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Synthesis and structure–activity relationship studies of theophylline analogs on population responses in the rat hippocampus in vitro

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ABSTRACT

We synthesized several theophylline analogs and tested the hypothesis that these compounds may be nootropic or cognitive enhancers by examining their effects on evoked population spikes recorded extracellularly in the CA1 region of the rat hippocampus. Whereas the length of the carbon chain on N7 had no effect, different size of the terminal lactam ring strongly influenced neuroactivity. Our results suggest that hexahydroazepin-2-one analogs have potential for further development as cognitive enhancers.

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1. Introduction

Dementia is a debilitating, chronic neurodegenerative disorder characterized by mild to severe impairment in cognitive function including memory, orientation, language, visual perception, and executive function.[1,2] These deficiencies affect the ability of sufferers, who are not unconscious, to perform activities of everyday living. Different types of dementia, with pathological and clinical features that may overlap, are recognized including Alzheimer's dementia or disease (AD), vascular dementia, dementia with Lewy bodies, and hippocampal sclerosis dementia.[3–6] AD, the most common type of dementia, has a complex pathophysiology that involves alteration of protein metabolism (β-amyloid and *tau* protein) and inappropriate neurotransmission including those mediated by glutamate, serotonin, dopamine, noradrenalin, and acetylcholine.[6,7] The most common neurochemical change found in brains of AD patients is a decrease in acetylcholine in cortical and subcortical regions such as the hippocampus, which are involved in higher cognitive function.[8–13] Currently, disease modifying agents (e.g., amyloid modifying agents) and symptomatic management agents[6,7,14] are two main pharmacotherapeutic approaches. The latter approach represents an essential part of current treatment of AD, and includes the anticholinesterase inhibitors donepezil, revastigmine, and galantamine.[2,15,16] However, therapy with these drugs provides only modest clinical relief of symptoms,[1,17] and does not stop or reverse disease progression. In addition, the long-term benefits of their use are unclear, and their use is often accompanied by intolerable side or adverse effects.[18–20] These drawbacks of the cholinesterase inhibitors have triggered a search for drugs characterized by reduced side effects, patient acceptance in long-term use and potential to inhibit the neuronal loss associated with AD and other dementias.[21] Recently, memantine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist with neuroprotective action and a potential to halt disease progression, has been approved for use in AD.[16]

In this study, neuronal responses to novel theophylline analogs in the hippocampus, a structure involved in learning and memory, have been investigated in order to identify possible candidates for further development as cognitive enhancers. The compounds tested were synthesized by chemically linking a purine-based compound (theophylline) with lactam rings through a carbon chain because both these chemical groups have each shown potential for cognitive enhancement. For example, methylxanthines that are naturally present in some foods have CNS actions that may influence memory.[22,23] Similarly, some derivatives of purines are currently undergoing clinical trials for use in AD patients because of their nootropic or cognitive enhancement effects.[24] As well, lactam rings are the primary structure of the racetams, which are also currently being used for memory enhancement.[25] The rationale for

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the synthesis of these compounds was therefore to see if a combination of a purine structure with a lactam ring would yield compounds with enhanced nootropic potential. Here, we report that some structural analogs of theophylline (a purine compound) synthesized by linking different lactam rings to this primary structure produce effects on hippocampal population responses that are consistent with cognitive enhancement while others have either no effect or produce the exact opposite effects.

2. Results

2.1. Chemistry

The target compounds were prepared in a two-step synthesis. Briefly, in the first step, *N*-(ω-chloroacyl)-lactams **1**–**6** were prepared by reacting ω-chloroacyl chlorides with various lengths of the chain (2-chloroacetyl chloride, 3-chloropropionyl chloride, 4-chlorobutyryl chloride) and five- to seven-membered lactams (Scheme 1). *N*-(ω-chloroacyl)-lactams were purified by vacuum distillation except for **4**, which was unstable under heating.[26] In the second step, theophylline analogs **7**–**12** were obtained by treatment of *N*-(ω-chloroacyl)-lactams with theophylline in *N*,*N*-dimethylformamide (Scheme 1, Fig. 1), and were re-crystallized from propan-2-ol.

Structures of final compounds were determined by using MS, IR, and ^{1}H, ^{13}C, and ^{15}N NMR spectroscopy. ^{1}H–^{13}C gs-HSQC, ^{1}H–^{13}C GSQMBC, ^{1}H–^{15}N gs-HMBC, and ^{1}H–^{15}N GSQMBC experiments were used to assign individual NMR resonances to the particular atoms. Generally, purine compounds can be *N*-alkylated at N3, N7, or N9 nitrogens due to the tautomerism phenomenon.[27] In this contribution, the position of *N*-alkylation of theophylline ring was unequivocally determined by using ^{1}H–^{13}C GSQMBC experiment.[28] Three bond correlation of H12 of acyl chain with C5 and C8 of purine ring in compounds **7**–**12** unambiguously confirmed the position of substituent at N7. This conclusion is further supported by analysis of $^{3}J_{H8-C5}$ (4.7–5.0 Hz) and $^{3}J_{H8-C4}$ coupling constants (12.7–13.1 Hz). The larger values of $^{3}J_{H8-C4}$ ($\sim$ 13 Hz) as compared to $^{3}J_{H8-C5}$ are characteristic of the N7-substituted purine derivatives.[29]

2.2. Electrophysiology

Theophylline analogs used in this study included compounds **7**–**12**. The results reported here were obtained from extracellular field recordings of populations spikes (PS) in 90 hippocampal slices. The PS recorded in the CA1 region was shown to be TTX-sensitive as they were abolished by bath application of 1 μM TTX (Fig. 2)

2.3. Effect of theophylline analogs on PS recorded in area CA1 of the hippocampus

Following the recording of stable PS for at least 15 min, all the theophylline analogs were diluted to the final desired concentration, and bath perfused for 5–6 min. Bath application of 10 μM compound **12** caused a consistent enhancement in the amplitude of PS by 56.5 ± 14.7% ($p < 0.05$, paired *t*-test, $n = 7$, Figs. 2 and 4). Maximal PS enhancement was observed after 6 min application, and recovery was not significant after more than 20 min washout of compound **12** as the effect was still significantly enhanced at 45.8 ± 22.7% ($p < 0.05$, paired *t*-test, $n = 7$, Fig. 2). This effect of compound **12** on PS amplitude was concentration-dependent with a minimum effective concentration of 0.1 μM and a maximum concentration of 100 μM with a calculated EC_{50} value of $\sim$2 μM (Fig. 4A). Similar to the effects of compound **12**, both compounds **10** and**11** caused enhancement of PS amplitude (42.4 ± 14.2% and 40.9 ± 14.0%, respectively, $p < 0.05$, paired *t*-test, $n = 4$ each, Fig. 4B). In contrast to the above analogs, compound **9** caused a concentration-dependent and reversible depression in the amplitude of PS (−25.9 ± 4.6%, $p < 0.05$, paired *t*-test, $n = 5$, Figs. 3 and 4). Recovery from depression was almost complete (91.1 ± 4.6%) and rapid (within 10 min of washout). The threshold concentration was 0.01 μM with a peak concentration of 10 μM, and a calculated EC_{50} value was 0.06 μM. Finally, the pyrrolidin-2-one derivatives **7** and **8** did not cause significant changes in the PS amplitude (6.4 ± 4.4%, $p > 0.05$, $n = 6/5$, Fig. 4).

