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Ligand-based modeling of Akt3 lead to potent dual Akt1/Akt3 inhibitor

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ABSTRACT

Akt1 and Akt3 are important serine/threonine-specific protein kinases involved in G2 phase required by cancer cells to maintain cell cycle and to prevent cell death. Accordingly, inhibitors of these kinases should have potent anti-cancer properties. This prompted us to use pharmacophore/QSAR modeling to identify optimal binding models and physicochemical descriptors that explain bioactivity variation within a set of 74 diverse Akt3 inhibitors. Two successful orthogonal pharmacophores were identified and further validated using receiver operating characteristic (ROC) curve analyses. The pharmacophoric models and associated QSAR equation were applied to screen the national cancer institute (NCI) list of compounds for new Akt3 inhibitors. Six hits showed significant experimental anti-Akt3 IC_{50} values, out of which one compound exhibited dual low micromolar anti-Akt1 and anti-Akt3 inhibitory profiles.

1. Introduction

Cancer is currently considered a major health problem [1]. The number of cancer patients is expected to reach 11.5 million worldwide by 2030 [2]. Protein kinases are a family of diverse enzymes involved in all aspects of cell division, differentiation and growth [3]. In fact, faulty protein kinases have been associated with variety of human cancers [4].

Akt, also known as protein kinase B (PKB), is a serine/threonine kinase that exists in three homologous human isoforms (Akt1, Akt2, and Akt3). Akt isozymes play significant roles in apoptotic pathways and signal transduction, and therefore, influence cellular survival and proliferation [5,6]. Akt over-expression exerts significant anti-apoptotic effects in many cell types [3–6]. Many cancers (e.g., breast, ovarian, prostate carcinomas and glioblastomas) were found to involve mutation or loss of the Akt negative regulator PTEN. 3–5 Accordingly, inhibition of Akt signaling is a promising approach towards managing many cancers [7–9].

Akt3 is the most frequently amplified Akt isoform in melanomas, ovarian, endometrial, and breast cancers [10,11,14–16]. Moreover, Akt3 plays critical role in tumor progression [12] and in rendering tumor cells resistant to apoptosis [13]. Additionally, Akt3 appears to be key factor in tumor resistance against anticancer chemotherapy [10,17].

Interest in finding anti-Akt inhibitors led to design of several potent inhibitors [4,5,7–9]. Fig. 1 shows the chemical structures of most prominent Akt inhibitors under clinical evaluation. The continuous interest in new Akt3 inhibitors [17,18] combined with lack of crystallographic structures of Akt3 co-crystallized with ligands and absence of ligand-based modeling efforts for new Akt3 inhibitors [19–21] prompted us to use QSAR-guided pharmacophore exploration [22–37] to identify putative ligand binding modes within Akt3 binding site. The resulting pharmacophores were used to mine for new Akt3 inhibitors. Six active Akt3 inhibitors were identified, of which one hit showed dual micro molar inhibition against Akt1/Akt3.

2. Material and methods

2.1. Data set and conformational analysis

The structures of 74 Akt3 inhibitors were collected from published literature [39–42]. The inhibitors were carefully gathered in such a way to make sure that they were bioassayed under similar conditions to allow proper QSAR correlation. The in vitro bioactivities of the collected inhibitors were expressed as the concentration of the test compound that results in 50% drop in the

