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Design, synthesis and biological evaluation of multifunctional ligands targeting opioid and bradykinin 2 receptors

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ABSTRACT

We report here the design and synthesis of novel multifunctional ligands that act as (μ/δ) opioid agonists and bradykinin 2 receptor antagonists. These multifunctional ligands were designed to interact with the multiple receptors to show an enhanced analgesic effect, with no opioid-induced tolerance. We designed our multifunctional ligands based on the well-known second generation bradykinin 2 receptor antagonist Hoe 140 (DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg-OH) and the opioid enkephalin analogues Tyr-DAla-Phe, Tyr-DAla-Gly-Phe and Tyr-Pro-Phe. We explored the conjugation of opioid pharmacophore to the Hoe 140 (DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg-OH) in various positions with and without a linker. These bifunctional ligands showed very good binding affinity towards the both μ and δ opioid receptors. Among these bifunctional ligands **8**, **11** and **12** showed excellent and balanced binding affinity at both μ and δ opioid receptors (0.5 nM, 2.0 nM; 0.3 nM, 2 nM; 2 nM and 3 nM), respectively. On the other hand these bifunctional ligands showed very weak and no binding affinity for rat brain bradykinin 2 receptors. Similarly, the Hoe 140 showed very low affinity (>10,000 nM and 9000 nM) against [^{3}H] BK binding in rat brain membranes and in HEK293 cells, respectively. In contrast, the Hoe 140 showed very good binding affinity in guinea pig ileum (0.43 nM) similar to that of previously reported. The bradykinin 2 receptors are known to be present in rat brain membrane, guinea pig ileum (GPI) and rabbit jugular vein. Previously the binding affinity of Hoe 140 for bradykinin 2 receptor was reported using guinea pig ileum. The above results suggest that the bradykinin 2 receptors present in rat brain membrane are a different sub type than the bradykinin 2 receptor present in guinea pig ileum (GPI).

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Chronic pain or prolonged pain is one of the most difficult pathologies to diagnose and treat effectively. It is estimated that over hundred million American adults live in chronic pain, according to a 2011 report from the prestigious Institute of Medicine an arm of the National Academy of Sciences.[1] Currently, opioids are the most common choice for chronic pain management. Although they provide pain relief in many patients, their major disadvantages stem from their side effect profile, which includes constipation, nausea, vomiting, and respiratory depression. Opioid receptors, which belong to the large family of G-protein coupled receptors, mediate antinociceptive effects in humans, and these are classified into three different type's mu (μ), delta (δ) and kappa (κ) receptors. Kinins are naturally occurring vasoactive peptides which are known to be important mediators of a variety of biological effects, including cardiovascular homeostasis, inflammation, and nociception.[2–4] The kinin family includes bradykinin, kallidin and their active metabolites des-Arg-bradykinin and des-Arg-kallidin, the biological actions of these kinins are mediated by two major G-protein coupled bradykinin receptors: B1 and B2.[5,6] Overproduction of kinins under pathophysiological conditions is associated with a number of disorders, including pain and inflammation. Therefore, antagonists of BR receptors may offer a novel approach to the treatment of pain. Bradykinin receptors act as mediators of inflammation and pain in the PNS and CNS. A systematic modification of natural agonist bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) led to the development of the most

Abbreviations: BK, bradykinin; BR, bradykinin receptor; CNS, central nervous system; DALKD, [des-Arg10, Leu9]-kallidin; Dap, 2,3-diaminopropionic acid; DIPEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; GPI, guinea pig ileum; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate; HCTU, 2-(6-chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate; HOBt, *N*-hydroxybenzotriazole; PNS, peripheral nervous system; RP-HPLC, reverse phase high performance liquid chromatography; rt, room temperature; SAR, structure–activity relationship; TFA, trifluoroacetic acid; TIS, triisopropylsilane.

