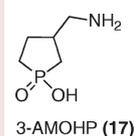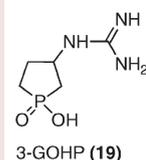# Novel Cyclic Phosphinic Acids as GABA_C ρ Receptor Antagonists: Design, Synthesis, and Pharmacology

Navnath Gavande,[†] Izumi Yamamoto,[†] Noeris K. Salam,[‡] Tu-Hoa Ai,[§] Peter M. Burden,[§] Graham A. R. Johnston,[§] Jane R. Hanrahan,[†] and Mary Chebib*,[†]

[†]Faculty of Pharmacy, The University of Sydney, NSW, Australia, [‡]Schrodinger, Inc., 8910 University Center Lane, Suite 270, San Diego, California, United States, and [§]Adrien Albert Laboratory, Department of Pharmacology, The University of Sydney, NSW, Australia

ABSTRACT   Understanding the role of GABA_C receptors in the central nervous system is limited due to a lack of specific ligands. Novel γ-aminobutyric acid (GABA) analogues based on 3-(aminomethyl)-1-oxo-1-hydroxy-phospholane **17** and 3-(guanido)-1-oxo-1-hydroxy-phospholane **19** were investigated to obtain selective GABA_C receptor antagonists. A compound of high potency (**19**, $K_B = 10$ μM) and selectivity (greater than 100 times at ρ_1 GABA_C receptors as compared to $\alpha_1\beta_2\gamma_{2L}$ GABA_A and GABA_{B(1b,2)} receptors) was obtained. The cyclic phosphinic acids (**17** and **19**) are novel lead agents for developing into more potent and selective GABA_C receptor antagonists with increased lipophilicity for future in vivo studies.

KEYWORDS γ-Aminobutyric acid, ligand-gated ion channels, GABA receptors, cyclic phosphinic acids, two-electrode voltage clamp, ρ_1 GABA_C homology model

GABA_C ρ_1 IC$_{50}$ = 19.91 μM
GABA_C ρ_2 IC$_{50}$ = 57.13 μM

3-AMOHP (**17**)

GABA_C ρ_1 IC$_{50}$ = 29.74 μM
                    (K_B = 10 μM)
GABA_C ρ_2 IC$_{50}$ = 51.31 μM

3-GOHP (**19**)

γ-Aminobutyric acid **1** (GABA **1**, Figure 1) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS) and is essential for the overall balance between neuronal excitation and inhibition.[1,2] GABA influences neurons via three major classes of receptors that are grouped on the basis of their subunit composition, gating properties, and pharmacological profiles: GABA_A, GABA_B, and GABA_C (GABA ρ) receptors. GABA_A and GABA_C receptors are ionotropic receptors, belonging to the Cys loop family of ligand-gated ion channels, which also incorporates nicotinic acetylcholine, strychnine-sensitive glycine, serotonin type 3, and some invertebrate anionic glutamate receptors.[1,2] Both GABA_A and GABA_C receptors are chloride channels that mediate fast synaptic inhibition when activated by GABA. In contrast, GABA_B receptors are members of the family 3 class metabotropic receptors; these receptors couple via G proteins (G_{i/o}) to interact with inwardly rectifying potassium (GIRK) and voltage-gated calcium channels, mediating slow, longer lasting synaptic inhibition by increasing potassium and decreasing calcium conductances.[3]

The ionotropic GABA_A receptors are transmembrane protein complexes composed of five heteropentameric subunits. So far, 16 human GABA_A receptor subunits have been identified, and they have been classified into α ($\alpha_1-\alpha_6$), β ($\beta_1-\beta_3$), γ ($\gamma_1-\gamma_3$), δ, ε, π, and θ. Although a wide range of different GABA_A receptor combinations exist in vivo, the most common is the $\alpha_1\beta_2\gamma_2$ combination, constituting approximately 18% of all GABA_A receptors in the human brain.[4] The GABA_B receptors consist of heterodimers, which are composed of two subunits, a ligand-binding subunit (GABA_{B1}), and a signal transduction subunit (GABA_{B2}).[3]

