Letters

Antiseizure Activity of Novel γ-Aminobutyric Acid (A) Receptor Subtype-Selective Benzodiazepine Analogues in Mice and Rat Models

Felix M. Rivas,[†,▽] James P. Stables,[‡] Lauren Murphree,[‡] Rahul V. Edwankar,[†] Chitra R. Edwankar,[†] Shengming Huang,[†] Hiteshkumar D. Jain,[†] Hao Zhou,[†] Samarpan Majumder,[§] Subramanian Sankar,[||] Bryan L. Roth,[§] Joachim Ramerstorfer,[⊥] Roman Furtmüller,[⊥] Werner Sieghart,[⊥] and James M. Cook*,[†]

Department of Chemistry and Biochemistry, University of Wisconsin—Milwaukee, Milwaukee, Wisconsin 53211, National Institute of Neurological Disorders and Stroke, Anticonvulsant Screening Program, Rockville, Maryland 20852, Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, Department of Biochemistry, CWRU Medical School, Cleveland, Ohio 44106, Center for Brain Research, Medical University Vienna, A-1090 Vienna, Austria

Received December 29, 2008

Abstract: The antiseizure activity of benzodiazepines (BDZs) **1**−**5** in mice and rats as animal models is described. These BDZs have selective efficacy for α2β3γ2 and α3β3γ2 $GABA_A$-receptors. Significant anticonvulsant activity with little or no motor impairment and therapeutic indexes (TI) of 2.8−44 (mice, ip) were observed for compounds **2**−**4** in the subcutaneous metrazole seizure (scMET) test. In rats, orally (po) the TI was >5 to 105. These compounds represent novel leads in the search for anticonvulsants devoid of sedative, ataxic, and amnestic side effects.

Many of the commonly used benzodiazepines (BDZs[a]) display good anticonvulsant activity against acutely elicited seizures induced with either maximal electroshock (MES) and pentylenetetrazole (MET).[1−3] The anticonvulsant actions of BDZs have been utilized clinically in patients to treat specific seizure types or conditions, i.e., akinetic, myoclonic, absence variant seizures as well as to help terminate status epilepticus or serial seizures.[2] BDZ diazepam when administered intravenously can be very effective for arresting status epilepticus.[6] However, oral administration of this drug is less effective because tolerance to the anticonvulsant effects develops within a relatively short period.[1,4] In addition to diazepam, other BDZs that have demonstrated anticonvulsant action are clonazepam, clorazepate, clobazam, lorazepam, midazolam, and nitrazepam.[5,6]

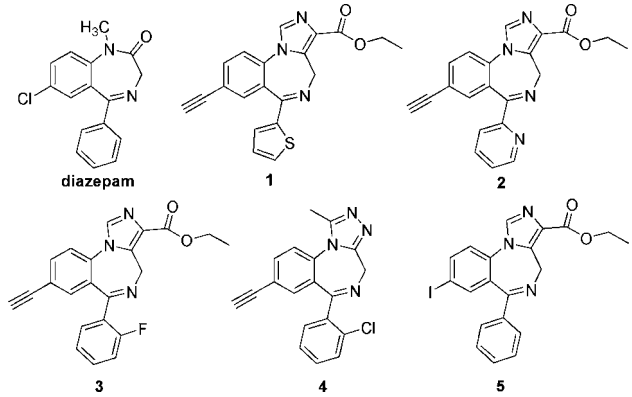

Figure 1. Benzodiazepines (BDZs) **1**−**5**.