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well-known second generation bradykinin 2 antagonist Hoe 140 (DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg-OH).[4] The Hoe 140 is currently used in Europe for the treatment of hereditary angioedema, a rare and life threatening disease.[7]

Lai et al.[8,9] reported a pronociceptive actions of dynorphin A via bradykinin receptors in rat brain membranes, dynorphin A activates bradykinin receptors to maintain neuropathic pain. They hypothesized that under chronic pain conditions, up-regulated dynorphin A (Dyn A) interacts with bradykinin receptors (BRs) in the spinal cord to promote hyperalgesia. Recently our group reported a dynorphin A analogues to inhibit the neuroexcitatory actions of dynorphin A through bradykinin receptors in the spinal cord.[10–12] Earlier our group successfully explored the bifunctional/bivalent ligand approach and discovered potent multifunctional ligands for opioid-neurokinin,[13,14] and opioid-cholecystokinin[15–17] receptors. Based on the above report and our previous experience in successful exploration of bifunctional/bivalent ligands, here we designed and synthesized novel multifunctional ligands that act as (μ/δ) opioid agonists and bradykinin receptor (B2 receptor) antagonists. Here we have taken a multidisciplinary approach to discover novel peptide and peptidomimetic ligands which have potent antinociceptive activities in both acute and neuropathic pain states. Our basic hypothesis is such that multivalent and bifunctional ligands will be particularly useful for neuropathic pain and for chronic pain without tolerance. We propose that the synergistic effect between opioid and bradykinin ligands will enhance the biological activity of the designed multifunctional ligands designed for both bradykinin and opioid receptors. Many potent opioid receptor agonists and bradykinin receptor antagonists are already discovered.[18–20] The endogenous neuropeptides enkephalins, β-endorphins, endomorphins and dynorphins which interact with the δ, μ and κ opioid receptors, respectively.[21] The subsequent elongation or truncation modifications of these endogenous peptides led to highly promising opioid agonists.[22,23] The opioid fragments for our multifunctional ligands (Tyr-DAla-Phe, Tyr-DAla-Gly-Phe and Tyr-Pro-Phe) which are potent well established mu/delta ligands. The systematic modification of natural agonist bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) led to the development of several bradykinin 2 antagonists including Hoe 140 (DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg-OH) and B-9430.[4] Similarly the structure activity studies have taken place on Hoe 140 and other peripheral bradykinin 2 receptor antagonists. Introduction of a back bone structural constraint, replacement of aromatic-aliphatic substitution and further gain in affinity and metabolic resistance, loss in residual agonist effect leads to the Hoe 140 other bradykinin 2 receptor antagonists.[24,25] Kyle et al. extensively studied the structure–activity relationships of Hoe 140 analogues as have other research groups.[26–32]

Our approach started with the linear multifunctional ligands that contain the agonist opioid pharmacophore and the antagonist pharmacophore for the bradykinin 2 receptor. Here we designed and synthesized novel bifunctional ligands based on the most well-known second generation bradykinin B2 receptor antagonist Hoe 140 (DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg-OH), with the opioid pharmacophore (opioid mu/delta agonist Tyr-DAla-Phe, Tyr-DAla-Gly-Phe and Tyr-Pro-Phe) conjugated to the N-terminus of Hoe 140. In our further designs, the opioid pharmacophore (Tyr-Dala-Gly-Phe) was conjugated to different possible positions of Hoe 140 with and without a linker. Our initial approach ligands (**3**–**8**) start with linear peptides that contain the agonist opioid pharmacophore and an antagonist pharmacophore for the bradykinin 2 receptor with and without linker. For the μ/δ agonist component we have chosen (Tyr-DAla-Phe, Tyr-DAla-Gly-Phe and Tyr-Pro-Phe) structure with the possible addition of an amino acid linker such as Met and Phe or other linkers such as β-alanine and 8-aminooctanoic acid conjugated to the N-terminus of Bradykinin 2 receptor antagonists Hoe 140. (see Figs. 1 and 2)

In bifunctional ligands (**9**–**11**) we conjugated the Dap (2,3-diaminopropionic acid) to the N-terminus of Hoe 140, and the opioid pharmacophore was attached to the side chain of the 2,3-diaminopropionic acid amino group with and without a linker. The ligands **12** and **13** were designed based on the biological activities of Ala scan analogues of Hoe 140.[30] In ligands **12** and **13** we replaced the 4th position hydroxyl proline and 7th position serine with Dap (2,3-diaminopropionic acid) in Hoe 140, and the opioid pharmacophore was attached to the side chain amino group of Dap.

