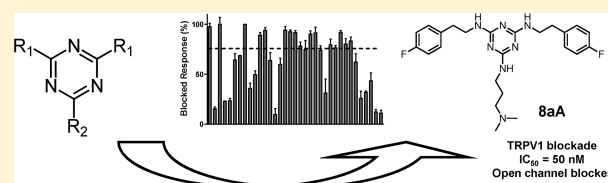


Triazine-Based Vanilloid 1 Receptor Open Channel Blockers:
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Supporting Information

ABSTRACT: The thermosensory transient receptor potential vanilloid 1 channel (TRPV1) is a polymodal receptor activated by physical and chemical stimuli. TRPV1 activity is drastically potentiated by proinflammatory agents released upon tissue damage. Given the pivotal role of TRPV1 in human pain, there is pressing need for improved TRPV1 antagonists, the development of which will require identification of new pharmacophore scaffolds. Uncompetitive antagonists acting as open-channel blockers might serve as activity-dependent blockers that preferentially modulate the activity of overactive channels, thus displaying fewer side effects than their competitive counterparts. Herein we report the design, synthesis, biological evaluation, and SAR analysis of a family of triazine-based compounds acting as TRPV1 uncompetitive antagonists. We identified the triazine **8aA** as a potent, pure antagonist that inhibits TRPV1 channel activity with nanomolar efficacy and strong voltage dependency. It represents a new class of activity-dependent TRPV1 antagonists and may serve as the basis for lead optimization in the development of new analgesics.



INTRODUCTION

Cloning of the vanilloid receptor subunit I channel (transient receptor potential vanilloid 1, TRPV1) has notably contributed to our current knowledge on the molecular and cellular mechanisms underlying chemical and thermal nociception and pain transduction.^{1,2} TRPV1 was the first identified member of a family of thermosensory receptors currently referred to as thermoTRPs, which encompass cold-, warm-, and heat-activated channels. TRPV1 is a capsaicin-operated nonselective cation channel with high Ca²⁺ permeability. It is also gated by noxious heat (≥ 42 °C), low pH, and different endogenous compounds (endocannabinoids, phorbols, etc.) and therefore can be considered as a molecular integrator of noxious stimuli in nociceptors. Genetic and pharmacological suppression of TRPV1 activity markedly reduces the thermal hyperalgesia characteristic of inflammatory pain.^{3–5} Notably, enhanced expression of this thermoTRP channel has been observed in various human chronic pathologies, including arthritis and cancer pain. TRPV1 is essential for peripheral sensitization of nociceptors upon tissue injury and/or inflammation produced by trauma, infection, surgery, burns, or diseases with an inflammatory component.^{6,7} Thus, TRPV1 has garnered great attention as a therapeutic target for pain management, including for neuropathic, postoperative, chronic, or bone cancer pain.^{8–10}

Validation of TRPV1 as a therapeutic target has prompted intensive drug discovery programs aimed at developing orally

active antagonists. Consequentially, numerous TRPV1 antagonists have been identified that block the receptor with high efficacy and potency (Figure 1). However, and rather disappointingly, despite the claimed therapeutic potential of these TRPV1 antagonists, very few candidates have progressed into clinical trials because of unpredicted side effects such as hyperthermia. Furthermore, complete blocking of TRPV1 in some models of chronic pain models apparently results in enhanced hypersensitivity.¹¹ These observations are consistent with its widespread distribution in neuronal and non-neuronal tissues, which suggests that it is involved in body functions other than nociception and pain (e.g., body temperature regulation).¹¹ Indiscriminate pharmacological blocking of the receptor with high affinity, quasi-irreversible, competitive vanilloid antagonists may be responsible for the observed side effects.¹¹ Thus, high affinity antagonists that bind to the receptor in an activity-independent manner should show limited therapeutic indices, since these compounds would interact with both resting and active channels. Taken together, these data support the need for a different class of antagonists that would either act on a specific mode of activation or be activity-dependent, primarily targeting over-activated receptors.

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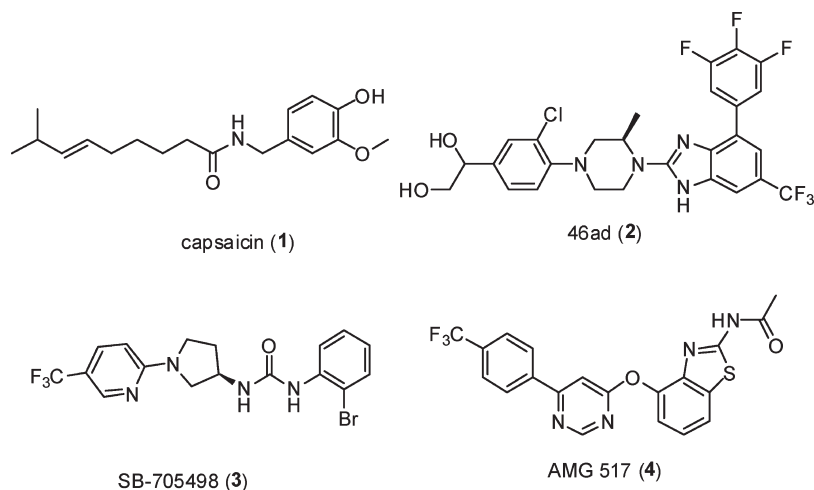


Figure 1. Relevant TRPV1 ligands: the agonist capsaicin (1) and competitive antagonists (2–4).

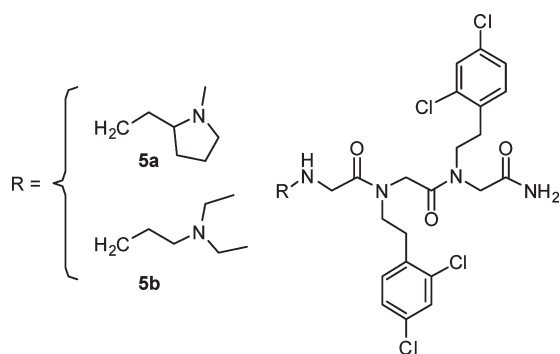


Figure 2. *N*-Alkylglycine trimers that block TRPV1.¹⁴

Uncompetitive antagonists are activity-dependent inhibitors that specifically bind to the agonist–receptor complex or to the open state of the channel.¹² Because of their interaction with active receptors, which enables them to preferentially block highly activated receptors while only interacting minimally with physiologically working or silent channels, these compounds have attracted sizable interest as potent and safe drugs. For example, memantine, an uncompetitive *L*-glutamate antagonist of the NMDA receptor, has been approved for the treatment of Alzheimer's disease.¹³ Accordingly, identification and validation of uncompetitive antagonists acting as open channel blockers of TRPV1 receptors warrant exploration as a strategy to develop selective analgesic drugs that would offer higher therapeutic indices than do currently known channel antagonists.

Open-channel blockers are antagonists that recognize a site within a channel's aqueous pore; therefore, they can only access the channel when it is open (i.e., in the conductive state). Furthermore, the accessible time is directly proportional to the time the channel remains in its open conformation. Therefore, the drug-binding site is more exposed in overactivated channels than in silent receptors or in channels that only briefly open upon receiving a nociceptive signal.

We have previously identified compounds with channel-blocking activity.^{14–16} These antagonists, particularly the *N*-alkylglycine trimers (peptoids) **5a** and **5b** (Figure 2), block TRPV1 with micromolar efficacy by interacting with the external vestibule of the ionic pore, at a site apparently located outside the membrane

electrical field at the pore entry.¹⁴ Although they are active *in vivo* in animal models of pain, their development was precluded because of unanticipated side effects arising when they were used at submicromolar concentration, namely, release of α -CGRP from nociceptive neurons. We have speculated that their binding site location was also accessible in the closed-channel state and that this could account for the observed side effects. Regardless, the chemical diversity surrounding the glycine oligomer scaffold in **5a** and **5b** constituted the basis of the present study.

The peptoids **5a** and **5b**, and other active TRPV1 blockers,^{4,15,16} share structural features that have been assumed to constitute a basic pharmacophore for TRPV1 blocking activity: two aryl moieties and one cationic group. Herein we report the design, synthesis, *in vitro* screening, and SAR analysis of a new family of TRPV1 antagonists built around a triazine scaffold based on said pharmacophore. Among these, triazine **8aA** blocked TRPV1 with submicromolar efficacy in a strongly voltage-dependent manner, consistently with the receptor binding-site being located deep within the membrane electric field.

RESULTS AND DISCUSSION

Design and Synthesis of New TRPV1 Blockers. To expand upon the available SAR data obtained from our previous hit compounds (the peptoids **5a** and **5b**), we designed compounds representing the chemical diversity shown in Figure 3. To modulate their TRPV1-blocking capacity, each compound was conferred with two identical arylalkyl substituents (except entry **j**, which can be compared as a cycloalkyl analogue) and one alkyl moiety (polar cationic, polar nonionizable, or apolar). The compounds were chiefly assembled from commercially available primary and secondary amines (see Experimental Section). Initially, four new peptoids were prepared (**5c–f**) using a previously reported synthetic methodology.¹⁷ However, preliminary evaluation revealed that they had significantly lower activity than did **5a** and **5b** (Table 1). This discrepancy was greatest for those compounds lacking the tertiary amine group, a trend that corroborates the proposed pharmacophore. Therefore, a second generation of compounds was developed, which contained a cationic guanidyl or trialkylamino group and in which the undesired conformational flexibility of the peptoid moiety was partially restricted by using piperazine-2,5-dione as

