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Review Article

Improvements in the Pharmacological Profile of Diazepam by KRM-II-81, an Imidazodiazepine Positive Allosteric Modulator of α 2/3-Containing $GABA_A$ Receptors: Preclinical Data Predict Enhanced Efficacy for Epilepsy, Chronic Pain, Anxiety, and Depression

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ABSTRACT

Enhancement of $GABA_A$ receptor inhibition has long been used in the treatment of anxiety beginning with meprobamate, diazepam, chlordiazepoxide, and alprazolam in present times. Positive allosteric modulation of $GABA_A$ receptors has thus proven its place in medical practice. Subsequent work focused on the design of compounds with reduced sedative liabilities. Several non-benzodiazepine $GABA_A$-positive allosteric modulator (PAM) compounds (MRK-409, TPA-023, TPA-023B, NS11821, AZD7325 and PF-06372865) were tested in early clinical trials but suffered from signs of sedation and motor impairment and only three compounds progressed to proof of concept studies (TPA-023, AZD7325 and PF-06372865). TPA-023 was terminated due to toxicity in preclinical species while AZD7325 and PF-06372865 did not achieve efficacy endpoints in clinical trials. All compounds tested in Phase-II trials produced some signs of sedation at the minimum effective dose. We highlight a new compound, KRM-II-81, that is an imidazodiazepine selective for $GABA_A$ receptors containing α 2/3 proteins. KRM-II-81 has demonstrated a reduced liability for motor-impairing and respiratory effects compared to non-selective agents. KRM-II-81 has shown efficacy in animal models of epilepsy and is active in models for which other standard-of-care antiepileptics are not active. KRM-II-81 also produces anxiolytic-like effects but with minimal sedation. In contrast to benzodiazepines like diazepam, KRM-II-81 also produces anti-nociceptive effects including reduction in pain responses in models of neuropathic pain. Unlike diazepam, KRM-II-81 displays antidepressant-like effects. KRM-II-81 dampens cortical excitability in mice with traumatic brain injury. Thus, KRM-II-81 is a newly discovered, non-benzodiazepine compound, which targets a selective population of $GABA_A$ receptors for improved therapeutic gain and reduced side effects.

ABBREVIATIONS

KRM-II-81: 5-(8-ethynyl-6-(pyridin-2-yl)-4*H*-benzo[*f*]imidazo[1,5-a][1,4]diazepin-3-yl)oxazole; PAM: Positive Allosteric Modulator

INTRODUCTION

The history of diazepam rests upon the backs of giants. The first rationally-designed anxiolytic drug, meprobamate (Miltown) (Figure 1) was discovered and championed by Frank Berger who modified (with Bernard Ludwig) the muscle relaxant mephenesin with the goal of reducing muscle-relaxing and sedative properties while augmenting anti-anxiety effects. Miltown was the first blockbuster drug and was, in the late 1950s, being used by many people in the United States [1].

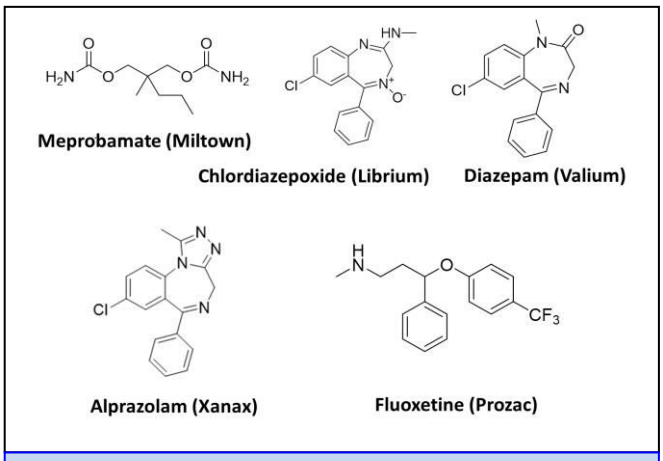

Figure 1: Structures of some classical anxiolytic drugs including the carbamate, meprobamate, the 1,4-benzodiazepines, chlordiazepoxide, diazepam, and alprazolam, and the selective serotonin uptake inhibitor, fluoxetine.

The carbamate, mebrobamate, led to the next generation of anxiolytic drugs - the 1,4-substituted benzodiazepines. In search of a drug to compete with meprobamate, Hoffmann La-Roche synthesized many compounds without finding improvement over meprobamate and the project was terminated by management. Months later, these compounds were slated for destruction when a lab technician noted that Ro 5-0690 had not been tested [2]. The head of medicinal chemistry, Leo Sternbach directed animal testing [3] and took the compound himself providing the first clinical data on chlordiazepoxide [2]. With the introduction of chlordiazepoxide (Librium) (Figure 1) into clinical practice with FDA approval in 1959, another generation of anxiolytic agents was born and, as with meprobamate, found widespread use for anxiety. Diazepam (Valium) (Figure 1) arose from the 1,4-substituted benzodiazepine chemical series and was approved for clinical use in 1965. By 1970, antianxiety drugs, mostly benzodiazepines, were used by 1 in 5 woman and 1 in 13 men in the United States [4]. Valium was and still is a highly valuable drug used for the treatment of anxiety and other disorders including acute convulsions. Despite its bad press for being addictive [5], and the reluctance of the medical community to prescribe it wholesale, it is still widely used and is sold over-the-counter in a number of countries and has been included in the World Health Organization's List of Essential Medicines. Valium as an anxiolytic has now been largely supplanted by another benzodiazepine, alprazolam [6,7] (Figure 1).

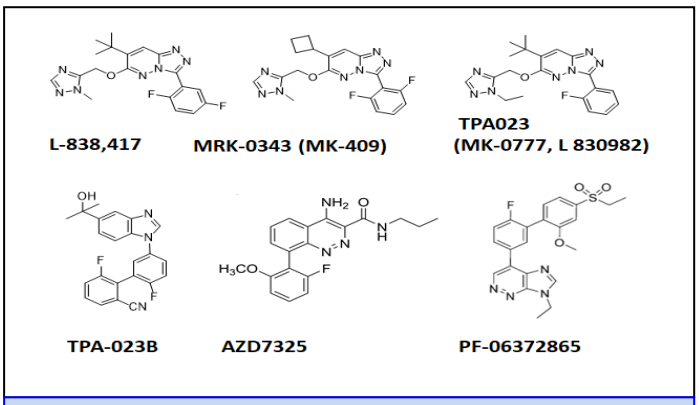

Figure 2: Structures of some compounds that potentiate α1-containing $GABA_A$ receptors less than that of other α -containing $GABA_A$ receptors.

Overall, this history demonstrates the huge demand for medicines that control anxiety, a disorder of high prevalence worldwide. In modern times, primarily due to concern for dependence and abuse of benzodiazepine anxiolytics, the first-line therapies for anxiety prescribed by most physicians in the United States are the antidepressant/anxiolytics that block reuptake of monoamines (e.g., selective serotonin uptake inhibitors or SSRIs like Prozac, Figure 1). Although there is ample clinical documentation of their ability to impact anxiety symptoms [8,9], the comparative magnitude of effect is often relatively small, it requires weeks of daily dosing to achieve full therapeutic benefit in responders [10-12] and can lead to adverse effects such as sexual dysfunction in some patients [13].

