borohydride. A Heck reaction was utilized to convert quinoxalinone **11** to the acrylate derivative **12**, which was subsequently reduced and cyclized to provide the key lactam **13**, in 49% overall yield (**11** → **13**). Deprotonation of **13** followed by addition of diethyl chlorophosphate gave the enol intermediate **14**, which was not isolated but directly converted to the desired analog **4** by the addition of 5-cyclopropyl-3-(isocyanomethyl)-1,2,4-oxadiazole[17] (**15**) and potassium *tert*-butoxide. The yields for this conversion were typically quite low in comparison to our previous work.[12,13] Presumably the greater ring strain imparted by the additional cyclic structure from N(5)−C(6) is responsible for the decreased efficiency observed for this transformation.

Scheme 1[a]

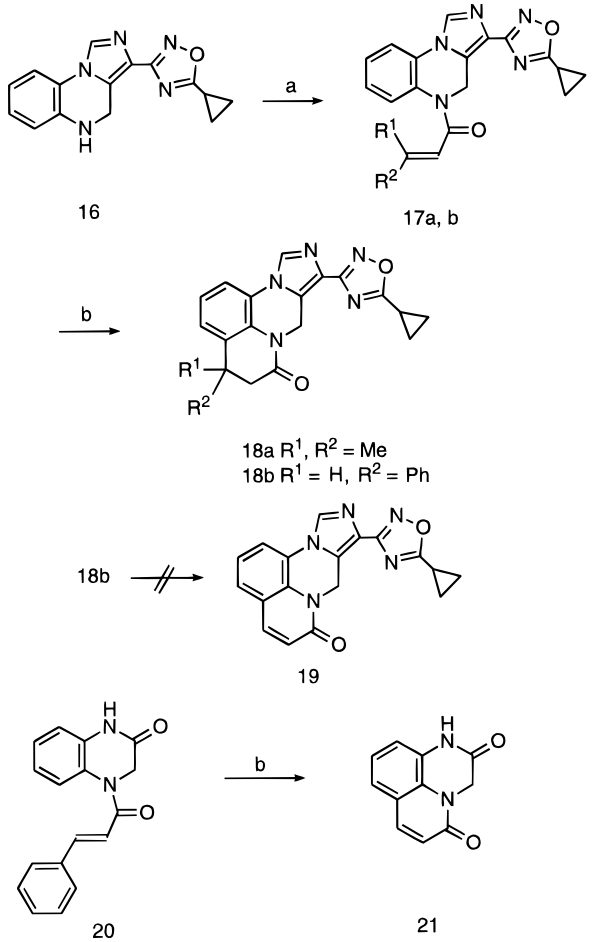


[a] (a) HgO (red), Br_2, CCl_4, *hν*; (b) $tBuNH_2$, EtOH, 150 °C; (c) $TiCl_3$, MeOH, NaOAc; (d) $ClCH_2COCl$, $EtNiPr_2$, THF; (e) NaI, CH_3CN, $EtNiPr_2$; (f) TFA; (g) $NaBH_4$, EtOH; (h) ethyl acrylate, $Pd(PPh_3)_4$, Et_3N; (i) H_2, Pd/C, TsOH, EtOH; (j) tBuOK, THF, diethyl chlorophosphate, tBuOK, 5-cyclopropyl-3-(isocyanomethyl)-1,2,4-oxadiazole (**15**).

Scheme 2 depicts the synthesis of the substituted tetracyclic lactam analogs **18a**,**b**. The imidazoquinoxaline[12,13] **16** was acylated with either 3,3-dimethylacryloyl chloride or cinnamoyl chloride to provide the desired amides **17a**,**b**. These amides were readily cyclized to the appropriate analogs **18a**,**b** under the influence of aluminum trichloride. The limits of this aluminum chloride-based cyclization were defined as the crotonyl amide (prepared by the acylation of **16** with crotonyl chloride) failed to cyclize to the lactam analog. It is also interesting to note that the product resulting from dearylation of **18b**, unsaturated lactam **19**, was not observed even upon prolonged reaction time and elevated temperatures. This is in contrast to the work of Manimaran[18] in which cinnamanilides readily underwent aluminum chloride cyclization and dearylation to provide unsaturated carbostyrils, as well as our own work where **20** cyclized and smoothly dearylated to provide **21** in 99% yield.

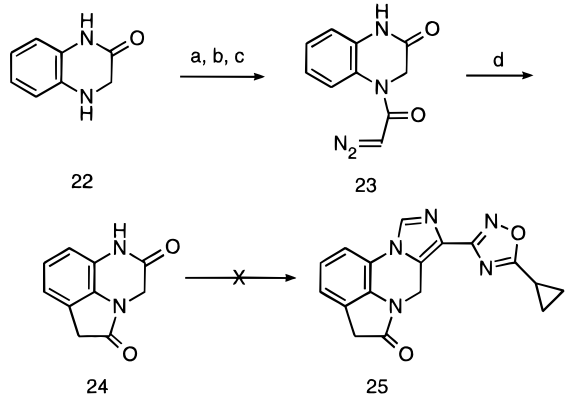
In an effort to prepare the five-membered lactam analog **25** which would serve as the "opposite" to **3**, the appropriate tricyclic precursor **24** was targeted (Scheme 3). Quinoxaline[13,19] **22** was converted to the diazo intermediate **23** through a diazo transfer process.[20,21] Decomposition of the diazo quinoxalinone **23** with rhodium(II) trifluoroacetate dimer[22] provided the desired lactam **24** in 60% yield. In contrast, decomposition of **23** with rhodium(II) acetate dimer furnished only minimal amounts (<10%) of lactam **24**. Unfortunately, all attempts to prepare the desired imidazoquinoxaline analog **25** failed, presumably due to the aforementioned ring strain of the lactam or poor solubility of **24** in the reaction conditions. Lactam **21** (Scheme 2) also failed to cyclize with isocyanide **15** under similar conditions.

Scheme 2[a]



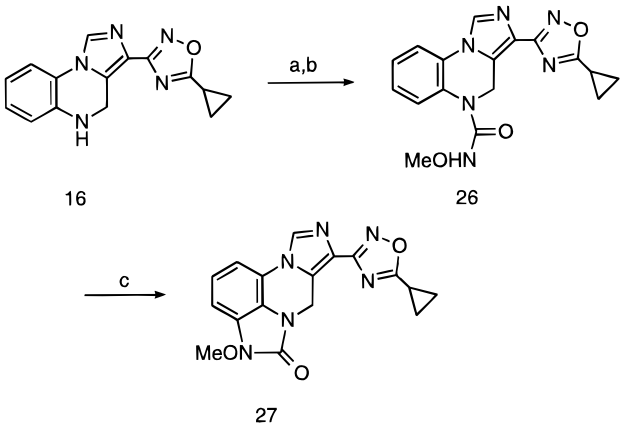
[a] (a) $R^1R^2C{=}CHCOCl$, DMAP, Et_3N, CH_2Cl_2; (b) $AlCl_3$, 1,2-dichlorobenzene, 100 °C.

Scheme 3[a]



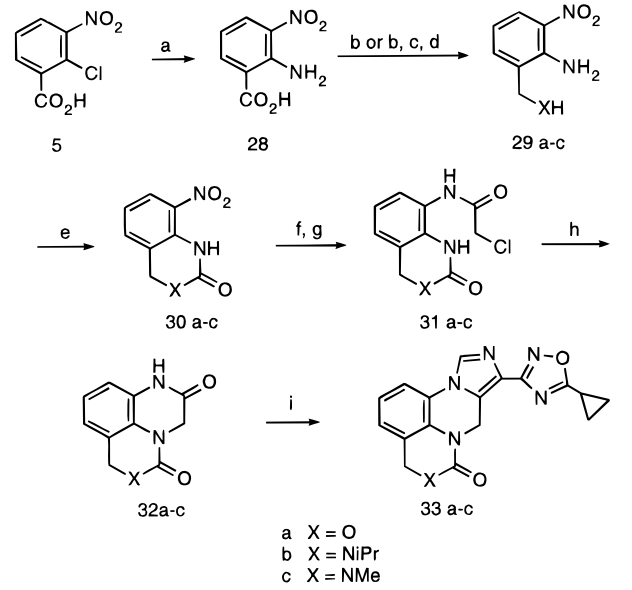
[a] (a) Diketene, DMAP, CH_2Cl_2; (b) MsN_3, Et_3N, CH_3CN; (c) 10% NaOH, THF; (d) $Rh_2(O_2CCF_3)_4$, CH_2Cl_2.

Scheme 4[a]



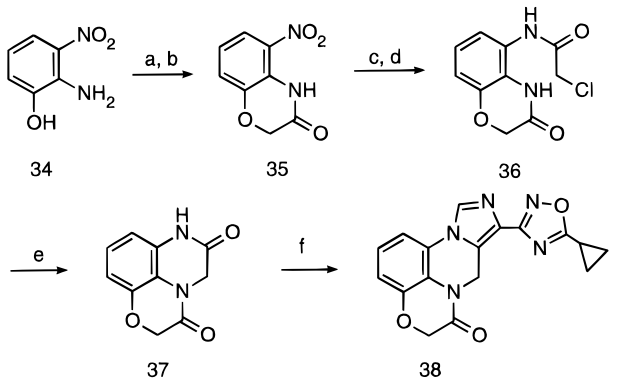
[a] (a) Triphosgene, EtNiPr$_2$, THF; (b) MeONH$_2$·HCl, EtNiPr$_2$; (c) PhI(CF$_3$CO$_2$)$_2$, CH$_2$Cl$_2$.

Scheme 5[a]



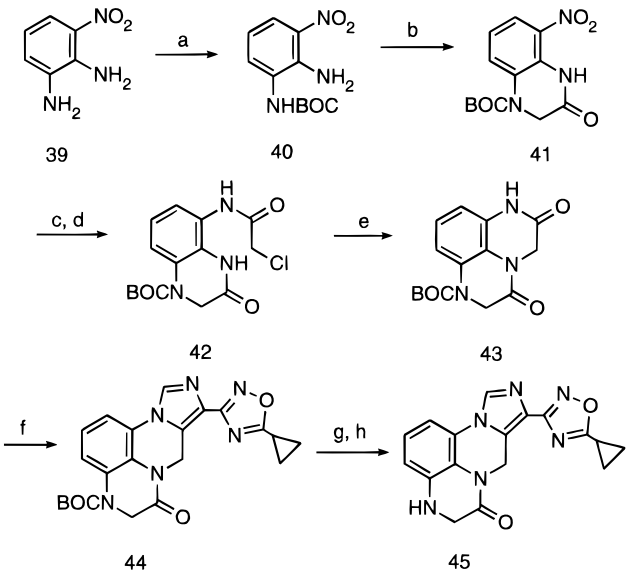
[a] (a) NH$_4$OH, CuCl; (b) BH$_3$DMS, THF; (c) MsCl, EtNiPr$_2$, THF; (d) NH$_2$R; (e) 1,1′-carbonyldiimidazole, THF; (f) H$_2$, 10% Pd/C, EtOH; (g) ClCH$_2$COCl, EtNiPr$_2$, THF; (h) tBuOK, THF; (i) tBuOK, THF−DMF, diethyl chlorophosphate, tBuOK, 5-cyclopropyl-3-(isocyanomethyl)-1,2,4-oxadiazole (**15**).

