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Medicinal chemistry of ρ GABA_C receptors

The inhibitory neurotransmitter, GABA, is a low-molecular-weight molecule that can achieve many low-energy conformations, which are recognized by GABA receptors and transporters. In this article, we assess the structure–activity relationship profiles of GABA analogs at the ionotropic ρ GABA_C receptor. Such studies have significantly contributed to the design and development of potent and selective agonists and antagonists for this subclass of GABA receptors. With these tools in hand, the role of ρ GABA_C receptors is slowly being realized. Of particular interest is the development of selective phosphinic acid analogs of GABA and their potential use in sleep disorders, inhibiting the development of myopia, and in improving learning and memory.

GABA is a flexible, low-molecular-weight molecule that can achieve a large number of low-energy conformations [1], that are recognized by three major classes of receptors in the mammalian brain: the ionotropic GABA_A and ρ GABA_C receptors, and the metabotropic GABA_B receptors. The GABAergic system is involved in a variety of physiological processes, such as memory [2,3], cognition [4,5], vision [6–9], pain management [10–12], sleep [13–15] and cardiovascular regulation [16,17]. Defects and deficits of this system have been implicated in the development of neurological and psychiatric conditions such as epilepsy [18,19], anxiety [2,20–22], mood disorders [23], schizophrenia [5,24,25], Alzheimer's disease [26,27] and Huntington's chorea [28,29]. Thus, agents that can modify the GABAergic system have the potential to become important therapies. This article concentrates on a subclass of ionotropic GABA receptors, the ρ GABA_C receptor, a topic of previous reviews [30–34].

The ρ GABA_C receptors belong to the nicotinicoid superfamily or cys-loop superfamily [35], which includes nicotinic acetylcholine receptors, GABA_A receptors (see review [36]), serotonin 5-HT₃ receptors, strychnine-sensitive glycine receptors and invertebrate anionic glutamate receptors. These receptors are all structurally related [35,37,38] possessing four transmembrane domains (TM1–4) connected by variable lengths of intracellular and extracellular loops. The TM2 domain of each subunit lines the ion pore and, in the case of ρ GABA_C receptors, is permeable to chloride ions. Each subunit contains a large extracellular N-terminal domain for ligand binding, as well as a short extracellular C-terminus [39,40]. Another common physical feature shared throughout the family members is the signature-conserved disulfide-bridged loop

in the extracellular domain, formed by a pair of cysteines at a 15-residue spacing at a fixed position [35,41,42].

Subunit composition, distribution & function of GABA_C receptors

The first indication of a possible new class of ionotropic GABA receptors arose from a study that evaluated *cis*- and *trans*-4-aminocrotonic acid (CACA and TACA, respectively; **FIGURE 1**) on the firing cat spinal interneurons [43]. It was found that CACA, a folded analog of GABA, depressed the firing of the interneurons and this action could not be antagonized by the GABA_A antagonist bicuculline; while the effect of TACA, the extended form of GABA, was antagonized by bicuculline [43]. This was followed by the discovery that CACA had no influence on the binding against radiolabelled baclofen in rat cerebellum. Results from these studies led to the term 'GABA_C receptors' for a class of 'bicuculline- and baclofen-insensitive receptors' [44].

It was not until the early 1990s that the so-called 'GABA_C receptor' was shown to be functional [45]. Isolated bovine retinal mRNA, when expressed in *Xenopus* oocytes, was found to form two receptors: a bicuculline-sensitive GABA_A receptor and a novel receptor type that was both bicuculline and baclofen insensitive [45]. Subsequently, Cutting and coworkers [46] cloned and sequenced a new subunit, termed 'rho 1 (ρ_1) subunit' from human retinal cDNA. The sequence of the ρ_1 subunit displayed approximately 30–38% amino acid similarity to previously identified GABA_A subunits and when expressed in *Xenopus* oocytes, was able to form a homooligomeric receptor, 50-times more sensitive to picrotoxin than GABA_A receptors [46].

Clarissa KL Ng¹, Hye-lim Kim¹, Navnath Gavande¹, Izumi Yamamoto¹, Rohan J Kumar¹, Kenneth N Mewett², Graham AR Johnston², Jane R Hanrahan¹ & Mary Chebib[†]

¹Faculty of Pharmacy A15, The University of Sydney, NSW, Australia 2006

²Adrien Albert Laboratory of Medicinal Chemistry, Department of Pharmacology D06, The University of Sydney, NSW, Australia 2006

[†]Author for correspondence:

Tel.: +61 293 518 584

Fax: +61 293 514 391

E-mail: mary.collins@sydney.edu.au

GABA	CACA	TACA
$EC_{50} (\alpha_1\beta_1\gamma_{2L}) = 16.5 \mu\text{M}$	$GABA_A = \text{inactive}$	$EC_{50} GABA_A = 133 \mu\text{M}$
$EC_{50} (GABA_B) = 2.3 \mu\text{M}$	$GABA_B = \text{inactive}$	$GABA_B = \text{inactive}$
$EC_{50} (\rho_1) = 1 \mu\text{M}$	$EC_{50} (\rho_1) = 74 \mu\text{M}$	$EC_{50} (\rho_1) = 0.6 \mu\text{M}$
$EC_{50} (\rho_2) = 0.8 \mu\text{M}$	$EC_{50} (\rho_2) = 70 \mu\text{M}$	$EC_{50} (\rho_2) = 0.4 \mu\text{M}$
$EC_{50} (\rho_3) = 4 \mu\text{M}$	$EC_{50} (\rho_3) = 139.4 \mu\text{M}$	$EC_{50} (\rho_3) = 3.8 \mu\text{M}$

Figure 1. GABA, CACA and TACA.

