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A Series of β-Carboline Alkaloids from the Seeds of *Peganum harmala* Show G-Quadruplex Interactions

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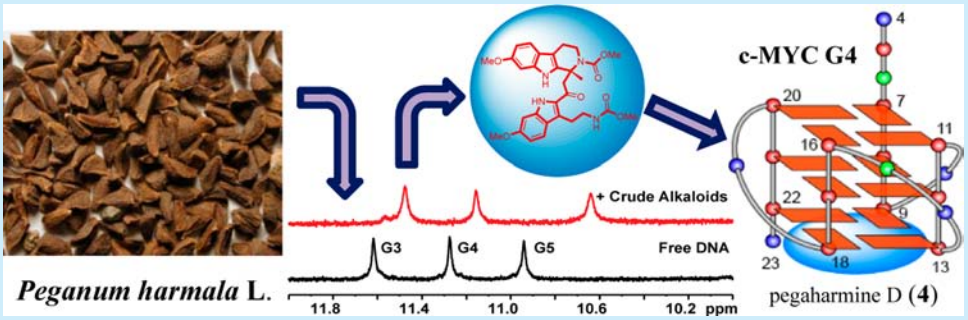
Supporting Information



ABSTRACT: In this study, we screened 17 medicinal plants for binding activity to G-quadruplex $d(TTGGGTT)_4$ by ^1H NMR spectroscopy and found that the crude extract of *Peganum harmala* L. seeds showed the most potential binding activity. Subsequently, ^1H NMR- and bioassay-guided isolation of the extract of *P. harmala* L. was performed to obtain four pairs of partially racemized β-carboline alkaloids, pegaharmines A–D (**1**–**4**). Their structures and absolute configurations were determined by extensive NMR analyses, X-ray crystallography, ECD calculations, and CD exciton chirality approaches. Interestingly, pegaharmine D (**4**), which showed the strongest G-quadruplex interaction, exhibited significant cytotoxic activity against three cancer cell lines. This work contributed a practical strategy for the discovery of novel G-quadruplex ligands from natural products and provided potential insights for using β-carboline alkaloids as anticancer lead compounds specifically targeting G-quadruplexes.

Natural products have long been an important source of therapeutic drug leads and continue to inspire new approaches in organic chemistry.[1] Indeed, ca. 50% of today's prescription drugs, and the majority of anticancer agents, can trace their original or underlying synthetic design principles to natural products.[1c,2] Famous antitumor drugs from natural products include paclitaxel, teniposide, camptothecin and vincristine.[1a] However, finding bioactive molecules from natural libraries is challenging due to their limited availability, structural complexity, and functional diversity.[1a,3] Recently, G-quadruplexes have emerged as attractive targets for anticancer drugs, with a novel mechanism of action. G-quadruplexes are four-stranded DNA secondary structures consisting of stacked G-tetrad planes and are found to play important regulatory roles in gene transcription and genome stability.[4] Small molecules which can stabilize or induce G-quadruplex formation have potential as antitumor agents through the alteration of oncogene expression levels or the disruption of telomere maintenance.[4] In this study, we screened 17 medicinal plants for novel G-quadruplex-interactive ligands. We found the crude extract from *Peganum harmala* L. seeds bound most potently to the G-quadruplex $d(TTGGGTT)_4$, with evident upfield shifts in the G-quadruplex characteristic imino region (δ_H = 10–12 ppm) of their ^1H NMR spectra, and showed specific cytotoxicity against the PC-3 cancer cell line with an IC_{50} value of 13.65 μg/mL (Figure S1 and Table S1).

P. harmala L., belonging to the medicinally important family of Zygophyllaceae, is mainly distributed in arid and semiarid regions of China, USA, Iran, and India, and is abundant in β-carboline alkaloids.[5] Its seeds, called "Luo-Tuo-Peng-Zi", are a traditional Chinese medicine frequently prescribed for the treatment of alimentary tract cancers and malaria in northwest China.[6] Using ^1H NMR and bioassay coguided fractionation of the crude extract from *P. harmala* L. seeds, we found that the crude alkaloids fraction displayed the strongest G-quadruplex binding activity and showed the most potent cytotoxic effects against HL-60, PC-3, and SGC-7901 cell lines, with IC_{50} values of 3.48, 10.59, and 11.53 μg/mL, respectively (Figure S2 and Table S2). Previous

