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Computational benchmarking of putative KIFC1 inhibitors

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Abstract

The centrosome in animal cells is instrumental in spindle pole formation, nucleation, proper alignment of microtubules during cell division, and distribution of chromosomes in each daughter cell. Centrosome amplification involving structural and numerical abnormalities in the centrosome can cause chromosomal instability and dysregulation of the cell cycle, leading to cancer development and metastasis. However, disturbances caused by centrosome amplification can also limit cancer cell survival by activating mitotic checkpoints and promoting mitotic catastrophe. As a smart escape, cancer cells cluster their surplus of centrosomes into pseudo-bipolar spindles and progress through the cell cycle. This phenomenon, known as centrosome clustering (CC), involves many proteins and has garnered considerable attention as a specific cancer cell-targeting weapon. The kinesin-14 motor protein KIFC1 is a minus end-directed motor protein that is involved in CC. Because KIFC1 is upregulated in various cancers and modulates oncogenic signaling cascades, it has emerged as a potential chemotherapeutic target. Many molecules have been identified as KIFC1 inhibitors because of their centrosome declustering activity in cancer cells. Despite the ever-increasing

Abbreviations: ADP, adenosine diphosphate; APC, anaphase-promoting complex; ATP, adenosine triphosphate; CA, centrosome amplification; CC, centrosome clustering; CENP, centromere protein; CPC, chromosome passenger complex; DNA, deoxyribonucleic acid; G2/M phase, growth 2/mitotic phase; GFP, green fluorescent protein; HTS, high-throughput screening; IC_{50}, Inhibition concentration for 50 percent cell death; ILK, integrin-linked kinase; KIFC1, kinesin family member C1; MDM2, mouse double minute 2 homolog; Mg-ADP, magnesium-ADP; MTOC, microtubule organizing center; nM, nanomolar; PCM, pericentriolar material; PLK, polo-like kinase; RNA, ribonucleic acid; SCCHN, squamous cell carcinoma of head and neck; STAT, signal transducer and activator of transcription; TNBC, triple-negative breast cancer; µM, micromolar.

literature in this field, there have been few efforts to review the progress. The current review aims to collate and present an in-depth analysis of known KIFC1 inhibitors and their biological activities. Additionally, we present computational docking data of putative KIFC1 inhibitors with their binding sites and binding affinities. This first-of-kind comparative analysis involving experimental biology, chemistry, and computational docking of different KIFC1 inhibitors may help guide decision-making in the selection and design of potent inhibitors.

KEYWORDS

centrosome amplification, centrosome declustering, KIFC1, KIFC1 inhibitors, molecular docking

1 | INTRODUCTION

The centrosome is a membrane-free organelle near the nucleus. It is composed of two perpendicularly organized centrioles surrounded by a pericentriolar matrix of different proteins. The centrosome undergoes duplication during the synthesis phase (S phase) of the cell cycle, and later during the mitosis phase, it assists in establishing microtubule spindle poles for executing chromatid separation.[1]

Abnormalities in the centrosome number or structure, known as centrosome amplification (CA), can promote carcinogenesis.[2] An excess of centrosomes pushes the cell to a spindle multipolarity stage, following syntelic (attachment of the kinetochore of both the sister chromatids to microtubules from single spindle pole) and merotelic (attachment of single kinetochore of a sister chromatid from microtubules coming out of both the spindle poles) attachments.[3] Spindle multipolarity can lead to low-grade aneuploidy and chromosomal instability, increasing intratumoral heterogeneity and the metastatic potential of cancer cells. Cell cycle checkpoints can prevent the multiplication of cancer cells harboring extra centrosomes by promoting mitotic catastrophe. However, cancer cells can overcome mitotic catastrophe by clustering extra centrosomes into two functional spindle poles, known as pseudo-bipolar spindles (Figure 1).[4] Since this phenomenon occurs exclusively in cancer cells, it can be exploited as a potential cancer-specific therapeutic target.

A gamut of proteins participates in centrosome clustering (CC).[5] Among these proteins, KIFC1 has garnered substantial attention. KIFC1 (also referred to as HSET, an ortholog in *Drosophila melanogaster*) is a kinesin-14 motor protein that moves along microtubules by hydrolyzing adenosine triphosphate (ATP), transporting vesicles and organelles. Structurally, it possesses three domains, viz-a-viz, a head/motor, a stalk, and a tail (Figure 2A). The motor domain possesses ATPase activity and is crucial for the protein's function. KIFC1 is overexpressed in various cancers, including ovarian cancer[6] and breast cancer.[7] In cancer cells, KIFC1 is pivotal for the clustering of supernumerary centrosomes, causing the formation of pseudo-bipolar spindles.[8] KIFC1 is considered nonessential in cells possessing a normal number of centrosomes[9]; thus, KIFC1 is an attractive, cancer-specific chemotherapeutic target.

Many small molecules (synthetic and natural) have been shown to target KIFC1 directly or indirectly. With the recent advances in homology-based inhibitor design, high-throughput screening (HTS), and plant metabolite screening, multiple new chemotypes have been identified as KIFC1 inhibitors over the last decade. Nevertheless, none of these inhibitors have progressed beyond preclinical studies, suggesting a poor understanding of the role of

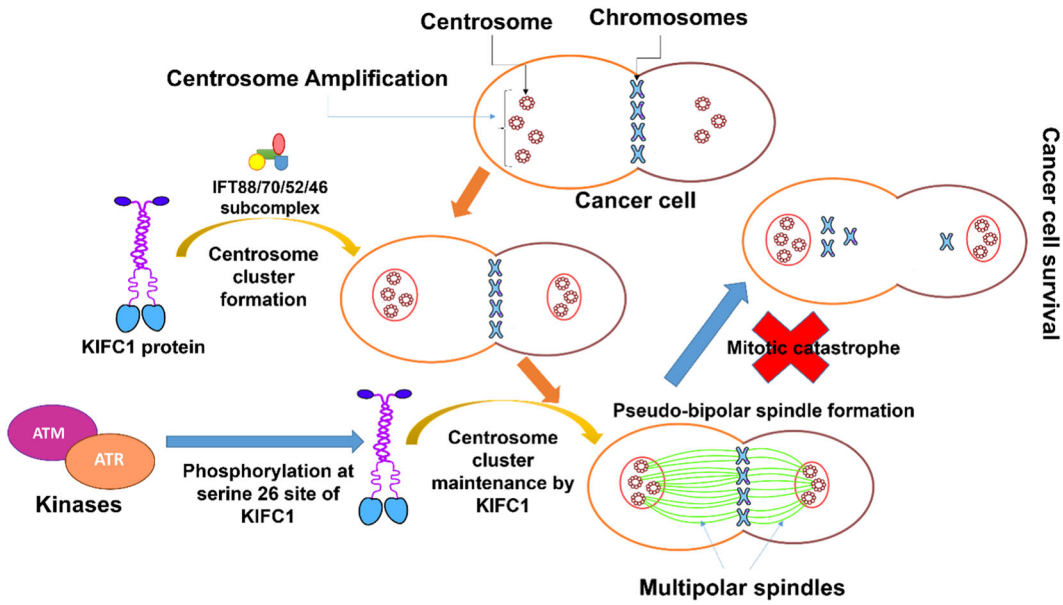

FIGURE 1 Pictorial representation of centrosome amplification, centrosome clustering, and the role of KIFC1 in centrosome clustering and cancer cell survival. KIFC1 has been shown to interact with IFT88/70/52/46 subcomplex of Intra Flagellar proteins at its motor domain to promote centrosome cluster formation and is also phosphorylated by ATM and ATR kinases at serine 26 position under stress conditions to encourage and sustain centrosome clustering. [Color figure can be viewed at wileyonlinelibrary.com]

KIFC1 inhibition in cancer. Despite extensive research on the downstream effects of KIFC1 inhibition, there have been no efforts to summarize these effects in a review article, hindering further development in this very important area. In this article, we summarize the progress in this field and describe the biological effects of each small molecule inhibitor. In addition, we outline the role of KIFC1 in CA and clustering in cancer cells.

Structural data on KIFC1 inhibitors and binding information across different inhibitor chemotypes remain limited. Therefore, we present data from a comprehensive docking analysis involving all known chemotypes and the best available KIFC1 crystal structure to benchmark the binding event. This computational analysis is an effort to give a direct means to compare the binding affinities of known KIFC1 inhibitors at three possible binding sites (i.e., two known allosteric sites and the adenosine diphosphate [ADP]-binding site) of the motor domain (Figure 2B). This docking analysis can also help identify pharmacophores across the inhibitor chemotypes and ultimately guide the design of improved KIFC1 inhibitors. Therefore, this data-driven review article may be instrumental in the development of novel anticancer compounds with increased potency, superior pharmacological profiles, and low toxicity.

To the best of our knowledge, this is a first-of-its-kind study that not only summarizes the developments in KIFC1 inhibition using small molecules but also provides a structural framework for the comparative analysis of existing KIFC1 inhibitors and the design of superior inhibitors.

