IN THE SEARCH OF AURORA-A KINASE INHIBITORS AS ANTITUMOR AGENTS

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Abstract. The use of cheminformatics in the process of choosing the appropriate leads is becoming essential in the academic environment. The role of Aurora-A kinase as a major regulator of the cellular processes and the potential of its inhibitors in the treatment of cancer renders their research a top priority in the oncology studies. In this paper, we performed a structural profiling of the Aurora-A inhibitors in the search for new leading structures in the development of future Aurora-A kinase inhibitors. Based on the statistical analyses performed, the important descriptors for the Aurora-A affinity were identified and a set of rules of thumbs were elaborated in order to screen structural databases for new Aurora-A inhibitors. Thus, the hydrogen bonding capacity and the presence of nitrogen atoms in pyrazole, pyrimidine, piperazine or piperidine scaffold are prerequisite keys in order to increase the target affinity of Aurora-A inhibitors.

Key words: aurora kinase, Reaxys, database filtering, property distribution.

INTRODUCTION

Aurora kinases represents a family of serine/threonine kinases that are close homologues of the prototypic yeast Ipll and *Drosophila* Aurora kinases. The three members of this kinase family have been identified so far in humans, referred to as Aurora-A, Aurora-B and Aurora-C kinases [6]. The three Aurora kinases have between 309 and 403 amino acids and share 67–76% amino acid sequence identity in their catalytic domains, but little similarity in their N-terminus chain [1]. The Aurora family are crucial regulators of essential cellular processes ranging from mitotic entry to cytokinesis. During the S-phase, Aurora-A starts to accumulate at the centrosomes, with expression peaking in late G2 [8, 10]. Aurora-A is chiefly associated with centrosome during interphase, and binds the poles and half-spindle during mitosis [18]. Aurora-B functions include regulation of chromosome interactions with microtubules, chromatid cohesion, spindle stability and cytokinesis [16]. Aurora-C is located to centrosomes only in telophase cells [7].

Overexpression of Aurora kinases induces aneuploidy and genomic instability, which plays a leading role in the pathogenesis of malignancy for many

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types of tumor. The overexpression of Aurora-A has been found to occur in breast, bladder, colon, ovarian, pancreatic and stomach cancers, while the Aurora-B overexpression occurs mainly in colon cancer [20]. For this reason, Aurora kinases are considered a high-value target for development of cancer therapeutics, with multiple agents currently in early-phase clinical trials [12].

ZM447439 was reported as the first Aurora kinase inhibitor in 2003 and inhibits both Aurora-A and Aurora-B [2]. Tozasertib (VX-680) is an ATP-binding pan-Aurora inhibitor, but it exerts the strongest inhibition on Aurora-A. The clinical trials for leukemia were discontinued due to the prolongation of the QT interval [19]. Danusertib (PHA-739358) is also a pan-Aurora kinase inhibitor, as well an inhibitor of ABL and Ret tyrosine kinases and it has shown efficacy in clinical trials in progressive solid cancers and BCR/ABL-positive leukemia [3]. Barasertib is a highly selective Aurora B inhibitor 3700 fold more selective for Aurora-B over Aurora-A, and demonstrated preliminary anti-acute myeloid leukemia activity in the clinical setting [5]. Alisertib is a selective Aurora-A inhibitor with over 200-fold higher selectivity for Aurora-A than Aurora-B and it undergoes a series of phase I, II and III clinical trials as antitumor therapy [4].

The structures of some important Aurora kinases inhibitors are presented in Figure 1.

