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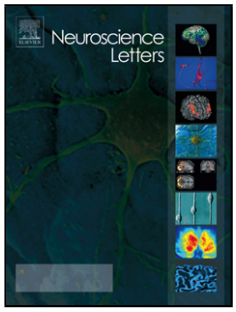

Design and development of 1,3,5-triazine-thiadiazole hybrids as potent adenosine A_2A receptor (A_2AR) antagonist for benefit in Parkinson's Disease

Anoop Masih, Saumya Singh, Amol Kumar Agnihotri, Sabeena Giri, Jitendra Kumar Shrivastava, Nidhi Pandey, Hans Raj Bhat, Udaya Pratap Singh

PII: S0304-3940(20)30492-4

DOI: https://doi.org/10.1016/j.neulet.2020.135222

Reference: NSL 135222

To appear in: *Neuroscience Letters*

Received Date: 22 May 2020

Revised Date: 25 June 2020

Accepted Date: 27 June 2020

Please cite this article as: Masih A, Singh S, Agnihotri AK, Giri S, Shrivastava JK, Pandey N, Bhat HR, Singh UP, Design and development of 1,3,5-triazine-thiadiazole hybrids as potent adenosine A_2A receptor (A_2AR) antagonist for benefit in Parkinson's Disease, *Neuroscience Letters* (2020), doi: https://doi.org/10.1016/j.neulet.2020.135222

Design and development of 1,3,5-triazine-thiadiazole hybrids as potent adenosine A_2A receptor (A_2AR) antagonist for benefit in Parkinson's Disease

Short Title: 1,3,5-triazine as A_2A receptor antagonist.

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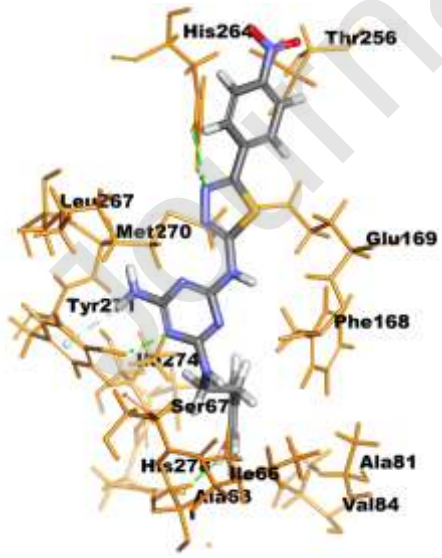
Graphical abstract



Highlights

- Discovery of 1,3,5-triazine-thiadiazole as novel A_2A receptor antagonist.
- Compound 7e as most potent A_2A receptor antagonist.
- Compound 7e found deeply buried into the active site of A_2A receptor.

Abstract

Various studies showed adenosine A_2A receptors (A_2ARs) antagonists have profound therapeutic efficacy in Parkinsons Disease (PD) by improving dopamine transmission, thus being active in reversing motor deficits and extrapyramidal symptoms related to the disease. Therefore, in the presents study, we have showed the development of novel 1,3,5-triazine-thiadiazole derivative as potent A_2AARs antagonist. In the radioligand binding assay, these molecules showed excellent binding affinity with A_2AR compared to A_1R, with significant selectivity. Results suggest, compound **7e** as most potent antagonist of A_2AR among the tested series. In docking analysis with A_2AR protein model, compound **7e** found to be deeply buried into the cavity of receptor lined via making numerous interatomic contacts with His264, Tyr271, His278, Glu169, Ala63, Val84, Ile274, Met270, Phe169. Collectively, our study demonstrated 1,3,5-triazine-thiadiazole hybrid as a highly effective scaffold for the design of new A_2A antagonists.

Keywords: Parkinson's disease, adenosine A_2A receptor, 1,3,5-triazine, antagonist, docking.

1. Introduction

Parkinson's disease (PD) is a long term, degenerative neurological disease mainly affecting motor function of the body due to loss of nerve cells producing dopamine [1]. Thus, depletion of dopamine results in tremor, rigidity, bradykinesia and postural instability. The current therapeutic approach to treat PD are mainly based on the dopaminergic drugs designed to replace the action of dopamine in the deplete striatum [2–4]. Despite their clinical benefit against PD, these drugs are associated with problematic adverse effects, such as, dyskinesia, sedation and compulsive behaviour and increases as disease progresses [5].

In recent year targeting the adenosine A_2A receptor (A_2AR) has emerged as a promising strategy for the treatment of Parkinson's diseases (PD) [6]. It belongs to the four subtypes of A-family of G-protein-coupled receptors (GPCRs), for instance, A_1, A_2A, A_2B and A_3, which elicit their specific function after interaction with neurotransmitter adenosine. The striatum of brain is the location of A_2AR, where it regulates the motor activity by modulating the dopamine

D2 receptor activation [7,8]. Consequently, inhibition of the interaction of adenosine with the A_2AR by antagonists may provide a potential treatment for Parkinson's disease by improving dopamine transmission, thus being active in reversing motor deficits and extrapyramidal symptoms related to the disease [9]. Various A_2AR antagonists have made their way to the clinical trials and advanced up to Phase III (Fig. 1) [10]. Despite their high potency, these inhibitors are associated with various drawbacks such as poor solubility and high toxicity.

