

Pyridines, pyridazines and guanines as CDK2 inhibitors: a review

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Abstract

Cell cycle progresses by the activation of cyclin and cdk complexes. They act as check points and regulate the transition of cell cycle from one phase to another. Cdk2 inhibitors decrease the kinase activities by blocking the transition from G1 to S phases. In this article, we present a review on purine based cdk2 inhibitors. The review covers in great detail the different structures and the effects on cdk2 inhibition by various substitutions. The substitutions that most greatly influence the orientation and binding towards the ATP binding site are discussed and the effects of several substituents that explored the active site region of cdk2 with bound inhibitor has provided a rationale to review four relatively new families of purine based cdk2 inhibitors such as imidazo[1,2-*a*]pyridines, imidazo[1,2-*b*]pyridazines, 1*H*-pyrazolo[3,4-*b*]pyridines and O⁶-substituted guanines, respectively. The orientation and hydrogen bond interactions of analogues with Leu83, Asp86 and Lys33 residues influence the binding and the major features responsible for effective cdk2 inhibitor discovery and design are presented.

Keywords: Cyclin dependent kinase, guanine, pyridine, pyridazine

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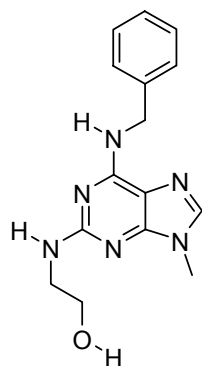
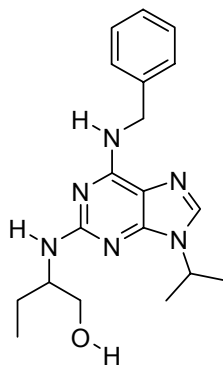
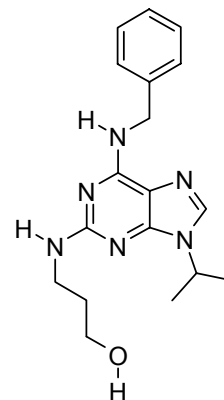
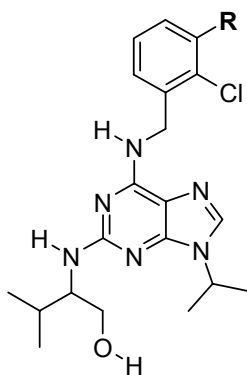
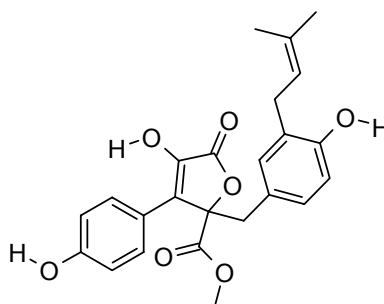
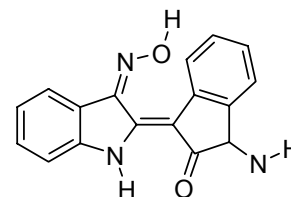
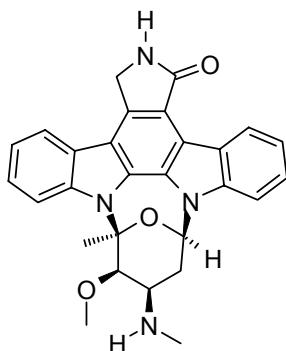
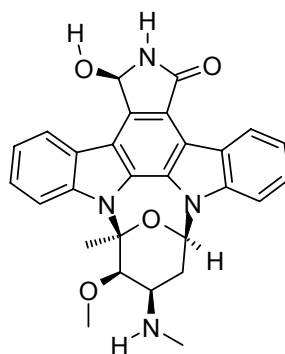
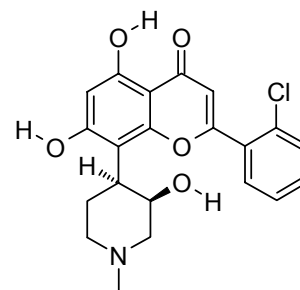
1. Introduction

Cell proliferation is a consequence of positive signals which promote cell division and negative signals which suppress the process. Key factors in this signaling cascade are a series of cyclin dependent kinases (cdks)¹. It has been shown that they are also required for replication of viruses that replicate only in dividing cells, such as adeno- and papillomaviruses as well as in non-dividing cells, such as HIV-1 and herpes simplex virus types 1 and 2 (HSV-1 and HSV-2)². Cyclin-dependent kinases are a family of serine/threonine kinases which play a crucial role in cell cycle control³ and are involved in diverse cellular processes, in regulation of cell division (cdks1, 2, 3, 4, 6 and 7), transcription (cdks7, 8 and 9) or maintenance of the structure of the cytoskeleton (cdk5)².

Cyclin dependent kinases control the cell cycle progression operating at the transition from G₂ to M, G₁ to S phases, and progression through S phase, regulated by a complex set of mechanisms, including the presence of activating cyclins, regulatory phosphorylations, and endogenous cdk inhibitors at checkpoints⁴. Cell cycle progresses by the activation of Cyclin and cdk complexes⁵. These cyclins and cdks function as check points regulating the transition from one phase of cell cycle to another. Structural studies have explored the active and inactive states of cdk2. Monomeric form was inactive, while association of Cyclin A with cdk2 and Thr160 phosphorylation results active cdk2⁶. Active complex phosphorylate and inactivate members of the retinoblastoma protein (Rb) family that are negative regulators of G₁ and S-phase progression, leading to induction of E2F-regulated gene expression and cell proliferation⁷. Cdk inhibitors decrease the kinase activities of the cyclin/ckd complexes, blocking the transition from G₁ to S phases⁸. Activation of cdk2 results in rotation of N- and C-terminal domains leading to a slight widening of ATP cleft⁹. The movement of PSTAIRE helix and Glu51 and the subsequent reorganization leads to reshaping of the phosphate-binding site^{10,11}.

Following the discovery of olomoucine and roscovitine (Figure 1) as selective ATP competitive cdk2 inhibitors, much effort has been devoted to find more specific and potent inhibitors because kinases within the cell share a high degree of sequence similarity at the active site. X-ray crystallographic analysis of a substantial number of cdk2/inhibitor complexes has elaborated the diverse binding modes of different inhibitors in atomic detail³. Several types of

cdk inhibitors, shown in Figure 1, have so far been described: staurosporine, UCN-01¹², flavopiridol (L86-8275)¹³, butyrolactone I¹⁴, other purine derivatives¹⁵⁻¹⁸, indirubin¹⁹, paullones²⁰ and others²¹⁻²³.

