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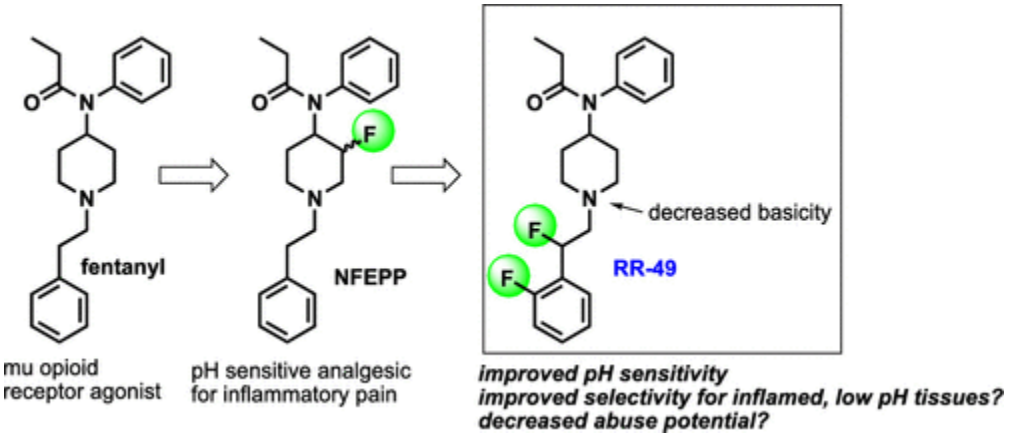
Abstract



The concept recently postulated by Stein and co-workers (*Science***2017**, *355*, 966) that mu opioid receptor (MOR) agonists possessing amines with attenuated basicity show pH-dependent activity and can selectively act at damaged, low pH tissues has been additionally supported by in vitro studies reported here. We synthesized and tested analogs of fentanyl possessing one or two fluorine atoms at the beta position of the phenethylamine side chain, with additional fluorines optionally added to the benzene ring of the side chain. These compounds were synthesized in 1 to 3 steps from commercial building blocks. The novel bis-fluorinated analog RR-49 showed superior pH sensitivity, with full efficacy relative to DAMGO, but with 19-fold higher potency (IC_{50}) in a MOR cAMP assay at pH 6.5 versus 7.4. Such compounds hold significant promise as analgesics for inflammatory pain with reduced abuse potential.

KEYWORDS:

Mu opioid receptor agonist, fentanyl, fluorination, pH-sensitive, analgesic, cAMP

Agonism of the mu opioid receptor (MOR), originally via the natural product morphine, has been utilized for millennia as the most effective form of analgesia for acute pain.(1) The potential for addiction and abuse with sustained use of narcotic MOR agonists, combined with the dangerous side effect of respiratory depression, has driven the present opioid epidemic, with more than 70,000 overdose deaths in the U.S. in 2017.(2) Fortunately, new approaches to MOR agonism have been investigated that could lead to analgesics with improved safety profiles.(3−7) Stein and co-workers have recently added an additional strategy worthy of careful consideration: the use of pH-dependent ligands designed to have a higher affinity for receptors in damaged tissues with lower pH. This strategy leverages the fact that a protonated amine is generally required in MOR agonists to form an ion pair with Asp147 of human MOR, as suggested by site-directed mutagenesis(8) and confirmed by the X-ray structure of BU72 bound to MOR.(9) A fluorinated version of fentanyl(10−12) (**1**, Chart 1) called (±)-*N*-(3-fluoro-1-phenethylpiperidine-4-yl)-*N*-phenyl propionamide (NFEPP) (**2**), was reported by Stein et al. to have increased affinity for MOR and activity in functional G_i-driven assays at pH 6.5 versus normal pH (7.4).(13) Notably, NFEPP was also reported to be effective in rat models of acute and/or persistent inflammatory pain and may have lower CNS-related side effects than fentanyl.(13,14) The relative stereochemistry of this compound was not disclosed.