One issue with the benzodiazepine anxiolytics that is key to understanding their therapeutic value as well as an aspect of their pharmacology that impedes therapeutic utility is the dose-

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dependent sedation that these compounds produce. While sedation is sometimes desired, sedation is a dose-limiting side-effect for some other indications. For example, although it is well-known that increasing inhibitory tone in the nervous system by amplifying GABA signaling is a critical mechanism for many neurological and psychiatric disorders, the 1,4 benzodiazepines are often not used because efficacious plasma levels cannot be achieved without undesirable sedative and motor-impairing effects. This major point will be elaborated below in the discussion of the comparative pharmacology of diazepam vs. a newly discovered GABAA receptor PAM.

Rational drug discovery efforts directed at creating improved GABAA receptor PAMs came from basic pharmacological data along with the discovery of the benzodiazepine receptor [14,15] and its role in potentiation of GABA currents [16]. This discovery enabled establishment of binding assays (using [3H]BZs to identify and optimize ligands for benzodiazepine receptor interaction [17]. Promising ligands were then evaluated in animal models for efficacy and reduced unwanted side effects (reviewed in [18]). At least four such compounds (bretazenil, abecarnil, alpidem, and ocinaplon) progressed into clinical trials due to their favorable preclinical profile but mostly discontinued due to sedation (bretazenil, abecarnil) or liver toxicity (ocinaplon) observed in humans. Alpidem was approved as an anxiolytic with relatively little sedation [19] but was later withdrawn due to high occurrence of hepatitis [20]. Based upon the ability of some compounds to produce anxiolytic-like effects without sedation in animal models (e.g., CL218-872), it was early hypothesized that multiple benzodiazepine receptors might exist that mediate anxiolytic versus sedative effects [21] The advent of molecular biology enabled further refinement in the search for anxio-selective drugs.

The GABAA receptor is a pentameric ligand-gated ion channel, allowing for various combinations of five different subunits, which are expressed humans as the following types: α 1-6, β1-3, γ1-3, ρ1-3, δ, ε, π, and θ [22,23]. Each functional GABAA receptor includes both an α and β subunit, and typically include α-, β-, and γ-subunits in a 2:2:1 ratio for functional activity. The particular α-subunit contributing to the benzodiazepine binding site of GABAA receptors defines the receptor's pharmacological properties; α1-subtype-containing GABAA receptors have been found to preferentially mediate the sedative, amnestic, ataxic effects of ligands as well as dependence [24-29], whereas α2- and α3-subtypes mediate anxiolytic effects [30,31] and pain therapeutics [32,33] and the α5-subtype has been implicated in memory function [34,35] . Such studies also directly demonstrated that when the α1-subtype was rendered insensitive to benzodiazepines, the therapeutic window of diazepam was markedly increased [30] while the anxiolytic efficacy of diazepam was retained [25]. In addition, analgesic efficacy, not previously observed with diazepam, was uncovered due to the decreased sedation and motor impairment that resulted from the deletion of its interactions with α1-containing GABAA receptors [36]. Based primarily on the data associating α1-containing GABAA receptors with sedation, substantial discovery effort over the last 15 years was directed at the identification and development of GABAA -receptor PAM anxiolytics, antiepileptics and analgesics with preference for α2 and α3 over α1-containing GABAA receptor subtypes [37].

SUBTYPE SELECTIVE GABAA PAMS

One of the first "selective" molecules reported was L–838,417 (Figure 2), a partial agonist at α2,3- and α5-containing receptors and a negative allosteric modulator at α1-containing receptors. L-838,417, produced anxiolytic-like effects in the elevated plus maze but did not impair motor activity [25,38]. Further drug discovery effort at Merck resulted in three compounds which were progressed into clinical studies; two analogs of L–838,417 (TPA-023 and MRK-409) (Figure 2) and a structurally unrelated TPA-023B (Figure 2) [39]. All three compounds were partial agonists at α2/3 subtypes with no substantial α1 efficacy in vitro [40]; they were all efficacious in animal models of anxiety without observed sedation [41]. Clinical data, however, presented a more complex picture - MRK-409, despite its minimal activity at α1 subtypes, produced sedation in man at relatively low (< 10%) levels of receptor occupancy [42]. Considering that the sedation liability of MRK-409 in man could be attributed to its residual partial agonist efficacy at the α1 subtype [43], a second compound in

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this series, TPA-023, was developed which lacked any appreciable efficacy at the α1 subtype. In Phase-II clinical trial, TPA-023 produced anxiolytic effects, however it also exhibited signs of sedation such as dizziness, drowsiness, and motor incoordination [41,44-46]. The sedative effects were however observed at relatively high levels of receptor occupancy (>50%) which was substantially higher than 24% reported for diazepam [47]. The clinical trial was terminated early due to preclinical toxicity issues (cataract formation in rodents) which prevented completion of the study and determination of a conclusive efficacy readout. It is possible that mild sedative effects of TPA-023 are at least in part due to the potentiation of GABAA α 2/3 subtypes as reported in a recent primate study [48]. The follower compound, TPA-023B, which similarly lacked α1 PAM activity in vitro (was an antagonist) produced weak signs of sedation in early clinical trials at approximately 50% receptor occupancy [49]. No human efficacy data were reported and clinical development of TPA-023B was terminated. The reasons for this decision were not publicly disclosed.

Two more recent α1-sparing, partial subtype-selective GABAA receptor PAMs are NS11821from Neurosearch (structure not disclosed) which primarily potentiates α2/3/5 subtypes and AZD7325 from Astra Zeneca (Figure 2) which exhibited good efficacy at α2/3 subtypes. In animal models both ligands produced a dose dependent reduction of anxiety-like behavior with less sedation, motor impairment, and memory impairment than diazepam or chlordiazepoxide [50,51]. In early clinical trials NS11821 displayed a small pharmacodynamic effect (a decrease in saccadic peak velocity) with weak signs of sedation (body sway and the visual analogue scale for alertness) and signs of memory impairment which may result from its activity at α5 subtypes [51]. No receptor occupancy (RO) was reported for NS11821 and the compound was not evaluated in a proof of concept clinical trial. AZD7325 required RO > 80% [50,52] to produce a pharmacodynamic response (saccadic peak velocity, EEG spectrum); however, this high level of receptor occupancy was not sufficient for achieving significant anxiolytic activity [50]. While AZD7325 produced lower cognitive and neurophysiological side effects than lorazepam, benzodiazepine-like side effects (dizziness, headache and somnolence) were reported at sub-anxiolytic doses [50].

The most recent GABAA receptor subtype selective compound (α2/3/5 vs α1) evaluated in the clinic was PF-06372865 (Figure 2) [53,54]. PF-06372865 was efficacious in an animal model of absence epilepsy [55] in multiple pain modalities in a Phase-I clinical trial [56], and in a small Phase-II trial for photosensitive epilepsy [57]. No severe side effects were reported, although sedation and dizziness were reported in half of the photosensitive epilepsy patients. When tested in a larger Phase-II trial for lower back pain, the ligand did not achieve the primary efficacy end point of reduction in pain intensity and produced benzodiazepine-like side effects including sedation and memory impairment [58]. In a clinical Phase-II trial for anxiety, PF-06372865 failed for lack of efficacy and for induction of side effects [59]. It is possible that the ~50% occupancy produced with the maximal dose of 7.5 mg was not sufficient for critical therapeutic effect [53]; however, the occurrence of somnolence, dizziness and memory impairment at this dose would preclude higher dosing. No further work on this compound has been reported.

In summary, all of the compounds with relative in vitro preference for α2/3 versus α1-containing GABAA receptors displayed efficacy in the absence of benzodiazepine-like side effect in animal models. However, both the efficacy and the side-effect profiles in humans were not as impressive as preclinical data forecasted. Benzodiazepine-like side effects were observed for all compounds in early clinical trials and only three compounds progressed to Phase-II (TPA-023, AZD7325 and PF-06372865) where they suffered from weak efficacy at the dose that started to produce side effects. These findings suggest that reduced activity at α1-containing GABAA receptors, while beneficial, has not been sufficient to create the desired therapeutic profile for the drug developers to date.

