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Review

The purines: Potent and versatile small molecule inhibitors and modulators of key biological targets

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Abstract—The goal of this review is to highlight the wide range of biological activities displayed by purines, with particular emphasis on new purine-based agents which find potential application as chemical-biology tools and/or therapeutic agents. The expanding interest in the biological properties of polyfunctionalized purine derivatives issues, in large part, from the development of rapid high-throughput screening essays for new protein targets, and the corresponding development of efficient synthetic methodology adapted to the construction of highly diverse purine libraries. Purine-based compounds have found new applications as inducers of interferon and lineage-committed cell dedifferentiation, agonists and antagonists of adenosine receptors, ligands of corticotropin-releasing hormone receptors, and as inhibitors of HSP90, Src kinase, p38α MAP kinase, sulfotransferases, phosphodiesterases, and Cdks. The scope of application of purines in biology is most certainly far from being exhausted. Testing purine derivatives against the multitude of biological targets for which small molecule probes have not yet been found should thus be a natural reflex.

Contents

Keywords: Purine; Inhibitor; Receptor; Agonist; Antagonist; Therapeutic agents.
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1. Introduction

Emil Fischer, Nobel laureate in chemistry in 1902, attributed the name purine to the fused imidazo[4,5-*d*]pyrimidine compound **1** in 1884 and achieved its synthesis in 1898.[1] He further showed through a series of elegant transformations that the natural substances adenine, xanthine, caffeine, uric acid, and guanine correspond to different hydroxyl and amino derivatives of this fundamental system (Fig. 1).[2]

Purines bearing functionality at one or more of the seven peripheral atoms which make up its bicyclic structure can be readily synthesized by well-established routes from monocyclic precursors.[3] For instance, N-acylation of 'diaminopyrimidines' such as **2** and **5** followed by ring closure provides access to 2,8-disubstituted adenines **4** and theophyllines/xanthines **7**, respectively (Scheme 1). Alternatively, construction of the six-membered ring as in **9** can be achieved through reaction of 5-aminoimidazole-4-carboxylic acid derivative **8** with isocyanates or the corresponding amides with orthoformates. When higher acid ester (R_1CO_2Me) derivatives are employed, the R_1 substituent is introduced at C-2 in the purine product **10** (Scheme 2).

Polyfunctionalized purines **12** substituted at the 2-, 6-, and 8-positions are also obtained through the reaction of suitably activated purine intermediates **11** with heteroatom and carbon nucleophiles through S_NAr-type substitution and transition metal catalyzed coupling reactions.[4–15] N-alkylation/acylation, or Vorbrüggen[16–18] and other electrophile based reactions, can also be used to introduce functionality onto the nitrogen atoms in the purine ring. An example of this methodology is the highly selective N-9 alkylation of purines under

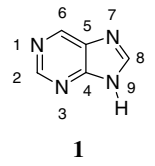

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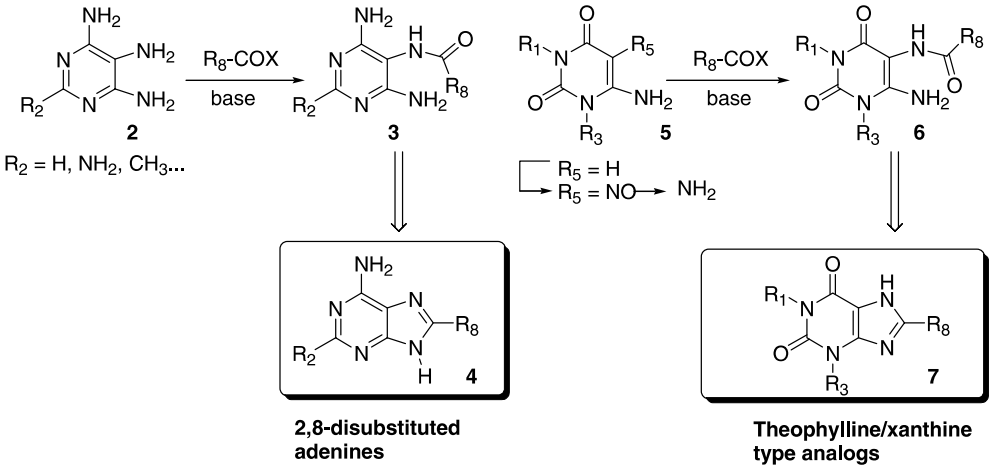
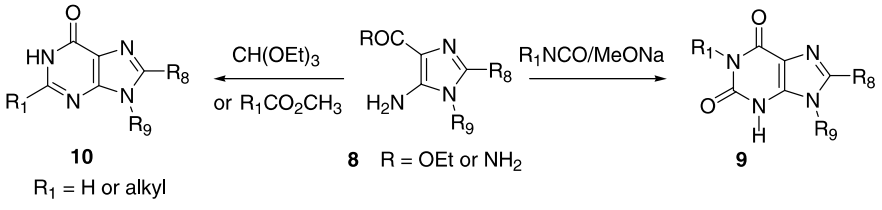
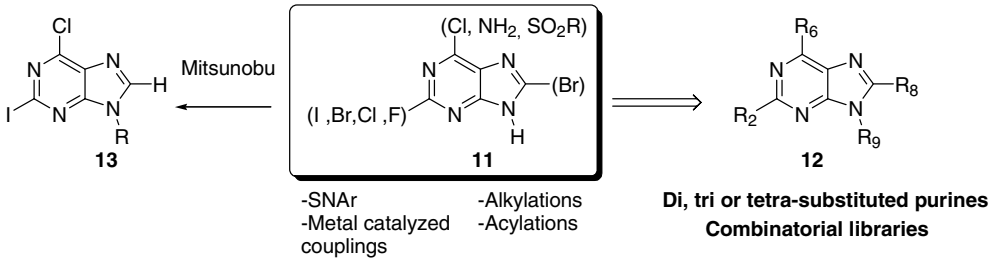
Figure 1.



Scheme 1.



Scheme 2.



Scheme 3.

Mitsunobu conditions (**11**–**13**) (Scheme 3).[11,15,19,20] These reactions have been incorporated into a number of strategies for the construction of both targeted and diversity driven compound libraries around the purine scaffold.[8–12,21] To illustrate this point, two solid-phase synthesis strategies to purine libraries which take into account the greater reactivity of the C-6 versus C-2 position in halo-substituted purines are presented in Scheme 4. In the first, diversity is introduced at the C-6 center through reaction of different benzylamino resins with intermediate **14**. The second approach is more flexible in that libraries of 2,6,9-trisubstituted purines are obtained from a single sulfur-linked purine resin **15**.[10,12]

As will be apparent in the following discussion, the screening of purine libraries against a wide variety of biological targets has contributed to opening new horizons for the development of purines as chemical-biology tools and hopefully as new therapeutic agents. Before entering into this discussion it should be recalled that a number of major purine-based drugs exist (Fig. 2) which find current application for the treatment of cancer (6-mercaptopurine **16**, thioguanine **17**),[22,23] systemic mastocytosis (2-chloro-2′-deoxy adenosine **18**),[22,24–26] viral infections such as Herpes and AIDS (acyclovir **19**, ganciclovir **20**, carbovir **21**, abacavir **22**, ddA **23**, and ddI **24**),[27,28] and organ rejection (azathioprine **25**).[29–32]

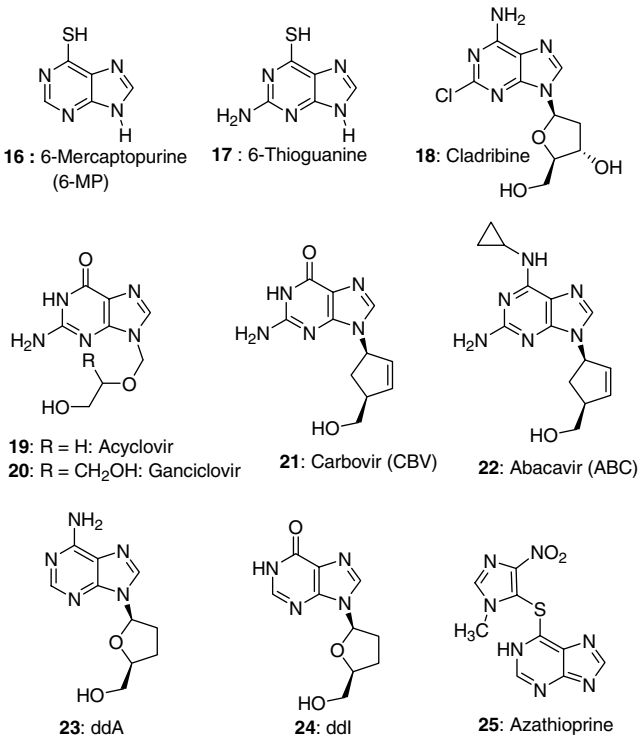
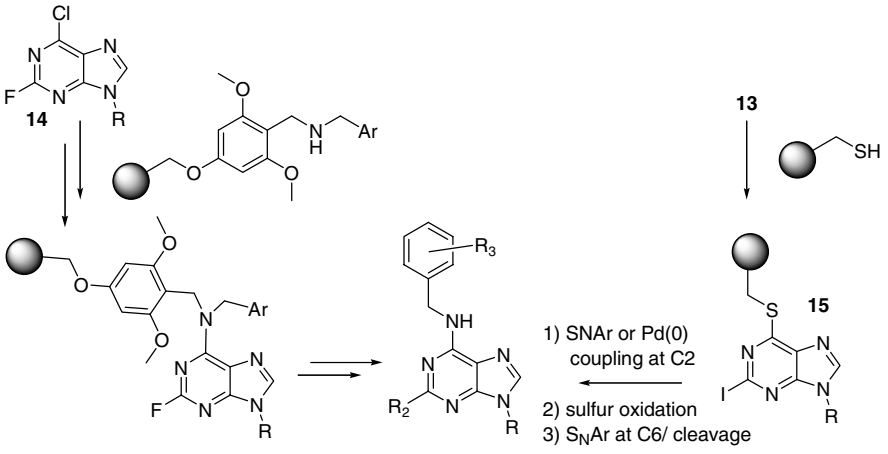


Figure 2.

