PART 1
CHAPTER 1
ADENOSINE RECEPTORS, AGONISTS AND ANTAGONISTS

ADENOSINE
<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 1 Introduction</td>
<td>4</td>
</tr>
<tr>
<td>1 2 Classification of Purinergic receptors</td>
<td>8</td>
</tr>
<tr>
<td>1 3 Adenosine receptors (A₁, A₂ &amp; A₃) in isolated tissues</td>
<td>9</td>
</tr>
<tr>
<td>1 4 Structure of Adenosine receptors</td>
<td>11</td>
</tr>
<tr>
<td>1.5 Pharmacokinetic properties of Adenosine</td>
<td>12</td>
</tr>
<tr>
<td>1 6 Signal Transduction pathway of Adenosine receptors</td>
<td>14</td>
</tr>
<tr>
<td>1 7 Biological function of Adenosine receptors</td>
<td>15</td>
</tr>
<tr>
<td>1 7 1 CNS</td>
<td>15</td>
</tr>
<tr>
<td>1.72 CVS</td>
<td>15</td>
</tr>
<tr>
<td>1 7 3 Renal system</td>
<td>16</td>
</tr>
<tr>
<td>1 7 4. Respiratory tract</td>
<td>16</td>
</tr>
<tr>
<td>1 8 Therapeutic implications</td>
<td>19</td>
</tr>
<tr>
<td>1 9 Adenosine receptor (A₁, A₂a, A₂b, A₃) agonists with structure approach</td>
<td>22</td>
</tr>
<tr>
<td>1 10 Adenosine receptor antagonants with structure approach</td>
<td>35</td>
</tr>
</tbody>
</table>
1.1 Introduction:

Adenosine receptors are expressed on the surface of nearly all cells. Activation of adenosine receptors produces cell-specific physiological responses that are potentially useful for the treatment of a variety of diseases including hypertension, myocardial and cerebral ischemia, arrhythmia, asthma, thrombosis, seizure, pain, anxiety, neurodegeneration, diabetes and inflammation\textsuperscript{1,2}

Despite the therapeutic potential of adenosinergic compounds, the only two drugs currently marketed are adenosine (1) as iv infusion for the treatment of arrhythmia, for controlled hypotension in aneurism, in surgery and Paroxysmal supraventricular tachycardia\textsuperscript{3}, under trade name Adenocard (USA) and Adrekar (Germany) and, the dipyridamol (2)\textsuperscript{4} adenosine transport blocker in the form of tablets and injection.

\[
\begin{array}{c}
1 \quad \text{NH}_2 \\
\text{HO} & \text{O} & \text{HO} & \text{OH} \\
\end{array}
\begin{array}{c}
2 \\
\text{N} & \text{(CH}_2\text{OH})_2 \\
\text{(HO}_2\text{C}_2\text{)}_2\text{N} & \text{N} \\
\text{HO} & \text{C} & \text{N} \\
\end{array}
\]

Potent extracellular effects of both adenosine (endogenous nucleoside) and adenine nucleotides (3, ATP and 4, AMP, 5, cAMP) were reported for the first time in 1929 by Drury & Szent-Gyorgi\textsuperscript{5} The word "purinergic" was coined in 1971\textsuperscript{6} to describe purine nucleotide, ATP, as modulators of neural and muscular functions. The transmitter named and classified the respective receptors as 'purinergic receptors' or 'purinoceptors' in 1978 by Burnstock\textsuperscript{7} The mechanism of action of these purines, Adenosine and ATP, as modulators of neural and muscular functions with discrete cell surface receptors is known as purinoceptors, in that they respond to purines
The first subdivision of purinoceptors into the P$_1$ & P$_2$ categories was based mainly on the criteria that nucleoside like adenosine activates P$_1$ purinoceptor, nucleotides like ATP stimulate P$_2$ purinoceptors. Adenosine acting at P$_1$ purinoceptors generally depresses the activity of particular tissue or organ, linked through the adenylate cyclase. ATP acting at P$_2$ purinoceptors has both inhibitory and stimulatory effects. Adenine nucleotides, particularly 5'-di and 5'-tri phosphates are nearly inactive at P$_1$ purinoceptors, while adenosine is very weak at P$_2$ purinoceptors. Adenosine 3',5'-cyclic monophosphate (cAMP, 5) acts as a second messenger at P$_1$ purinoceptors, while P$_2$ purinoceptors, activated by adenine nucleotides, induces the formation of prostaglandins. However, adenosine 5'-diphosphate (ADP, 6) acts as a powerful platelet-aggregating agent through inhibition of adenylate cyclase at ADP receptor. P$_1$ purinoceptors were further subclassified on the basis of either inhibition (A$_1$) or stimulation (A$_2$) of cAMP accumulation and are alternatively termed as Ri and Ra.
respectively\textsuperscript{9,10} $A_1$ and $A_2$ receptors are now operationally defined by pharmacological potencies of selective agonists and antagonists\textsuperscript{11,12,13,14}.

![Chemical structure of adenosine](image)

6

Stimulatory $A_2$ adenosine receptors have been further divided into two categories $A_{2a}$ and $A_{2b}$ based on the potencies of agonists in different tissues\textsuperscript{11}. High affinity $A_{2a}$ receptor is located in striatum\textsuperscript{12}, whereas low affinity $A_{2b}$ receptor in fibroblasts\textsuperscript{15,16}.

$A_3$ receptor is the recently found of this class, and its existence has been confirmed by cloning studies\textsuperscript{17,16}. The proposal for the role of this receptor to decrease the intracellular calcium levels still remains questionable\textsuperscript{19}.

Recently, Cornfield et al., claimed the existence of an $A_4$ purinoceptor basing on binding studies with the agonist 2-phenyladenosine (CV-1808, 7)\textsuperscript{20}.

![Chemical structure of 2-phenyladenosine](image)

7

In addition to the extracellular $A_1$ & $A_2$ and $A_3$ receptors, an intracellular P site\textsuperscript{21,22}, at which adenosine inhibits adenylate cyclase in adipocytes and is resistant to the occupation by xanthines, whereas methylxanthines are selective antagonists at $A_1$ & $A_2$ receptors. The P-site was so named because an intact purine moiety was thought to be required\textsuperscript{23}. The physiological relevance of the P-site remains unclear.
Adenosine receptors coupled to guanine nucleotide regulatory proteins (G<sub>i</sub> and G<sub>s</sub>), mediate the biological effects of adenosine on adenylate cyclase and other second messenger systems. Prolonged exposure to adenosine agonists leads to desensitization of adenosine receptor activation. A receptor for adenosine 5'-diphosphate (ADP) apparently distinct from adenosine and ATP receptors has been described in the platelet. ADP stimulates platelet aggregation, an action which is not shared by other naturally occurring nucleoside diphosphates.
1.2. Classification Of Purinergic Receptors:

![Diagram]

**Purinergic Receptors**

- $P_1$
- ADP Receptors
- $P_2$

(Adenosine Receptors)

- $A_1$
- $A_2$
- $A_3$

(ATP Receptors)

- $P$-site

- $A_{2a}$
- $A_{2b}$ (internal adenosine receptor)
1.3. Adenosine Receptors ($A_1$, $A_2$ & $A_3$) in Isolated Tissues:

In order to assign functional responses to the adenosine receptor subtypes and to map their distribution in different tissues, a number of isolated tissues and cell studies have been reported.

