Pharmacological differences of GABAergic compounds: a pharmacodynamic characterization

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CHAPTER 1

Introduction

BACKGROUND

GABAergic systems

The inhibitory neurotransmission in the vertebrate central nervous system (CNS) is primarily mediated by γ-aminobutyric acid (GABA). It is estimated that depending on the brain region about 20 to 50% of all central synapses use GABA as their transmitter [1]. The enhancement of neuronal inhibition by GABA is one of the most powerful therapeutic strategies for the treatment of diseases in which some form of CNS over-activation seems to play a role, such as generalized anxiety disorders, sleep disturbances, muscle spasms and seizure disorders (see table 1). Historically the $GABA_A$ receptor has been the target of many drug treatments. The earliest compounds were ions like bromide, then came barbiturates, and finally, from 1960s onwards, a number of benzodiazepines. Existing treatments are efficient but are often hampered by the presence of side effects. At present, the $GABA_A$ receptor is still a drug target of interest, and involved in the development of many novel treatments for various diseases, with an improved efficacy and a reduced adverse event profile. In this thesis, several studies are presented, which are devoted to various aspects of different GABAergic drugs. A range of methodologies have been used to describe relevant characteristics of GABAergic agents in different stages of development.

GABA and its receptor

The action of GABA is mostly mediated by two classes of receptors, GABA type A ($GABA_A$) and type B ($GABA_B$) receptors. In contrast to the $GABA_A$ receptor, the $GABA_B$ receptor is a metabotropic receptor that is present on pre- and postsynaptic neurons. The GABA type C receptors, which are comprised of proteins that are related to $GABA_A$ receptor subunits [2], are found primarily in the retina [3]. $GABA_B$ and $GABA_C$ will not be discussed further here. $GABA_A$ receptors are ligand-gated chloride ion channels which are not only stimulated by GABA but also by pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, steroids, anaesthetics, and anticonvulsants [4]. The $GABA_A$ receptor is a pentameric structure composed of five distinct glycoprotein subunits that span a lipid bilayer and form a cylindrical structure whose center constitutes an ion channel. Binding of GABA to its recognition sites on the receptor results in conformational changes that can lead to opening of the channel with a resulting influx of chloride into the cell [3,5]. The resulting hyperpolarisation of the post-synaptic cell membrane increases the inhibitory tone. Benzodiazepines do not

independently activate this process but rather facilitate the action of GABA by increasing the frequency of ion channel opening [2]. Other psychoactive drugs, including barbiturates, anaesthetic steroids and alcohol allosterically modify the receptor at different sites, and have the same effect of enhancing the neuronal inhibition [6] (see figure 1). Binding of an inverse agonist to the GABA receptor reduces the chloride flux in the absence of GABA [7] and decreases the inhibitory effects of GABA. Furthermore, there is a spectrum of efficacies that range from full-agonists, through partial agonist, antagonist and partial inverse agonist to full inverse agonist [8] (see figure 2).

Many possibilities in the pentameric composition of the GABA receptor are possible because of the heterogeneity of subunits [4,9]. There are several molecular families of mammalian subunits (α1-α6, β1- β4, y1- y4, δ, ε, π, ρ1- ρ3) [2] and the most receptors seem to be composed of two of four α subunits (1, 2, 3, or 5), two β subunits (2 or 3) and one y subunit [5]. Benzodiazepines only bind to $GABA_A$ receptors that include the α1, α2, α3 or α5 and not the α4 or α6 subunit. The benzodiazepine site is located at the interface between the α and y2 subunit. Both the affinity and efficacy of benzodiazepines is determined by the type of α and y subunits that are present in the receptor [5].

GABAERGIC DRUG DEVELOPMENT

Pharmacokinetic modification

As so many treatments involve the GABA receptor, the pharmacokinetic properties of a compound often determine the indication of the drug. For example, the differential use of benzodiazepines as muscle relaxants, hypnotics or anxiolytics is largely determined by the pharmacokinetic characteristics, like the dose, route of administration, effect compartment half-life and formation of active metabolites. In the prevention of epileptic seizures and anti-anxiety treatment, continuous treatment is pursued, so that compounds with long elimination half-lives of parent drug or active metabolites are of advantage. If on the other hand a benzodiazepine is taken as a hypnotic, the concentration should be high enough to cause sleep and the duration of action should be restricted to the night hence a compound with a short elimination half-life is preferred. Benzodiazepines for induction of anesthesia or interruption of status epilepticus should have pharmacokinetic properties that are compatible with high CNS-concentrations, a rapid onset and a limited duration of action. The method of changing the pharmacokinetic

properties among benzodiazepines has shown to be an effective approach, to adapt drugs with a similar pharmacological activity to different therapeutic indications.

