Design, synthesis and evaluation of novel sulfonamides as potential anticancer agents

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ABSTRACT

Based on modern literature data about biological activity of E7010 derivatives, a series of new sulfonamides as potential anticancer drugs were rationally designed by QSAR modeling methods. Classification learning QSAR models to predict the tubulin polymerization inhibition activity of novel sulfonamides as potential anticancer agents were created using the Online Chemical Modeling Environment (OCHEM) and are freely available online on OCHEM server at https://ochem.eu/article/107790. A series of sulfonamides with predicted activity were synthesized and tested against 60 human cancer cell lines with growth inhibition percent values. The highest antiproliferative activity against leukemia (cell lines K-562 and MOLT-4), non-small cell lung cancer (cell line NCI-H522), colon cancer (cell lines NT29 and SW-620), melanoma (cell lines MALME-3M and UACC-257), ovarian cancer (cell lines IGROV1 and OVCAR-3), renal cancer (cell lines ACHN and UO-31), breast cancer (cell line T-47D) was found for compounds 4–9. According to the docking results the compounds 4–9 induce cytotoxicity by the disruption of the microtubule dynamics by inhibiting tubulin polymerization via effective binding into colchicine domain, similar the E7010.

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1. Introduction

Microtubules, constructed from α and β-tubulin heterodimers, are one of the key structural elements of the cytoskeleton of eukaryotic cells (Perez, 2009). They play a very significant role in many cellular processes, such as the mitosis, the cellular form formation, the intracellular transport, the cell motility and they are one of the targets for anticancer drugs (Jordan et al., 1998). It is known that drugs-inhibitors of the microtubules function binds with each of three active sites of tubulin. The stabilizing drugs act to the sites including the vinca and colchicine domains. The destabilizing drugs affect the paclitaxel site of the microtubules. These agents stop the dividing cells in G2/M phase of the cell cycle causing the mitotic catastrophe and cell apoptosis (Lu et al., 2012). Microtubule-stabilizing and microtubule-destabilizing agents are widely used in modern chemotherapy of many types of cancer – in the treatment of leukemias, lymphomas, ovarian cancer, colon cancer and small cell lung cancer (Mustafa et al., 2017; Kaur et al., 2014; Dumontet and Jordan, 2010; Kumar et al., 2016; Dimitroulis and Stathopoulos, 2005).

Fig. 1 shows the structural diversity of microtubule inhibitors as antitumor agents that bind to the colchicine site similar to the biological active sulfonamide E7010 (Yoshino et al., 1992; Pareek et al., 2013). It is known that mechanism of antimitotic action of E7010 is the inhibition of tubulin polymerization by binding to the colchicine active site of β-tubulin (Yoshimatsu et al., 1997). Besides, E7010 is widely used in the drug discovery of new anticancer agents. Synthesis and biological tests of new E7010 derivatives led to the development of new antitumor sulfonamides, for example the E7070, which strongly inhibits the cell proliferation of various tumor lines (Owa et al., 1999).

The paper presents the results of QSAR analysis, biological testing and molecular docking of new synthesized potential tubulin inhibitors.

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2. Materials and methods

2.1. Methods of QSAR models development

2.1.1. Dataset

The data of tubulin inhibitors that perturb the dynamics of cellular microtubules was analyzed and the initial dataset for our analysis was collected from PubChem BioAssay, University of Pittsburgh Molecular Library Screening Center database (Anon, 2015). The set of 1625 active compounds and the set of 194 228 inactive compounds were analyzed for the similarity search to oxazole derivatives by Dice Index using Instant JChem program (Anon, 2016). As the results, the current dataset used in this QSAR study consisted of 1228 structures as antimitotic agents for further classification QSAR model creation wherein 613 among them were active and 615 compounds were inactive with the Dice Index in the range 0.5–0.6. Suchlike ratio of active and inactive compounds is a necessary condition for the creation of high-grade models. All molecules were processed using the Chemaxon standardizer and stored in SDF format (Breiman et al., 1984). The detailed information about structures and the corresponding activity of the compounds can be found in the Supplementary materials 1.