From the order of agonist potency observed, the adenosine receptors that mediate cardiac depression, inhibit renin secretion, cause vasoconstriction, bronchoconstriction, inhibition of lipolysis and neurotransmitter release have been called as $A_1$ receptors\(^{25}\).

$A_2$ receptors are found in neutrophils, on airway and vascular smooth muscle (where they mediate relaxation), on liver cells (mediate gluconeogenesis), on platelets (inhibition of aggregation).

Smooth muscle preparations that relax in the presence of adenosine appear to contain $A_2$ receptors. But structure activity studies using agonists and antagonists in smooth muscle preparations correlate less well with binding studies at $A_2$ sites. The interpretation of results are complicated by the presence of additional purine-sensitive sites that can also mediate relaxation but which are not blocked by xanthines\(^{26,27}\).

In addition, both $A_1$ & $A_2$ receptors can occur in the same smooth muscle preparation. The $A_1$ receptor generally mediating contraction and $A_2$ receptors are mediating relaxation\(^{28,29,30}\). In the rat colon and vas deferens $A_1$ and $A_2$ receptors are clearly at different anatomical locations. But this may not be the case in all other tissues in which the two sub-types co-exist.

The cloned dog $A_2$ receptor binds CGS 21680 (8) with high affinity and is presumably of the $A_{2a}$ type. Adenosine receptors with the expected characteristics of the $A_{2b}$ receptors have recently been cloned from rat brain\(^{31}\).
In 1992, Zhou & colleagues\textsuperscript{17} reported the cloning, expression and functional characterization of a novel A\textsubscript{3} adenosine receptor. It was suggested that the A\textsubscript{3} receptor was present on excitable tissues and mediated inhibition of transmitter release from central and peripheral neurones, and negative chronotropic and inotropic effects on the heart.

Cloned A\textsubscript{3} receptor can be considered an entity distinct from the established A\textsubscript{1} and A\textsubscript{2} receptor subtypes, concluded by IUPHAR purinoceptor subcommittee in a recent status report on purinoceptor nomenclature. These receptors A\textsubscript{1}, A\textsubscript{2a}, A\textsubscript{2b} and A\textsubscript{3} belong to the super family of G-protein.
1.4. Structure Of Adenosine Receptors:

A large number of receptors in the plasma membrane regulate distinct effector proteins through the mediation of a group of GTP binding proteins known as G-proteins. These act as intermediate between receptors and the effectors. The effectors include enzymes such as adenylyl cyclase and phospholipases C and Ca\(^{++}\), K\(^{+}\) or Na\(^{+}\) channels and certain transport proteins.

G-proteins are bound to the inner surface of the plasma membrane. These are heteroatomic molecules (subunits are designated as $\alpha$, $\beta$, and $\gamma$). These polypeptides have highly homologous guanine nucleotide binding domains and they are thought to have distinct domains for interaction with receptors and effectors.

Receptors $\rightarrow$ G-proteins $\rightarrow$ Effector

Based on molecular cloning and sequencing studies, adenosine receptors are part of the G-protein coupled receptor superfamily\(^{32}\). Sequence homology to other G-protein coupled receptors indicates that adenosine receptors have the classical seven transmembrane helix topology with an extracellular aminoterminal and a cytoplasmic carbonyl terminus containing phosphorylation site and a cytoplasmic domain that could interact with G-proteins.

Clusters of serine and threonine residues in the C-terminal segments, commonly seen in G-protein-linked receptors are absent in RDC-7 (A\(_1\)). Finally, with 326 amino acids, RDC-7 (A\(_1\)) is among the smallest members of the super family. RDC-7 (A\(_1\)) and RDC-3 (A\(_{2a}\)) are similar to a variety of other seven transmembrane helix proteins, but none of these is more than 30% identical to either of adenosine receptors.

The A\(_1\), A\(_{2a}\), A\(_{2b}\) and A\(_3\) purinoceptors are, moreover, fairly small having 328-362, 409-412, 322-328 and 317-320 amino acids, respectively. With an amino acid sequence homology of 49.5%, the A\(_3\) subtype is more closely related to the A\(_1\) than to the A\(_{2a}\) (43.2%) or A\(_{2b}\) (39.9%) subtypes.
1.5. Pharmacokinetic Properties Of Adenosine:

Adenosine is metabolised extremely rapidly in vivo, and as a result investigation of many standard pharmacokinetic variables is difficult or not possible.

Most of the endogenous adenosine appears to be bound by intracellular δ-adenosyl homocysteine hydrolase, the enzyme catalyzing reversible transmethylation of δ-adenosyl homocysteine to form adenosine and L-homocysteine$^{33,34}$. Adenosine is also formed via dephosphorylation of adenine nucleotides$^{35,36}$.

Adenosine is rapidly taken up from plasma by erythrocytes and blood vessel endothelial cells, and most metabolisms occur via cellular mechanisms (Scheme-1).

Adenosine has a very short half-life of less than 10 sec with a turnover of 1.5 μmol/L/min in vitro experimental studies$^{37}$, and appears to increase with increasing adenosine concentration, possibly as a result of substrate inhibition of metabolic enzymes and/or enzyme saturation$^{34,38}$.

At physiological concentrations (0.1 to 1.0 μmol/L) adenosine is predominantly metabolized to adenine 5'-monophosphate$^{38}$ and following administration of therapeutic doses, adenosine is deaminated to inosine$^{38,39,40}$.
Pathways Of Adenosine Metabolism

S-adenosyl homocysteine

- S-adenosine cysteine hydrolase
  - adenosine kinase

  Adenosine
  - S'-nucleotidase
  - Adenosine deaminase
  - Inosine
    - Purine nucleoside phosphorylase
      - Hypoxanthine
        - Xanthine oxidase
          - Xanthine
            - Xanthine oxidase
              - Uric acid

High energy phosphate pool

Adenosine-5'-monophosphate

Scheme - 1
1.6. Signal Transduction Pathway Of Adenosine Receptors:

P₁ receptors mediate the responses via multiple transduction mechanisms mediation of adenylate cyclase, phopholipase C (PLC), guanylate cyclase, potassium channels, calcium channels, and TNF-α. Adenylate cyclase (intracellular enzyme) inhibited by G₁-proteins and stimulated by G₅-proteins, in turn the intracellular cyclic AMP level is decreased or increased, leads to activation of protein kinase, which in turn phosphorylates diverse protein targets. The A₁ (G₁) and A₃ subtypes are linked to this enzyme in an inhibitory manner, and A₂ subtype (G₅) is positively linked to Adenylate cyclase.

Activation of the A₃ receptor has been shown to stimulate phospholipases C and D. The stimulation of Guanylate cyclase enzyme via a G₅ leads to accumulation of the intracellular second messenger cyclic GMP (cGMP), which activates specific protein kinases for the phosphorylation purpose. Ultimately, the intracellular Ca²⁺ level is reduced. This pathway is believed to account at least partly for A₂ receptor mediated vasodilators.