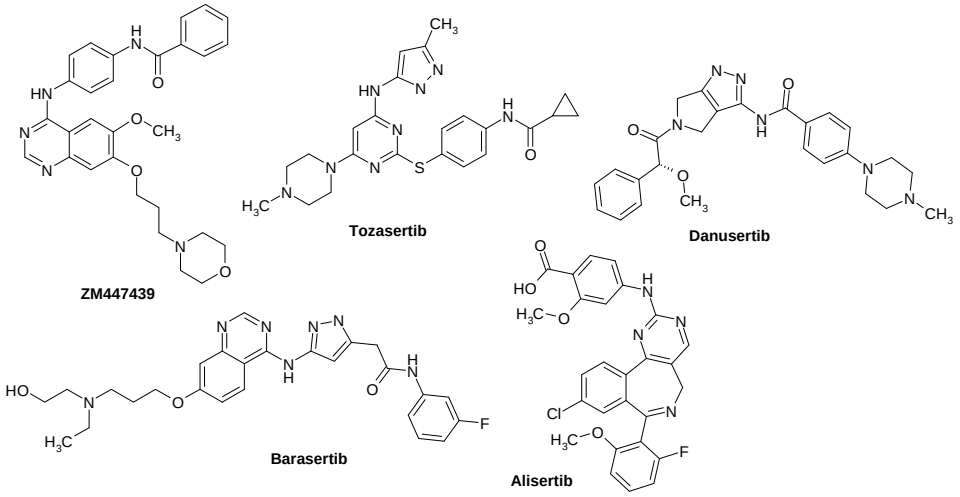

Fig. 1. Chemical structures of representative Aurora kinases inhibitors.

The potential of Aurora-A inhibitors in the treatment of cancer was demonstrated by several clinical trials, but no compound was yet approved as anticancer tumor.

Based on our previous research [13, 14], in the field of protein kinase inhibitors, this study was focused on the structural analysis of the Aurora-A inhibitors in the view of revealing the best ways to search for new leading structures in the development of future potent inhibitors.

MATERIALS AND METHODS

MATERIALS

The selection of compounds with certain target affinity and characteristic substructures from large chemical databases is an important step in determining structure-activity relationships [21]. A large number of virtual screening and data mining studies were performed using a variety of chemical databases [9, 11]. The main resource for obtaining freely-available bioassay data is the PubChem repository provided by the National Center for Biotechnology Information, but the data is not curated and is potentially erroneous [17].

Reaxys is a web-based chemistry database and its Medicinal Chemistry section contains over 5,400,000 substances and more than 26,000,000 bioactivity data points compiled from 320,000 medicinal chemistry publications and patents, fully indexed and normalized [22]. Reaxys database was used to link the screening results to chemical structures in order to identify structure-bioactivity relationships, to study their polypharmacology properties and derive the chemical profile of the Aurora inhibitors. The database was screened from 10 to 13 November 2014. The access to Reaxys was granted by the UMF Carol Davila's Library.

METHOD

In the all targets field the keywords used were "aurora-a" or "aura" for Aurora-A kinase, "aurora-b" or "aurb" for Aurora-B kinase and "aurora-c" or "aurc" for Aurora-C kinase target. The pX querylet was used to filter a desirable range of affinity between the compounds and the targets. The pX value represents the logarithmic inverse value of any affinity measure, like inhibitory concentration 50% (*IC*50), efficacy concentration 50% (*EC*50), lethal dose 50% (*LD*50) or inhibition constant (K_i) or dissociation constant (K_d). The Analysis View tool gives an overview of the results by grouping them into histograms based on a certain parameter and it was used to filter the compounds accordingly.

TARGET SELECTIVITY ANALYSIS

It is essential to evaluate the selectivity and promiscuity of a drug candidate in order to fully exploit their therapeutic potential and to minimize the toxic effects. To evaluate these properties, a straightforward approach is to investigate the bioactivity profile by screening each compound across a broad panel of similar targets, a process which could be very expensive when dealing with protein kinases [15]. The use of Reaxys allows the investigation of selectivity towards Aurora kinases and to derive target profiling information.

The query was performed for each Aurora target and the substances report was analyzed using the targets histograms. The percentage of selectivity was calculated as the number of compounds that interact with both two targets without any consideration of the affinity for the targets.

The queries on each type of Aurora kinase were merged in one single data set and the results were filtered to keep one isoform and exclude the others in order to estimate the homology of the targets.

PROPERTY DISTRIBUTION

The Reaxys Medicinal Chemistry database was filtered to find all the substances reported active on Aurora-A and the resulting structural set was analyzed in respect to the molecular descriptors distribution. In this study we used the molecular weight (*MV*), the calculated logarithm of the octanol/water partition coefficient (ClogP), the number of hydrogen bond donors (*HBD*), the number of hydrogen bond acceptors (*HBA*), and the number of rotatable bonds (*RTB*).

Using the substructure search feature we analyzed the frequency of various scaffolds in the structure of the Aurora-A kinase inhibitors. The scaffolds used were: benzene, pyridine, piperidine, pyrazine, piperazine, pyrimidine, pyrrole and pyrazole. The number of oxygen atoms (NO), the number of nitrogen atoms (*NN*), the number of halogens (NX) and their type were also computed.

