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Tuning Wasp Toxin Structure for Nicotinic Receptor Antagonism: Cyclohexylalanine-Containing Analogues as Potent and Voltage-Dependent Blockers

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The wasp venom constituent philanthotoxin-433 (PhTX-433, **1**)[1] is a polyamine toxin that antagonizes ionotropic glutamate receptors (iGluRs)[2] and nicotinic acetylcholine receptors (nAChRs)[3] nonselectively (Figure 1). Thus, similar potencies have been reported for PhTX-433 and its synthetic spermine-containing analogue PhTX-343 (**2**) toward a range of human and insect glutamate- and ACh-gated ion channels.[4] The broad selectivity observed for **1** is compatible with its natural role as a prey suppression tool. Attempts to improve selectivity toward human receptors, necessary for potential therapeutic applications, have been partly successful through manipulation of the parent polyamine structure, whereas no notable improvements in selectivity have yet been observed through structural modifications of the tyrosine head group. Thus, while PhTX-83 (**3**) has been shown to be a selective antagonist of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR),[5] PhTX-12 (**4**) is a selective human muscle-type nAChR antagonist, which is inactive toward AMPAR.[5,6] Herein, we report analogues in which the tyrosine moiety present in **1**–**4** has been replaced by cyclohexylalanine (Cha). These novel head-group analogues (compounds **5**–**10**) show pronounced selectivity toward human muscle-type nAChR, with one of them exhibiting unprecedented potency at nanomolar concentrations.

The synthesis of polyamine toxins is greatly facilitated by the use of solid-phase synthesis (SPS) strategies.[7] In particular, Fukuyama–Mitsunobu alkylation has been successfully applied as a means of stepwise construction of the polyamine moiety.[8–11] However, a large excess of reagents in three repetitive couplings is required to obtain satisfactory yields. Accordingly, a method that involves S_N2 amine alkylation was devised for the synthesis of **8** and **9** (Scheme 1). In preliminary alkylation experiments with various resin-bound sulfonates, mesylate proved superior, presumably owing to the absence of competing *trans* sulfonation, which becomes predominant in SPS with increasingly reactive sulfonates.[12] Hence, the mesylate displacement strategy[13,14] was chosen for the synthesis of **8** and **9**. Argopore Wang resin **13** was derivatized with a mono-Teoc-protected diamine (**11** or **12**; Scheme 1, Method A), and the resulting resins were treated successively with Boc_2O and Bu_4NF to give resin **15** or **16**, respectively. Peptide and acyl couplings completed the syntheses, with isolation of the required analogues **8** and **9** in 8–9% yield. Moreover, **9** was re-synthesized on a polystyrene trityl resin **14** by using a modified procedure in an attempt to improve the overall yield; instead of the repetitive polyamine chain elongation, the monoprotected diamine **12** was employed at a concentration of 1 M (Scheme 1, Method B),[13–15] to give **9** in 24% yield. Compounds **5**–**7** were obtained by using Fukuyama–Mitsunobu alkylation in isolated yields of 30%.[9] For the analogue **10**, which contains the symmetrical spermine moiety, it was more rational to apply the original[16] synthetic method in solution. Thus, tri-Boc spermine was coupled with Cha, protected with an Fmoc group and activated as a pentafluorophenyl (Pfp) ester, followed by deprotection and coupling with Pfp butanoate. All analogues **5**–**10**

