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GABA Receptors

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Historical Perspective

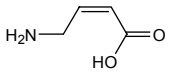
GABA is the major inhibitory amino acid transmitter of the mammalian central nervous system (CNS). Essentially all neurons in the brain respond to GABA and perhaps 20% use it as their primary transmitter.[1] Early electrophysiological studies, carried out using iontophoretic application of GABA to CNS neuronal preparations, showed it to produce inhibitory hyperpolarizing responses[2] that were blocked competitively by the alkaloid bicuculline.[3] However, in the late 1970s, Bowery and his colleagues, who were attempting to identify GABA receptors on peripheral nerve terminals, noted that GABA application reduced the evoked release of noradrenalin in the rat heart and that this effect was not blocked by bicuculline. This action of GABA was mimicked, however, by baclofen (Figure 1), a compound that was unable to produce rapid hyperpolarizing responses in central neurons. This newly identified receptor was named $GABA_B$ to differentiate it from the more familiar receptor type which became known as $GABA_A$.[4,5] Another bicuculline-insensitive receptor was first identified using the conformationally restricted GABA analog, CACA[6,7] (Figure 1). This receptor, previously termed $GABA_C$, has now been subsumed into the $GABA_A$ receptor class, on the recommendation of the IUPHAR Nomenclature Committee.[8]

The $GABA_A$ Receptor

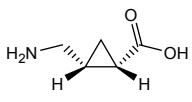
Distribution and Function

$GABA_A$ receptors are widely but differentially distributed within the CNS.[9] These receptors can be activated by a number of GABA isosteres, including muscimol and isoguvacine[10] (Figure 1). After radiolabeling, some of these ligands proved valuable in the early delineation of receptor distribution. Functionally, receptor

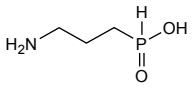
Figure 1 | Structures of selected GABA receptor agonists



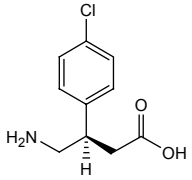
CACA
cis-Aminocrotonic acid



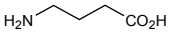
(1S,2*R*)-(+)-2-(Aminomethyl)-cyclopropane-1-carboxylate



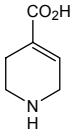
3-Aminopropyl phosphinic acid



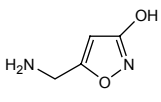
(*R*)-Baclofen
(Cat. No. 0796)



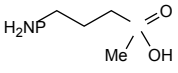
GABA
(Cat. No. 0344)



Isoguvacine
(Cat. No. 0235)



Muscimol
(Cat. No. 0289)



SKF 97541
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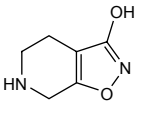


THIP
(Cat. No. 0807)

(**Bold** text denotes compounds available from Tocris at time of publication)

activation results in an increased membrane chloride conductance,[11,12] usually causing an influx of Cl^- and membrane hyperpolarization. In general, concentration-response curves exhibit positive cooperativity, which is consistent with the presence of at least two agonist binding sites on each receptor molecule.[13–15] On continued exposure to high agonist concentrations, the agonist-induced current decreases as a consequence of receptor desensitization.[16–18] Biophysical characterization of the receptor, carried out initially using noise analysis of neurons in primary culture, provided the first estimates of mean single channel conductance and average channel open times,[19] the latter of which varied with the nature of the activating agonist.[20] Development of single channel recording techniques[21] provided further detail on the nature of single channel events with the demonstration of multiple single channel conductances: 44, 30, 19 and 12pS,[22] the 30pS conductance being the most prevalent. Both channel opening times and opening frequency are dependent on agonist concentration and the competitive antagonist, bicuculline, reduces the conductance by modulating both of these parameters.[23,24] Other competitive antagonists include the pyridazinyl GABA derivative, SR 95531 (Figure 2). The receptor can also be blocked non-competitively by picrotoxin and a number of bicyclophosphates.[25] In addition, penicillin decreases channel open probability in a manner that is compatible with open channel block.[26]

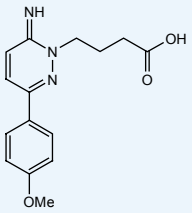
Receptor Diversity

Purification of the bovine brain receptor in the early 1980s revealed two major subunits of the $GABA_A$ receptor, which were named α and β. Elucidation of partial amino acid sequences of these subunits allowed subunit-specific monoclonal antibodies to be raised, thus providing the opportunity to explore the fine anatomical detail of receptor distribution.[27] The sequence data also facilitated the cloning of the first two $GABA_A$ receptor subunit isoforms.[28,29]

Subsequent molecular studies revealed a multiplicity of protein subunits that have now been divided into seven classes, based on the extent of similarities in their deduced amino acid sequences. Within these classes there are further subdivisions into subunit isoforms, some of which exhibit alternate splice variants. In man, six α-, three β-, three γ- and three ρ- subunit isoforms are presently known, together with single representatives of the δ, ε, π and θ classes. Within a single subunit class, the sequence homology is about 70% but between classes this falls to around 30%. Additional isoforms of some of these classes are known in other species.[30] In the earlier receptor nomenclature,

SR 95531 hydrobromide, a selective, competitive $GABA_A$ antagonist

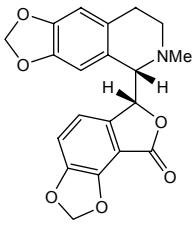
Cat. No. 1262



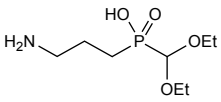
SR 95531 is a selective, competitive $GABA_A$ receptor antagonist that displaces [^{3}H]-GABA from rat brain membranes with a K_i value of 150 nM. Unlike bicuculline, SR 95531 selectively antagonizes GABA-induced Cl^- currents with little action on pentobarbitone-induced currents. The compound also acts as a low affinity glycine receptor antagonist.

Heaulme *et al.* (1986) *Brain Res.* **384** 224. **Uchida *et al.*** (1996) *Eur.J.Pharmacol.* **307** 89. **Beato *et al.*** (2007) *J.Physiol.* **580** 171.

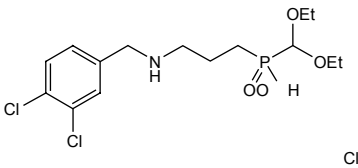
Figure 2 | Structures of selected GABA receptor agonists



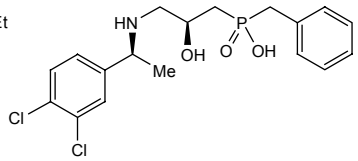
(+)-Bicuculline
(Cat. No. 0130)



CGP 35348
(Cat. No. 1245)



CGP 52432
(Cat. No. 1246)



CGP 55845
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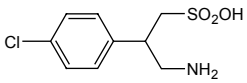
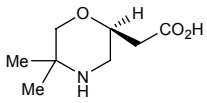
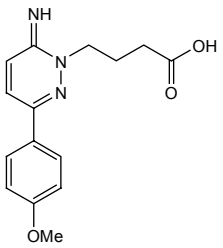


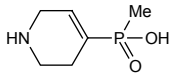
Saclofen
(Cat. No. 0246)



SCH 50911
(Cat. No. 0984)



SR 95531
(Cat. No. 1262)



TPMPA
(1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid
(Cat. No. 1040)

(**Bold** text denotes compounds available from Tocris at time of publication)

the three ρ-subunits were considered to define the $GABA_C$ receptor.[8]

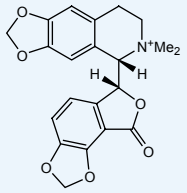
Deduced amino acid sequences from each of the subunits reveal homologies and a common structural organization which places them firmly within the so-called Cys-loop ligand-gated ion channel (LGIC) family. These receptors are pentamers of homologous subunits that assemble to form a central ion channel traversing the cell membrane. The archetypal member of the family is the peripheral nicotinic acetylcholine receptor (nAChR) with other members of the family including glycine and $5\text{-}HT_3$ receptors. Each subunit has a long amino terminal domain of more than 200 amino acids which carries the signature cys-cys loop. This extracellular domain is followed by four hydrophobic segments, each of which is about 20 amino acids long. These four segments, termed TM1–TM4, were predicted to form transmembrane domains with TM2 contributing to formation of the ion channel lining. Between TM3 and TM4 there is a large intracellular loop, which is the most divergent part of the sequence within the $GABA_A$ receptor subfamily.

Despite the plethora of receptor subunits, current evidence suggests that only a limited number of $GABA_A$ receptor subunit combinations are expressed *in vivo*.[31] Each subunit is encoded by a separate gene and a combination of *in situ* hybridization and immunohistochemical studies has revealed a distinct distribution of the various gene products in the CNS.[32,33] This is consistent with the idea that each receptor subtype, made up of different combinations of subunits, serves defined physiological roles. In turn, this provides valuable information for development of subtype-selective pharmaceutical agents. However, an added complexity is that the expression patterns of individual subunits are not immutable. These can change during development, in response to normal physiological cycles and also as a consequence of pharmacological intervention.[34–37]

Most receptors in the mammalian CNS comprise α-, β- and γ-subunits, with the most ubiquitous receptor subtype containing the α1, β2 and γ2 isoforms.[38] The recognition and functional

(–)-Bicuculline methochloride, a water-soluble $GABA_A$ antagonist

Cat. No. 0131



(–)-Bicuculline methochloride is a water soluble and more stable salt of (+)-bicuculline (Cat. No. 0130) that acts as a competitive $GABA_A$ receptor antagonist. The compound blocks inhibitory hyperpolarizing responses and reduces Cl^- conductance by modulating channel opening time and frequency.

Kemp *et al.* (1986) *Br.J.Pharmacol.* **87** 677. **MacDonald *et al.*** (1989) *J.Physiol.* **410** 479. **Seutin and Johnson** (1999) *TiPS* **20** 268.

characteristics of individual $GABA_A$ receptor subtypes have been explored extensively using recombinant receptors expressed in mammalian cells or *Xenopus* oocytes. Many mutagenesis studies have been carried out to determine the roles of individual subunits, peptide segments and specific amino acids in receptor function. It is clear that in order to interpret mutagenic results effectively at the molecular level, it is essential to have an accurate view of the overall structure of the receptor.

