AAT-009: 5-HT4 Partial Agonist

A Novel Therapy for Cognitive Dysfunction Syndrome in Pet Animals

ASKAT

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AskAt Inc.

Non-Confidential Information

Summary of CDS and AAT-009

Cognitive dysfunction syndrome (CDS)

- A cognitive dysfunction (CD) in senior pet animals causes abnormal behavioral symptoms such as change in life rhythm and activity, incontinence, and barking in the night etc., CDS, that harms QOL of the pets and their owners
- Mechanism of etiology of each CDS symptoms is not identified, donepezil/Aricept® which has cholinergic mechanism of action, was shown to improve certain CD-related behavioral symptoms in dogs

AAT-009, a brain-penetrative 5-HT4 receptor (5-HT4) partial agonist

- 5-HT4 agonist has pro-cognitive, antidepressant and anxiolytic efficacy mainly through a cholinergic mechanism in animals[1]
  - AAT-009 does not increase baseline brain ACh level unlike donepezil, but it enhances ACh signal only when cognitive behavior is triggered. This feature will contribute significantly to drug safety
- AAT-009 is expected to be a therapeutic for CD-related symptoms through cholinergic mechanism
  - A pro-cognitive and other CNS-related symptoms-suppressive efficacy of AAT-009 indicates the potential to normalize abnormal behavioral symptoms of CDS
  - Safety and tolerability of AAT-009 were confirmed in GLP and non-GLP studies in dogs as well as in a human Phase 2

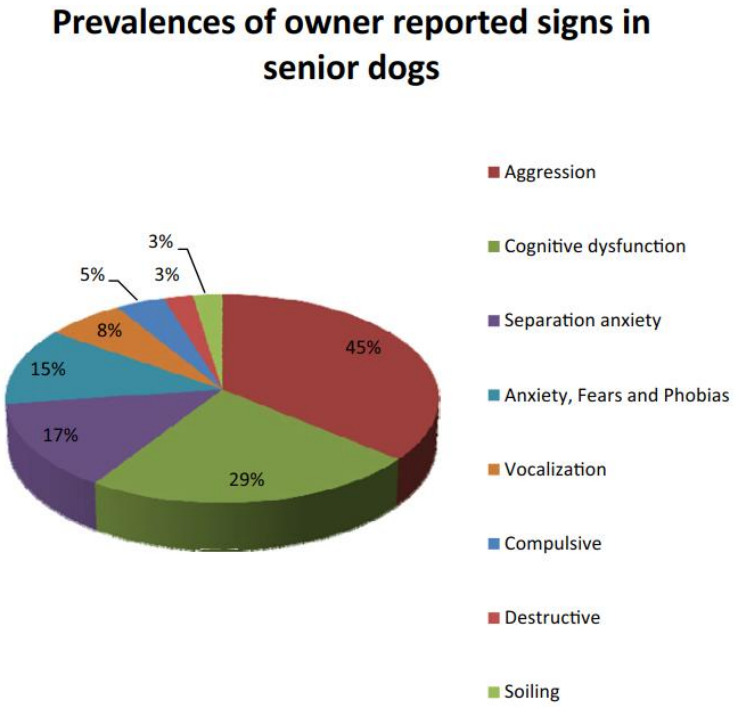
A small pilot field trial testing efficacy for specific behavioral conditions in CDS dogs can identify the endpoints in subsequent pivotal field study and Investigational New Animal Drug (INAD) strategy

1. *Int. J. Mol. Sci.* 2024, *25*(10), 5245; https://doi.org/10.3390/ijms25105245

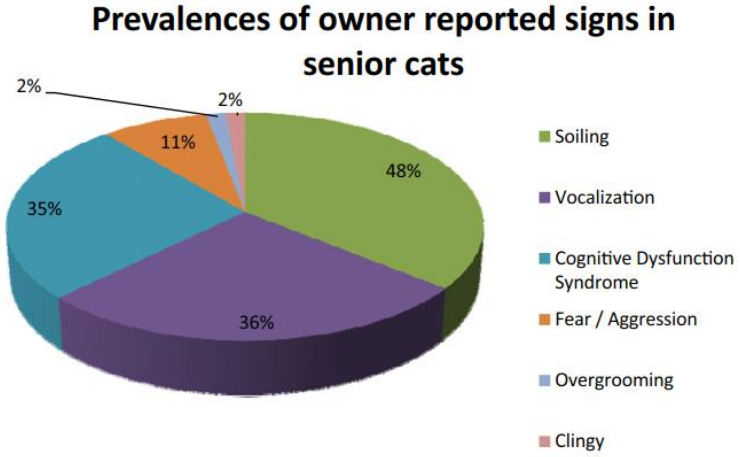
Prevalence and Signs of Cognitive Dysfunction Syndrome (CDS) in Pets

- The prevalence of CDS is extremely high in elderly dogs and cats [1,2]
  - 30% in 11- to 12-year-old dogs, and 70% in 15- to 16-year-old dogs develop CDS
  - ~35% in >11-year-old cats, and 50% in over 15-years develop CDS

1. MSD Veterinary Manual, Behavioral problems in Dogs and Cats
2. JAVMA 2001 218(11):1787-1791; Vet Rec 2021;e3, https://doi.org/10.1002/vetr.3



Fears and phobias (includes generalized anxiety), compulsive includes repetitive and stereotypic behavior; cognitive dysfunction includes disorientation, wandering, waking and anxious at night. Behavior signs were combined from 3 studies: a Spanish study of 270 dogs older than age 7 that were presented for behavior problems, 103 dogs referred to a veterinary behaviorist, and a search of the Veterinary Information Network (VIN) of 50 dogs aged 9 to 17 years.



Soiling includes marking, cognitive dysfunction includes disorientation, restless, wandering and night waking, and fear/aggression (includes fear and hiding). Behavior signs were combined from a VIN data search of 100 cats aged 12 to 22 years and 83 from 3 different behavior referral practices.

