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Subtype selective γ -Aminobutyric Acid Type A Receptor (GABA_AR) Modulators Acting at the Benzodiazepine Binding Site: An Update

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Abstract

γ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter within the central nervous system (CNS) with fast, trans-synaptic and modulatory extrasynaptic effects being mediated by the ionotropic GABA Type A receptors (GABA_ARs). These receptors are of particular interest since they are the molecular target of a number of pharmacological agents, of which the benzodiazepines (BZDs), such as diazepam, are the best described. The anxiolytic, sedating and myorelaxant effects of BZDs are mediated by separate populations of GABA_ARs containing either $\alpha 1$, $\alpha 2$, $\alpha 3$ or $\alpha 5$ subunits and the molecular dissection of the pharmacology of BZDs indicates that subtype-selective GABA_AR modulators will have novel pharmacological profiles. This is best exemplified by $\alpha 2/\alpha 3$ -GABA_AR positive allosteric modulators (PAMs) and $\alpha 5$ -GABA_AR negative allosteric modulators (NAMs), which were originally developed as non-sedating anxiolytics and cognition enhancers, respectively. This review aims to summarize the current state of the field of subtype-selective GABA_AR modulators acting via the BZD binding site and their potential clinical indications.

1. INTRODUCTION

1.1 γ -Aminobutyric Acid Type A Receptor (GABA_AR) structure and function. γ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter within the central nervous system (CNS). The effects of GABA are exerted via activation of either the ionotropic GABA type A or metabotropic GABA type B receptors (GABA_ARs and GABA_BRs). GABA_ARs are responsible for the point-to-point transfer of information across the synapse but they are also located extrasynaptically, where they mediate a more modulatory, tonic inhibitory tone.¹ They are members of the Cys-loop superfamily of ligand-gated ion channels² and contain a roughly 13-residue disulphide loop within the large N-terminal domain that is conserved within this family which also include the nicotinic acetylcholine, serotonin type 3 (5HT₃) and glycine receptors.³ When activated, GABA_ARs generally permit the flow of chloride ions from outside to inside the neuron (although this may be reversed early in development)⁴ thereby resulting in a hyperpolarisation of the membrane potential that reduces the probability of the neuron firing an action potential.

GABA_ARs are heteropentamers assembled from the 19 members (α 1-6, β 1-3, γ 1-3, δ , ϵ , θ , π and ρ 1-3) of the GABA_A protein family, with the most abundant forms comprising α , β and γ subunits in a 2:2:1 stoichiometry.^{5,6} The various (~25) different GABA_AR subtypes that occur in the mammalian brain^{7,8} have subcellular (i.e., synaptic or extrasynaptic), region- and circuit-specific distributions that suggest the different subtypes are associated with distinct CNS functions.⁹ The binding site for the orthosteric ligand (GABA) is found at the interface of the α and β subunits and hence there are typically two GABA binding sites per heteropentamer.^{6,10,11,12} In addition, GABA_ARs have recognition sites for a variety of allosteric modulators, including barbiturates, alcohol, benzodiazepines (BZDs), and neurosteroids,^{13,14,15} with structural aspects of these binding sites beginning to be elucidated.^{16,17,18}

Recently, high-resolution cryo-electron microscopy (cryo-EM) structures of the human α 1 β 2 γ 2 and α 1 β 3 γ 2 GABA_ARs have been reported.^{11,12,19} The analysis of the receptors in complexes with the orthosteric agonist GABA and the GABA binding site antagonist bicuculline as well as the open-channel blocker picrotoxin and BZD binding site ligands such as diazepam (Figure 1), alprazolam and flumazenil not only provide a detailed insight into the overall assembly and heteromeric interactions of GABA_ARs, but have also started to reveal the structural basis of receptor activation and allosteric

modulation. The focus of the present review will be on compounds that bind to the BZD site with more detailed descriptions regarding the other GABA_AR binding sites being available elsewhere.^{14,15,20,21}

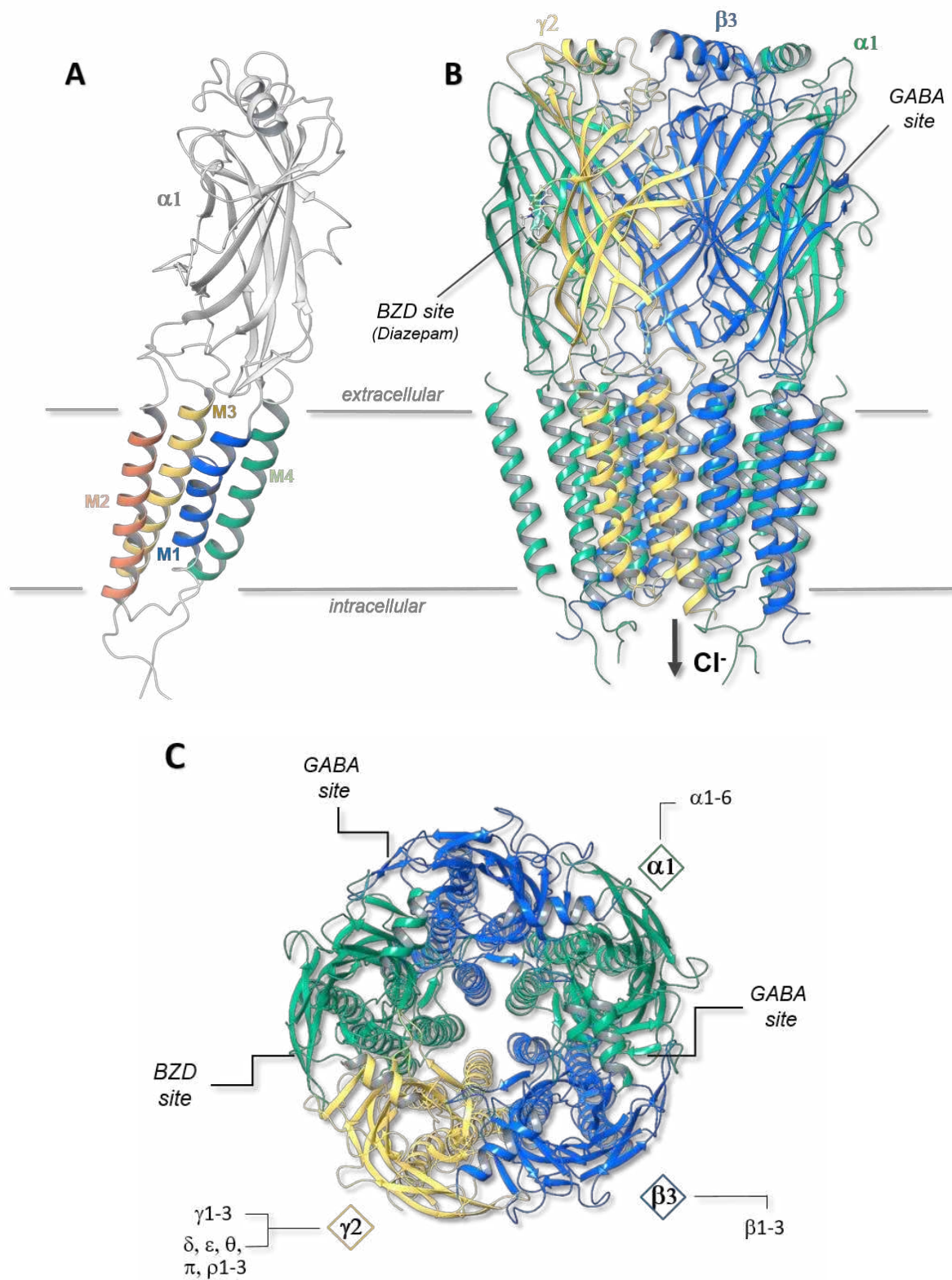


Figure 1. 3D-reconstruction of the $\alpha 1\beta 3\gamma 2$ -GABA_AR (bound to the channel blocker picrotoxin which is not shown in the figure, PDB:6HUP)¹⁹. Images were generated using Maestro Version 11.9.011, MMshare Version 4.5.011, Release 2019-1. The $\alpha 1$ subunits are colored in grey (**A**) or green (**B**, **C**), $\beta 3$ in blue and $\gamma 2$ in yellow. **A**) GABA_AR_s belong to the Cys-loop family of ligand-gated ion channels with each subunit being characterized by a long extracellular N-terminus, which contains the defining Cys-loop, and four trans-membrane domains (M1-4), two short loops that link M1-M2 and M2-M3, a longer intracellular loop between M3 and M4 (modulated by phosphorylation), and a small extracellular C-terminus. **B**) Side view: the heteropentameric arrangement of subunits in the 2:2:1 stoichiometry of α , β and γ subunits that comprise the most abundant form of GABA_AR, arranged around the central chloride-permeable channel pore, lined by the M2 regions of the different subunits. **C**) Top view: two GABA binding sites are found at the junctions between α and β subunits while the BZD site is located at the interface of a $\gamma 2$ and either an $\alpha 1$, $\alpha 2$, $\alpha 3$ or $\alpha 5$ (but not $\alpha 4$ or $\alpha 6$) subunit.^{10,11,12,17} The γ subunit may be replaced by a variety of less abundant subunits (e.g., δ , ϵ , θ or π) whereas the $\rho 1-3$ subunit can form homopentameric assemblies that are also known as GABA_C receptors.²² Not illustrated here are the multiple additional binding sites on the GABA_AR that occur, for example, within the transmembrane region, such as the anesthetic and neurosteroid binding sites, or within the ion channel pore itself, for example the picrotoxin (or convulsant) binding site.

1.2 BZDs are positive allosteric modulators of GABA_AR_s. Since the introduction of chlordiazepoxide and diazepam in the early 1960s, and thereafter multiple other analogues including alprazolam and lorazepam, the BZD class of drugs has been widely used in the clinic as anxiolytics, sedative-hypnotics, myorelaxants and anticonvulsants.²³ However, although the myorelaxant/sedative properties of BZDs can be useful in an anesthetic context, they represent significant side effects in the treatment of patients with generalized anxiety disorder (GAD). Moreover, prolonged BZD use can be accompanied by the development of physical dependence and a tolerance towards certain aspects of their pharmacology, which results, for example, in them being unsuitable for prophylactic use in epilepsy patients. Of the various modulators of GABA_AR function, BZDs are the best characterized based not only upon their clinical utility but also our understanding that the BZD binding site occurs at the $\alpha/\gamma 2$ subunit interface when the α subunit is either an $\alpha 1$, $\alpha 2$, $\alpha 3$ or $\alpha 5$ (but not $\alpha 4$ or $\alpha 6$).¹⁷ In addition, compounds that bind at this recognition site can modulate GABA_AR function in a bidirectional manner with compounds producing either positive or negative allosteric modulators (PAMs and NAMs) that result in a shift to the left or right, respectively, of the GABA concentration-effect curve. Hence, a PAM increases and a NAM decreases the apparent affinity of the GABA_AR for GABA. In addition, ligands such as flumazenil (**23**, Figure 9) may bind to the BZD binding site but be functionally silent in that they produce no physiological response. Such compounds can be described either as neutral

allosteric modulators or site silent binders at or competitive antagonists of the BZD site and it is this latter nomenclature that we will use in the current manuscript.

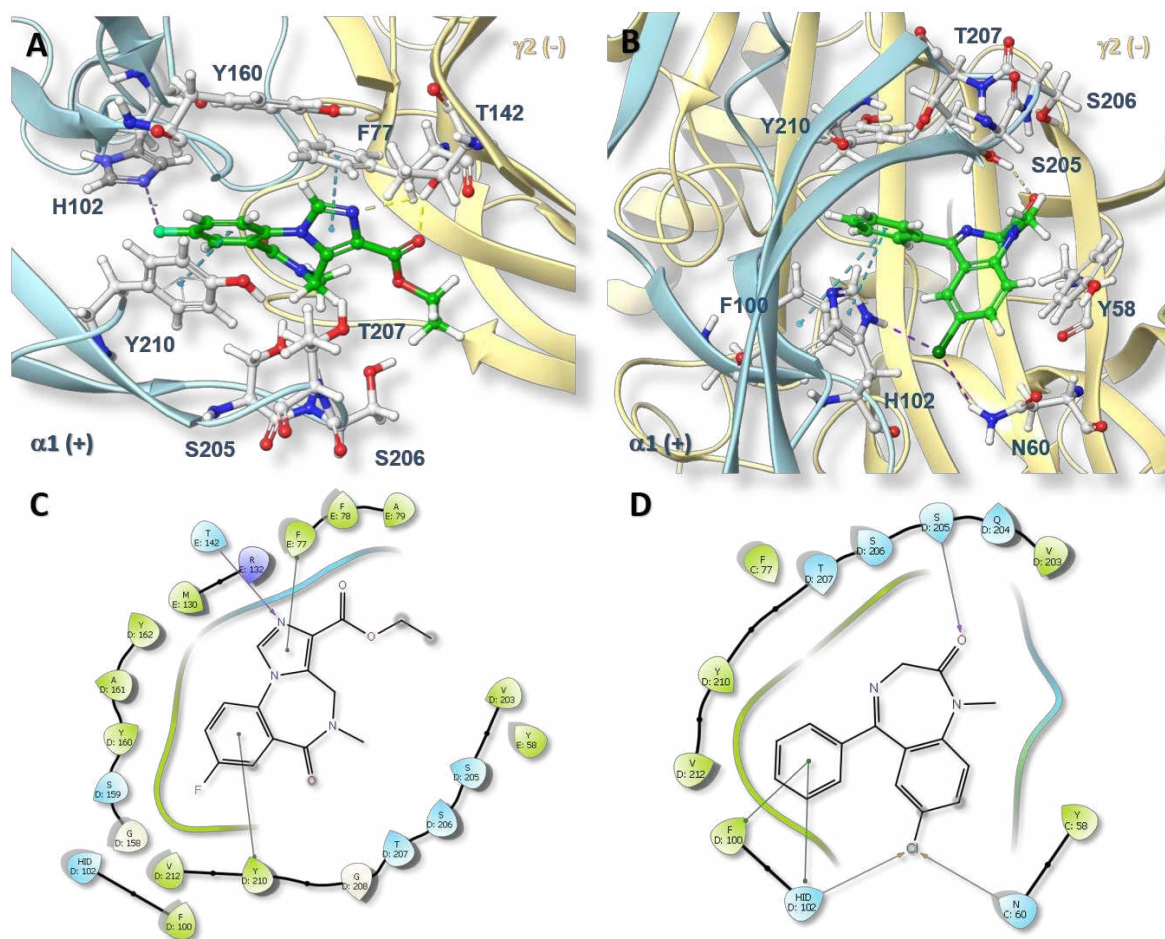


Figure 2. Details of the architecture and schematic representations of the main interactions of flumazenil (A and C, PDB:6D6U) and diazepam (B and D, PDB:6HUP) in the BZD binding site of $\alpha 1\beta 2\gamma 2$ and $\alpha 1\beta 3\gamma 2$ GABA_ARs, respectively. Images have been generated using Maestro Version 11.9.011, MMshare Version 4.5.011, Release 2019-1. **A, B)** $\alpha 1$ subunit is colored in light blue while $\gamma 2$ is golden. H-bonds are in yellow dots, halogen bonds in purple dots, π - π stacking in blue dots; **C, D)** Representations of the aminoacidic residues principally involved in the interaction with BZDs in a 3 Å-range. H-bonds are in purple arrows, halogen bonds in yellow arrows, π - π stacking in green arrows.

The so-called classical BZDs are non-selective PAMs that potentiate the effects of GABA at the different GABA_AR subtypes (i.e., $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ - and $\alpha 5$ -GABA_ARs) with equivalent affinity and efficacy.¹⁹ The structural characterization of GABA_ARs has opened up the way for the analysis of ligands interactions in the BZD binding site (Figure 2), although the correlation between specific interactions and the efficacy profile of GABA_ARs modulators has not yet been fully investigated.

1.3 Different GABA_AR subtypes have distinct functions. In order to develop compounds that retain efficacy but have a reduced side effect liability compared to BZDs, it is important to understand which of the various pharmacological features of BZDs can be attributed to specific subtypes of the GABA_AR. In this regard, molecular genetic studies with α -subunit knock-out and point-mutation mice and medicinal chemistry approaches that selectively target particular subtypes, have led to the general concept that α 1-GABA_ARs mediate the sedative effects of BZDs, whereas α 2- and/or α 3-GABA_ARs are associated with anxiolysis and α 5-GABA_ARs play a role in cognitive processes and learning.²⁴ Evidence that the α 1 subtype mediates sedation includes the fact that the non-BZD hypnotic Zolpidem (an imidazopyridine which nevertheless binds to the BZD recognition site) has higher affinity for α 1-GABA_ARs compared to other subtypes²⁵ and that mice genetically modified to render their α 1-GABA_ARs insensitive to BZDs show a much reduced sensitivity to the sedative effects of diazepam.²⁶ Similarly, it has been demonstrated that the α 2- and/or α 3-GABA_AR subtypes mediate anxiolysis^{27,28} while the preferential expression of α 5-GABA_ARs in the hippocampus²⁹ and the improved cognitive performance in mice with reduced α 5-GABA_AR expression³⁰ support the role of this subtype in cognition. These data have therefore formed the basis of hypotheses that PAMs that selectively modulate α 2/ α 3-GABA_ARs should be non-sedating anxiolytics, possibly with a reduced tolerance and dependence liability,³¹ whereas α 5-GABA_AR NAMs would enhance cognitive performance but without the anxiogenic and proconvulsant liabilities associated with non-selective NAMs, such as FG7142.³² More recently, data has emerged suggesting that α 5-GABA_AR PAMs might increase GABA-mediated inhibition and therefore reduce hippocampal activity in disorders such as schizophrenia that are associated with hippocampal hyperactivity.³³

GABA_AR subtype selectivity may be achieved in one of two ways: *i*) subtype-selective affinity, in which a compound binds with higher affinity for a particular subtype(s) relative to others but has equivalent efficacy at each subtype, albeit that the functional affinity (EC₅₀ values) reflect the binding affinity; *ii*) subtype-selective efficacy, in which a compound binds with equivalent affinity for each of the four (*i.e.*, α 1, α 2-, α 3- and α 5-containing) GABA_AR subtypes but only has functional effects at the subtype(s) of interest with no effects being observed at the other subtypes (at which the compound is a silent binder and behaves as an antagonist like flumazenil). In the present article, we will summarize

key aspects of representative examples of compounds with various selectivity profiles, being either efficacy-selective, affinity-selective or a combination of the two, highlighting in particular, those $\alpha 2/\alpha 3$ -GABA_AR PAMs and $\alpha 5$ -GABA_AR NAMs that have progressed into clinical studies over the last decade or so. What emerges clearly is that subtype-selective GABA_AR modulators have preclinical and clinical pharmacological profiles that are very different from the non-selective modulators, whether they be PAMs or NAMs. However, and aside from the general issues (such as preclinical toxicology and clinical pharmacokinetics and tolerability) that can halt the clinical development of any particular drug, there remains the challenge of selecting the correct dose for the right patient population but even in these areas lessons are being learned and progress is being made.

