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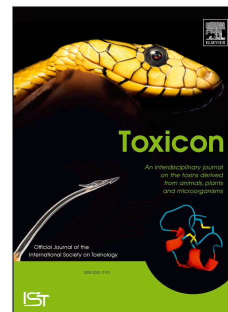

Isolation, chemical and functional characterization of several new K^+-channel blocking peptides from the venom of the scorpion *Centruroides tecomanus*

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1 Isolation, chemical and functional characterization of several new K^+-channel blocking
2 peptides from the venom of the scorpion *Centruroides tecomanus*
3
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22 Key words: amino acid sequence; *Centruroides*; ion-channel; scorpion toxin
23

24 **ABSTRACT**

25

26 Six new peptides were isolated from the venom of the Mexican scorpion *Centruroides*
27 *tecomanus*; their primary structures were determined and the effects on ion channels were
28 verified by patch-clamp experiments. Four are K^+-channel blockers of the α-KTx family,
29 containing 32 to 39 amino acid residues, cross-linked by three disulfide bonds. They all
30 block Kv1.2 in nanomolar concentrations and show various degree of selectivity over
31 Kv1.1, Kv1.3, Shaker and KCa3.1 channels. One peptide has 42 amino acids cross-linked
32 by four disulfides; it blocks ERG-channels and belongs to the γ-KTx family. The sixth
33 peptide has only 32 amino acid residues, three disulfide bonds and has no effect on the ion-
34 channels assayed. It also does not have antimicrobial activity. Systematic numbers were
35 assigned (time of elution on HPLC): α-KTx 10.4 (time 24.1); α-KTx 2.15 (time 26.2); α-
36 KTx 2.16 (time 23.8); α-KTx 2.17 (time 26.7) and γ-KTx 1.9 (elution time 29.6). A partial
37 proteomic analysis of the short chain basic peptides of this venom, which elutes on
38 carboxy-methyl-cellulose column fractionation, is included. The pharmacological
39 properties of the peptides described in this study may provide valuable tools for
40 understanding the structure-function relationship of K^+ channel blocking scorpion toxins.

41

42

43 ## 1. INTRODUCTION

44

45 Scorpion venom components display important pharmacological activities, which have
46 been used in the past for physiological characterization of ion channels, possible

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47 development of new antibiotics, anti-malaria agents, specific enzymatic functions and
48 development of anti-venoms, among other activities (Ortiz et al., 2015; Possani et al.,
49 1999). Recently, several publications dealing with proteomic and transcriptomic analysis of
50 scorpion venoms have been published, starting with (Schwartz et al., 2007) followed by
51 (Abdel-Rahman et al., 2013; Diego-Garcia et al., 2012; He et al., 2013; Luna-Ramirez et
52 al., 2015; Luna-Ramirez et al., 2013; Ma et al., 2012; Ma et al., 2010; Rendon-Anaya et al.,
53 2012; Smith et al., 2012), and others, revised in (Rendón-Anaya et al., 2014). Among the
54 main constituents of these venoms are proteins (enzymes such as hyaluronidase,
55 phospholipases, proteases with hydrolytic effects), peptides that block or modulate ion
56 channels (Na^+, K^+, Ca^{2+}, or Cl^- channels), free amino acids, biogenic amines, nucleotides,
57 carbohydrates, lipids and many small molecular weight components of unknown function
58 (see reviews (Ortiz et al., 2014; Possani et al., 1999; Rodriguez de la Vega and Possani,
59 2005; Smith and Alewood, 2014)). From the huge diversity of scorpions known (over 2,000
60 different species, as compiled by (Dunlop and Penney, 2012)), in the world, only a minor
61 fraction have been studied in some extent concerning the molecular biology of their
62 components and their corresponding activities. A few recent reviews in the literature can be
63 consulted on the subject (Bartok et al., 2015; Gurevitz et al., 2014; Martin-Eauclaire et al.,
64 2014; Santibanez-Lopez and Possani, 2015). México among the richest regions in the world
65 on scorpion diversity, only those belonging to the *Centruroides* and a few other genera,
66 such as *Hadrurus*, *Anuroctonus* and *Vaejovis* have been studied thus far. All the dangerous
67 species to humans in the country belong to the genus *Centruroides*. One of the most lethal
68 venoms is from the scorpion of the state of Colima, *Centruroides tecomanus*. From this
69 scorpion several peptides that impair proper function of Na^+-channels of excitable cells

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70 were previously isolated and characterized (Martin et al., 1988; Possani et al., 1980;
71 Ramirez et al., 1988), and more recently a proteomic and transcriptomic analysis was
72 published (Valdez-Velazquez et al., 2013). According to the transcriptomic analysis the
73 venom of *C. tecomanus* contains peptides whose amino acid sequence was similar to other
74 known scorpion toxins that block K^+-channels (KTx), but none of these putative peptides
75 have been formerly studied at the chemical and functional level. Here we describe the
76 isolation of six new peptides, whose complete primary structure was determined and the
77 specific effects on voltage-dependent K^+-channels were identified. Many peptides from
78 scorpion venom that inhibit potassium channels are known, which permitted the
79 establishment of a systematic nomenclature. Based on structural characteristics these
80 peptides are classified as: α, β, γ and κ KTx families (Rodriguez de la Vega and Possani,
81 2004; Tytgat et al., 1999). The largest group is the α-KTx. These toxins are typically 23-43
82 amino acid long oligopeptides with three or four disulfide bonds stabilizing their 3D
83 structure. Many of them show high specificity and uniquely high affinity to a targeted
84 channel. They generally possess a conserved amino acid pair called the essential dyad,
85 which consists of a lysine and another, usually aromatic residue in a distance of *circa* 7
86 Angstroms. (Dauplais et al., 1997). As the positively charged lysine intrudes the selectivity
87 filter of K^+ channels this dyad plays important role in the channel blocking ability. The
88 primary structure of the peptides, the arrangement of the dyad and its surrounding residues
89 will define the selectivity and affinity for the various types of K^+-channels. In this paper we
90 describe four new α-KTxs, one γ-KTx and an orphan peptide for which the function is thus
91 far unknown.
92

93

94

95 2. MATERIAL AND METHODS

96

97 *2.1 Venom source, chemical and reagents*

98 Scorpions collected in Colima State (Mexico) with official permission from SEMARNAT
99 (number MOR-IN-166-07-04) were milked for venom by electrical stimulation. The venom
100 was dissolved in water, centrifuged at 10,000 *g* for 20 minutes and the supernatant was
101 lyophilized and kept at -20 ºC until use. All chemicals and reagents were analytical grade
102 substances. Double distilled water was used through all the procedures.

103

104 *2.2 Purification of peptides*

105 The purification strategy initially described by our group was followed for the isolation of
106 the peptides, with small modifications. The soluble venom was separated by gel filtration
107 on Sephadex G-50 column, in 20 mM ammonium acetate buffer, pH 4.7, exactly as earlier
108 described (Ramirez et al., 1988). The toxic fraction II from Sephadex column was applied
109 to ion-exchange purification on carboxy-methyl-cellulose (CM-cellulose) resins. The
110 column was equilibrated with the same buffer, but eluted with a salt gradient from 0 to 0.5
111 M sodium chloride (the earlier gradient was from 0 to 0.4 M NaCl). The CM-cellulose
112 fractions of interest were further separate by high performance liquid chromatography
113 (HPLC), using an analytical C18 reverse-phase column (4.6 x 250 mm from Vydac,
114 Hysperia, CA. USA), not used in the earlier procedure. The pure peptides were obtained by
115 means of a linear gradient from solution A (0.12% trifluoroacetic acid (TFA) in water to

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116 60% solution B (0.10% TFA in acetonitrile), run for 60 min. The detection was monitored
117 by absorbance at λ = 230 nm and components were manually collected, freeze-dried using a
118 Savant SpeedVac dryer and used for chemical and functional characterization as described
119 below.
120
121 *2.3 Sequence by Edman degradation and mass spectrometry analysis*
122 Purity of peptides was verified by three means: elution profile from HPLC (single peaks),
123 mass spectrometry analysis (see below) and amino acid sequencing on a PPSQ-31A Protein
124 Sequencer from Shimadzu Scientific Instruments, Inc. (Columbia, Maryland, USA).
125 Usually a sample of native peptide (*circa* 0.5 nmoles) was directed loaded for sequence and
126 a reduced and alkylated sample of the same peptide was additionally sequenced for
127 identification of the cysteine residues. When necessary fractionation of clean peptides were
128 obtained by protease digestion or cyanogen bromide cleavage as described below and the
129 corresponding peptides were purified by HPLC and placed in the automatic Sequencer.
130 Mass spectrometry analysis of peptides was performed using a LCQ*Fleet* apparatus from
131 Thermo Fisher Scientific Inc. (San Jose, CA, USA).
132 The protein sequence data reported in this paper will appear in the UniProt Knowledgebase
133 under the accession numbers: C0HJW2 for α-KTx 10.4 (time 24.1); C0HJW1 for α-KTx
134 2.15 (time 26.2); C0HJW6 for α-KTx 2.16 (time 23.8); C0HJW5 for α-KTx 2.17 (time
135 26.7) and C0HJW3 for γ-KTx 1.9 (elution time 29.6).
136
137 *2.4 Alkylation, digestion and chemical cleavage*
138

