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Activity against *Trypanosoma cruzi* of New Analogues of Nifurtimox

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Ten new derivatives **1** structurally related to Nifurtimox have been synthesized from 5-nitrofurfural and the corresponding N-aminoheterocyclic compounds. Physical data, spectroscopic characteristics and biological properties have been examined. An unusual long-range coupling constant in the pyrazolyl derivative **1b** has been observed. Compounds in which the heterocyclic counterparts are 1,2,4-triazol-4-yl and pyridin-1-yl groups clearly show s superior activity against *Trypanosoma cruzi.*

Neue Nifurtimox-Analoge mit Wirkung gegen Trypanosoma cruzi

Zehn neue Derivate strukturbezogen auf Nifurtimox wurden aus 5-Nitrofurfural und den entspr. heterocyclischen N-amino Verbindungen hergestellt. Über ihre physikalischen und spektroskopischen Daten bzw. biologische Wirkung wird berichtet. Eine ungewöhnliche „long-range" Kopplungs Konstante wurde an der Pyrazolylverbindung **1b** beobachtet. Die Verbindungen, in denen der heterocyclischer Partner 1,2,4-Triazol-4-yl und Pyridin-1-yl Gruppierungen sind, zeigen eine eindeutig höhere Wirkung gegenüber *Trypanosoma cruzi* als Nifurtimox.

Introduction

Discovery of the antibacterial activity of nitrofuran derivatives lead to assay these class of compounds against protozoal infections and today Nifurtimox **1a** is the most important drug for the treatment of trypanosomiasis[2].

However, search of new agents able to equal or to improve the activity of Nifurtimox is continuing due to the enormous economic, social and politic impact that would suppose world trypanosomiasis control.

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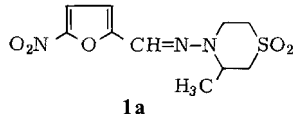
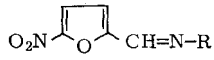
Our contribution to the development of this research consists in the synthesis and biological activity assays against *T. cruzi* of a series of new ten derivatives **1b–k** where R is a five or six membered heteroaromatic ring.



1a

R =



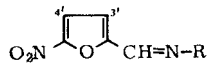
1

- **b** pyrazol-1-yl
- **c** 1,2,4-triazol-1-yl
- **d** 1,2,4-triazol-4-yl
- **e** benzimidazol-1-yl
- **f** benzotriazol-1-yl
- **g** indazol-1-yl
- **h** 3,5-bis(methylthio)-1,2,4-triazol-4-yl
- **i** 1-methyl-3-methylthio-1,2,4-triazol-4-yl-5-thione
- **j** 1-methyl-3-methylthio-1,2,4-triazol-4-yl-5-one
- **k** 4,6-diphenylpyridin-1-yl-2-one

Tab. 1: Yields, melting points and microanalytical data of the imino derivatives **1**

Compound No	Yield (%)	m.p. (°) (Solvent)	Molecular formulae	Calcd./Found C	H	N
1b	75	163–5 (ethanol-water)	$C_8H_6N_4O_3$ (206.1)	46.6 46.6	2.94 3.28	27.2 26.9
1c	79	188–90 (ethanol)	$C_7H_5N_5O_3$ (207.1)	40.6 40.8	2.43 2.32	33.8 34.2
1d	82	203 (dec) (ethanol)	$C_7H_5N_5O_3$ (207.1)	40.6 40.3	2.43 2.31	33.8 34.1
1e	82	225–8 (ethanol-water)	$C_{12}H_8N_4O_3$ (256.2)	56.2 56.0	3.15 3.14	21.2 21.6
1f	80	242–3 (ethanol)	$C_{11}H_7N_5O_3$ (257.2)	51.4 51.5	2.75 2.93	27.2 27.0
1g	82	233–5 (toluene)	$C_{12}H_8N_4O_3$ (256.2)	56.2 56.4	3.15 3.07	21.8 21.6
1h	76	151 (dec) (toluene)	$C_9H_9N_5O_3S_2$ (299.3)	36.1 36.0	3.03 3.48	23.4 23.3
1i	89	189–90 (ethanol)	$C_9H_9N_5O_3S_2$ (299.3)	36.1 35.9	3.03 3.06	23.4 23.1
1j	30	223–7 (ethanol)	$C_9H_9N_5O_4S_2$ (283.3)	38.1 38.0	3.21 3.41	24.7 24.5
1k	87	192–3 (ethanol-acetone)	$C_{22}H_{15}N_3O_4$ (385.4)	68.5 68.4	3.92 3.60	10.9 11.1

