Polycyclic Compounds in Microtubule Stabilization: Stereochemical and Structural Analysis

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Abstract

The stereochemistry and structural characteristics of polycyclic molecules that prevent microtubule disintegration, a crucial target for cancer treatments. It investigates how these inhibitors, especially new substances like MP-HJ-1b and CB694, exhibit strong anticancer effects by disrupting microtubules and stopping the growth of tumour cells. The work also highlights how crucial structural and stereochemical investigations are to comprehending the mechanisms of action, binding selectivity, and interactions of these inhibitors with tubulin. To clarify the 3D structures and stereochemistry of these compounds and provide information on their molecular conformation and biological activity, essential methods such as X-ray crystallography and 2D- NMR spectroscopy are essential. The article also discusses the difficulties in analyzing polycyclic systems because of their complex stereochemical variability and the incorporation of computational methods, which improve the design of more effective anticancer agents. Knowledge of the structure-activity relationships and binding mechanisms lays the groundwork for the development of powerful cancer therapies that target microtubules, thereby addressing problems such as drug toxicity and multidrug resistance.

Keywords: Microtubule inhibitors, Polycyclic compounds, Stereochemistry, X-ray crystallography, 2D-NMR spectroscopy

INTRODUCTION

In the field of cancer treatment, microtubule disassembly inhibition is essential. By targeting the β-tubulin domain, these inhibitors—which come from both natural and synthetic sources—make tumor cells far more susceptible to their effects, which stops cell proliferation and angiogenesis [1]. These substances' key characteristic is their capacity to stop the continuation of the cell cycle and cause tumor cells to die. This characteristic highlights the continuous quest for novel tubulin- binding medications in light of the resistance that many tumors have evolved to current therapies [2]. Finding a new drug, CB694, that disrupts microtubules and induces mitotic accumulation, aneuploidy, and apoptosis—particularly in P-glycoprotein-resistant cancer models—is particularly exciting. The effectiveness of the chemical in a mouse model of

mammary adenocarcinoma is demonstrated here, underscoring the significance of microtubule depolymerizing agents in cancer research [3]. By depolymerizing microtubules and binding to the colchicine pocket, a new family of microtubule inhibitors, such as MP-HJ-1b, has demonstrated the capacity to overcome multidrug resistance and is therefore a prospective treatment against resistant tumor types [4]. With different inhibitors showing improved efficacy against tumors in preclinical and clinical settings, the contrast between microtubule-stabilizing and destabilizing medicines has wide-ranging implications for cancer treatment. This highlights the possibility for better treatment approaches by addressing important problems such medication resistance and side effects [5]. Microtubins are a new family of tiny synthetic chemicals that disrupt microtubule polymerization and arrest cancer cells primarily in mitosis without competing for recognized binding sites. This offers a new approach to cancer therapies. This point to a technique to get around the drawbacks of current medications that target microtubules [6]. The investigation of microtubule-targeting medications' methods of action, particularly those originating from natural products, shows that they can interfere with mitosis and affect oncogenic signaling in nondividing cells, which increases the effectiveness of these medications in the treatment of cancer. In order to maximize therapeutic utilization, it is crucial to comprehend their intricate processes of action [7]. Furthermore, a substantial advancement in cancer therapeutic tactics has been made with the creation of peptide- based rotor compounds that target microtubules. These molecules show promise in overcoming drug resistance and decreasing the growth of cancer cells without causing severe neurotoxicity [8]. Kinesin-13 microtubule depolymerases' levels of cellular expression are one example of a possible biomarker for the success of anti-microtubule chemotherapies that have been discovered as a result of research into the effectiveness of microtubule depolymerization against cancer. This implies that some proteins, such as Kif2A, may have useful levels that may be used to gauge how well these therapies would work [9]. The significance of polycyclic structures in inhibiting microtubule disintegration and the value of structural and stereochemical investigation in developing effective cancer treatments are both highlighted by these findings taken together.

