Indian Journal of Chemistry
Vol. 51B, August 2012, pp 1105-1113

Design, microwave-assisted synthesis and *in silico* docking studies of new 4*H*-pyrimido[2,1-*b*]benzothiazole-2-arylamino-3-cyano-4-ones as possible adenosine A_{2B} receptor antagonists

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Received 10 August 2010; accepted (revised) 4 June 2012

A series of new 4*H*-pyrimido[2,1-*b*]benzothiazole-2-arylamino-3-cyano-4-ones **6a-g** have been designed and synthesized by the application of microwave-assisted organic synthesis (MAOS) technique. *In silico* docking studies have been carried out to gain an insight into the hypothetical binding motif of the title compounds using a homology model of A_{2B} adenosine receptor employing GOLD (CCDC, 4.0.1 version) software. The binding modes are proposed on the basis of molecular docking studies.

Keywords: 4*H*-pyrimido[2,1-*b*]benzothiazole-4-one, adenosine receptor antagonists, docking studies, microwave-assisted organic synthesis, GOLD

Adenosine receptors (ARs) belong to the super family of GPCRs which are divided into four subtypes, A_1, A_{2A}, A_{2B} and A_3 (Ref 1). With ubiquitous distribution in most mammalian and human tissues, ARs mediate a plethora of biological effects. It is well known that A_{2A} and A_{2B} receptors activate adenylate cyclase, while A_1 and A_3 receptors cause inhibition of lyase[2,3]. Sufficient evidence now supports the key role that adenosine and its A_{2B}.

ARs play in asthma and COPD[4-6]. These receptors mediate the synergistic effects of adenosine and allergen on human mast cells, which are believed to be involved in adenosine-induced bronchoconstriction in asthmatics[7-9]. The bronchodilating effect of theophylline and its structural analogues have been attributed to the antagonism of the A_{2B} ARs (Ref 10). As xanthine derivatives have several physico-chemical limitations in addition to the lack of selectivity in inhibition, several nonxanthines including fused-thiazole derivatives were synthesized and evaluated for A_{2B} ARs antagonistic activity in an effort to overcome these problems. Cai *et al*., reported some new substituted 2-acylaminothiazoles (compound **1**) which have been explored for affinity towards A_{2B} ARs (**Figure 1**) (Ref 11).

Biological activities of the compounds containing pyrimidine ring[12] have stimulated considerable interest to explore the synthesis of new and potentially useful compounds in which pyrimidine ring is fused with benzothiazole through a bridgehead nitrogen atom. Bioisosteric modification of the thiophene ring with differently substituted aromatic nucleus on sterically favoured region and introducing a nitrile function as additional H-bond acceptor to result new features on the designed heterofused pyrimidine derivatives (**Figure 2**).

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List of Abbreviations:
MAOS = Microwave-Assisted Organic Synthesis; ARs = Adenosine Receptors; GPCRs = G-protein-coupled receptors; COPD = Chronic Obstructive Pulmonary Disease; MW = Microwave; GOLD = Genetic optimization for ligand docking; GA = Genetic Algorithm; PRCG = Polack-Ribiere Conjugate Gradient; RMSD = Root Mean Square Deviation

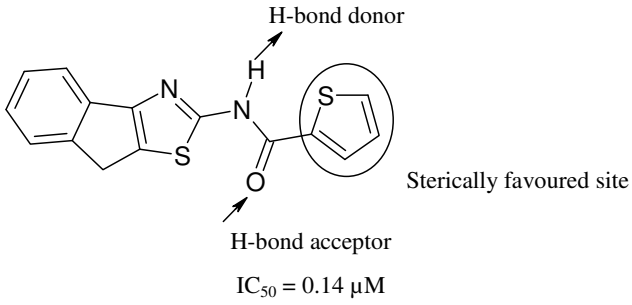


Figure 1 — Substituted 2-acylaminothiazoles (Compound **1**) (Ref 11)