2 | CENTROSOMES, CENTROSOME AMPLIFICATION (CA), AND, CENTROSOME CLUSTERING (CC)

In 1887, Theodore Boveri first described the centrosome as "the organ for cell division."[10] Since then, our understanding of the morphology and physiology of the centrosome has improved dramatically. The centrosome is a nonmembrane-bound organelle consisting of two perpendicularly oriented barrel-shaped

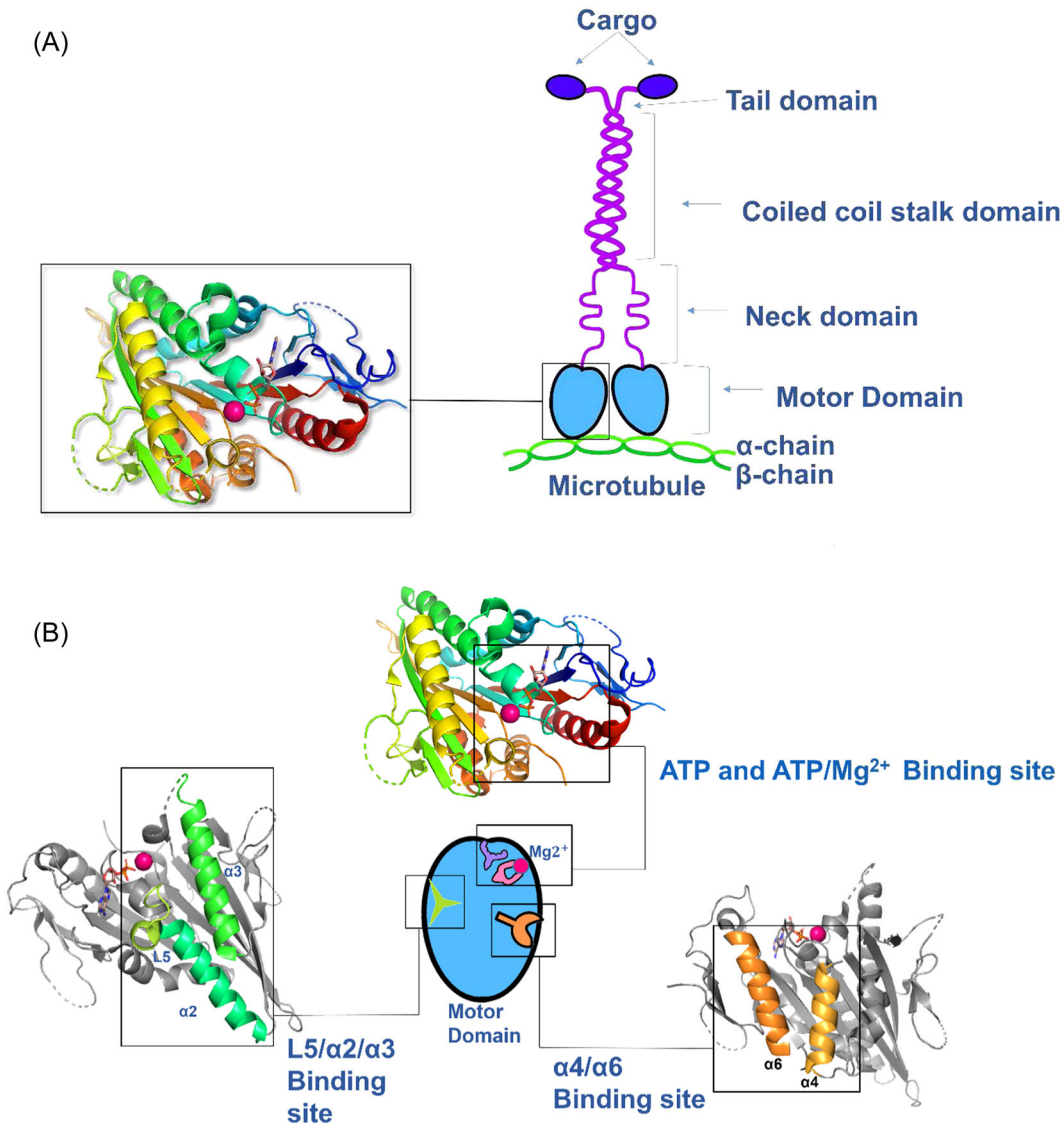

FIGURE 2 (A) KIFC1 structural components. (B) Four sites of KIFC1 motor domain viz-a-viz L5/α2/α3, α4/α6, ATP, and ATP/Mg^{2+} sites. ATP, adenosine triphosphate. [Color figure can be viewed at wileyonlinelibrary.com]

centrioles surrounded by a thick matrix of pericentriolar material (PCM), a hub to hundreds of proteins.[11] It regulates various cellular processes, including the organization of microtubules and actin cytoskeleton, spindle assembly, cell polarity, chromosome segregation, intracellular signaling, and cell motility.[12] The centrosome is duplicated during the S phase of the cell cycle, and its division is tightly regulated by numerous proteins.[13] A healthy cell has one centrosome until it reaches the mitotic stage, when centrosomes divide in sync with deoxyribonucleic acid (DNA) replication. After chromosome segregation, centrosomes are equally distributed to the daughter cells.[14] Aberrations in the number and structure of the centrosome can result in uncontrolled cell proliferation.[15]

CA signifies an overload of centrosomes due to numerical or structural abnormalities in the cell. Structural aberrations result from changes in the centrosome size or shape due to defects in centriole structure and the

amount of PCM surrounding it. On the other hand, numerical aberrations are caused by errors in centrosome duplication, de novo centrosome formation, cell–cell fusion, mitotic slippage, and aberrant cytokinesis.[2,16] The latter type of aberration can result from deregulation of certain proteins central to execution, processing, and termination of centriole duplication; these proteins include PLK4, Sas6, CP110, PLK1, and Aurora A.[16]

P53 negatively regulates PLK-4 and may promote CA directly or indirectly.[17] Consistent with this, alterations in the *TP53* gene (gene encoding for P53 protein) have been implicated in CA. In line with the crucial role of p53 in centrosome overload, p53-deficient mouse fibroblasts at interphase were found to harbor more than two centrosomes typically seen during bipolar division. When these cells underwent mitosis, more than half of them multiplied following spindle multipolarity, leading to chromosomal and genomic instability. It was also concluded that the absence of p53 promotes multiple, early centrosome duplication cycles during a single cell division. All the excess centrosomes had centrioles and retained their microtubule nucleating ability.[18] Squamous cell carcinomas of the head and neck (SCCHN) and breast ductal carcinomas display a high frequency of*TP53* mutations and are ideal models for investigating the role of p53 in CA in cancer cells. SCCHN and breast ductal cancer cells are prone to centrosome overload and chromosome instability due to *TP53* mutations and high levels of the p53 inhibitor MDM2.[19] Supernumerary centrosomes in cancer cells can lead to chromosome segregation errors and aneuploidy. The resulting genetic instability can enhance the malignant and metastatic potential of tumor cells.[20] Studies in organoids and Drosophila models revealed that CA could also increase cancer cell invasiveness, induce oxidative stress, and promote aberrant stem cell division.[21–23]

Cancer cells often cluster their excess centrosomes into a pseudo-bipolar spindle, a phenomenon also known as CC.[24] CC allows cancer cells to continue bipolar mitotic division with a surplus of centrosomes and maintain an optimal level of aneuploidy, which is essential for their survival, evolution, and metastasis.[4] A genome-wide RNA interference screening to identify proteins regulating CC revealed the crucial role of the chromosomal passenger complex (CPC; comprising Aurora B, INCENP, survivin, and borealin) and the Ndc80 complex (HEC1, SPC24, and SPC25) in CC in cancer cells with centrosome surplus. Depletion of CPC proteins promoted the formation of multipolar spindles due to alterations in microtubule–kinetochore interactions; however, these events did not cause mitotic arrest. In contrast, knockdown of Ndc80 complex proteins and centromere potein T resulted in mitotic arrest due to insufficient spindle tension and subsequent activation of SAC. Furthermore, silencing of shugoshin (prevents removal of centromeric Cohesin until Anaphase to ensure proper sister chromatid cohesion)[25] and haspin (a Histone H3 threonine-3 kinase, pivotal for cohesion binding in inner centromeres and sister chromatid association during Mitosis)[26] decreased chromatid cohesion, thereby dwindling spindle tension and causing mitotic arrest dependent on Aurora B kinase activity. Silencing of augmin complex proteins (FAM29A, HEI-C, and HAUS3) interfered with γ-tubulin localization to the spindle, leading to the loss of kinetochore microtubules. The resulting insufficient spindle tension activated SAC, which promoted mitotic arrest and apoptotic cell death.[5]

A recent study highlighted the role of STAT3 in transcription-independent CC and γ-tubulin activity at the centrosomes via Stathmin and PLK1 signaling.[27] Recruitment of excess PCM is considered a structural centrosome aberration, which can cause CA. Gopalakrishnan et al. showed that tubulin regulated microtubule nucleation and inhibited PCM recruitment via the centrosomal protein Sas4 (Drosophila homolog of CPAP protein). It has also been shown that perturbing the tubulin–CPAP interaction is critical to the CC process.[28,29] Another study underscored the role of the E3 ligase anaphase-promoting complex/cyclosome (APC/C) in CC through Eg5, which regulates spindle pole tension.[30] Integrin-linked kinase (ILK) also plays a key role in CC as it modulates the microtubule-regulating proteins TACC3 and ch-TOG in an Aurora A kinase-dependent manner.[31]

Kunotop et al. investigated the ability of chemical compounds to inhibit CC and found that the actin filament-severing protein cofilin was pivotal for CC. They also found that cofilin activation by SSH1 and SSH2 destabilized the cortical actin network, inducing spindle multipolarity.[32] This review focuses on the role of KIFC1 in CC.

3 | KIFC1: A MEMBER OF THE KINESIN-14 FAMILY

There are three large superfamilies of molecular motor proteins involved in intracellular transport: viz-a-viz kinesins, dyneins, and myosins. Phylogenetic analyses clustered kinesins into 14 families distributed in three categories (i.e., N-type, C-type, and M-type kinesins) based on the functional group in the motor domain.[33,34] The motor domain of kinesin has ATPase activity and hydrolyzes ATP to drive the transport of cargo along the microtubules.[35] Some members of the kinesin superfamily, including kinesin-1 (KSP) and kinesin-5 (Eg5), have been extensively studied for their structure, molecular activity, and inhibition using pharmacological agents.[36–38]

Alongside KIFC2 and KIFC3, KIFC1 is a member of kinesin-14 family proteins, which share a common C-terminal motor domain.[39] KIFC1 is composed of 663 amino acid residues, which form three distinct domains: a tail (1-138), a coiled-coil (141-297), and a head/motor domain (317-663). Biophysical profiling of the KIFC1 structure has shown that human KIFC1 is a dimeric kinesin containing a well-folded globular motor domain joined with an intrinsically disordered tail region by a continuous α-helical coiled-coil domain. Dimerization of the KIFC1 coiled-coil stalk domain is instrumental in determining the kinetic properties of KIFC1.[40] The crystal structure of full-length KIFC1 remains unresolved. However, the model structure of the entire motor domain recently came into light by AlphaFold predictions and the crystal structure of the motor domain in an Mg-ADP-bound state has been reported a few years back (Protein Data Bank [PDB] ID: 5WDH, 2.25 Å) (Supporting Information: Figure S1 and Figure 3), along with the motor domains of its homologs KIFC3 and Ncd. Sequence alignment studies have shown that KIFC1 shares 42.6% sequence identity with Ncd and 45.8% with KIFC3. KIFC1 and KIFC3 display similar structures, although differences in their structures may hold the key to their selective inhibition. The most significant difference lies in loop L11, which is shorter in KIFC3 than in KIFC1 (14 vs. 30 residues), while helix α4 is three times longer in KIFC3 than KIFC1. The structures of motors in KIFC1 and Ncd are also very similar, although the loop is longer in KIFC1 than in Ncd.