These and other developments based on the purine scaffold have largely inspired and directed parallel developments in the chemistry and biology of related heterocyclic systems, including pyrrolo-pyrimidines **26**[33–37] and **27**,[38] pyrazolo-pyrimidines **28**,[39–41] **29**,[42] and **30**,[43,44] imidazo-pyridines **31**,[45,46] **32**,[47] and **33**,[48] triazolo-pyrimidines **34**,[49] triazolo-triazines **35**,[50,51] triazolo-pyrazines **36**,[52] pyrazolo-pyrazine **37**,[53] and imidazo-pyrazine **38** (Fig. 3).[54–57]

From the biological standpoint, interest in purines has been considerably reinforced by analysis of the human genome. It is estimated that approximately 4–7% of all proteins encoded by the genome, including in particular, GPCRs (G-protein-coupled receptors), depend on purine nucleotides (ATP, GTP, cAMP, cGMP, NAD, FAD, PAPS. . .) as co-factors or co-substrates for their function,[58,59] making these proteins a large family of potential therapeutic targets. Seven percent may seem like a relatively small number, but in terms of the percentage of the drugable genome, it is significant. Synthetic purine derivatives thus possess great potential to interfere with important cellular functions. A further interesting pharmacological property of purine derivatives and analogs is that they can be transported across biological membranes by nucleobase active and passive transport systems, which have been characterized in a variety of mammalian cells.[60]

Recently, naturally occuring purines have been reviewed by Rosemeyer.[61] The goal of the present review is to bring to the reader's attention the scope of biological



Scheme 4.

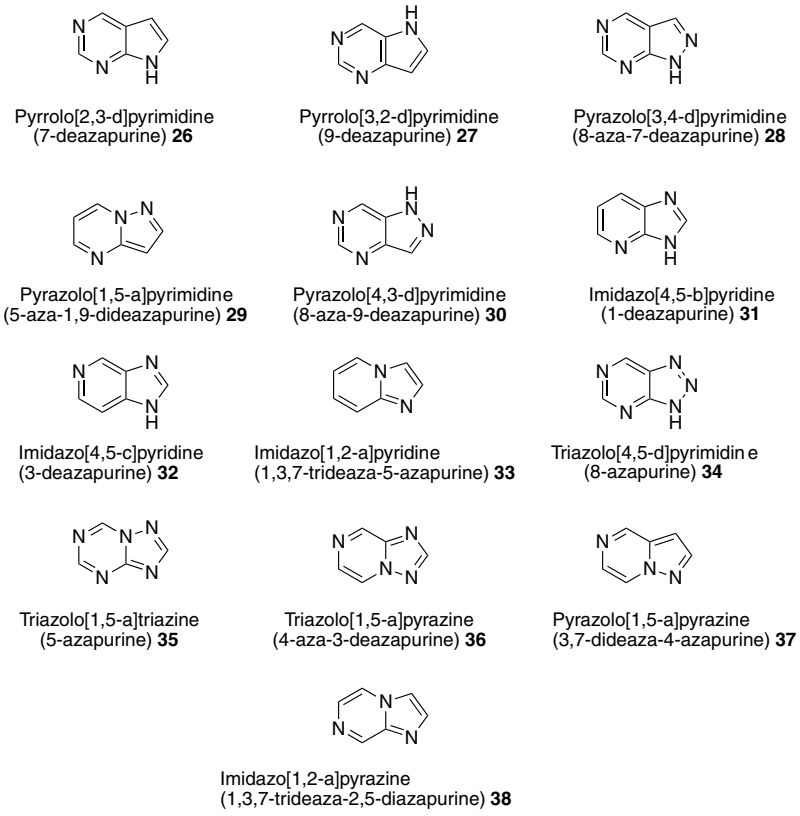


Figure 3.

activities displayed by synthetic purines, with particular attention devoted to new purine-based compounds reported during the past five years which have found potential therapeutic application as enzyme inhibitors and receptor agonists/antagonists.

2. Interferon inducers

Interferon (IFN) therapy for chronic hepatitis C is presently based on interferon-α alone or in combination with ribavirin. This treatment is associated with a loss of efficacy as a result of neutralizing antibodies against exogenous IFN. A new strategy under consideration for treating virus infections is to induce endogenous IFN synthesis with orally bioavailable small molecular-weight compounds such as imiquimod (Fig. 4).

This compound ultimately failed in clinical trials, but a new series of 2-substituted 8-hydroxyadenines has been identified, including the purine derivatives **39** and **40**, which are 100- and 30-fold, respectively, more potent than imiquimod and display excellent bioavailability.[62–64] The action mechanisms of these compounds are under investigation.

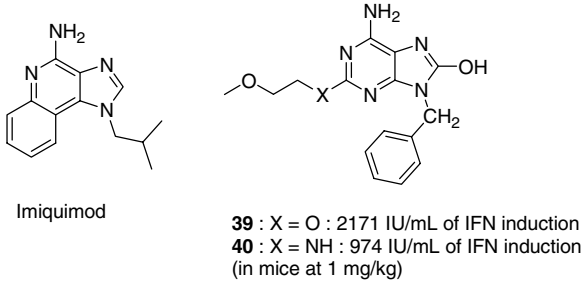


Figure 4.

3. Microtubule assembly inhibitors and reversal of differentiation

Schultz and collaborators have shown that myoseverin **41** is a potent microtubule-disassembling compound.[65] The low toxicity of myoseverin and analogs suggests that they might find use as cytostatic antitumor agents. Myoseverin was found to arrest the cell cycle at the G2/M transition by interfering with spindle assembly. The N9 cyclohexyl derivative **42** (myoseverin B) is an improved nontoxic inhibitor of microtubule assembly and is currently undergoing evaluation as an anticancer drug candidate.[65] In addition to being a purine-based microtubule inhibitor, myoseverin reverted terminal muscle-differentiated cells to a state that was responsive to environmental cues. Thus, it may find application in muscle regeneration and stem cell differentiation.[66]

In a subsequent in vitro screen of a 1561 compound library, 15 purine derivatives were identified that destabilized microtubules without targeting tubulin directly, resulting in small spindles. The potential target was NQO1, a NADP-dependent oxidoreductase which allowed the identification of a novel factor required for microtubule morphogenesis and cell division. The most potent compound diminutol **43** was studied in detail.

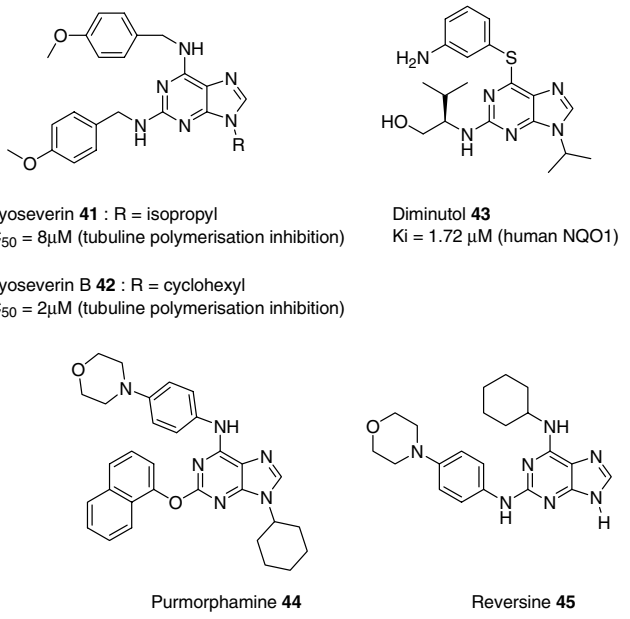


Figure 5.

It does not inhibit Cdk1, DNA replication or actin polymerization and seems to exhibit a specific effect on the microtubule cytoskeleton.[67] Interestingly, replacement of the C-6 thioether in **43** with an oxygen or a sulfonyl group resulted in a complete loss of activity (short microtubule induction).

By screening a much larger library of 100,000 small molecules, the Schultz group further discovered purmorphamine **44** and reversine **45**.

Purmorphamine,[68,69] a 2,6,9-trisubstituted purine, induces osteoblast differentiation of multipotent mesenchymal progenitor cells and lineage-committed preosteoblasts. Mesenchymal stem cells (MSC) are capable of differentiating into a number of cell lineages, such as bone, cartilage, adipose, muscle, stroma, and tendon, and play important roles in repair and regeneration. Reversine,[70] a 2,6-disubstituted-purine has the remarkable property of inducing myogenic lineage-committed cells to become multipotent mesenchymal progenitor cells which can proliferate and redifferentiate into bone (osteoblasts) and fat cells (adipocytes). So far, reversine is the first low-molecular-weight compound that can act as an external signal and induce lineage-committed mammalian cells to become multipotent progenitor cells. Although clinical application of small molecules like reversine is still speculative, such compounds are useful tools to understand dedifferentiation on a molecular level (Fig. 5).[71]

4. Inhibitors of Hsp90

Hsp90 is a chaperone required for the ATP-dependent refolding of denatured or unfolded proteins, and for the conformational maturation of oncogenic signaling proteins, including HER-2/ErbB2, Akt, Raf-1, Cdk4, cMet, Flt-3, Polo-1 kinase, Bcr-Abl, mutated p53...[72,73] Client proteins that are unchaperoned are ubiquitinated and subsequently degraded by the proteasome. As Hsp90 inhibition induces the proteasomal degradation of Hsp90 client proteins, Hsp90 represents a unique target for cancer therapeutics.[74,75]

