Journal of Molecular Structure 1133 (2017) 66–73

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Antitumor activity of 3,4-ethylenedioxythiophene derivatives and quantitative structure-activity relationship analysis

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ARTICLE INFO

Article history:
Received 31 August 2016
Received in revised form
24 November 2016
Accepted 24 November 2016
Available online 25 November 2016

Keywords:
Antitumor agents
Heterocycles
Computational chemistry
QSAR
Drug research
Cell culturing

ABSTRACT

The aim of this study was to evaluate nine newly synthesized amidine derivatives of 3,4- ethylenedioxythiophene (3,4-EDOT) for their cytotoxic activity against a panel of human cancer cell lines and to perform a quantitative structure–activity relationship (QSAR) analysis for the antitumor activity of a total of 27 3,4-ethylenedioxythiophene derivatives. Induction of apoptosis was investigated on the selected compounds, along with delivery options for the optimization of activity. The best obtained QSAR models include the following group of descriptors: BCUT, WHIM, 2D autocorrelations, 3D-MoRSE, GETAWAY descriptors, 2D frequency fingerprint and information indices. Obtained QSAR models should be relieved in elucidation of important physicochemical and structural requirements for this biological activity. Highly potent molecules have a symmetrical arrangement of substituents along the x axis, high frequency of distance between N and O atoms at topological distance 9, as well as between C and N atoms at topological distance 10, and more C atoms located at topological distances 6 and 3. Based on the conclusion given in the QSAR analysis, a new compound with possible great activity was proposed.

1. Introduction

Cancer is a major global health problem and its rates increase every year. In 2012, there were 14.1 million new cancer cases and 8.2 million deaths directly caused by cancer. Cancer treatment research is fundamental to improving outcomes for patients affected by the disease. These efforts include the development of more effective, more selective and less toxic treatments, such as targeted therapies, immunotherapies and cancer vaccines. As part of the research, special attention is paid to the discovery of new molecules that selectively bind to DNA [1,2]. Over the past few decades, a relatively large number of useful anticancer drugs have been discovered or rationally designed based on the principle of nucleic acids recognition [3,4].

Aromatic amidines are structural parts of numerous compounds of biological interest and form important medical and biochemical agents exhibiting a broad spectrum of significant antimicrobial activity [5–9] and potency, but so far they have been less investigated for their antitumor activity [10,11]. Although the mechanism of action of aromatic amidines has not been fully elucidated, it has been proven that their bioactivity is a direct result of DNA binding and subsequent inhibition of DNA-dependent enzymes or possibly direct inhibition of transcription [12,13]. Despite a broad-spectrum activity of these compounds, only pentamidine has been found to be of significant use in humans, although it displays several adverse side effects. Its toxicity, lack of oral availability and appearance of pentamidine resistance stimulate the development of additional drugs for treatment. A large number of pentamidine analogues have been synthesized and intended to replace the unstable and flexible alkyldiether linker with conformation-restricted five-member heterocycles. Further modifications of two aromatic moieties and terminal amidine group had the objective to improve useful therapeutic properties and reduce undesirable effects. For this reason, over time, our group has synthesized and tested a number of new analogues of pentamidine with stable 3,4-ethylenedioxythiophene as a central linker [7,14–16]. Benzene

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http://dx.doi.org/10.1016/j.molstruc.2016.11.074
0022-2860/

and/or benzimidazole rings were aromatic moieties and unsubstituted, alkyl-substituted or cyclic amidines were used as terminal groups. These structural modifications were meant to increase the stability of the complex formed with DNA minor groove and enhance the biological activity of the compounds. All synthesized compounds were tested on various human cancer cell lines and some of them showed highly significant inhibitory activity.

The aim of this study was to determine the antitumor activity of 27 3,4-EDOT derivatives against six carcinoma cell lines and derive a quantitative structure–activity relationship (QSAR) analysis. The goal of the QSAR analysis was to find out which physicochemical and quantum-chemical molecular properties influence enhanced antitumor activity.

2. Material and methods

2.1. Compounds

The synthesis, physical properties and antitumor activity of symmetrical EDOT's derivatives (**1**–**18**) have been described previously [14–16]. The synthesis and physical properties of asymmetrical compounds **19**–**27** were given earlier [7] and theirs antitumor activity is described in the present study. Structural details of all studied molecules are shown in Fig. 1.

2.2. Cell culturing and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test

Antitumor activity for compounds **19** through **27** was tested as follows: tumour cell line derived from MIAPaCa2, CaCo2, HEp2 and HeLa were grown in DMEM medium (Gibco, EU). The NCI H358 and AGS cell lines were grown in RPMI 1640 medium (Gibco, EU). Both media were supplemented with 10% heat-inactivated fetal bovine serum-FBS (Gibco, EU), 2 mM glutamine (Gibco, EU), 1 mM sodium pyruvate (Gibco, EU), 10 mM HEPES (Sigma-Aldrich, USA) and 100 U/0.1 mg antibiotic/antimycotic (Gibco, EU).

