RASĀYAN *J. Chem.*
Vol. 19 | No. 2 | 807-813| April-June | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

STRUCTURE-BASED KINASE INHIBITOR DESIGN: BINDING-MODE TAXONOMY, DFG-STATE TARGETING, AND ALLOSTERIC STRATEGIES

Venkatesh Kamath[1], Chandrashekhar K.S.[2], Aravinda Pai[3,✉] and Muddukrishna B.S.[4]

[1]Department of Pharmaceutical Biotechnology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal-576104, (Karnataka) India
[2] Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal-576104, (Karnataka) India
[3]Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal-576104, (Karnataka) India
[4]Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal-576104, (Karnataka) India
Corresponding author: aravind.pai@manipal.edu

ABSTRACT

Structure-based drug design (SBDD) has emerged as a transformative approach in the development of selective small-molecule kinase inhibitors, driven by advances in structural biology and computational chemistry. This review highlights the central role of kinase conformational dynamics-particularly the "DFG-in" and "DFG-out" states—in dictating inhibitor binding modes, selectivity, and mechanisms of resistance. By integrating insights from high-resolution crystallography, molecular docking, virtual screening, and recent geometric deep-learning-based methods, we summarise contemporary strategies that enable precise exploration of kinase active and allosteric pockets. Key trends discussed include the preferential targeting of the DFG-out conformation to improve specificity, the rise of allosteric modulators to overcome ATP-competitive limitations, and iterative structure-guided optimisation pipelines that accelerate lead discovery. We also examine emerging computational–experimental hybrid frameworks that enhance predictive accuracy, support exploration of undersampled chemical space, and enable the rational design of next-generation inhibitors with improved pharmacological profiles. Overall, this review underscores how modern SBDD methodologies continue to advance kinase inhibitor discovery by addressing longstanding challenges related to selectivity, resistance, and off-target effects, ultimately contributing to the development of safer and more effective therapeutic agents.

Keywords: Structure-Based Drug Design, Kinase Inhibitors, DFG-In/DFG-Out, Binding Modes, Allosteric Inhibition, Molecular Docking.

RASĀYAN *J. Chem.*, Vol. 19, No.2, 2026

INTRODUCTION

Protein kinases are an exciting class of enzymes that phosphorylate serine, threonine, or tyrosine residues in protein substrates, the process being an important post-translational modification. Kinases come in all variations, for they are involved in cell cycle progression, apoptosis, metabolism, and signal transduction processes.[5] It is of utmost importance to maintain the tight regulation of kinase activity; disturbance, brought about mostly by mutations and overexpression, will invariably lead to the diseased state, including cancer. The activation loop, containing the DFG motif, is one of the main structural features of kinases. With an active DFG-in conformation, the aspartate residue forms essential hydrogen bonds with and interacts with the metal ion of Mg^{2+}, thus stabilising the ATP binding conformation. It ensures that ATP is appropriately aligned and positioned for efficient transfer of the phosphoryl group. In contrast, the DFG-out conformation shifts the aspartate residue out of the ATP binding site, exposing a hydrophobic pocket that can be exploited by type II inhibitors.[6] The recent structural studies suggest that the stabilisation of the DFG-out conformation promotes selectivity, and hence this has come to be emphasised in the design of modern inhibitors. Apart from functioning catalytically, kinases possess

http://doi.org/10.31788/RJC.2026.1929489

regulatory domains that can modulate catalysis by the kinase domain.[7] Consequently, alterations in these domains can profoundly influence the overall conformation and behaviour of the protein. Curiously, some kinase inhibitors work from afar, i.e., allosterically, by binding to sites outside the ATP pocket.[8] Allosteric inhibition often carries the advantage of increased selectivity and can circumvent resistance introduced by mutations in the ATP-binding pocket. Hence, gaining an understanding of the active site and the extra regulatory sites of kinases is necessary when designing inhibitors (Fig.-1).

