

Design and Synthesis of Indole-Based Peptoids as Potent Noncompetitive Antagonists of Transient Receptor Potential Vanilloid 1

Martina Quintanar-Audelo,[†] Asia Fernández-Carvajal,[‡] Wim Van Den Nest,[§] Cristina Carreño,[§] Antonio Ferrer-Montiel,^{*,‡} and Fernando Albericio^{*,†,||}

IRB Barcelona, Barcelona Science Park, University of Barcelona, and Department of Organic Chemistry, 08028 Barcelona, Spain, Institute of Molecular and Cellular Biology, University Miguel Hernández, 03202 Alicante, Spain, and DiverDrugs SL, 08850 Gavà, Barcelona, Spain

Received May 26, 2007

The vanilloid receptor subunit 1, or transient receptor potential vanilloid 1 (TRPV1), integrates physical and chemical stimuli in the peripheral nervous system, playing a key role in inflammatory pain. Identification of potent TRPV1 antagonists is thus an important goal of current neuropharmacology. Herein, we describe the solid-phase synthesis of a series of indole-based peptoids (*N*-alkylglycines) and the biological activity of the peptoids as novel TRPV1 antagonists. The potency and selectivity of the compounds were determined by electrophysiological recordings in *Xenopus* oocytes. The most potent and selective noncompetitive TRPV1 antagonist of the series, compound **7**, represents an interesting pharmacophoric structure for analgesic lead optimization.

Introduction

Organisms require pain to survive and to maintain structural integrity, but sustained or chronic pain can result in side effects and in a decreased quality of life. Inflammatory pain is a response to tissue damage. It begins when noxious stimuli (thermal, chemical, or mechanical) excite sensory neurons called nociceptors.^{1–4} Nociceptors are sensitized by activation of PKC^a and PKA pathways, which leads to phosphorylation of sensory receptors involved in the nociceptive function.⁵ The resultant sensory signaling is conveyed to the spinal cord and, finally, to specific brain regions, leading to the sensation of pain.⁶ There is evidence that peripheral sensitization is mediated by hyperexcitation of TRPV1 receptors. Identification of TRPV1 as a molecular integrator of noxious stimuli that involves both thermal nociception and inflammatory hyperalgesia has validated it as a key therapeutic target for inflammatory pain.^{7–12}

TRPV1 was first reported by Caterina et al.¹³ as a capsaicin (**1**) receptor, a nonselective cation channel activated by vanilloids and lipids, and gated at temperatures above 43 °C. In addition, mild extracellular pH potentiates its activation by noxious heat and vanilloids, whereas strongly acidic conditions (pH < 6) directly activate the channel.^{14,15} Furthermore, the channel is notably sensitized by proalgesic substances released during inflammation.¹⁶ TRPV1 hyperstimulation by capsaicin leads to long-term desensitization of the sensory neurons and thus produces an analgesic effect. Capsaicin is the active component of various topical pain relievers and has been used to treat pain associated with diabetic neuropathy and arthritis.^{17,18} However, the clinical use of TRPV1 agonists (See Figure 1),

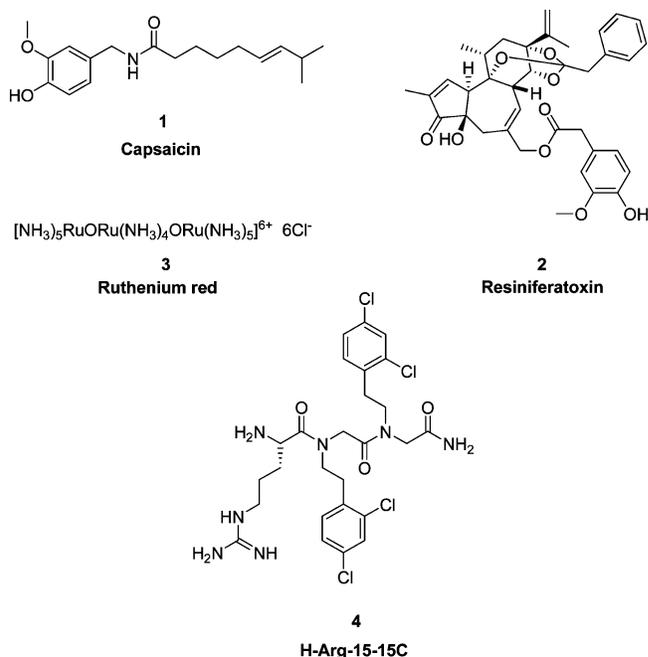


Figure 1. Chemical structures of TRPV1 agonists and antagonists.

such as **1** and resiniferatoxin (RTX, **2**), is limited due to side effects (burning sensation, irritation, and neurotoxicity) resulting from continuous influx of Ca^{2+} into the cells. Structure–activity relationship (SAR) studies of vanilloids have led to other potent drugs.¹⁹ However, these also exhibit irritant side effects. An alternative approach to pain relief is to block the TRPV1-mediated pain signaling pathways with receptor antagonists, a promising strategy for the development of novel analgesic drugs with potentially fewer side effects.²⁰ In addition, some TRPV1 antagonists have shown positive effects in animal models of neuropathic pain, which makes these kinds of compounds very attractive as analgesic molecules.²¹ During the past few years, several classes of competitive TRPV1 antagonists, either structurally related or not to **1** or **2**, have been described, and their chemistry and pharmacology have been reviewed.^{22–25} Many of these antagonists are focused on structures containing

* To whom correspondence should be addressed. A.F.-M.: phone, 0034 966658727; fax, 0034 966658758; e-mail, aferrer@umh.es. F.A.: phone, 0034 934037088; fax, 0034 934037126; e-mail, albericio@pcb.uv.es.

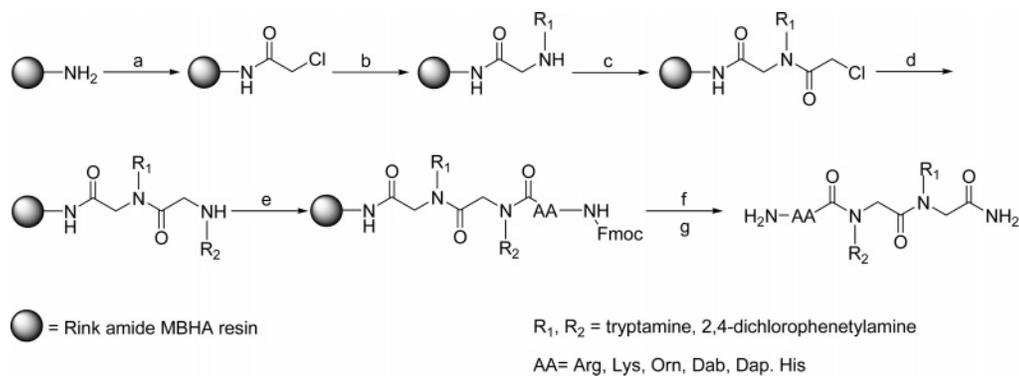
[†] IRB Barcelona, Barcelona Science Park, University of Barcelona.

[‡] University Miguel Hernández.

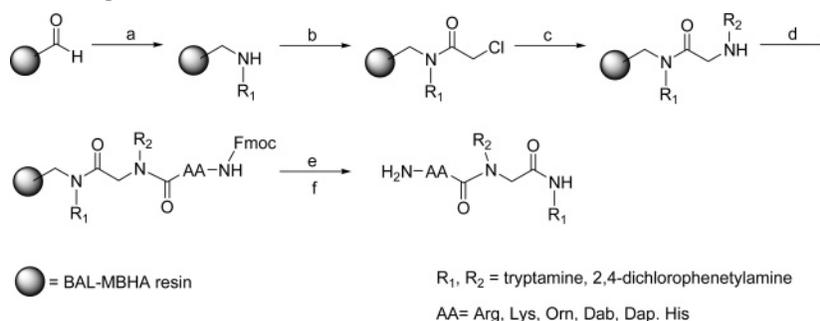
[§] DiverDrugs SL.

^{||} Department of Organic Chemistry, University of Barcelona.

^a Abbreviations: BAL, backbone amide linker; MTBD, 1,3,4,6,7,8-hexahydro-1-methyl-2H-pyrimido[1,2-*a*]pyrimidine; NMDA, *N*-methyl-D-aspartate; PKA, protein kinase A; PKC, protein kinase C; TES, 2-[(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid; TRPV1, transient receptor potential vanilloid 1; abbreviations used for amino acids follow the IUPAC–IUB Commission of Biochemical Nomenclature (Jones, J. H. J. *Pept. Sci.* **2003**, 9, 1–8).

Scheme 1. General Synthesis of Peptoids, Method A^a

^a Conditions: (a) ClCH₂COOH, DIC, DMF; (b) R₁-NH₂, Et₃N, DMSO/DMF; (c) ClCH₂COOH, DIC, DMF; (d) R₂-NH₂, Et₃N, DMSO/DMF; (e) Fmoc-AA-OH; DIC/HOBt, DMF; (f) piperidine/DMF; (g) TFA cocktail cleavage.

Scheme 2. General Synthesis of Peptoids, Method B^a

^a Conditions: (a) R₁-NH₂, NaBH₃CN, AcOH/DMF; (b) ClCH₂COOH, DIC, DMF; (c) R₂-NH₂, Et₃N, DMSO/DMF; (d) Fmoc-AA-OH, DIC/HOBt, DMF; (e) piperidine/DMF; (f) TFA cocktail cleavage.

