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# Antiseizure Activity of Novel Gamma-Aminobutyric Acid (A) Receptor Subtype-Selective Benzodiazepine Analogs 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 Furtmuller**[§], **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, NC 27599, Department of Biochemistry, CWRU Medical School, Cleveland, OH 44106, Center for Brain Research, Medical University Vienna, A-1090 Vienna, Austria*

[†]*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*

## 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) 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 administrated 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]

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

*To whom correspondence should be addressed. Phone: 414-229-5856, Fax: 414-229-5530, Email: E-mail: capncook@uwm.edu..

**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.

distributed within the brain. Electrophysiological changes attributed to certain 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 which limit their use as chronic anticonvulsant agents.[1-3] These side effects along with the issue of tolerance which 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 non-sedating 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 which 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, Mohler 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 analog **4**, but not to the control ligand **5**.

Examination of the initial anticonvulsant screen (Table 1, 100mg/kg) on ligands **1-5** (administered as free bases) at the 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 hours. 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 α1β3γ2 subtypes.[14,19]

The antiseizure activity in rat animal models for MES, scMET and toxicity showed that ligands **2-5** significantly increased the seizure threshold level of scMET in both oral (po) and intraperitoneal (ip) route of administration (Table 2). Using rats *via* the po routes the protection ranged from a median effective dose ($ED_{50}$) of 1.58mg/kg for **4** to 98.5mg/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 500mg/kg *via* either the po or ip routes of administration (Table 3). For 2′-pyrido analog **2** in rats, the protection was 100% dosed orally and 88% *via* the ip route after 0.5 hour. 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 4h at a lower dose of 15mg/kg. Ligand 8-iodo-imidazobenzodiazepine **5** showed no activity in mice dosed ip (data not shown). Since 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 50mg/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 admistration 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 difference 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 α2/α3 versus α1 subtypes and an efficacy at α1 that is lower than that displayed by diazepam. The efficacy level at α1 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 α1 (282%) appears to result in some minimal TOX for ligand **3** while ligand **2** (233%) had no TOX. Ligand **2** appears to have high enough efficacy at α2 and α3 to provide significant antiseizure activity with no toxicity in vivo (mice and rats) due to its lower efficacy at α1 subtypes compared to diazepam. Because of its simultaneous reduced efficacy at α1- and α5-$GABA_A$ 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]

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## aAbbreviations

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

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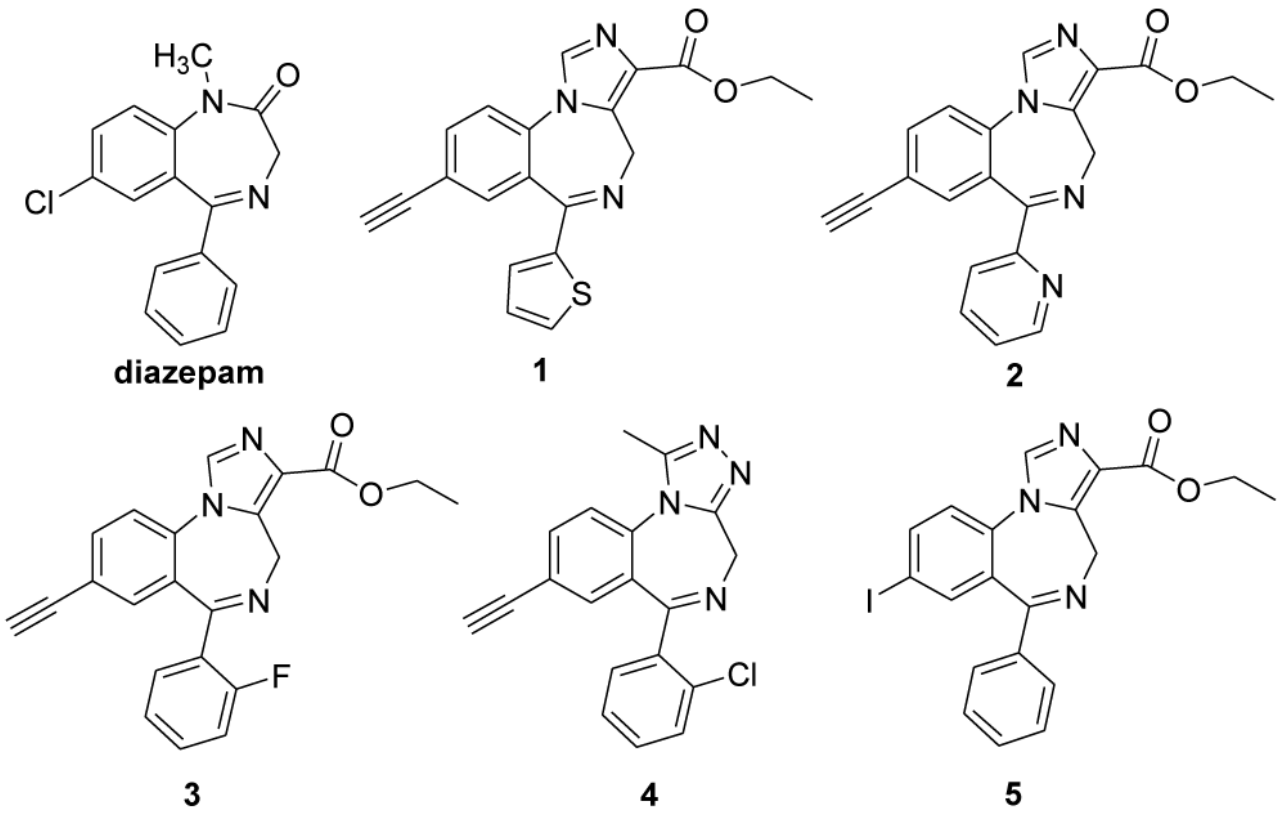


