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REVIEW ARTICLE

TYROSINE KINASE, AURORA KINASE AND LEUCINE AMINOPEPTIDASE AS ATTRACTIVE DRUG TARGETS IN ANTICANCER THERAPY - CHARACTERISATION OF THEIR INHIBITORS

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ABSTRACT

Cancers are the leading cause of deaths all over the world. Available anticancer agents used in clinics exhibit low therapeutic index and usually high toxicity. Wide spreading drug resistance of cancer cells induce a demanding need to search for new drug targets. Currently, many on-going studies on novel compounds with potent anticancer activity, high selectivity as well as new modes of action are conducted. In this work, we describe in details three enzyme groups, which are at present of extensive interest to medical researchers and pharmaceutical companies. These include receptor tyrosine kinases (e.g. EGFR enzymes) and non-receptor tyrosine kinases (Src enzymes), type A, B and C Aurora kinases and aminopeptidases, especially leucine aminopeptidase. We discuss classification of these enzymes, biochemistry as well as their role in the cell cycle under normal conditions and during cancerogenesis. Further on, the work describes enzyme inhibitors that are under *in vitro*, preclinical, clinical studies as well as drugs available on the market. Both, chemical structures of discovered inhibitors and the role of chemical moieties in novel drug design are discussed. Described enzymes play essential role in cell cycle, especially in mitosis (Aurora kinases), cell differentiation, growth and apoptosis (tyrosine kinases) as well as G_1/S transition (leucine aminopeptidase). In cancer cells, they are overexpressed and only their inhibition may stop tumor progression. This review presents the clinical outcomes of selected inhibitors and argues the safety of drug usage in human volunteers. Clinical studies of EGFR and Src kinase inhibitors in different tumors clearly show the need for molecular selection of patients (to those with mutations in genes coding EGFR and Src) to achieve positive clinical response. Current data indicates the great necessity for new anticancer treatment and actions to limit off-target activity.

Key words: *tyrosine kinase, Aurora kinase, leucine aminopeptidase, enzyme inhibitors*

STRESZCZENIE

Nowotwory stanowią jedną z głównych przyczyn zgonów na świecie. Dostępne w lecznictwie substancje przeciwnowotworowe charakteryzują się niskim indeksem terapeutycznym jak i wysoką toksycznością. Rozwijająca się oporność komórek nowotworowych na dostępne w terapii leki przyczynia się do konieczności poszukiwania nowych punktów uchwytu/miejsc docelowych (z ang. targets) dla potencjalnych substancji przeciwnowotworowych. Obecnie prowadzonych jest również wiele prac nad nowymi związkami przeciwnowotworowymi o wysokim potencjale terapeutycznym, nowym mechanizmie działania i/bądź wyższym indeksie selektywności. W pracy, autorzy skupili uwagę na trzech grupach enzymów, będących obecnie w obszarze zainteresowań współczesnej medycyny. Omówione zostały kinazy tyrozynowe na przykładzie enzymów EGFR i Src, kinazy Aurora typu A, B i C, a także aminopeptydazy na przykładzie aminopeptydazy leucynowej. Scharakteryzowano klasyfikację enzymów, ich rolę w cyklu komórkowym w warunkach fizjologicznych i procesie nowotworowym. Opisano również inhibitory enzymów, substancje będące w trakcie badań *in vitro*, przedklinicznych i klinicznych jak i leki wprowadzone na rynek farmaceutyczny. Zwrócono uwagę na budowę chemiczną inhibitorów enzymów i tym samym na kierunek poszukiwań nowych leków przeciwnowotworowych. Omówione enzymy w warunkach fizjologicznych odgrywają ważną rolę w cyklu komórkowym, zwłaszcza na etapie podziału mitotycznego. Jednakże w procesie nowotworowym dochodzi do ich nadekspresji. Zjawisko to można zahamować poprzez inhibicję aktywności enzymu. Autorzy omówili wpływ inhibitorów kinaz tyrozynowych, kinaz Aurora czy aminopeptydaz leucynowych na cykl komórkowy i bezpieczeństwo stosowania tych potencjalnych leków u ludzi. Dotychczasowe badania przedkliniczne i kliniczne inhibitorów kinazy tyrozynowej typu EGFR czy Src potwierdziły konieczność selekcji pacjentów, na tych z mutacją w genie kodującym dany enzym. Badania prowadzone na wybranej grupie chorych przynosiły oczekiwany pozytywny wynik. Wiele aspektów dotyczących nowych punktów uchwytu w terapii przeciwnowotworowej wciąż wymaga dalszych prac, aczkolwiek daje również nadzieję na odkrycie skutecznych i selektywnych leków.

Słowa kluczowe: *kinaza tyrozynowa, kinaza Aurora, aminopeptydaza leucynowa, inhibitory enzymów*

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INTRODUCTION

Cancer is a leading worldwide cause of deaths in humans. According to the data of the World Health Organization, 8.2 mln people die from cancer each year, which corresponds to 13% of the total number of deaths noted worldwide [3, 6]. Over the next two decades, a 70% increase of new cancer cases is expected. More than 100 cancer types have been determined to exist, each requiring unique diagnosis and treatment [3, 6]. There is a demanding need for new drugs with novel modes of action and fewer side-effects. Drugs currently used in cancer chemotherapy usually show high toxicity toward normal cells. Another undesired consequence is drug resistance which persists during cancer chemotherapy. Alternative therapeutic options are also still limited. Therefore, the growing trend toward targeted cancer therapy [22, 45], which is based on delivering drugs to particular enzymes, genes, proteins or peptides essential in carcinogenesis or which promote cancer growth. The effectiveness of such therapy lies in minimizing side effects in normal tissues [45].

There is great effort to identify crucial molecular targets of anticancer drug discovery. In general, the approach is based on sequencing various cancer genomes, which is often a complex and heterogeneous activity. Among known key enzyme targets for anticancer drugs are tyrosine kinases, Aurora kinases and leucine aminopeptidases. There are on-going intensive studies to discover the most potent and selective inhibitors against these enzymes. In this review, we present enzymes classification, overall structure, mode of action as well as chemical structures of inhibitors that are currently studied or have just been discovereware involved in the cell cycle, particularly in mitosis. They are involved in checkpoints regulation and their abnormal expression may disturb checkpoints functions [35]. Also, leucine aminopeptidase (LAP) plays a significant role in the cell cycle by promoting G_1 checkpoint progression. Aberrant LAP expression causes proliferation of cancer cells and metastasis [65]. Based on the data above, the current findings demonstrate that these enzymes represent potential "druggable targets" in cancer, controlling key oncogenic pathways.

TYROSINE KINASES

Tyrosine kinases (TKs) are a family of enzymes that transfer the phosphate group from adenosine triphosphate (ATP) to a tyrosine amino acid residue in a protein, subsequently triggering downstream molecular signaling [22, 47]. They are important mediators in signal transduction, leading to cell proliferation, differentiation, migration, metabolism and programmed cell death [47, 49]. TKs can be classified as receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs). The function of both is based on regulating other enzymes by phosphorylation. However, while RTKs (e.g. EGFR, PDGFR, FGFR) exist on the surface of the cell as part of cell membrane, NRTKs (e.g. SRC, ABL, FAK or Janus kinase) are located in the cytoplasm [47, 49].

RECEPTOR TYROSINE KINASES (RTKs)

RTKs constitute a protein superfamily that plays an important role in the control of cellular processes, including cell cycle, cell migration, survival, cell proliferation and differentiation [57]. The domain structure of RTKs consists of an extracellular hydrophylic ligand binding domain, which recognizes the ligand, a hydrophobic transmembrane domain and an intracellular domain essential in signal transduction processes [58]. The intracellular domain contains a conserved protein tyrosine kinase core and other regulatory sequences that are subjected to autophosphorylation or phosphorylation by heterologous protein kinases [57]. RTKs are activated by the ligand which binds to their extracellular domain. Ligands are extracellular signal molecules that induce receptor dimerization [39, 47]. There are several strategies in which ligands can achieve stable dimeric conformation – one ligand may bind to two receptors and form a dimer complex or two ligands can bind to two receptors. Then, when the dimer is phosphorylated, it is fully active and various proteins can attach to phosphorylated RTKs. This causes a series of signal transduction event. There are nearly 60 RTKs, divided into 20 subfamilies. Among them, EGFR/ErbB (class I), the receptor for insulin (class II), for PDGF (class III), for FGF (class IV), for VEGF (class V) and HGF (MET, class VI) are strongly associated with carcinogenesis [49, 58]. Many cancers are caused by mutated RTKs which are active without a signal molecule (ligand). In malignant cells, there is excessive production of tyrosine kinase receptors. High levels of receptor expression lead to formation of increased number of binding sites that are available to the ligand. This in effect causes a cascade of excessive signals.