Scheme 6[a]



[a] (a) BrCH$_2$CO$_2$Et, EtNiPr$_2$; (b) EtOH, K$_2$CO$_3$; (c) H$_2$, 10% Pd/C, EtOH; (d) ClCH$_2$COCl, EtNiPr$_2$, THF; (e) tBuOK, THF; (f) tBuOK, THF−DMF, diethyl chlorophosphate, tBuOK, 5-cyclopropyl-3-(isocyanomethyl)-1,2,4-oxadiazole (**15**).

Scheme 7[a]



[a] (a) (BOC)$_2$O, Et$_3$N, THF; (b) NaH, THF, BrCH$_2$CO$_2$Et; (c) H$_2$, 10% Pd/C, EtOAc; (d) ClCH$_2$COCl, THF, EtNiPr$_2$; (e) tBuOK, THF; (f) tBuOK, THF, diethyl chlorophosphate, DMF, tBuOK, 5-cyclopropyl-3-(isocyanomethyl)-1,2,4-oxadiazole (**15**); (g) TFA, CH$_2$Cl$_2$; (h) NaBH$_4$, MeOH.

While the synthesis of lactam **25** was unsuccessful, the five-membered cyclic urea analog **27** was prepared as shown in Scheme 4. Conversion of **16** to the methoxy urea **26** (via the carbamoyl chloride) and subsequent cyclization with [bis(trifluoroacetoxy)iodo]benzene following the general protocol outlined by Romero[23] provided **27**, albeit in low yield. Removal of the *O*-methoxy group was not possible due to the lability of the N−O bond of the oxadiazole.

The preparation of a variety of tetracycles which incorporate a heteroatom α to the carbonyl group is depicted in Scheme 5. Addition of ammonia to **5** gave **28** which was reduced with borane−methyl sulfide complex to provide alcohol **29a**. This alcohol was either converted directly to the cyclic carbamate **30a** with 1,1′-carbonyldiimidazole (CDI) or transformed to amines **29b**,**c** (via the mesylate), which were cyclized to give the cyclic ureas **30b**,**c**, respectively. Reduction of the nitro group of **30a**−**c** and acylation of the resulting amine with chloroacetyl chloride provided the chloroacetamides **31a**−**c**, which were readily cyclized with potassium *tert*-butoxide to provide the tricyclic intermediates **32a**−**c**. Conversion of these tricyclics to the final imidazo-annulated analogs **33a**−**c** followed the previously mentioned protocol, again in low yield.

Schemes 6 and 7 diagram the synthesis of analogs with a heteroatom substituted β to the carbonyl group. The initial step in the preparation of the oxygen-substituted analog was the reaction of 2-amino-3-nitrophenol (**34**) with ethyl bromoacetate to furnish lactam **35**. Conversion of this bicyclic lactam to the final analog **38** followed a sequence identical with the one previously described (Scheme 5). The β-amine analog was prepared in a similar manner after initial protection of 3-nitro-1,2-phenylenediamine (**39**) to provide BOC derivative **40** (Scheme 7). Treatment of **40** with 2 equiv of sodium hydride followed by ethyl bromoacetate provided amide **41** in excellent yield. Hydrogenation, acylation with chloroacetyl chloride, and cyclization provided the tricyclic intermediate **43** which was converted to imidazoquinoxaline **44** under standard conditions. The BOC group was removed with TFA to

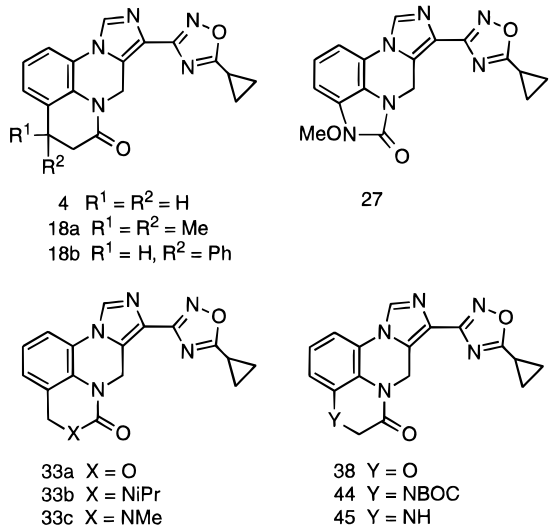


Figure 3. Imidazo[1,5-*a*]quinoxalines prepared.

provide a mixture of the desired analog **45** and the imine. Pure **45** was obtained upon sodium borohydride reduction of the crude reaction mixture.

Results and Discussion of Biological Evaluation

The binding affinity of the imidazo[1,5-*a*]quinoxalines to the benzodiazepine receptor in rat cortical membranes was determined by competition experiments with radiolabeled agonist [^{3}H]flunitrazepam.[24] The *in vitro* efficacy of these compounds was measured by two different methods. The TBPS ratio[25] was determined for each compound by measuring its effect on *tert*-butylbicyclophosphorothionate (TBPS) binding to the picrotoxin convulsant site on the $GABA_A$ chloride complex. The changes in TBPS binding presumably occur due to conformational changes in the chloride ionophore, allosterically caused by the binding of the test compound to the benzodiazepine receptor. The resultant value, expressed as a ratio of that for the test drug to that of diazepam, is 1 for a full agonist and 0 for an antagonist, with negative values for inverse agonists. A second and more direct measure of *in vitro* efficacy was determined by a Cl^- current assay.[25−27] The synaptic chloride conductance effected by GABA activating the $GABA_A$ receptor complex is modulated by ligands acting at the benzodiazepine receptor. Full agonists increase current, and antagonists have no effect, while inverse agonists decrease ion flow. The test compounds were compared to diazepam, and thus like the TBPS assay, a full agonist has a value of 1 or greater, with antagonists having no effect (0), while inverse agonists have negative values. To provide a quick measure of *in vivo* efficacy, most analogs were evaluated for their ability to antagonize metrazole-induced seizures (clonic and tonic).[28] While a direct measure of anticonvulsant activity, this assay is also predictive of anxiolytic properties as standard full agonist benzodiazepine agents such as diazepam, alprazolam, and zolpidem are extremely effective in this assay, as are partial agonists **1**, bretazenil, and abecarnil,[29] although to a lesser degree. The results of the *in vitro* and metrazole antagonism assays for the tetracyclic quinoxalines (Figure 3) are shown in Table 1.

For the most part, the excellent affinity observed for **3** (0.87 nM) was maintained for analogs within this series. The most direct analog of **3**, lactam **4**, had a K_i of 2.6 nM. Other modifications of the six-membered ring provided analogs such as the methyl- or isopropylureas (**33c** or **33b**) and amide **38**, which also had excellent affinity. However, affinity was decreased 10-fold for several close heteroatom analogs, specifically carbamate **33a** and amine **45**. Some steric bulk could be incorporated into the "bottom" ring, with *gem*-dimethyl groups (at the 4-position) tolerated quite well as **18a** had reasonable affinity (9.9 nM). Incorporation of a bulkier substituent at this site, such as the phenyl ring of **18b**, decreased affinity further, roughly 10-fold less than the parent ring system. Substitution with a *tert*-butyl carbamate was not tolerated, with **44** having quite poor affinity. The lone example containing a five-membered ring, **27**, had good affinity for the BzR.

Table 1. [^{3}H]Fnz Binding, TBPS Shift, Cl^- Current Changes, and Metrazole Antagonism Data for Imidazo[1,5-*a*]quinoxalines

compd	K_i (nM)[a]	[^{35}S]TBPS shift[b,c]	Cl^- current changes[b,c]	metrazole[d] ED_{50} (mg/kg, ip)
4	2.6	0.09	0.07	1.9 (1.1−1.9)
18a	9.9	0.75	1.1	0.20 (0.13−0.29)
18b	25	0.88	0.88	0.94 (0.5−1.7)
27	7.3	0.68	0.73	3.7 (2.2−6.2)
33a	21	0.52	0.35	>50
33b	1.2	0.41	0.65	>50
33c	3.7	0.08	0.05	>50
38	2.0	0.35		
44	579	0.63	0.60	25 (13.4−46.4)
45	19	0.58	0.39	4.4 (2.3−8.1)
1	1.6	0.06	0.04	1.6
2	1.0	0.69	0.68	21
3	0.87	1.15	0.29	1.1
diazepam	4.9	1.0 ± 0.2	1.0 ± 0.15	0.50 (0.38−1.2)

[a] Mean binding affinity against [^{3}H]flunitrazepam; see ref 24 and the Experimental Section for methods. The standard error was <±10% of the mean. [b] Diazepam is defined as a full agonist which gives a value of 1. Antagonists are defined as having a shift value of 0; partial agonists are intermediate. [c] See the Experimental Section. The standard error was <±10% of the mean. [d] Antagonism of metrazole-induced clonic convulsions in the rat after ip injection; see the Experimental Section.

By *in vitro* measurement, analogs within this series had efficacy spanning the range from antagonists to full agonists. A number of analogs had partial agonist properties quite similar to **2**. In general, efficacy was enhanced with increased substitution on the bottom ring. The most direct analog to **3**, lactam **4**, was an antagonist by both TBPS shift and chloride current measurement. The *N*-methylurea analog **33c** had similar values in these assays indicating antagonistic properties, similar to **1**. Increasing the size of the substituent to an isopropyl group resulted in significantly enhanced efficacy with **33b** being a partial agonist (TBPS, 0.41; Cl^-, 0.65). The two oxygen-substituted analogs **33a** and **38** and amine **45** were also partial agonists having similar TBPS shift ratios and Cl^- current values. Protection of **45** with a BOC group, **44**, resulted in enhanced chloride current, although the TBPS shift ratio was not appreciably affected. Efficacy was further enhanced through substitution at the 4-position with either a phenyl ring or through the incorporation of *gem*-dimethyl groups. Phenyl **18b** was nearly a full agonist by both the TBPS shift and chloride current assays, while **18a** had a similar TBPS shift ratio (0.75) and greater efficacy than diazepam in the Cl^- current screen (1.1). In the five-membered ring series, the *N*-methoxy lactam was a partial agonist having a

significantly lower TBPS shift ratio than **3** (1.15), although the Cl^- current was greater for **27**. It is quite interesting to note that all the relatively planar analogs were antagonists or partial agonists, whereas the two derivatives (**18a**,**b**) which contain out-of-plane groups were nearly full agonists. Most of the analogs from this series were evaluated for their ability to antagonize metrazole-induced seizures. For the most part, the activity observed in this assay was consistent with the *in vitro* results. The urea (six-membered) and carbamate analogs were all inactive, in accordance with their antagonist to weak partial agonist properties, although the lack of activity for **33b** is surprising. In contrast, all of the amides (regardless of the 4-substituent) and the five-membered analog were active as metrazole antagonists, consistent with the partial to full agonist properties observed in the TBPS and Cl^- current assays. However, a major exception proved to be **4**. This antagonist had exceptional potency in the metrazole assay (ED_{50} = 1.9 mg/kg), similar to that reported for the enantiomers of **3**. The other analogs in this assay had ED_{50} values more consistent with their *in vitro* properties, although **44** was quite active considering its affinity. In addition, both of the lactams containing 4-substituents were exceedingly potent with **18a** having an ED_{50} roughly 3-fold lower than that of diazepam. Interestingly, most of these analogs were significantly more effective than **2**, even though their *in vitro* properties were similar.

