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TRPV1 modulators: Structure–activity relationships using a rational combinatorial approach

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ABSTRACT

A discrete library of linear and hydantoin-containing dipeptide derivatives, based on the Lys-Trp(Nps) scaffold, was prepared by solid-phase synthesis. SAR studies indicated that potency for TRPV1 blockade and selectivity towards NMDA is mainly dictated by the side-chain length and the basic nature of α, ω-groups in the N-terminal residue. The 2-Nps moiety at position 2 of Trp indole ring is preferred over the 2-pyridine one.

Capsaicin, a pungent chemical present in hot peppers, specifically activates TRPV1, a membrane-bound, nonselective, transient receptor potential cation channel. Since its cloning in 1997,[1] TRPV1 receptor has generated significant interest because of its role in pain pathways.[1–6] TRPV1 is predominantly expressed in the nociceptors and is activated by a variety of endogenous stimuli that are known to be generated as a result of tissue injury and inflammation. These endogenous ligands include heat and low pH,[7] endocannabinoid anandamide,[8] and arachidonic acid metabolites.[9] The resulting excitation of sensitive pathways is followed by a refractary state referred as desensitization during which the neurons become unresponsive to capsaicin and to noxious endogenous stimuli. In addition, activation of this receptor can be potentiated by pro-nocicpetive mediators such as bradykinin, NGF and others.[10] These different activation pathways indicate that TRPV1 has a significant role in pathway pain and represents an intriguing novel target for the treatment of inflammation, cancer and for other disorders, such as urinary urge incontinence,[11] chronic cough,[12] irritable bowel syndrome,[13] and migraine.[14] In the last years a growing interest in developing TRPV1 agonist[15–17] and antagonist[18–25] molecules as potential analgesic drugs has been showed. Due to the side effects associated to the agonist agents, several pharmaceutical companies have also focused their attention on the discovery of potent and selective TRPV1 antagonists some of which are already in phase I/II clinical trials for the indications of chronic inflammatory pain and migraine.[26] In this regard, we have described a series of dipeptides containing 2-[(*o*-nitrophenyl)sulfenyl] substituted tryptophan (Trp) as a new class of TRPV1 channel blockade, showing selective analgesic activity.[27] These results prompted us to develop a methodology for the facile preparation of a library of TRPV1 ligands based on the 2-substituted Trp moiety, to further explore the structural requirements of TRPV1 receptor blockers.

The most convenient method for the preparation of libraries of molecules is the solid-phase mode,[28,29] which allows parallelization, easy workups, and give typically high yields by the use of large excess of reactants.[30] The rational design of the library was performed considering as scaffold a dipeptide composed by Trp and a ω-basic amino acid at the C- and N-termini position, respectively (Fig. 1). Single modifications were introduced at the 2-position of Trp, α- and ω-N-terminus, peptide backbone, and explored combinations between them. In particular, a pyridine moiety as a phenyl bioisostere onto the Trp indole ring and different amino

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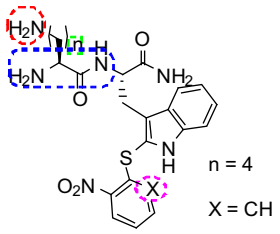


Figure 1. Starting scaffold for library generation.

acids such as Arg, Lys, diamino butyric acid (Dba) and diamino propionic acid (Dpa) were introduced. To explore further the role of basicity, fully pentasubstituted guanidinium groups and the methyl quaternary amino group were also introduced at the side-chain of the N-terminal residue. Finally, compounds with a free or acetylated α-NH_2, and cyclized backbone through a hydantoin structure were also considered to investigate the role of the N-terminus as well as the rigidity of the molecule.

