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Structural findings of phenylindoles as cytotoxic antimitotic agents in human breast cancer cell lines through multiple validated QSAR studies

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ABSTRACT

Antimitotic agents are potential compounds for the treatment of breast cancer. Cytotoxicity is one of the parameters required for anticancer activity. A validated comparative molecular modeling study was performed on a set of phenylindole derivatives through R-group QSAR (RQSAR), regression-based and linear discriminant analysis (LDA)-based 2D QSAR studies and kernel-based partial least square (KPLS) analyses as well as CoMSIA 3D-QSAR study. Antiproliferative activities against two breast cancer cell lines (MDA-MB-231 and MCF7) were separately used as dependent variables. The RQSAR analysis highlighted different E-state indices and pharmacophoric requirements of important substitutions. The best 2D-QSAR model is established on the basis of three machine learning tools – MLR, SVM and ANN. The 2D-QSAR models depicted importance of different structural, physicochemical and topological descriptors. While RQSAR analyses demonstrated the fingerprint requirements of various substitutions, the KPLS analyses showed these requirements for the entire molecule. The CoMSIA model further refines these interpretations and reveals how subtle variations in these structures may influence biological activities. Observations of different modeling techniques complied with each other. The current QSAR study may be used to design potential antimitotic agents. It also demonstrates the utilities of different molecular modeling tools to elucidate the SAR.

1. Introduction

Mitosis involves a series of mechanical events that produce two new nuclei containing identical copies of DNA in cell division. Because of the crucial role of the mitotic spindle in mitosis, the spindle fiber is a valuable target in cancer therapy for decades (Wood et al., 2001). In eukaryotic cells, microtubules play vital roles in a variety of fundamental cellular processes including mitosis, formation and maintenance of cell shape, regulation of motility, cell signaling and secretion as well as the separation of duplicated chromosomes during the cell division and intracellular transport (Amos, 2004; Downing and Nogales, 1998a; Honore et al., 2005; Pellegrini and Budman, 2005; Walczak, 2000). Microtubules are cytoskeletal filaments, consist of α- and β-tubulin heterodimers (Downing and Nogales, 1998b), involving dynamic polymerization as well as depolymerization transitions by the reversible addition of tubulin dimers at their end. Interference of this equilibrium process blocks proper microtubular function and ultimately leads to the cell death (Silvestri, 2013). Therefore, antimitotic agents become promising weapons for development of new anticancer drugs (Jordan and Wilson, 2004). Antimitotic agents help in mitotic arrest at the level of chromosomes, nuclear membrane and the mitotic spindle that result in a sharp increase in the proportion of cells in the G2/M phase of the cell cycle (Hamel and Covell, 2002). A variety of natural compounds were found to be tubulin polymerization inhibitors (Fig. S1), such as paclitaxel (Jordan and Wilson, 2004), vinblastine (Hadfield et al., 2003), docetaxel and vincristine (Kuppens, 2006), combrestatin A-4 (Hamel and Covell, 2002), colchicine (Dumontet and Jordan, 2010; Hamel and Covell, 2002) and podophyllotoxin (Dumontet and Jordan, 2010). Unfortunately, the majority of mitotic spindle blockers failed in clinical trials because of their poor therapeutic indices. Many compounds were observed to produce inadequate efficacy and high toxicity in clinical trials due to the chemical instability and unrecognized multiple protein target interactions (Budman, 1997; Goldspiel, 1997). Despite the clinical failures of the first generation spindle poisons, paclitaxel has gained a huge success as a broadly effective and commercially

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successful anticancer drug. This success led to extensive efforts in developing new effective antitubulin agents with improved pharmacokinetic profiles and selectivity. Interestingly, these tubulin binders were also found to interact with tumor endothelial cells leading to a rapid occlusion of tumor vasculature and results in necrotic cell death (Choi et al., 2013). Therefore, inhibition of tubulin polymerization is a choice of strategy for estrogen sensitive as well as hormone dependent breast cancers. As a result, a great interest still persists in designing new antimitotic agents with higher activity and less toxicity, and search for new antimitotic agents is still on. Cytotoxic phenylindole derivatives were found to be effective and promising potential antimitotic agents (Gastpar et al., 1998; Kaufmann et al., 2007; Pojarova et al., 2007; Vogel et al., 2008). The antiproliferative activities of these compounds were tested against two breast cancer cell lines: MDA-MB-231 and MCF7. The MDA-MB-231 cell line is a triple negative breast carcinoma (TNBC) cell line where three receptors – human epidermal receptor 2 (HER2), estrogen and progesterone receptors are not overexpressed. The MCF7 cell line retains several characteristics of differentiated mammary epithelium including ability to function estradiol via cytoplasmic estrogen receptors and the capability of forming domes. Over the years, quantitative structure activity relationship (QSAR) study has been utilized in modeling a wide range of biological and physicochemical activities (Lewis and Wood, 2014; Nantasenamat et al., 2010). To find the structural features for the higher antimitotic activity, validated comparative chemometric modeling was performed on phenylindole derivatives through R-group QSAR (RQSAR), regression based 2D-QSAR, linear discriminant analysis (LDA) based 2D-QSAR studies, kernel-based partial least square (KPLS) and 3D-QSAR CoMSIA studies. The aim of the work is not only to highlight the important structural requirement of these derivatives but also to investigate how well different QSAR-based molecular modeling tools predict the structure activity relationship (SAR) and how much interpretations of these tools may correlate with each other.

