1st International Electronic Conference on Medicinal Chemistry

2-27 November 2015

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Evaluation of Alkanediamide-linked Bisbenzamidines as Potential Antiparasitic Agents

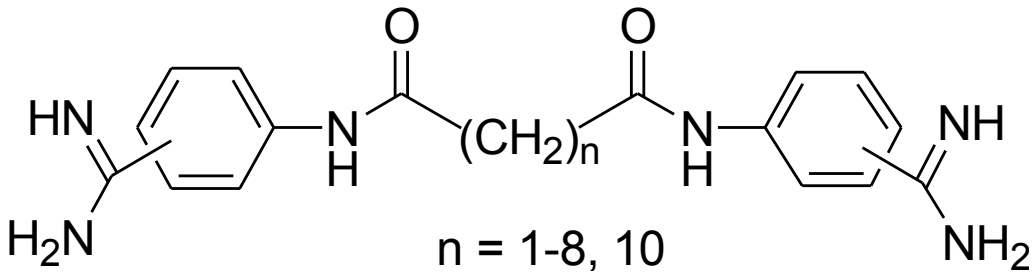
Jean J. Vanden Eynde[1], Annie Mayence[2], Madhusoodanan Mottamal[3], Cyrus J. Bacchi[4], Nigel Yarlett[4], Marcel Kaiser[5], Reto Brun[5], Tien L. Huang[3*]

[1]University of Mons-UMons, department of organic chemistry, B-7000 Mons, Belgium;
[2]Haute Ecole Provinciale de Hainaut Condorcet, B-7730 Saint-Ghislain, Belgium;
[3]Xavier University of Louisiana, College of Pharmacy, New Orleans, Louisiana, USA;
[4]Pace University, Haskins Laboratories, New York, USA;
[5]Parasite Chemotherapy, Swiss Tropical institute, Switzerland.

* Corresponding author: thuang@xula.edu

Evaluation of Alkanediamide-linked Bisbenzamidines as Potential Antiparasitic Agents

Graphical Abstract



7 compounds (**5, 6, 10, 11, 12, 14, 15**)

IC_{50} = 1-96 nM versus *T. brucei and P. falciparum*

Abstract: A series of 15 alkanediamide-linked bisbenzamidines and related analogs were synthesized and tested *in vitro* against two *Trypanosoma brucei* (Tb) strains: *T. b. brucei* (Tbb) and *T. b. rhodesiense (*Tbr*)*, two *Plasmodium falciparum (*Pf*)* strains: a chloroquine-sensitive strain (NF54) and a chloroquine-resistant strain (K1), *Trypanosoma cruzi (*Tc*)*, and *Leishmania donovani (*Ld*)*. The *in vitro* cytotoxicity was determined against rat myoblast cells (L6). Seven compounds (**5**, **6, 10, 11, 12, 14, 15**) showed high potency toward both strains of Tb and Pf with the inhibitory concentrations for 50% (IC_{50}) in the nanomolar range (IC_{50} = 1-96 nM). None of the tested derivatives was significantly active against Tc or Ld. Three of the more potent compounds (**5**, **6**, **11**) were evaluated *in vivo* in mice infected with the drug-sensitive (Lab 110 EATRO and KETRI 2002) or drug-resistant (KETRI 2538 and KETRI 1992) clinical isolates of *T. brucei*. Compounds **5** and **6** were highly effective in curing 100% mice infected with the drug-sensitive strains, including a drug-resistant strain KETRI 2538, but were ineffective against KETRI 1992. Thermal melting of DNA and molecular modeling studies indicate AT-rich DNA sequences in the minor grove as possible binding sites for these compounds.

Keywords: Bisbenzamidines; *Trypanosoma brucei*; *Plasmodium falciparum*, Antiparasitics; DNA

Introduction

Current Therapy of Parasitic Diseases (Human African Trypanosomiasis and Malaria)

Human African Trypanosomiasis (HAT)

- Transmitted by tsetse fly
- Two sub-species: *T. b. gambiense* & *T. b. rhodesiense*
- Causes "sleeping sickness"
- 50,000 deaths reported annually

Haemolymphatic stage (Early-stage)

- Pentamidine (1937) vs *T.b. gambiense*
- Suramin (1916) vs *T.b. rhodesiense*

Central nervous system stage (Late-stage)

- Melarsoprol (1946) vs both strains
- Eflornithine (1977) vs *T.b. gambiense*

Current Therapy of Parasitic Diseases (Human African Trypanosomiasis and Malaria)

Malaria

- Transmitted by female *Anopheles* mosquito
- Four sub-species: *Plasmodium falciparum* is the most lethal
- 450,000 deaths reported annually

Blood schizonticide

- Artemisinin (2015 Nobel Prize awarded to Youyou Tu for this discovery)
- Chloroquine
- Quinine
- Mefloquine
- Pyrimethamine

