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The Activity of Diguanidino and 'Reversed' Diamidino 2,5-Diarylfurans versus *Trypanosoma cruzi* and *Leishmania donovani*

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Abstract—The in vitro activity of 20 dicationic molecules containing either diguanidino or reversed amidine cationic groups were evaluated versus *Trypanosoma cruzi* and *Leishmania donovani*. The most active compounds were in the reversed amidine series and six exhibited IC_{50} values of less than 1 μmol versus *T. cruzi* and five gave similar values versus *L. donovani*.

Protozoal parasitic diseases have plagued mankind for centuries and continue to cause significant public health problems, particularly in rural developing countries. Two such diseases are Chagas' disease and leishmaniasis. Chagas' disease (American trypanosomiasis) is caused by *Trypanosoma cruzi* and is widespread in Middle and South America. Cases are reported from the southern United States to southern Argentina. Approximately 90 million people are at risk and it is estimated that 16–18 million are currently infected.[1] It is also estimated that approximately 500,000 new cases develop per year and that about 50,000 deaths annually are associated with Chagas' disease. Current recommended treatment for Chagas' disease is either nifurtimox or benznidazole, although the former is not readily available.[1–3] Both of these drugs give variable results when used against the acute form of the disease. No effective treatment exists for the chronic stage of the disease.

As many as 15 species of the *Leishmania* parasite give rise to human leishmanial disease.[4] The three main clinical variations of leishmaniasis are the cutaneous, mucocutaneous, and visceral forms. It is currently estimated that leishmaniasis affects people in 88 countries, with 350 million at risk of contracting the disease. Approximately 2 million new cases are estimated to develop annually.[5] For decades, Pentostam and Glucantime, pentavalent, antimonial compounds, have been used to treat leishmaniasis. Both are administered according to Sb (V) content and are thought to be equivalent in efficacy and toxicity. These drugs are given by injection, exhibit considerable toxicity and resistance to them is developing.[6] Amphotericin B has also been used to treat the visceral form of leishmaniasis.[4] However, it too has toxic side effects, is given by injection and is expensive. Miltefosine, an orally effective drug, is showing considerable promise in the treatment of both visceral and cutaneous leishmaniasis.[7,8] The diamidine pentamidine(4,4′-(pentamethylenedioxy)dibenzamidine) is also used for the treatment of leishmaniasis,[9,10] however it must be given by injection due to its lack of oral bioavailability, and exhibits a number of toxic side effects. From this brief survey of the current status of chemotherapy of these diseases, there is clearly a need for development of new effective therapies for both Chagas' disease and leishmaniasis.

Broad spectrum antimicrobial activity of aromatic dicationic compounds, for which pentamidine may be considered the prototype molecule, has been reported against a variety of organisms.[11–16] In the pentamidine type of molecules, the cationic centers are amidine groups. These type of compounds bind to the minor groove of DNA at AT sites.[17–19] This binding has been hypothesized to be important in the mode of anti-

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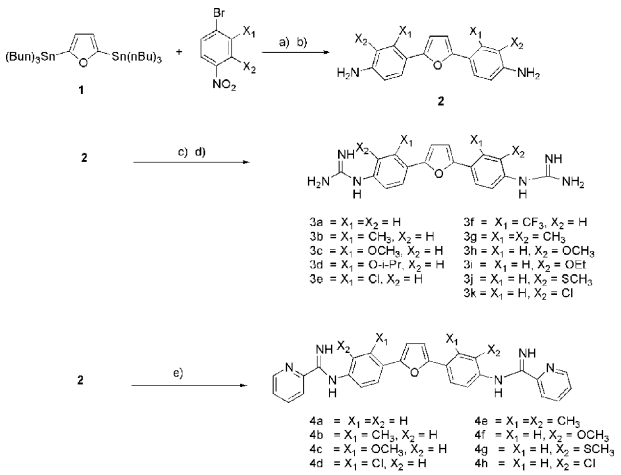
microbial action of these compounds possibly leading to inhibition of DNA dependent enzymes.[20–22] Other mechanisms of action have also been proposed for pentamidine, including disruption of polyamine metabolism[23] and disruption of mitochondrial membrane potential.[24] Diamidine compounds with improved efficacy and reduced toxicity compared to pentamidine have been developed.[15,25] Prodrug strategies for this class of compounds have provided orally effective molecules thus obviating an injection dosage regime, one of the major disadvantages of the pentamidine class of compounds.[26–28] A prodrug of furamidine [2,5-bis(4-amidinophenyl)furan] is currently undergoing Phase II clinical trials against Human African trypanosomiasis and *Pneumocystis carinii* pneumonia.