Among the heterocyclic molecules, 1,3,5-triazine is well known for diverse array of biological activities [11]. Since last decade, our group has been working on the development of novel derivatives of 1,3,5-triazine against cancer [12], diabetes, bacteria [13–15], fungus [16], malaria [17–19] and against cystic fibrosis [20]. Particularly against PD, ZM241385, a non-xanthine, 1,3,5-triazine based competitive A_2AR antagonist showed high affinity in the nano-molar range while it has low affinity at the other adenosine receptor subtypes (A_1, A_2B, and A_3) [21–23]. Tozadenant, an another A_2A receptor antagonist shown promising result in PD and advanced to Phase III trials [24–26]. However, the trial has been stopped due to death of five enrolled patients. Thus, discovery of novel A_2A receptor antagonist is worth to be investigated. The present study was undertaken to develop potent and effective 1,3,5-triazine-thiadiazole based A_2AR antagonist inspired by ZM241385 and tozadenant for possible benefit in PD (Fig. 1).

2. Experimental

The chemical used in present study were obtained Sigma Aldrich, USA, unless otherwise stated. The chemical used in the present study was procured from Sigma Aldrich (USA). The spectra of ^{1}H NMR and ^{13}C NMR were recorded on Bruker Avance 400 and Bruker Avance 100 Spectrophotometer, respectively. The chemical shifts are expressed in parts per million (ppm), and coupling constants are expressed in Hertz (Hz). The Low-resolution mass spectrum (MS) was recorded on a Waters ZQ LC/MS single quadrupole system equipped with an electrospray ionization (ESI) source. The elemental analysis of the final derivatives was performed on Vario Elemental analyser. The Thin-layer chromatography was performed on 0.25 mm Merck silica gel plates (60F-254) and visualized under UV light.

2.1. *The synthesis of compound **2**, **3**, **5** (**a-f**) and **6** (**a-f**) was performed as per the earlier reported procedures.*[14, 27]

2.2. *General procedure for the synthesis of title 1,3,5-triazine-thiadiazole derivatives **7** (**a-f**)*

Compound **3** (0.1 mol) was added into 50 mL of 1,4-dioxane at temperature 120–145℃. A solution of substituted thiadiazole **6** (**a–f**) (0.1 mol) in 35 mL of 1,4-dioxane was added slowly to above solution and stirred for 90 min followed by drop-wise addition of K_2CO_3 (0.1 mol). The reaction mixture was refluxed for 8-9 h at 135–145℃. The product was filtered and washed with cold water and re-crystallized with ethanol to afford the corresponding pure products **7** (**a-f**).

2.2.1. *4-(2-((4-amino-6-((5-phenyl-1,3,4-thiadiazol-2-yl)amino)-1,3,5-triazin-2-yl)amino)ethyl)phenol.* (**7a**)

Yield: 86%; MP: 219-220 ºC; MW: 406.47; R_f: 0.79; FT-IR (ν_{max}; cm^{-1} KBr): 3429 (OH stretching), 3328 (NH_2 stretching), 3279 (N–H stretching), 3083 (C-H stretching, Aromatic), 1668 (C=N Aromatic), 1618 (C=C stretching), 1543 (N–H bending, NH), 1464 (CH_2 bending, Aliphatic), 1151 (C-C stretching, Aromatic), 1038 (C–N stretching), 642 (C–S stretching), 605; ^{1}H-NMR (400MHz, DMSO-d_6, TMS) δ ppm: 9.06 (s, 1H, Ar-OH), 8.04 (d, 2H, *J* = 1.47 Hz, Ar-H), 7.56 (d, 2H, *J* = 1.11 Hz, Ar-H), 7.43 (t, 1H, *J* = 1.4 Hz, Ar-H), 7.05 (d, 2H, *J* = 1.08 Hz, Ar-H), 6.97 (s, 2H, NH_2), 6.71 (d, 2H, *J* = 2.53 Hz, Ar-H), 3.95 (s, 2H, 2 × NH)), 3.43 (t, 2H, *J* = 6.69 Hz, CH_2), 2.91 (t, 2H, *J* = 6.17 Hz, CH_2); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm:182.7, 174.4, 165.9, 159.4, 155.9, 152.8, 133.7, 132.1, 130.9, 130.2, 129.3, 128.8, 115.9, 44.5, 35.2; Mass: 407.48 (M+1); Elemental analysis for $C_{19}H_{18}N_8OS$: Calculated: C, 56.14; H, 4.46; N, 27.57; Found: C, 56.18; H, 4.42; N, 27.58.