After P₁ receptor stimulation and G-protein coupling, the opening of diverse K⁺ channels might lead to hyperpolarization and shortening of the action potential, as has been well documented for the A₁ receptor in atria, and reports on A₂ receptor related opening of K⁺-channels are also available.

A G-protein-mediated inactivation of ligand-gated Ca²⁺ channels and indirect closure of voltage dependent Ca²⁺ channels is another important pathway for A₁ receptor mediated responses.

The varied effects of A₃ receptor agonists appear to be dual and opposite, i.e., either cytoprotective or cytotoxic, depending on the level of receptor activation and the system studied. Activation of the A₃ receptor has been shown to stimulate phospholipases C and D and to inhibit adenylate cyclase. There may be an involvement of A₃ receptors in cancer. An A₃ agonist inhibited the release of potentially damaging TNF-α in activated macrophages, thus A₃ agonists may be protective in the models of inflammation.
1.7. Biological Function Of Adenosine Receptors:

1.7.1. CNS:

Most brain regions are rich in A₁ and A₂b receptors, the A₂a subtype is specifically located in the striatum, and the A₃ subtypes seems to be expressed less abundantly in the brain. Adenosine acts as a neuromodulator inhibiting, via prejunctional A₁ receptors, the release of various transmitters including excitatory amino acids, and also tends to limit the excitation of effector cells. Thus, adenosine reduces the neuronal activity and oxygen consumption. A₂ receptor activation leads to increase the cerebral blood flow.

Collectively, adenosine might be regarded as cerebroprotective agent, the production of which is increased, during ischaemic conditions.

Adenosine also exerts behavioral effects, the decrease of locomotor activity and psychomotor activity (sedative action) and is likely to be correlated with postsynaptic A₂a receptor stimulation.

Notably, a postsynaptic A₂a/D₂ interaction has been suggested in that adenosine agonists reduce dopamine binding. An anticonvulsive property of adenosine has also been described. In the spinal cord adenosine is involved in the analgesic effects of opioids.

1.7.2. CVS:

A₁ receptors are located on atrial and ventricular myocytes, while A₂a and A₂b subtypes are present on coronary smooth muscle and coronary endothelium, respectively. A₃ receptor is only moderate expressed in the heart. Adenosine exerts cardio depressive effects, i.e., a negative chronotropic, dromotropic and antiarrhythmic action via A₁ receptors, and inhibition of inotropic responses to β-adrenergic agonists, thus decreasing the oxygen consumption. On the other hand, adenosine increases the coronary blood flow through the mediation of A₂ receptor vasodilation thereby increasing oxygen supply. Adenosine is generally a potent vasodilator (A₂ & A₃) except in kidney and few other blood vessels. Peripheral vasodilation in combination with reduced cardiac output results in the effective...
lowering of blood pressure. In addition, adenosine is an endogenous inhibitor of platelet aggregation (A_{2a})^{31}, owing to increased cAMP levels.

1.7.3. Renal System:
Adenosine acts to reduce the glomerular filtration rate by A_{1} receptor mediated vasoconstriction of afferent and A_{2} receptor mediated vasodilation of efferent arterioles^{56}, resulting in an anti-diuretic effect, and also inhibits renin release via A_{1} receptors

1.7.4. Respiratory Tract:
While, adenosine exerts bronchodilation in healthy subjects (A_{2}), the response is bronchoconstriction^{57} in asthma patients. In asthmatics, adenosine is able to release histamine and leukotrienes as mast cell mediators. This effect of adenosine is weakly blocked by xanthines. Because, the A_{3} receptor, which is mainly xanthine insensitive, is abundantly expressed in lung it is suggested that it might be involved in the aetiology of asthma^{52}
Table 1: Adenosine Receptors And Actions

<table>
<thead>
<tr>
<th>S No</th>
<th>Tissue</th>
<th>Cell</th>
<th>Adenosine action</th>
<th>Receptor sub-type</th>
<th>Signal transduction pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vascular</td>
<td>Smooth muscle</td>
<td>Vasodilation</td>
<td>A2a, A2b</td>
<td>Adenylyl cyclase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endothelial</td>
<td>Increase permeability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Blood</td>
<td>Platelet</td>
<td>Aggregation inhibition</td>
<td>A2a</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monocyte</td>
<td>Inhibits superoxide generation</td>
<td>A2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lymphocyte</td>
<td>Inhibits proliferation</td>
<td>A2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Basophil</td>
<td>Inhibits histamine release</td>
<td>A2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neutrophil</td>
<td>Inhibits adhesion &amp; superoxide formation</td>
<td>A1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Promotes chemotaxis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Blood</td>
<td>Blood Platelet</td>
<td>Aggregation inhibition</td>
<td>A2a</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood Neutrophil</td>
<td>Inhibits superoxide generation</td>
<td>A2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood Lymphocyte</td>
<td>Inhibits proliferation</td>
<td>A2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood Basophil</td>
<td>Inhibits histamine release</td>
<td>A2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Heart</td>
<td>Atrioventricular nodal cell</td>
<td>Prolongs impulse Conduction</td>
<td>A1</td>
<td>K⁺</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sinus nodal cell</td>
<td>Decrease heart rate</td>
<td>A1</td>
<td>K⁺</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Atrial myocyte</td>
<td>Decrease contractility</td>
<td>A1</td>
<td>K⁺</td>
</tr>
<tr>
<td>4</td>
<td>Brain</td>
<td>Presynaptic neuron</td>
<td>Inhibits glutamate release</td>
<td>A1</td>
<td>Ca²⁺</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post synaptic neuron</td>
<td>Inhibits hyperpolarisation</td>
<td>A2</td>
<td>Adenylyl cyclase (AC)</td>
</tr>
<tr>
<td>5</td>
<td>Spinal cord</td>
<td>Nociceptive neuron</td>
<td>Antinociception</td>
<td>A2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Fat</td>
<td>Adipocyte</td>
<td>Inhibits lipolysis</td>
<td>A1</td>
<td>Ca²⁺</td>
</tr>
<tr>
<td>7</td>
<td>Kidney</td>
<td>Smooth muscle</td>
<td>Vasoconstriction</td>
<td>A1</td>
<td>Ca²⁺</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Juxtaglomerular cell</td>
<td>Vasodilation</td>
<td>A2</td>
<td>AC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inhibits renin release</td>
<td>A1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>GI tract</td>
<td>Gastric cell</td>
<td>Inhibits acid secretion</td>
<td>A1</td>
<td>AC</td>
</tr>
<tr>
<td>9</td>
<td>Liver muscle</td>
<td></td>
<td>Stimulation of gluconeogenesis, Vasocostriction</td>
<td>A2</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Skin</td>
<td></td>
<td>Vasoconstriction</td>
<td>A1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Trachea</td>
<td></td>
<td>Brochoconstriction</td>
<td>A1, A2</td>
<td></td>
</tr>
<tr>
<td>(Lungs)</td>
<td></td>
<td></td>
<td>Relaxation of airway smooth muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Immune system</td>
<td></td>
<td>Immunosuppression</td>
<td>A2</td>
<td></td>
</tr>
</tbody>
</table>
## Table - 2

