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Optimisation of in silico derived 2-aminobenzimidazole hits as unprecedented selective kappa opioid receptor agonists ☆

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ABSTRACT

Kappa opioid receptor (KOR) is an important mediator of pain signaling and it is targeted for the treatment of various pains. Pharmacophore based mining of databases led to the identification of 2-aminobenzimidazole derivative as KOR agonists with selectivity over the other opioid receptors DOR and MOR. A short SAR exploration with the objective of identifying more polar and hence less brain penetrant agonists is described herewith. Modeling studies of the recently published structures of KOR, DOR and MOR are used to explain the receptor selectivity. The synthesis, biological evaluation and SAR of novel benzimidazole derivatives as KOR agonists are described. The in vivo proof of principle for anti-nociceptive effect with a lead compound from this series is exemplified.

The kappa (KOR), delta (DOR) and mu (MOR) opioid receptors are closely related G-protein coupled receptors responsible for analgesic and other pharmacological effects exhibited by opioid-type drugs and endogenous opioid peptides. All three receptors appear to be present in the central and peripheral nervous system of many organisms including humans.[1,2] Although activation of all three receptor subtypes is known to produce antinociception, the majority of opioid drugs that are currently in clinical use as analgesic agents (e.g., morphine and fentanyl) are MOR agonists.[3] However, MOR stimulation is also responsible for the spectrum of unwanted side effects associated with opioids including respiratory depression, dependence liability, and inhibition of gastrointestinal motility.[4] It has been established that KOR agonists are capable of producing analgesia without the side effects common to morphine and other classical opioids.[5] KOR agonists have the potential for treatment of incisional pain, inflammatory pain, pruritus, burn injury pain,[6] neuropathic pain,[7] visceral pain including dysmenorrhea or gastrointestinal pain,[8] and rheumatoid arthritis.[9] The great potential of KOR agonists stimulated research which led to identification of KOR ligands with great structural diversity.[10–12]

Dynorphin A (**1**) is an endogenous ligand for KOR whereas the naturally occurring diterpenoid salvinorin A (**2**) is a unique example of non-nitrogenous KOR agonists (Fig. 1). Many promising small molecule KOR agonists have been identified at the discovery stage, but clinical studies of the first generation centrally acting KOR agonists (viz., spiradoline, bremazocine and enadoline) showed CNS liabilities such as sedation and dysphoria.[13] Further efforts towards peripherally restricted KOR agonist as second generation compounds also led to limited success.

Asimadoline (**3**), a small molecule KOR agonist, is currently in Phase III clinical trial for the treatment of patients with diarrhea-predominant irritable bowel syndrome (Fig. 1).[14] The tetra-peptide CR-845 (**4**) has shown positive results in a phase II trial for post-operative pain in women following hysterectomy. KOR agonists

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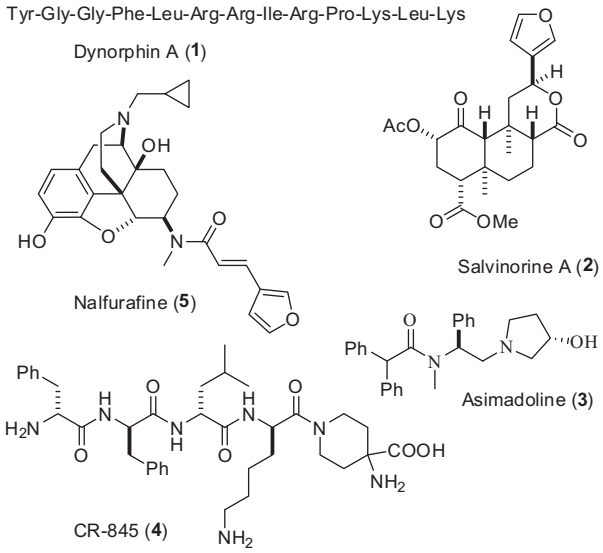


Figure 1. KOR agonists with structural diversity.

also have demonstrated in experimental and clinical studies antipruritic effects in antihistamine-resistant and antihistamine-sensitive pruritus in animals and humans.[15] Nalfurafin (**5**), a compound from the morphinan class, has been approved in Japan for the treatment of uremic pruritus in people undergoing hemodialysis.[15] Considering the wide potential of KOR agonism the search for an efficacious and safe KOR agonist is still in demand. We describe herein the identification of an unprecedented chemotype as KOR agonist by pharmacophore-based in silico screening, a brief SAR exploration and in vivo efficacy with a prototype compound.