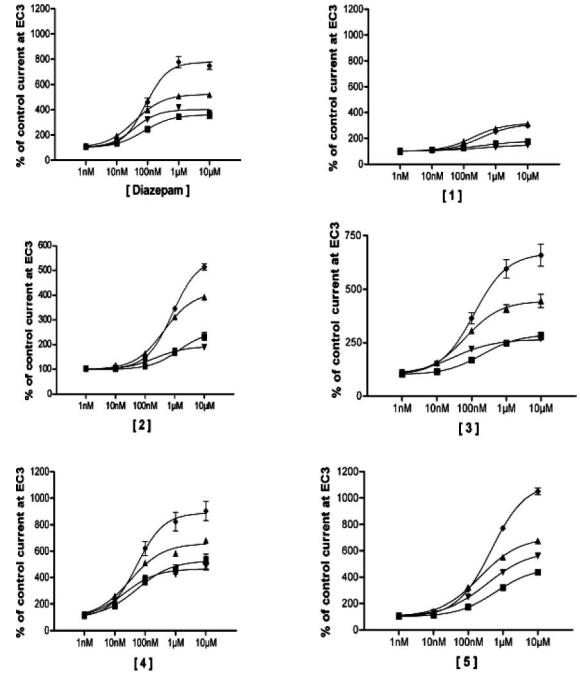

Figure 2. Dose−response curves for diazepam and **1**−**5** in oocytes expressing α1β3γ2 (■), α2β3γ2 (▲), α3β3γ2 (♦), or α5β3γ2 (▼) $GABA_A$ receptors. Values are presented as mean ± SEM of at least four oocytes from at least two batches. A concentration of 1 μM of diazepam resulted in 345 ± 27%, 508 ± 29%, 776 ± 44%, and 420 ± 12% of control current (at GABA EC_3) in α1β3γ2, α2β3γ2, α3β3γ2, and α5β3γ2 receptors, respectively. A concentration of 1 μM of **2** resulted in 167 ± 9%, 313 ± 9%, 346 ± 9%, and 174 ± 6% of control current (at GABA EC_3) in α1β3γ2, α2β3γ2, α3β3γ2, and α5β3γ2 receptors, respectively. A concentration of 1 μM of **3** resulted in 248 ± 14%, 410 ± 19%, 596 ± 43%, and 246 ± 4% of control current (at GABA EC_3) in α1β3γ2, α2β3γ2, α3β3γ2, and α5β3γ2 receptors, respectively. All these values were significantly different from the respective control currents ($p < 0.01$, Student's *t*-test).

In general, BDZs as a class offer many benefits as drug therapy.[7] For example, they are rapidly absorbed from the gastrointestinal tract and normally reach maximum blood concentrations within one to two hours of ingestion. They readily cross the blood−brain barrier and are rapidly distributed within the brain. Electrophysiological changes attributed to certain

* To whom correspondence should be addressed. Phone: 414-229-5856. Fax: 414-229-5530. E-mail: capncook@uwm.edu.
[†] University of Wisconsin—Milwaukee.
[‡] National Institute of Neurological Disorders and Stroke.
[§] University of North Carolina at Chapel Hill.
[||] CWRU Medical School-Cleveland.
[⊥] Center for Brain Research-Vienna.
[▽] Current address: Chicago State University, 9501, S King Drive/SCI 309, Chicago, IL 60628.

[a] Abbreviations: BDZ, benzodiazepine; $GABA_A$, γ-aminobutyric acid (A) receptor; MES, maximal electroshock seizure; scMET, subcutaneous metrazol seizure; TOX, motor impairment; ED_{50}, median effective dose; TD_{50}, median toxic dose; TI, therapeutic index; ip, intraperitoneal; po, oral; NINDS, National Institute of Neurological Disorders and Stroke; ASP, anticonvulsant screening program.

10.1021/jm801652d CCC: $40.75
Published on Web 03/10/2009

Table 1. Assessment of Antiseizure Activity on Benzodiazepine (BDZ) Ligand **2** at 100 mg/kg after 0.5 and 4.0 h in Mice via ip[a]

BDZ	time (h)	MES	mice ip scMET	TOX
2	0.5	0/3	3/5	0/8
2	4.0	0/3	0/1	0/4

[a] Results indicate number protected or toxic/number tested. Refer to Table 3 for abbreviations.