2.1.2. Machine learning methods

In the current work, we used the Online Chemical Modeling Environment (OCHEM) platform (Quinlan, 1993) to create high accuracy classification models for predicting the anticancer activity of oxazole derivatives. Five machine learning methods were used to build classification models via different types of descriptors.

2.1.2.1. WEKA-RF (Random Forest). Random Forests is an ensemble classifier consisting of many of individual learners. The method is specially devised to operate quickly over large data sets. Decision trees are a popular method for various machine learning tasks. This method commonly used for classification, regression and other tasks, that operate by constructing a multitude of decision trees at training time and outputting the class (Tetko, 2002). This method was used to determine the activity of the compounds into two classes according to the criterion of active/inactive.

2.1.2.2. WEKA-J48. WEKA-J48 is a Weka implementation of the C4.5 pruned decision tree. The method is classification-only. The C4.5 tree tries to recursively partition the data set into subsets by evaluating the normalized information gain (difference in entropy) resulting from choosing a descriptor for splitting the data. The descriptor with the highest information gain is used on every step (Tollenaere, 1990).

2.1.2.3. ASNN (Associative Neural Network) ASNN is a combination of an ensemble of feed-forward neural networks and the k-nearest neighbor technique (kNN). The method uses the correlation between ensemble responses as a measure of distance amid the analyzed cases for the kNN. It provides an improved prediction by the bias correction of the neural network ensemble. ASNN method is essential to design the models that are able to predict the activity with good accuracy (Zhokhova et al., 2007). The SuperSab algorithm was used to optimize the neural network weights (Haenlein and Kaplan, 2004). The number of neurons in the input layer of the ASNN corresponded to the number of selected descriptors. One hidden layer with five neurons was used in the calculations. The number of learning iterations for neural network training was 1000 and ensemble included 100 neural networks.

2.1.2.4. FSMLR (Fast Stepwise Stagewise Multivariate Linear Regression). It is a procedure for stage wise building of linear regression models by means of greedy descriptor selection. The method is specially designed to be compatible with the three-set approach based on the use of three different sets for learning (Hall and Kier, 1995).

2.1.2.5. PLS (Partial Least Squares). It is a statistical method that finds a linear regression model by projecting the predicted variables and the observable variables to a new space (Tetko and Tanchuk, 2002). The optimal number of latent variables was selected automatically and equaled 18.

2.1.3. Applicability domain estimation

QSAR prediction of biological and physico-chemical properties has limited value without an estimated applicability domain (AD) of a model. The AD of all created QSAR models was automatically detected by OCHER for each new molecule (i.e. whether a molecule inside or outside of AD). On the OCHER web site the DM which covers 95% of compounds from the training set is used to define AD of the model. As result, according to the OCHER prediction all test set compounds were inside the scope of the model.
2.1.4. Molecular descriptors

OChem support many unique software packages for calculation of different molecular descriptors. The certain types of molecular descriptors and their combinations were calculated using 6 descriptor packages which cover different representations of chemical structures for modeling physicochemical and biological properties through the machine learning methods.

E-State indices. Calculation combines electrotopological characteristics of the analyzed compounds (Adriana, 2017). E-State indices are 2D descriptors that separated on atom/bond type and the atom and bonds indices and counts were attracted. AlogPS descriptors (2D) predict logP (lipophilicity) and logS (water solubility) of chemical compounds for drug design (Anon, 2017a).

ADRIANA. Code uses a series of methods for the generation of 3D structures and comprises the calculation of molecular descriptors on chemical and physical base and molecular properties based on quick empirical models. This software package includes global molecular descriptors that represent a chemical structure by a structural, chemical or physicochemical feature or property of the molecule expressed by a single value (molecular weight, number of atoms, topological polar surface area, molecular dipole moment, number of donors and acceptors, molecular and ring complexity and size); topological descriptors, shape and size descriptors that characterize the size and the 3D shape of a molecule (molecular span, molecular diameter, molecular eccentricity, molecular radius of gyration, principal moment of inertia, molecular asphericity) and spatial descriptors (electronenegativity, charge, effective atom polarizability and others) (Cherkasov, 2005).

ChemAxon descriptors describe a big variety of descriptors that calculated physico-chemical and life-science related properties of chemicals (Steinbeck et al., 2006). All these descriptors ranging from 0D to 3D including elemental analysis, geometry, charge, partitioning, protonation and others were used in model building.

