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Structurally novel histamine H_3 receptor antagonists GSK207040 and GSK334429 improve scopolamine-induced memory impairment and capsaicin-induced secondary allodynia in rats

Andrew D. Medhurst [a,*], *Michael A. Briggs* [a], *Gordon Bruton* [a], *Andrew R. Calver* [a], *Iain Chessell* [a], *Barry Crook* [a], *John B. Davis* [a], *Robert P. Davis* [a], *Andrew G. Foley* [b], *Teresa Heslop* [a], *Warren D. Hirst* [a,1], *Stephen J. Medhurst* [a], *Sandrine Ociepka* [a], *Alison Ray* [a], *Ciaran M. Regan* [b], *Becky Sargent* [a], *Joanne Schogger* [a], *Tania O. Stean* [a], *Brenda K. Trail* [a], *Neil Upton* [a], *Trevor White* [a], *Barry Orlek* [a], *David M. Wilson* [a]

[a] *Neurology and GI Centre of Excellence for Drug Discovery, GlaxoSmithKline, Third Avenue, Harlow, Essex CM19 5AW, UK*
[b] *Berand Limited, UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland*

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ABSTRACT

GSK207040 (5-[(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)oxy]-*N*-methyl-2-pyrazinecarboxamide) and GSK334429 (1-(1-methylethyl)-4-({1-[6-(trifluoromethyl)-3-pyridinyl]-4-piperidinyl}carbonyl)hexahydro-1*H*-1,4-diazepine) are novel and selective non-imidazole histamine H_3 receptor antagonists from distinct chemical series with high affinity for human ($pK_i = 9.67 \pm 0.06$ and 9.49 ± 0.09, respectively) and rat ($pK_i = 9.08 \pm 0.16$ and 9.12 ± 0.14, respectively) H_3 receptors expressed in cerebral cortex. At the human recombinant H_3 receptor, GSK207040 and GSK334429 were potent functional antagonists ($pA_2 = 9.26 \pm 0.04$ and 8.84 ± 0.04, respectively versus H_3 agonist-induced changes in cAMP) and exhibited inverse agonist properties ($pIC_{50} = 9.20 \pm 0.36$ and 8.59 ± 0.04 versus basal GTPγS binding). Following oral administration, GSK207040 and GSK334429 potently inhibited cortical ex vivo [^{3}H]-*R*-α-methylhistamine binding ($ED_{50} = 0.03$ and 0.35 mg/kg, respectively). Functional antagonism of central H_3 receptors was demonstrated by blockade of *R*-α-methylhistamine-induced dipsogenia in rats ($ID_{50} = 0.02$ and 0.11 mg/kg p.o. for GSK207040 and GSK334429, respectively). In more pathophysiologically relevant pharmacodynamic models, GSK207040 (0.1, 0.3, 1 and 3 mg/kg p.o.) and GSK334429 (0.3, 1 and 3 mg/kg p.o.) significantly reversed amnesia induced by the cholinergic antagonist scopolamine in a passive avoidance paradigm. In addition, GSK207040 (0.1, 0.3 and 1 mg/kg p.o.) and GSK334429 (3 and 10 mg/kg p.o.) significantly reversed capsaicin-induced reductions in paw withdrawal threshold, suggesting for the first time that blockade of H_3 receptors may be able to reduce tactile allodynia. Novel H_3 receptor antagonists such as GSK207040 and GSK334429 may therefore have therapeutic potential not only in dementia but also in neuropathic pain.

* *Corresponding author*. Tel.: +44 1279 622004; fax: +44 1279 622555.
E-mail address: andy.medhurst@gsk.com (A.D. Medhurst).

[1] Current address: Discovery Neuroscience, Wyeth Research, Princeton, NJ 08543, USA.

Abbreviations: H_3 receptor, histamine H_3 receptor; PWT, paw withdrawal threshold

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1. Introduction

Histamine mediates its diverse biological effects via four histamine receptor subtypes designated H_1, H_2, H_3 and H_4 [1]. H_3 receptors are predominantly expressed in brain areas such as cerebral cortex, hippocampus, basal ganglia, thalamus and hypothalamus, as well as in spinal cord and peripheral ganglia [2–5]. These G-protein coupled receptors were first described as H_3 autoreceptors with their activation resulting in the inhibition of histamine synthesis and release from histaminergic neurons [6,7]. Subsequently, H_3 receptors were also shown to act as heteroreceptors with their stimulation leading to the inhibition of release of other neurotransmitters such acetylcholine, noradrenaline, dopamine and 5-HT from non-histaminergic neurons [8–10]. In contrast, blockade of H_3 receptors with selective antagonists can increase the release of several neurotransmitters including acetylcholine, histamine, noradrenaline, and dopamine [11–13].

Given their modulatory effects on multiple neurotransmitter systems, selective H_3 antagonists may have a number of potential therapeutic applications. For example, H_3 antagonists have been shown to improve performance in a number of rodent cognition models including object recognition, olfactory recognition, water maze, radial maze and passive avoidance [13–18], suggesting that they may be useful for the treatment of cognitive deficits in conditions such as Alzheimer's disease, other dementias, mild cognitive impairment and schizophrenia [19]. H_3 antagonists can also increase wakefulness in preclinical species, consistent with the pivotal role of histamine in the sleep-wake cycle [10], and indicative of a potential use in disorders of wakefulness and attention such as narcolepsy and attention deficit hyperactivity disorder [19]. Preclinical data also supports the potential use of H_3 antagonists in other disease indications including obesity [20].

Despite the discovery of the first H_3 antagonist nearly 20 years ago, clinical development of this class of agent has yet to be fully realised. First generation imidazole-based molecules such as thioperamide [7], clobenpropit [21,22] and ciproxifan [23] proved to be undevelopable for clinical use due to species differences in pharmacology, metabolic issues, lack of selectivity or poor brain penetration. More recently, following the molecular cloning of the H_3 receptor [24], several series of non-imidazole H_3 receptor antagonists have been developed [25–28], with examples such as ABT-239 [13,29], A-349821 [30], A-304121 and A-317920 [31,32] showing pro-cognitive effects in a number of rodent models. Other non-imidazole compounds including JNJ-5207852, NNC 38-1049 and SCH 79687 have been shown to exert either wake promoting effects [33], anti-obesity properties [20] and nasal decongestion in combination with H_1 antagonists [34], respectively. A number of non-imidazole H_3 antagonists are currently in clinical trials for various therapeutic indications, but to date no efficacy data has been reported in either healthy volunteers or patient populations [27].

A putative role for H_3 receptors in pain processes has recently been suggested, although data to support this is limited and somewhat conflicting [35]. Thioperamide was shown to produce an antinociceptive effect in a mouse hot plate test whilst the H_3 receptor agonist imetit caused allodynia [36]. In contrast, thioperamide reduced the analgesic effects of morphine whilst the H_3 agonist *R*-α-methylhistamine potentiated them [37]. Given the lack of selectivity of thioperamide, studies with more selective H_3 antagonists seems warranted to further understand the involvement of H_3 receptors in pain pathways, particularly since other agents that increase monoamine neurotransmitters (e.g. uptake blockers duloxetine and venlafaxine) are effective in neuropathic pain [38].