All of the analogs screened *in vitro* had an excellent correlation between the TBPS shift and Cl^- current assays. In addition, the correlation between *in vitro* efficacy and activity in the metrazole assay was usually quite good, except for **4**. This was also not the case for **3**, which had a TBPS shift ratio of 1.15 and a chloride current of 0.29 yet was exceptionally active in the metrazole assay. The potent activity of **3** in the metrazole assay is consistent with a full agonist profile as indicated by the TBPS shift assay, which is run across the native receptor population. However, the weak partial agonist properties measured for **3** in the Cl^- current assay in the $\alpha_1\beta_2\delta_2$ subtype indicate that the potent metrazole activity observed for this analog may, in fact, be effected by other $GABA_A$ receptor subtypes. Quite possibly the unique properties of **3** may be due to its interaction with the $\alpha_6\beta_2\delta_2$ subtype, where it is a full agonist. Given the close structural similarity to **3**, analogs from this series were also evaluated for their affinity to the $\alpha_6\beta_2\delta_2$ subtype[30,31] with the results shown in Table 2. However in contrast to **3**, none of the tetracyclic analogs examined had reasonable binding affinity (>589 nM) to the $\alpha_6\beta_2\delta_2$ subtype. Nonetheless, several compounds did interact with this subtype, with **18a** and **27** inhibiting [^{3}H]Ro 15-4513 binding by approximately 35% at 100 nM. Clearly, the additional cyclic structure of **4** and related analogs prevents effective interaction with the $\alpha_6\beta_2\delta_2$ subtype in contrast to **3**.

Molecular Conformations. The low-energy conformers of each of the tetracyclic compounds listed in Table 1 were determined by molecular mechanics methods. Calculations were performed using both the AMBER* and MM2* force fields, which were developed for the MacroModel[32] (version 4.5) system of programs as extensions of the original AMBER[33] and MM2[34] parameter sets. Initial structures to be minimized were generated using an internal coordinate Monte Carlo procedure[35] in which torsional angles around both acyclic rotatable bonds and bonds within the tetracyclic ring system were varied. For rotations about cyclic bonds, rings were temporarily opened to provide pseudo-acyclic structures, and torsional variations were allowed subject to ring closure constraints.[36] For each of the present compounds, structures obtained using the AMBER* and MM2* force fields did not differ significantly, and thus only the AMBER* results are discussed below.

Table 2. [^{3}H]Ro 15-4513 Binding in the $\alpha_6\beta_2\gamma_2$ Subtype

compd	% inhibition at 100 nM[a] ($\alpha_6\beta_2\gamma_2$)	K_i (nM)[a] ($\alpha_6\beta_2\gamma_2$)
4	0.0 ± 0.1	>589
18a	35.0 ± 0.9	>589
18b	13.6 ± 0.3	>589
27	36.4 ± 0.7	>589
33a	14.3 ± 0.7	>589
33b	20.9 ± 0.1	>589
33c	16.3 ± 0.2	>589
44	13.5 ± 0.5	>589
45	14.7 ± 0.1	>589
1		603
3		13.2
Ro 15-4513	86.6 ± 1.2	2.3 ± 0.4
diazepam		>7000

[a] The ability of the compounds to displace [^{3}H]Ro 15-4513 in membranes from Sf-9 insect cells expressing the $\alpha_6\beta_2\gamma_2$ subtype as described in the Experimental Section.

With the exception of **3**, the minimum energy conformer of all tetracyclic compounds contained a planar or near-planar ring system, with the oxadiazole substituent in a nearly coplanar orientation relative to the tetracyclic rings, as illustrated in Figure 4a for **4**. For compound **3**, Figure 4b shows that the oxadiazole remained in a near-planar orientation, while the five-membered saturated ring was projected well below the imidazoquinoxaline plane. For each molecule studied, the preferred orientation of the oxadiazole ring is that shown in Figure 4.

For analogs **18a**,**b** and **33b**, one of the substituents attached to the bottom ring was positioned in an out-of-plane orientation in the respective minimum energy structures, as shown in Figure 5. In **18b**, the phenyl substituent is shown in an axial position, which was favored energetically over the equatorial position. The BOC group in **44** was found to be out-of-plane as well, with several different conformations of this substituent providing the same minimum energy. For the remaining substituted analogs, **27**, and **33c**, the respective methoxy and methyl groups did not project significantly above or below the plane.

Pharmacophoric Models. In previous reports,[12,37] it was shown that the imidazoquinoxaline series of BzR ligands possess a number of features consistent with models of BzR recognition and activation proposed by Wermuth[38] and Gardner.[39] Similarly, in the present analogs the fused phenyl ring and the N_2 atom represent the aromatic region and hydrogen acceptor site (δ_1), respectively, which are considered to be important elements for receptor recognition. Wermuth also proposed a second hydrogen acceptor site (δ_2) positioned approximately 3.2 Å from δ_1. This element is also a feature in a number of other models such as those presented by Cook[40] and Loew,[41] in which larger $\delta_1-\delta_2$ separations are allowed in accordance with a proposed

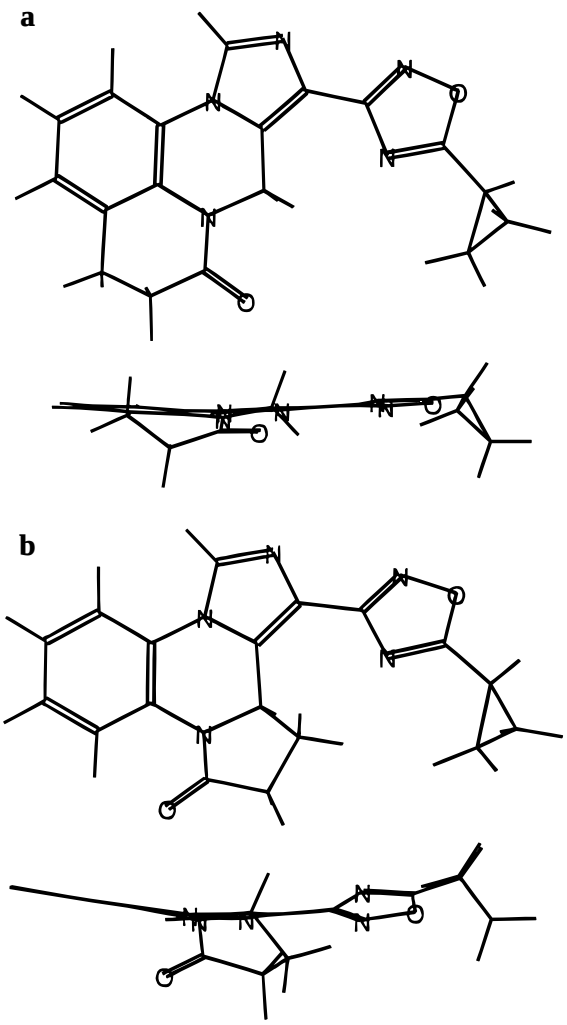


Figure 4. Molecular structures of the minimum energy conformer of (a) **4** and (b) **3**.

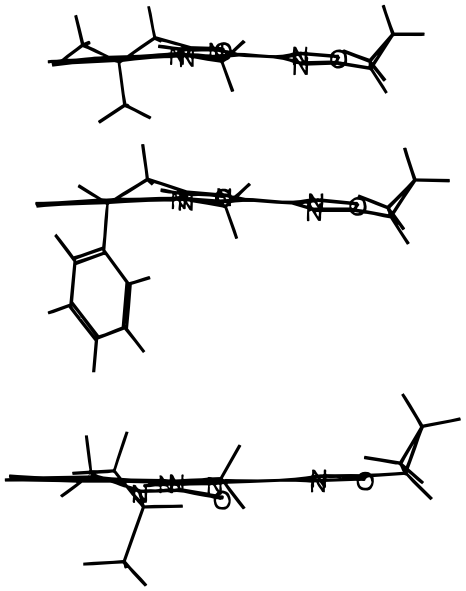


Figure 5. Edge views of the structures of **18a**,**b**, and **33b**.

more flexible binding site. Under this more relaxed condition, it is reasonable to assign the carbonyl group in **4** and related analogs as δ_2.

The oxadiazole group represents the "freely rotating aromatic" group cited by Wermuth, which can influence both binding affinity and efficacy. In a previous investigation of 3-substituted imidazoquinoxalines,[37] it was shown that binding affinity was enhanced provided that a planar 3-substituent could orient coplanar with the imidazoquinoxaline ring and was adversely affected by 3-substituents restrained in non-coplanar positions. In this study, a coplanar 3-substituent was found in all minimum energy structures, which correlates well with the generally high affinity of the present analogs.

According to Wermuth, an out-of-plane substituent (located below the bottom ring in the present analogs) represents an important structural feature which has been correlated with full agonist activity. Among the present structures, **18a**,**b** were full agonists with moderately out-of-plane regions. However, a number of analogs, such as **27** and **33a**, were partial agonists with planar structures, while others such as **33b** were also partial agonists with out-of plane groups. Thus, for the present analogs the connection between agonist activity and an out-of-plane region appears to be less well established than in other BzR ligands, although an out-of-plane substituent is required for full agonist activity.

Conclusions

Constraining the appended substituent of **2** into a tetracyclic imidazo[1,5-*a*]quinoxaline ring system provided a series of high-affinity ligands for the $GABA_A$/benzodiazepine receptor complex. Only the *tert*-butyl carbamate analog **44** had poor binding affinity. In addition, this constraint, which forces the carbonyl group into a relatively planar ring system, provided analogs with an antagonist to partial agonist intrinsic efficacy profile as indicated by TBPS shift and Cl^- current measurement. Only **18a**,**b**, which contain out-of-plane substituents at the 4-position, were nearly full agonists (*in vitro*). Most analogs were active in a metrazole antagonism assay consistent with anticonvulsant and possible anxiolytic activity. While the most effective analogs in this assay contained out-of-plane 4-substituents, several of the planar derivatives, including **4**, were surprisingly effective, especially considering their intrinsic efficacy. In contrast to **3**, none of the analogs reported herein had reasonable affinity for the diazepam insensitive $\alpha_6\beta_2\delta_2$ subtype. The dramatic selectivity differences observed between these two series helps to define the structural requirements of the $\alpha_6\beta_2\delta_2$ subtype. Clearly, the orientation of the carbonyl group (and added steric bulk) in **4** and related analogs prevents effective interaction with the $\alpha_6\beta_2\delta_2$ subtype in contrast to the nonplanar lactam ring of **3**. In addition, orientating the carbonyl group away from the aryl ring in a planar configuration provides analogs with partial agonist (or antagonist) properties which can, however, be overridden by bulky out-of-plane substituents to provide full agonists.

