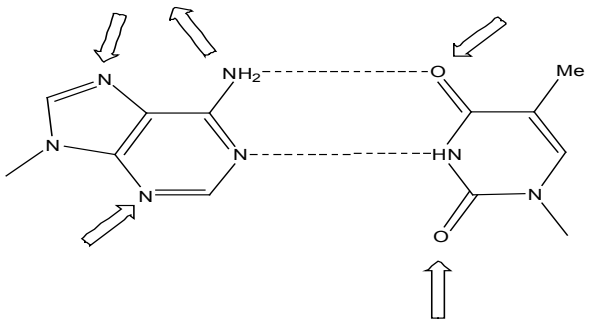
Minor DNA Groove Binding Agent Optimisation

Medicinal Chemistry
Rory Scanlon
Senior Freshman

A current mode of action for antitumor drugs is the non-covalent binding of drugs to the minor groove of DNA in order to deregulate the replication of the cell. The principal compounds that are used for this pathway are Distamycin and Netropsin. The traits of these structures which are thought to be useful are the amount of hydrogen bond donors and acceptors present as well as their positions. The main limitation of these compounds is their lack of anti-tumour activity. This has resulted in the development of many hybrid derivatives to optimise binding and specificity as well as to introduce anti-tumour activity. Drugs that bind to the minor groove often target cancer and have been proposed to be useful in disrupting the DNA of Protozoal diseases like Human African trypanosomiasis (HAT) and plasmodium diseases. The compounds that are useful for this pathway, as well as possibly cancer chemotherapy, are guanidine 2-aminoimidazoline diaromatic derivatives, which are related in structure to furamidine. This review focuses on some of the general compounds whose mode of action is to bind to the minor groove of DNA in a non-covalent manner and attempts to discuss optimum design by looking at the interactions of these ligands with the aim of increasing activity against protozoic diseases and cancer.

Introduction

DNA is often the target of drugs trying to interfere with basic cellular mechanisms, such as cell division, and for years DNA has been the target for chemotherapeutic action. (Henderson, D. *et al.* 1995). Often diseases such as cancer or HAT interfere with cellular processes at the DNA level and therefore treatment of such diseases employs the use of minor groove binding drugs. There are three main modes of action for drugs to bind to DNA - electrostatic interactions, van der Waal forces and hydrogen bonding. They mainly target the minor groove of DNA to cause cytotoxic effects (Khan G.S. *et al.* 2012). Small crescent shaped molecules are often the basic framework that is used in the design of these drugs (Khan, G.S. *et al.* 2012). This is because the concave shape of the ligand fits into the convex shape of the groove (Neidle, S. 2001). Hunt, R. *et al.* (2011) showed that linear ligands also bind in the minor groove, using analogues of DB921.



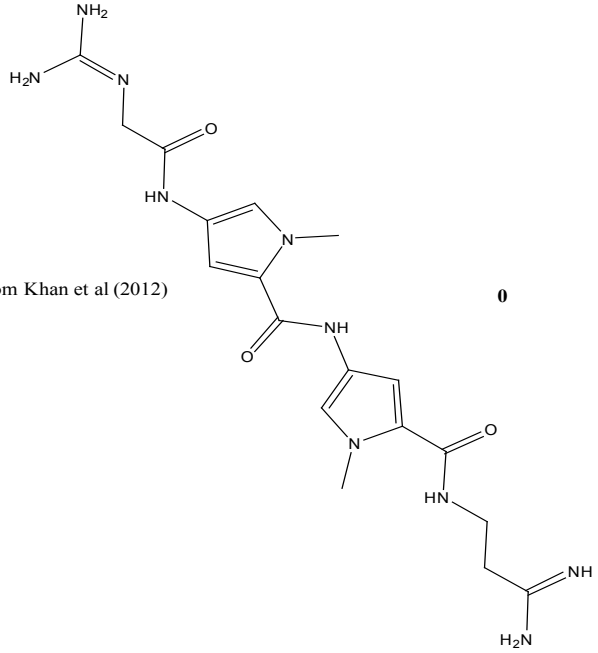
Schematic view of hydrogen bond donors and acceptors in the A:T base pair, adapted from Neidle S. (2001)

