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Research paper

Stabilizing parallel G-quadruplex DNA by a new class of ligands: Two non-planar alkaloids through interaction in lateral grooves

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A B S T R A C T

Human DNA sequences consisting of tandem guanine (G) nucleotides can fold into a four-stranded structure named G-quadruplex via Hoogsteen hydrogen bonding. As the sequences forming G-quadruplex exist in essential regions of eukaryotic chromosomes and are involved in many important biological processes, the study of their biological functions has currently become a hotspot. Compounds selectively binding and stabilizing G-quadruplex structures have the potential to inhibit telomerase activity or alter oncogene expression levels and thus may act as antitumor agents. Most of reported G-quadruplex ligands generally have planar structures which stabilize G-quadruplex by π–π stacking. However, based on a pharmacophore-based virtual screening two non-planar G-quadruplex ligands were found. These two ligands exhibit good capability for G-quadruplex stabilization and prefer binding to paralleled G-quadruplex rather than to duplex DNA. The binding of these ligands to G-quadruplex may result from groove binding at a 2:1 stoichiometry. These results have shown that planar structures are not essential for G-quadruplex stabilizers, which may represent a new class of G-quadruplex-targeted agents as potential antitumor drugs.

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1. Introduction

Human DNA sequences that consist of tandem guanine (G) nucleotides can fold into a four-stranded structure named G-quadruplex via Hoogsteen hydrogen bonding [1]. As the sequences forming G-quadruplex exist in essential regions of eukaryotic chromosomes and are involved in many important biological processes [2], the study of their biological functions has currently become a hotspot. Compounds selectively binding and stabilizing G-quadruplex structure have the potential to inhibit telomerase activity or alter oncogene expression levels and thus may act as antitumor agents [3–9]. A number of G-quadruplex ligands with the common feature of an extended aromatic ring system capable of interacting through extensive π–π stacking with G-tetrads [6,10,11] have been reported. For example, PIPER either forms a sandwich complex bound between the blunt ends of a quadruplex dimer formed from $[d(TTAGGG)]_4$, or intercalates by end-stacking at the GpT step of $[d(TTAGGGTTA)]_4$ [12]. Other compounds such as TmPyP4 and RHPS4, also interact with the G-quadruplex DNA by stacking with the G-tetrads planar [13,14]. Recent research on Distamycin A showed that ligand could interact with the G-quadruplex structures in the grooves [15]. As distamycin A is generally a planar molecule, it forms dimer to fit the size of the G-quadruplex grooves. Although Brassart's work showed that several non-planar steroids could bind to G-quadruplex structures [16], there is still no enough information about the structural bases of these novel non-planar G-quadruplex ligands and the molecular mechanism of the G-quadruplex–ligands interaction. Thus it is of interest to find more non-planar structural molecules capable of stabilizing G-quadruplex, which is the motivation of our present study. We investigated ligand-based pharmacophore for G-quadruplex ligands to enhance the process for new G-quadruplex ligands discovery which combined CATALYST HypoGen approach [17]. In this study, feature-based three-dimensional model for G-quadruplex ligands was developed and

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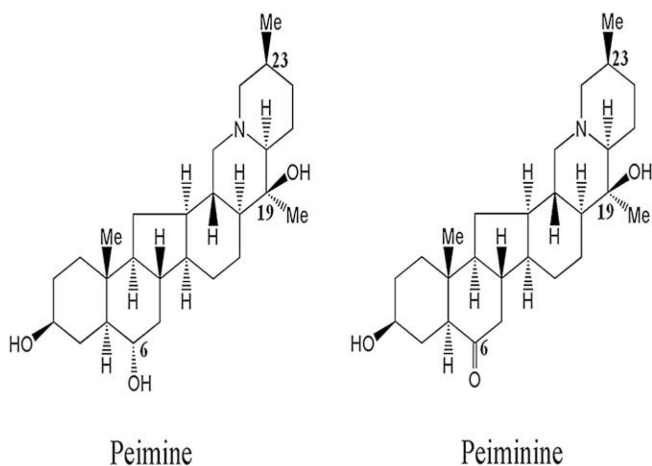
the model was then used as a search query for the Chinese Herbal Medicine Database (CHMD) in an attempt to find new classes of G-quadruplex ligands. Among the screening result, potential hit compounds were selected for further study. Herein, we report a successful discovery of two novel G-quadruplex ligands with non-planar structures, peimine and peiminine (Scheme 1) by virtual screening, which exhibit good capability for G-quadruplex stabilization through groove binding mode as determined by measuring the variant-temperature CD spectra, the proton NMR spectra and NOESY spectra.