Vet Clin Small Anim 42 (2012) 749 –768 doi:10.1016/j.cvsm.2012.04.003

Current Therapy for CDS and Positioning of AAT-009

- Current therapeutics
  - Selegiline (Anipryl®, Zoetis, US), nicergoline (Fitergol®, Merial Italia SPA, EU) and propentofylline (Vivitonin®, MSD AH, EU) are used to treat the clinical sign of CDS in dogs while no drugs for cats[1]
    - Selegiline is a monoamine oxidase B (MAO-B) inhibitor.
    - Nicergoline is an alpha 1 and alpha 2 adrenergic (α1/α2) agonist. [2]
    - Propentofylline is a phosphodiesterase (PDE) inhibitor. [1]
  - Existing drugs are effective only for a limited kinds of CDS symptoms and have not shown stable therapeutic effects
    - Selegiline exhibited improvement in sleep and housetraining, while no efficacy in orientation, responsiveness, or greeting[3], and the duration of efficacy was as short as 8 weeks for about half of the cases.
    - Nicergoline exhibited improvement in sleep, appetite and awareness, while no efficacy in incontinence/house training[2]
- AAT-009 may offer an effective treatment for abnormal behaviors that the existing drugs do not respond to
  - The mechanisms of the current drugs are adrenergic stimulation or PDE inhibition, while that of AAT-009 is cholinergic enhancement through 5-HT4 activation.
  - A study of donepezil, which has cholinergic mechanism shared with AAT-009, in CDS dogs improved behavioral symptoms such as life rhythm, recoil, walking condition, posture, and vocalization at night.

1. VetRecord 2021;e3 DOI: 10.1002/vetr.3
2. From the journal Proceedings of the First International Conference on Veterinary Behavioral Medicine, Birmingham, UK, April 1 & 2 1997; use of nicergoline in the reversal of behavioral changes due to ageing in dogs: a multicenter clinical field trials [1997]
3. FOI Anipryl https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/614

Mechanism of Action of AAT-009 and Dog Cognitive Dysfunctions

- AAT-009 activates brain ACh signaling through 5-HT4 signal activation of cholinergic neuron
  - 1 mg/kg PO single dosing increased hippocampus ACh level in the rats
  - 0.1 ~ 1 mg/kg PO single dosing demonstrated the enhancement of cognitive functions in mice behavioral models
  - Though donepezil increased baseline brain ACh level after dosing, AAT-009 amplified brain ACh signal associated with cognitive function. This feature will not produce the neurogenic side effects that are problematic with donepezil and contribute significantly to drug safety

- Brain ACh and dog CD-related symptoms
  - Cholinesterase inhibitor improved both memory and learning in aged dogs

    Progress in Neuro-Psychopharmacology & Biological Psychiatry 29 (2005) 411– 422
    Journal of Alzheimer's Disease 26 (2011) 143–155

  - Donepezil therapy improved abnormal behaviors in dogs with CDS (page 14)
    - Donepezil HCl (Aricept®) , 0.1 ~ 0.17 mg/kg QD, was administered to twelve dogs which had been diagnosed with CDS. After two weeks therapy, several behavioral scores were significantly improved.

      Journal of Animal Clinical Medicine 19 (2010) 91-93
      https://www.jstage.jst.go.jp/article/dobutsurinshoigaku/19/3/19_3_91/_article/-char/en

Acta Neuropathol 103 (2002) 228-236
Animals 11 (2021) 2584. doi: 10.3390/ani11092584
Front. Vet. Sci. 7 (2020) 551895. doi: 10.3389/fvets.2020.551895

Expected Position of AAT-009 in the Current Drug Therapy for CDS

- AAT-009 is expected to improve abnormal behavioral symptoms for which Aricept has shown efficacy

Drug	CDS Abnormal Behavioral Symptoms Reported to be Effective by the Drug Therapy
Aricept® * **(Cholinergic)**	• **Life rhythm** • **Recoil** • **Walking condition** • **Posture** • **Vocalization**
Anipryl® ** **Fitergol® **** **(Adrenergic mechanism)** **Vivitonin® **** **(PDE inhibitor)**	• **Orientation / awareness** • **Activity** • **Sleep** • **House training** • **Collapse/fits/ stroke-like symptom** • **Reduced appetite**

* Approved for Alzheimer's disease therapy in human
** Approved for dog CDS or CDS-related conditions

Pharmacology and Safety of AAT-009

- 5-HT4 signal is a modulator of hippocampal synaptic information processing and cognition
  - Pro-cognitive effects of 5-HT4 agonists were demonstrated in animals [1]
  - A 5-HT4 agonist PRX-03140 demonstrated therapeutic efficacy in AD patients [2]
  - Pro-cognitive effects of 5-HT4 agonist were shown in human[3,4]
- AAT-009 activates 5-HT4 signal *in vitro* and *in vivo*, and is highly penetrative to the brain
  - A partial agonist with potent efficacy *in vitro*; EC_{50} for human $5\text{-}HT_{4a}$ receptor is ~0.1 nM
  - Brain distribution; $AUC_{inf,brain}/AUC_{inf,plasma}$ = 8.84 (PO in Mice)
- AAT-009 significantly induces brain ACh level and inhibits Aβ accumulation
  - 0.1 ~ 1 mg/kg PO single dosing demonstrated the enhancement of cognitive functions in mice behavioral models
  - 1 mg/kg PO single dosing increased hippocampus ACh level in the rats
  - 1 and 10 mg/kg PO 3-week dosing inhibited Aβ accumulation in the Tg2576 mice brain
- Pharmacological efficacy of AAT-009 in GI and GLP safety studies were conducted in Dogs
- AAT-009 has limited potential of GI side effects, and no CV side effects in Dogs
- Dose of AAT-009 in dogs is estimated to be 0.1 – 0.3 mg/kg PO, QD which is lower than peripheral use

1 Neurobiology of Learning and Memory (**2017**) 8:145-153 https://doi.org/10.1016/j.nlm.2016.06.014
2. https://www.alzdiscovery.org/uploads/cognitive_vitality_media/PRX-03140-Cognitive-Vitality-For-Researchers.pdf
3. Translational Psychiatry (**2021**)11:497 ; https://doi.org/10.1038/s41398-021-01568-4
4. Psychological Medicine (**2020**) 50:2722-2730 https://doi.org/10.1017/S0033291719002836