139 For reduction, the pure peptides were dissolved in 200 mM TRIS-HCl buffer, pH 8.6
140 containing 1 mg/mL EDTA and 6 M guanidinium chloride, to which 2 mg of dithiotreitol
141 (DDT) was added. Nitrogen was bubbled to the solution for 5 min and stored 45 min at 55
142 ºC. Immediately after 2.5 mg of Iodoacetamide was added to the reacting vial, placed in the
143 dark and let stand for 30 min. Finally, the reduced and alkylated peptide was applied to a
144 C18 reverse-phase column for elimination of reagents (similar conditions than described
145 above). When the sequencing procedure showed that the full sequence was not completed,
146 the peptides were either treated with cyanogens bromide or cleaved with protease V8 from
147 *Staphylococcus aureus*. For enzymatic digestion the reduced and alkylated sample (usually
148 50 µg) was dissolved in 100 mM ammonium bicarbonate buffer, pH 7.8 and added 2 µg of
149 protease v8 (Mannheim, Germany) and incubated at 37 ºC over-night. The digested sample
150 was separated by HPLC using the same conditions used for purification of peptides and the
151 products identified on the Sequencer. Alternatively, the reduced and alkylated pure peptides
152 were chemically cleaved with cyanogen bromide. Normally, to 50 µg of peptide dissolved
153 in 100 µL of 70% formic acid it was added 5 mg of cyanogen bromide (Sigma-Aldrich, St.
154 Louis, MO, USA) until completely dissolved, sealed and covered from light and let stand
155 for 4 h at room temperature. Immediately after, 50 µL of octanol were added and the
156 solution was evaporated with a nitrogen stream. The products were also separated by HPLC
157 and sequenced as described above.
158

159 *2.5 Amino acid comparison of peptide sequences*

160

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161 *C. tecomanus* peptide sequences were compared with known sequences using NCBI
162 BLAST.
163
164 *2.6 Cell culture and manipulation*
165
166 Antimicrobial activity was assayed according to Ramirez-Carreto et al., (2015).
167 For electrophysiological experiments (see below) tsA201 cells (Shen et al., 1995) were
168 cultured under standard conditions, as described previously (Corzo et al., 2008). Human
169 peripheral lymphocytes were drawn from healthy volunteers. Mononuclear cells were
170 isolated using Ficoll-Hypaque density gradient separation technique and were grown in 24-
171 well culture plates in a 5% CO_2 incubator at 37°C in RPMI 1640 medium supplemented
172 with 10% fetal calf serum (Sigma-Aldrich), 100 µg/ml penicillin, 100 µg/ml streptomycin,
173 and 2 mM L-glutamine (density, 5 x 10^5 cells per ml) for 2 to 5 days. 5, 7.5 or 10 µg/ml
174 phytohemagglutinin A (Sigma-Aldrich) was added to the medium to increase K^+ channel
175 expression.
176 Kv1.1, Kv1.2, KCa3.1, Shaker-IR, Kv10.1 (EAG) and Kv11.1 (hERG) channel coding
177 vectors were transfected in tsA201 cells using Ca^{2+} phosphate transfection kit (Sigma-
178 Aldrich, Hungary) according to the manufacturer's instructions. hKv1.1 and hKv1.2 genes
179 are coded in pCMV6-GFP plasmid (OriGene Technologies, Rockville, MD), hKCa3.1 in
180 pEGFP-C1 vector (gift from H. Wulff, University of California, Davis CA).
181 Shaker-IR (inactivation ball deletion mutant) (gift from G. Yellen, Harvard Medical
182 School, Boston, MA), Kv10.1 (EAG, gift from L. Pardo, Max Planck Institute for
183 Experimental Medicine, Göttingen, Germany) and hKv11.1 (hERG, kind gift from SH.

184 Heinemann, Max-Plank-Gesellschaft, Jena, Germany) coding plasmids were co-transfected
185 with plasmid containing the gene of GFP in a molar ratio of 10:1 (Bartok et al., 2014).
186 For the measurements of hKv1.3 currents activated lymphocytes were used (Bartok et al.,
187 2014).
188
189 *2.7 Electrophysiology*
190
191 Measurements were carried out using patch-clamp technique in voltage-clamp mode in
192 whole-cell or outside-out patch configuration. For the recordings Axon Axopatch 200B or
193 multiclamp 700B amplifiers and Axon Digidata 1440 digitizer were used (Molecular
194 Devices, Sunnyvale, CA). Micropipettes were pulled from GC 150 F-15 borosilicate
195 capillaries (Harvard Apparatus Kent, UK) resulting in 3- to 5-MΩ resistance in the bath
196 solution. Generally the extracellular solution consisted of 145 mM NaCl, 5 mM KCl, 1 mM
197 $MgCl_2$, 2.5 mM $CaCl_2$, 5.5 mM glucose, 10 mM HEPES, pH 7.35. For the measurements of
198 hKv11.1 (hERG) channels the bath solution contained 5 mM KCl, 10 mM HEPES, 20 mM
199 glucose, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.1 mM $CdCl_2$, 140 mM choline-chloride, pH 7.35.
200 Bath solutions were supplemented with 0.1 mg/ml BSA when toxins were dissolved. The
201 osmolarity of the extracellular solutions was between 302 and 308 mOsM/L. For the
202 measurements of Kv1.1, Kv1.2, Kv1.3 and Shaker currents the pipette solution contained
203 140 mM KF, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES and 11 mM EGTA, pH 7.22. To
204 measure on Kv11.1 channels the intracellular solution consisted of 140 mM KCl, 10 mM
205 HEPES, 2mM $MgCl_2$ and 10 mM EGTA, pH 7.22, for the KCa3.1 recordings it contained
206 150 mM K-aspartate, 5mM HEPES, 10 mM EGTA, 8.7 mM $CaCl_2$, 2 mM $MgCl_2$, pH 7.22

207 resulting in 1 μM free Ca^{2+} in the solution to activate KCa3.1 channels (Grissmer et al.,
208 1993). The osmolarity of the intracellular solutions was 295 mOsM/L.
209 To measure hKv1.1, hKv1.2, hKv1.3 and Shaker currents 15 or 200 ms long depolarization
210 impulses were applied to +50 mV from a holding potential of -100 mV every 15 s. KCa3.1
211 currents were elicited every 15 s with voltage ramps to +50 mV from a holding potential of
212 -120 mV. For hKv11.1 channels, currents were evoked in every 30 s with a voltage step
213 from a holding potential of -80 mV to +20 mV followed by a step to -40 mV, during the
214 latter the peak current was measured.
215 To acquire and analyze the measured data pClamp10 software package was used. Current
216 traces were lowpass-filtered by the analog four-pole Bessel filters of the amplifiers. The
217 sampling frequency was 2-50 kHz, at least twice the filter cut-off frequency. The effect of
218 the toxins in a given concentration was determined as remaining current fraction ($RF = I/I_0$,
219 where I_0 is the peak current in the absence of the toxin and I is the peak current at
220 equilibrium block at a given toxin concentration). Points on the dose-response curves
221 represent the mean of 3-8 independent measurements where the error bars represent the
222 S.E.M. Data points were fitted with a two-parameter Hill equation, $RF = IC_{50}^H/($
223 $IC_{50}^H+[Tx]^H)$, where IC_{50} is the dissociation constant, H is the Hill coefficient and [Tx] is
224 the toxin concentration. To estimate IC_{50} from three measured toxin concentrations in case
225 of lower toxin affinity we used the Lineweaver-Burk analysis, where 1/RF was plotted as a
226 function of toxin concentration, fitting a straight line to the points, where Kd = 1/slope. To
227 estimate the IC_{50} using RF of a single toxin concentration H = 1 was used for the Hill
228 equation.
229

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230 **3. RESULTS**

231

232 *3.1 Chemical characterization*

233

234 For the first step of separation 220 mg of soluble venom protein were loaded in a Sephadex
235 G-50 column, following the original technique (Ramirez et al., 1988). Three well-defined
236 fractions were obtained (data not shown), which repeated exactly the same profile as earlier
237 described. Fraction II was toxic (Ramirez et al., 1988) and used for the second
238 chromatographic step. Fig.1A shows the CM-cellulose ion-exchange separation of this
239 fraction. At least 14 fractions were recovered, from which numbers 10, 11 and 12 were
240 additionally separated by HPLC as described in Material and Methods. Figures Fig. 1B to
241 Fig.1D show the profile of separation of these fractions. For the sake of clarity and
242 systematization of nomenclature the labeling of the components was based on the following
243 rationale: roman number II for the Sephadex G-50 column, numbers 10, 11 and 12 for the
244 sub-fractions obtained from CM-Cellulose column and the last number (third number
245 separated by point) for the pure peptides. Fraction II-10 applied into the HPLC system
246 separated at least 19 sub-fractions (Fig. 1B), which were analyzed by mass spectrometry.
247 Only components further characterized were labeled in Fig.1B to 1D, among the many
248 small peaks not well resolved in the figures due compression of the graphics. Sub-fractions
249 II-10.4, II-10.5, II-10.9 and II-10.10 were all homogeneous peptides with molecular masses
250 within the range expected for K^+-channel specific toxins. For this reason the full amino acid
251 sequence of these peptides were obtained, and their physiological effects on ion channels
252 were assayed as described below. Similarly, fraction II-11 subjected to HPLC fractionation

