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Article

Molecular Recognition of B-DNA Minor-Groove Binders: The Rigid Analogue Approach to Synthesise Antileishmaniasis Compounds. A Molecular Modeling Study[#]

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Bisamidinas aromáticas são compostos ativos frente a *Leishmania sp.* e *Pneumocystis carinii.* Embora o modo de ação não seja conhecido, tem sido sugerido a interação com o B-DNA. Dentro do planejamento racional de drogas baseado na estrutura do receptor, este artigo mostra o cálculo das energias de interação de alguns análogos da pentamidina e o B-DNA e estabelece bases racionais para a síntese de derivados rígidos ou semi-rígidos que contenham uma conformação farmacofórica "isoélica" ao B-DNA. Os resultados demonstram que quanto mais próxima da conformação farmacofórica, melhor a energia de interação com o B-DNA.

Aromatic bisamidines are active against *Leishmania sp.* and *Pneumocystis carinii.* The mode of action is not known, but it has been suggested that they bind to B-DNA minor grooves. Within the drug design based upon the receptor structure, we show the binding energies for some pentamidine analogues with B-DNA and results establish a common base for the synthesis of their rigid counterparts. This is done through the identification of pharmacophore conformation that is isohelical to B-DNA. Results also show the closer pharmacophoric conformation the better binding energies.

Keywords: *B-DNA, pentamidine, Pneumocystis carinii,* Leishmania sp

Introduction

The aromatic bis(amidine) compound pentamidine is currently in widespread clinical use for the treatment of *Peneumocystis carinii* pneumonia (PCP) in AIDS patients[1]. Its analogues are effective against a number of microbial infections including *Trypanosoma rhodesiensis, Giardia lamblia, Cryptosporidium parvum*, and also against leishmaniasis[2,3]. Pentamidine is still being used against antimony-resistant leishmaniasis[4]. Pentamidine analogues have a good correlation with DNA binding affinity, as estimated by stabilisation of the DNA helix to coil thermal denaturation transition (T_m). Footprinting studies have also shown that the molecule binds to AT-rich regions of duplex DNA[5]. Thus, the mode of antimicrobial action for these dicationic molecules has been linked to their selective binding to the minor-groove of DNA at AT rich sites and their ability to selectively interfere with the normal functioning of the parasite topoisomerases, though this has not been proven yet. In the antigiardial activity, for instance, the binding to DNA minor-groove and the resulting complex is thought to be responsible for inhibition of an ATP-dependent giardial topoisomerase. The X-ray crystal structure of pentamidine bound to the DNA sequence $d(CGCGAATTCGCG)_2$ has been determined and shows binding within the AT-rich region of the DNA minor groove[6]. The interest in the rational design and synthesis of sequence selective or specific DNA binding agents has been continuously sought. Although selective control of gene function is currently being assessed using synthetic oligonucleotides targeted toward either mRNA (antisense approach)[7-9] or duplex DNA (antigen strategy)[9], low molecular weight molecules that bind to double-stranded

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DNA in a nonintercalative, sequence-directed manner continue to be an alternative focus of attention[10]. Moreover, there is a great deal of interest in designing novel ligands for biologically important receptors based primarily on knowledge of the three-dimensional structure of the receptor. This structure-based ligand design has the potential to greatly increase the number of lead compounds discovered for the development of new therapeutic agents. This paradigm is cyclical in nature, and the information on the receptor is used to design putative ligands which are synthesised and then evaluated for binding to the targeted receptor. By the comparison of predicted versus observed binding properties, rationally selected ligand modifications can then be employed in subsequent design cycles to improve both the design methodology and the binding affinity and selectivity of its resulting ligands.

The toxicity and side effects associated with the use of pentamidine and its analogues have led to extensive investigations to identify a derivative of pentamidine which is more active and less toxic than the parent drug.

With the advent of the acquired immunodeficiency syndrome (AIDS), the therapeutic importance of pentamidine (PENTAM), has greatly increased[11-14]. Pentamidine is as effective as trimethoprim-sulfamethoxazole (TMP-SX), for the treatment of *Pneumocystis carinii* pneumonia in persons with AIDS. *P. carinii* pneumonia occurs in approximately 80% of human immunodeficiency virus (HIV)-infected individuals[15,16]. However, pentamidine is considered second-line in the treatment due to its toxicity. But, its widespread use has come with an increased awareness of its potential benefits and adverse effects. Thus, in an attempt to circumvent the toxicities associated with its use, the development of either newer drugs or methods for its delivery have been evaluated for both therapy and prevention of *P. carinii*.