There are two primary inhibitor binding sites in kinesins: L5/α2/α3 and α4/α6. L5/α2/α3 is the first binding pocket identified in kinesins and is known to bind a wide range of structurally different inhibitors. Inhibitors binding to this pocket can cause allosteric, ATP-noncompetitive effects by affecting the release of ADP from the motor without directly competing with ATP for binding. Park et al. evaluated the ability of L5/α2/α3 in KIFC1 and

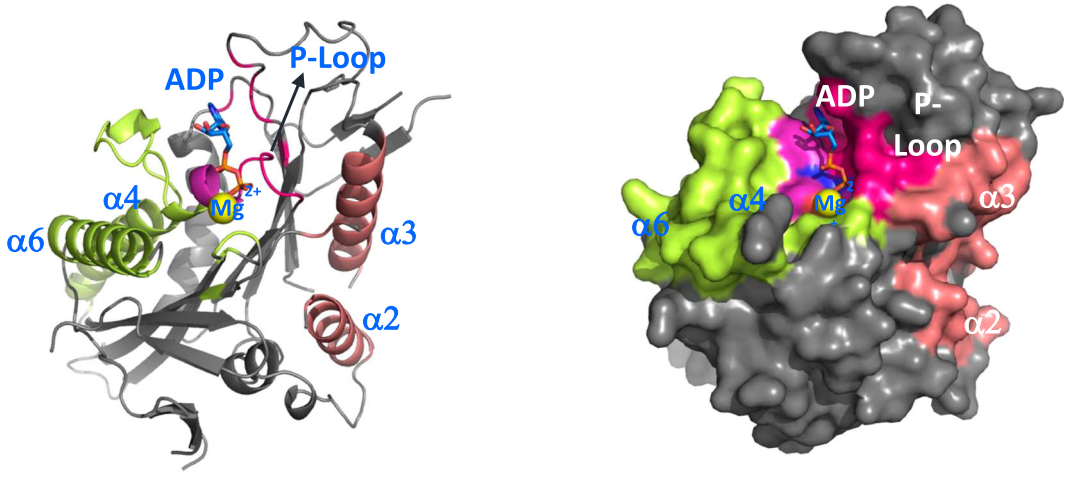

FIGURE 3 Ribbon models showing the experimental crystal structure of the motor domain of human KIFC1 (left, PDB ID: 5WDH). ADP binding pocket is shown in pink, the allosteric site α4/α6 in green, and L5/α2/α3 in orange. ADP, adenosine diphosphate; PDB, Protein Data Bank. [Color figure can be viewed at wileyonlinelibrary.com]

kinesin-5-ADP-ispinesib cocrystal (PDB: 4AP0) to bind small molecules. They found the size of L5/α2/α3 was greatly reduced in KIFC1 and that the domain maintained a closed and inaccessible to inhibitors conformation owing to the interaction of R521 in helix α3 with other residues to stabilize loop L5. Notably, the small opening at the lower front of this pocket lined with negatively charged residues (E431, D514) inhibited the binding of hydrophobic compounds. Although this site is relatively flexible in kinesins, the short length and conformational rigidity of L5 in KIFC1 make it largely inaccessible to inhibitors.[41]

The cleft formed at the interface of α4 and α6 represents the second allosteric binding site in KIFC1. The cleft lies on the opposite side of the L5/α2/α3 and the motor. The α4/α6 pocket in KIFC1 is wider than that in kinesin-5, making it accessible to small molecules. Interestingly, the site is lined with hydrophobic amino acids on the one side and at the bottom, whereas charged residues can be found on the other side. This type of residue arrangement enables a range of stabilizing interactions (e.g., hydrophobic and charge–charge interactions) between the protein and small molecules. The inhibitors binding to this pocket show an ATP-competitive effect, possibly due to the conformational change induced in the highly conserved P-loop, which hinders ATP binding.[41]

KIFC1 plays various pivotal roles in cells. It slides and crosslinks microtubules, coordinating spindle assembly and integration during the mitosis phase of the cell cycle.[42] It also functions as a motor protein that carries cargo toward the minus end of the cell. It has been implicated in active transportation of bare exogenous DNA to the eukaryotic cell nucleus by preferentially binding to the dsDNA.[43] Moreover, KIFC1 orchestrates spermiogenesis in invertebrates and regulates oocyte development and embryo gestation in humans and rhesus monkeys.[8] In association with nuclear factors, it assists in the formation and elongation of spermatid acrosome, an organelle pivotal to spermiogenesis.[44] In nonpolarized mammalian cells, the motor domain of KIFC1 recognizes and partially binds to the Golgi apparatus, regulating its positioning and structural maintenance. KIFC1 knockdown and overexpression of its motor domain led to profound disorganization of the Golgi. Evidence also suggests that KIFC1 functions as a linker between microtubules, MTOC, and the Golgi, ensuring the positioning of the Golgi in the vicinity of the nucleus.[45] In mouse liver cells, it interacts with KIF5B motor protein to facilitate bidirectional motion of early endocytic vesicles and mediate the fission of vesicle into two daughter vesicles.[46]

KIFC1 is dispersed throughout the cell during the interphase. The tail domain of KIFC1 possesses a nuclear localization signal, which drives the translocation of KIFC1 into the nucleus during the S phase of the cell cycle. KIFC1 is essential for cell growth and proliferation, probably because of its role in intercellular connections and cell adhesion forces. KIFC1 deficiency prolongs the S phase of the cell cycle and delays cell division. KIFC1 also maintains the membrane morphology of the nucleus and regulates chromatin distribution inside the nucleus.[42]

3.1 | The role of KIFC1 in cancer

HSET was first identified as a lead hit amongst CC causing genes in *D. melanogaster* by RNA interference screening of Drosophila cells containing centrosomes organized in a pseudo-bipolar spindle assembly. The genetic screening led to the identification of Ncd as a minus-end motor protein involved in centrosome coalescence. Silencing of the mammalian homolog of Ncd, KIFC1, increased the frequency of spindle multipolarity in cells possessing an overload of centrosomes, leading to reduced cell viability. On the contrary, KIFC1 depletion had no effects in normal diploid cells with bipolar spindle assembly.[9] A study showed that the differences in the effects of KIFC1 depletion among different cancer cell lines were mediated by a balance between centrosomal and acentrosomal forces. It has been proposed that KIFC1 is upregulated in cells with acentrosomal spindle assembly, facilitating centrosome agglomeration to form pseudo-bipolar spindles and directing acentrosomal MTOC into spindle assembly. In cancer cells, KIFC1 is regulated by the RanGTP pathway through its interaction with importin a/b, which bind to the NLS in the tail domain and trigger acentrosomal microtubule polymerization and organization in the vicinity of chromosomes, rendering cancer cells dependent on KIFC1. DNA damage signaling activation due to defective

cell cycle checkpoints in cancer cells has also been implicated in the regulation of acentrosomal microtubule nucleation and spindle organization.[47]

The role of KIFC1 in cancer development and progression has been assessed in numerous studies. KIFC1 is upregulated in breast cancer cells and has been proposed as a cancer-specific target in breast cancer. KIFC1 expression is higher in progesterone receptor (PR)-negative, estrogen receptor (ER)-negative, and triple-negative breast cancer (TNBC) than in other breast cancer subtypes, although the relationship between KIFC1 and HER2 remains elusive. Knockdown of KIFC1 greatly reduced cell viability in almost all breast cancer cell lines.[48] Immunohistochemical analysis of tissues from patients with hepatocellular carcinoma revealed the cytoplasmic abundance of KIFC1 and a strong association between KIFC1 levels, number of tumor nodes, and tumor size. Furthermore, KIFC1 overexpression was associated with poor overall survival (OS) and relapse-free survival. Notably, KIFC1 silencing significantly reduced cancer cell proliferation in vitro and tumor growth in vivo.[49] Consistently, KIFC1 knockdown reduced cell proliferation in endometrial cancer cells, and KIFC1 overexpression promoted cancer cell migration and invasion. The PI3K/AKT signaling cascade was found to mediate the ability of KIFC1 to promote cancer progression.[50]

Ovarian cancer cells exhibit prominent amplification of centrosomes and pseudo-bipolarity, which enables them to escape mitotic catastrophe. KIFC1 overexpression in ovarian carcinoma cells was associated well with the upregulation of genes driving CA and deregulating the cell cycle, supporting the crucial role of KIFC1 in the clustering of extra centrosomes. High KIFC1 levels were also associated with poor OS in patients with ovarian cancer. Evaluation of KIFC1 levels in multiple ovarian cancer cell lines showed that although KURAMOCHI cells possessed high CA, they had low KIFC1 levels. This finding indicated that KURAMOCHI cells did not undergo CC, leading to low cell viability and reduced tumorigenicity in vivo.[6,51]

KIFC1 has emerged as a critical regulator of CC. Chavali et al. showed that KIFC1 interacted with the PCM protein CEP215 to promote CC by stabilizing the centrosome-spindle pole connection.[52] In addition to this, it also interacts with other proteins that regulate CC. A recent study unraveled that the interaction of intraflagellar proteins (IFT) with KIFC1 was essential for CC (Figure 1). Silencing of IFT88 and IFT52 (individually and together) in various cancer cells with excess centrosomes promoted a stage of multipolar anaphase due to disruption of centrosome conglomeration. No such effect was noted in normal replicating cells lacking excess centrosomes. This study also provided evidence that KIFC1 and dynein work in sync with IFT proteins to maintain CC. Endogenous interaction between the IFT88/70/52/46 subcomplex and KIFC1 was identified at the motor domain of KIFC1; this interaction was confirmed by the reduction in GFP-KIFC1 turnover on mitotic spindle microtubules upon IFT88 knockdown. Centrosome dynamics analysis pointed toward an increase in multipolar spindles and the distance between centrosomes during mitosis due to IFT52 depletion. In cancer cells harboring supernumerary centrosomes, IFT88 depletion significantly reduced clustering and cell proliferation.[53] KIFC1 is indispensable to cancer cells for cell survival, proliferation, and clustering of excess centrosomes required for mitosis.

