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# Attenuation of scopolamine-induced deficits in navigational memory performance in rats by bis(7)-tacrine, a novel dimeric AChE inhibitor

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**KEY WORDS** cholinesterase inhibitors; tacrine; bis(7)-tacrine; memory; Morris water maze; scopolamine; Alzheimer disease

## ABSTRACT

**AIM**: To study the effects of 1,7-*N*-heptylene-bis-9,9′-amino-1,2,3,4-tetrahydroacridine [bis(7)-tacrine], a novel dimeric acetylcholinesterase inhibitor (AChEI) derived from 9-amino-1,2,3,4-tetrahydroaminoacridine (tacrine), on scopolamine-induced spatial memory impairment. **METHODS**: The effects of bis(7)-tacrine were investigated on the 5-d performance of young adult rats in the Morris water maze. The latency to find the platform in the water maze was measured to evaluate performance. Tacrine was used as a reference drug. **RESULTS**: Scopolamine (0.3 mg·kg$^{-1}$, ip) resulted in an increase in latency period (>100 % increase) as compared with saline treated controls. Both bis(7)-tacrine and tacrine lessened the increased latency induced by scopolamine to the level of saline control group. The relative potency of bis(7)-tacrine (0.35 μmol·kg$^{-1}$, ig or ip) to shorten the escape latency was 24 or 12 times of tacrine (8.52 μmol·kg$^{-1}$ ig, 4.26 μmol·kg$^{-1}$ ip) following ig or ip administration, respectively. There appeared to be an inverse bell-shape dose-dependent effect for both compounds tested. **CONCLUSION**: Bis(7)-tacrine is a more potent and orally active AChEI than tacrine, and has potential for the palliative treatment of Alzheimer disease.

## INTRODUCTION

Alzheimer disease (AD) is a devastating neurodegenerative disorder that is characterized by global cognitive decline and dramatic personality changes. In developed nations AD has become the fourth leading cause of death in adults, and the costs related to AD exceed those related to cancer and heart disease[1]. Due to cultural factors, it is quite likely that at present AD is under-diagnosed in China. But as the aging population continues to grow in the country, a more accurate picture of the extent of the disease should emerge. Development of an effective therapy would therefore have tremendous value from both scientific and economic standpoints.

Pathologically AD could at least be characterized by the degeneration of the central cholinergic system and the deposit of amyloid plagues in the brain[2]. Many possible approaches have been investigated in developing AD therapeutic drugs, but to date only the cholinergic therapy has proven efficacy. A number of cholinergic compounds have been used to treat this disease, such as acetylcholinesterase inhibitors (AChEI) and cholinergic agonists or precursors[3,4]. Encouraging improvement of memory has been reported with 9-amino-1,2,3,4-tetrahydroaminoacridine (tacrine or Cognex™

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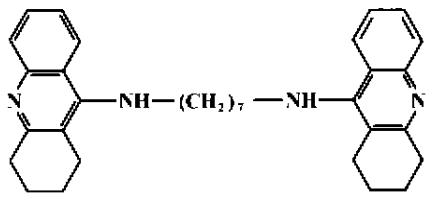
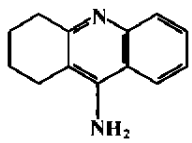
marketed by Parke-Davis in 1993), a centrally active, reversible AChEI that has been used to treat patients with AD[4]. However, tacrine is not an ideal drug for clinical use because of its low bioavailability, narrow therapeutic window, and hepatotoxicity[5,6]. Therefore much research has been directed towards the development of more potent and selective tacrine analogs.

Computer-aided rational drug design led to the synthesis and evaluation of heptylene-linked bis-tacrine, henceforth bis (7)-tacrine, which offers remarkable increases in AChE inhibition potency and selectivity compared to tacrine itself[7].




Bis (7) -tacrine




Tacrine

Subsequent synthesis and evaluation of short-tether homologs of bis(7)-tacrine confirmed that a seven-methylene tether optimized both AChE potency and selectivity[8]. The observed 150-fold increase in AChE potency was proposed to be due to simultaneous binding of the two tacrine units of bis(7)-tacrine to the catalytic site and the computer-determined low-affinity peripheral site near the opening of the active site gorge. Preliminary *in vivo* AChE inhibition studies have also demonstrated superior efficacy and selectivity of bis (7)-tacrine relative to tacrine[9]. To further clarify the therapeutic potential of bis(7)-tacrine for treatment of AD, this study was designed to compare effects of bis (7)-tacrine with tacrine for their abilities to reverse the scopolamine-induced amnesia in Morris water maze.