Structure and Function

When the sequence homologies of many subunits of the Cys-loop LGIC family were first revealed, it seemed reasonable to predict that all members would share the same structural organization as the *Torpedo* nAChR. This is the best characterized member of the family and it has been elegantly imaged using cryoelectron microscopy, most recently at a resolution of 4Å.[39] It is a pentamer of homologous subunits that are arranged pseudosymmetrically around an integral ion channel. Using electron microscopy to

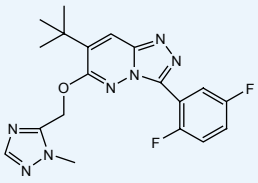
Table 1 | Comparative pharmacology of GABA receptors

Compound	$GABA_A$	$GABA_B$	$GABA_{A\text{-}\rho}$ (formerly $GABA_C$)	Reference
GABA	Agonist	Agonist	Agonist	–
Muscimol	Agonist	Inactive	Partial agonist	5, 7, 158
Isoguvacine	Agonist	Inactive	Antagonist	5, 7
THIP	Agonist	Inactive	Antagonist	5, 7
P4S	Agonist	Inactive	Antagonist	5, 7
TACA	Agonist	Inactive	Agonist	7
CACA	Inactive	Inactive	Partial agonist	7
(R)-Baclofen	Inactive	Agonist	Inactive	5, 7
Bicuculline	Antagonist	Inactive	Inactive	5, 7
Picrotoxin	Antagonist	Inactive	Antagonist	5, 7
CGP 35348	Inactive	Antagonist	Inactive	159
CGP 54626	Inactive	Antagonist	Inactive	159
CGP 64213	Inactive	Antagonist	Inactive	159
SCH 50911	Inactive	Antagonist	Inactive	159
TPMPA	Inactive	Inactive	Antagonist	7, 160, 161

(**Bold** text denotes compounds available from Tocris at time of publication)

L-838,417, a subtype-selective $GABA_A$ partial agonist

Cat. No. 3250



L-838,417 is a subtype-selective $GABA_A$ receptor partial agonist. It selectively binds to α1, α2, α3 and α5 subunits (K_i values are 0.79, 0.67, 0.67 and 2.25 nM respectively) but displays no efficacy at α1 (α1-sparing). The compound exhibits non-sedative anxiolytic, antinociceptive and anti-inflammatory activity *in vivo*.

McCabe *et al.* (2004) *Neuropharmacology* **46** 171. **McMahon and France** (2006) *Br.J.Pharmacol.* **147** 260. **Knabi *et al.*** (2008) *Nature* **451** 330.

image the purified porcine $GABA_A$ receptor by negative staining, a pentameric structure of similar diameter (about 8 nm across the pentamer) was revealed.[40] It is now believed that the most abundant α1β2γ2 $GABA_A$ receptor subtype comprises two copies each of the α1- and β2-subunits together with a single γ2-subunit.[41] The arrangement of the subunits within the pentamer was first studied by concatenation,[42] an approach that involves physical linking of the cDNAs encoding two or more subunits prior to their ectopic expression with other subunits. Such studies have demonstrated a pentameric subunit arrangement of β-α-β-α-γ lying in an anticlockwise direction when viewed from the outside of the cell.[43] With this information, it has been possible to use the 4Å structure of the *Torpedo* nAChR as a template to construct *in silico* models of this most common $GABA_A$ receptor subtype[44,45] (Figures 3a and 3b). These models provide a means to explore the similarities and differences in the structure and function of different members of the $GABA_A$ receptor subfamily.

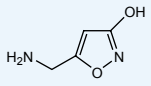
The exploration of ligand recognition in the extracellular domains of the Cys-loop family of receptors has been continuing for almost four decades. In 2001, rejuvenation of interest in this area came from a somewhat unexpected source. The structure of a water-soluble acetylcholine binding protein (AChBP) from *Lymnaea stagnalis* was determined at 2.7Å resolution,[46] a structure that soon proved to be a valuable homolog of the extracellular segment of the nAChR and other members of the family. This protein was the first to be used as a template to model the extracellular domain of the α1β2γ2 $GABA_A$ receptor.[47] Together with the plethora of mutagenic data available in the literature at the time, this model furnished the first direct structural evidence that was compatible with the long-standing idea that GABA recognition sites were located at the β-α interfaces. In addition, it rationalized a great deal of experimental data which suggested that an allosteric site for the classical benzodiazepines lies in a similar position at the adjacent α-γ interface. In the case of the GABA activation sites, the current consensus is that the primary determinants of agonist recognition are found within at least six non-contiguous stretches ('loops') of amino acids in the extracellular domains of each subunit, loops A–C being contributed by the 'principal' subunit (β) and loops D–F by the neighboring 'subordinate' subunit (α, Figure 3c). Sequence comparisons of the 'recognition loops' in different subunits of the receptor superfamily reveals some homology. However, it is the structural divergence within these loops that provides the exquisite acuity of ligand recognition which differentiates the family members.

The value of this *in silico* approach has proved significant. Not only does it allow visualization of the disposition of the amino acids involved in ligand recognition, but also, using theoretical ligand docking approaches, it becomes feasible to address receptor subtype-selective ligand design, an area that is of undoubted commercial interest.[48] Since binding sites for both neurotransmitters and allosteric modulators occur at subunit-subunit interfaces, we must again consider the importance of the subunit arrangement within each pentameric receptor. As discussed above, there is considerable theoretical and experimental evidence to assume that the subunit arrangement of the α1β2γ2 receptor is secure. However, there is no *a priori* reason to assume that less abundant receptors should adopt a similar pentameric architecture. What, for example, is the arrangement of subunits in receptor subtypes comprising αβδ-subunits? To address this question, atomic force microscopy (AFM) was recently used to investigate the α4β3δ subtype.[49] The subunits were C-terminally tagged with different epitopes and, after ectopic expression and decoration with the appropriate antibodies, the receptor-antibody complexes were visualized by AFM. The results suggested a similar arrangement to the α1β2γ2 subtype with the δ-subunit simply replacing the γ-subunit within the pentamer. However, the possibility of heterogeneity in receptor assembly cannot be excluded and, for example, results from concatenation studies suggest that the δ-subunit may be a little more promiscuous than first suggested.[50]

Comparison of the two receptor subtypes described above (α1β2γ2 and α4β3δ) is important from both a physiological and pharmacological perspective. During the last several years, it has become clear that there are two major types of $GABA_A$ receptor-mediated inhibitory responses i.e. phasic and tonic.[51] Phasic inhibition results from activation of $GABA_A$ receptors that are localized primarily to the synapse, such as the abundant α1β2γ2 subtype. Tonic transmission is mediated by less abundant extrasynaptic receptors, including the α4β3δ subtype, that are thought to be activated by the low concentrations of the natural agonist which escape the efficient re-uptake machinery found in both neurons and glia. There is currently considerable interest in developing drugs that have differential effects on these two forms of inhibitory neurotransmission. Although the natural

Muscimol, a potent $GABA_A$ agonist

Cat. No. 0289

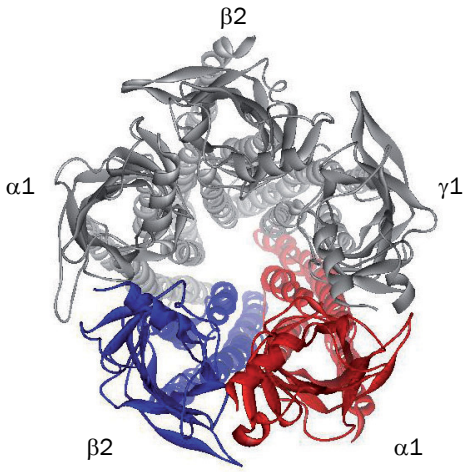


Muscimol is a potent $GABA_A$ receptor agonist and partial $GABA_C$ receptor agonist. The compound inhibits memory retention via central $GABA_A$ receptors and attenuates airway constriction via peripheral $GABA_A$ receptors.

Johnstone *et al.* (1996) *TiPS* **17** 319. **Gleason *et al.*** (2009) *J.Appl.Physiol.* **106** 1257. **Jafari-Sabet and Jannat-Dastjerdi** (2009) *Behav.Brain Res* **202** 5.

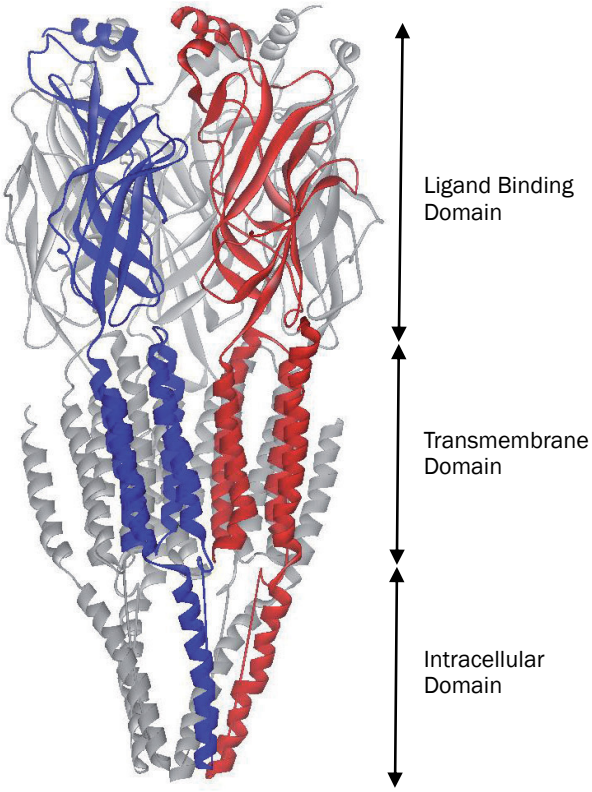
Figure 3 | Model of the $\alpha1\beta2\gamma2$ $GABA_A$ receptor structure

a) View from the synapse down the long axis



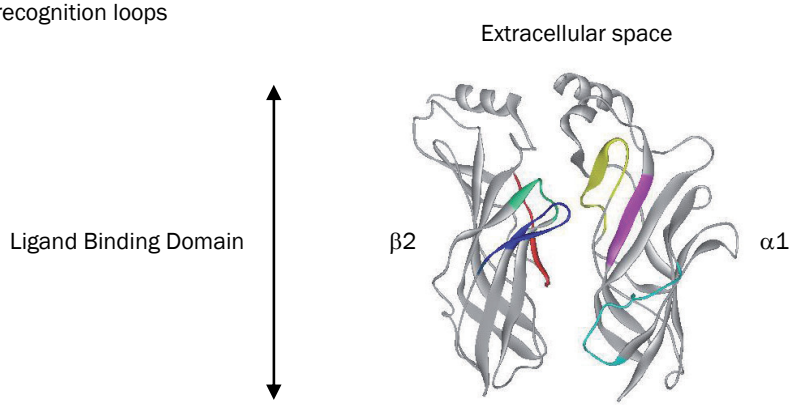
Two views of the receptor folding pattern are shown above. Each β2–α1 interface carries a GABA activation site. At one such interface the β2 subunit is colored blue and the α1 subunit is colored red; the other subunits are colored grey for simplicity.