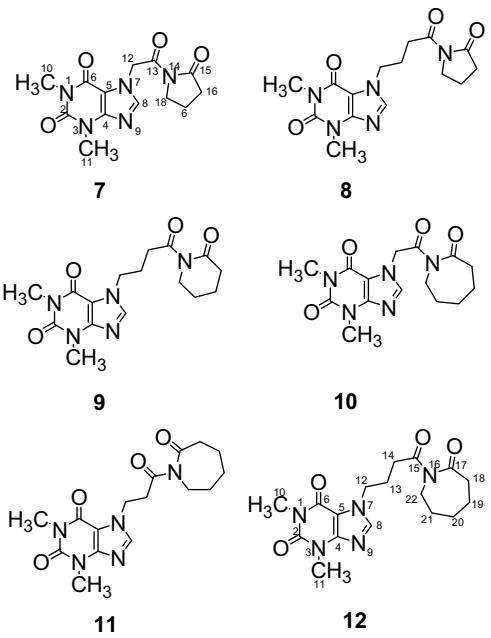
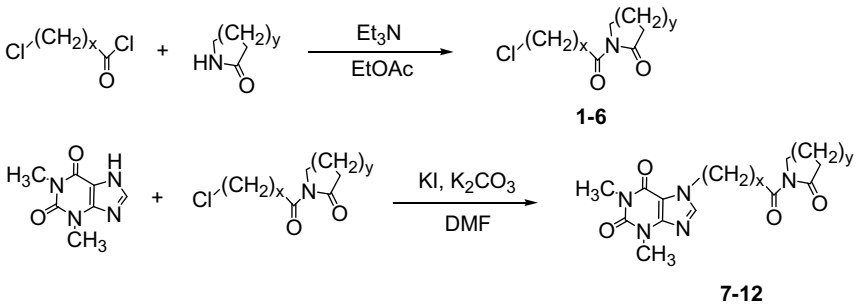


Figure 1. Structures of theophylline analogs with atom numbering.

2.4. Pharmacology of compound 12 effects on PS

To explore possible mechanisms by which the enhancers produced their effect on PS amplitude, we chose compound **12** as their



Scheme 1. Synthesis of intermediates **1**–**6** and theophylline analogs **7**–**12**; $x = 1$–3, $y = 1$–3.

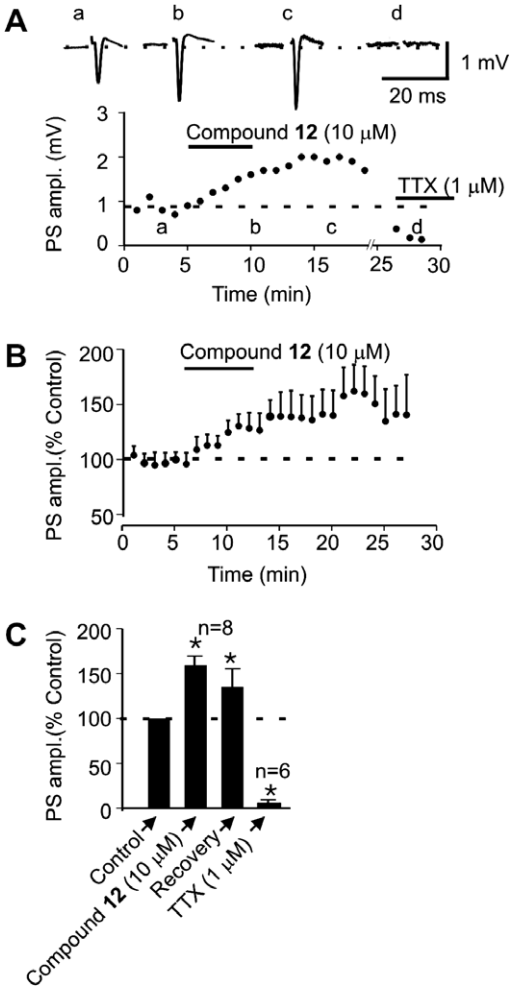


Figure 2. The theophylline analog compound **12** enhances evoked PS amplitude recorded in the cell body layer of area CA1 of the hippocampus. (A) A time-effect plot in a representative cell showing that compound **12** (10 μM) enhances the amplitude of TTX sensitive PS. Inserts are sample PS traces taken at the times indicated by alphabets in the graph. In this and in all time-effect plots, each filled circle represents the average response taken in a minute. The length of the solid line below each drug represents the duration of application, and the broken lines indicate baseline. (B) An average time-effect plot obtained from 8 slices showing the consistent effect of compound **12** in enhancing PS amplitude. (C) A bar graph summarizing the effect of 10 μM compound **12** ($n = 8$) and 1 μM TTX ($n = 6$) on evoked PS amplitude (In this figure and in all other figures, ∗indicates significance compared to control in aCSF at $p \leqslant 0.05$).

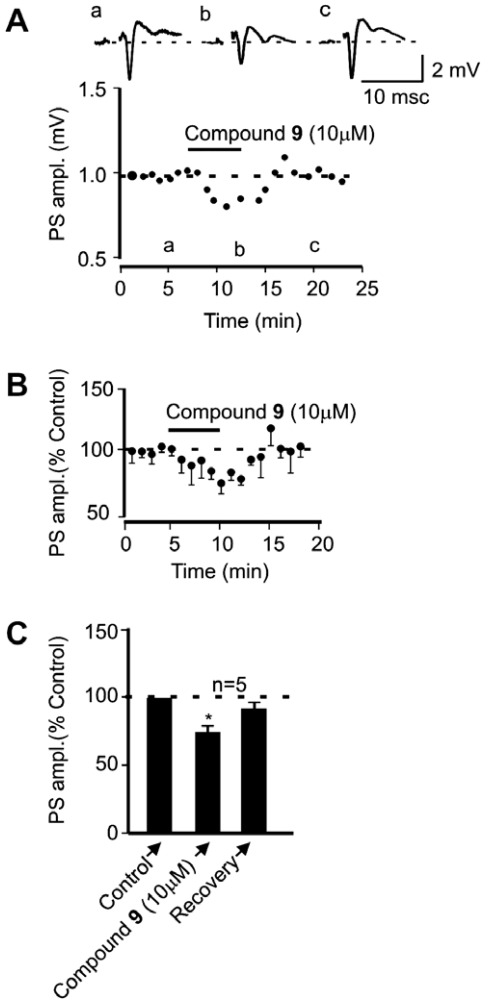


Figure 3. Another theophylline analog compound **9** depresses evoked PS amplitude recorded in the cell body layer of area CA1 of the hippocampus. (A) A time-effect plot in a representative cell showing that compound **9** (10 μM) reversibly depresses the amplitude of evoked PS. Inserts are sample PS traces taken at the times indicated by alphabets in the graph. (B) An average time-effect plot obtained from 5 slices showing the consistent effect of compound **9** in depressing PS amplitude. (C) A bar graph summarizing the effect of 10 μM compound **9** ($n = 5$) on evoked PS amplitude.

