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Design, Synthesis, and Pharmacological Characterization of Polyamine Toxin Derivatives: Potent Ligands for the Pore-Forming Region of AMPA Receptors

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Polyamine toxins, such as philanthotoxins, are low-molecular-weight compounds isolated from spiders and wasps, which modulate ligand-gated ion channels in the nervous system. Philanthotoxins bind to the pore-forming region of AMPA receptors, a subtype of glutamate receptors which are important for memory formation and are involved in neurodegenerative diseases. Previous studies have demonstrated that modification of the polyamine moiety of philanthotoxins can lead to very potent and highly selective ligands for the AMPA receptor, as exemplified with philanthotoxin-56. Much less attention has been paid to the importance of the aromatic head group of philanthotoxins, but herein we demonstrate that modification of this moiety leads to a significant improvement in potency relative to philanthotoxin-56 at cloned AMPA receptors. Interestingly, the incorporation of an adamantane moiety is particularly favorable, and the most potent compound has a K_i value of 2 nM, making it the most potent uncompetitive antagonist of AMPA receptors described to date. Such compounds are potentially useful as neuroprotective agents.

Introduction

In the brain, the communication between neurons, known as synaptic transmission, is facilitated by neurotransmitters. In the mammalian brain, fast synaptic transmission is mainly mediated by glutamate by binding to ionotropic glutamate (iGlu) receptors. iGlu receptors are important for the basic cellular and molecular mechanism by which memories are formed and stored, as characterized by long-term potentiation (LTP)[1,2] and long-term depression (LTD).[3] Moreover, the dysfunction of iGlu receptors is implicated in a wide range of neurological insults including chronic neurodegenerative disorders such as Alzheimer's disease.[4–6]

The iGlu receptors are classified into three subtypes according to selective activation by the agonists (*R*,*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA), *N*-methyl-D-aspartate (NMDA), and kainic acid. Herein we focus on the AMPA receptor subtype, which is distributed ubiquitously throughout the central nervous system (CNS) and is composed of various combinations of the four subunits designated GluR1–GluR4, which form the receptor by tetrameric assembly.[4–6] AMPA receptors can be classified according to their Ca^{2+} permeability; in the GluR1, GluR3, and GluR4 subunits a glutamine residue (Q0) is present at a site known as the Q/R site located in the pore-forming region of the receptor and allows high Ca^{2+} permeability (Figure 1). This residue has been edited in virtually all GluR2 subunits to arginine (R0), which leads to a drastic decrease in Ca^{2+} permeability of AMPA receptors. It is believed that Ca^{2+}-permeable AMPA receptors are important for the pathogenesis of neurological disorders,[7] and recent studies suggest that the abnormal editing of GluR2 subunits leads to an increase in the number of Ca^{2+}-permeable AMPA receptors and promotes amyotrophic lateral sclerosis, also known as Lou Gehrig's disease.[8–10] Thus, use of AMPA receptor antagonists is believed to be useful in treating neurodegenerative disorders and/or decreasing the amount of neurological damage associated with these disorders.[11–14]

Polyamine toxins, isolated from the venom of spiders and wasps, form a class of low molecular weight compounds that are nonselective antagonists of iGlu receptors.[15–17] Polyamine toxins block the receptors by an uncompetitive mechanism, that is, they require activation of the receptor before antagonism takes place, most likely by binding to the pore-forming region of the receptor (Figure 1). This mode of action is interesting from a drug-discovery perspective, as an uncompetitive antagonist of NMDA receptors, memantine (Ebixa), was recently launched for the management of patients with moderate to severe cases of Alzheimer's disease.[18]

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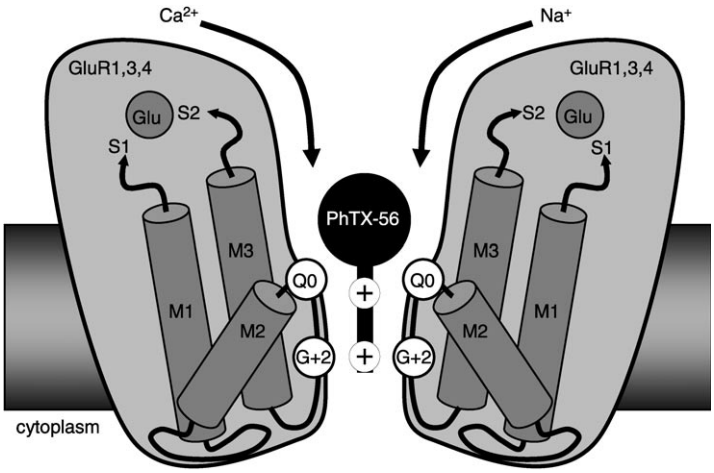


Figure 1. Suggested mode of binding of (*S*)-PhTX-56 (**3**) to the pore-forming region of AMPA receptors. Polyamine toxins such as **3** are believed to antagonize ionotropic receptors by binding to the pore-forming region, inside the ion channel. Antagonism of AMPA receptors only takes place when receptors are composed of GluR subunits 1, 3, and 4, whereas blockade is prevented if the GluR2 subunit is present. This selectivity is explained by editing of the Q/R site; in GluR subunits 1, 3, and 4, a glutamine (Q) residue is present, whereas virtually all GluR2 subunits are edited and contain an arginine (R) group. (Image adapted from ref. [16]).

The wasp toxin philanthotoxin-433 ((*S*)-PhTX-433, (**1**)) isolated from venom of the female digger wasp *Philanthus triangulum* is a prototypical example of polyamine toxins (Figure 2).[19]

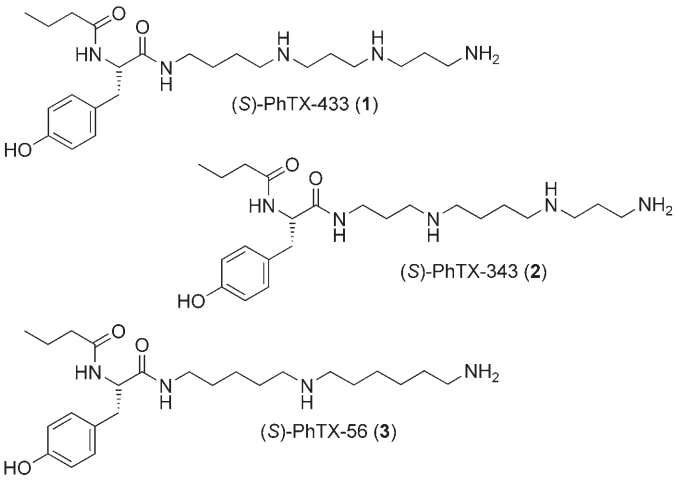


Figure 2. Structures of polyamine toxins: (*S*)-PhTX-433 (**1**) is the native philanthotoxin isolated from the wasp *Philanthus triangulum*, whereas (*S*)-PhTX-343 (**2**) and (*S*)-PhTX-56 (**3**) are derivatives, the latter being a very potent and highly selective AMPA receptor antagonist.

Toxin **1** and its synthetic analogue (*S*)-PhTX-343 (**2**) are antagonists of Ca^{2+}-permeable AMPA and kainate receptors, which make them useful pharmacological tools for the determination of the subunit composition of these receptors.[20,21] The potential of these compounds as drug candidates has so far been considered to be limited as a result of their nonspecific action toward other ionotropic receptors. Recently, however, it was demonstrated that modification of the polyamine portion of **2** led to selective antagonism of various ionotropic receptor classes.[22–25] The most striking example was philanthotoxin-56 ((*S*)-PhTX-56, (**3**)), which is a very potent and highly selective antagonist of Ca^{2+}-permeable AMPA receptors (Figure 2).[25]

The current hypothesis of how philanthotoxins interact with the AMPA receptor ion channel is based on structure–activity relationship (SAR) and mechanistic studies, as well as molecular modeling. Modeling studies by Tikhonov et al. led the authors to propose that philanthotoxins bind in a stretched out conformation[26] and that the terminal primary amino group of philanthotoxins interacts with main-chain carbonyl oxygen atoms that line the narrowest part of the AMPA receptor pore (Figure 1).[27] Thus, the polyamine portion is critical for specific interaction and seems to determine potency and specificity, whereas the aromatic head group (tyrosine-*N*-butyramide) probably interacts in a more nonspecific manner, but is important for anchoring the molecule to the receptor. These suggestions have been corroborated by a number of SAR studies, showing that modification of the polyamine portion of philanthotoxins has a dramatic effect on their biological activity.[28] In contrast, the effects of modifying the aromatic head group of philanthotoxins are ambiguous. It was shown that the biological activity of PhTX-343 derivatives was significantly improved by changing the butyramide moiety to 2-phenylacetamide,[23] whereas changing the tyrosine moiety without changing the *N*-butyramide portion had only minor effects on biological activity.[29] On the other hand, changes in the *N*-butyramide moiety of the selective AMPA receptor antagonist PhTX-83, a di-cationic philanthotoxin, did not lead to major changes in biological activity.[25]

