Journal of Medicinal Chemistry

Volume 43, Number 8 April 20, 2000

Perspective

GABA-Activated Ligand Gated Ion Channels: Medicinal Chemistry and Molecular Biology

Mary Chebib and Graham A. R. Johnston*

Adrien Albert Laboratory of Medicinal Chemistry, Department of Pharmacology, University of Sydney, Sydney, New South Wales 2006, Australia

Received August 24, 1999

1. Introduction

GABA (**1**, 4-aminobutanoic acid, γ-aminobutyric acid) is the major inhibitory neurotransmitter in the brain and is essential for the overall balance between neuronal excitation and inhibition. GABA influences neurons via a large number of receptor subtypes which are grouped on the basis of their pharmacology under three major classes of receptors: $GABA_A$, $GABA_B$, and $GABA_C$ receptors. $GABA_A$ and $GABA_C$ receptors are ligand gated ion channels, while $GABA_B$ receptors are G-protein coupled receptors. This Perspective compares and contrasts aspects of the medicinal chemistry and molecular biology of $GABA_A$ and $GABA_C$ receptors. The molecular diversity of these ligand gated ion channels represents important challenges for medicinal chemists in the design of subunit-specific therapeutic agents.

$GABA_A$ receptors were reviewed in the *Journal of Medicinal Chemistry* by Krogsgaard-Larsen and colleagues[1] in 1994 in a Perspective titled "$GABA_A$ Receptor Agonists, Partial Agonists, and Antagonists: Design and Therapeutic Prospects". There continues to be extensive investigations of $GABA_A$ receptors, particularly with respect to their modulation by important drugs, such as the benzodiazepines, ethanol, general anesthetics, barbiturates, and a wide range of chemically diverse substances. Johnston[2] has described $GABA_A$ receptors as "the most complicated of the superfamily of ligand-gated ion channels in terms of the large number of receptor subtypes and also the variety of ligands that interact with specific sites on the receptors." There are many excellent, extensive reviews on aspects of $GABA_A$ receptors.[1,3−10] $GABA_C$ receptors appear to be much simpler than $GABA_A$ receptors, but they have not been studied anywhere nearly as intensively as $GABA_A$ receptors.[11−16]

1.1. Ligand Gated Ion Channels. Ligand gated ion channels (LGICs) mediate fast synaptic transmission via the movement of ions through channels gated by neurotransmitters such as acetylcholine and GABA. These receptors are very different from G-protein coupled receptors (GPCRs) that were the subject of a recent Perspective in the *Journal of Medicinal Chemistry*.[17] The nicotinicoid superfamily of LGICs encompasses several families of receptors including nicotinic acetylcholine receptors, $5\text{-}HT_3$ receptors, $GABA_A$ receptors, $GABA_C$ receptors, strychnine-sensitive glycine receptors, and some invertebrate anionic glutamate receptors. Other superfamilies of LGICs include the excitatory glutamate receptors (with subfamilies of NMDA, AMPA, and kainate receptors) and the ATP receptors (which currently consist of a single family). From the nicotinicoid superfamily of LGICs, the most studied and best understood of this class of receptors is the nicotinic acetylcholine receptors, while the $GABA_A$ receptors appear to be the most complex and the $GABA_C$ receptors the simplest.[18]

Members of the nicotinicoid superfamily are considered to be pentamers composed of protein subunits likely to have been derived from a common ancestor.[18−20] As illustrated in Figure 1A, each subunit has a large extracellular N-terminal domain which incorporates

* To whom correspondence should be addressed. Tel: 61 2 9351 6117. Fax: 61 2 9351 3868. E-mail: grahamj@mail.usyd.edu.au.

10.1021/jm9904349 CCC: $19.00
Published on Web 03/30/2000

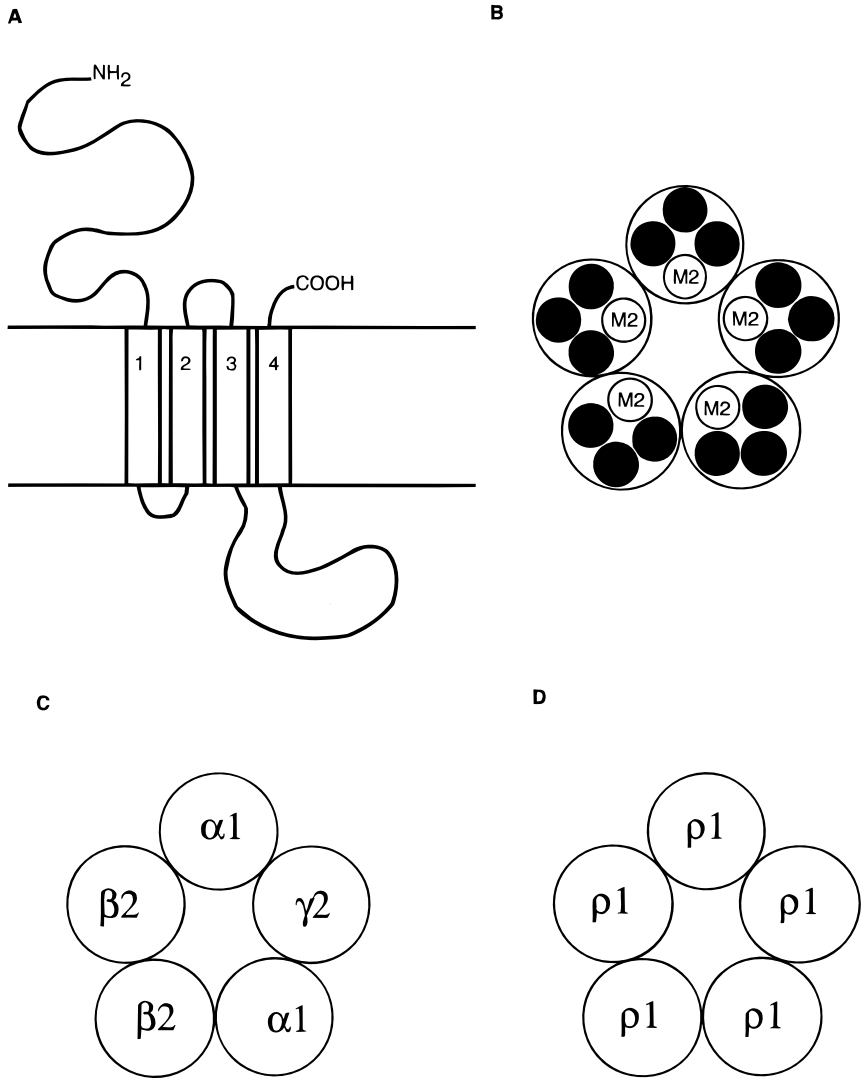


Figure 1. Representations of GABA-activated ligand gated ion channels: A, general structure of one of the five protein subunits showing four membrane spanning regions; B, pentameric arrangement of the protein subunits showing the second membrane spanning region lining the pore of the ion channel; C, heteromeric makeup of a $GABA_A$ receptor with two α1, two β2, and one γ2 protein subunits; and D, homomeric makeup of a $GABA_C$ receptor with five ρ1 protein subunits.

part of the agonist/antagonist-binding site, followed by three membrane spanning domains (M1−3), an intracellular loop of variable length and a fourth membrane spanning domain (M4), and the C-terminal end being extracellular. Each subunit arranges itself such that the second membrane spanning domain (M2) forms the wall of the channel pore (Figure 1B) and the overall charge of the domain determines whether the channel conducts anions or cations. Both $GABA_A$ and $GABA_C$ receptors are GABA-gated chloride ion channels causing inhibition of neuronal firing, with $GABA_A$ receptors being heteromeric, i.e., composed of different subunits (e.g. α1, β2, and γ2 subunits as illustrated in Figure 1C) and $GABA_C$ receptors being homomeric (e.g. composed exclusively of ρ1 subunits, Figure 1D). The cytoplasmic loop (Figure 1A), between the third and fourth transmembrane domains (M3 and M4), is believed to be the target for protein kinases, required for subcellular targeting and membrane clustering of the receptor.

The hierarchy within the nicotinicoid superfamily of LGICs as described by Le Novere and Changeux[18] is as follows: the first rank is the family defined by the endogenous ligand responsible for channel opening; the second rank is the subfamily defined by the ability to form endogenous receptors together; and the last rank is the tribe. In the present context of the GABA-activated LGIC family, $GABA_A$ and $GABA_C$ receptors are subfamilies, while the tribes are the protein subunits that show substantial sequence identity, e.g., the six α subunits, the four β subunits, etc., and the three ρ subunits.

The optimized alignment of the human $GABA_A$ β1 and $GABA_C$ ρ1 amino acid sequences from Le Novere and Changeux[18] shows overall 172 matching amino acids between these proteins equating to an optimized sequence identity of 36%. Similar matching between human $GABA_A$ α1 and human $GABA_C$ ρ1 sequences shows significantly less sequence identity: overall 128 matching amino acids, 28% optimized sequence identity, about 50% identity in the transmembrane spanning domains, and 44% identity in a 71-amino acid sequence of the N-terminal extracellular domain.

1.2. Recombinant Receptors: Complementary Structure−Activity Studies. The ability to express functional receptors composed of proteins of known amino acid sequence in suitable biological cells, e.g., frog

oocytes or human embryonic kidney (HEK) cells, that do not normally express such receptors has revolutionized our understanding of LGICs. Cloning studies have yielded a variety of sequence-related nucleotides that code for a variety of closely related membrane-bound LGIC receptors. When injected into appropriate cells, these cells will express functional LGIC recombinant receptors that can be studied by conventional electrophysiological recording techniques, e.g., two-electrode voltage clamp studies, or by radioligand binding studies. This technology enables structure−activity studies on a series of related compounds to be carried out on clearly defined receptor subtypes.

The native nucleotides coding for these receptors may be subjected to site-directed mutagenesis or cutting and splicing techniques to produce mutants or chimeras. On injection into appropriate cells, these nonnative nucleotides may produce mutant or chimeric receptors that have different functional properties to the native receptors. This technology thus enables systematic variation of the receptors themselves and can be used to delineate which amino acid residues are responsible for the pharmacological and physiological properties of the receptors.

Structure−activity relationship (SAR) studies involving structural variations of both the ligands and their receptor targets (termed complementary structure−activity studies)[21] play important roles in drug development, being vital to the discovery of drugs that interact selectively with particular native and mutant receptor subtypes. Inheritable mutations in the nicotinicoid superfamily of LGICs, including $GABA_A$ receptors, have been identified.[22]

1.3. How GABA Activates LGICs. Ligand binding to LGICs has been generally considered to be diffusion-limited, i.e., fast, with binding affinity being primarily determined by the rate of ligand dissociation. This appears to be the case for nicotinic LGICs where the binding of acetylcholine is almost as fast as that predicted if the rate-limiting step is the diffusion of acetylcholine into its binding site. Two recent studies on GABA-activated LGICs suggest, however, that GABA binds orders of magnitude more slowly to $GABA_A$ and $GABA_C$ receptors than expected for relatively free diffusion.

Jones et al.[23] used patch clamping of rat hippocampal neurons in culture to study what defines ligand affinity at these native $GABA_A$ receptors. Their results indicate that a ligand-specific energy barrier between unbound and bound states determines $GABA_A$ receptor selectivity. They quantitatively model this barrier by requiring the participation of movable elements within a flexible binding site. Their flexible binding-site model envisages the binding site behaving as a pair of mobile 'arms' attached to fixed 'anchor' sites by spring-like tethers. Binding is diffusion-limited only if the ligand is long enough to span the distance between these wells. Shorter agonists bind more slowly because the arms must move to accommodate them, which requires activation energy. The model explicitly requires the ligand to perform thermodynamic work and implies that only nondiffusion-limited ligands can be agonists because otherwise they cause no movement of the receptor. Successful agonist binding involves the coordinated motion of separate parts of the receptor, and variations of a fraction of an angstrom or a single hydrogen bond cause quite large changes in affinity.

Chang and Weiss[24] studied the relationship between agonist binding and channel gating by measuring radioligand binding of GABA agonists to single oocytes expressing recombinant human ρ1 $GABA_C$ receptors and directly correlating the binding kinetics with electrophysiological measurements. They showed that the association rate for GABA is 4 orders of magnitude slower than diffusion, indicating that GABA has restricted access to its binding site. They interpret their results in terms of a minimalist five-state model involving the sequential binding of three agonist molecules to the receptor and a transition to the open channel. Chang and Weiss[24] envisage the channel opening locking the agonist molecules onto the $GABA_C$ receptor. Colquhoun[25] has raised some problems of interpretation with this study. For example, there seems to be about 10 times as many binding sites as the number of receptors inferred from the maximum current. However, Colquhoun[25] is enthusiastic about the overall interpretation that the GABA agonist remains bound while the receptor is active, i.e., while the channel is open.

The $GABA_A$ receptor activation model of Jones et al.[23] involves ligand-induced changes in a dynamic receptor, while the $GABA_C$ receptor activation model of Chang and Weiss[24] involves the receptor changing between at least five different states in the presence of an activating ligand. Each model involves much slower binding of agonists than that allowed by diffusion and consequently much slower dissociation of bound agonists in order to accommodate what can be measured as equilibrium affinity constants. The five different states envisaged by Chang and Weiss[24] possibly equate to low-energy states of a dynamic receptor as envisaged by Jones et al.,[23] although there are significant differences between the channel properties of $GABA_A$ and $GABA_C$ receptors (see section 2.1).

2. Similarities and Differences between the $GABA_A$ and $GABA_C$ LGIC Subfamilies

$GABA_A$ and $GABA_C$ receptors are classified as subfamilies of the GABA LGIC family on the basis of their significant physiological, pharmacological, and molecular biological differences.

The protein subunits that make up the $GABA_A$ and $GABA_C$ LGIC subfamilies show up to 36% optimized sequence identity overall and up to 73% sequence identity in the transmembrane spanning regions.[18] They are clearly derived from common ancestral proteins in the nicotinicoid superfamily.

The two subfamilies can be distinguished on the basis of their different channel properties, the availability of selective agonists, antagonists, and modulators, the different chromosomal localization of the genes coding for the protein subunits of the two subfamilies and the reluctance of these subunits to coexpress in functional receptors, and the different proteins that anchor the protein complexes to the cytoskeleton. A summary of these differences follows (Table 1), some aspects being covered in more detail in later sections.

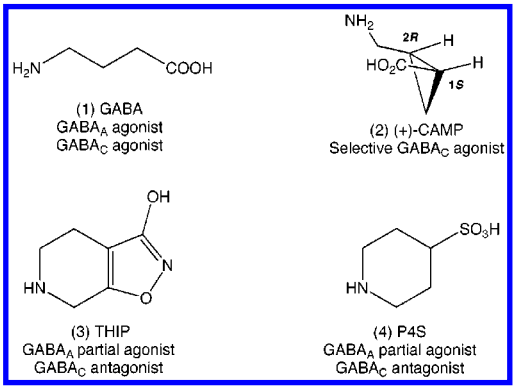
2.1. Channel Properties. The mean channel open time for $GABA_A$ receptors is less than that of $GABA_C$

Table 1. Differences between $GABA_A$ and $GABA_C$ Receptors

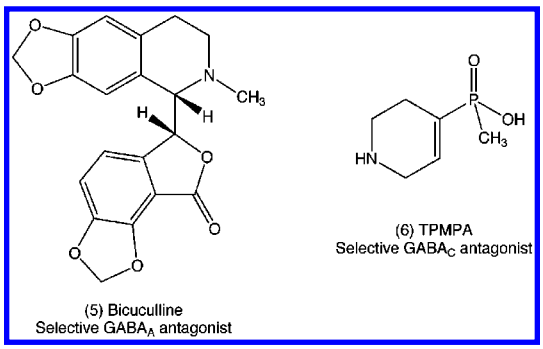
	$GABA_A$ receptors	$GABA_C$ receptors
channel type	Cl^- channel	Cl^- channel
conductance	27−30 pS	7−8 pS
mean channel open time	25−30 ms	150−200 ms
GABA concentrations	10−100 μM	1 μM
channel composition	heterooligomeric	homooligomeric
subunit composition	α1−6, β1−4, γ1−3, δ, ϵ, θ	ρ1−3
chromosomal location of coding genes	chromosomes 1, 4, 5, 15, and X	chromosomes 3 and 6
selective agonist	not known	(+)-CAMP
selective antagonist	bicuculline	TPMPA
potency order of common agonists	muscimol > GABA > TACA	TACA > GABA > muscimol
modulators	benzodiazepines, barbiturates, and steroids	not known
anchoring protein	GABARAP	MAP-1B

receptors. $GABA_A$ receptor gated chloride channels desensitize more readily and are activated and closed more quickly than $GABA_C$ receptor gated chloride channels.[26−28]

2.2. Selective Agonists. (+)-CAMP (**2**, 1*S*,2*R*-2-(aminomethyl)cyclopropanecarboxylic acid) is a selective agonist for $GABA_C$ receptors being inactive at $GABA_A$ receptors.[29] No equivalent selective $GABA_A$ agonist is yet known that does not also have a significant agonist/antagonist action at $GABA_C$ receptors. However, THIP (**3**, 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol) and P4S (**4**, piperidine-4-sulfonic acid**)** are partial agonists at $GABA_A$ receptors and competitive antagonists at $GABA_C$ receptors.[30]



2.3. Selective Antagonists. Bicuculline (**5**) and TPMPA (**6**, (1,2,5,6-tetrahydropyridin-4-ylphosphinic acid) are selective competitive antagonists for $GABA_A$ and $GABA_C$ receptors, respectively. Bicuculline is some 5000 times more potent as an antagonist at $GABA_A$ receptors than at $GABA_C$ receptors,[30] while TPMPA is at least 100 times more potent as an antagonist at $GABA_C$ receptors than at $GABA_A$ receptors.[31] Some phosphinic acid analogues of GABA that are inactive at $GABA_A$ receptors (and agonists at $GABA_B$ receptors) are potent $GABA_C$ antagonists.[30]



2.4. Modulators. $GABA_A$ receptors are modulated by barbiturates, benzodiazepines, and neurosteroids,[2] whereas $GABA_C$ receptors are insensitive to these agents.[12]

2.5. Subunit Expression. The protein subunits that can coexpress to make up recombinant $GABA_A$ receptors do not coexpress with any of the $GABA_C$ protein subunits to form functional heteromeric receptors. $GABA_A$ receptor protein subunits do not form functional homomeric receptors, whereas $GABA_C$ receptor protein subunits do. Distinct $GABA_A$ and $GABA_C$ receptor responses can be observed in the same native neurons[27] and in recombinant expression systems.[30]

2.6. Chromosomal Location of Coding Genes. The genes coding for $GABA_A$ subunit proteins show $\alpha\beta\gamma/\epsilon$ clustering on human chromosomes 1, 4, 5, 15, and X (see section 3.1), whereas the genes coding for $GABA_C$ subunit proteins ρ1 and ρ2 are found on human chromosome 6[32−34] and the gene for ρ3 is on human chromosome 3.[35]

2.7. Anchoring Proteins. Different proteins, $GABA_A$ receptor-associated protein (GABARAP)[36] and microtubule-associated protein 1B (MAP-1B),[37] anchor $GABA_A$ and $GABA_C$ subunits respectively to the cytoskeleton.