2. $\alpha 2/\alpha 3$ -GABA_AR PAMs

2.1 Background and Context. Although BZDs are effective anxiolytics in the treatment of GAD, around 50% of patients report a significant degree of sedation which increases the risk of accidents^{34,35} as well as falls, which are of particular concern in the elderly.^{36,37} Hence, there has long been a search for a non-sedating anxiolytic (“Valium without the sedation”) which has been described as a Holy Grail of psychopharmacology.³⁸ With the dissection of the pharmacological mechanism of BZDs, the potential of an $\alpha 2/\alpha 3$ -GABA_AR PAM to be anxiolytic in GAD yet devoid of sedative side effects became apparent. However, the medicinal chemistry challenge was how this might be achieved. Initial attempts to achieve significantly (>10-fold) higher affinity for $\alpha 2$ - and $\alpha 3$ - versus $\alpha 1$ -GABA_ARs proved unsuccessful, largely due to similarity of the BZD binding site at these different subtypes.³⁹

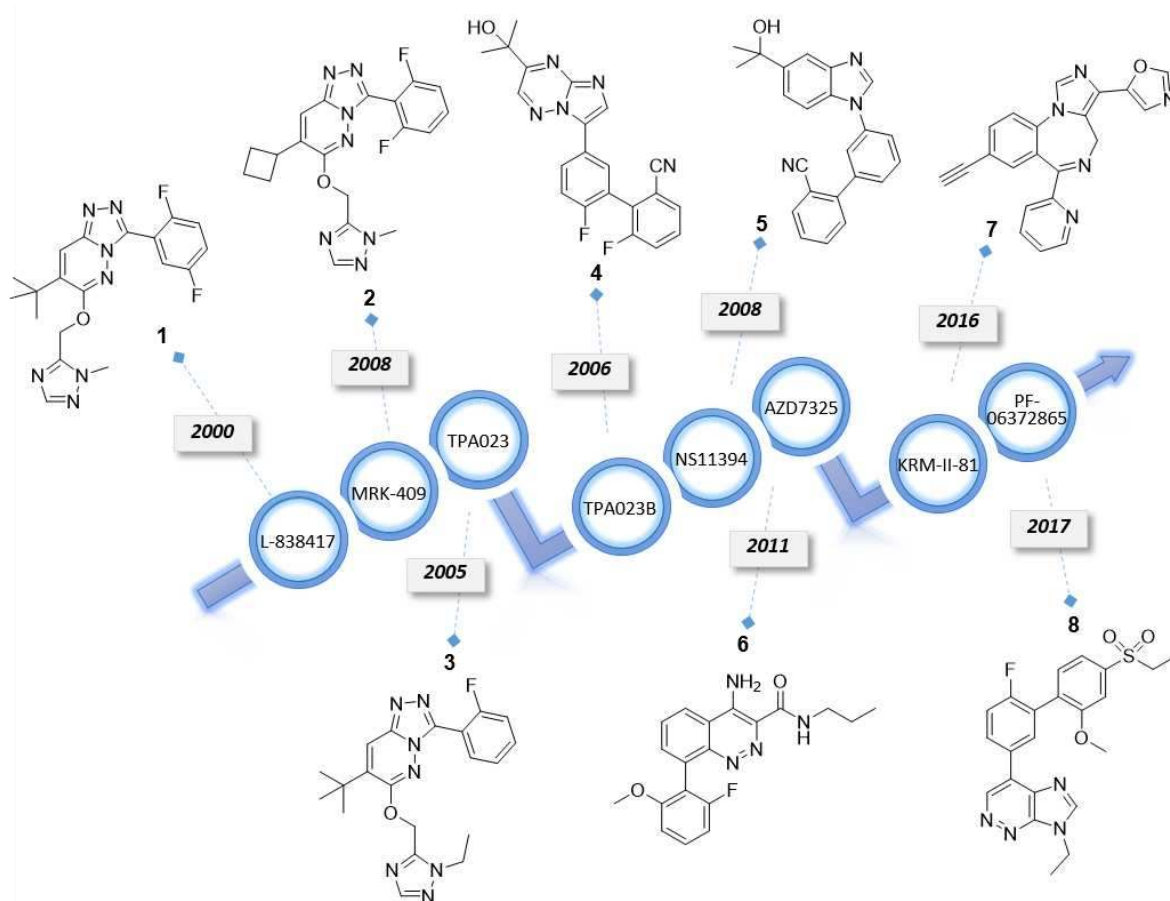


Figure 3. Structures and approximate chronological sequence of representative $\alpha 2/\alpha 3$ -GABA_AR functionally-selective agents. Compounds **1-8** are representatives of different classes of GABA_AR PAMs, starting with the first prototypic compound L-838417 (**1**) through to the most recently-described clinical candidate PF-06372865 (**8**, now known as CVL-865).

In contrast, the subtype-selective efficacy approach proved very fruitful with the identification of the prototypic $\alpha 2/\alpha 3$ -GABA_AR PAM L-838417⁴⁰ (**1**, Figure 3) being followed by a variety of other compounds all of which have PAM activity at the $\alpha 2$ - and $\alpha 3$ -containing subtypes but have lower efficacy or are largely functionally silent (*i.e.*, are antagonists) at $\alpha 1$ -GABA_ARs (see Table 1 and Figure 3). Of these compounds, several have progressed into clinical studies and been evaluated either in GAD (TPA023 and AZD7325, Figure 2) or in other indications that can be explored once the sedative effects associated with non-selective BZDs have been “dialed-out”. For example, PF-06372865 (now CVL-865, **8**, Figure 3) has been evaluated for efficacy against lower back pain⁴¹ and photosensitive epilepsy.⁴²

Table 1. Summary of the *in vitro* properties representative $\alpha 2/\alpha 3$ -GABA_AR PAMs

Compound	Structure	K _i (nM)				Relative Efficacy ^a			
		$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 5$	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 5$
Diazepam ²⁰	Benzodiazepine	16	17	17	15	1.0	1.0	1.0	1.0
PF-06372865^b	Imidazopyridazine	0.18	2.92	1.06	18.0	0.11	0.35	0.49	0.70
L-838417	Triazolopyridazine	0.79	0.67	0.67	2.25	0.02	0.43	0.43	0.39
MRK-409		0.22	0.40	0.21	0.23	0.18	0.23	0.45	0.18
TPA023		0.27	0.31	0.19	0.41	0.00	0.11	0.21	0.05
TPA023B	Imidazotriazine	0.73	2.0	1.8	1.1	0.03	0.38	0.50	0.37
AZD7325	Cinnoline	0.5	0.3	1.3	230	-0.01	0.19	0.17	0.11
AZD6280		0.5	5.0	31	1680	0.08	0.32	0.34	0.07
NS11394	Benzimidazole	0.41	0.84	0.50	0.12	0.08	0.26	0.52	0.78
NS16085		NR ^c				-0.10 ^d	0.27	0.47	0.05
NS11821	Unknown	1.6	9.7	3.8	2.5	0.04	0.17	0.40	0.41
HZ-166	Imidazobenzodiazepine	300	160	527	82	0.27	0.52	0.36	0.23
KRM-II-81 ^e		489	118	205	NR	0.35	0.66	0.77	NR
MP-III-024 ^e		NR	~1,000	~1,000	NR	25%	140%	129%	11%

^a Relative efficacy is the ability of a compound to increase the current produced by a submaximal (for example, an EC₃₋₅ or an EC₂₀) concentration of GABA relative to the potentiation observed for a non-selective BZD such as diazepam or chlordiazepoxide. However, in the case of MP-III-024 values are expressed as the % potentiation of a GABA EC₃ concentration. ^b Compounds in **bold** are those which progressed into clinical studies. ^c NR: not reported. ^d Negative relative efficacy values denote a NAM effect. ^e Affinity values are the functional affinity expressed as EC₅₀, which in the case of MP-III-024 were estimated by eye.

2.2 The imidazopyridazine PF-06372865 (now CVL-865). Based upon similarity searches and the molecular overlaying of known GABA_AR PAMs to establish a pharmacophore model, the Neusentis (Pfizer) group screened their in-house chemical collection to identify three general series with different heterocyclic cores (Series 1-3, Figure 4). The imidazopyridazine series (Series 2) was selected as the preferred chemotype based on an analysis of the affinity, functional selectivity, microsomal turnover and CNS penetration (estimated from an MDCK cell efflux assay).⁴³

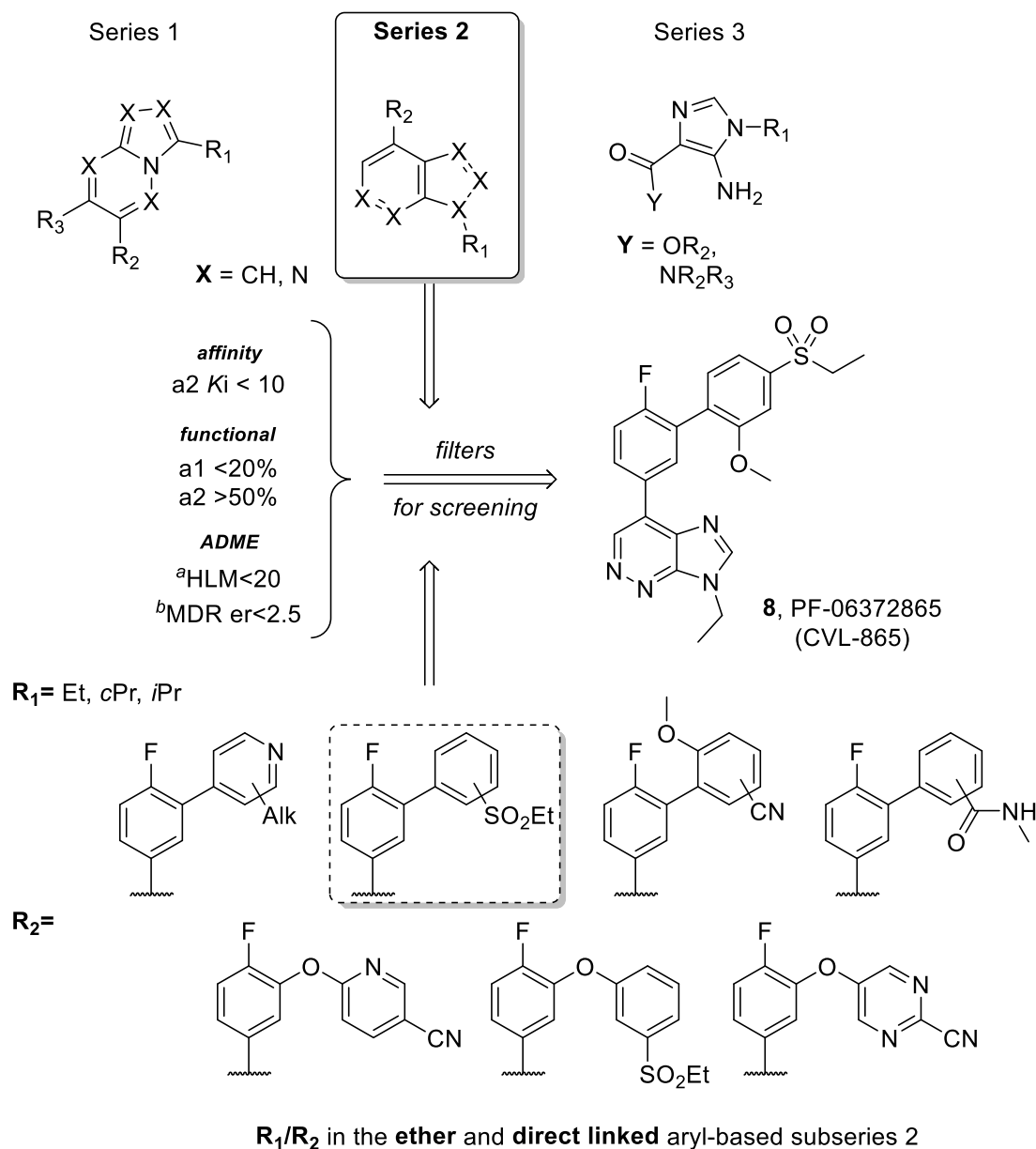


Figure 4. General structures of the 3 series identified by screening for novel α_2/α_3 -GABA_ARs PAM, with further optimization within Series 2 leading to imidazopyridazine PF-06372865 (**8**). ^aHLM Clint ($\mu\text{L min}^{-1} \text{mg}^{-1}$) *in vitro* human microsomal stability measurement. ^bMDR er: efflux ratio of test compound at 2 μM across confluent monolayers from MDR1-transfected MDCK cells.

A similar analysis was conducted on the direct- and ether-linked subseries within the imidazopyridazine chemotype and from this, the direct-linked scaffold was predicted to be far more likely to produce a compound with the desired functional selectivity.⁴⁴ In this subseries, two preclinical lead compounds were identified, from which PF-06372865 (now known as CVL-865, **8**, Figures 3 and 4) was selected as the clinical candidate.⁴⁴ PF-06372865 has 6-100-fold higher affinity for $\alpha 1$ - relative to $\alpha 2$ -, $\alpha 3$ - or $\alpha 5$ -GABA_ARs (Table 1) and is, as expected, devoid of affinity for $\alpha 4$ - and $\alpha 6$ -GABA_ARs.⁴⁵ Despite the higher affinity for the $\alpha 1$ subtype, PF-06372865 only very modest efficacy at this subtype (relative efficacy = 0.11), with efficacy at the $\alpha 2$ -, $\alpha 3$ - and $\alpha 5$ -GABA_AR subtypes being much higher (respective relative efficacy values of 0.35, 0.49 and 0.70). Although efficacy at the $\alpha 5$ subtype is higher than at $\alpha 2$ - and $\alpha 3$ -GABA_ARs, the lower affinity of PF-06372865 for $\alpha 5$ -GABA_ARs combined with the lower expression of this subtype within the brain mean that most of the *in vivo* pharmacological effects of PF-06372865 are likely mediated by $\alpha 2$ - and/or $\alpha 3$ -GABA_ARs.

PF-06372865 demonstrated excellent *in vivo* occupancy with the dose required to occupancy 50% of rat brain GABA_ARs (the Occ₅₀) varying from 0.3-1.0 mg/kg p.o., depending on brain region.⁴⁵ Implanted telemetry transmitters were used to show a dose-dependent increase in quantitative EEG (qEEG) beta frequency⁴⁵ that was comparable to the $\alpha 2/\alpha 3$ -GABA_AR PAM L-838417.^{46,47} PF-06372865 was anxiolytic in the elevated plus maze, analgesic in the chronic constriction injury model and was anticonvulsant in the mouse PTZ seizure and rat amygdala kindling models.⁴⁴ In addition, PF-06372865 dose-dependently reduced the number of spike-and-wave discharges in the genetic absence epilepsy rats from Strasbourg (GAERS).⁴⁸ The rat half-life of 5.5 h for PF-06372865 translated into a half-life in man of 6.0-8.9 h over a dose range of 0.04 to 100 mg in an oral suspension.⁴⁵ Doses of 10 and 65 mg gave whole brain GABA_AR occupancy of 69 and 89% in a [¹¹C]flumazenil PET study and there were dose-dependent effects on a number of pharmacodynamic parameters, including saccadic peak velocity, qEEG and a slight increase in body-sway, with the latter possibly being related to sedation and/or myorelaxation that was $\alpha 1$ -GABA_AR-mediated.⁴⁵

While a Phase 2 study in GAD was terminated early for business reasons with only 90 of the planned 384 subjects having been enrolled into the study,⁴⁹ PF-06372865 demonstrated robust efficacy in a small study of photosensitive epilepsy patients.⁴² Following single doses of drug (17.5 and 52.5

mg), participants had a reduction in the response to intermittent photic stimulation that was comparable to lorazepam, which served as the active comparator. Accordingly, PF-063728675 was licensed to Cerevel Therapeutics and is now being developed as an antiepileptic drug.

In healthy subjects, single 15 mg or 65 mg doses of PF-06372865 had an analgesic effect on pressure pain and the cold pressor task as judged by an increase in the pain tolerance threshold in the absence of any signs of sedation⁵⁰. However, PF-06372865 did not demonstrate efficacy in a Phase 2 study of chronic low back pain,⁴¹ although as noted by the authors, the doses selected (2.5-7.5 mg) may not have been sufficient to achieve adequate GABA_AR occupancy.

2.3 The 1,2,4-triazolo[4,3-*b*]pyridazine series: MRK-409 and TPA023. The triazolopyridazine series developed by Merck resulted not only in the identification of the prototypic pharmacological tool compound L-838417⁴⁰ (**1**, Figure 3) but also the clinical candidates MRK-409 and TPA023 (**2** and **3**, respectively, Figures 3 and 5). The starting point for this series was compound **9** (in which the triazolopyridazine core is highlighted in blue in Figure 5), which demonstrated a modest (<5-fold) $\alpha 2/\alpha 3$ -GABA_AR binding selectivity and was used as a hit for further optimization.⁵¹ The replacement of the 2-pyridyl moiety with a 1,2,4-alkyltriazole (**10**, Figure 5) was accompanied by a shift from affinity- to efficacy-selective compounds, and the replacement of the bicyclic scaffold with either a cyclobutyl or a *t*-butyl group producing a significant reduction of efficacy at $\alpha 1$ -GABA_ARs. Within the *t*-butyl subseries, compound **12** (Figure 5) had the desired $\alpha 2/\alpha 3$ -GABA_AR PAM and $\alpha 1$ antagonist efficacy profile but the unsubstituted phenyl group of this compound resulted in a high *in vitro* metabolism as measured in a microsome assay.

This *in vitro* metabolic liability observed in **12** was addressed by replacing the phenyl with a 2,5-difluorophenyl group and the resulting compound L-838417 was an antagonist at $\alpha 1$ -GABA_ARs and became the prototypic $\alpha 2/\alpha 3$ -GABA_AR efficacy-selective pharmacological tool PAM. Hence, L-838417 had similar binding affinity across the four GABA_AR subtypes and partial PAM efficacy at $\alpha 2$, $\alpha 3$ and $\alpha 5$ subtypes (with relative efficacy values ranging from 0.39-0.43; Table 1) but negligible efficacy at the $\alpha 1$ subtype.⁴⁰ This compound was characterized *in vivo* and demonstrated anxiolytic-like activity with no impairment in motor performance in rats and primates.^{39,52} Although this compound

was relatively stable in an *in vitro* metabolism assay, it had poor *in vivo* pharmacokinetics which prevented its further preclinical development.⁵³ Nevertheless, this compound was important in establishing the preclinical *in vivo* proof-of-concept that an $\alpha 2/\alpha 3$ -GABA_AR PAM was a non-sedating anxiolytic.

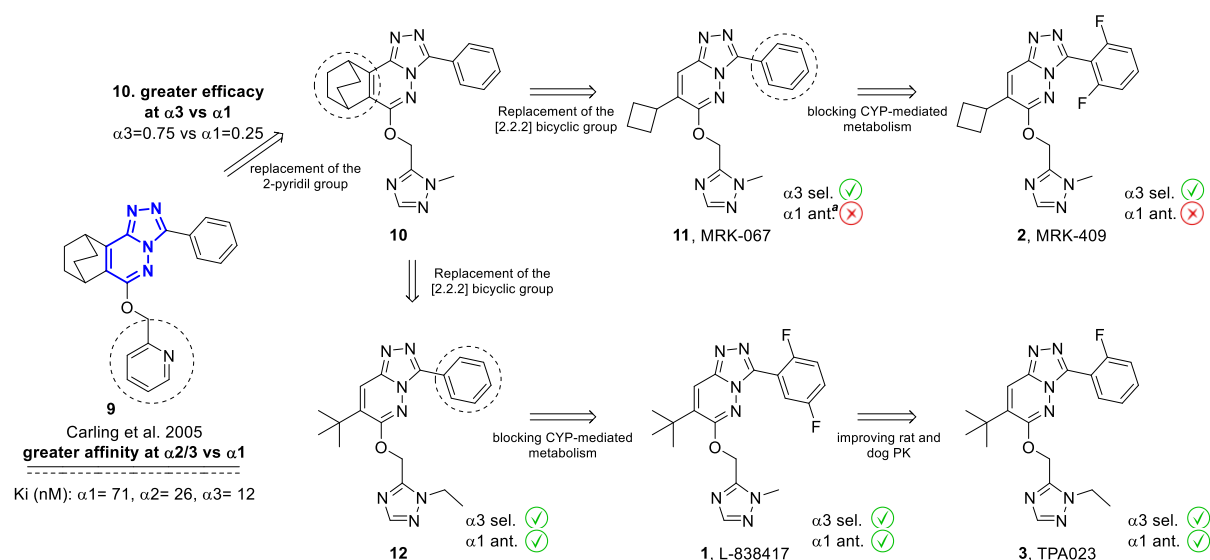


Figure 5. The discovery of MRK-409 (**2**) and TPA023 (**3**). The efficacy values are in comparison with the non-selective BZD chlordiazepoxide (which, by definition, has a relative efficacy at each subtype of 1.0). For clarity, efficacy values for $\alpha 2$ -GABA_AR (which generally closely track values at the $\alpha 3$ subtype) and $\alpha 5$ -GABA_AR have been omitted. ^aant. = antagonist (i.e., relative efficacy ~0.0).

Within the cyclobutyl subseries, MRK-067³⁹ (**11**, Figure 5) had higher efficacy at $\alpha 2$ - and $\alpha 3$ - compared to $\alpha 1$ -GABA_AR and proved to be a non-sedating anxiolytic in preclinical species. On the basis of these data, MRK-067 was selected as a preclinical candidate but further development was halted due to the demonstration of covalent binding due to CYP1A2-mediated metabolism on the unsubstituted phenyl ring; an issue that was addressed by the introduction of a 2,6-difluorophenyl group into MRK-409. This compound had equal affinity for all four ($\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ - and $\alpha 5$ -containing) GABA_AR subtypes, with K_i values ranging from 0.21 to 0.40 nM. The efficacy was greater at $\alpha 3$ - versus $\alpha 1$ -GABA_AR although, and as with MRK-067, there remained a modest degree of potentiation of GABA currents at this latter subtype (relative efficacy = 0.18) which although insufficient to cause sedation in preclinical species, ultimately resulted in sedation in man (see below).

MRK-409 readily entered the rat brain with the dose required for 50% *in vivo* GABA_AR occupancy (Occ₅₀) being 2.2 mg/kg p.o. and it was anxiolytic in unconditioned (rat elevated plus maze) and conditioned (rat fear-potentiated startle and conditioned suppression of drinking, and squirrel monkey conditioned emotional response) preclinical models of anxiety.⁵⁴ The compound did not cause sedation in the mouse rotarod or squirrel monkey lever pressing assays and therefore appeared to be non-sedating. On the basis of these data, MRK-409 (also described as MK-0343) progressed into the clinic where in single-ascending dose studies, a 2 mg dose unexpectedly produced marked sedation and the maximum tolerated dose was defined as 1 mg, at which dose [¹¹C]flumazenil positron emission tomography (PET) studies showed GABA_AR occupancy to be below the limits of detection (<10%).⁵⁵ As a result of these unexpected data, further development of MRK-409 as a non-sedating anxiolytic was halted. The failure of preclinical species to predict the sedative effects of MRK-409 in man was also experienced with the non-selective partial PAM bretazenil, which exhibited an anxi-selective profile in preclinical models but induced sedation in healthy volunteers.⁵⁶ It was assumed that - and for whatever reason - humans are particularly sensitive to even modest efficacy at α 1-GABA_ARs and that the relative efficacy of 0.18 for MRK-409 at this subtype was nevertheless still sufficient to produce pronounced sedation in man. This therefore defined the requirement for subsequent compounds to have zero intrinsic efficacy (*i.e.*, be an antagonist) at α 1-GABA_ARs.

