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Solid-phase synthesis of a library of amphipatic hydantoins. Discovery of new hits for TRPV1 blockade

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Abstract

Some heterocyclic systems, called privileged scaffolds, appear frequently in bioactive products and marketed drugs. The combination of a recognized privileged scaffold (hydantoin) and a functional group with high incidence in bioactive molecules (guanidine) guided the design of a library of amphipatic compounds, which allowed to discover novel TRPV1 ion channel blockers. The library was synthesized by parallel solid-phase synthesis from an orthogonally protected resin-bound Lys-Lys skeleton. Key steps of the synthetic procedure were the construction of the hydantoin ring, by reaction of the *N*-terminal amino group with N,N-disuccinimidyl carbonate (DSC) and subsequent base-induced cyclization, and the guanidinylation of the *C*-terminal Lys side-chain after removal of the Alloc protecting-group. The preliminary biological studies have allowed the identification of some of the key structural features directing the blockage of capsaicin-induced Ca^{2+} influx through TRPV1 channels, particularly, the strong preference showed for highly lipophilic acyl groups and substituted guanidine moieties. Active compounds based on this new pharmacophoric scaffold that display *in vitro* and *in vivo* inhibitory activity

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Author contribution

MR and FA conceived and designed the library and supervised its synthesis. GGN performed the library synthesis and characterization. RGM supervised the characterization. AFC and JMGR performed the biological assays. AFM designed and supervised the biological evaluation. CC supervised the synthesis of selected compounds and all biological data. RGM and MR co-wrote the manuscript.

ASSOCIATED CONTENT

Supporting Information. Detailed characterization of library members: Yields, purities, HPLC, MS, ^{1}H and ^{13}C-NMR data. Full data of TRPV1 and NMDA channel blockade. This material is available free of charge via the Internet at http://pubs.acs.org.

Keywords

Hydantoins; Guanidine groups; Solid-phase; TRPV1 blockers; Pain

Introduction

5,5-Diphenylhydantoin is being used since 1938 for the treatment of epilepsy due to its regulatory effect on the bioelectric activity of the nervous system.[1] Since then, many other hydantoin-derived compounds with a wide range of therapeutic applications have been discovered. For example, 5-arylidene derivatives have shown antituberculosis and antiproliferative activity,[2,3] whereas Pt(II) complexes with hydantoin ligands have proven to be very effective citotoxic agents.[4] Other examples include antiviral agents,[5] ligands for the glycine binding site of the NMDA receptor and for voltage-gated sodium channels,[6,7] as well as modulators of certain protein-protein interactions related to cell adhesion.[8,9] Thus, and due to the large variety of biological applications found for this family of compounds, the hydantoin scaffold is being considered a "privileged structure", term first coined by Evans in 1988 to define a single molecular framework able to provide ligands for a range of different biological targets.[10]

The impact of hydantoins in medicinal chemistry programs has triggered an extensive development of synthetic methodologies for the generation of libraries based on this heterocyclic skeleton.[11–13] Apart from some procedures in solution, using multicomponent reactions and fluorous synthesis,[14,15] most efforts to prepare hydantoin-based libraries have been carried out on solid-phase, following two main approaches: i) cyclization of an acyclic precursor with concomitant cleavage from the resin, and ii) cyclization prior to cleavage.

The vast majority of synthetic sequences, derived from the first approach,[16] comprise the attachment of an amino acid or dipeptide to the resin through the *C*-terminal carboxylate, further elaboration to an acyclic urea, and the final cyclative/cleavage step.[17–29] An alternative strategy used a urethane attachment of amino acids to the resin (through the α–NH_2 group), followed by base-catalyzed cyclization of linear adducts to form and detach final hydantoins.[30]

On the other hand, many different strategies have been described to develop methodologies following the second approach, which allow further on-bead transformations. They differ mainly in the nature of the reactive intermediate or in the activating agents used to facilitate the cyclization step. Thus, for example base-promoted cyclization of dipeptide-derived phenyl carbamates, thermal ring closure from dipeptide-isocyanate intermediates, and activation of the peptide *N*-terminal amino group by means of diphosgene, triphosgene, disuccinimide carbonate or carbonyldiimidazole have been successfully employed.[31–39]

The guanidine functional group is also a structural motif commonly found in natural products and in many therapeutically active compounds, including some marketed drugs, where it usually acts as a crucial pharmacophore entity. Thus, guanidine-containing derivatives have been shown to have antitumor,[40–42] antibiotic,[43,44] and antiviral properties.[45] Regarding the biological target, the guanidine group is frequent in both enzyme inhibitors[46,47] and ligands for different receptors and ion channels.[48–51] Therefore, and by analogy with the "privileged scaffolds", the guanidine moiety can be considered as a "privileged functional group" in the search for new bioactive compounds.

Based on the above mentioned considerations, we hypothesized that the combination of a "privileged structure" and the guanidine "privileged functional group" could be a practical approach for the discovery of new compounds of therapeutic relevance. Hence, using the hydantoin ring as central scaffold, we have prepared a discrete library with two points of diversity, which are decorated by an arrangement of differently substituted guanidine groups and diverse acyl moieties, to confer a certain amphipatic character to library compounds. We report herein the preparation of this collection of compounds and the identification of TRPV1 channel blockers with *in vitro* and *in vivo* activity. We focused on this receptor because its central role in pain transduction. In addition, we used in parallel the NMDA receptor with the aim of identifying compounds that preferentially block the TRPV1 over the glutamatergic receptor. The rationale of using the NMDA receptor is that displays a similar Ca^{2+} permeability as the TRPV1 channel.

Results and Discussion

A common Lys-Lys-derived hydantoin scaffold was envisaged to incorporate the elements of diversity at the Lys side-chains. The new chemset of 112 compounds resulted from a combination of 14 diverse acyl moieties and 8 different guanidine groups at *N*- and *C*-terminal side-chains, respectively (**1**{**G**1-8,**A**1-14}, Figure 1). As diversity elements at the *C*-terminal Lys side-chain, three sublibraries contain unsubstituted guanidine groups, connected through spacers with different flexibility (**G**{1-2}) or directly linked to the carbon side-chain (**G**3). The other five sublibraries possess different pentasubstituted guanidine moieties (**G**{4-8}) linked to the tetramethylene side-chain. To further incorporate diversity at the 5-(4-aminobutyl)-substituent of the hydantoin skeleton, we decided to acylate the amino group with carboxylic acids containing: short linear and branched aliphatic chains (**A**{1-4}), fatty-type saturated chains (**A**{5-6}), carbocyclic and aromatic moieties (**A**{7-11}), and linear and cyclic substituents incorporating primary and secondary amino groups (**A**{12-14}).