Pentamidine is an aromatic diamidine that was found to have significant anti-protozoa activity in studies of experimental African trypanosomes in 1938[17-19]. The pentamidine exhibited anti-protozoal activity when screened *in vitro* against *Leishmania mexicana amazonensis*[20].

The mode of action is still uncertain. It is known to interfere with numerous cellular process. It has been shown to bind to DNA in a nonintercalative manner to AT-rich regions of minor groove duplex DNA. This prediction has been confirmed by crystallographic and NMR analysis of pentamidine-dodecanucleotide complexes[21, 22]. The molecular modeling study for a representative set of pentamidine analogues was reported as a tool for predicting the binding energy with a model DNA in order to try and better understand their structure-activity relationships[23].

This study examines the applicability of the methodology in deriving new drug molecules that would be better binders than pentamidine itself, mainly those with constrained structures[24]. The crystallographic structure for the pentamidine [21] and also propamidine-dodecanucleotide complexes[25] were used as starting points for molecular modeling.

Methods

Initial structures for molecules were built using the MacroModel version 4.5 program[26]. This program was also used for manipulation and interactive docking manoeuvres using a Silicon Graphics 4D-20G. Atom-centred charges for each molecule were calculated from MNDO wavefunctions and derived MEP charges were fitted to the entire molecules. Additional AMBER force field[27] parameters required for the dicationic bisamidine ligands were set as follows: the Lennard-Jones energy was set to 10-12 and the distance dependent dielectric constant to 4. This avoids any hydrogen bonding to phosphate groups of the DNA strands. The results obtained for propamidine and pentamidine compare very well with those previously published and no explicit water or counter-ion were needed. The amidinium groups were set coplanar to phenyl rings. Calculated MEP charges and AMBER force field parameters are available from the authors upon request.

Initial coordinates for the 12-mer DNA duplex host were taken from the crystal structure of the pentamidine (1)-d(CGCGAATTCGCG)$_2$ complex[21] and were used to generate structures of equivalent 1:1 stoichiometry for each DNA-ligand complex. Coordinates for each drug molecule were superimposed upon those for the reference pentamidine ligand at its crystallographic 5'-AATT binding site within the DNA minor groove. Compounds which are essentially isostructural with pentamidine with respect to the disubstituted linker, afforded exact overlays but the others were manoeuvred to adopt the "isohelical" conformation of pentamidine.

The DNA-ligand complexes were initially regularised by steepest descent MM to reduce poor intermolecular steric contacts, by using (i) "belly"-type MM refinement (500 cycles) to minimise the energy of the bound ligand alone and (ii) MM minimisation (4000 cycles) by conjugate gradient and (iii) MM minimisation (4000 cycles) of the unrestrained complex to an RMS energy gradient of $\leq$ 0.05kJ/-mol. MD for each complex were subsequently performed for 40 ps (integration time step = 1.5 fs) at 300 K. All bonds were constrained using the SHAKE algorithm. Systems reached equilibrium at times $\leq$ 2 ps. Atomic coordinates were averaged for 100 structures and the final ones were subjected to a final MM relaxation to generate the refined complex.

Nonbonded energy terms were included up to 8.0 Å for van der Waals interactions and 20 Å for charge-charge electrostatics. The solvent effect was simulated by the use of a distance-dependent dielectric constant of the form $\varepsilon = 4r_{ij}$.[28]

Binding energies were calculated as[29]:

$$E_p(i) = E(i \text{ in bound conformation}) - E(i \text{ minimised})$$

$$E_p = Ep(drug) - Ep(DNA)$$

$$E_{int} = E(DNA + ligand)_{complex} - [E(DNA)_{idealised} + E(ligand)_{global\ minimum}]$$

$$E_{bind} = E_{int} + E_p \quad (1)$$

where Ep is the perturbation energy calculated as the difference of $E_p(i)$ for both drug and B-DNA bound and minimised conformations. E_{int}, reflects the interaction energy between the drug and B-DNA with no perturbation counting while E_{bind} does include the perturbation term.

Molecular modeling studies[30] have suggested that the minimum energy conformation for the benzamidine system is one in which the amidinium group is coplanar with respect to the phenyl ring. And, this is reflected in the crystallographic structure: the amidinium groups are twisted out of the planes of the phenyl rings by 3° at the 5'-end and -6° at the 3'-end.[21] However, it has been pointed out that due to steric clashes between ortho hydrogens of phenyl ring and the amidinium group would bring them both to be out of plane by 45° [23]. This is also in agreement with a search of the Cambridge Crystallographic Database that gives a mean of 30 ± 12° for the twisted angles[31]. Nevertheless, we have set the initial twisted angle to be 0° but allow torsion due to any interaction within the minor groove.