2.2.2. *4-(2-((4-amino-6-((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)amino)-1,3,5-triazin-2-yl)amino)ethyl)phenol.* (**7b**)

Yield: 79%; MP: 245-246 ºC; MW: 440.91; R_f: 0.69; FT-IR (ν_{max}; cm^{-1} KBr): 3424 (OH stretching), 3317 (NH_2 stretching), 3281 (N–H stretching), 3074 (C-H stretching, Aromatic), 1689 (C=N Aromatic), 1631 (C=C stretching), 1139 (C-C stretching, Aromatic), 1516 (N–H bending, NH), 1459 (CH_2 bending, Aliphatic), 1048 (C–N stretching), 793 (C-Cl stretching), 645 (C–S stretching); ^{1}H-NMR (400MHz, DMSO-d_6, TMS) δ ppm: 9.10 (s, 1H, Ar-OH), 7.74 (d, 2H, *J* = 1.35 Hz, Ar-H), 7.68 (d, 2H, *J* = 1.49 Hz, Ar-H), 7.05 (d, 2H, *J* = 1.05 Hz, Ar-H), 6.96 (s, 2H, NH_2), 6.71 (d, 2H, *J* = 2.58 Hz, Ar-H), 3.99 (s, 2H, 2 × NH)), 3.36 (t, 2H, *J* = 6.67 Hz, CH_2), 2.74 (t, 2H, *J* = 6.12 Hz, CH_2);^{13}C-NMR (100MHz, DMSO-d_6) δ ppm:182.7, 174.4, 165.8, 159.5, 155.9, 152.6, 134.4, 132.2, 131.7, 130.5, 129.4, 128.8, 115.3, 44.8, 35.6 ; Mass: 441.94 (M+1); Elemental analysis for $C_{19}H_{17}ClN_8OS$: Calculated: C, 51.76; H, 3.89; N, 25.41; Found: C, 51.75; H, 3.92; N, 25.40.

2.2.3. *4-(2-((4-amino-6-((5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)amino)-1,3,5-triazin-2-yl)amino)ethyl)phenol.* (**7c**)

Yield: 84%; MP: 224-225 ^{o}C; MW: 424.46; R_f: 0.62; FT-IR (ν_{max}; cm^{-1} KBr): 3431 (OH stretching), 3323 (NH_2 stretching), 3288 (N–H stretching), 3079 (C-H stretching, Aromatic), 1694 (C=N Aromatic), 1635 (C=C stretching), 1139 (C-C stretching, Aromatic), 1512 (N–H bending, NH), 1462 (CH2 bending, Aliphatic), 1162 (C-F stretching), 1042 (C–N stretching), 643 (C–S stretching); ^{1}H-NMR (400MHz, DMSO-d_6, TMS) δ ppm: 9.08 (s, 1H, Ar-OH), 7.72 (d, 2H, *J* = 1.49 Hz, Ar-H), 7.34 (d, 2H, *J* = 1.28 Hz, Ar-H), 7.03 (d, 2H, *J* = 1.05 Hz, Ar-H), 6.93 (s, 2H, NH_2), 6.71 (d, 2H, *J* = 2.52 Hz, Ar-H), 3.97 (s, 2H, 2 × NH)), 3.39 (t, 2H, *J* = 6.62 Hz, CH_2), 2.75 (t, 2H, *J* = 6.08 Hz, CH_2);^{13}C-NMR (100MHz,DMSO) δ,ppm:182.7, 174.5, 165.9, 162.9, 159.2, 155.8, 152.9, 132.4, 130.2, 129.5, 129.1, 116.2, 115.8, 44.5, 35.3, ; Mass: 425.49 (M+1); Elemental analysis for $C_{19}H_{17}FN_8OS$: Calculated: C, 53.76; H, 4.04; N, 26.40; Found: C, 53.79; H, 4.02; N, 26.38.

2.2.4. *4-(2-((4-amino-6-((5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)amino)-1,3,5-triazin-2-yl)amino)ethyl)phenol.* (**7d**)

Yield: 68%; MP: 256-257 ^{o}C; MW: 485.36; R_f: 0.75; FT-IR (ν_{max}; cm^{-1} KBr): 3435 (OH stretching), 3329 (NH_2 stretching), 3284 (N–H stretching), 3075 (C-H stretching, Aromatic), 1687 (C=N Aromatic), 1628 (C=C stretching), 1139 (C-C stretching, Aromatic), 1512 (N–H bending, NH), 1461 (CH_2 bending, Aliphatic), 1053 (C–N stretching), 764 (C-Br stretching), 632 (C–S stretching); ^{1}H-NMR (400MHz, DMSO-d_6, TMS) δ ppm: 9.04 (s, 1H, Ar-OH), 7.82 (d, 2H, *J* = 1.51 Hz, Ar-H), 7.72 (d, 2H, *J* = 1.48 Hz, Ar-H), 7.05 (d, 2H, *J* = 1.02 Hz, Ar-H), 6.95 (s, 2H, NH_2), 6.69 (d, 2H, *J* = 2.51 Hz, Ar-H), 3.98 (s, 2H, 2 × NH), 3.41 (t, 2H, *J* = 6.58 Hz, CH_2), 2.72 (t, 2H, *J* = 6.03 Hz, CH_2);^{13}C-NMR (100MHz, DMSO-d_6) δ ppm:182.7, 174.4, 165.8, 159.5, 155.9, 152.8, 132.6, 132.3, 132.1, 130.2, 129.7, 123.2, 115.7, 44.6, 35.3 ; Mass: 486.39 (M+1); Elemental analysis for $C_{19}H_{17}BrN_8OS$: Calculated: C, 47.02; H, 3.53; N, 23.09; Found: C, 47.01; H, 3.54; N, 23.07.

