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Mechanistic characterization of S 38093, a novel inverse agonist at histamine H3 receptors

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ABSTRACT

Histaminergic H3 inverse agonists, by stimulating central histamine release, represent attractive drug candidates to treat cognitive disorders. The present studies aimed to describe the mechanistic profile of S 38093 a novel H3 receptors inverse agonist.

S 38093 displays a moderate affinity for rat, mouse and human H3 receptors (Ki=8.8, 1.44 and 1.2 μM, respectively) with no affinity for other histaminergic receptors.

In cellular models, the compound was able to antagonize mice H3 receptors (K_B=0.65 μM) and to suppress cAMP decrease induced by an H3 agonist via human H3 receptors (K_B=0.11 μM). The antagonism properties of the compound were confirmed by electrophysiological studies on rat hippocampal slices (from 0.1 μM). In cells expressing a high H3 density, S 38093 behaved as a moderate inverse agonist at rat and human H3 receptors (EC_{50}=9 and 1.7 μM, respectively).

S 38093 was rapidly absorbed in mouse and rat (T_{max}=0.25–0.5 h), slowly in monkey (2 h), with a bioavailability ranging from 20% to 60% and $t_{1/2}$ ranging from 1.5 to 7.4 h. The compound was widely distributed with a moderate volume of distribution and low protein binding. The brain distribution of S 38093 was rapid and high.

In mice, S 38093 significantly increased *ex vivo* N-tele-Methylhistamine cerebral levels from 3 mg/kg p.o. and antagonized R-α-Methylhistamine-induced dipsogenia from 10 mg/kg i.p.

Taken together, these data suggest that S 38093, a novel H3 inverse agonist, is a good candidate for further *in vivo* evaluations, in particular in animal models of cognition.

1. Introduction

Histamine is an important neurotransmitter exerting its actions via four classes of molecularly and/or pharmacologically well-defined receptors, which differ in distribution, pharmacology, and function. The third histamine receptor subtype (H3), which was identified by Arrang et al. (1983), is widely expressed in the brain, particularly in areas involved in cognitive processes and arousal, such as the cerebral cortex, hippocampus, basal ganglia, and hypothalamus (Martinez-Mir et al., 1990; Pollard et al., 1993). The H3 receptor, a member of the large superfamily of G protein-coupled receptors, has mostly presynaptic localization and its activation leads to the inhibition of the synthesis and release of histamine (Arrang et al., 1983). Likely, presynaptic H3 receptors on heterologous nerve endings are thought to negatively regulate the release of neurotransmitters such as acetylcholine, serotonin, noradrenaline, and dopamine (Blandina et al., 1996; Brown et al., 2001).

The involvement of H3 receptors in different brain functions has led to the proposal of H3 antagonists/inverse agonists for different therapeutic applications, especially in the treatment of cognitive deficits in conditions such as Alzheimer's disease and other dementias as well as for disorders of sleep and attention such as narcolepsy and attention deficit hyperactivity disorder (Esbenshade et al., 2008; Celanire et al., 2009; Passani et al., 2004, 2009; Brioni et al., 2011).

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Consequently, a wide range of structurally diverse ligands has been proposed as pharmacological tools or as potential drug candidates (Leurs, 2005; Łażewska and Kieć-Kononowicz, 2014). The early synthetized potent histamine H3 antagonists/inverse agonists were imidazole ligands, they were found to be active in preclinical models but limited as drug candidates due for instance to interactions with the hERG channel, phospholipidosis, cytochrome P450 inhibition or poor blood-brain penetration (Łażewska and Kieć-Kononowicz, 2010). Consequently, researchers made many efforts to synthetize novel, potent and optimized H3 antagonists/inverse agonists, belonging to several different chemical groups such as piperidine, piperazine, pyrrolidine derivatives and cycloalkylamines for instance. Recent years brought the first results from clinical studies discussing the usefulness of the tested structures in specific diseases as well as providing information about adverse effects, the most commonly reported up to now being insomnia.

Under these circumstances, from a series of azabicyclic derivatives, S 38093 was selected as a novel H3 antagonist/inverse agonist. Herein, the mechanistic characterization and pharmacokinetic properties of S 38093 are presented. A separate publication (Panayi et al., 2017) presents the *in vivo* pharmacological profile of this novel compound.

2. Materials and methods

2.1. Drugs

Two forms of S 38093 were used either S 38093-1 (oxalate salt) or S 38093-2 (hydrochloride salt with a salt/base ratio of 1.126) (Fig. 1). S 38093-1 and S 38093-2 was provided by Servier. [^{125}I]-Iodoproxifan and [^{35}S]-GTPγS were obtained from Perkin Elmer (France). R-α-Methylhistamine was purchased from Sigma (France), ASCENT (France) (electrophysiology) or RBI (France) (dipsogenia model). Imetit and Thioperamide were obtained from Sigma-Aldrich (France).

2.2. Animals

Electrophysiological experiments were performed in the rat by Neuroservice (Aix-en-provence, France) in accordance to the French and European legislations for animals care.

Pharmacokinetic studies were carried out at Biologie Servier on mice supplied by Charles River Laboratories Domaine des Oncins, 69210 Saint Germain sur L'Arbresle, France and monkeys supplied by Centre de Recherches Primatologiques Ltd, 10, rue de l'Intendance, Port Louis, Mauritius and Noveprim Ltd, Le Vallon – Ferney S.E., Mahebourg, Mauritius, in accordance to the French and European legislations for animals care.