The epidermal growth factor receptor (EGFR) family, called "prototypical" RTKs [40], is regulated in humans by at least seven different activating ligands, including the epidermal growth factor (EGF), transforming growth factor-α (TGF-α), betacellulin (BTC) and others. The EGFR family is comprised of four structurally related receptors, EGFR (ErbB1/HER1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4) [40, 72]. EGFR (ErbB1/HER1) is a multiple domain glycoprotein (170 kDa) that consists of a typical for RTKs extracellular ligand-binding

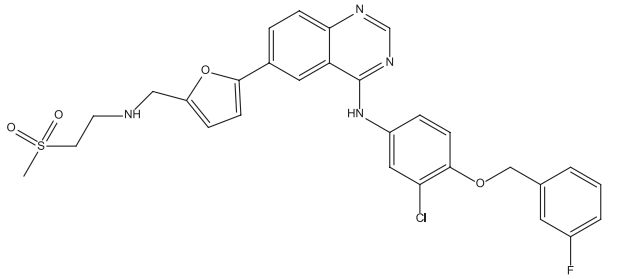
domain and intracellular tyrosine kinase domain separated by a transmembrane region [28, 51]. Endogenous ligands, such as EGF, can bind to EGFR and cause receptor homo- or heterodimerization. The receptor dimerization leads to autophosphorylation of the intracytoplasmic EGFR tyrosine kinase domain. Then, phosphorylated tyrosine kinase stimulates an intracellular signal transduction cascade [51].

Tyrosine kinase inhibitors (TKIs) that target the intracellular tyrosine kinase region include those, which mode of action is to interfere with ATP binding to the receptor, and other compounds which act at the substrate binding region [50].

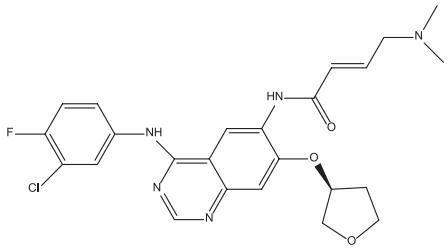
Based on the chemical structure, RTKIs can be divided as those possessing the quinazoline core (e.g. lapatinib, afatinib, gefitinib, erlotinib, etc.) and their derivatives (such as inhibitors with oxazolo [4,5-*g*] quinazolin-2(1*H*)-one moiety).

EGFR and HER2 are frequently overexpressed in breast, ovarian, prostate and colon cancers. It was also observed that dual EGFR/HER2 inhibitors can cause more potent inhibition than solely EGFR and HER2 inhibitors. There are some dual EGFR/HER2 inhibitors available on the market, such as lapatinib (*N*-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(5-(((2-(methylsulfonyl)ethyl)amino)methyl)furan-2-yl) quinazolin-4-amine, Figure 1) and afatinib (*S*,*E*)-*N*-(4-((3-chloro-4-fluorophenyl)amino)-7-(tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-4-(dimethylamino)but-2-enamide) (Figure 1) [72]. The quinoline moiety plays a significant role in EGFR/HER2 inhibitory activity. However, due to the toxicity of marketed drugs, further studies are carried out to discover new, more optimal drugs. In the work of *Yin* et al., authors found a series of novel dual EGFR/HER2 inhibitors with oxazolo[4,5-*g*] quinazolin-2(1*H*)-one moiety (Figure 1). Several newly discovered molecules showed more potent activity against the mentioned enzymes than the reference drug, lapatinib, and exhibited impressive results on cancer cell lines (human lung adenocarcinoma A549 and breast cancer SK-Br3) as well as lower toxicity [72].

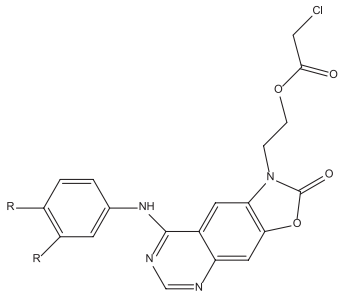
The introduction of EGFR inhibitors, such as gefitinib (*N*-(3-chloro-4-fluorophenyl)-7-methoxy-6-(3-morpholinopropoxy)quinazolin-4-amine, Figure 1), erlotinib (*N*-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine, Figure 1) and afatinib, to the market has changed the treatment of patients with advanced non-small-cell lung cancer (NSCLC) with mutations in the EGFR gene [53]. These drugs inhibit phosphorylation and tyrosine kinase activity of the intracellular ATP-binding domain of EGFR through competitive binding to this site. The



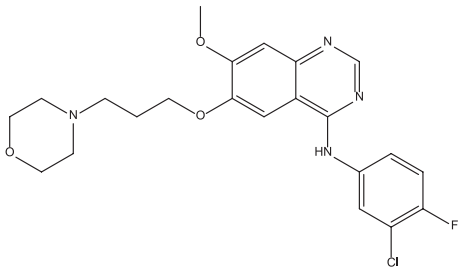
lapatinib



afatinib



oxazolo[4,5-*g*]quinazolin-2(1*H*)-one scaffold



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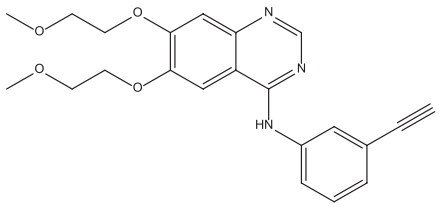


erlotinib

Figure 1. Chemical structures of RTKIs

response to EGFR-TKIs is associated with the presence of activating EGFR mutations in NSCLC. Different studies showed that EGFR-TKIs have high anti-tumor activity in EGFR mutation-positive patients [53].

In the study of *Rossi* et al., efficacy and safety of gefitinib and afatinib were compared in patients of different age. The trial was conducted on a group of NSCLC patients with mutation in exon 19 or 21. This group was divided into patients aged <70 and ≥70 years [54]. The overall results showed that elderly patients gave better response in comparison to the group aged <70 years. However, majority of patients experienced disease progression after first-line TKI treatment and none of them achieved a complete response [54].

At present, there are plenty of TKIs on the market that are widely used in clinical treatment of solid tumors and other types of cancers. In colon cancer, regorafenib is one of the current therapeutic solutions. Imatinib, sunitinib and regorafenib are used in therapy of gastrointestinal stromal tumors and chronic myeloid leukemia (imatinib). Sorafenib shows a positive response in treatment of hepatocellular carcinoma. Melanoma treatment is based on vemurafenib and dabrafenib [66]. As mentioned previously, in non-small cell lung cancer therapy, gefitinib, erlotinib, afatinib, ceritinib and crizotinib are among the therapeutic TKI representatives [51]. The therapeutic potential of pazotinib is described in ovarian cancer.

TKIs, as other medicines, induce side effects, especially gastrointestinal disorders, such as diarrhea, nausea and emesis. Moreover, these drugs tend to develop hypertension and renal disorders. They are metabolized in the liver and usually give interactions through CYP3A4 induction [66].

NON-RECEPTOR TYROSINE KINASES (NRTKs)

The family of NRTKs consists of nine main families, differing in the domain structure. The activation process of NRTKs is more complex in comparison to RTKs, requiring heterologous protein-protein interactions to enable transphosphorylation. The largest group of NRTKs is the Src family. It is divided into three main subfamilies: Lyn-related, Src-related and PTK6/Brk-related [24]. Members of the Src family display a conserved domain organization: a myristoylated N-terminal segment (S4 domain), SH3, SH2, linker, tyrosine kinase domains (SH1 domain containing Tyr416), a short C-terminal regulatory segment containing Tyr527 [20, 46, 52]. The C-terminal region bears an auto-inhibitory phosphorylation site [20]. The activity of Src enzymes is regulated by phosphorylation and intramolecular protein-protein interactions (in SH2 and SH3 regions) [20]. Both sites, Tyr416 and Tyr527, play major role in protein phosphorylation.

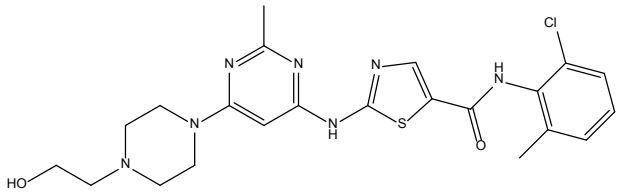
There are several ways in which Src kinases can be activated and many ways in which they can activate the process of carcinogenesis. Src kinases participate in a variety of signaling processes, including cell proliferation, T- and B-cells activation, cytoskeleton restructuring, cell movement and endocytosis [24, 52]. They are activated during the G_2/M cell cycle. Brain, osteoclasts and platelets express higher Src levels than other human tissues [52]. Elevated expression and activity of Src promote tumor growth and stimulate its migratory and invasive potential [20]. Src deregulation and overexpression has been linked to several human cancers, such as melanoma, breast, lung and colon cancer [20, 24]. There is clear evidence that Src elevation has an impact on progression of colon cancer; however, further studies should be conducted to determine this direct association. Similar data suggest elevated expression of Src in ovarian, esophageal, lung, head and neck as well as gastric cancers [20]. Imbalance between phosphorylation and dephosphorylation of the Src protein leads to drastic changes. Among processes that can contribute to carcinogenesis are: dephosphorylation of Tyr527, deletion or mutation of Tyr527, displacement of the SH3- and SH2-mediated intramolecular interactions or phosphorylation of Tyr416 [20].

