Biodegradable amphipathic peptide hydrogels as extended-release system for opioid peptides

Charlotte Martin,1 Maria Dumitrascuta,2 Morgane Mannes,1 Aquilino Lantero,2 Dominik Bucher,2 Katja Walker,2 Yannick Van Wanseele,3 Edith Oyen,1 Sophie Hernot,4 Ann Van Eeckhaut,3 Annemieke Madder,5\* Richard Hoogenboom,6\* Mariana Spetea2\* and Steven Ballet1\*

1 Research Group of Organic Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, Brussels, B-1050, Belgium

2 Department of Pharmaceutical Chemistry, Institute of Pharmacy and Center for Molecular Biosciences (CMBI), University of Innsbruck, Innrain 80-82, 6020 Innsbruck, Austria

3 Department of Pharmaceutical Chemistry, Drug Analysis and drug Information, Center for Neurosciences (C4N), Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium

4 In Vivo Cellular and Molecular Imaging, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium

5 Organic and Biomimetic Chemistry Research Group, Ghent University, Krijgslaan 281, 9000 Ghent, Belgium

6 Supramolecular Chemistry Group, Centre of Macromolecular Chemistry (CMaC), Ghent University, Krijgslaan 281, 9000 Ghent, Belgium

ABSTRACT: Chronic pain is currently treated with opioids that offer unsatisfactory long-term analgesia and produce serious side effects. There is a clear need for alternative therapies. Herein, peptide-based hydrogels are used as extended-release drug delivery carriers. Two different formulations were developed: either the drug is co-formulated within the hydrogel, or the drug is an integral part of the hydrogelator. Both strategies afford a prolonged and significant antinociception up to 72 hours after subcutaneous administration in mice.

INTRODUCTION

Chronic pain remains one of the main challenges in human medicine at the beginning of the third millennium, with ca. 20-30% of people worldwide suffering from chronic pain.1 Opioid analgesics are the most effective and widely prescribed drugs to treat moderate to severe pain,2 but their clinical use is often limited by serious side effects and complications, such as respiratory depression, constipation, nausea, sedation, development of analgesic tolerance and abuse liability.3 Furthermore, in the light of the current opioid epidemic, there is a central need to find innovative, effective and safe analgesics.4 To treat chronic pain, opioids can be administered as short-acting opioid (SAO) or long-acting opioid (LAO) formulations. The SAOs require repeated administration, with a duration of action of 2-3 hours, resulting in a poor and inconsistent pain relief with higher occurrence of adverse effects, while LAOs show a more gradual drug release in the bloodstream leading to prolonged effects of 8 up to 72 hours.5 Such LAOs use controlled drug delivery systems that provide a constant drug concentration in the systemic circulation, thus reducing the frequency of administration which in turn improves patient compliance.6 Although various extended-release (ER) formulations/technologies have been developed over the years, consistent pain relief is still poorly managed, stressing the need for innovative and superior treatments. Notably, only conventional opioids, including morphine, oxycodone, buprenorphine and fentanyl, all known to induce major side effects, are used to date in existing ER formulations.6

As an alternative to conventional opioid drugs displaying detrimental adverse effects, several endogenous and exogenous opioid peptides have been evaluated as potential analgesics over the years. One such peptide, dermorphin, is approximately 1000-times more potent in humans than morphine, while inducing less side effects, paralleled by longer analgesia.7 Inspired by these promising findings, dermorphin was used as a platform for the development of various analgesic peptides,8 and hence we envisaged them as useful leads for the design of peptidomimetic LAO formulations with better antinociceptive effects, as compared to morphine, and with a reduced propensity for side effects. In the domain of pain therapy, several controlled-delivery systems are available for hydrophobic analgesics,6 but versatile systems for hydrophilic drugs, such as painkilling peptides, are still lacking.