Development Overview for CDS Therapy

- Target Indication
  - Cognitive dysfunction syndrome (CDS) –related abnormal behaviors in dogs and cats [1]
    - » CDS is consisting of various abnormal behaviors and conditions that may result from different neuronal changes
- Unmet Medical Needs
  - Existing drugs are not sufficient for the treatment of CDS-related behavior
  - Drugs are needed to control abnormal behaviors that significantly impair the QOL of pets and their owners
- Mechanism Background of ACh signal control by 5-HT4 Agonist for CDS Therapy in Pets
  - Several 5-HT4 agonists exhibited therapeutic efficacy in CD-related symptoms in animals and humans [3]
  - Donepezil improved abnormal behaviors in dog CDS [4]
  - 5-HT4 receptor partial agonist PRX-03140 was shown efficacy in a clinical trial for Alzheimer's disease [2,3]
- Pre-clinical Data of AAT-009
  - Exhibits efficacy superior to donepezil in standard models of cognitive impairment in mice
  - Enhances memory task-dependent hippocampus ACh signal in mice
  - Decreases Aβ accumulation in Tg2576 mice
  - GI pharmacology and GLP safety studies are conducted in Dogs
  - Therapeutic dose in dogs is estimated to be 0.1 – 0.3 mg/kg PO, QD which is lower than peripheral use
- Intellectual Property of AAT-009
  - Dementia use (~2031 Feb + PTE)
  - Opportunity to file a new patents for animal health use

1. J Vet Intern Med 2013;27:822–829; 2. Animals 2021, 11, 2584; 3. https://www.alzdiscovery.org/uploads/cognitive_vitality_media/PRX-03140-Cognitive-Vitality-For-Researchers.pdf
4. J. Anim. Clin. Med., 19: 91-93, 2010

Field Study Strategy for CDS Therapy

- Background
  - AAT-009 and donepezil (DPZ) exhibited pro-cognitive effect in rodents by increasing brain ACh signal
  - Two-week DPZ therapy in CDS dogs improved scores of several types of abnormal behaviors [1]
- Hypothesis
  - AAT-009 has pro-cognitive efficacy and normalizes abnormal behaviors of CDS dogs
- Non-clinical safety tested in dogs
  - 1-month multiple dosing general toxicity : NOAEL = 6 mg/kg, PO
  - Negative in genotoxicity, no QTc changes up to 5 mg/kg in dog, no significant effects in dog purkinje assay
- Drug product development
  - PO drug product for human was developed
- A pilot field study will identify targeted behavioral symptoms
  - To identify behavioral symptoms to be focused on the pivotal field study
    - Dose of AAT-009 in dogs will be 0.1 – 0.3 mg/kg PO, QD
    - Test efficacy for the several CD-related behavioral conditions in CDS dogs based on the DPZ study [1]
    - Confirm safety in senior dogs
- Pivotal field study testing specific behavioral symptoms selected in a pilot field study
  - Therapy of abnormal behavioral conditions in CDS dogs

1. J. Anim. Clin. Med., 19: 91-93, 2010

IP Status

Patent	Int'l. Publication Number (Int'l. Application Number)	Int'l. Filing Date (Normal Expiration Date)	Status as of January 28, 2026
AAT-009 (Substance)	WO 2005/021539 (PCT/IB2004/002741)	August 20, 2004 (August 20, 2024)	Expired
AAT-009 (Use: Dementia)	WO 2011/099305 (PCT/JP2011/000793)	February 14, 2011 (February 14, 2031)	Granted: BR, CA, EP (FR, DE, GB, IT, ES), JP, KR, MX, RU, US

- Opportunity to file a new patents for animal health use

Competitor, 5-HT$_4$ Agonists for Dementia Therapy

As of June 2024

Phase 1	Phase 2	Phase 3, NDA	Launch
Usmarapride SUVN-D4010 [1] (Suven)	–	–	–

- No competitor of 5-HT4 agonist for animal CDS as far as we know
- Usmarapride, a 5-HT4 agonist, is a Phase 1 completed candidate for human AD use
  - Increase of ACh, cortical sAPPα, potentiation of pro-cognitive effects of donepezil, antidepressant-like effects are demonstrated in preclinical rodent models
  - Phase-1 clinical studies (NCT02575482, NCT03031574) completed [2]

1. http://www.suven.com/pdf/Usmarapride%20(SUVN-D4010),%20Cognitive%20Disorders.pdf
2. https://link.springer.com/article/10.1007/s40261-021-01027-4

APPENDICES

1. Behavioral Changes Related to CDS
2. Efficacy of Donepezil in Dog CDS
3. Current Drugs: Anipryl / Fitergol / Vivitonin

1. Behavioral Changes Related to CDS

CDS-related abnormal behavioral conditions in geriatric dogs and cats

Confusion/Spatial Disorientation

- Gets lost in familiar locations
- Goes to the wrong side of the door (where the hinge is)
- Gets stuck and can't navigate around or over obstacles

Relationships/Social Behavior

- Less interested in petting, interactions, greeting people or other dogs, etc.
- Needs constant contact, becomes overdependent and clingy

Activity-Increased or Repetitive

- Stares, fixates on or snaps at objects
- Paces or wanders about aimlessly
- Licks you, family members or objects a lot
- Vocalizes more
- Eats more food or eats more quickly

Activity-Decreased, Apathetic

- Explores less and responds less to things going on around him
- Grooms himself less
- Eats less

Anxiety/Increased Irritability

- Seems restless or agitated
- Anxious about being separated from family members
- Behaves more irritably in general

Sleep-Wake Cycles/Reversed Day-Night Schedule

- Sleeps restlessly, awakens at night
- Sleeps more during the day

Learning and Memory-House Soiling

- Eliminates indoors in random locations or in view of you or family members
- Eliminates indoors after returning from outside
- Eliminates in sleeping areas (for example, in his crate or on the couch or floor)
- Uses body language less (body postures and signals associated with feelings)
- Develops incontinence (accidental release of bladder)

J Vet Intern Med 2013;27:822–829
https://pets.webmd.com/dogs/guide/behavior-problems-older-dogs#9

