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# Novel heterocyclic-substituted benzofuran histamine $H_3$ receptor antagonists: In vitro properties, drug-likeness, and behavioral activity

*Marlon Cowart* *, *Gregory A. Gfesser, Kaitlin E. Browman, Ramin Faghih, Thomas R. Miller, Ivan Milicic, John L. Baranowski, Kathleen M. Krueger, David G. Witte, Angela L. Molesky, Victoria A. Komater, Michael J. Buckley, Gilbert J. Diaz, Gerard D. Gagne, Deliang Zhou, Xiaoqing Deng, Liping Pan, Ellen M. Roberts, Marilyn S. Diehl, Jill M. Wetter, Kennan C. Marsh, Gerard B. Fox, Jorge D. Brioni, Timothy A. Esbenshade, Arthur A. Hancock*

*GPRD_AP9a/216, Department of Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064-6123, United States*

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ABSTRACT

Three novel heterocyclic benzofurans A-688057 (**1**), A-687136 (**2**), and A-698418 (**3**) were profiled for their in vitro and in vivo properties as a new series of histamine $H_3$ receptor antagonists. The compounds were all found to have nanomolar potency in vitro at histamine $H_3$ receptors, and when profiled in vivo for CNS activity, all were found active in an animal behavioral model of attention. The compound with the most benign profile versus CNS side effects was selected for greater scrutiny of its in vitro properties and overall drug-likeness. This compound, A-688057, in addition to its potent and robust efficacy in two rodent behavioral models at blood levels ranging 0.2–19 nM, possessed other favorable features, including high selectivity for $H_3$ receptors ($H_3$, $K_i$ = 1.5 nM) versus off-target receptors and channels (including the hERG $K^+$ channel, $K_i$ > 9000 nM), low molecular weight (295), high solubility, moderate lipophilicity ($logD_{pH7.4}$ = 2.05), and good CNS penetration (blood/brain 3.4×). In vitro toxicological tests indicated low potential for phospholipidosis, genotoxicity, and $CYP_{450}$ inhibition. Even though pharmacokinetic testing uncovered only moderate to poor oral bioavailability in rat (26%), dog (30%), and monkey (8%), and only moderate blood half-lives after i.v. administration ($t_{1/2}$ in rat of 2.9 h, 1.7 h in dog, 1.8 h in monkey), suggesting poor human pharmacokinetics, the data overall indicated that A-688057 has an excellent profile for use as a pharmacological tool compound.



## 1. Introduction

The field of study of the histamine $H_3$ receptor began with the 1983 report by Arrang et al. [1] on the discovery and characterization of a then-novel histamine receptor with pharmacology distinct from the previously characterized $H_1$ and $H_2$ receptors. In the early years of the field (the 1980s and 1990s), the characterization of the pharmacology and physiological role of the histamine $H_3$ receptor was enabled by the availability of potent imidazole-based $H_3$ ligands such as

* *Corresponding author*. Tel.: +1 847 938 8170; fax: +1 847 937 9195.
E-mail address: marlon.d.cowart@abbott.com (M. Cowart).
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ciproxifan (**4**) [2] and thioperamide (**5**) [3], compounds that have become pharmacological reference standards. Another major milestone in the field was the cloning of the receptor and identification the human [4] histamine $H_3$ receptor, and other species isoforms, which enabled subsequent interspecies comparisons [5]. The receptor has been found localized predominantly in the central and peripheral nervous system [6]. It was found that when activated, the $H_3$ receptor can suppress the release of stimulatory neurotransmitters (negative modulation), neurotransmitters that include not only histamine but also dopamine, acetylcholine, norepinephrine, serotonin, glutamate, and others. Taken together, these and other findings suggested the potential utility of histamine $H_3$ receptor antagonists in the treatment of CNS diseases.

The initial work in this field was facilitated by the early availability of tool compounds suitable for animal experiments. These include ciproxifan (**4**) and thioperamide (**5**), compounds that are still in wide use today for pharmacological investigations. These compounds are readily available from commercial sources, and have a number of favorable properties, such as potent activity at rodent $H_3$ receptors, and a history of in vivo use. Later, other imidazole-based compounds were described with activity in animal models, including SCH-79876 (**6**) [7], and GT-2331 (**7**) [8]. More recently, other more diverse structures based on non-imidazoles have been highlighted as histamine $H_3$ antagonists, prominently GSK-198254 (**8**) [9], BF-2649 (**9**) [10], as well as ABT-239 (**10**) [11] (Fig. 1). A summary of some of the problems encountered in developing high-quality compounds such as ABT-239 has been described [12]. With a view to enhancing diversity in the benzofuran series genus [13], and addressing specific challenges previously raised, three new heterocyclic-substituted benzofurans were made, and their in vitro and in vivo properties are detailed here: A-688057 (**1**, (*R*)-4-(2-(2-(2-methylpyrrolidin-1-yl)ethyl)benzofuran-5-yl)-1*H*-pyrazole), A-687136 (**2**, (*R*)-2-(2-(2-(2-methylpyrrolidin-1-yl)ethyl)benzofuran-5-yl)pyrazine), and A-698418 (**3**, (*R*)-5-(2-(2-(2-methylpyrrolidin-1-yl)ethyl)benzofuran-5-yl)nicotinonitrile).

## 2. Materials and methods

### 2.1. Drugs

Synthesis of A-688057, (*R*)-4-(2-(2-(2-methylpyrrolidin-1-yl)ethyl)benzofuran-5-yl)-1*H*-pyrazole (**1**). A slurry of 75.5 g (245 mmol) of (*R*)-1-(2-(5-bromobenzofuran-2-yl)ethyl)-2-methylpyrrolidine [11], 117 g (268 mmol) of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-trityl-1*H*-pyrazole [14], and 3.51 g (10.0 mmol) of biphenyl-2-yldicyclohexylphosphine was stirred in 0.55 L of dioxane. To this was added in turn 7.02 g (10.0 mmol) of $Pd(Ph_3P)_2Cl_2$ and 0.55 L ethanol, followed by slow addition of 0.28 L of 1 M aqueous sodium carbonate. The resulting suspension was stirred for 3 days, heated at 60 °C for 2 h, cooled to ambient temperature, and the reaction liquid phase separated from solids by filtration and retained. Filtered solids were washed with water and 2:3 water/ethanol, then extracted by washing with ethanol, then $CH_2Cl_2$. The ethanol filtrate was combined with the reaction liquid phase and partially concentrated to produce a solid, which was collected by filtration and washed with ethanol; the collected solids were dissolved in $CH_2Cl_2$, filtered through basic alumina. The combined $CH_2Cl_2$ filtrates were passed through neutral alumina, concentrated, and dried under vacuum to give 99.9 g of (*R*)-4-(2-(2-(2-methylpyrrolidin-1-yl)ethyl)benzofuran-5-yl)-1-trityl-1*H*-pyrazole as a tan powder, MS (ESI) *m*/*z* 538 $(M + H)^+$.