**1, Olomoucine****2, Roscovitine****3, Bohemine****4a, R=H; Purvalanol A**
4b, R=COOH; Purvalanol B**Butyrolactone I****Indirubin-3'-monoxime****Staurosporine****UCN-01****Flavopiridol****Figure 1.** Cyclin dependent kinase inhibitors.

Based on the studies that explored the active site region of cdk2 with bound inhibitor, and the role of cdk2 in cell cycle progression and proliferation, cdk2 acts as a potential therapeutic target in several proliferative diseases, including cancer¹¹. Development of successful small molecule cdk2 inhibitors as anticancer agents has provided a rationale to review four relatively new families of purine based cdk2 inhibitors with emphasis on substituted groups and their influence towards activity and selectivity at the ATP binding site. Therefore, analogues with sulfonamide and amino substituted imidazo[1,2-*a*]pyridines, imidazo[1,2-*b*]pyridazines, 4-, 5- or 6-substituted 1H-Pyrazolo[3,4-*b*]pyridines and O⁶ - substituted guanines are reviewed to explore the effect of substituents on cdk2 inhibition.

2. Purines

Cytokinin derived purine analogues yielded the first cdk-selective protein kinase inhibitors²⁴. The first cdk inhibitor discovered was dimethylaminopurine²⁵ shown to inhibit cdk1 activity (IC₅₀: 120 μM). A derivative, isopentenyladenine was somewhat more selective than dimethylaminopurine [26]. Later on, a moderately potent cdk2 inhibitor, olomoucine **1**¹⁵ was developed followed by roscovitine **2**^{16, 27-29} and bohemine **3**³⁰ with improved inhibition (Figure 1). The purvalanols (**4a** and **4b**) with 6-anilino purine substituents are the most potent cdk2 inhibitors³¹. 2-(1R-isopropyl-2-hydroxyethylamino)-6-(3-chloro-4-carboxyphenylamino)-9-isopropylpurine (purvalanol B), has an IC₅₀ of 6 nM against cdk2-cyclin A complex, which corresponds to a 1000-fold increase over olomoucine and a 30-fold increase over flavopiridol³².

2.1 Imidazo[1,2-*a*]pyridines

The importance of imidazo[1,2-*a*]pyridine^{33,34} group and SAR were reported^{35,36} to investigate the binding site selectivity between cdk2 and cdk4 (Figure 2). The affinity of compound **5** (IC₅₀: 2.9 μM) with ATP binding site residues, depicted in Figure 2, pdb code: 1oiq, resulted in three hydrogen-bonding interactions between the pyrimidine N1 and Leu83 backbone NH, the 2-amido NH and Leu83 backbone carbonyl, and the imidazo[1,2-*a*]pyridine N1 and the Lys33 amino group, respectively. Structural analyses of cdk2 with compound **6** (IC₅₀: 0.032 μM) revealed the affinity of hydrophobic aniline ring towards hydrophobic surface formed by Phe82, Leu83, Leu134 and Ile10 side chains (Figure 2, pdb code: 1oir). Importance of purine scaffold as cdk2 inhibitors can be evidenced by the replacement of imidazo[1,2-*a*]pyridine ring with aniline ring, in compound **7**, resulted in loss of potency (IC₅₀: 32 μM).