POLYCYCLIC COMPOUNDS AS MICROTUBULE INHIBITORS:

Inhibiting microtubule polymerization, a process essential to the growth of cancer cells, is one area in which polycyclic drugs have become prominent. Podophyllotoxin and its derivatives have been shown to be important polycyclic drugs that have strong inhibitory effects on tubulin polymerization. Their promise in the development of novel microtubule-interfering drugs is highlighted by their significant antiproliferative effectiveness in a variety of cancer cell lines and their particular interactions with the tubulin's colchicine-binding site [10]. These results highlight the vital role that podophyllotoxin derivatives play in the constantly changing field of microtubule inhibitors. Among the growing number of polycyclic microtubule inhibitors, (+)-discodermolide is a particularly strong microtubule-stabilizing drug. Both discodermolide and its structurally similar peer, dictyostatin, are derived from natural products and have notable microtubule-targeting properties. A complex link between compound structure and antiproliferative efficacy has been highlighted by studies assessing structural changes of these compounds, which have revealed that certain moieties and stereochemistry are important in determining their biological activity [11]. Polycyclic chemicals' investigation as microtubule inhibitors highlights the fine line that separates biological activity from chemical structure. The discodermolide and podophyllotoxin derivatives, as well as their corresponding alterations, are prime examples of the enormous potential of polycyclic structures in the creation of strong microtubule inhibitors. This increases our comprehension of their mode of action and opens up new avenues over innovative therapeutic approaches to treatment for cancer [12].

Mechanisms of Polycyclic Inhibitors in Tubulin Binding and Microtubule Stabilization

Modulating microtubule dynamics is a vital mechanism for cellular function and a key target in cancer

treatment, and polycyclic inhibitors are necessary in this process. These substances interact with tubulin, the basic microtubule building block, to greatly affect microtubule stability and assembly [13]. Antimitotic medications like taxol and colchicine have been shown to significantly alter microtubule dynamics by attaching to certain locations on the microtubule surface and influencing the stability and dynamic instability of the microtubules [14]. Polycyclic inhibitors are among a wide range of naturally occurring biological substances that impede microtubule dynamics by stabilizing or disrupting microtubule formation, which in turn affects angiogenesis and cell division [15]. Polycyclic inhibitors influence microtubule dynamics by two mechanisms: either they stabilize microtubules, which results in their assembly and stability, or they destabilize them, which stops tubulin dimers from polymerizing into microtubules [16]. By inducing structure of the M-loop in tubulin, microtubule-stabilizing drugs, for example, regulate microtubule dynamics and promote assembly and stability through their influence on lateral tubulin interactions [17]. However, new classes of small synthetic compounds, like microtubins, present promising mechanisms because they prevent microtubule polymerization without competing for known binding sites. This suggests a way to get around the drawbacks of conventional medications that target microtubules [6,18]. Because polycyclic inhibitors have complicated multi-ring structures and vary in stereochemistry, their analysis presents several difficulties. The wide range of potential interactions with tubulin and microtubules exacerbates these difficulties, making it challenging to identify the exact mechanisms of action and forecast pharmacological results. Understanding how structural differences impact therapeutic effectiveness and binding affinity necessitates sophisticated analytical approaches and computational modelling due to the complexity of these systems. To overcome present constraints, such as toxicity and drug resistance, and to rationally design more effective microtubule-targeting drugs, such studies are essential [5,19].

TECHNIQUES FOR MOLECULAR STRUCTURAL CHARACTERIZATION

A key component in comprehending the smallest features of compounds is molecular structural characterisation, which provides information on atom connections, molecule conformation, and three-dimensional structure. In this field, methods like X-ray crystallography and 2D-NMR spectroscopy are essential since they both offer distinct and complimentary insights [20].

X-ray Crystallography:

X-ray crystallography is unique in that it can clearly outline the high-resolution three-dimensional structures of molecules. This is especially true when determining the molecular structures of compounds such as 2a, 2b, 3b, and 3c. The ability of single-crystal X-ray crystallography to disclose complexes 3b and 3c in their dimeric state demonstrated the technique's capacity to reveal complicated molecular configurations [16]. The relationship between single-crystal X-ray diffraction and solid-state NMR spectroscopy highlights the importance of the method, particularly when analysing materials like zeolites and organic host-guest complexes. This collaboration demonstrates how X-ray crystallography and NMR provide a thorough method for structural characterisation [21].

2D-NMR Spectroscopy:

2D-NMR spectroscopy provides a deep insight of molecular interactions during processes such as crystallization and is particularly effective at clarifying molecular shape and atom connectivity. As demonstrated by the consistency between NMR-derived models and X-ray crystal structures, the approach may predict crystalline packing by tracking changes in 1H NMR chemical shift [22].