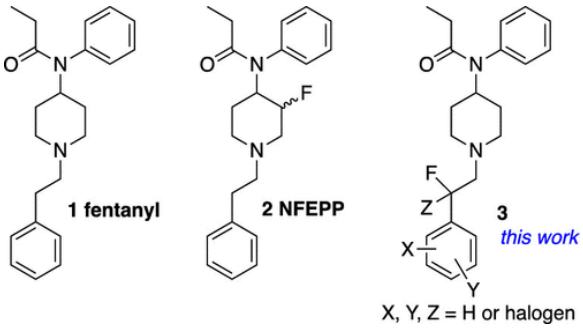
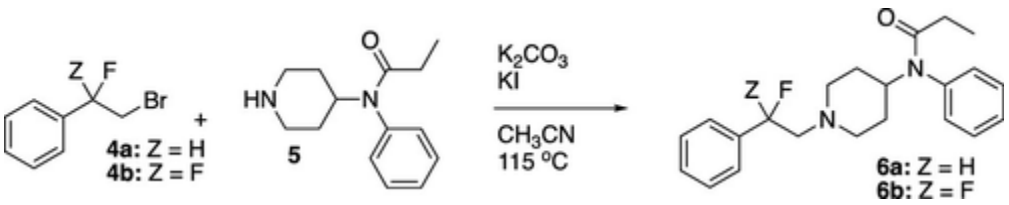


Chart 1. Fentanyl and Fluorinated Analogs

We reasoned that other MOR ligands with carefully attenuated basicities could be effective peripheral analgesics; such compounds (**3**, Chart 1) could be easier to prepare than NFEPP, are easily accessible in pure isomeric forms, and can be prepared via flexible synthetic routes that offer the opportunity to identify analogs with superior pH sensitivity and drug-like properties. The potential to selectively activate MORs at damaged peripheral sites at lower pH, over central MORs known to mediate undesirable effects such as euphoria and respiratory depression, makes this approach worthy of further investigation.

The introduction of pK_a-lowering electronegative fluorine atoms beta to amines is a well appreciated tactic that has been frequently used to improve drug-like properties.(15−17) We continued with this strategy applied to the fentanyl scaffold by aiming to place fluorine(s) beta to the piperidine nitrogen, but on the phenethyl side chain, rather than on the piperidine itself, as in NFEPP (Chart 1). We synthesized β-fluorofentanyl **6a** and β,β-difluorofentanyl **6b** via simple substitution reactions between the commercially available phenethyl halides **4a** and **4b** and piperidine **5** (Scheme 1). These analogs were compared to fentanyl and NFEPP in an MOR assay in transfected HEK 293 cells measuring relative cAMP concentrations. In this assay, MOR agonists induce G_i-mediated inhibition of adenylyl cyclase, and the resulting changes in cAMP concentration are measured indirectly via changes in luminescence from a luciferase enzyme engineered with a cAMP binding region.(18)



Scheme 1. One-Step Synthesis of β-Fluorofentanyls

In this assay, NFEPP and **6a** performed nearly identically, with both acting as full agonists (data not shown) relative to fentanyl, both with IC_{50}s of 0.71 nM at pH 6.5. As a measure of pH sensitivity, we define the “pH ratio”, which is simply the ratio of IC_{50}s at pH 7.4 and 6.5. Importantly, both NFEPP and **6a** showed significantly higher potencies at pH 6.5 than 7.4, with pH ratios of 10.6 and 8.2, respectively, compared to fentanyl with a measured pH ratio of 1.9 (Figure 1 and Table1). The bis-fluorinated compound **6b**, with a calculated pK_a of 5.5, was expected to be only weakly active even at low pH, and this was consistent with the experimental results, with an IC_{50} nearly 4 orders of magnitude higher than **6a** (Table1). During the course of this work, Stein reported *in vitro* and *in vivo* analgesia data with **6a** (aka FF3), though details of its synthesis and characterization were not disclosed. A pH ratio was reported to be 4.9 for **6a** in an MOR GTPγS assay.(19)