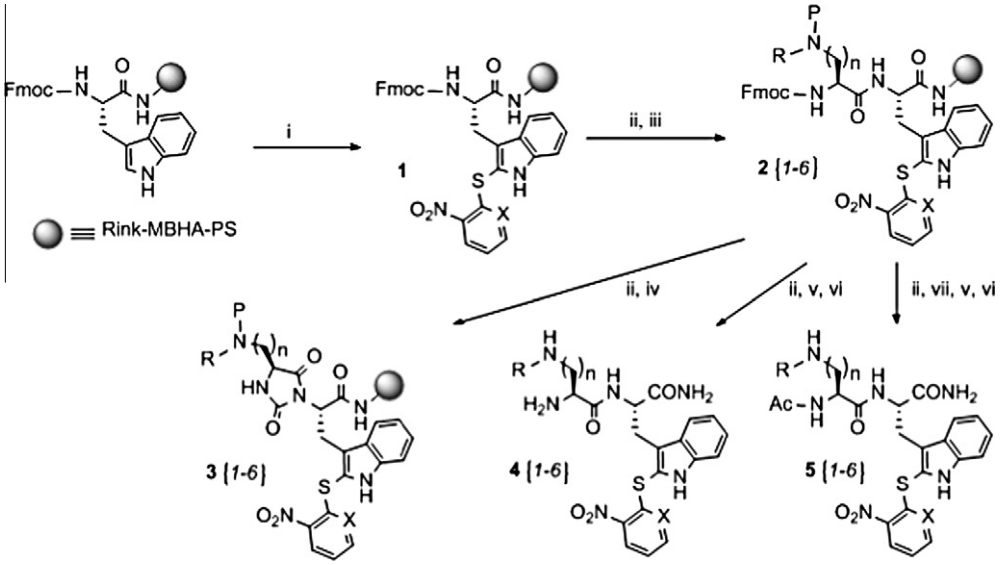
The library was synthesized by solid phase using Rink-*p*-methylbenzhydrylamine (MBHA)-polystyrene (PS) resin and fluorenylmethoxycarbonyl (Fmoc) based chemistry.[31] Rink-MBHA-resin was acylated with Fmoc-Trp-OH, with unprotected side-chain, using *N*,*N*-diisopropylcarbodiimide (DIPCDI) in the presence of aza-hydroxybenzotriazole (HOAt). Modification of Trp indole ring at position 2 was carried with the corresponding sulfenyl derivatives, 2-[(*o*-nitrophenyl)sulfenyl] chloride (NpsCl) or 2-[(*o*-nitropyridinyl)sulfenyl] chloride (NpysCl). Initial trials carried out using DMF as a solvent resulted in incomplete substitution, but the use of HOAc/DMF under Ar atmosphere achieved total conversion to give intermediates **1** (Scheme 1). After removal of the Fmoc group, resin **1** was divided in aliquots and Fmoc-Arg(Pmc)-OH, Fmoc-Lys-OH, Fmoc-Dab-OH, and Fmoc-Dap-OH, the last three Fmoc-amino acids with the amino group of their side-chain protected either with allyloxycarbonyl (Alloc) or methyltrityl (Mtt) groups, were incorporated to provide resins **2** {1–6}. Most of the remaining conversions were carried out using standard conditions. Thus, formation of the hydantoin ring to resins **3** was performed in two steps, first reaction with *N*,*N*′-dissucinimidyl carbonate (DSC) in the presence of *N*,*N*-dimethylamino pyridine (DMAP) in DMF as a solvent, followed by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[32] Removal of Alloc or Mtt groups from resins **2** were carried out with tetrakis(triphenylphosphine)-palladium ($Pd(PPh_3)_4$) and phenylsilane ($PhSiH_3$) in DCM, or with 3% TFA-DCM, respectively. Cleavage with TFA in the presence of triethylsilane (TES)[35] afforded final compounds **4** {1–6} (Scheme 1). Acetyl derivatives **5** {1–6} were obtained from **2** by Fmoc-removal, followed by acetylation with Ac_2O in the presence of *N*,*N*-diisopropylethylamine (DIPEA) and cleavage, as above indicated.

