

ASSOCIATE EDITOR: ELIOT H. OHLSTEIN

International Union of Basic and Clinical Pharmacology. CVI: GABA_A Receptor Subtype- and Function-selective Ligands: Key Issues in Translation to Humans

Werner Sieghart and Miroslav M. Savić

Center for Brain Research, Medical University of Vienna, Vienna, Austria (W.S.) and Department of Pharmacology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia (M.M.S.)

Abstract	838
I. Introduction	838
A. γ -Aminobutyric Acid Type A Receptors and Their Heterogeneity	838
B. γ -Aminobutyric Acid Type A Receptor Structure and Pharmacology	839
C. Aim of the Present Review	840
II. Receptor Subtype-selective Binding Versus Subtype-selective Efficacy	841
III. Receptor Subtype-selective Efficacy—Dependence on the Conditions of Measurements	841
IV. Importance of Concentration-response Relationships of “Receptor Subtype-selective Ligands”	842
A. Referring Compound Actions to that of Standard Benzodiazepines Distorts Original Data	842
B. Maximal Efficacy Hides a Possible Subtype Selectivity at Lower Concentrations	843
C. Concentration of Compounds Eliciting In Vivo Effects	843
V. Currently Available Compounds Claimed to Be γ -Aminobutyric Acid Type A Receptor Subtype-Selective	843
A. Subtype-Selectivity Claimed for Incompletely Investigated Compounds	843
B. Compounds Claimed to Selectively Modulate $\alpha 1\beta\gamma 2$ Receptors	844
1. Zolpidem	844
2. Indiplon	845
3. Zaleplon, Zopiclone	845
4. β -CCt	846
5. Ro15-1788 (Flumazenil)	846
C. Compounds Claimed to Selectively Modulate $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$, and $\alpha 5\beta\gamma 2$ Receptors	846
1. L-838,417	847
2. TPA-023B	847
3. NS11394, NS16085	847
D. Compounds Claimed to Selectively Modulate $\alpha 2\beta\gamma 2$ and $\alpha 3\beta\gamma 2$ Receptors	848
1. Compound 4	848
2. Pyridazine Series of $\alpha 2\beta\gamma 2$ - and $\alpha 3\beta\gamma 2$ -selective Compounds	848
3. SL-651,498	848
4. TPA023	848
5. MRK-409	849
6. SH-053-2'-N (HZ166), MP-III-024, KRM-II-81	850
7. Baicalin	851
8. 6-Hydroxyflavone	851

Address correspondence to: Werner Sieghart, Department of Molecular Neurosciences, Center for Brain Research, Medical University of Vienna, Spitalgasse 4, A-1090 Vienna, Austria. E-mail: Werner.Sieghart@meduniwien.ac.at

In part, financial support was provided by the Ministry of Education, Science and Technological Development, R. Serbia – Grant No. 175076 (MMS).
<https://doi.org/10.1124/pr.117.014449>

9. Fa131.....	851
10. AZD7325.....	851
E. Compounds Claimed to Selectively Modulate $\alpha 3\beta\gamma 2$ Receptors.....	852
1. TP003.....	852
2. YT-III-31.....	853
3. $\alpha 3IA$	853
F. Compounds Claimed to Selectively Modulate $\alpha 5\beta\gamma 2$ Receptors.....	853
1. SH-053-R-CH3-2'F, MP-III-022.....	854
2. $\alpha 5IA$	854
3. $\alpha 5IA-II$	854
4. MRK-016.....	855
5. L-655,708.....	855
6. PWZ-029.....	856
7. RY-024.....	856
8. RO4938581.....	856
9. TB21007.....	857
10. S44819.....	857
11. XLi-093.....	857
G. Compounds Preferentially Modulating $\alpha 4\beta\gamma 2$ and/or $\alpha 6\beta\gamma 2$ Receptors.....	858
1. CMD-45.....	858
2. XHe-III-74.....	858
3. PZ-II-029 (Compound 6), LAU159, LAU463.....	859
4. (+)ROD188.....	859
5. Amiloride.....	860
6. Furosemide.....	860
H. Benzodiazepine Site Antagonist Actions.....	860
I. Compounds Claimed to Selectively Modulate $\beta 1$ -containing GABA _A Receptors.....	861
1. Salicylidene Salicylhydrazide.....	861
2. Fragrant Dioxane Derivatives.....	861
3. Pyrazoloquinolinones.....	862
J. Compounds Claimed to Selectively Modulate $\beta 2/3$ -containing GABA _A Receptors.....	863
1. Loreclezole, Etomidate, and Others.....	863
2. Etifoxine and Polyacetylene Compounds.....	864
3. $\beta 2/3$ -Selective Enaminones (Compound 2-261).....	864
4. Function of $\beta 2$ or $\beta 3$ Subunit-containing GABA _A Receptors.....	864
K. Compounds Claimed To Modulate Selectively δ -Containing GABA _A Receptors.....	864
1. γ -Aminobutyric Acid Site Ligands.....	865
a. THIP (gaboxadol).....	865
b. Thio-4-PIOL.....	865
c. Thio-THIP.....	865
d. GABA site antagonists.....	865
2. Neurosteroids and Tracazolate.....	866
3. Ketamine.....	866
4. DS2.....	866
5. Methaqualone.....	866
L. Compounds Modulating $\gamma 1$ - or $\gamma 3$ -Containing GABA _A Receptors.....	867
M. Compounds Modulating ε -, θ -, or π -Containing GABA _A Receptors.....	867
N. Compounds Modulating ρ -Containing GABA _A Receptors.....	868
VI. Discrepancy between the In Vivo Effects of Drugs in Rodent and Human Studies.....	868
A. Receptors Containing $\beta 1$ -Subunits Might Contribute to the Sedative Action of Benzodiazepine Site Ligands.....	869
B. Receptors Containing $\gamma 1$ or $\gamma 3$ Subunits, or Those Containing $\alpha 4$, $\alpha 6$, or δ Subunits, Might Contribute to the In Vivo Effects of Benzodiazepine Site Ligands.....	870

ABBREVIATIONS: DBI, diazepam binding inhibitor; GABA, γ -aminobutyric acid; GABA_A-receptor, GABA type A receptor; GABA_B-receptor, GABA type B receptor; KCC2, K⁺-Cl⁻ cotransporter 2; NKCC1, Na⁺-K⁺-2Cl⁻ cotransporter 1; TSPO, translocator protein (18 kDa).

C. Additional Interaction with Targets Different from GABA _A Receptors Could Also Contribute to the Separation of Anxiolytic and Sedative Properties of Ligands	870
D. A Low Potency and Efficacy of Compounds Might Enhance Their Receptor Subtype-selective Actions	870
VII. Outlook	871
Acknowledgments	872
References	872

Abstract—GABA_A receptors are the major inhibitory transmitter receptors in the brain. They are ligand-gated chloride channels and the site of action of benzodiazepines, barbiturates, neuroactive steroids, anesthetics, and convulsants. GABA_A receptors are composed of five subunits that can belong to different subunit classes. The existence of 19 homologous subunits and their distinct regional, cellular, and subcellular distribution gives rise to a large number of GABA_A receptor subtypes with distinct pharmacology, which modulate different functions of the brain. A variety of compounds have been identified that were claimed to modulate selectively individual GABA_A receptor subtypes. However, many of these compounds have only incompletely been investigated or, in addition to a preferential modulation of a receptor subtype, also modulate other subtypes at similar concentrations. Although their differential

efficacy at distinct receptor subtypes reduced side effects in behavioral experiments in rodents, the exact receptor subtypes mediating their behavioral effects cannot be unequivocally delineated. In addition, the discrepant in vivo effects of some of these compounds in rodents and man raised doubts on the applicability of the concept of receptor subtype selectivity as a guide for the development of clinically useful drugs. Here, we provide an up-to-date review on the currently available GABA_A receptor subtype-selective ligands. We present data on their actual activity at GABA_A receptor subtypes, discuss the translational aspect of subtype-selective drugs, and make proposals for the future development of ligands with better anxioreactivity in humans. Finally, we discuss possible ways to strengthen the conclusions of behavioral studies with the currently available drugs.

I. Introduction

A. γ -Aminobutyric Acid Type A Receptors and Their Heterogeneity

γ -Aminobutyric acid (GABA) is the major inhibitory transmitter of the central nervous system and exerts its action via two types of receptors, GABA_A and GABA_B receptors. Whereas GABA_B receptors are metabotropic receptors that activate inwardly rectifying K⁺ channels and/or inhibit high voltage-activated Ca²⁺ channels (Bettler et al., 2004), GABA_A receptors belong to the Cys-loop family of pentameric ligand-gated ion channels that includes the nicotinic acetylcholine-, serotonin type 3-, and strychnine-sensitive glycine receptors (Galzi and Changeux, 1994; Olsen and Sieghart, 2008), a Zn²⁺-activated ion channel (Davies et al., 2003), and an invertebrate glutamate-gated chloride channel (Hibbs and Gouaux, 2011). GABA_A receptors are anion-selective channels, and increased chloride permeability generally reduces neuronal excitability. But these receptors also can conduct other anions with variable permeability ratios relative to chloride. For instance, HCO₃⁻ flux could be physiologically relevant under certain conditions (Kaila et al., 1997; Olsen and Sieghart, 2008).

Twenty distinct GABA_A receptor subunit genes (6 α , 4 β , 3 γ , δ , ϵ , π , θ , 3 ρ) have been identified in the vertebrate nervous system, including a putative β 4 subunit gene that originally was identified in chicken (Bateson et al., 1991), but also has been demonstrated in humans (Levin et al., 1996; Berezhnoy et al., 2007). These genes as well as several alternatively spliced isoforms of the respective subunits, for instance γ 2L and γ 2S, give rise to a

possible enormous heterogeneity of GABA_A receptor subtypes (Barnard et al., 1998; Berezhnoy et al., 2007; Olsen and Sieghart, 2008). Recently, the extent of heterogeneity has been extensively discussed together with criteria for the unequivocal identification of native GABA_A receptor subtypes. So far, no sufficient new information has been accumulated that would justify the addition of a new member to the list of 26 GABA_A receptor subtypes “identified” or “existing with a certain probability” (Olsen and Sieghart, 2008). The majority of GABA_A receptors is composed of two α , two β , and one γ subunits ($\alpha\beta\gamma$ receptors, for receptor nomenclature, see Alexander et al., 2015; Olsen and Sieghart, 2008). In these receptors, alternating α and β subunits are connected by a γ subunit (Tretter et al., 1997). Depending on the subunit composition and arrangement, these receptors exhibit different pharmacology, and due to their distinct regional and cellular distribution (Wisden et al., 1992; Fritschy and Mohler, 1995; Pirker et al., 2000; Hörtnagl et al., 2013) each receptor subtype also contributes to the modulation of distinct functions of the brain (Sieghart, 1995; Olsen and Sieghart, 2008). Whereas GABA_A receptors composed of α 1 β γ 2, α 2 β γ 2, α 3 β γ 2 are predominantly located synaptically and contribute to phasic inhibition, receptors composed of α 4 β γ 2, α 5 β γ 2, α 6 β γ 2, or $\alpha\beta\epsilon$ subunits are partially located extrasynaptically and therefore, contribute to both phasic and tonic inhibition of neurons (Wagner et al., 2005; Glykys and Mody, 2007a; Belujon et al., 2009; Chen et al., 2017). In contrast, GABA_A receptors composed of $\alpha\beta$ or $\alpha\beta\delta$ subunits seem to be exclusively located extrasynaptically and hence only exert a tonic inhibition of neurons (Brickley and Mody, 2012).

In addition, at least some GABA_A receptor subtypes not only can be modulated by GABA but also exhibit some spontaneous gating in the absence of GABA and thus also contribute to tonic inhibition (Włodarczyk et al., 2013). These include $\alpha 6\beta 2\gamma 2$ (Knoflach et al., 1996), $\alpha 6\beta 2\delta$ (Hadley and Amin, 2007); $\alpha 4\beta 3\gamma 2$ and $\alpha 4\beta 3\delta$ (Tang et al., 2010); $\alpha 4\beta 1\delta$, $\alpha 4\beta 2\delta$, $\alpha 6\beta 1\delta$, $\alpha 6\beta 3\delta$, $\alpha 1\beta 3\delta$ (Karim et al., 2012b); or receptors containing an ε subunit (Neelands et al., 1999; Wagner et al., 2005). Furthermore, rat or murine homo-oligomeric $\beta 1$ receptors exhibit spontaneous gating that could be inhibited by picrotoxin (Sigel et al., 1989; Krishek et al., 1996) and modulated by pentobarbital and propofol (Krishek et al., 1996). These receptors, however, were insensitive to GABA. In contrast, bovine (Krishek et al., 1996) or human $\beta 1$ receptors (Sanna et al., 1995) that also exhibit spontaneous gating were GABA sensitive. GABA sensitivity of homo-oligomeric $\beta 1$ receptors thus seems to be species dependent. Homo-oligomeric $\beta 3$ receptors are also spontaneously open ion channels that are insensitive to GABA (Cestari et al., 1996; Woollorton et al., 1997) and can be modulated by various allosteric modulators, such as pentobarbital, etomidate, alphaxalone, propofol, and chlormethiazole (Slany et al., 1995; Woollorton et al., 1997). Interestingly, however, these receptors could be further activated by histamine (Saras et al., 2008; Seeger et al., 2012). Homo-oligomeric $\beta 3$ receptors are thus not GABA-gated but histamine-gated channels (Saras et al., 2008; Hoerbelt et al., 2016). In contrast to the receptors mentioned above and composed of $\alpha\beta\gamma 2$ or $\alpha\beta\delta$ subunits that were classified as “identified in the nervous system,” receptors containing ε subunits were classified as “tentatively occurring in the brain” (Olsen and Sieghart, 2008). Homo-oligomeric $\beta 1$ - or $\beta 3$ -containing receptors so far have not been demonstrated in the brain due to a lack of specific tools that could identify them among a variety of hetero-oligomeric $\alpha\beta$, $\alpha\beta\gamma$, or $\alpha\beta\delta$ receptors. However, especially homo-oligomeric $\beta 3$ receptors are easily formed in recombinant expression systems (Slany et al., 1995; Cestari et al., 1996; Miller and Aricescu, 2014) and there is no reason to believe that they cannot also be formed in the nervous system (Hoerbelt et al., 2016). In any case, drugs that can modulate spontaneously open receptors will also modulate tonic inhibition.

It has to be kept in mind, however, that depending on the chloride concentration in the inside of neurons, that is enhanced by the Na⁺-K⁺-2Cl[−] cotransporter NKCC1 and reduced by the K⁺-Cl[−] cotransporter KCC2, opening of the GABA_A receptor associated Cl[−] channel can elicit a depolarization and hyperpolarization, respectively. During early development of the nervous system NKCC1 predominates, resulting in a high Cl[−] concentration in the inside of neurons and thus in a Cl[−] efflux on activation of GABA_A receptors (Ben-Ari et al., 2012; Kaila et al., 2014). At this time, GABA is an excitatory transmitter. During the establishment of the excitatory glutamate system in the brain the expression of

KCC2 increases, the Cl[−] concentration in the inside of neurons decreases and activation of GABA_A receptors causes Cl[−] influx and thus a hyperpolarization. In the adult brain, activation of GABA_A receptors in most cases results in a hyperpolarization, and thus GABA_A receptors become the major inhibitory transmitter receptors in the nervous system. But even then, depolarizing actions of GABA can also occur in some neurons or even in individual compartments of neurons, depending on the subcellular localization of NKCC1 and KCC2 as well as on the intracellular distribution of large impermeable anions that are predicted to cause a discrete balance of chloride ions (Astorga et al., 2015; Knoflach et al., 2016).

B. γ -Aminobutyric Acid Type A Receptor Structure and Pharmacology

GABA_A receptors are the site of action of a variety of pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, anesthetics, neuroactive steroids, and convulsants (Sieghart, 1995; Berezhnoy et al., 2007). Whereas GABA exerts its channel opening effects via two binding sites located at the two extracellular interfaces of β and α subunits ($\beta+\alpha$ – interfaces), (Smith and Olsen, 1995), benzodiazepines bind to the homologous interface of an α and a γ subunit ($\alpha+\gamma$ – interface) (Sigel and Buhr, 1997; Ernst et al., 2003). Ligands acting via the benzodiazepine binding site cannot directly open the GABA_A receptor-associated chloride channel, but only allosterically enhance (positive allosteric modulators) or reduce (negative allosteric modulators) GABA-induced currents. A third group of benzodiazepine site ligands does not significantly change the conformation of GABA_A receptors. These ligands are antagonists (silent, neutral, or null modulators) at the benzodiazepine binding site. They exhibit no direct effects at GABA-induced currents but are able to block the action of positive or negative allosteric modulators acting at this site.

In addition to the GABA and the benzodiazepine binding sites there are multiple other binding sites at GABA_A receptors. At least 16 solvent accessible spaces have been identified in the extracellular and transmembrane domain of a GABA_A receptor structural model (Ernst et al., 2005). Five are located at the five extracellular interfaces between subunits, among those the benzodiazepine- and the two GABA-binding sites, five at the transmembrane interfaces, five are located within the four-helix-bundles forming the transmembrane domain of individual subunits, and at least one is located within the channel pore, mediating the action of some convulsants (Ernst et al., 2005). Furthermore, ligand-bound crystal structures of bacterial homologs of GABA_A receptors (GLIC, isolated from *Gloeobacter violaceus*; ELIC, isolated from *Erwinia chrysanthemi*), the structures of a glutamate-gated channel (isolated from the nematode *Caenorhabditis elegans*) for an

overview, see Sieghart, 2015; and the recently published crystal structure of the homo-oligomeric $\beta 3$ GABA_A receptor (Miller and Aricescu, 2014) indicated the existence of additional ligand-binding sites in the transmembrane and extracellular domains (Sieghart, 2015; Puthenkalam et al., 2016). All these solvent accessible spaces could function as drug binding pockets. They differ from each other in their size and their hydrophilic and hydrophobic properties, depending on the types of amino acid residues contributing to their formation (Ernst et al., 2005). In addition, neighboring subunits can influence the conformation of the pockets via subunit-subunit interactions, indicating that even pockets formed by the four-transmembrane helices of two identical α or β subunits within GABA_A receptors could be different from each other. And some of the pockets might accommodate more than one drug at distinct positions (Sieghart, 2015; Puthenkalam et al., 2016). In addition, binding of a drug to its pocket(s) can influence the conformation of other pockets of the receptors, resulting in allosteric interactions with other drugs (Puthenkalam et al., 2016). There are multiple examples for such allosteric interactions of drugs in GABA_A receptors (Sieghart, 1995).

Possible drug binding sites in the intracellular domain of GABA_A receptors so far could not be investigated because the structure of the intracellular domain of these receptors currently is not known. In addition, drugs could bind to the surface of GABA_A receptors and by that influence their flexibility (Baur et al., 2005, 2013). And binding sites of ions such as Zn^{2+} , Cu^{2+} , or La^{3+} in many cases are formed by specific amino acid residues that differ in distinct receptor subtypes (Sieghart, 2015). In contrast to the GABA and the benzodiazepine binding sites that are located in the extracellular domain of GABA_A receptors, barbiturates, anesthetics, and neuroactive steroids seem to bind to sites in the transmembrane domain (Sieghart, 2015; Forman and Miller, 2016; Feng and Forman, 2018). These compounds not only allosterically modulate GABA-induced currents but at higher concentrations also can directly activate the ion channel intrinsic to GABA_A receptors in the absence of GABA. In recent years, more than 100 compounds have been identified that are able to modulate GABA-induced currents or to activate directly GABA_A receptors via binding sites different from the benzodiazepine or the GABA binding site, respectively. For most of these compounds, their binding sites at GABA_A receptors so far have not been identified.

Drugs that exclusively modulate GABA-induced currents in an allosteric way are limited in their activity to those GABA_A receptors that are active in a certain task and therefore have less adverse effects than GABA site agonists or drugs that are able directly to open the chloride channel of most if not all GABA_A receptors. Benzodiazepines were the first compounds identified only to allosterically modulate GABA_A receptors, and due to their anxiolytic, anticonvulsant, sedative, hypnotic,

and muscle relaxant action and their clinical importance, much of the ensuing effort was directed toward the development of benzodiazepine site ligands with a more selective action.

The classic benzodiazepines possess comparable affinities and efficacies for GABA_A receptor subtypes composed of $\alpha 1\beta\gamma 2$, $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$, or $\alpha 5\beta\gamma 2$ subunits and thus produce comparable behavioral effects. Over the years, however, a variety of benzodiazepine binding site ligands from different structural classes have been developed that are able to differentially interact with these diazepam-sensitive $\alpha 1\beta\gamma 2$, $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$, or $\alpha 5\beta\gamma 2$ receptor subtypes. Their reduced side effect profile in behavioral experiments in rodents seemed to confirm the molecular genetic evidence indicating that $\alpha 1\beta\gamma 2$ receptors partially mediate the sedative, anterograde amnesic, and anticonvulsive actions, and $\alpha 2\beta\gamma 2$ receptors partially mediate the anxiolytic-like action (Löw et al., 2000). $\alpha 2\beta\gamma 2$ together with $\alpha 3\beta\gamma 2$ receptors seem to mediate some antinociceptive and muscle relaxant actions (Crestani et al., 2001; Knabl et al., 2008; Ralvenius et al., 2015), and $\alpha 5\beta\gamma 2$ receptors mediate the cognitive effects of benzodiazepine site ligands (Collinson et al., 2002; Crestani et al., 2002; Rudolph and Möhler, 2014). Moreover, from such experiments it was concluded that selective targeting of distinct GABA_A receptor subtypes not only may provide an anxiolytic-like effect without sedation, but also unveil additional effects, which can be starting points for the development of an innovative treatment of pain, cognitive disorders, stroke, schizophrenia, depression, or Down syndrome (Rudolph and Knoflach, 2011; Rudolph and Möhler, 2014).

C. Aim of the Present Review

Unfortunately, however, many of the compounds claimed to be GABA_A receptor subtype selective have only been incompletely investigated or, in addition to a preferential modulation of one receptor subtype, also significantly modulate other receptor subtypes at similar concentrations. Although their differential efficacy at distinct receptor subtypes reduced side effects and was beneficial for certain applications, the exact receptor subtype(s) mediating the behavioral effects of such compounds cannot be unequivocally delineated. Uncritical acceptance of a compound as being subtype selective, therefore, leads to poorly supported conclusions on GABA_A receptor subtypes, eliciting compound-induced behavior, that add confusion to the literature. In addition, the discrepant in vivo effects of some of these ligands in rodents and humans recently raised doubts on the applicability of the concept of receptor subtype-selectivity as a guide for the development of clinically useful drugs (Skolnick, 2012).

Here, we provide an up-to-date review on the currently available GABA_A receptor subtype-selective ligands. In sections II, III, and IV, we discuss methodological aspects important for the development of such drugs.

In *section V* we critically discuss compounds claimed to be GABA_A receptor subtype-selective and provide information on their actual interaction with various receptors, on their behavioral actions *in vivo*, as well as on their receptor occupancy during their behavioral actions, if available. Since presenting all the reasonably characterized compounds with some receptor subtype selectivity would have been a highly repetitive endeavor, compounds discussed were selected either because of their frequent use in behavioral and pharmacological studies, or because they are the currently most selective compounds within a compound class for which a reasonable data set is available. Most of these ligands exert their actions via the benzodiazepine binding site. Some of them, however, act via other allosteric binding sites at GABA_A receptors. And a few ligands have been identified that can be used to selectively activate or inhibit certain GABA_A receptor subtypes by acting via their GABA binding site. In *section VI* we discuss the translational aspect of subtype-selective drugs as exemplified by the discrepant *in vivo* effects of some of these ligands in rodents and man (Skolnick, 2012). In *section VII* we make proposals for the future development of ligands with improved anxiolytic selectivity as well as discuss possible ways to strengthen the conclusions of behavioral studies with the currently available “receptor subtype-selective drugs.”

II. Receptor Subtype-selective Binding Versus Subtype-selective Efficacy

Since the discovery of the high affinity “central” benzodiazepine binding site in the brain (Braestrup and Squires, 1977; Möhler and Okada, 1977) that later on turned out to be an allosteric modulatory binding site at GABA_A receptors (Karobath and Sperk, 1979; Sieghart and Karobath, 1980), more than 100 distinct compound classes have been identified that could inhibit high affinity binding of [³H]diazepam, [³H]flunitrazepam, or [³H]flumazenil to brain membranes. Most of these compounds have never been investigated for a possible GABA_A receptor subtype selectivity because receptor subtypes and techniques to investigate them were not available at the time of their synthesis. Evidence for a molecular heterogeneity of GABA_A receptors was available soon after the discovery of the benzodiazepine binding site (Sieghart and Karobath, 1980). However, recombinant receptor subtypes could only be expressed and investigated several years later after the individual subunits had been cloned and sequenced (Schofield et al., 1987; Levitan et al., 1988; Pritchett et al., 1989). In those early days, electrophysiological investigations of recombinant GABA_A receptors were only rarely performed and putative receptor subtype-selectivity of drugs was deduced from their differential inhibition of [³H]flunitrazepam or [³H]flumazenil binding at the recombinant diazepam-sensitive $\alpha 1, 2, 3, 5\beta\gamma 2$ or diazepam-insensitive $\alpha 4, 6\beta\gamma 2$ GABA_A receptor subtypes, respectively (McKay et al., 2004;

Selleri et al., 2003, 2005; Yin et al., 2010), or from two or three of these receptor subtypes, only (Guerrini et al., 2007; Lager et al., 2008). However, there are multiple examples of both, drugs exhibiting a comparable affinity but a differential efficacy and drugs exhibiting a differential affinity but a comparable efficacy for modulating GABA-induced currents of all these receptors (Atack, 2010a,b; Vinkers et al., 2010). It has to be kept in mind that it is the potency and efficacy of a drug for modulating GABA_A receptor subtypes that is decisively important for its action and not its affinity for a certain binding site (Korpi et al., 2002).

This conclusion is strengthened by increasing evidence that most if not all currently available drugs can interact with several binding sites at the same GABA_A receptor subtype (Sieghart, 2015; Puthenkalam et al., 2016). Interaction of the drug with some of these binding sites can be silent, whereas interaction with other binding sites can induce or stabilize a conformational change eliciting some drug effects. The most impressive examples for such a pattern are the pyrazoloquinolinones, such as CGS 9895, which more than three decades ago were identified as high affinity ligands for the benzodiazepine site of GABA_A receptors (Bennett, 1987). These compounds exhibited anxiolytic and anticonvulsant effects but produced less sedative and muscle relaxant effects than benzodiazepines. Only recently it was demonstrated that CGS 9895 and most of its structural analogs are high affinity silent modulators (null modulators, antagonists) at the benzodiazepine binding site but mediate their low potency action at various GABA_A receptor subtypes via a novel binding site at the previously not investigated $\alpha + \beta$ -interface of GABA_A receptors (Ramerstorfer et al., 2011; Varagic et al., 2013b). Thus it is the functional selectivity resulting from the interaction of a drug with all its binding sites at the respective receptor subtype and at the concentration used, which is important for the action of a drug. But of course, a compound highly selective for the benzodiazepine site of a certain GABA_A receptor subtype might also exhibit a high functional selectivity for this subtype if its action primarily is mediated via the benzodiazepine site.

III. Receptor Subtype-selective Efficacy—Dependence on the Conditions of Measurements

A possible functional receptor subtype selectivity can only be identified by a comparison of drug effects on GABA-induced currents elicited at various GABA_A receptor subtypes. Unfortunately, however, depending on the expression system used for generating recombinant GABA_A receptor subtypes (*Xenopus laevis* oocytes or cell line type, permanent or transient transfection, ratio of cDNAs used for transfection, etc.), in addition to the desired receptor subtype, homo- or heterooligomeric combinations of subunits can be formed, some of which

might not occur in vivo (Olsen and Sieghart, 2008; Boileau et al., 2010; You et al., 2010). Depending on the exact mixture of recombinant receptors formed in the expression system the measured pharmacological properties can be different. And differences in electrophysiological protocols between laboratories also can impact efficacy and potency estimates (de Lucas et al., 2015). Finally, the GABA concentration used for measuring allosteric modulation of GABA-induced currents in recombinantly expressed receptors, strongly influences the extent of modulation observed. In different studies, GABA concentrations eliciting 3%–5% (EC_3 , Baur and Sigel, 2007; Ramerstorfer et al., 2010), 10% (EC_{10} ; Popik et al., 2006), 20% (EC_{20} ; Carling et al., 2006), or even 50% (EC_{50} ; Harvey et al., 2002; Yin et al., 2010) of the maximal currents were used, making comparison of the data from individual studies even more difficult.

While synaptic GABA_A receptors are only partially saturated by GABA released from single vesicles in the course of miniature inhibitory postsynaptic currents (Perrais and Ropert, 1999; Hájos et al., 2000; Rumpel and Behrends, 2000), they are completely saturated by GABA released by repetitive action potentials. GABA concentrations within the synaptic cleft were estimated to rapidly rise to 1.5–3 mM and decay within a few hundred microseconds (Mozrzymas et al., 2003). When receptors are saturated by GABA, allosteric modulators no longer can enhance the current amplitude but only prolong the action of GABA. To estimate the action of a drug under these conditions, one would have to increase the time of measurement. However, the extent of GABA-induced current modulation would then strongly be influenced also by the desensitization of the receptor, a phenomenon more dominant at higher GABA concentrations and longer measurement times (Jones and Westbrook, 1995). This effect will be even more dramatic with drugs that allosterically accelerate current decay (Dillon et al., 1993, 1995; Simeone et al., 2017).

GABA_A receptors have a high density at synapses, but synapses constitute only a small part of the cell surface. Despite their lower density at the extrasynaptic membrane, the overall abundance of extrasynaptic receptors is higher than that of synaptic receptors (Nusser and Mody, 2002; Kasugai et al., 2010). Since GABA_A receptors are inserted into the membrane at extrasynaptic sites (Bogdanov et al., 2006), all GABA_A receptor subtypes, even the synaptic receptors, are also, at least temporarily, present extrasynaptically. The GABA concentrations acting at extrasynaptic GABA_A receptors (0.2–2.5 μ M; Glykys and Mody, 2007b) activate extrasynaptic as well as synaptic receptors, although to a different extent (Mortensen et al., 2012; Karim et al., 2013). For the majority of recombinant $\alpha\beta\gamma 2$ GABA_A receptors, such concentrations are between GABA EC_3 and EC_{40} . Some, but not all $\alpha\beta$ and $\alpha\beta\delta$ receptors, however, are more sensitive to GABA. In these receptors, 0.2 μ M GABA concentrations already

elicit 10%–50% and 2.5 μ M GABA concentrations 20%–80% of the maximal GABA-induced currents (Mortensen et al., 2012; Karim et al., 2013). Nevertheless, it has been demonstrated that even $\alpha 1\beta 2\gamma 2$ receptors, which exhibit a quite low GABA sensitivity, can be activated by 0.5 μ M GABA, and the resulting GABA EC_1 currents can be modulated by allosteric modulators (Li and Akk, 2015).

In addition, measurements at low GABA concentrations (GABA EC_3) have technical advantages over measurements at higher GABA concentrations. For instance, allosteric modulation measured as a percentage of increase in the GABA-induced current in the presence of a drug is much stronger at GABA EC_3 than at higher GABA concentrations, because it is referred to a smaller GABA-induced current. Differences in the efficacy of a drug for modulating GABA-induced currents at different receptor subtypes are thus also more evident at low than at high GABA concentrations. Measurements are also more reproducible, because GABA EC_3 is not in the linear range of GABA stimulation. Slight differences in the GABA concentration from the actual GABA EC_3 thus do not matter as much as similar variations at GABA EC_{10} , EC_{20} , or EC_{50} . Given all these arguments, it is suggested to measure allosteric modulation of GABA_A receptors at GABA EC_3 whenever technically feasible to increase the sensitivity of measurements and to allow a better comparison of pharmacological data.

IV. Importance of Concentration-response Relationships of “Receptor Subtype-selective Ligands”

A. Referring Compound Actions to that of Standard Benzodiazepines Distorts Original Data

In an effort to compare the actions of compounds at various receptor subtypes with those of chlordiazepoxide (Blackaby et al., 2006; Carling et al., 2006; Jennings et al., 2006), diazepam (Griebel et al., 2001; Alhambra et al., 2011; de Lucas et al., 2015), or zolpidem (Griebel et al., 2001), their efficacy was often also given relative to the maximal efficacy of these reference drugs measured in the same oocyte or cell culture system. Although such presentation allows for an immediate estimation of whether the compound exhibits a stronger or weaker effect at a receptor subtype than the reference compound, it also distorts the actual efficacy of the compound. For instance, if a compound exhibits a comparable efficacy at two receptor subtypes, its relative efficacy at these receptor subtypes will be different if the efficacy of the reference compound is different at these receptors. This is the case, for instance, for diazepam, which exhibits a higher maximal efficacy for $\alpha 3\beta 3\gamma 2$ than for $\alpha 2\beta 3\gamma 2$ or for $\alpha 1\beta 3\gamma 2$ and $\alpha 5\beta 3\gamma 2$ receptors (Puia et al., 1991; Ramerstorfer et al., 2010). Furthermore, data presented relative to diazepam cannot be compared with those presented relative to chlordiazepoxide or zolpidem in the

absence of information on the efficacy of the reference compounds used to calculate the results. Thus, unfortunately, important information is lost due to such data presentation, and the actions of the examined compounds cannot be compared with those of other ligands. To fully benefit from a comparison with standard benzodiazepines, the authors should also provide the original concentration-response curves of the compounds at the individual receptor subtypes, as for instance in Dias et al. (2005), Atack et al. (2006b, 2011b), Ren et al. (2010), and Christian et al. (2015).

B. Maximal Efficacy Hides a Possible Subtype Selectivity at Lower Concentrations

An apparent lack of receptor subtype selectivity of most of the published “receptor subtype-selective” compounds, however, can be deduced from three recent reviews that compared their affinity for the benzodiazepine binding site and their maximal relative efficacy at various GABA_A receptor subtypes (Atack, 2010a,b; Vinkers et al., 2010). From these overviews it is clear that even compounds that preferentially modulate a certain receptor subtype exhibit substantial activity also at one or more other receptor subtypes when the maximal efficacy at each receptor subtype is attained. It has been established previously that, depending on the behavioral task involved, a 1%–25% *in vivo* GABA_A receptor occupancy by diazepam (Gardner, 1992; Lippa et al., 2005) may be sufficient to elicit discernible anxiolytic or other behavioral effects. A 15%–30% receptor occupancy (Atack et al., 2010) or an efficacy greater than 0.1 relative to 1 μ M diazepam (Alhambra et al., 2011) elicits sedation and sleep. Thus even small effects of drugs at receptor subtypes must not be ignored. A 10% or 20% increase in living costs significantly matters in daily life, and a 10% or 20% enhancement of GABA-induced currents probably also will be able to elicit some but not all effects that can be elicited by a stronger enhancement of GABA currents. The *in vivo* effects of a drug therefore cannot be exclusively assigned to that receptor which can be modulated by the drug with the highest efficacy.

C. Concentration of Compounds Eliciting In Vivo Effects

The maximal efficacy of a drug is usually measured at a very high drug concentration. In many cases, concentrations 1000-fold the K_i value estimated from benzodiazepine binding studies were used, resulting in drug concentrations of 1–10 μ M (Street et al., 2004; Carling et al., 2006; Goodacre et al., 2006). Such concentrations, at least for benzodiazepine site ligands, only rarely can be achieved in the brain after pharmacological (rather than toxicological) doses. It has been demonstrated that the oral dose of diazepam required to occupy 50% of all diazepam-sensitive GABA_A receptors in rodents was as low as 1 to 2 mg/kg, resulting in total brain

concentrations of 1 μ mol/l and above (Greenblatt and Sethy, 1990; Müller Herde et al., 2017). But only the unbound (free) fraction of the total drug concentration in the brain may bind to receptor sites, and it has been demonstrated that the free concentrations of drugs are best correlated with their pharmacological effects in the brain (Hammarlund-Udenaes, 2010). The unbound fraction of benzodiazepine site ligands can differ substantially and amounts to 3.6% for diazepam or 47.1% for zaleplon (Summerfield et al., 2007). Under therapeutic conditions, benzodiazepines thus usually achieve free brain concentrations far below 1 μ M.

The free drug concentration at the receptor easily can be estimated from *in vivo* receptor occupancy data of compounds and their concentration-response curves at individual recombinant receptor subtypes. Assuming that a 50% receptor occupancy under therapeutic conditions corresponds with the concentration of the compound that generates 50% of its maximal effect at the most abundant GABA_A receptor subtype ($\alpha 1\beta\gamma 2$) in the brain, the active concentration of the compound at this receptor and in the brain, as well as the extent of modulation of the individual receptor subtypes under these conditions, can be estimated. The data discussed below indicate that most of the compounds so far investigated are eliciting their *in vivo* effects at low nanomolar concentrations. It is then clear that most if not all receptor subtypes are fully modulated by the drug at a close to 100% receptor occupancy. Therefore, it is reasonable to investigate the receptor subtype selectivity in a concentration range that also can be achieved in the brain *in vivo* and that elicits the behavioral actions of the drug. The best and most reliable way to measure receptor subtype selectivity, thus, is a comparison of the functional concentration-response curves for all possible receptor subtypes, because there might be concentration ranges of a compound in which it exhibits high receptor subtype selectivity. Only by knowing these concentrations and the doses required for achieving these free concentrations in the brain, it is possible selectively to modulate the respective receptor subtype(s) in *in vitro* and *in vivo* experiments, respectively.

V. Currently Available Compounds Claimed to Be γ -Aminobutyric Acid Type A Receptor Subtype-Selective

A. Subtype-Selectivity Claimed for Incompletely Investigated Compounds

Even when the effects of compounds at recombinant GABA_A receptor subtypes were investigated by electrophysiological studies, sometimes not all diazepam-sensitive receptor subtypes were investigated and in many cases only the maximal ligand effects, but no concentration-response curves, were shown at the individual receptor subtypes. Receptor subtype selectivity

was then claimed when a compound exhibited a higher positive or negative maximal efficacy at the given receptor as compared with other receptors, although other receptors also were significantly modulated by the compound (Street et al., 2004; Goodacre et al., 2006). In addition, a receptor subtype selectivity was even claimed based on a relatively selective binding to the benzodiazepine site of a receptor subtype and a single maximal efficacy at this one receptor (Achermann et al., 2009; Buettelmann et al., 2009).