prototype and 10 μM as a suitable concentration because its effects at this concentration were robust. We first tested if compound **12** employed cholinergic mechanism to enhance the PS amplitude because alteration in acetylcholine and cholinergic neurons/terminals is implicated in the pathophysiology of dementias.[2,6] We tested for a role of the cholinergic system by using d-tubocurarine (d-TC) and atropine, nicotinic (nAChR) and muscarinic (mAChR) acetylcholine receptor antagonist, respectively, as both these receptors are reported to be involved in AD, a common cause of dementia.[30–33] Bath application of atropine (100 μM) did not produce a significant effect on PS amplitude (8.2 ± 15.3%, $p > 0.05$, paired t-test; $n = 5$, Fig. 5A). In the presence of atropine, compound **12** (10 μM) was no longer able to cause an enhancement in PS amplitude (0.2 ± 2.8%, $p > 0.05$ compared to the effect in atropine alone, paired t-test; $n = 5$, Fig. 5A). Similar to atropine, d-TC by itself did not change PS amplitude (1.8 ± 6.4%, $p > 0.05$, paired t-test; $n = 6$, Fig. 5B) but blocked the ability of compound **12** to enhance the PS (−2.5 ± 5.0%, $p > 0.05$ compared to the effect in d-TC alone, paired t-test; $n = 6$, Fig. 5B).

Further, we tested if compound **12** employed other mechanisms (non-cholinergic) to produce the enhancement. In the hippocampus and other brain regions, blockade of $GABA_B$ receptors results in disinhibition and increase in PS.[34,35] Therefore we tested if the potent $GABA_B$ receptor antagonist, CGP 55845 (CGP;[36]), would block or occlude the effect of compound **12** since it has been reported to cause PS enhancement in the CA1 region.[35] Bath application of 1 μM CGP predictably enhanced the PS amplitude by 55.4 ± 15.5%, $p < 0.05$, paired t-test; $n = 6$ (Fig. 5C). Compound **12** was applied at the peak of this CGP effect, and it further enhanced the PS amplitude by 32.7 ± 6.9%, $p < 0.05$ compared to baseline in the presence of CGP, paired t-test; $n = 6$ (Fig. 5C). Another neuromodulator in the CNS is adenosine[37], and blockade of adenosine A1 receptors also causes disinhibition and enhancement in synaptic responses. We therefore tested if blockade of A1 receptors would occlude the compound **12** effect. The relatively selective A1 receptor antagonist, CPT (1 μM), enhanced PS amplitude by itself (76.5 ± 18.3%, $p < 0.05$, paired t-test; $n = 6$, Fig. 5C). In the first two slices tested, at the peak of this CPT effect, compound **12** produced very little enhancement in the PS amplitude. Since the CPT effect was very big, we suppose that this may be a saturating effect

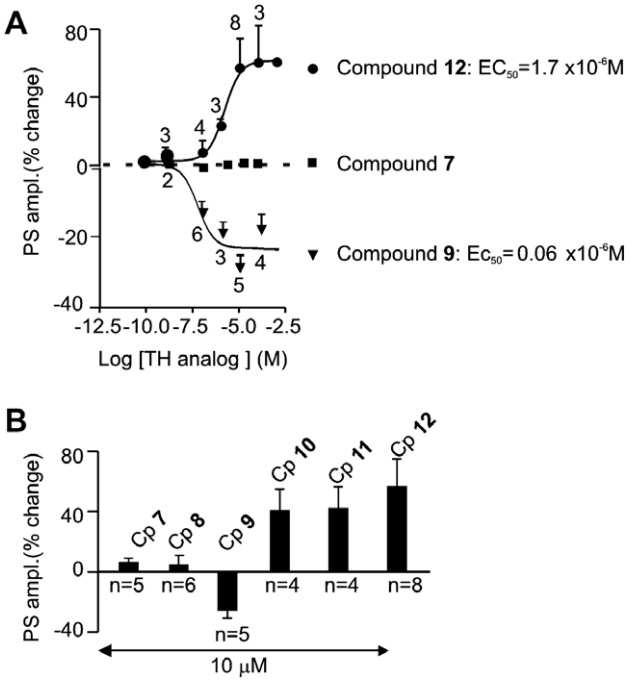


Figure 4. The effects of theophylline analogs on evoked PS are concentration-dependent. (A) Concentration–response curves generated by applying different concentrations of 3 different theophylline analogs (compounds **7**, **9**, and **12**). Each point on the graph has an n value of 3–8 slices (except compound **7**, where $n = 1$). (B) An integrated bar graph showing the effects of 10 μM of all TH-analogs tested. The n values of each compound are shown below the bar.

on the response rather than pharmacological antagonism. To examine the possibility that CPT actually blocked the effect of compound **12**, in other four slices, at the peak of the CPT effect, the stimulus strength was decreased to produce responses close to the initial baseline response recorded in normal aCSF. Once this new baseline stabilized, subsequent application of compound **12** produced an enhancement of 47.9 ± 22.7% ($n = 4$; Fig. 5C) in the PS amplitude, an effect similar to that observed for compound **12** in aCSF alone ($p > 0.05$, unpaired t-test).

2.5. Pharmacology of compound 9 effects on PS

To investigate the mechanism by which compound **9** produced the observed depression in PS amplitude, we tested if it employed mechanisms known to modulate synaptic responses in the CNS. In the hippocampus, GABA, acting on presynaptic $GABA_B$ receptors, is known to cause depression in synaptic and cellular responses.[38,39] To test if compound **9** employed $GABA_B$ receptors or GABAergic mechanism to cause PS suppression, we pretreated slices with 1 μM CGP,[36] a potent $GABA_B$ receptor antagonist. Bath application of CGP for 8 min caused an enhancement in the PS amplitude (53.8 ± 15.5%, $p < 0.05$; $n = 7$; Fig. 6A and C). At the peak of this CGP effect, concurrent application of 10 μM compound **9** no longer produced a significant depression in the PS amplitude (10.6 ± 11.5%, $p > 0.05$ compared to baseline in the presence of CGP; $n = 7$; Fig. 6A and C). To determine if the action of compound 9 was mediated by adenosine acting as an intermediate to suppress the PS amplitude, we blocked adenosine A1 receptors with 1 μM CPT, and then tested compound **9** to determine if the PS amplitude was suppressed in the presence of this antagonist. Similar to CGP above, CPT also caused an enhancement in PS amplitude (51.6 ± 26.7%, $p < 0.05$; $n = 5$; Fig. 6B and C) but did not block the depressing effect of 10 μM compound **9** on PS amplitude (−27.6 ± 6.5, $p < 0.05$ compared to baseline in the presence of CPT; $n = 5$; Fig. 6B and C).