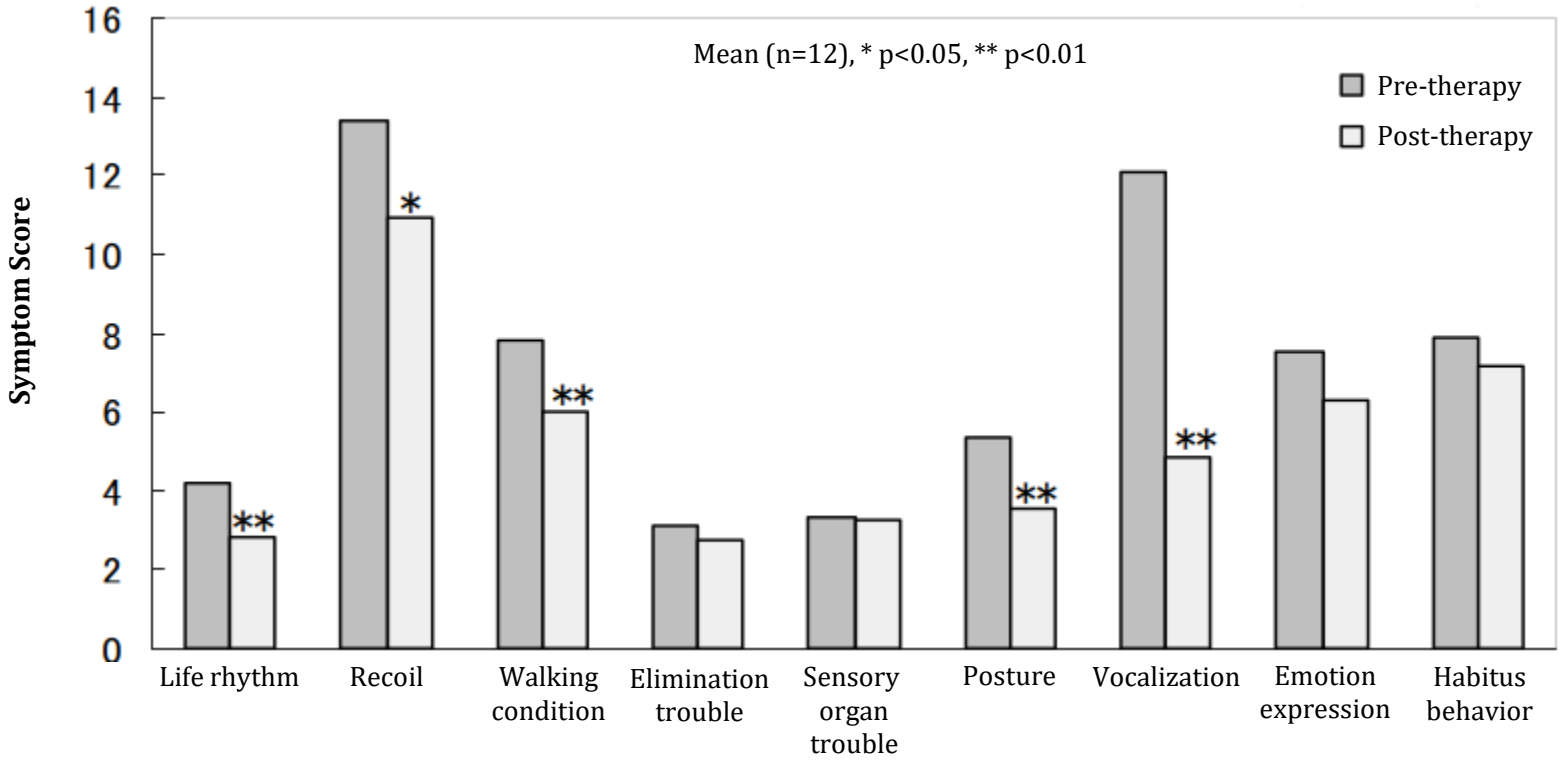
2. Donepezil Therapy Improved Abnormal Behaviors in Dog CDS

Efficacy of Donepezil Hydrochloride in Canine Cognitive Dysfunction Syndrome

Matsunami N., Koizumi K., Fukatsu C., Yasuda K., and Fukatsu K.

J. Anim. Clin. Med., 19: 91-93, 2010 (Japanese), https://www.jstage.jst.go.jp/article/dobutsurinshoigaku/19/3/19_3_91/_pdf/-char/en

Donepezil HCl (Aricept) , 0.1 ~ 0.17 mg/kg QD, was administered to twelve dogs which had been diagnosed as showing CDS. In two weeks, the total score counted by the criteria was improved and particularly the score of "midnight vocalization" showed a predominant improvement.



3. Current Drugs for CDS

Drug		Anypril[1] / Zoetis	Fitergol[2] / Merial Italia SPA	Vivitonine[3] / MSD
Country / Area		US	EU	EU and Australia
Indication		Clinical signs associated with canine cognitive dysfunction syndrome (CDS) and control of clinical signs associated with uncomplicated canine pituitary dependent hyperadrenocorticism (PDH).	Behavioral disorders related to dog senescence; adynamy, asthenia, fatigue, reduced activity and attention, loss of appetite, locomotor disorders of vascular origin, cerebral insufficiency of vascular origin, etc.	Conditions associated with the aging process in dogs, such as senility, lethargy, tiredness, apathy, stiff gait, difficulty in standing up and walking, lack of appetite, wasting, alopecia, dull coat.
Animal		Dog	Dog	Dog
Mechanism		Monoamine oxidase (MAO)-B inhibitor	Alpha 1 and alpha 2 adrenergic agonist	Phosphodiesterase (PDE) inhibitor
Pharmacology		Enhancing dopamine and other catecholamines in the cortex and hippocampus and by decreasing free radical load	Increase cerebral blood flow and oxgen supply, neuroprotective effects on neurons, and inhibits platelet aggregation	Increase the blood flow, particularly of the heart and skeletal muscle, and the brain and therefore its oxygen supply, without increasing the brain's glucose demand.
Field Trials		Study 1: Placebo controlled, multi-site, dose range clinical field trial	Multi-centre field trial in the UK	
	Animals and Diagnosis	Study 1 199 client-owned dogs with more than 3 symptoms of CDS Study 2 73 client-owned dogs with CDS with clinical or behavioral signs	The trial included 109 dogs showing behavioral changes associated with ageing.	
	Dosage and Study Duration	Study 1 0.2 (N=65) and 1.0 (N=67) mg/kg, placebo (N=67) QD, PO. 4 weeks + 8 weeks (1.0 mg/kg) Study 2 0.5 mg/kg PO. QD, 1.0 mg/kg only for 3 dogs PO, QD. for 3 months	0.5 mg/kg QD. PO., for 1 month.	
	Endopoints	Study 1 The owners' assessments of changes in behavior: orientation, activity, sleep pattern, housetraining, responsiveness, and greeting behavior. Study 2 Changes in the behavious and clinical signs	A full clinical examination was carried out by the veterinary surgeon at the start of the trial, including a questionnaire on all aspects of the behavior and medical history of the dog to be completed in conjunction with the owner. At the end of the one-month trial, the owner was asked to return the dog for a further full examination and comparison of clinical signs with the previous month.	
	Results	Study 1 Significant improvements were observed in sleep pattern, housetraining, and activity in 4 and 12 week therapy. The duration of effect may be as short as 8 weeks in about 50% of the cases. Study 2	89 dogs completed the trial. Overall improvement was 76.5%. Efficacy was observed for activity, sleep disorders, episodes of collapse/fits/stroke-like symptoms, house training, appetite loss, awareness.	