### A₃ Receptor And Actions

<table>
<thead>
<tr>
<th>S No</th>
<th>Tissue</th>
<th>Cell</th>
<th>Adenosine action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vascular</td>
<td>Smoothmuscle</td>
<td>Vasodilation</td>
</tr>
<tr>
<td>2</td>
<td>Heart</td>
<td>Myocyte</td>
<td>-ve chronotropic &amp; inotropic effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cardioprotective (in case of hypoxia)</td>
</tr>
<tr>
<td>3</td>
<td>Brain</td>
<td>Neuron</td>
<td>Inhibits transmitter release from central and peripheral neurons</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inhibits Nitric oxide synthase, Cerebroprotective</td>
</tr>
<tr>
<td>4</td>
<td>Trachea</td>
<td></td>
<td>In healthy person - Bronchodilation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>In asthma person - Bronchoconstriction</td>
</tr>
<tr>
<td>5</td>
<td>General</td>
<td>Inhibits inflammation</td>
<td>Inhibits release of tumor necrosis factor-α (TNF-α)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(a)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b)</td>
<td>As anti-cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c)</td>
<td>cytoprotective</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d)</td>
<td>Decreases locomotory action on A₃ stimulation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e)</td>
<td>Enhances the release of inflammatory mediators from mast cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(f)</td>
<td>Shows &quot;behavioral depressant&quot;action</td>
</tr>
</tbody>
</table>
1.8. Therapeutic Implications:

Currently, the major clinical use of adenosine is in the acute treatment and diagnosis of supraventricular arrhythmia\(^58\). Given a wide variety of effects elicited by the purine, many more therapeutic applications can be envisaged. Agonists with appropriate selectivity for \(A_1\), \(A_{2a}\), \(A_{2b}\) or \(A_3\) receptors could be beneficial in a variety of metabolic, CNS & cardiovascular disorders.

In addition to a direct ligand approach (agonists or antagonists), an indirect approach using agents that act to potentiate the actions of endogenous adenosine might thus be regarded as “site and event-specific” agents, and often seem more valuable because of fewer side effects.

Adenosine plays an important role in neurodegenerative diseases such as Parkinson’s and Alzheimer’s disease involving excitatory amino acids\(^54\). Importantly, in Alzheimer patients, the \(A_1\) receptor density is reduced whereas coupling to G proteins is preserved\(^59\). The administration of \(A_1\) agonists has been found to protect against neurodegeneration\(^54\). Adenosine antagonists appear, on the other hand, to be beneficial in the treatment of these disorders\(^60\). \(A_{2a}\) selective antagonists are promising as antiparkinsonian agents because of their indirect enhancement of dopamine binding. \(A_1\) receptor antagonists are being developed as cognition enhancers with potential use in Alzheimer’s disease. As has been shown for carbamazepine, chronic administration of \(A_1\) receptor antagonists will be useful in the treatment of seizures (epilepsy) and might also serve as a prophylactic of affective diseases\(^61\). \(A_1\) selective agonists in combination with hypnoanalgesics might, furthermore, render pain therapy more effective and probably safer, because activation of adenosine receptors in the dorsal horn of the spinal cord inhibits transmission induced by pain mediators (e.g., substance P)\(^54\).

Adenosine is of diagnostic use in arrhythmia and valuable in cardiac preconditioning\(^58\). \(A_2\) receptor agonists and uptake blockers (e.g., dipyridamole\(^2\)) are potent coronary vasodilators and hypotensive and inhibitors of platelet aggregation. Acute treatment with \(A_1\) receptor agonists or indirect agents like adenosine uptake blocker or long-term treatment with adenosine antagonists is useful in stroke therapy\(^{54,60,62}\). Selective \(A_{2a}\) receptor agonists might also serve as antithrombotics, owing to inhibition of platelet aggregation.
Selective A1 receptor antagonists are being developed as novel diuretics with potential protective action against acute renal failure63.

The development of A3 selective antagonist might be an intriguing novel approach in asthma therapy due to the secretion of histamine and leukotrienes from mast cells involving A3 receptor stimulation52.

Apart from the above therapeutic implications, it should be pointed out that patients with an inherited adenosine deaminase enzyme (ADA) deficiency (often observed in patients with severe combined immunodeficiency) might profit from an ADA gene replacement therapy64.

ADA inhibitors have potential importance as anticancer or antiviral agents55. The potent lymphocytotoxic ADA inhibitor, deoxycoformycin (9) has recently been approved for the treatment of haircell leukaemia in Germany66.

Recently, the important benefits of adenosine agonists are to specifically increase endogenous extracellular adenosine levels in the ischemic region by enhancing adenosine production or by inhibiting adenosine utilisation. These agents are now known as "adenosine regulating agents" or ARA's and are pharmacologically silent except in tissue undergoing ischemias or some other condition that involves net ATP catabolism. The first compound identified with these properties was 5-amino-1β-D-ribofuranosylimidazole-4-carboxamide or AICA ribosides (10). This AICA riboside selectively increase adenosine levels in blood from ischemic regions of a canine heart. They don't have any cardiac or haemodynamic effects in non-ischemic regions. The site and event specificity of AICA results from its ability to increase extracellular adenosine concentrations only at site of net ATP breakdown and from
the short half life of adenosine, which confines the pharmacological actions of adenosine to the site of production.

Agents have been reported to protect the heart from ischemic damage by enhancement of extracellular adenosine. Based on the pathways and enzymes involved in adenosine production and utilisation, several potential ARA target exist. Adenosine deaminase and the adenosine transporter represent two possible sites based on their role in adenosine metabolites. ADA inhibitors have been reported to be effective in models of global and regional ischemia.

Two potent and selective ADA inhibitors, namely 2'-deoxycoformycin and erythro-9(2-hydroxy-3-nonyl)adenine (EHNA, 11) were found to improve recovery of contractile function in buffer perfused hearts undergoing in global ischemia. Adenosine transport inhibitors have also been studied as potential cardio-protective agents.
1.9. Adenosine Receptor (A₁, A₂₆, A₂₉, A₃) Agonists With Structure Approach:

Affinity of adenosine cannot be measured directly due to its rapid degradation by adenosine deaminase, a necessary additive in adenosine receptor binding assays. Therefore, most of the information available on the pharmacological actions of adenosine has been generated with a rather small number of stable adenosine analogues with binding affinities at the A₁, A₂₆, A₂₉, A₃ receptors. Few alterations of the ribose moiety are tolerated at the adenosine receptor binding site. Except for a limited number of modifications of 5' position, the intact ribose moiety generally is required for adenosine agonist activity.

Inversion of the glycosidic bond results in the loss of receptor affinity. Most modifications at the 2' and 3' positions of the ribose ring or inversions of chiral centres on the ribose diminish adenosine receptor binding. For example, 2'-deoxy-2'-fluoroadenosine (12) and adenosine 9-β-D-arabinoside (13) are inactive agonists or antagonists at A₂ receptors.

9-Methyladenine and 9-phenyl-7-deazaadenine are adenosine antagonist, because they lack ribose moiety.

Recently, 5'-deoxy-5'-methylthioadenosine (14) was found to be an agonist at A₁ receptors and at putative A₂ receptors that relax smooth muscle.
The 5'-carboxylate derivative of adenosine (15) and several esters are active in vivo at cardiovascular A₂ receptors⁷², but are inactive at fibroblast A₂ receptors coupled to adenylate cyclase⁵⁹.