The GABA_C receptor has distinct pharmacology, physiology, and subunit composition to that of GABA_A and GABA_B receptors.[1,5,6] Consisting of homo-oligomeric or pseudohomo-oligomeric pentameric compositions of ρ subunits (ρ_1, ρ_2, and ρ_3 subunits) in mammals,[5,7] the receptors are highly expressed in many parts of the brain, including the superior colliculus,[8] cerebellum,[9] hippocampus (high ρ_2 subunit expression),[10] and, most prominently, the retina (high ρ_1 subunit expression).[11]

The first selective GABA_C receptor antagonist developed was 1,2,5,6-tetrahydropyridine-4-yl-methyl-phosphinic acid **3** (TPMPA **3**, Figure 1).[12] TPMPA **3** has been shown to enhance memory in chicks,[13] inhibit myopia development in chicks,[14] modulate the sleep-waking behavior in rats,[15] and exert influence on the lateral nucleus of the amygdala (LA).[16] However, TPMPA **3** probably does not cross the blood brain barrier, and there have been no reports delineating the CNS effects of TPMPA **3** upon systemic administration.

Recently, we have synthesized a series of conformationally restricted 3-aminocyclopentane/cyclopentene alkylphosphinic acids from a structure–function study of various aminocyclopentane/cyclopentene phosphinic acid analogues of GABA, and these conformationally restricted alkyl phosphinic acids are highly potent and selective for GABA_C receptors.[17] (S)-4-ACPBPA **6** is the most potent and selective GABA_C receptor antagonist of this series. Interestingly, (S)-4-ACPBPA
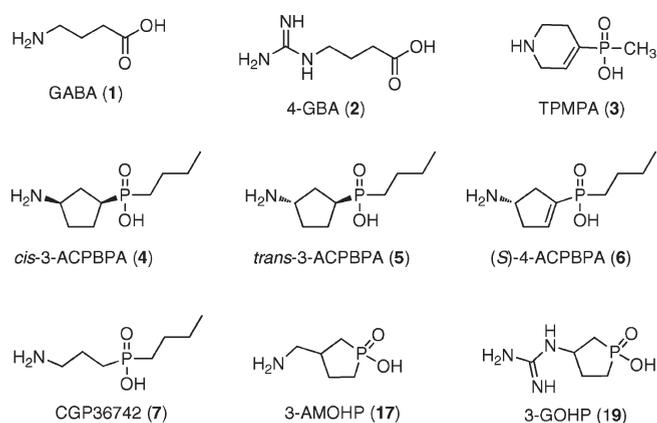
Figure 1. Structures of GABA (**1**), compounds active at GABA receptors (**2**−**7**), and synthesized novel 3-AMOHP (**17**) and 3-GOHP (**19**).

Scheme 1. Synthesis of Compounds **15**−**17**[a]

[a] Reagents and conditions: (a) (*i*-PrO)$_4$Si, CH$_3$CN, reflux for 2 h, 86%. (b) CH$_2$=CHCOOCH$_3$, NaOMe, room temperature for 24 h, 89%. (c) CH$_2$=CHCOOCH$_3$, NaOPr^i, *i*-PrOH, room temperature, 95%. (d) *n*-BuLi, THF, 0 °C to reflux for 4 h, 86% or KOtBu, toluene, 0 °C for 5 h, 83%. (e) 3 N HCl, *i*-PrOH, reflux for 12 h, 49%. (f) Tl(NO$_3$)$_3$·3H$_2$O, CH$_2$Cl$_2$, room temperature for 48 h, 86%. (g) ClCOOCH$_2$CH$_3$, Et$_3$N, THF, −10 °C for 1 h; NaBH$_4$ 0−5 °C, 4 h, 79%. (h) 3 N HCl, reflux for 8 h, 60%. (i) 3 N HCl, reflux for 5 h, 68%. (j) DEAD, 1 M NH$_3$ in benzene, PPh$_3$, THF, 12 h; 3 N HCl, reflux for 3 h, 55%.