The biological interest in polyamine toxins and polyamines in general has nourished an interest in the efficient synthesis of these compounds and their derivatives.[15–17] Particularly, the use of solid-phase synthesis has greatly facilitated the synthesis of these compounds.[15,16,30] A successful strategy has been the application of the Fukuyama amination, in which an amine group is protected and activated as a nitrobenzene sulfonamide, which is subsequently alkylated either by conventional alkylation or by a Mitsunobu reaction.[15,16,30] We previously showed how a small library of philanthotoxins can be synthesized by a sequential strategy using Fukuyama amination under Mitsunobu conditions,[31] and the methodology has since been used for the synthesis of several philanthotoxin analogues[25] including labeled analogues of PhTX-56.[32] Recently, a slightly modified version was presented as a more convenient reaction sequence by using N-protected aminoalkyl halides rather than amino alcohols.[33]

In the study reported herein, we investigated the effect of changing both the tyrosine and butyramide portions of a di-cationic philanthotoxin such as (*S*)-PhTX-56 (**3**). A small (40-member) library of PhTX-56 derivatives was designed in which eight different amino acids and five different acids were coupled to the same polyamine portion. We speculated that the

overall polarity of philanthotoxins could be a factor influencing their activity; hence, amino acids and acids were selected to cover a wide range in hydrophobicity (calculated Clog *P*). Aside investigations of the effects on biological activity, such compounds would also be useful in determining the feasibility of introducing fluorescent groups, which are generally quite bulky, in place of the aromatic head group without a loss in biological activity. This would allow the preparation of potent fluorescent analogues of PhTX-56 which would be excellent tools for dynamic studies of Ca^{2+}-permeable AMPA receptors.

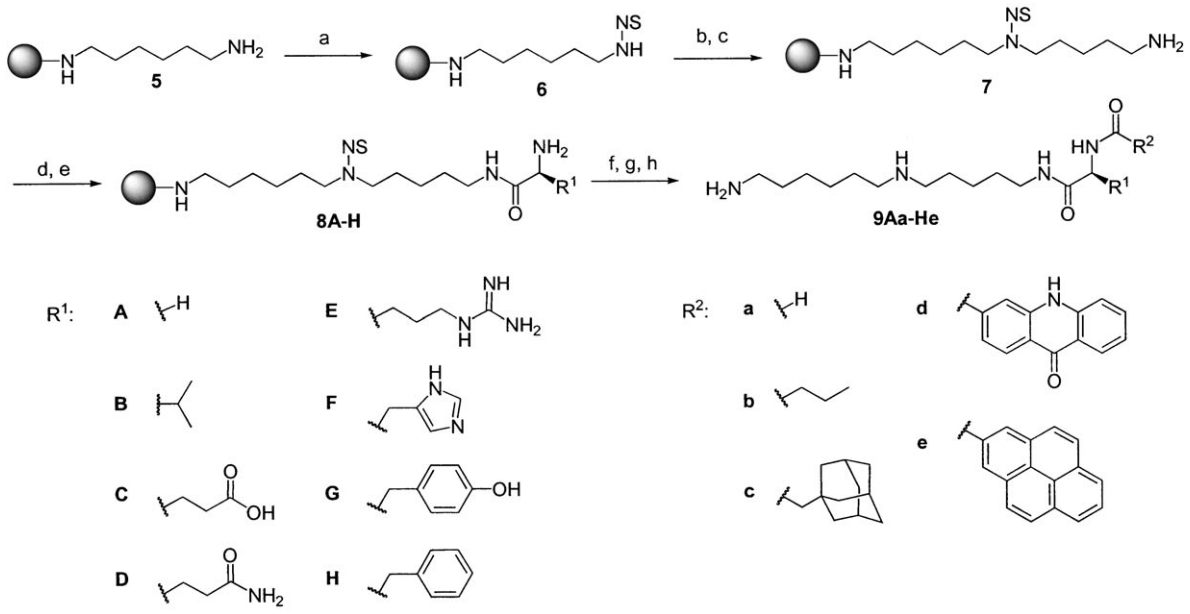
Results

Chemistry

Herein we describe the design and synthesis of a 40-member library of PhTX-56 derivatives in which the aromatic head group is modified. These compounds can be divided into eight groups based on the amino acid moiety incorporated, ranging from the nonpolar glycine, valine, and phenylalanine residues, to the polar but non-ionic glutamine, the acidic residues tyrosine and glutamic acid, as well as the basic amino acids arginine and histidine. Within these eight groups, similar *N*-acyl substitutions were made ranging from reaction with formic acid, butyric acid, adamantan-1-yl-acetic acid, 9-oxo-9,10-dihydroacridine-3-carboxylic acid, and pyrene-2-carboxylic acid. The compounds were synthesized by a parallel synthetic approach with solid-phase methodology and semi-automated equipment (MiniBlock, Mettler Toledo), and the crude products were purified by preparative HPLC–MS using intelligent fraction collection.

The preparation of philanthotoxin analogues **9Aa**–**He** is shown in Scheme 1. Briefly, resin-bound 1,6-hexanediamine **5** reacted with *o*-nitrobenzenesulfonyl chloride to give the corresponding *o*-nitrobenzene sulfonamide **6**. This was then combined with the *N*-2-(trimethylsilyl)ethoxycarbonyl (*N*-Teoc)-protected 5-aminopentan-1-ol in a modified Mitsunobu reaction, using 1,1′-(azadicarbonyl)dipiperidine (ADDP) and tributylphosphine (Bu_3P) as redox reagents. Subsequently, the Teoc protecting group was removed by treatment with tetrabutyl ammonium fluoride (TBAF) to give the resin-bound polyamine precursor **7**. This precursor was allowed to react with eight different amino acids **A**–**H**: (*S*)-*N*-Fmoc-*O*-(*tert*-butyl)tyrosine, (*S*)-*N*-Fmoc-*N*-Pbf-arginine, (*S*)-*N*-Fmoc-*N*-trityl-histidine, (*S*)-*N*-Fmoc-phenylalanine, (*S*)-*N*-Fmoc-*O*-(*tert*-butyl)glutamic acid, (*S*)-*N*-Fmoc-glutamine, (*S*)-*N*-Fmoc-valine, and *N*-Fmoc-glycine (Fmoc = 9-fluorenylmethyloxycarbonyl). This was carried out by using *N*-(dimethylamino)-1*H*-1,2,3-triazol[4,5-b]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) and collidine as coupling reagents to ensure high stereochemical purity (>99% *ee*). Removal of the Fmoc group was followed by coupling with five different acids **a**–**e**: formic acid, butyric acid, adamantan-1-yl-acetic acid, 9-oxo-9,10-dihydroacridine-3-carboxylic acid, and pyrene-2-carboxylic acid. Finally, the nitrobenzene sulfonamide group was removed, and the compounds were cleaved from the resin with concomitant deprotection of the amino acids to provide the compounds as crude products. These were purified by preparative HPLC–MS using intelligent fraction collection to provide 37 of the planned 40 compounds **9Aa**–**He** in low to good yields (3–19% in eight steps from **5**). The compounds that were not successfully synthesized were **9Ce**, **9Ea**, and **9Eb**.

All compounds were characterized by ^1H NMR spectroscopy and MS, and purity was checked by HPLC and evaporative light scattering (ELS). The latter technique is able to detect polyamine impurities, which cannot be detected by ultraviolet



Scheme 1. Reagents and conditions: a) *o*-nitrobenzenesulfonyl chloride, DIEA; b) 2-(trimethylsilyl)ethyl-5-hydroxypentylcarbamate, Bu_3P, ADDP; c) TBAF; d) amino acids **A**–**H**, HATU, collidine; e) piperidine (20%) in *N*,*N*-dimethylformamide (DMF); f) acids **a**–**e**, HATU, collidine; g) $HSCH_2CH_2OH$, DBU; h) trifluoroacetic acid (TFA), triisopropylsilane, CH_2Cl_2, H_2O.

light. All compounds were within 96–100% purity as determined by HPLC–ELS.