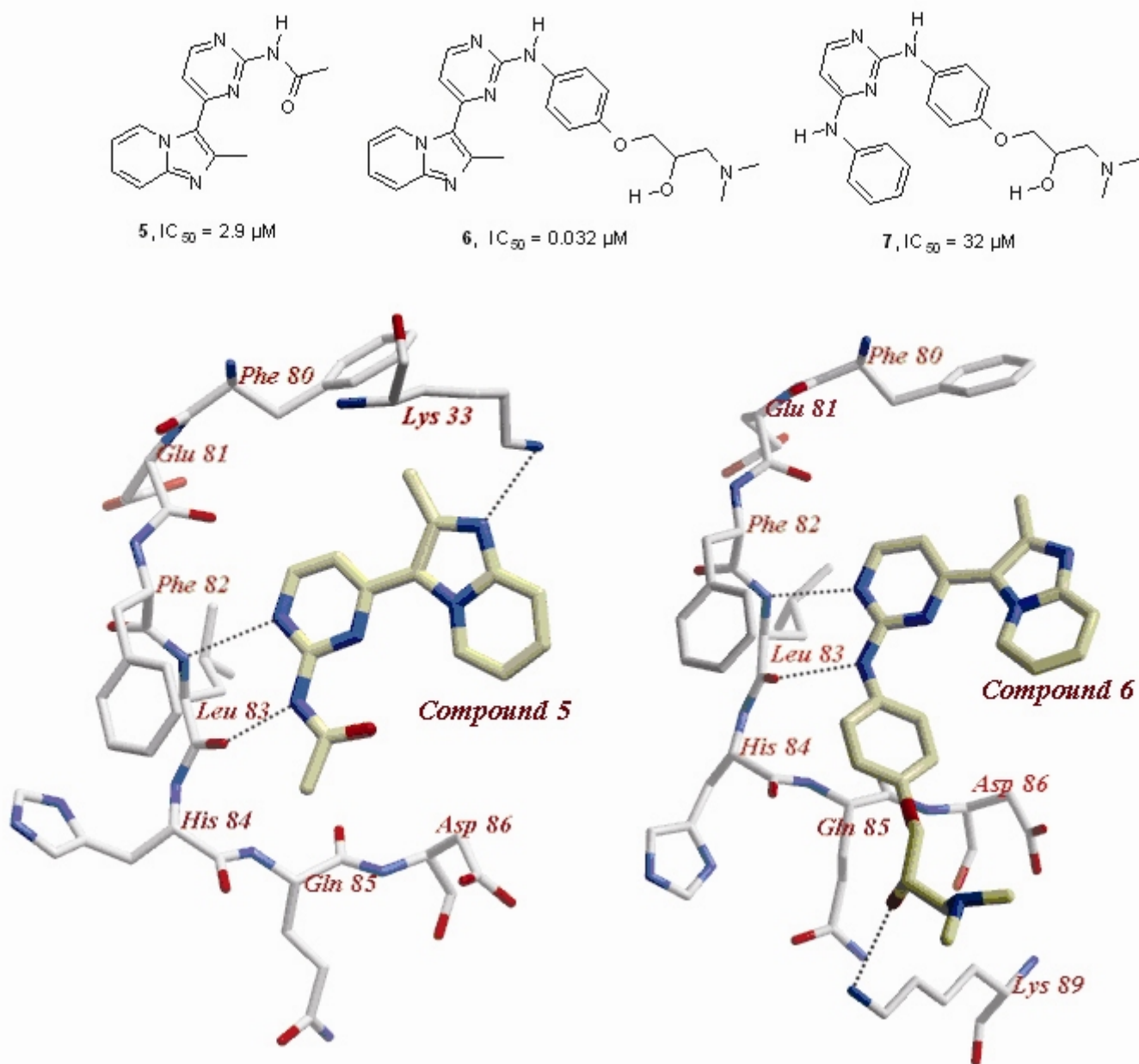


Figure 2. Imidazo[1,2-*a*]pyridine class of inhibitors (values of IC_{50} refer to the affinity of the compound with ATP). Binding of imidazo[1,2-*a*]pyridine analogues, compounds **5** and **6** bound with selected residues (labeled and drawn in stick representation) of pdb proteins 1oiq and 1oir. Compound **5** makes three hydrogen bond interactions with Leu83 and Lys33 residues and compound **6** displaying three hydrogen bond interactions with Leu83 and Lys 89 respectively. Hydrogen bonds are indicated by dotted lines.

2.1.1 Effect of sulfonamide substitution

Structural studies showed that the aniline group substitution with sulfonamide was beneficial for cdk2 activity³⁷. Compounds **8** and **9** with methyl group substitution on imidazo[1,2-*a*]pyridine ring displays the importance of sulfonamide substitution. A 4-sulfonamide substituted compound **8** resulted in an increased potency over unsubstituted compound **9** (IC₅₀: 0.038 μM and IC₅₀: 0.21 μM). Compound **10**, devoid of methyl group substitution on imidazo[1,2-*a*]pyridine ring displayed tremendous activity (IC₅₀: <0.003 μM). This was due to the hydrogen bonds formed by para-sulfonamide group with backbone NH and carboxylic side chain of Asp86 (Figure 3, pdb code: 1oit). Comparatively, further substitution on sulfamoyl group showed no profound effect on cdk2 activity for compounds **11-13** (IC₅₀: 0.005 μM, <0.003 μM and 0.004 μM), which demonstrates that the substituted NH of sulfonamide was not essential for cdk2 activity. As given in Figure-3, substitutions at 5-position of pyrimidine (R1) and imidazo[1,2-*a*]pyridine ring (R2) showed that small substituents (**14, 15** and **16, 17, 18**; IC₅₀: <0.003 μM) are tolerated with similar activity.

2.1.2 Substituted 2-aminoimidazo[1,2-*a*]pyridines

SAR studies of 2-aminoimidazo[1,2-*a*]pyridine compounds revealed the presence of at least one substituent (R³) on aryl group at position 3 (Figure 4) would increase potency^{38,39}.

2.1.2.1 Effect of electron-withdrawing and electron-donating groups on aryl ring (R³).

Figure 4 shows that a disubstituted electron-withdrawing fluorine at positions 2 and 6 on aryl ring (**19**, IC₅₀: 0.122 μM) proved to be most active cdk2 inhibitor than unsubstituted derivative **20** (IC₅₀: 0.324 μM). Electron-donating methoxy group at position 2 on aryl ring enhanced activity for compound **21** (IC₅₀: 0.068 μM) and electron-withdrawing fluorine and chlorine groups at positions 2 and 6 reduced activity, respectively, for compounds **22** (IC₅₀: 0.121 μM) and **23** (IC₅₀: 0.102 μM). A five-fold increase in activity observed for 2,6-disubstitution with electron-withdrawing fluorine groups on aryl ring (**24** (IC₅₀: 0.026 μM) and **25** (IC₅₀: 0.029 μM)) attached with a thioketone linker at position 3 on imidazo[1,2-*a*]pyridine ring shows that this moiety was more preferred than the ketone group.