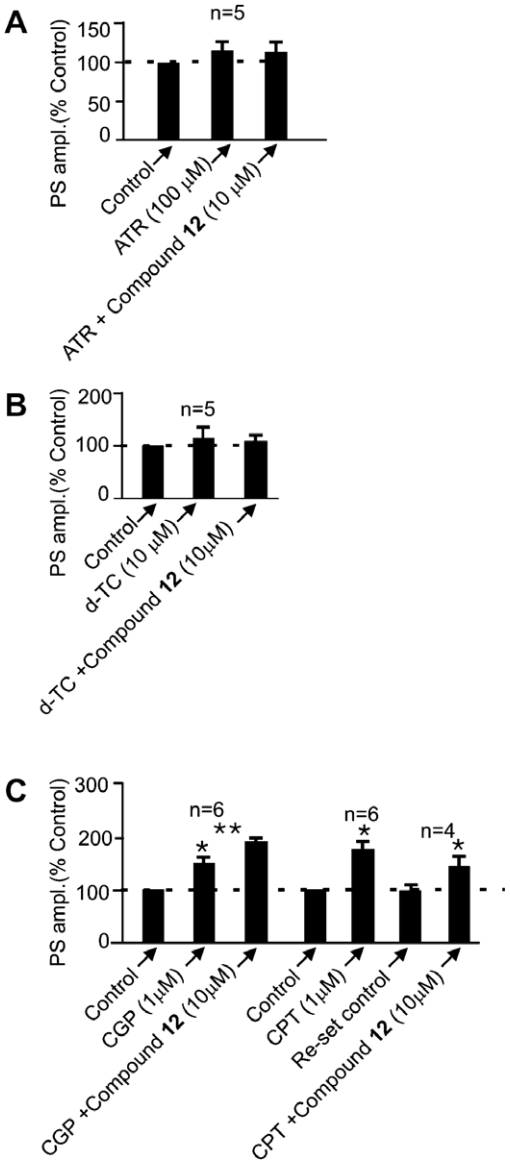


Figure 5. Compound **12** effects on PS amplitude are blocked by atropine and d-tubocurarine but not by CGP55845 or CPT. (A) Bar graph summarizing the lack of effect of compound **12** ($n = 5$) on PS amplitude when applied after pretreatment of slices with atropine (ATR; 100 μM). (B) Similar to A, pretreatment of slices with d-tubocurarine (d-TC; 10 μM) also prevented compound **12** from enhancing the PS amplitude ($n = 5$). (C) In contrast to the effect of ATR and d-TC shown in A and B, pretreatment of slices with both CGP (1 μM; $n = 6$) and CPT (1 μM; $n = 4$) enhanced the PS amplitude but did not prevent compound **12** from further enhancing the PS amplitude. (**indicates significance at $p \leqslant 0.05$ compared to the response in the presence of CGP).

3. Discussion

We report here the results of a study on the effects of different derivatives of theophylline on neuronal responses in the rat hippocampus, a region involved in memory processing and implicated in the pathophysiology of dementias. These compounds were synthesized for use as possible nootropic agents or cognitive enhancers in patients with dementias such as AD. Our results reveal that terminal ring substitution of theophylline at position 7 determined the type of effect on hippocampal PS recorded in vitro. All chain length substituents with hexahydroazepin-2-one (compounds **10**–**12**) caused similar enhancements in population spike amplitude regardless of the carbon chain length ($x = 1$–3) between the ring and the theophylline skeleton. In contrast, piperidin-2-one ring

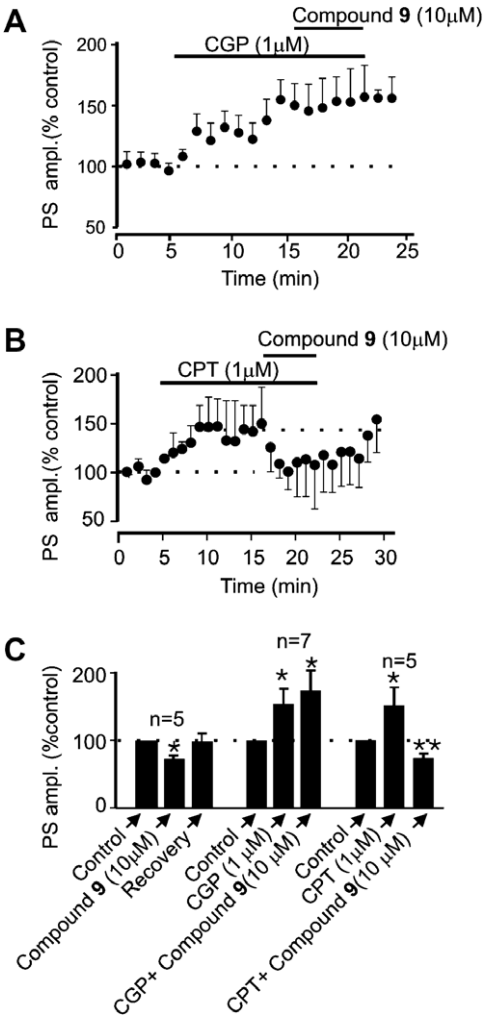


Figure 6. The effect of compound **9** on PS amplitude is blocked by CGP but not by CPT. (A) An average time-effect plot showing that pre-treatment of slices with CGP (1 μM) enhances PS amplitude and blocks the ability of compound **9** to depress PS ($n = 7$). (B) Another average time-effect plot showing that pre-treatment of slices with CPT (1 μM) also enhances PS amplitude, but in contrast to the effect of CGP above does not block the ability of compound **9** to depress PS amplitude ($n = 5$). (C) Summary bar graphs showing that the compound **9**-induced PS amplitude depression is blocked in the presence of CGP, but not CPT. Note that the effect of compound **9** alone is from Fig. 3 for comparison only. (∗∗indicates significance at $p \leqslant 0.05$ compared to the response in the presence of CPT).

substitution (compound **9**) caused a suppression of PS amplitude while pyrrolidin-2-one substituted compounds (compounds **7** and **8**) produced no detected neuroactivity in the hippocampus. Our data further indicate that the enhancers of PS employ cholinergic mechanisms to do this and hence supports the hypothesis that hexahydroazepin-2-one derivatives may be useful as a cognitive enhancer in conditions such as AD where neuronal communication in the hippocampus and some cortical regions is diminished due to a decline in cholinergic inputs or activity or both.[1,6,32,33,40] Our data also indicate that piperidin-2-one and pyrrolidin-2-one derivatives are not good candidates for use as cognitive enhancers. However, piperidin-2-one derivatives may have potential utility in other CNS disorders characterized by excessive excitation such as epilepsy.