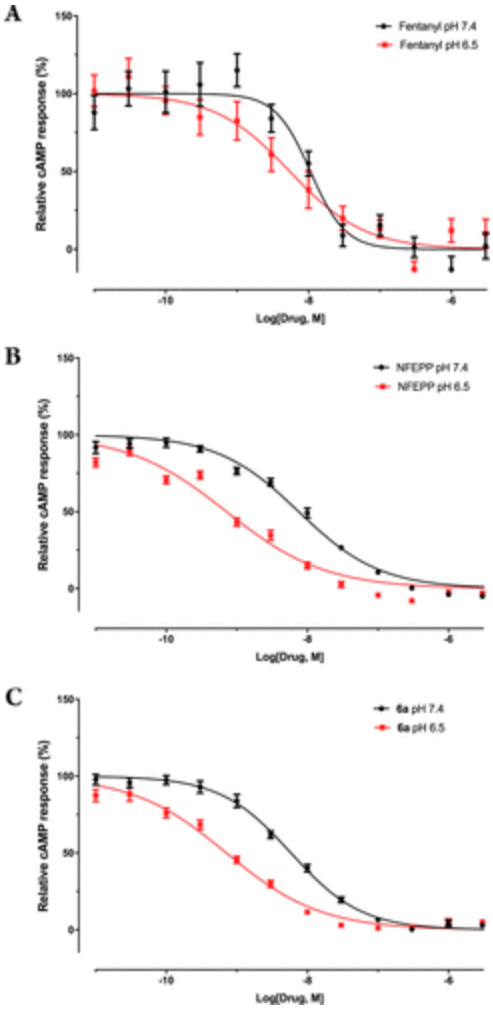


Figure 1. Concentration–response curves of (A) fentanyl; (B) NFEPP; and (C) **6a** in GloSensor MOR cAMP assay. Data was individually normalized to each compound to show % response.

Table 1. Summary of MOR Agonist Activities[a]

compound	calcd pK_a[b] (exptl)	pIC_{50} (pH 7.4)[c]	pIC_{50} (pH 6.5)[c]	pH ratio[d]
DAMGO		9.10 ± 0.07	8.71 ± 0.07	0.4
fentanyl (1)	8.77 (8.4)	7.97 ± 0.08	8.26 ± 0.12	1.9
NFEPP (2)	7.30 (6.82)	8.12 ± 0.03	9.15 ± 0.04	10.6
6a	7.18 (7.22)	8.23 ± 0.03	9.15 ± 0.04	8.2
6b	5.48	4.50 ± 0.07	5.24 ± 0.06	5.4
RR-49 (12a)	6.60	6.98 ± 0.07	8.26 ± 0.07	19.0
12b	6.64	7.11 ± 0.09	8.19 ± 0.07	12.0
12c	6.94	6.99 ± 0.07	7.60 ± 0.06	4.0
12d	6.38	7.05 ± 0.11	7.93 ± 0.07	9.0

[a]Agonist activities were measured with a GloSensor cAMP assay with HEK 293 cells in 384-well plates transiently expressing MOR. See Supporting Info for full details. Results were independently normalized, with 0% and 100% activity defined for each compound as the top and bottom of curves fit with 4-parameter nonlinear regression (GraphPad Prism v. 8).

[b]pK_a of protonated amine calculated with ChemAxon Marvin v.18.3.

