RSC Advances

PAPER

View Article Online
View Journal | View Issue



Cite this: *RSC Adv.*, 2017, **7**, 20015

# Synthesis and biological evaluation of fentanyl acrylic derivatives†

Shaohua Li,[ab] Devora Cohen-Karni,[ab] Marina Kovaliov,[ab] Nestor Tomycz,[b] Boyle Cheng,[b] Donald Whiting[b] and Saadyah Averick *[ab]

Received 1st February 2017
Accepted 24th March 2017

DOI: 10.1039/c7ra01346a

rsc.li/rsc-advances

The synthesis of novel fentanyl acrylate derivatives *via* bromo-fentanyl using Heck coupling is described. The synthesis is concise and represents an efficient and useful method for functionalizing fentanyl for medicinal chemistry investigations. The agonistic and analgesic activities are evaluated by Mu opioid receptor activation and hot plate assays in mice.



Pain has become the most common reason for patients to seek advice from healthcare professionals. Opioids and their synthetic analogs belong to the most potent analgesics currently used for moderate to severe chronic pain treatment.[1–3] The primary opioid receptor involved in pain perception is the Mu opioid receptor (MOR), making Mu opioid agonists, such as morphine, methadone, codeine and fentanyl commonly used opioids.[4–8] A tremendous amount of synthetic work has been reported with the aim of altering the potency, selectivity, and bioavailability of these opioids.[6,8–13]

Fentanyl is a potent synthetic MOR agonist that belongs to the class of compounds known as 4-anilidopiperidines.[14–19] Its use is generally restricted to post-surgical acute pain management and transdermal patches due to the high risk for overdoes and death.[20–28] Due to fentanyl's hydrophobicity it has a short therapeutic half-life of 15 minutes which creates challenges in its use for managing post-surgical pain.[29–32] Although fentanyl analogues have been synthesized and evaluated, new fentanyl derivatives with improved half-life and lower risk for overdose are still needed.

An apparent gap in the literature regarding PEG-conjugates of fentanyl encouraged us to investigate a parent derivative with a synthetic handle to facilitate the preparation of a small fentanyl derivative library with diverse functional groups and properties.[15,33,34] Considering recent achievements in PEG-drug conjugates including opioids, PEG-fentanyl derivatives have the potential for improved properties by increasing hydrophilicity and molecular weight.[35–38] Therefore, herein we report the synthesis of a fentanyl-aryl halide derivative: Fen-Br that allows for the synthesis of novel PEO(PEG)-fentanyl conjugates and also provides a unique linking group chemistry while activating MOR both *in vitro* and *in vivo*.

We prepared Fen-Br derivative in one step from commercially available starting materials. Initial derivatives in our campaign were prepared by direct palladium catalysed cross-coupling of acrylates with Fen-Br using $Pd(OAc)_2$ and dppe. Upon refinement of the reaction conditions, Fen-Br was coupled with *tert*-butyl acrylate (Fen-Acry-*t*Bu) followed by trifluoroacetic acid mediated hydrolysis to yield Fen-Acry-OH. The use Fen-Acry-OH dramatically simplifies both synthesis and purification of the desired derivatives. A small library of fentanyl derivatives was then prepared (Scheme 1). The commonality in the library is a shared linking group between the fentanyl and the new moiety.

We prepared a small library of fentanyl analogs by utilizing a novel "rigid" acrylate linking group between fentanyl and the hydrophilic moiety. The unique acrylate linking chemistry allowed for the retention of Mu opioid receptor agonist properties both *in vitro* and *in vivo* while dramatically increasing molecular weight, polar surface area, and hydrophilicity.

Fen-Br was prepared from commercially available reagents by a substitution reaction of norfentanyl and bromoethyl phenyl bromide. Pure Fen-Br was isolated as HCl salt by precipitation from ethyl ether, with a 95% yield in gram scale. Fen-Br was characterized by $^1$H NMR and RP-HPLC, as shown in Fig. 1a. Identical peaks at ~7.38, 7.08, and 7.02 ppm are attributed to the phenyl protons (full $^1$H NMR spectrum is presented in Fig. S1 in ESI†).