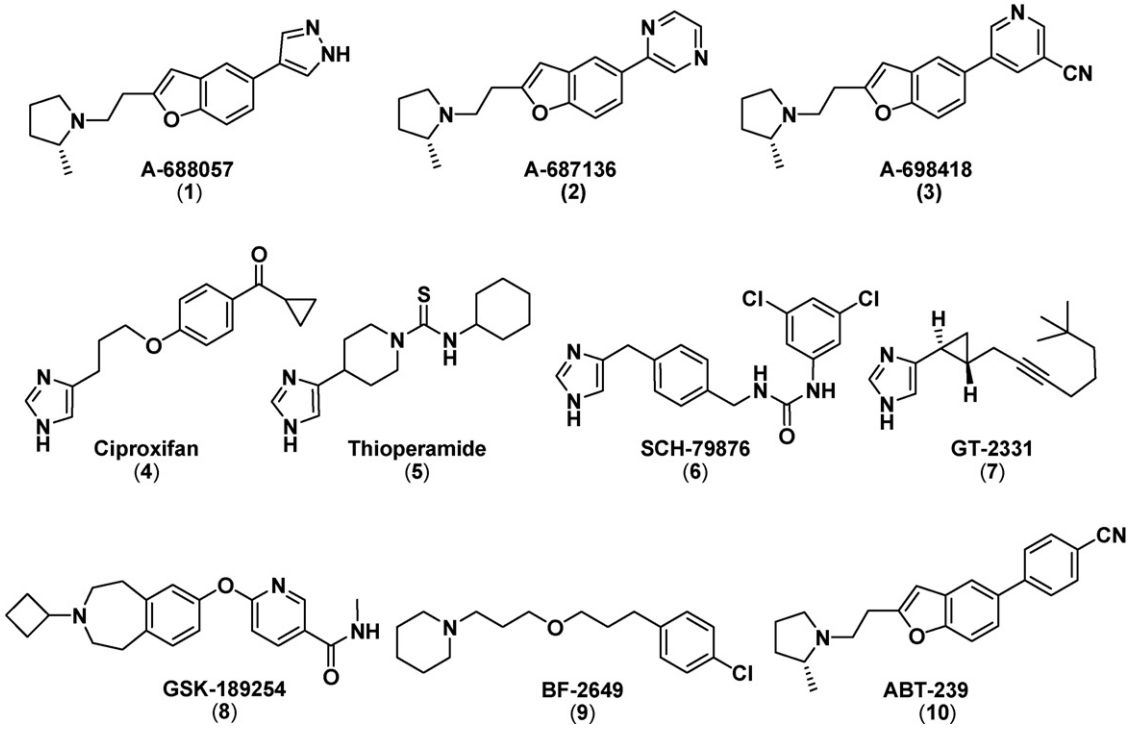


**Fig. 1 – Chemical structures of histamine $H_3$ receptor antagonists.**

This was suspended in 300 mL of 88% aqueous formic acid, stirred 70 min, and poured into 1200 mL 0.1 M aqueous HCl. Two hundred milliliter of 3:1 hexanes/ethyl acetate was added, and the biphasic mixture thoroughly mixed and then filtered through Celite. Twice, the collected solids were stirred with more 0.1 M aqueous HCl and refiltered with a 0.1 M aqueous HCl rinse. The three filtrates were combined and washed twice with 3:1 hexanes/ethyl acetate. The aqueous phase was filtered, cooled in a water–ice bath, and slowly adjusted to pH 11 with solid NaOH. The resulting suspension was filtered and collected solids were washed with water. The solids were then boiled in aqueous ethanol and filtered hot with an aqueous ethanol rinse, diluted with water and partially concentrated. The solution was then decanted from a small amount of a sticky gum, and further concentrated to give a solid precipitate which was collected by filtration and washed with water. These solids were then reboiled in aqueous ethanol and treated similarly as before. Drying under vacuum afforded 31.4 g of a light beige solid as the free base monohydrate, m.p.: 167–169. $^1$H NMR (300 MHz, $CD_3OD$) δ: 1.17 (d, 3H), 1.45 (m, 1H), 1.74–1.84 (m, 2H), 2.00 (m, 1H), 2.27 (m, 1H), 2.40–2.56 (m, 2H), 3.01 (m, 2H), 3.19–3.3 (m, 2H), 6.54 (s, 1H), 7.39 (d, 1H), 7.43 (dd, 1H), 7.68 (d, 1H), 7.92 (s, 2H); $^{13}$C NMR (75 MHz, $d_6$-DMSO) δ: 18.9, 21.3, 27.5, 32.4, 51.1, 53.0, 59.0, 102.4, 110.7, 116.5, 121.0, 121.5, 125.0 (b), 127.7, 129.1, 136.1 (b), 152.6, 158.4; MS (ESI) *m/z*: 296 $(M + H)^+$. Anal. Calcd. ($C_{18}H_{21}N_3O{\cdot}H_2O$): C, 68.98; H, 7.40; N, 13.41. Found: C, 69.22; H, 7.42; N, 13.49. The free base was converted to the monophosphate salt by stirring with one equivalent of $H_3PO_4$ in $CH_3OH$. The salt was crystallized and collected by filtration, rinsed with $CH_3OH$, and dried under vacuum. Anal. Calcd. ($C_{18}H_{21}N_3O{\cdot}H_3PO_4{\cdot}0.2H_2O$): C, 54.46; H, 6.20; N, 10.58. Found: C, 54.46; H, 5.84; N, 10.30.

Synthesis of A-687136, (*R*)-4-(2-(2-(2-methylpyrrolidin-1-yl)ethyl)benzofuran-5-yl)-1*H*-pyrazole (**2**). A solution of 2.31 g (7.5 mmol) of (*R*)-1-(2-(5-bromobenzofuran-2-yl)ethyl)-2-methylpyrrolidine [11], 2.29 g (9 mmol) of 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi(1,3,2-dioxaborolane), 2.16 g (22 mmol) of potassium acetate, and 245 mg (0.3 mmol) of 1.1′-bis(diphenylphosphino)ferrocene palladium dichloride dichloromethane in 30 mL of dioxane was heated at 70 °C for 21 h, then cooled, filtered through Celite, concentrated in vacuo, purified by chromatography on silica gel, eluting with 1:1 ethyl acetate:dichloromethane, and concentrated under vacuum to give (*R*)-2-methyl-1-(2-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzofuran-2-yl)ethyl)pyrrolidine (3.5 g) as a brown syrup. Next, a mixture of 0.9 g (2.5 mmol) of the (*R*)-2-methyl-1-(2-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzofuran-2-yl)ethyl)pyrrolidine, 0.72 g (3.5 mmol) of iodopyrazine, and 70 mg (0.1 mmol) of bis(triphenylphosphine) palladium dichloride in a mixture of 15 mL of isopropanol and 2.5 mL of 2 M aqueous sodium carbonate was heated at 55 °C for 12 h. After cooling, the mixture was filtered through silica gel, concentrated in vacuo, and purified by chromatography on silica gel, eluting with a gradient of 0–5% methanol in dichloromethane to give a light brown solid as the product (0.19 g, 24%). $^1$H NMR (300 MHz, $CD_3OD$) δ: 1.18 (d, 3H), 1.43 (m, 1H), 1.8 (m, 2H), 2.00 (m, 1H), 2.30 (m, 1H), 2.6 (m, 2H), 3.05 (m, 2H), 3.20–3.30 (m, 2H), 6.45 (s, 1H), 7.55 (d, 1H), 7.95 (dd, 1H), 8.24 (d, 1H), 8.46 (d, 1H), 8.62 (m, 1H), 9.16 (d, 1H); MS (ESI) *m/z*: 308 $(M + H)^+$.

Synthesis of A-698418, (*R*)-5-(2-(2-(2-methylpyrrolidin-1-yl)ethyl)benzofuran-5-yl)nicotinonitrile (**3**). A mixture of 0.14 g (0.39 mmol) of the (*R*)-2-methyl-1-(2-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzofuran-2-yl)ethyl)pyrrolidine, 0.18 g (1 mmol) of bromonicotinonitrile, and 14 mg (0.02 mmol) of bis(triphenylphosphine) palladium dichloride in a mixture of 2 mL of isopropanol and 0.4 mL of 2 M aqueous sodium carbonate was heated at 50 °C for 15 h. After cooling, the mixture was partitioned between dichloromethane and water, and the organic phase dried ($Na_2SO_4$) and purified by chromatography on silica gel, eluting first with 1:1 ethyl acetate:dichloromethane, then a gradient of 2–5% methanol:-dichloromethane to give a light orange solid as the product (91 mg, 70%). $^1$H NMR (300 MHz, $CD_3OD$) δ: 1.18 (d, 3H), 1.45 (m, 1H), 1.8 (m, 2H), 2.30 (m, 1H), 2.5 (m, 2H), 3.05 (m, 2H), 6.65 (s, 1H), 7.55 (s, 1H), 7.88 (m, 1H), 8.43 (m, 1H), 8.84 (d, 1H), 9.09 (d, 1H); MS (ESI) *m/z*: 332 $(M + H)^+$.

