Synthesis, DNA binding and Antitrypanosomal Activity of Benzimidazole Analogues of DAPI

Abdelbasset A. Farahat, Cheree Bennett-Vaughn, Ekaterina M. Mineva, Arvind Kumar, Tanja Wenzler, Reto Brun, Yang Liu, W. David Wilson, David W. Boykin

PII: S0960-894X(16)31146-5
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.11.006
Reference: BMCL 24401

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 27 September 2016
Revised Date: 31 October 2016
Accepted Date: 1 November 2016

Please cite this article as: Farahat, A.A., Bennett-Vaughn, C., Mineva, E.M., Kumar, A., Wenzler, T., Brun, R., Liu, Y., David Wilson, W., Boykin, D.W., Synthesis, DNA binding and Antitrypanosomal Activity of Benzimidazole Analogues of DAPI, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.11.006

Synthesis, DNA binding and Antitrypanosomal Activity of Benzimidazole Analogues of DAPI

Abdelbasset A. Farahat[a,b] *, Cheree Bennett-Vaughn[a], Ekaterina M. Mineva[a], Arvind Kumar[a], Tanja Wenzler[c,d], Reto Brun[c,d], Yang Liu[a], W. David Wilson[a] and David W. Boykin[a].

[a]Department of Chemistry, Georgia State University, Atlanta, GA, 30303.

[b]Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.

[c]Swiss Tropical and Public Health Institute, Basel, 4002, Switzerland,

[d]University of Basel, Basel, 4003, Switzerland

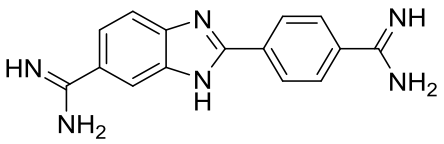
* Fax: 0014044135505, E-mail: afarahat@gsu.edu

Abstract

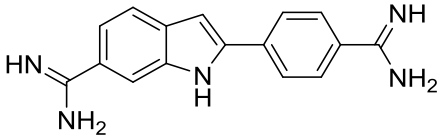
A series of novel benzimidazole diamidines were prepared from the corresponding dicyano analogues either by applying Pinner methodology (**5 a-c, 10 and 13 a**) or by making amidoximes intermediates that were reduced to the corresponding amidines (**15 a-c**). The new amidines were evaluated *in vitro* against the protozoan parasite *Trypanosoma brucei rhodesiense* (*T. b. r.*). The thiophene analogue **5b** and the *N*-methyl compound **15a** showed superior antitrypanosomal activity compared to that of the parent **I**.

Keywords. Benzimidazole diamidines, DAPI, DNA minor groove binders, antitrypanosomal activity.

Benzimidazole analogues have been extensively studied as AT specific DNA minor groove binders [1]. Benzimidazole containing drugs have been used as analgesic, anti-inflammatory [2], antihistaminic [3], anthelmintic [4], antiulcer [5], and antiamoebic [6] agents. Also, benzimidazole containing compounds have shown good antiprotozoal activity[7b,c]. It has been estimated that over 50 million people are at risk for contracting Human African Trypanosomiasis (HAT) in more than 30 African countries and there remains a clear need to develop new safe, efficacious and affordable drugs [8]. Over sixty years ago, 5-amidino-2-(*p*-amidinophenyl)benzimidazole **(I)** **(**Figure 1) was reported to show significant activity against several African trypanosome strains and it was more active than the related benzothiazole and benzoxazole analogues [9]. However, since that report this system has received little study. The closely related structural analogue 4', 6-diamidino-2-phenylindole, DAPI, (Figure 1), an extensively studied minor groove DNA binder, was developed as a diamidino compound related to Berenil and Stilbamidine, to be used as an antitrypanosomal agent [10]. Recently, we have demonstrated that modifications of DAPI could lead to more active antiprotozoal agents [11]. Based on the above findings, we report the preparation of three types of benzimidazole diamidine compounds, by replacing the phenyl moiety with heterocycles (**5 a-c**), and by substitution of the benzimidazole NH of **I** with an NOH group (**10**) or by *N*-alkyl groups (1**5 a-d**).