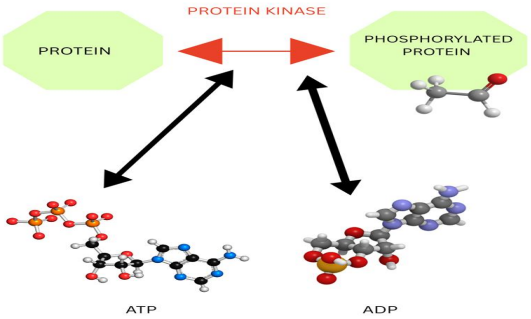

Fig.-1: Mechanism of Kinase Binding

EXPERIMENTAL

Structure-Based Design Techniques

Structure-based drug design, or SBDD, has transformed enzyme-targeted drug discovery by using detailed atomic-level information about kinases. This process begins with obtaining structural data from X-ray crystallography or nowadays more so from computational means via AlphaFold2. A high-resolution structure enables the medicinal chemists to identify the key sites of interaction at or near that site, which may be conserved or unique to the kinase domain.[10] Drug-like chemicals in a biochemical environment form the chemical space that should be sampled through docking and virtual screening in a structure-based drug design approach. Molecular docking is thus the prediction of the interaction of a small molecule with the active or allosteric site of a kinase, which implies that it simulates the binding orientations and affinity. These predictions form the basis for rational lead identification and optimisation. Geometric deep learning algorithms were recently introduced, which would enable molecular docking in highly complex and flexible biochemical environments.[11] SBDD ideally uses cascades for synthesis and biological evaluation. Structure-based design techniques allow the chemist to capitalise on differences in the non-conserved area of the kinase active-site to further the selectivity.[12] For example, small yet mechanistically significant modifications on the inhibitor scaffold can be exploited to favour inhibitor binding to the hydrophobic pocket that is available in the DFG-out conformation. The dynamic interplay between the treatment of experimental structural results and computational modelling has become inseparable in modern drug design: it allows researchers to hypothesise binding affinities with greater computed probability and with more comprehensible theories for the observed pharmacological profiles. Moreover, improvements in high-throughput biophysical and biochemical screening approaches have also sped up the design process. This method of screening, when coupled with SBDD, allowed rapid identification of interactions in compound libraries that otherwise would have been made through in-silico analyses alone.[13] In such a fashion, an iterative approach with such a docking algorithm shall be used to make drug discovery pipelines more productive, for which more integrated platforms that correlate docking outputs with experimental binding data are increasingly being sought after (Fig.-2).

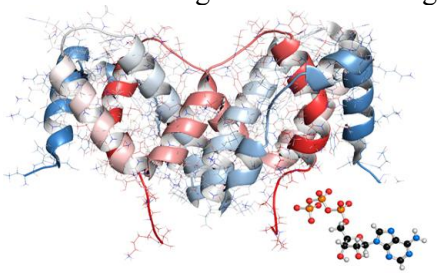

Fig.-2: Protein Ligand Interactions at the Active Site

RESULTS AND DISCUSSION

Binding Mode Classification

A key point when optimising kinase inhibitors is the classification of their binding modes. Kinase inhibitors have generally been classified according to how they bind to the DFG motif of kinases. The name DFG-in conveys the conformation generally associated with the occupation of the ATP-binding pocket by type I inhibitors.[14] Type I inhibitors compete directly with ATP, binding only when the kinase is in its active form. Conversely, type II inhibitors are designed to bind to unphosphorylated DFG-out conformers. In this DFG-out state, the position of the aspartate residue changes, creating a hydrophobic pocket adjacent to the ATP site; this allows for extra interactions that may increase binding affinity and specificity.[15] The design of type II inhibitors has been paramount to building selective molecules, as they help to reduce off-target effects that occur when competing with ATP, which is abundantly present inside cells, by exploiting the unique structural state of DFG-out. Kinase inhibitors using allosteric binding sites, aside from the classical two binding modes, are increasingly being developed.[16] Allosteric inhibitors do not bind to the ATP pocket but instead bind to other regions of the kinase molecule to induce conformational changes to inactivate the enzyme. This approach has expanded the therapeutic application of kinase inhibitors in tackling drug resistance. Since allosteric sites are less conserved among kinases, this approach also holds promise for yielding better selectivity profiles.[17] Furthermore, the implementation of structure-based drug design, which involves identifying prospective allosteric pockets, accelerates the development of novel modulators to inhibit kinase activity while also blocking compensatory signalling pathways commonly associated with resistance. Because docking simulations are typical methods for predicting binding interactions, the various docking studies reveal that inhibitors can have binding modes that correspond to particular kinases, either in DFG-in or DFG-out conformations. As these simulations explain, however, different factors such as ligand flexibility and water-mediated interactions assume huge importance when dealing with real inhibitors and their effective design[18] (Fig.-3).