Tissue schizonticide

- Primaquine

Limitations of Current Therapy

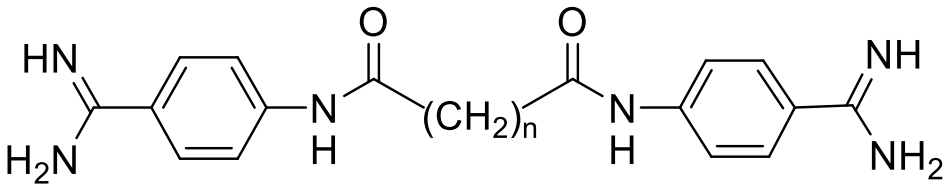
- Unacceptable Toxicity
- Variable efficacy
- Parenteral route of administration
- Drug resistance by parasites

Therefore, new drugs with improved biological properties are urgently needed for the chemotherapy of HAT and malaria.

Research Goals

- Design and synthesize novel bisbenzamidines and related analogs with promising antifungal and/or antiparasitic activity
- Test compounds against *Pneumocystis jirovecii*, *Trypanosoma brucei*, *Leishmania donovani*, and *Plasmodium falciparum*
- Improve efficacy, reduce toxicity, enhance oral activity and increase CNS permeation of lead compounds

Drug Design Rationale



5 : n = 3
6 : n = 4

Compounds **5** and **6** demonstrated excellent *in vitro* and *in vivo* activity against *Pneumocystis carinii* and are lead compounds for the synthesis of an expanded series of alkanediamide-linked bisbenzamidines reported in this study.

Anti-*Pneumocystis carinii* activity

- Vanden Eynde, J.J.; Mayence, A.; Huang, T.L.; Collins, M.S.; Rebholz, S.; Walzer, P.W.; Cushion, M.T. Novel bisbenzamidines as potential drug candidates for the treatment of *Pneumocystis carinii* pneumonia. *Bioorg. Med.Chem.Lett*. **2004**, *14*, 4545–4548.

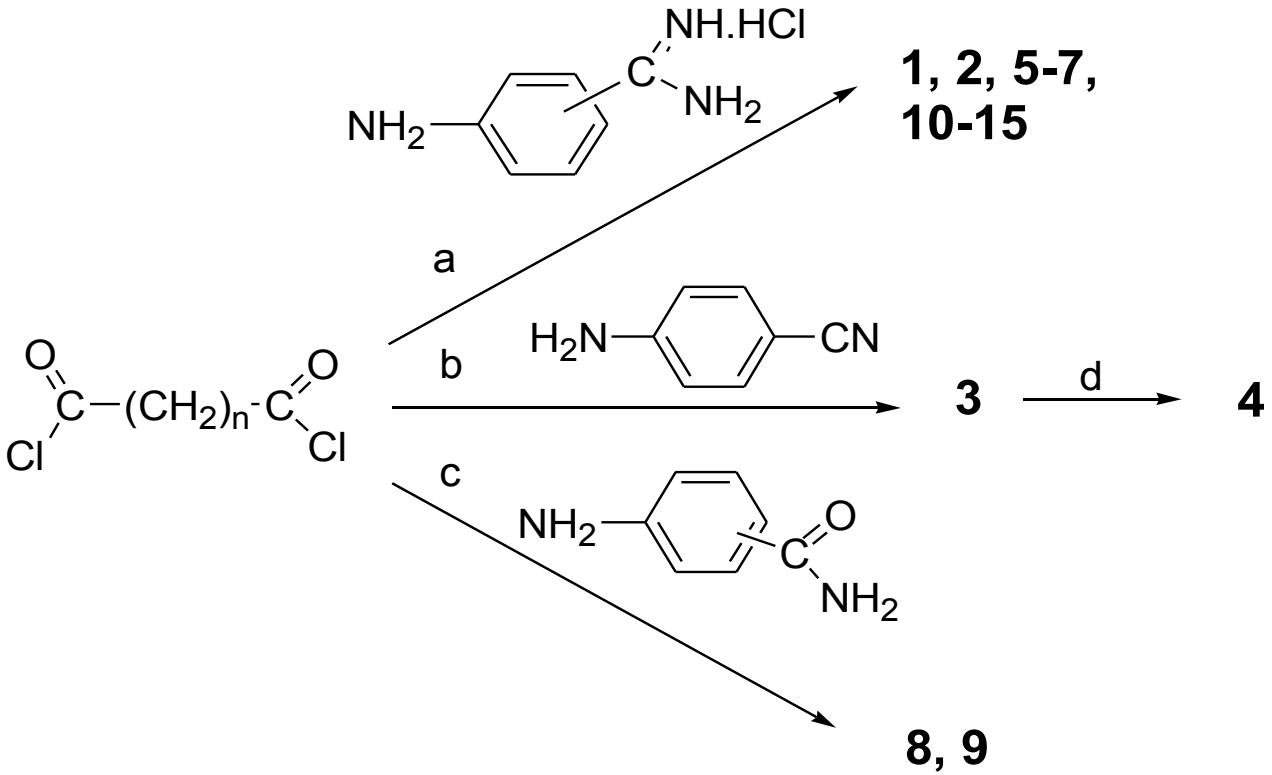
- Cushion, M.T.; Walzer, P.D.; Ashbaug, A.; Rehbolz, S.; Brubkaker, R.; Vanden Eynde, J.J.; Mayence, A.; Huang, T.L. *In vitro* selection and *in vivo* efficacy of piperazine- and alkanediamide-linked bisbenzamidines against *Pneumocystis* pneumonia in mice. *Antimicrob. Agents Chemother.***2006**, *50*, 2337–2343.