Important in this context is the observation that the ansamysins geldanamycin and 17-AAG, the first Hsp90 inhibitors identified, were found to exhibit a 100-fold higher binding affinity for Hsp90 derived from tumor cells over Hsp90 from normal cells. Unlike the two ansamysins, which are only moderately active and insoluble, the purine derivatives PU3 **46** and Pu24FCl **47** are potent drug-like small molecule inhibitors of Hsp90.[76,77]

PU3 **46** was designed to fit into the HSP90 α-ADP/ATP-binding site, its 8-trimethoxyphenyl substituent reaching into the phosphate binding region. The 3D structure of the N-terminal domain of HSP90β-PU3 **46** complex was found to be similar to an earlier computer generated model, lacking only the interaction with Lys58 that geldanamycin was able to establish through the methoxy group on the benzoquinone ring.[78]

The synthesis of a series of 8-arylsulfanyl analogs of PU3 was more recently undertaken to study the influence of the aryl moiety on activity. The 8-arylsulfanyl adenine derivatives **48**, **49**, and **50** retained the activity of the methylene-linked compounds **46** and **47**. As the synthesis of these sulfanyl compounds was more amenable to analog production, extensive SAR studies were thus made. Compound **51** is the most potent and selective purine-based HSP90 inhibitor to date [IC_{50} = 30 nM, SI (tumor vs normal cells) = 730- to 3200-fold depending on the cell type] (Fig. 6).[73]

Cancer cells being particularly sensitive to HSP90 inhibition, inducing cell cycle arrest and apoptosis, the

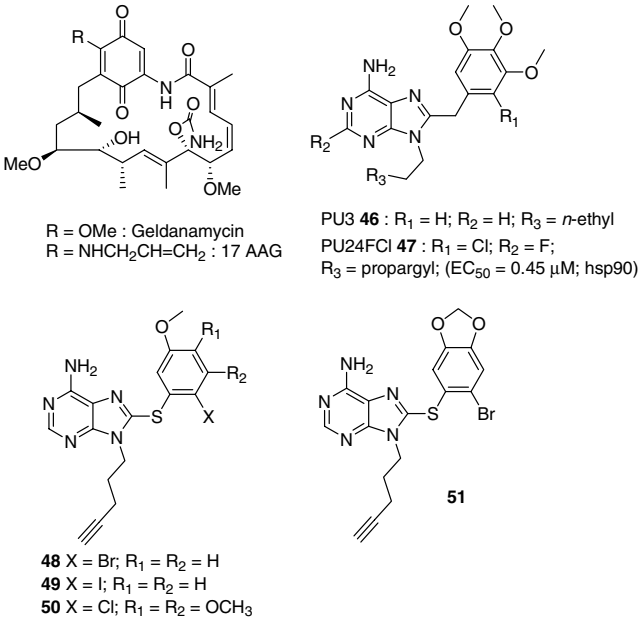


Figure 6.

development of improved synthetic inhibitors of HSP90 is an important objective.[79] A therapeutic advantage of HSP90 inhibitors may result from the simultaneous and combined effects on multiple oncogenic proteins that are responsible for tumor progression.

5. Antimycobacterial purines

Despite the availability of very potent antitubercular agents, tuberculosis remains of great concern due to the development of highly drug-resistant forms of the disease. Promising new agents have been identified through the screening of libraries of 6-thio-N9-substituted purines.[80,81]

In particular, (6-decylsulfanyl-purin-9-yl)-acetic acid ethyl ester **52** and the dodecyl derivative **53** exhibited MIC values of 1.56 and 0.78 μg/mL, respectively, against the Mtb $H_{37}Rv$ strain.[82] Compounds **54** and **55** were also found highly active against *M. tuberculosis* (MIC = 1.56 and 0.39 μg/mL, respectively) with low toxicity against mammalian cells. Their mechanism of action is not yet known.[83] Anti-mycobacterium tuberculosis activity has also been reported for Agelasine F (**56**), a 7,9-dialkylpurinium salt isolated from marine sponges (*Agelas* sp.). This activity was accentuated for analogs **57** and **58** obtained by synthesis (Fig. 7).[84]

6. Inhibitors of leukotriene A_4 hydrolase

Leukotriene A_4 hydrolase is a key enzyme in the biosynthesis of leukotriene B4 (LTB4).

This enzyme is a potent pro-inflammatory mediator implicated in the pathogenesis of a number of diseases, including inflammatory bowel disease, psoriasis, rheumatoid arthritis and asthma. In a recent study at Pharmacia, a series of imidazopyridines and imidazopyrimidines (purines) like **59** were tested and shown to exhibit improved activities over the known clinical candidates SC-57461A and SC-56938 (Fig. 8).[85]

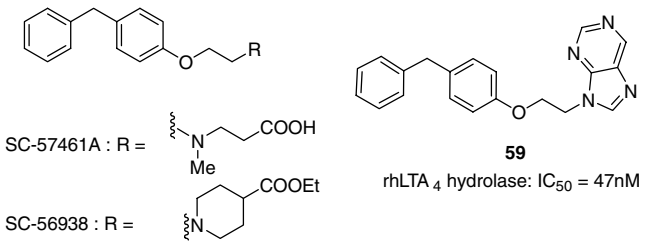


Figure 8.

7. Sulfotransferase inhibitors

Sulfotransferases catalyze the transfer of a sulfonate group from the universal sulfate donor PAPS (3′-phosphoadenosine-5′-phosphosulfate) to a hydroxy or amino group of an acceptor. This family of enzymes (10 members known to date) plays a role in molecular recognition, detoxification, hormone regulation, drug processing, and modulation of receptor binding. A primary screen of a purine library by Bertozzi and coworkers[86] revealed the 2,6,9-trisubstituted purine **60** to be an inhibitor of carbohydrate sulfotransferase NodH from *Rhizobium meliloti*. Although this molecule also displays activity against Cdk2 and p38/MAP kinase, it provided the incentive for the subsequent screening of a much larger 35,000 member library of purine and pyrimidine analogs against sulfotransferases (ST). This led to the discovery of the potent and selective estrogen sulfotransferase (EST) purine inhibitor NG38 **61**

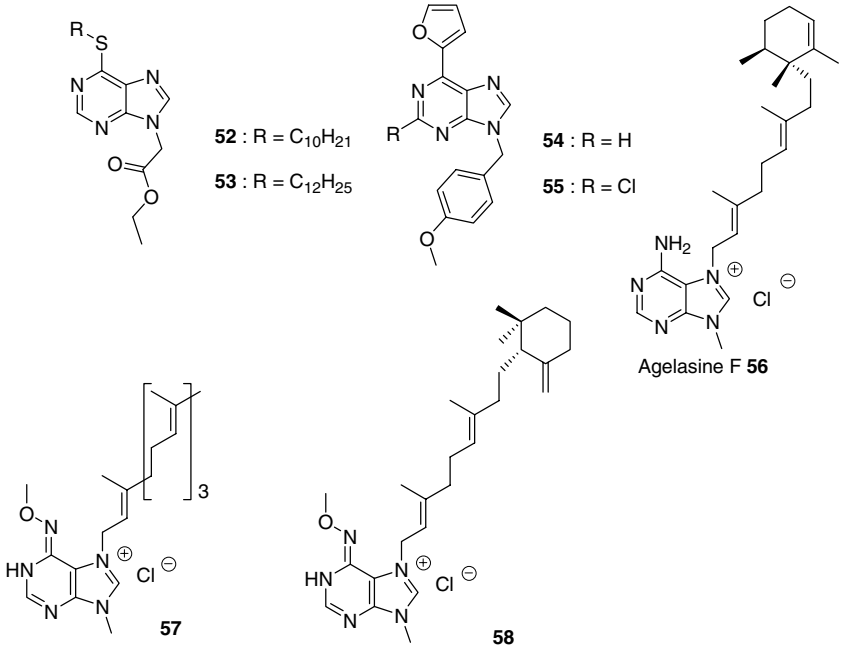


Figure 7.

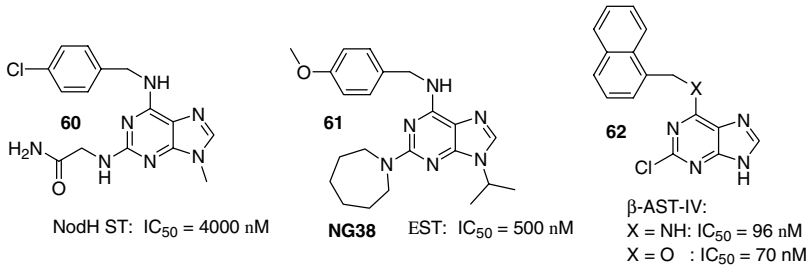


Figure 9.

(IC_{50} = 500 nM versus IC_{50} = 4000 nM against Cdk1),[87,88] and to (2-chloro-9*H*-purin-6-yl)-naphthalen-1-ylmethyl-amine **62**, a specific inhibitor of β-aryl-sulfotransferase-IV (β-AST-IV) (IC_{50} = 96 nM) (Fig. 9). Sulfotransferases are interesting tools for the study of cancer metastasis and potential targets for anti-inflammatory therapy.

8. Inhibitors of the cysteine protease cathepsin K

Cathepsin K (Cat K) is a cysteine protease that is highly expressed by osteoclast and bone tissue. The primary role of this enzyme is to effect type I collagen degradation, one of the main constituents of bone matrix. In a high-throughput screen of a purine library, researchers from Novartis identified several interesting hits, including compound **63**, a novel low nanomolar inhibitor of cathepsin K (Fig. 10). The potency and specificity of this 2,6,9-trisubstituted purine appears to be dependent on the nature of the substituents at the ortho position of the 6-anilino motif. Interest in these inhibitors results from the crucial role cathepsin K plays in bone matrix resorption.[89] Cat K represents a promising therapeutic target for the treatment of osteoporosis.[90]

9. Phosphodiesterase inhibitors

Phosphodiesterases (PDEs) hydrolyze the second messenger molecules cAMP and cGMP, that are themselves formed by adenylate and guanylate cyclases, respectively. These second messengers are involved in a variety of cellular responses to extracellular agents such as hormones and neurotransmitters. There are at least, 11 families of PDEs, some of which (PDE4, 7, and 8) are specific for cAMP and others (PDE5, 6, and 9) for cGMP.