2.8. IUPHAR Recommendations Regarding the Nomenclature of Subtypes of $GABA_A$ Receptors. The International Union of Pharmacology (IUPHAR) has formed a Nomenclature Committee to attempt to rationalize the nomenclature of pharmacologically important receptors. Most of the receptor nomenclature reports thus far have been concerned with GPCRs. The IUPHAR report on $GABA_A$ receptors[8] was the first major report on LGICs. It was a task that the committee appeared to take on with some trepidation. They state that the 'combinatorial principle of receptor construction introduces a higher order of complexity. This complexity renders the recognition of the structures of receptor subtypes in their natural setting extremely difficult (in fact, at present, usually unattainable). Thus, it is not possible to construct a classification comparable with the comprehensive scheme for native receptor subtypes obtained in the previous articles of this series.' The IUPHAR committee notes that theirs is "a provisional version" of recommendations and recognizes "its limitations are considerable".

The proposals made by the IUPHAR committee largely address the issue of the wide variety of effects of benzodiazepines on $GABA_A$ receptors. The major criteria for their classification are the pharmacological effects of various benzodiazepines together with the protein subunit composition of recombinant receptors

that exhibit specific responses to benzodiazepines and related drugs. They identify six major types of benzodiazepine responses and thus recommend that the subtypes of the $GABA_A$ receptor should be designated as a series: $GABA_{A1}$, $GABA_{A2}$, $GABA_{A3}$, $GABA_{A4}$, $GABA_{A5}$, and $GABA_{A6}$. They then address the issue that not all $GABA_A$ receptors are influenced by benzodiazepines and propose that benzodiazepine-insensitive $GABA_A$ receptors be known as $GABA_{A0}$ receptors.

The IUPHAR committee noted the sequence similarities between $GABA_A$ receptor subunits and what are currently classified as $GABA_C$ receptor subunits and recommended that the latter be reclassified as benzodiazepine-insensitive $GABA_A$ receptors. Thus what are now widely referred to in the literature as $GABA_C$ receptors would be classified as $GABA_{A0r}$ receptors − 0 for benzodiazepine-insensitivity and r for being comprised of a ρ subunit. The justification for this appears to be solely the sequence similarities between the $GABA_A$ receptor protein subunits and the $GABA_C$ receptor subunits as discussed above in section 2. They note that "selective antagonists have been the most powerful operational tools for discriminating subtypes in other receptor classes"; unfortunately they appear to have overlooked the discovery of TPMPA (**6**) as a selective $GABA_C$ receptor antagonist in 1996.[38]

$GABA_A$ and $GABA_C$ receptors are clearly distinguished on the basis of the selective antagonists bicuculline (**5**) and TPMPA (**6**) for $GABA_A$ and $GABA_C$ receptors, respectively. Furthermore, the IUPHAR classification minimizes the significance of the reluctance of $GABA_C$ receptor subunits to coexpress with $GABA_A$ receptor subunits in functional recombinant receptors, the clear evidence in the retina for functionally distinct native $GABA_A$ and $GABA_C$ receptors on the same neurons, and the substantial physiological and pharmacological differences between $GABA_A$ and $GABA_C$ receptors.

The IUPHAR classification as applied to benzodiazepine-sensitive, bicuculline-sensitive GABA-activated LGICs is a welcome initiative. However, we reject the inclusion of $GABA_C$ receptors in the $GABA_A$ receptor classification as provisionally recommended by Barnard et al.[8] and prefer the classification on $GABA_A$ and $GABA_C$ receptors as distinct subfamilies of the family of GABA-activated LGICs (as used by Darlison and Albrecht,[4] Le Novere and Changeux,[18] and Bormann[39]).

We consider that the principles enunciated by Barnard et al.[8] for the $GABA_A$ receptor subfamily could well be applied to the $GABA_C$ receptor subfamily as TPMPA-sensitive, bicuculline-insensitive GABA-activated LGICs being called $GABA_{Cr1}$, $GABA_{Cr2}$, or $GABA_{Cr3}$ receptors to designate them being composed of ρ1, ρ2, or ρ3 subunits.

3. $GABA_A$ Receptors

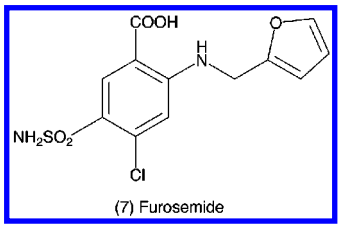
3.1. $GABA_A$ Receptor Subunits. $GABA_A$ receptors are considered heterooligomeric receptors. To date, at least 16 human $GABA_A$ receptor proteins have been described, and these have been classified under five distinct subfamilies of protein subunits termed α, β, γ, δ, and ϵ. There are six α subunits, four β subunits with two splice variants, three γ subunits with two splice variants, one δ subunit, and one ϵ subunit.[2,21,40] Recently a θ subunit has been described.[41] There is approximately 30% sequence identity between the subunit classes and approximately 70% sequence identity between subunit subtypes. If all possible combinations of isoforms containing 2α, 2β, and one other class were represented in native pentameric $GABA_A$ receptors, then more than 2000 different $GABA_A$ receptors could exist in vivo.[2] Barnard et al.[8] suggested a maximum of the order of 800 combinations. It is quite unlikely that these extraordinary numbers of $GABA_A$ receptor subtypes exist in vivo; however, a significant number do exist that can be differentiated pharmacologically as well as by subunit composition.

McKernan and Whiting[42] concluded that the number of major subtypes of $GABA_A$ receptors was probably less than 10, with the $\alpha1\beta2\gamma2$ subtype accounting for 43%, $\alpha2\beta2/3\gamma2$ 18%, and $\alpha3\beta n\gamma2/\gamma3$ 17% on the basis of immunoprecipitation studies, but this has been challenged by Sigel and Kannenberg[43] in that receptor subtypes that are difficult to solubilize, e.g., anchored receptors, are under-represented in immunoprecipitation studies. Barnard et al.[8] discuss the difficulties of a variety of methods for determining $GABA_A$ receptor subtypes in situ, while Darlison and Albrecht[4] address the issue of "$GABA_A$ Receptor Subtypes: Which, Where and Why?" Subunit mutant and receptor gene knockout animals can provide evidence of the importance of particular subunits of $GABA_A$ receptors.[44−46] Clearly, the availability of a range of subunit-specific drugs would greatly aid in our understanding of the relative importance of the various $GABA_A$ receptor subunit proteins and their preferred combinations.

3.2. Structural Diversity of Chemicals Acting on $GABA_A$ Receptors. Johnston[2] proposed that there were at least 11 different structurally specific sites on $GABA_A$ receptors. The 11 sites were (1) agonist sites, which also recognize competitive antagonists; (2) picrotoxinin sites, which also interact with γ-butyrolactones, caprolactams, and some insecticides; (3) sedative−hypnotic barbiturate sites; (4) neuroactive steroid sites; (5) benzodiazepine sites; (6) ethanol sites; (7) stereoselective sites for inhalation anesthetics; (8) furosemide sites; (9) sites for Zn^{2+} ions; (10) sites for other divalent cations; and (11) sites for La^{3+} ions. In addition, 3 other possible sites were noted: (a) phospholipid sites; (b) phosphorylation sites involving specific protein kinase activities; and (c) sites involved in the interaction of $GABA_A$ receptors and microtubules that may anchor receptor clusters at postsynaptic membranes. Furthermore, it was clear that several of the proposed sites, e.g., the benzodiazepine and steroid sites, could be further subdivided.

The structural diversity of chemicals acting on $GABA_A$ receptors suggests that these receptors do indeed have a large number of sites that recognize specific chemical patterns. Some of the sites may be associated with specific protein subunits; e.g., the diuretic furosemide (**7**) appears to act on sites specifically associated with α6 subunits[47] − indeed an important medicinal chemistry target is the discovery of subunit-specific agents. Studies on chimeric α1 and α6 subunits and site-directed mutagenesis of these and β2/3 subunits indicate that particular amino acid residues in transmembrane

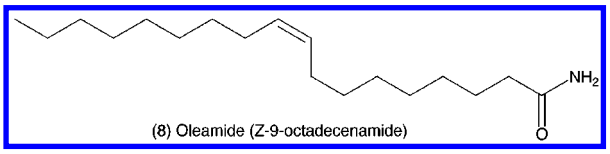
domains 1 and 2 are important in the specificity of the GABA receptor antagonist action of furosemide.[48]



(7) Furosemide

There has been significant recent interest in a number of structural classes of chemicals that influence $GABA_A$ function. These include endogenous substances found in vertebrates, natural products found in herbal preparations, and new chemical entities.

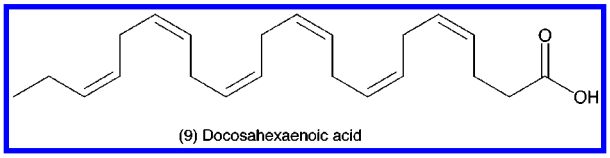
3.2.1. Oleamide and Related Fatty Acid Derivatives. The brain lipid, oleamide (**8**) (*Z*-9-octadecenamide), accumulates in the CSF of sleep-derived cats suggesting that it may be an endogenous sleep-inducing factor. Oleamide enhanced the effects of GABA on rat cultured cortical neurons with an EC_{50} of 15 μM.[49] In studies on recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes, oleamide enhanced the effects of GABA only on benzodiazepine-sensitive receptors containing a γ2 subunit, but its enhancing action was not sensitive to the specific benzodiazepine antagonist flumazenil.[49,50] Oleamide and related compounds are being intensively investigated for use in sleep therapy.



(8) Oleamide (Z-9-octadecenamide)

Oleic acid does not directly influence $GABA_A$ receptor function, nor does the endogenous cannabinoid anandamide (*N*-arachidonylethanolamide).[51]

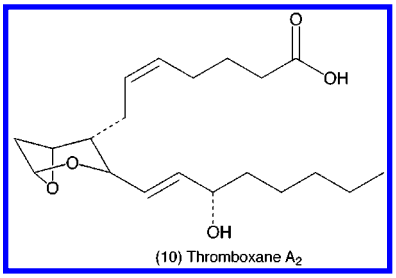
Docosahexaenoic acid (**9**) has complex effects of recombinant $GABA_A$ receptors, accelerating desensitization at 0.1 μM, enhancing peak amplitude at 1 μM, and gradually suppressing peak amplitude at 3 μM. Oleic acid had no effect, while arachidonic acid mimicked the effect of docosahexaenoic acid.[52] 13-L-Hydroxylinoleic acid and a variety of food additives enhanced the action of GABA on recombinant $GABA_A$ receptors.[53]



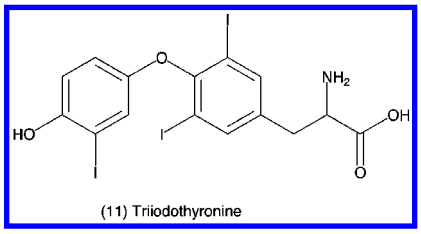
(9) Docosahexaenoic acid

The arachidonic acid metabolite, thromboxane A_2 (**10**), and several analogues inhibit the action of GABA on $GABA_A$ receptors.[54]

3.2.2. Thyroid Hormones. Thyroid hormones antagonize $GABA_A$ receptor function. The most potent is L-triiodothyronine (**11**), a noncompetitive inhibitor with an IC_{50} of 7 μM.[55] Studies on recombinant $GABA_A$ receptors show that the effect requires only α1 and β2 subunits and that it was not blocked by the benzodiazepine antagonist flumazenil. This work suggests a nongenomic action of thyroid hormones, which are known to be released from nerve terminals, on $GABA_A$ receptors. L-Triiodothyronine also had a direct channel-gating action (EC_{50} 23 μM) that was blocked by picrotoxin but insensitive to bicuculline.



(10) Thromboxane A_2



(11) Triiodothyronine

3.2.3. Peptides and Proteins. The peptide hormone, somatostatin-14, and GABA are known to be colocalized in some CNS neurons and to modulate reciprocally each other's release. Studies on TBPS binding to $GABA_A$ receptors indicate that somatostatin interacts with these receptors in the micromolar range,[56] but functional studies on $GABA_A$ receptors have yet to be reported.

Waglerin-1, a 22-amino acid peptide purified from the venom of Wagler's pit viper, modulated the action of GABA on hypothalamic neurons.[57] It enhanced the action of GABA on 78 and suppressed on 44 of 141 neurons examined. The potentiation could be blocked by the benzodiazepine antagonist flumazenil. The suppression was competitive and positively correlated with inhibition of GABA responses by Zn^{2+} ions, suggestive that waglerin-1 is a competitive antagonist of $GABA_A$ receptors that lack γ subunits. This suggests that waglerin has subunit-specific actions on $GABA_A$ receptors, acting as a benzodiazepine-like positive modulator on some receptors and as a competitive antagonist on other receptors.

Amyloid-β protein is the major component of the amyloid deposition that characterizes Alzheimer's disease. Studies on *Apylsia* neurons have shown that amyloid-β fragments 1−40 and 25−35, but not 1−16, inhibit GABA-activated responses, indicating that this activity resides in amino acid residues 17−40 of amyloid-β.[58] Studies on mammalian $GABA_A$ receptors have yet to be reported.

Insulin promotes the rapid translocation of $GABA_A$ receptors from the intracellular compartment to the plasma membrane in transfected HEK cells.[59] This requires the presence of the β2 subunit. In CNS neurons, insulin increases the expression of $GABA_A$ receptors on postsynaptic and dendritic membranes.

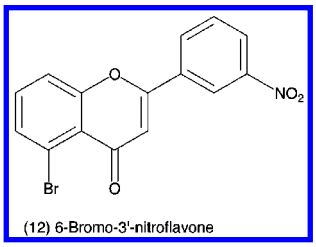
A new cellular protein, GABARAP, that links $GABA_A$ receptors via γ2 subunits to the cytoskeleton has been identified.[36] GABARAP binds to $GABA_A$ receptors in vitro and in vivo. Its highly positive charged N-terminus features a putative tubulin-binding motif suggesting a mechanism for the targeting and clustering of $GABA_A$ receptors at synapses.

The microtubule depolymerizing agent, colchicine, is a competitive antagonist of recombinant $\alpha1\beta2\gamma2L$ $GABA_A$ receptors expressed in *Xenopus* oocytes.[60] Other depolymerizing agents, such as nocodazole and vinblastine, did not influence these $GABA_A$ receptors, and the polymerizing agent, taxol, did not affect colchicine inhibition of GABA responses.

3.2.4. Nitric Oxide and Nitrous Oxide. The diffusible endogenous factor, nitric oxide, directly influences recombinant $GABA_A$ receptor function. It acted as a GABA antagonist on $\alpha1\beta2\gamma2s$ receptors, while on $\alpha1\beta2$ receptors it was inactive at low concentrations and enhanced GABA action at high concentrations.[61] Nitric oxide also has indirect actions on $GABA_A$ receptors acting via protein kinase G and A.[62]

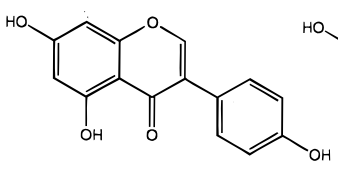
The gaseous anesthetic, nitrous oxide, enhances the action of GABA on $GABA_A$ receptors in a manner similar to other anesthetic agents.[63]

3.2.5. Flavonoids. Neuroactive flavonoids have been described as a new family of benzodiazepine receptor ligands.[64,65] Flavonoids isolated from plants used in traditional medicine as tranquilizers have specific actions on the benzodiazepine sites of $GABA_A$ receptors. Unlike the benzodiazepines, they have anxiolytic effects not associated with myorelaxant, sedative, or amnesic actions. Combinatorial chemistry has yielded synthetic flavonoids such as 6,3′-dinitroflavone and 6-bromo-3′-nitroflavone (**12**) that have high affinity (K_i 1.5−30 nM) for $GABA_A$ receptors.[65] SAR studies on recombinant $GABA_A$ receptors indicate that 6-methylflavone is a benzodiazepine antagonist.[66] Molecular modeling analysis of 3′- and 4′-substituted flavones has allowed further development of a comprehensive pharmacophore model of the interaction of benzodiazepines and related compounds with $GABA_A$ receptors.[67]

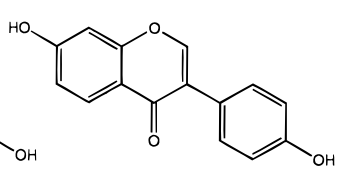


(12) 6-Bromo-3'-nitroflavone

The isoflavones, genistein (**13**) and daidzein (**14**), have been shown to directly inhibit the action of GABA on recombinant $GABA_A$ receptors; this action is independent of the well-known tyrosine kinase inhibitory action of genistein (**13**), since daidzein (**14**) is not a tyrosine kinase inhibitor.[68]

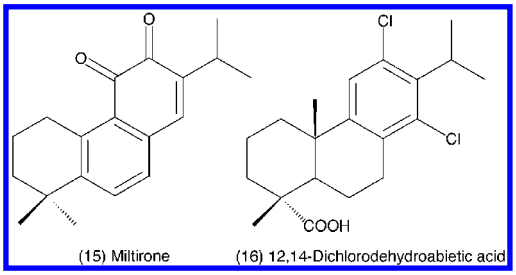


(13) Genistein



(14) Daidzein

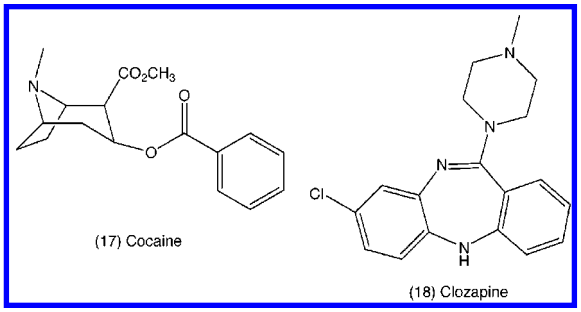
3.2.6. Terpenoids. A range of terpenoids are known to modulate $GABA_A$ receptors. Miltirone (**15**) is the most potent of a series of diterpene quinolones from the Chinese medicinal herb *Salvia miltriorrhiza* that positively modulate $GABA_A$ receptors.[69] Quantitative structure−activity relationship (QSAR) studies have been carried out on 28 picrotoxane terpenoids, including picrodendrin, that noncompetitively block rat brain $GABA_A$ receptors, to reveal significant differences between the ionotropic GABA receptors in rat brain and houseflies.[70] 12,14-Dichlorodehydroabietic acid (**16**), a fish toxin which is produced from the diterpene carboxylic acid dehydroabietic acid in the bleaching of wood pulp, is a potent noncompetitive antagonist of $GABA_A$ receptors.[71]



(15) Miltirone (16) 12,14-Dichlorodehydroabietic acid

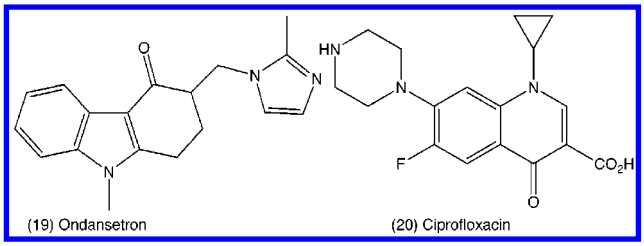
3.2.7. Miscellaneous Therapeutic Agents. A variety of therapeutic agents known for their effects on other receptors also have actions on $GABA_A$ receptors. These actions on $GABA_A$ receptors may contribute to side effects of the therapeutic agents and provide structural leads for the development of more specific agents.

