Bibliographical References.

1. Bunnage, M. E. Getting pharmaceutical R&D back on target. *Nat. Chem. Biol.* **2011,** 7, 335-339.
2. Drews, J. Drug Discovery: A Historical Perspective. *Science* **2000,** 287, 1960-1964.
3. Swinney, D. C.; Anthony, J. How were new medicines discovered? *Nat. Rev. Drug Discov.* **2011,** 10, 507-519.
4. Paul, S. M.; Mytelka, D. S.; Dunwiddie, C. T.; Persinger, C. C.; Munos, B. H.; Lindborg, S. R.; Schacht, A. L. How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat. Rev. Drug Discov.* **2010,** 9, 203-214.
5. *Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research*. The National Academies Press: **2011**.
6. Editorial: The need of a taxonomy. *Pain* **1979,** 6, 247-252.
7. Kreitler, S. Taxonomy and classification of pain. In *The Handbook of Chronic Pain*, PP., R., Ed. Nova Biomedical Books: **2007**.
8. Loeser, D.; Butler, H.; Richard, C.; Turk, C. *Bonica's Management of Pain*. Lippincott Williams & Wilkins Publishers: **2001**.
9. Vargas-Schaffer, G. Is the WHO analgesic ladder still valid? Twenty-four years of experience. *Can. Fam. Physician* **2010,** 55.
10. Shelemay, K.; Coakley, S. *Pain and its transformations: the interface of biology and culture*. Harvard University Press: Cambridge, **2007**.
11. Craig, A. D. PAIN MECHANISMS: Labeled Lines Versus Convergence in Central Processing. *Annu. Rev. Neurosci.* **2003,** 26, 1-30.
12. Tracey, I. Imaging pain. *Br. J. Anaesth.* **2008,** 101, 32-39.
13. Flórez, J. Fármacos analgésicos opioides. In *Farmacología humana*, Masson, S. A., Ed. **1998**; 435-452.
14. Velasco, A.; Lorenzo, P.; Serrano, J.; De Andrés-Trelles, F. Analgésicos opiáceos. In *Farmacología básica y clínica*, Interamericana, M. H., Ed. **1993**; 446-470.
15. Tsuboi, K.; Sugimoto, Y.; Ichikawa, A. Prostanoid receptor subtypes. *Prostag. Oth. Lipid M.* **2002,** 68-69, 535-556.
16. Sima, L.; Fang, W. X.; Wu, X. M.; Li, F. Efficacy of oxycodone/paracetamol for patients with bone-cancer pain: a multicenter, randomized, double-blinded, placebo-controlled trial. *J. Clin. Pharm. Ther.* **2012,** 37, 27-31.
17. Finnerup, N. B.; Sindrup, S. H.; Jensen, T. S. The evidence for pharmacological treatment of neuropathic pain. *Pain* **2010,** 150, 573-581.
18. Venkatachalam, K.; Montell, C. TRP Channels. *Annu. Rev. Biochem.* **2007,** 76, 387-417.
19. Cheng, W.; Yang, F.; Takanishi, C. L.; Zheng, J. Thermosensitive TRPV Channel Subunits Coassemble into Heteromeric Channels with Intermediate Conductance and Gating Properties. *J. Gen. Physiol.* **2007,** 129, 191-207.
20. Planells-Cases, R.; Valente, P.; Ferrer-Montiel, A.; Qin, F.; Szallasi, A. Complex Regulation of TRPV1 and Related Thermo-TRPs: Implications for Therapeutic Intervention. In *Transient Receptor Potential Channels*, Islam, M. S., Ed. Springer Netherlands: **2011**; 704, 491-515.
21. Moran, M. M.; McAlexander, M. A.; Biró, T.; Szallasi, A. Transient receptor potential channels as therapeutic targets. *Nat. Rev. Drug Discov.* **2011,** 10, 601-620.
22. Szallasi, A.; Blumberg, P. M. Vanilloid (Capsaicin) Receptors and Mechanisms. *Pharmacol. Rev.* **1999,** 51, 159-212.
23. Szallasi, A. After a decade of intravesical vanilloid therapy: still more questions than answers. *Lancet Neurol.* **2002,** 1, 167-72.
24. Szallasi, A.; Cortright, D. N.; Blum, C. A.; Eid, S. R. The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. *Nat. Rev. Drug Discov.* **2007,** 6, 357-372.

25. Szallasi, A.; Gunthorpe, M. Peripheral TRPV1 Receptors As Targets for Drug Development: New Molecules and Mechanisms. *Curr. Pharm. Des.* **2008,** 14, 32-41.
26. Vennekens, R.; Owsianik, G.; Nilius, B. Vanilloid Transient Receptor Potential Cation Channels: An Overview. *Curr. Pharm. Des.* **2008,** 14, 18-31.
27. Lishko, P. V.; Procko, E.; Jin, X.; Phelps, C. B.; Gaudet, R. The Ankyrin Repeats of TRPV1 Bind Multiple Ligands and Modulate Channel Sensitivity. *Neuron* **2007,** 54, 905-918.
28. Moiseenkova-Bell, V. Y.; Stanciu, L. A.; Serysheva, I. I.; Tobe, B. J.; Wensel, T. G. Structure of TRPV1 channel revealed by electron cryomicroscopy. *Proc. Natl. Acad. Sci. U.S.A.* **2008,** 105, 7451-7455.
29. Liao, M.; Cao, E.; Julius, D.; Cheng, Y. Structure of the TRPV1 ion channel determined by electron cryo-microscopy. *Nature* **2013,** 504, 107-112.
30. Xiaorui, C.; Qinghua, W.; Fengyun, N.; Jianpeng, M. Structure of the full-length Shaker potassium channel Kv1.2 by normal-mode-based X-ray crystallographic refinement. *Proc. Natl. Acad. Sci. U.S.A.* **2010,** 107, 11352–11357.
31. Uysal, S.; Vásquez, V.; Tereshko, V.; Esaki, K.; Fellouse, F. A.; Sidhu, S. S.; Koide, S.; Perozo, E.; Kossiakoff, A. Crystal structure of full-length KcsA in its closed conformation. *Proc. Natl. Acad. Sci. U.S.A.* **2009,** 106, 6644–6649.
32. Lee, J.; Lee, Y.; Ryu, H.; Kang, D.; Lee, J.; Lazar, J.; Pearce, L.; Pavlyukovets, V.; Blumberg, P.; Choi, S. Structural insights into transient receptor potential vanilloid type 1 (TRPV1) from homology modeling, flexible docking, and mutational studies. *J. Comput. Aid. Mol. Des.* **2011,** 25, 317-327.
33. Fernández-Ballester, G.; Ferrer-Montiel, A. Molecular Modeling of the Full-length Human TRPV1 Channel in Closed and Desensitized States. *J. Membr. Biol.* **2008,** 223, 161-172.
34. Ryu, S.; Liu, B.; Yao, J.; Fu, Q.; Qin, F. Uncoupling Proton Activation of Vanilloid Receptor TRPV1. *J. Neurosci.* **2007,** 27, 12797-12807.
35. Brauchi, S.; Orta, G.; Mascayano, C.; Salazar, M.; Raddatz, N.; Urbina, H.; Rosenmann, E.; Gonzalez-Nilo, F.; Latorre, R. Dissection of the components for PIP2 activation and thermosensation in TRP channels. *Proc. Natl. Acad. Sci. U.S.A.* **2007,** 104, 10246-10251.
36. Aneiros, E.; Cao, L.; Papakosta, M.; Stevens, E. B.; Phillips, S.; Grimm, C. The biophysical and molecular basis of TRPV1 proton gating. *Embo. J.* **2011,** 30, 994-1002.
37. Brauchi, S.; Orta, G.; Salazar, M.; Rosenmann, E.; Latorre, R. A Hot-Sensing Cold Receptor: C-Terminal Domain Determines Thermosensation in Transient Receptor Potential Channels. *J. Neurosci.* **2006,** 26, 4835-4840.
38. Bhave, G.; Zhu, W.; Wang, H.; Brasier, D. J.; Oxford, G. S.; Gereau Iv, R. W. cAMP-Dependent Protein Kinase Regulates Desensitization of the Capsaicin Receptor (VR1) by Direct Phosphorylation. *Neuron* **2002,** 35, 721-731.
39. Jung, J.; Shin, J. S.; Lee, S.-Y.; Hwang, S. W.; Koo, J.; Cho, H.; Oh, U. Phosphorylation of Vanilloid Receptor 1 by Ca2+/Calmodulin-dependent Kinase II Regulates Its Vanilloid Binding. *J. Biol. Chem.* **2004,** 279, 7048-7054.
40. Woo, D.; Jung, S.; Zhu, M.; Park, C.-K.; Kim, Y.; Oh, S.; Lee, C. J. Direct activation of Transient Receptor Potential Vanilloid 1 (TRPV1) by Diacylglycerol (DAG). *Mol. Pain* **2008,** 4, 42.
41. Rathee, P. K.; Distler, C.; Obreja, O.; Neuhuber, W.; Wang, G. K.; Wang, S.-Y.; Nau, C.; Kress, M. PKA/AKAP/VR-1 Module: A Common Link of Gs-Mediated Signaling to Thermal Hyperalgesia. *J. Neurosci.* **2002,** 22, 4740-4745.
42. Lee, J.; Saloman, J. L.; Weiland, G.; Auh, Q. S.; Chung, M.-K.; Ro, J. Y. Functional interactions between NMDA receptors and TRPV1 in trigeminal sensory neurons mediate mechanical hyperalgesia in the rat masseter muscle. *Pain* **2012,** 153, 1514-1524.

43. Jeske, N. A.; Diogenes, A.; Ruparel, N. B.; Fehrenbacher, J. C.; Henry, M.; Akopian, A. N.; Hargreaves, K. M. A-kinase anchoring protein mediates TRPV1 thermal hyperalgesia through PKA phosphorylation of TRPV1. *Pain* **2008,** 138, 604-616.
44. Lee, J.; Chung, M.-K.; Ro, J. Y. Activation of NMDA receptors leads to phosphorylation of TRPV1 S800 by protein kinase C and A-Kinase anchoring protein 150 in rat trigeminal ganglia. *Biochem. Bioph. Res. Commun.* **2012,** 424, 358-363.
45. Huang, J.; Zhang, X.; McNaughton, P. A. Modulation of temperature-sensitive TRP channels. *Semin. Cell Dev. Biol.* **2006,** 17, 638-645.
46. Scholz, J.; Woolf, C. J. Can we conquer pain? *Nat. Neurosci.* **2002,** 5, 1062 - 1067.
47. Messeguer, A.; Planells-Cases, R.; Ferrer-Montiel, A. Physiology and Pharmacology of the Vanilloid Receptor. *Curr Neuropharmacol.* **2006** 4, 1–15.
48. Gautam, B.; Gereau, W. R. I. Posttranslational mechanisms of peripheral sensitization. *Dev. Neurobiol.* **2004,** 61, 88-106.
49. Mohapatra, D. P.; Nau, C. Desensitization of Capsaicin-activated Currents in the Vanilloid Receptor TRPV1 Is Decreased by the Cyclic AMP-dependent Protein Kinase Pathway. *J. Biol. Chem.* **2003,** 278, 50080-50090.
50. Sanz-Salvador, L.; Andrés-Borderia, A.; Ferrer-Montiel, A.; Planells-Cases, R. Agonist- and Ca2+-dependent Desensitization of TRPV1 Channel Targets the Receptor to Lysosomes for Degradation. *J. Biol. Chem.* **2012,** 287, 19462-19471.
51. Jordt, S.-E.; Julius, D. Molecular Basis for Species-Specific Sensitivity to "Hot" Chili Peppers. *Cell* **2002,** 108, 421-430.
52. Gavva, N. R.; Klionsky, L.; Qu, Y.; Shi, L.; Tamir, R.; Edenson, S.; Zhang, T. J.; Viswanadhan, V. N.; Toth, A.; Pearce, L. V.; Vanderah, T. W.; Porreca, F.; Blumberg, P. M.; Lile, J.; Sun, Y.; Wild, K.; Louis, J.-C.; Treanor, J. J. S. Molecular determinants of vanilloid sensitivity in TRPV1. *J. Biol. Chem.* **2004,** 279, 20283-20295.
53. Jung, J.; Hwang, S. W.; Kwak, J.; Lee, S.-Y.; Kang, C.-J.; Kim, W. B.; Kim, D.; Oh, U. Capsaicin Binds to the Intracellular Domain of the Capsaicin-Activated Ion Channel. *J. Neurosci.* **1999,** 19, 529-538.
54. Raisinghani, M.; Pabbidi, R. M.; Premkumar, L. S. Activation of transient receptor potential vanilloid 1 (TRPV1) by resiniferatoxin. *J. Physiol.* **2005,** 567, 771-786.
55. Szallasi, A. Vanilloid Receptor Ligands: Hopes and Realities for the Future. *Drugs Aging* **2001,** 18, 561-573.
56. Wallace, M. S.; Pappagallo, M. Qutenza: a capsaicin 8% patch for the management of postherpetic neuralgia. *Expert. Rev. Neurother.* **2011,** 11, 15-27.
57. Appendino, G.; Minassi, A.; Morello, A. S.; De Petrocellis, L.; Di Marzo, V. N-Acylvanillamides: Development of an Expeditious Synthesis and Discovery of New Acyl Templates for Powerful Activation of the Vanilloid Receptor. *J. Med. Chem.* **2002,** 45, 3739-3745.
58. Walpole, C. S. J.; Bevan, S.; Bovermann, G.; Boelsterli, J. J.; Breckenridge, R.; Davies, J. W.; Hughes, G. A.; James, I.; Oberer, L. The Discovery of Capsazepine, the First Competitive Antagonist of the Sensory Neuron Excitants Capsaicin and Resiniferatoxin. *J. Med. Chem.* **1994,** 37, 1942-1954.
59. Douat, J.; Vachon, P.; Beaudry, F. Characterization of in vitro metabolism of capsazepine, a vanilloid transient receptor potential channel antagonist, by liquid chromatography quadrupole ion trap mass spectrometry. *Biomed. Chromatogr.* **2011,** 25, 479-492.
60. Wahl, P.; Foged, C.; Tullin, S.; Thomsen, C. Iodo-Resiniferatoxin, a New Potent Vanilloid Receptor Antagonist. *Mol. pharmacol.* **2001,** 59, 9-15.
61. Lim, K. S.; Kang, D. W.; Kim, Y. S.; Kim, M. S.; Park, S.-G.; Choi, S.; Pearce, L. V.; Blumberg, P. M.; Lee, J. Receptor activity and conformational analysis of 5'-