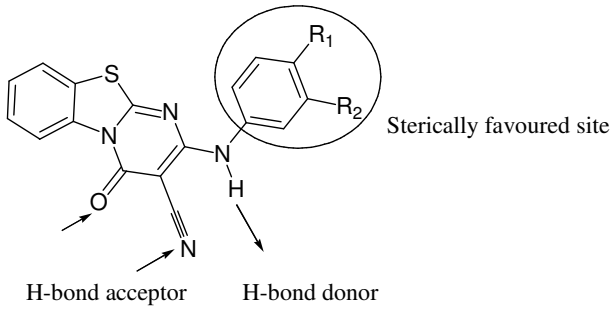


Figure 2 — Designed fused-pyrimidines **6a-f**

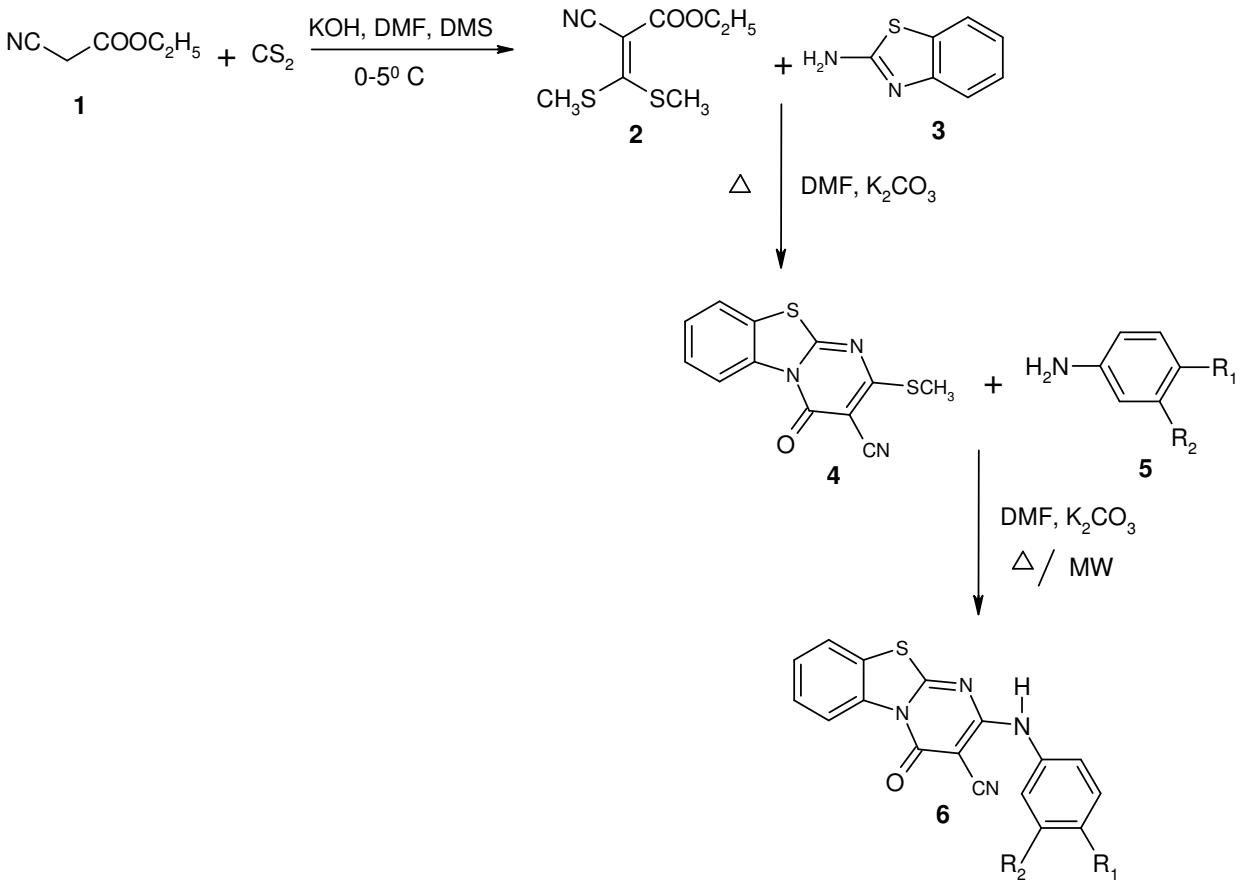
A literature survey revealed few reports on the synthesis of fused pyrimido benzothiazole derivatives[13,14]. Wade *et al*., reported the synthesis and antiallergic activity of acidic derivatives of 4*H*-pyrimido[2,1-*b*]benzazole-4-ones by the condensation of 2-aminobenzothiazole, 2-amino benzoxazole and 2-amino-1-methyl benzimidazole independently with 2-aminofumarate and diethylethoxymethylene malonate[15,16]. Synthesis of these compounds requires passing of a stream of nitrogen gas to result in 2 or 3-substituted derivatives. 4*H*-Pyrimido[2,1-*b*]benzothiazole-2-thiomethyl-3-cyano-4-one **4**, a key intermediate was synthesized by following a reported procedure[17]. Depending on the substituents on arylamines used, this reaction could provide different 2,3-disubstituted benzothiazole-fused pyrimidines in reasonably good yields. Hence it was applied as a convenient method to synthesize a series of 4*H*-pyrimido[2,1-*b*]benzothiazole-2-arylamino-3-cyano-4-ones **6a-g** (**Scheme I**).

