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Anti-trypanosomal compounds. Part II. Novel amidinium sulfinic compounds and phosphorylated heterocycles as anti-trypanosomal agents

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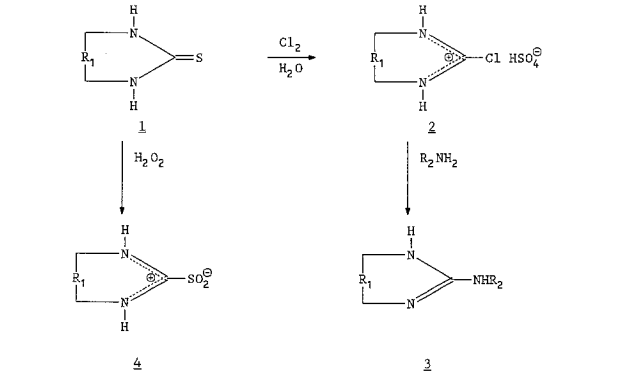
Introduction

In the course of our work on the design of active molecules against African trypanosomiasis, we are developing two different approaches: one based on glycolytic enzyme inhibition owing to the essential character of this metabolism [1, 2] and a second based on more systematic investigations of new structures, designed by varying and combining functionalities for which a biological activity has been recognized. Along these lines, we looked for possible new drugs in the field of cyclic amidines, hydantoins and derivatives, such as sulfinic amidinium compounds, phosphohydantoins and phosphoureas. These compounds were tested *in vitro* against *Trypanosoma equiperdum* and some of them on infected mice.



3a–d **4a–c**

a : R_1 = CH_2-CH_2 a : R_1 = CH_2-CH_2 ; R_2 = C_6H_4OH a : R_1 = CH_2-CH_2

b : R_1 = CH_2-CH_2 ; R_2 = $C_6H_4SO_2NH_2$

b : R_1 =  c : R_1 = CH_2-CH_2 ; R_2 = $C_6H_4SO_3^-Na^+$ b : R_1 = 

d : R_1 = CH_2-CH_2 ; R_2 = CH_2COOH c : R_1 = $COCH_2$

Scheme 1.

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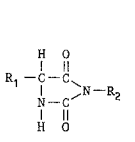
Chemistry

A first set of compounds was obtained by using the reactivity of chloroamidinium ions, prepared by chlorination in water of thioureas. This route allows easy access to the cyclic amidine structure [3], the amidinyl group being a part of certain trypanocides [4]. The reaction is indicated in Scheme 1.



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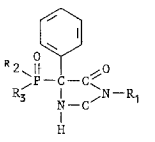

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5a–d **6a–e**

a : R_1 = C_6H_5 ; R_2 = H

b : R_1 = H ; R_2 = C_6H_5

c : R_1 = CH_3 ; R_2 = C_6H_5

d : R_1 = CH_6H_5 ; R_2 = C_6H_5

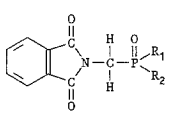
a : R_1 = C_6H_5 ; R_2 = CH_3O ; R_3 = CH_3O

b : R_1 = C_6H_5 ; R_2 = OH ; R_3 = OH

c : R_1 = H ; R_2 = C_6H_5 ; R_3 = C_6H_5

d : R_1 = H ; R_2 = CH_3O ; R_3 = CH_3O

e : R_1 = C_6H_5 ; R_2 = C_6H_5 ; R_3 = C_6H_5



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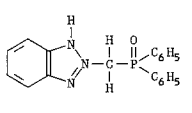

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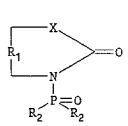

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7a–c **9a–c**

a : R_1 = CH_3O ; R_2 = CH_3O

b : R_1 = CH_3O ; R_2 = C_6H_5O

c : R_1 = OH ; R_2 = OH

a : X = NH ; R_1 = CH_2CH_2 ; R_2 = C_6H_5O

b : X = NH ; R_1 =  ; R_2 = C_6H_5O

c : X = O ; R_1 =  ; R_2 = C_6H_5O

Such compounds can undergo nucleophilic attack at the C_2 carbon atom; this process allows the attachment at C_2 of different moieties. Two chloroamidinium **2a** and **2b** and the corresponding derivatives **3a, 3b, 3c** and **3d** were thus synthesized.