6 is a conformationally restricted analogue of the orally active GABA$_{B/C}$ receptor antagonist 3-aminopropyl-*n*-butylphosphinic acid **7** (CGP36742 or SGS742) (GABA$_B$, GABA$_C$, and GABA$_A$: IC$_{50}$ = 38, 62, and 508 μM, respectively). CGP36742 or SGS742 reached phase II clinical trials, enhancing memory in mildly memory-impaired subjects.[18] However, because CGP36742 (SGS742) is approximately equipotent on GABA$_B$ as it is on GABA$_C$ receptors, it is not clear whether the pharmacological effects of CGP36742 are due to its GABA$_B$ or GABA$_C$ activity. In continuation of our efforts to identify the role of GABA$_C$ receptors in the CNS, we discovered that the selective GABA$_C$ receptor antagonists, *cis*- and *trans*-(3-aminocyclopentanyl)-*n*-butylphosphinic acid (*cis*-3-ACPBPA **4** and *trans*-3-ACPBPA **5**; Figure 1), inhibit myopia progression in chicks and facilitate learning and memory in the Morris Water Maze task in rats.[19]

The discovery of potent and selective GABA$_C$ antagonists emphasizes some important pharmacological differences between these GABA receptor families. Thus, GABA$_C$ receptors may be of clinical and pharmacological interest as potential therapeutic targets for myopia, in enhancing cognition and managing memory-related disorders, and its presence in the amygdala might be an alternative target for the development of antianxiety drugs.[16]

To date, the structural manipulations made in developing GABA analogues for the GABA$_C$ receptor have mainly been confined to the carboxylic acid end of the molecule or restricting the conformations of the flexible GABA backbone. As the structure−activity-relationship (SAR) profile of selective GABA$_C$ receptor ligands is limited, there is a need to develop more structurally diverse GABA$_C$ receptor ligands to understand the physiological role of these receptors. During the course of our ongoing research, we envisioned a novel template for the GABA$_C$ receptor by modifying the terminal nitrogen to incorporate a guanidino functional group, which is known to act at GABA receptors[20] along with restraining the phosphinic acid moiety (bioisostere of the carboxylic acid). Therefore, in the present study, we report the design, synthesis, and pharmacological evaluation of synthetically challenging cyclic phosphinic acid analogues as GABA$_C$

receptor antagonists. The cyclic phosphinic acid nucleus has not been previously used for GABA analogues, and only one paper has described similar cyclic phosphinic acid derivatives as glutamate receptor agonists.[21]

The synthesis of 3-(aminomethyl)-1-oxo-1-hydroxy-phospholane **17** (3-AMOHP) is depicted in Scheme 1. The intermediate isopropoxy-1-oxophosphorinan-4-one **12** was prepared in five steps from water-free hypophosphoric acid, with few modifications as previously described by Verkade et al.[22] Hypophosphoric acid was treated with commercially available tetraisopropyl orthosilicate to afford isopropyl phosphinate **8**, which was treated with methyl acrylate to provide diester **9**. The diester **9** was treated with methyl acrylate in isopropanol to afford triester **10**, which was cyclized in the presence of base to afford compound **11**. The selective hydrolysis of compound **11** is a key step to afford intermediate

isopropoxy-1-oxophosphorinan-4-one **12**. The selectivity relies on the difference in reactivity of the carboxylic and phosphinic esters. We investigated several different reaction conditions (such as different concentrations of HCl in isopropanol at room/elevated temperature), and the best condition, which gave a reasonable yield of the intermediate **12**, was the treatment of the compound **11** with 3 N hydrochloric acid in the presence of isopropanol.