BIASED BENZODIAZEPINE RECEPTOR LIGANDS

Even though benzodiazepines as a class act at all γ subunit containing GABAA receptors (α1,2,3,5), some compounds display less sedation than others. One such compound is clobazam [60,61], whose milder sedative liability could have contributed to its approval as an add-on therapy for Lennox-Gastaut syndrome [62]. A small proof of concept clinical trial

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also reported reduction of capsaicin-induced hyperalgesia with clobazam [63]. The activity of clobazam might be due, at least in part, to buildup of its active metabolite, N-desmethyl-clobazam. The metabolite exhibits functional selectivity for α2,3,5 GABAA receptor subtypes and is less efficacious at α1 subtype [64]. N-desmethyl-clobazam was further evaluated in invivo in animal models of pain where it produced significant analgesia without sedation [64] The authors of the study filed a patent for clinical use of N-desmethyl-clobazam for chronic pain [65] but the compound has not, to our knowledge, been evaluated in a clinical setting. The discovery of compounds which retain the beneficial properties of benzodiazepines but cause less sedation continues to be an exciting proposition.

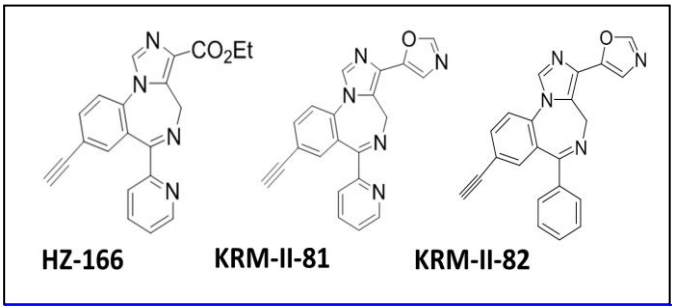

Figure 3: Structure of HZ-166 and the ester bioisosters, KRM-II-81 and KRM-II-82. HZ-166 and KRM-II-81 are selective for α2/3-containing $GABA_A$ receptors while KRM-II-82 is not selective.

Cook and associates synthesized HZ-166 (Figure 3), a non-benzodiazepine molecule [imidizodiazepine] GABAA receptor PAM with preference for α2 and α3 versus α1-containing GABAA receptor subtypes [66,67], The selectivity of HZ-166 compared to diazepam is shown in (Figure 4). HZ-166 was efficacious in animal models of pain and produced no overt sedation or tolerance [68]. Further progression of HZ-166 was prevented by its poor pharmacokinetic properties resulting from the ester functionality rendering the compound liable to metabolic deactivation through ester hydrolysis (e.g., Poe et al. [67]). This liability led to SAR optimization and the synthesis of several HZ-166 analogs with improved pharmacokinetic properties. One such analog, KRM-II-81 (Figure 3) was discovered in 2016 when Poe and colleagues created the oxazole bio-isostere of HZ-166, KRM-II-81, by a straightforward synthetic route (Figure 5). KRM-II-81 retained selectivity for GABAA α2/3 receptors over GABAA α1 receptors expressed in oocytes [33,69] (Figure 4). In contrast, diazepam does not largely discriminate among GABAA receptor configurations (Figure 4). Similar observations were reported for GABAA receptors expressed in mammalian cells where both the potency and efficacy of KRM-II-81KRM-II-81 at the α1 subtype were lower than at α2 and 3 subtypes [67]. Conversely, the reverse was reported for zolpidem with higher efficacy and potency at the α1 subtype [70].

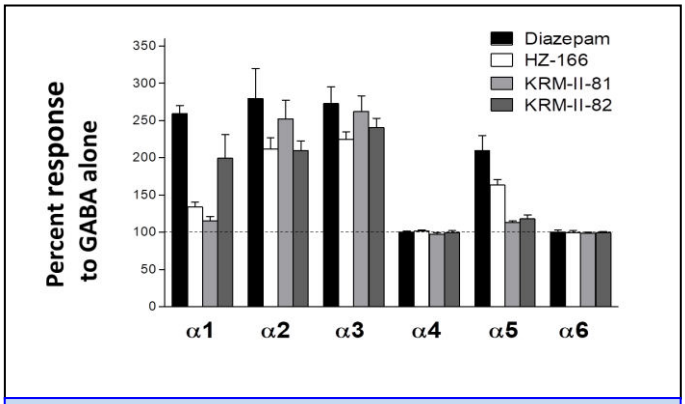

Figure 4: Average enhancement of the current evoked by GABA EC_3 by 0.1 μM (α1, α2, α3, α5) or 1 μM (α4, α6) of the modulator indicated. The response was divided by the peak response to GABA alone for each cell. The dashed line at 100% indicates the response to GABA alone. Bars represent mean + SEM (n = 4−8). Cells were transiently transfected with one of the α subtypes, as indicated, along with β3 and γ2L, and voltage clamped at −50 mV. Data for KRM-II-81 and diazepam are replotted from Lewter et al. [33]; data for KRM-II-82 are replotted from Methuku et al. [69].

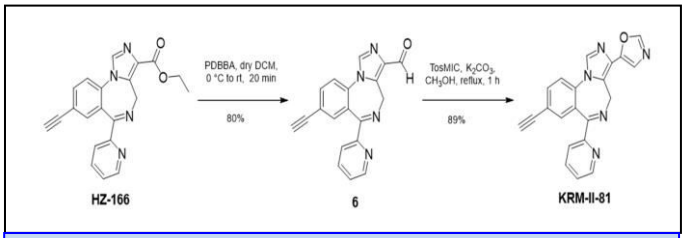

Figure 5. Synthetic scheme for KRM-II-81 from HZ-166.

The physiological relevance of the effects of KRM-II-81was further demonstrated in isolated dorsal root ganglion neurons where KRM-II-81 potentiated native GABA currents [71].

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Surprisingly the 30nM potency of KRM-II-81 in native cells exceeded the potency reported for recombinant cells [67] and oocytes [33] with the mechanism of potency differences remaining unexplored. For predicting behavioral end points, data from isolated neurons may not be sufficient in view of neuronal network complexity. For that reason, the effects of KRM-II-81 were tested on hyper-excited networks of cortical neurons recorded with a microelectrode array. KRM-II-81 reduced the frequency of neuronal firing and bursting [72] thus demonstrating the relevance of KRM-II-81 as a potential antiepileptic drug. The anticonvulsant action of KRM-II-81 in vitro was confirmed by microelectrode recordings from slices obtained from freshly excised cortex from epileptic patients where KRM-II-81 suppressed epileptiform activity.

As a GABAA receptor PAM, KRM-II-81 produced a host of effects that suggest its viability as a therapeutic for epilepsy [72,73], pain [33,71], anxiety [67,74], depression [69], and traumatic brain injury [75]. The discussion to follow will provide data to illustrate these biological activities of KRM-II-81 and those that differentiate the effects of KRM-II-81 from that of the non-α-selective compounds diazepam, chlordiazepoxide, and alprazolam. Both diazepam and KRM-II-81 produce anticonvulsant effects in rodent models [72]. However, under some conditions, diazepam was less efficacious. For example, KRM-II-81 increased the seizure threshold to pentylenetetrazol to a greater extent than diazepam (Figure 6, left panel) and increased the after-discharge threshold more than diazepam in amygdala-kindled rats (Figure 6, right panel). In both assays, HZ-166 was inactive (Figure 6). KRM-II-81 exhibited broad efficacy as an anticonvulsant drug in a host of seizure-provocation models [72] and in models of pharmaco-resistant epilepsy where some standard-of-care antiepileptic medicines are ineffective [75].