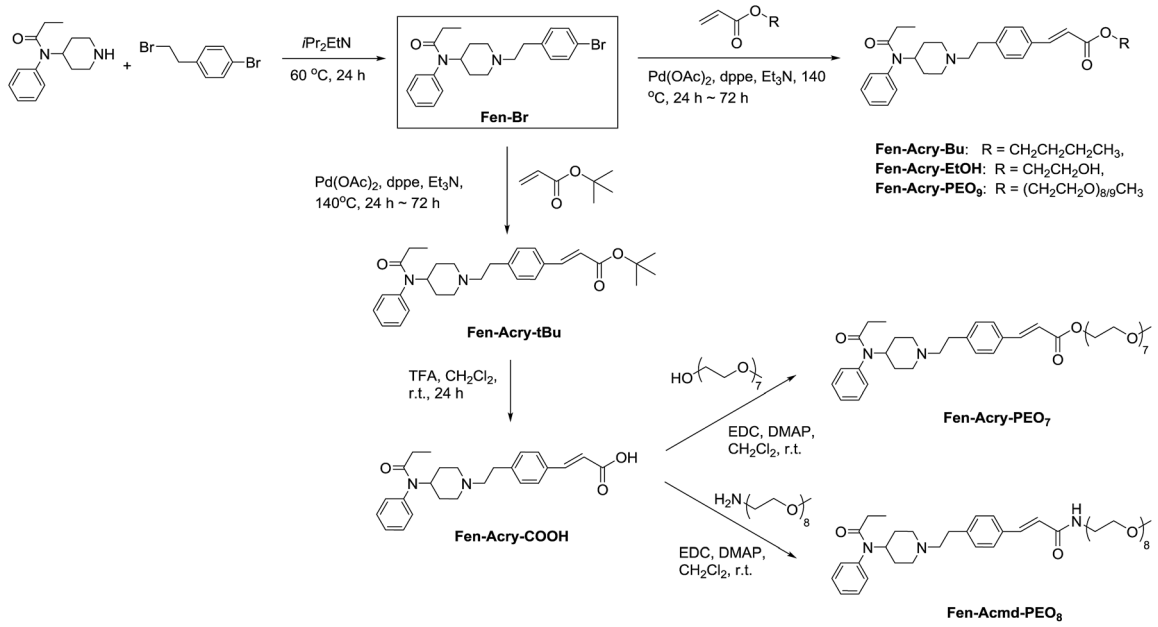
The initial synthetic approach was performed by directly reacting Fen-Br and acrylates using $Pd(OAc)_2$/dppe. Fen-Acry-EtOH, Fen-Acry-Bu, Fen-Acry-*t*Bu, and Fen-Acry-$PEO_9$ were obtained with good yields and high purity.

The initial synthetic was performed by directly reacting Fen-Br and acrylates using $Pd(OAc)_2$ and dppe. Fen-Acry-EtOH, Fen-Acry-Bu, Fen-Acry-*t*Bu, and Fen-Acry-$PEO_9$ were obtained with good yields and high purity. However, this synthetic route requires preparation of variable acrylates prior to the Heck

[a]*Neuroscience Disruptive Research Lab, Allegheny Health Network Research Institute, Allegheny General Hospital, Pittsburgh, Pennsylvania 15212, USA. E-mail: Saadyah.Averick@ahn.org; Tel: +1-412-359-4943*

[b]*Neuroscience Institute, Allegheny Health Network, Allegheny General Hospital, Pittsburgh, Pennsylvania 15212, USA*

† Electronic supplementary information (ESI) available: $^1$H-NMR spectra, RP-HPLC traces, reaction conditions, and methods. See DOI: 10.1039/c7ra01346a




Scheme 1 Synthesis of Fen-Br and its derivatives through Heck coupling reactions.