2. Materials and methods

2.1. Virtual screening

A series of 1,4-disubstituted anthraquinone derivatives [18] showing cytotoxicity against the telomerase positive rat glioma C6 cells [19] were chosen to comprise the training set (Supplementary material). The biological activity of these compounds is supposed to be due to the stabilization of the G-quadruplex and further induces telomerase inhibition. The activities for the training set of the 38 compounds covers 3 orders of magnitude (0.07 μM $\geq IC_{50} \geq$ 103 μM). The pharmacophore model construction and following compound database screening were carried out using the CATALYST software package (version 4.11, Accelrys Inc., San Diego, CA). All of the molecules were built up and minimized using modified CHARMM force field in the CATALYST package. Catalyst can generate a conformational model that represents the flexibility of a molecule using a Monte-Carlo-like algorithm together with poling. The conformational model emphasizes broad coverage of conformational space and consists of a representative set of conformers taken from the range of energetically reasonable conformations of the molecule. These conformational models could be used to fit the compound to a functional features comprised hypothesis. Therefore, the minimized structures were then used as the starting points for conformational analysis with confirm module. For each molecule, a maximum of 250 conformers that lie within 20 kcal mol^{-1} from the calculated potential minimum was considered for the model generation. The diverse conformational generation for each molecule ensured maximum coverage of the conformational space. While generating the HypoGen model, a minimum number of features involving hydrogen bond acceptor and donor, hydrophobic and positive ionizable moieties were specified on the basis of common feature identification.



Scheme 1. Structural formulas of Peimine and Peiminine.

The pharmacophore hypotheses derived from the training set is expected to qualitatively filter off the inactive molecules and recognize molecules with high activity. To identify new G-quadruplex stabilizers, we carried out virtual screening on the Chinese Herbal Medicine compounds 3D Database [20], using the fast flexible search approach implemented within CATALYST. The Chinese Herbal Medicine compounds 3D Database contains 9820 structures which derived from 2073 Chinese Traditional Medicine herbs records from 296 families and these records cover most of the Chinese Herbs. For the pre-generated Catalyst 3D database which contains 9820 molecule structures, 250 conformers were specified as the maximum number of conformers of each molecule in the compound database to ensure maximum coverage of the conformational space. The fast fit method was chosen to proceed the database searching.

2.2. General materials

DNA Hum7 d(TTAGGGT), M24 $d(TTAGGG)_4$, c-kit d(CGGGCGGG CGCGAGGGAGGGG), H24A $d(TTGGG(TTAGGG)_3A)$, H22 d(AGGG $(TTAGGG)_3)$, Dup duplex DNA sequence d(GGCATAGTGCGTGGGC GTTAGC) and its complementary strand were purchased from Tsingke Biotechnology Ltd. (Beijing, China), purified by C18 column. Peimine and Peiminine were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The 3-(trimethylsilyl) propionic acid-D_4 sodium salt (TSP) and DMSO-D_6 were purchased from Sigma Co. (USA). Other chemicals were of analytical reagent grades and ultra-pure water was used throughout the experiments.

2.3. Temperature-dependent CD experiments

To form parallel stranded G-quadruplex and duplex DNA, the buffer solution used was K-PO_4 buffer solution containing 17.2 mM K^+ and with 1 mM EDTA. Here, 17.2 mM K^+ is chosen because of the following two reasons: (1) G-quadruplex could be formed under both 17.2 mM K^+ and 100 mM K^+ conditions and their CD spectral patterns are almost identical; (2) Tetramolecular G-quadruplex structure of $[d(TTAGGGT)]_4$ is very stable when $[K^+]$ is more than 100 mM, making it difficult to evaluate the effect of these molecules on the stability of G-quadruplex. Parallel stranded G-quadruplexes were formed using the sequence Hum7 d(TTAGGGT) (5 μM in tetramolecular quadruplex), H24A $d(TTGGG(TTAGGG)_3A)$ (5 μM in single strand), c-kit d(CGGGCGGGCGCGAGGGAGGGG) (5 μM in single strand). Mixed-type G-quadruplex were formed using the sequence M24 $d(TTAGGG)_4$ (5 μM in single strand). Duplex DNA was formed using the sequence d(GGCATAGTGCGTGGGCGTTAGC) hybridized with its complementary strand (5 μM in duplex) and expressed as Dup in this text.

To form other types of G-quadruplex DNA structure, the buffer solution used was Na-PO_4 buffer solution containing 17.2 mM Na^+ and with 1 mM EDTA. The G-quadruplex was formed using the sequence H22 $d(AGGG(TTAGGG)_3)$ (5 μM in single strand).

The peimine and peiminine were initially dissolved as a 10 mM stock solution in DMSO. The molar ratio of [drug]/[DNA] was 10:1 in the CD experiments. The solution was equilibrated at room temperature for 24 h before measurements.

The formation of $[d(TTAGGGT)]_4$ was confirmed by circular dichroism (CD). Circular dichroism measurements were carried out on a Jasco-815 spectropolarimeter in a 1 cm path-length cell at room temperature. Spectra were collected with scan speed of 500 nm/min and response time of 0.5 s. Each spectrum was the average of five scans and corrected by buffer solution.

To determine the melting point, the CD melting curves were measured by monitoring characteristic signals of the quadruplexes

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