KIFC1 is also abundant in the nucleus of prostate cancer cells. KIFC1 inhibition using AZ82 (0.5 μM) inhibited tumor growth and prostate cancer cell proliferation. Additionally, KIFC1 inhibition induced multipolar mitosis and centrosome declustering, leading to apoptotic cell death.[54] Recently, Fan et al. provided evidence to support the role of KIFC1 in drug resistance and tumor recurrence.[55] They found that under DNA damage conditions, ATM and ATR kinases selectively phosphorylated KIFC1 at Ser-26, thereby promoting CC and cancer cell survival (Figure 1). These events promoted resistance to chemotherapy and increased the risk of tumor recurrence. These findings emphasize the clinical relevance of KIFC1 as a prognostic marker and a therapeutic target. KIFC1 harbors multifaceted role in cancer cells and has been reported to facilitate cancer cell progression via CC-independent mechanisms. Pannu et al. revealed for the first time that KIFC1 stabilizes survivin (an antiapoptotic protein) by interfering with its poly ubiquitination-dependent proteolysis process. Survivin accumulation supports over-expression of Aurora B kinase and in turn, phosphorylated Histon H3 (cell proliferation marker). KIFC1 overexpression led to enhanced cell cycle kinetics as observed by elevated levels of cyclins, compromised spindle

assembly checkpoint (SAC) function, by disturbing Mad1/Mad2 balance, and upregulation of HIF-1α (Hypoxia Inducible Factor-1α).[7]

3.2 | Role of KIFC1 as prognostic marker in a variety of cancers

KIFC1 protein is overexpressed in a variety of cancers and has fewer deleterious effects on normal cell, if inhibited, thus making it an attractive cancer-specific marker. There have been some studies to fathom the prognostic significance of KIFC1 to identify potential cancer biomarkers, making early detection possible. A metareview by Sun et al. involving 11 studies and 2424 patients gives systematic insights into the prognostic role of KIFC1 and unravel the impact that KIFC1 overexpression has on OS, relapse-free survival (RFS), and clinicopathological traits of cancer patients. The study revealed significant correlation between KIFC1 overexpression and poor OS in univariate analysis and poor RFS in univariate and multivariate analysis. Upregulated levels of KIFC1 also showed correlation to advanced tumor, nodes, metastasis (TNM) stage.[56] Another study focused on determining the prognostic significance of KIFC1 in hepatocellular carcinoma unravels that overexpression of KIFC1 is associated significantly with recurrence, metastasis, disease-free survival and OS rates.[57] Another interesting study in a racial dependence manner and using Immunohistochemistry approach, it sheds light on nuclear KIFC1 as biomarker of poor prognosis in African American (AA) women. Through a multivariate Cox model, the authors concluded that high nuclear KIFC1 weighted index has strong association with worse OS, distant metastasis-free survival, and progression-free survival in AA TNBC compared to white TNBC. KIFC1 knockdown notably impaired the migration in AA TNBC than white TNBC samples.[58] Li et al. observed that higher KIFC1 expression correlates not only with aggressive clinicopathologic parameters but is also linked with poor survival prognosis in renal cell carcinoma.[59] KIFC1 overexpression shows association with tumor aggressiveness, advanced tumor stage and grade and poor patient survival in ovarian adenocarcinoma.[60] Furthermore, upregulated KIFC1 levels have been shown to be poor prognostic marker in prostate cancer[61] and KIFC1 shows significant correlation with tumor size, lymph node and distant metastasis and depth of invasion in gastric cancer.[62]

4 | SMALL MOLECULE INHIBITORS OF KIFC1

Despite the immense potential of inhibiting KIFC1 to selectively eliminate cancer cells, there are currently no clinically approved KIFC1 inhibitors. However, a range of structurally different small molecules have been reported to inhibit KIFC1 (Figure 4). These molecules belong to nine different chemotypes, and some of them are known to also bind to other proteins. Hence, there is a dire need for novel small molecule inhibitors of KIFC1. In this section, we review small molecules that have been reported to inhibit KIFC1 including their discovery, biological profiling, structure–activity relationships, binding insight, and so on. A concise summary of the same has also been added in a tabular format in Supporting Information: Table S2.

4.1 | AZ82

AZ82 is the first small molecule reported to selectively inhibit KIFC1.[63] AZ82 was discovered through HTS of over a million compounds, followed by iterative medicinal chemistry optimization. The HTS hits identified by a malachite green (MG) ATPase assay were validated by a pyruvate kinase/lactate dehydrogenase-coupled assay, which led to the identification of a phenylalanine-containing compound 1a with modest activity (IC_{50}: 14–18 μM). The compound was subjected to multipronged optimization using a robust late-stage diversification strategy of

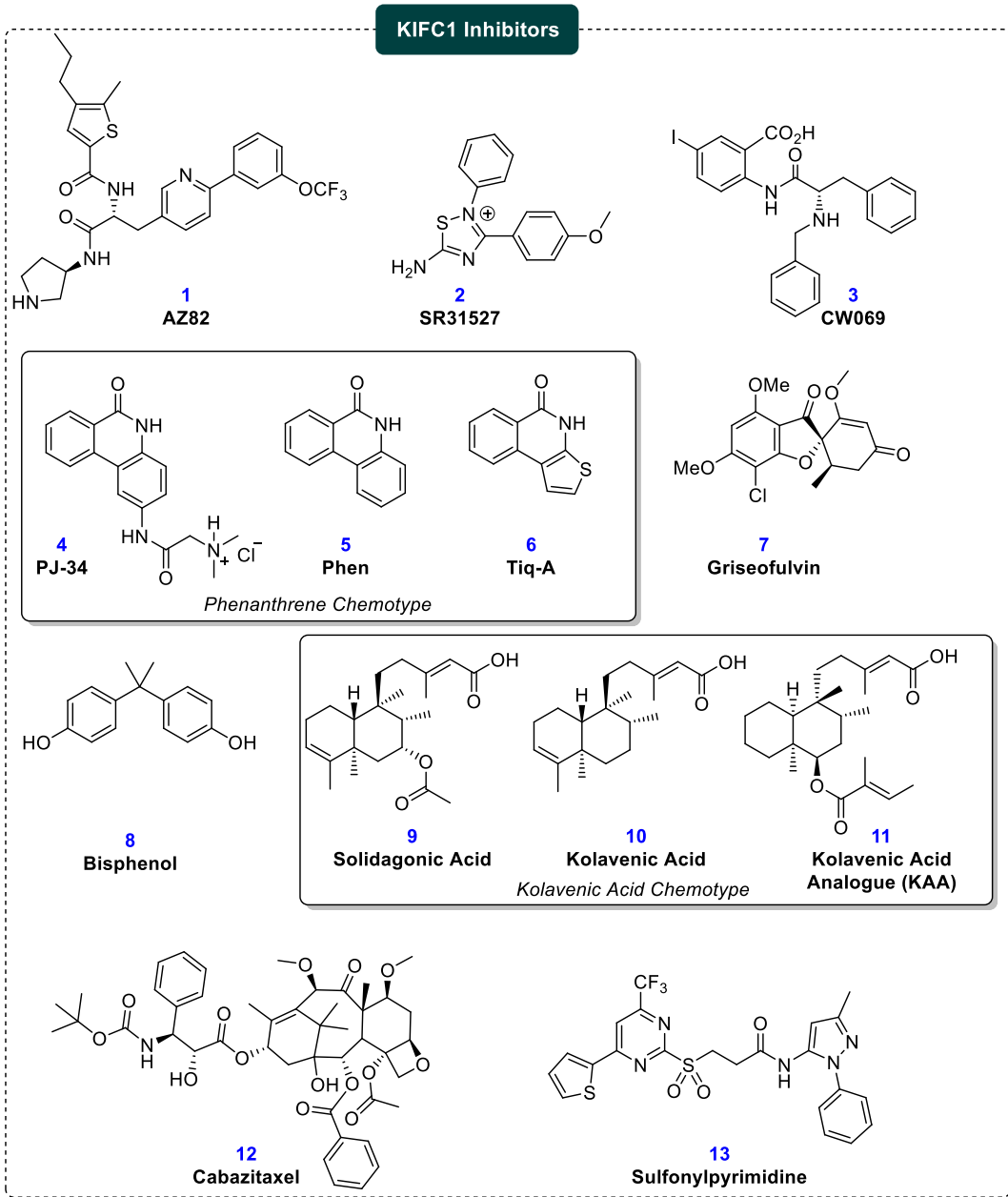

FIGURE 4 Small molecule inhibitors of KIFC1 [Color figure can be viewed at wileyonlinelibrary.com]

peripheral groups (R^{1-3}) (Figure 5).[64] The phenylalanine core was retained to improve the potency and the physicochemical properties of the compound. Although R^2 and R^3 groups were found to be difficult to replace in the initial HTS hits, R^1 was far more tractable, and its replacement contributed to the improved potency of the molecule. Among various R^1 substituents, Me-Pr-substituted thiophene carboxamide 1b was found to be the most optimal. Screening of multiple substituents to replace the 3-CF_3Ph in the initial hit led to a marginally better R^2 substituent, 3-OCF_3Ph. Replacement of phenyl with 2-pyridyl in the phenylalanine core along with the introduction of 3-aminopyrrolidine as R^3 led to AZ82, a compound with optimal potency (IC_{50}: 0.31 μM) and physicochemical

