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Original article

Synthesis and adenosine receptor binding studies of some novel triazolothienopyrimidines

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Abstract

A new series of 5-alkyl/aryl-8,9-dimethyl/8,9,10,11-tetrahydro[1]benzothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine-3(2*H*)-thiones (**4a**–**k**) have been synthesized through a facile cyclization reaction of 4-hydrazino-2-alkyl/aryl-5,6-dimethyl/5,6,7,8-tetrahydro[1]benzothieno [2,3-*d*]pyrimidines (**3a**–**k**) using carbon disulphide under basic conditions. 4-Hydrazino-2-alkyl/aryl-5,6-dimethyl/5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidines (**3a**–**k**) were prepared by replacing the chloro group of 4-chloro-2-substituted-5,6-dimethyl/5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidines (**2a**–**k**) with hydrazine hydrate which were obtained by a known one-pot synthesis. The affinities of these compounds for adenosine A_1/A_{2A} receptors were determined at 1 μM concentration. The test compounds which exhibited more than 20% inhibition were selected and further screened at six different concentration levels to estimate their EC_{50}/K_i values. The most potent compounds in the series were **4c** and **4d** having an ethyl side chain at C_5 position with dimethyl and cyclohexyl substitution at the C_8–C_9 positions, exhibiting K_i values of 2.1 and 1.1 μM, respectively, at A_1ARs. The SAR indicates that by increasing or decreasing the alkyl chain length at C_5 led to reduced affinity. The remaining aryl/arylalkyl derivatives of the series were inactive showing that a simple alkyl side chain at C_5 is necessary for these ligands to bind at A_1ARs. However, none of the compounds showed inhibition on A_{2A} receptors at 1 μM concentration indicating their selectivity. This communication describes the design, synthesis and evaluation of these new molecules.

Keywords: Synthesis; Triazolopyrimidines; Adenosine A_1 receptor antagonists; Triazolothienopyrimidines; Radioligand binding assays

1. Introduction

Adenosine is an endogenous neuromodulator which mediates its biological effects by interacting with four adenosine receptor (AR) subtypes namely A_1, A_{2A}, A_{2B} and A_3 identified and cloned from various mammalian tissues [1–3]. Adenosine A_1 receptors (A_1AR) play significant role in a number of varied biological effects. These receptors are found both pre- and post-synaptically and are involved in the sedative, anxiolytic and locomotor-depressant effects of adenosine in the central nervous system [4–7]. Abundant evidence reveals an important role for A_1ARs in protection of heart [8–10], lung and brain [11] from ischemia reperfusion injury. Like A_1 receptors, the A_{2A} receptors have been implicated in a wide range of

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biological events. Because of the interaction between dopamine and A_{2A} receptors and as these receptors are primarily expressed in striatum, antagonists of A_{2A} receptors have potential therapeutic utility in alleviating Parkinson's disease [12].

Despite a large number of potential therapeutic applications, relatively few compounds entered clinical trials [13,14]. This could be due to a poor pharmacokinetic profile of some of the known AR antagonists, including low water solubility and/or low central nervous system penetration. As a consequence, a lot of interest has been generated in recent years to find ligands of ARs with high degree of selectivity. A large number of studies are aimed at designing new ligands which are free from undesirable side effects.

The naturally occurring xanthines, caffeine and theophylline are the prototypic AR antagonists. Several attempts have been made to improve the potency and selectivity of xanthine-based compounds which resulted in the synthesis and screening of a large number of new analogues. Of them, DPCPX and KW3802 (Fig. 1) are the standard xanthine A_1AR antagonists. However, these compounds failed in clinical trials as they lack acceptable pharmacokinetic profiles. This gave an impetus for the design of new xanthine-based, non-xanthine heterocyclic analogues and test their efficacy and selectivity towards ARs. Literature reveals that a wide variety of nitrogen containing heterocycles have been synthesized as possible A_1/A_{2A} selective antagonists (A_1AR) [15,16]. Particularly some tricyclic heteroaromatic (six-six-five ring systems) compounds (Fig. 2) like triazolo[1,5-*c*]quinazoline, triazolo[4,3-*a*]quinoxaline and imidazo[4,5-*c*]quinoline [17–20] derivatives were shown to be selective ligands with potent antagonistic activity at A_1ARs. They were designed as agents with potential therapeutic utility as cognitive enhancers, agents with antidementia properties, as psychostimulants, antidepressant drugs and ameliorants of cerebral function [21]. Several of these A_1AR antagonists were demonstrated with promising therapeutic potential for renal and cardiac failure [22,23]. Sarges et al. suggested that CP-68247 [24], a triazolo[4,3-*a*]quinoxaline derivative binds to ARs, by mimicking adenosine derivatives (Fig. 3).