Oncogenes, in other words, genes involved in biochemical points (called checkpoints) that control transitions in the cell cycle, can mutate or be overexpressed [35]. The v-Src oncogene was first isolated from the transforming virus, Rous Sarcoma Virus. It lacks the cellular protein region (c-Src) that contains Tyr527, what makes it continually active [4]. The v-Src has a growth-promoting effect in fibroblasts. In comparison to normal cells, v-Src cells suppress expression of the cyclin-dependent kinase (CDK) inhibitor p27, leading to more rapid transit of the G_1 phase of the cell cycle and a failure to enter the quiescent state when deprived of serum mitogenes [20].

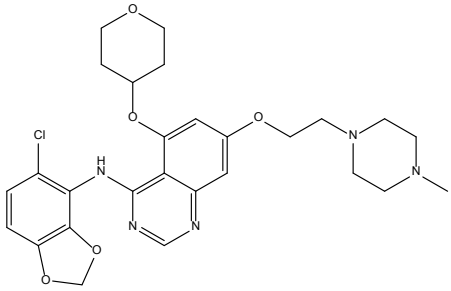
Until now, several Src inhibitors were tested in clinical trials, e.g. dasatinib, saracatinib and bosutinib [74]. Their chemical structures are more diverse than RTKIs and are based on 4-(piperazin-1-yl)pyrimidine (dasatinib), 7-(2-(piperazin-1-yl)ethoxy)quinazoline (saracatinib) and 7-(3-(piperazin-1-yl)propoxy)quinoline (bosutinib). Bosutinib, 4-((2,4-dichloro-5-methoxyphenyl)amino)-6-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy) quinolone -3-carbonitrile (Figure 2) was tested on breast cancer cells. Study of *Vultur* et al. showed that this compound causes decreased cell motility and invasion as well as increased cell-cell adhesion [67]. Phase I clinical trial of bosutinib on patients with breast cancer was completed in 2011. At present, there is an ongoing phase I trial for bosutinib in combination with another anticancer agent (inotuzumab ozogamicin) in the Philadelphia-chromosome (PC) positive acute

lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML) [2]. Besides evident activity in pre-clinical studies, candidates for Src inhibitors did not show potent efficacy when tested in monotherapy of various types of solid tumors in clinical trials [74]. The Src inhibitor, saracatinib (AZD-0530, (*N*-(5-chlorobenzo[*d*][1,3]dioxol-4-yl)-7-(2-(4-methylpiperazin-1-yl)ethoxy)-5-(tetrahydro-2*H*-pyran-4-yl)oxy)quinazolin-4-amine (Figure 2), showed promising activities when tested in pre-clinical models of biliary tract carcinomas (BTC) [11]. In the study of *Cavalloni* et al., this compound exhibited reduced cell migration and proliferation *in vitro* and delayed tumor growth in human BTC xenografts *in vivo* [11]. Saracatinib was also tested on patients with colorectal cancer. However, in this trial patients were not selected as those with tumors with activated Src signaling and those without activated Src. This accounts for the unsatisfactory results of the study. Also, in trials with pancreatic cancer patients, Src inhibitors did not show high activity. However, it has been hypothesized that activated Src signaling maybe a biomarker for successful targeting of Src and positive clinical outcomes [74]. The recent study of *Heusschen* et al. showed that Src inhibition by saracatinib limits the development of osteolytic bone disease in multiple myeloma [29]. The *in vitro* data revealed an inhibitory effect of the tested drug on osteoclast differentiation, polarization and resorptive function [29]. There is also an ongoing phase II clinical trial on saracatinib versus placebo in cancer-induced pain SarCaBon [2].

Another chemical moiety, 1*H*-pyrazolo[3,4-*d*]pyrimidine, is a core structure in Src inhibitors, such as PP2. PP2 (1-(tert-butyl)-3-(4-chlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine, Figure 2) was also investigated in *in vitro* and *in vivo* research regarding malignant glioma cells U251 [17]. The effect of chemoradiotherapy with PP2 and temolozomide on cancer cells was tested using clonogenic assays and *in vivo* brain tumor model. PP2 enhanced radioselectivity of malignant glioma cells and suppressed invasion and migration of U251 cells. However, the authors did not



dasatinib



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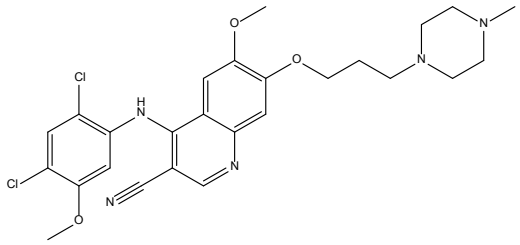
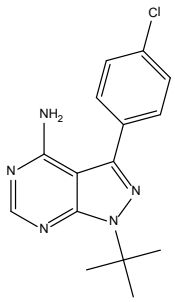


bosutinib



PP2

Figure 2. Chemical structures of NRTKIs

observe a significant decrease in tumor volume [17].

In another study performed by *Formisano* et al., Src inhibitors (saracatinib, dasatinib and bosutinib) were tested on NSCLC models [19]. Obtained results showed that all tested compounds directly inhibited EGFR, which is a well-characterized mutated oncogene in NSCLC. Among the three inhibitors tested in an *in vitro* kinase assay, saracatinib presented the most potent activity towards EGFR, whereas dasatinib (*N*-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-yl)amino)thiazole-5-carboxamide, Figure 2) was least effective. Saracatinib also showed impressive results on erlotinib-sensitive cells containing EGFR-activating mutants and *in vivo* HCC827 tumor xenografts (where it was more active than dasatinib). Saracatinib mode of action was based on Src inhibition and by EGFR activation reduction. Conducted study demonstrated that Src inhibitors may act through various mechanisms in NSCLC [19]. Taking together all of the above, it is clear that Src inhibitors present antiproliferative

activity in various cancers. Disappointing results from a series of clinical trials (resulting mainly from the lack of proper molecular patient selection) delayed the development of Src inhibitors. However, studies on patients with Src-activated signaling should be continued due to the great beneficial effect of these inhibitors.

AURORA KINASES

Aurora enzymes are type serine/threonine (Ser/Thr) kinases, which serve as important mitotic regulators with essential role in the regulation of cell division, from mitotic entry to cytokinesis [21, 33, 35]. In mammals, three enzyme types (A, B and C) are distinguished. They contain two domains: catalytic, at the COOH-terminus, and regulatory, at the NH_2-terminus. Kinases A, B and C have identical catalytic domains, but various regulatory domains. Moreover, each Aurora kinase differs in its subcellular location.

AURORA A KINASE

Aurora A is a 403-amino acid enzyme with a molecular weight of 46 kDa [5, 14], which demands phosphorylation for its activation [7]. The presence of phosphoric groups at residues Ser51, Thr288 and Ser342 of the activation motif is essential for the proper function of this enzyme [7]. Aurora A kinase is found mainly on centrosomes, spindle poles and transiently along the spindle microtubules as cells progress mitosis [14, 33]. The human Aurora A kinase maps to chromosome 20q13.2 [7]. The level of the enzyme is regulated by different factors, such as the anaphase-promoting complex (APC) [10] and phosphorylation [7]. Its production is raised at the G_2/M transition and decreases at M/G_1 transition of cell cycle [10]. The enzyme controls correct development of various phases of mitosis (centrosome maturation and separation, mitotic entry, bipolar spindle assembly, chromosome alignment on the metaphase plate and cytokinesis) [7, 14, 34]. Cell growth in normal conditions depends on the balance between Aurora A and other factors, such as Chfr (a mitotic checkpoint protein), BRCA1 and p53 (tumor suppressor) [21]. Several studies indicated that this enzyme suppresses BRCA1 and BRCA2 [34, 70], which are human genes encoding tumor suppressor proteins engaged in repair of damaged DNA [1]. In certain cancer types, such as ovarian, pancreatic and breast, there is a negative correlation between Aurora A kinase expression and BRCA2 [70]. *Yang* et al. suggested that Aurora A kinase and BRCA2 expression ratio can be a valuable tool in predicting ovarian cancer outcomes [70]. The enzyme is overexpressed in some types of cancer (solid tumors, such as breast, bladder and ovarian cancers as well as gastrointestinal cancers [34]) and is considered to play a crucial role in the inactivation of apoptosis of cancer cells, oncogenic transformation through development of centrosome amplification and chromosomal instability (CIN) [14, 61]. As mentioned, Aurora A kinase plays important role in gastrointestinal cancers. The enzyme was previously described to promote activation of the AKT pro-survival signaling pathway (signal transduction pathway that promotes survival and growth in response to extracellular signals) as well as NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells; complex that controls DNA transcription) and STAT3 (signal transducer and activator of transcription) pathways [34, 71]. Recent research proved that dual Aurora A and JAK2 kinase (Janus kinase important in tumor survival) blockade effectively suppresses malignant transformation. Depletion of both of these enzymes is effective at inhibiting anchorage-dependent and –independent growth and invasion and at inducing apoptosis [71].