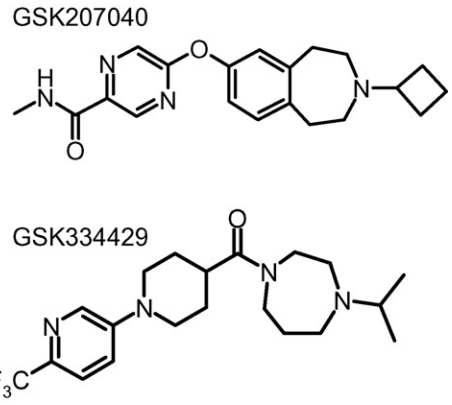

Fig. 1 – Chemical structures of GSK207040 (5-[(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)oxy]-*N*-methyl-2-pyrazinecarboxamide) and GSK334429 (1-(1-methylethyl)-4-({1-[6-(trifluoromethyl)-3-pyridinyl]-4-piperidinyl}carbonyl)hexahydro-1*H*-1,4-diazepine).

In the current study, we describe the in vitro and in vivo pharmacological properties of GSK207040 (5-[(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)oxy]-*N*-methyl-2-pyrazinecarboxamide) [39] and GSK334429 (1-(1-methylethyl)-4-({1-[6-(trifluoromethyl)-3-pyridinyl]-4-piperidinyl}carbonyl)-hexahydro-1*H*-1,4-diazepine) [40], exemplars from two novel series of highly potent and selective non-imidazole H_3 receptor antagonists whose chemical structures are shown in Fig. 1. In particular, the potential efficacy of these novel H_3 antagonists was explored in rodent models of cognition and pain.

2. Materials and methods

2.1. Animals

All experimental procedures involving animals (except passive avoidance) were conducted in compliance with the Home Office Guidance on the operation of the Animals (Scientific Procedures) Act 1986 under the authority granted in personal and project licenses, and was reviewed and approved by the GlaxoSmithKline Procedures Review Panel. Passive avoidance studies were approved by the Animal Research Ethics Committee of University College Dublin and carried out under licence issued by the Irish Department of Health.

2.2. Drugs

GSK207040 (5-[(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)oxy]-*N*-methyl-2-pyrazinecarboxamide) and GSK334429 (1-(1-methylethyl)-4-({1-[6-(trifluoromethyl)-3-

pyridinyl]-4-piperidinyl}carbonyl)hexahydro-1*H*-1,4-diazepine) were synthesised at GlaxoSmithKline, Harlow, UK. *R*-α-methylhistamine, imetit, thioperamide and clobenpropit were obtained from Tocris Bioscience. All tissue culture media, supplements and other chemicals were purchased from Invitrogen Life Technologies or Sigma Chemicals unless otherwise stated.

2.3. H_3 receptor cloning and preparation of membranes

H_3 receptors were cloned by polymerase chain reaction from human, rat and mouse brain cDNAs. Translated protein sequences from these clones were identical to previously published sequences for human [41], rat [42] and mouse [43] H_3 receptors.

Human embryonic kidney 293 cells stably expressing the macrophage scavenger receptor class II (HEK-293-MSR-II) were maintained in minimum essential medium (MEM) supplemented with Earle's salts, 2 mM L-glutamine, 1% non-essential amino acids, 400 μg/ml geneticin and 10% foetal bovine serum at 37 °C, 5% CO_2 in a humidified environment. Lipofectamine 2000 was used to transiently transfect exponentially growing cells with human, rat or mouse recombinant H_3 receptor cDNA according to the manufacturer's instructions (Invitrogen Life Technologies) and cells were then incubated under normal growth conditions for 24 h. Cells were harvested in phosphate buffered saline (PBS), pelleted by centrifugation (200 × *g*, 5 min, room temperature), the supernatant discarded and the pellets stored at −80 °C prior to membrane preparation. Membranes were prepared by homogenising cell pellets in ice-cold 50 mM Tris–HCl buffer (pH 7.7 at 25 °C) for approximately 10 s using a Jankel Ultra-Turrax homogeniser. The homogenates were centrifuged at 50,000 × *g* for 20 min at 4 °C and the resulting pellet was re-homogenised and centrifuged as above. The membranes were finally resuspended in the same buffer at a concentration of approximately 4 mg protein/ml and stored at −80 °C until use.

Cerebral cortical tissues from human (three non-identifiable individuals aged 17–93 years, whose cause of death was non-neurological, from Peterborough Hospital, UK, approved by a local ethics committee), rat (male Sprague Dawley, 200–250 g, Charles River), mouse (CD1 25–40 g, Charles River), dog (Beagle, 10–15 kg, ~20 months old, bred by GlaxoSmithKline) or pig (Yorkshire/Landrace, 40 kg) were homogenised and membranes prepared as described above for the HEK-293-MSR-II cells, with the inclusion of an additional wash step following incubation of the homogenate at 37 °C for 20 min after the first centrifugation.

2.4. Radioligand binding

Binding studies were carried out on membranes derived from cerebral cortical tissues or HEK-293-MSR-II cells transiently transfected with H_3 receptors (see above) in 50 mM Tris–HCl (pH 7.7 at 25 °C) containing 5 mM EDTA using methodology similar to that described previously [22,44]. Assays consisted of 50 μl of displacing compound or buffer, 400 μl of membrane suspension (corresponding to approximately 15 μg protein/well for the recombinant cells and 50 μg protein/well for the brain tissue) and 50 μl of *R*(−)α-methyl[imidazole-2,5(*n*)-^{3}H]histamine dihydrochloride ([^{3}H]-*R*-α-methylhistamine; specific activity, 24 Ci mmol^{-1}, Amersham Biosciences).

In competition binding experiments, 10 concentrations of the competing ligands were tested (concentration range 0.03 nM–1 μM), with a final [^{3}H]-*R*-α-methylhistamine concentration of 2 nM. Incubations with [^{3}H]-*R*-α-methylhistamine were for 45 min at 30 °C and non-specific binding was determined in the presence of 10 μM imetit. The experiments were terminated by rapid filtration through Whatman GF/B filters (pre-soaked in 0.3%, v/v polyethyleneimine (PEI)), and then the filters washed through with 4× 5 ml of ice-cold buffer containing 50 mM Tris–HCl (pH 7.7 at 25 °C), 5 mM $MgCl_2$. Filters were dried and added to vials each containing 4 ml Ultima Gold MV scintillation fluid (Hewlett Packard) and radioactivity determined by liquid scintillation spectrometry using a Packard Tri-Carb 2500TR liquid scintillation counter. Protein concentrations were determined using the Bradford assay method (Bio-Rad protein assay kit, Bio-Rad, York, UK) with bovine serum albumin as a standard. The concentration of drug inhibiting specific radioligand binding by 50% (IC_{50}) was determined by iterative curve fitting [45]. pK_i values (the negative $\log_{10}$ of the molar K_i) for receptor binding were then determined from the IC_{50} values using the Cheng and Prusoff approximation [46].