During the last decade, MW irradiation has become a handy and valuable tool for preparative organic chemists. Its versatile utility need not be overemphasized as more and more applications are being studied for a wide variety of syntheses[18-27]. A large number of organic reactions can be carried out under MW irradiation often giving higher yields with shorter reaction times and milder conditions. Furthermore, reactions under MW have the great advantage of using minimal or no organic solvents ('solvent free'), thus making such reactions more environment friendly while generating few side products. The successful application of MW activation to the nucleophilic substitution of arylamines with replaceable thiomethyl group was investigated and the results are described in the present study.

The structure-based drug design requires the knowledge of 3D structure of ARs. As the 3D structure of the A_{2B} AR is yet to be known, homology models based on rhodopsin structure have been employed[28]. Recently, the crystal structure of A_{2A} AR has been determined[29] which can be utilized as a suitable template for building A_{2B} AR homology model. The sequence identity between A_{2A} and A_{2B} AR subtypes amounts to 56% higher than in bovine rhodopsin (23%) and human β_2-adrenergic receptor (31%). Hence it was thought appropriate to evaluate the synthesized compounds *in silico* by conducting docking studies employing A_{2A} AR based model. As part of the ongoing studies to develop fused-pyrimidines of biological interest, herein is reported the design, microwave-assisted synthesis and *in silico* evaluation of seven title compounds **6a-g** as possible adenosine A_{2B} receptor antagonists.

Results and Discussion

The key intermediate **4** was prepared by following a known method and was characterized based on the reported data[17a]. The nucleophilic substitution of thiomethyl group on **4** with different aryl amines **5a-g** resulted in the formation of crystalline product exhibiting marked deviation in TLC profiles. The final compounds **6a-g** were characterized based on physical and spectral (IR, ^{1}H NMR and MS) data. The conspicuous presence of NH, C≡N and C=O signals at 3241, 2211 and 1681 cm^{-1} respectively in the IR spectrum of **6b** indicated the formation of the desired product. Further its ^{1}H NMR spectrum recorded a D_2O exchangeable signal at δ 9.8 attributable to the NH proton besides retaining the aromatic protons appearing at δ 7.1 to 8.7. The compound was finally confirmed as 4*H*-pyrimido[2,1-*b*]benzothiazole-2-(4-fluoroanilino)-3-cyano-4-one **6b** by its mass spectrum where the molecular ion (M^+) was recorded as base peak (100%) at *m/z* 336. Similarly, **6a-c** and **6e-g** were characterized based on their spectral data. As **6d**



5a, 6a: R_1=R_2=H
5b, 6b: R_1=F, R_2=H
5c, 6c: R_1=F, R_2=Cl
5d, 6d: R_1=CH_3, R_2=H
5e, 6e: R_1=Cl, R_2=H
5f, 6f: R_1=OCH_3, R_2=H
5g, 6g: R_1= NO_2, R_2=H

Scheme I — Synthesis of 4*H*-pyrimido[2,1-*b*]benzothiazole-2-arylamino-3-cyano-4-one, **6a-g**

is a known compound[17a], its details are not included. However **6d** is also included in the present *in silico* studies.

Under classical heating conditions, these reactions have certain disadvantages like long reaction times (8-10 h), high energy consumption, lower yield and the need for large amounts of solvents for purification. However, the present investigations report the syntheses of final compounds **6a-g** employing MW irradiation technique. The reactions have been carried out using a Catalyst Microwave Reactor, under constant irradiation power and by varying the temperature (the so-called "power control"). The best results were obtained when full power of the magnetron (700 W) was used. The details of the optimized conditions employed, under MW irradiation as well as under classical heating are presented in **Table I**. A comparative analysis of the data obtained leads to the conclusion that the use of MW resulted in a remarkable acceleration of the reactions, with the reaction times decreasing dramatically, from hours to minutes (12 to 15 min). While optimizing the conditions, it was interesting to note that the reactions could be carried out at considerably lower temperatures (in most cases by 10 to 30 °C). It was also of interest that, in some cases, under MW irradiation the yields were higher (substantially, by almost 20%).

