INTRODUCTION

Akt kinase, which is also known as Protein Kinase B (PKB), is one of the key members of extended serine/threonine protein kinase family. This kinase plays a crucial role in multiple cellular processes such as glucose metabolism, cell proliferation, apoptosis, transcription and cell migration. However, it should be noted, that targeting the kinase activity has revealed itself to be a challenge due to non-selectivity of the compounds towards other kinases. To date, three main Akt isoforms (Akt1, Akt2, and Akt3) have been identified in human cells. The first member is deeply involved in cellular survival pathways by inhibiting apoptotic processes. It is also able to induce protein synthesis pathways, and is therefore a key signaling protein in the cellular pathways that lead to skeletal muscle hypertrophy, and general tissue growth. Since it can block apoptosis, and thereby promote cell survival, Akt1 has been implicated as a major factor in many types of cancer. Akt (now also called Akt1) was originally identified as the oncogene in the transforming retrovirus AKT8. The second isoform, Akt2, is an important signaling molecule in the Insulin signaling pathway. It is required to induce glucose transport. The role of Akt3 is less clear, though it appears to be predominantly expressed in brain. Akt is associated with tumor cell survival, proliferation, and invasiveness. The activation of Akt is also one of the most frequent alterations observed in human cancer and tumor cells. Tumor cells that have constantly active Akt may depend on Akt for survival. Therefore, understanding Akt and its pathways is important for the creation of better therapies to treat a range of diseases, especially cancer. In the past decade, the upstream signals that lead to Akt activation, the downstream substrates that exert the effects of Akt, and the secondary binding proteins that regulate Akt activation have been well documented. Among them, phosphatidylinositol 3-kinase (PI3K) is the most studied enzyme and several PI3K inhibitors have reached clinical trials. For example, the constitutive activation of the PI3K/Akt pathway has been confirmed as an essential step toward cell transformation. Inhibition of Akt kinase restores DNA damage processing and Chk1 activation after
irradiation in late G2 \[^1\]. This data demonstrates a previously unrecognized role for Akt in cell cycle regulation of DNA repair and checkpoint activation. Because Akt/PKB is frequently activated in many tumor types, these findings have important implications for the evolution and therapy of such cancers. It has also been described that PKB plays a critical role in regulation of migration of various cell types \[^2\].

1. Structural and functional bases of Akt kinase

Akt was originally identified as the human homologue of the viral oncogene v-akt from the transforming retrovirus AKT8, which was isolated from an AKR mouse T-cell lymphoma \[^3\]. Akt is now classified as a family of kinases that bears significant homology to both protein kinase A (PKA) and protein kinase C (PKC). In mammalian cells, Akt has three closely related and highly conserved (>80% sequence identity) cellular homologues, designated as Akt1/PKB\(\alpha\), Akt2/PKB\(\beta\), and Akt3/PKB\(\gamma\) (Figure 1). The three isoforms of human (mouse) Akt are located at chromosomes 14q32 (12F1-2), 19q13 (7B1), and 1q44 (1H4-6), respectively \[^4\]. Each Akt family member contains an N-terminal pleckstrin homology (PH) domain, a central kinase domain, and a carboxyl-terminal regulatory domain that contains the hydrophobic motif (HM). The HM is a characteristic of AGC kinases (for PKA, protein kinase G [PKG], and PKC), which in mammalian cells include Akt, p70 ribosomal S6 kinase (S6K1), and serum and glucocorticoid-inducible kinase (SGK) \[^5\]. Analysis of mice lacking either individual Akt isoforms or various combinations of Akt isoforms has indicated that the Akt1 isoform has a dominant role in embryonic development, fetal growth, and fetal survival, whereas Akt2 and Akt3 have non-redundant functions in glucose homoeostasis and postnatal brain development, respectively \[^6\].
Aberrant activation of the Akt pathway has been widely implicated in many cancers \cite{7}. Elevated Akt activation in human cancers can result from enhanced activation phosphorylation of Akt on the Ser473 site, owing to enhanced PI3K activation resulting from: (1) the mutation or amplification/over-expression of growth factor receptors (such as Her-2/neu and EGFR), somatic mutations of Ras oncogenes, somatic mutations as well as amplification/overexpression of PI3K (either PI3CA or the p85 subunit); (2) loss of function mutations (somatic or germ line) and decreased expression (loss of heterozygosity or methylation) of a 3'-phosphatase with tensin homology or mutated in multiple advanced cancers (PTEN/MMAC1), which converts the lipid second messenger phosphatidylinositol-3,4,5- triphosphate (PIP3) to phosphatidylinositol- 4,5-biphosphate (PIP2) and thus shuts off PI3K signaling \cite{8}.

