Research for New Antichagasic Drugs

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A series of ten 1-[(5-nitrothenylidene)amino]azoles has been synthesized by the reaction of 5-nitrothiophene-2-carbaldehyde with 1-aminopyrazole, 1-aminoimidazole, 1- and 4- amino-1,2,4-triazoles, 1-aminoindole, 1- and 2-aminoindazoles, 1-aminobenzimidazole and 1- and 2-aminobenzotriazoles. Physical data, spectroscopic characteristics and biological properties of all the derivatives have been examined. The antiprotozoal activity has been tested against *Trypanosoma cruzi*, comparative to Nifurtimox (Lampit).

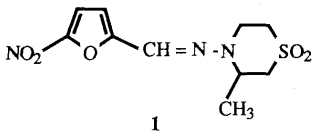
Keywords 1-[(5-nitrothenylidene)amino]azole; synthesis; ^{1}H-NMR; ^{13}C-NMR; antitrypanosomal activity

Nifurtimox or tetrahydro-3-methyl-4[(5-nitrofurfuryl-idene)]-2*H*-thiazine-1,1-dioxide (nfx) is one of the most effective drugs used in the treatment of Chagas' disease (American trypanosomiasis). However its use is limited due to its mutagenicity, side effects and non curative action in certain cases. Besides, none of the few compounds which have reached the stage of clinical trial in Chagas' disease are considered to be safe, effective, convenient and inexpensive chemotherapeutic agents for extensive use in man, not even to prevent transmission during blood transfusion.[1)]

In these circumstances, the development of new compound alternatives to the currently used nfx is a research area of great interest due to the economic, social and political impact that the control of trypanosomiasis would have.

Chemistry Recently, we have synthesized and examined the antitrypanosomal activity of new derivatives structurally related to nfx **1**, where the thiazine 1,1-dioxide ring of this latter drug was replaced by a *N*-heterocyclic residue. The compounds in which the heterocyclic counterparts were 1,2,4-triazol-4-yl and pyridin-1-yl groups clearly showed superior *in vitro* activity against *T. cruzi*.[2)]

The present work was undertaken in order to examine in a further step what would be the effect on the biological activity when the furan ring of series **2** was replaced by a thiophene.



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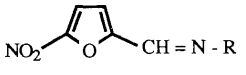

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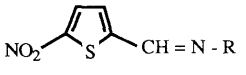


3

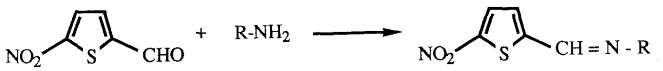
a : pyrazol-1-yl
b : imidazol-1-yl
c : 1, 2, 4-triazol-4-yl
d : 1, 2, 4-triazol-1-yl
e : indol-1-yl
f : indazol-1-yl
g : indazol-2-yl
h : benzimidazol-1-yl
i : benzotriazol-1-yl
j : benzotriazol-2-yl

Chart 1

According to the results shown in Table I, the title compounds **3** (**a** to **j**) were obtained from the *N*-aminoderivatives **4** by reaction with 5-nitrothiophene-2-carbaldehyde **5** in boiling toluene with catalytic amounts of *p*-toluene sulfonic acid, the yields ranging from 63 to 98%.

1-Aminopyrazole **4a**,[3)] 1-aminoimidazole **4b**,[4)] 1-amino-1,2,4-triazole **4d**,[5)] 1-aminoindole **4e**,[6)] 1-amino- **4f** and 2-aminoindazoles **4g**,[7)] 1-aminobenzimidazole **4h**,[5)] 1-amino- **4i** and 2-aminobenzotriazoles **4j**,[8)] were prepared according to the literature from the corresponding azoles with hydroxylamine-*O*-sulfonic acid. 5-Nitrothiophene-2-carbaldehyde **5** was synthesized by nitration of thiophene-2-carbaldehyde with fuming nitric acid and anhydrous acetic