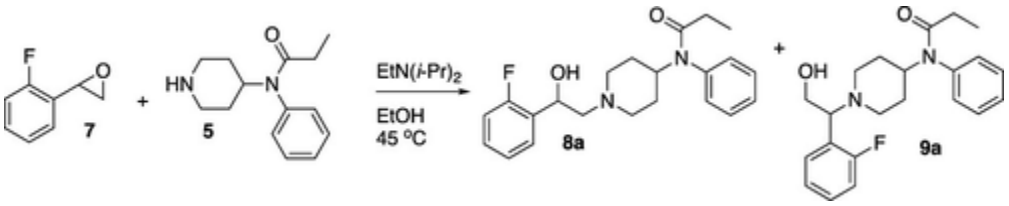
[c]pIC_{50} = −log(IC_{50}). Uncertainty is indicated by SEM for the curve fitting to a minimum of 16 measurements.

[d]pH ratio = IC_{50} (pH 7.4)/IC_{50} (pH 6.5).

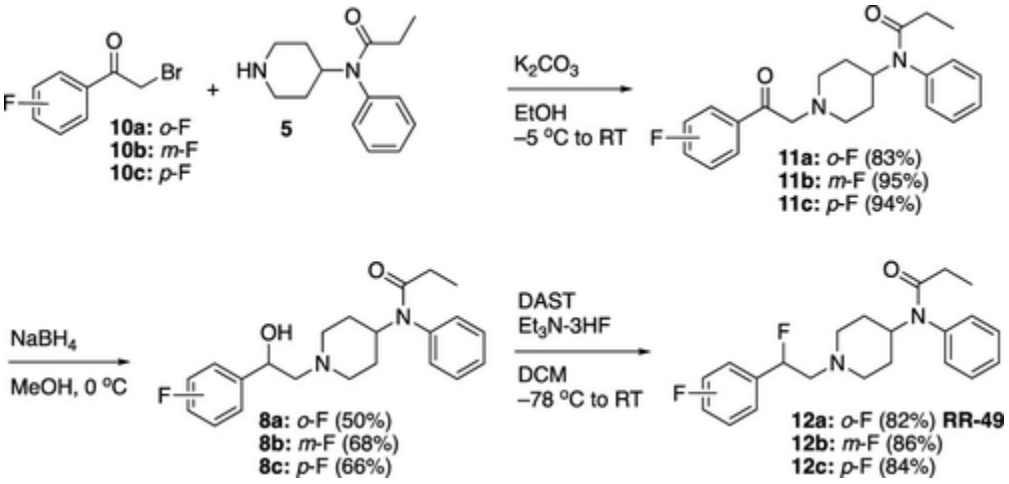
Stein reported that **6a** showed impressive dose-dependent analgesia in rat models of inflammatory pain, though of limited duration. Unfortunately, it also showed similar conditioned place preference to fentanyl, suggesting that it could suffer from similar abuse liabilities,(19) which is likely also an issue with NFEPP. We reasoned that molecules with pK_as less than **6a** (measured by Stein to be 7.22)(19) but greater than **6b** (calculated to be 5.48, Table1) could offer substantially better selectivity for inflamed tissues and a larger therapeutic window.

With these promising results in hand for **6a**, we aimed to prepare molecules with amine pK_as closer to 6.5, i.e., the pH that may be present in inflamed tissues. To do this, we simply added fluorine(s) to the benzene of the phenethyl side chain. Since the required alkyl halides were not commercially available, we pursued alternative routes that could also support the preparation of enantiopure materials, if desired. Since styrene oxides are potentially available in highly enantioenriched forms via asymmetric epoxidation methods, we first attempted epoxide opening reactions with piperidine **5**, with alcohol products that could be subsequently converted to the desired fluorides in a single step.