Synthesis

The orthogonally protected Fmoc-Lys(Mtt)-Lys(Alloc)-PS (**2**) was prepared by conventional SPPS methods on a Fmoc-Rink amide-MBHA-Polystyrene resin (Scheme 1). Removal of the Fmoc group from this dipeptidyl resin, followed by treatment with DSC in the presence of DBU allowed the cyclization to the hydantoin scaffold,[38–39] and thus the formation of the key resin-bound intermediate **3**, characterized by HPLC-MS after cleavage. The selective removal of the Alloc protecting group, using $Pd(PPh_3)_4/PhSiH_3$, afforded a free amino at the *C*-terminal Lys side-chain, used for the incorporation of the guanidine moieties. Thus, acylation with the hydrochloride salt of 4-guanidinobenzoic acid and 5-guanidinovaleric acid, in the presence of DIPCDI/HOBt as coupling agents, gave resins **4**{1-2}. On the other hand, direct guanidinylation with various guanidinylating agents (**G**{3-8}),[52] bearing different substituents, led to the dipeptidyl chemset resins **5**{3-8}. Aliquots of every member of the chemsets **4** and **5** were subjected to cleavage and HPLC-MS analysis, revealing a >80% conversion in all cases. Next, each resin, **4**{**G**1-2} and **5**{**G**3-8}, was treated with the acidic cocktail TES:TFA:DCM (1:3:96) for Mtt protecting group removal, and divided into 14 portions for the incorporation of the second element of diversity (acyl groups, **A**{1-14}). Coupling reactions to chemsets **6** and **7** were performed with 1.5 equiv. of the reagent chemset **A**{1-14 } in the presence of DIPCDI/HOBt. Finally, all members of chemsets **6** and **7** were cleaved from the resin with TFA.

The expected final compounds **1**{**G**1-8,**A**1-14} were obtained in variable yields, ranging from low to moderate for sublibraries **1**{1,1-14} and **1**{2,1-14}, and from moderate to good for the other subset of compounds, **1**{3-8,1-14} (see supporting information, Table S1, for details). Ninety (80.4%) of the 112 compounds were isolated in ≥80% purity, and a further

14.3% in 70–80% purity. The remaining hydantoin derivatives (5.3%) showed purities that range from 48 to 68%.

It is interesting to note that main components in library **1**{7,1-14} are the diguanidinyated compounds indicated in Figure 2 (>70%), while the expected monoguanidinylated derivatives were in minor proportion within the mixtures (5–15%). The presence of a second guanidine group was determined by MS, and its location at position 1 of the hydantoin ring was assigned from the ^{1}H NMR spectra (see the chemical shifts of contiguous H5 protons, Table S9 of supporting information). The cleavage of an aliquot of the **5**{7} resin corroborated the incorporation of the second guanidine moiety during the guanidinylation reaction, a side-reaction that was also observed, but in much lower extent, during guanidinylation of resin **3** with **G**4 (8%). Only compounds **1**{7,12} and **1**{7,14} were obtained as monoguanidinylated products in high purity (>80%). This result indicates that treatment of resins **7**{7,12} and **7**{7,14} with piperidine for the Fmoc removal, prior to cleavage, reverted the di- to the monoguanidylated derivative.

All final compounds **1**{**G**1-8,**A**1-14} were obtained as an approximately 1:1 mixtures of diastereoisomers, due to epimerization during the hydantoin ring formation under the DBU basic medium. The Cα of the C-terminal Lys residue is most likely being epimerized, since it was demonstrated that epimerization takes place at the *C*-terminal residue on related hydantoin-derived Phe-Phe model peptides.[53] Although the use of the more reactive carbonylating agent CDI in combination with the weaker base DIEA could avoid epimerization,[39] we decided to use the epimerizing method in our combinatorial program to initially explore a greater diversity.

Screening as TRPV1 and NMDA Channel Blockers

In the search for new bioactive chemical entities, compounds of library **1** were assayed as blockers of the capsaicin-induced channel activity of TRPV1, and of the glutamate-evoked activity of NMDA, both heterogously expressed in *Xenopus laevis* oocytes.

TRPV1 is a neuronal receptor that integrates thermal and chemical stimuli in the peripheral nervous system.[54] From a therapeutic point of view, TRPV1 antagonists have shown efficacy in reducing nociception from inflammatory and neuropathic pain in animal models.[55–587] NMDA receptors are non-specific cation channels that directly contribute to excitatory synaptic transmission, playing a key role in a wide range of physiologic and pathologic processes, such as excitotoxicity.[59] The interest in developing safe and effective NMDA channel blockers comes from the pivotal role of glutamate and NMDA receptors in mediating multiple neurodegenerative CNS disorders.

To identify activity-dependent TRPV1 channel blockers, we used used saturating concentrations of capsaicin, and a hyperpolarized membrane potential. Similar conditions were used for the NMDA receptor by using saturating concentrations of L-glutamate and glycine. The full set of compounds blocked the capsaicin-evoked currents from TRPV1 and the L-glutamate/glycine responses from NMDA receptors to different extents (Figures S3 and S4 in Supporting Information). Noteworthy, some library members significantly inhibited the TRPV1 activity induced by capsaicin, without significantly affecting NMDA receptor function (Figures S3 and S4 in SI). Note that some of the compounds produced in increase of the response probably by binding to an allosteric site that potentiates the activity of the agonist used.

An inspection of the active components of the library provides preliminar information on the structural requirements for TRPV1 channel blockers based on this structure. Thus, highly lipophylic fatty-type substituents, like palmitoyl (**A**5) and litocholyl (**A**8), as well as mnitrocinnamyl (**A**10) are preferred on the 5-(4-aminobutyl) chain. In addition, compounds with substituted guanidine groups (**G**{4-8}) at the C-terminal Lys side-chain showed better TRPV1 blockade than free guanidine-derived analogues (**G**{1-3}). In the last case, the distance between the guanidine and acyl moieties is important for the antagonist potency, with better results for derivatives with the guanidine group directly linked to the Lys side-chain, such as **1**{3,5}, **1**{3,6} or **1**{3,8}, than for the corresponding analogues **1**{1-2} with longer distances between the diversity elements.