This work describes new peptide-based hydrogels for chronic pain management by using them as biomaterial formulations for the extended release of opioid peptides. Among various types of hydrogels, peptide-based hydrogels are highly attractive due to their biocompatibility, biodegradability and cytocompatibility, as they are made from natural building blocks, and their ease of synthesis and purification.9 Hence, they found widespread use in biomedical applications, such as tissue engineering and drug delivery.10 Recently, we designed a new family of short amphipathic peptide-based hydrogels, which form thixotropic injectable hydrogels upon dissolution in aqueous solutions.11 The efficacy of the hydrogel networks as controlled drug delivery platform was demonstrated for morphine, showing extended antinociceptive effect up to 72 hours after subcutaneous (sc) administration in mice.12 In this study, we established the use of peptide hydrogels for the ER of opioid peptides, which is to the best of our knowledge unprecedented proof that amphipathic peptide hydrogels are compatible with peptide cargoes.

RESULTS AND DISCUSSION

In this study, two strategies were targeted: i) the analgesic drug is encapsulated within the hydrogel network (i.e. a ‘co-formulation’), and ii) the analgesic pharmacophore is covalently linked to the hydrogelator, resulting in an analgesic hydrogel conjugate (hereafter called ‘biogel’ formulation) (Figure 1). While the conjugation of drugs to macromolecule carriers (such as PEG) has been used to improve pharmacokinetic profiles, to our knowledge, the design of prodrugs solely based on biodegradable self-assembling peptides is unprecedented.

While in the co-formulation the drug loading and release depend on diffusion and complexation/desorption processes, guided by the interaction of the drug with the hydrogel’s fibers, the biogel concept presents advantages such as a lowered drug release rate *via* implementation of a covalent linkage between the opioid pharmacophore and the peptide hydrogelator, thus limiting the risk of burst release, and protecting the peptide drug from rapid clearance.13



**Figure 1.** Illustration of self-assembling of supramolecular hydrogels based on amphipathic peptide sequences. Both the ‘co-formulation’ and ‘biogel’ strategies are depicted.

Herein, the opioid pharmacophores **1** to **5** (Table 1) were designed based on dermorphin (H-Tyr-DAla-Phe-Gly-Tyr-Pro-Ser-NH2) and endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH2), two endogenous opioid peptides, displaying high potency and selectivity for the µ-opioid receptor (MOR), the primary target for effective analgesia.14 Since it was demonstrated that the *N*-terminal tetrapeptide of opioid peptides is commonly accepted to be the minimum sequence required for high opioid activity, *N*-terminal [Dmt1,D-Xaa2]tetrapeptide analogues were designed. While **3** is an optimized and balanced MOR/δ-opioid receptor (DOR) agonist,15 opioid peptides **4** and **5** correspond to the [Dmt1]endomorphin-2 and [Dmt1]endomorphin-1 sequences, respectively. All opioid peptides, **1** to **5**, showed very high affinity to the MOR, with **1** and **5** demonstrating picomolar affinity (Table S2).

Generally, all opioid peptides displayed decreased affinities to the DOR and κ-opioid (KOR) receptors, thus providing MOR selectivity. Also, the opioid receptor binding affinity of **3** established in this study is in line with the profile recently described.16 On the basis of functional activity at the human MOR as determined in the [35S]GTPγS binding assay, **1** to **5** were very potent agonists (EC50 values ranging from 0.10 to 0.86 nM), and showed high efficacy acting as full agonists, except for **4**, which was a potent MOR partial agonist (Table S3).

Accumulated evidence indicates that MOR-mediated antinociception results from G protein-mediated signaling, while β-arrestin2 signaling pathways promote the unwanted effects of opioids.17 The concept of biased agonism at the MOR has gained significance to drug discovery, where the development of G protein-biased MOR agonists may deliver the desired analgesia while avoiding the side effects. To verify MOR biased agonism of the opioid peptides (**1** to **5**) towards activation of G protein- over β-arrestin-2-mediated signaling, we compared their functional activity, i.e. potency and efficacy, across two functional assays that measure G protein coupling (the [35S]GTPγS binding assay) and β-arrestin-2 translocation (the DiscoveRx PathHunter β-arrestin2 recruitment assay) at the human MOR (Table S3). We established that opioid peptides **1** to **5** activate G protein with high potency as full MOR agonists, while displaying much lower potencies in inducing β-arrestin2 recruitment, thus stimulating the MOR in a manner that is preferentially biased toward G protein signaling.