Amplification, over-expression, and somatic mutation (at a very low frequency, ~2%) of Akt itself also contribute to the elevated expression of Akt in human cancers \cite{9}. Additionally, altered expression of PHLPP, a Ser473-specific protein phosphatase, may also affect Akt activity as reduced PHLPP in certain cancer cell lines correlates with Akt activity \cite{10}. Gene amplifications and mutations of Akt and other molecules in the PI3K/Akt pathway were discussed in a recent review by Brugge et al \cite{11}.

Akt is activated through receptor tyrosine kinase pathways, such as those of platelet-derived growth factor receptor (PDGF-R), insulin, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and insulin-like growth factor I (IGF-I) (Figure 2) \cite{12}. In the absence of growth factor

**Figure 1.** Akt domains and comparison of Akt isoforms (% of homology). Chromosome location of each Akt isoform in human as well as reported phosphorylation sites in Akt1 are also depicted.
stimulation in quiescent cells, all three isoforms of the Akt kinase are catalytically inactive. Growth factor stimulation activates Akt through a PI3K-dependent process \[^{[13]}\].

**Figure 2.** Cellular signaling around Akt and Akt substrates regulated major cellular processes.

It should be especially noted, that full activation of Akt is a multi-step process, and the final step is to phosphorylate Akt on two sites, Thr308 (for human Akt1) and Ser473 (for Akt1), via PDK1 and PDK2, respectively \[^{[14]}\]. Point mutants at these two sites with Alanine (T308A and S473A for Akt1) show little activity, even after stimulation with insulin or IGF-I, while the phosphorylation-mimicking mutant (T308D/S473D) shows constitutive kinase activation, indicating that Thr308 and Ser473 are necessary and sufficient for the full activation of Akt. Interestingly, in serum-starved cells, Akt is constitutively phosphorylated at Ser124 and Thr450, which are independent of PI3K, and neither serum starvation nor treatment of cells with the PI3K inhibitor Wortmannin interferes with phosphorylation at Ser124 and Thr450. In addition, inactive mutation of Ser124 and Thr450 into Ala (Ser124A and T450A) only marginally inhibits the activation of Akt by growth factors. Therefore, it
has been proposed that phosphorylation of Akt on these sites is the first step for full activation of Akt
\[15\].

The PH domain containing protein PDK1 is the only known mammalian isoform of the Thr308- Akt kinase \[16\]. The C-terminal PH domain of PDK1 binds phospholipids, keeping it constitutively localized at the plasma membrane. Upon growth factor stimulation and PIP3 production by PI3Ks, Akt interacts with these phospholipids, causing Akt to translocate to the inner membrane, where PDK1 is located. The interaction of the Akt PH domain with 3'-phosphoinositides is thought to impose conformational changes in Akt, exposing its two main phosphorylation sites at the kinase domain (T308 for Akt1) and the HM of the C-terminal (S473 for Akt1). The direct homodimerization of the two PH domains between Akt and PDK1 might also mediate protein proximity and subsequently phosphorylate Thr-308 in Akt, which stabilizes the activation loop in an active conformation and renders Ser473 phosphorylation by the rapamycin-insensitive mTORC2, resulting in full activation of Akt kinase. In certain contexts, kinases including PDK1, ataxia telangectasia mutated (ATM), DNA-dependent protein kinase (DNA-PK), integrin-linked kinase (ILK), PKCζ/ι, PKC-related kinase-2 (PRK2), mitogen-activated protein kinase-associated protein kinase-2 (MAPKAP-K2), mTOR, and Akt itself were implicated in the phosphorylation of Akt at Ser473 before mTORC2 was identified as a Ser473 kinase \[17\]. In a later phase, activated Akt is translocated to the nucleus, where several of its substrates are located (see Figure 2).