CACA: *Cis*-aminocrotonic acid; TACA: *Trans*-4-aminocrotonic acid.

These results were further supported by studies carried out by Feigenspan *et al.* on rat retinal bipolar cells [47], and Qian *et al.* on rod-driven horizontal cells isolated from white perch retina [48].

To date, five different subtypes of the ρ subunit have been cloned from different species, which include human (ρ_{1-3}), mouse (ρ_{1-2}), rat (ρ_{1-3}), chicken (ρ_{1-2}) and white perch (ρ_{1A-B} , ρ_{2A-B} and ρ_3 ; see the review by Enz [49]). The ρ subunits share approximately 70% to over 90% amino acid similarity within the subunit family and between different species [50–52].

Most ρ subunits form functional homopentameric chloride channels [46,51,53–57]. This concept is supported by various Hill coefficient studies of both native and recombinant receptors that show Hill coefficients of 3–4, indicating that at least three GABA molecules are required to activate the receptor [8,50,58]. Studies on recombinant and native receptors have also provided evidence that GABA_C receptors may consist of a combination of ρ_1 , ρ_2 and/or ρ_3 subunits, forming ‘pseudo-homomeric’ GABA_C receptors [49,59,60]. Although some studies have reported the possible coassembly of the ρ subunits with other GABA_A subunits, for example the α_1 and γ_2 subunits, this remains controversial and it is still unknown if these heteromeric receptors exist *in vitro* [8,61–65].

Immunohistochemical and electrophysiological studies have shown that, in most species, the ρ_1 subunits are predominately expressed in bipolar cells [66–71] and horizontal cells in the retina; while the ρ_2 subunits are found not only in the retina but also in the spinal cord [72] and in several brain regions, such as the hippocampus, cortex, pituitary, cerebellum and thalamus [49,60]. The ρ_3 subunits are reported to be found mainly in the retina and in all regions of the brain, except the superior colliculus [59,73]. GABA_C receptors have also been found outside the CNS, such as the GI tract [74,75].

McCall *et al.* found that the absence of the ρ_1 subunit in knock-out mice led to the abolishment of the GABA-evoked response in the mouse retina normally mediated by GABA_C receptors [76], and signaling from rod bipolar cells to third order cells was altered [76,77], while Schlicker *et al.* observed an alteration, rather than a complete elimination, in GABA_C-mediated responses in the superior colliculus, suggesting that ρ_2 or ρ_3 GABA_C receptors are functional in this tissue [77]. In addition, Zheng and colleagues demonstrated a role for GABA_C receptors in maintaining both homeostasis and balance of retinal neurotransmitter function as knockout of the retinal ρ_1 GABA_C subunit led to changes in vascular permeability similar to the pathological changes induced by retinal hypoxic conditions [78]. Interestingly, Zheng and coworkers saw a decrease in pain threshold in ρ_1 -mutant mice [79]. Knock-out studies also showed that ρ_1 GABA_C receptors mediate inhibitory modulation on the olfactory bulb [80]. Knock-out studies on the GABA_C receptors in mice also suggest the involvement of these receptors in pain pathways [201].

Both ρ_1 and ρ_2 GABA_C receptors are found in the hippocampus where there is evidence for a functional role, as extrasynaptic receptors activated via spillover of synaptically released GABA [81] and in paired-pulse depression of inhibitory postsynaptic currents [82]. GABA_C receptors may also be involved in the regulation of thyrotropin release from the pituitary [83] and in synaptic transmission in the spinal cord [72]. GABA_C receptors have also been described on neurons in the gastrointestinal system [75] where they may increase the release of nitric oxide from nonadrenergic, noncholinergic inhibitory neurons [84].

The localization of the various ρ GABA_C receptors, knock-out and pharmacological studies show the receptor’s critical roles in visual processing and myopia development [85,86], olfactory senses [80], learning and memory [3,87], sleep patterns [87,88], nociception [79] and hormone secretion [89].

Agonists at ρ GABA_C receptors

The pharmacology of ρ GABA_C receptors is distinctive and seemingly less complex compared with that of GABA_A receptors, possibly due to its simpler subunit composition. GABA_C receptors are approximately eightfold more sensitive to GABA than GABA_A receptors [31], and have a smaller chloride conductance

(8 vs 27 pS); a longer mean channel-opening time (150 vs 25 ms); and are not prone to desensitization [33,49,90,91]. Over the last 15 years, conformationally restricted analogs of GABA have been employed to investigate the preferred conformation of GABA at these 'novel' receptors, thereby assisting with the characterization of the binding site.

The known GABA_A agonist muscimol is not selective as it is found to be a potent partial agonist at ρ GABA_C receptors [53,92], while isoguvacine also acts as weak partial agonist at ρ GABA_C receptors, with a higher efficacy at ρ_2 than at ρ_1 receptors [92]. CACA is a partial agonist at both ρ_1 and ρ_2 GABA_C receptors with little activity at GABA_A receptors. However, it acts as a substrate for GABA uptake transporters that are sensitive to β -alanine and nipecotic acid [93]. CACA activates GABA_C receptors with an efficacy approximately one-quarter of that of GABA. By contrast, CACA only weakly activates homomeric ρ_3 receptors [94]. TACA, an isomer of CACA and an analog of GABA in the 'extended' form, is a nonselective agonist at ρ_1 and ρ_2 receptors that is almost equipotent to GABA [95]. TACA is the most potent GABA_C agonist described to date, being approximately 120 and 40-times more potent than CACA at homomeric ρ_1 and ρ_2 , and ρ_3 recombinant receptors expressed in *Xenopus* oocytes, respectively [33,94]. However, TACA is also a potent GABA_A receptor agonist [53,92].