The $GABA_A$ receptor antagonist action (IC_{50} 127 μM) of cocaine (**17**) may contribute to cocaine-induced seizures.[72] Clozapine (**18**) and some other antipsychotic drugs may preferentially block the same subset of $GABA_A$ receptors.[73] The dehydro derivative of the antidepressant, pirlindole, is a $GABA_A$ receptor antagonist (EC_{50} 12 μM).[74]



(17) Cocaine (18) Clozapine

The 5-HT_3 receptor antagonist, ondansetron (**19**), is a noncompetitive antagonist (IC_{50} 10 μM) of $GABA_A$ receptors; this action may be the basis of ondansetron-induced seizures.[75]

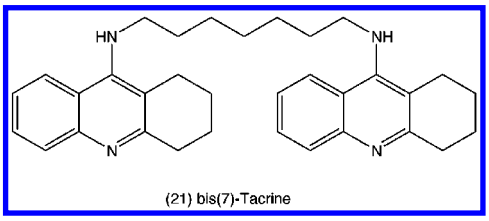


(19) Ondansetron (20) Ciprofloxacin

Quinolone antibacterial agents, such as ciprofloxacin (**20**), that have convulsant side effects are $GABA_A$ receptor antagonists; the increased incidence of convulsions induced by these quinolones administered concomitantly with nonsteroidal antiinflammatories, such

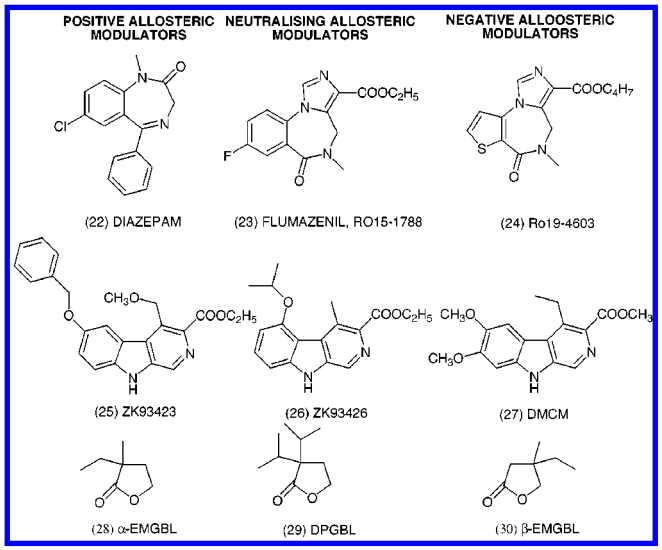
as felbinac, is consistent with such antiflammatories potentiating the $GABA_A$ receptor antagonist action of the quinolones.[76,77]

bis(7)-Tacrine (1,7-*N*,*N*′-heptylenebis(9,9′-amino-1,2,3,4-tetrahydroacridine)) (**21**) is a potential Alzheimer's disease drug on the basis of its superior acetylcholinesterase inhibition and memory-enhancing potency relative to tacrine. It is a competitive $GABA_A$ receptor antagonist (IC_{50} 6 μM), some 18 times more potent than tacrine on these receptors.[78]



(21) bis(7)-Tacrine

3.3. Benzodiazepines and Related Compounds as Allosteric Modulators of a Subset of $GABA_A$ Receptors. The discovery in 1975 that the therapeutic effects of the then most widely prescribed of all drugs, the benzodiazepines, were related to modulation of GABA receptors[79,80] continues to have a profound influence on the study of $GABA_A$ receptors. A range of diverse chemicals have been found to interact with what became known as benzodiazepine receptors, including diazepam (**22**), flumazenil (**23**), some β-carbolines (**25**−**27**), and γ-butyrolactones (**28**−**30**). New concepts about receptor modulation were realized ('inverse agonists', positive and negative modulators) and new generations of drugs developed (e.g. the imidazopyridine, zolpidem (**31**)).



GABA receptors became known as GABA/Bz receptors, and subtypes of these receptors were described (BZ_1 and BZ_2). These terms should no longer be used as we now know that (a) benzodiazepines act on many other receptors unrelated to $GABA_A$ receptors, (b) benzodiazepines interact with only one of several regulatory sites on $GABA_A$ receptors, and (c) $GABA_A$ receptors exist that are insensitive to modulation by benzodiazepines.[8]

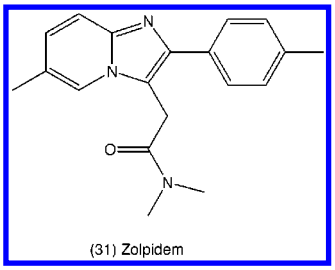
Benzodiazepines have no direct action on mammalian $GABA_A$ receptors. They act to enhance the action of GABA by increasing the frequency of GABA-activated channel openings[81] and by increasing channel conductance.[82] The discovery of agents, such as 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (**27**, DMCM), that decreased the action of GABA on $GABA_A$ receptors led to the concept of benzodiazepine 'inverse agonists'.[83] Interestingly, DMCM (**27**) was initially described as having 'negative efficacy' at benzodiazepine receptors[84] − this is a more accurate terminology than 'inverse agonist' as strictly benzodiazepines are allosteric modulators of $GABA_A$ receptors rather than agonists or 'inverse agonists'. True inverse agonists have been described in constitutively active G-protein coupled receptors, such as β2-adrenoceptors.[85]

Puia et al.[86] introduced the terms 'positive allosteric modulator' and 'negative allosteric modulator' to accurately describe what are commonly but incorrectly described as 'agonists' and 'inverse agonists' when referring to the actions of benzodiazepines on $GABA_A$ receptors. Johnston[2] introduced the term 'neutralizing allosteric modulator' for benzodiazepine 'antagonists', such as flumazenil (**23**, Ro15-1788). The term 'allosteric' is justified on the basis of GABA agonists and competitive antagonists binding to $GABA_A$ receptors at sites different from those that bind benzodiazepines and related modulators. The classification of these modulatory actions should be made on the basis of functional assays in which the concentrations of agonists and modulators are systematically varied. This enables the relative potencies and efficacies of positive and negative allosteric modulators to be established and thus to classify some modulators as, e.g., partial positive allosteric modulators, if their maximal modulation of agonist responses is less than that of other modulators.

The effects of benzodiazepines on $GABA_A$ receptors are complexly dependent on subunit composition as excellently reviewed by Barnard et al.[8] In general, the γ2 subunit is required for the most widely observed effects of benzodiazepines on native $GABA_A$ receptors, consistent with γ2 subunits being much more abundant in brain than γ1 and γ3 subunits. Positive allosteric modulation is reduced when γ2 is replaced by γ1 or γ3 in recombinant $GABA_A$ receptors and negative allosteric modulators become positive allosteric modulators.[86,87] In addition, flumazenil (**23**) changes from a neutralizing allosteric modulator to a positive allosteric modulator when γ2 is replaced by γ1.[88] If γ is replaced by δ or ϵ, the $GABA_A$ receptors are insensitive to benzodiazepines.[89−91]

The importance of the γ2 subunit in $GABA_A$ receptors for benzodiazepine effects is demonstrated by transgenic mice that lack the gene coding for γ2 subunits and whose $GABA_A$ receptors are insensitive to benzodiazepines.[92] A single amino acid on the γ2 subunit has a profound influence on the effects of the benzodiazepines.[93] Mutating threonine 142 to serine changes the action of flumazenil from a neutralizing to a positive allosteric modulator and doubles the potencies of diazepam (**22**), flunitrazepam, and clonazepam as positive allosteric modulators, whereas responses to zolpidem (**31**) and alpidem are halved by this mutation. The agonist effects of GABA and the modulatory effects of barbiturates and neurosteroids were not influenced by this mutation. The nature of the α subunit in $GABA_A$

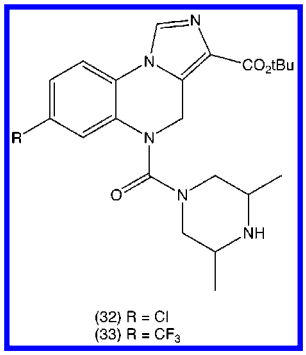
receptors also has a major influence on benzodiazepine action (see Barnard et al.[8]), whereas the β subunit has little influence.[94]



(31) Zolpidem

The development of subunit-specific modulators of $GABA_A$ receptors is an important medicinal chemistry target with the objective of discovering better therapeutic agents that do not have either the unwanted side effects or the full range of therapeutic actions of the traditional benzodiazepines. These side effects include dependence, potentiation of the effects of ethanol, mild ataxia, and mild amnesia. Agents selective for one of the four major therapeutic actions of benzodiazepines, i.e., anxiolytic, sedative−hypnotic, anticonvulsant, and muscle-relaxant actions, are much sought after. The widely prescribed sedative−hypnotic drug, zolpidem (**31**), is an imidazopyridine that has only minor anxiolytic, muscle relaxant, and anticonvulsant properties and may bind selectively to some benzodiazepine sites on particular $GABA_A$ receptor subtypes,[95,96] though its amnesic side effects appear to be similar to those of traditional benzodiazepines.[97]

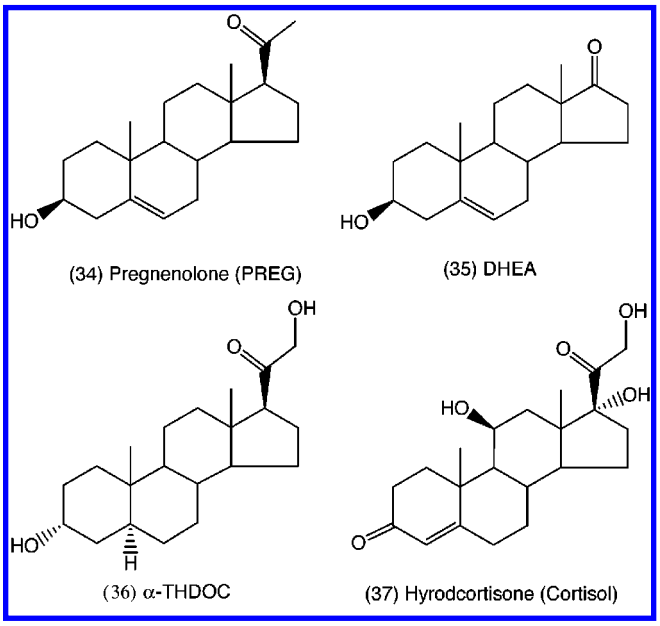
There have been many extensive QSAR studies on benzodiazepines and related compounds using activity data generated from studies on what we now know to be mixed populations of $GABA_A$ receptors.[98−100] Some recent studies have used activity data using recombinant $GABA_A$ receptors of known subunit composition. Thus, Huang et al.[101] measured the binding affinities for a series of imidazobenzodiazepines at five different $GABA_A$ receptors and carried out QSAR studies using comparative molecular field analysis (CoMFA). Jacobsen et al.[102] carried out binding and functional studies on a series of 61 piperazine imidazo[1,5-*a*]quinoxaline ureas on $\alpha1\beta2\gamma2$ recombinant $GABA_A$ receptors to develop two urea-based compounds (**32**, **33**) that were partial positive allosteric modulators with good anxiolytic properties and minimal benzodiazepine-type side effects.



(32) R = Cl
(33) R = CF_3

3.4. Steroid Modulation of $GABA_A$ Receptors. Neuroactive steroids are potent, selective allosteric modulators of $GABA_A$ receptors. These steroids include neurosteroids that are synthesized in the brain and may serve as endogenous anxiolytic and analgesic agents, steroids such as corticosteroids that are produced elsewhere in the body, and a variety of synthetic steroids. Studies on recombinant $GABA_A$ receptors provide evidence for a subunit-selective heterogeneity of steroid sensitivity,[103,104] although steroid modulation appears to be a general property of most $GABA_A$ receptors unlike benzodiazepine modulation.[2] Neuroactive steroids also modulate NMDA receptors[105] and calcium channels associated with G-protein receptors.[106]

Neurosteroids synthesized in the brain and neuroactive steroids imported into the brain from the periphery represent important endogenous agents for influencing brain function by modulating the activation of $GABA_A$ receptors.[107] They include pregnenolone (**34**, PREG), dehydroepiandrosterone (**35**, DHEA), their sulfates, and their reduced metabolites such as 3α,21-dihydroxy-5α-pregnan-20-one (**36**, α-THDOC). The brain levels of α-THDOC (**36**), among the most potent known steroid modulators of $GABA_A$ receptors, increase during stress; this increase is prevented by adrenalectomy indicating that the adrenals are the source of α-THDOC.[108] The adrenal steroids, hydrocortisone (**37**, cortisol) and cortisone, are also potent modulators of $GABA_A$ receptors.[109,110]

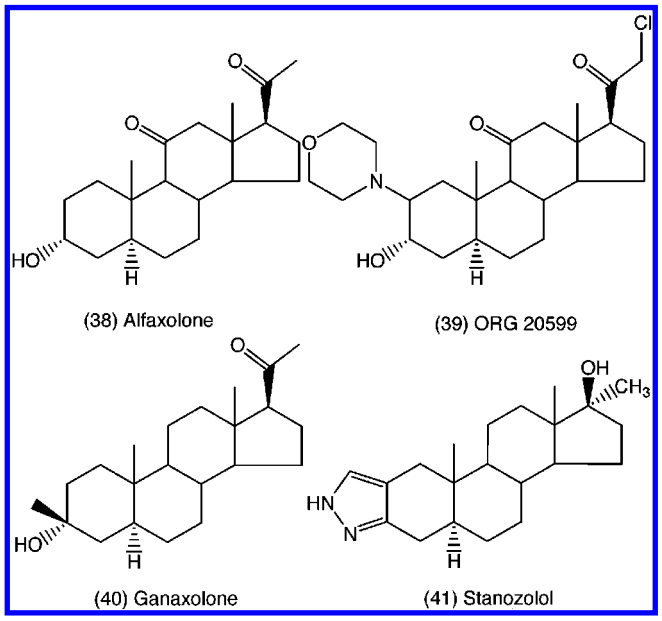


(34) Pregnenolone (PREG)
(35) DHEA
(36) α-THDOC
(37) Hyrodcortisone (Cortisol)

Steroid modulation of $GABA_A$ receptors is enantioselective indicating that steroids act at specific sites on $GABA_A$ receptors rather than via nonenantioselective interactions with membrane lipids.[111] Nilsson et al.,[112] however, found that this was not true for all steroids, observing enantioselectivity for DHEA (**35**) but not for pregnenolone sulfate or 3α,5β-3-hydroxypregnan-20-one sulfate. Some evidence has been produced to indicate that the neuroactive steroids might modulate $GABA_A$ receptors via the picrotoxinin site in the chloride channel,[113] but studies on mutant $GABA_A$ receptors that are insensitive to picrotoxinin show that PREG sulfate and DHEA sulfate do not require a functional picrotoxinin site to modulate $GABA_A$ receptors.[114] Although many of the actions of neuroactive steroids are similar to those of barbiturates on $GABA_A$ receptors, steroids and barbiturates interact with different sites on $GABA_A$ recep-

tors.[115] Neuroactive steroids appear to modulate $GABA_A$ receptors only from the extracellular domain, being inactive on intracellular administration.[116]

Synthetic steroids that modulate $GABA_A$ receptors show promise as therapeutic agents. The steroid anesthetic alfaxolone (**38**, 3α-hydroxy-5α-dihydropregnane-11,20-dione) is no longer used in human medicine but has provided a lead for the development of new steroid derivatives. Thus, CCD-3693, an orally active analogue of pregnanolone, shows promise as a sedative−hypnotic agent;[117] some water-soluble 2β-morpholinyl steroids (e.g. **39**) are intravenous anesthetic agents,[118] and the steroid analogue, ganaxolone (**40**), is an orally active anticonvulsant.[119]



Anabolic steroids used to enhance sporting performance are known to modulate $GABA_A$ receptors. The steroids include DHEA (**35**, an endogenous steroid whose use is permitted in major league baseball but is banned in many other sports) and stanozolol (**41**).[113,120]

Many neuroactive steroids have bidirectional activity on $GABA_A$ receptors, acting as positive allosteric modulators at low concentrations and as negative allosteric modulators at higher concentrations (e.g. PREG and its sulfate[121]). Epipregnanolone (3β-hydroxy-5β-pregnan-20-one) is able to block the positive modulating action of neuroactive steroids suggesting that the bidirectional activity results from interactions with more than one class of modulatory site for steroids on $GABA_A$ receptors.[122,123] This is supported by the opposing effects of some 3α-hydroxy (positive modulation) and 3β-hydroxy (negative modulation) steroids.[124] Steroid partial positive modulators ('partial agonists'), such as 3α,21-dihydroxy-5β-pregnan-20-one, have been noted.[125]

4. $GABA_C$ Receptors

4.1. $GABA_C$ Receptor Subunits. $GABA_C$ receptors are considered to be both homooligomeric and pseudoheterooligomeric receptors comprised of ρ subunits. To date, up to five different ρ subunits have been cloned, including two from human retinal cDNA library (ρ1 and ρ2)[32,126] with two ρ1 splice variants,[127] three from rat (ρ1−3),[128−131] two from chick (ρ1 and ρ2),[132] five from white perch (ρ1−5),[133] and two from mouse (ρ1 and ρ2)[134] retinae. These subunits are often referred to as $GABA_A$ receptor subunits because they show 30−40% sequence identity with other $GABA_A$ receptor subunits.[21] This level of subunit similarity is comparable with $GABA_A$ receptor subunits; however, it is also comparable with other LGIC subunits such as glycine and nicotinic acetylcholine receptor subunits. The ρ subunits have approximately 60−70% sequence identity between each other [32,130,135,136] and have between 85% and 99% sequence identity among species.

