Allosteric Targeting of Aurora A Kinase Using Small Molecules: A Step Forward Towards Next Generation Medicines?

Resmi C. Panicker[a,b,†], Anthony G. Coyne[c,†] and Rajavel Srinivasan[a,*]

[a]*School of Pharmaceutical Science and Technology, Tianjin University, 92 Weijin Road, Nankai District, Tianjin-3000072;* [b]*Department of Chemistry, NSS Hindu College, Mahatma Gandhi University, Changanacherry-686102, Kerala, India;* [b]*University Chemical Laboratory, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom;* †*These authors contributed equally to this work.*

Abstract: Aurora A (AurA) kinase is a key mitotic protein essential for carrying out numerous cellular functions. Overexpression or malfunction of this enzyme results in numerous human diseases most notably in cancer. Several small molecule inhibitors targeting the ATP binding site of this enzyme are in various stages of clinical development. However, ATP binding site inhibitors can result in selectivity problems often leading to undesirable off-target effects. Moreover, these drugs are prone to drug resistance problem rendering them unfit for long-term administration. Allosteric inhibition of kinases using small molecules is an alternative strategy to target these enzymes and these could serve as the seeds for next generation medicines and minimize any selectivity problems associated with ATP binding site inhibitors. This review discusses the developments in the non-ATP site binding small molecule inhibitors of AurA and their prospect as future therapeutics and tools for chemical biology.

Keywords: Aurora A, AURKA, TPX2, AurA-TPX2 inhibition, kinase inhibitors, allosteric inhibition, protein-protein interaction, Type IV inhibitors.

1. INTRODUCTION

Kinases play a crucial role in signal transduction and numerous cellular processes [1, 2]. Aberrant regulation of kinases leads to various human diseases. Kinases have been targeted to treat a range of diseases such as cancer [3-5], diabetes [6, 7], rheumatoid arthritis [8, 9], idiopathic pulmonary fibrosis [10], neurological [11, 12], immune disorders [13], and even cardiovascular diseases [14]. As of September 2016, a total of 33 small molecule inhibitors targeting various kinases have been approved by the FDA with around 20 of these drugs approved since 2010 [15, 16]. This is the testimony of how crucial targeting these enzymes are in the present drug discovery arena. The majority of these approved drugs target the ATP binding site (active site), which is highly conserved within different families of kinases [17, 18]. Targeting the ATP binding site of the kinases can result in poor selectivity and can cause side-effects for the patients. In addition, there can be the emergence of drug resistance due to kinase active site mutation [19, 20]. One way of tackling this problem is by designing inhibitor which could binds at a distant allosteric site on a kinase and able to arrest the kinase activity. These type of inhibitors are referred to as type IV inhibitors [17, 21, 22]. Unlike the enzyme active site which are characterized by deep and hydrophobic pockets, allosteric sites are often shallow pockets which makes them difficult to target using small molecules as the interaction area is minimal with small molecules unlike the natural protein ligands or peptides [23-26].

*Address correspondence to this author at the School of Pharmaceutical Science and Technology, Tianjin University, Building 24, No. 92, Weijin Road, Nankai district, Tianjin - 300072, P. R. China; Tel/Fax: +86-22-87401830; E-mails: rajavels@tju.edu.cn

Generally, kinases possess multiple allosteric sites which could be used to bind and interact with different other protein partners. Bivalent phage display approach [xx] and in situ screening methods [xx] can be used to identify allosteric sites which are close to the active sites of enzymes. Targeting distant allosteric sites would be more beneficial in coming up with highly selective inhibitors which are less prone to resistance problems. Those allosteric sites could be identified and targeted by small molecules by two most common approaches. (1) High-throughput screening (HTS) of a large library of compounds and (2) Fragment-based approaches. HTS followed by Structure-Activity Relationship (SAR) studies remains the most common choice to identify allosteric inhibitors. Inhibitors targeting the allosteric sites of Mitogen-Activated Protein Kinases (MAPK) discovered by this approach are in various stages of clinical trials [27,28]. Fragment-based approaches using X-ray crystallography [29] and tethering are more rational ways to identify allosteric ligands. 3-phosphoinositide-dependent protein kinase-1 (PDK1) has been subjected to allosteric inhibition by using disulfide-based tethering approach [30]. Recently, computational method [30], vibrational spectroscopic technique (discussed in 1.2.2), etc. to identify distant allosteric sites are also beginning to appear. Apart from MAPK and PDK1 there are only a handful of other kinases which have been successfully targeted at distant allosteric sites using small molecules, one such kinase is Aurora A (AurA).

Aurora Kinases are the family of three Serine/Threonine kinases (A, B and C) that play key roles in cell division [31, 32]. AurA is associated in regulating the early stages of the mitosis, which includes centrosome maturation and separation, mitotic entry and bipolar spindle formation, and has a major role in upholding the genomic integrity of the daughter cells [33-38]. AurA is frequently overexpressed in diverse human cancers such as breast, pancreatic, ovarian, and gastric [39]. It is also known to deregulate a crucial tumour suppressor protein p53 [40]. Moreover, overexpression of AurA induces resistance to Taxol, a first-line chemotherapeutic agent [41]. Recently it has been suspected that AurA could contribute to glioblastoma tumor recurrence [42]. All of the above mentioned factors suggest that AurA has potential as an excellent target for cancer treatment and necessitates the need for novel therapeutic agents to selectively target AurA with fewer off-target effects. A number of ATP competitive inhibitors of AurA are already in various stages of clinical development [5, 43]. However, all these small molecules target the highly homologous ATP active site on the kinase and may result in selectivity and drug-resistance problems. Recently, there is an interest aimed at targeting allosteric inhibition sites of AurA kinase and this review summarizes the small-molecule inhibitors which have been developed to target allosteric sites of AurA kinase.