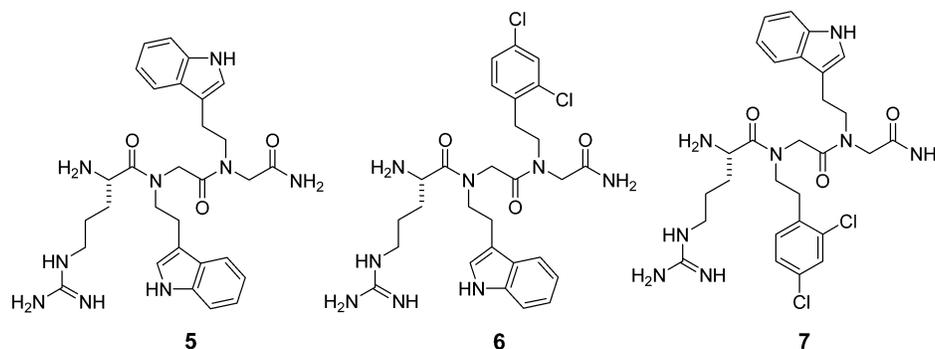


Figure 2. Chemical structures of indole-based analogs of peptoid 4.

thiourea, urea, and amide groups.²⁶ However, further studies are required to assess their clinical efficacy.

Noncompetitive antagonists interact with additional binding sites on the receptor structure, thereby preventing opening of the receptor by an agonist or blocking its aqueous pore. The first noncompetitive TRPV1 antagonist discovered was the trinuclear polyamine complex ruthenium red (**3**) (Figure 1), which binds to the pore region of the channel with high potency (IC₅₀ = 0.14 μM). However, its poor selectivity seems to underlie its proconvulsive activity in animal models, which precludes its clinical development.²⁵ Certain arginine-rich peptides that block recombinant TRPV1 channels expressed in *Xenopus* oocytes with micromolar potency have also been described, but they are toxic.²⁷ *N*-Alkylglycines, or peptoids, were the first small molecules identified as noncompetitive TRPV1 antagonists.²⁸ These compounds have advantages over arginine-rich peptides, such as higher proteolytic stability.²⁹

Compound H-Arg-15-15C (**4**) (Figure 1) has been described as a good scaffold for analgesic lead optimization, as it presents submicromolar potency and *in vivo* analgesic and anti-inflammatory activities. However, its receptor selectivity is not optimal.³⁰

Herein we report the synthesis of noncompetitive antagonists of TRPV1 by analoging of peptoid **4**. A library of 20 peptoids was constructed on solid-phase, some of which were identified through biological assays as having higher potency than **4** and an increase in the receptor selectivity.

Results and Discussion

Design and Synthesis of the Library. On the basis of the previous synthesis of **4**,³⁰ we designed a series of analogs to improve its potency. Studies of the pore region of the channel suggest that a charged or polar side chain could be crucial to activity, due to the presence of a cation binding site right at the

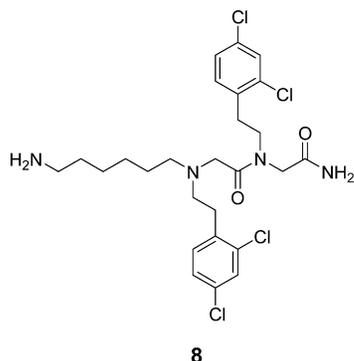


Figure 3. Chemical structure of peptoid **8**.

pore vestibule.^{15a} Moreover, it could be complementary with the presence of hydrophobic groups that may stabilize the structure in the pore entrance. Several modifications were therefore introduced on the basis of these two characteristics: (i) substitution of 2,4-dichlorophenethyl group with a different hydrophobic planar group such as indole; (ii) incorporation of different amino acids at the N-terminus to evaluate the effect of polar and charged groups of different lengths; (iii) deletion of the acetamide group at the C-terminus; (iv) substitution of the N-terminal amide group with a tertiary amine group; and (v) incorporation of a cyclic group into the structure, while the hydrophobic groups and a charged side chain are maintained.

All peptoids were prepared via the submonomer method,³¹ using two general routes starting on Rink amide MBHA (method A; see Scheme 1) and BAL-MBHA (method B; see Scheme 2) resins. In method A, the primary amine of Rink linker is acylated with chloroacetic acid and *N,N'*-diisopropylcarbodiimide (DIC), and then the chloroacetyl group undergoes nucleophilic substitution with a primary amine in basic media to yield a secondary amine. In method B, the aldehyde group of BAL linker reacts with a primary amine by reductive amination.³² The syntheses then proceed similarly: acylation of the corresponding amines with chloroacetic acid and DIC, followed by nucleophilic substitution with tryptamine or 2,4-dichlorophenethylamine in DMSO/DMF. A Fmoc-protected amino acid is then coupled using DIC/HOBt, and after deprotection of the Fmoc group, the compounds are cleaved from the resin with an optimized TFA cocktail.

Incorporation of different hydrophobic groups using method A (Scheme 1), afforded derivatives **5**–**7** (Figure 2), which contain indole and 2,4-dichlorophenethyl moieties. In the synthesis of peptoid **8** (Figure 3), the amide bond on the N-terminus was deleted via reductive amination with Fmoc-6-aminohexanal. Fmoc-6-aminohexanal was prepared by reduction of the corresponding amino acid with NaBH₄ to provide the alcohol (99% yield) which, upon Swern oxidation, afforded the desired amino aldehyde (90%).³³

Removal of the acetamide group at the C-terminus from the chemical structures of peptoids **4** and **5** results in the chemical structures of peptoids **9** and **10** (Figure 4), respectively. This modification was achieved by using BAL linker in method B (Scheme 2).

Several amino acids (Lys, Orn, Dab, Dpr, His) were evaluated in the N-terminal position (**11**–**15**) (Figure 4) to determine the effects of the length and polarity of the side chain on biological activity. Cleavage of these peptoids from the resin with TFA yielded complex mixtures, possible due to the reactivity of the deprotected indole group. Therefore, optimization of TFA

Table 1. Acidolytic Cleavage Cocktails (v/v)

mixture 1 (94:2.5:2.5:1)	mixture 2 (95:5)	mixture 3 (95:5)	mixture 4 (49:49:2)	mixture 5 (88:5:5:2)
TFA	TFA	TFA	TFA	TFA
H ₂ O	H ₂ O	H ₂ O	DCM	H ₂ O
EDT			anisole	phenol
<i>i</i> -Pr ₃ SiH				<i>i</i> -Pr ₃ SiH
ether washes	ether washes	THF washes	ether washes	ether washes

Table 2. HPLC Data (%) of Crude Products Obtained with Different Cleavage Cocktails^a

peptoid	mixture 1	mixture 2	mixture 3	mixture 4	mixture 5
10	19	32	34	67	38
11	25	43	45	61	60
12	19	58	33	80	38
13	20	26	40	70	21
14	15	46	50	57	34
15	22	40	46	64	59

^a HPLC systems: (A) H₂O–TFA 99.955:0.045, (B) ACN–TFA 99.964:0.036; gradient 10% B to 90% B in 15 min. Compounds were detected by UV absorption at 220 nm.

cocktails was necessary to minimize side reactions and improve the purity of the crude products. Five TFA cocktails (Table 1) with different scavengers and washing methods were evaluated. For all compounds, the best results were obtained with TFA–DCM–anisole (49:49:2, v/v) (Table 2), providing purities between 60% and 80%. Anisole was the best scavenger for the Boc group, offering 3–4-fold higher purity of crude products.

Incorporation of a cyclic group into the peptoid was achieved as described in Scheme 3 for the synthesis of compound **16**. Starting with BAL-MBHA resin and after reductive amination with 2,4-dichlorophenethylamine, Alloc-Amp(Fmoc)-OH was coupled with DIC/HOAt. Orthogonal protection of this amino acid allowed incorporation of a 2,4-dichlorophenyl group at the α -amine group by reductive amination, and coupling of Fmoc-Arg(Pbf)-OH at the γ -amine group with DIC/HOAt. The compound was cleaved from the resin with HF, as cleavage with TFA did not yield the desired product. The presence of the α -amino group in the cyclic moiety may explain the increased stability of the bond between the BAL linker and the peptoid.³⁴

Peptoids **17**–**22** (Figure 5) are derivatives of **11** prepared by different *N*-alkylations at the ϵ -amine of the lysine side chain. Peptoids **17** and **18** were obtained by exhaustive alkylation with DIEA and an excess of MeI and EtI, respectively. Monoalkylation was achieved using Miller's method:³⁵ protecting the free amine with *o*-nitrobenzenesulfonyl chloride in basic media, followed by deprotonation and selective monoalkylation with methyl *p*-nitrobenzenesulfonate or EtI in DMF. Selective removal of the sulfonamide with β -mercaptoethanol and DBU in DMF afforded peptoids **19** and **20**. The preparation of dialkylated derivatives (**21** and **22**) was first evaluated using consecutive reductive aminations, which did not proceed successfully. Thus, the amines were first monoalkylated using Miller's method, obtaining **21** and **22** via reductive amination with formaldehyde or acetaldehyde, respectively.