In vitro electrophysiology

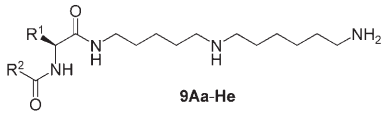
The 37 PhTX-56 derivatives (compounds **9Aa**–**He**) were tested using two-electrode voltage-clamped *Xenopus laevis* oocytes expressing homomeric AMPA receptors composed of GluR1 flop subunits, and the oocytes were clamped at a holding potential (V_H) of −70 mV. The K_i values are calculated from normalized responses in the interval [0.4;0.6] (Table 1). Each compound was tested on oocytes from three different frogs, thus resulting in a total of 18–27 measurements for each compound.

In general, the compounds showed a remarkable variation in activity, with K_i values ranging from 2 nM to ca. 20 μM, thus demonstrating a greater than 10000-fold difference in activity. The two most potent compounds were adamantylacetamide derivatives with basic amino acids arginine and histidine, compounds **9Ec** and **9Fc**, with K_i values of 4.0 and 2.2 nM, respectively. Similarly, compounds with basic amino acids but with other bulky substituents, such as **9Ed**, **9Ee**, and **9Fd** were also very potent, with K_i values of 12.6, 29.4, and 14.1 nM, respectively. However, adamantylacetamide derivatives with glycine (compound **9Ac**) and valine (compound **9Bc**) as amino acids were also quite potent, with K_i values of 9 and 28 nM respectively. Increasing the bulk and hydrophobicity of the amino acid moiety while maintaining the adamantane substituent, as in compounds **9Gc** and **9Hc**, either did not affect or slightly decreased affinity, as shown by K_i values of 36 and 91 nM, respectively. Thus, substitution with adamantylacetamide is particularly favorable even in comparison with the two other hydrophobic substituents, 9-oxo-9,10-dihydroacridine and pyrene, and is almost independent of the nature of the amino acid. On the other hand, the presence of *N*-formamide or *N*-butyramide is generally less favorable than larger groups.

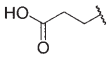
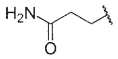
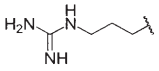
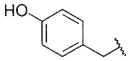
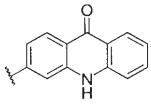
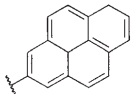
Current traces for **9Ac** and **9Fc** are shown in Figure 3, and indicate that blockade by **9Ac** at a concentration of 100 nM is faster than blockade by **9Fc** at 10 nM, although the potency of **9Fc** is slightly higher than that of **9Ac**, with K_i values of 2.2 and 9 nM, respectively. This suggests a potential difference between the blocking profiles of the two compounds. The dose–response curves for the same compounds are shown in Figure 4.

Interestingly, the presence of a glutamic acid moiety severely decreased activity, and led to the least potent group of compounds, **9Ca**–**9Cd**, with K_i values from 1.57 to 18.4 μM, regardless of *N*-acyl substitution. Decreasing the polarity of the amino acid by replacing glutamate with glutamine had a significant impact on activity. Although compounds **9Db** and **9De** were still relatively weak antagonists, with K_i values in the low μM range, compound **9Da** was 100-fold more potent than the corresponding glutamate analogue **9Ca**, and again, the adamantylacetamide derivative **9Dc** was the most potent with a K_i value of 63 nM. Notably, in the presence of a histidine moiety, there was a 1000-fold difference in K_i values even among derivatives with hydrophobic substituents; compounds **9Fc** and **9Fd** were very potent, with K_i values of 2 and 14 nM,

Table 1. Biological activities of PhTX-56 derivatives.[a]



9Aa-He

R^2 \ R^1 =	**A:** 	**B:** 	**C:** 	**D:** 	**E:** 	**F:** 	**G:** 	**H:** 
a: 	1.21 ± 0.518	0.366 ± 0.171	18.1 ± 7.87	0.208 ± 0.097	NT	1.32 ± 0.628	0.031 ± 0.013	0.039 ± 0.017
b: 	0.439 ± 0.21	1.22 ± 0.512	18.4 ± 8.63	2.63 ± 1.08	NT	0.228 ± 0.102	0.014 ± 0.004	0.143 ± 0.062
c: 	0.009 ± 0.004	0.028 ± 0.013	1.57 ± 0.719	0.063 ± 0.026	0.004 ± 0.002	0.002 ± 0.001	0.036 ± 0.017	0.091 ± 0.041
d: 	0.241 ± 0.103	0.298 ± 0.136	4.46 ± 2.57	0.321 ± 0.151	0.013 ± 0.006	0.014 ± 0.006	0.033 ± 0.015	0.069 ± 0.036
e: 	0.085 ± 0.041	0.143 ± 0.065	NT	2.03 ± 0.983	0.029 ± 0.013	1.68 ± 0.680	0.387 ± 0.178	1.91 ± 0.863

[a] Reported as K_i [μM]: inhibition of the current elicited by 100 μM glutamate by simultaneous co-application of the antagonist in oocytes injected with GluR1 flop RNA. Values represent the mean ± SD of measurements from three different oocytes, and for each compound six to nine measurements were performed at each oocyte, thus a total of 18–27 measurements for each value.

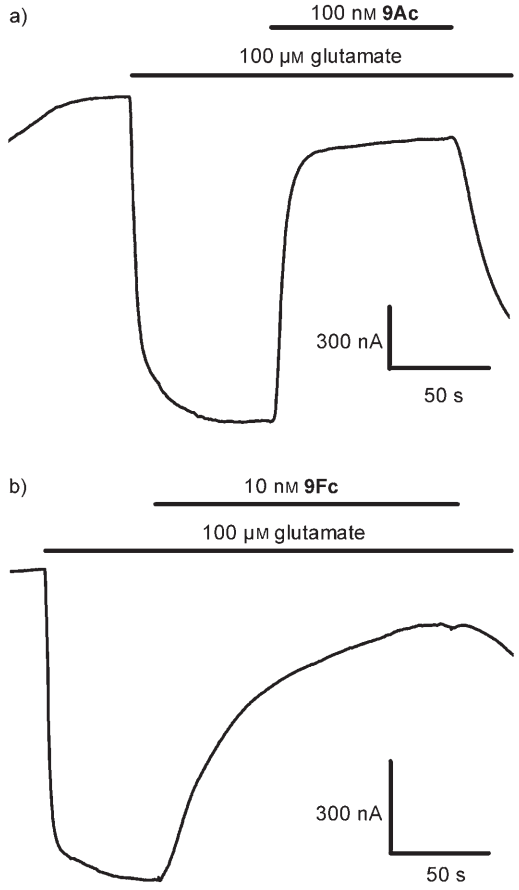


Figure 3. Current traces for compounds a) **9Ac** and a) **9Fc** recorded from a two-electrode, voltage-clamped *X. laevis* oocyte expressing GluR1 flop. The addition of 100 μM glutamate induces a current through the AMPA receptor, which is blocked by either **9Ac** (100 nM) or **9Fc** (10 nM). Notably, blockade by **9Fc** is somewhat slower than blockade by **9Ac**, which suggests a slower channel-blocking rate for **9Fc** relative to **9Ac**.

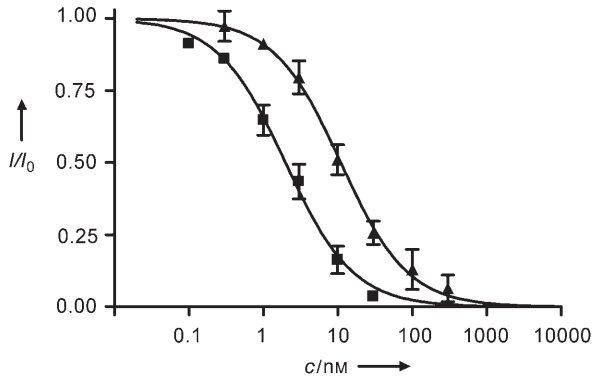


Figure 4. Concentration–inhibition data for compounds **9Ac** (▲) and **9Fc** (■) on homomeric GluR1 flop receptors at $V_H = -70$ mV, for which bars represent SD. Values of log K_i for **9Ac** and **9Fc** are -7.96 ± 0.05 and -8.70 ± 0.04, thus giving $K_i = 10.98$ and 1.98 nM, respectively. In each case, the Hill slope is ≈ 1 (0.97 ± 0.1 and 0.95 ± 0.08 for **9Ac** and **9Fc**, respectively).

respectively, whereas the pyrene carboxylic amide derivative **9Fe** had a K_i value of 1.68 μM.

Voltage dependency was investigated for three selected compounds, **9Cc**, **9Dc**, and **9Ec** (Table 2), which are structurally similar and differ only in the amino acid moiety, yet which have variation in biological activity. The voltage dependence is investigated by testing the compounds at different holding potentials, and provides information on the mode of interaction. The three compounds were investigated at $V_H = -100$ and -60 mV (Table 2), and they all showed a similar degree of voltage dependence, which suggests they share an uncompetitive mode of action.