1. Anipril FOI
2. Fitergol
3. Vivitonin MSD AH

AAT-009 Information

5-HT_4 Receptor Partial Agonist
AAT-009

1. Compound Information

- Compound code
  - AAT-009
- Structure
  - Small molecule
- Intellectual properties
  - Dementia use patent is granted in the major country including the seven major markets (7MM)
- Chemistry, manufacturing and control (CMC)
  - Active Pharmaceutical Ingredient:
    - No major issue in the bulk campaign to provide ~1.8 kg
    - No significant stability issue have been observed (52 weeks, 25 ˚C/60%RH)
  - Drug product:
    - Highly soluble and permeable
    - Film coated tablets for clinical studies
    - No stability issue (12 weeks, 25 ˚C/60%RH)

2. Non-clinical Pharmacology

- Binding affinities to human 5-HT subtypes

K_i (nM)	AAT-009	PRX-03140 [1]
5-HT_4	0.90*	31**
5-HT_{1A}	>4900	>5000
5-HT_{1B}	>5600	>5000$
5-HT_{1D}	>4300	>5000$$
5-HT_{2A}	>5800	>5000
5-HT_3	1700***	>5000
5-HT_7	>4800	>5000

* 5-HT_{4d}, ** 5-HT_{4a}, *** 5-HT_{3A}, $ Rat, $$ Bovine

Highly selective and potent affinity to the human 5-HT_4 receptor

2. Non-clinical Pharmacology

- Functional activities

Assay		AAT-009		PRX-03140	
		EC_{50} (nM)	E_{max}* (%)	EC_{50} (nM)	E_{max} (%)
Human 5-HT_4 cAMP formation	5-HT_{4a}[1]	0.092	86	15–93[2]	41–53[2]
	5-HT_{4b}[1]	0.19	101	18–42[2]	39–59[2]
	5-HT_{4d}	1.3	70	NT**	NT
Rat TMM ***		2.0	77	NT	NT

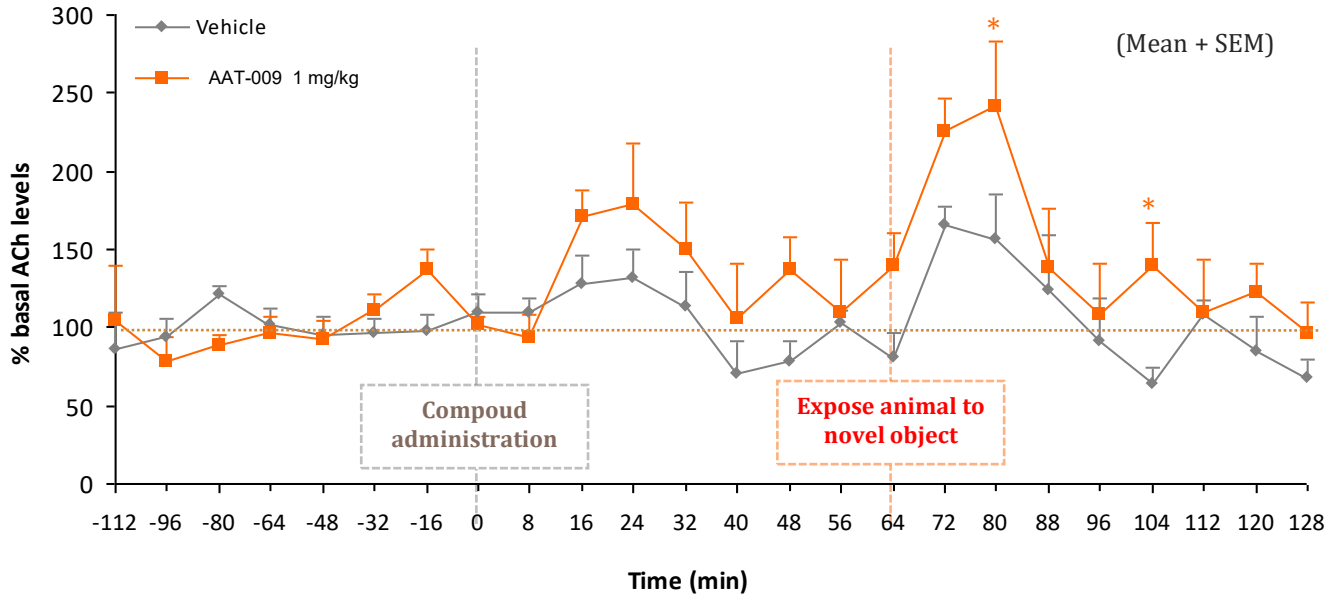
* E_{max}: % of 5-HT maximal response
** NT: Not tested
*** TMM: Tunica Muscularis Mucosae (inhibition of 5-HT-induced contraction)

AAT-009 demonstrated potent agonist activity on human and rat 5-HT_4 receptors and was >100-fold more potent than PRX-03140

