Bioorganic & Medicinal Chemistry Letters 22 (2012) 2200–2203

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

SAR and biological evaluation of 3-azabicyclo[3.1.0]hexane derivatives as μ opioid ligands

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ARTICLE INFO

Article history:
Received 19 December 2011
Revised 24 January 2012
Accepted 25 January 2012
Available online 2 February 2012

Keywords:
μ Opioid
Opioid

ABSTRACT

3-Azabicyclo[3.1.0]hexane compounds were designed as novel achiral μ opioid receptor ligands for the treatment of pruritus in dogs. In this paper, we describe the SAR of this class of opioid ligand, highlighting changes to the lead structure which led to compounds having picomolar binding affinity, selective for the μ receptor over δ and κ subtypes. Some subtleties of functional activity will also be described.

Pruritus is intense itch, often associated with flea allergy dermatitis and atopic dermatitis in dogs. Excessive scratching in response to itch can result in wounds then liable to secondary bacterial or fungal infection, causing further inflammation and itch sensation. Pruritus in dogs is a major cause for veterinary referral. Current therapies include corticosteroids, antihistamines, essential fatty acid dietary supplements and various emollients. However there is a continuing need for alternative improved treatments of pruritus. We desired a safe non steroidal agent which rapidly blocked the itch sensation, suitable for once or twice a day oral dosing.

μ-Opiate antagonists have been reported to be antipruritic.[1,2] Previously we have described the design and synthesis of a novel achiral class of potent μ opioid receptor antagonists, such as **1** (Fig. 1) which produced rapid and dramatic reduction in pruritic behaviour in dogs with flea allergy dermatitis.[3]

Compound **1** was shown to be an antagonist with a pA2 of 7.97 against μ in an isolated guinea-pig myenteric plexus-longitudinal preparation. In the mouse tail flick model at 10 mg/kg *s.c.* **1** showed antagonism of morphine induced anti-nociception.[3]

The key element of the design in this class was the 'trans' relationship across the cyclopropyl ring between the phenyl and the fused five-membered rings to lock the molecule with the phenyl ring into the 'equatorial' position (Fig. 2). Unlike the phenylpiperidine class of opioid ligands, there is no longer required further piperidine substitution to favour the phenyl equatorial conformer, thus allowing a symmetrical achiral series which demonstrated full opioid antagonist pharmacology.

As the project developed, we wanted to identify an agent in the series which had, among other desired properties, increased potency and ideally subtype selectivity for the dog μ opioid receptor. We still wanted an achiral compound with low potential cost of goods. Several hundred library and singleton compounds were prepared which varied in three key regions; 'Head', 'Quaternary

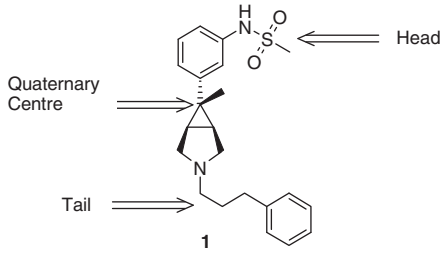

Figure 1. Example of 3-azabicyclo[3.1.0]hexane class of μ opioid receptor antagonists.

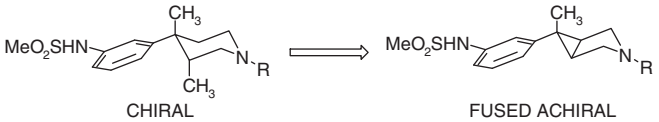

Figure 2. Design of locked geometry in 3-azabicyclo[3.1.0]hexane core.