4.2. Composition of $GABA_C$ Receptors. ρ Subunits do not coassemble with the classical $GABA_A$ receptor subunits. Coexpression of ρ subunits with the $GABA_A$ receptor subunits α1, β2, and γ2 expressed in *Xenopus* oocytes and with the β subunit of the glycine receptor in HEK 293 cells showed no detectable alterations in whole cell currents.[14] Furthermore, coimmunoprecipitation experiments using human ρ1 and ρ2 subunits with α1, α5, and β1 $GABA_A$ receptor subunits showed no interaction between these subunits in vitro.[137] Thus, ρ subunits do not combine with the classical $GABA_A$ receptor subunits. It has been shown that the N-terminal region of the subunits directs the assembly of either ρ or $GABA_A$ receptor subunits into homooligomeric $GABA_C$ and heterooligomeric $GABA_A$ receptors.[137] Thus, $GABA_C$ receptors are physically and functionally different from $GABA_A$ or glycine receptor subunits.

The human ρ1 and ρ2 subunits form homooligomeric receptors when expressed in oocytes, with different physiological yet similar pharmacological properties. Whole cell currents generated by human ρ2 receptors are an order of magnitude lower than currents generated by ρ1 receptors,[136,138] yet they have a pharmacological profile similar to that of expressed ρ1 receptors. This suggests that the amino acid residues important for most aspects of GABA receptor agonist action may not be among those that differ between ρ1 and ρ2 subunits, including the 20% amino acid difference in the N-terminal region.[135] Chimera constructs between human ρ1 and ρ2 subunits have shown that the N-terminus of the ρ1 subunit contains subunit assembly signals that aid ρ1 subunits to form more efficient homooligomeric receptors than ρ2 subunits.[139,140]

Even though there is high sequence identity between the rat and human ρ2 subunits, the rat ρ2 subunit does not appear to form homooligomeric receptors when expressed in *Xenopus* oocytes. However, it can assemble with the rat ρ1 subunit to form pseudoheterooligomeric receptors in oocytes, with similar pharmacology to native $GABA_C$ receptors in the rat retina.[128] The rat ρ3 subunit forms both homooligomeric[141] as well as pseudoheterooligomeric receptors by assembling with other ρ subunits. The increasing evidence that rat and human ρ1 and ρ2 subunits, as well as rat ρ3 subunits, combine to form pseudoheterooligomeric receptors[128,142] with physical properties distinct from homooligomeric receptors indicates that $GABA_C$ receptors are heterogeneous in vivo.

4.3. Site-Directed Mutagenesis Studies on $GABA_C$ Receptors. Site-directed mutagenesis studies have been used to study the structure−function of ρ1 $GABA_C$ receptors. The β2 $GABA_A$ receptor subunit has been shown to have the highest sequence identity with the

ρ1 $GABA_C$ receptor subunit (approximately 50%). Thus, much of the site-directed mutagenesis studies of ρ1 $GABA_C$ receptors have been directed at comparisons with similar studies on the β2 $GABA_A$ receptor subunit.[28] Five amino acid residues (Y198, Y200, Y241, T244, and Y247), located between the N-terminal extracellular cysteine loop and the first transmembrane spanning domain, were shown to be involved in GABA-mediated activation at homooligomeric ρ1 $GABA_C$ receptors.[28] The amino acid residues were conservatively mutated, and these mutations significantly impaired GABA-mediated activation. These residues were grouped in two domains that corresponded in position to the putative agonist-binding domains previously identified for the β2 subunit of the $\alpha1\beta2\gamma2$ GABA channels. Y198, T244, and Y247 correspond directly with the amino acid residues identified for GABA activation in the β2 subunit, while Y200 and Y241 do not.[28] The latter residues correspond to important residues identified for glycine receptor activation.

A single amino acid residue in the M2 region which corresponds to threonine 314 (T314) has been shown to confer picrotoxinin sensitivity to rat ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes.[128] $GABA_C$ receptors in the rat retina are insensitive to picrotoxinin, while ρ1 $GABA_C$ receptors, expressed in *Xenopus* oocytes, are sensitive to picrotoxinin. However, coexpressing ρ1 and ρ2 subunits in oocytes produces a novel GABA receptor with similar pharmacology to native $GABA_C$ receptors in the rat retina indicating that pseudoheterooligomeric $GABA_C$ receptors may exist in vivo. Mutation of T314 to a methionine on the ρ1 subunit resulted in a receptor that was insensitive to picrotoxinin.

$GABA_A$ receptors containing a β subunit are potentiated by the presence of low concentrations of barbiturate anesthetics, while $GABA_C$ receptors are not. Chimeric constructs using β2 and ρ1 subunits were made to identify the region(s) of the β subunit that confers barbiturate sensitivity, and these were found to be located on the M3 domain. Further studies using site-directed mutagenesis identified a single amino acid residue, tryptophan 328 (W328), which is located on the M3 domain to confer barbiturate sensitivity of the ρ1 subunit when replaced by a hydrophobic residue.[143,144]

4.4. Channel Properties of $GABA_C$ Receptors. $GABA_C$ receptors differ from $GABA_A$ receptors in many significant ways. $GABA_C$ receptors are more sensitive than $GABA_A$ receptors to GABA. In studies using single-channel electrophysiological recordings with outside-out patches from rat retinal bipolar cells, $GABA_C$ receptors conducted less current and were 7 times more sensitive to GABA receptors than $GABA_A$ receptors (at $GABA_C$ receptors, EC_{50} 4 μM; at $GABA_A$ receptors, EC_{50} 27 μM).[26] The mean channel open time for $GABA_C$ receptors in bipolar cells is approximately 5 times longer than for $GABA_A$ receptors.[26] $GABA_C$ receptors have slower activation times. They do not readily desensitize with maintained GABA application and have slower closing times than $GABA_A$ receptors consisting of α1, β2, and γ2 subunits expressed in *Xenopus* oocytes.[28]

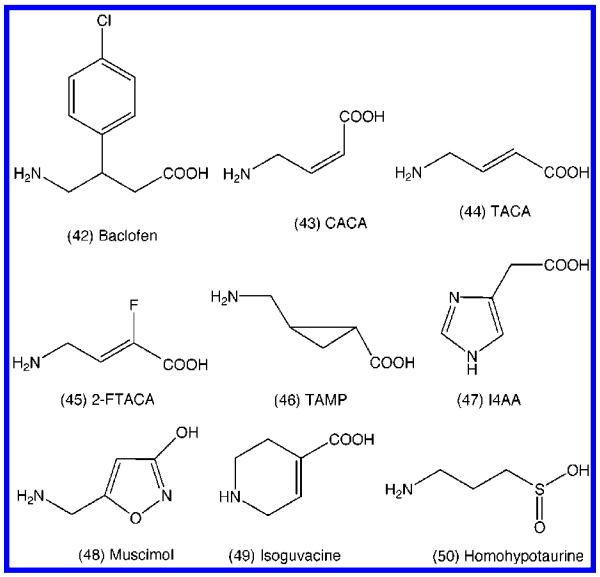
4.5. Distribution of $GABA_C$ Receptor Subunits. The use of reverse transcriptase PCR, Northern blot, and in situ hybridization techniques has shown that rat ρ1 and ρ2 mRNAs are located predominantly in the retina. However, rat ρ2 mRNA is also found in most brain areas.[145−151] The ρ3 mRNA expression pattern is different from the expression pattern of ρ1 and ρ2 mRNAs in rat brain. ρ3 mRNA has lower expression levels in the retina and stronger expression levels in the hippocampus.[150] RT-PCR and in situ hybridization have also been used to determine the expression patterns of ρ1 and ρ2 subunits in the brains of 1-day-old chicks.[132,152] Transcripts were present in the cerebellum, optic tectum, epithalamus, and nucleus pretectalis. Furthermore, mRNA patterns show that the genes expressing ρ1 and ρ2 subunit exist on different populations of cells.[152] RT-PCR showed that human ρ subunits are heterogeneously expressed in the CNS. Whereas the ρ1 subunit is confined mainly to the retina, the ρ2 subunit is present in all brain regions tested, including the cerebellum, thalamus, and temporal and frontal cortices.[142]

Immunohistochemical studies have shown that ρ subunits are localized to rod bipolar cell axons where the GABAergic amacrine cells form synapses in the rat retina.[14,16,145−147,149,153] Clusters of these subunits have been shown to occur on postsynaptic sites. Many other receptor subunits including $GABA_A$ and glycine receptor subunits are also found on rod bipolar cell axons; however, these do not colocalize with ρ subunits.[149] The distribution of $GABA_C$ receptors in the retina and in other parts of the visual pathway indicates that these receptors are involved in the temporal processing of visual information.[154,155]

4.6. Compounds Acting on $GABA_C$ Receptors. The initial studies on GABA receptor classification began when the naturally occurring alkaloid, bicuculline (**5**), and its derivative, bicuculline methochloride, were shown to block the inhibitory actions of GABA in the spinal cord.[156−160] By 1981, it had been shown that baclofen (**42**), a lipophilic analogue of GABA that crosses the blood−brain barrier, had similar agonist effects as GABA in the CNS. However, these effects could not be blocked by bicuculline (**5**). Thus, evidence was emerging that GABA receptors were heterogeneous and that not all GABA receptors were antagonized by bicuculline (**5**). The $GABA_A$/$GABA_B$ classification was developed to distinguish GABA receptors that were (a) inhibited by bicuculline and insensitive to baclofen (**42**) and (b) activated by baclofen (**42**) and insensitive to bicuculline (**5**). These bicuculline-resistant receptors were classified as $GABA_B$ receptors and the original bicuculline-sensitive receptors were termed $GABA_A$ receptors.

At this time, a series of conformationally restricted analogues of GABA, which included CACA (**43**, *cis*-4-aminocrotonic acid) and TACA (**44**, *trans*-4-aminocrotonic acid), were tested for their depressant effects on GABA receptors located on spinal interneurons of the cat under pentobarbitone anesthesia.[160] CACA and TACA were designed to reduce the number of conformations that GABA can attain in order to identify which conformations bind to GABA receptors in the spinal cord. CACA (**43**) is a conformationally restricted analogue of GABA held in a folded conformation, while TACA (**44**) is a conformationally restricted analogue of GABA held in the extended conformation. It was shown that the depressant effect of TACA (**44**) but not CACA (**43**) was inhibited by bicuculline methochloride.[160]

CACA (**43**) continued to depress the firing of spinal interneurons in the presence of bicuculline methochloride. Furthermore, the glycine receptor antagonist, strychnine, did not inhibit the depressant effect of CACA. Thus, CACA had no effect on glycine receptors in the spinal cord.[160] Andrews and Johnston[161] therefore postulated that folded conformations of GABA activated a population of GABA receptors that were insensitive to bicuculline and extended conformations of GABA activated GABA receptors that were sensitive to bicuculline. CACA (**43**) was also shown to depress the firing of Renshaw cells in the cat spinal cord, yet baclofen (**42**) had relatively little effect on these cells. It seemed likely, therefore, that CACA (**43**) and baclofen (**42**) acted at different receptors. In addition to CACA and baclofen, several other conformationally restricted analogues of GABA have been shown to have bicuculline-insensitive depressant actions in the spinal cord. These include (±)-CAMP ((±)-*cis*-2-(aminomethyl)cyclopropanecarboxylic acid),[162] T2ACHC (*trans*-2-aminocyclohexanecarboxylic acid),[163] and T3ACHC (*trans*-3-aminocyclohexanecarboxylic acid).[163]



Both CACA and (±)-CAMP failed to displace the binding of [^{3}H]baclofen to rat cerebellar membranes.[164] On the basis of these results, the existence of a class of bicuculline-insensitive binding sites for GABA that are insensitive to baclofen was proposed, and these sites could be termed $GABA_C$ receptors.[164] For some time, the $GABA_C$ receptor classification was not accepted and the term non-A non-B was used instead to describe bicuculline-insensitive, baclofen-insensitive receptor sites.[165] However, studies of the effects of CACA in a variety of neuronal preparations link CACA to receptors that are activated by GABA and are distinct from the classical $GABA_A$ and $GABA_B$ receptors.[27,30,166,167] Thus, popularity of classifying these receptors as $GABA_C$ receptors has grown.

Expression of poly(A)$^+$ RNA from mammalian retinae injected into *Xenopus* oocytes generated two pharmacologically distinct GABA receptors, consisting of a bicuculline-sensitive component ($GABA_A$ component) and a bicuculline-insensitive and baclofen-insensitive component ($GABA_C$ component).[30] The $GABA_A$ component was blocked by bicuculline, and the remaining current ($GABA_C$ component) was used to determine a structure−activity profile. The most potent $GABA_C$ receptor agonists in this study were found to be TACA (**44**, K_D 0.6 μM) and GABA (K_D 1.7 μM),[30] while CACA (**43**) was shown to be a moderately potent, selective partial agonist.[30]

$GABA_C$ receptors were detected electrophysiologically in rod bipolar cells from rat retina. These receptors were insensitive to bicuculline (**5**) and baclofen (**42**) but were activated by CACA (**43**) after coapplication of GABA (**1**) with 100 μM bicuculline (**5**) to abolish the $GABA_A$ component.[27] It was found that $GABA_C$ receptors were more sensitive to GABA and the channel opened for longer periods with maintained agonist application, compared to $GABA_A$ receptors.[26]

$GABA_C$ receptors were also detected in white perch retina on rod-driven horizontal cells (H4) but not bipolar cells, which were shown to contain $GABA_A$ receptors.[167] Differences between the two receptor types were detected by applying GABA to both types of cells. First, the bipolar cells showed rapid desensitization, while at rod-driven horizontal cells desensitization was not observed.[167] Further studies showed that $GABA_C$ receptors were found on cone-driven but not rod-driven horizontal cells isolated from catfish retinae[168] as well as in bass retinal bipolar cells.[169] GABA receptors on bipolar terminals in tiger salamander retinae were also shown to conduct chloride ions with similar pharmacology to $GABA_C$ receptors.[170,171]

For a long time, CACA (**43**) was believed to be a selective partial agonist for $GABA_C$ receptors. Unlike TACA (**44**), it is neither a substrate for nor an inhibitor of GABA:2-oxoglutarate aminotransferase in extracts of rat brain mitochondria, nor does it influence the activity of glutamate decarboxylase in rat brain extracts.[12] However, uptake studies have shown that CACA (**43**) acts as a weak inhibitor of β-alanine uptake in rat cerebral cortex slices.[172] CACA (**43**) is a weak substrate for a transporter that transports GABA, β-alanine, and nipecotic acid in glial cells isolated from guinea-pig retina.[173] Furthermore, CACA (**43**) stimulates the passive release of [^{3}H]GABA and [^{3}H]β-alanine by heteroexchange from slices of rat cerebellum, cerebral cortex, and spinal cord, without influencing potassium-evoked release.[174] CACA (**43**) is only 10 times weaker as a substrate for the transporter than as a partial agonist at the $GABA_C$ receptors. This transporter may be related to the GAT-3 protein, a GABA transporter located predominantly on glial cells in the CNS.[174]

To date, the most selective agonist at $GABA_C$ receptors is (±)-CAMP, more specifically (+)-CAMP (**2**),[29] while TPMPA (**6**) is the most selective antagonist at these receptors.[31,38]

4.6.1. $GABA_C$ Receptor Agonists and Partial Agonists. SAR studies have been carried out on $GABA_C$ receptors in a variety of systems, which include $GABA_C$ receptors on rod horizontal cells of white perch retina as well as bovine retinal RNA[30] and human homooligomeric ρ1 and ρ2 cRNAs expressed in *Xenopus* oocytes.[135,138,175−177] From these studies, GABA (**1**), TACA (**44**), 2-FTACA (**45**, 2-fluoro-*trans*-4-aminocrotonic acid), and (±)-CAMP were shown to be agonists, while TAMP (**46**, *trans*-2-(aminomethyl)cyclopropanecarboxylic acid), CACA (**43**), imidazole-4-acetic acid (**47**,

I4AA), muscimol (**48**), isoguvacine (**49**), and homohypotaurine (**50**) were shown to be partial agonists at $GABA_C$ receptors. The order of agonist/partial agonist potency at $GABA_C$ receptors can be summarized as follows: TACA > GABA > muscimol ≈ 2-FTACA > homohypotaurine > I4AA > TAMP ≫ (±)-CAMP ≈ CACA > isoguvacine. At $GABA_A$ receptors, the order of potency is muscimol ≫ GABA ≈ TACA > isoguvacine ≫> CACA. From these SAR studies, it appears that the partially folded conformation of GABA that activates $GABA_C$ receptors is similar to the conformation which TACA, CACA, and (±)-CAMP can attain. However, a more extended conformation of GABA is required to activate $GABA_A$ receptors, and this conformation is inaccessible to CACA.

CACA (**43**), which was the first compound to distinguish $GABA_C$ receptors from $GABA_A$ receptors, is a moderate partial agonist at human ρ1 and ρ2 receptors expressed in *Xenopus* oocytes (EC_{50}(ρ1) 74 μM; EC_{50}-(ρ2) 70 μM) and shows 70−80% of the efficacy of GABA.[135,175] The potency and efficacy of CACA at these receptors is similar to those found in $GABA_C$ receptors derived from bovine retinal RNA expressed in *Xenopus* oocytes[30] but not to those found in white perch retinae using patch clamp electrophysiology. These results indicate that $GABA_C$ receptors in the retina may consist of ρ subunits and are heterogeneous. Other differences were also observed with the pharmacology of native $GABA_C$ receptors in white perch retina compared to those expressed in oocytes. Isoguvacine (**49**) is an agonist in the perch retina, while in *Xenopus* oocytes it is a partial agonist; I4AA (**47**) is a pure antagonist in the retina but a partial agonist in oocytes; THIP (**3**) is less potent as an antagonist in the retina than in oocytes; and muscimol (**48**) has a lower efficacy in the retina than in oocytes. The differences in the pharmacology observed in the white perch retina and in oocytes may be attributed to differences in species, receptor heterogeneity, or phosphorylation states that may influence pharmacological actions.

Muscimol (**48**) is an analogue of GABA where the carboxylic acid moiety of GABA is replaced by an isoxazole ring system. It has two low-energy conformations, one in an extended and one in a partially folded conformation. Muscimol (**48**) is widely used as a pharmacological tool to study $GABA_A$ receptors. However, muscimol is a potent partial agonist, albeit weaker than GABA, at both human ρ1 (K_D 2.3 μM) and ρ2 (K_D 1.4 μM) $GABA_C$ receptors expressed in oocytes,[135,175] bovine retinal RNA expressed in oocytes,[30] and rod horizontal cells of white perch retina.[167] Muscimol is more potent at $GABA_C$ than $GABA_A$ receptors expressed in oocytes.[30,135,175] Therefore, one requires caution when using muscimol (**48**) to study $GABA_A$ receptors.