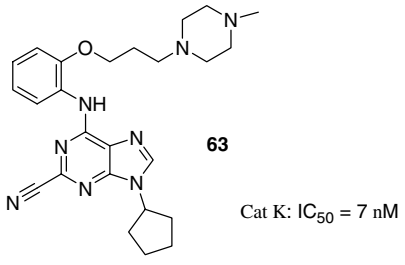


Figure 10.

Additional family members (PDE1-3, 10, and 11) have dual specificity. In addition, PDE subtypes occur within the different families, as, for example, the five subtypes PDE4A, PDE4B, PDE4D2, PDE4D3, and PDE4D5 for PDE4. A number of potent PDE4 inhibitors have been developed such as the 2-iodopurine **66**.[91] Recently the 3,8-disubstituted adenine compounds **64** and **65** were shown to be highly potent and selective inhibitors of PDE4B, PDE4D3, and PDE4D5.[92] PDE4 inhibitors[91] have demonstrated efficacy in models of dermatitis, rheumatoid arthritis, multiple sclerosis, autoimmune diseases, and various gastrointestinal and neurologic diseases.

The 1,2,3,7,8-pentasubstituted purines **67**, **68**, and **69** are specific nanomolar inhibitors of PDE5.[93,94] Some of these PDE5 inhibitors are equipotent to, and more selective than, the approved drugs Sildenafil, Vardenafil, and Tadalafil, such as **67** which showed good oral bioavailability in both rat and dog with oral efficacy in the anesthetized dog erectile function model.[95] Other 8-aryl xanthine derivatives such as **70** and **71** function as potent PDE5 inhibitors (Fig. 11).[96]

Aberrant regulation of cAMP levels is involved in asthma, inflammation, immuno-related disorders, allergy, and cancer. PDEs are thus connected to a wide range of physiological functions and continue to be a major target for drug development. Inhibitors of PDE7 have been suggested to have broad application for the treatment of lymphoid malignancies,[97] T-cell-dependent diseases such as asthma, rheumatoid arthritis,[98] and as immunosuppressants. 6-*para*-Sulfonamidylbenzylamino-substituted 9-ethylpurine **72** is a potent and specific inhibitor of PDE7 (PDE1/PDE7 = 200; PDE3/PDE7 > 5000)[99] but poorly soluble in water. Other PDE7 inhibitors are therefore needed for application as immunosuppressants and antiasthmatics.

Compound **73** is one of the most potent inhibitor of PDE1[94] known to date. Inhibition of cGMP hydrolysis by PDE1 and/or PDE5 has been shown to reduce blood pressure in hypertensive animals.[94]

Among the twenty crystal structures of PDEs available to date, it is interesting to note that the nonselective purine PDE inhibitor IBMX **74** has been co-crystallized with PDEs 3, 4, 5, and 9 (Fig. 12). Its ability to interact

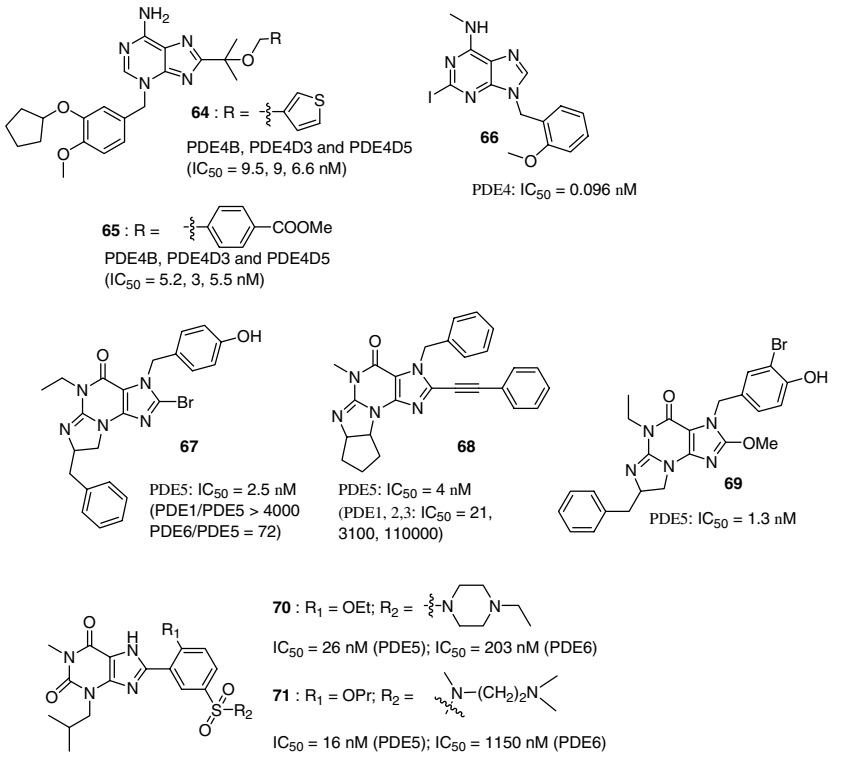


Figure 11.

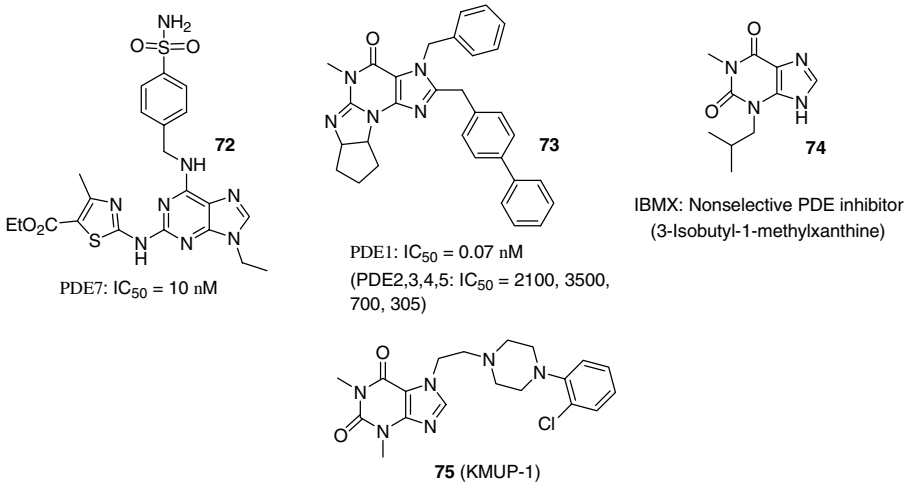


Figure 12.

with the binding site in more than one orientation has been suggested to contribute to its lack of selectivity. Reducing the number of binding orientations is considered important to the design of more selective inhibitors.[100]

It should also be noted that less specific PDE inhibitors are interesting tools to study the effects of simultaneous inhibition of several PDEs. For instance, the xanthine derivative KMUP-1 **75** has bronchodilator effects in guinea pig, which could be due to activation of the adenylyl cyclase pathway and also to inhibition of the PDE3 and 4 enzymes.[101]

10. Adenosine receptor ligands

Adenosine receptors (AR) belong to the superfamily of purine receptors which are currently subdivided into P_1 (adenosine) and P_2 (ATP, ADP, and other nucleotides) receptors. Four receptor subtypes for adenosine have been characterized. The subtypes A_1 and A_{2A} exhibit high affinity for adenosine, while the two other known subtypes A_{2B} an A_3 are low-affinity receptors. Activation of A_1 and A_3 receptors inhibits adenylate cyclase through G_i coupling, while activation of A_{2A} and A_{2B} receptors stimulates adenylate cyclase through G_s coupling, controlling intracellular cyclic AMP levels.

Adenosine receptor agonists are often closely related in structure to the natural ligand, adenosine. Thus, β-L-adenosine and α-D-adenosine exhibit virtually no affinity at A_1, A_{2A} or A_3 receptors. Modification of the 2′- or 3′-hydroxyls results in great loss of receptor binding affinity. Only modified adenosines such as for instance, **76**, **77** or **78**, obtained by substitution at N6 or C2 of the purine heterocycle or at the 5′ position of the ribose moiety, are potent and selective adenosine agonists.[102] Baraldi and co-workers[103] have described in 2004 the selective affinity of various *N*6-[4-(substituted)sulfonamidophenylcarbamoyl]adenosine-5′-uronamides including **79** and **80** toward the AR. Small alkyl groups increased the selectivity of the A_3 AR agonists (Fig. 13).

In contrast to agonists, adenosine receptor antagonists are more diverse in structure. They lack the ribose moiety which is essential for agonist activity. The first AR antagonists reported were the naturally occuring xanthines caffeine and theophylline. However, they exhibit weak affinity and subtype selectivity at ARs. Structure–activity relationships of AR agonists and antagonists were reviewed by Jacobson as early as 1992, and the basis of the structural modifications of adenosine and xanthine, as AR agonists and antagonists, respectively, was established.[104]

Substitutions at N1, N3, N7, and C8 of xanthine contribute to potency and selectivity at A_1 and A_2 receptors. In particular, N1 and N3 substitution (sometimes N7) combined with C8 substitution in xanthine has led to the development of the potent and selective A_1 and A_2 AR antagonists **81**–**83**, **86**, and **87** (Fig. 14).