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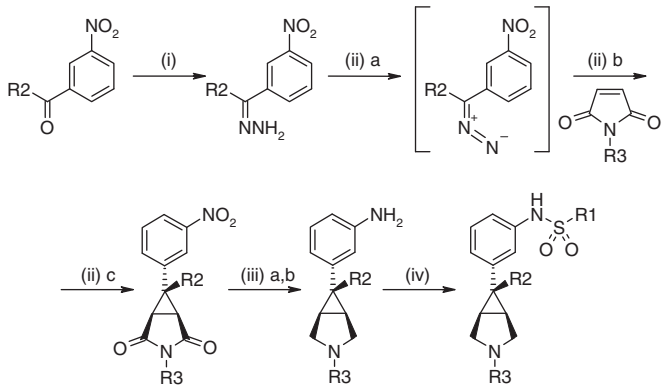
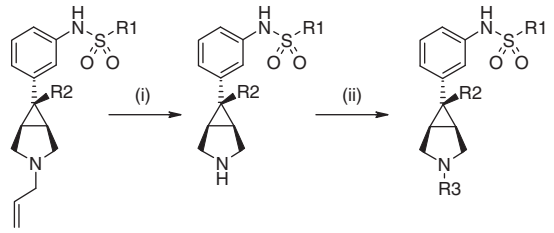
doi:10.1016/j.bmcl.2012.01.099



Scheme 1. Reagents and conditions: (i) H_2NNH_2, industrial methylated spirit, water, reflux; (ii) (a) MnO_2, dioxane, 20 °C; (b) dioxane, 20 °C; (c) reflux; (iii) (a) Fe, $CaCl_2$, EtOH, water, reflux; (b) $LiAlH_4$, THF, −15 °C; (iv) DCM, NEt_3, $R1SO_2Cl$.



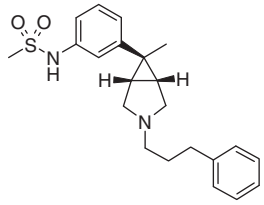
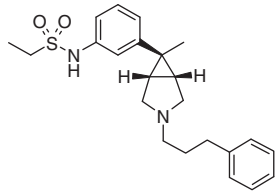
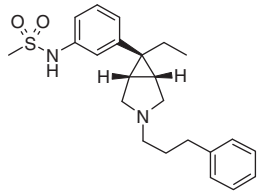
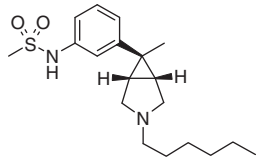
Scheme 2. Reagents and conditions: (i) 1,3-dimethylbarbituric acid, $Pd(PPh_3)_4$, DCM, reflux; (ii) (a) alkyl halide or (b) amidation then reduction.

centre' and 'Tail' denoted in Figure 1. These included a range of sulfonamides, heterocyclic bioisosteric replacements of the sulfonamide, various substituents at the quaternary centre and many 'tail' variations.[4,5] The core template enabled significant SAR variation with a large majority of compounds acting as antagonists. In this communication, we will outline a small selection of some of the opioid receptor binding structure–activity relationships in this series leading to compounds with picomolar activities, and highlight some functional subtleties that emerged during our investigations.

Compounds were made according to the general reaction Schemes 1 and 2. The chemistry utilised to vary the 'quaternary centre' and the 'head' (Scheme 1) followed that previously described.[3–5] Further variation at the 'tail' was afforded via removal of an allyl protecting group to reveal versatile intermediate amines, which were elaborated with alkylation or acylation/reduction procedures (Scheme 2).

Compounds were tested in dog μ, κ and δ opioid receptor binding assays (Table1).[6] In general, variation on the head group from initial example **1** did little to improve binding potency, with the methyl sulfonamide being overall optimum. On the whole at the quaternary position, ethyl and then methyl were optimum, with ethyl being in most cases superior as exemplified by pairs **1** versus **3** and **12** versus **13**. Where the addition of extra lipophilic binding made the greatest impact was with a methyleneindane substituent as the tail, for example, examples **12**, **13** and **15** with significant increase in lipophilic efficiency (LipE), a measure of lipophilicity per unit of in vitro potency.[7] The increase in LipE seen in these examples due to optimally placed lipophilicity could be explained in part due to conformational constraint of the 'tail group' whereby