In a next stage, these opioid peptides **1**-**5** (coded as **OP1-5**) were either co-formulated or covalently linked to recently described hexapeptide hydrogelators **14**-**16** (coded as **GEL1-4**)11-12 giving way to a set of biogel sequences (Table 1).

Table 1: Opioid pharmacophores (OP), Biogel sequences, and selected hydrogelator sequences (GEL)

|  |  |  |
| --- | --- | --- |
| n ° | Code | Sequence |
| 1 | OP1 | H-Dmt-DArg-Phe-Phe-NH2 |
| 2 | OP2 | H-Dmt-DLys-Phe-Phe-NH2 |
| 3 | OP3 | H-Dmt-DArg-Aba-βAla-NH2 |
| 4 | OP4 | H-Dmt-Pro-Phe-Phe-NH2 |
| 5 | OP5 | H-Dmt-Pro-Trp-Phe-NH2 |
| 6 | OP1-GEL2 | H-Dmt-DArg-Phe-Phe-Gln-Phe-Gln-Phe-Lys-NH2 |
| 7 | OP1-GEL4 | H-Dmt-DArg-Phe-Phe-Gln-β3hPhe-Phe-Gln-Phe-Lys-NH2 |
| 8 | OP2-GEL1 | H-Dmt-DLys-Phe-Phe-Glu-Phe-Gln-Phe-Lys-NH2 |
| 9 | OP2-GEL2 | H-Dmt-DLys-Phe-Phe-Gln-Phe-Gln-Phe-Lys-NH2 |
| 10 | OP3-GEL2 | H-Dmt-DArg-Aba-βAla-Phe-Gln-Phe-Gln-Phe-Lys-NH2 |
| 11 | OP4-GEL1 | H-Dmt-Pro-Phe-Phe-Glu-Phe-Gln-Phe-Lys-NH2 |
| 12 | OP4-GEL2 | H-Dmt-Pro-Phe-Phe-Gln-Phe-Gln-Phe-Lys-NH2 |
| 13 | OP5-GEL2 | H-Dmt-Pro-Trp-Phe-Gln-Phe-Gln-Phe-Lys-NH2 |
| 14 | GEL1 | H-Phe-Glu-Phe-Gln-Phe-Lys-NH2 |
| 15 | GEL2 | H-Phe-Gln-Phe-Gln-Phe-Lys-NH2 |
| 16 | GEL4 | H-Phe-Glu-β3hPhe-Phe-Gln-Phe-Lys-NH2 |

The biogel conjugates were constructed by linking the opioid part to the peptide hydrogelator *via* an amide bond. The resulting biogel sequences can be considered as prodrugs, that release active pharmacophores after proteolytic degradation, and wherein the hydrogelators were selected based on our recent findings related to thixotropic behavior, as well as extended *in vivo* release properties.11-12



**Figure 2.** Co-formulation and biogel strategies for the extended release profile of opioid peptide **1**. (**a**) Comparison of antinociceptive activity of **1** co-formulated with hydrogelator **15 (GEL2**) or conjugated to hydrogelator **15** resulting in biogel **6**, in the tail-flick test after sc administration in mice. Groups of mice received **1** (0.56 µmol/mouse) co-formulated with **15** (2% v/w), **6** (2% v/w), **15** (2% v/w) alone (control), or saline (control) in a volume of 150 µL per mouse. (**b**) *In vitro* degradation profile of **6** (in solution) in human plasma. Antinociceptive activity of **6** and its metabolites **17**-**19** (**Met-1 to -3**, respectively) in the tail-flick test after sc administration in mice. Groups of mice received solutions of **6** (3.0 µmol/kg), **17** (3.9 µmol/kg), **18** (4.2 µmol/kg), **19** (6.8 µmol/kg), or saline (control) (right panel). Antinociceptive response, as %MPE, was measured over time, and area-under-the curve (AUC) values for the respective time curves were calculated. Data are the mean ± SEM of 6-8 mice per group. \**P* < 0.05 vs. respective control groups, #*P* < 0.05 vs. **6** group, two-way ANOVA followed by Bonferroni *post hoc* test.