## 2.2. *In vitro assays*

### 2.2.1. *Competition binding*

Assays for determination of $H_3$ receptor binding affinity and inverse agonism were carried using membranes expressing human $H_3$ (in HEK) or rat $H_3$ receptors (in C6 cells) [15]. Membranes were prepared by homogenization in cold buffer (pH 7.4, 50 mM Tris–HCl, 5 mM EDTA, 10 mM $MgCl_2$, and protease inhibitors) followed by centrifugation at 40,000 × *g* for 20 min at 4 °C, and the resulting pellet was resuspended in buffer containing 50 mM Tris–HCl, pH 7.4, 5 mM EDTA, and 10 mM $MgCl_2$. Before freezing and storage, glycerol and BSA were added to give final concentration of 10% glycerol and 1% bovine serum albumin.

$K_i$ values were determined in competition binding assays using [$^3$H]N-α-methylhistamine at a concentration of 0.5–1.0 nM, in the presence of increasing test drug concentrations (11 concentrations spaced over a 5-log unit range), all for 30 min at 25 °C in a final volume of 0.5 mL of pH 7.4 buffer containing 50 mM Tris 5 mM EDTA. Nonspecific binding was defined with 10 μM thioperamide. Binding reactions were terminated by suction under vacuum through polyethylenimine (0.3%) presoaked Unifilter plates followed by three rapid washes with 2 mL of binding buffer. Bound radiolabel was quantitated by liquid scintillation counting, and $IC_{50}$ values and Hill slopes determined by Hill transformation. p$K_i$ values were determined as p$K_i$ ± S.E.M. by the Cheng–Prusoff equation using GraphPad Prism (GraphPad Software, San Diego).

### 2.2.2. *Inverse agonism*

Inverse agonism was assessed in [$^{35}$S] GTPγS binding assays in membranes diluted in assay buffer (pH 7.4, 25 mM HEPES, 2.5 mM $MgCl_2$, and 75 mM NaCl, pH 7.4) [16]. Ten microgram of membrane protein was incubated in a 96-well deep-well block in the presence of 5.0 μM unlabeled GDP, 0.5 nM [$^{35}$S] GTPγS, in the presence of test drug, with incubation at 37 °C for 20 min. Assays were terminated by the addition of cold buffer (50 mM Tris–HCl, 75 mM NaCl, and 2.5 mM $MgCl_2$, pH 7.6), followed by vacuum filtration onto a Packard Unifilter 96-well GF/B plates (PerkinElmer Life and Analytical Sciences). After extensive washing, the plates were dried, Microscint-20 was added, and

bound [$^{35}$S] GTPγS was quantitated by liquid scintillation counting on a Topcount (PerkinElmer Life and Analytical Sciences). The percentage of [$^{35}$S] GTPγS bound in each sample was calculated (relative to that bound to control samples incubated in the absence of histamine $H_3$ receptor ligands). Triplicate determinations were obtained at each drug concentration, and the data were analyzed using GraphPad Prism to obtain $EC_{50}$ values and Hill slopes. The mean ± S.E.M. was determined using data from at least three independent experiments.

### 2.3. Behavioral tests

#### 2.3.1. General observation test

The general observation test for drugs was carried out as previously described [17], using adult male CD-1 mice from Charles River (Portage, MI) at 20–25 g body weight. Mice were separated into groups of three and placed into observation cages (23 cm× 21 cm× 20 cm). Mice were then injected with vehicle or test drug and continuously observed for adverse behaviors, such as tremor and seizure activity (including Straub tail, wild running, clonus, and tonus) and general changes in activity levels, piloerection, ptosis, monitoring closely for 1 h following injection, then intermittently over the ensuing 2, 3, 6, and 24 h. All subjective observations (e.g., activity) in drug-treated mice were made with constant reference to a cage of vehicle-treated control mice. Body temperature was recorded 0.25, 0.5, 1, 2, 3, 6, and 24 h following drug administration, and a decrease of 2 °C or more was considered hypothermia. Drug effects were most severe during the first hour, and observed effects were listed in order of severity at each dose noted at any time during the first hour following drug injection.

#### 2.3.2. Five-trial avoidance acquisition

The effectiveness of drugs in a five-trial, repeated acquisition avoidance animal model of attention and impulsivity was carried out as previously described [17,18] in male SHR (spontaneous hypertensive rat) pups. Pups were obtained from Harlan Labs at postnatal day 7 and housed up to 12 per cage (average of two litters) and fostered with Long–Evans lactating females prior to testing on postnatal days 20–24 (at body weights ranged from 35 to 50 g). In the test, after s.c. dosing with test drug or vehicle, pups were trained to avoid a mild foot shock (0.1 mA, 1 s duration) delivered after the animals voluntarily transferred from a brightly lit compartment to a darkened compartment (which they normally prefer) using as test apparatus a computer controlled Gemini inhibitory avoidance system (San Diego Instruments). After the first trial, the pup was removed and returned to its home cage and the transfer latency (in seconds) was noted. After approximately 60 s, the same pup was again placed in the brightly illuminated compartment, and the training process was repeated in like manner, until a total of five trials had been conducted, with the transfer latencies recorded for each trial. A maximum test time of 60 s was used in the first trial and 180 s for each of the four subsequent trials. Drug or saline vehicle was administered by subcutaneous injection 30 min prior to testing. As aversive stimulus, mild foot shock was calibrated to ensure intertribal consistency using a V-212 oscilloscope (20 MHz; Hitachi, San Jose, CA) and a 100 kΩ resistor. Pups ($n$ = 10–16) were not habituated to the avoidance apparatus before the first trial to avoid potentially confounding latent inhibitory effects. As positive control, ciproxifan (3 mg/kg) was used, and the experimenter was blinded as to treatment. Separate controls did not detect any effect of drug on sensitivity to the foot shock nor nonspecific drug effects on transfer latency in the absence of the aversive stimulus.

#### 2.3.3. Social recognition memory

The social recognition memory test was carried out in adult male Sprague–Dawley rats ($n$ = 9–10 for each dose; 350–450 g) [17]. The test measures social recognition memory of a prior exposure to a conspecific juvenile. Adult rats were placed into fresh test cages and allowed to habituate to the cage for 30 min. Then, an unfamiliar juvenile was introduced, and the overall investigation duration of the juvenile by the adult (grooming, sniffing, and close following) was recorded for a 5 min period. Test drug or saline vehicle was administered to the adult rat immediately after the first exposure period by intraperitoneal injection. After the first exposure, both adult and juvenile were removed to their respective holding cages. After 90 min, the adult was returned to the original test cage, and the same juvenile reintroduced after 30 min, after which the overall investigation duration was noted and again recorded during a second 5 min period. Social memory was quantified by determining the ratio of investigation duration (RID) of the second to the first investigation periods. To control for nonspecific effects, immediately after the second investigation period, a new, unfamiliar juvenile was introduced to the same adult rat for a third 5-min period, and overall investigation duration was recorded once again; and assessed by determining the RID (third to the first investigation periods) for the unfamiliar juvenile. A-688057 produced no nonspecific effects on RID.