Table 1
Dog μ, κ and δ opioid receptor binding for compounds **1–15**

Compound	Structure	Dog μ binding K_i, nM[a]	Dog κ binding K_i, nM[a]	Dog δ binding K_i, nM[a]	% Decrease of twitch tension @ 1 μ M[c]	Human μ binding K_i, nM[a,b]	μ antagonist GTP-γ S K_i, nM[a]	*c*Log*P*	LE[d]	LipE[e]
1		4.1	94	183	<10	4.8	3.5	3.6	0.39	4.1
2		37	219	2900	—	—	—	4.1	0.36	3.3
3		1.5	67	79	<10	—	—	4.1	0.4	4.1
4		14	270	869	<10	—	—	4.0	0.44	3.8

(*continued on next page*)

Table 1 (*continued*)

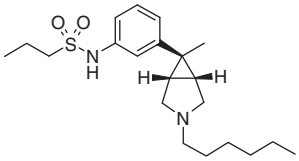
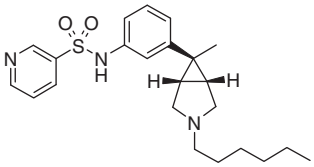
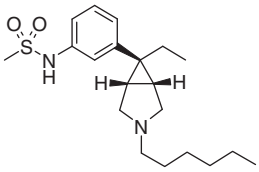
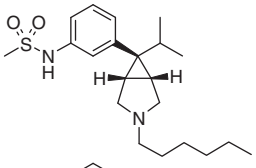
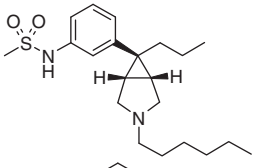
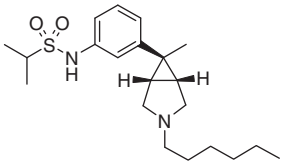
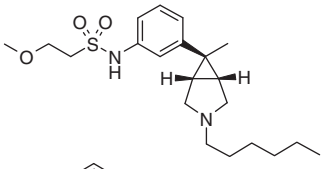
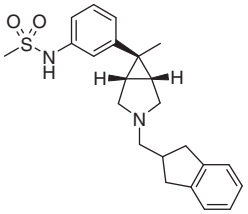
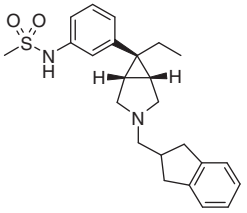
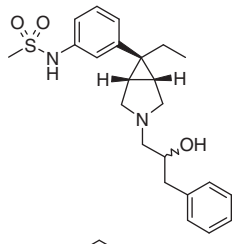
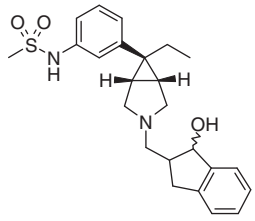
Compound	Structure	Dog μ binding K_i, nM[a]	Dog κ binding K_i, nM[a]	Dog δ binding K_i, nM[a]	% Decrease of twitch tension @ 1 μ M[c]	Human μ binding K_i, nM[a,b]	μ antagonist GTP-γ S K_i, nM[a]	*c*Log *P*	LE[d]	LipE[e]
5		104	1330	>10,000	—	—	—	5.0	0.37	2.0
6		71	1530	>10,000	—	—	—	5.1	0.34	2.0
7		50	1340	>10,000	—	—	—	4.2	0.40	2.8
8		3	48	308	32	—	—	4.9	0.45	3.6
9		10	328	66	<10	—	—	5.0	0.42	3.0
10		14	791	1580	18	—	—	4.8	0.41	3.1
11		10	172	464	>10	—	—	4.1	0.41	3.9
12		0.55	51	384	<10	0.81	0.3	3.6	0.44	5.4
13		0.2	22	29	—	—	—	4.1	0.45	5.5

Table 1 (*continued*)