B. Compounds Claimed to Selectively Modulate $\alpha 1\beta\gamma 2$ Receptors

Combined molecular genetic and pharmacological approaches demonstrated that $\alpha 1\beta\gamma 2$ GABA_A receptors partially mediate the sedative, anticonvulsant, and anterograde amnesic properties of diazepam (Rudolph et al., 1999; McKernan et al., 2000; Ralvenius et al., 2015). Drugs selectively modulating $\alpha 1\beta\gamma 2$ GABA_A receptors should thus exhibit sedative and anticonvulsant properties.

1. Zolpidem. The imidazopyridine zolpidem (Fig. 1) exhibits a 10-fold higher affinity for the benzodiazepine binding site of $\alpha 1\beta\gamma 2$ than for that of $\alpha 2\beta\gamma 2$ or $\alpha 3\beta\gamma 2$ receptors and an exceptionally low affinity for $\alpha 5\beta\gamma 2$ receptors (Sieghart, 1995). From that it was concluded that zolpidem is an $\alpha 1\beta\gamma 2$ -selective compound. However, from the concentration-response curves of zolpidem (Fig. 1) it can be deduced that there is only a small concentration range (between 1 and 30 nM) in which zolpidem only modulates recombinant GABA_A receptors composed of $\alpha 1\beta\gamma 2$ receptors (Ramerstorfer et al., 2010).

At 100 nM concentrations, zolpidem already significantly enhanced GABA-induced currents at $\alpha 2\beta 3\gamma 2$ or $\alpha 3\beta 3\gamma 2$ receptors from 100% to 132% or 121%, respectively, and at concentrations above 100 nM, which presumably are achievable in vivo at a moderate to high zolpidem dose, zolpidem only preferentially, but not selectively, modulates $\alpha 1\beta\gamma 2$ receptors. So, by carefully controlling the concentration of zolpidem, for instance in electrophysiological experiments using cell cultures or

brain slices, or by local application of zolpidem in in vivo experiments, it is possible to use this drug for a selective modulation of $\alpha 1\beta\gamma 2$ receptors. As expected, due to its preferential action at $\alpha 1$ -containing receptors, zolpidem exhibits sedative, hypnotic, and anticonvulsive actions in rodents and humans. The muscle relaxant action of zolpidem might be mediated by $\alpha 2$ - and $\alpha 3$ -containing GABA_A receptors (Ralvenius et al., 2015) while any anxiolytic-like action of zolpidem is behaviorally nonspecific and confounded by sedation (Savić et al., 2004).

Interestingly, zolpidem at clinically relevant concentrations was recently demonstrated to enhance GABA-induced currents in $\alpha 1\beta 3$ receptors composed of $3\alpha 1$ and $2\beta 3$ subunits in a flumazenil-sensitive manner (Che Has et al., 2016). These receptors contain an $\alpha 1$ - $\alpha 1$ interface and thus differ from $\alpha 1\beta 3$ receptors composed of $2\alpha 1$ and $3\beta 3$ subunits that contain a $\beta 3$ - $\beta 3$ interface. Diazepam also was able to modulate the $3\alpha 1:2\beta 3$ -containing $\alpha 1\beta 3$ receptors, but the efficacy of diazepam was significantly lower for this receptor than that of zolpidem, and no modulation by either zolpidem or diazepam was detected at the $2\alpha 1:3\beta 3$ receptor. To the best of our knowledge this is the first example of a stoichiometry-dependent action of a drug. Results indicate that zolpidem is acting via a binding site at the $\alpha 1+\alpha 1$ - interface, which obviously mimics the classic $\alpha 1+\gamma 2$ - benzodiazepine site. Receptors composed of $\alpha 1\beta 3$ subunits are expressed in the rat brain (Mortensen and Smart, 2006; Olsen and Sieghart, 2008). Studies on recombinant $\alpha 1\beta 3$ receptors have indicated that they exhibit a stoichiometry of $2\alpha 1:3\beta 3$ subunits (Tretter et al., 1997; Baumann et al., 2001). However, other studies have indicated that recombinant $\alpha 1\beta 2$ (Boileau et al., 2005) or $\alpha 6\beta 2$ (Im et al., 1995) receptors might be composed of 3α and 2β subunits. As discussed in Che Has et al. (2016), zolpidem is not a typical GABA_A receptor hypnotic. Unlike benzodiazepines, zolpidem modulates tonic GABA currents in the rat dorsal motor nucleus of the vagus (Gao and Smith, 2010), exhibits residual effects in mice carrying the point mutation $\gamma 2F77I$ that drastically reduces interaction of zolpidem with the benzodiazepine binding site of GABA_A receptors (Cope et al., 2005; Ramerstorfer et al., 2010), and improves speech as well as cognitive and motor functions in human patients with severe brain injury (Che Has et al., 2016). The receptors by which zolpidem mediates these effects are not known. It is thus quite possible that $\alpha 1\beta 3$ receptors composed of $3\alpha 1:2\beta 2$ subunits might mediate at least some of these effects. In any case, these surprising observations on the action of zolpidem at $\alpha 1\beta 3$ receptors add to previous observations on the existence of additional benzodiazepine binding sites at GABA_A receptors (Sieghart, 2015). In addition, they indicate that not all actions of benzodiazepines or benzodiazepine site ligands can be explained by their allosteric modulation of the classic benzodiazepine binding site at the $\alpha+\gamma$ - interface of $\alpha\beta\gamma 2$ GABA_A receptors. It has

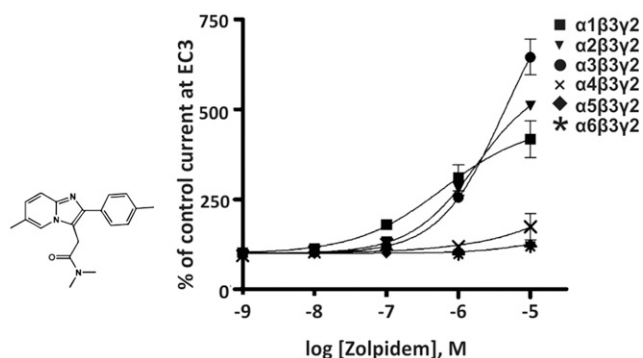


Fig. 1. Chemical structure and concentration-response curves of zolpidem at various recombinant GABA_A receptor subtypes from the rat expressed in *Xenopus laevis* oocytes and measured at GABA EC₃. The concentration-response curve of $\alpha 5\beta 3\gamma 2$ receptors is overlapping with that of $\alpha 6\beta 3\gamma 2$ receptors. Modified from Ramerstorfer et al., *Eur J Pharmacol*, 636,18-27, Elsevier, 2010.

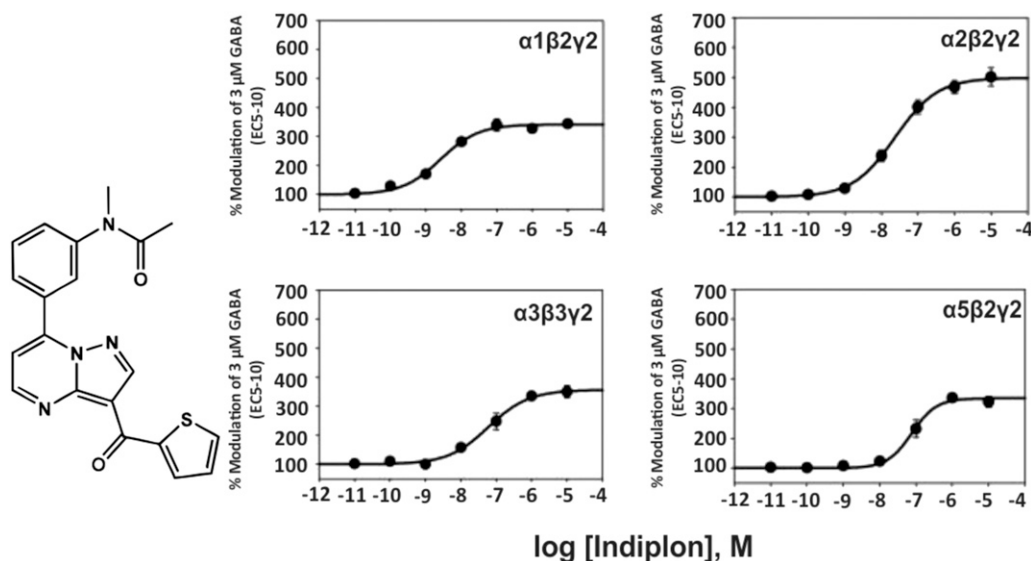


Fig. 2. Chemical structure and concentration-response curves of indiplon at various recombinant GABA_A receptor subtypes from the rat expressed in HEK 293 cells. GABA concentration was 3 μ M (GABA EC₅₋₁₀) for all recombinant receptors, and data were measured by patch-clamp recording. The EC₅₀ values for allosteric modulation of $\alpha 1\beta 2\gamma 2$, $\alpha 2\beta 2\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 5\beta 2\gamma 2$ receptors by indiplon were 2.6, 24, 60, and 77 nM, respectively. Figure modified from (Petroski et al., 2006).

to be kept in mind that these compounds in addition to their interaction with the benzodiazepine site might be able to elicit some of their effects via other GABA_A receptor subtypes that do not carry that site, or even via other effector systems.

2. Indiplon. The pyrazolopyrimidine indiplon (Fig. 2) has a nanomolar affinity for the benzodiazepine binding site of GABA_A receptors in various rat brain regions (Sullivan et al., 2004) and was claimed to exhibit a 10-fold selectivity for $\alpha 1\beta 2\gamma 2$ over $\alpha 2$ -, $\alpha 3$ -, or $\alpha 5$ -containing receptors in electrophysiological experiments at recombinant GABA_A

receptors from the rat (Petroski et al., 2006). But the concentration-response curves in Fig. 2 indicate that this at best is true up to 1 nM concentration. At 10 nM concentrations, all receptor subtypes investigated become positively modulated by that compound. At high concentrations, indiplon is a high efficacy positive allosteric modulator (full benzodiazepine site agonist) at all GABA_A receptor subtypes investigated. Indiplon exhibits sedative-hypnotic properties in rodents and humans.

3. Zaleplon, Zopiclone. The structurally related pyrazolopyrimidine zaleplon (Fig. 3) exhibits a much lower

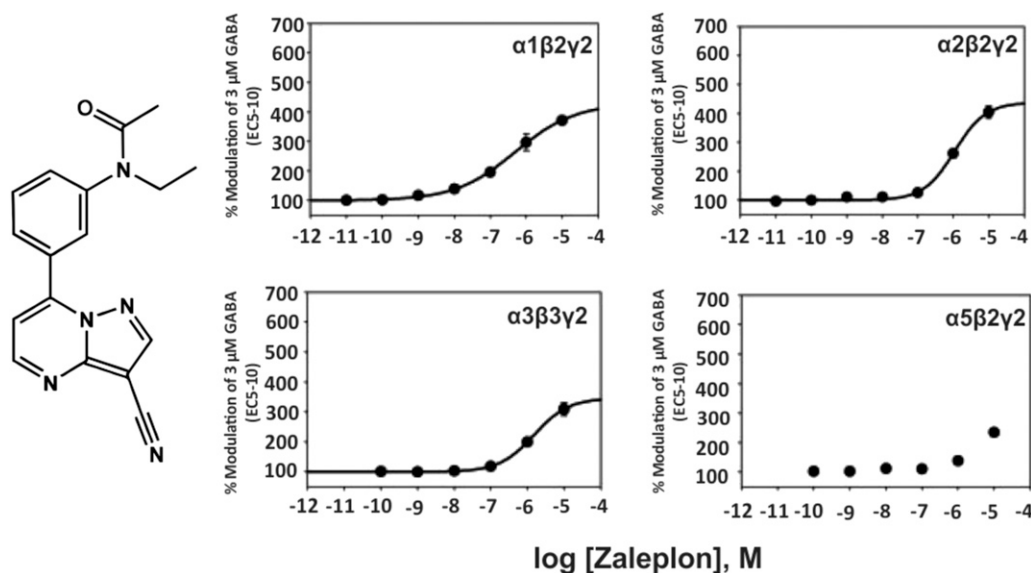


Fig. 3. Chemical structure and concentration-response curves of zaleplon at various recombinant GABA_A receptor subtypes from the rat expressed in HEK 293 cells. GABA concentration was 3 μ M (GABA EC₅₋₁₀) for all recombinant receptors, and data were measured by patch-clamp recording. The EC₅₀ values for allosteric modulation of $\alpha 1\beta 2\gamma 2$, $\alpha 2\beta 2\gamma 2$, $\alpha 3\beta 3\gamma 2$, or $\alpha 5\beta 2\gamma 2$ receptors by zaleplon were 499, 1098, 1514, or >3000 nM, respectively. Figure modified from (Petroski et al., 2006).

affinity for the benzodiazepine binding site of recombinant $\alpha 1\beta 2\gamma 2$, $\alpha 2\beta 1\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$ receptors from rat (K_i of 66, 830, 710, 1780 nM, respectively) (Dämgén and Lüddens, 1999) and, due to its lower potency in electrophysiological experiments, seems to be $\alpha 1$ -selective up to 100 nM, while at 1 μ M all receptor subtypes are modulated by zaleplon (Petroski et al., 2006). At 10 μ M concentration it is a full benzodiazepine site agonist at $\alpha 1\beta 3\gamma 2$ receptors and a partial agonist at $\alpha 5\beta 3\gamma 2$ receptors. Similar to zolpidem and indiplon, zaleplon exhibits sedative-hypnotic properties in rodents and humans.

The structurally dissimilar cyclopyrrolone derivative zopiclone, the third so-called “Z” drug after zolpidem and zaleplon, apparently does not exert significant effects up to 10 nM concentrations at recombinant $\alpha 1\beta 2\gamma 2$, $\alpha 2\beta 2\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 5\beta 2\gamma 2$ receptors from rat (Petroski et al., 2006). At 100 nM concentration, however, it modulates $\alpha 1\beta 2\gamma 2$ receptors to about 300% of GABA EC_{5-10} , and $\alpha 5\beta 2\gamma 2$ to about 250%. The allosteric modulation by 100 nM of zopiclone of recombinant $\alpha 2\beta 2\gamma 2$ or $\alpha 3\beta 3\gamma 2$ receptors, however, was below 200% of GABA EC_{5-10} . The EC_{50} values for the allosteric modulation of $\alpha 1\beta 2\gamma 2$, $\alpha 2\beta 2\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 5\beta 2\gamma 2$ receptors by zopiclone, were 158, 598, 1187, and 146 nM, respectively (Petroski et al., 2006). Zopiclone thus is not GABA_A receptor subtype-selective at all. It only preferentially modulates $\alpha 1\beta 2\gamma 2$ and $\alpha 5\beta 2\gamma 2$ receptors but also significantly modulates the other GABA_A receptor subtypes at a similar concentration. Similar to the other “Z” drugs, zopiclone exhibits sedative-hypnotic properties in rodents and humans. But this compound also exhibits anxiolytic, anticonvulsant, and muscle relaxant properties.

4. β -CCt. The β -carboline-3-carboxylate-t-butyl ester (Fig. 4) was described as a mixed benzodiazepine agonist-antagonist ligand with >10-fold binding selectivity for $\alpha 1$ over $\alpha 2$, and $\alpha 3$ receptors and >110-fold selectivity for $\alpha 1$ - over $\alpha 5$ -containing receptors (June et al., 2003; Yin et al., 2010). To the best of our knowledge, no concentration-response curves have been published for β -CCt, but from the bar graphs in Fig. 4, it can be deduced that this compound, even at high concentrations, acts

more or less as a null modulator (antagonist) at $\alpha 1$ - and $\alpha 2$ -containing receptors, as a weak positive modulator at $\alpha 3$ and $\alpha 4$ receptors, and a negative modulator at $\alpha 5$ receptors. Thus, despite its at least 10-fold selectivity for $\alpha 1$ receptors in benzodiazepine binding studies, part of the effects of β -CCt are presumably exerted via other GABA_A receptor subtypes. Looking at these data, conclusions that the anti-ethanol actions of this compound are primarily mediated via $\alpha 1$ -containing receptors (June et al., 2003; Yin et al., 2010) seem not to be sufficiently supported. Nevertheless, in behavioral studies, β -CCt demonstrated clear differences when directly compared with flumazenil (Savić et al., 2004), and it is still used as one of the rare “selective” antagonists at the benzodiazepine binding site (see section V.H).

5. Ro15-1788 (Flumazenil). As can be seen in Fig. 4, the imidazobenzodiazepine flumazenil (Ro15-1788) (Fig. 5), which originally was assumed to be a general antagonist at the benzodiazepine site of all GABA_A receptor subtypes, acts as an antagonist only at $\alpha 1\beta 3\gamma 2$, but not at other GABA_A receptor subtypes. This conclusion is supported by the concentration-response curves of Fig. 5, indicating that flumazenil, like other imidazobenzodiazepines, is also a positive allosteric modulator at $\alpha 4\beta 3\gamma 2$ and $\alpha 6\beta 3\gamma 2$ receptors (Ramerstorfer et al., 2010). The relatively weak positive or negative allosteric modulation of this compound at these other receptors as well as changes in GABA_A receptor expression and activity during benzodiazepine treatment or disease states can at least partially (see also section V.H) explain a variety of surprising observations when flumazenil was used in humans as an antagonist of benzodiazepine actions (Nutt, 1983; Hulse et al., 2015).

C. Compounds Claimed to Selectively Modulate $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, and $\alpha 5\beta 3\gamma 2$ Receptors

Since GABA_A receptors composed of $\alpha 1\beta 3\gamma 2$ subunits seemed to mediate the sedative effects of benzodiazepine site ligands, the development of drugs with reduced or

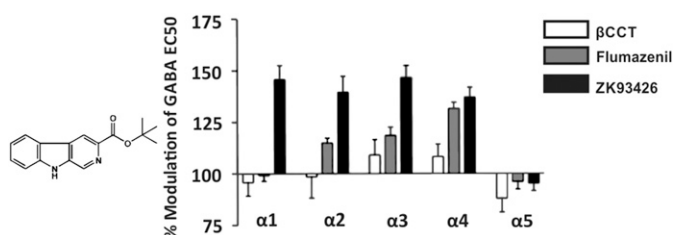


Fig. 4. Chemical structure of β -CCt and bar graph showing the maximal efficacy of this compound as well as that of flumazenil or the β -carboline ZK93426 in modulating rat recombinant $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 4\beta 3\gamma 2$, and $\alpha 5\beta 3\gamma 2$ GABA_A receptor subtypes expressed in *Xenopus laevis* oocytes. A saturating concentration (1–10 μ M) of β -CCt, flumazenil, or ZK93426 was coapplied over voltage-clamped oocytes along with an EC_{50} of GABA. Modified from Yin et al. (2010), *Bioorg Med Chem*, 18,7548-7564, Elsevier, 2010.

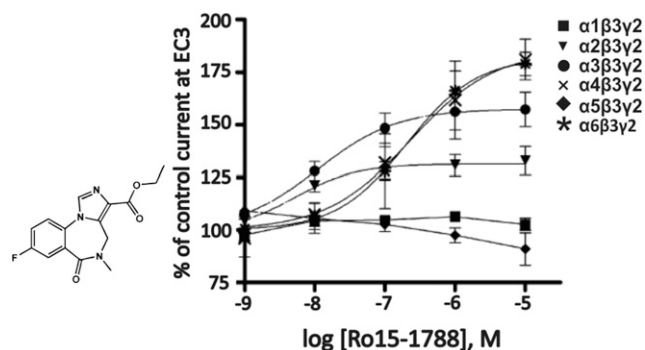


Fig. 5. Chemical structure and concentration-response curves of Ro15-1788 (flumazenil) at various recombinant GABA_A receptor subtypes from the rat expressed in *Xenopus laevis* oocytes and measured at GABA EC_3 . Modified from Ramerstorfer et al., *Eur J Pharmacol*, 636,18-27, Elsevier, 2010.

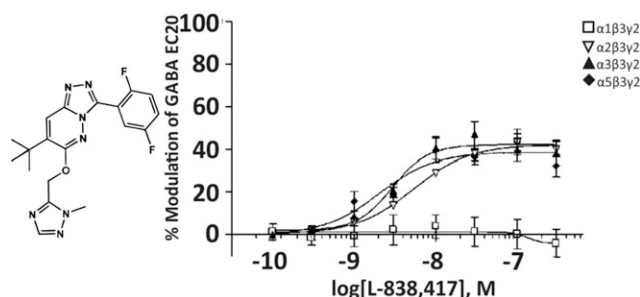


Fig. 6. Chemical structure and concentration-response curves of L-838,417 at various human recombinant GABA_A receptor subtypes at GABA EC₂₀ expressed in Ltk cells. Figure modified from (McKernan et al., 2000), with permission by Springer Nature.

eliminated modulation of these receptors became a high priority of pharmaceutical industry.

1. L-838,417. The triazolopyridazine L-838,417 (Fig. 6) has a similar low nanomolar affinity for the benzodiazepine binding site of recombinant human GABA_A receptors composed of α1,2,3,5β3γ2 subunits and expressed in Ltk cells. The affinity for α4β3γ2 or α6β3γ2 receptors was 267 or 2183 nM, respectively. Concentration-response curves indicate that L-838,417 is a positive allosteric modulator with nanomolar potency at α2β3γ2, α3β3γ2, and α5β3γ2 receptors, but is devoid of modulatory actions at α1β3γ2 receptors (Fig. 6). Due to the low affinity at α4- and α6-containing receptors, physiologic effects of this compound possibly mediated through these subtypes were not investigated (McKernan et al., 2000). In contrast to diazepam, L-838,417 did not impair the motor performance of wild-type mice on the rotarod, but it enhanced locomotor activity. This is a sign of behavioral disinhibition that might have been caused by a positive allosteric modulation of α2-containing GABA_A receptors under conditions where it is not opposed by a simultaneous modulation of α1 receptors (Ralvenius et al., 2015). At doses that occupied less than 50% of the benzodiazepine binding sites, L-838,417 retained anticonvulsant activity in mice and anxiolytic-like activity in rat. The data indicated that, in rodents, a compound with no efficacy at the α1 subtype does not produce sedation, but retains its anxiolytic-like properties (McKernan et al., 2000).

2. TPA-023B. The imidazotriazine TPA-023B (Fig. 7) exhibits a similar low nanomolar affinity for the benzodiazepine binding site of human GABA_A receptor subtypes composed of α1,2,3,5β3γ2 subunits and expressed in Ltk cells. The affinity for α4β3γ2 or α6β3γ2 receptors was 3300 or 4700 nM, respectively. In electrophysiological experiments, it is a comparably strong modulator of GABA EC₂₀ at α2β3γ2, α3β3γ2, and α5β3γ2 receptors and only weakly modulates α1β3γ2 receptors (Attack et al., 2011a). The high receptor occupancy in the rat at a dose that generates significant anxiolytic-like effects (87% at 1 mg/kg) indicates that all these receptors are fully modulated in vivo at a low dose of the drug already. Rotarod performance of rats was not significantly impaired by TPA-023B, even at a dose (10 mg/kg) that

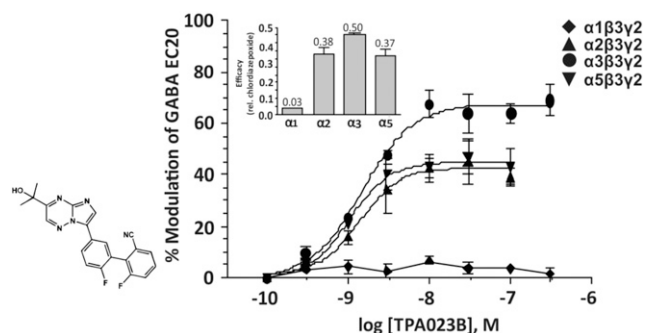


Fig. 7. Chemical structure and concentration-response curves of TPA-023B at various human recombinant GABA_A receptor subtypes stably expressed in Ltk cells and measured at GABA EC₂₀. Inset shows the maximum efficacy at each receptor subtype relative to that produced by 3 μM of the nonselective full agonist chlordiazepoxide (CDP). Three micromolars chlordiazepoxide potentiated GABA EC₂₀ currents by 105% ± 6%. Figure modified from Attack et al. (2011a), with permission by SAGE Publishing.

gave essentially complete receptor occupancy. Other studies indicated that TPA-023B is a nonsedating anxiolytic in primates (Attack et al., 2011a). The inset in Fig. 7 indicates that TPA-023B exhibits less than half of the maximal efficacy of chlordiazepoxide at α2, α3, and α5 receptors and about 3% of the maximal efficacy of chlordiazepoxide at α1 receptors.

3. NS11394, NS16085. The [3'-(5-(1-hydroxy-1-methyl-ethyl)-benzoimidazol-1-yl)-biphenyl-2-carbonitrile] NS11394 (Fig. 8) exhibited a subnanomolar affinity for the benzodiazepine site of human α1-, α2-, α3-, and α5β3γ2 receptors, whereas the affinity for α4β3γ2 and α6β3γ2 receptors was weak (*K_i* of 324 and 1009 nM, respectively). Based on oocyte electrophysiology with human GABA_A receptors (GABA EC₅₋₂₅) relative to 0.5 μM diazepam, NS11394 was claimed to exhibit a functional selectivity profile at GABA_A receptors of the α5 > α3 > α2 > α1 order (Mirza et al., 2008). Nevertheless, the concentration-response curves indicate that, despite its distinct efficacy for the individual receptor subtypes, this compound cannot selectively modulate a single GABA_A receptor subtype at any concentration. NS11394 behaved as an anxiolytic-like compound with

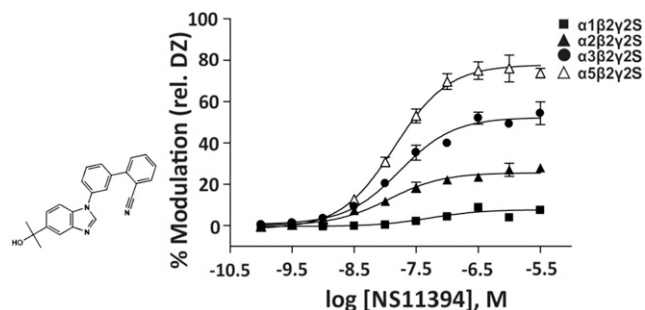


Fig. 8. Chemical structure and concentration-response curves of NS11394 at various human recombinant GABA_A receptor subtypes expressed in *Xenopus laevis* oocytes and measured at GABA EC₅₋₂₅. Data are expressed relative to the effects of 0.5 μM diazepam on the same oocyte (Christian et al., 2015) (rel. DZ). Figure modified from Mirza et al. (2008).

a reduced side-effect profile in rat and mouse, even at full receptor occupancy (Mirza et al., 2008).

The structurally related compound NS16085 (de Lucas et al., 2015), is $\alpha 2/\alpha 3$ selective up to a concentration of 3 nM, where it exerts about 10% of the actions of 0.5 μ M diazepam at these receptors. In addition, it is a weak negative allosteric modulator at $\alpha 1$ -containing receptors and a marginally positive allosteric modulator at $\alpha 5$ -containing receptors. Both NS16085 and NS11394 were demonstrated to exert an analgesic action by depressing activity-dependent spinal sensitization after inflammatory injury. These data indicate that potentiation of $\alpha 2$ - and $\alpha 3$ -GABA_A receptors is sufficient and that positive modulation at $\alpha 5$ -containing GABA_A receptors as with NS11394 is not necessary for inducing this pharmacological effect (de Lucas et al., 2015).

D. Compounds Claimed to Selectively Modulate $\alpha 2\beta\gamma 2$ and $\alpha 3\beta\gamma 2$ Receptors

Molecular genetic and pharmacological evidence indicated that $\alpha 2\beta\gamma 2$ receptors predominantly mediate the anxiolytic effects of diazepam (Löw et al., 2000; Behlke et al., 2016; Engin et al., 2016). In addition, $\alpha 2\beta\gamma 2$ and $\alpha 3\beta\gamma 2$ receptors also exhibit antihyperalgesic actions (Knabl et al., 2008; Ralvenius et al., 2015) and at higher concentrations also seem to mediate the muscle relaxant effects of diazepam. Because $\alpha 1\beta\gamma 2$ receptors seemed to mediate the sedative effects (Rudolph et al., 1999; McKernan et al., 2000) and $\alpha 5\beta\gamma 2$ receptors the cognition-impairing effects of classic benzodiazepines (Collinson et al., 2002; Crestani et al., 2002), avoiding modulation of both of these receptor types was another major goal of pharmaceutical industry.

1. Compound 4. The first compound claimed to be $\alpha 2$ selective was compound 4, a modified quinolone antibiotic that exhibited anxiolytic-like properties but did not cause sedation. It produced stronger effects than L-838,417 at $\alpha 2$ -GABA_A receptors but did not act via the benzodiazepine binding site (Johnstone et al., 2004). Unfortunately, however, only $\alpha 1\beta 2\gamma 2$ and $\alpha 2\beta 2\gamma 2$ receptors were investigated in this study, and to the best of our knowledge, no further information on this compound is available in the literature.

2. Pyridazine Series of $\alpha 2\beta\gamma 2$ - and $\alpha 3\beta\gamma 2$ -selective Compounds. In addition to the compounds mentioned in the previous section, some anxiolytic-like pyridazine compounds with no overt signs of ataxia or sedation have been identified that depending on their exact structure exhibit $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$, and $\alpha 5\beta\gamma 2$ selectivity (compound 15); $\alpha 2\beta\gamma 2$ and $\alpha 3\beta\gamma 2$ selectivity (compound 14, Fig. 9); or $\alpha 3\beta\gamma 2$ selectivity (compound 16). These ligands exhibit no modulatory activity at the $\alpha 1$ subtype, a good central nervous system penetration and receptor occupancy, and excellent oral bioavailability (Lewis et al., 2006). To the best of our knowledge, no additional studies have been reported on these compounds.

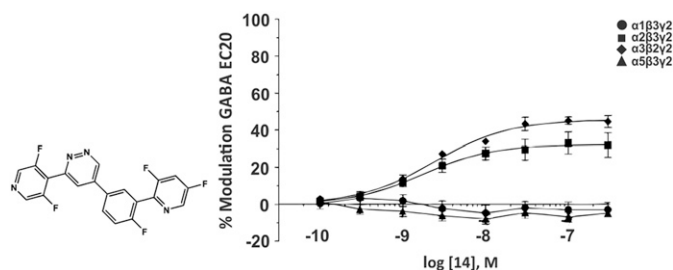


Fig. 9. Chemical structure and concentration-response curves of compound 14 at various recombinant human GABA_A receptor subtypes stably expressed in L(tk-) cells measured at GABA EC₂₀. Figure modified from Lewis et al. (2006), with permission of The American Chemical Society.

3. SL-651,498. The pyridoindole derivative SL-651,498 (Fig. 10) exhibited a differential affinity for the benzodiazepine site of rat $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, or $\alpha 5\beta 3\gamma 2$ receptors (K_i of 17, 73, 80, or 215 nM, respectively) and based on electrophysiological measurements was claimed to be an $\alpha 2$ -, $\alpha 3$ -selective positive allosteric modulator (Griebel et al., 2001). But the functional concentration-response curves at various GABA_A receptor subtypes, relative to zolpidem ($\alpha 1\beta 2\gamma 2$) or diazepam ($\alpha 2\beta 2\gamma 2$, $\alpha 3\beta 2\gamma 2$, and $\alpha 5\beta 3\gamma 2$), provide evidence that this compound is only $\alpha 2, \alpha 3$ selective up to a 10 nM concentration. In rats, this compound administered at 1–10 mg/kg elicited anxiolytic-like activity similar to that of diazepam and induced muscle weakness, ataxia, or sedation at substantially higher doses (≥ 30 mg/kg) (Griebel et al., 2001).

4. TPA023. The triazolopyridazine TPA023 (Fig. 11) has a subnanomolar affinity for the benzodiazepine binding site of $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, or $\alpha 5\beta 3\gamma 2$ receptors and a reduced affinity for $\alpha 4\beta 3\gamma 2$ or $\alpha 6\beta 3\gamma 2$ receptors (K_i of 60 or 418 nM, respectively). It was claimed to be selective for $\alpha 2\beta 3\gamma 2$ and $\alpha 3\beta 3\gamma 2$ receptors (Atack et al., 2006b).

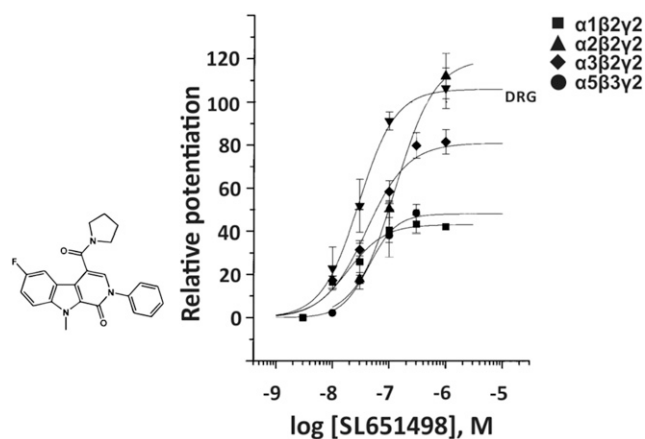


Fig. 10. Chemical structure and concentration-response curves of SL-651,498 at various rat recombinant GABA_A receptor subtypes stably expressed in HEK 293 cells measured using whole cell patch-clamp at GABA EC₅₋₁₀ and presented relative to zolpidem ($\alpha 1\beta 2\gamma 2$) or diazepam ($\alpha 2\beta 2\gamma 2$, $\alpha 3\beta 2\gamma 2$, and $\alpha 5\beta 3\gamma 2$). DRG indicates the concentration-response curve of SL-651,498 at dorsal root ganglia neurons in culture that contain exclusively native $\alpha 2$ -containing GABA_A receptors. Figure modified from Griebel et al. (2001).

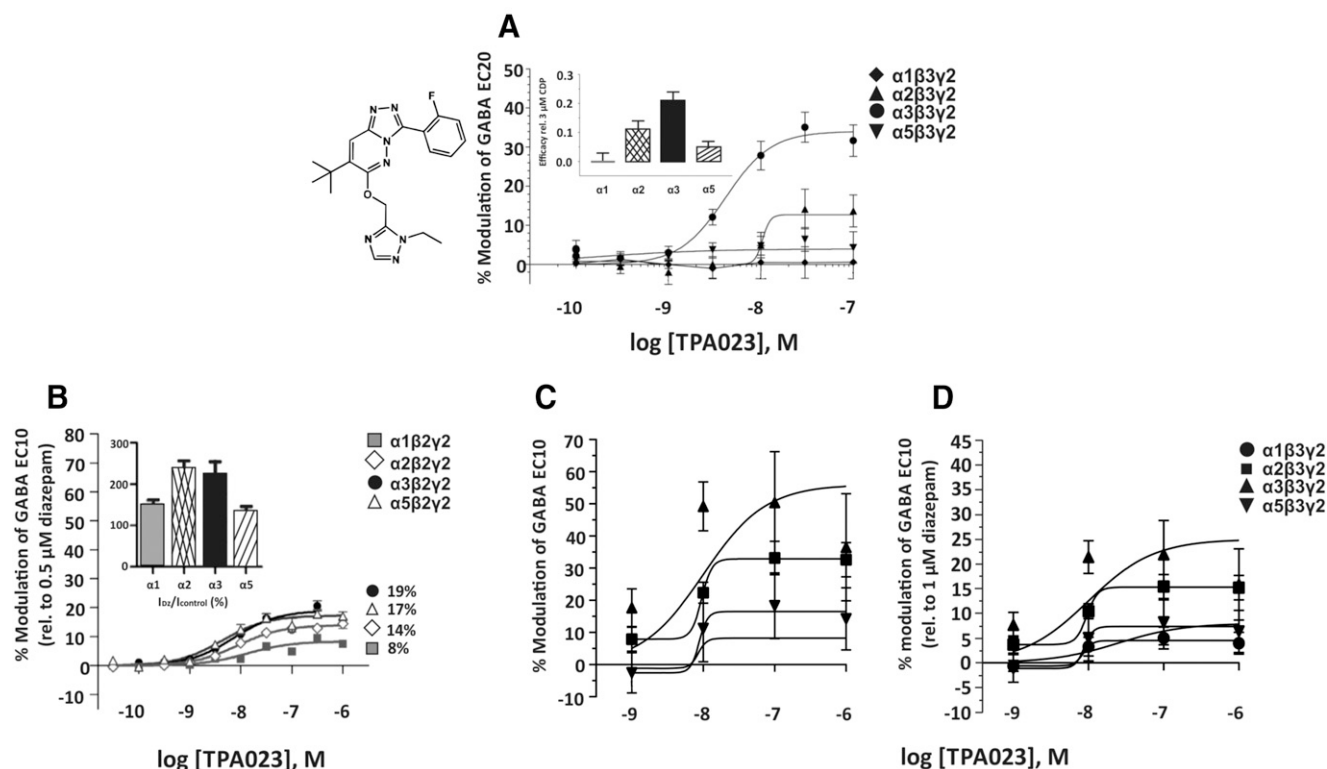


Fig. 11. Chemical structure and concentration-response curves of TPA023. (A) Figure modified from Attack et al. (2006b), generated at various recombinant human GABA_A receptor subtypes (containing β3 subunits) stably expressed in L(tk-) cells measured at GABA EC₂₀. Inset shows the maximum efficacy of TPA023 at each subtype relative to that produced by 3 μM of the nonselective full agonist chlordiazepoxide (CDP). (B) Figure modified from de Lucas et al. (2015), Biochemical Pharmacology, 93, 370-379, Elsevier, 2015, generated at various recombinant human GABA_A receptor subtypes (containing β2 subunits) expressed in *Xenopus laevis* oocytes measured at GABA EC₁₀ relative to 0.5 μM diazepam in the same oocyte. Inset shows % of modulation of GABA EC₁₀ by 0.5 μM diazepam at the individual receptors. The percentages given at the right of the curves represent the E_{max} elicited by TPA023 as % of the effect of 0.5 μM diazepam (de Lucas et al., 2015). (C and D) Figures modified from Christian et al. (2015) with permission by the American Physiological Society, generated with TPA023 at various recombinant human GABA_A receptor subtypes (containing β3 subunits) expressed in *Xenopus laevis* oocytes and measured at GABA EC₁₀. (C) Data represent % modulation of GABA EC₁₀ by TPA023. (D) Data represent % modulation of GABA EC₁₀ by TPA023, and normalized to the current potentiation produced by 1 μM diazepam that elicited a near maximal response.