halogenated resiniferatoxin analogs as TRPV1 ligands. *Bioorg. Med. Chem. Lett.* **2011,** 21, 299-302.
62. Gavva, N. R.; Treanor, J. J. S.; Garami, A.; Fang, L.; Surapaneni, S.; Akrami, A.; Alvarez, F.; Bak, A.; Darling, M.; Gore, A.; Jang, G. R.; Kesslak, J. P.; Ni, L.; Norman, M. H.; Palluconi, G.; Rose, M. J.; Salfi, M.; Tan, E.; Romanovsky, A. A.; Banfield, C.; Davar, G. Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. *Pain* **2008,** 136, 202-210.
63. Wong, G. Y.; Gavva, N. R. Therapeutic potential of vanilloid receptor TRPV1 agonists and antagonists as analgesics: Recent advances and setbacks. *Brain Res. Rev.* **2009,** 60, 267-277.
64. Li, S.; Bode, A. M.; Zhu, F.; Liu, K.; Zhang, J.; Kim, M. O.; Reddy, K.; Zykova, T.; Ma, W.-y.; Carper, A. L.; Langfald, A. K.; Dong, Z. TRPV1-antagonist AMG9810 promotes mouse skin tumorigenesis through EGFR/Akt signaling. *Carcinogenesis* **2011,** 32, 779-785.
65. Baraldi, P. G.; Borea, P. A.; Geppetti, P.; Trevisani, M. Biarylcarboxyarylamides as Vanilloid-1 Receptor Modulators. Patent WO2008006480 **2008**.
66. Watabiki, T.; Kiso, T.; Kuramochi, T.; Yonezawa, K.; Tsuji, N.; Kohara, A.; Kakimoto, S.; Aoki, T.; Matsuoka, N. Amelioration of Neuropathic Pain by Novel Transient Receptor Potential Vanilloid 1 Antagonist AS1928370 in Rats without Hyperthermic Effect. *J. Pharmacol. Exp. Ther.* **2011,** 336, 743-750.
67. Napoletano, M.; Pavani, M. G.; Fruttarolo, F.; Trevisani, M. Nicotinamide *N*-Oxide derivatives as vanilloid-1 receptor modulators. Patent WO2009043582. **2009**.
68. Kort, M. E.; Kym, P. R. TRPV1 Antagonists: Clinical Setbacks and Prospects for Future Development. *Progr. Med. Chem.* **2012,** 51, 57-70.
69. García-Martínez, C.; Humet, M.; Planells-Cases, R.; Gomis, A.; Caprini, M.; Viana, F.; De la Peña, E.; Sanchez-Baeza, F.; Carbonell, T.; De Felipe, C.; Pérez-Payá, E.; Belmonte, C.; Messeguer, A.; Ferrer-Montiel, A. V. Attenuation of thermal nociception and hyperalgesia by VR1 blockers. *Proc. Natl. Acad. Sci. U.S.A.* **2002,** 99, 2374-2379.
70. Quintanar-Audelo, M.; Fernández-Carvajal, A.; Van Den Nest, W.; Carreño, C.; Ferrer-Montiel, A.; Albericio, F. Design and Synthesis of Indole-Based Peptoids as Potent Noncompetitive Antagonists of Transient Receptor Potential Vanilloid 1. *J. Med. Chem.* **2007,** 50, 6133-6143.
71. Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Wang, L.; Rosenberg, S. H.; Marlowe, C. K. Peptoids: a modular approach to drug discovery. *Proc. Natl. Acad. Sci. U.S.A.* **1992,** 89, 9367-9371.
72. Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. Efficient method for the preparation of peptoids [oligo(N-substituted glycines)] by submonomer solid-phase synthesis. *J. Am. Chem. Soc.* **1992,** 114, 10646-10647.
73. Zuckermann, R. N.; Martin, E. J.; Spellmeyer, D. C.; Stauber, G. B.; Shoemaker, K. R.; Kerr, J. M.; Figliozzi, G. M.; Goff, D. A.; Siani, M. A. Discovery of Nanomolar Ligands for 7-Transmembrane G-Protein-Coupled Receptors from a Diverse N-(Substituted)glycine Peptoid Library. *J. Med. Chem.* **1994,** 37, 2678-2685.
74. Humet, M.; Carbonell, T.; Masip, I.; Sánchez-Baeza, F.; Mora, P.; Cantón, E.; Gobernado, M.; Abad, C.; Pérez-Payá, E.; Messeguer, A. A Positional Scanning Combinatorial Library of Peptoids As a Source of Biological Active Molecules: Identification of Antimicrobials. *J. Comb. Chem.* **2003,** 5, 597-605.
75. Brown, N. J.; Johansson, J.; Barron, A. E. Biomimicry of Surfactant Protein C. *Acc. Chem. Res.* **2008,** 41, 1409-1417.
76. Nguyen, J. T.; Turck, C. W.; Cohen, F. E.; Zuckermann, R. N.; Lim, W. A. Exploiting the Basis of Proline Recognition by SH3 and WW Domains: Design of N-Substituted Inhibitors. *Science* **1998,** 282, 2088-2092.

77. Udugamasooriya, D. G.; Dineen, S. P.; Brekken, R. A.; Kodadek, T. A Peptoid "Antibody Surrogate" That Antagonizes VEGF Receptor 2 Activity. *J. Am. Chem. Soc.* **2008,** 130, 5744-5752.
78. Reddy, M. M.; Wilson, R.; Wilson, J.; Connell, S.; Gocke, A.; Hynan, L.; German, D.; Kodadek, T. Identification of Candidate IgG Biomarkers for Alzheimer's Disease via Combinatorial Library Screening. *Cell* **2011,** 144, 132-142.
79. Mondragón, L.; Orzáez, M.; Sanclimens, G.; Moure, A.; Armiñán, A.; Sepúlveda, P.; Messeguer, A.; Vicent, M. J.; Pérez-Payá, E. Modulation of Cellular Apoptosis with Apoptotic Protease-Activating Factor 1 (Apaf-1) Inhibitors. *J. Med. Chem.* **2008,** 51, 521-529.
80. Márquez, N.; Sancho, R.; Macho, A.; Moure, A.; Masip, I.; Messeguer, A.; Muñoz, E. Anti-Tat and anti-HIV activities of trimers of n-alkylglycines. *Biochem. Pharmacol.* **2006,** 71, 596-604.
81. Montolio, M.; Messeguer, J.; Masip, I.; Guijarro, P.; Gavin, R.; Antonio del Río, J.; Messeguer, A.; Soriano, E. A Semaphorin 3A Inhibitor Blocks Axonal Chemorepulsion and Enhances Axon Regeneration. *Chemistry & Biology* **2009,** 16, 691-701.
82. Ferrer-Montiel, A.; García-Martínez, C.; Morenilla-Palao, C.; García-Sanz, N.; Fernández-Carvajal, A.; Fernández-Ballester, G.; Planells-Cases, R. Molecular architecture of the vanilloid receptor. *FEBS J.* **2004,** 271, 1820-1826.
83. García-Martínez, C.; Fernández-Carvajal, A.; Valenzuela, B.; Gomis, A.; Van Den Nest, W.; Ferroni, S.; Carreño, C.; Belmonte, C.; Ferrer-Montiel, A. Design and Characterization of a Noncompetitive Antagonist of the Transient Receptor Potential Vanilloid Subunit 1 Channel With In Vivo Analgesic and Anti-inflammatory Activity. *J. Pain* **2006,** 7, 735-746.
84. Bonache, M.; García-Martinez, C.; García de Diego, L.; Carreño, C.; Pérez de Vega, M.; García-López, M.; Ferrer-Montiel, A.; González-Muñiz, R. Old Molecules for New Receptors: Trp(Nps) Dipeptide Derivatives as Vanilloid TRPV1 Channel Blockers. *ChemMedChem* **2006,** 1, 429-438.
85. de Vega, M. J. P.; García-López, M. T.; Zaccaro, L.; Royo, M.; Albericio, F.; Fernández-Carvajal, A.; Ferrer-Montiel, A.; González-Muñiz, R. Solid-Phase Synthesis of New Trp(Nps)-Containing Dipeptide Derivatives as TRPV1 Channel Blockers. *Molecules* **2010,** 15, 4924-4933.
86. Zaccaro, L.; García-López, M. T.; González-Muñiz, R.; García-Martínez, C.; Ferrer-Montiel, A.; Albericio, F.; Royo, M. TRPV1 modulators: Structure, activity relationships using a rational combinatorial approach. *Bioorg. Med. Chem. Lett.* **2011,** 21, 3541-3545.
87. Gerona-Navarro, G.; González-Muñiz, R.; Fernández-Carvajal, A.; González-Ros, J. M.; Ferrer-Montiel, A.; Carreño, C.; Albericio, F.; Royo, M. Solid-Phase Synthesis of a Library of Amphipatic Hydantoins. Discovery of New Hits for TRPV1 Blockade. *ACS Comb. Sci.* **2011,** 13, 458-465.
88. Moure, A. "Modulación química de rutas de señalización celular: Optimización de hits identificados a partir de quimiotecas". PhD Thesis. Universidad de Barcelona, Barcelona, **2009**.
89. Scheper, J.; Guerra-Rebollo, M.; Sanclimens, G.; Moure, A.; Masip, I.; González-Ruiz, D.; Rubio, N.; Crosas, B.; Meca-Cortés, Ó.; Loukili, N.; Plans, V.; Morreale, A.; Blanco, J.; Ortiz, A. R.; Messeguer, Á.; Thomson, T. M. Protein-Protein Interaction Antagonists as Novel Inhibitors of Non-Canonical Polyubiquitylation. *PLoS One* **2010,** 5, e11403.
90. Figliozzi, G. M.; Goldsmith, R.; Ng, S. C.; Banville, S. C.; Zuckermann, R. N.; John, N. A. Synthesis of N-substituted glycine peptoid libraries. *Method. Enzymol.* **1996,** 267, 437-447.

91. Hancock, W. S.; Battersby, J. E. A new micro-test for the detection of incomplete coupling reactions in solid-phase peptide synthesis using 2,4,6-trinitrobenzene-sulphonic acid. *Anal. Biochem.* **1976,** 71, 260-264.
92. Van der Plas, S. E.; De Clercq, P. J.; Madder, A. Fast and easy detection of aromatic amines on solid support. *Tetrahedron Lett.* **2007,** 48, 2587-2589.
93. Baumgartner, W.; Islas, L.; Sigworth, F. J. Two-Microelectrode Voltage Clamp of Xenopus Oocytes: Voltage Errors and Compensation for Local Current Flow. *Biophys. J.* **1999,** 77, 1980-1991.
94. Saleh, M.; Abbott, S.; Perron, V.; Lauzon, C.; Penney, C.; Zacharie, B. Synthesis and antimicrobial activity of 2-fluorophenyl-4,6-disubstituted [1,3,5]triazines. *Bioorg. Med. Chem. Lett.* **2010,** 20, 945-949.
95. Menicagli, R.; Samaritani, S.; Signore, G.; Vaglini, F.; Dalla Via, L. In Vitro Cytotoxic Activities of 2-Alkyl-4,6-diheteroalkyl-1,3,5-triazines: New Molecules in Anticancer Research. *J. Med. Chem.* **2004,** 47, 4649-4652.
96. Zheng, M.; Xu, C.; Ma, J.; Sun, Y.; Du, F.; Liu, H.; Lin, L.; Li, C.; Ding, J.; Chen, K.; Jiang, H. Synthesis and antitumor evaluation of a novel series of triaminotriazine derivatives. *Bioorg. Med. Chem.* **2007,** 15, 1815-1827.
97. Xiong, Y.-Z.; Chen, F.-E.; Balzarini, J.; De Clercq, E.; Pannecouque, C. Non-nucleoside HIV-1 reverse transcriptase inhibitors. Part 11: Structural modulations of diaryltriazines with potent anti-HIV activity. *Eur. J. Med. Chem.* **2008,** 43, 1230-1236.
98. Mooibroek, T. J.; Gamez, P. The s-triazine ring, a remarkable unit to generate supramolecular interactions. *Inorg. Chim. Acta* **2007,** 360, 381-404.
99. Shie, J.; Fang, J. Microwave-Assisted One-Pot Tandem Reactions for Direct Conversion of Primary Alcohols and Aldehydes to Triazines and Tetrazoles in Aqueous Media. *J. Org. Chem.* **2007,** 72, 3141-3144.
100. Oudir, S.; Rigo, B.; Hénichart, J.; Gautret, P. A Convenient Method for the Conversion of a Carboxy Group into a 4,6-Dimethoxy-1,3,5-triazine Group: Application to N-Benzylpyroglutamic Acids. *Synthesis* **2006,** 17, 2845-2848.
101. Uccello-Barretta, G.; Samaritani, S.; Menicagli, R.; Salvadori, P. 2,4,6-Tri[(S)-1'-methylbenzylamino]-1,3,5-triazine: a new NMR chiral solvating agent for 3,5-dinitrophenyl derivatives; an attempt at a chiral discrimination rationale. *Tetrahedron-Asymmetr.* **2000,** 11, 3901-3912.
102. Díaz-Ortiz, A.; Elguero, J.; De la Hoz, A.; Jiménez, A.; Moreno, A.; Moreno, S.; Sánchez-Migallón, A. Microwave-Assisted Synthesis and Dynamic Behaviour of Tris(1pyrazolyl)-1,3,5-triazine-2,4,6-triamines. *QSAR Comb. Sci.* **2005,** 24, 649-659.
103. Bork, J. T.; Lee, J. W.; Khersonsky, S. M.; Moon, H. S.; Chang, Y. T. Novel Orthogonal Strategy toward Solid-Phase Synthesis of 1,3,5-Substituted Triazines. *Org. Lett.* **2003,** 5, 117-120.
104. Huthmacher, K.; Most, D. Cyanuric Acid and Cyanuric Chloride. In *Ullmann's Encyclopedia of Industrial Chemistry*, 5th ed.; VCH: Weinheim, **2000**.
105. Shaw, R. A.; Ward, P. 1011. 1,3,5-Triazines. Part V. The reactions of cyanuric chloride with NN-dialkyl-anilines, -toluidines, and -xylidines. *J. Chem. Soc.* **1965**, 5446-5451.
106. Thurston, J. T.; Dudley, J. R.; Kaiser, D. W.; Hechenbleikner, I.; Schaefer, F. C.; Holm-Hansen, D. Cyanuric Chloride Derivatives. I. Aminochloro-s-triazines. *J. Am. Chem. Soc.* **1951,** 73, 2981-2983.
107. Kaiser, D. W.; Thurston, J. T.; Dudley, J. R.; Schaefer, F. C.; Hechenbleikner, I.; Holm-Hansen, D. Cyanuric Chloride Derivatives. II. Substituted Melamines. *J. Am. Chem. Soc.* **1951,** 73, 2984-2986.
108. Dudley, J. R.; Thurston, J. T.; Schaefer, F. C.; Holm-Hansen, D.; Hull, C. J.; Adams, P. Cyanuric Chloride Derivatives. III. Alkoxy-s-triazines. *J. Am. Chem. Soc.* **1951,** 73, 2986-2990.