Finally, to evaluate the effect of guanidinium deactivation, we synthesized compounds **23** and **24** (Figure 6), with a nitro and a tosyl moiety, respectively, both of which are stable to cleavage conditions, as arginine protecting groups. The synthesis was performed as described above for peptoid **10** but using Fmoc-Arg(NO₂)-OH and Fmoc-Arg(Tos)-OH, respectively, at the N-terminal position.

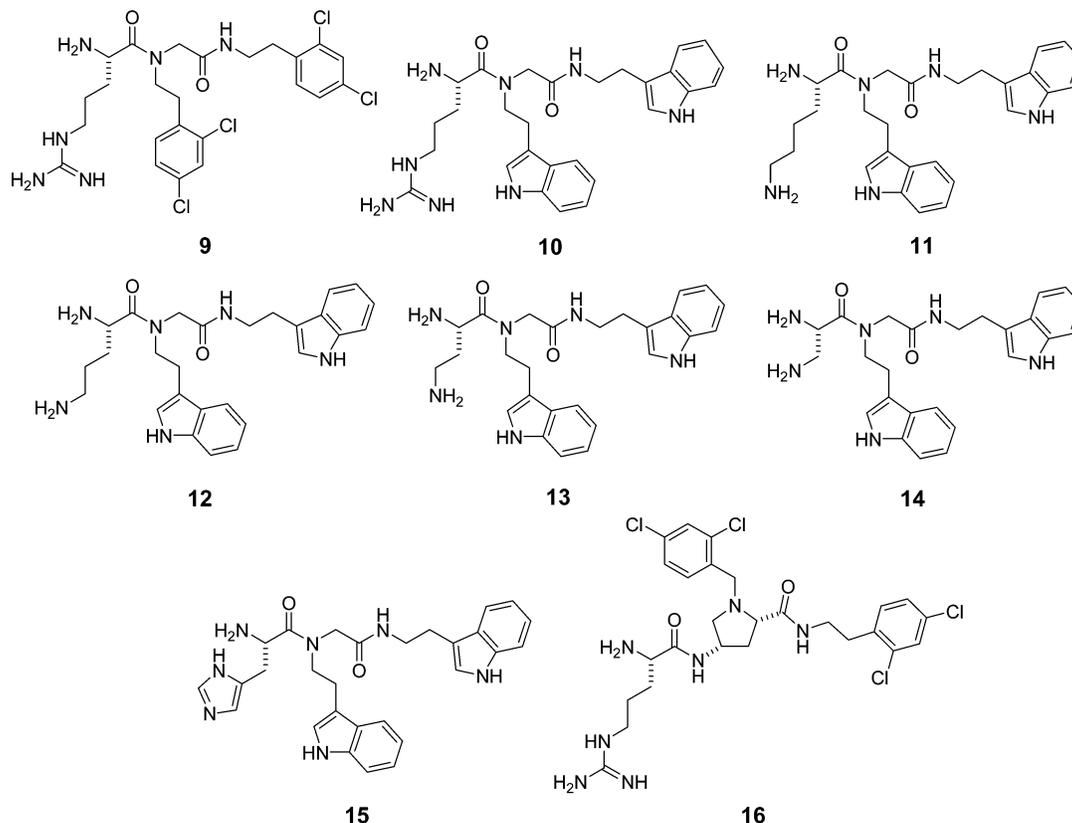
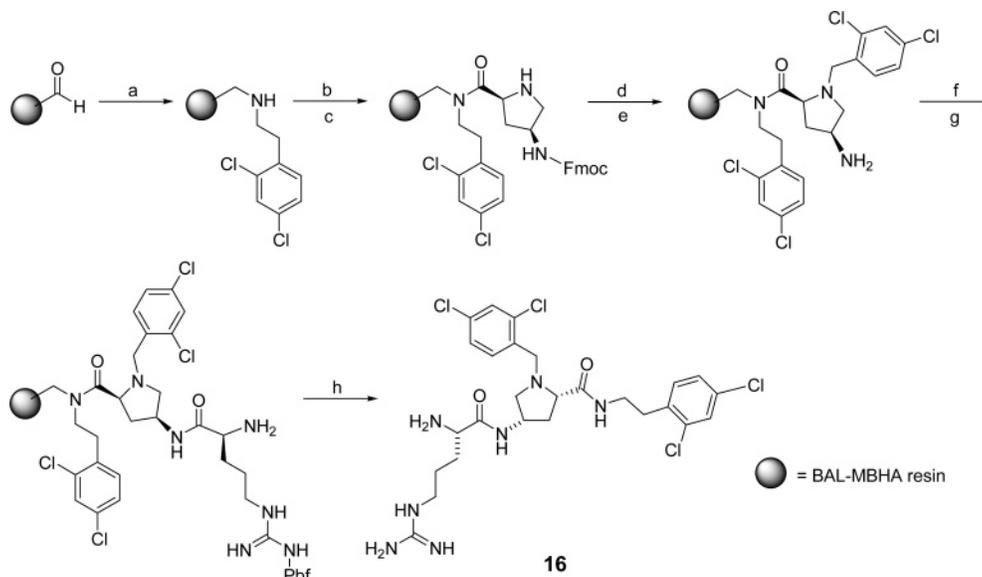


Figure 4. Analogs of peptoid **10** with different N-terminal amino acids.

Scheme 3. Synthesis of Compound **16**^a



^a Conditions: (a) 2,4-dichlorophenethylamine, NaBH₃CN, AcOH/DMF; (b) Fmoc-Arg(Pbf)-OH, DIC/HOAt, DMF; (c) (i) PhSiH₃, Pd(PPh₃)₄, DCM, (ii) sodium diethyldithiocarbamate, DMF; (d) 2,4-dichlorobenzaldehyde, NaBH₃CN, AcOH/DMF; (e) piperidine/DMF; (f) Fmoc-Arg(Pbf)-OH, DIC/HOAt, DMF; (g) piperidine/DMF; (h) 10% anisole/HF, -78 °C.

Biological Results. The ability of the 20 peptoids (**5–24**) to block capsaicin-mediated activation of TRPV1 channel was first evaluated at 10 μ M. The capacity of each peptoid to block the activity of TRPV1 heterologously expressed in *Xenopus* oocytes was determined using a two microelectrode voltage-clamp amplifier at \sim 20 °C. The channel activity was elicited with 10 μ M capsaicin at a holding potential of -40 mV, and then the corresponding compounds were infused.^{36–38} The results of this

first screening are shown in Figure 7. The receptor selectivity of each peptoid was first evaluated by determining the extent of blockade of the *N*-methyl-D-aspartate receptor (NMDA) at 10 μ M (Figure 8).

Those peptoids with only one indole group in their structure (**6** and **7**) showed the highest blocking activity. The introduction of a second indole group (**5**) leads to a decreased activity, but an increased selectivity, compared to the parent compound **4**.

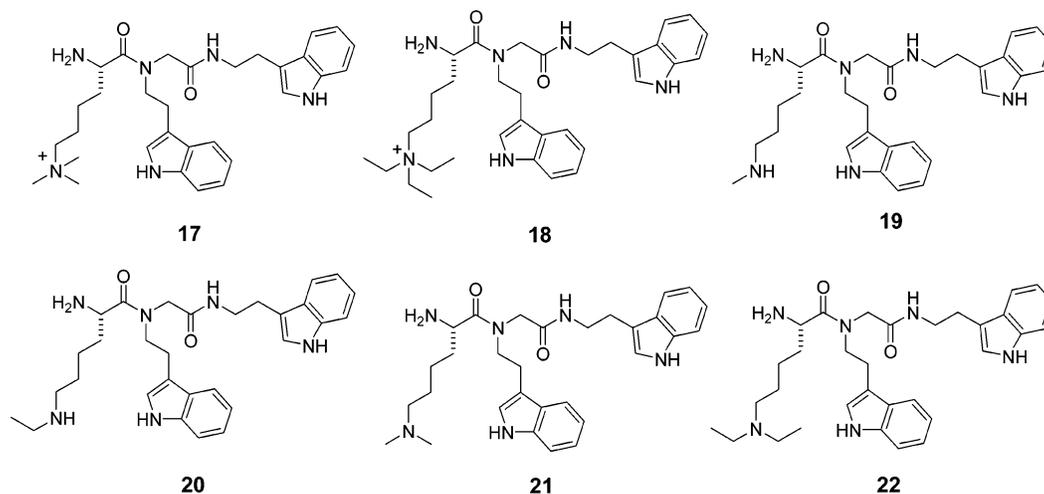


Figure 5. N^{ϵ} -Alkylated analogs of peptoid 11.

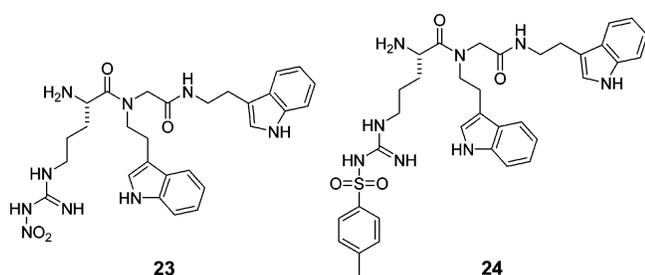


Figure 6. Chemical structure of peptoids 23 and 24.