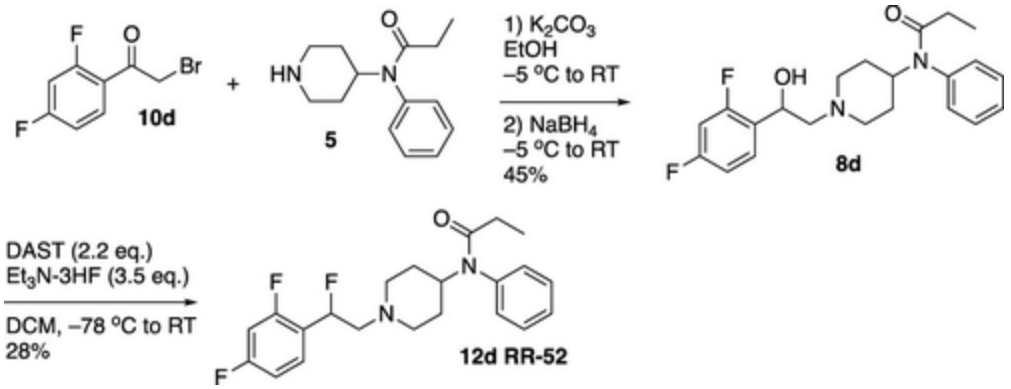
Somewhat unexpectedly, the ring opening reactions under basic conditions yielded regioisomeric products that were surprisingly difficult to separate. For example, treatment of epoxide **7** with **5** generated a mixture of the desired secondary alcohol **8a** and undesired primary alcohol **9a** (Scheme 2). Alternatively, piperidine **5** was cleanly alkylated with α-bromoacetophenones **10**, then the resulting ketones **11** reduced with sodium borohydride to generate the desired amino alcohols **8** (Scheme 3). Finally, these were converted to the final β-fluorofentanyl analogs **12** by treatment with both DAST and Et_3N-3HF, which presumably protonates the basic amine to minimize formation of fluoroamine byproducts. The 2,5-difluorophenyl analog **12d** was generated without isolation of the intermediate ketone **11d**, which was particularly unstable (Scheme 4).



Scheme 2. Epoxide Opening Reaction



Scheme 3. Three-Step Synthesis of β-Fluorofentanyls



Scheme 4. Synthesis of **12d**

The analogs **12a**–**d** were tested in the GloSensor assay along with the standard MOR agonist DAMGO and fentanyl (Figure 2; Table1). As expected, the pH ratio tracks with the amine pK_a, as the *ortho*-fluoro analog **12a** (called RR-49), with its lower pK_a value of 6.60, showed better pH sensitivity (pH ratio = 19.0, Figure 3) relative to the *para*-fluoro **12c** (calcd pK_a = 6.94; pH ratio = 4.0). However, the 2,4-difluoro analog **12d** is the exception to the trend, as it had a lower pH ratio than **12a** (9.0). We cannot rule out the fact that a fluorine in the *para* position may have additional effects on receptor activation.