The second group of amidinium type compounds were obtained by oxidation of the same thioureas using hydrogen peroxide; this reaction, described in the literature for acyclic thioureas [5, 6], can be extended to cyclic thioureas and also to thiohydantoins. Three compounds of this class were synthesized **4a, 4b** and **4c**.

Such formamidinium sulfinic acids exhibit anti-mitotic properties [7] and were therefore considered valuable for testing as trypanocides.

Another group of tested compounds was hydantoins and phosphorylated derivatives owing to the biological activity known for the two types of compounds and particularly the latter, bearing some similarity with active ureido phosphonates [4, 8–11]. These hydantoins were described elsewhere [12]. They correspond to structures **5** and **6** respectively.

Other phosphorylated compounds, corresponding to structures **7, 8** and **9**, were also synthesized and tested.

Results and Discussion

Results obtained with these compounds and with two standard drugs, pentamidine and α-(difluoromethyl)ornithine (DFMO) are summarized in Table I.

Concerning first amidine derivatives **2a–3d**, none of them exhibits an activity in the range of concentrations used. The substitutions on an amidine group including a fast metabolized amino acid in **3d**, do not provide any improvement.

Compounds of the second group have, on the contrary, an *in vitro* activity, particularly **4b**, which produces total depletion of trypanosomes within 24 h, at 10^{-5} M. Other formamidinium sulfinic compounds have proven anti-mitotic activity [7, 14]. This feature has also been noted for other compounds, such as ethidium bromide, which behaves as both an anti-cancer agent and a trypanocide [15], or the drug α-(difluoromethyl)ornithine thought to be a suicidal inhibitor of ornithine decarboxylase in trypanosomes, which also behaves as an anti-tumor agent [16]. This dual activity may be based on common features in trypanosomes and cancer cells and particularly the fact that both are rapidly dividing systems [17]. Therefore, compounds such as the intercalating ethidium bromide may act on replication enzymes of the trypanosome, as formamidium sulfinic acid might be trypanocidal as well as anti-mitotic agent.

Concerning the hydantoins **5a–5d** and the phosphorylated compounds tested, results are as follows: hydantoins are totally inactive in the range of concentrations tested as are their phosphorylated derivatives, with esterified groups or as free acid except **5a** and **6e** which are weakly active. Phosphoderivatives of ureas **9a–9c** are also inactive. In contrast, an activity is found for the phosphorylated derivatives of phthalimide **7a–7c** and benzimidazole **8** in the 5×10^{-5} M range of concentrations. For these compounds, we verified that the starting material, phthalimide or benzimidazole, was not or only very slightly active.

When compounds **3a, 3c, 4a, 4b, 7a, 7b** and **8** were tested *in vivo*, on mice-infected with *T. equiperdum*, no activity was found. But **4a, 4b** and **4c** exhibit high activity on glycolytic enzymes in trypanosomes (M. Willson, J.J. Périé and F.R. Opperdoes, in preparation), particu-

Table I. Trypanocidal activity against *Trypanosoma equiperdum in vitro*.