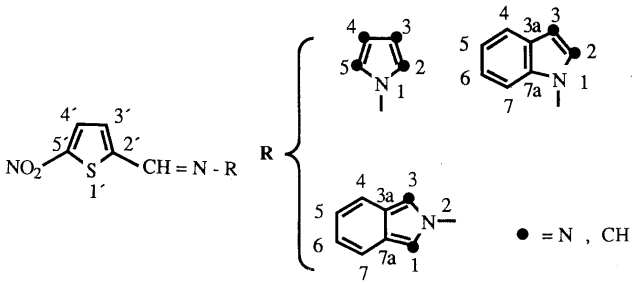
TABLE I. Yields, Melting Points and Microanalytical Data



5 **4** **3**

Compd.	Yield (%)	mp (°C) (Solvent)	Formula (Mol. weight)	Calcd (Found) C	H	N
3a	87	172—174 (Acetone–water)	$C_8H_6N_4O_2S$ (222.2)	43.23 (43.28	2.72 2.94	25.22 24.96)
3b	80	149—150 (Chloroform–ethanol)	$C_8H_6N_4O_2S$ (222.2)	43.23 (43.41	2.72 2.65	25.22 25.54)
3c	98	204—206 (Toluene)	$C_7H_5N_5O_2S$ (223.2)	37.66 (37.54	2.26 2.04	31.38 31.62)
3d	85	210 (dec.) (Chloroform)	$C_7H_5N_5O_2S$ (223.2)	37.66 (37.84	2.26 2.85	31.38 31.10)
3e	90	191—193 (Ethyl acetate–hexane)	$C_{13}H_9N_3O_2S$ (271.3)	57.55 (57.64	3.34 3.37	15.49 15.67)
3f	63	251—252 (Chloroform)	$C_{12}H_8N_4O_2S$ (272.3)	52.93 (52.72	2.96 2.90	20.58 20.77)
3g	83	226—228 (Chloroform)	$C_{12}H_8N_4O_2S$ (272.3)	52.93 (53.01	2.96 3.05	20.58 20.35)
3h	81	225—227 (Ethanol–water)	$C_{12}H_8N_4O_2S$ (272.3)	52.93 (53.20	2.96 3.12	20.58 20.39)
3i	85	242—243 (Toluene)	$C_{11}H_7N_5O_2S$ (273.3)	48.34 (48.19	2.58 2.71	25.63 25.65)
3j	85	262—265 (Ethanol)	$C_{11}H_7N_5O_2S$ (273.3)	48.34 (48.53	2.58 2.46	25.63 25.52)

TABLE II. ^{1}H-NMR Data[a,b] (Chemical Shifts, δ, ppm Relative to Internal TMS) in Hexadeuteriodimethylsulphoxide



Compound	–CH=N–	H3′	H4′	H2	H3	H4	H5	H6	Hz
3a	9.38 (s)	7.80 (dd)	8.18 (d)	—	7.72 (dd)	6.53 (dd)	8.10 (dd)		
3b	9.18 (s)	7.63 (d)	8.18 (d)	8.17 (s)	—	7.93 (s)	7.12 (s)		
3c	9.30 (s)	7.70 (d)	8.17 (d)	—	9.11 (s)	—	9.11 (s)		
3d	9.40 (s)	7.87 (d)	8.15 (d)	—	8.18 (s)	—	8.90 (s)		
3e	9.08 (s)	7.52 (d)	8.14 (d)	8.16 (d)	6.82 (d)	7.60 (dd)	7.17 (m)	7.30 (m)	7.70 (dd)
3f	9.33 (s)	7.75 (d)	8.13 (d)	—	8.36 (d)	7.85 (dd)	7.31 (ddd)	7.58 (ddd)	7.81 (dd)
3g	9.78 (s)	7.94 (d)	8.20 (d)	—	8.67 (d)	7.75 (dd)	7.12 (ddd)	7.38 (ddd)	7.63 (dd)
3h	9.41 (s)	7.70 (d)	8.17 (d)	8.89 (s)	—	7.73 (dd)	7.33 (ddd)	7.41 (ddd)	7.83 (dd)
3i	9.86 (s)	7.96 (d)	8.23 (d)	—	—	8.15 (dd)	7.54 (ddd)	7.72 (ddd)	7.95 (dd)
3j	9.91 (s)	8.04 (d)	8.20 (d)	—	—	7.96 (m)	7.54 (m)	7.54 (m)	7.96 (m)

a) Apparent multiplicity is given in the table: s, singlet; d, doublet; m, multiplet. *b*) δ values for 5-nitrothiophene-2-carbaldehyde are: CHO, 10.20 (s); H3′, 8.20 (d); H4′, 8.80 (d).