Table 2. Assessment of scMET Antiseizure Activity on Benzodiazepine (BDZ) Analogues after 0.5 and 4.0 h in Rat via po and ip Admistration

BDZ	time (h)	MES[a]	rat po scMET[b]	TOX	rat ip scMET[b]	TOX
2	0.5	1/4	4/4	0/4	7/8	0/8
2	4.0	0/4	0/4	0/4	5/8[d]	0/8[d]
3	0.5	0/4	3/4	0/4	3/4	0/4
3	4.0	1/4	4/4	0/4	3/4	0/4
4	0.5	0/4	4/4 3/4[c]	0/4	NT[e]	NT[e]
4	4.0	0/4	3/4 2/4[c]	0/4	NT[e]	NT[e]
5	0.5	NT[e]	2/6	0/6	1/4	0/4
5	4.0	NT[e]	5/6[d]	0/6[d]	2/4	0/4

[a] Dose of 30 mg/kg. [b] Dose of 50 mg/kg. [c] Dose of 15 mg/kg. [d] After 1 h of dosing. [e] NT = not tested. Results indicate number protected or toxic/number tested. Refer to Table 3 for abbreviations.

BDZs can be detected as early as five minutes after intravenous injection.[8] At clinically relevant doses, the BDZs do not induce significant liver microsomal enzymes that often can result in drug−drug interactions.[9]

In general, they lack serious toxicity even when overdosed.[1,4] Unfortunately, BDZs produce many side effects such as drowsiness, somnolence, fatigue, ataxia, lethargy, sedation, muscle relaxation, amnesia, and tolerance to the anticonvulsant effects that limit their use as chronic anticonvulsant agents.[1−3] These side effects along with the issue of tolerance that develops from the extended use of these agents both in animal models and patients has been studied in detail.[1−6,10]

Much work has been done in the search for new BDZs with improved pharmacological profiles; it has been suggested that partial agonists at the γ-aminobutyric acid (A) receptor ($GABA_A$) would reduce and possibly eliminate the unwanted side effects.[11] However, these preclinical properties did not translate into clinical agents sufficiently free of side effects and tolerance liability.[12−14] An alternative approach is to develop nonsedating anticonvulsants that target specific $GABA_A$ receptor subtype(s) involved in mediation of the anticonvulsant action but not the sedative action.[15,16] This selectivity for $GABA_A$ receptor subtypes may be achieved by selective efficacy.[14] Those ligands that are agonists with subtype selectivity for α2- and α3-$GABA_A$ receptors that also have reduced agonistic and/or exhibit antagonistic activity at α1-$GABA_A$ receptors should provide ligands with anticonvulsant properties but with reduced sedative, ataxic, and amnestic side effects.[15,16] Among the ligands reported with α2 and/or α3 subtype selectivity are pyrazolo-quinolinones,[17] pyrazoles,[18] pyridazines,[19] pyridoindolones,[20,21] pyridones,[22] tetrahydroimidazo-pyrido-pyrimidinones,[23] triazolo-phthalazines,[24] pyrazolopyridinones,[25] imidazopyrimidines, and triazines.[26]

Recently, it has been shown that tolerance (in part) to the anticonvulsant effects of diazepam is mediated by an interaction at the α1-subtype.[27] Moreover, Rijnsoever, Möhler et al.[28] have shown that manifestation of tolerance to the motor-depressant action of diazepam depends on the chronic activation of two competitive mechanisms orchestrated by α1- and α5-$GABA_A$ receptors, respectively. They also demonstrated that tolerance to the sedative action of diazepam was accompanied by a 15% reduction of α5-$GABA_A$ receptors in the dentate gyrus.[28,29]

Because the BDZ scaffold is generally nontoxic with good logP properties, efforts have centered here on a selected group of novel 8-substituted triazolo- and imidazobenzodiazepines as shown in Figure 1,[30] which exhibit low efficacy at α1- and α5-subtypes. The dose−response curves for the stimulation of GABA-induced currents by diazepam and BDZs **1**−**5** in oocytes, which expressed $GABA_A$ receptors of the subtypes α1β3γ2, α2β3γ2, α3β3γ2, and α5β3γ2, are illustrated in Figure 2. It is clear the efficacy at α1β3γ2 and α5β3γ2 subtypes is low, especially for ligands **2** and **3**, as compared to diazepam. Although the efficacy at α1 and α5 are low for **1**, the potency also remains too low (for useful or serious consideration). The acetylene−halogen switch employed for **1**−**3** was also extended to triazolam analogue **4** but not to the control ligand **5**.