The minor groove is regarded as being devoid of many molecules compared to the major groove and is therefore a great target for these drugs (Khan G.S. *et al.* 2012). Drugs which target the minor groove bond favourably in this position because of an entropic increase due to the displacement of water bound in the minor groove. (Neidle, S 2001). Distamycin and Netropsin are often the primary compounds used and they have been shown to preferentially bond to the adenine and thymine base pairs of DNA (Wartell, R.M. *et al.* 1974). Their mode of action is to bind to the minor groove temporarily and alter the structure of DNA, thus changing

its function. This has an advantage over covalent bonding as the binding is temporary and minimizes risk of damage to the DNA (Khan, G.S. *et al.* 2012).

Main Body

When a compound binds to the minor groove it displaces the spine of hydration in the groove. This is driven by hydrophobic forces and results in an increase in entropy. In response to this DNA counter-ions are released to maintain electric neutrality and the compound is incorporated into the minor groove. The counter-ion release manifests as a large entropic increase that favours binding. Once this occurs van der Waal forces and hydrogen bonding are exhibited between the compound and A-T sequences. Netropsin is unable to bind to G-C pairs because of the amino group at position two on guanine and binds exclusively to double stranded DNA. This demonstrates the specificity and shape optimisation of such compounds. (Wartell, R.M. *et al.* 1974).



adapted from Khan et al (2012)

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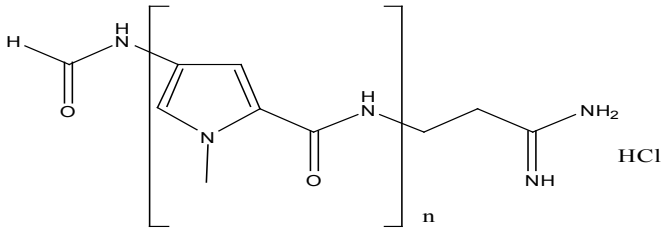
Netropsin is a molecule which consists of two pyrrolecarbamoyl units with an amidino group at each end of the molecule (Khan, G.S. *et al.* 2012).

To synthesise new compounds which were better in their affinity for minor groove binding, the number of pyrrole units were increased up to six frames. This provided larger specificity for longer chains which are high in amounts of A-T sequences and thus increased a drug's potency (D'Allessio, R. *et al.* 1994, Bailly, C. *et al.* 1998, Baraldi, P.G *et al.* 2007). An increase by six units appears to be the limit as in an experiment by Bailly, C. et al. (1998) the twisting of the DNA helix didn't allow a seventh unit to be attached to the compound in the hopes of increasing binding. To overcome the amino group on guanine - which acts as a hydrogen bond donor (HBD) - a solution was to incorporate a hydrogen bond acceptor (HBA) in place of one of the pyrrole units. The most effective functional group was an imidazole where the nitrogen that was not bound to the methyl unit would function as the HBA (Lown, J.W. 1988).

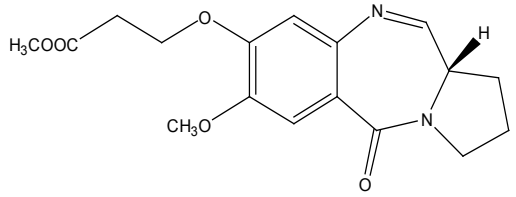
Distamycin is distinguished by the presence of only one cationic guanidinium group in its structure - which has at least one more pyrrolecarbamoyl unit - and by having a longer binding site in DNA (Neidle, S. 2001). In Distamycin A, the compound is characterized by three oligopeptidic pyrrolecarbamoyl units ending with an amidino group. It has a lack of antitumour activity but specifically binds to AATT segments.



(Distamycin A), n=3 **1**
n=4
n=5

Adapted from Baraldi P.G. et al (2007)

Baraldi, P.G *et al.* (2007) used Distamycin A and a homologue with four pyrrole units as a vector. They attached various alkylating agents to improve its chemotherapeutic action. These include a pyrrolo[2,1-c][1,4] benzodiazepines (PBD) group attached at the formyl end of a variable pyrrole chain unit of Distamycin (Baraldi, P.G *et al.* 1999).