Results and Discussion

Chemistry

The synthesis of these compounds are shown in Scheme 1. Starting with the appropriate diacid chlorides and aminobenzamidines or aminobenzonitrile or aminobenzamide, the final products **1-3**, **5-15** were easily obtained in fair yields (40-90%). The bisbenzamidoxime **4** was readily obtained by reacting the bisnitrile **3** with hydroxylamine in 90% yield. The chemical structures and purity of the synthesized products were confirmed by spectral data (^{1}H NMR, FTIR, HRMS) and elemental analyses.

Synthesis of Target Compounds



Scheme 1. General procedures for the synthesis of compounds **1**-**15**.
Reagents and conditions: (a) DMF, pyridine, reflux, 30 min – 2 h; (b) and (c): Dioxane, room temp., stirred overnight; (d) Hydroxylamine, DMSO, 70 °C, 17 h.

Pharmacology: *In Vitro* Results (Table 1)

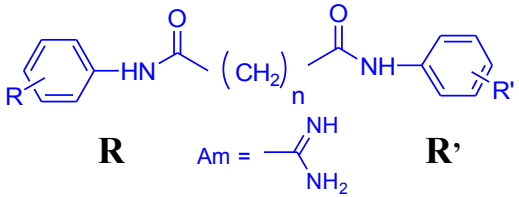
- Seven compounds (**5, 6, 10, 11, 12, 14, 15**) showed high potency against both strains of Tb and Pf with IC_{50}s in the nanomolar ranges (1-96 nM) irrespective of the length of the central methylene chain (n = 3, 4, 5, 6, 8, 10).

- Terminal basic amidinium groups were essential for activity. Replacement with terminal nitrile **3**, amidoxime **4** or amide groups **8** and **9** resulted in inactive compounds.

- None of the tested compounds showed significant activity against *T. cruzi* or *L. donovani*.

- Selectivity index (SI = ratio of cytotoxic L6 cells to antiparasitic IC_{50} values) showed **6** to be most selective against Tb with SI > 50,000, whereas, compounds **1** and **5** were most selective against Pf with SI > 33,000.

Table 1. *In vitro* antiparasitic and cytotoxic properties of alkanediamide-linked bisbenzamidines and analogs

#	n	 R	R'	IC_{50} (μM)[a]						
				T.b. brucei[b]	*T. b. rhod*[c]	*T. Cruzi*	*L. donovani*	*P. falcip* NF54[d]	*P. falcip* K1[e]	L6 cells
1	1	p-Am	p-Am	ND	ND	> 100	> 100	**0.008**	**0.003**	> 100
2	2	p-Am	p-Am	9.0	2.19	> 100	> 100	ND	0.29	28.0
3	3	p-CN	p-CN	6.50	7.90	> 100	> 100	ND	44.8	> 100
4	3	p-C(=NOH)NH2	p-C(=NOH)NH2	7.30	10.0	>100	>100	ND	3.16	>100
5	3	p-Am	p-Am	**0.009**	**0.096**	> 100	> 100	**0.002**	**0.002**	69.0
6	4	p-Am	p-Am	**0.003**	**0.002**	> 100	> 100	**0.004**	**0.018**	> 100
7	4	m-Am	m-Am	0.041	0.021	> 100	> 100	ND	0.38	> 100
8	4	p-CONH2	p-CONH2	2.80	1.10	> 100	> 100	ND	> 100	> 100
9	4	m-CONH2	m-CONH2	ND	1.97	> 100	> 100	ND	> 100	46.4
10	4	p-Am	m-Am	**0.012**	**0.007**	> 100	> 100	**0.015**	**0.004**	> 100
11	5	p-Am	p-Am	**0.002**	**0.002**	> 100	> 100	**0.002**	**0.002**	43.0
12	6	p-Am	p-Am	**0.003**	**0.001**	> 100	> 100	**0.002**	**0.006**	38.4
13	7	p-Am	p-Am	0.400	0.240	99.5	67.4	**0.002**	**0.008**	49.2
14	8	p-Am	p-Am	**0.002**	**0.004**	76.4	68.5	**0.014**	**0.012**	78.6
15	10	p-Am	p-Am	0.008	0.007	80.9	10.7	0.032	0.015	80.2
ref				0.002 [f]	0.002 [f]	1.13 [g]	0.44 [h]	0.006 [i]	0.18 [i]	0.010 [j]