In all behavioral tests (general observation, inhibitory avoidance, social recognition memory), animals were housed in a quiet room under conditions of 12 h lights on (at 6:00 a.m.), followed by 12 h lights off, with food and water available ad libitum. All testing was carried out in the light phase, and all experiments were conducted in accordance with Abbott Animal Care and Use Committee and National Institutes of Health Guide for Care and Use of Laboratory Animals guidelines in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

## 3. Results and discussion

### 3.1. $H_3$ receptor binding potency

High potency for the molecular target is a prima facie requirement for compounds targeted as potential therapeutics. As seen in Table 1, the new compounds **1**–**3** all were highly potent at both rat and human $H_3$ receptors, with nanomolar or better $K_i$ values. Although there are substantial similarities between rodent and human receptors, differences have been noted that rationalize the relative species affinity of compounds [5]. Historically, imidazole-based compounds have often been found to have higher potency at rodent $H_3$

**Table 1 – Comparative in vitro potency of the heterocyclic histamine $H_3$ antagonist ligands (1–3) and reference antagonists in binding assays and functional assays of inverse agonism**

| | Binding $pK_i$ ($K_i$, nM) | | Inverse agonism GTPγS $pEC_{50}$ ($EC_{50}$, nM) | |
|---|---|---|---|---|
| | Rat | Human | Rat | Human |
| (**1**) A-688057 | 8.50 ± 0.07 (3.1) | 9.31 ± 0.07 (0.5) | 7.78 ± 0.09 (16.5) | 8.81 ± 0.12 (1.5) |
| (**2**) A-687136 | 8.78 ± 0.11 (1.6) | 9.84 ± 0.07 (0.14) | 8.10 ± 0.11 (7.9) | 9.11 ± 0.1 (0.78) |
| (**3**) A-698418 | 9.62 ± 0.12 (0.24) | 10.1 ± 0.08 (0.08) | N.D. | 9.51 ± 0.12 (0.31) |
| (**4**) Ciproxifan | 9.29 ± 0.09 (0.51) | 7.20 ± 0.05 (63) | 8.87 ± 0.16 (1.4) | 7.05 ± 0.17 (89) |
| (**5**) Thioperamide | 8.44 ± 0.07 (3.6) | 7.14 ± 0.06 (72) | 8.07 ± 0.07 (8.5) | 6.85 ± 0.1 (141) |
| (**10**) ABT-239 | 8.87 ± 0.04 (1.4) | 9.35 ± 0.04 (0.5) | 7.76 ± 0.2 (17) | 8.90 ± 0.06 (1.29) |

$pK_i$ and $pEC_{50}$ values represent a mean of three or more experiments ± S.E.M.

receptors than human. For example, ciproxifan (**4**) is 120× more, and thioperamide (**5**) is 20× more potent at the rat than human receptor in binding assays. In contrast, the SAR of non-imidazole-based compounds has shown that this class very often has greater affinity for human than for rat receptors [11]. Indeed, it could be said that for most non-imidazole series, achieving high potency at rat receptors is the more difficult task. This is significant because compounds are typically evaluated preclinically in rodent models, and thus potency at rodent $H_3$ is at least as important a property as potency at human $H_3$. The three new compounds showed a species binding profile typical of non-imidazole compounds, having higher potency at human than rat receptors, with respective rat and human $H_3$ receptor $K_i$ values of 3.1 and 0.5 nM for A-688057, 1.6 and 0.14 nM for A-687136, and 0.24 and 0.08 nM for A-698418, the last compound having extraordinarily high potency. Optimally, compounds have high potency at all species subtypes, as exemplified by ABT-239 (human $K_i$ = 0.5 nM, rat $K_i$ = 1.4 nM) and other benzofuran analogs [11], by GSK-189254 (human $K_i$ = 0.2 nM, rat $K_i$ = 2.5 nM) [9], and by BF-2649 (human $K_i$ = 2.7 nM, rat $K_i$ = 17 nM) [10].

### 3.2. *Selectivity for $H_3$ receptors*

The potency of a compound at the target receptor (here, $H_3$ species isoforms) is obviously an important property promoting therapeutic activity, but the specificity versus other sites is also important. The off-target activity of the new compounds was determined by screening at a battery of >70 non-$H_3$ molecular sites ranging from GPCRs to kinases in assays run at Abbott Laboratories, and in a commercial screen (Cerep ExpresSprofile, Paris, France). For A-688057, interactions at non-$H_3$ sites were generally weak ($IC_{50}$ or $K_i$ > 10 μM), but with some exceptions seen in binding to adrenergic receptors ($\alpha_{2C}$ = 138 nM, $\alpha_{1a}$ = 436 nM) and the dopamine transporter (DAT $K_i$ = 141 nM). Selectivity was however still high for these targets, for example 45-fold selective for rat $H_3$ versus DAT, and 282-fold human $H_3$ receptor versus DAT. The weak interactions at adrenergic receptors were judged unlikely to have a negative impact on behavioral activity, as other behaviorally active compounds also bind to these sites (ABT-239 is 60 nM at $\alpha_{2C}$ adrenergic; ciproxifan is 63 nM at $\alpha_{2C}$). Although the selectivity for human $H_3$ versus the dopamine transporter was high (282-fold), it must be cautioned that there is still the possibility for behavioral consequences (e.g., addictive liability) that might emerge at higher drug levels if the drug at these concentrations is able to inhibit the dopamine transporter, considering that cocaine and methylphenidate have addictive liability, and are known to be active at the dopamine transporter at behaviorally effective levels. The profile for A-687136 at non-$H_3$ sites was somewhat different than for A-688057, with the sites showing interactions with $IC_{50}$ or $K_i$ values <1 μM being adrenergic receptors ($\alpha_{2C}$ = 257 nM, $\alpha_{1a}$ = 513 nM, and $\alpha_{2b}$ = 813 nM) and the muscarinic $M_2$ receptor ($K_i$ 646 nM). However, A-687136 showed no interaction with the dopamine transporter ($K_i$ > 10 μM). Similar to compounds **1** and **2**, the selectivity profile for A-698418 (**3**) at non-$H_3$ sites indicated activity at adrenergic receptor sites (subtypes $\alpha_{2C}$ = 224 nM, $\alpha_{2a}$ = 339 nM, $\alpha_{1a}$ = 178 nM). Also, for A-698418, a weak interaction was observed at the dopamine transporter ($K_i$ = 309 nM) as well as the norepinephrine transporter ($K_i$ = 525 nM).

ABT-239 has been noted as having the potential to prolong $QT_c$ [12]; in anesthetized dogs, at circulating blood levels of 158 ng/mL (474 nM), $QT_c$ was prolonged in all animals at multiples as low as 30-fold over the blood levels found efficacious in rat behavioral models (0.22–4.7 ng/mL; 0.67–14 nM). Compounds **1–3** were assessed in an in vitro assay for inhibition of the hERG $K^+$ channel by competitive inhibition of $^3$H-dofetilide binding carried out as described by Diaz et al. [28]. A-688057 (**1**) had a $K_i$ of >9000 nM, A-687136 (**2**) had a $K_i$ of >10,000 nM, and A-698418 had a $K_i$ of 1122 ± 230 nM. Thus, all three compounds had higher selectivity and showed significantly less interaction with the hERG channel than ABT-239, which showed a $K_i$ of 195 nM [12]. Furthermore, because A-688057 was ultimately found (vide infra) to be efficacious in animal behavioral models at blood concentrations of 0.2–19 nM (comparable to the blood levels of ABT-239 found effective) yet interacts weakly or not at all with hERG channels (A-688057 hERG $K_i$ of >9000 nM, compared to ABT-239 $K_i$ of 195 nM), A-688057 appears to have lower potential to induce $QT_c$ prolongation in vivo.

### 3.3. *Functional assessment of antagonism and inverse agonism*

In assays measuring inverse agonism by drug-dependent inhibition of constitutive GTPγS binding (Table 1) [16], the compounds were potent, with A-688057 (**1**) having $EC_{50}$ values of 16.5 and 1.5 nM at rat and human $H_3$ receptors, respectively, A-687136 (**2**) having 7.9 and 0.78 nM $EC_{50}$ values at rat and human receptors, respectively, and A-698418 (**3**), the most potent of all, with an $EC_{50}$ of 0.31 nM at the human $H_3$ receptor. As might be expected, the rank order of inverse agonism

potency of compounds **1–3** paralleled the histamine $H_3$ receptor binding affinity. For most therapeutics targeting a receptor antagonist, it might seem a simple assumption that antagonists would not differentiate themselves from each other, and that aside from potency, one compound might be as good as any other. However, it has been demonstrated that the histamine $H_3$ receptor exhibits a substantial degree of constitutive activity [19,20], which necessitates consideration of an additional factor: the possibility that a compound might act not just as an antagonist but also as an 'inverse agonist'. Inverse agonism is a term that denotes the ability of a compound to not just block the effect of endogenous receptor activator, e.g., histamine, but an additional ability to reduce the agonist-independent activation of the receptor. This phenomenon arises not solely as a property of the synthetic ligand, but is also dependent on the presence and degree of intrinsic activity of the target receptor [21–23]. The phenomenon of potentially complex pharmacology has been well recognized with certain imidazole-based $H_3$ ligands, such as GT-2331. This compound was first described as an antagonist [8], but was found in many subsequent studies to have significant agonist properties in many systems [24,25]. Another example in the imidazole class is proxyfan, which also shows system-dependent pharmacology, and has been christened a protean agonist [26]. Thus, when assessing the in vitro properties of a compound, it is important to characterize the in vitro properties of the compound not just in binding assays, but in a variety functional assays capable of characterizing antagonism and inverse agonism. With the exception of GT-2331 (**7**), the compounds in Fig. 1 are reported to be inverse agonists. Where data for non-imidazoles have been reported, they have been found to be antagonists and inverse agonists, consistent with our observations of the properties of **1–3**.