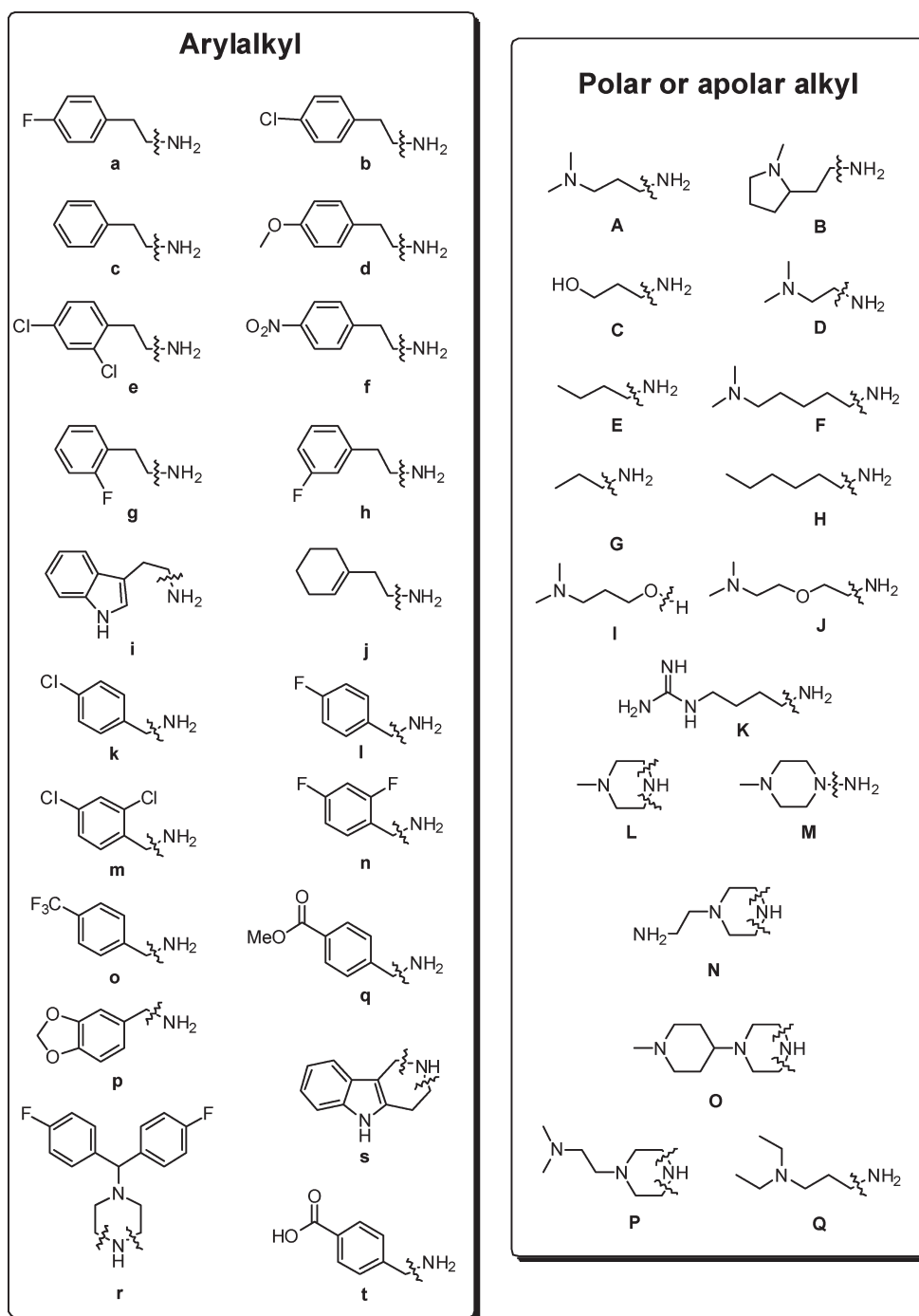


Figure 3. Formal sources of chemical diversity incorporated in the new series of TRPV1 blockers. The wavy lines indicate the bond(s) of attachment to the corresponding scaffold.

scaffold (**6a–f**, Table 1). This strategy has been successful to increase the selectivity against other pharmaceutical targets studied in our group,^{18,19} but in this case **6a–f** showed poor activity as TRPV1 antagonists (Table 1). These new heterocyclic compounds were synthesized using a methodology developed in our laboratory.¹⁸

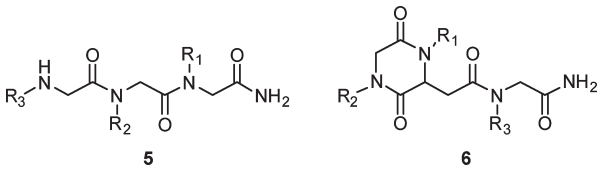
We reasoned that although satisfying the requirements of the pharmacophore, the suboptimal 3D arrangement of structural features imposed by scaffold **6** could be responsible for the modest or null activity observed. Furthermore, the structure of

scaffold **6** still holds a relatively high degree of flexibility and on the other hand it contains a chiral center and three nonequivalent positions for substitution. These features render an undesirable number of possibilities for different combinations of $R_1–R_3$. Therefore, we turned to a more rigid and symmetric scaffold: 2,4,6-trisubstituted-1,3,5-triazine (**8**, Scheme 1), which has been harnessed to generate compounds with antimicrobial,²⁰ anticancer,^{21,22} or HIV-1 reverse transcriptase inhibitory activities.²³

The initial results for some of the new compounds (e.g., **8eA**; see below) were promising, leading us to design and synthesize a

library of 35 triazines (Table 2, Figure S2). Preparation of triazine derivatives using the readily available 2,4,6-trichlorotriazine as starting material is well documented.^{24,25} We used the synthetic sequence shown in Scheme 1 and various optimized workup

Table 1. Structure of Peptoids 5a–f and Piperazine-2,5-diones 6a–f and Their Corresponding Activities against TRPV1 (Expressed as % Blocking at 10 μ M)^a



compd	R ₁	R ₂	R ₃	% block	compd	R ₁	R ₂	R ₃	% block
5a	e	e	B	99	6a	e	e	K	30
5b	e	e	Q	99	6b	e	e	B	40
5c	e	e	A	55	6c	e	Q	e	3
5d	b	b	A	45	6d	b	b	K	55
5e	e	e	E	34	6e	c	B	e	28
5f	e	e	C	32	6f	e	B	e	3

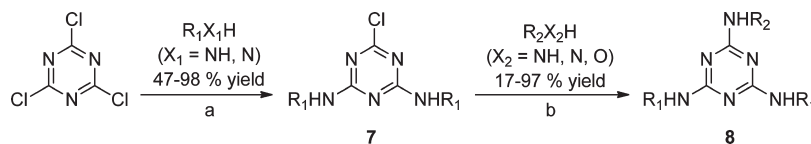
^aThe substituents R₁–R₃ refer to the groups shown in Figure 3.

protocols (see Experimental Section) to efficiently obtain the desired compounds.

Thus, for the first step the selected amine was used in excess (4 equiv) to facilitate substitution and to further elimination of the acid that is released via formation of the corresponding amine hydrochloride. In most cases, the desired disubstituted products 7 were separated from their corresponding monosubstituted derivatives by exploiting the different solubility of the two species in alcoholic solvents. Introduction of the third substituent required high temperature, for which microwave activation proved invaluable. The reagent amines, already converted into their corresponding hydrochlorides, were eliminated by simply washing the crude reaction mixture with water. The workup procedures developed for the library synthesis obviated use of chromatography for most of the products, enabling facile preparation of selected triazines (e.g., 8aA) on gram scale.

Biological Evaluation. The inhibitory activity of each triazine was evaluated by voltage-clamp against rat TRPV1 channels heterologously expressed in amphibian oocytes.²⁶ As illustrated in Figure 4, instillation of capsaicin onto oocytes expressing TRPV1 channels held at -60 mV generated a large inward current that was rapidly blocked in a dose dependent-manner by application of a single triazine compound. Note the different blocking efficacies of the three triazines displayed (8cA, 8bA, and 8aA), of which 8aA was the most potent (at 10 μ M it completely

Scheme 1. General Synthesis of the Trisubstituted Triazines 8^a



^aReaction conditions: (a) R₁ = arylalkyl (Figure 3), microwave irradiation at 70 °C (max potency 90 W), 10 min, THF; (b) R₂ = polar or apolar alkyl (Figure 3), microwave irradiation at 100 °C (max potency 110 W), 20 min, THF.

Table 2. Structures of Triazines 8 and Their Determined TRPV1 Blocking Activity^a

compd	X ₁ -R ₁	X ₂ -R ₂	IC ₅₀ (μ M) ^b	compd	X ₁ -R ₁	X ₂ -R ₂	IC ₅₀ (μ M) ^b
8aA	NH-a	NH-A	0.05 \pm 0.007	8eA	NH-e	NH-A	1.21 \pm 0.2
8aC	NH-a	NH-C	1488 \pm 774 ^c	8fA	NH-f	NH-A	1.63 \pm 0.37
8aF	NH-a	NH-F	0.1 \pm 0.04	8gA	NH-g	NH-A	0.45 \pm 0.07
8aG	NH-a	NH-G	2173 \pm 1745 ^c	8hA	NH-h	NH-A	0.30 \pm 0.04
8aH	NH-a	NH-H	5761 \pm 6716 ^c	8iA	NH-i	NH-A	2.33 \pm 1.23
8aI	NH-a	O-I	6.14 \pm 0.7	8jA	NH-j	NH-A	218 \pm 104
8aJ	NH-a	NH-J	7.05 \pm 2.99	8kA	NH-k	NH-A	2.62 \pm 0.94
8aK	NH-a	NH-K	0.55 \pm 0.06	8lA	NH-l	NH-A	3.06 \pm 0.22
8aO	NH-a	N-O	70.9 \pm 9.1	8mA	NH-m	NH-A	0.84 \pm 0.18
8aP	NH-a	N-P	18.6 \pm 6	8nA	NH-n	NH-A	3.30 \pm 0.61
8bA	NH-b	NH-A	0.50 \pm 0.15	8oA	NH-o	NH-A	0.66 \pm 0.11
8bB	NH-b	NH-B	1.2 \pm 0.03	8pA	NH-p	NH-A	4.98 \pm 0.67
8bD	NH-b	NH-D	6.7 \pm 2.4	8qA	NH-q	NH-A	69.1 \pm 29.6
8bL	NH-b	N-L	1.4 $\times 10^7$ \pm 5.8 $\times 10^4$ ^c	8rA	N-r	NH-A	56.4 \pm 14
8bM	NH-b	NH-M	6.4 \pm 2.4	8sA	N-s	NH-A	7.0 \pm 2.9
8bN	NH-b	N-N	0.96 \pm 0.21	8tA	NH-t	NH-A	178 \pm 47
8cA	NH-c	NH-A	0.67 \pm 0.06	8tE	NH-t	NH-E	229 \pm 23
8dA	NH-d	NH-A	0.97 \pm 0.19				

^aSubstitutions in X₁-R₁ and X₂-R₂ refer to the groups shown in Figure 3 (see also Figure S2). ^bValues derived from the corresponding dose response curves. Responses were recorded at -60 mV and normalized with respect to that elicited by 10 μ M capsaicin. Data are shown as the mean \pm SD, with $n \geq 6$. ^cExtrapolated IC₅₀ values.