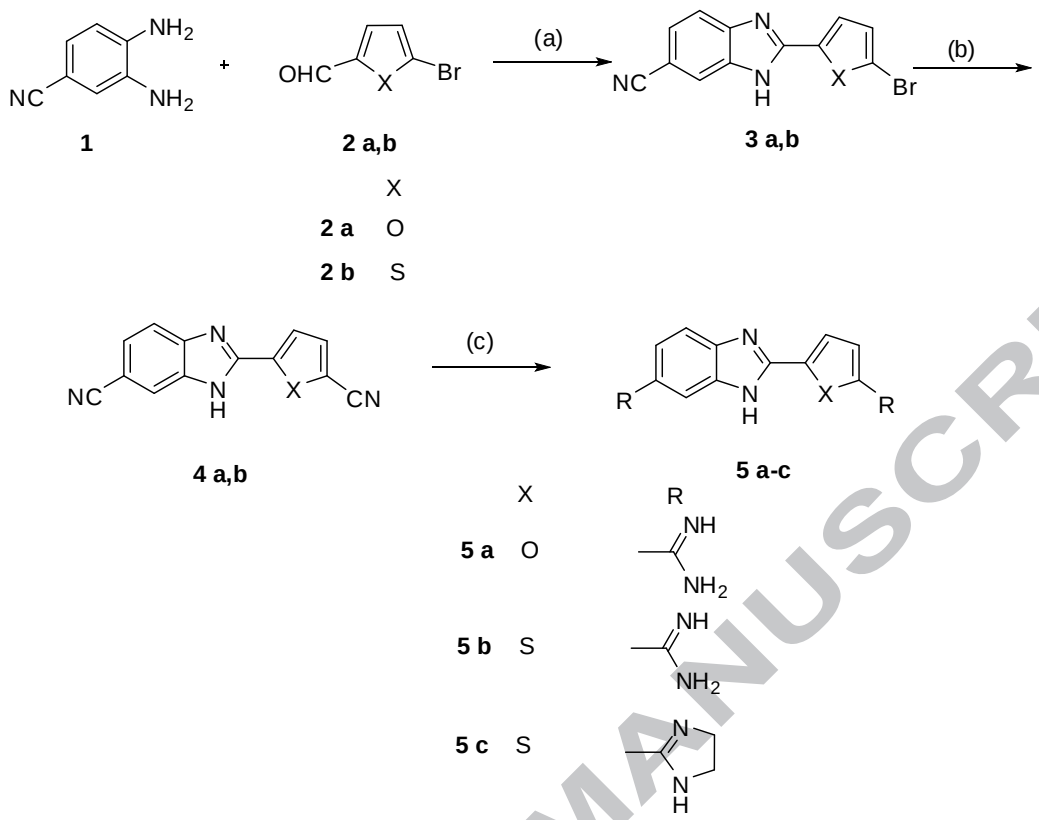
I



DAPI

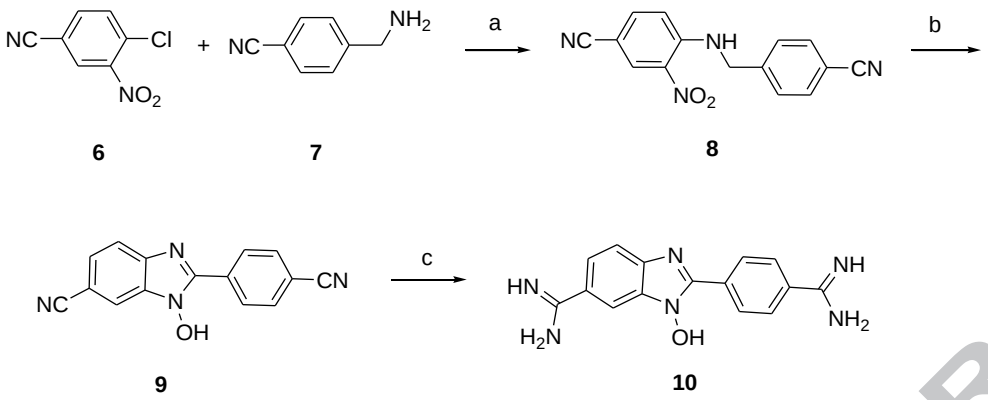
Figure 1. Parent benzimidazole and DAPI

Scheme 1 outlines the synthesis of the diamidines **5 a-c.** The diamine **1** underwent oxidative cyclization by coupling with the aldehydes **2 a, b** in the presence of 1, 4-benzoquinone in ethanol [7a] or sodium bisulphite in dimethylformamide [7b] to give the benzimidazole derivatives **3 a, b** in good yields. The bromo compounds **3 a, b** were converted to the corresponding cyano derivatives **4 a, b** by heating at 130-140 ^{o}C overnight with copper cyanide in dimethylformamide under a nitrogen atmosphere [12]. Stirring the dinitriles **4 a, b** in ethanolic HCl according to Pinner methodology [7c], with rigorous exclusion of water, afforded the corresponding bis-imidate ester hydrochloride which was allowed to react with