109. Skoog, D. A.; Donald, M. W.; Holler, F. J.; Stanley, R. C. Molecular absortion spectroscopy. In *Fundamentals of analytical chemistry* 8ed.; Thomson Brooks: New York, **2009**.
110. Kamiński, Z. J. Triazine-based condensing reagents. *J. Pept. Sci.* **2000,** 55, 140-164.
111. Samaritani, S.; Peluso, P.; Malanga, C.; Menicagli, R. Selective Amination of Cyanuric Chloride in the Presence of 18-Crown-6. *Eur. J. Org. Chem.* **2002**, 1551-1555.
112. Srinivas, K.; Srinivas, U.; Jayathirtha, V.; Rao, K.; Bhanuprakash, K. Synthesis and Antibacterial Activity of 2,4,6-Tri-substituted s-Triazines. *Bioorg Med Chem Lett.* **2005,** 36.
113. Afonso, C.; Lourenco, N.; Rosatella, A. Synthesis of 2,4,6-Tri-substituted-1,3,5-Triazines. *Molecules* **2006,** 11, 81-102.
114. Sagar, A. D.; Patil, D. S.; Bandgar, B. P. Microwave Assisted Synthesis of Triaryl Cyanurates. *Synth. Commun.* **2000,** 30, 1719-1723.
115. Masip, I.; Cortés, N.; Abad, M.-J.; Guardiola, M.; Pérez-Payá, E.; Ferragut, J.; Ferrer-Montiel, A.; Messeguer, A. Design and synthesis of an optimized positional scanning library of peptoids: identification of novel multidrug resistance reversal agents. *Bioorg. Med. Chem.* **2005,** 13, 1923-1929.
116. Messeguer, J.; Cortés, N.; García-Sanz, N.; Navarro-Vendrell, G.; Ferrer-Montiel, A.; Messeguer, A. Synthesis of a Positional Scanning Library of Pentamers of N-Alkylglycines Assisted by Microwave Activation and Validation via the Identification of Trypsin Inhibitors. *J. Comb. Chem.* **2008,** 10, 974-980.
117. Giguere, R. J.; Bray, T. L.; Duncan, S. M.; Majetich, G. Application of commercial microwave ovens to organic synthesis. *Tetrahedron Lett.* **1986,** 27, 4945-4948.
118. Kappe, C. O.; Dallinger, D. The impact of microwave synthesis on drug discovery. *Nat. Rev. Drug. Discov.* **2006,** 5, 51-63.
119. Zhou, Y.; Sun, Z.; Froelich, J. M.; Hermann, T.; Wall, D. Structure-activity relationships of novel antibacterial translation inhibitors: 3,5-Diamino-piperidinyl triazines. *Bioorg. Med. Chem. Lett.* **2006,** 16, 5451-5456.
120. Gupton, J. T.; Andrews, S. A. *β*-dimethylaminomethylenation: *N,N*-Dimethyl-*N'*-*p*-tolylformamidine. *Org. Synth.* **1986,** 64, 85.
121. Soylu, O.; Uzun, S.; Can, M. The investigation of acid effect on chemical polymerization of indole. *Colloid Polym. Sci.* **2011,** 289, 903-909.
122. Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M. Diprotected Triflylguanidines: A New Class of Guanidinylation Reagents. *J. Org. Chem.* **1998,** 63, 3804-3805.
123. Birkett, H. E.; Cherryman, J. C.; Chippendale, A. M.; Evans, J. S. O.; Harris, R. K.; James, M.; King, I. J.; McPherson, G. J. Structural investigations of three triazines: solution-state NMR studies of internal rotation and structural information from solid-state NMR, plus a full structure determination from powder x-ray diffraction in one case. *Magn. Reson. Chem.* **2003,** 41, 324-336.
124. Birkett, H. E.; Cherryman, J. C.; Margaret Chippendale, A.; Hazendonk, P.; Harris, R. K. Molecular modelling studies of side-chain rotation in substituted triazine rings. *J. Mol. Struct.* **2002,** 602-603, 59-70.
125. Diaz-Ortiz, A.; Elguero, J.; Foces-Foces, C.; Hoz, A. d. l.; Moreno, A.; Moreno, S.; Sanchez-Migallon, A.; Valiente, G. Synthesis, structural determination and dynamic behavior of 2-chloro-4,6-bis(pyrazolylamino)-1,3,5-triazines. *Org. Biomol. Chem.* **2003,** 1, 4451-4457.
126. Ghiviriga, I.; Oniciu, D. C. Steric hindrance to the solvation of melamines and consequences for non-covalent synthesis. *Chem. Commun.* **2002**, 2718-9.

127. Becke, A. D. Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **1993,** 98, 5648-52.
128. Frisch, M. J.; Pople, J. A.; Binkley, J. S. Self-consistent molecular orbital methods. 25. Supplementary functions for Gaussian basis sets. *J. Chem. Phys.* **1984,** 80, 3265-9.
129. Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B: Condens. Matter* **1988,** 37, 785-9.
130. Schrödinger. *Suite 2012*, Schrödinger, LLC: New York, NY, **2012**.
131. Marten, B.; Kim, K.; Cortis, C.; Friesner, R. A.; Murphy, R. B.; Ringnalda, M. N.; Sitkoff, D.; Honig, B. A New Model For Calculation of Solvation Free Energies: Correction of Self-Consistent Reaction Field Continuum Dielectric Theory for Short Range Hydrogen-Bonding Effects. *J. Phys. Chem.* **1996,** 100, 11775-11788.
132. Tannor, D. J.; Marten, B.; Murphy, R.; Friesner, R. A.; Sitkoff, D.; Nicholls, A.; Honig, B.; Ringnalda, M.; Goddard, W. A., III. Accurate First Principles Calculation of Molecular Charge Distributions and Solvation Energies from Ab Initio Quantum Mechanics and Continuum Dielectric Theory. *J. Am. Chem. Soc.* **1994,** 116, 11875-82.
133. Gutowsky, H. S.; Holm, C. H. Rate Processes and Nuclear Magnetic Resonance Spectra. II. Hindered Internal Rotation of Amides. *J. Chem. Phys.* **1956,** 25, 1228-1234.
134. Bain, A. D. Chemical exchange in NMR. *Prog. Nucl. Mag. Res. Sp.* **2003,** 43, 63-103.
135. Mnova. *v. 9.0.0-12821*, Mestrelab Research, S.L. (http://www.mestrelab.com): Santiago de Compostela, **2014**.
136. Lu, J.; Ma, D.; Hu, J.; Tang, W.; Zhu, D. Nuclear magnetic resonance spectroscopic studies of pyridine methyl derivatives binding to cytochrome c. *J. Chem. Soc. Dalton* **1998**, 2267-2274.
137. Perrin, C. L.; Dwyer, T. J. Application of two-dimensional NMR to kinetics of chemical exchange. *Chem. Rev.* **1990,** 90, 935-967.
138. Pons, M.; Millet, O. Dynamic NMR studies of supramolecular complexes. *Prog. Nucl. Mag. Res. Sp.* **2001,** 38, 267-324.
139. Zolnai, Z.; Juranić, N.; Vikić-Topić, D.; Macura, S. Quantitative Determination of Magnetization Exchange Rate Constants from a Series of Two-Dimensional Exchange NMR Spectra. *J. Chem. Inf. Model.* **2000,** 40, 611-621.
140. Cobas, J. C.; Martin-Pastor, M. *EXSYCalc v. 1.0* (http://desoft03.usc.es/mmartin/software.html), Mestrelab Research, S.L. (http://www.mestrelab.com): Santiago de Compostela, **2004**.
141. Tominaga, M.; Tominaga, T. Structure and function of TRPV1. *Pflugers Arch.* **2005,** 451, 143-150.
142. Neelands, T.; Jarvis, M.; Han, P.; Faltynek, C.; Surowy, C. Acidification of rat TRPV1 alters the kinetics of capsaicin responses. *Mol. Pain* **2005,** 1, 28.
143. Lukacs, V.; Rives, J.-M.; Sun, X.; Zakharian, E.; Rohacs, T. Promiscuous Activation of Transient Receptor Potential Vanilloid 1 (TRPV1) Channels by Negatively Charged Intracellular Lipids: the key role of endogenous phosphoinositides in maintaining channel activity. *J. Biol. Chem.* **2013,** 288, 35003-35013.
144. Ambrosino, P.; Soldovieri, M. V.; Russo, C.; Taglialatela, M. Activation and desensitization of TRPV1 channels in sensory neurons by the PPARα agonist palmitoylethanolamide. *Br. J. Pharmacol.* **2013,** 168, 1430-1444.
145. Valente, P.; Fernández-Carvajal, A.; Camprubí-Robles, M.; Gomis, A.; Quirce, S.; Viana, F.; Fernández-Ballester, G.; González-Ros, J. M.; Belmonte, C.; Planells-Cases, R.; Ferrer-Montiel, A. Membrane-tethered peptides patterned after the TRP

domain (TRPducins) selectively inhibit TRPV1 channel activity. *FASEB J.* **2011,** 25, 1628-1640.
146. Ferrer-Montiel, A. V.; Montal, M. Engineering the NMDA Receptor Channel Lining. *NMDA Receptor Protocols* **1999,** 128, 167-178.
147. Vidal-Mosquera, M.; Fernández-Carvajal, A.; Moure, A.; Valente, P.; Planells-Cases, R.; González-Ros, J. M.; Bujons, J.; Ferrer-Montiel, A.; Messeguer, A. Triazine-Based Vanilloid 1 Receptor Open Channel Blockers: Design, Synthesis, Evaluation, and SAR Analysis. *J. Med. Chem.* **2011,** 54, 7441-7452.
148. Mellor, I. R.; Ogilvie, J.; Pluteanu, F.; Clothier, R. H.; Parker, T. L.; Rosini, M.; Minarini, A.; Tumiatti, V.; Melchiorre, C. Methoctramine analogues inhibit responses to capsaicin and protons in rat dorsal root ganglion neurons. *Eur. J. Pharmacol.* **2004,** 505, 37-50.
149. Kitaguchi, T.; Swartz, K. J. An Inhibitor of TRPV1 Channels Isolated from Funnel Web Spider Venom *Biochemistry* **2005,** 44, 15544-15549.
150. Lardner, A. The effects of extracellular pH on immune function. *J. Leukocyte Biol.* **2001,** 69, 522-530.
151. Woolf, C. J.; Ma, Q. Nociceptors—Noxious Stimulus Detectors. *Neuron* **2007,** 55, 353-364.
152. Caterina, M. J.; Leffler, A.; Malmberg, A. B.; Martin, W. J.; Trafton, J.; Petersen-Zeitz, K. R.; Koltzenburg, M.; Basbaum, A. I.; Julius, D. Impaired Nociception and Pain Sensation in Mice Lacking the Capsaicin Receptor. *Science* **2000,** 288, 306-313.
153. Walder, R. Y.; Radhakrishnan, R.; Loo, L.; Rasmussen, L. A.; Mohapatra, D. P.; Wilson, S. P.; Sluka, K. A. TRPV1 is important for mechanical and heat sensitivity in uninjured animals and development of heat hypersensitivity after muscle inflammation. *Pain* **2012,** 153, 1664-1672.
154. Woodhull, A. M. Ionic Blockage of Sodium Channels in Nerve. *J. Gen. Physiol.* **1973,** 61, 687-708.
155. Bautista, D. M.; Siemens, J.; Glazer, J. M.; Tsuruda, P. R.; Basbaum, A. I.; Stucky, C. L.; Jordt, S.-E.; Julius, D. The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature* **2007,** 448, 204-208.
156. Andersen, H. H.; Olsen, R. V.; Møller, H. G.; Eskelund, P. W.; Gazerani, P.; Arendt-Nielsen, L. A review of topical high-concentration L-menthol as a translational model of cold allodynia and hyperalgesia. *European Journal of Pain* **2014,** 18, 315-325.
157. Zorumski, C. F.; Izumi, Y. NMDA receptors and metaplasticity: Mechanisms and possible roles in neuropsychiatric disorders. *Neurosci. Biobehav. R.* **2012,** 36, 989-1000.
158. Meyer, D.; Bonhoeffer, T.; Scheuss, V. Balance and Stability of Synaptic Structures during Synaptic Plasticity. *Neuron* **2014,** 82, 430-443.
159. Sanguinetti, M. C.; Jiang, C.; Curran, M. E.; Keating, M. T. A mechanistic link between an inherited and an acquird cardiac arrthytmia: HERG encodes the IKr potassium channel. *Cell* **1995,** 81, 299-307.
160. Viskin, S. Long QT syndromes and torsade de pointes. *Lancet* **1999,** 354, 1625-1633.
161. Milnes, J. T.; Crociani, O.; Arcangeli, A.; Hancox, J. C.; Witchel, H. J. Blockade of HERG potassium currents by fluvoxamine: incomplete attenuation by S6 mutations at F656 or Y652. *Br. J. Pharmacol.* **2003,** 139, 887-898.
162. The state of ion channel research in 2004. *Nat. Rev. Drug Discov.* **2004,** 3, 239-278.
163. Projean, D.; Lessard, E.; Ducharme, M. P.; Ducharme, J. Use of Freund's Complete Adjuvant (FCA) in inflammatory pain models: Consequences on the metabolism and pharmacokinetics of the non-peptidic delta receptor agonist SNC80 in the rat. *Xenobiotica* **2007,** 37, 870-883.

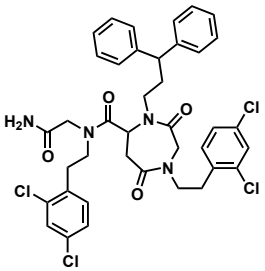
164. Garcia-Martinez, C.; Morenilla-Palao, C.; Planells-Cases, R.; Merino, J. M.; Ferrer-Montiel, A. Identification of an aspartic residue in the P-loop of the vanilloid receptor that modulates pore properties. *J. Biol. Chem.* **2000**.
165. Ferrer-Montiel, A.; Fernández-Carvajal, A.; González, J.; Belmonte, C.; Viana, F.; Gomis, A.; Messeguer, A.; Bujons, J.; Vidal-Mosquera, M. Triazine derivatives and their uses as TRPV1 inhibitors. **2012**.
166. Carbó-Dorca, R., Robert, D., Amat, L., Girones, X., Besalu, E. . *Molecular Quantum Similarity in QSAR and Drug Design*. Springer-Verlag: Berlin, Heidelberg, **2000**; 73, 123.
167. F., G. O. *Pharmacophore Perception, Development and Use in Drug Design*. International University Line: La Jolla, CA. , **2000**; 2, 560.
168. Vogt, M.; Bajorath, J. Predicting the Performance of Fingerprint Similarity Searching. *Methods Mol. Biol.* **2011,** 672, 159-173.
169. Myint, K.-Z.; Wang, L.; Tong, Q.; Xie, X.-Q. Molecular Fingerprint-Based Artificial Neural Networks QSAR for Ligand Biological Activity Predictions. *Mol Pharm.* **2012,** 9, 2912-2923.
170. Nantasenamat C, I.-N.-A. C., Naenna T, Prachayasittikul V A practical overview of quantitative structure-activity relationship. . *Excli J.* **2009,** 8, 74-88.
171. Kubinyi, H. *QSAR: Hansch Analysis and Related Approaches*. VCH: Weinheim, **1993**.
172. Goodford, P. J. A computational procedure for determining energetically favorable binding sites on biologically important macromolecules. *J. Med. Chem.* **1985,** 28, 849-857.
173. Kubinyi, H. *3D QSAR in Drug Design. Theory, Methods and Applications*. ESCOM: Leiden, **1993**.
174. Klebe, G.; Abraham, U. On the prediction of binding properties of drug molecules by comparative molecular field analysis. *J. Med. Chem.* **1993,** 36, 70-80.
175. Cramer, R. D.; Patterson, D. E.; Bunce, J. D. Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins. *J. Am. Chem. Soc.* **1988,** 110, 5959-5967.
176. Cramer, R. D., III; DePriest, S. A.; Patterson, D. E.; Hecht, P. The Developing Practice of Comparative Molecular Field Analysis. In *3D QSAR in Drug Design* Kubinyi, H., Ed. ESCOM: Leiden, **1993**; 443-485.
177. Klebe, G. Comparative Molecular Similarity Indices Analysis: CoMSIA. In *3D QSAR in Drug Design: Volume 3: Recent Advances*, Kubinyi, H.; Folkers, G.; Martin, Y. C., Eds. Kluwer Academic Publishers: New York, Boston, Dordrecht, London, Moscow, **1998**.
178. Klebe, G.; Abraham, U. Comparative Molecular Similarity Index Analysis (CoMSIA) to study hydrogen-bonding properties and to score combinatorial libraries. *J. Comput.-Aided Mol. Des.* **1999,** 13, 1-10.
179. Klebe, G.; Abraham, U.; Mietzner, T. Molecular Similarity Indices in a Comparative Analysis (CoMSIA) of Drug Molecules to Correlate and Predict Their Biological Activity. *J. Med. Chem.* **1994,** 37, 4130-4146.
180. Viswanadhan, V. N.; Sun, Y.; Norman, M. H. Three-Dimensional Quantitative Structure−Activity Relationships and Activity Predictions of Human TRPV1 Channel Antagonists: Comparative Molecular Field Analysis and Comparative Molecular Similarity Index Analysis of Cinnamides. *J. Med. Chem.* **2007,** 50, 5608-5619.
181. *SYBYL 7.3*, Tripos International: St. Louis, Missouri, **2008**.
182. Kubinyi, H.; Folkers, G.; Martin, Y. C.; Klebe, G. Comparative Molecular Similarity Indices Analysis: CoMSIA. In *3D QSAR in Drug Design*, Springer Netherlands: **2002**; 3, 87-104.