The ability of A-688057 to act as a histamine $H_3$ antagonist was demonstrated in the well known *ex vivo* guinea pig ileum assay (where antagonists are able to dose-dependently antagonize the action of the $H_3$ agonist RAMH in blocking electrically induced twitches in ileum strips) [16,27]. In the guinea pig ileum assay, the compound showed very potent functional antagonism, with a $pA_2$ of $8.08 \pm 0.69$ (Fig. 2). Consistent with this, using a different functional assay [16], wherein compounds are assessed for the ability to block the histamine-dependent inhibition of $^3$H-histamine release in $K^+$ depolarized synaptosomes, A-688057 demonstrated functional antagonism with a $K_b$ of 1.1 nM. By comparison, ABT-239 is much weaker in this assay, with a $K_b$ of only 18.6 nM. When A-687136 was tested in the guinea pig ileum assay, it was found to be even more potent than A-688057, with a $pA_2$ of $8.86 \pm 0.50$.

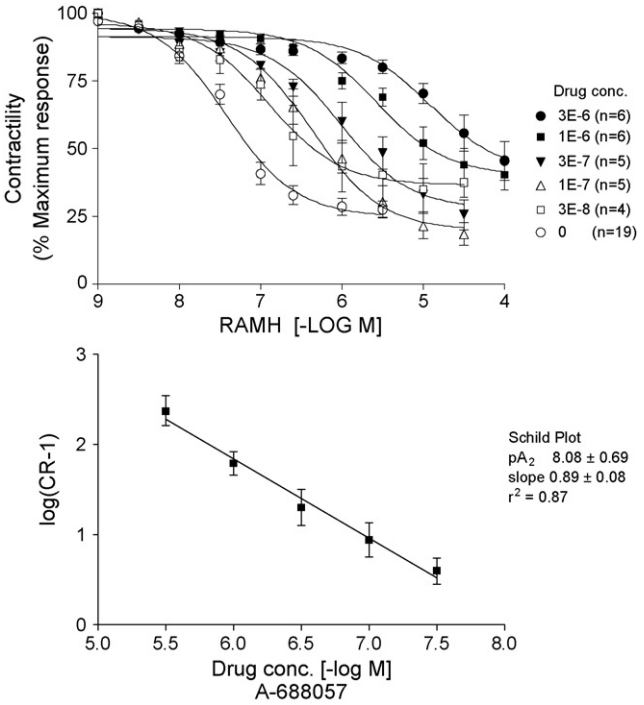


**Fig. 2 – Demonstration of competitive antagonism of $H_3$ agonist (RAMH, (*R*)-α-methylhistamine)-induced inhibition of electrical field-stimulated contraction of guinea pig ileum, carried out as described [35,37]. Ileal tissue strips were incubated with varying concentrations of A-688057 for 30 min prior to determination of RAMH concentration response curves; as can be seen, A-688057 antagonizes the effect of the $H_3$ agonist RAMH in a concentration-dependent manner. Schild transformation of the shifts in concentration response curves reveals that the $pA_2$ is 8.08, with a slope of 0.89 ($n = 26$).**

### 3.4. *Behavioral profile*

Histamine $H_3$ antagonists have been shown effective in animal models relevant to a number of human diseases, a topic that has been reviewed recently [12,29–31]. Early in the field, histamine $H_3$ antagonists were found efficacious in animal models of attention, impulsivity, cognition, and memory [17,18,32,33], and the research described here focuses on the effects of the new compounds in animal models of these conditions, particularly an animal model of ADHD. There are, however, other diseases and conditions that, based on animal models, have been projected to benefit from the administration of histamine $H_3$ receptor antagonists including narcolepsy and sleep disorders [31,34], obesity [35], ADHD [17,18], schizophrenia [36], epilepsy [37], and allergic rhinitis [7].

### 3.5. *General observation test for CNS side effects*

Before behavioral profiling of the compounds for efficacy in models of cognition, attention, and memory, the compounds were examined for their general effects on animal behavior at high doses, with the motivation of probing the compounds for their propensity to produce CNS side effects that could interfere with determination of efficacy. The observations are shown in Table 2, where for each compound at each dose the CNS effects are listed in order of severity, with effects such as seizure judged more notable than piloerection or mild tremor. Compounds were administered by i.p. injection to mice, after which the animals were closely watched for 1 h; the occurrence of any behavior during the first hour is noted in the table. At 2.8 μmol/kg (0.9 mg/kg), two of the compounds, A-

**Table 2 – Behaviors observed during the first hour following drug injection**

| Compound | Dose | | | | | |
|---|---|---|---|---|---|---|
| | Vehicle | 2.8 μmol/kg | 8.4 μmol/kg | 28 μmol/kg | 84 μmol/kg | 280 μmol/kg |
| Observation (number of animals exhibiting) | | | | | | |
| **A-688057** | No effect | No effect | Piloerection (2)<br>Ptosis (1) | Piloerection (2) | **Tremor** (2)<br>**Ataxic** (1)<br>Hypoactivity (3)<br>Ptosis (1) | **Lethality** (2) |
| **A-687136** | No effect | No effect | Piloerection (1) | **Tremor** (3)<br>Piloerection (1) | **Straub tail** (1)<br>**Tremor** (3)<br>**Hyperreactivity** (3)<br>Piloerection (1)<br>Ptosis (3) | **Straub tail** (3)<br>**Tremor** (3)<br>**Hyperreactivity** (1) |
| **A-698418** | No effect | **Hyperreactivity** (2)<br>Piloerection (1) | **Straub tail** (2)<br>**Tremor** (3)<br>**Hyperreactivity** (3)<br>Piloerection (2)<br>Ptosis (2) | **Seizure** (3)<br>**Straub tail** (3)<br>**Tremor** (3)<br>**Hyperreactivity** (3)<br>**Wild running** (3)<br>Piloerection (2)<br>Ptosis (2) | **Lethality** (1)<br>**Seizure** (2)<br>**Straub tail** (2)<br>**Tremor** (2)<br>**Hyperreactivity** (2)<br>**Wild running** (2)<br>Piloerection (2)<br>Ptosis (2) | **Lethality** (3)<br>**Seizure** (3)<br>**Straub tail** (3)<br>**Tremor** (3)<br>**Wild running** (3) |

Note: These are reports of any of the behaviors during the first hour following drug injection to $n = 3$ animals. Effects are listed in order of severity at each dose, and the number of animals exhibiting the behavior is shown in paranthesis.
Effects are listed in order of severity at each dose.