Table 2. Voltage dependence of selected PhTX-56 derivatives.[a]

Compd	$-\log K_i$ (M) $V_H = -60$ mV	$V_H = -100$ mV	$V_{H_{-100}} - V_{H_{-60}}$
9Cc	5.138 ± 0.063	6.626 ± 0.031	1.488
9Dc	6.192 ± 0.059	7.551 ± 0.117	1.359
9Ec	7.946 ± 0.078	9.398 ± 0.036	1.452

[a] Inhibition of the current elicited by 100 μM glutamate by simultaneous co-application of the antagonist in oocytes injected with GluR1 flop RNA at $V_H = -60$ mV and -100 mV. Values represent the mean $\pm$SD.

Discussion

A small 40-member library of PhTX-56 derivatives was designed in which the polyamine moiety remained unchanged, whereas eight different amino acids and five different acids were coupled to the polyamine. The compounds were synthesized with solid-phase methodology, and 37 compounds were successfully prepared. These compounds were investigated by electrophysiology for their ability to antagonize cloned AMPA receptors composed of GluR1 subunits.

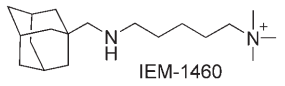
Previously, it was demonstrated that changes in the polyamine moiety result in dramatic changes in activity,[22–25] whereas studies in modification of the aromatic head group have given ambiguous results.[23,25,29] In this study, both the tyrosine and the *N*-acyl portions of the head group were varied simultaneously, and the structural variation in terms of Clog *P* values, for example, were quite large, as the compounds span more than eight orders of magnitude (data not shown).

The building blocks were selected partially on the basis of Clog *P*, as we hypothesized that there is a correlation between polarity and biological activity. However, plotting the biological activity ($-\log K_i$) as a function of the Clog *P* value reveals only a weak correlation ($R^2 = 0.2739$) between increasing Clog *P* and increasing biological activity (data not shown). If only adamantane derivatives are taken into account, the correlation between $-\log K_i$ and Clog *P* improves ($R^2 = 0.3497$) if Clog *P* values above 4 are not included in the plot (data not shown), as at higher Clog *P* values, the K_i decreases.

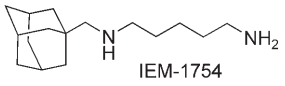
In this study, we were intrigued to observe up to 10000-fold difference in biological activity between the most and least potent derivative, suggesting an increased importance of the head group in philanthotoxins. In this series of compounds, the *N*-acyl group seems to be the major determinant of biological activity, although very polar amino acids such as glutamate diminish activity independently of the *N*-acyl group. Formamide and butyramide are generally the least favorable *N*-acyl

groups, whereas substitution with bulkier and more hydrophobic substitutions improves activity. Remarkably, substitution with adamantylacetamide is a particularly favorable, almost regardless of the nature of the amino acid.

Interestingly, di-cationic adamantane derivatives such as {5-[(adamantan-1-ylmethyl)amino]pentyl}trimethylammonium (IEM-1460) and N^1-adamantan-1-ylmethylpentane-1,5-diamine (IEM-1754) were described as AMPA receptor ligands by Magazanik and colleagues.[34] IEM-1460 is a semipotent antagonist



IEM-1460



IEM-1754

of Ca^{2+}-permeable AMPA receptors ($IC_{50} = 1.6\ \mu M$) and seems to antagonize AMPA receptors in a manner similar to that of philanthotoxins. It has been shown that the distance between the hydrophobic adamantane moiety and the terminal ammonium group is crucial for activity at AMPA receptors.[35–37] Modeling of IEM-1460 in a GluR1 homology model suggested that the hydrophobic adamantane moiety interacts with Q0, which lacks accessible nucleophilic atoms, whereas the terminal ammonium group binds to the oxygen atom of the backbone carbonyl group of glycine (G + 2), in much the same way as PhTX-343 is believed to bind to the AMPA receptor pore region.[27] Thus the adamantane derivatives prepared in this study, such as **9Ac**, **9Ec**, and **9Fc** can be considered as hybrid molecules between (*S*)-PhTX-56 (**3**) and IEM-1460, but are significantly more potent than either of the lead structures. It is therefore tempting to speculate that the adamantane moiety binds to a separate and particularly favorable hydrophobic region which confers high potency. These novel derivatives open up new possibilities for fine-tuned structure–activity relationships of these molecules.

One of the objectives of this study was to investigate whether it would be possible to replace the native aromatic head group with fluorescent groups, which are usually inherently bulky and hydrophobic. The results do not provide an unambiguous answer; clearly a bulky group such as adamantane improves biological activity, whereas flat structures such as pyrene improves activity in some cases. Synthesis of fluorescent PhTX-56 derivatives is in progress in our research group, and subsequent biological testing will reveal whether such compounds retain potent AMPA receptor antagonism.

From a drug-discovery perspective, these novel derivatives might prove interesting, although a number of features such as selectivity, penetration of the blood–brain barrier, and in vivo behavior remain to be tested. However, the hypothesis that antagonists of Ca^{2+}-permeable AMPA receptors could be a novel way to treat neurodegenerative disorders could be examined by using these compounds.[11–14]

Conclusions

A small library of polyamine toxin derivatives containing eight different amino acids and five different *N*-acyl groups were designed and synthesized on solid phase. The 37 derivatives were tested as AMPA receptor antagonists by using electrophysiology on *Xenopus* oocytes expressing homomeric GluR1 AMPA receptors. In contrast to previous studies, changes in the aromatic head group had a major influence on biological activity, as differences in activity among the novel derivatives were as great as 10000-fold.

Interestingly, the most potent compounds had an adamantane moiety in the *N*-acyl region, whereas the specific nature of the amino acid moiety plays a less important role. A combination of basic amino acid moieties, histidine and arginine, and the adamantane group, as in compounds **9Fc** and **9Ec**, resulted in the most potent derivatives. Compound **9Fc** had a K_i value of 2.18 ± 1 nM, and is thereby the most potent uncompetitive AMPA receptor antagonist described to date.

We have provided a novel, highly potent AMPA receptor antagonist that opens up exciting possibilities for future studies. Structure–activity studies exploring the remarkable effect of the adamantane group might lead to even more potent compounds, and combining compound **9Fc** with a fluorescent group could provide an important tool for dynamic studies of Ca^{2+}-permeable AMPA receptors. Finally, compounds such as **9Fc** might be explored as neuroprotective drug candidates, and could definitely be used to examine the hypothesis that blockade of Ca^{2+}-permeable AMPA receptors is an advantageous principle for neuroprotection.

Experimental Section

Chemistry, general: Resin-bound diamines (trityl chloride resin, 1% divinylbenzene, 200–400 mesh) and (*S*)-*N*-Fmoc-protected amino acids were obtained from Novabiochem (Läufelingen, Switzerland). All other starting materials were obtained commercially from Aldrich or Fluka. All starting materials and solvents were used without further purification except DMF, which was stored over molecular sieves (3 Å) and THF, which was distilled under N_2 from Na/benzophenone. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury spectrometer at 300 MHz or on a Varian Gemini 200 BB at 300 MHz with CD_3OD as solvent. Chemical shifts are reported in ppm (δ). Coupling constants (J) are given in Hz. Multiplicities of ^{1}H NMR signals are given as follows: s, singlet; bs, broad singlet; d, doublet; t, triplet; dt, doublet of triplets; q, quartet; p, pentet; hex, sextet; h, septet; m, multiplet. Analytical and preparative HPLC–MS was performed on a Sciex API150ex instrument equipped with an atmospheric pressure chemical ionization (APCI) ion source. The HPLC system consisted of two Shimadzu LC10 ADvp pumps. UV/Vis traces were obtained with a Gilson UV/VIS 155 UV detector operating at $\lambda = 254$ nm. Evaporative light scattering (ELS) traces were obtained with Sedere Sedex 55 light scattering detector and were used for estimation of the purity of the final products. Analytical HPLC–MS was performed on a 50× 4.6 mm YMC RP18 column using a mixture of A: water with 0.05% TFA and B: acetonitrile with 5% water and 0.035% TFA at a flow rate of 2 mL min^{-1}. Preparative HPLC–MS (split-flow MS detection) was run with injections of 500 μL (20 mg sample in 1.0 mL DMSO)

onto a Phenomenex Synergi Hydro column (4 mm, 21.20×50 mm) eluted with the same solvent gradient at 22.7 mL min^{-1}.

