The Effects of Conformational Constraints in the Polyamine Moiety of Philanthotoxins on AMPAR Inhibition

Henrik Franzyk, John W. Grzeskowiak, Denis B. Tikhonov, Jerzy W. Jaroszewski, Ian R. Mellor

Philanthotoxin-433 (PhTX-433) is a known potent inhibitor of ionotropic glutamate receptors and analogues have been synthesized to identify more potent and selective antagonists. Here, we report on the synthesis of four PhTXs with a cyclopropane moiety introduced into their polyamine chain, and on their inhibition of an AMPA receptor subtype by using two-electrode voltage-clamp on Xenopus oocytes expressing the GluA1flop subunit. All analogues were more potent than PhTX-343 with trans-cyclopropyl-PhTX-343 being the most potent (~28-fold) and cis-cyclopropyl-PhTX-343 least potent (~4-fold). Both cis- and trans-cyclopropyl-PhTX-444 had intermediate potency (both ~12-fold). Molecular modelling indicates that a cyclopropane moiety confers a favourable steric constraint to the polyamine part but this is compromised by a cis conformation due to enhanced intramolecular folding. Elongated PhTX-444 analogues alleviate this to some extent but optimal positioning of the amines is not permitted.

Introduction

Philanthotoxin-433 (PhTX-433, 1); the numerals indicate the number of melanin groups spacing the nitrogenos in the polyamine moiety; Figure 1) is a toxin found naturally in the venom of the solitary wasp, Philanthus triangulum. PhTX-433 and many of its synthetic analogues have been shown to have non-competitive inhibitory effects at both ionotropic glutamate receptors and nicotinic acetylcholine receptors. In that respect PhTXs are attractive molecules to investigate further given that both of these receptor types are accepted as valid drug targets for a variety of neurodegenerative and other disorders of the central nervous system. The modular butyryl-tyrosyl-thermospermine composition of 1 has allowed for efficient generation of many synthetic analogues demonstrating the importance of all of these structure segments. PhTXs 1, 2 and an array of other analogues have been shown to produce powerful voltage-dependent inhibition of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) currents suggesting a binding mode with the polyamine inserted deeply within the pore region of the ion channel. This hypothesis is supported by the observation that AMPARs containing the GluA2 subunit with arginine at the “Q/R” site in the selectivity filter of the pore exhibit drastically reduced inhibition by PhTX-343 and other polyamine-containing molecules.

In the last two decades advanced methodologies for solid-phase synthesis (SPS) of polyamines have been developed, however, no examples of SPS of cyclopropane-containing polyamine derivatives have been reported. The commonly used solution-phase method for obtaining polyamines displaying a cyclopropane moiety is alkilation of mesylenesulfonamides with mesitylenesulfonates of cyclopropane diols, but this is not readily transferred into an SPS protocol due to the harsh conditions required for deprotonation of the sulfonamide and the risk of cross-linking the resin due to the bifunctional building block. Cyclopropane-trans-1,2-dicarboxylic acid may be readily obtained from the corresponding ethyl diester and the cis-anhydride 3-oxabicyclo[3.1.0]hexane-2,4-dione was commercially available, and therefore, we chose an approach involving on-resin reduction of the diamide corresponding to the desired 4,4’-dimethoxytrityl-protected polyamine.

In the present work we focus on incorporating unprecedented structural variations of the polyamine moiety present in both 1 and its well-studied close structural analogue, PhTX-343, and examine how these influence the inhibitory effects of the resulting PhTX analogues on a specific subunit, GluA1flop, present in members of the AMPAR subdivision of the ionotropic glutamate receptor family. This subunit is characteristic of a calcium-permeable and polyamine-sensitive subtype of AMPARs, with the flop splice variant (a 38 amino acid region upstream of the fourth transmembrane region) being upregulated in place of the flip splice variant during early development to become dominating in adult CNS. Constraints were introduced in the central region of the polyamine moiety in an attempt to assess the importance of rigidity in this region. This was achieved by solid-phase synthesis incorporating cyclopropane moieties into...
the polyamine chain to give trans- and cis-analogues with 3-4:3 or 4-4:4 (i.e. PhTX-343- or PhTX-444-like) spacing between the nitrogens (3–6 = trans-343, trans-444, cis-343, cis-444, respectively; Figure 1). Analogues 2–6 were tested for inhibitory potency against homomeric rat GluA1flop AMPA receptors expressed in Xenopus oocytes by measurement of their reduction of currents evoked by stimulation with kainic acid (KA; 100 μM) by using a two-electrode voltage-clamp at a holding potential of ~80 mV. Although KA is a weaker agonist of AMPARs it is often used instead of AMPA or L-Glu in these assays to avoid the problem of rapid receptor desensitization.