## MATERIALS AND METHODS

**Rats** Sprague-Dawley rats (♂, weighing 240 g ± *s* 20 g) were supplied by Animal Care Facility of The Hong Kong University of Science & Technology. The rats were housed individually in a climatically controlled room in a 12-h light/dark cycle. Rats were allowed an access to water and food *ad lib*.

**Drugs** Bis(7)-tacrine (bis-hydrochloride salt) was prepared according to the literature method[8], and recystallized from 10 % methanol-water. Elemental analysis matched the dihydrate and HPLC analysis indicated greater than 99.5 % purity. Tacrine (hydrochloride salt) was purchased from Sigma. These compounds were all dissolved in saline and administered ig or ip in a volume of 1 mL·kg$^{-1}$ body weight, respectively 30 min before daily training. Scopolamine hydrobromide (Sigma Chemical Co) dissolved in saline was also administered ip 30 min before daily training in a volume of 1 mL·kg$^{-1}$ body weight.

**Apparatus** The water maze apparatus was a circular, black painted stainless steel tank with white symbols such as triangles, circles, waves, and rectangles on the wall. It was 150 cm in diameter, 60 cm deep, and filled to height of 35 cm with water at room temperature (20 ± 1) ℃ to cover an invisible black platform (diameter 10 cm). The tank was divided into four equal quadrants. The platform was submerged approximately 15 mm below the surface of the water and remained in one location for the entire training period. The starting locations were called north, east, south and west, and they were arbitrarily at equal distances on the pool rim. Swimming activity of each rat was

monitored by a video camera linked to a computer through an image analyzer.

**Procedure** Each rat received 2 trials per day for 4 consecutive days. A trial started when a rat, held facing the pool wall, was immersed in the water. The rat was then allowed 60 s to search for the platform; if the rat failed to escape within this time, it was placed on the platform. Regardless of whether the rat found the platform or not, it remained there for 10 s. There was a 30-s recovery period between trials. The two trials were started from the two points located farthest from the platform respectively. The probe trial (without platform) was assessed on the fifth day of behavioral testing (one experiment), and the time spent in the quadrant where the escape platform had been set during training was recorded.

**Statistics** Because both latency and swim distance data revealed similar group differences, only latency data were presented in the text. The two-way ANOVA with repeated measures was used to analyze latency values which were calculated as the mean latency periods for each animal. The one-way ANOVA followed by Duncan multiple group comparison was used to analyze group differences of the data collected during probe trials.

## RESULTS

**Effects of bis(7)-tacrine** Spatial memory of rats treated with saline (control), scopolamine, or a combination of bis(7)-tacrine and scopolamine was tested in the Morris water maze. The latency to find the platform in the water maze was used to evaluate performance. It was observed that scopolamine (0.3 $mg \cdot kg^{-1}$, ip) significantly increased escape latencies in swimming compared with both saline ig + ip [$F(1,7) = 49.6$, $P < 0.01$] and saline ip alone [$F(1,8) = 12.0$, $P < 0.01$] treated group. Analysis of the latencies in training trials with ig and ip administrations of bis(7)-tacrine revealed significant overall group effects [$F(4,28) = 3.9$ for ig administration, $F(4,32) = 3.7$ for ip administration, all $P < 0.05$]. Bis(7)-tacrine significantly shortened the escape latencies prolonged by scopolamine with ig doses from 0.18 to 0.35 $\mu mol \cdot kg^{-1}$ [0.18 $\mu mol \cdot kg^{-1}$: $F(1,7) = 6.1$, $P < 0.05$; 0.35 $\mu mol \cdot kg^{-1}$: $F(1,7) = 6.6$, $P < 0.05$], and at ip dose of 0.35 $\mu mol \cdot kg^{-1}$ [$F(1,8) = 19.6$, $P < 0.01$] (Fig 1). The dose-response curves were bell-shaped with the maximal improvement at 0.35 $\mu mol \cdot kg^{-1}$ ig and ip for bis(7)-tacrine. It was apparent that bis(7)-tacrine whether given ig or ip had almost the equivalent improving effects on scopolamine-induced memory impairments in water maze.