We have previously described a physicogenetic methodology of identifying binding pocket-related GPCRs and associated ligands to construct pharmacophore models to mine commercial and in-house data bases to extract ligands for the GPCR of interest.[13] In the case of the kappa opioid receptor we identified receptors which are related also from a conventional phylogenetic relationship, that is, in particular the other opioid receptors and monoaminergic receptors. The challenge lies in the construction of a search query specific enough for the KOR, which was based on the construction of a homology model, build from the recently disclosed adrenergic β2-receptor, to refine the pharmacophore model with guidance from interactions of ligands to the binding pocket-related GPCRs as described previously.[16] According to our physicogenetic classification system,[16] and the Schwartz numbering system[17] the minor amino acid variations between the receptors in TM II:13, V:05 and VI:20 belong to the same groups, whereas the variations in TM II:23, V:08, VI:23 and VII:02 in the binding pockets are distinctly different and offer opportunities to design in receptor selectivity (Fig. 2C). Today the situation would have been quite different with access to crystalline structures of all the opioid receptors with bound ligands which allows for identification of minimal differences towards DOR and MOR.[19–21] A more detailed analysis based on the actual crystal structures will follow (vide infra). As a second step we utilized known ligands for the MOR and DOR in the construction of discriminative filters.

An exhaustive in silico search of available chemical vendor libraries containing over 10^7 compounds complemented with a GPCR-biased in-house library was conducted. The accepted compounds were filtered using a neural network for druglikeness[22] followed by rejection of compounds displaying high similarity to DOR and MOR ligands extracted from the Integrity data base with potencies <1 μM (Tannimoto coefficient >0.8 using UNITY fingerprints). Finally, 2616 compounds (45% commercial and 55% in-house) were selected using clustering based on 2D UNITY finger prints to maximize the chemical diversity of the extracted set of compounds. The compounds were screened in a KOR IP1 HTRF assay (COS-7 cells transiently transfected with the human KOR receptor cDNA)[23] at 10 μM concentration and 374 primary hits showed >50% activation of KOR compared to control.[24,25] The high hit rate (>14.3%) demonstrated the robustness of the developed pharmacophore model. These compounds were subjected to in vitro dose response studies and counter screen against the closest related receptors, that is, DOR and MOR using protocols[18] analogous to KOR IP1. This exercise led to identification of 39 compounds from 12 distinct chemical classes having EC_{50} values between 10 and 500 nM. Based on the chemical attractiveness, excellent in vitro potency (39 nM) and selectivity over DOR (200-fold) and MOR (1000-fold) one such compound **6** (Fig. 3) was chosen for further SAR studies.

To access the structural basis for ligand binding, we docked compound **6** into the KOR, DOR and MOR structures.[19–21,26] The predicted binding pose of compound **6** (yellow sticks) in the KOR structure are shown in Figure 2A and B. For comparison, subtype selective ligands in complex with recently published crystal structures of KOR, MOR and DOR (Protein Data Bank codes 4DJH, 4DKL, and 4EJ4) are shown in the Supplementary material. Analysis of compound **6** binding to the KOR, in combination with SAR results, suggest that $Val108^{2.53}$ (Ala and Ala; II:13), $Val118^{2.63}$ (Asn and Lys; II:23), $Met226^{5.38}$ (Leu and Thr; V:08), $Ile294^{6.55}$ (Val and Val; VI:20), and in particular $Tyr312^{7.35}$ (Trp and Leu; VII:02) contribute to the subtype selectivity of compound **6** (corresponding amino acids in MOR and DOR are shown in parentheses followed by the generic pocket numbering as indicated in bottom of Fig. 2C). Thus, changes in the $Val118^{2.63}$ side chain, where larger hydrophilic residues, $Asn^{2.63}$ and $Lys^{2.63}$ are found in MOR and DOR, respectively, are likely to introduce unfavorable contacts with the benzimidazole moiety located between TM II, -III and -VII. The remaining two hydrophobic side chains replacements, Val to Ala at position 2.53 and Ile to Val at position 6.55, may cause a reduction of the hydrophobic contact between compound **6** and the receptor. The *N*-ethylpyrrolidine moiety reaches deep into the orthosteric ligand binding pocket to form hydrophobic interaction with the conserved $Trp287^{6.48}$ side chain (the 'rotamer toggle switch'),[27] possibly playing a critical role in the pharmacological properties of this ligand. The carboxyl side chain of $Asp138^{3.32}$ III:08 are simultaneously engaged in charge–charge interactions with the positively charged *N*-ethylpyrrolidine moiety and hydrogen bond interaction with the central amide linker. Additionally, changing $Tyr312^{7.35}$ to the $Trp^{7.35}$ and $Leu^{7.35}$ residues found in MOR and DOR, respectively, are likely to result in the loss of an important polar interaction with the central amide linker in compound **6**, a predicted key interaction for the chemical series. Lastly, the *p*-methyl benzamide binds in a predominantly hydrophobic pocket between TM III, -V and VI.