All the final compounds **6a-g** were evaluated *in silico* (docking) to recognize their hypothetical binding mode using a molecular (homology) model of A_{2B} AR (Ref 30-33). To investigate and validate the

Table I — Synthesis of 4*H*-pyrimido[2,1-*b*]benzothiazole-2-arylamino-3-cyano-4-one **6a-g** under microwave and classical heating conditions, in liquid phase

Compd	Classical			Microwave		
	Reaction Time (hr)	Reaction Temp (°C)	Yield (%)	Reaction Time (min)	Reaction Temp (°C)	Yield (%)
6a	10	150	58	12	120	72
6b	10	150	60	12	120	70
6c	9	150	61	12	120	82
6d	8	150	52	15	120	74
6e	8	150	59	15	120	71
6f	9	150	58	15	120	73
6g	8	150	61	15	120	79

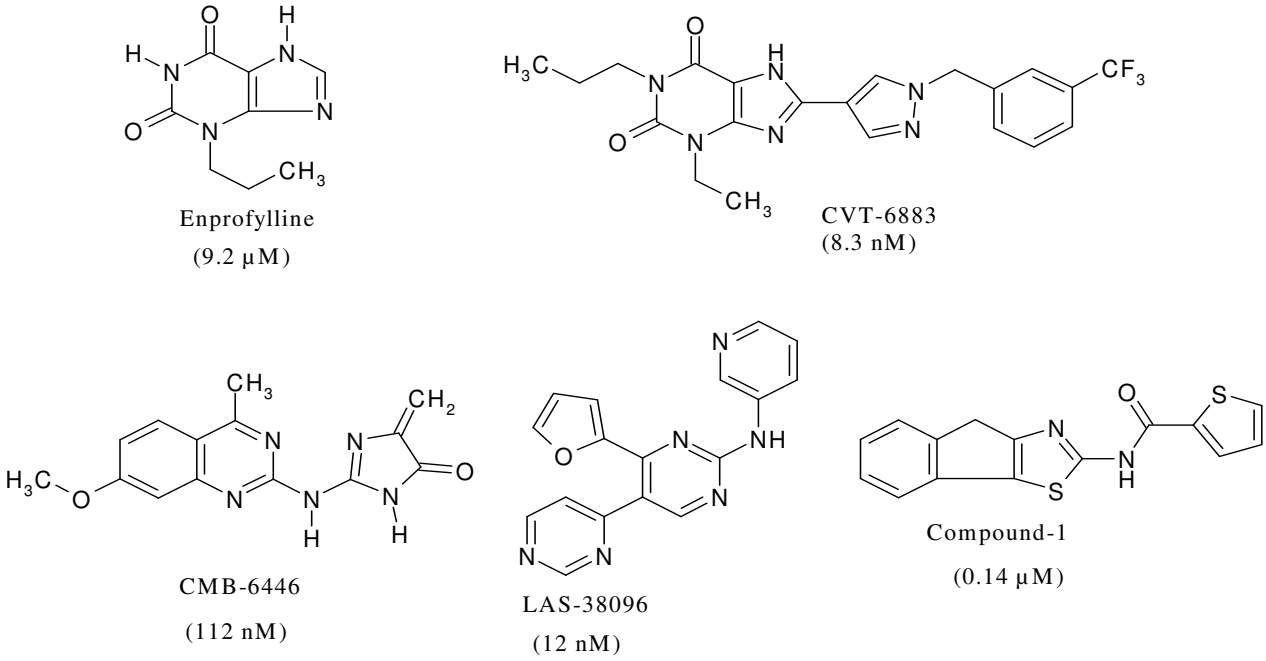


Figure 3 — The A_{2B} AR antagonists used as reference standard and the values in parenthesis indicates their binding affinity towards A_{2B} AR

data to scrutinize the ability of molecular docking, some of the reference ligands (**Figure 3**) were also docked onto the active-site of the receptor (**Figure 4**) using GOLD docking program (CCDC, 4.0.1 version)[34].