A few of these active compounds were selected, resynthesized and evaluated as mixture of enantiomers for *in vivo* activity. Basically, we evaluated the effect attenuating the burning sensation evoked by intraplantar capsaicin administration,[60] which is evidenced by the duration of shaking and licking. In parallel, we evaluated the effect on the thermal nociception by measuring the latency to a response in a hot plate at 52°C. As illustrated in Figure 3, intraperitoneal administration of selected compounds did not affect the thermal nociception as evidenced by the similar latency times of animals administered with vehicle and compounds, indicating that TRPV1 blockers do not affect temperature sensitivity in physiological conditions. In contrast, compounds significantly decreased the burning sensation of intraplantar capsaicin application by reducing the duration of the flinching and licking of the inflamed paw. Administration of capsaicin induces an acute inflammatory state that results in a nocifensive response. Therefore, these findings imply that selected hits display anti-inflammatory activity *in vivo* by attenuating TRPV1 function in nociceptor terminals; although a synergistic effect on NMDA receptors cannot been completely discarded because of their weak interaction with this ion channel. In addition, because these compounds act as non-competitive channel blockers, they may not display the hyperthermic effects observed for competitive antagonists. Further optimization using medicinal chemistry is necessary to clearly unveil the therapeutic potential of these hits and to evolve leads for drug development.

Conclusions

A resin-bound Fmoc-Lys(Mtt)-Lys(Alloc) dipeptide derivative was used as key intermediate for the generation of a hydantoin library with two points of diversity. After the base-promoted cyclization of the corresponding N^{α}-DSC-activated intermediate to the hydantoin ring, the orthogonal deprotection of Lys side-chains allowed the parallel and successive incorporation of the diversity elements through simple reactions. This library, joining the privileged scaffold hydantoin and a series of differently substituted guanidine and diverse acyl groups, allowed the discovery of new hits for TRPV1 ion channel blockade, and to establish the first structural requirements for activity within this series.

Experimental Section

Preparation of resin-bound dipeptide 2

The Fmoc-AM-MBHA-PS resin (3.5 mmol) was swollen in DCM and DMF, and treated with 20% piperidine in DMF (1 × 1 min, 3 × 5 min, 1 × 10 min) for the removal of the Fmoc group, and washed with DMF (5 × 0.5 min), DCM (5 × 0.5 min) and DMF (5 × 0.5 min). Then, Fmoc-Lys(Alloc)-OH (2.375 g, 5.25 mmol), DIPCDI (0.82 mL, 5.25 mmol), and HOBt (0.71 g, 5.25 mmol) were added in DMF (15 mL) and shaken for 6 h. This coupling reaction was repeated overnight. After removing the Fmoc group in the above indicated conditions, Fmoc-Lys(Mtt)-OH (3.28 g, 5.25 mmol),), DIPCDI (0.82 mL, 5.25 mmol), and HOBt (0.71 g, 5.25 mmol) were added in DMF (15 mL) and shaken for 12 h. The second coupling was repeated twice with 1 equivalent of the amino acid derivative and coupling agents. Couplings were monitored by the Kaiser ninhydrin test.

Synthesis of Hydantoin intermediate 3

Resin **2** (3.5 mmol) was first treated with 20% piperidine in DMF (1 × 5 min, 1 × 20 min), washed with DMF (5 × 0.5 min), DCM (5 × 0.5 min) and DMF (5 × 0.5 min), and reacted with DSC (5 equiv.) and DMAP (0.5 equiv.) in DMF (minimum volume possible). The mixture was shaken for 3 h, washed with DMF (5 × 0.5 min.) and DCM (5 × 0.5 min.), and completion was monitored by the ninhydrin test. Then the resin was treated with DBU in DMF (1:4 v/v 1 × 1 min., 1 × 20 min.), and washed as previously indicated. An aliquot portion of the resin was cleaved to the expected product, (4″-Amino)butyl-3-(5′-allyloxycarbonylamino-1′-carbamoyl)pentylhydantoin, which was analyzed as follows: HPLC t_R = 12.50 min (>90%); ESI-MS: 406.2 $(M+Na)^+$. At this point the resin was divided into 8 batches for further modifications.

Preparation of resins 4{1-2}

Batches 1 and 2 of resin **3** (0.43 mmol each) were individually reacted with $Pd(PPh_3)_4$ (0.1 equiv.) and $PhSiH_3$ (24 equiv.) in DCM under Ar, to remove the Alloc group. The reaction mixtures were shaken under Ar for 20 min and washed with DCM. The resulting resins were swollen in DMF and treated with **G**1 and **G**2 (1.1 mmol), repectively, DIPCDI (1.1 mmol) and HOBt (1.1 mmol). The couplings were accomplished for 12 h, and repeated twice with 1 equiv. of each reagent for 12 h, until negative ninhydrin test. HPLC and MS analysis of the cleavage products indicated a >86% conversion to the expected guanidine-derived hydantoin intermediates in both cases.

Preparation of resins 5{3-8}

After removing the Alloc group in bathes 3-8 of resin 3 (0.43 mmol each), as above indicated, DIEA (2.16 mmol in the case of G3 and 1.2 mmol in all other cases) and the corresponding guanidinylating agent (G{3-8}, 2.16 mmol for G3 and 1.2 mmol for others) were successively added. For the preparation of resin 5{3}, the guanidinylation reaction was carried out for 12 h and repeated twice in the same conditions. For the synthesis of resins 5{4-8}, the reaction was shorter (3 h), and it needed to be repeated once with half equivalents of each reagent. This reaction was monitored by the Kaiser test. In all cases, after cleavage of aliquot portions, good conversions were observed in HPLC and the $(M+1)^+$ of the corresponding guanidinylated intermediates were found in MS experiments. The only exception was compound 5{7}, for which the main product corresponded to a diguanidinylated derivative with $(M+1)^+$ = 601.9.