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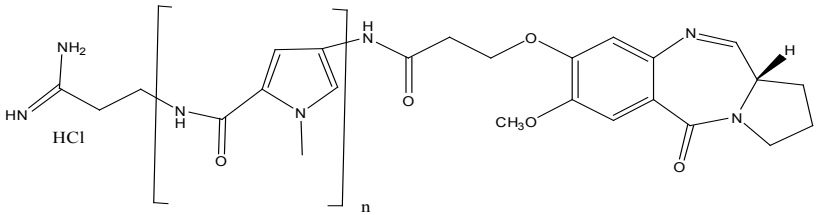
pyrrolo[2,1-c][1,4] benzodiazepines (PBD) group. Adapted from Baraldi P.G. et al 1999



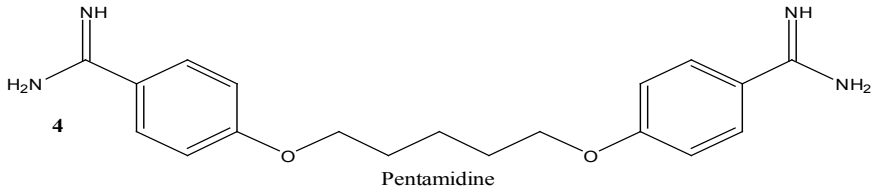
n=1, **3a**
n=2, **3b**
n=3, **3c**
n=4, **3d**

(PBD) group attached at the formyl end of a variable pyrrole chain unit of Distamycin. Adapted from Baraldi P.G. at al (1999)

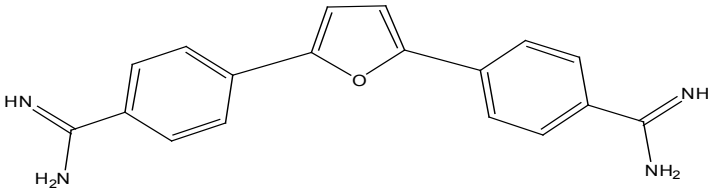
These compounds were shown to have a much lower IC_{50} (μM) in vitro against T-lymphoblastoid Jurkat cells and human chronic myeloid leukaemia K562 cells than Distamycin A itself. The substituent chains showed a lower IC_{50} when they were longer. This meant that there were extra possibilities for hydrogen bond donation (Baraldi, P.G *et al.* 1999).

This minor groove binding property of compounds has been considered not only for drugs looking to treat cancer but also for those aiming to treat parasitic diseases such as HAT. Little investment has been made into new clinical drugs that combat HAT and other tropical diseases. In 2003, less than 1% of all new chemical substances distributed treated tropical parasitic diseases,

with some minor exceptions (Fairlamb, A.H 2003). HAT is thought to be one of the most neglected diseases in terms of distribution, according to the WHO. Plasmodium derived species, which cause diseases such as malaria, are one of the exceptions to the distribution problem but there has been development of resistant strains of these diseases. In both of these cases new drugs need to be developed to respond to the problem. Pentamidine has historically been used to treat HAT but has been seen to have levels of toxicity that proved it largely unsatisfactory for treatment (Fairlamb, A.H 2003). Even the arsenic based drug melanosporol, which treats late stage HAT when it breaches the CNS, is toxic (Tabernero, L. *et al.* 1996). 2,5-Bis(4-guanylphenyl)furans were seen to have antiprotozoal activity against the acute form of HAT, *Trypanosoma brucei rhodesiense (T.b.rhodesiense)*, much like Pentamidine but with higher levels of activity (Das, B.P *et al.* 1977) and also lower levels of toxicity (Neidle, S. 2001)