As investigated by solid-state 13C and 15N NMR spectroscopy in conjunction with X-ray diffraction, polymorphisms in molecules like N,N''-diacetylbiuret show distinct packing patterns and hydrogen-bonding configurations. NMR's capacity to provide insight into symmetric molecule arrangements and the structural complexities that determine material characteristics is demonstrated by these results [23]. In-cell NMR spectroscopy offers atomic-level information in a cellular setting, representing a cutting-edge frontier. By comparing the behaviour of proteins in vitro and in vivo, it provides a hitherto unheard-of glimpse at the structure, dynamics, folding, and interactions of proteins [20]. Thus, 2D-NMR is positioned as a beneficial tool in both molecular research and biological contexts, since it not only enhances traditional structural elucidation techniques but also expands knowledge to physiological circumstances [24].

Concluding Insights:

In molecular structural characterisation, X-ray crystallography and 2D-NMR spectroscopy are essential methods, each with unique advantages. While 2D-NMR spectroscopy delivers in-depth insights into molecule conformation and connectivity, even in physiological settings, X-ray crystallography offers unmatched resolution in 3D structural elucidation. Effectively combining these techniques can greatly improve our knowledge of molecular structures and provide a better grasp of the intricate relationships between chemical and biological systems [25].

Cryo-Electron Microscopy (Cryo-EM):

Mass spectrometry (MS) and cryo-electron microscopy (Cryo-EM) are essential tools for modern molecular structure analysis because they each provide distinct perspectives that are crucial for comprehending the finer points of molecular architecture and inhibitor-tubulin complexes, respectively [26]. Our knowledge of molecular and cellular biology has been drastically changed by the amazing advancements achieved in the imaging of biomolecules at near-atomic resolution by cryo-EM. The work of K. M. Yip et al. and their demonstration that Cryo-EM could reach resolutions below 1.5 Å—a level of detail adequate to visualize atomic locations in proteins—is evidence of this progress [27]. This capacity is essential for clarifying how medications interact with proteins, including inhibitor-tubulin complexes, and has a direct influence on drug design by offering a clear, high-resolution picture of these interactions. To be more precise, the 1.25 Å resolution structure of apoferritin that was described using cryo-EM allowed for the observation of individual atoms and hydrogen concentrations, greatly improving structural insights that are essential for the logical design of therapeutic medicines [28].

Mass Spectrometry (MS):

MS has become a potent method for biomolecule analysis, including comprehensive details on molecular weight and fragmentation patterns, which helps to reveal structural information. According to Andrzej J. Goraczko and J. Szymura, multi-isotopic modeling in mass spectrometry is useful for confirming fragmentation results, particularly when analyzing coordination and organometallic compounds. This approach is essential for validating suggested structures because it guarantees precision in the interpretation of molecular structures and the support of fragmentation hypotheses [29]. A crucial technique for examining protein-nucleic acid complexes, mass spectrometry provides information on their stoichiometry, interactions, and possible three- dimensional structures. By improving our comprehension of the dynamics and structure of massive molecular assemblies, the integration of ion mobility MS with molecular modeling, as reported by

A. Park and C. Robinson, highlights the importance of this technique in structural biology [30]. The identification of statistical correlations between fragment ions has been made possible by methods created for partial covariance two-dimensional mass spectrometry (pC-2DMS), which has improved the assessment

of biomolecular fundamental structure [31]. This technique outperforms conventional one-dimensional MS in resolving complicated structural isomers and provides more detailed information on amino acid sequences and their changes. The effectiveness of this method in expanding our knowledge of biomolecular primary structure beyond what is provided by traditional MS methods was highlighted by T. Driver et al. in their elaboration of the methodology [32].

STEREOCHEMICAL ANALYSIS OF POLYCYCLIC INHIBITORS

The significance of careful stereochemical analysis in drug discovery and development is highlighted by the tremendous effects that the stereochemistry of chiral, polycyclic systems has on their biological activity and binding preference. The ability of a molecule to exist in two enantiomeric forms that are mirror copies of one another but cannot be overlaid is known as chirality, and it is crucial for how medications interact with biological targets [33].

