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Research paper

Novel CLK1 inhibitors based on *N*-aryloxazol-2-amine skeleton - A possible way to dual VEGFR2 TK/CLK ligands

Miroslav Murár [a], Juraj Dobiaš [a], Peter Šramel [a, c], Gabriela Addová [b], Gilles Hanquet [c], Andrej Boháč [a, d, *]

[a] Department of Organic Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská Dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia
[b] Institute of Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská Dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia
[c] Université de Strasbourg, Ecole Européenne de Chimie, Polyméres et Matériaux (ECPM) Laboratoire de Synthése et Catalyse (UMR CNRS 7509), 25, Rue Becquerel, F-67087 Strasbourg, France
[d] Biomagi, Ltd., Mamateyova 26, 851 04 Bratislava, Slovakia

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ABSTRACT

Background: Inhibitors of CLK protein kinases suppress cell growth and induce apoptosis by modulating pre-mRNA splicing in cancer. CLK family kinases are also involved in alternative splicing and RNA processing in *Duchenne muscular dystrophy*, *Alzheimer's disease*, *HIV-1*, and *influenza virus*. Small inhibitors are valuable tools for better understanding the molecular mechanisms of splicing and may serve as seeds for a novel class of therapeutics.

Achievements: Here we describe a discovery of four novel CLK1 inhibitors possessing *N*-aryloxazol-2-amine skeleton. Their activity against CLK1 (IC_{50}: 20, 30, 40 and 80 nM) and some other CMGC kinases, predicted CLK binding poses, synthesis and physico-chemical characteristics are also stated. Additionally analysis of all PDB available CLK structures and interactions of their ligands was performed. There are only few powerful dual CLK/VEGFR inhibitors known in the literature. We proposed that our inhibitors have similar binding places and interactions in CLK1, 3 and VEGFR2 TK mostly due to the joint *N*-aryloxazol-2-amine pharmacophoric fragment. One of our *N*-aryloxazol-2-amines already proved a good activity against both VEGFR2 and CLK1 enzymes (23/80 nM, resp). We proposed that the presented class of compounds has a potential to be developed in dual VEGFR2/CLK clinical compounds with prospective synergy to treat cancer.

1. Introduction

Alternative splicing is a regulated process during the gene expression that greatly increases the biodiversity of proteins and allows the human genome to direct the synthesis of many more proteins (50–100 000) than would be expected from human 20 000 protein-coding genes [1]. There is a growing recognition of the importance of protein kinases in the control of alternative splicing. Splicing requires reversible phosphorylation of serine/arginine-rich (SR) proteins in eukaryotic mRNA. These phosphorylation events are dependent on SR proteins and cdc2-like kinase (CLK) families [2]. The CLKs are an evolutionarily conserved group belonging to the CMGC Ser/Thr protein kinase family from Human Kinome [3,4] (for CMGC part see supplementary material (chapter: *CLKs in the Human Kinome*). CLKs are primarily localized to the cytoplasm and nucleus [5]. CLKs are part of LAMMER PK family possessing identity of the motif "EHLAMMERILG" in their kinase subdomain (see supplementary material: chapter *Superimposed CLKs with marked LAMMER subdomain*). This motif was reported to be essential for kinase activity [6]. CLKs (CDC2-like or LAMMER kinases) are dual specific kinases that have been shown to autophosphorylate on serine, threonine and tyrosine residues and phosphorylate exogenous substrates on serine and threonine residues. The CLK family kinases are found in diverse species, from yeast to human. A critical role of the CLK family kinases is the regulation of mRNA splicing. CLK have shown to interact with, and phosphorylate, serine- and arginine-rich (SR) proteins [7,8]. SR

* Corresponding author. Biomagi, Ltd., Mamateyova 26, 851 04 Bratislava, Slovakia.
E-mail address: andrej.bohac@fns.uniba.sk (A. Boháč).

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proteins are splicing factors that regulate the assembly of the spliceosome, a macromolecular complex where RNA splicing occurs in nucleus [9]. CLKs can co-exist as full-length catalytically active and alternatively-spliced truncated inactive forms [10]. The CLK family consists of four isoforms CLK1-4 exhibiting the typical protein kinase fold (see supplementary material: chapter *Graphical abstract*). CLK1-4 isoforms possess different length of amino acids (AAs) chain: Clk1 (484 AAs), Clk2 (499 AAs), Clk3 (638 AAs), Clk4 (481 AAs) [11]. CLK1 regulates its own splicing [12]. CLKs inhibitors suppress cell growth and induce apoptosis by modulating pre-mRNA splicing in cancer [13]. CLK family kinases are also involved in alternative splicing and RNA processing in *Duchenne muscular dystrophy, Alzheimer's disease, HIV-1*, and *influenza virus* [14]. CLK1 is involved in the pathophysiology of *Alzheimer's* disease, hence the inhibition of CLK1 can be used as a therapeutic strategy for it [9]. CLK1 in the host cells is responsible for alternative splicing of the M2 gene of influenza virus during influenza infection and replication. Therefore CLK1 inhibitors may have potential in anti-influenza drug screening [15]. CLK1 has been shown to interact and phosphorylate other protein kinases as well as protein phosphatases [12]. Human CLK2 links cell cycle progression, apoptosis and telomere length regulation [16]. CLK2 acts as a suppressor of hepatic gluconeogenesis and glucose output [17]. CLK2 is overexpressed in breast tumours. Downregulation of CLK2 inhibits breast cancer growth [18]. CLK3 is a protein kinase with a non-conserved *N*-terminal domain.

CLK small molecule inhibitors are valuable tools for better understanding the molecular mechanisms of splicing and may serve as seeds for a novel class of therapeutics [2,14].

The limitations of many mono-kinase inhibitors can be overcome by agents with multi-target action by increasing their potency, due to the synergistic effect. A review was published in 2015 about the most recent examples of multi-kinase inhibitors [19]. Some dual inhibitors for distanced kinases CLK1 and CK1 were developed recently based on pyrido[3′, 2′:4, 5]thieno[3, 2-*d*]pyrimidin-4-amine skeleton [20].

Table 1
hu-CLK structures published in PDB database.