In order to understand how the distribution of the descriptors is influenced by the affinity towards the Aurora-A target, the compounds were divided into the following subsets: pX > 1, pX > 3, pX > 6 and pX > 9. Each set contains only compounds with a pX value higher than the one mentioned in its title.

STRONG INHIBITORS *VERSUS* WEAK INHIBITORS

The set of Aurora-A inhibitors was divided into 2 subsets based on their pX values. The first set contains weak Aurora-A inhibitors with pX values in the range of 1 to 4, and the second consists of strong inhibitors with pX values in the range of 8 to 12. The two sets were compared based on their molecular descriptors distribution in order to understand the most important structural feature for a good Aurora-A kinase affinity.

RESULTS AND DISCUSSION

The search of compounds reported active on Aurora-A target returned 43099 substances, most of them interacting with a wide range of other targets. The query on Aurora-B target returned 23510 compounds and 3548 substances on Aurora-C. The histogram analysis of the targets spectrum is presented in Table 1.

The results show that most of Aurora kinases inhibitors are active on other types of protein kinases. For each set of Aurora kinases inhibitors the most 15 common targets belong to the protein kinases family. Approximately 45% of the set of Aurora-A inhibitors interact also with Aurora-B. In the cases of Aurora-B and Aurora-C inhibitors the cross affinity with Aurora-A is up to 83% and 85%, respectively.

Table 1

Target distribution for Aurora kinases inhibitors

Aurora-A inhibitors		Aurora-B inhibitors		Aurora-C inhibitors	
Target	%	Target	%	Target	%
aurb	45.3	aura	83.0	aura	85.1
cdk2	30.5	kdr	26.8	aurb	84.3
kdr	29.0	met	25.7	kdr	56.7
src	28.0	src	24.3	jak2	34.2
igf1r	25.8	flt3	23.2	egfr	34.2
flt3	23.8	cdk2	22.5	jak3	33.7
egfr	23.5	chk1	21.5	tie2	33.0
met	22.8	igf1r	21.0	flt3	31.3
gsk3beta	22.5	egfr	20.5	rock1	30.6
abl	19.2	abl	18.7	src	30.2
lck	18.7	gsk3beta	17.9	igf1r	30.2
pka	18.0	lck	17.8	lck	29.2
cdk5	17.2	jak3	16.5	chk1	28.9
kit	16.9	chk2	15.7	btk	28.6

The merged set of Aurora kinase inhibitors contains 47000 compounds. Using a Venn diagram the selectivity of a substances for a single or more types of Aurora kinases was measured and represented in Figure 2 as number of compounds. Close to 49.3% of all the compounds are active on Aurora-A, and not on Aurora-B or Aurora-C, whereas only 8.2% of the set are selective for Aurora-B and 0.87% for Aurora-C.

The homology between a pair of Aurora kinases was estimated calculating the ratio of the number of inhibitors in the intersection set *versus* the number of compounds in the union of the sets. The highest similarity was found between Aurora type A and B, with a percentage of 49.7 shared inhibitors, followed by types B and C with a percentage of 34.1. The similarity between types A and C was the smallest with 20.9% of shared compounds.

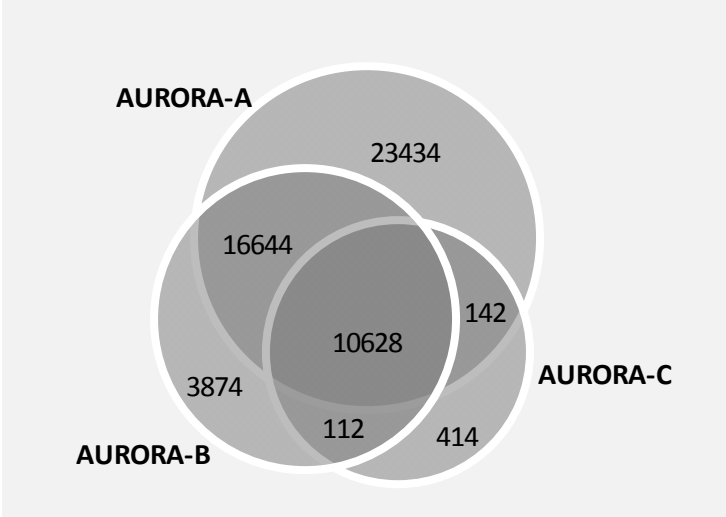

Fig. 2. Venn diagram of Aurora kinases inhibitors.