ethanol saturated with ammonia gas to give the diamidines **5 a, b** or to react with ethylene diamine in ethanol to give the diimidazoline compound **5 c**.



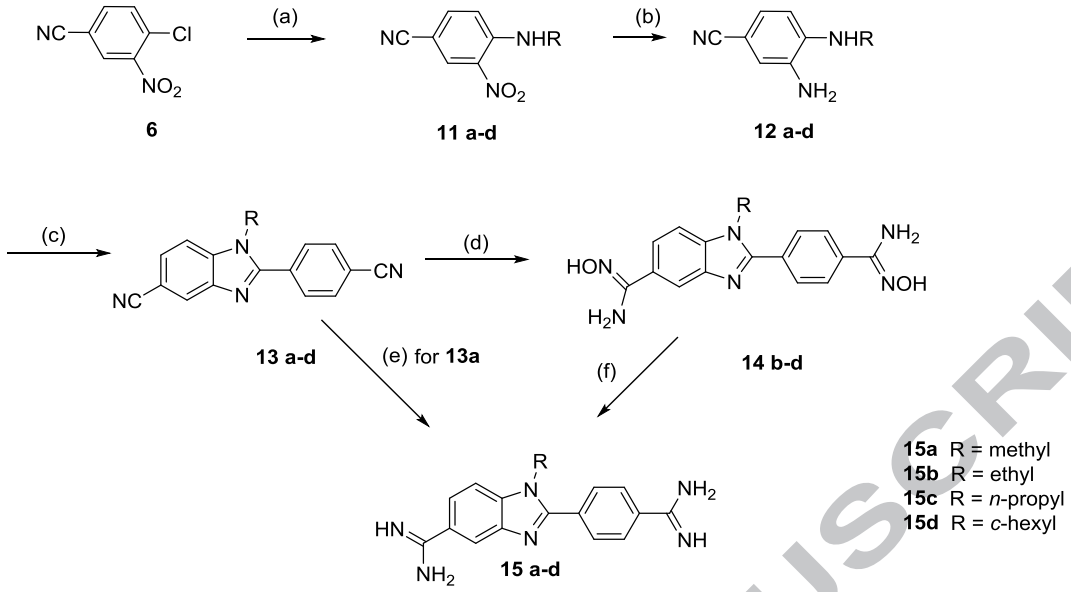
Scheme 1. Reagents and conditions (a) i-1,4-benzoquinone, EtOH, or ii- Sodium bisulphite, DMF; (b) CuCN, DMF; (c) i-HCl gas/EtOH, ii-NH_3 gas/EtOH or $NH_2(CH_2)_2NH_2$/EtOH.

Scheme 2 gives the synthetic approach used to prepare the N-hydroxy diamidino benzimidazole **10** starting with the activated chlorobenzene derivative **6** which on reaction with the p-cyanobenzylamine **7** in dioxane in the presence of potassium carbonate gives **8.** The dicyano derivative **8** was cyclized by heating in a sodium methoxide/ methanol solution to afford the *N*-hydroxybenzimidazole **9**. The diamidine **10** was obtained according to Pinner methodology discussed before.