**Results and Discussion**

**Solid-phase synthesis of philantotoxin analogues**

First, cyclopropane-trans-1,2-dicarboxylic acid diethyl ester (7) was converted into the corresponding pentafluorophenyl diester 8 (Scheme 1) via alkaline hydrolysis and subsequent transesterification by using pentafluorophenyl trifluoroacetate–pyridine (1:1) in DMF.[15,17] Building blocks 9a and 9b were obtained upon condensation of pentafluorophenyl diester 8 with one equivalent of the appropriate 2-(trimethylsilyl)ethoxycarbonyl (Teoc)-monoprotected diamine.[18]

In order to minimize cross-linking of the 2-chlorotrityl chloride resin, it was loaded with the appropriate Teoc-monoprotected diamine (Scheme 2). A prolonged reaction time of two days enabled a cost-efficient use of these selectively protected diamines as only 10% excess was required. The resulting resin-bound diamines 10a/10b were then coupled with building blocks 9a/9b to give Teoc-protected diamide intermediates 11a/11b that were subjected to exchange of the N-protection group followed by borane reduction to yield the 4,4'-dimethoxytrityl (Dmt)-protected polyamine intermediates 12a/12b. The thus formed secondary amino functionalities were Boc-protected, and then the Dmt group on the terminal primary amine was removed under weakly acidic conditions to give selectively protected resin-bound polyamines 13a/13b. Successive acylation with the activated ester, Fmoc-Tyr(Bu)-OPfp, Fmoc deprotection, acylation with pentafluorophenyl butanolate,[18] and cleavage from the linker afforded the crude trans-cyclopropane-containing target philantotoxins that readily were pulsed by reversed-phase preparative HPLC to give PhTXs 3 and 4 as the tris(TFA) salts.

![Scheme 1](image)

**Scheme 1.** Reagents and conditions: a) NaOH, H₂O–EtOH; b) CF₃COOPfp (2.5 equiv), pyridine (2.5 equiv), DMF, 19 h; c) TeocNH(CH₂)₂NH₂ or TeocNH(CH₂)₃NH₂ (1 equiv), DIPEA (2 equiv), CH₂Cl₂, 19 h. DIPEA = disopropylethylamine, Pfp = pentafluorophenyl, Teoc = 2-(trimethylsilyl)ethoxycarbonyl.

![Scheme 2](image)

**Scheme 2.** Reagents and conditions: a) TeocNH(CH₂)₂NH₂ or TeocNH(CH₂)₃NH₂ (1.1 equiv), DIPEA (5 equiv), CH₂Cl₂, 2 days then CH₂Cl₂–MeOH–DIPEA (85:15:5), 2 × 10 min; b) TBAF (3 equiv), DMF, 50 °C, 1 h then room temperature for 3 h; c) Compound 9a or 9b (2 equiv), HODHBI (1 equiv), DIPEA (2 equiv), DMF, 16 h; d) TBAF (5 equiv), DMF, 55 °C, 2 × 15 min; e) Dmt-Cl (6 equiv), DIPEA (6 equiv), DMF, 16 h; f) TBAF (5 equiv), DMF, 55 °C, 2 × 15 min; g) Dmt-Cl (6 equiv), DIPEA (6 equiv), DMF, 16 h; h) 0.1M chloroacetic acid, CH₂Cl₂, 4 × 30 min; i) Fmoc-Tyr(Bu)-OPfp (3 equiv), HODHBI (1 equiv), DIPEA (3 equiv), DMF, 2 × 16 h; j) 20% piperidine–DMF, 2 × 10 min; k) CH₃COOPfp (3 equiv), HODHBI (1 equiv), DIPEA (3 equiv), DMF, 16 h.; l) TFA–CH₂Cl₂ (1:1), 2 h. TBAF: tetrabutyrammonium fluoride; Dmt-Cl: 4,4'-dimethoxytrityl chloride; HODHBI: 3-hydroxy-1,2,3-benzotriazin-4(3H)-one.
SPS of the corresponding cis-cyclopropane-containing PhTXs also started from resin-bound diamines 10a/10b, but in this case these were acylated with the cis- anhydride 3-oxabicyclo[3.1.0]-hexane-2,4-dione to give intermediate resins 14a/14b. Elongation with the respective Teoc-monoprotected diamines under DIC/HOBt amide coupling conditions followed by removal of the Teoc group furnished the unprotected diamides 15a/15b. Introduction of the Dmt-protecting group and subsequent borane reduction of the amide functionalities gave rise to resins 16a/16b that upon Boc-protection and Dmt removal afforded primary amines 17a/17b that were acylated and cleaved from the resin as described for the trans-analogues to yield PhTXs 5 and 6 as the corresponding tris(TFA) salts upon purification.