Following the unexpected failure in the clinic of MRK-409, attention turned back to the *t*-butyl subseries and the poor *in vivo* pharmacokinetics of L-838417 was addressed primarily by the replacement of the 2-methyl-1,2,4-triazole with the ethyl analogue which resulted in the identification of TPA023^{51,57} (also known as MK-0777). This compound has equivalent affinity (ranging from 0.19 to 0.41 nM) for the α 1-, α 2-, α 3- and α 5-GABA_AR subtypes and has efficacy at α 2- and α 3-GABA_ARs, with respective relative efficacy values of 0.11 and 0.21, but 0.0 efficacy at the α 1 subtype (Table 1). Like MRK-409, TPA023 gave excellent target engagement in rats with an Occ₅₀ of 0.42 mg/kg p.o. and a plasma concentration required to give 50% occupancy (plasma EC₅₀) of 25 ng/mL.⁵⁸ TPA023 demonstrated anxiolytic-like effects but no sedative action in rats and primates^{59,60} and the compound progressed into preclinical toxicology studies, during which cataract formation in dogs was observed in high-dose, long-term studies (3 mo. at 100 mg/kg p.o. or 1 y. at 30 mg/kg p.o.). However, since there

was a very large safety margin, the compound progressed into the clinic and in Phase 1 healthy volunteers, TPA023 was generally well-tolerated with the maximum tolerated doses being defined by adverse events such as light headedness, dizziness and drowsiness. Single doses of 2 mg TPA023 produced $\geq 50\%$ GABA_AR occupancy in a [¹¹C]flumazenil PET study with no signs of sedation and plasma concentrations of 9 ng/mL corresponding to 50% occupancy.⁵⁸ The pharmacodynamic effects of TPA023 were compared to lorazepam in a healthy male volunteers,⁶¹ using saccadic peak velocity (SPV) or body sway as markers of GABAergic pharmacology.⁶² TPA023 produced a significant dose-dependent reduction in SPV but no effects were observed on body sway or either a visual analogue scales (VAS) of alertness or a variety of cognitive function tests. In contrast, while lorazepam likewise increased the SPV, it also produced a marked increase in body sway and impaired alertness and cognitive function.⁶¹ Next, three Phase 2a studies were initiated in GAD, with each study being a two-arm (placebo versus TPA023), 125-patient per arm double-blind, randomized controlled trial, using the Hamilton Anxiety (HAM-A) rating scale as the primary end point. However, shortly after each of the trials had commenced, cataract formation was reported in high dose, long-term (2-year) rodent carcinogenicity studies. Despite the fact that the therapeutic margin had not decreased and was still defined by the dog studies conducted prior to the commencement of the Phase 1 studies, the decision was nevertheless taken to halt development of TPA023. A *post-hoc* analysis of the combined data from the three terminated studies showed a significantly greater decrease in the HAM-A scores of TPA023-versus placebo-treated patients, consistent with an anxiolytic-like effect of the compound in man.⁵⁷ Although development of TPA023 in GAD was stopped, the compound was thereafter evaluated in an exploratory study in schizophrenia, in which the drug showed a trend towards improving cognitive symptoms⁶³ that were not confirmed in subsequent a larger study.⁶⁴ In addition, TPA023 had no effect in essential tremor.⁶⁵

2.4 The imidazotriazine compound TPA023B. Based upon the benzimidazole starting point NS-2710,⁶⁶ the imidazotriazine TPA023B (**4**, Figures 3 and 6) was identified by a sequential modification of the benzimidazole core. Hence, the nitrogen atoms in the benzimidazole core were rearranged to produce the imidazopyridine series (typified by compound **14**, Figure 6) which had a suitable *in vitro*

profile but generally poor rat pharmacokinetics. This issue was addressed by introducing an imidazopyridine core (**15**, Figure 6) which gave good rat but generally poor dog pharmacokinetics. Notable exceptions within this series were MRK-623 and MRK-898 which progressed into preclinical development pending the outcome of the clinical studies with the lead and back-up compound (TPA023 and TPA023B, respectively). The addition of a further nitrogen into the imidazopyrimidine core resulted in the imidazotriazine series (**16**, Figure 6) which resulted in good rat and dog pharmacokinetics with further optimization resulting in the identification of TPA023B.⁶⁷ Interestingly, the structure-activity relationship (SAR) could be largely retained across series, despite their different core structures.

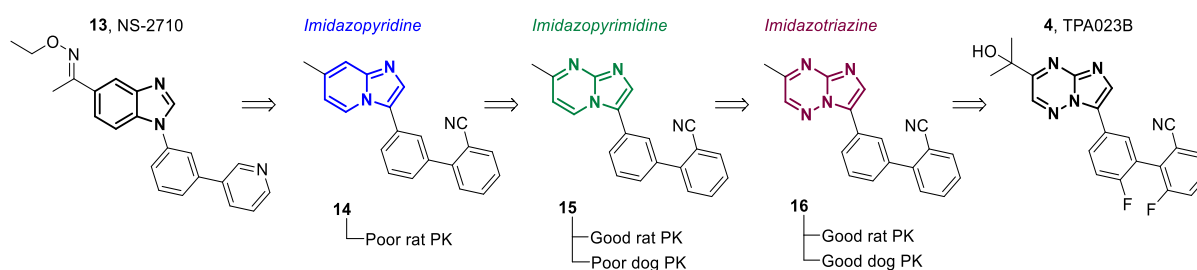


Figure 6. The NS-2710 core modifications leading to the discovery of TPA023B. The sequential addition of nitrogen atoms to the core addressed poor rat and then dog *in vivo* pharmacokinetics.

As with the triazolopyridazine clinical candidates MRK-409 and TPA023, TPA023B had similar affinity for the four GABA_AR subtypes (K_i values ranging from 0.7 to 2.0 nM, Table 1) and while the respective α_2 - and α_3 -GABA_AR relative efficacy values of 0.38 and 0.50 were higher than the corresponding values of 0.11 and 0.21 for TPA023, it was nevertheless possible to retain the lack of potentiation at the α_1 subtype. TPA023B was particularly potent at occupying rat brain GABA_AR receptors with an Occ_{50} of 0.09 mg/kg p.o. and a plasma EC_{50} of 19 ng/mL and was a non-sedating anxiolytic in rodents and primates.⁶⁸ In man, the compound was well tolerated with the maximum tolerated single dose in healthy young men was defined as 2 mg based upon observations of fatigue, tiredness and somnolence/drowsiness at a dose of 3 mg. In a [¹¹C]flumazenil PET study, single doses of 1.5 mg of TPA023B did not produce sedation at cortical GABA_AR occupancies of 52% and 46% measured 5 and 24 h after dosing, respectively, with the plasma EC_{50} value being 5.8 ng/mL.⁶⁹

Therefore, and consistent with TPA023, TPA023B did not produce a marked sedation even at relatively high levels of occupancy (roughly 50%). However, this compound did not advance into further clinical trials as a consequence of the project being terminated for business reasons. Nevertheless, TPA023B continues to be a very useful preclinical tool compound and has been used, for example, to define the role of $\alpha 2/\alpha 3$ -GABA_AR PAMs in reducing itch.⁷⁰

2.5 Cinnolines AZD7325 and AZD6280. Initial studies examining the GABA_AR modulatory effects of cinnoline- (the prototypic example of which is ICI 198,256)⁷¹ and related quinoline-based compounds were halted due to the low bioavailability of these molecules, along with their potential to form reactive metabolites.⁷² However, these compounds were chosen by AstraZeneca as good starting points for their renewed interest in developing subtype-selective GABA_AR modulators.^{73,72} The exemplars of the cinnoline series and related quinoline structures (**17** and **18**, Figure 7) had high efficacy for $\alpha 1$ -GABA_ARs while at the same time being metabolically unstable.

Changes to the cinnoline series were focused around the aryl group in the 8 position and the amidic portion. A few aliphatic chains were tolerated in the amide functionality, mainly short linear tethers and their equivalent carbocycles, with the *n*-propyl sequence giving the best results in terms of $\alpha 2$ - vs $\alpha 1$ -GABA_AR functional selectivity. With the *n*-propylamide in place, the 8-aryl cinnolines were of particular interest since they retained $\alpha 2$ -GABA_AR binding affinity but depending upon the substituents on the aromatic ring, a range of efficacies at the $\alpha 1$ subtype were observed. Nitrogen-containing heterocyclic replacements of the phenyl group did not confer any advantage and therefore the 2-fluoro-6-methoxyphenyl and 2,5-dimethoxyphenyl analogues, AZD7325 and AZD6280 respectively (**6** and **20**, Figure 7), were selected for clinical development.

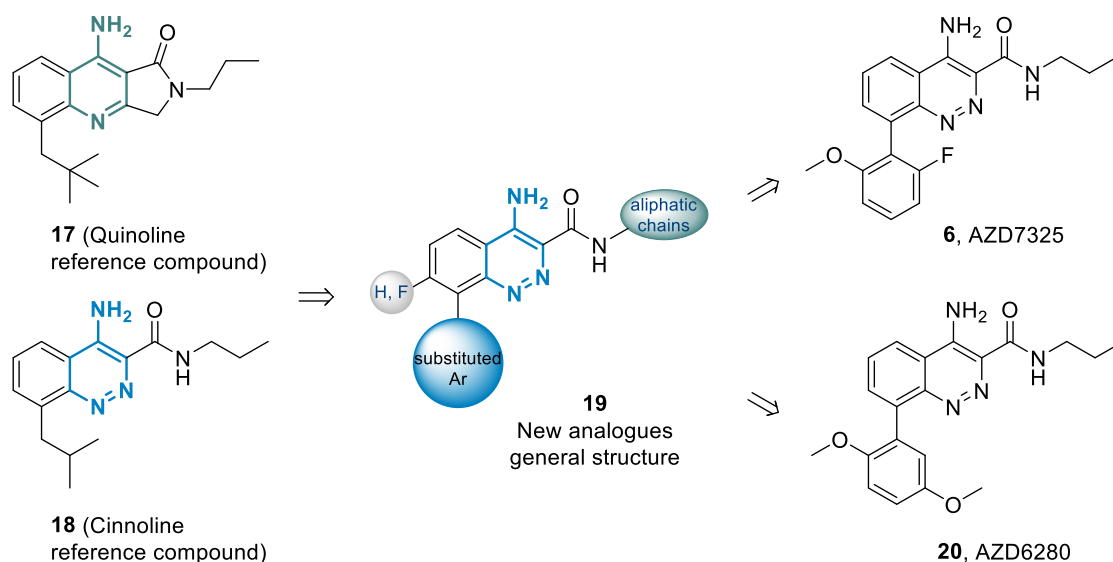
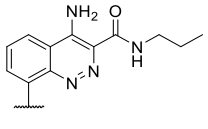
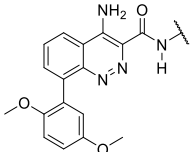
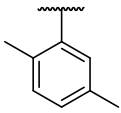

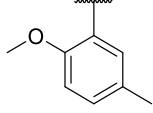
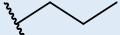
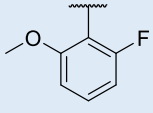

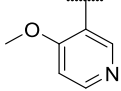

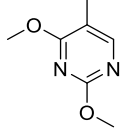



Figure 7. Structures of the quinoline- (**17**) and cinnoline-based (**18**) AZ starting points and general structure of the cinnoline-based new compounds (**19**). The SAR around this nucleus led to the identification of the clinical candidates AZD7325 and AZD6280 (**6** and **20**, respectively).

AZD7325 has high affinity for α 1-, α 2- and α 3-GABA_ARs (K_i of 0.5, 0.3 and 1.3 nM, respectively) but (and like PF-06372865) affinity was lower at α 5-GABA_ARs ($K_i = 230$ nM).^{72,47} It displayed no and low efficacy at α 1- and α 5-containing GABA_ARs, respectively and partial efficacy at the α 2- and α 3-subtypes. AZD7325 gave good rat brain GABA_AR occupancy with an Occ₅₀ in the region of 1-2 μ mol/kg s.c. as well as a robust anxiolytic effect in a punished responding task. There was sedation in the unpunished responding assay and a significant, dose-dependent increase in the EEG β and γ spectral power band.⁴⁷ AZD7325 was also anxiolytic in a mouse model in which a mutation in the collybistin-binding region of the α 2 subunit produces an anxiogenic phenotype⁷⁴ and it also protects against seizures in a mouse model of Dravet syndrome⁷⁵ as well as attenuating the impairment of prepulse inhibition and reversing dendritic abnormalities produced by siRNA knockdown of DISC1.⁷⁶

Preclinical *in vitro* studies highlighted the potential for AZD7325 to be an inducer of CYP3A4 and CYP1A2. However, in man co-administration studies of AZD7325 with the CYP3A4 substrate midazolam and the CYP1A2 substrate caffeine showed that there was a limited potential for AZD7325-mediated drug-drug interactions at clinically-relevant doses.⁷⁷ In a single ascending dose study, 0.2 to 100 mg were well tolerated with a dose of 20 mg corresponding to an average occupancy in a [¹¹C]flumazenil PET study of 83% in the occipital cortex and the cerebellum.⁷⁸

Table 2. Summary of the *in-vitro* affinity/efficacy of the cinnoline-based series

<i>8-aryl substitutions^a</i>		<i>N-alkyl chains^b</i>	
	$\alpha 2$ pKi $\alpha 1$ Rel. Eff. ^c $\alpha 2$ Rel. Eff.		$\alpha 2$ pKi $\alpha 1$ Rel. Eff. ^c $\alpha 2$ Rel. Eff.
	8.26 0.28 0.38		7.53 0.01 0.23
	8.21 0.13 0.52	 AZD6280	7.82 0.09 0.36
 AZD7325	9.51 -0.01 0.19		7.25 -0.02 0.20
	8.65 0.18 0.29		8.00 0.14 NR ^d
	8.13 0.10 0.54		7.58 0.10 0.28

^aSAR around 8-aryl and heterocycle substitutions of cinnoline N-propylamides. ^bSAR of amide substitutions of 8-(2,5-dimethoxyphenyl)-cinnolines. ^c Efficacy is relative to the potentiation observed for the non-selective BZD diazepam. ^dNR: not reported.

Doses of 2 and 10 mg AZD7325 were safe and well-tolerated in a pharmacodynamic study in healthy controls and although the 10 mg dose produced a reduction on delta (2-4 Hz) and theta (4-7.5 Hz), the effects on a variety of other different pharmacodynamic markers was relatively modest.⁷⁹ AZD7325 was then evaluated in two, 4-week Phase 2a studies in GAD. In the first study (Clinicaltrials.gov identifier NCT00808249), AZD7325 was dosed at either 2 or 5 mg twice a day or 10 mg once a day whereas in the second (NCT00807937) doses of 5 or 15 mg were administered twice a day. While neither study achieved its primary end-point (significantly greater change in the HAM-A rating scale relative to placebo), the placebo response rate was considered to be high and the 10 mg

dose reduced other anxiety endpoints and the depression MADRS score.⁸⁰ Moreover, both studies were confounded by a high rate of non-compliance (as defined by the absence of drug in plasma samples removed for pharmacokinetic analyses) and *post-hoc* analyses with data from non-compliant subjects removed demonstrated a clear anxiolytic-like effect (Dr. Alan Cross, personal communication). Although AstraZeneca terminated their internal efforts, AZD7325 has been made available to investigators as part of the AstraZeneca Open Innovation initiative and is currently under investigation in a small, 15-participant study of the effects of drug (5 or 15 mg twice a day for adults with fragile X syndrome (NCT03140813) as well as in a study in 100 adults with autism spectrum disorder (NCT03678129) in which single doses of 10 or 20 mg will be used to study the effects on brain neurochemistry and functional connectivity measured using magnetic resonance imaging.

The second clinical candidate AZD6280⁸¹ differed from AZD7325 in that although both compounds had a similar (0.5 nM) affinity at α 1-GABA_ARs, AZD6280 has lower affinity for the α 2-, α 3- and α 5-GABA_AR subtypes but has greater intrinsic efficacy at the α 2 and α 3 subtypes compared to AZD7325 (Table 1). The lower affinity of AZD6280 is presumably in part responsible for its approximately 10-fold lower potency in a rat [³H]flumazenil occupancy assay relative to AZD7325⁴⁷ as well as a human [¹¹C]flumazenil PET study.⁷⁸ Nevertheless, and presumably because of its greater intrinsic efficacy, 10 and 40 mg doses of AZD6280 were able to increase plasma prolactin levels in healthy male volunteers, as was a 2 mg dose of lorazepam whereas 2 and 10 mg doses of AZD7325 were without effect.⁸² These same 10 and 40 mg doses of AZD6280 were tested in a variety of additional pharmacodynamic readouts and while the effects were generally not to the same extent as those seen with lorazepam, they were nevertheless more marked than observed with AZD7325.^{79,81} No further clinical studies for this compound have been reported.

2.6 Benzimidazole NS11394 and related compounds. The Danish company NeuroSearch (subsequently renamed Aniona and then Saniona) have a long heritage in the field of benzimidazole-based GABA_AR PAMs typified by NS-2710 (**13**, Figures 6 and 8), NS11394 (**5**, Figure 3 and 8) and NS16085 (**21**, Figure 8). They have also described the clinical candidate NS11821, which although no structure has been publically disclosed is assumed to be of this same chemotype (Figure 8).⁸³

NS11394 has roughly equivalent, subnanomolar affinity (ranging from 0.1-0.8 nM, Table 1) for the different GABA_AR subtypes and had relative efficacy values at the α 1-, α 2-, α 3- and α 5-subtypes of 0.08, 0.26, 0.52 and 0.78, respectively. The maximal ED₅₀ for occupying rat brain GABA_ARs was 0.69 mg/kg p.o.⁸³ and NS11394 was shown to be anxiolytic in different assays at doses ranging from 0.1 and 0.3 mg/kg with only a slight degree of impairment on rotarod performance and locomotor activity at doses (\geq 60 mg/kg) that are $>$ 100-fold higher than anxiolytic doses.⁸³ There is considerable evidence to suggest that α 2/ α 3-GABA_AR PAMs may exert analgesic effects in the spinal cord, probably related to the α 2 subtype.^{84,85,86} In this regard, NS11394 has also been shown to be analgesic in a variety of inflammatory and neuropathic pain models.⁸⁷ NS11394 unexpectedly also caused a worsening of symptoms in the *dt^{sc}* mutant hamster model of dystonia,⁸⁸ in contrast to clonazepam which demonstrated a marked beneficial effect and zolpidem which showed a modest improvement.⁸⁹

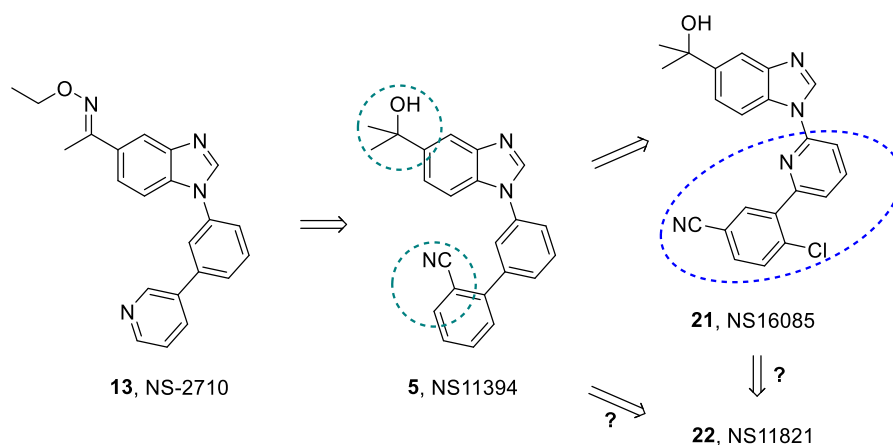


Figure 8. From NS-2710 to the optimized benzimidazole-based NS-compounds. Two-point modification in NS-2710 (characterized by a modest degree of α 3 vs α 1 selective efficacy) resulted in compound NS11394 (**5**), endowed with selectivity profile in the order of α 5 $>$ α 3 $>$ α 2 $>$ α 1, and higher α 3-GABA_ARs efficacy. Further modification to the diaryl-system yielded NS16085 (**21**), exhibiting negligible activity at α 5-GABA_ARs. SAR analysis around NS11821 (**22**) remains undisclosed.