b) View perpendicular to the long axis

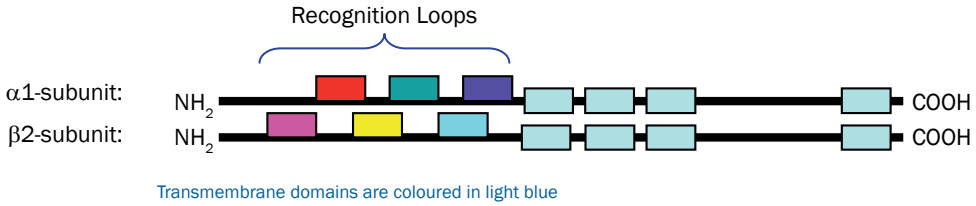


c) Schematic representation of the ligand binding domain

i) Juxtaposition of the recognition loops



ii) Sequence format



Transmembrane domains are coloured in light blue

The structural representations shown are compatible with **Mokrab *et al.*** (2007) where further detail can be found.[45]

agonist, GABA, appears to be a full agonist at the α1β2γ2 receptor, its conformationally restricted analog, THIP (also known as gaboxadol, Figure 1), is a partial agonist. In contrast, THIP is a full agonist at the α4β3δ receptor where GABA acts as a partial agonist. Interestingly THIP exhibits hypnotic properties[52] which are functionally quite distinct from those seen with the most widely used hypnotics, namely zopiclone and the α1-selective agents, zolpidem and zaleplon. The latter compounds facilitate phasic inhibition by interacting with classical benzodiazepine-sensitive receptors at the synapse. THIP appears to produce its effects by modulating tonic inhibition mediated by extrasynaptic receptors,[53] which may also be selective targets for general anesthetics.

Modulators of $GABA_A$ Receptor Function

The Benzodiazepines

The therapeutic importance of the benzodiazepines has been a significant impetus to $GABA_A$ receptor research. Classical benzodiazepines potentiate agonist-mediated activation of the $GABA_A$ receptor by causing a parallel leftward shift of the GABA concentration-response curve. In 1976, the discovery of saturable, high affinity binding sites for [^{3}H]-diazepam in the brain[54,55] provided an important experimental tool for their study. All of the overt effects of the benzodiazepines: sedative, anxiolytic, anticonvulsant, muscle relaxant and amnestic, are mediated by $GABA_A$ receptors. However, not all the $GABA_A$ receptors recognize the benzodiazepines. The particular α-subunit isoform present within an individual $GABA_A$ receptor subtype is the primary determinant of benzodiazepine recognition (Table 2). If the α1-subunit of the most common $GABA_A$ receptor (α1β2γ2) is replaced by α4 or α6 the receptor fails to recognize the classical benzodiazepines. It is now clear from both biochemical and mutational analysis that this insensitivity can be attributed to a single amino acid substitution in the extracellular N-terminal domain: a histidine (H101) in the α1-, α2-, α3- and α5-subunits is replaced by an arginine residue in α4 and α6.[56,57] When receptors containing the former subunits are expressed with a β- and γ2-subunit, all are recognized by the classical benzodiazepines. However, several agents differentiate between the subtypes on the basis of the particular α-subunit isoform present in the pentamer. The first of these compounds to be identified was the triazolopyridazine CL 21887258 (Figure 4), which is related to the recently introduced hypnotic, zaleplon. Similarly β-carboline- 3-carboxylic acid esters also show a preference for certain α-subunit-containing receptors.[59] Zolpidem (Figure 4), currently the most widely prescribed hypnotic in the USA, has been shown to have high affinity for α1-containing receptors, lower affinity for receptors carrying α2 or α3 very low affinity for those containing α[560,61] (Table 2) and no observable interaction with receptors which contain the α4- or α6-subunits.

Using knockin (KI) technology, the importance of the α-subunit histidine-arginine substitution has been turned into an advantage. The exquisite specificity of this switch dictates that, by replacing the α1 histidine with an arginine (H101R) in the germ line, the KI adult animals will differ from their wild-type counterparts only in the ability of their α1-containing receptors to recognize the benzodiazepines. Thus, it was expected that characterization of the knockin mouse phenotype would allow the complex pharmacological effects of benzodiazepines to be dissected based on their interactions with specific $GABA_A$ receptor subtypes. Extensions of this approach have proved particularly valuable; it is now clear that the α1-subunit is responsible for the sedative, anterograde amnestic and some of the anticonvulsant effects of the benzodiazepines,[62,63] whereas the α2-subunit has been associated with their anxiolytic actions.[64] Not all of the results are clear cut; for example, the pharmacodynamic profile of the α3-selective ligand, TP003, suggests a contribution of this subunit to both anxiolytic and anticonvulsant effects.[65,66] Also, receptors containing the α5-subunit have been implicated in learning and memory processes.[67] This approach has been significantly advanced recently using conditional knockin studies, which have revealed selective changes in the ability of $GABA_A$ receptors within particular cell groups to recognize the hypnotic, zolpidem.[68] Unfortunately, attempts to delineate the functional importance of individual $GABA_A$ receptor subunits using gene knockout technology have proved frustrating. It is clear that ablation of subunit expression frequently results in compensatory changes in the expression of other subunits, providing significant challenges in assigning specific responsibility for the resulting phenotype.[38]

Perhaps one of the most interesting phenomenological observations to arise from studies of benzodiazepine interactions with the $GABA_A$ receptors has been the development of the inverse agonist concept. Non-benzodiazepine ligands were

Table 2 | Affinity of benzodiazepine site ligands for $GABA_A$ receptor subtypes

Compound	α1β2γ2	α2β2γ2	α3β2γ2	α4β2γ2	α5β2γ2	α6β2γ2
Diazepam	16.1	16.9	17.0	>10,000	14.9	>10,000
Clonazepam	1.3	1.7	2.0	–	–	>10,000
Triazolam	1.8	1.2	3.0	–	1.2	–
Bretazenil	1.2	1.2	1.3	–	2.4	–
Flumazenil	1.0	1.1	1.5	107	0.4	90
Ro 15-4513	10.4	5.5	7.8	5.0	0.5	5.1
CL 218872	130	1820	1530	>10,000	490	>10,000
β-CCM	1.7	6.5	4.1	–	27	2050
Zolpidem	17	291	357	–	>15,000	–

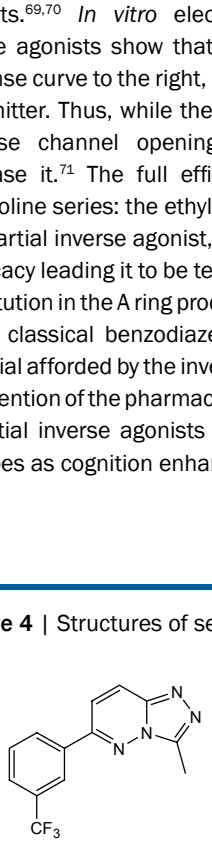
(**Bold** text denotes compounds available from Tocris at time of publication)

discovered that were able to displace a radiolabeled benzodiazepine from its binding sites. One of the first of these was ethyl β-carboline-3-carboxylate (β-CCE) which was shown to have effects that were diametrically opposed to those of the classical benzodiazepines e.g. it is proconvulsant. This led to a new terminology; β-CCE became known as an inverse agonist with the classical benzodiazepines then being classified as agonists.[69,70] *In vitro* electrophysiological experiments using inverse agonists show that they shift the GABA concentration-response curve to the right, decreasing the potency of the natural transmitter. Thus, while the agonist benzodiazepine site ligands increase channel opening frequency, the inverse agonists decrease it.[71] The full efficacy spectrum is found within the β-carboline series: the ethyl ester is proconvulsant and thus acts as a partial inverse agonist, the propyl ester is essentially devoid of efficacy leading it to be termed an antagonist,[72] while aromatic substitution in the A ring produces agonists with similar properties to the classical benzodiazepines[73] (Figure 4). The therapeutic potential afforded by the inverse agonist concept has not escaped the attention of the pharmaceutical industry with the development of partial inverse agonists selective for α5-containing receptor subtypes as cognition enhancers.[74]

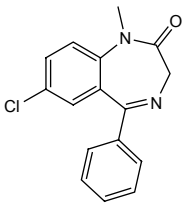
Steroids

The observation that 5α-pregnan-3α-ol-11,20-dione (alphaxalone; Figure 5), a synthetic steroidal anesthetic, was able to enhance stimulation-evoked inhibition produced by $GABA_A$ receptor agonists in rat cuneate nucleus slices,[75] was the first evidence for allosteric steroid sites on these receptors. Subsequent voltage clamp studies conducted on both neurons and adrenomedullary chromaffin cells[76,77] confirmed the stereoselective activity of the progesterone metabolites 5α-pregnan-3α-ol-20-one (allopregnanolone), 5β-pregnan-3α-ol-20-one (pregnanolone) and 5α-pregnan-3α,21-diol-20-one (allotetrahydrodeoxycorticosterone). Mechanistically, the action of these compounds appeared to be similar to that of the barbiturates which, at low concentrations, potentiate the effects of GABA by increasing channel open times and, at higher concentrations, directly activate the receptor.[78–82] Later studies revealed that the sites of barbiturate and steroid action are distinct.[83] Conserved residues within the α- and β-subunit membrane spanning domains of the α1β2γ2 receptor, which are important for both steroid facilitation and direct activation, have been identified.[84]

