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8-(Furan-2-yl)-3-phenethylthiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidine-2(3H)-thione as novel, selective and potent adenosine A_{2A} receptor antagonist

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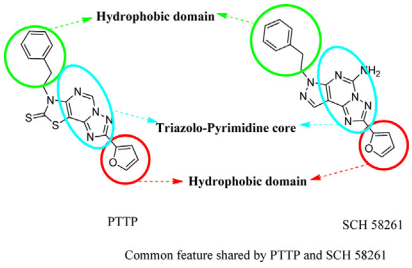
HIGHLIGHTS

- PTTP showed high binding affinity and selectivity for adenosine A_{2A} receptor.
- Compound exhibited potent A_{2A} receptor antagonist property in cAMP functional assay.
- PTTP successfully reduced haloperidol and NECA induced motor impairment in mice.
- Compound did not show neurotoxicity in rotarod test at the dose 20 mg/kg.

GRAPHICAL ABSTRACT



Common feature shared by PTTP and SCH 58261

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ABSTRACT

Antagonism of the human A_{2A} receptor has been implicated to alleviate the symptoms associated with Parkinson's disease. The present finding reveals the potential of PTTP (8-(furan-2-yl)-3-phenethylthiazolo[1,2,4]triazolo[1,5-c]pyrimidine-2(3H)-thione) as novel and potent $A_{2A}R$ antagonist. In radioligand binding assay, PTTP showed significantly high binding affinity (K_i 6.3 nM) and selectivity with $A_{2A}R$ ($A_1R/A_{2A}R = 4603$) which was comparable to the results of docking analysis ($K_i = 1.6$ nM, $\Delta G = -14.52$ Kcal/mol). PTTP antagonized (0.46 pmol/ml) the effect of NECA-induced increase in cAMP concentration (0.65 pmol/ml) better than SCH58261 (0.55 pmol/ml) in HEK293 T cells. Haloperidol and NECA-induced mice pre-treated with PTTP at 10 mg/kg showed attenuation in catalepsy and akinesia without significant neurotoxicity in rotarod test at 20 mg/kg. Essentially, novel compound demonstrated remarkable potential as $A_{2A}R$ antagonist in the therapy of PD.

1. Introduction

Parkinson's disease (PD) is a severe neurological disorder characterized by gradual depletion of striatal dopamine levels due to degeneration of dopaminergic neurons in nigrostriatal pathway [1]. Deregulations of striatal function leads to the classic motor symptoms of PD, such as resting tremor, muscular rigidity, akinesia, and bradykinesia. Symptomatic relief for management of PD involves L-dopa treatment to supplement dopamine [2].

The common side effects of L-dopa therapy are nausea, somnolence, dizziness, and headache; however, serious adverse reactions may include confusion, hallucinations, delusions, agitation, and psychosis [3]. The adenosine receptors have emerged as exciting non-DA target in the therapy of PD [4]. Adenosine interacts with four adenosine receptor subtypes viz. A_1, A_{2A}, A_{2B}, and A_3 belonging to the family of G-protein coupled receptor [5].

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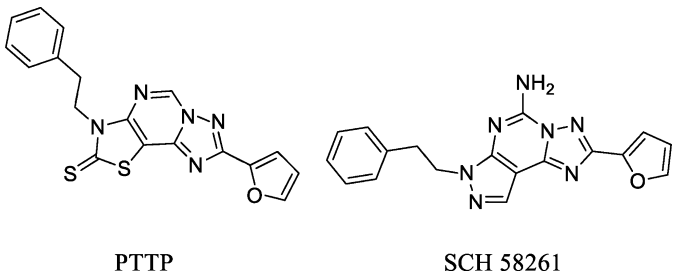

Fig. 1. Chemical structure of PTTP and standard drug SCH 58261.

Adenosine A_{2A} receptors (A_{2A}Rs) are highly expressed in the striatum, and predominantly located post-synaptically in D_2 dopamine (DA) receptor (D_2Rs)-expressing striatopallidal projecting neurons [6]. A_{2A}R antagonists facilitate DA receptor signaling to control motor function in animal models of DA deregulation and might provide therapeutic benefit in PD patients [7].

Numerous structurally different A_{2A}R antagonists such as xanthine compounds viz. methylxanthines, caffeine and theophylline were synthesized, however, were unsuccessful in clinical trials such as KW6002 [8]. Non-xanthine compounds viz. SCH58261 and ZM241385 have been reported to possess strong binding affinity with A_{2A}R and are currently in clinical trials [9]. Essentially, development of novel A_{2A}R antagonists with high binding affinity and selectivity for A_{2A}R is needed.