The results of the molecular docking of the enprofylline, one of the most potent but not selective A_{2B} antagonists, suggest that three amino acid residues of the receptor directly interact with the ligand: Ser92, Asn282, and Trp247. The Ser92 formed H-bond with a carbonyl group at the 2-position of the xanthine ring at a distance of 2.0578 Å, while Trp247 seems to be essential for binding because of a π-π interaction[35]. These results are in agreement with the available data on the site-directed mutagenesis obtained for ARs[36]. CVT-6883, a potent highly selective A_{2B} AR antagonist is located inside the hydrophobic pocket formed by Thr89, His251, and Val250. The *n*-propyl chains lie inside two hydrophobic pockets formed by (i) Leu195, Met198, and Ala244 and (ii) Leu49, Asp53, Asn286, and Pro287. Additionally, Trp247 and Phe243 are involved in ligand binding *via* π-π interactions with the phenylxanthine moiety. Further, the fluoro group of trifluoromethyl group interacts with Asn254 by H-bond formation at a distance of 2.1160 Å.

Some of the nonxanthine derivatives (CMB-6446, LAS-38096 and compound **1**) were also docked onto

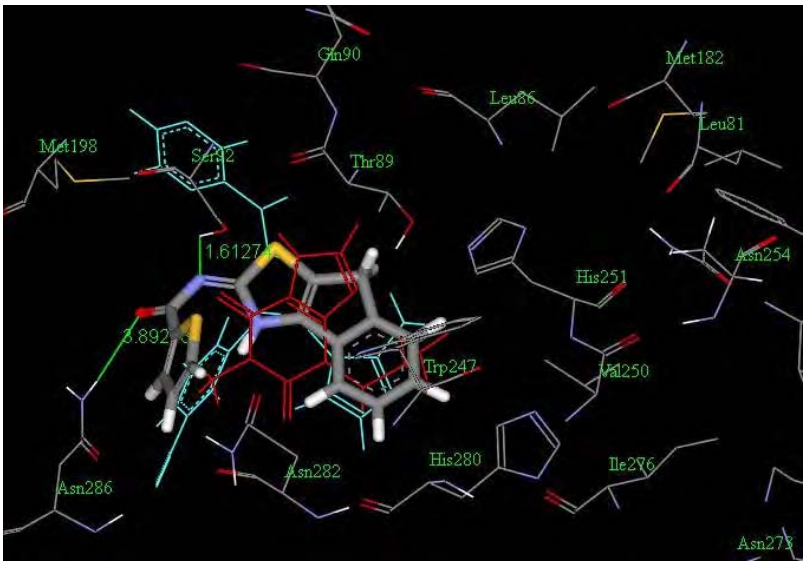


Figure 4 — The A_{2B} AR antagonists enprofylline (red coloured, wire frame) and compound 1 (atom coloured, stick model) used as reference standards and docked into the active-site of A_{2B} AR. Hydrogen atoms are hidden for clarity

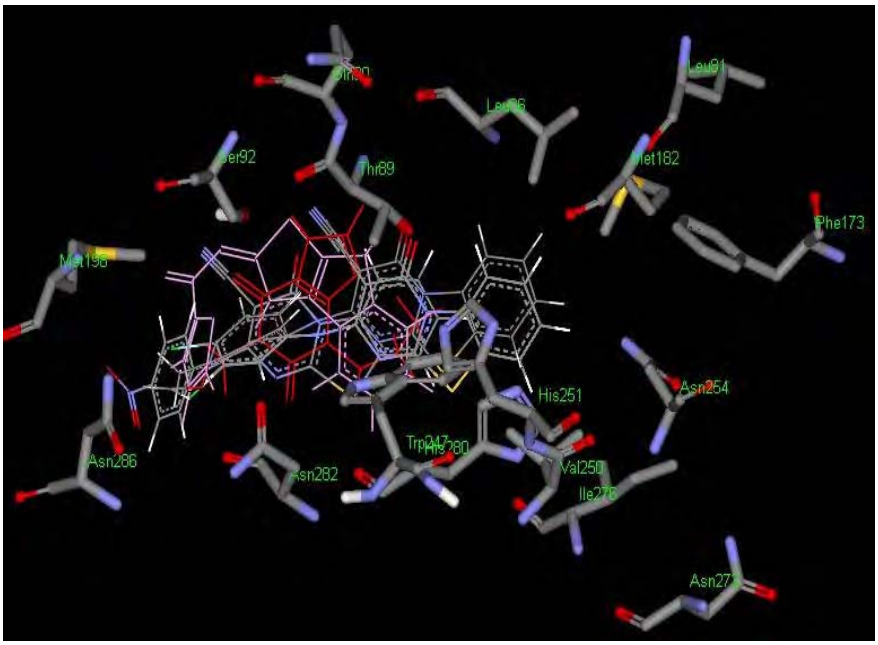


Figure 5 — All of the synthesized ligands docked into the active-site of A_{2B} AR. Ligands are in atom coloured, wire-frame model and enprofylline in red coloured wire frame model, amino acid residues are shown in atom coloured stick model. Hydrogen atoms are hidden for clarity