2.2.5. *4-(2-((4-amino-6-((5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)amino)-1,3,5-triazin-2-yl)amino)ethyl)phenol*. (**7e**)

Yield: 86%; MP: 261-263 ^{o}C; MW: 451.47; R_f: 0.81; FT-IR (ν_{max}; cm^{-1} KBr): 3436 (OH stretching), 3325 (NH_2 stretching), 3289 (N–H stretching), 3078 (C-H stretching, Aromatic), 1692 (C=N Aromatic), 1634 (C=C stretching), 1548 (NO_2 stretching), 1141 (C-C stretching, Aromatic), 1512 (N–H bending, NH), 1457 (CH_2 bending, Aliphatic), 1056 (C–N stretching), 644 (C–S stretching); ^{1}H-NMR (400MHz, DMSO-d_6, TMS) δ ppm: 9.07 (s, 1H, Ar-OH), 8.26 (d, 2H, *J* = 1.94 Hz, Ar-H), 7.78 (d, 2H, *J* = 1.56 Hz, Ar-H), 7.01 (d, 2H, *J* = 1.03 Hz, Ar-H),

6.94 (s, 2H, NH_2), 6.72 (d, 2H, *J* = 2.54 Hz, Ar-H), 3.96 (s, 2H, 2 × NH)), 3.44 (t, 2H, *J* = 6.52 Hz, CH_2), 2.78 (t, 2H, *J* = 6.05 Hz, CH_2);^{13}C-NMR (100MHz, DMSO-d_6) δ ppm:182.3, 174.8, 165.8, 159.5, 155.4, 152.9, 147.9, 139.5, 132.1, 130.4, 128.5, 124.4, 115.9, 44.4, 35.5, ; Mass: 452.47 (M+1); Elemental analysis for $C_{19}H_{17}N_9O_3S$: Calculated: C, 50.55; H, 3.80; N, 27.92; Found: C, 50.58; H, 3.75; N, 27.95.

2.2.6. *4-(2-((4-amino-6-((5-(p-tolyl)-1,3,4-thiadiazol-2-yl)amino)-1,3,5-triazin-2-yl)amino)ethyl)phenol.* (**7f**)

Yield: 72%; MP: 237-238 ºC; MW: 420.50; R_f: 0.84; FT-IR (ν_{max}; cm^{-1} KBr): 3438 (OH stretching), 3327 (NH_2 stretching), 3295 (N–H stretching), 3081 (C-H stretching, Aromatic), 2958 (alkyl C-H stretching), 1693 (C=N Aromatic), 1621 (C=C stretching), 1143 (C-C stretching, Aromatic), 1512 (N–H bending, NH), 1458 (CH2 bending, Aliphatic), 1052 (C–N stretching), 643 (C–S stretching); ^{1}H-NMR (400MHz, DMSO-d_6, TMS) δ ppm: 9.04 (s, 1H, Ar-OH), 7.74 (d, 2H, *J* = 1.57 Hz, Ar-H), 7.24 (d, 2H, *J* = 1.16 Hz, Ar-H), 7.03 (d, 2H, *J* = 1.02 Hz, Ar-H), 6.97 (s, 2H, NH_2), 6.72 (d, 2H, *J* = 2.51 Hz, Ar-H), 3.98 (s, 2H, 2xNH)), 3.42 (t, 2H, *J* = 6.51 Hz, CH_2), 2.72 (t, 2H, *J* = 6.07 Hz, CH_2);2.23 (s, 3H, CH_3); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm:182.7, 174.3, 165.9, 159.5, 155.8, 152.9, 132.2, 131.8, 130.6, 130.4, 129.5, 127.4, 115.9, 44.5, 35.3, 21.3 ; Mass: 421.51 (M+1); Elemental analysis for $C_{20}H_{20}N_8OS$: Calculated: C, 57.13; H, 4.79; N, 26.65; Found: C, 57.11; H, 4.80; N, 26.64.

2.3. *Physicochemical Property*

All compounds were analyzed for physiochemical properties, such as logP value, solubility in water, hydrogen bonder donar/acceptor, molecular weight and total polar surface area (TPSA) via molispiration (http://www.molispiration.com) software.

2.5. *Radioligand binding assays*

[^{3}H]ZM241385 and [^{3}H]DPCPX binding assays for adenosine A_2AR and A_1Rs, respectively, were performed in HEK293 cells. Briefly, 10 µg HEK293 cell membranes isolated from stably transfected HEK293 cells with human A_2AR and A_1R cDNA were incubated with different concentrations (1 pM to 1 lM) of compound **7e** and 1 nM [3 H]ZM241385 in 200 µl incubation buffer containing 50 mM Tris–Cl, 1 mM EDTA, pH 7.4 and 2.5 U/ml adenosine deaminase. Adenosine A_1R assays were performed on 10 µg of HEK293 cell membranes expressing human adenosine A_1Rs and 1 nM [^{3}H]DPCPX in 200 µl incubation buffer. Reactions were carried out for 60 min at 26℃ and were terminated by rapid filtration over 96-well plates

equipped with GF/B filters (Millipore, USA). Filters were washed three times with 300 µl of cold washing buffer containing 50 mM Tris–Cl, 10 mM $MgCl_2$, pH 7.4, air dried, and radioactivity retained on filters were counted in 1450 LSC & Luminescence counter (Wallac Microbeta Trilux, Perkin–Elmer, USA). Nonspecific binding for adenosine A_2AR and A_1R were determined in the presence of 50 µM NECA and 50 µM CPA, respectively. Assays were performed in duplicates and compounds were tested twice. Data were fitted in one site competition-binding model for IC_{50} determination using the program GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA) and K_i values were calculated using Cheng and Prusoff formula.

