Benzodiazepines on trial: a research strategy for their rehabilitation

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Ataxia, sedation, amnesia, ethanol and barbiturate potentiation, tolerance, dependence, and the potential for drug abuse plague the clinical use of anxiolytic benzodiazepines. Benzodiazepine and non-benzodiazepine ligands that are in current clinical use act as full allosteric modulators of GABA-gated Cl^- channels, and on chronic administration trigger compensatory changes in the subunit expression of $GABA_A$ receptors. In these putative abnormal receptors, full allosteric modulators have low intrinsic activity and potency, and tolerance and dependence ensue. In this review, **Erminio Costa and Alessandro Guidotti** discuss the development of partial allosteric modulators, such as imidazenil, which have high potency and low intrinsic activity at GABA-gated Cl^- channels. Since in animals tolerant to full allosteric modulators imidazenil also fails to show cross-tolerance, it is an example of a new type of anxiolytic and anticonvulsant drug acting at $GABA_A$ receptors via benzodiazepine recognition sites.

Almost 20 years ago, it was discovered that anxiolytic, tranquillizing benzodiazepines specifically facilitate GABA-mediated neurotransmission in the CNS (Refs 1, 2). The high-affinity recognition sites through which benzodiazepines exert their action are located on the extracellular domain of the $GABA_A$ receptor, an ionotropic receptor that is a transmembrane, heteroligomeric, neuronal protein probably comprising five subunits that define a Cl^- channel[3–5]. The benzodiazepine recognition site is located predominantly, or perhaps exclusively, in the α-subunit of $GABA_A$ receptors. Although activation of this site fails to gate the Cl^- channels, it is a modulatory element for the effect of GABA on these channels. The term 'allosteric' can be applied to this benzodiazepine recognition site because it is physically distinct from the GABA recognition site.

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$GABA_A$ receptors are assembled from subunits of various molecular forms derived from four subunit families (α, β, γ and δ), each encoded by multiple genes[3–5]. Since 16 genes are currently known to encode the subunits that are pentamerically[5] assembled in $GABA_A$ receptors, an astonishing structural diversity of these receptors may be expressed in the CNS. Barnard[5] has proposed that more than 800 structurally different $GABA_A$ receptors are theoretically compatible with the mRNAs detected in various CNS structures that encode the subunits for this receptor. The relative quantities of these mRNAs not only differ in various brain structures[6,7] but can change in the same structure during ontogenesis[8–10] and also under several experimental conditions such as tolerance to benzodiazepines[11–14]. GABA potency and the benzodiazepine and non-benzodiazepine modulators of GABA action at $GABA_A$ receptors are influenced by the structure of the subunits assembled to form the $GABA_A$ receptor (see Box 1). The $GABA_C$ receptor includes another type of Cl^- channel gated by GABA; this type of receptor differs from the $GABA_A$ receptor in that it probably comprises only two molecular forms of the ρ-subunit family[3]. The $GABA_C$ receptor family is probably phylogenetically older than the $GABA_A$ receptor family. Often $GABA_C$ receptors are homomeric, are expressed abundantly in the retina, and are resistant to the positive allosteric modulation induced by benzodiazepines and to isosteric inhibition by bicuculline[3].

Ligands for benzodiazepine recognition sites

One class of benzodiazepine and non-benzodiazepine high-affinity ligands for the benzodiazepine recognition sites associated with $GABA_A$ receptors can increase the opening frequency of Cl^- channels elicited by GABA; these ligands are called positive allosteric modulators or agonists. Another class can decrease this opening frequency and are known as negative allosteric modulators or inverse agonists[4,15]. A third class of high-affinity ligands for benzodiazepine recognition sites fails to modulate the action of GABA and are called 'receptor antagonists'; because of their high affinity for benzodiazepine recognition sites, they can prevent GABA modulation elicited by positive or negative allosteric modulators[15]. A fourth class of ligands for benzodiazepine recognition sites is unable to elicit either a maximal amplification or maximal attenuation of GABA action at different $GABA_A$ receptors on which the ligands have been tested; this class comprises what are known as partial positive or partial negative allosteric modulators (partial agonists or partial inverse agonists, respectively)[15,16] (Table 1). Despite their limited intrinsic activity, some partial allosteric modulators have a high affinity for benzodiazepine recognition sites and can prevent the further amplification of GABA-mediated responses by agonists with high intrinsic activity at these sites[15] (Table 1).