Further various triazolopyrazolopyrimidines (five-six-five ring systems) [25] have been explored for affinity towards ARs, which showed better affinity/selectivity. These observations led us to design these novel molecules by replacing pyrazole ring from its bioisosteric thiophene which resulted in triazolothienopyrimidines. These were also found to be bioisosteres of triazoloquinoxalines in a fused system. The proposed structures have three lipophilic pockets (one of which can be filled better with cycloalkyl moiety) along with hydrogen donor–acceptor motif (Fig. 4) as per proposed pharmacophore model for A_1AR binding [26]. Hence it was felt worthwhile to synthesize a novel series of 5-alkyl/aryl-8,9-dimethyl/8,9,10,11-tetrahydro[1]benzothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine-3(2*H*)-thiones (**4a–k**) as potential A_1AR antagonists.

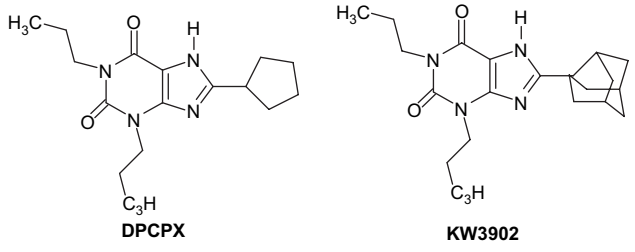


Fig. 1. Standard xanthine-based adenosine A_1 receptor antagonists.

2. Results and discussion

2.1. Chemistry

The synthetic route to the target compounds is outlined in the Scheme 1. 2-Amino-3-carbethoxy-4,5-dimethylthiophene, 2-amino-3-carbethoxy-4,5,6,7-tetrahydro[1]benzothiophene [27,28], 4-chloro-2-substituted-5,6-dimethylthieno[2,3-*d*]pyrimidines and 4-chloro-2-substituted-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidines **2a–k** [29] were prepared following reported procedures. The chlorine atom in **2a–k** was replaced by hydrazine by treating **2a–k** with hydrazine hydrate (99%) to yield 4-hydrazino-2-alkyl/aryl-5,6-dimethylthieno[2,3-*d*]pyrimidines and 4-hydrazino-2-alkyl/aryl-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidines **3a–k** in good yields [30]. The prominent peaks (3400–3200 cm^{-1}) for primary and secondary amino groups in the IR spectra indicated the formation of the expected product. The ^{1}H NMR spectrum showed a triplet at δ 6.4–6.6 ppm and a doublet at δ 3.8 ppm for NH and NH_2 groups and the presence of characteristic signals for dimethyl and cyclohexyl groups confirmed the product formation. Furthermore, the mass spectrum showed a prominent molecular ion peak [M^+] as the base peak and fragmentation pattern characteristic to its structure. The absence of an isotopic peak ($M^+ + 2$) also confirmed the replacement of the chloro group in the product.

Compounds **3a–k** were condensed with carbon disulphide under basic conditions using ethanol as solvent to yield the title compounds. The products thus obtained were purified and characterized as 5-alkyl/aryl-8,9-dimethylthieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine-3(2*H*)-thiones and 5-alkyl/aryl-8,9,10,11-tetrahydro[1]benzothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine-3(2*H*)-thiones **4a–k** on the basis of their analytical and spectral data. The IR spectra showed characteristic peaks (C=S) at 1200 cm^{-1} [weak] and 3090 cm^{-1} (*sec*-amino group). The disappearance of the primary amino proton signal and the appearance of the secondary amino signal at δ 14.0 in PMR spectrum indicated the product formation. Furthermore, the mass spectrum showed molecular ion peak [M^+] as the base peak and fragmentation pattern characteristic to its structure. The elemental analyses of all the newly synthesized compounds confirmed their formation.