Compound	MW	Activity (μM)*
Pentamidine		active at 0.01
DFMO		active at 10
2a	285	inactive at 70
2b	347	inactive at 57
3a	177	inactive at 113
3b	244	inactive at 82
3c	263	inactive at 76
3d	165	inactive at 120
4a	134	active at 150
4b	182	active at 10
4c	132	active at 150
5a	176	active at 144
5b	176	inactive at 113
5c	114	inactive at 175
5d	252	active at 80
6a	360	inactive at 150
6b	332	inactive at 160
6c	345	inactive at 158
6d	284	inactive at 70
6e	385	active at 150
7a	269	active at 74
7b	315	active at 63
7c	241	active at 83
8	328	active at 61
9a	191	inactive at 150
9b	323	inactive at 150
9c	324	inactive at 150

*Minimum concentration for complete depletion of *T. equiperdum* population.

larly glycerol-phosphate dehydrogenase and phosphoglycerol kinase.

We are therefore now considering structural modifications on compounds of series **4** with the aim to make them active *in vivo*.

Experimental protocols

Chemistry

Melting points were obtained using a Buchi apparatus. The IR spectra were recorded on Perkin–Elmer 284 and 68 instruments, the ^{31}P NMR spectra on a Bruker AC 80 spectrometer operating at 32.4 MHz and using 85% H_3PO_4 as an external reference. The ^{13}C NMR spectra was recorded on a Bruker WH 90 spectrometer operating at 22.6 MHz using tetramethyl silane (TMS) as an internal reference. Elemental analysis for C, H, N, S and P were performed by the Microanalytical Service of CNRS Vernaison, France.

Sample preparation of amidine derivatives (Scheme 1)

4,5-Dihydro-2-chloroimidazolium hydrogenosulfate ***2a.*** This synthesis was performed according to Trani [3], as was 2-chloro-benzimidazolium hydrogenosulfate **2b**, starting from benzimidazole and using a 2:1 mixture of water and acetone instead of pure water as the solvent. Recrystallized in methanol; yield 65%; mp: 163°C. IR (KBr cm^{-1}) ν_{NH}: 3120; $\nu_{C=N}$: 1620. ^1H NMR (DMSO-d_6) δ 3.97 (s, 4H); 12.0 (s, 3H). Anal. $C_7H_6N_2Cl{\cdot}HSO_4$ (C, H, N, Cl).

2-[4-Hydroxy-anilino]-4-5-dihydro-imidazol ***3a.*** To 15 mmol (4.27 g) of **2a** dissolved in 50 ml of dimethyl formamide (DMF), were added 15 mmol (1.63 g) of 4-hydroxyaniline and 3 eq. of triethylamine. The solution was refluxed for 3 h; the solid which separated was filtered off, washed with hexane, and recrystallized in ethanol. Yield 57%; mp: 187°C. IR (KBr, cm^{-1}) ν_{OH}: 3400; ν_{NH}: 3300–3180; $\nu_{C=N}$: 1650. ^1H NMR (D_2O; ref. disiloxysilazane (DSS)) δ 3.60 (s, 4H); 7.0 (d, 2H); 7.4 (d, 2H) corresponding to the AA'BB' protons of the aromatic ring (J = 8 Hz). Anal. $C_9H_{11}N_3O$ (C, H, N).

2-[4-Aminosulfonyl-aniline]4,5-dihydro imidazol ***3b.*** To 15 mmol (4.27 g) of **2a** dissolved in 50 ml of DMF were added 1 eq. of 4-aminosulfonyl-aniline (2.58 g), 3 eq. of potassium carbonate (6.21 g) and 0.5 g of catalyst triethylbenzylammonium chloride. The mixture was refluxed for 3 h, then the remaining solid was filtered off. After partial evaporation of the solvent (30 ml), an oily product was precipitated by addition of methanol, which then crystallized by cooling: recrystallized in ethyl acetate. Yield: 42%; mp: 244°C. IR (KBr, cm^{-1}) ν_{NH}: 3320 and 3280; ν_{CN}: 1610; ν_{SO_2}: 1320 and 1160. ^1H NMR (DMSO-d_6) δ 3.6 (s, 4H); 6.2 (m, 4H, exchanged in D_2O); 7.2–8 (2d, 4H; J = 8 Hz). Anal. $C_7H_{12}N_3O_2S$ (C, H, N, S).