Collectively, this evidence distinguishes the notion of affinity from that of efficacy in benzodiazepine pharmacology. In fact, a ligand for a benzodiazepine recognition site can have a high affinity but may possess low intrinsic activity and act as a partial allosteric modulator. (This concept is discussed in Box 1 with reference to channel kinetics.) It should be noted that although a number of compounds have been classed as partial allosteric modulators they actually belong to another class of benzodiazepine recognition site

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Table 1. Classification criteria for positive allosteric modulators of GABA action

Allosteric modulator	Maximal Cl^- current amplification in $GABA_A$ receptors	Cognition impairment	Antagonism of cognitive impairment	Ethanol and barbiturate potentiation	Ataxia, sedation	Classification	Refs
Imidazenil	NRM	None	Yes	None	None	PAM	33, 36, 37, 40
Ro198022	?	?	(?)	None	None	PAM	43, 44
Divaplon	NRM	Weak	Weak (?)	None	None	PAM	45, 46
Bretazenil	NRM	None	Yes	Weak[a]	Weak[a]	PAM	15, 32, 35
CGS20625	?	?	?	Weak	Weak	PAM?	47
Abecarnil	In some subtypes	?	No	Weak	Weak	SAM	45, 48
Y23684	In some subtypes	?	?	Weak	Weak	SAM	49, 50
CL218872	In some subtypes	?	?	Weak	Weak	SAM	49, 50
Alpidem	In some subtypes	?	?	Weak	Weak	SAM	26, 28
Zolpidem	In some subtypes	None	No	Yes	Yes	SAM	26, 28
Zopiclone	?	?	?	?	Yes	SAM?	32
Triazolam	In many subtypes	Yes	No	Yes	Yes	FAM	15, 28
Alprazolam	In many subtypes	Yes	No	Yes	Yes	FAM	15, 28
Diazepam	In many subtypes	Yes	No	Yes	Yes	FAM	15, 28

PAM, partial allosteric modulator; SAM, selective allosteric modulator; FAM, full allosteric modulator; NRM, never reaching maximal amplification; ?, additional data needed.
[a]Observed with high doses of the modulator, suggesting the possible formation of a metabolite with SAM activity.

ligands called selective allosteric modulators. This class includes abecarnil, Y23684, alpidem and zolpidem (Table 1), ligands for which there is clear-cut evidence of their selective allosteric modulator profile on native or recombinant $GABA_A$ receptors. In this review, the term 'partial allosteric modulator' refers to ligands that (1) have a high affinity for benzodiazepine recognition sites on $GABA_A$ receptors, (2) fail to activate maximal GABA action in every $GABA_A$ receptor subtype on which they have been tested, (3) antagonize the cognitive action, sedation and ataxia elicited by full allosteric modulators, (4) fail to potentiate ethanol and barbiturates, (5) fail to cause cognitive impairing effects or ataxia, and (6) maintain anxiolytic and anti-convulsant activity. These criteria certainly apply to imidazenil and may also apply to Ro198022 and divaplon (Table 1).

Drawbacks in the therapeutic use of benzodiazepines

The narrow margin between benzodiazepine doses required to elicit therapeutic effects and those that elicit unwanted side-effects has created an increasingly negative attitude towards the safety of benzodiazepines as therapeutic agents in anxiety, mood disorders and epilepsy[17]. This cautionary frame of mind is bolstered by reports that the benzodiazepines presently available for therapeutic use induce dizziness, vertigo, amnesia, ataxia, dysarthria, and a tendency for high tolerance and physical dependence with the subsequent potential for drug abuse[17]. In elderly patients, ataxia may lead to falls that in these patients incur a higher risk of fractures.

Benzodiazepine-induced cognitive impairment

The mechanisms of memory impairment elicited by benzodiazepines should be discussed with respect to the role of GABA in the columnar organization of neocortical function that was first proposed by Mountcastle[18]. This theory suggests that cortical neurones are functionally connected across layers in columns approximately 500 μm in diameter. This intracolumnal connectivity is regulated by a 'time-related' afferent stimulation that synchronizes the activity of columnar neurones and thereby facilitates their functional assembly. Ultimately, the number of neurones that are functionally interacting at any one time appears to be modulated by GABAergic synaptic links between basket, axo-axonic or chandelier cells and neocortical or hippocampal pyramidal cells[19]. Thus, GABA contributes to cortical projection field dimensions and facilitates other interactions in functional neuronal assemblies of the neo- and limbic cortex[19].

Disrupting the role of GABAergic synapses in synchronizing neuronal assemblies by antagonizing or facilitating GABAergic transmission alters information processing and generates changes in direction and orientation selectivity[20]. Basket cells innervate the somata and the proximal dendrites of cortical pyramidal cells whereas the axo-axonic cells almost exclusively innervate the initial axon segments of this type of neurone. Thus, more than 90% of the synapses impinging on neocortical pyramidal neuronal somata are GABAergic[21]. As a result, GABAergic transmission has a physiological role in the regulation of functional neuronal assemblies; in turn, benzodiazepine-elicited amnesia may occur because benzodiazepine-induced amplification of GABAergic

Box 1. Benzodiazepine action

The GABA-induced changes in the equilibrium kinetics of the Cl^- channel of $GABA_A$ receptors are the target for the action of positive or negative modulators acting at high-affinity binding sites for benzodiazepines located on $GABA_A$ receptors[1]. In the GABA-gated channels of cortical cells from rat brain tissue in culture, the main conductance of the open state approaches 31 pS, although less frequent events with a conductance of 17–20 pS can also be recorded. Studies with recombinant receptors and with transgenic mice in which the expression of the γ_2-subunit of the $GABA_A$ receptor is suppressed infer that the main conductance of the open state is reduced in the absence of this γ_2-subunit[2,3]. It is interesting to note that in these channels, the positive allosteric modulation of benzodiazepines on GABA action is absent[3].