However, this compound already produces a subnanomolar subtle positive modulation at α5β3γ2 receptors. At 1 nM it starts to positively modulate α3β3γ2 and at 10 nM concentrations also α2β3γ2 receptors. Thus there is no concentration where this compound exhibits pure α2/α3 selectivity. This conclusion is supported by other researchers (Christian et al., 2015; de Lucas et al., 2015), who identified even stronger interactions of this compound with other receptor subtypes and indicated that in their hands TPA023 exhibited only marginal selectivity; see Fig. 11B (de Lucas et al., 2015) or Fig. 11, C and D (Christian et al., 2015). In rats, a 50% occupancy of TPA023 corresponded to an oral dose of 0.42 mg/kg. TPA023 produced anxiolytic-like effects in rodents at minimal effective doses of 1–3 mg/kg corresponding to 70%–88% occupancy, while there was no appreciable sedation up to 30 mg/kg (Attack et al., 2006b). The inset in Fig. 11A indicates that the maximal efficacy elicited by TPA023 relative to 3 μM chlordiazepoxide is quite weak at the various GABA_A receptor subtypes. This conclusion is confirmed by Fig. 11B, which presents modulation of GABA EC₁₀ by TPA023 relative to the modulation by 0.5 μM diazepam, or by Fig. 11D, which presents

modulation of GABA EC₁₀ by TPA023 relative to 1 μM diazepam.

5. MRK-409. The structurally related triazolopyridazine MRK-409 (Fig. 12) has a comparable subnanomolar

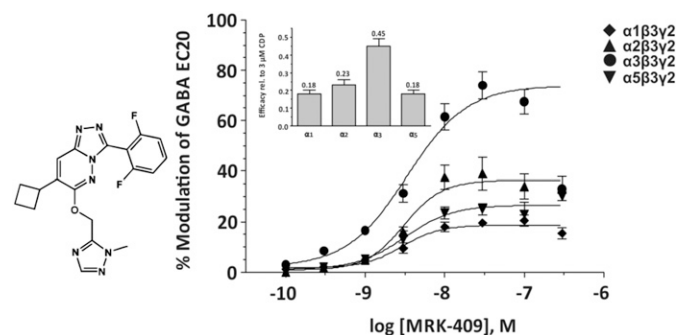


Fig. 12. Chemical structure and concentration-response curves of MRK-409 at various recombinant human GABA_A receptor subtypes stably expressed in mouse L(tk-) cells, measured using whole cell patch-clamp electrophysiology at GABA EC₂₀. Inset shows maximal potentiation at each cell type expressed relative to that produced by 3 μM of the nonselective full benzodiazepine site agonist chlordiazepoxide. Figure modified from Attack et al. (2011b) with permission by SAGE Publishing.

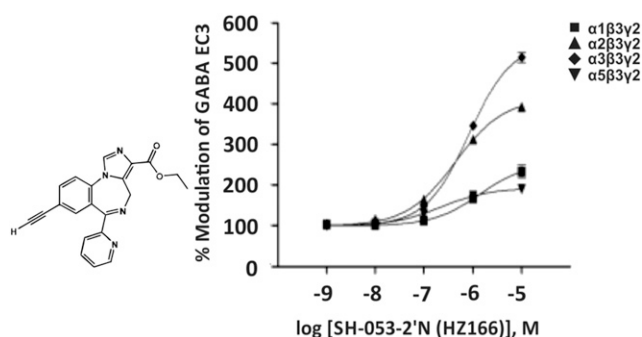


Fig. 13. Chemical structure and concentration-response curves of SH-053-2'-N (HZ166) at various rat recombinant GABA_A receptor subtypes expressed in *Xenopus laevis* oocytes and measured at GABA EC₃. Figure modified from Rivas et al. (2009) with permission of The American Chemical Society.

affinity for the benzodiazepine binding site of recombinant human $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 5\beta 3\gamma 2$ GABA_A receptor subtypes and a lower affinity for $\alpha 4\beta 3\gamma 2$ or $\alpha 6\beta 3\gamma 2$ receptors ($K_i = 78$ or 980 nM, respectively). In electrophysiological studies it is relatively selective for $\alpha 3$ receptors compared with $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, and $\alpha 5\beta 3\gamma 2$ receptors at subnanomolar but not at higher concentrations (Fig. 12). MRK-409 produced anxiolytic-like activity in rodents and primates, with minimum effective doses corresponding to occupancies from 35% to 65%, depending on the particular model used, and first overt signs of sedation at occupancies greater than 90% (Atack et al., 2011b). The inset in Fig. 12 indicates that MRK-409,

relative to $3 \mu\text{M}$ chlordiazepoxide, behaves as a weak to moderate partial agonist at the benzodiazepine binding site of diazepam-sensitive GABA_A receptor subtypes.

6. SH-053-2'-N (HZ166), MP-III-024, KRM-II-81. The imidazobenzodiazepine SH-053-2'-N (Fig. 13) exhibits a moderate affinity for the benzodiazepine site of GABA_A receptor subtypes (K_i of 300, 160, 527, or 82 nM for $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, or $\alpha 5\beta 3\gamma 2$ receptors, respectively) (Fischer et al., 2010) and was claimed to be an $\alpha 2/\alpha 3$ GABA_A receptor-selective benzodiazepine. MP-III-024, the methyl ester analog of the ethyl ester HZ166, exhibited a slightly lower efficacy than HZ166, but a similar preferential activity at $\alpha 2\beta 3\gamma 2$ and $\alpha 3\beta 3\gamma 2$ receptors (Fischer et al., 2017). KRM-II-81, a derivative of HZ166 carrying an oxazole ring instead of the ethyl ester of HZ166, exhibited a higher potency and efficacy for $\alpha 2\beta 3\gamma 2$ and $\alpha 3\beta 3\gamma 2$ receptors (Lewter et al., 2017). However, the respective concentration-response curves indicate that all three of these compounds already at 100 nM concentrations significantly modulate $\alpha 1$ and $\alpha 5$ receptors in addition to $\alpha 2/\alpha 3$ receptors (Rivas et al., 2009; Fischer et al., 2010, 2017; Lewter et al., 2017) (Fig. 13). MP-III-024 and KRM-II-81 exhibited significant antinociceptive effects (Fischer et al., 2017; Lewter et al., 2017). HZ166 produced some anticonvulsive (Rivas et al., 2009), anxiolytic-like (Savić et al., 2010), and antihyperalgesic effects (Di Lio et al., 2011), while it was devoid of sedation and motor impairment in rodents in some (Rivas et al., 2009; Di Lio et al., 2011),

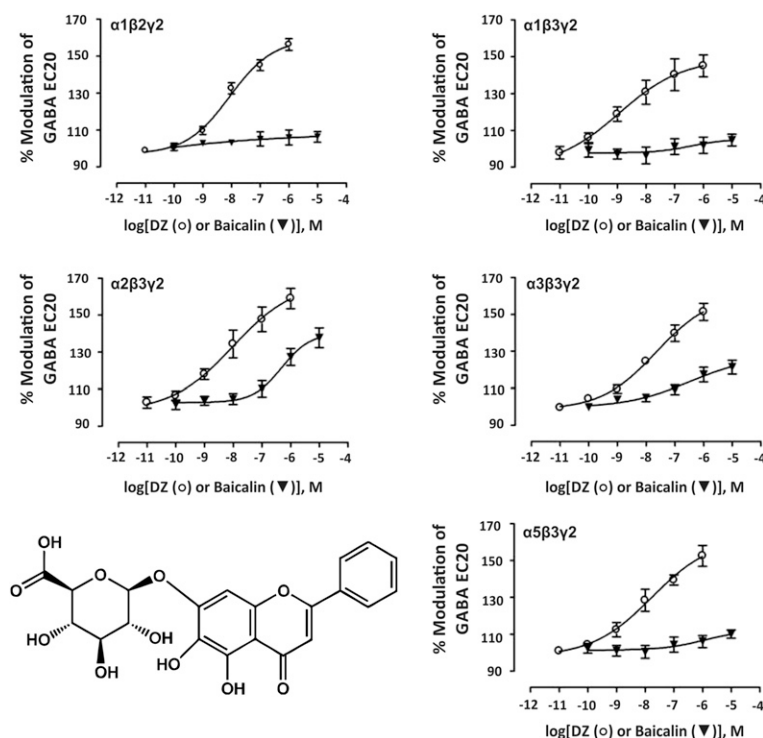


Fig. 14. Chemical structure of baicalin and concentration-response curves of diazepam (DZ, open circles) and baicalin (filled triangles) on various human recombinant GABA_A receptor subtypes expressed in HEK 293T cells under whole cell patch-clamp at GABA EC₂₀. Modified from Wang et al., *Neuropharmacology*, 55:1231–1237, Elsevier, 2008.

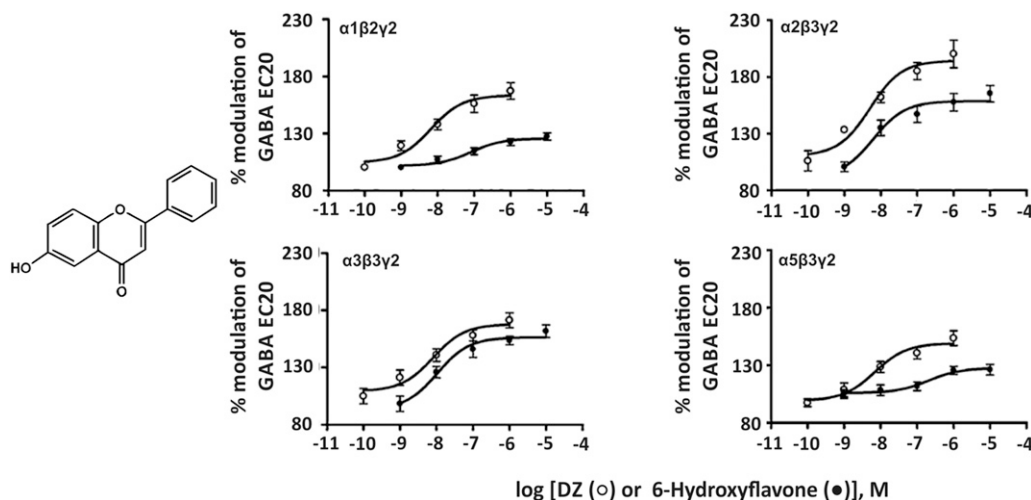


Fig. 15. Chemical structure of 6-hydroxyflavone and concentration-effect curves of diazepam (DZ, open circles) and 6-hydroxyflavone (filled circles) on human recombinant GABA_A receptor subtypes expressed in HEK 293T cells under whole cell patch-clamp at GABA EC₂₀. Modified from Ren et al., *Biochemical Pharmacology* 79: 1337–1344, Elsevier, 2010.

but not all, studies (Savić et al., 2010). In the latter study, the magnitude of the hypolocomotor effect of 30 mg/kg SH-053-2'-N in rats was somewhere in the middle between the effects of two tested doses of diazepam (1.25 and 2.5 mg/kg) (Savić et al., 2010).

7. Baicalin. A variety of flavonoids have been demonstrated to interact with the benzodiazepine site of GABA_A receptors and some of them also have some preferences for certain GABA_A receptor subtypes (Furmueller et al., 2008; Wang et al., 2008; Karim et al., 2012a). The flavonoid baicalin (Fig. 14) has been isolated from the traditional Chinese herb Huangqin, the dry root of *Scutellaria baicalensis* Georgi, and interacts with the benzodiazepine binding site of GABA_A receptors with a poor K_i value of 77.1 μ M. It produced anxiolytic-like effects in a Vogel conflict test and elevated plus maze test, and was devoid of sedation, myorelaxation, anticonvulsant, amnesic, and motor incoordination effects. In whole cell patch-clamp studies at 1 μ M concentrations, baicalin showed significant preference for $\alpha 2$ - and $\alpha 3$ -containing GABA_A receptor subtypes (Wang et al., 2008). However, baicalin in addition also is a known prolyl endopeptidase inhibitor (Tarragó et al., 2008) and induces apoptosis in pancreatic cancer cells (Takahashi et al., 2011).

8. 6-Hydroxyflavone. The flavonoid 6-hydroxyflavone (Fig. 15) has a quite moderate affinity of 1.3–4.9 μ M for the benzodiazepine binding site of human diazepam-sensitive GABA_A receptor subtypes expressed in HEK 293T cells (Ren et al., 2010) and preferentially modulates $\alpha 2$ - and $\alpha 3$ -containing GABA_A receptors in electrophysiological experiments. This compound produced anxiolytic-like effects in mice without the side effects observed with classic benzodiazepines (Ren et al., 2010). It should be kept in mind, however, that flavonoids can mediate their effects via multiple binding sites at GABA_A receptors (Hanrahan et al., 2015) and that the

actions of flavonoids are not necessarily limited to GABA_A receptors. Depending on their structure they also can interact with nicotinic acetylcholine receptors, serotonin type 3A receptors, glutamate AMPA/kainate receptors and others (Johnston and Beart, 2004).

9. Fa131. The flavan-3-ol derivative Fa131 (Fig. 16) does not inhibit [³H]flunitrazepam binding to rat cortical membranes. However, at low micromolar concentrations it is a positive allosteric modulator of human recombinant $\alpha 1,2,3,5\beta 2\gamma 2$ L and $\alpha 1\beta 2$ receptors expressed in *Xenopus laevis* oocytes, and this enhancement is not mediated via the benzodiazepine site, as it could not be blocked by the benzodiazepine site antagonist flumazenil (Fernandez et al., 2008). Fa131 preferentially modulates $\alpha 2$ receptors below 3 μ M concentrations and induces anxiolytic-like but no sedative effects in rodents. This compound highlights the potential of targeting nonbenzodiazepine allosteric sites in the search for new anxiolytic drugs.

10. AZD7325. The cinnoline-carboxamide AZD7325 (Fig. 17) was claimed to be an $\alpha 2/\alpha 3$ GABA_A receptor-

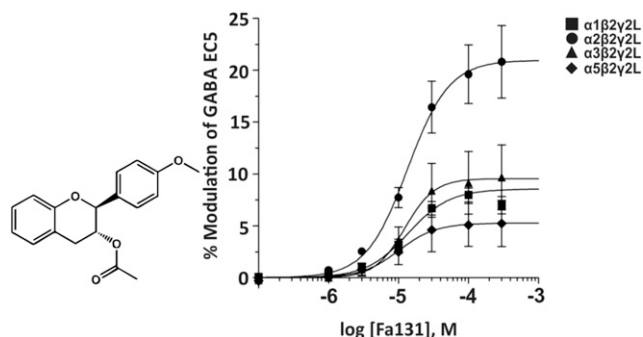


Fig. 16. Chemical structure and concentration-response curves of Fa131 at various human recombinant GABA_A receptor subtypes expressed in *Xenopus laevis* oocytes and measured at GABA EC₅. Modified from Fernandez et al., *Neuropharmacology*, 55: 900–907, Elsevier, 2008.

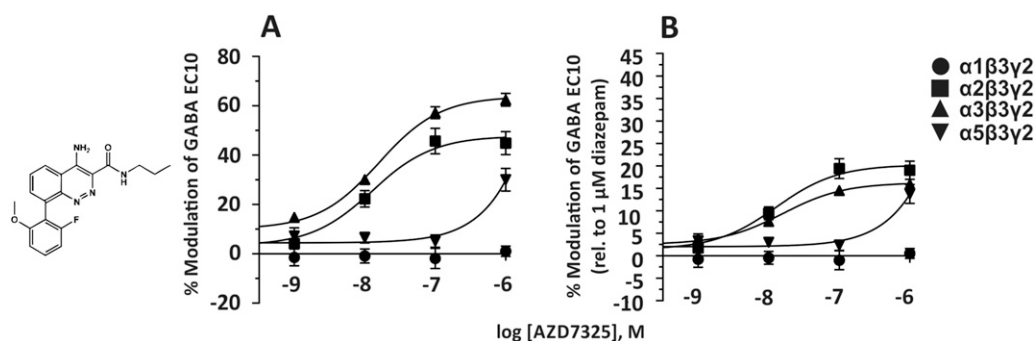


Fig. 17. Chemical structure and concentration-response curves of AZD7325 at various human recombinant GABA_A receptor subtypes expressed in *Xenopus laevis* oocytes and measured at GABA EC₁₀. (A) % Modulation of GABA EC₁₀. (B) The same data relative to the current potentiation produced by 1 μM diazepam, which elicited near maximal response at all receptor subtypes investigated. Figure modified from Christian et al. (2015) with permission by the The American Physiological Society.

selective partial modulator acting via the benzodiazepine site (Christian et al., 2015; Jucaite et al., 2017). Concentration-response curves at GABA EC₁₀ and human $\alpha\beta\gamma 2$ receptors expressed in *Xenopus laevis* oocytes indicate, however, that $\alpha 5$ receptors also are modulated to a small extent already at nanomolar concentrations (Christian et al., 2015). Positron emission tomography studies demonstrated that up to 80% receptor occupancy could be reached in the human brain by AZD7325 for its anxiolytic effects without overt sedation or cognitive impairment. The declared lack of side effects in humans can be explained by an insufficient modulation of $\alpha 1$ - and $\alpha 5$ -containing receptors under these conditions (Jucaite et al., 2017).

E. Compounds Claimed to Selectively Modulate $\alpha 3\beta\gamma 2$ Receptors

So far, no reasonably investigated compound has been identified that modulates only $\alpha 2\beta\gamma 2$ GABA_A receptors with a certain selectivity, but several compounds have been identified that were claimed to selectively modulate $\alpha 3\beta\gamma 2$ receptors.

1. **TP003.** The imidazopyridin-3-yl-biphenyl-2-carbonitrile TP003 (Fig. 18) has a comparable subnanomolar affinity for the benzodiazepine binding site of recombinant human $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, and $\alpha 5\beta\gamma 2$ GABA_A receptors (K_i between 0.3 and 0.5 nM), and a low affinity for $\alpha 4$ - or $\alpha 6\beta\gamma 2$ receptors (K_i of 2.4 or 1.8 μM, respectively) and was declared to be an $\alpha 3\beta\gamma 2$ -selective positive allosteric modulator (Dias et al., 2005). The complete functional concentration-response curves (at GABA EC₂₀, referred to the potentiation of 3 μM chlordiazepoxide) indicate, however, that due to the exceptionally high potency of this compound this at best holds true only at concentrations up to 1 nM (Fig. 18A); even before 10 nM, TP003 additionally modulates $\alpha 2\beta\gamma 2$ and $\alpha 5\beta\gamma 2$ receptors. More recently, stronger effects of TP003 at various receptor subtypes were demonstrated (de Lucas et al., 2015) (Fig. 18B) when GABA EC₁₀ was used and it was concluded that TP003 exhibits only marginal receptor subtype selectivity.

In Table 1 of Christian et al. (2015), data are presented indicating that TP003 exhibits no selectivity at all for $\alpha 3$ -containing GABA_A receptor subtypes.

Nevertheless, based on the apparent selectivity of TP003 for $\alpha 3$ -containing receptors and on experiments

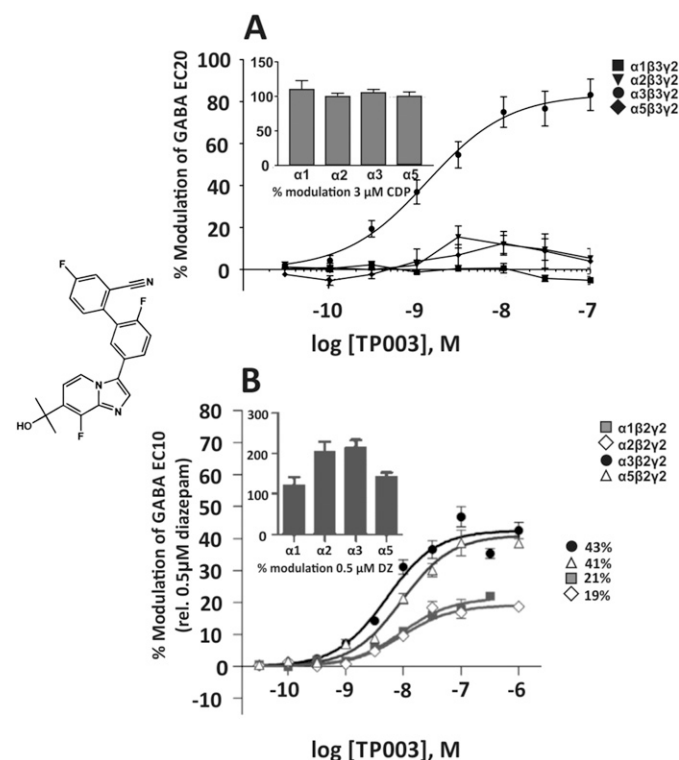


Fig. 18. Chemical structure and concentration-response curves of TP003. (A) Figure modified from Dias et al. (2005) with permission of the Society of Neuroscience, generated at various human $\alpha\beta\gamma 2$ recombinant GABA_A receptor subtypes stably expressed in L(tk-) cells and measured at GABA EC₂₀. Data presented are referred to the potentiation of 3 μM chlordiazepoxide. The inset histogram shows % modulation of GABA EC₂₀ by 3 μM chlordiazepoxide (CDP) at these receptor subtypes. (B) Figure modified from de Lucas et al., *Biochemical Pharmacology* 93, 370–379, Elsevier, 2015, generated at various human recombinant GABA_A receptor subtypes (containing $\beta 2$ subunits in this case) expressed in *Xenopus laevis* oocytes and measured at GABA EC₁₀ relative to 0.5 μM diazepam in the same oocyte. Inset shows % of modulation of GABA EC₁₀ by 0.5 μM diazepam at the individual receptors. The percentages given at the right of the curves represent the E_{max} elicited by TP003 as % of the effect of 0.5 μM diazepam.

indicating that TP003 generated anxiolytic-like effects even in mice with a point mutation that renders $\alpha 2$ -containing receptors benzodiazepine insensitive (Dias et al., 2005), it was claimed that $\alpha 3$ -containing receptors have a significant role in mediating the anxiolytic effects of benzodiazepines. However, in contrast to diazepam, which not only is inactive at the $\alpha \beta \gamma 2$ GABA_A receptor subtypes carrying the point mutation $\alpha 2^{H101R}$ but also at receptors carrying the mutations $\alpha 1^{H101R}$, $\alpha 3^{H126R}$, or $\alpha 5^{H105R}$ that are also used for demonstrating the function of the respective receptor subtypes in the brain (Rudolph and Knoflach, 2011; Rudolph and Möhler, 2014), other ligands of the benzodiazepine site, such as bretazenil or Ro15-4513, are even more active at all these point-mutated receptors (Benson et al., 1998). The anxiolytic effects of TP003 in $\alpha 2$ -point-mutated mice thus cannot be interpreted in the absence of evidence that TP003 is really inactive at recombinant $\alpha 2^{H101R} \beta \gamma 2$ receptors. Moreover, due to the only marginal selectivity of TP003, the involvement of other receptor subtypes such as $\alpha 5$ -containing receptors in anxiolytic-like activity (Behlke et al., 2016), as well as the absence of molecular genetic evidence indicating an involvement of $\alpha 3 \beta 2 \gamma 2$ receptors in the anxiolytic-like action (Löw et al., 2000), it has to be concluded that an anxiolytic-like role of $\alpha 3$ -containing GABA_A receptors is not supported by the available data.

2. YT-III-31. The imidazobenzodiazepine YT-III-31 (Fig. 19) preferentially modulates the recombinant $\alpha 3 \beta 3 \gamma 2$ GABA_A receptor subtype (Namjoshi et al., 2013). Although the concentration-response curves at first sight seem to point to a high selectivity for $\alpha 3$ receptors, this compound also significantly modulates most investigated receptor subtypes at 10 nM concentrations. In rats, YT-III-31 produced anxiolytic-like actions in a narrow dose range (<10 mg/kg) but sedation at higher doses (Batinić et al., 2018). The concentration-response curves of Fig. 19 predict moderate potentiation of $\alpha 3$ as well as $\alpha 2$ and $\alpha 5$ receptors before sedative effects mediated via $\alpha 1$ receptors are activated.

3. $\alpha 3$ IA. The pyridone $\alpha 3$ IA (Fig. 20) has a modest affinity for the benzodiazepine binding sites of $\alpha 1$ -, $\alpha 2$ -,

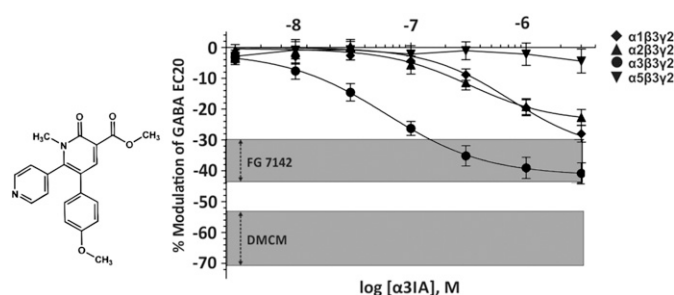


Fig. 20. Chemical structure and concentration-response curves of $\alpha 3$ IA at various human recombinant $\alpha \beta \gamma 2$ GABA_A receptor subtypes stably expressed in L(tk-) mouse fibroblast cells and measured at GABA EC₂₀. The shaded areas represent the range of inverse agonist efficacies across subtypes for the nonselective partial inverse agonists FG7142 or the nonselective full inverse agonist DMCm. Figure modified from Attack et al. (2005). Reprinted with permission of John Wiley & Sons, Inc.

$\alpha 3$ -, or $\alpha 5 \beta 3 \gamma 2$ receptors ($K_i = 1029, 323, 82$, or 410 nM, respectively, and a very weak affinity for that of $\alpha 4$ - or $\alpha 6 \beta 3 \gamma 2$ receptors ($K_i > 10,000$ nM) and was claimed to be an $\alpha 3 \beta 3 \gamma 2$ receptor-selective negative allosteric modulator (Attack et al., 2005). Concentration-response curves measured at GABA EC₂₀, however, indicate that this only holds true up to a concentration of 30 nM, as at 100 nM concentrations, receptors composed of $\alpha 1 \beta 3 \gamma 2$ and $\alpha 2 \beta 3 \gamma 2$ subunits also become negatively modulated by this drug. At doses that produce relatively low levels of occupancy (12%) in the rat cerebellum, a brain region that contains predominantly $\alpha 1$ - and $\alpha 6$ -containing GABA_A receptors and only 2% of $\alpha 3$ - and 7% of $\alpha 2$ -containing receptors (Pörtl et al., 2003), this compound elicited an anxiogenic-like effect similar to FG7142, and this effect could be blocked by the benzodiazepine site antagonist flumazenil (Attack et al., 2005). From the concentration-response curves in Fig. 20 it can be concluded that the concentration eliciting a 12% occupancy of $\alpha 1$ receptors amounts to >100 nM $\alpha 3$ IA. At that concentration, $\alpha 3$ IA elicits a similar negative allosteric modulation at $\alpha 3 \beta 3 \gamma 2$ receptors as the anxiogenic compound FG7142.

F. Compounds Claimed to Selectively Modulate $\alpha 5 \beta \gamma 2$ Receptors

A combination of molecular genetic and pharmacological approaches indicated that $\alpha 5 \beta \gamma 2$ receptors mediate the unwanted cognitive effects of diazepam and that a negative modulation of these receptors enhances learning and memory (Collinson et al., 2002; Crestani et al., 2002). Other experiments indicated that reducing excessive tonic inhibition by negative allosteric modulators at $\alpha 5 \beta \gamma 2$ receptors may promote functional recovery from stroke (Clarkson et al., 2010). Therefore, the main effort of researchers was directed to the development of negative allosteric modulators of $\alpha 5$ -containing GABA_A receptors on cognition in the aging brain (Koh et al., 2013), in schizophrenia (Gill and Grace, 2014), or in neuropsychiatric disorders characterized by cognitive deficits due

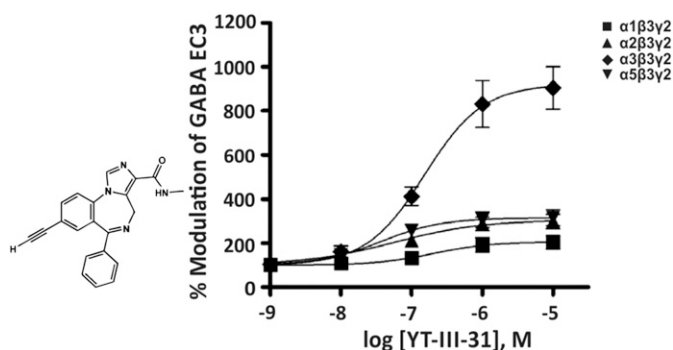


Fig. 19. Chemical structure and concentration-response curves of YT-III-31 at various rat recombinant GABA_A receptor subtypes expressed in *Xenopus laevis* oocytes and measured at GABA EC₃. Modified from Namjoshi et al., *Bioorg Med Chem*, 21, 93–101, Elsevier, 2013.

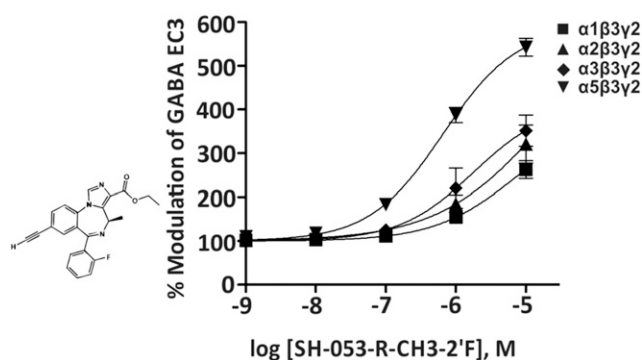


Fig. 21. Chemical structure and concentration-response curves of SH-053-R-CH3-2'F at various rat recombinant GABA_A receptor subtypes expressed in *Xenopus laevis* oocytes and measured at GABA EC₃. Modified from Savić et al., *Prog Neuropsychopharmacol Biol Psychiatry*, 34:376–386, Elsevier, 2010.

to impaired memory interference management (Engin et al., 2015). These observations point to a more complex bidirectional modulation of cognition by $\alpha 5$ -containing GABA_A receptors and provide new impetus also to develop selective positive allosteric modulators at $\alpha 5\beta\gamma 2$ receptors. This impetus is further enhanced by recent findings that positive allosteric modulators at $\alpha 5$ -containing GABA_A receptors have beneficial effects in the treatment of asthma (Gallos et al., 2015) or medulloblastomas (Jonas et al., 2016).

1. SH-053-R-CH3-2'F, MP-III-022. One of the first relatively $\alpha 5\beta\gamma 2$ -selective positive allosteric modulators reported with concentration-response curves was the imidazobenzodiazepine SH-053-R-CH3-2'F (Savić et al., 2010) (Fig. 21). This compound has a moderate affinity for the benzodiazepine binding site of $\alpha 5$ receptors ($K_i = 95.2$ nM) and a low affinity for $\alpha 1$ -, $\alpha 2$ -, or $\alpha 3\beta\gamma 2$ receptors ($K_i = 759, 948$, or 768 nM, respectively) (Fischer et al., 2010). In electrophysiological experiments, SH-053-R-CH3-2'F is selective for $\alpha 5$ receptors up to a 30 nM concentration. At 100 nM concentration, this compound significantly enhances GABA EC₃ currents at $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, and $\alpha 5\beta 3\gamma 2$ receptors from 100% to 111%, 124%, 125%, and 183%, respectively (Fischer et al., 2010; Savić et al., 2010). At doses up to 30 mg/kg, SH-053-R-CH3-2'F depressed locomotion but did not induce cognitive impairment or anxiolytic-like activity (Savić et al., 2010). In addition, it was demonstrated to relax precontracted intact airway smooth muscle cells (Gallos et al., 2015).

Ester to amide substitution in SH-053-R-CH3-2'F led to MP-III-022, with improved selectivity, efficacy, and kinetic behavior as a positive modulator of GABA_A receptors containing the $\alpha 5$ subunit (Stamenić et al., 2016). While at doses 1–10 mg/kg it was devoid of ataxia, sedation, or an influence on the extent of anxiety-related behavior in rats, at the dose of 10 mg/kg MP-III-022 caused a strong positive modulation of $\alpha 5\beta\gamma 2$ receptors and mild, but significant, muscle relaxation (Stamenić et al., 2016).

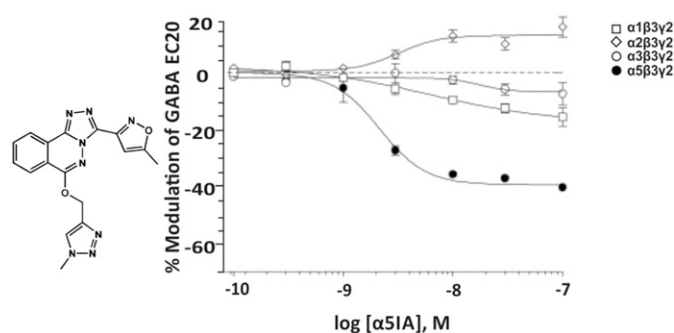


Fig. 22. Chemical structure and concentration-response curves of $\alpha 5$ IA at various human recombinant $\alpha\beta\gamma 2$ GABA_A receptor subtypes stably expressed in L(tk-) mouse fibroblast cells and measured at GABA EC₂₀. Figure modified from Dawson et al. (2006).

2. $\alpha 5$ IA. The triazolopyridazine $\alpha 5$ IA (Fig. 22) was demonstrated to bind with equivalent subnanomolar affinity (0.5–0.9 nM) to the benzodiazepine binding site of recombinant human GABA_A receptors containing $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, or $\alpha 5\beta\gamma 2$ subunits and possessed much lower affinity ($K_i = 60$ or 418 nM, respectively) for receptors containing $\alpha 4$ or $\alpha 6\beta\gamma 2$ subunits. $\alpha 5$ IA was declared to be an $\alpha 5$ receptor-selective negative allosteric modulator (Dawson et al., 2006), but the data presented for human recombinant $\alpha\beta\gamma 2$ GABA_A receptors indicate that there was no concentration where this compound acted exclusively via $\alpha 5$ receptors (Fig. 22). Interestingly, however, data obtained from different expression systems [*Xenopus* oocytes or L(tk-) cells stably expressing the same human receptors), depending on the receptor subtype investigated were found to be significantly different (Dawson et al., 2006). A possible additional action of this compound via $\alpha 4\beta\gamma 2$ receptors was not investigated. Other studies indicated that with oral doses of 0.1, 1.0, and 10 mg/kg, GABA_A receptor occupancies in Sprague-Dawley rat brain were 27%, 79%, and 87%, respectively, 2 hours after oral administration (Atack et al., 2009a). This compound significantly enhanced performance in a rat hippocampal-dependent test of learning and memory with a minimum effective oral dose of 0.3 mg/kg. It was not convulsant or anxiogenic in rodents and did not impair performance in the mouse rotarod test (Dawson et al., 2006).

3. $\alpha 5$ IA-II. The structurally related triazolopyridazine $\alpha 5$ IA-II (Fig. 23) exhibited a comparably high affinity for the benzodiazepine site of $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, or $\alpha 5\beta\gamma 2$ receptors ($K_i = 1.4, 2.7, 1.4$, or 0.8 nM, respectively). The affinity for $\alpha 4$ - or $\alpha 6\beta\gamma 2$ receptors was not investigated. $\alpha 5$ IA-II was claimed to be a selective negative allosteric modulator at $\alpha 5\beta\gamma 2$ receptors (Collinson et al., 2006). Nevertheless, there is no concentration where this compound modulates only $\alpha 5\beta\gamma 2$ receptors. Occupancy studies indicated that a dose of 1 mg/kg $\alpha 5$ IA-II produced sustained and high level occupancy in rats, with maximum occupancy (80%) being achieved within 15 minutes of dosing. Assuming that receptor occupation parallels receptor modulation, this indicates that an in vivo

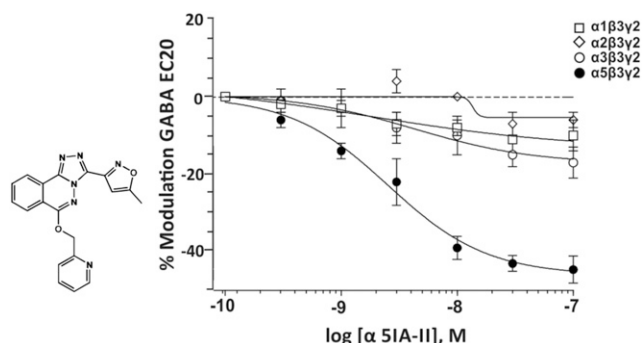


Fig. 23. Chemical structure and concentration-response curves of a5IA-II at various human recombinant GABA_A receptor subtypes stably expressed in L(tk-) mouse fibroblast cells and measured at GABA EC₂₀. Adapted with permission, Springer, *Psychopharmacology* (Berl), Collinson et al. (2006).

application of this drug at 1 mg/kg probably is able to modulate not only $\alpha 5\beta 3\gamma 2$ receptors. Behavioral studies indicated that $\alpha 5$ IA-II induced an enhancement of cognitive performance in the Morris water maze, affecting encoding and recall but not the consolidation phases of performance (Collinson et al., 2006).

4. MRK-016. The pyrazolotriazine MRK-016 (Fig. 24) exhibited a high and essentially equivalent affinity for the benzodiazepine binding site of human recombinant GABA_A receptors composed of $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, or $\alpha 5\beta 3\gamma 2$ subunits (K_i value range, 0.83–1.4 nM). The affinity was much lower for $\alpha 4$ - or $\alpha 6\beta 3\gamma 2$ receptors (K_i = 400 or 4100 nM, respectively). MRK-016 was declared to be a selective negative modulator at $\alpha 5$ receptors (Attack et al., 2009b). However, there is no concentration where this compound only modulates $\alpha 5\beta 3\gamma 2$ receptors (Fig. 24). Other experiments indicated that the maximal occupancies of GABA_A receptors in the rat brain after 1, 3, or 10 mg/kg orally, measured 0.5 hour after dosing, were 79%, 81%, and 91%, respectively, again suggesting that $\alpha 5\beta 3\gamma 2$ receptors were not exclusively modulated in vivo under these conditions (Attack et al., 2009b). MRK-016 increased long-term potentiation in mouse hippocampal slices to a greater extent than $\alpha 5$ IA, consistent with its greater $\alpha 5$ -inverse agonism, and enhanced cognitive performance in the Morris water maze. In mice,

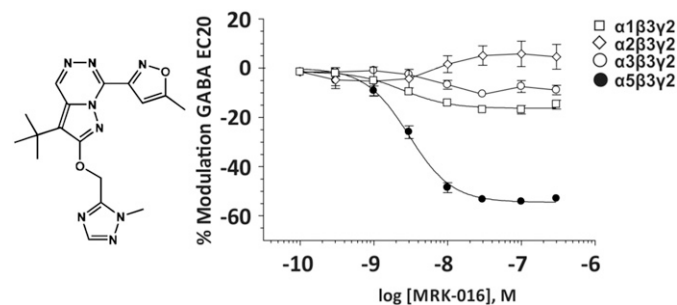


Fig. 24. Chemical structure and concentration-response curves of MRK-016 at various human recombinant GABA_A receptor subtypes stably expressed in L(tk-) mouse fibroblast cells and measured at GABA EC₂₀. Figure modified from Attack et al. (2009b).

it was not anxiogenic, not proconvulsant, and did not produce kindling. Nevertheless, it was poorly tolerated in elderly subjects, and this precluded its further development (Attack et al., 2009b). Given the recent evidence that negative allosteric modulators at $\alpha 5$ -containing GABA_A receptors improve cognitive function in young, but not in aged, rats (Koh et al., 2013), it is not surprising that this extremely strong negative allosteric modulator at $\alpha 5$ receptors was poorly tolerated in elderly subjects.