Although GABA is known to be an integral biological mediator of pain, diazepam and other 1,4-benzodiazepines are generally not used to control pain [76]. It is argued that the sedative liabilities of diazepam do not allow sufficient dosing to produce therapeutic benefit [25,36,43,77,78]. In an animal model of inflammatory pain, diazepam is not active in reducing formalin-induced pain behaviors while KRM-II-81 is (Figure 7). KRM-II-81 is also effective in reducing pain in other rodent models of inflammatory pain [33] and in models of neuropathic pain [71].

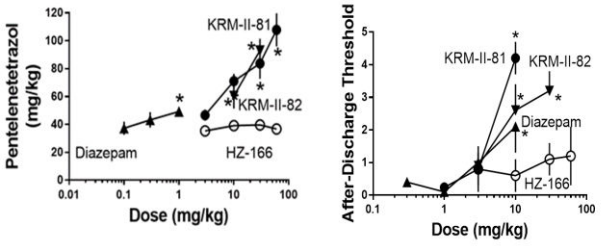

Figure 6. Left Panel. Comparative effects of HZ-166, KRM-II-81, KRM-II-82, and diazepam against convulsions induced by pentylenetetrazole (PTZ, i.v.) in rats. Data show the dose of PTZ required to induce convulsions as a function of drug dose (mean ± SEM, n=8). * $p<0.05$ compared to vehicle by Dunnett's test after ANOVA. Right Panel. Comparative effects of HZ-166, KRM-II-81, and diazepam on after-discharge thresholds in amygdala-kindled rats. The scale for the after-discharge threshold (ADT) is scale-adjusted to capture the stimulation scale change required to observe a seizure from the previous baseline to the ADT scored on test day. The average scale adjusted ADT was approximately 0.63 mA in vehicle treated rats. * $p<0.05$ compared to vehicle control (n=8/group) by Dunnett's test after ANOVA Reprinted from Neuropharmacology, Vol. 137, Witkin et al. [72], Bioisosteres of ethyl 8-ethynyl-6-(pyridin-2-yl)-4H-benzo[f]imidazo [1,5-a][1,4]diazepine-3-carboxylate (HZ-166) as novel α2,3 selective potentiators of $GABA_A$ receptors: Improved bioavailability enhances anticonvulsant efficacy.Pages 332–343, Copyright © 2018, with permission from Elsevier Ltd.

Diazepam is a known anxiolytic [79]. The GABAA receptor α2/3 mechanism is also effective in producing anxiolytic-like effects. For example, KRM-II-81, like the anxiolytic chlordiazepoxide, decreased marble-burying in mice (Figure 8, left panel). However, in contrast to KRM-II-81, chlordiazepoxide impaired motor performance of these mice on a rotarod (Figure 8, right panel).

Benzodiazepine anxiolytic drugs are not generally used for the treatment of Major Depressive Disorder (MDD). KRM-II-81 was active in the forced-swim test in mice, a model that detects antidepressant drugs (Figure 9). In contrast, diazepam was not active under these conditions. However, if the motor-impairing effects of diazepam are prevented by the α1-containing

GABAA receptor antagonist, B-CCt [74], then diazepam showed an antidepressant-like signal comparable to that of KRM-II-81 (Figure 9).

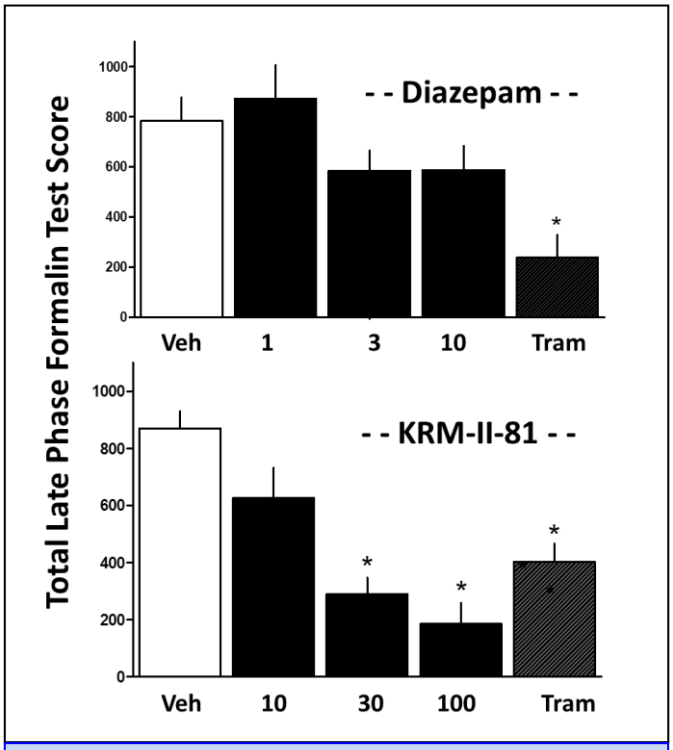

Figure 7: Comparison of effects of diazepam and KRM-II-81 on pain behaviors in the late phase of the formalin assay in rats. Effects of tramadol (Tram, 80 mg/kg) are shown as a positive control. Each point represents the mean + SEM of the same 8 rats *p<0.05 compared to vehicle control data by Dunnett's test after ANOVA. Data are extracted and replotted from Witkin et al. [71].

That α1-containing GABAA receptors are responsible for the motor-impacting effects of GABAA receptor PAMs was further supported by data comparing KRM-II-81 with KRM-II-82. KRM-II-82 impaired rotarod performance of mice at 30 mg/kg whereas KRM-II-81 did not (Figure 10). KRM-II-82 potentiated current in GABAA receptors containing α1 subunits, whereas KRM-II-81 did not (Figure 4).

Side effects of drugs are only important when considered in relationship to their therapeutic or efficacious doses or exposure levels. Diazepam and KRM-II-81 can be contrasted based upon a 'therapeutic index'. For example, when doses that impair motor performance are compared to the doses that produce efficacy in rodent seizure models, KRM-II-81 showed a larger separation or protective index than that of diazepam across a host of assays (Figure 11). A similar separation in respiratory side-effects has been reported between KRM-II-81 and the anxiolytic alprazolam (Figure 12).

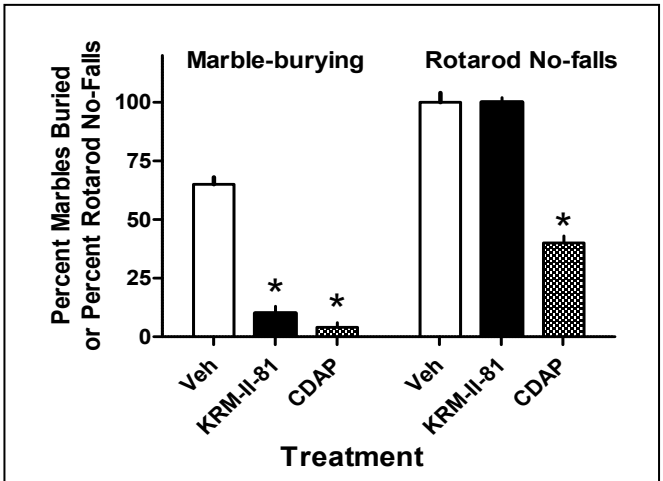

Figure 8. Left Panel. Effects of KRM-II-81 and chlordiazepoxide (CDAP) on marble-burying in mice. Data represent the mean + SEM (n=10). * p<0.05 compared to vehicle control by Dunnett's test after ANOVA. Right Panel. Effects of KRM-II-81 and chlordiazepoxide on rotarod performance of mice. Data represent the mean ± SEM (n=10). KRM-II-81 and chlordiazepoxide were given at 30 mg/kg. * p<0.05 compared to vehicle control by Dunnett's test after ANOVA. Data are extracted and replotted from Li et al. and Poe et al. [67].