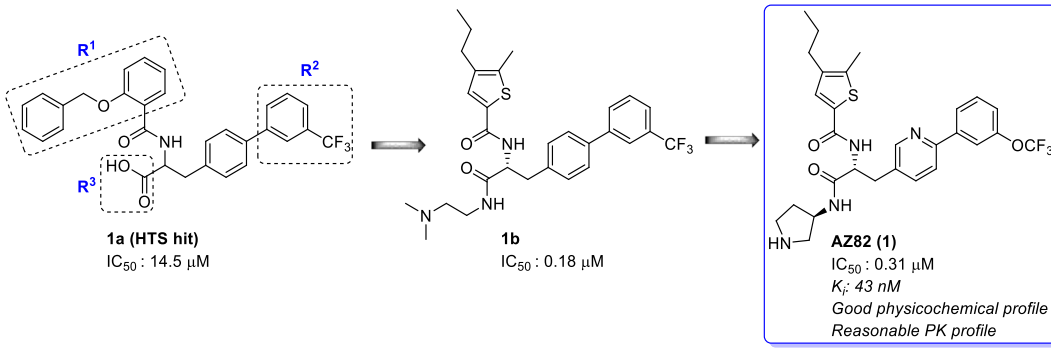

FIGURE 5 Optimization of phenylalanine-containing compounds leading to the development of AZ82. [Color figure can be viewed at wileyonlinelibrary.com]

properties (e.g., solubility and plasma protein binding). Following intravenous and oral administration in rats and mice, AZ82 showed negligible bioavailability, low solubility, and high cellular efflux, contributing to its failure to reach the market. However, substantial drug exposure was reported in mice intraperitoneally injected with the drug (30 mg/kg).[64]

AZ82 was used by Wu et al. in a proof-of-concept study to assess whether KIFC1 inhibition can be a viable strategy to selectively target cancer cells.[63] Using equilibrium dialysis/mass spectrometry analysis followed by a cosedimentation assay, Wu et al. showed that AZ82 had a higher affinity for the microtubule-KIFC1 complex than for KIFC1 alone. By binding to the KIFC1- microtubules complex, AZ82 locked KIFC1 in a stronger affinity state to microtubules, thereby preventing ADP release and compromising the ATPase activity of KIFC1. Fluorescent nucleotide exchange experiments using mant-ATP confirmed the function of AZ82 as a microtubule-noncompetitive and ATP-competitive inhibitor of KIFC1 with a K_i of 0.043 μM. Notably, this mode of inhibition is similar to that of the centromere protein (CENP)-E inhibitor GSK923295. Wu et al. also investigated the effects of AZ82 on cells and found that AZ82 could negate the cellular effects of plus-end-directed Eg5, which is known to induce monopolar spindle formation. They observed that treatment with the Eg5 inhibitor AZD4877 followed by AZ82 treatment restored bipolar spindle formation in HeLa cells. In aneuploid BT-549 cells, AZ82 treatment induced the formation of multipolar spindles and mitotic catastrophe, confirming its declustering effect on supernumerary centrosomes.[63]

Despite evidence supporting AZ82 as a promising lead molecule, the modest pharmacokinetic (PK) profile of AZ82 hinders its clinical translation. Furthermore, at a concentration above 4 μM, AZ82 showed nonspecific cytotoxic effects.[63] This off-target toxicity was further confirmed by Yukawa et al. in a fission yeast cellular assay. Specifically, AZ82 decreased the viability of yeast cells despite the absence of KIFC1 in the cells.[65] However, no morphological changes were detected. Interestingly, AZ82 at 10 μM rescued cell death mediated by KIFC1 overexpression in yeast cells, further validating its binding to KIFC1 even though nonspecifically.[65]

The precise mode of binding of AZ82 to KIFC1 has also been explored. Based on its similarity to other kinesin inhibitors, AZ82 was initially proposed to bind to the L5/α2/α3 binding site of KIFC1.[41] However, based on the crystal structure of KIFC1 (PDB: 5WDH) and because of the presence of a biaryl motif and a trifluoromethyl group in AZ82, Park et al. hypothesized that AZ82 likely binds the α4/α6 cleft rather than the L5/α2/α3 pocket of KIFC1. They were able to validate this hypothesis by docking studies, which showed that during the binding of AZ82 to KIFC1, the residues Y409, L599, and F656 underwent significant movement to form the binding pocket. The trifluoromethoxy group was found to interact with Y409, whereas pyridine in the core was found to interact with F656. The authors also explained the selectivity of AZ82 toward KIFC1 rather than KIFC3 or Ncd based on the predicted free energy change (ΔG = −8.1 kcal/mol for KIFC1, −5.4 kcal/mol for KIFC3, and −7.9 kcal/mol for Ncd)[41] (Figure 6).

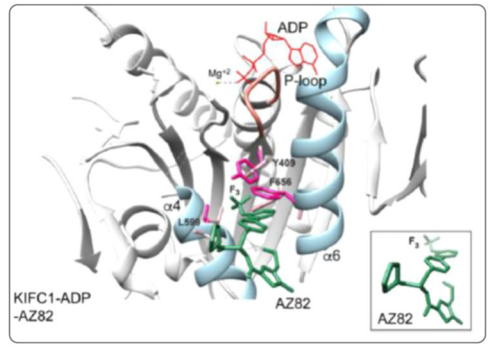

FIGURE 6 AZ82 docked into the α4/α6 cleft of KIFC1 (PDB: 5WDH). AZ82, dark green; KIFC1 helices α4 and α6, pale blue; P-loop, coral; ADP, red; Mg^{+2}, yellow-green. Inset, AZ82 conformation after docking (Reproduced with permission from Park et al.[41] Copyright 2017 Scientific Reports). ADP, adenosine diphosphate. [Color figure can be viewed at wileyonlinelibrary.com]

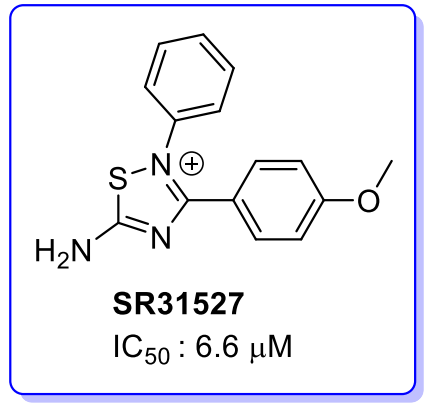

FIGURE 7 The structure of SR31527 [Color figure can be viewed at wileyonlinelibrary.com]

4.2 | SR31527

The 1,2,4-thiadiazole derivative SR31527 (Figure 7) was identified as a KIFC1 inhibitor that targets the microtubule-stimulated ATPase activity of KIFC1.[65] Among other HTS hits, SR31527 had the most potent activity (IC_{50}: 6.6 μM) and favorable structural features. To assess whether SR31527 binds KIFC1 only or the complex KIFC1-microtubules, Zhang et al. performed a binding assay using biolayer interferometry and found that SR31527 displayed direct binding to KIFC1 with a K_d of 25.4 nM. The binding of SR31527 to KIFC1 was confirmed by saturation-transfer difference nuclear magnetic resonance. To gain further structural insights into the binding of SR31527 to KIFC1, Zhang et al. carried out a docking experiment based on the similarity of motor domains among kinesins. The S2 site (α4/α6 cleft) of KIFC1 had the highest docking score; hence, the authors concluded that this cleft was the most likely binding site, with aromatic rings of the inhibitor fitting well into the hydrophobic pockets through π–π stacking interactions with Tyr^{100} and Phe^{347}. It is important to note that this binding site is common between AZ82 and SR31527.

Functional characterization of SR31527 in TNBC cell lines (MDA-MB-231, BT549, and MDA-MB-435) demonstrated that the inhibitor promoted spindle multipolarity, reduced cell viability in a concentration-dependent

manner (IC_{50}: 20–33 μM), and inhibited colony formation (6.25–25 μM). Although treatment of normal human lung fibroblasts with similar concentrations of SR31527 had no profound effects, higher concentrations of the drug (100 μM) displayed significant cytotoxicity.[66] The cytotoxicity of SR31527 due to off-target effects was further confirmed in a fission yeast cell assay using cells without KIFC1 overexpression.[65]

4.3 | CW069

CW069 was discovered around the same time as AZ82. Similar molecules often bind to similar targets. A KIFC1 binding model based on the high sequence similarity (>80%) between KSP and KIFC1 was developed using ~500 known KSP ligands in the ChEMBL database. After in silico screening of 20 million compounds, approximately 200 compounds were identified, which were triaged to 50 compounds based on the bioactivity model. An in vitro ATPase enzymatic assay of these compounds led to the discovery of the phenylalanine derivative CW069 as another small molecule that allosterically inhibits KIFC1. CW069 displayed an IC_{50} value of 75 ± 20 μM in vitro and was selective for KIFC1 over KSP.[67]

Extensive computational studies were employed to ascertain various aspects of CW069-KIFC1 binding. Energy minimization studies of KIFC1 bound to CW069 revealed that a dynamic conformational change was required around loop L5 to create a cavity opening ~2–3 Å to ensure ligand binding. The dynamic nature of loop L5 was independently confirmed from a 1-μs all-atom molecular dynamics simulation. H-bond interactions between the carboxylate moiety of CW069 and the Arg521 residue of KIFC1 loop L5, as well as interactions between the carboxylate and amine groups of CW069 with the respective backbone amide and carbonyl groups of Gly423 and Leu517 residues of KIFC1 were found to be crucial for CW069-KIFC1 binding (Figure 8). The specificity of CW069 for KIFC1 was also experimentally demonstrated by differential scanning fluorimetry, which revealed a maximum ΔT_m of −8.0°C as a result of ligand binding. A value of ΔT_m lesser than zero also suggests that KIFC1 is destabilized as a result of CW069 binding.[67]

CW069 treatment significantly increased the number of multipolar spindles in N1E-115 cells with supernumerary centrosomes (control 30% vs. 98% at 100 μM). The antiproliferative effects of CW069 on N1E-115 cells were significantly more potent than those on normal human dermal fibroblasts cells. KIFC1 is known to antagonize the activity of the related kinesin KSP during spindle formation.[68] Evaluation of the mitotic duration in HeLa cells showed that KIFC1inhibition using CW069 suppressed mitotic arrest and restored bipolar spindle

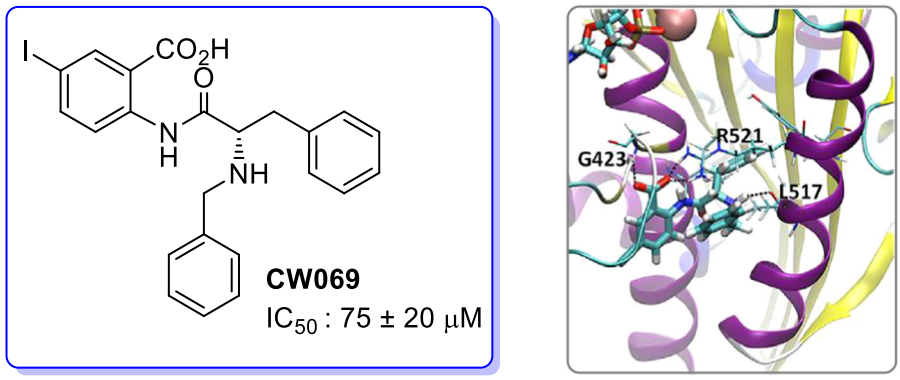

FIGURE 8 Structure of CW069 (left) and binding pose of CW069 into the loop L5 of KIFC1 based on computational modeling (Reproduced with permission from Watts et al.[67] Copyright 2013 ScienceDirect). [Color figure can be viewed at wileyonlinelibrary.com]

formation in cells treated with the KSP inhibitor monastrol. These data confirm the specificity of CW069 for KIFC1.[67]