Allosteric modulators of adenosine receptors (ARs) are of potential clinical use, and over the years a lot of effort has been made to find specific ligands of each AR subtype. Theophylline, a nonselective inhibitor of PDE and a nonselective AR antagonist has been used for the treatment of asthma.[105] In order to better define the role of the A_{2B} AR in asthma,[105] selective A_{2B} adenosine receptor antagonists such as **81** have been developed. In this perspective, the theophylline analog **82**, as well as the more metabolically stable analog **83**, have been synthesized.[105]

Antagonism of A_2 may produce a novel type of antidiabetic agent.[106] In this context, both highly potent A_{2A} receptor agonists and selective antagonists (**81**, **82**, and **83**) of human A_{2B} AR have been found.[107] Recently, the search for A_{2A} receptor antagonists[108] led to the discovery of the 8-styrylxanthine derivative KW-6002 (**86**).[109,110]

This compound exhibits antiparkinsonian activity in the parkinsonian monkey without producing hyperactivity and provoking dyskinesia.

The antiparkinsonian activity of KW-6002 is attributable to A_{2A} AR blockade, since an adenosine A_{2A} agonist reversed the antiparkinsonian actions of this drug.[111] These studies suggest that A_{2A} receptor antagonists also have strong potential to be a new class of antisymptomatic drugs for Parkinson's disease and other neurodegenerative disorders (Huntington's disease).[111,112]

Other nonxanthine antagonists of AR A_{2A} have also been found such as the 2,8,9-trisubstituted adenine ST1535 **92**, which is able to increase significantly the locomotor activity in rats after oral administration. A very potent and selective antagonist of A_3 adenosine receptor **87** has also been reported this year.[113] More interesting, the 6,8,9-trisubstituted purines **84** and **85** (Fig. 14) (i.e., molecules not bearing *N*-alkyl substituents) were found to be antagonists of adenosine A1 receptor.[114] The previously identified dedifferentiating

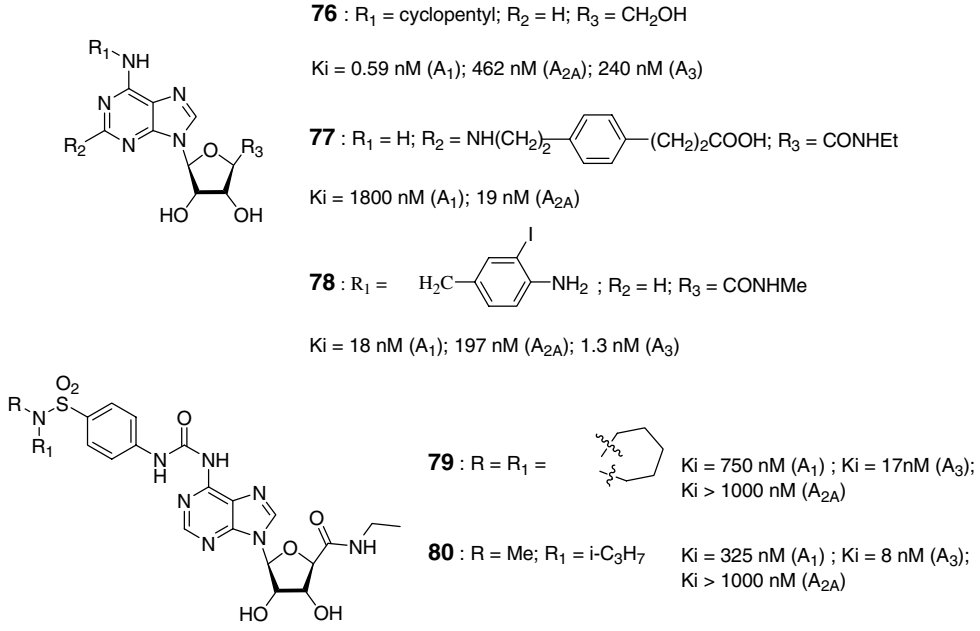


Figure 13.

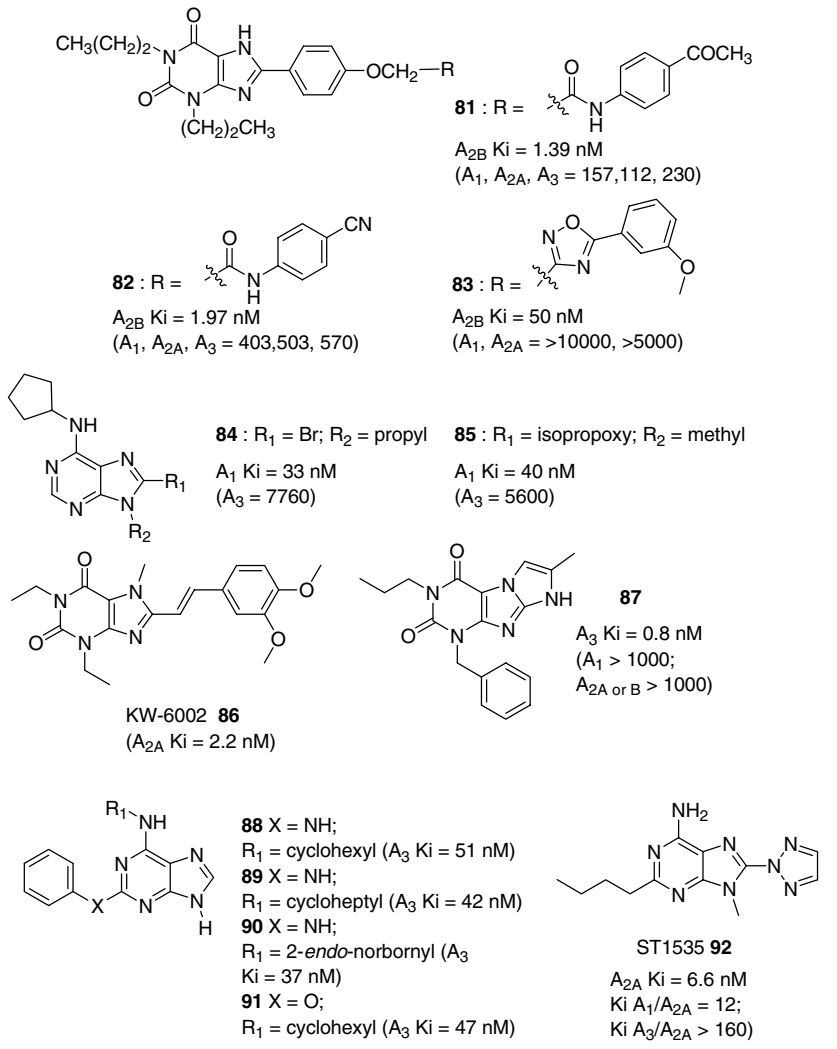


Figure 14.

agent reversine (**45**, Fig. 5), a 2,6-disubstituted purine, was also found to be a moderately potent antagonist of the human A_3 AR (K_i value of 0.66 μM). This led Jacobson and collaborators[115] to explore the SAR of related derivatives. The most selective compound **91** exhibited more than 200 fold selectivity toward A_3 relative to A1 and A_{2A} ARs. However, the authors were not able to demonstrate any connection between A_3 AR antagonist effects and myoblast dedifferentiation. Adenosine A_3 receptors are involved in several pathophysiological processes such as inflammation, cancer, and glaucoma.

11. Corticotropin-releasing hormone receptor (CRH-R1) modulators

Corticotropin-releasing hormone is a major modulator of the body's response to stress. Preclinical studies on CRF (corticotropin-releasing factor) agonists and antagonists suggested that abnormal CRF secretion or synthesis may underlie the pathologies of neuropsychiatric disorders (anxiety, depression, Parkinson's disease, Alzheimer's disease, alcohol withdrawal...).

Considerable progress toward the identification of non-peptide modulators of CRF function has been made over the last **10** years.[116] Optimization of the pharmacokinetic profiles of a series of pyrrolopyridines led to the discovery of 2,6,9-trisubstituted purine **93** and other 2,6,7,9-tetrasubstituted purinones (Fig. 15).[117] These small molecule CRF1 antagonists may be potential anxiolytic and antidepressant agents.

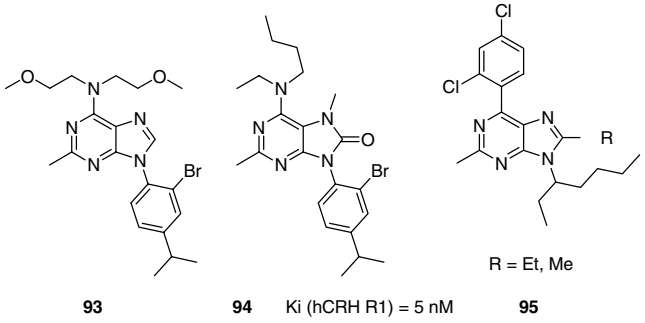


Figure 15.