Bursts of channel opening are defined as groups of high-frequency, repeated channel opening events separated by periods when the channel is closed. They consist of repeated openings of the same open state, and the duration of the burst increases as the single-channel, open-time constant increases. A variety of structurally different molecules can act on the benzodiazepine recognition sites located on $GABA_A$ receptors[4]. Benzodiazepines, like flunitrazepam, and their pharmacological congeners, unlike barbiturates and neurosteroids, fail to change the open-time constant of $GABA_A$ receptor channels but increase the probability of their opening. Benzodiazepines acting as positive allosteric modulators increase the frequency of channel opening in $GABA_A$ receptors without altering the conductance or open burst duration of the channels, as predicted from their inability to alter the channel open-time constant[1].

It is important to stress that the action of benzodiazepines with a partial allosteric modulator profile on single-channel kinetics has never been studied. Most information on benzodiazepine-induced kinetic modifications of single-channel opening elicited by GABA comes from studies with diazepam[5] and flunitrazepam[1]. The actions of both of these drugs are consistent with the hypothesis that they increase the probability that receptors ligated by only one GABA molecule but not those that are bi-ligated, will open. Diazepam very likely reduces the probability that bi-ligated GABA receptors become desensitized, and because of this action this drug might indirectly increase the receptor gating frequency elicited by GABA.

Exactly the contrary might be expected to occur in the presence of negative allosteric modulators (inverse agonists), which, by reducing the receptor affinity for GABA, decrease the opening probability of the GABA mono-ligated receptors and may also increase the probability that the bi-ligated receptor becomes desensitized. DMCM, a β-carboline derivative with inverse agonist activity, fails to decrease the main conductance of the open channel but reduces channel opening frequency[1,5]. It is obvious that the modification of single-channel properties of recombinant receptors by benzodiazepines that are full or partial allosteric modulators requires further study.

It has been clearly shown that the subunit composition of recombinant $GABA_A$ receptors is a determinant for the responsiveness of the benzodiazepine recognition site to various ligands; however, the ligand structure also has an important role[6,7]. As shown in Table 1, the presence of a γ_2-subunit is an absolute requirement for an optimal modulation of GABA efficacy by positive and negative modulators of GABA action at recombinant $GABA_A$ receptors. The presence of a γ_2-subunit in a $GABA_A$ receptor optimizes the positive modulatory capacity of diazepam, clonazepam, alpidem and zolpidem, but co-expression of the α_6- and the γ_2-subunits reduces the modulatory ability of diazepam and clonazepam and almosts obliterates that of alpidem and zolpidem (two imidazopyridine derivatives).

Table 2 shows that the presence of the α_6-subunit in a $GABA_A$ receptor virtually obliterates the activity of all benzodiazepines tested, despite the presence of γ_2-subunits. In contrast, the negative modulatory activity of

Table 1. Maximal modification of Cl^- current at recombinant $GABA_A$ receptors by positive and negative benzodiazepine modulators

Subunit	Diazepam	Clonazepam	Alpidem	Zolpidem	DMCM	BCCM	EC_{50} (μM)
β_1	0	0	0	0	0	0	ND
α_1, β_1	0	0	0	0	0	0	1.5
α_1, β_1, γ_2	+150	+130	+320	+230	−50	−45	4.5
α_2, β_1, γ_2	+280	+220	+300	+210	−50	−43	7.5
α_3, β_1, γ_2	+400	+300	+210	+280	−75	−38	15
α_5, β_1, γ_2	+100	+60	+10	+15	−40	−70	2.4
α_1, β_1, γ_1	+70	+60	+40	+45	+70	+50	1.2
α_2, β_1, γ_1	+70	+30	+5	−5	−29	+85	ND
α_3, β_1, γ_1	+100	+40	−25	+5	+2	−45	ND
α_5, β_1, γ_1	+50	+42	+15	+10	+18	−2	ND

Data taken from Refs 6, 7 and 9. ND, not determined.

on GABA-gated Cl⁻ channels

Table 2. Maximal amplification of Cl⁻ current at recombinant $GABA_A$ receptors by benzodiazepines