All peptides were synthesized by solid phase peptide synthesis using the Fmoc/tBu strategy with HCTU as a coupling reagent on a Wang resin followed by cleavage using 92.5% trifluoroacetic acid cocktail solution containing 2.5% TIS, 2.5% water and 2.5% 1,2-ethanedithiol to quench the highly stabilized carbocation's released from permanent protecting groups to give the crude peptides, and the crude peptides were precipitated by addition of cold ether. Final purification is performed by semi-preparative RP-HPLC with 10–40% acetonitrile gradient in water containing 0.1% trifluoroacetic acid in 40 min, 3 mL/min. The Dap (2,3-diaminopropionoic acid) containing enkephalin peptide analogues **1** and **2** were prepared by solution phase peptide synthesis following the N^{α}-Boc strategy by utilizing HBTU as the coupling reagent with DIPEA as base and acetonitrile as a solvent. By utilizing the enkephalin peptide derivatives **1** and **2** in solid phase peptide synthesis the following multifunctional ligands **9**, **10**, **11**, **12** and **13** were synthesized.

The biological evaluation of synthesized bifunctional peptide derivatives were performed as previously described. The binding affinities of the synthesized bifunctional peptide derivatives for the human δ-opioid receptors and rat μ-opioid receptors were determined by competitive binding against ([^{3}H]DPDPE) and ([^{3}H]DAMGO) in cells that stably express these receptors.[33] Binding affinities of multifunctional peptide analogues **3**, **4**, **5**, **6** and **Hoe 140** at the bradykinin 2 receptors were determined by radio-ligand competition analysis using [^{3}H] BK in rat brain membranes according to the procedure described earlier.[10] The antagonist action of the peptide derivatives **4**, **5** and **6** were determined for tissue bioassays using GPI in the presence of naloxone to block all the opioid-mediated activities (the detailed experimental procedures are available in the Experimental section).

These multifunctional ligands showed good to excellent in vitro binding affinity both at μ and δ opioid receptors but weak binding affinity at the bradykinin 2 receptors. The linear bifunctional peptide analogues **3**, **4** and **5** in which the opioid pharmacophore is directly conjugated to the N-terminus of the bradykinin pharmacophore showed moderate to weak μ opioid binding affinity (2300 nM, 70 nM and 480 nM), respectively, and showed very weak δ opioid binding affinity. In peptide ligand **6** the opioid

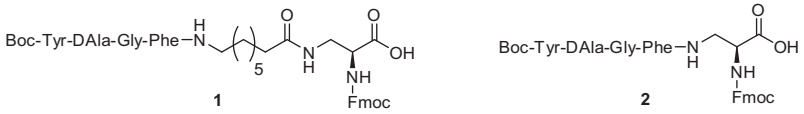


Figure 1. Opioid pharmacophore attached to the 2,3-diaminopropionoic acid with and without linker.

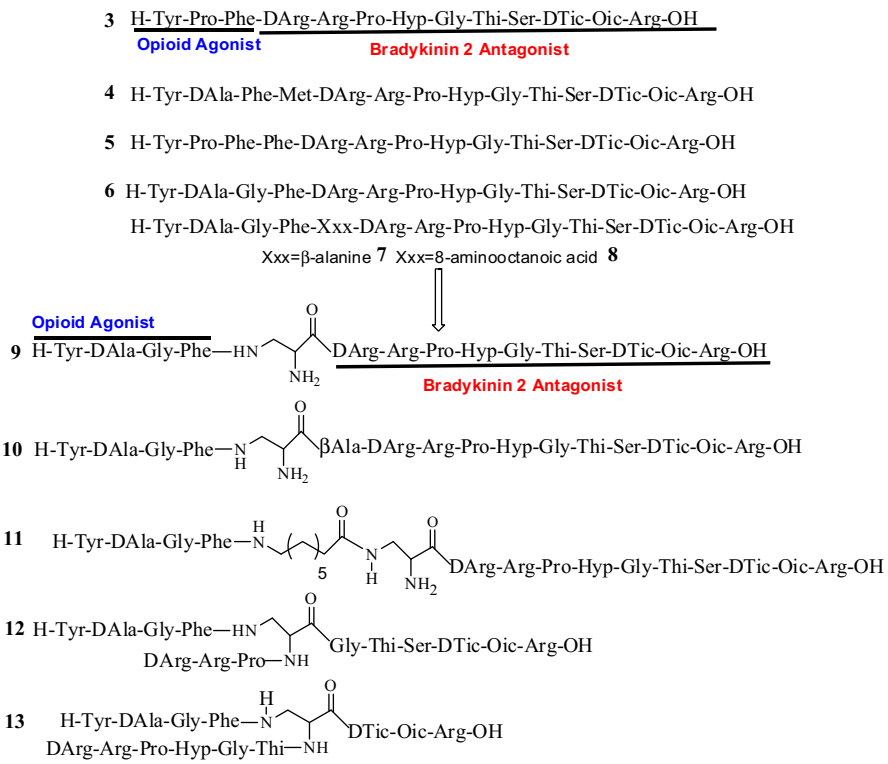


Figure 2. Sequences of multifunctional ligands **3**–**13**.