183. Jolliffe, I. T. *Principal Component Analysis*. Springer-Velag: New York, **2002**; 489.
184. Bush, B. L.; Nachbar, R. B. Sample-distance partial least squares: PLS optimized for many variables, with application to CoMFA. *J. Comput.-Aided Mol. Des.* **1993,** 7, 587-619.
185. Zhang, L. T., K. C.; Du, L.; Fang, H.; Li, M.; Xu, W. How to generate reliable and predictive CoMFA models. *Curr Med Chem* **2011,** 18, 923-930.
186. Golbraikh, A.; Tropsha, A. Beware of q2! *J Mol Graph Model* **2002,** 20, 269-276.
187. Jara-Oseguera, A.; Llorente, I.; Rosenbaum, T.; Islas, L. D. Properties of the Inner Pore Region of TRPV1 Channels Revealed by Block with Quaternary Ammoniums. *J. Gen. Physiol.* **2008,** 132, 547-562.
188. Long, S. B.; Campbell, E. B.; MacKinnon, R. Crystal Structure of a Mammalian Voltage-Dependent Shaker Family K+ Channel. *Science* **2005,** 309, 897-903.
189. Long, S. B.; Campbell, E. B.; MacKinnon, R. Voltage Sensor of Kv1.2: Structural Basis of Electromechanical Coupling. *Science* **2005,** 309, 903-908.
190. Halgren, T. A. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J. Comput. Chem.* **1996,** 17, 490-519.
191. Halgren, T. A. MMFF VI. MMFF94s option for energy minimization studies. *J. Comput. Chem.* **1999,** 20, 720-729.
192. Halgren, T. A. MMFF VII. Characterization of MMFF94, MMFF94s, and other widely available force fields for conformational energies and for intermolecular-interaction energies and geometries. *J. Comput. Chem.* **1999,** 20, 730-748.
193. *MOE, version 2009.10*, Chemical Computing Group: Montreal, **2009**.
194. Labute, P.; Williams, C.; Feher, M.; Sourial, E.; Schmidt, J. M. Flexible Alignment of Small Molecules. *J. Med. Chem.* **2001,** 44, 1483-1490.
195. Liu, Z.; Hartwig, J. F. Mild, Rhodium-Catalyzed Intramolecular Hydroamination of Unactivated Terminal and Internal Alkenes with Primary and Secondary Amines. *J. Am. Chem. Soc.* **2008,** 130, 1570-1571.
196. Hesp, K. D.; Stradiotto, M. Intramolecular Hydroamination of Unactivated Alkenes with Secondary Alkyl- and Arylamines Employing [Ir(COD)Cl]2 as a Catalyst Precursor. *Org. Lett.* **2009,** 11, 1449-1452.
197. Zhang, J.; Yang, C.-G.; He, C. Gold(I)-Catalyzed Intra- and Intermolecular Hydroamination of Unactivated Olefins. *J. Am. Chem. Soc.* **2006,** 128, 1798-1799.
198. Davoren, J. E.; Gray, D. L.; Harris, A. R.; Nason, D. M.; Xu, W. Remarkable [3+2] Annulations of Electron-Rich Olefins with Unstabilized Azomethine Ylides. *Synlett* **2010,** 2010, 2490-2492.
199. Srihari, P.; Yaragorla, S. R.; Basu, D.; Chandrasekhar, S. Tris(pentafluorophenyl)borane-Catalyzed Synthesis of *N*-Benzyl Pyrrolidines. *Synthesis* **2006,** 2006, 2646-2648.
200. Fukuzawa, S.-i.; Oki, H. Highly Enantioselective Asymmetric 1,3-Dipolar Cycloaddition of Azomethine Ylide Catalyzed by a Copper(I)/ClickFerrophos Complex. *Org. Lett.* **2008,** 10, 1747-1750.
201. Specker, E.; Böttcher, J.; Brass, S.; Heine, A.; Lilie, H.; Schoop, A.; Müller, G.; Griebenow, N.; Klebe, G. Unexpected Novel Binding Mode of Pyrrolidine-Based Aspartyl Protease Inhibitors: Design, Synthesis and Crystal Structure in Complex with HIV Protease. *ChemMedChem* **2006,** 1, 106-117.
202. March, J. *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*. Wiley-Interscience: New Jersey, **1992**.
203. Ohshima, T.; Hayashi, Y.; Agura, K.; Fujii, Y.; Yoshiyama, A.; Mashima, K. Sodium methoxide: a simple but highly efficient catalyst for the direct amidation of esters. *Chem. Commun.* **2012,** 48, 5434-5436.

204. Movassaghi, M.; Schmidt, M. A. N-Heterocyclic Carbene-Catalyzed Amidation of Unactivated Esters with Amino Alcohols. *Org. lett.* **2005,** 7, 2453-2456.
205. Matsumoto, K.; Hashimoto, S.; Uchida, T.; Okamoto, T. Direct aminolysis of nonactivated and thermally unstable esters at high pressure. *Chemische Berichte* **1989,** 122, 1357-1363.
206. Sridharan, V.; Ruiz, M.; Menéndez, J. C. Mild and High-Yielding Synthesis of ß-Keto Esters and ß-Ketoamides. *Synthesis* **2010,** 2010, 1053-1057.
207. Rezazgui, O.; Boëns, B.; Teste, K.; Vergnaud, J.; Trouillas, P.; Zerrouki, R. One-pot and catalyst-free amidation of ester: a matter of non-bonding interactions. *Tetrahedron Lett.* **2011,** 52, 6796-6799.
208. Vollhardt, K. P. C. S., N.E. Organic Chemistry: Structure and Function. In W.H. Freeman: New York, NY, USA, **1999**; 342-343.
209. Pu, Y.-M.; Grieme, T.; Gupta, A.; Plata, D.; Bhatia, A. V.; Cowart, M.; Ku, Y.-Y. A Facile and Scaleable Synthesis of ABT-239, A Benzofuranoid H3 Antagonist. *Org. Process. Res. Dev.* **2004,** 9, 45-50.
210. Lane, C. F. Reduction of organic compounds with diborane. *Chem. Rev.* **1976,** 76, 773-799.
211. Yoon, N. M.; Pak, C. S.; Brown Herbert, C.; Krishnamurthy, S.; Stocky, T. P. Selective reductions. XIX. Rapid reaction of carboxylic acids with borane-tetrahydrofuran. Remarkably convenient procedure for the selective conversion of carboxylic acids to the corresponding alcohols in the presence of other functional groups. *J. Org. Chem.* **1973,** 38, 2786-2792.
212. Zaidlewicz, M.; Brown, H. C.; Neelamkavil, S. F. Borane–Tetrahydrofuran. In *e-EROS Encyclopedia of Reagents for Organic Synthesis*, **2008**.
213. Amedio, J. C.; Bernard, P. J.; Fountain, M.; Wagenen, G. V. A Practical Manufacturing Synthesis of 1-(R)-Hydroxymethyl-Dtpa: An Important Intermediate in the Synthesis of MRI Contrast Agents. *Synth. Commun.* **1999,** 29, 2377-2391.
214. Nagarajan, S.; Ganem, B. Chemistry of naturally occurring polyamines. 10. Nonmetabolizable derivatives of spermine and spermidine. *J. Org. Chem.* **1986,** 51, 4856-4861.
215. Threadgill, M. D.; Webb, P. Selective Reductions of 1-(Carbonyl)Substituted 2-Nitro-Imidazoles with Alkali Metal Borohydrides and with Borane. Tetrahydrofuran. *Synth. Commun.* **1990,** 20, 2319-2325.
216. Valente, P.; Garcia-Sanz, N.; Gomis, A.; Fernandez-Carvajal, A.; Fernandez-Ballester, G.; Viana, F.; Belmonte, C.; Ferrer-Montiel, A. Identification of molecular determinants of channel gating in the transient receptor potential box of vanilloid receptor I. *FASEB J.* **2008,** 22, 3298-3309.
217. Ferrer-Montiel, A. V.; Merino, J. M.; Planells-Cases, R.; Sun, W.; Montal, M. Structural determinants of the blocker binding site in glutamate and NMDA receptor channels. *Neuropharmacology* **1998,** 37, 139-147.
218. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* **1983,** 65, 55-63.
219. Heppelmann, B.; Pawlak, M. Inhibitory effect of somatostatin on the mechanosensitivity of articular afferents in normal and inflamed knee joints of the rat. *PAIN* **1997,** 73, 377-382.
220. *Epik, version 2.1*, Schrödinger, L.L.C.: New York, NY, **2010**.
221. Greenwood, J.; Calkins, D.; Sullivan, A.; Shelley, J. Towards the comprehensive, rapid, and accurate prediction of the favorable tautomeric states of drug-like molecules in aqueous solution. *J. Comput.-Aided Mol. Des.* **2010,** 24, 591-604.

222. Shelley, J.; Cholleti, A.; Frye, L.; Greenwood, J.; Timlin, M.; Uchimaya, M. Epik: a software program for pK a prediction and protonation state generation for drug-like molecules. *J. Comput.-Aided Mol. Des.* **2007,** 21, 681-691.

Annex 1 - Molecule index.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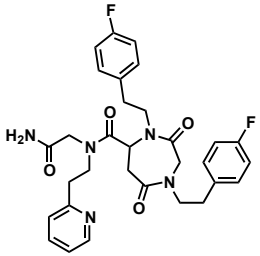
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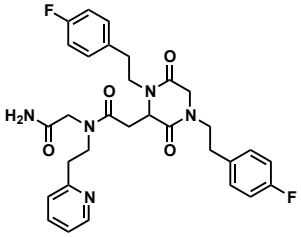
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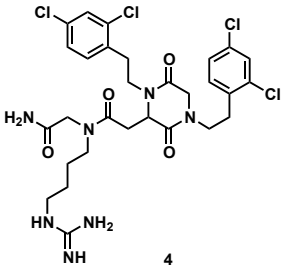
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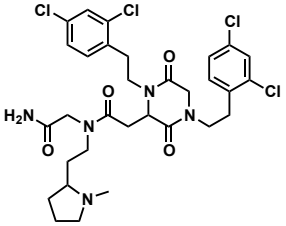
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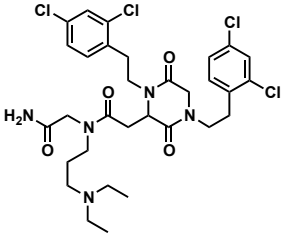
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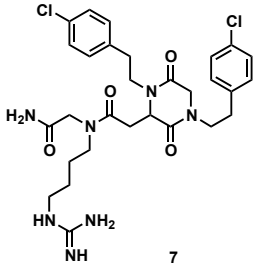
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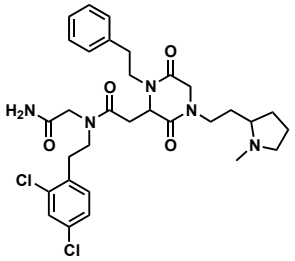
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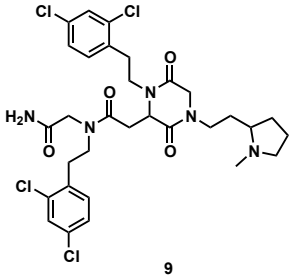
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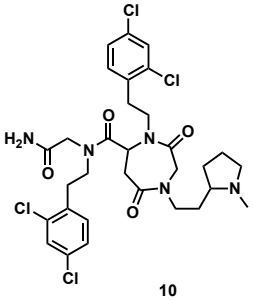
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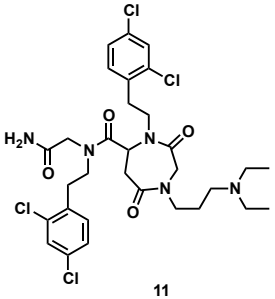
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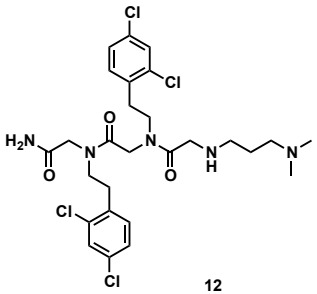
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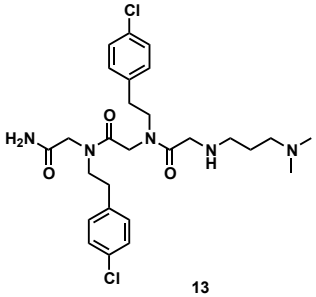
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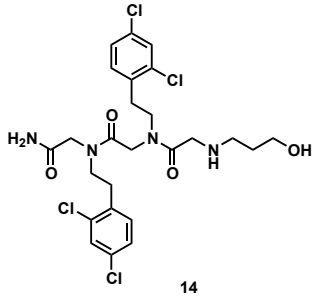
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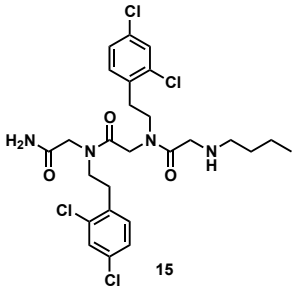
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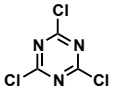
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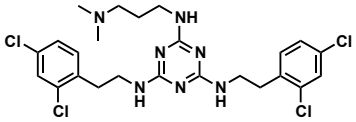
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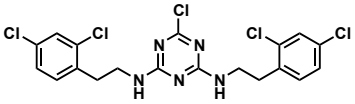
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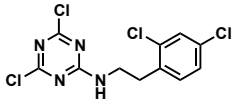
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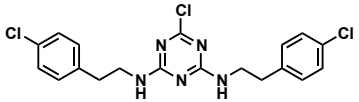
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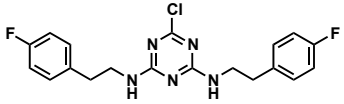
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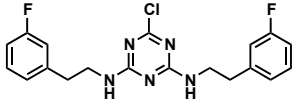
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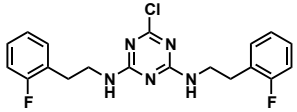
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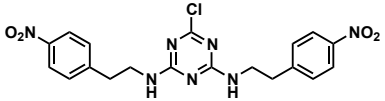
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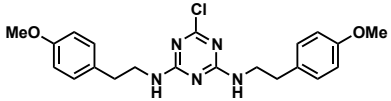
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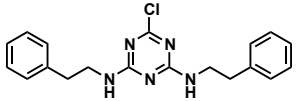
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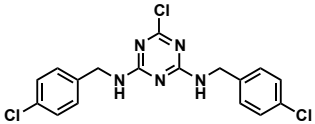
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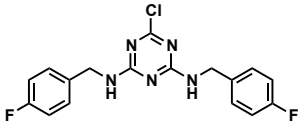
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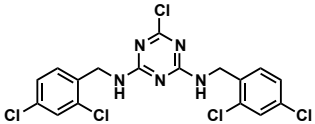
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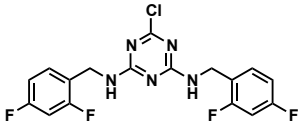
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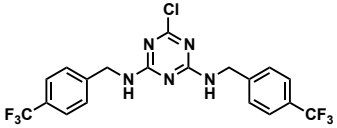
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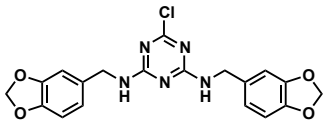
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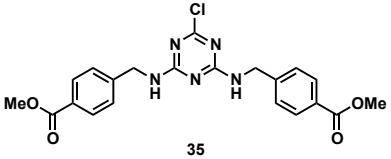
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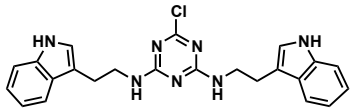
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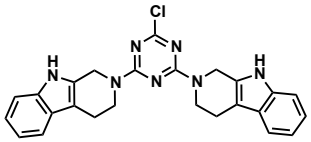
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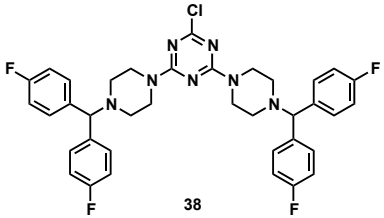
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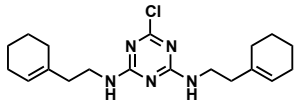
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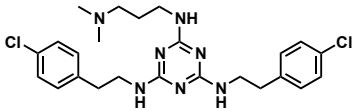
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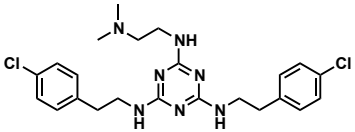
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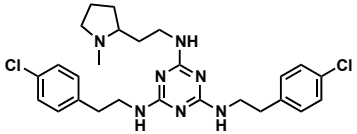
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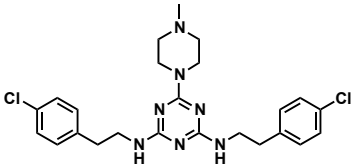
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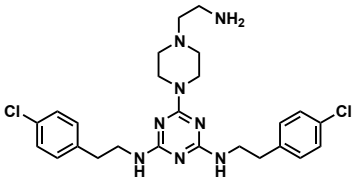
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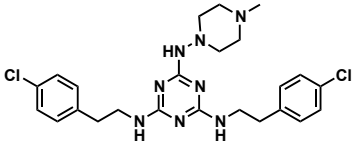
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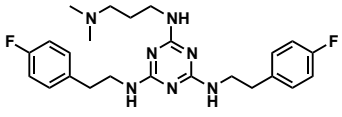
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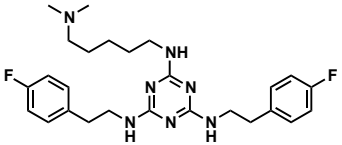
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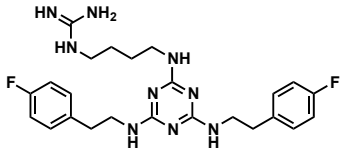
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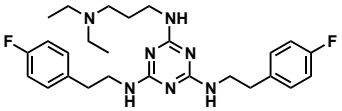
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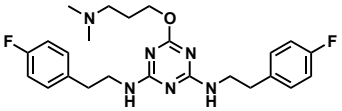
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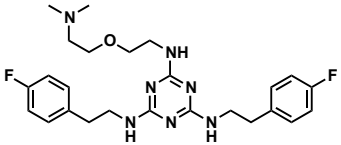
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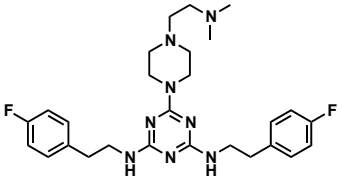
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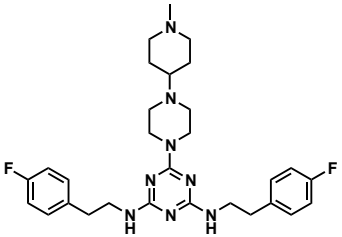
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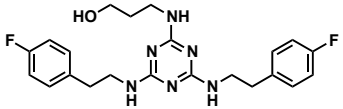
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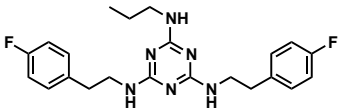
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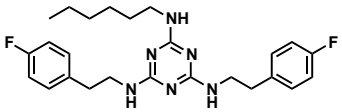
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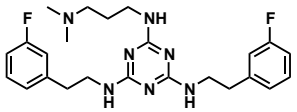
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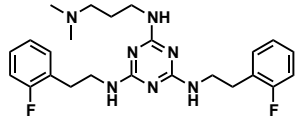
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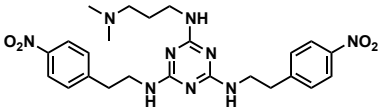
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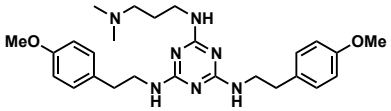
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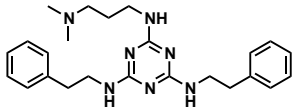
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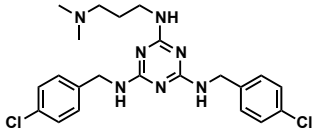
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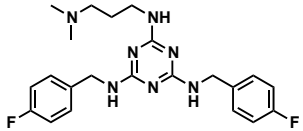
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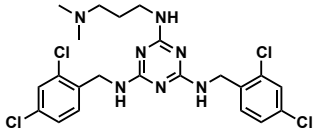
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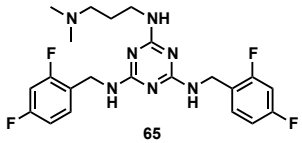
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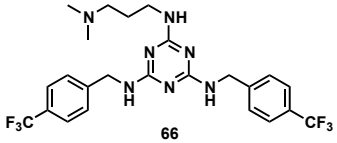
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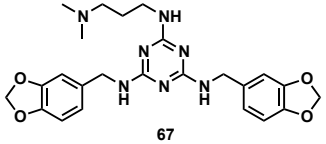
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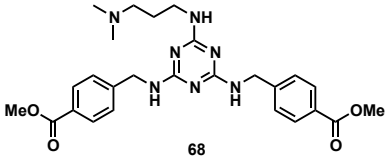
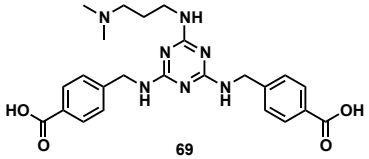