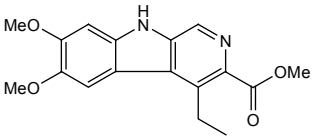
Figure 4 | Structures of selected benzodiazepine site ligands



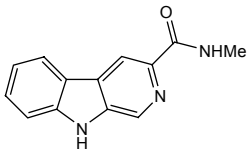
CL 218872
(Cat. No. 1709)



Diazepam
(Cat. No. 2805)



DMCM
(Cat. No. 3083)



FG 7142
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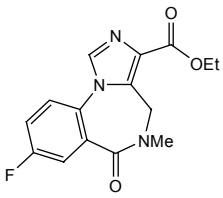

Flumazenil
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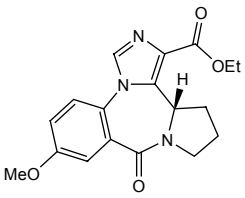
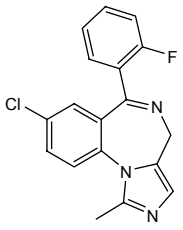
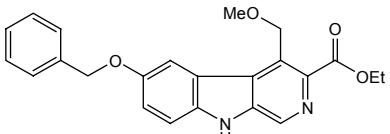


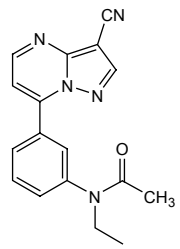
L-655,708
(Cat. No. 1327)



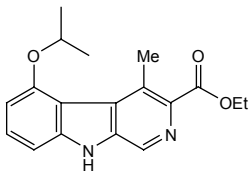
Midazolam
(Cat. No. 2832)



ZK 93423
(Cat. No. 1994)



Zaleplon
(Cat. No. 3655)



ZK 93426
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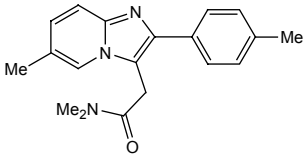
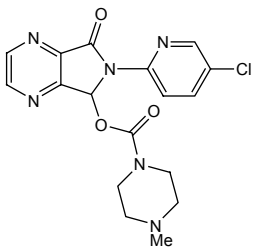


Zolpidem
(Cat. No. 0655)

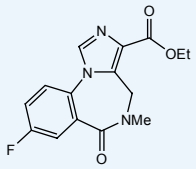


Zopiclone
(Cat. No. 1094)

(**Bold** text denotes compounds available from Tocris at time of publication)

Flumazenil, a benzodiazepine antagonist

Cat. No. 1328



Flumazenil is a benzodiazepine antagonist that is nonselective for α1, α2, α3 or α5-containing $GABA_A$ receptors. The compound reverses benzodiazepine sedation and is centrally active upon systemic administration *in vivo*.

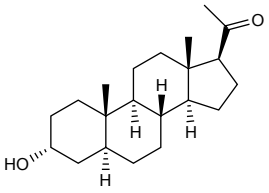
Polc *et al.* (1981) *Naunyn-Schmied.Arch.Pharmacol.* **316** 317. **Atack *et al.*** (1999) *Neuropsychopharmacology* **20** 255. **Doble** (1999) *J.Psychopharmacol.* **13** S11.

Studies with ectopically expressed receptors comprising αβγ-subunits demonstrated limited impact of subunit composition on the functional effects of the steroids.[85] At putative extrasynaptic receptors, where δ replaces the γ-subunit, there is evidence for increased steroid potency.[86] However, these observations may be explained, at least in part, by the reduced efficacy of the endogenous neurotransmitter, GABA, at these receptor subtypes.[87] There have been many literature reports to demonstrate that the potency of steroids varies in different brain regions and there is also evidence to suggest that the observed effects may be influenced by changes in receptor phosphorylation and modulation of enzymatic activity in the steroid metabolic pathways.[88] This functional complexity is amplified further by normal physiological fluctuations in steroid levels associated with, for example, pregnancy and the ovarian cycle. These can lead to altered patterns of subunit expression that may contribute to the mood swings that are associated with these events.[89,37]

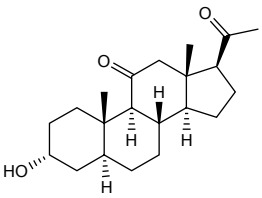
General Anesthetics

It is now clear that $GABA_A$ receptors play a significant role in general anesthesia. Many of the receptor subtypes are sensitive to clinically relevant concentrations of general anesthetics and exhibit the appropriate stereospecificity. The characteristics of these agents are diffuse; they exhibit sedative, hypnotic, analgesic and amnestic properties in addition to producing a loss of mobility.[90] This multiplicity of effects, together with their structural diversity, has meant that it is very difficult to dissect the actions of general anesthetics at the molecular level. One agent, ketamine, mainly affects glutamatergic excitatory responses mediated by NMDA receptors and there is no evidence that the older anesthetics, nitrous oxide and xenon, modulate GABAergic inhibition. The evidence for interactions of other inhalational and intravenous agents with the $GABA_A$ receptors continues to grow. Since general anesthetics are hydrophobic and need to access the CNS, it is perhaps not surprising that they target hydrophobic pockets within the transmembrane domains of the receptor. Initial evidence suggested that the inhalational anesthetics favored the α-subunits[91] while *in vitro* and *in vivo* evidence has accumulated to suggest that intravenous anesthetics interact with the β-subunits.[92,93] Over the past decade it has become increasingly clear that significant effects of the general anesthetics occur not by their ability to potentiate the fast phasic inhibition mediated by synaptically located receptors but as a result of their effects on receptors that are located extrasynaptically. The extrasynaptic α5β3γ2 receptor in the hippocampus is probably associated with the amnestic actions of many of these agents,[94] while those receptors containing δ-subunits in the ventrobasal thalamic nucleus provide the intriguing link between the reversible loss of consciousness in man, a sleep-related phenomenon that is a primary characteristic induced by the general anesthetics.[95] It is clear that the diversity

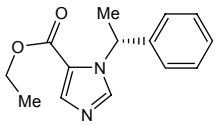
Figure 5 | Structures of selected compounds active at allosteric sites of $GABA_A$ receptors



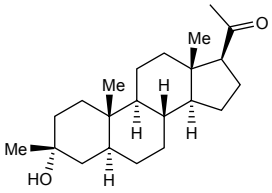
Allopregnanolone
3α-hydroxy-5α-pregnan-20-one
(Cat. No. 3653)



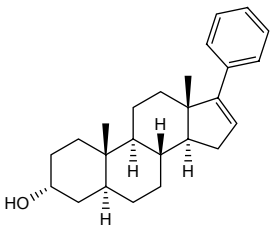
Alphaxalone
(Cat. No. 3046)



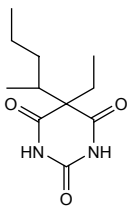
Etomidate
(Cat. No. 1471)



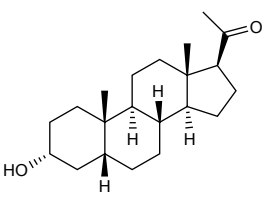
Ganaxolone
(Cat. No. 2531)



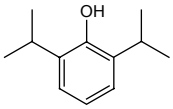
17-PA
(Cat. No. 2681)



Pentobarbitone



Pregnanolone
3α-hydroxy-5β-pregnan-20-one
(Cat. No. 3652)

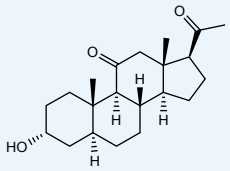


Propofol
(Cat. No. 0939)

(**Bold** text denotes compounds available from Tocris at time of publication)

Alphaxalone, a direct activator and potentiator of $GABA_A$

Cat. No. 3046



Alphaxalone is a neurosteroid anesthetic that directly activates and potentiates the $GABA_A$ receptor-activated membrane current (I_{GABA}). Efficacy, but not potency, of this compound is determined by the alpha subunit of the receptor (EC_{50} values are 1.4, 1.8, 2.1, 2.4 and 2.5 μM for α1β1γ3, α1β1γ1, β1γ1, α2β1γ2L and α1β1γ2L isoforms respectively).

Maitra *et al.* (1999) *Brain Res.* **819** 75. **Wegner *et al.*** (2007) *Neuropharmacology* **52** 672. **Zecharia *et al.*** (2009) *J.Neurosci.* **29** 2177.

of agents and targets provide valuable clues that must be addressed systematically to optimize the potential for development of novel anesthetic agents.[96]

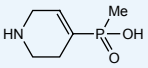
Alcohol

The receptors responsible for the pharmacological effects of ethanol have been the subject of much speculation but there are only a small number of putative targets that are responsive to low concentrations of ethanol (< 20 mM). Initial observations of altered ethanol-induced behaviors in δ-subunit knockout mice[97] were followed by *in vitro* studies of recombinant $GABA_A$ receptors. There was significant excitement when it was reported that agonist activation of δ-containing $GABA_A$ receptors could be facilitated by 10 mM ethanol;[98,99] however, replication of this response has not proved possible and these discrepancies remain unexplained.[100] It has been suggested that many of the *in vivo* effects of ethanol may be attributed to indirect effects arising from its ability to increase levels of several endogenous steroids which, in turn, can potentiate $GABA_A$ receptor-mediated responses. Evidence in favor of this idea comes from observations that, not only do the consequential effects of ethanol correlate well with an increase in steroid levels, but they are also inhibited by blockers of steroid synthesis.[101]

Phosphorylation may again play a significant role since it has been noted that in PKCδ knockout mice, the pharmacological effects of ethanol are reduced as are the ataxic responses to both pentobarbital and pregnanolone. Since the flunitrazepam response remained intact in these animals, it was suggested that the overt effects were mediated by benzodiazepine insensitive $GABA_A$ receptors. Supplementary studies showed that the PKCδ-dependent effects of ethanol could be observed in ectopically expressed α4β3δ receptors.[102] Thus, assignment of the effects of ethanol to specific $GABA_A$ receptors remains enigmatic.