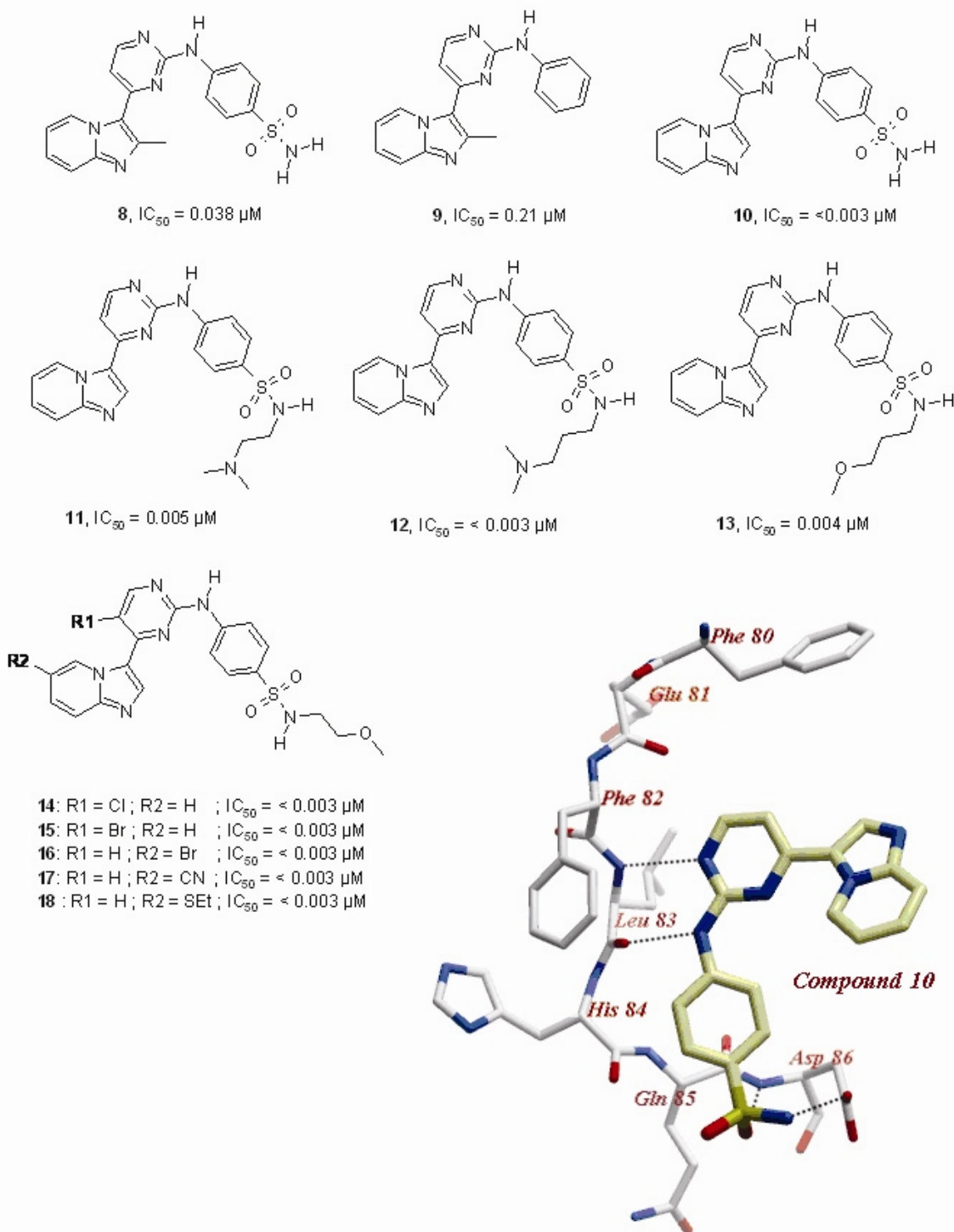


Figure 3. Sulfonamide substituted imidazo[1,2-*a*]pyridine analogues. Binding of sulfonamide group substituted imidazo[1,2-*a*]pyridine analogue, compound **10** displaying four hydrogen bond interactions with Leu83 and Asp86 residues of 1o1t. Hydrogen bonds are indicated by dotted lines.

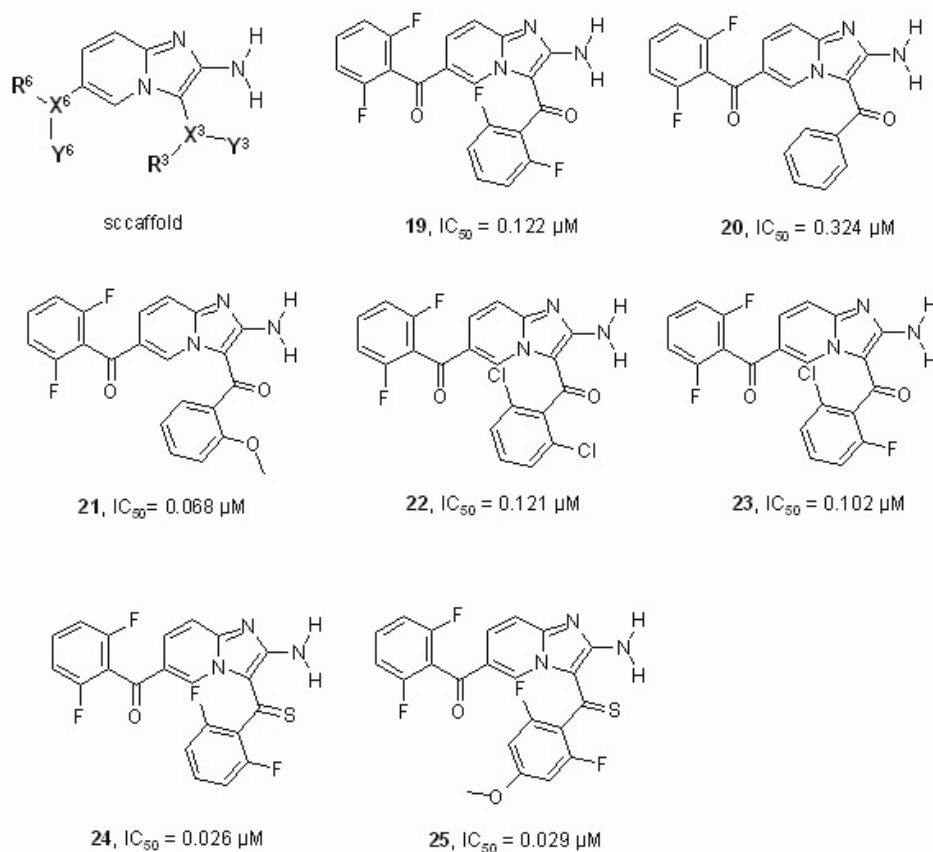


Figure 4. 2-amino substituted imidazo[1,2-*a*]pyridines.

2.2 Imidazo[1,2-*b*]pyridazines

The sulfonamide substituted imidazo[1,2-*a*]pyridine core structure³⁷ was modified as imidazo[1,2-*b*]pyridazines⁴⁰ to reduce the lipophilicity. Compounds **26** (IC_{50} : $<0.003 \mu\text{M}$) and **27** (IC_{50} : $0.003 \mu\text{M}$) (Figure 5) are characterized as potent and selective inhibitors. The binding mode of this series of compounds showed a significant dependence on the presence of 4-sulfonamide substituent on the phenylamino group. The imidazo[1,2-*b*]pyridazine ring is located, in the active site, in an inverted orientation relative to the sulfonamide substituted imidazo[1,2-*a*]pyridine (compare Figure 5 and Figure 2). The H-bonding interaction between N1 of imidazole ring and Lys33 amino group was important for cdk2 activity³⁶, characteristic feature deficient in binding mode of imidazo[1,2-*b*]pyridazine compound **28** (Figure 5, pdb code: 1urw, IC_{50} : $<0.003 \mu\text{M}$) compared with imidazo[1,2-*a*]pyridine compound **10** (Figure 4, IC_{50} : $<0.003 \mu\text{M}$). In either case, the key hydrogen bonding interactions and the activity are similar and structural studies⁴⁰ reveal that this change in binding conformation was probably due to the electrostatic repulsion between N4 of imidazo[1,2-*b*]pyridazine and N3 pyrimidine ring of compound **28**.