Primarily based on their diverse pharmacokinetic properties, benzodiazepines have been in widespread use for more than 40 years, as drugs for conditions like anxiety, epilepsy, sleep disorders, mania, muscle spasms and anesthesia [10]. Benzodiazepines have a safer mechanism of action compared to earlier $GABA_A$-agonists like barbiturates and bromide derivatives, since they only enhance the action of GABA while barbiturates can directly activate the $GABA_A$ receptor in the absence of GABA, making them less safe in case of an overdose. The disadvantages of benzodiazepines are the side effects, like sedation, postural instability, memory impairment and the potential development of tolerance, abuse and dependence after long-term use. Depending on the clinical setting, the various pharmacological attributes of the benzodiazepines may be either beneficial or a liability. For example, the myorelaxant and cognitive impairing properties may be beneficial when they are used as premedication for anesthesia [11,12], but are clearly disadvantageous for everyday activities when given for other indications. The sedative/hypnotic properties are useful for treating sleep disorders, but are undesirable for an anxiolytic [11]. Patients with anxiety disorders, who are a large part of the benzodiazepine consumers, are particularly prone to experience side effects [13]. Benzodiazepines are also often used in the elderly population as hypnotics or tranquilizers, while particularly in this group the side effects are associated with higher incidences of falls [14] and cognitive impairment [15,16]. Therefore, a clear medical need remains for the development of improved therapies that are more efficacious, easy to use, and better tolerated than those already marketed. There is a limit to how this can be achieved with modifications of the pharmacokinetic properties of benzodiazepines. Consequently, $GABA_A$-ergic drugs with distinct pharmacological characteristics have been designed.

Pharmacological modification

As there was still need for more therapeutic selectivity and a larger therapeutic window, more GABAergic agents were developed to improve the side effect profile.

In the late 1980s and the early 1990s, non-selective, partial agonists were launched with equivalent affinity for all $GABA_A$ subtypes but lower efficacies [17]. Their development was based on the assumption that neurons mediating anticonvulsant and anti-anxiety effects have a higher receptor reserve than neurons mediating

other unwanted effects. Pre-clinical profiles showed that they all demonstrated a margin between doses that produce anxiolysis and sedation that is superior to that associated with the non-selective full agonists such as diazepam [18]. For some of these agents, like bretazenil and pazinaclone, the sedative effects could not be differentiated from anxiolytic effects [19-22]. For other non-selective partial agonists, the development fate is unknown [8].

The development of GABAergic compounds has not been limited to partial agonists but also other compounds that directly or indirectly affect GABA or its receptor.

Vigabatrin elevates brain GABA levels by inhibiting the enzyme GABA transaminase which is responsible for intracellular GABA catabolism [23]. In contrast, tiagabine elevates synaptic GABA levels by inhibiting the GABA uptake transporter, GAT1, and preventing the uptake of GABA into neurons and glia [23]. Neuroactive steroids allosterically modulate the $GABA_A$ receptor and were seen as a potential therapeutic use in neurological and psychiatric disorders [24]. So far, ganaxolone has shown to be effective in the treatment of epilepsy [25]. Ethanol also modulates the $GABA_A$ receptor and elicits, in a dose-dependent manner, an array of central depressant effects.

Recently, several GABA analogues have been synthesized, but interestingly none of these actually influence the GABA-binding site on the GABA receptor. Tiagabine affects the $GABA_A$ receptor by inhibiting GAT1. GABApentin and pregabalin are chemically related lipophilic GABA-analogues, which do not mimic GABA at $GABA_A$ or $GABA_B$ receptors, nor do they augment $GABA_A$ responses like benzodiazepines or barbiturates [26]. Pregabalin rather seems to bind primarily to the $\alpha 2\delta$ subunit of voltage-gated calcium channels in the CNS. Binding to these channels induces release of neurotransmitters at many sites in the CNS to attenuate abnormal hyperexcitability and abnormal synchronization of neuronal networks, thereby providing anticonvulsant and analgesic effects [27]. The details of the mechanism of reducing the neurotransmitters remain to be defined. Pregabalin was originally launched for the treatment of neuropathic pain and epilepsy, and has recently also been registered as an anxiolytic [28]. In this thesis, possible sleep improving properties of pregabalin are investigated.