Chebib *et al.* investigated the activities of various mono- and di-substituted TACA analogs on ρ_1 GABA_C receptors [96]. It was found that a fluoro substituent at the C2 position (*trans*-4-amino-2-fluorobut-2-enoic acid) caused a reduction in agonist activity when compared with GABA and TACA [96]. A methyl substituent at the same position (*trans*-4-amino-2-methylbut-2-enoic acid [2-MeTACA]) gives rise to a competitive antagonist at ρ_1 receptors, while it acts as a partial agonist at ρ_2 receptors [92], and inactive at ρ_3 receptors [94]. Thus, 2-MeTACA can be used to differentiate between ρ_1 and ρ_2 receptors [92].

The saturated, 2-methyl-substituted analog (*S*)-4-amino-2-methylbutanoic acid ((*S*)-2-MeGABA) is a full but moderate agonist at ρ GABA_C receptors [97,98]. The hydroxylated GABA analogs (*S*)- and (*R*)-4-amino-3-hydroxybutanoic acid also act as full agonists at ρ_1 GABA_C receptors in a stereoselective manner [99]. The sulfinic analog of GABA, homohypotaurine, is a potent partial agonist at ρ_1 receptors [96].

The introduction of a cyclopropane moiety to the GABA backbone gave rise to the conformationally restricted analogs (\pm)-*cis*-2-(aminomethyl)cyclopropanecarboxylic acid ([\pm]-CAMP), and (\pm)-*trans*-2-(aminomethyl)cyclopropanecarboxylic acid ([\pm]-TAMP). The racemic mixtures were first synthesized and their activities studied by Allan *et al.* [100]. (\pm)-TAMP, corresponding to the 'extended' form of GABA, was found to be a potent and bicuculline-sensitive depressant of firing of cat spinal neurons. (\pm)-CAMP, the 'folded' form, was less potent than (\pm)-TAMP [100]. The enantiomers were isolated and further studied by Duke *et al.* [101] and Crittenden *et al.* [97] at ρ GABA_C receptors expressed in *Xenopus* oocytes. It was revealed that the full agonist activity exhibited by (\pm)-CAMP at ρ GABA_C receptors resides in (+)-CAMP, while (–)-CAMP acts as an antagonist at the same receptors (**FIGURE 2**). Both (+)-TAMP and (–)-TAMP are partial agonists at ρ GABA_C receptors with significant agonist activities at the GABA_A receptors. The superior selectivity of (+)-CAMP for ρ GABA_C receptors over (+)- and (–)-TAMP further demonstrates that the 'folded' form of GABA is preferred at these receptors.

The effects of a series of racemic cyclopentane analogs of GABA were evaluated for their ability to inhibit the firing of hippocampal pyramidal neurons in rats [102] and motoneurons on isolated frog spinal cord [103]. The (\pm)-*trans*-3-aminocyclopentane carboxylic acids ([\pm]-3-TACP) rather than the (\pm)-*cis*-isomers ([\pm]-3-CACP) were the most potent depressants of neuronal firing and the effects were bicuculline sensitive. Syntheses of the pure enantiomers were later reported by Allan *et al.* [104] and these compounds were subsequently investigated for their effects on GABA_A receptors in guinea pig ileum, in binding studies using rat brain membranes,

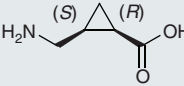

	
(+)-CAMP	(–)-CAMP
IC ₅₀ ($\alpha_1\beta_1\gamma_{2L}$) = >1 mM	IC ₅₀ ($\alpha_1\beta_1\gamma_{2L}$) = >1 mM
EC ₅₀ (ρ_1) = 39.7 μ M	IC ₅₀ (ρ_1) = 890 μ M
EC ₅₀ (ρ_2) = 16.6 μ M	IC ₅₀ (ρ_2) = 400 μ M
EC ₅₀ (ρ_3) = 28.1 μ M	IC ₅₀ (ρ_3) = 178 μ M

Figure 2. (+)- and (–)-CAMP.

CAMP: *Cis*-2-(aminomethyl)cyclopropanecarboxylic acid.

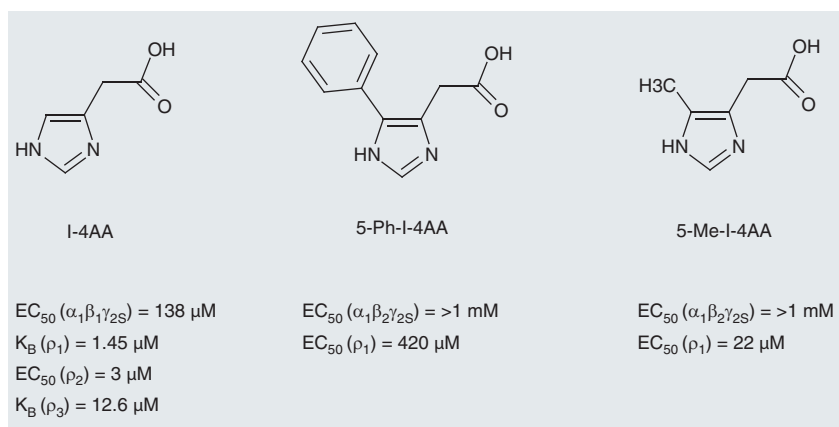


Figure 3. I-4AA, 5-Ph I-4AA and 5-Me I-4AA.
I-4AA: Imidazole-4-acetic acid.