The N-terminus (8) and C-terminus (9 and 10) modifications retain the activity of the parent compound but result in a lower selectivity. All of the analogs with different N-terminal amino acids (11–15) were less active and selective than the parent compound. This result indicates the importance of the guanidinium group for activity, whose replacement with a primary amine leads to 4-fold lower activity. On the other hand, the introduction of a rigid moiety into the peptoid skeleton (16) does not markedly affect activity but results in a lower selectivity. Regarding the *N*-alkylated peptoids (17–22), the screening revealed a relationship between activity and the basicity of the side chain of the N-terminal amino acid. Although these analogs were less active than the parent compound, comparison to peptoid 11 indicated that the more basic the amine groups are, the higher the observed activity is (18 >

19 > 20 > 21 > 22). Finally, the low activity of peptoids 23 and 24 is also consistent with the fact that activity is at least partly dependent on the presence of a free guanidinium group.

These results agree with the reported molecular architecture of the pore region of the TRPV1 channel.^{15a} The location of three acidic residues (Asp646, Glu648, and Glu651) close to the permeation pathway ensures a strong negative electrostatic potential, which is required to attract positive groups. This fact is consistent with the high activity observed for peptoids containing a positive charge, such as a guanidinium group (6, 7, 9, 10, and 16), at the opposite site of hydrophobic groups. Essentially, the presence of these hydrophobic groups seems required for peptoid interaction at the outer hydrophobic entrance of the channel, whereas the positive charge fits into the internal region of the channel. The combination of these two interactions allows the peptoids to act as noncompetitive antagonists by blocking the pore entrance and consequently inhibiting TRPV1 activity.

Dose–response curves were performed for the most active compounds (5–10 and 16) and representative curves are shown in Figure 9. The corresponding IC_{50} values were determined (Table 3) and showed that five of these peptoids have similar or higher potency than 4. For comparison, the blockade potency of the noncompetitive TRPV1 antagonist ruthenium red has been included. As seen, this compound is a potent TRPV1 blocker but lacks receptor selectivity. Peptoid 7 exhibited the highest

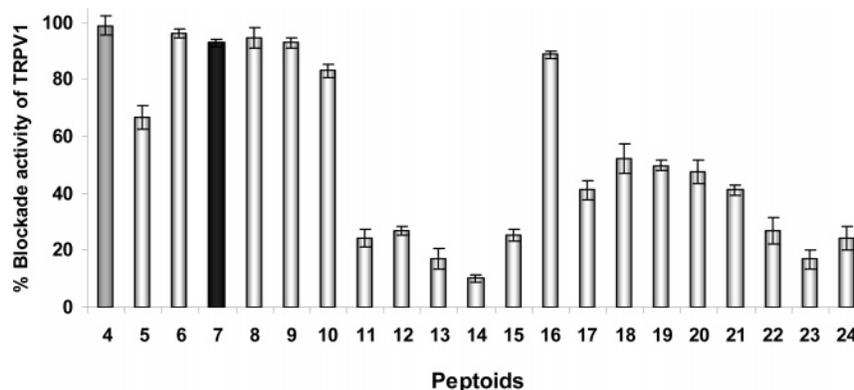


Figure 7. Blockade activity of TRPV1 channels expressed in *Xenopus* oocytes. The channels were activated with 10 μ M capsaicin and incubated with peptoids at 10 μ M. Oocytes were perfused at 20 $^{\circ}$ C with Ringer buffer pH 7.4 and held at -40 mV. Percentages were normalized with respect to the value obtained in the absence of peptoids. Each point represents the mean \pm SEM, $n \geq 4$.

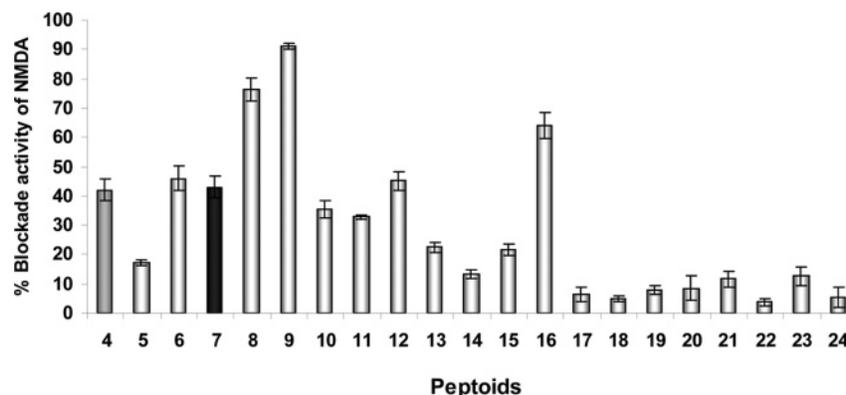


Figure 8. Blockade activity of NMDA channels expressed in *Xenopus* oocytes. The channels were activated with 100 μM L-Glu/20 μM Gly and exposed to 10 μM peptides. Oocytes were perfused with Ringer buffer pH 7.4 and held at -40 mV. All measurements were performed at 20 $^{\circ}\text{C}$. Percentages were normalized with respect to that obtained in the absence of peptides. Each point represents the mean \pm SEM, $n \geq 4$.

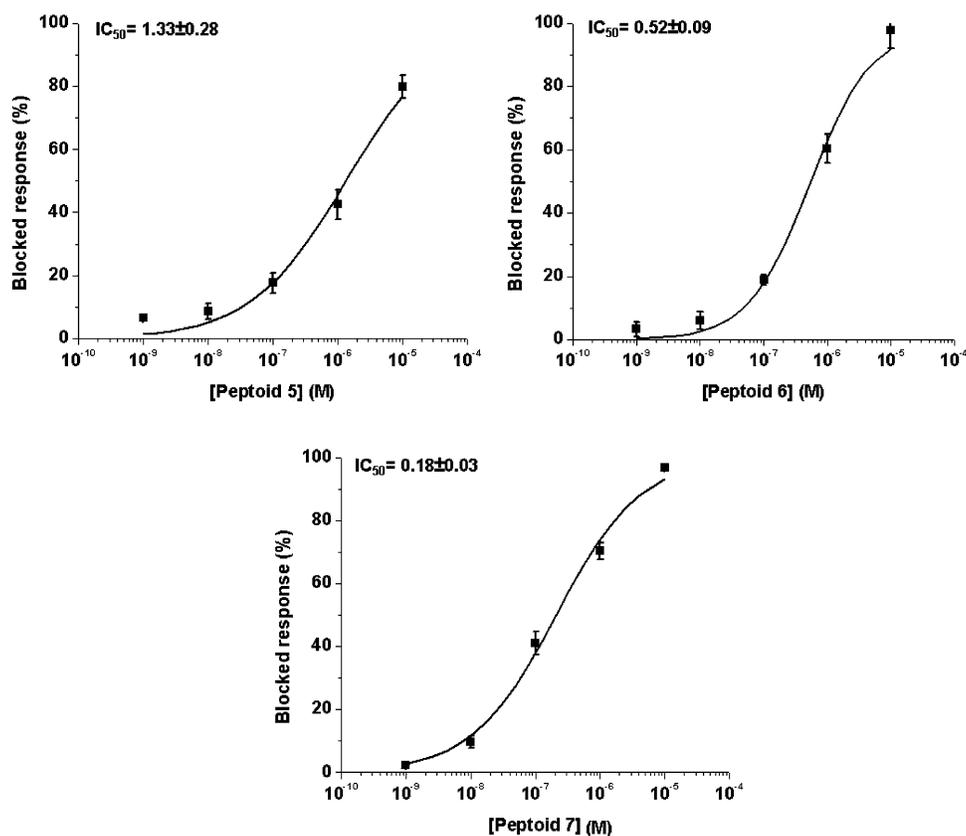


Figure 9. Dose–response curves of blockade activity of capsaicin-activated TRPV1 channels expressed in *Xenopus* oocytes for representative peptides 5–7. Channels were activated with 10 μM capsaicin in Ringer buffer at pH 7.4 and held at -40 mV. Responses were normalized with respect to that obtained in the absence of peptides. Each point represents the mean \pm SEM, $n \geq 4$; solid lines denote the best fit to the Hill equation.

antagonistic activity ($\text{IC}_{50} = 0.18 \mu\text{M}$) of our peptides, which is ≈ 4 -fold stronger than **4** ($\text{IC}_{50} = 0.7 \mu\text{M}$). Noteworthy, peptide **7** also exhibited improved receptor selectivity with respect to the parent peptide **4**, as evidenced by the 10-fold higher IC_{50} value for blockade of the NMDA receptor (Table 3). In addition, this peptide did not antagonize the ion channel activity of voltage-gated ion channels [$\text{IC}_{50} > 100 \mu\text{M}$ for K^+ channel subtype Kv1.1, Na^+ channel subtype SKN4A, and a1H T-type Ca^{2+} channel (data not shown)].²⁸ Thus, the modification that leads to peptide **7** produced a noncompetitive antagonist with higher blockade potency and selectivity than the starting peptide **4**.