To evaluate the clinical implications of KIFC1 inhibition, Sekino et al. investigated the role of KIFC1 in docetaxel (DTX) resistance in prostate cancer cell lines and the potential of KIFC1 inhibitors to resensitize cells to DTX treatment, the standard-of-care for prostate cancer treatments.[69] They found that CW069 treatment decreased cell viability in parental and resistant cell lines. Notably, the IC_{50} value was lower in resistant cells than in parental cells, consistent with the high levels of KIFC1 in resistant cells. Importantly, CW069 augmented the cytotoxic effects of DTX in DTX-resistant cell lines.[69] These findings open new avenues for exploring KIFC1 inhibition in combination with chemotherapy for the treatment of prostate cancer. Compared with AZ82 and SR31527, CW069 displayed low cytotoxicity in fission yeast cells (without KIFC1 upregulation) due to its inability to permeate the cells.[65] Nonetheless, the moderate potency of CW069 raises the need for the identification of analogs with increased potency and KIFC1 selectivity.

4.4 | Phenanthrene derivatives

Poly (ADP-ribose) polymerase (PARP) is a nuclear protein activated in response to DNA damage. PARP plays a crucial role in DNA damage repair, and numerous PARP inhibitors have been tested in the clinic for their ability to eradicate cancer cells.[70] Among the various PARP inhibitor chemotypes, a class of potent inhibitors derived from phenanthrene was found to selectively eradicate breast cancer cells. The most potent among those molecules were PJ-34, Tiq-A, and Phen (Figure 9). Notably, PJ-34 at a concentration of 10 μM eradicated 99% of MCF-7 cells within 48-72 h. Flow cytometry analysis revealed that PJ-34-treated cancer cells underwent G2/M arrest and cell death. The same concentration of PJ-34 did not affect cell survival in normal human mammary epithelial cells (MCF-10A), which underwent only a transient cell cycle arrest. Moreover, treatment of xenografted nude mice with PJ-34 strongly suppressed tumor formation.[71]

Investigations into the mechanisms of action of PJ-34 revealed that PJ-34 exerted selective cytotoxicity in cancer cells through the declustering of supernumerary centrosomes. As a consequence of declustering, cells displayed distorted multipolar spindles and abnormal chromosome segregation leading to mitotic catastrophe. Confocal imaging showed that PJ-34 did not affect the structure of centrosomes. PJ-34 exerted similar effects in other cancer cells with supernumerary centrosomes, including lung (H1299), colon (DLD-1), ovarian (HeyA8), and pancreatic (Panc1) cells. Nonphenanthrene PARP inhibitors, including BSI-201 and ABT888, completely lacked centrosome declustering activity.[72] The cytotoxic effects of PJ-34 on multicentrosomal MDA-MB-231 cells were documented by confocal live imaging. PJ-34 at 20 μM declustered the extra centrosomes and induced the

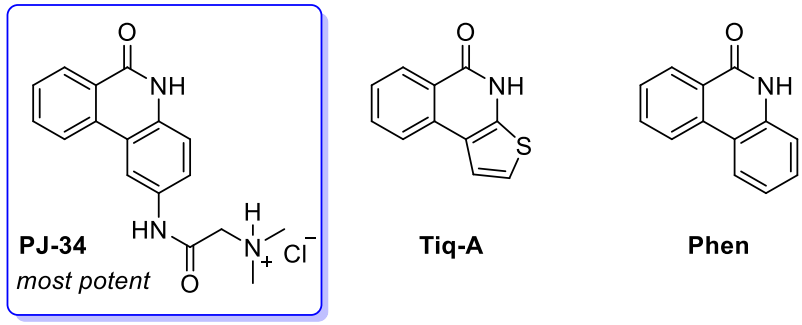

FIGURE 9 Chemical structures of phenanthrene derivatives with activity against KIFC1 [Color figure can be viewed at wileyonlinelibrary.com]

formation of multipolar spindles, leading to mitotic catastrophe and cell death.[73] Consistently, Li et al. showed that PJ-34 promoted spindle multipolarity in different cancer cell lines, suppressing colony formation and promoting cell death due to declustering of extra centrosomes.[48] Unlike other nonphenanthrene PARP-1 inhibitors, PJ-34 at 20 μM declustered the extra centrosomes, distorted mitotic spindles, and induced cell death in a dose-dependent pattern in PARP-1-deficient nontumor cells harboring supernumerary centrosomes. The potent declustering activity of PJ-34 in PARP-1-deficient nontumor cells harboring supernumerary centrosomes highlight the non-PARP-dependent declustering effect of phenanthrene inhibitors.[73]

Despite mounting evidence to support the potential of PJ-34 as a centrosome declustering agent, its targets remain unclear. The findings of two recent studies suggest that PJ-34 inhibits KIFC1 along with other proteins. Li et al. showed that PJ-34 (7–56 μM) significantly downregulated KIFC1 in breast cancer cells.[48] Another study demonstrated that the phenanthrene class of PARP inhibitors prevented the posttranslational modification of kinesins, including KIFC1, KIF18A, and NuMa, as indicated by the shift in their isoelectric point (pI).[74] NuMa is a nonmotor protein that regulates the structure of spindle poles.[75] PJ-34 abolished the binding of NuMa to kinesins and α-tubulin, although the binding of kinesins to the microtubules remained unaffected. The same effects were not observed for nonphenanthrene PARP-1 inhibitors. This mechanism of inhibition underscores the ability of PJ-34 to attenuate the poly-ADP-ribosylation of NuMA, a posttranslational modification necessary for the binding of NuMA to spindle proteins. Additionally, PJ-34 inhibited tankyrase 1, which is required for the poly-ADP-ribosylation of NuMA. The inhibition of the serine/threonine kinase PIM1 was also identified as one of the mechanisms by which PJ-34 altered the function of NuMa. Binding of tankyrase 1 to the centrosome outside the spindle pole could be one of the mechanisms leading to centrosome declustering in cells with supernumerary centrosomes and distorted spindles. Phenanthrene may exert a similar centrosome declustering effect by binding to scattered tankyrase 1 polymers localized to the centrosome.[74]

4.5 | Griseofulvin

Griseofulvin is a metabolic byproduct first extracted from *Penicillium griseofulvum* and is used to treat fungal infections in humans.[76] Griseofulvin as a bioactive compound class has been studied extensively.[77] Considering the ability of griseofulvin to inhibit cell division, Weber et al. investigated its interaction with microtubules in vitro.[78] However, its ability to cause mitotic arrest by interfering with tubulin polymerization could not be proved. In an unbiased screening of a fungal extract library, griseofulvin was identified as a centrosome declustering agent. The extracts were chosen using a chemotaxonomic approach to ensure diversity and were screened in a cell-based phenotypic assay using squamous cell carcinoma cells. The centrosome declustering ability of griseofulvin was also tested in four human cancer cell lines (SCC114, HeLa, U2OS, MCF-7) and normal fibroblasts. Expectedly, griseofulvin promoted multipolar mitosis, inhibited cell proliferation, induced G2/M cell cycle arrest, and promoted apoptosis in a concentration-dependent manner in all the cancer cell lines. In contrast, no such effect was seen in normal fibroblasts.[79] Continuous time-lapse microscopy revealed a higher percentage of apoptosis in multipolar SCC114 cells (80%) than in bipolar SCC114 cells (20%) after treatment with griseofulvin. Interestingly, griseofulvin strongly blocked centrosome coalescence during interphase in a concentration-dependent fashion, without altering the localization of centrosomal motor proteins (e.g., dynein) and nonmotor proteins (e.g., NuMa).[80]

A total of 35 griseofulvin analogs were synthesized and tested for their centrosome declustering potential. The 2′-position and the 4′-position were the most amenable for modification to improve the potency of the compound (Figure 10). Analogs with 2′-position substitutions, including enol ether and methoxy groups, exhibited enhanced centrosome declustering potential. Interestingly, the introduction of bulkier groups at this position (e.g., 2′-benzyloxy analog) displayed maximum activity and lowest IC_{50} values while maintaining optimum lipophilicity required for cell transport. The 4′ position was tagged with sp2-hybridized groups, including oxime and hydrazine (Figure 10).[79]

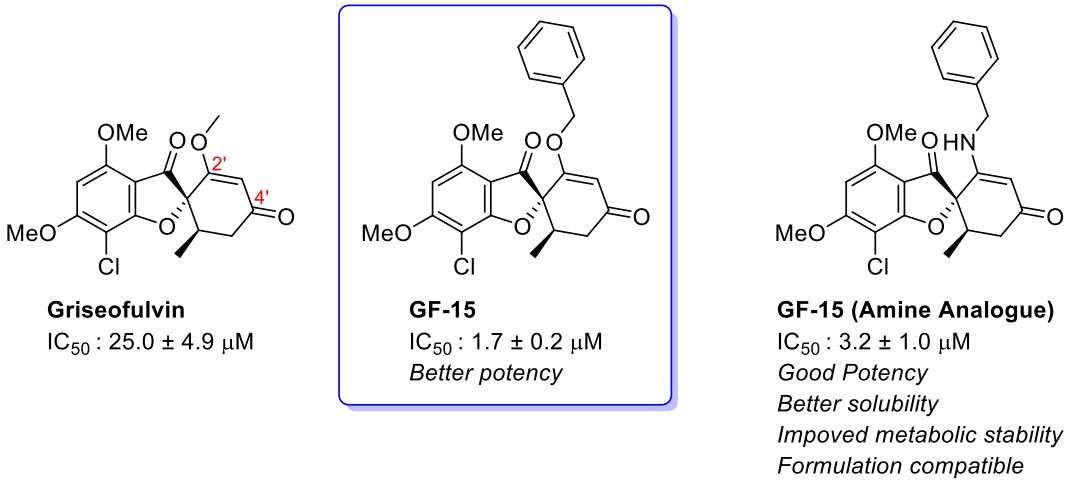

FIGURE 10 Structure of griseofulvin and its two most optimized analogs, GF-15 and the amine analog of GF-15. [Color figure can be viewed at wileyonlinelibrary.com]