the active-site of the receptor and interacted favourably with the amino acid residues. Methoxy oxygen of CMB-6446 (amino substituted quinazoline derivative) exhibited H-bond interaction with Asn286 at a distance of 2.2530 Å and -NH at 2nd position interacted with Ser92 at a distance of 2.8606 Å. LAS-38096, a pyridinyl-bipyrimidine derivative, exhibited H-bond interaction between pyridinyl nitrogen and –NH of Asn282 at a distance of 2.0043 Å. Furan oxygen showed H-bond interaction with Ser92 at a distance of 1.1029 Å. The pyrimidine ring orientation in nonxanthine derivatives was observed

relative to that of pyrimidine ring of xanthine derivatives. Compound **1** (a ring fused-thiazole derivative)[11] exhibited H-bond interaction between side chain –N and hydroxyl group of Ser92 at a distance of 1.1627 Å and ring NH as well as amide oxygen were located in the vicinity of residues of Asn282 and Asn286.

The synthesized ligands **6a-g** were also docked onto the active-site and interacted with crucial amino-acid residues (**Figure 5**). Ligand **6a** (unsubstituted aromatic derivative) exhibited two weaker H-bond interactions: (i) between ring sulphur and His280, (ii) -N of CN and –OH of Ser90. Carbonyl oxygen of the fused-ring was located in proximity to Thr89 and the tricyclic system settled well within the cavity formed by the residues of Leu86, Val250, His251, Asn254 and Ile276. The other derivatives **6b-f** also exhibited similar interaction as observed for that of **6a**, except differing in their substituent interaction with crucial residues. Compound **6b** (4-fluoro derivative) exhibited vdW interaction with Asn282 and Asn286 while **6c** (4-fluoro-3-chloro derivative) showed strong H-bond interaction with Asn282 at a distance of 2.0152 Å. Hydrophobic interaction was observed for **6d** (4-methyl derivative) but H-bond interaction was not exhibited. Compound **6e** (4-chloro derivative) exhibited strong H-bond interaction with Asn286 at a distance of 2.2936 Å and **6f** (4-methoxy derivative) showed strong H-bond interaction with Asn286 at a distance of 2.1413 Å. Compound **6g** (4-nitro derivative) exhibited strong H-bond interaction with Asn286 at a distance of 2.1413 Å. The proposed binding motif for the synthesized ligands was found to be in agreement with xanthine derivatives.

Experimental Section

Melting points were recorded in open capillaries on Casiaa Siamea (VMP-AM) melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer FT-IR 240-C spectrophotometer using KBr discs. ^{1}H NMR spectra were recorded on Bruker Avance II 400 MHz spectrometer in DMSO-d_6 using TMS as internal standard. Mass spectra (ESI) were recorded on Waters Micromass Q-TOF Micro. All the reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F_{254} (mesh) (E. Merck, Mumbai) and spots were visualized under UV light (254 nm). All the solvents and general chemicals used were of analytical grade and commercially available (E. Merck, Mumbai) and were used as received.

Molecular modeling studies were conducted using Dell Precision work station T3400 running Intel Core2 Duo Processor, 4GB RAM, 250 GB hard disk, and NVidia Quodro FX 4500 graphics card. GOLD (Genetic optimization for ligand docking) module (CCDC, 4.0.1 version) was employed for the docking studies.

Synthesis

Ethyl-2-cyano-3,3-bismethyl thioacrylate, 2:

Compound **2** was prepared following a known method[37].

4*H*-Pyrimido[2,1-*b*]benzothiazole-2-thiomethyl-3-cyano-4-one, 4:

Compound **4** was prepared following a known method[17a].