The substitution of 5'-COOH by N-methyl (16) or N-Ethyl (17)⁷³ enhances A₂ activity as a vasodilator⁷² or as inhibitor of platelet aggregation⁷⁴,⁷⁵. Carboxamidoadenosines are the most active agonists on the human A₂b receptor⁷⁶. It was found that besides, N-methyl (16), several analogues displayed selectivity toward A₃ receptor⁷⁷.
5'-N-Ethylcarboxamido adenosine (NECA, 17) and the closely related cyclopropyl analog, CPCA (18) are among the most potent known adenosine agonists at A2 receptors. However, NECA (17) is a potent agonist with high affinity for both A1 and A2 sites and is not A2 selective, since it is very potent at A1 receptors. It binds to rat and human A1, A2a and A3 receptors with Ki values in the nanomolar range. Although far from selective with respect to the other subtypes, NECA was the most potent carboxamide on the A2b receptor with low micromolar range. Recently it was reported that 5'-carboxamidoadenosines without modifications on other positions are the most active A2b agonists.

Simple alkyl groups larger than propyl greatly diminish biological activity at the coronary A2 adenosine receptors. The 5-N-cyclopropyl and or equivalent size of ethyl group of NECA are well tolerated in the pocket of A2 receptor. Derivatives with a small 5'-N-substituent on the carboxamido function, such as ethyl in NECA, were found to strongly induce coronary vasodilation in dogs. On all receptor subtypes relatively small substituents on the carboxamido moiety were optimal.

5'-N,N-Dialkylaronamide groups are not tolerated in this binding region and diadenosine uronamide derivatives joined by N-alkyl chains ten or more in length were active in coronary vasodilation.

The adenine moiety is more amenable to substitutions than the ribose moiety. Thus, 2-Chloroadenosine (19), in comparison to adenosine, is highly potent at coronary A2 receptor.
2-Alkyne derivatives of adenosine are more hypotensive (A₂) than cardiac depressant (A₁).

2-Phenylaminoadenosine (CV-1808, 20) and its analog 2-(4-methoxyphenyl) adenosine (CV-1674, 21) were reported as A₂ selective agonists. Thus large alkylamino or arylamino substituents at the 2-position are accepted better by A₂ receptors than by A₁ receptors.

A combination of modification of the 5'-position and C₂ substitution has led to potent and selective A₂a receptor agonist 2-[p-(2-carboxyethyl)-phenylethylamino]-NECA (CGS 21680). A 2-substituted derivative of NECA, by the introduction of hydrophilic carboxyalkyl substituent in the C-2-position, CGS 21680 (8) is 140 fold A₂ selective as an adenosine agonist than 2-(arylalkylamino)adenosine-5'-uronamides.
Substitution of $N^6$-amino group of adenosine by an alkyl, arylalkyl or aryl group often results in selectivity of the agonists for $A_1$ receptors over $A_2$ sites. Thus, $N^6$-cyclohexyladenosine (CHA, 22) is a high affinity ligand at $A_1$ sites, and shows a selective $A_1$ agonist activity. $N^6$-cyclopentyl analog (CPA, 23) has the greatest $A_1$ selectivity at central adenosine receptors. Recent results indicating that the 1-deaza derivatives of adenosines showed 10-fold less affinity for $A_1$ receptors than the corresponding adenosine derivatives. The chloro substitution at C2-position showed slightly higher affinity than the 2-unsubstituted counterparts. The 2'-deoxy derivatives bind to the $A_1$ receptor with affinities in the high nanomolar range.

Certain, $N^6$-(2-phenyl ethyl) derivatives (24) are highly potent ligands at the $A_2$ receptor. At the coronary $A_2$ receptor exhibit strong potency.
Olsson et al studied N<sup>6</sup>-substituted 5'-carboxamidoadenosine derivatives on their ability to inhibit or stimulate adenylate cyclase via A<sub>1</sub> & A<sub>2</sub> receptors and concluded that analogues with small substituents showed the highest activities<sup>81</sup>

N<sup>6</sup>-(2,2-Diphenylethyl)adenosine (C1936, 25) having K<sub>i</sub> values at A<sub>1</sub> and A<sub>2</sub> receptors of 68 and 25 mM, respectively<sup>82</sup> has an antipsychotic activity in behavioral tests

N<sup>6</sup>-(2-(3,5-Dimethoxyphenyl)-2-(2-methylphenyl)-ethyl)adenosine (DPMA, 26) and its 5'-uronamide are A<sub>2</sub> selective agonists<sup>83</sup> The bridged derivative (27)<sup>84</sup> of C1936 is also a very potent A<sub>2</sub> agonist
N\textsuperscript{6}-Alkyladenosines that also contain the 5'-uronamide modification tend to lose the high potency at A\textsubscript{2} receptors, typical of 5'-uronamide derivatives\textsuperscript{83,85}, but tend to enhance the potency at A\textsubscript{1} receptors and to enhance activity at A\textsubscript{1} receptors favoring subsistent at C-2 position along with N-6-alkyl substitution increases A\textsubscript{1} selectivity\textsuperscript{86}. EMD 28422 a diastereomeric pair of N\textsuperscript{6}-2-(4-chlorophenyl)-bicyclo[2,2,2]octyladenosines, is a potent modulator at central benzodiazepine receptors, and is a weak nonselective agonist at adenosine receptors\textsuperscript{87}

An adenosine amine congener, (ADAC, 28) is a highly potent agonist ligand at A\textsubscript{1} receptors, with a K\textsubscript{d} of 0.85 nM

\[
\text{NH-C}_6\text{H}_4\text{-CH}_2\text{CONH-C}_6\text{H}_4\text{-CH}_2\text{CONH(CH}_2)_2\text{NH}_2
\]

28 (ADAC)

Naturally occurring marine product 1-methylisoguanosine (29) acts as an adenosine receptor agonist in the relaxation of striated muscle\textsuperscript{88} and in the central nervous
In adenylate cyclase systems, it is several times more potent at $A_1$ receptors (adipocytes) than at $A_2$ receptors.

Recently, selective $A_2$ receptor agonist activity has been reported in 2-[2-(4-methylphenyl)ethoxy]adenosine (MPEA, 30) and 2-[(2-cyclohexyl ethyl) amino] adenosine (CGS 22492).

2-(1-Hexyln)adenosine-5'-N-cyclopropylarnamide is reported to exhibit the most potent affinity to the $A_2$ receptor with a $K_i$ of 26 nM. The N-alkyl substituents of 5'-uranoamide derivatives did not seem to potentiate the $A_2$ binding affinity but drastically reduced the $A_1$ affinity compared with 2-(1-hexynyl)adenosine (2-HA, 31). A decrease in the carbon number of the N-alkyl substituent at the 5'-carboxamide gradually reduced the $A_2$ affinity.
Derivatives of 2-Alkynyl adenosine-5'-ethyluronamide, (4-hydroxy-1-butynyl) (32) and the 4-(2-tetrahydro-2H-pyranloxy)-1-butynyl appear to be very potent in inducing vasorelaxation without appreciable effect on heart rate. The 2-alkynyl derivatives of 5'-N-ethyluronamides retained the A₂ affinity whereas the A₁ affinity was attenuated, resulting in an up to 10-fold increase in A₂ selectivity. Presence of α-hydroxyl group in the alkynyl side chain caused a greater increase in antiaggregatory activity than either NECA or HE-NECA.