pharmacophore (Tyr-DAla-Gly-Phe) structure related to biphalin was directly conjugated to the Hoe 140 and it showed very good μ opioid binding affinity (10 nM) and weak binding affinity (1700 nM) at the δ opioid receptor. In further designs we maintained the biphalin core opioid pharmacophore (Tyr-DAla-Gly-Phe). The ligand **7** possessing a shorter linker (β-alanine) between the opioid pharmacophore and Hoe 140 showed very good binding affinity (1.3 nM and 36 nM) at both μ and δ opioid receptors, respectively. The ligand **8** which has a longer linker (8-aminooctanoic acid) in between the two pharmacophores showed very good balanced binding affinity (0.5 nM and 2 nM) at μ and δ opioid receptor, and 18 fold increase in δ binding affinity comparative with ligand **7** which has a β-alanine linker between two pharmacophores**.** This is almost a 20 fold increase in μ opioid binding affinity and 850 fold increases in δ opioid binding affinity in comparison with the **6** which is lacking a linker in between the two pharmacophores.

The peptide ligands **9**, **10** and **11** showed similar excellent binding affinity (0.6 nM, 0.5 nM and 0.3 nM) towards the μ opioid receptor. Similarly these ligands showed good δ binding affinity (38 nM, 7 nM and 2 nM) as well. The ligands **9**, **10** and **11** were designed based on the opioid pharmacophore maintained towards the N-terminus of Hoe 140 via the side chain amino group of Dap (2,3-diaminopropionic acid) with and without a linker. The linker between the Dap (2,3-diaminopropionic acid) side chain amino group and opioid pharmacophore does not have much impact on the opioid binding affinity. In ligands **12** and **13** the 4th position hydroxyl proline (Hyp[4]) and seventh position serine (Ser[7]) in Hoe 140 were replaced with Dap, and the side chain amino group of Dap conjugated to the opioid pharmacophore (Tyr-DAla-Gly-Phe). The ligands **12** and **13** showed very good binding affinity (2 nM and 6.5 nM) at μ opioid receptor, respectively. Similarly these ligands showed very good binding affinity (0.2 nM and 2 nM) at δ opioid receptor. Among all the multifunctional ligands, the ligands **8** and **11** showed excellent balanced binding affinities at both μ and δ opioid receptors which contained a longer spacer like 8-aminooctanoic acid between opioid and bradykinin pharmacophore (Table 1).

On the other hand the multifunctional ligands **3**, **4**, **5**, **6** and **Hoe 140** showed very weak or no binding affinity at the rat brain membrane bradykinin 2 receptor. Hoe 140 (DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg-OH) is a well-studied second generation Bradykinin 2 receptor antagonist.[4] The bradykinin 2 receptors are known to be present in rat brain, guinea pig ileum (GPI) and rabbit jugular vein.[34,35] In the past, binding affinity of Hoe 140 for the bradykinin 2 receptors were reported using the guinea pig ileum (1 nM).[36] Here we designed our multifunctional ligands based on Hoe 140 and these ligands were tested for the binding using rat brain membranes. These ligands showed very weak or no binding affinity towards the bradykinin 2 receptors. Similarly, the Hoe 140 showed very low affinity (>10,000 nM and 9000 nM) against [^{3}H] BK binding in rat brain membranes and in membranes isolated from transfected HEK293 cells that express hB2 cells, respectively. On the other hand the Hoe 140 showed very good binding affinity in membranes isolated from the terminal segment of guinea pig ileum (0.43 nM) similar to that of previously reported.[36] The most probable reason for the poor binding affinity of Hoe 140 and other bifunctional ligands at the bradykinin 2 receptor, the bradykinin 2 receptors present in rat brain membranes are different from the B2 receptors present in the guinea pig ileum. Further evidence for this hypothesis the bifunctional ligands **4**, **5** and **6** exhibit very good potent antagonist activities (1.8 nM, 1.9 nM and 1.7 nM) in GPI assay, respectively. Heterogeneity of B2 receptors has been suggested in the literature since the 80s. For example Regoli[34,35] and coworkers found that a B2 receptor in the rabbit vein jugular and a B2 receptor in the guinea pig ileum showed much different potencies for B2 antagonists.