A further aspect of our studies was the identification of more polar compounds (lower log*P* and higher polar surface area (PSA)) with retained potency in an effort to identify compounds with lower propensity to pass the blood–brain barrier (BBB) and thereby identify compounds with a reduced central action. We have previously used this strategy to develop CB1 antagonists with such properties.[28,29] Compound **6** is a good starting point with a relatively low lipophilicity (chromatographically determined log*D*[28] of 1.9, calculated values log*P* of 3.8 and log*D* of 2.8) albeit with a too low PSA of 50 $Å^2$.

The synthesis of compounds was fairly straight forward as mentioned in Scheme 1. S_NAr reaction of compounds **I** with amines **II** followed by reduction of nitro to amine and cyanogen bromide mediated cyclization led to the formation of compounds **III**. Coupling of various acids with **III** afforded final compounds.

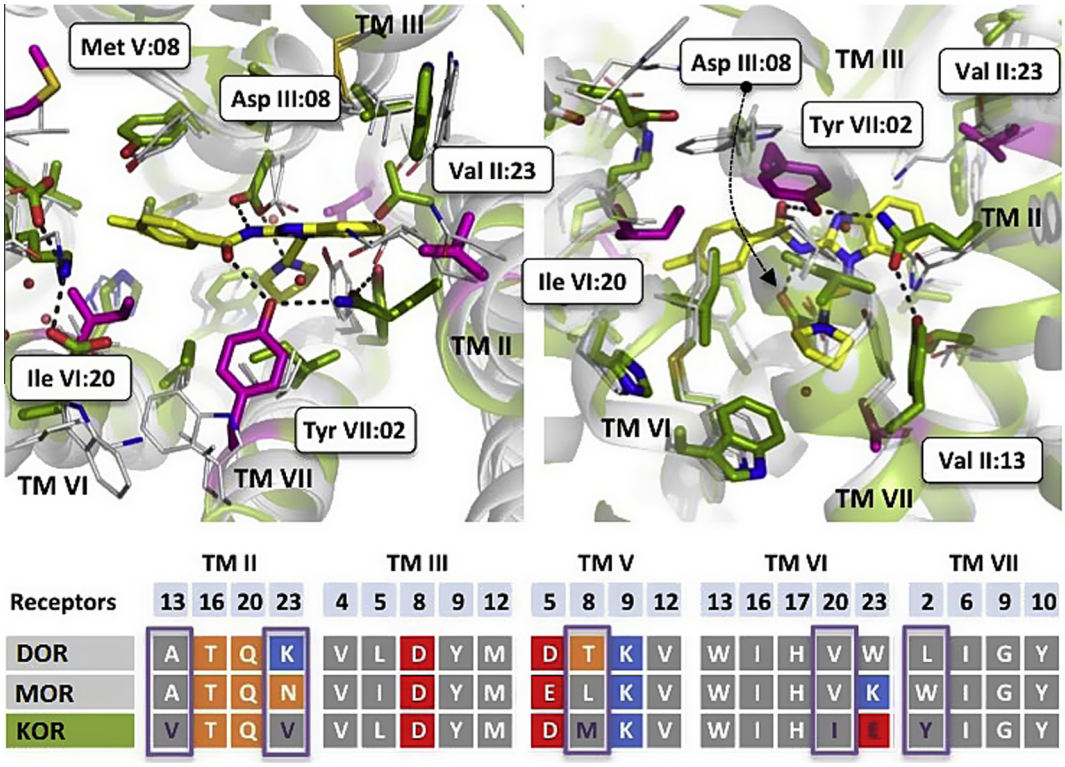


Figure 2. Extracellular view (A: top left) and side view (B: top right) of the predicted binding pose of compound **6** (yellow sticks) to the human KOR (green). Dark grey dashed lines show polar interactions. The MOR and DOR structures (PDBID: 4DKL and 4EJ4) are shown in grey for comparison. Among many contacts, compound **6** interacts with—or are in close proximity—to five residues (purple sticks) in the KOR binding pocket that differ in the MOR and DOR highlighted by purple rectangles in the pseudo-sequence alignment (C: bottom) of binding pocket residues in the three opioid subtype receptors. The residues predicted to contribute to the subtype selectivity are: Val108$^{2.53}$ (II:13), Val118$^{2.63}$ (II:23), Met226$^{5.38}$ (V:08), Ile294$^{6.55}$ (VI:20), and Tyr312$^{7.35}$ (VII:02). The two GPCR numbering annotations by Schwartz[17] and Ballesteros[18] are shown for clarity as described in detail in Ref. 16b.