General procedure for the preparation of resin-bound intermediates 6 and 7

Syringes containing resins **4**{1-2} and **5**{3-8} were swollen in DCM and treated with TES:TFA:DCM (1:3:96, 3 × 10 min, and 2 × 15 min) for the removal of the Mtt group. After washing with DMF (5 × 0.5 min), DCM (5 × 0.5 min) and DMF (5 × 0.5 min), a neutralization step was carried out with 5% DIEA in DCM (3 × 0.5 min) followed by a final washing with DCM and drying under vacuum. Each resulting resin was then divided into 14 sub-batches for the incorporation of the selected carboxylic acids. For this purpose, resins of each sub-batch were treated with the corresponding carboxylic acids (1.5 equiv.), HOBt (1.5 equiv.) and DIPCDI (1.5 equiv.). When required, the coupling reaction was repeated until the Kaiser test was negative.

General procedure for the cleavage from the resin: Synthesis of final compounds 1

Cleavage from the resins was carried out by treatment with neat TFA (1 mL/100 mg of resin) for 1.5 h. After filtration, the acidic filtrate was evaporated by a N_2 or Ar stream, and the resultant residue was triturated with Et_2O and centrifuged. The resulting solid residue was separated, dissolved in H_2O or H_2O/ACN mixtures and lyophilized. All library members were characterized by HPLC, MS and ^{1}H NMR, while ^{13}C NMR data was recorded for some representative compounds (see supporting information for details).

Recombinant rat TRPV1 and NMDAR channels expression in *Xenopus* oocytes and channel blockade

All the procedures have been described in detail elsewhere[60]. Whole-cell currents from rat TRPV1-injected oocytes were recorded in Mg^{2+}-Ringer's solution (in mM: 10 Hepes pH 7.4, 115 NaCl, 2.8 KCl, 0.1 $BaCl_2$, 2.0 $MgCl_2$) with a two-microelectrode voltage-clamp amplifier at 20°C. TRPV1 channels were activated by application of 10 μM capsaicin in absence or presence of individual compounds at a holding potential (V_h) of −80 mV. Receptor selectivity was evaluated on heterologously expressed NMDA receptors (rat NR1:NR2A). Recombinant NMDA receptor responses were activated with 100 μM L-glutamate plus 20 μM glycine in the absence and presence of the compound in normal Ringer solution (in mM: 10 Hepes pH 7.4, 115 NaCl, 2.8 KCl, 1.8 $BaCl_2$), and at a holding of −80 mV.

Behavioural nociception assays

Adult male ICR mice were habituated to the test environment for 24 h in plexiglass chambers prior the nociception assays. Thermal nociception was studied using a hot plate at 52°C. The response latency for paw shaking or licking or jumping was measured[60].

Capsaicin-induced hyperalgesia

Capsaicin (10 μL at 0.06% in 10% ethanol, 10% Tween 80 and 80% saline) was injected intradermally into the heel pad with a 0.3 mm diameter needle attached to a Hamilton syringe. The duration time of licking and shaking the paw in response to the injection was recorded[60].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Ac_2O	acetic anhydride
ACN	acetonitrile
Alloc	allyloxycarbonyl
CDI	1,1′-carbonyldiimidazole
CNS	central nervous system

DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DIPCDI	N,N′-diisopropylcarbodiimide
DIEA	diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	dimethylformamide
DSC	*N*,*N*′-Disuccinimidyl carbonate
Fmoc	Fluorenylmethoxycarbonyl
HOBt	hydroxybenzotriazole
NMDA	N-methyl D-aspartate
Mtt	4-Methyltrityl
Fmoc-Rink amide-MBHA-PS or Fmoc-AM-MBHA-resin	4-(2′,4′dimethoxyphenyl-Fmoc-aminomethyl)-pheoxyacetamido *p*-methylbenhidrylamine polystyrene resin
HPLC	high performance liquid chromatography
HPLC-MS	high performance liquid chromatography-mass spectrometry
MS	mass spectrometry
ESI-MS	electrospray ionization mass spectrometry
SPPS	solid-phase peptide synthesis
TES	triethyl silane
TFA	trifluoroacetic acid
TRVP1	transient receptor potential cation channel, subfamily V, member 1. Abbreviations used for amino acids follow the IUPAC-IUB Commission of Biochemical Nomenclature in Jones, J.H. *J. Pept. Sci.*, **2003**, *9*, 1–8

References

1. López, CA.; Trigo, GG. Advances in Heterocyclic Chemistry. Brown, C.; Davinson, RM., editors. Vol. 38. Academic Press; New York: 1985. p. 177-228.
2. Kiec-Kononowicz K, Szymanska E. Antimycobacterial activity of 5-arylidene derivatives of hydantoin. Il Farmaco. 2002; 57:909–916. [PubMed: 12484539]
3. Carmi C, Cavazzoni A, Zuliani V, Lodola A, Bordi F, Plazzi PV, Alfieri RR, Petroni PG, Mor M. 5-Benzylidene-hydantoins as new EGFR inhibitors with antiproliferative activity. Bioorg Med Chem Lett. 2006; 16:4021–4025. [PubMed: 16713265]
4. Bakalova A, Buyukliev R, Tcholakova I, Momekov G, Kostantinov S, Karavanova M. Synthesis, physicochemical investigation and cytotoxic activity of new Pt(II) complexes with hydantoin ligands. Eur J Med Chem. 2003; 38:627–632. [PubMed: 12832135]
5. Opacic N, Barbaric M, Zorc B, Cetina M, Nagl A, Frkovic D, Kralj M, Pavelic K, Balzarini J, Andrei G, Snoeck R, De Clercq E, Raic-Malic S, Mintas M. Peptidyl 3-substituted 1-hydroxyureas as isosteric analogues of succinylhydroxamate MMP inhibitors. J Med Chem. 2005; 48:475–482. [PubMed: 15658861]