3.1. Structural requirements for PS enhancement

The results of this study reveal that the main requirement for enhancing PS amplitude in the hippocampus is the nature of the ring on the substituent at nitrogen 7 in the theophylline structure since compounds **10**–**12** all of which have the hexahydroazepin-2-one ring produced statistically indistinguishable enhancement in PS amplitude at 10 μM; ∼56%; ∼42%; and ∼41% enhancement, respectively. The only structural difference among these derivatives being the length of the carbon chain (x) between the nitrogen at position 7 of the theophylline ring and the nitrogen at position 1 of the hexahydroazepin-2-one, a 7-membered ring ($y = 3$) common to compound **12**: $x = 3$; compound **11**: $x = 2$; and compound **10**: $x = 1$. Thus, the carbon chain length is not an essential requirement for enhancement in hippocampal neuronal activity. Furthermore, the same carbon chain length ($x = 3$) with a different terminal ring structure (6-membered ring; $y = 2$), such as in compound **9**, produced the opposite effect on hippocampal neuronal activity, while another derivative compound **7** with the same chain length ($x = 1$) as contained in compound **10** but with a different terminal ring (5-membered ring; $y = 1$) had no detectable neuroactivity. Finally, compound **8** with a bridging carbon chain length of $x = 3$, but with the 5-membered pyrrolidin-2-one ring ($y = 1$) as in compound **7** ($x = 1$), also had no effect on hippocampal neuronal activity. To summarize our findings, the above data indicate that the length of the bridging carbon chain is not essential for neuroactivity in this series of theophylline derivatives. Furthermore, the data suggest that the pharmacophore for enhancing PS amplitude resides in the hexahydroazepin-2-one ring. The nitrogen in this 7-membered ring ($y = 3$) may possess the ideal configuration to be protonated in solution to generate a quaternary compound such as that contained in the natural transmitter ACh, possibly endowing the analogs with this ring substitution with an ability to interact at the acetylcholine binding site on cholinergic receptors.

3.2. Mechanism(s) of action of enhancers to increase PS amplitude

As indicated above, all of the enhancers produce almost identical increases in PS amplitude at 10 μM. Because the compound **12** effect appeared to be more robust, compound **12** was chosen as a prototype of the enhancers to investigate the possible mechanism(s) by which they produced enhancement in PS amplitude. Because of the well-known role of the cholinergic system in the pathophysiology of dementias,[1,6,32,33,40] we tested to see if compound **12** employed this system in the hippocampus to cause PS enhancement. Both nAChRs and mAChRs are present in the CNS[41–43] and are reported to be involved in cognitive processes, and drugs are being developed that target both these receptors.[33,40] In these studies, we showed that the enhancing effect of compound **12** on PS amplitude was blocked by atropine, a mAChR antagonist.[44,45] Furthermore, they were also blocked by d-tubocurarine, a nAChR antagonist.[46]

The above findings indicate that compound **12** and possibly the other PS enhancers employ cholinergic mechanisms to enhance PS amplitude. The likely site of action is either at the muscarinic or nicotinic receptors or at both sites. Presynaptic muscarinic receptor activation is reported to depress excitatory synaptic transmission in the hippocampus.[47–49] Since acetylcholine is reported to produce a tonic effect in the hippocampus and other regions[50–52] blockade of mAChRs would be expected to reverse a muscarinic tonic inhibition leading to excitation. Interestingly, although we did not unmask a tonic mAChR-mediated effect with atropine, the latter nonetheless blocked the effect of compound **12**. This suggests that cholinesterase activity in our preparation is so efficient that released ACh has no chance of activating its receptors. This would tend to rule out blockade of mAChR as the mechanism by which compound **12** is enhancing the PS. On the other hand, nAChRs are reported to cause excitation by increasing glutamate release[53,54] and activation of these receptors by compound **12** and

the other enhancers can thus lead to the observed effects in this study. Another possible nAChR-dependent mechanism through which compound **12** may cause the enhancement will be as an allosteric potentiating ligand at the nAChRs such as that reported for galantamine.[55,56]

Since our evidence indicates the involvement of both cholinergic receptors in the action of compound **12** to enhance PS amplitude, a more plausible mechanism of action of these enhancers may be to alter the levels of endogenous acetylcholine, which then acts non-selectively to produce the observed effect. If this were the case, the enhancing effect may reflect a predominant nAChR activity in normal conditions so that excitation is observed. Similar dual effects of ACh have been reported in the nucleus accumbens whereby the effect of nAChR was only unmasked after blockade of mAChR.[51] Such an indirect action to enhance ACh levels could explain why both mAChR and nAChR receptor blockades are both effective in preventing the effects of the enhancers. This preliminary study is unable to dissect out the exact mechanism or what contribution each receptor subtype makes to the final effect. The evidence, however, clearly rules out the involvement of other well-known neuromodulators, such as adenosine[57] and GABA[58] in the action of compound **12** to enhance the PS amplitude.

The PS depression caused by compound **9** on the other hand was blocked by CGP, a $GABA_B$ receptor antagonist but not by CPT, an adenosine A1 receptor antagonist, indicating that the compound **9**-induced PS depression was either direct through activation of $GABA_B$ receptors or indirect through increasing the levels of endogenous extracellular GABA. Further studies are required to determine if this analog is a $GABA_B$ receptor agonist, a GABA reuptake inhibitor, or a GABA degradatory enzyme inhibitor, all of which can cause depression in excitatory synaptic transmission. Thus, the piperidin-2-one analogs may have a role in CNS disorders characterized by excessive excitation.

4. Conclusion

The results of this study indicate that the hexahydroazepin-2-one analogs of theophylline have actions on hippocampal neuronal activity that are consistent with cognitive enhancement, and hence have the greatest potential for use as cognitive enhancers or nootropic agents, whereas the piperidin-2-one and pyrrolidin-2-one derivatives have no potential in the management of memory and cognitive impairment associated with dementias. Indeed, the piperidin-2-one analogs may exacerbate memory and cognitive deficits in dementia patients but may be useful in other CNS disorders such as epilepsy. Our results also show that structural modifications of methylxanthines can yield products with different pharmacological actions from those commonly associated with methylxanthine analogs, for example, adenosine receptor antagonism.

5. Experimental

5.1. Synthesis of theophylline analogs

Starting materials were obtained from the commercial suppliers, and were used without further purification. Reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (Merck 60 F 254), and were visualized with UV light or iodine vapor. ESI-MS spectra were determined on an Agilent LC-MSD 1100 VL Series. IR spectra were recorded on a Nicolet Impact 410 FT spectrometer using KBr discs, UV spectra were obtained on a HP 8453 spectrophotometer. Melting points were determined on an electrothermal melting point apparatus, and are uncorrected. NMR spectra were obtained on a Varian Gemini spectrometer operating at frequencies of 199.98 MHz (^{1}H), 50.29 MHz (^{13}C), and a Bruker Avance 300 spectrometer operating at frequencies of 300.13 MHz (^{1}H), 75.48 MHz (^{13}C), and 30.41 MHz (^{15}N). NMR samples were prepared by dissolving individual compounds in $CDCl_3$ (550 μL). ^{1}H and ^{13}C chemical shifts (δ) were referenced relative to the signal of solvent [δ = 7.27 ppm for ^{1}H (residual $CHCl_3$); δ = 77.23 ppm for ^{13}C ($CDCl_3$)]. ^{15}N NMR chemical shifts determined by ^{1}H–^{15}N correlation experiments are reported relative to liquid ammonia.[59,60] The ^{1}H–^{13}C GSQMBC NMR experiment was adjusted for a long-range coupling of 7.5 Hz. ^{1}H–^{15}N GSQMBC and ^{1}H–^{15}N gs-HMBC NMR experiments were adjusted for couplings ranging from 9 to 11 Hz. Chemical shifts are reported in ppm, and indirect spin–spin coupling constants (J) are reported in hertz (Hz).