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chemical studies also suggested the alkaloids are the main antitumor constituents in *P. harmala* L.[5] Further detailed isolation of these crude alkaloids led to the discovery of four pairs of partially racemized β-carboline alkaloids, pegaharmines A-D (**1**–**4**), characterized by the rare carbon skeleton of a tetrahydro-β-carboline fused with five-membered amide ring.[7] Two possible biosynthetic precursors, pegaharmine E (**5**) and harmaline, as well as the main alkaloid, harmine, were also identified. Notably, the partially racemized property was discovered unexpectedly, resulting from opposite absolute configurations determined by ECD calculations and X-ray crystallography. This phenomenon was further demonstrated by chiral HPLC and the formation was deduced by Pictet-Spengler reaction.

The antiproliferative activities and G-quadruplex binding activities were evaluated for the newly discovered and previously known β-carboline alkaloids. Significantly, the G-quadruplex interactions appeared to be positively correlated with the cytotoxic activities of these β-carboline alkaloids. The most cytotoxic compound **4** showed the strongest binding with the $d(TTGGGTT)_4$ G-quadruplex. Further investigation showed that compound **4** could selectively bind to the biologically relevant intramolecular parallel G-quadruplexes formed in the promoter regions of c-MYC,[4c] BCL-2,[8] and VEGF[9] genes, but not to the hybrid-type (3 + 1) human telomeric G-quadruplexes, or double- and single-stranded DNA. Here, the isolation, structural elucidation, biosynthetic considerations, biological evaluations, and G-quadruplex interactions of these alkaloids are presented.

Pegaharmine A (**1**) was initially obtained as a white amorphous powder. The ^{1}H NMR spectrum (Table S3) showed signals assigned to eight aromatic protons, one methoxyl group [δ_H 3.69 (3H, s)], one methyl group [δ_H 1.82 (3H, s)], one broad NH singlet (δ_H 8.07), three methylenes, and one methine, which were consistent with the HSQC experiment. The ^{13}C NMR spectrum (Table S3) displayed 24 discrete carbon resonance lines that were classified by HSQC experiment. Further detailed 2D NMR spectroscopic analyses enabled the construction of the planar structure of **1** (Figure 1). The full details of the structural assignment are described in the Supporting Information.

The relative configuration of **1** was determined based on the NOESY spectrum, and the Chem3D modeling suggested a conformation for compound **1** (Figure 2, left). The absolute

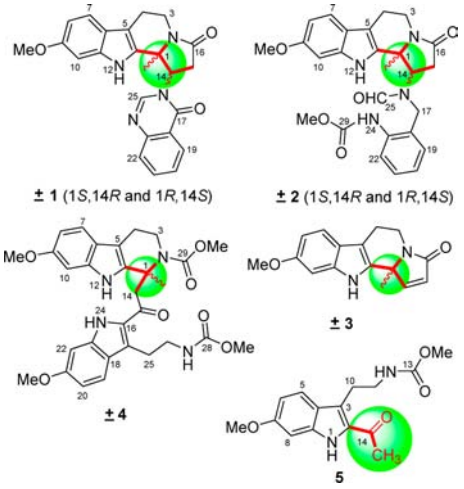

Figure 1. Structures of **1**–**5**.

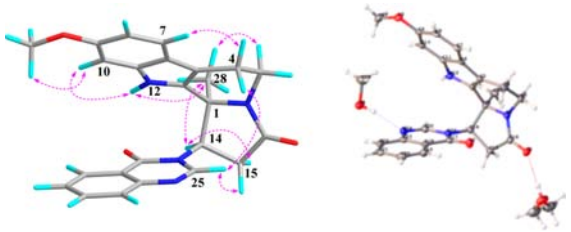

Figure 2. Selected NOESY (left, with hydrogens shown) and X-ray structure (right) of pegaharmine A (**1**).

