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ANTIPARASITIC ACTIVITY OF CERTAIN ISOQUINOLINE ALKALOIDS AND THEIR HYPOTHETICAL COMPLEXES WITH OLIGONUCLEOTIDES

M. A. Turabekova,[1,2*] V. I. Vinogradova,[2] B. F. Rasulev,[2] M. G. Levkovich,[2] K. Werbovetz,[3] J. Capers,[3] and N. D. Abdullaev[2]

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*The antiparasitic activity of two tetrahydrodiisoquinoline alkaloids **2**, **3**, β-allocryptopine (**4**), protopine (**5**), and a substituted phenylethylamine **6** was studied. Compounds **2** and **6** inhibited the growth of the parasite* Leishmania donovani. *The capability of the examined compounds to bind DNA was estimated by molecular modeling. It has been shown that binding occurs in the small groove and primarily at the AT-enriched part of the oligonucleotide.*

Key words: antiparasitic activity, alkaloids, molecular docking.

Protoberberine compounds include an important class of isoquinoline alkaloids (IQA) that exhibit a broad spectrum of pharmacological activity [1, 2]. The type of IQA is currently of great interest because of the characteristic manifestation of high cytotoxicity and antiparasite activity (for example, berberine is known in folk medicine as a powerful agent for treating many parasite-borne diseases) [3]. Considering the demand for new promising antiparasite and antitumor preparations [4, 5], the present work is very timely.

The antitumor activity of berberine (**1**) and its cytotoxic analogs is related to their ability to inactivate topoisomerase I (and topoisomerase II in certain instances). It is assumed that the key step in this process is the specific binding of the alkaloid to DNA that stabilizes the ternary complex DNA—topoisomerase—alkaloid [6-8]. Under normal conditions (without alkaloid), topoisomerase I performs one of its key tasks related to the cleavage of two DNA chains (introduces a single-strand break into the DNA chain), thereby preparing for the subsequent processes of replication and transcription. Fluorescence analysis and NMR spectroscopy have now established that berberine exhibits high affinity toward DNA [9]. Studies of the interaction of protoberberine alkaloids with oligonucleotides confirm the hypothesis that the alkaloid binds primarily to the small groove (SG) of the DNA helix [7, 9, 10].

The mechanism of the antiparasite activity of berberine and its analogs cannot be unambiguously determined by experimental data. In particular, it cannot be determined if this is a consequence of binding with kinetoplast DNA. However, it has been reported that compounds that are cytotoxic or have the capability to interact with human DNA typically exhibit antiparasite activity. The interaction with the SG or intercalation are usually examined because both of these types of interaction affect DNA and/or topoisomerase of parasites [11-13]. The alkaloid camptothecine, on the basis of which many antitumor preparations have been created and tested clinically, provides an example. Camptothecine, like berberine, forms ternary complexes with DNA and topoisomerase. it has been shown experimentally that camptothecine can form covalent DNA—topoisomerase complexes with human (nuclear) DNA and kinetoplast DNA isolated from trypanosomes, *leischmania*, and other parasites [6].

1) Mirzo Ulugbek National University of Uzbekistan, Tashkent, Republic of Uzbekistan, e-mail: malohathon@yahoo.com; 2) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent,Republic of Uzbekistan, fax (99871) 120 64 75; 3) College of Pharmacy, The Ohio State University, Columbus, USA. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 270-274, May-June, 2008. Original article submitted July 16, 2007.

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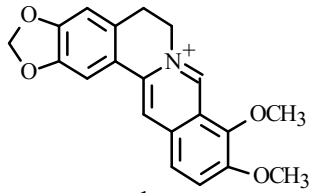
Berberine (**1**) is the most studied of several diisoquinoline alkaloids that are quaternary ammonium bases. This makes it an interesting subject for theoretical studies [14]. Preliminary computer docking studies of berberine and some of its analogs have been published. Molcular docking is the procedure of incorporating a ligand (most often a low-molecular-weight compound) into the active center of a receptor (i.e., biomacromolecules such as proteins and DNA). The goal of the search is to establish the most stable position of the studied molecule in the receptor, i.e., the energetically favorable ligand—receptor complex. The sequential search for such hypothetical stable complexes with the biological target and the evaluation of their quality can for most low-molecular-weight compounds reveal the factors that enhance the ligand—receptor interaction, i.e., increase the apparent activity. For example, the role of substituents and the position of the rings in berberine and certain of its derivatives in the docking to various chains of oligonucleotides has been investigated [6]. A hypothetical model of the ternary DNA—topoisomerase—alkaloid complex has also been studied for four protoberberine alkaloids [15].