(1) Highly expressed in brain
(2) Neuropharmacology, **53** (2007) 563-573

2. Non-clinical Pharmacology

- Change of hippocampus ACh level in rats (Microdialysis)



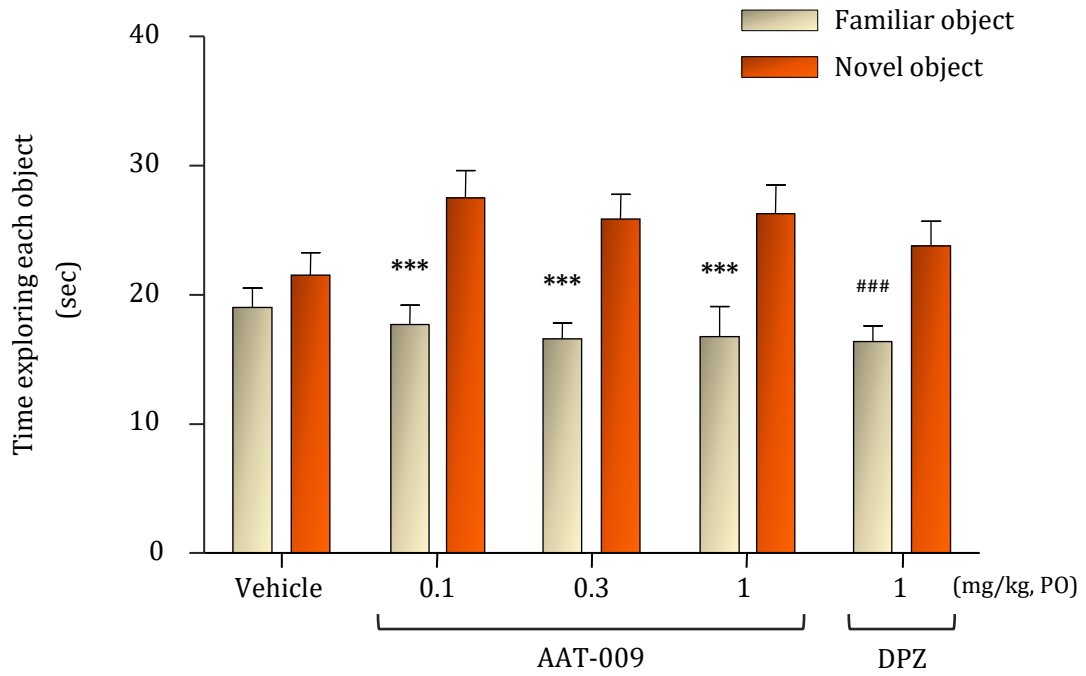
During the test periods, probe-implanted SD rats (N = 9) were placed in test arena. AAT-009 (1 mg/kg, PO) or vehicle were dosed at Time 0, and then rats were exposed to a novel object at Time 64. Although there was no difference of general effect on ACh levels between AAT-009 and vehicle, AAT-009 induced a significantly higher ACh level compared to vehicle group when animals were exposed to a novel object at Time 80 and 104.

*P<0.05; vs. vehicle (two-way ANOVA for repeated measurements followed by Student Newman Keuls post-hoc test)

AAT-009 stimulated hippocampus ACh release during a novelty-seeking behavior

2. Non-clinical Pharmacology

- Novel object recognition test – Time exploring each object



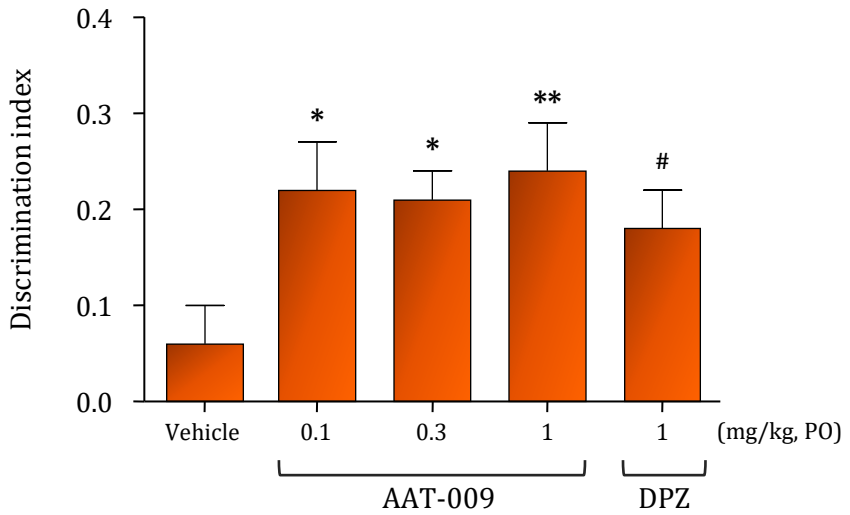
Each value represents the mean + SEM (N = 15)

AAT-009: two-way ANOVA followed by Tukey's multiple comparison test (***P<0.001; vs. novel object)
Donepezil (DPZ): paired t-test (###P<0.001; vs. novel object)

Significant difference of time spent exploring the novel and familiar objects was demonstrated for drug treated groups

