Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 24, pp. 3079-3084, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00541-2

Aromatic Guanyl Hydrazones: Synthesis, Structural Studies and *in vitro* Activity against *Trypanosoma cruzi*

J. C. Messeder, L. W. Tinoco and J. D. Figueroa-Villar*

Departamento de Química, Instituto Militar de Engenharia, Praça General Tibúrcio 80, 22290-270, Rio de Janeiro, Brasil

E. M. Souza, R. Santa Rita and S. L. de Castro

Departamento de Ultraestrutura e Biologia Celular. Instituto Oswaldo Cruz, 21045-900, Rio de Janeiro, Brasil

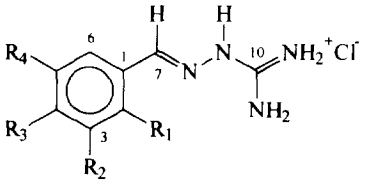
Abstract. A series of 22 aromatic guanyl hydrazones, prepared by condensation of several aldehydes with aminoguanidine hydrochloride, were fully characterized by NMR techniques and tested *in vitro* against the trypomastigote form of *Trypanosoma cruzi*, the causative agent of Chagas disease. Most of the compounds, especially those without hydrogen bonding groups and possessing *ortho*-substitution, were significantly more active than crystal violet (ID_{50} 536 μM). The most active compound has an ID_{50} value of 17 μM (25 times more potent than gentian violet).

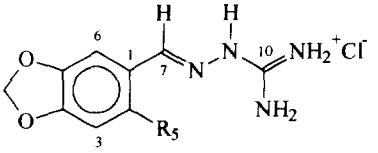
Chagas disease, caused by *Trypanosoma cruzi*, is responsible for much pain and suffering in South America, where about 12 million people are infected with this parasite.[1,2] The current chemotherapy against Chagas disease is still very inadequate. The two principal drugs in use are nifurtimox, whose mechanism of action may involve superoxide anion, which is toxic to the parasite,[3] and benznidazol, which acts on the respiratory system of the parasite and also inhibits DNA synthesis.[4] Both drugs are inefficient to treat chronic Chagas disease, which can be regarded as a disease with no cure. Infection with *T. cruzi* is normally transmitted by a hematophagous insect of the subfamily *Triatominae*. The second way of infection is through transfusion of contaminated blood, which may be the principal way of transmission in some areas. The lack of control of blood quality could spread Chagas disease to areas outside the endemic zones. Although blood contaminated with *T. cruzi* may be used for transfusion after treatment with gentian violet, some restrictions regarding undesirable effects of this compound on the erythrocytes have been reported.[5]

The bisguanyl hydrazone of methyl glyoxal (MGBG) and analogues were first reported as antileukemic agents.[6,7] Further research led to the discovery of the action of MGBG against African trypanosomiasis.[8] More recently, the trypanocidal activity of pentamidine and analogues led Ulrich and Cerami to prepare and study several aromatic and heterocyclic bisguanyl hydrazones which were active against *T. brucei*.[9,10] Although the

guanyl hydrazones have been investigated as therapeutic agents against African trypanosomiasis, there are no reports on their use against *T. cruzi*. This may be due to the significant differences in metabolism and biological cycles between the causative agents of American and African trypanosomiases. In this work, a series of aromatic guanyl hydrazones, shown in Table 1, were prepared, characterized, and tested *in vitro* against the trypomastigote forms of *T. cruzi*.

Table 1. Synthesis of aromatic guanyl hydrazones.