[a]: Each value is the average of at least two determinations.[b,c]: The *Trypanosoma brucei brucei* strain was Lab 110 and the *Trypanosoma brucei rhodesiense* strain was KETRI 243 [d,e]: The *Plasmodium falciparum* strains NF 54 and K1 are chloroquine-sensitive and –resistant strains respectively. [f]: pentamidine; [g]: benznidazole; [h]: miltefosine; [i]: chloroquine; [j]: podophyllotoxin. ND not done.

Pharmacology: *In Vivo* Results (Table 2)

- *In vivo* efficacy of compounds **5, 6** and **11** were evaluated in mice infected with drug-susceptible (Lab 110 EATRO and KETRI 2002) and drug-resistant (KETRI 2538 and 1992) strains of *T. brucei*.

- All 3 compounds were effective in curing mice infected with Lab 110 EATRO or KETRI 2002 strains at several doses. Compound **11** was the most effective since it cured the mice at lower doses.

- Compounds **5** and **6** were effective in curing mice infected with KETRI 2538 at several doses. However, these compounds including the reference drug pentamidine, were ineffective in curing mice infected with KETRI 1992.

Table 2. *In vivo* trypanocidal activity of selected compounds in mice[a]

Clinical isolate	Compound #	Dosage (mg/kg/day)	Mean survival (days)	No. of mice cured/ total (%)
Lab 110 EATRO[b]	None	-	5.0	0/3
	Pentamidine	1.0, 2.5, 5, 10	>30	5/5 (100)[c]
	5	1.0	6.0	0/3
		2.5	11.3	0/3
		5	10	2/3 (66)
		10	>30	3/3 (100)
		15	>30	3/3 (100)
	6	1.0	6.7	0/3
		2.5	10.0	0/3
		5	>30	3/3 (100)
		10	>30	3/3 (100)
		15	>30	3/3 (100)
	11	1.0, 2.5, 5, 10	>30	3/3 (100) [c]
KETRI 2002[b]	None	-	9.0	0/3
	Pentamidine	1.0, 5, 10	>30	5/5 (100)[c]
	5	10, 15, 25	>30	3/3 (100)[c]
	6	10, 15, 25	>30	3/3 (100)[c]
	11	1.0, 2.5, 5, 10	>30	3/3 (100)[c]

Table 2. Continued

Clinical isolate	Compound #	Dosage (mg/kg/day)	Mean survival (days)	No. of mice cured/ total (%)
KETRI 2538[b]	None		4.3	0/3
	Pentamidine	1.0, 5, 10	>30	5/5 (100)[c]
	5	10, 15, 25	>30	3/3 (100)[c]
	6	5, 10, 15	>30	3/3 (100)[c]
KETRI 1992[b]	None	-	7.4	0/5
	Pentamidine	1	12.6	0/3
		5	17.0	0/3
		10	22.2	0/3
	5	10	17.5	0/3
		15	14.5	0/3
		25	25	0/3

[a] *In vivo* efficacy of compounds given via i.p route vs. several clinical isolates of *T. brucei*. Mice were infected with 250,000 parasites and dosing commenced 24 h. post infection. Mice were separated into groups of three and injected i.p once a day for 3 days unless otherwise noted. Infected untreated controls were used for each experiment. Mice were considered cured if surviving more than 30 days beyond death of the last control without parasites in tail vein blood smears. Mean survival (in days) of animals dying of trypanosomias is exclusive of cured animals.[b] Trpanosome strains. *T. b. brucei* Lab 110 EATRO strain is susceptible to standard trypanocides including the diamidines. The following are clinical isolates of *T. b. rhodesiense*: KETRI 243, 2002, 2538 and 1992. Strain 2002 is susceptible to standard trypanocides including the diamidines. Strains refractory to DFMO are KETRI 243 and 2538. Strains refractory to arsenical drugs are KETRI 243, 1992 and 2538. Strains refractory to diamidines are KETRI 243 and 1992 (see Bacchi et al., 1990 for details). [c] All doses cured. Groups of 3 or 5 animals used for all doses.

DNA Binding Affinity

- Thermal melting studies indicate that active compounds have good binding affinity for calf thymus DNA and poly(dA-dT). Binding to poly(dA-dT) were consistently stronger (Table 3 – thermal melting data) which is in agreement with literature data that aromatic diamidines prefer AT-rich DNA sequences in the minor grove of parasitic organisms.