Therefore, our goal was to study the capability of **2**-**6** to inhibit the growth of parasites *Leishmania donovani*. The capability of these compounds to interact intermolecularly with the SG of several oligonucleotides was evaluated by molecular docking. We examined simplified models of alkaloid—DNA complexes because there is no information on the actual structure of the berberine—DNA—topoisomerase complex and the calculations cannot be compared with experimental results. Such an approach is justified for two reasons. First, such systems have been well studied experimentally. Second, the activity of berberine toward topoisomerase I is known to be determined mainly by its interaction with DNA, the result of which is unwinding to a certain extent of the DNA helices (for example, to an angle of 11° [8]).



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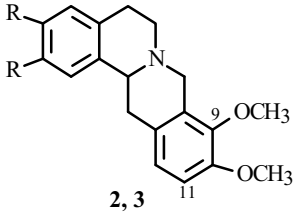

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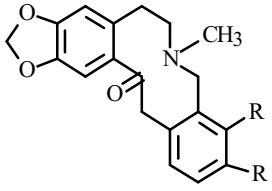

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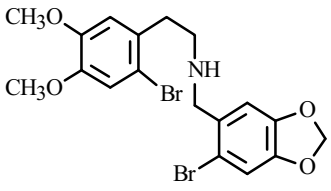

6

2, **5**: R-R = OCH_2O; **3**, **4**: R = OCH_3

Pharmacological Studies. Four isoquinoline alkaloids [tetrahydroberberine (**2**), tetrahydropalmatine (**3**), allocryptopine (**4**), protopine (**5**)] and *N*-(3′,4′-methylenedioxy-6′-bromobenzyl)-2,3-dimethoxy-6-bromophenylethylamine (**6**) were selected for evaluation of the capability to inhibit growth of *L. donovai*. A procedure using an axenic culture of *L. donovani* as amastigotes for rapid preliminary screening of a large series of compounds for antileishmania activity [16] was developed. Two compounds with moderate activity were found. These were tetrahydroberberine (**2**) (IC_{50} 71.3 μg/mL) and amine **6** (IC_{50} 63.5 μg/mL).

The antileishmania activity of **2**-**6** was:

Compound	Inhibition of L. donovani, IC_{50}, μg/mL
2	71.3
3	>100
4	>100
5	>100
6	63.5
Pentamidine, μM	1.6
GB-II-5, μM	4.8

Preliminary *in vivo* tests of the antiparasite activity of certain protoberberine alkaloids toward models of two types of leishmania (*L. donovani* and *L. braziliensis*) in Guinea pigs have been published [15]. It was shown that **2** was less toxic but had better antileishmania properties than its precursor **1**. It was also reported that palmatine, despite its structure that is similar to **1**, was inactive as an antiparasite. Our *in vitro* tests also found moderate activity for **2** and no antiparasite activity for tetrahydropalmatine **3**. It has also been reported that certain monomeric isoquinoline alkaloids with antileishmania activity also typically inhibit the growth of the parasite *Plasmodium falciparum* that causes malaria [17]. Although it was found that protopine and allocryptopine in addition to protoberberine and aporphine derivatives possessed antiprotozoa activity [17], our results did not indicate antileishmania activity for **4** and **5**.

TABLE 1. Docking Energies (kcal/mol) of **1**-**6** to SG of AT- and CG-Oligonucleotides

Compound	AT-oligonucleotide	AT-oligonucleotide+Na^+	CG-oligonucleotide	CG-oligonucleotide+Na^+
1*	-4.760	-4.621	-4.512	-4.570
1**	8.693	-8.811	10.878	-4.044
2*	-4.70	-4.762	-4.622	-4.627
2**	10.152	-3.666	15.961	3.677
3*	-4.564	-4.550	-4.436	-4.451
3**	-5.489	-18.146	3.934	-13.007
4*	-4.909	-4.494	-4.915	-4.902
4**	29.968	15.817	38.933	20.115
5*	-4.869	-4.857	-4.851	-4.871
5**	32.189	79.007	38.075	19.139
6*	-4.197	-4.165	-3.997	-4.032
6**	-3.758	-18.942	7.007	-10.495

*Docking of alkaloid to SG of oligonucleotide using ArgusDock procedure.