Examination of the initial anticonvulsant screen (Table 1, 100 mg/kg) on ligands **1**−**5** (administered as free bases) at the

Table 3. Quantification of Antiseizure Activity ED_{50} MES, ED_{50} scMET, TD_{50} TOX, and Therapeutic Index (TI) via ip and po Routes[a]

	mice ip			rat po			TI mice ip	TI rat po
entry	ED_{50} MES[d]	ED_{50} scMET[d]	TD_{50} TOX[d]	ED_{50} MES[d]	ED_{50} scMET[d]	TD_{50} TOX[d]	TD_{50}/ED_{50} (scMET)	TD_{50}/ED_{50} (scMET)
2	>300	**16.28**	**>500**	>250	**98.5**	**>500**	**>30.2**	**≫5**[c]
3	>200	**8.87**	**>400**	>250	**23.72**	**>500**	**>44**	**>21.1**
4	>6	1.027	2.875	>150	**1.58**	**166.25**	2.8	**>105**
carbamazepine[b]	7.81	>50	45.4	5.35	>250	364	<0.9	1.5
clonazepam[b]	25.6	0.02	0.26	7.86	0.61	2.38	13	3.9
phenytoin[b]	5.64	>50	41.0	28.1	>500	>1000	<0.82	2.0

[a] MES, maximal electroshock induced seizures; scMET, subcutaneous pentylenetetrazole induced seizures; TOX, observed minimal muscular or neurological impairment as indicated by rotorod paradigm (mice) or abnormal, uncoordinated gait (rats); TI, therapeutic index = TD_{50}/ED_{50}; ED_{50}, median effective dose; TD_{50}, median toxic dose; ip, intraperitoneal; po, oral. [b] Refer to ref 32. [c] A higher dose was not tested because 500 mg/kg was clearly not sedating. [d] All values are in mg/kg.

Table 4. Preliminary Hippocampal Kindling Screen-Rats ip

	seizure score				afterdischarge duration (s)				
	pre-drug		drug		pre-drug		drug		
BDZ	L	H	L	H	L	H	L	H	TOX[d]
2[a]	4	5	3		47	61	59		0/2[e]
3[b]	5		3		30	41	38		0/2[e]
4[c]	5		3		29	41	92		0/2[f]

[a] Dose of 100 mg/kg. [b] Dose of 50 mg/kg. [c] Dose of 10 mg/kg. [d] After 1 h of dosing. [e] Dose of 30 mg/kg. [f] Dose of 3 mg/kg. L = low, H = high. Refer to Tables 2 and 3 for abbreviations.

National Institute of Neurological Disorders and Stroke (NINDS) under the Anticonvulsant Screening Program (ASP) indicated that the 8-acetyleno-2′-pyridoimidazobenzodiazepine **2** had the most significant antiseizure profile in mice when administrated ip. It raised the seizure threshold level induced by subcutaneous metrazole (scMET) in 60% of mice (3/5) with no motor impairment, as indicated by the rotorod paradigm test (TOX). Ligand **2** also appeared to have a relatively rapid onset and short duration of action because the antiseizure protection was absent after 4.0 h. Toxicity in this study was based on motor impairement (locomotor, rotorod). Ligand **2** lacked activity against MES induced seizures in keeping with low efficacy of **2** at $\alpha1\beta3\gamma2$ subtypes.[14,19]