1.1. Targeting the AurA-TPX2 interaction:

Allosteric sites on kinases are often used for interacting with other protein partners, and by targeting these, selective modulation of the kinases could be achieved [16, 22]. AurA kinase is known to bind and interact with various protein partners to carry out its cellular functions. Among them the interaction with Targeting Protein for Xenopus kinesin-like protein 2 (TPX2) is structurally and biochemically well studied [44, 45]. This interaction is crucial for the successful mitotic progression. Abolishing TPX2 function in cells prevents the localization of AurA to the spindles during mitosis and resulted in apoptosis [46]. TPX2 is a larger protein than AurA, however, residues 7-43 of TPX2 has shown to be adequate for binding and activating AurA [44, 45, 47]. Residues 7-21 bind at the N-terminal lobe of AurA and responsible for anchoring the TPX2 mainly through hydrophobic interactions whilst TPX2 residues 30-43 interact with the activation loop of the kinase. Upon TPX2 binding, AurA attains the active conformation necessary for catalysis [44]. Hence by targeting the AurA-TPX2 interaction using small molecules, one could selectively abolish the kinase activity. A number of research groups have reported targeting this interaction and these are reviews below.

1.1.1 Lewis' Patent on HTS to identify non-ATP competitive AurA inhibitors

One of the first high-throughput screening (HTS) campaigns to identify non-ATP competitive AurA inhibitors was carried out by Lewis and co-workers at EMBL, Heidelberg [48]. They identified a number of non-ATP competitive compounds based on aromatic amides and urea derivatives capable of inhibiting the AurA kinase activity both *in vitro* and in HeLa cells, and one of those compounds was validated by TPX2 competition assay to bind at the TPX2 binding site on AurA kinase.

In their study, a commercial library of 53,000 compounds were screened for Aur A kinase activity. Standard luciferase assay for measuring kinase activity was employed to find out the active compounds [49]. The active compounds identified in the screening were then subjected to a commercial Z'Lyte Kinase™ assay (a Fluorescence Resonance Energy Transfer-based biochemical assay) to ascertain if they were ATP competitive. Dose-response experiments were performed and the IC_{50} values for the active compounds were measured at 3 different ATP concentrations of 20, 200 and 1000 μM. The criterion that set was if the IC_{50} value of the compound increased by more than 5 folds per log10 increase in ATP concentration, then the particular compound was considered to be ATP competitive. Four compounds (compounds **1** to **4**) were identified to disobey this criteria and were considered to be non-competitive with ATP (Fig. **1**). Three out of these four compounds posses a common 4,4'-bipyridine scaffold (compounds **1**, **2** and **3**) with a urea linkage whilst the last hit was a nicotinoyl derivative with amide linkages (compound **4**). Data for compound **3** showed that the IC_{50} values remained almost unchanged even at 1000 μM concentration of ATP. The next step to determine where these compounds were binding was to perform a competition assay with recombinant TPX2 protein which was carried out to ascertain if the ATP non-competitive compounds bind at TPX2 binding site on AurA. Compound **1** was found to compete with TPX2 recombinant peptide in the competition assay where it's IC_{50} value increased from 4.8 μM (in the absence of recombinant TPX2) to 78.8 μM (in the presence of recombinant TPX2) suggesting that the compound is competing with TPX2 and could be binding at the TPX2 binding site on the AurA. Furthermore, in a cell-based assay

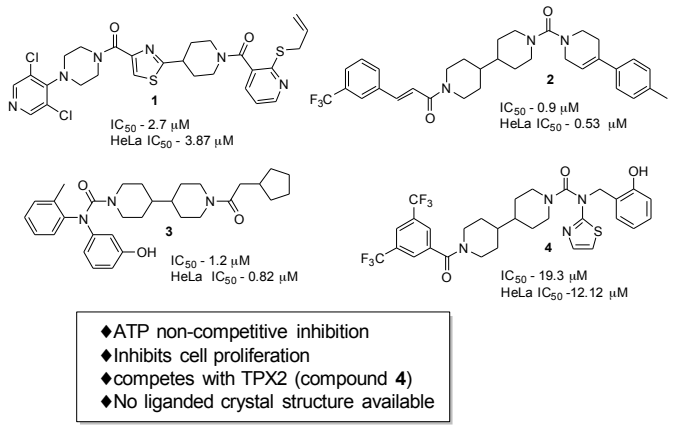

Fig. (1). Non-ATP competitive AurA inhibitors

compounds (**1-4**) were shown to inhibit cell proliferation by inhibiting AurA kinase activity. All the 4 compounds were showing IC_{50} of low μM values in HeLa cells. From this study, the 4 compounds (**1- 4**) identified were shown to be ATP non-competitive and could abolish AurA kinase activity but their binding site is still unclear due to the lack of any X-ray crystal structure data of the protein-inhibitor complex.