12. Inhibition of nucleoside transport

Nucleoside transport proteins (NTPs) control the transport of hydrophilic nucleosides such as adenosine across the cell membrane. NTPs are subdivided into two families, the concentrative nucleoside transporters (CNT), which drive the nucleoside flow against the concentration gradient, and the equilibrative nucleoside transporters (ENT), which drive the nucleoside flow following their concentration gradient. The ENT are themselves divided into four subtypes labeled ENT1 to 4. ENT1 and ENT2 are either sensitive or insensitive to inhibition by NBTI **96** (Fig. 16).[118]

Unlike their mammalian host, most parasites lack the pathways for de novo purine biosynthesis and rely on salvage pathways to meet their purine demands. In addition, nucleoside transport in parasite-infected cells is insensitive to mammalian NTP inhibitors such as **96**.[119]

However, certain nucleoside transport inhibitors have been reported to exert antimalarial and antitoxoplasmosis effects on their own. The differences between parasites or parasite-infected host cells and uninfected host cells in sensitivity and chemotherapeutic efficacy of mammalian nucleoside transport inhibitors can be exploited for selective treatment against parasites or parasite-infected cells. Inhibition of ENT1 results in an increase of extracellular concentration of adenosine, and consequently to an occupancy of the adenosine A1 receptor through which adenosine exerts numerous physiological effects, such as counteracting pain. Inhibition of nucleoside transport proteins has been found as an important strategy for the treatment of ischemic heart diseases, stroke, and host tissue protection in chemotherapy.[120] However, the therapeutic application of NBTI **96** has been limited due to its poor pharmacological profile, its toxicity, and its lack of in vivo efficacy. Compounds **97**–**99** are inhibitors of ENT-1 with improved pharmacological properties. The presence of a bulky susbtituent at the C-8 position of the purine ring (**99**) increased the inhibitory effect significantly.[118,121]

13. Kinase inhibitors

13.1. Src tyrosine kinase inhibition

Src is a nonreceptor protein tyrosine kinase which is activated by specific cellular receptors and functions as an early transduction signal. Many different compound family types have been identified which competitively inhibit Src kinase activity through binding in the ATP site. However, much of the current effort in this area has been focused on finding bone targeted Src-kinase inhibitors, since Src has been implicated in regulating the function of mature osteoclasts. The purine type Src inhibitor NVP-AAK980 **100** has been reported to influence bone remodeling.[122] ARIAD pharmaceuticals has also been active in this domain, demonstrating that bisphosphonate compounds **101** and **102** target bone and are potent Src inhibitors (IC_{50} = 41 and 10 nM, respectively) (Fig. 17).[122,123]

The bisphosphonate motif is known to exhibit exceptional affinity for the inorganic component of bone, and its positioning on the *para*-position of the 6-anilino substituent in **101**/**102** places it on the easily accessible outside surface of the ATP-binding region. These compounds may find application against osteosarcoma and various bone-related disorders (osteoporosis).

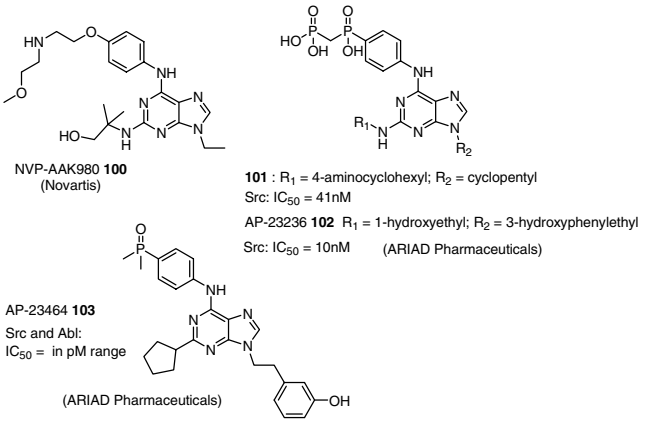


Figure 17.

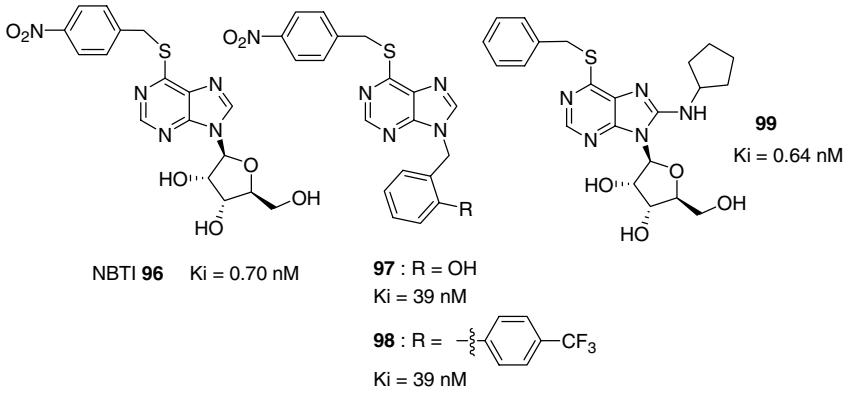


Figure 16.

The phosphine oxide AP-23464 **103**[124] is also an exciting new purine compound, in that it is a selective and highly potent (IC_{50} < 1 nM) inhibitor of both Src and Abl kinases, with specificity for these enzymes versus more than 40 others tested.[125,126] It thus has therapeutic potential for the treatment of Gleevec-resistant chronic myelogenous leukemia (CML).[124]Furthermore, AP23464 **103** was very recently shown to strongly inhibit the kinase activity of the D816V activation-loop mutant of c-kit, both in vitro (IC_{50} = 53 nM) and in vivo, and to induce cell-cycle arrest and apoptosis in cells expressing this mutation. This molecule is considered to be a promising candidate for clinical development for the treatment of systemic mastocytosis (SM) and acute myelogenous leukemia (AML).[127]

13.2. P38α MAP kinase inhibitors

More than a dozen MAP kinases have been cloned. The best characterized of these are extracellular-signal-regulated kinases (ERKs), the c-Jun N-terminal kinases (JNKs), and p38 (or CSBP2). Significant interest in the MAP kinases as drug targets arose from the discovery that specific inhibitors of p38 block the production of proinflammatory cytokines (TNF-α, Il-1).[128] This has important implications for the treatment of asthma and other inflammatory disorders such as rheumatoid arthritis and Crohn's disease.[129] Using database screening and structure-based design strategies GlaxoSmithKline chemists identified the novel p38α inhibitors **104** and **105** (IC_{50} = 82 and 16 nM, respectively) (Fig. 18) in which the C-6 anilino nitrogen is present as an urea. From the X-ray structure for the p38α-**104** complex, one sees that the crucial role of the carbamoyl motif is to capture the H-bonding contact with Met 109 which is important in the binding of the pyridinylimidazole type SB inhibitors in the ATP site (Fig. 18).[128]

An additional H-bond with the carbonyl oxygen of His 107 is observed for the purine inhibitors, mimicking the interaction of the C-6 NH_2 of ATP with the kinase. Binding of the C-2 *o*-fluorophenyl substituent into a hydrophobic pocket of the kinase was suggested to impart selectivity for p38α. Olomoucine **107**, the archetypical Cdk1, 2, and 5 inhibitor, cannot capture these contacts and is consequently inactive against P38α MAP kinase and only weakly active against the closely related MAP kinase ERK2.[128,130]

13.3. Inhibitors of Inositol-1,4,5-trisphosphate-3-kinase

To further demonstrate the usefulness of a purine library that was initially constructed to target cyclin-dependent kinases, Schultz and co-workers identified the 2,6-disubstituted purine **106** as one of several interesting new leads against IP3K (IC_{50} = 10–200 μM) (Fig. 19).[131] IP3K phosphorylates inositol 1,4,5-trisphosphate (IP3) to inositol 1,3,4,5-tetrakisphosphate (IP4) and plays an important role in the regulation of intracellular calcium concentration. Specific inhibitors of IP3K will be useful tools to study the role of this enzyme in transmembrane signal transduction and intracellular calcium metabolism.

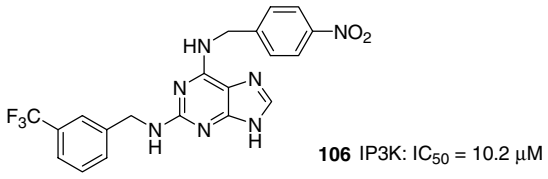
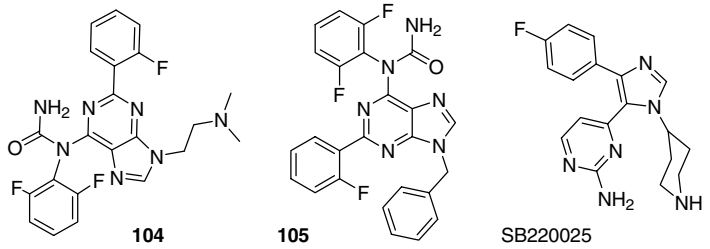
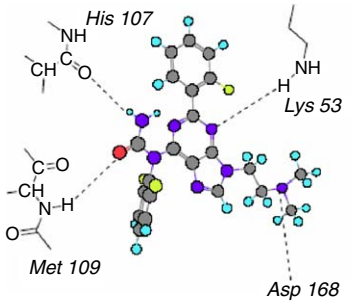
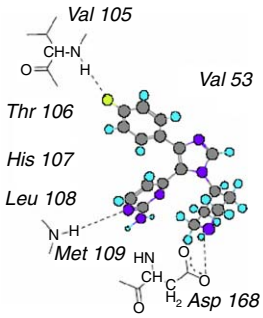


Figure 19.





p38α MAP kinase-purine **104** complex



p38α MAP kinase-SB220025 complex

Figure 18.

13.4. Cyclin-dependent kinase inhibitors

Cyclin-dependent kinases (Cdks) are Ser/Thr kinases which, in association with the requisite cyclin, play a key role as regulators of the different phases of the cell cycle. To date, 13 Cdks and 25 cyclins have been discovered and some of the substrates phosphorylated by the Cdks have been identified.[132,133] The cellular activity of Cdks is, itself, regulated by a number of mechanisms including phosphorylations by other kinases, dephosphorylations by phosphatases, and the binding of inhibitory peptides that belong to the $p16^{INK4A}$ and $p21^{CIP}$/$p27^{KIP}$ families.

These natural peptide inhibitors are frequently mutated or deleted in tumors. This observation, and others, has progressively given rise to the idea that Cdk inhibition by small molecules may be an effective strategy to treat cancer.[134,135]

The biological response to Cdk inhibitors is complex, but selective in tumor cells. Due to their intact G1/S checkpoints, normal cells are shown to be reversibly blocked by Cdk inhibitors in either the G1 phase or in the G2/M transition. In transformed cells, where these checkpoints are defective or absent, the presence of Cdk inhibitors leads these cells to apoptosis.[133,135–137]

The discovery of olomoucine[138] **107** and roscovitine **108**[139] triggered intense interest in the development of 2,6,9-trisubstituted purines as selective inhibitors of Cdk1, 2, and 5. Examples of optimized N-6 anilino/benzylamino inhibitors (**109-115**), discovered for the most part through screening of large libraries of 2,6,9-trisubstituted purines, are shown in Figure 20.[19,132,133,139–151]

Purvalanol A **109** is one of the most potent compounds in this group, but its pharmacological profile may be inferior to that for roscovitine (**108**).[152] Being more orally available, roscovitine (Seliciclib or CYC202) is currently in phase II clinical trials in combination with gemcitabine/cisplatin for nonsmall cell lung cancer and as a single agent for cell malignancies including multiple myeloma.[153]

Hoechst Marion Roussel[154] synthesized several hundred 2-[*trans*-(4-aminocyclohexylamino]-6,9-disubstituted purines and screened them against Cdks.