coupling reaction, which would complicate the purification of the products. An alternate route was designed and performed by synthesizing Fen-Acry-*t*Bu by Heck coupling reaction followed by deprotection of the *tert*-butyl group. The deprotection by TFA afforded Fen-Acry-OH in quantitative yield as determined by proton NMR and RP-HPLC analysis (Fig. 1b). New proton shifts at ~7.6 and ~6.4 ppm were observed for the acrylate functional group. The Fen-Acry-OH also exhibited higher polarity by eluting earlier in RP-HPLC analysis (Fig. 1b). The resulting compound Fen-Acry-OH was used in a variety of additional coupling reactions to afford esters and amides. As a demonstration of concept we prepared Fen-Acry-$PEO_7$ and Fen-Acmd-$PEO_8$.

To quantify the influence of chemical modification of fentanyl on its physiochemical and biological properties we computationally characterized the library for impacts on molecular weight, polar surface area, and log *P*. The impact on Mu opioid agonist activity was determined by measuring changes in cAMP levels in a live cell assay with CHO cells expressing the MOR (CHO-MORs) (Fig. 2). Our results indicate that the incorporation of the "rigid" linking group allows for a dramatic increase (1.2–3 times) in molecular weight and hydrophilicity while still ensuring Mu opioid agonist activity (Table 1).

MOR activation assays were used to determine the $EC_{50}$ values of the Fen-Br derivatives. cAMP inhibition studies were performed by incubation of the Fen-Br derivatives at a range of concentrations ($10^{-12}$ to $10^{-4}$ M), with forskolin treated CHO-MOR cells. cAMP levels were determined using a cAMP-Glo kit from Promega using manufactures instructions. DAMGO ($EC_{50}$ 2.71 ± 0.07 nM), morphine ($EC_{50}$ 24.03 ± 0.401 nM) and fentanyl ($EC_{50}$ 1.58 ± 0.04 nM) were used as references.

All compounds were tested and most of their $EC_{50}$ values were determined within the range of 6–50 nM, excluding Fen-Acry-OH. Fen-Acry-$PEO_7$, Fen-Acry-$PEO_9$ and Fen-Acmd-$PEO_8$ have $EC_{50}$ of 16.4, 7.4 and 10.0 nM respectively, which are an

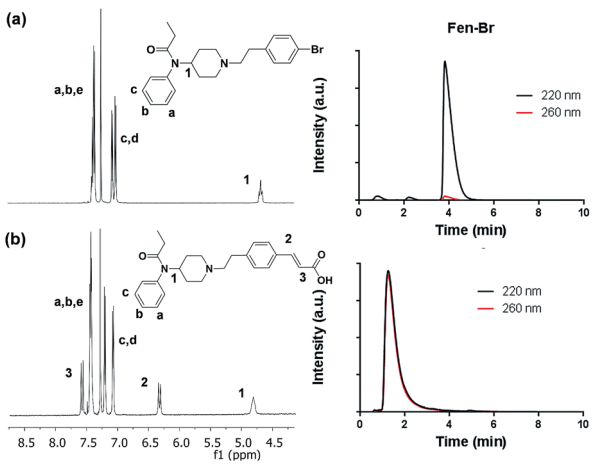



Fig. 1 $^1$H NMR and RP-HPLC spectra of (a) Fen-Br and (b) Fen-Acry-OH.