2.2. Pharmacology

2.2.1. Radioligand binding studies

The synthesized compounds, **4a–k** were tested for their ability to displace [^{3}H]2-chloro-N^6-cyclohexyladenosine ([^{3}H]CCPA)

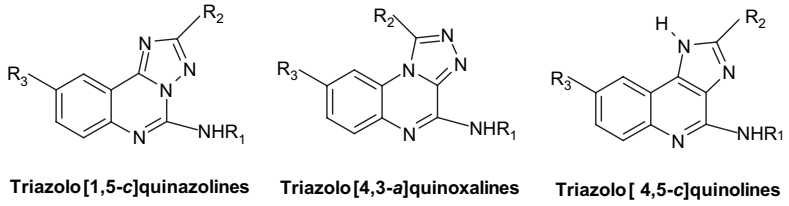


Fig. 2. Basic structures of representative tricyclic xanthine-based non-xanthine adenosine antagonists.

from A_1ARs and [^{3}H]MSX-2 from A_{2A}AR in rat cortical and striatal membranes, respectively. The A_1 and A_{2A} receptor binding affinities for compounds **4a–k** are expressed as K_i values or in percent inhibition of radioligand binding (at 1.0 μM concentration). The binding affinities data of compounds **4a–k** are presented in Table 1.

None of the compounds showed affinity towards A_{2A}ARs at 1.0 μM concentration. This gives a clue that the compounds fit fairly well into the proposed pharmacophoric model for A_1AR's.

Alkyl derivatives of the series (**4b**, **4c**, **4d**) showed affinity towards A_1ARs *i.e.*, the percentage of inhibition at 1.0 μM concentration was found to be 26, 21 and 56%, respectively, whereas the aryl and arylalkyl derivatives did not bind at the receptor site. This may be due to the increase in bulkiness of the lipophilic site which may result in lesser interaction. The results are in agreement with the fact that DPCPX, the archetypical A_1AR antagonist also has alkyl side chains in its structure (Fig. 1).

Those compounds which showed 20% inhibition were further screened at six different concentrations ranging from 0.01 μM–10 μM to determine their EC_{50} and K_i values. The most potent compounds in the series were **4c** and **4d** having an ethyl side chain at the C_5 position with dimethyl and cyclohexyl substitution at the C_8–C_9 positions, exhibiting K_i values of 2.1 and 1.1 μM, respectively, for A_1ARs.

Of the alkyl derivatives (methyl, ethyl and propyl derivatives), ethyl derivatives, **4c** and **4d** showed inhibition at micromolar ranges. Increasing or decreasing the length of the side chain (replacement of ethyl group by methyl or propyl) at C_5 led to reduced affinity indicating ethyl group at C_5 position as optimal structural requirement for the compounds to bind to A_1ARs.

Out of the 8,9,10,11-terahydrobenzo series (**4b**, **4d**, **4f**, **4h**, **4j**, **4k**) and 8,9-dimethyl series (**4a**, **4c**, **4e**, **4g**, **4i**) the former showed more affinity towards A_1ARs which is in agreement with the fact that one of the lipophilic pockets when filled with cycloalkyl moiety increases the affinity. As a whole this study reveals that the compounds presently synthesized, although weakly active at A_1ARs, are fairly selective. Further optimization may potentially result in better affinity.

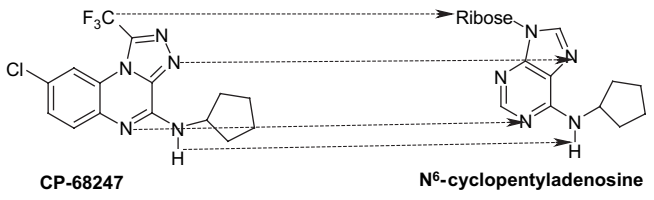


Fig. 3. Putative binding mode of CP-68247: overlap with N^6-cyclopentyladenosine.

3. Conclusion

In conclusion, we designed a set of novel triazolothienopyrimidines based on the adenosine A_1 receptor pharmacophoric model. The designed compounds were successfully synthesized in quantitative yields. The compounds were screened for their affinity towards A_1 and A_{2A} receptors and showed no affinity towards A_{2A}ARs. This indicates the validity of our design as they fit well in the proposed pharmacophoric model. Alkyl derivatives showed affinity at micromolar ranges towards A_1ARs out of which, ethyl derivative with a fused cyclohexyl ring system at C_{8-9} showed highest affinity with selectivity towards A_1ARs. Hence it is worthwhile to attempt at fusing triazolothienopyrimidines with bulky cycloalkyl rings and to evaluate them for affinity. Further efforts are currently being taken up to optimize the lead structure and the results of which will be the basis of our future research endeavor.