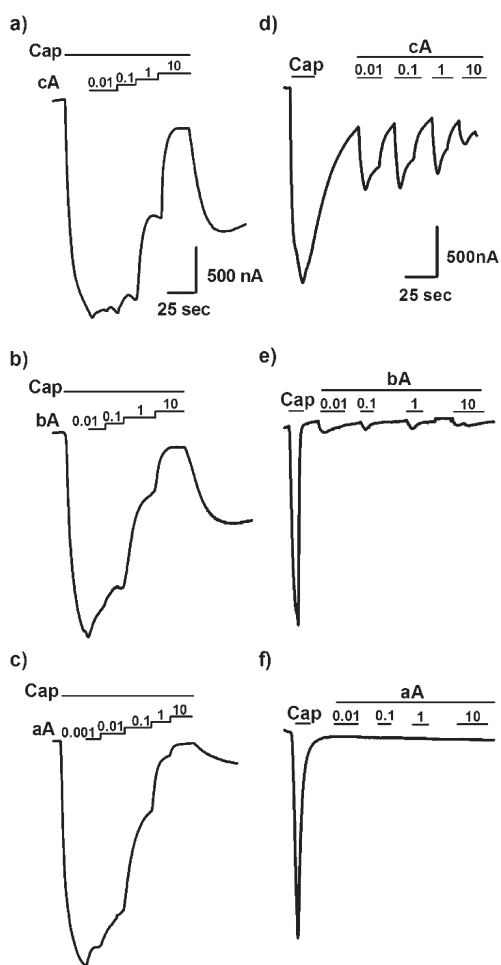


Figure 4. Representative ionic currents showing the blocking of capsaicin-evoked responses (a–c) and triazine-induced TRPV1 activity (d–f) of compounds **8cA** (a, d), **8bA** (b, e), and **8aA** (c, f) at the different concentrations (μM) indicated. Ionic currents were elicited from oocytes heterologously expressing TRPV1. The holding potential was -60 mV. The concentration of capsaicin (Cap) was 10 μM . Ionic currents were measured in Mg^{2+} Ringer buffer. The horizontal bars indicate the experimental paradigm used for agonist stimulation and channel blocking.

abrogated the capsaicin response). The blocking was reversible, as evidenced by the recovery of capsaicin currents after the triazines were washed away.

The blocking activity of each triazine in the library is summarized in Figure 5a, which shows that 15 compounds blocked more than 75% of the channel activity. Because the triazines **8** can be protonated at physiological pH (see Figure S1 for calculated pK_a) and known charged blockers have previously displayed agonistic activity, we also sought to determine whether these compounds actually activate the TRPV1 channel. We observed that some of the triazines exhibited significant agonist activities, whereas others, such as the triazine **8aA**, did not (Figure 5b). Complete screening of the library revealed that only a few of the triazines are “pure” blockers.

To further substantiate the TRPV1-blocking activity of the triazines **8**, we obtained the corresponding dose response curves. Figure 5c shows representative results for three triazines (**8aA**, **8bA**, and **8aP**), and Table 2 summarizes the IC_{50} values for each triazine from the library. The triazine **8aA** showed the highest potency, with

a noteworthy IC_{50} value of 50 ± 10 nM ($n_{\text{H}} = 0.9 \pm 0.1$), and most of the triazines exhibited inhibitory micromolar IC_{50} .

Blocking Mechanism of the Triazine 8aA. The above results pointed to the triazine **8aA** (Figure 6) as the most promising antagonist, as it exhibited high blocking potency without significant agonistic effects. Therefore, we next investigated the blocking mechanism of **8aA**.

Voltage dependency of inhibitory activity is a hallmark of open-channel blockers that sense the membrane electric field, exerting their activity within a defined range of voltages. The blocking efficacy of positively charged channel blockers is clearly stronger at negative membrane potentials than at positive ones ($V \geq 0$ mV). As depicted in Figure 6a, **8aA** blocks TRPV1 at negative membrane potentials, yet it is nearly inactive at depolarized membrane potentials, indicating that its activity is strongly voltage-dependent. Indeed, plotting the blocking ratio against the voltage provides a curve that could be approximated by a Woodhull model that yields an electric distance $\delta = 0.36$ for the binding site within the membrane electric field (Figure 6b). This value is consistent with the interaction site being located relatively deep within the aqueous pore of the channel and with an uncompetitive mechanism of channel blocking. That **8aA** acts as an open-channel blocker was further corroborated by the finding that the EC_{50} of capsaicin was not altered by the presence of **8aA**, indicating that this compound is not a competitive antagonist of capsaicin (data not shown).

Regarding channel selectivity, the IC_{50} values found for the triazine **8aA** when screened against two related ion channels (TRPM8 and NMDAR [NR1:NR2A]) were 7.5 ± 1.5 and 9.2 ± 2.1 μM , respectively (compared to 0.05 ± 0.007 μM , against TRPV1; see Table 2). The blocking percentages at 1 μM and 10 μM **8aA** were 37% and 55%, respectively, against TRPM8 and were 25% and 51%, respectively, against NMDAR. Together, these data indicate that **8aA** exhibits more than 150-fold blocking activity against TRPV1 over other ion channels that have similar permeability.

Structure–Activity Relationship (SAR) Analysis. We extracted qualitative SAR information by analyzing the aforementioned results. First, and in agreement with the postulated pharmacophore, replacing the tertiary amine with a polar non-ionizable group or an apolar alkyl moiety either in the peptoids (compare **5c** with **5e** or **5f**) or the triazines (compare **8aA** with **8aC**, **8aG**, or **8aH**) caused a loss in activity (up to 40000-fold). In contrast, changes in the length of the alkyl chain that supports the tertiary amine in the triazine series had a relatively minor effect (compare **8aA** with **8aF** or compare **8bA** with **8bD**). Similarly, changing the tertiary amine group (compare **8bA** with **8bB**) or replacing it with a guanidyl group (compare **8aF** with **8aK**) caused only a minor decrease in activity. Regarding the atom that links the dimethylaminopropyl moiety to the triazine, replacing nitrogen with oxygen (compare **8aA** with **8aI**) resulted in a substantial decrease in activity (~ 120 -fold), suggesting that the nitrogen atom is important for binding. Introducing an oxygen atom into the chain that supports the trimethylamino group (compare **8aF** with **8aJ**) caused a similar decrease in activity. Conformational restriction of the flexibility of the alkyl chain that contains the ionizable group, induced by introducing a cycloalkyl linker, also rendered a decrease in activity (compare **8aF** with **8aP** or **8bA** with **8bN**), which was greater (**8aO**) or much greater (**8bL**) for the most restricted analogues, thereby suggesting that steric interactions could be determinant for the activity.

Regarding the arylalkyl moieties, the >300 -fold decrease in activity of **8jA** relative to **8cA** indicated the importance of the

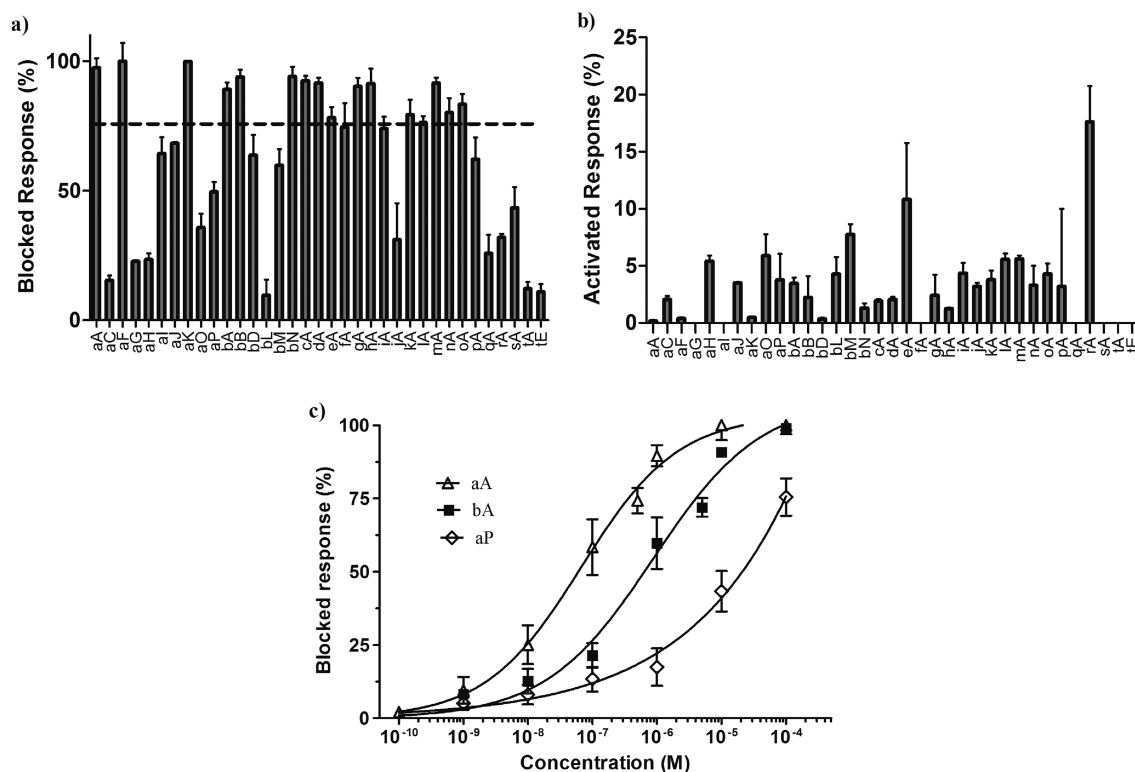


Figure 5. Extent of TRPV1 blocking and activation by the triazines **8**. (a) Blocked response of capsaicin-evoked current at 10 μ M triazine and (b) ionic currents activated at 10 μ M triazine in oocytes heterologously expressing TRPV1. (c) Representative dose–response curves displaying the blocking of capsaicin-evoked currents at different concentrations of the triazines **8aA**, **8bA**, and **8aP**. The solid lines depict the Michaelis–Menten binding isotherms that fit the blocking activity.

aromatic groups. Substituting the aromatic moiety and/or modifying the length of the alkyl linker seemed less important: compounds **8bA**, **8cA**, **8dA**, **8eA**, **8fA**, **8gA**, **8hA**, **8kA**, **8lA**, **8mA**, **8nA**, and **8oA** showed activities that are within 1 order of magnitude of each other. The exceptions to these trends were the compounds featuring a carboxylic acid (**8tA**) or a carboxylic ester (**8qA**), which showed a ≥ 100 -fold decrease in activity relative to their unsubstituted counterpart (**8cA**). The compounds containing either larger bicyclic aromatic systems (**8iA** or **8pA**) or rigid fused tricyclic systems (**8sA**), and even the bulkiest compound, the quite rigid bis-aromatic substituted triazine **8rA**, did not show such a large loss in activity, which suggests that these moieties might occupy sites on the TRPV1 receptor that are relatively open and sterically unrestricted. Regardless, defining the role of the aromatic substituents and of the triazine ring in binding is not trivial. This could be expected, since most of the compounds contain the triazine core and two aromatic rings, which, as previously stated, show little influence on activity when their substituents are modified. However, the low activity of **8jA**, the only compound lacking aromatic substituents, suggests that these aromatic groups must be involved in binding. These results also support the premise that high TRPV1-blocking activity requires a positively charged group.