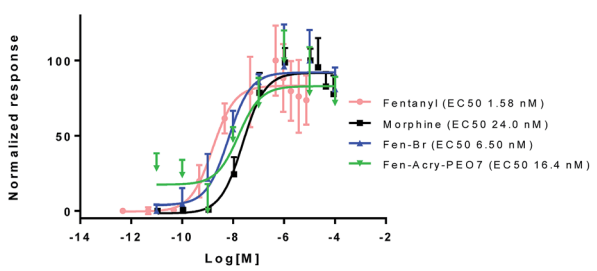
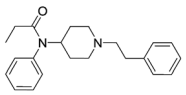
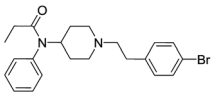
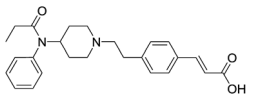
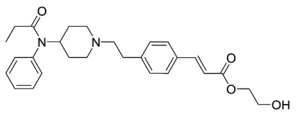
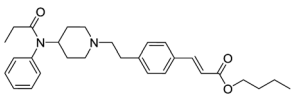
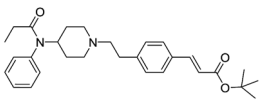
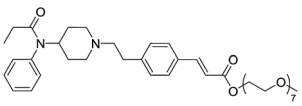
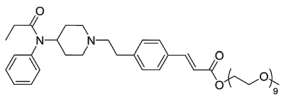
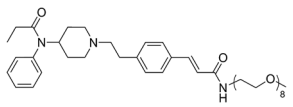



Fig. 2 Activation of MOR by synthesized fentanyl analogs using cAMP-Glo™ Assay.

Table 1 Summary of fentanyl derivatives and properties

| Name | Structure | Predicted PSA | PSA/PSA-fentanyl | log *P* | MW (g mol$^{-1}$) | MW/fentanyl | $EC_{50}$ (nM) |
|---|---|---|---|---|---|---|---|
| Fentanyl |  | 23.55 | 1.00 | 3.79 | 336.48 | 1.0 | 1.58 ± 0.04 |
| Fen-Br |  | 23.55 | 1.00 | 4.60 | 415.38 | 1.2 | 6.54 ± 0.15 |
| Fen-Acry-OH |  | 60.85 | 2.58 | 3.69 | 406.53 | 1.2 | 129.50 ± 3.28 |
| Fen-Acry-EtOH |  | 70.08 | 2.98 | 3.44 | 450.58 | 1.3 | 22.86 ± 0.85 |
| Fen-Acry-Bu |  | 49.85 | 2.12 | 5.82 | 462.63 | 1.4 | 15.01 ± 0.43 |
| Fen-Acry-*t*Bu |  | 49.85 | 2.12 | 5.57 | 462.63 | 1.4 | 53.53 ± 1.61 |
| Fen-Acry-$PEO_7$ |  | 114.49 | 4.86 | 2.95 | 728.92 | 2.2 | 16.43 ± 0.72 |
| Fen-Acry-$PEO_9$ |  | 132.96 | 5.65 | 2.55 | 817.03 | 2.4 | 7.40 ± 0.93 |
| Fen-Acmd-$PEO_8$ |  | 126.49 | 5.37 | 2.41 | 772.40 | 2.3 | 10.02 ± 1.21 |

order of magnitude less effective than fentanyl and still more active than morphine ($EC_{50}$ 24.03 ± 0.401 nM) (Fig. 2). In addition, their relatively low Clog *P*, and increased weight are predicted to have longer circulation half-lives with the potential for minimal blood brain barrier (BBB) permeability. Our assay shows that the Fen-Acry-OH is two orders of magnitude less active than fentanyl. This derivative would be deprotonated at physiological pH thus likely to carry a negative charge which may be a contributor for poor activation of MOR. Fen-Acry-*t*Bu has relatively weaker interaction with the receptor ($EC_{50}$ 53.53 ± 1.61) possibly due to steric hindrance of the *tert*-butyl bulky group. With the preparation of Fen-Acry-$PEO_9$ we have identified a strong lead compound for further evaluation. Although Fen-Acmd-$PEO_8$ shows good activity ($EC_{50}$ 10.02 ± 1.21 nM) we decided to concentrate on acrylate analogs in this communication due to their preferable synthesis and purification conditions. We selected seven acrylate derivatives for *in vivo* studies. Despite the high $EC_{50}$ of Fen-Acry-OH we decided to test it as a potential metabolite of PEG-fentanyl conjugates.