**Scheme 3.** Reagents and conditions: a) 3-oxabicyclo[3.1.0]-hexane-2,4-dione (4 equiv), DIPEA (2 equiv), DMF, 16 h; b) TeocNH(CH2)NH2 (m = 3 or 4; 4 equiv), HOBt (4 equiv), DIC (4 equiv), DMF, 16 h; c) TBAF (5 equiv), DMF, 55 ºC, 2 × 15 min; d) Dmt-Cl (6 equiv), DIPEA (6 equiv), CH2Cl2, 3.5 h then CH2Cl2–MeOH–DIPEA (85:15:5); e) 1M BH4–THF (20 equiv), THF, reflux for 16 h; f) BocO (10 equiv), DIPEA (10 equiv), CH2Cl2, 16 h; g) 0.1M chloroacetic acid, CH2Cl2, 4 × 30 min; h) Fmoc-Tyr(Bu)-OPfp (3 equiv), HODhBt (1 equiv), DIPEA (3 equiv), DMF, 16 h; i) 20% piperidine–DMF, 2 × 10 min; j) COCl2 (6 equiv), CH2Cl2, 16 h; k) TFA–CH2Cl2 (1:1), 2 h.

**Receptor inhibition by phanlantoxtins**

PhTX analogues 2-6 were all able to cause potent inhibition of currents evoked by exposure of oocytes to 100 μM KA (Figure 2) with their IC50 values given in Table 1. The present study is the first to report on PhTX-343 inhibition of homomeric rat GluA1flop channels while previous reports have focused on rat GluA1flop or AMPARs expressed from mRNA extracted from rat brain. We found that the IC50 for GluA1flop inhibition is similar to that for GluA1flop12] but is about 10-fold higher than that observed for KA-activated channels expressed from rat brain mRNA.[4,10] The subunit combination of the latter is not known, but presumably it contains other AMPAR subunits that may be more sensitive to PhTX 2. All of the cyclopropane-containing PhTX analogues (3-6) were significantly more potent inhibitors than 2, with the trans-configured 3 being the most potent; nearly 30-fold more so than the corresponding straight-chain PhTX 2.

**Figure 2.** A) Current observed in response to exposure to 100 μM KA, and its inhibition following addition of 1 μM PhTX analogue 3 to an oocyte expressing GluA1flop at V0 = -80 mV. B) Concentration-inhibition curves for compounds 2-6 in their inhibition of GluA1flop currents evoked by 100 μM KA. Points are mean ± SEM (n = 4-7 oocytes). IC50 values estimated from curve fits (Hill equation) are given in Table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 ± SEM (μM)</th>
<th>n (oocytes)</th>
<th>Relative potency</th>
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<tr>
<td>2</td>
<td>6.77 ± 1.41</td>
<td>6</td>
<td>1.0</td>
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<tr>
<td>3</td>
<td>0.24 ± 0.04</td>
<td>6</td>
<td>28.2</td>
</tr>
<tr>
<td>4</td>
<td>0.54 ± 0.12</td>
<td>7</td>
<td>12.5</td>
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<tr>
<td>5</td>
<td>1.79 ± 0.50</td>
<td>4</td>
<td>3.8</td>
</tr>
<tr>
<td>6</td>
<td>0.57 ± 0.06</td>
<td>6</td>
<td>11.9</td>
</tr>
</tbody>
</table>

[a] Number of replicates each representing individual oocytes. [b] Ratio between IC50 values for PhTX 2 and each of the other PhTXs. [c] P < 0.0001 (significance of difference by an extra sum of squares F-test compared to 2). [d] P < 0.001 (significance of difference by an extra sum of squares F-test compared to 2).
Modelling studies on philanthotoxins

It is well known that the potency of a philanthotoxin analogue is influenced by changes in the length of the polyamine moiety as well as by the number and distribution of the positively charged amino groups as these factors determine the overall shape and electronic properties of the resulting molecule via intramolecular H-bonding.\(^3, 19, 20\)