688057 (**1**) and A-687136 (**3**), showed no observable adverse effect on animal behavior, a profile similar to ABT-239 and earlier compounds [17]. Some effects did appear at 8.4 μmol/kg (2.7 mg/kg) and 28 μmol/kg (9 mg/kg), but even these were relatively benign (piloerection and ptosis for A-688057 and tremor for A-687136). More severe adverse effects were seen however at higher doses of 84 and 280 μmol/kg. One compound, A-698418 (**2**), did have an unusually severe side effect profile, eliciting adverse CNS effects (hyperreactivity and piloerection) at very low doses. Even at 2.8 μmol/kg (0.9 mg/kg), hyperreactivity was prominent, progressing to tremor and Straub tail even at 8.4 μmol/kg (2.7 mg/kg). The reasons for the potency of A-698418 in inducing such adverse CNS effects are unknown. It should however be noted that this compound contains a meta cyano functional group on the heterocycle; in a series of previously reported aromatic substituted benzofuran histamine $H_3$ antagonists [11], the compound most potent in inducing CNS side effects also happened to contain a meta cyano functional group.

Overall, A-688057 was the cleanest of the three new compounds versus induction of overt side effects after acute administration; this compound was more benign than even ABT-239, which at 84 μmol/kg s.c. induced tremors, Straub tail, and hypoactivity. One utility proposed for histamine $H_3$ receptor antagonists has been treatment of obesity; [35] interestingly, for A-688057, in a chronic study (30 days) administering the compound to rats at the maximum tolerated dose (limited by CNS effects), very substantial blood exposures could be achieved (end of study 3500 ng/mL, 11,800 nM) without inducing weight loss.

### 3.6. *Efficacy in an a model of attention and impulsivity*

The new compounds were tested and found efficacious in an animal model assessing components of attention, impulsivity, and learning in a five-trial inhibitory avoidance paradigm in SHR (spontaneous hypertensive rat) pups (Fig. 3) [17,18]. Several histamine $H_3$ antagonists are known to be active in this model, with high potency noted in early benzofuran-based compounds, maximal efficacy being noted at doses of 0.03–0.3 mg/kg [9]. Other structural classes of histamine $H_3$ receptor antagonists have also proven effective, including thioperamide, ciproxifan, A-304121, and A-349821, although these were found to be less potent than benzofuran-based compounds (1–10 mg/kg) [11].

In this model, SHR pups are placed in a brightly lit chamber; vehicle-treated animals will transfer (escape) to a darkened chamber when permitted the opportunity. When this transference is paired with a mild aversive stimulus (foot shock), animals learn to avoid the darkened chamber over the course of five trials (Fig. 3a). When animals were pretreated with histamine $H_3$ receptor antagonists (A-688057, A-687136, A-698418, and ciproxifan), there was an increase in the acquisition of the avoidance behavior compared to vehicle-treated controls. This acquisition can be depicted more clearly (Fig. 3b) by summing the latencies to enter the darkened chamber during the learning trials (trials 2–5). All the histamine $H_3$ antagonists significantly increased acquisition of the avoidance behavior as compared to vehicle-treated controls ($p < 0.05$; Mann–Whitney; $n = 10$–12 animals/dose). A-688057 was effective at 0.1 and 0.3 mg/kg, comparable in potency to ABT-239, which is efficacious in this model at 0.1 mg/kg s.c. [11,17]. A-687136 (0.3 mg/kg) was effective also in increasing avoidance acquisition in the model, as was A-698418 at the very low dose of 0.01 mg/kg, s.c., making this last compound the most potent we have reported in this model. The observation of efficacy and potency in this model, combined with its relatively benign profile for CNS side effects in the general observation test motivated more detailed investigation of A-688057. To determine the drug levels

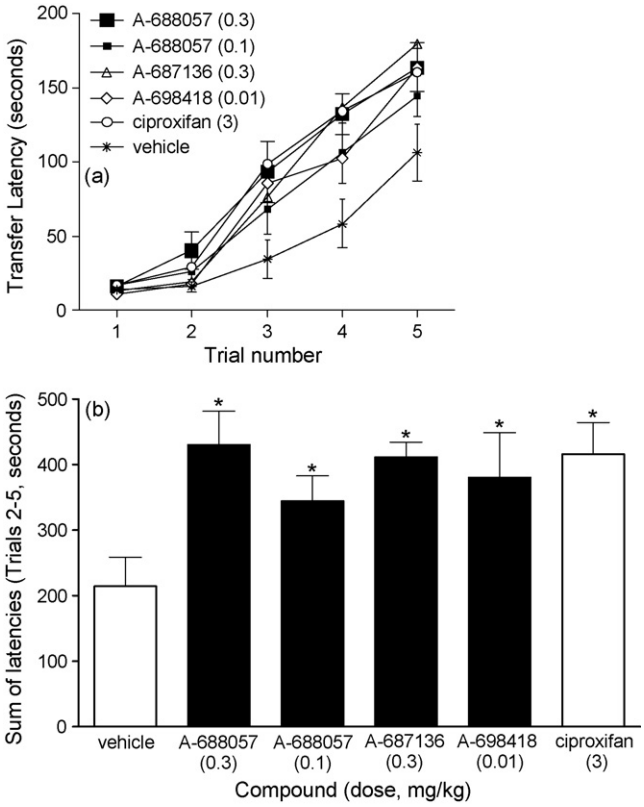


**Fig. 3 – Efficacy of histamine $H_3$ receptor antagonists in a model of attention and impulsivity, the five-trial inhibitory avoidance acquisition paradigm. Spontaneously hypertensive rat pups were dosed subcutaneously with either vehicle or drugs (A-688057 (1) at 0.1 and 0.3 mg/kg; A-687136 (2) at 0.3 mg/kg; A-698418 (3) at 0.01 mg/kg; ciproxifan at 3.0 mg/kg). (a) Compared to vehicle-treated control animals, all the histamine $H_3$ receptor antagonists increased acquisition of the avoidance over the five trials, as illustrated by the increase in time latency to transfer from the light chamber to the darkened chamber. (b) The acquisition of avoidance was expressed as the sum of the time latencies in the trials 2–5, the learning trials. All responses to the tested histamine $H_3$ receptor antagonists were significant ($p < 0.05$) versus vehicle-treated animals. Values represent mean ± S.E.M.**

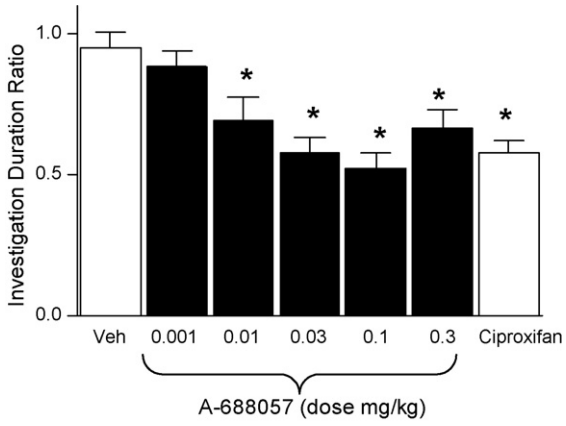


**Fig. 4 – Effect of A-688057 and ciproxifan in the social recognition memory test in adult rats. Drugs or vehicle were administered to adult rats by intraperitoneal injection. Thirty minutes later, adults were exposed to a juvenile animal, and the duration of the initial social investigation of the juvenile by the adult recorded, as described previously [20]. In vehicle-treated adults, 120 min after the initial exposure, adults forget the initial encounter and reinvestigate the juvenile for the same amount of time as the initial encounter, so that the investigation duration ratio (time spent on second contact/first contact) is 1. However, with increasing doses of A-688057 or the reference $H_3$ antagonist ciproxifan (1 mg/kg), recall of the encounter is improved, with a consequent reduction in the time spent reinvestigating the juvenile, so that the investigation duration ratio is reduced. A ratio of 0.6 indicates a significant degree of social recognition; such a ratio is seen in untreated rats when there is only a short time delay, e.g., 30 min, between the initial encounter and re-exposure. Compared to vehicle-treated adults, A-688057 and ciproxifan were both effective in reducing the investigation duration ratio, expressed as mean ± S.E.M.**

producing this efficacy, parallel experiments were carried out in 'satellite' groups of animals dosed and sampled under conditions identical to those used in the behavior study, which determined that the blood levels of A-688057 were 8.4 nM for the 0.1 mg/kg dose and 19 nM for the 0.3 mg/kg dose. Control experiments showed that A-688057 (dosed 0.03–3 mg/kg s.c.) had no effect on sensitivity to the shock stimulus.