Subunit	Triazolam	Clonazepam	Diazepam	Bretazenil	Imidazenil	EC_{50} (μM)
$\alpha_1, \beta_1, \gamma_3$	70	ND	40	50	45	0.60
$\alpha_1, \beta_1, \gamma_2$	250	130	150	95	80	4.5
$\alpha_1, \beta_1, \gamma_1$	140	60	70	60	60	1.2
$\alpha_2, \beta_1, \gamma_2$	210	300	400	150	140	15
$\alpha_5, \beta_1, \gamma_2$	90	60	100	50	45	2.4
$\alpha_6, \beta_1, \gamma_2$	50	ND	10	9	9	0.50
$\alpha_3, \beta_2, \gamma_2$	175	ND	125	80	75	4.5
$\alpha_1, \beta_3, \gamma_2$	185	ND	120	60	55	1.7

Data taken from Refs 6, 7, 9, 10. ND, not determined.

DMCM and BCCM remains virtually unchanged no matter which α- subunit co-exists with the γ_2- subunits (Table 1). The presence of the γ_1-subunit reduces the positive modulation by imidazopyridines more markedly than that by the benzodiazepines. Interestingly, the negative modulatory activity by DMCM and BCCM is positive in receptors that co-express γ_1- and α_1-subunits. This positive modulation does not occur when the γ_1-subunit is co-expressed with α_3- subunits. While the positive modulation by the two imidazopyridines is maximal when the γ_2-subunit is co-expressed with α_1-, α_2- and α_3- subunits, both drugs fail to act in the presence of the α_5-subunit. Clearly, the responses of all the positive allosteric modulators reported in Table 1 are maximized when the α_3- and γ_2- subunits are co-expressed.

Differences in the positive modulatory activity of benzodiazepines were observed in Cl⁻ currents recorded in the whole-cell mode from native receptors of neurones isolated from substantia nigra slices including pars reticulata and pars compacta neurones[8]. The positive modulation of GABA action by benzodiazepines in neurones of the pars reticulata was smaller than that in pars compacta neurones[8]. In native receptors of pars reticulata neurones, diazepam was less efficacious than flunitrazepam or zolpidem. To find out if these differences in efficacy were related to GABA potency, GABA EC_{50} values were measured in a number of different recombinant $GABA_A$ receptor subtypes (Tables 1 and 2). It appears that GABA potency is unaffected by the presence of either γ_1- or γ_2-subunits. In contrast, the greatest efficacy of positive and negative allosteric modulators was recorded in recombinant receptors co-expressing α_3-, β_1- and γ_2-subunits, in which GABA potency is low. Similar evidence was collected in recombinant receptors derived from transfections including both γ_2- and δ-subunits; in these experiments, the inclusion of the δ-subunit reduces GABA potency but enhances diazepam efficacy.

The data reported in Table 2 provisionally suggest, that triazolam, diazepam and clonazepam maximize GABA action in most of the recombinant receptors so far tested; therefore, they appear to act as full allosteric modulators[9]. On the other hand, although alpidem and zolpidem facilitated GABA action in most receptors tested, they did not maximize GABA action in every receptor; therefore, they act as selective allosteric modulators. In contrast, bretazenil and imidazenil positively modulated GABA action in every receptor tested but with consistently marginal efficacy, and can thus be called partial allosteric modulators[9,10]. Imidazenil also acts as a partial allosteric modulator of GABA action *in vivo*[10] (see text).

On the basis of the data available, it is impossible to be sure that the differences between a full and a selective allosteric modulator are a realistic representation of tests performed in every structurally different $GABA_A$ receptor expressed in the brain. Therefore, this review considers only two main classes of ligands for benzodiazepine recognition sites: the partial allosteric modulators (Table 2), which cannot maximize GABA action; and the full and selective allosteric modulators, which can maximize GABA action. The second class includes compounds like diazepam, triazolam and alprazolam, which maximize GABA action in a great number of structurally different $GABA_A$ receptors so far tested, and also abecarnil, Y23684, CL218872, zolpidem and alpidem, which can maximize GABA action but in fewer structurally different $GABA_A$ receptors compared with triazolam.

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Table 2. Effect of long-term treatment (over 14 days) with triazolam, diazepam or imidazenil on their anticonvulsant efficacy in rats

Drug (μmol kg^{-1})	Threshold dose of bicuculline to cause convulsions (μmol kg^{-1})	
	After long-term vehicle treatment	After long-term benzodiazepine treatment
Vehicle	1.1±0.1	1.1±0.1
Triazolam (5.8)	3.3±0.2	1.2±0.2[a]
Diazepam (17.6)	3.4±0.3	1.3±0.1[a]
Imidazenil (2.85)	3.4±0.3	3.4±0.3

[a] $p<0.01$ when compared with long-term, vehicle-treated rats (Duncan's multiple range test). For details, see Ref. 33

transmission disorganizes the formation of such functional neuronal assemblies. Although the amnesic action of benzodiazepines can be exploited as premedication in surgical anaesthesia, it clearly imposes an important limitation when these drugs are administered continuously; for example, benzodiazepine amnesia can interfere with the normal life of patients being treated for anxiety. In general, anxiolytic benzodiazepines have no important effects on short-term or procedural memory but do interfere with episodic memory[22].