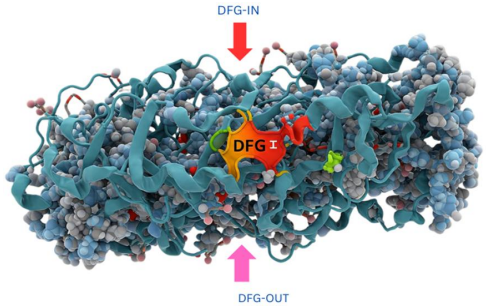

Fig.-3: Kinase Binding Modes

Emerging Trends in Kinase Inhibitor Development

Thanks largely to advances made in structural biology and computational chemistry, the last decade has seen some incredible breakthroughs in kinase-inhibitor development. One exciting trend that is forming is the reinvigorated emphasis on developing compounds selectively targeting the DFG-out conformation to overcome limitations of the classic ATP-competitive inhibitors. Investigations into DFG-out targeting resulted in the discovery of inhibitors with higher potency and selectivity.[19] For example, studies of Aurora A kinase have found that the judicious introduction of substituents such as halogen atoms or nitrile groups may induce the DFG-out conformation, which leads to effective stabilisation of the inactive form of the enzyme.[20] This approach increases target specificity and circumvents resistance mechanisms arising from mutations in the active site. The next noteworthy trend is the emergence of allosteric inhibitors. The therapeutic potential of allosteric inhibition is underscored by its ability to fine-tune kinase activity by binding to sites different from the ATP pocket.[21] Allosteric inhibitors are useful in limiting undesired off-target effects and also provide novel means of overcoming mutation-driven resistance to ATP-competitive inhibitors. For instance, SBDD and molecular docking approaches have been invaluable in the discovery and validation of novel allosteric sites, leading to the progression of type III and IV inhibitors.[22] This review aligns with Sustainable Development Goal 3 (Good Health and Well-Being) by

consolidating structure-guided strategies that can accelerate the discovery of safer, more effective targeted therapeutics.

The incredibly fast-paced evolution of computational sciences is performing an upheaval in drug discovery. One of the highlights amongst these developments is the integration of molecular docking and geometry-based deep learning to screen colossal libraries of compounds against extremely tight time frames. Such a mix has increased the predictive power of classical docking methods to give more clarity and details into binding affinities of molecules and their conformity to different shapes.[23] These computational techniques speed up the discovery of new drugs, helping us to explore chemical space in a better way, thereby revealing some new inhibitors that would have been missed by standard screening. Additionally, the updating of high-resolution structural data has completely breathed new life into iterative structure-guided design. Today, researchers seamlessly incorporate computational predictions into the experimental pipeline, thus helping in rapid fine-tuning of inhibitor scaffolds, optimisation of binding interactions, and reduction of chances of off-target effects.[24] This integrated approach of in silico methods with in vitro assays turned out to be remarkably efficient for tailor-making inhibitors to meet very specific structural traits of target kinases. In dealing with resistance to kinase inhibitors being a big challenge in the clinics, more focus has been placed on strategies that can directly confront this problem. For instance, design strategies that can reinforce inhibitors within the binding pockets through strong hydrogen bonding and hydrophobic interactions have gained extensive attention, which is crucial when mutations destabilise the structural integrity of the active site. Also, improvements in computational modelling of kinases in both their active and inactive states act as further help in identifying compounds that remain effective as resistance patterns evolve[25] (Table-1).