5. L-655,708. The imidazobenzodiazepine L-655,708 (Fig. 25) exhibited a 30–70-fold selectivity for $\alpha 5$ - compared with $\alpha 1$ -, $\alpha 2$ -, and $\alpha 3\beta 3\gamma 2$ receptors (K_i of 1, 70, 48, and 31 nM, respectively) in benzodiazepine binding studies and was described as a highly selective weak negative allosteric modulator at $\alpha 5\beta 3\gamma 2$ receptors as indicated by its concentration-response curves (Attack et al., 2006a). However, due to the exceptionally high potency of this compound at $\alpha 5\beta 3\gamma 2$ receptors, this is true up to only a 10 nM concentration (Fig. 25A). L-655,708 enhanced long-term potentiation in a mouse hippocampal slice model. Under conditions where it achieved 75% occupancy of $\alpha 5$ and 22% occupancy of $\alpha 1$, $\alpha 2$, and $\alpha 3$ receptors, L-655,708 enhanced cognition in the Morris water maze, but was devoid of proconvulsant activity.

The data presented, however, are different from our own data presented in Fig. 25B (Ramerstorfer et al., 2010).

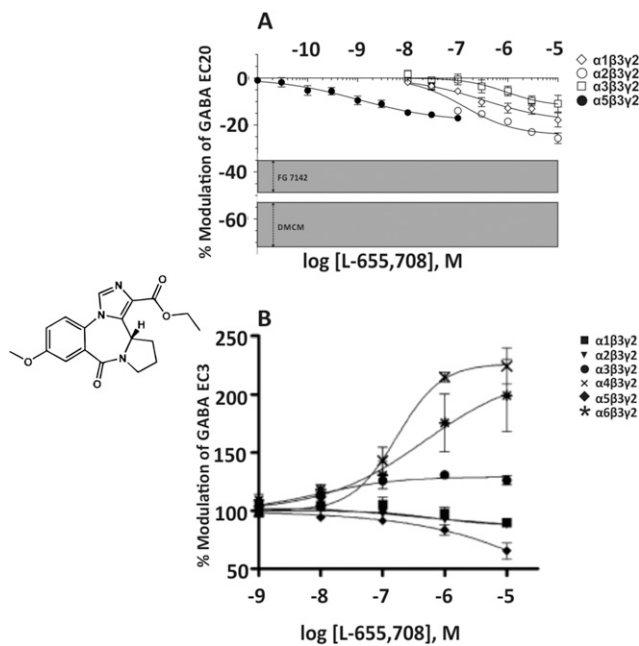


Fig. 25. Chemical structure and concentration-response curves of L-655,708. (A) Modified from Attack et al., *Neuropharmacology*, 51: 1023–1029, Elsevier, 2006a. Data were generated at various human recombinant GABA_A receptor subtypes stably expressed in L(tk-) mouse fibroblast cells and measured at GABA EC₂₀. The shaded areas represent the range of inverse agonist efficacies across subtypes for the nonselective partial inverse agonists FG7142 or the nonselective full inverse agonist DMCM. (B) Figure modified from Ramerstorfer et al., *Eur J Pharmacol*, 636,18–27, Elsevier, 2010. Data were generated at various rat recombinant GABA_A receptor subtypes expressed in *Xenopus laevis* oocytes and measured at GABA EC₃. The concentration-response curve of $\alpha 2\beta 3\gamma 2$ receptors is overlapping with that of $\alpha 1\beta 3\gamma 2$ receptors.

The opposite direction of $\alpha 3\beta 3\gamma 2$ receptor-modulation by this compound in the two data sets is especially puzzling. Presumably, the concentration of applied GABA (EC_{20} vs. EC_3) or the exact experimental conditions affected the final results. It can be assumed that at low GABA concentrations, predominantly one of the two GABA binding sites of $GABA_A$ receptors is occupied. At higher GABA concentrations, increasingly both sites at the receptors become occupied by GABA. Interaction of a compound with a receptor containing two occupied GABA sites might cause a conformational change different from that with only one site occupied, opening the possibility that extent and direction of the allosteric modulation by some compounds, depending on the receptor subtype investigated, could also depend on the GABA concentration used. However, so far, this possibility has not been systematically investigated for any compound. In any case, this compound, similar to other imidazobenzodiazepines, additionally exhibits significant positive modulatory effects at $\alpha 4\beta 3\gamma 2$ and $\alpha 6\beta 3\gamma 2$ receptors (Fig. 25B) that were not investigated in previous studies and could have contributed to the behavioral effects observed.

6. PWZ-029. The imidazobenzodiazepine PWZ-029 (Fig. 26) exhibited a moderate affinity for the benzodiazepine binding site of $\alpha 5\beta 3\gamma 2$ receptors (K_i of 38.8 nM) and K_i values of >300 nM for all other $GABA_A$ receptors investigated. In electrophysiological studies it was demonstrated to be a relatively selective negative allosteric modulator at $\alpha 5\beta 3\gamma 2$ receptors up to a concentration of 100 nM (Harris et al., 2008; Savić et al., 2008a), while this compound modulates other receptor subtypes by less than 10%. At concentrations >100 nM this compound exhibits weak positive modulatory effects at $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 4\beta 3\gamma 2$ or $\alpha 6\beta 3\gamma 2$ receptors (Harris et al., 2008). This compound was able to attenuate scopolamine-induced contextual memory impairment in mice (Harris et al., 2008) and improved passive avoidance learning in rats (Savić et al., 2008a), indicating that it facilitates some aspects of cognitive performance.

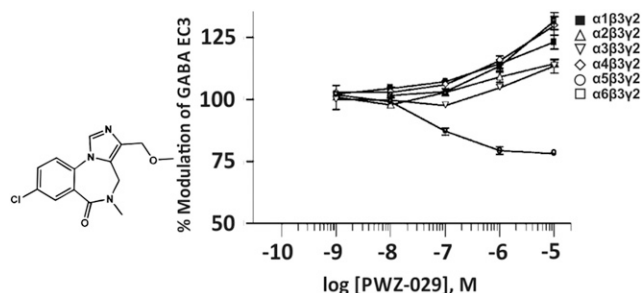


Fig. 26. Chemical structure and concentration-response curves of PWZ-029 at various rat recombinant $GABA_A$ receptors expressed in *Xenopus laevis* oocytes and measured at $GABA_{EC3}$. The concentration-response curves at $\alpha 4\beta 3\gamma 2$ and $\alpha 6\beta 3\gamma 2$ receptors were measured at $GABA_{EC20}$. Figure modified from Harris et al. (2008) with permission of The American Chemical Society.

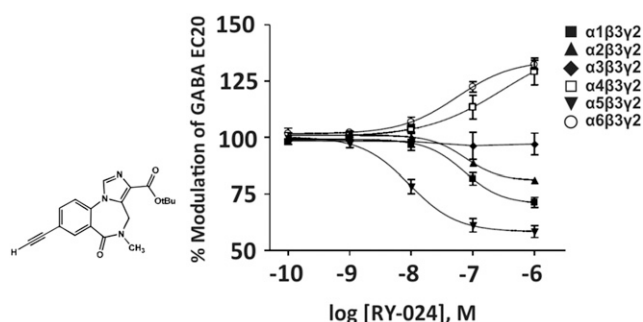


Fig. 27. Chemical structure and concentration-response curves of RY024 at various rat recombinant $GABA_A$ receptors expressed in *Xenopus laevis* oocytes and measured at $GABA_{EC20}$. Figure modified from Harris et al. (2008) with permission of The American Chemical Society.

7. RY-024. The imidazobenzodiazepine RY-024 (Fig. 27) exhibits nanomolar affinities for the benzodiazepine site of $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, $\alpha 5$ -, $\alpha 6\beta 3\gamma 2$ receptors (K_i values of 26.9, 26.3, 18.7, 0.4, 5.1 nM, respectively) and a unique spectrum of actions at these receptor subtypes (Harris et al., 2008). It is a negative allosteric modulator at $\alpha 5\beta 3\gamma 2$, $\alpha 1\beta 3\gamma 2$, and $\alpha 2\beta 3\gamma 2$; has no action via $\alpha 3\beta 3\gamma 2$; and is a positive allosteric modulator at $\alpha 4\beta 3\gamma 2$ and $\alpha 6\beta 3\gamma 2$ receptors. Due to its high potency for $\alpha 5$ receptors, this compound only up to a 10 nM concentration can be considered a more or less selective $\alpha 5\beta 3\gamma 2$ -negative modulator. Nevertheless, based on receptor binding studies only, which indicated an about 70-fold selectivity of this compound for $\alpha 5$ over $\alpha 1$ receptors, in experiments with RY024 it was claimed that $\alpha 5$ -containing receptors play an important role in regulating the reinforcing, motor-impairing, and sedative effects of alcohol in outbred rats (McKay et al., 2004).

8. RO4938581. The imidazobenzodiazepine RO4938581 (Fig. 28) has a high affinity (K_i of 4.6 nM) for the benzodiazepine site of rat $\alpha 5\beta 3\gamma 2$ receptors and a much lower affinity for that of $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, or $\alpha 3\beta 3\gamma 2$ receptors (K_i values of 174, 185, or 80 nM, respectively) and was also suggested to be an $\alpha 5$ -selective negative allosteric modulator (Ballard et al., 2009). The incomplete presentation of the concentration-response curves at

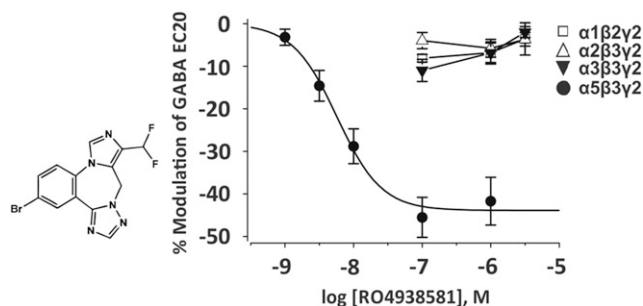


Fig. 28. Chemical structure and concentration-response curves of RO4938581 at various rat recombinant $GABA_A$ receptor subtypes stably expressed in HEK 293 cells and measured using the whole cell patch-clamp technique at $GABA_{EC20}$. Adapted with permission, Springer, *Psychopharmacology (Berl)*, Ballard et al. (2009).

the remaining receptor subtypes as well as the missing data for $\alpha 4\beta 3\gamma 2$ and $\alpha 6\beta 3\gamma 2$ receptors cast doubt on that claim. The degree of $\alpha 5$ receptor occupancy in the rat hippocampus produced by RO4938581 at oral doses of 0.1, 1.0, and 10 mg/kg was 30%, 74%, and 90%, respectively (Ballard et al., 2009). In experiments with mice, rats, and monkeys, this compound enhanced hippocampal long-term potentiation and was cognition enhancing at 30% receptor occupancy in the rat, while being devoid of anxiety-like and proconvulsant actions (Ballard et al., 2009).

9. TB21007. The 6,6-dimethyl-3-(2-hydroxyethyl)-thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one (TB21007) (Fig. 29, compound 43 in Chambers et al. (2003)) exhibits a 10- to 13-times selectivity for $\alpha 5\beta 3\gamma 2$ receptors in benzodiazepine binding studies (K_i of 20, 16, 20, and 1.6 nM, for human $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, and $\alpha 5\beta 3\gamma 2$ receptors, respectively) and acts as a strong negative allosteric modulator at the $\alpha 5\beta 3\gamma 2$ receptor subtype (Chambers et al., 2003). Concentration-response curves, however, indicate that due to the exceptionally high potency of this compound at $\alpha 5\beta 3\gamma 2$ receptors and the only 10–13-fold difference of its affinity and potency for $\alpha 5\beta 3\gamma 2$ and $\alpha 1\beta 3\gamma 2$ receptors, this compound is $\alpha 5$ -selective at only subnanomolar concentrations. TB21007 improved memory performance of young rats but not of rats exhibiting age-related memory impairment in a radial arm maze task (Koh et al., 2013). Interestingly, in rats with age-related memory impairment some poorly characterized positive allosteric modulators at $\alpha 5$ -containing receptors were effective in improving memory performance [compound 6 in van Niel et al. (2005) and compound 44 in Chambers et al. (2003)].

10. S44819. Recently, a substituted 8-methyl-5-[1-benzothiophen-2-yl]-1,9-dihydro-2*H*-[1,3]oxazolo[4,5-*h*][2,3]benzodiazepin-2-one (S44819) was claimed to be an $\alpha 5$ -GABA_A receptor-selective antagonist competitively inhibiting the action of GABA at these receptors (Ling et al., 2015; Etherington et al., 2017) (Fig. 30). This compound enhanced object recognition memory, long-term potentiation, blocked tonic current mediated by extrasynaptic $\alpha 5$ -GABA_A receptors, but had no effect

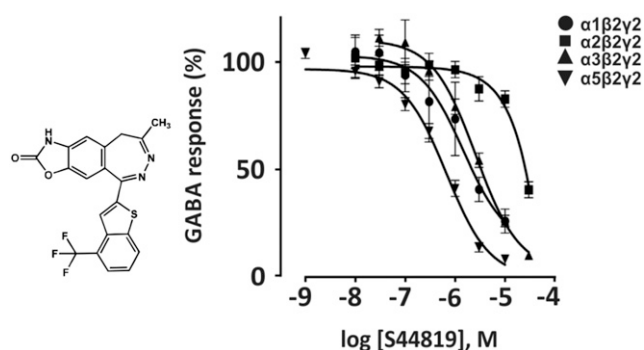


Fig. 30. Chemical structure and concentration-response curves of S44819 at various recombinant GABA_A receptor subtypes, stably expressed in HEK 293 cells and investigated by use of the fluorometric imaging plate reader dye assay at 1.6 μ M GABA (EC30–50). Recombinant receptors were composed of the human α -subunit isoforms, together with the rat $\beta 2$ S and rat $\gamma 2$ L subunits. Modified from Etherington et al., *Neuropharmacology*, 125,353–364, Elsevier, 2017.

on synaptic GABA_A receptors and reversed scopolamine-induced impairment of spatial working memory in the eight-arm radial maze. Data presented indicate, however, that S44819 exhibited only marginal receptor subtype selectivity. It preferentially inhibited GABA-induced currents at $\alpha 5\beta 2\gamma 2$ receptors in the concentration range of 1 nM to 10 μ M, with a binding constant of 221 nM. It required about threefold higher concentrations to inhibit $\alpha 1\beta 2\gamma 2$ or $\alpha 3\beta 2\gamma 2$ receptors to the same extent, with few effects on $\alpha 2\beta 2\gamma 2$ receptors up to 10 μ M concentrations (Etherington et al., 2017). This compound thus selectively inhibits $\alpha 5\beta 2\gamma 2$ receptors up to only 100 nM concentration in recombinant receptors. Nevertheless, it is the first in class compound that uniquely acts as a relatively potent, competitive, and selective antagonist of recombinant as well as native $\alpha 5$ -GABA_A receptors. Its apparent selectivity in vivo cannot be explained by its only weak selectivity for $\alpha 5\beta 3\gamma 2$ receptors. Its selectivity presumably is enhanced by the low GABA concentration acting at extrasynaptic $\alpha 5\beta \gamma 2$ receptors that can be more easily overcome by the competitive GABA-site antagonist than the much higher GABA concentrations acting at synaptic $\alpha 1\beta \gamma 2$ or $\alpha 3\beta \gamma 2$ receptors (Ling et al., 2015). In addition, the low potency of this compound also might contribute to its selectivity, because concentrations sufficient to inhibit significantly synaptic receptors might not be easily achieved in the brain.

11. XLi-093. The bivalent imidazobenzodiazepine XLi-093 (Fig. 31) has been identified as a selective antagonist at the benzodiazepine site of $\alpha 5\beta 3\gamma 2$ GABA_A receptors. XLi-093 exhibits an affinity of 15 nM for the benzodiazepine site of $\alpha 5\beta 3\gamma 2$ GABA_A receptors and a 60-fold to >100-fold lower affinity for $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, $\alpha 4$ -, or $\alpha 6\beta 3\gamma 2$ receptors. In electrophysiological experiments, it has been identified as a potent antagonist of the effects of diazepam at rat $\alpha 5\beta 3\gamma 2$ GABA_A receptors but not at $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, or $\alpha 3\beta 3\gamma 2$ GABA_A receptors (Li et al., 2003). This compound could be a valuable tool for

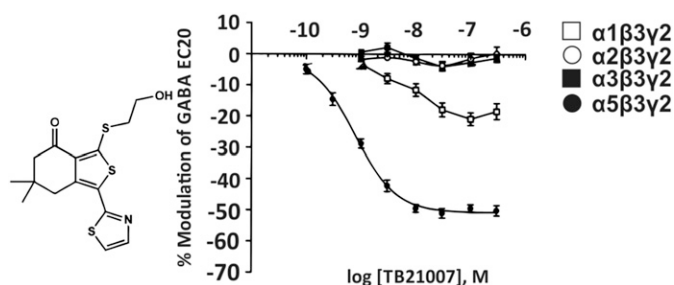


Fig. 29. Chemical structure and concentration-response curves of TB21007 at various human recombinant GABA_A receptor subtypes stably expressed in L(tk-) mouse fibroblast cells and measured at GABA EC20. Figure modified from Chambers et al. (2003) with permission of The American Chemical Society.

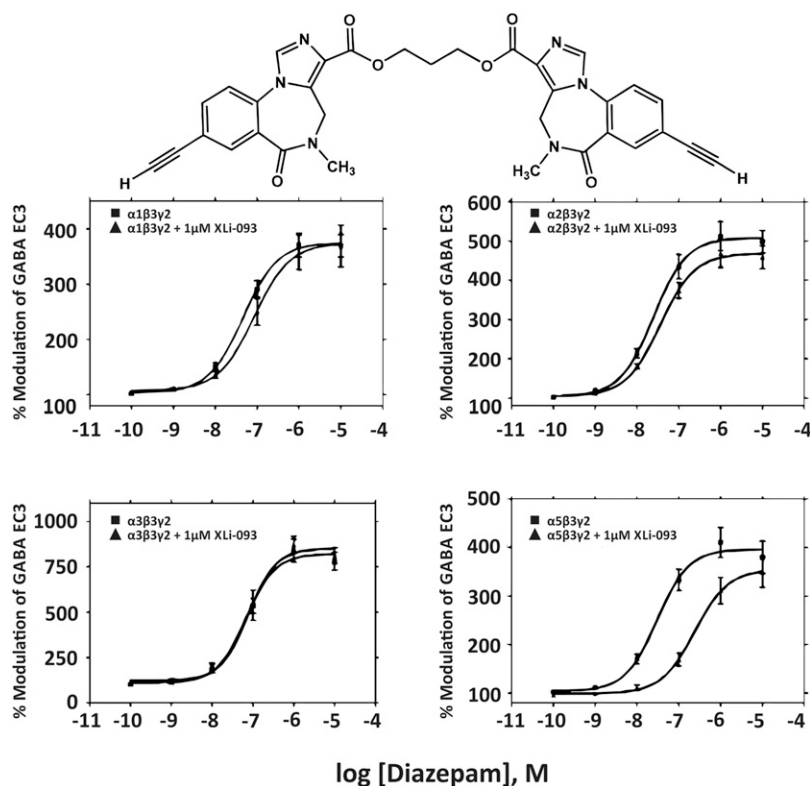


Fig. 31. Chemical structure of XLI-093 and its inhibition of the effects of diazepam at the diazepam-sensitive GABA_A receptor subtypes. The concentration-response curves show the effects of diazepam in the absence or presence of 1 μM XLI-093. Inhibition is shown by the right-shift of the diazepam concentration-response curve. Figure modified from Li et al. (2003) with permission of The American Chemical Society.

validating effects of compounds mediated via α5β3γ2 GABA_A receptors.

G. Compounds Preferentially Modulating α4βγ2 and/or α6βγ2 Receptors

GABA_A receptor subtypes composed of α6βγ2 (Nusser et al., 1998) and presumably also those composed of α4βγ2 are located synaptically as well as extrasynaptically and thereby contribute to phasic and tonic inhibition in the central nervous system, respectively. Whereas α4βγ2 receptors are located predominantly in the forebrain, hippocampus, and thalamus (Benke et al., 1997; Bencsits et al., 1999), GABA_A receptors composed of α6βγ2 are predominantly located in the cerebellar granule cells (Nusser et al., 1998; Pirker et al., 2000). Currently, not much is known about the function of α4βγ2 or α6βγ2 receptors.

1. CMD-45. The imidazobenzodiazepine CMD-45 (Fig. 32) preferentially enhances the action of GABA at α4β3γ2- and α6β3γ2-containing recombinant GABA_A receptors, presumably by acting via the benzodiazepine binding site. But this compound also modulates α1β3γ2, α2β3γ2, α3β3γ2, and α5β3γ2 receptors to a smaller extent. It was recently demonstrated that human airway smooth muscle cells among GABA_A receptor α subunits express only α4 and α5 subunits (Gallos et al., 2015) and that CMD-45 is able to significantly relax precontracted human airway smooth muscle cells ex vivo (Yocum et al.,

2016). This seems to indicate that selective targeting of α4-containing GABA_A receptors with inhaled ligands may be a novel therapeutic pathway to treat bronchoconstriction while avoiding sedative effects in the central nervous system, which are largely mediated by α1- and possibly also α3-containing GABA_A receptors (Ralvenius et al., 2015; Behlke et al., 2016; Yocum et al., 2016).

2. XHe-III-74. The structurally similar imidazobenzodiazepine XHe-III-74 (Fig. 33) exhibits a comparable preferential efficacy for the modulation of α4β3γ2 and α6β3γ2, but exhibits a weaker effect at α1β3γ2

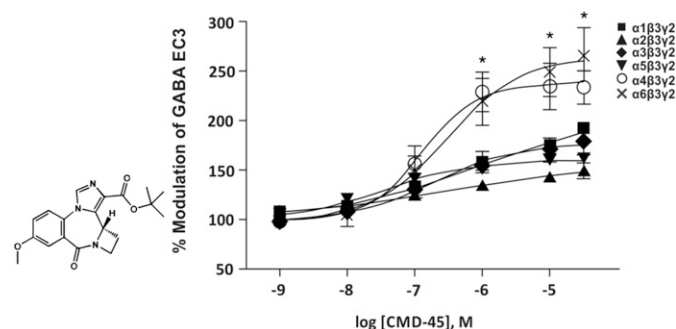


Fig. 32. Chemical structure of CMD-45 and concentration-response curves at various recombinant GABA_A receptor subtypes from the rat expressed in *Xenopus laevis* oocytes and measured at GABA EC₃. *Data for α4β3γ2 and α6β3γ2 are significantly different from those of α1β3γ2 receptors. Figure modified from Yocum et al. (2016) with permission of The American Thoracic Society. Copyright © 2016.

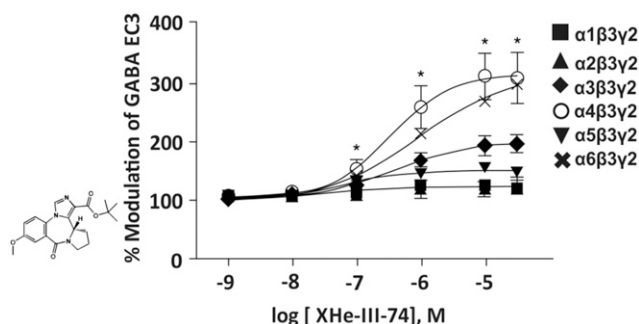


Fig. 33. Chemical structure and concentration-response curves of XHe-III-74 at various recombinant GABA_A receptor subtypes from the rat expressed in *Xenopus laevis* oocytes and measured at GABA EC₃. *Data for $\alpha 4\beta 3\gamma 2$ and $\alpha 6\beta 3\gamma 2$ are significantly different from those of $\alpha 1\beta 3\gamma 2$ receptors. Figure modified from Yocum et al. (2016) with permission of The American Thoracic Society. Copyright © 2016.

receptors than CMD-45. Similar to CMD-45, it significantly relaxed precontracted human airway smooth muscle cells ex vivo and reduced respiratory system resistance in an asthmatic mouse model in vivo (Yocum et al., 2016). These results were confirmed and extended by using the structurally similar XHe-III-74 ethyl ester and XHe-III-74 acid (Forkuo et al., 2016), but the respective concentration-response curves of the two latter compounds were only incompletely published.

3. *PZ-II-029* (Compound 6), *LAU159*, *LAU463*. The pyrazoloquinolinone PZ-II-029 (compound 6) (Fig. 34), as well as other structurally related compounds, are high affinity null modulators at the benzodiazepine binding sites of various GABA_A receptor subtypes but in addition also positively modulate $\alpha 6\beta 3\gamma 2$ receptors with low potency and exceptionally high selectivity via the $\alpha 6+\beta 3-$ interface (Varagic et al., 2013a). Recently, the structurally related LAU159 (8-chloro-2-(3-methoxyphenyl)-2H-pyrazolo[4,3-c]quinolin-3(5H)-one) has been demonstrated to show the highest functional selectivity for positive modulation at $\alpha 6\beta 3\gamma 2$ receptors with nearly no residual activity at the other $\alpha 1-5\beta 3\gamma 2$ receptors up to 10 μ M concentrations (Treven et al., 2018). PZ-II-029 and LAU159, together with LAU463 (7-bromo-

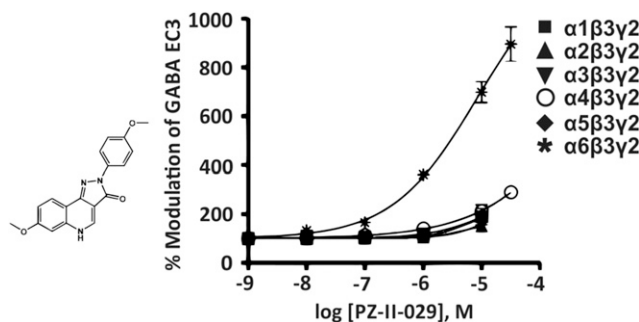


Fig. 34. Chemical structure and concentration-response curves of PZ-II-029 (compound 6) at a variety of recombinant GABA_A receptor subtypes from the rat expressed in *Xenopus laevis* oocytes and measured at GABA EC₃. Figure modified from Varagic et al. (2013a). Reprinted with permission of John Wiley & Sons, Inc.

2-(4-methoxyphenyl)-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one), another $\alpha 6\beta 3\gamma 2$ receptor-selective compound, were recently used as lead compounds for the development of a variety of deuterated $\alpha 6\beta 3\gamma 2$ receptor-selective compounds with increased metabolic stability and bioavailability (Knutson et al., 2018). These compounds achieve 100–300 nM concentrations in the rat brain in in vivo studies at 10 mg/kg (Knutson et al., 2018), and due to their low potency, this is sufficient to selectively modulate $\alpha 6\beta 3\gamma 2$ receptors. The recent demonstration that $\alpha 6\beta 3\gamma 2$ receptors not only occur in the cerebellar granule cells, the cochlea nucleus, olfactory bulb, spinal cord, and retina (Gutiérrez et al., 1996) but also in the trigeminal ganglia (Hayasaki et al., 2006), striatum (Leggio et al., 2015) and in the hippocampus (Yang et al., 2016), suggests that PZ-II-029, LAU159, and its congeners might have interesting applications in diseases in which this receptor subtype plays a role. This conclusion is supported by recent reports suggesting that $\alpha 6$ -containing GABA_A receptors may play a role in neuropsychiatric disorders with sensorimotor gating deficits, such as tic disorders, certain symptoms of schizophrenia, obsessive compulsive disorders, attention deficit disorders, and Huntington's chorea (Liao et al., 2016; Chiou et al., 2018), as well as in depression (Yang et al., 2016), trigeminal orofacial pain (Puri et al., 2012; Kramer and Bellinger, 2013), trigeminal neuropathy, and migraine (PCT/US2016/035761).

4. *(+)-ROD188*. Bicuculline is a competitive antagonist at the GABA binding site of GABA_A receptors. The bicuculline derivative *(+)-ROD188* (Fig. 35) is an allosteric modulator of GABA_A receptors (Thomet et al., 2000). Surprisingly, this compound does not interact with the GABA binding site but competitively inhibits [³H]Ro15-1788 binding with an IC₅₀ value of 33.1 μ M. Electrophysiological experiments, however, indicated that the positive allosteric modulation of GABA_A receptors by *(+)-ROD188* was inhibited only partially (29%) by Ro15-1788, indicating that most of the effects of this compound are mediated by a so far unidentified second

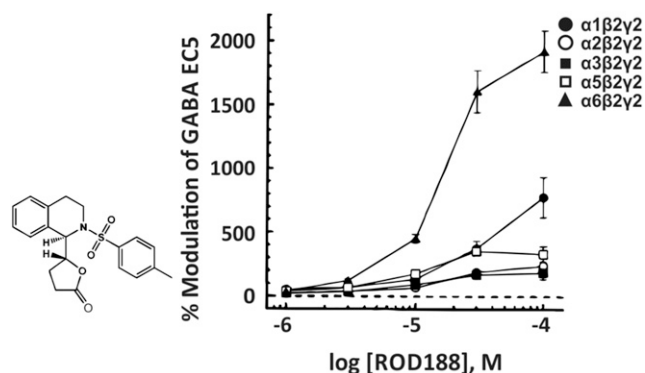


Fig. 35. Chemical structure and concentration-response curves of *(+)-ROD188* at various recombinant GABA_A receptor subtypes from the rat expressed in *Xenopus laevis* oocytes and measured at GABA EC₅. Figure modified from Thomet et al. (2000). Reprinted with permission of John Wiley & Sons, Inc.

site of action different from the benzodiazepine binding site. Concentration response curves shown in Fig. 35 indicate that this compound preferentially modulates $\alpha 6\beta 2\gamma 2$ receptors. The in vivo effects of this compound so far have not been investigated. In a subsequent study, some structural analogs of (+)ROD188 also induced a preferential modulation of $\alpha 6\beta 3\gamma 2$ receptors (Ramerstorfer et al., 2015). The absence of any direct effects at GABA_A receptors, as well as their potential selectivity for receptor subtypes, make this compound class suitable for drug discovery programs.

5. Amiloride. The diuretic amiloride (Fisher, 2002) is an antagonist at $\alpha 6\beta 3\gamma 2$ receptors, with no actions at $\alpha 1$ – $5\beta 3\gamma 2$ receptors up to 30 μ M concentrations (Fig. 36). It seems to act competitively via the GABA binding site and in addition seems to be an open channel blocker, and the $\alpha 6$ -subunit seems to confer higher affinity for both sites. Amiloride inhibition was only dependent on the $\alpha 6$ subunit and not influenced by the type of β or γ subunit or the presence of a δ subunit in GABA_A receptors (Fisher, 2002). However, its in vivo application is limited by the fact that amiloride does not readily cross the blood-brain barrier, by its inhibitory action at Na⁺ channels and transporters, and its additional modulation of other transmitter receptors (Fisher, 2002).

6. Furosemide. The diuretic furosemide (Fig. 37) is another relatively selective antagonist at $\alpha 6\beta 2/3\gamma 2$, $\alpha 6\beta 2/3$, and $\alpha 6\beta 2/3\delta$ receptors, with no interaction with receptor subtypes containing other α subunits up to 3 μ M concentrations. But furosemide does not interact with $\beta 1$ -containing receptors (Korpi et al., 1995; Wafford et al., 1996; Korpi and Lüddens, 1997) and presumably interacts with a binding site within the transmembrane domain of $\alpha 6$ subunits (Thompson et al., 1999), which is different from that of amiloride (Fisher, 2002). As with amiloride, its in vivo application is limited due to its diuretic properties.

H. Benzodiazepine Site Antagonist Actions

Currently, there are only a few compounds that are antagonists (null modulators) at the benzodiazepine

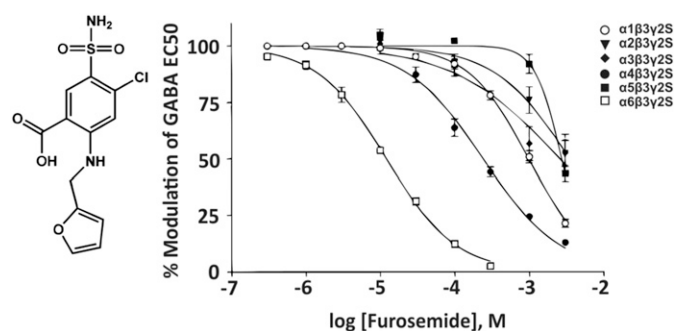


Fig. 37. Chemical structure and concentration-response curves of furosemide showing its inhibition of a GABA EC₅₀ response at human GABA_A receptors expressed in *Xenopus laevis* oocytes. Figure modified from Thompson et al. (1999).

site of GABA_A receptor subtypes. β -CCt (Fig. 4, see section V.B.4) is an antagonist at $\alpha 1$, and less avidly, at $\alpha 2$ receptors, but is a weak positive modulator at $\alpha 3$ and $\alpha 4$ and a negative modulator at $\alpha 5$ receptors (June et al., 2003; Yin et al., 2010). Flumazenil (Ro15-1788) (Figs. 4 and 5, see section V.B.5), is an antagonist at $\alpha 1$ receptors only, but a positive allosteric modulator at $\alpha 2$ -, $\alpha 3$ -, $\alpha 4$ -, and $\alpha 6$ -containing receptors and a weakly negative modulator of $\alpha 5\beta 3\gamma 2$ receptors (Ramerstorfer et al., 2010). The bivalent imidazobenzodiazepine XLI-093 (Fig. 31, see section V. F. 11) is a selective antagonist at the benzodiazepine site of $\alpha 5\beta 3\gamma 2$ GABA_A receptors (Li et al., 2003), but unfortunately its actions at $\alpha 4\beta \gamma 2$ and $\alpha 6\beta \gamma 2$ receptors have not been investigated. Since imidazobenzodiazepines usually can bind to the benzodiazepine site of $\alpha 4\beta \gamma 2$ and $\alpha 6\beta \gamma 2$ receptors, some actions of XLI-093 at these receptors can be expected. PZ-II-029 (Fig. 34, see section V.G.3) or LAU159 is a high affinity antagonist at the benzodiazepine site of $\alpha 1$ – $6\beta \gamma 2$ receptors, but low potency positive modulators of $\alpha 6\beta \gamma 2$ receptors by an additional action via the $\alpha + \beta$ -interface of GABA_A receptors (Varagic et al., 2013a).

It could be concluded that these compounds on systemic or local application are only silent at receptors for which they are null modulators, but in addition modulate other receptor subtypes according to their respective potency and efficacy. In addition, all these compounds are able to antagonize the actions of strongly

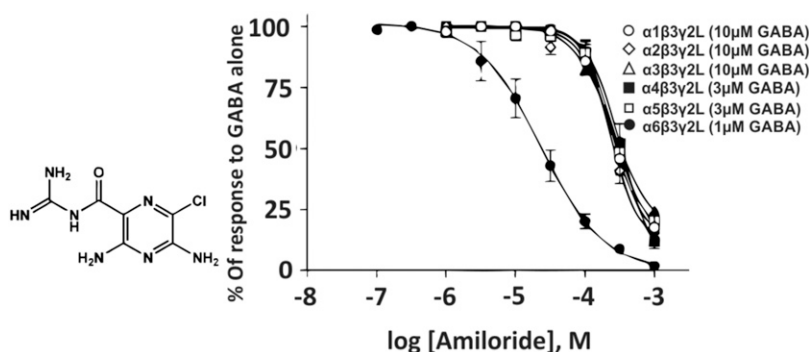


Fig. 36. Chemical structure and concentration-response curves of amiloride showing its inhibition of the currents elicited by 1 ($\alpha 6$), 3 ($\alpha 4, \alpha 5$), or 10 μ M ($\alpha 1, \alpha 2, \alpha 3$) GABA at various rat GABA_A receptor subtypes recombinantly expressed in mouse fibroblast cell line L929. Figure modified from Fisher (2002).

positive or negative allosteric modulators at the benzodiazepine site, at all receptor subtypes at which they exhibit a null modulatory, weakly positive, or weakly negative allosteric modulation.

But null modulators at the benzodiazepine site also may exert additional actions in the brain. More than 30 years ago, in addition to other compounds, a peptide was identified in the brain that is able to inhibit the binding of diazepam to brain membranes (diazepam binding inhibitor, DBI (Costa and Guidotti, 1985). DBI and other compounds with similar actions might thus be endogenous modulators of GABA_A receptors acting via the benzodiazepine site. Recently, new evidence for the existence and function of endogenous ligands for the benzodiazepine binding site has been accumulated (Möhler, 2014), and it seems clear now that endozepines, such as DBI or octadecaneuropeptide, might negatively or positively modulate the function of GABA_A receptors via the benzodiazepine site and by that modulate, for instance, neurogenesis or thalamic oscillations, respectively. Flumazenil seems to be able to inhibit the actions of these endogenous modulators (Möhler, 2014), and similar effects can also be expected from other benzodiazepine site null modulators. Thus antagonists at the benzodiazepine site are not necessarily silent but might interfere with the actions of endogenous modulators in the brain, and this could contribute to their spectrum of action in vivo (Hulse et al., 2015). Because the receptor subtype selectivity of endogenous modulators of the benzodiazepine site of GABA_A receptors currently is not known, the receptor subtype(s) mediating the behavioral action of a benzodiazepine site antagonist via blockade of endogenous modulators cannot be delineated. In addition, it is not possible to distinguish between effects of benzodiazepine site antagonists elicited by their direct modulation of some GABA_A receptor subtypes and effects elicited by blockade of endogenous ligands.