4. Experimental

Analytical TLC was performed on Silica Gel F_{254} plates (Merck) with visualization by UV (254 nm) chamber with protective filters (Wiswo Instruments, Mumbai). Melting points were determined in open capillaries on a Gallenkemp Melting Point Apparatus and are uncorrected. The IR spectra (KBr, υ_{max}, cm^{-1}) were run on Perkin Elmer FTIR Spectrophotometer. Proton (^{1}H) NMR spectra were recorded on a AMX-400 and chemical shifts are expressed as δ values (ppm) downfield from tetramethylsilane (TMS) using either $CDCl_3$

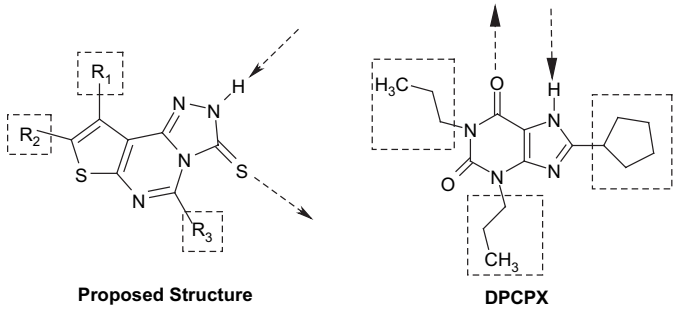
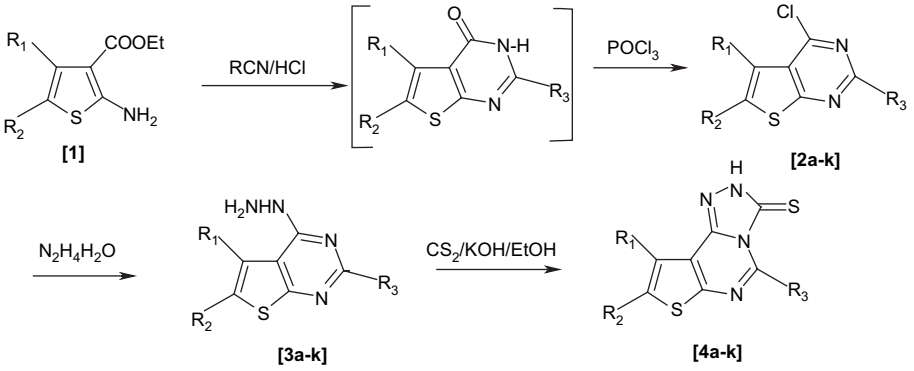


Fig. 4. Comparison of proposed structure with published pharmacophore model (DPCPX) for A_1 receptor antagonists. Dashed arrows indicate hydrogen bond acceptor–donor motifs; dashed squares indicate lipophilic pockets.



	R1	R2	R3		R1	R2	R3
4a	Me	Me	Me	**4f**	-$(CH_2)_4$-		Pr
4b	-$(CH_2)_4$-		Me	**4g**	Me	Me	Ph
4c	Me	Me	Et	**4h**	-$(CH_2)_4$-		Ph
4d	-$(CH_2)_4$-		Et	**4i**	Me	Me	Bz
4e	Me	Me	Pr	**4j**	-$(CH_2)_4$-		Bz
				4k	-$(CH_2)_4$-		4-Pyridyl

Scheme 1. Synthesis of some novel 5-alkyl/aryl-8,9-dimethyl/8,9,10,11-tetrahydro[1]benzothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine-3(2*H*)-thiones.

or DMSO-d_6 as solvents. Mass [EI-MS (70 eV)] spectra were recorded on Autospec Mass spectrometer. Elemental analyses were performed on Carlo Erba 1108 elemental analyzer. All the chemicals were obtained from E. Merck (Mumbai) of analytical grade, and were used without further purification. Graph Pad PRISM [Version 3.0 from San Diego, CA) was used for biological data analyses.

4.1. Chemistry

4.1.1. General procedure for the preparation of 4-hydrazino-2-alkyl/aryl-5,6-dimethyl/5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidines (**3a–k**)

To a warm solution of 2-alkyl/aryl-4-chloro-5,6-dimethyl/5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidines [10 mmol] (**2a–k**)] in ethanol (95%, 20 ml) was added a solution of hydrazine hydrate [99%, 4.3 g, 100 mmol] dropwise and heated under reflux for 2 h. Then the reaction mixture was cooled and poured onto crushed ice. The solid precipitate thus obtained was filtered, dried and recrystallized from ethanol (95%).