6. Jansen M, Potschka H, Brandt C, Löscher W, Dannhardt G. Hydantoin-substituted 4,6-dichloroindole-2-carboxylic acids as ligands with high affinity for the glycine binding site of the NMDA receptor. J Med Chem. 2003; 46:64–73. [PubMed: 12502360]
7. Zha C, Brown GB, Brouillette WJ. Synthesis and SAR studies for hydantoins and analogues as volltage-gated sodium channel ligands. J Med Chem. 2004; 47:6519–6528. [PubMed: 15588087]
8. Last-Barney K, Davidson W, Cardozo M, Frye LL, Grygon CA, Hopkins JL, Jeanfavre DD, Pav S, Quian C, Stevenson JM, Tong L, Zindell R, Kelly TA. Binding site elucidation of hydantoin-based antagonists of LFA-1 using multidisciplinary technologies: evidence for the allosteric inhibition of a protein-protein interaction. J Am Chem Soc. 2001; 123:5643–5650. [PubMed: 11403595]
9. Potin D, Launay M, Nicolai E, Fabreguette M, Malabre P, Caussade F, Besse D, Skala S, Stetsko DK, Todderud G, Reno BR, Cheney DL, Chang CJ, Sheriff S, Hollenbaugh DL, Barrish JC, Iwanowicz EJ, Suchard SJ, Dhar TGM. De novo design, synthesis, and in vitro activity of LFA-1 antagonists based on a bicyclic[5.5]hydantoin scaffold. Bioorg Med Chem Lett. 2005; 15:1161–1164. [PubMed: 15686933]
10. Evans BE, Rittle KE, Bock MG, DiPardo RM, Freidinger RM, Whitter WL, Lundell GF, Veber DF, Anderson PS, Chang RSL, Lotti VJ, Cerino DJ, Chen TB, Kling PJ, Kunkel KA, Springer JP, Hirsfield J. Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists. J Med Chem. 1988; 31:2235–2246. [PubMed: 2848124]
11. Meusel M, Gütschow M. Recent Developments in Hydantoin Chemistry. A Review. Org Prep Proced Int. 2004; 36:391–443.
12. Reyes S, Burgess K. On Formation of Thiohydantoins from Amino Acids under Acylation Conditions. J Org Chem. 2006; 71:2507. [PubMed: 16526805]
13. Murray RG, Whitehead D, Le Strat F, Conway SJ. Facile one-pot synthesis of 5-substituted hydantoins. Org Biomol Chem. 2008; 6:988. [PubMed: 18327322]
14. Hulme C, Ma L, Romano JJ, Morton G, Tang SY, Cherrier MP, Choi S, Salvino J, Labaudiniere R. Novel applications of convertible isonitriles for the synthesis of mono and bicyclic -lactams via a UDC strategy. Tetrahedron Lett. 2000; 41:1889–1893.
15. Zhang W, Lu Y, Chen HT, Zeng L, Kassel DB. Fluorous Mixture Synthesis of Two Libraries with Hydantoin- and Benzodiazepinedione-Fused Heterocyclic Scaffolds. J Comb Chem. 2006; 8:687–695. [PubMed: 16961407]
16. DeWitt HS, Kiely JS, Stankovic CJ, Schroeder MC, Cody DMR, Pavia MR. "Diversomers": an approach to nonpeptide, nonoligomeric chemical diversity. Proc Natl AcadSci USA. 1993; 90:6909–6913.
17. Mattews J, Rivero RA. Base-Promoted Solid-Phase Synthesis of Substituted Hydantoins and Thiohydantoins. J Org Chem. 1997; 62:6090–6092.
18. Kim SW, Ahn SY, Koh JS, Lee JH, Ro S, Cho HY. Solid phase synthesis of hydantoin library using a novel cyclization and traceless cleavage step. Tetrahedron Lett. 1997; 38:4603–4606.
19. Kim SW, Koh JS, Lee EJ, Ro S. Solid phase synthesis of benzamidine and butylamine-derived hydantoin libraries. Mol Diver. 1998; 3:129–132.
20. Boeijen A, Kruijtzer JAW, Liskamp RMJ. Combinatorial chemistry of hydantoins. Bioorg Med Chem Lett. 1998; 8:2375–2380. [PubMed: 9873544]
21. Karnbrock W, Deeg M, Gerhardt J, Rapp W. Solid phase synthesis of hydantoins by thermal cyclization and screening of reaction conditions using APOS 1200. Mol Diver. 1998; 3:165–171.
22. Gong YD, Najdi S, Olmstead MM, Kurth MJ. Solid-Phase Synthesis: Intramolecular Azomethine Ylide Cycloaddition (→Proline) and Carbanilide Cyclization (→Hydantoin) Reactions. J Org Chem. 1998; 63:3081–3086.
23. Park KH, Kurth MJ. An uncatalyzed cyclo-elimination process for the release of N_3-alkylated hydantoins from solid-phase: synthesis of novel isoxazoloimidazolidinediones. Tetrahedron Lett. 1999; 40:5841–5844.
24. Huang W, Cheng S, Sun W. 2-Polystyrylsulfonyl ethanol supports for the solid-phase syntheses of hydantoins and ureas. Tetrahedron Lett. 2001; 42:1973–1974.
25. Park KH, Ehrler J, Spoerri H, Kurth MJ. Preparation of a 990-Member Chemical Compound Library of Hydantoin- and Isoxazoline-Containing Heterocycles Using Multipin Technology. J Comb Chem. 2001; 3:171–176. [PubMed: 11300857]