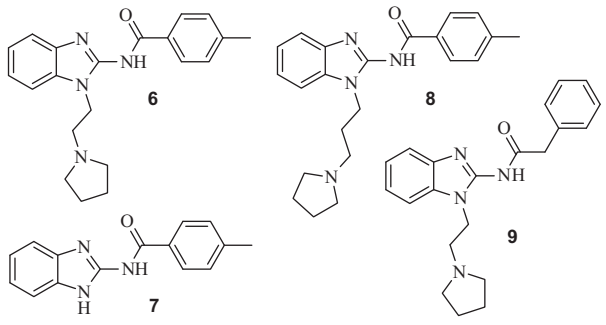
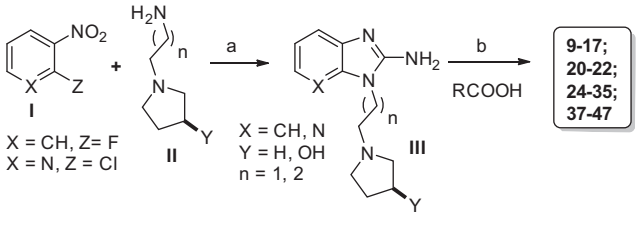


Figure 3. Initial KOR agonist in silico screening hit **6** and related structures **7**–**9**.



Scheme 1. Reagents and conditions: (a) (i) DMF, K_2CO_3, rt, 16 h, (ii) 10% Pd/C, H_2, MeOH, rt, 16 h, (iii) BrCN, EtOH, CH_2Cl_2, rt, 16 h; (b) EDCI, HOBt, DCM, rt, 16 h.

Compound **6** and **8** were prepared from **7** by N-alkylation. Compounds **18**, **19**, **23** and **36** were obtained by hydrolysis of the corresponding esters.

The potency of the test compounds was determined using KOR IP1 assay[25] and the results are summarized in Tables 1 and 2. The select compounds were counter screened using DOR and MOR assays[25] and the result is presented in Table 3. Initially we investigated three compounds **7**–**9** (Fig. 3) to understand their impact on SAR and selectivity. Compound **7** (the truncated version of **6** without southern *N*-ethylpyrrolidine) was tested and found to be inactive (Table 1). Extending the methylene spacer from two to three carbons as in compound **8** led to significant decrease in potency. Similarly replacing benzamide with phenylacetamide as in **9** also led to decrease in potency. Based on these observations we decided to explore SAR on the eastern part of the molecule. Initially we scanned through various polar and non-polar substitutions on benzamides and the results are summarized in Table 1. The halogenated derivatives *p*-chloro (**12**) and *m*,*p*-dichloro (**30**) benzamides displayed single digit agonist potency on KOR. The selectivity over MOR and DOR is also excellent (>200) as exemplified with **30** (Table 3). In general *ortho*-substituents are inferior to other positions (**11** vs **10**, and **14** vs **13** and **15**) unless in the case of *o*-hydroxy (**24**), capable of forming intramolecular hydrogen bonds that is reasonably accepted (cf. **10**). The *p*-fluoro (**10**) is having lipophilicity and potency equal to **6** but the somewhat less lipophilic *m*-cyano derivative **15** is displaying slightly reduced potency. Both compounds showed reduced but still reasonable selectivity over DOR and MOR (Table 2). However, most of the larger polar groups were not tolerated. Thus, carboxylic acid ester (**16**, **17**), ketone (**20**, **21**), mesylate (**22**), sulfonamide (**25**, **28**), sulfone (**26**) and pyrazole (**29**) derivatives suffered from significantly reduced potencies (Table 1). In every instance acidic groups (**18**, **19**, **23** and **27**) which would assist in driving PSA and lipophilicity further in the desired direction were detrimental to KOR potency.

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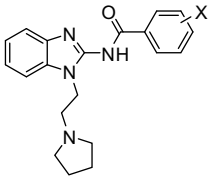
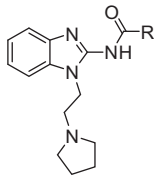
Table 1
Human KOR agonist potency of benzamide derivatives

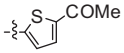
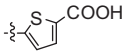
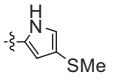
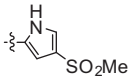


Compound	X	KOR EC_{50}[a] (nM)
3[b]		0.5
6[c]		39
7[c]		2000
8[c]		878
9[c]		1400
10	*p*-F	33
11	*o*-F	423
12	*p*-Cl	7
13	*p*-CN	355
14	*o*-CN	1225
15	*m*-CN	72
16	*p*-COOMe	1550
17	*m*-COOMe	4250
18	*p*-COOH	>10,000
19	*m*-COOH	>10,000
20	*p*-COMe	537
21	*m*-COMe	970
22	*m*-OMs	478
23	*m*-OCH_2COOH	>10,000
24	*p*-F, *o*-OH	118
25	*p*-SO_2NH_2	2950
26	*m*-SO_2Me	2400
27	*m*-2-Tetrazolyl	>10,000
28	*m*-SO_2NH_2, *p*-Cl	1550
29	*m*-*N*-Pyrazolyl, *p*-Cl	1800
30	*m*,*p*-diCl	7

[a] Values are mean of at least two experiments.
[b] Refer Figure 1 for structure.
[c] Refer Figure 3 for structures.