Comp. n°	R_1	R_2	R_3	R_4	R_5	Yield	m. p. (°C)*
1	H	H	H	H	-	64	144-5
2	NO_2	H	H	H	-	47	284-7
3	NH_2	H	H	H	-	17	210(d)
4	OMe	H	H	H	-	71	232-4
5	OEt	H	H	H	-	33	234-6
6	OH	H	H	H	-	80	258-60
7	OH	OEt	H	H	-	47	245-8
8	OH	NO_2	H	H	-	75	245(d)
9	OMe	OMe	H	H	-	43	230-2
10	OMe	H	OMe	H	-	29	205-7
11	OMe	H	H	OMe	-	67	157-9
12	H	NO_2	H	H	-	87	270-3
13	H	OMe	OH	H	-	36	165-8
14	H	H	NO_2	H	-	64	216(d)
15	H	H	CO_2H	H	-	79	280(d)
16	H	H	Cl	H	-	37	138-40
17	H	H	Me	H	-	82	149-51
18	H	H	Me_2N	H	-	68	70(d)
19	-	-	-	-	H	43	158-60
20	-	-	-	-	NO_2	83	295(d)
21	-	-	-	-	NH_2	66	245(d)
22	-	-	-	-	Br	81	250-2

* Melting points are uncorrected.

The aromatic guanyl hydrazones were prepared by direct reaction of the respective aldehydes with aminoguanidine hydrochloride in refluxing 95% ethanol and using concentrated HCl as catalyst.[10] For the less reactive aldehydes, the reaction was carried out in a Dean Stark apparatus in dry toluene or benzene with *p*-toluene sulfonic acid as catalyst. The guanyl hydrazones were usually isolated as crystalline products after cooling to room temperature, and recrystallized two times from an appropriate solvent, usually 95% ethanol. The yields reported in Table 1 correspond to purified products and were not optimized. All the compounds were obtained as their hydrochlorides and are soluble in DMSO. The structures of the products were determined by spectroscopic techniques, principally nuclear magnetic resonance (NMR) and high resolution electron impact mass spectroscopy (HREIMS). A complete NMR assignment is summarized in Tables 2 and 3.

Table 2. ^{1}H NMR Chemical Shift Assignment for the Aromatic Guanyl Hydrazones

Comp. n°	^{1}H NMR Chemical Shift (ppm)							
	H2	H3	H4	H5	H6	H7	H9	H11
1	7.85	7.43	7.44	7.43	7.85	8.13	11.62	7.70
2	-	8.09	7.69	7.82	8.42	8.55	12.10	7.89
3	-	6.79	7.12	6.57	7.19	8.16	11.28	7.65
4	-	7.06	7.42	6.97	8.09	8.44	12.25	7.75
5	-	7.05	7.40	6.96	8.10	8.45	11.80	7.70
6	-	6.93	7.20	6.78	7.90	8.40	12.00	7.74
7	-	-	6.90	6.70	7.50	8.40	*	8.10
8	-	-	8.30	7.00	8.00	8.40	12.00	7.90
9	-	-	7.13	7.70	7.12	8.40	11.68	7.68
10	-	8.02	-	6.58	6.58	8.36	11.85	7.68
11	-	7.12	7.70	-	7.12	8.40	12.10	7.80
12	8.70	-	8.22	7.69	8.25	8.15	12.80	8.06
13	7.50	-	-	6.80	7.10	8.10	12.00	7.90
14	8.11	8.22	-	8.22	8.11	8.20	11.92	7.91
15	7.96	7.96	-	7.96	7.96	8.19	*	8.01
16	7.53	7.86	-	7.86	7.53	8.18	12.14	7.83
17	7.52	7.02	-	7.02	7.52	7.95	11.78	7.60
18	7.67	6.82	-	6.82	7.67	7.98	11.33	7.55
19	7.17	6.93	-	-	7.64	8.08	12.10	7.80
20	-	7.63	-	-	7.97	8.53	12.28	7.95
21	-	6.39	-	-	6.84	8.04	11.08	7.50
22	-	7.20	-	-	7.90	8.40	12.30	8.00

* not observed.