Synthesis of PhTX-56 analogues (9Aa–He), general procedure: Resin-bound diaminohexane (0.16 mmol) was suspended in CH_2Cl_2 (4 mL). Diisopropylethylamine (DIEA) (0.96 mmol) and *o*-nitrobenzenesulfonyl chloride (0.64 mmol) were added successively, and the reaction mixture was stirred under nitrogen at room temperature for 3 h. The resin was drained and washed with DMF (3×2.5 mL), CH_2Cl_2 (3×2.5 mL), MeOH (3×2.5 mL), and again with CH_2Cl_2 (3×2.5 mL) before drying under vacuum. The above resin was suspended in dry THF/CH_2Cl_2 (1:1, 2 mL) under nitrogen. A solution of 2-(trimethylsilyl)ethyl 5-hydroxypentylcarbamate (0.8 mmol) in dry THF/CH_2Cl_2 (1:1, 1 mL), tributylphosphine (0.8 mmol), and a solution of 1,1′-(azadicarbonyl)dipiperidine (ADDP) in dry THF/CH_2Cl_2 (1:1, 1 mL) were added successively. The mixture was stirred at room temperature under nitrogen for 3 h. The resin was drained and washed with DMF (3×2.5 mL), CH_2Cl_2 (3×2.5 mL), MeOH (3×2.5 mL), and again with CH_2Cl_2 (3×2.5 mL) before drying under vacuum. The procedure was repeated twice, and the resulting resin was dried under vacuum. The above resin was suspended in dry THF (5 mL) under nitrogen at 50 °C. A solution of TBAF (1 M in THF, 0.64 mmol) was added slowly, and the mixture was stirred at 50 °C for 30 min. The resin was drained and washed with DMF (3×2.5 mL), CH_2Cl_2 (3×2.5 mL), MeOH (3×2.5 mL), and again with CH_2Cl_2 (3×2.5 mL) before drying under vacuum. A solution of the appropriate (*S*)-*N*-Fmoc-protected amino acid (**A**–**H**, 0.48 mmol) and HATU (0.48 mmol) in DMF (1 mL), followed by a solution of collidine (0.72 mmol) in DMF (0.5 mL) was added to the resin. The mixture was stirred at room temperature for 2 h, and the resin was subsequently washed with DMF (3×2.5 mL), CH_2Cl_2 (3×2.5 mL), MeOH (3×2.5 mL), and again with CH_2Cl_2 (3×2.5 mL). The product was treated with piperidine in DMF (20% *v/v*, 2 mL) and the mixture was agitated for 3 min at room temperature. The resulting resin was washed with DMF (3×2.5 mL) and treated again with piperidine in DMF (20% *v/v*, 2 mL) for 20 min. It was then washed with DMF (3×2.5 mL), CH_2Cl_2 (3×2.5 mL), MeOH (3×2.5 mL), and again with CH_2Cl_2 (3×2.5 mL) before drying under vacuum. A solution of the appropriate acid (**a**–**e,** 0.48 mmol) and HATU (0.48 mmol) in DMF (1 mL), followed by a solution of collidine (0.72 mmol) in DMF (0.5 mL) was added to the resin. The mixture was agitated at room temperature for 2 h. The resin was drained and washed with DMF (3×2.5 mL), CH_2Cl_2 (3×2.5 mL), MeOH (3×2.5 mL), and again with CH_2Cl_2 (3×2.5 mL) before drying under vacuum. The resin was treated with DBU (0.6 mmol) in DMF (1 mL) and mercaptoethanol (0.6 mmol) in DMF (1 mL) for 30 min. The resin was drained and washed with DMF (5×2.5 mL). The procedure was repeated three more times. The resin was washed with DMF (3×2.5 mL), CH_2Cl_2 (3×2.5 mL), MeOH (3×2.5 mL), and again with CH_2Cl_2 (3×2.5 mL) before treatment with a solution of CH_2Cl_2/TFA/triisopropylsilane/H_2O (47.5:47.5:2.5:2.5 *v/v*, 2 mL) for 2 h. The resin was drained and washed with MeOH (2 mL) and CH_2Cl_2 (2 mL). The solutions of the cleaved product and the washings were combined, evaporated under vacuum, and purified by preparative HPLC to give the final products.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-2-formylaminoacetamide (9Aa): Yellow oil (yield: 2.2 mg, 4%); ^{1}H NMR: δ = 1.31–1.74 (m, 14H), 2.88–3.00 (m, 6H), 3.18–3.26 (m, 2H), 3.83 ppm (s, 2H); ES$^+$ MS: *m/z*: 287.3 $[M+H]^+$; HPLC–ELS: 99.8%.

(*S*)-*N*-{[5-(6-Aminohexylamino)pentylcarbamoyl]methyl}butyramide (9Ab): Colorless oil (yield: 1.6 mg, 3%); ^{1}H NMR: δ = 0.94 (t, *J* = 7.3, 3H), 1.35–1.72 (m, 16H), 2.23 (t, *J* = 7.3, 2H), 2.88–3.00 (m, 6H), 3.20 (t, *J* = 6.7, 2H), 3.76 ppm (s, 2H); ES$^+$ MS: *m/z*: 329.0 $[M+H]^+$; HPLC–ELS: 99.5%.

(*S*)-2-Adamantan-1-yl-*N*-{[5-(6-aminohexylamino)pentylcarbamoyl]methyl}acetamide (9Ac): Yellow oil (yield: 1.3 mg, 3%); ^{1}H NMR: δ = 1.36–1.76 (m, 27H), 1.94 (bs, 2H), 1.99 (s, 2H), 2.88–3.00 (m, 6H), 3.20 (t, *J* = 6.7, 2H), 3.75 ppm (s, 2H); ES$^+$ MS: *m/z*: 435.2 $[M+H]^+$; HPLC–ELS: 99.9%.

(*S*)-*N*-{[5-(6-Aminohexylamino)pentylcarbamoyl]methyl}-2-(9-oxo-9,10-dihydroacridin-3-yl)acetamide (9Ad): Yellow crystals (yield: 1.9 mg, 3%); ^{1}H NMR: δ = 1.30–1.72 (m, 14H), 2.84–2.99 (m, 6H), 3.31–3.35 (m, 2H), 4.09 (s, 2H), 7.29–7.36 (m, 2H), 7.61 (d, *J* = 8.5, 1H), 7.72–7.77 (m, 1H), 8.17 (dd, *J* = 7.4 and 1.4, 1H), 8.31 (dd, *J* = 8.3 and 1.1, 1H), 8.51 ppm (dd, *J* = 8.0 and 1.4, 1H); ES$^+$ MS: *m/z*: 480.0 $[M+H]^+$; HPLC–ELS: 99.7%.

(*S*)-*N*-{[5-(6-Aminohexylamino)pentylcarbamoyl]methyl}-2-pyren-2-yl-acetamide (9Ae): Yellow oil (yield: 2.5 mg, 3%); ^{1}H NMR: δ = 1.06–1.54 (m, 14H), 2.50 (t, *J* = 8.0, 2H), 2.58–2.62 (m, 2H), 2.79 (t, *J* = 7.7, 2H), 3.16 (t, *J* = 6.6, 2H), 3.80 (s, 2H), 4.35 (s, 2H), 7.96 (d, *J* = 8.0, 1H), 8.00 (d, *J* = 7.7, 1H), 8.04 (s, 2H), 8.11–8.19 (m, 4H), 8.26 ppm (d, *J* = 9.4, 1H); ES$^+$ MS: *m/z*: 501.0 $[M+H]^+$; HPLC–ELS: 99.9%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-2-formylamino-3-methylbutyramide (9Ba): Yellow oil (yield: 5.1 mg, 8%): ^{1}H NMR: δ = 0.94 (dd, *J* = 6.9 and 2.2, 6H), 1.36–1.72 (m, 14H), 2.02–2.08 (m, 1H), 2.84–3.00 (m, 6H), 3.16–3.24 (m, 2H), 4.12 ppm (d, *J* = 6.9, 1H); ES$^+$ MS: *m/z*: 329.0 $[M+H]^+$; HPLC–ELS: 98.2%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-2-butyrylamino-3-methylbutyramide (9Bb): Colorless oil (yield: 8.5 mg, 12%); ^{1}H NMR: δ = 0.92–0.96 (m, 9H), 1.34–1.68 (m, 16H), 2.00 (h, *J* = 7.0, 1H), 2.22 (td, *J* = 10.2 and 3.2, 2H), 2.88–3.00 (m, 6H), 3.15–3.23 (m, 2H), 4.00 ppm (t, *J* = 7.7, 1H); ES$^+$ MS: *m/z*: 371.0 $[M+H]^+$; HPLC–ELS: 100%.

