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Discovery and synthesis of a new class of opioid ligand having a 3-azabicyclo[3.1.0]hexane core. An example of a 'magic methyl' giving a 35-fold improvement in binding

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ABSTRACT

In looking for a novel achiral μ opioid receptor antagonist for the treatment of pruritus, we designed and synthesised azabicyclo[3.1.0]hexane compounds as a new class of opioid ligand. During optimisation, an addition of a single methyl resulted in a 35-fold improvement in binding. An early example from the series had excellent μ opioid receptor antagonist antagonist activity and was very effective in an in vivo pruritus study.

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 nonsteroidal 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] We had demonstrated that examples from our lead 4-phenylpiperidine opioid series such as **1** (Fig. 1) produce rapid and dramatic reduction in pruritic behaviour in dogs with flea allergy dermatitis.[3]

As the treatment was indicated for the veterinary market, low cost of compound manufacture was highly desirable. Due to a lengthy synthesis of **1** the projected cost of manufacture was high. Therefore we desired a novel more potent μ-selective ligand, preferably achiral with a simpler more cost effective synthesis. The compound would need to have no opiate agonism pharmacology to avoid any abuse potential.

Our design was influenced by literature which discussed the 4-phenylpiperidine μ opioid ligand series pharmacology.[4,5] The evidence presented suggested that if the phenyl ring adopted an axial position in a piperidine chair conformer then agonist pharmacology was observed, however if the phenyl ring adopted and preferred an equatorial position, then the compound acted as an antagonist. The *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine template is described as having this phenyl equatorial preferred conformation. This lower energy equatorial conformation was driven by the *trans* dimethyl substituents at the 3 and 4 positions. Deletion of the '3 methyl' resulted in compounds with a weak preference for phenyl axial conformation and some agonist pharmacology.

We designed 3-azabicyclo[3.1.0]hexane targets (Fig. 2) with a '*trans*' relationship across the cyclopropyl ring between the phenyl ring and the fused five-membered ring to lock the molecule in the proposed 'equatorial antagonist' geometry. This enabled deletion of the methyl corresponding to the 3 substituent in the piperidine series to produce a symmetrical achiral compound.

Two synthetic strategies were adopted to access the substituted 3-azabicyclo[3.1.0]hexane templates; both paying careful attention to the desired fused ring geometry. The first was intended to enable variation on the basic nitrogen as late as possible (Scheme 1).

The key step (i) forming the cyclopropane ring was a rhodium(II)acetate catalysed reaction of ethyldiazoacetate with the appropriate *trans* cinnamyl halide. Yields with the chlorides were

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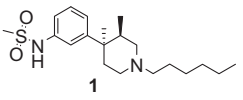


Figure 1. 4-Phenyl piperidine series lead μ opioid receptor antagonist.

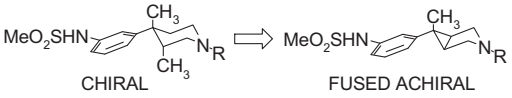


Figure 2. Design of locked geometry in 3-azabicyclo[3.1.0]hexane core.

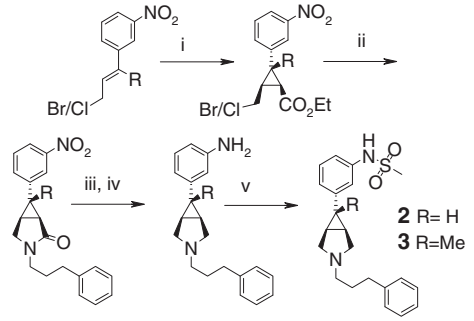
significantly higher than the bromides.[6] This carbenoid addition gave the (racemic) major product cyclopropane with desired stereochemistry[7]: that is, with 'retention' of the starting cinnamyl olefin geometry, and the ethylcarboxylate *trans* to the bulkier aromatic ring. Ring closure with 3-phenylpropylamine formed the racemic lactam. Reduction then sulfonylation afforded the final target compounds **2** and **3**.

This route was not particularly amenable to large scale up due to the potentially hazardous ethyldiazoacetate step so the second synthetic strategy (Scheme 2) was adopted and developed for bulk scale up. This route forms the key cyclopropane ring of the bicycle by the thermal extrusion of nitrogen from a pyrazoline intermediate.