Table 1
Binding affinity of compounds **4a–k** at rat A_1 and A_{2A} adenosine receptors

Compd. no.	R_1	R_2	R_3	A_1AR[a] % inhibition at 1.0 μM conc.	$K_i \pm$ SEM μM
4a	Me	Me	Me	4	–
4b	$-(CH_2)_4-$		Me	26	4.40 ± 1.1
4c	Me	Me	Et	21	2.10 ± 0.4
4d	$-(CH_2)_4-$		Et	56	1.17 ± 0.4
4e	Me	Me	Pr	13	–
4f	$-(CH_2)_4-$		Pr	0	–
4g	Me	Me	Ph	0	–
4h	$-(CH_2)_4-$		Ph	0	–
4i	Me	Me	Bz	0	–
4j	$-(CH_2)_4-$		Bz	0	–
4k	$-(CH_2)_4-$		4-Pyridyl	0	–
DPCPX	–		–	–	0.5 ± 0.2 (nM)

[a] All the test compounds failed to show any $A_{2A}AR$ inhibition up to 1 μM and DPCPX showed K_i values at 157 ± 6.0 nM on rat A_{2A} receptors.

*4.1.1.1. 4-Hydrazino-2-ethyl-5,6-dimethylthieno[2,3-d]pyrimidine (**3c**).* M.p. 109–110 °C; yield 71%; IR (KBr) υ_{max}: 3190, 3320 (NH_2), 2910 (CH_3CH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.2–1.4 (t, 3H, $J = 8.7$ Hz, CH_2CH_3), 2.42 (s, 6H, CH_3), 3.35–3.40 (q, 2H, $J = 8.7$ Hz, CH_2CH_3), 3.65–3.85 (d, 2H, $J = 5.6$ Hz, $NHNH_2$, D_2O exchangeable), 6.5–6.7 (t, 1H, $J = 5.7$ Hz, $NHNH_2$, D_2O exchangeable) ppm. EI-MS [*m/z*, %]: 222 [M^+ 100]. Anal. Calcd for $C_{10}H_{14}N_4S$: C, 54.05; H, 6.30; N, 25.22. Found: C, 54.25; H, 6.55; N, 25.52.

*4.1.1.2. 4-Hydrazino-2-ethyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine (**3d**).* M.p. 159–160 °C; yield 75%; IR (KBr) υ_{max}: 3190, 3320 (NH_2), 2910 (CH_3CH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.2–1.4 (t, 3H, $J = 7.9$ Hz, CH_3), 1.8–2.0 (m, 4H, CH_2 at 6 and 7), 2.7–3.0 (m, 6H, $-CH_2$ at 2, 5 and 8), 3.6–3.8 (d. 2H, $J = 5.79$ Hz, $-NH_2$, D_2O exchangeable), 6.4–6.6 (t, 1H, $J = 6.0$ Hz, NH, D_2O exchangeable) ppm; EI-MS [*m/z*, %]: 248 [M^+ 100]. Anal. Calcd for $C_{12}H_{16}N_4S$: C, 58.06; H, 6.45; N, 22.58. Found: C, 58.35; H, 6.85; N, 22.88.

*4.1.1.3. 4-Hydrazino-2-propyl-5,6-dimethylthieno[2,3-d]pyrimidine (**3e**).* M.p. 102–103 °C; yield 77%; IR (KBr) υ_{max}: 3190, 3320 (NH_2), 2910 (alk) cm^{-1}; ^1H NMR ($CDCl_3$,) δ: 1.10–1.12 (t, 3H, $J = 11.1$ Hz, CH_3), 2.25 (s, 6H, CH_3), 2.42–2.52 (m, 2H, $-CH_2CH_2CH_3$), 3.01–3.05 (m, 2H, $-CH_2CH_2CH_3$), 3.62–3.82 (d. 2H, $J = 5.8$ Hz, $-NHNH_2$, D_2O exchangeable), 6.6–6.8 (t, 1H, $J = 6.0$ Hz, $NHNH_2$, D_2O exchangeable) ppm; EI-MS [*m/z*, %]: 236 [M^+ 100]. Anal. Calcd for $C_{11}H_{16}N_4S$: C, 55.93; H, 6.77; N, 23.72. Found: C, 56.13; H, 7.13; N, 24.10.