Inductive descriptors are used for modeling of different physicochemical and biological properties. These molecular parameters are easily accessible from electro-negativities and covalent radii of the constituent atoms and inter-atomic distances and can reflect a variety of aspects of intra- and intermolecular interactions (Anon, 2017b). The electro-negativity-based, hardness-based, softness-based and charge-based descriptors from all inductive were involved in the construction of QSAR models.

CDK descriptors include 204 molecular descriptors of 5 types (Chokkappagari et al., 2014), among which the following types have been selected: topological, geometrical, constitutional and electronic descriptors. Unsupservised filtering of descriptors was executed to descriptor sets before using it for the model developing. The descriptors with fewer than 2 unique values were eliminated and grouped, that have pair-wise correlations Pearson’s correlation coefficient \( R \) larger 0.95. Moreover, descriptors with variance smaller than 0.01 were excluded. All structures were standardized and optimized with structure generator Corina (Nitulescu et al., 2010), which used by pharmaceutical and chemical companies to convert their 2D structures into 3D. Detailed information about all used descriptors can be found on the OChem website.

2.1.5. Validation of QSAR models

The accuracy of designed QSAR models was evaluated using 5-fold cross-validation method. The OChem provides with a variety of statistical instruments to analyze the performance of models and to find outliers in the training and validation sets. The performance of created classification QSAR models was evaluated with such statistical measures applicable to binary classification models as accuracy (AC), precision (Pr), sensitivity (Sn) and specificity (Sp).

The main parameter accuracy is merely the percentage of correctly classified samples.

\[
AC = \frac{(TP + TN)}{(TP + FP + TN + FN)},
\]

here TP, FP, TN and FN define true positives, false positives, true negatives and false negatives, respectively.

Another parameter is balanced accuracy (BA). It is the averaged accuracy for each class. This parameter is important for imbalanced datasets, which have significantly different number of samples in different classes. In our case, the accuracy and balanced accuracy of all classification QSAR models are identical.

\[
BA = 0.5 \times \frac{(Sn + Sp)}{TN/(TP + FN)},
\]

Class hit rate is a measure that is applicable to a single class in a classification model and denotes a ratio of instances of a specific class that were correctly identified as belonging to this class. For binary classification tasks class hit rate for positive class is called sensitivity, and for negative class – specificity.

Precision can be calculated as ratio between the cases correctly identified to a total number of cases belonging to this class. Sensitivity/Specificity or true positive/negative accuracy can be calculated as:

\[
Sn = \frac{TP}{(TP + FN)}, \quad Sp = \frac{TN}{(TN + FP)}.
\]

All these parameters of classification models accuracy are described particularly in OChem website and presented in Supplementary materials 1.

2.2. Chemistry

The reactions were followed by TLC (Silica gel, aluminum sheets 60 F254; Merck). Melting points were recorded on a Fisher-Johns apparatus. IR spectra were recorded on a Vertex-70 spectrometer in KBr pellets. 1H, 13C NMR spectra were recorded on a Varian Mercury spectrometer (400 or 500 and 125 MHz, respectively) in CDCl\(_3\) or DMSO-\(d_6\), with TMS as internal standard. Multicities were described using the following abbreviations: s = singlet, bs = broad singlet, d = doublet, t = triplet and m = multiplet. Mass spectra were recorded on an Agilent 1100 Series LC–MS system, equipped with diode array and mass selective detector Agilent LC/MSD SL (atmospheric pressure chemical ionization). Elemental analysis was performed in the Analytical Laboratory of the Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine. Reagents and solvents from commercial sources were used. (Supplementary materials 2)

2.3. Anticancer screening methodology

The human tumor cell lines of the cancer-screening panel were grown in RPMI 1640 medium. For a screening, cells were inoculated into 96 well plates in at cell densities ranging from 5000 to 40000. After inoculation, the plates were incubated at 37 °C for 24 h prior to addition of testing compounds. After 24 h, few plates of each cell line were treated with trichloroacetic acid, to measure cell population for each cell line at the time of drug addition (T2). Testing compounds were solubilized in DMSO. Following drug addition, the plates were incubated for an additional 48 h at 37 °C. For adherent cells, the assay was terminated by the addition of cold TCA and incubated for 60 min at 4 °C. (Supplementary materials 3)