All these agents were developed to affect the action of GABA or its receptor using different approaches to improve the side effect profile. This thesis describes several ways to show how the pharmacological improvements are reflected in potential therapeutic advantages in humans. It is shown that studies in healthy volunteers can demonstrate distinctive pharmacodynamic characteristics of novel GABAergic drugs in comparison to existing treatments. In the early

stages of development, the clinical relevance of these improved pharmacological characteristics is not always clear, particularly if the pathophysiology and the involvement of GABAergic systems are incompletely understood. In such cases, studies in patients can explore the potential therapeutic usefulness of innovative GABA-ergic agents, and the role of GABAergic mechanism in the disease. This thesis describes how these different strategies were explored in a range of studies with different GABAergic or GABA-like drugs in healthy volunteers and patients.

THE ASSESSMENT OF PHARMACODYNAMIC EFFECTS OF NEWLY DESIGNED $GABA_A$-ERGIC AGENTS IN EARLY PHASE DRUG DEVELOPMENT

New development of subtype selective (partial) agonists

The insights into the complexity of the $GABA_A$ receptor family and the identification of the subtypes modulated by benzodiazepines raised the possibility that some of the clinical properties of benzodiazepines might be mediated through different receptor subtypes. The different receptor subtypes are located at diverse brain areas with the $\alpha 1$ subtype present in most brain areas and $\alpha 5$ only in the hippocampus. Several preclinical studies were undertaken to elucidate the different pharmacological effects of the discrete $GABA_A$ receptor subtypes. The anxiolytic effect of benzodiazepines is thought to be mediated by $GABA_A$ $\alpha 2$ receptors [29,30], and recently more emphasis is given to $GABA_A$ $\alpha 3$ [8,31,32]. These two subtypes are also believed to be associated with muscle relaxation [33,34]. The widespread $\alpha 1$ subtype appears to be involved in the sedative effects of generalized CNS-depression [30,35-38]. The hippocampal $\alpha 5$ subtypes could have a role in memory [39]. These findings have stimulated the development of compounds that are selective for a certain subtype to cause specific pharmacological effects or conversely don't bind to subtypes to avoid undesirable effects. This selectivity could be achieved by selective affinity or efficacy for the receptor subtype involved with a certain function (see figure 3). Zolpidem and zaleplon are examples of compounds with a higher affinity for the $\alpha 1$ subtype, and both are registered as selective hypnotics [40]. Additionally, imidazo[1,2-a] pyrimidines with selectivity for the $\alpha 2,3$ subtype have been developed as anxiolytics with putatively reduced sedative properties [41].

Pharmacodynamic measurements in early drug development

An important question is whether the pre-clinical differentiating pharmacological characteristics of these novel agents are reflected by a similar distinctive profile in humans. Unfortunately, the functional relevance of the different $GABA_A$ receptor subtypes has not yet been determined in human health and disease, which thwarts the direct evaluation of pharmacological properties of subtype-selective $GABA_A$-agonists in early clinical development. Benzodiazepines have shown effects on a wide range of pharmacodynamic measurements including saccadic eye movements, smooth pursuit performance, body sway, adaptive tracking, memory testing and Visual Analogue Scales (VAS) of alertness, contentedness and calmness [19,42-45]. It is not unreasonable to assume that these rather diverse effects of benzodiazepines in some way reflect the variations in $GABA_A$ receptor subtypes. By inference, it seems plausible that subjective alertness and impairment of body sway in humans are related to α1-stimulation. Reduction of saccadic peak velocity has been shown to be closely related to the anxiolytic potencies of benzodiazepines [46], and could thus reflect α2,3-activity. Memory effects could be related to α5-receptor subtypes. The effects of different compounds with different binding and efficacy profiles on this CNS-test battery could therefore provide an accurate impression of their selectivity. Knowledge about the pharmacodynamic profile of these selective agents is primarily helpful in the prediction of side effects. Secondly, measurement of pharmacodynamic parameters might be useful in the determination of a biomarker for the therapeutic efficacy. In this thesis, the pharmacodynamic profile of four different $GABA_A$ subtype selective agents has been investigated. Chapter 2, 3 and 4 of this thesis describe studies that have been performed with α2,3 selective (partial) $GABA_A$ agonists TPA023, MK-0343 and SL65.1498 that showed promising differential effects in the pre-clinical phase. In these studies, the pharmacodynamic effects have been determined and compared to the effects of the full agonist lorazepam in healthy volunteers. Another selective compound in this thesis is the hypnotic zolpidem, which is selective for the α1 subtype. Its pharmacodynamic and pharmacokinetic/pharmacodynamic effects are described in Chapter 5. One subject developed florid pseudo-hallucinations during this study. A comparison of the detailed pharmacokinetic and pharmacodynamic profiles of the selective α1-agonist between this subject and the other healthy volunteers, allowed us to describe several aspects of zolpidem-induced pseudo-hallucinations in Chapter 6.