The phospholane-1-oxide carboxylic acid **13** was synthesized from keto phosphinane-1-oxide **12** via ring contraction along with oxidation reaction in one step.[23] This observation may be of synthetic utility for five-membered heterocycle formation; as to the authors knowledge, it is the first demonstration of direct one-step access to heterocyclic five-membered carboxylic acid from heterocyclic

six-membered ketone. The carboxylic acid **13** was reduced to primary alcohol **14** using $NaBH_4$ through in situ mixed carbonic−carboxylic acid anhydride. Finally, the amine was generated via a one pot Mitsunobu−Staudinger reaction, followed by phosphinate ester hydrolysis using aqueous HCl and the crude product purified via ion-exchange chromatography and recrystallization to give the free amine **17** (Scheme 1). Compounds **15** and **16** were prepared by hydrolysis of corresponding phosphinate esters using aqueous HCl.

The syntheses of 3-(amino)-1-oxo-1-hydroxy-phospholane (3-AOHP) **18** and 3-(guanido)-1-oxo-1-hydroxy-phospholane (3-GOHP) **19** are depicted in Scheme 2. Modified Curtius rearrangement of the acid **13** and hydrolysis of the intermediate isocyanates with aqueous HCl afforded the amine **18**. Compound **19** was prepared by guanylation of amine **18** using formamidinesulfinic acid in the presence of a base.

The functional characterization of the cyclic phosphinic acids at GABA receptors was performed using two-electrode voltage clamp on recombinant human GABA receptors expressed in *Xenopus* oocytes.[17,24] The cyclic phosphinic acids (**15−19**) were evaluated for activity alone and in the presence of GABA on $GABA_A$ ($\alpha_1\beta_2\gamma_{2L}$), $GABA_B$ (1b/2), and $GABA_C$ (ρ_1 and ρ_2) receptors to determine whether they behave as agonists, antagonists, or modulators. It has previously been observed that orientations of the acid, backbone, and amine group are important for antagonist activity at GABA receptors, but the distance between acid−amine counterparts and the use of alkyl phosphinic acids as a bioisostere of a carboxylic acid are also an important criterion to develop selective $GABA_C$ antagonists.[17]

As shown in Table 1, we have examined the effects of cyclic phosphinic acids (**15−19**) on $GABA_A$, $GABA_B$, and $GABA_C$ receptors. Compounds **15** and **16**, which lack the terminal nitrogen showed no effects at 100 μM on all three GABA receptors.

Scheme 2. Synthesis of Compounds 18 and 19[a]
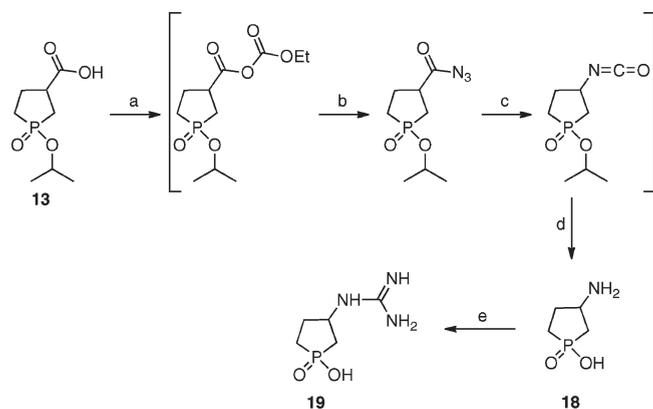
[a] Reagents and conditions: (a) ClCOOCH₂CH₃, Et₃N, acetone, −10 °C for 1 h. (b) NaN₃, H₂O, 2 h. (c) Toluene, reflux for 2 h. (d) 3 N HCl, reflux for 3 h, 48 %. (e) Formamidinesulfinic acid, NaOH, H₂O, room temperature for 12 h, 76 %.