5.1.1. General procedure for *N*-(ω-chloroacyl)-lactames

The solution of a proper lactame (107 mmol) in ethyl acetate (10 mL), ω-chloroacyl chloride (90 mmol) was added, and the mixture was stirred at 30 °C for 20 min. The solution of triethylamine (11.2 mL, 80 mmol) in ethyl acetate (40 mL) was added dropwise in the course of 1.5 h, and the reaction mixture was heated at 50 °C for 1 h. The solid precipitate was filtered off, and the filtrate was treated with water and 3% solution of sodium bicarbonate. Organic layer was dried over sodium sulfate, filtered and evaporated to dryness under vacuum.

5.1.2. *N*-(2-Chloroacetyl)-pyrrolidin-2-one (1)

The title compound was prepared by a reaction of pyrrolidin-2-one and 2-chloroacetyl chloride. Crude product was suspended in diisopropyl ether (100 mL), and the mixture was refluxed for 5 min, filtered, and the filtrate was let to crystallize at −15 °C. White crystals were washed with diisopropyl ether, and were dried at ambient condition. Yield: 8.99 g, 66.2%; mp 42–45 °C; ^{1}H NMR and ^{13}C NMR spectra were identical with those published previously.[61]

5.1.3. *N*-(4-Chlorobutyryl)-pyrrolidin-2-one (2)

The title compound was prepared by a reaction of pyrrolidin-2-one and 4-chlorobutyryl chloride. Crude product obtained was distilled under vacuum (88.0–89.5 °C, 33 Pa) to provide colorless liquid. Yield: 13.01 g, 76.5%; ^{1}H NMR (200 MHz) δ 3.64 (t, J = 6.9 Hz, 2H, NCH_2 pyrrolidin-2-one), 3.08 (t, J = 7.0 Hz, 2H, NCH_2), 2.53 (t, J = 6.9 Hz, 2H, CH_2CO pyrrolidin-2-one), 2.37 (t, J = 7.0 Hz, 2H, CH_2CO), 1.96–2.21 (m, 4H, $CH_2CH_2CH_2$ pyrrolidin-2-one, $CH_2CH_2CH_2$); ^{13}C NMR (50 MHz) δ 175.32, 173.06, 45.33, 44.16, 33.93, 33.55, 27.08, 17.21.

5.1.4. *N*-(4-Chlorobutyryl)-piperidin-2-one (3)

The title compound was prepared by a reaction of piperidin-2-one and 4-chlorobutyryl chloride. Crude product obtained was distilled under vacuum (87.5–90.5 °C, 20 Pa) to provide colorless liquid. Yield: 12.92 g, 70.5%; ^{1}H NMR (200 MHz) δ 3.50–3.70 (m, 2H, J = 6.9 Hz, NCH_2 piperidin-2-one), 3.26 (t, J = 7.0 Hz, 2H, NCH_2), 2.50 (t, J = 6.9 Hz, 2H, CH_2CO), 2.30-2.50 (m, 2H, J = 7.0 Hz, CH_2CO piperidin-2-one), 2.30-2.50 (m, 6H, $CH_2CH_2CH_2CH_2$ piperidin-2-on, $CH_2CH_2CH_2$); ^{13}C NMR (50 MHz) δ 177.40, 174.49, 44.45, 43.97, 39.09, 33.51, 28.76, 23.20, 21.86.

5.1.5. *N*-(2-Chloroacetyl)-hexahydroazepin-2-one (4)

The title compound was prepared by a reaction of hexahydroazepin-2-one and 2-chloroacetyl chloride. Yield: 16.51 g, 81.4% of brown liquid; ^{1}H NMR and ^{13}C NMR spectra were identical with the literature data.[26]

5.1.6. *N*-(3-Chloropropionyl)-hexahydroazepin-2-one (5)

The title compound was prepared by a reaction of hexahydroazepin-2-one and 3-chloropropionyl chloride. Crude product

obtained was distilled under vacuum (86.0–89.0 °C, 13 Pa) to provide colorless liquid. Yield: 10.51 g, 58.0%; ^{1}H NMR (200 MHz) δ 3.85–4.00 (m, 2H, NCH_2 hexahydroazepin-2-one), 3.80 (t, J = 6.8 Hz, 2H, CH_2Cl), 3.36 (t, J = 6.8 Hz, 2H, $ClCH_2CH_2$), 2.60–2.75 (m, 2H, $COCH_2$ hexahydroazepin-2-one), 1.50-1.90 (m, 6H, $CH_2CH_2CH_2CH_2CH_2$ hexahydroazepin-2-one); ^{13}C NMR (50 MHz) δ 177.69, 172.73, 43.27, 42.11, 39.54, 39.46, 29.05, 28.46, 23.58.

5.1.7. *N*-(4-Chlorobutyryl)-hexahydroazepin-2-one (6)

The title compound was prepared by a reaction of hexahydroazepin-2-one and 4-chlorobutyryl chloride. Crude product obtained was distilled under vacuum (88.0–95.0 °C, 35 Pa) to provide colorless liquid. Yield: 12.18 g, 76.6%; ^{1}H NMR (200 MHz) δ 3.80–4.00 (m, 2H, NCH_2 hexahydroazepin-2-one), 3.61 (t, J = 7.0 Hz, 2H, NCH_2), 3.05 (t, J = 7.0 Hz, 2H, CH_2CO), 2.65–2.70 (m, 2H, CH_2CO hexahydroazepin-2-one), 2.12 (qi J = 7.0 Hz, 2H, $CH_2CH_2CH_2$), 1.50-1.90 (m, 6H, $CH_2CH_2CH_2CH_2CH_2$ azepan-2-one); ^{13}C NMR (50 MHz) δ 178.48, 175.00, 43.32, 43.27, 39.75, 36.28, 29.08, 28.52, 28.01, 23.69.

5.1.8. General procedure for 1,3-dimethyl-7-(ω-oxo-ω-(1-azacycloalkan-2-one-1-yl)- alkyl)-3,7-dihydro-1*H*-purine-2,6-diones

The suspension of theophylline (2.00 g, 11 mmol), proper *N*-(ω-chloroacyl)-lactame (12 mmol), potassium carbonate (2.30 g, 17 mmol) and potassium iodide (0.37 g, 2 mmol) in *N*,*N*-dimethylformamide (10.0 mL) was heated at 80 °C for 4 h. Reaction mixture was diluted with water (50.0 mL), and aqueous solution was extracted with chloroform. Chloroform solution was treated with 1M sodium hydroxide, dried over sodium sulfate, and evaporated to dryness under vacuum. Acetone (3 mL) was added to residuum, and the solution was let to crystallize at −15 °C. Crystals were filtered, and were re-crystallized from propan-2-ol.