The *in vivo* antinociceptive properties of selected acrylate derivatives were evaluated using an *in vivo* hot plate withdrawal assay (Fig. 3). Hot plate withdrawal assay is commonly used to corroborate analgesia due to its sensitivity and is largely employed to evaluate opioids. All animal care was in compliance with the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication no. 86-23) and approved by Institutional Animal Care and Use Committee (IACUC) at Allegheny Health Network. Male CD-1 mice ($n$ = 10) weighing 30 g were dosed subcutaneously 30 minutes prior to placement on a 55 °C hot plate and withdrawal latencies were measured (jumping or hind-paw licking) within a 30 second time frame. The maximum possible effects (MPE) at a 95% confidence interval were calculated according to the following equation: $MPE = 100 \times (time_{latency} - time_{saline})/(30\ s - time_{saline})$.

The MPE for Fen-Br and Fen-Acry-Bu were 73% at 2.4 mg kg$^{-1}$ and 94% at 0.54 mg kg$^{-1}$ respectively compared to an MPE

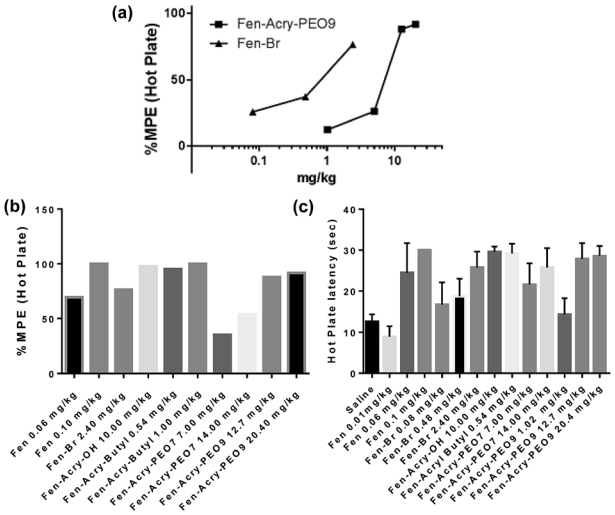



Fig. 3 Hot plate test: (a) each point shows the % of MPE induced by Fen-Br and Fen-Acry-$PEO_9$; (b) each column shows the % of MPE induced by Fen-acrylate derivatives; (c) each column indicates latency of withdrawal (s) mean ± SEM ($n = 10$) at 30 min post dose of Fen-acrylate derivatives in different concentrations.

of 65% at 0.06 mg $kg^{-1}$ of fentanyl. The PEG-fentanyl hydrophobic conjugates Fen-Acry-$PEO_9$ and Fen-Acry-$PEO_7$ had antinociceptive properties with MPE of 87% at 12.7 mg $kg^{-1}$ and 54% at 14.0 mg $kg^{-1}$. These results indicate that acrylate linking group can be used to prepare compounds with acceptable *in vivo* activity. The high dose required for the Fen-Acry-$PEO_9$ can be explained by the relatively low permeability of these compounds as well as being restricted to peripheral MORs which prevents neural MOR binding. All the tested compounds exhibit two orders of magnitude lower activity than fentanyl, and are still well within the range of known opioids,[4,39] introducing a new family of active compounds. Notably, the rigid linking group chemistry has activity that translates well from *in vitro* to *in vivo* and is not dependent on the polarity of the linked group *i.e.* butyl *vs.* PEO.

While opioids elicit their therapeutic effect by binding both central and peripheral receptors within the human nervous system and some soft tissues, the binding of opioids across the BBB to the brainstem neuronal receptors is the primary mechanism of opioid addiction.[40–42] Our novel fentanyl derivatives have been designed to have an intrinsically lower BBB permeability than the parent compound due to an increased molecular weight, polar surface area, and hydrophilicity. In this manuscript we report the synthesis and characterization of these novel hydrophilic opioid receptor agonists and in future work we will study the BBB permeability in a rodent model.