Biogels **6**, **9**, **10**, **12** and **13** are based on the hexapeptide hydrogelator H-Phe-Gln-Phe-Gln-Phe-Lys-NH2 (**15**) and biogel **8** and **11** on H-Phe-Glu-Phe-Gln-Phe-Lys-NH2 (**14**)with Glu in position two instead of Gln, while biogel **7** contains a mixed α/β sequence, H-Phe-Gln-β3hPhe-Phe-Gln-Phe-Lys-NH2 (**16**)(Table 1). The latter hydrogel was designed to stabilize the peptide sequence against enzymatic degradation, in view of a potential extended release of the drug by slowing down its degradation.12 In order to evaluate the *in vivo* stability of the peptide hydrogels, *in vivo* SPECT/CT imaging experiments in mice were performed (Figures S3.6). The degradation of the hydrogel was followed by determination of the hydrogels’ volume at the site of the injection. The selected hydrogelators **14** and **15** showed a slow *in vivo* degradation profile, with around 20% of the hydrogel still present 72 hours after sc injection in mice (ESI, Figure S3.6B).

The *in vitro* pharmacological profiles of the opioid pharmacophores **1** to **5** and biogels **6** to **13** (Table 1) were evaluated and compared in terms of binding affinities and functional activities at the human opioid receptors (See ESI, Tables S2 and S3). Covalent attachment of the opioid peptides **1** to **5** to recently reported peptide hydrogelators,11-12 resulting in biogel **6** to **13** (Table 1) considerably decreased affinity, selectivity and agonist potency at the MOR. In the series of biogel sequences, affinities in the nanomolar range were shown at the MOR (Ki values ranging from 5 to 141 nM), with biogels **6**, **7** and **9** being the most active and potent agonists *in vitro* (Table S3). Biogels **8**, **11**, **12** and **13** displayed moderate MOR affinity and low agonist potency, some of them even showing partial agonism at the MOR. This lower activity of the biogels enhances their safety as prodrugs as they only become active after metabolic degradation (*vide infra*).

Central goals in chronic pain control are to provide analgesia of adequate efficacy and duration, and to balance the patient’s pain relief, to limit potential harmful consequences of opioids, and to improve the overall quality of life. In the current study, we assessed and compared antinociceptive properties of the opioid peptides in solution, co-formulated with hydrogels, and as biogels (Figure 2a, Figure S3.4A, Figure S3.4B and Figure S3.4C). Antinociception was evaluated in a model of acute thermal nociception, the tail-flick test, after systemic sc administration in mice. When administered in solution, compounds **1** to **3** produced a significant antinociceptive effect (up to 7 hours for **1**, 8 hours for **2** and 4 hours for **3**, Figure S3.4B). Opioid peptides **4** and **5** had a much shorter duration of the antinociceptive effect without reaching the desired 80% Maximum Possible Effect (%MPE).

We then evaluated the co-formulation and biogel strategies in terms of their extended release profile of the embedded/attached opioid peptides. Compound **1** co-formulated with hydrogelator **15** or conjugated to hydrogelator **15** (to give biogel **6**) produced prolonged and significant antinociception up to 72 hours when sc administered to mice (Figure 2a). Moreover, no significant differences were noted when comparing the effect of the two formulation strategies, supporting the previously proposed hypothesis that the controlled-drug release is based on hydrogel fiber erosion, rather than the proteolytic degradation of the hydrogelating sequences.12 On the other hand, when **1** is conjugated to a stabilized hydrogelator sequence **16** resulting in biogel **7**, a noticeable difference was found with **7** being significantly less efficacious in inducing an antinociceptive response with a much shorter duration action (up to 3 hours) (Figure S3.4A, panel a). The reduced potency of **7** may be explained by its increased proteolytic stability due to the presence of a mixed α/β sequence obstructing its metabolic degradation into the active form, *vide infra* (Table 1). In contrast, a promising effect was shown for opioid peptide **2** co-formulated with hydrogelator **15**, or either conjugated to hydrogelator **14** (biogel **8**) or to hydogelator **15** (biogel **9**), producing prolonged and significant antinociception up to 72 and 24 hours, respectively (Figure S3.4A, panel b). When comparing **2** formulated in **8** or **9**, sc administered to mice, there was no significant difference in the antinociceptive effect. When co-formulated with hydrogelator **15**, the more evolved peptidomimetic **3** was also highly effective, with a significant antinociceptive effect up to 72 hours post-administration (Figure S3.4C). As **4** and **5** were established not to be very efficacious in causing an antinociceptive response and their corresponding biogels **11**, **12** and **13** exhibited fairly weak binding and agonist potencies at the MOR (Tables S2 and S3), eventually due to the rapid cleavage of the Dmt1-Pro2, no formulations of these peptides were investigated *in vivo* for antinociceptive properties.