and in GABA uptake using rat brain cortical slices [105]. The activities of the resolved isomers were also studied on recombinant ρ_1 and ρ_2 GABA_C receptors [97,106] and were found to have a range of activities as either agonists or antagonists, indicating that the compounds were not selective for either GABA_A or GABA_C receptors. Nevertheless, these molecules became leads for the development of selective ρ GABA_C antagonists [85,107].

A series of cyclopentane carboxylic acid analogs of GABA was also found to be antagonists at ρ GABA_C receptors [106]. However, increasing the size of the cyclopentane ring to a cyclohexane ring or reducing the size to a cyclobutane ring led to analogs with markedly reduced activity at either the GABA_A or GABA_C receptors [102,103,107,108].

Imidazole-4-acetic acid (I-4AA; **Figure 3**), a naturally occurring bioisostere of GABA, possesses a mixed pharmacological profile at the various ρ receptors: at ρ_1 receptors, I-4AA is a potent antagonist possessing low intrinsic activity; at ρ_2 receptors, it is a potent partial agonist with a 12-fold higher intrinsic activity than at

ρ_1 receptors; while it was found to be a potent antagonist at ρ_3 receptors. Thus, I-4AA can be used to distinguish between the ρ_1 , ρ_2 and ρ_3 receptors [34,92,94,109]. More recently, substituted analogs of I-4AA analogs have been developed. Among the series of analogs synthesized, more lipophilic and selective agonists were identified for the GABA_C receptor (**Figure 3**). It was found that substituents in the 5-position were tolerated while the same substituent in the 2-position led to inactive molecules [110]. **Table 1** summarizes the activities of selective agonists known for the ρ GABA_C receptor.

Antagonists at ρ GABA_C receptors

There are reports showing that some GABA_A receptor antagonists are also antagonists at ρ GABA_C receptors including GABAzine (SR-95531), while other reports show certain GABA_A receptor agonists act as antagonists at GABA_C receptors. These include isonipepic acid, 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol (THIP; gaboxadol) and piperidin-4-ylsulfonic acid. Picrotoxin is a noncompetitive GABA_C and GABA_A antagonist [33].

Krehen *et al.* evaluated a series of THIP analogs and found that iso-THIP is a more potent GABA_C antagonist than THIP, while aza-THIP was found to be a moderate, competitive antagonist at human ρ_1 GABA_C receptors that is virtually inactive at GABA_A receptors [111].

The methyl-substituted TACA analog, 2-MeTACA, is a competitive ρ_1 GABA_C antagonist. The (*R*)-isomer of methyl-substituted GABA, (*R*)-2-MeGABA is also a moderate antagonist at ρ_1 and ρ_2 GABA_C receptors [97]. The GABA homolog δ -aminovaleric acid, a GABA_B antagonist, and the isothiuronium CACA analog Z-3-([aminoiminomethyl]thio) prop-2-enoic acid, a GABA_A agonist [112], are both moderate GABA_C antagonists [33,113].

A series of cyclopentene analogs of GABA was synthesized [114] and their activities at GABA receptors studied [105,106]. Double-bond insertion at the C2–3 position gives the *cis*-pair of isomers (+) and (–)-*cis*-4-aminocyclopent-2-ene-1-carboxylic acid ((±)-4-CACPCA), and the *trans*-pair of isomers (+) and (–)-*trans*-4-aminocyclopent-2-ene-1-carboxylic acid ((±)-4-TACPCA). The *cis* isomers were found to be weak antagonists at human ρ_1 and ρ_2 GABA_C receptors expressed in *Xenopus* oocytes [106]. Double-bond insertion specifically at the C1–2 position restricts the movement of the carboxylic acid group and thus gives the isomers

Table 1. Summary of the activities of GABA_C-selective agonists.

Compound	GABA _A	GABA _C	GABA _B	Ref.
CACA	Inactive <500 μM	Partial agonist ρ_1 $EC_{50} = 74 \mu M$ ρ_2 $EC_{50} = 70 \mu M$ ρ_3 $EC_{50} = 139 \mu M$	Inactive	[94,96]
(+)-CAMP	Weak antagonist	Full agonist ρ_1 $EC_{50} = 40 \mu M$ ρ_2 $EC_{50} = 17 \mu M$	Inactive	[101]
5-Me-I-4AA	> 1mM	ρ_1 $EC_{50} = 22 \mu M$	Not determined	[110]

CACA: Cis-aminocrotonic acid; CAMP: Cis-2-(aminomethyl)cyclopropanecarboxylic acid.

(+)-4-aminocyclopent-1-enyl carboxylic acid ([+]-4-ACPCA) and (-)-4-aminocyclopent-1-enyl carboxylic acid ([-]-4-ACPCA). (+)-4-ACPCA is a GABA_C antagonist; while (-)-4-ACPCA is largely inactive. This suggests that the (*S*)-configuration at the amino terminal is preferred at ρ GABA_C receptors [97,106].