Scheme 2. Reagents and conditions a) K_2CO_3, dioxane; b) MeOH, NaOMe; c) i-HCl gas/EtOH, ii-NH_3 gas/EtOH.

Scheme 3 describes the synthesis of the diamidino benzimidazoles **15 a-d**. Alkylation of the activated chlorobenzene derivative **6** was achieved by heating with an excess of the alkylamines to give **11 a-d** in good yields [13]. The nitro compounds **11 a-d** were hydrogenated at 50 psi using 10% Pd/C to afford the diamines **12 a-d** [13]. The diamines **12 a-d** underwent oxidative cyclization by coupling with p-cyanobenzaldehyde in the presence of sodium bisulphite in dimethylformamide [7b] to give the benzimidazole derivatives **13 a-d** in good yield. The bisnitrile **13a** was converted to the diamidine **15a** using the Pinner approach described above. The dinitriles **13 b-d** were allowed to react at room temperature for 24 h with a mixture of hydroxylamine hydrochloride and potassium tert-butoxide in DMSO solution to furnish the amidoximes **14 b-d** in excellent yield. Acetate salts of the diamidines **15 b-d** were obtained from their respective diamidoximes **14 b-d**, through the corresponding bis-*O*-acetoxyamidoximes, followed by hydrogenation in glacial acetic acid/ethanol in the presence of 10% Pd/C [7b].



Scheme 3. Reagents and conditions (a) RNH_2 ;(b) Pd/C, H_2, EtOH; (c) i- 4-cyanobenzaldehyde, sodium bisulphite, DMF; (d) NH_2OH-HCl/KO-*t*-Bu, DMSO; (e) i - HCl gas/EtOH, ii -NH_3 gas/EtOH (f) i- AcOH/Ac_2O, ii- Pd/C, H_2, AcOH/EtOH.

Table 1 contains the DNA binding affinities for the new diamidino analogues of 5-amidino-2-(*p*-amidinophenyl)benzimidazole (**I)** as well as the *in vitro* activity for these compounds against *T. b. r.* For comparative purposes, data for **I** and DAPI are included. The thermal melting increase ΔTm (Tm of complex – Tm of free DNA) is a rapid and reliable method for ranking binding affinities for large numbers of aryldiamidines [14]. The ΔTm values for the complexes between poly (dA-dT) and the new analogues are quite varied and ranges from 1.1 to 26^{o}C. The thiophene compounds (**5b and 5c**) exhibit high ΔTm values (25.2 and 26.0 ^{o}C) comparable to that of DAPI and **I**. Interestingly, **5a**, the furan isostere of **5b**, gives a ΔTm value of only 10.1 ^{o}C. This result is consistent with other DNA minor groove binders which have shown stronger affinities for thiophene analogues than their furan counterparts [15,16]. The weaker DNA binding affinity of the furan analogue **5a** is likely due to the poorer fit with the minor groove as a result of its greater curvature than that of **5b** (see Figure 2).The *N*-alkyl

benzimidiazoles **15a-d** all show relatively low ΔTm values ranging from 8.0 to 2.0 ^{o}C. The drop in ΔTm values is consistent with the loss of the benzimidazole NH which has been shown to play a key role in the binding affinity of these type minor groove binders [16]. Notably, the ΔTm values roughly decrease with increasing size of the alkyl group. This interesting result maybe be due to steric interactions between the alkyl groups and the walls of the groove and/or may arise from the protrusion of the alkyl groups out into solvent which may disrupt of waters of hydration in the minor groove. Finally, the *N*-OH compound **10** has low binding (ΔTm 1.1 ^{o}C) to poly (dA-dT). This result is counterintuitive as the NOH group provides another potential H-bond donor. This result suggests that the *N*-OH faces the floor of the groove and prevents the required close approach of the diamidino groups to the floor of the groove and thereby eliminates important H-bonding interactions between the amidine NHs and the thymine carbonyl groups.