To study the drug release mechanism from the biogel conjugates, *in vitro* biostability experiments were performed in human plasma at physiological (37°C) temperature. From this study, the half-life of each sequence was calculated and the major metabolites identified at different time-points. Comparison of the half-life values highlighted that the presence of Dmt at the *N*-terminal end of the sequence (**6**, t1/2 = 16 min) does not increase the stability of the sequence, compared to the Tyr analogue (t1/2 = 18 min), while insertion of a β3homo residue within the hydrogelator sequence (biogel **7**) gives rise to a highly stable peptide (t1/2 = 123 min). To understand the metabolic degradation profile of these biogel sequences, the structures of the main metabolites were determined by LC-MS analysis of collected samples from the plasma stability experiments. The results showed that in human plasma, the biogels are degraded from the *C*- to *N*-terminus, with preferential amide cleavage at the Gln5-Phe4 or Glu5-Phe4 site. From this degradation mechanism, three main metabolites were identified corresponding to the hepta-, penta- and tripeptides. For example, in case of **6**, the main metabolites are **17**, **18** and **19** (Figure 2b). Regarding biogels **7** and **10**, that contain unnatural amino acids, the degradation pattern seems to be similar. However, the presence of β3hPhe and βAla residues, respectively, is suggested to prevent the release of an active pharmacophore, able to induce a biological effect *in vivo*.

Among the three metabolites of **6** (Figure 2b), **18** was identified as the most potent MOR agonist *in vitro*, being ca. 130-times more active than **6** (Table S3). Overall, the rank order of affinities/agonist potencies of the Biogel **6** metabolites at the human MOR *in vitro* was **18** > **19** > **17** ≈ **6**. *In vivo*, they all produced potent antinociceptive effect in the tail-flick test after sc administration in mice. While **17** and **18** displayed a similar time-course of the antinociceptive response to **6** when given at equianalgesic doses and a relatively short effect (up to 3-4 hours), **19** showed an extremely long duration of action for such a short, non-formulated peptide (up to 8 hours, *P* < 0.05 vs. saline- and **6** treated mice, two-way ANOVA with Bonferroni *post hoc* test) (Figure 2b).

CONCLUSIONS

Two peptide-based hydrogel strategies were deployed to achieve effective and prolonged antinociception. Herein, the released opioid compounds consisted of potent opioid tetrapeptides, when applying the co-formulation method, or truncated peptide-derivatives resulting from the proteolytic degradation of the gelator segment in the biogel sequences. The degradation profile of one biogel sequence **6**, unveiled the discovery of an ultrapotent and long-acting tripeptidic metabolite **19**. The biodegradability of the gelator sequence is extremely favorable, since conjugation to the described hydrogelators can be regarded as a prodrug approach, applicable to many other biologically active sequences, which would gain therapeutic potential when existing in a controlled-release format.