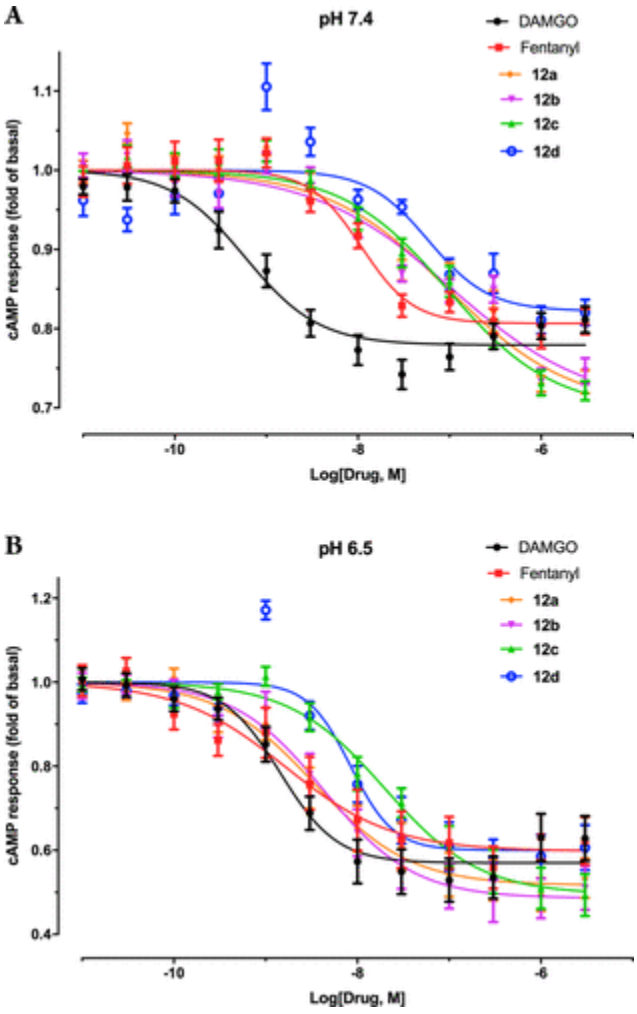


Figure 2. Concentration–response curves (fold activity) of DAMGO, fentanyl, and agonists **12a**–**d** in the GloSensor MOR cAMP assay at pH 7.4 (A) and 6.5 (B). Results were normalized with basal activity as 1.0 and analyzed in Prism using the built-in 4-parameter logistic function.

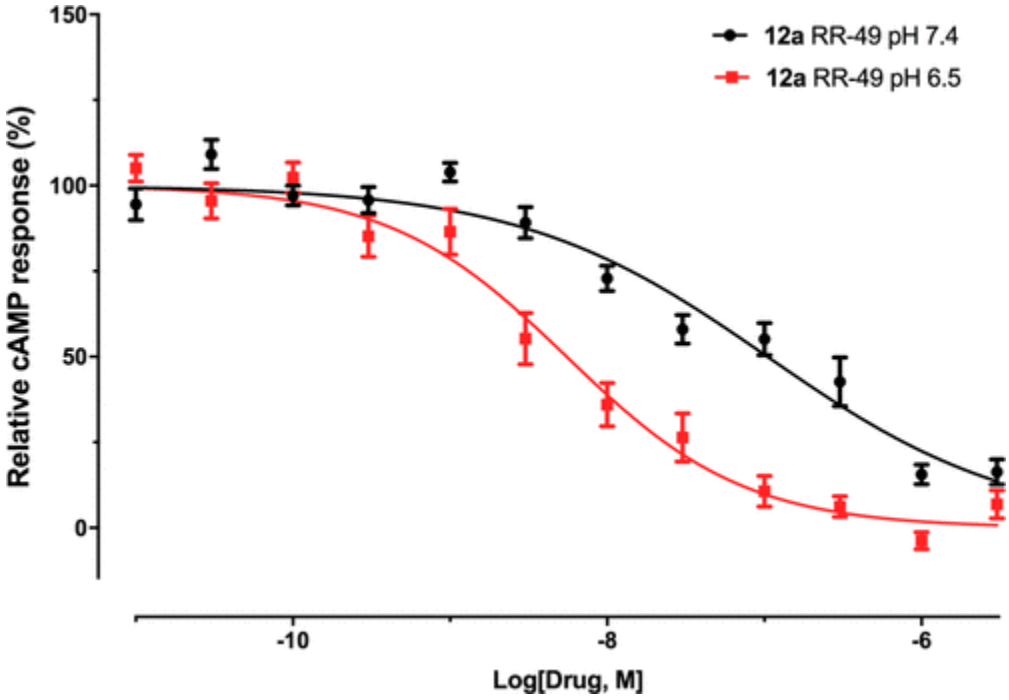


Figure 3. pH-dependent activity of **12a** (RR-49) (% response).

In summary, we have identified **12a** (RR-49) as a more highly pH-sensitive MOR agonist than the prior reported compounds NFEPP (**2**) and **6a**. Compound **12a** also maintains full agonist activity relative to DAMGO and fentanyl, as estimated in Figure 2. *In vivo* studies are underway to confirm that it may impart lower CNS effects and abuse liabilities than prior compounds of this class. pH-sensitive MOR agonists may represent a promising strategy for the treatment of inflammatory pain with decreased risks relative to current opioids.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.9b00335.