Tab. 2: Proton nuclear magnetic resonance of the imino derivatives **1** in DMSO-d_6 (δ in ppm)



Compound No	-CH=N-	$H_{3'}$	$H_{4'}$	H_2	H_3	H_4	H_5	H_6	H_7	Other protons
1b	9.11	7.42	7.74	–	7.68	6.48	8.12	–	–	–
		$J_{3'4'} = 3.9$			$J_{34} = 1.9$ $^6J = 0.8$[a]		$J_{35} = 0.9$ $J_{45} = 2.5$			
1c	9.12	7.48	7.72	–	8.18	–	9.05	–	–	–
		$J_{3'4'} = 3.9$								
1d	9.11	7.41	7.74	–	9.18	–	9.18	–	–	–
		$J_{3'4'} = 3.9$								
1e	9.15	7.41	7.82	8.86	–	7.57-7.77(m)	7.24–7.39 (m)		7.57-7.77(m)	–
		$J_{3'4'} = 3.9$								
1f	9.53	7.65	7.88	–	–	8.17	7.56	7.77	7.96	–
		$J_{3'4'} = 4.0$					$J_{45} = 8.4, J_{56} = 8.0, J_{67} = 7.9$			
1g	9.09	7.15–7.87(m)		–	8.36	←	7.15–7.87(m)		→	–
1h	8.82	7.61	7.80	–	–	–	–	–	–	SMe: 2.58
		$J_{3'4'} = 4.0$								
1i	10.54	7.63	7.83	–	–	–	–	–	–	SMe: 2.54 NMe: 3.65
		$J_{3'4'} = 4.0$								
1j	9.69	7.84	8.13	–	–	–	–	–	–	SMe: 2.84 NMe: 3.66
		$J_{3'4'} = 3.85$								
1k[b]	9.70	6.57	6.82	–	7.17 7.74(m)	–	6.83	–	–	Ph : 7.17–7.74 (m)
		$J_{3'4'} = 3.9$								

a) Long range coupling with the proton of the -CH=N- group.
b) Due to solubility reasons this spectrum was recorded in $CDCl_3$.

Chemistry

The title compounds **1** were synthesized by condensation of the N-aminoderivative with 5-nitrofurfural **2** in boiling toluene with catalytic amounts of *p*-toluene sulfonic acid, the yields ranging from 70 to 90 %. 1-amino pyrazole, 1-amino-1,2,4-triazole[3], 1-aminobenzimidazole[3], 1-aminobenzotriazole[4], and 1-aminoindazole[5], were prepared from the corresponding azoles with hydroxylamine-O-sulfonic acid[6] according to the literature. Commercial 4-amino-1,2,4-triazole was used without further purification.

4-Amino-3,5-bis(methylthio)-1,2,4-triazole[7], 1-methyl-3-methylthio-4-amino-1,2,4-triazole-5-thione[8], 1-methyl-3-methylthio-4-amino-1,2,4-triazole-5-one[9], and 1-amino-4,6-diphenylpyridin-2-one[10] have been prepared by ring-closure synthetic methods as previously described[8,9].

The structure of the new iminoderivatives **1** has been established without ambiguity by their physical properties (see Table 1 for yields, m p.s and microanalytical data) and spectroscopic characteristics (see Table 2). A longrange coupling constant between the H_3 proton of the pyrazol-1-yl moiety and the proton of the imino group –C<u>H</u>=N– of 0.8 Hz was observed in compound **1b**.

Biology

Trypanosoma cruzi is the aethiological agent of Chaga's disease or American trypanosomiasis which affect 10^7 people in Central and South America.

In vitro assays results of the products **1b–k** against *T. cruzi*[11] epimastigote forms are expressed in growth inhibition percentages (see Table 3) as previously described[12].

From the data in Table 3 it can be seen that the most active compounds are those bearing pyrazol-1-yl, 1,2,4-triazol-1-yl and 1,2,4-triazol-4-yl, benzimidazol-1-yl and pyridin-1-yl groups, which even accomplish at 100 µg/ml total lysis of the culture in 48–72 h.