The supernatant was discarded, and the plates were washed and dried. Sulforhodamine B (SRB) solution in 1% acetic acid was
added to each well, and plates were incubated. Then unbound dye is removed by threatening with acetic acid. Bound stain was solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. Percentage growth inhibition (GI) was calculated as:

\[ GI = \left( \frac{\text{Ti} - \text{Tz}}{\text{C} - \text{Tz}} \right) \times 100\% \text{ at Ti} \geq \text{Tz} \]

\[ GI = \left( \frac{\text{Ti} - \text{Tz}}{\text{Tz}} \right) \times 100\% \text{ at Ti} < \text{Tz} \]

Ti – absorption after treatment with tested compound, Tz – absorption before treatment with tested compound, C – absorption of control.

2.4. Molecular docking

The AutoDock Tools 1.5.6 (ADT) (Sanner, 1999) was applied to prepare the docking compatible structure formats of the protein, ligands and grid box creation. The methodology for conducting molecular docking was similar to that used earlier (Semenyuta et al., 2016; Trush et al., 2017). From the RCSB Protein Data Bank, the crystal structure of the colchicine–tubulin complex (PDB code: 1SA0) was used (Berman et al., 2002). The structure of B-subunit of β-tubulin was selected and stored as a PDB file by Accelrys DS (ver. 2.5.5) (Anon, 2017c). The only polar hydrogens in ADT we added, using the no Bond Order method and renumbered atoms to include new hydrogens. The partial charges were calculated and added by using Gasteiger method, the prepared file was saved in PDBQT format. The structures of ligands (8, 9 and 11) were created and optimized by using the ChemAxon Marvin Sketch 5.3.7 program (Anon, 2017d) and saved in Mol2 format. Torsions angles and partial charges of ligands were changed by ADT and saved the resulting files in PDBQT format. The AutoGrid software was used for the grid map preparation using the grid box. The box center \((x=117.219, y=90.180, z=6.290)\) was set to the center of colchicine in the B-subunit 1SA0. The grid of 30°30'30 points with grid spacing of 0.375 Å was used. The Auto Dock Vina 1.1.2 was applied to perform docking simulations (Trott and Olson, 2010) The one ligand docking was in progress approximately 3–5 min. All operations were performed on a Windows XP SP3 computer with an Intel Core i3 CPU (3.20 GHz) and 2 GB of RAM. Accelrys DS software package was used for illustration and to study protein–ligand interactions (Trush et al., 2017).

3. Results and discussion

3.1. QSAR modeling of oxazole derivatives anticancer activity

In the current study, the initial set of 1228 tubulin inhibitors was used for QSAR modeling of antimitotic activity. In the preliminary stage of our analysis, many classification QSAR models were built using different machine learning methods and a big variety of descriptors accordingly the selected methods. As result, five final classification QSAR models were built with good quality and high accuracy and used in this study for virtual screening of newagents with potent inhibitory activity against β-tubulin. The best constructed models included 624 descriptors from 6 descriptor packages such as E-State indices, ALogPS, ADRIANA.Code, ChemAxon, Inductive and CDR descriptor by particular selected method (WEKA-RF, WEKA-J48, ASNN, FSMLR, PLS). Consensus model, built on the base of averaging of five individual models, was applied for the selection of promising anticancer agents. The results are summarized in Table 1, Figs. 1–6 in the Supplementary materials 1.

According to the statistical significance presented in Table 1, all classification QSAR models have shown the similar results in terms of precision, sensitivity, specificity and accuracy. The accuracies for the training sets were in the range 88.2–94.4% for the individual QSAR models, and the accuracy of consensus model was 93.2%.

In the next step of our analysis due to the described above calculation, a virtual set of 1,3-oxazole-based sulfonamides was generated for anticancer activity testing. Via Instant JChem program the Dice Index for these compounds was calculated in relation to the training set. Consequently, all derivatives were similar to the most of compounds in the training set (DI similarity > 0.5) and their activity prediction is realistic and applicable. As result, the initial dataset was reduced to six compounds.