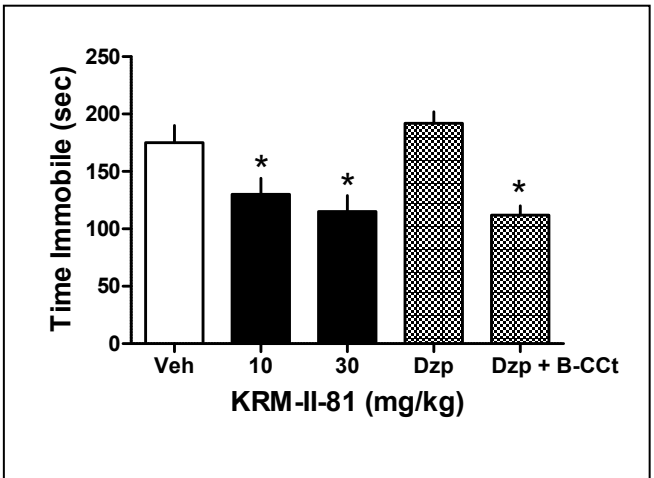

Figure 9: Effects of KRM-II-81 (10 and 30 mg/kg, i.p.) and diazepam (22 mg/kg, p.o.) alone or in the presence of B-CCt (10 mg/kg) on immobility time in the forced-swim test in mice. Data represent the mean + SEM (n=7-8). * p<0.05 compared to vehicle control by Dunnett's test after ANOVA. Data are extracted and replotted from Methuku et al. [69].

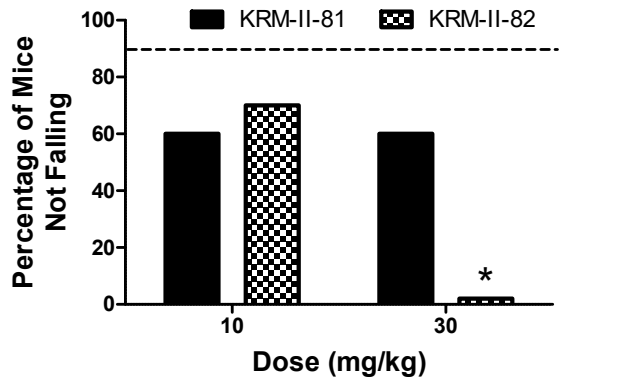

Figure 10: Effects of KRM-II-81 and KRM-II-82 on rotarod performance of mice. Data represent the mean (n=10). * $p<0.05$ compared to vehicle control. Data are extracted and replotted from Poe et al. [67].

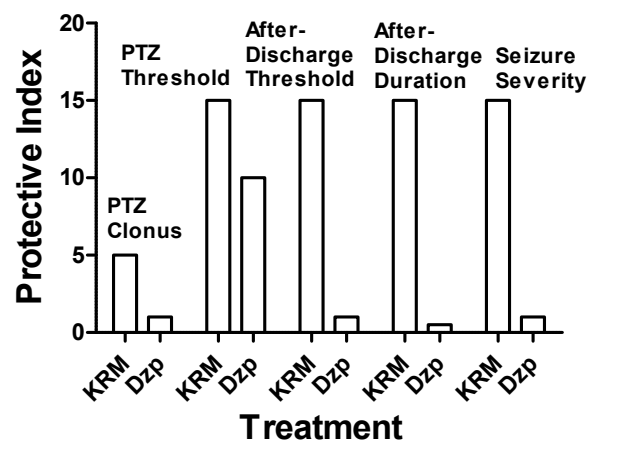

Figure 11: Protective indices for different anticonvulsant measures in rats. The protective index is the ratio [minimal effective dose impairing motor performance/minimal effective anticonvulsant dose]. KRM: KRM-II-81; Dzp: diazepam. Data are extracted and replotted from Witkin et al., [72].

The idea that α protein composition of GABAA receptors can guide pharmacological effects has been key to the rational discovery of the compounds discussed above. However, it should be recognized that there are aspects of the biology and pharmacology of GABAA receptors that are not fully understood. The mechanism of sedation appears to be more complex than the singular reliance on α1 proteins. For example, a recent study in primates demonstrated that anxiolytic compounds with functional selectivity for α2, α3, and/or α5 GABAA receptors can produce a mild-type of sedation through the activation of the same receptors subtypes [48]. In addition there is evidence that some of the sedation and ataxia mediated by drugs that fell out of the clinic was mediated by positive modulation at α5/β3/γ2 subtypes [80-82]. The non-sedating profile of KRM-II-81 is therefore consistent with the lack of α5 GABAA receptor potentiation observed invitro [33,69]. A different study using α1-subtype diazepam insensitive mice [α1 (H101R) mice] found no difference in the effect of diazepam on sleep EEG between the mutant and the wild type mice [83]. Considering that sleep can be used as sedation biomarker [18] the data suggest that the α1 subtype of GABAA receptor is not the exclusive driver of sedation.

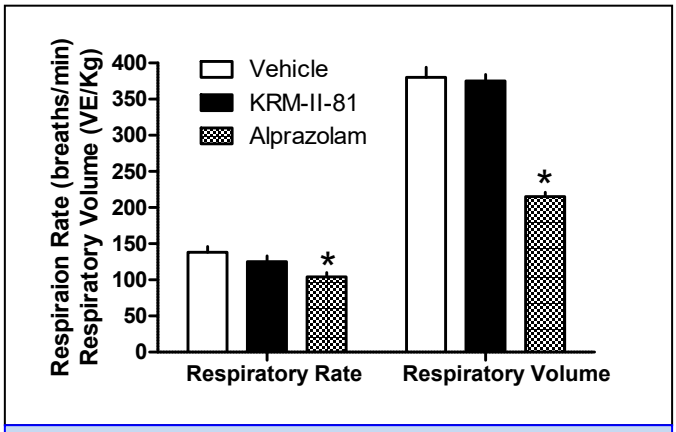

Figure 12: Effects of KRM-II-81 compared to alprazolam on respiration in rats. Both compounds were dosed at 3.2 mg/kg. Data are means + SEM (n=8). Respiration rate was measured as breaths/min and respiratory volume was tidal volume/Kg. * $p<0.05$ compared to vehicle control by Dunnett's test after ANOVA. Data are extracted and replotted from Witkin et al. [71].

We suggest that there are likely multiple routes and mechanisms by which one can design molecules to achieve medically-targeted effects while reducing side-effects. KRM-II-81 appears to achieve this end through its selectivity to α2/3-containing GABAA receptors. Ocinaplon, in contrast, does not selectively target these α proteins but has been shown in the clinic to have a reduced sedative liability [84,85]. Other methods may also exist for optimized drug efficacy/side-effect balances within this system. For example, β2/3-subunit

subtype-selective GABAA receptor PAMs have been shown to produce reduced motor-impacting effects [86-88].

Finally, while lower sedative effects are desirable, their elimination is not necessarily required for an improved therapeutic agent. The benzodiazepine, alprazolam, is highly prescribed for anxiety despite sedative properties. Other areas of neurological and psychiatric practice are in such need of improved medications, that improved efficacy, as predicted for KRM-II-81, are likely sufficient for driving their development. For example, despite its dose-dependent induction of motor-impairment that includes falling, perampanel (Fycompa) is used as a newer antiepileptic drug [89]. And for pain, the opioids that have produced devastating health and morbidity consequences [90] are in clear need of replacement where efficacy with improved safety, as predicted for KRM-II-81, would be key developmental drivers.