STRUCTURAL DESCRIPTORS DISTRIBUTION

The set of 43099 Aurora-A inhibitors present values of ClogP ranging from 5.73 up to 10.88 and with an average of 3.64. About 70 percent of the data values are within one standard deviation of the mean. The distribution of ClogP was analyzed for subsets formed based on pX values. In the subsets with a higher pX value, the ClogP has approximately the same average, but the ranging domain gets shorter and the standard deviation smaller. Figure 3 presents the distribution of ClogP in all the pX subsets.

The number of hydrogen bond donors ranges from 0 up to 32 and the number of hydrogen bond acceptors from 0 to 81. *HBA* has an average close to 7 and about 78 percent of the compounds are within one standard deviation of the mean, with values in the range of 5 to 9. In the subsets with higher pX values, the average value is closer to 8. *HBD* has an average close to 2.5 and about 70 percent of the compounds have 2 or 3 hydrogen bond donors. The compounds with a higher affinity for Aurora-A have in average 3 *HBD*. In Figure 4 the *HBA* and *HBD* histograms are plotted together.

The smallest *MV* value recorded is 118.1 g/mol, the highest is 3088.33 g/mol and the average value is 441 g/mol. About 70 percent of the compounds are within one standard deviation of the mean, with values in the range of 350 to 540. In Figure 5 is presented the histogram of the *MV* values distribution in the set of Aurora-A inhibitors.

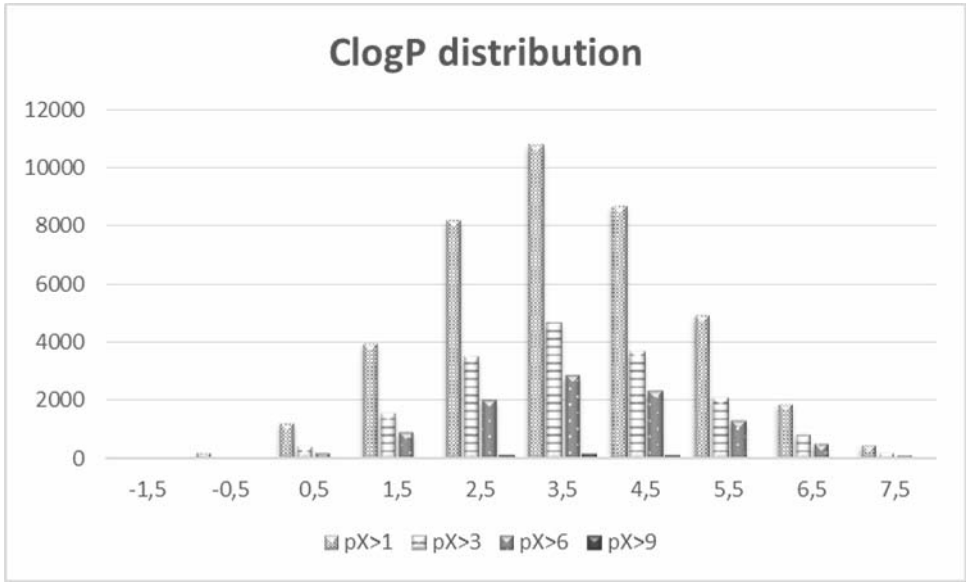

Fig. 3. Histograms of ClogP distribution in sets with various pX values.