The analgesic efficacy of NS11394 can be attributed to the α 2- and/or α 3-GABA_AR subtype(s) by comparison with NS16085. Hence, this latter compound has α 2/ α 3-GABA_AR efficacy comparable to NS11394 but is devoid of effects at α 5-GABA_ARs (relative efficacy = 0.05 vs 0.78 for NS11394). Nevertheless, NS16085 retained analgesic efficacy, indicating that α 5-GABA_ARs do not play a role in analgesia.⁹⁰

Although neither NS11394 nor NS16085 progressed into clinical development, the presumably structurally-related analogue, NS11821 was evaluated in man.⁹¹ NS11821 has high and relatively non-selective affinity for the different GABA_AR subtypes (K_i values ranging from 1.6-9.7 nM; Table 1), weak to moderate PAM efficacy at the α_2 , α_3 and α_5 subtypes (relative efficacy values of 0.17, 0.40 and 0.41, respectively) but negligible effects at the α_1 subtype (relative efficacy of 0.04). It demonstrated anxiolytic-like activity with a minimum effective dose of 3 mg/kg, without affecting rotarod performance.⁹¹ In a Phase 1 pharmacodynamic study, NS11821 was relatively safe and well-tolerated with the highest dose tested (600 mg, corresponding to a C_{max} of 1,430 ng/mL) producing nausea, vomiting, fatigue, dizziness and/or somnolence in $\geq 50\%$ of six subjects. Lower single doses of NS11821 (i.e., 10, 30 and 75 mg) did not demonstrate and marked pharmacodynamic effects. However, the higher doses of 150, 300 and 600 mg all produced statistically significant effects on a variety of parameters, with effects on saccadic peak velocity being observed at the 300 and 600 mg doses. Most notably, the 600 mg dose showed impairments in alertness, was associated with feeling high impairments and altered the EEG beta bands of the power spectrum. The increase in the time taken to achieve peak plasma drug concentrations between the 15 mg and 600 mg doses (respective T_{max} values of 0.5 and 4.0 h) suggested a complex absorption pattern that was thought to be associated with the low solubility of the compound.⁹¹ Accordingly, it was suggested that modifications in the formulation of NS11821 might result in improved bioavailability and dose-proportionality.

2.7 Imidazobenzodiazepines. Based upon an imidazobenzodiazepine core typified by flumazenil (**23**, Figure 9), bretazenil and imidazenil, Cook and colleagues at the University of Wisconsin described a compound, HZ-166 (**26**, Figure 9) that had relatively low and non-selective affinity for the different GABA_AR subtypes (K_i values ranging from 82-527 nM) and produced greater potentiation at α_2 - and α_3 - compared to α_1 - and α_5 -GABA_ARs.⁹² However, when the values are expressed relative to the potentiation produced by diazepam, HZ-166 was relatively non-selective (Table 1).⁹³ Nevertheless, HZ166 was not only anticonvulsant but it also was a non-sedating anxiolytic in rhesus monkeys.^{92,93} The related compounds XHe-II-053 and JY-XHe-053 (**24** and **25**, Figure 9) had slightly or markedly higher GABA_AR affinity, respectively, than HZ-166 but again neither had subtype-selective efficacy⁹³

(Table 1) and interestingly, JY-XHe-053 was not anxiolytic in the elevated plus maze.⁹⁴ Replacement of the ethyl ester of HZ-166 with a methyl ester resulted in a compound MP-III-024 (**27**, Figure 9) which had an improved *in vitro* metabolic stability and demonstrates analgesic effects but no sedation in mice.⁹⁵

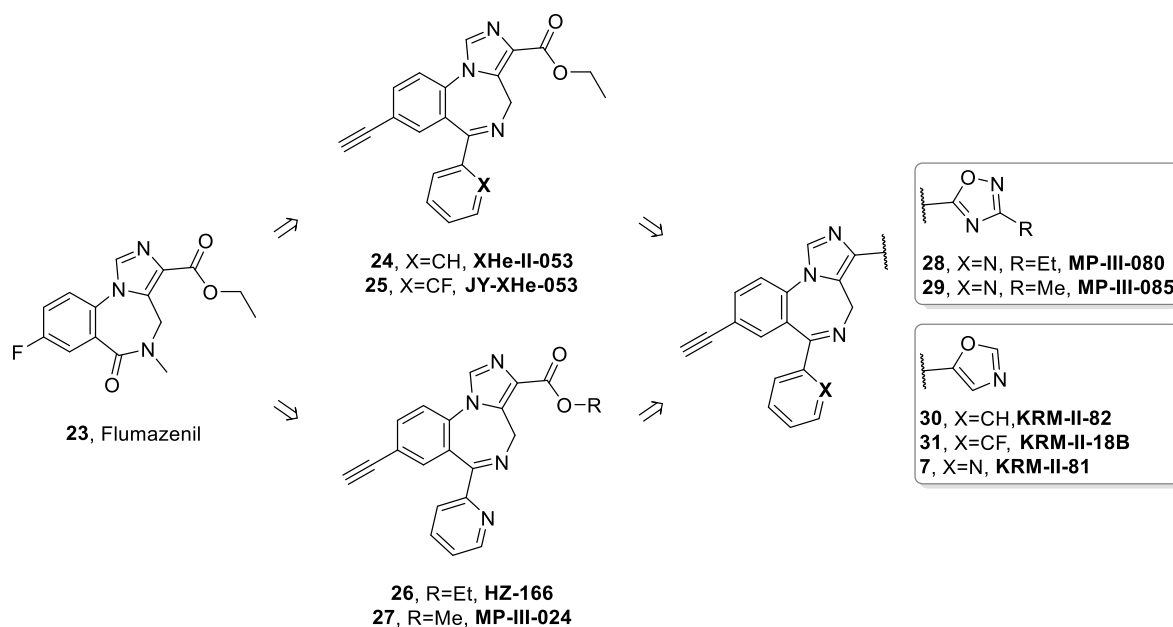


Figure 9. Examples of imidazobenzodiazepines $\alpha 2/\alpha 3$ -GABA_AR PAMs related to the prototypic non-selective antagonist flumazenil. The ester-containing analogues (**24-27**) have poor metabolic stability and the ester to oxazole/oxadiazole bioisosteric replacement afforded a new series of compounds (**7, 28-31**) with optimized profiles and anxiolytic, analgesic, anticonvulsant and antidepressant activities.

The ester-containing compound XHe-II-053 was reported to have been progressed into Phase 1 clinical trials by Bristol-Myers Squibb. However, the compound was rapidly transformed into the metabolite XHe-II-053-acid by liver enzymes,⁹⁶ resulting in the search for more metabolically-stable analogues. Since the ester group in these first generation imidazobenzodiazepines is a metabolic liability, compounds were synthesized in which either an oxazole or (alkyl)oxadiazole (**28** and **29**, Figure 9) were used as a more metabolically-stable ester bioisostere.^{96,97,98} The oxazoles subseries includes the compounds KRM-II-82, KRM-II-18B and KRM-II-81 (**30, 31** and **7**, Figure 9), of which the latter is the best described.⁹⁷ Hence, KRM-II-81 has a EC₅₀ and relative efficacy at the $\alpha 1$ -, $\alpha 2$ - and $\alpha 3$ -subtypes in the range of 118-489 nM and 0.35-0.77, respectively, with negligible efficacy at $\alpha 5$ -GABA_AR efficacy being detected⁹⁷ (Table 1). This compound demonstrates anxiolytic, analgesic and

anticonvulsant effects.^{97,98,99,100} As regards the compounds with an oxadiazole replacement of the ester group, then *in vitro* metabolic stability is improved⁹⁶ and a representative compound from this class, MP-III-80 (**28**, Figure 9), was shown to have antidepressant-like activity.¹⁰¹ Since these compounds all possess varying degrees of efficacy at $\alpha 1$ -GABA_ARs, they are probably best described as being $\alpha 2/\alpha 3$ -GABA_AR preferring to distinguish them from compounds that lack any efficacy at the $\alpha 1$ subtype which are therefore $\alpha 2/\alpha 3$ -GABA_AR selective.

3. $\alpha 5$ -GABA_AR NAMs

3.1 Background and Context. As above-mentioned, $\alpha 5$ -GABA_ARs are preferentially expressed within the hippocampus²⁹ which has a well-established role in learning and memory, resulting in the hypothesis that $\alpha 5$ -GABA_AR NAMs should enhance cognition.¹⁰² However, an increased activity in the hippocampal formation in the aging brain,¹⁰³ schizophrenia patients³³ or neuropsychiatric disorders with cognitive deficits,¹⁰⁴ has recently highlighted the therapeutic potential of $\alpha 5$ -GABA_AR PAMs^{105, 106} which will be addressed separately below. While the BZD binding sites at $\alpha 1$ -, $\alpha 2$ -, and $\alpha 3$ -GABA_ARs have a high degree of similarity, thus making it difficult to find compounds with binding affinities that differentiate between these subtypes, $\alpha 5$ GABA_ARs appear to have an allosteric site that is more distinct from the other subtypes.¹⁰⁷ Accordingly, the identification of ligands with $\alpha 5$ -GABA_AR-selective affinity appears to be more achievable compared to $\alpha 2/\alpha 3$ -GABA_AR binding-selective modulators. For example, the NAM L-655708 (**33**, Figure 10)¹⁰⁸ has 50-100-fold higher affinity for $\alpha 5$ - versus $\alpha 1$ -, $\alpha 2$ - and $\alpha 3$ -GABA_ARs whereas conversely zolpidem has moderate to high affinity for $\alpha 1$ -, $\alpha 2$ - and $\alpha 3$ -GABA_ARs (ranging from 17-360 nM) but no affinity for the $\alpha 5$ subtype.²⁰

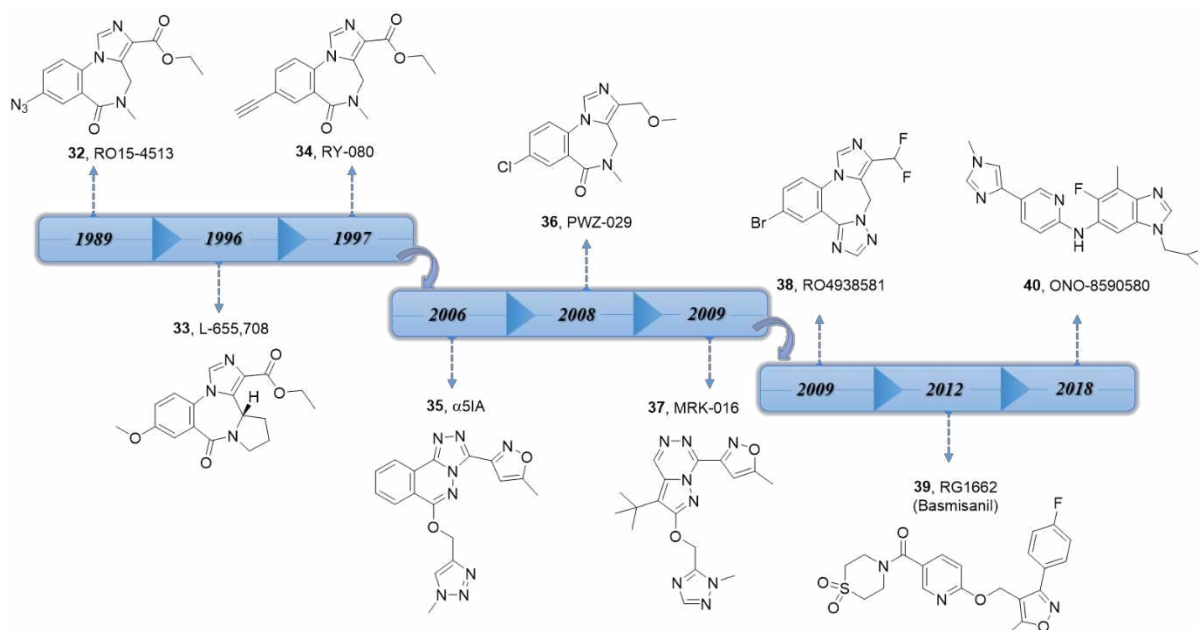


Figure 10. Structures and chronological sequence of the description of various $\alpha 5$ -GABA_AR NAMs. Compounds **32-40** are representatives of different classes of NAMs, starting with the imidazobenzodiazepines and moving to the recently-described compounds Basmisanil (RG1662; **39**) and ONO-8590580 (**40**). Of these compounds, $\alpha 5$ IA (**35**), MRK-016 (**37**) Basmisanil (**39**) have all progressed into clinical studies.

Table 3. Summary of the *in vitro* properties of $\alpha 5$ -GABA_AR NAMs

Compound	Structure	K _i (nM)				Efficacy ^a			
		$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 5$	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 5$
RO4882224	Imidazotriazolo-benzodiazepine	15	24	14	2.0	-3%	-9%	-11%	-40%
RO4938581		174	185	80	4.6	-3%	-4%	2%	-35%
Basmisanil	Isoxazole-Pyridine	985	502	489	4.7	-4%	-6%	-2%	-39%
$\alpha 5$IA^b	Triazolopyridazine	0.88	0.58	0.61	0.66	-0.25	0.10	-0.11	-0.70
MRK-016	Pyrazolotriazine	0.83	0.85	0.77	1.4	-0.16	0.06	-0.09	-0.55
PWZ-029	Imidazo-benzodiazepine	362	180	328	6	20%	15%	45%	-20%
ONO-8590580	Benzimidazole	140	32	24	7.9	~0%	~0%	~0%	-44%

^a Efficacy is expressed either as a % reduction in the GABA-induced current or is expressed relative to the reduction in GABA current produced by NAM, the prototypic non-selective GABA_AR NAM.

^b Compounds in **bold** are those which progressed into clinical studies.

3.2 Roche compounds. The imidazotriazolo-benzodiazepines RO4882224 (**38**, Figure 10) and RO4938581 were identified following optimization of hits from a screening campaign.^{109,110} RO4882224 has a moderate (7-12-fold) and RO4938581 an appreciable (17-40-fold) $\alpha 5$ - versus $\alpha 1$ -, $\alpha 2$ - or $\alpha 3$ -GABA_AR binding selectivity. In addition, both compounds also had a marked $\alpha 5$ -GABA_AR NAM efficacy selectivity (Table 3), making them $\alpha 5$ binding and efficacy selective. Both compounds attenuated a scopolamine-induced deficit in cognitive performance in the rat delayed matching to position test¹¹⁰ and RO4938581 improved executive function in monkeys¹¹⁰ and rescued behavioral, anatomical and biochemical abnormalities in a mouse model of Down syndrome (DS).^{111,112} Although both RO4882224 and RO4938581 were selected as clinical candidates for further development,¹⁰⁹ no further details regarding their preclinical or clinical development have been reported and it could have been presumably halted due to preclinical hepatotoxicity issues associated with these compounds.

An isoxazole-pyridine series was optimized to produce the clinical candidate Basmisanil (RG1662, **39**, Figure 10). Although no peer-reviewed publication has appeared describing this compound, it is described in the patent US8835425(B2) as compound R1.¹¹³ Hence, Basmisanil not only has 100-200-fold $\alpha 5$ - versus $\alpha 1$ - $\alpha 2$ - and $\alpha 3$ -GABA_AR binding selectivity (Table 3), but it is also has $\alpha 5$ -selective NAM efficacy such that, and like RO4882224 and RO4938581, it also has $\alpha 5$ subtype binding and efficacy selectivity. In a [¹¹C]RO15-4513 PET study, doses of 20-1000 mg RG1662 produced a dose- and exposure-proportional reduction in radiotracer uptake and a maximum receptor occupancy of around 80%, with the plasma drug concentration required to produce 50% occupancy being in the region of 750 ng/mL.¹¹⁴ Basmisanil was described as attenuating the deficits in long-term potentiation and performance in the Morris water maze seen in Ts65Dn mice¹¹³ and presumably on this basis was progressed into Phase 2 clinical studies in adolescents and adults with DS. However, RG1662 did not show any efficacy in the cognitive or behavioral primary endpoints and the compound is now being assessed for its effects on cognitive performance in schizophrenia (NCT02953639).

3.3 Merck compounds. The lead $\alpha 5$ -GABA_AR NAM compound described by the Merck group was the triazolophthalazine $\alpha 5$ IA (**35**, Figures 10 and 11).¹¹⁵ This compound shared a common ancestry with the $\alpha 2/\alpha 3$ -GABA_AR PAM TPA023 and optimization of the parent compound (Figure 11) resulted in

the identification of $\alpha 5$ IA. It showed equivalent subnanomolar affinity across the different GABA_AR subtypes (ranging from 0.58-0.88 nM; Table 3) but had much higher NAM efficacy at the $\alpha 5$ compared to the $\alpha 1$ -, $\alpha 2$ - and $\alpha 3$ -GABA_AR subtypes (Table 3), displaying a relative efficacy of -0.70 compared to the prototypic non-selective GABA_AR NAM DMCM. $\alpha 5$ IA also demonstrated good *in vivo* GABA_AR occupancy in mice and rats with Occ₅₀ values of 0.12 and 0.25 mg/kg p.o. in mice and rats,¹¹⁵ respectively, as well as in primates.¹¹⁶

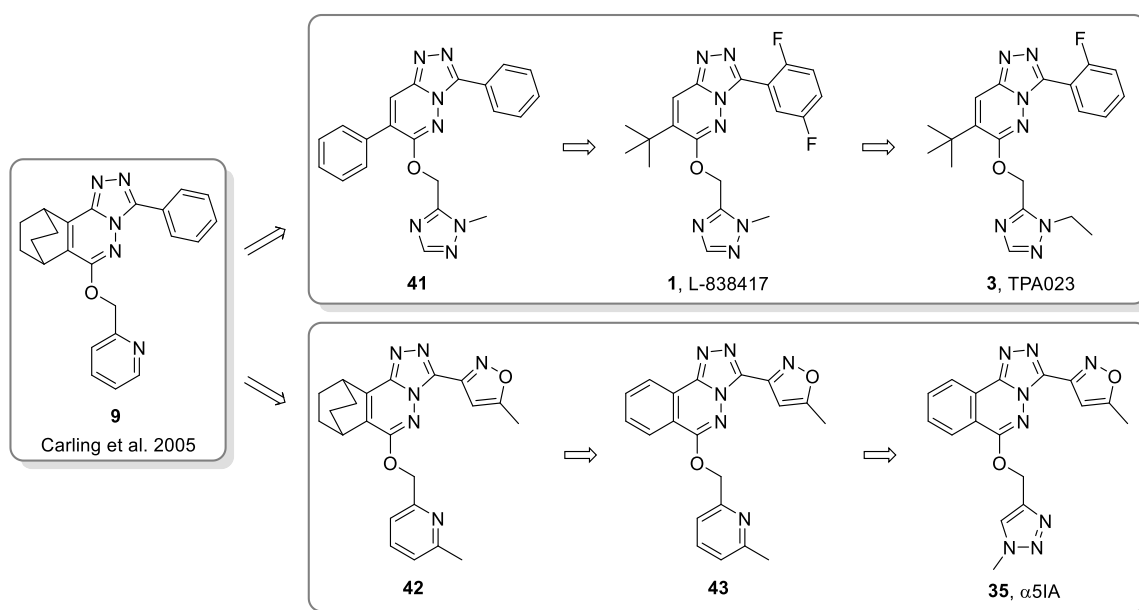


Figure 11. The SAR analysis leading to the PAM TPA023 (**3**) and to the NAM $\alpha 5$ IA (**35**), starting from the common ancestry triazolopyridazine analogue (**9**). These compounds illustrate the concept that different selectivity profiles ($\alpha 2/\alpha 3$ -GABA_AR PAMs or $\alpha 5$ -GABA_AR NAMs) can be developed from a common chemical starting point.