TABLE III. ^{13}C-NMR Data[a] (Chemical Shifts, δ, in ppm Relative to Internal TMS) in Hexadeuteriodimethylsulphoxide

Compound	C2′	C3′	H4′	H5′	–CH=	C2	C3	C4	C5	C6	C7	C3a	C7a
3a	143.9	132.7	130.4	152.2	143.5	—	139.1	107.6	130.0	—			
3b	143.8	132.1	130.3	152.2	146.5	136.5	—	129.1	112.8	—			
3c	142.4	133.3	130.2	153.0	151.3	—	139.1	—	139.1	—			
3d	142.1	133.2	129.5	152.8	147.1	—	149.8	—	142.6	—			
3e	146.4	129.5	130.6	150.8	139.0	119.1	105.9	121.1	121.7	123.7	110.3	126.8	135.7
3f	144.8	130.6	129.9	151.2	139.2	—	134.8	122.6	121.3	128.2	109.7	123.4	137.4
3g	142.9	134.3	130.3	152.8	148.3	—	123.9	121.2	122.4	128.2	117.0	121.4	146.2
3h	144.4	131.6	130.3	152.0	145.6	137.7	—	120.1	123.3	124.2	110.9	141.7	131.4
3i	142.8	133.5	129.9	153.0	146.8	—	—	119.6	125.2	129.2	110.2	144.6	130.0
3j	141.4	135.5	129.9	154.1	152.5	—	—	118.0	128.2	128.2	118.0	142.9	142.9

See Table II for structural formula numbering. *a*) δ values for 5-nitrothiophene-2-carbaldehyde are: C2′, 146.6; C3′, 135.6; C4, 130.0; C5′, 156.0; CHO, 185.8.

anhydride.[9]

Structures of compounds **3a**—**j** were confirmed by proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{3}C-NMR) spectroscopy (Tables II and III). Assignments of all the signals to the different protons and carbon atoms were achieved on the basis of previous data existing in the literature[2,10] and two dimensional NMR (2D-NMR) techniques [highest occupied molecular orbital (HOMO) (^{1}H–^{1}H) and heteronuclear (^{1}H–^{13}C) correlated experiments][11] for **3e**, **3f**, **3g**, and **3h**. A detailed analysis of the molecular structures of **1**, **2a** and **3a**, in solution by ^{1}H- and ^{13}C-NMR, and in solid state by X-ray crystallography, has been recently published by us.[12]

Chemical shift and coupling constant values (H3′/H4′ 4.0; C3′/H3 177.0; C3′/H4′ 2.5; C4′/H4′ 179.5; C4′/H3′ 4.3 Hz) for the thiophene counterpart were in accordance with the literature data.[13]

The main features observed in the NMR spectra of the azole groups in derivatives **3** are similar to the ones encountered for the furan analogues **2**.[2,10a] Attention must be paid to the fact that an error slipped by in reference 10*a* concerning C3 of the furan ring and C2 of the indole group in 1-[(5-nitrofurfurylidene)amino]indole. ^{13}C-NMR data were reversed, C3 appearing at 114.8 ppm, with *J* values of 184.1 and 7.6 Hz.

Biological Results To determine the activity of compounds (**3a**—**j**) against the Y strain[14] of *Trypanosoma* (*S.*) *cruzi*, three types of tests were carried out: i) *In vitro* tests, directed to determine the trypanocidal activity on *T. cruzi* cultures.[15] ii) *In vivo* tests, to know the possible chemotherapic action of the new synthesis compounds.[16] iii) Mixed tests, based on the previous study of the compounds activity on infected stored blood and posterior inoculation of this blood to recipient animals.[17]

Nifurtimox was used as the reference drug in all of the experiments.

In vitro activity was assayed on epimastigote culture during their exponential growth in liver infusion tryptose (LIT) medium supplemented with 10% fetal calf serum (FCS) (Chart 2). NUNCLON plaques were filled with 2.5 ml/well of an initial culture. Three wells were used for every dose assayed. Compounds were dissolved in phosphate-buffered saline (PBS) containing 0.2% dimethylsulfoxide (DMSO). At this concentration no adverse

effects attributable to the DMSO were noted.