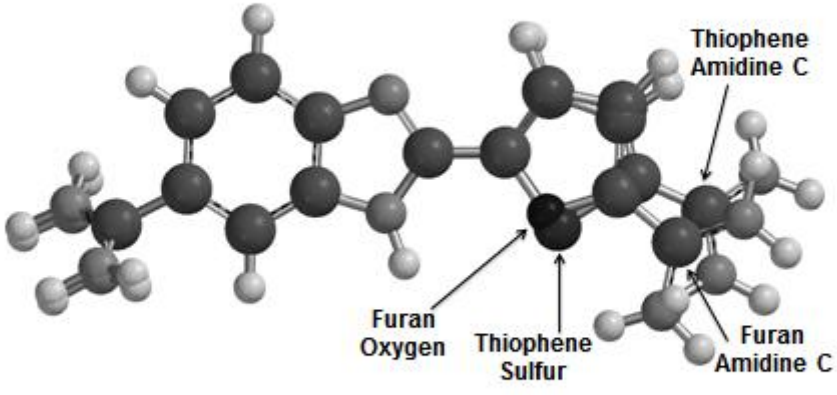
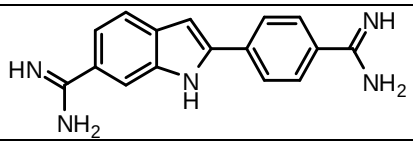
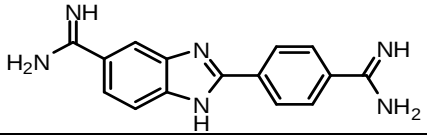
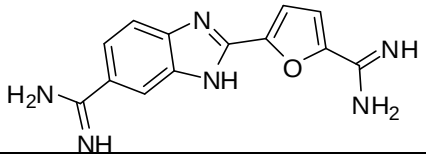
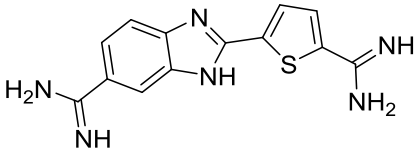
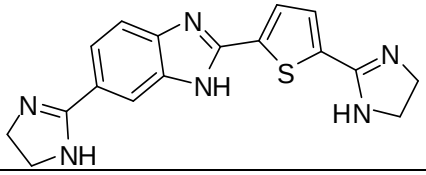
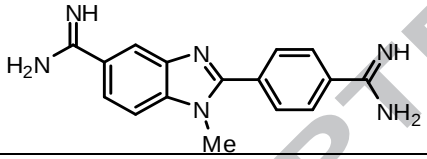
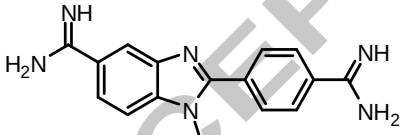
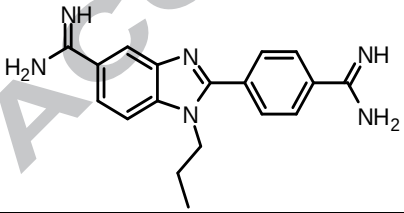
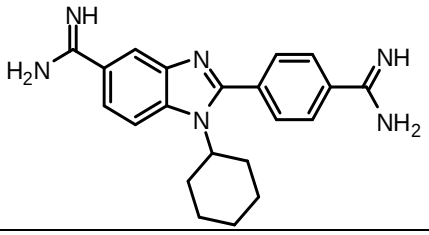
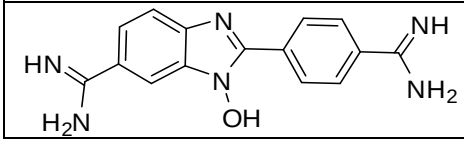


Figure 2. The furan **5 a** and thiophene **5 b** were energy minimized by geometry optimization in the Spartan 10 software package with the molecular mechanics MMFF force field. The benzimidazole group are quite constant in structure and are overlayed to compare the structural changes at the furan and thiophene groups and amidines.