It is assumed that Aurora A kinase is a 'druggable' target in cancer as it controls essential oncogenic pathways. Its inhibitors can be divided into various groups based on their chemical core, such as *N*-(5-methyl-1*H*-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl) pyrimidin-4-amines (e.g. tozasertib, ENMD-2076) and 9-chloro-5*H*-benzo[*c*]pyrimido[4,5-*e*]azepines (e.g. alisertib, MLN8054) or *N*-(pyridin-2-yl)thiazol-2-amine (MK-5108). The first registered Aurora A kinase inhibitor was tozasertib (MK-0457, UX-680, *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl) cyclopropane carbo xamide, Figure 3), which showed promising activities towards Aurora kinases: K_i of 0.6 nM against Aurora A, 18nM against Aurora B and 5nM against Aurora C. It was withdrawn from the market due to cardiovascular side effects, such as QTc prolongation [9]. However, several new Aurora A kinase inhibitors were investigated and tested in clinical trials. Among them, MLN 8054 [41] (4-((9-chloro-7-(2,6-difluorophenyl)-5*H*-benzo[*c*]pyrimido[4,5-*e*]azepin-2-yl)amino)benzoic acid, Figure 3), which was shown to inhibit proliferation of various cultured tumor cell lines and demonstrated potent oral antitumor activity in mice bearing human tumor xenografts. It delayed G_2/M progression in cultured human tumor cells [41]. When tested in phase I clinical trial in patients with advanced solid tumors, the drug candidate caused reversible somnolence, which prevented adequate dose establishment [16]. MLN 8054 was further chemically modified (to limit its toxicity) to alisertib (MLN 8237, 4-((9-chloro-7-(2-fluoro-6-methoxyphenyl)-5*H*-benzo[*c*]pyrimido[4,5-*e*]azepin-2-yl)amino)-2-methoxybenzoic acid, Figure 3). At present, the latter compound undergoes several clinical trials [44]. Up to now, therapies with alisertib alone or in combination with known chemotherapeutic agents

have been conducted on patients with hematological and solid tumor malignancies. Also, the efficacy of alisertib is clinically tested on patients with non-small cell lung cancer, advanced solid tumors, lymphoma, ovarian carcinoma, fallopian tube cancer, peritoneal cancer, breast carcinoma, small cell prostate cancer and others [2]. Its activity was also evaluated in soft tissue sarcomas [44]. *Nair* et al. demonstrated that alisertib at nanomolar concentrations is a potent inhibitor of Aurora A and induces apoptosis. At micromolar concentration, it also inhibits Aurora B-induced polyploidy [44]. A novel Aurora A kinase inhibitor, TAS-119, entered clinical trials: one as a monotherapy, second in combination with taxane-based chemotherapy (phase I clinical trials) [2, 14]. Moreover, two other Aurora A kinase inhibitors are in clinical development: ENMD-2076 ((*E*)-*N*-(5-methyl-1*H*-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl)-2-styrylpyrimidin-4-amine) and MK-5108 ((1*R*,4*R*)-4-(3-chloro-2-fluorophenoxy)-1-((6-(thiazol-2-ylamino) pyridin-2-yl)methyl)cyclohexanecarboxylic acid) [2, 34]. Both chemical structures are presented on Figure 3.

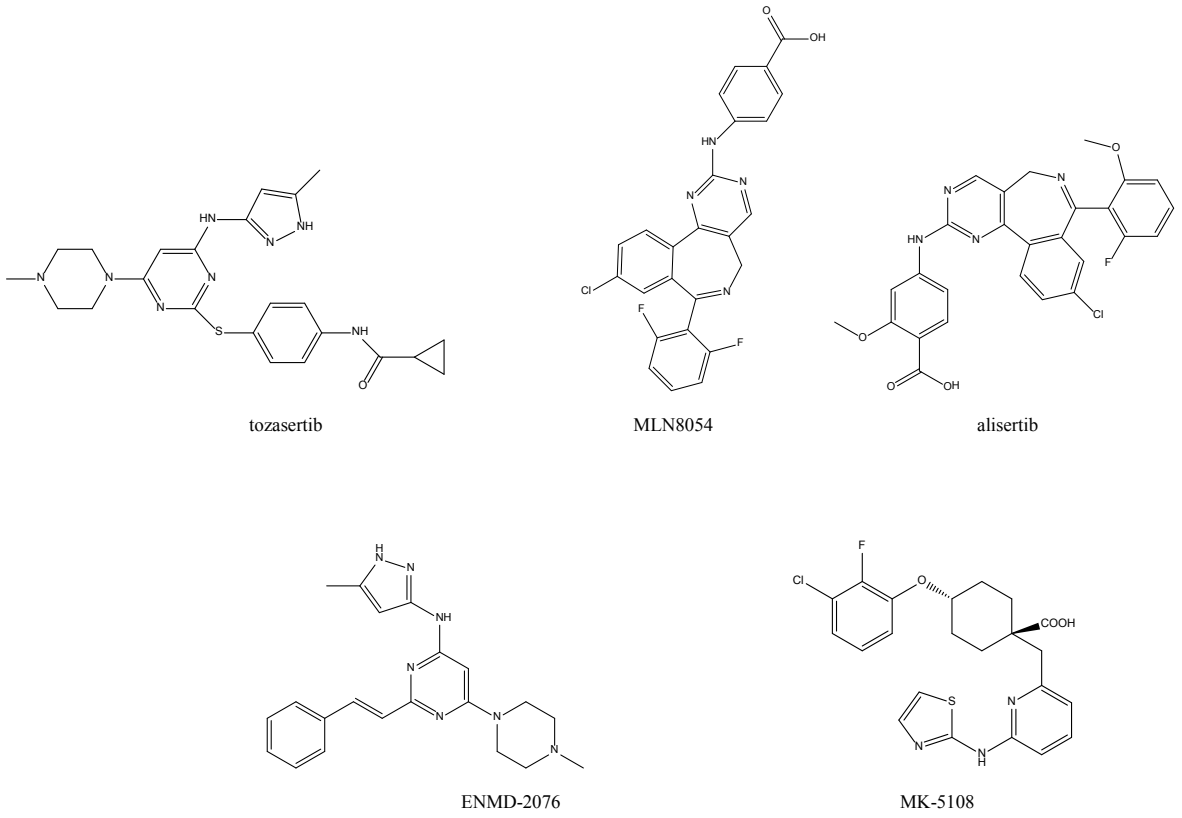


Figure 3. Chemical structures of Aurora A kinase inhibitors

AURORA B KINASE

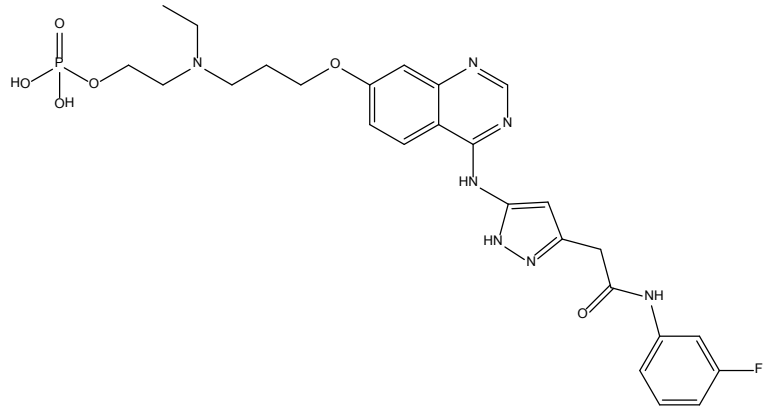
Aurora B, known as a chromosome passenger protein, is 344-amino acid enzyme with a molecular mass of 39 kDa [5]. It is localized in centromeres in early mitosis and then in the spindle mid-zone in anaphase [23, 36]. It is associated with the chromosomal passenger complex (CPC)[33]. The enzyme is activated by auto-phosphorylation of Thr232 in the T-loop and requires interaction with CPC proteins [33]. Aurora B kinase is required for histone H3 phosphorylation, which then helps in chromatin condensation and separation. The enzyme also takes part in chromosome bi-orientation, the spindle assembly checkpoint (SAC) and cytokinesis [15, 23, 36]. It maps to chromosome 17q13 [21]. Overexpression of Aurora B kinase leads to multi-nucleation and polyploidy as well as defects in chromosome segregation and in cytokinesis [21].