GF-15 showed an EC_{50} value of 900 nM and, compared with the parent compound, GF-15 exhibited a 27-fold increase in multipolar spindle-inducing activity. The improved potency of GF-15 was attributed to better cellular uptake of the compound. GF-15 treatment was further shown to significantly reduce the tension across sister kinetochores, suppress CC, and activate the SAC, ultimately causing multipolar anaphase and cell death. Intraperitoneal administration of GF-15 in mice resulted in a dose-dependent increase in multipolar mitosis and reduction in tumor size while causing negligible toxicity.[81] In adrenocortical cells harboring numerical and structural chromosomal abnormalities, griseofulvin treatment resulted in a drastic decrease in cell viability and proliferation, accompanied by a dose-dependent increase in the levels of proapoptotic markers.[82] Although the precise cellular target of griseofulvin remains unknown, its effects suggest that it may target KIFC1. Therefore, we assessed the effects of griseofulvin on the ATPase activity of KIFC1 in colon cancer cells (data unpublished). Interestingly, griseofulvin and the KIFC1 inhibitor CW069 (at 50 and 100 μM) displayed similar values of KIFC1 percentage inhibition and mean ligand efficiency for KIFC1 loop L5. The binding energy distribution chart generated from docking studies confirmed that griseofulvin and CW069 exhibited similar binding energies with high clustering (high dispersion from the mean). The ability of griseofulvin to target KIFC1 warrants further validation.

4.6 | Solidagonic acid (SA), kolavenic acid analog (KAA), and kolavenic acid (KA)

Fission yeast cells overexpressing KIFC1 have emerged as a robust tool for phenotypic screening of potential KIFC1 inhibitors. Because fission yeast cells show genetic lethality upon KIFC1 overexpression, molecules that restore the growth of these cells may inhibit KIFC1.[65] Methanol extracts from the root of *Solidago altissima* exhibited strong dose-dependent growth-restoring activity in fission yeast cells. Isolation and characterization of biologically active molecules from these extracts led to the identification of three compounds: SA, KAA, and KA (Figure 11). Although all three molecules increased the growth of yeast colonies, KAA was the most potent. These compounds rescued the viability of YA8 yeast cells (KIFC1 overexpressing cells) by more than two-fold and restored their bipolar spindle assembly, as reflected by the drastic decrease in cells harboring monopolar spindles. Intriguingly, when these three molecules were tested for their centrosome declustering ability in breast cancer cells with supernumerary centrosomes and KIFC1overexpression, only KAA treatment (at 20 and 40 μM) increased the percentage of multipolar spindles.[83] These findings could be explained by the unique cis-decalin skeleton of KAA, which differs

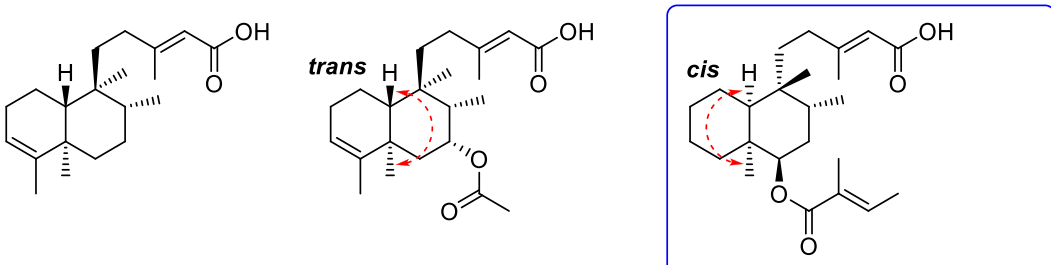

FIGURE 11 Structures of the three natural compounds from the plant *Solidago altissima*: kolavenic acid, solidagonic acid, and kolavenic acid analog. [Color figure can be viewed at wileyonlinelibrary.com]

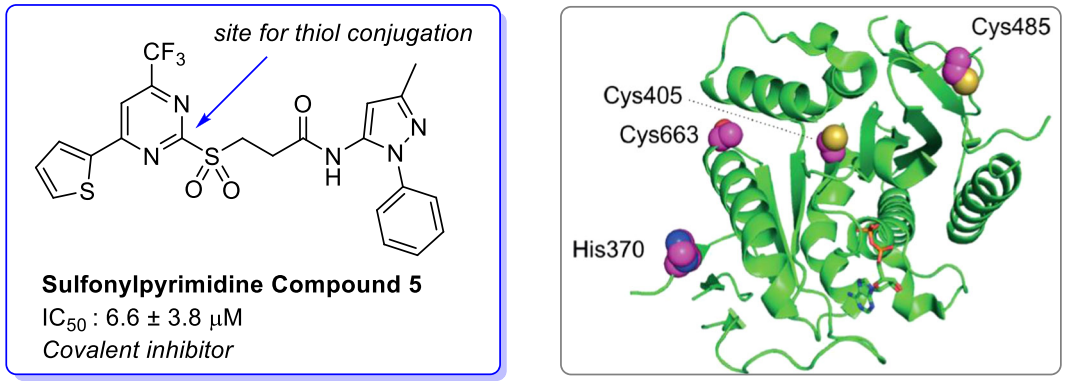

FIGURE 12 Structure of the sulfonyl pyrimidine compound 5 (left) and mapping of the alkylated amino acid residues in the structure of KIFC1 (right) (Reproduced with permission from Förster et al.[84] under license number 5155420237882 Copyright 2019 *European Journal of Organic Chemistry*). [Color figure can be viewed at wileyonlinelibrary.com]

profoundly from the transdecalin scaffold of SA and KA. Being structurally differentiated from other known synthetic KIFC1 inhibitors, KAA should be further investigated to identify its binding site on KIFC1. Further functional and structural characterization of these molecules is warranted.

4.7 | 2-Sulfonyl pyrimidines

While most of the KIFC1 inhibitors reported to date function as reversible inhibitors, Förster et al. recently identified a sulfonyl pyrimidine scaffold that covalently modified KIFC1 (Figure 12). Hits obtained from a screen of 148000 compounds were optimized through rigorous structure–activity relationship studies, which showed that the 2-sulfonyl and 4-CF_3 groups were essential for KIFC1 inhibition. Although multiple compounds were found to be active in a primary assay, only compound 5 (IC_{50}: 6.6 ± 3.8 μM) induced the accumulation of mitotic breast cancer cells with multipolar mitotic spindles. Treatment of HeLa cells with an Eg5 inhibitor combined with S-trityl-L-

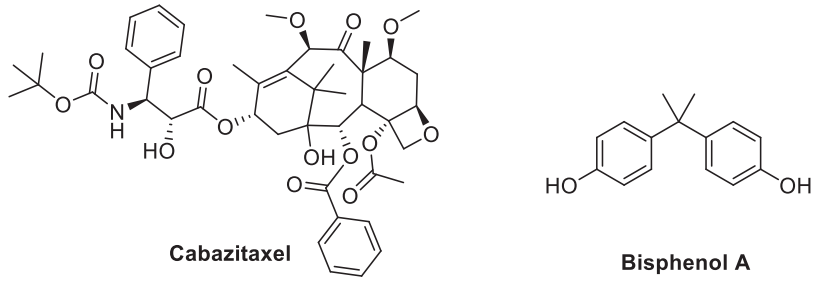

FIGURE 13 Chemical structure of Cabazitaxel and bisphenol A

cysteine and compound 5 reversed the monopolar morphology to bipolar spindle morphology, indicating the direct interaction of compound 5 with KIFC1. Nanoscale liquid chromatography coupled to tandem mass spectrometry (nano-high performance liquid chromatography - mass spectrometry [HPLC-MS/MS]) revealed that two prominent Cys residues (Cys485 and Cys663) mapped away from the binding site but near the surface of the protein were modified with a pyrimidine electrophilic warhead (Figure 12).[84]

4.8 | Cabazitaxel and bisphenol A

Chronic exposure of androgen-sensitive castration-resistant prostate cancer cells to the taxane Cabazitaxel significantly downregulated KIFC1 after an initial ephemeral upregulation owing to enhanced CC and CA accompanied by severe multinucleation culminating into mono-astral spindle formation (Figure 13).[85] The bisphenol compounds bisphenol A and bisphenol F were evaluated for their effect on spindle stability during meiotic division in oocytes (Figure 13). Treatment with both bisphenol compounds disrupted fully assembled spindles, hampered microtubule-chromosome attachments, and decreased KIFC1 levels along microtubules. These findings could explain the indispensable role of KIFC1 in microtubule crosslinking and spindle pole formation during meiosis.[86] These findings also support the notion that bisphenol compounds may downregulate KIFC1. The interaction between bisphenol compounds and KIFC1 merits further investigation.