PhTX analogues can adopt three general low-energy structures, “head and tail” (HT; extended), “semi-folded” (SF) and “folded” (F) (as depicted in Figure 3), depending on whether H-bonding involves the first, second, or third amino group, respectively. The HT structure is believed to be the most active at AMPARs due to previous observations showing that the mono-cationic analogues are virtually inactive at AMPARs.\(^4, 14\) In terms of general shape mono-cationic analogues are “folded” because they do not possess a “tail” at all. Our modelling studies show that for all compounds the vast majority (99.8%) of low-energy conformations possessed at least one intramolecular H-bond. Ensembles of low-energy conformations obtained for the PhTX analogues studied contained all three types of structures (HT, SF and F) within 3 kcal mol\(^{-1}\) from the apparent energy minimum (0.5-1.0 kcal mol\(^{-1}\)). To reveal the conformational preferences of the compounds we compared the relative numbers of conformations belonging to the different types within these ensembles. Compound 2 was found to be distributed as 85% HT (Figure 3A), 13% SF and 2% F (Figure 3B). This was unchanged for 3 so the additional hydrocarbon bulk, which in fact becomes part of the head group and relocating the tyrosyl group (Figure 3C), appears to be responsible for the increased potency of 3. This is reminiscent of analogues where short hydrocarbon chains were introduced to the central section of the polyamine moiety resulting in more potent analogues at insect quisqualate sensitive ionotropic glutamate receptors.\(^9\)

The cis-analogue of 3 (i.e. compound 5) was less potent as the cis arrangement caused the molecule to adopt a greater proportion of SF structure (44%) (Figure 3D) at the expense of HT (54%). The cause of this effect is that in the cis form both amino groups occur at the same side of the bulk and rigid cyclopropane ring. While the trans configuration dictates a rather large distance between amino groups (6.2 Å in the lowest-energy structure), the cis configuration results in a significantly smaller distance (4.6 Å). As a result, the probability that the second amino group is involved in intramolecular H-bonding (and thus the structure is semi-folded) is larger for the cis than the trans configuration (Figure 3G).

PhTX analogues 4 and 6 showed intermediate potency between 3 and 5 since the additional carbon in the chain between the head group and the first amine functionality allowed for more flexibility and hence a higher proportion of SF structures (Figure 3; E-F). The SF structures for these analogues are likely to be more active because of the longer chain between the second and terminal amine groups (giving it a more HT-like structure).

Conclusion

Introduction of a cyclopropane moiety between the first and second amino groups of PhTX-343 proved beneficial for AMPAR antagonism, but less so for the cis-analogue due to its greater tendency to become semi-folded as inferred by modeling studies. The positive (for trans-analogues) and negative (for cis-analogues) effects of this structural alteration are to some extent obscured by the increased spacing of the amide and the first amine functionality as well as of the second and third amines, such that both PhTX-444 analogues display similar potency.

Experimental Section

General procedures. Unless otherwise stated, starting materials and solvents were purchased from commercial suppliers (H-Tyr(Bu)-OH from Novabiochem, reagents and solvents from Sigma-Aldrich, Fluka, or Lancaster) and used as received. CH\(_2\)Cl\(_2\) was distilled from P\(_2\)O\(_5\) and stored over 4 Å molecular sieves. THF was distilled from Na/benzophenone immediately before use. \(^1\)H NMR and \(^13\)C NMR spectra were recorded at 400.14 MHz and 100.62 MHz, respectively, on a Bruker AMX 400 spectrometer, or at 300.06 MHz and 75.45 MHz, respectively, on a Varian Mercury Plus spectrometer, using CDCl\(_3\) or CD\(_2\)OD as solvents and tetramethylsilane (TMS) as internal standard. Coupling constants (\(J\) values) are given in hertz (Hz), and multiplicities of \(^1\)H NMR signals are reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet, sx, sextet; m, multiplet; br, broad. High-resolution mass spectrometry (HRMS) measurements were performed on a Bruker APEX Qe Fourier transform mass spectrometer equipped with a 9.4 tesla superconducting cryomagnet, and an external electrospray ion source (Apollo II source). The spectra were externally calibrated with an arginine cluster in positive
mode. The samples were dissolved in MeOH, introduced into the electrospray ion source using a syringe pump with a flow of 2 μL/min. Solid-phase reactions were performed in Teflon filter vessels on a Scansys PLS 4 × 6 Organic Synthesizer equipped with a heating block. Preparative HPLC system consisted of an Agilent 1100 system with 2 preparative pump units, a UV detector, and a Phenomenex Luna C18(2) (5 μm) column (25 × 2.12 cm). Linear elution gradients were composed by mixing solvent A (MeCN–H₂O–TFA 5:95:0.1) and solvent B (MeCN–H₂O–TFA 95:5:0.1) at a flow rate of 20 mL/min. Analytical HPLC was performed on a Shimadzu system consisting of an SCL-10A VP controller, an SIL-10AF VP autosampler, an LC-10AT VP pump, a SPD-M10A VP diode array detector, and a CTO-10AC VP column oven, using a Phenomenex Luna C18(2) (3μ) column (50 × 4.6 mm). The HPLC system was controlled by Shimadzu Class VP. Linear elution was performed with two different solvent systems (total flow of 0.8 mL/min). Solvent A = MeCN–H₂O–TFA 10:90:0.1 and solvent B = MeCN–H₂O–TFA 90:10:0.1; t = 0-5 min 0-40% B, t = 5-30 min 40-100% B). The purities of target compounds 3-6 were determined (from UV absorption integration at λ = 215 nm) and were within the ranges 97-99%.