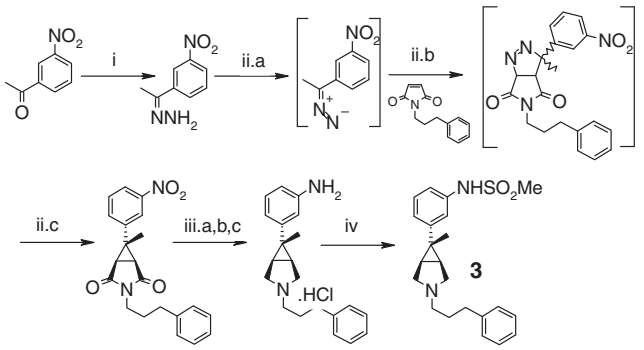
1-(3-Nitrophenyl)-1-ethanone hydrazone is oxidised with manganese dioxide to the diazo intermediate; this undergoes a 3+2 cycloaddition with 1-(3-phenylpropyl)-1*H*-pyrrole-2,4-dione to form a pyrazoline intermediate; thermolysis results in an diradical closure to form the cyclopropane ring with excellent selectivity for the desired geometry—the bulkier phenyl *trans* to the succinimide ring. Reduction then sulfonylation afforded **3** in multigram quantities.

Using this route the projected cost of bulk synthesis of **3** was a quarter of that projected for **1**. Furthermore later this route was developed further enabling several versatile late stage intermediates suitable for making analogues in parallel.[8] Compound **2** has also been resynthesised using this methodology.

Both **2** and **3** were potent μ-selective ligands (Table 1).[9,10] **2** was not potent enough at the time to warrant any further investigation. Pleasingly **3** confirmed as a full opiate receptor antagonist with a pA_2 of 7.97 against μ in an isolated guinea-pig myenteric plexus-longitudinal experiment preparation.[11] In the mouse tail flick model[12] at 10 mg/kg *s.c.* **3** showed antagonism of morphine induced



Scheme 1. First synthetic strategy to key 3-azabicyclo[3.1.0] targets **2** and **3**. Reagents and conditions: (i) $N_2CH_2CO_2Et$, DCM, $Rh_2(OAc)_4$, 20 °C; (ii) 3-phenylpropylamine DMF, $NaHCO_3$, 150 °C; (iii) $LiAlH_4$ in THF, reflux; (iv) Raney Ni, hydrogen (1 atm), EtOH, 20 °C; (v) MsCl, DCM, pyridine, 0–20 °C.



Scheme 2. Second synthetic strategy to key 3-azabicyclo[3.1.0] target **3**. Reagents and conditions: (i) H_2NNH_2, IMS, water, reflux, 82%, (ii.a) MnO_2, dioxan, 20 °C, (ii.b) dioxan 20 °C, (ii.c) reflux, 65%; (iii.a) $NaBH_4$, $BF_3{\cdot}O(Et)_2$, EtOAc, 0–65 °C, (iii.b) 5% Pd/C, hydrogen (1 atm), EtOAc, 20 °C, (iii.c) HCl, 90%; (iv) MsCl.

Table 1
Human and dog μ receptor binding for compounds **2** and **3**, with binding for dog κ and δ opiate receptor

Compds	Human μ binding K_i[a] (nM)	Dog μ binding K_i[a] (nM)	Dog κ binding K_i[a] (nM)	Dog δ binding K_i[a] (nM)
2	168 (±57.9)	379[b]	1576[b]	>10000[b]
3	4.79 (±1.92)	4.1 (±2.3)	94 (±35)	183 (±103)

[a] Values are means of five or more experiments, standard deviation is given in parentheses.
[b] Mean of two experiments.

anti-nociception. This indeed demonstrated that this novel achiral series could offer potent μ antagonist pharmacology.

What was quite remarkable in the comparison between compounds **2** and **3**, was that in making a single change from a hydrogen to a methyl, a binding affinity improvement of 35 was observed in the human μ receptor—a truly magic methyl, and a greater than 35-fold difference was observed in the μ and δ dog receptors (note $n = 2$ for **2** in the dog binding assays). A methyl group with its lipophilic surface well buried into a protein hydrophobic pocket is 'expected' to provide up to 1.5 kcal mol^{-1} binding energy and so give up to a 10-fold improvement in binding energy.[13] In this case, comparing **2** with **3** something significantly extra was occurring. We postulated that the added methyl impacted the rotational freedom of the phenyl ring at the 6 position on the 3-azabicylo[3.1.0] core, giving rise to a preferred lower energy tortional angle, thus reducing rotational entropic loss on binding. To understand this further we modelled the energy profiles of these systems with the rotation of this phenyl ring.[14] The flexible phenpropyl chain could adopt a variety of conformations. In the modelling this was truncated to methyl so as to focus on the energy differences due to the rotation of the phenyl ring. The results were very interesting, and showed a distinct energy profile difference between the compounds (Fig. 3).