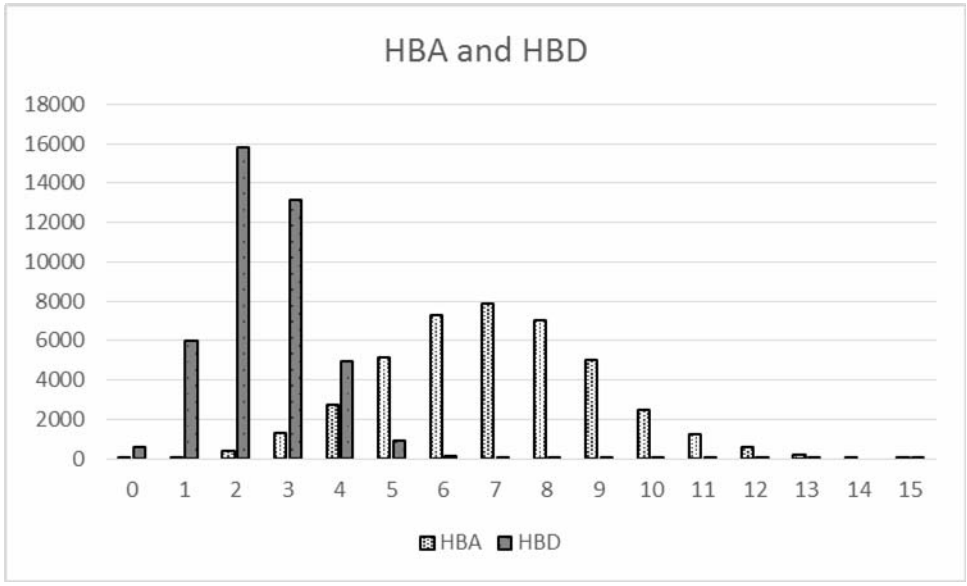

Fig. 4. Histograms of *HBA* and *HBD* distribution in Aurora-A inhibitors.

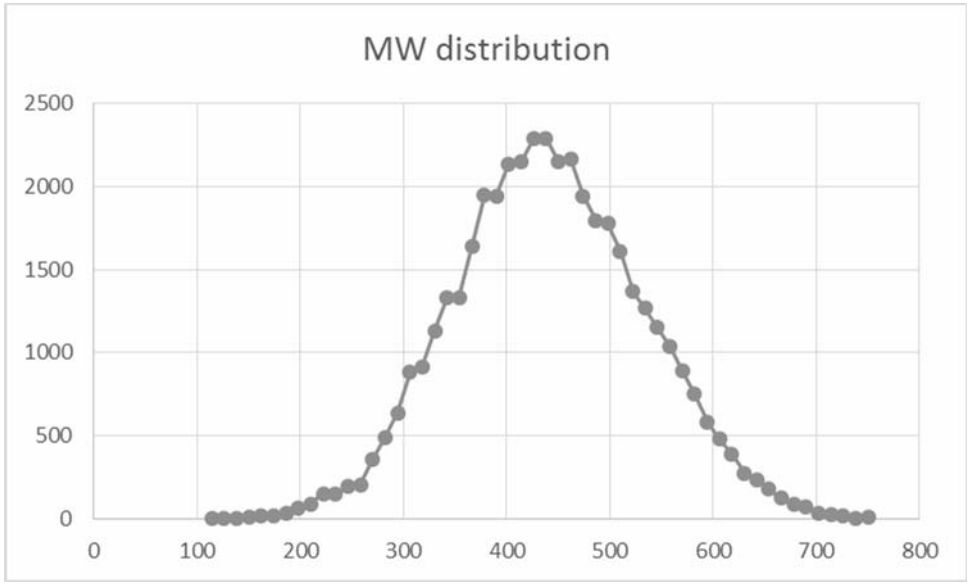

Fig. 5. Histograms of *MV* distribution in Aurora-A inhibitors.

STRONG INHIBITORS *VERSUS* WEAK INHIBITORS

The group of weak Aurora-A inhibitors (Gw) consists of 3067 compounds and the group of strong inhibitors (Gs) comprises 1893 compounds.

The statistical analysis of the 2 subsets ClogP values reveals a higher average value for the Gs set and a smaller standard deviation compared with the Gw set. The most important difference in the two sets is that all the strong inhibitors have a ClogP value over 0.

The *HBA* values are significantly higher in the Gs group, with an average close to 8, compared with the weak inhibitors group that have in average 6 *HBA*. The vast majority of the compounds from the Gs set has at least 4 *HBA* in their structure. In Figure 6 are presented the 2 histograms of the *HBA* values over the Gw and Gs sets.

In the Gs set the average *HBD* is close to 3, whereas in the Gw set the median value is approximately 2. Unlike the *HBA*, the *HBD* is not a very relevant descriptor in relation to Aurora-A affinity.

The analysis of the *MV* distribution in the two sets shows a higher average molecular weight for the strong inhibitors and a smaller standard deviation compared to the weak inhibitors. Only 10.2% of the compounds of the Gs set have a *MV* under 400 g/mol. The data indicates that *MW* > 400 could be used as a rule of thumb in the design of new potent Aurora-A kinase inhibitors.