I. Compounds Claimed to Selectively Modulate β 1-containing GABA_A Receptors

The majority of GABA_A receptors is composed of two α , two β , and one γ or one δ subunit (Barnard et al., 1998; Olsen and Sieghart, 2008), and there is also evidence for the existence of receptors composed of $\alpha\beta$ subunits in the brain (Mortensen and Smart, 2006; Olsen and Sieghart, 2008). The latter receptors might be composed of two α and three β subunits (Tretter et al., 1997; Baumann et al., 2001), but different stoichiometries seem to be possible (Im et al., 1995; Boileau et al., 2005; Che Has et al., 2016). Receptors containing ε , θ , or π subunits also seem to contain β subunits (Olsen and Sieghart, 2008). Depending on the type of β subunit present in receptors, their regional, cellular, and subcellular distribution, as well as their function in the brain, might be different. Drugs that are able to selectively modulate receptors containing a specific β subunit only will thus exhibit more specific properties

and less unwanted effects than those that cannot differentiate between receptors containing different β subunits. In addition, there is ample evidence for the existence of two different α and/or two different β subunits in native GABA_A receptors (Jechlinger et al., 1998; Benke et al., 2004; Olsen and Sieghart, 2008). Depending on the types of α and β subunits within the receptors, their stoichiometry, and their subunit arrangement, the receptors contain distinct subunit interfaces and thus, exhibit distinct pharmacological properties that might allow for selectively targeting the respective receptor subtypes (Che Has et al., 2016). However, the pharmacology of receptor subtypes containing two different α or β subunits so far has not been sufficiently investigated. Depending on their regional, cellular, and subcellular location in the brain, they contribute to different types of behavior (Ralvenius et al., 2015). It thus can be expected that the behavioral selectivity of compounds can be increased if they not only can distinguish between different α but also between different β subunits or even between receptors containing different α and β subunit combinations. First evidence for this assumption is provided by the in vivo actions of compounds that can distinguish between receptors containing different types of β subunits.

1. Salicylidene Salicylhydrazide. By screening approximately 10,000 compounds from a structurally diverse screening library for their activity on $\alpha 2\beta 1\gamma 1\theta$, $\alpha 3\beta 3\gamma 2s$, and $\alpha 4\beta 3\gamma 2s$ GABA_A receptors at a single concentration (8 μ M) and using a high-throughput voltage/ion probe reader assay, salicylidene salicylhydrazide (Fig. 38), was identified as a potent selective inhibitor of $\alpha 2\beta 1\gamma 1\theta$, with a maximum inhibition of 56% and an IC₅₀ of about 32 nM (Thompson et al., 2004). By using patch-clamp electrophysiological techniques, it was then demonstrated that the compound in a limited set of receptor subtypes was selective for the $\beta 1$ subunit. The extent of inhibition was modulated by $\alpha 1$ and $\alpha 2$, $\gamma 1$, and $\gamma 2$, but was not dependent on the presence of θ subunits. Salicylidene salicylhydrazide produced incomplete inhibition on all $\beta 1$ -containing subtypes investigated and, hence, seemed not to act within the ion channel but via a so far unidentified binding site (Thompson et al., 2004). Although there are only limited biologic data with salicylidene salicylhydrazide, it is a known chelator of metal ions and in vitro has comparable cytotoxicity to cisplatin. In addition, this compound also exhibited poor in vivo pharmacokinetics and thus seems to be of limited use for the investigation of the function of $\beta 1$ subunit-containing receptors (Thompson et al., 2004).

2. Fragrant Dioxane Derivatives. By screening several libraries of odorants, fragrant (1,3)-dioxane derivatives were identified (Fig. 38) that enhance the action of GABA with six times higher potency at $\beta 1$ subunit-containing compared with the $\beta 2$ or $\beta 3$ subunit-containing GABA_A receptors (Sergeeva et al., 2010). In the limited set of recombinant receptors investigated, the effects of the fragrant dioxane derivatives depended

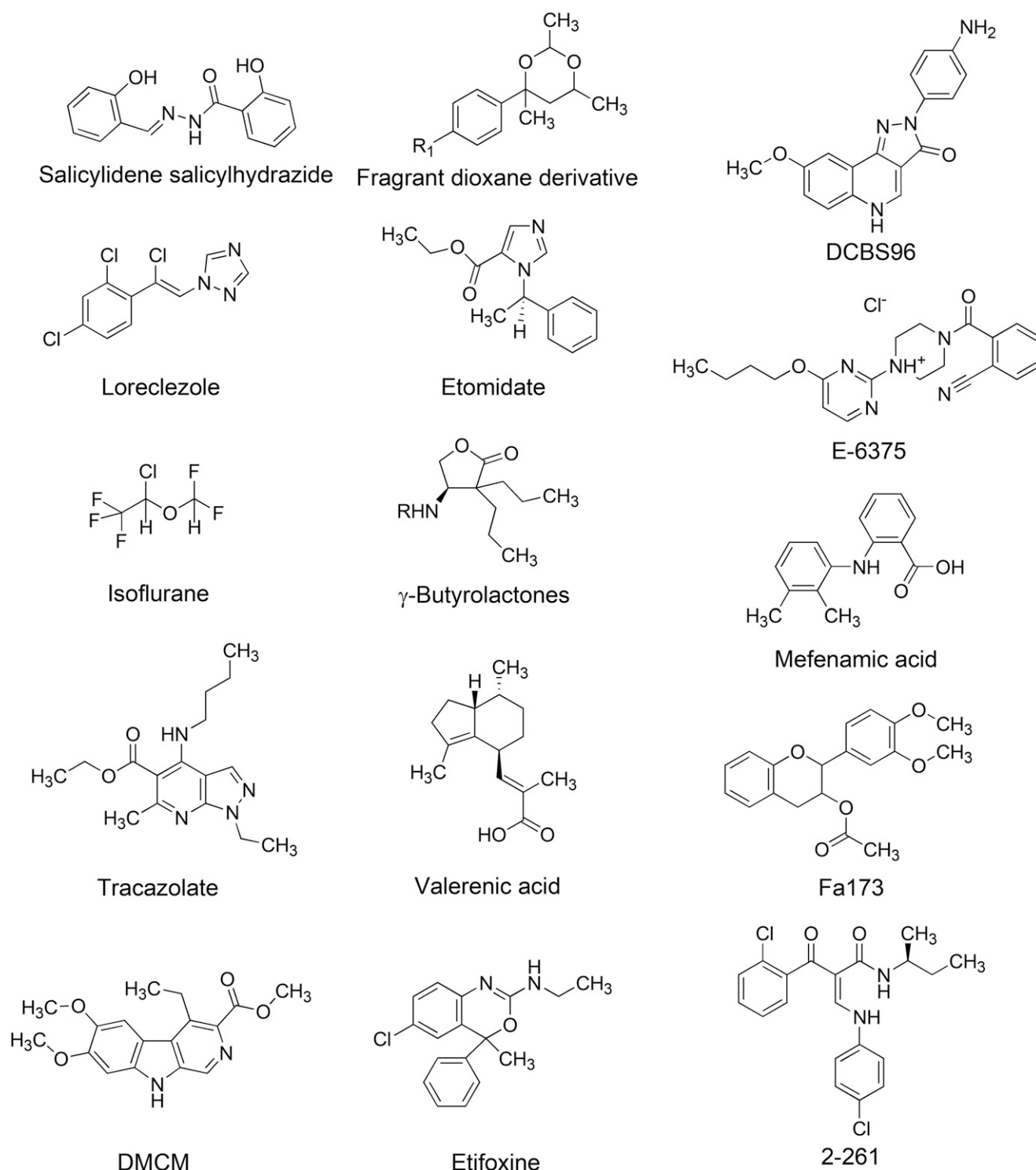


Fig. 38. Compounds selective for $\beta 1$ - or $\beta 2/3$ -containing GABA_A receptors.

only on the type of β subunit, were independent of the γ subunit, and obviously were similar in $\alpha 1$ - or $\alpha 2$ -containing receptors. These compounds act via a so far unidentified binding site at GABA_A receptors and up to a 10 μ M concentration selectively modulated $\alpha 1\beta 1\gamma 2$ receptors. With the help of these compounds, $\beta 1$ subunits were identified in synapses that modulate wake-promoting

histaminergic neurons in the posterior hypothalamus, indicating that $\beta 1$ -containing receptors might regulate wakefulness and sleep (Sergeeva et al., 2010).

3. Pyrazoloquinolinones. Pyrazoloquinolinones, such as CGS 9895 or PZ-II-029 (compound 6; see section V.G.3) in many cases are high-affinity antagonists (null modulators) at the benzodiazepine binding site ($\alpha + \gamma 2$ – interface)

of GABA_A receptors and exert their low-potency allosteric modulatory action of GABA_A receptors via an additional binding site at the $\alpha+\beta$ - interface (Ramerstorfer et al., 2011; Varagic et al., 2013b). Since both α and β subunits contribute to the latter interface, the action of these compounds is strongly dependent on the α as well as the β subunit type. Whereas CGS 9895 and PZ-II-029 preferentially modulate GABA_A receptors containing $\beta 2$ or $\beta 3$ subunits, some other pyrazoloquinolinones, such as PZ-II-028 (compound 11 of Varagic et al., 2013b), as well as some structural analogs thereof, preferentially or exclusively (DCBS96, Fig. 38) modulate GABA_A receptors containing $\beta 1$ subunits (Simeone et al., 2017). Unfortunately, however, the so far investigated compounds show only limited α selectivity and, thus, can only be used for investigating the effects of $\beta 1$ subunit-containing receptors irrespective of the associated α subunit type.

J. Compounds Claimed to Selectively Modulate $\beta 2/3$ -containing GABA_A Receptors

The majority of GABA_A receptors contain $\beta 2$ and/or $\beta 3$ subunits (Sieghart and Sperk, 2002), and so far, no compounds could be identified that can distinguish between receptors containing one or the other β subunit type, with the possible exception of Thio-THIP (Fig. 39; see section V.K.1.c). However, molecular genetic techniques provided evidence that $\beta 2$ - or $\beta 3$ -containing GABA_A receptors contribute to distinct behavioral actions of anesthetics.

1. Loreclezole, Etomidate, and Others. A variety of compounds have been claimed to be $\beta 2/3$ selective (Fig. 38), such as the anticonvulsant loreclezole (Wafford et al., 1994; Wingrove et al., 1994), the intravenous general anesthetics etomidate (Belelli et al., 1997; Hill-Venning et al., 1997) or E-6375 (Pau et al., 2003), the inhalation anesthetic isoflurane (Li et al., 2010), some γ -butyrolactones (El Hadri et al., 2002), mefenamic acid (Halliwell et al., 1999) and certain other nonsteroidal anti-inflammatory agents (Smith et al., 2004), the anxiolytic and anticonvulsant tracazolate (Thompson et al., 2002b; Smith et al., 2004), the anticonvulsant sesquiterpenoid valerenic acid and its derivatives (Khom et al., 2007, 2016), furosemide (Fig. 37) (Thompson et al., 1999), or the anxiolytic-like flavanoid Fa131 (Fig. 16) (Fernandez et al., 2012). All of these compounds, with the exception of etomidate, for which binding sites have been identified in the two $\beta+\alpha$ - transmembrane interfaces of $\alpha 1\beta 2/3\gamma 2$ receptors (Feng and Forman, 2018), are allosteric modulators at GABA_A receptors acting via so far unidentified binding sites. But actually they are not $\beta 2/3$ selective at all, and only preferentially modulate $\beta 2/3$ -containing GABA_A receptors. In addition, they also modulate $\beta 1$ -containing receptors and sometimes to a quite significant extent (Gee et al., 2010).

Loreclezole was the first one of these compounds identified, and it was demonstrated that the preferential interaction of loreclezole with $\beta 2$ - or $\beta 3$ -containing

GABA_A receptors depended on a single asparagine residue in the second transmembrane domain of β subunits ($\beta 2$ Asn289 or $\beta 3$ Asn290, when numbered including the signal peptide, or Asn265 in the $\beta 2$ and $\beta 3$ subunit, numbering of the mature subunits) that was different from the serine at the homologous position in $\beta 1$ subunits (Wingrove et al., 1994). Since the $\beta 2/3$ selectivity of all the above mentioned compounds depended on the same amino acid residues, all these compounds were claimed to act via this “loreclezole binding site.” These residues, however, are not necessarily near the respective binding sites but might be involved in the signal transduction of the drug effects to the ion channel.

Interestingly, the flavanoid Fa173 (Fig. 38) was demonstrated to be an antagonist of the positive modulatory actions of the flavanoid Fa131 (Fig. 16; see section V.D.9), as well as of etomidate (Fig. 38), loreclezole (Fig. 38), and the GABA-potentiating effects induced by high, but not low, concentrations of diazepam at $\alpha 1\beta 2$ and $\alpha 1\beta 2\gamma 2L$ receptors. The action of all these compounds could be reduced by the point mutation $\beta 2$ Asn265Ser (Fernandez et al., 2012). These results reinforce the idea that these compounds either share their binding pocket or activation domains. Fa173, however, did not block the modulatory actions of the neuroactive steroid 5α -pregnan- 3α -ol-20-one, the barbiturate thiopental, and the anesthetic propofol at GABA-induced currents, suggesting that these compounds act via a binding site different from that of loreclezole and etomidate (Fernandez et al., 2012). Further investigations using this antagonistic compound might answer the question whether the effects of Fa173 occur through competition for the loreclezole, etomidate, flavanoid site (loreclezole site), or via another allosteric site, blocking the signal transduction of these compounds. In any case, Fa173 represents a lead compound in the development of novel antagonists at GABA_A receptors (Fernandez et al., 2012).

The convulsant β -carboline DMCM (Fig. 38) is a benzodiazepine site ligand with negative allosteric modulatory actions at most GABA_A receptor subtypes below 1 μ M concentration. At higher concentrations, this compound turns into a positive allosteric modulator, suggesting interaction with a second binding site at GABA_A receptors (Ramerstorfer et al., 2010). At $\alpha 6\beta 3\gamma 2$ receptors, however, DMCM does not exhibit a negative modulation of GABA-induced currents and is a positive allosteric modulator above 100 nM concentrations (Ramerstorfer et al., 2010). This positive allosteric modulation by $>1 \mu$ M DMCM at all receptor subtypes investigated was not inhibited by the benzodiazepine site antagonist Ro15-1788, was only observed at $\beta 2$ - or $\beta 3$ -containing receptors and was dependent on the presence of $\beta 3$ Asn290 (numbering including signal peptide) (Stevenson et al., 1995). Therefore, DMCM not only interacts with the benzodiazepine site at nanomolar concentrations but also with the “loreclezole site” at $>1 \mu$ M concentrations.

2. *Etifoxine and Polyacetylene Compounds.* In contrast to the compounds mentioned above, the anxiolytic-like and anticonvulsant etifoxine (Fig. 38) (Schlichter et al., 2000; Hamon et al., 2003) or some polyacetylene compounds (Baur et al., 2005) might mediate their preferential modulation of $\beta 2$ - or $\beta 3$ -containing receptors via an unidentified site different from the "lorecleazole site." Interestingly, etifoxine might elicit its anxiolytic-like action at least partially via an additional interaction with the so called "peripheral benzodiazepine receptor," now called the 18-kDa translocator protein (TSPO) that seems to be involved in the regulation of neurosteroid synthesis and is also discussed as a target for anxiolytic drugs (Costa et al., 2012; Nothdurfter et al., 2012).

3. *$\beta 2/3$ -Selective Enaminones (Compound 2-261).* Empirical observations of the $\beta 2/3$ selective compounds lorecleazole, mefenamic acid, tracazolate, and etifoxine (section V.J.1) in both animals and humans provided anecdotal evidence for the possibility that the degree of activation of $\beta 1$ subunit-containing GABA_A receptors may contribute to their sedative/ataxic properties (Gee et al., 2010). Based on this hypothesis, several enaminones were investigated for their positive allosteric modulation of $\alpha 1\beta 1\gamma 2$ or $\alpha 1\beta 2\gamma 2$ GABA_A receptors. These compounds allosterically modulate GABA_A receptors via a so far unidentified binding site and compared with other $\beta 2/3$ -selective compounds, such as lorecleazole, tracazolate, or etomidate, exert a dramatically reduced maximal efficacy at $\alpha 1\beta 1\gamma 2$ over $\alpha 1\beta 2\gamma 2$ GABA_A receptors (Gee et al., 2010). GABA-dependent modulation by the prototypic compound 2-261 (Fig. 38) at $\beta 2/3$ -containing receptors did not appear to be strongly dependent on the type of α subunits. This compound showed equivalent maximal modulation at $\alpha 1$ -, $\alpha 2$ -, or $\alpha 3\beta 2/3\gamma 2$ receptors. Compound 2-261 produced anxiolytic-like actions with reduced ataxic or sleep-inducing effects (Gee et al., 2010; Yanovsky et al., 2012), and the degree of sedation/ataxia induced by different enaminones or other $\beta 2/3$ -selective compounds (section V.J.1) correlated with their activity at $\alpha 1\beta 1\gamma 2$ receptors. Compounds with high efficacy at $\beta 1$ -containing GABA_A receptors induced rotarod failures, whereas those with low activity at these receptors did not. In addition, it was demonstrated that compounds that reach brain levels associated with >47% stimulation of the $\alpha 1\beta 1\gamma 2$ receptor elicit rotarod deficits regardless of the potency of the compound (Gee et al., 2010).

4. *Function of $\beta 2$ or $\beta 3$ Subunit-containing GABA_A Receptors.* Given the relatively low selectivity of the $\beta 2/3$ -selective compounds, their inability to distinguish between $\beta 2$ - or $\beta 3$ -containing receptors, their differential interaction with various GABA_A receptor subtypes, and their widely different effects in vivo, these compounds could not be used to delineate a possible function of $\beta 2$ - or $\beta 3$ -containing receptors in the brain. Nevertheless, using mice harboring point mutated $\beta 2$ or $\beta 3$ subunits that drastically reduced the effects of etomidate,

lorecleazole, or of the inhalation anesthetic isoflurane, either in $\beta 2$ ($\beta 2\text{Asn265Ser}$) (Reynolds et al., 2003; Groves et al., 2006) or $\beta 3$ ($\beta 3\text{Asn265Meth}$) (Jurd et al., 2003; Lambert et al., 2005) containing receptors, some attribution of the in vivo functions of $\beta 2$ or $\beta 3$ subunit-containing receptors was still possible. The anticonvulsant effects of lorecleazole seem at least partially to be mediated via $\beta 2$ -containing receptors (Reynolds et al., 2003; Belelli et al., 2005; Zeller et al., 2005; Groves et al., 2006). The hypothermic and cardiac depressant effects of etomidate seem to be predominantly mediated by $\beta 2$ -containing receptors (Zeller et al., 2005), suggesting that avoiding $\beta 2$ -containing receptor modulation should lead to an improved recovery after anesthesia (Cirone et al., 2004). The sedative action of etomidate (action on locomotor activity) is mediated by $\beta 2$ -containing GABA_A receptors (Reynolds et al., 2003). The hypnotic actions of etomidate and propofol (loss of righting reflex) seem to be mediated via both $\beta 3$ and $\beta 2$ receptors (Jurd et al., 2003; Reynolds et al., 2003; Zeller et al., 2005). In contrast, both the immobilizing action of etomidate and of propofol and their induction of respiratory depression seem to be mediated via $\beta 3$ -containing receptors (Jurd et al., 2003; Reynolds et al., 2003; Zeller et al., 2005, 2007). The anterograde amnesic action of propofol is independent of $\beta 3$ -containing receptors (Zeller et al., 2007). The type(s) of α subunits present in receptors mediating the individual effects of anesthetics, however, could not be identified by these experiments. In addition, these experiments could not answer the question whether the respective receptors were of the $\alpha\beta$, $\alpha\beta\gamma$, $\alpha\beta\delta$, $\alpha\beta\epsilon$ type or whether they contained $\gamma 1$, $\gamma 2$, or $\gamma 3$ subunits.

K. Compounds Claimed To Modulate Selectively δ -Containing GABA_A Receptors

GABA_A receptors containing δ subunits seem to be exclusively located extrasynaptically (Nusser et al., 1998) and contribute to the tonic inhibition of neurons in the brain that modulates both cell and network behavior (Farrant and Nusser, 2005). They seem to be dynamically expressed during ovarian cycle, stress, and puberty (Shen et al., 2017), and evidence has been accumulated that drugs selectively modulating δ -containing GABA_A receptors might be beneficial for a variety of human disorders (Brickley and Mody, 2012). However, studies with concatenated $\alpha 1\beta 3\delta$ GABA_A receptors have indicated that there might be several possibilities for the incorporation of a δ subunit in recombinant receptors (Kaur et al., 2009; Eaton et al., 2014; Botzolakis et al., 2016; Wongsamitkul et al., 2016). Depending on the subunit stoichiometry and arrangement, these receptors exhibit distinct properties. This conclusion is supported by the finding that nonconcatenated $\alpha 6\beta\delta$ (Hadley and Amin, 2007) and $\alpha 4\beta 1/3\delta$ (Karim et al., 2012b) receptors can be activated by GABA with a nanomolar and a micromolar potency and that the latter

receptor might form a novel GABA-binding site at the δ subunit interface (Karim et al., 2012b). The mixture of recombinant receptors formed will thus determine the pharmacological properties measured (Hartiadi et al., 2016). If receptors with distinct stoichiometry and subunit arrangement are also present in the brain, they represent novel receptor subtypes that, depending on their regional, cellular, and subcellular distribution, will modulate distinct brain functions.

1. γ -Aminobutyric Acid Site Ligands. GABA and orthosteric GABA-site agonists, such as muscimol, isoguvacine, or THIP, exhibit up to 100-fold difference in their potency and efficacy for activation of various GABA_A receptor subtypes (Fig. 39A) (Ebert et al., 1994; Ducić et al., 1995; Frølund et al., 2002; Mortensen et al., 2012; Karim et al., 2013).

a. THIP (gaboxadol). The GABA agonist THIP (gaboxadol, Fig. 39A) has been shown to be devoid of the neurotoxic properties of muscimol and, in contrast to muscimol, is metabolically stable. It is less potent than GABA but approximately 10 times more potent at δ -containing receptors than at γ 2S-containing receptors (Brown et al., 2002; Frølund et al., 2002) and in contrast to GABA that is a partial agonist at δ -containing receptors (Bianchi and Macdonald, 2003; Meera et al., 2011), it is a full agonist at these receptors and thus elicits a markedly larger response than GABA. This view, however, was recently challenged by demonstrating that THIP at α 6 β 3 δ receptors exhibits a high potency but low efficacy, whereas at the contaminating α 6 β 3 receptors it exhibits a low potency high efficacy modulation (Meera et al., 2011). The previously observed high efficacy modulation by THIP of α 6 β 3 δ receptors might thus have been mediated by simultaneously formed α 6 β 3 receptors, which not only might be present in recombinant expression systems but also in the brain. Due to its sleep-inducing and analgesic action (Krogsgaard-Larsen et al., 2004; Wafford and Ebert, 2006), gaboxadol was developed as a hypnotic drug with no tolerance to sleep EEG and sedative effects after repeated daily dosing (Ebert et al., 2008), but its clinical development was abandoned due to safety concerns. In any case, this compound provided some insight on the role of δ -containing GABA_A receptors in sedation and sleep (Krogsgaard-Larsen et al., 2004; Herd et al., 2009). It has to be kept in mind, however, that THIP is also an antagonist at ρ -containing GABA_A receptors (Chebib, 2004). The interpretation of the *in vivo* results of this compound may therefore be more equivocal than previously assumed.

b. Thio-4-PIOL. The GABA site agonist Thio-4-PIOL (Fig. 39A) displayed substantial partial agonist activity of up to 30% relative to the effect of GABA at the human extrasynaptic GABA_A receptor subtypes composed of α 5 β 3 γ 2s, α 4 β 3 δ , and α 6 β 3 δ , and somewhat lower efficacies (4%–12%) at the corresponding α 5 β 2 γ 2s, α 4 β 2 δ , and α 6 β 2 δ subtypes (Hoestgaard-Jensen et al., 2013). In

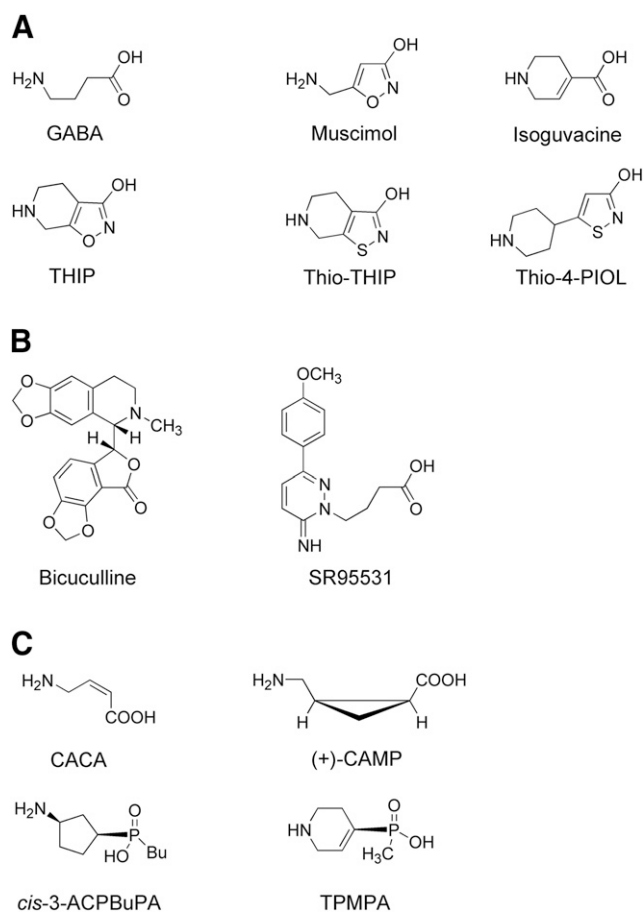


Fig. 39. GABA-site ligands. (A) GABA-site agonists at $\alpha\beta\gamma$ receptors. (B) GABA-site antagonists at $\alpha\beta\gamma$ receptors. (C) GABA-site agonists and antagonists at ρ -containing receptors.

contrast, it was an antagonist at the synaptic GABA_A receptors composed of α 1 β 2,3 γ 2S, α 2 β 2,3 γ 2S, and α 3 β 2,3 γ 2S (maximal responses of 0%–4% of the GABA current). Thio-4-PIOL thus possibly could be used for exploring the physiologic roles of native synaptic and extrasynaptic GABA_A receptors (Hoestgaard-Jensen et al., 2013).

c. Thio-THIP. The GABA site agonist Thio-THIP (Fig. 39A) displayed weak antagonistic activity at α 1,2,5 β 2,3 γ 2s and ρ 1 receptors and partial agonism at α 6 β 2,3 δ receptors. It also exhibited a pronounced agonism at α 4 β 1 δ and α 4 β 3 δ and a negligible activity at α 4 β 2 δ receptors. Thio-THIP is thus the first published ligand capable of discriminating between β 2- and β 3-containing receptor subtypes and could be a valuable tool for the exploration of native α 4 β δ GABA_A receptors (Hoestgaard-Jensen et al., 2014).

d. GABA site antagonists. While the potency of GABA site agonists and modulators of GABA_A receptors varies with subunit composition, the potency of GABA site antagonists such as bicuculline, SR95531 (Fig. 39B), and others is largely independent of receptor subunit composition (Lüddens and Korpi, 1995; Ebert et al., 1997; Frølund et al., 2002; Johnston, 2013).

Recently, however, the compound S44819 was claimed to be an orthosteric, competitive, $\alpha 5$ -GABA_A receptor-selective antagonist (Ling et al., 2015; Etherington et al., 2017; see Fig. 30, section V.F.10). Although this compound exhibited only an about threefold selectivity for $\alpha 5\beta 3\gamma 2$ over $\alpha 1\beta 3\gamma 2$ or $\alpha 3\beta 3\gamma 2$ receptors when recombinantly expressed, its selectivity in vivo is enhanced due to the low extrasynaptic GABA concentrations at $\alpha 5\beta 3\gamma 2$ receptors, which can be more easily overcome by this antagonist than the high synaptic GABA concentrations at $\alpha 1\beta 3\gamma 2$ or $\alpha 3\beta 3\gamma 2$ receptors (Ling et al., 2015; see section V.F.10). In addition, the low potency of this compound also might have contributed to its selectivity in vivo, because concentrations sufficient to inhibit significantly fully saturated synaptic receptors might not be easily achieved in the brain. So far the action of S44819 at δ -containing receptors has not been investigated. It can be assumed, however, that it also will preferentially inhibit $\alpha 5\beta \delta$ over other δ -containing receptors.

In addition, the diuretic amiloride (Fig. 36; see section V.G.5) is a relative selective antagonist at $\alpha 6\beta 1-3\gamma 1-3$, or $\alpha 6\beta 3\delta$ receptors and seems to mediate its action primarily as a competitive antagonist of the GABA binding site of these receptors (Fisher, 2002).

2. Neurosteroids and Tracazolate. The anxiolytic, sedative, and anticonvulsant neurosteroids seem to act via several neurosteroid binding sites in the transmembrane domain of GABA_A receptors that still have not been unequivocally identified and might differ in different receptor subtypes (Seljeset et al., 2015; Lavery et al., 2017). They are able to modulate δ -containing receptors to a much stronger extent than other GABA_A receptors presumably by shifting the physiologic GABA-induced partial agonist activation of δ -containing GABA_A receptors from low- to high-efficacy gating patterns (Belelli et al., 2002; Bianchi and Macdonald, 2003; but see also Meera et al., 2011). The same seems to hold true for the nonsedative anxiolytic-like and anticonvulsant pyrazolopyridine tracazolate (Fig. 38) that seems to act via a so far unidentified binding site at GABA_A receptors (Thompson et al., 2002b; Zheleznova et al., 2008). This compound, however, is also interacting with adenosine receptors and phosphodiesterases (Thompson et al., 2002b).

3. Ketamine. The chiral arylcyclohexylamine (Fig. 40) is a dissociative anesthetic capable of inducing analgesia, psychomimetic behavior, and a catatonic state of unconsciousness. It noncompetitively inhibits NMDA receptors, and at anesthetically relevant concentrations is also a positive allosteric modulator at GABA_A receptors. Up to concentrations of 20 μ M ketamine exhibits some selectivity for $\alpha 6\beta 2\delta$ - and $\alpha 6\beta 3\delta$ -containing receptors compared with a limited set of other GABA_A receptor subtypes ($\alpha 1\beta 2\gamma 2$, $\alpha 1\beta 2$, $\alpha 1\beta 2\delta$, $\alpha 4\beta 2\gamma 2$, $\alpha 4\beta 2\delta$, $\alpha 6\beta 2\gamma 2$) (Hevers et al., 2008). At higher concentrations, ketamine directly activates both $\alpha 6\beta 2\delta$ - and $\alpha 6\beta 3\delta$ -receptor subtypes.

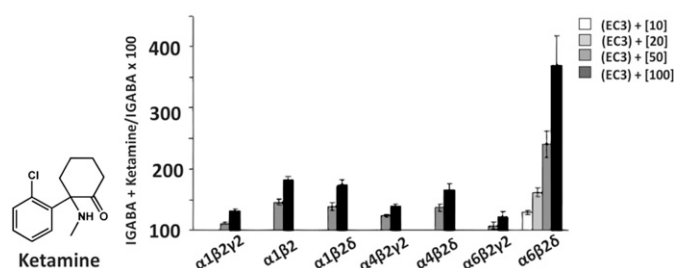


Fig. 40. Chemical structure of ketamine and bar graphs representing the average of ketamine-dependent potentiation (at 10, 20, 50, and 100 μ M ketamine) of the indicated rat recombinant receptors at GABA EC₃. Figure modified from Hevers et al. (2008) with permission of the Society of Neuroscience.

4. DS2. The imidazopyridine DS2 (Fig. 41) is a relatively selective positive modulator of δ -containing receptors (Wafford et al., 2009). It exhibits weak effects at $\alpha 1\beta 2\gamma 2$, $\alpha 2\beta 2\gamma 2$, $\alpha 3\beta 2\gamma 2$, and $\alpha 5\beta 2\gamma 2$ receptors, but strongly modulates $\alpha 4\beta 1\delta$, $\alpha 4\beta 2\delta$, $\alpha 4\beta 3\delta$, and $\alpha 6\beta 2\delta$ receptors (Jensen et al., 2013). It interacts with a novel binding site at GABA_A receptors that so far has not been identified. The bar graph in Fig. 41 shows the extremely strong potentiation of GABA-induced currents by this compound at δ -containing GABA_A receptors. Unfortunately, however, in vivo experiments with this compound are hampered because of its very poor brain penetration. Nevertheless, this compound can be used in slice experiments to clarify the function of δ -containing GABA_A receptors (Ye et al., 2013).

5. Methaqualone. The sedative-hypnotic and recreational drug methaqualone (Fig. 42) was demonstrated to be a positive allosteric modulator at human $\alpha 1,2,3,5\beta 2,3\gamma 2$ s GABA_A receptors, whereas it displayed highly diverse functionalities at the $\alpha 4,6\beta 1,2,3\delta$ GABA_A receptor subtypes, ranging from inactivity ($\alpha 4\beta 1\delta$)

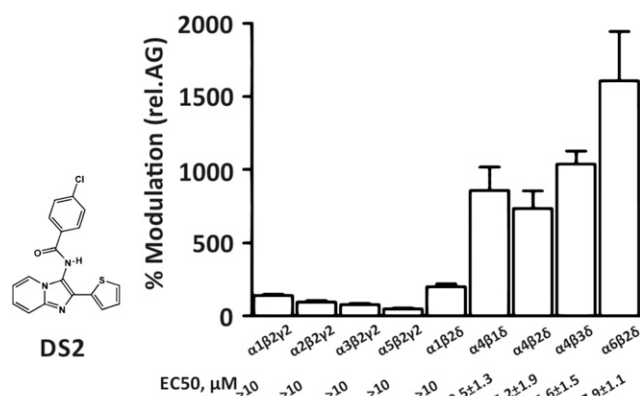


Fig. 41. Chemical structure of DS2 and bar graphs indicating the modulation by 10 μ M DS2 of the indicated human recombinant receptor subtypes expressed in *Xenopus laevis* oocytes and stimulated by GABA EC₅–20 (for $\alpha 1$ – $5\beta 2\gamma 2$) or GABA EC₂₀–50 for $\alpha 1\beta 2\delta$ and $\alpha 4\beta 1$ – 3δ and $\alpha 6\beta 2\delta$ relative to the action of the agonist GABA (rel.AG). In addition, EC₅₀ values of DS2 were determined from full concentration-response curves and given below the indicated receptor subtypes. Unfortunately, most of these full concentration-response curves were not published. Figure modified from Jensen et al. (2013). Reprinted with permission of John Wiley & Sons, Inc.

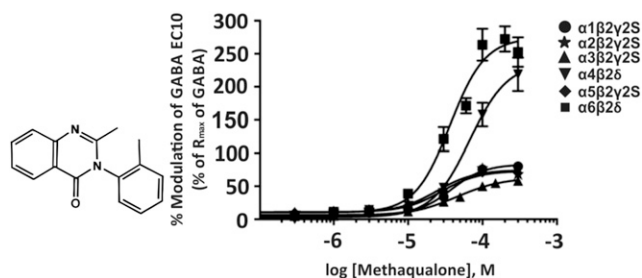


Fig. 42. Chemical structure and concentration-response curves of methaqualone at various human GABA_A receptors expressed in *Xenopus* oocytes, measured at GABA EC₁₀. Data were normalized to the maximal response elicited by GABA on each oocyte. Figure modified from Hammer et al. (2015).

through negative ($\alpha 6\beta 1\delta$) or positive allosteric modulation ($\alpha 4\beta 2\delta$, $\alpha 6\beta 2,3\delta$) to extremely strong effects at $\alpha 4\beta 3\delta$ (Hammer et al., 2015). Methaqualone was proposed to interact with the transmembrane $\beta + \alpha$ -interface, possibly targeting a site overlapping with that of the general anesthetic etomidate. Methaqualone exhibited negligible activities at numerous neurotransmitter receptors and transporters, suggesting that it is a selective GABA_A receptor modulator. In addition, the doses producing significant *in vivo* effects in assays for locomotion and anticonvulsant activity correlated fairly well with its potencies as a modulator of recombinant GABA_A receptors (Hammer et al., 2015).

L. Compounds Modulating $\gamma 1$ - or $\gamma 3$ -Containing GABA_A Receptors

GABA_A receptors containing $\gamma 2$ subunits are the major GABA_A receptor subtypes in the brain. Sixty to seventy percent of all GABA_A receptors contain this subunit (Sieghart and Sperk, 2002), and most of the compounds discussed so far exert their actions at least partially via these receptors. GABA_A receptors containing $\gamma 1$ or $\gamma 3$ subunits have not been investigated as extensively as those containing $\gamma 2$ subunits, presumably because of their low abundance in the brain. Three to eleven percent or 3%–14% of all GABA_A receptors contain $\gamma 1$ or $\gamma 3$ subunits, respectively (Pirker et al., 2000; Sieghart and Sperk, 2002). The $\gamma 1$ subunit is absent or only weakly expressed in most rat brain regions but is relatively enriched in the basal ganglia, the septal and basal forebrain region, the amygdala, in some thalamic and hypothalamic areas, cerebellum, pons, and medulla. The $\gamma 3$ subunit is weakly expressed and diffusely distributed all over the rat brain (Wisden et al., 1992; Pirker et al., 2000). Nevertheless, despite their relatively low abundance, these receptors might have important regulatory functions in the brain. In the absence of any information on their abundance and regional and cellular distribution in the human brain, their possible importance for regulating the function of the human central nervous system cannot be estimated. Since these receptors contain most (if not all) drug binding sites that are

also present on $\gamma 2$ -containing receptors, they can also be modulated by most, if not all the compounds modulating receptors containing $\gamma 2$ subunits. In any case, $\gamma 1$ -containing (Ymer et al., 1990; Puia et al., 1991; Wafford et al., 1993; Belelli et al., 2002; Khom et al., 2006) or $\gamma 3$ -containing receptors (Knoflach et al., 1991; Herb et al., 1992; Graham et al., 1996; Sur et al., 1998; Davies et al., 2000; Lippa et al., 2005) also have a benzodiazepine binding site and thus can be modulated by benzodiazepine site ligands (Sieghart, 1995), although in most cases with a lower potency and/or efficacy as judged by the limited number of compounds investigated. So far, no compounds have been identified that selectively modulate $\gamma 1$ or $\gamma 3$ subunit-containing receptors.