The guanyl hydrazone moiety gives characteristic signals in both ^{1}H and ^{13}C NMR spectra, for all compounds. The terminal NH_2 hydrogens appear as broad singlets between 8.1 and 7.5 ppm, while the imine hydrogen is always above 7.9 ppm. The guanidine carbon resonates almost invariably around 155 ppm along the series. As expected, the chemical shift of the imine carbon is more sensitive to the nature and position of the ring subtituents, but it appears regularly between 140 and 150 ppm. Long range HETCOR[11] and coupled ^{13}C spectra support the ^{1}H and ^{13}C NMR assignments (Tables 2 and 3, respectively).

Table 3. ^{13}C NMR Chemical Shifts Assignment for the Aromatic Guanyl Hydrazones

Comp. n°	^{13}C NMR Chemical Shifts (ppm)							
	C1	C2	C3	C4	C5	C6	C7	C10
1	138.1	133.5	132.4	135.3	132.3	133.5	151.7	159.9
2	127.8	148.3	124.7	131.2	133.7	128.6	142.6	155.1
3	113.9	147.4	115.7	131.3	115.3	132.9	151.6	154.4
4	121.3	157.8	111.1	132.2	120.6	126.4	142.0	155.4
5	121.4	157.2	112.8	132.2	120.6	126.4	142.5	155.0
6	132.4	157.0	116.7	127.1	119.8	119.9	143.9	155.6
7	120.3	146.3	147.1	114.2	119.1	118.4	143.2	155.4
8	124.5	151.1	136.7	133.6	119.9	127.1	141.2	155.3
9	126.8	152.6	148.2	114.9	117.8	124.4	142.8	155.0
10	127.6	162.9	98.0	159.3	106.6	114.1	142.2	155.1
11	126.9	152.6	124.3	117.0	148.1	114.7	142.3	155.1
12	135.6	121.8	148.3	124.7	130.3	133.9	144.7	155.6
13	124.9	115.3	148.1	149.4	109.7	122.7	147.0	155.3
14	139.8	128.8	124.0	148.2	124.0	128.8	144.7	155.4
15	132.3	127.6	129.6	137.5	129.6	127.6	145.4	155.5
16	132.4	128.7	129.2	135.0	129.2	128.7	145.5	155.5
17	130.8	127.7	129.5	140.6	129.5	127.7	147.1	155.4
18	121.9	129.1	112.6	151.2	112.6	129.1	147.6	154.9
19	128.0	124.4	108.3	149.4	148.1	105.4	146.4	155.4
20	124.8	143.5	105.1	149.2	152.0	106.3	142.4	155.4
21	106.0	145.4	96.1	150.4	138.2	109.6	150.6	154.2
22	116.0	125.8	112.4	150.2	147.9	106.6	144.8	155.3

The bioassays against *Trypanosoma cruzi* were carried out using the trypomastigote (blood) form of the parasite obtained from mice inoculated intraperitoneally with 10^5 Y strain trypomastigotes.[12] The stock solutions of the compounds were prepared in Dulbecco's modified Eagle medium (DME) containing no more than 1% dimethyl sulfoxide (DMSO) to help dissolution of the drugs. The parasites were isolated from infected

blood at the parasitemia peak and ressupended in DME containing 20% blood to a parasite concentration of 2-5x10^6 cells/ml. All the tests were carried out in triplicate by mixing 100 μL of cell suspension with an equal volume of the desired drug solution to make final drug concentrations ranging from 2.0 to 4800 μM, and incubating at 4 °C for 24 hours. We observed no deleterious effect of the drugs to erythrocytes afte 24h at 4 °C. Untreated and crystal violet-treated parasites were used as controls. The results were analyzed graphically by plotting the *T. cruzi* inhibition percentage against the drug concentration. The values of ID_{50}, the drug concentration (μM) necessary to kill 50% of the parasites, were obtained by linear and polynomial regression analysis.[13] The results are summarized in Table 4.

Table 4. Values of ID_{50} and total lysis for compounds **1** to **22**.