5.1.9. 1,3-Dimethyl-7-(2-oxo-2-(2-oxopyrrolidine-1-yl)-ethyl)-3, 7-dihydro-1*H*-purine-2,6-dione (7)

The title compound was prepared by a reaction of theophylline and **1**. Yield: 1.35 g, 40.1%; mp 209–212 °C; ^{1}H NMR (300 MHz) δ 7.55 (s, 1H, aromatic H), 5.61 (s, 2H, NCH_2), 3.87 (t, J = 7.1 Hz, 2H, NCH_2 pyrrolidin-2-one), 3.61 (s, 3H, N^3CH_3), 3.37 (s, 3H, N^1CH_3), 2.68 (t, J = 8.1 Hz, 2H, $COCH_2$ pyrrolidin-2-one), 2.16 (qi, J = 7.2 Hz, 2H, $CH_2CH_2CH_2$ pyrrolidin-2-one); ^{13}C NMR (75 MHz) δ 176.16 (C15), 167.15 (C13), 155.29 (C6), 151.69 (C2), 148.59 (C4), 141.94 (C8), 107.39 (C5), 50.94 (C12), 45.22 (C18), 32.91 (C16), 29.76 (C11), 27.81 (C10), 17.62 (C17). ^{15}N NMR (30 MHz) δ 232.7 (N9), 168.6 (N14), 155.2 (N7), 151.8 (N1), 114.8 (N3); IR (cm^{-1}) 3116, 2963, 1744, 1700, 1673, 1550, 1456, 1407, 1369, 1294, 1274, 1236, 1192; MS m/z 328.2 ($M-Na^+$); UV λ_{max} (methanol) 208 nm, 274 nm.

5.1.10. 1,3-Dimethyl-7-(4-oxo-4-(2-oxopyrrolidine-1-yl)-butyl)-3, 7-dihydro-1*H*-purine-2,6-dione (8)

The title compound was prepared by a reaction of theophylline and **2**. Yield: 1.61 g, 44.0%; mp 136–137 °C; ^{1}H NMR (300 MHz) δ 7.57 (s, 1H, aromatic H), 4.38 (t, J = 7.3 Hz, 2H, NCH_2), 3.81 (t, J = 7.0 Hz, 2H, NCH_2 pyrrolidin-2-one), 3.59 (s, 3H, N^3CH_3), 3.41 (s, 3H, N^1CH_3), 2.90 (t, J = 7.0 Hz, 2H, CH_2CO pyrrolidin-2-one), 2.60 (t, J = 8.0 Hz, 2H, CH_2CO), 2.24 (m, 2H, $CH_2CH_2CH_2$), 2.06 (qi, J = 7.1 Hz, 2H $CH_2CH_2CH_2$ pyrrolidin-2-one); ^{13}C NMR (75 MHz) δ 175.34 (C17), 172.76 (C15), 154.98 (C6), 151.72 (C2), 149.04 (C4), 140.96 (C8), 106.94 (C5), 46.27 (C12), 45.39 (C20), 33.49 (C14), 33.15 (C18), 29.69 (C11), 27.89 (C10), 25.35 (C13), 17.21 (C19); ^{15}N NMR (30 MHz) δ 230.6 (N9), 170.9 (N16), 168.0 (N7), 151.6 (N1), 114.6 (N3); IR (cm^{-1}) 3109, 2956, 1741, 1707, 1666, 1549, 1475, 1403, 1362, 1294, 1222, 1191; MS m/z 356.2 ($M-Na^+$); UV λ_{max} (methanol) 207 nm, 274 nm.

5.1.11. 1,3-Dimethyl-7-(4-oxo-4-(2-oxopiperidine-1-yl)-butyl)-3,7-dihydro-1*H*-purine-2,6-dione (9)

The title compound was prepared by a reaction of theophylline and **3**. Yield: 1.04 g, 27.2%; mp 124–126 °C; ^{1}H NMR (300 MHz) δ 7.54 (s, 1H, aromatic H), 4.62 (t, J = 6.4 Hz, 2H, NCH_2), 3.69–3.74 (m, 2H, NCH_2 piperidin-2-one), 3.59 (s, 3H, N^3CH_3), 3.40 (s, 3H, N^1CH_3), 2.94 (t, J = 6.3 Hz, 2H, CH_2CO), 2.47–2.60 (m, 2H, $COCH_2$ piperidin-2-one), 2.23 (qi, J = 7.2 Hz, 2H, $CH_2CH_2CH_2$), 1.76–1.86 (m, 4H, $CH_2CH_2CH_2CH_2$ piperidin-2-one); ^{13}C NMR (75 MHz) δ 175.36 (C15), 173.27 (C17), 155.05 (C6), 151.69 (C2), 148.96 (C4), 140.95 (C8), 107.02 (C5), 46.34 (C12), 43.93 (C21), 35.86 (C14), 34.76 (C18), 29.65 (C11), 27.86 (C10), 26.18 (C13), 22.34 (C20), 20.14 (C19). ^{15}N NMR (30 MHz) δ 230.5 (N9), 170.5 (N16), 168.2 (N7), 151.6 (N1), 114.7 (N3); IR (cm^{-1}) 3116, 2956, 1706, 1704, 1666, 1548, 1475, 1359, 1291, 1201, 1162; MS m/z 370.3 ($M-Na^+$); UV λ_{max} (methanol) 205 nm, 274 nm.

5.1.12. 1,3-Dimethyl-7-(2-oxo-2-(2-oxoazepan-1-yl)-ethyl)-3,7-dihydro-1*H*-purine-2,6-dione (10)

The title compound was prepared by a reaction of theophylline and **4**. The reaction mixture was heated at 50 °C for 4 h. Yield: 1.34 g, 36.2%; mp 148–151 °C; ^{1}H NMR (300 MHz) δ 7.57 (s, 1H, aromatic H), 5.51 (s, 2H, NCH_2), 3.80–4.00 (m, 2H, NCH_2 hexahydroazepin-2-one), 3.61 (s, 3H, N^3CH_3), 3.36 (s, 3H, N^1CH_3), 2.72–2.84 (m, 2H, $COCH_2$ hexahydroazepin-2-one), 1.65–2.05 (m, 6H, $CH_2CH_2CH_2CH_2$ hexahydroazepin-2-one); ^{13}C NMR (75 MHz) δ 178.18 (C15), 169.68 (C13), 155.24 (C6), 151.76 (C2), 148.64 (C4), 142.05 (C8), 107.28 (C5), 52.78 (C12), 44.07 (C20), 39.21 (C16), 29.71 (C19), 29.13 (C11), 28.15 (C10), 27.77 (C17), 23.52 (C18); ^{15}N NMR (30 MHz) δ 231.8 (N9), 174.5 (N14), 158.8 (N7), 151.7 (N1), 114.7 (N3); IR (cm^{-1}) 3113, 2943, 1704, 1659, 1550, 1475, 1376, 1253, 1192, 1158; MS m/z 356.2 ($M-Na^+$); UV λ_{max} (methanol) 206 nm, 274 nm.