M. Compounds Modulating ε -, θ -, or π -Containing GABA_A Receptors

Not much is known about ε -containing GABA_A receptors (Davies et al., 1997a, 2001; Whiting et al., 1997; Neelands et al., 1999; Moragues et al., 2000, 2002, 2003; Thompson et al., 2002a; Maksay et al., 2003; Sergeeva et al., 2005; Bollan et al., 2008) and even less about GABA_A receptors containing θ (Bonnert et al., 1999; Sinkkonen et al., 2000; Moragues et al., 2002; Ranna et al., 2006) or π subunits (Hedblom and Kirkness, 1997; Neelands and Macdonald, 1999; Jin et al., 2005). The overall abundance of these receptors in the brain so far has not been investigated. The cDNA sequences of the ε and θ subunits were divergent in mouse, rat, and human tissues (Sinkkonen et al., 2000; Davies et al., 2002; Thompson et al., 2002a), and due to discrepant data in the literature, many researchers might have hesitated to investigate receptors containing these subunits. Receptors containing ε , θ , or π subunits are present in peripheral tissues as well as in the brain (Sieghart and Sperk, 2002), as indicated by *in situ* hybridization studies. The ε subunit has been demonstrated to be expressed by neurons located in septal and preoptic areas as well as in various hypothalamic nuclei, amygdala and thalamus, and the mRNA was also detected in major neuronal groups with broad-range influence, such as the cholinergic (basal nucleus), dopaminergic (substantia nigra compacta), serotonergic (raphe nuclei), and noradrenergic (locus coeruleus systems) neurons (Moragues et al., 2000, 2002, 2003). The θ subunit showed strikingly overlapping expression patterns with ε subunits throughout the brain. The π subunit was detected in several peripheral human tissues as well as in the brain (hippocampus and temporal cortex) (Hedblom and Kirkness, 1997; Neelands and Macdonald, 1999). But so far no study investigating the detailed regional distribution of π subunits in the brain has been published.

ε , θ , and π subunits can combine with other GABA_A receptor subunits in recombinant expression systems, resulting in receptors with unique subunit composition and pharmacological properties. As with δ subunits, ε

subunits might form receptors that differ in their subunit stoichiometry and arrangement (Wagner et al., 2005; Ranna et al., 2006; Bollan et al., 2008). However, the actual subunit composition of native GABA_A receptors containing these subunits is not known and has not been extensively investigated due to a lack of subunit-specific antibodies for immunoprecipitation experiments. No compounds have been identified yet that selectively modulate these receptors.

N. Compounds Modulating ρ -Containing GABA_A Receptors

ρ -Containing receptors form homo-oligomers or hetero-oligomers with other ρ subunits or possibly even with some other GABA_A receptor or glycine receptor subunits (Qian and Ripps, 1999; Pan et al., 2000; Hartmann et al., 2004; Milligan et al., 2004; Frazao et al., 2007). Originally, ρ -containing receptors were named GABA_C receptors because of some differences in their pharmacology compared with GABA_A receptors (Chebib, 2004; Martínez-Delgado et al., 2010; Ng et al., 2011; Naffaa et al., 2017). Due to the sequence homology of ρ -subunits with other GABA_A receptor subunits as well as the structural homology of ρ -containing receptors with $\alpha\beta\gamma$ GABA_A receptors, the International Union of Pharmacology nomenclature commission decided that ρ -containing receptors belong to the GABA_A receptor family and should be classified as such (Barnard et al., 1998; Olsen and Sieghart, 2008). This is even more justified considering homo-oligomeric $\beta 3$ receptors. These receptors share many pharmacological properties with $\alpha\beta$ or $\alpha\beta\gamma 2$ receptors (Slany et al., 1995; Zezula et al., 1996; Davies et al., 1997b; Woollorton et al., 1997), but are activated by histamine and not by GABA or muscimol (Saras et al., 2008; Hoerbelt et al., 2016). A distinct pharmacology thus cannot be used as a criterion for a distinct receptor nomenclature (Barnard et al., 1998; Olsen and Sieghart, 2008). Although homo-oligomeric $\beta 3$ receptors so far have not been identified in the brain due to the lack of selective ligands and the abundant expression of $\beta 3$ subunits as constituents of $\alpha\beta\gamma 2$ GABA_A receptors, their easy formation in recombinant expression systems that recently culminated in the first crystal structure of a GABA_A receptor subtype (Miller and Aricescu, 2014), indicates that these receptors probably are expressed also in the brain.

GABA_A receptors containing ρ subunits originally were identified in the retina (Cutting et al., 1991; Enz et al., 1996; Enz and Cutting, 1998), but later were also identified in many areas of the mammalian brain (Boue-Grabot et al., 1998; Wegelius et al., 1998; López-Chávez et al., 2005; Martínez-Delgado et al., 2010; Naffaa et al., 2017). However, the overall abundance of these subunits relative to other GABA_A receptor subunits so far has not been investigated. The localization of the various ρ -containing GABA_A receptors, as well as knockout and pharmacological studies, indicate that

these receptors might play a role in visual processing and myopia development, olfactory senses, learning and memory, sleep patterns, nociception, and hormone secretion (Ng et al., 2011). Over time, a variety of GABA site agonists, such as CACA (*cis*-4-aminocrotonic acid; Fig. 39C), (+)-CAMP [(+)-*cis*-2-aminomethylcyclopropane carboxylic acid; Fig. 39C] or *cis*-3-ACPBuPA (*cis*-3-aminocyclopentanylbutoyl-phosphinic acid; Fig. 39C), and antagonists, such as TPMPA (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid; Fig. 39C), were identified that selectively activated and inhibited, respectively, ρ -containing GABA_A receptors compared with heteromeric GABA_A receptors or metabotropic GABA_B receptors (Chebib, 2004; Ng et al., 2011). In addition, some ligands with unique pharmacological profiles have been identified that show selectivity for one ρ subtype over others (Ng et al., 2011; Naffaa et al., 2017).

VI. Discrepancy between the In Vivo Effects of Drugs in Rodent and Human Studies

Subtype-selective drugs are important tools for in vitro cell culture or brain slice studies to clarify a possible contribution of a receptor subtype to the measured effect or in preclinical studies for the investigation of the role of receptor subtypes in the regulation of physiologic functions. But the main motivation for the development of such compounds is the hope to finally identify more selective drugs with fewer side effects and especially, anxiolytic drugs lacking sedation, hypnotic activity, and ataxia. One of the main obstacles in the development of such drugs, however, was recently addressed (Skolnick, 2012): compounds claimed to exhibit selectivity for $\alpha 2/\alpha 3$ -containing GABA_A receptor subtypes, such as MRK 409 - (Atack et al., 2011b) or TPA023B (Atack et al., 2011a), and demonstrating a wide dose separation in their anxiolytic-like and sedative effects in rodents, in human studies exhibited actions more or less similar to those of the classic benzodiazepines, eliciting tiredness, drowsiness, and dizziness and at higher doses marked sedation in addition to their anxiolytic effects (Skolnick, 2012). Although the anxiolytic effects of TPA023 were significantly superior to placebo in three separate Phase II studies on generalized anxiety disorder without inducing sedation, in a study with 12 healthy volunteers this compound induced signs of sedation (including drowsiness and dizziness) in some volunteers and elicited a reduced saccadic peak velocity similar to a comparable dose of lorazepam as a possible additional indicator of a sedative action (de Haas et al., 2007; Skolnick, 2012). On the contrary, compounds such as ocinaplon or alpidem, which were not GABA_A receptor subtype selective at all in recombinant receptor studies, reportedly behaved as anxiolytics with no sedative component in both rodents and humans (Musch et al., 1988; Lippa et al., 2005; Czobor et al., 2010; Skolnick, 2012). Based on these apparently paradoxical results, the concept of receptor subtype

selectivity as a basis for generating drugs with reduced side effects was seriously questioned, and the enthusiasm of the pharmaceutical industry for the development of GABA_A receptor subtype-selective drugs was dramatically reduced (Skolnick, 2012).

Given the discussion above, it is evident, however, that none of the drugs currently available is truly receptor subtype selective. By accepting the finding that even a subtle modulation of a receptor subtype can influence behavior, it has to be concluded that the effects of these “selective” drugs cannot be attributed exclusively to the receptor for which they possess the highest affinity or efficacy. In addition, evidence is accumulating that not only $\alpha 2$ -containing receptor subtypes, but also $\alpha 5$ - (Botta et al., 2015; Behlke et al., 2016)- or $\alpha 4$ - and δ -containing receptor subtypes (Marowsky and Vogt, 2014) contribute to the modulation of anxiety. The anxiolytic but reduced sedative effects of the $\alpha 2/\alpha 3$ -selective drugs in rodents might thus have been caused by an optimally balanced modulation of various receptor subtypes involved in the regulation of anxiety and sedation in these species. The comparable anxiolytic but relatively increased sedative properties of these drugs in humans could then suggest that GABA_A receptor subtypes modulating anxiety might be similar, but receptors mediating sedation might be different in rodents and man (Skolnick, 2012).

The validity of animal models used in anxiety and depression research is not universally accepted. Especially scientists in the field of neurodevelopmental and psychiatric disorders often indicate that nonhuman animals can never express the full range of abilities and disabilities that characterize humans (Garner et al., 2009; Blanchard et al., 2013). While this may be and probably is true, it does not compromise the translational validity of at least some animal models used in anxiety studies. In recent years, fear conditioning models from non-human animal research have been substantiated and extended in humans, using neuropsychological and neuroimaging methodologies (Delgado et al., 2006). Recently, the open field test has been demonstrated to be related to human agoraphobic fear (Walz et al., 2016).

In addition, it is generally accepted that biology is similar in different mammalian species, although the complexity of their brains and the exact regulation of individual functions might be different. Apparently, the discrepancy in the behavioral effects of GABA_A receptor subtype-preferring drugs in rodents and humans is observed rather in their sedative than anxiolytic-like effects, although animal models of sedation are much less disputed than those of anxiety research. It is thus more probable that additional neuronal systems are involved in the regulation of tiredness, drowsiness, dizziness, and sedation in the more complex and better controlled human brain, thus increasing the overall inhibition by the drugs applied. This possibility is supported by evidence indicating that the regional

distribution of GABA_A receptor subunits is at least partially different in rodent and human brains (Waldvogel et al., 1999; Loup et al., 2000; Stojanovic et al., 2016; Stefanits et al., 2018). In addition, it has been demonstrated that $\alpha 1\beta\gamma 2$ receptors are expressed in human (Hellsten et al., 2010), but not reliably in rodent, noradrenergic locus coeruleus neurons (Luque et al., 1994; Chen et al., 1999). Since locus coeruleus neurons are involved in regulation of vigilance states, species differences in the expression profile of $\alpha 1\beta\gamma 2$ receptors in these neurons might thus contribute to the increased sedative effects of $\alpha 2/\alpha 3$ preferring benzodiazepine site ligands in humans, if these compounds also exert a certain degree of activity at $\alpha 1$ receptors (Hellsten et al., 2010). A positive modulation of $\alpha 5$ receptors (Savić et al., 2008b) or $\alpha 3$ receptors (Behlke et al., 2016) might also contribute to sedation. Enhanced expression of such receptors in humans might thus also contribute to the enhanced adverse effects of insufficiently selective GABA_A receptor ligands. Therefore, compounds that are really receptor subtype selective and that completely avoid the majority of receptors mediating sedation, might dramatically improve the situation and increase the separation between their anxiolytic and sedative action also in the human brain.

A. Receptors Containing $\beta 1$ -Subunits Might Contribute to the Sedative Action of Benzodiazepine Site Ligands

It is generally assumed that the type of β subunit in a receptor subtype does not significantly influence the action of benzodiazepine site ligands (Hadingham et al., 1993). However, this does not hold true for all benzodiazepine site ligands (Sieghart, 1995; Stevenson et al., 1995; Gee et al., 2010; Varagic et al., 2013a). A possible involvement of $\beta 1$ subunit-containing GABA_A receptors in the regulation of wakefulness was suggested by the use of the $\beta 1$ -preferring fragrant dioxane derivatives (*section V.I.2*) (Sergeeva et al., 2010). The importance of $\alpha 1\beta 1\gamma 2$ GABA_A receptors for eliciting ataxia, sedation, and sleep was then further emphasized by studies using $\beta 2/3$ -selective enaminones (Gee et al., 2010) (*section V.J.3*). These compounds allosterically modulate GABA_A receptors via a so far unidentified binding site and compared with other $\beta 2/3$ selective compounds, such as loreclezole, tracazolate, or etomidate, exert a dramatically reduced maximal efficacy at $\alpha 1\beta 1\gamma 2$ over $\alpha 1\beta 2\gamma 2$ GABA_A receptors (Gee et al., 2010). These enaminones produced anxiolytic-like actions with reduced ataxic or sleep-inducing effects (Gee et al., 2010; Yanovsky et al., 2012) and also reduced abuse liability or memory impairment (Yanovsky et al., 2012; Yoshimura et al., 2014).

The activity of loreclezole and other $\beta 2/3$ -selective compounds at $\beta 1$ -containing receptors might have been still too high (Gee et al., 2010) to avoid sedative side effects. In addition, $\beta 2$ - or $\beta 3$ -containing receptors might also contribute to the sedative actions of these

compounds (Belelli et al., 2005; Groves et al., 2006). Nevertheless, the extent of modulation of $\alpha 1\beta 1\gamma 2$ receptors might have contributed to the extent of the sedative and ataxic effects of the currently available drugs. Ocinaaplone exhibits a lower modulation of $\alpha 1\beta 1\gamma 2$ receptors than bretazenil or diazepam (Gee et al., 2010). It might thus be interesting to investigate whether the sedative and ataxic effects of ocinaaplone, MRK 409, TPA 023, and TPA 023B or of any other drug for which preclinical as well as clinical data are available, correlate with their efficacy at $\beta 1$ -containing GABA_A receptors.

GABA_A receptors containing $\beta 1$ subunits are expressed in the brain stem and arousal-related areas of the rat brain, for instance in the reticular thalamic nucleus, which is an important area for modulating sleep (Pirker et al., 2000; Gee et al., 2010). They are relatively minor receptor subtypes compared with those containing $\beta 2$ or $\beta 3$ subunits. Depending on the brain region investigated, about 20%–30%, 65%, or 55% of all GABA_A receptors contain $\beta 1$, $\beta 2$ or $\beta 3$ subunits, respectively (Sieghart and Sperk, 2002). The finding that these percentages add up to >100% indicates a significant colocalization of $\beta 1$, $\beta 2$, or $\beta 3$ subunits in GABA_A receptors (Jechlinger et al., 1998). A possible contribution of a minor GABA_A receptor subtype to the sedative effects of MRK 409 in humans was suggested by the extremely low receptor occupancy (<10%) of this compound under conditions that caused sedation in young healthy male volunteers (Atack et al., 2011b). This might have been caused by an especially high potency or efficacy of this compound for a low abundance receptor subtype mediating sedation in man. To investigate a possible contribution of $\beta 1$ -containing receptors to the sedative effects of MRK 409 and to possibly identify the GABA_A receptor subtype involved, the effects of MRK 409 at recombinant GABA_A receptors composed of $\alpha 1$ – $\beta 1\gamma 1$ – $\beta 3$ should be investigated and compared with the respective receptors containing $\beta 2$ or $\beta 3$ subunits.

B. Receptors Containing $\gamma 1$ or $\gamma 3$ Subunits, or Those Containing $\alpha 4$, $\alpha 6$, or δ Subunits, Might Contribute to the In Vivo Effects of Benzodiazepine Site Ligands

Benzodiazepine site ligands not only interact with GABA_A receptors containing $\gamma 2$ subunits, but also with those containing $\gamma 1$ (Puia et al., 1991; Wafford et al., 1993; Sieghart, 1995; Khom et al., 2006) or $\gamma 3$ subunits (Herb et al., 1992; Sieghart, 1995; Davies et al., 2000; Lippa et al., 2005). But so far, a possible contribution of such receptors to the spectrum of in vivo actions of benzodiazepine site ligands was not considered and no compounds have been identified that selectively modulate $\gamma 1$ or $\gamma 3$ receptors. Although $\gamma 1$ or $\gamma 3$ receptors are much less abundant in the mammalian brain than those containing $\gamma 2$ subunits (Pirker et al., 2000; Sieghart and Sperk, 2002), they might have important regulatory functions. The presence of $\gamma 1$ receptors in the amygdala (Pirker et al., 2000; Esmaeili et al., 2009) might suggest

their possible involvement in the regulation of anxiety, and ligands interacting with such receptors might mediate some of their effects via this mechanism and this could enhance their anxiolytic relative to their sedative effects.

Imidazobenzodiazepines (Figs. 5, 25–29, 31–33), pyrazoloquinolinones (Fig. 34), β -carboline, such as DMCM (Fig. 38) and other structural classes of benzodiazepine site ligands, not only modulate the diazepam-sensitive $\alpha 1, \alpha 2, \alpha 3, \alpha 5\beta \gamma 2$ receptors, but also $\alpha 4$ and $\alpha 6$ subunit containing GABA_A receptors (Sieghart, 1995; Stevenson et al., 1995; Ramerstorfer et al., 2010; Varagic et al., 2013a), and thus effects mediated by these drugs via these receptor subtypes cannot be ignored. The recent identification of a pyrazoloquinolinone modulating $\alpha 1\beta 3\delta$, $\alpha 4\beta 3\delta$, and $\alpha 6\beta 3\delta$ receptors (Mirheydari et al., 2014) together with the observation that extrasynaptic receptors containing $\alpha 4$ and δ subunits seem to be involved in inducing sedation and sleep (Belelli and Lambert, 2005; Belelli et al., 2005) and possibly also anxiety (Marowsky and Vogt, 2014) indicate that modulation of such receptors can contribute to the overall action of such compounds. A possible modulation of such receptors cannot be excluded before appropriate experiments have been performed.

C. Additional Interaction with Targets Different from GABA_A Receptors Could Also Contribute to the Separation of Anxiolytic and Sedative Properties of Ligands

Alpidem, similar to the structurally related zolpidem, potently and efficiently modulates $\alpha 1$ -, $\alpha 2$ -, and $\alpha 3$ -containing receptors, and exhibits no effects at $\alpha 5$ receptors (Puia et al., 1991; Costa and Guidotti, 1996). Whereas alpidem has anxiolytic but low sedative effects, zolpidem is a sedative hypnotic drug in humans. Both of these drugs, however, exhibit a similar modulatory effect at GABA_A receptors containing $\beta 1$ subunits (Puia et al., 1991; Costa and Guidotti, 1996). But in contrast to zolpidem, alpidem also has a high affinity for TSPO, which seems to be involved in the regulation of neurosteroid synthesis and is also discussed as a target for anxiolytic drugs (Costa et al., 2012; Nothdurfter et al., 2012). Similarly, the anxiolytic pyrazolopyridine CGS 20625 not only acts via GABA_A receptors but additionally exhibits some interaction with TSPO, known previously as the “peripheral benzodiazepine receptor” (Williams et al., 1989). Therefore, the wider separation of the anxiolytic over the sedative action of CGS 20625 or of alpidem compared with zolpidem, might have been caused by an additional interaction of CGS 20625 or alpidem with TSPO.

D. A Low Potency and Efficacy of Compounds Might Enhance Their Receptor Subtype-selective Actions

But how can ocinaaplone act as a selective anxiolytic in rodents and humans (Czobor et al., 2010), although it

seems not to be receptor subtype selective at all (Lippa et al., 2005; Berezhnoy et al., 2008)? Ocinalon is a low potency and low efficacy allosteric modulator. This is in contrast to the previously investigated high potency, low efficacy allosteric modulators such as bretazenil and abecarnil (Ramerstorfer et al., 2010), MRK 409 (Fig. 12; section V.D.5), TPA023 (Fig. 11; section V.D.4), or TPA023B (Fig. 7; section V.C.2), which lost their anxiolytic action observed in rodents when applied in humans (Skolnick, 2012). Due to their high potency, the latter drugs probably achieved brain concentrations eliciting their maximal efficacy at all receptor subtypes under conditions of in vivo application and by that sufficiently activated also those receptors mediating sedation in humans. The potency of ocinalon for modulating GABA_A receptors, however, is about 100–1000-fold lower than that of diazepam, depending on the receptor subtype investigated, and in addition this compound exhibits lower efficacy (Berezhnoy et al., 2008). This indicates that an at least 100–1000-fold higher concentration of ocinalon has to be reached at GABA_A receptors to elicit a modulation similar to that of diazepam. It is quite possible that concentrations sufficient to modulate GABA_A receptors mediating sedation cannot be achieved by this drug.

Therefore, the low potency and low efficacy of ocinalon, possibly combined with an intrinsic inability to sufficiently interact with those receptor subtypes mediating sedation at the concentrations achieved, might have added up to the favorable profile of this compound in humans. If more compounds with low potency and low efficacy, but with receptor subtype selectivity, such as 6-hydroxyflavone (Fig. 15; section V.D.8 (Ren et al., 2010), baicalin (Fig. 14; section V.D.7) (Wang et al., 2008), AZD7325 (Fig. 17; section V.D.10) (Christian et al., 2015), PZ-II-029 (Fig. 34; section V.G.3) (Varagic et al., 2013a), LAU159 (Treven et al., 2018), ELB138 (imepitoin), or ELB139 (Rabe et al., 2007; Rundfeldt and Löscher, 2014), confirm this hypothesis in clinical investigations by demonstrating a wider separation of anxiolytic and sedative properties in humans, pharmaceutical companies might have to radically change their strategy for the development of more selective drugs: Instead of developing high potency, high efficacy drugs with receptor subtype selectivity, the development of low potency, low efficacy drugs with receptor subtype selectivity might be more promising for eliciting selective actions. Since drug efficacy plays a critical role in determining the degree of GABA_A receptor uncoupling, and, perhaps in the development of tolerance and dependence (Primus et al., 1996), low efficacy drugs might have an added benefit in a long-term treatment of patients.

VII. Outlook

Given the data summarized above, it is evident that many of the compounds claimed to be selective for a

specific $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, or $\alpha 5\beta 3\gamma 2$ receptor subtypes are only marginally selective and that their limited selectivity is even further reduced by their high potency. Generating compounds with reduced potency and efficacy but high receptor subtype-selectivity seems to be a way out of this problem. Such compounds, in addition to their receptor subtype selectivity, might not achieve concentrations in the brain that sufficiently modulate receptor subtypes mediating sedative effects. Investigating such compounds in a clinical setting is also the only possibility to clarify whether it is their high receptor subtype selectivity that results in high functional selectivity or whether functional selectivity is achieved by an optimal balance of activity at various receptor subtypes.

In addition, the “receptor subtype selectivity” of benzodiazepine site ligands so far was predominantly investigated at the “diazepam-sensitive” $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, and $\alpha 5\beta 3\gamma 2$ receptors only, although most if not all of these drugs additionally can modulate receptors containing the same α , but different β or γ , subunits. All these receptors exhibit a distinct regional, cellular, and subcellular distribution in the brain and can be assumed to exhibit distinct behavioral effects, but the investigation of their function so far has been neglected. The same holds true for receptors containing $\alpha 4$ or $\alpha 6$ subunits. Certain structural classes of benzodiazepine site ligands can also modulate these or even δ -containing receptors. Therefore, all of these receptor subtypes have to be considered and investigated when claiming a “receptor subtype selectivity” of a drug. Additional interaction with such so far not investigated receptors can shift the balance between anxiolytic and sedative effects. Although an investigation of all these additional receptor subtypes seems to be a tremendous task, it seems to be feasible using high-throughput electrophysiological measurements (Trumbull et al., 2003) or fluorometric imaging plate readers (FLIPR) using membrane potential red dye, which redistributes across the plasma membrane in a voltage-dependent manner (Nik et al., 2017). In addition, the already available compounds with limited receptor subtype selectivity and their congeners, together with structural models of individual GABA_A receptor subtypes (Miller and Aricescu, 2014), receptor subtype-specific pharmacophore models (Clayton et al., 2007, 2015), and structure-based drug design (Richter et al., 2012), will facilitate the development of new and more selective drugs. Recent binding site mapping in models of GABA_A receptors has identified additional binding sites in these receptors that possibly also can be exploited for the development of receptor subtype-selective drugs (Ernst and Sieghart, 2015; Puthenkalam et al., 2016).

Finally, to strengthen the conclusions from biologic datasets generated by available compounds with limited or unclear receptor subtype selectivity, compounds from the same structural class exhibiting a similar as well as dissimilar selectivity profile together with compounds

selectively antagonizing the actions of the investigated drug at the receptor supposedly mediating its *in vivo* action, should be included into the protocol. In addition, positive controls (compounds from a different structural class that are also active at the respective receptor subtype) or negative controls (compounds exhibiting no activity at the respective receptor subtype) should also be included into the protocol, if available. The present review can serve as a guide for the selection of adequate positive or negative controls. Furthermore, the data obtained with compounds preferentially interacting with a certain receptor subtype have to be carefully interpreted by considering all the known interactions of the compound at the drug concentrations achieved in the brain. After all, a possible additional modulation by the drug of other GABA_A receptor subtypes or binding sites or of different transmitter systems has to be acknowledged in the discussion of the data.

Acknowledgments

We thank the countless contributors to the work cited in this review, without whom the current understanding of the physiological and pharmacological role of GABA_A receptor subtypes would not be possible. In addition, we want to thank Dr. Aleksandar Obradović, Dr. Bojan Marković, and Dr. Nebojša Cekić for their expert assistance in the preparation of the modified figures.

Authorship Contribution

Wrote or contributed to the writing of the manuscript: Sieghart, Savić.

References

- Achermann G, Ballard TM, Blasco F, Broutin PE, Büttelmann B, Fischer H, Graf M, Hernandez MC, Hilty P, Knoflach F, et al. (2009) Discovery of the imidazo[1,5-a][1,2,4]-triazolo[1,5-d][1,4]benzodiazepine scaffold as a novel, potent and selective GABA(A) alpha5 inverse agonist series. *Bioorg Med Chem Lett* **19**:5746–5752.
- Alexander SP, Peters JA, Kelly E, Marrion N, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Southan C, and Davies JA; CGTP Collaborators (2015) The Concise Guide to Pharmacology 2015/16: ligand-gated ion channels. *Br J Pharmacol* **172**: 5870–5903.
- Alhambra C, Becker C, Blake T, Chang AH, Damewood JR Jr, Daniels T, Dembofsky BT, Gurley DA, Hall JE, Herzog KJ, et al. (2011) Development and SAR of functionally selective allosteric modulators of GABA_A receptors. *Bioorg Med Chem* **19**: 2927–2938.
- Astorga G, Bao J, Marty A, Augustine GJ, Franconville R, Jalil A, Bradley J, and Llano I (2015) An excitatory GABA loop operating *in vivo*. *Front Cell Neurosci* **9**:275.
- Atack JR (2010a) Development of subtype-selective GABA_A receptor compounds for the treatment of anxiety, sleep disorders and epilepsy. In *GABA and Sleep, Molecular, Functional and Clinical Aspects*, pp 25–72, Springer, Basel, Switzerland.
- Atack JR (2010b) Preclinical and clinical pharmacology of the GABA_A receptor alpha5 subtype-selective inverse agonist alpha5IA. *Pharmacol Ther* **125**:11–26.
- Atack JR, Bayley PJ, Seabrook GR, Wafford KA, McKernan RM, and Dawson GR (2006a) L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for alpha5-containing GABA_A receptors. *Neuropharmacology* **51**:1023–1029.
- Atack JR, Eng WS, Gibson RE, Ryan C, Francis B, Sohal B, Dawson GR, Hargreaves RJ, and Burns HD (2009a) The plasma-occupancy relationship of the novel GABA_A receptor benzodiazepine site ligand, alpha5IA, is similar in rats and primates. *Br J Pharmacol* **157**:796–803.
- Atack JR, Hallett DJ, Tye SJ, Wafford KA, Ryan C, Sanabria-Bohórquez SM, Eng WS, Gibson RE, Burns HD, Dawson GR, et al. (2011a) Preclinical and clinical pharmacology of TPA023B, a GABA_A receptor alpha2/alpha3 subtype-selective partial agonist. *J Psychopharmacol* **25**:329–344.
- Atack JR, Hutson PH, Collinson N, Marshall G, Bentley G, Moyes C, Cook SM, Collins I, Wafford K, McKernan RM, et al. (2005) Anxiogenic properties of an inverse agonist selective for alpha3 subunit-containing GABA_A receptors. *Br J Pharmacol* **144**:357–366.
- Atack JR, Maubach KA, Wafford KA, O'Connor D, Rodrigues AD, Evans DC, Tattersall FD, Chambers MS, MacLeod AM, Eng WS, et al. (2009b) *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 alpha5 subtype-selective inverse agonist. *J Pharmacol Exp Ther* **331**:470–484.
- Atack JR, Wafford KA, Street LJ, Dawson GR, Tye SJ, Van Laere K, Bormans G, Sanabria-Bohórquez SM, De Lepeleire I, de Hoon JN, et al. (2011b) 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* **25**:314–328.
- Atack JR, Wafford KA, Tye SJ, Cook SM, Sohal B, Pike A, Sur C, Melillo D, Bristow L, Bromidge F, et al. (2006b) TPA023 [7-(1,1-dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine], an agonist selective for alpha2- and alpha3-containing GABA_A receptors, is a non-sedating anxiolytic in rodents and primates. *J Pharmacol Exp Ther* **316**:410–422.
- Atack JR, Wong DF, Fryer TD, Ryan C, Sanabria S, Zhou Y, Dannals RF, Eng WS, Gibson RE, Burns HD, et al. (2010) 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-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine (TPA023) in rats, primates, and humans. *J Pharmacol Exp Ther* **332**:17–25.
- Ballard TM, Knoflach F, Prinssen E, Borroni E, Vivian JA, Basile J, Gasser R, Moreau JL, Wettstein JG, Buettelmann B, et al. (2009) RO4938581, a novel cognitive enhancer acting at GABA_A alpha5 subunit-containing receptors. *Psychopharmacology (Berl)* **202**:207–223.
- Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, Braestrup C, Bateson AN, and Langer SZ (1998) International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acid_A receptors: classification on the basis of subunit structure and receptor function. *Pharmacol Rev* **50**:291–313.
- Bateson AN, Lasham A, and Darlison MG (1991) gamma-Aminobutyric acid_A receptor heterogeneity is increased by alternative splicing of a novel beta-subunit gene transcript. *J Neurochem* **56**:1437–1440.
- Batinčić B, Stanković T, Stephen MR, Kodali R, Tiruveedhula VV, Li G, Scholze P, Marković BD, Obradović AL, Ernst M, et al. (2018) Attaining *in vivo* selectivity of positive modulation of alpha3-gamma2 GABA(A) receptors in rats: A hard task! *Eur Neuropsychopharmacol* **28**:903–914.
- Baumann SW, Baur R, and Sigel E (2001) Subunit arrangement of gamma-aminobutyric acid type A receptors. *J Biol Chem* **276**:36275–36280.
- Baur R, Kiehl M, Richter L, Ernst M, Ecker GF, and Sigel E (2013) Molecular analysis of the site for 2-arachidonylglycerol (2-AG) on the beta2 subunit of GABA(A) receptors. *J Neurochem* **126**:29–36.
- Baur R and Sigel E (2007) Replacement of histidine in position 105 in the alpha5 subunit by cysteine stimulates zolpidem sensitivity of alpha5-gamma2 GABA(A) receptors. *J Neurochem* **103**:2556–2564.
- Baur R, Simmen U, Senn M, Séquin U, and Sigel E (2005) Novel plant substances acting as beta subunit isoform-selective positive allosteric modulators of GABA_A receptors. *Mol Pharmacol* **68**:787–792.
- Behlke LM, Foster RA, Liu J, Benke D, Benham RS, Nathanson AJ, Yee BK, Zeilhofer HU, Engin E, and Rudolph U (2016) A pharmacogenetic 'restriction-of-function' approach reveals evidence for anxiolytic-like actions mediated by alpha5-containing GABA_A receptors in mice. *Neuropsychopharmacology* **41**:2492–2501.
- Belelli D, Casula A, Ling A, and Lambert JJ (2002) The influence of subunit composition on the interaction of neurosteroids with GABA(A) receptors. *Neuropharmacology* **43**:651–661.
- Belelli D and Lambert JJ (2005) Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci* **6**:565–575.
- Belelli D, Lambert JJ, Peters JA, Wafford K, and Whiting PJ (1997) The interaction of the general anesthetic etomidate with the gamma-aminobutyric acid type A receptor is influenced by a single amino acid. *Proc Natl Acad Sci USA* **94**: 11031–11036.
- Belelli D, Peden DR, Rosahl TW, Wafford KA, and Lambert JJ (2005) Extrasynaptic GABA_A receptors of thalamocortical neurons: a molecular target for hypnotics. *J Neurosci* **25**:11513–11520.
- Belujon P, Baufretton J, Grandoso L, Boué-Grabot E, Batten TF, Ugedo L, Garret M, and Taupignon AI (2009) Inhibitory transmission in locus coeruleus neurons expressing GABA_A receptor epsilon subunit has a number of unique properties. *J Neurophysiol* **102**:2312–2325.
- Ben-Ari Y, Woodin MA, Sernagor E, Cancedda L, Vinay L, Rivera C, Legendre P, Luhmann HJ, Bordey A, Wenner P, et al. (2012) Refuting the challenges of the developmental shift of polarity of GABA actions: GABA more exciting than ever! *Front Cell Neurosci* **6**:35.
- Bencsits E, Ebert V, Tretter V, and Sieghart W (1999) A significant part of native gamma-aminobutyric acid_A receptors containing alpha4 subunits do not contain gamma or delta subunits. *J Biol Chem* **274**:19613–19616.
- Benke D, Fakitsas P, Roggenmoser C, Michel C, Rudolph U, and Mohler H (2004) Analysis of the presence and abundance of GABA_A receptors containing two different types of alpha subunits in murine brain using point-mutated alpha subunits. *J Biol Chem* **279**:43654–43660.
- Benke D, Michel C, and Mohler H (1997) GABA(A) receptors containing the alpha4-subunit: prevalence, distribution, pharmacology, and subunit architecture *in situ*. *J Neurochem* **69**:806–814.
- Bennett DA (1987) Pharmacology of the pyrazolo-type compounds: agonist, antagonist and inverse agonist actions. *Physiol Behav* **41**:241–245.
- Benson JA, Löw K, Keist R, Mohler H, and Rudolph U (1998) Pharmacology of recombinant gamma-aminobutyric acid_A receptors rendered diazepam-insensitive by point-mutated alpha-subunits. *FEBS Lett* **431**:400–404.
- Berezchnoy D, Gravielle MC, Downing S, Kostakis E, Basile AS, Skolnick P, Gibbs TT, and Farb DH (2008) Pharmacological properties of DOV 315,090, an ocinaplon metabolite. *BMC Pharmacol* **8**:11.
- Berezchnoy D, Gravielle MC, and Farb DH (2007) Pharmacology of the GABA_A receptor, in *Handbook of Contemporary Neuropharmacology* (Sibley DR, Hanin I, Kuhar M, and Skolnick P, eds) pp 465–568, John Wiley & Sons, Inc., Hoboken, NJ.
- Bettler B, Kaupmann K, Mosbacher J, and Gassmann M (2004) Molecular structure and physiological functions of GABA(B) receptors. *Physiol Rev* **84**:835–867.
- Bianchi MT and Macdonald RL (2003) Neurosteroids shift partial agonist activation of GABA(A) receptor channels from low- to high-efficacy gating patterns. *J Neurosci* **23**:10934–10943.