In summary, KRM-II-81 produces biological effects in rodent models suggesting its therapeutic value in several neurological and psychiatric disorders. At the same time, KRM-II-81 can be differentiated from that of GABAA receptor modulators that are not selective for α2/3-containing GABAA receptor PAMs. The unique and improved efficacy of KRM-II-81 in models of epilepsy and in neuropathic pain is promising. The compound awaits clinical data to begin to evaluate the therapeutic potential of this novel GABAA receptor PAM.

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Conflict of interest

Authors report no conflict of interest with the exception that James M. Cook is a patent holder for the invention of KRM-II-81. The University of Wisconsin-Milwaukee owns the patent.

REFERENCES

1. Tone A. (2009). The age of anxiety: A history of America's turbulent affair with tranquilizers. New York, NY, US: Basic Books.
2. Winters RW. (2016). Accidental Medical Discoveries: How Tenacity and Pure Dumb Luck Changed the World, 1 edition. Skyhorse Publishing.
3. Randall LO, Schallek W, Heise GA, Keith EF, Bagdon RE. (1960). The Psychosedative Properties of Methaminodiazepoxide. J Pharmacol Exp Ther. 129:163-171.
4. Parry HJ, Balter MB, Mellinger GD, Cisin IH, Manheimer DI. (1973). National Patterns of Psychotherapeutic Drug Use. Arch Gen Psychiatry. 28:769-783.
5. Hollister LE, Bennett JL, Kimbell I, Savage C, Overall JE. (1963). Diazepam in newly admitted schizophrenics. Dis Nerv Syst. 24:746-750.
6. Aden GC, Thein SG. (1980). Alprazolam compared to diazepam and placebo in the treatment of anxiety. J Clin Psychiatry. 41:245-248.
7. Maletzky BM (1980) Anxiolytic efficacy of alprazolam compared to diazepam and placebo. J Int Med Res 8:139143.
8. Birmaher B, Waterman GS, Ryan N, Cully M, Balach L, et al. (1994). Fluoxetine for Childhood Anxiety Disorders. J Am Acad Child Adolesc Psychiatry. 33:993-999.
9. Baldwin D, Woods R, Lawson R, Taylor D. (2011). Efficacy of drug treatments for generalised anxiety disorder: systematic review and meta-analysis. BMJ. 342:1199.
10. Katz MM, Koslow SH, Frazer A. (1996). Onset of antidepressant activity: reexamining the structure of depression and multiple actions of drugs. Depress Anxiety. 4:257-267.
11. Michelson D, Allgulander C, Dantendorfer K, Knezevic A, Maierhofer D, et al. (2001). Efficacy of usual antidepressant dosing regimens of fluoxetine in panic disorder: Randomised, placebo-controlled trial. Br J Psychiatry. 179:514-518.
12. Perez V, Puiigdemont D, Gilaberte I, Alvarez E, Artigas F, et al. (2001). Augmentation of fluoxetine's antidepressant action by pindolol: analysis of clinical, pharmacokinetic,

and methodologic factors. J Clin Psychopharmacol. 21:36-45.

13. Montejo-Gonzalez AL, Llorca G, Izquierdo JA, Ledesma A, Bousono M, et al. (1997). SSRI-induced sexual dysfunction: fluoxetine, paroxetine, sertraline, and fluvoxamine in a prospective, multicenter, and descriptive clinical study of 344 patients. J Sex Marital Ther. 23:176-194.
14. Braestrup C, Squires RF. (1977). Specific benzodiazepine receptors in rat brain characterized by high-affinity (3H)diazepam binding. Proc Natl Acad Sci USA. 74:3805-3809.
15. Haefely WE. (1989). Pharmacology of the benzodiazepine receptor. Eur Arch Psychiatr Neurol Sci. 238:294-301.
16. Choi DW, Farb DH, Fischbach GD. (1977). Chlordiazepoxide selectively augments GABA action in spinal cord cell cultures. Nature. 269:342-344.
17. Williamson MJ, Paul SM, Skolnick P. (1978). Labelling of benzodiazepine receptors in vivo. Nature. 275: 551-553.
18. Skolnick P. (2012). Anxioselective anxiolytics: on a quest for the Holy Grail. Trends Pharmacol Sci. 33: 611-620.
19. Musch B, Morselli PL, Priore P. (1988). Clinical studies with the new anxiolytic alpidem in anxious patients: an overview of the European experiences. Pharmacol Biochem Behav. 29:803-806.
20. Baty V, Denis B, Goudot C, Bas V, Renkes P, et al. (1994). [Hepatitis induced by alpidem (Ananxyl). Four cases, one of them fatal]. Gastroenterol Clin Biol. 18:1129-1131.
21. Klepner CA, Lippa AS, Benson DI, Sano MC, Beer B. (1979). Resolution of two biochemically and pharmacologically distinct benzodiazepine receptors. Pharmacol Biochem Behav. 11:457-462.
22. Hevers W, Lüddens H (1998) The diversity of GABAA receptors. Pharmacological and electrophysiological properties of GABAA channel subtypes. Mol Neurobiol 18:3586.
23. Olsen RW, Sieghart W (2009) GABA A receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology 56:141148.
24. Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, et al. (1999). Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature. 401:796-800.
25. McKernan RM et al. (2000). Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. Nat Neurosci. 3: 587-592.
26. Wafford KA. (2005). GABAA receptor subtypes: any clues to the mechanism of benzodiazepine dependence? Current Opinion in Pharmacology. 5:47-52.
27. Licata SC, Platt DM, Cook JM, Van Linn ML, Rowlett JK. (2009). Contribution of alpha1 subunit-containing gamma-aminobutyric acidA (GABAA) receptors to motor-impairing effects of benzodiazepines in squirrel monkeys. Psychopharmacology (Berl). 203:539-546.
28. Ator NA, Atack JR, Hargreaves RJ, Burns HD, Dawson GR. (2010). Reducing Abuse Liability of GABAA/Benzodiazepine Ligands via Selective Partial Agonist Efficacy at α1 and α2/3 Subtypes. J Pharmacol Exp Ther. 332:4-16.
29. Tan KR, Brown M, Labouebe G, Yvon C, Creton C, et al. (2010). Neural bases for addictive properties of benzodiazepines. Nature. 463:769-774.
30. Low K, Crestani F, Keist R, Benke D, Brunig I, et al. (2000). Molecular and neuronal substrate for the selective attenuation of anxiety. Science. 290:131-134.
31. Rudolph U, Mohler H. (2014). GABAA receptor subtypes: Therapeutic potential in Down syndrome, affective disorders, schizophrenia, and autism. Annu Rev Pharmacol Toxicol. 54:483-507.
32. Dias R. et al. (2005). Evidence for a Significant Role of α3-Containing GABAA Receptors in Mediating the Anxiolytic Effects of Benzodiazepines. J Neurosci. 25:10682-10688.
33. Lewter LA, Fisher JL, Siemian JN, Methuku KR, Poe MM, et al. (2017). Antinociceptive effects of a novel α2/α3 subtype selective GABAA receptor positive allosteric modulator. ACS Chem Neurosci. 8:1305-1312.
34. Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, et al. (2002). Enhanced learning and memory and altered GABAergic synaptic transmission in mice

lacking the alpha 5 subunit of the GABAA receptor. J Neurosci. 22: 5572-5580.

35. Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, et al. (2006). An inverse agonist selective for alpha5 subunit-containing GABAA receptors enhances cognition. J Pharmacol Exp Ther. 316:1335-1345.
36. Ralvenius WT, Benke D, Acuna MA, Rudolph U, Zeilhofer HU. (2015). Analgesia and unwanted benzodiazepine effects in point-mutated mice expressing only one benzodiazepine-sensitive GABAA receptor subtype. Nat Commun. 6: 6803.
37. Rudolph U, Knoflach F. (2011). Beyond classical benzodiazepines: novel therapeutic potential of GABA A receptor subtypes. Nat Rev Drug Discov. 10:685-697.
38. Carling RW,Madin A, Guiblin A, Russell MG, Moore KW, et al. (2005). 7-(1,1-Dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine: a functionally selective gamma-aminobutyric acid(A) (GABA(A)) alpha2/alpha3-subtype selective agonist that exhibits potent anxiolytic activity but is not sedating in animal models. J Med Chem. 48:7089-7092.
39. Atack JR. (2011). GABAA receptor subtype-selective modulators. I. α2/α3-selective agonists as non-sedating anxiolytics. Curr Top Med Chem. 11:1176-1202.
40. Atack JR, Wafford KA, Tye SJ, Cook SM, Sohal B, et al. (2006). TPA023 [7-(1,1-dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine], an agonist selective for alpha2- and alpha3-containing GABAA receptors, is a nonsedating anxiolytic in rodents and primates. J Pharmacol Exp Ther. 316:410-422.
41. Atack JR. (2009). Subtype-selective GABA(A) receptor modulation yields a novel pharmacological profile: the design and development of TPA023. Adv Pharmacol. 57:137-185.
42. de Haas SL, de Visser SJ, van der Post JP, Schoemaker RC, van Dyck K, et al. (2008). Pharmacodynamic and pharmacokinetic effects of MK-0343, a GABA(A) alpha2,3 subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. J Psychopharmacol. 22:24-32.
43. Atack JR. (2010). GABAA receptor alpha2/alpha3 subtype-selective modulators as potential nonsedating anxiolytics. Curr Top Behav Neurosci. 2:331-360.
44. de Haas SL, de Visser SJ, van der Post JP, de Smet M, Schoemaker RC, et al. (2007). Pharmacodynamic and pharmacokinetic effects of TPA023, a GABA(A) alpha(2,3) subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. J Psychopharmacol. 21:374-383.
45. de Haas SL, Zoethout RWM, Van Dyck K, De Smet M, Rosen LB, et al. (2012). The effects of TPA023, a GABAAα2,3 subtype-selective partial agonist, on essential tremor in comparison to alcohol. J Psychopharmacol. 26: 282-291.
46. Atack JR,Wafford KA, Tye SJ, Cook SM, Sohal B, et al. (2010). Benzodiazepine binding site occupancy by the novel GABAA receptor subtype-selective drug 7-(1,1-dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine (TPA023) in rats, primates, and humans. J Pharmacol Exp Ther. 332:17-25.
47. Fujita M, Woods SW, Verhoeff NP, Abi-Dargham A, Baldwin RM, et al. (1999). Changes of benzodiazepine receptors during chronic benzodiazepine administration in humans. Eur J Pharmacol 368:161-172.
48. Duke AN, Meng Z, Platt DM, Atack JR, Dawson GR, et al. (2018). Evidence That Sedative Effects of Benzodiazepines Involve Unexpected GABAA Receptor Subtypes: Quantitative Observation Studies in Rhesus Monkeys. J Pharmacol Exp Ther. 366:145-157.
49. Atack JR, Hallett DJ, Tye S, Wafford KA, Ryan C, et al. (2011). Preclinical and clinical pharmacology of TPA023B, a GABAA receptor α2/α3 subtype-selective partial agonist. J Psychopharmacol. 25:329-344.
50. Chen X, Jacobs G, Kam M de, Jaeger J, Lappalainen J, et al. (2014). The central nervous system effects of the partial GABA-Aα2,3-selective receptor modulator AZD7325 in comparison with lorazepam in healthy males. Br J Clin Pharmacol. 78: 1298-1314.
51. Zuiker RGJA, Chen X, Osterberg O, Mirza NR, Muglia P, et al. (2016). NS11821, a partial subtype-selective

GABAA agonist, elicits selective effects on the central nervous system in randomized controlled trial with healthy subjects. J Psychopharmacol. 30:253-262.

52. Jucaite A, Cselenyi Z, Lappalainen J, McCarthy DJ, Lee C-M, et al. (2017). GABAA receptor occupancy by subtype selective GABAAα2,3 modulators: PET studies in humans. Psychopharmacology (Berl). 234:707-716.
53. Nickolls SA, Gurrell R, van Amerongen G, Kammonen J, Cao L, et al. (2018). Pharmacology in translation: the preclinical and early clinical profile of the novel α2/3 functionally selective GABAA receptor positive allosteric modulator PF-06372865. Br J Pharmacol. 175:708-725.
54. Owen RM,Blakemore D, Cao L, Flanagan N, Fish R, et al. (2019). Design and Identification of a Novel, Functionally Subtype Selective GABAA Positive Allosteric Modulator (PF-06372865). J Med Chem. 62:5773-5796.
55. Duveau V, Buhl DL, Evrard A, Ruggiero C, Mande-Niedergang B, et al. (2018). Pronounced antiepileptic activity of the subtype-selective GABAA-positive allosteric modulator PF-06372865 in the GAERS absence epilepsy model. CNS Neurosci Ther. 25:255-260.
56. van Amerongen G, Siebenga PS, Gurrell R, Dua P, Whitlock M, et al. (2019). Analgesic potential of PF-06372865, an α2/α3/α5 subtype-selective GABAA partial agonist, in humans. Br J Anaesth. 123: 194-203.
57. Gurrell R, Gorman D, Whitlock M, Ogden A, Reynolds DS, et al. (2019). Photosensitive epilepsy: Robust clinical efficacy of a selective GABA potentiator. Neurology. 92:1786-1795.
58. Gurrell R, Dua P, Feng G, Sudworth M, Whitlock M, et al. (2018). A randomised, placebo-controlled clinical trial with the α2/3/5 subunit selective GABAA positive allosteric modulator PF-06372865 in patients with chronic low back pain. Pain. 159:1742-1751.
59. Simen A, Whitlock M, Qiu R, Miceli J, Zumpano L, et al. (2019). An 8-Week, Randomized, Phase 2, Double-Blind, Sequential Parallel-Group Comparison Study of Two Dose Levels of the GABAA Positive Allosteric Modulator PF-06372865 Compared With Placebo as an Adjunctive Treatment in Outpatients With Inadequate Response to Standard of Care for Generalized Anxiety Disorder. J Clin Psychopharmacol. 39:20-27.
60. Wildin JD, Pleuvry BJ, Mawer GE, Onon T, Millington L. (1990). Respiratory and sedative effects of clobazam and clonazepam in volunteers. Br J Clin Pharmacol. 29:169-177.
61. Sankar R. (2012). GABA(A) receptor physiology and its relationship to the mechanism of action of the 1,5-benzodiazepine clobazam. CNS Drugs. 26:229-244.
62. Ng YT, Conry JA, Drummond R, Stolle J, Weinberg MA, et al. (2011). Randomized, phase III study results of clobazam in Lennox-Gastaut syndrome. Neurology. 77:1473-1481.
63. Besson M, Matthey A, Daali Y, Poncet A, Vuilleumier P, et al. (2015). GABAergic modulation in central sensitization in humans: a randomized placebo-controlled pharmacokinetic-pharmacodynamic study comparing clobazam with clonazepam in healthy volunteers. Pain. 156:397-404.
64. Ralvenius WT, Desmeules J, Besson M, Zeilhofer HU, Daali Y, et al. (2016). Use of n-desmethylclobazam in the treatment of chronic pain disorders and related methods.
65. RALVENIUS WT, DESMEULES J, BESSON M, Zeilhofer HU, DAALI Y, MATTHEY A. (2016). Use of n-desmethylclobazam in the treatment of chronic pain disorders and related methods. Available at: https://patents.google.com/patent/EP3064208A1/en [Accessed January 9, 2020].
66. Rivas FM, Stables JP, Murphree L, Edwankar RV, Edwankar CR, et al. (2009). Antiseizure activity of novel gamma-aminobutyric acid (A) receptor subtype-selective benzodiazepine analogues in mice and rat models. J Med Chem. 52:1795-1798.
67. Poe MM, Methuku KR, Li G, Verma AR, Teske KA, et al. (2016). Synthesis and characterization of a novel gamma-aminobutyric acid type A (GABAA) receptor ligand that combines outstanding metabolic stability, pharmacokinetics, and anxiolytic efficacy. J Med Chem. 59:10800-10806.
68. Di Lio A, Benke D, Besson M, Desmeules J, Daali Y, et al. (2011). HZ166, a Novel GABAA Receptor Subtype-