68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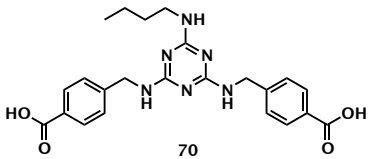
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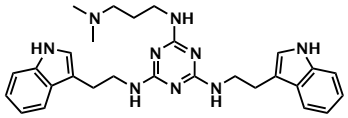
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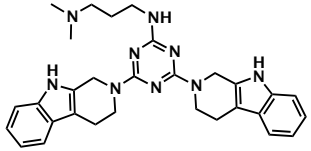
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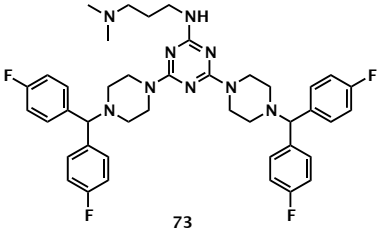
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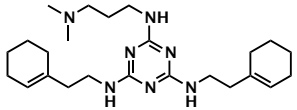
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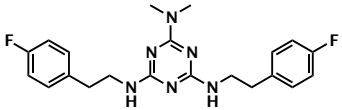
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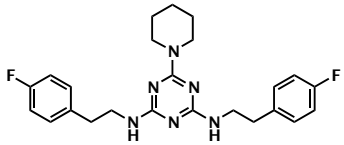
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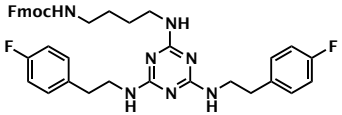
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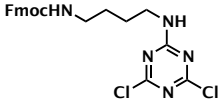
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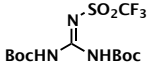
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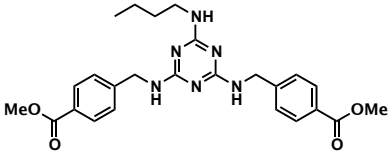
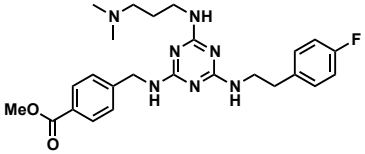

81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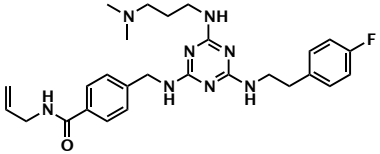
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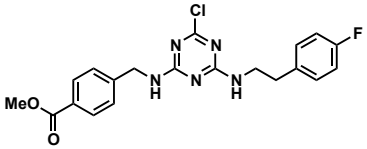
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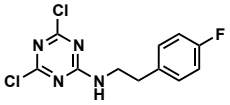
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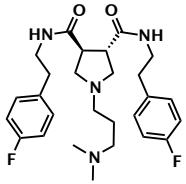
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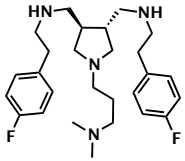
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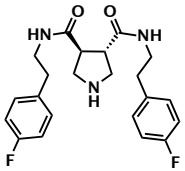
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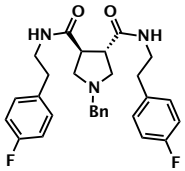
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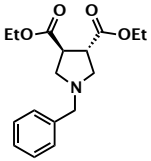
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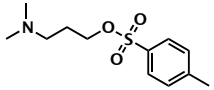
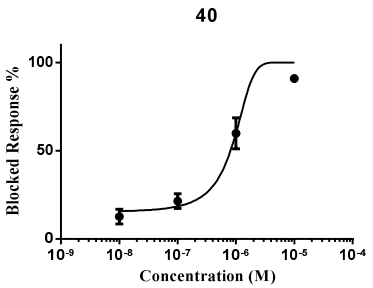
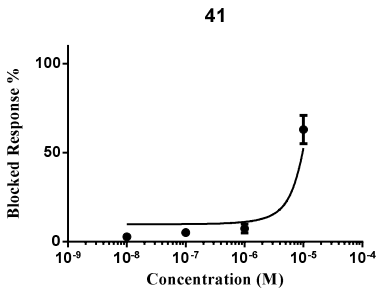
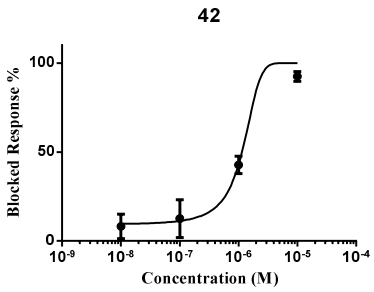
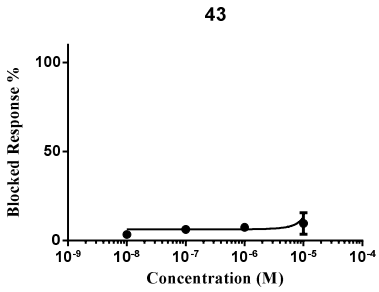

91

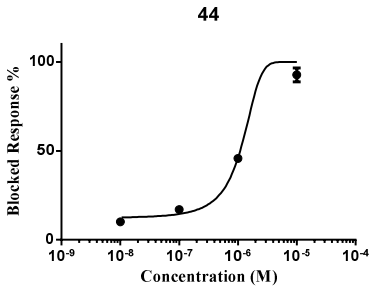
Annex 2 – Dose-Response curves.