During the initial characterization of ρ GABA_C receptors, Woodward *et al.* found that certain phosphinic acid analogs of GABA, which are potent GABA_B receptor agonists, are also potent ρ GABA_C receptor antagonists, while the phosphonic acid analogs were less potent [113]. Consequently, phosphinic acid analogs of GABA, CACA and TACA that were originally developed as potent GABA_B receptor ligands [115,116], were subsequently studied at ρ_1 GABA_C receptors expressed in *Xenopus* oocytes [108]. The bioisosteres of GABA, 3-aminopropylphosphinic acid (3-APPA; **Figure 4**) and 3-aminopropyl(methyl)phosphinic acid (3-APMPA; **Figure 4**) are among the most potent competitive antagonists at ρ_1 GABA_C receptors. Introduction of unsaturation between C2 and C3 appear to reduce the affinity for GABA receptors, while methyl phosphinic acid analogs are more potent and selective at GABA_C over GABA_A receptors than the H-phosphinic acid counterparts. Nonetheless, despite their activity for the GABA_C receptor, these compounds are also potent GABA_B agonists or antagonists. The poor affinity of phosphinic acids for GABA_A receptors indicates such a bioisostere could be used to differentiate the GABA_C receptors from the GABA_A receptors.

Despite the reduced activity of the phosphonic acid analog of GABA, phosphonic diesters were recently prepared and alkylphosphonic acid 2-aminoethyl esters were identified as a novel class of potent ρ GABA_C receptor antagonists with no activity at recombinant $\alpha_1\beta_2\gamma_2$ GABA_A receptors [117]. Whether this class of molecules is active on GABA_B receptors remains to be determined.

The first selective GABA_C antagonist, (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA; **Figure 5**) was developed by Murata *et al.* [118], followed by an improved synthetic route by Hanrahan *et al.* [119] and Dumond *et al.* [120]. The structure development was based on the observation that isoguvacine is an agonist at both GABA_A and GABA_C receptors but virtually inactive at GABA_B receptors. In stark contrast, 3-APMPA, the methyl phosphinic acid analog of GABA exhibits agonist effects at GABA_B and antagonist effects at GABA_C receptors but is inactive

3-APPA	3-APMPA	SGS742 (CGP36742)
EC ₅₀ (GABA _A) = 938 μ M	GABA _A = inactive	IC ₅₀ (GABA _A) = 508 μ M
EC ₅₀ (GABA _B) = 0.8 μ M	EC ₅₀ (GABA _B) = 0.15 μ M	IC ₅₀ (GABA _B) = 38 μ M
IC ₅₀ (ρ_1) = 2.47 μ M	IC ₅₀ (ρ_1) = 0.75 μ M	IC ₅₀ (ρ_1) = 62.5 μ M

Figure 4. The phosphinic acid analogs of GABA, 3-APPA, 3-APMPA and SGS742 or CGP36742.

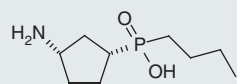
APMPA: Aminopropyl(methyl)phosphinic acid; APPA: Aminopropylphosphinic acid; SGS742 (or CGP36742): (3-aminopropyl)-*N*-butylphosphinic acid.

at GABA_A receptors. Therefore, it was reasoned that the incorporation of a tetrahydropyridine ring and the replacement of the carboxylic acid moiety with a methyl phosphinic acid moiety would yield a compound that is inactive at both GABA_A and GABA_B receptors. TPMPA was found to be a potent antagonist at the ρ_1 GABA_C receptors, demonstrating a more than 100-fold selectivity for GABA_C over GABA_A receptors, where it is a weak antagonist. It is approximately 500-times more selective at inhibiting GABA_C than activating GABA_B receptors [118,121]. The saturated piperidine analog of TPMPA, (piperidin-4-yl)methylphosphinic acid (P4MPA; **Figure 5**), is also a superior antagonist that is more selective for ρ GABA_C receptors than both GABA_A and GABA_B receptors [122]. The selenic acid analog of P4MPA, piperidin-4-ylseleninic acid (SEPI, **Figure 5**) is also a potent antagonist at ρ_1 GABA_C receptors [123]. These results suggest that phosphinic and selenic acid analogs of GABA constricted by a ring system are likely to be one of the preferred

TPMPA	P4MPA	SEPI
K _B ($\alpha_1\beta_2\gamma_2$) = 320 μ M	K _B ($\alpha_1\beta_2\gamma_2$) = >100 μ M	EC ₅₀ ($\alpha_1\beta_3\gamma_2$) = 200 μ M
EC ₅₀ (GABA _B) = ~500 μ M	K _B (GABA _B) = >1 mM	GABA _B = inactive
K _B (ρ_1) = 2.3 μ M	K _B (ρ_1) = 6.0 μ M	K _i (ρ_1) = 0.95 μ M
K _B (ρ_2) = 14.9 μ M	K _B (ρ_2) = 4.2 μ M	
K _B (ρ_3) = 4.5 μ M	K _B (ρ_3) = 10.2 μ M	

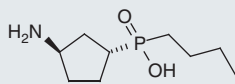
Figure 5. TPMPA, P4MPA and SEPI.

P4MPA: (Piperidin-4-yl)methylphosphinic acid; SEPI: Piperidin-4-ylseleninic acid; TPMPA: (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid.