Mechanistically, activation of Akt will profoundly affect cellular processes by phosphorylating numerous Akt substrates (listed and reviewed in \[18\]). Consensus motif analysis indicates that there are potentially thousands of cellular substrates for Akt; about 50 of them have been characterized so far \[19\]. Through phosphorylation, Akt may either positively or negatively affect the functions of these substrates, alter their subcellular localization, or modify their protein stabilities. A major outcome of Akt activation is toward cell survival and cell growth; many Akt substrates play important roles. Among those substrates are: (1) regulators of cell survival or cell death, such as Bad, caspase-9, ASK1, apoptosis signal-regulating kinase 1 (ASK1), forkhead box O transcription factors (FoxOs), Bim1, FasL, inhibitor of nuclear factor-κB kinase (IKK-NFkB), and p53; (2) regulators of cells cycle progression; such as p21, p27, cyclin D1, and glycogen synthase kinase-3β (GSK-3β); (3) regulators of protein synthesis or cell growth, such as tuberous sclerosis complexes 1 and 2 (TSC1/2), mTOR, elongation-initiation factor 4E binding protein-1 (4E-BP1), and S6K; (4) regulators of angiogenesis, such as mTOR and hypoxia-inducible factor-1 (HIF-1); and (5) regulators of cell
metabolism, such as glucose transporter 1 (Glut1), GSK3, and a Ras homologue enriched in brain (RheB) (summarized in Figure 2). Akt-mediated regulation of hormone receptors such as estrogen receptor (ER) and androgen receptor (AR) are not listed in Figure 2. Positive and negative regulators of Akt-interacting proteins have also been identified. These include oncogenes identified in human T-cell leukemia (Tcl1 and Tcl1 family members); JNK interacting protein 1 (JIP1); growth factor receptorbinding protein-10 (Grb10); Ras GTPase-activation protein (RasGAP); Hsp90/Cdc37 molecular chaperone or co-chaperone complex; a tribbles homologue 3 (TRB3); adaptor protein containing PH domain, PTB domain, and Leucine- zipper motif (APPL); C-terminal modulator protein (CTMP); Akt phosphorylation enhancer or hook-related protein-1 (APE); SH3 domaincontaining protein, Src and Arg-binding protein 2γ (ArgBP2γ); breast tumor kinase (Btk); prohibin 2 (PHB2); and pleckstrin homology-like domain, family A, member 3 (PHLDA3) \[20\] (see also Figure 2).

2. Small-molecule inhibitors of the Akt-signaling network

To date, more than 700 Akt kinase inhibitors have been discovered. These include both the protein-based agents as well as a wide range of small-molecule compounds (Figure 3). At least 14 molecules are currently being evaluated in advanced clinical trials or already launched in pharmaceutical market.
Figure 3. Representative examples of small-molecule Akt inhibitors currently evaluated in advanced clinical trials.