As τ varies the difference between the maximum and minimum energy where R = H is 1.1 kcal mol^{-1}, suggesting unrestricted rotation of this aromatic ring. However for R = Me this range is 3.99 kcal mol^{-1}, giving a higher barrier to rotation. For the range of angles 45°–135° and 210°–315° the relative difference of R = Me to R = H is up to 2.5 kcal mol^{-1}. If the tortional angle required for receptor binding exists in one of these regions, then with such relative energy differences it seems reasonable to propose that the methyl group does contribute to binding by influencing the population of rotational isomers as well as lipophilic binding. While tempting to postulate that the angle of τ in binding at the

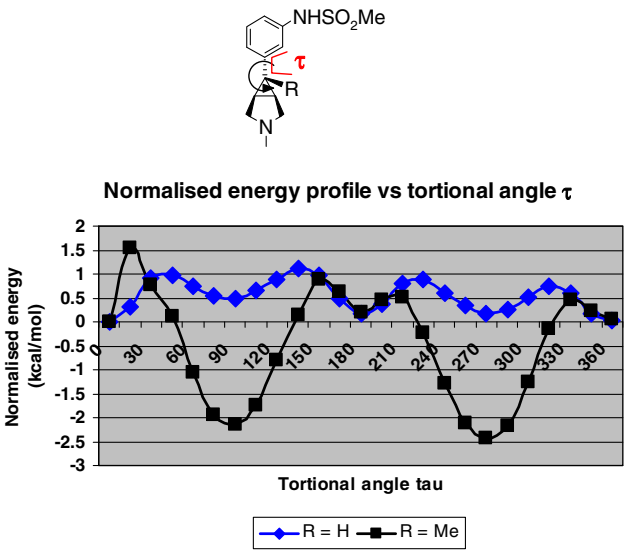


Figure 3. Normalised comparison of lowest energy state profile with variation in τ between model compounds.

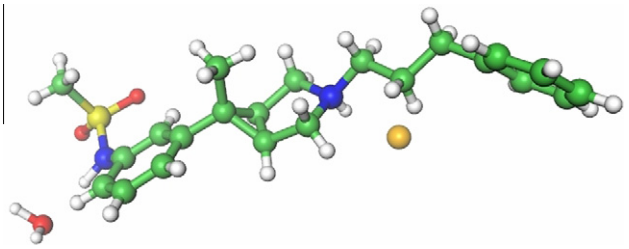


Figure 4. X-ray crystal structure of HCl salt monohydrate of **3**.

receptor is close to that of the phenol ring in classical 'rigid' ligands such as morphine or naltrexone, one truly cannot be sure.

The structure of **3** was further confirmed by X-ray crystallography of the hydrochloride salt.[15] (Fig. 4) It is interesting to note that for the crystal structure τ = 103°.

Further NMR studies on various salts were unable to reveal any clear stereo isomeric differences at the charged nitrogen atom between compounds **2** and **3**.

On further profiling **3** was negative in the Ames and CHO micronucleus genetic toxicology in vitro assays. Its pharmacokinetic profile in rat and dog showed it suitable for oral dosing. With a dose of 2 mg/kg po **3** produced a rapid and dramatic reduction in pruritic behaviour in dogs with flea allergy dermatitis which lasted for 12 hours (Fig. 5).[16]

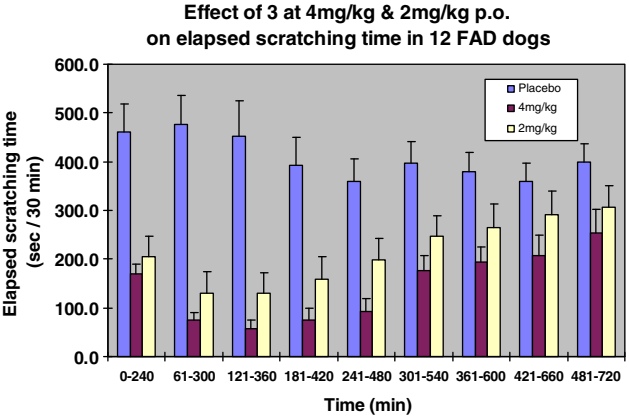


Figure 5. Antipruritic activity of **3** in dogs with flea allergy dermatitis. Showing mean of 12 FAD dogs ± SEM.