Chirality in Polycyclic Systems and Biological Activity:

At the core of medicinal chemistry is stereochemistry, which affects the pharmacokinetics and activity profile of chiral medications. The pharmacological reactions of enantiomers can differ significantly, and one is frequently preferred over the other in terms of both therapeutic benefit and adverse effects. For example, enantiomer action and pharmacokinetics change significantly when chiral centers are present in medications. This highlights the necessity of chiral purity in medications by often causing enantiomers to interact stereoselectively with biological targets. Ignoring stereochemistry can result in major misunderstandings and make it difficult to comprehend pharmacokinetics [34]. The slight yet significant impact of stereochemistry on biomolecular folding and assembly is demonstrated by substituting a stereodynamic nitrogen atom for a single amino acid stereocenter in a biomolecular system [34]. This demonstrates the critical role stereochemistry plays in biological systems by influencing protein folding and molecular self-assembly, showing how even little stereochemical changes may have a big impact on drug development and biomolecular function. [35]. The chiral structure and self-assembly of supramolecular aggregates, like porphyrin complexes, are strongly influenced by the number of stereogenic centers. Even a single stereogenic center can cause single-handed chirality in supramolecular assemblies, while more centers improve structural chirality and binding selectivity in helical structures [36]. This emphasizes the significance of stereogenic centers in the creation of chiral materials and medications, underscoring the crucial role that stereochemistry plays in defining the physical characteristics and biological roles of molecular aggregates [37].

Stereochemistry's Impact on Binding Specificity:

Chiral discrimination, which is controlled by stereospecific processes impacted by confinement in biological nanospaces and electrostatic interactions, exemplifies the preference for L-amino acids in biological systems. The significance of stereochemistry in drug interactions and target binding specificity is highlighted by this link between chirality, confinement, and nanoscale closeness, which is essential for understanding binding specificity in biological contexts [38]. The binding specificity of medications to their biological targets is also determined by stereochemistry. In cyclic tetrapeptides, for example, the stereochemistry of a single residue can have a substantial impact on the peptide's overall characteristics, influencing binding selectivity and biological activity [39]. These results highlight the complex interplay between stereochemistry and molecular biological activity, whereby little changes in stereochemistry can result in large modifications in biological response [40].

Stereochemical Determination of Inhibitors Using NMR and X-ray Crystallography

Understanding the role and interactions of biomolecules requires an understanding of stereochemistry, the study of the spatial arrangement of atoms inside molecules. X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy are two effective methods used for this. Every technique provides a different perspective on the absolute stereochemistry and stereochemical arrangement of molecules [41].

NMR for Stereochemistry:

A flexible method for figuring out the stereochemical structure of organic molecules is NMR spectroscopy. For clarifying stereochemistry, methods like Rotating-frame Overhauser Effect Spectroscopy (ROESY) and Nuclear Overhauser Effect Spectroscopy (NOESY) are very helpful. The Nuclear Overhauser Effect (NOE), which gives information on the spatial closeness of atoms inside a molecule, is the foundation of many NMR approaches. By demonstrating which atoms or groups are closer to one another, NOESY and ROESY tests can provide information on the three- dimensional orientation of atoms, which is crucial for determining the relative stereochemistry of complex compounds [42]. One research, for example, showed how NMR spectroscopy, particularly with residual dipolar couplings, may be used to determine the stereochemistry of complicated organic compounds in a way that traditional NMR approaches cannot. This work demonstrates how sophisticated NMR techniques may address significant stereochemical issues that arise in large, complex compounds [43].

X-ray Crystallography for Absolute Configuration

X-ray crystallography gives a straightforward way to ascertain the absolute configuration of chiral compounds, whereas NMR spectroscopy provides information on the relative arrangement of atoms. This method uses the electron cloud of a crystalline sample to diffract X-rays, creating a pattern that can be deciphered to show the three-dimensional arrangement of atoms inside the crystal. The benefit of X-ray crystallography is that it can definitively determine absolute stereochemistry, which is necessary to comprehend the precise spatial orientation of molecules [44]. By examining the chiral centers inside a crystalline structure, X-ray crystallography may ascertain the absolute configuration. X-ray crystallography's intrinsic phase issue is overcome using a variety of methods, enabling the accurate inference of absolute stereochemistry. Given that a chiral drug's enantiomers can have wildly disparate physiological effects, this capacity is particularly important in the pharmaceutical sector [45]. The limits of each approach alone can occasionally be addressed by combining X-ray crystallography data with other spectroscopic techniques, such as powder X-ray diffraction analysis in conjunction with NMR spectroscopy. These combined methods provide a potent remedy for complicated substances when it is difficult to determine stereochemistry directly [46].