For instance, GSK-690693 is a small-molecule ATP-competitive Akt kinase inhibitor (IC\textsubscript{50} = 2, 13 and 9 nM for Akt-1, -2 and -3, respectively) that also inhibits the phosphorylation of several proteins downstream of Akt, including GSK3-beta, PRAS40 and FOXO1/FOXO3a \cite{21}. It was also found to suppress the proliferation of multiple human tumor cell lines, including breast carcinoma BT-474 and SKBR-3, and prostate cancer LNCaP (IC\textsubscript{50} = 0.021-0.298 µM). This compound (0.47 or 0.94 µM for 16 h) showed an additive effect of up to approx. 50% cell death in human head and neck cancer HN5 cells when administered following Lapatinib treatment (3.75 or 7.5 µM for 24 h). A single intraperitoneal dose of the compound in mice bearing human BT-474 tumors dose- and time-dependently inhibited GSK3-beta phosphorylation and, at 30mg/kg i.p., q.i.d. for 21 days, it exhibited antitumor activity in mice bearing BT474, HCC1954 and MDA-MB-453 breast cancer, SK-OV-3 ovarian cancer, and LNCaP prostate
cancer xenografts (64%, 75%, 33%, 58% and 61% inhibition, respectively). GSK-690693 is currently being described as promising anticancer agent.

Perifosine [octadecyl-(1,1-dimethyl-piperidinio-4-yl)-phosphate] is a synthetic novel alkylphospholipid with promising anticancer activity. This compound targets cell membranes and inhibits Akt activation with high potency. Thus, it was shown that baseline phosphorylation of Akt in multiple myeloma (MM) cells was completely inhibited by Perifosine in a time- and dose-dependent fashion, without inhibiting phosphoinositide-dependent protein kinase 1 phosphorylation \([^{22}]\). Perifosine induces significant cytotoxicity in both MM cell lines and patient MM cells resistant to conventional therapeutic agents, whereas it does not induce cytotoxicity in peripheral blood mononuclear cells. Neither exogenous interleukin-6 (IL-6) nor insulin-like growth factor 1 (IGF-1) overcomes Perifosine-induced cytotoxicity. Importantly, Perifosine induces apoptosis even of MM cells adherent to bone marrow stromal cells. Perifosine triggers c-Jun N-terminal kinase (JNK) activation, followed by caspase-8/9 and poly (ADP)ribose polymerase cleavage. Inhibition of JNK abrogates perifosine-induced cytotoxicity, suggesting that JNK plays an essential role in perifosine-induced apoptosis. Interestingly, phosphorylation of extracellular signal-related kinase (ERK) is increased by perifosine; conversely, MEK inhibitor synergistically enhances Perifosine-induced cytotoxicity in MM cells. Furthermore, Perifosine augments Dexamethasone, Doxorubicin, Melphalan, and Bortezomib-induced MM cell cytotoxicity. Finally, it demonstrates significant antitumor activity in a human plasmacytoma mouse model, associated with down-regulation of Akt phosphorylation in cancer cells.

It has also been found that Perifosine, at 30 \(\mu\)M concentration, decrease AKT phosphorylation and increase apoptosis in all four NB cell lines \(\textit{in vitro} \) \([^{23}]\). Thus, Perifosine-treated mice bearing xenograft NB tumors had longer survival than untreated mice (untreated vs treated, median survival: AS, 13 days, 95% confidence interval [CI] = 11 to 16 days vs not reached, P = .003; NGP, 22 days, 95% CI = 20 to 26 days vs not reached, P = .013; BE2, 24 days, 95% CI = 21 to 27 days vs not reached, P < .001; and KCNR, 18 days, 95% CI = 18 to 21 days vs not reached, P < .001). Perifosine treatment induced regression in AS tumors, growth inhibition in BE2 tumors, and slower growth in NGP and KCNR tumors. Inhibition of AKT phosphorylation and induction of caspase-dependent apoptosis were noted in tumors of Perifosinetreated mice in all four \(\textit{in vivo} \) NB tumor models. Therefore, Li et al \([^{24}]\) have suggested that Perifosine, inhibiting the activation of AKT, represents an
effective cytotoxic agent in NB cells \textit{in vitro} and \textit{in vivo}. These studies support the future clinical evaluation of Perifosine for the treatment of NB tumors as well as MM.