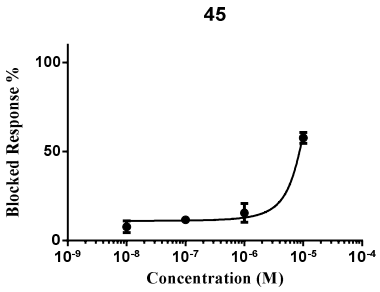

40
Blocked Response %
100
50
0
10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

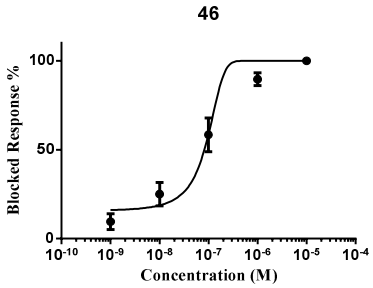

41
Blocked Response %
100
50
0
10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

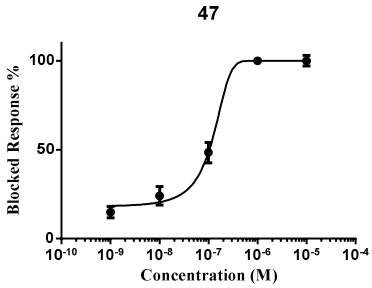

42
Blocked Response %
100
50
0
10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

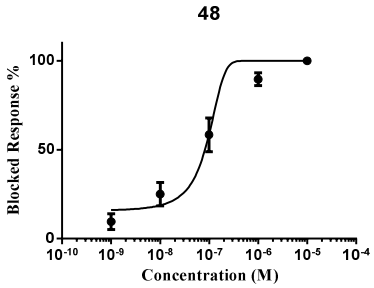

43
Blocked Response %
100
50
0
10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

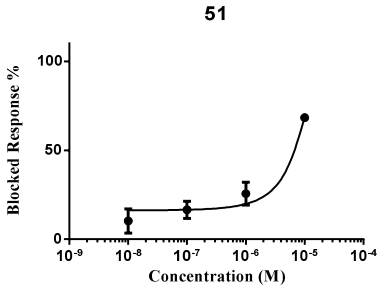

44
Blocked Response %
100
50
0
10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

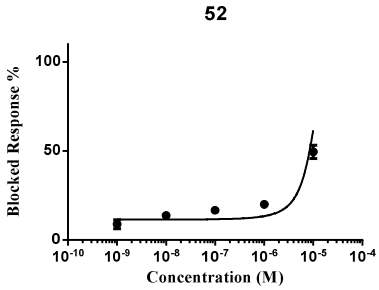

45
Blocked Response %
100
50
0
10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

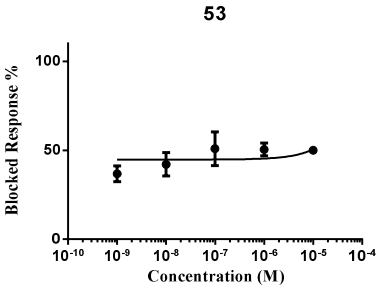

46
Blocked Response %
100
50
0
10-10 10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

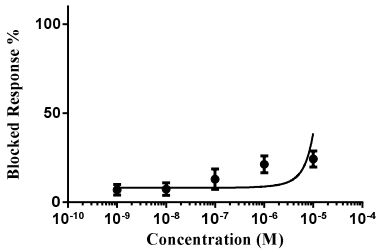

47
Blocked Response %
100
50
0
10-10 10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

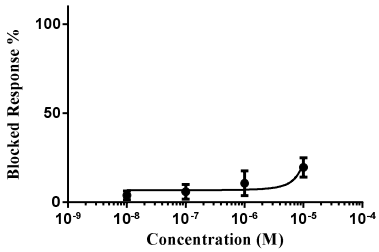

48
Blocked Response %
100
50
0
10-10 10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

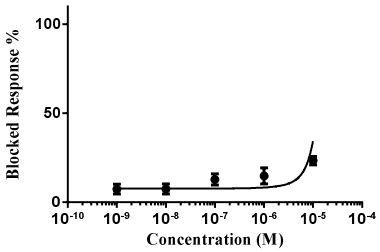

51
Blocked Response %
100
50
0
10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

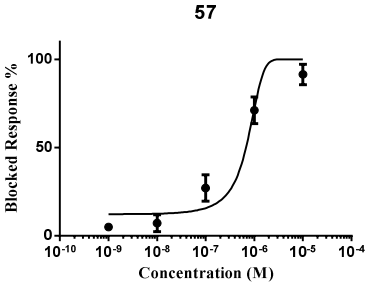

52
Blocked Response %
100
50
0
10-10 10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

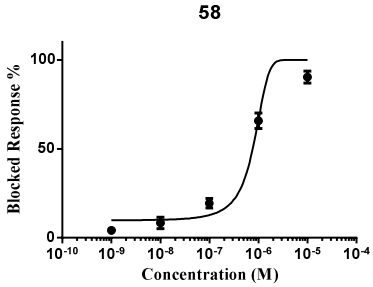

53
Blocked Response %
100
50
0
10-10 10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

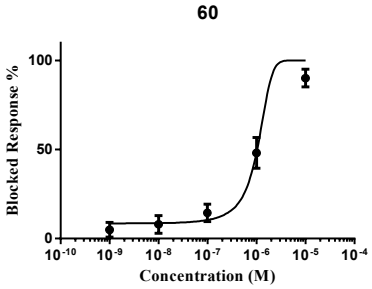

54
Blocked Response %
100
50
0
10-10 10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

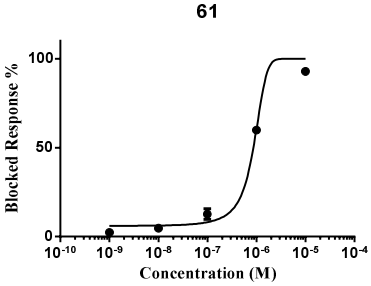

55
Blocked Response %
100
50
0
10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

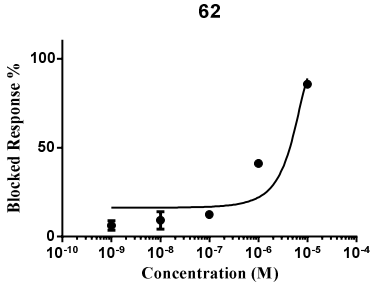

56
Blocked Response %
100
50
0
10-10 10-9 10-8 10-7 10-6 10-5 10-4
Concentration (M)

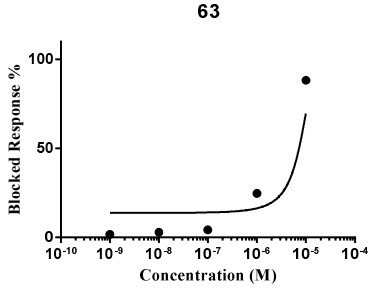

57
Blocked Response %
Concentration (M)

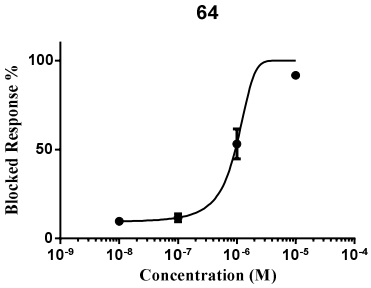

58
Blocked Response %
Concentration (M)

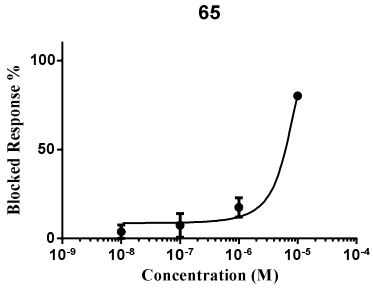

60
Blocked Response %
Concentration (M)

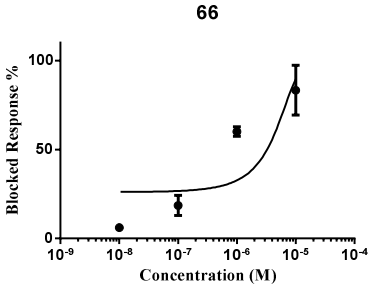

61
Blocked Response %
Concentration (M)

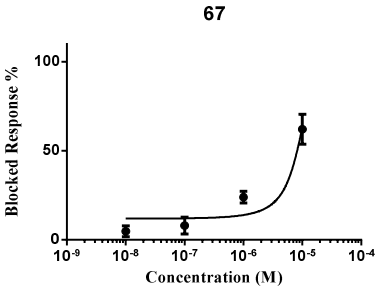

62
Blocked Response %
Concentration (M)

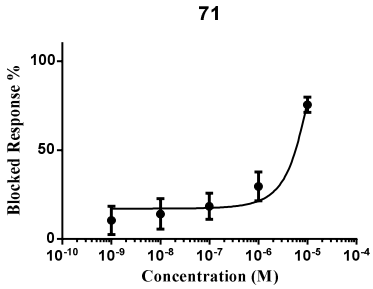

63
Blocked Response %
Concentration (M)

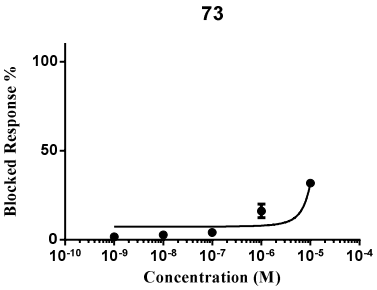

64
Blocked Response %
Concentration (M)

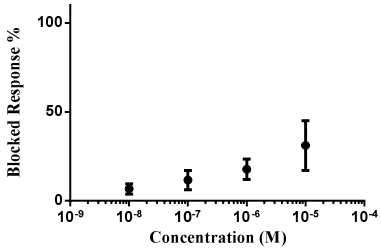

65
Blocked Response %
Concentration (M)


66
Blocked Response %
Concentration (M)

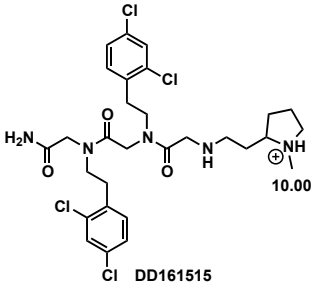

67
Blocked Response %
Concentration (M)


71
Blocked Response %
Concentration (M)

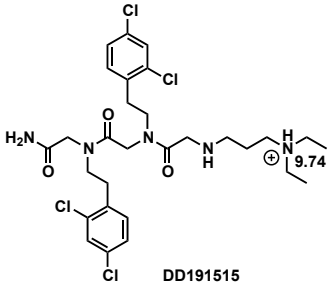

73
Blocked Response %
Concentration (M)


74
Blocked Response %
Concentration (M)

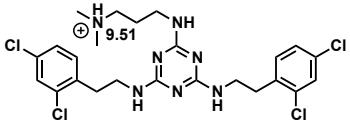
Annex 3 - Evaluated pKa for synthesized molecules.



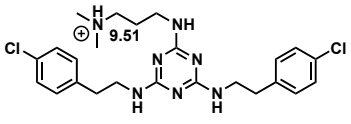
DD161515



DD191515



17



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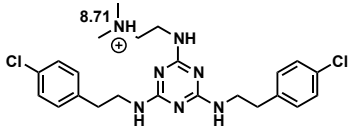

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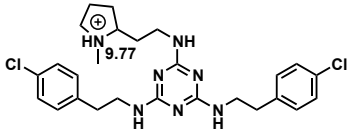

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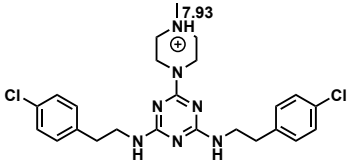

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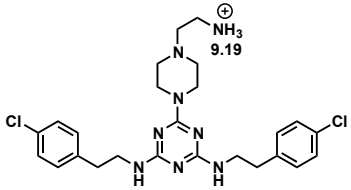

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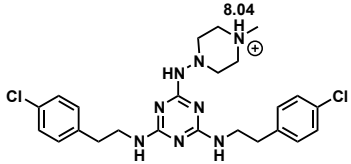

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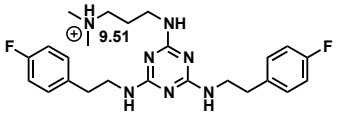

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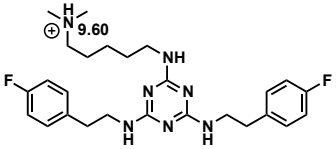

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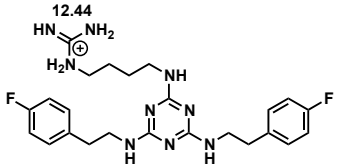

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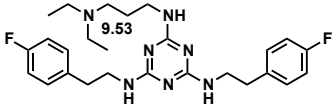

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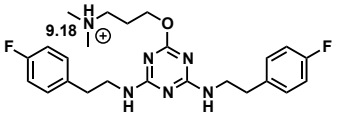

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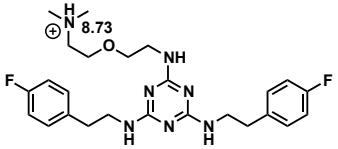

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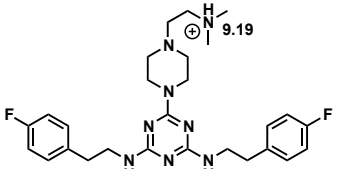

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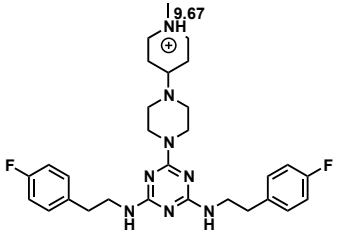

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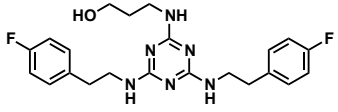

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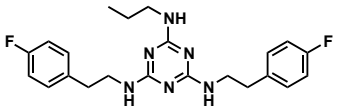

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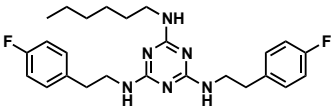

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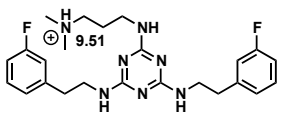
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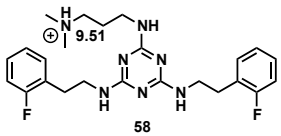
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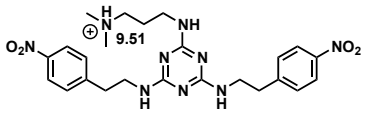
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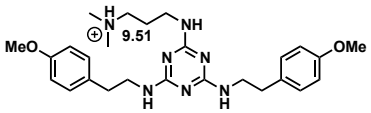
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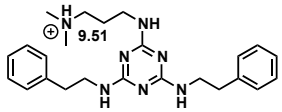
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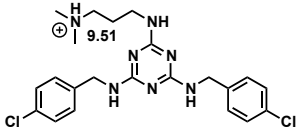
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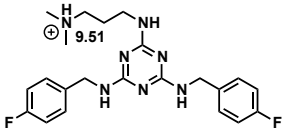
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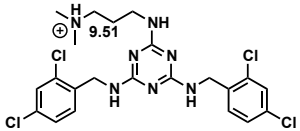
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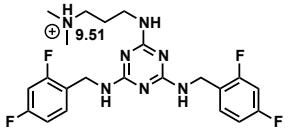
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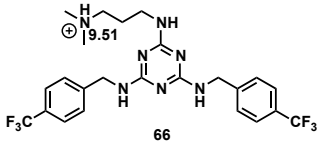
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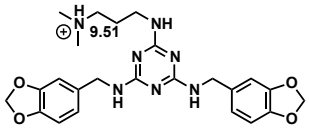
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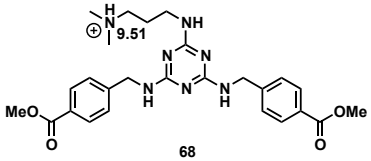
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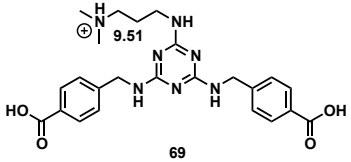
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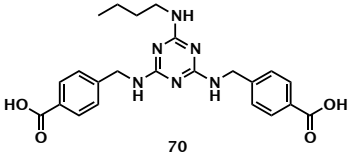
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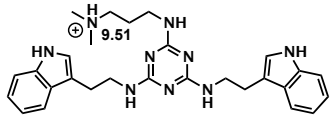
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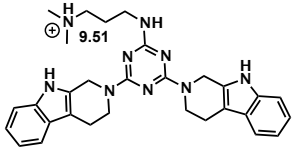
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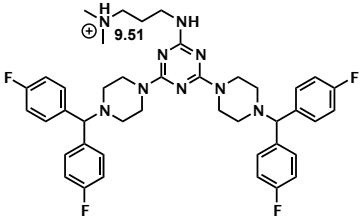
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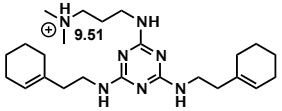
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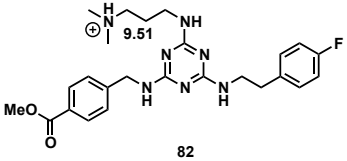
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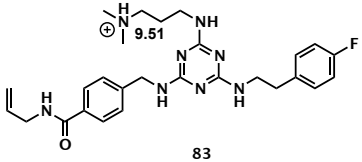
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Annex 4 – Published article.

Journal of Medicinal Chemistry

ARTICLE

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Triazine-Based Vanilloid 1 Receptor Open Channel Blockers: Design, Synthesis, Evaluation, and SAR Analysis†

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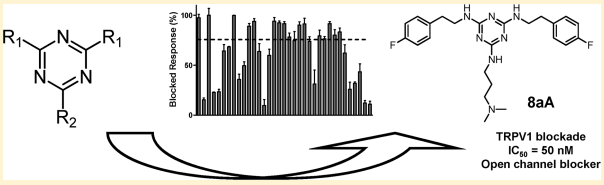
S Supporting Information

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 Ca^{2+} 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 overactivated receptors.

Received: March 15, 2011
Published: September 27, 2011

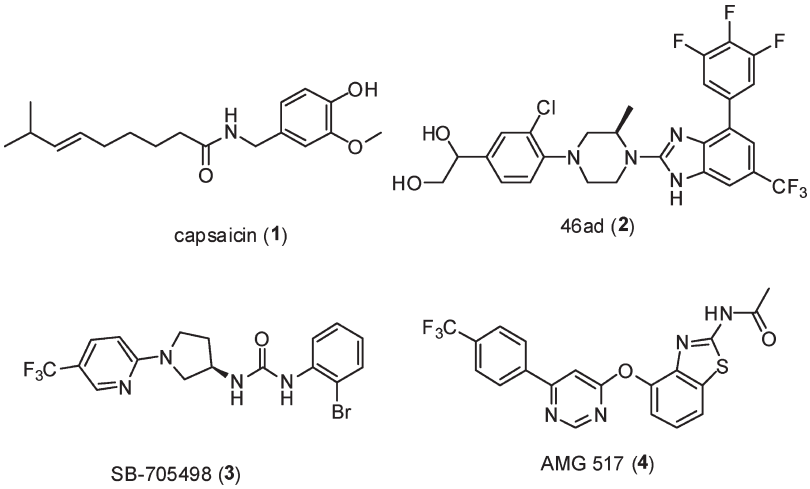


Figure 1. Relevant TRPV1 ligands: the agonist capsaicin (**1**) and competitive antagonists (**2**−**4**).