Experimental Section

Mass spectra, infrared spectra, and elemental analyses were performed by the Physical and Analytical Chemistry Department, Upjohn Laboratories, The Upjohn Co. ^{1}H NMR spectra were recorded on a Bruker 300 MHz instrument. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. THF was dried over sodium and distilled from benzophenone ketyl. DMF was dried over 3Å molecular sieves, and acetonitrile was distilled from calcium hydride.

When an aqueous workup is indicated (organic solvent, brine, drying agent), the procedure was to quench the reaction with H_2O, extract the aqueous layer several times with the

organic solvent, wash the combined organic solvents with H_2O and brine, dry the organic layer with the indicated drying agent, filter off the drying agent, and remove the solvent under reduced pressure. When a basic workup is indicated, the procedure is analogous to the aqueous workup; however, the indicated base was used.

3-Bromo-2-chloronitrobenzene (6). A mixture of 2-chloro-3-nitrobenzoic acid (**5**; 15.0 g, 74.4 mmol), red mercury oxide (24.2 g, 112 mmol), and CCl_4 (350 mL) was irradiated with a 100 W light bulb and heated to reflux. Bromine (5.75 mL, 112 mmol) was added dropwise over 30 min, and the reaction mixture was heated at reflux for 3.5 h. After cooling to room temperature, aqueous $NaHCO_3$ was added and the mixture was stirred vigorously for 20 min. The mixture was filtered, and the solids were washed with excess CH_2Cl_2. The organic fractions were combined and washed with $NaHCO_3$, H_2O, and brine, and dried ($MgSO_4$). The mixture was filtered and concentrated to provide 16.8 g (96%) of **6** as a crystalline, pale yellow solid (mp 56−58 °C): IR (mineral oil) 1527, 1427, 1053, 794 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (dd, J = 8.1, 1.5 Hz, ArH), 7.73 (dd, J = 8.1, 1.5 Hz, ArH), 7.31 (apparent t, J = 8.1 Hz, ArH); MS (EI) m/e 237, 235, 191, 179, 110. Anal. ($C_6H_3BrClNO_2$) C, H, Br, Cl, N.

2-Bromo-*N*-*tert*-butyl-6-nitroaniline (7). A bomb was charged with a mixture of **6** (10.0 g, 42.3 mmol), *tert*-butylamine (40 mL), and EtOH (20 mL). The mixture was heated at 150 °C for 4 days. After cooling to −78 °C, the bomb was vented and opened. After a basic workup (ethyl acetate, $NaHCO_3$, H_2O, brine, $MgSO_4$) the residue was purified by flash chromatography (4:1 hexane:ethyl acetate) to provide 10.8 g (93%) of **7** as a yellow oil: IR (neat) 3360, 2969, 1531, 1442, 1365, 1347, 1192 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (dd, J = 8.0, 1.6 Hz, ArH), 7.71 (dd, J = 8.1, 1.5 Hz, ArH), 7.01 (apparent t, J = 8.0 Hz, ArH), 4.46 (s, NH), 1.21 (s, tBuN); MS (EI) m/e 272, 259, 257, 218, 216, 172; HRMS (EI) calcd for $C_{10}H_{13}BrN_2O_2$ (M^+) 272.0161, found 272.0172.

3′-Bromo-2′-(*tert*-butylamino)-2-chloroacetanilide (8). To a mixture of **7** (10.5 g, 38.4 mmol), sodium acetate (174 g, 2.12 mol), MeOH (150 mL), and H_2O (75 mL) was added aqueous titanium trichloride (20%, 250 mL) over a period of 20 min. After the mixture had stirred for 2 h at room temperature, the reaction was quenched by the slow addition of a saturated aqueous $NaHCO_3$ solution. The mixture was extracted with ethyl acetate, and the combined organic layers were washed with $NaHCO_3$, H_2O, and brine and dried ($MgSO_4$). The mixture was filtered and concentrated to provide 9.10 g (97%) of the amine intermediate as a pale yellow oil: IR (neat) 3450, 3353, 2970, 1602, 1572, 1468, 1216 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92 (dd, J = 8.1, 1.4 Hz, ArH), 6.75 (apparent t, J = 7.9 Hz, ArH), 6.62 (dd, J = 8.3, 1.4 Hz, ArH), 4.11 (br s, NH_2), 3.05 (br s, N**H**tBu), 1.28 (s, $C(CH_3)_3$); MS (EI) m/e 244, 242, 229, 188, 186; HRMS (EI) calcd for $C_{10}H_{15}BrN_2$ (M^+) 242.0419, found 242.0424.

A solution of the above intermediate (8.75 g, 36.0 mmol) and diisopropylethylamine (6.90 mL, 39.5 mmol) in THF (150 mL) was cooled to −60 °C. Chloroacetyl chloride (3.0 mL, 38 mmol) was added dropwise and the solution was allowed to stir for 3 h at −60 °C and for 16 h at room temperature. After a basic workup (ethyl acetate, $NaHCO_3$, H_2O, brine, $MgSO_4$) the residue was purified by flash chromatography ($CHCl_3$) to provide 8.90 g (77%) of **8** as a light brown liquid: IR (neat) 3322, 2973, 1687, 1578, 1516, 1426 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.70 (br s, CONH), 8.34 (d, J = 8.1 Hz, ArH), 7.34 (d, J = 7.1 Hz, ArH), 7.02 (apparent t, J = 8.1 Hz, ArH), 4.20 (s, $COCH_2$), 3.15 (br s, N**H**tBu), 1.26 (s, $C(CH_3)_3$); MS (EI) m/e 318, 305, 303, 215, 213, 185; HRMS (EI) calcd for $C_{12}H_{16}BrClN_2O$ (M^+) 318.0135, found 318.0134.

5-Bromo-4-*tert*-butyl-1,2,3,4-tetrahydroquinoxalin-2-one (9). A mixture of **8** (8.55 g, 26.7 mmol), diisopropylethylamine (6.00 mL, 34.4 mmol), sodium iodide (0.570 g, 3.80 mmol), and acetonitrile (100 mL) was heated at reflux for 22 h. After cooling to room temperature, concentration, and a basic workup (ethyl acetate, $NaHCO_3$, brine, $MgSO_4$), the residue was purified by flash chromatography (9:1 ethyl acetate:MeOH) to provide 5.95 g (79%) of **9** as an off-white solid (mp 278−280 °C): IR (mineral oil) 3045, 1689, 1572, 1427, 1365 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.49 (br s, CONH), 7.26 (d, J = 6.4 Hz, ArH), 6.96 (apparent t, J = 7.9 Hz, ArH), 6.84 (d, J = 7.9 Hz, ArH), 3.72 (ABq, J_{AB} = 17.3 Hz, $\Delta\nu$ = 159 Hz, NCH_2), 1.29 (s, $C(CH_3)_3$); MS (EI) m/e 282, 267, 228, 226, 199, 197, 118. Anal. ($C_{12}H_{15}BrN_2O$) C, H, Br, N.

5-Bromo-1,2,3,4-tetrahydroquinoxalin-2-one (11). To a solution of trifluoroacetic acid (50 mL) at 0 °C was added **9** (4.50 g, 15.9 mmol) over a period of 5 min. The solution was allowed to warm to room temperature and stir for 20 h. The solution was concentrated under reduced pressure and subjected to a basic workup (CH_2Cl_2, $NaHCO_3$, brine, $MgSO_4$) to give a 6:1 mixture[42] of **11** and **10**. The crude products were combined with MeOH (20 mL), EtOH (20 mL), and sodium borohydride (0.20 g, 5.3 mmol). After stirring for 6 h at room temperature, the mixture was concentrated under reduced pressure and diluted with CH_2Cl_2, and the reaction was quenched with aqueous ammonium chloride. After an aqueous workup (CH_2Cl_2, H_2O, $MgSO_4$) the material was purified by recrystallization (ethyl acetate) to provide 2.36 g (65%) of **11** as a pale yellow solid (mp 194−195 °C): IR (mineral oil) 3392, 1688, 1501, 1293 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.46 (s, CONH), 7.03 (d, J = 8.0 Hz, ArH), 6.72 (d, J = 7.6 Hz, ArH), 6.53 (apparent t, J = 7.9 Hz, ArH), 5.68 (br s, NH), 3.79 (s, NCH_2); MS (EI) m/e 228, 226, 199, 197, 118, 90. Anal. ($C_8H_7BrN_2O$) C, H, Br, N.

3-(1,2,3,4-Tetrahydro-2-oxo-5-quinoxalinyl)-(*E*)-2-propenoic Acid Ethyl Ester (12). A solution of **11** (1.67 g, 7.35 mmol), ethyl acrylate (1.60 mL, 14.8 mmol), triethylamine (1.95 mL, 14.0 mmol), and tetrakis(triphenylphosphine)-palladium(0) (0.15 g) in DMF (10 mL) was heated at 120 °C for 6 h. After cooling to room temperature and a basic workup (CH_2Cl_2, $NaHCO_3$, brine, $MgSO_4$), the residue was recrystallized from ethyl acetate to provide 0.945 g of **12** as an orange crystalline solid. The recrystallization filtrates were concentrated, and the residue was purified by flash chromatography (9:1 CH_2Cl_2:MeOH) to provide an additional 0.20 g (1.15 g total, 63%) of **12** (mp 227−230 °C): IR (mineral oil) 3409, 1691, 1684, 1616, 1316, 1189 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.38 (s CONH), 7.89 (d, J = 15.6 Hz, CH), 7.21 (d, J = 7.8 Hz, ArH), 6.77 (d, J = 7.5 Hz, ArH), 6.61 (apparent t, J = 7.8 Hz, ArH), 6.45 (s, NH), 6.42 (d, J = 15.6 Hz, CH), 4.18 (q, J = 7.1 Hz, $OC\mathbf{H}_2CH_3$), 3.78 (narrow m, NCH_2), 1.26 (t, J = 7.1 Hz, $OCH_2C\mathbf{H}_3$); MS (EI) m/e 246, 201, 171, 145, 143, 117. Anal. ($C_{13}H_{14}N_2O_3$) H, N; C: calcd, 63.40; found, 62.92.

6,7-Dihydro-1*H*,5*H*-pyrido[1,2,3-*de*]quinoxaline-2,5(3*H*)-dione (13). A mixture of **12** (0.73 g, 2.96 mmol), *p*-toluenesulfonic acid monohydrate (0.15 g), palladium on charcoal (5%, 0.33 g), ethanol (50 mL), and methanol (50 mL) was hydrogenated (40 psi) for 40 h at room temperature. The mixture was filtered, and the solids were washed with copious amounts of methanol and CH_2Cl_2. The filtrates were combined and concentrated under reduced pressure. The residue was purified by flash chromatography (9:1 CH_2Cl_2:MeOH) to provide 0.47 g (79%) of **13** as a white solid (mp 262−264 °C): IR (mineral oil) 1682, 1665, 1615, 1603, 1490, 1444, 1406 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.71 (s, CONH), 6.75−6.95 (m, ArH, 3 H), 4.33 (s, NCH_2), 2.88 (t, J = 7.9 Hz, 2 H), 2.58 (t, J = 8.0 Hz, 2 H); MS (EI) m/e 202, 173, 159, 145. Anal. ($C_{11}H_{10}N_2O_2 \cdot (H_2O)_{1/10}$) C, H, N.