**Dipentafluorophenyl Cyclopropane-trans-1,2-dicarboxylate (8):** Diethyl trans-1,2-cyclopropanedicarboxylate (7, 5.15 g, 27.7 mmol) was subjected to hydrolysis with NaOH (5.16 g, 4.66 × 27.7 mmol) in H₂O–EtOH (2:1, 75 mL) for 4 h at room temperature. The reaction mixture was diluted with H₂O (200 mL) and then washed with Et₂O (100 mL), which was extracted back with H₂O (50 mL). The combined aqueous phases were concentrated to dryness in vacuo. The residue was partially dissolved in EtOAc (100 mL), which then was filtered through a layer of Na₂SO₄. The filtrate was evaporated to give the crude diacid (2.98 g, 83%). The diacid (1.40 g, 10.8 mmol) was treated with CF₂COOPt (4.64 mL, 2.5 × 10.8 mmol) and pyridine (2.18 mL, 2.5 × 10.8 mmol) in dry DMF (15 mL) for 19 h under N₂. The reaction mixture was diluted with EtOAc (250 mL), and then extracted successively with 0.1 M HCl (3 × 150 mL), saturated aqueous NaHCO₃ (3 × 150 mL), and brine (150 mL). Upon drying (Na₂SO₄ the solvent was removed to give 8 (4.86 g, 98%).

**1H NMR (300 MHz, CDCl₃):** δ = 1.81 (t, J = 7.5 Hz, 2H), 2.65 (t, J = 7.5 Hz, 2H), 3.31 (m, 4H), 3.15 (m, 4H), 4.14 (br s, 2H), 2.82 (m, 4H), 2.52 (m, 4H). HRMS [M + Na]⁺ calculated for C₁₂H₁₀F₄O₄Na: 251.0554. Found: 251.0552.

**Protected Monoamines (9a/9b) from Dipentafluorophenyl Cyclopropane-trans-1,2-carboxylate:** The diPfp ester (1.78 g, 3.85 mmol) was dissolved in dry CH₂Cl₂ (25 mL), and then the appropriate Teoc-bound diamines (3.85 mmol) and DIPEA (10 equiv) in dry CH₂Cl₂ (10 mL) were added. After 19 h, the reaction mixture was diluted with EtOAc (150 mL), and then washed with 0.1 M HCl–brine (3 × 150 mL), H₂O (100 mL), sat NaHCO₃ (3 × 150 mL), and brine (150 mL). The organic layer was dried (Na₂SO₄) and then filtered to give 10-(56% yield) as a white solid. The crude diPfp ester (1.78 g, 3.85 mmol), DIPEA (10 equiv), 9a (1.10 g, 56%), HRMS [M + Na]⁺ calculated for C₁₂H₁₀F₄O₄Na: 251.0554. Found: 251.0552.
38.6, 45.6, 46.0/45.9, 52.1, 52.2, 57.1, 116.2, 117.6, 128.7, 131.1, 157.2, 162.1, 175.1/175.0, 176.0; *signals with high intensity (splitting due to presence of diastereomers); **two distinct close signals. HRMS m/z [M+H]⁺ calcld for C₇₇H₈₁NO₁₅: 1280.9, 1311.1, 1571.1, 1624.1, 1739.1, 1758.1. HRMS m/z [M+H⁺] calcld for C₂₀H₂₆N₂O₂: 476.35952, found 476.35956.