Tolerance liability to benzodiazepines

When benzodiazepines are administered for a long period of time, the onset of tolerance to these drugs is a significant drawback that has virtually prevented their use in the treatment of epilepsy. This tolerance is characterized by a downregulation of GABAergic transmission[11] and by a reduction in benzodiazepine-induced amplification of GABA-elicited Cl^- currents[23]. Interesting shifts in the expression of mRNAs encoding α- and γ-subunits of $GABA_A$ receptors have been detected in specific brain areas of tolerant rats[11–14]. Using a 14-day treatment schedule to induce tolerance to diazepam in rats, the amount of mRNA α_1-, γ_2S- and γ_2L-subunits decreases while that of α_5 increases in the fronto-parietal motor cortex and in the hippocampus[14] (Table 2). Perhaps rearrangements of the $GABA_A$ receptor subunit assembly occur to form $GABA_A$ receptor subtypes with a decreased sensitivity to benzodiazepine modulation. Interestingly, rats treated chronically with anticonvulsant doses of imidazenil (a partial allosteric modulator) that were equivalent to those of diazepam that cause tolerance elicited neither tolerance nor changes in the expression of the mRNA encoding α_1-, γ_2- and α_5-subunits[14].

Several lines of independent investigation suggest that a regulated dynamic state may control the expression and assembly of $GABA_A$ receptor subunits[24,25]. Perhaps this regulated flexibility can act to attenuate the consequences of an enduring amplification of GABA-gated Cl^- currents elicited by protracted treatment with full allosteric modulators. Since GABA has different potencies in $GABA_A$ receptors assembled from different subunits[25] (see Box 1), and since the extent of GABA signalling amplification by benzodiazepines requires the presence of certain α- and γ-subunits[16,26–29] (see Box 1), changes in subunit assembly were investigated to see if they occur to compensate for a persistent increase in synaptic GABA concentration or for the enduring maximal amplification of GABA currents elicited by chronic benzodiazepine treatment[11–14].

Research strategies to obtain safer benzodiazepine ligands

Ligands modulating a specific $GABA_A$ receptor subtype

The diversity of $GABA_A$ receptor subtypes expressed in the CNS has prompted speculation that the symptoms of anxiety, mood disorders and perhaps even those of epilepsy might be related to structural abnormalities of $GABA_A$ receptors. If the subunit composition of these abnormal receptors was known, new families of benzodiazepine ligands that selectively modulate these unusual $GABA_A$ receptor subtypes might be designed.

Currently, there is only one known instance of a point mutation in a $GABA_A$ receptor subunit that is associated with a behavioural abnormality[29]. The α_6-subunit, which is expressed predominantly (or perhaps exclusively) in cerebellar granule cells[3,4,10], confers to $GABA_A$ receptors an almost negligible affinity for benzodiazepine ligands with anxiolytic activity[30] (Table 2 in Box 1). By exchanging a portion of the large extracellular domain of the α_6-subunit with that of the α_1-subunit, the resultant chimeric $GABA_A$ receptor has an increased affinity for benzodiazepines[31]. Moreover, the substitution of an arginine residue for a histidine residue at position 100 of the α_6-subunit appears to be the key to restoring the sensitivity of GABA receptors comprising the canonic α_6-subunit to benzodiazepines[30,31]. Interestingly, the inbred ethanol-intolerant rat line, in contrast to the ethanol-tolerant line, is highly sensitive to the diazepam-induced impairment of postural reflexes[29]. In this ethanol-intolerant strain, there is a histidine residue in position 100 of the α_6-subunit[29].

Allelic variants of the $GABA_A$ receptor subunit families have only been described for the human β_1- and the rat α_1-subunits[31]. While the β_1 variant did not have functional consequences, the α_1 variant, in which Phe64 is changed to Leu, produced a sharp decrease in the affinities of GABA and GABA agonists for $GABA_A$ receptors[31]. Collectively, this evidence suggests that putative allelic variants of subunit structures are associated with changes in the sensitivity of $GABA_A$ receptors to GABA and benzodiazepines. Thus, when the technology becomes available to investigate the stoichiometry and subunit composition of putative abnormal $GABA_A$ receptor subtypes associated with neuropsychopathology, benzodiazepines can hopefully be designed that are specific for these abnormal receptors.