26. Lamothe M, Lannuzel M, Perez M. Solid-Phase Preparation of Hydantoins through a New Cyclization/Cleavage Step. J Comb Chem. 2002; 4:73–78. [PubMed: 11831884]
27. Alsina J, Scott WL, O'Donnell MJ. Solid-phase synthesis of α-substituted proline hydantoins analogs. Tetrahedron Lett. 2005; 46:3131–3135.
28. Kuster GJT, van Berkom LWA, Kalmoua M, van Loevezijn A, Sliedregt LAJM, van Steen BJ, Kruse CG, Rutjes FPJT, Scheeren HW. Synthesis of Spirohydantoins and Spiro-2,5-diketopiperazines via Resin-Bound Cyclic α,α-Disubstituted α-Amino Esters. J Comb Chem. 2006; 8:85–94. [PubMed: 16398558]
29. Yeh WP, Chang WJ, Sun ML, Sun CM. Microwave-assisted traceless synthesis of hydantion-fused β-carboline scaffold. Tetrahedron. 2007; 63:11809–11816.
30. Dressman BA, Spangle LA, Kaldor SW. Solid phase synthesis of hydantoins using a carbamate linker and a novel cyclization/cleavage step. Tetrahedron Lett. 1996; 37:937–940.
31. Xiao XY, Ngu K, Chao C, Patel DV. Selective Solid Phase Synthesis of Ureas and Hydantoins from Common Phenyl Carbamate Intermediates. J Org Chem. 1997; 62:6968–6973.
32. Chong PY, Petillo PA. Solid phase hydantoin synthesis: An efficient and direct conversion of Fmoc-protected dipeptides to hydantoins. Tetrahedron Lett. 1999; 40:2493–2496.
33. Bhalay G, Cowell D, Hone ND, Scobie M, Baxter AD. Multiple solid-phase synthesis of hydantoins and thiohydantoins. Mol Diver. 1997; 3:195–198.
34. Nefzi A, Dooley C, Ostresh JM, Houghten RA. Combinatorial chemistry: From peptides and peptidomimetics to small organic and heterocyclic compounds. Bioorg Med Chem Lett. 1998; 8:2273–2278. [PubMed: 9873527]
35. Nefzi A, Ostresh JM, Giulianotti M, Houghten RA. Efficient solid phase synthesis of 3,5-disubstituted hydantoins. Tetrahedron Lett. 1998; 39:8199–8202.
36. Nefzi A, Giulianotti M, Truong L, Rattan S, Ostresh JM, Houghten RA. Solid-Phase Synthesis of Linear Ureas Tethered to Hydantoins and Thiohydantoins. J Comb Chem. 2002; 4:175–178. [PubMed: 11886293]
37. Severinsen R, Lau JF, Bondensgaard K, Hansen BS, Begtrup M, Ankersen M. Parallel solid-phase synthesis of disubstituted (5-biphenyltetrazolyl) hydantoins and thiohydantoins targeting the growth hormone secretagogue receptor. Bioorg Med Chem Lett. 2004; 14:317–320. [PubMed: 14698149]
38. Royo M, Van der Nest W, Del Fresno M, Frieden A, Yahalom D, Rosenblatt M, Chorev M, Albericio F. Solid-phase syntheses of constrained RGD scaffolds and their binding to the $\alpha_v\beta_3$ integrin receptor. Tetrahedron Lett. 2001; 42:7387–7391.
39. Vazquez J, García-Jareño A, Mondragón L, Rubio-Martínez J, Pérez-Payá E, Albericio F. Conformationally Restricted Hydantoin-Based Peptidomimetics as Inhibitors of Caspase-3 with Basic Groups Allowed at the S_3 Enzyme Subsite. ChemMedChem. 2008; 3:979–985. [PubMed: 18393268]
40. Ekelund S, Nygren P, Larsson R. Guanidino-containing drugs in cancer chemotherapy: biochemical and clinical pharmacology. Biochem Pharmacol. 2001; 61:1183–1193. [PubMed: 11322922]
41. Ravaud A, Cerny T, Terret C, Wanders J, Bui BN, Hess D, Droz JP, Fumoleau P, Twelves C. Phase I study and pharmacokinetic of CHS-828, a guanidino-containing compound, administered orally as a single dose every 3 weeks in solid tumours: An ECSG/EORTC study. Eur J Cancer. 2005; 41:702–707. [PubMed: 15763645]
42. Sienczyk M, Oleksyszyn J. Inhibition of trypsin and urokinase by Cbz-amino(4-guanidinophenyl)methanephosphonate aromatic ester derivatives: The influence of the ester group on their biological activity. Bioorg Med Chem Lett. 2006; 16:2886–2890. [PubMed: 16546384]
43. Katsura Y, Nishino Y, Inoue Y, Sakane K, Matsumoto Y, Morinaga C, Ishikawa H, Takasugi H. Anti-*Helicobacter pylori* agents. 5. 2-(substituted guanidino)-4-arylthiazoles and Aryloxazole Analogues. J Med Chem. 2002; 45:143–150. [PubMed: 11754586]
44. Oh CH, Cho JH. Synthesis and biological evaluation of 1β-methylcarbapenems having guanidino moieties. Eur J Med Chem. 2006; 41:50–55. [PubMed: 16260069]

45. Masuda T, Yoshida S, Arai M, Kaneko S, Yamashita M, Honda T. Synthesis and anti-influenza evaluation of polyvalent pialidase inhibitors bearing 4-guanidino-Neu5Ac2en derivatives. Chem Pharm Bull. 2003; 51:1386–1398. [PubMed: 14646315]
46. Huang H, Martasek P, Roma LJ, Silverman RB. Synthesis and evaluation of peptidomimetics as selective inhibitors and active site probes of Nitric Oxide Synthases. J Med Chem. 2000; 43:2938–2945. [PubMed: 10956202]
47. Lam PYS, Clark CG, Li R, Pinto DJP, Orwat MJ, Galemmo RA, Fevig JM, Teleha CA, Alexander RS, Smallwood AM, Rossi KA, Wright MR, Bai SA, He K, Luettgen JM, Wong PC, Knabb RM, Wexler RR. Structure-based design of novel guanidine/benzamidine mimics: potent and orally bioavailable factor Xa inhibitors as novel anticoagulants. J Med Chem. 2003; 46:4405–4418. [PubMed: 14521405]
48. Soyka R, Guth BD, Weisenberger HM, Luger P, Müller TH. Guanidine derivatives as combined thromboxane A_2 receptor antagonists and synthase Inhibitors. J Med Chem. 1999; 42:1235–1249. [PubMed: 10197967]
49. Lack SL, Chaovignac C, Grundt P, Miller CN, Wood S, Traynor JR, Lewis JW, Husbands SM. Guanidino N-substituted and N,N-disubstituted derivatives of the κ-opioid antagonist GNTI. J Med Chem. 2003; 46:5505–5511. [PubMed: 14640558]
50. Suagse K, Horikawa M, Sugiyama M, Ishiguro M. Restriction of a peptide turn conformation and Conformational analysis of guanidino group using arginine-proline fused amino acids: application to mini Atrial Natriuretic peptide on binding to the receptor. J Med Chem. 2004; 47:489–492. [PubMed: 14711318]
51. Lee S, Yi KY, Hwang SK, Lee BH, Yoo SE, Lee K. (5-Arylfuran-2-ylcarbonyl)guanidines as cardioprotectives through the inhibition of Na^+/H^+ exchanger isoform-1. J Med Chem. 2005; 48:2882–2891. [PubMed: 15828827]
52. Del Fresno M, El-Faham A, Carpino LA, Royo M, Albericio F. Substituted guanidines: introducing diversity in combinatorial chemistry. Org Lett. 2000; 2:3539–3542. [PubMed: 11073639]
53. Vázquez J, Royo M, Albericio F. Re-evaluation of a solid-phase hydantoin synthesis. Lett Org Chem. 2004; 1:224–226.
54. Caterina MJ, Julius D. The Vanilloid receptor: a molecular gateway to the pain pathway. Annu Rev Neurosci. 2001; 24:487–517. [PubMed: 11283319]
55. Jara-Oseguera A, Simon SA, Rosenbaum T. TRPV1: on the road to pain relief. Curr Mol Pharmacol. 2008; 1:255–269. [PubMed: 20021438]
56. Szallasi A, Cortright DN, Blum CA, Eid SR. The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. Nat Rev Drug Discov. 2007; 6:357–372. [PubMed: 17464295]
57. Messeguer A, Planells-Cases R, Ferrer-Montiel A. Physiology and pharmacology of the vanilloid receptor. Curr Neuropharmacol. 2006; 4:1–5. [PubMed: 18615132]
58. Garcia-Martinez 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 non competitive antagonist of the TRVP1channel with in vivo analgesic and anti-inflammatory activity. J Pain. 2006; 7:735–746. [PubMed: 17018334]
59. Albensi BC. The NMDA receptor/ion channel complex: a drug target for modulating synaptic plasticity and excitotoxicity. Curr Pharm Des. 2007; 13:3185–3194. [PubMed: 18045168]
60. 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. Attenuation of thermal nociception and hyperalgesia by VR1 blockers. Proc Natl Acad Sci USA. 2002; 99:2374–2379. [PubMed: 11854530]