Cells were grown on 37 °C, with 5% CO_2 gas in humidified CO_2 incubator (IGO 150 CELLlife™, JOUAN, Thermo Fisher Scientific, Waltham, MA, USA). A trypan blue dye exclusion method was used to assess cell viability. Tested compounds were dissolved in DMSO (dimethyl sulfoxide) as a 1×10^{-2} M (mol dm^{-3}) stock solution. Working dilutions were prepared at a concentration range 10^{-3}–10^{-6} M.

For the MTT test [17] cells were seeded on 96 micro well flat bottom plates (Greiner, Austria) at 2×10^4 cells/ml. After 72 h of incubation with the tested compounds MTT (Merck, Germany) was added. DMSO (Merck, Germany) was used to dissolve the formed MTT-formazane crystals. Absorbance was measured at 595 nm on Elisa micro plate reader (iMark, BIO RAD, Hercules, CA, USA). All experiments were performed three times in triplicates. The IC_{50} values, defined as the concentration of compound achieving 50% of cell growth inhibition, were calculated, and used to compare cytotoxicity among the compounds.

2.3. Compound delivery

Two delivery systems were tested on compounds **19** and **20**, liposome and albumin based, respectively. Lipofectamine (Sigma, EU) was used to form liposomes containing selected compounds. Selected compounds were diluted in OptiMEM (Gibco, EU) to the concentration of 2×10^{-5} mol dm^{-3} and then, mixed with equal volumes of 10% Lipofectamine in OptiMEM according to the manufacturer's instructions. Final concentration of Lipofectamine was 5% and compound concentration was 10^{-5} mol dm^{-3}. Fresh liposomes were made for every treatment.

Albumin particles were made by solvatation as previously described [18,19]. Briefly, albumin, fraction V (Roche, Switzerland) was dissolved in sterile water to a concentration of 100 mg/mL, while the selected compound was dissolved to target concentration of 10^{-5} mol dm^{-3} in ethanol. Compound solution was then added dropwise to the albumin solution under constant mixing with final ethanol to albumin volume ratio of 2:1. Glutaraldehyde (8%, v/v, Sigma, EU) was added to enable cross-linking and the solution was left for 24 h with constant mixing. After 24 h particles were centrifuged at 600×*g* for 10 min and the pellet was resuspended in water in an ultrasound water bath for 10–15 min. Samples were washed 3 times before the resulting solution was stored at 4 °C. The both delivery systems were tested on CML cells (K562) using MTT test as described above.

2.4. Apoptosis induction

To determine apoptosis induction after treatment by selected compounds we measured changes in mitochondrial membrane potential using the Mitochondrial Membrane Potential Kit (Sigma, EU). In brief, K562 cells were plated in 6-well plates (5×10^5 cells/mL) and treated with selected compounds at the concentration 10^{-5} M for 24 h. Cells were then stained according to the kit protocol and analysed by flow cytometry (FacsCanto II, BD Biosciences, USA) using Flowlogic software (Inivai Technologies).

2.5. Regression analysis and validation of models

The dataset used for building QSAR models consists of 27 molecules whose antitumor activity was measured and described in present and our previously published papers [14–16]. The synthesis and physical properties of compounds have been described previously [7,14–16]. Antitumor activities expressed as IC_{50} (μM), were converted in the form of the logarithm ($logIC_{50}$) and presented in Table 1. For the inactive compounds whose IC_{50} values are estimated as ">200", log IC_{50} was set to 2.40.

The 3D structures were optimized using molecular mechanics force fields (MM+) using the HyperChem 8.0 Subsequently, all structures were submitted to geometry optimization using the semi-empirical AM1 method and several physico-chemical and quantum-chemical descriptors were calculated by HyperChem 7.0 (HyperCube, Inc., Gainesville, FL).

The 2D and 3D molecular descriptors used in this study were calculated by applying the online software Parameter Client (Virtual Computational Chemistry Laboratory, http://146.107.217.178/lab/pclient/) an electronic remote version of the Dragon program [20]. Seventeen groups of Dragon's descriptors were used to generate QSAR models: constitutional, topological, walk and path counts, connectivity, information, 2D autocorrelations, edge adjacency, BCUT (Burden eigenvalues), topological charge, eigenvalue-based, geometrical, RDF (Radial Distribution Function), 3D-MoRSE (3D-molecular representation of structures based on electron diffraction), WHIM (WeigHted Covariance Matrices), GETAWAY (Geometry, Topology, and Atom-Weights AssemblY) descriptors, functional group counts, and molecular properties [21].

The selection of descriptors based on the best-subset method and the multiple regression analysis (MLR) was performed with the use of STATISTICA 12 (StatSoft, Inc. Tulsa, USA). The number of descriptors (*I*) in the multiple regression equation was limited to two, in consideration of the fact that the number of compounds in the training set was 22.

Prior to splitting a data set on training and a test set, the initial number of 1280 calculated molecular descriptors and physicochemical properties were reduced to 35 descriptors. The procedure started with the elimination of variables that are weakly correlated