1.1.2 Conti's Patent on computational modeling of allosteric AurA inhibitors

Conti, Bayliss and co-workers were the first to disclose the X-ray crystal structures of the phosphorylated human AurA fragment (122-403) and also with a ligand bound (human TPX2 residues 1-43). They were among the pioneers to elucidate the structural basis of AurA activation by TPX(1-43) peptide [44]. In a patent, based on the X-ray crystal structures and using computational modeling studies, they have designed compounds with the aim of allosteric inhibition of AurA kinase [50]. They have shown a few indole and indene derived compounds could act as inhibitors of AurA kinase and claim that these compounds induces conformational change on the protein and therefore decreasing the kinase activity. Further they claim that some of these molecules or at least a portion of the molecules bind with one or more residues on the TPX2 binding region on AurA (Fig. **2**). However no crystal structure of these small molecules- AurA complexes were reported and have not been deposited in the PDB. In a cell based assay the indoles **5** and **6** are specific towards Aurora Kinase and it does not interfere with the activities of Protein Kinase A (PKA). Conversely, other than PKA the effect of these small molecules on the activities of other kinases were not compared.

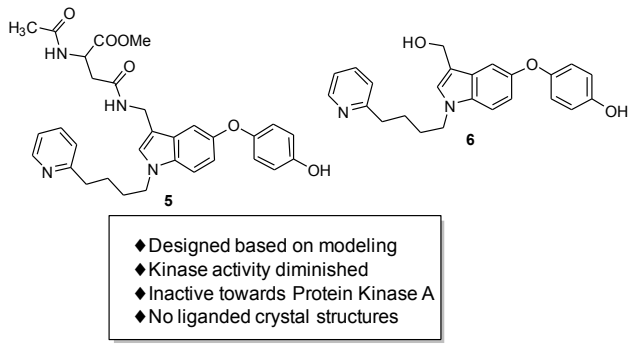

Fig. (2). Non-competitive AurA inhibitors designed based on modeling

1.1.3 Cambridge work on AurA-TPX2 inhibitors

A team of research groups at the University of Cambridge has successfully developed inhibitors of the protein-protein interaction between AurA and TPX2(1-43) peptide [51]. The compounds inhibit AurA activity by a novel mechanism as elucidated by crystal structures, *in vitro* and *in vivo* assays. A library of 170,000 rationally selected compounds [52] was screened in order to identify novel PPI inhibitors between the AurA and TPX2 interaction using an in-house developed FP assay. The assay made use of TAMRA-labeled TPX2(1-43) peptide which binds with a K_d of 8 nM to AurA(123-403) segment in fluorescence anisotropy assay. Fifteen compounds were found as hits and in order to make sure that the compounds are ATP non-competitive, the screening was performed by blocking the ATP-site of the kinase using a potent non-selective kinase inhibitor JNJ-7706621 (JNJ) [53]. JNJ does not affect the binding of TPX2(1-43) peptide and stabilizes AurA. It was found that various hit compounds were able to bind with AurA even in the presence of excess JNJ suggesting that the compounds are non-ATP competitive. Compound **7** was identified to be the most promising hit with a ligand efficiency (L_{Eff}) of 0.25 and a K_d of 10.6 μM (ITC) with AurA which was in line with K_i of 8.9 μM determined in FP assay (Fig. **3**). A brief SAR study was carried out by modification of the substituents on the quinoline ring in compound **7** and from the study the team identified compound **8** to be more ligand efficient (L_{Eff} = 0.36) and potent compound with a K_d of 3.77 μM (ITC).

A X-ray crystal structure of the ligand-protein complex is the gold standard for pinpointing the binding site of the ligand with the protein. The Cambridge team is the first to obtain and publish the X-ray crystal structure of TPX2 site inhibitor-AurA complex (2.05 Å - 2.86 Å). The X-ray crystal structure revealed compound **8** was positioned inside a hydrophobic pocket (termed as 'Y' pocket) on AurA where the two tyrosine residues in the **Y**S**Y** motif (Y = Tyrosine, S = Serine) of TPX2 binds. The binding of the ligand is characterized by the interaction of the quinoline unit and phenyl ring which form hydrophobic interaction in the so called 'Y'-pocket as defined by residues L178, V182, V206 and L208. The bromine substituent on the phenyl ring helped to reinforce the contact . Further, the carboxylic acid group on compound **8** is engaged in ionic interaction with K166 and H201. The binding of the ligand induced local conformational changes to the protein with the electron density of the activation loop remain largely undefined. The αC helix and the glycine rich G-loop also changed conformation and it should be noted that this loop plays key roles in AurA kinsase activity. In the *in vitro* kinase assays, compound **8** showed a dose dependent inhibition of the kinase activity with a K_i of 3.7 μM. The kinetic profile showed that the K_m value of ATP remained unchanged suggesting an allosteric mode of inhibition. In cell based assay compound **8** caused mislocalisation of AurA from the bipolar spindle and also resulted in decreased T288 phosphorylation - an indication of decreased AurA activity. All the above results clearly suggest that the 'Y' pocket is indeed druggable and inhibitors targeting this site may allosterically inhibit the kinase activity by a novel mechanism opening up new avenues for specifically targeting AurA.