Among Aurora B inhibitors, no distinguishable chemical core that is evidently crucial for inhibitory activity has been identified. Inhibitors barasertib (AZD1152, Figure 4) and BI 811283 have been examined in clinical trials. Barasertib (2-(ethyl(3-((4-((3-(2-((3-fluorophenyl)amino)-2-oxoethyl)-1*H*-pyrazol-5-yl)amino)quinazolin-7-yl)oxy)propyl) amino)ethyl dihydrogen phosphate) was shown to be a highly specific inhibitor of Aurora B (0.37 nM). It was tested on patients with large B-cell lymphoma (phase II trial, completed in 2013), patients with AML (phase I trial, completed in 2009) and patients with advanced solid malignancies (phase I trial, completed in 2008) [2]. In phase II trial, the activity of barasertib was compared with low dose cytarabine in patients with AML; however the survival benefit was not achieved [59]. In prior studies, it was shown that barasertib is rapidly converted into AZD1152-HQPA, an active compound with advanced pharmacokinetic properties

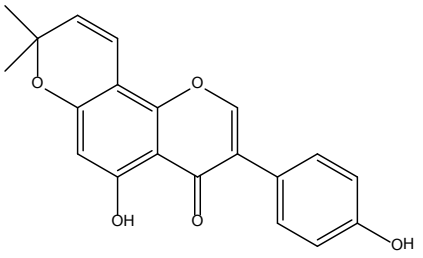
in human plasma [33].

Another Aurora B inhibitor, BI 811283, was tested in phase II in combination with cytarabine in previously untreated AML patients unqualified for intensive treatment (trial completed in 2015 [2]). In contrast, phase I was performed in patients with various solid tumors (completed in 2014) [2].

There is an ongoing search for new inhibitors of Aurora kinases. Recently, a new inhibitor, Derrone, was described by *Hoang* et al. [31]. The chemical structure of this compound, 5-hydroxy-3-(4-hydroxyphenyl)-8,8-dimethylpyrano[2,3-*f*]chromen-4(8*H*)-one, is presented on Figure 4. Derrone was isolated from a natural plant source, *Erythrina orientalis* L. Murr. It showed inhibitory activity towards Aurora kinases A (IC_{50}=22.3 μM) and B (IC_{50}=6μM), being more selective towards the latter. Derrone inhibited phosphorylation of histone H3 on Ser10 (a natural



barasertib



Derrone

Figure 4. Chemical structures of Aurora B kinase inhibitors

substrate of Aurora B, used as an indicator of Aurora B inhibition) both in the kinase assay and at the cellular level [31].

AURORA C KINASE

Aurora C kinase is less known in comparison to the two previously mentioned Aurora kinases, A and B. The human enzyme is a protein comprising 309 amino acids and a molecular mass of 35kDa [5]. It maps to chromosome 19q13 [21]. The enzyme shows 83% similarity to Aurora kinase B and 71% - to enzyme A. However, Aurora C kinase lacks the N-terminal domain, found in both enzymes [48]. The enzyme is essential in mitosis and centrosome function. In comparison to the two other mentioned kinases, which have various localizations in the body, Aurora C kinase is expressed mainly by cells undergoing meiosis (sperm and oocyte) [18, 48]. Normal somatic cells usually show low expression of this enzyme. The localization of Aurora C kinase in spermatophytes and oocytes is dynamic [48]. *Fellmeth* et al. found that human oocytes express three splice variants of the enzyme with different functions, however, all additive in meiosis [18].

There are several studies concerning enzyme overexpression in certain types of cancers cells, such as HeLa, HepG2, MDA-MD-453 and Hu H7. Mutations in Aurora C kinase were described to be linked with male infertility and cancer [18]. However, it has not been proven that Aurora C kinase plays role in carcinogenesis, thus, further investigation is needed [21, 35]. Probably, enzyme overexpression in mitotic cells leads to centrosome amplification and multi-nucleation [48].

LEUCINE AMINOPEPTIDASE (LAP)

Aminopeptidases are a class of zinc metalloenzymes that catalyze the cleavage of amino acids nearby the N-terminus of polypeptides. They are widely distributed in the natural environment, in plants, animals, fungi and bacteria [30, 62] and seem to be important for both, prokaryotic and eukaryotic cells. Aminopeptidases are involved in a variety of biological processes as protein maturation, angiogenesis [56], antigen presentation, neuropeptide and hormone processing, pregnancy and reproduction, protein turnover, memory, inflammation, tumor growth, cancer and metastasis, blood pressure and hypertension [43, 62].

Among medically important aminopeptidases, there are (i) leucine aminopeptidase (α-aminoacyl-peptide hydrolase, LAP, EC 3.4.11.1), which belongs to the M17 family, (ii) alanyl aminopeptidase (also known as aminopeptidase N, APN or CD13; E.C.3.4.11.2), (iii) leucine aminopeptidase 3 (LAP3) and (iv) cystyl aminopeptidase (α-aminoacyl-peptide hydrolase, oxytocinase, EC 3.4.11.3) from the M1 family [43, 55].

Leucine aminopeptidase removes most effectively the leucine amino acid (Leu) and other hydrophobic residues from peptide substrate analogs. In its substrate spectrum, there are also other amino acids, such as phenylalanine (Phe). This cytosolic enzyme is of utmost importance in various biological processes. Its altered activity is observed in pathological disorders, such as cancers, cataracts, cystic fibrosis as well as in ageing [25, 60, 64]. Among LAP enzymes, bovine lens leucine aminopeptidase (blLAP) is one of the best described [60], although leucine aminopeptidase from porcine kidney is the only one commercially available. blLAP is a hexamer, consisting of six identical protomers, 54kDa each (487 amino acids). There are two zinc ions (Zn488 and Zn489) in the blLAP active site and interactions with these ions are substantial for the enzyme catalytic activity, substrate binding and activation as well as inhibitory activity of compounds [25, 60].

Alanyl aminopeptidase (APN) is located in cellular membrane and most favourably cleaves peptide bonds with alanine (Ala) at N-terminus of polypeptide. However, peptides with Leu, Phe, tyrosine (Tyr), arginine (Arg), methionine (Met), lysine (Lys), tryptophan (Trp), glycine (Gly), glutamine (Gln), Ser and histidine (His) are also substrates for APN [8].

Numerous studies have been conducted on the role of leucine aminopeptidases in the cell cycle. They are mostly devoted to cell surface leucine aminopeptidase 3 (LAP3) [26, 65, 68, 73]. LAP3 is a neutral protease which belongs to M1 family. The work of *He* et al. showed that LAP3 could promote proliferation of glioma cancer cells U87 and U251 and inhibition of this enzyme might be involved in cell cycle arrest at G_0/G_1 phase [26]. In the study of *Zhang* et al., authors reported that LAP3 was able to promote G_1/S transition [73]. Overexpression of LAP3 promoted esophageal squamous cell proliferation and migration abilities *in vitro*. It was also observed that LAP controlled cell cycle progression through the activation of cyclin-dependent kinases CDK2, CDK4, CDK6 and cyclin A [73]. *Tian* et al. examined the biological function of LAP and its influence on hepatocellular carcinoma (HCC) [65]. LAP3 promoted HCC cells proliferation and its overexpression accelerated G_1/S phase transition. In another work, LAP3 was suggested to be involved in regulation of tumor invasion [68]. The study of *Wang* et al. showed that inhibition of the enzyme caused up-regulation of the p38 MAPK/Hsp27 pathway and down-regulation of fascin protein expression which resulted in suppression of ovarian cancer cell invasion [68]. The function of the mammalian p38 mitogen-activated protein kinases MAPK is to regulate the cell cycle checkpoints at G_0, G_1/S and G_2/M transitions [13, 27]. The small heat shock protein - HSP27, is believed to be a physiological substrate for these kinases. Phosphorylation of serine residues on HSP27 play a role in actin cytoskeleton remodeling during cellular stress and growth [27]. Fascin, an actin-bundling protein, organizes actin filaments into tightly packed bundles. Its high level is observed in various transformed cells [69]. On the other hand, down-regulation of fascin results in a decrease of cell proliferation.