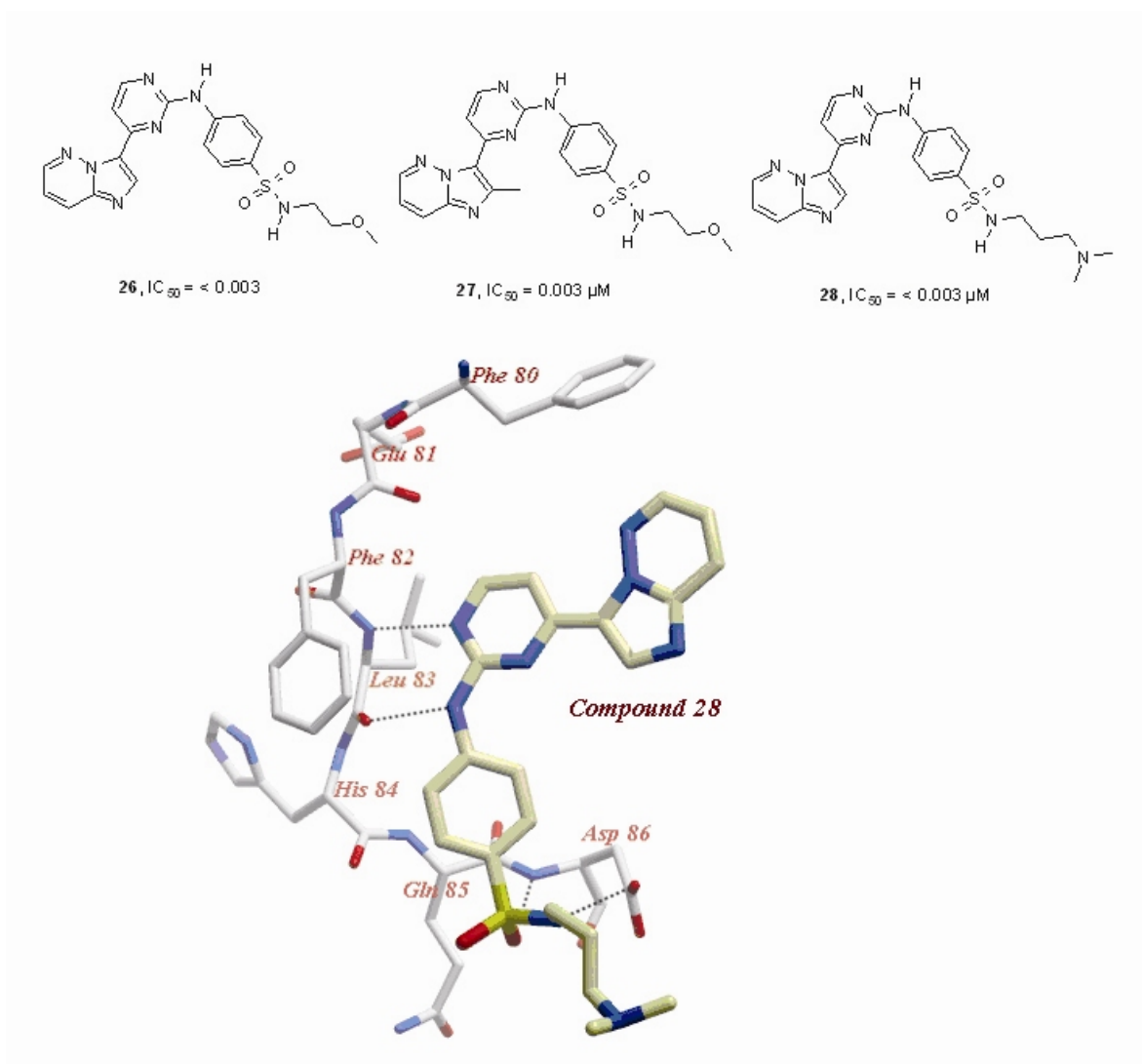


Figure 5. Imidazo[1,2-*b*]pyridazine class of inhibitors as potential cdk2 agents. Compound **28**, a 4-sulfonamide substituent on the phenylamino moiety of imidazo[1,2-*b*]pyridazine displaying four hydrogen bond interactions with Leu83 and Asp86 of 1urw. Hydrogen bonds are indicated by dotted lines. The imidazo[1,2-*b*]pyridazine ring was in an inverted orientation comparative to the imidazo[1,2-*a*]pyridine ring (Figure 2).

2.3 Pyrazolo[3,4-*b*]pyridines

Pyrazolo[3,4-*b*]pyridines were recognized as a new class of cdk2 inhibitors with potent in vitro activity⁴¹. Screening efforts revealed 1H-pyrazolo[3,4-*b*]pyridine derivatives **29** (SQ-67563; IC_{50} : 0.11 μM) and **30** (SQ-67454; IC_{50} : 0.18 μM) (Figure 6) as potent inhibitors of cdk2. N-ethyl analogue, **31** (IC_{50} : >25 μM) did not inhibit cdk2, which indicates that N-Et at this position was critical to binding.

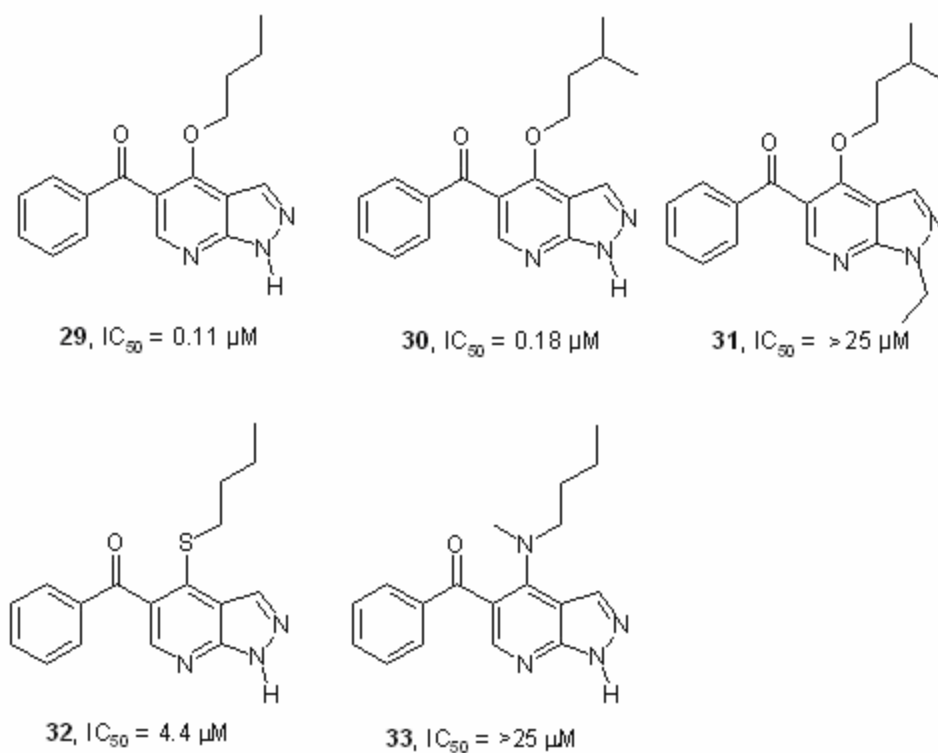


Figure 6. 4-Substituted 1H-pyrazolo[3,4-b]pyridine derivatives.