(±)-*cis*-3-ACPBuPA

GABA_A ($\alpha_1\beta_2\gamma_{2L}$) = weak antagonist
GABA_B = weak antagonist
IC₅₀ (ρ_1) = 5.06 μ M
IC₅₀ (ρ_2) = 11.08 μ M



(±)-*trans*-3-ACPBuPA

GABA_A ($\alpha_1\beta_2\gamma_{2L}$) = weak antagonist
GABA_B = weak antagonist
IC₅₀ (ρ_1) = 72.58 μ M
IC₅₀ (ρ_2) = 189.7 μ M

Figure 6. The (±)-*cis*- and (±)-*trans*-3-ACPBuPA.
ACPBuPA: (Aminocyclopentane)methylphosphinic acid.

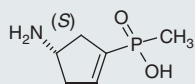
conformations to achieve selective antagonist effects at GABA_C receptors.

The investigation of conformationally restricted analogs targeting the GABA_C receptors continued with the study of a series of alkyl-substituted phosphinic acid cyclopentane analogs. Hanrahan *et al.* reported the syntheses of the methyl-substituted (±)-*cis/trans*-3-aminocyclopentane)methylphosphinic acid, ([±]-*cis* and [±]-*trans*-3-ACPMMA, respectively; **FIGURE 6**), and the butyl-substituted (±)-*cis/trans*-3-aminocyclopentane)butylphosphinic acid ([±]-*cis* and [±]-*trans*-3-ACPBuPA, respectively; **FIGURE 6**) [124]. All of the phosphinic acid analogs are potent GABA_C receptor antagonists, with the general trend of being more potent at human ρ_1 and ρ_2 receptors and slightly less potent at rat

ρ_3 receptors expressed in *Xenopus* oocytes. They are also weak-to-moderate GABA_A receptor antagonists and weak GABA_B agonists [122]. It was found that (±)-*cis*-3-ACPMMA were slightly more potent and, in general, more selective than (±)-*trans*-3-ACPMMA at GABA_C receptors. A larger substituent on the phosphinic acid moiety led to an increase in selectivity for the GABA_C receptor, as activities at both GABA_A and GABA_B receptors were dramatically diminished, as shown by the *N*-butyl substituted analogs (**FIGURE 6**). However, the larger substituent also caused a reduction in antagonist activity at the same receptor, as compared with the methyl-substituted analogs. The abolishment of activities at both GABA_A and GABA_B receptors could be attributed to the introduction of the cyclopentane ring system and the phosphinic acid moiety, a similar rationale behind the synthesis of TPMMA. Furthermore, the *cis* compounds were also observed to be more potent than the *trans* compounds [125].

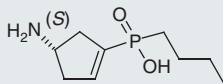
As seen previously, the introduction of unsaturation to the cyclopentane carboxylic acid analogs led to a series of cyclopentene carboxylic acid analogs that converted the compounds from being agonists to potent antagonists. The phosphinic acid moiety, as seen with the straight chain analogs of GABA and the cyclopentane phosphinic acid analogs, also converts the compounds into potent ρ GABA_C antagonists. Furthermore, a large alkyl substitution on the phosphinic acid moiety appears to increase a compound's selectivity for the GABA_C receptors, as shown by the cyclopentane butyl phosphinic acid compounds. Therefore, a similar approach was undertaken with some of the cyclopentene analogs, where the carboxylic acid group is replaced with an alkyl phosphinic acid moiety, and the double bond is fixed at the α position of an alkyl phosphinic acid. The syntheses of alkyl-substituted 4-aminocyclopent-1-enyl phosphinic acid analogs (4-ACPXPA, where X indicates the alkyl substituent at phosphinic acid; **FIGURE 7**) as racemic mixtures and resolved enantiomers, along with their activities at GABA receptors have been reported [107].

In general, all of these analogs are antagonists at both $\alpha_1\beta_2\gamma_{2L}$ GABA_A and ρ_1 GABA_C receptors, with some showing high selectivity for GABA_C receptors. These compounds also displayed weak agonist effects at GABA_B receptors. An increase in the length of the alkyl substituent led to an increase in selectivity for GABA_C receptors, although potencies are reduced. Increasing



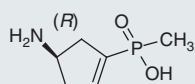
(S)-4-ACPMMA

GABA_A ($\alpha_1\beta_2\gamma_{2L}$) = weak antagonist
EC₅₀ GABA_B = 52.2 μ M
K_B (ρ_1) = 0.78 μ M



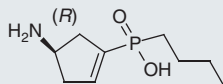
(S)-4-ACPBuPA

GABA_A ($\alpha_1\beta_2\gamma_{2L}$) = weak antagonist
GABA_B = weak antagonist
K_B (ρ_1) = 4.97 μ M



(R)-4-ACPMMA

GABA_A ($\alpha_1\beta_2\gamma_{2L}$) = inactive
EC₅₀ GABA_B = 71.9 μ M
K_B (ρ_1) = 18.9 μ M



(R)-4-ACPBuPA

GABA_A ($\alpha_1\beta_2\gamma_{2L}$) = inactive
GABA_B = weak antagonist
K_B (ρ_1) = 59.3 μ M

Figure 7. (S)- and (R)-4-ACPMMA and (S)- and (R)-4-ACPBuPA.
ACPBuPA: (Aminocyclopentane)methylphosphinic acid.
ACPMMA: (Aminocyclopentane)methylphosphinic acid.

the bulkiness of the substituent, such as the isopropyl- and benzyl-substituted compounds, causes a major reduction in activity at GABA_C receptors. Interestingly, the isopropyl compound was found to be more selective and potent at the GABA_A receptor. It was revealed from the resolved isomers that the (*S*)-enantiomer is more potent than the (*R*)-enantiomer.