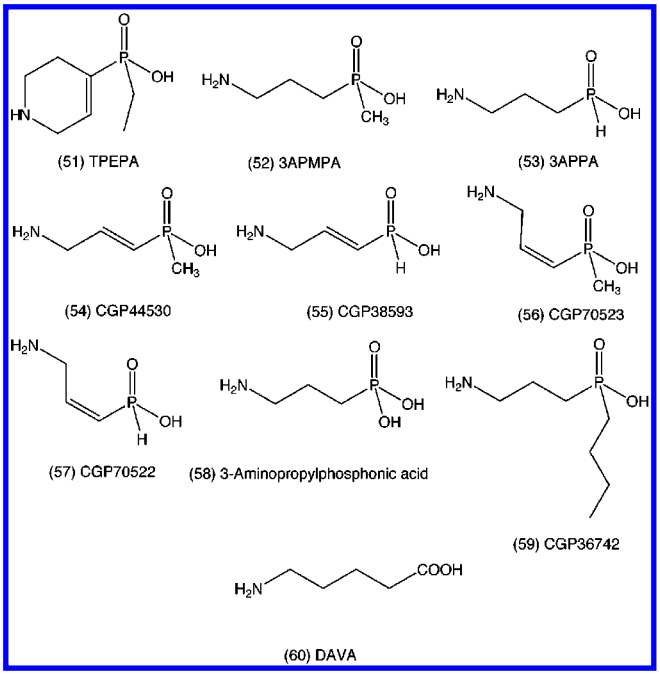
The sulfinic acid analogue of GABA, homohypotaurine (**50**), is a potent partial agonist at ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes (EC_{50} 4.6 μM), albeit weaker than GABA, activating 69% of the maximal response produced by GABA.[177] Thus, the sulfinic acid group appears to reduce the ability of homohypotaurine to activate the channel of ρ1 $GABA_C$ receptors in oocytes.

TACA (**44**) is approximately 120 times more potent than CACA (**43**) at homooligomeric ρ1 and ρ2 $GABA_C$ receptors expressed in *Xenopus* oocytes. It has a high efficacy, activating 95% of the maximal response produced by GABA, and is the most potent agonist at $GABA_C$ receptors expressed in oocytes (EC_{50}(ρ1) 0.6 μM; EC_{50}(ρ2) 0.4 μM[135,175]). Halogeno and methyl substituents on the C2, C3, and C4 positions of TACA produced a marked reduction in both the efficacy and affinity of the compound for ρ1 $GABA_C$ receptors expressed in oocytes.[177] Out of the C2, C3, and C4 positions, only the C2 position of TACA tolerated any form of substitution. 2-FTACA (**45**), substituted with a fluoro group at the C2 position, is a potent agonist (EC_{50} 2.43 μM), albeit weaker than the parent compound, TACA (EC_{50} 0.44 μM), at human ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes. It has a high efficacy, activating 90% of the maximal response produced by GABA.[177] Other substituents including a methyl group at the C2 position markedly reduced both the affinity and activity of the compound for this receptor. Substituents on the C2 position of GABA, including chloro, methyl, and methylene substituents, also reduced the affinity and activity for human ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes. These results indicate that larger substituents on the C2 position may interact unfavorably with the receptor protein when undergoing receptor activation.[177]

TAMP (**46**) is a moderately potent partial agonist with low efficacy at ρ1 and ρ2 $GABA_C$ receptors expressed in *Xenopus* oocytes (EC_{50}(ρ1) 20 μM; EC_{50}(ρ2) 17.5 μM[135,175]). TAMP activates ρ1 and ρ2 $GABA_C$ receptors by 25% and 43% of the maximal response produced by GABA, respectively.[135,175] In contrast to TAMP, (±)-CAMP is a moderately potent full agonist at ρ1 and ρ2 $GABA_C$ receptors (EC_{50}(ρ1) 68.0 μM; EC_{50}(ρ2) 34.5 μM[135,175]). CAMP has no effect in inhibiting [^{3}H]GABA uptake in cortical slices and thus may be the most selective agonist at $GABA_C$ receptors.[162]

4.6.2. Competitive $GABA_C$ Receptor Antagonists. TPMPA (**6**) and TPEPA (**51**, 1,2,5,6-tetrahydropyridin-4-ylethylphosphinic acid) are the most selective $GABA_C$ receptor antagonists to date. These compounds were developed from the observation that isoguvacine, a conformationally restricted analogue of TACA held in a partially folded conformation, has activity at both $GABA_A$ and $GABA_C$ receptors but no activity at $GABA_B$ receptors. Thus, the tetrahydropyridine ring distinguishes $GABA_B$ from $GABA_C$ receptors but does not distinguish $GABA_A$ from $GABA_C$ receptors. As discussed previously, the methylphosphinic acid group can distinguish $GABA_C$ from $GABA_A$ receptors. Thus by incorporating a tetrahydropyridine ring system with either a methylphosphinic or ethylphosphinic moiety, potent and selective antagonists at $GABA_C$ receptor were developed.[31,38] TPMPA is twice as potent as TPEPA, which further establishes that an increase in the alkyl group on the alkylphosphinic acid reduces $GABA_C$ receptor affinity. TPMPA is more that 100 times more selective in blocking ρ1 $GABA_C$ receptors than in blocking $GABA_A$ receptors from rat cerebral cortex poly-(A^+) RNA expressed in *Xenopus* oocytes.[31] At $GABA_B$ receptors, TPMPA is a weak agonist when measured by whole-cell patch recordings from pyramidal neurons in rat hippocampal slices.[31] TPMPA is more than 500 times more selective in blocking ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes than in activating $GABA_B$

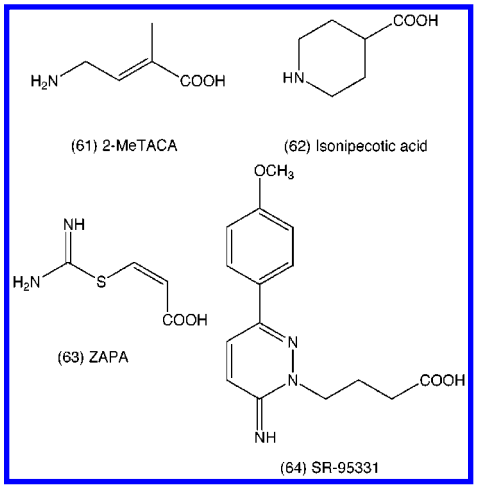
receptors.[31] Furthermore, TPMPA was found to be 8 times weaker at ρ2 than at ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes.[138] TPMPA has been shown to block responses in the neonatal rat spinal cord providing evidence for the involvement of $GABA_C$ receptors in synaptic transmission in this tissue.[178]



From SAR studies, many $GABA_C$ receptor antagonists have been identified. These include a variety of phosphinic, methylphosphinic, and phosphonic acids (**52**−**59**), DAVA (**60**, δ-aminovaleric acid, K_B 20 μM), 2-MeTACA (**61**, 2-methyl-*trans*-4-aminocrotonic acid, K_B 45 μM), P4S (**4**, piperidine-4-sulfonic acid, K_B 81 μM), 3-aminopropylphosphonic acid (**58**, K_B 10 μM), isonipecotic acid (**62**, K_B >100 μM), and THIP (**3**, K_B 32 μM). Other compounds such as strychnine (K_B 69 μM), ZAPA (**63**, *Z*-3-[(aminoiminomethyl)thio]prop-2-enoic acid, K_B 19 μM), and SR-95331 (**64**, gabazine, 2-(3-carboxypropyl)-3-amino-6-(*p*-methoxyphenyl)pyridazinium bromide, K_B 35 μM) are also moderately potent $GABA_C$ receptor antagonists at bovine retinal poly(A)$^+$ RNA expressed in *Xenopus* oocytes.[30] The order of potency for competitive antagonists at $GABA_C$ receptors is as follows: methylphosphinic acids ≥ phosphinic acids > 3-APA > ZAPA ≈ DAVA > THIP ≈ SR-95331 > 2-MeTACA > strychnine > P4S > isonipecotic acid.

P4S (**4**) is the sulfonic acid analogue of isoguvacine. It was first developed as a $GABA_A$ receptor ligand and appears to have similar potency as GABA in activating $GABA_A$ receptors from rat cortex RNA expressed in *Xenopus* oocytes (K_D 104 μM).[30] However, this compound is a partial agonist, activating rat brain $GABA_A$ receptors expressed in *Xenopus* oocytes by <30% of the currents produced by GABA.[30] P4S produced no response on its own but was shown to be a moderately potent antagonist at $GABA_C$ receptors from retinal RNA expressed in oocytes.[30]

Phosphonic acid analogues of GABA, such as 3-aminopropylphosphonic acid (**58**), differentiate between $GABA_A$ and $GABA_C$ receptors. 3-Aminopropylphosphonic acid is a moderately potent inhibitor of $GABA_C$



receptors. This compound has no clear action as an agonist or antagonist at $GABA_A$ receptors from rat cortex RNA expressed in *Xenopus* oocytes.[30] However, 3-aminopropylphosphonic acid is not selective for $GABA_C$ receptors. It has weak depressant effects on the CNS that is insensitive to bicuculline, and it acts as a partial agonist at peripheral $GABA_B$ receptors. Like 3-aminopropylphosphonic acid, 4-aminobutylphosphonic acid had little effect on $GABA_A$ receptors. It is a weak inhibitor of $GABA_B$ receptors but only has weak inhibitory effects on $GABA_C$ receptors. 2-Aminoethylphosphonic acid is largely inactive at $GABA_C$ receptors.[30]

To date, the most potent classes of $GABA_C$ receptor antagonists are the methylphosphinic acid analogues, followed by the phosphinic acid analogues, of GABA. The most potent $GABA_C$ antagonist is 3APMPA (**52**, (3-aminopropyl)methylphosphinic acid). The phosphinic acid analogue of GABA, 3APPA (**53**, 3-aminopropylphosphinic acid), is approximately 3 times weaker than 3APMPA ((3-aminopropyl)methylphosphinic acid). Thus, the methyl group may either bind to a small hydrophobic pocket on the binding site and/or alter the pK_A to improve binding. Similar results were observed with the methylphosphinic and phosphinic acid analogues of TACA, CGP44530 (**54**, (*E*-3-aminopropen-1-yl)methylphosphinic acid) and CGP38593 (**55**, *E*-3-aminopropen-1-ylphosphinic acid) respectively, and the methylphosphinic and phosphinic acid analogues of CACA, CGP70523 (**56**, (*Z*-3-aminopropen-1-yl)methylphosphinic acid) and CGP70522 (**57**, *Z*-3-aminopropen-1-ylphosphinic acid) respectively.[176] The methylphosphinic acids CGP44530 (**54**) and CGP70523 (**56**) were more potent antagonists than the corresponding phosphinic acid analogues CGP38593 (**55**) and CGP70522 (**57**). However, the unsaturated methylphosphinic and phosphinic acid analogues were weaker than the corresponding saturated analogues.[176]

3APMPA (**52**), 3APPA (**53**), CGP44530 (**54**), CGP38593 (**55**), and CGP70523 (**56**) are not selective $GABA_C$ receptor antagonists as they all activate $GABA_B$ receptors. However, the methylphosphinic and phosphinic acid moieties dramatically reduce affinity for $GABA_A$ receptors. Thus, the methylphosphinic and phosphinic acid substituents are able to differentiate $GABA_A$ from $GABA_C$ receptors but not from $GABA_B$ receptors. This could be attributed to the different pK_A activity between the carboxylic and phosphinic acids, as well as the orientation which the different acid groups can attain.

The phosphinic acid moiety is tetrahedral, while the carboxylic acid moiety is planar. The relative lack of selectivity of the methylphosphinic and phosphinic acid analogues of TACA and particularly of CACA is unexpected but may be explained by the higher degree of rotational freedom between the C2 and C3 bonds and between the phosphorus and C1 bonds. Thus, these compounds are more likely to attain conformations that bind to both $GABA_B$ and $GABA_C$ receptors.[176]

An increase in the size of the alkyl group on the alkylphosphinic acid moiety reduces the affinity at $GABA_C$ receptors. CGP36742 (**59**, (3-aminopropyl)butylphosphinic acid) is a moderately potent antagonist, while CGP35348, the diethoxymethyl analogue of GABA, has no effect at $GABA_C$ receptors.[176]

4.6.3. Noncompetitive $GABA_C$ Receptor Antagonists. Picrotoxinin and the related chloride channel inhibitor, *t*-butylbicyclophosphorothionate (TBPS), blocked homooligomeric ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes. However, these compounds were 30 and 250 times weaker at $GABA_C$ than $GABA_A$ receptors expressed in *Xenopus* oocytes.[26] The picrotoxinin sensitivity of $GABA_C$ receptors derived from ρ1 and ρ2 subunits has been found to be dependent on agonist concentration, as picrotoxinin may be a use-dependent antagonist interacting with open chloride channels. Furthermore, studies have shown that a single amino acid residue in the M2 region of the ρ2 subunit confers picrotoxinin sensitivity.[128,179,180] Coexpression of ρ1 and ρ2 subunits in *Xenopus* oocytes gives rise to functional receptors with picrotoxinin insensitivity[128] similar to native $GABA_C$ receptors in rat retina.[26] Thus pseudoheteromeric as well as homomeric $GABA_C$ receptors may exist in vivo.

4.6.4. $GABA_C$ Receptor Modulators. $GABA_C$ receptors are not modulated by benzodiazepines, steroids, or barbiturates, and in fact little is known about $GABA_C$ receptor modulators. $GABA_C$ receptors in rat bipolar cells have been shown to be modulated by protein kinase C.[181] Furthermore, low micromolar concentrations of glycine have also been shown to modulate the effect of a submaximal concentration of GABA at ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes.[182] However, at millimolar concentrations, glycine directly activates these receptors. Glycine modulation of GABAergic synapses may play an important role in synaptic transmission.[182]

4.7. Differences between $GABA_C$ Receptor Subtypes. Some SAR differences between human ρ1 and ρ2 receptor subtypes have been noted. These include the intrinsic activity (calculated as a percentage of the maximum whole cell current produced by a maximum dose of GABA) of the partial agonist, I4AA (**47**), being 7-fold higher at ρ2 than ρ1 receptors.[135] I4AA is an analogue of GABA where the amino group of GABA has been replaced by an imidazole ring. Due to a resonance charge distribution between the two nitrogens of the imidazole ring system, there is a weakening of the charge at the basic end of the molecule. I4AA is a moderately potent partial agonist (EC_{50} 16 μM), with low intrinsic activity (I_m 3%) at ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes.[175] However, it is a more potent antagonist at this receptor (K_B 1.5 μM), with an affinity similar to that of GABA at ρ1 $GABA_C$ receptors.[138] I4AA was shown to activate ρ2 $GABA_C$ receptors expressed in *Xenopus* oocytes. It is a potent partial agonist (EC_{50} 3 μM) with a higher intrinsic activity for the ρ2 (I_m 38%) than the ρ1 $GABA_C$ receptors.[135,138,175]

2-MeTACA (**61**) is a moderately potent inhibitor of ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes (K_B 45 μM). In contrast, 2-MeTACA (**61**) activates the human ρ2 $GABA_C$ receptor subtype (EC_{50} 101 μM) by 34% of the maximal response produced by GABA.[138] Although receptor binding was less favorable at the ρ2 compared to the ρ1 $GABA_C$ receptors, steric interaction between the C2 substituent and the receptor protein may play an important role in activation. Thus, 2-MeTACA (**61**) can pharmacologically distinguish between the human ρ1 and ρ2 homooligomeric $GABA_C$ receptor subtypes.[138]

5. Future Directions

While great progress has been made in our understanding of the medicinal chemistry and molecular biology of GABA-activated ligand gated ion channels over the past decade, this has been achieved without significant input from combinatorial chemistry, high-throughput screening, or structure-based drug design. We can expect important advances from these key strategies in the future, with structural leads coming from the side effects of other drugs and from herbal extracts. In addition, genomics will reveal links between specific GABA receptor gene products and neurological disorders, which will provide specific targets for drug development via complementary SAR studies.

While some classic combinatorial chemistry was carried out on benzodiazepines, this did not lead to better modulators of GABA-activated ligand gated ion channels but rather to novel agents that interact with other classes of receptors, e.g., cholecystokinin receptors.[183] The only reported use of combinatorial chemistry for the development of new GABA receptor agents has been the generation of small libraries of flavones by solution-phase combinatorial chemistry that have yielded novel flavone derivatives that modulate GABA receptors.[184] The great chemical diversity of agents acting on GABA-activated ligand gated ion channels described in this Perspective should provide significant opportunities for the application of combinatorial chemistry to the discovery of new agents with selective actions on the various subtypes of these receptors.

The high-throughput screening of agents acting on ligand gated ion channel receptors has proved difficult. Limited success has been achieved using binding assays, but functional assays are needed to differentiate between agonists, partial agonists, antagonists, and modulators of these receptors. High-throughput screening functional assays have been described for ligand gated cation channel receptors such as nicotinic acetylcholine receptors[185] and the NMDA subtypes of ionotropic glutamate receptors,[186] but no high-throughput functional assays have been described thus far for ligand gated anion channel receptors such as ionotropic GABA receptors. The development of such assays is highly desirable.

An important strategic question for high-throughput functional assays for GABA-activated ligand gated ion channels is what protein subunit combinations should

be used in the primary screens? Given that the discovery of subunit-specific GABA receptor ligands is a major goal, it may be that particular target receptor subtypes will have to be selected on the basis of, for example, association with a specific genetic disorder. To achieve the desired specificity, only compounds that have no or very little interaction with the ubiquitous subtypes of GABA receptors will be of interest. This means that new structural classes of test compounds will need to be developed, e.g., by screening diverse combinatorial libraries, by following structural leads from the side effects of other drugs such as the apparent selectivity of the diuretic furosemide (**7**) for particular amino acids in α6 GABA receptor subunits,[48] and from herbal extracts. Many herbal extracts have been reported to influence the activity of GABA-activated ligand gated ion channels: e.g., extracts of Ginkgo biloba[187] and ginseng.[188]

Structure-based drug design depends on the availability of good structural data for the target receptor. High-resolution structural information on ligand-gated ion channels is rather sparse, but the determination of the crystal structure of a ligand-binding region of an ionotropic glutamate receptor in a complex with the agonist kainate[189] suggests that similar information will be soon forthcoming for GABA-activated ligand gated ion channels. Molecular modeling of receptor sites is another approach which has been applied to nicotinic acetylcholine receptors[190] and to GABA-activated ligand gated ion channels,[191] while methods are being developed for predicting the location of ligand-binding sites on protein surfaces.[192] The relatively simple homomeric $GABA_C$ receptors would appear to be excellent targets for high-resolution structural data by crystal studies and molecular modeling.