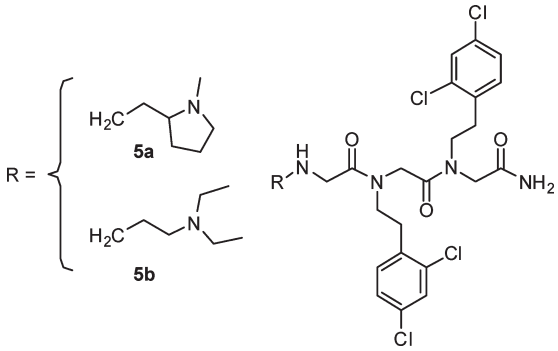


Figure 2. *N*-Alkylglycine trimers that block TRPV1.[14]

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 TRV1 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

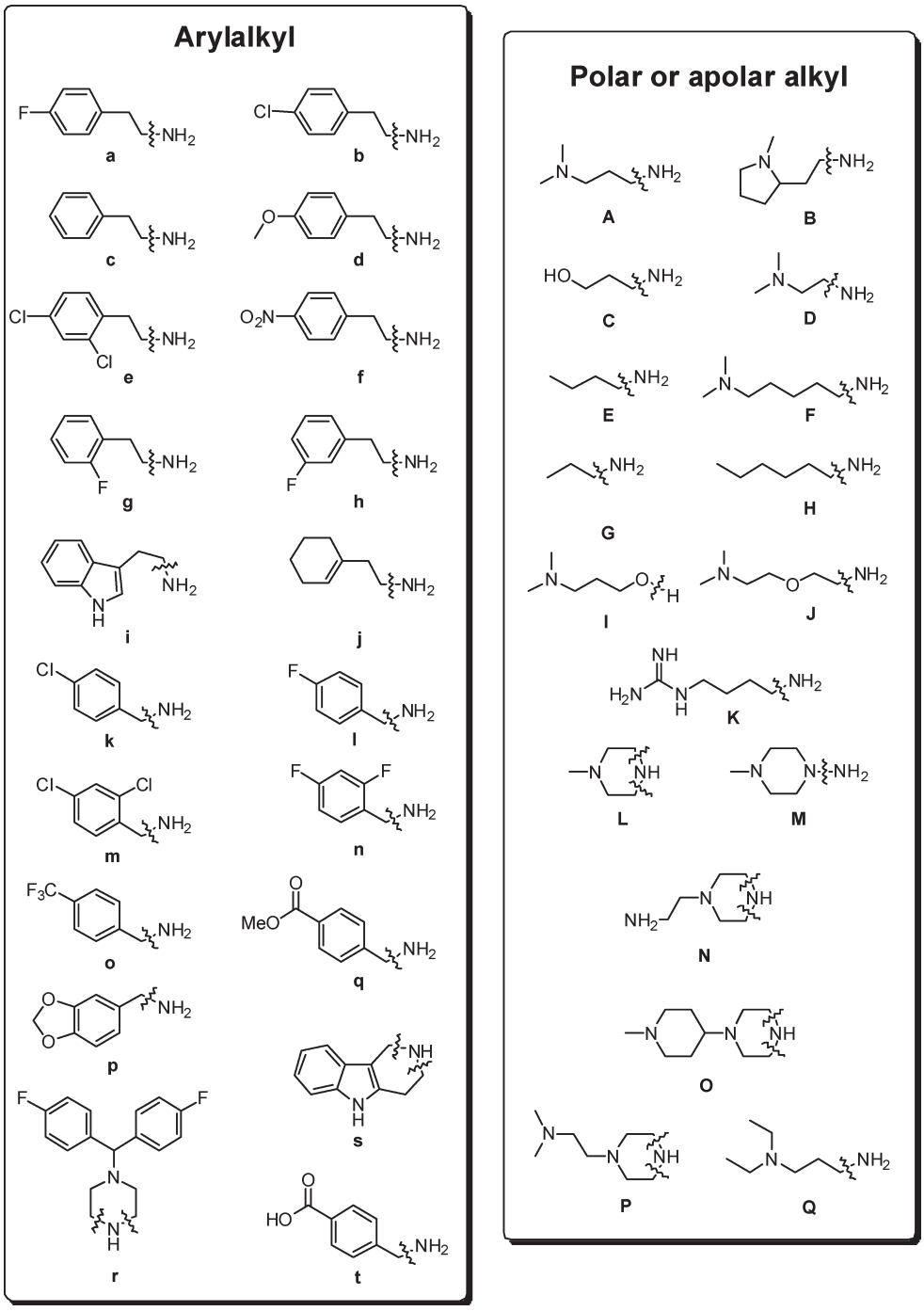


Figure 3. Formal sources of chemical diversity incorporated in the new series of TRPV1 blockers. The wavy lines indicate the bond(s) of attachment to the corresponding scaffold.

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]

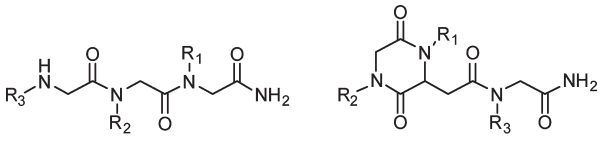
The initial results for some of the new compounds (e.g., **8eA**; see below) were promising, leading us to design and synthesize a

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 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 μM it completely

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 μM)[a]

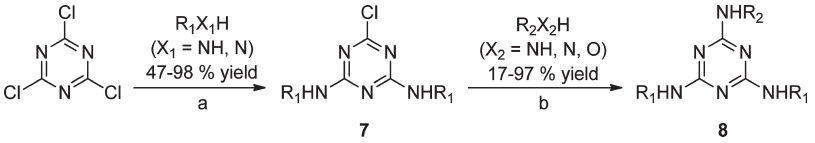


5 **6**

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

[a] The substituents R_1−R_3 refer to the groups shown in Figure 3.

Scheme 1. General Synthesis of the Trisubstituted Triazines 8[a]



[a] Reaction conditions: (a) R_1 = arylalkyl (Figure 3), microwave irradiation at 70 °C (max potency 90 W), 10 min, THF; (b) R_2 = 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} (μM)[b]	compd	X_1-R_1	X_2-R_2	IC_{50} (μM)[b]
8aA	NH-a	NH-A	0.05 ± 0.007	**8eA**	NH-e	NH-A	1.21 ± 0.2
8aC	NH-a	NH-C	1488 ± 774[c]	**8fA**	NH-f	NH-A	1.63 ± 0.37
8aF	NH-a	NH-F	0.1 ± 0.04	**8gA**	NH-g	NH-A	0.45 ± 0.07
8aG	NH-a	NH-G	2173 ± 1745[c]	**8hA**	NH-h	NH-A	0.30 ± 0.04
8aH	NH-a	NH-H	5761 ± 6716[c]	**8iA**	NH-i	NH-A	2.33 ± 1.23
8aI	NH-a	O-I	6.14 ± 0.7	**8jA**	NH-j	NH-A	218 ± 104
8aJ	NH-a	NH-J	7.05 ± 2.99	**8kA**	NH-k	NH-A	2.62 ± 0.94
8aK	NH-a	NH-K	0.55 ± 0.06	**8lA**	NH-l	NH-A	3.06 ± 0.22
8aO	NH-a	N-O	70.9 ± 9.1	**8mA**	NH-m	NH-A	0.84 ± 0.18
8aP	NH-a	N-P	18.6 ± 6	**8nA**	NH-n	NH-A	3.30 ± 0.61
8bA	NH-b	NH-A	0.50 ± 0.15	**8oA**	NH-o	NH-A	0.66 ± 0.11
8bB	NH-b	NH-B	1.2 ± 0.03	**8pA**	NH-p	NH-A	4.98 ± 0.67
8bD	NH-b	NH-D	6.7 ± 2.4	**8qA**	NH-q	NH-A	69.1 ± 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 ± 14
8bM	NH-b	NH-M	6.4 ± 2.4	**8sA**	N-s	NH-A	7.0 ± 2.9
8bN	NH-b	N-N	0.96 ± 0.21	**8tA**	NH-t	NH-A	178 ± 47
8cA	NH-c	NH-A	0.67 ± 0.06	**8tE**	NH-t	NH-E	229 ± 23
8dA	NH-d	NH-A	0.97 ± 0.19				

[a] Substitutions in X_1-R_1 and X_2-R_2 refer to the groups shown in Figure 3 (see also Figure S2). [b] Values derived from the corresponding dose response curves. Responses were recorded at −60 mV and normalized with respect to that elicited by 10 μM capsaicin. Data are shown as the mean ± SD, with $n \geq 6$. [c] Extrapolated IC_{50} values.

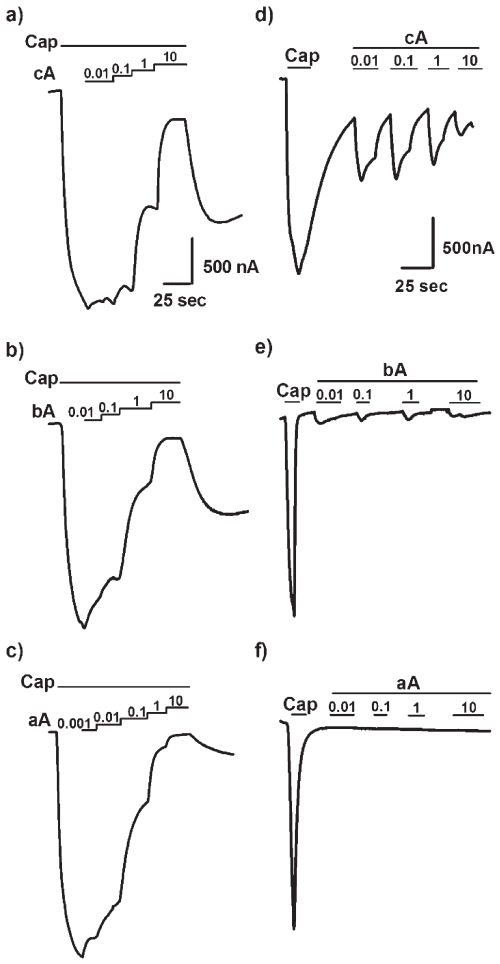


Figure 4. Representative ionic currents showing the blocking of capsaicin-evoked responses (a−c) and triazine-induced TRPV1 activity (d−f) of compounds **8cA** (a, d), **8bA** (b, e), and **8aA** (c, f) at the different concentrations (μM) indicated. Ionic currents were elicited from oocytes heterologously 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_H = 0.9 ± 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 voltages, 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 δ = 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 at 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

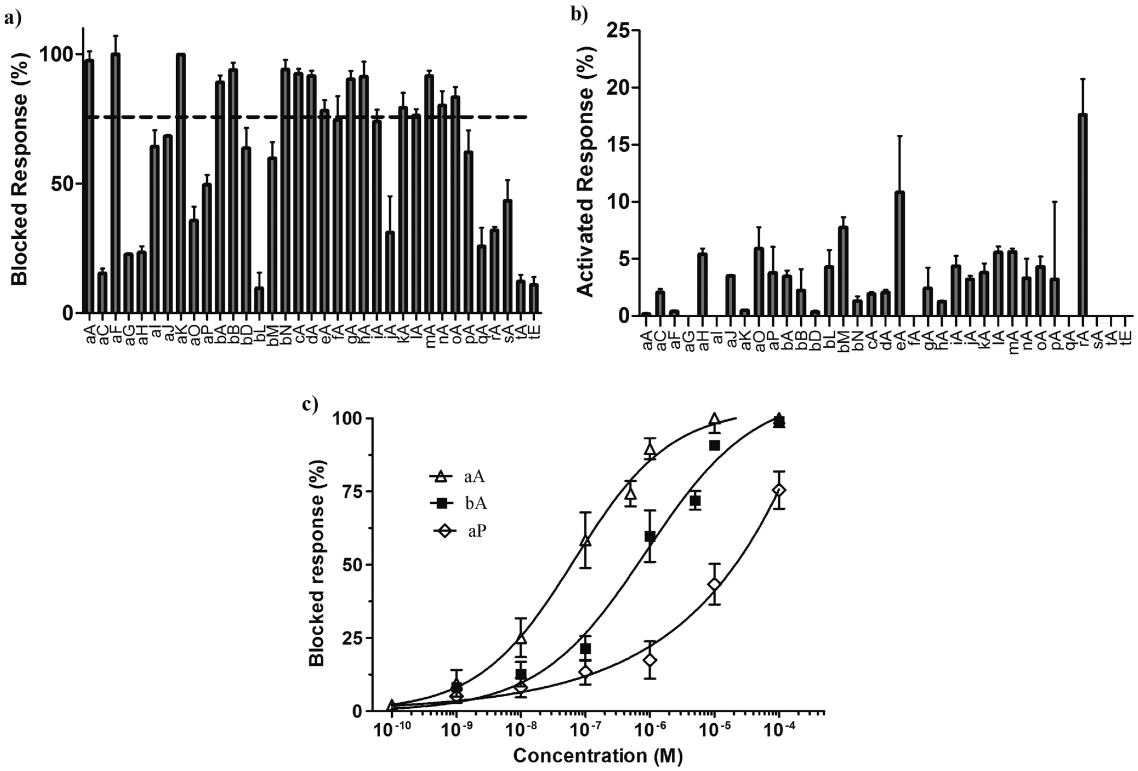


Figure 5. Extent of TRPV1 blocking and activation by the triazines **8**. (a) Blocked response of capsaicin-evoked current at 10 μM triazine and (b) ionic currents activated at 10 μM triazine in oocytes heterologously expressing TRPV1. (c) Representative dose−response curves displaying the blocking of 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-trisubstitued-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 μm, 4.6 mm × 150 mm), with CH_3CN-H_2O mixtures containing 0.1% TFA at 1 mL/min as mobile phase and with

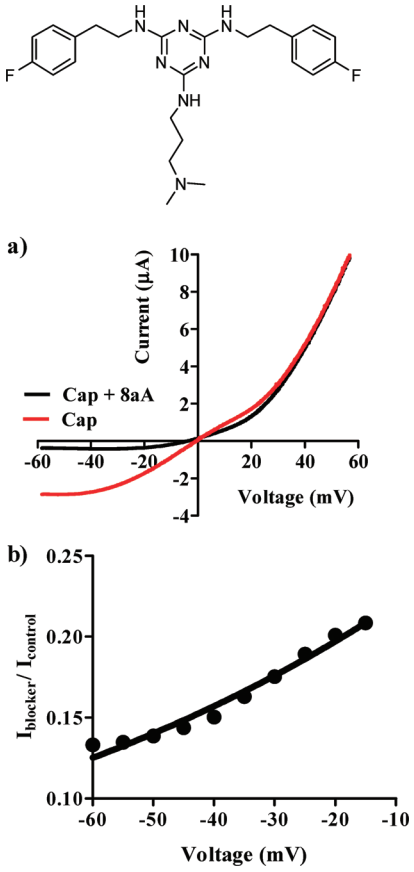


Figure 6. Voltage dependency of TRPV1 blocking by the triazine **8aA** (a). Representative ionic currents were evoked by 10 μM capsaicin using a linear ramp from −60 to +60 mV in the absence (red line) or presence (black line) of **8aA** at 10 μM. (b) Fractional blocking of TRPV1 by **8aA** as a function 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 μm, 47 mm × 300 mm), CH_3CN-H_2O 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 (^{1}H 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 ^{1}H 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 °C) or in the presence of ∼7% trifluoroacetic acid (TFA; broad signal at ∼10.5 ppm in ^{1}H 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 μm, 2.1 mm × 100 mm), with CH_3CN-H_2O mixtures containing 20 mM HCOOH as mobile phases. The purity of compounds **8** was checked by reverse phase HPLC and confirmed to be ≥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 guanidinylated using the protocol described by Feichtinger et al.[30] (see Supporting Information).

***N*-Alkyglycine 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. ^{1}H NMR (CD_3CN, 40 °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 °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 $C_{27}H_{35}C_{l4}N_5O$ (M + 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. ^{1}H NMR (CD_3CN, 40 °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 °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 $C_{27}H_{37}Cl_2N_5O_3$ (M + H), 550.2374; found, 550.2352.

[*N*-Butylglycyl]-[*N*-(2′,4′-dichlorophenethyl)glycyl]-*N*-(2′,4′-dichlorophenethyl)glycinamide (5e). Yield: 72 mg, 37%. ^{1}H 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 $C_{26}H_{32}Cl_4N_4O_3$ (M + 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. ^{1}H 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 $C_{25}H_{30}Cl_4N_4O_4$ (M + 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)aminocarbonylmethyl]-1,4-bis(2′,4′-dichlorophenethyl)piperazine-2,5-dione (6a). Yield: 10 mg, 6%. ^{1}H NMR (DMSO-d_6, 40 °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, 10 H), 1.62 (m, 2H), 1.54 (m, 2H). ^{13}C NMR (DMSO-d_6, 40 °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 $C_{29}H_{36}Cl_4N_7O_3$ (M + 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%. ^{1}H 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 $C_{31}H_{39}Cl_4N_5O_4$ (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%. ^{1}H 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 $C_{31}H_{40}Cl_4N_5O_4$ (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%. ^{1}H 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 $C_{29}H_{38}Cl_2N_7O_4$ (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%. ^{1}H 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 $C_{31}H_{41}Cl_2N_5O_4$ (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%. ^{1}H 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: $C_{31}H_{39}Cl_4N_5O_4$ (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. ^{1}H 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 $C_{24}H_{31}N_7F_2$ (M + H), 456.2687; found, 456.2668.