The antiseizure activity in rat animal models for MES, scMET, and TOX showed that ligands **2**−**5** significantly increased the seizure threshold level of scMET in both oral (po) and intraperitoneal (ip) routes of administration (Table 2). Using rats via the po route, the protection ranged from a median effective dose (ED_{50}) of 1.58 mg/kg for **4** to 98.5 mg/kg for **2**, with the ED_{50} for **3** falling in the middle (Table 3). For **2** and **3**, no TOX was observed in rats that were dosed up to 500 mg/kg via either the po or ip routes of administration (Table 3). For 2′-pyrido analogue **2** in rats, the protection was 100% dosed orally and 88% via the ip route after 0.5 h (Table 2). After four hours, ligand **2** offered no protection with po dosing but maintained 63% protection via ip dosing. Imidazobenzodiazepine **3** exhibited similar protection orally and ip but for a longer duration as compared to **2**. Ligand **4** was the most potent of all the ligands tested orally in rats (Table 2), with 50% protection over a period of 4 h at a lower dose of 15 mg/kg. Ligand 8-iodoimidazobenzodiazepine **5** showed no activity in mice dosed ip (data not shown). Because the calculated logP for **5** (4.59) was significantly greater than **2** (2.48), it is possible that **5** crosses the blood−brain barrier more rapidly than **2**, reaches a maximum effective concentration more rapidly, and is consequently metabolized more rapidly when admistered ip. Even though **2** would be expected to be more bioavailable (especially) po, it may not cross the blood−brain barrier as rapidly as **5**. The ligand **5** was not subjected to quantification of antiseizure activity, but activity was evident (5/6) at 50 mg/kg in rats dosed orally with no observed TOX at that dose.

The quantitative antiseizure effects of BDZs **2**, **3**, and **4** are shown in Table 3. Imidazobenzodiazepine **2** was much more active in the scMET seizure model than in MES, which suggested that it may have potential use for the treatment of absence and myoclonic seizures.[31] The ED_{50} for scMET for ligand **2** was smaller than that of carbamazepine and phenytoin. Moreover, the median toxic (sedating) dose (TD_{50}) for **2** (>500 mg/kg) in mice ip provided a calculated therapeutic index (TI) greater than 30 in mice ip. Similarly, **3** showed better activity against scMET than MES in mice ip and rat po, with ED_{50}s smaller than those reported for carbamazepine and phenytoin (Table 3). However in the MES, both carbamazepine and phenytoin have better ED_{50}s than ligand **3**. The TD_{50} of **3** was >400 mg/kg in both tests, which provided a calculated TI of 44 in mice ip and 21 in rats orally (Table 3). Triazolobenzodiazepine **4** showed the most potent activity of the ligands tested for scMET in mice and rats. However, only in rats via oral administration was a significant separation of protective effects and motor impairment found (Tables 2, 3).

To further characterize the anticonvulsant activity of some of these novel BDZs, a hippocampal kindling screen was performed on **2**−**4**. The hippocampal kindling screen is a useful adjunct to the traditional MES and scMET tests for identification of a substance's potential utility for treating complex partial seizures.[32] BDZs **2**−**4** appeared to block the kindle motor seizure as shown by the reduction of the seizure score from 4−5 to 3 (Table 4). No toxic effects were observed as indicated by the lack of motor impairment on the rats tested.

It is clear from the rat po data (Table 2) that **2** has a shorter half-life than **3**, presumably because of differences in esterase enzyme interactions with the two molecules. Because the half-lives of such esters in primates and humans would be much longer, ligands **2**−**4** represent potential anticonvulsant agents with little or no side effects. Certainly, the efficacy profiles of **2** and **3** are consistent with this finding.

In conclusion, these novel BDZs possess significant antiseizure activity in the scMET test in mice and rats and showed minimal TOX. Therefore, ligands **2** and **3** appear to provide antiseizure effects with minimal or no TOX by maintaining a good selectivity between $\alpha2/\alpha3$ versus $\alpha1$ subtypes and an efficacy at $\alpha1$ that is lower than that displayed by diazepam. The efficacy level at $\alpha1$ appears to be of critical importance to avoid motor impairment in mice and rats, as predicted by Möhler et al.[28] This was demonstrated by the fact that a slightly higher efficacy at $\alpha1$ (248%) appears to result in some minimal TOX for ligand **3**, while ligand **2** (167%) had no TOX. Ligand **2** appears to have high enough efficacy at $\alpha2$ and $\alpha3$ to provide significant antiseizure activity with no toxicity in vivo (mice and rats) due to its lower efficacy at $\alpha1$ subtypes compared to diazepam. Because of its simultaneous reduced efficacy at $\alpha1$- and $\alpha5$-$GABA_A$ receptors, ligand **2** represents an important potential anticonvulsant agent. Recent data from NINDS indicates that, on chronic dosing (5 days), tolerance to the anticonvulsant effects of ligand **2** did not develop.[33]

