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Group-based QSAR and molecular dynamics mechanistic analysis revealing the mode of action of novel piperidinone derived protein–protein inhibitors of p53-MDM2

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ABSTRACT

Tumour suppressor p53 is known to play a central role in prevention of tumour development, DNA repair, senescence and apoptosis which is in normal cells maintained by negative feedback regulator MDM2 (Murine Double Minute 2). In case of dysfunctioning of this regulatory loop, tumour development starts thus resulting in cancerous condition. Inhibition of p53-MDM2 binding would result in activation of the tumour suppressor. In this study, a novel robust fragment-based QSAR model has been developed for piperidinone derived compounds experimentally known to inhibit p53-MDM2 interaction. The QSAR model developed showed satisfactory statistical parameters for the experimentally reported dataset (r^2 = 0.9415, q^2 = 0.8958, pred_r^2 = 0.8894 and *F*-test = 112.7314), thus judging the robustness of the model. Low standard error values (r^2_se = 0.3003, q^2_se = 0.4009 and pred_r^2_se = 0.3315) confirmed the accuracy of the developed model. The regression equation obtained constituted three descriptors (R_2-DeltaEpsilonA, R_1-RotatableBondCount and R_2-SssOCount), two of which had positive contribution while third showed negative correlation. Based on the developed QSAR model, a combinatorial library was generated and activities of the compounds were predicted. These compounds were docked with MDM2 and two top scoring compounds with binding affinities of −10.13 and −9.80 kcal/mol were selected. The binding modes of actions of these complexes were analyzed using molecular dynamics simulations. Analysis of the developed fragment-based QSAR model revealed that addition of unsaturated electronegative groups at R_2 site and groups with more rotatable bonds at R_1 improved the inhibitory activity of these potent lead compounds. The detailed analysis carried out in this study provides a considerable basis for the design and development of novel piperidinone-based lead molecules against cancer and also provides mechanistic insights into their mode of actions.

1. Introduction

Cancer is a major health problem caused by unregulated growth and division of cells. Normal cells turn into cancerous cells due to many reasons, major one being mutations in the critical genes. According to the world cancer report, 12.7 million new cases diagnosed and reported in 2008 are expected to rise to 21 million by 2030 [1]. Better diagnosis as well as improving survival rates has played an important role in increasing number of cancer survivors. Side effects of the conventional treatment plans such as decrease in bone density, cardiovascular diseases, cognitive deficits, fatigue, physical and emotional distress, infertility and pulmonary dysfunction etc. in addition to drug resistance has posed a need for more effective and less toxic anticancer therapeutics [2,3].

As compared to conventional drug development methods, in silico methods are a good and viable alternative for identification and development of novel drug leads. One such in silico method is ligand-based drug design which establishes a quantitative structure-activity relationship (QSAR) between the inhibitory activity and structure of the inhibitors. Group-based QSAR (GQSAR) is a new fragment based method that allows studying the relationship of variation in the biological response with molecular fragments of interest by evaluating fragment-dependent

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descriptors [4]. Unlike the conventional QSAR methods, GQSAR can be developed for both congeneric (template-based approach) as well as non-congeneric series (user-defined scheme) of compounds to obtain site specific clues, which has to be optimized for designing new molecules and quantitatively predicting their activity. This approach provides robust models in terms of the contribution of each individual substituent site that can be applied to create a combinatorial library by substituting different entities at these sites and then predicting their activities. This approach has been exploited in this study to develop novel piperidinone derived inhibitors with enhanced inhibitory effect on the basis of mathematical QSAR model.

New treatment methods aim at molecular targets such as oncogenes and tumour suppressors that are known to be involved in the progress of human cancers [5]. Tumour suppressor p53 is one such target which regulates the cell cycle, apoptosis, DNA repair, senescence and angiogenesis [6–8]. In normal cells, activity and concentration levels of p53 are regulated by MDM2 which is a ubiquitously expressed protein and plays an important role in tissue development. p53 and MDM2 mutually control their cellular levels by an autoregulatory feedback loop. When the level of MDM2 increases, it binds to p53 and inactivates it by directly blocking p53 transactivation domain. Dysfunction of this regulatory loop results in tumour formation. Thus, by inhibiting MDM2, p53 would fail to bind with MDM2 resulting in stabilization and activation of the tumour suppressor, ultimately leading to cell cycle arrest or programmed cell death (apoptosis) of cancer cells. Recently, cis-imidazoline derived MDM2 antagonists (nutlins) were identified in an in vivo study which focuses on activation of p53 tumour suppression pathway [9]. Drugs known to inhibit MDM2 were designed to block the hydrophobic cleft where three critical residues of p53 (Phe19, Trp23, Leu26) are known to bind, thus inhibiting the protein–protein interaction [9–12]. Based on the binding mode of already known inhibitors, a new series of piperidinone-derived compounds was identified experimentally which showed high inhibitory activity against p53-MDM2 interaction [13]. The binding affinity of these piperidinone-derived compounds for MDM2 improved through conformational change in both the piperidinone ring and the appended N-alkyl substituent.