Final compounds **6**–**9** were obtained from hydantoin-resins **3** as indicated in Scheme 2. Thus, the sequential removal of the side-chain protecting group, guanidilation reaction by treatment with uromium salts, such as 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo-[4,5-*b*]pyridinium hexafluorophosphate 3-oxide (HATU)[32] and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-bis(tetramethylene) uronium hexa-fluorophosphate (HBPyU),[33] and acid cleavage afforded compounds **6** and **7**. A similar sequence, but changing the guanidylation by a permethylation step (using CH_3I), resulted in derivatives **8**. Finally, analogues **9** were prepared by simple side-chain protecting group removal and cleavage from the resin.

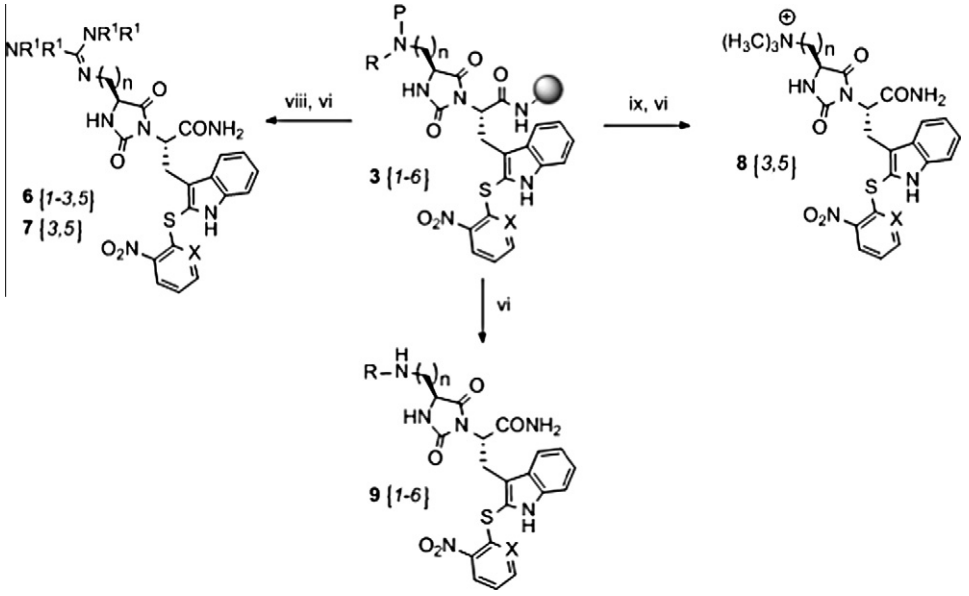
Application of the above sequences of reactions to resins **2** allowed the preparation of final dipeptide derivatives **11**–**13** and their acetylated counterparts **15**–**17** (Scheme 3). In this case, special attention is required for the guanidylation and permethylation reactions in the presence of Fmoc groups. If these reactions are carried out in the presence of DIPEA, both reactions take place at both amino functions, due to partial removal of the Fmoc group under reaction conditions.[34] In fact, compound **18** {3} was obtained as the main product due to double guanidinium group incorporation on to the resin bound intermediate **10** {3} with HBPyU/DIPEA in DMF.[35]

The activity and selectivity of the library members were tested on recombinant TRPV1 and NMDA channels expressed in Xenopus oocytes by electrophysiological experiments.[27] The results establish some general requirements that determine the potency for the TRPV1 blockade and selectivity towards NMDA (Tables 1 and 2).