It is presumed that aminopeptidase inhibition influences mammalian target of rapamycin (mTOR). mTOR is a protein kinase known as a master regulator of protein synthesis, cell growth and proliferation through the control of translation, transcription, mRNA turnover, protein stability, actin cytoskeletal organization and autophagy [32]. It consists of two complexes: mTORC1 and mTORC2. Data suggests that leucine is essential for mTORC1 activation and its deprivation due to LAP inhibition makes mTORC1 inactive [30].

There are two types of LAP inhibitors: amino acid or peptide analogues. Among the first group are: aminoaldehydes, α-aminoboronic acid analogues, phosphonic acid analogues of amino acids, hydroxamate amino acid analogues, chloromethyl ketone amino acid analogues as well as thiol analogues of amino acids. L-leucinal is one of the described aminoaldehyde LAP inhibitors. The crystal structure of the enzyme-inhibitor complex was determined by *Sträter* et al. [60]. Aminoaldehydes are strong LAP inhibitors. L-leucinal was shown to inhibit LAP activity with an inhibition constant of K_i = 60 nM [60].

The second group of LAP inhibitors comprises: bestatin and amastatin analogues, sulfur-containing analogues of bestatin, phosphonic analogues of peptides, ketomethylene peptide analogues as well as other peptide LAP inhibitors. Bestatin, a dipeptide analogue of PheLeu ([(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-(*S*)-leucine, Figure 5) is the first clinically approved aminopeptidase inhibitor (trade name Ubenimex). This drug was originally discovered as an immune-modulating agent [30]. However, further research showed that it strongly inhibits LAP and APN. Bestatin was shown to be a stronger inhibitor towards aminopeptidases with two metal ions in the active center (LAP, K_i= 9 nM) than those with a single metal ion (APN, K_i=3.03 μM) [43]. It binds to each protomer of blLAP [63]. Thus, six bestatin equivalents are bound per LAP hexamer [64]. Amastatin is a tripeptide analogue ([(2*S*,3*R*)-3-amino-2-hydroxy-5-methylhexanoyl-*S*-valyl-*S*-valyl-*S*-aspartic acid], Figure 5) and it is a strong inhibitor of LAP as well as APN [25].

Among aminopeptidases inhibitors, both amino acid and peptide analogues, are those containing phosphorus (Figure 5). Although the phosphorus/phosphinate group is a rather weak zinc-complexing

moiety, their tetrahedral shape mimics the high energy transition state of the peptide bond hydrolysis [43]. For instance, isosters of hydrophobic aliphatic amino acids such as valine (Figure 5) and leucine are potent LAP inhibitors, K_i of 0.15 and 0.23 μM, respectively [38, 43]. Other, aminopeptidase inhibitors worth mentioning are amino acid-derived hydroxamates.

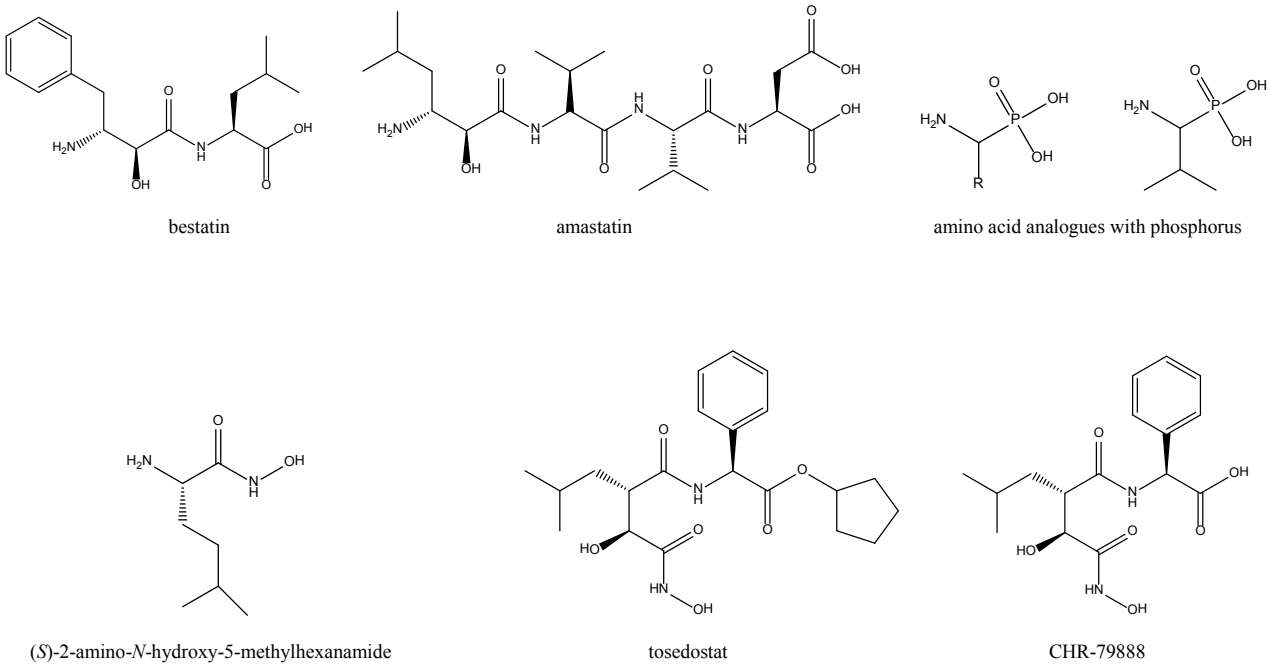


Figure 5. Chemical structures of LAP inhibitors

Hydroxamic acids are analogues of carboxylic acids and amides. They exhibit a close structural similarity to the substrates/products of the peptide bond hydrolysis. They also have metal-complexing properties. One of α-aminohydroxamates, (*S*)-2-amino-*N*-hydroxy-5-methylhexanamide (Figure 5), showed LAP inhibitory activity of K_i= 14 μM [12, 43].

Among the recent developments on aminopeptidase inhibitors is the study on tosedostat (CHR-2797; (*S*)-cyclopentyl 2-((*S*)-2-((*S*)-1-hydroxy-2-(hydroxyamino)-2-oxoethyl)-4-methylpentanamido)-2-phenylacetate, which is currently in phase II clinical trials on acute myeloid leukemia (AML) [42]. Tosedostat is a prodrug converted into a pharmacologically active acid product, CHR-79888 ((*S*)-2-((*S*)-2-((*S*)-1-hydroxy-2-(hydroxyamino)-2-oxoethyl)-4-methylpentanamido)-2-phenylacetic acid) [37]. Chemical structures of tosedostat and CHR-79888 are presented on Figure 5. Tosedostat is a potent inhibitor of various aminopeptidases, including leucine aminopeptidase (IC_{50}=100 nM) (active compound CHR-79888 IC_{50}=30 nM). It exerts antiproliferative activity against histiocytic lymphoma U-937 (IC_{50}=10 nM), acute myelogenous leukemia KG-1 (IC_{50}=15 nM) and HNT-34 (IC_{50}=35 nM), promyelocytic leukemia HL-60 (IC_{50}=30 nM) and myelomonoblastic leukemia GDM-1 (IC_{50}=15 nM) [37]. Currently, there is an ongoing phase II clinical trial of tosedostat with cytarabine or decitabine in newly diagnosed older patients with AML or high-risk myelodysplastic syndrome [42]. Another study recruits patients with metastatic pancreatic adenocarcinoma to be treated with tosedostat with capecytabine [2].

CONCLUSIONS

There is a demanding need for the new molecular drug targets. In the present study, the authors review enzymes, such as RTKs, NRTKs, Aurora kinases and LAP, that are currently actively explored as novel targets, and characterize their potential inhibitors. TK and Aurora kinase enzyme inhibitors present chemical scaffolds mainly comprised of piperazine, pyrimidine, quinoline or quinazoline, whereas LAP inhibitors are mainly amino acid or peptide analogues. First clinical trials on TK or Aurora kinase inhibitors were rather disappointing, indicating that much should be done to explore the role of the enzymes in cancer pathophysiology. A proper clinical study design based on selection of patients with mutations in genes coding for the mentioned enzymes caused positive outcomes. Even when clinical responses to the new drug candidates were rather poor, the combination of such compounds as RTKI, NRTKI, Aurora kinase or LAP inhibitors with other anticancer agents is considered a promising strategy of increasing their antitumor activity. The discovered enzyme inhibitors may serve as model compounds in searching of new "hits" with