The A₃ adenosine receptor is the most recent identified among the three major subtypes of adenosine receptors, A₁, A₂ and A₃. 1,3-Dibutylxanthine-7-riboside (33) is a partial agonist at A₃ adenosine receptors whereas 1,3-dipentylxanthine-7-riboside (34) was slightly selective for A₃ receptors. The affinity of xanthine-7-ribosides at A₃ receptors depend on the 1,3-dialkyl substituents in the order Pent > Bu >> Hx > pr = Me.
2-Thio vs 2-oxo substitution increased potency at all the three subtypes of adenosine receptors and slightly increased $A_3$ vs $A_1$ selectivity.

$N^6$-(3-iodobenzyl)adenosine (35) is 2-fold selective for $A_3$ Vs $A_1$ or $A_2$ receptors, thus it is the first mono substituted adenosine analog having any $A_3$ selectivity, and 2-substitution (36) (both small and sterically bulky) is well tolerated at $A_3$-receptors. The substitution at 2-position by chloro had a $k_i$ value of 1.4nm and moderate selectivity for $A_3$ receptors and its $A_3$ affinity-enhancing effects are additive with effects of uronamides at the 5'-position and a 3-iodobenzyl group at the $N^6$-position. The substituents –Cl, -NHCH$_3$, -SCH$_3$, -OCH$_3$ at 2-position favoured $A_3$ receptors.

$5'$-(Alkythio)substituted analogues of $N^6$-benzyl and $N^6$-(3-iodobenzyl)adenosine have been synthesized recently and evaluated for their affinities at adenosine receptor subtypes. The compounds proved to be selective for the adenosine $A_3$ receptor and displayed affinities in nanomolar range. The compound 37 has
displayed the highest affinity for the A<sub>3</sub> receptor with Ki value 27.7nM. The compound LUF 5403 (N<sup>6</sup>-benzyl with 5'-methylthio analog, 38) maintained a reasonable affinity and had a high selectivity for the A<sub>3</sub> receptor whereas LUF 5411 (N<sup>6</sup>-iodobenzyl with 5'-n-propylthio, 39) had the highest affinity and highest selectivity for the A<sub>3</sub> receptor with Ki value 44.3nM. Thus, partial agonists have been obtained with N<sup>6</sup>,5'-disubstituted adenosine derivatives<sup>97</sup>.

\[ \text{37, } R=H, \text{ } R_1=CH_3 \]
\[ \text{39, } R=I, \text{ } R_1=n-C_3H_7 \]

A 3'-deoxy analogue of a highly A<sub>3</sub>-selective adenosine derivative (40) retained selectivity in binding with receptor. It showed a full agonist property by inhibiting the adenylcyclase mediated via cloned rat A<sub>3</sub> receptors expressed in Chinese hamster ovary cells. This compound was reported to be an agonist at A<sub>1</sub> receptors, a low - efficacy agonist at A<sub>2a</sub> receptors, and an antagonist at A<sub>2b</sub> receptors. The 3'-OH and 4'-CH<sub>2</sub>OH groups of adenosine are not required for activation at A<sub>3</sub> receptors. A number of 2',3'-dideoxy and 9-acyclic substituted adenosines appear to inhibit adenyl cyclase at the allosteric "P site"<sup>68</sup>. There is evidence that at A<sub>1</sub> receptors 2',3'-dideoxy adenosines and other truncated ribose analogues act as antagonists or partial agonists<sup>68,99,100,101</sup>. A tetrahydrofuran derivative (41) had a Ki value of 3.5 μM at A<sub>3</sub> receptors and 4.1 μM at A<sub>2a</sub> receptor<sup>98</sup>.  

32
Adenosine derivatives bearing N$_6$-(3-iodobenzyl) group is reported to enhance the affinity of adenosine-5'-uronamide analogs (42) as agonists at A$_3$ adenosine receptors$^{102}$.

IB-MECA (43) is a highly potent A$_3$ agonist and 50 fold selective for A$_3$ vs either A$_1$ or A$_2$ receptors$^{103}$ Modification at 5'-methyluronamide and the N$_6$-benzylgroup, either alone or in combination increases the affinity in binding to A$_3$ receptors related to A$_1$ and A$_{2a}$ receptors$^{104}$.
1.10. Adenosine Receptor Antagonists With Structure Approach:

Most of the reported adenosine receptor antagonists are analogs of caffeine (44) and theophylline (45), i.e. xanthine derivatives. Modifications at the N-1, N-3, N-7 and C-8 positions of xanthines have resulted in better selective antagonist. At the 2-position, replacement of oxygen with sulfur is tolerated for adenosine receptors, even for 8-substituted analogs\(^{105}\)

Besides these xanthine antagonists, a number of non-xanthine adenosine antagonists\(^{106,107}\), including triazoloquinazolines, e.g., CGS 15943 (46)\(^{108}\), 9-methylaolenines (47)\(^{109}\) pyrazolotriazolopyrimidines, SCH 58261 (48)\(^{110}\), triazolotriazines ZM 241385 (49)\(^{111}\) have been reported but are not selective.
Recently, a novel class of non-nitrogen containing heterocycles, the tetrahydrobenzothiophenes has been reported as antagonists of adenosine receptors in micromolar range, e.g., Ethyl-3-(benzylthio)-4-oxo-4,5,6,7-tetrahydrobenzo[e] thiophene-1-carboxylate (BTH₄, 50). Its 1-methylpropylthioether derivative was 29 fold more selective for A₁ vs A₂a receptors. Earlier, there were only two reports of non-nitrogen containing natural products, the protein tyrosine kinase inhibitor genistein and benzofurancarbaldehyde derivatives.

Among the xanthine derivatives, theophylline (45) is the most potent naturally occurring antagonist at adenosine receptors. Caffeine (44) is significantly less potent. However, its metabolite paraxanthine is nearly as potent as theophylline. These are non-selective for adenosine subtypes. Theobromine (51) considerably
less potent is somewhat $A_1$ selective. The 8-chlorotheophylline (52) is 5-fold more potent antagonist than the parent caffeine.

Enprofylline (53) is a more potent smooth muscle relaxant and antiasthmatic drug than theophylline but with fewer side effects$^{115}$ This is due to its inactivity as an adenosine antagonist and at higher plasma concentrations it acts as competitive antagonists at both $A_1$ and $A_2$ receptors$^{116,117}$.

3-Isobutyl-1-methylxanthine (IBMX, 54) is a potent phosphodiesterase inhibitor and is a non-selective competitive antagonist at adenosine receptors$^{116}$

Certain N-alkyl modifications in a series of caffeine analogs have resulted in slight $A_2$ selectivity$^{13}$
Replacement of the methyl group at the N-1 position of xanthine (by n-propyl, allyl or propargyl groups) or N-7 position (by allyl, or propargyl groups) increases $A_2$ selectivity between 3-10 fold.$^{118}$

Modifications of more than one N-alkyl residue of the caffeine molecule not only has failed to enhance selectivity by additively, but also in some cases eliminated the $A_2$ selectivity.