CONCLUSION

A small library of 2,4,6-trisubstituted-1,3,5-triazines designed to satisfy the requirements of a simple pharmacophore model for TRPV1 blocking was synthesized and screened in vitro, and the

results were evaluated for SAR data. The triazines act as uncompetitive TRPV1 antagonists. Several compounds with activities in the submicromolar range were identified. The triazine **8aA** was the most potent compound, exhibiting an IC_{50} of 50 nM. Moreover, TRPV1 blocking by **8aA** is strongly voltage dependent, which is characteristic of open-channel blockers. Furthermore, the fast blocking kinetics observed suggest that **8aA** acts from the extracellular side of the channel.

The triazine **8aA** is among the strongest TRPV1 open channel blockers described to date.^{27,28} Unlike competitive antagonists, uncompetitive inhibitors only interact with the open channel by recognizing a binding site located deep within the pore pathway. This activity-dependent blocking enables preferential abrogation of pathologically sensitized channels and implies only marginal interaction with physiologically working receptors. Consequently, uncompetitive antagonists exhibit fewer side effects than their competitive counterparts.

Owing to its high efficacy and selectivity and to its blocking mechanism, the triazine **8aA** represents a novel class of synthetic TRPV1 modulators and may provide a basis on which to develop uncompetitive antagonists for other thermoTRPs (e.g., TRPA1 and TRPM8) implicated in human diseases.

EXPERIMENTAL SECTION

Chemistry. All solvents were obtained from VWR (Barcelona, Spain). HPLC analyses were run on a Hewlett-Packard series 1100 (UV detector 1315A) modular system using an Xterra MS RP18 (Waters) column (5 μ m, 4.6 mm \times 150 mm), with CH_3CN-H_2O mixtures containing 0.1% TFA at 1 mL/min as mobile phase and with

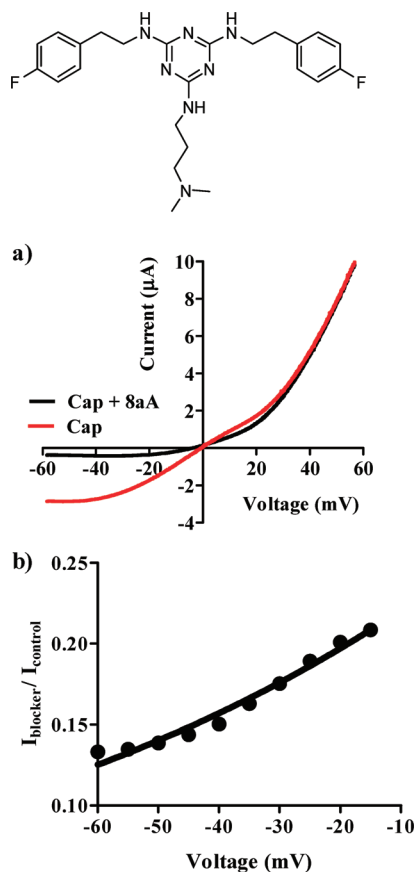


Figure 6. Voltage dependency of TRPV1 blocking by the triazine **8aA** (a). Representative ionic currents were evoked by 10 μ M capsaicin using a linear ramp from -60 to $+60$ mV in the absence (red line) or presence (black line) of **8aA** at 10 μ M. (b) Fractional blocking of TRPV1 by **8aA** as a function of the voltage. The solid line depicts the fitting to the Woodhull model, which for the **8aA** binding site yields an electric distance (δ) value of 0.36 within the membrane electric field.

monitoring at 220 nm. Compounds were purified from the crude reaction mixture by semipreparative HPLC using an X-terra RP18 (Waters) column (15–20 μ m, 47 mm \times 300 mm), $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ mixtures containing 0.1% TFA as mobile phases at a flow rate of 10 mL/min. Unless otherwise stated, NMR spectra were recorded in CDCl_3 on a Varian Inova 400 apparatus (^1H NMR, 400 MHz; ^{13}C NMR, 100 MHz). Chemical shifts are given in ppm (δ) relative to the CDCl_3 signal (7.24 ppm for ^1H NMR and 77.23 ppm for ^{13}C NMR), and coupling constants (J) are reported in hertz (Hz). To improve solubility, some spectra were recorded above ambient temperature (≤ 48 $^\circ\text{C}$) or in the presence of $\sim 7\%$ trifluoroacetic acid (TFA; broad signal at ~ 10.5 ppm in ^1H NMR and two quadruplets at 160.00 [$J = 39$] and 116.72 ppm [$J = 290$] in ^{13}C NMR). Some of the described compounds exhibit unexpectedly complex NMR spectra because of the presence of different conformers in equilibrium in solution. Thus, in some cases, the spectra of these compounds are given as absorption ranges rather than discrete signals and are denoted with the abbreviation “confs”. High resolution mass spectra (HRMS) were recorded at the Mass Spectrometry Service of IQAC-CSIC (Barcelona, Spain) with an Aquity UPLC (Waters) chromatograph coupled to a LCT Premier Xe TOF detector (Waters), using an Aquity UPLC BEH C18 (Waters) column (1.7 μ m, 2.1 mm \times 100 mm), with $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ mixtures containing 20 mM HCOOH as mobile phases. The purity of compounds **8** was checked by reverse phase HPLC and confirmed to be $\geq 95\%$.

Most compounds used as diversity sources (**9**, Figure 3) were commercially available from Sigma-Aldrich, except amine **9F**, which was from Matrix Scientific (Columbia, SC, U.S.). Amine **9J** was synthesized from 5-dimethylamino-3-oxapentanol in 25% overall yield using the Mitsunobu approach.²⁹ Amine **9K** was only a formal source of diversity, since technically, *N*-Fmoc-1,4-butanediamine was used to prepare the corresponding triazine or piperazinedione, which, after deprotection of the Fmoc group on the primary amino group, was guanidylated using the protocol described by Feichtinger et al.³⁰ (see Supporting Information).

***N*-Alkylglycine Trimers 5.** These compounds were synthesized following the general procedure reported by our laboratory for *N*-alkylglycine oligomers.³¹

[*N*-(3'-(*N,N*-Dimethylamino)propyl)glycyl]-[*N*-(2',4'-dichlorophenethyl)glycyl]-*N*-(2',4'-dichlorophenethyl)glycinamide (5c**).** Yield: 100 mg, 52%, white powder. ^1H NMR (CD_3CN , 40 $^\circ\text{C}$) (confs): 7.36–7.12 (m, 8H), 4.15–3.77 (m, 6H), 3.57–2.97 (m, 10H), 2.86–2.63 (m, 8H), 1.27–1.145 (m, 2H). ^{13}C NMR (CD_3CN , 40 $^\circ\text{C}$) (confs): 169.43–169.09, 167.49–167.16, 139.17–138.94, 138.53–138.22, 131.95–131.61, 129.75–129.19, 55.29, 50.81–49.82, 48.80–48.48, 45.77, 43.58, 34.50–33.51, 22.25–21.83. HRMS calcd for $\text{C}_{27}\text{H}_{35}\text{Cl}_4\text{N}_5\text{O}$ ($M + \text{H}$), 618.1589; found, 618.1572.

[*N*-(3'-(*N,N*-Dimethylamino)propyl)glycyl]-[*N*-(4'-chlorophenethyl)glycyl]-*N*-(4'-chlorophenethyl)glycinamide (5d**).** Yield: 82 mg, 45%, white powder. ^1H NMR (CD_3CN , 40 $^\circ\text{C}$) (confs): 7.36–7.16 (m, 8H), 4.12–3.68 (m, 6H), 3.57–3.29 (m, 4H), 3.18–2.99 (m, 6H), 2.87–2.64 (m, 8H), 1.27–1.15 (m, 2H). ^{13}C NMR (CD_3CN , 40 $^\circ\text{C}$) (confs): 169.39–169.23, 168.91–168.82, 167.44–167.01, 136.70–136.42, 135.78–135.45, 133.62–133.23, 130.17–129.81, 128.66–128.22, 55.30, 50.71–50.30, 49.07–48.02, 45.74–45.20, 43.61, 32.67–32.58, 32.26–32.07, 31.57–31.37, 22.59–21.76, 20.84–19.97. HRMS calcd for $\text{C}_{27}\text{H}_{37}\text{Cl}_2\text{N}_5\text{O}_3$ ($M + \text{H}$), 550.2374; found, 550.2352.

[*N*-Butylglycyl]-[*N*-(2',4'-dichlorophenethyl)glycyl]-*N*-(2',4'-dichlorophenethyl)glycinamide (5e**).** Yield: 72 mg, 37%. ^1H NMR (CD_3CN) (confs): 7.49–7.20 (m, 6H), 4.15–3.36 (m, 10H), 3.06–2.79 (m, 6H), 1.67–1.55 (m, 2H), 1.40–1.14 (m, 2H), 0.95–0.85 (s, 3H). ^{13}C NMR (CD_3CN) (confs): 173.02–172.35, 169.72–169.20, 168.15–167.80, 137.02–135.87, 134.35–133.68, 130.48–130.12, 128.95–128.54, 50.25–48.62, 33.04–31.70, 28.81, 20.72, 14.14. HRMS calcd for $\text{C}_{26}\text{H}_{32}\text{Cl}_4\text{N}_4\text{O}_3$ ($M + \text{H}$), 589.1309; found, 589.1311.

[*N*-(3'-Hydroxypropyl)glycyl]-[*N*-(2',4'-dichlorophenethyl)glycyl]-*N*-(2',4'-dichlorophenethyl)glycinamide (5f**).** Yield: 55 mg, 28%, white powder. ^1H NMR (CD_3CN) (confs): 7.50–7.20 (m, 6H), 4.14–3.82 (m, 4H), 3.67–3.56 (m, 3H), 3.13–2.83 (m, 4H), 1.87–1.83 (m, 8H), 1.28–1.24 (s, 2H). ^{13}C NMR (CD_3CN) (confs): 172.50–171.63, 169.69–169.18, 168.04–167.45, 137.16–135.95, 134.43–133.76, 130.56–130.26, 129.04–128.60, 61.54, 51.07–48.92, 33.08–32.91, 32.60–32.45, 31.95–31.79, 30.75–30.18, 29.10. HRMS calcd for $\text{C}_{25}\text{H}_{30}\text{Cl}_4\text{N}_4\text{O}_4$ ($M + \text{H}$), 591.1107; found, 591.1099.