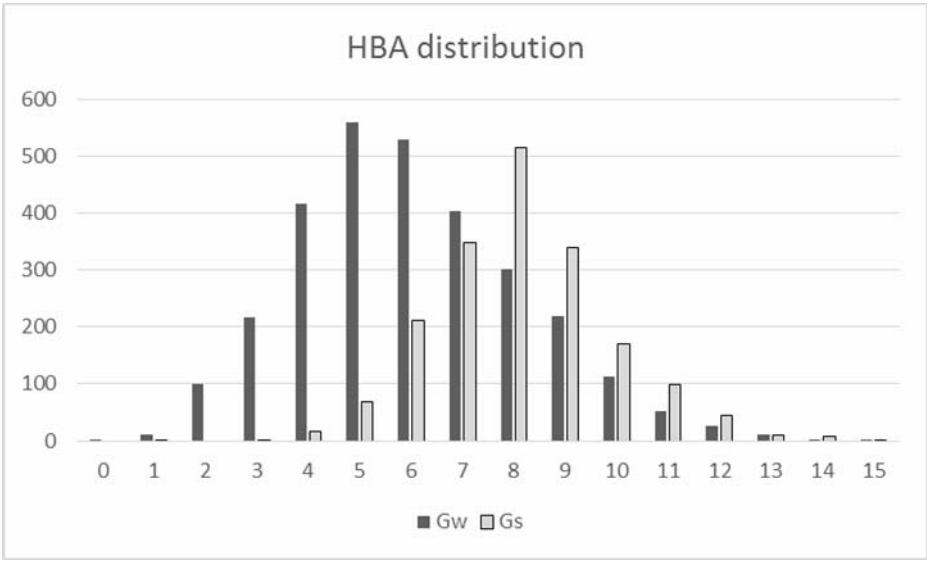

Fig. 6. Histograms of *HBA* distribution in the weak (Gw) and strong (Gs) groups of Aurora-A inhibitors.

In Figure 7 are presented the *MV* distributions for the weak (Gw) and strong (Gs) groups of Aurora-A inhibitors.

The substances with a strong affinity for the Aurora-A kinase have in average 7.5 *RTB* in their structure, whereas for the weak inhibitors the average is close to 5.5. Almost 60% of the Gs group have between 6 and 9 *RTB*, while 60% of the compounds of the Gw set have in their structure a number of 4 to 9 *RTB* .

The number of nitrogen atoms is significantly higher in the structures of the Gs set, with an average close to 6, compared with the Gw set where the compounds have an average 4.5 *NN*. Only 12.6% of the Gs compounds have the NA under 5. As a rule of thumb, a *NN* value of 5 and higher increases the chances of finding a potent Aurora-A inhibitor. The number of oxygen atoms is not very important for the affinity, but it can be used as a filter. Almost 91% of the strong inhibitors have in their structure a NO between 1 and 4. A compound with 5 or more oxygen atoms in its molecule is highly likely to have a weak affinity on Aurora-A kinase.

In the set of strong Aurora-A inhibitors 54.8% contain in their structure at least one halogen, compared with the Gw set where a halogen is present in only 32% of the compounds. The presence of at least one fluorine atom in the structure of a compound seems to improve their affinity towards the Aurora-A target.

The analysis of the heterocyclic moieties reveals the pyrazole ring as the best choice to increase the target affinity. Approximately 46% of the Gs compound have in their structure a pyrazole ring, whereas 18% of the Gw set. Pyrimidine, piperazine and piperidine are also good choices, whereas the pyridine ring is more common in the structures of the weak Aurora-A inhibitors.