configuration of **1** was first determined by ECD calculations[10] and CD exciton chirality[5c] approaches to be (1*R*,14*S*). Simulated ECD spectra of (1*R*,14*S*)-**1** using time-dependent density functional theory (TDDFT) matched well with the experimental data, assigning the absolute configuration of **1** (Figure S10). Moreover, the experimental ECD spectrum showed a positive exciton chirality according to a positive Cotton effect at 230 nm ($\Delta\varepsilon$ + 3.1) and a negative Cotton effect at 217 nm ($\Delta\varepsilon$ − 2.2), which further confirmed the configuration of 1*R* and 14*S* (Figure S9). Fortunately, a suitable quality single-crystal X-ray diffraction using Cu Kα radiation was obtained, which unambiguously confirmed the proposed structure of pegaharmine A (**1**) (Figure 2, right, CCDC 1476718). Unexpectedly, the absolute configuration was determined to be (1*S*,14*R*)-**1** in the X-ray crystal structure, which is reversed from the one assigned by the ECD calculations and CD exciton chirality approaches. This phenomenon indicated compound **1** has a partially racemic nature, as further supported by its weak optical activity [$[\alpha]^{20}_D$ +5.4 (*c* 0.12, MeOH)]. Subsequent HPLC of **1** on a chiral stationary phase led to the separation of two enantiomers, (+)-**1** and (−)-**1**, with a ratio of 54:46, which were opposite in terms of their CD curves and optical rotations. The ECD spectrum calculated for (1*S*,14*R*)-**1** agreed well with that measured for (−)-**1** (Figure S11, **1a**), and that calculated for (1*R*, 14*S*)-**1** matched well with that measured for (+)-**1** (Figure S11, **1b**). Thus, the structures for a pair of partially racemic enantiomers were determined and depicted in Figure S11. Notably, the conformation of **1** determined by X-ray crystallography was also different from the one assigned by NOESY experiment, with the quinazolinone moiety rotated ca. 180° (Figure 2). This observation suggested that, with the same absolute configuration, different stable conformations existed in solution vs crystal (Figures S10 and S11). Significantly, this partial racemization phenomenon points out the possibility that some reported natural products whose chiralities have been determined by ECD spectrum and optical rotation could in fact be partially racemic mixtures.

The structures of the four remaining pegaharmines B–E (**2**–**5**) were determined by NMR analyses and comparison with **1** (Supporting Information p 24). Full details on the structural assignment can be found in Tables S3 and S4.

The unreported partially racemized status found in these novel β-carboline alkaloids inspired us to further investigate their biosynthetic pathways. Strictosidine synthase (STR1), a "Pictet–Spenglerase," which stereoselectively converts the substrates tryptamine and secologanin to the β-carboline alkaloid, 3α-(*S*)-strictosidine, is the central enzyme in the biosynthesis of the β-carboline products, especially for the monoterpene indole alkaloids.[11] However, in the case of pegaharmine C (**3**), the important biosynthetic precursor of **1** and **2**, the observed partially racemic modification motivated us to further under-

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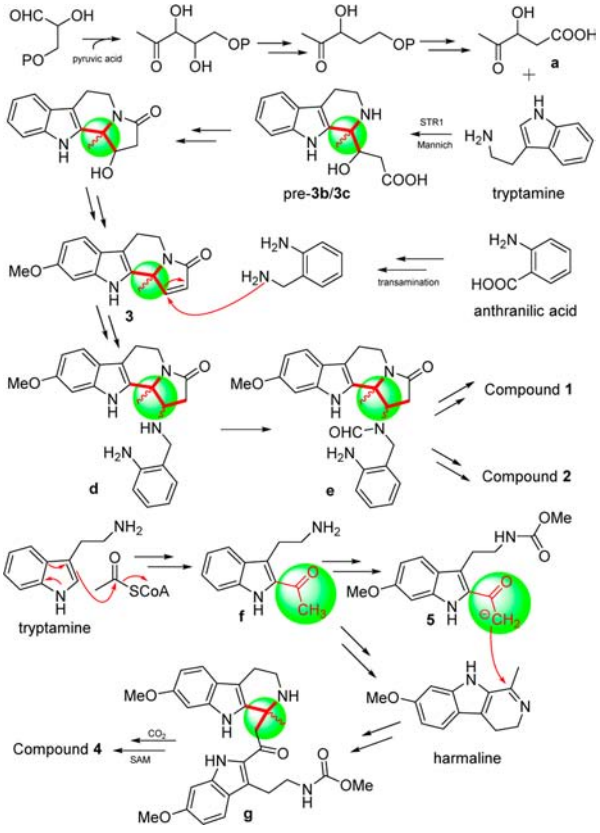
stand the structure of STR1 and to clarify the possible biogenetic mechanism. Through the biosynthetic considerations combined with the molecular docking analysis (Supporting Information p 17−20), we proposed that STR1 nonstereoselectively converted tryptamine and 3-hydroxy-4-oxopentanoic acid to the β-carboline products pre-**3b** and **3c** (Figure S7). They are eventually converted to the central biosynthetic intermediate pegaharmine C (**3**), with the (1*R*)-enantiomer slightly exceeding the (1*S*)-one. Moreover, it should be noted that the Pictet−Spengler condensation enzyme in the genus *Peganum* might differ from STR1 found in *Rauvolfia serpentine*,[11] and detailed information on the architecture of STR1 from the genus *Peganum* requires further investigation. Finally, biosynthetic pathways have been proposed to account for the racemic nature of these β-carboline alkaloids as well as the ability to incorporate different amines into the core structure (Scheme 1). Of note, the