2. Non-clinical Pharmacology

- Novel object recognition test – Discrimination index



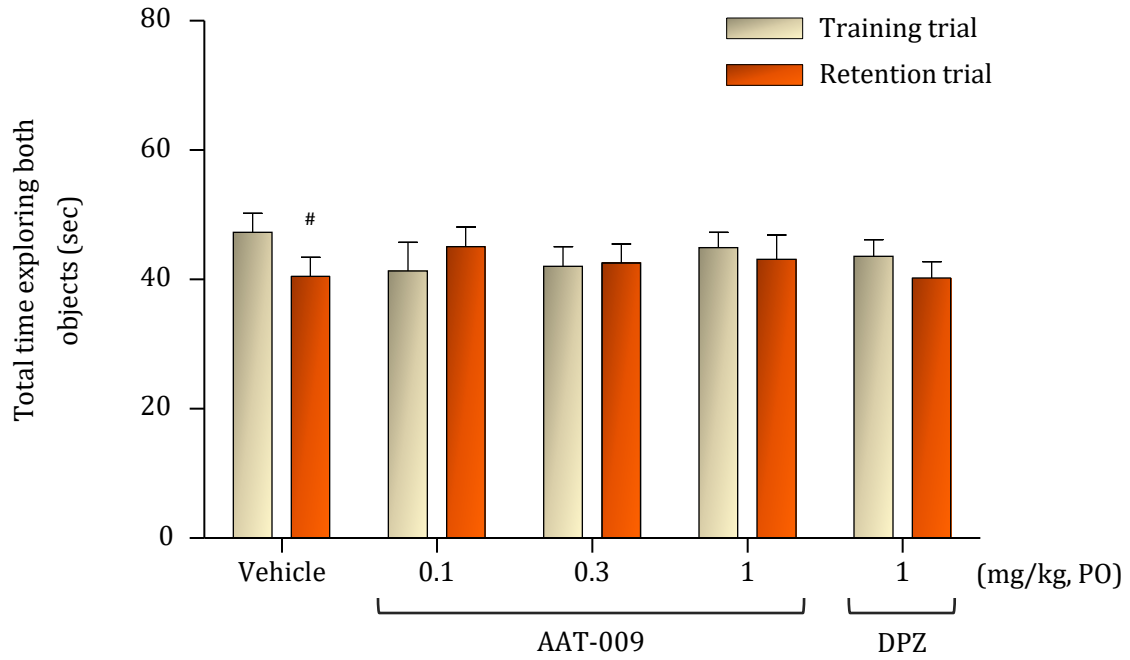
Discrimination index is defined as the difference in exploration time for the objects divided by total exploration time.
Each value represents the mean + SEM (N = 15).

AAT-009: Bartlett's test followed by Dunnett's multiple comparison test (*P<0.05, **P<0.01; vs. vehicle)
Donepezil (DPZ): F-test followed by Student's t-test ($^{\#}P$<0.05; vs. vehicle)

AAT-009 significantly enhanced novel object recognition at
0.1 ～ 1 mg/kg, PO, in rats

2. Non-clinical Pharmacology

- Novel object recognition test – Total exploring time



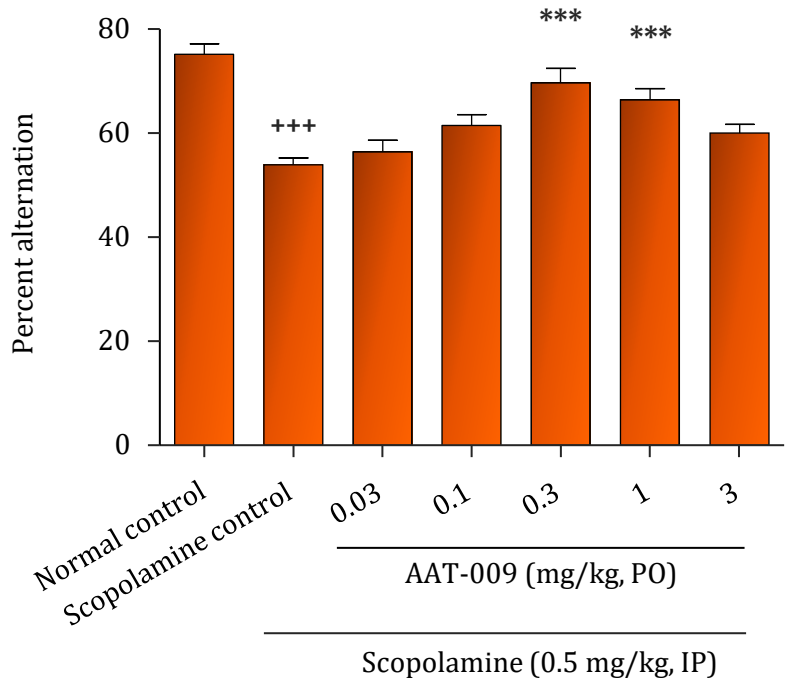
Each value represents the mean + SEM (N = 15).

AAT-009: two-way ANOVA followed by Tukey's multiple comparison test (vs. vehicle, training vs. retention)
Donepezil (DPZ): unpaired t-test (vs. vehicle), paired t-test (training vs. retention)
Vehicle: paired t-test ($^{\#}P<0.05$; vs. training trial)

Total time spent exploring was similar in all treatment groups and trials

2. Non-clinical Pharmacology

- Scopolamine-induced impairment of spontaneous alternation in rats



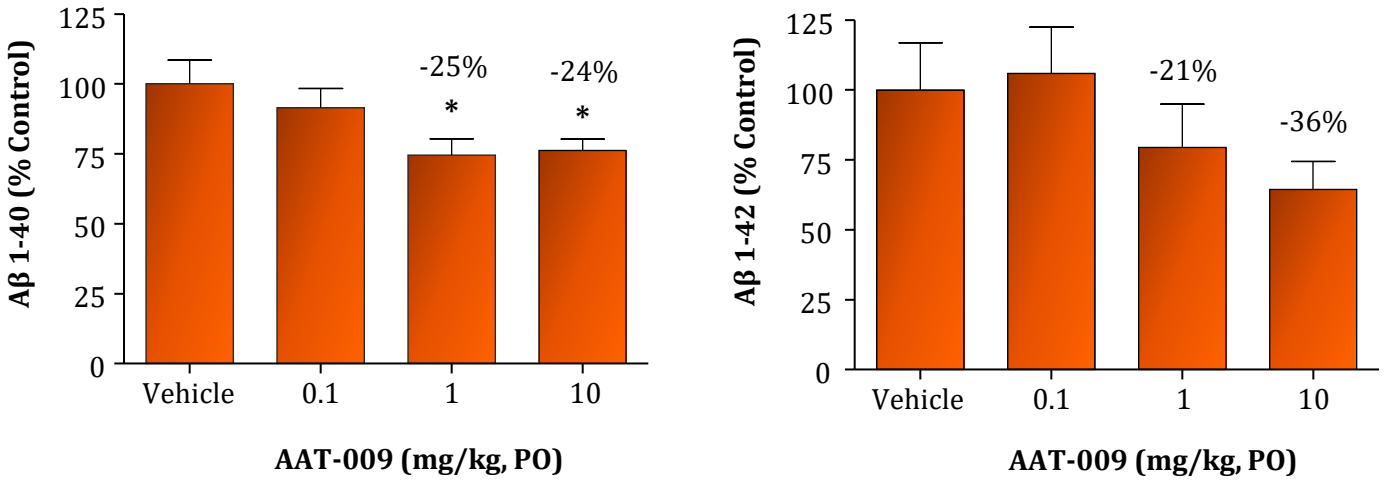
Each value represents the mean + SEM (N = 15).