In this study, we carried out fragment based QSAR study on a congeneric set of piperidinone derivatives. The aim of this study was to generate a reliable GQSAR model of piperidinone derived p53-MDM2 interaction inhibiting compounds which maps the variation of biological activity of the chosen compounds as a function of their site-specific molecular fragments. This model predicted the relationship between inhibitory activity and electronic and chemical properties of the derived compounds with high correlation. Our GQSAR model identified descriptors evaluated specifically for the fragments of piperidinone derivatives which are necessary for exhibiting anticancer activity. In addition to QSAR, we also provide detailed insights into the mode of binding of this class of compounds. In this study an attempt has been made to understand the effect of various substituents at two substitution sites in a common template derived from experimentally reported dataset by predicting the inhibitory activity of synthesized combinatorial library of piperidinone derived molecules and then virtually docking it with MDM2. After molecular dynamics simulations of the ligand bound protein complexes, ADME properties of these ligands were predicted for further analysis. This analysis would help in providing novel piperidinone-based lead molecules for the development of anticancer drugs. This study will enhance understanding and provide insight into mechanism of action of piperidinone derivatives as potent anticancer therapeutics in terms of structural requirements needed for drug development.

2. Materials and methods

2.1. Selection and preparation of data set

A dataset of 23 previously reported piperidinone-derived compounds [13] was drawn using MarvinSketch [14]. The 2D compounds were converted into 3D using VlifeEngine module of VLifeMDS ver.4.3 [15]. These 3D compounds were energy minimized using force field batch minimization module of VlifeEngine and then used for developing the GQSAR model.

2.2. Calculation of descriptors for GQSAR model

The GQSAR module of VLifeMDS was used for building fragment based QSAR model. Using modify utility of VLifeMDS, a template for the congeneric set of piperidinone derived compounds was created by keeping a common moiety and substituting groups at the two sites (named R_1 and R_2) of substitution. The optimized set of compounds and the template created were selected for fragment-based GQSAR model building. The inhibitory activity values of the molecules in the form of pIC_{50} were manually incorporated in VLife MDS platform and the physico-chemical 2D descriptors were calculated for different substituents present at both substitution sites (i.e. at fragments R_1 and R_2) of the dataset molecules (Supplementary Table 1). Since the same descriptors were calculated for various groups at two different sites, a particular nomenclature was applied for naming a descriptor at a particular position. For example R_1_RotatableBondCount signifies the number of rotatable bonds at R_1 substitution site. Invariable columns were removed leaving a total of 174 from 343 descriptors for QSAR analysis. The training and test sets were selected manually keeping a uniform distribution of compounds in the two sets based on their pIC_{50} values. Six molecules, namely 7, 8, 14, 15, 18 and 20 were chosen as test set, while the remaining 17 were selected as training set. The unicolumn statistics of the selected training and test sets were calculated and analyzed.

Supplementary Table 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jmgm.2014.04.015.

2.3. Building the GQSAR model of piperidinone-derived compounds

For this study, out of various model building methods stepwise forward partial least square regression method was implemented. The stepwise forward variable selection algorithm began by developing a trial model one step at a time with only one independent variable. At each step independent variables were added one by one and then the fitness of the model was evaluated using the PLS (partial least square) cross-validation method. Accordingly, the model was constantly changed from the previous one by adding or removing a predictor variable. This method continued until there are no more significant variables left outside the model. The advanced variable selection and model building wizard was utilized for this purpose with cross correlation limit as 0.7, *F*-test as 4, term selection criteria as r^2, variance cut-off as 0.1 and number of random iterations as 100.

2.4. Validation of the developed GQSAR model

The 'goodness of fit' of the developed model was analyzed using different statistical parameters, such as r^2, q^2, pred_r^2, *F*-test and standard error [16]. A model is considered to be robust when it fulfils the conditions: $r^2 > 0.6$, $q^2 > 0.6$ and pred_$r^2 > 0.5$ [16,17]. The *F*-test denotes the ratio of the variance observed by the model and the variance due to error in regression. High *F*-test value indicates that the model is statistically significant which shows a negligible