(*S*)-2-(2-Adamantan-1-yl-acetylamino)-*N*-[5-(6-aminohexylamino)-pentyl]-3-methylbutyramide (9Bc): Colorless oil (yield: 10.3 mg, 12%); ^{1}H NMR: δ = 0.95 (t, *J* = 7.0, 6H), 1.40–1.73 (m, 28H), 1.91–2.05 (m, 3H), 2.88–3.00 (m, 7H), 3.15–3.25 (m, 2H), 3.97 (dd, *J* = 9.4 and 6.1, 1H), 7.15–7.24 ppm (m, 5H); ES$^+$ MS: *m/z*: 477.1 $[M+H]^+$; HPLC–ELS: 100%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-3-methyl-2-[2-(9-oxo-9,10-dihydroacridin-3-yl)acetylamino]butyramide (9Bd): Yellow oil (yield: 5.2 mg, 6%); ^{1}H NMR: δ = 1.06 (dd, *J* = 6.9 and 4.7, 6H), 1.36–1.72 (m, 14H), 2.22 (hex, *J* = 6.9, 1H), 2.78–2.91 (m, 6H), 3.13–3.46 (m, 2H), 4.35 (d, *J* = 7.7, 1H), 7.28–7.36 (m, 2H), 7.62 (d, *J* = 10.2, 1H), 7.72–7.77 (m, 1H), 8.17 (dd, *J* = 7.2 and 1.5, 1H), 8.30 (dd, *J* = 7.7 and 1.1, 1H), 8.50 ppm (dd, *J* = 8.0 and 1.5, 1H); ES$^+$ MS: *m/z*: 522.2 $[M+H]^+$; HPLC–ELS: 99.8%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-3-methyl-2-(2-pyren-2-yl-acetylamino)butyramide (9Be): Brown oil (yield: 9.0 mg, 10%); ^{1}H NMR: δ = 0.96 (dd, *J* = 10.5 and 6.9, 6H), 1.04–1.57 (m, 14H), 2.06 (hex, *J* = 6.9, 1H), 2.33–2.57 (m, 4H), 2.80 (t, *J* = 7.6, 2H), 3.08–3.25 (m, 2H), 4.03 (dd, *J* = 7.7 and 4.9, 1H), 4.34 (s, 2H), 7.93 (d, *J* = 8.0, 1H), 7.99 (d, *J* = 7.4, 1H), 8.03 (s, 2H), 8.09–8.19 (m, 3H), 8.24–8.30 ppm (m, 2H); ES$^+$ MS: *m/z*: 543.1 $[M+H]^+$; HPLC–ELS: 99.8%.

(*S*)-4-[5-(6-Aminohexylamino)pentylcarbamoyl]-4-formylamino-butyric acid (9Ca): Colorless oil (yield: 6.3 mg, 9%); ^{1}H NMR: δ = 1.36–1.70 (m, 14H), 1.98–2.11 (m, 2H), 2.35 (t, *J* = 7.0, 2H), 2.87–2.99 (m, 6H), 3.19 (t, *J* = 7.0, 2H), 4.33 ppm (dd, *J* = 8.3 and 5.6, 1H); ES$^+$ MS: *m/z*: 359.3 $[M+H]^+$; HPLC–ELS: 98.2%.

(*S*)-4-[5-(6-Aminohexylamino)pentylcarbamoyl]-4-butyrylamino-butyric acid (9Cb): Colorless oil (yield: 3.9 mg, 6%); ^{1}H NMR: δ = 0.93 (t, J = 7.3, 3H), 1.40–1.68 (m, 16H), 2.21 (t, J = 7.3, 2H), 2.36 (t, J = 7.1, 2H), 2.85–3.00 (m, 6H), 3.17–3.22 (m, 2H), 4.25 ppm (dd, J = 9.5 and 5.7, 1H); ES$^+$ MS: m/z: 401.2 $[M+H]^+$; HPLC–ELS: 98.9%.

(*S*)-4-(2-Adamantan-1-yl-acetylamino)-4-[5-(6-aminohexylamino)-pentylcarbamoyl] butyric acid (9Cc): Yellow oil (yield: 12.8 mg, 15%); ^{1}H NMR: δ = 1.52–1.57 (m, 29H), 2.05–2.23 (m, 4H), 2.57 (t, J = 8.2, 2H), 3.07–3.19 (m, 6H), 3.87 (t, J = 6.8, 2H), 4.44 ppm (dd, J = 9.1 and 5.6, 1H); ES$^+$ MS: m/z: 507.3 $[M+H]^+$; HPLC–ELS: 99.9%.

(*S*)-4-[5-(6-Aminohexylamino)pentylcarbamoyl]-4-[2-(9-oxo-9,10-dihydro-acridin-3-yl)acetylamino]butyric acid (9Cd): Yellow oil (yield: 11.1 mg, 12%); ^{1}H NMR: δ = 1.21–1.67 (m, 14H), 2.01–2.25 (m, 2H), 2.49 (t, J = 7.6, 2H), 2.79–2.91 (m, 6H), 3.19–3.33 (m, 2H), 4.61 (dd, J = 9.4 and 5.3, 1H), 7.27–7.33 (m, 2H), 7.58 (d, J = 8.2, 1H), 7.70–7.75 (m, 1H), 8.19 (dd, J = 7.3 and 1.5, 1H), 2.28 (dd, J = 8.2 and 1.2, 1H), 8.48 ppm (dd, J = 8.2 and 1.5, 1H); ES$^+$ MS: m/z: 552.0 $[M+H]^+$; HPLC–ELS: 99.0%.

(*S*)-2-Formylaminopentanedioic acid 5-amide 1-{[5-(6-aminohexylamino)pentyl]amide} (9Da): Colorless oil (yield: 12.4 mg, 18%); ^{1}H NMR: δ = 1.31–1.65 (m, 14H), 1.90–2.05 (m, 2H), 2.49 (t, J = 5.6, 2H), 2.86–2.97 (m, 6H), 3.18 (t, J = 7.0, 2H), 4.28 ppm (dd, J = 9.1 and 5.6, 1H); ES$^+$ MS: m/z: 358.1 $[M+H]^+$; HPLC–ELS: 96.9%.

(*S*)-2-Butyrylaminopentanedioic acid 5-amide 1-{[5-(6-aminohexylamino)pentyl]amide} (9Db): Colorless oil (yield: 3.2 mg, 5%); ^{1}H NMR: δ = 0.94 (t, J = 7.3, 3H), 1.39–2.05 (m, 16H), 2.22 (t, J = 7.4, 2H), 2.28 (t, J = 7.4, 2H), 2.81–3.00 (m, 8H), 3.19 (t, J = 6.6, 2H), 4.20 ppm (dd, J = 8.8 and 5.0, 1H); ES$^+$ MS: m/z: 400.0 $[M+H]^+$; HPLC–ELS: 96.1%.

(*S*)-2-(2-Adamantan-1-yl-acetylamino)pentanedioic acid 5-amide 1-{[5-(6-aminohexylamino)pentyl]amide} (9Dc): Yellow oil (yield: 5.3 mg, 6%); ^{1}H NMR: δ = 1.37–1.75 (m, 27H), 1.85–2.02 (m, 6H), 2.29 (t, J = 7.4, 2H), 2.88–3.00 (m, 6H), 3.19 (t, J = 6.6, 2H), 4.20 ppm (dd, J = 8.9 and 5.5, 1H); ES$^+$ MS: m/z: 506.1 $[M+H]^+$; HPLC–ELS: 99.7%.

(*S*)-2-[2-(9-Oxo-9,10-dihydro-acridin-3-yl)acetylamino]pentanedioic acid 5-amide 1-{[5-(6-aminohexylamino)pentyl]amide} (9Dd): Yellow oil (yield: 7.9 mg, 8%); ^{1}H NMR: δ = 1.22–1.72 (m, 14H), 2.05–2.28 (m, 2H), 2.44 (t, J = 7.4, 2H), 2.74–2.96 (m, 6H), 3.19–3.40 (m, 2H), 4.54 (dd, J = 9.4 and 4.7, 1H), 7.30–7.38 (m, 2H), 7.62 (d, J = 8.3, 1H), 7.76 (t, J = 8.3, 1H), 8.24 (d, J = 7.7, 1H), 8.32 (d, J = 7.7, 1H), 8.53 ppm (d, J = 8.3, 1H); ES$^+$ MS: m/z: 551.0 $[M+H]^+$; HPLC–ELS: 100%.