Piperazine-2,5-diones 6. These compounds were synthesized following a procedure developed in our laboratory.¹⁸

3-[*N*-Aminocarbonylmethyl-*N*-(4'-guanidylbutyl)amino-carbonylmethyl]-1,4-bis(2',4'-dichlorophenethyl)piperazine-2,5-dione (6a**).** Yield: 10 mg, 6%. ^1H NMR ($\text{DMSO}-d_6$, 40 $^\circ\text{C}$) (confs): 7.48–7.45 (m, 2H), 7.39–7.27 (m, 4H), 4.30–4.25 (m, 2H), 4.20–3.90 (m, 4H), 3.85–3.80 (m, 2H), 3.74–3.44 (m, 3H), 3.30–2.95 (m, 10H), 1.62 (m, 2H), 1.54 (m, 2H). ^{13}C NMR ($\text{DMSO}-d_6$, 40 $^\circ\text{C}$) (confs): 173.6, 173.0, 172.1, 171.3, 168.4, 168.2, 167.0, 166.9, 156.6, 156.3, 136.5, 136.0, 134.4, 133.5, 133.4, 130.3, 128.7, 128.6, 58.8, 58.5, 51.6, 51.4, 51.2, 50.1, 49.5, 48.8, 48.1, 47.2, 45.2, 45.0, 42.3, 42.2, 35.0, 34.7, 31.3, 31.2, 30.9, 29.8, 27.1, 27.0, 26.8, 25.7. HRMS calcd for $\text{C}_{29}\text{H}_{36}\text{Cl}_4\text{N}_7\text{O}_3$ ($M + \text{H}$), 686.1583; found, 686.1592.

3-[*N*-Aminocarbonylmethyl-*N*-(2'-(1''-methyl-2''-pyrrolidinyl)ethyl)aminocarbonylmethyl]-1,4-bis(2',4'-dichlorophenethyl)piperazine-2,5-dione (6b**).** Yield: 27 mg, 24%. ^1H NMR

(DMSO- d_6 , 40 °C) (confs): 7.51–7.49 (m, 2H), 7.40–7.27 (m, 4H), 4.12–3.60 (m, 9H), 3.50–3.30 (m, 3H), 3.19–3.02 (m, 2H), 2.98–2.70 (m, 9H), 2.30–2.10 (m, 2H), 1.98–1.79 (m, 2H), 1.70–1.50 (m, 2H). ^{13}C NMR (DMSO- d_6 , 40 °C) (confs): 170.6–169.3, 166.2–166.0, 164.0–164.3, 135.6–135.5, 134.2, 132.6–132.4, 132.0, 128.7, 127.5, 66.7–65.5, 66.1–66.0, 56.9–55.4, 50.4–48.5, 46.0–45.2, 44.2–41.1, 40.0–38.0, 34.8–33.7, 29.8–28.3, 21.4–21.1. HRMS calcd for $\text{C}_{31}\text{H}_{39}\text{Cl}_4\text{N}_5\text{O}_4$ (M + H), 684.1678; found, 684.1689.

3-[*N*-Aminocarbonylmethyl-*N*-(2',4'-dichlorophenethyl)-aminocarbonylmethyl]-1-[3'-(*N,N*-diethylamino)propyl]-4-(2',4'-dichlorophenethyl)piperazine-2,5-dione (6c). Yield: 54 mg, 21%. ^1H NMR (DMSO- d_6 , 40 °C) (confs): 7.56–7.30 (m, 6H), 4.17–4.11 (m, 2H), 3.97–3.72 (m, 3H), 3.69–3.31 (m, 6H), 3.23–2.81 (m, 12H), 1.91 (m, 2H), 1.19 (t, $J = 7.1$ Hz, 6H). ^{13}C NMR (DMSO- d_6 , 40 °C) (confs): 170.8–169.9, 167.3–167.1, 164.6, 135.5, 135.4, 135.3, 134.8, 133.9, 133.8, 132.2–131.6, 128.6–128.3, 127.3–127.1, 57.0, 50.9, 50.1, 50.0, 49.5, 49.3, 48.7, 47.3, 47.0, 43.4, 43.0, 34.5, 33.9, 31.9, 30.9, 30.4, 21.6, 21.4, 9.3. HRMS calcd for $\text{C}_{31}\text{H}_{40}\text{Cl}_4\text{N}_5\text{O}_4$ (M + H), 686.1834; found, 686.1849.

3-[*N*-Aminocarbonylmethyl-*N*-(4'-guanidylbutyl)aminocarbonylmethyl]-1,4-bis(4'-chlorophenethyl)piperazine-2,5-dione (6d). Yield: 13 mg, 9%. ^1H NMR (DMSO- d_6 , 40 °C) (confs): 7.34–7.31 (m, 4H), 7.26–7.21 (m, 4H), 4.14–3.73 (m, 6H), 3.48 (m, 2H), 3.29 (m, 1H), 3.23 (m, 1H), 3.16–3.05 (m, 3H), 2.93–2.61 (m, 6H), 1.48 (m, 2H), 1.40 (m, 2H). ^{13}C NMR (DMSO- d_6 , 40 °C) (confs): 169.9, 169.8, 169.6, 168.9, 165.9, 165.8, 164.0, 163.9, 156.6, 156.5, 137.7, 130.7, 130.4–130.3, 130.7, 56.5, 56.2, 49.8, 49.7, 49.6, 48.1, 47.9, 46.4, 46.3, 46.1, 44.5, 44.4, 40.4, 40.3, 34.0, 33.8, 31.7, 31.6, 31.2, 28.7, 25.6, 25.5, 24.9, 23.9. HRMS calcd for $\text{C}_{29}\text{H}_{38}\text{Cl}_2\text{N}_7\text{O}_4$ (M + H), 618.2362; found, 618.2367.

3-[*N*-Aminocarbonylmethyl-*N*-(2',4'-dichlorophenethyl)-aminocarbonylmethyl]-1-[2'-(1''-methyl-2''-pyrrolidinyl)ethyl]-4-(phenethyl)piperazine-2,5-dione (6e). Yield: 32 mg, 16%. ^1H NMR (DMSO- d_6 , 40 °C) (confs): 7.56–7.37 (m, 2H), 7.30–7.28 (m, 3H), 7.24–7.20 (m, 3H), 4.20–4.03 (m, 2H), 3.99–3.74 (m, 5H), 3.67–3.27 (m, 4H), 3.35 (m, 2H), 3.27 (s, 3H), 3.15–2.79 (m, 6H), 2.72–2.63 (m, 1H), 2.37–2.20 (m, 2H), 2.03–1.81 (m, 2H), 1.67–1.51 (m, 2H). ^{13}C NMR (DMSO- d_6 , 40 °C) (confs): 173.0–169.3, 166.4–166.2, 163.7, 138.8, 138.7, 135.7, 135.0, 134.0, 132.8, 132.1, 131.7, 128.8, 128.6, 127.5–127.4, 126.3–126.2, 69.2, 66.2–62.6, 56.3–55.4, 50.5, 48.8, 48.1, 48.0, 46.5, 44.8–44.5, 41.7–41.3, 38.6–38.4, 33.7–32.4, 31.2–30.2, 29.0, 28.6–28.4, 24.4–22.0. HRMS calcd for $\text{C}_{31}\text{H}_{41}\text{Cl}_2\text{N}_5\text{O}_4$ (M + H), 616.2457; found, 616.2445.

3-[*N*-Aminocarbonylmethyl-*N*-(2',4'-dichlorophenethyl)-aminocarbonylmethyl]-1-[2'-(1''-methyl-2''-pyrrolidinyl)ethyl]-4-[2',4'-dichlorophenethyl]piperazine-2,5-dione (6f). Yield: 45 mg, 19%. ^1H NMR (DMSO- d_6 , 40 °C) (confs): 7.58–7.45 (m, 3H), 7.40–7.34 (m, 3H), 4.18–4.03 (m, 4H), 4.00–3.60 (m, 12 H), 3.52 (m, 1H), 3.19 (m, 1H), 3.07–2.93 (m, 7H), 2.82–2.63 (m, 6H), 2.35–2.25 (m, 2H), 1.95–1.86 (m, 2H), 1.68–1.51 (m, 2H). ^{13}C NMR (DMSO- d_6 , 40 °C) (confs): 170.1–169.0, 166.3–166.1, 163.9–163.8, 135.6–135.0, 134.0–133.9, 132.8–131.7, 128.6–128.5, 127.5–127.3, 66.0–65.8, 65.4, 56.2–55.1, 50.2–46.4, 42.8–41.3, 38.7–38.3, 33.8–33.0, 31.1–30.1, 29.6, 29.0, 27.2–26.5, 21.1–20.8. HRMS calcd for: $\text{C}_{31}\text{H}_{39}\text{Cl}_4\text{N}_5\text{O}_4$ (M + H), 684.1678; found, 684.1644.

Trisubstituted Triazines 8. General Procedure. These compounds were synthesized following the procedure shown in Scheme 1. Thus, a solution of 2,4,6-trichlorotriazine (0.5 mmol, 1 equiv) in THF (4 mL) was allowed to react with the corresponding amine (2 mmol, 4 equiv) under microwave activation for 10 min at 70 °C (90 W closed system). Then the crude reaction mixture was poured into H_2O (20 mL), heated for 10 min at 60 °C, and filtered. The precipitate was subjected to the same treatment and the insoluble material was washed with cold

absolute ethanol and dried to give the expected disubstituted triazine 7. For analytical data on compounds 7, see Supporting Information.

Then a suspension of the disubstituted triazine 7 (0.5 mmol, 1 equiv) in THF (5 mL) was allowed to react with the corresponding amine (2 mmol, 4 equiv) for 20 min at 100 °C under microwave activation (110 W, closed system). The crude reaction mixture was diluted in EtOAc (20 mL) and washed with H_2O (20 mL), brine (20 mL) and dried over MgSO_4 . Elimination of solvents yielded the expected trisubstituted triazine 8. If higher purity than that obtained by this procedure was required, the residue obtained after elimination of solvent was purified by semipreparative HPLC using mixtures of CH_3CN and H_2O containing 0.1% CF_3COOH . The collected fractions were evaporated under vacuum, redissolved in EtOAc, washed with saturated solution aqueous NaHCO_3 (20 mL) and brine (20 mL), and finally dried over MgSO_4 . Elimination of solvent afforded the pure desired trisubstituted triazine 8. Syntheses of triazines 8aF, 8aI, 8aK, 8tA, 8tE are described in detail in the Supporting Information.

2,4-Bis(4'-fluorophenethylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8aA). Yield: 2.28 g, 73%, colorless oil. ^1H NMR (CDCl_3 , 48 °C): 7.11 (dd, $J_1 = 8$, $J_2 = 5$, 4H), 7.00–6.83 (m, 4H), 3.54 (d, $J_1 = 6$, $J_2 = 13$, 4H), 3.43–3.32 (m, 2H), 2.79 (t, $J = 7$, 4H), 2.32 (t, $J = 7$, 2H), 2.19 (s, 6H), 1.79–1.60 (m, 2H). ^{13}C NMR (CDCl_3 , 48 °C): 166.39, 161.75 (d, $J = 244$), 135.30 (d, $J = 3$), 130.28 (d, $J = 8$), 115.37 (d, $J = 21.2$), 57.91, 45.60, 42.24, 39.64, 35.53, 27.71. HRMS calcd for $\text{C}_{24}\text{H}_{31}\text{N}_7\text{F}_2$ (M + H), 456.2687; found, 456.2668.