*4.1.1.4. 4-Hydrazino-2-propyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine (**3f**).* M.p. 128–130 °C; yield 82%; IR (KBr)

υ_{max}: 3190, 3320 (NH_2), 2910 (alk) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.0–1.1 (t, 3H, $J = 10.9$ Hz, $CH_3CH_2CH_2$–), 1.7–2.0 (m, 4H, CH_2 at 6 and 7), 2.45–2.55 (m, 2H, $-CH_2CH_2CH_3$), 2.75–2.80 (m, 2H, CH_2 at 5 and 8), 3.85–4.05 (m, 2H, $-CH_2CH_2CH_3$), 4.20–4.30 (d. 2H, $J = 5.5$ Hz, $-NHNH_2$, D_2O exchangeable), 6.6–6.8 (t, 1H, $J = 5.6$ Hz, $NHNH_2$, D_2O exchangeable) ppm; EI-MS [*m/z*, %]: 262 [M^+ 100]. Anal. Calcd for $C_{13}H_{18}N_4S$: C, 59.54; H, 6.87; N, 21.37. Found: C, 59.85; H, 7.05; N, 21.60.

*4.1.2. General procedure for the preparation of 5-alkyl/aryl-8,9-dimethyl/8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thiones (**4a–k**)*

2-Alkyl/aryl-4-hydrazino-5,6-dimethyl/5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidines (**3a–k**) [10 mmol] and carbon disulphide [7.6 g, 10 ml, 100 mmol] in 10% alcoholic potassium hydroxide (20 ml) solution was heated under reflux for 5 h. The reaction mixture was cooled and added onto crushed ice. The solid product thus obtained was filtered, dried and recrystallized from chloroform–ethanol (1:1) mixture.

*4.1.2.1. 5-Methyl-8,9-dimethylthieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (**4a**).* M.p. 275–276 °C; yield 69%; IR (KBr) υ_{max}: 3190 (NH), 2910 (CH_3), 1200 (C=S), 1290 (C–N) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.2 (s, 3H, CH_3 at 5), 2.45 (s, 6H CH_3 at 8 and 9), 11.4 (s, 1H, N*H*, D_2O exchangeable) ppm; EI-MS [*m/z*, %]: 250 [M^+ 100]. Anal. Calcd for $C_{10}H_{10}N_4S_2$: C, 48.00; H, 4.00; N, 22.40. Found: C, 48.25; H, 4.30; N, 22.75.

*4.1.2.2. 5-Methyl-8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (**4b**).* M.p. 280–281 °C; yield 76%; IR (KBr) υ_{max}: 3190 (NH), 2910 (CH_3), 1200 (C=S), 1290 (C–N) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.9 (m, 4H, CH_2 at 9 and 10), 2.35 (s, 1H CH_3), 2.7–3.0 (m, 4H, CH_2 at 8 and 11), 11.1 (s, 1H, N*H*, D_2O exchangeable) ppm; EI-MS [*m/z*, %]: 276 [M^+ 100]. Anal. Calcd for $C_{12}H_{12}N_4S_2$: C, 52.17; H, 4.34; N, 20.28. Found: C, 52.45; H, 4.66; N, 20.50.

*4.1.2.3. 5-Ethyl-8,9-dimethylthieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (**4c**).* M.p. 180–181 °C; yield 68%; IR (KBr) υ_{max}: 3190 (NH), 2910 (CH_3), 1200 (C=S), 1290 (C–N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.2–1.4 (t, 3H, $J = 8.69$ Hz, CH_2CH_3), 2.35 (s, 6H CH_3 at 8 and 9), 3.4 (t, 2H, $J = 8.69$ Hz, CH_2CH_3), 11.1 (s, 1H, N*H*, D_2O exchangeable) ppm; EI-MS [*m/z*, %]: 264 [M^+ 100]. Anal. Calcd for $C_{11}H_{12}N_4S_2$: C, 50.76; H, 4.61; N, 21.53. Found: C, 50.95; H, 4.75; N, 21.65.

