NOVEL SYMMETRIC BIS-BENZIMIDAZOLES: SYNTHESIS, DNA/RNA BINDING AND ANTITRIPANOSOMAL ACTIVITY

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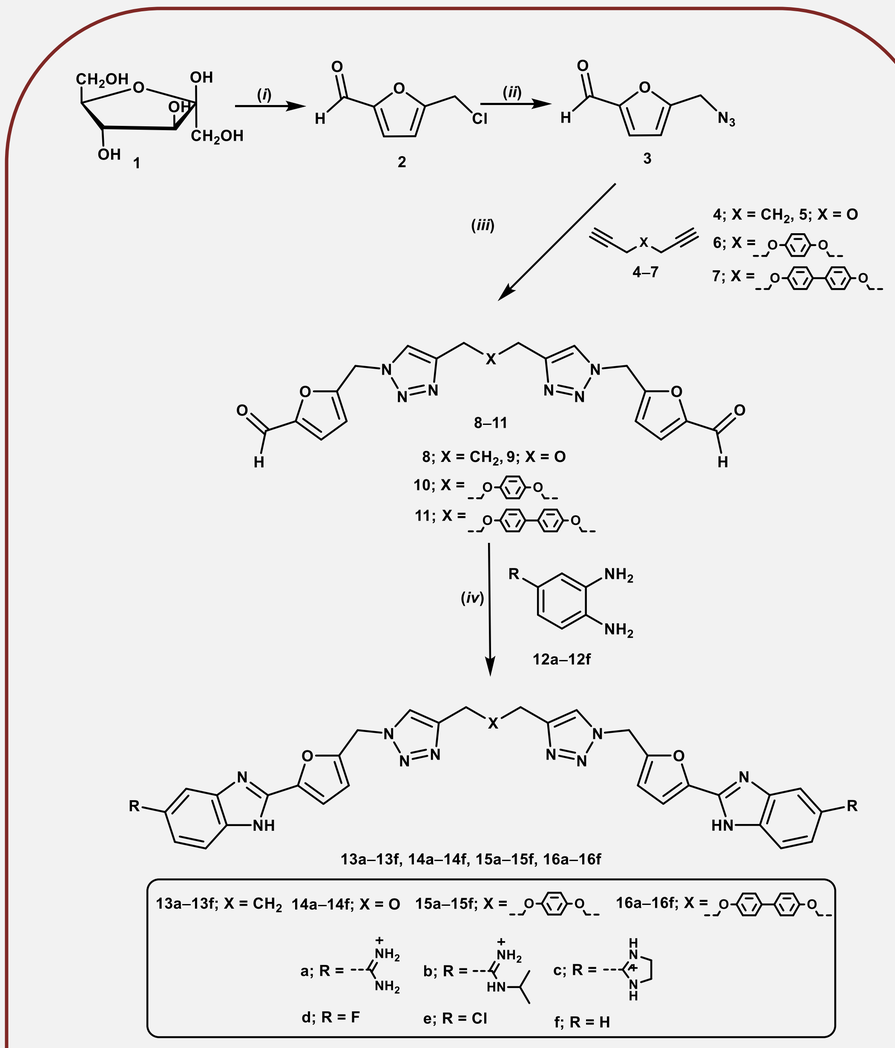
INTRODUCTION

Human African trypanosomiasis (HAT), or sleeping sickness, is one of the most deadly neglected tropical diseases (NTDs), with 65 million people at risk in 36 countries [1]. Current therapies for HAT are unsatisfactory and under threat from emerging resistance [2]. This has prompted the search of benzimidazole derivatives that are more efficacious for combating this fatal infection. In continuation of our recent work on the development of aromatic amidines as DNA-binding ligands and anti-trypanosomal agents [3], we aimed to expand the benzimidazole scaffold to 5-membered furyl and 1,2,3-triazolyl moieties that may adopt helical topology to approximately match the curvature of DNA in the minor groove.



Sheme 1. *Reagents and conditions*: (*i*): propargyl bromide, K_2CO_3, EtOH, reflux, 24 h; (*ii*): azide, $CuSO_4$, Cu(0), DMF, *t*-BuOH : H_2O = 1 : 1, 80 °C, 1.5 h, MW/US; (*iii*): 1,2-phenyldiamine (**4**–**8**), $NaHSO_3$, EtOH, reflux, 6h.