In addition to pentamidine, many of these diamidine molecules exhibit promising activity against *Leishmania*. The antileishmanial activity of several such dicationic molecules was reported some time ago[29–31] and more recently several new dicationic molecules have shown improved in vitro activity against *L. donovani* compared to pentamidine.[32] A report on the in vitro activity of mono-amidines against *T. cruzi* has appeared.[33] Evaluations of dicationic compounds against *T. cruzi* appear to be limited to pentamidine and related analogues, molecules which have shown only very limited in vivo activity.[34,35]

As part of a study to determine the influence of the structure of the cationic center on the antimicrobial properties of these minor groove binding molecules, we are studying replacement of the amidine unit by guanidine and reversed amidine units.[36] Although antimicrobial activity has been reported for various classes of guanidino compounds,[12] this type of cationic compounds has not been extensively studied as antiprotozoan agents. Compounds containing the other modification of the cationic center being investigated are referred to as 'reversed' amidines. In the reversed amidines, the imino group is attached to an 'anilino' nitrogen in contrast to the original amidino compounds in which the imino group is directly attached to an aryl ring. We have recently shown that a group of 2,5-diarylfurans containing modified cationic centers has significant in vitro antifungal activity.[36] We now report the in vitro evaluation of 20 dicationic molecules with modified cationic groups against *T. cruzi* and *L. donovani*.

The syntheses of the diguanidino compounds and the 'reversed' amidines were achieved starting with 2,5-bis(tri-*n*-butylstannyl)furan using the approach which we previously described[36] and which is outlined in Scheme 1. The 2,5-bis(4-aminophenyl)furan analogues **2** were obtained in good yields and served as the key common intermediates for both sets of target compounds **3** and **4**. The two-step conversion of **2** into the diguanidino compounds **3** was achieved in good yields. The yields of the diguanidino compounds bearing substituents ortho to the guanidino groups were somewhat lower (~50%) than the other analogues. The reaction of **2** with a *S*-(2-naphthylmethyl)thioimidate gave the



Scheme 1. (a) $Pd(PPh_3)_4$, 1,4-dioxane; (b) H_2, Pd/C, EtOAc, EtOH or $SnCl_2$ dihydrate, EtOH, DMSO; (c) *S*-methyl-di-Bocthiourea, $HgCl_2$, TEA, DMF; (d) HCl, CH_2Cl_2, EtOH; (e) *S*-(2-naphthylmethyl)-2-pyridylthiomidate, MeCN, EtOH.

reversed amidines **4** in a straightforward manner. The yields for the reversed amidines were also lower (~30%) when a substituent was *ortho* to the amidino unit.