2.6. *Molecular docking analysis*

The X-ray crystal structure of human A_2A adenosine receptor co-crystallized with ligand ZM241385 at 2.6 Å resolution (PDB: 3EML) was downloaded from protein data bank. All molecular modelling calculations and docking studies were carried out using BIOVIA Discovery Studio software (Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 2018, San Diego, USA: Dassault Systèmes, 2016) running on a Windows 7 PC.

Binding site sphere determination

The protein–ligand complex obtained from the protein data bank was prepared for docking as follows. Initially, co-crystallized ligand and water molecules were deleted from the protein file. Automatic protein preparation module was used for preparation of protein for docking using CHARMm forcefield. The binding site sphere has been defined automatically by the software over the co-crystallized ligand ZM241385.

Preparation of target compounds for docking

The compound to be dock was exported to Discovery Studio 3.0 for docking after sketching on Chem Draw 10.0. The hydrogen atoms were added to the exported compound and their standard geometry, optical isomers and 3D conformations were automatically generated.

Docking analysis

Docking was performed using CDOCKER protocol in BIOVIA Discovery Studio software keeping the parameters at default. The best scoring pose of the docked compounds was recognized. Receptor–ligand interactions of the complexes were examined in 2D and 3D styles.

Results and discussion

Chemistry

The synthesis of title molecules was achieved *via* three synthetic pathways as shown in scheme 1. Initially, in step 1, mono-substituted 4,6-dichloro-1,3,5-triazin-2-amine (**2**) was achieved by reaction of 2,4,6-trichloro-1,3,5-triazine (**1**) with ammonia in presence of activating base. The compound **2** was further reacted with 4-(2-aminoethyl) phenol in presence of triethylamine and DMF to afford compound **3.** In the step 2, different substituted methyl benzoate **4** (**a-f**) was reacted with thiosemicarbazide to furnish corresponding thiosemicarbazide derivatives **5** (**a-f**), which on subsequent reaction with sulphuric acid to form different substituted thiadiazoles **6** (**a-f**). In the final step, compound **3** was allowed to react with substituted thiadiazoles **6** (**a-f**) to afford title compounds **7**(**a-f**) in good yield.

All the synthesized compounds were characterized by different spectroscopic methods, such as FT-IR, ^{1}H NMR, ^{13}C NMR, Mass and elemental analysis. The FT-IR peaks observed at 3424-3438 cm^{-1} is due to the presence of OH group linked to phenyl ring. Absorption bands at 3323-3328 cm^{-1} suggested as stretching vibration of NH_2 group at 1,3,5-triazine. Another peak at 3279-3295 cm^{-1} attributed to NH group at 1,3,5-triazine ring. Furthermore, absorption bands appeared at 3074-3083 cm^{-1} due to stretching vibration of C-H of aromatic ring. Another peak at 1668-1693 cm^{-1} corresponds to stretching vibrations of aromatic C=N group. Triazine C-N group appeared at 1038-1056 cm^{-1}. The aromatic NO_2 group appeared at 1548 cm^{-1}. Another peak at 1162 cm^{-1} corresponds to the presence of fluoro linked to phenyl ring. The phenyl bromine group appears at 764 cm^{-1}. The thiadiazole C-S group appears at 632-645 cm^{-1}. The ^{1}H-NMR spectrums of final synthesized compounds **7**(**a-f**) shown singlet peak at 9.04-9.10 due to the presence of aromatic hydrogen. The proton in the phenyl ring appeared as doublet as 8.26-7.82 ppm. The 1,3,5-triazine NH_2 proton appears at 6.93-6.97 ppm as singlet. The proton in the triazine ring linked with NH group appeared at 3.95-3.99 as singlet peak. Furthermore, the resonance at 3.44-3.36 ppm suggested the presence of C-H proton of aliphatic chain with triplet peak. Another, aliphatic CH_2 proton appears at 2.72-2.91 ppm as triplet peak. The ^{13}C-NMR resonance peaks appear at 182.9- 159.1 ppm due the 1,3,5-triazine ring carbon atom. The thiadiazole carbon appeared at 152.4-152.7 ppm. Furthermore, the resonance at 44.1-35.2 ppm due to the presence of carbon of aliphatic CH_2 side chain. Another resonance peak appears at 155.7- 115.8 ppm due phenyl ring carbon atoms. The mass spectroscopy and elemental analysis data were found in aggrement with the proposed structure of compounds **7** (**a-f**).