9-(5-Cyclopropyl-1,2,4-oxadiazol-3-yl)-4,5-dihydro-6*H*,8*H*-imidazo[1,5-*a*]pyrido[1,2,3-*de*]quinoxalin-6-one (4). A solution of **13** (0.370 g, 1.83 mmol) in THF (15 mL) and DMF (8 mL) was cooled to −65 °C, and potassium *tert*-butoxide (1.0 M in THF, 2.10 mL, 2.10 mmol) was added. The mixture was allowed to warm to 15 °C over 2.5 h followed by cooling to −40 °C. Diethyl chlorophosphate (0.30 mL, 2.1 mmol) was added, and the mixture was allowed to stir for 2 h while slowly warming to 15 °C. The mixture was cooled to −40 °C, and isocyanide **15** (0.360 g, 2.41 mmol) was added followed by the dropwise addition of potassium *tert*-butoxide (1.0 M in THF, 2.30 mL, 2.30 mmol). The mixture was allowed to warm to room temperature and stir for 16 h. After the reaction was quenched with aqueous ammonium chloride and after an aqueous workup (ethyl acetate, $MgSO_4$), the residue was purified by flash chromatography (9:1 CH_2Cl_2:MeOH) to provide 0.201 g (33%) of **4** as a white powder (mp 257−259

°C): IR (mineral oil) 1661, 1577, 1505, 1395, 1319, 1210, 1188 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, ArH), 7.4−7.5 (m, ArH), 7.1−7.15 (m, ArH, 2 H), 5.40 (s, NCH_2), 3.03 (dd, J = 8.4, 6.9 Hz, 2 H), 2.73 (dd, J = 8.4, 6.9 Hz, 2 H), 2.2−2.35 (m, CH), 1.2−1.4 (m, CH_2CH_2); MS (EI) *m/e* 333, 292, 265, 69; HRMS (EI) calcd for $C_{18}H_{15}N_5O_2$ (M^+) 333.1226, found 333.1246. Anal. ($C_{18}H_{15}N_5O_2 \cdot (H_2O)_{1/2}$) C, H, N.

3-(5-Cyclopropyl-1,2,4-oxadiazol-3-yl)-4,5-dihydro-5-(1-oxo-3-methyl-2-butenyl)imidazo[1,5-*a*]quinoxaline (17a). A solution of **16** (0.53 g, 1.9 mmol), triethylamine (0.40 mL, 2.9 mmol), 4-(dimethylamino)pyridine (0.047 g), CH_2Cl_2 (20 mL), and DMF (4 mL) was cooled to 0 °C. A solution of 3,3-dimethylacryloyl chloride (0.30 mL, 2.7 mmol) in CH_2Cl_2 (4 mL) was added dropwise, and the reaction mixture was allowed to stir for 16 h at room temperature. After a basic workup (CH_2Cl_2, $NaHCO_3$, H_2O, brine, $MgSO_4$), the residue was purified by flash chromatography (ethyl acetate) to provide 0.44 g (64%) of **17a** as an off-white solid (mp 74−77 °C): IR (mineral oil) 1659, 1576, 1507, 1498, 1365, 1165 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.13 (s, ArH), 7.20−7.60 (m, ArH, 4 H), 5.87 (s, 0.5 H, rotamer), 5.15−5.40 (m, 1.5 H, rotamer), 4.70−4.95 (m, 0.5 H, rotamer), 3.15−3.30 (br s, 0.5 H, rotamer), 2.20−2.35 (m, CH), 2.12 (s, CCH_3, 2 H, rotamer), 1.86 (s, CCH_3, 2 H, rotamer), 1.60−1.90 (m, 2 H, rotamer), 1.20−1.40 (m, CH_2CH_2); MS (EI) *m/e* 361, 292, 279, 238, 210, 195, 102; HRMS (EI) calcd for $C_{20}H_{19}N_5O_2$ (M^+) 361.1539, found 361.1560. Anal. ($C_{20}H_{19}N_5O_2 \cdot (H_2O)_{1/5}$) C, H, N.

9-(5-Cyclopropyl-1,2,4-oxadiazol-3-yl)-4,5-dihydro-4,4-dimethyl-6*H*,8*H*-imidazo[1,5-*a*]pyrido[1,2,3-*de*]quinoxalin-6-one (18a). A mixture of **17a** (0.310 g, 0.858 mmol), freshly sublimed aluminum trichloride (3.80 g, 28.5 mmol), and 1,2-dichlorobenzene (10 mL) was heated at 100 °C for 2.5 h. The mixture was allowed to cool to room temperature, and the reaction was quenched by the slow addition of H_2O. After an aqueous workup (CH_2Cl_2, H_2O, brine, $MgSO_4$), the residue was purified by flash chromatography (19:1 CH_2Cl_2:MeOH) to provide 0.195 g (63%) of **18a** as a white powder (mp 199−202 °C): IR (mineral oil) 1672, 1574, 1364, 1319 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.13 (s, ArH), 7.47 (d, J = 7.7 Hz, ArH), 7.15−7.30 (m, ArH, 2 H), 5.40 (s, NCH_2), 2.58 (s, $COCH_2$), 2.20−2.35 (m, CH), 1.36 (s, $C(CH_3)_2$, 6 H), 1.20−1.35 (m, CH_2CH_2); MS (EI) *m/e* 361, 292, 277, 263, 250, 235. Anal. ($C_{20}H_{19}N_5O_2 \cdot (H_2O)_{1/5}$) C, H, N.

1,2,3,4-Tetrahydro-4-(1-oxo-3-phenyl-(*E*)-2-propenyl)-quinoxalin-2-one (20). Cinnamoyl chloride (4.66 g, 28.0 mmol) was added in portions over 10 min to a mixture of **22** (3.15 g, 21.3 mmol), K_2CO_3 (5.00 g, 36.2 mmol), H_2O (20 mL), and acetone (10 mL) at 0 °C. The mixture was stirred at 0 °C for 20 min and at room temperature for 3 h. The mixture was poured into ice water. The solids were filtered, washed with H_2O and hot CH_2Cl_2, and dried to give 5.10 g (86%) of **20** as a cream-colored solid (mp 271−273 °C): IR (mineral oil) 1690, 1659, 1503, 1361, 751 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 10.82 (s, NH), 7.50−7.75 (m, 3 H), 7.15−7.45 (m, 5 H), 6.85−7.15 (m, 3 H), 4.46 (s, NCH_2CO); MS (EI) *m/e* 278, 131, 103; HRMS (EI) calcd for $C_{17}H_{14}N_2O_2$ (M^+) 278.0155, found 278.1068.

1*H*,5*H*-Pyrido[1,2,3-*de*]quinoxaline-2,5(3*H*)-dione (21). A mixture of **20** (2.22 g, 7.98 mmol), freshly sublimed aluminum trichloride (10.0 g, 75.0 mmol), and 1,2-dichlorobenzene (20.0 mL) was heated at 120 °C for 4 h and allowed to cool to room temperature. The mixture was poured into 100 mL of ice−water and allowed to stand for 1 h. The resultant precipitate was filtered, washed with copious amounts of water and hexane, and dried *in vacuo* to give 1.56 g (98%) of **21** as an off-white solid (mp >300 °C): IR (mineral oil) 1693, 1632, 1593, 1566, 1404 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 11.04 (s, NH), 7.94 (d, J = 9.6 Hz, C**H**=CH), 7.33 (d, J = 6.6 Hz, ArH), 7.16 (apparent t, J = 7.7 Hz, ArH), 7.06 (d, J = 7.3 Hz, ArH), 6.65 (d, J = 9.5 Hz, CH=C**H**), 4.52 (s, NCH_2CO); MS (EI) *m/e* 200, 171. Anal. ($C_{11}H_8N_2O_2 \cdot (H_2O)_{4/5}$) C, N; H: calcd, 4.51; found, 4.05.

4-(2-Diazoacetyl)-1,2,3,4-tetrahydroquinoxalin-2-one (23). A mixture of **22** (1.48 g, 9.99 mmol), diketene (1.00 mL, 13.0 mmol), 4-(dimethylamino)pyridine (0.020 g), and CH_2Cl_2 (20 mL) was heated at reflux for 2 h. After cooling to room temperature and an aqueous workup (CH_2Cl_2, H_2O, brine, $MgSO_4$), a semisolid was obtained which solidified overnight to provide 1.55 g (67%) of the acylated intermediate as a white solid (mp 139−142 °C). To a solution of the above intermediate (1.20 g, 5.17 mmol) and triethylamine (1.00 mL, 7.17 mmol) in acetonitrile (25 mL) was added mesyl azide[21] (1.00 g, 8.26 mmol). The mixture was allowed to stir for 8 h at room temperature followed by a basic workup (ethyl acetate, $NaHCO_3$, H_2O, brine, $MgSO_4$). The resulting residue was triturated with diethyl ether−hexane to provide 1.22 g (91%) of the diazo compound as a yellow solid (mp 158−160 °C).

To a solution of the above diazo intermediate (1.25 g, 4.84 mmol) in THF (25 mL) was added 10% NaOH (5 mL). After stirring for 1 h at room temperature, the mixture was subjected to an aqueous workup (ethyl acetate, brine, $MgSO_4$), and the residue was triturated with diethyl ether to provide 0.720 g (69%) of **23** as a pale yellow powder (mp 185 °C dec): IR (mineral oil) 2135, 1686, 1622, 1501 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 10.74 (s, CONH), 7.45 (d, J = 7.8 Hz, ArH), 7.20 (apparent t, J = 6.9 Hz, ArH), 7.00−7.10 (m, ArH, 2 H), 5.92 (s, 1 H), 4.30 (s, NCH_2); MS (EI) *m/e* 216, 188, 160, 148, 131, 118; HRMS (EI) calcd for $C_{10}H_8N_4O_2$ (M^+) 216.0647, found 216.0661.

1*H*-Pyrrolo[1,2,3-*de*]quinoxaline-2,5(3*H*,6*H*)-dione (24). To a solution of rhodium(II) trifluoroacetate dimer[22] (40.0 mg, 0.061 mmol) in CH_2Cl_2 (50 mL) was added a slurry of **23** (390 mg, 1.80 mmol) in CH_2Cl_2 (50 mL) over 20 min at room temperature. After an additional 10 min the solvent was removed under reduced pressure, and the residue was triturated with diethyl ether−hexane to give a solid which was further purified by flash chromatography (9:1 CH_2Cl_2:MeOH) to provide 0.204 g (60%) of **24** as an off-white solid (mp 222−226 °C): IR (mineral oil) 1718, 1713, 1684, 1647, 1502 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 10.68 (s, CONH), 6.8−6.9 (m, ArH, 2 H), 6.65−6.75 (m ArH), 4.31 (s, 2 H), 3.59 (s, 2 H); MS (EI) *m/e* 188, 159, 131. Anal. ($C_{10}H_8N_2O_2 \cdot (H_2O)_{1/4}$) C, H, N.