Then, all the selected compounds were screened using OCHEM classification models for their inhibitory activity of tubulin polymerization. The results of the prediction activity by all developed QSAR models are shown in Table 2.

Six compounds were selected for synthesis and biological testing (Table 2) in accordance with the results of the analysis based on the consensus prediction. Fig. 2 shows the predicted active structures of E7010 derivatives with including the oxazole, pyrazole and piperidine rings, which as is well-known are responsible for high anticancer activity (Wang et al., 2015).

### Table 1

<table>
<thead>
<tr>
<th>Model</th>
<th>Method</th>
<th>Precision (active)</th>
<th>Precision (inactive)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>WEKA-RF</td>
<td>0.90</td>
<td>0.87</td>
<td>0.87</td>
<td>0.91</td>
<td>88.8 ± 0.9</td>
</tr>
<tr>
<td>2.</td>
<td>WEKA-J48</td>
<td>0.87</td>
<td>0.89</td>
<td>0.89</td>
<td>0.87</td>
<td>88.2 ± 0.9</td>
</tr>
<tr>
<td>3</td>
<td>ASNN</td>
<td>0.94</td>
<td>0.95</td>
<td>0.95</td>
<td>0.94</td>
<td>94.4 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>FSMLR</td>
<td>0.86</td>
<td>0.92</td>
<td>0.90</td>
<td>0.86</td>
<td>87.6 ± 1.0</td>
</tr>
<tr>
<td>5</td>
<td>PLS</td>
<td>0.90</td>
<td>0.88</td>
<td>0.86</td>
<td>0.91</td>
<td>88.6 ± 0.9</td>
</tr>
<tr>
<td>Consensus</td>
<td>0.93</td>
<td>0.93</td>
<td>0.93</td>
<td>0.93</td>
<td>0.93</td>
<td>93.2 ± 0.7</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
<th>Consensus Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>NA</td>
<td>A</td>
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<tr>
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<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>

\( A \) – compound predicted as active.

\( NA \) – compound predicted as inactive.
3.2. Chemistry

The synthesis of selected compounds was accomplished by the route illustrated in Scheme 1.

4-Cyano-1,3-oxazole-5-sulfonamides 4–7 were prepared by refluxing of 2-aryl-4-cyano-1,3-oxazole-5-sulfonyl chlorides (Kornienko et al., 2012) 1 or 2 and corresponding amine. Methyl 5-((5-amino-3-phenyl-1H-pyrazol-1-yl)sulfonyl)-2-phenyloxazole-4-carboxylate 8 and methyl 5-((5-amino-3-methyl-1H-pyrazol-1-yl)sulfonyl)-2-phenyloxazole-4-carboxylate 9 were prepared analogous to the previous sulfonamides 4–7 by refluxing of methyl 5-chlorosulfonyl-2-phenyloxazole-4-carboxylate 3 with 3-methyl- or 3-phenyl-1H-pyrazol-5-amine and triethylamine in dioxane according to the method (Kornienko et al., 2014).

The structure and composition of all obtained sulfonamides 4–9 have been in good accordance with data of elemental analysis, 1H, 13C NMR, IR spectroscopy and chromatography–mass spectrometry. All CH$_2$ and CH-proton signals are visible in the 1H NMR spectrum. Signal of the OH group of 6 at 4.79 ppm, NH$_2$ group (8, 9) 6.32–6.55 ppm. The intensive absorption bands of SO$_2$-group appeared at 1147–1158 cm$^{-1}$ and 1353–1402 cm$^{-1}$ in the IR spectra. Also, the broad intensive bands at 1731–1732 cm$^{-1}$ corresponded to esters C=O bond of 8 and 9 and intensive bands at 2247–2252 cm$^{-1}$ corresponded to CN group of 4–7 were observed. (Supplementary materials 2)

3.3. In vitro evaluation of the anticancer activity

All the synthesized compounds 4–9 were submitted to National Cancer Institute (NCI) for in vitro anticancer screening. Primary one dose assay was performed in full NCI 60 cell panel representing leukemia, melanoma and cancers of lung, colon, brain, breast, ovary, kidney and prostate in accordance with the protocol of the NCI, USA. (DTP, 2017; Monks et al., 1991; Boyd and Paull, 1995; Boyd and Teicher, 1997; Shoemaker, 2006) The compounds were added at a single concentration ($10^{-5}$ M) and the cultures were incubated for 48 h. Endpoint determinations were made with a protein binding dye, sulforhodamine B. Results for each compound were reported as a mean graph of the percent growth inhibition of the treated cells when compared to the untreated control cells. (Supplementary materials 3).