Table 2
Human KOR agonist potency of heteroaromatic derivatives



Compound	R	KOR EC_{50}[a] (nM)
31		2050
32		70
33		152
34		1050
35		734
36		>10,000
37		22
38		122
39		8
40		1440
41		765
42		1
43		31
44		2
45[b]		10
46[b]		37
47[b]		395

[a] Values are mean of at least two experiments.
[b] Refer Figure 4 for structures.

We then explored amides of a small set of heterocycles replacing benzamides and the results are summarized in Table 2. Both the pyridyl amides **32** and **33** showed moderate potency and good selectivity (**32**, Table 3) whereas thiophenes (**34** and **35**) were less tolerated. The corresponding carboxylic acid **36** was as expected inactive. On the other hand the more polar pyrrole amide **39** with log*P* of 2.7, log*D* of 1.8 and PSA of 66 (Table 3) was a very potent agonist displaying 8 nM potency in the hKOR assay and several 100-fold selectivity over MOR and DOR (Table 3). Replacing pyrrole NH with NMe (**39** vs **41**) resulted in a ~100 fold drop in potency, indicating a critical role of the N–H to stabilize a more coplanar conformation. Surprisingly the pyrrole to pyrazole modification as in **40** was not accommodated despite the tautomeric possibility to resemble **39**. When thiophene was replaced with the larger and more lipophilic benzothiophene (log*P* 4.4/log*D* 3.5/PSA 50), a significant improvement in KOR potency was observed (**34** vs **37**) with a retained selectivity over the other opioid receptors. The corresponding benzothiazole **38** had reduced activity on all three opioid receptors (Table 3). Following a similar pattern indole derivative **44** displayed a higher KOR potency of 2 nM compared to the plain pyrrole **39** but at the expense of a lower selectivity (<100) over DOR and MOR (Table 2).

Another modification of the pyrrole with a lower lipophilicity than the indole **44** was the thiomethyl substituted pyrrole **42** (log*P* 3.4/log*D* 2.5/PSA70). The sulfide **42** showed a very high KOR agonist potency of 1 nM and a moderate selectivity over DOR (10-fold) and high selectivity over MOR (200-fold). At this stage we were interested to introduce further polarity onto this scaffold. When sulfide **42** was transformed to sulfone **43** (log*P* 2.1/log*D* 1.5/PSA 100) the later showed somewhat reduced potency KOR potency and reduced opioid receptor selectivity (Tables 2 and 3). However, compared to the benzamidesulfone **26** this is a higher potency than expected once again indicating the positive conformational influence of the pyrrole system.

Another way of affecting the polarity is by manipulation of the basic side chain, Thus, incorporation of a hydroxyl functionality on the pyrrolidine as in **45** (Fig. 4) is completely tolerated as it displayed equipotency to corresponding compound **12**. This derivative also showed 200-fold selectivity over the other two opioid receptors and had improved polarity indexes (log*P* 3.1/log*D* 2.7/PSA 70). Alternatively, replacement of benzimidazole (**12**) with the slightly more polar imidazopyridine (**46**) led to little loss in potency (Table 2). Hybridizing the structural features of **43** and **46** into **47** provided the most polar compound (log*P* 1.6/log*D* 1.0/PSA 113). It showed an additional 10-fold drop in potency to KOR and the selectivity over DOR and MOR was significantly reduced.

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Table 3
Opioid receptor selectivity and physicochemical profile of selected compounds

Compound	KOR EC_{50} (nM)	DOR EC_{50} (nM)	MOR EC_{50} (nM)	log P[a]	log D[a]	tPSA[a]	M_NO[a]
3	0.5	8000	60,000	3.58	3.42	43.8	4
6	39	7800	39,000	3.75	2.83	50.2	5
10	33	1800	2400	3.79	2.90	50.2	5
12	7	2000	6100	4.10	3.10	50.2	5
15	72	5500	7800	3.29	2.45	73.9	6
30	7	1700	1800	4.67	3.70	50.2	5
32	70	100,000	100,000	2.87	2.05	63.1	6
37	22	5200	3300	4.40	3.47	50.2	5
38	122	100,000	100,000	3.72	2.95	63.1	6
39	8	2800	6800	2.66	1.83	65.9	6
42	1	12	255	3.36	2.45	69.9	6
43	31	72	3800	2.12	1.46	100.1	8
44	2	82	150	3.96	3.19	66.0	6
45	10	1850	1750	3.10	2.70	70.4	6
46	37	37,000	52,500	3.26	2.41	63.1	6
47	395	1150	34,500	1.55	1.00	113.0	9

[a] $S + \log P$, $S + \log D$ (pH 7.4), tPSA and number of nitrogen and oxygen were calculated with MedChem Designer(TM) version 2.0.0.34. Simulations Plus, Inc.