Scheme 1. Hypothetical Biogenetic Pathways of Pegaharmines A−E (1−5)



known alkaloid harmaline could also be generated from the unreported intermediate **f** by a rational Schiff reaction, which is consistent with the reaction mechanism by which compound **1** was derived from the intermediate **e**.

The antiproliferative activities of these β-carboline alkaloids against cancer cell lines were assessed using the trypan blue method and the MTT method with 5-fluorouracil (5-Fu) as a positive control (Table S5).[5c,d] Pegaharmine D (**4**) showed significant cytotoxic activities against HL-60, PC-3, and SGC-7901 cell lines, with IC_{50} values of 3.81 ± 0.32, 11.52 ± 0.62, and 15.16 ± 0.42 μM, respectively. However, two monomers, pegaharmine E (**5**) and harmaline, were inactive. Additionally, pegaharmines A (**1**) and B (**2**) did not exhibit significant cytotoxicity against the three cancer cell lines, though their common biosynthetic precursor, pegaharmine C (**3**), showed moderate cytotoxic activity against HL-60 and SGC-7901 cell lines. These data indicated the dimerization and extended electron-deficient π-aromatic surface may be important for the antitumor activity of **4**; however, a constrained conformation could abolish such activity, as shown for **1** and **2**.

We then investigated the binding activities of these alkaloids with the G-quadruplex formed by $d(TTGGGTT)_4$ via ^{1}H NMR titration experiments (Figure 3). The G-quadruplex formed by

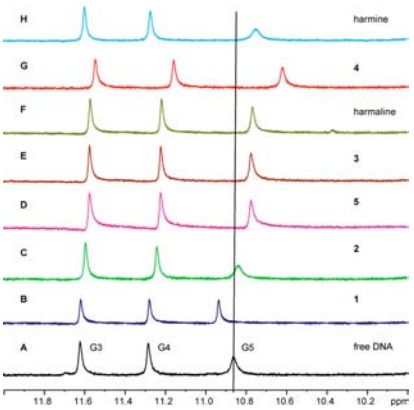


Figure 3. ^{1}H NMR spectra (δ = 10.0−12.0 ppm) showing imino protons signals of G3, G4, and G5 of $d(TTGGGTT)_4$ (G4DNA, 0.25 mM) in the absence (A) and presence of 0.25 mM of different compounds of *Peganum harmala*. **1** (B); **2** (C); **5** (D); **3** (E); harmaline (F); **4** (G); and harmine (H).

$d(TTGGGTT)_4$ is a simple but straightforward system for the screening of G-quadruplex-interactive ligands.[12] Three imino proton signals, i.e., G3, G4, and G5, were observed in the δ_H = 10.0−12.0 ppm region for the free $d(TTGGGTT)_4$, characteristic of G-quadruplex formation (Figure 3A).[11a] Adding 1.0 equiv of various pure β-carboline alkaloids differentially affected the ^{1}H NMR spectra (spectra B−H), indicating the varying extent of G-quadruplex interactions. The β-carboline alkaloids appeared to bind at the 3′-end G-tetrad of the $d(TTGGGTT)_4$ G-quadruplex as indicated by the G5 imino proton having the largest change in chemical shift. Intriguingly, the G-quadruplex interactions appeared to be positively correlated with antiproliferative activities in cancer cell lines, suggesting that these β-carboline alkaloids may exert their anticancer effects through G-quadruplex interactions. For example, the most cytotoxic pegaharmine D (**4**) appeared to be the most potent G-quadruplex interactive compound, as shown by the significant upfield shift of its imino protons, as compared to those of the less active compounds **1** and **2**.