(*S*)-2-(2-Pyren-2-yl-acetylamino)pentanedioic acid 5-amide 1-{[5-(6-aminohexylamino)pentyl]amide} (9De): Yellow crystals (yield: 6.4 mg, 7%); ^{1}H NMR: δ = 1.00–1.53 (m, 14H), 1.95–2.10 (m, 2H), 2.28–2.55 (m, 6H), 2.79 (t, J = 7.7, 2H), 3.09–3.25 (m, 2H), 4.23 (dd, J = 8.6 and 5.6, 1H), 4.35 (s, 2H), 7.95 (d, J = 8.0, 1H), 8.01 (d, J = 7.4, 1H), 8.05 (s, 2H), 8.13–8.21 (m, 4H), 8.27 ppm (d, J = 9.4, 1H); ES$^+$ MS: m/z: 572.1 $[M+H]^+$; HPLC–ELS: 99.9%.

(*S*)-2-(2-Adamantan-1-yl-acetylamino)-5-guanidinopentanoic acid [5-(6-aminohexylamino)pentyl]amide (9Ec): Colorless oil (yield: 3.1 mg, 3%); ^{1}H NMR: δ = 1.42–1.72 (m, 31H), 1.94–2.02 (m, 4H), 2.88–3.00 (m, 8H), 3.13–3.22 (m, 2H), 4.25 ppm (dd, J = 8.0 and 5.6, 1H); ES$^+$ MS: m/z: 534.1 $[M+H]^+$; HPLC–ELS: 99.1%.

(*S*)-5-Guanidino-2-[2-(9-oxo-9,10-dihydroacridin-3-yl)acetylamino]pentanoic acid [5-(6-aminohexylamino)pentyl]amide (9Ed): Yellow oil (yield: 9.7 mg, 9%); ^{1}H NMR: δ = 1.27–1.85 (m, 18H), 2.57 (t, J = 6.5, 2H), 2.78–2.89 (m, 6H), 3.10–3.43 (m, 2H), 4.60 (dd, J = 8.8 and 5.7, 1H), 7.30–7.38 (m, 2H), 7.62 (d, J = 8.0, 1H), 7.73–7.79 (m, 1H), 8.23 (dd, J = 7.4 and 1.7, 1H), 8.31 (dd, J = 8.3 and 1.4, 1H), 8.53 ppm (dd, J = 8.0 and 1.4, 1H); ES$^+$ MS: m/z: 579.3 $[M+H]^+$; HPLC–ELS: 100%.

(*S*)-5-Guanidino-2-(2-pyren-2-yl-acetylamino)pentanoic acid [5-(6-aminohexylamino)pentyl]amide (9Ee): Yellow oil (yield: 2.1 mg, 3%); ^{1}H NMR: δ = 1.32–1.87 (m, 16H), 2.43–2.59 (m, 4H), 2.81 (t, J = 7.5, 2H), 2.92–3.24 (m, 6H), 4.25 (dd, J = 8.3 and 5.7, 1H), 4.35 (s, 2H), 7.96 (d, J = 8.0, 1H), 8.01 (d, J = 7.4, 1H), 8.06 (s, 2H), 8.13–8.21 (m, 4H), 8.28 ppm (d, J = 9.4, 1H); ES$^+$ MS: m/z: 600.4 $[M+H]^+$; HPLC–ELS: 99.5%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-2-formylamino-3-(3H-imidazol-4-yl)propionamide (9Fa): Pale yellow oil (yield: 16.3 mg, 19%); ^{1}H NMR: δ = 1.30–1.69 (m, 14H), 2.86–2.98 (m, 8H), 3.11–3.20 (m, 2H), 4.67 (dd, J = 8.5 and 5.6, 1H), 7.30 (s, 1H), 8.04 ppm (s, 1H); ES$^+$ MS: m/z: 367.2 $[M+H]^+$; HPLC–ELS: 99.7%.

(*S*)-*N*-1-[5-(6-Aminohexylamino)pentylcarbamoyl]-2-(1*H*-imidazol-4-yl)ethylbutyramide (9Fb): Colorless oil (yield: 3.1 mg, 5%); ^{1}H NMR: δ = 0.85 (t, J = 7.3, 3H), 1.34–1.67 (m, 16H), 2.17 (td, J = 8.9 and 1.2, 2H), 2.88–3.24 (m, 10H), 4.63 (dd, J = 9.4 and 5.8, 1H), 7.27 (s, 1H), 8.75 ppm (s, 1H); ES$^+$ MS: m/z: 409.4 $[M+H]^+$; HPLC–ELS: 96.1%.

(*S*)-2-(2-Adamantan-1-yl-acetylamino)-*N*-[5-(6-aminohexylamino)-pentyl]-3-(3H-imidazol-4-yl)propionamide (9Fc): Yellow oil (yield: 2.8 mg, 4%); ^{1}H NMR: δ = 1.35–1.72 (m, 29H), 1.88–1.94 (m, 5H), 2.87–3.25 (m, 7H), 4.65 (dd, J = 9.7 and 5.6, 1H), 7.32 (s, 1H), 8.81 ppm (d, J = 1.4, 1H); ES$^+$ MS: m/z: 515.2 $[M+H]^+$; HPLC–ELS: 99.2%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-3-(3H-imidazol-4-yl)-2-[2-(9-oxo-9,10-dihydroacridin-3-yl)acetylamino]propionamide (9Fd): Yellow crystals (yield: 9.1 mg, 8%); ^{1}H NMR: δ = 1.33–1.66 (m, 14H), 2.82–2.91 (m, 8H), 3.32–3.47 (m, 2H), 5.00 (dd, J = 9.4 and 5.5, 1H), 7.28–7.37 (m, 3H), 7.55 (d, J = 8.3, 1H), 7.71–7.78 (m, 1H), 8.20 (dd, J = 7.4 and 1.7, 2H), 8.51 (dd, J = 8.0 and 1.4, 1H), 8.78 ppm (d, J = 1.4, 1H); ES$^+$ MS: m/z: 560.0 $[M+H]^+$; HPLC–ELS: 97.9%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-3-(3H-imidazol-4-yl)-2-(2-pyren-2-yl-acetylamino)propionamide (9Fe): Yellow oil (yield: 8.3 mg, 7%); ^{1}H NMR: δ = 1.18–1.61 (m, 16H), 2.57–2.62 (m, 2H), 2.85 (t, J = 7.6, 2H), 3.03–3.24 (m, 4H), 4.30 (s, 2H), 4.64 (dd, J = 9.1 and 5.6, 1H), 7.19 (s, 1H), 7.89 (d, J = 7.9, 1H), 8.01 (t, J = 7.6, 1H), 8.06 (s, 2H), 8.13 (dd, J = 10.8 and 8.5, 1H), 8.18–8.22 (m, 4H), 8.62 ppm (d, J = 1.2, 1H); ES$^+$ MS: m/z: 581.1 $[M+H]^+$; HPLC–ELS: 99.9%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-2-formylamino-3-(4-hydroxyphenyl)propionamide (9Ga): Yellow oil (yield: 4.7 mg, 6%); ^{1}H NMR: δ = 1.14–1.76 (m, 14H), 2.78–3.17 (m, 10H), 4.47 (t, J = 7.4, 1H), 6.68 (d, J = 8.5, 2H), 7.01 ppm (d, J = 8.5, 2H); ES$^+$ MS: m/z: 393.0 $[M+H]^+$; HPLC–ELS: 96.6%.

(*S*)-*N*-1-[5-(6-Aminohexylamino)pentylcarbamoyl]-2-(4-hydroxyphenyl)ethylbutyramide (9Gb, PhTX-56):[25] Colorless oil (yield: 6.3 mg, 8%); ^{1}H NMR: δ = 0.84 (t, J = 7.3, 3H), 1.36–1.68 (m, 16H), 2.14 (t, J = 7.3, 2H), 2.77 (dd, J = 13.5 and 8.2, 1H), 2.88–3.16 (m, 9H), 4.40 (t, J = 7.6, 1H), 6.66 (d, J = 8.2, 2H), 7.01 ppm (d, J = 8.4, 2H); ES$^+$ MS: m/z: 435.1 $[M+H]^+$; HPLC–ELS: 99.8%.