MOR cAMP assay protocol; synthetic protocols; characterization data (^{1}H NMR, ^{13}C NMR, and LC–MS chromatograms) (PDF) ml9b00335_si_001.pdf (4.27 MB)

Author Contributions

Designed compounds: C.D. Developed synthetic routes: C.D., R.R. Synthesized and characterized compounds: R.R. Performed assays: X.-P.H. Processed and analyzed data: X.-P.H., B.L.R., C.D. Wrote the manuscript: C.D. Prepared Supporting Info: R.R., C.D. Edited the manuscript: X.-P.H., B.L.R.

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An earlier version of this manuscript was submitted to the preprint server ChemRxiv.(20)

The authors declare the following competing financial interest(s): A patent application including this work has been submitted with C.D. and R.R. as inventors.

Terms & Conditions

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ABBREVIATIONS	
cAMP	**cyclic adenosine monophosphate**
CNS	**central nervous system**
DAMGO	**H-Tyr-D-Ala-Gly-N-MePhe-Gly-ol**
DAST	**diethylaminosulfur trifluoride**
DCM	**dichloromethane**
DIEA	***N*,*N*-diisopropylethylamine**
GPCR	**G-protein coupled receptor**
HEK	**human embryonic kidney**
IC_{50}	**half-maximal inhibitory concentration**
MOR	**mu opioid receptor**
SEM	**standard error of the mean**

References

1 Pasternak, G. W.; Pan, Y.-X. Mu Opioids and Their Receptors: Evolution of a Concept. *Pharmacol. Rev.* **2013**, *65*, 1257– 1317, DOI: 10.1124/pr.112.007138

2 U.S. Centers for Disease Control and Prevention. https://wonder.cdc.gov/.

3 Siuda, E. R.; Carr, R.; Rominger, D. H.; Violin, J. D. Biased Mu-Opioid Receptor Ligands: a Promising New Generation of Pain Therapeutics. *Curr. Opin. Pharmacol.* **2017**, *32*, 77– 84, DOI: 10.1016/j.coph.2016.11.007

4 Yekkirala, A. S.; Roberson, D. P.; Bean, B. P.; Woolf, C. J. Breaking Barriers to Novel Analgesic Drug Development. *Nat. Rev. Drug Discovery* **2017**, *16*, 545– 564, DOI: 10.1038/nrd.2017.87

5 Chan, H. C. S.; McCarthy, D.; Li, J.; Palczewski, K.; Yuan, S. Designing Safer Analgesics via M-Opioid Receptor Pathways. *Trends Pharmacol. Sci.* **2017**, *38*, 1016– 1037, DOI: 10.1016/j.tips.2017.08.004

6 Cunningham, C. W.; Elballa, W. M.; Vold, S. U. Bifunctional Opioid Receptor Ligands as Novel Analgesics. *Neuropharmacology* **2019**, *151*, 195– 207, DOI: 10.1016/j.neuropharm.2019.03.006

7 Roth, B. L. Molecular Pharmacology of Metabotropic Receptors Targeted by Neuropsychiatric Drugs. *Nat. Struct. Mol. Biol.* **2019**, *26*, 535– 544, DOI: 10.1038/s41594-019-0252-8

8 Surratt, C. K.; Johnson, P. S.; Moriwaki, A.; Seidleck, B. K.; Blaschak, C. J.; Wang, J. B.; Uhl, G. R. Mu Opiate Receptor. Charged Transmembrane Domain Amino Acids Are Critical for Agonist Recognition and Intrinsic Activity. *J. Biol. Chem.* **1994**, *269*, 20548– 20553