It improved performance in a delayed-matching-to-place version of the Morris water maze,¹¹⁷ and had no proconvulsant effect in the pentylenetetrazole assay and nor anxiogenic effect in the rat elevated plus maze assay. In man, $\alpha 5$ IA was safe and well tolerated¹¹⁸ and demonstrated good levels of target engagement in a [¹¹C]flumazenil PET study with a single 2 mg dose producing GABA_AR occupancy in the region of 50% and a plasma EC₅₀ of 10 ng/mL.¹¹⁹ However, further development of this compound was halted due to the occurrence of renal toxicity in preclinical species. The toxicity was caused by the deposition of crystals of the insoluble, hydroxymethyl isoxazole metabolite of $\alpha 5$ IA that made it unsuitable for long-term dosing in man.¹¹⁸ Nevertheless, the compound was suitable for short-term

dosing and in an experimental medicine paradigm in healthy volunteers in which an impairment in list recall was induced by alcohol consumption, $\alpha 5IA$ showed a significant improvement in list recall compared to placebo-treated subjects.¹²⁰

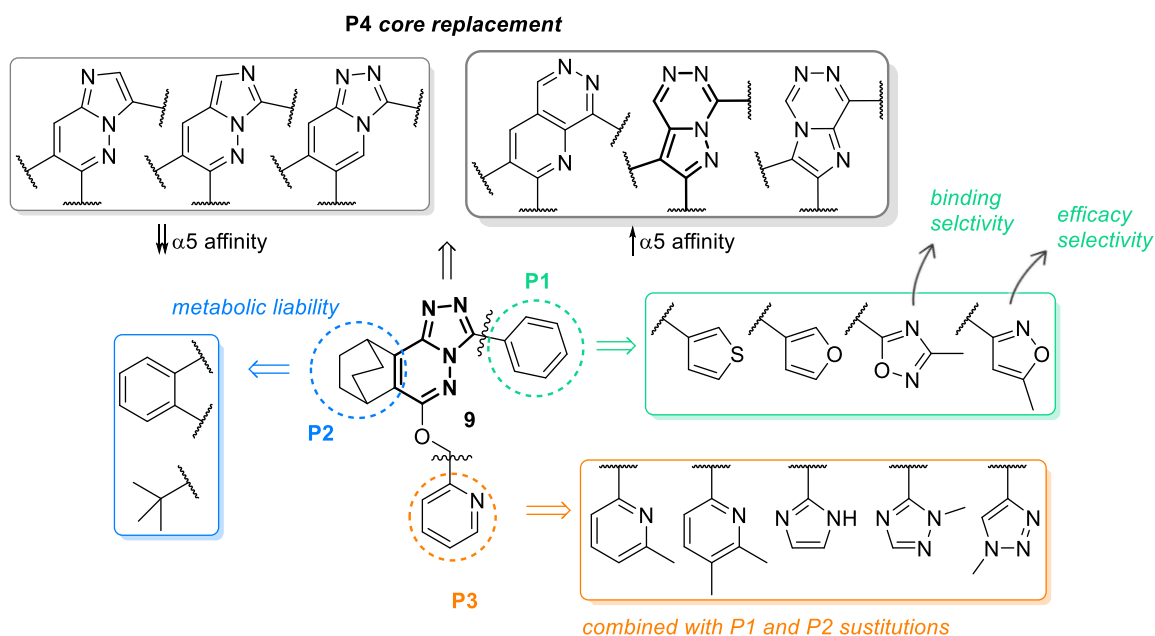


Figure 12. The SAR study around compound **9** leading to $\alpha 5AI$ and MRK-016. **P1:** replacement of the phenyl group at C-3 position with oxadiazole and isoxazole gave lead intermediates for binding and efficacy selectivity, respectively; **P2:** replacement of the [2.2.2]-bicyclic ring system with a benzo-fused ring addressed metabolic stability issues but did not improve oral bioavailability. **P3:** initial substitution of 6-methylpyridin-2-yl group with a dimethyl pyridyl moiety in the [2.2.2]-bicyclic ring system gave the maximum binding selectivity in the series. The following combination of 1-methyl-1,2,3-triazol-4-yl with the benzo-fused ring led to the identification of $\alpha 5AI$ (**35**, Figures 9 and 10). **P4:** different heterocyclic cores have been explored. By merging the isoxazole and triazole groups in a pyrazolotriazine core with a *tert*-butyl moiety, MRK-016 (**37**, Figure 9) was identified.

By replacing the triazolophthalazine core of $\alpha 5IA$ with a pyrazolotriazine while retaining the isoxazole and triazole groups and replacing the bicyclic ring system in $\alpha 5IA$ with a *tert*-butyl group (Figure 12), the back-up compound MRK-016 (**37**, Figure 10) was identified.¹²¹ This molecule has high and equivalent affinity across the GABA_AR subtypes (0.77-1.4 nM), with $\alpha 5$ -GABA_AR selective efficacy profile similar to that of $\alpha 5IA$ except that MRK-016 has great NAM activity at the $\alpha 5$ subtype (relative efficacy = -0.96). MRK-016 gave good receptor occupancy after oral dosing in rats, with the Occ₅₀ being 0.39 mg/kg p.o. and a corresponding rat plasma EC₅₀ value of 15 ng/mL. It was able to

enhanced cognitive performance in the delayed matching-to-position version of the Morris water maze and was not proconvulsant or anxiogenic. MRK-016 has also been shown to protect against lipopolysaccharide-induced deficits in memory acquisition and consolidation and restore the behavioral expression of fear in lipopolysaccharide-treated animals, an effect that may be attributed to increased brain-derived neurotrophic factor mRNA expression.^{122,123} Finally, MRK-016 has antidepressant-like activity in a variety of preclinical assays.^{124,125,126}

Although MRK-016 had a short half-life in preclinical species, it had a lower rate of *in vitro* turnover in human hepatocytes and this translated into a half-life in man of 3.5 h. Based on plasma drug concentrations and the occupancy EC₅₀ value in rhesus monkey (21 ng/mL) the maximum tolerated dose of 5 mg in young healthy controls was estimated to correspond to 75% GABA_AR occupancy.¹²¹ However, MRK-016 studies in man were halted due to poor tolerability in the healthy elderly volunteers as well as variable pharmacokinetic,¹²¹ and based upon these data clinical development of this drug was terminated.

3.4 Imidazobenzodiazepines. Based upon the structure of RO15-4513 (**32**, Figure 10), which has 10-20-fold higher affinity for $\alpha 5$ - versus $\alpha 1$ -, $\alpha 2$ - and $\alpha 3$ -GABA_ARs, Cook and colleagues synthesized a series of $\alpha 5$ binding-selective compounds typified by RY-080 (**34**, Figure 10), but which also included RY-023, RY-024 and RY-079.¹²⁷ This in turn led to the identification of PWZ-029¹²⁸ (**36**, Figure 10) which has 30-60-fold binding selectivity for $\alpha 5$ - versus $\alpha 1$ -, $\alpha 2$ and $\alpha 3$ -GABA_ARs (Table 3).^{128,129} This compound has a degree of PAM activity and the $\alpha 1$, $\alpha 2$ and $\alpha 3$ subtypes but modest NAM activity at $\alpha 5$ -GABA_ARs¹²⁸ such that it is best described as a low-efficacy $\alpha 5$ -GABA_AR NAM whereas RY-023, RY-024 and RY-080 are classified as high-efficacy $\alpha 5$ -selective compounds¹³⁰. Hence, RY-080 attenuates GABA-induced currents at the $\alpha 5$ subtype by around 36% whereas the corresponding value for PWZ-029 was only 17%.¹³¹ The relatively weak $\alpha 5$ -GABA_AR NAM activity of PWZ-029 is reflected in the lower efficacy of this compound in the mouse forced-swim assay relative to RY-080.¹³¹ PWZ-029 improved learning in a rat passive but not active avoidance task at a dose, 5 mg/kg i.p., that was just below those at which an impairment in locomotor activity was observed (10 and 20 mg/kg).¹²⁸ Despite its relatively weak $\alpha 5$ -GABA_AR NAM activity, PWZ-029 was nevertheless able to enhance

performance in a rhesus monkey object retrieval task that was either “difficult” or in which performance was impaired by scopolamine.¹²⁹ All these imidazobenzodiazepines appear to be preclinical tools with none having been described to date as having entered clinical or preclinical development.

3.5 Other compounds. The benzimidazole ONO-8590580 (**40**, Figures 10 and 13) was recently described as an $\alpha 5$ -GABA_AR NAM.¹³² The affinity of this compound for the $\alpha 5$ subtype is 7.9 nM, which is around 3-30-fold higher than at $\alpha 1$ -, $\alpha 2$ - or $\alpha 3$ -GABA_ARs.¹³² This modest binding selectivity is accompanied by efficacy selectivity such that ONO-8590580 attenuated a GABA EC₂₀ current by 44% whereas there was negligible effects at the other subtypes. The compound gave good hippocampal $\alpha 5$ -GABA_AR occupancy, with the Occ₅₀ being 1.9 mg/kg p.o. and in rat behavioral assays, ONO-8590580 was able to attenuate MK-801- or scopolamine-induced deficits in performance in the passive avoidance and radial arm maze assays but was not anxiogenic nor proconvulsant.¹³² The development status of this or related compounds has not been disclosed but neither this compound nor the $\alpha 5$ -GABA_AR NAM mechanism is listed in the company pipeline.

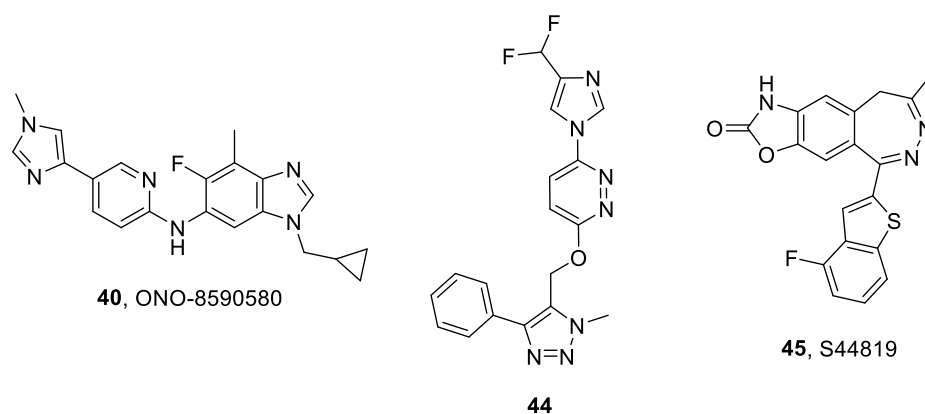


Figure 13. Other compounds. ONO-8590580 and a Saniona compound described in WO 2014/001280 A1 (**44**), both of which are $\alpha 5$ -GABA_AR PAMs acting at the BZD binding site, along with S44819 which is an $\alpha 5$ -GABA_AR subtype selective antagonist acting via the GABA (rather than the BZD) recognition site.

The Danish company Saniona have published a series of single compound patents (WO 2014/001278-1282 A1) in which five compounds (typified by **44**, Figure 13) all shared a common triazole core and had $\alpha 5$ -GABA_AR affinities ranging from 0.39-87 nM and a reduction of a GABA-induced current (NAM activity) of between -18 to -39% at this subtype. No selectivity data was

presented and none of these compounds have been described in peer-reviewed journals. Finally, Servier have described a benzothiophene compound, S44819 (Egis-13529) which modulates GABA_AR function by selectively antagonizing the agonist (GABA) binding site of $\alpha 5$ -GABA_AR.¹³³ It is therefore mechanistically different from the other compounds described in this section and is currently being evaluated as a therapy to assist post-stroke recovery (NCT02877615).¹³⁴

4. $\alpha 5$ -GABA_AR PAMs

There is an increasing awareness that hippocampal hyperactivity may be a core feature of disorders such as schizophrenia.^{135,33} This hyperactivity is thought to result in an increased output from the hippocampus that results in the increased activity of dopaminergic neurons that underlies the disease. Moreover, a dysfunction in the regulation of overlapping memories is a core feature of schizophrenia that is thought to be associated with $\alpha 5$ -GABA_AR in the hippocampus.¹⁰⁴ These data collectively suggest that an $\alpha 5$ -GABA_AR PAM may represent a novel approach to the treatment of schizophrenia.^{33,106}

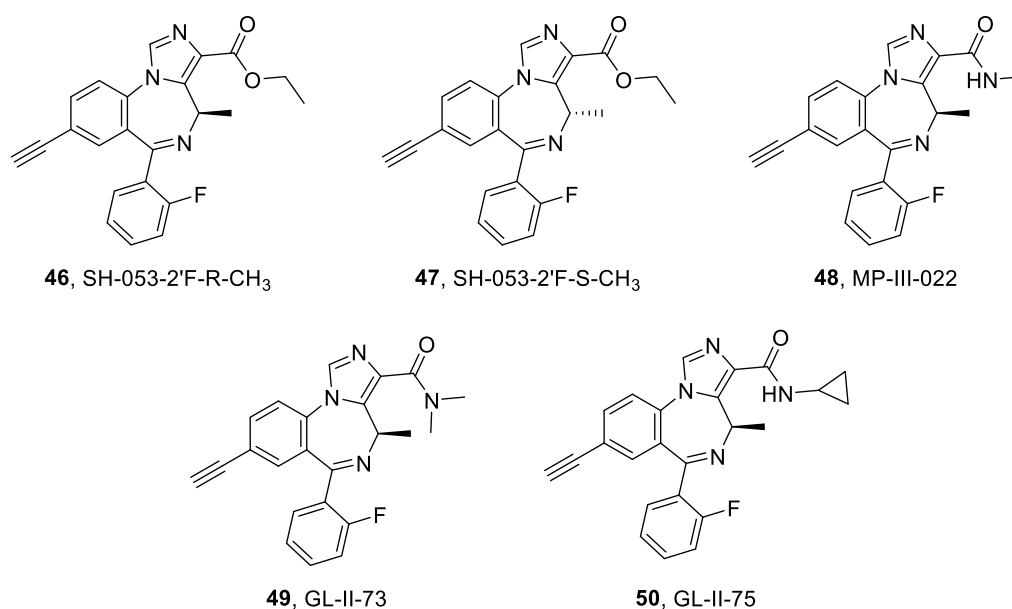


Figure 14. Structure of imidazobenzodiazepines described as being $\alpha 5$ -GABA_AR selective PAMs

During the characterization of the imidazobenzodiazepines previously described as $\alpha 2/\alpha 3$ -GABA_AR selective PAMs (see above), Cook and colleagues described the properties of SH-053-2'F-R-CH₃ and SH-053-2'F-S-CH₃ (**46** and **47**, Figure 14) the 4-methyl stereoisomers of JY-XHe-053.⁹³ Both these compounds had modest affinity for $\alpha 5$ -GABA_ARs, (95 and 19 nM, for the R and S isomers, respectively) that was generally in the region of around 10-fold higher than at the $\alpha 1$, $\alpha 2$ and $\alpha 3$ subtypes (Table 4).⁹³

Table 4. Summary of the *in vitro* properties of $\alpha 5$ -GABA_AR PAMs

Compound	Structure	K _i (nM)				Relative Efficacy ^a			
		$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 5$	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 5$
SH-053-2'F-R-CH ₃	Imidazo- benzodiazepine	759	948	769	95	0.25	0.19	0.18	1.19
SH-053-2'F-S-CH ₃		468	33	292	19	0.30	0.57	0.31	1.19
MP-III-022		850	360	660	55	0.11	0.50	0.20	2.48
GL-II-073		55,000	30,000	63,000	5,000	0.65	^b NR	NR	1.46
GL-II-074		1,060	809	1,250	83	1.09	NR	NR	1.39
GL-II-075		542	789	480	79	1.22	NR	NR	1.23

^a The efficacy is calculated based upon the potentiation of an EC₃₋₅ GABA concentration of a 1 μ M concentration of drug relative to the potentiation produced by diazepam, either at a concentration of 1 μ M⁹³ or the average maximum potentiation.¹³⁵ ^bNR = not reported.

SH-053-2'F-R-CH₃ and SH-053-2'F-S-CH₃ have varying degrees of efficacy at $\alpha 1$ -, $\alpha 2$ - and $\alpha 3$ -GABA_ARs, with relative efficacy values ranging from 0.18 to 0.57, but most striking, both compounds have efficacy at the $\alpha 5$ subtype that was greater than diazepam. The administration of methylazoxymethanol acetate (MAM) to pregnant rats results a changes in the offspring that result in a spontaneous dopamine activity within the ventral tegmental area (VTA) that is associated with altered activation of the ventral hippocampus. In this neurodevelopmental of schizophrenia, SH-053-2'F-R-CH₃ reduced the number of spontaneously active dopamine neurons within the VTA, whether the compound was administered systemically or infused directly into the hippocampus.¹³⁶ Moreover, SH-053-2'F-R-CH₃ was able to attenuate the increased sensitivity to amphetamine observed in MAM rats.¹³⁶ In a rhesus monkey conflict assay, SH-053-2'F-R-CH₃ and SH-053-2'F-S-CH₃ produced variable effects in individual animals, with the R but not S isomer achieving a significant but modest overall anxiolytic effect without showing signs of sedation.⁹³

The replacement of the ester group in SH-053-2'F-R-CH₃ with an amide resulted in the identification of MP-III-022 (**48**, Figure 14) which has a binding affinity across all four subtypes and efficacy at the α 1- and α 3-subtypes comparable to the ester.¹³⁷ However, MP-III-022 has an increased efficacy at the α 2 subtype and efficacy at α 5-GABA_ARs was around twice that of SH-053-2'F-R-CH₃, with a relative efficacy of 2.48 compared to diazepam.^{93,137} The amide replacement did indeed increase the metabolic stability of MP-III-022 relative to SH-053-2'F-R-CH₃ in an *in vitro* rat plasma stability assay. However, this *in vitro* metabolic stability was not reflected *in vivo* with the ester having an half-life after i.p. dosing, (75 min) that was twice that of the amide.¹³⁷ MP-III-022 caused a degree muscle relaxation but did not cause sedation, affect anxiety levels or alter the pentylenetetrazole seizure threshold.¹³⁷

An additional series of amide analogues of SH-053-2'F-R-CH₃, GL-II-73 (**49**, Figure 14), GL-II-74 and GL-II-75 (**50**, Figure 14) have been described (together with a fourth compound GL-II-76, but no *in vitro* binding or efficacy data were provided).¹³⁸ These compounds have a moderate (6-15-fold) binding selectivity for α 5- over α 1-, α 2- and α 3-GABA_ARs, with GL-II-74 and GL-II-75 having a modest α 5 affinity (~80 nM) whereas GL-II-73 is very low affinity (5 μ M). At a concentration of 1 μ M, all compounds had PAM activity at all four subtypes with both GL-II-73 and GL-II-74 having efficacy at the α 1 and α 5 subtypes that was comparable to or greater than diazepam. All three compounds were able to show effects in the mouse forced-swim test with GL-II-73 and GL-II-74 (but not GL-II-75) having anxiolytic activity in the mouse elevated plus maze with GL-II-73 and GL-II-75. Finally, and despite it is much lower GABA_AR affinity, GL-II-73 was able to reverse stress- and age-related impairments of working memory to a much greater extent than GL-II-75.¹³⁸ The data for GL-II-73 have been further highlighted¹³⁹ but in the absence of target engagement (GABA_AR occupancy) data, these results are difficult to interpret. While the collective data for putative α 5-GABA_AR PAMs make the potential therapeutic relevance of α 5-GABA_AR PAMs unclear, it is nevertheless worth noting that Roche currently have a drug of this mechanism, RG7816, listed in their pipeline as currently undergoing Phase 1 clinical studies with a therapeutic indication of autism spectrum disorder. Although no further details are currently available regarding RG7816, this is probably the same as RO7017773, for which a [¹¹C]RO15-4513 PET study has been conducted in healthy volunteers (NCT03507569).

5. CONCLUSIONS AND PERSPECTIVES

It is now approaching 60 years since the introduction of BZDs with their GABAergic, or more specifically GABA_AR-mediated mechanism of action began to emerge in the mid-1970s.¹⁴⁰ Nevertheless, there continues to be considerable interest in the modulation of GABA_ARs for a variety of CNS and other indications,¹⁴¹ most recently fueled by the approval of Brexanolone as a neurosteroid antidepressant.¹⁴² However, the main focus on the development of novel GABA_AR modulating drugs has been on compounds that act via the BZD binding site. Since the start of the millennium there has been a convergence of elegant transgenic mouse and subtype-selective pharmacology to provide the robust preclinical data that underpins the $\alpha 2/\alpha 3$ -GABA_AR PAM non-sedating anxiolytic and $\alpha 5$ -GABA_AR NAMs cognition enhancer hypotheses and it has been rewarding to see representative molecules from each of these mechanisms progress into the clinic. However, as with all clinical studies, particularly within the neuroscience therapeutic area, the challenge remains in translating preclinical science and pharmacology into clinical efficacy and thereafter patient benefit. In this regard, the use of [¹¹C]flumazenil or [¹¹C]RO15-4513 PET has proved very useful in establishing clinical target engagement (GABA_AR occupancy) and selecting a clinical dose. Yet despite this, questions can remain regarding the selection of a dose sufficient to test the hypothesis (e.g., PF-06372865 in the chronic back pain study) and the correct patient population for establishing efficacy of the mechanism (e.g., is DS the most appropriate patient population for evaluating the cognition-enhancing effects of Basmisnil?). Layered on top of these issues are the generic issues that plague drug development and halt further development such as unexpected preclinical toxicity (e.g., cataract formation with TPA023, crystallization of an insoluble metabolite causing renal toxicity with $\alpha 5$ IA), variable pharmacokinetics and/or poor tolerability (MRK-016) or poor patient compliance (AZD7325). Finally, there are the pharma company strategic and/or business decisions that result in what the scientists involved in the area are always bound to consider the premature termination of their favorite GABA_AR modulator projects. The bigger picture, however, is that we have now reached a stage in the GABA_AR subtype-selective modulator story where we have moved on from preclinical data and are now able to discuss clinical studies. While there is still a way to go until such compounds, whether they be $\alpha 2/\alpha 3$ -GABA_AR PAMs or $\alpha 5$ -GABA_AR NAMs (or even PAMs) finally reach the market, there is little doubt that such

molecules have pharmacological profiles that are very distinct from those of BZDs themselves.¹⁴³ In addition, GABA_AR modulators may be evaluated using experimental medicine paradigms or a small but well characterized patient population. In this latter regard, the use of a small number (seven) of photosensitive epilepsy patients to demonstrate efficacy of PF-06372865 is a particularly elegant example⁴² which has redirected the drug, now known as CVL-865, towards being developed as an antiepileptic drug. Hence, despite the occasional setbacks, the GABA_AR BZD site subtype-selective modulator field continues to advance forward towards the goal of delivering novel medicines to patients.