Pharmacogenomics is regarded as one of the 'hottest' fields in biotechnology today.[193] Genetic polymorphisms in receptors and other drug targets are being linked to individual differences in drug efficacy, metabolism, and side effects.[194] The era of 'one drug fits all patients' is seen as about to give way to individualized therapy matching a patient's unique genetic makeup with an optimally effective drug.[193] High-throughput analysis of differential gene expression, via 'DNA chips' and other technology, is now a reality.[195] While mutations of receptors in disease states appear to be relatively uncommon, inheritable mutations $GABA_A$ receptors have been identified.[22] Disruption of the gene coding for the β3 $GABA_A$ receptor subunit has been associated with Angelman syndrome, a neurodevelopmental disorder characterized by mental retardation and epilepsy.[196] Knockout mice that lack this gene exhibit electroencephalograph abnormalities, seizures, and behavioral characteristics typically associated with Angelman syndrome.[196] Rett syndrome, an X-linked dominant neurodevelopmental disorder, may involve the gene coding for the α3 $GABA_A$ receptor subunit,[197] while a number of studies have linked polymorphisms in the genes coding for the α6, β1, β2, and γ2 $GABA_A$ receptor subunits with alcohol dependence syndrome and Korsakoff's psychosis.[198,199] The genes coding for ρ1 and ρ2 $GABA_C$ receptor subunits are located in a region of chromosome 6q that contains loci for inherited disorders of the eye.[35] These studies indicate that genetic disorders of GABA-activated ligand gated ion channels may represent key therapeutic targets and that the development of drugs acting on abnormal GABA receptor gene products is an important endeavor. Site-directed mutagenesis studies of recombinant GABA receptors show that changing even a single amino acid residue can have major effects on drug sensitivity. For example, changing T328 to a hydrophobic amino acid confers barbiturate sensitivity on the ρ1 $GABA_C$ subunit.[143,144] Thus, meaningful pharmacogenomics in relation to drugs acting on GABA-activated ligand gated ion channels is a distinct possibility, with complementary SAR studies[21] involving structural variations of both the ligands and their receptor targets playing a vital role.

6. Conclusion

This Perspective highlights the remarkable structural diversity of natural and synthetic chemicals that interact with $GABA_A$ receptors to alter their function in the brain. While medicinal chemistry studies on $GABA_C$ receptors are just beginning, it is already clear that $GABA_C$ receptors are very different from $GABA_A$ receptors in terms of agonist and antagonist structural profiles. Both subfamilies of GABA-activated ligand-gated ion channels are targets for drug development, with major advances likely to result from the interactive application of combinatorial chemistry, high-throughput screening, structure-based drug design, and genomics.

Acknowledgment. The authors are grateful to their many colleagues who have contributed to their studies including Robin Allan, Peter Burden, Gary Cutting, Rujee Duke, Wolfgang Froestl, Jane Hanrahan, Jeremy Greenwood, Povl Krogsgaard-Larsen, Ken Mewett, Dorte Petersen, Ron Quinn, Hue Tran, George Uhl, Robert Vandenberg, and Paul Whiting and to the Australian National Health and Medical Research Council and Polychip Pharmaceuticals for financial support.

Biographies

Mary Chebib received a Ph.D. degree in organic chemistry in 1994 from Griffith University under the supervision of Ronald Quinn. She is currently a Senior Research Officer funded by the National Health and Medical Research Council of Australia in the Adrien Albert Laboratory of Medicinal Chemistry, University of Sydney.

Graham A. R. Johnston received a Ph.D. degree in organic chemistry in 1963 from the University of Cambridge under the supervision of Lord Todd. Following a postdoctoral year at the University of California, Berkeley with Melvin Calvin, he spent 15 years at the John Curtin School of Medical Research, Australian National University. In 1980, he took up the Chair of Pharmacology at the University of Sydney and founded what is now the Adrien Albert Laboratory of Medicinal Chemistry.

References

(1) Krogsgaard-Larsen, P.; Frølund, B.; Jørgensen, F. S.; Schousboe, A. $GABA_A$ receptor agonists, partial agonists, and antagonists. Design and therapeutic prospects. *J. Med. Chem.* **1994**, *37*, 2489−2505.
(2) Johnston, G. A. R. $GABA_A$ receptor pharmacology. *Pharmacol. Ther.* **1996**, *69*, 173−198.
(3) Sieghart, W. Structure and pharmacology of γ-aminobutyric $acid_A$ receptor subtypes. *Pharmacol. Rev.* **1995**, *47*, 181−234.
(4) Darlison, M. G.; Albrecht, B. E. $GABA_A$ receptor subtypes: which, where and why? *Sem. Neurosci.* **1995**, *7*, 115−126.
(5) Rabow, L. E.; Russek, S. J.; Farb, D. H. From ion currents to genomic analysis − recent advances in $GABA_A$ receptor research. *Synapse* **1995**, *21*, 189−274.

(6) Krogsgaard-Larsen, P.; Frølund, B.; Ebert, B. GABA-A receptor agonists, partial agonists, and antagonists. In *The GABA Receptors*, 2nd ed.; Enna, S. J., Bowery, N. G., Eds.; Humana: Totowa, NJ, 1997; pp 37−81.

(7) Korpi, E. R.; Mattila, M. J.; Wisden, W.; Luddens, H. $GABA_A$ receptor subtypes − clinical efficacy and selectivity of benzodiazepine site ligands. *Ann. Med.* **1997**, *29*, 275−282.

(8) Barnard, E. A.; Skolnick, P.; Olsen, R. W.; Möhler, H.; Sieghart, W.; Biggio, G.; Braestrup, C.; Bateson, A. N.; Langer, S. Z. International Union of Pharmacology − XV − Subtypes of γ-aminobutyric $acid_A$ receptors − classification on the basis of subunit structure and receptor function. *Pharmacol. Rev.* **1998**, *50*, 291−313.

(9) Enna, S. J.; Bowery, N. G. *The GABA Receptors*, 2nd ed.; Humana Press: Totowa, NJ, 1997; p 332.

(10) Mehta, A. K.; Ticku, M. K. An update on $GABA_A$ receptors. *Brain Res. Rev.* **1999**, *29*, 196−217.

(11) Bormann, J.; Feigenspan, A. $GABA_C$ receptors. *Trends Neurosci.* **1995**, *18*, 515−519.

(12) Johnston, G. A. R. $GABA_C$ receptors: relatively simple transmitter-gated ion channels? *Trends Pharmacol. Sci.* **1996**, *17*, 319−323.

(13) Johnston, G. A. R. Molecular biology, pharmacology and physiology of $GABA_C$ receptors. In *The GABA Receptors*, 2nd ed.; Enna, S. J., Bowery, N. G., Eds.; Humana: Totowa, NJ, 1997; pp 297−323.

(14) Enz, R.; Cutting, G. R. Molecular composition of $GABA_C$ receptors. *Vision Res.* **1998**, *38*, 1431−1441.

(15) Feigenspan, A.; Bormann, J. GABA-gated Cl^- channels in the rat retina. *Prog. Ret. Eye Res.* **1998**, *17*, 99−126.

(16) Wassle, H.; Koulen, P.; Brandstatter, J. H.; Fletcher, E. L.; Becker, C. M. Glycine and GABA receptors in the mammalian retina. *Vision Res.* **1998**, *38*, 1411−1430.

(17) Bikker, J. A.; Trumpp-Kallmeyer, S.; Humblet, C. G-Protein Coupled Receptors: Models, Mutagenesis, and Drug Design. *J. Med. Chem.* **1998**, *41*, 2911−2927.

(18) Le Novere, N.; Changeux, J. P. The ligand gated ion channel database. *Nucl. Acids Res.* **1999**, *27*, 340−342.

(19) Le Novere, N.; Changeux, J. P. Molecular evolution of the nicotinic acetylcholine receptor: an example of multigene family in excitable cells. *J. Mol. Evol.* **1995**, *40*, 155−172.

(20) Ortells, M. O.; Lunt, G. G. Evolutionary history of the ligand-gated ion-channel superfamily of receptors. *Trends Neurosci.* **1995**, *18*, 121−127.

(21) Johnston, G. A. R.; Chebib, M.; Duke, R. K.; Mewett, K. N.; Mitrovic, A. D.; Vandenberg, R. J. Medicinal chemistry and molecular pharmacology of GABA receptors and glutamate transporters − complementary structure−activity relationships. *Drug Dev. Res.* **1999**, *46*, 255−260.

(22) Vafa, B.; Schofield, P. R. Heritable mutations in the glycine, $GABA_A$, and nicotinic acetylcholine receptors provide new insights into the ligand-gated ion channel superfamily. *Int. Rev. Neurobiol.* **1998**, *42*, 285−332.

(23) Jones, M. V.; Sahara, Y.; Dzubay, J. A.; Westbrook, G. L. Defining affinity with the $GABA_A$ receptor. *J. Neurosci.* **1998**, *18*, 8590−8604.

(24) Chang, Y.; Weiss, D. S. Channel opening locks agonist onto the $GABA_C$ receptor. *Nature Neurosci.* **1999**, *2*, 219−225.

(25) Colquhoun, D. GABA and the single oocytes: relating binding to gating. *Nature Neurosci.* **1999**, *2*, 201−202.

(26) Feigenspan, A.; Bormann, J. Differential pharmacology of $GABA_A$ and $GABA_C$ receptors on rat retinal bipolar cells. *Eur. J. Pharmacol.* **1994**, *288*, 97−104.

(27) Feigenspan, A.; Wassle, H.; Bormann, J. Pharmacology of GABA receptor Cl^- channels in rat retinal bipolar cells. *Nature* **1993**, *361*, 159−162.

(28) Amin, J.; Weiss, D. S. Homomeric rho 1 GABA channels: activation properties and domains. *Recept. Chann.* **1994**, *2*, 227−36.

(29) Duke, R. K.; Allan, R. D.; Chebib, M.; Greenwood, J. R.; Johnston, G. A. R. Resolution and conformational analysis of diastereoisomeric esters of cis- and trans-2-(aminomethyl)-1-carboxycyclopropanes. *Tetrahedron Asymm.* **1998**, *9*, 2533−2548 and unpublished observations.

(30) Woodward, R. M.; Polenzani, L.; Miledi, R. Characterization of bicuculline/baclofen-insensitive (rho-like) gamma-aminobutyric acid receptors expressed in *Xenopus* oocytes. II. Pharmacology of gamma-aminobutyric $acid_A$ and gamma-aminobutyric $acid_B$ receptor agonists and antagonists. *Mol. Pharmacol.* **1993**, *43*, 609−625.

(31) Ragozzino, D.; Woodward, R. M.; Murata, Y.; Eusebi, F.; Overman, L. E.; Miledi, R. Design and in vitro pharmacology of a selective γ-aminobutyric $acid_C$ receptor antagonist. *Mol. Pharmacol.* **1996**, *50*, 1024−1030.

(32) Cutting, G. R.; Curristin, S.; Zoghbi, H.; O'Hara, B.; Seldin, M. F.; Uhl, G. R. Identification of a putative gamma-aminobutyric acid (GABA) receptor subunit rho2 cDNA and colocalization of the genes encoding rho2 (GABRR2) and rho1 (GABRR1) to human chromosome 6q14-q21 and mouse chromosome 4. *Genomics* **1992**, *12*, 801−806.

(33) McLean, P. J.; Farb, D.; Russek, S. J. Mapping of the α4 subunit gene (GABARA4) to human chromosome 4 defines an α2-α4-β1-γ1 gene cluster: Further evidence that modern $GABA_A$ receptor gene clusters are derived from an ancestral gene cluster. *Genomics* **1995**, *26*, 580−586.

(34) Russek, S. J. Evolution of GABA(A) receptor diversity in the human genome. *Gene* **1999**, *227*, 213−222.

(35) Bailey, M.; Albrecht, B. E.; Johnson, K. J.; Darlison, M. G. Genetic linkage and radiation hybrid mapping of the three human GABA(C) receptor rho subunit genes: GABRR1, GABRR2 and GABRR3. *Biochim. Biophys. Acta, Gene Struct. Express.* **1999**, *1447*, 307−312.

(36) Wang, H. B.; Bedford, F. K.; Brandon, N. J.; Moss, S. J.; Olsen, R. W. $GABA_A$-receptor-associated protein links $GABA_A$ receptors and the cytoskeleton. *Nature* **1999**, *397*, 69−72.

(37) Hanley, J. G.; Koulen, P.; Bedford, F.; Gordon-Weeks, P. R.; Moss, S. J. The protein MAP-1B links $GABA_C$ receptors to the cytoskeleton at retinal synapses. *Nature* **1999**, *397*, 66−69.

(38) Murata, Y.; Woodward, R. M.; Miledi, R.; Overman, L. E. The first selective antagonist for a $GABA_C$ receptor. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2073−2076.

(39) Bormann, J. The 'ABC' of GABA receptors. *Trends Pharmacol. Sci.* **2000**, *21*, 16−19.

(40) Davies, P. A.; Hanna, M. C.; Hales, T. G.; Kirkness, E. F. Insensitivity to anaesthetic agents conferred by a class of $GABA_A$ receptor subunit. *Nature* **1997**, *385*, 820−823.

(41) Bonnert, T. P.; McKernan, R. M.; Farrar, S.; le, B. B.; Heavens, R. P.; Smith, D. W.; Hewson, L.; Rigby, M. R.; Sirinathsinghji, D.; Brown, N.; Wafford, K. A.; Whiting, P. J. theta, a novel gamma-aminobutyric acid type A receptor subunit. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9891−9896.

(42) McKernan, R. M.; Whiting, P. J. Which $GABA_A$-receptor subtypes really occur in the brain? *Trends Neurosci.* **1996**, *19*, 139−143.

(43) Sigel, E.; Kannenberg, K. $GABA_A$-receptor subtypes. *Trends Neurosci.* **1996**, *19*, 386.

(44) Homanics, G. E.; Ferguson, C.; Quinlan, J. J.; Daggett, J.; Snyder, K.; Lagenaur, C.; Mi, Z. P.; Wang, X. H.; Grayson, D. R.; Firestone, L. L. Gene knockout of the α6 subunit of the γ-aminobutyric acid type A receptor − lack of effect on responses to ethanol, pentobarbital, and general anesthetics. *Mol. Pharmacol.* **1997**, *51*, 588−596.

(45) Homanics, G. E.; Delorey, T. M.; Firestone, L. L.; Quinlan, J. J.; Handforth, A.; Harrison, N. L.; Krasowski, M. D.; Rick, C.; Korpi, E. R.; Makela, R.; Brilliant, M. H.; Hagiwara, N.; Ferguson, C.; Snyder, K.; Olsen, R. W. Mice devoid of γ-aminobutyrate type A receptor β3 subunit have epilepsy, cleft palate, and hypersensitive behavior. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4143−4148.

(46) Möhler, H.; Luscher, B.; Fritschy, J. M.; Benke, D.; Benson, J.; Rudolph, U. $GABA_A$-receptor assembly in vivo − lessons from subunit mutant mice. *Life Sci.* **1998**, *62*, 1611−1615.

(47) Korpi, E. R.; Luddens, H. Furosemide interactions with brain $GABA_A$ receptors. *Br. J. Pharmacol.* **1997**, *120*, 741−748.

(48) Thompson, S. A.; Arden, S. A.; Marshall, G.; Wingrove, P. B.; Whiting, P. J.; Wafford, K. A. Residues in transmembrane domains I and II determine gamma-aminobutyric acid type A(A) receptor subtype-selective antagonism by furosemide. *Mol. Pharmacol.* **1999**, *55*, 993−999.

(49) Lees, G.; Edwards, M. D.; Hassoni, A. A.; Ganellin, C. R.; Galanakis, D. Modulation of $GABA_A$ receptors and inhibitory synaptic currents by the endogenous CNS sleep regulator *cis*-9,10-octadecenoamide (COA). *Br. J. Pharmacol.* **1998**, *124*, 873−882.

(50) Yost, C. S.; Hampson, A. J.; Leonoudakis, D.; Koblin, D. D.; Bornheim, L. M.; Gray, A. T. Oleamide potentiates benzodiazepine-sensitive γ-aminobutyric acid receptor activity but does not alter minimum alveolar anesthetic concentration. *Anesthesia Analgesia* **1998**, *86*, 1294−1300.

(51) Kimura, T.; Ohta, T.; Watanabe, K.; Yoshimura, H.; Yamamoto, I. Anandamide, an endogenous cannabinoid receptor ligand, also interacts with 5-hydroxytryptamine (5-HT) receptors. *Biol. Pharm. Bull.* **1998**, *21*, 224−226.

(52) Nabekura, J.; Noguchi, K.; Witt, M. R.; Nielsen, M.; Akaike, N. Functional modulation of human recombinant γ-aminobutyric acid type a receptor by docosahexaenoic acid. *J. Biol. Chem.* **1998**, *273*, 11056−11061.

(53) Aoshima, H.; Tenpaku, Y. Modulation of GABA receptors expressed in Xenopus oocytes by 13-l-hydoxylinoleic acid and food additives. *Biosci. Biochem. Technol.* **1997**, *61*, 2051−2057.