2,4-Bis(4'-fluorophenethylamino)-6-(3'-hydroxypropylamino)-1,3,5-triazine (8aC). Yield: 100 mg, 91%, colorless oil. ^1H NMR (CDCl_3 , 48 °C): 7.14 (dd, $J_1 = 8$, $J_2 = 6$, 4H), 6.96 (t, $J = 9$, 4H), 3.70–3.35 (m, 5H), 2.82 (t, $J = 7$, 4H), 1.68 (s, 2H). ^{13}C NMR (CDCl_3 , 48 °C): 166.67, 166.00, 161.87 (d, $J = 244.3$), 135.05 (d, $J = 3.0$), 130.33 (d, $J = 7.8$), 115.51 (d, $J = 21.2$), 58.63, 42.25, 36.87, 35.47, 33.34. HRMS calcd for $\text{C}_{22}\text{H}_{26}\text{N}_6\text{OF}_2$ (M + H), 429.2214; found, 429.2207.

2,4-Bis(4'-fluorophenethylamino)-6-(5'-(*N,N*-dimethylamino)pentylamino)-1,3,5-triazine (8aF). Yield: 48 mg, 23%, yellow oil. ^1H NMR (CDCl_3 , 48 °C): 7.15–7.12 (m, 4H), 6.97–6.96 (m, 4H), 3.60–3.52 (m, 4H), 3.37–3.32 (m, 2H), 2.85–2.79 (m, 4H), 2.28–2.25 (m, 2H), 2.21 (s, 6H), 1.62–1.45 (m, 4H), 1.41–1.32 (m, 2H). ^{13}C NMR (CDCl_3 , 48 °C): 161.84 (d, $J = 244$), 135.21 (d, $J = 2$), 130.35 (d, $J = 8$), 115.49 (d, $J = 21$), 59.82, 45.48, 42.26, 35.56, 30.01, 27.41, 24.96. HRMS calcd for $\text{C}_{26}\text{H}_{35}\text{N}_7\text{F}_2$ (M + H), 484.3000; found, 484.2992.

2,4-Bis(4'-fluorophenethylamino)-6-propylamino-1,3,5-triazine (8aG). Yield: 84 mg, 53%, colorless oil. ^1H NMR (CDCl_3 , 7% TFA): 7.15–6.97 (m, 8H), 3.51–3.36 (m, 4H), 3.48–3.40 (m, 2H), 2.91 (t, $J = 7$, 4H), 1.66 (m, 2H), 1.00–0.95 (m, 3H). ^{13}C NMR (CDCl_3 , 7% TFA): 162.21 (d, $J = 254$), 154.47, 152.45, 152.11, 133.08, 130.261, 115.85 (d, $J = 21$), 44.31, 43.55, 34.29, 22.14, 11.15. HRMS calcd for $\text{C}_{22}\text{H}_{26}\text{N}_6\text{F}_2$ (M + H), 413.2265; found, 413.2279.

2,4-Bis(4'-fluorophenethylamino)-6-hexylamino-1,3,5-triazine (8aH). Yield: 85 mg, 73%, colorless oil. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.15–6.97 (m, 8H), 3.76–3.62 (m, 4H), 3.47–3.39 (m, 2H), 2.91–2.88 (m, 4H), 1.64–1.58 (m, 2H), 1.33–1.28 (m, 6H), 0.92–0.84 (bb, 3H). ^{13}C NMR (CDCl_3 , 7% TFA) (confs): 161.97 (d, $J = 244$), 154.96, 153.96, 153.46, 153.070, 133.212, 130.07 (d, $J = 8$), 115.70 (d, $J = 21$), 42.97, 42.15, 34.34, 31.30, 28.51, 26.40, 22.45, 13.84. HRMS calcd for $\text{C}_{25}\text{H}_{32}\text{N}_6\text{F}_2$ (M + H), 455.2734; found, 455.2739.

2,4-Bis(4'-fluorophenethylamino)-6-(3'-(*N,N*-dimethylamino)propoxy)-1,3,5-triazine (8aI). Yield: 90 mg, 38%, colorless oil. ^1H NMR (CDCl_3 , 48 °C): 7.17–7.09 (m, 4H), 7.00–6.92 (m, 4H), 4.37–4.25 (bs, 2H), 3.67–3.55 (bs, 4H), 2.88–2.76 (bs, 4H), 2.61–2.52 (m, 2H), 2.34 s (6H), 2.25–1.92 (bb, 2H). ^{13}C NMR (CDCl_3 , 48 °C): 167.38, 161.87 (d, $J = 245$), 134.81, 130.34 (d, $J = 8$), 115.56 (d, $J = 21$), 60.52, 56.46, 45.11, 42.28, 35.33, 25.43. HRMS calcd for $\text{C}_{24}\text{H}_{30}\text{N}_6\text{F}_2\text{O}$ (M + H), 457.2527; found, 457.2515.

2,4-Bis(4'-fluorophenethylamino)-6-(2'-(2''-(*N,N*-dimethylamino)ethoxy)ethylamino)-1,3,5-triazine (8aJ). Yield: 50 mg, 23%, yellow oil. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.15–7.09 (m, 4H), 7.00–6.94 (m, 4H), 3.82–3.61 (m, 10H), 3.36 (m, 2H), 3.00–2.82 (m, 10H). ^{13}C NMR (CDCl_3 , 7% TFA) (confs): 161.95 (d, $J = 243$), 155.18, 153.89, 133.32, 130.08 (d, $J = 8$), 115.65 (d, $J = 21$), 68.82, 63.80, 58.11, 43.88, 42.99, 40.99, 34.33. HRMS calcd for $\text{C}_{25}\text{H}_{33}\text{N}_7\text{F}_2\text{O}$ (M + H), 486.2793; found, 486.2796.

2,4-Bis(4'-fluorophenethylamino)-6-(4'-guanidylbutylamino)-1,3,5-triazine (8aK). Yield: 60 mg, 23%, colorless oil. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.17–7.09 (m, 4H), 7.03–6.95 (m, 4H), 3.74–3.60 (m, 4H), 3.54–3.15 (m, 4H), 2.94–2.83 (m, 4H), 1.76–1.59 (m, 4H). ^{13}C NMR (CD_3CN , 7% TFA) (confs): 162.84 (d, $J = 240$), 158.30, 156.54, 136.23, 131.75 (d, $J = 8$), 116.20 (d, $J = 21$), 43.30, 42.47, 41.27, 35.376, 30.16, 26.83, 26.79. HRMS calcd for $\text{C}_{24}\text{H}_{31}\text{N}_9\text{F}_2$ (M + H), 484.2749; found, 484.2729.

2,4-Bis(4'-fluorophenethylamino)-6-(4'-(1''-methylpiperidin-4''-yl)piperazin-1'-yl)-1,3,5-triazine (8aO). Yield: 250 mg, 95%, yellow oil. ^1H NMR (CDCl_3 , 48 °C): 7.17–7.14 (m, 4H), 6.99–6.96 (m, 4H), 3.82–3.77 (m, 4H), 3.62–3.56 (m, 4H), 3.36–3.29 (m, 2H), 2.85 (t, $J = 14$, 4H), 2.65–2.45 (m, 10 H), 2.06–1.94 (m, 4H). ^{13}C NMR (CDCl_3 , 48 °C): 161.87 (d, $J = 244$ Hz), 134.70, 130.32 (d, $J = 8$ Hz), 115.52 (d, $J = 21$ Hz), 59.25, 54.06, 49.12, 44.35, 43.86, 42.33, 35.16, 26.22. HRMS calcd for $\text{C}_{29}\text{H}_{38}\text{N}_8\text{F}_2$ (M + H), 537.3265; found, 537.3209.

2,4-Bis(4'-fluorophenethylamino)-6-(4'-(2''-(*N,N*-dimethylamino)ethyl)piperazin-1'-yl)-1,3,5-triazine (8aP). Yield: 192 mg, 73%, yellow oil. ^1H NMR (CDCl_3 , 48 °C): 7.15–7.12 (m, 4H), 6.97–6.94 (m, 4H), 3.79–3.72 (bs, 4H), 3.60–3.53 (m, 4H), 2.85–2.79 (m, 4H), 2.53–2.43 (m, 8H), 2.27 (s, 6H). ^{13}C NMR (CDCl_3 , 48 °C): 166.15, 164.99, 161.62 (d, $J = 244$), 135.12 (d, $J = 3$), 130.25 (d, $J = 8$), 115.38 (d, $J = 21$), 56.88, 56.83, 53.67, 45.98, 42.96, 42.20, 35.38. HRMS calcd for $\text{C}_{27}\text{H}_{36}\text{N}_8\text{F}_2$ (M + H), 511.3109; found, 511.3092.

2,4-Bis(4'-chlorophenethylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8bA). Yield: 64 mg, 71%, white powder. ^1H NMR (CD_3CN , 7% TFA) (confs): 7.33–7.20 (m, 8H), 3.65–3.52 (m, 4H), 3.48–3.38 (m, 2H), 3.12–3.04 (m, 2H), 2.89–2.83 (m, 4H), 2.79–2.75 (m, 6H), 1.98–1.90 (m, 2H). ^{13}C NMR (CDCl_3 , 7% TFA) (confs): 163.32, 155.57–154.78, 136.50, 132.71, 129.97, 128.852, 55.93, 43.57, 42.34, 37.39, 34.63, 24.33. HRMS calcd for $\text{C}_{24}\text{H}_{31}\text{N}_7\text{Cl}_2$ (M + H), 488.2096; found, 488.2103.

2,4-Bis(4'-chlorophenethylamino)-6-(2'-(1''-methylpyrrolidin-2''-yl)ethylamino)-1,3,5-triazine (8bB). Yield: 70 mg, 60%, colorless oil. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.30–7.10 (m, 8H), 3.91–3.08 (m, 8H), 2.97–2.70 (8H), 2.36–1.76 (m, 6H). ^{13}C NMR (CDCl_3 , 7% TFA) (confs): 162.35, 155.43, 154.80, 136.30, 132.86, 129.94, 128.93, 68.23, 56.78, 42.50, 40.66, 38.00, 34.59, 29.60, 29.32, 21.55. HRMS calcd for $\text{C}_{26}\text{H}_{33}\text{N}_7\text{Cl}_2$ (M + H), 514.2253; found, 514.2230.