After 6 d of incubation at 28°C, doubled counts with Neubauer chambers were made. The percentage of growth inhibition was calculated as follows:

$$\% \text{ growth inhibition} = \left(1 - \frac{\text{growth of experimental culture}}{\text{growth of control culture}}\right) \times 100$$

In vivo activity was determined (Chart 3) by treatment of infected mice and subsequent determination of parasitaemia and mortality rates in animals. NMRI mice were infected by intraperitoneally (i.p.) injection with 100000 trypanosomes (tryp) obtained from donor animals. Compounds were dissolved in PBS containing 16% ethyleneglycol and administered at 50 mg/kg body weight/d during 3 to 7 postinfection days by i.p. injection.

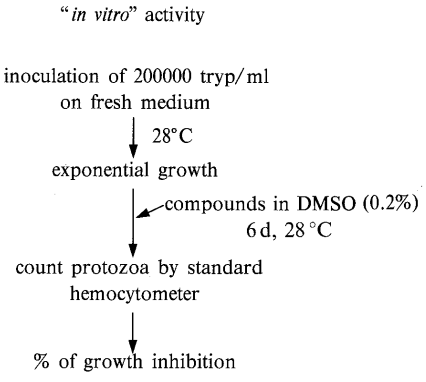


Chart 2

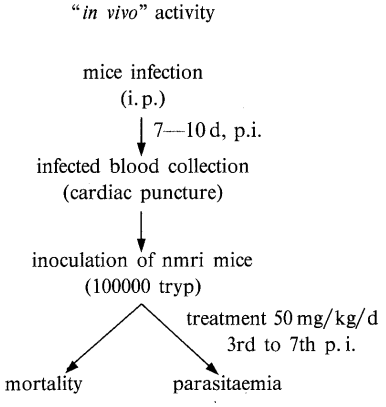


Chart 3

Chemoprophylactic activity of the compounds was studied using infected blood obtained from donor mice and stored in the same conditions as in blood banks (Chart 4). Compounds were dissolved as above and were added at 1 mg/ml to tubes containing infected blood with 250000 trypomastigotes/ml. Tubes were incubated at 4 °C on a shaker placed into a dark room. After incubation times, groups of mice were inoculated (i.p. injection) with 0.5 ml of the stored blood. Parasitaemia and mortality rates were followed in the animals.

As shown in Fig. 1 for *in vitro* activity a complete growth inhibition was obtained at 10 μg/ml with nfx, and compounds named **3b**, **3c** and **3e**. At this concentration level, **3d** and **3h** derivatives were able to only partially inhibit the growth.

In vivo treatment with the reference drug nfx produced a delay in the parasitaemia and reduced the parasitaemia levels. Survival of these animals with nfx (Table IV) was higher than the one obtained in animals treated with the new compounds. All of the new compounds **3** assayed showed parasitaemia curves similar to the control ones.

The treatment of infected stored blood with products named **3c**, **3i** and nfx delayed the beginning of parasitaemia in recipient animals. However, none of the compounds produced a significative reduction in the parasitaemia peaks and only some of them (Table V) prolonged the

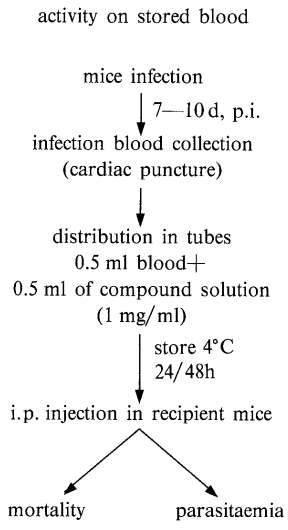
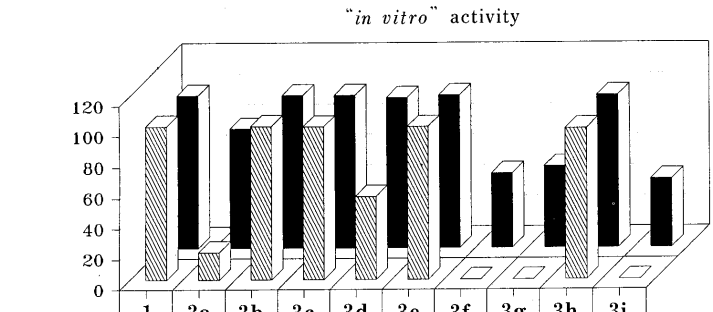