2.5. cAMP accumulation

Human embryonic kidney 293 cells stably expressing G-protein Gαo (HEK-293-Gαo) were maintained in similar conditions to HEK-293-MSR-II cells but without 1% non-essential amino acids. Exponentially growing cells were transduced with BacMam virus [47,48] encoding the human recombinant H_3 receptor as follows. Cells were detached from flasks in PBS and collected by centrifugation at 200 × *g* for 5 min at room temperature. The cells were then resuspended in growth media containing virus at a multiplicity of infection (m.o.i.) of 50, re-plated and then incubated under normal growth conditions for 24 h. Cyclic AMP levels in cells were determined by radioimmunoassay (FlashPlate® Adenylyl Cyclase Activation Assay System, Perkin Elmer) as previously described [49]. Cells were washed once with PBS and centrifuged at 200 × *g* for 5 min at room temperature. The supernatant was discarded and the pellet resuspended in manufacturer's stimulation buffer and washed by centrifugation (as above). The pellet was then resuspended in stimulation buffer containing IBMX at a cell density of 2 million cells/ml and 100,000 cells added to the appropriate wells of the FlashPlates®. The wells contained 10 μM forskolin together with a concentration range of the H_3 agonist imetit (1 pM–100 μM) in the absence or presence of GSK207040 (3–100 nM) or GSK334429 (30–300 nM). Plates were incubated for 30 min at 37 °C, before the addition of the manufacturer's detection buffer containing [^{125}I]cAMP-tracer (0.16 μCi/ml) to the wells. Plates were covered and left for 24 h at room temperature prior to counting on a Packard TopCount scintillation counter. Drug concentration–response curves from cAMP accumulation assays were fitted using Grafit 5.0.8 (Erithacus Software Ltd.), to a four-parameter logistic equation constraining the E_{max} of each curve to 100%. The pA_2 for antagonism was determined by Schild analysis [50] of the data where, for a

reversible competitive antagonist, provided the slope is not significantly different from unity, the $pA_2 = pK_B$.

2.6. GTPγS binding

GTPγS binding assays similar to those previously described [51] were used to investigate potential inverse agonist properties of GSK207040 and GSK334429 in HEK-293-Gαo cells transduced with human H_3 receptor-encoding BacMam virus as described above but using a m.o.i. of 100. Following overnight incubation, cells were collected into 10 ml PBS and spun at 200 × *g* for 5 min. After removal of the supernatant, the pellet was resuspended and homogenised in 20 mM HEPES (pH 7.4) containing 3 mM $MgCl_2$, and 100 mM NaCl, centrifuged at 50,000 × *g* for 20 min, then homogenised and centrifuged again. The membrane pellet was then resuspended and assayed for protein concentration. Cell membranes were diluted to ~1 mg/ml in assay buffer (20 mM HEPES, 100 mM NaCl, 10 mM $MgCl_2$, pH 7.4) and incubated with wheat germ agglutinin scintillation proximity assay (SPA) beads (Amersham Biosciences) for 45 min, after which GDP (40 μM) was added. Various concentrations of GSK207040 and GSK334429 (0.03 nM–1 μM in half log increments) were added to a 96-well plate along with 10 μl assay buffer. Non-specific binding was determined by the inclusion of 0.6 mM GTPγS. Sixty microliters (~55 μg protein/well) of membranes/SPA beads/GDP mix was then added to each well and the plate incubated on an orbital shaker for 30 min at room temperature. [^{35}S]-GTPγS (0.3 nM) was then added to each well, the plate incubated on the shaker for a further 30 min, and bound [^{35}S]-GTPγS was determined by scintillation counting on a Wallac 1450 Microbeta Trilux counter. From GTPγS binding studies, pIC_{50} values were generated from dose–response curves using Grafit 5.0.8 (Erithacus Software Ltd.).

2.7. Ex vivo binding

Ex vivo binding studies were carried out based on methodology previously reported [49,52]. Adult male rats (Lister hooded 200–250 g, Charles River, UK) received vehicle (1%, w/v aqueous methylcellulose), GSK207040 (0.01, 0.03, 0.1, 0.3, 1 or 3 mg/kg) or GSK334429 (0.03, 0.1, 0.3, 1, 3 and 10 mg/kg) by oral gavage ($n = 3$ per group) and were sacrificed 2 or 3 h later. Terminal blood samples were collected and brains rapidly removed. Half brain cerebral cortex tissue was dissected for ex vivo binding and half brain for pharmacokinetic analysis of brain concentrations of each compound. All dissected tissue samples were snap frozen in liquid nitrogen, and stored at −80 °C until use. The tissues were rapidly thawed and homogenised in approximately 30 volumes of ice-cold buffer containing 50 mM Tris–HCl (pH 7.7 at 25 °C) and 5 mM EDTA. This crude homogenate (600–800 μg/well) was then used to measure H_3 receptor binding as described earlier with [^{3}H]-R-α-methylhistamine as radioligand. Diluted blood (50 μl + 50 μl water) or brain (25 μl + 25 μl water) samples were extracted using protein precipitation with acetonitrile. The samples were prepared, vortex mixed and centrifuged, and the resultant supernatant analysed for GSK207040 and GSK334429 content by reverse phase HPLC/MS/MS using a heat assisted electrospray interface. In ex vivo binding studies, specific radioactivity in the samples was corrected for protein, and data were expressed as inhibition of H_3 binding (% control) as a surrogate measure for H_3 receptor occupancy. ED_{50} values (dose required to produce a 50% reduction in ex vivo radioligand binding) were determined by plotting the $\log_{10}$ of the oral dose against % specific binding using GRAFIT 5.0.8 software (Erithacus Software Ltd.).

2.8. Pharmacokinetic analysis

Pharmacokinetic studies with GSK207040 and GSK3344429 were conducted in conscious male Sprague Dawley rats. Each study was a cross-over design with a period of at least 2 days between dose administrations with each animal receiving the same compound on both dosing occasions. Animals were prepared with a cannula inserted via the femoral vein into the vena cava (for compound administration) and via the jugular vein (for blood sampling), as described previously [53]. The cannulae were exteriorised at the back of the neck and the animals placed in jackets with tethers and housed in plastic-bottom cages in facilities with a 12-h dark/light cycle. Each animal had free access to food and water. Following post-operative recovery, animals received an intravenous infusion of GSK207040 ($n = 3$; weight range 294–347 g) or GSK334429 ($n = 3$; weight range 328–365 g) administered at a nominal dose level of 1 mg free base/kg for 1 h via the femoral vein cannula (10 ml/h/kg). Compounds were dissolved in 2% (w/v) DMSO and 10% (w/v) Kleptose in 0.9% (w/v) aqueous saline (GSK207040) or 0.9% (w/v) aqueous saline (GSK334429) at a target concentration of 0.1 mg free base/ml and filtered with a 0.22 mm Millex-GV filter prior to administration. Following a washout of at least 2 days, the same rats received a single oral administration of GSK207040 or GSK334429 at a nominal dose level of 2 mg free base/kg. Compounds were formulated in 1% (w/v) aqueous methylcellulose at a target concentration of 0.4 mg free base/ml. Serial blood samples were collected from the jugular vein cannula pre-dose and at intervals up to 12 h (GSK207040) or 24 h (GSK334429) after the start of the intravenous infusion and up to 30 h post-oral dose. Diluted samples (1:1 with deionised water) were analysed for GSK207040 or GSK334429 concentrations using a method based on protein precipitation and HPLC-MS/MS analysis. The lower limit of quantification was 5 ng/ml (0.013–0.014 μM). Non-compartmental pharmacokinetic parameters were obtained from the blood concentration–time curves using WinNonlin Professional v3.2 (Pharsight Corp., CA, USA). Oral bioavailability was calculated as the ratio of the area under the blood concentration versus time curve (AUC) after oral and intravenous doses after normalising for dose.

2.9. Drinking assay

The effect of GSK207040 and GSK334429 on H_3 receptor agonist-induced water intake in male Lister Hooded rats (370–500 g, Harlan, UK) was tested in a dipsogenia model similar to that described previously [23,54]. Groups of individually housed rats with access to water via identical bottles and spouts received either vehicle (1% aqueous methylcellulose), GSK207040 (0.01, 0.03 and 0.1 mg/kg p.o.)

or GSK334429 (0.01, 0.03, 0.1 and 0.3 mg/kg p.o.) followed 2 h later by either vehicle (0.9% saline, 2 ml/kg) or R-α-methylhistamine (2.5 mg/kg s.c.). Water consumption was determined in each animal for 60 min after administration of the second treatment by weighing each bottle to the nearest 0.1 g. These data were used to determine ID_{50} values (dose of antagonist required to inhibit water consumption by 50% of R-α-methylhistamine control) using ALLFIT analysis [55]. Statistical differences from the R-α-methylhistamine alone treated group were determined using one-way ANOVA followed by Dunnett's t-test according to Statistica 6.1 (StatSoft).