TPMPA, a selective $GABA_{A-\rho}$ antagonist

Cat. No. 1040



TPMPA is a selective, competitive $GABA_{A-\rho}$ antagonist which exhibits only minimal effects on $GABA_A$ and $GABA_B$ receptors (K_b values are 2.1 μM (antagonist), 320 μM (antagonist) and EC_{50} ~ 500 μM (weak agonist) respectively). It displays 8-fold selectivity for human recombinant ρ1 receptors over ρ2 receptors and blocks the paired-pulse depression component of inhibitory post-synaptic currents *in vitro*.

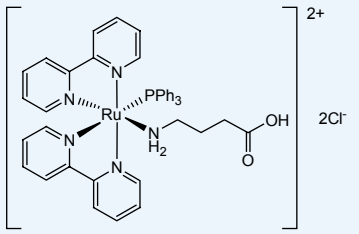
Murata *et al.* (1996) *Bioorg.Med.Chem.Lett.* 6 2073. **Chebib *et al.*** (1998) *Eur. J.Pharmacol.* **357** 227. **Xu *et al.*** (2009) *Exp.Neurol.* **216** 243.

$GABA_{A-\rho}$ Receptors (Previously Classified as $GABA_C$)

Although the receptors that were originally designated as $GABA_C$ are now considered to be members of the $GABA_A$ family,[8] it is useful to highlight their distinguishing features. These receptors were originally classified on the basis of their unique pharmacology. The natural agonist, GABA, was reported to be about an order of magnitude more potent at this subclass than at other $GABA_A$ receptors and, although CACA activated this receptor, this agent was not recognized by either the $GABA_A$ or $GABA_B$ classes (Figure 1). $GABA_C$ receptor responses were not inhibited by bicuculline but, like the $GABA_A$ receptors, they were blocked by picrotoxin. A selective $GABA_C$ receptor antagonist, (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid7 (TPMPA, Figure 2) was later identified. Additional pharmacological differences from the $GABA_A$ receptors included its lack of modulation by the benzodiazepines, barbiturates or neuroactive steroids. Receptors displaying these characteristics were shown to have a restricted distribution, initially being found in the spinal cord and subsequently in the retina,[6,103] the source from which the first ρ-subunit was cloned.[104] Three homologous ρ-subunits, ρ1 to ρ3, have now been identified. These can be expressed as either homomers or heteromers[105,106] and the ectopically expressed receptors exhibit the pharmacological characteristics of the elusive $GABA_C$ receptors. There is only limited evidence that the ρ-subunits co-assemble with any of the other $GABA_A$ receptor subunits.[107] The genes encoding the ρ1- and ρ2-subunits are found on chromosome 6 in humans, and are thus distinct from the clusters of $GABA_A$ receptor subunit genes which are

RuBi-GABA, excitable by visible wavelength

Cat. No. 1040

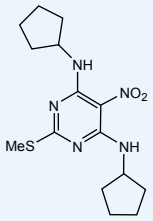


RuBi-GABA (Ruthenium-bipyridine-triphenylphosphine caged GABA) is excited by visible wavelengths and has two-photon uncaging capabilities. It provides greater tissue penetration, less phototoxicity, faster photorelease kinetics and better spatial resolution than UV light-sensitive caged compounds. Following photolysis, the compound produces GABA receptor-mediated currents in pyramidal neurons *in vitro* and displays no effect on endogenous GABAergic or glutamatergic transmission at concentrations effective for uncaging.

Zayat *et al.* (2003) *J.Am.Chem.Soc.* **125** 882. **Nikolenko *et al.*** (2005) *Chem. Comm.* **7** 1752. **Rial Verde *et al.*** (2008) *Front.Neural Circuits* **2** 2.

GS 39783, a positive modulator at $GABA_B$ receptors

Cat. No. 2001



GS 39783 is a positive allosteric modulator of $GABA_B$ receptor function. It potentiates the effects of GABA on [^{35}S]GTPγS binding at recombinant and native $GABA_B$ receptors (EC_{50} values are 2.1 and 3.1 μM respectively). The compound decreases cocaine self-administration, blocks the rewarding properties of nicotine and produces anxiolytic-like activity without the side effects associated with baclofen or benzodiazepines *in vivo*.

Urwyler *et al.* (2003) *J.Pharmacol.Exp.Ther.* **307** 322. **Cryan *et al.*** (2004) *J.Pharmacol.Exp.Ther.* **310** 952. **Mombereau *et al.*** (2007) *J.Pharmacol.Exp. Ther.* **321** 172.

found on chromosomes 4, 5, 15 and X with the exception of δ, which is found on chromosome 1. The ρ-subunit sequences display between 30 and 38% homology to the $GABA_A$ receptor subunits at the amino acid level but, interestingly, in the important TM2 region of the sequence, they show greater homology to the glycine α-subunits than to any of the $GABA_A$ receptor subunits.

The $GABA_B$ Receptor

The other major class of GABA receptors is the metabotropic (G-protein-coupled) $GABA_B$ receptor. These exhibit a distinct ligand recognition profile to the $GABA_A$ receptor family,[4] and are differentially distributed within the mammalian CNS.[108] Functionally they inhibit adenylyl cyclase activity[109] and presynaptic calcium channels, decreasing transmitter release,[110] and activate postsynaptic potassium channels, producing the late inhibitory postsynaptic potential.[111]

Distribution and Function

The initial observation that GABA inhibited the release of noradrenalin from rat atria *in vitro*, an effect not blocked by bicuculline methobromide but mimicked by (*R*)-baclofen, provided the seminal evidence to distinguish the $GABA_B$ receptor from more familiar members of the $GABA_A$ receptor family.[4] Subsequent studies, using both functional and radioligand binding techniques, have further refined the structure-activity profile at $GABA_B$ receptors[112] (Figures 1 and 2). Although the receptor is widely distributed within the mammalian CNS it is generally found at lower densities than $GABA_A$ receptors, and exhibits a distinct distribution: the highest concentrations being found in the molecular layer of the cerebellum, the frontal cortex and certain thalamic nuclei.[108] The receptor is also found in the periphery where its activation modulates autonomic control of the intestine and decreases esophageal reflux.[112,113] The receptor is coupled to adenylyl cyclase via G_i and G_o proteins. While the consequences remain poorly defined, activation of presynaptic $GABA_B$ receptors also leads to the inhibition of high voltage-activated Ca^{2+} channels, an effect that is mediated by the G protein βγ subunits. This results in decreased transmitter release and possibly also limits synaptic vesicle recruitment to the active zone.[114]

GABA activation of postsynaptic $GABA_B$ receptors produces hyperpolarization via the modulation of inwardly rectifying $K_{IR}3$ type K^+ channels[115] that mediate the late phase of the inhibitory postsynaptic potential.

Molecular Characterization

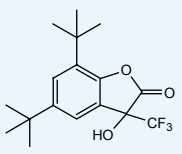
The molecular characterization of the $GABA_B$ receptor was achieved in 1997 when the availability of specific high affinity antagonists allowed the expression cloning of the $GABA_{B1}$ subunit.[116] Subsequent studies demonstrated that while this protein showed many of the expected characteristics, when expressed ectopically, it coupled poorly to its effector machinery, and exhibited a remarkably low affinity for agonists compared to the native receptor; it appeared to be retained within the endoplasmic reticulum.[117] Subsequent studies revealed the identity of an additional subunit, $GABA_{B2}$,[118–120] which interacts with the $GABA_{B1}$ subunit C-terminus, masking the ER retention signal of the $GABA_{B1}$ subunit[121] and facilitating the trafficking of the $GABA_{B1}$ subunit to the cell surface. This provided the first secure evidence of receptor dimerization.

The $GABA_B$ receptor belongs to the class C of the GPCRs, together with the metabotropic glutamate receptors mGlu1–8 and the calcium-sensing receptor.[122] Each subunit comprises a large N-terminal extracellular domain exhibiting the venus fly-trap motif, followed by 7-transmembrane helices and an intracellular C-terminus. Two splice variants for the $GABA_{B1}$ subunit are known; they are encoded by the same gene and arise by alternate promoter usage to produce $GABA_{B1a}$ and $GABA_{B1b}$.[123] These differ only in their N-terminal domains, $GABA_{B1a}$ contains a repeat of a conserved protein binding motif, so-called 'sushi domains', that are lacking in $GABA_{B1b}$; the first 147 amino acids of $GABA_{B1a}$ are replaced by 18 amino acids in $GABA_{B1b}$.[124]

While both subunits within the $GABA_B$ heterodimer exhibit the venus fly-trap motif at the extracellular N-terminus, it is the $GABA_{B1}$ subunit that is responsible for both agonist and antagonist recognition; the residues responsible are not conserved in the $GABA_{B2}$ subunit.[125] Within this recognition domain there is also a serine residue that appears to be responsible for the ability of the

Rac BHFF, a potent, selective $GABA_B$ positive allosteric modulator

Cat. No. 3313



Rac BHFF is a potent and selective $GABA_B$ receptor positive allosteric modulator that increases the potency and efficacy of GABA (> 15-fold and > 149% respectively). The compound exhibits anxiolytic activity *in vivo* and is orally active.