Table-1: Updated Classification of Kinase Inhibitors Based on Their Binding Modes

Binding-mode type	Kinase conformation / binding pocket	Competitive with ATP?	Reversibility	Key molecular contacts	Representative inhibitors
Type I	Active "DFG-in"; kinase hinge & adenine pocket	Yes	Reversible	Hinge H-bonds, hydrophobic pocket near gatekeeper	Gefitinib, Dasatinib, Crizotinib
Type I½	Inactive "DFG-in, αC-out" (intermediate)	Yes	Reversible	Hinge + extension into the back pocket	Lapatinib, Vemurafenib
Type II	Inactive "DFG-out" + adjacent allosteric back pocket	Yes (occupies ATP site & back cleft)	Reversible	Hinge H-bonds plus salt-bridge to Glu/DFG Asp	Imatinib, Sorafenib
Type III	Allosteric pocket next to, but not overlapping, the ATP site	No	Reversible	Unique hydrophobic cavity; avoids hinge	Trametinib, Cobimetinib, LIMK2 inhibitor 3
Type IV	The distal allosteric site is away from the catalytic cleft	No	Reversible	Regulatory or inter-domain pocket (often PH or FKBP site)	Everolimus (mTOR), Rapamycin
Type V	Bivalent inhibitors bridging two distinct sites (e.g., ATP + substrate or allosteric)	Partially	Reversible	Dual pharmacophore tethered by a linker	Staurosporine–peptide PKA inhibitor, EGFR ATP–allosteric bivalents
Type VI	Covalent bond to nucleophilic residue (usually	Irrelevant after bond formation	Irreversible (or slowly reversible)	Electrophilic "warhead" forms a covalent adduct	Afatinib, Ibrutinib, Osimertinib

	Cys) within ATP pocket				
Type VII	Non-classical allosteric inhibitors binding extracellular or juxtamembrane regions	No	Mostly irreversible	Blocks ligand binding or receptor dimerisation	SSR128129E (FGFR), DDR2 ECD inhibitor WRG-28

Future Perspectives

Looking forward, hybridisation of the newest computational and experimental methods will generate innovations in kinase inhibitor design. An exciting direction remains the continuing enhancements in computational chemistry methods involving geometric deep learning-based molecular docking. Such techniques have already demonstrated the merit of quickly and accurately predicting binding modes, including the slight differences that exist between DFG-in and DFG-out conformations.[26] Future endeavours will investigate more choice machine learning paradigms that would not only predict binding affinity but simultaneously couple with data regarding pharmacokinetics and toxicity derived from such structural data. Another interesting strategy involves integrated experimental assays combining high-throughput screens with biophysical characterisation tools that may advance, thanks to cryo-EM and NMR spectroscopy, to study the dynamic conformational changes of kinases.[27] Real-time conformational dynamics may alter the landscape of understanding the binding of inhibitors to both active kinases and inactive kinase states, which will steer the future generation of inhibitors. Moreover, the continued pursuit of allosteric inhibition is a critical future direction. Beyond the inherent limitations of ATP-competitive inhibitors, allosteric-binding-site-targeting drugs could show improved selectivity and reduced side effects. Enhanced knowledge of the allosteric network within kinases, supported by a combination of structural investigations and computational simulations, will probably unveil more regulatory sites for therapeutic targeting. Additionally, combinatorial approaches utilising both ATP-competitive and allosteric inhibitory mechanisms might arise as potent means of countering complex resistance mechanisms.[28] The future of medicinal chemistry involves elevating structure-based design by drawing upon knowledge from several sectors. For instance, a blending of systems biology with structural and computational chemistry will provide fresh solutions to acknowledge the behaviour of kinase networks in cellular conditions. At the same time, new experimental models and in vivo imaging techniques will be able to give clearer views of inhibitor action at biological levels and bring about the assessment of laboratory findings into applications.[29] In addition, next-generation sequencing and proteomics will provide great refinement to our understanding of the structure-function relationships of kinases. Such developments will characterise, in detail, kinase mutations and post-translational modifications that influence drug binding. Complemented by SBDD, this information will empower the designers to create inhibitors that are highly selective yet robust to adaptive changes toward cancer cells or other systems of disease. The next generation of kinase inhibitor development is an integrative science where advances in computational, chemical, and biological sciences are interwoven to produce successful treatment paradigms.[30]