**Figure 1.**
Benzodiazepines (BDZs) **1-5**.

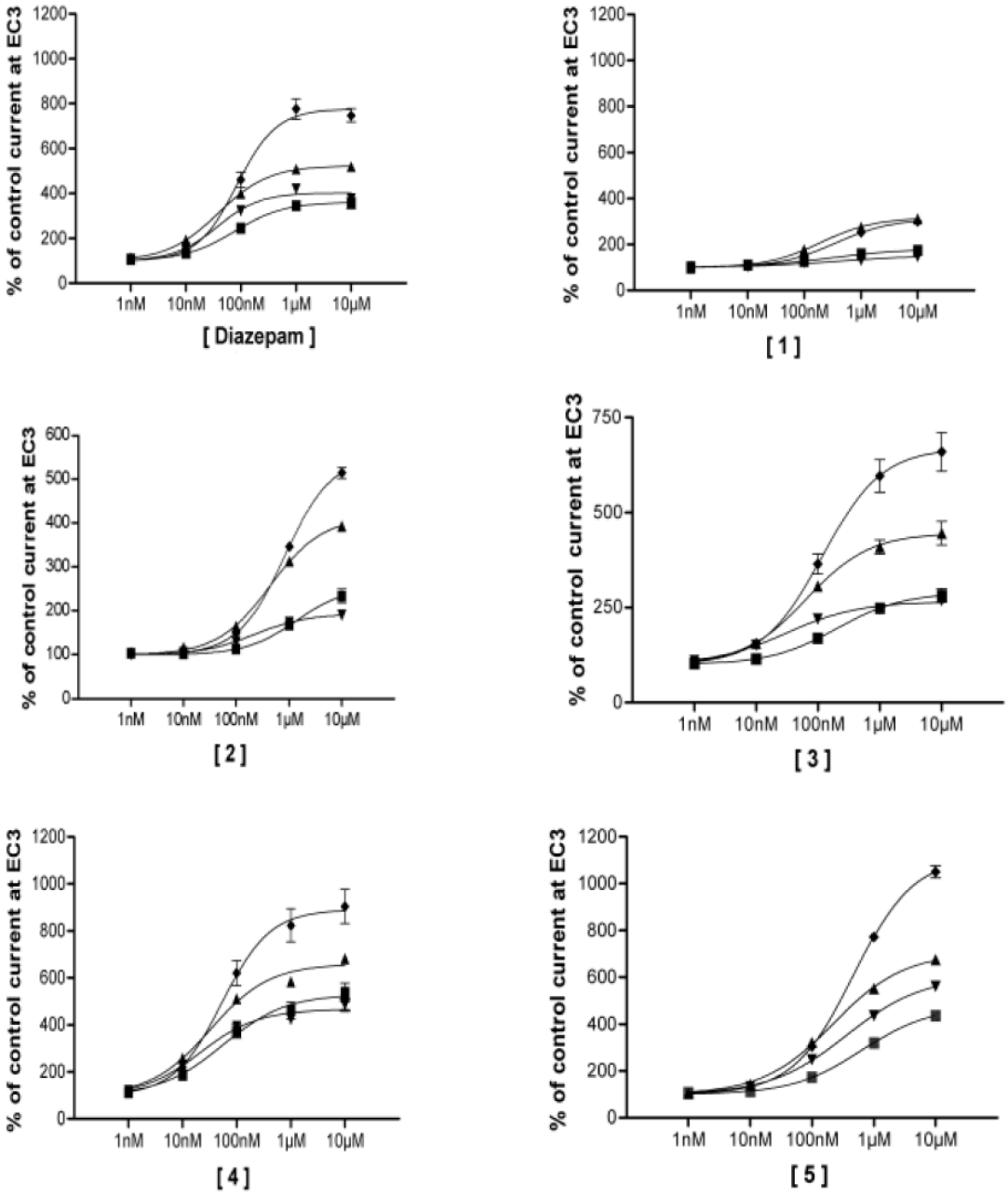


**Figure 2.**

Dose response curves for diazepam and **1-5** in oocytes expressing α1β3γ2 (■), α2β3γ3 (▲), 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 1μM 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 1μM 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 1μM 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).

**Table 1**

Assessment of Antiseizure Activity on Benzodiazepine (BDZ) Ligand **2** at 100mg/kg after 0.5 and 4.0 h in Mice *via* IP

| BDZ | Time (hour) | MES | Mice IP scMET | TOX |
|---|---|---|---|---|
| **2** | 0.5 | 0/3 | 3/5 | 0/8 |
| **2** | 4.0 | 0/3 | 0/1 | 0/4 |

Results indicate number protected or toxic/number tested.

Refer to Table 3 for abbreviations.

**Table 2**

Assessment of scMET Antiseizure Activity on Benzodiazepine (BDZ) Analogs 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.

**Table 3**

Quantification of Antiseizure Activity $ED_{50}$ MES, $ED_{50}$ scMET, $TD_{50}$ TOX, and Therapeutic Index (TI) via IP and PO routes

| Entry | Mice IP | | | Rat PO | | | TI Mice IP | TI Rat PO |
|---|---|---|---|---|---|---|---|---|