These results will be further expounded upon in future publications focusing on the preparation of a library of hydrophilic opioid derivatives with full SAR tables for different Fen-Br isomers and different length PEO oligomers. Furthermore, we found that assuming a constant blood volume of 75 ml $kg^{-1}$ (calculated in mol $L^{-1}$) the fentanyl and Fen-Br derivate exhibit an $MPE_{50}$ about 3000 times their $EC_{50}$ data as generated in cells. This data can be used to assist in predicting *in vivo* activity of novel fentanyl derivatives.

## Conclusions

In conclusions, a series of fentanyl derivatives containing "rigid" linkers were synthesized from a new parent compound, Fen-Br, through Heck Pd catalyzed cross coupling. These compounds were evaluated *in vitro* by Mu opioid receptor activation in a live cell cAMP inhibition assay and most resulted in $EC_{50}$ values within the range of 6–50 nM. Optimized hydrophilic compounds present lower $EC_{50}$ values (higher activity) than morphine. Select compounds were evaluated *in vivo* in a hot plate withdrawal assay, demonstrating positive results. Future studies will explore different rigid linking groups accessible through Pd catalyzed cross coupling reactions (*i.e.* acrylamide, alkene, alkyne) as well as the effect on different length PEO on MOR activation.

## Acknowledgements

We gratefully thank the Allegheny Health Network Research Institute and the Neuroscience Institute for start-up funds to conduct this research.

## Notes and references

1 I. Kissin, *Anesth. Analg.*, 2010, **110**, 780–789.
2 J. Woodcock, J. Witter and R. A. Dionne, *Nat. Rev. Drug Discovery*, 2007, **6**, 703–710.
3 D. M. Zimmerman and J. D. Leander, *J. Med. Chem.*, 1990, **33**, 895–902.
4 C. Andrews and C. Prys-Roberts, *J. Clin. Anesthesiol.*, 1983, **1**, 97–112.
5 P. W. H. Peng and A. N. Sandler, *Anesthesiology*, 1999, **90**, 576–599.
6 R. R. Petrov, R. S. Vardanyan, Y. S. Lee, S. W. Ma, P. Davis, L. J. Begay, J. Y. Lai, F. Porreca and V. J. Hruby, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 4946–4950.
7 A. Poklis, *J. Toxicol., Clin. Toxicol.*, 1995, **33**, 439–447.
8 R. S. Vardanyan and V. J. Hruby, *Future Med. Chem.*, 2014, **6**, 385–412.
9 P. A. Janssen, C. J. Niemegeers and J. G. Dony, *Arzneimittelforschung*, 1963, **13**, 502–507.
10 W. B. Wright, H. J. Brabander and R. A. Hardy, *J. Org. Chem.*, 1961, **26**, 485–490.
11 L. V. Kudzma, S. A. Severnak, M. J. Benvenga, E. F. Ezell, M. H. Ossipov, V. V. Knight, F. G. Rudo, H. K. Spencer and T. C. Spaulding, *J. Med. Chem.*, 1989, **32**, 2534–2542.
12 R. Vardanyan, V. K. Kumirov, G. S. Nichol, P. Davis, E. Liktor-Busa, D. Rankin, E. Varga, T. Vanderah, F. Porreca, J. Lai and V. J. Hruby, *Bioorg. Med. Chem.*, 2011, **19**, 6135–6142.
13 P. T. Bremer, A. Kimishima, J. E. Schlosburg, B. Zhou, K. C. Collins and K. D. Janda, *Angew. Chem.*, 2016, **55**, 3772–3775.
14 M. P. Davis, *Expert Rev. Neurother.*, 2011, **11**, 1197–1216.