2.3.1 Effect of 4-position substitution

Replacement of oxygen with either nitrogen or sulfur at position 4 (**32**, **33**; IC_{50} : $4.4 \mu\text{M}$, $>25 \mu\text{M}$) resulted in a significant loss of cdk2 activity. Straight chain, branched and cyclic alkoxy groups are tolerated as they extend into the space occupied by the ribose of ATP without any specific contacts with the protein. Polar substituents like hydroxyl and dimethylamino resulted in loss of activity⁴¹.

2.3.2 Effect of 5-position substitution

Substitution at 5-keto position⁴² with small heterocycles, such as 2-furanyl, 2-thienyl and 2-thiazolyl exhibited comparable cdk1 and cdk2 inhibitory potency to phenyl analogue **29** (SQ-67563). Replacement of 5-keto position (Figure 7) by sulfoxide, compound **34**, and sulfone, compound **35**, showed moderate inhibition with IC_{50} values 0.34 and $0.52 \mu\text{M}$, respectively, indicating either sulfone or sulfoxide may be substituted for the carbonyl group.

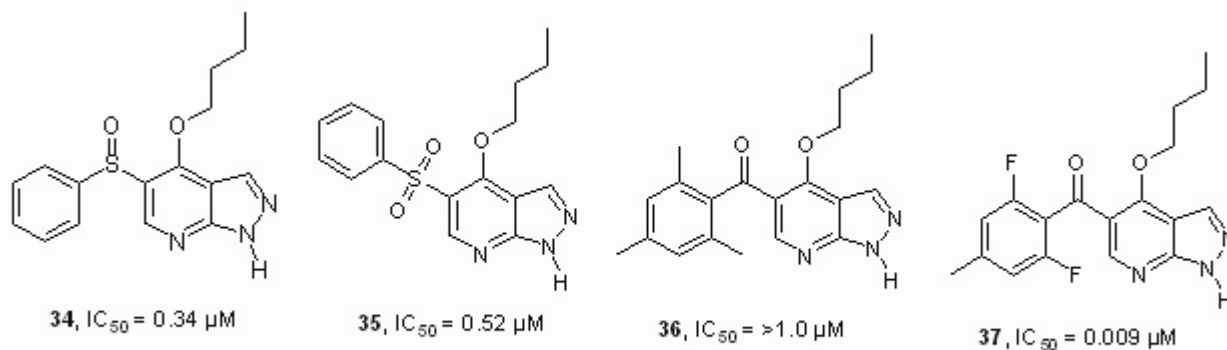


Figure 7. 5-position substituted 1H-pyrazolo[3,4-*b*]pyridine analogues as potent and selective cdk2 inhibitors.

An ortho fluoro substitution on 5-keto phenyl ring represents a key for potent inhibitory activity⁴². The 2,4,6-trimethylphenyl analogue, **36** (IC_{50} : $>1.0\mu\text{M}$) resulted as a poor cdk2 inhibitor in contrast with 2,6-difluoro-4-methyl analogue, **37** (IC_{50} : $0.009\mu\text{M}$).

2.3.3 Effect of 6-position substitution

GSK-3 class of inhibitors, 6-aryl-pyrazolo[3,4-*b*]pyridines⁴³, (Figure 8) homologous to cdk2 inhibitors were tested for specificity as they both share a high degree of similarity. Substitution at position 6 with small heterocycles, such as 2-pyrrolyl, 2-furanyl and 2-thienyl exhibited greater potency than the corresponding phenyl analogue.

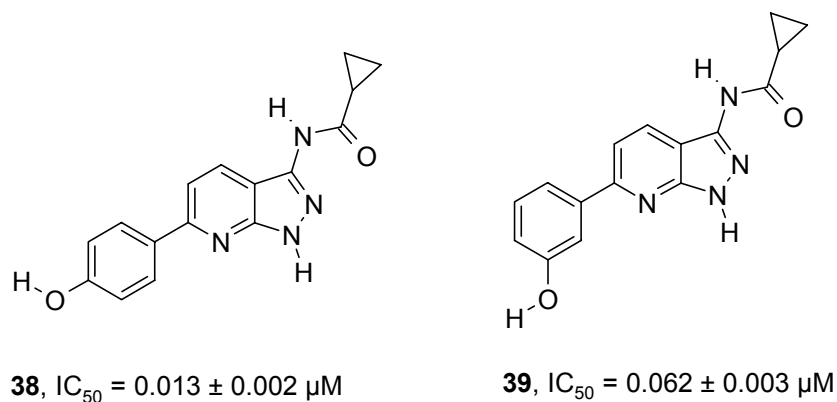


Figure 8. 6-aryl-pyrazolo[3,4-*b*]pyridines as cdk2 inhibitors.

Incorporation of a hydroxyl group either at *para* (**38**; cdk2 IC_{50} : $0.013 \pm 0.002 \mu\text{M}$) or *meta* (**39**; cdk2 IC_{50} : $0.062 \pm 0.003 \mu\text{M}$) position of the C6-phenyl ring resulted in an increase in cdk-2 inhibition.

2.4 O⁶ - substituted guanines

ATP ribose binding domain was probed with ATP competitive O⁶ - substituted guanine derivatives⁴⁴. An increase in inhibition observed with increase in chain length (Figure 9) from O⁶ - methylguanine to O⁶ - pentylguanine and for O⁶-alkoxyalkyl purine derivatives **40** and **41** (IC₅₀: 15 ± 2 μM, 16 ± 1 μM), respectively. Cyclohexylmethoxypurine, NU2058 showed similar inhibition (**42**, IC₅₀: 17 ± 2 μM) as observed with other 6-alkoxyalkylpurine derivatives. Replacement of the cyclohexyl group of NU2058 with a phenyl group in compound **43**, (O⁶ - benzylguanine) resulted in a 2-3 fold reduction in activity (IC₅₀: 35 ± 6 μM), whereas substitution on the O⁶-benzyl group (**44**, **45** and **46**) resulted in loss of potency (% inhibition: 52 ± 7, 52 ± 3 at 100 μM and 49 ± 14 at 10 μM). This might be due to the substituents unable to form favorable hydrogen bonds within the ribose binding domain. SAR studies suggest that a broad range of substituents are tolerated at the O⁶-position, but none resulted in appreciable activity or specificity as seen with NU2058, which suggests that the hydrogen bonds formed by the substituents within the ribose pocket contributed greatly towards potency and selectivity.