2. Materials and methods

2.1. Dataset

A modeling set containing eighty phenylindole derivatives (Gastpar et al., 1998; Kaufmann et al., 2007; Pojarova et al., 2007; Vogel et al., 2008) were considered for the chemometric studies. The general structure of the phenylindole derivatives with arbitrary numbering is shown in Fig. 1.

Antiproliferative activities of these phenylindoles against hormone-independent human MDA-MB-231 breast cancer cell line as well as estrogen-sensitive MCF7 breast cancer cell line were used for the chemometric modeling studies. Structures and the antimitotic activities of these phenylindole derivatives are provided in the supplementary materials (Table S1).

2.2. Selection of training and test sets

The training and the test sets were selected by using the k-means cluster analysis (k-MCA) technique (Halder et al., 2013; Tropsha, 2003), keeping an account on the maximum structural variation as well as the activity variations for both of these datasets. While computing clusters, both the observed activities and physicochemical descriptors (AlogP, molecular fractional polar surface area, molecular weight, number of hydrogen bond acceptor, hydrogen bond donor, rotatable bonds, aromatic rings and molecular fragments) were taken into consideration (Halder et al., 2013). The test set was designed with 25% members of the dataset and remaining compounds were treated as the training set. Since more than one chemometric analyses were performed in the current study, multiple splitting was avoided. Rather, the principal component analysis (PCA) technique (Tropsha, 2003) was performed to check the uniformity of the test set in terms of structural and biological variation. The selected training and the test set combination was used in all chemometric analyses performed in the current work.

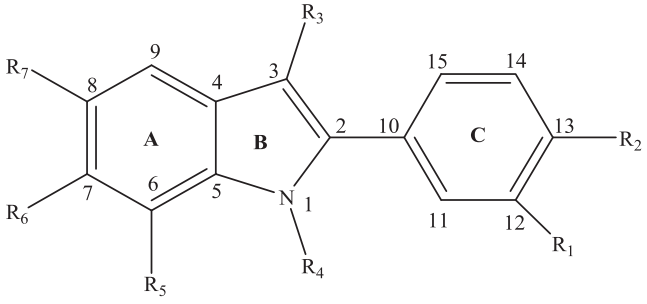

Fig. 1. General structure of phenylindole derivatives with arbitrary numbering.

2.3. R-group QSAR analyses

The R-group analysis (Chen et al., 2013) is an extension of Free-Wilson analyses which is combined with the bitmap fingerprint-based QSAR method. This method may be utilized to elucidate SAR from the congeneric series of compounds. It is effective in identifying scaffolds, attachment points and R-groups at each point. It also provides information regarding the function of each substitution for determination of the biological activities. Since the compounds of interest in the current work have a fixed scaffold (Fig. 1), the R-group analysis was performed to identify roles of important substitutions. Two R-group based QSAR (RQSAR) analyses – (a) pharmacophore-based RQSAR (*Pharm-RQSAR*) and (b) E-state-based RQSAR (*Estate-RQSAR*) studies were performed by *R group analysis* tool (Canvas, 2013). In the *Pharm-RQSAR* analysis, models were developed on the basis of pharmacophore feature counts present in the R groups (substitutions) as the independent variables. Different types of pharmacophore features to be analyzed are hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic (H), negatively charged (N), positively charged (P) and ring aromatic (R). After completion of calculations, the R groups were labeled with these pharmacophore features, colored by significance (Red for the positive contribution and blue for the negative contribution and gray for inconclusive contributions. If a feature is missing in a position, a lower case letter is used for the pharmacophore feature type.). Similarly for the Estate RQSAR, the E-state atom types present in R-groups at each position were used as the independent variables. The attachment points were labeled with a list of letters representing the E-state atom types, colored by significance (same as the *Pharm-RQSAR* analysis). In current analyses, predicted activities of the *Pharm-RQSAR* and the *Estate-RQSAR* were compared with the experimental activities to understand roles of substitutions in determination of the observed activity. The correlation coefficient was calculated from the values of the observed and the predicted activities.

2.4. Regression based 2D-QSAR study

In the present work, both regression (Adhikari et al., 2014, 2013a, 2013b) and classification QSAR analyses (Kar and Roy, 2013a,b; Nandy et al., 2014, 2013) were performed so that more information could be extracted regarding the structural and physicochemical requirements.

2.4.1. Calculation and selection of descriptors

Different descriptors were calculated for the QSAR analysis. All the geometrics of phenylindole derivatives were completely

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