*4.1.2.4. 5-Ethyl-8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (**4d**).* M.p. 244–245 °C; yield 72%; IR (KBr) υ_{max}: 3190 (NH), 2910 (CH_3), 1200 (C=S), 1290 (C–N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.1 (t, 3H $J = 4.4$ Hz, CH_3), 1.7–2.0 (m, 4H, CH_2 at 9 and 10), 2.8–3.0 (m, 4H, CH_2 at 8 and 11), 3.9–4.0 (q, 2H, $J = 4.24$ Hz, $-CH_2CH_3$), 14.0 (s, 1H, N*H*, D_2O exchangeable) ppm; EI-MS [*m/z*, %]: 290 [M^+ 100]. Anal. Calcd for $C_{13}H_{14}N_4S_2$: C, 53.79; H, 7.3; N, 19.3. Found: C, 54.04; H, 7.26; N, 19.12.

*4.1.2.5. 5-Propyl-8,9-dimethylthieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (**4e**).* M.p. 198–200 °C; yield 65%; IR (KBr) υ_{max}: 3190 (NH), 2910 (CH_3), 1200 (C=S), 1290 (C–N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.10–1.13 (t, 3H, $J = 10.9$ Hz, $CH_3CH_2CH_2$–), 2.28 (s, 6H, CH_3), 2.45–2.55 (m, 2H, $CH_3CH_2CH_2$–), 3.05–3.15 (m, 2H, $CH_3CH_2CH_2$–), 11.2 (s, 1H, N*H*, D_2O exchangeable) ppm; EI-MS [*m/z*, %]: 278 [M^+ 100]. Anal. Calcd for $C_{12}H_{14}N_4S_2$: C, 51.79; H, 5.03; N, 20.14. Found: C, 52.05; H, 5.25; N, 20.35.

*4.1.2.6. 5-Propyl-8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (**4f**).* M.p. > 300 °C; yield 74%; IR (KBr) υ_{max}: 3190 (NH), 2910 (CH_3), 1200 (C=S), 1290 (C–N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.0–1.1 (t, 3H, $J = 11.1$ Hz, $CH_3CH_2CH_2$), 1.7–2.0 (m, 4H, CH_2 at 9 and 10), 2.45–2.55 (m, 2H, $CH_3CH_2CH_2$), 2.75–2.80 (t, 2H, $J = 8.3$ Hz, CH_2 at 5), 2.85–3.05 (t, 2H, $J = 8.3$ Hz, CH_2 at 8), 3.85–4.0 (m, 2H, $CH_3CH_2CH_2$), 14.0 (s, 1H, N*H*, D_2O exchangeable) ppm; EI-MS [*m/z*, %]: 304 [M^+ 50]. Anal. Calcd for $C_{14}H_{16}N_4S_2$: C, 55.26; H, 5.26; N, 18.42. Found: C, 55.50; H, 5.55; N, 18.65.

*4.1.2.7. 5-Phenyl-8,9-dimethylthieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (**4g**).* M.p. > 300 °C; yield 80%; IR (KBr) υ_{max}: 3190 (NH), 2910 (CH_3), 1200 (C=S), 1290 (C–N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.4 (s, 6H, CH_3 at 8 and 9), 7.2–7.6 (m, 5H, C_6H_5), 14.2 (s, 1H, N*H*, D_2O exchangeable) ppm; EI-MS [*m/z*, %]: 312 [M^+ 100]. Anal. Calcd for $C_{15}H_{12}N_4S_2$: C, 57.69; H, 3.84; N, 17.9. Found: C, 57.84; H, 4.12; N, 18.15.

*4.1.2.8. 5-Phenyl-8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (**4h**).* M.p. > 300 °C; yield 80%; IR (KBr) υ_{max}: 3190 (NH), 2910 (CH_3), 1200 (C=S), 1290 (C–N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.5–1.7 (m, 4H, CH_2 at 9 and 10), 2.5–2.7 (m, 4H, CH_2 at 8 and 11), 7.2–7.6 (m, 5H, Ar*H*), 14.3 (s, 1H, N*H*, D_2O exchangeable) ppm; EI-MS [*m/z*, %]: 339 [M^+100]. Anal. Calcd for $C_{15}H_{12}N_4S_2$: C, 60.35; H, 4.14; N, 16.56. Found: C, 60.60; H, 4.45; N, 16.95.