It is also possible that receptors with α_1-subunits are related to sedation caused by benzodiazepines. Nonsedative anxiolytic ligands for benzodiazepine recognition

sites, or other anxiolytics acting on $GABA_A$ receptors, could be designed by eliminating those compounds that amplify GABA-elicited currents on receptors that include the α_1-subunit and instead investigating the activity of compounds acting on $GABA_A$ receptors lacking α_1- but including α_3- and α_5-subunits. This screening strategy may produce selective allosteric modulators that are nonsedative but it cannot predict a priori if these modulators will be devoid of the ability to affect cognition. Table 1 shows that currently available modulators of this type fail to be completely devoid of sedation, ataxia and ethanol or barbiturate potentiating action.

The use of partial allosteric modulators

An ideal, therapeutically advantageous benzodiazepine ligand should be fully effective as an anxiolytic and an antiepileptic and yet be devoid of undesirable side-effects and of tolerance and dependence liability. Encouraged by the reports of Haefely and colleagues[15,32] that bretazenil, a ligand for benzodiazepine sites with a partial allosteric modulator profile, fulfils some of the criteria for an ideal tranquillizing, anxiolytic drug, we turned our attention to developing new modulators of this type with a pharmacokinetic profile even more appropriate to clinical use than that of bretazenil.

Our working hypothesis intended to test whether a drug with a partial allosteric modulator profile, characterized by a consistent low efficacy at most $GABA_A$ receptors, can modestly increase the GABAergic tone, keep the synaptic GABAergic strength in balance, and conserve a buffering reserve in this synaptic function for prompt use in an emergency[28]. We hypothesize that a potent benzodiazepine with a full allosteric modulator profile, which promptly elicits a maximal amplification of GABA action at most $GABA_A$ receptor subtypes, obliterates the ability of the receptor to produce a graded response to different amounts of transmitter released from GABAergic terminals[28]. Presumably, this then triggers compensatory changes in $GABA_A$ receptor structure that reduce the sensitivity of the receptor to modulation by the drug, leading to tolerance and perhaps dependence. In contrast, such changes in receptor sensitivity to benzodiazepines may not occur when partial allosteric modulators are administered[28]. The 'ideal' partial allosteric modulator should possess a high affinity and low intrinsic activity for most $GABA_A$ receptors, it should not produce metabolites with a full allosteric modulator profile, and it should have a good bioavailability and an appropriate half-life.

As discussed above, Table 1 lists ligands for benzodiazepine recognition sites with a putative partial allosteric modulator profile. In this list, the compounds that so far comply closest with the criteria for an ideal modulator of this type include imidazenil, Ro198022, divaplon and CGS20625. However, although the partial allosteric modulator activity of imidazenil has been established in a large number of tests used to define an ideal modulator of this type, the activity of Ro198022, divaplon and CGS20625 needs to be studied in further tests. Bretazenil,

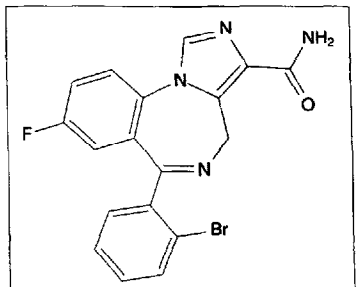

Fig. 1. Structure of imidazenil.

which is a partial allosteric modulator *in vitro* but has a limited tolerance liability *in vivo* in humans and rats[33–35], cannot be considered as an ideal partial allosteric modulator because it is rapidly metabolized into a full allosteric modulator and may induce sedation[35,36].

Pharmacological profile of imidazenil

The ligand for benzodiazepine recognition sites that is most like the ideal partial allosteric modulator (as defined above) is imidazenil[37,38] (Fig. 1). This drug has a greater affinity for benzodiazepine recognition sites than bretazenil and maintains an intrinsic activity clearly lower than that of full allosteric modulators in almost every $GABA_A$ receptor in which it has been tested (Table 2 in Box 1). In rats and mice, imidazenil has full anxiolytic and anticonvulsant action[37–39] but because it displays a low intrinsic activity for modulating $GABA_A$ receptors, it occupies a greater number of receptors than diazepam in order to elicit comparable pharmacological responses

Table 3. Receptor occupancy and EC_{50} values following treatment with imidazenil and diazepam in rats

Test	Diazepam		Imidazenil	
	EC_{50}[a]	Receptor occupancy (%)[b]	EC_{50}[a]	Receptor occupancy (%)[b]
Anticonflict	2.0 (1.4–2.8)	53	2.9 (1.0–5.2)	98
Ethanol potentiation	1.9 (1.8–2.4)	47	>50	100
Thiopent potentiation	1.7 (1.2–2.5)	51	>60	100
Decreased activity	2.6 (2.2–2.9)	63	>60	100
Ataxia	2.4 (2.0–3.0)	60	>60	100

[a] EC_{50} values are μmol kg^{-1}, i.v., with confidence limits in parentheses.
[b] Receptor occupancy was calculated *ex vivo* as a measure of displacement of flumazenil binding using different doses of diazepam and imidazenil.
Data taken from Ref. 37.