Table 1. Pharmacological Data

compd	human $GABA_C$ receptors IC_{50} (95% CI)/K_B (μM) ρ_1	ρ_2	human $GABA_{B\ (1b/2)}$ receptors EC_{50} (μM) or % inhibition	human $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors K_B (μM) or % inhibition
TPMPA (**3**)	IC_{50} = 2.22 (1.32−6.09) μM K_B = 2.1 μM[a]	IC_{50} = 22.09 (18.87−25.86) μM K_B = 14.9 μM[b]	EC_{50} = ∼500 μM[a]	K_B = 320 μM[a]
(*S*)-4-ACPBPA (**6**)	K_B = 4.97 μM[c]		22.5 ± 1.9 %[c,d]	24.2 ± 1.7 %[c,e]
CGP36742 (**7**)	IC_{50} = 62 μM[f]		IC_{50} = 38 μM[f]	IC_{50} = 508 μM[f]
15	inactive at 100 μM		inactive at 100 μM	inactive at 100 μM
16	inactive at 100 μM		inactive at 100 μM	inactive at 100 μM
17	IC_{50} = 19.91 (16.79−23.60) μM	IC_{50} = 57.13 (54.92−63.39) μM	weak agonist [g]	inactive at 600 μM
18	inactive at 600 μM		9.76 ± 4.6 % [h]	inactive at 600 μM
19	IC_{50} = 29.74 (26.64−35.42) μM K_B = 10 ± 1.6 μM[i]	IC_{50} = 51.31 (47.30−55.40) μM	inactive at 600 μM	29.21 ± 2.3 % [e]