	Protein structure[a]	Kinase/ligand complex structure[a]
hu-CLK1[b]	none	PDB: 2VAG (**V25**; IC_{50} = 19.7 nM; 2007, 1.80 Å, diphosphorylated kinase) [22] PDB: 1Z57 (**DBQ**;[c] 2005, 1.70 Å) [2]
hu-CLK2[b]	none	PDB: 3NR9 (**NR9**; 2010, 2.89 Å) [23]
hu-CLK3[b]	PDB: 2EU9 (2005, 1.53 Å) [2] PDB: 2EXE (2005, 2.35 Å, phosphorylated) [24][e]	PDB: 3RAW (**3RA**;[d] 2011, 2.09 Å) [25] PDB: 2WU6 (**DKI**; IC_{50} = 29.2 nM; 2009, 1.92 Å) [22] PDB: 2WU7 (**V25**; IC_{50} = 530 nM; 2009, 2.25 Å) [22]
hu-CLK4	none	none

[a] The data are described in the following order: PDB: code (**ligand code,** it's activity; year of deposition in PDB DB; X-ray resolution; notes, if any).
[b] All CLK kinase structures in PDB are in an active DFG-IN conformation: DFG fragment (Asp-Phe-Gly triade and their isoform specific primary sequence numbers: 325–327 (hu-CLK1), 327–329 (hu-CLK2), 320–322 (hu-CLK3).
[c] Also known as 10*Z*-Hymenialdisine.
[d] Also known as leucettine (L41).
[e] This is an incomplete structure of CLK3.

2. Results and discussion

2.1. Analysis of hu-CLKs structures in PDB

With the aim to perform docking experiments we analysed CLKs structures in the PDB database [21]. Currently, there are 8 X-ray structures of human CLK1-3: two hu-CLK3 proteins and six CLK1-3/ligand complexes. No structure of hu-CLK4 was published. According to our analysis, all CLK structures in PDB are in an active DFG-IN kinase conformation (Table 1).

2.2. Analysis of hu-CLKs PDB ligand interactions

To understand the ligand/CLK bindings, interactions diagrams of all inhibitors (2×**V25**, **DBQ**, **DKI**, **NR9** and **3RA**) from PDB hu-CLK complexes were composed. The ligand structures on diagrams are drawn uniformly according their positions in an *Africa*-like kinase perspective [26]. Some biological activities of CLK PDB inhibitors are listed too [27]. The data for all CLKs PDB ligands can be found in supplementary material (chapter: *Analysis of hu-CLKs PDB ligand interactions*). Here we present an example of analysis for ligand **V25** from both complexes CLK1 (PDB: 2VAG) and CLK3 (PDB: 2WU7). (Fig. 1**).**

2.2.1. (*E*)-Ethyl 3-(2-amino-1-cyanovinyl)-6,7-dichloro-1-methyl-1*H*-indole-2-carboxylate (***V25***) in complex with hu-CLK1 and ***3***

For compound **V25** [SciFinder CAS: 1354037-26-5; Reaxys RRN: 21739116] the following IC_{50} activities were described in the literature: hu-CLK1 (19.7 nM), hu-CLK3 (530 nM) and hu-DYRK1A (55.2 nM) [27].

2.3. Interaction diagrams

See Fig. 1.

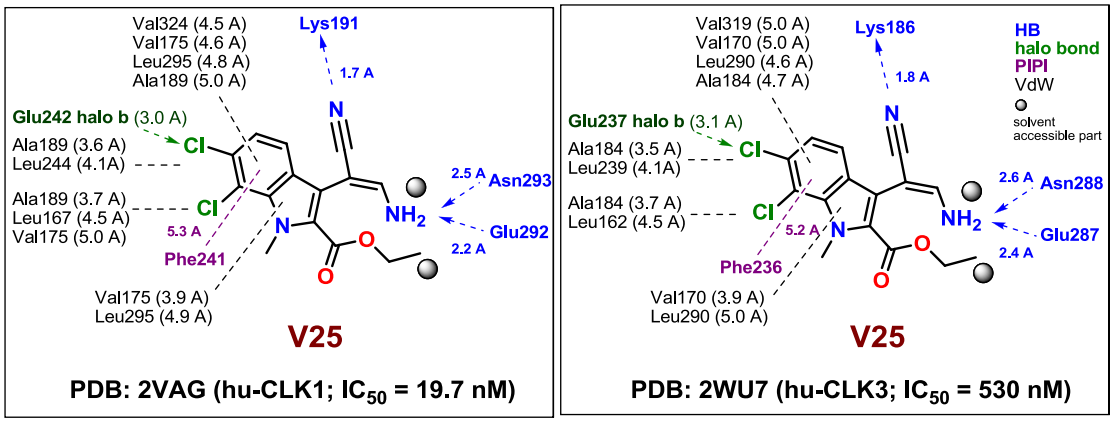


Fig. 1. The structure and intermolecular interactions of **V25** determined in hu-CLK1 and 3 complexes.

2.4. CLKs inhibitors

In order to know how many inhibitors exist, CLKs abbreviations and synonyms [28] were collected and used to search biological active CLK compounds in the Reaxys DB [27]. The search resulted in the amount of inhibitors depicted for each kinase in parenthesis: CLK1 (176), CLK2 (108), CLK3 (84), CLK4 (261). By combining the above hit sets, we found 388 known compounds connected with CLKs activities.

2.5. Clinical drugs as CLKs inhibitors

There are only few clinical drugs able to inhibit CLK kinases. The drugs CLK activities were mostly determined by searching their side effects [29] by screening pharmaceutical compounds against 456 human kinases (*Kinome scan*). The determined drug CLK affinities are as follows: CLK1 (sunitinib K_D = 22 nM, bosutinib K_D = 600 nM, nilotinib K_D = 2100 nM); CLK2 (sunitinib K_D = 20 nM, ruxolitinib K_D = 460 nM, bosutinib K_D = 1700 nM); CLK3 (bosutinib K_D = 300 nM); CLK4 (sunitinib K_D = 29 nM, ruxolitinib K_D = 1700 nM, imatinib K_D = 2100 nM). The above drugs were developed for different primary target(s) as CLKs. From them only sunitinib was developed against VEGFR2 TK. The dual CLK and VEGFR2 activities of sunitnib are interesting because both CLKs and VEGFR2 inhibitors are important as potential anticancer drugs.