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253 resulted in at least 27 sub-fractions (Fig.1C). Fraction II-12 separated 15 components, as
254 shown in Fig.1D. Peptides eluted as II-12.5 and II-12.8 were homogeneous and were
255 completely sequenced and used for physiological assays. It is interesting to observe that
256 among the various sub-fractions obtained from fraction II-11 (the most diverse fraction,
257 showing many components) contained identical peptides that were eluted either in sub-
258 fraction II-10 or II-12. As expected there are cross-contaminations of these small basic
259 peptides when separated by HPLC. Peptide eluted as number II-11.10 is identical to
260 component II-10.5 and peptide II-11.11 is identical to component II-12.8. In summary,
261 from fraction II-10 to II-12 six peptides were obtained in pure form, sequenced and assayed
262 for pharmacological action. Many other components were identified by mass spectrometry
263 analysis (Table 1S), but were not further studied, for three main reasons: i) the molecular
264 weight correspond to peptides expected to modify normal Na^+-channel functions, such as
265 peptide II-10.16 in Fig.1B, peptides II-11.20, II-11.21 and II-11.22 in Fig.1C and peptides
266 II-12.11, II-12.12 and II-12.13 in Fig.1D (see supplementary Table 1); ii) the molecular
267 mass was under the expected values for a K^+-channel toxin, such as peptide II-10.1, peptide
268 II-10.2 in Fig.1B; peptides II-11.4 and II-11.5 in Fig.1C, and peptide II-12.2 in Fig.1D (see
269 supplementary Table 1); iii) the quantity of material obtained was too low, not enough for
270 fully chemical and functional analysis (labeled ND or NI in supplementary Table 1). The
271 six purified and characterized peptides are in very small quantities in the venom, less than
272 1%. The percentage yields of these peptides after purification as shown in Fig.1B were:
273 peptide II-10.4 (0.2%), peptide II-10.5 (0.09%), peptide II-10.9 (0.05%), peptide II-10.10
274 (0.04%), whereas for peptides II-12.5 and II-12.8 of Fig.1D were only 0.16% and 0.07%,
275 respectively. The complete primary structures of peptides II-10.4, II-10.5, II-10.10 were

276 obtained directly by Edman degradation of native peptide, confirmed after reduction and
277 alkylation, without need for cleavage, whereas peptides II-12.5 and II-12.8 were chemically
278 treated with cyanogen bromide. Peptide II-10.9 was digested with protease V8, as described
279 in Material and Methods. Fig.2 shows the amino acid sequences determined, as well as the
280 molecular weights found: II-10.4 (3804.2 Da); II-10.5 (4124.0 Da); II-10.9 (4874.0 Da); II-
281 10.10 (3907.0 Da); II-12.5 (4339.9 Da) and II-12.8 (4211.9 Da).
282
283 *3.2 Electrophysiological characterization*
284
285 Among the K^+ channels involved in the screening, the voltage-dependent K^+-channel Kv1.2
286 is the main target of the α-KTx peptides isolated from the *C. tecomanus* venom. Toxins II-
287 10.4 (elution time 24.12), II-10.5 (elution time 26.22), II-12.5 (elution time 23.84) and II-
288 12.8 (elution time 26.75) inhibited Kv1.2 currents reversibly (Fig.3 A-D) with IC_{50} values
289 3.6 nM (H = 1.1), 0.3 nM (H = 1.1), 0.7 nM (H = 1.2) and 2.9 nM (H = 0.9) respectively
290 (Fig.3 E). The insets to the Figs. 6A-D show, that the blocking effect of peptides II-12.5
291 and II-12.8 were completely reversible during the time-scale of the wash-out period
292 whereas very slow and incomplete relieve of the block is observed for peptides II-10.4 and
293 II-10.5 upon washing the recording chamber with toxin-free solution. The effect of these
294 venom components on whole-cell Kv1.1, Kv1.3, Shaker-IR and KCa3.1 currents was also
295 determined in order to characterize the selectivity of the peptides.
296 Toxin II-10.4 slightly inhibited Kv1.3 currents (RF = 0.88) in 10 nM concentration whereas
297 it was not effective on the other ion channels included in this study (data not shown).

298 Single-point estimation of the IC_{50} using RF=0.88 at 10 nM and assuming 1:1
299 channel:toxin stoichiometry gives IC_{50} = 72 nM for the inhibition of Kv1.3.
300 On the other hand, 10 nM toxin II-10.5 reversibly inhibited the whole-cell Kv1.3, Shaker
301 and KCa3.1 (Fig.4 A-C) currents but not the Kv1.1 channels (data not shown). IC_{50} values
302 were the following: 8.3 nM (H = 0.8) for Kv1.3, 22.0 nM for Shaker-IR and 6.7 nM (H =
303 1.1) for KCa3.1 (Fig.4 D, E).
304 The peptide eluting at 23.84 min (II-12.5) inhibited Kv1.3 reversibly with IC_{50} = 26.2 nM
305 (Fig. 5.). It slightly blocked KCa3.1 (RF = 0.83) in 10 nM which results in an estimated
306 IC_{50} = 56 nM using a single-point estimation of the IC_{50} (data not shown). Neither Kv1.1
307 nor Shaker K^+ channels were inhibited by peptide II-12.5 (data not shown)
308 Toxin II-12.8 (eluting time 26.75) inhibited Kv1.1 reversibly with an IC_{50} = 4.8 nM (H =
309 0.8) (Fig. 6.) whereas other channels included in this study (Kv1.3, Shaker and KCa3.1)
310 were not affected by the peptide.
311 The peptide eluting at 29.61 min (toxin II-10.9), which belongs to the γ-KTx family,
312 inhibited the Kv11.1 (hERG) channel with IC_{50} = 16.9 nM, whereas this peptide did not
313 block the closely related Kv10.1 channel at 10 nM concentration (Fig 7.). Toxin II-10.9 did
314 not inhibit the other channels tested (Kv1.1, Kv1.2, Kv1.3, Shaker, KCa3.1) at 10 nM
315 concentration (data not shown).
316 The pharmacological data obtained for Kv1.1, Kv1.2, Kv1.3, Shaker and KCa3.1 are
317 summarized in Fig. 8 where the remaining current fraction at equilibrium block is shown in
318 the presence of 10 nM II-10.4, II-10.5, II-10.9, II-10.10, II-12.5 and II-12.8. As pointed out
319 above analysis of the current inhibition at significantly higher peptide concentrations was
320 unrealistic due to the limited amount of the peptides. Peptide II-10.10 was also assayed for

321 possible antimicrobial activity and was shown to be inactive using up 10 µM concentration
322 (data not shown),
323
324
325
326 **4. DISCUSSION**
327
328 The source of the venoms reported in this paper was a scorpion species earlier known as a
329 subspecies of *Centruroides limpidus* (Possani et al., 1980) but was recently renamed simply
330 as *Centruroides tecomanus,* instead of *Centruroides limpidus tecomanus* (Valdez-
331 Velazquez et al., 2013). Earlier studies indicated that the venom of this scorpion is a rich
332 source of peptides that interfere with the normal function of Na^+-channels and the venom is
333 very toxic to mammals (Martin et al., 1988; Ramirez et al., 1988). Actually, a recent
334 publication regarding the proteomic and transcriptomic analysis of this venom reported the
335 presence of 24 different amino acid sequences of peptides belonging to the Na^+-channel
336 specific toxin family (Valdez-Velazquez et al., 2013). In the same communication only four
337 putative K^+-channels blockers of the subfamily α-KTx were identified. The proteomic
338 analysis reported the identity of peptide II-10.4 (molecular weight 3804 Da), peptide II-
339 10.5 (molecular weight 4124 Da) and peptide II-12.8 (molecular weight 4212 Da), as
340 shown in Table 1 of Valdez-Velazquez et al (Valdez-Velazquez et al., 2013). The amino
341 acid sequence of peptide II-10.4 determined here (Fig. 2) is identical to the expected
342 sequence inferred from the transcriptomic analysis (see component Ct33, of Fig. 5 of the
343 publication by (Valdez-Velasquez et al., 2013), except that an extra segment of six residues

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344 was shown at the N-terminal segment, plus an additional Lysine at the C-terminal region of
345 component Ct33 identified from the transcriptome, compared to II-10.4. These differences
346 are assumed to be a postranslational modification of the peptide. Another almost identical
347 peptide to Ct27 of Valdez-Velazquez et al. 2013 paper was toxin II-12.8. Here the toxin II-
348 12.8 shows an Alanine instead of Valine in position 25 found in Ct27. The extra Glycine in
349 the transcriptome analysis of Ct27 means that very likely our toxin II-12-8 is amidated at
350 the C-terminal residue, also due to posttranslational processing during maturation of the
351 peptide. These small differences are not contradictory and somehow were expected, since
352 the transcriptomic analysis is not exhaustive and was conducted with venomous gland of
353 only two scorpions (4 glands, each scorpion has a pair of glands), whereas the venom
354 separation for this communication was obtained from several hundred individuals (220 mg,
355 the first Sephadex column separation corresponds to the extraction of at least 100
356 scorpions). Yet, the amount of the pure peptides obtained here are under 1% of the soluble
357 venom used. Variations certainly occur among individual scorpions and also possibly to
358 genre differences. The soluble venom used was obtained from male and female scorpions
359 together. The systematic numbers assigned to these purified peptides were done according
360 to previous information available in the UniProt Knowledgebase data. The corresponding
361 systematic numbers are indicated in the section of Material and Methods.
362
363 Guided by the primary amino acid sequences of the peptides we aimed to characterize their
364 K^+ channel blocking potencies, find similarities to other known peptides and compare their
365 selectivity (Table 1). Due to the limited amount of the purified natural peptides we involved
366 the following ion channels in this study: Three closely related voltage gated channels,