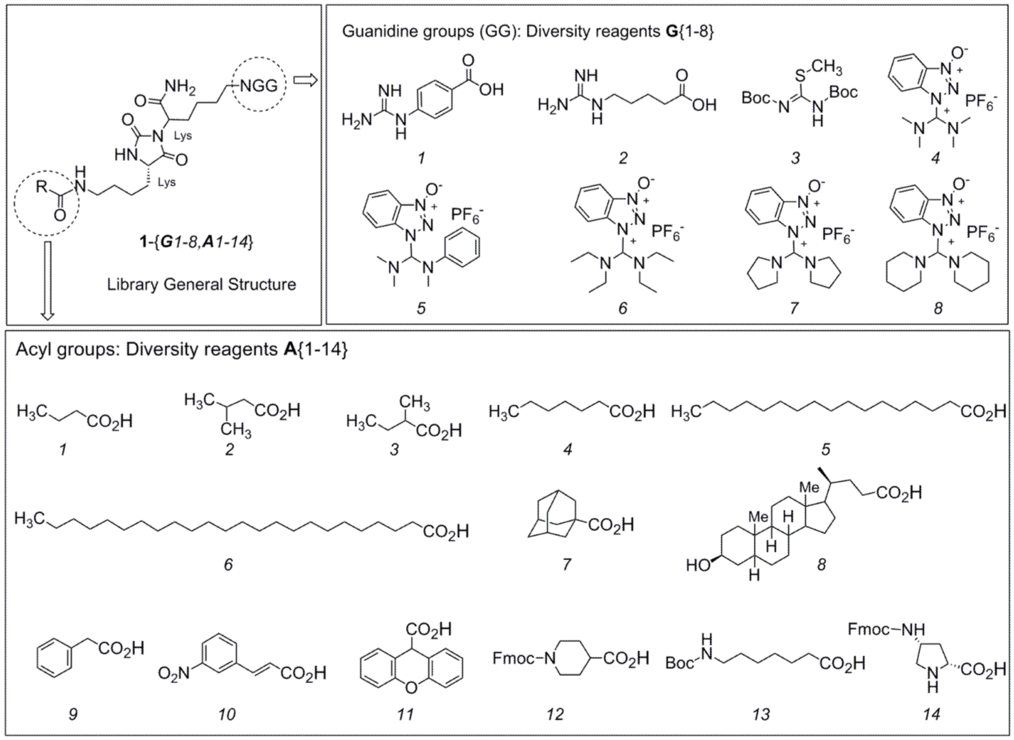
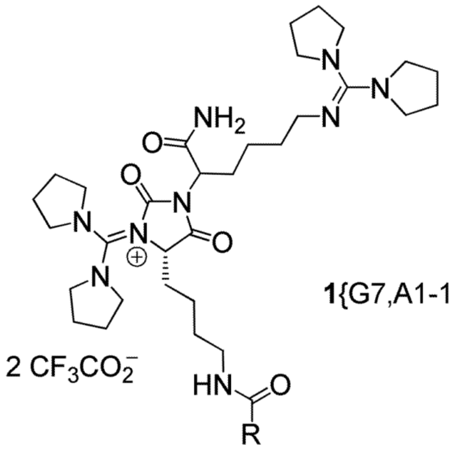
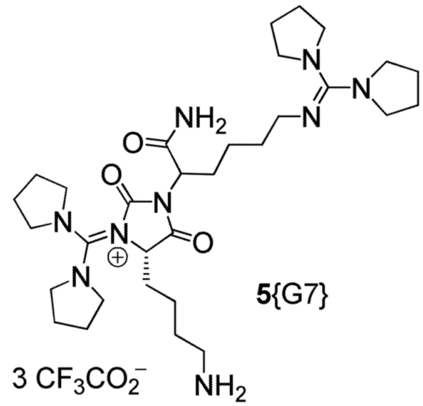


Figure 1.
General structure of the hydantoin library and selected building blocks



1{G7,A1-11,13}



5{G7}

Figure 2.

