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Development of highly predictive 3D-QSAR CoMSIA models for anthraquinone and acridone derivatives as telomerase inhibitors targeting G-quadruplex DNA telomere

Vishal P. Zambre, Prashant R. Murumkar, Rajani Giridhar, Mange Ram Yadav*

Pharmacy Department, Faculty of Technology and Engineering, Kalabhavan, The M.S. University of Baroda, Vadodara 390001, Gujarat, India

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ABSTRACT

G-quadruplex structures of DNA represent a potentially useful target for anticancer drugs. Telomerase enzyme, involved in immortalization of cancer cells is inhibited by stabilization of G-quadruplex at the ends of chromosomes. Anthraquinone and acridone derivatives are promising G-quadruplex ligands as telomerase inhibitors. So far, optimization of these ligands remained hampered due to the lack of creditable quantitative structure–activity relationships. To understand the structural basis of anthraquinone and acridone derivatives, a predictive 3D-QSAR model has been developed for the first time for telomerase inhibitory activity of G4 ligands, employing comparative molecular similarity indices analysis (CoMSIA). Considering the proposition that the basic nitrogens in these compounds should exist in protonated form at physiological pH the protonated forms of the reported compounds were analyzed and investigated. The QSAR model from conformational template Conf1 exhibited best correlative and predictive properties. The actual predictive abilities of the QSAR model were thoroughly validated through an external validation test set of compounds. The statistics indicate a significantly high prediction power of the best model (r^2, 0.721), supporting the proposed molecular mechanism of DNA G-quadruplex ligands.

1. Introduction

Telomerase is a complex ribonucleoprotein reveres transcriptase enzyme. RNA template (hTR) and a catalytic protein domain (hTERT) are two major components of human telomerase [1]. Telomeric DNA consists of repetitive guanine-rich sequences which forms secondary structures based on reverse Hoogsteen-type base pairing involving four guanines in a planar arrangement termed G-tetrad [2,3]. Telomere length progressively shortens in somatic cells with successive rounds of cell division, leading eventually to senescence and apoptosis. In contrast telomere length is maintained in cancer cells and is a major factor in immortalization and tumorigenesis [4]. This happens almost always under activation of the enzyme telomerase [5] which has made it a highly selective target for antitumor drug design [6,7]. Various strategies have been proposed to induce telomerase inhibition [8]. A promising strategy is based on the inhibition of telomerase by interacting with G-quadruplex DNA.

G-quadruplex DNA interactive compounds inhibit telomerase *in vitro* by stabilizing single stranded 3′-telomere ends as a quadruplex [9,10]. The best example is a ligand called quarfloxacin (formerly CX-3543) that is now in phase-II clinical trial as an anticancer agent [11].

Substituted acridines and anthraquinones have been reported previously [12,13] to stabilize G-quadruplex. Campbell et al. reported structural basis of DNA quadruplex recognition by an acridine drug (BRACO-19) [14] and also discussed issues related to selectivity in ligand recognition of G-quadruplex loop [15]. Acridone derivatives are another class of ligands that stabilize the G-quadruplex structures possessing comparable telomerase inhibitory activity to acridine derivatives [16].

Harrison et al. [16], Read et al. [17], and Perry et al. [18,19] carried out structure–activity relationship studies of telomerase inhibitors. Effects of amide bond direction on modulation of G-quadruplex recognition and telomerase inhibition by substituted anthracenedione derivatives have studied [20]. Recently, Cuenca et al. postulated that the incorporation of aromatic side chains to the acridone core in the 4 and 5 position would enhance G-quadruplex affinity, although these derivatives did not show telomerase inhibitory activity [21]. A three-dimensional structure–activity relationship (3D-QSAR) as rational basis for the G-quadruplex ligand optimization for anthraquinones and acridones remains unreported. With 3D-QSAR analysis it is possible to analyze the probable structural elements affecting the biological activity of compounds. We have been involved in the computer aided designing of novel acridine derivatives as telomerase inhibitors

* Corresponding author. Tel.: +91 265 2434187; fax: +91 265 2418927.
E-mail addresses: mryadav11@yahoo.co.in, mryadav-phar@msu.ac.in (M.R. Yadav).

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using CoMFA (comparative molecular field analysis) and CoMSIA (comparative molecular similarity indices analysis) approaches [22]. Various other targets like tumor-necrosis alpha converting enzyme (TACE) [23,24], farnesyltransferase [25–27] and COX-II (cyclooxygenase-II) [28] have been successfully exploited for rational design of novel enzyme inhibitors. Structural requirements for anthraquinone and acridone derivatives in terms of physicochemical descriptors have been studied in this laboratory for their G-quadruplex stabilizing telomerase inhibitory activity using 3D-QSAR techniques. In this paper, we report 3D-QSAR CoMSIA [29] studies from a panel of 61 telomerase inhibitors obtained from literature [16–19]. Considering that the basic nitrogens in these compounds should remain protonated at physiological pH, the protonated forms were analyzed and investigated in the study. Some remarkable observations were made during this study in relation to the structures of these molecules and their putative binding site of G-quadruplex. All these findings are reported in this work, which is a first successful attempt of its kind for lead optimization using 3D-QSAR CoMSIA technique for telomerase inhibitory activity of anthraquinone and acridone derivatives. The model so developed showed high correlation and prediction of G-quadruplex ligands with telomerase inhibitory activity specifying that telomerase inhibition and G-quadruplex stabilization was directly related. This strongly supported the proposed unified mechanism of telomerase inhibition. CoMSIA studies obtained with hydrophobic, H-bond acceptor and H-bond donor fields (HAD) afforded the best model with good predictive power outlining the dominant role of these fields for telomerase inhibitory activity of the studied G-quadruplex stabilizers. CoMFA [30] studies were also performed for the chosen series of compounds prior to the CoMSIA studies. The CoMFA models (data not shown) obtained were statistically inferior to CoMSIA models, which further confirmed the involvement of hydrophobic interactions as dominant field for anthraquinone and acridone derivatives as telomerase inhibitors.