Compound	Structure	Dog μ binding K_i, nM[a]	Dog κ binding K_i, nM[a]	Dog δ binding K_i, nM[a]	% Decrease of twitch tension @ 1 μ M[c]	Human μ binding K_i, nM[a,b]	μ antagonist GTP-γ S K_i, nM[a]	*c*Log*P*	LE[d]	LipE[e]
14		2.6	70	595	—	6.6	2.5	3.0	0.40	5.6
15		0.07	4.5	19	—	0.1	0.08	2.5	0.46	7.7

[a] Values are means of two or more experiments.
[b] See Ref. 9 for details.
[c] Human binding K_i determined from [^{3}H]DAMGO filter binding assay.
[d] LE = Ligand efficiency (from dog μ binding).
[e] LipE calculated from *c*Log*P* and dog μ binding data (LipE = pK_i − *c*Log*P*).

the methyleneindane has fewer rotational bonds than the *n*-propylbenzene.

As a strategy to minimize *c*Log*P* for the benefit of overall physicochemical properties, it was pleasing that a hydroxyl on the phenpropyl tail chain **14**, and on the methyleneindane **15** were well tolerated and indeed provided a compound **15** with picomolar activity, improved LipE and slightly improved ligand efficiency (LE).[8] In terms of subtype selectivity, few significant improvements were seen across the examples made, however the selectivity seen in **1** was maintained.

To confirm functional activity, some of the compounds were tested further in the isolated guinea-pig myenteric plexus-longitudinal electrical field stimulation (EFS) preparation or in a μ antagonist GTP-γ S binding assay.[9] In the EFS functional assay, compounds were administered first to the tissue, followed by a cumulative full dose response with the μ receptor agonist DAMGO. A decrease of greater than 10% of twitch tension, caused by the effect of the compound alone at concentration of 1 μM, was considered as 'possible agonist like activity' (full agonist DAMGO gave 100% reduction). The large majority of compounds tested in this 3-azabicyclo[3.1.0]hexane series showed no agonist activity (<10%). However in the project there were a few rare examples of where, as the molecules grew in size in particular locations, a hint of 'possible agonist like activity' was observed, such as examples **8**, **10** and **11**. As such, because of concern of abuse potential with full or even potential weak partial agonists, such compounds progressed no further in our studies. Compounds **12**, **14** and **15** behaved as antagonists in the human antagonist GTP γS assay and showed potency similar to that seen in the other binding assays.

In summary the SAR of some analogues in an achiral 3-azabicyclo[3.1.0]hexane series of μ opioid receptor ligands has been described. Substituents at the basic nitrogen in particular the methyleneindane scaffold, as exemplified by compounds **13** and **15**, provided binding improvements giving picomolar activity. Significantly, this was achieved by a big increase in LipE for compound **15**. Further exploration of this SAR in this series was then exploited to provide a compound for clinical evaluation.[10]

Acknowledgments

The authors thank Adrian Foster, Steve Gibson, Bill Howson, Richard Armer and Jinee Patel for their contributions to this work.

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9. Guinea-pig myenteric plexus-longitudinal muscle strips were prepared following the procedure from Henderson, G.; Hughes, J.; Kosterlitz, H. W. *Br. J. Pharmacol.* **1975**, *53*, 505, attached to an isometric transducer and an electrode. The tissues were then placed under a 1 g tension and left to equilibrate for 1 h, washed with Kreb's solution every 15 min, maintained at 37 °C throughout with constant supply of 95% O_2/5% CO_2. After equilibration, the tissues were rebalanced and then electrically stimulated using frequency of 0.1 Hz and 1 ms pulse width. When all the tissues were stabilised, supramaximal voltage was determined. This voltage was used for the rest of the experiment. The tissues were then washed and left for 20 min. 1 μM of the kappa antagonist nor-Binaltorphimine was then added and left for 10 min. 1 μM of captopril, a peptidase inhibitor, was added to prevent the breakdown of DAMGO. When the twitches plateaued, a dose range of test compound was added. After a further 10 min, a cumulative dose response was constructed for the mu agonist DAMGO (each concentration 3 min contact time with 1 min recording). pA2 for 1 = 7.97 (±0.26 SD) based on $n = 3$).
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