4*H*-Pyrimido[2,1-*b*]benzothiazole-2-arylamino-3-cyano-4-one, 6:

General procedure under classical heating

A mixture of **4** (1 mmol) and various arylamines (1 mmol) in 10 mL of DMF and catalytic quantity of anhydrous K_2CO_3 was heated independently under reflux for 8-10 h. The reaction mixture was cooled to RT and poured into ice-cold water. The separated solid product was filtered, washed with water and purified by recrystallization from ethanol to yield the title compounds.

General procedure under microwave irradiation

Caution! It is hazardous to rapidly heat reactions under MW irradiation. Therefore, caution should be exercised when conducting reactions of this type.

A mixture of **4** (1 mmol) and various arylamines (1 mmol) in 10 mL of DMF and catalytic quantity of anhydrous K_2CO_3 was placed in the reaction vessel (Pyrex glass or quartz). The tubes were then placed in the MW cell and heated for the appropriate time (**Table I**). Stirring of the reaction mixture is desirable. Once the heating cycle is complete (monitored by TLC for each 30 seconds), the tube was cooled to ambient temperature, removed from the reactor, and poured into ice-cold water, then processed as indicated under classical heating.

4*H*-Pyrimido[2,1-*b*]benzothiazole-2-(anilino)-3-cyano-4-one, 6a:

Yellow solid, m.p. 318°C; IR: 3261 (NH), 2220 (C≡N), 1691 cm^{-1} (C=O); ^{1}H NMR: δ 7.1–8.9 (m, 9H, Ar-H), 9.1 (s, 1H, -NH, exchangeable with D_2O).

4*H*-Pyrimido[2,1-*b*]benzothiazole-2-(4-fluoro-anilino)-3-cyano-4-one, 6b:

Yellow solid, m.p. >300°C; IR: 3241 (NH), 2211 (C≡N), 1681 cm^{-1} (C=O); ^1H NMR: δ 7.1–8.7 (m, 8H, Ar-H), 9.8 (s, 1H, -NH, exchangeable with D_2O).

4*H*-Pyrimido[2,1-*b*]benzothiazole-2-(4-fluoro-3-chloroanilino)-3-cyano-4-one, 6c:

White solid, m.p. 325°C; IR: 3269 (NH), 2217 (C≡N), 1699 cm^{-1} (C=O); ^1H NMR: δ 7.3–8.8 (m, 7H, Ar-H), 9.9 (s, 1H, -NH, exchangeable with D_2O).

4*H*-Pyrimido[2,1-*b*]benzothiazole-2-(4-methyl-anilino)-3-cyano-4-one, 6d:

The characterization data was in consonance with the literature report[17a].

4*H*-Pyrimido[2,1-*b*]benzothiazole-2-(4-chloro-anilino)-3-cyano-4-one, 6e:

White solid, m.p. 237-40°C (Lit. 235-38°C)[17a]; IR: 3272 (NH), 2217 (C≡N), 1689 cm^{-1} (C=O); ^1H NMR: δ 7.0–8.9 (m, 8H, Ar-H), 9.6 (s, 1H, -NH, exchangeable with D_2O); ESI-MS: *m/z* 352 (M^+), 354 (M^++2).

4*H*-Pyrimido[2,1-*b*]benzothiazole-2-(4-methoxy-anilino)-3-cyano-4-one, 6f:

Yellow solid, m.p. 288-92°C (Lit. 285-90°C)[17a]; IR: 3271 (NH), 2219 (C≡N), 1691 cm^{-1} (C=O); ^1H NMR: δ 3.7 (s, 3H, OCH_3), 7.1–8.8 (m, 8H, Ar-H), 9.3 (s, 1H, -NH, exchangeable with D_2O).

4*H*-Pyrimido[2,1-*b*]benzothiazole-2-(4-nitroani-lino)-3-cyano-4-one, 6g:

Yellow solid, m.p. >300°C (Lit. 340°C)[17a]; IR: 3260 (NH), 2215 (C≡N), 1699 cm^{-1} (C=O); ^1H NMR: δ 7.3–8.9 (m, 8H, Ar-H), 9.9 (s, 1H, -NH, exchangeable with D_2O).

Docking Protocol

Homology model of A_{2B} Adenosine Receptor (A_{2B} AR)

Like most other transmembrane GPCRs, the high-resolution A_{2B} AR crystal structure has not been solved to date, thus only homology model can be used to perform docking studies. In the present paper, A_{2B} AR homology model has been used to generate by Swiss-model (automated protein structure homology-modeling) server[30-33], accessible *via* the ExPASy web server, or from the program Deep View (Swiss PDB-Viewer). The model utilized the crystal structure of A_{2A} AR as an appropriate template.