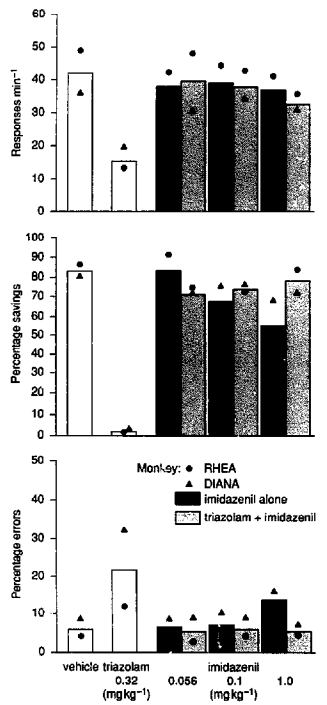

Fig. 2. Effect of varying doses (intramuscular administration) of imidazenil alone or in combination with triazolam (0.32 mg kg^{-1}) on overall response rate, percentage savings and percentage errors on delayed performance in patas monkeys. The columns indicate the mean values for at least ten doses of vehicle and two determinations each for triazolam or imidazenil alone and in combination. The closed symbols represent the mean values for the individual subjects after the respective treatments. Data modified from Ref. 36.

(Table 3). Although imidazenil has a pharmacological profile different from that of classical benzodiazepines, it shares with them a low overall toxicity. However, unlike the classical drugs it is virtually devoid of sedative action, ataxia and muscle relaxation (Table 3). Most interestingly, it fails to potentiate the sedative effects of barbiturates and ethanol (Table 3).

The half-life of imidazenil in rats (following the administration of 2 mg kg^{-1} i.v.) is 92 min. An unidentified metabolite becomes detectable in the blood during its metabolism and the area under the curve for this metabolite is about 30% of that of the parent compound. The absorption of [^{14}C]imidazenil after oral administration (2 mg kg^{-1}) is very rapid and the blood levels peak in about 40 min. The half-life of the drug is about 3 h, but by this route of administration the ^{14}C-labelled plasma metabolites are virtually undetectable. Within 24 h, 82% of detectable imidazenil radioactivity has been excreted in the faeces and 12% in the urine. When compared with bretazenil, imidazenil has a longer-lasting action due to its slower rate of metabolism[37,38]. Imidazenil does not cause overt sedative effects in monkeys receiving doses 100 times greater than those required to inhibit the amnesic effect of triazolam; in contrast, the sedative liability of bretazenil in monkeys is much greater than that of imidazenil[36,40].

Lack of tolerance and dependence liability in rats

To test the tolerance liability of imidazenil, it was administered to rats over 14 days in a treatment schedule including three daily oral doses and four periodical dosage increases (Table 2)[33]. We compared the onset of tolerance to the anti-bicuculline activity of imidazenil to that of equipotent doses of triazolam and diazepam: these two drugs elicited tolerance at the end of this treatment schedule, whereas imidazenil did not. These differences in tolerance liability cannot be ascribed to selective changes in disposition rates[33] of diazepam and triazolam. We also found that the rats that became tolerant to diazepam were not tolerant to a challenge with an anticonvulsant dose of imidazenil[33]. This suggests that if diazepam tolerance is related to a rearrangement of subunit assembly in some $GABA_A$ receptors, then the low intrinsic amplification of the GABA action elicited by imidazenil on every receptor subtype tested[37] probably also applies to the newly formed receptors in the benzodiazepine-tolerant rats. The validity of such a mechanism in preventing such cross-tolerance requires further experimental verification.

Using a constant and equipotent dosage treatment schedule of diazepam and imidazenil in other experiments on rats, the onset of diazepam tolerance occurred 5 days after treatment began, whereas imidazenil tolerance failed to occur after 180 days[41]. Similarly, chronic administration (three times daily for 30 days) of an anticonvulsant dose of imidazenil (0.1 mg kg^{-1}, i.p.) in mice induced neither tolerance nor $GABA_A$ receptor down-regulation[39].