Physiochemical properties

The physiochemical and molecular properties such as, partition coefficient (LogP), total polar surface area (TPSA), and molecular weight (m.w.) has a significant impact on both pharmacokinetic and pharmacodynamic process, as well as drug safety [28]. LogP value signifies unique hydrophobic/lipophilic balance of the molecule and numerous studies suggested a strong correlation exist between lipophilicity and activity, where lipophilic nature of the compound has an easy access to CNS via increased permeability to BBB (blood brain barrier) [29]. On the other hand, TPSA is defined as the surface sum over all polar atoms or molecules, primarily oxygen and nitrogen, which also counting their attached hydrogen atoms. The molecules with a polar surface area of greater than 140 angstroms squared tend to be poor at permeating cell membranes. The molecular weight is another parameter which significantly affect the drug action, and higher molecular weight have less chances to reach CNS [30]. Therefore, in the present study the designed molecules were assessed for determination of their molecular and physicochemical properties, and it has been found that none of the synthesized molecules disobeyed the Lipinski's rule of 5 (Ro5). These results suggest that designed molecules have easy access to CNS.

Radioligand binding assay

The results of the radioligand binding assay of the synthesized molecules are tabulated in table 1. It has been found that designed compounds showed considerable binding affinities against both $A_{2A}R$ and A_1R receptor subtypes. Among the tested derivative, compound **7a** showed least binding affinity against both the tested receptors. However, upon insertion of *para*-chloro (**7b**), the binding affinity was found to be boosted significantly against $A_{2A}R$, with mild improvement against A_1R receptor. The selectivity index was found reduced in the case of compound **7c** having *para*-fluoro with mild reduction in binding affinity against A_2AR. Moreover, compound **7c** showed mild improvement in affinity against A_1R. In the case of compound **7d** (*para*-bromo), the binding affinity was found reduced significantly against both the tested receptors with a mild drop in SI also. Among the tested analogues, compound **7e** (*para*-nitro) was found most potent for both receptor sub-types with more selectivity towards $A_{2A}R$ as confirmed by SI. However, the binding affinity was found reduced significantly with drop in SI in the case of compound **7f** having *para*-methyl group. The structure-activity relationship of the designed analogues sugest that, electron withdrawing substituent found more potent than their electron donating counterparts. Among electron withdrawing groups, non-halogen substituent (NO2)

found more active than halogen counterparts for both iso-forms of receptor. Moreover, the activity was found significantly reduced in the case of non-substituted derrivative, suggesting substitution favours bioactivity..

Table 1: Radioligand binding and selectivity study of target compounds.

Entry	Substituent	Ki (A_2AR) nM	Ki (A_1R) nM	A_1R / $A_{2A}R$
7a	H	127.2	712.7	5.6
7b	4-Cl	44.6	520.4	11.66
7c	4-F	59.3	456.5	6.69
7d	4-Br	71.2	505.2	7.09
7e	4-NO_2	32.1	322.3	10.04
7f	4-CH_3	89.2	569.1	6.38

Docking analysis

In current scenario of drug discovery, molecular docking is one of the most widely accepted tool and it have irreplaceable role in structure-based drug design. It allows predicting the binding-conformation of small molecule ligands to the appropriate target binding site [31]. In view of excellent binding affinity of compound **7e** against A_2A receptor, it is necessary to perform docking analysis with A_2A protein model to predict its orientation in receptor cavity. The X-ray crystal structure of $A_{2A}R$ (pdb id: 3EML) co-crystallized to ZM241385 at 3.5 Å was selected to perform docking with compound **7e**. To perform docking analysis, the ligand ZM241385 was removed and protein was prepared according to the default protocol of Discovery Studio 3.0. The ligand **7e** was minimized and allowed to be docked into the active site of A_2A receptor via CDOCKER protocol. Analysis of docking results as shown in Fig. 2 and 3, compound **7e** found to be efficiently dock into the active site of A_2A receptor having perpendicular binding pocket lined by His264, Glu169, Asn253 and Val84. As shown in Fig. 4, the phenyl-thiadiazole core of the molecule **7e** was found to be deeply buried into the cavity of receptor lined with Cys262, Thr256 and Pro260, via formation of H-bond with His264 and pi-anion with Glu169. The 1,3,5-triazine central core forms two H-bond with Tyr271 via engaging amine and its neighbouring nitrogen. It also bind with Met270 via pi-alkyl interaction, which further found similar in the case of thiadiazole nucleus. The phenyl containing hydroxy group form numerous pi-bonds with neighbouring residues, such as, pi-pi stacking with Phe168

and Pi-alkyl with Ile274 and Val84. Moreover, the terminal *hydroxy* group of compound **7e** form one H-bond with His278. Compound 7e also showed scoring function with A_2A receptor, Table 2. Collectively, our docking results confirmed that compound **7e** bind efficiently with A_2A receptor probably due to excellent docking interaction and scoring functions (Table 2).

Table 2: Interacting residues and scoring of compound 7e in A_2A receptor.

Compound	Interacting residues with type of interaction				Scoring function	
7e	H-bond	Pi-Anion	Pi-Alkyl	Pi-Pi stacking	CDOCKER energy:	CDOCKER Interaction energy
	His264, Tyr271, His278	Glu169	Ala63, Val84, Ile274, Met270	Phe169	45.66	46.21

Figure 2

Figure 3

Figure 4

Conclusion

This paper report results for our research program aiming to identify potent A_2A receptor antagonists. The present study demonstrated the development of novel 1,3,5-triazine-thiadiazole hybrids as potent and selective A_2A receptor antagonists via integration of computational tools, synthetic chemistry and pharmacological assays. Currently, we are working on the structural optimization of this novel scaffold to provide insights into the structural requirements for future development of new anti-parkinsonian agents.