2.6. VEGFR2 TK drugs

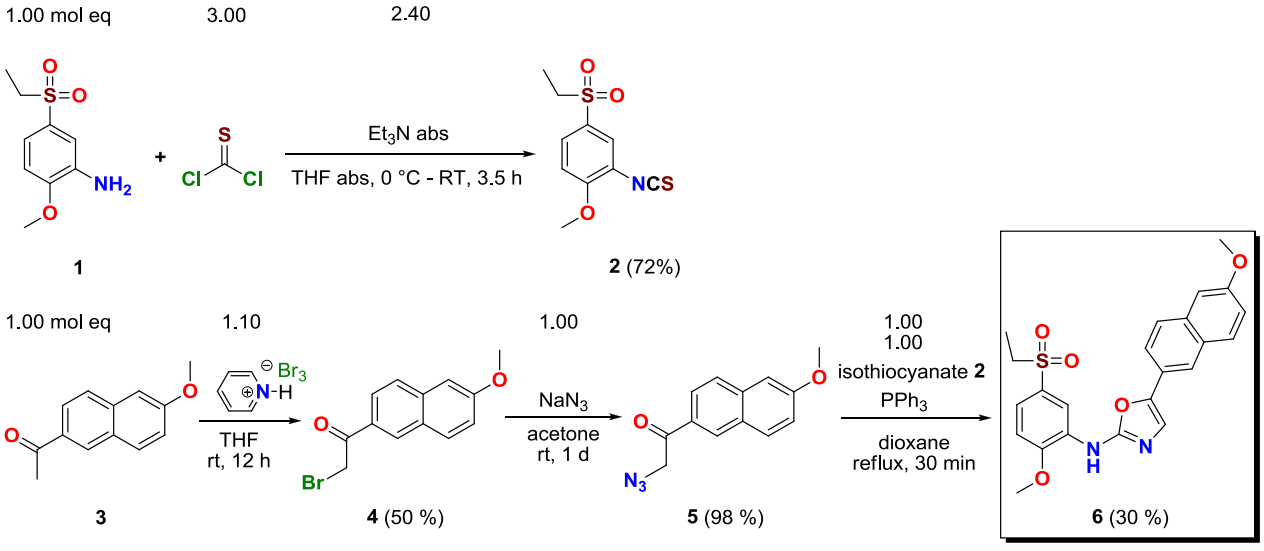
VEGFR2 TK (also named as KDR kinase) is an important receptor for VEGF signalling. VEGF is a key mediator of angiogenesis in cancer [30]. Similarly as for CLKs above we identified 6299 active compounds against VEGFR1-3 in Reaxys DB. Within recent ten years pharmaceutical companies produced nine approved anticancer drugs inhibiting VEGFR2 TK [31]. Their structures, year of approval and name of owning pharma company are published in supplementary material (chapter: *A list of VEGFR2 approved drugs*).

2.7. Synthesis of CLK1 inhibitors **6**, **10**, **21** and **29**

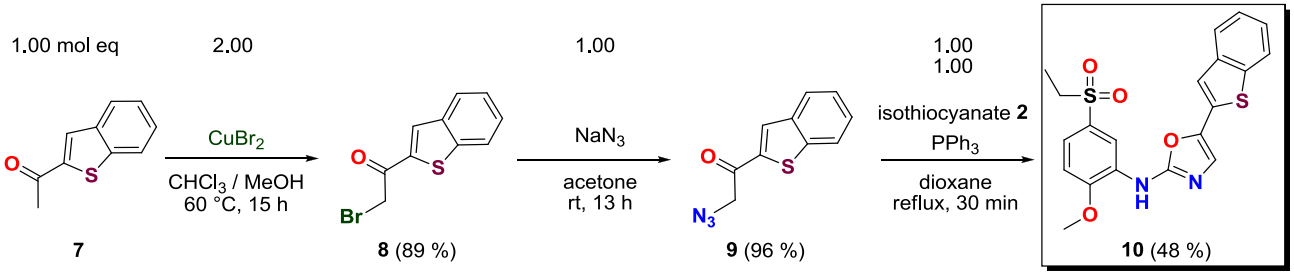
CLK1 inhibitors **6**, **10**, **21** and **29** and their intermediates were prepared according the procedures depicted on Schemes 1–4. Synthetic details, characterisation and figures of spectra of all compounds are stated in supplementary material (chapter: *Supplementary Experimental*).

2.8. Biological screening

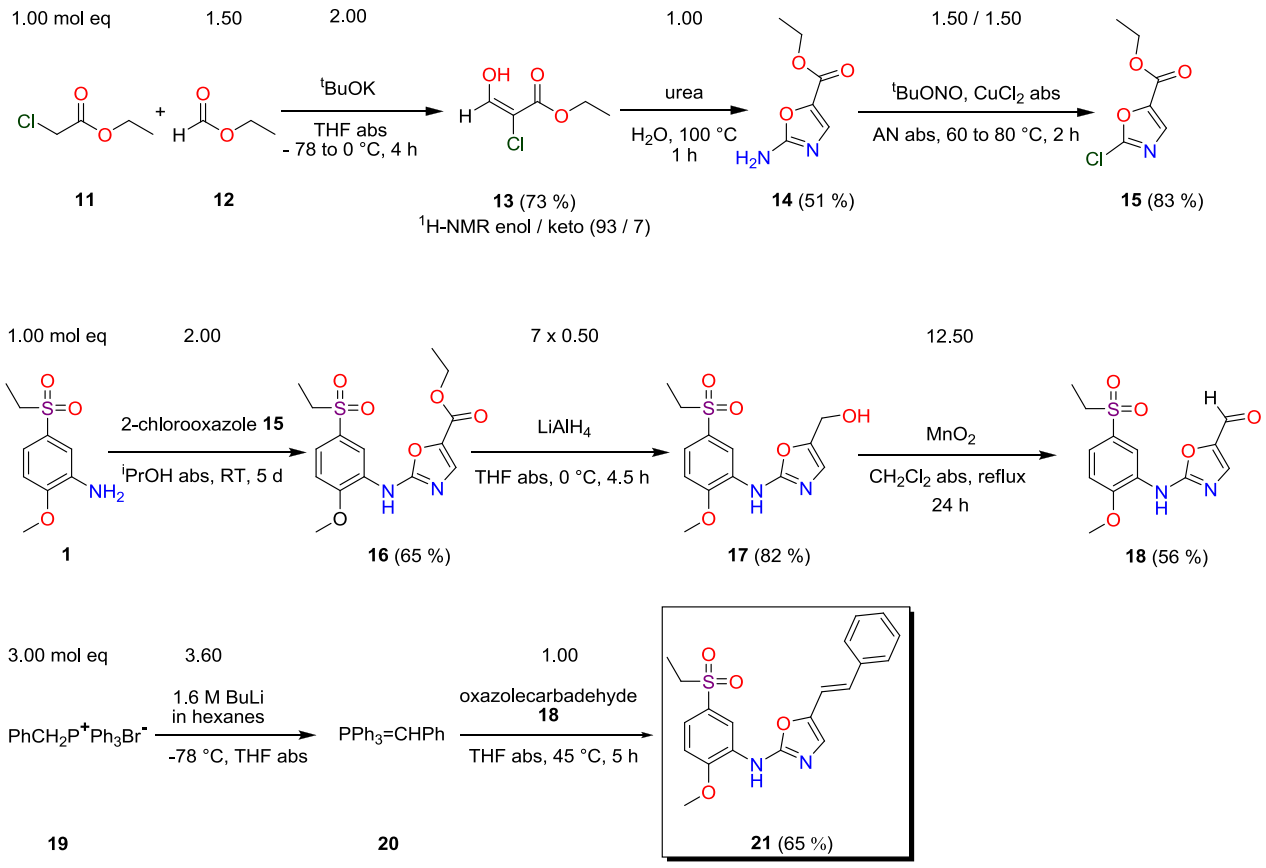
Compounds **6**, **10**, **21** and **29** previously developed to inhibit VEGFR2 TK were screened on eleventh protein kinases by radiometric protein kinase assay in 10 semi-logarithmic concentrations on a panel of eleventh protein kinases: CDK2/CyclinA (cyclin-dependant kinase); CDK5/p25; CDK9/CyclinT; PIM1 (proto-oncogene serine/threonine-protein kinase); DYRK1A (dual specificity tyrosine-phosphorylation-regulated kinase); GSK3α/β (glycogen synthase kinase); GSK3; CLK1 (CDC2-like or LAMMER family dual specificity protein kinase); CK1δ/ε (caseine kinase 1); CK1; HASPIN (haploid germ cell-specific nuclear protein kinase); AURKB (Aurora kinase B); RIPK3 (receptor-interacting serine/threonine-protein