5.1.13. 1,3-Dimethyl-7-(3-oxo-3-(2-oxoazepan-1-yl)-propyl)-3,7-dihydro-1*H*-purine-2,6-dione (11)

The title compound was prepared by a reaction of theophylline and **5**. Yield: 3.31 g, 85.8%; mp 146–147 °C; ^{1}H NMR (300 MHz) δ 7.70 (s, 1H, aromatic H), 4.62 (t, J = 6.8 Hz, 2H, NCH_2), 3.80–3.95 (m, 2H, NCH_2 hexahydroazepin-2-one), 3.49 (t, J = 6.9 Hz, 2H, CH_2CO), 3.59 (s, 3H, N^3CH_3), 3.40 (s, 3H, N^1CH_3), 2.62–2.78 (m, 2H, $COCH_2$ hexahydroazepin-2-one), 1.55–1.95 (m, 6H, $CH_2CH_2CH_2CH_2CH_2$ hexahydroazepin-2-one); ^{13}C NMR (75 MHz) δ 177.50 (C16), 172.97 (C14), 155.11 (C6), 151.75 (C2), 148.96 (C4), 142.15 (C8), 106.86 (C5), 43.30 (C12), 42.81 (C21), 40.39 (C13), 39.56 (C17), 29.69 (C11), 29.01 (C20), 27.91 (C10), 27.92 (C18), 23.63 (C19); ^{15}N NMR (30 MHz) δ 230.4 (N9), 176.5 (N15), 166.3 (N7), 151.5 (N1), 114.6 (N3); IR (cm^{-1}) 3123, 2935, 2857, 1700, 1668, 1547, 1475, 1387, 1260, 1185, 1151; MS m/z 370.2 ($M-Na^+$); UV λ_{max} (methanol) 206 nm, 274 nm.

5.1.14. 1,3-Dimethyl-7-(4-oxo-4-(2-oxoazepan-1-yl)-butyl)-3,7-dihydro-1*H*-purine-2,6-dione (12)

The title compound was prepared by a reaction of theophylline and **6**. Yield: 1.54 g, 38.4%; mp 108–110 °C; ^{1}H NMR (300 MHz) δ 7.58 (s, 1H, aromatic H), 4.38 (t, J = 6.7 Hz, 2H, NCH_2), 3.85–3.95 (m, 2H, NCH_2 hexahydroazepin-2-one), 3.59 (s, 3H, N^3CH_3), 3.41 (s, 3H, N^1CH_3), 2.91 (t, J = 6.9 Hz, 2H, CH_2CO), 2.65–2.75 (m, 2H, $COCH_2$ hexahydroazepin-2-one), 2.24 (qi, J = 6.8 Hz, 2H $CH_2CH_2CH_2$), 1.55–1.95 (m, 6H, $CH_2CH_2CH_2CH_2CH_2$ hexahydroazepin-2-one); ^{13}C NMR (75 MHz) δ 177.56 (C17), 174.65 (C15), 155.05 (C6), 151.70 (C2), 148.97 (C4), 140.97 (C8), 107.03 (C5), 43.34 (C12), 43.29 (C22), 39.60 (C18), 35.53 (C14), 29.65 (C11), 29.02 (C21), 28.44 (C19), 27.86 (C10), 26.63 (C20), 26.30 (C13); ^{15}N NMR (30 MHz) δ 230.6 (N9), 176.7 (N16), 168.5 (N7), 151.5 (N1), 114.6 (N3); IR (cm^{-1}) 3099, 2943, 2853, 1707, 1656, 1550, 1478,

1383, 1332, 1185, 1151; MS m/z 384.3 ($M-Na^+$); UV gλ_{max} (methanol) 205 nm, 274 nm.

5.2. Animal experiments

All animals used in this study were male Sprague–Dawley rats supplied by Kuwait University Animal Resource Centre. All experiments were done in accordance with guidelines on humane handling of experimental animals as established by the Canadian Council on Animal Care. The procedures were designed to minimize animal suffering, and the smallest number of animals necessary to produce the required results was used.

5.3. Slice preparation

Extracellular electrophysiological experiments were performed in coronal hippocampal slices generated from rats (75–150 g) using previously published techniques and methods.[62,63] Briefly, rats were deeply anesthetized with halothane, and were killed by quick decapitation. The brains were quickly removed and placed in ice cold (4 °C) artificial cerebrospinal fluid (aCSF) bubbled continuously with 95% O_2 and 5% CO_2 (Carbogen). The composition of the aCSF used for dissection, storage, and PS recording was (in mM) 120 NaCl, 3.3 KCl, 1.2 $MgSO_4$, 1.3 $CaCl_2$, 1.23 $NaHPO_4$, 25 $NaHCO_3$, and 10 D-glucose. 400 μm thick coronal slices containing the hippocampus were cut from a block of brain tissue in ice-cold aCSF using Leica VT 1000S (Leica Microsystems, Wetzlar, Germany) or OTS-4000 (Electron Microscopy Sciences, Hatfield PA, USA) tissue slicers. Prior to recording, slices were incubated for 1 h in aCSF, which was continuously bubbled with carbogen at room temperature. Slices were trimmed and suspended on nylon mesh in a 500 μL capacity recording chamber. Bath temperature was tightly maintained at 29–31 °C to ensure that changes in responses were not due to variation in temperature.[64] Slices were perfused at a flow rate of 2–3 mL/min with aCSF that was bubbled with carbogen.

5.4. Population spike recordings

An extracellular field recording glass electrode (3–15 MΩ) filled with 3 M NaCl was placed in the *stratum pyramidale* of area CA1, and bipolar stimulating electrodes were placed in the *stratum radiatum* near area CA1 to activate Schaffer collateral/commissural fibers. A PS was recorded by stimulating these afferents, and by moving the field recording electrode around until an optimal PS could be recorded. These responses were monitored for at least 30 min and were shown to be stable prior to the application of any drug.

5.5. Data acquisition, analysis, and statistics

All recordings were made using an Axopatch 1D amplifier and pClamp software (Clampex 8, Axon instruments) in current clamp mode at sampling rates of 50 kHz, filtered at 1 kHz, digitized and stored for off-line analysis. Each stored PS trace was an average of five successively triggered responses elicited at 10 s intervals. The amplitude of the PS was measured from the peak of the positive going wave to the tip of the negative going wave. Drugs were perfused between 5 and 20 min depending on the type of experiment. All data are expressed as mean ± standard error (SE). Statistical significance of all measures was determined using Student's t-test (paired or unpaired where appropriate) and was considered significant at $p \leqslant 0.05$ using SigmaStat® (Systat Software Inc., San Jose, CA, USA). PS amplitudes were normalized by taking the mean of 4–5 responses prior to drug application, and by dividing the rest of the values by this mean. These values were used for average plots and bar graphs. Graphical representations were done using GraphPad Prism® (GraphPad Software, San Diego, CA, USA), SigmaPlot® (Systat Software Inc., San Jose, CA, USA), and CorelDraw® (Corel Corp., Ottawa, ON, Canada) softwares.

5.6. Chemicals and drugs

All the theophylline analogs were synthesized in-house, and were dissolved in dimethyl sulfoxide. Stock solutions of 10 mM were prepared, aliquoted, and stored at −20 °C and were used within 2 weeks. Tetrodotoxin, atropine, 8-cyclopentyl-theophylline, and d-tubocurarine and all salts used in the preparation of aCSF and recoding solutions were obtained from Sigma Company (Steinheim, Germany). CGP55845 was obtained from Tocris (Bristol, UK). All drugs and chemicals were diluted by at least 1000-fold with aCSF to the desired concentration, and were applied by bath perfusion.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.07.045.

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