Malherbe et al (2008) Br.J.Pharmacol. 154 797.

receptor to sense Ca^{2+} concentrations.[126] While the $GABA_{B2}$ subunit is not primarily responsible for agonist recognition, its presence markedly increases the agonist affinity of the $GABA_{B1}$ subunit.[127] The $GABA_{B2}$ subunit mediates G protein-coupling, the second intracellular loop being particularly important,[128,129] although it is clear that $GABA_{B1}$ is important in facilitating this process. It is the $GABA_{B2}$ subunit that appears to be the interaction site for an increasing family of positive allosteric modulators[130] (Figure 6), where binding occurs within the transmembrane domain[131] to augment agonist tone while exhibiting no direct agonist activity.[132]

The restricted molecular heterogeneity found in the $GABA_B$ receptor population has proved a significant frustration, since ectopic expression studies have failed to provide support for the varied functional responses ascribed to these receptors *in vivo*.[133] Knockout studies targeting $GABA_{B1}$ or $GABA_{B2}$ have not relieved these difficulties, both deletions producing similar phenotypes, although each compromised the expression of the conjugate subunit.[134,135] Functional distinctions between the $GABA_{B1}$ subunit isoforms have started to emerge suggesting that the $GABA_{B1a}$ isoform is primarily associated with the heteroceptors controlling glutamate release.[136–139] It has been suggested that this differential cellular localization may be associated with the presence of the sushi repeats, present in $GABA_{B1a}$ but not in $GABA_{B1b}$, that are known to be important in protein-protein interactions in other environments.[140] Interestingly it has recently been shown that a soluble truncated form of the $GABA_{B1a}$ subunit, named $GABA_{B1j}$, exhibits nanomolar affinity for neuronal membranes. It is identical to the first 157 amino acids of the $GABA_{B1a}$ subunit and contains the sushi repeats together with a 72 amino acid C-terminal extension with no homology to other known proteins. In its presence both basal and stimulated glutamate release are decreased, but $GABA_B$ receptor function at presynaptic autoreceptors or postsynaptic receptors remains unaffected.[141]

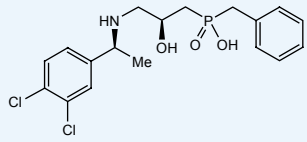
Clinical Potential

Baclofen remains the only clinically available agent that targets the $GABA_B$ receptor. It was introduced into clinical practice in 1972 long before the discovery of the $GABA_B$ receptor and remains the intervention of choice in spasticity associated with multiple sclerosis and cerebral palsy. Baclofen exhibits a challenging side effect profile on systemic administration, producing drowsiness, nausea, muscle weakness and mental confusion largely due to poor brain penetration necessitating the use of high oral doses.[142] The muscle relaxant effects mediated within the spinal cord can be secured by intrathecal administration, allowing a marked reduction in dose, thus limiting the systemic effects and the development of tolerance.[143] Baclofen also exhibits antinociceptive properties at the spinal level, which again allows local administration,[144] however significant analgesia is also mediated within the ventrobasal nucleus of the thalamus,[145] a site which requires systemic administration. The analgesic effects of baclofen currently have limited application in humans.[146]

The early specific $GABA_B$ receptor antagonists suffered from a limited potency, with phaclofen, for example, displaying an affinity of only 100 μM. A number of selective, high affinity and systemically active antagonists are now available (Figure 2), that may have significant clinical potential in absence epilepsy.[142] Mice overexpressing the $GABA_{B1a}$ isoform exhibit characteristics associated with atypical absence epilepsy.[147] In contrast, recent reports suggest that impaired $GABA_B$ receptor function may contribute to repetitive firing in human temporal lobe epilepsy tissue.[148] The first exploration of $GABA_B$ receptor antagonists clinically was an open trial with SGS 742 (CGP 36742) in mild cognitive deficit in man.[149] While the initial results appeared promising further clinical reports have not reached the literature. Recent studies suggest that mechanistically phospho-protein

CGP 55845, a potent, seletive $GABA_B$ antagonist

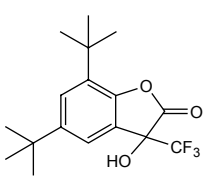
Cat. No. 1248



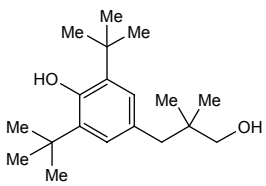
CGP 55845 is a potent, selective $GABA_B$ receptor antagonist (IC_{50} = 5 nM) that prevents agonist binding (pK_i = 8.35) and inhibits GABA and glutamate release (pEC_{50} values are 8.08 and 7.85 respectively). The compound inhibits $GABA_B$ receptor responses to baclofen (IC_{50} = 130 nM in an isoproterenol assay) and potentiates the hypoglycemic response to glucose *in vitro*.

Waldmeier *et al.* (1994) *Br.J.Pharmacol.* **113** 1515. **Cunninghan and Enna** (1996) *Brain Res.* **720** 220. **Zhang *et al.*** (2009) *J.Physiol.* **578** 735.

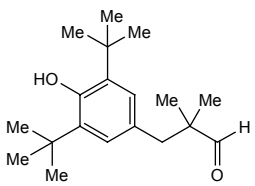
Figure 6 | Structures of selected allosteric modulators of the $GABA_B$ receptor



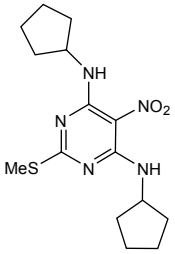
rac BHFF
(Cat. No. 3313)



CGP 7930
(Cat. No. 1513)



CGP 13501
(Cat. No. 1514)

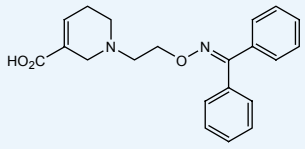


GS 39783
(Cat. No. 2001)

(**Bold** text denotes compounds available from Tocris at time of publication)

NNC 711, a selective inhibitor of GAT-1

Cat. No. 1779



NNC 711 is a potent and selective inhibitor of GABA uptake by GAT-1 (IC_{50} values are 0.04, 171, 1700 and 622 µM for hGAT-1, rGAT-2, hGAT-3 and hBGT-1 respectively). The inhibitor displays anticonvulsant activity following systemic administration *in vivo*.

Suzdak *et al.* (1992) *Eur.J.Pharmacol.* **223** 189. **Borden *et al.*** (1994) *Eur. J.Pharmacol.* **269** 219. O'Connell ***et al.*** (2001) *Eur.J.Pharmacol.* **424** 37.

kinase A (pPKA) plays a significant role in the effects of this antagonist in the Morris water maze.[150]

It has been known for some time that $GABA_B$ receptor activation effectively reduces the craving for addictive drugs, first demonstrated as a reduction in cocaine self-administration in rats,[151] and similar findings have emerged with other drugs of abuse.[142] Positive allosteric modulators at the receptor may prove to be a more attractive means of control. These agents could reasonably be expected to facilitate $GABA_B$ receptor mediated tone circumventing the side effect profile associated with the use of systemic agonists. Indeed, recent studies suggest that compounds of this type significantly reduce cocaine self-administration in rats[152,153] (Figure 5), with similar approaches providing some support for their potential as anxiolytics.[154]

The $GABA_B$ receptors remain somewhat enigmatic, promissory notes of significant therapeutic potential have not thus far materialized and it has been argued that the lack of readily differentiable receptor subtypes has limited the opportunity for discrete drug targeting. Evidence for their functional importance continues to expand with recent studies highlighting their impact on both the tegmental pedunculopontine nucleus, important in the acute rewarding effects of the opiates[155] and orexin neurons, associated with sleep/ wakefulness cycles.[156] Clinical applications remain elusive; perhaps the allosteric modulators may yet prove as valuable in the modulation of tonic activity at the $GABA_B$ receptors as the benzodiazepines within the $GABA_A$ receptor family. Recent development of novel $GABA_B$ receptor agonists, the structure of which effectively restricts them to the peripheral compartment, are currently under investigation for intervention in gastroesophageal reflux in man, after proving efficacious and importantly devoid of the baclofen-associated central side effects in preclinical studies.[157]

Conclusions

Despite the overwhelming representation of the GPCRs in the human genome, it is the ionotropic receptor for the major inhibitory neurotransmitter GABA that has achieved the most visibility to date. Its serendipitous exploitation within the clinical arena has stimulated a plethora of intriguing insights into the mechanisms by which communication within the nervous system is achieved and the nuances of modulation which provide opportunities for further refinement of pharmacological intervention. The paucity of molecular heterogeneity exhibited by the $GABA_B$ receptors has proved problematic for specific drug targeting. The functional characterization of this receptor in the mammalian system was predicted to lead to significant clinical developments. Although this goal has not yet been achieved, recent research provides novel opportunities for therapeutic intervention. The next decade will undoubtedly prove exciting.

References

1. **Somogyi *et al.*** (1998) *Brain Res.Rev.* **26** 113.
2. **Krnjevic and Schwartz** (1967) *Exp.Brain Res.* **3** 320.
3. **Curtis *et al.*** (1970) *Nature* **226** 1222.
4. **Bowery *et al.*** (1980) *Nature* **283** 92.
5. **Hill and Bowery** (1981) *Nature* **290** 149.
6. **Johnston *et al.*** (1975) *J.Neurochem.* **24** 157.
7. **Johnston** (1996) *TiPS* **17** 319.
8. **Barnard *et al.*** (1998) *Pharmacol.Rev.* **65** 68-76.
9. **Palacios *et al.*** (1981) *Brain Res.* **222** 285.
10. **Ebert *et al.*** (1997) *Mol.Pharmacol.* **52** 1150.
11. **Curtis *et al.*** (1968) *Exp.Brain Res.* **5** 1.
12. **Kelly *et al.*** (1969) *Exp.Brain Res.* **7** 11.
13. **Dichter** (1980) *Brain.Res.* **190** 111.
14. **Sakmann *et al.*** (1983) *J.Neural Transm.* **18** 83.
15. **Bormann and Clapham** (1985) *Proc.Natl.Acad.Sci.USA* **82** 2168.
16. **Adams and Brown** (1975) *J.Physiol.* **250** 85.
17. **Akaike *et al.*** (1987) *J.Physiol.* **391** 219.
18. **Mathers** (1987) *Synapse* **1** 96.
19. **Barker *et al.*** (1982) *J.Physiol.* **322** 365.
20. **Barker and Mathers** (1981) *Science* **212** 358.
21. **Hamill *et al.*** (1981) *Pflugers Arch.* **391** 85.
22. **Bormann *et al.*** (1987) *J.Physiol.* **385** 243.
23. **Macdonald *et al.*** (1989) *J.Physiol.* **410** 479.
24. **Twyman *et al.*** (1990) *J.Physiol.* **423** 193.
25. **Bowery *et al.*** (1976) *Br.J.Pharmacol.* **57** 435.
26. **Twyman *et al.*** (1992) *J.Physiol.* **445** 97.
27. **Richards *et al.*** (1987) *J.Neurosci.* **7** 1866.
28. **Stephenson and Barnard** (1986) in Benzodiazepine/GABA chloride channels: structural and functional properties. p261 Alan R Liss.
29. **Schofield *et al.*** (1987) *Nature* **328** 221.
30. **Hevers and Luddens** (1998) Mol.Neurobiol. **18** 35.
31. **Olsen and Sieghart** (2008) *Pharmacol.Rev.* **60** 243.
32. **Wisden *et al.*** (1992) *J.Neurosci.* **12** 1040.
33. **Pirker *et al.*** (2000) *Neuroscience* **101** 815.
34. **Laurie** (1992) *J.Neurosci.* **12** 4151.
35. **Holt *et al.*** (1996) *Neuropharmacology* **35** 1457.
36. **Smith *et al.*** (1998) *J.Neurosci.* **18** 5275.
37. **Griffiths and Lovick** (2005) *Neuroscience* **136** 457.
38. **Rudolph and Mohler** (2004) *Ann.Rev.Pharmacol.Toxicol.* **44** 475.
39. **Unwin** (2005) *J.Mol.Biol.* **346** 967.
40. **Nayeem *et al.*** (1994) *J.Neurochem.* **62** 815.
41. **Farrar *et al.*** (1999) *J.Biol.Chem.* **274** 10100.
42. **Im** (1995) *J.Biol.Chem.* **270** 26063.
43. **Baumann** (2002) *J.Biol.Chem.* **277** 46020.
44. **Ernst *et al.*** (2005) *Mol.Pharmacol.* **68** 1291.
45. **Mokrab *et al.*** (2007) *J.Mol.Graph Model.* **26** 760.
46. **Brejc *et al.*** (2001) *Nature* **411** 269.
47. **Cromer *et al.*** (2002) *TiBS* **27** 280.
48. **Clayton *et al.*** (2007) *Curr.Med.Chem.* **14** 2755.
49. **Barrera *et al.*** (2008) *Mol.Pharmacol.* **73** 960.
50. **Kaur** (2009) *J.Biol.Chem.* **284** 7889.
51. **Farrant and Nusser** (2005) *Nature Neurosci.Rev.* **6** 215.
52. **Krogsgaard-Larson *et al.*** (2004) *Biochem.Pharmacol.* **68** 1573.