*4.1.2.9. 5-Benzyl-8,9-dimethylthieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (**4i**).* M.p. 165–167 °C; yield 73%; IR (KBr) υ_{max}: 3190 (NH), 2910 (CH_3), 1200 (C=S), 1290 (C–N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.5 (s, 6H, CH_3), 3.2 (S, 2H, CH_2Ph), 7.28–7.67 (m, 5H, C_6H_5), 14.1 (s, 1H, N*H*, D_2O exchangeable) ppm; EI-MS [*m/z*, %] (70 eV): 326 [M^+ 25], 294 [M^+–SH, 100]. Anal. Calcd for $C_{16}H_{14}N_4S_2$: C, 58.89; H, 4.29; N, 17.17. Found: C, 59.16; H, 4.55; N, 17.35.

*4.1.2.10. 5-Benzyl-8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (**4j**).* M.p. 232–234 °C; yield 75%; IR (KBr) υ_{max}: 3190 (NH), 2910 (CH_3), 1200 (C=S), 1290 (C–N) cm^{-1}; ^1H NMR (DMSO-d_6) δ:

1.3–1.5 (m, 4H, CH_2 at 9 and 10), 2.2–2.5 (m, 4H, CH_2 at 8 and 11), 3.35 (s, 2H, CH_2Ph), 7.25–7.8 (m, 5H, Ar*H*), 14.0 (s, 1H, N*H*, D_2O exchangeable) ppm; EI-MS [*m*/*z*, %] (70 eV): 352 [M^+ 100]. Anal. Calcd for $C_{18}H_{16}N_4S_2$: C, 61.36; H, 4.54; N, 15.90. Found: C, 61.50; H, 4.85; N, 16.27.

*4.1.2.11. 5-Pyridyl-8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (**4k**).* M.p. 238–240 °C; yield 67%; IR (KBr) υ_{max}: 3190 (NH), 2910 (CH_3), 1200 (C=S), 1290 (C–N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.6–1.9 (m, 4H, CH_2 at 9 and 10), 2.4–2.8 (m, 4H, CH_2 at 8 and 11), 7.65–8.25 (m, 4H, Ar*H*), 14.4 (s, 1H, N*H*, D_2O exchangeable) ppm; EI-MS [*m*/*z*, %]: 339 [M^+ 100]. Anal. Calcd for $C_{16}H_{13}N_5S_2$: C, 56.63; H, 3.83; N, 20.64. Found: C, 56.95; H, 4.10; N, 20.89.

4.2. Radioligand binding studies

4.2.1. Materials

[^{3}H]CCPA was obtained from NEN Life Sciences (48.6 Ci/mmol) and [^{3}H]MSX-2 from Amersham (85 Ci/mmol).

4.2.2. Membrane preparations

Frozen rat brains were obtained from Pel Freez, Rogers, AR, USA. Rat brains were dissected to obtain cortical membrane preparations for A_1 and striatal membrane preparations for A_{2A} assays as described [31,32].

4.2.3. Radioligand binding assays

Binding assays for A_1 and A_{2A} were performed essentially as described in the literature [33,34]. Stock solutions of the compounds were prepared in dimethyl sulphoxide (DMSO); the final concentration of DMSO in the assay was 2.5%. The radioligand concentrations were [^{3}H] CCPA: 0.5 nM (rat A_1) and [^{3}H] MSX-2: 1.0 nM (rat A_{2A}).

Drug solution (50 μl) was added into a 96-well plate followed by 50 μl of radioligand solution and 100 μl (30 μg/vial) of protein suspension of rat brain cortex (A_1) or rat brain striatal membranes (A_{2A}) and incubated at room temperature for 90/30 min. CADO (2-chloroadenosine)/NECA [5′-(*N*-ethylcarbamoyl)adenosine] was used for nonspecific binding for A_1 and A_{2A}AR binding studies, respectively. Incubation was terminated by rapid filtration over Whatman GF/B filters using a Brandell cell harvester (Brandell, Gaithersburg, MD). To the filter plate scintillation liquid was added (40 μl/vial – Microscent-20) and incubated at room temperature for 10 h and radioactivity was measured by a scintillation counter. Those compounds whose percentage inhibition was found to be more than 20% at a test concentration of 1 μM were taken and a full concentration–inhibition curve was determined.

4.2.4. Data analysis

Data were analyzed using Graph Pad PRISM version 3.0 (San Diego, CA). For nonlinear regression analysis, the Cheng–Prushoff equation and a K_D value of 0.5 nM for [^{3}H] CCPA at rat A_1ARs was used to calculate K_i values from EC_{50} values.

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