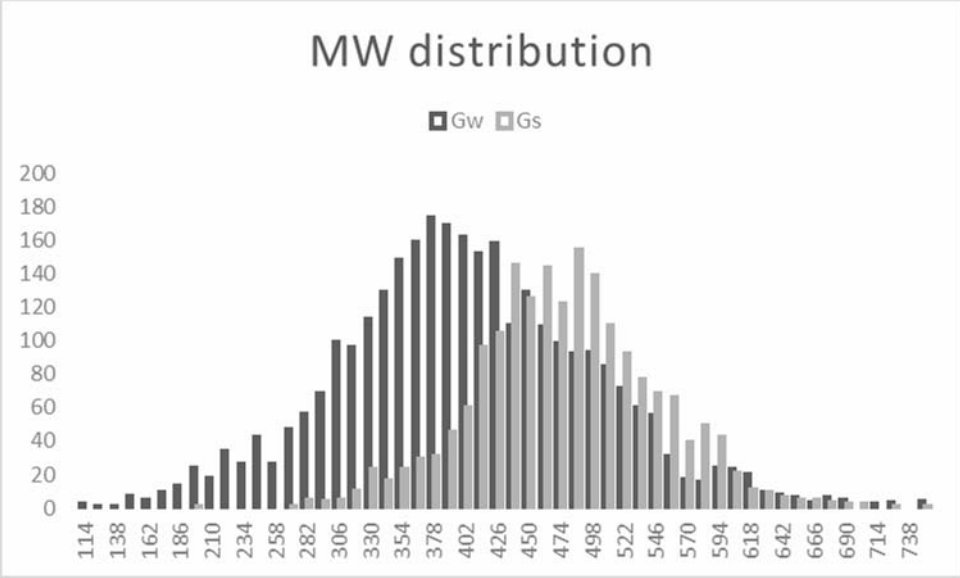

Fig. 7. Histograms of *MV* distribution in the weak (Gw) and strong (Gs) groups of Aurora-A inhibitors.

In order to find the most important structural patterns in a good Aurora-A kinase inhibitors we selected the compound with a pX value higher than 6. This set was analyzed based on each descriptor distribution and the following rules were used to count the number of compounds in each subset:

1) ClogP between 2 and 5.
2) *HBA* between 7 and 9.
3) *HBD* between 2 and 4.
4) *MV* between 396 and 516.
5) *RTB* between 2 and 5.

The rules were applied in pair conjunction in order to filter the database and to weigh their relevance in the discrimination of potent Aurora-A kinase inhibitors. The *RTB* values proved to be a poor descriptor. The best results were obtained when using ClogP and *HBD*.

Based on the structural analyses we consider that the best chances to find a potent Aurora-A kinase inhibitor is by using the following rules of thumb:

1) ClogP between 2 and 5.
2) *HBD* between 2 and 4.
3) *HBA* between 5 and 10.
4) *NN* between 5 and 10.

The Reaxys database was filtered based on these rules and returned 425933 results. Of these results, 15277 were recorded as Aurora-A kinase inhibitors, representing close to 3.6% of the filters results. This percent is highly increased if

the compounds contain in their structure at least one pyrazole, pyrimidine, piperazine or piperidine scaffold.

CONCLUSIONS

This research analyzed the structural patterns of the Aurora-A inhibitors in the view of finding leading structures for the development of future Aurora-A kinase inhibitors as potent anticancer therapies.

Using the Reaxys Medicinal Chemistry database the substances recorded to act on Aurora-A kinases were analyzed in respect to their selectivity towards the related Aurora-B and Aurora-C kinase, as well on other protein kinases.

The structural patterns of the Aurora-A inhibitors were analyzed, demonstrating the high importance of the hydrogen bonding capacity and of the presence of a minimum 5 nitrogen atoms. The integration of the nitrogen atoms in a pyrazole, pyrimidine, piperazine or piperidine scaffold was demonstrated to increase the target affinity.