A series of linear bifunctional peptides were designed and synthesized based on the well-known second generation bradykinin 2 antagonist Hoe 140 and opioid agonist enkephalin analogues. For

Table 1
Binding affinities of multifunctional peptide derivatives at μ/δ opioid receptors and Bradykinin receptor antagonists

Compd	Binding K_i (nM)				B2R, [^{3}H]BK K_i[a] (nM)	B2 antagonist activity GPI (nM)
	[b]log IC_{50}	MOR (μ) [^{3}H]DAMGO	[b]log IC_{50}	DOR (δ) [^{3}H]DPDPE		
3	−5.29 ± 0.21	2300		>10,000	>10,000	n.d.
4	−6.81 ± 0.14	70	−4.77 ± 0.10	6900	5200	1.8 ± 0.2
5	−5.97 ± 0.08	480		>10,000	>10,000	1.9 ± 0.3
6	−7.64 ± 0.18	10	−5.38 ± 0.08	1700	>10,000	1.7 ± 0.2
7	−8.51 ± 0.05	1.3	−7.04 ± 0.10	36	n.d.	n.d.
8	−8.91 ± 0.07	0.5	−8.33 ± 0.06	2	n.d.	n.d.
9	−8.85 ± 0.15	0.6	−7.03 ± 0.08	38	n.d.	n.d.
10	−8.94 ± 0.07	0.5	−7.77 ± 0.10	7	n.d.	n.d.
11	−9.09 ± 0.05	0.3	−8.29 ± 0.07	2	n.d.	n.d.
12	−8.32 ± 0.05	2	−8.17 ± 0.03	3	n.d.	n.d.
13	−7.81 ± 0.09	6.5	−9.22 ± 0.27	0.2	n.d.	n.d.
Hoe 140					9000/>10,000/0.43[c]	

n.d.: not determined.
[a] Competition assays were carried out using rat brain membranes.
[b] Logarithmic values determined from the nonlinear regression analysis of data collected from at least two independent experiments in duplicate.
[c] Binding affinities of Hoe140 against [^{3}H] bradykinin in membranes isolated from transfected HEK293 cells that express hB2, from rat brain and guinea pig ileum assays, respectively.

conjugation of the opioid pharmacophore to the Hoe 140 (bradykinin pharmacophore) various positions well explored. These bifunctional ligands showed excellent in vitro binding affinity at both μ and δ opioid receptors. On the other hand these bifunctional ligands and Hoe 140 itself showed weak or no binding affinity towards the rat brain bradykinin 2 receptors. Bradykinin 2 receptors are known to be present in rat brain, guinea pig ileum (GPI) and rabbit jugular vein.[34,35] In previous studies the binding affinity of Hoe 140 for bradykinin 2 receptors was reported using the guinea pig ileum (1 nM). Hoe 140 and the bifunctional ligands which we designed based on Hoe 140 were tested for the binding affinity using rat brain membranes containing the bradykinin receptors and they showed very weak or no binding affinity towards the bradykinin 2 receptor. Based on the above results we suggested that the bradykinin 2 receptors present in rat brain membranes are different from bradykinin 2 receptors present in guinea pig ileum (GPI). We are working further on to explore the different subtypes of bradykinin 2 receptors.

Radio ligand Competition Binding Assay: Binding affinities of bifunctional peptide analogues at the BRs were determined by radioligand competition analysis using [^{3}H] BK in rat brain membranes or in transfected HEK293 cells expressing the human B2R. Crude rat brain membranes were pelleted and resuspended in 50 mM Tris buffer containing 50 μg/mL bacitracin, 10 μM captopril, 100 μM PMSF, and 5 mg/mL bovine serum albumin (BSA). Ten concentrations of a test compound were each incubated with 50 μg of membranes and [^{3}H]DALKD (1 nM, 76.0 Ci/mmol) or [^{3}H]BK (1 nM, 85.4 Ci/mmol) at 25 °C for 2 h. Nonspecific binding was defined by that in the presence of 10 μM KD in all assays. Reactions were terminated by rapid filtration through Whatman GF/B filters presoaked in 1% polyethylenimine, followed with four washes of 2 mL each of cold saline. Radioactivity was determined by liquid scintillation counting in a Beckman LS5000 TD. Data were analyzed by nonlinear least-squares analysis using GraphPad Prism 4. Logarithmic values were determined from nonlinear regression analysis of data collected from at least two independent experiments.