367 Kv1.1, which is expressed in chondrocytes, brain, heart and skeletal muscle (Beckh and
368 Pongs, 1990; Gutman et al., 2005; Varga et al., 2011), Kv1.2, which is present in neurons,
369 heart and smooth muscle (Bakondi et al., 2008; Gutman et al., 2005; Rusznak et al., 2008)
370 and Kv1.3, which plays key role in the activation of lymphocytes (Chandy et al., 2004;
371 Panyi et al., 2006); Kv10.1 (EAG), with oncogenic relevance (Pardo et al., 1999); Kv11.1
372 (ERG) which pays key role in the cardiac action potential (Sanguinetti et al., 1995);
373 KCa3.1, which is also present in lymphocytes (Chandy et al., 2004; Panyi et al., 2006); and
374 the *Drosophila* Shaker, which is frequently used in structure-function studies (Panyi and
375 Deutsch, 2006). Interestingly all four α-KTx peptides inhibited Kv1.2 in nanomolar
376 concentration (Fig. 8). Peptide II-10.4 shows 97% sequence identity with cobatoxin
377 (CoTx1), isolated from the venom of *Centruroides noxius*, which also inhibits Kv1.2 with
378 IC_{50} = 27nM (Table 1). CoTx also inhibits Kv1.1 (IC_{50} = 24.4 μM), Kv1.3 (IC_{50} = 5.3 μM),
379 Shaker (IC_{50} = 1 μM) and KCa3.1 (IC_{50} = 7.1 μM) channels, however these values are
380 more than 100 fold higher than that of Kv1.2; therefore CoTx fulfills the criteria of
381 selectivity to Kv1.2 (Giangiacomo et al., 2004). Toxin II-10.4 inhibited Kv1.3 with an
382 estimated IC_{50} = 72 nM, however due to the limited amount of natural peptide we could not
383 determine IC_{50} on Kv1.3 with higher accuracy nor taking measurements on the other
384 channels in micromolar concentration to compare peptide II-10.4 with CoTx.
385 Among the peptides described II-10.5 is the highest affinity inhibitor of Kv1.2 (IC_{50} = 0.3
386 nM) however this peptide also blocked the Kv1.3, Shaker-IR and KCa3.1 channels in
387 nanomolar concentration. Peptide II-10.5 has 92% sequence identity with Css20 (from the
388 venom of *Centruroides suffusus suffusus*), which inhibits Kv1.2 (IC_{50} = 1.26 nM) and
389 Kv1.3 (IC_{50} = 7.21 nM) channels but has no effect on Kv1.1, Shaker-IR, KCa3.1 or Kv11.1

390 channels in 10 nM (Corzo et al., 2008). Toxin II-10.5 is also highly similar to Ce3 (89%)
391 (*Centruroides elegans*) and CIITx1 (81%) (*Centruroides limpidus*). Ce3 was shown to
392 inhibit Kv1.3 (IC_{50} = 366 nM) but was not effective on Shaker-IR and KCa3.1 channels in
393 10 nM (Olamendi-Portugal et al., 2005) whereas CIITx1 was not tested specifically on any
394 of the channels involved in this study; thus its selectivity profile is still unknown (Martin et
395 al., 1994).
396 Toxin II-12.5 inhibits Kv1.2, Kv1.3 and KCa3.1 channels in nanomolar concentrations. The
397 amino acid sequence of II-12.5 is 97% identical to Ce2, 95% to Ce1 and 87% to Ce4 (all
398 three isolated from *Centruroides elegans*). Ce2, Ce1 and Ce4 all inhibit Kv1.3 with half-
399 inhibiting concentrations of 0.25 nM, 0.71 nM and 0.98 nM, respectively and neither of
400 them inhibited Shaker-IR nor KCa3.1 in 10 nM, however the effect of the peptides on
401 Kv1.1 and Kv1.2 is unknown (Olamendi-Portugal et al., 2005). Margatoxin (MgTx, from
402 *Centruroides margaritatus*) (Garcia-Calvo et al., 1993), which was shown to inhibit Kv1.1
403 Kv1.2 and Kv1.3 channels with IC_{50} values 4.7 nM, 6.4 pM and 11.7 pM, respectively but
404 does not inhibit Shaker-IR, Kv11.1 and KCa3.1 in 1nM concentration (Bartok et al., 2014),
405 shows 81% identity to II-12.5.
406 Component II-12.8 has a sequence similar to Ce5 (92%), Ce4 (90%), Ce2 (89%), Ce1
407 (86%) and MgTx (81%). Interestingly, the essential dyad of II-12.8 contains a serine in
408 addition to the conserved lysine (Dauplais et al., 1997). This type of dyad is also present in
409 Ce5. Peptide II-12.8 inhibits Kv1.1 and Kv1.2 but not Kv1.3, whereas Ce5 is effective on
410 Kv1.3 (IC50 = 69 nM); unfortunately Ce5 was not tested on Kv1.1 and Kv1.2 (Olamendi-
411 Portugal et al., 2005).
412

413 The analysis of the sequence of II-10.9 shows 90% sequence similarity to CnErg1, which is
414 a potent inhibitor of Kv11.1 (IC_{50} =8.5 nM) (Torres et al., 2003). So far the amino acid
415 sequence of 29 γ-KTx peptides have been published, but only 5 of them were proved to
416 inhibit Kv11.1, the others were classified to be in this family solely based on the primary
417 amino acid sequence. Here we report a novel peptide, belonging to the group of γ-KTx,
418 which shows several thousand-fold selectivity for Kv11.1 over the closely related Kv10.1
419 and the other channels included in this study (Kv1.1, Kv1.2, Kv1.3, Shaker, KCa3.1).
420 Although II-10.10 has a cysteine pattern similar to that of many α-KTx peptides (e.g.
421 CoTx1, Fig. 9), the essential dyad, a characteristic pattern required for the interaction with
422 K^+ channels (Dauplais et al., 1997) cannot be identified. Although a positively charged
423 amino acid (R) is present in II-10-10 in an equivalent position to K21 of CoTx1 the amino
424 acid, which corresponds to Y30 in CoTx1, the part of the dyad which is usually
425 hydrophobic and frequently aromatic in K^+ channel blocker peptides cannot be identified in
426 II-10.10. Since peptide II-10.10 did not inhibit any of the tested ion channels in 10 nM
427 concentration (Fig. 8), and its primary amino acid sequence shows no significant similarity
428 to any of the sequences in protein databases (pdb, swissprot, etc.) its function remains
429 unknown. In addition this peptide was also assayed for possible antimicrobial activity with
430 negative results.
431 Overall, when compared to the 133 known α-KTx peptides (reviewed in (Bartok et al.,
432 2015)), the novel α-KTx peptides isolated from *Centruroides tecomanus,* described in this
433 article show the highest sequence identities to peptides isolated from other species of the
434 *Centruroides* genus. Most of the peptides mentioned above can inhibit multiple ion
435 channels, however, the detailed selectivity profile for many of the peptides is yet to be

436 determined. Since all four α-KTx peptides of *C. tecomanus* inhibit Kv1.2 with high affinity
437 but their additional target ion channels are different, and due to the high sequence identity
438 of peptides II-10.5, II-12.5 and II-12.8, further studies involving docking simulations, the
439 mutagenesis and synthesis of these peptides may provide valuable information of the
440 molecular and structural determinants of the ion channel selectivity of K^+ channel blocking
441 scorpion toxins.

442

443 5. CONCLUSIONS

444

445 The venom from the Mexican scorpion *C. tecomanus* contains four different peptides
446 composed of 32-39 amino acid residues, cross-linked by three disulfide bonds, that impair
447 normal function of Kv1.2, Kv1.1, Kv1.3, Shaker and KCa3.1 ion-channels, at nanomolar
448 concentrations. They belong to the known α-KTx family of scorpion peptides. Another
449 peptide with 42 amino acid residues, cross-linked by four disulfide bonds, belonging to the
450 γ-KTx family, block ERG-channel. A sixth new peptide with 32 amino acid residues, three
451 disulfide bonds, is a peptide of unknown function, which does not affect the K^+-channels
452 assayed in this report. The pharmacological properties of the peptides described here will
453 certainly be valuable tools for studies aimed at understanding the structure-function
454 relationship of K^+ channels.

455

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463 verifying the antimicrobial effects of peptide II-10.10.