The inspection of the structure of these peptides revealed that deletion of the acetamide group at the C-terminus of the parent compound leads to a higher potency, as reflected in peptides **9** ($\text{IC}_{50} = 0.17 \mu\text{M}$), **5**, and **10**. Interestingly, peptide **8** has an IC_{50} of 0.40 μM , despite the fact that it lacks a guanidinium group. This result can be partially explained by considering the absence of an amide bond in the N-terminal position of **8**, which enables a different structural conformation. However, we also observed that a more rigid structural conformation does not improve potency, since the introduction of a cyclic moiety into the peptide skeleton (**16**) does not greatly affect activity. Despite the high potency of peptides **8**, **9**, and **16**, their poor receptor

Table 3. Blockade Efficacy and Selectivity of Most Active Peptoids^a

peptoid	IC ₅₀ (μM)	
	TRPV1	NMDA
RR	0.14 ± 0.09	0.5 ± 0.2
4	0.7 ± 0.1	15 ± 2
5	1.33 ± 0.28	207 ± 55
6	0.52 ± 0.09	17 ± 3
7	0.18 ± 0.03	166 ± 12
8	0.4 ± 0.1	3 ± 1
9	0.17 ± 0.01	0.8 ± 0.4
10	1.1 ± 0.1	18 ± 4
16	0.7 ± 0.2	8 ± 2

^a TRPV1 ion channel activity was elicited with 10 μM capsaicin, and NMDA receptor activity was evoked with 100 μM L-Glu/20 μM Gly. Oocytes were held at -40 mV and continuously perfused with Ringer's solution at 20 °C. IC₅₀ values were obtained from the fit of dose-response curves to the Hill equation. Values are given as mean ± SEM, *n* ≥ 4. RR denotes ruthenium red.

selectivity (Table 3) led us to exclude them from further pharmacological assays.

Conclusions

A series of analogs of peptoid **4** was synthesized in order to obtain SAR information of noncompetitive TRPV1 antagonist activity, as well as requirements for receptor selectivity. A 20-member library was synthesized entirely on solid-phase using Rink amide MBHA and BAL-MBHA resins via the submonomer method. Optimization of cleavage conditions led to the identification of anisole as the best scavenger to minimize the side reactions derived from an unprotected indole group. The inhibitory activity of the analogs toward TRPV1 and NMDA was assayed in *Xenopus* oocytes, and five peptoids more potent than the original peptoid **4** were identified. These studies revealed that the combination of two different hydrophobic groups (indole and 2,4-dichlorophenethyl) and the presence of the guanidinium group in the N-terminal amino acid are favorable to activity. Deactivation of the guanidinium group or substitution with a primary amine significantly decreased the inhibitory activity. These results substantiate the relevance of a positive charge to enhance the interaction with the outer pore region of the TRPV1 channel, where acidic residues are known to be located. The mechanism of action of these peptoids may imply interaction of the hydrophobic moieties with the hydrophobic vestibule of the channel and concomitant interaction of the positive charge with the outer pore region of the channel. Other chemical modifications, such as elimination of the acetamide group in the C-terminus or the introduction of a tertiary amine at the N-terminus, increased the potency but decreased the selectivity vs NMDA receptor.

Peptoid **7** is the most potent and selective noncompetitive antagonist of TRPV1 based on the *N*-alkylglycine structure described to date. Its ≈4-fold stronger potency and 10-fold higher selectivity than the parent peptoid **4** converts this noncompetitive antagonist to an interesting pharmacophoric scaffold for analgesic lead development.

Experimental Section

Materials and Equipment. Protected amino acids and HOBt were obtained from IrisBiotech (Martdrewitz, Germany). DIC, trifluoroacetic acid, and acetaldehyde were purchased from Fluka Chemika (Busch, Switzerland). 2,4-Dichlorobenzaldehyde and tryptamine were obtained from Acros (Fair Lawn, NJ). Capsaicin was from Sigma (St Louis, MO). *N*-Ethyl-diisopropylamine and HPLC grade formic acid were purchased from Merck (Darmstadt, Germany). Solid supports were supplied by Calbiochem-Novabio-

chem AG (Darmstadt, Germany). HF was obtained from Air Products and Chemicals, Inc. (Allentown, Canada), and related equipment was obtained from Peptide Institute (Minoh, Osaka, Japan). Other chemicals were purchased from Aldrich (Milwaukee, WI) and the solvents were obtained from SDS (Peypin, France) and were used as received, except THF, which was distilled over sodium. Analytical RP-HPLC was performed using Waters (Milford, MA) chromatography systems with reverse-phase Symmetry C₁₈ (150 × 3.9 mm, 5 μm) columns. Semipreparative RP-HPLC was performed on a Waters (Milford, MA) chromatography system using a Symmetry C₁₈ (100 × 30 mm, 5 μm) column. Compounds were detected by UV absorption at 220 nm. Mass spectra were obtained by RP-HPLC, UV-vis detector, and ZQ Electrospray mass detector from Waters (Milford, MA). High-resolution mass spectra (HRMS) were obtained in an LC/MSD TOF from Agilent Technologies (Unitat d'Espectrometria de Masses, Serveis Científico-Tècnics, University of Barcelona). ¹H NMR were recorded on a Mercury 400 spectrometer (Unitat de RMN, Serveis Científico-Tècnics, University of Barcelona). Electrophysiological recordings were performed with an electrode voltage clamp amplifier [TEC 10CD, NPI Electronic (Tamm, Germany)]. Electrodes were made with hematocrit glass capillaries and pulled with P-97 puller of Sutter Ins. Co. (Novato, CA).

General Procedures. Solid-phase syntheses were performed in polypropylene syringes equipped with a polyethylene porous disk. Solvents and soluble reagents were removed by suction. Washes between deprotection, coupling, and subsequent deprotection steps were carried out with DMF (5 × 1 min) and DCM (5 × 1 min) using 10 mL of solvent/g per wash. All final compounds were purified to ≥95% purity by semipreparative RP-HPLC using gradients of acetonitrile (0.05% TFA) and Milli-Q water (0.1% TFA) at 20 mL/min. The purity of the compounds was determined by analytical RP-HPLC using a gradient of acetonitrile (0.036% TFA) and Milli-Q water (0.045% TFA) at 1 mL/min. High-resolution mass spectrometry (HRMS) was run in ES positive ionization mode. ¹H NMR spectra were obtained with a Varian-Mercury 400 MHz spectrometer. Chemical shifts are reported in part per million (ppm, δ units), and coupling constants are given in hertz (Hz).

Coupling of Amino Acids and BAL Linker. Protected amino acid or BAL linker (0.63 mmol) in DMF, HOBt (85 mg, 0.63 mmol) and DIC (98 μL, 0.63 mmol) were added to the resin and allowed to react with automatic shaking for 1 h. The solvent was removed by filtration and the resin was washed as indicated above. Incorporation of the BAL linker to the MBHA resin was run overnight and confirmed by the Kaiser test.³⁹

Removal of Protecting Groups. Fmoc: piperidine/DMF (2:8, v/v) (2 × 10 min), washes with DMF (5 × 1 min). Alloc: Pd(PPh₃)₄ (0.1 mmol), PhSiH₃ (10 mmol) in anhydrous DCM (3 × 15 min) under an N₂ atmosphere, washes with 0.02 M sodium diethyldithiocarbamate/DMF (3 × 15 min).

Reductive Amination. The corresponding amine (2.1 mmol) was dissolved with AcOH/DMF (1:99, v/v) and added to the resin. NaBH₃CN (132 mg, 2.1 mmol) was then added, and the mixture was allowed to react at room temperature for 6 h. The resin was washed with DMF and DCM.

Preparation of Fmoc-6-aminoheptanal. Fmoc-6-aminoheptanoic acid (1 g, 2.8 mmol) was dissolved in anhydrous THF at -78 °C in N₂ atmosphere. NMM (372 μL, 3.36 mmol) and isobutyl chloroformate (440 μL, 3.36 mmol) were then added, and the solution was warmed to 0 °C for 40 min. The temperature was lowered to -78 °C, a methanolic solution of NaBH₄ (214 mg, 5.6 mmol) was added, and the reaction mixture was maintained at -78 °C for 2 h. The reaction was then gradually brought to room temperature and quenched with aqueous ammonium chloride. The crude mixture was extracted with EtOAc, and the organic phase was washed with NaHCO₃ and brine, dried, and concentrated in vacuo to afford the corresponding alcohol as a white solid (955 mg, 99% yield). This alcohol was then oxidized to the target aldehyde as follows: Oxalyl chloride (268 μL, 3 mmol) was dissolved in anhydrous DCM, and the resulting solution was treated

with DMSO (240 μL , 3.36 mmol) at $-78\text{ }^\circ\text{C}$ for 30 min. A DCM solution of the alcohol was then added, and the mixture was allowed to react at $-78\text{ }^\circ\text{C}$ in N_2 atmosphere for 1 h. Triethylamine (1.2 mL, 8.4 mmol) was then added, and after 1 h, the reaction was brought to room temperature and then concentrated in vacuo. The crude was purified by flash chromatography with hexane/EtOAc (1:1) to obtain a white solid (860 mg, 90% yield).