- Blackaby WP, Atack JR, Bromidge F, Castro JL, Goodacre SC, Hallett DJ, Lewis RT, Marshall GR, Pike A, Smith AJ, et al. (2006) Imidazo[1,2-*a*]pyrimidines as functionally selective GABA(A) ligands. *Bioorg Med Chem Lett* **16**:1175–1179.
- Blanchard DC, Summers CH, and Blanchard RJ (2013) The role of behavior in translational models for psychopathology: functionality and dysfunctional behaviors. *Neurosci Biobehav Rev* **37**:1567–1577.
- Bogdanov Y, Michels G, Armstrong-Gold C, Haydon PG, Lindstrom J, Pangalos M, and Moss SJ (2006) Synaptic GABA_A receptors are directly recruited from their extrasynaptic counterparts. *EMBO J* **25**:4381–4389.
- Boileau AJ, Pearce RA, and Czajkowski C (2005) Tandem subunits effectively constrain GABA_A receptor stoichiometry and recapitulate receptor kinetics but are insensitive to GABA_A receptor-associated protein. *J Neurosci* **25**:11219–11230.
- Boileau AJ, Pearce RA, and Czajkowski C (2010) The short splice variant of the gamma 2 subunit acts as an external modulator of GABA(A) receptor function. *J Neurosci* **30**:4895–4903.
- Bollan KA, Baur R, Hales TG, Sigel E, and Connolly CN (2008) The promiscuous role of the epsilon subunit in GABA_A receptor biogenesis. *Mol Cell Neurosci* **37**:610–621.
- Bonnert TP, McKernan RM, Farrar S, le Bourdellès B, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghi DJS, Brown N, et al. (1999) theta, a novel gamma-aminobutyric acid type A receptor subunit. *Proc Natl Acad Sci USA* **96**:9891–9896.
- Botta P, Demmou L, Kasugai Y, Markovic M, Xu C, Fadok JP, Lu T, Poe MM, Xu L, Cook JM, et al. (2015) Regulating anxiety with extrasynaptic inhibition. *Nat Neurosci* **18**:1493–1500.
- Botzakis EJ, Gurba KN, Lagrange AH, Feng HJ, Stanic AK, Hu N, and Macdonald RL (2016) Comparison of γ -aminobutyric acid, type A (GABA_A), receptor $\alpha\beta\gamma$ and $\alpha\beta\delta$ expression using flow cytometry and electrophysiology: evidence for alternative subunit stoichiometries and arrangements. *J Biol Chem* **291**:20440–20461.
- Boue-Grabot E, Roudbaraki M, Bascles L, Tramu G, Bloch B, and Garret M (1998) Expression of GABA receptor rho subunits in rat brain. *J Neurochem* **70**:899–907.
- Braestrup C and Squires RF (1977) Specific benzodiazepine receptors in rat brain characterized by high-affinity (3H)diazepam binding. *Proc Natl Acad Sci USA* **74**:3805–3809.
- Brickley SG and Mody I (2012) Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. *Neuron* **73**:23–34.
- Brown N, Kerby J, Bonnert TP, Whiting PJ, and Wafford KA (2002) Pharmacological characterization of a novel cell line expressing human $\alpha(4)\beta(3)\delta$ GABA(A) receptors. *Br J Pharmacol* **136**:965–974.
- Buettelmann B, Ballard TM, Gasser R, Fischer H, Hernandez MC, Knoflach F, Knust H, Stadler H, Thomas AW, and Trube G (2009) Imidazo[1,5-*a*][1,2,4]-triazolo[1,5-*d*] [1,4]benzodiazepines as potent and highly selective GABA_A $\alpha(5)$ inverse agonists with potential for the treatment of cognitive dysfunction. *Bioorg Med Chem Lett* **19**:5958–5961.
- Carling RW, Russell MG, Moore KW, Mitchinson A, Guiblin A, Smith A, Wafford KA, Marshall G, Atack JR, and Street LJ (2006) 2,3,7-Trisubstituted pyrazolo[1,5-*d*] [1,2,4]triazines: functionally selective GABA_A $\alpha(3)$ -subtype agonists. *Bioorg Med Chem Lett* **16**:3550–3554.
- Cestari IN, Uchida I, Li L, Burt D, and Yang J (1996) The agonistic action of pentobarbital on GABA_A beta-subunit homomeric receptors. *Neuroreport* **7**:943–947.
- Chambers MS, Atack JR, Broughton HB, Collinson N, Cook S, Dawson GR, Hobbs SC, Marshall G, Maubach KA, Pillai GV, et al. (2003) Identification of a novel, selective GABA(A) $\alpha(5)$ receptor inverse agonist which enhances cognition. *J Med Chem* **46**:2227–2240.
- Chebib M (2004) GABAC receptor ion channels. *Clin Exp Pharmacol Physiol* **31**:800–804.
- Che Has AT, Absalom N, van Nieuwenhuijzen PS, Clarkson AN, Ahring PK, and Chebib M (2016) Zolpidem is a potent stoichiometry-selective modulator of $\alpha(1)\beta(3)$ GABA_A receptors: evidence of a novel benzodiazepine site in the $\alpha(1)\alpha(1)$ interface. *Sci Rep* **6**:28674.
- Chen CL, Yang YR, and Chiu TH (1999) Activation of rat locus coeruleus neuron GABA(A) receptors by propofol and its potentiation by pentobarbital or alfaxalone. *Eur J Pharmacol* **386**:201–210.
- Chen X, Keramidas A, and Lynch JW (2017) Physiological and pharmacological properties of inhibitory postsynaptic currents mediated by $\alpha(5)\beta(1)\gamma(2)$, $\alpha(5)\beta(2)\gamma(2)$ and $\alpha(5)\beta(3)\gamma(2)$ GABA_A receptors. *Neuropharmacology* **125**:243–253.
- Chiou LC, Lee HJ, Ernst M, Huang WJ, Chou JF, Chen HL, Mouri A, Chen LC, Treven M, Mamiya T, et al. (2018) Cerebellar $\alpha(6)$ -subunit-containing GABA_A receptors: a novel therapeutic target for disrupted prepulse inhibition in neuropsychiatric disorders. *Br J Pharmacol* **175**:2414–2427.
- Christian EP, Snyder DH, Song W, Gurley DA, Smolka J, Maier DL, Ding M, Gharahdaghi F, Liu XF, Chopra M, et al. (2015) EEG- β/γ spectral power elevation in rat: a translatable biomarker elicited by GABA(A $\alpha(2/3)$)-positive allosteric modulators at nonseparating anxiolytic doses. *J Neurophysiol* **113**:116–131.
- Cirone J, Rosahl TW, Reynolds DS, Newman RJ, O'Meara GF, Hutson PH, and Wafford KA (2004) Gamma-aminobutyric acid type A receptor beta 2 subunit mediates the hypothermic effect of etomidate in mice. *Anesthesiology* **100**:1438–1445.
- Clarkson AN, Huang BS, Macisaac SE, Mody I, and Carmichael ST (2010) Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. *Nature* **468**:305–309.
- Clayton T, Chen JL, Ernst M, Richter L, Cromer BA, Morton CJ, Ng H, Kaczorowski CC, Helmstetter FJ, Furtmüller R, et al. (2007) An updated unified pharmacophore model of the benzodiazepine binding site on gamma-aminobutyric acid(a) receptors: correlation with comparative models. *Curr Med Chem* **14**:2755–2775.
- Clayton T, Poe MM, Rallapalli S, Biawat P, Savić MM, Rowlett JK, Gallos G, Emala CW, Kaczorowski CC, Stafford DC, et al. (2015) A review of the updated pharmacophore for the alpha 5 GABA(A) benzodiazepine receptor model. *Int J Med Chem* **2015**:430248.
- Collinson N, Atack JR, Laughton P, Dawson GR, and Stephens DN (2006) An inverse agonist selective for $\alpha(5)$ subunit-containing GABA_A receptors improves encoding and recall but not consolidation in the Morris water maze. *Psychopharmacology (Berl)* **188**:619–628.
- Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, et al. (2002) Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABA_A receptor. *J Neurosci* **22**:5572–5580.
- Cope DW, Halbsguth C, Karayannis T, Wulff P, Ferraguti F, Hoeger H, Leppä E, Linden AM, Oberto A, Ogris W, et al. (2005) Loss of zolpidem efficacy in the hippocampus of mice with the GABA_A receptor gamma2 F77I point mutation. *Eur J Neurosci* **21**:3002–3016.
- Costa B, Da Pozzo E, and Martini C (2012) Translocator protein as a promising target for novel anxiolytics. *Curr Top Med Chem* **12**:270–285.
- Costa E and Guidotti A (1985) Endogenous ligands for benzodiazepine recognition sites. *Biochem Pharmacol* **34**:3399–3403.
- Costa E and Guidotti A (1996) Benzodiazepines on trial: a research strategy for their rehabilitation. *Trends Pharmacol Sci* **17**:192–200.
- Crestani F, Keist R, Fritschy J-M, Benke D, Vogt K, Prut L, Blüthmann H, Möhler H, and Rudolph U (2002) Trace fear conditioning involves hippocampal $\alpha(5)$ GABA(A) receptors. *Proc Natl Acad Sci USA* **99**:8980–8985.
- Crestani F, Löw K, Keist R, Mandelli M, Möhler H, and Rudolph U (2001) Molecular targets for the myorelaxant action of diazepam. *Mol Pharmacol* **59**:442–445.
- Cutting GR, Lu L, O'Hara BF, Kasch LM, Montrose-Rafizadeh C, Donovan DM, Shimada S, Antonarakis SE, Guggino WB, Uhl GR, et al. (1991) Cloning of the gamma-aminobutyric acid (GABA) rho 1 cDNA: a GABA receptor subunit highly expressed in the retina. *Proc Natl Acad Sci USA* **88**:2673–2677.
- Czobor P, Skolnick P, Beer B, and Lippa A (2010) A multicenter, placebo-controlled, double-blind, randomized study of efficacy and safety of ocinaplon (DOV 273,547) in generalized anxiety disorder. *CNS Neurosci Ther* **16**:63–75.
- Dämlgen K and Lüddens H (1999) Zaleplon displays a selectivity to recombinant GABA_A receptors different from zolpidem, zopiclone and benzodiazepines. *Neurosci Res Commun* **25**:139–148.
- Davies M, Newell JG, Derry JM, Martin IL, and Dunn SM (2000) Characterization of the interaction of zopiclone with gamma-aminobutyric acid type A receptors. *Mol Pharmacol* **58**:756–762.
- Davies PA, Hanna MC, Hales TG, and Kirkness EF (1997a) Insensitivity to anaesthetic agents conferred by a class of GABA(A) receptor subunit. *Nature* **385**:820–823.
- Davies PA, Kirkness EF, and Hales TG (1997b) Modulation by general anaesthetics of rat GABA_A receptors comprised of alpha 1 beta 3 and beta 3 subunits expressed in human embryonic kidney 293 cells. *Br J Pharmacol* **120**:899–909.
- Davies PA, Kirkness EF, and Hales TG (2001) Evidence for the formation of functionally distinct alphabeta gamma epsilon GABA(A) receptors. *J Physiol* **537**:101–113.
- Davies PA, McCartney MR, Wang W, Hales TG, and Kirkness EF (2002) Alternative transcripts of the GABA(A) receptor epsilon subunit in human and rat. *Neuropharmacology* **43**:467–475.
- Davies PA, Wang W, Hales TG, and Kirkness EF (2003) A novel class of ligand-gated ion channel is activated by Zn²⁺. *J Biol Chem* **278**:712–717.
- Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, MacLeod AM, Choudhury HI, McDonald LM, Pillai G, Rycroft W, et al. (2006) An inverse agonist selective for $\alpha(5)$ subunit-containing GABA_A receptors enhances cognition. *J Pharmacol Exp Ther* **316**:1335–1345.
- de Haas SL, de Visser SJ, van der Post JP, de Smet M, Schoemaker RC, Rijnbeek B, Cohen AF, Vega JM, Agrawal NG, Goel TV, et al. (2007) Pharmacodynamic and pharmacokinetic effects of TPA023, a GABA(A) $\alpha(2/3)$ subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. *J Psychopharmacol* **21**:374–383.
- Delgado MR, Olsson A, and Phelps EA (2006) Extending animal models of fear conditioning to humans. *Biol Psychol* **73**:39–48.
- de Lucas AG, Ahring PK, Larsen JS, Rivera-Arconada I, Lopez-Garcia JA, Mirza NR, and Munro G (2015) 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* **93**:370–379.
- Dias R, Sheppard WF, Fradley RL, Garrett EM, Stanley JL, Tye SJ, Goodacre S, Lincoln RJ, Cook SM, Conley R, et al. (2005) Evidence for a significant role of alpha 3-containing GABA_A receptors in mediating the anxiolytic effects of benzodiazepines. *J Neurosci* **25**:10682–10688.
- Di Lio A, Benke D, Besson M, Desmeules J, Daali Y, Wang ZJ, Edwankar R, Cook JM, and Zeilhofer HU (2011) HZ166, a novel GABA_A receptor subtype-selective benzodiazepine site ligand, is antihyperalgesic in mouse models of inflammatory and neuropathic pain. *Neuropharmacology* **60**:626–632.
- Dillon GH, Im HK, Hamilton BJ, Carter DB, Gammill RB, Judge TM, and Im WB (1993) U-93631 causes rapid decay of gamma-aminobutyric acid-induced chloride currents in recombinant rat gamma-aminobutyric acid type A receptors. *Mol Pharmacol* **44**:860–865.
- Dillon GH, Im WB, Carter DB, and McKinley DD (1995) Enhancement by GABA of the association rate of picrotoxin and tert-butylbicyclophosphorothionate to the rat cloned alpha 1 beta 2 gamma 2 GABA_A receptor subtype. *Br J Pharmacol* **115**:539–545.
- Ducic I, Caruncho HJ, Zhu WJ, Vicini S, and Costa E (1995) gamma-Aminobutyric acid gating of Cl⁻ channels in recombinant GABA_A receptors. *J Pharmacol Exp Ther* **272**:438–445.
- Eaton MM, Bracamontes J, Shu HJ, Li P, Mennerick S, Steinbach JH, and Akk G (2014) γ -aminobutyric acid type A $\alpha(4)$, $\beta(2)$, and δ subunits assemble to produce more than one functionally distinct receptor type. *Mol Pharmacol* **86**:647–656.
- Ebert B, Anderson NJ, Cremers TJ, Rasmussen S, Vogel V, Fahay JM, and Sánchez C (2008) Gaboxadol -- a different hypnotic profile with no tolerance to sleep EEG and

- sedative effects after repeated daily dosing. *Pharmacol Biochem Behav* **90**: 113–122.
- Ebert B, Thompson SA, Saounatsou K, McKernan R, Krogsgaard-Larsen P, and Wafford KA (1997) Differences in agonist/antagonist binding affinity and receptor transduction using recombinant human gamma-aminobutyric acid type A receptors. *Mol Pharmacol* **52**:1150–1156.
- Ebert B, Wafford KA, Whiting PJ, Krogsgaard-Larsen P, and Kemp JA (1994) Molecular pharmacology of gamma-aminobutyric acid type A receptor agonists and partial agonists in oocytes injected with different alpha, beta, and gamma receptor subunit combinations. *Mol Pharmacol* **46**:957–963.
- El Hadri A, Abouabdellah A, Thomet U, Baur R, Furtmüller R, Sigel E, Sieghart W, and Dodd RH (2002) N-Substituted 4-amino-3,3-dipropyl-2(3H)-furanones: new positive allosteric modulators of the GABA(A) receptor sharing electrophysiological properties with the anticonvulsant loreclezole. *J Med Chem* **45**:2824–2831.
- Engin E, Smith KS, Gao Y, Nagy D, Foster RA, Tsvetkov E, Keist R, Crestani F, Fritschy JM, Bolshakov VY, et al. (2016) Modulation of anxiety and fear via distinct intrahippocampal circuits. *eLife* **5**:e14120.
- Engin E, Zarnowska ED, Benke D, Tsvetkov E, Sigal M, Keist R, Bolshakov VY, Pearce RA, and Rudolph U (2015) Tonic inhibitory control of dentate gyrus granule cells by $\alpha 5$ -containing GABAA receptors reduces memory interference. *J Neurosci* **35**:13698–13712.
- Enz R, Brandstätter JH, Wässle H, and Bormann J (1996) Immunocytochemical localization of the GABAC receptor rho subunits in the mammalian retina. *J Neurosci* **16**:4479–4490.
- Enz R and Cutting GR (1998) Molecular composition of GABAC receptors. *Vision Res* **38**:1431–1441.
- Ernst M, Brauchart D, Borech S, and Sieghart W (2003) Comparative modeling of GABA(A) receptors: limits, insights, future developments. *Neuroscience* **119**: 933–943.
- Ernst M, Bruckner S, Borech S, and Sieghart W (2005) Comparative models of GABAA receptor extracellular and transmembrane domains: important insights in pharmacology and function. *Mol Pharmacol* **68**:1291–1300.
- Ernst M and Sieghart W (2015) GABAA receptor subtypes: structural variety raises hope for new therapy concepts. *e-Neuroforum* **6**:97–103.
- Esmaili A, Lynch JW, and Sah P (2009) GABAA receptors containing gamma1 subunits contribute to inhibitory transmission in the central amygdala. *J Neurophysiol* **101**:341–349.
- Etherington LA, Mihalik B, Pálvölgyi A, Ling I, Pallagi K, Kertész S, Varga P, Gunn BG, Brown AR, Livesey MR, et al. (2017) Selective inhibition of extra-synaptic $\alpha 5$ -GABA_A receptors by S44819, a new therapeutic agent. *Neuropharmacology* **125**: 353–364.
- Farrant M and Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci* **6**:215–229.
- Feng HJ and Forman SA (2018) Comparison of $\alpha 2\delta$ and $\alpha 3\gamma$ GABAA receptors: allosteric modulation and identification of subunit arrangement by site-selective general anesthetics. *Pharmacol Res* **133**:289–300.
- Fernandez SP, Karim N, Mewett KN, Chebib M, Johnston GA, and Hanrahan JR (2012) Flavan-3-ol esters: new agents for exploring modulatory sites on GABA(A) receptors. *Br J Pharmacol* **165**:965–977.
- Fernandez SP, Mewett KN, Hanrahan JR, Chebib M, and Johnston GA (2008) Flavan-3-ol derivatives are positive modulators of GABA(A) receptors with higher efficacy for the $\alpha 2$ subtype and anxiolytic action in mice. *Neuropharmacology* **55**:900–907.
- Fischer BD, Licata SC, Edwankar RV, Wang ZJ, Huang S, He X, Yu J, Zhou H, Johnson EM Jr, Cook JM, et al. (2010) Anxiolytic-like effects of 8-acetylene imidazobenzodiazepines in a rhesus monkey conflict procedure. *Neuropharmacology* **59**:612–618.
- Fischer BD, Schlitt RJ, Hamade BZ, Rehman S, Ernst M, Poe MM, Li G, Kodali R, Arnold LA, and Cook JM (2017) Pharmacological and antihyperalgesic properties of the novel $\alpha 2/3$ preferring GABA_A receptor ligand MP-III-024. *Brain Res Bull* **131**:62–69.
- Fisher JL (2002) Amiloride inhibition of gamma-aminobutyric acid(A) receptors depends upon the alpha subunit subtype. *Mol Pharmacol* **61**:1322–1328.
- Forkuo GS, Guthrie ML, Yuan NY, Nieman AN, Kodali R, Jahan R, Stephen MR, Yocum GT, Treven M, Poe MM, et al. (2016) Development of GABAA receptor subtype-selective imidazobenzodiazepines as novel asthma treatments. *Mol Pharm* **13**:2026–2038.
- Forman SA and Miller KW (2016) Mapping general anesthetic sites in heteromeric γ -aminobutyric acid type A receptors reveals a potential for targeting receptor subtypes. *Anesth Analg* **123**:1263–1273.
- Frazao R, Nogueira MI, and Wässle H (2007) Colocalization of synaptic GABA(C)-receptors with GABA (A)-receptors and glycine-receptors in the rodent central nervous system. *Cell Tissue Res* **330**:1–15.
- Fritschy JM and Möhler H (1995) GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *J Comp Neurol* **359**:154–194.
- Frølund B, Ebert B, Kristiansen U, Liljefors T, and Krogsgaard-Larsen P (2002) GABA(A) receptor ligands and their therapeutic potentials. *Curr Top Med Chem* **2**: 817–832.
- Furtmueller R, Furtmueller B, Ramerstorfer J, Paladini AC, Wasowski C, Marder M, Huck S, and Sieghart W (2008) 6,3'-Dinitroflavone is a low efficacy modulator of GABA(A) receptors. *Eur J Pharmacol* **591**:142–146.
- Gallos G, Yocum GT, Siviski ME, Yim PD, Fu XW, Poe MM, Cook JM, Harrison N, Perez-Zoghbi J, and Emala CW Sr (2015) Selective targeting of the $\alpha 5$ -subunit of GABAA receptors relaxes airway smooth muscle and inhibits cellular calcium handling. *Am J Physiol Lung Cell Mol Physiol* **308**:L931–L942.
- Galzi J-L and Changeux J-P (1994) Neurotransmitter-gated ion channels as unconventional allosteric proteins. *Curr Opin Struct Biol* **4**:554–565.
- Gao H and Smith BN (2010) Zolpidem modulation of phasic and tonic GABA currents in the rat dorsal motor nucleus of the vagus. *Neuropharmacology* **58**:1220–1227.
- Gardner CR (1992) A review of recently-developed ligands for neuronal benzodiazepine receptors and their pharmacological activities. *Prog Neuropsychopharmacol Biol Psychiatry* **16**:755–781.
- Garner M, Möhler H, Stein DJ, Mueggler T, and Baldwin DS (2009) Research in anxiety disorders: from the bench to the bedside. *Eur Neuropsychopharmacol* **19**: 381–390.
- Gee KW, Tran MB, Hogenkamp DJ, Johnstone TB, Bagnara RE, Yoshimura RF, Huang JC, Belluzzi JD, and Whitemore ER (2010) Limiting activity at beta1-subunit-containing GABAA receptor subtypes reduces ataxia. *J Pharmacol Exp Ther* **332**:1040–1053.
- Gill KM and Grace AA (2014) The role of $\alpha 5$ GABAA receptor agonists in the treatment of cognitive deficits in schizophrenia. *Curr Pharm Des* **20**:5069–5076.
- Glykys J and Mody I (2007a) Activation of GABAA receptors: views from outside the synaptic cleft. *Neuron* **56**:763–770.
- Glykys J and Mody I (2007b) The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. *J Physiol* **582**:1163–1178.
- Goodacre SC, Street LJ, Hallett DJ, Crawforth JM, Kelly S, Owens AP, Blackaby WP, Lewis RT, Stanley J, Smith AJ, et al. (2006) Imidazo[1,2-a]pyrimidines as functionally selective and orally bioavailable GABA(A) $\alpha 2/\alpha 3$ binding site agonists for the treatment of anxiety disorders. *J Med Chem* **49**:35–38.
- Graham D, Faure C, Besnard F, and Langner SZ (1996) Pharmacological profile of benzodiazepine site ligands with recombinant GABAA receptor subtypes. *Eur Neuropsychopharmacol* **6**:119–125.
- Greenblatt DJ and Sethy VH (1990) Benzodiazepine concentrations in brain directly reflect receptor occupancy: studies of diazepam, lorazepam, and oxazepam. *Psychopharmacology (Berl)* **102**:373–378.
- Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Decobert M, Françon D, Avenet P, Depoortere H, Tan S, et al. (2001) SL651498: an anxiolytic compound with functional selectivity for $\alpha 2$ - and $\alpha 3$ -containing gamma-aminobutyric acid(A) (GABA(A)) receptors. *J Pharmacol Exp Ther* **298**:753–768.
- Groves JO, Guscott MR, Hallett DJ, Rosahl TW, Pike A, Davies A, Wafford KA, and Reynolds DS (2006) The role of GABABeta2 subunit-containing receptors in mediating the anticonvulsant and sedative effects of loreclezole. *Eur J Neurosci* **24**: 167–174.
- Guerrini G, Ciciani G, Cambi G, Bruni F, Selli S, Besnard F, Montali M, Martini C, Ghelardini C, Galeotti N, et al. (2007) Novel 3-iodo-8-ethoxypyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide as promising lead for design of $\alpha 5$ -inverse agonist useful tools for therapy of mnemonic damage. *Bioorg Med Chem* **15**:2573–2586.
- Gutiérrez A, Khan ZU, and De Blas AL (1996) Immunocytochemical localization of the alpha 6 subunit of the gamma-aminobutyric acidA receptor in the rat nervous system. *J Comp Neurol* **365**:504–510.
- Hadingham KL, Wingrove PB, Wafford KA, Bain C, Kemp JA, Palmer KJ, Wilson AW, Wilcox AS, Sikela JM, Ragan CI, et al. (1993) Role of the beta subunit in determining the pharmacology of human gamma-aminobutyric acid type A receptors. *Mol Pharmacol* **44**:1211–1218.
- Hadley SH and Amin J (2007) Rat $\alpha 6\beta 2\delta$ GABAA receptors exhibit two distinct and separable agonist affinities. *J Physiol* **581**:1001–1018.
- Hájos N, Nusser Z, Rancz EA, Freund TF, and Mody I (2000) Cell type- and synapse-specific variability in synaptic GABAA receptor occupancy. *Eur J Neurosci* **12**: 810–818.
- Halliwell RF, Thomas P, Patten D, James CH, Martinez-Torres A, Milei R, and Smart TG (1999) Subunit-selective modulation of GABAA receptors by the non-steroidal anti-inflammatory agent, mefenamic acid. *Eur J Neurosci* **11**:2897–2905.
- Hammarlund-Udenaes M (2010) Active-site concentrations of chemicals - are they a better predictor of effect than plasma/organ/tissue concentrations? *Basic Clin Pharmacol Toxicol* **106**:215–220.
- Hammer H, Bader BM, Ehner C, Bundgaard C, Bunch L, Hoestgaard-Jensen K, Schroeder OH, Bastlund JF, Gramowski-Voß A, and Jensen AA (2015) A Multifaceted GABAA receptor modulator: functional properties and mechanism of action of the sedative-hypnotic and recreational drug methaqualone (Quaalude). *Mol Pharmacol* **88**:401–420.
- Hamon A, Morel A, Hue B, Verleye M, and Gillardin JM (2003) The modulatory effects of the anxiolytic etifoxine on GABA(A) receptors are mediated by the beta subunit. *Neuropharmacology* **45**:293–303.
- Hanrahan JR, Chebib M, and Johnston GA (2015) Interactions of flavonoids with ionotropic GABA receptors. *Adv Pharmacol* **72**:189–200.
- Harris D, Clayton T, Cook J, Sahbaie P, Halliwell RF, Furtmüller R, Huck S, Sieghart W, and DeLorey TM (2008) Selective influence on contextual memory: physicochemical properties associated with selectivity of benzodiazepine ligands at GABAA receptors containing the $\alpha 5$ subunit. *J Med Chem* **51**:3788–3803.
- Hartadi LY, Ahring PK, Chebib M, and Absalom NL (2016) High and low GABA sensitivity $\alpha 4\beta 2\delta$ GABAA receptors are expressed in *Xenopus laevis* oocytes with divergent stoichiometries. *Biochem Pharmacol* **103**:98–108.
- Hartmann K, Stief F, Draguhn A, and Frahm C (2004) Ionotropic GABA receptors with mixed pharmacological properties of GABAA and GABAC receptors. *Eur J Pharmacol* **497**:139–146.
- Harvey SC, Foster KL, McKay PF, Carroll MR, Seyoum R, Woods JE II, Grey C, Jones CM, McCane S, Cummings R, et al. (2002) The GABA(A) receptor $\alpha 1$ subtype in the ventral pallidum regulates alcohol-seeking behaviors. *J Neurosci* **22**: 3765–3775.
- Hayasaka H, Sohma Y, Kanbara K, Maemura K, Kubota T, and Watanabe M (2006) A local GABAergic system within rat trigeminal ganglion cells. *Eur J Neurosci* **23**: 745–757.
- Hedblom E and Kirkness EF (1997) A novel class of GABAA receptor subunit in tissues of the reproductive system. *J Biol Chem* **272**:15346–15350.
- Hellsten KS, Sinkkonen ST, Hyde TM, Kleinman JE, Särkioja T, Maksimow A, Uusi-Oukari M, and Korpi ER (2010) Human locus coeruleus neurons express the GABA (A) receptor gamma2 subunit gene and produce benzodiazepine binding. *Neurosci Lett* **477**:77–81.

- Herb A, Wisden W, Lüddens H, Puia G, Vicini S, and Seeburg PH (1992) The third gamma subunit of the gamma-aminobutyric acid type A receptor family. *Proc Natl Acad Sci USA* **89**:1433–1437.
- Herd MB, Foister N, Chandra D, Peden DR, Homanics GE, Brown VJ, Balfour DJ, Lambert JJ, and Belelli D (2009) Inhibition of thalamic excitability by 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridine-3-ol: a selective role for delta-GABA(A) receptors. *Eur J Neurosci* **29**:1177–1187.
- Hevers W, Hadley SH, Lüddens H, and Amin J (2008) Ketamine, but not phencyclidine, selectively modulates cerebellar GABA(A) receptors containing alpha6 and delta subunits. *J Neurosci* **28**:5383–5393.
- Hibbs RE and Gouaux E (2011) Principles of activation and permeation in an anion-selective Cys-loop receptor. *Nature* **474**:54–60.
- Hill-Venning C, Belelli D, Peters JA, and Lambert JJ (1997) Subunit-dependent interaction of the general anaesthetic etomidate with the gamma-aminobutyric acid type A receptor. *Br J Pharmacol* **120**:749–756.
- Hoerbel P, Ramerstorfer J, Ernst M, Sieghart W, Thomson JL, Hough LB, and Fleck MW (2016) Mutagenesis and computational docking studies support the existence of a histamine binding site at the extracellular $\beta 3 + \beta 3$ -interface of homooligomeric $\beta 3$ GABAA receptors. *Neuropharmacology* **108**:252–263.
- Hoestgaard-Jensen K, Dalby NO, Krall J, Hammer H, Krogsgaard-Larsen P, Frølund B, and Jensen AA (2014) Probing $\alpha 4\beta 8$ GABAA receptor heterogeneity: differential regional effects of a functionally selective $\alpha 4\beta 8/\alpha 4\beta 3\delta$ receptor agonist on tonic and phasic inhibition in rat brain. *J Neurosci* **34**:16256–16272.
- Hoestgaard-Jensen K, O'Connor RM, Dalby NO, Simonsen C, Finger BC, Golubeva A, Hammer H, Bergmann ML, Kristiansen U, Krogsgaard-Larsen P, et al. (2013) The orthosteric GABAA receptor ligand Thio-4-PIOL displays distinctly different functional properties at synaptic and extrasynaptic receptors. *Br J Pharmacol* **170**:919–932.
- Hörtnagl H, Tasan RO, Wieselthaler A, Kirchmair E, Sieghart W, and Sperk G (2013) Patterns of mRNA and protein expression for 12 GABAA receptor subunits in the mouse brain. *Neuroscience* **236**:345–372.
- Hulse G, Kelty E, Hood S, Norman A, Basso MR, and Reece AS (2015) Novel indications for benzodiazepine antagonist flumazenil in GABA mediated pathological conditions of the central nervous system. *Curr Pharm Des* **21**:3325–3342.
- Im WB, Pregenzer JF, Binder JA, Dillon GH, and Alberts GL (1995) Chloride channel expression with the tandem construct of alpha 6-beta 2 GABAA receptor subunit requires a monomeric subunit of alpha 6 or gamma 2. *J Biol Chem* **270**:26063–26066.
- Jechlinger M, Pelz R, Tretter V, Klausberger T, and Sieghart W (1998) Subunit composition and quantitative importance of hetero-oligomeric receptors: GABAA receptors containing alpha6 subunits. *J Neurosci* **18**:2449–2457.
- Jennings AS, Lewis RT, Russell MG, Hallett DJ, Street LJ, Castro JL, Attack JR, Cook SM, Lincoln R, Stanley J, et al. (2006) Imidazo[1,2-b][1,2,4]triazines as alpha2/alpha3 subtype selective GABA A agonists for the treatment of anxiety. *Bioorg Med Chem Lett* **16**:1477–1480.
- Jensen ML, Wafford KA, Brown AR, Belelli D, Lambert JJ, and Mirza NR (2013) A study of subunit selectivity, mechanism and site of action of the delta selective compound 2 (DS2) at human recombinant and rodent native GABA(A) receptors. *Br J Pharmacol* **168**:1118–1132.
- Jin N, Narasaraaju T, Kolliputi N, Chen J, and Liu L (2005) Differential expression of GABAA receptor pi subunit in cultured rat alveolar epithelial cells. *Cell Tissue Res* **321**:173–183.
- Johnston GA (2013) Advantages of an antagonist: bicuculline and other GABA antagonists. *Br J Pharmacol* **169**:328–336.
- Johnston GA and Beart PM (2004) Flavonoids: some of the wisdom of sage? *Br J Pharmacol* **142**:809–810.
- Johnstone TB, Hogenkamp DJ, Coyne L, Su J, Halliwell RF, Tran MB, Yoshimura RF, Li WY, Wang J, and Gee KW (2004) Modifying quinolone antibiotics yields new anxiolytics. *Nat Med* **10**:31–32.
- Jonas O, Calligaris D, Methuku KR, Poe MM, Francois JP, Tranchese F, Changelian A, Sieghart W, Ernst M, Krummel DA, et al. (2016) First in vivo testing of compounds targeting group 3 medulloblastomas using an implantable microdevice as a new paradigm for drug development. *J Biomed Nanotechnol* **12**:1297–1302.
- Jones MV and Westbrook GL (1995) Desensitized states prolong GABAA channel responses to brief agonist pulses. *Neuron* **15**:181–191.
- Jucaite A, Csélenyi Z, Lappalainen J, McCarthy DJ, Lee CM, Nyberg S, Várnás K, Stenkrona P, Hallidin C, Cross A, et al. (2017) GABA_A receptor occupancy by subtype selective GABA_{A α 2,3} modulators: PET studies in humans. *Psychopharmacology (Berl)* **234**:707–716.
- June HL, Foster KL, McKay PF, Seyoum R, Woods JE, Harvey SC, Eiler WJ, Grey C, Carroll MR, McCane S, et al. (2003) The reinforcing properties of alcohol are mediated by GABA(A1) receptors in the ventral pallidum. *Neuropsychopharmacology* **28**:2124–2137.
- Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F, Zaugg M, Vogt KE, Ledermann B, Antkowiak B, et al. (2003) General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit. *FASEB J* **17**:250–252.
- Kaila K, Lamsa K, Smirnov S, Taira T, and Voipio J (1997) Long-lasting GABA-mediated depolarization evoked by high-frequency stimulation in pyramidal neurons of rat hippocampal slice is attributable to a network-driven, bicarbonate-dependent K⁺ transient. *J Neurosci* **17**:7662–7672.
- Kaila K, Price TJ, Payne JA, Puskarjov M, and Voipio J (2014) Cation-chloride cotransporters in neuronal development, plasticity and disease. *Nat Rev Neurosci* **15**:637–654.
- Karim N, Curmi J, Gavande N, Johnston GA, Hanrahan JR, Tierney ML, and Chebib M (2012a) 2'-Methoxy-6-methylflavone: a novel anxiolytic and sedative with subtype selective activating and modulating actions at GABA(A) receptors. *Br J Pharmacol* **165**:880–896.
- Karim N, Wellendorph P, Absalom N, Bang LH, Jensen ML, Hansen MM, Lee HJ, Johnston GA, Hanrahan JR, and Chebib M (2012b) Low nanomolar GABA effects at extrasynaptic $\alpha 4\beta 1/\beta 3\delta$ GABA(A) receptor subtypes indicate a different binding mode for GABA at these receptors. *Biochem Pharmacol* **84**:549–557.
- Karim N, Wellendorph P, Absalom N, Johnston GA, Hanrahan JR, and Chebib M (2013) Potency of GABA at human recombinant GABA(A) receptors expressed in *Xenopus* oocytes: a mini review. *Amino Acids* **44**:1139–1149.
- Karobath M and Sperk G (1979) Stimulation of benzodiazepine receptor binding by gamma-aminobutyric acid. *Proc Natl Acad Sci USA* **76**:1004–1006.
- Kasugai Y, Swinny JD, Roberts JD, Dalezios Y, Fukazawa Y, Sieghart W, Shigemoto R, and Somogyi P (2010) Quantitative localisation of synaptic and extrasynaptic GABAA receptor subunits on hippocampal pyramidal cells by freeze-fracture replica immunolabelling. *Eur J Neurosci* **32**:1868–1888.
- Kaur KH, Baur R, and Sigel E (2009) Unanticipated structural and functional properties of delta-subunit-containing GABAA receptors. *J Biol Chem* **284**:7889–7896.
- Khom S, Baburin I, Timin E, Hohaues A, Trauner G, Kopp B, and Hering S (2007) Valerenic acid potentiates and inhibits GABA(A) receptors: molecular mechanism and subunit specificity. *Neuropharmacology* **53**:178–187.
- Khom S, Baburin I, Timin EN, Hohaues A, Sieghart W, and Hering S (2006) Pharmacological properties of GABAA receptors containing gamma1 subunits. *Mol Pharmacol* **69**:640–649.
- Khom S, Hintersteiner J, Luger D, Haider M, Pototschnig G, Mihovilovic MD, Schwarzer C, and Hering S (2016) Analysis of β -subunit-dependent GABAA receptor modulation and behavioral effects of valerenic acid derivatives. *J Pharmacol Exp Ther* **357**:580–590.
- Knabl J, Witschi R, Hösl K, Reinold H, Zeilhofer UB, Ahmadi S, Brockhaus J, Sergejeva M, Hess A, Brune K, et al. (2008) Reversal of pathological pain through specific spinal GABAA receptor subtypes. *Nature* **451**:330–334.
- Knoflach F, Benke D, Wang Y, Scheurer L, Lüddens H, Hamilton BJ, Carter DB, Mohler H, and Benson JA (1996) Pharmacological modulation of the diazepam-insensitive recombinant gamma-aminobutyric acid A receptors alpha 4 beta 2 gamma 2 and alpha 6 beta 2 gamma 2. *Mol Pharmacol* **50**:1253–1261.
- Knoflach F, Hernandez MC, and Bertrand D (2016) GABAA receptor-mediated neurotransmission: not so simple after all. *Biochem Pharmacol* **115**:10–17.
- Knoflach F, Rhyner T, Villa M, Kellenberger S, Drescher U, Malherbe P, Sigel E, and Möhler H (1991) The gamma 3-subunit of the GABAA-receptor confers sensitivity to benzodiazepine receptor ligands. *FEBS Lett* **293**:191–194.
- Knutson DE, Kodali R, Divović B, Treven M, Stephen MR, Zahn NM, Dobrićić V, Huber AT, Meirrelles MA, Verma RS, et al. (2018) Design and synthesis of novel deuterated ligands functionally selective for the γ -aminobutyric acid type A receptor (GABA_A) $\alpha 6$ subtype with improved metabolic stability and enhanced bioavailability. *J Med Chem* **61**:2422–2446.
- Koh MT, Rosenzweig-Lipson S, and Gallagher M (2013) Selective GABA(A) $\alpha 5$ positive allosteric modulators improve cognitive function in aged rats with memory impairment. *Neuropharmacology* **64**:145–152.
- Korpi ER, Gründer G, and Lüddens H (2002) Drug interactions at GABA(A) receptors. *Prog Neurobiol* **67**:113–159.
- Korpi ER, Kuner T, Seeburg PH, and Lüddens H (1995) Selective antagonist for the cerebellar granule cell-specific gamma-aminobutyric acid type A receptor. *Mol Pharmacol* **47**:283–289.
- Korpi ER and Lüddens H (1997) Furosemide interactions with brain GABAA receptors. *Br J Pharmacol* **120**:741–748.
- Kramer PR and Bellinger LL (2013) Reduced GABAA receptor $\alpha 6$ expression in the trigeminal ganglion enhanced myofascial nociceptive response. *Neuroscience* **245**:1–11.
- Krishek BJ, Moss SJ, and Smart TG (1996) Homomeric beta 1 gamma-aminobutyric acid A receptor-ion channels: evaluation of pharmacological and physiological properties. *Mol Pharmacol* **49**:494–504.
- Krogsgaard-Larsen P, Frølund B, Liljefors T, and Ebert B (2004) GABA(A) agonists and partial agonists: THIP (Gaboxadol) as a non-opioid analgesic and a novel type of hypnotic. *Biochem Pharmacol* **68**:1573–1580.
- Lager E, Nilsson J, Østergaard Nielsen E, Nielsen M, Liljefors T, and Sterner O (2008) Affinity of 3-acyl substituted 4-quinolones at the benzodiazepine site of GABA(A) receptors. *Bioorg Med Chem* **16**:6936–6948.
- Lambert S, Arras M, Vogt KE, and Rudolph U (2005) Isoflurane-induced surgical tolerance mediated only in part by beta3-containing GABA(A) receptors. *Eur J Pharmacol* **516**:23–27.
- Laverty D, Thomas P, Field M, Andersen OJ, Gold MG, Biggin PC, Gielen M, and Smart TG (2017) Crystal structures of a GABA_A-receptor chimera reveal new endogenous neurosteroid-binding sites. *Nat Struct Mol Biol* **24**:977–985.
- Leggio GM, Torrisi SA, Castorina A, Platania CB, Impellizzeri AA, Fidilio A, Caraci F, Bucolo C, Drago F, and Salomone S (2015) Dopamine D3 receptor-dependent changes in alpha6 GABAA subunit expression in striatum modulate anxiety-like behaviour: responsiveness and tolerance to diazepam. *Eur Neuropsychopharmacol* **25**:1427–1436.
- Levin ML, Chatterjee A, Pragliola A, Worley KC, Wehnert M, Zhuchenko O, Smith RF, Lee CC, and Herman GE (1996) A comparative transcription map of the murine bare patches (Bpa) and striated (Str) critical regions and human Xq28. *Genome Res* **6**:465–477.
- Levitan ES, Schofield PR, Burt DR, Rhee LM, Wisden W, Köhler M, Fujita N, Rodriguez HF, Stephenson A, Darlison MG, et al. (1988) Structural and functional basis for GABAA receptor heterogeneity. *Nature* **335**:76–79.
- Lewis RT, Blackaby WP, Blackburn T, Jennings AS, Pike A, Wilson RA, Hallett DJ, Cook SM, Ferris P, Marshall GR, et al. (2006) A pyridazine series of alpha2/alpha3 subtype selective GABA A agonists for the treatment of anxiety. *J Med Chem* **49**:2600–2610.
- Lewter LA, Fisher JL, Siemian JN, Methuku KR, Poe MM, Cook JM, and Li JX (2017) Antinociceptive effects of a novel $\alpha 2/\alpha 3$ -subtype selective GABA_A receptor positive allosteric modulator. *ACS Chem Neurosci* **8**:1305–1312.