1,3-Oxazole-5-sulfonamides exhibited high cytotoxic and cytostatic activity against tested cell lines (Table 3). With regard to the effectivity against some individual cell lines 4-cyano-N-(2-hydroxyethyl)-N-methyl-2-(4-methylphenyl)-1,3-oxazole-5-sulfonamide (6) and N-[2-(4-chlorophenyl)-2-piperidin-1-ylethyl]-4-cyano-2-phenyl-1,3-oxazole-5-sulfonamide (7) showed high cytotoxic activity against all cell lines of Leukemia (K-562 (25.01 and 8.67%), HL-60 (8.18% for compound 7) SR (10.83 and 12.17%), Non-Small Cell Lung Cancer NCI-H522 (17.5% for compound 6), and Colon Cancer SW-620 (35.14 and 26.52% respectively) and moderate cytostatic activity against HL-60(TB) (−18.14%) and

![Scheme 1](image1.png)

**Scheme 1.** Reagents and conditions: (a) amine, Et$_3$N, dioxane, reflux, 2 h, then r.t., 12 h.

![Image 2](image2.png)

**Fig. 2.** Designing and QSAR analysis results of the sulfonamides structures as active inhibitors of tubulin-derivatives E7010.
The results of high anticancer activity of compounds 4-9 induced our studying of their antimitotic action mechanism by using molecular docking. Molecular docking studies of substances 4-9 showed the formation of a stable protein-ligand complexes with $E = -7.7, -7.8, -6.8, -8.3, -7.3, -8.4$ kcal/mol respectively, depicted in Figs. 3-7.

Molecular docking of ligands 5 and 4 illustrates many common parameters. This is the formation of one hydrogen bond with length 3.5 Å between the phenyl ring and the S-H group Cys241 and Pi-alkyl interaction with length 3.4 Å between the phenyl ring and Leu255. Also Six compounds, the formation of Pi-alkyl and alkyl bonds with length 3.8–5.4 Å between the phenyl ring of ligand and amino acid residues Leu255, Ala250, between the oxazole ring and Cys241, Ala250, Ala354, Leu255, between the aminocido acid Lys352 and piperidine ring and ligand methyl group was registered. The alkyl bond between the methyl group of ligand and Met259, Pi-alkyl interaction between the oxazole ring and Leu248 and the absence of Pi-alkyl bond between the oxazole ring and Leu255 is the feature of molecular docking of compound 5.

Molecular docking studies of substance 6 show the formation two hydrogen bonds with length 3.2 Å between the hydroxyl group and the NH$_2$-group of Asn258 and with length 3.6 Å between the phenyl ring and SH-group Cys241. There are also two Pi-alkyl interactions with length 3.4 Å between the phenyl ring and Leu255 and with length 3.8 Å between the oxazole ring and Leu248. Also, it should be noted the formation of Pi-alkyl and alkyl bonds with length 4.5–5.4 Å between methyl group of ligand and amino acid residues Leu255, Leu242, Val328, between the phenyl ring and Leu242, Ala250 and between the oxazole ring and Ala354, Cys241.

Molecular docking studies of substance 7, in contrast to substance 6, indicates the forming two hydrogen bond with length 3.6 Å between the phenyl ring and the NH$_2$-group of Asn258 and with length 3.7 Å between the phenyl ring and SH-group Cys241, and also one Pi-alkyl interaction with length 3.4 Å between the phenyl ring and Leu255. We should also mention the formation the Pi-alkyl and alkyl interactions with length 4.4–5.4 Å between chlorine atom of ligand and amino acid residues Val328, Leu255, Leu242, and between the first phenyl ring and Leu242, Ala250, and between Lys352 and the oxazole ring and the second phenyl ring of ligand.