Results and Discussion

Binding of pentamidine ant its analogues

Table 1 shows the calculated binding enthalpies and other component energies for each minimised complex structure. The E_{bind} values for the pentamidine analogues compare quite well with those previously published[23] and the same pattern of interactions can be drawn. Thus, we shall not have to further analyse this.

Binding of furan and its analogues

How molecules interact with the minor groove of the nucleic acids has intrigued chemists and biologists. They can be summarised as follows[32]: (i) genome regulatory proteins show affinity for non-specific sites; (ii) this non-specific binding is largely 'electrostatic' in nature; (iii) specific binding involves the interaction of a matrix of DNA hydrogen bond donors and acceptors, located in the groove; (iv) close van der Waals contacts. Small molecules primarily bind in the narrow minor groove of B-DNA whereas larger molecules such as proteins utilise the wide major groove. The extensively studied antitumor antibiotics netropsin and distamycin bind to the minor groove of DNA at AT sequences[33]. These molecules can be regarded

Table 1. Binding energies for study compounds (E in kJ Mol^{-1})

Ligand	$\Delta E_{(bind)}$	$E_{(int)}$	E (DNA - ligand)	E (DNA) (bound)	E Ligand	E Ligand (bound)	$Ep_{(l)}$	$Ep_{(DNA)}$	Ep
pentamidine	**-293.37**	**-292.83**	**-1,333.94**	**-1,079.90**	**51.58**	**63.83**	**12.25**	**12.79**	**-0.54**
propamidine	-302.22	-297.82	-1,346.95	-1,079.60	43.56	52.25	8.69	13.09	-4.40
isost21C	-302.78	-298.36	-1,364.49	-1,079.04	26.56	35.79	9.23	13.65	-4.42
isost23C	-302.80	-298.79	-1,364.92	-1,079.40	26.56	35.84	9.28	13.29	-4.01
imidisoC	-342.65	-324.80	-1,267.96	-1,068.01	149.53	156.36	6.83	24.68	-17.85
imidtautoC	-339.11	-326.59	-1,286.80	-1,078.29	132.48	134.36	1.88	14.40	-12.52
imidtautome	-339.31	-325.65	-1,278.70	-1,075.43	139.64	143.24	3.60	17.26	-13.66
imiddna1C	-311.12	-296.90	-1,252.52	-1,074.91	137.07	140.63	3.56	17.78	-14.22
imiddna2C	-315.35	-299.86	-1,255.48	-1,068.19	137.07	146.08	9.01	24.50	-15.49
imidCH	-334.00	-323.26	-1,267.87	-1,077.89	148.08	152.14	4.06	14.80	-10.74
furanC2C	**-311.31**	**-301.19**	**-1,264.75**	**-1,079.13**	**129.13**	**132.57**	**3.44**	**13.56**	**-10.12**
furan	-325.36	-296.66	-1,266.47	-1,054.46	122.88	132.41	9.53	38.23	-28.70
py1	-347.16	-327.10	-1,271.24	-1,068.36	148.55	152.82	4.27	24.33	-20.06
py2	-346.59	-330.25	-1,272.33	-1,076.95	150.61	154.01	3.40	19.74	-16.34

(i) E = kJ/mol. (ii) $E_{(DNA)}$= -1.092.69 kJ/mol = idealised B-DNA energy; (iii) $\delta E_{(bind)}$= Energy for binding (includes the perturbation term); $E_{(int)}$ = Energy with no perturbation term; $E_{(DNA\text{-}ligand)}$= output Energy for the complex; $E_{(DNAbound)}$= B-DNA energy in the bound conformation; E_{ligand} = Energy for the lowest drug conformer; $E_{ligandbound}$ = Energy for bound drug to B-DNA; $Ep_{(l)}$, $Ep_{(DNA)}$ and Ep perturbation energies calculated according to Eq. 1.