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REFERENCES

1. BISCHOFF, J.R., G.D. PLOWMAN, The Aurora/Ipl1p kinase family: regulators of chromosome segregation and cytokinesis, *Trends Cell Biol.* 1999, **9**(11), 454–459.
2. GADEA, B.B., J.V. RUDERMAN, Aurora kinase inhibitor ZM447439 blocks chromosome-induced spindle assembly, the completion of chromosome condensation, and the establishment of the spindle integrity checkpoint in Xenopus egg extracts, *Mol. Biol. Cell*, 2005, **16**(3), 1305–1318.
3. GONTAREWICZ, A, T.H. BRÜMMENDORF, Danusertib (formerly PHA-739358) – a novel combined pan-Aurora kinase and third generation Bcr-Abl tyrosine kinase inhibitor, *Recent Results Cancer Res*., 2010, **184**, 199–214.
4. HILTON, J.F., G.I. SHAPIRO, Aurora kinase inhibition as an anticancer strategy, *J. Clin. Oncol*., 2014, **32**(1), 57–59.
5. KANTARJIAN, H.M., M.A. SEKERES, V. RIBRAG, P. ROUSSELOT, G. GARCIA-MANERO, E.J. JABBOUR, KATE OWEN, P.K. STOCKMAN, S.D. OLIVER, Phase I study assessing the safety and tolerability of barasertib (AZD1152) with low-dose cytosine arabinoside in elderly patients with AML, *Clin. Lymphoma Myeloma Leuk.*, 2013, **13**(5), 559–567.
6. KATAYAMA, H., W.R. BRINKLEY, S. SEN, The Aurora kinases: role in cell transformation and tumorigenesis, *Cancer Metast. Rev*., 2003, **22**, 451–464.
7. KE, Y.W., Z. DOU, J. ZHANG, X.B. YAO, Function and regulation of Aurora/Ipl1p kinase family in cell division, *Cell Res.*, 2003, **13**, 69–81.
8. KIM, J.-T., S.H. JUNG, S.Y. KANG, C.-K. RYU, N.S. KANG, The discovery of Aurora kinase inhibitor by multi-docking-based virtual screening, *Int. J. Mol. Sci.*, 2014, **15**, 20403–20412.
9. LI, Q., T. CHENG, Y. WANG, S.H. BRYANT, PubChem as a public resource for drug discovery, *Drug Discov. Today.*, **15**(23–24), 1052–1057.

10. MAHEN, R., A.R. VENKITARAMAN, Pattern formation in centrosome assembly, *Curr. Opin. Cell Biol.*, 2012, **24**(1), 14–23.
11. MCCARTHY, ALICE, The NIH Molecular Libraries Program: identifying chemical probes for new medicines, *Chem. Biol.*, 2010, **17**(6), 549–550.
12. NIKONOVA, A.S., I. ASTSATUROV, I.G. SEREBRIISKII, R.L. DUNBRACK JR, E.A. GOLEMIS, Aurora A kinase (AURKA) in normal and pathological cell division, *Cell Mol. Life Sci.*, 2013, **70**(4), 661–687.
13. NITULESCU, G.M., C. DRAGHICI, O.T. OLARU, New potential antitumor pyrazole derivatives: synthesis and cytotoxic evaluation, *Int. J. Mol. Sci.*, 2013, **14**(11), 21805–21818.
14. NITULESCU, G.M., C. DRAGHICI, A.V. MISSIR, Synthesis of new pyrazole derivatives and their anticancer evaluation, *Eur. J. Med. Chem.*, 2010, **45**(11), 4914–4919.
15. RIX, U., G. SUPERTI-FURGA, Target profiling of small molecules by chemical proteomics, *Nat. Chem. Biol.*, 2009, **5**(9), 616–624.
16. RUCHAUD, S., M. CARMENA, W.C. EARNSHAW, Chromosomal passengers: conducting cell division. *Nat. Rev. Mol. Cell Biol.*, 2007, **8**(10), 798–812.
17. SCHIERZ, AMANDA, Virtual screening of bioassay data, *J. Cheminform*., 2009, **21**(1), 1-12.
18. STENOIEN, D.L., S. SEN, M.A. MANCINI, B.R. BRINKLEY, Dynamic association of a tumor amplified kinase, Aurora-A, with the centrosome and mitotic spindle, *Cell Motil. Cytoskeleton*, 2003, **55**(2), 134–146.
19. TYLER, REBECCA K., NATALIA SHPIRO, R. MARQUEZ, P.A. EYERS, VX-680 inhibits Aurora A and Aurora B kinase activity in human cells, *Cell. Cycle*, 2007, **6**(22), 2846–2854.
20. UMENE, K., K. BANNO, I. KISU, M. YANOKURA, Y. NOGAMI, K. TSUJI, K. MASUDA, A. UEKI, Y. KOBAYASHI, W. YAMAGAMI, H. NOMURA, E. TOMINAGA, N. SUSUMU, D. AOKI, Aurora kinase inhibitors: Potential molecular-targeted drugs for gynecologic malignant tumors, *Biomed. Rep.*, 2013, **1**(3), 335–340.
21. WANG, Y., XIAO J., T.O. SUZEK, ZHANG J., WANG J., S.H. BRYANT, PubChem: a public information system for analyzing bioactivities of small molecules, *Nucleic Acids Res.*, 2009, **37**(suppl 2), W623–W633.
22. ***Reaxys, version 2.18608; Elsevier; 2014; RRN 969209 (accessed, 2014).