\textbf{Concept and Applications}

Akt-targeted library design at CDL involves:

- \textit{A combined profiling methodology that provides a consensus score and decision based on various advanced computational tools:}

1. Unique morphing and funneling procedures in designing novel potential Akt-ligands with high IP value. We apply CDL’s proprietary Chemosoft\textsuperscript{TM} software and commercially available solutions from Accelrys, MOE, Daylight and other platforms.
2. Neural Network tools for target-library profiling, in particular Self-organizing Kohonen Maps, performed in SmartMining Software.
3. 3D-molecular docking approach to focused library design.
4. Computational-based `\textit{in silico}` ADME/Tox assessment for novel compounds includes prediction of human CYP P450-mediated metabolism and toxicity as well as many pharmacokinetic parameters, such as Brain-Blood Barrier (BBB) permeability, Human Intestinal Absorption (HIA), Plasma Protein binding (PPB), Plasma half-life time (T\textsubscript{1/2}), Volume of distribution in human plasma (V\textsubscript{d}), etc.

The fundamentals for these applications are described in a series of our recent articles on the design of exploratory small molecule chemistry for bioscreening [for related data visit ChemDiv. Inc. online source: \texttt{www.chemdiv.com}].

- \textit{Synthesis, biological evaluation and SAR study for the selected structures:}

1. High-throughput synthesis with multiple parallel library validation. Synthetic protocols, building blocks and chemical strategies are available.
2. Library activity validation via bioscreening; SAR is implemented in the next library generation.
We practice a multi-step approach for building Akt-focused library:

Virtual screening

(1) Small-molecule Akt inhibitors (more than 700 cmpds) are compiled into the unique knowledge base (the reference ligand space). Among these compounds, several agents are currently being evaluated in advanced clinical trials or already launched in pharmaceutical market. The knowledge base has further been analyzed for the pharmacophore hypotheses (not shown here) as well as specific bioisosteric rules used in the subsequent morphing procedures. The reference ligand space is used as a source for searching novel chemotypes via a variety of structure morphing procedures: bioisosteric, pharmacophore, 2D and 3D similarity, the “bit-string” code, etc. A series of consecutive funneling procedures are applied to enhance the target-specific relevance of novel compounds. During this step, we address the compound’s leadlikeness (enforcing partial Rule of 3 compliance), the availability of unique R-groups, the pre-synthetic analysis of privileged templates, the IP potential, the feasibility of high-throughput chemistry. The resulting database includes more that 150K “high-score” compounds from ChemDiv store.

(2) At the second stage of our Akt-targeted library design, we have collected a 22K-compound database of known drugs and compounds entered into clinical trials; their structures and assignments were mainly obtained from Prous Integrity Database \([25]\). Each compound in this database has been characterized by a defined profile of target-specific activity, focused against 1 of more than 100 different protein targets, including Akt kinases. The database was then filtered based on MW (not more than 800). Molecular features encoding the relevant physicochemical and topological properties of compounds were calculated from 2D molecular representations and selected by PCA. These molecular descriptors encode the most significant molecular features, such as molecular size, lipophilicity, H-binding capacity, flexibility, and molecular topology (see below). Taken in combination, they define both pharmacokinetic and pharmacodynamic behavior of compounds and are effective for property-based classification of targetspecific groups of active agents. However, it should be noted that for each particular target-specific activity group, another, more optimal set of descriptors can be found, which provides better classification ability.

Self-organizing Kohonen mapping
Self-organizing Kohonen maps [26] belong to a class of neural networks known as competitive learning or self-organizing networks which in turn are based on unsupervised learning rule. They were originally developed to model the ability of the brain to store complex information as a reduced set of salient facts without loss of information about their interrelationships. High-dimensional data is mapped onto a two-dimensional rectangular or hexagonal lattice of neurons in such a way as to preserve the topology of the original space. This methodology has successfully been used in various medicinal chemistry applications [for review, see: 27].