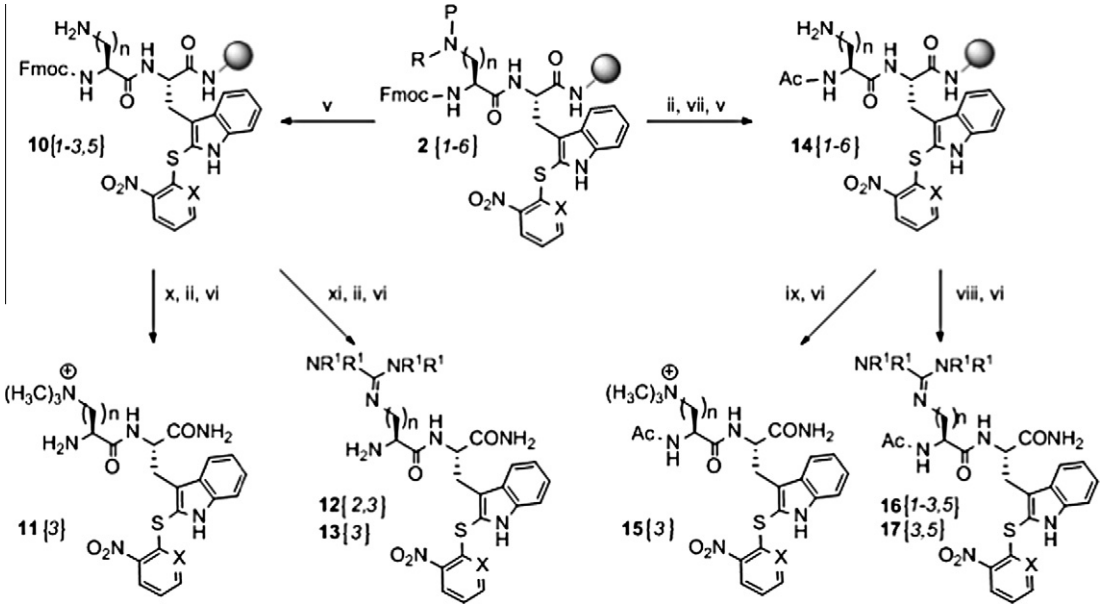
For linear peptide derivatives (Table 1), the TRPV1 blocking potency appears to be directly related to the increase in the basicity of ω-N side-chain functional groups, with the activity



Scheme 1. Reagents and conditions: (i) X = CH, NpsCl in HOAc/DMF; X = N, NPysCl in HOAc/DMF; (ii) piperidine/DMF; (iii) Fmoc-protected amino acid (see Table 1)-DIPCDI-HOAt-DIPEA in DMF; (iv) (a) DSC-DMAP in DMF; (b) DBU-DMF; (v) P = Alloc, $Pd(PPh_3)_4$–$PhSiH_3$ in DCM, P = Mtt, TFA/DCM; (vi) TFA/TES/H_2O; (vii) Ac_2O/DIPEA in DMF. P = side-chain protecting group. R = H for Dap, Dab, and Lys, R = $C(NH)NH_2$ for Arg.



Scheme 2. Reagents and conditions: (vi) TFA/TES/H_2O; (viii) $R^1 = CH_3$, HATU-DIPEA in DMF; $R^1 = C_4H_8$, HBPyU-DIPEA in DMF; (ix) CH_3I-DIPEA in DMF. P = side-chain protecting group. R = H for Dap, Dab, and Lys, R = C(NH)NH_2 for Arg.

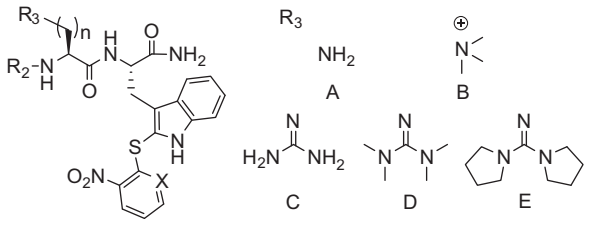


Scheme 3. Reagents and conditions: (ii) Piperidine/DMF; (v) P = Alloc, $Pd(PPh_3)_4^-PhSiH_3$ in DCM, P = Mtt, TFA/DCM; (vi) TFA/TES/H_2O; (vii) Ac_2O/DIPEA in DMF; (viii) $R^1 = CH_3$, HATU-DIPEA in DMF; $R^1 = C_4H_8$, HBPyU-DIPEA in DMF; (ix) CH_3I-DIPEA in DMF; (x) (a) DIPEA/DMF, (b) CH_3I in DMF; (xi) (a) DIPEA/DMF, (b) $R^1 = CH_3$, HATU in DMF; $R^1 = C_4H_8$, HBPyU in DMF. P = side-chain protecting group. R = H for Dap, Dab, and Lys, R = C(NH)NH_2 for Arg.