2.10. Passive avoidance

The ability of acute treatment of GSK207040 and GSK334429 to influence memory consolidation in male Wistar rats (350–400 g; Biomedical facility, University College, Dublin) was investigated in a passive avoidance paradigm in which some animals were administered scopolamine to induce a cholinergic deficit [56]. Following a habituation period of 5 days, where animals were housed in pairs, vehicle (1% aqueous methylcellulose), GSK207040 (0.1, 0.3, 1 and 3 mg/kg p.o.) or GSK334429 (0.3, 1 and 3 mg/kg p.o.) was administered to rats 2 h prior to training ($n = 6$ per group). Analysis of spontaneous behaviour in an open-field apparatus was performed 2 days before training and immediately prior to training as previously described using a 5 min evaluation period each time [57]. Rats were then trained in a single-trial, step-through, light–dark passive avoidance paradigm as previously described [57]. Briefly, on the day of training, animals were placed into the light compartment of the apparatus. Their latency to enter the dark chamber was recorded, and having completely entered the dark compartment, a scrambled foot shock (0.5 mA, 5 s duration) was administered to the animal, which immediately returned to the light compartment. Animals were rendered amnesic of the task by administration of scopolamine (0.8 mg/kg i.p.) 6 h after training. Recall of the inhibitory stimulus was evaluated 24 h post-training by returning the animal into the light chamber and recording their latency to enter the dark chamber and a criterion time of 600 s was employed. In the passive avoidance study, data were analysed by Mann–Whitney U-test and by ANOVA followed by the Bonferroni post hoc test. In all cases, values of $P \leq 0.05$ were deemed to be significant.

2.11. Capsaicin-induced secondary allodynia

Injection of capsaicin into the plantar surface of the heel of the rat hindpaw produces a reduction in paw withdrawal thresholds, to a tactile stimulus in the footpad, distal to the site of injection (secondary allodynia). GSK207040 and GSK334429 were tested for their ability to reverse capsaicin-induced secondary allodynia based on methodology previously described [58]. In one study male Random Hooded rats (160–200 g, B&K Universal) were dosed orally with GSK207040 (0.1, 0.3 and 1 mg/kg) or vehicle (saline) 60 min before being lightly anaesthetised with isoflurane and injected intraplantar with capsaicin (Sigma UK; 10 μg of capsaicin dissolved in 10 μl solution of 10% ethanol, 10% Tween, 80% saline) into the heel of the left hind paw [59]. In another study, Random Hooded rats (160–200 g, B&K Universal) were dosed orally with GSK334429 (1, 3 and 10 mg/kg) or vehicle (1% methylcellulose) 30 min before the intraplantar injection of capsaicin under anaesthetic as described above. In both studies, 15 min after capsaicin administration, rats were placed in observation boxes (2 rats/box) on a raised metallic mesh platform for habituation prior to testing. Using a Dynamic Plantar Aesthesiometer (Ugo Basille, Italy), paw withdrawal thresholds (PWTs, g) were obtained by presenting the fine blunt probe of the anaesthesiometer at a rate of 50 g over 10 s to the plantar surface of the paw distal to the capsaicin injection site. Withdrawal thresholds of ipsi- and contra-lateral paws were compared and readings were taken at 22, 25 and 30 min post-capsaicin injection. The study was performed with the operator blind to which treatment each group received. Data are presented as mean ± S.E.M. for the three PWT readings taken and $n = 5$ per dosing group. The percentage inhibition was calculated from the mean PWTs using the formula: (ipsi drug − ipsi vehicle/contra vehicle − ipsi vehicle) × 100.

Statistical analysis was carried out on the PWTs and compared all drug dose groups with the vehicle treated rats using ANOVA followed by a Dunnett's post hoc test, where $P < 0.05$ was considered significant.

2.12. Mechanical nociception

To assess the effect of H_3 antagonists on PWTs of naïve rats to a noxious mechanical stimulus, paw pressure assessments were carried out using an analgesymeter (Ugo Basille, Italy) 1 h before and 2 h after oral administration of GSK207040 (1, 3 and 10 mg/kg p.o.) or vehicle (1% methylcellulose). Methods were conducted according to Randall and Selitto [60] with an increasing mechanical pressure applied to the left hind paw of Lister hooded rats (200–230 g, Charles River, UK) until paw withdrawal occurred, at which point the force (g) was recorded. A cut-off was set at 500 g and data are presented as PWT (g) with seven animals in each dosing group. To obtain similar baseline readings between groups the 'pre-compound' withdrawal thresholds of all rats were ranked and randomised according to a Latin square and placed into four groups with similar PWTs. The study was performed with the operator blind to which treatment each group received. Statistical analysis was carried out on PWTs, where post-compound readings were compared to pre-compound readings using ANOVA followed by a Duncan's post hoc test, where $P < 0.05$ was considered significant. In both pain studies the number of animals and intensity of noxious stimuli were the minimum necessary to demonstrate consistent effects of drug treatments.

2.13. CNS side effect profiling

The effects of GSK207040 (3 and 25 mg/kg p.o.) and GSK334429 (3.5 and 35 mg/kg p.o.) on general behaviour were evaluated in male Sprague Dawley rats (250–300 g, Charles River, UK) using the Laboratory Animal Behaviour and Observational, Registration and Analysis System (LABORAS) as described previously [61]. Using the same doses, both compounds were tested for potential sedative effects in male Sprague Dawley rats (250–300 g, Charles River, UK) using a standard accelerating rotarod test [62] and potential pro-convulsant liability in

male Sprague Dawley rats (100–150 g Charles River, UK) using the maximal electroshock seizure threshold test [4,63].

3. Results

3.1. *In vitro characterisation of GSK207040 and GSK334429*

Radioligand binding studies with the selective H_3 receptor radioligand [^{3}H]-R-α-methylhistamine revealed that GSK207040 and GSK334429 had high affinity for human and rat recombinant H_3 receptors expressed in HEK-293-MSR-II cells, and native H_3 receptors expressed in human, rat, mouse, dog and pig cerebral cortex. pK_i values for GSK207040, GSK334429 and reference H_3 antagonists thioperamide, clobenpropit and ciproxifan are shown in Table 1. Generally, GSK207040 and GSK334429 exhibited very similar affinity for recombinant or cerebral cortex H_3 receptors, whilst both antagonists exhibited somewhat higher affinity for human and pig H_3 receptors compared to rat, mouse and dog H_3 receptors. GSK207040 and GSK334429 were >1000-fold selective for the H_3 receptor over histamine H_1 ($pK_b < 5.8$; FLIPR assay), H_2 ($pK_b < 5.5$; cAMP assay) and H_4 ($pK_b < 5.5$; FLIPR assay) receptors (data not shown), and showed minimal activity (<30% inhibition at 1 μM) when tested in a commercial battery of approximately 50 receptors, ion channels and other drug targets (CEREP, Celle L'Evescault, France).