Various molecular descriptors were calculated for Kohonen modeling using SmartMining software. As a result of specific selection procedure, at the output, an experimental set consisted of 7 molecular descriptors including Zagreb index, E-state indexes for the following structural fragments: >C-, -CH2-, CH3, the number of H-bond donors, HB2 (a structural descriptor which encodes the strength of H-bond acceptors following an empirical rule) and LogP was determined. This set was then used for Kohonen map generation. A Kohonen SOM of 22K pharmaceutical leads and drugs, including a set of Akt inhibitors, generated as a result of the unsupervised learning procedure is depicted in Fig. 4. It shows that the studied compounds occupy a wide area on the map, which can be characterized as the area of drug-likeness. Distribution of various target-specific groups of ligands in the Kohonen map demonstrates that most of these groups have distinct locations in specific regions of the map (Fig. 5a-e). A possible explanation of these differences is in that, as a rule, receptors of one type share a structurally conserved ligand-binding site. The structure of this site determines molecular properties that a receptor-selective ligand should possess to properly bind the site. These properties include specific spatial, lipophilic, and H-binding parameters, as well as other features influencing the pharmacodynamic characteristics. Therefore, every group of active ligand molecules can be characterized by a unique combination of physicochemical parameters differentiating it from other target-specific groups of ligands. Another explanation of the observed phenomenon can be related to different pharmacokinetic requirements to drugs acting on different biotargets. The described algorithm represents an effective procedure for selection of target-focused compound subsets compatible with high throughput in silico evaluation of large virtual chemical space.
Whenever a large enough set of active ligands is available for a particular receptor, the quantitative discrimination function can be generated allowing selection of a series of compounds to be assayed against the target. It is important to note that focusing on physicochemical rather than structural features makes this approach complementary to any available ligand structure similarity technique. The predictive ability of the model constructed towards Akt-active agents was approx. 80%. Therefore, this model can be satisfactorily used for targeted-library design and rational compound selection. We have used this model for rational compound selection. Thus, the generated library (more than 150K structures form ChemDiv store) has been tested and structures have been classified in different groups in accordance to the predicted activity, in particular against Akt kinase. As a result, the model provides 35K “high score” compounds.
**Fig. 5.** Distribution of nine large target-specific groups of pharmaceutical agents within the Kohonen map:
(a) GPCR agonists/antagonists (5432 compounds); (b) matrix metalloproteinase inhibitors (120 compounds); (c) tyrosine kinase inhibitors (175 compounds); (d) caspase inhibitors (50 compounds); (e) NMDA receptor agonists/antagonists (150 compounds); (f) potassium channel blockers/activators (302 compounds); (g) reverse transcriptase inhibitors (160 compounds); (h) serine protease inhibitors (531 compounds); (i) p38 MAPK inhibitors (100 compounds); (j) histamine receptor antagonists (168 compounds); (k) lipoxygenase inhibitors (114 compounds); (l) Akt kinase inhibitors (677 compounds)

(3) 3D-molecular docking study has also been provided for several chemotypes included in the 35K Aktbiased group. Currently, a sufficient amount of X-ray data is available from some sources, including PDB protein data bank. Therefore, the model has been developed based on the crystallographic data reported for several Akt isoforms. Key aspects of this model are presented below. For modeling we have used commercial MolSoft software package, ver. 3.6-li.

Recently, Bencsik and colleagues have reported the discovery and synthesis of a novel series of dihydrothieno- (see the picture below) and dihydrofuropyrimidines as potent pan Akt inhibitors [28]. Utilizing an available SAR and analysis of the amino acid sequences in the binding site authors have designed small-molecule inhibitors displaying increased PKA and general kinase selectivity with improved tolerability compared to the progenitor pyrrolopyrimidine. A representative dihydrothieno compound was advanced into a PC3-NCI prostate mouse tumor model in which it demonstrated a dosedependent reduction in tumor growth and stasis when dosed orally daily at 200 mg/kg.
Kallan et al. [29] have reported a novel series of spirochromane pan-Akt inhibitors (see the example below). SAR optimization furnished compounds with improved enzyme potencies and excellent selectivity over the related AGC kinase PKA. Attempted replacement of the phenol hinge binder provided compounds with excellent Akt enzyme and cell activities but greatly diminished selectivity over PKA.