In continuation of our earlier work [9], the tricyclic core thiazolo-triazolo-pyrimidine possessing N-3-phenylethyl side chain in thiazole ring was synthesized to give novel compound 8-(furan-2-yl)-3-phenethylthiazolo[1,2,4]triazolo[1,5-c]pyrimidine-2(3H)-thione (PTTP) as potential A_{2A}R antagonist (Fig. 1). The present work describes the pre-clinical studies of PTTP as a potent and selective A_{2A}R antagonist. The plausible interaction of PTTP with A_{2A}R was studied by docking analysis. In vitro radioligand-binding assay using membranes isolated from transfected HEK293T cells was carried to evaluate the binding affinity and selectivity of PTTP for A_{2A}R. cAMP functional assay was performed to confirm the antagonistic property of PTTP in NECA-induced HEK293T cells. To evaluate in vivo efficacy of PTTP, haloperidol and NECA induced catalepsy and akinesia was studied, and toxicity was assessed in rotarod test.

2. Materials and method

2.1. Docking analysis

The crystal structure of A_{2A}R and PTTP with standard A_{2A}R antagonists (e.g., SCH58261 and ZM 241385) was set up for docking analysis with standard protocol according to the reported method [9]. Docking simulations were performed with Auto Dock3.0.5.

2.2. Radioligand binding assay

Radioligand binding assay for A_{2A}R and A_1R were carried with [^{3}H] ZM241385, [^{3}H] DPCPX and PTTP using the membranes isolated from HEK293T cells transfected with A_1R and A_{2A}R respectively as described earlier [10,11].

2.3. Functional assay

In the functional assay, cAMP concentrations were determined using direct cAMP EIA kit (ENZO Life sciences) as reported earlier [10,11].

2.4. Haloperidol and NECA-induced catalepsy and akinesia

Adult Swiss white male mice (4–6 weeks, 20–30 g) were provided from National Institute of Communicable Diseases (NICD), Delhi, India. The experimental protocol met the National Guidelines on the "Proper Care and Use of Animals in Laboratory Research (Indian National Science Academy, New Delhi). The animals were divided into 9 groups and in each group 6 mice were used for experiments. SCH58261 (10 mg/kg), PTTP (5, 10, 15 and 20 mg/kg) were administered intraperitoneal (ip) to each mice of the assigned group (Groups 5–9) respectively. Saline and 1% acacia in saline and PTTP (20 mg/kg) alone were injected to three control groups (Groups 1–3). After 30 min of pre-treatment, haloperidol (2.5 mg/kg)/NECA (0.5 mg/kg) was injected (ip) to one group (Group 4) and all pre-treated groups. Catalepsy and akinesia score was measured at different time intervals (0, 30, 60, 90 and 120 min).

The inability of an animal to correct an externally imposed posture (Catalepsy score) was measured at different time intervals with both limbs on a square wooden block (3 cm high) by placing the animals on a flat horizontal surface. The length of time that animals held the bar without any voluntary movement was recorded, with a cutoff time of 3 min.

Akinesia was measured by noting the latency in seconds (s) of the animals to move all four limbs and the test was terminated if latency exceeded 180 s. Each animal was initially acclimatized for 5 min on a wooden elevated (30 cm) platform (40 cm × 40 cm) used for measuring akinesia in mice. Using a stopwatch, the time taken (s) by the animal to move all the four limbs was recorded [9].

2.5. Neurotoxicity study

The neurological toxicity test (NT) induced by a compound was detected in mice using standardized rotarod test. Control mice, when placed on the rod can maintain their balance for prolong time period. The acute motor impairment can be demonstrated by the inability of the animal to maintain balance for 3 min in each three successive trial [12].

2.6. Drugs

The drugs [^{3}H] ZM 241385 and [^{3}H] DPCPX were procured from American Radiolabeled Chemicals, St. Louis, USA, and Haloperidol, SCH58261 and NECA from Sigma Chemicals Co. (St. Louis, MO), respectively. PTTP was synthesized in our laboratory (see Supplementary materials). Haloperidol, SCH58261, NECA and PTTP were dissolved in 1% acacia in saline.

3. Results

3.1. Docking analysis

Structure-based approaches have become vital components of modern drug design. The pose of antagonists binding with the amino acid residues in the receptor could be used for design of receptor selective antagonists. The docking analysis of PTTP and SCH58261 with A_{2A}R showed that both shared identical binding motif inside the trans-membranes (TMs) region and extracellular loops (ECL2) of the human A_{2A}R, and the interactions were comparable to the A_{2A} R co-crystallized ZM241385. In the binding cavity of A_{2A}R, PTTP and SCH58261 were oriented toward the amino acid residues N253, M177 and V178 extending H-bond interaction with amide moiety of N253 (Fig. 2). The receptor residues F168, E169 from ECL2 were involved in aromatic stacking and hydrophobic polar interaction with the phenyl ethyl moiety of PTTP. The furan ring of SCH58261 and PTTP formed H-bond interactions with the residues F183, H250 and hydrophobic interactions with V84, L85,