In our efforts to identify promising antileishmanial agents from a large group of dicationic compounds, we have employed an axenic assay using *L. donovani* amastigote-like parasites to quickly screen for intrinsic antileishmanial activity.[32,37] This initial screening assay indicated that the guanidines possessed good antileishmanial activity, while the reversed amidines displayed excellent antileishmanial activity. These compounds thus merited testing in the *Leishmania*-infected macrophage assay, which is more labor-intensive but also a better predictor of in vivo antileishmanial potential. The structure–activity relationships that have emerged differ somewhat, which is not surprising considering the differences between the two assays. For example, the axenic amastigotes are cultured alone in medium at pH 5.5, while amastigotes in macrophages reside within a membrane-bound acidic vacuole.[38] Thus, a prospective antileishmanial agent would need to cross one membrane in the axenic amastigote assay and three membranes in the infected macrophage model. These assays generally agree, in that they show the guanidines are moderately potent (Table 1) and the reversed amidines are highly potent (Table 2) against *L. donovani* in vitro. Note that seven of the eight reversed amidines reported here have IC_{50} values in the axenic amastigote assay that are below 1 μM, while only two of the 58 compounds reported in our previous work with diverse classes of diamidines[30] had IC_{50} values below 1 μM in this assay. The fact that the reversed amidines were identified as potent antileishmanials by the initial axenic amastigote assay highlights the utility of this screening approach.

To obtain a qualitative evaluation of the DNA binding affinity of these drug candidates, melting temperatures were measured for the compounds in Tables 1 and 2 bound to poly(dA-dT) and the Dickerson–Drew dodec-

Table 1. In vitro anti- *L. donovani* and *T. cruzi* activities, DNA binding affinities and cell toxicities of diguanidinium dications **3a–3k**

Compd	*L.d.* (amastigote) IC_{50}, μM[a]	*L.d.* (macrophage) IC_{50}, μM[a]	*T.c.* IC_{50}, μM[a]	Cell toxicity IC_{50}, μM[a]	ΔT_m[b] (AT)	ΔT_m[c] (oligo)
3a	10.9	>24	86.0	143.0	21.6	10.8
3b	3.3	>6	3.2	14.6	17.8	6.9
3c	18.7	5.2	1.3	20.0	15.2	2.8
3d	9.65	>6	35.0	16.0	12.0	1.5
3e	6.2	>6.8	27.0	14.2	26.1	4.7
3f	14.8	>5.6	31.2	11.3	5.9	0
3g	4.2	>19	2.7	33.4	3.8	1.3
3h	14.5	>29.0	29.0	39.6	15.4	6.2
3i	11.2	>40	152	74.0	16.5	6.8
3j	22.6	>19	114	68.0	12.2	2.6
3k	>50	>6.3	44.0	35.7	12.7	2.6
Penta[d]	2.6	9.3	7.1	4.2	12.8	4.8
Furam[e]	2.7	ND	23.3	6.4	25.0	11.7
Milt[f]		1.0				
Benzn[g]			1.27			

[a]See refs 32, 37, 39, 40 IC_{50} values are the mean of two independent assays.
[b]Increase in thermal melting of poly(dA.dT)$_2$ see ref 25.
[c]Increase in thermal melting of d(CGCGAATTCGCG)$_2$ see ref 25.
[d]Pentamidine.
[e]Furamidine.
[f]Miltefosine.
[g]Benznidazole.

Table 2. In vitro anti- *L. donovani* and *T. cruzi* activities, DNA binding affinities and cell toxicities of reversed amidine dications **4a–4h**

Compd	*L.d.* (amastigote) IC_{50}, μM[a]	*L.d.* (macrophage) IC_{50}, μM[a]	*T.c.* IC_{50}, μM[a]	Cell toxicity IC_{50}, μM[a]	ΔT_m[b] (AT)	ΔT_m[c] (oligo)
4a	0.55	1.45	9.6	17.1	19.6	7.5
4b	0.38	0.6	0.21	11.3	22.6	8.9
4c	0.10	0.48	0.57	12.7	19.0	7.8
4d	0.66	0.13	0.49	8.0	5.2	0
4e	1.14	3.6	0.91	60.6	13.8	4.2
4f	0.29	0.16	1.0	7.9	15.2	6.6
4g	0.35	0.15	0.15	7.3	1.0	0
4h	0.51	ND	0.5	128	0.6	1.5
Phen[d]	>100	>8	13.7	3.8	28.0	15.0
Penta[e]	2.6	9.3	7.1	4.2	12.8	4.8
Furam[f]	2.7	ND	23.3	6.4	25.0	11.7
Milt[g]		1.0				
Benzn[h]			1.27			