- A good correlation between the *in vitro* antiparasitic activity and DNA binding affinity for this series of compounds was observed (Table 4 –last column on right and Figure 1). The Pearson correlation (R) between the experimental pIC_{50} values of *T. b. brucei*, *T. b*. *rhodesiense*, *P. falciparum* K1 and the ΔT_m values of poly(dA-dT) were determined to be 0.73, 0.79, and 0.67 respectively. This suggests that DNA in these parasites is a potential target for these compounds.

Table 3. Docking of alkanediamide-linked bisbenzamidines and analogs to various DNA duplexes with specific central sequences, *in vitro* antiparasitic activities and thermal melting temperatures using Poly(dA-dT) and CT-DNA.

#	Central DNA sequences of the duplex and the docking scores			pIC_{50} for 3 cell lines			Thermal Melting	
	AAATTT	*AAAGTTT*	*AAAGCTTT*	*T.b. brucei*[a]	*T. b. rhod*[b]	*P. falcip* K1[c]	ΔTm, °C Poly(dA:dT)[d]	ΔTm, °C CT-.DNA[e]
1	4.71	4.79	5.74	ND	ND	8.523	ND	ND
2	6.26	6.01	7.16	5.046	5.660	6.538	11.5	8.1
3	5.88	5.95	5.03	5.187	5.102	4.349	0.15	-0.2
4	6.82	5.37	7.16	5.137	5.000	5.500	0.0	0.7
5	6.48	6.62	6.94	8.046	7.018	8.699	11.4	5.6
6	7.28	7.79	7.92	8.523	8.699	7.745	14.4	8.7
7	7.01	8.46	6.98	7.387	7.678	6.420	13.8	ND
8	1.99	5.09	5.01	5.553	5.959	4.000	0.00	ND
9	6.45	6.12	6.08	ND	5.706	4.000	0.00	ND
10	7.12	7.59	8.07	7.921	8.155	8.398	ND	ND
11	6.85	8.91	8.54	8.699	8.699	8.699	10.7	6.4
12	6.49	8.42	8.35	8.523	9.000	8.222	13.2	7.1
13	9.19	9.3	8.79	6.398	6.620	8.097	7.5	4.6
14	8.68	10.53	11.1	8.699	8.398	7.921	10.0	6.6
15	9.57	10.06	8.99	8.097	8.155	7.824	8.5	ND
16[f]	6.99	8.83	8.64	8.699	8.699	6.745	21.6	11.6

[a,b,c.] *Trypanosoma brucei brucei*, *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum* K1strains respectively. [d,e]: Poly(dA-dT) DNA and Calf Thymus DNA respectively. [f.] Pentamidine; ND, not done.

Correlation Between Modeling and *In Vitro* Data

Table 4. Pearson's correlation (R) between the experimental values (pIC_{50} and ΔT_m) and the docking scores. The values in brackets are the p-values.

	Correlation coefficient R between the docking scores and the pIC_{50} or ΔT_m				Correlation between pIC_{50} and ΔT_m
	AAATTT	AAAGTTT	AAAGCTTT		ΔT_m
T.b. brucei	0.43 (0.1269)	0.75 (0.0021)	0.68 (0.0076)		0.73 (0.0048)
T. b. rhod	0.39 (0.1495)	0.78 (0.0007)	0.70 (0.0038)		0.79 (0.0008)
P. falcip K1	0.48 (0.0595)	0.51 (0.0424)	0.62 (0.0104)		0.67 (0.0087)
ΔTm, °C Poly(dA-dT)	0.35 (0.2179)	0.58 (0.0282)	0.56 (0.0381)		