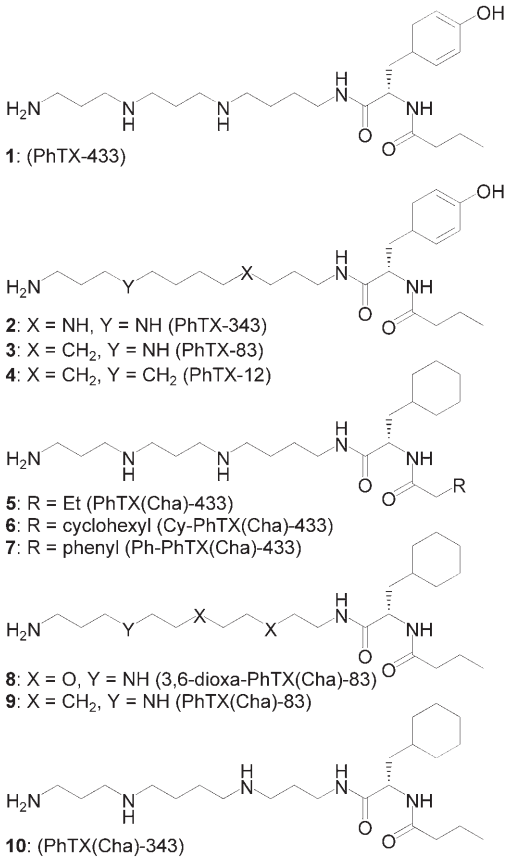

Figure 1. Structures of philanthotoxins **1**–**10**.

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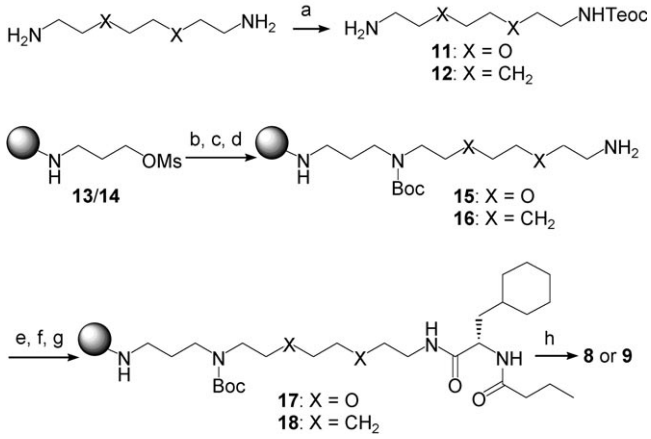

Scheme 1. Reagents and conditions: a) 2-(trimethylsilyl)ethyl *p*-nitrophenylcarbonate (0.2 equiv), MeOH/CH_2Cl_2 (1:1). Method A (Argopore Wang resin, **13**): b) **11** or **12** (0.2 M, 5 equiv), NMP, 70 °C, 16 h (repeated for 3 h); c) Boc_2O (5 equiv), iPr_2EtN (5 equiv), CH_2Cl_2, room temperature, N_2; d) Bu_4NF (5 equiv), DMF, 50 °C; e) (*S*)-N^{α}-Fmoc-Cha-OPfp (3 equiv), iPr_2EtN (2.6 equiv), HODhbt (1 equiv), DMF, room temperature, N_2; f) 20% piperidine in DMF; g) $C_3H_7COOPfp$ (3 equiv), iPr_2EtN (2.6 equiv), HODhbt (1 equiv), DMF, room temperature, N_2; h) TFA/CH_2Cl_2 (1:1); yields: **8** (8%) or **9** (9%). Method B (polystyrene trityl resin, **14**): b) **12** (1.0 M, 10 equiv), DMF, 50 °C, 6 h; c)–h) were performed as above; yield: **9** (24%). Boc = *tert*-butoxycarbonyl, DMF = *N*,*N*-dimethylformamide, Fmoc = 9-fluorenylmethoxycarbonyl, HODhbt = 3,4-dihydro-5-hydroxy-4-oxo-1,2,3-benzotriazine, Ms = methanesulfonate, NMP = *N*-methylpyrrolidinone, Teoc = 2-(trimethylsilyl)ethoxycarbonyl, TFA = trifluoroacetic acid.

were purified by preparative reversed-phase HPLC or reversed-phase vacuum liquid chromatography (VLC).[16]