**Effects of tacrine** Behavioral experiments of tacrine were performed similarly as that of bis(7)-tacrine. The latencies were also significantly increased by scopolamine (0.3 $mg \cdot kg^{-1}$, ip) compared with both saline ig plus ip [$F(1,7) = 18.9$, $P < 0.01$] and saline ip alone [$F(1,8) = 12.4$, $P < 0.01$], respectively. In the analysis of escape latencies, significant overall group effects were observed with ig and ip administration of tacrine [$F(4,28) = 4.0$ for ig, $P < 0.05$; $F(4,32) = 7.5$ for ip, $P < 0.01$]. The escape latencies were significantly shortened by the treatment of oral tacrine 8.52 - 17.04 $\mu mol \cdot kg^{-1}$ [8.52 $\mu mol \cdot kg^{-1}$: $F(1,7) = 6.8$, $P < 0.05$; 17.04 $\mu mol \cdot kg^{-1}$: $F(1,7) = 41.9$, $P < 0.01$] or tacrine 4.26 $\mu mol \cdot kg^{-1}$ ip [$F(1,8) = 13.2$, $P < 0.01$] compared with administration of scopolamine (Fig 1). The bell-shaped dose-response curves were also found for tacrine with the most effective dose at 4.26 $\mu mol \cdot kg^{-1}$ ip and 8.52 $\mu mol \cdot kg^{-1}$ ig. In contrast to bis(7)-tacrine, tacrine required higher doses when administered ig than administered ip to achieve similar attenuations of the scopolamine-induced memory deficits.

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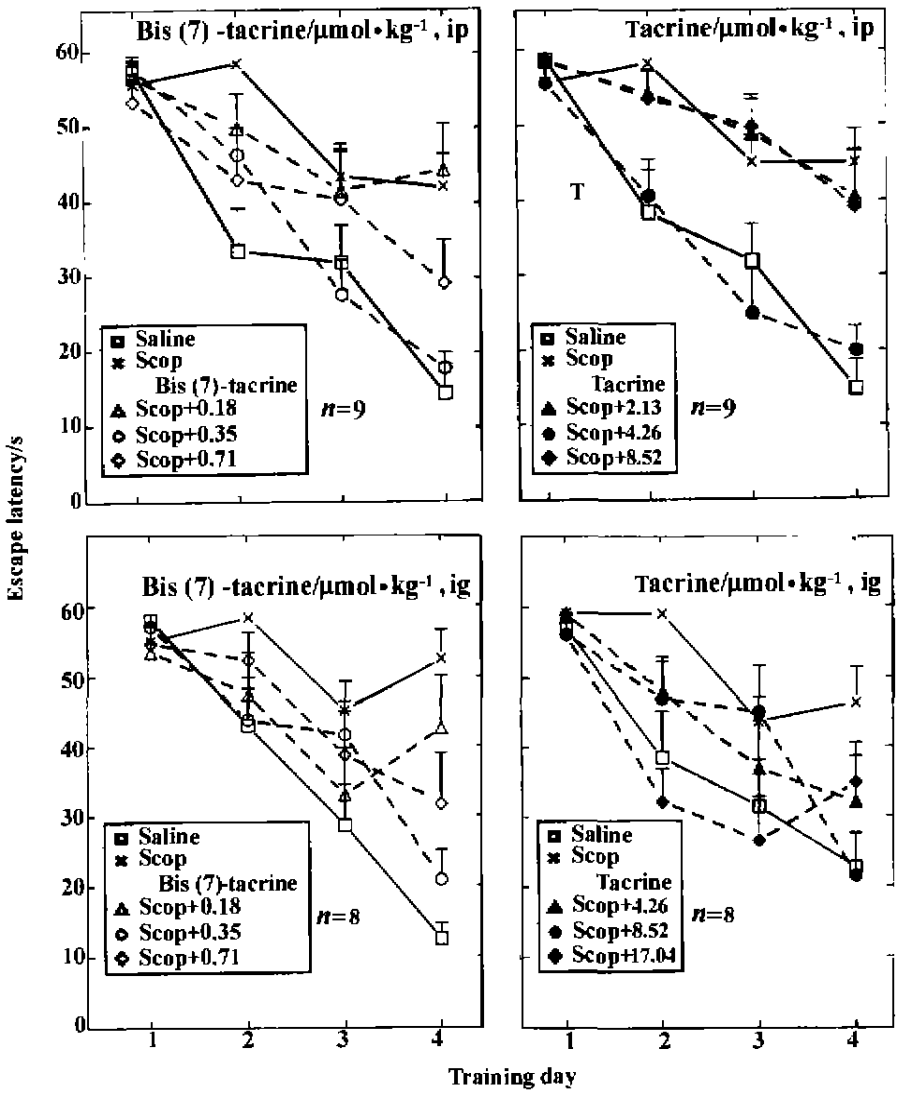