Molecular docking studies of substance 8 demonstrate the formation of one hydrogen bond with length 3.8 Å between the phenyl ring and SH-group Cys241, and also of two Pi-alkyl interactions with length 3.5–3.8 Å between the phenyl ring and Leu255, and pyrazole ring and Leu248. Besides the formation of the Pi-alkyl and alkyl interactions with length 4.3–5.4 Å between phenyl, oxazole, pyrazole rings atom of ligand and amino acid residues Cys241, Leu255, Leu242, Ala250, Lys352, Met259, Ala354 are represented.

Molecular docking of compound 9, in contrast to ligands 8 and 7, illustrates the formation of three hydrogen bonds with length 3.2 Å between the carboxyl group and the NH$_2$-group of Leu255.
and with length 4.1 Å between phenyl ring and Asn258, and with length 4.2 Å between the oxazole ring and the S-H group Cys241, and also Pi-σ interaction with length 3.5 Å between the oxazole ring and Leu255. Also it should be noted the formation of a number Pi-alkyl and alkyl bonds with length 3.6–5.0 Å between methyl group of ligand and the amino acid residues Leu255, Ala250, Cys241, and between the oxazole ring and Ala250 and between the aminoacid Lys352 and phenyl and oxazole rings of ligand. In addition, Fig. 7 shows the formation of the intramolecular hydrogen bond between sulfoxide and amino groups of the ligand.

High stability of ligand-protein complexes formed by compounds 4–9 with binding affinity $E = -7.7, -7.8, -6.8, -8.3, -7.3, -8.4$ kcal/mol respectively is shown. Moreover, amino acid residues Cys241, Asn258, Leu255, Leu248, Val238, Leu242, Ala250, Lys352, Met259, Ala354 performs the key role in the binding of these compounds in the active site of tubulin.
4. Conclusions

In summary, a series of 1,3-oxazole-based sulfonamides – tubulin inhibitors as E7010 derivatives were rationally designed using classification QSAR models. The created models demonstrated a good stability, robustness and predictive ability. They can be used to find the new tubulin inhibitors as potential anti-cancer agents. A series of novel potential microtubule inhibitors with predicted activity were synthesized and sulfonamides 4-9 showed the high anticancer activity against leukemia (cells lines K-562 and MOLT-4), non-small cell lung cancer (cell line NCI-H522), colon cancer (cells lines NT29 and SW-620), melanoma (cells lines MALME–3 M and UACC-257), ovarian cancer (cells lines IGROV1 and OVCAR-3), renal cancer (cells lines ACHN and UO-31), breast

Fig. 5. Molecular docking of compound 7 into the active site of tubulin (PDB ID: 1SA0). The left part of the figure shows the secondary structure of β-subunit of β-tubulin and the binding position of compound 7 to the protein. The bonds and interactions substance 7 in the active site of tubulin are demonstrated on the right part of the figure.

Fig. 6. Molecular docking of compound 8 into the active site of tubulin (PDB ID: 1SA0). The left part of the figure shows the secondary structure of β-subunit of β-tubulin and the binding position of compound 8 to the protein. The bonds and interactions substance 8 in the active site of tubulin are demonstrated on the right part.
cancer (cell line T-47D). Molecular docking study showed that the main role in the binding of these compounds into the active site of tubulin is performed by amino acid residues Cys241, Asn258, Leu255, Leu248, Val238, Leu242, Ala250, Lys352, Met259, Ala354. The high stability of this complexes is ensured by H- bonds, Pi-σ bonds, Pi-alkyl and alkyl interactions and is confirmed by high predicted binding affinity with E = −7.7, −7.8, −6.8, −8.3, −7.3, −8.4 kcal/mol. The formation of stable ligand-protein complexes of compounds 4–9 is correlated with their high antitumor activity. The highest anticancer activity of compounds 4,5 can be related to the presence of the piperidine ring containing the methyl group on positions 3 or 4 in their structure. The less pronounced antitumor activity of compound 7 in comparison with compounds 4, 5 pays an attention also. The compound 7 contains the piperidine ring as well as compounds 4, 5 but located at a greater distance from the sulfonamide group and does not have the methyl group. Thus, sulfonamides 4–9 can be considered as promising candidates in the development of new anticancer agents with a specific molecular mechanism of action.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.compbiolchem.2018.04.006.

References