Bradykinin Antagonist assay (GPI): Guinea pig isolated ileum/longitudinal muscle with myenteric plexus male Hartley guinea pigs under CO_2 anesthesia were sacrificed by decapitation and a non-terminal portion of the ileum removed. The longitudinal muscle with myenteric plexus (LMMP) was carefully separated from the circular muscle and cut into strips as described previously.[37] These tissues were tied to gold chains with suture silk and mounted between platinum wire electrodes in 20 mL organ baths at a tension of 1 g and bathed in oxygenated (95/5 O_2:CO_2) kreb's bicarbonate buffer at 37 °C. They were stimulated electrically (0.1 Hz, 0.4 ms duration) at supramaximal voltage. Following an equilibration period, compounds were added cumulatively to the bath in volumes of 14–60:1 until maximum inhibition was reached. A dose–response curve of PL-017 was constructed to determine tissue integrity before analog testing. Agonist and antagonist testing's compounds were tested as agonists by adding cumulatively to the bath until a full dose–response curve was constructed or to a concentration of 1 M. Compounds were tested as antagonists by adding to the bath 2 min before beginning the repeat agonist's dose response curves of bradykinin with 1 μm naloxone added to inhibit any opioid activity.

Analysis: percentage inhibition was calculated using the average tissue contraction height for one minute preceding the addition of the agonists divided by the contraction height 3 min after exposure to the dose of agonist. IC_{50} values represent the mean of not less than four tissues. IC_{50} and E_{max} estimates were determined by computerized nonlinear least-squares analysis (FlashCalc).

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Supplementary data

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References and notes

1. According to the report of Institute of Medicine in June **2011** (*National Academy of Sciences*).
2. Leeb-Lundberg, L. M.; Marceau, F.; Muller-Esterl, W.; Pettibone, D. J.; Zuraw, B. L. *Pharmacol. Rev.* **2005**, *57*, 27.
3. Regoli, D.; Barabé, J. *Pharmacol. Rev.* **1980**, *32*, 1.
4. Dziadulewicz, E. K. *Ann. Rep. Med. Chem.* **2004**, *39*, 113.
5. Marceau, F.; Regoli, D. *Nat. Rev. Drug Discovery* **2004**, *3*, 845.
6. Bock, M. G.; Longmore, J. *Curr. Opin. Chem. Biol.* **2001**, *5*, 626.
7. Bork, K.; Yasothan, U.; Kirkpatrick, P. *Icatibant Nat. Rev. Drug Discovery* **2008**, *7*, 801.
8. Lai, J.; Luo, M.-C.; Chen, Q.; Ma, S.; Gardell, L. R.; Ossipov, M. H.; Porreca, F. *Nat. Neurosci.* **2006**, *9*, 1534.
9. Lai, J.; Luo, M.-C.; Chen, Q.; Porreca, F. *Neurosci. Lett.* **2008**, *437*, 175.