**Fig 1. Effects of ip or ig bis(7)-tacrine and tacrine on scopolamine (0.3 mg·kg$^{-1}$, ip)-induced performance deficits. Two trials were given during each training day. $n$ = number of rats in each group. $\bar{x} \pm s$.**

**Probe trials** The percentage (%) of the total distance swum in the training quadrant was used to estimate the performance in probe trials. The group effects in the probe test were significant for bis(7)-tacrine [$F(4,35) = 2.7$ for ig; $F(4,40) = 3.7$ for ip, all $P < 0.05$] and tacrine [$F(4,33) = 2.7$ for ig; $F(4,40) = 3.7$ for ip, all $P < 0.05$]. (Fig 2)

The scopolamine treated groups exerted significantly lower percentage in the training quadrant than the values of saline groups. The percentage was increased significantly by the two AChEI at the doses of 0.35 μmol·kg$^{-1}$ ig or 0.35 – 0.71 μmol·kg$^{-1}$ ip for bis(7)-tacrine and 8.52 μmol·kg$^{-1}$ ig or 4.26 μmol·kg$^{-1}$ ip for tacrine. (Fig 2)

## DISCUSSION

In order to evaluate the effects of new compounds on learning and memory, many experimental amnesia models have been used. One of the most widely employed paradigms to