X-ray structure of NU2058 bound to monomeric cdk2 revealed that it forms a triplet of hydrogen bonds within the ATP binding site between residues in the hinge region of cdk2 and the NH₂, N3, and N9 nitrogen atoms of the inhibitor⁴³. Comparison of structures (Figure 9) of NU2058 (pdb code: 1e1v) and olomoucine (pdb code: 1w0x) bound to monomeric cdk2 revealed that NU2058 represents a different class of inhibitor to olomoucine [15] (IC₅₀: 7 μM) as the two compounds bind in different orientations⁴⁵. Extending this observation, SAR studies for a series of N²-substituted O⁶-cyclohexylmethylguanine derivatives^{46, 47} resulted in the discovery of potent inhibitor NU6102 (IC₅₀: 0.0054 ± 0.001 μM) (Figure 10, pdb code: 1h1s).

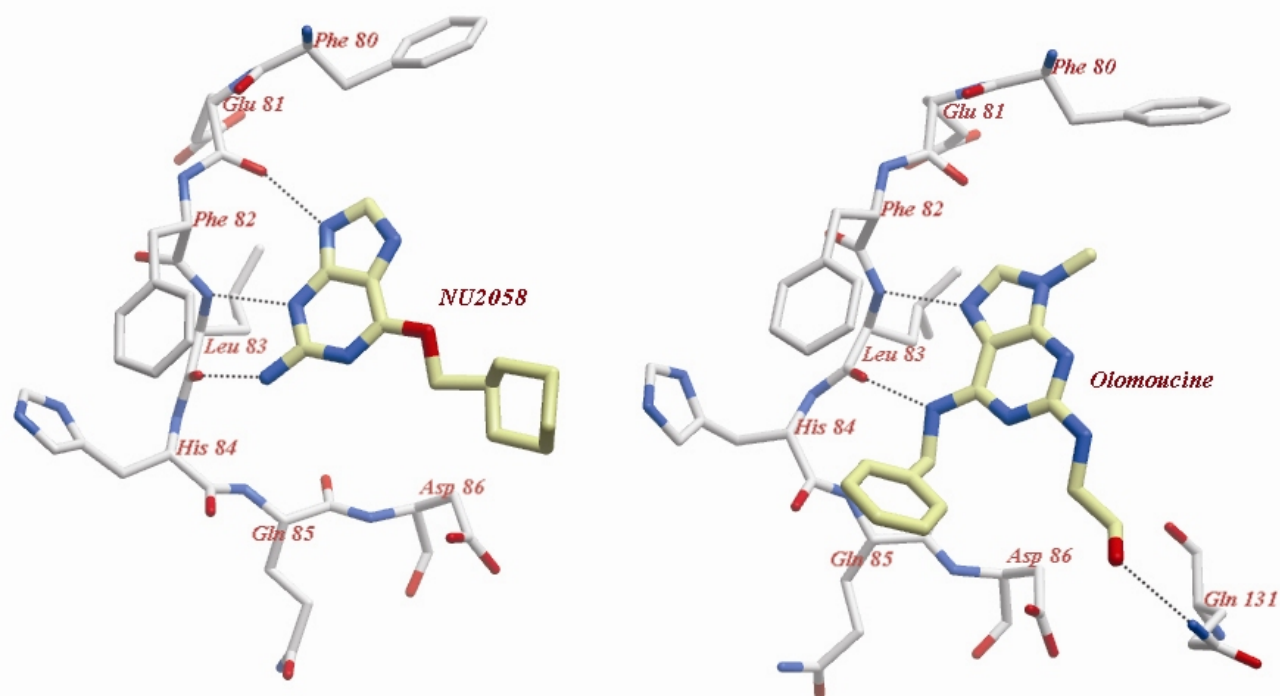
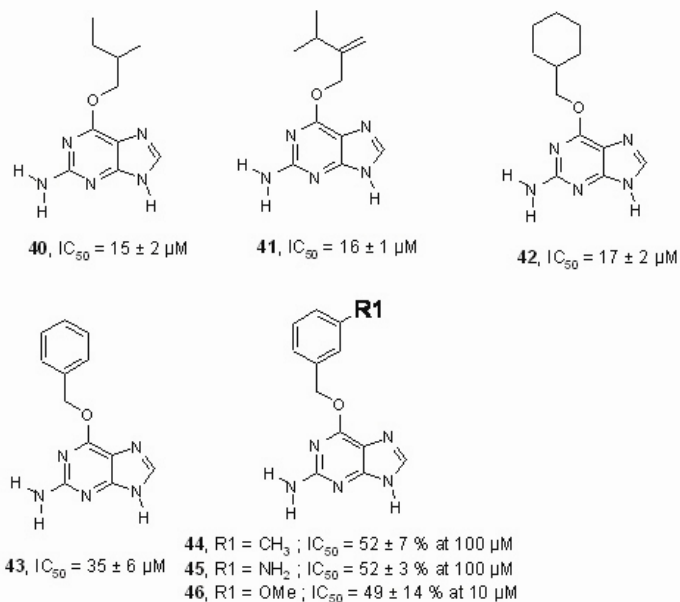


Figure 9. O⁶-substituted guanine derivatives. Comparison of structures of NU2058 (Thr160 phosphorylated cdk2/cyclinA, pdb code 1e1v) and olomoucine (1w0x) bound to monomeric cdk2 represents binding in different orientations. Hydrogen bonds are indicated by dotted lines.