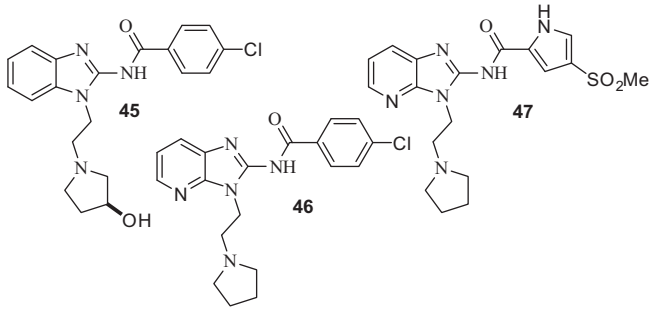

Figure 4. KOR agonists (**45**–**47**) with structural diversity.

Table 4
Pharmacokinetic profile[a] of compounds **3** and **42** in SAM[b]

Compound	Oral dose (mg/kg)	AUC_{0-t} (ng*h/ml)	C_{max} (ng/ml)	$t_{1/2}$ (h)
3	30	493	507	0.5
42	10	334	250	2

[a] AUC: area under the curve—a measure of the exposure to the drug; C_{max}: the maximum concentration recorded.
[b] Swiss albino mice.

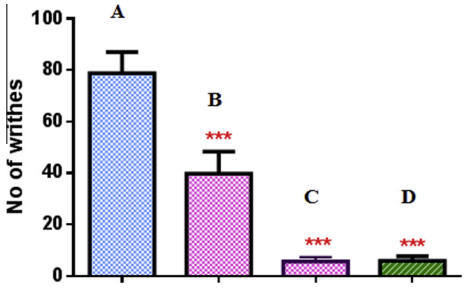

Figure 5. Anti-nociceptive effect of **42** (1 mg/kg-B and 10 mg/kg-C) and asimadoline (10 mg/kg-D) versus vehicle control animal (A) in mice acetic acid writhing model ($n = 12$).

As discussed above a number of highly potent KOR agonists (such as **12**, **30**, **39**, **42**, **44** and **45**) with low nanomolar potency and good selectivity over the other two opioid receptors have been identified. At this stage we were interested in evaluating the potential of this compound series by performing a few animal experiments. The compound **42** showed good PK profile in mice (Table 4) and was evaluated further to establish proof of concept in an acetic acid induced writhing model. On oral administration **42** showed dose dependent anti-nociceptive effect at 1 and 10 mg/kg doses as shown in Figure 5.[30,31]

In summary, the in silico screening approach led us to discover a benzimidazole derived novel series of potent KOR agonists which displayed good selectivity over the other opioid receptors DOR and MOR. Structural modifications helped to understand the initial SAR and identify regions for incorporating polar motifs. A representative molecule **42** showed significant anti-nociceptive effect in acetic acid induced mice writhing model. Further optimization of this series using the SAR information obtained will be disclosed elsewhere.

Acknowledgments

The authors thank the analytical chemists of Discovery Research for their support to generate spectral data of all compounds.

Supplementary data

Supplementary data (synthesis and characterization data of compounds and the details on the receptor models of ligands bound to the KOR, DOR and MOR receptors are provided) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.12.064.

References and notes

1. Minami, M.; Satoh, M. *Neurosci. Res.* **1995**, *23*, 121.
2. Satoh, M.; Minami, M. *Pharmacol. Ther.* **1995**, *68*, 343.
3. Millan, M. J. *Pain* **1986**, *27*, 303.
4. Martin, W. R. *Pharmacol. Rev.* **1983**, *35*, 283.
5. Martin, W. R.; Eades, C. G.; Thompson, J. A.; Huppler, R. E.; Gilbert, P. E. *J. Pharmacol. Exp. Ther.* **1976**, *197*, 517.
6. (a) Field, M. J.; Carnell, A. J.; Gonzalez, M. I.; McCleary, S.; Oles, R. J.; Smith, R.; Hughes, J.; Singh, L. *Pain* **1999**, *80*, 383; (b) Wilson, J. L.; Nayanar, V.; Walker, J. S. *Br. J. Pharmacol.* **1996**, *118*, 1754.
7. (a) Catheline, G.; Guilbaud, G.; Kayser, V. *Eur. J. Pharmacol.* **1998**, *357*, 171; (b) Walker, J.; Catheline, G.; Guilbaud, G.; Kayser, V. *Pain* **1999**, *83*, 509; (c) Przewlocki, R.; Przewlocka, B. *Eur. J. Pharmacol.* **2001**, *429*, 79.
8. (a) Delgado-Aros, S.; Chial, H. J.; Camilleri, M.; Szarka, L. A.; Weber, F. T.; Jacob, J.; Ferber, I.; McKinzie, S.; Burton, D. D.; Zinsmeister, A. R. *Am. J. Physiol.* **2002**, *284*, G558; (b) Kamp, E. H.; Carter, R.; Jones, W.; Tillman, S. R.; Gebhart, G. F. *Am. J. Physiol.* **2003**, *284*, G434.
9. (a) Endoh, T.; Tajima, A.; Suzuki, T.; Kamei, J.; Suzuki, T.; Narita, M.; Tseng, L.; Nagase, H. *Eur. J. Pharmacol.* **2000**, *387*, 133; (b) Spetea, M.; Rydelius, G.;