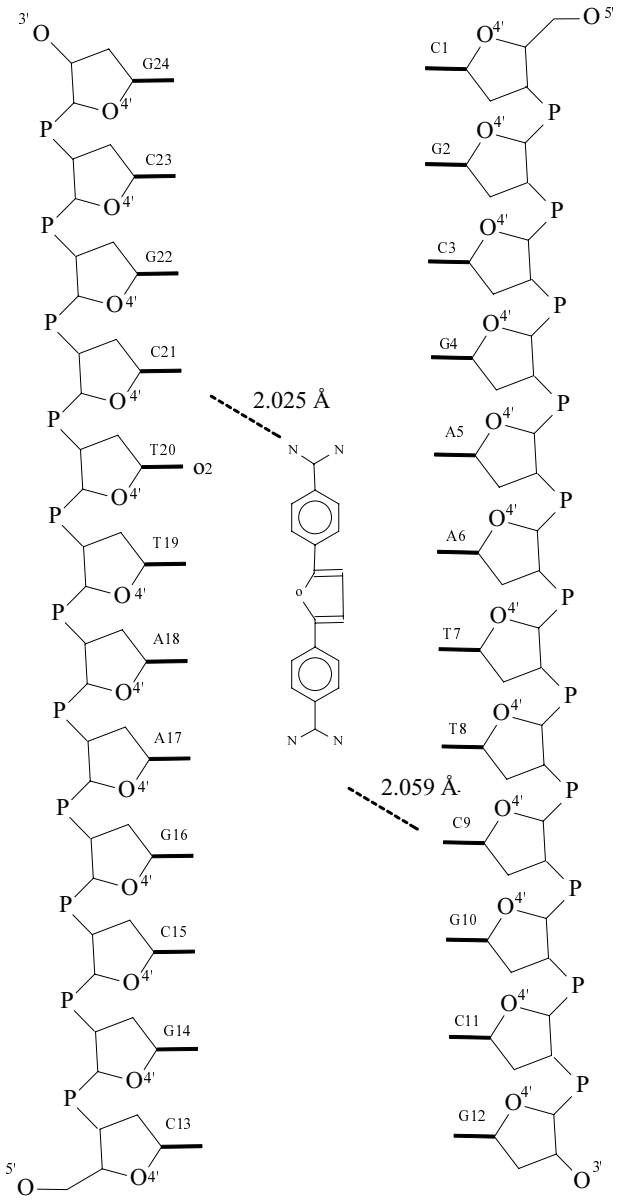

Figure 1. Mode of binding for Furan C2C.

as being assembled with cationic ends responsible for electrostatic interactions and NH groups resulting in hydrogen bonding that would be needed for binding though not being essential. The electrostatic attraction of doubly charged ends reads the AT sequence which is the deepest negative potential well in the minor groove of DNA[34]. Nevertheless, we have shown[23b] that for a set of 37 bisamidines active against *Leishmania mexicana amazonensis* the QSAR model does include the minimal electrostatic potential as being important for binding to AT sequence of B-DNA. The replacement of the pyrrole rings with imidazole moieties alters the recognition from AT to GC base pairs. By examining the molecular models of a number of aryldiamidines it seems that they fall into a particular spatial class.

Depending upon the spacer group the affinity of both ends can also alter the recognition. The shape-dependence has also been recognised. For instance, the two phenyl rings of the bound pentamidine molecule can be twisted by 35° with respect to each other[21]. Other studies suggest that binders have a 'concave-face' to allow 'isohelicity' with the minor groove[35,36].

Our interest in the design of sequence specific DNA binding agents has led us to explore the molecular recognition properties just outlined.

As a "bench test" we shall discuss the results obtained so far for compound furan C2C. However, similar conclusions can be drawn for the other ligands. The furan C2C is a better binder than pentamidine according to our calculations. The 'furan spacer group' spans the two aromatic amidines only to an extent of 5 Å as measured between the two *ipso* phenyl positions, Fig. 1. Thus, binding of the shorter furan analogue is predicted to be more favourable by 17.9 kJ/mol relative to pentamidine. Boykin *et al.* have recently shown that in fact this furan derivative is a better binder to AT DNA than pentamidine[37]. The furan C2C lies across the upper AT base pairs. The furan C2C is almost planar. The furan ring is twisted by *ca.* 7° out of the