The *in vitro* activity against *T. b. r.* of the parent benzimidazole **I** is slightly less than that of the related indole DAPI (IC_{50} values 31 and 18 nM, respectively). Four of the eight new diamidines where highly active and gave IC_{50} values less than 50 nM. The replacement of the phenyl group of **I** with furan (**5a**) results in an increase in IC_{50} (48 nM), however replacement with a thiophene (**5b**) provides a decrease in IC_{50} (19 nM). Replacement of the amidines of **5b** with imidazolines **5c** results in a loss of activity (IC_{50} = 67 nM). Such a decline in activity on replacement of amidino groups with cyclic amidines has been observed in other systems [7c]. With the exception of the *N*-methyl analogue **15a**, which is the most active compound in this set (IC_{50} = 15 nM and a selectivity index IC_{50}-L6 / IC50-*T.b.r* of SI > 10,000), all of the other *N*-alkyl compounds are less active than **I**. The *N*-cyclohexyl analog **15d** is significantly less active than **I** with an IC_{50} value of 529 nM while compound **10** is inactive with an IC_{50} value of 8,433 nM. The two most active compounds *in vitro* **5b** and **15a**, which also had reasonable selectivity indices, were advanced to the stringent STB900 mouse model for HAT [19]. These compounds were evaluated at the low screening dose of 5 mg/kg. Neither compound showed significant *in vivo* activity. The *in vivo* results for **5b** and **15a** are likely due to poor PK profiles.

Table 1. DNA biding affinities and biological data for the benzimidazole analogues.

Compound	Number	ΔTm (C^o)[a]	T.b.r (nM)[b]	Cytotoxicity (μM)[c]	Selectivity index[d]
	DAPI	>27	18	0.86	48
	I	24.7	31	26.3	848
	5a	10.1	48	150.5	3229
	5b	25.2	19	26.7	1405
	5c	26.0	67	129.2	1925
	15a	8.0	15	>169	>11266
	15b	5.5	64	>207	>3234
	15c	2.5	31	>137	>4419

	15d	2.0	529	>162	>306
	10	1.1	8433	>210	>25

[a] Increase in thermal melting of poly(dA-dT)$_n$ in ^{o}C [17].

[b] IC_{50} values obtained against the STIB900 strain of *T. b. r. The IC_{50} values are the average of two independent assays, the lower IC_{50} varied by less than 50% from the higher IC_{50}.* [18].

[c] Cytotoxicity (IC_{50}) was evaluated using cultured L6 rat myoblast cells [18]. *The IC_{50} values are the average of two independent assays, the lower IC_{50} varied by less than 50% from the higher IC_{50}.*

[d] Selectivity index for T. b. r. expressed as the ratio: IC50 (L6)/IC50 (T.b.r.).

Several new analogues of **I** have been synthesized. The thiophene analogues **5b** and **5c** show DNA affinities comparable to **I** and DAPI. All the other compounds showed lower binding affinities. The failure of the N-hydroxy benzimidazole **10** to bind to the minor grove provides new insights regarding placement of substituents which face the floor of the minor groove. The thiophene analogue **5b** and the *N*-methyl compound **15a** show superior *in vitro* antitrypanosomal activity compared to that of the parent, **I**, and despite the low *in vivo* activity, this study provides excellent lead compounds for optimization of PK properties.

Acknowledgments. This work was supported by an award from the Bill and Melinda Gates Foundation through the Consortium for Parasitic Drug Development (RB, WDW, DWB).

Supplementary data.

Supplementary data (complete characterizations of all the new compounds associated with this article can be found, in the online version of this journal.

References

1. (a) Czarny, A.; Boykin, D. W.; Wood, A. A.; Nunn, C. M.; Neidle, S.; Zhao, M.; Wilson, W. D. *J. Am. Chem. Soc*. **1995**, 117, 4716.
(b) Czarny, A.; Wilson, W. D.; Boykin, D. W. *J. Heterocyclic. Chem.* **1996**, 33, 1393.

2. Achar, K. C. S.; Hosamani, K. M.; Seetharamareddy, H. R. *Eur. J. Med. Chem.* **2010**, 45, 2048.

3. Goker, H.; Ayhan-Kilcigil, G.; Tuncbilek, M.; Kus, C.; Ertan, R.; Kendi, E.; Ozbey, S., Fort, M.; Garcia, C.; Farre, A. J. *Heterocycles*, **1999** 51, 2561.