Selective Benzodiazepine Site Ligand, Is Antihyperalgesic in Mouse Models of Inflammatory and Neuropathic Pain. Neuropharmacology. 60:626-632.

69. Methuku KR, Li X, Cerne R, Gleason SD, Schkeryantz JM, et al. (2018) An antidepressant-related pharmacological signature for positive allosteric modulators of α2/3-containing GABAA receptors. Pharmacol Biochem Behav. 170:9-13.
70. Mohler H, Benke D, Mertens S, Fritschy JM. (1992). GABAA-receptor subtypes differing in alpha-subunit composition display unique pharmacological properties. Adv Biochem Psychopharmacol. 47:41-53.
71. Witkin JM,Cerne R, Davis PG, Freeman KB, do Carmo JM, et al. (2019a). The α2,3-selective potentiator of GABAA receptors, KRM-II-81, reduces nociceptive-associated behaviors induced by formalin and spinal nerve ligation in rats. Pharmacol Biochem Behav. 180:22-31.
72. Witkin JM,Smith JL, Ping X, Gleason SD, Poe MM, et al. (2018). Bioisosteres of ethyl 8-ethynyl-6-(pyridin-2-yl)-4H-benzo[f]imidazo [1,5-a][1,4]diazepine-3-carboxylate (HZ-166) as novel alpha 2,3 selective potentiators of GABAA receptors: Improved bioavailability enhances anticonvulsant efficacy. Neuropharmacology. 137:332-343.
73. Witkin JM, Ping X, Cerne R, Mouser C, Jin X, et al. (2019b). The value of human epileptic tissue in the characterization and development of novel antiepileptic drugs: The example of CERC-611 and KRM-II-81. Brain Res. 1722:146356.
74. Witkin JM, Cerne R, Wakulchik M, S J, Gleason SD, et al. (2017). Further evaluation of the potential anxiolytic activity of imidazo[1,5-a][1,4]diazepin agents selective for α2/3-containing GABAA receptors. Pharmacol Biochem Behav. 157: 35-40.
75. Witkin JM, Li G, Golani LK, Xiong W, Smith JL, et al. (2019c). The positive allosteric modulator of α2/3-containing GABAA receptors, KRM-II-81, is active in pharmaco-resistant models of epilepsy and reduces hyperexcitability after traumatic brain injury. J Pharmacol Exp Ther.
76. Chou R, Deyo R, Friedly J, Skelly A, Weimer M, et al. (2017). Systemic Pharmacologic Therapies for Low Back Pain: A Systematic Review for an American College of Physicians Clinical Practice Guideline. Ann Intern Med. 166:480-492.
77. Knabl J, Zeilhofer UB, Crestani F, Rudolph U, Zeilhofer HU. (2009). Genuine antihyperalgesia by systemic diazepam revealed by experiments in GABAA receptor point-mutated mice. Pain 141:233-238.
78. Munro G, Ahring PK, Mirza NR. (2009). Developing analgesics by enhancing spinal inhibition after injury: GABAA receptor subtypes as novel targets. Trends in Pharmacological Sciences. 30:453-459.
79. Calcaterra NE, Barrow JC. (2014). Classics in Chemical Neuroscience: Diazepam (Valium). ACS Chem Neurosci. 5:253-260.
80. Milić M, Divljaković J, Rallapalli S, van Linn ML, Timić T, Cook JM, Savić MM (2012) The role of α1 and α5 subunit-containing GABAA receptors in motor impairment induced by benzodiazepines in rats. Behav Pharmacol 23:191197.
81. Savic MM, Huang S, Furtmuller R, Clayton T, Huck S, et al. (2008). Are GABAA receptors containing alpha5 subunits contributing to the sedative properties of benzodiazepine site agonists? Neuropsychopharmacology. 33:332-339.
82. Savic MM, Majumder S, Huang S, Edwankar RV, Furtmuller R, et al. (2010) Novel positive allosteric modulators of GABAA receptors: do subtle differences in activity at alpha1 plus alpha5 versus alpha2 plus alpha3 subunits account for dissimilarities in behavioral effects in rats? Prog Neuropsychopharmacol Biol Psychiatry. 34:376-386.
83. Tobler I, Kopp C, Deboer T, Rudolph U. (2001). Diazepam-induced changes in sleep: role of the alpha 1 GABA(A) receptor subtype. Proc Natl Acad Sci USA. 98:6464-6469.
84. Lippa A, Czobor P, Stark J, Beer B, Kostakis E, et al. (2005). Selective anxiolysis produced by ocinaplon, a GABA(A) receptor modulator. Proc Natl Acad Sci USA. 102:7380-7385.
85. Czobor P, Skolnick P, Beer B, Lippa A. (2010). A multicenter, placebo-controlled, double-blind, randomized study of efficacy and safety of ocinaplon (DOV 273,547)

in generalized anxiety disorder. CNS Neurosci Ther. 16:63-75.

86. Gee KW, Tran MB, Hogenkamp DJ, Johnstone TB, Bagnera RE, Yoshimura RF, et al. (2010). Limiting Activity at β1-Subunit-Containing GABAA Receptor Subtypes Reduces Ataxia. J Pharmacol Exp Ther 332: 1040-1053.
87. Yoshimura RF, Tran MB, Hogenkamp DJ, Johnstone TB, Xie JY, et al. (2014). Limited central side effects of a β-subunit subtype-selective GABAA receptor allosteric modulator. J Psychopharmacol (Oxford) 28:472-478.
88. Johnstone TBC, Xie JY, Qu C, Wasiak DJ, Hogenkamp DJ, et al. (2019). Positive allosteric modulators of nonbenzodiazepine γ-aminobutyric acidA receptor subtypes for the treatment of chronic pain. Pain 160:198-209.
89. Youn SE, Kim SH, Ko A, Lee SH, Lee YM, et al. (2018). Adverse Events During Perampanel Adjunctive Therapy in Intractable Epilepsy. J Clin Neurol. 14:296-302.
90. Coussens NP, Sittampalam GS, Jonson SG, Hall MD, Gorby HE, et al. (2019). The Opioid Crisis and the Future of Addiction and Pain Therapeutics. J Pharmacol Exp Ther. 371:396-408.