Search for biomarkers to predict pharmacological selectivity

In preclinical research, different animal models are used to quantify various effects of GABAergic drugs on memory, sedation, anxiety and muscle tension. These studies are used to predict the functional selectivity of novel compounds in drug development [35,47,48]. Clearly, such an approach would also be very helpful in the early clinical phases of development. However, no clear a priori hypothesis can be formulated, to predict the anticipated effect profile for a certain subtype-selective $GABA_A$ agonist. The different studies described in Chapters 2, 3 and 4 and previous CHDR-studies with benzodiazepines allowed us to evaluate the relationships between the pharmacological characteristics of different GABAergic compounds, and their distinctive CNS-effect profiles. The relationships between body sway and visual analogue scales (VAS) of alertness relative to saccadic peak velocity (SPV) were compared among different GABAergic drugs. SPV was chosen because in clinical studies, this eye movement parameter has been shown to be closely associated with anxiolytic and sedative effects of benzodiazepines [46] and sedative effects of other drugs and circumstances [43,44,49]. VAS alertness and body sway reflect other functional aspects of GABAergic stimulation (subjective sedation and postural instability). Chapter 7 describes how the relative effect relationships differed among GABAergic compounds with distinct pharmacological characteristics. This provided a first step in the charting of selective CNS-biomarkers for $GABA_A$ receptor subtypes in healthy humans.

THE EXPLORATION OF PHARMACODYNAMIC EFFECTS TO IDENTIFY NOVEL INDICATIONS

The studies presented in Chapters 2, 3 and 4 suggest that the selectivity for certain $GABA_A$ receptor subtypes is also present in humans. These pharmacological properties can be demonstrated in healthy volunteers, but such studies provide limited indications for the therapeutic relevance of subtype selectivity. Several studies were performed in patients, to explore potential therapeutic effects of novel GABAergic or GABA-like compounds.

As described in the previous section, the α2,3 subtypes are associated with both anxiolysis and muscle relaxation [33]. It was decided to investigate the clinical effects of TPA023 in essential tremor,

a neurological condition that increases with anxiety and improves with muscle relaxation. Essential tremor (ET) typically shows a postural and kinetic tremor between 4-12 Hz [50]. Benzodiazepines, barbiturates (primidone) and alcohol –all GABAergic compounds [51] have a well-determined therapeutic efficacy on ET [52], which is limited by a partial response and by side effects. Although the pathophysiology of ET is unknown, the clear effects of various GABAergic drugs suggest that certain $GABA_A$ receptor subtypes may be involved. Chapter 8 describes the effect of the $\alpha 2,3$ selective partial $GABA_A$ agonists TPA023 on essential tremor in comparison to that of ethanol, of which the activity is largely mediated by the $GABA_A$ receptor [53]. Laboratory tremography was used to determine the effects on tremor and pharmacodynamic CNS effects were also assessed in this patient group.

Giving a subunit-selective agent to this patient group could reveal the role of the different $GABA_A$ receptor subtypes in attenuating this type of tremor and consequently provide a new class of successful drugs for this disorder with potential fewer side effects.