### 3.7. *Efficacy in a model of short-term memory*

Histamine $H_3$ antagonists have been shown to enhance learning in other tests, including an assay of short-term memory, social recognition memory in adult rats [17,30]. In this task, carried out as previously described, adult rats spend time investigating an unfamiliar juvenile animal after it is first introduced; after familiarization, the time spent on investigating is reduced by a maximum of about half, a learning response which is retained through 30 min. However, after more time elapses, 2 h in this assay, the adults will reinvestigate the juvenile as if the juvenile were novel. For example, in Fig. 4, when the responses of the adult animals are expressed as the investigation duration ratio (social investigation duration at the 2 h time point divided by the investigation duration at the first introduction), it is seen that vehicle-treated adults seem to have no memory of the initial encounter so that the investigation duration ratio is about 1. However, after treatment with increasing doses of A-688057, there is an enhancement in the memory of the adults, expressed as a reduction of the investigation duration ratio. Optimal efficacy was obtained at doses of 0.03, 0.1, and 0.3 mg/kg dose, with maximal blood concentrations of A-688057 measured at 0.2, 0.71, and 3.1 nM, respectively. Other histamine $H_3$ antagonists in the benzofuran and other classes have been shown capable of achieving a comparable degree of efficacy,

**Table 3 – Pharmacokinetic properties of compounds in animals**

| Species | | Administration | | | | | |
|---|---|---|---|---|---|---|---|
| | | i.v. | | | p.o. | | |
| | | $t_{1/2}$ | $V_\beta$ | $Cl_b$ | $C_{max}$ | $T_{max}$ | $F_{p.o.}$ |
| A-688057 | Rat | 2.9 | 15.9 | 3.4 | 31 | 0.5 | 26% |
| A-688057 | Dog | 1.7 | 3.2 | 1.3 | 101 | 0.8 | 30% |
| A-688057 | Monkey | 1.8 | 4.5 | 1.7 | 14 | 4.0 | 8% |
| A-687136 | Rat | 2.6 | 12.2 | 2.7 | 25 | 0.4 | 17% |
| A-698418 | Rat | 2.3 | 9.2 | 2.8 | 8 | 1.3 | 14% |
| ABT-239 | Rat | 5.3 | 11.6 | 1.5 | 42 | 4.7 | 53% |
| ABT-239 | Dog | 8.3 | 9.3 | 0.8 | 93 | 1.8 | 74% |
| ABT-239 | Monkey | 29.2 | 12.7 | 0.3 | 104 | 5.0 | 89% |

PK properties were determined after administration of 1 mg/kg of drug to $n = 3$ animals. $t_{1/2}$ is half-life in hours; $V_\beta$ is volume of distribution (L/h); $CL_b$ is clearance (L/(h kg)); $F_{p.o.}$ is percent oral bioavailability (p.o. AUC/i.v. AUC); $C_{max}$ (ng/mL); $T_{max}$ (hours).

with potency being the greatest for ABT-239 at 0.01 mg/kg, and generally somewhat less with other compounds.

### 3.8. *Pharmacokinetic properties*

The new compounds were probed for their pharmacokinetic properties in animals (Table 3). The properties of ABT-239 are also shown for comparison. The oral bioavailability of A-688057 was fair in rat (26%) and dog (30%), but poor in monkey (8%). Administered i.v., the $t_{1/2}$ was moderate in rat (2.9 h), though this value was more than adequate to enable testing in the rodent behavioral models. However, the half-lives were shorter in dog (1.7 h) and monkey (1.8 h); to the extent that these shorter half-lives in the larger animals translate similarly into humans, a relatively short half-life in humans was deemed also likely. The oral bioavailabilities of A-687136 and A-698418 in rat were poor, 17 and 14%, respectively, though the half-lives in rat (2.6 and 2.3 h) were adequate for behavioral testing. The optimal pharmacokinetic profile of an $H_3$ antagonist may depend somewhat on the therapeutic indication. As our primary behavioral model and profiling of the present compounds targeted attention deficit disorder, much longer half-lives and better pharmacokinetic properties were targeted than were obtained with A-688057, and especially A-687136 and A-698418 in contrast to the excellent pharmacokinetic properties seen with ABT-239, a compound with high oral bioavailability (53–89%), slow clearance, and relatively longer $t_{1/2}$ (5.3–29.2 h). Although none of the new compounds had the targeted ideal pharmacokinetic profile, their behavioral efficacy justified their potential as histamine $H_3$ antagonist tool compounds. The compound with the most benign side effect profile in the general observation test, A-688057 was examined more closely for its overall drug-like properties.

### 3.9. *Physicochemical properties*

Physicochemical properties of drugs strongly influence 'drug-likeness' by controlling specific pharmacological properties such as membrane permeability and CNS penetration [38]. The lipophilicity of a compound affects the affinity for plasma proteins, as the major proteins in the blood exhibit an affinity for lipophilic substances. Lipophilicity is assessed as the octanol/water partition coefficient. With A-688057, the measured octanol–water partition coefficient $logD_{pH7.4}$ was 2.05, a favorable value for a CNS drug. Low molecular weight has been cited as an important consideration for CNS drugs, and should ideally be less than 350 amu. A-688057 meets this criterion, having a molecular weight of only 295. As a compound of moderate polarity and low molecular weight, the water solubility is high, with the free base hydrate found to have a water solubility of 0.9 mg/mL at pH 7. The $pK_a$ was $9.87 \pm 0.28$, well within the range expected for a monobasic compound. As a whole, the physicochemical properties of the compound were judged likely to favor a high free fraction of drug in vivo and minimal binding to plasma protein; this was confirmed by equilibrium dialysis experiments that showed only a moderate amount of binding to plasma proteins in all species (Table 4).

### 3.10. *Brain penetration and membrane penetration*

As A-688057 was targeted toward CNS diseases, its ability to permeate brain tissue was assessed. Dosed at 1 mg/kg i.p., brain concentrations were high (199 and 73 ng/g at 0.5 and 1 h), and the brain/blood ratio was found to be 3.4×, indicating efficient CNS penetration, a favorable profile likely arising as a consequence of the compound's moderate lipophilicity. Also consistent with the physicochemical properties of low molecular weight and moderate lipophilicity, membrane permeability was very high, $26.85 \times 10^{-6}$ cm/s ($\pm 1.35 \times 10^{-6}$ cm/s, $n = 3$), as assessed in Caco-2 membranes at pH 7.4 at drug concentration of 5 μM.

### 3.11. *Potential for drug–drug interactions*

Early imidazole-based histamine $H_3$ receptor antagonists such as thioperamide, GT-2331, and ciproxifan were found potent at

**Table 4 – Percent of A-688057 (1) bound to plasma protein, expressed as mean ± S.D.**

| Mouse | Rat | Dog | Monkey | Human |
|---|---|---|---|---|
| 63.6 ± 1.4 | 64.8 ± 0.2 | 64.5 ± 1.9 | 79.2 ± 1.1 | 87.5 ± 1.4 |