The antagonism of the Cha-containing toxins **5**–**10** on human muscle-type nAChR expressed in TE671 cells[17] was determined electrophysiologically by whole-cell patch-clamp experiments at three holding potentials ($V_H = -100$, -50, and $+50$ mV). The IC_{50} values obtained are compared in Table 1 with the published values for **2**–**4**. All synthesized Cha-containing analogues were significantly more potent than PhTX-343 (**3**) at nAChR at negative (physiologically relevant) holding potentials (Table 1), whereas the potency at AMPAR was similar to that of **3** (data not shown). Compounds **7**–**9** were approximately equipotent at $V_H = -100$ mV with PhTX-12 (**4**), obtained by the removal of both inner amino functionalities from the polyamine chain of the natural toxin PhTX-433 (**1**).[5,6] However, PhTX(Cha)-83 (**9**) was 39-fold more potent than PhTX-343 (**2**), and 8-fold more potent than PhTX-83 (**3**) (Table 1). Even more notable, PhTX(Cha)-343 (**10**) was 277-fold more potent than **2**, and 16-fold more potent than **4** (Table 1). Therefore, **10** is the most potent channel blocker identified so far for the human muscle-type nAChR.

Interestingly, in contrast to **4** and its homologues,[5,6] and similarly to PhTX-343 (**2**), the antagonism of **9** and **10** was voltage-dependent (Table 1). The voltage-dependent antagonism is believed to arise from blockage of an open-channel state of the receptor by ligand binding to a site deep inside the transmembrane pore.[18,19] Binding of a PhTX-343 analogue near the channel gate and close to the chlorpromazine binding site[20] was previously suggested from the results of photoaffinity labeling experiments.[3a] On the other hand, PhTX-12 (**4**) and its homologues are believed to bind to the channel vestibule, near the extracellular entrance to the pore.[3c,18,19] The significance of analogues such as **10** is thus related not only to their potency at nAChR relative to AMPAR, but primarily to the fact that their increased affinity comes from modification of the natural toxin head group, resulting in derivatives that apparently bind to the same binding site as **2**. Although the antagonism by **10** was voltage-dependent, there was still potent inhibition at $+50$ mV, at which the open-channel block should be absent. This perhaps suggests that **10** can also interact strongly with the shallow site.[18,19] Further structure–activity studies, delineation of pharmacological profiles on other types of ionotropic receptors, and mechanistic studies of the binding mode of these novel analogues to nAChR are in progress in our laboratories.

Table 1. Data for the inhibition of the nicotinic acetylcholine receptor.

	Compd	IC_{50} [μM][a]		
		−100 mV	−50 mV	+50 mV
2	PhTX-343[b]	16.6 ± 0.24 (43)	102 ± 6 (36)	⩾100
3	PhTX-83[b]	3.31 ± 0.18 (13)	8.91 ± 0.52 (14)	20.5 ± 1.6 (17)
4	PhTX-12[b]	0.93 ± 0.09 (29)	1.53 ± 0.11 (23)	1.67 ± 0.16 (22)
5	PhTX(Cha)-433	3.50 ± 0.12 (10)	3.73 ± 0.34 (12)	15.5 ± 1.6 (11)
6	Cy-PhTX-(Cha)-433	1.73 ± 0.26 (5)	3.84 ± 0.44 (11)	≈9 (8)[c]
7	Ph-PhTX-(Cha)-433	0.53 ± 0.01 (8)	1.19 ± 0.034 (10)	2.66 ± 0.18 (8)
8	3,6-dioxa-PhTX(Cha)-83	0.99 ± 0.47 (6)	1.75 ± 0.56 (8)	>100 (6)
9	PhTX(Cha)-83	0.43 ± 0.17 (15)	2.45 ± 0.64 (12)	>100 (6)
10	PhTX(Cha)-343	0.06 ± 0.03 (9)	0.27 ± 0.14 (14)	0.60 ± 0.33 (10)

[a] Values are ± SE for the inhibition of nAChR activated by 10 μM acetylcholine at holding potentials of: $V_H = -100$, -50, and $+50$ mV; values in parentheses: number of cells. [b] Values from reference [5]. [c] An estimate, as some potentiation at low concentrations (< 1 μM) was observed, which compromised the sigmoidal curve-fit.

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