53. **Drasbek and Jensen** (2006) *Cereb.Cortex* **16** 1134.
54. **Squires and Braestrup** (1976) *Nature* **266** 732.
55. **Mohler and Okada** (1977) *Science* **198** 849.
56. **Wieland** *et al.* (1992) *J.Biol.Chem.* **267** 1426.
57. **Duncalfe** *et al.* (1996) *J.Biol.Chem.* **271** 9209.
58. **Squires** *et al.* (1979) *Pharmacol.Biochem.Behav.* **10** 825.
59. **Nielsen and Braestrup** (1980) *Nature* **286** 606.
60. **Hadingham** *et al.* (1993) *Mol.Pharmacol.* **43** 970.
61. **Sanger** *et al.* (1994) *Neurosci.Behav.Rev.* **18** 355.
62. **Rudolph** *et al.* (1999) *Nature* **401** 796.
63. **McKernan** *et al.* (2000) *Nature Neurosci.* **3** 587.
64. **Low** *et al.* (2000) *Science* **290** 131.
65. **Dias** *et al.* (2005) *J.Neurosci.* **25** 10682.
66. **Fradley** *et al.* (2007) *J.Psychopharmacol.* **21** 384.
67. **Collinson** *et al.* (2000) *Eur.J.Neurosci.* **12** 171.
68. **Wulff** *et al.* (2007) *Nature Neurosci.* **10** 923.
69. **Tenen and Hirsch** (1980) *Nature* **288** 609.
70. **Cowan** *et al.* (1981) *Nature* **290** 54.
71. **Barker** *et al.* (1984) in Actions and interactions of GABA and the benzodiazepines. p203 Publ Raven Press.
72. **Smiechen** *et al.* (1993) in Anxiolytic β-carbolines: from molecular biology to the clinic. p7 Publ Springer Verlag.
73. **Stephens** *et al.* (1987) *Brain Res.Bull.* **19** 309.
74. **Sternfield** *et al.* (2004) *J.Med.Chem.* **47** 2176.
75. **Harrison and Simmonds** (1984) *Brain.Res.* **323** 287.
76. **Cottrell** *et al.* (1987) *Br.J.Pharmacol.* **90** 491.
77. **Harrison** *et al.* (1987) *J.Neurosci.* **7** 604.
78. **Study and Barker** (1981) *Proc.Natl.Acad.Sci.USA* **78** 7180.
79. **Twyman** *et al.* (1989) *Ann.Neurol.* **25** 213.
80. **Callachan** *et al.* (1987) *Neurosci.Letts.* **29** S21.
81. **Nicoll** *et al.* (1975) *Nature* **258** 625.
82. **Simmonds** (1981) *Br.J.Pharmacol* **73** 739.
83. **Peters** *et al.* (1988) *Br.J.Pharmacol.* **94** 1257.
84. **Hosie** *et al.* 2006 *Nature* **444** 486.
85. **Belelli** *et al.* (2006) *Neuroscience* **138** 821.
86. **Brown** *et al.* (2002) *Br.J.Pharmacol.* **136** 965.
87. **Bianchi and MacDonald** (2003) *J.Neurosci.* **23** 10934.
88. **Herd** *et al.* (2007) *Pharmacol.Ther.* **116** 20.
89. **Koksma** *et al.* (2003) *J.Neurosci.* **23** 788.
90. **Bonin and Orser** (2008) *Pharmacol.Biochem.Behav.* **90** 105.
91. **Nishikawa** *et al.* (2002) *Neuropharmacology* **42** 337 .
92. **Belelli** *et al.* (1997) *Proc.Natl.Acad.Sci.USA* **94** 11031.
93. **Jurd** *et al.* (2003) *FASEB J.* **17** 250.
94. **Cheng** *et al.* (2006) *J.Neurosci.* **26** 3713.
95. **Franks** (2008) *Nature Neurosci.Rev.* **9** 370.
96. **Zeller** *et al.* (2007) *BMC Pharmacol.* **7** 2.
97. **Mihalek** *et al.* (2001) *Alcohol Clin.Exp.Res.* **25** 1708.
98. **Sundstrom-Poromaa** (2002) *Nature Neurosci.* **5** 721.
99. **Wallner** *et al.* (2003) *Proc.Natl.Acad.Sci.USA* **100** 15218.
100. **Borghese** *et al.* (2006) *J.Pharmacol.Exp.Ther.* **316** 1360.
101. **Morrow** *et al.* (2001) *Brain Res.Rev.* **37** 98.
102. **Choi** *et al.* (2008) *J.Neurosci.* **28** 11890.
103. **Polenzani** *et al.* (1991) *Proc.Natl.Acad.Sci.USA* **88** 4318.
104. **Cutting** *et al.* (1991) *Proc.Natl.Acad.Sci.USA* **88** 2673.
105. **Enz and Cutting** (1998) *Vision Res.* **38** 1431.
106. **Enz and Cutting** (1999) *Eur.J.Neurosci.* **11** 41.
107. **Sieghart & Ernst** (2005) *Curr.Med.Chem.Cent.Nerv.Sys. Agents* **5** 217.
108. **Bowery** *et al.* (1987) *Neuroscience* **20** 365.
109. **Hill** (1985) *Br.J.Pharmacol.* **84** 249.
110. **Dunlap and Fishbach** (1981) *J.Physiol.* **317** 519.
111. **Newberry and Nicoll** (1985) *J.Physiol.* **360** 161.
112. **Bowery** *et al.* (2002) *Pharmacol.Rev.* **54** 247.
113. **Ong and Kerr** (1990) *Life Sci.* **46** 1489.
114. **Sakaba and Neher** (2003) *Nature* **424** 775.
115. **Luscher** *et al.* (1997) *Neuron* **19** 687.
116. **Kaupmann** *et al.* (1997) *Nature* **368** 239.
117. **Couve** et (1998) *J.Biol.Chem.* **273** 26361.
118. **Jones** *et al.* (1998) *Nature* **396** 674.
119. **Kaupmann** *et al.* (1998) *Nature* **396** 683.
120. **White** *et al.* (1998) *Nature* **396** 679.
121. **Bettler** *et al.* (2004) *Physiol.Rev.* 84 835.
122. **Pin** *et al.* (2007) *Pharmacol.Rev.* **59** 5.
123. **Steiger** *et al.* (2004) *J.Neurosci.* **24** 6115.
124. **Blein** (2004) *J.Biol.Chem.* **279** 48292.
125. **Kniazeff** *et al.* (2002) *J.Neurosci.* **22** 7352.
126. **Galvez** *et al.* (2000) *Mol.Pharmacol.* **57** 419.
127. **Galvez** *et al.* (2001) *EMBO J.* **20** 2152.
128. **Robbins** *et al.* (2001) *J.Neurosci.* **21** 8043.
129. **Duthey** *et al.* (2002) *J.Biol.Chem.* **277** 3236.
130. **Urwyler** *et al.* (2001) *Mol.Pharmacol.* **60** 963.
131. **Dupuis** *et al.* (2006) *Mol.Pharmacol.* **70** 2027.
132. **Pin** *et al.* (2005) *FEBS J.* **272** 2947.
133. **Ullrich and Bettler** (2007) *Curr.Opin.Neurobiol.* **17** 298.
134. **Schuler** *et al.* (2001) *Neuron* **31** 47.
135. **Gassmann** *et al.* (2004) *J.Neurosci.* **24** 6086.
136. **Vigot** *et al.* (2006) *Neuron* **50** 589.
137. **Perez-Garci** *et al.* (2006) *Neuron* **50** 603.
138. **Waldmeier** *et al.* (2008) *J.Neural Transm.* **115** 1401.
139. **Guetg** *et al.* (2009) *J.Neurosci.* **29** 1414.
140. **Grace** *et al.* (2004) *Proc.Natl.Acad.Sci.USA* **101** 12836.
141. **Tiao** *et al.* (2008) *J.Biol.Chem.* **283** 31005.
142. **Bowery** (2006) *Curr.Opin.Pharmacol.* **6** 37.
143. **Penn** (1989) *N.Engl.J.Med.* **320** 1517.
144. **Ataka** *et al.* (2000) *Pain* **86** 273.
145. **Potes** *et al.* (2006) *J.Neurosci.Res.* **83** 515.
146. **Pan** *et al.* (2008) *Pharmacol.Ther.* **117** 141.
147. **Wu** *et al.* (2007) *Neurobiol.Dis.* **26** 439.
148. **Teichgräber** *et al.* (2009) *Epilepsia* 2009 **50** 1697.
149. **Froestl** *et al.* (2004) *Biochem.Pharmacol.* **68** 1479.
150. **Sunyer** *et al.* (2009) *Hippocampus* **19** 90.
151. **Roberts and Andrews** (1997) *Psychopharmacology* **131** 271.
152. **Smith** *et al.* (2004) *Psychopharmacology* **173** 105.
153. **Slattery** *et al.* (2005) *Neuropsychopharmacology* **30** 2065.
154. **Malherbe** *et al.* (2008) *Br.J.Pharmacol.* **154** 797.
155. **Heinmiller** *et al.* (2009) *Behav.Neurosci.* **123** 145.
156. **Matsuki** *et al.* (2009) *Proc.Natl.Acad.Sci.USA* **106** 4459.
157. **Alstermark** *et al.* (2008) *J.Med.Chem.* **51** 4315.
158. **Zhang** *et al.* (2001) *TiPS* **22** 121.
159. **Bowery and Enna** (2000) *J.Pharmacol.Exp.Ther.* **292** 2.
160. **Murata** *et al.* (1996) *Bioorg.Med.Chem.Lett.* **6** 2071.
161. **Ragozzino** *et al.* (1996) *Mol.Pharmacol.* **50** 1024.
162. **Sieghart** (1995) *Pharmacol.Rev.* **47** 181.