following the order NH_2<$N(Me)_3^+$<$(NMe_2)_2C{=}N$ <(pyrrolidine)$_2C{=}N$. Similarly, the basic character of the α–NH_2 group is required for TRPV1 antagonist activity, since in all cases its acetylation reduces considerably the percentage of blockade. In addition, these derivatives maintain or even increase the ability to block NMDA ion channel with respect to their free amine analogues, thus compromising selectivity. Remarkably, compound **18** {3} resulting from the double guanidylation of both α- and ε-amino groups of Lys, was the most potent TRPV1-blocking compound within this library, while maintains a nice selectivity. With the only exception of H-Dap-, H-Dab-, and H-Lys-derivatives **4** {1–3}, an increase in the side-chain length results in both the enhancement of TRPV1 activity and selectivity versus NMDA. In contrast, replacement of the Nps moiety at position 2 of the indole ring by its pyridine bioisostere Npys generally reduces the ability for the TRPV1 blockade as well as selectivity.

The conformational restriction of the peptide backbone through $N^{\alpha},N^{\alpha'}$-cyclization to an hydantoin ring provided interesting results (Table 2). Thus, despite Lys α–NH_2 acylated, most compounds increase the blocking properties on TRPV1 ion channels in comparison to their acetylated linear analogues, indicating a positive role of the backbone constraint. Again, a tendency to increase activity and selectivity with the length and basicity of the N-terminal side-chain is observed, and the Nps moiety is preferred over Npys,

Table 1
Blockade of TRPV1 and NMDA receptors by Xaa-Trp(Nps) and Xaa-Trp(Npys) linear derivatives



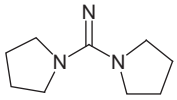
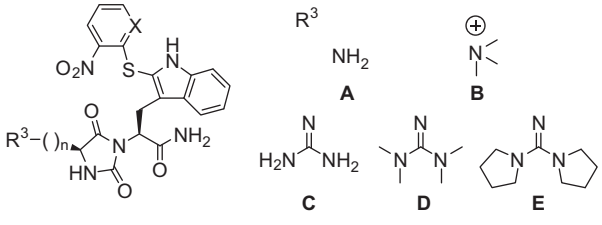
Compd	R^2	R^3	X	*n*	Activity (% blockade at 10 μM)	
					TRPV1 (1 μM)	NMDA
4 {1}	H	**A**	CH	1	39.3	24.0
4 {2}	H	**A**	CH	2	20.6	29.7
4 {3}	H	**A**	CH	4	26.3	13.5
4 {4}	H	**C**	CH	3	62.8 (18.7)	7.7
4 {5}	H	**A**	N	4	17.0	23.6
4 {6}	H	**C**	N	3	44.8	20.6
5 {1}	Ac	**A**	CH	1	14.6	27.0
5 {2}	Ac	**A**	CH	2	27.6	27.2
5 {3}	Ac	**A**	CH	4	14.3	15.4
5 {4}	Ac	**C**	CH	3	29.2	25.8
5 {5}	Ac	**A**	N	4	12.7	13.6
5 {6}	Ac	**C**	N	3	23.9	26.3
11 {3}	H	**B**	CH	4	36.7	5.5
12 {2}	H	**E**	CH	2	76.1 (23.5)	13.9
12 {3}	H	**E**	CH	4	55.8	8.3
13 {3}	H	**D**	CH	4	49.7 (21.1)	69.7
15 {3}	Ac	**B**	CH	4	29.0	32.2
16 {1}	Ac	**E**	CH	1	49.9	30.3
16 {2}	Ac	**E**	CH	2	42.4	9.6
16 {3}	Ac	**E**	CH	4	44.0	6.4
16 {5}	Ac	**E**	N	4	16.6	30.2
17 {3}	Ac	**D**	CH	4	19.9	6.1
17 {5}	Ac	**D**	N	4	12.0	20.6
18 {3}		**E**	CH	4	97.2 (27.3)	6.8