10. Lee, Y. S.; Muthu, D.; Hall, S. M.; Ramos-Colon, C.; Rankin, D.; Hu, J.; Sandweiss, A. J.; De Felice, M.; Xie, J. Y.; Todd, W.; Vanderah, T. W.; Porreca, F.; Lai, J.; Hruby, V. J. *J. Am. Chem. Soc.* **2014**, *136*, 6608.
11. Lee, Y. S.; Rankin, D.; Hall, S. M.; Ramos-Colon, C.; Ortiz, J. J.; Kupp, R.; Porreca, F.; Lai, J.; Hruby, V. J. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4976.
12. Lee, Y. S.; Hall, S. M.; Ramos-Colon, C.; Remesic, M.; Lebaron, L.; Nguyen, A.; Rankin, D.; Porreca, F.; Lai, J.; Hruby, V. J. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 30.
13. Yamamoto, T.; Nair, P.; Davis, P.; Ma, S. W.; Navratilova, E.; Moye, M.; Tumati, S.; Vanderah, T. W.; Lai, J.; Porreca, F.; Yamamura, H. I.; Hruby, V. J. *J. Med. Chem.* **2007**, *50*, 2779.
14. Yamamoto, T.; Nair, P.; Jacobsen, N. E.; Davis, P.; Ma, S. W.; Navratilova, E.; Lai, J.; Yamamura, H. I.; Vanderah, T. W.; Porreca, F.; Hruby, V. J. *J. Med. Chem.* **2008**, *51*, 6334.
15. Lee, Y. S.; Agnes, R. S.; Badghishi, H.; Davis, P.; Ma, S.-W.; Lai, J.; Porreca, F.; Hruby, V. J. *J. Med. Chem.* **2006**, *49*, 1773.
16. Agnes, R. S.; Lee, Y. S.; Davis, P.; Ma, S. W.; Badghisi, H.; Porreca, F.; Lai, J.; Hruby, V. J. *J. Med. Chem.* **2006**, *49*, 2868.
17. Brabez, N.; Lynch, R. M.; Xu, L.; Gillies, R. J.; Chassaing, G.; Lavielle, S.; Hruby, V. J. *J. Med. Chem.* **2011**, *54*, 7375.
18. Kyle, D. J.; Martin, J. A.; Farmer, S. G.; Burch, R. M. *J. Med. Chem.* **1991**, *34*, 1230.
19. Alcaro, M. C.; Vinci, V.; D'Ursi,c, A. M.; Scrima, M.; Chelli, M.; Giuliani, S.; Meini, S.; Giacomo, M. D.; Colombob, L.; Papinia, A. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2387.
20. Stewart, J. M. *Biopolymers* **1995**, *37*, 143.
21. Pasternak, G. W.; Goodman, R.; Synder, S. H. *Life Sci.* **1975**, *16*, 1765.
22. Janecka, A.; Fichna, J.; Janecki, T. *Curr. Top. Med. Chem.* **2004**, *4*, 1.
23. Giri, A. K.; Hruby, V. J. *Expert Opin. Investig. Drugs* **2014**, *23*, 227.
24. Vavrek, R. J.; Stewart, J. M. *Peptides* **1985**, *6*, 161.
25. Stewart, J. M. *Peptides* **2004**, *25*, 527.
26. Kyle, D. J. *Curr. Pharm. Design* **1995**, *1*, 233.
27. Burch, R. M.; Farmer, S. G.; Steranka, L. R. *Med. Res. Rev.* **1990**, *10*, 237.
28. Filizola, M.; Llorens, O.; CartenõÂ-Farina, M.; Perez, J. J. *Bioorg. Med. Chem.* **1998**, *6*, 1491.
29. Stewart, J. M.; Gera, L.; York, E. J.; Chan, D. C.; Bunn, P. *Immunopharmacology* **1999**, *43*, 155.
30. Quartara, L.; Ricci, R.; Meini, S.; Patacchini, R.; Giolitti, A.; Amadesi, S.; Rizzi, C.; Rizzi, A.; Varani, K.; Borea, P. A.; Carlo A .Maggi, C. A.; Regoli, D. *Eur. J. Med. Chem.* **2000**, *1001*, 35.
31. Dziadulewicz, E. K.; Brown, M. C.; Dunstan, A. R.; Lee, W.; Said, N. B.; Garratt, P. J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 463.
32. Mavunkel, B. J.; Lu, Z.; Goehring, R. R.; Lu, S.; Chakravarty, S.; Perumattam, J.; Novotny, E. A.; Connolly, M.; Valentine, H.; Kyle, D. J. *J. Med. Chem.* **1996**, *39*, 3169.
33. Misicka, A.; Lipkowski, A. W.; Horvath, R.; Davis, P.; Kramer, T. H.; Yamamura, H. I.; Hruby, V. J. *Life Sci.* **1992**, *1025*, 51.
34. Regoli, D.; Gobeil, F.; Nguyen, Q. T.; Jukic, D.; Seoane, P. R.; Salvino, J. M.; Sawutz, D. *Life Sci.* **1994**, *10*, 735.
35. Regoli, D.; Allogho, S. N.; Rizzi, A.; Gobeil, F. J. *Eur. J. Pharmacol.* **1998**, *348*, 1.
36. Hock, F. J.; Wirth, K.; Albus, U.; Linz, W.; Gerhards, H. J.; Wiemer, G.; Henke, St.; Breipohl, G.; Knoig, W.; Knolle, J.; Sholkens, B. A. *Br. J. Pharmacol.* **1991**, *102*, 769.
37. Porreca, F.; Burks, T. F. J. *Pharmacol. Exp. Ther.* **1983**, *227*, 22.