4 Pentamidine



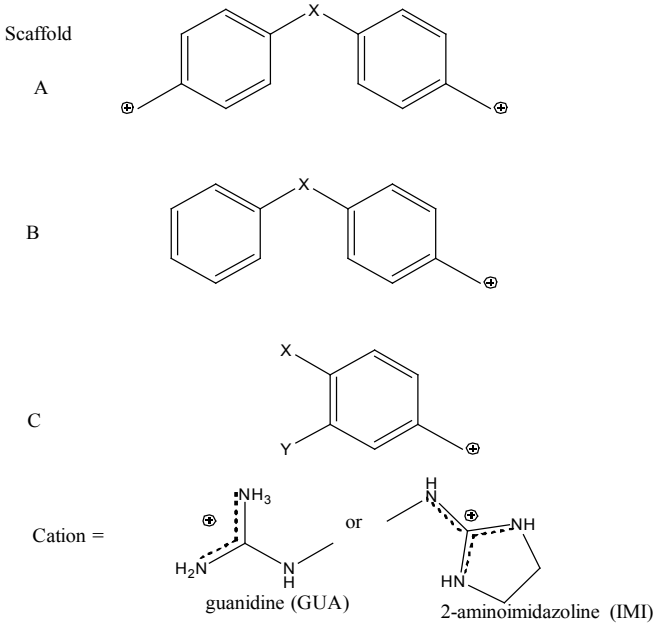
5 Furamidine

Adapted from Rozas et al 2013

The 2,5-Bis(4-guanylphenyl)furans' main mode of action is to bind to A-T rich sequences, which causes an inhibition of key DNA-dependant enzymes. The principle enzyme to be inhibited is topoisomerase which replicates the kDNA within the Protozoan

mitochondria. The specific mode of inhibition is reported to be at the first stage of topoisomerase action - site recognition - and it has been hypothesised by Dykstra, C.C *et al.* (1994) and Shapiro, T.A. *et al.* (1990) that the drugs that cause this inhibition linearize the DNA.

Bis guanidine and bis 2-aminoimidazolinediphenyl compounds have been shown to be analogous to 2,5-Bis(4-guanylphenyl)furans structurally and, as a result, are similar to Distamycin and Netropsin in the function of groove binding (Rodríguez, F. *et al.* 2008). Both the guanidine and 2-aminoimidazoline groups function as cations. The ligands tested in a study by Rodríguez, F. *et al.* exhibited activity against *T.b.rhodesiense* as well as *Plasmodium Falciparum* (*P.Falciparum*). The pathway taken was through the P2 transporter into the protozoan, a new vector, which has previously been resistant to most of this class of compounds, and minimizes the development of cross resistance. The importance of some binding interactions of parts of the ligands tested were demonstrated by testing the activity of different scaffolds which differed by the presence of single parts (Rodríguez, F. *et al.* 2008).



Adapted from (Rodríguez, F. et al 2008)

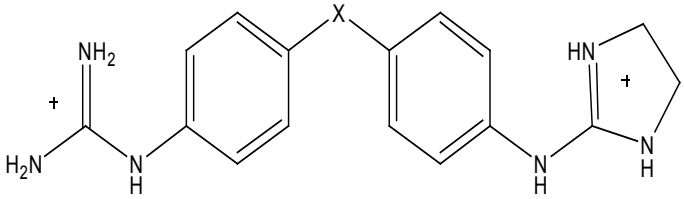
Table 1							
Compound	Scaffold	Cation (R)	X	Y	IC_{50}(μM)		
					T.b.rhodesiense	*P.Falciparum*	Cytotoxicity L6-cells
6a	A	GUA	NH	-	0.022	0.018	0.65
6b	B	GUA	NH	-	4.8	1.6	49.5
6c	C	GUA	NH_2	H	311.2	>26	131.8
7a	A	GUA	CH_2	-	0.161	0.032	2.8
7b	B	GUA	CH_2	-	15.9	3.8	43.9
7c	C	GUA	CH_3	H	163.7	>27	>484
8a	A	IMI	CH_2	-	0.897	0.0157	63.6
8b	B	IMI	CH_2	-	4.9	1.7	73
8c	C	IMI	CH_3	H	262.4	>23	>425

Adapted from (Rodríguez, F. et al. 2008)