3-(5-Cyclopropyl-1,2,4-oxadiazol-3-yl)-*N*-methoxy-imidazo[1,5-*a*]quinoxaline-5(4*H*)-carboxamide (26). A solution of **16** (1.33 g, 4.76 mmol) and diisopropylethylamine (0.95 mL, 5.5 mmol) in THF (40 mL) was cooled to 0 °C. Triphosgene (0.510 g, 1.72 mmol) was added, and the solution was allowed to stir for 3 h while warming to room temperature. The solution was then cooled to 0 °C, diisopropylethylamine (2.0 mL, 11 mmol) and methoxylamine hydrochloride (0.530 g, 6.35 mmol) were added, and the mixture was allowed to warm to room temperature and stir for 16 h. After a basic workup (ethyl acetate, $NaHCO_3$, brine, $MgSO_4$), the residue was purified by flash chromatography (ethyl acetate) to provide 1.10 g (66%) of **26** as a white solid (mp 207−209 °C): IR (mineral oil) 1672, 1571, 1500, 1482, 1279 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1 H), 8.02 (s, 1 H), 7.60−7.70 (m, ArH), 7.45−7.55 (m, ArH), 7.25−7.35 (m, ArH, 2 H), 5.20 (s, NCH_2), 3.79 (s, OCH_3), 2.15−2.30 (m, CH), 1.20−1.40 (m, CH_2CH_2); MS (EI) *m/e* 352, 278, 238, 210, 195; HRMS (EI) calcd for $C_{17}H_{16}N_6O_3$ (M^+) 352.1284, found 352.1306. Anal. ($C_{17}H_{16}N_6O_3$) C, H, N.

8-(5-Cyclopropyl-1,2,4-oxadiazol-3-yl)-4-methoxy-7*H*-diimidazo[1,5-*a*:1′,5′,4′-*de*]quinoxalin-5(4*H*)-one (27). A solution of **26** (0.62 g, 1.76 mmol) in CH_2Cl_2 (15 mL) was cooled to −40 °C. A solution of [bis(trifluoroacetoxy)iodo]benzene (0.820 g, 1.91 mmol) in CH_2Cl_2 (5 mL) was added dropwise, and the reaction mixture was allowed to stir for 3 h while warming to 5 °C. After a basic workup (CH_2Cl_2, Na_2CO_3, brine, $MgSO_4$) the residue was purified by flash chromatography (90:9:1 ethyl acetate:CH_2Cl_2:MeOH) to provide 0.16 g (26%) of **27** as a white solid (mp 232−234 °C): IR (mineral oil) 1718, 1521, 1508 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.81 (s, ArH), 7.61 (d, J = 7.7 Hz, ArH), 7.10−7.20 (m, 2 H, ArH), 5.32 (s, NCH_2), 4.03 (s, $NOCH_3$), 2.35−2.50 (m, CH), 1.15−1.35 (m, CH_2CH_2); MS (EI) *m/e* 350, 319, 281, 250, 236. Anal. ($C_{17}H_{14}N_6O_3 \cdot (H_2O)_{1/5}$) C, H, N.

2-Amino-3-nitrobenzoic Acid (28). A solution of 2-chloro-3-nitrobenzoic acid (**5**; 5.00 g, 24.8 mmol), ammonium hydroxide (25.0 mL), and copper(I) chloride (50 mg) was heated in a bomb at 125 °C for 20 h and allowed to cool to room temperature. The solid residue was dissolved in water and the solution acidified with 3 N HCl. The resultant orange

precipitate was filtered, washed, and dried to provide 4.51 g (100%) of **28** as a yellow powder (mp 201−203 °C): IR (mineral oil) 3478, 3345, 1692, 1677, 1571, 1559, 1520, 1512, 1442, 1271, 1259, 1130 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$−MeOD) δ 8.38 (d, J = 8.5 Hz, ArH), 8.30 (d, J = 6.0 Hz, ArH), 6.66 (apparent t, J = 8.0 Hz, ArH); MS (EI) *m/e* 182, 164.

2-Amino-3-nitrobenzyl Alcohol (29a). Borane−methyl sulfide complex (10.0 M, 1.20 mL, 12.0 mmol) was added to a mixture of **28** (1.05 g, 5.76 mmol) and THF (17.4 mL). The mixture was stirred for 3 h at room temperature and 16 h at reflux. After the mixture had cooled to room temperature, the reaction was quenched with 10% HCl. Basic workup (CH_2Cl_2, $NaHCO_3$, $MgSO_4$) gave 882 mg (91%) of **29a** as an orange solid homogeneous by TLC analysis (mp 100−101 °C): IR (mineral oil) 3488, 3466, 3365, 3346, 1641, 1515, 1428, 1250, 1015, 745 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, J = 7.2 Hz, ArH), 7.30 (d, J = 7.7 Hz, ArH), 6.85 (br s, NH_2), 6.65 (apparent t, J = 7.9 Hz, ArH), 4.78 (s, CH_2O); MS (EI) *m/e* 168, 150, 121, 105.

1,4-Dihydro-8-nitro-2*H*-3,1-benzoxazin-2-one (30a). A solution of **29a** (1.70 g, 10.1 mmol), THF (86.0 mL), and 1,1′-carbonyldiimidazole (1.81 g, 11.2 mmol) was heated at reflux for 48 h. After cooling to room temperature, aqueous workup (CH_2Cl_2, $MgSO_4$) provided **30a** as a solid. Recrystallization from ethyl acetate−hexane (two lots) gave 1.84 g (94%) of **30a** as an orange powder (mp 181−182.5 °C): IR (mineral oil) 3314, 1779, 1765, 1619, 1530, 1466, 1351, 1243, 1228, 1061, 765, 736 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.68 (br s, NH), 8.22 (d, J = 8.2 Hz, ArH), 7.46 (d, J = 7.3 Hz, ArH), 7.19 (apparent t, J = 8.0 Hz, ArH), 5.37 (s, OCH_2); MS (EI) *m/e* 194, 150.

8-(2-Chloroacetamido)-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (31a). A mixture of **30a** (195 mg, 1.00 mmol), ethanol (20.0 mL), and 10% Pd/C (40 mg) was hydrogenated (44 psi) at room temperature for 4.5 h. The mixture was filtered, the residue was washed with ethanol, MeOH, and CH_2Cl_2, and the combined filtrates were concentrated to provide 149 mg (91%) of the amine intermediate as a white powder (mp 198−200 °C): IR (mineral oil) 3465, 1713, 1697, 1633, 1414, 1289, 1048 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.13 (s, NH), 6.89 (apparent t, J = 7.7 Hz, ArH), 6.72 (d, J = 8.4 Hz, ArH), 6.57 (d, J = 7.7 Hz, ArH), 5.29 (s, OCH_2); MS (EI) *m/e* 164, 120, 105, 93.

Chloroacetyl chloride (0.39 mL, 4.9 mmol) was added to a solution of the above intermediate (708 mg, 4.31 mmol), diisopropylethylamine (1.71 mL, 9.82 mmol), and THF (50.0 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and for 16 h at room temperature. The mixture was diluted with 75 mL of water, filtered, and washed several times with water and ethyl acetate to give 612 mg of **31a** as a white powder. Aqueous workup (ethyl acetate, $MgSO_4$) of the filtrate provided an additional 218 mg (830 mg total, 80%) of **31a** (mp 255−257 °C): IR (mineral oil) 1715, 1704, 1679, 1555, 1475, 1459, 1267, 1062, 780 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.69 (s, NH, 2 H), 7.27 (d, J = 7.7 Hz, ArH), 7.10 (d, J = 7.7 Hz, ArH), 7.02 (apparent t, J = 7.7 Hz, ArH), 5.29 (s, CH_2O), 4.29 (s, $COCH_2Cl$); MS (EI) *m/e* 240, 191, 147.

1*H*,5*H*,7*H*-Pyrazino[3,2,1-*ij*][3,1]benzoxazine-2,5(3*H*)-dione (32a). Potassium *tert*-butoxide (1.0 M in THF, 3.50 mL, 3.50 mmol) was added to a mixture of **31a** (822 mg, 3.42 mmol) and THF (70.0 mL) at 0 °C. The mixture was stirred for 1 h at 0 °C and for 16 h at room temperature. The reaction was quenched with aqueous ammonium chloride. Aqueous workup (ethyl acetate, $MgSO_4$) and trituration of the resultant solid with ether provided 482 mg (69%) of **32a** as a light yellow powder (mp 255−256 °C): IR (mineral oil) 1705, 1630, 1504, 1410, 1271 cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6) δ 9.80 (br s, NH), 7.05 (apparent t, J = 7.6 Hz, ArH), 6.98 (d, J = 6.4 Hz, ArH), 6.92 (d, J = 6.3 Hz, ArH), 5.32 (s, OCH_2), 4.48 (s, NCH_2); MS (EI) *m/e* 204, 160, 131.

9-(5-Cyclopropyl-1,2,4-oxadiazol-3-yl)-4*H*,6*H*,8*H*-imidazo[1′,5′:4,5]pyrazino[3,2,1-*ij*][3,1]benzoxazin-6-one (33a). Potassium *tert*-butoxide (1.0 M in THF, 2.30 mL, 2.30 mmol) was added to a mixture of **32a** (425 mg, 2.08 mmol), THF (2.10 mL), and DMF (1.30 mL) at 0 °C. The resultant thick mixture was allowed to warm to room temperature and was stirred for 30 min. After cooling to −20 °C, diethyl chlorophosphate (0.39 mL, 2.7 mmol) was added and the mixture was allowed to warm to room temperature. THF (4.0 mL) and DMF (1.0 mL) were added to the mixture to form a solution which was stirred at room temperature for 45 min. After cooling to −78 °C, a solution of isocyanide **15** (339 mg, 2.27 mmol) and THF (0.80 mL) was added followed by dropwise addition of potassium *tert*-butoxide (1.0 M in THF, 2.30 mL, 2.30 mmol) over 5 min. The reddish solution was stirred at −78 °C for 30 min and was allowed to warm to room temperature over 2 h. After the mixture stirred at room temperature for an additional 2 h, the reaction was quenched with aqueous ammonium chloride. Aqueous workup (ethyl acetate, $MgSO_4$) and purification by flash chromatography (ethyl acetate) gave 223 mg (32%) of **33a**. An analytical sample was prepared by recrystallization from hot ethyl acetate−hexane to provide a light yellow powder (mp 235−237 °C): IR (mineral oil) 1710, 1574, 1510, 1456, 1419, 1399, 1377, 1293, 1201 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (s, ArH), 7.58 (d, J = 7.7 Hz, ArH), 7.22 (apparent t, J = 7.7 Hz, ArH), 7.09 (d, J = 7.7 Hz, ArH), 5.48 (s, NCH_2), 5.34 (s, OCH_2), 2.2−2.35 (m, C**H**CH_2), 1.2−1.4 (m, CH_2CH_2); MS (EI) *m/e* 335, 291, 266, 222, 207. Anal. ($C_{17}H_{13}N_5O_3$·($C_4H_8O_2$)$_{1/2}$·(H_2O)$_{1/4}$) C, H, N.