Diguanidylated compounds obtained for **G**7 sublibrary and cleavage product of **5**{**G**7}

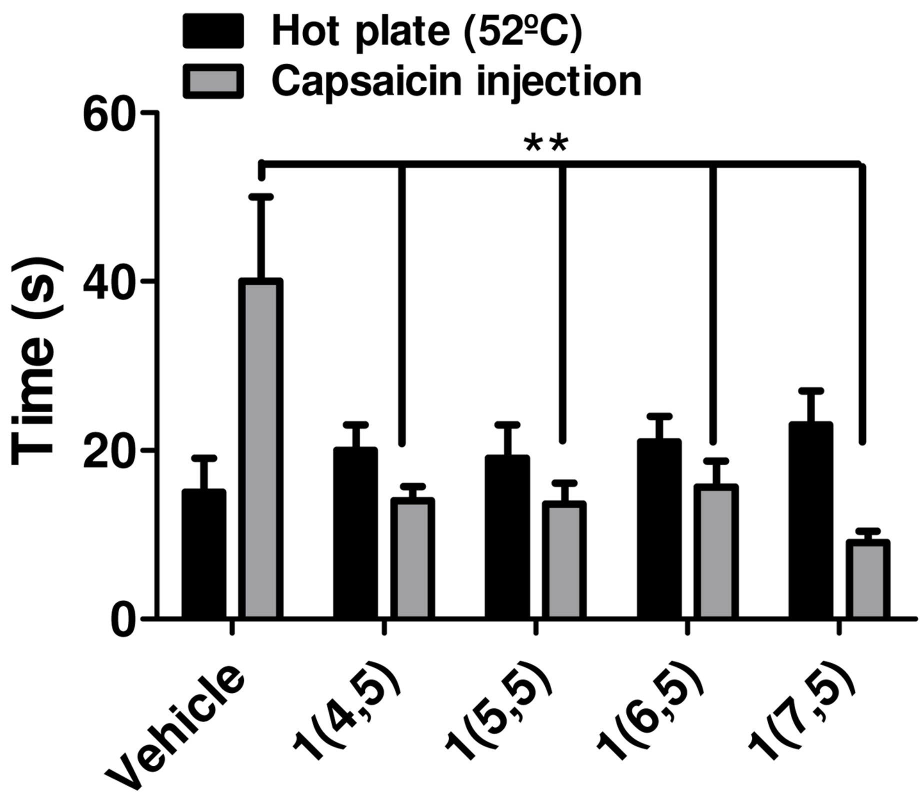
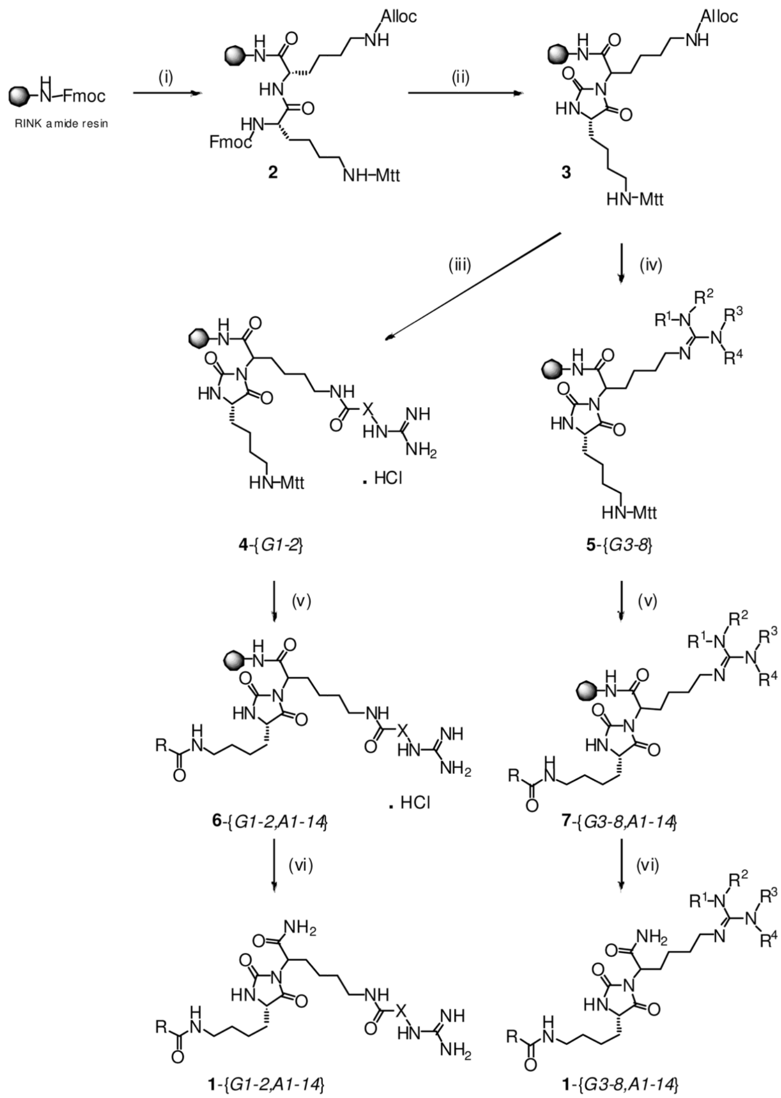


Figure 3.

In vivo activity of **1**{**G**1-8,**A**1-14} library compounds with significant TRVP1 blockade properties and . a) Black bars show latency time to the first response of animals in a hot plate at 52°C[42]. Mice were administered ip with saline (vehicle) or 30 mg/Kg of the compounds. b) Grey bars show duration time of the burning sensation evoked by intraplantar administration of capsaicin, measured as licking and shaking the paw[59]. Compound was administered ip at 30 mg/Kg. Data are given as mean±sem, with n≥6.



Scheme 1.

Reagents and conditions: (i) (a) Piperidine/DMF (1:4); (b) Fmoc-Lys(Alloc)-OH (1.5 equiv.), DIPCDI (1.5 equiv.), HOBt (1.5 equiv.), DMF; (c) Piperidine/DMF (1:4); (d) Fmoc-Lys(Mtt)-OH (1.5 equiv.), DIPCDI (1.5 equiv.), HOBt (1.5 equiv.), DMF. (ii) (a) Piperidine/DMF (1:4), DSC (5 equiv.), DMAP (0.5 equiv.); (b) 5% DBU/DMF. (iii) (a) (a) Pd[PPh_3]$_4$, $PhSiH_3$; (b) HCl.H_2N(NH)CNH-X-CO_2H (1.5 equiv.), DIPCDI (1.5 equiv.), HOBt (1.5 equiv.), DMF. (iv) (a) Pd [PPh_3]$_4$, $PhSiH_3$; (b) Guanidylation reaction. (v) (a) TES:TFA:DCM (1:3:96); (b) RCO_2H (1.5 equiv.), DIPCI (1.5 equiv.), HOBt (1.5 equiv.), DMF. (vi) TFA.