Compound **3** was mainly metabolized by the dog CYP2D15 enzyme acting as both substrate but unfortunately inhibitor. On dose elevation **3** showed nonlinear pharmacokinetics and did not show a sufficient safety margin over side effects to warrant full development.

In summary a novel achiral 3-azabicyclo[3.1.0]hexane series of μ opioid receptor ligands has been described. This series was designed to be locked in a defined geometry, so that chiral substituents were not necessary to provide a clean opiate antagonist profile. The difference in binding between analogues with hydrogen and methyl at the 6 position was 35-fold. We propose this is due to lipophilic binding and the influence over the rotation of the 6 position phenyl ring. The SAR in this series will be detailed further.[8]

Acknowledgments

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References and notes

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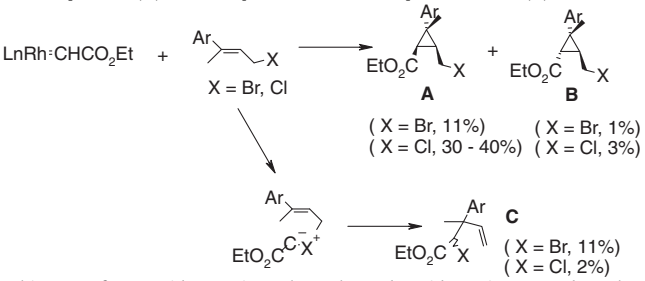
6. First attempts with cinnamyl bromides gave poor isolated yields (11%) of desired product (A) and an equal amount of side product olefin (C).



This arose from a side reaction where the carbenoid species reacted on the bromine to form an unwanted ylide intermediate (see Doyle, M. P.; Tamblyn, W. H.; Bagheri, V. *J. Org. Chem.* **1981**, *46*, 5094), which underwent a 2,3-sigmatropic rearrangement giving (C). This problem was circumvented by using the cinnamyl chloride analogue. Chlorine being less nucleophilic than bromine did not form the ylide intermediate so readily, resulting in higher yield, 40% of (A) with only 3% (C). Only traces of the non-desired isomer (B) were detected and easily removed by column chromatography.
7. For all intermediates in both synthetic routes, the coupling constants of the protons in the cyclopropyl ring were entirely consistent with the desired stereochemistry. For example, where R = H the coupling constant for this with the neighbouring '*trans*' protons *trans* in the cyclopropyl ring were always below 4.5 Hz; In **3** J = 3.2 Hz. Furthermore, 2D ROESY data further confirms relative stereochemistry.
8. Manuscript in preparation.
9. Dog receptor binding protocols for μ, κ and δ described in Banks, B. J.; Crook, R. J.; Gibson, S. P.; Lunn, G.; Pettman, A. J. *PCT. Int. Appl.*, WO20039089, 1999.
10. Human binding K_i determined from [3H]DAMGO filter binding assay.
11. 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 1msec 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 compound **3** was added. After a further 10 min, a cumulative dose response was constructed for the μ agonist DAMGO (each concentration 3 min contact time with 1 min recording). pA2 for **3** = 7.97 (±0.26 std. dev) based on $n = 3$.

12. Seven male ICR mice (17–19 g) were injected with vehicle (5% DMSO:5% Emulphur :90% Saline) or test compound (*s.c.*). Fifteen minutes later, each was injected with vehicle (saline) or morphine sulfate (3.2 mg/kg: *s.c.*). Thirty minutes later analgesia was assessed via tail flick. Latency to remove tail from a heated light source was recorded in seconds (12 s max). Data are presented as a % reversal from Veh/Morp [(Test compound mean − Vehicle/Morphine)/(Vehicle/Morphine − Vehicle/Vehicle)]. Compound **3** gave 89% at 10 mg/kg *s.c.*
13. Kuntz, I. D.; Chen, K.; Sharp, K. A.; Kollmann, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9997.
14. Energies were calculated using implicit models for solvent and quantum mechanics; the self-consistent reaction field (SCRF) model using B3LYP/6–31G** as implemented in Jaguar7.5 release 207. The SCRF method is able to model both aqueous and nonaqueous solvents, which permits the calculation of both gas to water and gas to octanol solvation energies. Jaguar 7.5 release 207 Schrodinger: Portland, OR, 2008. See: http://www.schrodinger.com/.
15. Cambridge Crystallographic Data Centre deposition number CCDC 819853.
16. A colony of mixed breed flea allergic dogs was challenged with fleas. Pruritic behaviour was recorded on video tape and evaluated by trained observers. Duration of pruritic activity is determined by measuring time spent in actual pruritic behaviour (scratching, rubbing, and licking) and is recorded as elapsed time.