Affinity to $A_1$ receptors was enhanced by homologation of 1,3-dialkyl substituents of theophylline.$^{119}$ 1,3-Diethylxanthine (55) and 1,3-dipropylxanthine (56) analogs were more potent than theophylline at $A_1$ receptors, but do not increase $A_2$ receptor affinity.

There is an apparent size limitation for Alkyl groups at the 1- and 3-positions, particularly at $A_2$ receptors. Thus, 1-isoamyl-3-isobutylxanthine (57) is inactive at central $A_2$ receptors, and is equipotent to theophylline at brain $A_2$ adenosine receptors. However, substitution of the 1,3-dipropyl groups with 1,3-diethyl groups retained $A_{2b}$ receptor selectivity while 1,3-di(cyclohexylmethyl) groups greatly reduced affinity at adenosine receptors.$^{120}$ Such $A_{2b}$ receptor antagonists have therapeutic potential as antiasthamtic agents. The structure activity relationships of 8-phenyl-1,3-di-(n-propyl)xanthine derivatives in binding to recombinant human $A_{2b}$ adenosine receptors in HEK-293 cells and at other adenosine receptor subtypes were explored.$^{120}$ The compound 58 was 400, 245 and 123 fold selective for $A_{2b}$ receptors versus human $A_1$/$A_{2a}$/$A_3$ receptors, respectively.
Considerable substitution at C-8 position is possible without loss of competitive binding at adenosine receptors.

8-Alkyl substitutions are better tolerated than 8-halosubstituted theophylline\textsuperscript{122} 8-cyclopentyl theophylline (CPT, 59)\textsuperscript{11} and the 1,3-dipropyl analog (DPCPX, 60)\textsuperscript{122,123} are highly A\textsubscript{1} selective. The A\textsubscript{1} receptor-selective antagonist DPCPX (60) has been evaluated in guinea pig smooth muscle and cardiac tissues, and shows high affinity in the cardiac tissues but not in the smooth muscles\textsuperscript{124} Recent reports have demonstrated that 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) and related xanthines, which are potent and selective adenosine receptor A\textsubscript{1} antagonists, have diuretic and saluretic properties\textsuperscript{125}.
The inclusion of unsaturation, as in (61) or halogen groups on the cyclopentyl ring reduces the potency in A1 receptor binding\textsuperscript{126}.

![Image of molecule 61](image.png)

Various 8-aryl substituents greatly increase the potency of 1,3-dialkyl xanthines, and substitution on 8-phenyl moiety (62) is well tolerated at the receptors\textsuperscript{127,128}.

![Image of molecule 62](image.png)

Effects of substitution at 1,3 and 7 positions and on the 8-phenyl substituent are not independent. 8-Phenyl caffeine is only slightly more potent than caffeine at both A\textsubscript{1} and A\textsubscript{2} receptors \textsuperscript{129}.

1,3-Dipropylxanthines having different hydrophobic moieties at C-8-position (63, 64, 65) increase the A\textsubscript{2} selectivity \textsuperscript{130}. Recent reports have demonstrated that 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX) and related xanthines, which are potent and selective adenosine receptor A\textsubscript{1} antagonist, have diuretic and saliuretic properties\textsuperscript{125}.

1,3-Dipropyl-8-phenyltheophylline (63) is 1100 times more potent than theophylline at A\textsubscript{1} receptors and 47 times more potent than A\textsubscript{2} receptors \textsuperscript{127}.

1,3-Dipropyl-8-(2-amino-4-chlorophenyl) xanthine (PACX, 66) is a ligand having a very high potency at A\textsubscript{1} receptor combined with a 37 fold margin of selectivity for A\textsubscript{1} receptors \textsuperscript{11}.
High $A_1$ potency of 8-phenylxanthines is enhanced by the presence of electron donating substituents in orthoposition than the para position$^{13,131}$

A potent analog reported in the sulfonamide series is as 8-(4-N-(3-dimethylamino propyl)sulfanamido)phenyl-1,3-dipropyl xanthine (PD 113297, 67)$^{11}$. Bristol and Badger$^{132}$ have patented a series of these derivatives.

A change from methyl to propyl on the 3-position of 8-phenyltheophylline gave a 7-fold increase in affinity for the $A_2$ site, but a similar changes in 1-position caused no increase in $A_2$ affinity$^{133}$. Introduction of a quaternary carbon and the conformationally restricted cyclopentyl moiety into the 8-position of xanthines enhanced the adenosine $A_1$ antagonism$^{109}$.
1,3-Dipropyl-8(3-noradamantyl)xanthine (KW 3902, 68) was identified to be a selective and the most potent A₁ receptor antagonist reported to date\textsuperscript{134}

7,8-Dihydro-8-ethyl-2-(3-noradamantyl)-4-propyl-1H-imidazo[2,1-j]purin-5-(6H)-one (69), a non-xanthine type heterocycle a derivative of KW-3902, showed greater water solubility than recently reported KFM-19 (70) A₁ antagonist\textsuperscript{135}

8-Cycloalkyl increases affinity of caffeine and 1,3-dipropyl-7-methylxanthine at the A₂ receptor\textsuperscript{129}

7-Methyl substitution did not alter the affinity at A₁ and A₂ receptors in 8-(2-phenylethyl), (E)-styril and (E)-cinnamylxanthines\textsuperscript{130}

Introduction of the (E)-3,4-dimethoxy styril (KW-6002, 71) or (E)-3,4,5-trimethoxy styril group (72) into the 8-position of 1,3-dialkyl-7-methylxanthines enhanced the A₂ antagonism\textsuperscript{136}
An 8-(p-(trifluromethyl)phenyl)substituent (73) increases the affinity of 1,3-disubstituted 8-phenylxanthines at A\textsubscript{2a} adenosine receptors, with little effect on affinity at A\textsubscript{1} adenosine receptors. 8-(Trifluromethyl) substitution (74) markedly reduces the affinity at both A\textsubscript{2a} and A\textsubscript{1} adenosine receptors\textsuperscript{137}.

(R)-Enantiomers of several 8-substituted-1,3-dipropylxanthines are significantly more potent than the corresponding S-enantiomers. The most potent compound at A\textsubscript{1} receptor was (R)-3,7-dihydro-8-(1-methyl-2-phenylethyl)-1,3-dipropyl-1H-purine-2,6-dione, MDL 102, 503 (75) and a more selective compound was (R)-3,7-dihydro-8-(1-phenylpropyl)-1,3-dipropyl-1H-purine-2,6-dione MDL 102,234 (76)\textsuperscript{138}.
A series of derivatives of 7-deazapurines with varying substituents in the 2-, 6-, and 9-position was synthesized in an attempt to improve the adenosine receptor affinity and $A_1$ or $A_2$ selectivity. The results indicate that 7-dezahypoxanthines have a potential for $A_2$ selectivity, while all 7-deazaadenines are $A_1$ selective. Introduction of a phenyl residue in the 2-position of 7-deazaadenines increases $A_1$ activity tremendously. 2-(p-Chlorophenyl)-7,8-dimethyl-9-phenyl-7-deazaadenine (77) is potent and specific for $A_1$ receptors of rat brain with $Ki$ value 122nM having no affinity for the $A_2$ receptors of rat striatum.