Scheme 1. Synthetic pathway to final naphthalenyloxazolamine **6**.



Scheme 2. Synthetic pathway to required benzothiophenyloxazolamine **10**.

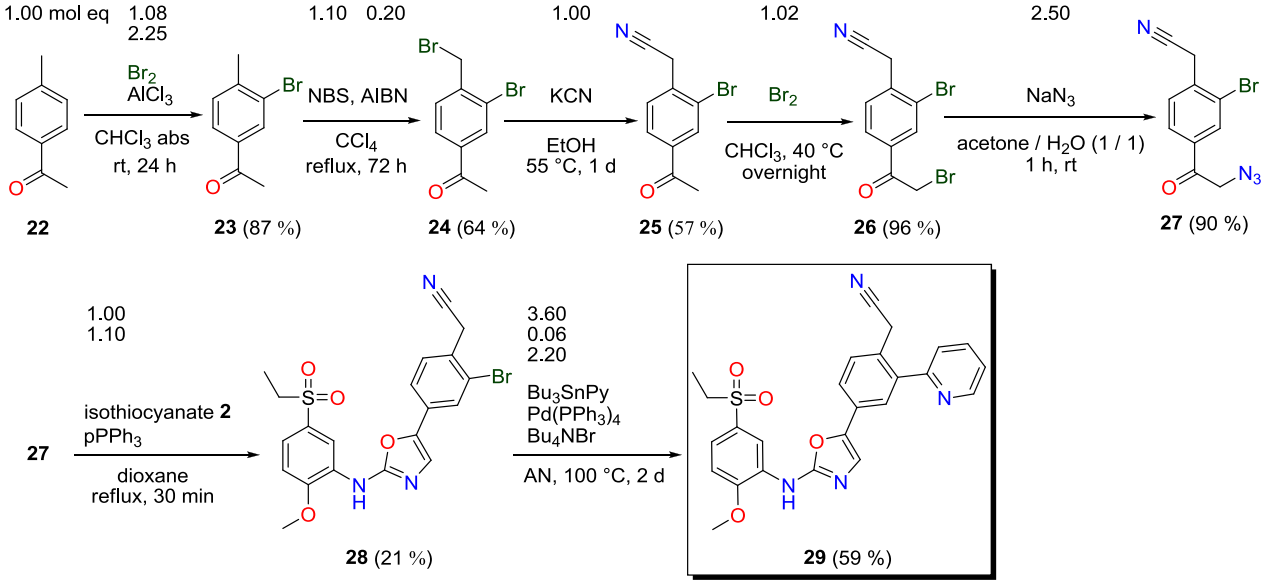


Scheme 3. Synthesis of final styryloxazolamine **21**.

kinase) and TLK (tousled-like kinase). The IC_{50} activities were obtained by Dr. Stéphane Bach and Thomas Robert (Station Biologique de Roscoff, Place Georges Teissier, CS 90074, 29688 Roscoff cedex, France). All compounds **6**, **10**, **21** and **29** were showed to be powerful CLK1 inhibitors. The results are given in Fig. 2. (see also the Supplementary material chapter *Biological activity assay*) Compound **29** was screened likewise on VEGFR2 TK activity. Surprisingly, this compound performed dual VEGFR2/CLK1 kinase activity even though VEGFR2 is not relative kinase to CLKs and belongs to distant TK subgroup in the Human Kinome [4].

2.9. Predicted CLK1 inhibitors binding poses

In order to find binding poses for CLK1 inhibitors (**6**, **10**, **21** and



Scheme 4. Synthetic pathway to aminooxazolephenylacetonitrile **29**.

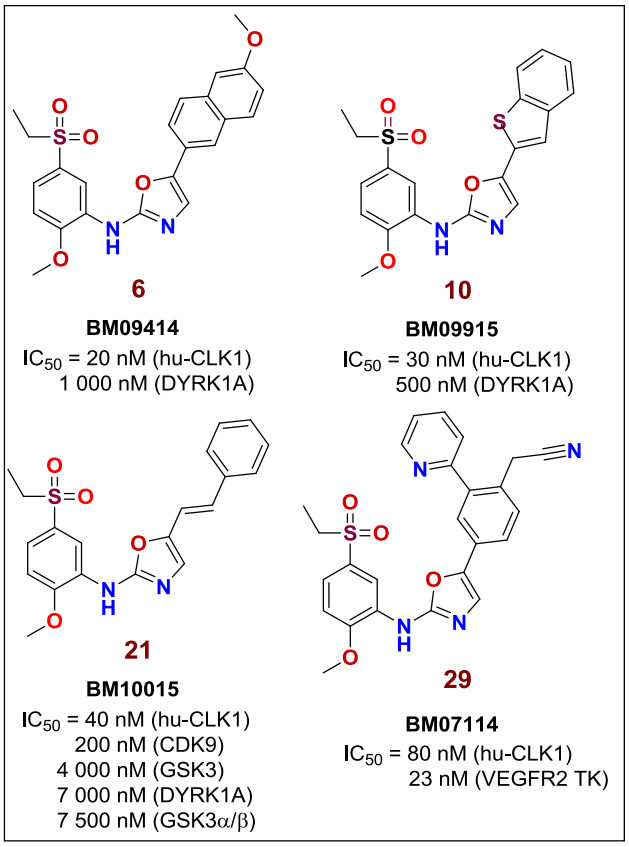


Fig. 2. Novel hu-CLK1 inhibitors **6**, **10**, **21**, **29** and their biological activities determined against some CMGC protein kinases together with VEGFR2 TK activity for **29**.