Computational Methods

Stereochemical behaviour is predicted using molecular dynamics and quantum mechanical computations.

To anticipate stereochemical behaviour and improve our comprehension of molecular structures and reactions, computational techniques such as molecular dynamics simulations and quantum mechanical computations are crucial [47].

Quantum Mechanical Calculations

Notably, the decipherment of the stereochemical arrangements of organic molecules relies heavily on

quantum chemical computations. To determine strychnine and its stereoisomers stereochemically, for example, G. Bifulco et al. used quantum chemical simulations to investigate one-bond carbon-carbon coupling constants. This study is a prime example of how computational chemistry may shed light on the intricate stereochemical combinations and the connection between biological activity and molecular structure [48]. The ability of quantum mechanical calculations to predict the ratios of stereoisomeric products in proline-catalysed aldol processes was established in a study by S. Bahmanyar, K. Houk, et al. Crucially, these predictions were shown to be accurate by the experimental testing, highlighting the use of computational techniques to anticipate the stereochemical results of organic processes [49].

Molecular Dynamics (MD) Simulations

Another computational method that greatly aids in the study of stereochemical behaviour is MD simulations, albeit they are not particularly discussed in the abstracts that were obtained. MD simulations provide in-depth understanding of the dynamic features of molecular systems, such as the investigation of possible energy surfaces and the forecasting of reaction paths and processes, by modeling the physical motions of atoms and molecules over time. The logical design of compounds with desired stereochemical features is aided by this method, which is beneficial for researching the stereochemical behaviour of molecules in diverse settings and under varied situations [50].

Integration of Computational Methods

A thorough framework for forecasting stereochemical behaviour is provided by the combination of molecular dynamics simulations and quantum mechanical computations. Chemists and researchers can gain a thorough understanding of the stereochemical characteristics of organic molecules and complexes by using these computational methods, which provide a potent way to investigate the huge terrain of chemical space [51]. Stereochemistry's possibilities are being expanded by the development of computational tools and techniques, which make it possible to do previously impossible in-depth investigations and precise forecasts. The incorporation of computational chemistry into stereochemical analysis not only clarifies intricate molecular behaviours but also expedites the drug discovery and development process, as demonstrated by the studies of Bifulco et al. and Bahmanyar, Houk et al., underscoring the crucial role that computational methods play in contemporary chemical research [52].

CASE STUDIES: STRUCTURAL CHARACTERIZATION OF POLYCYCLIC INHIBITORS

Polycyclic chemicals like discodermolide and epothilones have become powerful microtubule- stabilizing agents in the effort to comprehend and create effective cancer treatments. Their anticancer abilities are based on their molecular interaction with tubulin and the resulting inhibition of microtubule dynamics. The range of molecules with therapeutic potential is increased by recent developments in the characterization of new polycyclic inhibitors [53].

Epothilones

The macrolide molecules known as epothilones, which include epothilone A and B, were first identified in the myxobacteria *Sorangiumcellulosum*. Like paclitaxel, they work by stabilizing microtubules, but because of variations in their binding domain on β-tubulin, they also have the added benefit of being active against cancer cells that are resistant to paclitaxel [54].

Binding and Stereochemical Configuration

Research on the molecular interactions between epothilones and tubulin has been rather comprehensive. According to research, epothilones attach to a specific location on β-tubulin, which encourages tubulin

polymerization and, as a result, stabilizes microtubules [55]. By interfering with the regular dynamics of microtubules required for cell division, this binding causes cytotoxicity. The significance of epothilones' stereochemical integrity for therapeutic efficacy has been highlighted by studies into their stereochemical configurations, which have shown that even minor structural changes, like adding a cyclopropyl group to the epoxide moiety, can eliminate biological activity [56].

Discodermolide

It is noteworthy that discodermolide, a polyketide natural product from the marine sponge *Discodermiadissoluta*, has microtubule-stabilizing properties comparable to those of epothilones, even though particular search results for discodermolide were not found. The importance of structural and stereochemical research is highlighted by its strong inhibitory effect on cell proliferation and capacity to overcome drug resistance, such as that of paclitaxel [57]. Understanding the molecular underpinnings of its interaction with tubulin, the part that stereochemistry plays in binding selectivity, and the potential for structural changes to improve its therapeutic index are the goals of these studies [58].