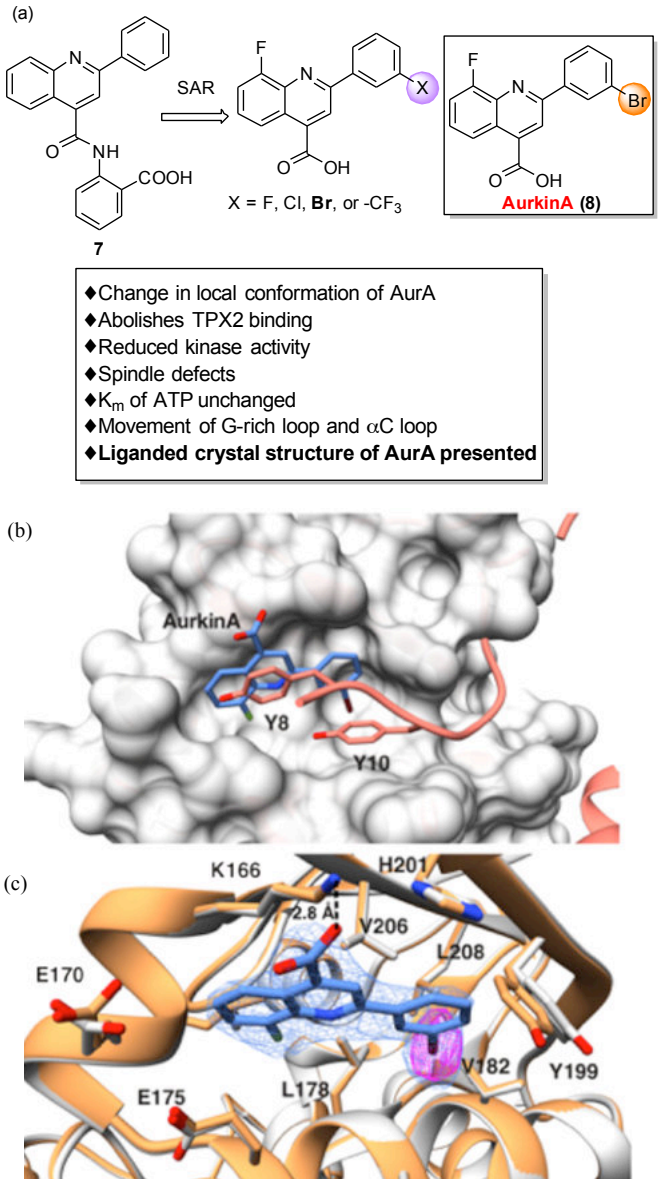

Fig. (3). Work from the Cambridge group (a) Structures of AurA-TPX2 inhibitors identified by the Cambridge group. (b) Crystal structure of the Y-pocket of AurA showing the binding of AurkinA (blue) overlayed with TPX2(8-11) fragment (orange) (c) Interactions of AurkinA in the Y-pocket of AurA [Figures (3b) and (3c) were reprinted from Scientific Reports, DOI: 10.1038/srep28528 which has been distributed under a Creative Commons CC-BY license]

1.2. Other Allosteric modulators of AurA

Apart from TPX2 protein, AurA binds and gets activated by various other protein partners such as Ajuba [54], Bora [55], N-Myc [55a], etc. This provide opportunities to design small molecules which could modulate AurA functions selectively. A few selective modulators of AurA has been recently reported and reviews below.

1.2.1 Anarcardic acid as selective modulator of AurA

There has been numerous reports on upstream regulators of AurA such as TPX2, Ajuba and Bora which promote phosphorylation and increase kinase activity of Aurora kinase A (AurA). Kishore and co-workers reported for the first time, a small-molecule natural product compound that could enhance the activity of Aurora Kinases, specifically AurA [56]. They adopted an *in silico* approach using Hex 4.5 software to screen natural product compounds from various resources. The search led to the finding of Anacardic Acid (AA) isolated from the nutshell liquid of *Anacardium occidentale* [57]. Anacardic acid is a salicylic acid derivative with an alkyl chain (Fig. **4**). The validation of docking experiments and the measurement of dose-response confirmatory assays were performed by measuring the extent of histone H3 phosphorylation by AurA and Aurora kinase B (AurB) in the presence or absence of AA. Though AA showed interaction with both AurA (amino acid residues 126-129) and AurB (amino acid residues 142-154) in the initial docking studies, it eventually turned out that only AurA gets augmentation in activity by 5 fold upon AA binding and not AurB.

The authors then investigated the detailed mechanistic aspects of this specific activity enhancement upon AurA-AA interaction. The effect of equilibrium binding of ATP and

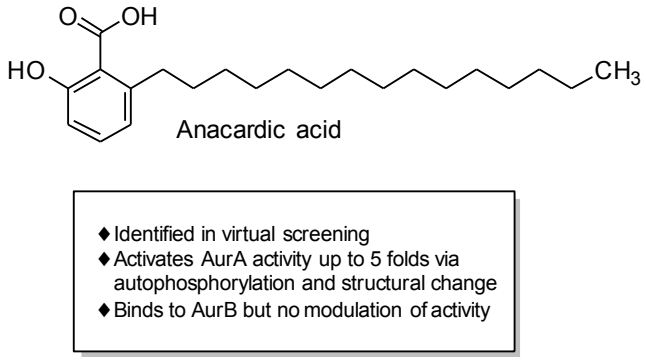

Fig. (4). Anacardic acid: Selective modulator of AurA.

AA to AurA was analysed (measuring the association constant (K_a) of ATP to AurA in presence of AA and vice versa) by spectrofluorometric assays. It was concluded that AA induces a structural change in the AurA, which in turn enhances the ATP recruitment by the enzyme thereby activating the process of phosphorylation. It was also found that AA enhances AurA activity by increasing the V_{max} of the reaction as well (from the kinetic analysis of AA mediated activation of AurA). This data and further investigations point to AurA kinase activity enhancement is through enhancement of autophosphorylation. To conclude, AA mediated alteration of enzyme structure and activation of autophosphorylation are the underlying mechanisms for inducing AurA activity. The role of AurA during mitosis has already been defined to a large extent. As AurA is a target

molecule for the development of antineoplastic drugs that attempt to halt cell proliferation, it would be more interesting to find out the functional groups of AA responsible for the activation of AurA and to establish its further link between over-expression of AurA and cancer.