Kumar *et al.* also synthesized and studied the activities of the methyl-substituted cyclobutane phosphinic acid analogs *cis/trans*-3-amino-cyclobutane)methylphosphinic acid (*cis/trans*-3-ACBMPA), and evaluated their effects on recombinant GABA receptors. They were found to be weak ρ_1 GABA_C antagonists and were inactive at GABA_B receptors [107].

Recently guanidino analogs have been shown to be potent ρ_1 GABA_C receptor antagonists [126] indicating that structural manipulation of the amino-terminal region of GABA can be modified in the search for selective agents. Indeed combining the guanidine moiety with a cyclic phosphinic acid (3-[guanido]-1-oxo-1-hydroxy-phospholane; **Figure 8**) led to a potent and selective GABA_C antagonist as opposed to the amino analog, 3-(aminomethyl)-1-oxo-1-hydroxy-phospholane [127]. Furthermore, 'muscimol-biotin' derivatives retain ρ_1 GABA_C activity [128]. This is the first amide reported to show activity at these receptors and it can be tethered directly to the receptor, an invaluable tool for visualizing the receptor on cell surfaces [129]. **Table 2** summarizes the activities selective antagonists known for the GABA_C receptor.

Modulators of ρ GABA_C receptor activity

GABA_C receptors are insensitive to many GABA_A allosteric modulators, such as benzodiazepines and barbiturates [31]; however, some GABA_A modulators also allosterically modulate GABA_C receptors [34,93]. Zinc (Zn²⁺) and some divalent cations were found to be negative modulators of GABA_C receptors; while lanthanides act as positive modulators at ρ_1 GABA_C receptors expressed in *Xenopus* oocytes [130,131]. Synthetic neurosteroids have been shown to exert both positive and negative modulation at the ρ_1 GABA_C receptors in a stereoselective manner [94,132,133]. Recently, loreclezole and (+)-ROD-188, both positive modulators at GABA_A receptors, were found to be negative modulators at ρ_1 GABA_C receptors [93,134]. In addition, a limited number of flavonoids have also been found to be modulators of GABA_C receptors [135].

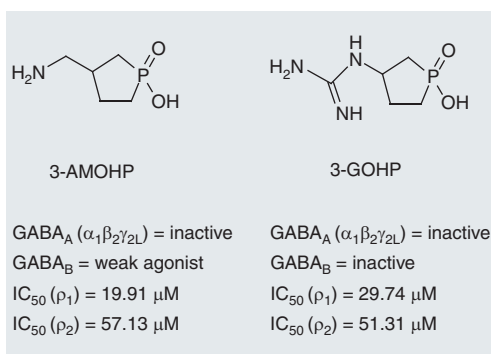


Figure 8. 3-AMOHP and 3-GOHP.

3-AMOHP: 3-(aminomethyl)-1-oxo-1-hydroxy-phospholane; 3-GOHP: 3-(guanido)-1-oxo-1-hydroxy-phospholane.

GABA_C agents as future therapies

(1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid and related GABA_C antagonists have been patented for the treatment of myopia [136]. TPMPA but not GABA_B antagonists inhibited the form-deprived myopia by displaying parallel inhibition of retinal elongation in the axial and equatorial dimensions. Both TPMPA and P4MPA have been used to study the role of GABA_C receptors in short-term memory formation in young chicks using a single-trial passive and discriminated avoidance task. In these studies, both TPMPA and P4MPA enhanced weakly reinforced memory in a dose- and time-dependent manner [3].

Table 2. Summary of the activities of selective GABA_C antagonists.

Compound	GABA _A	GABA _C	GABA _B	Ref.
TPMPA	Weak antagonist $K_B = 320 \mu\text{M}$	Antagonist $\rho_1 K_B = 2.1 \mu\text{M}$ $\rho_2 K_B = 14.9 \mu\text{M}$ $\rho_3 K_B = 4.5 \mu\text{M}$	Weak agonist $\text{EC}_{50} \sim 500 \mu\text{M}$	[92,94,121]
P4MPA	$K_B > 100 \mu\text{M}$	$\rho_1 K_B = 6 \mu\text{M}$ $\rho_2 K_B = 4.2 \mu\text{M}$ $\rho_3 K_B = 10.2 \mu\text{M}$	$> 1 \text{ mM}$	[92,94]
SEPI	$\text{IC}_{50} = 200 \mu\text{M}$	$\rho_1 K_i = 0.95 \mu\text{M}$	Inactive	[123]
(\pm)- <i>cis</i> -ACPBuPA	Inactive $< 300 \mu\text{M}$	$\rho_1 \text{IC}_{50} = 5 \mu\text{M}$	Weak antagonist	[85]
(\pm)- <i>trans</i> -ACPBuPA	Inactive $< 300 \mu\text{M}$	$\rho_1 \text{IC}_{50} = 73 \mu\text{M}$	Weak antagonist	[85]
(<i>R</i>)-ACPBuPA	Inactive at $600 \mu\text{M}$	$\rho_1 K_B = 59.3 \mu\text{M}$	Inactive $< 300 \mu\text{M}$	[107]
(<i>S</i>)-ACPBuPA	Inactive $< 600 \mu\text{M}$	$\rho_1 K_B = 4.97 \mu\text{M}$	Inactive $< 300 \mu\text{M}$	[107]
3-GOHP	Inactive $< 1 \text{ mM}$	$\rho_1 \text{IC}_{50} = 30.0 \mu\text{M}$	Inactive $< 600 \mu\text{M}$	[127]

3-GOHP: 3-(guanido)-1-oxo-1-hydroxy-phospholane; ACPBuPA: (Aminocyclopentane)methylphosphinic acid; K_B : Equilibrium dissociation constant for the competitive antagonist; K_i : Inhibitory constant of the antagonist; P4MPA: (Piperidin-4-yl)methylphosphinic acid; SEPI: Piperidin-4-ylseleninic acid; TPMPA: (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid.