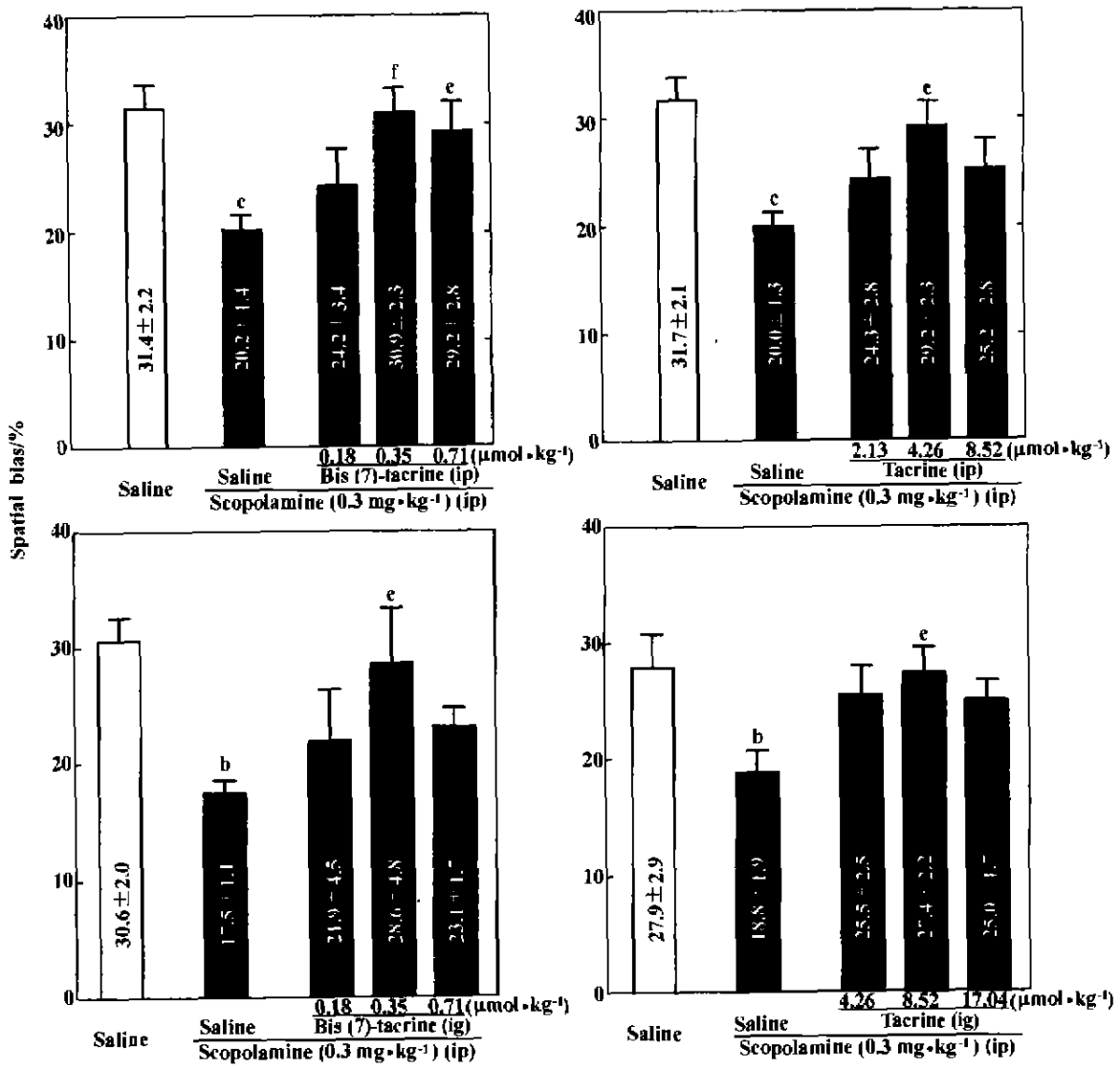


**Fig 2. Effects of ip or ig bis(7)-tacrine and tacrine on spatial bias (% of total distance swum in the training quadrant during spatial probe trial). The platform was removed. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* saline control. $^eP < 0.05$, $^fP < 0.01$ *vs* scopolamine.**

assess learning and memory in rodents is the Morris water maze spatial navigational task[10]. It is well known that scopolamine, an ACh muscarinic receptor antagonist, reduces the memory-cognitive function in animals and humans[11]. The impaired acquisition of water maze task in animals pretreated with scopolamine is consistent with a large body of research supporting a role for cholinergic mechanisms in the performance in tasks assessing spatial learning and memory[12,13]. It is also confirmed in the present study that scopolamine-treated animals have a disruption of their behavioral strategy since these rats circled more around the pool whereas saline-treated rats swam more directly to reach the platform in the later training days. Scopolamine resulted in a highly significant increase in latency period (more than 100 % increase) as compared to saline treated controls.

However, when either bis(7)-tacrine or tacrine were concurrently administered with

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scopolamine, significant attenuation of the scopolamine-induced decrement was observed. Latencies of animals treated with both scopolamine and bis(7)-tacrine or tacrine were shorter than scopolamine-treated subjects but longer than saline controls, with an inverted U-shape dose-response pattern, which was characteristic of many psychopharmacological studies of memory[14]. The present experiments also indicated that a lower dose of bis(7)-tacrine than of tacrine was required to ameliorate the memory deficits. The relative potency of bis (7)-tacrine to shorten the escape latency was 24 or 12 times of tacrine following oral or intraperitoneal administration, respectively. Furthermore, the efficacy of bis(7)-tacrine was quite similar with these two administration routes, while tacrine administered orally showed much lower efficacy than that given intraperitoneally. Parallel results have also been obtained for the inhibition of rat brain AChE in our previous studies[8,9]. These findings indicated that, in addition to its highly potent, selective AChE inhibition, bis (7)-tacrine had improved bioavailability and ability to pass through the blood brain barrier than tacrine.

Despite the fact that the neurodegeneration found in AD is likely to be a multifactorial process involving neurochemical and genetic factors[15,16], cholinesterase inhibition remains the only therapeutic approach to demonstrate clinical efficacy in patients suffering from AD[17] Thus a novel AChEI which is orally active, penetrable to reach the synaptic area, and efficacious continues to be a goal to successfully treat AD patients. Previous findings and the present results indicate that bis (7)-tacrine distinguishes itself from tacrine based on its biochemical, pharmacological and behavioral profiles, and may be advantageous in the symptomatic treatment of AD.

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## 新型胆碱酯酶抑制剂双(7)-他克林减弱东莨菪碱引起的大鼠空间记忆障碍

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**关键词** 胆碱酯酶抑制剂; 他克林; 双(7)-他克林; 记忆; Morris 水迷宫; 东莨菪碱; 阿尔采末病

**目的**: 研究他克林的双体衍生物双(7)-他克林对东莨菪碱引起的大鼠记忆障碍的影响. **方法**: 采用大鼠 Morris 水迷宫固定平台的程序研究空间记忆. 以他克林为对照药. **结果**: 东莨菪碱(0.3 mg·kg$^{-1}$, ip)使大鼠到达平台的潜伏期明显长于生理盐水对照组. 双(7)-他克林(0.35 μmol·kg$^{-1}$, ig 或 ip)和他克林(8.52 μmol·kg$^{-1}$ ig; 4.26 μmol·kg$^{-1}$ ip)均可对抗东莨菪碱导致的空间记忆障碍; 在灌胃及腹腔注射途径下, 双(7)-他克林的效价, 分别强于他克林 24 及 12 倍. **结论**: 双(7)-他克林明显改善东莨菪碱导致的空间记忆障碍, 其作用强于他克林.

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