Xu et al. [30] described the design and synthesis of novel bicyclic spiro sulfonamides as potent Akt inhibitors (see the example below). Through structure-based rational design, authors have successfully improved PKA selectivity of previously disclosed spiro-chromanes. Representative compounds showed favorable Akt potency while exhibiting up to 1000-fold selectivity against PKA.

Based on the HTS approach Lippa and colleagues [31] have revealed novel pyrrolopyrimidine inhibitors of the Akt kinase. X-ray co-crystal structures of two lead series results in the understanding of key binding interactions, the design of new lead series, and enhanced potency. For instance, the X-Ray representations of two hits: N-[2-(5-methyl-4H-1,2,4-triazol-3-yl)phenyl]-7H-pyrrolo[2,3-d]pyrimidin-4-amine (IC$_{50} = 151$ nM) and 5-(5-chloro-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-4,5,6,7-tetrahydro-1Himidazo[4,5-c]pyridine (IC$_{50} = 42$ nM) are shown below in details. It should be noted,
that among active compounds, spiro-indoline derivative is found to have an Akt1 kinase IC(50) of 2.4±/-0.6 nM, Akt cell potency of 50±/-19 nM, and provides 68% inhibition of tumor growth in a mouse xenograft model (50 mg/kg, qd, po).

We have used the X-Ray data and SARs listed above for the development of our 3D-molecular docking model with sufficient predictive power for reliable classification of compounds toward Akt kinase. As an example, fig. 6 shows a “high-score” compound (ID: 7639-0144) in the ATP active binding site of the studied target. As shown in the figure, the compound favourably maintains key interactions within the site which have been revealed by X-Ray for the reference structure.
Figure 6. (a) N-1,3-benzodioxol-5-yl-9H-purin-6-amine (ID: 7639-0144, yellow) in the active ATP-binding site of Akt kinase (docking results); (b) reference compound [32] (orange, crystallographic data), and (c) docking results (cyan)

At the output, we have selected more than 16K “high score” compounds for further evaluation.

(4) We also consider the ADME/Tox issues (such as HIA and BBB-permeability, plasma protein binding, cytochrome P450 substrate and inhibition potential) and key physico-chemical properties (such as DMSO and water solubility, stability and ionization potential, etc.). The funneling procedures can be carried out by Akt-specific neural networks, fragment and property-based models. Diversity of the final selection is optimized using proprietary algorithms.

Synthesis and biological evaluation

(4) Novel Akt-targeted library is synthesized according to the above criteria.
(5) Compounds targeted against Akt are planned to be validated by bioscreening in collaboration with academic institutions.

Our strategy has proven to be efficient for generation of kinase-targeted libraries. The higher hit rate over diverse libraries, along with identification of novel active chemotypes with optimized diversity and ADME properties, has been shown in multiple studies. Using the computational approaches listed above we have compiled Akt-focused library consisted of more than 16K small-molecule compounds targeted specifically against different Akt isoforms. Representative examples of the biased compounds is shown in Fig. 7.
Here, we provide rapid and efficient tools for follow-up chemistry on discovered hits, including single isomer chemistry, stereoselective synthesis and racemic mixture separation. Targeted library is updated quarterly based on a “cache” principle. Older scaffolds/compounds are replaced by templates resulting from our in-house development (unique chemistry, literature data, computational approaches) while the overall size of the library remains the same (ca. 15-17K compounds). As a result, the library is renewed each year, proprietary compounds comprising 55-75% of the entire set. Clients are invited to participate in the template selection process prior to launch of our synthetic effort.

References