(1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid has also been used to study GABA_C receptor function in the retina [137], cerebral cortex zone [138], cerebellum [139], hippocampus [82], lateral geniculate nucleus [77], superior colliculus [140], spinal cord [72], anterior pituitary [141] and duodenum [84].

(1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid has been used to study the involvement of ρ GABA_C receptors in sleep–waking behavior [142] and in antinociception in the periphery [143]. In the study by Arnaud *et al.*, vehicle and various amounts of TPMPA were randomly infused in the fourth ventricle of the rat [142]. TPMPA induced an increase of waking, which was the consequence of enhancement of both active and quiet wakefulness whereas total slow wave sleep and paradoxical sleep were decreased. It was suggested that ρ GABA_C receptor modulators could be potential medications acting at low doses with fewer side effects.

It is not clear whether or not TPMPA crosses the blood–brain barrier on systemic administration but it can be used to delineate the role

of ρ GABA_C receptors in the periphery. Few researchers focus on GABA receptors outside the brain and it is pertinent that research is carried out in these areas to delineate the role they play there. However, the development of prodrugs for TPMPA along with analogs that either are more lipophilic or can ride the transporters will be of significant benefit to study these receptors centrally.

Finally SGS742, (3-aminopropyl)-*N*-butylphosphinic acid (also known as CGP36742; **FIGURE 4**), is one of a range of phosphinic acid analogs of GABA that act as GABA_C antagonists [108]. It was developed as an orally active GABA_B receptor antagonist [116] and showed therapeutic potential for the treatment of cognitive deficits, petit mal epilepsy and depression [136], reaching Phase II trials for the treatment of cognitive impairment due to Alzheimer's disease. The discovery that it was also a ρ GABA_C receptor antagonist approximately half as potent as at GABA_B receptors [108] led to the development of cyclopentane analogs in which the conformational flexibility of the

Executive summary

Subunit composition, distribution & function of GABA_C receptors

- The ρ GABA_C receptor is a homopentameric chloride channel belonging to the cys-loop class of receptors.
- Three subtypes exist in mammals (ρ_{1-3}) and predominantly reside in the retina, although there is growing evidence that this subunit exists throughout the brain and periphery.
- Knock-out mice models and pharmacological studies show that the ρ GABA_C receptors play a role in myopia development, visual processes, and learning and memory.

Agonists at ρ GABA_C receptors

- The ρ GABA_C receptors have a unique pharmacology. These receptors are not blocked by bicuculline or activated by baclofen.
- Selective agonists include *cis*-aminocrotonic acid (CACA), (+)-(aminomethyl)cyclopropanecarboxylic acid ([+]-CAMP) and the imidazole-4-acetic acid (I-4AA) derivatives, 5-Ph I-4AA and 5-Me I-4AA.
- The I-4AA derivatives are more lipophilic than other selective agonists and may be important molecules to ascertain the physiological role of ρ GABA_C receptors *in vivo*.

Antagonists at ρ GABA_C receptors

- Selective antagonists for the ρ GABA_C receptor include 1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA), and substituted 4-aminocyclopent-1-enyl and 3-aminocyclopentanyl alkylphosphinic acids.
- Studies show that the phosphinic bioisostere imparts selectivity to GABA_C over GABA_A receptors and the conformationally restricted rings systems, such as piperidine and cyclopentanes impart selectivity for GABA_C over GABA_B receptor.

GABA_C agents as future therapies

- SGS742, the butylphosphinic acid analog of GABA, is a potent antagonist at both GABA_B and GABA_C receptors.
- SGS742 reached Phase II clinical trials for Alzheimer's disease. It is not clear whether the enhanced learning and memory exerted by SGS742 is via the GABA_B or GABA_C receptor.
- Evidence is growing to suggest that ρ GABA_C-selective antagonists, including TPMPA and (\pm)-*cis*-3-(aminocyclopentane)methylphosphinic acid, enhance learning and memory.
- TPMPA and (\pm)-*cis*-3-(aminocyclopentane)methylphosphinic acid also inhibit myopia development.
- Future studies are required to ascertain the mechanism of action of ρ GABA_C receptors in both myopia development and learning and memory.
- The role of ρ GABA_C receptors in the periphery remains unexplored.

3-aminopropyl moiety was constrained. These cyclopentane analogs were inactive at GABA_B receptors but retained the GABA_C receptor antagonist activity of SGS742. Of particular interest is (\pm)-*cis*-3-ACPBuPA, which was shown to be a selective GABA_C antagonist that enhanced learning and memory following intraperitoneal injection in rats and inhibited the development of myopia on intravitreal injection in chicks [85]. (\pm)-*cis*-3-ACPBuPA and related cyclopentane and cyclopentene analogs have been patented for use in enhancing cognitive activity [202,203]. Thus, selective GABA_C receptor ligands may become important agents for

therapy not only of myopia and learning, and memory-related disorders but also the treatment of pain and sleep disorders.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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