Correlation Between pIC_{50} and ΔT_M

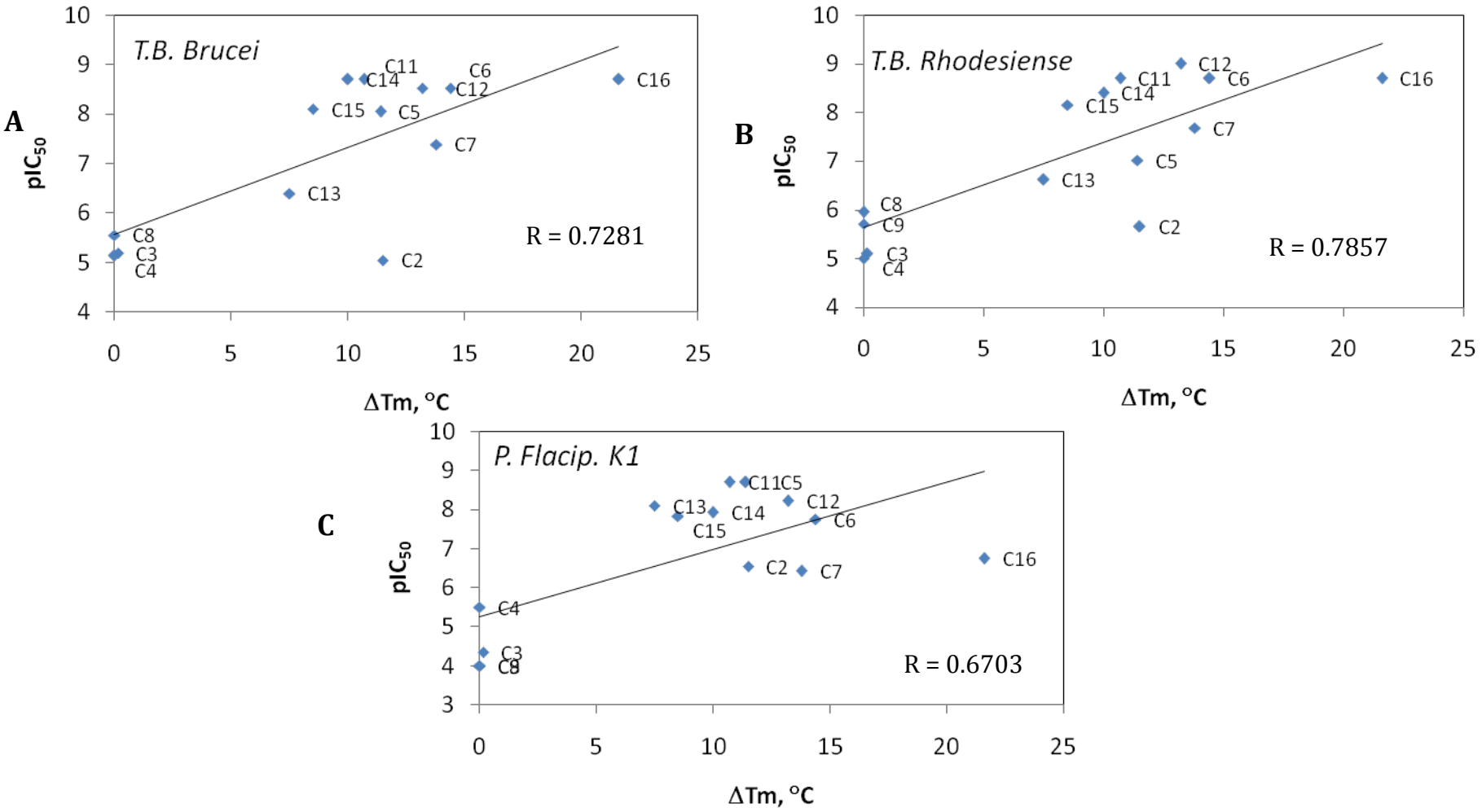


Figure 1. Correlation between the experimental pIC_{50} in different cell lines and ΔT_m with poly(dA-dT). (A) *Trypanosoma brucei brucei*, (B) *Trypanosoma brucei rhodesiense* and (C) *Plasmodium flaciparum* K1 cell lines. The p-values for the correlations are 0.0048, 0.0008 and 0.0087 for Tbb, Tbr and Pf K1 respectively.

Molecular Modeling Results

- To better understand the binding interactions between the compounds and DNA, three AT-rich DNA duplexes were selected for docking studies. The docking scores and correlation with pIC_{50} values are shown in Tables 3, 4 and Figure 2.

- A good correlation (R = 0.75 and 0.78) between the pIC_{50} data of *T. b. brucei* and *T. b. rhodesiense* and the docking scores with the DNA sequence AAAGTTT was observed suggesting that binding to this specific DNA sequence might be important in the anti-trypanosomal activity of the compounds (Table 4, Figure 2).

- A good correlation (R = 0.62) between the pIC_{50} data of *P. falciparum* and the docking scores with the DNA sequence AAAGCTTT was observed suggesting that binding to this specific DNA sequence might be important in the anti-plasmodial activity of the compounds (Table 4, Figure 2).