Pharmacokinetic studies on rats were performed at Northwick Park Institute for Medical Research, Northwick Park and St Mark's NHS trust, Harrow, Middlesex HA1 3UJ, United Kingdom, in accordance to the European legislations for animals care.

Liquid scintillation counting and cerebral autoradiography were carried out in the rat by Charles River Discovery Research Services (Kuopio, Finland) according to the National Institute of Health guidelines for the care and use of laboratory animals, and approved by the National Laboratory Animal Board, Finland.

Dipsogenia study and neurochemical determination of histamine turnover were conduced in the mice at the Institut de Recherches Servier (IdRs, Croissy-sur-Seine, France). Protocols were reviewed and approved by the local Ethical Committee of IdRs for these two studies.

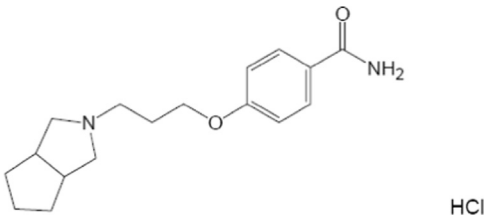

Fig. 1. Chemical structure of S 38093-2.

2.3. Radioligands binding assays

Radioligands binding assays were performed to evaluate the affinities of S 38093 at rat, mouse and human H3 receptors as well as at cloned human H1, H2 and H4 histamine receptor subtypes to determine H3 receptor selectivity of S 38093 versus other histaminergic receptors.

For the mouse H3 receptors binding evaluation, membrane preparation of Chinese Hamster Ovarian (CHO) cells transfected with mouse H3 recombinant receptors were incubated for 60 min at 20 °C in the presence of S 38093-1 diluted half-logarithmically from 100 μM to 1 nM (DMS 10% in water) and [125I]-Iodoproxifan at 25pM. Non-specific binding was estimated in the presence of Clobenpropit (1 μM).

Membrane preparation of rat cerebral cortex or CHO cells transfected with human H3 recombinant receptors were incubated 60 min at 22 °C with S 38093-2 at 3×10^{-8}, 1×10^{-7}, 3×10^{-7}, 1×10^{-6}, 3×10^{-6}, 1×10^{-5} and 3×10^{-5} M in presence of the specific radiolabeled ligand [^{3}H]Nα-Methylhistamine (1 nM). Non-specific binding was determined in presence of 1–5 μM of R-α-Methylhistamine. Membrane preparation of HEK-293 cells transfected with human H1 or H4 recombinant receptors was incubated 60 min at 22 °C with S 38093-2 at 1×10^{-7} and 1×10^{-5} M in presence of the specific radiolabeled ligand [3H]pyrilamine (1 nM) or [3H]histamine (10 nM), respectively, and the non-specific binding ligand pyrilamine (1 μM) or imetit (1 μM), respectively. Membrane preparation of CHO cells transfected with human H2 recombinant receptor were incubated 120 min at 22 °C with S 38093-2 at 1×10^{-7} and 1×10^{-5} M in presence of the specific radiolabeled ligand [125I]APT (0.075 nM) and non-specific binding ligand tiotidine(100 μM). Binding of S 38093-2 to rat and human H3 receptors and to human H1, H2 and H4 receptors was detected by scintillation counting for IC_{50}/Ki determination. The specific ligand binding to the receptors is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabeled ligand. Each experiment was made in duplicate (n=2).

2.4. [^{35}S]GTPγS binding assay

Guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate binding assay was used to investigate antagonist properties of S 38093-1 in CHO cells stably expressing the mouse H3 receptor in the presence of the H3 agonist R-α-methylHistamine (n=5 independent experiments).

CHO-mH3 cells were amplified in HAM F12 Glutamax +10% FCS culture medium. Cells were collected in PBS and pelleted before freezing at −80 °C. Membranes were prepared by cell disruption with a polytron (3×10 s) in Na2HPO4 37.8 mM+KH2PO4 12.2 mM buffer, pH 7.4, followed by slow speed centrifugation (140*g*, 10 min, 4 °C) to eliminate fragments. Supernatant was submitted to ultracentrifugation (23,000*g*, 30 min, 4 °C) and microsomal pellet was resuspended in 10 v/w of buffer. Protein concentration was estimated by Lowry method. Membrane Kd and Bmax were determined by Scatchard plot analysis, after [^{125}I]-Iodoproxifan saturation. For GTPγS assays, S 38093-1 was diluted logarithmically from 10 μM to 100 pM in DMSO 10% in water. Duplicates of S 38093-1 in serial dilution (25 μl)+R-α-Methylhistamine at 300 nM final concentration (25 μl)+microsomal membranes at 23 fmol/ml [^{125}I]-Iodoproxifan in Hepes 20 mM pH 7.4, NaCl 100 mM, MgCl2 3 mM, GDP 3 μM, Saponine 20 μg/ml (150 μl) were incubated in 96 wells, 2 ml polypropylene plates (Costar), for 30 min. Then, 50 μl of [^{35}S]-GTPγS at 0.16 nM was added for 30 min incubation. Non-specific binding was estimated in the presence of GTPγS (Sigma) at 10 μM. Incubation plates were filtrated on 96 wells, GF-B plates (Perkin Elmer) moisten with water and rinsed twice with