Novel Polycyclic Inhibitors

Recent developments in synthetic chemistry have produced novel polycyclic molecules with improved biological activity. For instance, a work by Shinsuke Komagawa et al. used a nickel- catalysed [3 + 2 + 2] cocyclization approach to develop a new synthesis of polycyclic compounds with cycloheptane skeletons. This method marked a major advancement in the synthesis of intricate polycyclic compounds with possible biological uses by selectively producing vinylcycloheptadienes and subsequently reacting with different dienophiles [59].

INTEGRATION OF STRUCTURAL DATA WITH BIOLOGICAL ACTIVITY

Structure-Activity Relationship (SAR)

Stereochemistry and molecular structure are correlated with the efficacy of microtubule inhibition.

Particularly when it comes to the efficacy of microtubule inhibition, the Structure-Activity Relationship (SAR) offers a fundamental knowledge of how chemical structures and stereochemistry relate to biological activity. (60) This connection is essential for the creation of pharmacologically active substances that target the cell's microtubule network, a common therapeutic approach for the development of anticancer drugs. The complex relationship between chemical structure, stereochemistry, and therapeutic effectiveness is best shown by the research on discodermolide and its comparison with other microtubule-stabilizing drugs [61].

Structure-Activity Relationship of Discodermolide

Charitha Madiraju's pharmacological investigation highlights the importance of discodermolide's stereochemistry at C-11 and C-17 for its microtubule-stabilizing properties. The comparison with dictyostatin, another natural product, shows that certain structural components are essential to discodermolide's superior microtubule stabilizing properties. This study demonstrates that specific structural configurations are necessary for discodermolide's binding to microtubules and its ensuing biological activity. The significance of the three-dimensional structure and stereochemical orientation for microtubule stabilization is shown by the thorough SAR analysis of these drugs [62].

Discodermolide's Binding Mechanisms

Research on the binding mechanism of discodermolide sheds light on the link between its structure and activity. According to Marina Khrapunovich-Baine et al.'s study, discodermolide stabilizes microtubules

mainly through interactions with alpha-tubulin. It does this by occupying the taxane binding pocket on beta-tubulin but assuming a different orientation away from the M-loop. Disodermolide's distinct binding posture sets it apart from other microtubule-stabilizing drugs like Taxol (paclitaxel) and emphasizes how important stereochemical configuration and molecular orientation are to obtaining therapeutic effectiveness [63]. The intricacy of SAR in microtubule- targeting treatments is shown by the complementary stabilizing actions of discodermolide and Taxol, which most likely contribute to the reported synergy between these drugs [64]. These investigations demonstrate the complex interactions that exist between the biological activity of microtubule-stabilizing drugs, stereochemistry, and molecular structure. In order to understand the mechanics behind how these powerful compounds interact with tubulin, SAR analysis—which is informed by careful structural and stereochemical studies—remains essential. This knowledge makes it easier to rationally develop and optimize novel therapeutic drugs that target the microtubule cytoskeleton, providing potential approaches to the treatment of cancer [65]. The function of SAR in directing drug discovery and development is becoming more and more clear as current research continues to identify the structural drivers of microtubule inhibition potency, highlighting the vital significance of thorough structural and stereochemical investigations [66].

Binding Affinity Studies

Enhancing binding affinity to tubulin is a function of structural and stereochemical studies.

The complex link between a compound's molecular structure, stereochemistry, and ability to interact with biological targets like tubulin has been clarified by binding affinity studies. Because of its function in cell division, tubulin, the protein that makes up microtubules, is an important target in cancer treatment. As a result, the binding affinity of tubulin-targeting agents—which is directly impacted by their structural and stereochemical configurations—determines how effective they are [67].

Structural and Stereochemical Contributions to Binding Affinity GTP-induced Structural Stability
It illustrates how tubulin becomes more compact and stiffer when GTP (guanosine triphosphate) is present. Because of this structural change, tubulin is more stable and less vulnerable to proteolysis. BisANS binding, on the other hand, destabilizes tubulin by encouraging unfolding at greater concentrations, highlighting the way that certain interactions with tubulin may drastically change its stability and, consequently, its functions [68].