CHEMISTRY

A one-pot route in biphasic mixture HCl-H_3PO_4/$CHCl_3$ was applied for efficient conversion of D-fructose to 5-chloromethylfurfural, which then in reaction with sodium azide, gave rise to 5-azidomethylfurfural. The key precursors, symmetric bis-triazolyl aldehydes were synthesized by Cu(I)-catalyzed 1,3-cycloaddition of the azide with corresponding terminal bis-alkynes, using microwave irradiation. Condensation of various *o*-phenylenediamines with bis-(1,2,3-triazolyl) aldehydes using $NaHSO_3$ or *p*-benzoquinone, as an oxidative reagent, afforded the target bis-benzimidazole derivatives with 5-amidino- (**13a–13c**, **14a–14c**, **15a–15c** and **16a–16c)**, 5-fluoro- (**13d–16d**) and 5-chloro-substituted (**13e–16e**), as well as non-substituted benzimidazoles (**13f–16f**).

INTERACTIONS WITH DNA/RNA

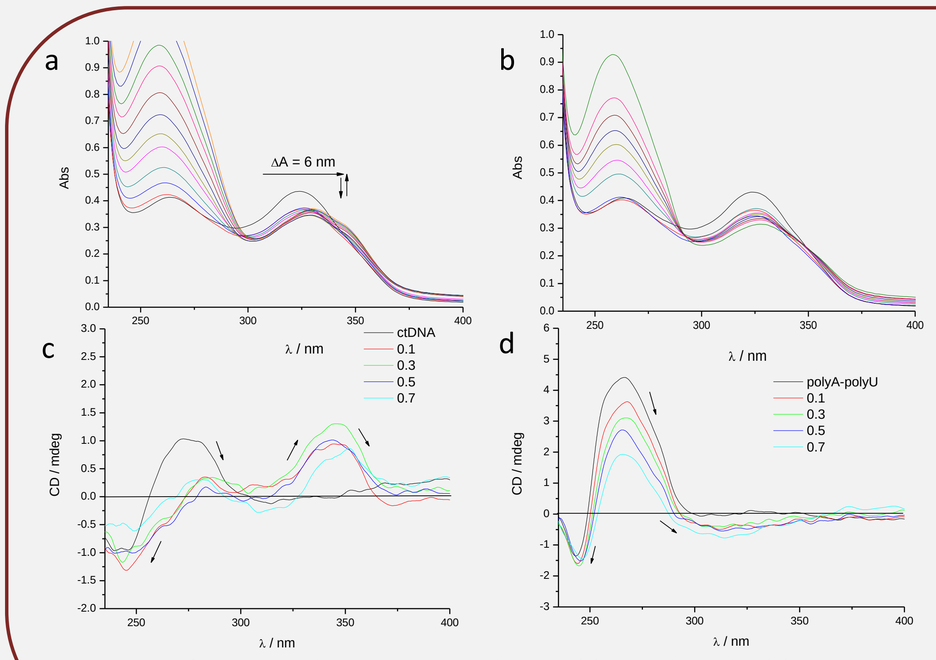


Figure 1. UV/Vis spectra of compound **15c** with ctDNA (a) and polyA-polyU (b) and CD spectra of compound **15c** with ctDNA (c) and polyA-polyU (d) .

Interactions of prepared compounds with ctDNA and polyA-polyU were investigated by UV-Vis and CD spectroscopy.
Amidine derivatives **13a–13c**, **14a–14c** and **15a–15c** showed higher stabilization compared to corresponding non-amidines.
The positive ICD band suggests that the compounds are positioned along the minor groove, identifying this as the dominant binding mode with ctDNA.
The ICD spectra of imidazoline derivatives **13c**, **14c**, **15a-c** and **16a-c** showed a negative signal at > 300 nm, which may indicate intercalation as a possible binding mode with polyA-polyU polinucleotide.

BIOLOGICAL EVALUATIONS

Table 1. Antitrypanosomal activity[a] of simetrical bis-benzimidazoles *Trypanosoma brucei* strain

Compd	T. brucei IC_{50} /μM	T. brucei IC_{90} /μM	L6 cells IC_{50} /μM	SI
13a	3.6 ± 0.4	5.5 ± 0.2	198 ± 3	55
13b	5.1 ± 0.2	8.2 ± 1.0	104 ± 3	20
13c	3.9 ± 0.3	5.4 ± 0.1	216 ± 21	55
13d	4.0 ± 0.2	8.9 ± 0.6	221 ± 12	55
13e	3.0 ± 0.3	7.2 ± 0.7	>300	>100
13f	>15	-	-	-
14a	3.3 ± 0.3	4.7 ± 0.1	88.6 ± 2.1	25
14b	7.9 ± 0.1	10.8 ± 0.1	-	-
14c	3.8 ± 1.0	6.0 ± 0.4	122 ± 3	30
14d	7.9 ± 0.3	>25	-	-
14e	6.4 ± 0.6	11.8 ± 1.1	-	-
14f	>15	-	-	-

