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DNA-binding Properties and Topoisomerase-I Inhibitory Activities of Natural and Synthesized Protoberberine Alkaloids

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ABSTRACT

The specific and noncovalent interactions of small organic molecules with DNA are important in medicinal chemistry since they are useful for elucidating the mechanisms of anti-tumor and anti-virus drugs and for developing promising chemotherapeutic agents. Naturally-occurring protoberberine alkaloids possess various pharmacological activities including anti-microbial, anti-cancer, and topoisomerase inhibitory activities. It was reported that DNA-binding played important role in their bioactivity. However, these natural alkaloids showed modest DNA-binding affinity and non-significant sequence selectivity. In order to improve the DNA-binding affinity and sequence selectivity of these protoberberines with the aim of obtaining promising lead compounds, a series of investigations were carried out. The results were summarized as the following:

(1) Six novel berberine homodimers (**9-14**, linked with 2-7 methylenes, respectively) were successfully synthesized. Their binding affinities with calf thymus (CT) DNA and three double-stranded oligodeoxynucleotides ($d(AAGAATTCTT)_2$, $d(AAGCATGCTT)_2$, and $d(TAAGAATTCTTA)_2$) were investigated by fluorescence spectrometric titration. Compared with the monomeric parent compound berberine, these berberine dimers showed greatly enhanced binding affinities up to approximately 100-fold and showed a prominent structure-activity relationship (SAR) when they bound with short double-stranded DNAs. In all cases, ber-$(CH_2)_3$-ber exhibits the highest affinity, indicating that the propyl chain may be the most suitable linker to bridge the two berberine units. In addition, canadine showed no binding affinity with CT DNA, suggesting the quaternary ammonium cation and planar structure are important for the intercalative binding of protoberberine alkaloids with double-stranded DNA.

(2) Three demethylated protoberberine alkaloids, *i.e.*, berberrubine, jatrorubine, and palmatrubine, showed much higher EB displacement ratio than their parent compounds berberine, jatrorrhizine, and palmatine, respectively, suggesting that partially demethylation is an effective way to improve the DNA-binding affinities for these protoberberine alkaloids. The comparative study provided scientific evidence to explain why the antibiotic activities of processed Rhizoma Coptidis (制黄連) and processed Cortex Phellodendri (制黄柏) are stronger than those of the crude herbs..

(3) Both mono- and dimeric berberines form intercalating complexes with double-stranded DNA which was manifested through spectrophotometric titration and competitive EB displacement experiment.

(4) Dimeric protoberberines showed much stronger topoisomerase-I inhibitory activity than their natural congeners or mono-modified berberines, in analogy to the SAR discovered in DNA-binding studies. It demonstrated that these dimers are promising compounds which can exhibit anticancer activity. Protoberberine monomers were proved to inhibit topoisomerase-I through a mechanism of stabilizing enzyme-mediated DNA 'cleavable complex' as camptothecin does. Dimeric berberines at low concentration exhibited similar mechanism as that of CPT. At concentration above 200

μM, obvious inhibition of the relaxation activity of topoisomerase-I was observed. This is probably due to the strong binding affinity of these dimeric protoberberines toward plasmid DNA.

In conclusion, this study is an investigation on the noncovalent interactions between the active components from Chinese herbal medicines and double-stranded DNA using medicinal, bioorganic, and biochemical methods. The results obtained in this research provided substantial evidence for exploring the action mechanism of these alkaloids at molecular level, and demonstrated that structural modifications of small organic molecules from Chinese herbal medicines is a useful tool for new drug discovery and development.

TABLE OF CONTENTS