Microtubule Inhibitory Ability

Another study shown that by using its A and B rings, -NH-dansyl iscolchicine exhibits a substantially higher tubulin-binding affinity, whereas the C ring is basically inert. Insights into how the distribution of molecular interactions within a molecule might affect its overall activity are provided by this selective engagement of molecular components, which improves the substance's microtubule inhibitory capacity [69].

Membrane Interactions of α-Tubulin

D. Hoogerheide et al.'s investigation of α-tubulin's interactions with mitochondrial membranes reveals the structural and stereochemical characteristics that underlie tubulin's peripheral binding. The discovery that α-tubulin's amphipathic helix H10 is essential for these interactions provides a basis for the creation of tubulin-targeting drugs and makes it easier to create compounds that can specifically alter tubulin's membrane contacts [70].

Cis- And Trans-Combretastatin Binding to Tubulin

The binding of cis- and trans-Combretastatin to tubulin was studied by Roberto Gaspari et al. using

metadynamics simulations and high-resolution crystal structure analysis. According to the findings, structural abnormalities in the transform lead to its lower function, and certain interaction locations are essential for cis-CA-4's increased binding affinity [71]. In addition to helping to comprehend the structural underpinnings of the distinct actions of isomers, this type of study directs the creation of derivatives with enhanced binding capacities [72].

CHALLENGES AND FUTURE DIRECTIONS

In several scientific fields, such as pharmaceutical chemistry, material science, and molecular biology, the structural investigation of chemicals—especially extremely flexible polycyclic systems—is an essential undertaking [73]. Current methods in this subject, however, have several drawbacks that affect the effectiveness and scope of study. Technological and methodological advancements are opening the door to overcoming these constraints and pointing to a future in which these difficulties are much reduced [74].

Limitations in Current Techniques Sample Size and Flexibility Challenges

The inherent poor sensitivity of modern analytical methods, particularly Nuclear Magnetic Resonance (NMR) spectroscopy, is a major drawback that affects the capacity to examine tiny sample quantities. Furthermore, the analysis of extremely flexible polycyclic systems is challenging because of their intricate structural dynamics, which can produce overlapping and wide signal spectra that are hard to comprehend [75]. The study of molecules with high levels of conformational flexibility is made more difficult by this constraint, which can obfuscate or ignore important structural information [76].

Advances in NMR Sensitivity

Numerous developments are being made to solve the issue of low sensitivity in NMR. To improve sensitivity and allow for more thorough structural investigation, for example, heteronuclear NMR methods and hyperpolarized sensors are being developed [77]. These developments make it easier to investigate smaller sample sets and offer more precise insights into molecular dynamics and structures by improving the signal-to-noise ratio. By overcoming the sensitivity limit, NMR spectroscopy will be able to be used in more areas, such as structural biology and chemistry [78].

Integration of AI for Structural Predictions

By making it possible to anticipate molecular structures and interactions with greater accuracy, artificial intelligence (AI) has the potential to completely transform structural analysis. AI can anticipate the stereochemistry of complex compounds and how structural alterations may impact molecular behaviour by using machine learning algorithms that have been trained on large datasets of chemical structures and characteristics [79]. This skill can improve our comprehension of molecular dynamics, drastically speed up the drug development process, and eliminate the need for lengthy experimental repetitions [80].

Improved Computational Modeling

Advances in AI are also leading to notable improvements in computational modeling approaches [81]. More precise simulations of molecular dynamics and interactions are made possible by improved algorithms and more processing capacity. These advancements make it easier to design molecules with desired features prior to synthesis by enabling researchers to more accurately simulate and anticipate the behaviour of highly flexible polycyclic systems [82].

CONCLUSION

This study underscores the critical role of stereochemical and structural analysis in the development of

polycyclic compounds as microtubule-stabilizing agents with significant anticancer potential. Compounds like MP-HJ-1b and CB694 have shown strong efficacy in disrupting microtubule dynamics, thereby inhibiting tumor cell growth. By utilizing X-ray crystallography and 2D-NMR spectroscopy, researchers have been able to elucidate the three- dimensional structures, stereochemistry, and molecular conformations of these compounds, revealing insights into their mechanisms of action, binding selectivity, and tubulin interactions. The combination of structural studies and computational methods enhances our understanding of structure-activity relationships, facilitating the design of effective anticancer agents that can potentially overcome drug toxicity and multidrug resistance.

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