Comp. n°	ID_{50} (μM)	Total lysis (μM)	Comp. n°	ID_{50} (μM)	Total lysis (μM)	Comp. n°	ID_{50} (μM)	Total lysis (μM)
1	204.9	400-500	9	22.5	75-100	17	103.4	250-300
2	87.5	150-200	10	33.8	100-150	18	202.1	500-600
3	260.7	500-600	11	41.4	150-200	19	273.9	700-1000
4	86.3	150-200	12	182.6	500-700	20	17.1	40-50
5	34.7	75-100	13	>4800	-	21	220.6	500-600
6	166.5	300-400	14	55.9	150-200	22	23.7	50-75
7	97.8	150-200	15	573.0	1200-1400	CV	536	
8	876.2	>1400	16	27.4	100-150			

Most of the synthesized aromatic guanyl hydrazones were significantly more active than crystal violet (CV) (ID_{50} 536 μM). The most active compound (20) has an ID_{50} value of 17 μM (25 times more potent than CV). A qualitative structure-activity relationship was established simply by analyzing the data and grouping the compounds according to the position of the substituents in the aromatic ring and to their ability to form hydrogen bonds. The compounds were also classified into five groups according to their activity. The high activity group was formed by the compounds with $ID_{50} < 105$ μM, the active group with $105 \leq ID_{50} < 205$, the medium activity group with $205 \leq ID_{50} < 300$, the low activity group with $300 \leq ID_{50} < 500$ and the very low activity group with $500 \leq ID_{50}$. All the compounds in the low and very low activity groups have substituents with exchangeable hydrogens, and this percentage decreases as the activity of the group goes up. Thus, among the 11 most active compounds only one (9%) has exchangeable hydrogens. If the analysis is carried out according to the aromatic ring substitution pattern, it is observed that 71% of the compounds in the medium and high activity groups are *ortho*-substituted.

These results suggest that the noncoplanarity of the aromatic ring with the guanyl hydrazone side chain and the lack of hydrogen bond forming substituents may be important for activity. There were no correlations between the electronic nature of the substituents and activity. Analysis of these results by statistical methodologies (QSAR) is in progress, as well as the design and synthesis of a second generation of anti-Chagas guanyl hydrazones.

Acknowledgements

We are grateful to the Chemistry Department of The University of Alberta for the HREIMS, and PADCT/CNPq for financial support.

References

1. Brener, Z. *Pharmacol. Therap.*, **1979**, *7*, 71.
2. De Castro, S. L. *Acta. Trop.* **1993**, *53*, 83.
3. Gutteridge, W. E. *British Med. Bull.* **1985**, *41*, 162.
4. Marr, J. J.; Docampo, R. *Rev. Infec. Diseases* **1986**, *8*, 884.
5. Thomas, S. M.; McPhee, D. E. *Mutation Res.* **1984**, *140*, 165.
6. Mihich, E. *Cancer Res.* **1963**, *23*, 1357.
7. Podrebarac, E. G.; Nyberg, W. J.; French, F. A.; Cheng, C. C. *J. Med. Chem.* **1963**, *6*, 283.
8. Chang, K. P.; Steiger, R. F.; Dave, C.; Cheng, W. C. *J. Protozool.* **1978**, *25*, 145.
9. Ulrich, P. C.; Grady, R. W.; Cerami, A. *Drug Dev. Res.* **1982**, *2*, 219.
10. Ulrich, P. C.; Cerami, A. *J. Med. Chem.* **1984**, *27*, 35.
11. Freeman, R.; Morris, G. A. *J. Chem. Soc. Chem. Commun.* **1978**, 684.
12. Silva, L. H. P.; Nussenszweig, V. *Folia Clin. Biol.* **1953**, *20*, 191.
13. Chaterjee, S.; Price, B. In *Regression Analysis by Example*; John Wiley & Sons: New York, 1977.

(*Received in USA* 12 *May* 1995; *accepted* 15 *November* 1995)