CONCLUSION

Structure-based drug design has revolutionised the field of kinase inhibitor development, offering a firm ground for the creation of selective small molecules. This review has emphasised the paramount importance of the DFG motif in kinase conformational dynamics, especially concerning the "DFG-in" and "DFG-out" states, and considered how these conformations affect the binding mechanisms of type I and type II inhibitors. The advent of molecular docking, virtual screening, and geometric deep learning has enhanced classical SBDD approaches and become a turning point in the discovery and optimisation of kinase inhibitors. In addition to these, exploring allosteric inhibition might provide a novel way to overcome the limitations of ATP-competitive inhibitors. Allosteric inhibitors present opportunities for reduced off-target effects and greater ability to circumvent resistance, opening up an exciting new chapter in medicinal chemistry. Future studies will continue to enhance inhibitor design and expedite the clinical translation of these compounds, thanks to the continuous development in computational and experimental

techniques. To conclude, newer trends of the last decade, such as targeting DFG-out conformation, the employment of allosteric inhibition, and modern molecular docking techniques, have brought a paradigm shift in the area of kinase inhibitor development. To address the increasing complexity of kinase biology and the growing menace of drug resistance, future work should focus on newer computational methods and novel experimental assays towards improving the accuracy and speed of SBDD. The conjoined efforts are sure to lead us towards the next-generation kinase inhibitors with better efficacy, safety, and clinical utility. In line with SDG-3 (Good Health and Well-Being), these advances collectively support the development of targeted therapies with improved safety and clinical impact.

ACKNOWLEDGMENTS

The Authors Acknowledge Manipal Academy of Higher Education for the support provided.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Venkatesh Kamath https://orcid.org/0000-0001-6508-7823X
Chandrashekhar K.S. https://orcid.org/0000-0003-3667-1781
Aravinda Pai https://orcid.org/0000-0003-2010-9479
Muddukrishna B.S. https://orcid.org/0000-0001-6447-5759

Cite this article as: V. Kamath *et al.*, *Rasayan J. Chem.*, 19(2), 807-813(2026), https://doi.org/10.31788/RJC.2026.1929489.

REFERENCE

1. K.S. Bhullar, N.O. Lagarón, E.M. McGowan, I. Parmar, A. Jha, B.P. Hubbard, H.V. Rupasinghe, *Molecular Cancer*, **17(1)**,48(2018), https://doi.org/10.1186/s12943-018-0804-2
2. A.J. Bridges, *Chemical Reviews*, **101(8)**, 2541(2001), https://doi.org/10.1021/cr000250y
3. L.N. Johnson, *Quarterly Reviews of Biophysics,* **42(1)**,1(2009), https://doi.org/10.1017/S0033583508004745
4. Y. Meng, Y.L. Lin, B. Roux, *The Journal of Physical Chemistry B*, **119(4)**, 1443(2015), https://doi.org/10.1021/jp511792a
5. L.N. Johnson, *Biochemical Society Transactions*, **37(4)**, 627(2009), https://doi.org/10.1042/BST0370627
6. P.M. Ung, A. Schlessinger, *ACS Chemical Biology*, **10(1)**,269(2015), https://doi.org/10.1021/cb500696t
7. Z. Zhao, H. Wu, L. Wang, Y. Liu, S. Knapp, Q. Liu, N.S. Grey, *ACS Chemical Biology*, **9(6)**,1230(2014), https://doi.org/10.1021/cb500129t
8. C. Arter, L. Trask, S. Ward, S. Yeoh, R. Bayliss, *Journal of Biological Chemistry*, **298(8)**,102247(2022), https://doi.org/10.1016/j.jbc.2022.102247
9. V.R. Mingione, Y. Paung, I.R. Outhwaite, M.A. Seeliger, *Biochemical Society Transactions*, **51(1)**, 373(2023), https://doi.org/10.1042/BST20220940
10. H. Wei, J.A. McCammon, *npj Drug Discovery*, **1**, Article 1(2024), https://doi.org/10.1038/s44386-024-00001-2
11. L.G. Ferreira, R.N. Dos Santos, G. Oliva, A.D. Andricopulo, *Molecules,* **20(7)**,13384(2015), https://doi.org/10.3390/molecules200713384