5 | COMPUTATIONAL DOCKING OF KIFC1 INHIBITORS

This extensive survey of the reported inhibitors of KIFC1 supports the feasibility of targeting KIFC1 with small molecules to promote centrosome declustering in cancer cells with supernumerary centrosomes. However, most of these inhibitors show moderate potency and high micromolar IC_{50} activity. One of the reasons for the suboptimal potency of KIFC1 inhibitors is the lack of structural data, hindering the structure-based design of potent KIFC1 inhibitors. Despite occasional efforts to fill this void, most structure-based design studies are based on obsolete protein structural information or variable homology models using other motor proteins as the backbone. While these strategies may help in the discovery of novel inhibitors, they offer limited structural insight required to further optimize the potency and physicochemical properties of the initial hits. The fact that KIFC1 harbors at least two binding sites further complicates the efforts to obtain reliable binding information in the absence of concrete structural data. To overcome these challenges, we performed a comprehensive computational benchmarking of all the known inhibitors of KIFC1 using the most resolved protein structural information (PDB ID: 5WDH). This analysis aimed to provide a strong structural basis for each KIFC1 inhibitor chemotype, which can be further optimized in structure-based studies. This benchmarking also aimed to rank all available KIFC1 inhibitor

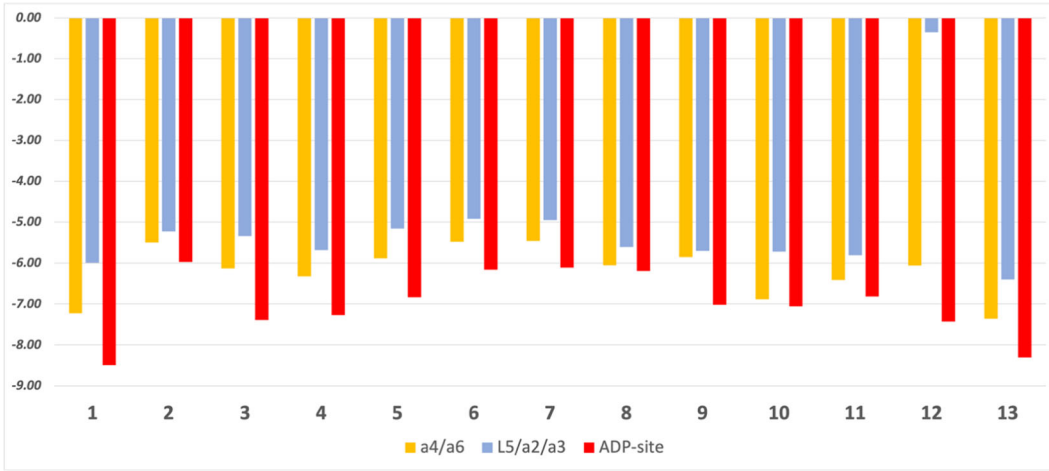

FIGURE 14 Binding affinity values for each KIFC1 ligand[1–13] against different sites: allosteric site a4/a6, L5/a2/a3, and ADP-binding site. The top-5 binding poses for each molecule were considered for the calculation of the average binding affinity score (TOP-5 E_a, avg). 1—AZ82, 2—SR31527, 3—CW069, 4—PJ-34, 5—Phen, 6—Tiq-A, 7—Griseofulvin, 8—Bisphenol A, 9—Solidagonic acid, 10—kolavenic acid analog (KAA), 11—kolavenic acid (KA), 12—Cabazitaxel, 13—2-Sulfonylpyrimidine. The individual binding poses for each inhibitor in each of the three binding sites are mentioned in Supporting Information: Figures S2–18. ADP, adenosine diphosphate. [Color figure can be viewed at wileyonlinelibrary.com]

chemotypes and compare their binding modes to help design novel chemotypes that bind and inhibit KIFC1 (Supporting Information: Table S1).

We chose 13 putative KIFC1 inhibitors across nine different chemotypes as shown in Figure 4 and performed rigid docking against the KIFC1 motor domain (PDB: 5WDH) using AutoDockVina[87] (Supporting Information: Figure S2–18). For each of the ligands, we performed docking against three different sites as shown in Figure 3 and Supporting Information: S1: (1) allosteric α2/α3 site; (2) allosteric L5/α4/α6 site; and (3) the ATP-binding site, as picked from AutoDockVina tool. A 20 × 20 × 20 Å conformational search area was used for each site. Although there are no reports of any inhibitors binding to the ADP-binding site of the protein, we included this site in our analysis to gauge the potential of known inhibitors to bind at this site. For each ligand docking, the top-20 conformations (based on affinity energy scoring) were saved, and the top-5 energy values of each confirmation were averaged for the analysis. The crystal structure of the docking receptor was obtained from the PDB (PDB-ID: 5WDH).[41] The addition of hydrogen atoms to the receptor was done using AMBER's reduce program[88] and AutoDock Tools.[87] The docking results indicated that all molecules exhibited their strongest binding toward the ADP-binding site, followed by the α4/α6 site and the L5/α2/α3 site (Figure 14 and Supporting Information: Table S1).

AZ82 is the most well-characterized KIFC1 inhibitor, both experimentally and computationally. Hence, we compared the reported docking results for AZ82 with our benchmarking results to validate the performance of our method (Figure 15 and Supporting Information: Figure S2). As can be seen from the docked images, there is good concordance in the results with respect to the molecular conformation and interaction during the binding of the inhibitor to the α4/α6 site.

Various interesting observations can be made from our docking data. Importantly, the binding energy values suggest that almost all inhibitors preferably bind to the α4/α6 (allosteric-1) site rather than the L5/α2/α3 site (Supporting Information: Table S1). This trend is consistent with the larger size of α4/α6 than L5/α2/α3, which remains largely inaccessible to inhibitors. This fact is most apparent for cabazitaxel,[12] which has the largest molecular size among all inhibitors and shows the worst binding affinity (<1 kcal/mol) for L5/α2/α3 (Supporting

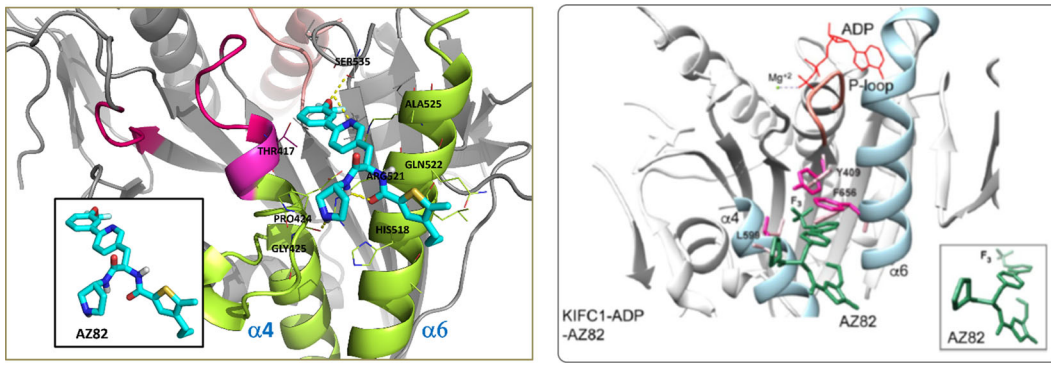

FIGURE 15 Comparison of binding poses of AZ82 into the α4/α6 site in our docking study (left) with that of Park et al.[41] (right). [Color figure can be viewed at wileyonlinelibrary.com]

Information: Figure S17). Our data also suggest that CW069[3] binds with stronger affinity at the α4/α6 site than the L5/α2/α3 site (Supporting Information: Figure S13). Interestingly, CW069 has been proposed to bind to the L5/α2/α3 site in the KSP-derived homology model of KIFC1.[67] Furthermore, these data indicate AZ82 as the most potent KIFC1 inhibitor among all the reported KIFC1 inhibitors, with a binding energy value of −7.26 kcal/mol.

Our results also provide some novel insights into a few KIFC1 inhibitors. For example, it demonstrates that 2-sulfonyl pyrimidine[13] has one of the best binding affinities (−7.36 kcal/mol) among all inhibitors despite being a covalent inhibitor (Supporting Information: Table S1). This finding suggests that the reported activity of 2-sulfonyl pyrimidine[13] might also have resulted from its reversible binding to KIFC1, in addition to its covalent binding. Moreover, this chemotype can potentially be repurposed to function as a reversible inhibitor with minor structural modifications.

Surprisingly, KAA[10] showed a binding energy value (−6.90 kcal/mol) only moderately inferior to that of AZ82,[1] despite the large difference in their reported IC_{50} values.[63,83] This unexpected finding indicates that KAA[10] could be a promising lead for further optimization, especially given the fact that KAA is natural product which generally have favorable physicochemical properties to begin with. Phen,[5] SA,[9] SR31527,[2] and griseofulvin[7] showed similar binding affinities, consistent with their reported IC_{50} (double-digit micromolar range). Furthermore, our findings suggest that PJ-34 is the most potent among the three phenanthrene class inhibitors. Notably, all inhibitors show better binding to the ADP-site of KIFC1 than its α4/α6 site, even though this mode of binding is not considered physiologically possible. Further studies are warranted to confirm this finding and determine its potential implications.

6 | CONCLUSION AND FUTURE PROSPECTS

In this article, we summarized the evolving literature centered around targeting KIFC1 as a promising selective chemotherapeutic option for cancer treatment. While outlining the physiological evidence for targeting KIFC1 in cancer, we focused on detailing small molecule inhibitors that have been reported to bind KIFC1. We went one step ahead and presented the results of a computational benchmarking study, which involved an exhaustive docking analysis of all putative KIFC1 inhibitors across all the possible binding sites on KIFC1. This analysis provided novel insights into the binding potential of KIFC1 inhibitors and has presented some tantalizing possibilities in the future design of potent and highly selective KIFC1 inhibitors.

We believe that this review and benchmarking results will help overcome challenges in the transition of small molecule inhibitors from the bench to the bedside. Most KIFC1 inhibitors were designed based on the structure of

inhibitors of other motor proteins, leading to the lack of inhibitors that strongly bind KIFC1 with little or no binding to other kinesins and motor proteins. This lack of selectively contributes to the high toxicity of current KIFC1 inhibitors and hinders their clinical translation. Exhaustive profiling of KIFC1 inhibitors in nonmalignant cells may help predict clinical outcomes and potential adverse events.

Additionally, the extent of medicinal chemistry efforts required to improve the moderate potency of KIFC1 inhibitors is another challenge that contributes to the lack of clinically approved KIFC1 inhibitors. Generating extensive structural data by cocrystalizing KIFC1 with inhibitors of different chemotypes may help overcome this challenge. Computational benchmarking and other computational docking strategies, such as the incorporation of the inherent dynamics using molecular dynamics simulations, may also assist in the design of novel inhibitor chemotypes and help overcome challenges in their clinical translation. The use of targeted protein degradation and other emerging technologies to optimize lead compounds may also accelerate progress in this field.

AUTHOR CONTRIBUTIONS

Conceptualization: Nivya Sharma, Rishikesh Narayan, and Ritu Aneja. *Manuscript writing and editing*: Nivya Sharma and Rishikesh Narayan. *Computational data generation*: Dani Setiawan and Rishikesh Narayan. *Review, editing, and proof-reading the manuscript*: Nivya Sharma, Rishikesh Narayan, Dani Setiawan, Donald Hamelberg, and Ritu Aneja. *Supervision*: Ritu Aneja. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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