1.2.2 Felodipine: uncompetitive inhibitor of AurA

Karthigeyan and co-workers demonstrated that SERS (surface enhanced Raman spectroscopy) could be used as a technique to determine the binding site of small molecules on the Aurora A protein [58]. SERS is a vibrational spectroscopy technique that is highly sensitive where it can detect molecules at the single molecule level [59]. This technique is widely used in the area of nanotechnology however it has only recently been applied to the study of both protein-protein and protein-ligand interactions [60-62].

The authors were interested in the development of a selective Aurora A inhibitor by trying to differentiate these from Aurora B. They discovered that the drug felodipine could act as a selective inhibitor of Aurora A under physiological conditions. Felodipine is a dihydropyridine compounds that is used as an antihypertensive drug for high blood pressure and can inhibit the H3 phosphorylation by Aurora A in a dose dependant manner however with negligible effect on Aurora B. In order to obtain a suitable SERS spectra citrate capped silver nanoparticles were attached to the positive charged regions of the kinase proteins by electrostatic attraction at physiological pH. A significant change in the SERS spectra of Aurora A complexed with felodipine compared to that of Aurora B was observed, which was attributed to a change in orientation in the Aurora A attachment on the silver nanoparticle (Fig. **5**). The key peaks in the SERS spectrum are from the aromatic amino acids Phe, Tyr, His and Trp amide bands. It was hypothesised the felodipine was a surface binding ligand and the most significant effect observed upon felodipine binding to Aurora A was the amide I band which changed from 1,620cm^{-1} to 1,647 cm^{-1}. Upon binding of a know Aurora A and Aurora B inhibitor, reversine, no change in this amide band was observed, however two new peaks were observed at 1,297 and 1,369 cm^{-1} in Aurora A-reversine complex and 1,293 and 1,376 cm^{-1} for Aurora B-reversine complex. Under competition experiments it was shown that reversine was not competitive with felodipine suggesting an alternative binding site.

These results suggest that the felodipine is binding at a new site on Aurora A. In order to confirm this further MD simulations and molecular docking suggested that felodipine causes a number of conformational changes that lined the active site which may affect the ATP binding to Aurora A. Further experimental evidence for the binding of felodipine, point mutations were carried out on Aurora A and this confirmed the binding mechanisms predicted by these techniques. The results obtained from SERS, molecular docking and MD simulations suggest that felodipine is an uncompetitive inhibitor of Aurora A. This study also shows that the technique such as SERS can distinguish between surface binding and competitive binding of small molecule's of Aurora A kinase.

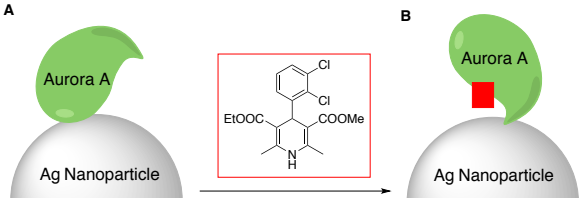

Fig. (5). A: mode of attachment of Aurora A to Ag nanoparticle. B: Change in orientation of Aurora A to Ag nanoparticle upon complexation with felodipine (red box).

CONCLUSION AND OUTLOOK

Allosteric inhibition of AurA has been shown to be an alternative strategy for inhibition as it is expected to have fewer off-target effects when compared with the ATP binding site targeting strategies. These allosteric site binding inhibitors could prove to be less prone to drug resistance and may also find utility in the reversal of taxane resistance, a major clinical problem in the cancer treatment. Apart from the potential clinical uses, these molecules could act also as valuable chemical tools to uncover a wide range of biological roles of AurA and its protein partners. New techniques such as fragment-based approaches, SERS-methods are evolving to identify allosteric site binding ligands thus overcoming the general difficulties associated with the identification of such ligands. Research towards allosteric inhibition of AurA is rapidly growing, and expected to deliver fruitful outcome in terms of novel therapeutics and understanding of the cellular mechanism of AurA. This strategy of allosteric-targeting could also be amenable to identify selective modulators for other classes of therapeutically important protein targets.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENT

RS gratefully acknowledges the School of Pharmaceutical Science and Technology (SPST), Tianjin University for the funding support (Start-up fund No: 290-0903071002).

REFERENCES

[1] Shokat, K.; Velleca, M. Novel chemical genetic approaches to the discovery of signal transduction inhibitors. *Drug Discov. Today*, **2002**, *7*(16), 872-879.

[2] Johnson, L.N.; Lewis, R.J. Structural basis for control by phosphorylation. *Chem. Rev.*, **2001**, *101*(8), 2209-2242.

[3] Lapenna, S.; Giordano, A. Cell cycle kinases as therapeutic targets for cancer. *Nat. Rev. Drug Discov.,* **2009***, 8*(7), 547-566.

[4] Keen, N.; Taylor, S. Aurora-kinase inhibitors as anticancer agents. *Nat. Rev. Cancer*, **2004**, *4*(12), 927-936.

[5] Bavetsias, V.; Linardopoulos, S. Aurora kinase inhibitors: Current status and outlook. *Front. Oncol*., **2015**, *5*, 278.

[6] Coghlan, M.P.; Smith, D.M. Introduction to the Kinases in Diabetes Biochemical Society Focused Meeting: are protein kinases good targets for antidiabetic drugs? *Biochem. Soc. Trans*., **2005**, *33*(Pt 2), 339-342.