EXPERIMENTAL SECTION

Peptide Synthesis. The syntheses were performed manually using standard Fmoc-based solid phase peptide synthesis (SPPS). Rink amide AM resin was used for the synthesis of all sequences (OP, Biogels and GELs) with exception of the three metabolites (MET) that were prepared starting from commercially available preloaded Wang resin. Amino acids (3 equiv) were coupled using HBTU (3 equiv) and DIPEA (5 equiv) in DMF for 40 minutes. The coupling of Boc *N*-protected Dmt (1.5 equiv) required the use of DIC (1.5 equiv) and HOBt (1.5 equiv) in DMF as coupling mixture with a reaction time of 2 hours. Fmoc removal was performed using a 20% 4-methylpiperidine in DMF solution for 2 times (5 and 15 min, respectively). After completion of the sequences, the peptides were cleaved from de resin by treatment with a cleavage cocktail constituted of TFA/TIPS/H2O (95:2.5:2.5) for 3 hours at room temperature. After filtration, the solvent was evaporated in vacuo, and the residue dissolved in water/acetonitrile (1:1) and lyophilized. Crude peptides were purified by preparative reverse phase HPLC, to yield pure peptide as a white powder after lyophilization, with a purity > 95%, as assessed by analytical HPLC. Details about peptide purification are provided in the Electronic Supporting Information.

Peptide Gelation. Peptide gelation was performed, as previously reported.12 Details are included in the Supporting Information.

Pharmacology. *In vitro* opioid receptor activities. Binding assays were conducted on human opioid receptors stably transfected into CHO cells according to the published procedures.18 Binding of [35S]GTPγS to membranes from CHO cells stably expressing the human MOR (CHO-hMOR) was conducted according to the published procedure.18 The measurement of MOR stimulated β-arrestin2 recruitment was performed using the DiscoveRx PathHunter® β-arrestin2 assay (DiscoveRx, Birmingham, UK) according to the manufacturer’s protocol and published procedures.19 Details are reported in the Supporting Information.

Nociceptive assessment. The radiant heat tail-flick test was used to assess antinociceptive effects of the test peptides after sc administration in mice using an UB 37360 Ugo Basile analgesiometer (Ugo Basile s.r.l., Varese, Italy), as described previously.20 Details are described in the Supporting Information.

Stability studies. *In vitro* proteolytic stability tests were performed using human plasma and the *in vivo* stability of hydrogel networks composed of GEL1 to 4 was controlled through *in vivo* imaging as previously reported.21 Details are included in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

The supporting information is available free of charge on the ACS Publications website.

Additional chemical and pharmacological information (PDF)

Molecular formula strings (CSV)

AUTHOR INFORMATION

Corresponding Authors

E-mail addresses: annemieke.madder@ugent.be (A. Madder ORCID: 0000-0003-0179-7608), [richard.hoogenboom@ugent.be](mailto:richard.hoogenboom@ugent.be) (R. Hoogenboom ORCID: 0000-0001-7398-2058), mariana.spetea@uibk.ac.at (M. Spetea ORCID: 0000-0002-2379-5358), steven.ballet@vub.be (S. Ballet ORCID: 0000-0003-4123-1641).

**Notes**The authors declare no competing financial interests.

ACKNOWLEDGEMENTS

We thank the Strategic Research Program of the VUB, the Research Foundation Flanders (FWO Vlaanderen, grant G.0517.13) and the Austrian Science Fund (FWF: I 2463-B21) for the financial support. Edith Oyen and Yannick Van Wanseele are research fellows of the Research Foundation Flanders (FWO Vlaanderen). Carina De Rijck is acknowledged for her technical assistance. This work was also supported by the Scientific Research Network (WOG) “Supramolecular Chemistry and Materials” of the Research Foundation-Flanders.

ABBREVIATIONS USED

%MPE, percentage of maximum possible effect; Aba, 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one; CHO, Chinese hamster ovary; DIC, *N,N′-*diisopropylcarbodiimide; DIPEA, *N,N*-diisopropylethylamine; Dmt, 2´,6’-dimethyltyrosine; DOR, δ-opioid receptor; ER, extended release; hMOR, human µ-opioid receptor; HBTU, *N,N,N′,N′-*Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole hydrate; KOR, κ-opioid receptor; LAO, long-acting opioid; MOR, µ-opioid receptor; SAO, short-acting opioid; SEM, standard error of the mean; SPECT-CT, single-photon emission computed tomography-X-ray computed tomography.

REFERENCES

1. Kapur, B. M.; Lala, P. K.; Shaw, J. L. Pharmacogenetics of chronic pain management *Clin. Biochem.* **2014,** *47*, 1169-1187.