4. Mavrova, A. T.; Anichina, K. K.; Vuchev, D. I.; Tesnov, J. A.; Kondeva, M. S.; Micheva, M. K. *Bioorg. Med. Chem*. **2005** 13, 5550.

5. Reddy, M. S.; Anisetti, R. N.; Prasad, K. D.; Sannigrahi, S.; Reddy, P. A. *Pharm. Chem. J*. **2011** 44, 642.

6. Rice, C. R.; Colon, B. L.; Alp, M.; Goker, H.; Boykin, D.W.; Kyle, D. E. *Antimircob. Agents Chemother*. **2015**, 59, 2037.

7. Laughlin, S.; Wang. S.; Farahat. A. A.; Kumar, A.; Boykin, D. W.; Wilson, W. D. *Chem. A Eur. J.* **2015**. 21, 5528. (b) Ismail, M. A.; Batista-Parra, A.; Miao, Y.; Wilson, W. D.; Wenzler, T.; Brun, R.; Boykin, D. W. *Bioorg. Med. Chem.* **2005**, 13, 6718. (c) Boykin, D. W. ; Kumar, A.; Xiao, G.; Wilson, W. D.; Bender, B. C.; McCurdy, D. R.;. Hall, J. E.; Tidwell, R.R. *J. Med. Chem*. **1998**, 41, 124.

8. World Health Organization. Human African Trypanosomiasis (sleeping sickness). Fact sheet No. 259. http://www.who.int/mediacentre/factsheets/fs259/en/.

9. Bower, J. D.; Stephens, F. F.; Wibberley, D. G. *J. Chem. Soc.* **1950**, 3341.

10. Dann, O.; Bergen, G.; Demant, E.; Vol, G. *Liebigs Ann. Chem.* **1971**, 749, 68.

11. Farahat, A. A.; Kumar, A.; Say, M.; Barghash, A. E-D. M.; Goda, F. E.; Eisa, H. M.; Wenzler, T.; Brun, R.; Liu, Y.; Mickelson, L.; Wilson, W. D.; Boykin, D. W. *Bioorg. Med. Chem*. **2010**, 18, 557.

12. Jo. Y. W.; Im, W. B.; Rhee, J. K.; Shim, M. J.; Kim, W. B.; Choi, E. C. *Bioorg. Med. Chem*. **2004**, 12, 5909.

13. Goker, H.; Kus, C.; Boykin, D. W.; Yildiz, S.; Altanlar, N. *Bioorg. Med. Chem*. **2002**, 10, 2589.

14. Wilson, W. D.; Tanious, F. A.; Fernandez-Saiz, M.; Rigl, C. T. *Methods in Mol. Biol.* **1997**, 90, 219.

15. (a) Ismail, M. A.; El Bialy, S.A.; Brun, R.; Wenzler, T.; Nanjunda, R.; Wilson, W.D.; Boykin, D. W. *Bioorg. Med. Chem.* **2011,** 19, 978. (b) Farahat, A. A.; Kumar, A.; Barghash, A. E-D. M.; Goda, F. E.; Eisa, H. M.; Boykin, D. W. *J. Heterocyclic Chem*. **2010**, 47, 167.

16. Mallena, S.; Lee, M. P. H.; Bailly, C.; Neidle, S.; Kumar, A.; Boykin, D. W.; Wilson, W. D. *J. Am. Chem. Soc.* **2004**, 126, 13659.

17. Branowska, D.; Farahat, A. A.; Kumar, A.; Wenzler, T.; Brun, R.; Liu, Y.; Wilson, W. D.; Boykin, D. W. *Bioorg. Med. Chem*. **2010**, 18, 3551.