9 Huang, W.; Manglik, A.; Venkatakrishnan, A. J.; Laeremans, T.; Feinberg, E. N.; Sanborn, A. L.; Kato, H. E.; Livingston, K. E.; Thorsen, T. S.; Kling, R. C.; Granier, S.; Gmeiner, P.; Husbands, S. M.; Traynor, J. R.; Weis, W. I.; Steyaert, J.; Dror, R. O.; Kobilka, B. K. Structural Insights Into M-Opioid Receptor Activation. *Nature* **2015**, *524*, 315– 321, DOI: 10.1038/nature14886

10 Janssen, P. A. A. Review of the Chemical Features Associated with Strong Morphine-Like Activity. *Br. J. Anaesth.* **1962**, *34*, 260– 268, DOI: 10.1093/bja/34.4.260

11 Janssen, P. A. J.; Gardocki, J. F. Method for Producing Analgesia. U.S. 3,141,823.

12 Vardanyan, R. S.; Hruby, V. J. Fentanyl-Related Compounds and Derivatives: Current Status and Future Prospects for Pharmaceutical Applications. *Future Med. Chem.* **2014**, *6*, 385– 412, DOI: 10.4155/fmc.13.215

13 Spahn, V.; Del Vecchio, G.; Labuz, D.; Rodriguez-Gaztelumendi, A.; Massaly, N.; Temp, J.; Durmaz, V.; Sabri, P.; Reidelbach, M.; Machelska, H.; Weber, M.; Stein, C. A Nontoxic Pain Killer Designed by Modeling of Pathological Receptor Conformations. *Science* **2017**, *355*, 966– 969, DOI: 10.1126/science.aai8636

14 Rodriguez-Gaztelumendi, A.; Spahn, V.; Labuz, D.; Machelska, H.; Stein, C. Analgesic Effects of a Novel pH-Dependent M-Opioid Receptor Agonist in Models of Neuropathic and Abdominal Pain. *Pain* **2018**, *159*, 2277– 2284, DOI: 10.1097/j.pain.0000000000001328

15 Müller, K.; Faeh, C.; Diederich, F. Fluorine in Pharmaceuticals: Looking Beyond Intuition. *Science* **2007**, *317*, 1881– 1886, DOI: 10.1126/science.1131943

16 Hagmann, W. K. The Many Roles for Fluorine in Medicinal Chemistry. *J. Med. Chem.* **2008**, *51*, 4359– 4369, DOI: 10.1021/jm800219f

17 Gillis, E. P.; Eastman, K. J.; Hill, M. D.; Donnelly, D. J.; Meanwell, N. A. Applications of Fluorine in Medicinal Chemistry. *J. Med. Chem.* **2015**, *58*, 8315– 8359, DOI: 10.1021/acs.jmedchem.5b00258

18 Binkowski, B. F.; Butler, B. L.; Stecha, P. F.; Eggers, C. T.; Otto, P.; Zimmerman, K.; Vidugiris, G.; Wood, M. G.; Encell, L. P.; Fan, F.; Wood, K. V. A Luminescent Biosensor with Increased Dynamic Range for Intracellular cAMP. *ACS Chem. Biol.* **2011**, *6*, 1193– 1197, DOI: 10.1021/cb200248h

19 Spahn, V.; Del Vecchio, G.; Rodriguez-Gaztelumendi, A.; Temp, J.; Labuz, D.; Kloner, M.; Reidelbach, M.; Machelska, H.; Weber, M.; Stein, C. Opioid Receptor Signaling, Analgesic and Side Effects Induced by a Computationally Designed pH-Dependent Agonist. *Sci. Rep.* **2018**, *8*, 8965, DOI: 10.1038/s41598-018-27313-4

20 Rosas, R., Jr.; Huang, X.-P.; Roth, B. L.; Dockendorff, C. B-Fluorofentanyls Are pH-Sensitive Mu Opioid Receptor Agonists. *ChemRxiv* **2019**, DOI: 10.26434/chemrxiv.9036752.v1