2. Skolnick, P.; Volkow, N. D. Re-energizing the development of pain therapeutics in light of the opioid epidemic *Neuron.* **2016,** *92*, 294-297.

3. Benyamin, R.; Trescot, A. M.; Datta, S.; Buenaventura, R. M.; Adlaka, R.; Sehgal, N.; Glaser, S. E.; Vallejo, R. Opioid complications and side effects *Pain Physician* **2008,** *11*, S105-S120.

4. (a) Jones, M. R.; Viswanath, O.; Peck, J.; Kaye, A. D.; Gill, J. S.; Simopoulos, T. T. A brief history of the opioid epidemic and strategies for pain medicine *Pain and Therapy* **2018,** *7*, 13-21; (b) Drieu la Rochelle, A.; Guillemyn, K.; Dumitrascuta, M.; Martin, C.; Utard, V.; Quillet, R.; Schneider, S.; Daubeuf, F.; Willemse, T.; Mampuys, P.; Maes, B. U. W.; Frossard, N.; Bihel, F.; Spetea, M.; Simonin, F.; Ballet, S. A bifunctional-biased mu-opioid agonist–neuropeptide FF receptor antagonist as analgesic with improved acute and chronic side effects *Pain* **2018,** *159*, 1705-1718.

5. Fine, P. G.; Mahajan, G.; McPherson, M. L. Long-acting opioids and short-acting opioids: appropriate use in chronic pain management *Pain Med.* **2009,** *10*, S79-S88.

6. Martin, C.; De Baerdemaeker, A.; Poelaert, J.; Madder, A.; Hoogenboom, R.; Ballet, S. Controlled-release of opioids for improved pain management *Mater. Today* **2016,** *19*, 491-502.

7. Basso, N.; Marcelli, M.; Ginaldi, A.; De Marco, M. Intrathecal dermorphine in postoperative analgesia *Peptides* **1985,** *6*, 177-179.

8. (a) Schiller, P. W.; Nguyen, T. M. D.; Berezowska, I.; Dupuis, S.; Weltrowska, G.; Chung, N. N.; Lemieux, C. Synthesis and in vitro opioid activity profiles of DALDA analogues *Eur. J. Med. Chem.* **2000,** *35*, 895-901; (b) Bai, L.; Li, Z.; Chen, J.; Chung, N. N.; Wilkes, B. C.; Li, T.; Schiller, P. W. [Dmt1]DALDA analogues with enhanced μ opioid agonist potency and with a mixed μ/κ opioid activity profile *Biorg. Med. Chem.* **2014,** *22*, 2333-2338; (c) Mizoguchi, H.; Watanabe, C.; Watanabe, H.; Moriyama, K.; Sato, B.; Ohwada, K.; Yonezawa, A.; Sakurada, T.; Sakurada, S. Involvement of endogenous opioid peptides in the antinociception induced by the novel dermorphin tetrapeptide analog amidino-TAPA *Eur. J. Pharmacol.* **2007,** *560*, 150-159.

9. Dasgupta, A.; Mondal, J. H.; Das, D. Peptide hydrogels *RSC Advances* **2013,** *3*, 9117-9149.

10. Altunbas, A.; Pochan, D. J. Peptide-Based and Polypeptide-Based Hydrogels for Drug Delivery and Tissue Engineering. In *Peptide-Based Materials*; Springer: 2011; pp 135-167.

11. Martin, C.; Oyen, E.; Mangelschots, J.; Bibian, M.; Ben Haddou, T.; Andrade, J.; Gardiner, J.; Van Mele, B.; Madder, A.; Hoogenboom, R.; Spetea, M.; Ballet, S. Injectable peptide hydrogels for controlled-release of opioids *MedChemComm* **2016,** *7*, 542-549.

12. Martin, C.; Oyen, E.; Van Wanseele, Y.; Haddou, T. B.; Schmidhammer, H.; Andrade, J.; Waddington, L.; Van Eeckhaut, A.; Van Mele, B.; Gardiner, J.; Hoogenboom, R.; Madder, A.; Spetea, M.; Ballet, S. Injectable peptide-based hydrogel formulations for the extended in vivo release of opioids *Mater. Today Chem.* **2017,** *3*, 49-59.