(54) Schwartzbloom, R. D.; Cook, T. A.; Yu, X. Inhibition of GABA-gated chloride channels in brain by the arachidonic acid metabolite, thromboxane A_2. *Neuropharmacology* **1996**, *35*, 1347−1353.
(55) Chapell, R.; Martin, J.; Machu, T. K.; Leidenheimer, N. J. Direct channel-gating and modulatory effects of triiodothyronine on recombinant $GABA_A$ receptors. *Eur. J. Pharmacol.* **1998**, *349*, 115−121.
(56) Vincens, M.; Mauvaisjarvis, F.; Behar, S. A novel recognition site for somatostatin-14 on the $GABA_A$ receptor complex. *Eur. J. Pharmacol.* **1998**, *344*, R1−R2.
(57) Ye, J. H.; McArdle, J. J. Waglerin-1 modulates γ-aminobutyric acid activated current of murine hypothalamic neurons. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 74−80.
(58) Sawada, M.; Ichinose, M. Amyloid beta proteins reduce the GABA-induced Cl^- current in identified *Aplysia* neurons. *Neurosci. Lett.* **1996**, *213*, 213−215.
(59) Wan, Q.; Xiong, Z. G.; Man, Y. H.; Ackerley, C. A.; Braunton, J.; Lu, W. Y.; Becker, L. E.; Macdonald, J. F.; Wang, Y. T. Recruitment of functional $GABA_A$ receptors to postsynaptic domains by insulin. *Nature* **1997**, *388*, 686−690.
(60) Bueno, O. F.; Leidenheimer, N. J. Colchicine inhibits $GABA_A$ receptors independently of microtubule depolymerization. *Neuropharmacology* **1998**, *37*, 383−390.
(61) Fukami, S.; Uchida, I.; Mashimo, T.; Takenoshita, M.; Yoshiya, I. Gamma subunit dependent modulation by nitric oxide (NO) in recombinant $GABA_A$ receptor. *NeuroReport* **1998**, *9*, 1089−1092.
(62) Wexler, E. M.; Stanton, P. K.; Nawy, S. Nitric oxide depresses $GABA_A$ receptor function via coactivation of cGMP-dependent kinase and phosphodiesterase. *J. Neurosci.* **1998**, *18*, 2342−2349.
(63) Dzoljic, M.; Vanduijn, B. Nitrous oxide-induced enhancement of γ-aminobutyric $acid_A$-mediated chloride currents in acutely dissociated hippocampal neurons. *Anesthesiology* **1998**, *88*, 473−480.
(64) Medina, J. H.; Viola, H.; Wolfman, C.; Marder, M.; Wasowski, C.; Calvo, D.; Paladini, A. C. Overview − flavonoids − a new family of benzodiazepine receptor ligands. *Neurochem. Res.* **1997**, *22*, 419−425.
(65) Medina, J. H.; Viola, H.; Wolfman, C.; Marder, M.; Wasowski, C.; Calvo, D.; Paladini, A. C. Neuroactive flavonoids − new ligands for the benzodiazepine receptors. *Phytomedicine* **1998**, *5*, 235−243.
(66) Ai, J. L.; Dekermendjian, K.; Wang, X. M.; Nielsen, M.; Witt, M. R. 6-Methylflavone, a benzodiazepine receptor ligand with antagonistic properties on rat brain and human recombinant $GABA_A$ receptors in vitro. *Drug Dev. Res.* **1997**, *41*, 99−106.
(67) Dekermendjian, K.; Kahnberg, P.; Witt, M. R.; Sterner, O.; Nielsen, M.; Liljefors, T. Structure−activity relationships and molecular modeling analysis of flavonoids binding to the benzodiazepine site of the rat brain GABA(A) receptor complex. *J. Med. Chem.* **1999**, *42*, 4343−4350.
(68) Huang, R. Q.; Fang, M. J.; Dillon, G. H. The tyrosine kinase inhibitor genistein directly inhibits GABA(A) receptors. *Mol. Brain Res.* **1999**, *67*, 177−183.
(69) Lee, C. M.; Wong, H. N.; Chui, K. Y.; Choang, T. F.; Hon, P. M.; Chang, H. M. Miltirone, a central benzodiazepine receptor partial agonist from a Chinese medicinal herb *Salvia miltiorrhiza*. *Neurosci. Lett.* **1991**, *127*, 237−241.
(70) Ozoe, Y.; Akamatsu, M.; Higata, T.; Ikeda, I.; Mochida, K.; Koike, K.; Ohmoto, T.; Nikaido, T. Picrodendrin and related terpenoid antagonists reveal structural differences between ionotropic GABA receptors of mammals and insects. *Bioorg. Med. Chem.* **1998**, *6*, 481−492.
(71) Nicholson, R. A.; Lees, G.; Zheng, J.; Verdon, B. Inhibition of GABA-gated chloride channels by 12,14-dichlorodehydroabietic acid in mammalian brain. *Br. J. Pharmacol.* **1999**, *126*, 1123−1132.
(72) Ye, J. H.; Liu, P. L.; Wu, W. H.; McArdle, J. J. Cocaine depresses $GABA_A$ current of hippocampal neurons. *Brain Res.* **1997**, *770*, 169−175.
(73) Squires, R. F.; Saederup, E. Clozapine and several other antipsychotic/antidepressant drugs preferentially block the same core fraction of $GABA_A$ receptors. *Neurochem. Res.* **1998**, *23*, 1283−1290.
(74) Medvedev, A. E.; Shvedov, V. I.; Chulkova, T. M.; Fedotova, O. A.; Saederup, E.; Squires, R. F. The influence of the antidepressant pirlindole and its dehydroderivative on the activity of monoamine oxidase A and $GABA_A$ receptor binding. *J. Neur. Transm., Suppl.* **1998**, *52*, 337−342.
(75) Ye, J. H.; Hunt, T.; Wu, W. H.; McArdle, J. J. Ondansetron modulates $GABA_A$ current of rat central nervous system neurons. *Eur. J. Pharmacol.* **1997**, *337*, 87−94.
(76) Green, M. A.; Halliwell, R. F. Selective antagonism of the $GABA_A$ receptor by ciprofloxacin and biphenylacetic acid. *Br. J. Pharmacol.* **1997**, *122*, 584−590.
(77) Kawakami, J.; Yamamoto, K.; Asanuma, A.; Yanagisawa, K.; Sawada, Y.; Iga, T. Inhibitory effect of new quinolones on $GABA_A$ receptor-mediated response and its potentiation with felbinac in *Xenopus* oocytes injected with mouse-brain mRNA − correlation with convulsive potency in vivo. *Toxicol. Appl. Pharmacol.* **1997**, *145*, 246−254.
(78) Li, C. Y.; Wang, H.; Xue, H.; Carlier, P. R.; Hui, K. M.; Pang, Y. P.; Li, Z. W.; Han, Y. F. Bis(7)-tacrine, a novel dimeric AChE inhibitor, is a potent $GABA_A$ receptor antagonist. *NeuroReport* **1999**, *10*, 795−800.
(79) Costa, E.; Guidotti, A.; Mao, C. C. Evidence for the involvement of GABA in the action of benzodiazepines. *Adv. Biochem. Psychopharmacol.* **1975**, *14*, 113−130.
(80) Haefely, W.; Kucsar, A.; Mohler, H.; Lieri, L.; Polc, P.; Schaffner, R. Possible involvement of GABA in the central actions of benzodiazepines. *Adv. Biochem. Psychopharmacol.* **1975**, *14*, 131−151.
(81) Rogers, C. J.; Twyman, R. E.; Macdonald, R. L. Benzodiazepine and β-carboline regulation of single GABA(A) receptor channels of mouse spinal neurones in culture. *J. Physiol.* **1994**, *475*, 69−82.
(82) Eghbali, M.; Curmi, J. P.; Birnir, B.; Gage, P. W. Hippocampal $GABA_A$ channel conductance increased by diazepam. *Nature* **1997**, *388*, 71−75.
(83) Petersen, E. N.; Jensen, L. H.; Honoré, T.; Braestrup, C. Differential pharmacological effects of benzodiazepine receptor inverse agonists. *Adv. Biochem. Psychopharmacol.* **1983**, *38*, 57−64.
(84) Braestrup, C.; Nielsen, M.; Honoré, T. Benzodiazepine receptor ligands with positive and negative efficacy. *Adv. Biochem. Psychopharmacol.* **1983**, *37*, 237−245.
(85) Bond, R.; Leff, P.; Johnson, T. D.; Milano, C. A.; Rockman, H. A.; McMinn, T. R.; Apparsundaram, S.; Hyek, M. F.; Kenakin, T. P.; Allen, L. F.; Lefkovitz, R. J. Physiological effects of inverse agonists in transgenic mice with myocardial overexpression of the β_2-adrenoceptor. *Nature* **1995**, *374*, 272−276.
(86) Puia, G.; Vicini, S.; Seeburg, P. H.; Costa, E. Influence of recombinant γ-aminobutyric $acid_A$ receptor subunit composition on the action of allosteric modulators of γ-aminobutyric acid-gated Cl^- currents. *Mol. Pharmacol.* **1991**, *39*, 691−696.
(87) Giusti, P.; Ducic, I.; Puia, G.; Arban, R.; Walser, A.; Guidotti, A.; Costa, E. Imidazenil: A new partial positive allosteric modulator of γ-aminobutyric acid (GABA) action at $GABA_A$ receptors. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 1018−1028.
(88) Wafford, K. A.; Whiting, P. J.; Kemp, J. A. Differences in affinity and efficacy of benzodiazepine receptor ligands at recombinant γ-aminobutyric $acid_A$ receptor subtypes. *Mol. Pharmacol.* **1993**, *43*, 240−244.
(89) Wisden, W.; Seeburg, P. H. $GABA_A$ receptor channels: From subunits to functional entities. *Curr. Opin. Neurobiol.* **1992**, *2*, 263−269.
(90) Saxena, N. C.; Macdonald, R. L. Assembly of $GABA_A$ receptor subunits: role of the δ-subunit. *J. Neurosci.* **1996**, *14*, 7077−7086.
(91) Neelands, T. R.; Fisher, J. L.; Bianchi, M.; Macdonald, R. L. Spontaneous and γ-aminobutyric acid (GABA)-activated $GABA_A$ receptor channels formed by epsilon subunit-containing isoforms. *Mol. Pharmacol.* **1999**, *55*, 168−178.
(92) Günther, U.; Benson, J. A.; Benke, D.; Fritschy, J.-M.; Reyes, G.; Knoflach, F.; Cretani, F.; Aguzzi, A.; Arigoni, M.; Lang, Y.; Möhler, H.; Luscher, B. Benzodiazepine-insensitive mice generated by targeted disruption of the γ2 subunit gene of γ-aminobutyric acid type A receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7749−7753.
(93) Mihic, S. J.; Whiting, P. J.; Klein, R. L.; Wafford, K. A.; Harris, R. A. A single amino acid of the human γ-aminobutyric acid type A receptor γ2 subunit determines benzodiazepine efficacy. *J. Biol. Chem.* **1994**, *269*, 32768−32773.
(94) Hadingham, K. L.; Wingrove, P.; Le Bourdelles, B.; Palmer, K. J.; Ragan, C. I.; Whiting, P. J. Cloning of cDNA encoding human α2 and α3 γ-aminobutyric $acid_A$ receptor subunits and characterization of the benzodiazepine pharmacology of recombinant α1-, α2-, α3-, and α5-containing human γ-aminobutyric $acid_A$ receptors. *Mol. Pharmacol.* **1993**, *43*, 253−259.
(95) Salva, P.; Costa, J. Clinical pharmacokinetics and pharmacodynamics if zolpidem − therapeutic implications. *Clin. Pharmacokin.* **1995**, *29*, 142−153.
(96) Graham, D.; Faure, C.; Besnard, F.; Langer, S. Z. Pharmacological profile of benzodiazepine site ligands with recombinant $GABA_A$ receptor subtypes. *Eur. Neuropsychopharmacol.* **1996**, *6*, 119−125.
(97) Mintzer, M. Z.; Griffiths, R. R. Selective effects of zolpidem on human memory functions. *J. Psychopharmacol.* **1999**, *13*, 18−31.
(98) Maddalena, D. J.; Johnston, G. A. R. Prediction of receptor properties and binding affinity of ligands to benzodiazepine/$GABA_A$ receptors using artificial neural networks. *J. Med. Chem.* **1995**, *38*, 715−724.

(99) So, S. S.; Karplus, M. Genetic neural networks for quantitative structure−activity relationships − improvements and application of benzodiazepine affinity for benzodiazepine/$GABA_A$ receptors. *J. Med. Chem.* **1996**, *39*, 5246−5256.
(100) Winkler, D. A.; Burden, F. R. Holographic QSAR of benzodiazepines. *Quant. Struct. Act. Relat.* **1998**, *17*, 224−231.
(101) Huang, Q.; Liu, R. Y.; Zhang, P. W.; He, X. H.; Mckernan, R.; Gan, T.; Bennett, D. W.; Cook, J. M. Predictive models for $GABA_A$/benzodiazepine receptor subtypes − studies of quantitative structure−activity relationships for imidazobenzodiazepines at five recombinant $GABA_A$/benzodiazepine receptor subtypes [alpha-*x*-beta-3-gamma-2 ($x = 1-3$, 5, and 6)] via comparative molecular field analysis. *J. Med. Chem.* **1998**, *41*, 4130−4142.
(102) Jacobsen, E. J.; Stelzer, L. S.; TenBrink, R. E.; Belonga, K. L.; Carter, D. B.; Im, H. K.; Im, W. B.; Sethy, V. H.; Tang, A. H.; VonVoigtlander, P. F.; Petke, J. D.; Zhong, W. Z.; Mickelson, J. W. Piperazine imidazo[1,5-a]quinoxaline ureas as high-affinity $GABA_A$ligands of dual functionality. *J. Med. Chem.* **1999**, *42*, 1123−1144.
(103) Maitra, R.; Reynolds, J. N. Modulation of $GABA_A$ receptor function by neuroactive steroids: evidence for heterogeneity of steroid sensitivity of recombinant $GABA_A$ receptor isoforms. *Can. J. Physiol. Pharmacol.* **1998**, *76*, 909−920.
(104) Maitra, R.; Reynolds, J. N. Subunit dependent modulation of $GABA_A$ receptor function by neuroactive steroids. *Brain Res.* **1999**, *819*, 75−82.
(105) Park-Chung, M.; Wu, F. S.; Farb, D. H. 3-α-Hydroxy-5β-pregnan-20-one sulfate: a negative modulator of the NMDA-induced current in cultured neurons. *Mol. Pharmacol.* **1994**, *46*, 146−150.
(106) ffrench-Mullen, J. M.; Danks, P.; Spence, K. T. Neurosteroids modulate calcium currents in hippocampal CA1 neurons via a pertussis toxin-sensitive G-protein-coupled mechanism. *J. Neurosci.* **1994**, *14*, 1963−1977.
(107) Baulieu, E. E. Neurosteroids: A novel function of the brain. *Psychoneuroendocrinology* **1998**, *23*, 963−987.
(108) Orchinik, M.; Weiland, N. G.; McEwen, B. S. Adrenalectomy selectively regulates $GABA_A$ receptor subunit expression in the hippocampus. *Mol. Cell. Neurosci.* **1994**, *5*, 451−458.
(109) Ong, J.; Kerr, D. I.; Johnston, G. A. Cortisol: a potent biphasic modulator at $GABA_A$-receptor complexes in the guinea pig isolated ileum. *Neurosci. Lett.* **1987**, *82*, 101−106.
(110) Ong, J.; Kerr, D. I.; Capper, H. R.; Johnston, G. A. Cortisone: a potent $GABA_A$ antagonist in the guinea-pig isolated ileum. *J. Pharm. Pharmacol.* **1990**, *42*, 662−664.
(111) Zorumski, C. F.; Mennerick, S. J.; Covey, D. F. Enantioselective modulation of gabaergic synaptic transmission by steroids and benz[*e*]indenes in hippocampal microcultures. *Synapse* **1998**, *29*, 162−171.
(112) Nilsson, K. R.; Zorumski, C. F.; Covey, D. F. Neurosteroid analogues − 6 − the synthesis and $GABA_A$ receptor pharmacology of enantiomers of dehydroepiandrosterone sulfate, pregnenolone sulfate, and (3α,5β)-3-hydroxypregnan-20-one sulfate. *J. Med. Chem.* **1998**, *41*, 2604−2613.
(113) Sousa, A.; Ticku, M. K. Interactions of the neurosteroid dehydroepiandrosterone sulfate with the $GABA_A$ receptor complex reveals that it may act via the picrotoxin site. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 827−833.
(114) Shen, W. X.; Mennerick, S.; Zorumski, E. C.; Covey, D. F.; Zorumski, C. F. Pregnenolone sulfate and dehydroepiandrosterone sulfate inhibit GABA-gated chloride currents in Xenopus oocytes expressing picrotoxin-insensitive GABA(A) receptors. *Neuropharmacology* **1999**, *38*, 267−271.
(115) Kerr, D. I. B.; Ong, J. GABA agonists and antagonists. *Med. Res. Rev.* **1992**, *12*, 593−636.
(116) Lambert, J. J.; Peters, J. A.; Sturgess, N. C.; Hales, T. G. Steroid modulation of the $GABA_A$ receptor complex: electrophysiological studies. In *Steroids and Neuronal Activity*; Chadwick, D., Widdows, K., Eds.; Wiley: Chichester, 1990; pp 56−71.
(117) Edgar, D. M.; Seidel, W. F.; Gee, K. W.; Lan, N. C.; Field, G.; Xia, H. J.; Hawkinson, J. E.; Wieland, S.; Carter, R. B.; Wood, P. L. Ccd-3693 − an orally bioavailable analogue of the endogenous neuroactive steroid, pregnanolone, demonstrates potent sedative hypnotic actions in the rat. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 420−429.
(118) Anderson, A.; Boyd, A. C.; Byford, A.; Campbell, A. C.; Gemmell, D. K.; Hamilton, N. M.; Hill, D. R.; Hillvenning, C.; Lambert, J. J.; Maidment, M. S.; May, V.; Marshall, R. J.; Peters, J. A.; Rees, D. C.; Stevenson, D.; Sundaram, H. Anesthetic activity of novel water-soluble 2-β-morpholinyl steroids and their modulatory effects at $GABA_A$ receptors. *J. Med. Chem.* **1997**, *40*, 1668−1681.
(119) Monaghan, E. P.; Navalta, L. A.; Shum, L.; Ashbrook, D. W.; Lee, D. A. Initial human experience with ganaxolone, a neuroactive steroid with antiepileptic activity. *Epilepsia* **1997**, *38*, 1026−1031.
(120) Masonis, A. E. T.; McCarthy, M. P. Effects of the androgenic/anabolic steroid stanozolol on $GABA_A$ receptor function − GABA-stimulated $Cl^{36(-)}$ influx and [S^{35}] TBPS binding. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 186−193.
(121) Ong, J.; Kerr, D. I.; Johnston, G. A. Pregnenolone and its sulphate modulate $GABA_A$-receptor-mediated contractile responses in the guinea-pig isolated ileum. *Eur. J. Pharmacol.* **1987**, *142*, 461−464.
(122) Prince, R. J.; Simmonds, M. A. Differential antagonism by epipregnanolone of alphaxalone and pregnanolone potentiation of [^{3}H]flunitrazepam binding suggests more than one class of binding site for steroids at $GABA_A$ receptors. *Neuropharmacology* **1993**, *32*, 59−63.
(123) Park, C. M.; Malayev, A.; Purdy, R. H.; Gibbs, T. T.; Farb, D. H. Sulfated and unsulfated steroids modulate gamma-aminobutyric acid(A) receptor function through distinct sites. *Brain Res.* **1999**, *830*, 72−87.
(124) Eletr, M.; Akwa, Y.; Robel, P.; Baulieu, E. E. Opposing effects of different steroid sulfates on $GABA_A$ receptor-mediated chloride uptake. *Brain Res.* **1998**, *790*, 334−338.
(125) Xue, B. G.; Whittemore, E. R.; Park, C. H.; Woodward, R. M.; Lan, N. C.; Gee, K. W. Partial agonism by 3α,21-dihydroxy-5β-pregnan-20-one at the γ-aminobutyric acid, receptor neurosteroid site. *J. Pharmacol. Exp. Ther.* **1997**, *281*, 1095−1101.
(126) Cutting, G. R.; Lu, L.; O'Hara, B. F.; Kasch, L. M.; Montrose-Rafizadeh, C.; Donovan, D. M.; Shimada, S.; Antonarakis, S. E.; Guggino, W. B.; Uhl, G. R. Cloning of the γ-aminobutyric acid (GABA) ρ1 cDNA: a GABA receptor subunit highly expressed in the retina. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2673−2677.
(127) Martineztorres, A.; Vazquez, A. E.; Panicker, M. M.; Miledi, R. Cloning and functional expression of alternative spliced variants of the ρ1 γ-aminobutyrate receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4019−4022.
(128) Zhang, D.; Pan, Z. H.; Zhang, X.; Brideau, A. D.; Lipton, S. A. Cloning of a γ-aminobutyric acid type C receptor subunit in rat retina with a methionine residue critical for picrotoxinin channel block. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11756−11760.
(129) Ogurusu, T.; Taira, H.; Shingai, R. Identification of $GABA_A$ receptor subunits in rat retina: cloning of the rat $GABA_A$ receptor ρ2-subunit cDNA. *J. Neurochem.* **1995**, *65*, 964−968.
(130) Ogurusu, T.; Shingai, R. Cloning of a putative γ-aminobutyric acid (GABA) receptor subunit ρ3 cDNA. *Biochim. Biophys. Acta* **1996**, *1305*, 15−18.
(131) Wegelius, K.; Reeben, M.; Rivera, C.; Kaila, K.; Saarma, M.; Pasternack, M. The ρ1 GABA receptor cloned from rat retina is down-modulated by protons. *NeuroReport* **1996**, *7*, 2005−2009.
(132) Albrecht, B. E.; Darlison, M. G. Localization of the ρ1- and ρ2-subunit messenger RNAs in chick retina by in situ hybridization predicts the existence of γ-aminobutyric acid type C receptor subtypes. *Neurosci. Lett.* **1995**, *189*, 155−158.
(133) Qian, H.; Hyatt, G.; Schanzer, A.; Hazra, R.; Hackam, A. S.; Cutting, G. R.; Dowling, J. E. A comparison of $GABA_C$ and ρ subunit receptors from the white perch retina. *Vis. Neurosci.* **1997**, *14*, 843−851.
(134) Greka, A.; Koolen, J. A.; Lipton, S. A.; Zhang, D. Cloning and characterization of mouse $GABA_C$ receptor subunits. *NeuroReport* **1998**, *9*, 229−232.
(135) Kusama, T.; Wang, T. L.; Guggino, W. B.; Cutting, G. R.; Uhl, G. R. GABA ρ2 receptor pharmacological profile: GABA recognition site similarities to ρ1. *Eur. J. Pharmacol.* **1993**, *245*, 83−84.
(136) Wang, T. L.; Guggino, W. B.; Cutting, G. R. A novel γ-aminobutyric acid receptor subunit (ρ2) cloned from human retina forms bicuculline-insensitive homooligomeric receptors in *Xenopus* oocytes. *J. Neurosci.* **1994**, *14*, 6524−6531.
(137) Hackam, A. S.; Wang, T. L.; Guggino, W. B.; Cutting, G. R. Sequences in the amino termini of GABA ρ and $GABA_A$ subunits specify their selective interaction in vitro. *J. Neurochem.* **1998**, *70*, 40−46.
(138) Chebib, M.; Mewett, K. N.; Johnston, G. A. R. $GABA_C$ receptor antagonists differentiate between human ρ1 and ρ2 receptors expressed in *Xenopus* oocytes. *Eur. J. Pharmacol.* **1998**, *357*, 227−234.
(139) Hackam, A. S.; Wang, T. L.; Guggino, W. B.; Cutting, G. R. A 100 amino acid region in the GABA ρ1 subunit confers robust homooligomeric expression. *NeuroReport* **1997**, *8*, 1425−1430.
(140) Hackam, A. S.; Wang, T. L.; Guggino, W. B.; Cutting, G. R. The N-terminal domain of human GABA receptor rho1 subunits contains signals for homooligomeric and heterooligomeric interaction. *J. Biol. Chem.* **1997**, *272*, 13750−13757.
(141) Shingai, R.; Yanagi, K.; Fukushima, T.; Sakata, K.; Ogurusu, T. Functional expression of GABA rho 3 receptors in Xenopus oocytes. *Neurosci. Res.* **1996**, *26*, 387−390.
(142) Enz, R.; Cutting, G. R. $GABA_C$ receptor ρ subunits are heterogeneously expressed in the human CNS and form homo- and heterooligomers with distinct physical properties. *Eur. J. Neurosci.* **1999**, *11*, 41−50.