(*S*)-2-(2-Adamantan-1-yl-acetylamino)-*N*-[5-(6-aminohexylamino)-pentyl]-3-(4-hydroxyphenyl)propionamide (9Gc): Pale yellow oil (yield: 6.3 mg, 7%); ^{1}H NMR: δ = 1.27–1.68 (m, 27H), 1.83–1.95 (m, 6H), 2.72 (dd, J = 14.6 and 9.0, 1H), 2.86–3.02 (m, 7H), 3.08–3.22 (m, 2H), 4.44 (dd, J = 9.4 and 6.3, 1H), 6.66 (d, J = 8.3, 2H),

7.03 ppm (d, $J=8.4$, 2H); ES^+ MS: *m/z*: 541.2 $[M+H]^+$; HPLC–ELS: 99.9%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-3-(4-hydroxyphenyl)-2-[2-(9-oxo-9,10-dihydroacridin-3-yl)acetylamino]propionamide (9Gd): Yellow oil (yield: 5.3, 5%); ^1H NMR: $\delta=1.25$–1.69 (m, 14H), 2.84–2.92 (m, 6H), 2.99 (dd, $J=13.8$ and 8.5, 1H), 3.13 (dd, $J=13.8$ and 7.2, 1H), 3.17–3.25 (m, 2H), 4.76 (dd, $J=8.5$ and 7.2, 1H), 6.79 (d, $J=8.5$, 2H), 7.12 (d, $J=8.5$, 2H), 7.28 (t, $J=7.8$, 2H), 7.53 (d, $J=8.3$, 1H), 7.69–7.75 (m, 1H), 8.09 (dd, $J=7.4$ and 1.4, 1H), 8.27 (dd, $J=8.3$ and 1.1, 1H), 8.46 ppm (dd, $J=8.3$ and 1.7, 1H); ES^+ MS: *m/z*: 586.2 $[M+H]^+$; HPLC–ELS: 99.7%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-3-(4-hydroxyphenyl)-2-(2-pyren-2-yl-acetylamino)propionamide (9Ge): Yellow oil (yield: 7.8 mg, 8%); ^1H NMR: $\delta=1.08$–1.56 (m, 14H), 2.54–2.62 (m, 2H), 2.80–3.17 (m, 6H), 4.26 (s, 2H), 4.41–4.48 (m, 1H), 6.62 (d, $J=8.3$, 2H), 6.95 (d, $J=8.03$, 2H), 7.81 (d, $J=7.7$, 1H), 7.87–7.96 (m, 1H), 8.00 (d, $J=7.4$, 1H), 8.03 (s, 2H), 8.08–8.12 (m, 2H), 8.17 ppm (d, $J=8.0$, 2H); ES^+ MS: *m/z*: 607.3 $[M+H]^+$; HPLC–ELS: 99.8%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-2-formylamino-3-phenyl-propionamide (9Ha): Brown oil (yield: 3.1 mg, 5%); ^1H NMR: $\delta=$ 1.36–1.68 (m, 14H), 2.86–3.19 (m, 10H), 4.55 (t, $J=7.4$, 1H), 7.14–7.32 ppm (m, 5H); ES^+ MS: *m/z*: 377.0 $[M+H]^+$; HPLC–ELS: 98.5%.

(*S*)-*N*-1-[5-(6-Aminohexylamino)pentyl-carbamoyl]-2-phenylethyl-butyramide (9Hb): Colorless oil (yield: 5.2 mg, 7%); ^1H NMR: $\delta=$ 0.81 (t, $J=7.3$, 3H), 1.39–1.75 (m, 16H), 2.13 (t, $J=7.3$, 2H), 2.81–3.15 (m, 10H), 4.48 (t, $J=7.1$, 1H), 7.17–7.27 ppm (m, 5H); ES^+ MS: *m/z*: 419.1 $[M+H]^+$; HPLC–ELS: 99.6%.

(*S*)-2-(2-Adamantan-1-yl-acetylamino)-*N*-[5-(6-aminohexylamino)-pentyl]-3-phenylpropionamide (9Hc): Pale yellow oil (yield: 10.3 mg, 11%); ^1H NMR: $\delta=1.26$–1.67 (m, 28H), 1.77–1.83 (m, 3H), 1.88 (d, $J=9.0$, 1H), 2.76–3.00 (m, 7H), 3.06–3.18 (m, 2H), 4.53 (dd, $J=9.9$ and 5.8, 1H), 7.15–7.24 ppm (m, 5H); ES^+ MS: *m/z*: 525.1 $[M+H]^+$; HPLC–ELS: 99.9%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-2-[2-(9-oxo-9,10-dihydro-acridin-3-yl)acetylamino]-3-phenylpropionamide (9Hd): Yellow crystals (yield: 11.5 mg, 12%); ^1H NMR: $\delta=1.11$–1.56 (m, 14H), 2.47–2.60 (m, 4H), 2.78–2.93 (m, 4H), 3.05 (dd, $J=13.5$ and 6.1, 1H), 3.11–3.16 (m, 1H), 4.24 (d, $J=3.3$, 2H), 4.51 (dd, $J=9.6$ and 5.5, 1H), 7.01–7.10 (m, 5H), 7.81 (d, $J=8.0$, 1H), 7.94–8.10 (m, 7H), 8.16 ppm (d, $J=7.5$, 2H); ES^+ MS: *m/z*: 570.1 $[M+H]^+$; HPLC–ELS: 99.9%.

(*S*)-*N*-[5-(6-Aminohexylamino)pentyl]-3-phenyl-2-(2-pyren-2-yl-acetylamino)propionamide (9He): Yellow crystals (yield: 9.5 mg, 10%); ^1H NMR: $\delta=1.32$–1.71 (m, 14H), 2.82–2.92 (m, 6H), 3.08 (dd, $J=13.8$ and 9.1, 1H), 3.16–3.26 (m, 3H), 4.84 (dd, $J=10.2$ and 4.9, 1H), 7.14–7.35 (m, 7H), 7.50 (d, $J=8.3$, 1H), 7.67–7.73 (m, 1H), 8.06 (dd, $J=7.4$ and 1.7, 2H), 8.24 (dd, $J=8.5$ and 1.4, 1H), 8.43 ppm (dd, $J=8.0$ and 1.4, 1H); ES^+ MS: *m/z*: 591.1 $[M+H]^+$; HPLC–ELS: 99.9%.

In vitro cRNA transcription: The construct AMPAR1 flop was inserted into the pGEMHE[38] oocyte expression vector. DNA (1 μg) was linearized with the appropriate enzyme. Run-off transcription was performed by using MEGAscript Kit (Ambion).

Electrophysiology: A female frog (*Xenopus laevis*) was anesthetized in a solution of 0.2% MS 222 (3-aminobenzoic acid ethyl ester, Sigma–Aldrich) for 15–30 min, and three to five ovarian lobes were surgically removed. The follicle layer was removed by washing twice in Barth's saline (88.0 mM NaCl, 1.0 mM KCl, 2.4 mM $NaHCO_3$, 15.0 mM HEPES pH 7.5, 0.30 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 100 U mL^{-1} penicillin), once in OR-2 (82.5 mM NaCl, 2.0 mM KCl, 1.0 mM $MgCl_2$, 5.0 mM HEPES pH 7.5), followed by treatment with collagenase A (1 mg mL^{-1} in OR-2) for 2–3 h at room temperature. Oocytes at stage 4–5 were selected and injected the following day with 50 nl (5–50 ng) cRNA. The oocytes were kept at 18 °C in Barth's saline before recordings were performed 4–7 days after injection using a two-electrode voltage clamp (OC-725C, Warner Instruments, Hamden, CT, USA). The recording solution was low-Ca^{2+} Ringer (LCR; 115 mM NaCl, 0.1 mM $CaCl_2$, 2.5 mM KCl, 1.8 mM $MgCl_2$, 10 mM HEPES pH 7.5). The LCR buffer was chosen to prevent activation of the endogenous Ca^{2+}-activated Cl^- channels. The oocytes were clamped at −70 mV. Electrodes (borosilicate glass capillaries; o.d. = 1.5 mm; i.d. = 1.17 mm, with inner filament; Harvard Apparatus, Kent, UK) were filled with 3 M KCl and exhibited resistance around 0.5 MΩ.

Data analysis: The data were acquired with Clampex 8.0 (Axon instruments, Union City, CA, USA). All responses were normalized to the average of a glutamate response applied before and after the channel blocker and glutamate co-application. Normalized responses in the interval [0.4;0.6] were used to calculate the K_i (equilibrium dissociation constant) for the channel blocker. At high glutamate concentration (100 μM), at which all ion channels are open, the proportion of ion channels occupied by the channel blocker is given by the Hill–Langmuir equation. It was assumed that the philanthotoxins block the ion channel in the re-entrant loop region, and therefore elicit minor effects on the equilibrium constants between the inactive, active, and desensitized states. Hence, the equilibrium dissociation constants for the philanthotoxins can be expressed as: $K_i=([PhTX]\times I_{normal})/(1-I_{normal})$. Mean and SD values were calculated assuming a Gaussian distribution of K_i.

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