18. Bakunov. S. A.; Bakunova, S. M.; Wenzler, T.; Ghebru, M.; Werbovetz,

K. A.; Brun, R.; Tidwell, R. R., *J. Med. Chem.* **2010**, 53, 254.

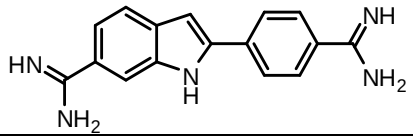
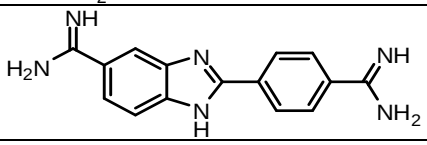
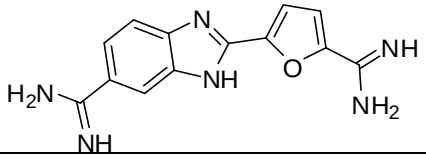
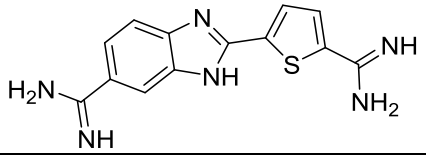
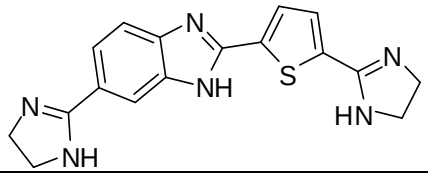
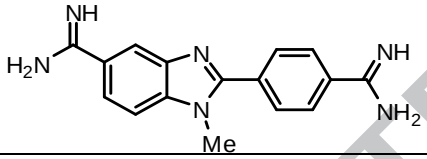
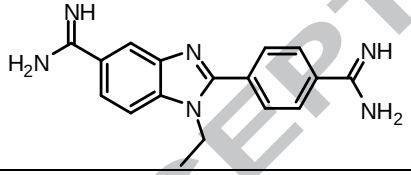
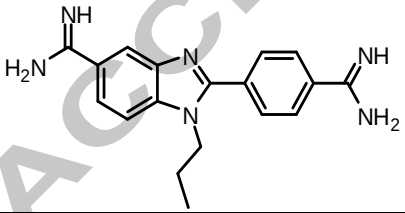
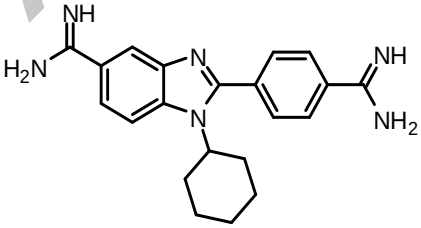
19. Wenzler, T.; Boykin, D. W.; Ismail, M. A.; Hall, J. E.; Tidwell, R. R.; Brun, R. *Antimicrob. Agents Chemother*. **2009**, 53,4185.

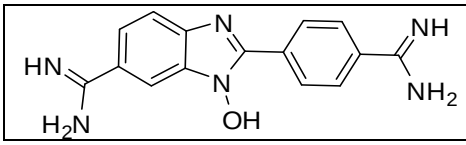
Highlights:

1- New analogues of the antitrypanosomal agent DAPI were made.

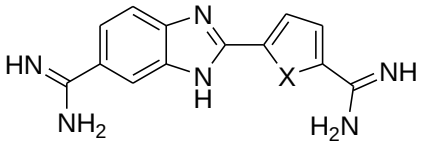
2- ΔTm of some of the final amidines was superior than that of DAPI.

4- Compounds **5b** and **15a** showed superior antitrypanosomal activity compared to the parent **I.**

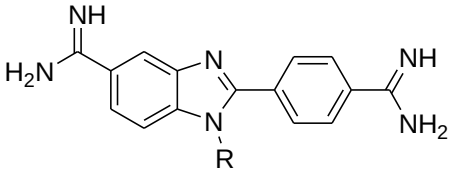
Compound	Number	ΔTm (C°)[a]	T.b.r (nM)[b]	Cytotoxicity (μM)[c]	Selectivity index [d]
	DAPI	>27	18	0.86	48
	I	24.7	31	26.3	848
	5a	10.1	48	150.5	3229
	5b	25.2	19	26.7	1405
	5c	26.0	67	129.2	1925
	15a	8.0	15	>169	>11266
	15b	5.5	64	>207	>3234
	15c	2.5	31	>137	>4419
	15d	2.0	529	>162	>306

	10	1.1	8433	>210	>25

Graphical Abstract



X = O, S



R = CH_3, CH_2CH_3, $CH_2CH_2CH_3$, cyclohexyl