For example, compounds **119**–**123** are highly active inhibitors of Cdk2/cyclin E and generally exhibit 5–100 times lower activity against Cdk4/cyclin D1. Some of these compounds are characterized by the presence of acyclic chains on the 6-position nitrogen, rather than an anilino or benzylamino group, as in the aforementioned analogs **107**–**115** (Fig. 20). Albani Molecular Research[155] has also synthesized a sizable 2,6,9-trisubstituted purine library which contains molecules such as **124**–**127** (Fig. 21) which are moderate Cdk inhibitors, but very potent antiproliferative agents against a number of human transformed cell lines. Although these compounds retain substituents at C-2 and N-9 found in other highly active Cdk inhibitors, the incorporation of various bis-aryl or bis-heteroaryl substituents at

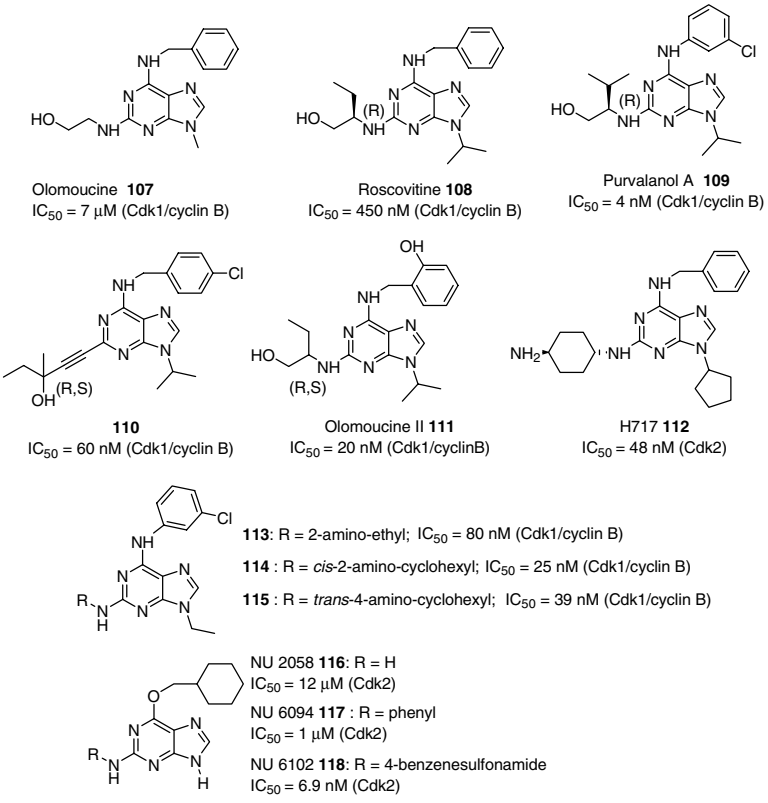


Figure 20.

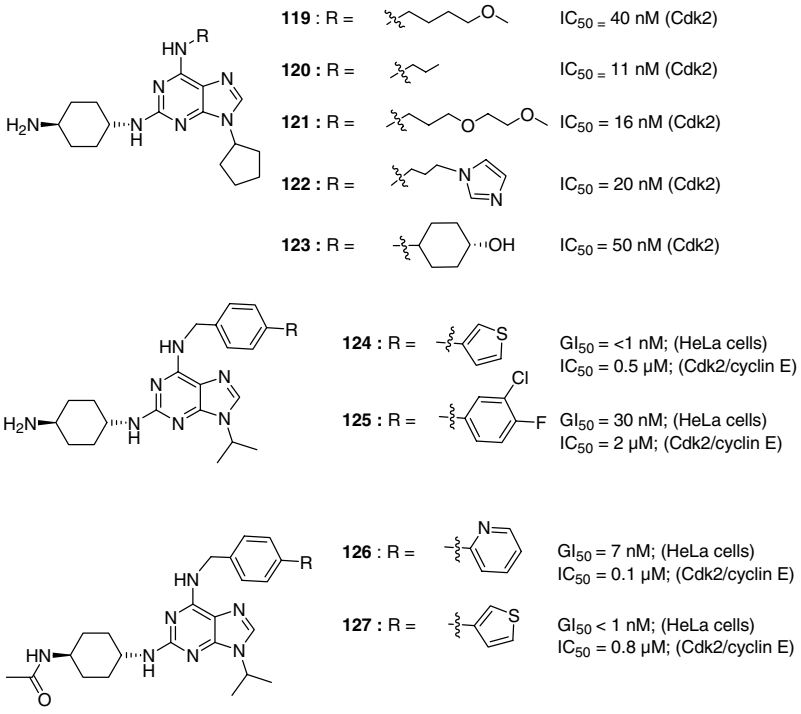


Figure 21.

position 6 appears to attenuate their activity. One can therefore ask whether the potent cytotoxic effect displayed by these purines against tumor cell lines is linked to their anti-Cdk activity. Further work on these compounds will most probably shed light on this question.

The co-crystal structure of olomoucine and roscovitine with Cdk2 revealed[139] that, in order to preserve important H-bonding contacts, the adenine ring in the inhibitors is oriented differently (i.e., rotated approximatively 160°) than the adenine motif in the ATP-Cdk2 complex. These structures also brought to light the existence of a hydrophobic pocket into which the aromatic ring of the 6-anilino/benzylamino substituent fits. This region is not used to bind ATP, but is accessible to many of the different families of Cdk inhibitors which have been developed.[150,156]

Recently, a new family of *O*-alkylguanine-based Cdk inhibitors was discovered which lack the seemingly crucial secondary amine substituent at C-6. From the crystal structure of the initial lead compound **116** (NU2058) with Cdk2 it was determined that it binds in the ATP site in yet another orientation.[149,151] As illustrated in Figure 22, roscovitine (**108**) forms H-bonds to Leu 83 via the C-6 nitrogen substituent and N-7.

In the NU series, this important donor–acceptor interaction is conserved by flipping the adenine ring such that the amino group at C-2 and the nitrogen N-3 interact with the backbone carbonyl and NH of Leu 83. Additional H-bonding interactions of the NU compounds with ATP-site residues involved N-9 and the backbone C=O of Glu 81 and N-7 with the side chain amino group of Lys 33. Optimization of the NU compounds led to the 2-anilino analog **117** and ultimately to the *para*-sulfonamidoaniline analog **118** which is a highly potent Cdk inhibitor. The presence of the *para*-sulfonamide group in this molecule permits two further H-bond interactions involving asp 86.

Cdks are considered to be prime targets for the development of selective inhibitors to treat cancer. They may also find application for the treatment of neurodegenerative disorders[157] (Alzheimer's and Parkinson diseases), stroke, ALS, cardiovascular diseases (restenosis), viral infections (HIV, Herpes, cytomegalovirus, and papillomavirus), and various parasites (*Plasmodium*, Trypanosomes, and *Leishmania*).[133] CYC202 has completed phase I trials and is also being explored for use in glomerulonephritis, an inflammatory disease associated with kidney cell proliferation. CYC 202 (roscovitine, **108)** is presently in phase II trial for lung cancer and hematological malignancies.[134]

14. The problem of the specificity of purine inhibitors

From the activities observed for the many purine-based inhibitors described in this review, it is clear that by modulating the position and the nature of the substituents on the purine ring it is possible to optimize potency and selectivity within a given enzyme family and with respect to other protein targets. To illustrate the latter point, C-8-substituted purines have been developed as HSP90 (**46**–**51**) and phosphodiesterase inhibitors (**64**, **65**, **68**, **70**, **71**, **73**) and as adenosine receptor antagonists (**81**–**83**, **86**, **92**). In contrast, kinase inhibitors bearing functionality at C-8 are currently unknown. Moravec

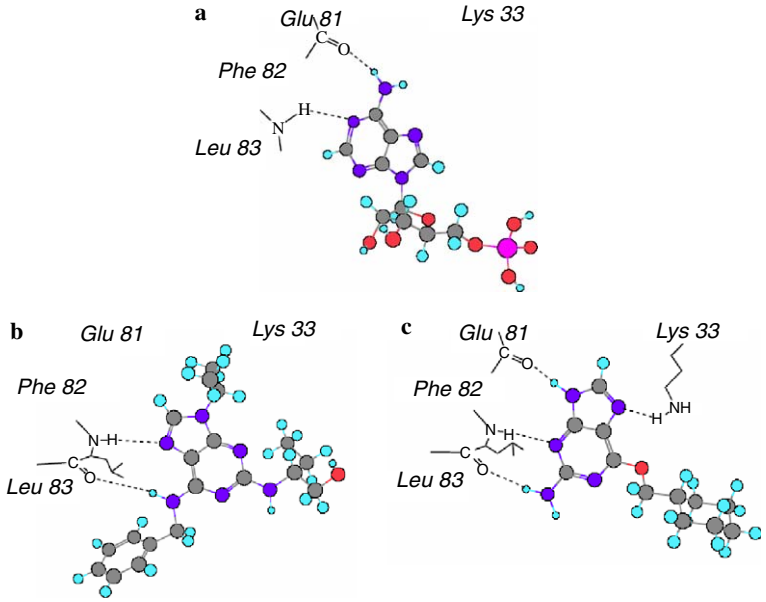


Figure 22. Schematic representation of (a) ATP; (b) roscovitine; and (c) NU2058 in the Cdk2 ATP binding pocket. Only Cdk2 residues Glu 81 to Leu 83 and Lys 33 are included for clarity. The orientation of the ATP-binding site remains constant.