(R)-7,8-Dimethyl-2-phenyl-9-(1-phenylethyl)-7-deazaadenine (78) is highly $A_1$ selective (790 fold) and is 30-35 times more potent at $A_1$ receptors than its $S$-enantiomer. Chloro substitution of the 2-phenyl ring appeared to improve solubility as well as the solubility over $A_1$ affinity ratio of 9-phenyl 7-deazaadenines.
1,3-Dimethyl (79) and 1,3-dipropyl-8-azaxanthines (80) substituted at N^8 or N^7 position usually increase the affinity of xanthines for the adenosine receptors^{140}

Introduction of methyl group at 8-position of 8-azatheophylline restored the antagonist activity at A_2 receptors, while 8-cyclohexyl moiety increased affinity at the both receptors^{140}

The 7-cyclopentyl-1,3-dipropyl-8-azatheophylline (81) appears to be one of the most potent and selective among 7-alkyl-substituted xanthines^{140}

Recently, imidazodiazepine diones (82) have been reported as a new class of adenosine receptor antagonists, with low activity at adenosine receptors than the
corresponding xanthines, due to the lack of planarity for the fused rings of the imidazodiazepinediones\textsuperscript{141}

\[
\text{82}
\]

9-Methyladenine (83)\textsuperscript{142} were shown to be adenosine antagonists and a series of $N_9$-alkyl derivatives were shown to be adenosine antagonists

\[
\text{83}
\]

$N^6$-Substituted 9-methyl adenosines (84-88)\textsuperscript{109} are potent antagonists of the activation of $A_1$ adenosine receptors. An $N^6$-cyclopentyl substituent increased the potency at the $A_1$ receptor and decreased potency at the $A_2$ receptor; the potency varies directly with the hydrophobicity of the substituent (cyclopentyl $>$ phenyl $>$ tetrahydrohexyl $>$ ethyl $>$ methyl $>$ 2-hydroxy ethyl)
A pyrazolopyrimidine derivative DJB-KK (89) containing thio substituent is more potent than theophylline as an antagonist at A<sub>1</sub> and A<sub>2</sub> adenosine receptors.

![Chemical Structure of DJB-KK (89)](image)

A (E)-(2R)-1-[3-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)acryloyl]-2-piperidineethanol (FK 453, 90), a novel non-xanthine adenosine A<sub>1</sub> receptor antagonist as a diuretic and renal vasodilator in several animal species including humans has been described. The oral bioavailability was poor due to rapid first-pass effect in the liver and due to poor solubility in water. The drug was modified in which heterocyclic groups like pyridazinones were introduced instead of the acryloyl moiety of FK-453. Pyridazinones (91 & 92) has c logP values of 1.95 and 2.48 with potent diuretic activity and were actually soluble in water as sodium salts at a dose level of 3.2 and 0.1 mg/Kg respectively. The compound FK-838 (92) was 10 times more potent than 91. These derivatives are highly selective A<sub>1</sub>-receptor antagonists useful for hypertension and renal failure. The compound 6-oxo-3-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)-1(6H)-pyridazinedibutanoic acid (FK-838) is in phase 2 clinical trials.

![Chemical Structures of FK-453 (90), FK-838 (92)](image)

90, R= -(CH<sub>2</sub>)<sub>2</sub>COOH, 91, R= -(CH<sub>2</sub>)<sub>3</sub>COOH, 92, R= -(CH<sub>2</sub>)<sub>3</sub>COOH
Alloxaxine (93), a nitrogen tricyclic (benzo\[g\]\)pteridino-2,4-dione) is more potent than theophylline in competitive inhibition of binding to both \(A_1\) and \(A_2\) adenosine receptors\(^{67}\)

\[
\text{\begin{center}
\includegraphics[width=0.3\textwidth]{alloxaxine.png}
\end{center}}
\]

\[93\]

A phenyl-substituted pyrazoloquinoline derivative (CGS 8216, 94) acted as a potent benzodiazepine antagonist and as a moderately potent adenosine antagonist\(^{144}\)

\[
\text{\begin{center}
\includegraphics[width=0.3\textwidth]{phenylsubstituted.png}
\end{center}}
\]

\[94\]

The imidazopyridine derivative (95) and related analogs act as adenosine antagonists\(^{107}\)

\[
\text{\begin{center}
\includegraphics[width=0.3\textwidth]{imidazopyridine.png}
\end{center}}
\]

\[95\]

The 4-aminopyrazolo[3,4-\(d\)]pyrimidine, APPP (96) is a potent nonselective adenosine antagonist of low water solubility

\[
\text{\begin{center}
\includegraphics[width=0.3\textwidth]{4aminopyrazolo.png}
\end{center}}
\]

\[96\]

\(A_2\alpha\) adenosine receptors have been proposed as novel therapeutics for Parkinson’s disease and may also be active as cognition enhancer, neuroprotective, antiallergic and analgesics
A number of xanthines have been developed as $A_{2a}$ adenosine receptor antagonists. A major problem of all high affinity $A_{2a}$ antagonists has been their low water solubility, which limits their usefulness especially for their in vivo studies.

Recently, water-soluble phosphate prodrugs (97 & 98) of 1-propragyl-8-styrylxanthine derivatives (99 and 100) have been reported as $A_{2a}$ selective adenosine receptor antagonists\textsuperscript{145}. The prodrugs were stable in aqueous solutions (pH 7) and were readily cleaved to liberate the active prodrug.
The classical antagonists known for the adenosine A_1 and A_2a receptors are xanthine derivatives, but at adenosine A_3 receptors, these compounds have shown rather low affinities and optimisation has not led to truly selective ligands\textsuperscript{146}. Recently, non-xanthine structures with high affinity at the adenosine A_3 receptor have been reported\textsuperscript{147,148,149,150}.

4-(Phenylethynyl)-6-phenyl-1,4-dihydropyridine derivatives (101) are selective antagonists at human A_3 adenosine receptors, with K_i values in radioligand binding assay Vs [\textsuperscript{125}] AB-MECA in the submicromolar range have been reported recently\textsuperscript{48}.

Synthesis and A_1, A_2 & A_3 receptor binding activities of some novel 4-Amino-6-benzylamino-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3-a]quinoxalin-1-one (102 & 103) bearing different substituents on the 2-phenyl and/or 4-amino moiety have been reported recently\textsuperscript{151}.

Earlier, 4-Amino-6-benzylamino-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3-a]quinoxalin-1-one (104) has been found to be potent selective A_2a vs A_1 antagonist while its 6-
desbenzyl derivative was two times more selective A₁ vs A₂a antagonist. A series of triazolo[3,4-a]quinoxalins bearing different substituents on the 2-phenyl and 4-amino groups of the parent (102 & 103) have been synthesized and screened for activity at adenosine receptor subtypes A₁, A₂a & A₃. Radio ligand binding assays at bovine A₁ & A₂a and cloned human A₃ adenosine receptor have allowed elucidation of the structural requirements for binding of these novel tricyclic derivatives at each receptor subtype. The SAR of these triazoloquinazolines has been studied in detail151.

102 R= -H
104 R=-CH₂C₆H₅

:\[ \text{Diagram of compounds 102 and 103} \]