$^{+++}P<0.001$; vs. Normal control (Student's t-test), $^{***}P<0.001$; vs. Scopolamine control (Dunnett's test)
No significant change of total arm entry between each treatment group was observed.

AAT-009 significantly recovered scopolamine-induced impairment of spontaneous alternation at 0.3 and 1 mg/kg, PO

2. Non-clinical Pharmacology

- Aβ level in Tg2576 mice



Tg2576 mice (31-weeks old at the beginning of the experiment) were dosed orally twice a day with AAT-009 at doses of 0.1, 1 and 10 mg/kg for 3 weeks.

Each value represents the mean + SEM (N = 9–10).

*P<0.05; vs. vehicle control (one-way ANOVA followed by Dunnett's post-hoc analysis)

AAT-009 dose-dependently reduced $A\beta_{1-40}$ and $A\beta_{1-42}$ in the cortex

3. Non-clinical Pharmacokinetics

- Oral bioavailability
  - Rats: 19.9–27.9%, Dogs: 44.1–63.4 %
- Brain penetration in rats
  - $AUC_{inf,brain}/AUC_{inf,plasma}$ = 8.84 (1 mg/kg, PO)
  - Higher brain penetration than PRX-03140 (1 hr after dosing)

Compounds (1 mg/kg SC)	Brain conc. (ng/g)	Brain/plasma ratio
AAT-009	175	3.24
PRX-03140	70.1	1.54

- Drug-drug interaction
  - IC_{50}: >30 μM against cytochrome P450 enzymes (1A2, 2C9, 2C19, 2D6, 3A4)
- Major clearance route
  - Hepatic metabolism: confirmed by rats

AAT-009 exhibited high CNS penetration

3. Non-clinical Pharmacokinetics

Study Type and Description		Route of Administration	Species/Cell line
Absorption	Single dose	IV/PO	Rat, Dog
	Plasma conc. in tox study	PO	Rat, Dog (1-month tox)
		PO	Rabbit (Range finding study)
Distribution	Tissue distribution	PO	Rat (Radioactivity)
	Plasma protein binding	-	Rat, Dog, Human (Radioactivity)
	Red blood cell partitioning (blood/plasma ratio)	-	Rat, Dog, Monkey, Human
	Permeability, mechanism, P-glycoprotein substrate properties	-	Caco-2
	Brain penetration	PO/SC	Rat

3. Non-clinical Pharmacokinetics

Study Type and Description		Route of Administration	Species/Cell line
Metabolism	In vitro metabolism in liver microsomes	-	Rat, Dog, Monkey, Human
	In vitro metabolism in hepatocytes	-	Rat, Dog, Human
	In vitro metabolism to identify CYP	-	Human
	Isolation and identification of the metabolite	-	Rat, Dog, Human liver microsomes and recombinant CYPs
		PO	Rat, Dog (Radioactivity)
	Inhibition of drug metabolizing enzymes (DDI)	-	Human
Excretion	Excretion	PO	Rat, Dog (Radioactivity)
	Urine excretion	IV	Rat, Dog (LCMS)
	Biliary excretion	IV	Rat (Radioactivity)

4. Non-clinical Safety

- Genotoxicity
  - Negative in all core-battery studies (Ames, HLA* and in vivo micronucleus)
- Cardiovascular Toxicity
  - No significant effects observed in dog purkinje assay
  - No QTc changes observed in dog at a dose up to 5 mg/kg
  - I_{herg} IC_{50} values for AAT-009 and PRX-03140 were 8.64 μM and 1.59 μM, respectively
- General Toxicity
  - 2-week oral dose range-finding toxicity study in dogs
    - GI-related evidences, emesis, salivation, and slight decrease in food consumption, were observed by 30 mg/kg
    - Dose-related clinical signs including convulsions and an increase in QTc interval was observed at 30 mg/kg
  - 1-month oral toxicity study in dogs
    - GI-related evidences, transient diarrhea and slight decrease in food consumption were observed at 20 mg/kg, and emesis was observed at 12 and 20 mg/kg
    - Clinical signs including convulsions, decreases in body weight and food consumption, changes in clinical pathology parameter and an increase in QTc interval at ≥12 mg/kg
    - NOAEL** was 6 mg/kg/day

* HLA: human lymphocyte aberrations, ** NOAEL: no observed adverse effect level

4. Non-clinical Safety

- Toxicology

Study Type and Duration		Route of Administration	Species/Cell line
Acute		PO	Rat
Multiple	7-Day range-finding	PO	Rat
	2-Week range-finding	PO	Rat, Dog
	1-Month	PO	Rat, Dog
Genotoxicity	Ames	-	Microbial
	In vitro cytogenetic	-	Human lymphocytes
	In vivo cytogenetic	PO	Rat
Reproductive*	Range-finding for teratology	PO	Rat
	Range-finding for teratology	PO	Rabbit

* This is not anticipated to be critical for AD therapy.

4. Non-clinical Safety

- Safety Pharmacology

Study Type and Description		Route of Administration	Species/Cell line
Cardiovascular system	Haemodynamics Electrocardiograph	PO	Dog
	Purkinje fiber	-	Dog
	Human ERG assay	-	Human ERG
Central nervous system	Rotarod Locomotor activity	PO	Rat
Respiratory system	Plethysmography	PO	Rat
Renal/urinary system	Excretion of fluid and electrolytes	PO	Rat
Gastrointestinal system	Propulsive activity	PO	Rat
Metabolites/isomers/ finished products	$5\text{-}HT_3$ antagonistic activity of the metabolite	-	Human/ HEK293
	Effect of the metabolite on the Bezold-Jarisch reflex	IV	Rat