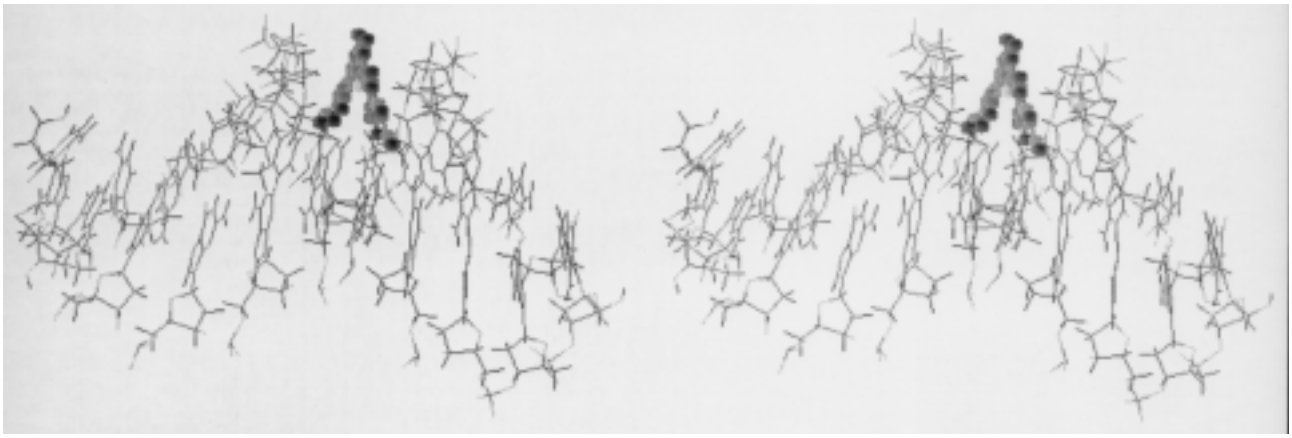

Figure 2. Stereoview of furan C2C within the minor groove.

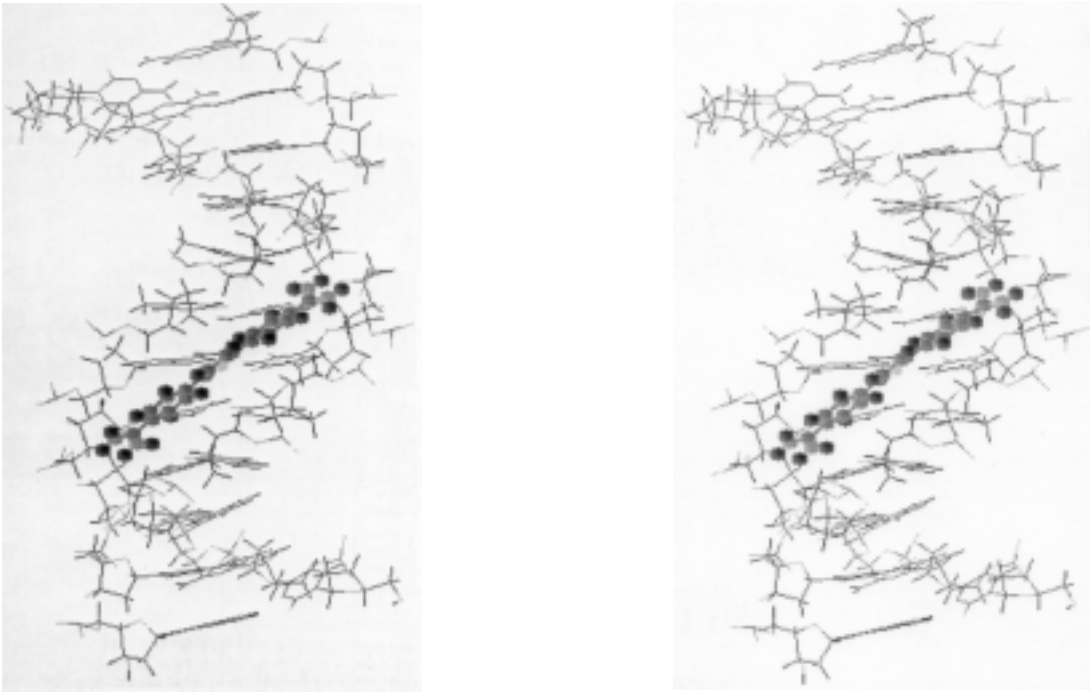

Figure 3. Stereoview of furan C2C showing the "isohelicity" to B-DNA.

bisamidine planes. However, there is a higher chain torsion in those angles due to an interaction of the amidinium groups within the groove. The two torsion angles are 14 and 19°. The two dihedral angles for the amidinium groups are 11 and 14°, Figs. 2 and 3. These twists bring the drug molecule to match the pitch of the DNA and results in a good isohelical fit to the minor groove[36].

The crystal structure of the complex between the furan derivative and d(CGCGAATTCGCG)$_2$ has been described[38,39] and it is in agreement with the findings we have made in this paper.

Conclusions

It seems that the above data can be regarded as a good way of ranking these drug molecules as they bind to B-DNA. Some of them have shown T_m values that are better than pentamidine, *i.e.* these drugs may bind better than the latter. It is encouraging to continue within this area of study and the method outlined can be used in order to allow a better insight into the binding prior to the synthesis of such compounds. Moreover, it can be recalled that the more rigid the linker between the two bisamidine rings the better the binding. Thus, we are pursuing this in the synthesis of new ligands with this characteristic, and some preliminary results have already shown this to be the case.

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