464

465

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467 **7. REFERENCES**

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603

604 **FIGURE LEGENDS**

605 **Figure 1: Venom fractionation and purification of peptides**

606 **A. CM-Cellulose ion-exchange separation.** The fraction II from Sephadex G-50 gel
607 filtration (Ramírez et al., 1988) of *C. tecomanus* venom (132 mg) was applied to a column
608 (0.9 x 30 cm) equilibrated with 20 mM ammonium acetate buffer, pH 4.7. The material was
609 eluted with a linear salt gradient from 0 to 0.5 M NaCl run in the same buffer. At the end of
610 the run a 1.0 M NaCl solution was applied to the column. Final recovery was about 80%.

611 **B. HPLC separation of fraction II-10. F**raction II-10 (190 µg) was applied to a C18
612 reverse-phase analytical column and separated with a linear gradient from solution A (water
613 in 0.12% TFA) to 60% solution B (acetonitrile in 0.10% TFA) run for 60 min. Note that
614 only the peak components further analyzed are indicates, among many others, which are
615 not evident due to compression of the figures.

616 **C. HPLC separation of Fraction II-11.** Fraction II-10 (130 μg) was applied to a C18
617 reverse-phase analytical column and separated with a linear gradient from solution A (water
618 in 0.12% TFA) to 60% solution B (acetonitrile in 0.10% TFA) run for 60 min.

619 **D. HPLC separation of Fraction II-12.** Fraction II-10 (380 μg) was applied to a C18
620 reverse-phase analytical column and separated with a linear gradient from solution A (water
621 in 0.12% TFA) to 60% solution B (acetonitrile in 0.10% TFA) run for 60 min.

622

623 **Figure 2: Sequence of the new peptides from the venom of *C. tecomanus***

624 First column shows the systematic number of the six peptides. Numbers on top of the
625 sequences correspond to the position in the primary structure. Direct Sequence indicate the
626 segment of the primary structure directly obtained from the sequencer and CnBr and V8
627 digestion indicate the segment of peptides, whose primary structure determination required
628 cleavage and separation by HPLC as indicated in Material and Methods. Last column
629 indicates the elution time on the HPLC columns for each one of the peptides sequenced.

630

631 **Figure 3: Novel α-KTx peptides inhibit human Kv1.2 channels**

632 **A-D,** Inhibition of the hKv1.2 current. Currents were recorded in outside-out patch
633 configuration in tsA201 cells transiently transfected with the gene encoding the hKv1.2
634 channel. The patches were depolarized to +50 mV from a holding potential of -100 mV
635 every 15 s. The traces show the K^+ current before the application of the toxin (control) and

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636 after reaching equilibrium block in the presence of 10 nM II-10.4 (A), II-10.5 (B), II-12.5
637 (C) and II-12.8. Insets: Peak K^+ currents were determined and plotted as a function of time.
638 Grey bars mark the presence of the corresponding toxin in the extracellular solution.

639 **E,** Concentration-dependence of the current block. The remaining fraction of the Kv1.2
640 current was calculated as I/I_0, where I_0 and I are the peak K^+ currents measured in the
641 control solution and upon reaching equilibrium block during bath perfusion with the test
642 solution containing peptide II-10.4 (filled diamond), II-10.5 (filled circle), II-12.5 (filled
643 triangle) and II-12.8 (filled square) at indicated concentrations. The voltage protocol and
644 other experimental conditions were the same as in A-D. The superimposed solid lines are
645 the Hill equations fitted to the data points (see Material and Methods). The best fit yielded
646 IC_{50} values of 3.6 nM (H = 1.1) for II-10.4, 0.3 nM (H = 1.1) for II-10.5, 0.7 nM (H = 1.2)
647 for II-12.5 and 2.9 nM (H = 0.9) for II-12.8.

648

649 **Figure 4: Effect of peptide II-10.5 on Kv1.3, Shaker-IR and KCa3.1 channels**

650 **A-C,** Currents were recorded in whole cell configuration in activate human lymphocytes
651 (A) or outside-out patch configuration in tsA201 cells transiently transfected with the gene
652 encoding the indicated channel (B, C). The traces show the K^+ current before the
653 application of the toxin (control) and after reaching equilibrium block in the presence of 10
654 nM II-10.5. Insets: Peak K^+ currents were determined and plotted as a function of time.
655 Grey bars mark the presence of 10 nM II-10.5 in the extracellular solution.

656

657 **Figure 5: Effect of peptide II-12.5 on the Kv1.3 channel**

658 **A,** Currents were measured in whole cell configuration in activated human lymphocytes.
659 The traces show the K^+ current before the application of the toxin (control) and after
660 reaching equilibrium block in the presence of 10 nM II-12.5. Inset: Peak K^+ currents were
661 determined and plotted as a function of time. Grey bar marks the presence of 10 nM II-12.5
662 in the extracellular solution. B, The remaining fraction of the current was calculated as
663 described in Fig.6 and the Materials and Methods. IC_{50} on was determined by the
664 Lineweaver-Burk method resulted in IC_{50} = 26.2 nM.

665

666 **Figure 6: Effect of peptide II-12.8 on the Kv1.1 channel**

667 A, Currents were recorded in outside-out patch configuration in tsA201 cells transiently
668 transfected with the gene encoding the hKv1.1 channel. The patches were depolarized to
669 +50 mV from a holding potential of -100 mV every 15 s. The traces show the K^+ current
670 before the application of the toxin (control) and after reaching equilibrium block in the
671 presence of 10 nM II-12.8. Inset: Peak K^+ currents were determined and plotted as a
672 function of time. Grey bar marks the presence of the 10 nM toxin in the extracellular
673 solution. B, The remaining fraction of the current was calculated as described in Fig.3 and
674 the Materials and Methods. The superimposed solid line is the Hill equation fitted to the
675 data points (see Material and Methods). The best fit yielded IC_{50} value of 4.8 nM (H = 0.8).

676

677 **Figure 7: Effect of II-10.9 on Kv11.1 (hERG) and Kv10.1 (EAG).**

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678 A, C, Currents were recorded in whole cell (A) or outside-out patch (C) configuration in
679 tsA201 cells transiently transfected with the gene encoding the indicated channel. Currents
680 were evoked wit voltage protocols described in Material and Methods. The traces show the
681 K^+ current before the application of the toxin (control) and after reaching equilibrium block
682 in the presence of 10 nM II-10.9. Insets: Peak K^+ currents were determined and plotted as a
683 function of time. Grey bars mark the presence of toxin II-10.9 in 10 nM concentration in
684 the extracellular solution. B, The remaining fraction of the current was calculated as
685 described in Fig.3 and the Materials and Methods. IC_{50} on was determined by the
686 Lineweaver-Burk method resulted in IC_{50} = 16.9 nM

687

688 **Figure 8: Comparison of the selectivity of the *C. tecomanus* toxins.**

689 Effect of the peptides were measured in 10 nM concentration on Kv1.1, Kv1.2, Kv1.3,
690 Shaker and KCa3.1 channels as described previously. The remaining fraction of the current
691 was calculated as described in Fig.3 and the Materials and Methods.

692

693 **Figure 9: Characteristic patterns in the *C. tecomanus* peptides**

694 A, The primary amino acid sequences of II-10.10 and CoTx1 were aligned by pairing their
695 cysteine residues (shown in boxes). The position of the two amino acids required for K^+-
696 channel inhibition in CoTx are labeled as dyad. B, The primary amino acid sequences of II-
697 10.5, II-12.5 and II-12.8 were aligned by matching their cysteine residues. Different
698 residues in the equivalent positions are shown in bold letters.

699

700 **Table 1: Blast analysis of the novel *C. tecomanus* toxins**

701 Sections in the table contain the name, accession number and sequence of peptides with
702 high sequence identity to II-10.4 (A), II-10.5 (B), II-12.5 (C), II-12.8 (D) and II-10.9 (E),
703 and their IC_{50} values measured on the listed channels. Missing values represent that effect
704 on a given channel is not determined (ND) or the peptide does not block the channel (-).