Credit author statment

Anoop Masih, Saumya Singh, Amol Kumar Agnihotri, Sabeena Giri, Jitendra Kumar Shrivastava, Nidhi Pandey: Performed experiments, data analysis and interpretation, statistical analysis and docking analysis.

Hans Raj Bhat: Data analysis and interpretation; Writing - review & editing.

Udaya Pratap Singh: Conceptualization Funding acquisition; Investigation; Writing - review & editing.

All authors approved the final version of the manuscript.

Conflict of Interest

Authors declare no conflict of interest.

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Figure captions

Scheme 1: Synthesis of 1,3,5-triazine-thiadiazole hybrid derivatives. Reagents and condition: a) liq. NH3, 0 ℃, stirring, 2-3 h, $NaHCO_3$; b) K_2CO_3, *p*-hydroxy phenyl ethyl amine, 40-45 ℃; c) reflux; d) H_2SO_4, heating, 50-70℃; e) K_2CO_3, reflux, 8-9 h, 135–145℃.

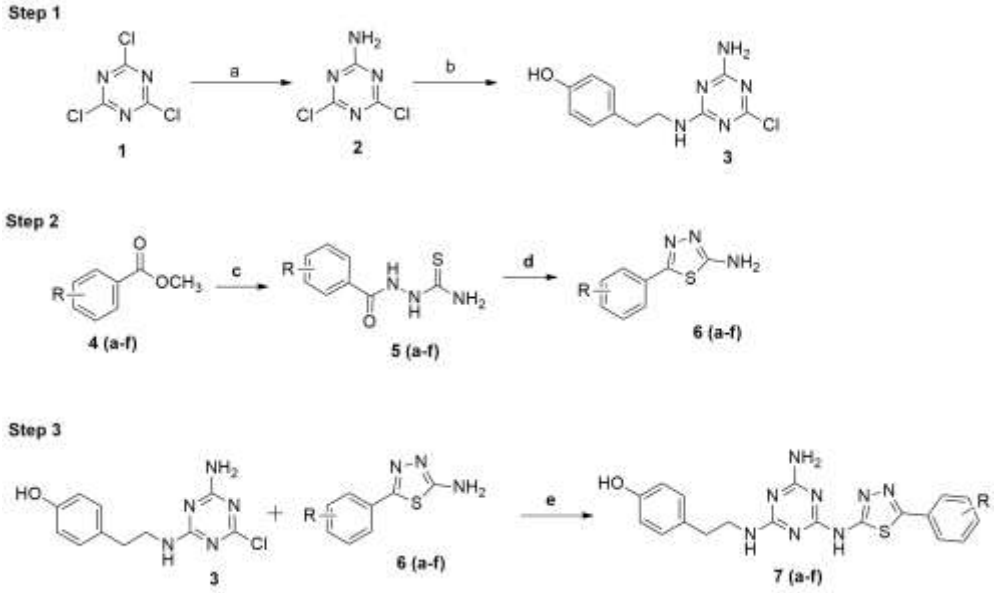

Figure 1: Various A_2A receptor antagonists.