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Dr. Samuele Maramai received his master in Chemistry and Pharmaceutical Technologies cum laude in 2008 at the University of Siena, working on the synthesis of cholinesterase inhibitors and multi-target directed ligands for Alzheimer disease. After a master in Drug Design and Synthesis, he got a PhD in pharmaceutical sciences in 2012, working on selective inhibitors of enzymes of the endocannabinoid system. He has been a post-doctoral researcher at the University of Siena until 2016, when he joined the Sussex Drug Discovery Centre as a Research Fellow in Medicinal Chemistry, under the supervision of Prof. John Atack and Prof. Simon Ward. He possesses a wide expertise on neuroscience drug discovery as well as a long experience in the research of proapoptotic and anticancer agents.

Dr. Mohamed Bencheikroun holds a Master in Organic Chemistry (2010, Université Paris-Sud) and a Doctorate in Medicinal Chemistry (2014, Université de Franche-Comté). In 2015-2016, he did a

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Prof. Simon E. Ward has an MA in Natural Sciences and PhD in synthetic organic chemistry from Cambridge University, UK. He has a wide-ranging experience of drug discovery in both major pharmaceutical companies, biotech & academia (GlaxoSmithKline, BioCrea GmbH, Knoll Pharmaceuticals, Vernalis, Chiroscience, University of Sussex, Cardiff University) and specialist experience of discovering and developing drugs for patients with neurological and psychiatric illness and against cancers. In 2010 he joined the University of Sussex to initiate a translational drug discovery group, which successfully grew into a center with over 60 staff and students working on neuroscience and oncology targets. He currently is the Sêr Cymru Professor in Translational Drug Discovery and Director of the newly established Medicines Discovery Institute at Cardiff University.

Prof. John R. Atack is a molecular pharmacologist with over 25 years of drug discovery experience, primarily in the field of neuroscience. He got his PhD in neurochemical pathology in the Department of Pathology of Newcastle General Hospital. From there, he joined the Merck Sharp and Dohme Neuroscience Research Centre and from 2006 to 2012, he was part of Janssen Pharmaceuticals where he worked on a variety of aspects of neuroscience drug discovery. He joined the University of Sussex in 2012 to help establish, with Prof. Simon Ward, the Sussex Drug Discovery Centre, focusing on neuroscience drug discovery and ion channel pharmacology. In summer 2017, he moved to Cardiff University as Professor and Co-Director of the new Medicines Discovery Institute.

ABBREVIATIONS USED

BZD: benzodiazepine; CNS: Central Nervous System; Cryo-EM: cryo-electron microscopy; CYP: Cytochrome P450; DS: Down syndrome; EC₅₀: half maximal effective concentration; EEG: electroencephalogram; GABA: γ -aminobutyric acid; GABA_AR, GABA Type A receptor; GAD: generalized anxiety disorder; HAM-A: Hamilton Anxiety; NAM: negative allosteric modulator; Occ₅₀: dose required to occupancy 50% of brain receptors; PAM: positive allosteric modulator; PET: positron emission tomography; SAR: Structure-Activity Relationship.

References

- (1) Lorenz-Guertin, J. M.; Jacob, T. C. GABA Type A Receptor Trafficking and the Architecture of Synaptic Inhibition. *Dev. Neurobiol.* **2018**, *78* (3), 238–270.
- (2) Miller, P. S.; Smart, T. G. Binding, Activation and Modulation of Cys-Loop Receptors. *Trends Pharmacol. Sci.* **2010**, *31* (4), 161–174.
- (3) Sparling, B. A.; Di Mauro, E. F. Progress in the Discovery of Small Molecule Modulators of the Cys-Loop Superfamily Receptors. *Bioorg. Med. Chem. Lett.* **2017**, *27* (15), 3207–3218.
- (4) Kaila, K.; Price, T. J.; Payne, J. A.; Puskarjov, M.; Voipio, J. Cation-Chloride Cotransporters in Neuronal Development, Plasticity and Disease. *Nat. Rev. Neurosci.* **2014**, *15* (10), 637–654.
- (5) Alexander, S. P.; Peters, J. A.; Kelly, E.; Marrion, N. V.; Faccenda, E.; Harding, S. D.; Pawson, A. J.; Sharman, J. L.; Southan, C.; Davies, J. A. THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Ligand-Gated Ion Channels. *Br. J. Pharmacol.* **2017**, *174* (S1), S130–S159.
- (6) Sigel, E.; Steinmann, M. E. Structure, Function, and Modulation of GABA_A Receptors. *J. Biol. Chem.* **2012**, *287* (48), 40224–40231.
- (7) Olsen, R. W.; Sieghart, W. International Union of Pharmacology. LXX. Subtypes of γ -Aminobutyric Acid A Receptors: Classification on the Basis of Subunit Composition, Pharmacology, and Function. Update. *Pharmacol. Rev.* **2008**, *60* (3), 243–260.
- (8) Sieghart, W.; Savić, M. M. International Union of Basic and Clinical Pharmacology. CVI: GABAA Receptor Subtype- and Function-Selective Ligands: Key Issues in Translation to Humans. *Pharmacol. Rev.* **2018**, *70* (4), 836–878.
- (9) Engin, E.; Benham, R. S.; Rudolph, U. An Emerging Circuit Pharmacology of GABA_A Receptors. *Trends Pharmacol. Sci.* **2018**, *39* (8), 710–732.
- (10) Ernst, M.; Bruckner, S.; Boresch, S.; Sieghart, W. Comparative Models of GABA_A Receptor Extracellular and Transmembrane Domains: Important Insights in Pharmacology and Function. *Mol. Pharmacol.* **2005**, *68* (5), 1291–1300.

- (11) Lavery, D.; Desai, R.; Uchański, T.; Masiulis, S.; Stec, W. J.; Malinauskas, T.; Zivanov, J.; Pardon, E.; Steyaert, J.; Miller, K. W.; Aricescu, A. R. Cryo-EM Structure of the Human $\alpha 1\beta 3\gamma 2$ GABA A Receptor in a Lipid Bilayer. *Nature* **2019**, *565* (7740), 516–520.
- (12) Masiulis, S.; Desai, R.; Uchański, T.; Martin, I. S.; Lavery, D.; Karia, D.; Malinauskas, T.; Zivanov, J.; Pardon, E.; Kotecha, A.; Steyaert, J.; Miller, K. W.; Aricescu, A.R. GABA A Receptor Signalling Mechanisms Revealed by Structural Pharmacology. *Nature* **2019**, *565* (7740), 454–459.
- (13) Johnston, G. A. R. GABA_A Receptor Pharmacology. *Pharmacol. Ther.* **1996**, *69* (3), 173–198.
- (14) Sieghart, W. Chapter Three - Allosteric Modulation of GABA_A Receptors via Multiple Drug-Binding Sites. In *Advances in Pharmacology*; Rudolph, U., Ed.; Diversity and Functions of GABA Receptors: A Tribute to Hanns Möhler, Part A; Academic Press (Cambridge, USA), 2015; Vol. 72, pp 53–96.
- (15) Olsen, R. W. GABA_A Receptor: Positive and Negative Allosteric Modulators. *Neuropharmacology* **2018**, *136*, 10–22.
- (16) Miller, P. S.; Scott, S.; Masiulis, S.; De Colibus, L.; Pardon, E.; Steyaert, J.; Aricescu, A. R. Structural Basis for GABA_A Receptor Potentiation by Neurosteroids. *Nat. Struct. Mol. Biol.* **2017**, *24* (11), 986–992.
- (17) Sigel, E.; Buhr, A. The Benzodiazepine Binding Site of GABA_A Receptors. *Trends Pharmacol. Sci.* **1997**, *18* (11), 425–429.
- (18) Scott, S.; Aricescu, A. R. A Structural Perspective on GABA_A Receptor Pharmacology. *Curr. Opin. Struct. Biol.* **2019**, *54*, 189–197.
- (19) Zhu, S.; Noviello, C. M.; Teng, J.; Walsh Jr, R. M.; Kim, J. J.; Hibbs, R.E. Structure of a Human Synaptic GABA A Receptor. *Nature* **2018**, *559*, 67–72.
- (20) Sieghart, W. Structure and Pharmacology of Gamma-Aminobutyric Acid A Receptor Subtypes. *Pharmacol. Rev.* **1995**, *47* (2), 181–234.
- (21) Solomon, V. R.; Tallapragada, V. J.; Chebib, M.; Johnston, G. A. R.; Hanrahan, J. R. GABA Allosteric Modulators: An Overview of Recent Developments in Non-Benzodiazepine Modulators. *Eur. J. Med. Chem.* **2019**, *171*, 434–461.

- (22) Naffaa, M. M.; Hung, S.; Chebib, M.; Johnston, G. A. R.; Hanrahan, J. R. GABA- ρ Receptors: Distinctive Functions and Molecular Pharmacology. *Br. J. Pharmacol.* **2017**, *174* (13), 1881–1894.
- (23) Sternbach, L. H. The Benzodiazepine Story. *J. Med. Chem.* **1979**, *22* (1), 1–7.
- (24) Rudolph, U.; Möhler, H. GABA-Based Therapeutic Approaches: GABA_A Receptor Subtype Functions. *Curr. Opin. Pharmacol.* **2006**, *6* (1), 18–23.
- (25) Hevers, W.; Lüddens, H. The Diversity of GABA_A Receptors. Pharmacological and Electrophysiological Properties of GABA_A Channel Subtypes. *Mol. Neurobiol.* **1998**, *18* (1), 35–86.
- (26) Rudolph, U.; Crestani, F.; Benke, D.; Benson, J. A.; Fritschy, J.-M.; Martin, J. R.; Bluethmann, H.; Möhler, H. Benzodiazepine Actions Mediated by Specific γ -Aminobutyric Acid A Receptor Subtypes. *Nature* **1999**, *401*, 796–800.
- (27) Löw, K.; Crestani, F.; Keist, R.; Benke, D.; Brünig, I.; Benson, J. A.; Fritschy, J.-M.; Rüllicke, T.; Bluethmann, H.; Möhler, H.; Rudolph, U. Molecular and Neuronal Substrate for the Selective Attenuation of Anxiety. *Science* **2000**, *290* (5489), 131–134.
- (28) Atack, J. R.; J. R.; Hutson, P. H.; Collinson, N.; Marshall, G.; Bentley, G.; Moyes, C.; Cook, S. M.; Collins, I.; Wafford, K.; McKernan, R. M.; Dawson, G.R. Anxiogenic Properties of an Inverse Agonist Selective for α 3 Subunit-Containing GABA_A Receptors. *Br. J. Pharmacol.* **2005**, *144* (3), 357–366.
- (29) Fritschy, J.M.; Mohler, H. GABA_A-receptor Heterogeneity in the Adult Rat Brain: Differential Regional and Cellular Distribution of Seven Major Subunits. *J. Comp. Neurol.* **1995**, *359* (1), 154–194.
- (30) Crestani, F.; Keist, R.; Fritschy, J.-M.; Benke, D.; Vogt, K.; Prut, L.; Blüthmann, H.; Möhler, H.; Rudolph, U. Trace Fear Conditioning Involves Hippocampal α 5 GABA_A Receptors. *Proc. Natl. Acad. Sci.* **2002**, *99* (13), 8980–8985.

- (31) Cheng, T.; Wallace, D. M.; Ponteri, B.; Tuli, M. Valium without Dependence? Individual GABA_A Receptor Subtype Contribution toward Benzodiazepine Addiction, Tolerance, and Therapeutic Effects. *Neuropsychiatr. Dis. Treat.* **2018**, *14*, 1351–1361.
- (32) Evans, A. K.; Lowry, C. A. Pharmacology of the β -Carboline FG-7142, a Partial Inverse Agonist at the Benzodiazepine Allosteric Site of the GABA_A Receptor: Neurochemical, Neurophysiological, and Behavioral Effects. *CNS Drug Rev.* **2007**, *13* (4), 475–501.
- (33) Gill, K. M.; Grace, A. A. The Role of α 5 GABA_A Receptor Agonists in the Treatment of Cognitive Deficits in Schizophrenia. *Curr. Pharm. Des.* **2014**, *20* (31), 5069–5076.
- (34) Neutel, C. I. Risk of Traffic Accident Injury after a Prescription for a Benzodiazepine. *Ann. Epidemiol.* **1995**, *5* (3), 239–244.
- (35) Engeland, A.; Skurtveit, S.; Mørland, J. Risk of Road Traffic Accidents Associated With the Prescription of Drugs: A Registry-Based Cohort Study. *Ann. Epidemiol.* **2007**, *17* (8), 597–602.
- (36) Cumming, R. G.; Conteur, D. G. L. Benzodiazepines and Risk of Hip Fractures in Older People. *CNS Drugs* **2003**, *17* (11), 825–837.
- (37) Madhusoodanan, S.; Bogunovic, O. J. Safety of Benzodiazepines in the Geriatric Population. *Expert Opin. Drug Saf.* **2004**, *3* (5), 485–493.
- (38) Skolnick, P. Anxiolytic: On a Quest for the Holy Grail. *Trends Pharmacol. Sci.* **2012**, *33* (11), 611–620.
- (39) Atack, J. R. GABA_A Receptor Subtype-Selective Modulators. I. α 2/ α 3-Selective Agonists as Non-Sedating Anxiolytics. *Curr. Top. Med. Chem.* **2011**, *11* (9), 1176–1202.
- (40) McKernan, R. M.; Rosahl, T. W.; Reynolds, D. S.; Sur, C.; Wafford, K. A.; Atack, J. R.; Farrar, S.; Myers, J.; Cook, G.; Ferris, P.; Garrett, L.; Bristow, L.; Marshall, G.; Macaulay, A.; Brown, N.; Howell, O.; Moore, K.W.; Carling, R.W.; Street, L.J.; Castro, J.L.; Ragan, C.I.; Dawson, G.R.; Whiting, P.J. Sedative but Not Anxiolytic Properties of Benzodiazepines Are Mediated by the GABA A Receptor α 1 Subtype. *Nat. Neurosci.* **2000**, *3* (6), 587–592.
- (41) Gurrell, R.; Dua, P.; Feng, G.; Sudworth, M.; Whitlock, M.; Reynolds, D.; Butt, R. A Randomised, Placebo-Controlled Clinical Trial with the α 2/3/5 Subunit Selective GABA_A

- Positive Allosteric Modulator PF-06372865 in Patients with Chronic Low Back Pain. *Pain* **2018**, *159* (9), 1742–1751.
- (42) Gurrell, R.; Gorman, D.; Whitlock, M.; Ogden, A.; Reynolds, D. S.; DiVentura, B.; Abou-Khalil, B.; Gelfand, M.; Pollard, J.; Hogan, R. E.; Krauss, G.; Sperling, M.; Vazquez, B.; Wechsler, R.T.; Friedman, D.; Butt, R.P.; French, J. Photosensitive Epilepsy: Robust Clinical Efficacy of a Selective GABA Potentiator. *Neurology* **2019**, *92* (15), 1786–1795.
- (43) Di, L.; Whitney-Pickett, C.; Umland, J. P.; Zhang, H.; Zhang, X.; Gebhard, D. F.; Lai, Y.; Federico, J. J.; Davidson, R. E.; Smith, R.; Reyner, E. L.; Lee, C.; Feng, B.; Rotter, C.; Varma, M. V.; Kempshall, S.; Fenner, K.; El-Kattan, A. F.; Liston, T. E.; Troutman, M. D. Development of a New Permeability Assay Using Low-efflux MDCKII Cells. *J. Pharm. Sci.* **2011**, *100* (11), 4974–4985.
- (44) Owen, R. M.; Blakemore, D.; Cao, L.; Flanagan, N.; Fish, R.; Gibson, K. R.; Gurrell, R.; Huh, C. W.; Kammonen, J.; Mortimer-Cassen, E.; Nickolls, S.a.; Omoto, K.; Owen, D.; Pike, A.; Pryde, D.C.; Reynolds, D.S.; Roeloffs, R.; Rose, C.; Stead, C.; Takeuchi, M.; Warmus, J.S.; Watsonet, C. Design and Identification of a Novel, Functionally Subtype Selective GABA_A Positive Allosteric Modulator (PF-06372865). *J. Med. Chem.* **2019**, *62* (12), 5773–5796.
- (45) Nickolls, S. A.; Gurrell, R.; Amerongen, G. van; Kammonen, J.; Cao, L.; Brown, A. R.; Stead, C.; Mead, A.; Watson, C.; Hsu, C.; Owen, R.M.; Pike, A.; Fish, R.L.; Chen, L.; Qiu, R.; Morris, E.D.; Feng, G.; Whitlock, M.; Gorman, D.; van Gerven, J.; Reynolds, D.S.; Dua, P.; Butt, R.P. Pharmacology in Translation: The Preclinical and Early Clinical Profile of the Novel α 2/3 Functionally Selective GABA_A Receptor Positive Allosteric Modulator PF-06372865. *Br. J. Pharmacol.* **2018**, *175* (4), 708–725.
- (46) Nickolls, S.; Mace, H.; Fish, R.; Edye, M.; Gurrell, R.; Ivarsson, M.; Pitcher, T.; Tanimoto-Mori, S.; Richardson, D.; Sweatman, C.; Nicholson, J. Ward, C.; Jinks, J.; Bell, C.; Young, K.; Rees, H.; Mos, A.; Kinloch, R.; McMurray, G. A Comparison of the α 2/3/5 Selective Positive Allosteric Modulators L-838,417 and TPA023 in Preclinical Models of Inflammatory and Neuropathic Pain. *Adv. Pharmacol. Sci.* **2011**, 608912.

- (47) Christian, E. P.; Snyder, D. H.; Song, W.; Gurley, D. A.; Smolka, J.; Maier, D. L.; Ding, M.; Gharahdaghi, F.; Liu, X. F.; Chopra, M.; Ribadeneira, M.; Chapdelaine, M.J.; Dudley, A.; Arriza, J.L.; Maciag, C.; Quirk, M.C.; Doherty, J.J. EEG- β/γ Spectral Power Elevation in Rat: A Translatable Biomarker Elicited by GABA_A $\alpha 2/3$ -Positive Allosteric Modulators at Nonsedating Anxiolytic Doses. *J. Neurophysiol.* **2015**, *113* (1), 116–131.
- (48) Duveau, V.; Buhl, D. L.; Evrard, A.; Ruggiero, C.; Mandé-Niedergang, B.; Roucard, C.; Gurrell, R. Pronounced Antiepileptic Activity of the Subtype-Selective GABA_A-Positive Allosteric Modulator PF-06372865 in the GAERS Absence Epilepsy Model. *CNS Neurosci. Ther.* **2019**, *25* (2), 255–260.
- (49) Simen, A.; Whitlock, M.; Qiu, R.; Miceli, J.; Zumpano, L.; Metz, M. D.; Dua, P.; Binneman, B. An 8-Week, Randomized, Phase 2, Double-Blind, Sequential Parallel-Group Comparison Study of Two Dose Levels of the GABA_A 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.* **2019**, *39* (1), 20–27.
- (50) van Amerongen, G.; Siebenga, P. S.; Gurrell, R.; Dua, P.; Whitlock, M.; Gorman, D.; Okkerse, P.; Hay, J. L.; Butt, R. P.; Groeneveld, G. J. Analgesic Potential of PF-06372865, an $\alpha 2/\alpha 3/\alpha 5$ Subtype-Selective GABA_A Partial Agonist, in Humans. *Br. J. Anaesth.* **2019**, *123* (2), 194–203.
- (51) Carling, R. W.; Madin, A.; Guiblin, A.; Russell, M. G. N.; Moore, K. W.; Mitchinson, A.; Sohal, B.; Pike, A.; Cook, S. M.; Ragan, I. C.; McKernan, R.M., Quirk, K.; Ferris, P.; Marshall, G.; Thompson, S.A.; Wafford, K.A.; Dawson, G.R.; Atack, J.R.; Harrison, T.; Castro, J.L.; Street, L.J. 7-(1,1-Dimethylethyl)-6-(2-Ethyl-2H-1,2,4-Triazol-3-Yl-methoxy)-3-(2-Fluorophenyl)-1,2,4-Triazolo[4,3-b]Pyridazine: A Functionally Selective γ -Aminobutyric Acid A (GABA_A) $\alpha 2/\alpha 3$ -Subtype Selective Agonist That Exhibits Potent Anxiolytic Activity but Is Not Sedating in Animal Models. *J. Med. Chem.* **2005**, *48* (23), 7089–7092.
- (52) Rowlett, J. K.; Platt, D. M.; Lelas, S.; Atack, J. R.; Dawson, G. R. Different GABA_A Receptor Subtypes Mediate the Anxiolytic, Abuse-Related, and Motor Effects of Benzodiazepine-Like Drugs in Primates. *Proc. Natl. Acad. Sci.* **2005**, *102* (3), 915–920.