29), docking experiments [32] on seven CLK proteins from PDB DB were performed. An incomplete 2EXE structure of CLK3 was excluded (Table 1). Most of the PDB CLK proteins showed to be unfavourable for docking experiments due to their inappropriate kinase conformation. Only the kinase variant from hu-CLK3 (2WU6) significantly outperformed the other models by docking scores and predicted poses for all ligands (**6**, **10**, **21** and **29**). As can be seen from ligands superimpositions especially the poses of their joint *N*-aryloxazole-2-amine parts were almost identical (Fig. 3).

The most active inhibitor **6** (IC_{50} = 20 nM) was predicted to form three hydrogen bonds (HBs) with two amino acids Leu239 and Asn242 (both from a kinase hinge region) and one ligand specific HB between -OMe group and Lys186. Moreover, *N*-aryloxazole-2-amine ligands showed their poses and binding interactions to be similar in both hu-CLK3 (2WU6) and VEGFR-2 TK (1Y6A) (Figs. 4 and 7).

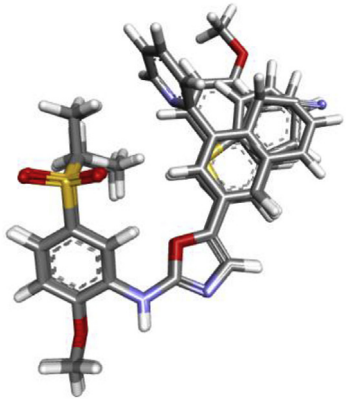


Fig. 3. The superimposed poses of inhibitors **6**, **10**, **21** and **29** from aligned complexes obtained after ligands docking in CLK3 (2WU6) protein conformer.

As mentioned above, both available CLK1 (1Z57, 2VAG) were not suitable for docking of our CLK1 inhibitors. In order to investigate problematic amino acid residues predicted CLK3 (2WU6)/(**6, 10, 21** and **29**) complexes were aligned with both CLK1 protein conformers. Then we could recognise that the most important unfavourable ligand interactions in CLK1 (2VAG) were between an amino group of *N*-aryloxazol-2-amines and too closely located carbonyl group of Leu244 from a hinge region. Due to the above repulsion the structures (**6**, **10**, **21** and **29**) could not be stabilized in good position in CLK1 (2VAG) (Fig. 5). In cases of **6** and **29** also some steric clashes with Phe172 were seen but they disappeared after a problematic ligand group rotation.

In the other CLK1 (1Z57) conformer, the most important unfavourable interactions were identified between the oxygen from -SO_2Et group of the ligand and too close positioned carboxylate oxygen from a hinge Asp250. This interaction could not be eliminated by simple changing the conformation of -SO_2Et group. The problematic Asp250 in CLK1 (1Z57) is replaced by Glu245 in CLK3 (2WU6). Even though positions of Asp250 and Glu245 are similar, the problematic carboxylate in larger Glu245 is folded out of the -SO_2Et ligand group excluding so unfavourable interactions in CLK3 compared to CLK1 (Fig. 6). Similarly as in CLK1 (2VAG) also in CLK1 (1Z57) the inhibitors **6** and **29** possess some clashes also with Phe172. (see Table 2).

To conclude the above observation, a different conformation of CLK1 kinase will be required to find the correct binding poses for *N*-aryloxazole-2-amine ligands with respect to their CLK1 activity. Therefore we aligned CLK1 proteins (1Z57, 2VAG) with **6**/CLK3 (2WU6) complex. In order to enable improved ligand docking to CLK1 the geometry minimization was carried out on both CLK1 (1Z57, 2VAG) conformers with ligand **6** from CLK3 (2WU6) complex. This approach resulted in CLK1 optimized protein models (1Z57opt, 2VAGopt) with significantly improved performance to give docked CLK1 inhibitors (**6**, **10**, **21** and **29**) positioned to keep essential hinge hydrogen bonds and docking scores that followed their IC_{50} values. These models will be used for further CLK1 ligands development. (see supplementary material, chapter: *Predicted inhibitors binding poses in CLK1 optimized protein*).

2.10. *N*-aryloxazole-2-amines in VEGFR2 and CLK

Compound **29** inhibits both kinases VEGFR2 (IC_{50} = 23 nM) and CLK1 (IC_{50} = 80 nM). The reason for a dual kinase activity of **29** can be explained by its similar binding pose and interactions in each kinase where the pair of hinge amino acid residues (Cys917/Asn921 in VEGFR2; Leu239/Asn242 in CLK3 or Leu244/Ser247 in CLK1) fixes a pharmacophoric *N*-arylooxazole-2-amine group in an ATP binding pocket. The interaction diagrams for ligands **AAZ**/VEGFR2 and **6**/CLK3 were composed. (Fig. 7).

2.11. Dual VEGFR/CLK active compounds

With aim to determine the amount of dual VEGFR/CLK inhibitors an intersection between previously found 388 CLKs inhibitors and 6299 VEGFRs TKIs in Reaxys DB was performed. This experiment resulted in 85 dual modulators with activities mostly determined between 1 and 10 μM concentrations. Many of duals were uncovered by searching target-off activities through Kinome scan for another biomacromolecule against that compounds were primarily developed. As we have seen, the activities of many of them were not very important or they were unbalanced for both

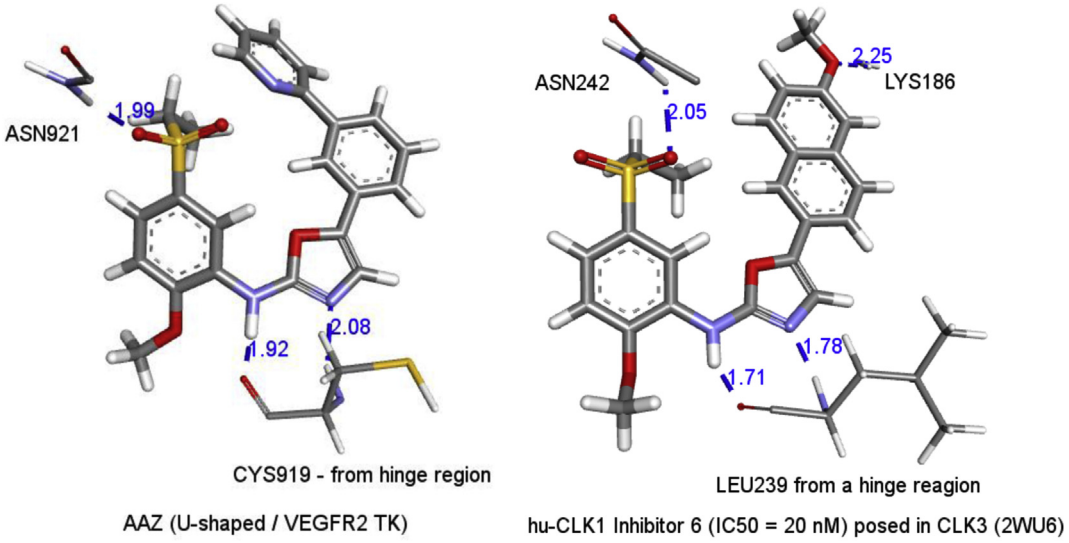


Fig. 4. Interactions of **AAZ** in VEGFR2 TK (1Y6A) and **6** in CLK3 (2WU6) to demonstrate similar binding interactions of *N*-aryloxazol-2-amine in both kinases.