- Li GD, Chiara DC, Cohen JB, and Olsen RW (2010) Numerous classes of general anesthetics inhibit etomidate binding to gamma-aminobutyric acid type A (GABAA) receptors. *J Biol Chem* **285**:8615–8620.
- Li P and Akk G (2015) Synaptic-type $\alpha 1\beta 2\gamma 2$ GABAA receptors produce large persistent currents in the presence of ambient GABA and anesthetic drugs. *Mol Pharmacol* **87**:776–781.
- Li X, Cao H, Zhang C, Furtmueller R, Fuchs K, Huck S, Sieghart W, Deschamps J, and Cook JM (2003) Synthesis, in vitro affinity, and efficacy of a bis 8-ethynyl-4H-imidazo[1,5a]-[1,4]benzodiazepine analogue, the first bivalent $\alpha 5$ subtype selective BzR/GABA(A) antagonist. *J Med Chem* **46**:5567–5570.
- Liao YH, Lee HJ, Huang WJ, Fan PC, and Chiou LC (2016) Hispidulin alleviated methamphetamine-induced hyperlocomotion by acting at $\alpha 6$ subunit-containing GABAA receptors in the cerebellum. *Psychopharmacology (Berl)* **233**:3187–3199.
- Ling I, Mihalik B, Etherington LA, Kapus G, Pálvölgyi A, Gígler G, Kertész S, Gaál A, Pallagi K, Kiricsi P, et al. (2015) A novel GABA(A) $\alpha 5$ receptor inhibitor with therapeutic potential. *Eur J Pharmacol* **764**:497–507.
- Lippa A, Czobor P, Stark J, Beer B, Kostakis E, Gravielle M, Bandyopadhyay S, Russek SJ, Gibbs TT, Farb DH, et al. (2005) Selective anxiolysis produced by ocinaplon, a GABA(A) receptor modulator. *Proc Natl Acad Sci USA* **102**:7380–7385.
- López-Chávez A, Milei R, and Martínez-Torres A (2005) Cloning and functional expression of the bovine GABA(C) $\rho 2$ subunit. Molecular evidence of a widespread distribution in the CNS. *Neurosci Res* **53**:421–427.
- Loup F, Wieser HG, Yonekawa Y, Aguzzi A, and Fritschy JM (2000) Selective alterations in GABAA receptor subtypes in human temporal lobe epilepsy. *J Neurosci* **20**:5401–5419.
- Löw K, Crestani F, Keist R, Benke D, Brünig I, Benson JA, Fritschy JM, Rülcke T, Bluethmann H, Möhler H, et al. (2000) Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* **290**:131–134.
- Lüddens H and Korpi ER (1995) GABA antagonists differentiate between recombinant GABA/benzodiazepine receptor subtypes. *J Neurosci* **15**:6957–6962.
- Luque JM, Malherbe P, and Richards JG (1994) Localization of GABAA receptor subunit mRNAs in the rat locus coeruleus. *Brain Res Mol Brain Res* **24**:219–226.
- Maksay G, Thompson SA, and Wafford KA (2003) The pharmacology of spontaneously open $\alpha 1$ $\beta 3$ $\gamma 2$ GABA A receptor-ionophores. *Neuropharmacology* **44**:994–1002.
- Marowsky A and Vogt KE (2014) Delta-subunit-containing GABAA-receptors mediate tonic inhibition in paracapsular cells of the mouse amygdala. *Front Neural Circuits* **8**:27.
- Martínez-Delgado G, Estrada-Mondragón A, Milei R, and Martínez-Torres A (2010) An update on GABA ρ receptors. *Curr Neuropharmacol* **8**:422–433.
- McKay PF, Foster KL, Mason D, Cummings R, Garcia M, Williams LS, Grey C, McCane S, He X, Cook JM, et al. (2004) A high affinity ligand for GABAA receptor containing $\alpha 5$ subunit antagonizes ethanol's neurobehavioral effects in Long-Evans rats. *Psychopharmacology (Berl)* **172**:455–462.
- McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, Farrar S, Myers J, Cook G, Ferris P, et al. (2000) Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor $\alpha 1$ subtype. *Nat Neurosci* **3**:587–592.
- Meera P, Wallner M, and Otis TS (2011) Molecular basis for the high THIP/gaboxadol sensitivity of extrasynaptic GABA(A) receptors. *J Neurophysiol* **106**:2057–2064.
- Miller PS and Aricescu AR (2014) Crystal structure of a human GABAA receptor. *Nature* **512**:270–275.
- Milligan CJ, Buckley NJ, Garret M, Deuchars J, and Deuchars SA (2004) Evidence for inhibition mediated by coassembly of GABAA and GABAC receptor subunits in native central neurons. *J Neurosci* **24**:7241–7250.
- Mirheydari P, Ramerstorfer J, Varagic Z, Scholze P, Wimmer L, Mihovilovic MM, Sieghart W, and Ernst M (2014) Unexpected properties of δ -containing GABAA receptors in response to ligands interacting with the $\alpha + \beta$ -site. *Neurochem Res* **39**:1057–1067.
- Mirza NR, Larsen JS, Mathiasen C, Jacobsen TA, Munro G, Erichsen HK, Nielsen AN, Troelsen KB, Nielsen EO, and Ahning PK (2008) NS11394 [3'-(5-[1-hydroxy-1-methyl-ethyl]-benzimidazol-1-yl)-biphenyl-2-carbonitrile], a unique subtype-selective GABAA receptor positive allosteric modulator: in vitro actions, pharmacokinetic properties and in vivo anxiolytic efficacy. *J Pharmacol Exp Ther* **327**:954–968.
- Möhler H (2014) Endogenous benzodiazepine site peptide ligands operating bidirectionally in vivo in neurogenesis and thalamic oscillations. *Neurochem Res* **39**:1032–1036.
- Möhler H and Okada T (1977) Benzodiazepine receptor: demonstration in the central nervous system. *Science* **198**:849–851.
- Moragues N, Ciofi P, Lafon P, Odessa MF, Tramu G, and Garret M (2000) cDNA cloning and expression of a gamma-aminobutyric acid A receptor epsilon-subunit in rat brain. *Eur J Neurosci* **12**:4318–4330.
- Moragues N, Ciofi P, Lafon P, Tramu G, and Garret M (2003) GABAA receptor epsilon subunit expression in identified peptidergic neurons of the rat hypothalamus. *Brain Res* **967**:285–289.
- Moragues N, Ciofi P, Tramu G, and Garret M (2002) Localisation of GABA(A) receptor epsilon-subunit in cholinergic and aminergic neurones and evidence for co-distribution with the theta-subunit in rat brain. *Neuroscience* **111**:657–669.
- Mortensen M, Patel B, and Smart TG (2012) GABA potency at GABA(A) receptors found in synaptic and extrasynaptic zones. *Front Cell Neurosci* **6**:1.
- Mortensen M and Smart TG (2006) Extrasynaptic $\alpha 6$ subunit GABAA receptors on rat hippocampal pyramidal neurons. *J Physiol* **577**:841–856.
- Mozzrymas JW, Zarnowska ED, Pytel M, and Mercik K (2003) Modulation of GABA(A) receptors by hydrogen ions reveals synaptic GABA transient and a crucial role of the desensitization process [published correction appears in *J Neurosci* 2003;23]. *J Neurosci* **23**:7981–7992.
- Müller Herde A, Benke D, Ralvenius WT, Mu L, Schibli R, Zeilhofer HU, and Krämer SD (2017) GABA ρ receptor subtypes in the mouse brain: regional mapping and diazepam receptor occupancy by in vivo [18 F]flumazenil PET. *Neuroimage* **150**:279–291.
- Musch B, Morselli PL, and Priore P (1988) Clinical studies with the new anxiolytic alpidem in anxious patients: an overview of the European experiences. *Pharmacol Biochem Behav* **29**:803–806.
- Naffaa MM, Hung S, Chebib M, Johnston GAR, and Hanrahan JR (2017) GABA- ρ receptors: distinctive functions and molecular pharmacology. *Br J Pharmacol* **174**:1881–1894.
- Namjoshi OA, Wang ZJ, Rallapalli SK, Johnson EM Jr, Johnson YT, Ng H, Ramerstorfer J, Varagic Z, Sieghart W, Majumder S, et al. (2013) Search for $\alpha 3\beta 2\gamma 2$ subtype selective ligands that are stable on human liver microsomes. *Bioorg Med Chem* **21**:93–101.
- Neelands TR, Fisher JL, Bianchi M, and Macdonald RL (1999) Spontaneous and gamma-aminobutyric acid (GABA)-activated GABA(A) receptor channels formed by epsilon subunit-containing isoforms. *Mol Pharmacol* **55**:168–178.
- Neelands TR and Macdonald RL (1999) Incorporation of the pi subunit into functional gamma-aminobutyric acid(A) receptors. *Mol Pharmacol* **56**:598–610.
- Ng CK, Kim HL, Gavande N, Yamamoto I, Kumar RJ, Mewett KN, Johnston GA, Hanrahan JR, and Chebib M (2011) Medicinal chemistry of ρ GABAC receptors. *Future Med Chem* **3**:197–209.
- Nik AM, Pressly B, Singh V, Antrobus S, Hulsizer S, Rogawski MA, Wulff H, and Pessah IN (2017) Rapid throughput analysis of GABA ρ receptor subtype modulators and blockers using DiSBAC $_1(3)$ membrane potential red dye. *Mol Pharmacol* **92**:88–99.
- Nothdurfter C, Rammes G, Baghai TC, Schüle C, Schumacher M, Papadopoulos V, and Rupprecht R (2012) Translocator protein (18 kDa) as a target for novel anxiolytics with a favourable side-effect profile. *J Neuroendocrinol* **24**:82–92.
- Nusser Z and Mody I (2002) Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J Neurophysiol* **87**:2624–2628.
- Nusser Z, Sieghart W, and Somogyi P (1998) Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Neurosci* **18**:1693–1703.
- Nutt D (1983) Pharmacological and behavioural studies of benzodiazepine antagonists and contragonists. *Adv Biochem Psychopharmacol* **38**:153–173.
- Olsen RW and Sieghart W (2008) International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol Rev* **60**:243–260.
- Pan ZH, Zhang D, Zhang X, and Lipton SA (2000) Evidence for coassembly of mutant GABAC $\rho 1$ with GABAA $\gamma 2$, glycine $\alpha 1$ and glycine $\alpha 2$ receptor subunits in vitro. *Eur J Neurosci* **12**:3137–3145.
- Pau D, Bellelli D, Callachan H, Peden DR, Dunlop JI, Peters JA, Guitart X, Gutierrez B, and Lambert JJ (2003) GABAA receptor modulation by the novel intravenous general anaesthetic E-6375. *Neuropharmacology* **45**:1029–1040.
- Perrais D and Ropert N (1999) Effect of zolpidem on miniature IPSCs and occupancy of postsynaptic GABAA receptors in central synapses. *J Neurosci* **19**:578–588.
- Petroski RE, Pomeroy JE, Das R, Bowman H, Yang W, Chen AP, and Foster AC (2006) Indiplon is a high-affinity positive allosteric modulator with selectivity for $\alpha 1$ subunit-containing GABAA receptors. *J Pharmacol Exp Ther* **317**:369–377.
- Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, and Sperk G (2000) GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience* **101**:815–850.
- Pörtl A, Hauer B, Fuchs K, Tretter V, and Sieghart W (2003) Subunit composition and quantitative importance of GABA(A) receptor subtypes in the cerebellum of mouse and rat. *J Neurochem* **87**:1444–1455.
- Popik P, Kostakis E, Krawczyk M, Nowak G, Szewczyk B, Krieter P, Chen Z, Russek SJ, Gibbs TT, Farb DH, et al. (2006) The anxiolytic agent 7-(2-chloropyridin-4-yl)pyrazolo-[1,5-a]-pyrimidin-3-yl(pyridin-2-yl)methanone (DOV 51892) is more efficacious than diazepam at enhancing GABA-gated currents at $\alpha 1$ subunit-containing GABAA receptors. *J Pharmacol Exp Ther* **319**:1244–1252.
- Primus RJ, Yu J, Xu J, Hartnett C, Meyyappan M, Kostas C, Ramabhadran TV, and Gallager DW (1996) Allosteric uncoupling after chronic benzodiazepine exposure of recombinant gamma-aminobutyric acid(A) receptors expressed in Sf9 cells: ligand efficacy and subtype selectivity. *J Pharmacol Exp Ther* **276**:882–890.
- Pritchett DB, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR, and Seeburg PH (1989) Importance of a novel GABAA receptor subunit for benzodiazepine pharmacology. *Nature* **338**:582–585.
- Puia G, Vicini S, Seeburg PH, and Costa E (1991) Influence of recombinant gamma-aminobutyric acid-A receptor subunit composition on the action of allosteric modulators of gamma-aminobutyric acid-gated Cl $^{-}$ currents. *Mol Pharmacol* **39**:691–696.
- Puri J, Vinodhini P, Reuben J, Bellinger LL, Ailing L, Peng YB, and Kramer PR (2012) Reduced GABA(A) receptor $\alpha 6$ expression in the trigeminal ganglion alters inflammatory TMJ hypersensitivity. *Neuroscience* **213**:179–190.
- Puthenkalam R, Hieckel M, Simeone X, Suwattanasophon C, Feldbauer RV, Ecker GF, and Ernst M (2016) Structural studies of GABAA receptor binding sites: which experimental structure tells us what? *Front Mol Neurosci* **9**:44.
- Qian H and Ripps H (1999) Response kinetics and pharmacological properties of heteromeric receptors formed by coassembly of GABA ρ - and gamma 2-subunits. *Proc Biol Sci* **266**:2419–2425.
- Rabe H, Kronbach C, Rundfeldt C, and Lüddens H (2007) The novel anxiolytic ELB139 displays selectivity to recombinant GABA(A) receptors different from diazepam. *Neuropharmacology* **52**:796–801.
- Ralvenius WT, Benke D, Acuña MA, Rudolph U, and Zeilhofer HU (2015) Analgesia and unwanted benzodiazepine effects in point-mutated mice expressing only one benzodiazepine-sensitive GABAA receptor subtype. *Nat Commun* **6**:6803.
- Ramerstorfer J, Foppa V, Thiery H, Hermange P, Janody S, Berger ML, Dodd RH, and Sieghart W (2015) GABA(A) receptor subtype-selectivity of novel bicuculline derivatives. *Curr Med Chem* **22**:771–780.

- Ramerstorfer J, Furtmüller R, Sarto-Jackson I, Varagic Z, Sieghart W, and Ernst M (2011) The GABAA receptor alpha+beta- interface: a novel target for subtype selective drugs. *J Neurosci* **31**:870–877.
- Ramerstorfer J, Furtmüller R, Vogel E, Huck S, and Sieghart W (2010) The point mutation gamma 2F77I changes the potency and efficacy of benzodiazepine site ligands in different GABAA receptor subtypes. *Eur J Pharmacol* **636**:18–27.
- Ranna M, Sinkkonen ST, Möykkynen T, Uusi-Oukari M, and Korpi ER (2006) Impact of epsilon and theta subunits on pharmacological properties of alpha3beta1 GABAA receptors expressed in *Xenopus* oocytes. *BMC Pharmacol* **6**:1.
- Ren L, Wang F, Xu Z, Chan WM, Zhao C, and Xue H (2010) GABA(A) receptor subtype selectivity underlying anxiolytic effect of 6-hydroxyflavone. *Biochem Pharmacol* **79**:1337–1344.
- Reynolds DS, Rosahl TW, Cirone J, O'Meara GF, Haythornthwaite A, Newman RJ, Myers J, Sur C, Howell O, Rutter AR, et al. (2003) Sedation and anesthesia mediated by distinct GABA(A) receptor isoforms. *J Neurosci* **23**:8608–8617.
- Richter L, de Graaf C, Sieghart W, Varagic Z, Mözinger M, de Esch IJ, Ecker GF, and Ernst M (2012) Diazepam-bound GABAA receptor models identify new benzodiazepine binding-site ligands. *Nat Chem Biol* **8**:455–464.
- Rivas FM, Stables JP, Murphree L, Edwankar RV, Edwankar CR, Huang S, Jain HD, Zhou H, Majumder S, Sankar S, et al. (2009) Antiseizure activity of novel gamma-aminobutyric acid (A) receptor subtype-selective benzodiazepine analogues in mice and rat models. *J Med Chem* **52**:1795–1798.
- Rudolph U, Crestani F, Benke D, Brünig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H, and Möhler H (1999) Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. *Nature* **401**:796–800.
- Rudolph U and Knoflach F (2011) Beyond classical benzodiazepines: novel therapeutic potential of GABAA receptor subtypes. *Nat Rev Drug Discov* **10**:685–697.
- Rudolph U and Möhler H (2014) GABAA receptor subtypes: therapeutic potential in Down syndrome, affective disorders, schizophrenia, and autism. *Annu Rev Pharmacol Toxicol* **54**:483–507.
- Rumpel E and Behrendts JC (2000) Postsynaptic receptor occupancy during evoked transmission at striatal GABAergic synapses in vitro. *J Neurophysiol* **84**:771–779.
- Rundfeldt C and Löscher W (2014) The pharmacology of imepitoin: the first partial benzodiazepine receptor agonist developed for the treatment of epilepsy. *CNS Drugs* **28**:29–43.
- Sanna E, Garau F, and Harris RA (1995) Novel properties of homomeric beta 1 gamma-aminobutyric acid type A receptors: actions of the anesthetics propofol and pentobarbital. *Mol Pharmacol* **47**:213–217.
- Saras A, Hisselmann G, Vogt-Eisele AK, Erlkamp KS, Kletke O, Pusch H, and Hatt H (2008) Histamine action on vertebrate GABAA receptors: direct channel gating and potentiation of GABA responses. *J Biol Chem* **283**:10470–10475.
- Savić MM, Clayton T, Furtmüller R, Gavrilović I, Samardžić J, Savić S, Huck S, Sieghart W, and Cook JM (2008a) PWZ-029, a compound with moderate inverse agonist functional selectivity at GABA(A) receptors containing alpha5 subunits, improves passive, but not active, avoidance learning in rats. *Brain Res* **1208**:150–159.
- Savić MM, Huang S, Furtmüller R, Clayton T, Huck S, Obradović DI, Ugresić ND, Sieghart W, Bokonić DR, and Cook JM (2008b) Are GABAA receptors containing alpha5 subunits contributing to the sedative properties of benzodiazepine site agonists? *Neuropsychopharmacology* **33**:332–339.
- Savić MM, Majumder S, Huang S, Edwankar RV, Furtmüller R, Joksimović S, Clayton T Sr, Ramerstorfer J, Milinković MM, Roth BL, et al. (2010) Novel positive allosteric modulators of GABAA receptors: do subtle differences in activity at alpha1 plus alpha5 versus alpha2 plus alpha3 subunits account for dissimilarities in behavioral effects in rats? *Prog Neuropsychopharmacol Biol Psychiatry* **34**:376–386.
- Savić MM, Obradović DI, Ugresić ND, Cook JM, Yin W, and Bokonić DR (2004) Bidirectional effects of benzodiazepine binding site ligands in the elevated plus-maze: differential antagonism by flumazenil and beta-CCt. *Pharmacol Biochem Behav* **79**:279–290.
- Schlichter R, Rybalchenko V, Poisbeau P, Verleye M, and Gillardin J (2000) Modulation of GABAergic synaptic transmission by the non-benzodiazepine anxiolytic etifoxine. *Neuropharmacology* **39**:1523–1535.
- Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rodriguez H, Rhee LM, Ramachandran J, Reale V, Glencorse TA, et al. (1987) Sequence and functional expression of the GABA A receptor shows a ligand-gated receptor superfamily. *Nature* **328**:221–227.
- Seeger C, Christopeit T, Fuchs K, Grote K, Sieghart W, and Danielson UH (2012) Histaminergic pharmacology of homo-oligomeric beta 3 gamma-aminobutyric acid type A receptors characterized by surface plasmon resonance biosensor technology. *Biochem Pharmacol* **84**:341–351.
- Seljeset S, Laverty D, and Smart TG (2015) Inhibitory neurosteroids and the GABAA receptor. *Adv Pharmacol* **72**:165–187.
- Selleri S, Bruni F, Costagli C, Costanzo A, Guerrini G, Ciciani G, Gratteri P, Besnard F, Costa B, Montali M, et al. (2005) A novel selective GABA(A) alpha1 receptor agonist displaying sedative and anxiolytic-like properties in rodents. *J Med Chem* **48**:6756–6760.
- Selleri S, Bruni F, Costagli C, Costanzo A, Guerrini G, Ciciani G, Gratteri P, Bonaccini C, Malmberg Aiello P, Besnard F, et al. (2003) Synthesis and benzodiazepine receptor affinity of pyrazolo[1,5-*a*]pyrimidine derivatives. 3. New 6-(3-thienyl) series as alpha 1 selective ligands. *J Med Chem* **46**:310–313.
- Sergeeva OA, Andreeva N, Garret M, Scherer A, and Haas HL (2005) Pharmacological properties of GABAA receptors in rat hypothalamic neurons expressing the epsilon-subunit. *J Neurosci* **25**:88–95.
- Sergeeva OA, Kletke O, Kragler A, Poppek A, Fleischer W, Schubring SR, Görg B, Haas HL, Zhu XR, Lübbert H, et al. (2010) Fragrant dioxane derivatives identify beta1-subunit-containing GABAA receptors. *J Biol Chem* **285**:23985–23993.
- Shen H, Sabaliauskas N, Yang L, Aoki C, and Smith SS (2017) Role of alpha4-containing GABAA receptors in limiting synaptic plasticity and spatial learning of female mice during the pubertal period. *Brain Res* **1654** (Pt B):116–122.
- Sieghart W (1995) Structure and pharmacology of gamma-aminobutyric acid(A) receptor subtypes. *Pharmacol Rev* **47**:181–234.
- Sieghart W (2015) Allosteric modulation of GABAA receptors via multiple drug-binding sites. *Adv Pharmacol* **72**:53–96.
- Sieghart W and Karobath M (1980) Molecular heterogeneity of benzodiazepine receptors. *Nature* **286**:285–287.
- Sieghart W and Sperk G (2002) Subunit composition, distribution and function of GABA(A) receptor subtypes. *Curr Top Med Chem* **2**:795–816.
- Sigel E, Baur R, Malherbe P, and Möhler H (1989) The rat beta 1-subunit of the GABAA receptor forms a picrotoxin-sensitive anion channel open in the absence of GABA. *FEBS Lett* **257**:377–379.
- Sigel E and Buhr A (1997) The benzodiazepine binding site of GABA(A) receptors. *Trends Pharmacol Sci* **18**:425–429.
- Simeone X, Siebert DCB, Bampali K, Varagic Z, Treven M, Rehman S, Pyszkowski J, Holzinger R, Steudle F, Scholze P, et al. (2017) Molecular tools for GABA(A) receptors: high affinity ligands for beta1-containing subtypes. *Sci Rep* **7**:5674.
- Sinkkonen ST, Hanna MC, Kirkness EF, and Korpi ER (2000) GABA(A) receptor epsilon and theta subunits display unusual structural variation between species and are enriched in the rat locus ceruleus. *J Neurosci* **20**:3588–3595.
- Skolnick P (2012) Anxiolytic anxiolytics: on a quest for the Holy Grail. *Trends Pharmacol Sci* **33**:611–620.
- Slany A, Zezula J, Tretter V, and Sieghart W (1995) Rat beta 3 subunits expressed in human embryonic kidney 293 cells form high affinity [35S]t-butylbicyclophosphorothionate binding sites modulated by several allosteric ligands of gamma-aminobutyric acid type A receptors. *Mol Pharmacol* **48**:385–391.
- Smith AJ, Oxley B, Malpas S, Pillai GV, and Simpson PB (2004) Compounds exhibiting selective efficacy for different beta subunits of human recombinant gamma-aminobutyric acid A receptors. *J Pharmacol Exp Ther* **311**:601–609.
- Smith GB and Olsen RW (1995) Functional domains of GABAA receptors. *Trends Pharmacol Sci* **16**:162–168.
- Stamenić TT, Poe MM, Rehman S, Santrać A, Divović B, Scholze P, Ernst M, Cook JM, and Savić MM (2016) Ester to amide substitution improves selectivity, efficacy and kinetic behavior of a benzodiazepine positive modulator of GABA(A) receptors containing the alpha5 subunit. *Eur J Pharmacol* **791**:433–443.
- Stefanits H, Milenkovic I, Mahr N, Pataria E, Hainfellner JA, Kovacs GG, Sieghart W, Yilmazer-Hanke D, and Czech T (2018) GABA(A) receptor subunits in the human amygdala and hippocampus: immunohistochemical distribution of 7 subunits. *J Comp Neurol* **526**:324–348.
- Stevenson A, Wingrove PB, Whiting PJ, and Wafford KA (1995) beta-Carboline gamma-aminobutyric acid(A) receptor inverse agonists modulate gamma-aminobutyric acid via the loreclezole binding site as well as the benzodiazepine site. *Mol Pharmacol* **48**:965–969.
- Stojanovic T, Capo I, Aronica E, Adle-Biasette H, Höger H, Sieghart W, Kovacs GG, and Milenkovic I (2016) The alpha1, alpha2, alpha3, and gamma2 subunits of GABAA receptors show characteristic spatial and temporal expression patterns in rhombencephalic structures during normal human brain development. *J Comp Neurol* **524**:1805–1824.
- Street LJ, Sternfeld F, Jelley RA, Reeve AJ, Carling RW, Moore KW, McKernan RM, Sohal B, Cook S, Pike A, et al. (2004) Synthesis and biological evaluation of 3-heterocyclyl-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-*a*]phthalazines and analogues as subtype-selective inverse agonists for the GABA(A)alpha5 benzodiazepine binding site. *J Med Chem* **47**:3642–3657.
- Sullivan SK, Petroski RE, Verge G, Gross RS, Foster AC, and Grigoriadis DE (2004) Characterization of the interaction of indiplon, a novel pyrazolopyrimidine sedative-hypnotic, with the GABAA receptor. *J Pharmacol Exp Ther* **311**:537–546.
- Summerfield SG, Read K, Begley DJ, Obradović T, Hidalgo LJ, Coggon S, Lewis AV, Porter RA, and Jeffrey P (2007) Central nervous system drug disposition: the relationship between in situ brain permeability and brain free fraction. *J Pharmacol Exp Ther* **322**:205–213.
- Sur C, Quirk K, Dewar D, Atack J, and McKernan R (1998) Rat and human hippocampal alpha5 subunit-containing gamma-aminobutyric acid(A) receptors have alpha5 beta3 gamma2 pharmacological characteristics. *Mol Pharmacol* **54**:928–933.
- Takahashi H, Chen MC, Pham H, Angst E, King JC, Park J, Brovmann EY, Ishiguro H, Harris DM, Reber HA, et al. (2011) Baicalein, a component of *Scutellaria baicalensis*, induces apoptosis by Mcl-1 down-regulation in human pancreatic cancer cells. *Biochim Biophys Acta* **1813**:1465–1474.
- Tang X, Hernandez CC, and Macdonald RL (2010) Modulation of spontaneous and GABA-evoked tonic alpha4beta3delta and alpha4beta3gamma2L GABAA receptor currents by protein kinase A. *J Neurophysiol* **103**:1007–1019.
- Tarragó T, Kichik N, Claassen B, Prades R, Teixidó M, and Giralte E (2008) Baicalin, a prodrug able to reach the CNS, is a prolyl oligopeptidase inhibitor. *Bioorg Med Chem* **16**:7516–7524.
- Thomet U, Baur R, Razet R, Dodd RH, Furtmüller R, Sieghart W, and Sigel E (2000) A novel positive allosteric modulator of the GABA(A) receptor: the action of (+)-ROD188. *Br J Pharmacol* **131**:843–850.
- Thompson SA, Arden SA, Marshall G, Wingrove PB, Whiting PJ, and Wafford KA (1999) Residues in transmembrane domains I and II determine gamma-aminobutyric acid type A receptor subtype-selective antagonism by furosemide. *Mol Pharmacol* **55**:993–999.
- Thompson SA, Bonnert TP, Cagetti E, Whiting PJ, and Wafford KA (2002a) Overexpression of the GABA(A) receptor epsilon subunit results in insensitivity to anaesthetics. *Neuropharmacology* **43**:662–668.
- Thompson SA, Wheat L, Brown NA, Wingrove PB, Pillai GV, Whiting PJ, Adkins C, Woodward CH, Smith AJ, Simpson PB, et al. (2004) Salicylidene salicylhydrazide, a selective inhibitor of beta 1-containing GABAA receptors. *Br J Pharmacol* **142**:97–106.
- Thompson S-A, Wingrove PB, Connolly L, Whiting PJ, and Wafford KA (2002b) Tracazolate reveals a novel type of allosteric interaction with recombinant gamma-aminobutyric acid(A) receptors. *Mol Pharmacol* **61**:861–869.

- Tretter V, Ehya N, Fuchs K, and Sieghart W (1997) Stoichiometry and assembly of a recombinant GABAA receptor subtype. *J Neurosci* **17**:2728–2737.
- Treven M, Siebert DCB, Holzinger R, Bampali K, Fabjan J, Varagic Z, Wimmer L, Steudle F, Scholze P, Schnürch M, et al. (2018) Towards functional selectivity for $\alpha\beta\gamma 2$ GABAA receptors: a series of novel pyrazoloquinolinones. *Br J Pharmacol* **175**:419–428.
- Trumbull JD, Maslana ES, McKenna DG, Nemcek TA, Niforatos W, Pan JY, Parihar AS, Shieh CC, Wilkins JA, Briggs CA, et al. (2003) High throughput electrophysiology using a fully automated, multiplexed recording system. *Receptors Channels* **9**:19–28.
- van Niel MB, Wilson K, Adkins CH, Atack JR, Castro JL, Clarke DE, Fletcher S, Gerhard U, Mackey MM, Malpas S, et al. (2005) A new pyridazine series of GABAA $\alpha 5$ ligands. *J Med Chem* **48**:6004–6011.
- Varagic Z, Ramerstorfer J, Huang S, Rallapalli S, Sarto-Jackson I, Cook J, Sieghart W, and Ernst M (2013a) Subtype selectivity of $\alpha + \beta$ - site ligands of GABAA receptors: identification of the first highly specific positive modulators at $\alpha\beta 2/3\gamma 2$ receptors. *Br J Pharmacol* **169**:384–399.
- Varagic Z, Wimmer L, Schnürch M, Mihovilovic MD, Huang S, Rallapalli S, Cook JM, Mirheydari P, Ecker GF, Sieghart W, et al. (2013b) Identification of novel positive allosteric modulators and null modulators at the GABAA receptor $\alpha + \beta$ - interface. *Br J Pharmacol* **169**:371–383.
- Vinkers CH, Mirza NR, Olivier B, and Kahn RS (2010) The inhibitory GABA system as a therapeutic target for cognitive symptoms in schizophrenia: investigational agents in the pipeline. *Expert Opin Investig Drugs* **19**:1217–1233.
- Wafford KA, Bain CJ, Quirk K, McKernan RM, Wingrove PB, Whiting PJ, and Kemp JA (1994) A novel allosteric modulatory site on the GABAA receptor beta subunit. *Neuron* **12**:775–782.
- Wafford KA, Bain CJ, Whiting PJ, and Kemp JA (1993) Functional comparison of the role of gamma subunits in recombinant human gamma-aminobutyric acidA/benzodiazepine receptors. *Mol Pharmacol* **44**:437–442.
- Wafford KA and Ebert B (2006) Gaboxadol—a new awakening in sleep. *Curr Opin Pharmacol* **6**:30–36.
- Wafford KA, Thompson SA, Thomas D, Sikela J, Wilcox AS, and Whiting PJ (1996) Functional characterization of human gamma-aminobutyric acidA receptors containing the alpha 4 subunit. *Mol Pharmacol* **50**:670–678.
- Wafford KA, van Niel MB, Ma QP, Horridge E, Herd MB, Peden DR, Belelli D, and Lambert JJ (2009) Novel compounds selectively enhance delta subunit containing GABA A receptors and increase tonic currents in thalamus. *Neuropharmacology* **56**:182–189.
- Wagner DA, Goldschin-Ohm MP, Hales TG, and Jones MV (2005) Kinetics and spontaneous open probability conferred by the epsilon subunit of the GABAA receptor. *J Neurosci* **25**:10462–10468.
- Waldvogel HJ, Kubota Y, Fritschy J, Mohler H, and Faull RL (1999) Regional and cellular localisation of GABA(A) receptor subunits in the human basal ganglia: an autoradiographic and immunohistochemical study. *J Comp Neurol* **415**:313–340.
- Walz N, Mühlberger A, and Pauli P (2016) A human open field test reveals thigmotaxis related to agoraphobic fear. *Biol Psychiatry* **80**:390–397.
- Wang F, Xu Z, Ren L, Tsang SY, and Xue H (2008) GABA A receptor subtype selectivity underlying selective anxiolytic effect of baicalin. *Neuropharmacology* **55**:1231–1237.
- Wegielis K, Pasternack M, Hiltunen JO, Rivera C, Kaila K, Saarma M, and Reebien M (1998) Distribution of GABA receptor rho subunit transcripts in the rat brain. *Eur J Neurosci* **10**:350–357.
- Whiting PJ, McAllister G, Vassilatis D, Bonnert TP, Heavens RP, Smith DW, Hewson L, O'Donnell R, Rigby MR, Sirinathsinghji DJ, et al. (1997) Neuronally restricted RNA splicing regulates the expression of a novel GABAA receptor subunit conferring atypical functional properties.[erratum to be published]. *J Neurosci* **17**:5027–5037.
- Williams M, Bennett DA, Loo PS, Braunwalder AF, Amrick CL, Wilson DE, Thompson TN, Schmutz M, Yokoyama N, and Wasley JW (1989) CGS 20625, a novel pyrazolopyridine anxiolytic. *J Pharmacol Exp Ther* **248**:89–96.
- Wingrove PB, Wafford KA, Bain C, and Whiting PJ (1994) The modulatory action of loreclezole at the gamma-aminobutyric acid type A receptor is determined by a single amino acid in the beta 2 and beta 3 subunit. *Proc Natl Acad Sci USA* **91**:4569–4573.
- Wisden W, Laurie DJ, Monyer H, and Seeburg PH (1992) The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J Neurosci* **12**:1040–1062.
- Wlodarczyk AI, Sylantsev S, Herd MB, Kersanté F, Lambert JJ, Rusakov DA, Linthorst AC, Semyanov A, Belelli D, Pavlov I, et al. (2013) GABA-independent GABAA receptor openings maintain tonic currents. *J Neurosci* **33**:3905–3914.
- Wongsamitkul N, Baur R, and Sigel E (2016) Toward understanding functional properties and subunit arrangement of $\alpha 4\beta 2$ γ -aminobutyric acid, type A (GABAA) receptors. *J Biol Chem* **291**:18474–18483.
- Wooltorton JR, Moss SJ, and Smart TG (1997) Pharmacological and physiological characterization of murine homomeric beta3 GABA(A) receptors. *Eur J Neurosci* **9**:2225–2235.
- Yang L, Xu T, Zhang K, Wei Z, Li X, Huang M, Rose GM, and Cai X (2016) The essential role of hippocampal alpha6 subunit-containing GABAA receptors in maternal separation stress-induced adolescent depressive behaviors. *Behav Brain Res* **313**:135–143.
- Yanovsky Y, Schubring S, Fleischer W, Gisselmann G, Zhu XR, Lübbert H, Hatt H, Rudolph U, Haas HL, and Sergeeva OA (2012) GABAA receptors involved in sleep and anaesthesia: $\beta 1$ - versus $\beta 3$ -containing assemblies. *Pflugers Arch* **463**:187–199.
- Ye Z, McGee TP, Houston CM, and Brickley SG (2013) The contribution of δ subunit-containing GABAA receptors to phasic and tonic conductance changes in cerebellum, thalamus and neocortex. *Front Neural Circuits* **7**:203.
- Yin W, Majumder S, Clayton T, Petrou S, VanLinn ML, Namjoshi OA, Ma C, Cromer BA, Roth BL, Platt DM, et al. (2010) Design, synthesis, and subtype selectivity of 3,6-disubstituted β -carboline at Bz/GABA(A)ergic receptors. SAR and studies directed toward agents for treatment of alcohol abuse. *Bioorg Med Chem* **18**:7548–7564.
- Ymer S, Draguhn A, Wisden W, Werner P, Keinänen K, Schofield PR, Sprengel R, Pritchett DB, and Seeburg PH (1990) Structural and functional characterization of the gamma 1 subunit of GABAA/benzodiazepine receptors. *EMBO J* **9**:3261–3267.
- Yocum GT, Gallos G, Zhang Y, Jahan R, Stephen MR, Varagic Z, Puthenkalam R, Ernst M, Cook JM, and Emala CW (2016) Targeting the γ -aminobutyric acid A receptor $\alpha 4$ subunit in airway smooth muscle to alleviate bronchoconstriction. *Am J Respir Cell Mol Biol* **54**:546–553.
- Yoshimura RF, Tran MB, Hogenkamp DJ, Johnstone TB, Xie JY, Porreca F, and Gee KW (2014) Limited central side effects of a β -subunit subtype-selective GABAA receptor allosteric modulator. *J Psychopharmacol* **28**:472–478.
- You H, Kozuska JL, Paulsen IM, and Dunn SM (2010) Benzodiazepine modulation of the rat GABAA receptor $\alpha 4\beta 3\gamma 2$ subtype expressed in *Xenopus* oocytes. *Neuropharmacology* **59**:527–533.
- Zeller A, Arras M, Jurd R, and Rudolph U (2007) Mapping the contribution of beta3-containing GABAA receptors to volatile and intravenous general anesthetic actions. *BMC Pharmacol* **7**:2.
- Zeller A, Arras M, Lazaris A, Jurd R, and Rudolph U (2005) Distinct molecular targets for the central respiratory and cardiac actions of the general anesthetics etomidate and propofol. *FASEB J* **19**:1677–1679.
- Zeuzula J, Slany A, and Sieghart W (1996) Interaction of allosteric ligands with GABAA receptors containing one, two, or three different subunits. *Eur J Pharmacol* **301**:207–214.
- Zheleznova N, Sedelnikova A, and Weiss DS (2008) $\alpha 1\beta 2\delta$, a silent GABAA receptor: recruitment by trazolol and neurosteroids. *Br J Pharmacol* **153**:1062–1071.