[a]See refs 32, 37, 39, 40 IC_{50} values are the mean of two independent assays.
[b]Increase in thermal melting of poly(dA.dT)$_2$ see ref 25.
[c]Increase in thermal melting of d(CGCGAATTCGCG)$_2$ see ref 25.
[d]**4a** with the pyridyl groups replaced by phenyl groups.
[e]Pentamidine.
[f]Furamidine.
[g]Miltefosine.
[h]Benznidazole.

amer d(CGCGAATTCGCG)$_2$. The difference in T_m values between the drug–DNA complexes and free DNA in solution provides a useful tool to assess the interaction strength of the molecules with DNA.

The effect of substitutents on the DNA affinities of these types of molecules has been commented on previously,[34] however in this report we add results for molecules in which a substitutent is placed ortho to the cationic center. Generally, examination of the data obtained with poly (dA–dT) for the diguanidiums in Table 1 shows that substituents located either *ortho* or *meta* to the guanidinium groups (cf. **3b–3d** or **3 h**, **3i**) cause a similar modest lowering of the ΔT_m value except for the cases of **3e** with a moderate electron withdrawing chloro group, **3f** with a strong electron withdrawing trifluoromethyl group and **3g** which contains multiple substitution. Generally, a similar pattern of substitutent effects is seen from the results when the Dickerson–Drew dodecamer is employed. In general, the ΔT_m values for the pyridyl reversed amidines (Table 2) with substituents ortho to the cationic center are lower than the other analogues. This reduction in affinity seems likely to be due to a combination of steric and electronic effects. The good antiprotozoan activity (vide infra) of the compounds which exhibit low DNA affinities

suggests a mode of action for which DNA binding does not play a major role. In general, the DNA affinities for the substituted compounds in both Tables are reduced compared to the parent molecules (**3a** and **4a**). However, the DNA affinities for most of these molecules are similar to that of pentamidine.

The antimicrobial activities of the diguanidino compounds are presented in Table 1. The activities of the diguanidiniums are only modest. Only **3b** and **3c** show IC_{50} values which are comparable to those of pentamidine and furamidine. Three of the diguanidino analogues (**3b**, **3c**, **3g**) show activity against *T. cruzi* which is comparable to that of pentamidine and superior to that of furamidine.

The series of 2-pyridyl reversed amidines exhibits considerable antimicrobial activity against both protozoan parasites (Table 2). Again, there is a reasonably good correlation between the antileishmanial activities observed for these compounds from both in vitro models. Both models indicate IC_{50} values of less than 1 μmol for five molecules (**4b**, **4c**, **4d**, **4f, 4g**). The most active compound (**4d**) is found to be at least 170 times more active than pentamidine in the macrophage model. It is noteworthy that the replacement of the pyridyl rings with phenyl rings results in significant loss of activity although the DNA binding affinity is improved. Good in vitro activity versus *T. cruzi* is also noted for the 2-pyridyl reversed amidines, with six of these analogues showing IC_{50} values of less than 1 μmol. The best compound **4g** is 40-fold more active than pentamidine and exhibits a selectivity index of near 50.

These studies identify two new classes of dicationic compounds with activity against *Leishmania* and *T. cruzi*. The reversed amidines are particularly interesting in that they are far superior to pentamidine in antiparasitic activity in vitro. For example, pentamidine has little measurable activity against intracellular *Leishmania*, while the reversed amidines reduce parasite burdens in infected macrophages at sub-micromolar concentrations. The task remains to identify compounds from these structural classes with acceptable in vivo toxicity profiles that possess oral activity in animal models.

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