2,4-Bis(4′-fluorophenethylamino)-6-(3′-hydroxypropyl-amino)-1,3,5-triazine (8aC). Yield: 100 mg, 91%, colorless oil. ^{1}H 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 $C_{22}H_{26}N_6OF_2$ (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. ^{1}H 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 $C_{26}H_{35}N_7F_2$ (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. ^{1}H 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 $C_{22}H_{26}N_6F_2$ (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. ^{1}H 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 $C_{25}H_{32}N_6F_2$ (M + H), 455.2734; found, 455.2739.

2,4-Bis(4′-fluorophenethylamino)-6-(3′-(*N*,*N*-dimethyl-amino)propoxy)-1,3,5-triazine (8aI). Yield: 90 mg, 38%, colorless oil. ^{1}H 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 $C_{24}H_{30}N_6F_2O$ (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. ^{1}H 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 $C_{25}H_{33}N_7F_2O$ (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. ^{1}H 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 $C_{24}H_{31}N_9F_2$ (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. ^{1}H 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 $C_{29}H_{38}N_8F_2$ (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. ^{1}H 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 $C_{27}H_{36}N_8F_2$ (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. ^{1}H NMR (CD_3CN_3, 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 $C_{24}H_{31}N_7Cl_2$ (M + H), 488.2096; found, 488.2103.

2,4-Bis(4′-chlorophenethylamino)-6-(2′-(1″-methylpyrolidin-2″-yl)ethylamino)-1,3,5-triazine (8bB). Yield: 70 mg, 60%, colorless oil. ^{1}H 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 $C_{26}H_{33}N_7Cl_2$ (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. ^{1}H 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 $C_{23}H_{29}N_7Cl_2$ (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. ^{1}H 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 $C_{24}H_{29}N_7Cl_2$ (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. ^{1}H 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 $C_{24}H_{30}N_8Cl_2$ (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. ^{1}H 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 $C_{25}H_{32}N_8Cl_2$ (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. ^{1}H 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 $C_{24}H_{33}N_7$ (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. ^{1}H 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 $C_{26}H_{37}N_7O_2$ (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. ^{1}H 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 $C_{24}H_{29}N_7Cl_4$ (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. ^{1}H 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 $C_{24}H_{31}N_9O_4$ (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. ^{1}H 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 $C_{24}H_{31}N_7F_2$ (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. ^{1}H 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 $C_{24}H_{31}N_7F_2$ (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. ^{1}H 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 $C_{28}H_{35}N_9$ (M + H), 498.3094; found, 498.3084.

2,4-Bis(2′-(1″-cyc1ohexenyl)ethylamino)-6-(3′-(*N*,*N*-dimethylamino)propylamino)-1,3,5-triazine (8jA). Yield: 220 mg, 95%, white solid. ^{1}H 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 $C_{24}H_{41}N_7$ (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. ^{1}H 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 $C_{22}H_{27}N_7Cl_2$ (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. ^{1}H 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 $C_{22}H_{27}N_7F_2$ (M + H), 428.2374; found, 428.2394.

2,4-Bis(2′,4′-dichlorobenzylamino)-6-(3′-(*N*,*N*-dimethylamino)propylamino)-1,3,5-triazine (8 mA). Yield: 38 mg, 20%, white powder. ^{1}H 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 $C_{22}H_{25}N_7Cl_4$ (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. ^{1}H 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 $C_{22}H_{25}N_7F_4$ (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. ^{1}H 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 $C_{24}H_{27}N_7F_6$ (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. ^{1}H 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, 8 H), 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 $C_{24}H_{29}N_7O_4$ (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. ^{1}H 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 $C_{28}H_{33}N_7O_4$ (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. ^{1}H 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 $C_{42}H_{47}N_9F_4$ (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. ^{1}H 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 $C_{30}H_{35}N_9$ (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. ^{1}H NMR (C_5D_5N, 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 (C_5D_5N, 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 $C_{24}H_{29}N_7O_4$ (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. ^{1}H NMR (C_5D_5N, 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 (C_5D_5N, 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 $C_{23}H_{26}N_6O_4$ (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:

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

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 equation[32] with a nonlinear least-squares regression algorithm using GraphPad Prism 5 software.

■ ASSOCIATED CONTENT

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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■ ACKNOWLEDGMENT

This work was supported by grants from Spanish Ministry of Science and Innovation (Grant SAF2008-00048 to A.M., Grant BFU2009-08346 to A.F.-M., CONSOLIDER-INGENIO 2010 (Grant CSD2008-00005) to A.F.-M., J.M.G.-R., and A.M.), from Fundació La Marató de TV3 (to A.F.-M. and A.M.), and from PROMETEO/ 2010/046 from the GVA to A.F.-M.

■ 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

■ REFERENCES

(1) Szallasi, A.; Blumberg, P. M. Vanilloid (capsaicin) receptors and mechanisms. *Pharmacol. Rev.* **1999**, *51*, 159–212.

(2) Szallasi, A.; Fowler, C. J. After a decade of intravesical vanilloid therapy: still more questions than answers. *Lancet Neurol.* **2002**, *1*, 167–172.

(3) Messeguer, A.; Planells-Cases, R.; Ferrer-Montiel, A. Physiology and pharmacology of the vanilloid receptor. *Curr Neuropharmacol.* **2006**, *4*, 1–15.

(4) García-Martínez, C.; Fernández-Carvajal, A.; Valenzuela, B.; Gomis, A.; Van Den Nest, W.; Ferroni, S.; Carreño, C.; Belmonte, C.; Ferrer-Montiel, A. Design and characterization of a noncompetitive antagonist of the transient receptor potential vanilloid subunit 1 channel with in vivo analgesic and anti-inflammatory activity. *J. Pain* **2006**, *7*, 735–746.

(5) Valente, P.; Garcia-Sanz, N.; Gomis, A.; Fernandez-Carvajal, A.; Fernandez-Ballester, G.; Viana, F.; Belmonte, C.; Ferrer-Montiel, A. Identification of molecular determinants of channel gating in the transient receptor potential box of vanilloid receptor I. *FASEB J.* **2008**, *22*, 3298–3309.

(6) Szallasi, A.; Cortright, D. N.; Blum, C. A.; Eid, S. R. The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. *Nat. Rev. Drug Discovery* **2007**, *6*, 357–372.

(7) Szallasi, A. G.; Martin, J. Peripheral TRPV1 receptors as targets for drug development: new molecules and mechanisms. *Curr. Pharm. Des.* **2008**, *14*, 32–41.

(8) Kim, H. Y.; Park, C.-K.; Cho, I.-H.; Jung, S. J.; Kim, J. S.; Oh, S. B. Differential changes in TRPV1 expression after trigeminal sensory nerve injury. *J. Pain* **2008**, *9*, 280–288.

(9) Culshaw, A. J.; Bevan, S.; Christiansen, M.; Copp, P.; Davis, A.; Davis, C.; Dyson, A.; Dziadulewicz, E. K.; Edwards, L.; Eggelte, H.; Fox, A.; Gentry, C.; Groarke, A.; Hallett, A.; Hart, T. W.; Hughes, G. A.; Knights, S.; Kotsonis, P.; Lee, W.; Lyothier, I.; McBryde, A.; McIntyre, P.; Paloumbis, G.; Panesar, M.; Patel, S.; Seiler, M. P.; Yaqoob, M.; Zimmermann, K. Identification and biological characterization of 6-aryl-7-isopropylquinazolinones as novel TRPV1 antagonists that are effective in models of chronic pain. *J. Med. Chem.* **2006**, *49*, 471–474.

(10) Ghilardi, J. R.; Rohrich, H.; Lindsay, T. H.; Sevcik, M. A.; Schwei, M. J.; Kubota, K.; Halvorson, K. G.; Poblete, J.; Chaplan, S. R.; Dubin, A. E.; Carruthers, N. I.; Swanson, D.; Kuskowski, M.; Flores, C. M.; Julius, D.; Mantyh, P. W. Selective blockade of the capsaicin receptor TRPV1 attenuates bone cancer pain. *J. Neurosci.* **2005**, *25*, 3126–3131.

(11) Gavva, N. R.; Treanor, J. J. S.; Garami, A.; Fang, L.; Surapaneni, S.; Akrami, A.; Alvarez, F.; Bak, A.; Darling, M.; Gore, A.; Jang, G. R.; Kesslak, J. P.; Ni, L.; Norman, M. H.; Palluconi, G.; Rose, M. J.; Salfi, M.; Tan, E.; Romanovsky, A. A.; Banfield, C.; Davar, G. Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. *Pain* **2008**, *136*, 202–210.

(12) Planells-Cases, R.; Garcia-Martinez, C.; Royo, M.; Perez-Paya, E.; Carreno, C.; Albericio, F.; Messeguer, A.; Ferrer-Montiel, A. Small molecules targeting the vanilloid receptor complex as drugs for inflammatory pain. *Drugs Future* **2003**, *28*, 787–797.

(13) Johnson, J. W.; Kotermanski, S. E. Mechanism of action of memantine. *Curr. Opin. Pharmacol.* **2006**, *6*, 61–67.

(14) García-Martínez, C.; Humet, M.; Planells-Cases, R.; Gomis, A.; Caprini, M.; Viana, F.; Peña, E. D. l.; Sanchez-Baeza, F.; Carbonell, T.; Felipe, C. D.; Pérez-Payá, E.; Belmonte, C.; Messeguer, A.; Ferrer-Montiel, A. Attenuation of thermal nociception and hyperalgesia by VR1 blockers. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2374–2379.

(15) Quintanar-Audelo, M.; Fernández-Carvajal, A.; Van Den Nest, W.; Carreño, C.; Ferrer-Montiel, A.; Albericio, F. Design and synthesis of indole-based peptoids as potent noncompetitive antagonists of transient receptor potential vanilloid 1. *J. Med. Chem.* **2007**, *50*, 6133–6143.

(16) Bonache, M.; García-Martinez, C.; García de Diego, L.; Carreño, C.; Pérez de Vega, M.; García-López, M.; Ferrer-Montiel, A.; González-Muñiz, R. Old molecules for new receptors: Trp(Nps) dipeptide derivatives as vanilloid TRPV1 channel blockers. *ChemMedChem* **2006**, *1*, 429–438.

(17) Masip, I.; Cortés, N.; Abad, M.-J.; Guardiola, M.; Pérez-Payá, E.; Ferragut, J.; Ferrer-Montiel, A.; Messeguer, A. Design and synthesis of an optimized positional scanning library of peptoids: identification of novel multidrug resistance reversal agents. *Bioorg. Med. Chem.* **2005**, *13*, 1923–1929.

(18) Moure, A.; Sanclimens, G.; Bujons, J.; Masip, I.; Alvarez-Larena, A.; Pérez-Payá, E.; Alfonso, I.; Messeguer, A. Chemical modulation of peptoids: synthesis and conformational studies on partially constrained derivatives. *Chem.—Eur. J.* **2011**, *17*, 7927–7939.

(19) Scheper, J.; Guerra-Rebollo, M.; Sanclimens, G.; Moure, A.; Masip, I.; González-Ruiz, D.; Rubio, N.; Crosas, B.; Meca-Cortés, Ó.; Loukili, N.; Plans, V.; Morreale, A.; Blanco, J.; Ortiz, A. R.; Messeguer, Á.; Thomson, T. M. Protein−protein interaction antagonists as novel inhibitors of non-canonical polyubiquitylation. *PLoS One* **2010**, *5*, e11403.

(20) Saleh, M.; Abbott, S.; Perron, V.; Lauzon, C.; Penney, C.; Zacharie, B. Synthesis and antimicrobial activity of 2-fluorophenyl-4,6-disubstituted [1,3,5]triazines. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 945–949.

(21) Menicagli, R.; Samaritani, S.; Signore, G.; Vaglini, F.; Dalla Via, L. In vitro cytotoxic activities of 2-alkyl-4,6-diheteroalkyl-1,3,5-triazines: new molecules in anticancer research. *J. Med. Chem.* **2004**, *47*, 4649–4652.

(22) Zheng, M.; Xu, C.; Ma, J.; Sun, Y.; Du, F.; Liu, H.; Lin, L.; Li, C.; Ding, J.; Chen, K.; Jiang, H. Synthesis and antitumor evaluation of a novel series of triaminotriazine derivatives. *Bioorg. Med. Chem.* **2007**, *15*, 1815–1827.

(23) Xiong, Y.-Z.; Chen, F.-E.; Balzarini, J.; De Clercq, E.; Pannecouque, C. Non-nucleoside HIV-1 reverse transcriptase inhibitors. Part 11: Structural modulations of diaryltriazines with potent anti-HIV activity. *Eur. J. Med. Chem.* **2008**, *43*, 1230–1236.

(24) Uccello-Barretta, G.; Samaritani, S.; Menicagli, R.; Salvadori, P. 2,4,6-Tri[(*S*)-1′-methylbenzylamino]-1,3,5-triazine: a new NMR chiral solvating agent for 3,5-dinitrophenyl derivatives; an attempt at a chiral discrimination rationale. *Tetrahedron: Asymmetry* **2000**, *11*, 3901–3912.

(25) Díaz-Ortiz, A.; Elguero, J.; DelaHoz, A.; Jiménez, A.; Moreno, A.; Moreno, S.; Sánchez-Migallón, A. Microwave-Assisted Synthesis and Dynamic Behaviour of Tris(1pyrazolyl)-1,3,5-triazine-2,4,6-triamines. *QSAR Comb. Sci.* **2005**, *24*, 649–659.

(26) Baumgartner, W.; Islas, L.; Sigworth, F. J. Two-microelectrode voltage clamp of Xenopus oocytes: voltage errors and compensation for local current flow. *Biophys. J.* **1999**, *77*, 1980–1991.

(27) Mellor, I. R.; Ogilvie, J.; Pluteanu, F.; Clothier, R. H.; Parker, T. L.; Rosini, M.; Minarini, A.; Tumiatti, V.; Melchiorre, C. Methoctramine analogues inhibit responses to capsaicin and protons in rat dorsal root ganglion neurons. *Eur. J. Pharmacol.* **2004**, *505*, 37–50.

(28) Kitaguchi, T.; Swartz, K. J. An inhibitor of TRPV1 channels isolated from funnel web spider venom. *Biochemistry* **2005**, *44*, 15544–15549.

(29) Swamy, K. C. K.; Kumar, N. N. B.; Balaraman, E.; Kumar, K. V. P. P. Mitsunobu and related reactions: advances and applications. *Chem. Rev.* **2009**, *109*, 2551–2651.

(30) Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M. Diprotected triflylguanidines: a new class of guanidinylation reagents. *J. Org. Chem.* **1998**, *63*, 3804–3805.

(31) Messeguer, J.; Cortés, N.; García-Sanz, N.; Navarro-Vendrell, G.; Ferrer-Montiel, A.; Messeguer, A. Synthesis of a positional scanning library of pentamers of *N*-alkylglycines assisted by microwave activation and validation via the identification of trypsin inhibitors. *J. Comb. Chem.* **2008**, *10*, 974–980.

(32) Ferrer-Montiel, A. V.; Merino, J. M.; Planells-Cases, R.; Sun, W.; Montal, M. Structural determinants of the blocker binding site in glutamate and NMDA receptor channels. *Neuropharmacology* **1998**, *37*, 139–147.