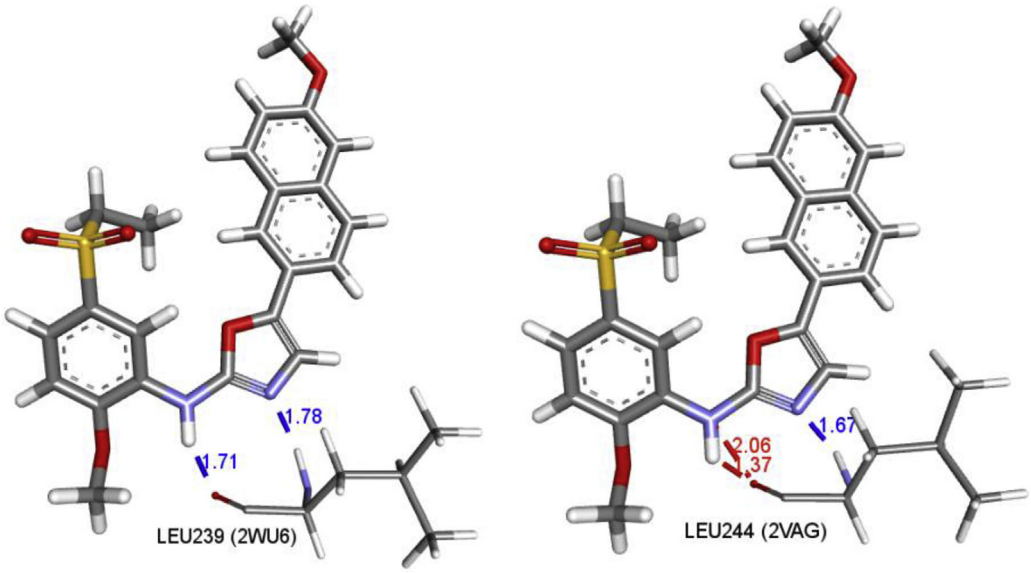


Fig. 5. In the left: the predicted pose of inhibitor **6** in CLK3 (2WU6) showing two hydrogen bonds (1.7 and 1.8 Å, in blue) with a hinge Leu239. In the right: Inhibitor **6** positioned in CLK-1 (2VAG) possessing unfavourable interactions with Leu244 (2.06 Å electrostatic repulsion, 1.4 Å steric clash, both in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

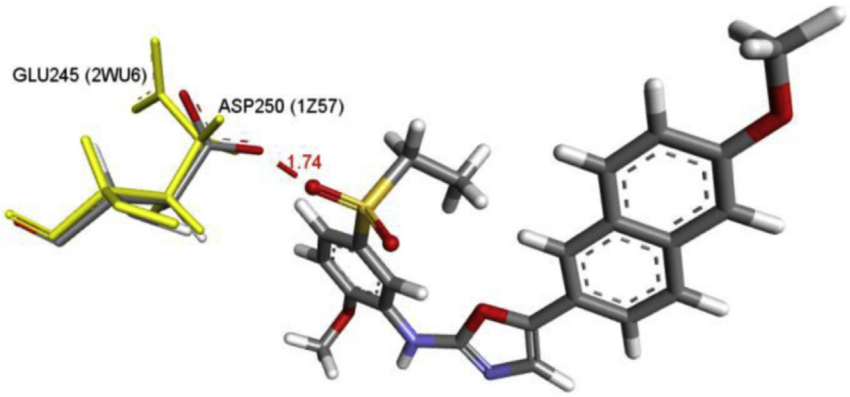


Fig. 6. A complex **6**/CLK3 (2WU6), obtained after docking, was aligned with CLK1 (1Z57) kinase. A hinge Glu245 from CLK3 (2WU6) is replaced by Asp250 (yellow) in CLK1 (1Z57) and forms unfavourable interaction with oxygen from -SO_2Et group of **6**. Observed strong electrostatic repulsion (1.7 Å, red) is hindering ligand **6** to be docked in CLK1 (1Z57) in an appropriate position. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

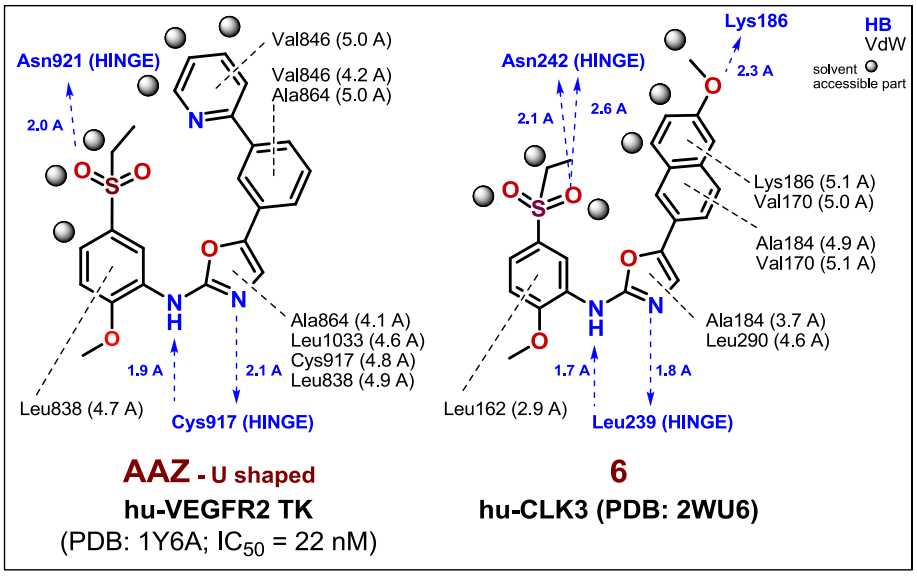


Fig. 7. The interactions of **AAZ** ligand with VEGFR2 TK and **6** in CLK3 are very similar especially for their joint *N*-aryloxazole-2-amine part.