Acknowledgment. We thank the NIMH for support of this work (MH-46851), and the Lynde and Harry Bradley Foundation. F.M.R. is grateful to the NIH for a Postdoctoral Fellowship.

Supporting Information Available: The characterization of compounds **1**−**5**.[30] This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Rogawski, M. A. Principles of Antiepileptic Drug Action. In *Antiepileptic Drugs*, 5th ed.; Levy, R. H., Mattson, R. H., Meldrum, B. S., Perucca, E., Eds.; Lippincott Williams and Wilkins: Philadelphia, 2002; pp 3−22.
(2) MacDonald, R. L. Benzodiazepines Mechanisms of Action. In *Antiepileptic Drugs*, 5th ed.; Levy, R. H., Mattson, R. H., Meldrum, B. S., Perucca, E., Eds.; Lippincott Williams and Wilkins: Philadelphia, 2002; pp 179−186.
(3) Killam, E. K., Suria, A. Benzodiazepines. In *Antiepileptic Drugs: Mechanisms of Action*; Glaser, G. H., Penry, J. K., Woodbury, D. M., Eds.;Advances in Neurology Vol. *27*; Raven Press: New York, 1980; pp 597−615.
(4) Hillestad, L.; Hansen, T.; Melsom, H. Diazepam Metabolism in Normal Man. II. Serum Concentration and Clinical Effect after Oral Administration and Cumulation. *Clin. Pharmacol. Ther.* **1974**, *16*, 485–489.
(5) De Sarro, G.; Gambardella, A.; De Sarro, A. Benzodiazepines in Pathology of Epilepsy. In *Benzodiazepine*; Biggio, G., Eds.; Springer-Verlag: Milan, 2000; pp 259−279.
(6) Shorvon, S. D. The Use of Clobazam, Midazolam, and Nitrazepam in Epilepsy. *Epilepsia* **1998**, *39*, S15–S23.
(7) Gorman, J. M. Benzodiazepines: Taking the Good with the Bad and the Ugly. *CNS Spectrums* **2005**, *10*, 14–15.
(8) Garattini, S.; Mussini, E.; Marcucci, F.; Guaitani, A. Metabolic Studies on Benzodiazepines in Various Animal Species. In *The Benzodiazepines*; Garattini, S., Mussini, E., Randall, L. O.; Raven Press: New York, 1973; pp 75−97.
(9) Stevenson, I. H.; Browning, M.; Crooks, J.; O'Malley, K. Changes in Human Drug Metabolism after Long-Term Exposure to Hypnotics. *Br. Med. J.* **1972**, *4*, 322–324.
(10) Söederpalm, B.; Eriksson, E.; Engel, J. A. Anticonflict and Rotarod Impairing Effects of Alprazolam and Diazepam in Rat After Acute