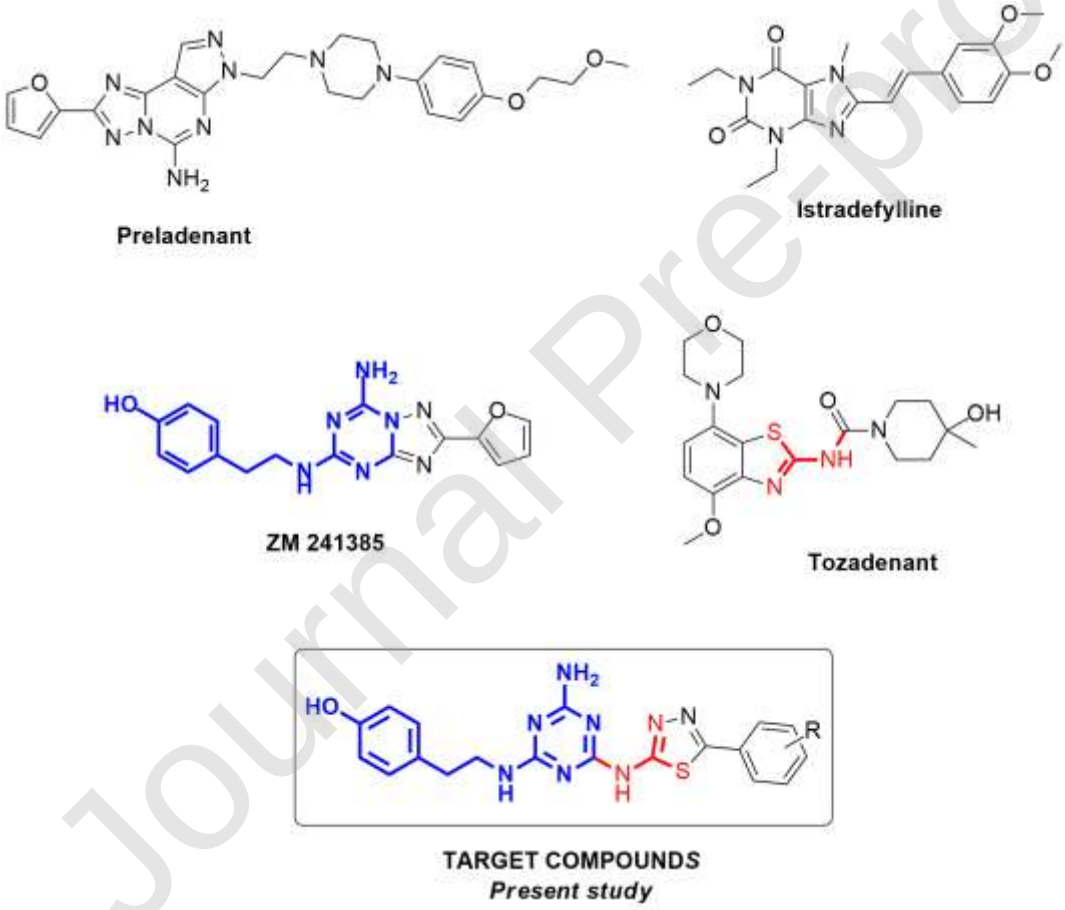

Figure 2: Orientation of compound **7e** after docking into the active site of A_2A receptor (Ligand **7e** shown as ball and stick, protein shown as solid ribbon; pdb: 3EML).

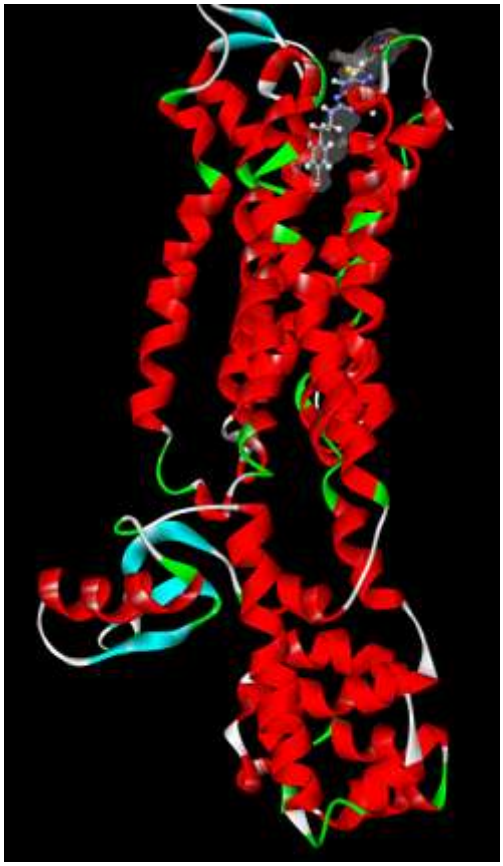

Figure 3: Docked pose of compound **7e** along with neighbouring and interacting amino acid residues of A_2A receptor.

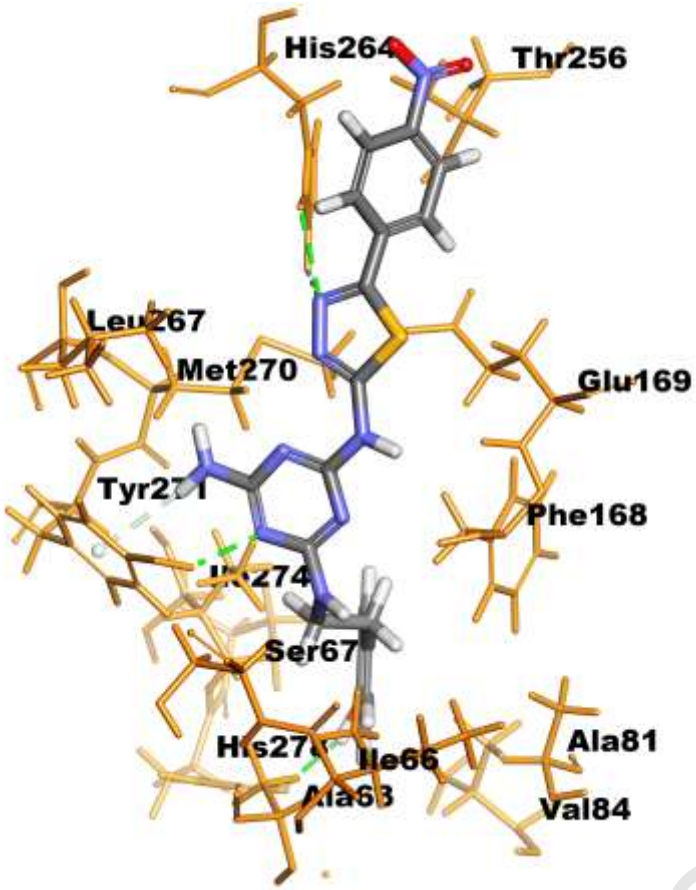

Figure 4: 2-D interaction diagram of compound 7e in the active site of A_2A receptor.