[a] Data from ref 12. [b] Data from ref 24. [c] Data from ref 17. [d] Percentage inhibition by 300 μM compound of the current produced by a submaximal does of GABA (3 μM, EC_{50}). Data are the means ± SEMs (n = 3−5 oocytes). [e] Percentage inhibition by 600 μM compound of the current produced by a submaximal does of GABA (30 μM, EC_{50}). Data are the means ± SEMs (n = 3 oocytes). [f] Data from ref 18. [g] Figure 2A showing the weak agonist effects of 3-AMOHP **17**. [h] Percentage inhibition by 600 μM compound of the current produced by a submaximal does of GABA (3 μM, EC_{50}). Data are the means ± SEMs (n = 3 oocytes). [i] The K_B value is the mean ± SEM.
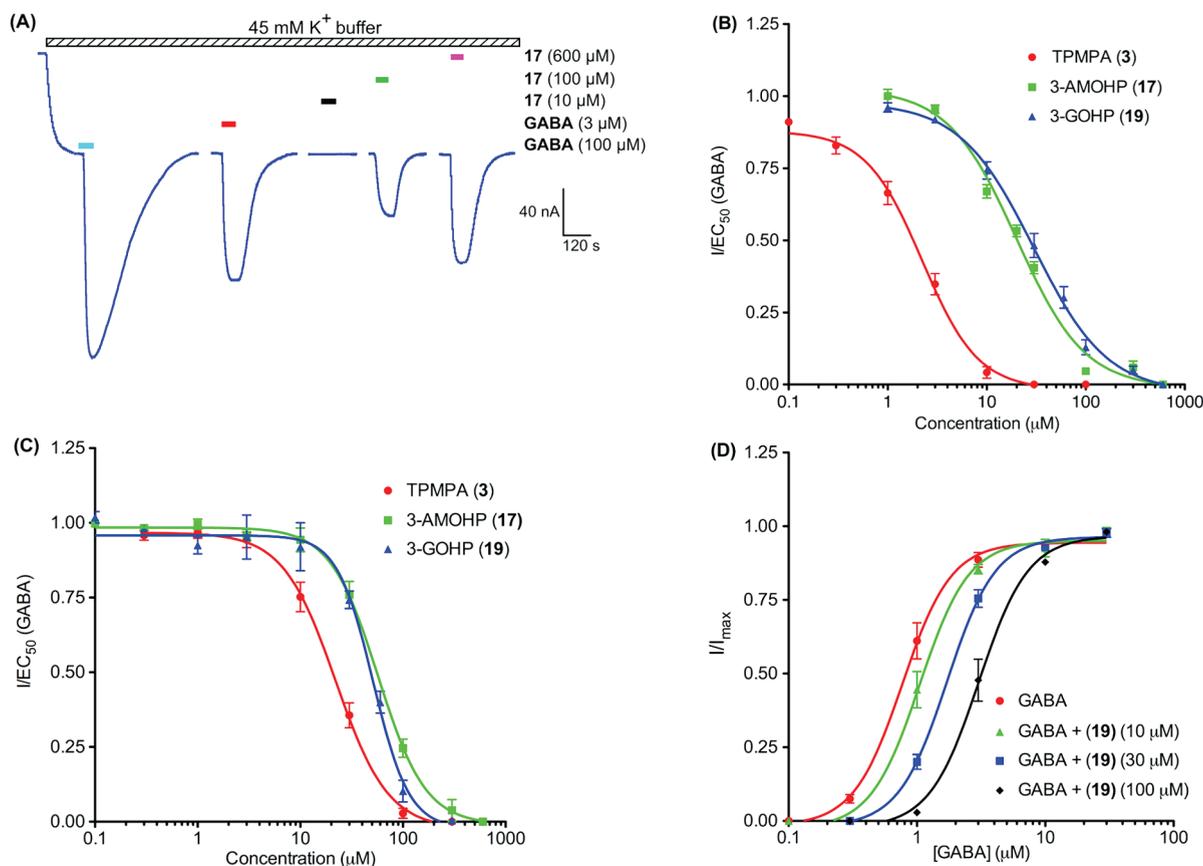
Figure 2. (A) Sample current trace showing the effects of 3-AMOHP **17** on human GABA$_{B(1b/2)}$ receptors coexpressed with GIRK1/4 channels in *Xenopus* oocytes using 45 mM K$^+$ buffer (forward hatched bar). 3-AMOHP **17** had no effect at 10 μM (black bar) but activated GABA$_B$ receptors at 100 (green bar) and 600 μM (purple bar) in a concentration-dependent manner, indicating weak agonist effects at this receptor. (B) Inhibitory concentration−response curves for TPMPA **3** (red dot, $n = 4$), 3-AMOHP **17** (green square, $n = 3$), and 3-GOHP **19** (blue triangle, $n = 4$) against GABA (1 μM) at human ρ_1 GABA receptors expressed in *Xenopus* oocytes. Data are the means $\pm$ SEMs ($n = 3-4$ oocytes). (C) Inhibitory concentration−response curves for TPMPA **3** (red dot, $n = 3$), 3-AMOHP **17** (green square, $n = 4$), and 3-GOHP **19** (blue triangle, $n = 4$) against GABA (1 μM) at human ρ_2 GABA receptors expressed in *Xenopus* oocytes. Data are the means $\pm$ SEMs ($n = 3-4$ oocytes). (D) Concentration−response curves of GABA alone (red dot, $n = 3$) and GABA in the presence of 10 (green triangle, $n = 4$), 30 (blue square, $n = 3$), and 100 μM ($\blacklozenge$, $n = 4$) **19** at human $\rho1$ GABAC receptors expressed in *Xenopus* oocytes. Data are the means $\pm$ SEMs ($n = 3-4$ oocytes).