Because the cytotoxic activities of β-carbolines appear to correlate with G-quadruplex interactions, we further investigated the interactions of these alkaloids with the monomeric intramolecular G-quadruplexes formed in the promoter regions of c-MYC,[4c] BCL-2,[8,13] VEGF,[9] and PDGFR-β[14] genes, and telomeric G-quadruplexes,[15] using ^{1}H NMR titration experiments. Interestingly, pegaharmine D (**4**) appeared to selectively bind to the biologically relevant parallel G-quadruplexes, such as those formed in the c-MYC[4c] (Figure 4), BCL-2 1245,[8] and VEGF[9] promoters; however, it did not bind to the (3 + 1) hybrid G-quadruplexes such as telomeric G-quadruplexes[15] and the BCL-2 mid G-quadruplex[13] (Figures S74−S78). In addition, compound **4** appeared to bind at the 3′-end G-tetrad of parallel G-quadruplexes, such as the G9-G13-G18-G22 tetrad in the c-

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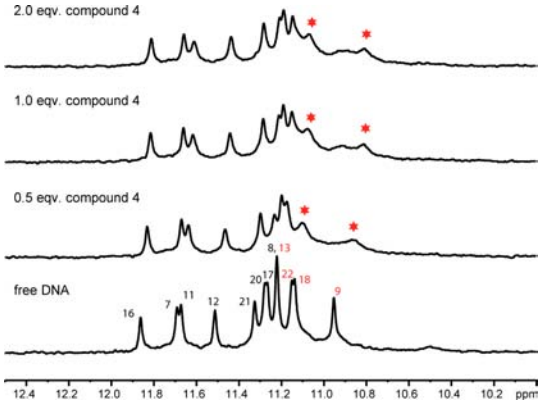

Figure 4. Imino proton region of the ^{1}H NMR titration spectra of the c-MYC G-quadruplex with compound **4**. The imino protons of the 3′-end G-tetrad are in red.

MYC G-quadruplex as shown by clear shift/broadening of imino proton signals (Figure 4), which is similar to its binding with the tetrameric d(TTGGGTT)$_4$ G-quadruplex. A CD melting study showed that **4** increased the melting temperature of the c-MYC G-quadruplex by 5 °C, but did not show a significant increase in the melting temperatures of VEGF and BCL-2 1245 G-quadruplexes (Figures S79−S81).

We further tested whether **4** could bind to double- or single-stranded DNA by ^{1}H NMR, which is an important consideration for G-quadruplex-targeting ligands. We used the self-complementary sequence d(TCGCGA)$_2$ as a model system for ds-DNA and d(CGCGTAA)$_2$ and d(CGCGTTT)$_2$ DNA which contain a single-stranded 3′-end overhang of −TAA or TTT, respectively, as a model system for 3′ single-stranded DNA. We found that compound **4** did not appear to bind double-stranded or single-stranded DNA even at the higher concentration of 150 μM. In contrast, harmine with a single-fused ring system showed obvious binding to ds-DNA (Figures S70−S73).

G-quadruplex structures formed in human oncogenic promoter regions may be an effective strategy for cancer therapy; thus, there is considerable interest in discovering novel molecules targeting these G-quadruplexes. In this study, we showed a practical strategy for the discovery of novel G-quadruplex-interactive ligands from natural products and presented the first report on the interactions of a class of β-carboline alkaloids with biologically relevant intramolecular parallel G-quadruplexes. Specifically, pegaharmine D (**4**) showed clear binding to the parallel monomeric promoter G-quadruplexes at the 3′-end G-tetrad, but not to the (3 + 1) hybrid intramolecular G-quadruplexes, or double- and single-stranded DNA. The quadruplex binding activity appears to correlate positively with the anticancer activity, which may present a novel mechanism of action for the β-carboline alkaloids. Moreover, these novel molecules provide insights into the biosynthetic pathway of β-carboline alkaloids, which implies that biogenetic mechanisms for creating distinct enantiomers may be widely expressed in this plant.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01560.

Experimental detail and characterization data (PDF)
X-ray data of compound **1** (CIF)

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Notes

The authors declare no competing financial interest.

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