Table 2
A summary for *N*-aryloxazole2-amine **6** interactions favourable for CLK3 (2WU6) kinase and problematic for both CLK1s (2VAG, 1Z57) proteins.

	oxazole-2-amine of **6**	-SO_2Et of **6**
CLK3 (2WU6)	hinge-Leu239 (CO, NH: 1.7, 1.8 Å)	hinge-Asn242 ($CONH_2$: 2.1 Å)
CLK1 (2VAG)	hinge-Leu244 (NH: 1.7 Å) UI: Leu244 (CO) (1.4, 2.1 Å)	hinge-Ser247 (NH: 2.5 Å)
CLK1 (1Z57)	hinge-Leu244 (CO, NH) (2 × 1.8 Å)	hinge-Ser247 (NH: 2.5 Å) UI Asp250 (COO^-: 1.7 Å)

UI: unfavourable interactions in CLK3, CLK1 isoforms possessing some replaced AAs residues (Leu239 to Leu244 and Asn242 to Ser 247, resp.).

kinases. A list of Reaxys codes (RRN) for 85 dual inhibitors are given in supplementary material (chapter: *Reaxys RRN codes of 85 compounds possessing dual VEGFR/CLK activities*). Among 85 structures there are about 7 with more balanced CLKs/VEGFRs activities (RRN: 18647647, 20385666, 20278959, 22546881, 20385602, 21237166 and 22449988.). One structure, a triazole-staurosporine like compound (RRN: 18647647, CAS: [1359754-81-6]) possess submicromolar bivalent activities to VEGFR2 and CLK2 (200/350 nM, resp.) [33]. We found also that sunitinib (RRN: 15426924, [CAS: 341031-54-7]) a multi-targeted tyrosine kinase ligand (PDGF, VEGFR2 TK) has strong ability to inhibit CLKs. K_D affinities for sunitinib are listed here: CLK1 (22 nM) CLK2 (20 nM), CLK4 (29 nM) [29]. There are only few powerful and activity balanced dual VEGFR2/CLKs inhibitors described in the literature. Such compounds could be advantageous for the development of clinical drugs treating tumours by two VEGFR2 and CLKs independent pathways. From this point of view *N*-aryloxazol-2-amine CLK1 inhibitors (**6**, **10**, **21** and **29**) previously designed as VEGFR2 TKIs seem to be a good starting point for such development.

3. Conclusions

Four novel *N*-aryloxazol-2-amine CLK1 inhibitors (**6**, **10**, **21** and **29**) were identified and their ATP binding poses in CLK kinase were predicted. Compound **29** has comparable activity for CLK1 and VEGFR2 TK. The observed dual inhibition of **29** was explained by similar binding interactions of its pharmacophoric *N*-(5-(ethylsulfonyl)-2-methoxyphenyl)oxazol-2-amine fragment that has joint affinities to CLK1 and VEGFR2 kinases *via* hinge amino acid residues HBs. We found that there are only few dual VEGFR/CLK inhibitors with comparable and strong activities on both targets. Such dual kinase compounds could be advantageous for development of clinical compounds targeting tumours by two independent and synergy pathways. Therefore presented *N*-aryloxazole-2-amine CLK1 inhibitors (**6**, **10**, **21** and **29**) can be used in the further study of the molecular mechanisms of splicing and seem to be also good seeds for development a novel class of VEGFR2/CLKs dual kinase therapeutics.