In functional assays with HEK-293-Gαo cells expressing the human H_3 receptor, GSK207040 (3, 10, 30 and 100 nM) and GSK334429 (30, 100 and 300 nM) produced dose-dependent rightward shifts in the concentration–effect curve to the H_3 agonist imetit with $pA_2 = 9.26 \pm 0.04$ and 8.84 ± 0.04, respectively ($n = 3$). GSK207040 and GSK334429 also exhibited inverse agonist properties at the human H_3 receptor, a common observation with other structurally distinct H_3 antagonists [29]. Basal GTPγS binding (in the absence of H_3 agonist) was inhibited by GSK207040 and GSK334429 (0.03 nM–1 μM) in a dose-dependent manner with $pIC_{50} = 9.20 \pm 0.36$ and 8.59 ± 0.04, respectively ($n = 3$).

3.2. *Ex vivo binding and drug exposure levels*

GSK207040 (0.01, 0.03, 0.1, 0.3 and 1 mg/kg p.o. 2 h post-dose) and GSK334429 (0.03, 0.1, 0.3, 1 and 3 mg/kg p.o. 3 h post-dose) dose-dependently inhibited ex vivo [^{3}H]-R-α-methylhistamine binding in the rat cortex (Fig. 2; $n = 3$) with a maximum inhibition of 100 and 84%, respectively. Iterative curve fitting of this data gave estimated mean ED_{50} values of 0.03 and 0.35 mg/kg, respectively. In other studies, >95% inhibition of ex vivo binding was observed with GSK207040 (3 mg/kg) and GSK334429 (10 mg/kg), whilst time course studies revealed little difference in occupancy (>70–80%) between 30 min and 8 h post-dose with GSK207040 (3 mg/kg) and GSK334429 (1 mg/kg; data not shown). Inhibition of ex vivo binding was also observed with the reference H_3 antagonists ciproxifan (1, 3 and 10 mg/kg p.o. gave 85, 95 and 97% inhibition) and thioperamide (1, 3 and 10 mg/kg p.o. gave 45, 54 and 80% inhibition). Following oral dosing of GSK207040 (0.1–3 mg/kg), brain concentrations ranged from 0.095 ± 0.009 to 2.964 ± 0.352 μM and blood concentrations from 0.026 ± 0.001 to 1.025 ± 0.088 μM, corresponding to a brain:blood concentration ratio in the range 3–4:1. With

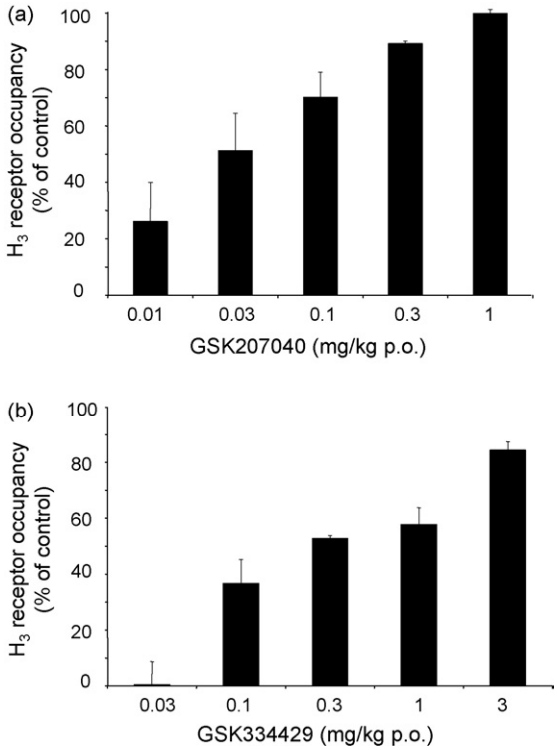

Fig. 2 – Inhibition of [^{3}H]-R-α-methylhistamine ex vivo binding in the rat cortex following oral administration of (a) GSK207040 (0.01–1 mg/kg; 2 h post-dose) and (b) GSK334429 (0.03–3 mg/kg; 3 h post-dose). Data points represent mean ± S.E.M. from three rats per group and are expressed as H_3 receptor occupancy (% of control).

Table 1 – Binding affinities (pK_i; $n \geq 3$) for GSK207040, GSK334429 and reference H_3 receptor antagonists at recombinant and cortical H_3 receptors

	Human cortex	Rat cortex	Mouse cortex	Dog cortex	Pig cortex	Human recombinant	Rat recombinant	Mouse recombinant
GSK207040	9.67 ± 0.06	9.08 ± 0.16	8.85 ± 0.04	8.35 ($n = 2$)	9.52 ($n = 2$)	9.72 ± 0.01	8.95 ± 0.12	8.92 ± 0.15
GSK334429	9.49 ± 0.09	9.12 ± 0.14	9.19 ± 0.02	8.97 ± 0.19	9.56 ± 0.06	9.91 ± 0.17	9.23 ± 0.08	9.15 ± 0.18
Thioperamide	n.d.	8.48 ± 0.01	8.30 ± 0.15	7.91 ± 0.10	8.25 ± 0.09	7.12 ± 0.10	8.62 ± 0.06	8.49 ± 0.05
Clobenpropit	9.11 ± 0.11	9.53 ± 0.11	9.57 ± 0.09	9.84 ± 0.12	10.08 ± 0.20	9.47 ± 0.19	9.59 ± 0.09	9.70 ± 0.08
Ciproxifan	6.72 ± 0.14	8.91 ± 0.04	9.10 ± 0.16	7.46 ± 0.10	8.03 ± 0.16	6.96 ± 0.06	9.29 ± 0.05	9.08 ± 0.08

Table 2 – Pharmacokinetic properties of GSK207040 and GSK334429 in the rat

Dose route	Parameter	GSK207040, mean (±S.D.) ($n = 3$)	GSK334429, mean (±S.D.) ($n = 3$)
Intravenous (1 mg/kg)	CLb (ml/min/kg)	28 ± 5	23 ± 4
	Vss (l/kg)	5.4 ± 0.4	3.1 ± 0.2
	C_{max} (μM)	0.594 ± 0.090	0.783 ± 0.047
	$t_{1/2}$ (h)	2.6 ± 0.5	2.0 ± 0.3
	MRT (h)	3.3 ± 0.6	2.3 ± 0.6
Oral (2 mg/kg)	C_{max} (μM)	0.443 ± 0.066	0.903 ($n = 2$)
	T_{max} (h)	0.8 (0.8–2.0)	1.5 ($n = 2$)
	$t_{1/2}$ (h)	2.9 ± 0.7	2.1 ($n = 2$)
	Fpo (%)	88 ± 16	91 ($n = 2$; 89, 94)

increasing dose (0.1–10 mg/kg), brain concentrations of GSK334429 increased from 0.069 ± 0.020 to 2.063 ± 0.202 μM and blood concentrations from 0.134 ± 0.012 to 2.358 ± 0.325 μM, corresponding to a brain:blood concentration ratio in the range 0.5–0.8:1. Brain and blood concentrations of GSK207040 and GSK334429 exhibited a linear relationship with dose across the range tested in efficacy models.

3.3. *Pharmacokinetic properties of GSK207040 and GSK334429*

The pharmacokinetics of GSK207040 and GSK334429 were evaluated in male Sprague Dawley rats. Following a 1 h intravenous infusion of GSK207040 at a target dose of 1 mg free base/kg to the rat, GSK207040 had a moderate blood clearance of 28 ± 5 ml/min/kg (*ca.* 30% liver blood flow) with a steady-state volume of distribution (Vss) of 5.4 ± 0.4 l/kg indicating distribution of GSK207040 into tissues, and a terminal half-life of 2.6 ± 0.5 h (Table 2). Mean residence time (MRT) was *ca.* 3.3 h. Following oral administration of GSK207040 at a target dose of 2 mg free base/kg, peak blood concentrations of GSK207040 of 0.443 ± 0.066 μM were achieved between 0.8 and 2.0 h after dosing with an oral half-life of 2.9 ± 0.7 h. The oral bioavailability of GSK207040 in the rat was 88 ± 16% (Table 2).