and Subchronic Administration. *Prog. Neuropsychopharmacol. Biol. Psych.* **1989**, *13*, 269–283.
(11) Rogawski, M. A. Epilepsy. In *Neurotherapeutics: Emerging Strategies*, 1st ed.; Pullan, L. M., Patel, J., Eds.; Humana Press: Totowa, NJ, 1996; pp 193−273.
(12) Korpi, E. R.; Mattila, M. J.; Wisden, W.; Luddens, H. $GABA_A$-Receptor Subtypes: Clinical Efficacy and Selectivity of Benzodiazepine Site Ligands. *Ann. Med.* **1997**, *29*, 275–282.
(13) Korpi, E. R.; Gründer, G.; Lüddens, H. Drug interactions at $GABA_A$ receptors. *Prog. Neurobiol.* **2002**, *67*, 113–159.
(14) Atack, J. R. Anxioselective Compounds Acting at the $GABA_A$ Receptor Benzodiazepine Binding Site. *Curr. Drug Targets* **2003**, *2*, 213–232.
(15) Sieghart, W.; Ernst, M. Heterogeneity of $GABA_A$ Receptors: Revived Interest in the Development of Subtype-Selective Drugs. *Curr. Med. Chem.* **2005**, *5*, 217–242, and references cited therein.
(16) Möhler, H.; Fritschy, J.-M.; Crestani, F.; Hensch, T.; Rudolph, U. Specific $GABA_A$ Circuits in Brain Development and Therapy. *Biochem. Pharmacol.* **2004**, *68*, 1685–1690.
(17) Yu, S.; He, X.; Ma, C.; McKernan, R.; Cook, J. M. Studies in Search of α2 Selective Ligands for $GABA_A$/BzR Receptor Subtypes Part I. Evidence for the Conservation of Pharmacophoric Descriptors for DS Subtypes. *Med. Chem. Res.* **1999**, *9*, 186–202.
(18) Loughhead, D. G.; Novakovic, S.; O'Yang, C.; Putman, D. G.; Soth, M. Preparation of Pyrazolopyridines and other Heterocycle-Fused Pyrazoles as $GABA_A$ α2 Subtype Selective Receptor Modulators. PCT Int. Appl. WO 2005077363 A1, 2005.
(19) Atack, J. R.; Wafford, K. A.; Tye, S. J.; Cook, S. M.; Sohal, B.; Pike, A.; Sur, C.; Melillo, D.; Bristow, L.; Bromidge, F.; Ragan, I.; Kerby, J.; Street, L.; Carling, R.; Castro, J. L.; Whiting, P.; Dawson, G. R.; McKernan, R. M. TPA023 [7-(1,1-Dimethylethyl)-6-(2-ethyl-2*H*-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-*b*]pyridazine], an Agonist Selective for α2- and α3-Containing $GABA_A$ Receptors, is a Nonsedating Anxiolytic in Rodents and Primates. *J. Pharmacol. Exp. Ther.* **2006**, *316*, 410–422.
(20) Griebel, G.; Perrault, G.; Simiand, J.; Cohen, C.; Granger, P.; Decobert, M.; Françon, D.; Avenet, P.; Depoortere, H.; Tan, S.; Oblin, A.; Schoemaker, H.; Evanno, Y.; Sevrin, M.; George, P.; Scatton, B. SL651498: An Anxioselective Compound with Functional Selectivity for α_2- and α_3-Containing γ-Aminobutyric $Acid_A$ ($GABA_A$) Receptors. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 753–768.
(21) Licata, S. C.; Platt, D. M.; Cook, J. M.; Sarma, P. V. V. S.; Griebel, G.; Rowlett, J. K. Contribution of $GABA_A$ Receptor Subtypes to the Anxiolytic-Like, Motor, and Discriminative Stimulus Effects of Benzodiazepines: Studies with the Functionally Selective Ligand SL651498 [6-Fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1*H*-pyridol[3,4-*b*]indol-1-one]. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 1118–1125.
(22) Crawforth, J.; Atack, J. R.; Cook, S. M.; Gibson, K. R.; Nadin, A.; Owens, A. P.; Pike, A.; Rowley, M.; Smith, A. J.; Sohal, B.; Sternfeld, F.; Wafford, K.; Street, L. J. Tricyclic Pyridones as Functionally Selective Human $GABA_A\alpha_{2/3}$ Receptor-Ion Channel Ligands. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1679–1682.