Nylander, I.; Ahmed, M.; Bileviciute-Ljungar, I.; Lundeberg, T.; Svensson, S.; Kreieberg, A. *Eur. J. Pharmacol.* **2002**, *435*, 245.
10. Frankowski, K. J.; Hedrick, M. P.; Gosalia, P.; Li, K.; Shi, S.; Whipple, D.; Ghosh, P.; Prisinzano, T. E.; Schoenen, F. J.; Su, Y.; Vasile, S.; Sergienko, E.; Gray, W.; Hariharan, S.; Milan, L.; Heynen-Genel, S.; Mangravita-Novo, A.; Vicchiarelli, M.; Smith, L. H.; Streicher, J. M.; Caron, M. G.; Barak, L. S.; Bohn, L. M.; Chung, T. D.; Aubé, J. *ACS Chem. Neurosci.* **2012**, *3*, 221.
11. For recent reviews on KOR agonists, see: (a) Aldrich, J. V.; McLaughlin, J. P. *AAPS J.* **2009**, *11*, 312; (b) Yamaotsu, N.; Hirono, S. *Top. Curr. Chem.* **2011**, *299*, 277; (c) Cunningham, C. W.; Rothman, R. B.; Prisinzano, T. E. *Pharmacol. Rev.* **2011**, *63*, 316.
12. KOR antagonists have potential as pharmacotherapies for the treatment of depression, anxiety, addictive disorders and other psychiatric conditions produced or exacerbated by stress. For a recent review on KOR antagonists, see: Urbano, M.; Guerrero, M.; Rosen, H.; Roberts, E. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2021.
13. (a) Pande, A.; Pyke, R. E.; Greiner, M.; Cooper, S. A.; Benjamin, R.; Peirce, M. W. *Clin. Neuropharmacol.* **1996**, *19*, 92; (b) Pande, A.; Pyke, R. E.; Greiner, M.; Wideman, G. L.; Benjamin, R.; Pierce, M. W. *Clin. Neuropharmacol.* **1996**, *19*, 451.
14. (a) Mangel, A. W.; Williams, V. S. *Expert Opin. Investig. Drugs* **2010**, *19*, 1257; (b) Mangel, A. W.; Hicks, G. A. *Clin. Exp. Gastroenterol.* **2012**, *5*, 1.
15. (a) Phan, N. Q.; Lotts, T.; Antal, A.; Bernhard, J. D.; Ständer, S. *Acta Derm. Venereol.* **2012**, *92*, 555; (b) Togashi, Y.; Umeuchi, H.; Okano, K.; Ando, N.; Yoshizawa, Y.; Honda, T.; Kawamura, K.; Endoh, T.; Utsumi, J.; Kamei, J.; Tanaka, T.; Nagase, H. *Eur. J. Pharmacol.* **2002**, *435*, 259; (c) Inui, S. *Expert Opin. Pharmacother.* **2012**, *13*, 1507.
16. (a) Frimurer, T. M.; Ulven, T.; Elling, C. E.; Gerlach, L. O.; Kostenis, E.; Högberg, T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3707; (b) Frimurer, T. M.; Högberg, T. *Curr. Top. Med. Chem.* **2011**, *11*, 1882.
17. Schwartz, T. W.; Gether, U.; Scambye, H. T.; Hjort, S. A. *Curr. Pharm. Des.* **1995**, *1*, 325.
18. Ballesteros, J. A.; Weinstein, H. *Methods Neurosci.* **1995**, *25*, 366.
19. Huixian, W.; Wacker, D.; Mileni, M.; Katritch, V.; Han, G. W.; Vardy, E.; Liu, W. *Nature* **2012**, *485*, 327.
20. Fenalti, G.; Giguere, P. M.; Katritch, V.; Huang, X. P.; Thompson, A. A.; Cherezov, V.; Roth, B. L.; Stevens, R. C. *Nature* **2014**, *506*, 191.
21. Manglik, A.; Kruse, A. C.; Kobilka, T. S.; Thian, F. S.; Mathiesen, J. M.; Sunahara, R. K.; Pardo, L.; Weis, W. I.; Kobilka, B. K.; Granier, S. *Nature* **2012**, *485*, 321.
22. Frimurer, T. M.; Bywater, R.; Naerum, L.; Lauritsen, L. N.; Brunak, S. *J. Chem. Inf. Comput. Sci.* **2000**, *40*, 1315.
23. Trinquet, E.; Bouhelal, R.; Dietz, M. *Expert Opin. Drug Discov.* **2011**, *6*, 981.
24. *HTRF IP1 assay:* The IP-One HTRFÒ technology has thoroughly been described in the CisBio assay kit manual. The production of intracellular IP1 will generate a competition between unlabeled IP1 and exogenously added d2-labled IP1 for terbium cryptate-labeled anti-IP1 antibodies.