***N*-(2-Amino-3-nitrobenzyl)isopropylamine (29b).** A solution of methanesulfonyl chloride (69.0 mg, 0.602 mmol) in THF (0.50 mL) was added to a solution of **29a** (100 mg, 0.595 mmol) and diisopropylethylamine (0.12 mL, 0.69 mmol) in THF (4.0 mL) at 0 °C. After 2.5 h at 0 °C, isopropylamine (0.51 mL, 6.0 mmol) was added. The solution was stirred for an additional 1 h at 0 °C and allowed to warm to room temperature. After 24 h, basic workup (ethyl acetate, $NaHCO_3$, $MgSO_4$) and purification by flash chromatography (2:1 hexane:ethyl acetate) gave 88.0 mg (71%) of **29b** as an orange oil: IR (neat) 3451, 3230, 2966, 1622, 1576, 1518, 1453, 1440, 1355, 1333, 1257, 743 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, J = 8.4 Hz, ArH), 7.72 (br s, NH_2), 7.23 (d, J = 6.9 Hz, ArH), 6.57 (dd, J = 8.7, 7.0 Hz, ArH), 3.90 (s, NCH_2), 2.7−2.9 (m, N**C**H$(CH_3)_2$), 1.12 (d, J = 6.3 Hz, CH(C**H**$_3$)$_2$); MS (EI) *m/e* 209, 192, 162, 151.

3,4-Dihydro-5-nitro-2*H*-1,4-benzoxazin-3-one (35). A mixture of 2-amino-3-nitrophenol (**34**; 3.00 g, 19.5 mmol), diisopropylethylamine (4.80 mL, 27.6 mmol), and ethyl bromoacetate (6.0 mL, 54 mmol) was heated at 140 °C for 5 h. The resultant solution was allowed to cool to room temperature. Aqueous workup ($CHCl_3$, $MgSO_4$) and purification by flash chromatography (3:1 hexane:ethyl acetate) gave 4.22 g of a 4:1 mixture[43] of the desired lactam and uncyclized ester intermediate. The crude material was combined with ethanol (300 mL) and K_2CO_3 (300 mg, 2.17 mmol). The mixture was heated at reflux for 72 h. After cooling to room temperature, concentration and aqueous workup (CH_2Cl_2, $MgSO_4$) gave 3.27 g of **35**. Purification by flash chromatography (3:1 hexane:EtOAc) gave 2.59 g (68%) of the product as a yellow solid. Recrystallization from ethyl acetate−hexane provided orange needles (mp 82−85 °C): IR (mineral oil) 3293, 1731, 1538, 1532, 1490, 1344, 1302, 1249, 1180, 735 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.07 (br s, NH), 7.94 (d, J = 8.5 Hz, ArH), 7.32 (d, J = 7.7 Hz, ArH), 7.09 (apparent t, J = 8.3 Hz, ArH), 4.71 (s, OCH_2CO); MS (EI) *m/e* 194.

5*H*-Pyrazino[1,2,3-*de*]-1,4-benzoxazine-3,6(2*H*,7*H*)-dione (37). A mixture of **35** (1.00 g, 5.15 mmol), 10% Pd/C (330 mg), and ethanol (140 mL) was hydrogenated (39 psi) at room temperature for 16 h. The mixture was filtered, the residue was washed with ethanol several times, and the combined filtrates were concentrated to provide 679 mg (80%) of the amine intermediate as a white powder (mp 211−215 °C): IR (mineral oil) 3448, 3350, 3217, 1698, 1687, 1650, 1614, 1451, 1412 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.80 (apparent t, J = 8.1 Hz, ArH), 6.44 (apparent t, J = 7.7 Hz, ArH, 2 H), 4.54 (s, OC**H**$_2$CON), 2.57 (s, NH_2, NH); MS (EI) *m/e* 164, 135.

Chloroacetyl chloride (0.37 mL, 4.6 mmol) was added to a mixture of the above amine (679 mg, 4.14 mmol), THF (55.0 mL), and diisopropylethylamine (1.80 mL, 10.3 mmol) at 0 °C. The mixture was stirred for 1 h at 0 °C and for 16 h at room temperature. Basic workup (ethyl acetate, $NaHCO_3$, $MgSO_4$) gave 921 mg of **36**, sufficiently pure to be carried on crude.

Spectral features for **36**: ^{1}H NMR (300 MHz, acetone-d_6) δ 9.2−9.4 (m, 1 H), 6.8−7.1 (m, ArH, 3 H), 4.54 (s, 2 H), 4.35 (s, 2 H).

Potassium *tert*-butoxide (1.0 M in THF, 4.2 mL, 4.2 mmol) was added to a mixture of the crude chloride **36** (921 mg) and THF (60 mL) at 0 °C. The mixture was stirred for 1 h at 0 °C and for 16 h at room temperature. Aqueous workup (ethyl acetate, $MgSO_4$) and trituration of the residue several times with ethyl acetate provided 310 mg (37%) of **37** as a light brown powder (mp 285−287 °C): IR (mineral oil) 1691, 1674, 1616, 1506, 1400, 1390, 1240 cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6) δ 9.77 (br s, NH), 6.95 (apparent t, J = 8.1 Hz, ArH), 6.71 (d, J = 7.7 Hz, ArH), 6.66 (d, J = 7.7 Hz, ArH), 4.66 (s, OCH_2), 4.43 (s, $COCH_2N$); MS (EI) *m*/*e* 204, 175, 147.

9-(5-Cyclopropyl-1,2,4-oxadiazol-3-yl)-8*H*-imidazo[1′,5′:4,5]pyrazino[1,2,3-*de*][1,4]benzoxazin-6(5*H*)-one (38). Potassium *tert*-butoxide (1.0 M in THF, 2.50 mL, 2.50 mmol) was added to a mixture of **37** (467 mg, 2.29 mmol), THF (4.0 mL), and DMF (4.0 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 30 min. After cooling to −20 °C, diethyl chlorophosphate (0.43 mL, 3.0 mmol) was added, and the mixture was allowed to warm to room temperature. The resultant solution was stirred at room temperature for 40 min and then cooled to −78 °C. A solution of isocyanide **15** (372 mg, 2.49 mmol) in THF (0.80 mL) was added. Potassium *tert*-butoxide (2.50 mL, 2.50 mmol) was then added dropwise over several minutes to form a red solution. This solution was stirred at −78 °C for 30 min and allowed to warm to room temperature over 1.5 h. After the mixture stirred for an additional 3 h at room temperature, the reaction was quenched with aqueous ammonium chloride. Aqueous workup (ethyl acetate and then $CHCl_3$, $MgSO_4$) and purification by flash chromatography (10:1 CH_2Cl_2:acetone) gave 85 mg (11%) of **38** as a white powder. An analytical sample was prepared by recrystallization from MeOH−ether to provide translucent needles (mp 267−268 °C): IR (mineral oil) 1689, 1519, 1507, 1424, 1415, 1402, 1207 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (s, ArH), 7.28 (d, J = 7.2 Hz, ArH), 7.12 (apparent t, J = 8.2 Hz, ArH), 6.95 (d, J = 7.4 Hz, ArH), 5.40 (s, CH_2N), 4.71 (s, OCH_2), 2.15−2.35 (m, C**H**CH_2), 1.2−1.4 (m, CH_2CH_2); MS (EI) *m*/*e* 335, 266, 238, 223, 174. Anal. ($C_{17}H_{13}N_5O_3 \cdot (H_2O)_{1/4}$) C, H, N.

(2-Amino-3-nitrophenyl)carbamic Acid 1,1-Dimethylethyl Ester (40). A solution of 3-nitro-1,2-phenylenediamine (**39**; 5.75 g, 37.5 mmol), di-*tert*-butyl dicarbonate ($(BOC)_2O$; 10.2 g, 46.7 mmol), and triethylamine (5.40 mL, 38.7 mmol) in THF (50 mL) was heated at reflux for 2 days. An additional 3.00 g of $(BOC)_2O$ (60.5 mmol total) was added, and the solution was heated at reflux for an additional 16 h. After cooling to room temperature and a basic workup (CH_2Cl_2, $NaHCO_3$, brine, $MgSO_4$), the residue was purified by flash chromatography (ethyl acetate) to provide 9.25 g (97%) of **40** as a yellow-orange solid (mp 138−140 °C): IR (mineral oil) 1686, 1533, 1509, 1449, 1279, 1255, 1158 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, J = 8.7 Hz, ArH), 7.48 (d, J = 7.4 Hz, ArH), 6.71 (apparent t, J = 7.7 Hz, ArH), 6.26 (br s, NH_2), 6.01 (br s, N**H**CO_2tBu), 1.52 (s, OtBu); MS (EI) *m*/*e* 253, 197, 180, 153. Anal. ($C_{11}H_{15}N_3O_4$) H, N; C: calcd, 52.17; found, 51.76.

3,4-Dihydro-5-nitro-3-oxo-1(2*H*)-quinoxalinecarboxylic Acid 1,1-Dimethylethyl Ester (41). A mixture of sodium hydride (60% in mineral oil, 2.00 g, 50.0 mmol) and THF (200 mL) was cooled to −60 °C. Amine **40** (6.08 g, 24.0 mmol) was added in portions over 10 min, and the mixture was allowed to stir for 0.5 h at −60 °C and for 10 min at 0 °C. The mixture was cooled to −60 °C, and ethyl bromoacetate (2.73 mL, 24.6 mmol) was added dropwise. The mixture was then allowed to slowly warm to room temperature and stir for 16 h, at which time the reaction was quenched by the addition of aqueous ammonium chloride. After an aqueous workup (ethyl acetate, brine, $MgSO_4$), the residue was recrystallized from CH_2Cl_2−hexane to provide 6.00 g (85%) of **41** as a pale yellow powder (mp 118−121 °C): IR (mineral oil) 1722, 1710, 1485, 1365, 1153 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.23 (br s, NHCO), 8.08 (dd, J = 8.5, 1.3 Hz, ArH), 7.96 (d, J = 7.7 Hz, ArH), 7.18 (apparent t, J = 8.3 Hz, ArH), 4.44 (s, NCH_2-CO), 1.54 (s, OtBu); MS (EI) *m*/*e* 293, 237, 220, 193. Anal. ($C_{13}H_{15}N_3O_5$) C, H, N.

5-(2-Chloroacetamido)-3,4-dihydro-3-oxo-1(2*H*)-quinoxalinecarboxylic Acid 1,1-Dimethylethyl Ester (42). A mixture of **41** (3.11 g, 10.6 mmol), Pd/C (5%, 0.85 g), and ethyl acetate (120 mL) was hydrogenated (40 psi) for 3 h at room temperature. The solids were filtered off and washed with excess ethyl acetate. The organic fractions were combined, and diisopropylethylamine (2.10 mL, 12.1 mmol) was added. After cooling to −55 °C, a solution of chloroacetyl chloride (0.95 mL, 12 mmol) in THF (15 mL) was added dropwise. The mixture was allowed to stir for 3 h while warming to 0 °C. After a basic workup (ethyl acetate, $NaHCO_3$, H_2O, brine, $MgSO_4$), the residue was triturated with diethyl ether to provide 3.40 g (94%) of **42** as a tan solid (mp 178−180 °C): IR (mineral oil) 1710, 1674, 1663, cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.45 (s, 1 H), 8.19 (s, 1 H), 7.50−7.55 (m, ArH), 7.05−7.20 (m, 2 H, ArH), 4.36 (s, 2 H), 4.29 (s, 2 H), 1.53 (s, $OC(CH_3)_3$); MS (EI) *m*/*e* 339, 283, 239, 162. Anal. ($C_{15}H_{18}ClN_3O_4$) C, H, N.