General Synthesis of Peptoids: Method A. Peptoids 5–7. Starting with 0.65 mmol/g Rink Amide MBHA resin (250 mg, 0.16 mmol) the first step was deprotection of the Fmoc group, thereafter acylation was carried out with chloroacetic acid (90 mg, 0.96 mmol) in the presence of DIC (77 μL , 0.48 mmol) in DMF for 1 h. The reaction was followed by the Kaiser test. After washing with DMF, the amination was performed with tryptamine or 2,4-dichlorophenethylamine (0.64 mmol), and triethylamine (89 μL , 0.64 mmol) in DMF. The reaction was run for 4 h and followed by the De Clercq test.⁴⁰ Acylation and amination steps (tryptamine or 2,4-dichlorophenethylamine) were successively repeated using the same conditions described above. After washing with DMF and DCM, the corresponding amino acids were coupled via the above-mentioned procedure.

Method B: Peptoids 9–24 (Except for 16). The BAL handle (0.63 mmol) was coupled to 300 mg of MBHA resin (0.7 mmol/g). Reductive amination of the handle was performed with a primary amine (tryptamine or 2,4-dichlorophenethylamine, 2.1 mmol) as described above, and the reaction was followed by the aldehydes test.⁴¹ The resulting amine was then acylated with chloroacetic acid (119 mg, 1.26 mmol) in the presence of DIC (99 μL , 0.63 mmol) in DMF for 1 h. After washing with DMF, the amination was performed with tryptamine or 2,4-dichlorophenethylamine (0.84 mmol) and triethylamine (117 μL , 0.84 mmol) in DMF, and the reaction was left for 4 h. After washing, the corresponding amino acids were incorporated via the above-mentioned procedure.

Synthesis of Peptoid 8. Peptoid 8 was synthesized via method A with 2,4-dichlorophenethylamine for amination. In the last step, instead of coupling an amino acid, a reductive amination was performed with Fmoc-aminohexanal (542 mg, 1.6 mmol) and NaBH_3CN (100 mg, 1.6 mmol) dissolved in AcOH/DMF (1:99 v/v) under agitation overnight. After Fmoc deprotection, the peptoid was cleaved from the resin with $\text{TFA}/\text{H}_2\text{O}$ (95:5 v/v).

Synthesis of Peptoid 16. Peptoid 16 was synthesized starting with 1.1 mmol/g BAL-aminomethyl resin (380 mg, 0.41 mmol) followed by reductive amination with 2,4-dichlorophenethylamine (630 μL , 4.1 mmol) and NaBH_3CN (263 mg, 4.1 mmol) as describe above. Next, Alloc-Amp(Fmoc)-OH (265 mg, 0.83 mmol) was then coupled with HOAt (114 mg, 0.83 mmol) and DIC (130 μL , 0.82 mmol) in DMF for 6 h. After washing with DMF and DCM, the Alloc group was eliminated as describe above. Reductive amination with 2,4-dichlorobenzaldehyde (731 mg, 4.1 mmol) and NaB_3HCN (263 mg, 4.1 mmol) was carried out in AcOH/DMF (1:99 v/v) overnight. After washing and deprotection of Fmoc group, Fmoc-Arg(Pbf)-OH (343 mg, 1.23 mmol) was incorporated in DMF using the general procedure. After Fmoc deprotection, cleavage from the resin was performed with 10% anisole in HF for 1 h at $-78\text{ }^\circ\text{C}$. The HF was evaporated, and the resin was washed with methyl *tert*-butyl ether (3 \times 5 min). The product was extracted with AcOH and acetonitrile and then lyophilized.

General Procedure for *N*-Alkylation of Lysine Side Chains (17–22). The basic structure was synthesized as peptoid 11, in which an *N*-terminus Boc-Lys(Fmoc)-OH (295 mg, 0.63 mmol) was incorporated with HOBt (85 mg, 0.63 mmol) and DIC (98 μL , 0.63 mmol) as coupling reagents. *N*-Alkylations were performed after Fmoc deprotection and washing with DMF and DCM.

Exhaustive alkylation was carried out with MeI (156 μL , 2.1 mmol) and DIEA (277 μL , 2.1 mmol) in DMF for 4 h to obtain peptoid 17. Peptoid 18 was synthesized with the same procedure using EtI (327 mg, 2.1 mmol) as alkylating reagent.

Monoalkylation to obtain 19 and 20 was performed via Miller's method. First, the amine group was protected by treatment with *o*-nitrobenzenesulfonyl chloride (139 mg, 0.63 mmol) and DIEA (138

μL , 1 mmol) in DCM for 2 h. The reaction was followed by the Kaiser test. After several washes, *N*-alkylation was carried out with MeI (62 μL , 0.84 mmol) or EtI (68 μL , 0.84 mmol) in the presence of MTBD (91 μL , 0.63 mmol) in DMF for 30 min. After washing, the protecting group was eliminated with β -mercaptoethanol (147 μL , 2.1 mmol) and DBU (140 μL , 1 mmol) in DMF (2 \times 20 min).

Dialkylation to obtain compounds 21 and 22 was performed in two steps: a first alkylation achieved via Miller's method as describe above and then a second alkylation via reductive amination. The latter comprised acetaldehyde (118 μL , 2.1 mmol) or formaldehyde (90 μL , 2.1 mmol) and NaBH_3CN (132 mg, 2.1 mmol) in AcOH/DMF overnight and was followed by the De Clercq test.

Cleavage of Peptoids from the Resin. Removal of the side chain protecting groups and cleavage of the peptoids from the resin were accomplished by treatment with a mixture of cold TFA (10 mL/g of resin) for 1 h, allowing the slurry to achieve room temperature. Compounds 8 and 17–24 were treated with $\text{TFA}-\text{H}_2\text{O}$ (95:5, v/v) for 1 h; compounds 5–7 and 9 were treated with $\text{TFA}-\text{phenol}-\text{H}_2\text{O}-i\text{Pr}_3\text{SiH}$ (88:5:5:2, v/v) for 2 h; and compounds 10–15 were treated with $\text{TFA}-\text{DCM}-\text{anisole}$ (49:49:2, v/v) for 1 h. The crude peptoids were concentrated in vacuo, washed with ether, dissolved in water, and then lyophilized to yield white powders.

Characterization of Peptoids. [L-Arginyl]-[N-[2-(indol-3-yl)ethyl]glycyl]-N-[(2-(indol-3-yl)ethyl)] Glycinamide (5). Yield: 4 mg, 5%. HRMS: m/z found ($M + H$)⁺ 574.3248, calcd ($M + H$)⁺ 574.3247. ¹H NMR ($\text{DMSO}-d_6$): δ 1.45 (m, 2H), 1.59 (m, 2H), 2.89 (m, 2H), 3.03 (m, 4H), 3.47 (m, 2H), 3.6 (m, 2H), 4.05 (m, 4H), 4.12 (m, 1H), 6.98 (m, 2H), 7.04 (m, 2H), 7.13 (m, 1H), 7.17 (m, 1H), 7.23 (m, 1H), 7.32 (m, 2H), 7.53 (m, 1H), 7.60 (m, 1H), 8.15 (m, 3H), 10.81–10.93 (m, 2H). HPLC [gradient 0% to 100% MeCN (+0.036% TFA) in H_2O (+0.045% TFA) in 15 min]: t_R = 6.9 min.

[L-Arginyl]-[N-[2-(indol-3-yl)ethyl]glycyl]-N-(2,4-dichlorophenethyl) Glycinamide (6). Yield: 4 mg, 5%. HRMS: m/z found ($M + H$)⁺ 603.2355, calcd ($M + H$)⁺ 603.2382. ¹H NMR ($\text{DMSO}-d_6$): δ 1.31 (m, 2H), 1.43 (m, 2H), 2.86 (m, 2H), 2.9 (m, 2H), 3.11 (m, 2H), 3.48 (m, 2H), 3.56 (m, 2H), 4.07 (m, 4H), 4.21 (m, 1H), 7.14 (m, 4H), 7.3 (m, 2H), 7.36 (m, 2H), 7.52 (m, 2H), 8.08 (m, 2H), 8.18 (m, 3H), 10.82 (br s, 1H). HPLC [gradient 20% to 100% MeCN (+0.036% TFA) in H_2O (+0.045% TFA) in 15 min]: t_R = 7.4 min.

[L-Arginyl]-[N-(2,4-dichlorophenethyl)glycyl]-N-[2-(indol-3-yl)ethyl]glycinamide (7). Yield: 5 mg, 6%. HRMS: m/z found ($M + H$)⁺ 603.2344, calcd ($M + H$)⁺ 603.2397. ¹H NMR ($\text{DMSO}-d_6$): δ 1.3 (m, 2H), 1.45 (m, 2H), 2.89 (m, 2H), 2.91 (m, 2H), 3.1 (m, 2H), 3.5 (m, 2H), 3.59 (m, 2H), 4.08 (m, 4H), 4.21 (m, 1H), 7.14 (m, 4H), 7.3 (m, 4H), 7.32 (m, 2H), 7.53 (m, 2H), 8.16 (m, 3H), 10.85 (br s, 1H). HPLC [gradient 20% to 100% MeCN (+0.036% TFA) in H_2O (+0.045% TFA) in 15 min]: t_R = 5.0 min.