Following a 1 h intravenous infusion of GSK334429 at a target dose of 1 mg free base/kg to the rat, GSK334429 had a low blood clearance of 23 ± 4 ml/min/kg (*ca.* 25% liver blood flow) with a steady-state volume of distribution (Vss) of 3.1 ± 0.2 l/kg, indicating distribution of GSK334429 into tissues, and a terminal half-life of 2.0 ± 0.3 h. Mean residence time (MRT) was *ca.* 2.3 h. Following oral administration of GSK334429 at a target dose of 2 mg free base/kg, peak blood concentrations of GSK334429 of between 0.827 and 0.978 μM were achieved at 1.5 h after dosing. The oral bioavailability of GSK334429 in the rat ranged between 89 and 94% (mean *ca.* 91%) with a terminal half-life following oral administration of between 1.9 and 2.4 h (Table 2).

3.4. *Dipsogenia*

R-α-Methylhistamine (2.5 mg/kg s.c.) produced a significant increase in water consumption in the rat dipsogenia model. Oral administration of GSK207040 (0.01–0.1 mg/kg p.o.) and GSK334429 (0.01–0.3 mg/kg p.o.) dose-dependently inhibited the dipsogenic response to *R*-α-methylhistamine with complete reversal being observed at 0.1 and 0.3 mg/kg, respectively (Fig. 3). Iterative curve fitting of the data yielded ID_{50} values of 0.02 and 0.11 mg/kg, respectively. The standard H_3 antagonists ciproxifan (10 mg/kg) and thioperamide (5 mg/kg) completely inhibited the dipsogenic response to *R*-α-methylhistamine.

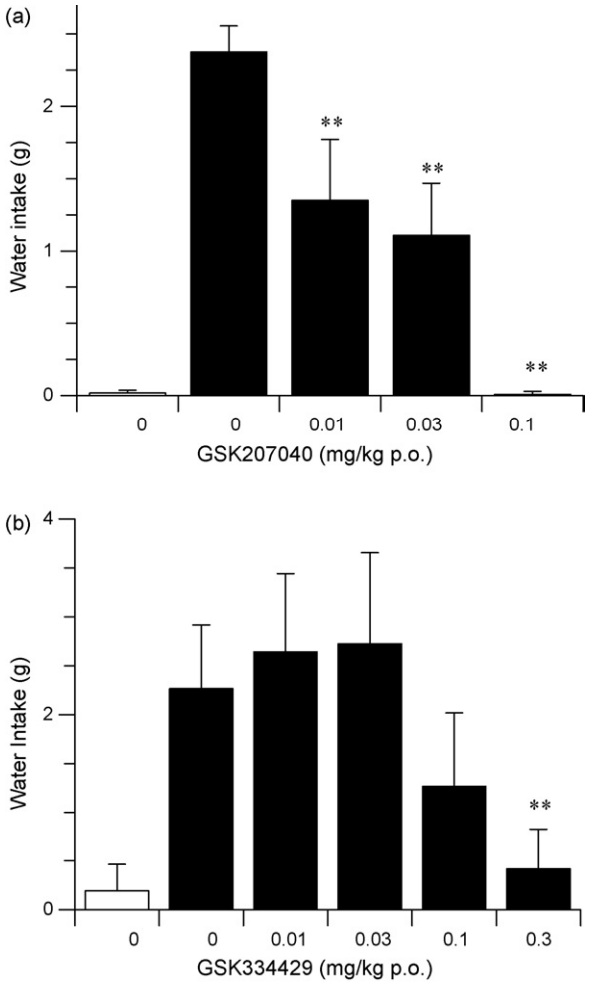

Fig. 3 – Effect of (a) GSK207040 (0.01, 0.03 and 0.1 mg/kg p.o.) and (b) GSK334429 (0.01, 0.03, 0.1 and 0.3 mg/kg p.o.) on water intake induced by the H_3 agonist *R*-α-methylhistamine (2.5 mg/kg s.c.) in Hooded Lister rats. Open bars to the left show basal water intake and black bars show water intake following *R*-α-methylhistamine administration in the presence and absence of increasing doses of GSK207040 and GSK334429. $^{*}P < 0.05$ compared to *R*-α-methylhistamine alone group ($n = 6$).

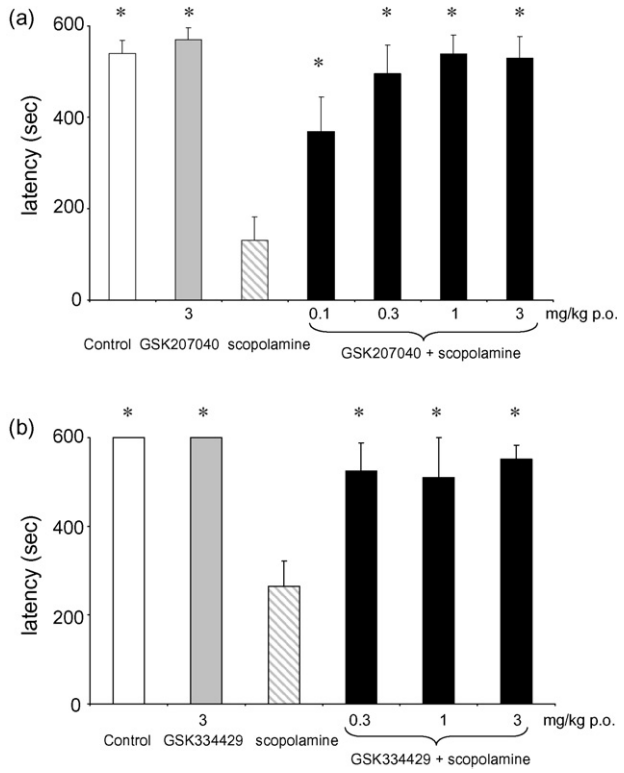

Fig. 4 – Effect of (a) GSK207040 (0.1, 0.3, 1 and 3 mg/kg p.o., $n = 6$) and (b) GSK334429 (0.3, 1 and 3 mg/kg p.o., $n = 6$) on scopolamine-induced amnesia of a passive avoidance response. Data represent the mean ± S.E.M. avoidance latency at the 24 h recall time. A significant difference from scopolamine alone response (hatched bars) is indicated with asterisk (Mann–Whitney *U*-test analysis; $P < 0.05$).

3.5. *Passive avoidance*

Vehicle treated control animals had significantly increased 24 h recall latencies compared to scopolamine treated animals (Mann–Whitney *U*-test analysis), consistent with an amnesic effect of scopolamine. GSK207040 reversed this scopolamine-induced amnesia in a dose-dependent manner, with significant effects being observed after administration of 0.1, 0.3, 1 and 3 mg/kg (Fig. 4a; $F(4, 29) = 9.2$; $P = 0.0001$). Similarly, GSK334429 (0.3, 1 and 3 mg/kg p.o.) significantly reversed the detrimental effects of scopolamine treatment on memory consolidation (Fig. 4b; $F(3, 23) = 4.3$; $P = 0.017$). When test compounds were administered alone (where animals were treated with vehicle instead of scopolamine at the 6 h post-training time point), both GSK207040 and GSK334429 (3 mg/kg) gave recall latencies which were significantly higher than the scopolamine treated animals (Mann–Whitney *U*-test analysis), and no different from vehicle treated control animals. The analysis of spontaneous exploratory behaviour in an open-field apparatus, for 2 days before training and again immediately prior to training, indicated that all animal groups underwent typical habituation to the open-field arena and no drug-induced changes in exploratory activity were observed (data not shown).