2,3,6,7-Tetrahydro-3,6-dioxo-1*H*,5*H*-pyrazino[1,2,3-*de*]-quinoxaline-1-carboxylic Acid 1,1-Dimethylethyl Ester (43). A solution of **42** (2.26 g, 6.65 mmol) in THF (60 mL) was cooled to −30 °C, and potassium *tert*-butoxide (1.0 M in THF, 7.00 mL, 7.00 mmol) was added dropwise. The mixture was allowed to stir for 1 h at −30 °C and for 3 h at room temperature. The mixture was then diluted with CH_2Cl_2 and the reaction quenched with aqueous ammonium chloride. After an aqueous workup (CH_2Cl_2, brine, $MgSO_4$), the residue was triturated with diethyl ether to provide 1.75 g (87%) of **43** as an off-white solid (mp 174−176 °C): IR (mineral oil) 1716, 1690, 1683, 1411 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.83 (s, CONH), 7.30−7.40 (m, ArH), 7.06 (apparent t, J = 8.1 Hz, ArH), 6.75 (d, J = 7.8 Hz, ArH), 4.58 (s, 2 H), 4.44 (s, 2 H), 1.54 (s, $OC(CH_3)_3$); MS (EI) *m*/*e* 303, 247, 203, 174, 146. Anal. ($C_{15}H_{17}N_3O_4$) C, H, N.

9-(5-Cyclopropyl-1,2,4-oxadiazol-3-yl)-5,6-dihydro-6-oxo-4*H*,8*H*-imidazo[1,5-]pyrazino[1,2,3-*de*]quinoxaline-4-carboxylic Acid 1,1-Dimethylethyl Ester (44). A solution of **43** (1.03 g, 3.40 mmol) in THF (30 mL) was cooled to −50 °C, and potassium *tert*-butoxide (1.0 M in THF, 4.10 mL, 4.10 mmol) was added. The mixture was stirred for 0.5 h at −50 °C and for 2 h at 0 °C followed by cooling to −50 °C. Diethyl chlorophosphate (0.60 mL, 4.2 mmol) was added, and the mixture was allowed to stir for 0.5 h at −50 °C and for 2 h at 0 °C. DMF (4 mL) was added, and the mixture was cooled to −50 °C. The isocyanide **15** (0.660 g, 4.43 mmol) was then added followed by the dropwise addition of potassium *tert*-butoxide (1.0 M in THF, 4.40 mL, 4.40 mmol). The mixture was allowed to warm to room temperature and stir for 16 h. After the reaction was quenched with aqueous ammonium chloride and after an aqueous workup (ethyl acetate, brine, $MgSO_4$), the residue was purified by flash chromatography (4:1 ethyl acetate:hexane) to provide 0.33 g (22%) of **44** as a white powder (mp 211−213 °C): IR (mineral oil) 1716, 1693, 1334, 1169 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (s, ArH), 7.60−7.70 (m, ArH), 7.38 (d, J = 7.7 Hz, ArH), 7.22 (apparent t, J = 8.3 Hz, ArH), 5.40 (s, NCH_2Ar), 4.44 (s, NCH_2CO), 2.25−2.35 (m, CH), 1.57 (s, $OC(CH_3)_3$), 1.25−1.40 (m, CH_2CH_2); MS (EI) *m*/*e* 434, 334, 265, 237, 222. Anal. ($C_{22}H_{22}N_6O_4 \cdot (H_2O)_{1/2}$) C, H, N.

9-(5-Cyclopropyl-1,2,4-oxadiazol-3-yl)-4,5-dihydro-6*H*,8*H*-imidazo[1, 5-*a*]pyrazino[1,2,3-*de*]quinoxalin-6-one (45). A solution of **44** (50.0 mg, 0.115 mmol) in CH_2Cl_2 (5 mL) was cooled to 0 °C. TFA (3 mL) was added, and the solution was allowed to warm to room temperature and stir for 2 h. After concentration and basic workup (CH_2Cl_2, 15% NaOH, $MgSO_4$), the residue was combined with MeOH (3 mL) and sodium borohydride (0.005 g) and the mixture stirred for 2 h at room temperature. The mixture was concentrated under reduced pressure and subjected to an aqueous workup (CH_2Cl_2, $MgSO_4$) to provide 0.020 g (52%) of **45** as a yellow solid (mp 215−217 °C): IR (mineral oil) 1686, 1581, 1501, 1392, 1290 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6/$CDCl_3$) δ 8.20 (s, ArH), 6.95−7.10 (m, 2 H, ArH), 6.73 (d, J = 7.7 Hz, ArH), 5.52 (s, NH), 5.35 (s, NCH_2Ar), 3.97 (s, NCH_2CO), 2.25−2.35 (m, CH),

1.20−1.40 (m, CH_2CH_2); MS (EI) *m*/*e* 334, 306, 292, 265, 237, 222; HRMS (EI) calcd for $C_{17}H_{14}N_6O_2$ (M^+) 334.1178, found 334.1186.

$GABA_A$ Receptor Expression and Membrane Preparation. DNA manipulations and general baculovirus methods (Sf-9 cell cultivation, infection, isolation, purification of recombinant viruses) were performed as described elsewhere.[30] The Sf-9 cells were infected at a multiplicity of infection of three plaque-forming units of viruses: AcNPV-α_1, α_3, or -α_6, AcNPV-β_2, and AcNPV-γ_2. Infected cells were used for electrophysiological measurements at 48 h postinfection or for membrane preparations at 60 h postinfection. The stable cell lines expressing α_1 or α_3, α_6, β_2, and γ_2 subunits of $GABA_A$ were derived by transfection of plasmids containing cDNA and a plasmid encoding G418 resistance into human kidney cells (A293 cells) as described elsewhere.[31] After 2 weeks of selection in 1 mg/mL G418, cells positive for all three $GABA_A$ receptor mRNAs by Northern blotting were used for electrophysiology to measure GABA-induced Cl^- currents. For equilibrium binding measurements, Sf-9 cells infected with baculovirus-carrying cDNAs for α_1 or α_3, α_6, β_2, and γ_2 subunits were harvested in 2 L batches 60 h postinfection. The membranes were prepared following the procedure described previously.[30] Briefly, the membranes were prepared in normal saline after homogenization with a Polytron PT 3000 homogenizer (Brinkman) for 4 min. Unbroken cells and large nuclei aggregates were removed by centrifugation at 1000*g* for 10 min. Then the membranes were recovered with the second centrifugation of the supernatant at 40000*g* for 50 min. The membranes were resuspended to a final concentration of 5 mg/mL in a solution containing 300 mM sucrose, 5 mM Tris/HCl, pH 7.5, and glycerol to a final concentration of 20% and stored at −80 °C. Equilibrium binding of [^{3}H]flunitrazepam or [^{3}H]Ro-4513 to the cloned $GABA_A$ receptors was measured in a 500 mL volume of normal saline containing 6 nM [^{3}H]flunitrazepam or [^{3}H]Ro-4513, varying concentrations of test ligands, and 50 mg of membrane protein. The mixture was incubated for 60 min at 4 °C, filtered over a Whatman glass fiber filter, and washed four times with cold normal saline. The filter was then counted for radioactivity in the presence of a scintillation cocktail (Insta Gel).

$GABA_A$ Receptor Binding. The *in vitro* binding affinity of the imidazo[1,5-*a*]quinoxalines for $GABA_A$ was determined as previously described with minor modification.[24] Freshly prepared rat cerebellar membranes were suspended in 300 mM sucrose and 10 nM Hepes/Tris, pH 7.4. Typically, the reaction medium contained 6 mM [^{3}H]flunitrazepam, 50 mg of membrane protein, test drugs at various concentrations or vehicle in 200 mL, 118 nM NaCl, 10 mM Hepes/Tris, pH 7.4, and 1 mM $MgCl_2$. The mixtures were incubated for 60 min at 4 °C. The amount of binding was determined with rapid filtration techniques using Whatman GF/B filters.

[^{35}S]-*tert*-Butyl bicyclophosphorothionate ([^{35}S]TBPS) Binding. Binding of [^{35}S]TBPS in the rat brain membranes was measured in the medium containing 2 nM [^{35}S]TBPS, unless specified otherwise, 50 mg of membrane proteins, 1 M NaCl, and 10 mM Tris/HCl, pH 7.4, in a total volume of 500 mL. Drugs were added in concentrated methanolic solutions, and the level of methanol did not exceed 0.2% and was maintained constant in all tubes. The mixtures were incubated for 120 min at 24 °C. The reaction mixtures were filtered over a Whatman GF/B filter under vacuum. The filters were washed three times with 4 mL of the reaction buffer without radioisotope and counted for radioactivity. Nonspecific binding was estimated in the presence of 2 mM unlabeled TBPS and subtracted to compute specific binding.[25]

Electrophysiology. The whole cell configuration of the patch clamp technique was used to record the GABA-mediated Cl^- currents in the A293 cells expressing the $\alpha_1\beta_2\gamma_2$ subtype, as described earlier.[27] Briefly, patch pipets made of borosilicate glass tubes were fire-polished and showed a tip resistance of 0.5−2 MΩ when filled with a solution containing (mM) 140 CsCl, 11 EGTA, 4 $MgCl_2$, 2 ATP, and 10 Hepes, pH 7.3. The cell-bathing external solution contained (mM) 135 NaCl, 5 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, and 5 Hepes, pH 7.2 (normal saline). GABA at the concentration of 5 mM in the external solution with or without indicated drugs was applied through a U-tube placed within 100 mm of the cells for 10 s, unless indicated otherwise. The current was recorded with an Axopatch 1D amplifier and a CV-4 headstage (Axon Instrument Co.). A Bh-1 bath headstage was used to compensate for changes in bath potentials. The currents were recorded with a Gould recorder 220. GABA currents were measured at the holding potential of −60 mV at room temperature (21−24 °C).

Metrazole Antagonism. Compounds were tested for their ability to antagonize metrazole-induced convulsions in mice after ip injection as described elsewhere.[28] Briefly, male CF-1 mice were injected with metrazole (85 mg/kg, sc), and convulsive seizure was elicited 15 min later with an auditory stimulation (5 dB for 10 s). Drugs tested for metrazole antagonism were injected ip 30 min before the metrazole challenge, 4 mice/dose at a 0.3 log dose interval. ED_{50}s for protection against tonic seizure were calculated by the method of Spearman−Karber (Finney, D. J. *Statistical Methods in Biological Assay*).

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