Chart 4



	1	3a	3b	3c	3d	3e	3f	3g	3h	3i
100 g/ml	100	77.7	100	100	98.3	100	48.4	52.9	99.9	44.7
10 g/ml	100	18	100	99.6	54.3	99.6	0	0	98.6	0

Fig. 1. % Growth Inhibition

▨, 10 g/ml; ■, 100 g/ml.

TABLE IV. *In Vivo* Tests. Survival Index (Day 30 Postinfection)

Compound	Survival index[a]
3b	0
3c	0.6
3d	1
3e	0.2
3f	0.8
3g	0.2
3h	1
3i	0.6
1 (nfx)	1.2

a) Survival of treated animals/survival of control animals.

TABLE V. Experiments on Stored Blood. Survival Index (Day 30 Postinfection)

Compound	Survival index[a]
3a	1
3b	1.5
3c	3
3d	1
3e	1.5
3f	2
3g	2.5
3h	1
3i	0.8
1 (nfx)	1.2

a) Survival of animals inoculated with treated blood/survival of animals inoculated with untreated blood.

survival of the animals.

Conclusion

From our results we can conclude that in the *in vitro* tests all the 1-[(5-nitrothenylidene)amino]azoles **3** show trypanocidal activity similar to Nifurtimox, the less active ones being indazole and benzotriazole derivatives. However the *in vivo* assays indicate that they were not able to completely erradicate the infection in treated mice. Furthermore, they did not present good chemoprophylactic activity as neither nfx **1** nor the new compounds **3** could completely kill the surviving trypanosomes in stored blood.

Experimental

Chemistry Melting points were determined on a capillary Büchi 512 apparatus and are uncorrected. The NMR spectra were taken with a Bruker AC 200, working at 200.13 MHz for ^{1}H and 50.32 MHz for ^{13}C. ^{1}H and ^{13}C chemical shifts (δ) are given from internal tetramethylsilane with an accuracy of ± 0.01 and ± 0.1 ppm, respectively. Coupling constants (J) were measured with digital resolutions of 0.2 and 0.6 Hz. The 2D-experiments were run according to the following data acquisition parameters: (i) for the 2D (^{1}H–^{1}H) COSY, F1 domain (SI1: 1K; SW1; 853 Hz) F2 domain (SI2; 2K; SW2; 1706 Hz), D1 : 1s RD: Os; PW : Os; NS (number of transients per FID): 16; DS (number of preparatory dummy transients per FID): 2. (ii) for the 2D (^{1}H–^{13}C) COSY, F1 domain (SI1; 256W; SW1 : 822 Hz), F2 domain (SI2 : 2K; SW2 : 10000 Hz), D1 : 1s, RD : 0s; NS : 160; DS : 0. All the 2D experiments were processed with a sine bell window.[11] Analyses were carried out using in-house facilities. Chromatographic purifications were performed through columns at normal pressure using silica gel Merck 60 (70—230 mesh). Thiophene-2-carbaldehyde, azoles, hydroxylamine-*O*-sulfonic acid and 4-amino-1,2,4-triazole were purchased from Aldrich.

Condensation of *N*-Aminoazoles (4) with 5-Nitrothiophene-2-carbaldehyde (5) Equimolar amounts (0.005 mol) of the *N*-amino compounds **4** and **5** in 40—50 ml of toluene were heated under reflux with *p*-toluene sulfonic acid. After 5—10 h the solvent was evaporated off and the crude products were purified by crystallization in derivatives **3a**, **3c**, **3i** and **3j** (Table I). In the other imino compounds, purification was achieved by column chromatography with the appropriate eluent: **3b** (chloroform–ethanol, 9 : 1), **3d** (chloroform), **3e** (ethyl acetate–hexane, 1 : 3), **3f** (chloroform), **3g** (chloroform), and **3h** (chloroform–ethanol, 95 : 5).

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