**Optimization of ligand-receptor complex in Amber force field.

Molecular Docking of Compounds in SG of Oligonucleotides. Typical preparations binding to the SG of DNA have several aromatic rings that can be pyrrole, furan, or benzene rings, condensed and/or freely rotating. The complex of DNA with the ligand binding to the SG is usually stabilized by Van-der-Waals forces, hydrophobic interaction, and formation of specific H-bonds between the ligand and the C-2 carbonyl of thymine or N-3 of adenine. The amino group of guanine in C—G base pairs interferes sterically with formation of H-bonds, analogous to ligand—AT complexes. This in turn is manifested as a significant effect on the three-dimensional geometry of the SG because it causes it to broaden. As a result, most low-molecular-weight preparations exhibit a high affinity to the narrower SG formed by AT bases. In contrast with intercalators, SG-binding preparations cause insignificant rearrangement and changes in the DNA helices [18].

Several oligonucleotide fragments constructed using the HyperChem program and consisting of only 10 AT (adenine and thymine) or CG (cytosine and guanine) pairs were used as the sample matrix. The first series of calculations consisted of automated docking of berberine with selected oligonucleotides using the ArgusDock algorithm in the ArgusLab program [19]. The ArgusDock algorithm places the ligand on the surface of the receptor region (or biological target) in a random order. Each successive position is independent of the previous three-dimensional orientation of the molecule and the binding energy. This approach is convenient for instances where there is no information on the possible binding site of the ligand to the receptor.

In the second series of calculations molecules **2**-**6** were examined. According to pharmacological data, only **2** and **6** showed moderate antiparasite activity (71.3 and 63.5 μg/mL, respectively). Molecular docking showed that the ArgusDock algorithm can dock the most energetically favorable sites of the ligand in the shortest time. Then, the resulting most stable ligand—receptor complex was also optimized in the Amber force field. Table 1 lists the calculation results. It can be seen that the presence or absence of Na^+ counterions does not substantially affect the docking results in ArgusDock. The docking energies using the ArgusDock algorithm for **1**-**6** are very similar. Neither the type of alkaloid nor the type of oligonucleotide changes this substantially. However, the results for the IQA—oligonucleotide complex, also performed using the Amber program, indicate clearly the need to consider the counterions in order to obtain correct results. For example, the positive energy for the berberine—AT system indicates that such a complex is unstable whereas the calculations for the **1**—AT(Na^+) system agree with the experimentally observed property of berberine to bind DNA. Next, it can be seen the the complex energy **2**—AT(Na^+) is greater than the energy of **1**—AT(Na^+), i.e., the complex of **1** is more stable than that of **2**. Therefore, **1** is more active. This also supports the experimental data for their antiparasite activity. The energies of AT— and CG—oligonucleotide complexes with **4** and **5** (compounds that do not affect growth of *L. donovani*) are also high, indicating the formation of such systems is improbable. The exception was **3**, which theoretically has affinity for DNA analogous to that of **2** but did not show substantial antiparasite activity either in our *in vitro* tests or in the literature [20]. Experimental data for palmatine (tetradehydrogenated analog of **3**) confirmed that it has high affinity for DNA [8, 21, 22]. The calculated energies for complexes of **6** showed high affinity for DNA. Formation of the **6**—AT complex is energetically more favorable than formation of **6**—GC. The high affinity of **6** for oligonucleotides can be explained by its high flexibility that enables it to situate itself favorably in the SG.