3.6. *Capsaicin-induced secondary allodynia*

Capsaicin induced a significant reduction in withdrawal threshold of the ipsilateral paw compared to the contralateral paw, when the mechanical stimulus was applied distally to the site of capsaicin injection, which is indicative of secondary allodynia. Mean PWTs ranged from >40 g in the contralateral to <15 g in the capsaicin injected ipsilateral paw. GSK207040 (0.1, 0.3, and 1 mg/kg p.o.) significantly inhibited capsaicin-induced secondary allodynia by 42 ± 5, 43 ± 8, and 65 ± 8%, respectively, compared to vehicle group ($P < 0.001$ for all

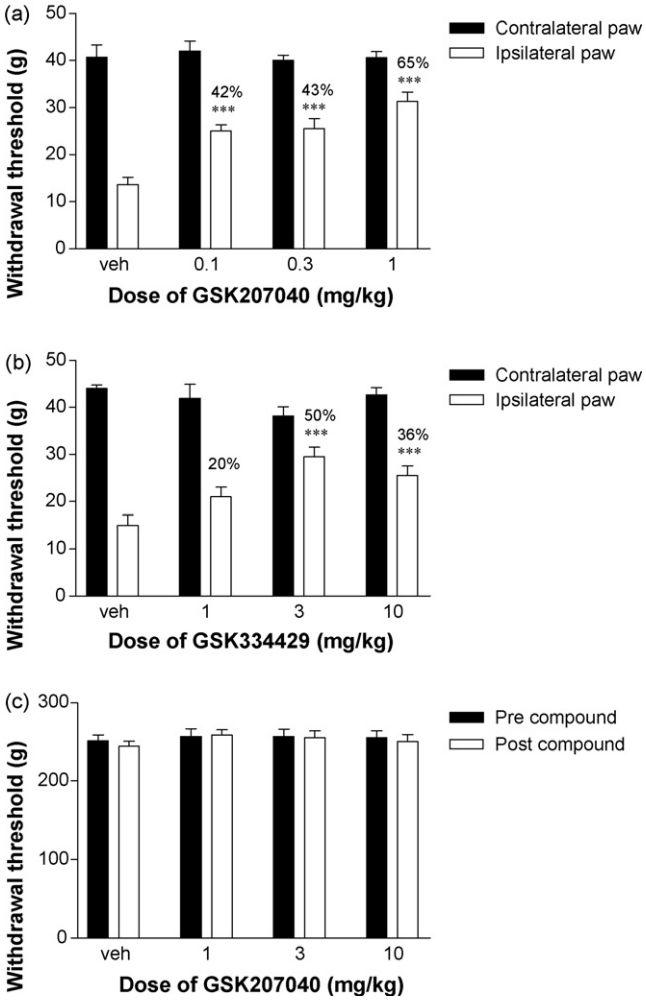

Fig. 5 – Effect of (a) GSK207040 (0.1, 0.3 and 1 mg/kg p.o. tested 90 min post-dose, $n = 5$ per dose group) and (b) GSK334429 (1, 3 and 10 mg/kg p.o. tested 60 min post-dose, $n = 5$ per dose group) on capsaicin-induced decreases in paw withdrawal threshold in ipsilateral (open bars) and contralateral (filled bars) paws. The percentages represent % inhibition of capsaicin-induced secondary allodynia. (c) Effect of GSK207040 (1, 3 and 10 mg/kg p.o. 120 min post-dose, $n = 7$ per dose group) on paw withdrawal threshold in naïve rats (pre-dose solid bars, post-dose open bars). $^{***}P < 0.001$, ANOVA followed by a Dunnett's post hoc test compared to the difference in ipsi- and contra-lateral paw thresholds in the vehicle treated group.

groups) when rats were tested 90 min post-drug administration (Fig. 5a). GSK334429 (3 and 10 mg/kg p.o.) also significantly inhibited capsaicin-induced secondary allodynia by 50 ± 7 and 36 ± 7%, respectively ($P < 0.001$) compared to vehicle when rats were tested 60 min post-drug administration but had no significant effect at 1 mg/kg p.o. (20 ± 7%) (Fig. 5b).

3.7. *Mechanical-induced antinociception testing*

GSK207040 (1, 3 and 10 mg/kg p.o.) had no effect on PWTs of naïve rats when tested 2 h post-dose. The mean PWT of all groups regardless of dose or timepoint ranged between 244 and 257 g with no overall significance observed (Fig. 5c).

3.8. *CNS side effect profiling*

Both GSK207040 (3 and 25 mg/kg p.o.; 3 h post-dose) and GSK334429 (3.5 and 35 mg/kg p.o.; 3 h post-dose) produced no overt dose related effects on any LABORAS-scored behaviour including grooming, eating, drinking, rearing, locomotor activity and immobility duration over a 30 min testing period. Small increases in grooming and immobility duration were observed but these were not dose related. GSK207040 (3 and 25 mg/kg p.o.; 1–4 h post-dose) and GSK334429 (3.5 and 35 mg/kg p.o.; 1–4 h post-dose) produced no impairment of rotarod performance throughout the testing period. In addition, GSK207040 (3 and 25 mg/kg p.o.; 3 h post-dose) and GSK334429 (3.5–35 mg/kg.; 3 h post-dose) showed no pro-convulsant liability in the maximal electroshock seizure threshold test in rats.

4. Discussion

GSK207040 is derived from a novel benzazepine series of non-imidazole H_3 receptor antagonists [39] structurally distinct from other recently described molecules [25–28]. GSK334429 is from another novel series of piperidine carboxamides [40], which also exhibit potent H_3 antagonist properties. Both antagonists exhibited sub-nanomolar affinity for the human H_3 receptor, similar affinity for the pig receptor, and slightly lower affinity for rat, mouse and dog H_3 receptors. Whilst these species differences are fairly minor, they are not unexpected, given previous reports showing that H_3 antagonists can show 10–100-fold differences in affinity between human and rodent receptors [31,64,65], observations we confirmed in the present study with the standard H_3 antagonists ciproxifan and thioperamide. It is well documented that these species differences in pharmacology are attributed to changes in two key amino acid residues in the third transmembrane domain of the receptor where Threonine-119 and Alanine-122 of the human sequence are replaced by Alanine-119 and Val-122 in the rat sequence [66,67].

In functional assays with the human recombinant H_3 receptor in vitro, GSK207040 and GSK334429 showed potent functional antagonist (cAMP assay) and inverse agonist (GTPγS assay) properties, with GSK207040 being slightly more potent (~0.5 log unit) than GSK334429 in both assays. Inverse agonism is a common property of H_3 antagonists in vitro when tested in recombinant systems with high constitutive activity [29–31]. Interestingly, constitutive activity of the H_3 receptor has also been demonstrated in the rodent brain both in vitro and in vivo [68,69], although it has yet to be observed in human brain tissue. However, it is believed that the inverse agonist properties of H_3 ligands may improve their therapeutic potential compared to neutral antagonists [69].