Pregabalin was originally launched for the treatment of neuropathic pain and epilepsy, and has currently also been registered as an anxiolytic [26]. Clinical studies showed that pregabalin did not only seem to improve neuropathic pain but also affected sleep interference scores that were part of these studies. This raised the question whether pregabalin, besides the indirect effect of sleep improvement as a consequence of pain relief, might have a direct sleep-modulating effect [54]. This possibly novel finding and consequently novel indication of the drug was a serendipitous discovery that was not based on pre-clinical assumptions as for the compounds described in the previous section. Subsequently, new studies in animals, healthy volunteers and patients with disturbed sleep were set up to verify the effects of pregabalin on sleep. The last part of this thesis describes efforts to identify a new potential indication for pregabalin, and to explore its effects on sleep disorders in patients with partial epilepsy.

As pregabalin was in development as adjuvant therapy in patients with partial epilepsy, it was thought that pregabalin could have beneficial effects on sleep in patients with partial epilepsy. However, the prevalence of sleep disturbance and the need for a sleep-improving agent in this patient group was unknown. A small number of articles about epilepsy and sleep had been published [55-59] which resulted in studies in which the effects of antiepileptic drugs on sleep were investigated [60]. However, before studies with pregabalin and sleep disturbed epilepsy patients were initiated, it was necessary to investigate the incidence of the problem and its effect on daily life in this patient group. Therefore, an inquiry study

was performed to investigate the prevalence of sleep disturbance in patients with partial epilepsy and its effects on quality of life. This study is described in Chapter 9 of this thesis. Based on the results of this inquiry study, a study to determine the effects of pregabalin on sleep disturbance seemed useful. Polysomnographic registrations and sleep questionnaires were used to determine the effects of pregabalin in patients with partial epilepsy, which is described in Chapter 10.

SUMMARY

This thesis describes different ways of exploring the pharmacological and therapeutic effects of novel GABAergic and GABA-like agents in humans. Systematic pharmacodynamic evaluations, using well-characterised positive controls, can confirm or refute the unique pharmacological properties of $GABA_A$ subtype selective drugs in healthy volunteers. Such studies can help to predict dosing regimens and therapeutic advantages of these drugs. The distribution of different $GABA_A$ receptor subtypes provides clues for their functional relevance. This knowledge can be used to optimise the desirable and undesirable effect profiles of selective GABAergic drugs. Very little is still known about the pathophysiological relevance of GABA-systems in CNS-disorders, although GABAergic treatments are in use for a wide range of clinical conditions. The availability of novel compounds with well defined pharmacological characteristics can clarify the involvement of these mechanisms in normal or abnormal physiology. This thesis hopes to show that carefully designed studies, using a range of CNS-measurement that reflect different GABAergic systems, can aid in the development of new GABAergic drugs, and help to unravel the role of the different GABAergic systems in health and disease.

Table 1 Overview of different GABA-receptor binding places, its ligands and indication of treatment

Direct GABA-receptor binding		
BENZODIAZEPINE BINDING PLACE	Benzodiazepines	Anxiety disorder
		Epilepsy
		Sleep disturbance
		Neuropathic pain
		Muscle spasm
		Essential tremor
		Anaesthesia
		Alcohol withdrawal
	Flumazenil	Benzodiazepine overdose
NEUROSTEROID BINDING PLACE	Ganaxolone	Epilepsy
ETHANOL BINDING PLACE	Ethanol	Essential tremor
BARBITURATE BINDING PLACE	Barbiturates	Anxiety disorder
		Epilepsy
		Sleep disturbance
		Anaesthesia
Indirect GABA-receptor activation		
BINDING α2δ-SUBUNIT CA-CHANNEL	Pregabalin	Epilepsy
		Generalized Anxiety disorder
		Neuropathic pain
	Gabapentin	Epilepsy
		Neuropathic pain
PRESYNAPTIC GAT-1 TRANSPORTER BLOCKADE	Tiagabine	Epilepsy
GABA-TRANSAMINASE DESTRUCTION	Vigabatrin	Epilepsy
OPENING K-CHANNEL	Retigabine	Epilepsy
DECREASES GLUTAMATE RELEASE	Lamotrigine	Epilepsy

Figure 1 Different binding places of a $GABA_A$ receptor.