2-[4-Sodium sulfonate anilino]-4,5-dihydro imidazol ***3c.*** To 15 mmol (4.27 g) of **2a** dissolved in 20 ml of water were added 1 eq. of sulfanilic acid in dichloromethane (30 ml) and 0.5 g of trimethylbenzyl ammonium chloride as the transfer agent, then 3 eq. of 5% NaOH in water. After 10 min, the precipitate formed was filtered off and washed twice with ether, then dried. Yield 66%; mp: >260°C. IR (KRr, cm^{-1}) ν_{NH}: 3310; $\nu_{C=N}$: 1625. ^1H NMR (D_2O); (ref DSS); δ 3.6 (s, 4H); 7–7.8 (2d, 4H). Anal. $C_9H_{10}N_3O_3SNa{\cdot}2\,H_2O$ (C, H, N, S).

2-[N-Glycil]4,5-dihydro imidazol ***3d.*** To 15 mmol (4.27 g) of **2a** dissolved in 20 ml of water were added 1 eq. of glycine (1.125 g) then 3 eq. of 5% NaOH in water. After 1 h the solution was acidified to pH 4 (3 N HCl); addition of ethanol allowed the formation of a solid, which was filtered off, washed twice with ether and recrystallized in methanol. Yield: 95%; mp: >260°C. IR (KBr, cm^{-1}) ν_{OH}: 3650–3300; ν_{NH}: 3190; $\nu_{C=N}$: 1690; $\nu_{C=O}$: 1710. ^1H NMR (DMSO-d_6): δ 3.8 (s, 4H); 4.1 (d, 2H); 8.5 (m, 3H exchanged in D_2O). Anal. $C_5H_9O_2N_3$ (C, H, N).

Hydantoins **5a–5d** were prepared according to classical [18, 19] or slightly modified procedures. Their phosphorylated derivatives **6a–6e** as well as the phosphophthalimides **7a–7c** were described elsewhere [12].

Diphenyl(benzimidazolyl)methylphosphine oxide **8** was obtained from a solution of 0.024 mol (3.9 g) of chloro-1-methylbenzimidazol and 0.024 mol (5.2 g) of methoxy diphenylphosphine in anhydrous toluene (sodium dried) refluxed for 2 h. After evaporation of the solvent and trituration of the remaining oil in chloroform and diethyl ether, a powder was filtered off and recrystallized in a 1/1 mixture of ethanol–benzene. Yield = 50%; mp: 97°C. ^{31}P NMR δ = + 30 ppm. IR (KBr, cm^{-1}) ν_{NH}: 3200–2700; $\nu_{C=N}$: 1620–1590; ν_{P-O}: 1184; ν_{P-C}: 1432.

Compounds **9a, 9b** and **9c** were described in an earlier paper [20].

Biological assays

All compounds were tested in a semi-defined medium for the cultivation of *Trypanosoma equiperdum*, 20 000 for each test [13]. The cultured trypanosomes had all the characteristics of the bloodstream forms including morphology, infectivity, antigenic variation and glucose metabolism. The standard medium consisted of Hepes-buffered minimum essential medium with Earle's salt, supplemented with 0.2 mM of 2-mercaptoethanol, 2 mM pyruvate and 10% heat-inactivated rabbit serum.

The values indicated for the trypanocidal activity correspond to the minimum amount required to obtain complete depletion of a *T. equiperdum* population. The test is run on different samples and the results are read by counting on the grid of a microscope after 4, 22 and 48 h for each sample. Active concentrations were determined by using different dilutions from 150 to 0.15 μM.

In addition, several compounds exhibiting *in vitro* activity, were tested *in vivo*. *T. equiperdum*-infected mice were injected subcutaneously with solutions of dimethyl sulfoxide (DMSO), at 3 concentrations 12, 50 and 100 mg/kg. Blood parasitemia was evaluated after 12 and 24 h.

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