- (53) Scott-Stevens, P.; Atack, J. R.; Sohal, B.; Worboys, P. Rodent Pharmacokinetics and Receptor Occupancy of the GABA_A Receptor Subtype Selective Benzodiazepine Site Ligand L-838417. *Biopharm. Drug Dispos.* **2005**, *26* (1), 13–20.
- (54) Atack, J.; Wafford, K.; Street, L.; Dawson, G.; Tye, S.; Van Laere, K.; Bormans, G.; Sanabria-Bohórquez, S.; De Lepeleire, I.; de Hoon, J.N.; Van Hecken, A.; Burns, H.D.; McKernan, R.M.; Murphy, M.G.; Hargreaves, R.J MRK-409 (MK-0343), a GABA A Receptor Subtype-Selective Partial Agonist, Is a Non-Sedating Anxiolytic in Preclinical Species but Causes Sedation in Humans. *J. Psychopharmacol. (Oxf.)* **2011**, *25* (3), 314–328.
- (55) de Haas, S. L.; de Visser, S. J.; van der Post, J. P.; Schoemaker, R. C.; van Dyck, K.; Murphy, M. G.; de Smet, M.; Vessey, L. K.; Ramakrishnan, R.; Xue, L.; Cohen, A.F.; van Gerven, J.M. Pharmacodynamic and Pharmacokinetic Effects of MK-0343, a GABA_A α 2,3 Subtype Selective Agonist, Compared to Lorazepam and Placebo in Healthy Male Volunteers. *J. Psychopharmacol. (Oxf.)* **2008**, *22* (1), 24–32.
- (56) Van Steveninck, A.L.; Gieschke, R.; Schoemaker, R. C.; Roncari, G.; Tuk, B.; Pieters, M. S. M.; Breimer, D. D.; Cohen, A. F. Pharmacokinetic and Pharmacodynamic Interactions of Bretazenil and Diazepam with Alcohol. *Br. J. Clin. Pharmacol.* **1996**, *41* (6), 565–573.
- (57) Atack, J. R. Subtype-Selective GABA_A Receptor Modulation Yields a Novel Pharmacological Profile: The Design and Development of TPA023. In *Advances in Pharmacology*; Enna, S. J., Williams, M., Eds.; Contemporary Aspects of Biomedical Research; Academic Press (Cambridge, USA), 2009; Vol. 57, pp 137–185.
- (58) Atack, J. R.; Wong, D. F.; Fryer, T. D.; Ryan, C.; Sanabria, S.; Zhou, Y.; Dannals, R. F.; Eng, W.; Gibson, R. E.; Burns, H. D.; Vega, J.M.; Vessy, L.; Scott-Stevens, P.; Beech, J.S.; Baron, J.C.; Sohal, B.; Schrag, M.L.; Aigbirhio, F.I.; McKernan, R.M.; Hargreaves, R.J. Benzodiazepine Binding Site Occupancy by the Novel GABA_A Receptor Subtype-Selective Drug 7-(1,1-Dimethylethyl)-6-(2-Ethyl-2H-1,2,4-Triazol-3-Yl-methoxy)-3-(2-Fluorophenyl)-1,2,4-Triazolo[4,3-b]Pyridazine (TPA023) in Rats, Primates, and Humans. *J. Pharmacol. Exp. Ther.* **2010**, *332* (1), 17–25.

- (59) 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-2H-1,2,4-Triazol-3-Yl-methoxy)-3-(2-Fluorophenyl)-1,2,4-Triazolo[4,3-b]Pyridazine], an Agonist Selective for α 2- and α 3-Containing GABAA Receptors, Is a Nonsedating Anxiolytic in Rodents and Primates. *J. Pharmacol. Exp. Ther.* **2006**, *316* (1), 410–422.
- (60) Atack, J. R. GABA_A Receptor Subtype-Selective Efficacy: TPA023, an α 2/ α 3 Selective Non-Sedating Anxiolytic and α 5IA, an α 5 Selective Cognition Enhancer. *CNS Neurosci. Ther.* **2008**, *14* (1), 25–35.
- (61) de Haas, S. L.; de Visser, S. J.; van der Post, J. P.; de Smet, M.; Schoemaker, R. C.; Rijnbeek, B.; Cohen, A. F.; Vega, J. M.; Agrawal, N. G. B.; Goel, T. V.; Simpson, R.C.; Pearson, L.K.; Li, S.; Hesney, M.; Murphy, M.G.; van Gerven, J.M. Pharmacodynamic and Pharmacokinetic Effects of TPA023, a GABAA α 2,3 Subtype-Selective Agonist, Compared to Lorazepam and Placebo in Healthy Volunteers. *J. Psychopharmacol. (Oxf.)* **2007**, *21* (4), 374–383.
- (62) de Visser, S. J.; van der Post, J. P.; de Waal, P. P.; Cornet, F.; Cohen, A. F.; van Gerven, J. M. A. Biomarkers for the Effects of Benzodiazepines in Healthy Volunteers. *Br. J. Clin. Pharmacol.* **2003**, *55* (1), 39–50.
- (63) Lewis, D. A.; Cho, R. Y.; Carter, C. S.; Eklund, K.; Forster, S.; Kelly, M. A.; Montrose, D. Subunit-Selective Modulation of GABA Type A Receptor Neurotransmission and Cognition in Schizophrenia. *Am. J. Psychiatry* **2008**, *165* (12), 1585–1593.
- (64) Buchanan, R. W.; Keefe, R. S. E.; Lieberman, J. A.; Barch, D. M.; Csernansky, J. G.; Goff, D. C.; Gold, J. M.; Green, M. F.; Jarskog, L. F.; Javitt, D. C.; Kimhy, D.; Kraus, M.S.; McEvoy, J.P.; Mesholam-Gately, R.I.; Seidman, L.J.; Ball, M.P.; McMahon, R.P.; Kern, R.S.; Robinson, J.; Marder, S.R. A Randomized Clinical Trial of MK-0777 for the Treatment of Cognitive Impairments in People with Schizophrenia. *Biol. Psychiatry* **2011**, *69* (5), 442–449.
- (65) de Haas, S.; Zoethout, R.; Van Dyck, K.; De Smet, M.; Rosen, L.; Murphy, M.; Gottesdiener, K.; Schoemaker, R.; Cohen, A.; van Gerven, J. The Effects of TPA023, a GABA_A α 2,3 Subtype-

- Selective Partial Agonist, on Essential Tremor in Comparison to Alcohol. *J. Psychopharmacol. (Oxf.)* **2012**, 26 (2), 282–291.
- (66) Teuber, L.; Waetjen, F.; Fukuda, Y.; Ichimaru, Y. Benzimidazole Compounds, Pharmaceutical Compositions Containing the Compounds and Their Use. WO9919323 (A1), Apr 22, 1999.
- (67) Russell, M. G. N.; Carling, R. W.; Street, L. J.; Hallett, D. J.; Goodacre, S.; Mezzogori, E.; Reader, M.; Cook, S. M.; Bromidge, F. A.; Newman, R.; Smith, A.J.; Wafford, K.A.; Marshall, G.R.; Reynolds, D.S.; Dias, R.; Ferris, P.; Stanley, J.; Lincoln, R.; Tye, S.J.; Sheppard, W.F.; Sohal, B.; Pike, A.; Dominguez, M.; Atack, J.R.; Castro, J.L. Discovery of Imidazo[1,2-b][1,2,4]Triazines as GABA_A α 2/3 Subtype Selective Agonists for the Treatment of Anxiety. *J. Med. Chem.* **2006**, 49 (4), 1235–1238.
- (68) Atack, J.; Hallett, D.; Tye, S.; Wafford, K.; Ryan, C.; Sanabria-Bohórquez, S.; Eng, W.; Gibson, R.; Burns, H.; Dawson, G.; Carling, R.W.; Street, L.J.; Pike, A.; De Lepeleire, I.; Van Laere, K.; Bormans, G.; de Hoon, J.N.; Van Hecken, A.; McKernan, R.M.; Murphy, M.G.; Hargreaves, R.J.; Preclinical and Clinical Pharmacology of TPA023B, a GABA A Receptor α 2/ α 3 Subtype-Selective Partial Agonist. *J. Psychopharmacol. (Oxf.)* **2011**, 25 (3), 329–344.
- (69) Van Laere, K.; Bormans, G.; Sanabria-Bohórquez, S. M.; de Groot, T.; Dupont, P.; De Lepeleire, I.; de Hoon, J.; Mortelmans, L.; Hargreaves, R. J.; Atack, J. R.; Burns, H. D.; In Vivo Characterization and Dynamic Receptor Occupancy Imaging of TPA023B, an α 2/ α 3/ α 5 Subtype Selective γ -Aminobutyric Acid–A Partial Agonist. *Biol. Psychiatry* **2008**, 64 (2), 153–161.
- (70) Ralvenius, W. T.; Neumann, E.; Pagani, M.; Acuña, M. A.; Wildner, H.; Benke, D.; Fischer, N.; Rostaher, A.; Schwager, S.; Detmar, M.; Frauenknecht, K.; Aguzzi, A.; Hubbs, J. L.; Rudolph, U.; Favrot, C.; Zeilhofer, H. U. Itch Suppression in Mice and Dogs by Modulation of Spinal A2 and α 3-GABA A Receptors. *Nat. Commun.* **2018**, 9 (1), 3230.
- (71) Patel, J. B.; Meiners, B. A.; Salama, A. I.; Malick, J. B.; Resch, J. F.; U'Prichard, D. C.; Giles, R. E.; Hesp, B.; Goldberg, M. E. A Novel Potent Non-Benzodiazepine Anxiolytic Agent with Reduced Dependence Liability: ICI 198, 256. *Prog. Clin. Biol. Res.* **1990**, 361, 483–488.

- (72) Alhambra, C.; Becker, C.; Blake, T.; Chang, A. (Hui-F.); Damewood, J. R.; Daniels, T.; Dembofsky, B. T.; Gurley, D. A.; Hall, J. E.; Herzog, K. J.; Horchler, C. L.; Ohnmacht, C. J.; Schmiesing, R. J.; Dudley, A.; Ribadeneira, M. D.; Knappenberger, K. S.; Maciag, C.; Stein, M. M.; Chopra, M.; Liu, X. F.; Christian, E. P.; Arriza, J. L.; Chapdelaine, M. J. Development and SAR of Functionally Selective Allosteric Modulators of GABA_A Receptors. *Bioorg. Med. Chem.* **2011**, *19* (9), 2927–2938.
- (73) Liu, X. F.; Chang, H.-F.; Schmiesing, R. J.; Wesolowski, S. S.; Knappenberger, K. S.; Arriza, J. L.; Chapdelaine, M. J. Developing Dual Functional Allosteric Modulators of GABA_A Receptors. *Bioorg. Med. Chem.* **2010**, *18* (23), 8374–8382.
- (74) Hines, R. M.; Maric, H. M.; Hines, D. J.; Modgil, A.; Panzanelli, P.; Nakamura, Y.; Nathanson, A. J.; Cross, A.; Deeb, T.; Brandon, N. J.; Davies, P.; Fritschy, J. M.; Schindelin, H.; Moss, S. J. Developmental Seizures and Mortality Result from Reducing GABA_A Receptor A2-Subunit Interaction with Collybistin. *Nat. Commun.* **2018**, *9*, 3130.
- (75) Nomura, T.; Hawkins, N. A.; Kearney, J. A.; George, A. L.; Contractor, A. Potentiating α 2 Subunit Containing Perisomatic GABA_A Receptors Protects against Seizures in a Mouse Model of Dravet Syndrome. *J. Physiol.* **2019**, *597* (16), 4293–4307.
- (76) Saito, A.; Taniguchi, Y.; Rannals, M. D.; Merfeld, E. B.; Ballinger, M. D.; Koga, M.; Ohtani, Y.; Gurley, D. A.; Sedlak, T. W.; Cross, A.; Moss, S. J.; Brandon, N. J.; Maher, B. J.; Kamiya, A. Early Postnatal GABA_A Receptor Modulation Reverses Deficits in Neuronal Maturation in a Conditional Neurodevelopmental Mouse Model of DISC1. *Mol. Psychiatry* **2016**, *21* (10), 1449–1459.
- (77) Zhou, D.; Sunzel, M.; Ribadeneira, M. D.; Smith, M. A.; Desai, D.; Lin, J.; Grimm, S. W. A Clinical Study to Assess CYP1A2 and CYP3A4 Induction by AZD7325, a Selective GABA_A Receptor Modulator – an in Vitro and in Vivo Comparison. *Br. J. Clin. Pharmacol.* **2012**, *74* (1), 98–108.
- (78) Jucaite, A.; Cselényi, Z.; Lappalainen, J.; McCarthy, D. J.; Lee, C.-M.; Nyberg, S.; Varnäs, K.; Stenkrona, P.; Halldin, C.; Cross, A.; Farde, L. GABA_A Receptor Occupancy by Subtype

- Selective GABA α 2,3 Modulators: PET Studies in Humans. *Psychopharmacology (Berl.)* **2017**, 234 (4), 707–716.
- (79) Chen, X.; Jacobs, G.; de Kam, M.; Jaeger, J.; Lappalainen, J.; Maruff, P.; Smith, M. A.; Cross, A. J.; Cohen, A.; van Gerven, J. The Central Nervous System Effects of the Partial GABA- α 2,3-Selective Receptor Modulator AZD7325 in Comparison with Lorazepam in Healthy Males. *Br. J. Clin. Pharmacol.* **2014**, 78 (6), 1298–1314.
- (80) AZD7325 | GABA A alpha 2 & 3 positive modulator | CNS | AstraZeneca Open Innovation <https://openinnovation.astrazeneca.com/azd7325.html> (accessed Jun 13, 2019).
- (81) Chen, X.; Jacobs, G.; de Kam, M. L.; Jaeger, J.; Lappalainen, J.; Maruff, P.; Smith, M. A.; Cross, A. J.; Cohen, A.; van Gerven, J. AZD6280, a Novel Partial γ -Aminobutyric Acid A Receptor Modulator, Demonstrates a Pharmacodynamically Selective Effect Profile in Healthy Male Volunteers. *J. Clin. Psychopharmacol.* **2015**, 35 (1), 22-33.
- (82) Te Beek, E. T.; Chen, X.; Jacobs, G. E.; Nahon, K. J.; de Kam, M. L.; Lappalainen, J.; Cross, A. J.; van Gerven, J. M. A.; Hay, J. L. The Effects of the Nonselective Benzodiazepine Lorazepam and the α 2/ α 3 Subunit-Selective GABA $_A$ Receptor Modulators AZD7325 and AZD6280 on Plasma Prolactin Levels. *Clin. Pharmacol. Drug Dev.* **2015**, 4 (2), 149–154.
- (83) Mirza, N. R.; Larsen, J. S.; Mathiasen, C.; Jacobsen, T. A.; Munro, G.; Erichsen, H. K.; Nielsen, A. N.; Troelsen, K. B.; Nielsen, E. Ø.; Ahring, P. K. NS11394 [3'-(5-(1-Hydroxy-1-Methyl-Ethyl)-Benzoimidazol-1-Yl)-Biphenyl-2-Carbonitrile], a Unique Subtype-Selective GABA $_A$ Receptor Positive Allosteric Modulator: In Vitro Actions, Pharmacokinetic Properties and in Vivo Anxiolytic Efficacy. *J. Pharmacol. Exp. Ther.* **2008**, 327 (3), 954–968.
- (84) Knabl, J.; Witschi, R.; Hösl, K.; Reinold, H.; Zeilhofer, U. B.; Ahmadi, S.; Brockhaus, J.; Sergejeva, M.; Hess, A.; Brune, K.; Fritschy, J.M.; Rudolph, U.; Möhler, H.; Zeilhofer, H.U. Reversal of Pathological Pain through Specific Spinal GABA $_A$ Receptor Subtypes. *Nature* **2008**, 451 (7176), 330–334.
- (85) Knabl, J.; Zeilhofer, U.; Crestani, F.; Rudolph, U.; Zeilhofer, H. Genuine Antihyperalgesia by Systemic Diazepam Revealed by Experiments in GABA $_A$ Receptor Point-Mutated Mice. *Pain* **2009**, 141 (3), 233–238.

- (86) Ralvenius, W. T.; Benke, D.; Acuña, M. A.; Rudolph, U.; Zeilhofer, H. U. Analgesia and Unwanted Benzodiazepine Effects in Point-Mutated Mice Expressing Only One Benzodiazepine-Sensitive GABA_A Receptor Subtype. *Nat. Commun.* **2015**, *6*, 6803.
- (87) Munro, G.; Lopez-Garcia, J. A.; Rivera-Arconada, I.; Erichsen, H. K.; Nielsen, E. Ø.; Larsen, J. S.; Ahring, P. K.; Mirza, N. R. Comparison of the Novel Subtype-Selective GABA_A Receptor-Positive Allosteric Modulator NS11394 [3'-[5-(1-Hydroxy-1-Methyl-Ethyl)-Benzoimidazol-1-Yl]-Biphenyl-2-Carbonitrile] with Diazepam, Zolpidem, Bretazenil, and Gaboxadol in Rat Models of Inflammatory and Neuropathic Pain. *J. Pharmacol. Exp. Ther.* **2008**, *327* (3), 969–981.
- (88) Spröte, C.; Richter, F.; Bauer, A.; Gerstenberger, J.; Richter, A. The $\alpha 2\beta 3\gamma 2$ GABA_A Receptor Preferring Agonist NS11394 Aggravates Dystonia in the Phenotypic Dtsz Model. *Eur. J. Pharmacol.* **2016**, *791*, 655–658.
- (89) Hofmann, M.; Kordás, K.; Gravius, A.; Bölcskei, K.; Parsons, C.; Dekundy, A.; Danysz, W.; Dézsi, L.; Wittko-Schneider, I.; Sághy, K.; Gyertyán, I.; Horváth C. Assessment of the Effects of NS11394 and L-838417, $\alpha 2/3$ Subunit-Selective GABA_A Receptor-Positive Allosteric Modulators, in Tests for Pain, Anxiety, Memory and Motor Function. *Behav. Pharmacol.* **2012**, *23* (8), 790–801.
- (90) de Lucas, A. G.; Ahring, P. K.; Larsen, J. S.; Rivera-Arconada, I.; Lopez-Garcia, J. A.; Mirza, N. R.; Munro, G. GABA_A $\alpha 5$ Subunit-Containing Receptors Do Not Contribute to Reversal of Inflammatory-Induced Spinal Sensitization as Indicated by the Unique Selectivity Profile of the GABA_A Receptor Allosteric Modulator NS16085. *Biochem. Pharmacol.* **2015**, *93* (3), 370–379.
- (91) Zuiker, R. G.; Chen, X.; Østerberg, O.; Mirza, N. R.; Muglia, P.; de Kam, M.; Klaassen, E. S.; van Gerven, J. M. NS11821, a Partial Subtype-Selective GABA_A Agonist, Elicits Selective Effects on the Central Nervous System in Randomized Controlled Trial with Healthy Subjects. *J. Psychopharmacol. (Oxf.)* **2016**, *30* (3), 253–262.
- (92) Rivas, F. M.; Stables, J. P.; Murphree, L.; Edwankar, R. V.; Edwankar, C. R.; Huang, S.; Jain, H. D.; Zhou, H.; Majumder, S.; Sankar, S.; Roth, B.L.; Ramerstorfer, J.; Furtmüller, R.; Sieghart,

- W.; Cook, J.M. Antiseizure Activity of Novel γ -Aminobutyric Acid (A) Receptor Subtype-Selective Benzodiazepine Analogues in Mice and Rat Models. *J. Med. Chem.* **2009**, *52* (7), 1795–1798.
- (93) Fischer, B. D.; Licata, S. C.; Edwankar, R. V.; Wang, Z.-J.; Huang, S.; He, X.; Yu, J.; Zhou, H.; Johnson, E. M.; Cook, J. M.; Furtmüller, R.; Ramerstorfer, J.; Sieghart, W.; Roth, B.L.; Majumder, S.; Rowlett, J.K. Anxiolytic-like Effects of 8-Acetylene Imidazobenzodiazepines in a Rhesus Monkey Conflict Procedure. *Neuropharmacology* **2010**, *59* (7–8), 612–618.
- (94) Savić, M. M.; Majumder, S.; Huang, S.; Edwankar, R. V.; Furtmüller, R.; Joksimović, S.; Clayton, T.; Ramerstorfer, J.; Milinković, M. M.; Roth, B. L.; Sieghart, W.; Cook, J.M. Novel Positive Allosteric Modulators of GABA_A Receptors: Do Subtle Differences in Activity at $\alpha 1$ plus $\alpha 5$ versus $\alpha 2$ plus $\alpha 3$ Subunits Account for Dissimilarities in Behavioral Effects in Rats? *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2010**, *34* (2), 376–386.
- (95) Fischer, B.D.; Schlitt, R.J.; Hamade, B.Z.; Rehman, S.; Ernst, M.; Poe, M. M.; Li, G.; Kodali, R.; Arnold, L. A.; Cook, J. M. Pharmacological and Antihyperalgesic Properties of the Novel $\alpha 2/3$ Preferring GABA_A Receptor Ligand MP-III-024. *Brain Res Bull.* **2017**, *131*, 62–69.
- (96) Namjoshi, O. A.; Wang, Z.; Rallapalli, S. K.; Johnson, E. M.; Johnson, Y.-T.; Ng, H.; Ramerstorfer, J.; Varagic, Z.; Sieghart, W.; Majumder, S.; Roth, B. L.; Rowlett, J. K.; Cook, J. M. Search for $\alpha 3\beta 2/3\gamma 2$ Subtype Selective Ligands That Are Stable on Human Liver Microsomes. *Bioorg. Med. Chem.* **2013**, *21* (1), 93–101.
- (97) Poe, M. M.; Methuku, K. R.; Li, G.; Verma, A. R.; Teske, K. A.; Stafford, D. C.; Arnold, L. A.; Cramer, J. W.; Jones, T. M.; Cerne, R.; Krambis, M. J.; Witkin, J. M.; Jambrina, E.; Rehman, S.; Ernst, M.; Cook, J. M.; Schkeryantz, J. M. Synthesis and Characterization of a Novel γ -Aminobutyric Acid Type A (GABA_A) Receptor Ligand That Combines Outstanding Metabolic Stability, Pharmacokinetics, and Anxiolytic Efficacy. *J. Med. Chem.* **2016**, *59* (23), 10800–10806.
- (98) Witkin, J. M.; Smith, J. L.; Ping, X.; Gleason, S. D.; Poe, M. M.; Li, G.; Jin, X.; Hobbs, J.; Schkeryantz, J. M.; McDermott, J. S.; Alatorre, A. I.; Siemian, J. N.; Cramer, J. W.; Airey, D.