[[N-(2,4-Dichlorophenethyl)-N-(6-aminohexyl)glycyl]-N-(2,4-dichlorophenethyl) Glycinamide (8). Yield: 24 mg, 19%. HRMS: m/z found ($M + H$)⁺ 577.1485, calcd ($M + H$)⁺ 577.1488. ¹H NMR ($\text{DMSO}-d_6$): δ 1.21–1.33 (m, 4H), 1.5–1.6 (m, 4H), 2.75 (m, 2H), 2.87 (m, 2H), 3.0 (m, 4H), 3.2 (m, 2H), 3.49 (m, 2H), 4.0 (m, 2H), 7.13 (br s, 1H), 7.27 (dd, J = 2.12, 8.24 Hz, 1H), 7.36 (m, 1H), 7.43 (m, 2H), 7.5 (m, 1H), 7.62 (m, 1H). HPLC [gradient 5% to 70% MeCN (+0.036% TFA) in H_2O (+0.045% TFA) in 15 min]: t_R = 10.0 min.

[L-Arginyl]-N-(2,4-dichlorophenethyl)glycine 2,4-(Dichlorophenethylamide) (9). Yield: 5 mg, 5%. HRMS: m/z found ($M + H$)⁺ 577.1230, calcd ($M + H$)⁺ 577.1236. ¹H NMR ($\text{DMSO}-d_6$): δ 1.49 (m, 2H), 1.66 (m, 2H), 2.86 (m, 6H), 3.39 (m, 2H), 3.51 (m, 2H), 3.91 (d, J = 9.2 Hz, 2H), 4.17 (m, 1H), 7.25 (d, J = 2.0 Hz, 1H), 7.32 (s, 2H), 7.37 (dd, J = 8.2, 2.0 Hz, 1H), 7.43 (dd, J = 8.2, 2.0 Hz, 1H), 7.54 (m, 1H), 7.57 (d, J = 2.0 Hz, 1H), 7.62 (d, J = 2.0 Hz, 1H), 8.15 (m, 5H). HPLC retention [gradient 5% to 100% MeCN (+0.036% TFA) in H_2O (+0.045% TFA) in 15 min]: t_R = 8.2 min.

[L-Arginyl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (10). Yield: 2 mg, 3%. HRMS: m/z found ($M + H$)⁺

517.3032, calcd (M + H)⁺ 517.3039. ¹H NMR (DMSO-*d*₆): δ 1.21 (m, 2H), 1.38 (m, 2H), 2.84 (m, 2H), 2.96 (m, 2H), 3.04 (m, 2H), 4.33 (m, 2H), 4.41 (m, 2H), 4.43 (m, 2H), 4.91 (m, 2H), 3.68 (br s, 1H), 4.48 (m, 3H), 4.91 (m, 1H), 6.94 (m, 5H), 7.06 (m, 2H), 7.09 (s, 1H), 7.51–7.58 (m, 2H), 9.21 (m, 2H), 10.82 (m, 2H). HPLC [gradient 25% to 55% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 15.5 min.

[L-Lysyl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (11). Yield: 5 mg, 6%. HRMS: *m/z* found (M + H)⁺ 489.2871, calcd (M + H)⁺ 489.2879. ¹H NMR (DMSO-*d*₆): δ 1.2 (m, 2H), 1.4 (m, 4H), 2.67 (m, 2H), 2.82 (m, 2H), 2.95 (m, 2H), 3.4 (m, 2H), 3.52 (m, 2H), 4.34 (m, 1H), 4.44 (m, 2H), 5.0 (br s, 1H), 6.96 (m, 2H), 7.08 (m, 4H), 7.18 (br s, 1H), 7.23 (m, 1H), 7.32 (d, *J* = 8.13 Hz, 1H), 7.5 (d, *J* = 8.26 Hz, 1H), 7.56 (m, 1H), 10.84 (m, 2H). HPLC [gradient 25% to 55% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 14.7 min.

[L-Ornityl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (12). Yield: 5 mg, 7%. HRMS: *m/z* found (M + H)⁺ 475.2814, calcd (M + H)⁺ 475.2821. ¹H NMR (DMSO-*d*₆): δ 1.58 (m, 2H), 1.69 (m, 2H), 2.86 (m, 4H), 2.73 (m, 2H), 3.41 (m, 2H), 3.50 (m, 2H), 3.9 (s, 2H), 4.12 (m, 1H), 6.97 (m, 2H), 7.05 (m, 2H), 7.12 (m, 2H), 7.22 (br s, 1H), 7.32 (m, 2H), 7.5 (d, *J* = 7.81 Hz, 1H), 7.57 (dd, *J* = 7.81, 19.9 Hz, 1H), 7.72 (br s, 2H), 8.23 (br s, 2H), 10.87 (m, 2H). HPLC [gradient 5% to 70% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 8.2 min.

[L-Diaminobutiryl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (13). Yield: 10 mg, 13%. HRMS: *m/z* found (M + H)⁺ 461.2661, calcd (M + H)⁺ 461.2665. ¹H NMR (DMSO-*d*₆): δ 2.49 (m, 4H), 2.88 (m, 6H), 3.28 (m, 2H), 3.72 (m, 2H), 4.13 (br s, 1H), 4.24 (m, 2H), 6.96 (m, 2H), 7.05 (m, 2H), 7.13 (m, 2H), 7.22 (br s, 1H), 7.33 (m, 2H), 7.49 (m, 1H), 7.57 (m, 1H), 8.12 (br s, 1H), 8.33 (br s, 1H), 8.47 (br s, 1H), 10.88 (m, 2H). HPLC [gradient 25% to 55% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 14.6 min.

[L-Diaminopropionyl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (14). Yield: 8 mg, 11%. HRMS: *m/z* found (M + H)⁺ 447.2505, calcd (M + H)⁺ 447.2508. ¹H NMR (DMSO-*d*₆): δ 0.83 (m, 2H), 2.97 (m, 2H), 3.06 (m, 2H), 3.14 (m, 2H), 3.46 (m, 2H), 3.95 (m, 2H), 4.24 (m, 2H), 6.96 (m, 2H), 7.05 (m, 2H), 7.13 (m, 1H), 7.2 (m, 1H), 7.32 (m, 2H), 7.53 (m, 2H), 8.04 (br s, 1H), 8.22 (br s, 1H), 8.33 (br s, 1H), 10.88 (m, 2H). HPLC [gradient 25% to 55% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 15.2 min.

[L-Histidyl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (15). Yield: 1 mg, 2%. HRMS: *m/z* found (M + H)⁺ 498.2610, calcd (M + H)⁺ 498.2617. ¹H NMR (DMSO-*d*₆): δ 2.85 (m, 2H), 2.99 (m, 2H), 3.1 (m, 1H), 3.22 (m, 1H), 3.41 (m, 2H), 3.49 (m, 2H), 3.85 (m, 2H), 4.2 (m, 1H), 6.96 (m, 2H), 7.05 (m, 2H), 7.11 (m, 1H), 7.14 (m, 1H), 7.2 (m, 1H), 7.32 (m, 2H), 7.37 (m, 1H), 7.49 (m, 2H), 8.4 (m, 1H), 8.59 (m, 1H), 8.91 (m, 1H), 10.88 (m, 2H). HPLC [gradient 5% to 70% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 8.3 min.

***cis*-(L-Arginyl)amino-N-(2,4-dichlorophenylmethyl)-L-proline 2,4-Dichlorophenethylamide (16).** Yield: 10 mg, 4%. HRMS: *m/z* found (M + H)⁺ 618.2335, calcd (M + H)⁺ 618.2339. ¹H NMR (DMSO-*d*₆): δ 1.21 (m, 2H), 1.42 (m, 2H), 1.63 (m, 2H), 2.75 (m, 4H), 3.07 (m, 2H), 3.3 (m, 2H), 3.72 (m, 3H), 4.2 (m, 2H), 7.22 (m, 2H), 7.37 (m, 1H), 7.47 (br s, 1H), 7.49 (m, 1H), 7.59 (m, 1H), 7.63 (m, 1H), 8.0 (br s, 1H), 8.12 (m, 2H), 8.64 (m, 1H). HPLC [gradient 20% to 100% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 5.4 min.

[N^ε-Trimethyl-L-lysyl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (17). Yield: 7 mg, 5%. HRMS: *m/z* found (M + H)⁺ 531.3443, calcd (M + H)⁺ 531.3448. ¹H NMR (DMSO-*d*₆): δ 1.21 (s, 9H), 1.28 (m, 2H), 1.47 (m, 2H), 1.63 (m, 2H), 2.82 (m, 2H), 2.86 (m, 2H), 3.15 (m, 2H), 3.43 (m, 2H), 3.53 (m, 2H), 4.19 (s, 1H), 4.23 (m, 2H), 6.9 (m, 2H), 7.06 (m, 2H), 7.14 (m, 2H), 7.32 (m, 2H), 7.54 (m, 2H), 8.16 (m, 3H), 10.86 (m, 2H). HPLC [gradient 0% to 90% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 7.7 min.

[N^ε-Triethyl-L-lysyl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (18). Yield: 12 mg, 11%. HRMS: *m/z* found (M + H)⁺ 573.3904, calcd (M + H)⁺ 573.3917. ¹H NMR (DMSO-*d*₆): δ 1.16 (m, 9H), 1.31 (m, 2H), 1.49 (m, 2H), 1.69 (m, 2H), 2.8 (m, 2H), 2.9 (m, 4H), 3.1 (m, 6H), 3.33 (m, 2H), 3.52 (m, 2H), 4.1 (m, 1H), 4.2 (m, 2H), 6.9 (m, 2H), 7.0 (m, 2H), 7.1 (m, 1H), 7.2 (m, 1H), 7.3 (m, 2H), 7.51 (dd, *J* = 3.65, 7.75 Hz, 1H), 7.58 (dd, *J* = 7.87, 14.15 Hz, 1H), 8.2 (br s, 1H), 10.85 (m, 2H). HPLC [gradient 10% to 80% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 7.1 min.