[7] Nandipati, K. C.; Subramanian, S.; Agrawal, D.K. Protein kinases: mechanisms and downstream targets in inflammation-mediated obesity and insulin resistance. *Mol. Cell Biochem*., **2016**, DOI:10.1007/s11010-016-2878-8

[8] Williams, N.K.; Bamert, R.S.; Patel, O.; Wang, C.; Walden, P.M.; Wilks, A.F.; Fantino, E.; Rossjohn, J.; Lucet, I.S. Dissecting specificity in the Janus kinases: the structures of JAK-specific inhibitors complexed to the JAK1 and JAK2 protein tyrosine kinase domains. *J. Mol. Biol*., **2009**, *387*(1), 219–232.

[9] Semerano, L.; Decker, P.; Clavel, G.; Boissier, M.C. Developments with investigational Janus kinase inhibitors for rheumatoid arthritis. *Expert Opin. Investig. Drugs*, **2016**, *25*(12), 1355-1359.

[10] Kutluk Cenik, B.; Ostapoff, K.T.; Gerber, D.E.; Brekken, R.A. BIBF 1120 (nintedanib), a triple angiokinase inhibitor, induces hypoxia but not EMT and blocks progression of preclinical models of lung and pancreatic cancer. *Mol. Cancer Ther*., **2013**, *12*(6), 992-1001.

[11] Chico, L.K.; Van Eldik, L.J.; Watterson, D.M. Targeting protein kinases in central nervous system disorders. *Nat. Rev. Drug Discov*., **2009**, *8*(11), 892-909.

[12] Zhuo, Z.H.; Sun, Y.Z.; Jin, P.N.; Li, F.Y.; Zhang, Y.L.; Wang, H.L. Selective targeting of MAPK family kinases JNK over p38 by rationally designed peptides as potential therapeutics for neurological disorders and epilepsy. *Mol. Biosyst*., **2016**, *12*(8), 2532-2540.

[13] Altman, A.; Kong, K.F. Protein kinase C inhibitors for immune disorders. *Drug Discov. Today*, **2014**, *19*(8), 1217-1221.

[14] Rask-Andersen, M.; Zhang, J.; Fabbro, D.; Schiöth, H.B. Advances in kinase targeting: current clinical use and clinical trials. *Trends Pharmacol. Sci*., **2014**, *35*(11), 604-620.

[15] U.S. Food and Drug Administration. Resources for Information on Approved Drugs. http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ (Accessed December 03, 2016).

[16] Wu, P.; Clausen, M.H.; Nielsen, T.E. Allosteric small-molecule kinase inhibitors. *Pharmacol. Ther*., **2015**, *156*, 59-68.

[17] Wu, P.; Nielsen, T.E.; Clausen, M.H.; FDA-approved small-molecule kinase inhibitors. *Trends Pharmacol. Sci*., **2015**, *36*(7), 422-439.

[18] Wu, P.; Nielsen, T.E.; Clausen, M.H. Small-molecule kinase inhibitors: an analysis of FDA-approved drugs. *Drug Discov. Today*, **2016**, *21*(1), 5-10.

[19] Gibbons, D.L.; Pricl, S.; Kantarjian, H.; Cortes, J.; Quintás-Cardama, A. The rise and fall of gatekeeper mutations? The BCR-ABL1 T315I paradigm. *Cancer*, **2012**, *118*(2), 293-299.

[20] Weisberg, E.; Manley, P.W.; Cowan-Jacob, S.W.; Hochhaus, A.; Griffin, J.D. Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia. *Nat. Rev. Cancer*, **2007**, *7*(5), 345-356.

[21] Zhang, J.; Adrian, F.J.; Jahnke, W.; Cowan-Jacob, S.W.; Li, A.G.; Iacob, R.E.; Sim, T.; Powers, J.; Dierks, C.; Sun, F.; Guo, G.R.; Ding, Q.; Okram, B.; Choi, Y.; Wojciechowski, A.; Deng, X.; Liu, G.; Fendrich, G.; Strauss, A.; Vajpai, N.; Grzesiek, S.; Tuntland, T.; Liu, Y.; Bursulaya, B.; Azam, M.; Manley, P.W.; Engen, J.R.; Daley, G.Q.; Warmuth, M.; Gray, N.S. Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. *Nature*, **2010**, 28, *463*(7280), 501-506.

[22] Cox, K.J.; Shomin, C.D.; Ghosh, I. Tinkering outside the kinase ATP box: allosteric (type IV) and bivalent (type V) inhibitors of protein kinases. *Future Med. Chem*., **2011**, *3*(1), 29-43.

[23] Wells, J.A.; McClendon, C.L. Reaching for high-hanging fruit in drug discovery at protein–protein interfaces. *Nature*, **2007**, *450*(7172), 1001-1009.

[24] Liu, F.; Wang, J.; Yang, L.; Liu, L.; Ding, S.; Fu, M.; Deng, L.; Gao, L.Q. Developing a fluorescence-coupled capillary electrophoresis based method to probe interactions between QDs and colorectal cancer targeting peptides. *Electrophoresis*, **2016**, *37*(15-16),2170-2174.

[25] Wang, J.; Zhang, C.; Liu, L.; Kalesh, K.A.; Qiu, L.; Ding, S.; Fu, M.; Gao, L.Q.; Jiang, P. A capillary electrophoresis method to explore the self-assembly of a novel polypeptide ligand with quantum dots. *Electrophoresis*, **2016**, *37*(15-16), 2156-2162.