It proved that both the acid and the amino counterparts are important for the ligand's affinity at these receptors. 3-AMOHP **17** was found to be a potent antagonist (IC$_{50}$ = 19.91 μM) at ρ_1 GABA$_C$ receptors, inactive (at 600 μM) at $\alpha_1\beta_2\gamma_{2L}$ GABA$_A$ receptors, and a weak agonist at GABA$_{B(1b/2)}$ receptors. Figure 2A shows the effect of 3-AMOHP **17** on GABA$_{B(1b/2)}$ receptors expressed in *Xenopus* oocytes. 3-AMOHP **17** shows activation itself at 100 (33.22 $\pm$ 4.65%) and 600 μM (45.17 $\pm$ 5.23%), indicating its weak agonist nature at GABA$_{B(1b/2)}$ receptors. 3-GOHP **19** was developed based on the observed activities of 4-guanidinobutanoic acid **2** (4-GBA), which is an antagonist at GABA$_A$ and GABA$_C$ receptors with no effects on GABA$_B$ receptors.[20] ω-Guanidino acids are known to act like GABA at GABA receptors, indicating that guanidino acids behave as though the guanidino group is equivalent to the amino functionality with a more basic nature.[20] 3-GOHP **19** is a potent and selective antagonist (K_B = 10.0 $\pm$ 1.6 μM) at the ρ_1 GABA$_C$ receptor. 3-GOHP **19** has reduced activity (29% inhibition at 600 μM) at $\alpha_1\beta_2\gamma_{2L}$ GABA$_A$ receptors and was inactive (at 600 μM) at GABA$_{B(1b/2)}$ receptors, indicating that substituting the amine functionality with a guanidino moiety is well

tolerated at the GABA$_C$ receptor ligand-binding site. 3-AOHP **18** was inactive at all three GABA receptors, which indicates that the distance between the acidic group and the terminal nitrogen of GABA receptor ligands appears to be important for ligand affinity at these receptors. Figure 2B shows the inhibitory concentration−response curves for the active analogues (TPMPA **3**, 3-AMOHP **17**, and 3-GOHP **19**) against GABA (1 μM) at ρ_1 GABA$_C$ receptors. The active compounds were further tested at human ρ_2 GABA$_C$ receptors. Figure 2C shows the inhibitory concentration−response curves for the TPMPA **3** (IC$_{50}$ = 22.09 μM), 3-AMOHP **17** (IC$_{50}$ = 57.13 μM), and 3-GOHP **19** (IC$_{50}$ = 51.31 μM) against GABA (1 μM) at ρ_2 GABA$_C$ receptors. Both compounds (**17** and **19**) are moderately potent antagonists at ρ_2 GABA$_C$ receptors. In addition, 3-GOHP **19** caused a parallel rightward shift of the GABA concentration−response curves in the presence of three antagonist concentrations, indicating its competitive nature (Figure 2D; K_B = 10.0 $\pm$ 1.6 μM).

To delineate the key interactions responsible for differences in binding affinity, the structures of TPMPA **3**, (S)-4-ACPBPA **6**, and each stereoisomer of 3-AMOHP **17**, 3-AOHP **18**, and 3-GOHP **19** were flexibly docked into the
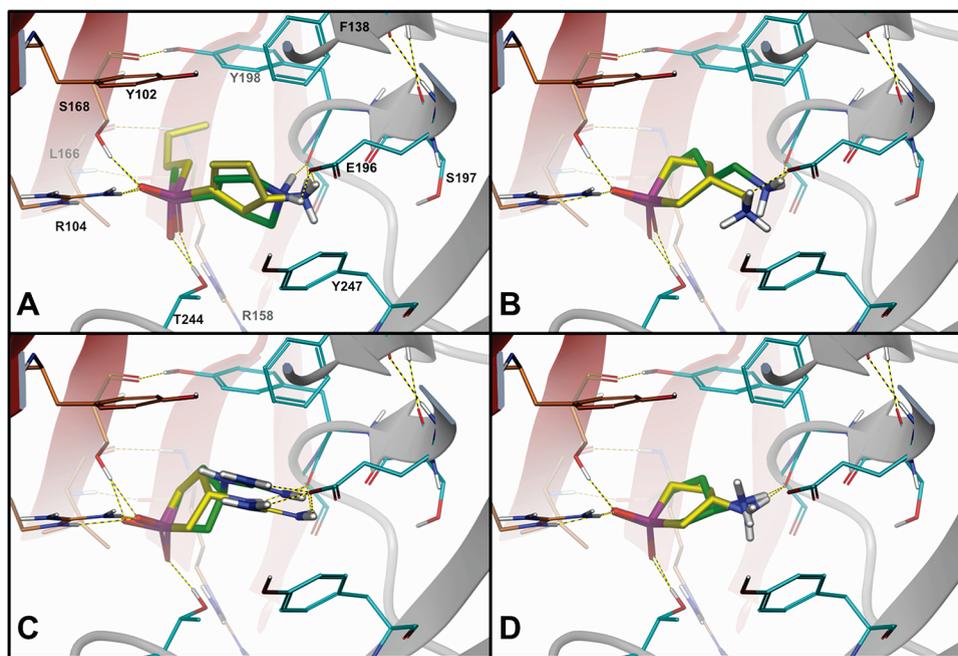
Figure 3. View of the ρ_1 GABA$_C$ ligand-binding site with predicted binding modes. (**A**) TPMPA **3** (green carbons) and (*S*)-4-ACPBPA **6** (yellow carbons). (**B**) Stereoisomer of 3-AMOHP **17**: (*R*)-3-AMOHP (green carbons) and (*S*)-3-AMOHP (yellow carbons). (**C**) Stereoisomer of 3-GOHP **19**: (*R*)-3-GOHP (green carbons) and (*S*)-3-GOHP (yellow carbons). (**D**) Stereoisomer of 3-AOHP **18**: (*R*)-3-AOHP (green carbons) and (*S*)-3-AOHP (yellow carbons).