- C.; Methuku, K. R.; Tiruveedhula, V. Jones, T. M.; Crawford, J.; Krambis, M. J.; Fisher, J. L.; Cook, J. M.; Cerne, R. 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 GABA_A Receptors: Improved Bioavailability Enhances Anticonvulsant Efficacy. *Neuropharmacology* **2018**, *137*, 332–343.
- (99) Lewter, L. A.; Fisher, J. L.; Siemian, J. N.; Methuku, K. R.; Poe, M. M.; Cook, J. M.; Li, J.-X. Antinociceptive Effects of a Novel α 2/ α 3-Subtype Selective GABA_A Receptor Positive Allosteric Modulator. *ACS Chem. Neurosci.* **2017**, *8* (6), 1305–1312.
- (100) Witkin, J. M.; Cerne, R.; Davis, P. G.; Freeman, K. B.; do Carmo, J. M.; Rowlett, J. K.; Methuku, K. R.; Okun, A.; Gleason, S. D.; Li, X.; Krambis, M. J.; Poe, M.; Li, G.; Schkeryantz, J. M.; Jahan, R.; Yang, L.; Guo, W.; Golani, L. K.; Anderson, W. H.; Catlow, J. T.; Jones, T. M.; Porreca, F.; Smith, J. L.; Knopp, K. L.; Cook, J. M. The α 2,3-Selective Potentiator of GABA_A Receptors, KRM-II-81, Reduces Nociceptive-Associated Behaviors Induced by Formalin and Spinal Nerve Ligation in Rats. *Pharmacol. Biochem. Behav.* **2019**, *180*, 22–31.
- (101) Methuku, K. R.; Li, X.; Cerne, R.; Gleason, S. D.; Schkeryantz, J. M.; Tiruveedhula, V. V. N. P. B.; Golani, L. K.; Li, G.; Poe, M. M.; Rahman, M. T.; Cook, J. M.; Fisher, J. L.; Witkin, J. M. An Antidepressant-Related Pharmacological Signature for Positive Allosteric Modulators of α 2/3-Containing GABA_A Receptors. *Pharmacol. Biochem. Behav.* **2018**, *170*, 9–13.
- (102) Atack, J. R. GABA_A Receptor Subtype-Selective Modulators. II. α 5-Selective Inverse Agonists for Cognition Enhancement. *Curr. Top. Med. Chem.* **2011**, *11* (9), 1203–1214.
- (103) Koh, M. T.; Rosenzweig-Lipson, S.; Gallagher, M. Selective GABA_A α 5 Positive Allosteric Modulators Improve Cognitive Function in Aged Rats with Memory Impairment. *Neuropharmacology* **2013**, *64* (1), 145–152.
- (104) Engin, E.; Zarnowska, E. D.; Benke, D.; Tsvetkov, E.; Sigal, M.; Keist, R.; Bolshakov, V. Y.; Pearce, R. A.; Rudolph, U. Tonic Inhibitory Control of Dentate Gyrus Granule Cells by α 5-Containing GABA_A Receptors Reduces Memory Interference. *J. Neurosci.* **2015**, *35* (40), 13698–13712.

- (105) Soh, M. S.; Lynch, J. W. Selective Modulators of $\alpha 5$ -Containing GABA_A Receptors and Their Therapeutic Significance. *Curr. Drug Targets* **2015**, *16* (7), 735–746.
- (106) Möhler, H.; Rudolph, U. Disinhibition, an Emerging Pharmacology of Learning and Memory. *Frontiers in Behavioral Neuroscience* **2017**, *11*, 101.
- (107) Lüscher, B. P.; Baur, R.; Goeldner, M.; Sigel, E. Influence of GABA_A Receptor α Subunit Isoforms on the Benzodiazepine Binding Site. *PLoS ONE* **2012**, *7* (7), 42101.
- (108) Quirk, K.; Blurton, P.; Fletcher, S.; Leeson, P.; Tang, F.; Mellilo, D.; Ragan, C. I.; McKernan, R. M. [3H]L-655,708, a Novel Ligand Selective for the Benzodiazepine Site of GABA_A Receptors Which Contain the Alpha 5 Subunit. *Neuropharmacology* **1996**, *35* (9–10), 1331–1335.
- (109) Knust, H.; Achermann, G.; Ballard, T.; Buettelmann, B.; Gasser, R.; Fischer, H.; Hernandez, M.-C.; Knoflach, F.; Koblet, A.; Stadler, H.; Thomas, A. W.; Trube, G.; Waldmeier, P. The Discovery and Unique Pharmacological Profile of RO4938581 and RO4882224 as Potent and Selective GABA_A $\alpha 5$ Inverse Agonists for the Treatment of Cognitive Dysfunction. *Bioorg. Med. Chem. Lett.* **2009**, *19* (20), 5940–5944.
- (110) Ballard, T. M.; Knoflach, F.; Prinssen, E.; Borroni, E.; Vivian, J. A.; Basile, J.; Gasser, R.; Moreau, J.-L.; Wettstein, J. G.; Buettelmann, B.; Knust, H.; Thomas, A. W.; Trube, G.; Hernandez, M. C. RO4938581, a Novel Cognitive Enhancer Acting at GABA_A $\alpha 5$ Subunit-Containing Receptors. *Psychopharmacology (Berl.)* **2009**, *202* (1), 207–223.
- (111) Martínez-Cué, C.; Martínez, P.; Rueda, N.; Vidal, R.; García, S.; Vidal, V.; Corrales, A.; Montero, J. A.; Pazos, Á.; Flórez, J.; Gasser, R.; Thomas, A. W.; Honer, M.; Knoflach, F.; Trejo, J.L.; Wettstein, J. G.; Hernández, M. C. Reducing GABA_A $\alpha 5$ Receptor-Mediated Inhibition Rescues Functional and Neuromorphological Deficits in a Mouse Model of Down Syndrome. *J. Neurosci.* **2013**, *33* (9), 3953–3966.
- (112) Block, A.; Ahmed, Md. M.; Rueda, N.; Hernandez, M.-C.; Martinez-Cué, C.; Gardiner, K. J. The GABA_A $\alpha 5$ -Selective Modulator, RO4938581, Rescues Protein Anomalies in the Ts65Dn Mouse Model of Down Syndrome. *Neuroscience* **2018**, *372*, 192–212.

- (113) Gasser, R.; Hernandez, M.-C.; Thomas, A. Use of Selective GABA_A Alpha 5 Negative Allosteric Modulators for the Treatment of Central Nervous System Conditions. US2012115839 (A1), May 10, 2012.
- (114) pA2 Online <http://www.pa2online.org/abstract/abstract.jsp?abid=32402> (accessed Aug 7, 2019).
- (115) Dawson, G. R.; Maubach, K. A.; Collinson, N.; Cobain, M.; Everitt, B. J.; MacLeod, A. M.; Choudhury, H. I.; McDonald, L. M.; Pillai, G.; Rycroft, W.; Smith, A. J.; Sternfeld, F.; Tattersall, F. D.; Wafford, K. A.; Reynolds, D. S.; Seabrook, G. R.; Atack, J. R. An Inverse Agonist Selective for $\alpha 5$ Subunit-Containing GABA_A Receptors Enhances Cognition. *J. Pharmacol. Exp. Ther.* **2006**, *316* (3), 1335–1345.
- (116) Atack, J. R.; Eng, W.; Gibson, R. E.; Ryan, C.; Francis, B.; Sohal, B.; Dawson, G. R.; Hargreaves, R. J.; Burns, H. D. The Plasma–Occupancy Relationship of the Novel GABA_A Receptor Benzodiazepine Site Ligand, $\alpha 5$ IA, Is Similar in Rats and Primates. *Br. J. Pharmacol.* **2009**, *157* (5), 796–803.
- (117) Steele, R. J.; Morris, R. G. Delay-Dependent Impairment of a Matching-to-Place Task with Chronic and Intrahippocampal Infusion of the NMDA-Antagonist D-AP5. *Hippocampus* **1999**, *9* (2), 118–136.
- (118) Atack, J. R. Preclinical and Clinical Pharmacology of the GABA_A Receptor $\alpha 5$ Subtype-Selective Inverse Agonist $\alpha 5$ IA. *Pharmacol. Ther.* **2010**, *125* (1), 11–26.
- (119) Eng, W.; Atack, J. R.; Bergstrom, M.; Sanabria, S.; Appel, L.; Dawson, G. R.; Sciberras, D.; Hargreaves, R. J.; Langstrom, B.; Burns, H. D. Occupancy of Human Brain GABA_A Receptors by the Novel $\alpha 5$ Subtype-Selective Benzodiazepine Site Inverse Agonist $\alpha 5$ IA as Measured Using [11C]Flumazenil PET Imaging. *Neuropharmacology* **2010**, *59* (7), 635–639.
- (120) Nutt, D. J.; Besson, M.; Wilson, S. J.; Dawson, G. R.; Lingford-Hughes, A. R. Blockade of Alcohol’s Amnestic Activity in Humans by an $\alpha 5$ Subtype Benzodiazepine Receptor Inverse Agonist. *Neuropharmacology* **2007**, *53* (7), 810–820.

- (121) Atack, J. R.; Maubach, K. A.; Wafford, K. A.; O'Connor, D.; Rodrigues, A. D.; Evans, D. C.; Tattersall, F. D.; Chambers, M. S.; MacLeod, A. M.; Eng, W.-S.; Ryan, C.; Hostetler, E.; Sanabria, S. M.; Gibson, R. E.; Krause, S.; Burns, H. D.; Hargreaves, R. J.; Agrawal, N.; McKernan, R. M.; Murphy, M. G.; Gingrich, K.; Dawson, G. R.; Musson, D. G.; Petty, K. J. In Vitro and in Vivo Properties of 3-Tert-Butyl-7-(5-Methylisoxazol-3-Yl)-2-(1-Methyl-1H-1,2,4-Triazol-5-Ylmethoxy)-Pyrazolo[1,5-d]-[1,2,4]Triazine (MRK-016), a GABA_A Receptor α 5 Subtype-Selective Inverse Agonist. *J. Pharmacol. Exp. Ther.* **2009**, *331* (2), 470–484.
- (122) Eimerbrink, M. J.; White, J. D.; Pendry, R. J.; Hodges, S. L.; Sadler, L. N.; Wiles, J. D.; Weintraub, M. K.; Chumley, M. J.; Boehm, G. W. Administration of the Inverse Benzodiazepine Agonist MRK-016 Rescues Acquisition and Memory Consolidation Following Peripheral Administration of Bacterial Endotoxin. *Behav. Brain Res.* **2015**, *288*, 50–53.
- (123) Eimerbrink, M. J.; Pendry, R. J.; Hodges, S. L.; Wiles, J. D.; Peterman, J. L.; White, J. D.; Hayes, H. B.; Chumley, M. J.; Boehm, G. W. The α 5-GABAAR Inverse Agonist MRK-016 Upregulates Hippocampal BDNF Expression and Prevents Cognitive Deficits in LPS-Treated Mice, despite Elevations in Hippocampal A β . *Behav. Brain Res.* **2019**, *359*, 871–877.
- (124) Fischell, J.; Van Dyke, A. M.; Kvarita, M. D.; LeGates, T. A.; Thompson, S. M. Rapid Antidepressant Action and Restoration of Excitatory Synaptic Strength After Chronic Stress by Negative Modulators of Alpha5-Containing GABA_A Receptors. *Neuropsychopharmacology* **2015**, *40* (11), 2499–2509.
- (125) Zanos, P.; Nelson, M. E.; Highland, J. N.; Krimmel, S. R.; Georgiou, P.; Gould, T. D.; Thompson, S. M. A Negative Allosteric Modulator for α 5 Subunit-Containing GABA Receptors Exerts a Rapid and Persistent Antidepressant-Like Action without the Side Effects of the NMDA Receptor Antagonist Ketamine in Mice. *eNeuro* **2017**, *4* (1), ENEURO.0285-16.2017.
- (126) Xiong, Z.; Zhang, K.; Ishima, T.; Ren, Q.; Chang, L.; Chen, J.; Hashimoto, K. Comparison of Rapid and Long-Lasting Antidepressant Effects of Negative Modulators of α 5-Containing GABA_A Receptors and (R)-ketamine in a Chronic Social Defeat Stress Model. *Pharmacol. Biochem. Behav.* **2018**, *175*, 139–145.

- (127) Liu, R.; Hu, R. J.; Zhang, P.; Skolnick, P.; Cook, J. M. Synthesis and Pharmacological Properties of Novel 8-Substituted Imidazobenzodiazepines: High-Affinity, Selective Probes for α 5-Containing GABA_A Receptors. *J. Med. Chem.* **1996**, *39* (9), 1928–1934.
- (128) Savić, M. M.; Clayton, T.; Furtmüller, R.; Gavrilović, I.; Samardžić, J.; Savić, S.; Huck, S.; Sieghart, W.; Cook, J. M. PWZ-029, a Compound with Moderate Inverse Agonist Functional Selectivity at GABA_A Receptors Containing α 5 Subunits, Improves Passive, but Not Active, Avoidance Learning in Rats. *Brain Res.* **2008**, *1208*, 150–159.
- (129) Clayton, T.; Poe, M. M.; Rallapalli, S.; Biawat, P.; Savić, M. M.; Rowlett, J. K.; Gallos, G.; Emala, C. W.; Kaczorowski, C. C.; Stafford, D. C.; Arnold, L. A.; Cook, J. M. A Review of the Updated Pharmacophore for the Alpha 5 GABA(A) Benzodiazepine Receptor Model. *Int. J. Med. Chem.* **2015**, *2015*, 430248.
- (130) Soto, P. L.; Ator, N. A.; Rallapalli, S. K.; Biawat, P.; Clayton, T.; Cook, J. M.; Weed, M. R. Allosteric Modulation of GABA_A Receptor Subtypes: Effects on Visual Recognition and Visuospatial Working Memory in Rhesus Monkeys. *Neuropsychopharmacology* **2013**, *38* (11), 2315–2325.
- (131) Xu, N. Z.; Ernst, M.; Treven, M.; Cerne, R.; Wakulchik, M.; Li, X.; Jones, T. M.; Gleason, S. D.; Morrow, D.; Schkeryantz, J. M.; Rahman, M. T.; Li, G.; Poe, M.M.; Cook, J. M.; Witkin, J. M. Negative Allosteric Modulation of Alpha 5-Containing GABA_A Receptors Engenders Antidepressant-like Effects and Selectively Prevents Age-Associated Hyperactivity in Tau-Depositing Mice. *Psychopharmacology (Berl.)* **2018**, *235* (4), 1151–1161.
- (132) Kawaharada, S.; Nakanishi, M.; Nakanishi, N.; Hazama, K.; Higashino, M.; Yasuhiro, T.; Lewis, A.; Clark, G. S.; Chambers, M. S.; Maidment, S. A.; Katsumata, S.; Kaneko, S. ONO-8590580, a Novel GABA_A α 5 Negative Allosteric Modulator Enhances Long-Term Potentiation and Improves Cognitive Deficits in Preclinical Models. *J. Pharmacol. Exp. Ther.* **2018**, *366* (1), 58–65.
- (133) Etherington, L.-A.; Mihalik, B.; Pálvölgyi, A.; Ling, I.; Pallagi, K.; Kertész, S.; Varga, P.; Gunn, B. G.; Brown, A. R.; Livesey, M. R.; Monteiro, O.; Belelli, D.; Barkóczy, J.; Spedding, M.;

- Gacsályi, I.; Antoni, F. A.; Lambert, J. J. Selective Inhibition of Extra-Synaptic $\alpha 5$ -GABA_A Receptors by S44819, a New Therapeutic Agent. *Neuropharmacology* **2017**, *125*, 353–364.
- (134) Gacsályi, I.; Móricz, K.; Gigler, G.; Megyeri, K.; Machado, P.; Antoni, F. A. Persistent Therapeutic Effect of a Novel $\alpha 5$ -GABA_A Receptor Antagonist in Rodent Preclinical Models of Vascular Cognitive Impairment. *Eur. J. Pharmacol.* **2018**, *834*, 118–125.
- (135) Heckers, S.; Konradi, C. GABAergic Mechanisms of Hippocampal Hyperactivity in Schizophrenia. *Schizophr. Res.* **2015**, *167* (1), 4–11.
- (136) Gill, K. M.; Lodge, D. J.; Cook, J. M.; Aras, S.; Grace, A. A. A Novel $\alpha 5$ GABA_AR-Positive Allosteric Modulator Reverses Hyperactivation of the Dopamine System in the MAM Model of Schizophrenia. *Neuropsychopharmacology* **2011**, *36* (9), 1903–1911.
- (137) Stamenić, T. T.; Poe, M. M.; Rehman, S.; Santrač, A.; Divović, B.; Scholze, P.; Ernst, M.; Cook, J. M.; Savić, M. M. Ester to Amide Substitution Improves Selectivity, Efficacy and Kinetic Behavior of a Benzodiazepine Positive Modulator of GABA_A Receptors Containing the $\alpha 5$ Subunit. *Eur. J. Pharmacol.* **2016**, *791*, 433–443.
- (138) Prevot, T. D.; Li, G.; Vidojevic, A.; Misquitta, K. A.; Fee, C.; Santrac, A.; Knutson, D. E.; Stephen, M. R.; Kodali, R.; Zahn, N. M.; Arnold, L. A.; Scholze, P.; Fisher, J. L.; Marković, B. D.; Banasr, M.; Cook, J. M.; Savic, M.; Sibille, E. Novel Benzodiazepine-Like Ligands with Various Anxiolytic, Antidepressant, or Pro-Cognitive Profiles. *Mol. Neuropsychiatry* **2019**, *5* (2), 84–97.
- (139) Prevot, T. D.; Li, G.; Vidojevic, A.; Misquitta, K. A.; Fee, C.; Santrac, A.; Knutson, D. E.; Stephen, M. R.; Kodali, R.; Zahn, N. M.; Arnold, L. A.; Scholze, P.; Fisher, J. L.; Marković, B. D.; Banasr, M.; Cook, J. M.; Savic, M.; Sibille, E. Novel Benzodiazepine-Like Ligands with Various Anxiolytic, Antidepressant, or Pro-Cognitive Profiles. *ACS Chem. Neurosci.* **2019**, *10* (5), 2088–2090.
- (140) Möhler, H. Chapter One - The Legacy of the Benzodiazepine Receptor: From Flumazenil to Enhancing Cognition in Down Syndrome and Social Interaction in Autism. In *Advances in*

Pharmacology; Rudolph, U., Ed.; Diversity and Functions of GABA Receptors: A Tribute to Hanns Möhler, Part A; Academic Press (Cambridge, USA), 2015; Vol. 72, pp 1–36.

- (141) Martin, C.; Lowery, D. Patent Watch: Selective GABA_A Receptor Modulators: Intellectual Property Landscape. *Nat. Rev. Drug Discov.* **2018**, *17*, 778–778.
- (142) Lüscher, B.; Möhler, H. Brexanolone, a Neurosteroid Antidepressant, Vindicates the GABAergic Deficit Hypothesis of Depression and May Foster Resilience. *F1000Research* **2019**, *8*. pii: F1000 Faculty Rev-751.
- (143) Chen, X.; Gerven, J. van; Cohen, A.; Jacobs, G. Human Pharmacology of Positive GABA-A Subtype-Selective Receptor Modulators for the Treatment of Anxiety. *Acta Pharmacol. Sin.* **2019**, *40* (5), 571–582.

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