[26] Fischer, G.; Rossmann, M.; Hyvönen, M. Alternative modulation of protein–protein interactions by small molecules. *Curr. Opin. Biotechnol*., **2015**, *35*, 78-85.

[27] Zhao, Y.; Adjei, A.A. The clinical development of MEK inhibitors. *Nat. Rev. Clin. Oncol*., **2014**, *11*(7), 385-400.

[28] Abe, H.; Kikuchi, S.; Hayakawa, K.; Iida, T.; Nagahashi, N.; Maeda, K.; Sakamoto, J.; Matsumoto, N.; Miura, T.; Matsumura, K.; Seki, N.; Inaba, T.; Kawasaki, H.; Yamaguchi, T.; Kakefuda, R.; Nanayama, T.; Kurachi, H.; Hori, Y.; Yoshida, T.; Kakegawa, J.; Watanabe, Y.; Gilmartin, A.G.; Richter, M.C.; Moss, K.G.; Laquerre, S.G. Discovery of a highly potent and selective MEK inhibitor: GSK1120212 (JTP-74057 DMSO solvate). *ACS Med. Chem. Lett*., **2011**, *2*(4), 320-324.

[29] Sadowsky, J.D.; Burlingame, M.A.; Wolan, D.W.; McClendon, C.L.; Jacobson, M.P.; Wells, J.A. Turning a protein kinase on or off from a single allosteric site via disulfide trapping. *Proc. Natl. Acad. Sci. U S A*, **2011**, *108*(15), 6056-6061.

[30] Kroon, E.; Schulze, J.O.; Süß, E.; Camacho, C.J.; Biondi, R.M.; Dömling, A. Discovery of a potent allosteric kinase modulator by combining computational and synthetic methods. *Angew. Chem. Int. Ed. Engl*., **2015**, *54*(47), 13933-13936.

[31] Carmena, M.; Earnshaw, W.C. The cellular geography of aurora kinases. *Nat. Rev. Mol. Cell Biol*., **2003**, *4*(11), 842-854.

[32] Goldenson, B.; Crispino, J.D. The aurora kinases in cell cycle and leukemia. *Oncogene*, **2015**, *34*(5), 537-545.

[33] Barr, A.R.; Gergely, F. Aurora-A: the maker and breaker of spindle poles. *J. Cell Sci*., **2007**, *120*(Pt 17), 2987-2996.

[34] Berdnik, D.; Knoblich, J.A. Drosophila Aurora-A is required for centrosome maturation and actin-dependent asymmetric protein localization during mitosis. *Curr. Biol*., **2002**, *12*(8), 640-647.

[35] Hannak, E.; Kirkham, M.; Hyman, A.A.; Oegema, K. Aurora-A kinase is required for centrosome maturation in Caenorhabditis elegans. *J. Cell Biol*., **2001**, *155*(7), 1109-1116.

[36] Giet, R.; McLean, D.; Descamps, S.; Lee, M.J.; Raff, J.W.; Prigent, C.; Glover, D.M. Drosophila Aurora A kinase is required to localize D-TACC to centrosomes and to regulate astral microtubules. *J. Cell Biol*., **2002**, *156*(3), 437-451.

[37] Cowley, D.O.; Rivera-Pérez, J.A.; Schliekelman, M.; He, Y.J.; Oliver, T.G.; Lu, L.; O'Quinn, R.; Salmon, E.D.; Magnuson, T.; Van Dyke, T. Aurora-A kinase is essential for bipolar spindle formation and early development. *Mol. Cell Biol*., **2009**, *29*(4), 1059-1071.

[38] Bird, A.W.; Hyman, A.A. Building a spindle of the correct length in human cells requires the interaction between TPX2 and Aurora A. *J. Cell Biol*., **2008**, *182*(2), 289-300.

[39] Gautschi, O.; Heighway, J.; Mack, P.C.; Purnell, P.R.; Lara, P.N.; Jr, Gandara, D.R. Aurora kinases as anticancer drug targets. *Clin. Cancer Res*., **2008**, *14*(6), 1639-1648.

[40] Liu, Q.; Kaneko, S.; Yang, L.; Feldman, R.I.; Nicosia, S.V.; Chen, J.; Cheng, J.Q. Aurora-A abrogation of p53 DNA binding and transactivation activity by phosphorylation of serine 215. *J. Biol. Chem*., **2004**, *279*(50), 52175-52182.

[41] Anand, S.; Penrhyn-Lowe, S.; Venkitaraman, A.R. AURORA-A amplification overrides the mitotic spindle assembly checkpoint, inducing resistance to Taxol. *Cancer Cell*, **2003**, *3*(1), 51–62.

[42] Willems, E.; Lombard, A.; Dedobbeleer, M.; Goffart, N.; Rogister, B. The Unexpected Roles of Aurora A Kinase in Gliobastoma Recurrences. *Targ. Oncol*., **2016**, DOI:10.1007/s11523-016-0457-2

[43] Boss, D.S.; Beijnen, J.H.; Schellens, J.H. Clinical experience with aurora kinase inhibitors: a review. *Oncologist*, **2009**, *14*(8), 780-793.

[44] Bayliss, R.; Sardon, T.; Vernos, I.; Conti, E. Structural basis of Aurora-A activation by TPX2 at the mitotic spindle. *Mol. Cell*, **2003**, *12*(4), 851-862.