(23) Albaugh, P. A.; Marshall, L.; Gregory, J.; White, G.; Hutchison, A.; Ross, P. C.; Gallagher, D. W.; Tallman, J. F.; Crago, M.; Cassella, J. V. Synthesis and Biological Evaluation of 7,8,9,10-Tetrahydroimidazo[1,2-*c*]pyrido[3,4-*e*]pyrimdin-5(6*H*)-ones as Functionally Selective Ligands of the Benzodiazepine Receptor Site on the $GABA_A$ Receptor. *J. Med. Chem.* **2002**, *45*, 5043–5051.
(24) Russell, M. G. N.; Carling, R. W.; Atack, J. R.; Bromidge, F. A.; Cook, S. M.; Hunt, P.; Isted, C.; Lucas, M.; McKernan, R. M.; Mitchinson, A.; Moore, K. W.; Narquizian, R.; Macaulay, A. J.; Thomas, D.; Thompson, S.-A.; Wafford, K. A.; Castro, J. L. Discovery of Functionally Selective 7,8,9,10-Tetrahydro-7,10-ethano-1,2,4-triazolo[3,4-*a*]phthalazines as $GABA_A$ Receptor Agonists at the α_3 Subunit. *J. Med. Chem.* **2005**, *48*, 1367–1383.
(25) Blackaby, W. P.; Atack, J. R.; Bromidge, F.; Lewis, R.; Russell, M. G. N.; Smith, A.; Wafford, K.; McKernan, R. M.; Street, L. J.; Castro, J. L. Pyrazolopyridinones as Functionally Selective $GABA_A$ Ligands. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4998–5002.
(26) Goodacre, S. C.; Street, L. J.; Hallett, D. J.; Crawforth, J. M.; Kelly, S.; Owens, A. P.; Blackaby, W. P.; Lewis, R. T.; Stanley, J.; Smith, A. J.; Ferris, P.; Sohal, B.; Cook, S. M.; Pike, A.; Brown, N.; Wafford, K. A.; Marshall, G.; Castro, J. L.; Atack, J. R. Imidazo[1,2-*a*]pyrimidines as Functionally Selective and Orally Bioavailable $GABA_A$ α2/α3 Binding Site Agonists for the Treatment of Anxiety Disorders. *J. Med. Chem.* **2006**, *49*, 35–38.
(27) Costa, E.; Auta, J.; Grayson, D. R.; Matsumoto, K.; Pappas, G. D.; Zhang, X.; Guidotti, A. $GABA_A$ Receptors and Benzodiazepines: A Role for Dendritic Resident Subunit mRNAs. *Neuropharmacology* **2002**, *43*, 925–937.
(28) van Rijnsoever, C.; Täuber, M.; Choulli, M. K.; Keist, R.; Rudolph, U.; Möhler, H.; Fritschy, J. M.; Crestani, F. Requirement of α_5-$GABA_A$ Receptors for the Development of Tolerance to the Sedative Action of Diazepam in Mice. *J. Neurosci.* **2004**, *24*, 6785–6790.
(29) Li, M.; Szabo, A.; Rosenberg, H. C. Down-Regulation of Benzodiazepine Binding to α5 Subunit-Containing γ-Aminobutyric $Acid_A$ Receptors in Tolerant Rat Brain Indicates Particular Involvement of the Hippocampal CA1 Region. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 689–696.
(30) CookJ. M. HuangQ. HeX. LiX. YuJ. HanD. Anxiolytic Agents with Reduced Sedative and Ataxic Effects. US Patent 7,119,1996, 2006.
(31) White, H. S. Preclinical Development of Antiepileptic Drugs: Past, Present, and Future Directions. *Epilepsia* **2003**, *44*, 2–8.
(32) White, S. H., Woodhead, J. H., Wilcox, K. S., Stables, J. P., Kupferberg, H. J., Wolf, H. H. Discovery and Preclinical Development of Antiepileptic Drugs. In *Antiepileptic Drugs*, 5th ed.; Levy, R. H., Mattson, R. H., Meldrum, B. S., Perucca, E., Eds.; Lippincott Williams and Wilkins: Philadelphia, 2002; pp 36−48.
(33) Edwankar, R. V.; Edwankar, C. R.; Stables, J. P.; Cook, J. M. Unpublished results.

JM801652D