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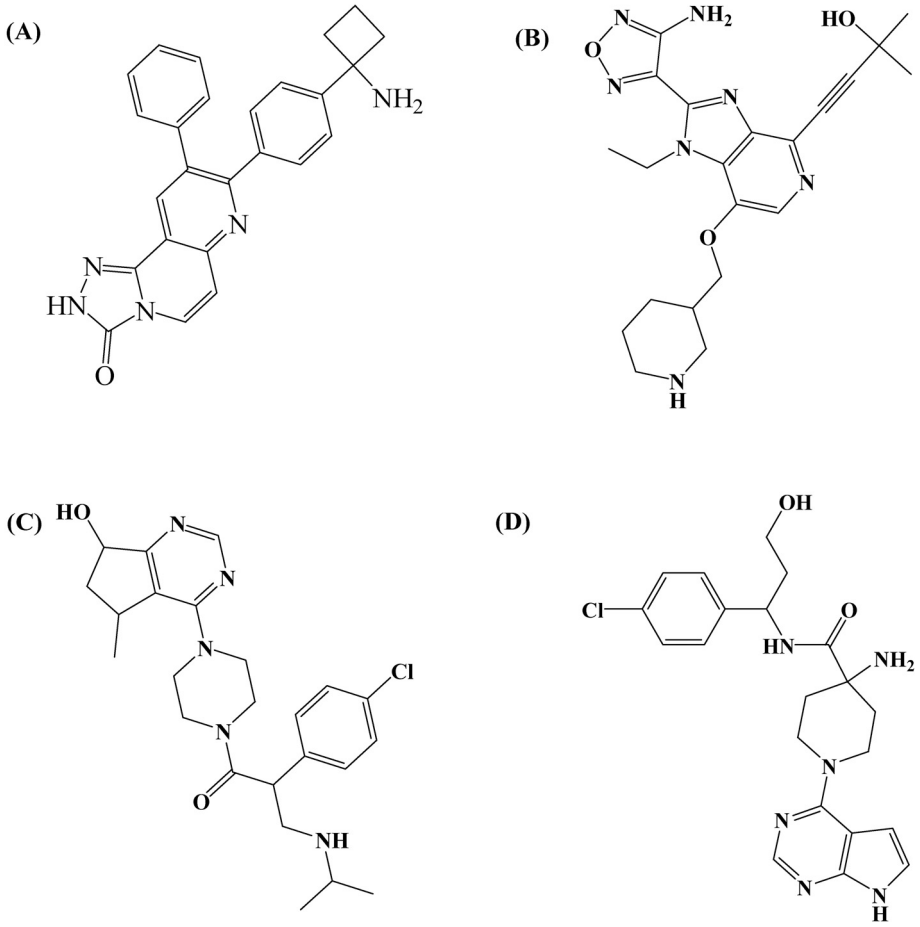

Fig. 1. Akt3 inhibitors under clinical evaluation: **(A)** MK2206 (IC_{50} = 65.0 nM), **(B)** GSK690693 (IC_{50} = 9.0 nM), **(C)** Ipatasertib (GDC-0068, IC_{50} = 8.0 nM), and **(D)** AZD5363 (IC_{50} = 8.0 nM).

activity of Akt enzyme, i.e., IC_{50}. Fig. 2 and Table A (under the Supplementary Materials) show the structures and IC_{50} values of the collected inhibitors. Six structurally diverse training subsets were selected from the collected compounds (table B under Supplementary Materials) and used to perform 48 automatic modeling runs to explore the pharmacophoric space of Akt3 inhibitors. Table C and sections **SM-1** and **SM-2** under Supplementary Materials describe the details of pharmacophore modeling using DiscoveryStudio software (Version 4.5), Biovea Inc. (www.biovea.com), USA [22–38,43–48]. Pharmacophore modeling requires conformational analysis of training compounds, exploration of their pharmacophoric space, assessment and clustering of the resulting pharmacophoric hypotheses (for full details see **SM-1**, **SM-2**, **SM-3**, and **SM-4** under Supplementary Materials) [22–38,43,44].

2.2. QSAR modeling

QSAR modeling commenced by dividing the collected compounds into two subsets: Training (60 compounds) and testing sets (14 compounds, *ca.* 20% of the dataset). The test molecules were selected as follows: all 74 collected Akt3 inhibitors (**1-74**, Fig. 2 and table A under Supplementary Materials) were ranked according to their IC_{50} values, and then every fifth compound was selected for the test set starting from the high-potency end. The selected test inhibitors are marked with asterisks in Table A under Supplementary Materials. Section **SM-3** under Supplementary Materials describes extensively the experimental details of QSAR modeling procedure [22–37,50,51].

2.3. Addition of exclusion volumes

HIPHOP-REFINE module of Discovery Studio was employed to add exclusion volumes to QSAR-selected pharmacophores to account for steric constrains of the binding pocket and improve ROC properties of the models [22–37]. Section **SM-4** under Supplementary Materials describes in details the experimental procedure and theoretical principles of HIPHOP-REFINE.

2.4. Receiver operating characteristic (ROC) curve analysis

QSAR-selected pharmacophores were further validated using receiver operator characteristic (ROC) curve analysis to test their abilities to discern active compounds from inactive decoys [52,53,58]. The reader is referred to supplementary materials section **SM-5** for additional details about ROC validation of QSAR-based pharmacophores. ROC analysis assesses the ability of a particular pharmacophore model to correctly distinguish active from inactive compounds in a testing list. It provides several success criteria for evaluation: Area under the curve (AUC) of the corresponding ROC

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