(143) Amin, J. A single hydrophobic residue confers barbiturate sensitivity to gamma-aminobutyric acid type C receptor. *Mol. Pharmacol.* **1999**, *55*, 411−423.
(144) Belelli, D.; Pau, D.; Cabras, G.; Peters, J. A.; Lambert, J. J. A single amino acid confers barbiturate sensitivity upon the GABA rho(1) receptor. *Br. J. Pharmacol.* **1999**, *127*, 601−604.
(145) Enz, R.; Brandstatter, J. H.; Hartveit, E.; Wassle, H.; Bormann, J. Expression of GABA receptor ρ1 and ρ2 subunits in the retina and brain of the rat. *Eur. J. Neurosci.* **1995**, *7*, 1495−1501.
(146) Enz, R.; Brandstatter, J. H.; Wassle, H.; Bormann, J. Immunocytochemical localization of the $GABA_C$ receptor ρ subunits in the mammalian retina. *J. Neurosci.* **1996**, *16*, 4479−4490.
(147) Koulen, P.; Brandstatter, J. H.; Kroger, S.; Enz, R.; Bormann, J.; Wassle, H. Immunocytochemical localization of the $GABA_C$ receptor ρ subunits in the cat, goldfish, and chicken retina. *J. Comp. Neurol.* **1997**, *380*, 520−532.
(148) Ogurusu, T.; Eguchi, G.; Shingai, R. Localization of γ-aminobutyric acid (GABA) receptor ρ3 subunit in rat retina. *NeuroReport* **1997**, *8*, 925−927.
(149) Koulen, P.; Brandstatter, J. H.; Enz, R.; Bormann, J.; Wassle, H. Synaptic clustering of $GABA_C$ receptor ρ-subunits in the rat retina. *Eur. J. Neurosci.* **1998**, *10*, 115−127.
(150) Boue-Grabot, E.; Roudbaraki, M.; Bascles, L.; Tramu, G.; Bloch, B.; Garret, M. Expression of GABA receptor ρ subunits in rat brain. *J. Neurochem.* **1998**, *70*, 899−907.
(151) Wegelius, K.; Pasternack, M.; Hiltunen, J. O.; Rivera, C.; Kaila, K.; Saarma, M.; Reeben, M. Distribution of GABA receptor rho subunit transcripts in the rat brain. *Eur. J. Neurosci.* **1998**, *10*, 350−357.
(152) Albrecht, B. E.; Breitenbach, U.; Stuhmer, T.; Harvey, R. J.; Darlison, M. G. In situ hybridization and reverse transcription-polymerase chain reaction studies on the expression of the $GABA_C$ receptor ρ1- and ρ2-subunit genes in avian and rat brain. *Eur. J. Neurosci.* **1997**, *9*, 2414−2422.
(153) Fletcher, E. L.; Koulen, P.; Wassle, H. $GABA_A$ and $GABA_C$ receptors on mammalian rod bipolar cells. *J. Comp. Neurol.* **1998**, *396*, 351−365.
(154) Dong, C. J.; Werblin, F. S. Temporal contrast enhancement via $GABA_C$ feedback at bipolar terminals in the tiger salamander retina. *J. Neurophys.* **1998**, *79*, 2171−2180.
(155) Lukasiewicz, P. D.; Shields, C. R. A diversity of GABA receptors in the retina. *Semin. Cell Dev. Biol.* **1998**, *9*, 293−299.
(156) Curtis, D. R.; Duggan, A. W.; Felix, D.; Johnston, G. A. R. Bicuculline and central GABA receptors. *Nature* **1970**, *228*, 676−677.
(157) Curtis, D. R.; Duggan, A. W.; Felix, D.; Johnston, G. A. R. GABA, bicuculline and central inhibition. *Nature* **1970**, *226*, 1222−1224.
(158) Curtis, D. R.; Duggan, A. W.; Felix, D.; Johnston, G. A. R. Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord of the cat. *Brain Res.* **1971**, *32*, 69−96.
(159) Curtis, D. R.; Duggan, A. W.; Felix, D.; Johnston, G. A. R.; McLennan, H. Antagonism between bicuculline and GABA in the cat brain. *Brain Res.* **1971**, *33*, 57−73.
(160) Johnston, G. A. R.; Beart, P. M.; Curtis, D. R.; Game, C. J.; McCulloch, R. M.; Maclachlan, R. M. Bicuculline methochloride as a GABA antagonist. *Nature − New Biol.* **1972**, *240*, 219−220.
(161) Andrews, P. R.; Johnston, G. A. R. GABA agonists and antagonists. *Biochem. Pharmacol.* **1979**, *28*, 2697−2702.
(162) Allan, R. D.; Curtis, D. R.; Headley, P. M.; Johnston, G. A. R.; Lodge, D.; Twitchin, B. The synthesis and activity of cis- and trans-2-(aminomethyl) cyclopropanecarboxylic acid as conformationally restricted analogues of GABA. *J. Neurochem.* **1980**, *34*, 652−656.
(163) Allan, R. D.; Bornstein, J. C.; Curtis, D. R.; Johnston, G. A. R.; Lodge, D. Selective antagonist activity of 5-aminohex-2-enedioic acid on amino acid excitation of cat spinal neurones. *Neurosci. Lett.* **1980**, *16*, 17−20.
(164) Drew, C. A.; Johnston, G. A. R.; Weatherby, R. P. Bicuculline-insensitive GABA receptors: studies on the binding of (−)-baclofen to rat cerebellar membranes. *Neurosci. Lett.* **1984**, *52*, 317−321.
(165) Drew, C. A.; Johnston, G. A. R. Bicuculline- and baclofen-insensitive gamma-aminobutyric acid binding to rat cerebellar membranes. *J. Neurochem.* **1992**, *58*, 1087−1092.
(166) Shimada, S.; Cutting, G.; Uhl, G. R. γ-Aminobutyric acid A or C receptor? γ-Aminobutyric acid ρ1 receptor RNA induces bicuculline-, barbiturate-, and benzodiazepine-insensitive gamma-aminobutyric acid responses in *Xenopus* oocytes. *Mol. Pharmacol.* **1992**, *41*, 683−687.
(167) Qian, H.; Dowling, J. E. Novel GABA responses from rod-driven retinal horizontal cells. *Nature* **1993**, *361*, 162−164.
(168) Dong, C. J.; Picaud, S. A.; Werblin, F. S. GABA transporters and $GABA_C$-like receptors on catfish cone- but not rod-driven horizontal cells. *J. Neurosci.* **1994**, *14*, 2648−2658.
(169) Qian, H.; Dowling, J. E. $GABA_A$ and $GABA_C$ receptors on hybrid bass retinal bipolar cells. *J. Neurophys.* **1995**, *74*, 1920−1928.
(170) Lukasiewicz, P. D.; Werblin, F. S. A novel GABA receptor modulates synaptic transmission from bipolar to ganglion and amacrine cells in the tiger salamander retina. *J. Neurosci.* **1994**, *14*, 1213−1223.
(171) Lukasiewicz, P. D.; Maple, B. R.; Werblin, F. S. A novel GABA receptor on bipolar cell terminals in the tiger salamander retina. *J. Neurosci.* **1994**, *14*, 1202−1212.
(172) Johnston, G. A.; Stephanson, A. L. Inhibitors of the glial uptake of β-alanine in rat brain slices. *Brain Res.* **1976**, *102*, 374−378.
(173) Biedermann, B.; Eberhardt, W.; Reichelt, W. GABA uptake into isolated retinal Muller glial cells of the guinea-pig detected electrophysiologically. *NeuroReport* **1994**, *5*, 438−440.
(174) Chebib, M.; Johnston, G. A. R. Stimulation of [^{3}H] GABA and β-[^{3}H] alanine release from rat brain slices by *cis*-4-aminocrotonic acid. *J. Neurochem.* **1997**, *68*, 786−794.
(175) Kusama, T.; Spivak, C. E.; Whiting, P.; Dawson, V. L.; Schaeffer, J. C.; Uhl, G. R. Pharmacology of GABA ρ1 and GABA α/β receptors expressed in *Xenopus* oocytes and COS cells. *Br. J. Pharmacol.* **1993**, *109*, 200−206.
(176) Chebib, M.; Vandenberg, R. J.; Froestl, W.; Johnston, G. A. R. Unsaturated phosphinic analogues of γ-aminobutyric acid as $GABA_C$ receptor antagonists. *Eur. J. Pharmacol.* **1997**, *329*, 223−229.
(177) Chebib, M.; Vandenberg, R. J.; Johnston, G. A. R. Analogues of γ-aminobutyric acid (GABA) and *trans*-4-aminocrotonic acid (TACA) substituted in the 2 position as $GABA_C$ receptor antagonists. *Br. J. Pharmacol.* **1997**, *122*, 1551−1560.
(178) Rozzo, A.; Ballerini, L.; Nistri, A. Antagonism by (1,2,5,6-tetrahydropyridine-4-yl) methylphosphinic acid of synaptic transmission in the neonatal rat spinal cord in vitro: An electrophysiological study. *Neuroscience* **1999**, *90*, 1085−1092.
(179) Enz, R.; Bormann, J. A single point mutation decreases picrotoxinin sensitivity of the human GABA receptor ρ1 subunit. *NeuroReport* **1995**, *6*, 1569−1572.
(180) Wang, T. L.; Hackam, A. S.; Guggino, W. B.; Cutting, G. R. A single amino acid in γ-aminobutyric acid ρ1 receptors affects competitive and noncompetitive components of picrotoxin inhibition. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11751−11755.
(181) Feigenspan, A.; Bormann, J. Modulation of $GABA_C$ receptors in rat retinal bipolar cells by protein kinase C. *J. Physiol.* **1994**, *481*, 325−330.
(182) Calvo, D. J.; Miledi, R. Activation of GABA ρ1 receptors by glycine and β-alanine. *NeuroReport* **1995**, *6*, 1118−1120.
(183) Bunin, B. A.; Plunkett, M. J.; Ellman, J. A. The combinatorial synthesis and chemical and biological evaluation of a 1,4-benzodiazepine library. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4708−4712.
(184) Marder, M.; Viola, H.; Bacigaluppo, J. A.; Colombo, M. I.; Wasowski, C.; Wolfman, C.; Medina, J. H.; Ruveda, E. A.; Paladini, A. C. Detection of benzodiazepine receptor ligands in small libraries of flavone derivatives synthesized by solution phase combinatorial chemistry. *Biochem. Biophys. Res. Commun.* **1998**, *249*, 481−485.
(185) Kuntzweiler, T. A.; Arneric, S. P.; Donnellyroberts, D. L. Rapid assessment of ligand actions with nicotinic acetylcholine receptors using calcium dynamics and flipr. *Drug Dev. Res.* **1998**, *44*, 14−20.
(186) Cunningham, M. D.; Cunningham, S. M. High-throughput functional detection of NMDA receptor activity. *NMDA Receptor Protocols* **1999**, *128*, 179−188.
(187) Sasaki, K.; Oota, I.; Wada, K.; Inomata, K.; Ohshika, H.; Haga, M. Effects of bilobalide, a sesquiterpene in Ginkgo biloba leaves, on population spikes in rat hippocampal slices. *Comp. Biochem. Physiol. C: Comp. Pharmacol. Toxicol.* **1999**, *124*, 315−321.
(188) Huong, N.; Matsumoto, K.; Yamasaki, K.; Watanabe, H. Involvement of supraspinal GABA receptors in majonoside-r2 suppression of clonidine-induced antinociception in mice. *Life Sci.* **1997**, *61*, 427−436.
(189) Armstrong, N.; Sun, Y.; Chen, G. Q.; Gouaux, E. Structure of a glutamate-receptor ligand-binding core in complex with kainate. *Nature* **1998**, *395*, 913−917.
(190) Ortells, M. O.; Barrantes, G. E.; Wood, C.; Lunt, G. G.; Barrantes, F. J. Molecular modelling of the nicotinic acetylcholine receptor transmembrane region in the open state. *Protein Eng.* **1997**, *10*, 511−517.
(191) Gready, J. E.; Ranganathan, S.; Schofield, P. R.; Matsuo, Y.; Nishikawa, K. Predicted structure of the extracellular region of ligand-gated ion-channel receptors shows sh2-like and sh3-like domains forming the ligand-binding site. *Protein Sci.* **1997**, *6*, 983−998.
(192) Bliznyuk, A. A.; Gready, J. E. Simple method for locating possible ligand binding sites on protein surfaces. *J. Comput. Chem.* **1999**, *20*, 983−988.
(193) Sadee, W. Pharmacogenomics. *Br. Med. J.* **1999**, *319*, 1286−1290.
(194) Evans, W. E.; Relling, M. V. Pharmacogenomics: Translating functional genomics into rational therapeutics [Review]. *Science* **1999**, *286*, 487−491.

(195) Carulli, J. P.; Artinger, M.; Swain, P. M.; Root, C. D.; Chee, L.; Tulig, C.; Guerin, J.; Osborne, M.; Stein, G.; Lian, J.; Lomedico, P. T. High throughput analysis of differential gene expression. *J. Cell. Biochem*. **1998**.

(196) DeLorey, T. M.; Olsen, R. W. GABA and epileptogenesis: comparing gabrb3 gene-deficient mice with Angelman syndrome in man. *Epilepsy Res.* **1999**.

(197) Amir, R.; Dahle, E.; Toriolo, D.; Zoghbi, H. Y. Candidate gene analysis in Rett syndrome and the identification of 21 SNPs in Xq. *Am. J. Med. Genet.* **1999**, *90*, 69−71.

(198) Loh, E. W.; Smith, I.; Murray, R.; McLaughlin, M.; McNulty, S.; Ball, D. Association between variants at the GABA(A)beta 2, GABA(A)alpha 6 and GABA(A)gamma 2 gene cluster and alcohol dependence in a Scottish population. *Mol. Psychiatry* **1999**, *4*, 539−544.

(199) Parsian, A.; Zhang, Z. H. Human chromosomes 11p15 and 4p12 and alcohol dependence: Possible association with the GABRB1 gene. *Am. J. Med. Genet.* **1999**, *88*, 533−538.

JM9904349