[N^ε-Methyl-L-lysyl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (19). Yield: 15 mg, 16%. HRMS: *m/z* found (M + H)⁺ 503.3130, calcd (M + H)⁺ 503.3134. ¹H NMR (DMSO-*d*₆): δ 1.21 (s, 3H), 1.28 (m, 2H), 1.47 (m, 2H), 1.63 (m, 2H), 2.8 (m, 6H), 3.39 (m, 2H), 3.59 (m, 2H), 4.09 (m, 2H), 4.16 (m, 1H), 6.96 (m, 2H), 7.05 (m, 2H), 7.14 (m, 2H), 7.33 (m, 2H), 7.5 (d, *J* = 7.93 Hz, 1H), 7.59 (dd, *J* = 7.87, 17.23 Hz, 1H), 8.17 (br s, 2H), 8.44 (br s, 2H), 10.88 (m, 2H). HPLC [gradient 10% to 80% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 6.8 min.

[N^ε-Ethyl-L-lysyl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (20). Yield: 13 mg, 15%. HRMS: *m/z* found (M + H)⁺ 517.3293, calcd (M + H)⁺ 517.3291. ¹H NMR (DMSO-*d*₆): δ 1.13 (m, 3H), 1.31 (m, 2H), 1.47 (m, 2H), 1.64 (m, 2H), 2.79 (m, 2H), 2.87 (m, 4H), 2.98 (m, 2H), 3.4 (m, 2H), 3.54 (m, 2H), 4.09 (m, 1H), 4.11 (br s, 2H), 6.9 (m, 2H), 7.05 (m, 2H), 7.1 (d, *J* = 2.1 Hz, 1H), 7.14 (br s, 1H), 7.32 (d, *J* = 8.09 Hz, 2H), 7.5 (d, *J* = 7.87 Hz, 1H), 7.57 (dd, *J* = 7.87, 17.41 Hz, 1H), 8.18 (m, 2H), 8.49 (m, 2H), 10.85 (m, 2H). HPLC [gradient 10% to 80% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 8.8 min.

[N^ε-Dimethyl-L-lysyl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (21). Yield: 9 mg, 10%. HRMS: *m/z* found (M + H)⁺ 517.3287, calcd (M + H)⁺ 517.3243. ¹H NMR (DMSO-*d*₆): δ 1.21 (s, 6H), 1.26 (m, 2H), 1.5 (m, 2H), 1.62 (m, 2H), 2.82 (m, 2H), 2.86 (m, 2H), 2.98 (m, 2H), 3.4 (m, 2H), 3.59 (m, 2H), 4.04 (m, 1H), 4.11 (s, 2H), 6.97 (m, 2H), 7.05 (m, 2H), 7.14 (m, 2H), 7.34 (m, 2H), 7.5 (d, *J* = 7.76 Hz, 1H), 7.57 (dd, *J* = 8.17, 16.64 Hz, 1H), 8.14 (m, 3H), 10.87 (m, 2H). HPLC [gradient 10% to 80% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 8.7 min.

[N^ε-Diethyl-L-lysyl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (22). Yield: 2 mg, 6%. HRMS: *m/z* found (M + H)⁺ 545.3598, calcd (M + H)⁺ 545.3604. ¹H NMR (DMSO-*d*₆): δ 1.14 (m, 6H), 1.31 (m, 2H), 1.50 (m, 2H), 1.65 (m, 2H), 2.81 (m, 2H), 2.87 (m, 4H), 3.0 (m, 4H), 3.4 (m, 2H), 3.5 (m, 2H), 4.1 (m, 1H), 4.2 (m, 2H), 6.9 (m, 2H), 7.0 (m, 2H), 7.11 (d, *J* = 12.1 Hz, 1H), 7.14 (m, 1H), 7.34 (m, 2H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.55 (dd, *J* = 8.2, 16.78 Hz, 1H), 8.15 (br s, 3H), 10.81 (m, 2H). HPLC [gradient 5% to 95% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 7.0 min.

[N^G-Nitro-L-arginyl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (23). Yield: 7 mg, 7%. HRMS: *m/z* found (M + H)⁺ 562.2881, calcd (M + H)⁺ 562.2890. ¹H NMR (DMSO-*d*₆): δ 1.5 (m, 2H), 1.64 (m, 2H), 2.85 (m, 2H), 3.0 (m, 2H), 3.12 (m, 2H), 3.45 (m, 2H), 3.6 (m, 2H), 4.11 (br s, 2H), 4.18 (m, 1H), 6.97 (m, 2H), 7.04 (m, 2H), 7.12 (m, 2H), 7.23 (d, *J* = 8.1 Hz, 1H), 7.33 (m, 2H), 7.5 (d, *J* = 7.92 Hz, 1H), 7.55 (dd, *J* = 8.12, 15.06 Hz, 1H), 8.17 (m, 4H), 8.43 (m, 1H), 10.81–10.92 (m, 2H). HPLC [gradient 10% to 80% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 8.1 min.

[N^G-Tosyl-L-arginyl]-N-[2-(indol-3-yl)ethyl]glycine 2-(Indol-3-yl)ethylamide (24). Yield: 9 mg, 8%. HRMS: *m/z* found (M + H)⁺ 671.3120, calcd (M + H)⁺ 671.3128. ¹H NMR (DMSO-*d*₆): δ 1.21 (s, 3H), 1.38 (m, 2H), 1.61 (m, 2H), 2.27 (m, 3H), 2.85 (m, 2H), 2.97 (m, 2H), 3.35 (m, 2H), 3.6 (m, 4H), 4.0 (m, 2H), 4.19 (m, 1H), 6.59 (m, 1H), 6.78 (m, 1H), 6.96 (m, 2H), 7.07 (m, 2H), 7.24 (m, 3H), 7.33 (m, 2H), 7.49 (m, 1H), 7.61 (m, 3H), 8.2 (br s, 2H), 10.8–10.86 (m, 2H). HPLC [gradient 0% to 90% MeCN (+0.036% TFA) in H₂O (+0.045% TFA) in 15 min]: *t*_R = 9.7 min.

Biological Assays. Electrophysiological Recordings. *Xenopus* oocytes were defolliculated using calcium-free Barth's solution, collagenase (2 mg/mL), and slow agitation (50–60 rpm) for 1–2 h. The oocytes were then stored at 18 °C for 12 h before injection. In vitro transcribed RNA encoding the rat TRPV1 or rat NR1 and NR2A subunits of the NMDA receptor (injected at 1:1 w/w) was injected into *Xenopus* oocytes (5–10 ng/oocyte). Electrophysiological recordings were made 2–4 days after injection. Electrophysiological recordings were performed using an electrode voltage clamp amplifier. Throughout the experiments, the oocytes were continuously perfused with an external Ringer's solution containing (in mM) 115 NaCl, 3 KCl, 0.1 BaCl₂, 2 MgCl₂·6H₂O, and 10 HEPES-Na⁺, pH 7.4 for TRPV1 experiments and 115 NaCl, 3 KCl, 2 BaCl₂ and 10 HEPES-Na⁺, pH 7.4 for NMDA experiments. Electrodes were filled with 1 M KCl buffered with 10 mM TES and typically had resistance of 300–500 kΩ. The currents were sampled at 4–5 Hz after filtering at 1 kHz. Leak subtraction was accomplished with two inverted quarter-amplitude prepulses that were scaled and subtracted from the test pulse (*P*/4). TRPV1 channels were activated by application of 10 μM of capsaicin in the absence or presence of the inhibitors of the channel at a holding potential (*V*_h) of –40 mV. For the selectivity assays, NMDA channels were activated with a solution of 100 μM L-glutamate and 20 μM glycine. Whole currents were recorded at ca. 20 °C. Responses were normalized with respect to that evoked in the absence of peptoids. Dose–response curves for blockade activity were fitted to the Hill equation

$$\frac{I}{I_{\max}} = \frac{1}{\left(1 + \frac{[\text{peptoid}]^{n_H}}{IC_{50}}\right)}$$

where IC₅₀ denotes the concentration of channel peptoid that inhibits half of the channel response obtained in its absence (*I*_{max}) and *n*_H denotes the Hill coefficient. Experimental data were processed using ORIGIN version 7.0 SMO (OriginLab Corp.).

Acknowledgment. The authors thank Laura García de Diego, for her work on some of the electrophysiological experiments, and Reme Torres, for mRNA preparation and oocytes injection and maintenance. This work was partially supported by funds from The Ministry of Education and Science (SAF2006-02580 to A.F.-M.) and Fundación Ramón Areces (to A.F.-M.). M.Q.-A. thanks *DURSI*, the Generalitat of Catalunya, and European Social Funds for a predoctoral (FI-IQUC) fellowship.

Supporting Information Available: A table containing HPLC data of all purified peptoids and dose–response curves of selected peptoids assayed in TRPV1 and NMDA receptors. This material is available free of charge via the Internet at <http://www.pubs.acs.org>.

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JM070612V