Compd	T. brucei IC_{50} /μM	T. brucei IC_{90} /μM	L6 cells IC_{50} /μM	SI
15a	1.3 ± 0.1	5.6 ± 0.4	39.6 ± 2.0	30
15b	1.5 ± 0.1	2.8 ± 0.4	121 ± 5	80
15c	**0.75 ± 0.15**	**1.5 ± 0.1**	**60.7 ± 3.1**	**80**
15d	3.4 ± 0.2	6.7 ± 1.0	219 ± 41	65
15e	1.4 ± 0.2	2.5 ± 0.4	>270	>190
15f	>15	-	-	-
16a	>10	-	-	-
16b	>10	-	-	-
16c	>10	-	-	-
16d	>15	-	-	-
16e	0.37 ± 0.06	9.7 ± 0.03	30.4 ± 6.1	80
16f	>10	-	-	-
Nifurtimox	4.4 ± 0.7[b]	-		

Results of the *in vitro* testing of novel symmetric bis-benzimidazoles and nifurtimox, as a reference drug, against bloodstream form *T. brucei* are summarized in Table 1. The cytotoxicity of the most active compounds (IC_{50} < 5 mM) was also assessed using the rat myoblast cell line L6. We found that imidazoline and 1,4-bis(oxymethylene)phenyl were favourable for strong antiprotozoal activity. Bis-benzimidazole imidazoline **15c**, was the most potent derivative with 6-fold higher activity than nifurtimox.

IN SILICO MOLECULAR BINDING

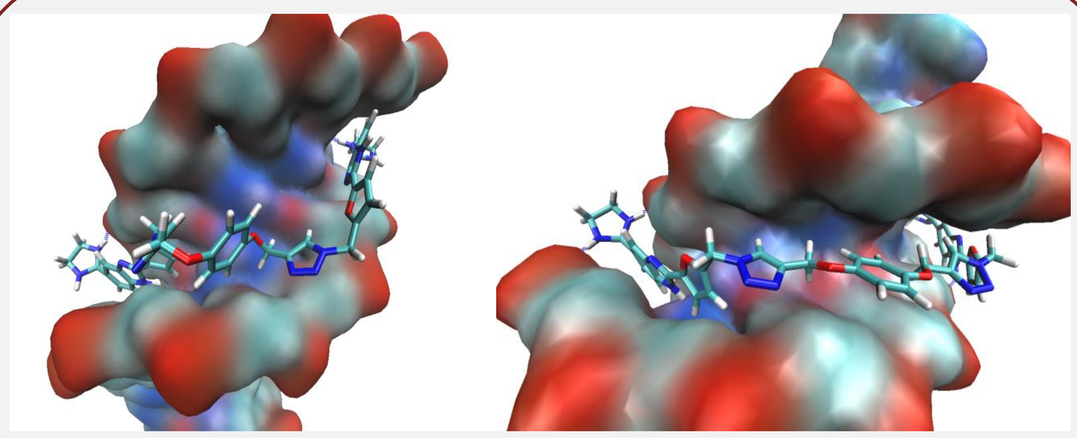


Figure 2. Complex of **15c** with 14 bp DNA d[(CTACCGATAAGCAG)]$_2$ (left) and 12 bp DNA d[(CGCGAATTCGCG)]$_2$ (right).

To verify suggested interactions of **15c**, the compound that exhibited the most potent trypanocidal activity, binding into the DNA minor groove was further analysed using *in silico* molecular studies. It was found that compound **15c** fits nicely into the minor groove of both B-DNA models. Molecular modelling studies revealed that compound **15c** could tightly bind within the *ct*DNA minor groove, whereas **15c** could not bind as efficiently to the much narrower pApU.

References

[1] M. Njoroge, N.M. Njuguna, P. Mutai, D.S. Ongarora, P.W. Smith, K. Chibale, Chem. Rev. 114 (2014) 11138–11163.
[2] C.P. Ward, P.E. Wong, R.J. Burchmore, H.P. de Koning, M.P. Barrett, Antimicrob. Agents Chemother. 55 (2011) 2352–2361.
[3] A. Bistrović, L. Krstulović, I. Stolić, D. Drenjančević, J. Talapko, J. M. Kelly, M. Bajić, S. Raić-Malić, J. Enzyme. Inhib. Med. Chem. 33 (2018) 271–285.

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