Preparation of protein

The molecular (homology) model of A_{2B} AR was taken and refined, prepared using Schrodinger protein preparation wizard tool (Glide)[38a], which performs the following steps: assigning of bond orders, addition of hydrogen atoms, optimization of hydrogen bonds by flipping amino side chains, correction of charges, and minimization of the protein complex. The tool neutralized the side chains that are not close to the binding cavity and do not participate in salt bridges. This step is then followed by restrained minimization, which reorients side chain hydroxyl groups and alleviates potential steric clashes. The complex obtained was minimized using OPLS_2005 force field[38b] with PRCG algorithm. The minimization was terminated at either completion of 5,000 steps (or) after the energy gradient converged below 0.05 kcal/mol.

Preparation of ligands

Structures of the ligands were sketched using built panel of Maestro and taken in .mae format. LigPrep is a utility of Schrodinger software suit that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers, steric isomers and perform a geometry minimization of the ligands. Molecular Mechanics Force Fields (OPLS_2005) with default settings were employed for the ligand minimization.

GOLD Docking

GOLD is a ligand-docking application that utilizes a GA to explore ligand conformation flexibility and orientation with partial flexibility of the protein, and satisfy ligand-binding requirements. One advantage of GOLD over many other docking algorithms is that it allows for both unconstrained ligand flexibility and partial flexibility of the binding pocket thus affording a more realistic environment for ligand–receptor associations.

For each of the 10 independent GA runs, a maximum number of 100 GA operations were performed. The standard set parameters were used in all the calculations. Default operator weights were used for crossover, mutation, and migration of 95, 95, and 10, respectively. Default cutoff values of 2.5 Å (for hydrogen bonds) and 4.0 Å (for vdW) were employed. Pop. Size = 100; max ops = 100,000; niche size = 2 were also employed. To further speed up the calculation, the GA docking was terminated when the top three solutions were within 1.5 Å RMSD of each other. GOLD scores each binding mode using a fitness function that accounts for the steric and

electrostatic complementarities between the ligand and receptor. The GOLD scoring function includes the terms for hydrogen-bonding, vdW and intra-molecular energies. The first ranked solutions of the ligands were taken for further observation of binding orientation and H-bond interactions.

Conclusion

A series of 4*H*-pyrimido[2,1-*b*]benzothiazole-2-arylamino-3-cyano-4-ones **6a-g** were designed based on bioisosteric replacement of known ligands. Herein is reported a comparative study of its syntheses under microwave irradiation and by classical heating in solvent. A fast, general, environment-friendly, and facile method under microwave irradiation is presented. The microwave irradiation provided a remarkable rate of acceleration for the nucleophilic substitution, and the reaction time decreased dramatically and in some cases (under MW irradiation) the yields are also substantially higher.

In silico docking studies aided to gain insight into the molecular interactions and binding mode of the target compounds into the receptor model. All the final compounds were docked well into the binding pocket of the target protein and interacted with the crucial amino acid residues. Among the synthesized ligands, the fluorinated derivative **6c** exhibited strong H-bond interactions which may be a potential lead as A_{2B} AR antagonist. The docking results revealed useful information to understand the interaction mode between fused pyrimidines with receptor model and will facilitate the next cycle of drug design (to explore the newer fluorinated pyrimidine-based lead molecules as potent A_{2B} AR antagonists). The *in vitro* affinity investigation of these compounds is in progress using radioligand binding studies. Efforts are currently being taken up to optimize the lead structure and the results of which will be the basis of future research endeavours.

Acknowledgements

The authors thankfully acknowledge the Coordinator, Centre with Potential for Excellence in Biomedical Sciences (CPEBS) and Chairman, University Institute of Pharmaceutical Sciences (UIPS) Panjab University (PU), Chandigarh for providing facilities. The authors gratefully acknowledge Mrs. Lauren Thomas, CCDC, Cambridge, UK for providing the GOLD software. CB, KR and BLN wish to thank the University Grants Commission (UGC), New Delhi for providing fellowships under UGC-RFSMS and DPK, VR and KVR thank CSIR, New Delhi for awarding Senior Research Fellowships. This work was supported by AICTE, New Delhi (RPS- 8023/2006-07).

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