705

706 **Supplementary Table 1: Mass spectrometry determination of peptides**

707 MW, indicates the molecular weight experimentally determined; ND, means not
708 determined; NI means not identified

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toxin name	accession	sequence	identity	Kv1.1	Kv1.2	Kv1.3	Shaker-IR	KCa3.1
A								
II-10.4		AVCVYRTCDKDCKRRGYRSGKCINNACKCYPYA		-	3.6 nM	~72 nM	-	-
CoTx1	O46028.1	AVCVYRTCDKDCKRRGYRSGKCINNACKCYPYG	97%	24.4 µM	27 nM	5.3 µM	1 µM	7.5 µM
B								
II-10.5		IFINVKCSSPQQCLKPCKKAFGQHAGGKCINGKCKCYP		-	0.3 nM	8.3 nM	12.4 nM	6.4 nM
Ce3	P0C163.1	IFINVKCSLPQQCLRPCKDRFGQHAGGKCINGKCKCYP	89%	ND	ND	366 nM	-	-
CllTx1	P45629.1	ITINVKCTSPQQCLRPCKDRFGQHAGGKCINGKCKCYP	87%	ND	ND	ND	ND	ND
Css20	P85529.1	IFINVKCSSPQQCLKPCKAAFGISAGGKCINGKCKCYP	92%	-	1.3 nM	7.2 nM	-	-
C								
II-12.5		TIINVKCTSPKQCLKPCKDLYGPHAGEKCMNGKCKCYKI		-	0.7 nM	26.2 nM	-	~56 nM
Ce2	P0C162.1	TIINVKCTSPKQCLKPCKDLYGPHAGAKCMNGKCKCY	97%	ND	ND	0.25 nM	-	-
Ce1	P0C161.1	TVINVKCTSPKQCLKPCKDLYGPHAGAKCMNGKCKCY	95%	ND	ND	0.71 nM	-	-
Ce4	P0C164.1	TIINVKCTSPKQCLLPCKEIYGIHAGAKCMNGKCKCYKI	87%	ND	ND	0.98 nM	-	-
MgTx	P40755.1	TIINVKCTSPKQCLPPCKAQFGQSAGAKCMNGKCKCYP	81%	4.7 nM	6.4 pM	11.7 pM	-	-
D								
II-12.8		TIINVKCTSPKQCLLPCKQIYGPHAGAKCMNGKCHCSKI		4.8 nM	2.9 nM	-	-	-
Ce5	P0C165.1	TIINVKCTSPKQCLPPCKEIYGRHAGAKCMNGKCHCSKI	92%	ND	ND	69 nM	-	-
Ce4	P0C164.1	TIINVKCTSPKQCLLPCKEIYGIHAGAKCMNGKCKCYKI	90%	ND	ND	0.98 nM	-	-
Ce2	P0C162.1	TIINVKCTSPKQCLKPCKDLYGPHAGAKCMNGKCKCY	89%	ND	ND	0.25 nM	-	-
Ce1	P0C161.1	TVINVKCTSPKQCLKPCKDLYGPHAGAKCMNGKCKCY	86%	ND	ND	0.71 nM	-	-
MgTx	P40755.1	TIINVKCTSPKQCLPPCKAQFGQSAGAKCMNGKCKCYP	81%	4.7nM	6.4pM	11.7 pM	-	-

toxin name	accession	sequence	identity	hERG
E				
II-10.9		DRDSCIDKSRCSKYGYYQECQDCCKKAGHNRGTCMFFKCKCA		16.9 nM
CnErg1	Q86QT3.1	DRDSCVDKSRCAKYGYYQECQDCCKNAGHNGGTCMFFKCKCA	90%	8.8 nM
CeErgTx5	P0C893.1	DRDSCIDKSRCSKYGYYQECQDCCKKAGHNGGTCMFFKCKCA	98%	ND

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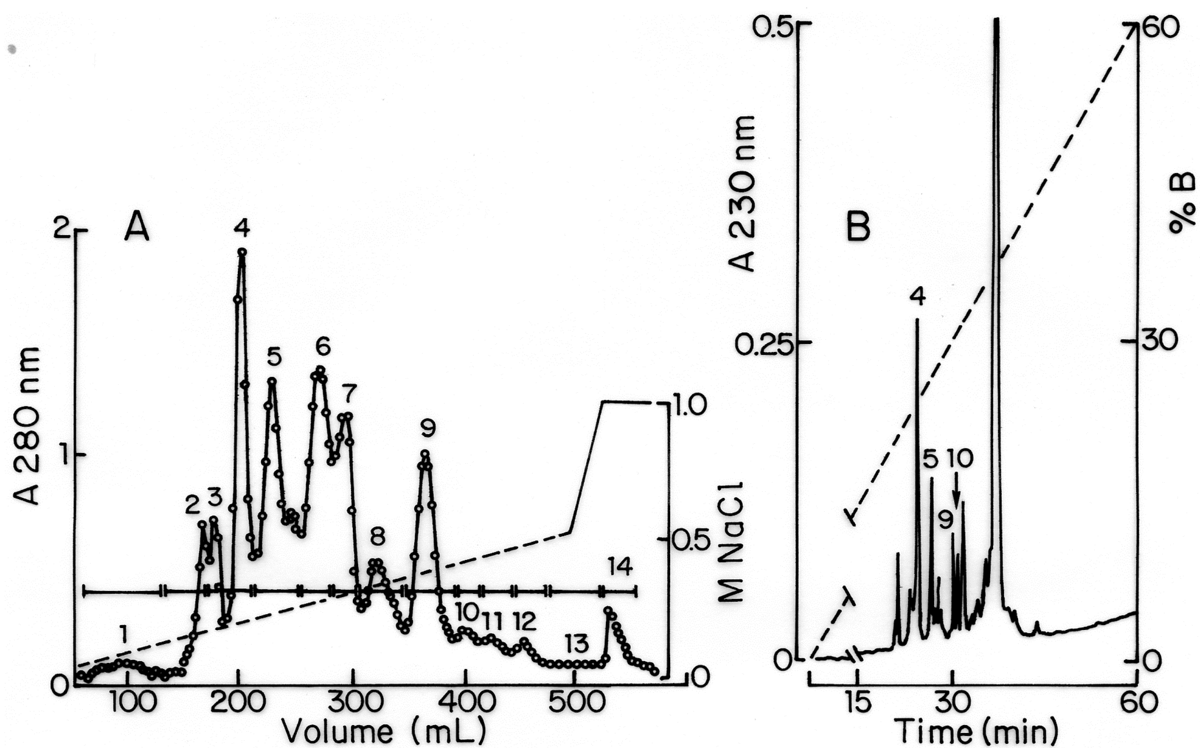

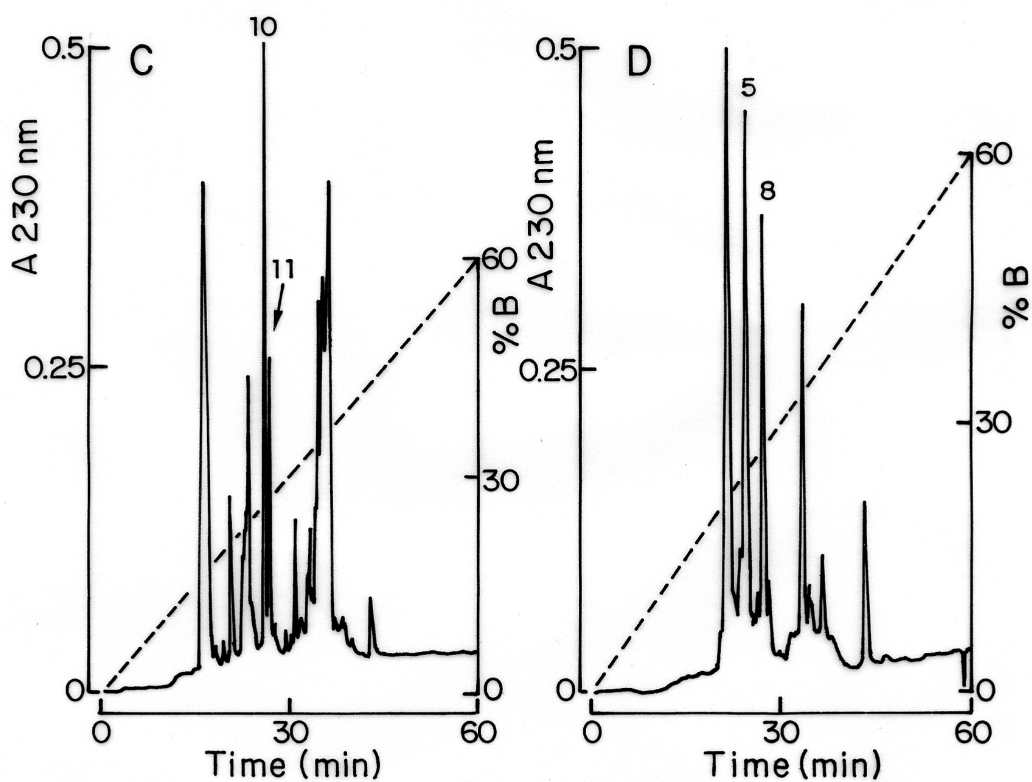

Peptides	Sequence	Molecular Weight (Da)
II-10.4	AVCVYRTCDKDCKRRGYRSGKCINNACKCYPYA :-----*direct sequence*-----------------------------→	3804.2
II-10.5	IFINVKCSSPQQCLKPCKKAFGQHAGGKCINGKCKCYP :-----*direct sequence*------------------------------------→	4124.0
II-10.9	DRDSCIDKSRCSKYGYYQECQDCCKKAGHNRGTCMFFKCKCA :-----*direct sequence*--------→ :----*V8 digestion*---------------→	4874.0
II-10.10	IRRYCDPRVCDRECLEKGKYFGRCIRDICKCN :-----*direct sequence*--------------------------→	3907.0
II-12.5	TIINVKCTSPKQCLKPCKDLYGPHAGEKCMNGKCKCYKI :-----*direct sequence*-----------------------→ :--*CnBr*----→	4339.9
II-12.8	TIINVKCTSPKQCLLPCKQIYGPHAGAKCMNGKCHCSKI :-----*direct sequence*-----------------------→ :--*CnBr*---→	4211.9