[45] Rennie, Y.K.; McIntyre, P.J.; Akindele, T.; Bayliss, R.; Jamieson, A.G. A TPX2 proteomimetic has enhanced affinity for Aurora-A due to hydrocarbon stapling of a helix. *ACS Chem. Biol*., **2016**, DOI: 10.1021/acschembio.6b00727

[46] Kufer, T.A.; Silljé, H.H.; Körner, R.; Gruss, O.J.; Meraldi, P.; Nigg, E.A. Human TPX2 is required for targeting Aurora-A kinase to the spindle. *J. Cell Biol*., **2002**, *158*(4), 617-623.

[47] Anderson, K.; Yang, J.; Koretke, K.; Nurse, K.; Calamari, A.; Kirkpatrick, R.B.; Patrick, D.; Silva, D.; Tummino, P.J.; Copeland, R.A.; Lai, Z. Binding of TPX2 to Aurora A alters substrate and inhibitor interactions. *Biochemistry*, **2007**, *46*(36), 10287-10295.

[48] Lewis, J. Aurora Kinase Inhibitors. WO2007115805, October 18, 2007.

[49] Garuti, L.; Roberti, M.; Bottegoni, G. Small molecule aurora kinases inhibitors, *Curr. Med. Chem*., **2009**, *16*(16), 1949-1963.

[50] Conti, E.; Bayliss, R.; Schultz, C.; Vernos, I.; Sardon, T. Crystals of an aurora-A TPX2 complex, TPX2 binding site of aurora-A, aurora-A ligands and their use. WO2005040368, May 06, 2005.

[51] Janeček, M.; Rossmann, M.; Sharma, P.; Emery, A.; Huggins, D.J.; Stockwell, S.R.; Stokes, J.E.; Tan, Y.S.; Almeida, E.G.; Hardwick, B.; Narvaez, A.J.; Hyvönen, M.; Spring, D.R.; McKenzie, G.J.; Venkitaraman, A.R. Allosteric modulation of AURKA kinase activity by a small-molecule inhibitor of its protein-protein interaction with TPX2. *Sci Rep*., **2016**, *6*, 29741.

[52] Huggins, D.J.; Venkitaraman, A.R.; Spring, D.R. Rational methods for the selection of diverse screening compounds. *ACS Chem.Biol.* **2011**, *6*(3), 208–217.

[53] Emanuel, S.; Rugg, C.A.; Gruninger, R.H.; Lin, R.; Fuentes-Pesquera, A.; Connolly, P.J.; Wetter, S.K.; Hollister, B.; Kruger, W.W.; Napier, C.; Jolliffe, L.; Middleton, S.A. The in vitro and in vivo effects of JNJ-7706621: a dual inhibitor of cyclin-dependent kinases and aurora kinases. *Cancer Res*., **2005**, *65*(19), 9038-9046.

[54] Hirota, T.; Kunitoku, N.; Sasayama, T.; Marumoto, T.; Zhang, D.; Nitta, M.; Hatakeyama, K.; Saya, H. Aurora-A and an interacting activator, the LIM protein Ajuba, are required for mitotic commitment in human cells. *Cell,* **2003**, *114*(5), 585–598.

[55] Hutterer, A.; Berdnik, D.; Wirtz-Peitz, F.; Zigman, M.; Schleiffer, A.; Knoblich, J.A. Mitotic activation of the kinase Aurora-A requires its binding partner Bora. *Dev. Cell,* **2006**, *11*(2), 147–157.

[56] Kishore, A.H.; Vedamurthy, B.M.; Mantelingu, K.; Agrawal, S.; Reddy, B.A.; Roy, S.; Rangappa, K.S.; Kundu, T.K. Specific small-molecule activator of Aurora kinase A induces autophosphorylation in a cell-free system. *J Med Chem*., **2008**, *51*(4), 792-797.

[57] Sullivan, J.T.; Richards, C.S.; Lloyd, H.A.; Krishna, G. Anacardic acid: molluscicide in cashew nut shell liquid. *Planta Med*., **1982**, *44*(3), 175-177.

[58] Karthigeyan, D.; Siddhanta, S.; Kishore, A. H.; Perumal, S.S.R.R.; Agren, H.; Sudevan, S.; Bhat, A.V.; Balasubramanyam, K.; Subbegowda, R.K.; Kundu, T.K.; Narayana, C. SERS and MD simulation studies of a kinase inhibitor demonstrate the emergence of a potential drug discovery tool. *Proc. Nat. Acad. Sci. USA*, **2014**, *111*(29), 10416-10412.

[59] Kumari, G.; Kandula, J.; Narayana, C. How far can we probe by SERS? *J. Phys Chem. C*, **2015**, *119*(23), 20057-20064.

[60] Pérez-Pineiro, R.; Correa-Durate, M.A.; Salgueirino, V.; Alvaraz-Pueblam R.A. SERS assisted ultra-fast peptidic screening: a new tool for drug discovery. *Nanoscale*, **2012**, *4*(1), 113-116.

[61] Costas, C.; López-Puente, V.; Bodelón, G.; González-Bello, C.; Pérez-Juste, J.; Pastoriza-Santos, I.; Liz-Marzán, L.M. Using surface enhanced Raman scattering to analyze the interactions of protein receptors with bacterial quorum sensing modulators. *ACS Nano*, **2015**, *9*(5), 5567-5576.

[62] Siddhanta, S.; Wróbel, M.S.; Barman, I. Integration of protein tethering in a rapid and label-free SERS screening platform for drugs of abuse. *Chem. Commun*., **2016**, *52*(58), 9016-9019.