2,4-Bis(4'-chlorophenethylamino)-6-(2'-(*N,N*-dimethylamino)ethylamino)-1,3,5-triazine (8bD). Yield: 102 mg, 71%, white powder. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.30–7.21 (m, 4H), 7.14–7.04 (m, 4H), 3.80–3.93 (m, 2H), 3.67–3.54 (m, 4H), 3.45–3.34 (m, 2H), 3.05–2.94 (m, 6H), 2.88–2.79 (m, 4H). ^{13}C NMR (CDCl_3 , 7% TFA): 154.86, 153.64, 135.96, 132.80, 129.89, 128.90, 57.02, 44.15, 42.59, 35.88, 34.42. HRMS calcd for $\text{C}_{23}\text{H}_{29}\text{N}_7\text{Cl}_2$ (M + H), 474.1940; found, 474.1946.

2,4-Bis(4'-chlorophenethylamino)-6-(4'-methylpiperazin-1'-yl)-1,3,5-triazine (8bL). Yield: 52 mg, 36%, white powder. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.26–7.24 (m, 4H), 7.10–7.08 (m, 4H), 4.74 (d, $J = 15$, 2H), 3.82 (d, $J = 6$, 2H), 3.64 (q, $J = 6$, 4H), 3.37 (t, $J = 13$, 2H), 3.02 (d, $J = 3.5$, 3H), 2.88–2.76 (d, $J = 3.5$, 6H). ^{13}C NMR

(CDCl_3 , 7% TFA) (confs): 162.01, 155.28, 136.56, 132.70, 130.10, 128.84, 53.99, 44.08, 42.51, 40.73, 34.94. HRMS calcd for $\text{C}_{24}\text{H}_{29}\text{N}_7\text{Cl}_2$ (M + H), 486.1940; found, 486.1986.

2,4-Bis(4'-chlorophenethylamino)-6-(4'-methylpiperazin-1'-ylamino)-1,3,5-triazine (8bM). Yield: 81 mg, 55%, white powder. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.29–7.04 (m, 8H), 3.77–3.43 (m, 10H), 3.16–3.01 (m, 2H), 2.93–2.82 (m, 7H). ^{13}C NMR (CDCl_3 , 7% TFA) (confs): 157.54, 154.29, 135.50, 133.43, 129.87, 129.20, 53.50, 51.60, 43.61, 43.24, 34.26. HRMS calcd $\text{C}_{24}\text{H}_{30}\text{N}_8\text{Cl}_2$ (M + H), 501.2049; found, 501.2058.

2,4-Bis(4'-chlorophenethylamino)-6-(4'-(2''-aminoethyl)piperazin-1'-yl)-1,3,5-triazine (8bN). Yield: 116 mg, 64%, white powder. ^1H NMR (CDCl_3 , 48 °C): 7.52–7.50 (m, 4H), 7.38–7.24 (m, 4H), 5.18–5.04 (bs, 2H), 4.10–3.95 (bsb, 4H), 3.91–3.74 (m, 4H), 3.19–3.04 (m, 6H), 2.80–2–66 (m, 6H). ^{13}C NMR (CDCl_3 , 48 °C): 166.43, 165.27, 138.10, 132.30, 130.24, 128.77, 77.48, 77.16, 76.84, 53.37, 43.28, 42.05, 38.87, 35.71, 30.54. HRMS calcd for $\text{C}_{25}\text{H}_{32}\text{N}_8\text{Cl}_2$ (M + H), 515.2205; found, 515.2204.

2,4-Bis(phenethylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8cA). Yield: 105 mg, 74%, colorless oil. ^1H NMR (CDCl_3 , 48 °C): 7.28–7.252 (m, 4), 7.20–7.17 (m, 6H), 3.61 (dd, $J_1 = 13$, $J_2 = 6$, 4H), 3.4–3.40 (m, 2H), 2.86 (t, $J = 7$, 4H), 2.41 (t, $J = 7$, 2H), 2.27 (s, 6H), 1.72–1.79 (m, 2H). ^{13}C NMR (CDCl_3 , 48 °C): 166.20, 139.65, 129.00, 128.74, 126.50, 57.86, 45.43, 42.28, 39.61, 36.40, 27.51. HRMS calcd for $\text{C}_{24}\text{H}_{33}\text{N}_7$ (M + H), 432.1703; found, 432.1703.

2,4-Bis(4'-methoxyphenethylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8dA). Yield: 87 mg, 57%, colorless oil. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.12–7.04 (m, 4H), 6.89–6.83 (m, 4H), 3.82 (s, 6H), 3.76–3.65 (m, 4H), 3.62–3.52 (m, 2H), 3.25–3.18 (m, 2H), 2.94–2.80 (m, 10H), 2.16–2.07 (m, 2H). ^{13}C NMR (CDCl_3 , 7% TFA) (confs): 158.35, 154.55, 130.07, 129.66, 114.44, 56.16, 55.94, 43.59, 42.94, 37.64, 34.25, 24.18. HRMS calcd for $\text{C}_{26}\text{H}_{37}\text{N}_7\text{O}_2$ (M + H), 480.3087; found, 480.3090.

2,4-Bis(2',4'-dichlorophenethylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8eA). Yield: 100 mg, 88%, yellow oil. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.39–7.36 (m, 2H), 7.20–7.12 (m, 4H), 3.73–3.60 (m, 4H), 3.57–3.48 (m, 2H), 3.22–3.14 (m, 2H), 3.02–2.96 (m, 4H), 2.9 (s, 6H), 2.11–2.05 (m, 2H). ^{13}C NMR (CDCl_3 , 7% TFA) (confs): 162.78, 155.60, 154.74, 134.77, 134.30, 133.50, 131.62, 129.43, 127.36, 55.73, 43.24, 40.39, 37.36, 32.64, 24.03. HRMS calcd for $\text{C}_{24}\text{H}_{29}\text{N}_7\text{Cl}_4$ (M + H), 556.1317; found, 556.1320.

2,4-Bis(4'-nitrophenethylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8fA). Yield: 224 mg, 97%, yellowish oil. ^1H NMR (CDCl_3 , 48 °C): 8.10 (d, $J = 8.7$, 4H), 7.34 (d, $J = 8.7$, 4H), 3.65–3.54 (m, 4H), 3.43–3.35 (m, 2H), 2.96 (t, $J = 7$, 4H), 2.47 (t, $J = 7$, 2H), 2.31 (s, 6H), 1.77 (m, 2H). ^{13}C NMR (CDCl_3 , 48 °C): 166.26, 147.53, 147.03, 129.82, 123.85, 57.59, 45.23, 41.65, 39.41, 36.31, 27.20. HRMS calcd for $\text{C}_{24}\text{H}_{31}\text{N}_9\text{O}_4$ (M + H), 510.2577; found, 510.2594.

2,4-Bis(2'-fluorophenethylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8gA). Yield: 143 mg, 57%, white solid. ^1H NMR (CDCl_3 , 48 °C): 7.25–7.15 (m, 4H), 7.11–6.99 (m, 4H), 3.76–3.65 (m, 4H), 3.63–3.50 (m, 2H), 3.28–3.18 (m, 2H), 2.97–2.92 (m, 10H), 2.16–2.07 (m, 2H). ^{13}C NMR (CDCl_3 , 48 °C): 163.87, 161.26 (d, $J = 243$), 155.89, 155.06, 153.91, 131.05, 128.85, 124.54, 124.33, 115.38 (d, $J = 22$), 56.00, 43.58, 41.48, 37.76, 28.51, 24.11. HRMS calcd for $\text{C}_{24}\text{H}_{31}\text{N}_7\text{F}_2$ (M + H), 456.2687; found, 456.2701.

2,4-Bis(3'-fluorophenethylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8hA). Yield: 133 mg, 53%, white solid. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.27–7.23 (m, 2H), 6.97–6.88 (m, 6H), 3.70–3.61 (m, 4H), 3.57–3.49 (m, 2H), 3.23–3.12 (m, 2H), 2.92–2.86 (m, 10H), 2.14–2.03 (m, 2H).

^{13}C NMR (CDCl_3 , 7% TFA) (confs): 163.88, 161.28 (d, $J = 236$), 155.88, 155.60, 131.00 (d, $J = 5$), 128.52, 125.00, 124.16, 115.27 (d, $J = 22$), 55.60, 43.26, 41.18, 37.11, 28.97, 24.32. HRMS calcd for $\text{C}_{24}\text{H}_{31}\text{N}_7\text{F}_2$ (M + H), 456.2687; found, 456.2705.

2,4-Bis(2'-(3''-indolyl)ethylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8iA). Yield: 20 mg, 34%, brown solid. ^1H NMR (CD_3CN , 48 °C): 9.03 (s, 2H), 7.62 (d, $J = 8$, 2H), 7.38 (d, $J = 8$, 2H), 7.12 (t, $J = 7$, 2H), 7.06 (s, 2H), 7.02 (t, $J = 7$, 2H), 3.69–3.572 (m, 4H), 3.40–3.30 (s, 2H), 2.99 (t, $J = 7$, 4H), 2.30 (t, $J = 7$, 2H), 2.16 (s, 6H), 1.73–1.63 (m, 2H). ^{13}C NMR (CD_3CN , 48 °C): 167.86, 138.03, 129.04, 123.77, 122.71, 120.05, 119.88, 114.38, 112.55, 58.76, 45.95, 42.38, 40.45, 28.76, 26.82. HRMS calcd for $\text{C}_{28}\text{H}_{35}\text{N}_9$ (M + H), 498.3094; found, 498.3084.

2,4-Bis(2'-(1''-cyclohexenyl)ethylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8jA). Yield: 220 mg, 95%, white solid. ^1H NMR (CDCl_3 , 48 °C): 5.46 (s, 2H), 3.35–3.47 (m, 6H), 2.34 (t, $J = 7.0$, 2H), 2.21 (s, 6H), 2.14 (t, $J = 8$, 2H), 2.00–1.86 (m, 8H), 1.70 (m, 2H), 1.62–1.50 (m, 8H). ^{13}C NMR (CDCl_3 , 48 °C): 166.24, 135.08, 123.50, 57.95, 45.65, 39.66, 38.84, 38.26, 28.29, 27.79, 25.48, 23.13, 22.64. HRMS calcd for $\text{C}_{24}\text{H}_{41}\text{N}_7$ (M + H), 428.3502; found, 428.3505.

2,4-Bis(4'-chlorobenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8kA). Yield: 110 mg, 68%, white oil. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.31–7.10 (m, 8H), 4.56–4.58 (m, 4H), 3.56–3.53 (m, 2H), 3.23–3.04 (m, 2H), 2.93–2.86 (m, 6H), 2.24–2.01 (2H). ^{13}C NMR (CDCl_3 , 7% TFA) (confs): 162.50, 155.10, 134.26, 129.17, 129.15, 56.01, 44.89, 43.74, 38.15, 24.09. HRMS calcd for $\text{C}_{22}\text{H}_{27}\text{N}_7\text{Cl}_2$ (M + H), 460.1783; found, 460.1779.