25. *KOR, DOR or MOR IP1 assay protocol:* COS-7 cells were transiently transfected with the human KOR/DOR/MOR receptor cDNA. One day following transfection cells were seeded in ½-area 96 well plates (Corning Costar, #675083) with 40,000 cells/well in DMEM medium supplemented with 10% fetal calf serum, 2 mM glutamine and 0.01 mg/ml gentamicin. The following day, media was aspirated and 50 μl Stimulation buffer (10 mM HEPES, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 4.2 mM KCl, 146 mM NaCl, 5.5 mM glucose, 50 mM LiCl, 0.1% BSA, pH 7.4) were added to each well. Test compounds were dissolved in DMSO in various concentrations and 1 μl was added to each well to stimulate cells. Following an incubation of about 60 min at 37 °C, 10 μl IP1-d2 (Cisbio) and 10 μl anti IP1-Cryptate (Cisbio) were added to each well. Plates were incubated at about 20–35 °C for a minimum of 60 min and counted on HTRF compatible Alpha-Fusion (Packard). Determinations were made in duplicates. EC50 values were calculated using AssayExplorer 3.2 (Symyx), a standard pharmacological data handling software.
26. The prediction of the binding mode of compound **6** to the KOR was performed using 'Internal Coordinate Mechanics' (Molsoft ICM 3.7-3b, Molsoft L.L.C., San Diego, CA, USA).[32,33] The structure of the human κ-opioid receptor (PDB code: 4DJH, solved to 2.9 Å resolution) in complex with JDTic[22] was obtained from the RCSB protein data bank (PDB). Crystallization water, co-crystal molecules were deleted and the structure was converted into an ICM object and subjected to 300 steps of Cartesian minimization. The ICM molecule editor was used to generate a 3D low energy structure of compound **6**. For the purpose of four-dimensional docking,[32] a stack of 21 receptor conformations generated by normal mode perturbation was used for docking.
27. Nygaard, R.; Frimurer, T. M.; Holst, B.; Rosenkilde, M. M.; Schwartz, T. W. *Trends Pharmacol. Sci.* **2009**, *30*, 249.
28. (a) Cooper, M.; Receveur, J.-M.; Bjurling, E.; Nørregaard, P. K.; Nielsen, P. A.; Sköld, N.; Högberg, T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 26; (b) Receveur, J. M.; Murray, A.; Linget, J. M.; Nørregaard, P. K.; Cooper, M.; Bjurling, E.; Nielsen, P. A.; Högberg, T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 453.
29. Sasmal, P. K.; Reddy, D. S.; Talwar, R.; Venkatesham, B.; Balasubrahmanyam, D.; Kannan, M.; Srinivas, P.; Shiva Kumar, K.; Neelima Devi, B.; Jadhav, V. P.; Khan, S. K.; Mohan, P.; Chaudhury, H.; Bhuniya, D.; Iqbal, J.; Chakrabarti, R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 562.
30. (a) Eckhardt, E. T.; Cheplovitz, F.; Lipo, M.; Govier, W. M. *Proc. Soc. Exp. Biol. Med.* **1958**, *98*, 186; (b) Le Bars, D.; Gozariu, M.; Cadden, S. W. *Animal Pharmacol. Rev.* **2001**, *53*, 597.
31. *Protocol for efficacy studies:* Swiss Albino mice (SAM) were acclimatized for 3 days in the experimental room and randomized into several groups. Feed was removed 2–3 h before dosing with compounds. The nociceptive response (abdominal contractions or writhes) was induced by dilute acetic acid. Acetic acid (0.6%, 10 ml/kg) was injected intra peritoneally in lower right abdominal quadrant (26 ½ gauge needle, 1 ml syringe) 30 min after treatment with compounds orally. Each animal was placed in a cylindrical transparent Perspex chamber (diameter and height: 1 feet × 1 feet). Animals were allowed to acclimatize in the chamber for 5 min after acetic acid injection and number of writhes (constriction of abdomen with extension of hind limb/s) was counted for next 25 min. For statistical analysis, one way ANOVA followed by Dunnett's test was applied using Sigma Stat software. The antinociceptive activity was expressed as a percentage of inhibition of writhes and calculated as follows: antinociception (%) = (1 − (no. of writhings of treatment/no. of mean writhings of control))*100.
32. Bottegoni, G.; Kufareva, I.; Totrov, M.; Abagyan, R. *J. Med. Chem.* **2009**, *52*, 397.
33. Timothy, V.; Philip, A.; Mosier, D.; Westkaemper, R. B.; Roth, B. L. *J. Biol. Chem.* **2007**, *282*, 3146.