Table 2
Blockade of TRPV1 and NMDA receptors by Xaa-Trp(Nps)- and Xaa-Trp(Npys)-derived hydantoins



Compd	R^3	X	*n*	Activity (% blockade at 10 μM)	
				TRPV1 (1 μM)	NMDA
6 {1}	**E**	CH	1	51.3	43.7
6 {2}	**E**	CH	2	56.3	12.0
6 {3}	**E**	CH	4	60.4	10.7
6 {5}	**E**	N	4	52.5	12.9
7 {3}	**D**	CH	4	13.0	9.3
7 {5}	**D**	N	4	40.9	10.8
8 {3}	**B**	CH	4	66.5 (17.4)	9.2
8 {5}	**B**	N	4	19.9	20.6
9 {1}	**A**	CH	1	29.9	18.4
9 {2}	**A**	CH	2	11.0	16.0
9 {3}	**A**	CH	4	15.4	10.5
9 {4}	**C**	CH	3	50.2	20.2
9 {5}	**A**	N	4	24.6	26.8
9 {6}	**C**	N	3	54.3	24.2

although the differences are smaller than for linear analogues. Derivative with a quaternary trimethylamino group at the Lys side-chain, **8** {3}, proved to be the most potent compound within the hydantoin series, with a blockade potency similar to that of the bispyrrolidine-containing guanidine derivative **6** {3}.

In conclusion, the solid-phase synthesis of a combinatorial library based on a Lys-Trp(Nps) peptide scaffold has contributed to a quick establishment of the minimal requirements for efficient TRPV1 ion channel blockade and selectivity over ionotropic NMDA receptor. Preferred structural issues are a side-chain length of 3-4-methylene groups and highly basic substituents at both α,ω-groups of the N-terminal residue, preferentially guanidine-type groups. For the C-terminal amino acid, the Nps moiety at position 2 of the Trp indole ring is superior to its pyridine bioisoster Npys. Conformational constrained hydantoin analogues also showed moderate to good inhibition of the Ca^{2+} influx through the TRPV1 channel, suggesting the hydantoin ring as a valuable scaffold for the search of new TRPV1 blockers. The best compound in this library, the diguanylated derivative **18** {3}, shows good activity and selectivity in vitro, excellent solubility properties, and represents a new scaffold within TRPV1 antagonists for further pharmacological characterization.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.141.