12. E. Lionta, G. Spyrou, D.K. Vassilatis, Z. Cournia, *Current Topics in Medicinal Chemistry*, **14(16)**,1923(2014), https://doi.org/10.2174/1568026614666140929124445
13. Q. An, L. Huang, C. Wang, D. Wang, Y. Tu, *Frontiers in Pharmacology*, **16**(2025), https://doi.org/10.3389/fphar.2025.1550158
14. M. Xu, L. Yu, B. Wan, L. Yu, Q. Huang, *PLoS ONE*, **6**,7(2011), https://doi.org/10.1371/journal.pone.0022644
15. I. Kufareva, R. Abagyan, *Journal of Medicinal Chemistry*, **51(24)**, 7921(2008), https://doi.org/10.1021/jm8010299
16. Q. Zhang, X. Zhang, Q. You, *Molecules*, **21(7)**,879(2016), https://doi.org/10.3390/molecules21070879
17. J. Li, C. Gong, H. Zhou, J. Liu, X. Xia, W. Ha, Y. Jiang, Q. Liu, H. Xiong, *International Journal of Molecular Sciences*, **25(10)**,5489(2024), https://doi.org/10.3390/ijms25105489
18. C. Arter, L. Trask, S. Ward, S. Yeoh, R. Bayliss, *Journal of Biological Chemistry*, **298(8)**, 102247(2022), https://doi.org/10.1016/j.jbc.2022.102247
19. P. Ranjitkar, A.M. Brock, D.J. Maly, *Chemistry & Biology*, **17(2)**, 195(2010), https://doi.org/10.1016/j.chembiol.2010.01.008
20. M.P. Martin, J.Y. Zhu, H.R. Lawrence, R. Pireddu, Y. Luo, R. Alam, S. Ozcan, S.M. Sebti, N.J. Lawrence, E. Schönbrunn, *ACS Chemical Biology*, **7(4)**, 698(2012), https://doi.org/10.1021/cb200508b
21. L. Li, C. Meyer, Z.W. Zhou, A. Elmezayen, K. Westover, *Journal of Molecular Biology*, **434(17)**,167626(2022), https://doi.org/10.1016/j.jmb.2022.167626
22. E. Lionta, G. Spyrou, D.K. Vassilatis, Z. Cournia, *Current Topics in Medicinal Chemistry,* **14(16)**,1923(2014), https://doi.org/10.2174/1568026614666140929124445
23. L. Pinzi, G. Rastelli, *International Journal of Molecular Sciences*, **20(18)**,4331(2019), https://doi.org/10.3390/ijms20184331
24. G. Sliwoski, S. Kothiwale, J. Meiler, E.W. Lowe Jr., *Pharmacological Reviews*, **66(1)**, 334(2013), https://doi.org/10.1124/pr.112.007336
25. C.M. Lovly, A.T. Shaw, *Clinical Cancer Research*, **20(9)**, 2249(2014), https://doi.org/10.1158/1078-0432.CCR-13-1610
26. P.H.M. Torres, A.C.R. Sodero, P. Jofily, F.P. Silva Jr., *International Journal of Molecular Sciences*, **20(18)**, 4574(2019), https://doi.org/10.3390/ijms20184574
27. A.I. Visan, I. Negut, *Life*, **14(2)**, 233(2024), https://doi.org/10.3390/life14020233
28. N.R. Gough, C.G. Kalodimos, *Current Opinion in Structural Biology*, **88**, 102890(2024), https://doi.org/10.1016/j.sbi.2024.102890
29. A.C. Kaushik, S. Sahi, D.Q. Wei, *Methods in Molecular Biology*, pp. 161(2022), https://doi.org/10.1007/978-1-0716-1767-0_9
30. L.K. Dogiparthi, S.P.N. Bukke, C. Thalluri, et al., *Discoveries in Applied Sciences*, **7**, Article 552(2025), https://doi.org/10.1234/das.2025.552

[RJC-9489/2026]