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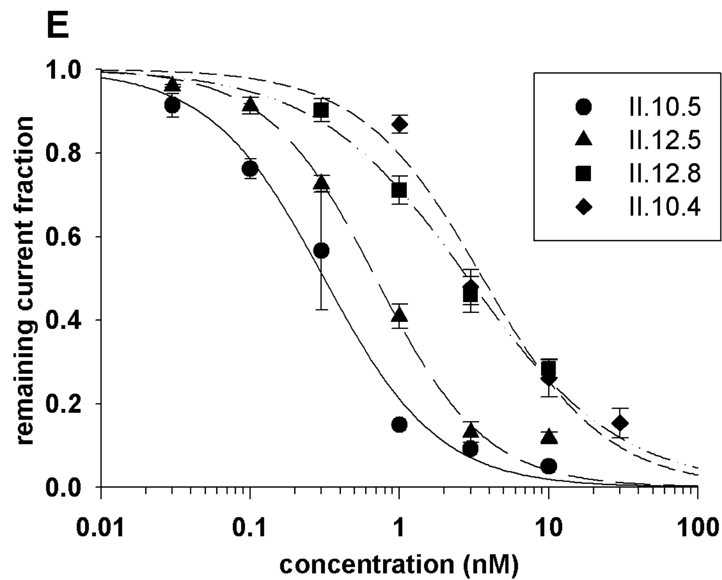
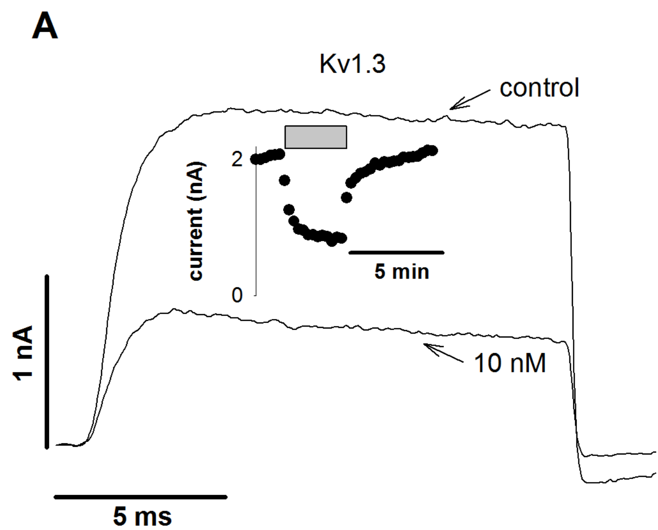
A
II-10.4
control
2.5
0.0
current (nA)
5 min
1 nA
10 nM
100 ms

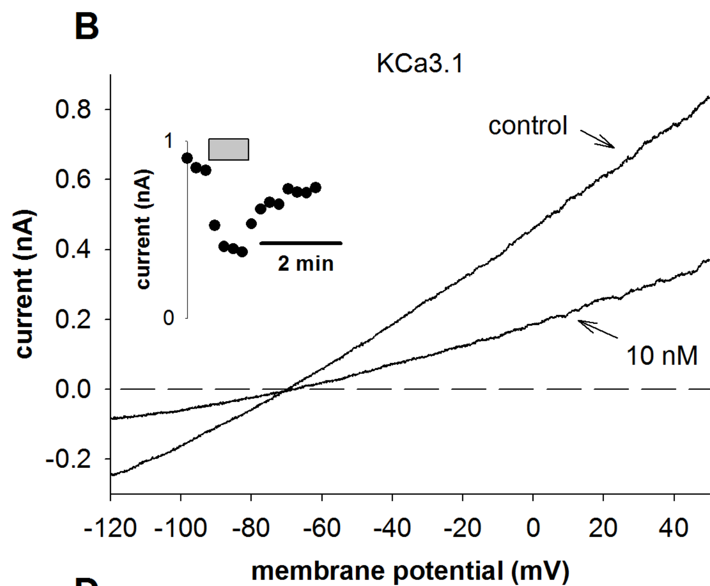
B
II-10.5
control
5
0
current (nA)
5 min
1 nA
10 nM
100 ms

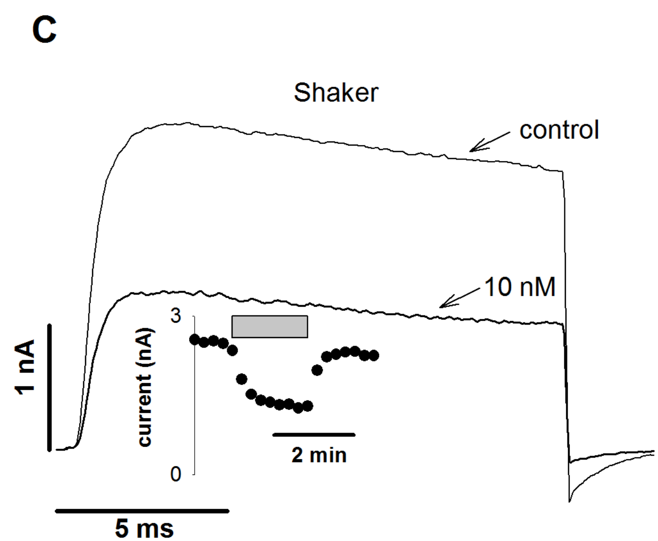
C
II-12.5
control
2.5
0.0
current (nA)
5 min
1 nA
10 nM
100 ms

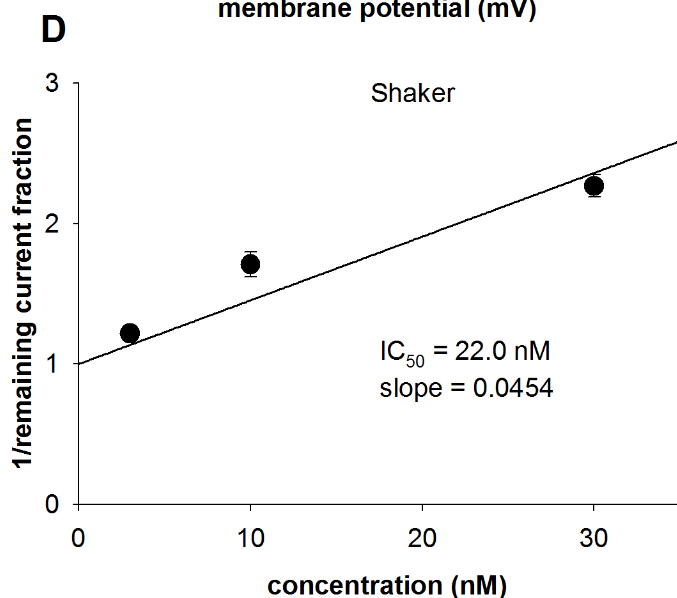
D
II-12.8
control
6
0
current (nA)
5 min
1 nA
10 nM
100 ms

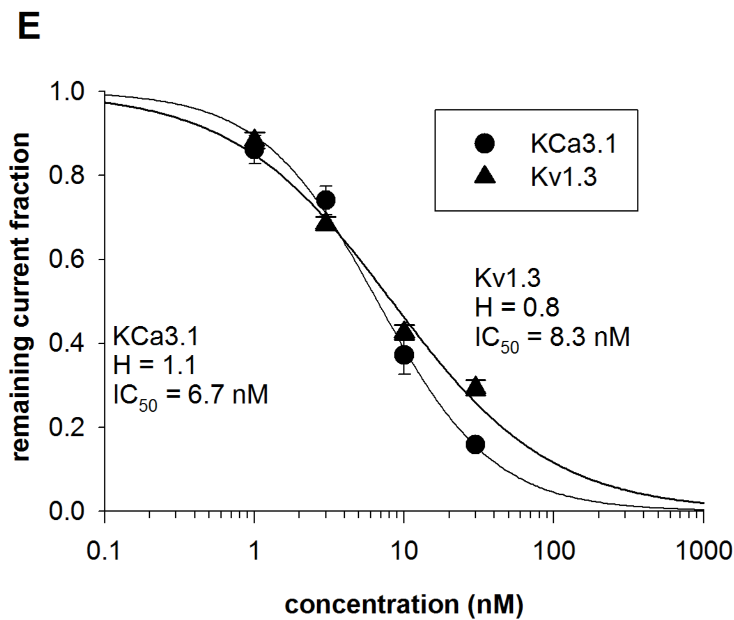



A
Kv1.3
control
10 nM
1 nA
5 ms
current (nA)
2
0
5 min


B
KCa3.1
control
10 nM
current (nA)
0.8
0.6
0.4
0.2
0.0
-0.2
1
0
2 min
-120 -100 -80 -60 -40 -20 0 20 40
membrane potential (mV)


C
Shaker
control
10 nM
1 nA
5 ms
current (nA)
3
0
2 min


D
Shaker
1/remaining current fraction
3
2
1
0
IC50 = 22.0 nM
slope = 0.0454
0 10 20 30
concentration (nM)


E
remaining current fraction
1.0
0.8
0.6
0.4
0.2
0.0
KCa3.1
Kv1.3
Kv1.3
H = 0.8
IC50 = 8.3 nM
KCa3.1
H = 1.1
IC50 = 6.7 nM
0.1 1 10 100 1000
concentration (nM)

A
control
10 nM
1 nA
5 ms
current (nA)
2.5
0.0
5 min
B
1/remaining current fraction
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
concentration (nM)
slope: 0.0382
IC_{50} = 26.2 nM

A

control

2

current (nA)