optimal activity and physicochemical properties as well as low toxicity.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. BRCA1 and BRCA2: Cancer Risk and Genetic Testing; Available from: http://www.cancer.gov/about-cancer/causes-prevention/genetics/brca-fact-sheet#q1. National Institute of Health (Accessed 09.08.2016).
2. Clinical Trials.gov. Available from: www.clinicaltrials.gov (Accessed 09.08.2016).
3. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. Available from: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx (Accessed 09.08.2016).
4. InterPro Protein Archive Src, proto-oncogene tyrosine-protein kinase. Available from: https://www.ebi.ac.uk/interpro/potm/2003_2/Page_2.htm. (Accessed 09.08.2016).
5. Uniprot database. Available from: www.uniprot.org. (Accessed 09.08.2016).
6. World Health Organization Media Centre Cancer Fact Sheet No 297. Available from: http://www.who.int/mediacentre/factsheets/fs297/en/ (Accessed 09.08.2016).
7. *Agnese V., Bazan V., Fiorentino F.P., Fanale D., Badalamenti G., Colucci G., Adamo V., Santini D., Russo A.*: The role of Aurora-A inhibitors in cancer therapy. Ann Oncol 2007;18 Suppl 6:vi47-vi52.
8. *Aoyagi T.*: Physiological roles of ectoenzymes indicated by the use of aminopeptidase inhibitors. In: *Taylor A. ed*. Aminopeptidases. Boston: Springer-Verlag Berlin Heidelberg GmbH,1996.
9. *Bebbington D., Binch H., Charrier J.D., Everitt S., Fraysse D., Golec J., Kay D., Knegtel R., Mak C., Mazzei F., Miller A., Mortimore M., O'Donnell M., Patel S., Pierard F., Pinder J., Pollard J., Ramaya S., Robinson D., Rutherford A., Studley J., Westcott J.*: The discovery of the potent aurora inhibitor MK-0457 (VX-680). Bioorg Med Chem Lett 2009;19:3586-3592.
10. *Castro A., Arlot-Bonnemains Y., Vigneron S., Labbe J.C., Prigent C., Lorca T.*: APC/Fizzy-Related targets Aurora-A kinase for proteolysis. EMBO Rep 2002;3:457-462.
11. *Cavalloni G., Peraldo-Neia C., Sarotto I., Gammaitoni L., Migliardi G., Soster M., Marchiò S., Aglietta M., Leone F.*: Antitumor Activity of Src Inhibitor Saracatinib (AZD-0530) in Preclinical Models of Biliary Tract Carcinomas. Mol Cancer Ther 2012;11:1528-1538.
12. *Chan W.W., Dennis P., Demmer W., Brand K.*: Inhibition of leucine aminopeptidase by amino acid hydroxamates. J Biol Chem 1982;257:7955-7957.
13. *Cuenda A., Rousseau S.*: p38 MAP-kinases pathway regulation, function and role in human diseases. Biochim Biophys Acta 2007;1773:1358-1375.
14. *D'Assoro A.B., Haddad T., Galanis E.*: Aurora-A Kinase as a Promising Therapeutic Target in Cancer. Front Oncol 2015;5:295-302.
15. *D'Avino P.P., Capalbo L.*: New Auroras on the Roles of the Chromosomal Passenger Complex in Cytokinesis: Implications for Cancer Therapies. Front Oncol 2015;5:221-226.
16. *Dees E.C., Infante J.R., Cohen R.B., O'Neil B.H., Jones S., von Mehren M., Danaee H., Lee Y., Ecsedy J., Manfredi M., Galvin K., Stringer B., Liu H., Eton O., Fingert H., Burris H.*: Phase 1 study of MLN8054, a selective inhibitor of Aurora A kinase in patients with advanced solid tumors. Cancer Chemother Pharmacol 2011;67:945-954.
17. *Eom K.Y., Cho B.J., Choi E.J., Kim J.H., Chie E.K., Wu H.G., Kim I.H., Paek S.H., Kim J.S., Kim I.A.*: The Effect of Chemoradiotherapy with SRC Tyrosine Kinase Inhibitor, PP2 and Temozolomide on Malignant Glioma Cells *In Vitro* and *In Vivo*. Cancer Res Treat 2016;48:687-697.
18. *Fellmeth J.E., Gordon D., Robins C.E., Scott R.T., Jr., Treff N.R., Schindler K.*: Expression and characterization of three Aurora kinase C splice variants found in human oocytes. Mol Hum Reprod 2015;21:633-644.
19. *Formisano L., D'Amato V., Servetto A., Brillante S., Raimondo L., Di M.C., Marciano R., Orsini R.C., Cosconati S., Randazzo A., Parsons S.J., Montuori N., Veneziani B.M., De P.S., Rosa R., Bianco R.*: Src inhibitors act through different mechanisms in Non-Small Cell Lung Cancer models depending on EGFR and RAS mutational status. Oncotarget 2015;6:26090-26103.
20. *Frame M.C.*: Src in cancer: deregulation and consequences for cell behaviour. Biochim Biophys Acta 2002;1602:114-130.
21. *Gavriilidis P., Giakoustidis A., Giakoustidis D.*: Aurora Kinases and Potential Medical Applications of Aurora Kinase Inhibitors: A Review. J Clin Med Res 2015;7:742-751.
22. *Gerber D.E.*: Targeted therapies: a new generation of cancer treatments. Am Fam Physician 2008;77:311-319.
23. *Girdler F., Gascoigne K.E., Eyers P.A., Hartmuth S., Crafter C., Foote K.M., Keen N.J., Taylor S.S.:* Validating Aurora B as an anti-cancer drug target. J Cell Sci 2006;119:3664-3675.
24. *Gocek E., Moulas A.N., Studzinski G.P.*: Non-receptor protein tyrosine kinases signaling pathways in normal and cancer cells. Crit Rev Clin Lab Sci 2014;51:125-137.
25. *Grembecka J., Kafarski P.*: Leucine aminopeptidase as a target for inhibitor design. Mini Rev Med Chem 2001;1:133-144.
26. *He X., Huang Q., Qiu X., Liu X., Sun G., Guo J., Ding*