2,4-Bis(4'-fluorobenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8lA). Yield: 105 mg, 72%, white solid. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.24–6.98 (m, 8H), 4.63–4.53 (m, 4H), 3.57–3.53 (m, 2H), 3.25–3.15 (m, 2H), 2.93–2.89 (m, 6H), 2.16–2.03 (m, 2H). ^{13}C NMR (CDCl_3 , 7% TFA) (confs): 162.80 (d, $J = 251$), 160.05, 154.73, 153.80, 131.49, 129.49, 115.93 (d, $J = 22$), 56.01, 45.05, 43.82, 38.29, 24.10. HRMS calcd for $\text{C}_{22}\text{H}_{27}\text{N}_7\text{F}_2$ (M + H), 428.2374; found, 428.2394.

2,4-Bis(2',4'-dichlorobenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8mA). Yield: 38 mg, 20%, white powder. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.41–7.05 (m, 6H), 4.67–4.61 (m, 4H), 3.55–3.46 (m, 2H), 3.17–3.10 (m, 2H), 2.90–2.86 (m, 6H), 2.12–2.05 (m, 2H). ^{13}C NMR (CDCl_3 , 7% TFA) (confs): 163.64, 155.95, 155.39, 134.40, 133.88, 132.59, 129.85, 129.48, 127.28, 55.92, 43.54, 42.18, 37.95, 24.23. HRMS calcd for $\text{C}_{22}\text{H}_{25}\text{N}_7\text{Cl}_4$ (M + H), 528.1004; found, 528.0992.

2,4-Bis(2',4'-difluorobenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8nA). Yield: 52 mg, 40%, white powder. ^1H NMR (CDCl_3 , 7% TFA) (confs): 7.29–7.18 (m, 2H), 6.88–6.79 (m, 4H), 4.67–4.59 (m, 4H), 3.65–3.47 (m, 2H), 3.22–3.17 (m, 2H), 2.91 (m, 6H), 2.14–2.05 (m, 2H). ^{13}C NMR (CDCl_3 , 7% TFA) (confs): 163 (dd, $J_1 = 197$, $J_2 = 12$), 163.02, 160.88 (dd, $J_1 = 197$, $J_2 = 12$), 155.39, 154.79, 131.08–130.66, 112.09–111.60, 104.56 (t, $J = 25$), 56.17, 43.80, 39.11, 37.94, 24.26. HRMS calcd for $\text{C}_{22}\text{H}_{25}\text{N}_7\text{F}_4$ (M + H), 464.2186; found, 464.2199.

2,4-Bis(4'-trifluoromethylbenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8oA). Yield: 220 mg, 96%, colorless oil. ^1H NMR (CDCl_3 , 48 °C): 7.54–7.39 (m, 8H), 4.58 (bs, 4H), 3.37 (dd, $J_1 = 12$, $J_2 = 6$, 2H), 2.39–2.122 (m, 8H), 1.67–1.60 (m, 2H). ^{13}C NMR (CDCl_3 , 48 °C): 166.47, 166.38, 144.07, 129.57 (q, $J = 32$), 127.67, 125.54 (q, $J = 4$), 124.39 (q, $J = 270$), 57.71, 45.41, 44.32, 38.57, 27.29. HRMS calcd for $\text{C}_{24}\text{H}_{27}\text{N}_7\text{F}_6$ (M + H), 528.2310; found, 528.2292.

2,4-Bis(3'-4'-methylenedioxybenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8pA). Yield: 226 mg, 94%, colorless oil. ^1H NMR (CDCl_3 , 48 °C): 6.84–6.70 (m, 6H), 5.92–5.88

(m, 4H), 4.48–4.40 (m, 4H), 3.48–3.34 (bs, 2H), 2.9–2.36 (m, 8H), 1.83–1.73 (m, 2H). ^{13}C NMR (CDCl_3 , 48 °C): 165.24, 148.06, 147.00, 133.41, 120.98, 108.48, 101.18, 77.55, 77.23, 76.91, 57.52, 44.89, 44.73, 39.40, 26.72. HRMS calcd for $\text{C}_{24}\text{H}_{29}\text{N}_7\text{O}_4$ (M + H), 480.2359; found, 480.2365.

2,4-Bis(4'-methoxycarbonylbenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8qA). Yield: 97 mg, 42%, colorless oil. ^1H NMR (CDCl_3 , 48 °C): 7.93 (d, $J = 8$, 4H), 7.31 (d, $J = 6$, 4H), 4.57 (d, $J = 5$, 4H), 3.88 (s, 6H), 3.35 (dd, $J_1 = 12$, $J_2 = 6$, 2H), 2.31 (t, $J = 6$, 2H), 2.19 (s, 6H), 1.66 (m, 2H). ^{13}C NMR (CDCl_3 , 48 °C): 167.11 C1, 166.58, 166.53, 145.20, 130.01, 129.24, 127.40, 57.96, 52.20, 45.61, 44.62, 39.81, 27.51. HRMS calcd for $\text{C}_{28}\text{H}_{33}\text{N}_7\text{O}_4$ (M + H), 508.2630; found, 508.2633.

2,4-Bis(4'-(bis(4''-fluorophenyl)methyl)piperazin-1'-yl)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8rA). Yield: 120 mg, 80%, white powder. ^1H NMR (CDCl_3 , 48 °C): 7.33 (dd, $J_1 = 8.7$, $J_2 = 5.4$, 8H), 6.96 (dd, $J_1 = 11.3$, $J_2 = 6.1$, 8H), 4.23 (s, 2H), 3.75–3.65 (m, 8H), 3.41 (dd, $J = 13$, 2H), 2.69 (t, $J = 9$, 2H), 2.48 (s, 6H), 2.38–2.28 (m, 8H), 1.91–1.85 (m, 2H). ^{13}C NMR (CDCl_3 , 48 °C): 166.10–166.84, 162.09 (d, $J = 247$), 138.11 (d, $J = 3.0$), 129.51 (d, $J = 7.8$), 115.60 (d, $J = 21$), 74.66, 56.87, 51.85, 44.21, 43.51, 38.60, 26.39. HRMS calcd for $\text{C}_{42}\text{H}_{47}\text{N}_9\text{F}_4$ (M + H), 754.3969; found, 754.3958.

2,4-Bis(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8sA). Yield: 80 mg, 63%, brownish oil. ^1H NMR (CD_3CN , 48 °C): 9.00 (s, 2H), 7.38 (dt, $J_1 = 22$, $J_2 = 7$, 4H), 7.06 (ddd, $J_1 = 15$, $J_2 = 14$, $J_3 = 7$, 4H), 4.98–4.89 (bs, 4H), 4.17–4.10 (bs, 4H), 3.544–3.34 (m, 2H), 2.81–2.75 (bs, 4H), 2.33 (t, $J = 7$, 2H), 2.21–2.15 (m, 6H), 1.74–1.67 (m, 2H). ^{13}C NMR (CD_3CN , 48 °C): 167.70, 167.00, 137.60, 133.27, 128.38, 122.18, 120.07, 118.71, 112.06, 109.45, 58.68, 45.90, 42.48, 42.35, 40.36, 28.64, 21.97. HRMS calcd for $\text{C}_{30}\text{H}_{35}\text{N}_9$ (M + H), 522.3094; found, 522.3079.

2,4-Bis(4'-hydroxycarbonylbenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (8tA). Yield: 160 mg, 61%, colorless oil. ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 48 °C): 8.38 (d, $J = 8$, 4H), 7.63 (d, $J = 8$, 4H), 4.87 (s, 4H), 3.61 (t, $J = 6$, 2H), 3.09 (t, $J = 7$, 2H), 2.70 (s, 6H), 2.15–2.05 (m, 2H). ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 7% TFA, 48 °C): 169.28, 164.91, 145.54, 131.85, 131.12, 130.49, 128.38, 128.05, 56.31, 45.15, 43.19, 38.74, 25.81. HRMS calcd for $\text{C}_{24}\text{H}_{29}\text{N}_7\text{O}_4$ (M + H), 480.2395; found, 480.2384.

2,4-Bis(4'-hydroxycarbonylbenzylamino)-6-(propylamino)-1,3,5-triazine (8tE). Yield: 25 mg, 27%, colorless oil. ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 48 °C): 8.37 (d, $J = 8$, 4H), 7.62 (d, $J = 8$, 4H), 4.88 (s, 4H), 3.56 (t, $J = 7$, 2H), 1.66–1.61 (m, 2H), 1.44–1.35 (m, 2H), 0.90 (t, $J = 7$, 3H). ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 48 °C): 169.32, 166.61, 146.20, 131.73, 131.12, 130.47, 128.40, 128.05, 45.21, 41.30, 32.76, 20.83, 14.42. HRMS calcd for $\text{C}_{23}\text{H}_{26}\text{N}_6\text{O}_4$ (M + H), 451.2094; found, 451.2087.

Biological Assays. Recombinant Rat TRPV1 Channels Expression in *Xenopus* oocytes and Channel Blocking. All the procedures have been described in detail elsewhere.^{4,5} Whole-cell currents from rat TRPV1-injected oocytes were recorded in Mg^{2+} Ringer's solution (10 mM HEPES, pH 7.4, 115 mM NaCl, 2.8 mM KCl, 0.1 mM BaCl_2 , 2.0 mM MgCl_2) with a two-microelectrode voltage-clamp amplifier at 20 °C. The TRPV1 channels were activated by applying 10 μM capsaicin in the absence or the presence of individual compounds at a holding potential (V_h) of -60 mV. Dose–response curves for individual peptoids were fitted to the Hill equation:

$$\frac{I}{I_{\max}} = \frac{1}{1 + \left(\frac{[\text{blocker}]}{\text{IC}_{50}}\right)^{n_{\text{H}}}}$$

where IC_{50} denotes the channel blocker concentration that inhibits half of the response obtained in its absence (I_{\max}) and n_{H} denotes the Hill

coefficient, which is an estimate of the number of drug binding sites. $I-V$ characteristics were recorded using a ramp protocol.^{5,32} Oocytes were depolarized from -60 to 60 mV in 5 s (25 mV/s). Leak currents were measured in the absence of agonist in the external bath medium and subtracted from the ionic current recorded in the presence of the ligand. Voltage dependence of channel blocking was studied as described in Ferrer-Montiel et al.³² Experimental data were fitted to either the Hill or Woodhull equation³² with a nonlinear least-squares regression algorithm using GraphPad Prism 5 software.

■ ASSOCIATED CONTENT

S Supporting Information. Full experimental details concerning concise product synthesis, NMR spectra of synthesized compounds, and full structures of triazines **8**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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■ DEDICATION

[†]Dedicated to the memory of Prof. Rafael Suau.

■ ABBREVIATIONS USED

DMEM, Dulbecco's modified Eagle's medium; DRG, dorsal root ganglion; FBS, fetal bovine serum; NGF, nerve growth factor; TRPV1, transient receptor potential vanilloid 1

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