ligand-binding site of a ρ_1 GABA$_C$ homology model (see the Supporting Information for details). Figures 3B,C shows the *S*- and *R*-stereoisomers of both 3-AMOHP **17** and 3-GOHP **19**. These compounds are predicted to bind similarly to TPMPA **3** and (*S*)-4-ACPBPA **6** (Figure 3A). The binding affinity is largely ascribed to various electrostatic interactions,[25−28] including (i) a salt bridge interaction between the phosphinic acid and the Arg104,[25] (ii) a salt bridge interaction between the basic amine/guanidino and the Glu196,[25] (iii) hydrogen bond contacts to Ser168 and Thr244 and to the backbone carbonyl of Tyr198,[26,27] and (iv) a cation···π attraction between the basic amine and the Tyr247, an experimentally determined interaction.[28] While similar binding energies are predicted for the structures of 3-AMOHP **17** and 3-GOHP **19** (see the Supporting Information), the larger guanidino group of 3-GOHP **19** incurs a slightly higher ligand strain penalty (~3−4 kcal/mol) than 3-AMOHP **17**, which may account for it is slightly decreased activity on ρ_1 GABA$_C$ activity. The complete inactivity of 3-AOHP **18** on ρ_1 GABA$_C$ is most likely due to the fact that neither the *S*- nor the *R*-stereoisomer can optimally span the width of the binding site (Figure 3D); therefore, they are unable to interact with Arg104 and Glu196 simultaneously, amino acids known to be important for GABA binding. Furthermore, they cannot establish a favorable cation···π attraction with Tyr247. Among all ligands docked, the largest difference is seen with (*S*)-4-ACPBPA **6**, which additionally inserts a butyl chain into a hydrophobic pocket enclosed by Tyr198, Leu166, and Arg158 (Cα, Cβ, Cγ, and Cδ atoms) (Figure 3A). This moiety potentially liberates thermodynamically unfavorable waters[29] and highlights a

region for further lead optimization. In conclusion, the synthesis of novel cyclic phosphinic acid template has been developed. The activity of these compounds has been investigated at the three major GABA receptor families, and 3-AMOHP **17** and 3-GOHP **19** (ω-guanidino acid) are discovered as potent and selective GABA$_C$ receptor antagonists. This is first demonstration of a ω-guanidino acid as a selective GABA$_C$ receptor antagonist. These results offer new knowledge of the architecture of GABA$_C$ receptor ligand's for studies regarding the binding site and receptor flexibility. In future studies, it might be useful to generate complete SARs of this novel template for developing into more potent and selective GABA$_C$ receptor antagonists.

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