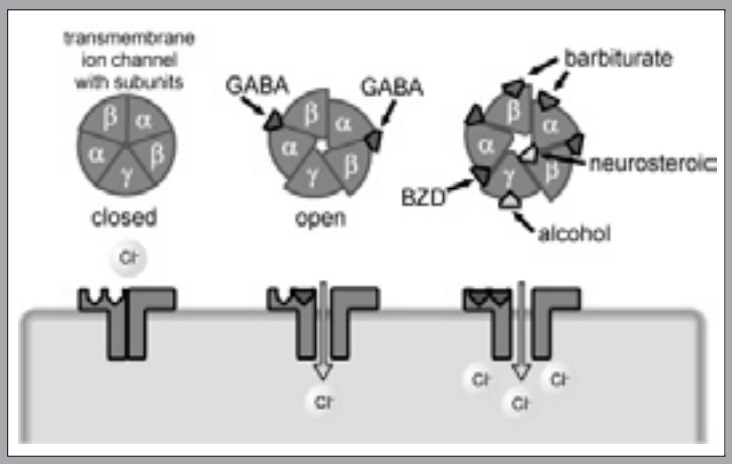


Figure 2 Schematic representation of the modulatory effects on GABA-mediated CL flux of BZ site with differing intrinsic efficacies.

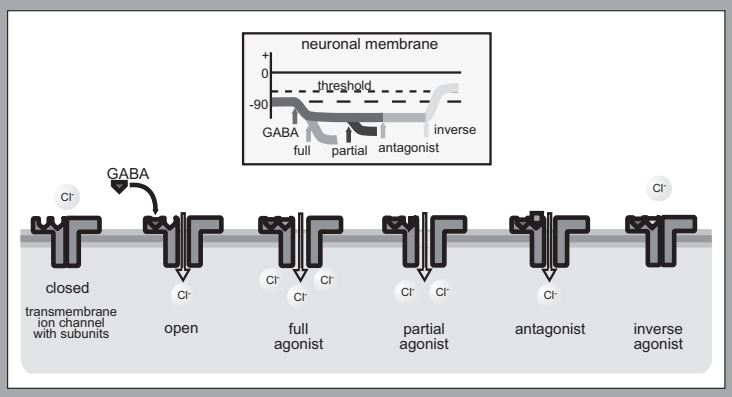
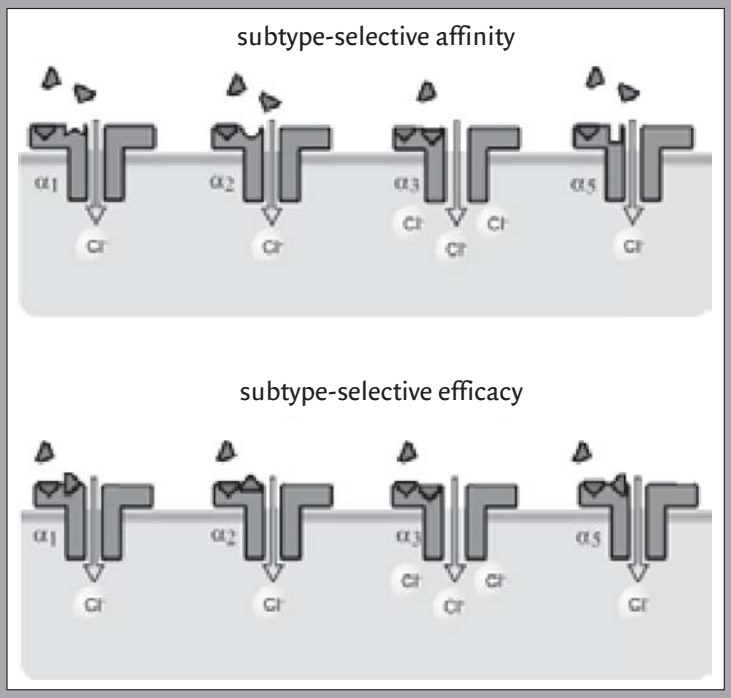


Figure 3 Strategies for developing subtype-selective compounds acting at the BZ site of the $GABA_A$ receptor.



A. Subtype selective affinity: a compound binds selectively to a particular receptor subtype, but not to other subtypes. In this example, the compound shows specific affinity and agonist efficacy for the α3-subtype, but because it can not bind to the other subtypes, will not alter GABA function at the α1-, α2-, α5-subtypes. B. Absolute subtype-selective efficacy: a compound binds to all four $GABA_A$ subtypes with equal affinity, but only shows efficacy at one particular subtype. In this example, the compound is a full agonist at the α3-subtype, a partial agonist at the α1-, α2- and α5-subtypes. TPA023 (not shown in Figure 3) is an antagonist at the α1-subtype and a partial agonist at α2- and α3-subtypes.

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