Molecules with scaffold A had IC_{50} values that were nanomolar in range but the other scaffolds (B and C) had values that were only micromolar, even when the same atom was used for (X), which indicated reduced activity. The bridging groups (X) that produced the lower IC_{50} values were amine, amide and ethane bridges, which were all similar in IC_{50} value. This was postulated to be due to their ability to function as similar HBDs, although the ethane bridging group is afforded more rotation and therefore has less ability as a HBD (Rodríguez, F. *et al.* 2008). Compounds with the 2-aminoimidazolinium cations showed higher selectivity based on the activity against *T.b.rhodesiense* than the corresponding guanidinium ones by having higher selective indexes for DNA consisting of A-T oligonucleotides. The 2-aminoimidazolinium derivatives had higher DNA denaturation temperature (ΔT_m) values and a relationship was elucidated between this and their DNA affinity (Rodríguez, F. *et al.* 2008). Many sources agree that higher ΔT_m values are characteristic of a molecule showing an increased affinity for binding to DNA. This is characteristic of stronger attractive forces (Dardonville, C. *et al.* 2006, Rodríguez, F. *et al.* 2008, Nagle, P.S *et al.* 2009, Nagle, P.S *et*

al. 2010)

Along this line of research new groups were later tested for their DNA binding affinity. These groups included a series of asymmetric diaromatic guanidinium/2-aminoimidazolinium dications. They all follow the basic shape of furamidine with synthetic changes made to the cationic arms of the molecule and bridging atom (X) (Nagle, P.S *et al.* 2009).



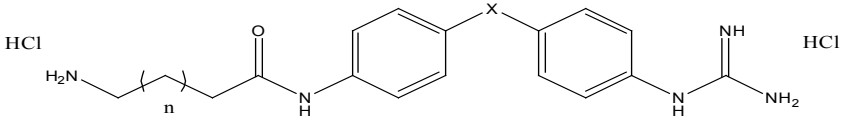
Adapted from (Nagle, P.S et al. 2009)

Again, the highest changes recorded for ΔT_m for diaromatic guanidinium/2-aminoimidazolinium dications were ones that had nitrogen atoms as the bridging group (X), along with a carbonyl group. The specificity was tested in a UV titration with separate carbonyl and nitrogen bridges. With a mixed amount of sequences both samples had UV shifts as the DNA was added, indicating the disappearance of the free substance. Addition of A-T oligonucleotides produced a stronger shift with the nitrogen bridging group representing the complex, implying a stronger binding (Nagle, P.S *et al.* 2010). Nitrogen is capable of forming strong hydrogen bonds by acting as a HBD. Oxygen and sulphur atoms function as HBAs. Therefore addition of more hydrogen bond donor groups could have helped increase affinity of these molecules (Nagle, P.S *et al.* 2009). SPR (Surface Plasmon Resonance) of these compounds with a methylene and nitrogen link confirmed the nitrogen atom as having a stronger binding constant for AATT oligonucleotides, which is what showed the best agreement with changes in denaturation temperature. The carbonyl linker also showed a lower binding constant for AATT but a higher one for CG sequences (Nagle, P.S *et al.* 2010).

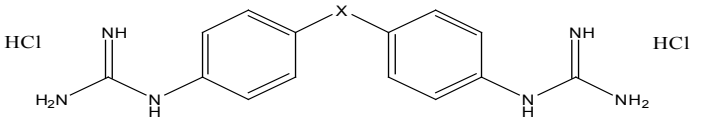
A comparative experiment was performed on Netropsin which showed a two-fold increase in affinity compared to the tested compounds. The large number of donor groups as well as acceptors

is thought to be responsible for Netropsin's performance. Repeated experiments in comparative conditions with increased amounts of A-T composition showed even bigger increases in temperature, revealing the increased affinity for these nucleotides (Nagle, P.S *et al.* 2009). In the case of methylene and ethylene, ethylene showed greater affinity from a higher temperature increase. The rotation afforded by the extra CH_2 could have been a contributing factor in increasing affinity (Nagle, P.S *et al.* 2009).