et al. showed, for instance, that the presence of a substituent at C-8 decreases dramatically anti-Cdk activity.[158] Similarly, in a study to explore the capacity of purine derivatives with aryl motifs at different ring positions to bind in the ATP site of 15 selected kinases, only marginal activity was detected for C-8 aryl-substituted compounds against only two kinases (Lck and p38 MAPK).[159]

Different strategies are being developed for kinase inhibition[136] (bisubstrate approach from small peptides and subsequent transformation of these molecules to peptidomimetics,[160] non competitive inhibitors targeting domains other than the ATP site,[161,162] SH2 domain inhibitors, peptidomimetics from protein–protein interactions[163]...). In this context, a vinyl sulfone derivative ON012380 has been identified as an ATP noncompetitive substrate inhibitor of a Gleevec-resistant form of Bcr-Abl.[161] However, targeting the catalytic site with ATP competitive inhibitors is, at the present time, the most effective approach for the development of kinase-directed drugs.[136] Selectivity is a major issue concerning this strategy, since many of the important residues in the ATP-binding site are conserved. The results of more than a decade of intense research have demonstrated, however, that it is possible to develop highly selective kinase inhibitors. Gleevec is now an approved drug for the treatment of CML and GISTs.[164] Several other ATP competitive protein kinase inhibitors are also being evaluated in advanced clinical trials.[136]

The structural basis for this selectivity has been provided by comparative crystallographic analysis. Between kinases there are specific amino acid replacements in the active site, and in the amino acid residues located at the exterior of the active site. These differences are found to influence recognition/binding of an inhibitor in that they give rise to changes in domain structure and in the flexibility of the ATP-binding pocket. Another factor is the existence of hydrophobic regions around the adenine ring which, although not used to bind ATP, are accessible to different inhibitor types.[165] The composition and volume of these hydrophobic pockets can be exploited to enhance selectivity, as can use of the sugar and phosphate-binding regions. Taking advantage of these considerations has permitted the design of highly potent and selective Cdk2 and Src inhibitors. For instance, due to a significant difference in the volume and composition of the specificity pocket (hydrophobic region 1) in Cdks and Src kinase the 2,6,9-trisubstituted purines purvalanol B and AP23137 selectively inhibit one system and not the other (Figure 23).

In the design and optimization of kinase inhibitors, a very pertinent question is just how far should one go to achieve selectivity. As it turns out, there is a non-negligible degree of redundancy/interconnection between signaling pathways. Use of a highly optimized compound that blocks a specific pathway may thus, in certain instances, have no therapeutic effect. Alternatively, it may result in upstream or downstream deregulation of undesired signaling events. In light of this situation, one current paradigm is to develop kinase inhibitors with the 'right combination' of activities against multiple targets. To address the issue of selectivity in a global sense requires technology to screen kinase inhibitors against all kinases (all proteins) in the cell.

Meijer and co-workers pioneered this pursuit, developing an affinity column approach in which immobilized purvalanol B was challenged with cell extracts coming from different sources.[167] More recently, a much more sensitive three-hybrid approach has been used to scan purvalanol B against the entire proteome. A total of 35 kinase interaction events were observed for this compound, 31 of which involve novel candidate kinase

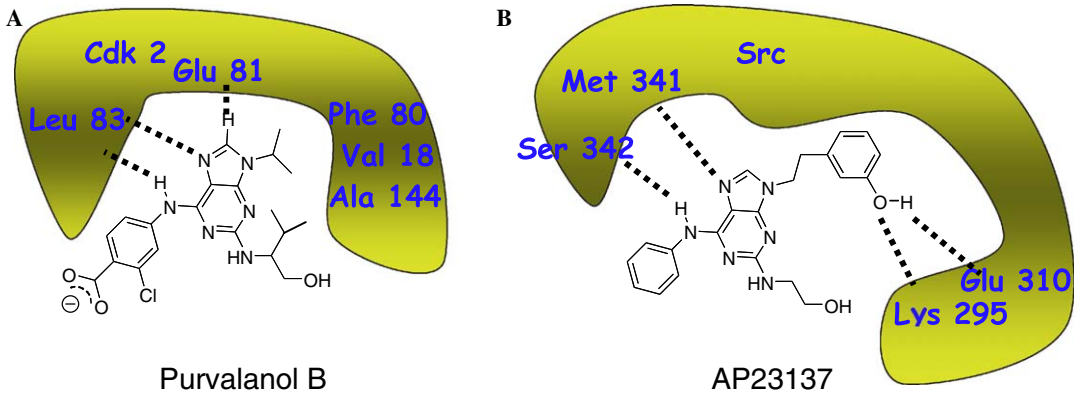


Figure 23. Schematic illustration of inhibitor interactions that can explain the selectivity of these drugs. (A) Binding of purvalanol B to CDK2 showing conserved H-bonding and interaction of its isopropyl group to a relatively small-sized hydrophobic pocket. (B) Binding of AP23137 to Src kinase showing conserved H-bonding (dotted lines) and interaction of its hydroxyphenylethyl group to a relatively large-sized hydrophobic pocket (with further H-bonding to the inhibitor's hydroxyl moiety).[166]

targets. At least 22 novel interactions were confirmed by secondary assays (affinity chromatography/LC–MS analysis of cell extracts, in vitro enzyme assays...).[168]

A completely different, and equally exciting, approach has also recently been developed by Zarrinkar and co-workers to measure the activity of small molecule kinase inhibitors against a panel of 119 protein kinases.[169] The unique innovation brought to this test is the use of a small set of immobilized probe ligands, which show affinity for a wide range of kinases, rather than ATP in the competition experiments with small molecules. Twenty kinase inhibitors, including roscovitine which is in clinical development, were evaluated to determine their selectivity profiles in the form of small molecule-kinase interaction maps. In addition to Cdk2 and Cdk5, roscovitine was found to inhibit eight other Cdk-like kinases. In this respect, it is a more selective kinase inhibitor than Gleevec.

15. Conclusion

The objective of this review has been to show the recent advances in the exploration of purine-based compounds as chemical-biology tools and to illustrate their potential as therapeutic agents to treat an impressively wide range of diseases. As purines (ATP, GTP, cAMP, cGMP, NAD, NADP, SAM, PAPS...) play an important role in life processes, it is a natural reflex to look at the effect

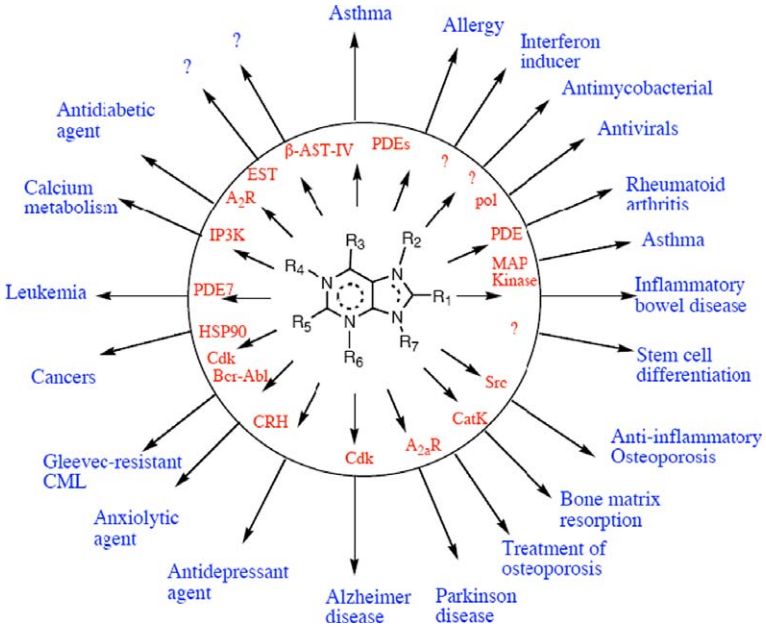


Figure 24. Potential applications of purine derivatives. Combinations of various substituents (1, 2, 3, 4...) on the different positions (R_1–R_7) of the purine ring lead to inhibitors of the different primary targets indicated in red. (PDEs, phosphodiestease; pol, DNA or RNA polymerase; Src, Src tyrosine kinase; CatK, cathepsin K = cysteine protease; $A_{2A}R$, adenosine receptorA_{2A}; Cdk, cyclin-dependent kinase; CRH, corticotropin-releasing hormone; HSP90, heat-shock protein-90; IP3K, inositol-1,4,5-triphosphate-3-kinase; EST, estrogen sulfotransferase β-AST, β-arylsulfotransferase); CML, chronic myeloid leukemia. Some potential therapeutic applications are noted at the outside of the circle. The question mark indicates that either the primary target or the possible application(s) is (are) not known.

of modification of substituents on the purine ring on biological activity. The chemistry of purines lends itself willingly to such manipulation, and as a consequence extensive and diverse libraries of purine derivatives have been prepared bearing different types and combinations of functionality at essentially all seven reactive centers on the exterior of the bicycle. Accompanying the development of methodology for purine library synthesis has been the development of new high-throughput screening assays against a myriad of new protein targets.

Increasing 'hands-on' access to X-ray crystal structures of these targets has also done much to accelerate the process of drug discovery, and in the present context, the development of purines as clinical candidates for drug development.

Great strides have thus been made over the past few years to exploit the biological properties of purines, and we now have in hand an impressive number of promising purine-based drug candidates for the treatment of cancer, rheumatoid arthritis, asthma, osteoporosis, diabetes, Parkinson's disease, and depression (Fig. 24).

There is every reason to believe that new and important therapeutic applications of purines are just waiting to be discovered.

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