**Table 5 – Percentage inhibition turnover velocity of $CYP_{450}$ enzymes, by 2 μM or 20 μM A-688057 in human liver microsomes**

| CYP | CYP-selective substrate and metabolic process measured | Percent inhibition of substrate metabolism by A-688057 | |
|---|---|---|---|
| | | 2 μM | 20 μM |
| 1A2 | Phenacetin O-deethylation | 0.0 | 19.9 |
| 2A6 | Coumarin 7-hydroxylation | 0.0 | 0.0 |
| 2C9 | S-Warfarin 7-hydroxylation | 11.2 | 45.0 |
| 2C19 | S-Mephenytoin 4′-hydroxylation | 0.0 | 26.5 |
| 2D6 | Dextromethorphan O-demethylation | 58.9 | 71.0 |
| 3A4 | Terfenadine hydroxylation/carboxylation | 11.8 | 50.7 |
| 2E1 | Chlorzoxaone 6-hydroxylation | 0.0 | 0.0 |

rat $H_3$ receptors, and were active in vivo. However, to develop a compound as a drug for the clinic, there are many important additional considerations. Celanire et al. [39] have recently reviewed the properties and status of the most advanced histamine $H_3$ receptor antagonists. An important consideration is whether the agent interferes with the metabolism or clearance of coadministered drugs. On this point, imidazoles often potently inhibit the cytochrome $P_{450}$ ($CYP_{450}$ or CYP) enzymes important for drug metabolism. While this property may reduce the metabolism of the compound itself in the body and thereby enhance its pharmacokinetic (PK) properties, it also can induce drug–drug interactions (DDI) in the clinic by reducing the metabolism of coadministered drugs. This liability has been observed in in vitro CYP inhibition studies on ciproxifan and thioperamide [40,41], and in vivo with coadministration studies with haloperidol and risperidone reported by our laboratory [42]. The significance of this potential liability has been noted with other imidazole-containing compounds [43,44]. Thus, caution is especially advised in interpreting studies where imidazole-containing $H_3$ antagonists are combined with other drugs; conclusions of pharmacological synergism or antagonism should be viewed with suspicion in the absence of an actual determination of in vivo drug levels, lest pharmacokinetic interactions be misinterpreted as pharmacological interactions. A-688057 was assessed for its ability to inhibit the microsomal metabolism of a battery of drugs predominantly metabolized by specific CYP subtypes (Table 5). A-688057 did not significantly block turnover via the major CYPs examined at 2 μM, except for some inhibition of the CYP 2D6 isoform, and only at a much higher concentration (20 μM) was the compound shown to significantly inhibit other CYP enzymes. This inhibition was hypothesized to be due to the heterocyclic moiety (pyrazole) on A-688057, since ABT-239 has an otherwise similar structure (but no aromatic heterocyclic moiety) and was found to not inhibit CYPs at all at 2 μM. A follow-up study of A-688057 established an $IC_{50}$ of 870 nM at CYP 2D6; it should be noted that this concentration is substantially above the behaviorally effective drug concentration of 0.2–19 nM previously discussed, greatly reducing the probability for significant drug–drug interactions.

### 3.12. *Toxicity in preclinical tests*

There are a number of early predictors of the potential toxicity of compounds. An earlier compound from our group, A-331440, was found to be genotoxic in an in vitro assay measuring clastogenic potential by micronucleus formation [45]. Unlike A-331440, in vitro tests on A-688057 did not uncover any potential for genotoxicity. Even at the highest drug concentrations tested (0.03–1 mM), there was no significant increase in multinucleated cells on drug treatment, as assessed in the micronucleus formation assay in V79 Chinese hamster lung cells, and in another parallel assay run in the presence of the S-9 fraction of Aroclor-induced rat liver. Likewise, no drug-induced signals of risk of mutagenicity by A-688057 or its metabolites were seen in the Ames test, run in *Salmonella typhimurium* strains TA98 and TA100; the same finding also held for the Ames test run in the presence of the S-9 fraction of Aroclor-induced rat liver. Micronucleus and Ames test assays were run as previously described [45].

Some of the especially potent early non-imidazole histamine $H_3$ receptor antagonists were diamines [39,46,47], compounds with two basic amine moieties in the molecule. Examples include Aplysamine and JNJ-5207852 [34], early Abbott compounds A-331440 [45] and A-304121 [12], and one subset of benzofuran antagonists [48]. We and others have noted that at least some members of this class of molecules

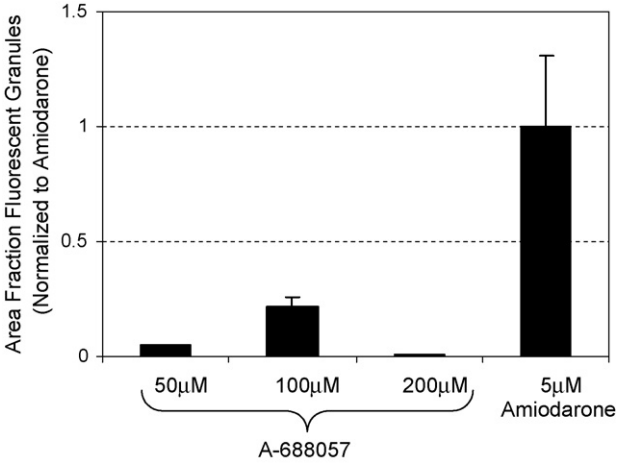


**Fig. 5 – In vitro assessment of potential to induce phospholipidosis in rat hepatocytes by A-688057. There was no diminution of cell viability up to 200 μM, and no increase in the number of fluorescent granules when A-688057 was incubated with fluorescent phospholipids [47]. In contrast, the positive control Amiodarone was a potent inducer of phospholipidosis.**

have an enhanced liability to induce phospholipidosis, as measured in in vivo or in in vitro assays [48,49]. This has been noted for lipophilic amines (otherwise known as CADs, or cationic amphiphilic drugs) in general [50]. This topic has been reviewed by Reasor et al. [51]. As can be seen in Fig. 5, A-688057, at all concentrations tested (50–200 μM), was found to not induce phospholipidosis in an in vitro assay [48,49], in contrast to the positive control (5 μM amiodarone).

## 4. Conclusions

Finding compounds with potent $H_3$ antagonism in in vitro assays has been demonstrated to be readily achievable, judging by the number of reports from academic and industrial groups [39]. All the new compounds described, A-688057, A-687136, and A-698418, are highly selective and potent in vitro at rat and human $H_3$ receptors. For all three of the new compounds, the high in vitro potency and selectivity for the target $H_3$ receptor also translated into in vivo efficacy in the primary behavioral model, five-trial inhibitory avoidance acquisition.

Of the three new compounds, A-688057 produced the fewest adverse effects in the general observation test, fewer even than ABT-239. Because of this, A-688057 was profiled more extensively. In addition to low nanomolar binding affinity at histamine $H_3$ receptors, potent functional antagonism and inverse agonism were demonstrated in vitro and in *ex vivo* assays. The compound showed good CNS penetration (blood/brain 3.4×), and excellent physicochemical properties (solubility, stability, crystallinity, lipophilicity). In vitro tests uncovered no concerns for potential toxicity and little propensity for drug–drug interactions. A notable feature of A-688057 was its low potential to inhibit the hERG $K^+$ channel ($K_i > 9000$ nM in vitro in the dofetilide competition binding assay), contrasting the much lower selectivity of ABT-239 for the hERG channel ($K_i = 195$ nM). A-688057 was effective in the five-trial inhibitory avoidance acquisition animal model at low doses (0.1 and 0.3 mg/kg), at circulating levels of 8.4 and 19 nM, respectively, at the time of behavioral testing. Taking into account the rat plasma protein binding (64.8% bound, 35.2% free), it can be estimated that behavioral efficacy was seen at free drug levels around 3–6.7 nM. These concentrations are comparable to the $K_i$ value of the compound for the rat histamine $H_3$ receptor ($K_i = 8.5$ nM). Using the method of Gillard et al. [52], it can be projected that at these concentrations, the fractional occupancy of the rat $H_3$ receptor ($K_i = 8.5$ nM) was minimally 26–44%.

Producing highly drug-like molecules is a demanding process. It requires that potent in vitro and in vivo activity be retained while at the same time optimizing for the many properties required of potential candidates for clinical use [12,39,46,47]. For most disease targets, excellent PK properties are sought to permit once daily drug dosing. On this point, all three of the new compounds (A-688057, A-687136, and A-698418) failed to satisfy this ideal; although their PK profiles were well suited for testing in acute rodent efficacy models, they failed to match the excellent oral bioavailability and long half-life seen with the earlier compound ABT-239 (Table 3). However, based on its overall profile of high potency and efficacy in behavioral models at doses much lower than doses producing CNS side effects, and its high in vitro selectivity, A-688057 was judged an excellent pharmacological tool compound useful for behavioral studies.

## Acknowledgements

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