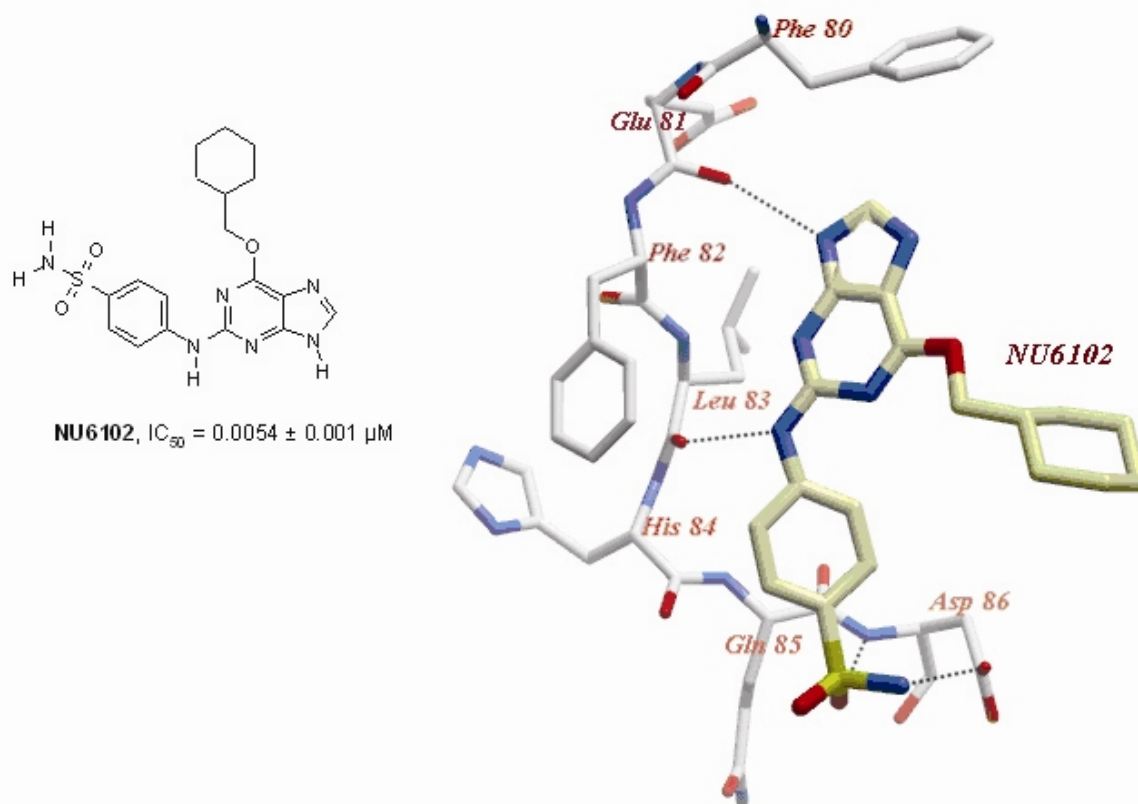


Figure 10. Crystal structure of NU6102 bound to cdk2 protein (1h1s) displaying four hydrogen bond interactions with Glu81, Leu83 and Asp86 exhibited potency and selectivity towards cdk2 inhibition.

3. Conclusions

A number of compounds with diverse substituents explored the potential active site region of cdk2 resulted in micromolar inhibition. The orientation and hydrogen bond interactions of analogues with Leu83, Asp86 and Lys33 greatly influence the binding for purine based cdk2 inhibitors and the major features responsible for effective cdk2 inhibitor discovery and design for four families are:

- presence of hydrophobic phenylamino moiety on imidazo[1,2-*a*]pyridines.
- presence of 4-sulfonamide substituent on the phenylamino moiety of imidazo[1,2-*b*]pyridazines to reduce lipophilicity
- 4-, 5- and 6- position heterocycle substitutions are well tolerated on pyrazolo[3,4-*b*]pyridines.
- increase in chain length from O⁶ - methylguanine to O⁶ - pentylguanine and presence of O⁶ - cyclohexyl group.

Therefore, these features form the basis to develop novel cdk2 analogues that would enhance nanomolar inhibition and drug discovery process.

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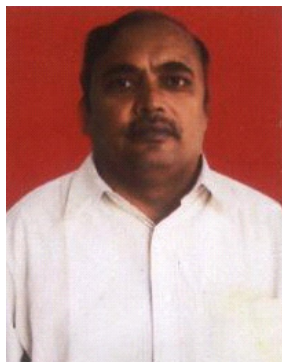


P Ajay Babu was born in Andhra Pradesh, India in 1973. He pursued his post-graduation in Bioinorganic Chemistry from College of Science and Technology, Andhra University in 1996. Being interested in Biotechnology, he pursued MTech Biotechnology from Jawaharlal Nehru Technological University in 1998. Since then he worked as Microbiologist (Hi-tech Pharmaceuticals), Analytical chemist (Dr. Reddy's Labs), Sr. Technical Team Member (Celestial Technologies) and Bioinformatics Faculty at Institute of Bioinformatics and Research Centre (ProGene Biosciences). Currently, he is pursuing his PhD from School for Biotechnology, Jawaharlal Nehru Technological University, Hyderabad. He played a key role in the design and maintenance of 1082 molecular biology databases at one interface, DoD2007 and DiaMedBase, a database of 389 medicinal plants related to diabetes. He published 11 papers in the field of bioinformatics and drug design.



Professor Laxmi Narasu was born in Andhra Pradesh, India in 1957. Her doctoral thesis entitled "Studies on larvicidal protein from *Bacillus sphaericus* 1593" was carried out in 1987 at Indian Institute of Science, Bangalore. For a brief period of two years, she was involved in working with eye lens proteins in Prof. D. Balasubramanian's lab at the Centre for Cellular and Molecular Biology, Hyderabad. After joining the Centre for Biotechnology, Jawaharlal Nehru Technological University, Hyderabad in April 1989, she devoted her time to establish plant tissue culture and lab facilities with a financial assistance of Rs.6 lakhs from mHRD and 3.00 lakhs from AICTE. Thereafter, tissue culture of *Artemisia annua*, an important medicinal plant that produces artemisinin and other compounds was initiated. Subsequently work was initiated on purification of the proteins involved in transformation of arteannuin B to artemisinin with a funding of 12.7 lakhs from DST. She was awarded with a 2.0 lakh 'AICTE career award for young teachers' in the year 1996 for the proposed work on transformed root cultures of

Artemisia annua. At present a few potential microorganisms that can carry out the same transformation reaction have been identified. Ten of her students have been awarded their Ph.D. degrees and more than six students are in different stages of completion. She has 26 years of research experience and her research interests include plant tissue culture of medicinal plants, environmental biotechnology and bioinformatics. In the area of plant tissue culture her group was successful in obtaining enhanced amounts of podophyllotoxin (anticancer compound) from transformed cultures of *Podophyllum hexandrum*. She published 35 papers in the fields of plant tissue culture, molecular biology and environmental biotechnology.



Professor Srinivas was born in Andhra Pradesh, India in 1962. He obtained his doctorate from Berhampur University in 2002 for his work in the field of Pharmacognosy and Phytochemistry. The work dealt with the biological screening of some folklore medicinal plants used in Arthritis, Diabetes and Liver disorders. He was Principal Investigator for AICTE sponsored R&D project 'Bioassay guided principles of medicinal plants having different biological activities and attempt to made formulations' during 1998-2000. He worked as a Lecturer (1991-94), Sr. Lecturer (1997-02) and obtained a Reader (2002-04) position at Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa. Since 2004, he accepted the position of Principal and Professor at Sri Venkateswara College of Pharmacy, Etcherla, Srikakulam. His research interests cover pharmacognosy, phytochemistry, medicinal chemistry, molecular modeling and drug design. Five PhDs have been awarded under his guidance and three students are at various stages of their research. He published 30 papers in the field of pharmacognosy, phytochemistry and medicinal plants.