References and notes

1. Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. *Nature* **1997**, *389*, 816.
2. Caterina, M. J.; Leffler, A.; Malmberg, A. B.; Martin, W. J.; Trafton, J.; Petersen-Zeitz, K. R.; Koltzenburg, M.; Basbaum, A. I.; Julius, D. *Science* **2000**, *288*, 306.
3. Cortright, D. N.; Krause, J. E.; Broom, D. C. *Biochim. Biophys. Acta* **2007**, *1772*, 978.
4. Palazzo, E.; Rossi, F.; Maione, S. *Mol. Cell. Endocrinol.* **2008**, *286*, S79.
5. Willis, W. D., Jr. *Exp. Brain Res.* **2009**, *196*, 5.
6. Cortright, D. N.; Szallasi, A. *Curr. Pharm. Des.* **2009**, *15*, 1736.
7. Tominaga, M.; Caterina, M. J.; Malmberg, A. B.; Rosen, T. A.; Gilbert, H.; Skinner, K.; Raumann, B. E.; Basbaum, A. I.; Julius, D. *Neuron* **1998**, *21*, 531.
8. Ross, R. A. *Br. J. Pharmacol.* **2003**, *140*, 790.
9. Hwang, S. W.; Cho, H.; Kwak, J.; Lee, S. Y.; Kang, C. J.; Jung, J.; Cho, S.; Min, K. H.; Suh, Y. G.; Kim, D.; Oh, U. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6155.
10. Huang, J.; Zhang, X.; McNaughton, P. A. *Curr. Neuropharmacol.* **2006**, *4*, 197.
11. Silva, C.; Silva, J.; Castro, H.; Reis, F.; Dinis, P.; Avelino, A.; Cruz, F. *BMC Urol.* **2007**, *7*, 9.
12. Brooks, S. M. *Lung* **2008**, *186*, S88.
13. Holzer, P. *Gut* **2008**, *57*, 882.
14. Shimizu, T. *Brain Nerve.* **2009**, *61*, 949.
15. Bley, K. R. *Expert. Opin. Investig. Drugs* **2004**, *13*, 1445.
16. Knotkova, H.; Pappagallo, M.; Szallasi, A. *Clin. J. Pain* **2008**, *24*, 142.

17. Conway, S. J. *Chem. Soc. Rev.* **2008**, *37*, 1530.
18. Pal, M.; Angaru, S.; Kodimuthali, A.; Dhingra, N. *Curr. Pharm. Des.* **2009**, *15*, 1008.
19. Broad, L. M.; Keding, S. J.; Blanco, M. J. *Curr. Top. Med. Chem.* **2008**, *8*, 1431.
20. Szallasi, A.; Cortright, D. N.; Blum, C. A.; Eid, S. R. *Nat. Rev. Drug Discov.* **2007**, *6*, 357.
21. Kym, P. R.; Kort, M. E.; Hutchins, C. W. *Biochem. Pharmacol.* **2009**, *78*, 211.
22. Szallasi, A.; Blumberg, P. M. *Pharmacol. Rev.* **1999**, *51*, 159.
23. Szallasi, A.; Appendino, G. *J. Med. Chem.* **2004**, *47*, 2717.
24. McLeod, R. L.; Correll, C. C.; Jia, Y.; Anthes, J. C. *Lung* **2008**, *186*, S59.
25. Gunthorpe, M. J.; Chizh, B. A. *Drug Discovery Today* **2009**, *14*, 56.
26. Wong, G. Y.; Gavva, N. R. *Brain Res. Rev.* **2009**, *60*, 267.
27. Bonache, M. A.; Garcia-Martinez, C.; Garcia de Diego, L.; Carreno, C.; Perez de Vega, M. J.; Garcia-Lopez, M. T.; Ferrer-Montiel, A.; Gonzalez-Muniz, R. *ChemMedChem* **2006**, *1*, 429.
28. *The Power of Functional Resins in Organic Chemistry*; Tulla-Puche, J., Albericio, F., Eds.; Wiley-VCH Verlag GmbH: KGaA, Weinheim (Germany), 2008.
29. Nandy, J. P.; Prakesch, M.; Khadem, S.; Reddy, P. T.; Sharma, U.; Arya, P. *Chem. Rev.* **2009**, *109*, 1999.
30. *Solid-Phase Synthesis. A Practical Guide.*; Kates, S. A., Albericio, F., Eds.; Marcel Dekker: New York (NY, USA), 2000.
31. Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.
32. Vazques, J.; Royo, M.; Albericio, F. *Lett. Org. Chem.* **2004**, *1*, 224.
33. Chen, S.; Xu, J. *Tetrahedron Lett.* **1992**, *33*, 647.
34. Farrera-Sinfreu, J.; Royo, M.; Albericio, F. *Tetrahedron Lett.* **2002**, *43*, 7813.
35. All final compounds were checked for purity by HPLC (monitored by UV absorption at 220 nm) and characterized by Maldi-Tof spectra (see Supplementary data). All compounds were assayed as crude materials.