15 R. Weibel, D. Reiss, L. Karchewski, O. Gardon, A. Matifas, D. Filliol, J. A. Becker, J. N. Wood, B. L. Kieffer and C. Gaveriaux-Ruff, *PLoS One*, 2013, **8**, e74706.
16 T. N. Riley, D. B. Hale and M. C. Wilson, *J. Pharm. Sci.*, 1973, **62**, 983–986.
17 F. Janssens, J. Torremans and P. A. Janssen, *J. Med. Chem.*, 1986, **29**, 2290–2297.
18 B. S. Lin, L. V. Kudzma and H. K. Spencer, *US Pat.*, 4 791 120 A, 1988.
19 J. R. Bagley and H. K. Spencer, *US Pat.*, 4 900 738 A, 1988.
20 G. Hadley, S. Derry, R. A. Moore and P. J. Wiffen, *Cochrane Database Syst Rev.*, 2013, **10**, Cd010270.
21 W. Jeal and P. Benfield, *Drugs*, 1997, **53**, 109–138.
22 R. B. Muijsers and A. J. Wagstaff, *Drugs*, 2001, **61**, 2289–2307.
23 R. Benyamin, A. Trescot, S. Datta, R. Buenaventura, R. Adlaka, N. Sehgal, S. Glaser and R. Vallejo, *Pain Physician*, 2008, **11**, S105–S120.
24 R. C. Dart, H. L. Surratt, T. J. Cicero, M. W. Parrino, S. G. Severtson, B. Bucher-Bartelson and J. L. Green, *N. Engl. J. Med.*, 2015, **372**, 241–248.
25 R. L. DuPont, *J. Psychoact. Drugs*, 2010, **42**, 127–132.
26 M. J. Edlund, B. C. Martin, J. E. Russo, A. DeVries, J. B. Braden and M. D. Sullivan, *Clin. J. Pain*, 2014, **30**, 557–564.
27 J. A. Gwira Baumblatt, C. Wiedeman, J. R. Dunn, W. Schaffner, L. J. Paulozzi and T. F. Jones, *JAMA Intern. Med.*, 2014, **174**, 796–801.
28 L. J. Paulozzi and G. W. Ryan, *Am. J. Prev. Med.*, 2006, **31**, 506–511.
29 L. E. Edinboro, A. Poklis, D. Trautman, S. Lowry, R. Backer and C. M. Harvey, *J. Forensic Sci.*, 1997, **42**, 741–743.
30 M. I. Jumbelic, *Am. J. Forensic Med. Pathol.*, 2010, **31**, 18–21.
31 C. Naumann, S. Erdine, A. Koulousakis, J.-P. Van Buyten and M. Schuchard, *Neuromodulation*, 1999, **2**, 92–107.
32 T. L. Yaksh, S. Hassenbusch, K. Burchiel, K. R. Hildebrand, L. M. Page and R. J. Coffey, *Pain Med.*, 2002, **3**, 300–312.
33 J. Riggs-Sauthier, B.-L. Deng and T. A. Riley, *US Pat.*, 8 946 285 B2, 2016.
34 J. Riggs-Sauthier, B. L. Deng and T. A. Riley, *EP Pat.*, 2 628 489 A1, 2013.
35 F. M. Veronese and G. Pasut, *Drug Discovery Today*, 2005, **10**, 1451–1458.
36 J. M. Harris and R. B. Chess, *Nat. Rev. Drug Discovery*, 2003, **2**, 214–221.
37 R. Greenwald, *J. Controlled Release*, 2001, **74**, 159–171.
38 X. Pang, H.-L. Du, H.-Q. Zhang, Y.-J. Zhai and G.-X. Zhai, *Drug Discovery Today*, 2013, **18**, 1316–1322.
39 R. O. Girón, R. Abalo, C. Goicoechea, M. I. Martín, L. F. Callado, C. Cano, P. Goya and N. Jagerovic, *Life Sci.*, 2002, **71**, 1023–1034.
40 D. F. Wu, Y. S. Kang, U. Bickel and W. M. Pardridge, *Drug Metab. Dispos.*, 1997, **25**, 768–771.
41 H. Pajouhesh and G. R. Lenz, *NeuroRx*, 2005, **2**, 541–553.
42 A. Reichel, *Chem. Biodiversity*, 2009, **6**, 2030–2049.