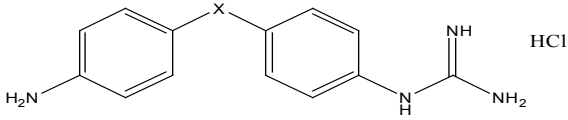
Based off this work of asymmetric diaromatic guanidinium/2-aminoimidazolinium derivatives, aminoalkyl derivatives of diaromatic guanidines were measured for their strength as DNA binders (McKeever, C. *et al.* 2013). The idea was to introduce an aliphatic chain on one of the aromatic groups which would help binding by displacing water molecules present in the minor groove. These hydrophobic interactions improve binding which could increase the antiprotozoal activity of the compounds (McKeever, C. *et al.* 2013).



n=1 X=CH2, CH2CH2, O, CO, NH **9a-e**
n=2 X=CH2, CH2CH2, O, CO, NH **10a-e**
n=5 X=CH2, CH2CH2, O, CO, NH **11a-e**
n=8 X=CH2, CH2CH2, O, CO, NH **12a-e**
n=9 X=CH2, CH2CH2, O, CO, NH **13a-e**



X=CH2, CH2CH2, O, CO, NH **14a-e**



X=CH2, CH2CH2, O, CO, NH **15a-e**

Adapted from McKeever, C. et al 2013

Data presented by Nagle, P.S *et al.* (2009) agree that while most compounds had an increase in the ΔT_m when tested in a random assortment of nucleotides, the figures reported for these derivatives were generally lower than those reported for the asymmetric diaromatic guanidinium/2-aminoimidazolinium derivatives. (McKeever, C. *et al.* 2013). I would suggest the aminoalkyl chain is not a suitable replacement for the aminoimidazole group if the objective is to bind to DNA. McKeever, C. *et al.* (2013) tested compounds without the amino alkyl side chain instead using guanidine group (14a-e). Some of this class reported the highest levels of binding in all the tested compounds. Compounds 15a-e, monoguanidinium-like derivatives in which an amino group replaces the aliphatic side chain showed very poor affinity toward DNA, probably due to their monocationic nature. This could imply the chain as the causative agent of this increased DNA binding in some cases. McKeever, C. *et al.* (2013) used wild type salmon sperm DNA so the specificity of these compounds for certain nucleotides wasn't tested. The highest value (ΔT_m=6) had oxygen and NH as their bridging groups and a chain of four methylene units (McKeever, C. *et al.* 2013). The optimum length of chain used is thought to be ones of four methylene units with n=2. These reported the best results for chained compounds and adding more units appeared to interfere with binding (McKeever, C. *et al.* 2013).

Conclusions

Considering the relationship between structural differences and DNA binding, one can see that the parts of the ligand that attach to DNA are important in determining the binding strength of the ligand and its therapeutic action. It is still a matter of discussion as to what interactions are the most important between electrostatic interactions, hydrogen bonding and van der Waal forces. Looking at the difference in IC_{50} based on the scaffolds, it would imply that the absence of a cation is more important than an aromatic ring capable of van der Waal interactions. 6b reported 218 times less activity than 6a whereas 6c was only 64 times less active (Rodríguez, F. *et al.* 2008). Looking at figures provided by McKeever, C. *et al.* (2013) and comparing the strength of binding between hydrogen bond

donor rich ligands (14a-e) and ones that have more van der Waal opportunities instead (9-13, a-e), it appears that maximising the number of hydrogen bonds present in a ligand is more important than the van der Waal forces supplied by an aliphatic chain (Tabernero, L. *et al.* 1996). While Rodríguez, F. *et al.* (2008) see ligand cationic groups as an important factor for binding, Tabernero, L. *et al.* (1996) say that the ligand cationic groups did not show a preference for hydrogen bonding. In this case the electrostatic interactions in the groove could be a compromise between both observations. In this review I mainly focused on compounds that bind specifically to A-T nucleotides. However they are not the only type that should be investigated. As discussed above, Lown, J.W. (1988) developed a range of compounds, termed lexitropsins, which allow binding to G-C pairs. Knowledge of how to optimise the right type of interaction is going to be important to elucidate ligands that may one day function as antitrypanosomal and antitumor drugs.

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