GABA Receptor Compounds Available from Tocris

Cat. No.	Product Name	Primary Action
$GABA_A$ Receptors		
Agonists		
0344	GABA	Endogenous agonist
0235	Isoguvacine hydrochloride	Selective $GABA_A$ agonist
3250	L-838,417	Subtype-selective $GABA_A$ partial agonist
0289	Muscimol	Potent $GABA_A$ agonist
0181	TACA	$GABA_A$ agonist. Also GABA-T substrate and GABA uptake inhibitor
0807	THIP hydrochloride	$GABA_A$ agonist
0180	ZAPA sulfate	Agonist at 'low affinity' $GABA_A$ receptor. More potent than GABA/muscimol
Antagonists		
0130	(+)-Bicuculline	Potent $GABA_A$ antagonist
2503	(−)-Bicuculline methiodide	Water-soluble $GABA_A$ antagonist
0109	(−)-Bicuculline methobromide	Water-soluble $GABA_A$ antagonist
0131	(−)-Bicuculline methochloride	Water-soluble $GABA_A$ antagonist
3109	Furosemide	$GABA_A$ receptor antagonist. Also $Na^+/2Cl^-/K^+$ cotransporter blocker
1128	Picrotoxin	$GABA_A$ receptor antagonist
2143	SCS	Selective $GABA_A$ receptor antagonist; β1-subunit-selective
1262	SR 95531 hydrobromide	Selective, competitive $GABA_A$ receptor antagonist
2905	TB 21007	α5-selective $GABA_A$ inverse agonist
2745	U 93631	$GABA_A$ receptor antagonist
Benzodiazepines		
3568	Bretazenil	Benzodiazepine partial agonist
0405	β-CCB	Benzodiazepine inverse agonist, putative endogenous ligand
2467	CGS 20625	Selective central benzodiazepine receptor partial agonist
0456	Chlormezanone	Skeletal muscle relaxant
1709	CL 218872	Benzodiazepine agonist
2805	Diazepam	Acts at the benzodiazepine modulatory site
0505	Dihydroergotoxine mesylate	Binds to $GABA_A$ receptor Cl^- channel; allosteric modulator of benzodiazepine site
3083	DMCM hydrochloride	Benzodiazepine inverse agonist
0554	FG 7142	Benzodiazepine inverse agonist
0658	FGIN-1-27	Potent, specific ligand for mitochondrial DBI receptor (TSPO)
0659	FGIN-1-43	Potent, specific ligand for mitochondrial DBI receptor
1328	Flumazenil	Benzodiazepine antagonist
0770	GBLD 345	High affinity benzodiazepine agonist
2174	Hispidulin	Partial positive allosteric modulator at the benzodiazepine receptor
1327	L-655,708	Selective for α5-containing $GABA_A$ receptors
3087	Lorazepam	Acts at the benzodiazepine modulatory site
2832	Midazolam hydrochloride	Benzodiazepine agonist
0670	PK 11195	Antagonist at peripheral benzodiazepine receptors
1997	Ro 15-4513	Benzodiazepine partial inverse agonist
1995	Ro 19-4603	Benzodiazepine inverse agonist
3655	Zaleplon	Benzodiazepine agonist
1994	ZK 93423 hydrochloride	Potent benzodiazepine agonist
1996	ZK 93426 hydrochloride	Potent, competitive benzodiazepine antagonist
0655	Zolpidem	Benzodiazepine agonist
1094	Zopiclone	Benzodiazepine agonist
Modulators		
3653	Allopregnanolone	$GABA_A$ receptor positive allosteric modulator
3046	Alphaxalone	Direct activator and potentiator of $GABA_A$
0881	Chlormethiazole hydrochloride	Potentiates $GABA_A$ receptor function
1471	Etomidate	GABA mimetic and GABA modulatory agent
2531	Ganaxolone	Potent, positive allosteric modulator of $GABA_A$ receptors
3597	Indiplon	Subtype-selective $GABA_A$ receptor positive allosteric modulator
1295	Loreclezole hydrochloride	Subtype-selective $GABA_A$ receptor modulator
2738	Org 20599	Positive allosteric modulator and direct agonist of $GABA_A$ receptors
2681	7-PA	Antagonist of neurosteroid potentiation and direct gating of $GABA_A$
3652	Pregnanolone	$GABA_A$ receptor positive allosteric modulator
0830	Primidone	Potentiates $GABA_A$ receptor function
1512	SB 205384	$GABA_A$ receptor modulator
3620	Topiramate	$GABA_A$ receptor positive allosteric modulator. Also GluR5 antagonist
1558	Tracazolate hydrochloride	Subtype-selective $GABA_A$ allosteric modulator
2734	U 89843A	Positive allosteric modulator of $GABA_A$ receptors
2733	U 90042	GABAA receptor ligand
3048	Valerenic acid	Positive allosteric modulator of $GABA_A$ receptors
$GABA_B$ Receptors		
Agonists		
0417	(*RS*)-Baclofen	Selective $GABA_B$ agonist
0796	(*R*)-Baclofen	Active enantiomer of Cat. No. 0417
0344	GABA	Endogenous agonist
0379	SKF 97541	Extremely potent $GABA_B$ agonist

Cat. No.	Product Name	Primary Action
Antagonists		
1245	CGP 35348	Brain penetrant, selective $GABA_B$ antagonist
1247	CGP 46381	Brain penetrant, selective $GABA_B$ antagonist
1246	CGP 52432	Potent, selective $GABA_B$ antagonist
1088	CGP 54626 hydrochloride	Potent, selective $GABA_B$ antagonist
1248	CGP 55845	Potent, selective $GABA_B$ antagonist
0245	2-Hydroxysaclofen	Selective $GABA_B$ antagonist, more potent than Cat. No. 0246
0178	Phaclofen	Selective $GABA_B$ antagonist
0246	Saclofen	Selective $GABA_B$ antagonist
0984	SCH 50911	Selective, competitive, orally active $GABA_B$
Modulators		
3313	rac BHFF	Potent, selective $GABA_B$ positive allosteric modulator
1513	CGP 7930	Positive modulator at $GABA_B$ receptors
1514	CGP 13501	Positive modulator at $GABA_B$ receptors
2001	GS 39783	Positive modulator at $GABA_B$ receptors
$GABA_{A-\rho}$ ($GABA_C$) Receptors		
Agonists		
0344	GABA	Endogenous agonist
0289	Muscimol	Partial $GABA_C$ agonist
0181	TACA	$GABA_C$ agonist
Antagonists		
0379	SKF 97541	$GABA_C$ antagonist. Also potent $GABA_B$ agonist
0807	THIP hydrochloride	$GABA_C$ antagonist
1040	TPMPA	Selective $GABA_C$ antagonist
0180	ZAPA sulfate	$GABA_C$ antagonist
GABA Transporters		
0206	β-Alanine	GABA uptake inhibitor (GAT-2 and -3). Also glycine receptor agonist
1296	CI 966	Selective inhibitor of GAT-1
0234	Guvacine	Specific GABA uptake inhibitor
0236	(±)-Nipecotic acid	GABA uptake inhibitor
2747	NNC 05-2090	GABA uptake inhibitor; moderately BGT-1 selective
1779	NNC 711	Selective inhibitor of GAT-1
0768	Riluzole	GABA uptake inhibitor. Also glutamate release inhibitor
1081	SKF 89976A	Potent GABA uptake inhibitor. Penetrates blood brain barrier
1561	(S)-SNAP 5114	GABA uptake inhibitor
0181	TACA	GABA uptake inhibitor. Also $GABA_A$ agonist and substrate for GABA-T
Miscellaneous GABA		
1814	N-ArachidonylGABA	Inhibits pain *in vivo*
0806	Gabapentin	Anticonvulsant. Increases brain GABA
0538	*trans*-4-Hydroxycrotonic acid	GHB receptor ligand
0386	3-Methyl-GABA	Activator of GABA amino-transferase
1811	Modafinil	Psychostimulant
0780	NCS-382	Antagonist of γ-hydroxybutyric acid
2687	Pentylenetetrazole	CNS stimulant
0939	Propofol	Potentiates $GABA_A$ receptor-mediated inhibition and inhibits glutamate receptor-mediated excitation
3400	RuBi-GABA	Caged GABA; excitable by visible wavelength
2815	Valproic acid, sodium salt	Increases GABA levels; anticonvulsant
0808	Vigabatrin	GABA-T inhibitor
2625	Zonisamide	Anticonvulsant, modulates GABA neurotransmission

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