SPS of Protected cis-Cyclopropane PhTx- Analogues: Resin-bound diamines (10a/10b, 0.55 mmol) were swelled in dry DMF for 30 min. The solvent was removed by suction, and a solution of 3-oxacibio[3.1]hexane-2,4-dione (4 equiv) and DiPEA (2 equiv) in dry DMF (7 mL/mmol resin) was added to the resin. After 16 h, the resin was drained and washed with DMF, MeOH, DMF, and dry CH₂Cl₂ (each 3 × 12 mL/mmol resin) for 16 h. The resin was then treated with Teoc-monoprotected diamine (4 equiv), HOBt (4 equiv), and DIC (3 equiv) in dry DMF (8 mL/mmol resin) for 16 h. The resin was drained and washed with DMF, MeOH, DMF, and dry CH₂Cl₂ (each 3 ×). Successive Teoc deprotection, introduction of Dmt protecting groups, boron reduction/complexation, and Boc protection were performed as described above for trans-analogues. Upon draining and wash with DMF, MeOH, DMF, and CH₂Cl₂ (each 3 ×), Dmt groups were removed with 0.1 M Ca(OAc)₂ in CH₂Cl₂ (11 mL/mmol resin, 4 × 30 min). The resin was drained and washed with DMF, MeOH, CH₂Cl₂, and dry DMF (each 3 ×). The resulting resin was eluted with the Tyr residues and peptides-terminated by introduction of the N-butyryl group as described above (using single couplings for 16 h). Cleavage of the product from the resin was performed with TFA–CH₂Cl₂ (1:1, 7 mL/mmol resin) for 2 h. The crude products were purified by preparative HPLC (>95% purity): 5 (220 mg) and 6 (64 mg). 5: ‘H NMR (300 MHz, methanol-d₄): δ = 0.56/0.58 (q, J = 5.8 Hz, 1H), 0.85/0.86 (t, J = 7.5 Hz, 3H), 1.11 (m, 1H), 1.39 (m, 2H), 1.54/1.56 (sx, J = 7.5 Hz, 2H), 1.82 (p, J = 6.6 Hz, 2H), 2.15-2.22 (br, 2H), 2.79-3.30 (br, 14H), 4.39/4.40 (t, J = 7.7 Hz, 1H), 6.72 (d, J = 8.4 Hz, 2H), 7.06/7.07 (d, J = 8.4 Hz, 2H). 6: ‘C NMR (75 MHz, methanol-d₄): δ = 10.2/10.5, 13.8/13.9, 14.0, 20.3, 25.4, 27.4, 36.7/36.8, 37.8, 38.0*, 38.6*, 45.7*/45.8*, 46.0*, 48.4 (2C), 57.0/57.2, 116.1/116.2, 118.0, 128.8, 131.1/131.2, 157.2*, 162.9, 169.1, 175.1*, 176.0; ppm; signals with high intensity (splitting due to presence of diastereomers); two distinct close signals. HRMS m/z [M+H⁺] calcld for C₇₇H₈₁N₂O₂: 1280.9, 1311.1, 1571.1, 1624.1, 1739.1, 1758.1. Analysis was performed using ZMM (ZMM Software Inc., Flamborough, Ontario, Canada). The non-bound interactions were calculated by using the AMBER force field with a cut-off distance of 8 Å. The hydration energy was calculated by using the implicit solvent method. Electrostatic interactions were calculated by using the distance-dependent dielectric function ϵ = 4r where (r) is the atom-atom distance. No specific energy terms were used for cation–cation interactions, which were accounted for with partial negative charges at the aromatic carbons. Bond lengths and valent angles were assigned standard values. Atomic charges at ligands were calculated by using the AM1 method in the MOPAC program. For each ligand, 100 starting conformations were generated by randomizing torsion angle values. The Monte Carlo minimization (MCM) method was used to optimize the structures. MCM of each model was performed until 600 consecutive energy minimizations did not decrease the energy of the apparent global minimum. For statistical analysis, the 1000 lowest energy conformations were selected.

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References


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In the search for improved inhibitors of GluA1flop AMPA receptors – a question is "may incorporation of cyclopropane moieties be beneficial – and what configuration is superior – cis or trans?" Synthesis, test and modelling of four analogues revealed the structure-activity features of this unprecedented subclass of inhibitors.

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