Inhibition of cognitive deficits induced by alprazolam and triazolam in monkeys

The effects of imidazenil compared with those of bretazenil, alprazolam and triazolam have also been studied in patas monkeys working on a complex behavioural task[36,40]. (Such a study should also be extended to divaplon, Ro198022 and CGS20625 to define their partial allosteric modulator profile.) In the repeated acquisition paradigm (learning), patas monkeys acquired a different chain of four responses during each experimental session

by responding sequentially on three keys in the presence of four discriminative stimuli (geometric forms or numerals). In the other paradigm (performance), the four-response chain was the same in each session. In both protocols, the responses were maintained by presenting food under a fixed-ratio schedule. Alprazolam (0.01–0.32 mg kg^{-1} per os) decreased the rate of response and increased the percentage of errors in both paradigms in a dose-related manner[10]. Learning was more susceptible to drug-induced modification than performance. In doses up to 1 mg kg^{-1}, imidazenil had virtually no effect on acquisition or performance[10]. When imidazenil (0.1 mg kg^{-1} per os) was administered 60 min before alprazolam (0.32 mg kg^{-1}), it almost completely antagonized the behavioural disruption caused by alprazolam; even 0.01 mg kg^{-1} imidazenil reduced the alprazolam-induced (0.32 mg kg^{-1}) disruption of acquisition and performance.

In another series of experiments in monkeys, the effects of imidazenil and triazolam on the retention of acquired discriminations were tested[36]. These studies demonstrated that imidazenil differs from drugs with a full allosteric modulator profile by its virtual inability to impair acquisition and memory in doses that are two orders of magnitude greater than the dose that inhibits the memory impairment caused by full allosteric modulators like triazolam (Fig. 2). The low liability of imidazenil to elicit tolerance and to disrupt learning and memory in the light of its potent anxiolytic and anticonvulsant actions suggests that its therapeutic potential for treating anxiety and epileptic disorders should be tested.

Closing remarks

Perhaps imidazenil is a prototype of a new generation of anxiolytic and anticonvulsant drugs that have minimal disruptive effects on learning and memory and are virtually devoid of the tolerance liability and other unwanted side-effects of classic benzodiazepines. However, imidazenil and other putative partial allosteric modulators (Ro198022 and divaplon) have not yet been tested in humans. Only bretazenil has been tested in humans and, as mentioned above, it causes sedation – presumably attributable to the formation of a sedative metabolite with a full allosteric modulator profile. Since the mechanisms inducing tolerance and dependence may differ among species, imidazenil should be assessed more thoroughly for its capacity to induce dependence.

We have tested[33] whether imidazenil, following abrupt withdrawal after its chronic administration, increases the behavioural impact of a mild electric shock in the Vogel conflict response. It is known that after chronic treatment with benzodiazepines that induce dependence liability, abrupt withdrawal reverses the action of flumazenil[42] at the $GABA_A$ receptor. Withdrawal of triazolam and diazepam facilitates the conflict response to a mild electric shock in the Vogel test in a manner that is potentiated by flumazenil[33]. In contrast, imidazenil fails to elicit a sensitization to the proconflict response in the presence or absence of flumazenil.

In another group of experiments[33], it was observed that if after treating the animals for 14 days with imidazenil or diazepam they are then kept drug-free for 24 h, the threshold dose of bicuculline needed to elicit convulsion is essentially equal in rats receiving either of the two drugs or the vehicle. However, after injecting flumazenil (16.5 μmol kg^{-1}, i.v.) this threshold dose of bicuculline is reduced significantly in rats that had previously undergone long-term treatment with diazepam but not in rats pretreated with either imidazenil or vehicle for the same length of time[33]. Thus, in support of Nutt's proposal to explain the mechanism of benzodiazepine tolerance[42], we suggest that a structural shift in $GABA_A$ receptors produced by chronic administration of full allosteric modulators might account for the appearance of tolerance and withdrawal symptoms associated with long-term treatment with these benzodiazepines.

If this hypothesis is correct, a persistent increase in GABAergic tone may be linked with changes in $GABA_A$ receptor subunit assembly, and tolerance and dependence liability may be considered as two homostatic, interdependent phenomena that are linked to putative $GABA_A$ receptor structure modifications. Perhaps such modifications are triggered as a compensatory mechanism in response to the over-activation of $GABA_A$ receptors that is caused by the long-lasting, maximal amplification of GABA action elicited by long-term treatment with full allosteric modulators.

In conclusion, imidazenil appears to be a benzodiazepine that is related to classical benzodiazepines chemically but not pharmacologically. Imidazenil is not a new benzodiazepine but it is one with a new and ideal pharmacological profile in mice, rats, dogs and monkeys that predicts a clinical anxiolytic and anticonvulsant activation via a partial allosteric modulation of $GABA_A$ receptors.

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Chemical names

CGS20625: 2-(4-methoxy-phenyl)-2,3,5,6,7,8,9,10-octahydrocyclohepta(*b*)pyrazolo(3,4-*d*)pyridin-3-one

Ro198022: (R)-1-[(10-chloro-4-oxo-3-phenyl-4*H*-benzo[a]-quinolizin-1-yl)carbonyl-2-pyrrodinemethanol

Y23684: (±)-2-(4-chlorophenyl)-5,6-dihydro-[1]benzothiepino[5,4-c]pyridazin-3(2*H*)-one 7-oxide