0

5 min

1 nA

10 nM

5 ms

B

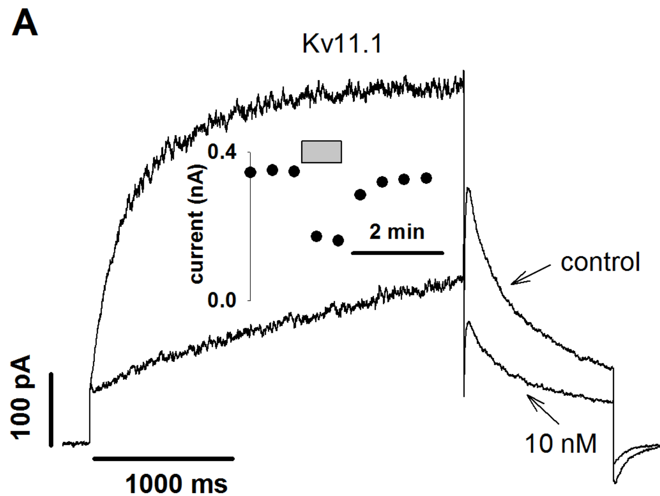
remaining fraction

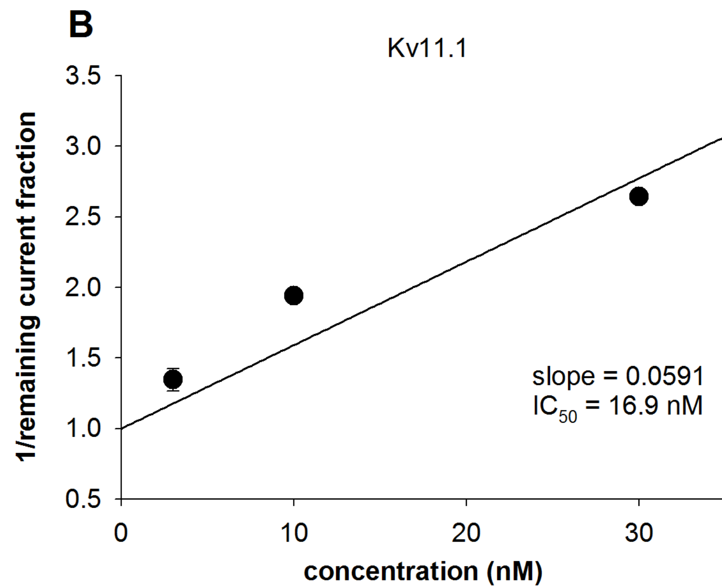
1.0
0.8
0.6
0.4
0.2
0.0

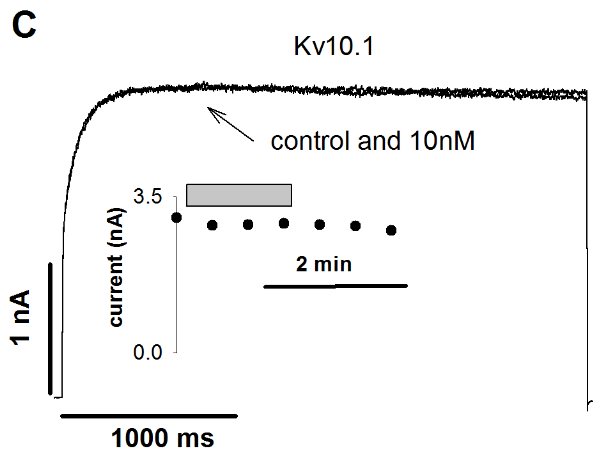
H = 0.8
IC_{50} = 4.8 nM

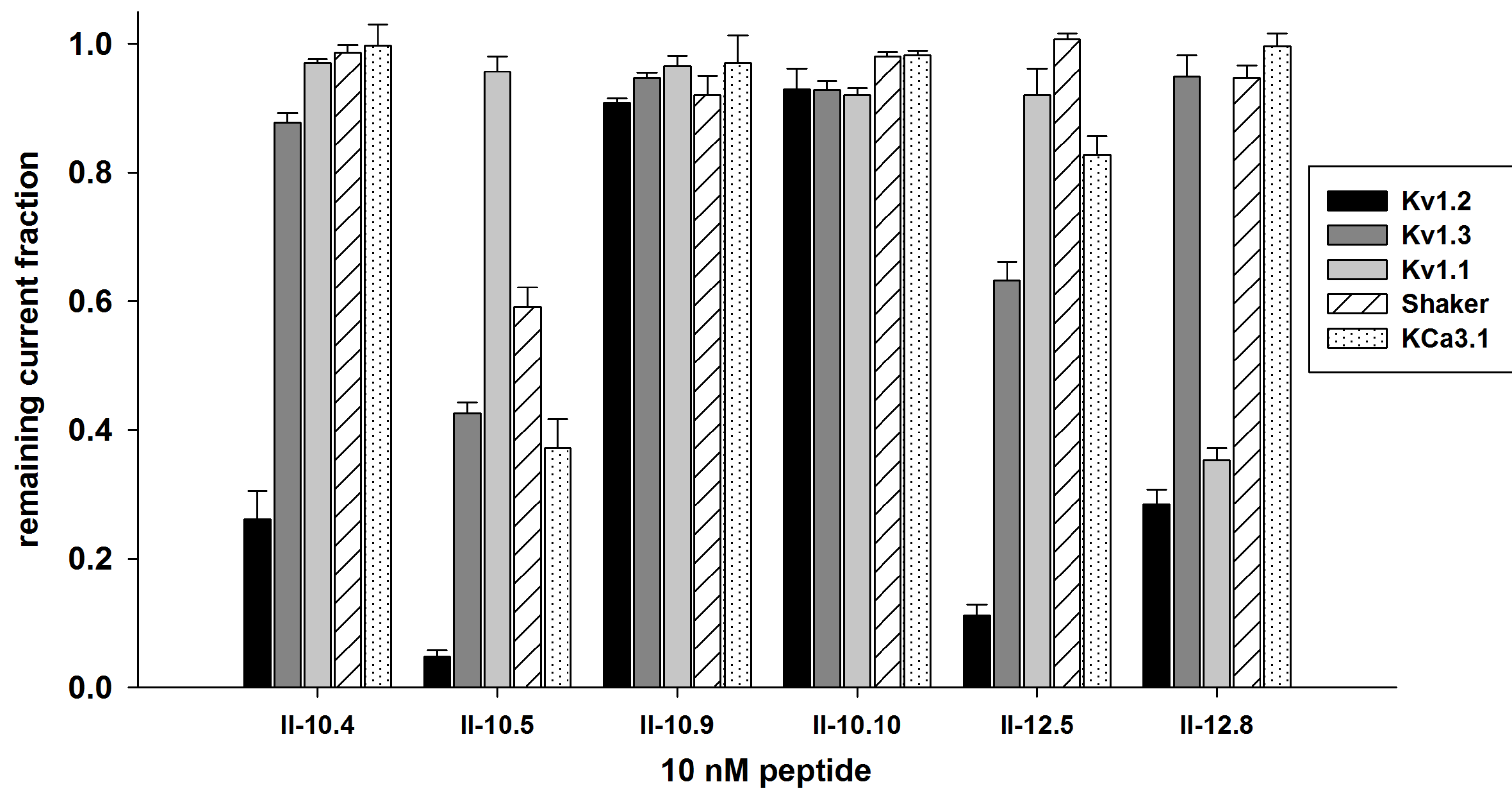
0.1
1
10
100

concentration (nM)


A
Kv11.1
0.4
0.0
current (nA)
2 min
control
10 nM
100 pA
1000 ms


B
Kv11.1
1/remaining current fraction
concentration (nM)
slope = 0.0591
IC50 = 16.9 nM


C
Kv10.1
control and 10nM
3.5
0.0
current (nA)
2 min
1 nA
1000 ms



remaining current fraction
0.0
0.2
0.4
0.6
0.8
1.0
II-10.4
II-10.5
II-10.9
II-10.10
II-12.5
II-12.8
10 nM peptide
Kv1.2
Kv1.3
Kv1.1
Shaker
KCa3.1

A

II-10.10 IRRYCDPRYCDRECLEKGKYFGRCIRDICKCN

CoTx1 AVCVYRTCDKDCKRRGYRSGKCINNACKCYPYG

dyad

B

II-10.5 **IF**INVKC**S**SP**Q**QCL**K**PCK**KAF**G**Q**HAG**G**KC**I**NGKC**K**C**YP**

II-12.5 **TI**INVKC**T**SP**K**QCL**K**PCK**DLY**G**P**HAG**E**KC**M**NGKC**K**C**YKI**

II-12.8 **TI**INVKC**T**SP**K**QCL**L**PCK**QIY**G**P**HAG**A**KC**M**NGKC**H**C**SKI**

Highlights

1. Four new peptides of the α-KTx family of scorpion venom components are described.
2. A novel blocking peptide that blocks regular function of ERG-channels is reported.
3. A 32 amino acid long peptide with no apparent pharmacological action on K^+-channels was found.
4. For the first time it is demonstrated that venom from *C. tecomanus* also has peptides that block ion-channel function.

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Ethical Statement

The authors of this manuscript declare that there are no ethical problems with this manuscript.

All authors have read and agreed with the content of the manuscript and also agree with the order of the authors.

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