Correlation Between Modeling and *In Vitro* and Thermal Data

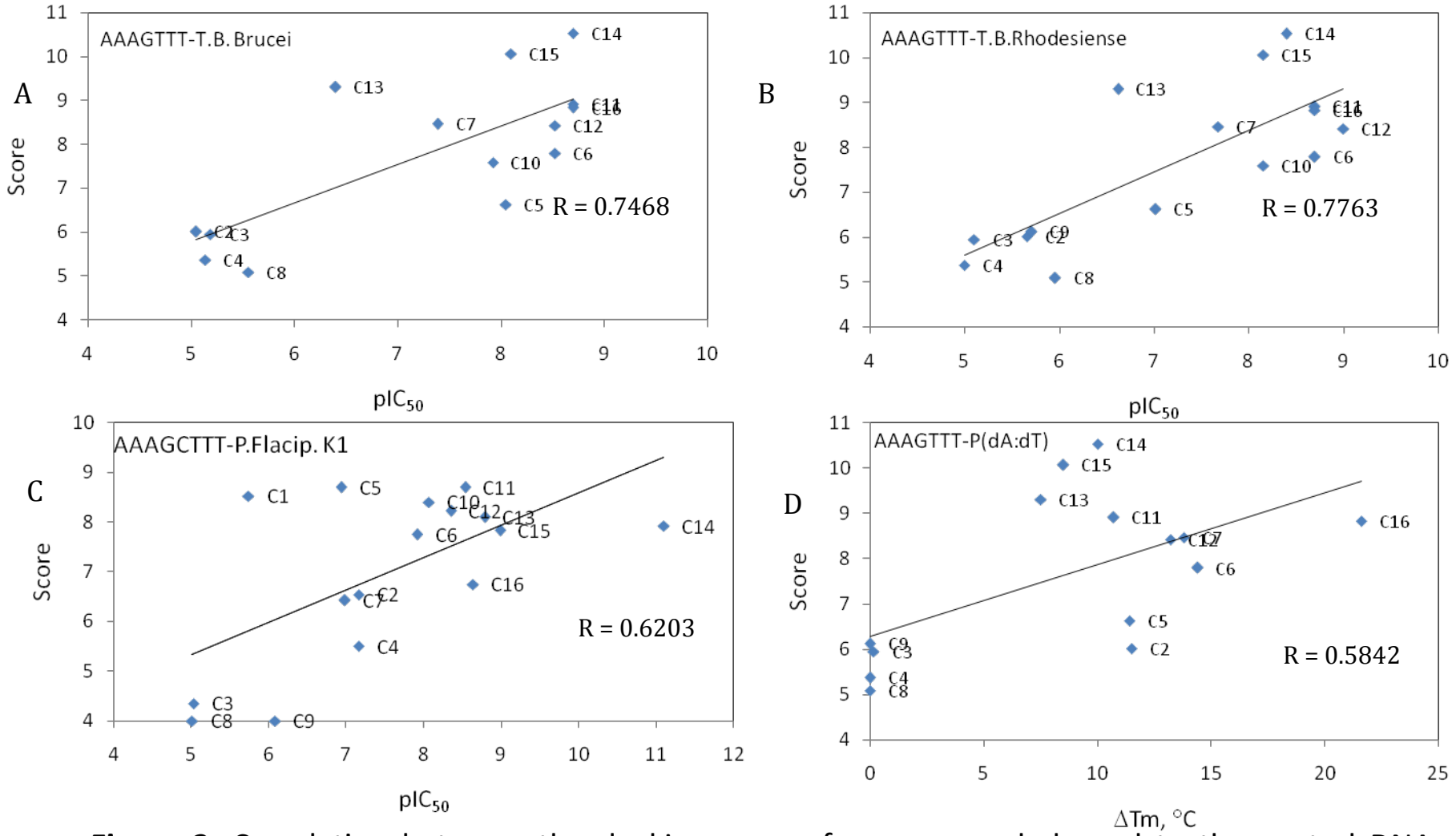


Figure 2. Correlation between the docking scores for compounds bound to the central DNA sequence site and the pIC_{50} in different cell lines or ΔT_m with poly(dA-dT). The p-values for the correlations are 0.0021, 0.0007, 0.0104 and 0.0282 for (A) T.b. brucei, (B) T.b. rhod., (C) P. flacip.K1 and (D) Poly(dA-dT) respectively.

Molecular Modeling Results

•In Figure 3, molecular models with all compounds (A), **8** (B), and **11** (C) docked into the minor groove of 5'-d(CCAAAGTTTGC)-3' duplex is shown.

•Conformation of compounds adopted a shape that closely matches the minor groove shape to form stable DNA duplex-ligand complexes (A).

•Binding mode of the less active compound **8** with DNA indicate two hydrogen bond interactions between the two nitrogens of the alkanediamide linker in **8** with N3 of Adenine 5 and O2 of Thymine 18 of DNA (B).

•Binding mode of the more active compound **11** with DNA indicate four hydrogen bond interactions with DNA. This include the two hydrogen bonds formed between the two nitrogens of the alkanediamide linker of **11** with N3 of Adenine 5 and O2 of Thymine 7 of DNA. In addition, the terminal bisamidinium nitrogens of **11** also form hydrogen bonds with the O3' of the pentose sugars attached to Adenine 5 and Cytosine 17 of DNA (C). Thus, the more active compounds form additional hydrogen bonding interactions with the nucleotides of DNA.