2. Materials and methods

2.1. Data set

The molecular structures and activities of 61 telomerase inhibitors were taken from the literature [16–19]. The telomerase inhibitory activity of compounds is reported as EC_{50} values in the micro molar (μM) range. The selected compounds cover a wide range of biological activity (0.2–50 μM) and diverse structural features. The reported EC_{50} values were converted into $-\log(EC_{50})$, i.e. pEC_{50} for use in the QSAR studies. The whole set of 61 compounds (Table 1) was divided into training set of 49 and test set of 12 compounds in the process of model refinement for all CoMSIA models reported herein. In the training set, most potent, moderately active and lowly active compounds were included to spread the activity range. The test set compounds were selected in such a manner that at least one structural analog remained in the training set.

2.2. Molecular modeling

A Silicon Graphics Fuel workstation with IRIX 6.5 operating system running SYBYL 7.0 [31] was used for three-dimensional structure building and molecular modeling studies. Three different molecular alignments were carried out in the present study. Initially, two molecular alignments, based on MacroModel Monte Carlo conformational search-derived templates, were derived using centroid and atom-based alignment rule in SYBYL. The two conformational search-based templates were derived as follows. The most active compound **61** was constructed in SYBYL. Its structure was minimized, and then used as a starting point for a Monte

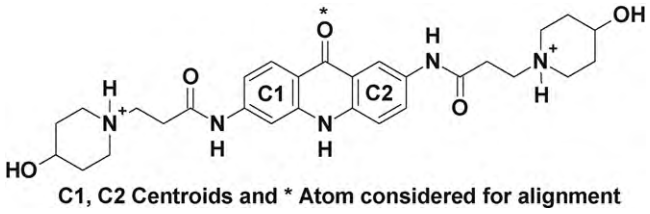

Fig. 1. Template for alignment.

Carlo conformational search employing MacroModel version 7.0 (Schrödinger, Inc.). The conformational search was carried out using MMFF94 force field in MacroModel for 5000 iterations using water as a solvent. The global minimum conformation, designated as Conf1, and the second lowest energy conformation from the global minimum, designated as Conf2 were used as templates to construct the rest of the molecules. A third alignment was derived as discussed below:

The conformation of a disubstituted acridine derivative (biologically active moiety) was extracted from the co-crystallized structure of G-quadruplex PDB 1L1H [32]. Conf1 of the most active compound **61** was superimposed on the conformation of the acridine derivative, extracted from the co-crystallized structure. As a measure of superimposition reliability, the rmsd between these two conformations was taken into the consideration (0.9 Å obtained herein). It was interesting to note that the resulting conformation (conf3) of compound **61** fully superimposed (rmsd 0.00 Å) on conformation conf1. Hence, conformations conf1 and conf2 only were used as templates for further study. The partial atomic charges required for the electrostatic interactions were computed by the semi empirical molecular orbital method using Molecular Orbital PACkage (MOPAC) [33] with Austin Model 1 (AM1) Hamiltonian [34]. The centroids and atoms considered for alignment are marked with C1, C2 and an asterisk (*), respectively in Fig. 1. The superimposition of all the compounds on template (compound **61**) is shown in Fig. 2.

2.3. pK_a value estimation

Pallas 3.7.1.1 software from CompuDrug International Inc. [35] was used to calculate pK_a values for side chain substituent piperazine, in compounds **11**, **50**, and **54** (Table 2). This program provides an indispensable resource for predicting acidic and basic pK_a values before synthesis in QSAR studies.

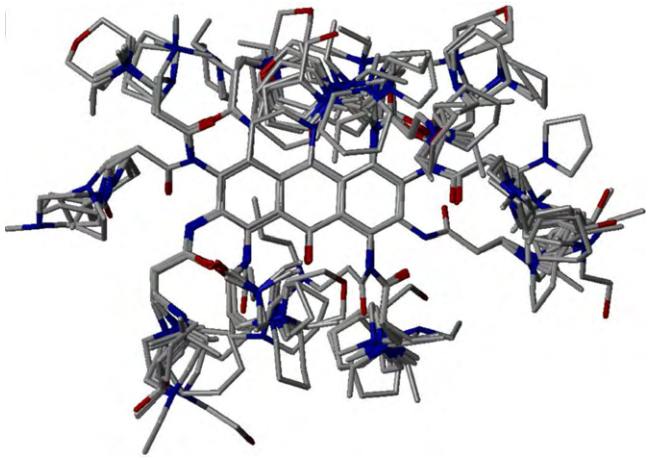

Fig. 2. Alignment of all compounds.