13. Ma, W.; Cheetham, A. G.; Cui, H. Building nanostructures with drugs *Nano Today* **2016,** *11*, 13-30.

14. (a) Zadina, J. E.; Hackler, L.; Ge, L.-J.; Kastin, A. J. A potent and selective endogenous agonist for the µ-opiate receptor *Nature* **1997,** *386*, 499; (b) Erspamer, V.; Melchiorri, P.; Falconieri-Erspamer, G.; Negri, L.; Corsi, R.; Severini, C.; Barra, D.; Simmaco, M.; Kreil, G. Deltorphins: a family of naturally occurring peptides with high affinity and selectivity for delta opioid binding sites *Proc. Natl. Acad. Sci* **1989,** *86*, 5188-5192.

15. Guillemyn, K.; Kleczkowska, P.; Lesniak, A.; Dyniewicz, J.; Van der Poorten, O.; Van den Eynde, I.; Keresztes, A.; Varga, E.; Lai, J.; Porreca, F.; Chung, N. N.; Lemieux, C.; Mika, J.; Rojewska, E.; Makuch, W.; Van Duppen, J.; Przewlocka, B.; Vanden Broeck, J.; Lipkowski, A. W.; Schiller, P. W.; Tourwe, D.; Ballet, S. Synthesis and biological evaluation of compact, conformationally constrained bifunctional opioid agonist - Neurokinin-1 antagonist peptidomimetics *Eur. J. Med. Chem.* **2015,** *92*, 64-77.

16. Guillemyn, K.; Starnowska, J.; Lagard, C.; Dyniewicz, J.; Rojewska, E.; Mika, J.; Chung, N. N.; Utard, V.; Kosson, P.; Lipkowski, A. W.; Chevillard, L.; Arranz-Gibert, P.; Teixido, M.; Megarbane, B.; Tourwe, D.; Simonin, F.; Przewlocka, B.; Schiller, P. W.; Ballet, S. Bifunctional peptide-based opioid agonist nociceptin antagonist ligands for dual treatment of acute and neuropathic pain *J. Med. Chem.* **2016,** *59*, 3777-3792.

17. Madariaga-Mazón, A.; Marmolejo-Valencia, A. F.; Li, Y.; Toll, L.; Houghten, R. A.; Martinez-Mayorga, K. Mu-opioid receptor biased ligands: A safer and painless discovery of analgesics? *Drug Discov. Today* **2017,** *22*, 1719-1729.

18. Dumitrascuta, M.; Ben Haddou, T.; Guerrieri, E.; Noha, S. M.; Schläfer, L.; Schmidhammer, H.; Spetea, M. Synthesis, pharmacology, and molecular docking studies on 6-desoxo-N-methylmorphinans as potent μ-opioid receptor agonists *J. Med. Chem.* **2017,** *60*, 9407-9412.

19. Spetea, M.; Eans, S. O.; Ganno, M. L.; Lantero, A.; Mairegger, M.; Toll, L.; Schmidhammer, H.; McLaughlin, J. P. Selective κ receptor partial agonist HS666 produces potent antinociception without inducing aversion after i.c.v. administration in mice *Br. J. Pharmacol.* **2017,** *174*, 2444-2456.

20. Novoa, A.; Van Dorpe, S.; Wynendaele, E.; Spetea, M.; Bracke, N.; Stalmans, S.; Betti, C.; Chung, N. N.; Lemieux, C.; Zuegg, J. Variation of the net charge, lipophilicity, and side chain flexibility in Dmt1-DALDA: effect on opioid activity and biodistribution *J. Med. Chem.* **2012,** *55*, 9549-9561.

21. Oyen, E.; Martin, C.; Caveliers, V.; Madder, A.; Van Mele, B.; Hoogenboom, R.; Hernot, S.; Ballet, S. In vivo imaging of the stability and sustained cargo release of an injectable amphipathic peptide-based hydrogel *Biomacromolecules* **2017,** *18*, 994-1001.

Table of Contents (TOC)