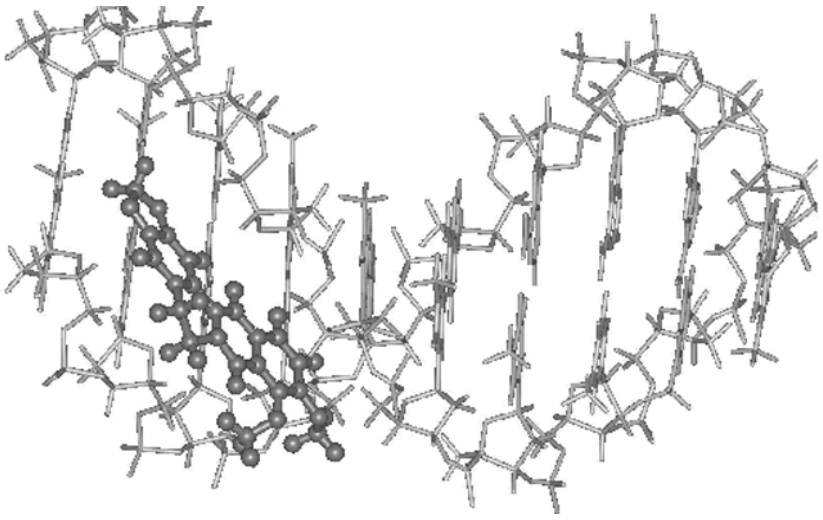

Fig. 1. Model of $(dAT)_{10}$ oligonucleotide complex with berberine bound in the SG.

It is important to note the effect of the C-9 substituent on the affinity for DNA of the protoberberine alkaloids. Thus, some studies note a decreased ability to bind DNA and; therefore, to inhibit topoisomerase if the methoxy is shifted from the 11-position to the 9-position [8]. However, other studies found that addition to C-9 of bulky functional groups increases the probability of forming additional H-bonds and electrostatic interactions that strengthen the ligand—DNA complexes [10]. The ligand in one of the first calculated models of protoberberine alkaloids with DNA is bound through intercalation, i.e., incorporated between parallel nucleotide bases [8]. Low-molecular-weight compounds in the ligand—oligonucleotide complexes calculated herein are incorporated exclusively into the SG of DNA. This agrees with published models [9]. The principal difference between our models and those published [9] is that the molecule is oriented not toward the external part of the complex but is drawn into the inner region (Fig. 1) for binding of berberine and its analogs to C5–C6–N^+–C8.

In certain instances, the experimental and theoretical study of the binding specifics of protoberberine-type molecules with a certain type of oligonucleotides indicates that the molecules prefer to bind to the AT-portion of DNA [8, 9] or in general lack any specificity for oligonucleotides with a different sequence [21, 22].

The overall trend in energies of complexes that were calculated using the Amber program taking into account Na^+ indicates that protoberberine-type alkaloids **1**-**3** and amine **6** bind primarily to AT—oligonucleotide. They typically have less affinity for GC—oligonucleotide (**1**, **3**, **6**) or none at all (**2**). For **4** and **5**, which showed no affinity for AT, formation of ligand—GC complexes is also improbable.

Thus, the capability of **2**-**6** to suppress growth of *L. donovani in vitro* was studied experimentally. Compounds **2** and **6** possessed moderate antiparasite activity. In addition, calculations estimated the formation of hypothetical complexes of **1**-**6** with AT— and GC—oligonucleotides. Our calculations showed that berberine and its analogs form rather stable alkaloid—oligonucleotide complexes in which they are bound primarily to AT-enriched portions of DNA.

EXPERIMENTAL

The procedure for cultivating *L. donovani* and conditions for performing the biological screening have been described in detail [17]. The standards were the medicinal preparation pentamidine [23, 24], which is used to treat parasite diseases, and GB-II-5 (a new platform compound based on orysalin with high antitrypanosome and antileishmania activities) [25].

Molecular structures of **1**-**6** were constructed and optimized preliminarily in the Amber force field (molecular mechanics) with subsequent re-optimization using the AM1 semi-empirical approach (Polak-Ribiere calculation algorithm, gradient 0.1) in the HyperChem 6.01 program set [26]; construction of AT and GC oligonucleotides, using the HyperChem 6.01 program set that allows sodium ions to be included automatically as counterions in the examined oligonucleotide systems. Docking calculations were performed using the ArgusDock procedure integrated into the ArgusLab 4.0 program set [19]. The

binding energy of the ligand to the receptor region was calculated by minimizing the total energy of the liagnd—receptor region system (SG of DNA) using the AScore scoring function. The ligand—DNA complexes obtained from the docking calculations were further optimized in the Amber force field.

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