4. Experimental

The syntheses of biologically active compounds **6, 10, 21** and **29** and their intermediates are described in the Supplementary material *(chapter: Supplementary Experimental)*.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2016.11.003. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] D.L. Black, Mechanisms of alternative pre-messenger RNA splicing, Annu. Rev. Biochem. 72 (2003) 291–336, http://dx.doi.org/10.1146/annurev.biochem.72.121801.161720.
[2] A.N. Bullock, S. Das, J.E. Debreczeni, P. Rellos, O. Fedorov, F.H. Niesen, K. Guo, E. Papagrigoriou, A.L. Amos, S. Cho, B.E. Turk, G. Ghosh, S. Knapp, Kinase domain insertions define distinct roles of CLK kinases in SR protein phosphorylation, Structure 17 (2009) 352–362, http://dx.doi.org/10.1016/j.str.2008.12.023.
[3] G. Manning, D.B. Whyte, R. Martinez, T. Hunter, S. Sudarsanam, The protein kinase complement of the human genome, Science 298 (2002) 1912–1934, http://dx.doi.org/10.1126/science.1075762.
[4] Cell Signaling Technology Interactive Human Kinome Tree. http://www.cellsignal.com/contents/science-protein-kinases/protein-kinases-interactive-human-kinome/kinases-human-kinome (accessed 22.06.16).
[5] G. Sessa, V. Raz, S. Savaldi, R. Fluhr, PK12, a plant dual specificity protein kinase of the LAMMER family, is regulated by the hormone ethylene, Plant Cell 8 (1996) 2223–2234.
[6] S. Savaldi-Goldstein, G. Sessa, R. Fluhr, The ethylene inducible PK12 kinase mediates the phosphorylation of SR splicing factors, Plant J. 21 (2000) 91–96.
[7] O. Nayler, S. Stamm, A. Ullrich, Characterization and comparison of four serine- and arginine-rich (SR) protein kinases, Biochem. J. 326 (1997) 693–700.
[8] (a) K. Colwill, T. Pawson, B. Andrews, J. Prasad, J.L. Manley, J.C. Bell, P.I. Duncan, The Clk/Sty protein kinase phosphorylates SR splicing factors and regulates their intranuclear distribution, EMBO J. 15 (1996) 265–275;
(b) J. Prasad, J.L. Manley, Mol. Cell Biol. 23 (2003) 4139–4149;
(c) M. Muraki, B. Ohkawara, T. Hosoya, H. Onogi, J. Koizumi, T. Koizumi, K. Sumi, J.-I. Yomoda, M.V. Murray, H. Kimura, K. Furuichi, H. Shibuya, A.R. Krainer, M. Suzuki, M. Hagiwara, Manipulation of alternative splicing by a newly developed inhibitor of clks, J. Biol. Chem. 279 (2004) 24246–24254, http://dx.doi.org/10.1074/jbc.M314298200.
[9] P. Jain, C. Karthikeyan, N.S. Moorthy, D.K. Waiker, A.K. Jain, P. Trivedi, Human CDC2-like kinase 1 (CLK1): a novel target for Alzheimer's disease, Curr. Drug Targets 15 (2014) 539–550.
[10] J. Hanes, H. von der Kammer, J. Klaudiny, K.H. Scheit, Characterization by cDNA cloning of two new human protein kinases: evidence by sequence comparison of a new family of mammalian protein kinases, J. Mol. Biol. 244 (1994) 665–672, http://dx.doi.org/10.1006/jmbi.1994.1763.
[11] The UniProt Database. http://www.uniprot.org/ (assessed 03.04.16).
[12] A. García-Sacristán, M.J. Fernández-Nestosa, P. Hernández, J.B. Schvartzman, D.B. Krimer, Protein kinase clk/STY is differentially regulated during erythroleukemia cell differentiation: a bias toward the skipped splice variant characterizes postcommitment stages, Cell Res. 15 (2005) 495–503, http://dx.doi.org/10.1038/sj.cr.7290319.
[13] S. Araki, R. Dairiki, Y. Nakayama, A. Murai, R. Miyashita, M. Iwatani, T. Nomura, O. Nakanishi, Inhibitors of CLK protein kinases suppress cell growth and induce apoptosis by modulating pre-mRNA splicing, PLoS One 10 (2015) e0116929, http://dx.doi.org/10.1371/journal.pone.0116929 eCollection 2015.
[14] Shinsuke Araki, Ryo Dairiki, Yusuke Nakayama, Aiko Murai, Risa Miyashita, Misa Iwatani, Toshiyuki Nomura, Osamu nakanishi inhibitors of CLK protein kinases suppress cell growth and induce apoptosis by modulating pre-mRNA splicing, PLoS One 10 (2015) e0116929, http://dx.doi.org/10.1371/journal.pone.0116929.
[15] B.Z. Mian, L. Chao, F. Jian-Song, L. Wen-Wen, L. Ai-Lin, Z. Li-Shu, D. Guan-Hua, Drug discovery of host CLK1 inhibitors for Influenza treatment, Molecules 20 (2015) 19735–19747.
[16] N. Jiang, C.Y. Bénard, H. Kébir, E.A. Shoubridge, S. Hekimi, CLK2 may serve as a link between cell cycle progression, apoptosis, and telomere length regulation, J. Biol. Chem. 278 (2003) 21678–21684.
[17] PhosphoSitePlus Cell Signaling Technology. http://www.phosphosite.org/proteinAction.do?id=810&showAllSites=true (assessed 26.06.2016).
[18] T. Yoshida, J.H. Kim, K. Carver, Y. Su, S. Weremowicz, L. Mulvey, S. Yamamoto, C. Brennan, S. Mei, H. Long, J. Yao, K. Polyak, CLK2 is an oncogenic kinase and splicing regulator in breast Cancer, Cancer Res. 75 (2015) 1516–1526, http://dx.doi.org/10.1158/0008-5472.CAN-14-2443.
[19] L. Garuti, M. Roberti, G. Bottegoni, Multi-kinase inhibitors, Curr. Med. Chem. 22 (2015) 695–712, http://dx.doi.org/10.2174/0929867321666141216125528.
[20] Y. Loidreau, E. Deau, P. Marchand, M.-R. Nourrisson, C. Logé, G. Coadou, N. Loaëc, L. Meijer, T. Besson, Synthesis and molecular modelling studies of 8-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines as multitarget Ser/Thr kinases inhibitors, Eur. J. Med. Chem. 92 (2015) 124–134, http://dx.doi.org/10.1016/j.ejmech.2014.12.038.
[21] RCSB Protein Data Bank. http://www.rcsb.org (accessed 22.06.16).
[22] O. Fedorov, K. Huber, A. Eisenreich, P. Filippakopoulos, O. King, A.N. Bullock, D. Szklarczyk, L.J. Jensen, D. Fabbro, J. Trappe, U. Rauch, F. Bracher, S. Knapp, Specific clk inhibitors from a novel chemotype for regulation of alternative splicing, Chem. Biol. 18 (2011) 67–76, http://dx.doi.org/10.1016/j.chembiol.2010.11.009.
[23] A. Chaikuad, P. Savitsky, T. Krojer, J. Muniz, P. Filippakopoulos, P. Rellos, T. Keates, O. Fedorov, A. Pike, J. Eswaran, G. Berridge, C. Phillips, Y. Zhang, F. von Delft, J. Weigelt, C. Arrowsmith, A. Edwards, C. Bountra, S. Knapp, (Structural Genomics Consortium) S to be published, http://dx.doi.org/10.2210/pdb3nr9/pdb.
[24] E. Papagrigoriou, P. Rellos, S. Das, A. Bullock, L.J. Ball, A. Turnbull, O. Fedorov, C. Johansson, E. Ugochukwu, F. Sobott, F. von Delft, A. Edwards, M. Sundstrom, J. Weigelt, C. Arrowsmith, S. Knapp, Crystal structure of the phosphorylated CLK3 to be published, http://dx.doi.org/10.2210/pdb2exe/pdb.
[25] P. Filippakopoulos, O. Fedorov, O. King, A. Bullock, J.R.C. Muniz, F. von Delft, C.H. Arrowsmith, A.M. Edwards, J. Weigelt, C. Bountra, S. Knapp, Crystal Structure of human CDC-like kinase 3 isoform with a benzo-dioxol ligand (Structural Genomics Consortium) to be published, http://dx.doi.org/10.2210/pdb3raw/pdb.
[26] L. Lintnerová, M. García-Caballero, F. Gregáň, M. Melicherčík, A.R. Quesada, J. Dobiaš, J. Lác, M. Sališová, A. Boháč, A development of chimeric VEGFR2 TK inhibitor based on two ligand conformers from PDB: 1Y6A complex - Medicinal chemistry consequences of a TKs analysis, Eur. J. Med. Chem. 72 (2014) 146–159, http://dx.doi.org/10.1016/j.ejmech.2013.11.023.
[27] Reaxys Database. https://www.reaxys.com (accessed 22.06.16).
[28] The UniProt Database. http://www.uniprot.org/ (accessed 26.06.16).
[29] The Targets and their Inhibitors. http://www.guidetopharmacology.org/GRAC/searchPage.jsp (accessed 26.06.16).
[30] P. Carmeliet, VEGF as a key mediator of angiogenesis in cancer, Oncology 69 (Suppl. 3) (2005) 4–10, http://dx.doi.org/10.1159/000088478.
[31] IUPHAR/BPS Guide to Pharmacology. http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1813 (accessed 31.01.16).
[32] J.J. Irwin, B.K. Shoichet, M.M. Mysinger, N. Huang, F. Colizzi, P. Wassam, Y. Cao, Automated docking screens: a feasibility study, J. Med. Chem. 52 (2009) 5712–5720, http://dx.doi.org/10.1021/jm9006966. Software Dock Blaster, http://blaster.docking.org/ (assessed 26.06.16).
[33] G. Gu, H. Wang, P. Liu, C. Fu, Z. Li, X. Cao, Y. Li, Q. Fang, F. Xu, J. Shen, P.G. Wang, Discovery and structural insight of a highly selective protein kinase inhibitor hit through click chemistry, Chem. Commun. 48 (2012) 2788–2790, http://dx.doi.org/10.1039/c1cc15851a.