Following oral dosing in rats, GSK207040 and GSK334429 potently inhibited ex vivo H_3 receptor binding in the cerebral cortex, indicative of brain penetration and CNS receptor occupancy. This was supported by data in a model of dipsogenia [54,70], showing that both antagonists were potent at reversing H_3 agonist-induced drinking, consistent with in vivo functional blockade of central H_3 receptors following oral dosing. GSK207040 appeared somewhat more potent than GSK334429 in both the ex vivo binding and drinking assays, consistent with the in vitro functional data, and increased brain penetration.

The effects of GSK207040 and GSK334429 were also studied in more pathophysiologically relevant pharmacodynamic assays using doses that completely blocked the dipsogenia response and inhibited ex vivo binding by >50%. In the passive avoidance paradigm, a model of fear motivated memory consolidation involving primarily the amygdala and hippocampus [56,57], GSK207040 and GSK334429 reversed amnesia induced by the cholinergic antagonist scopolamine, consistent with facilitation of cholinergic neurotransmission following blockade of H_3 heteroreceptors. Other H_3 antagonists including thioperamide, clobenpropit and FUB 181 have also been shown to reverse the effects of scopolamine in passive avoidance [15,16], whilst in other variations of inhibitory avoidance, H_3 antagonists have shown efficacy in senescence accelerated mice [14], and spontaneously hypertensive rat pups [71]. Interestingly, the amnesic effects of scopolamine in passive avoidance are not observed in H_3 knock-out mice, supporting a role for H_3 receptors in cholinergic function [72]. Taken together, these data suggest that clinical studies using scopolamine to induce deficits in cognitive function similar to previous investigations [73,74] could be a useful approach for validating pro-cognitive effects of H_3 antagonists such as GSK207040 and GSK334429 in humans. The present data is also in agreement with the effects of H_3 antagonists on performance in other rodent models of cognitive function which have been reviewed in detail [17,71]. In addition, preliminary studies suggest that GSK207040 and GSK334429 also show efficacy in an object recognition paradigm at similar doses to those active in passive avoidance when dosed 2 h before testing (unpublished observations).

Few studies have investigated the effects of imidazole and non-imidazole H_3 receptor antagonists in preclinical pain models and the data that is published is somewhat confusing [35]. Thioperamide produced an antinociceptive effect in the mouse hot plate test whilst the H_3 receptor agonist imetit caused allodynia [36]. However another H_3 receptor agonist immepip attenuated both phases of the formalin-induced licking test [75]. In contrast, thioperamide blocked morphine-induced analgesia whilst the H_3 agonist R-α-methylhistamine caused potentiation of morphine's effect [37]. Interpretation of this data is complicated by the fact that thioperamide has similar functional potency at H_4 and H_3 receptors [1,76], and up until now there have been no reports of any data generated with

more selective non-imidazole H_3 antagonists. In the current study, we chose to investigate the effects of GSK207040 and GSK334429 in the capsaicin model of secondary allodynia/hyperalgesia rather than in general nociception models, because this model is thought to exhibit characteristics of one of the underlying mechanism of neuropathic pain, namely central sensitisation [59,77]. In addition, capsaicin is used to induce mechanical hypersensitivity and tactile allodynia in clinical studies [78]. Injection of capsaicin into the heel of the rat hindpaw produces a secondary allodynia in the footpad distal to the site of injection by mechanisms involving sensitisation of second order dorsal horn neurons in the spinal cord [59]. In the present study, GSK207040 and GSK334429 produced a significant inhibition of capsaicin-induced decreases in paw withdrawal threshold. To our knowledge, this is the first study to show effects of H_3 receptor antagonists on capsaicin-induced secondary allodynia, supporting the hypothesis that H_3 receptor blockade may potentially improve tactile allodynia in patients with neuropathic pain. It could be hypothesised that the positive effects observed with H_3 antagonists in this study could be due to increases in monoamines following H_3 receptor blockade, given that drugs such as the monoamine uptake blockers duloxetine and venlafaxine, that can also increase monamines through other mechanisms, also show efficacious effects in neuropathic pain models as well as in patients [38,77].

The lack of effect with GSK207040 on paw withdrawal thresholds in naïve animals, not subjected to capsaicin treatment, suggests that the effects on secondary allodynia observed with these H_3 receptor antagonists are not due to modulation of normal antinociceptive responses. In addition, preliminary studies showed that GSK207040 had no effect on Freunds Complete Adjuvant-induced mechanical hyperalgesia in rats when tested at doses that were efficacious in the capsaicin model (unpublished observations). Further studies are warranted in additional clinically relevant animal models to determine the full potential of H_3 antagonists in neuropathic pain.

The positive effects of GSK207040 and GSK334429 in the rat passive avoidance and allodynia models are unlikely to be due to non-specific effects on general behaviour as both compounds showed little activity in LABORAS, accelerating rotarod and maximal electroshock tests at doses up to 25 and 35 mg/kg, respectively. Given the high selectivity of GSK207040 and GSK334429 it is likely that these efficacious effects are due to H_3 receptor blockade rather than off-target activity. In addition, it is unlikely that the positive effects in passive avoidance are due to the 'analgesic' properties of GSK207040 and GSK334429 rendering the rats unable to feel the footshock, because administration of GSK207040 or GSK334429 alone did not reduce 24 h recall latency.

Whilst collectively the current studies support positive effects of the novel H_3 antagonists GSK207040 and GSK334429 in a number of models, further consideration of the pharmacokinetic/pharmacodynamic relationship between the various assays is warranted. Efficacious doses in the tested cognition and pain models were somewhat higher than the minimum effective doses in the ex vivo binding and dipsogenia assays. This may be because complete functional blockade of H_3 receptors is required to observe robust efficacy in the tested cognition and pain models that involve complex pathways and multiple neurotransmitters. In contrast, the dipsogenia and ex vivo binding assays reflect specific interactions between an H_3 agonist and antagonist and therefore less H_3 receptor blockade may be required for an effect to be observed, particularly if the agonist is occupying only a small fraction of the receptors. Estimated efficacious brain concentrations of GSK207040 and GSK334429 were also higher than rat cortex binding affinities, probably due to non-specific binding in the brain and/or because of methodology differences. Affinities were determined in cerebral cortex membranes and reflect specific competition binding at the H_3 receptor. The brain concentrations of each drug were measured in whole brain samples (unbound and bound drug), and the drug concentration at the receptor level is unknown. Plasma protein binding is low for GSK334429 (66%) and is likely to be similar for GSK207040 (based on data for similar compounds in the same chemical series) and so this is unlikely to completely account for the mismatches observed. It should be noted that these pharmacokinetic/pharmacodynamic discrepancies are not unique to GSK207040 and GSK334429. For example, higher doses of the H_3 antagonists ciproxifan and JNJ-5207852 are required in efficacy models compared to dipsogenia or ex vivo binding assays [33,70]. Despite the high affinity of ciproxifan for rat cortex H_3 receptors ($pK_i = 8.9$, i.e. comparable to GSK207040 and GSK334429), a dose of 3 mg/kg has routinely been used to demonstrate positive effects in cognition assays [13]. We have demonstrated that this dose results in brain concentrations of $\sim$3 μM at 2 h following oral dosing, suggesting similar mismatches between pK_i and brain drug concentrations to those observed with GSK207040 and GSK334429 (unpublished observation).

In conclusion, the current study has shown that GSK207040 and GSK334429, two potent and selective novel non-imidazole H_3 antagonists from different chemical series, can reverse scopolamine-induced amnesia and capsaicin-induced secondary allodynia. These data suggest that H_3 antagonists such as GSK207040 and GSK334429 may have therapeutic potential for the symptomatic treatment of dementia and neuropathic pain.

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