Models of DNA-Drug (Bisbenzamidines) Complexes

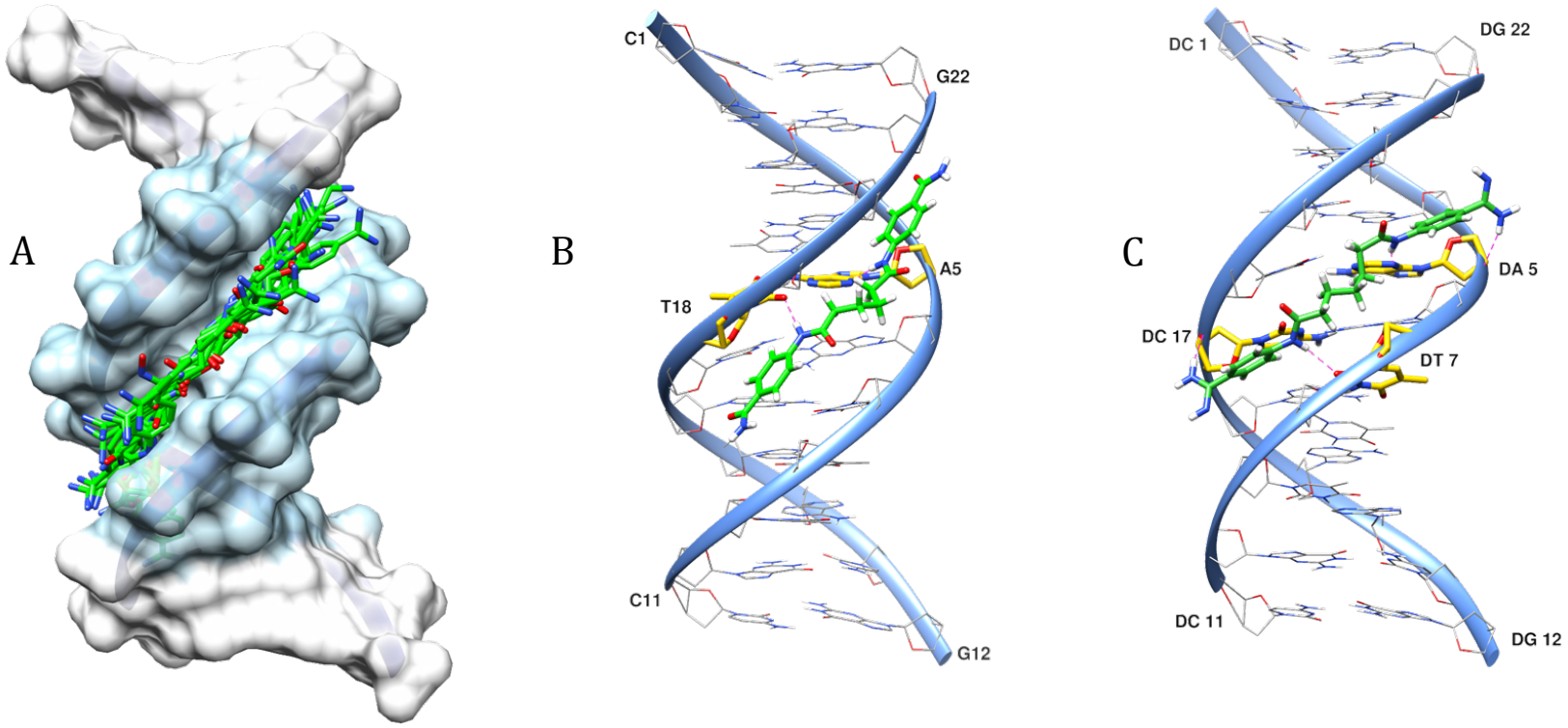


Figure 3. Molecular models for alkanediamide-linked bisbenzamidines and analogs docked into the minor groove of 5'-d(CCAAAGTTTGC)-3' duplex. (A) Top ranked binding poses of compounds **1**-**16** based on the docking scores. The DNA is represented as blue ribbon as well as in molecular surface with the central –AAAGTTT- sequence site in light blue and the remaining in white. Detailed views from the minor groove of the hydrogen bond interactions between the central –AAAGTTT- sequence site and compound **8** (B) and compound **11** (C). All the compounds and nucelotides that form hydrogen bonds with **8** and **11** are shown in stick models. Carbon, oxygen and nitrogen atoms of all the compounds are colored in green, red and blue respectively. The carbon atoms of the nucleotides that form hydrogen bonds with the compounds **8** and **11** are shown in yellow.

Conclusions

- A series of alkanediamide-linked bisbenzamidines were synthesized and several were highly effective against drug-susceptible and drug-resistant strains of *Trypanosoma brucei* and *Plasmodium falciparum*.

- The synthesized compounds showed minimal toxicity in rat myoblast cells (L6) and in efficacy studies.

- Three compounds (**5**, **6**, **11**) were effective in curing mice infected with the drug-sensitive (Lab 110 EATRO and KETRI 2002) clinical isolates of *T. brucei*. Compounds **5** and **6** were also effective in curing mice infected with the drug-resistant strain KETRI 2538, but were ineffective against KETRI 1992.

- Thermal melting of DNA and molecular modeling studies indicate AT-rich DNA sequences in the minor grove as possible binding sites for these compounds.

Acknowledgments

Generous funding from the National Institutes of Health (Grant No. 2G12MD007595) is gratefully acknowledged.