Z., Yang L., Ban N., Tao T., Wang D.: LAP3 promotes glioma progression by regulating proliferation, migration and invasion of glioma cells. Int J Biol Macromol 2015;72:1081-1089.
27. *Hedges J.C., Dechert M.A., Yamboliev I.A., Martin J.L., Hickey E., Weber L.A., Gerthoffer W.T.*: A role for p38(MAPK)/HSP27 pathway in smooth muscle cell migration. J Biol Chem 1999;274:24211-24219.
28. *Herbst R.S.*: Review of epidermal growth factor receptor biology. Int J Radiat Oncol Biol Phys 2004;59:21-26.
29. *Heusschen R., Muller J., Binsfeld M., Marty C., Plougonven E., Dubois S., Mahli N., Moermans K., Carmeliet G., Leonard A., Baron F., Beguin Y., Menu E., Cohen-Solal M., Caers J.*: SRC kinase inhibition with saracatinib limits the development of osteolytic bone disease in multiple myeloma. Oncotarget 2016;7:30712-30729.
30. *Hitzerd S.M., Verbrugge S.E., Ossenkoppele G., Jansen G., Peters G.J.*: Positioning of aminopeptidase inhibitors in next generation cancer therapy. Amino Acids 2014;46:793-808.
31. *Hoang N.T., Phuong T.T., Nguyen T.T., Tran Y.T., Nguyen A.T., Nguyen T.L., Bui K.T.*: *In vitro* Characterization of Derrone as an Aurora Kinase Inhibitor. Biol Pharm Bull 2016;39:935-945.
32. *Ikenoue T., Hong S., Inoki K.*: Monitoring mammalian target of rapamycin (mTOR) activity. Methods Enzymol 2009;452:165-178.
33. *Katayama H., Sen S.*: Aurora kinase inhibitors as anticancer molecules. Biochim Biophys Acta 2010;1799:829-839.
34. *Katsha A., Belkhiri A., Goff L. El-Rifai W.*: Aurora kinase A in gastrointestinal cancers: time to target. Mol Cancer 2015;14:106-118.
35. *Kollareddy M., Dzubak P., Zheleva D., Hajduch M.*: Aurora kinases: structure, functions and their association with cancer. Acta Univ Palacki Olomuc Fac Med 2008;152:27-33.
36. *Kollareddy M., Zheleva D., Dzubak P., Brahmkshatriya P.S., Lepsik M., Hajduch M.*: Aurora kinase inhibitors: progress towards the clinic. Invest New Drugs 2012;30:2411-2432.
37. *Krige D., Needham L.A., Bawden L.J., Flores N., Farmer H., Miles L.E., Stone E., Callaghan J., Chandler S., Clark V.L., Kirwin-Jones P., Legris V., Owen J., Patel T., Wood S., Box G., Laber D., Odedra R., Wright A., Wood L.M., Eccles S.A., Bone E.A., Ayscough A., Drummond A.H.*: CHR-2797: an antiproliferative aminopeptidase inhibitor that leads to amino acid deprivation in human leukemic cells. Cancer Res 2008;68:6669-6679.
38. *Lejczak B., Kafarski P., Zygmunt J.*: Inhibition of aminopeptidases by aminophosphonates. Biochemistry 1989;28:3549-3555.
39. *Lemmon M.A., Schlessinger J.*: Cell signaling by receptor tyrosine kinases. Cell 2010; 141:1117-1134.
40. *Lemmon M.A., Schlessinger J., Ferguson K.M.*: The EGFR family: not so prototypical receptor tyrosine kinases. Cold Spring Harb Perspect Biol 2014; 6:a020768.
41. *Manfredi M.G., Ecsedy J.A., Meetze K.A., Balani S.K., Burenkova O., Chen W., Galvin K.M., Hoar K.M., Huck J.J., LeRoy P.J., Ray E.T., Sells T.B., Stringer B., Stroud S.G., Vos T.J., Weatherhead G.S., Wysong D.R., Zhang M., Bolen J.B., Claiborne C.F.*: Antitumor activity of MLN8054, an orally active small-molecule inhibitor of Aurora A kinase. Proc Natl Acad Sci USA. 2007;104:4106-4111.
42. *Mawad R., Becker P.S., Hendrie P., Scott B., Wood B.L., Dean C., Sandhu V., Deeg H.J., Walter R., Wang L., Myint H., Singer J.W., Estey E., Pagel J.M.*: Phase II study of tosedostat with cytarabine or decitabine in newly diagnosed older patients with acute myeloid leukaemia or high-risk MDS. Br J Haematol 2016;172:238-245.
43. *Mucha A., Drag M., Dalton J.P., Kafarski P.*: Metallo-aminopeptidase inhibitors. Biochimie 2010;92:1509-1529.
44. *Nair J.S., Schwartz G.K.*: MLN-8237: A dual inhibitor of aurora A and B in soft tissue sarcomas. Oncotarget 2016;7:12893-12903.
45. *Padma V.V.*: An overview of targeted cancer therapy. Biomedicine (Taipei) 2015; 5:19-24.
46. *Parsons S.J., Parsons J.T.*: Src family kinases, key regulators of signal transduction. Oncogene 2004;23:7906-7909.
47. *Paul M.K., Mukhopadhyay A.K.*: Tyrosine kinase - Role and significance in Cancer. Int J Med Sci 2004;1:101-115.
48. *Quartuccio S.M., Schindler K.*: Functions of Aurora kinase C in meiosis and cancer. Front Cell Dev Biol 2015;3:50-57.
49. *Raval S.H., Singh R.D., Joshi D.V., Patel H.B., Mody S.K.*: Recent developments in receptor tyrosine kinases targeted anticancer therapy. Vet World 2016;9:80-90.
50. *Raymond E., Faivre S., Armand J.P.*: Epidermal growth factor receptor tyrosine kinase as a target for anticancer therapy. Drugs 2000;60 Suppl 1:15-23.
51. *Roengvoraphoj M., Tsongalis G.J., Dragnev K.H., Rigas J.R.*: Epidermal growth factor receptor tyrosine kinase inhibitors as initial therapy for non-small cell lung cancer: focus on epidermal growth factor receptor mutation testing and mutation-positive patients. Cancer Treat Rev 2013;39:839-850.
52. *Roskoski R., Jr.*: Src protein-tyrosine kinase structure and regulation. Biochem Biophys Res Commun 2004;324:1155-1164.
53. *Rossi A., Pasquale R., Esposito C., Normanno N.*: Should epidermal growth factor receptor tyrosine kinase inhibitors be considered ideal drugs for the treatment of selected advanced non-small cell lung cancer patients? Cancer Treat Rev 2013;39:489-497.
54. *Rossi S., D'Argento E., Schinzari G., Dadduzio V., Di N., V, Cassano A., Barone C.*: Are TKIs favourable for the elderly with non-small-cell lung cancer? Oncotarget 2016;7:46871-46877.
55. *Sanderink G.J., Artur Y., Siest G.*: Human Aminopeptidases: A Review of the literature. J Clin Chem Clin Biochem 1988;26:795-807.
56. *Sato Y.*: Role of Aminopeptidase in Angiogenesis. Biol Pharm Bull 2004;27:772-776.

57. *Schlessinger J.*: Cell signaling by receptor tyrosine kinases. Cell 2000;103:211-225.
58. *Segaliny A.I., Tellez-Gabriel M., Heymann M.F., Heymann D.*: Receptor tyrosine kinases: Characterisation, mechanism of action and therapeutic interests for bone cancers. J Bone Oncol 2015;4:1-12.
59. *Shafer D., Grant S.*: Update on rational targeted therapy in AML. Blood Rev 2016;30:275-283.
60. *Strater N., Lipscomb W.N.*: Two-metal ion mechanism of bovine lens leucine aminopeptidase: active site solvent structure and binding mode of L-leucinal, a gem-diolate transition state analogue, by X-ray crystallography. Biochemistry 1995;34:14792-14800.
61. *Sun C., Chan F., Briassouli P., Linardopoulos S.*: Aurora kinase inhibition downregulates NF-kappaB and sensitises tumour cells to chemotherapeutic agents. Biochem Biophys Res Commun 2007;352:220-225.
62. *Taylor A.*: Aminopeptidasaes, Occurrence, Regulation and Nomenclature. In: *Taylor A*. ed. Aminopeptidases. Austin, Texas, USA: R.G. Landes Company,1996.
63. *Taylor A., Peltier C.Z., Torre F.J., Hakamian N.*: Inhibition of bovine lens leucine aminopeptidase by bestatin: number of binding sites and slow binding of this inhibitor. Biochemistry 1993;32:784-790.
64. *Taylor A., Sanford D., Nowell T.*: Structure and function of bovine lens aminopeptidase and comparison with homologous aminopeptidases. In: *Taylor A*. ed. Aminopeptidases. Austin, Texas, USA: R.G. Landes Company,1996.
65. *Tian S.Y., Chen S.H., Shao B.F., Cai H.Y., Zhou Y., Zhou Y.L., Xu A.B.*: Expression of leucine aminopeptidase 3 (LAP3) correlates with prognosis and malignant development of human hepatocellular carcinoma (HCC). Int J Clin Exp Pathol 2014;7:3752-3762.
66. *Vergoulidou M.*: More than a Decade of Tyrosine Kinase Inhibitors in the Treatment of Solid Tumors: What We Have Learned and What the Future Holds. Biomark Insights 2015; 10:33-40.
67. *Vultur A., Buettner R., Kowolik C., Liang W., Smith D., Boschelli F., Jove R.*: SKI-606 (bosutinib), a novel Src kinase inhibitor, suppresses migration and invasion of human breast cancer cells. Mol Cancer Ther 2008;7:1185-1194.
68. *Wang X, Shi L, Deng Y, Qu M, Mao S, Xu L, Xu W, Fang C.*: Inhibition of leucine aminopeptidase 3 suppresses invasion of ovarian cancer cells through down-regulation of fascin and MMP-2/9. Eur J Pharmacol 2015;768:116-122.
69. *Xie J.J., Xu L.Y., Zhang H.H., Cai W.J., Mai R.Q., Xie Y.M., Yang Z.M., Niu Y.D., Shen Z.Y., Li E.M.*: Role of fascin in the proliferation and invasiveness of esophageal carcinoma cells. Biochem Biophys Res Commun 2005;337:355-362.
70. *Yang G., Chang B., Yang F., Guo X., Cai K.Q., Xiao X.S., Wang H., Sen S., Hung M.C., Mills G.B., Chang S., Multani A.S., Mercado-Uribe I., Liu J.*: Aurora kinase A promotes ovarian tumorigenesis through dysregulation of the cell cycle and suppression of BRCA2. Clin Cancer Res 2010;16:3171-3181.
71. *Yang H., Lawrence H.R., Kazi A., Gevariya H., Patel R., Luo Y., Rix U., Schonbrunn E., Lawrence N.J., Sebti S.M.*: Dual Aurora A and JAK2 kinase blockade effectively suppresses malignant transformation. Oncotarget 2014;5:2947-2961.
72. *Yin S., Tang C., Wang B., Zhang Y., Zhou L., Xue L., Zhang C.*: Design, synthesis and biological evaluation of novel EGFR/HER2 dual inhibitors bearing a oxazolo[4,5-g]quinazolin-2(*1H*)-one scaffold. Eur J Med Chem 2016;120:26-36.
73. *Zhang S., Yang X., Shi H., Li M., Xue Q., Ren H., Yao L., Chen X., Zhang J., Wang H.*: Overexpression of leucine aminopeptidase 3 contributes to malignant development of human esophageal squamous cell carcinoma. J Mol Histol 2014;45:283-292.
74. *Zhang S., Yu D.*: Targeting Src family kinases in anti-cancer therapies: turning promise into triumph. Trends Pharmacol Sci 2012;33:122-128.

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