| | **$ED_{50}$ MES[c]** | **$ED_{50}$ scMET[c]** | **$TD_{50}$ TOX[c]** | **$ED_{50}$ MES[c]** | **$ED_{50}$ scMET[c]** | **$TD_{50}$ TOX[c]** | **$TD_{50}/ED_{50}$ (scMET)** | **$TD_{50}/ED_{50}$ (scMET)** |
| **2** | >300 | **16.28** | >**500** | >250 | **98.5** | >**500** | >**30.2** | >>**5**[b] |
| **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[a] | 7.81 | >50 | 45.4 | 5.35 | >250 | 364 | <0.9 | 1.5 |
| Clonazepam[a] | 25.6 | 0.02 | 0.26 | 7.86 | 0.61 | 2.38 | 13 | 3.9 |
| Phenytoin[a] | 5.64 | >50 | 41.0 | 28.1 | >500 | >1000 | <0.82 | 2.0 |

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.

[a] Refer to reference [32].

[b] A higher dose was not tested, since 500 mg/kg was clearly not sedating.

[c] All values are in mg/kg.

**Table 4**

Preliminary Hippocampal Kindling Screen-Rats IP

| BDZ | Seizure Score | | Afterdischarge Duration (secs) | | TOX[d] |
|---|---|---|---|---|---|
| | Pre-Drug L - H | Drug L - H | Pre-Drug L - H | Drug L - H | |
| **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 | 29 | 0/2[f] |

[a] Dose of 100mg/kg.

[b] Dose of 50mg/kg.

[c] Dose of 10mg/kg.

[d] After 1h of dosing.

[e] Dose of 30mg/kg.

[f] Dose of 3mg/kg. L = Low, H = High. Refer to Table 2 and 3 for abbreviations.