Tab. 3: Growth inhibiton percentages of *T. cruzi* culture epimastigote forms[a]

Hours	24			48			72		
Drug/Concentration (µg/ml)	100	10	1	100	10	1	100	10	1
1b	54.7	0	0	100	55.8	34.5	100	65.0	36.6
1c	54.7	0	0	100	58.4	33.3	100	75.9	50.0
1d	61.3	11.8	0	100	24.6	14.2	100	64.5	5.8
1e	51.8	0	0	100	40.2	33.3	100	67.7	31.6
1f	24.5	0	0	15.5	10.7	5.4	82.1	59.0	24.1
1g	19.9	9.1	0	40.8	37.2	26.8	39.7	20.7	14.9
1h	100	100	35.1	100	100	47.8	100	100	57.4
1i	100	19.1	9.8	100	44.7	26.3	100	80.3	19.8
1j	100	100	23.3	100	100	48.8	100	100	57.9
1k	100	100	25.8	100	100	76.9	100	100	81.9
Nifurtimox	33.5	19.7	10.8	53.4	44.3	39.6	82.9	79.8	50.9

a) Average values of five experiments.

Acknowledgements

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Experimental Part

Chemistry

MP: Gallenkamp capillary apparatus (uncorr.). ^{1}H-NMR spectra: Varian EM-390. IR spectra: Perkin-Elmer 257 spectrophotometer.

1-Aminopyrazole

Pyrazole (0.08 moles) and KOH (0.20 moles) in 230 ml water were heated at 70°. Hydroxylamine-O-sulfonic acid (0.10 moles) was slowly added with stirring during 45 min. After 1 h more of heating a dark brown reaction mixture was obtained. Continuous extraction with ethyl ether afforded a mixture of pyrazole [^{1}H-NMR (DMSO-d_6): δ (ppm) = 6.42 (1H), 7.79 (2H)] and 1-aminopyrazole [^{1}H-NMR (DMSO-d_6): δ (ppm) (ppm) = 6.26 (1H), 7.50 (1H), 7.62 (1H)] (1:1) which was used without further purification.

General procedure for the condensation of 5-nitrofurfural **(2)** *with N-amino heterocyclic compounds.*

Equimolar amounts of the N-amino compound and **2** in a minimal volume of toluene were heated under reflux with of p-toluenesulfonic acid. After 5–10 h of reaction the imino derivatives **1** precipitated from the cooled reaction mixture (see Table 1).

Biology

Parasites were grown at 28° in liquid MTL medium supplemented with 10 % of inactivated foetal calf serum[13]. From exponential growing cultures, 100 µl samples were taken and placed into 96 wells microtiter plates, adjusted to 5 × 10^5 flagellates/ml. Final concentration solutions of 100, 10 and 1 µg/ml of each product were used. The concentration of the solvent DMSO was about 0.2 % which is not toxic for the parasites.

Antiparasitic activity tests were realized at 24, 48 and 72 h after addition of the products. Cell growth was measured by applying a colorimetric method using a Kontron STL 210 spectrophotometer[14]).

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11 The strain employed in this study comes from a human clinical case isolated at the „Instituto de Malariología" (Maracay, Venezuelae) and was donated in 1975 to us by Dr. Blazquez (C.N.M.V.I., Madrid, Spain). Since this time the strain has been maintained as described by A. Osuna, G. Ortega, F. Gamarro, S. Castanys and M. L. Mascaró, Int. J. Parasitol. *14*, 253 (1984).
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H_2-Antihistaminika, 34. Mitt.[1)]

1,3,4-Oxadiazol-2,5-diamine mit H_2-antagonistischer Aktivität

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Es wird über die Synthese und H_2-antagonistische Wirksamkeit von mono- und disubstituierten 1,3,4-Oxadiazol-2,5-diaminen mit Piperidinomethylphenoxypropylseitenkette sowie deren methylierte Derivate berichtet.

H_2-Antihistaminics, XXXIV: 1,3,4-Oxadiazole-2,5-diamines with H_2-Antagonistic Activity

Syntheses and H_2-antagonistic activities of mono- and disubstituted 1,3,4-oxadiazole-2,5-diamines with a [(piperidinomethyl)phenoxy]propyl substituent and of their methyl derivatives are reported.

Trotz vielfältiger Strukturvariationen vereinen die meisten H_2-Rezeptor-Antagonisten in ihrer Struktur einen basischen bzw. basisch substituierten (Hetero)Aromaten, der über eine flexible Kette mit der sogenannten „polaren Gruppe" verbunden ist. Durch Verknüpfung des Piperidinomethylphenoxypropyl-Strukturelementes[2)] mit einem N-Methyl- bzw. N-Phenyl-1,3,4-oxadiazol-2,5-diamin-Baustein als Strukturvariante der „polaren Gruppe" hatten wir Verbindungen mit ausgeprägter H_2-antagonistischer Wirkung erhalten[3)]. Dies veranlaßte uns, in der vorliegenden Arbeit das monosubstituierte 1,3,4-Oxadiazol-2,5-diamin mit entspr. Seitenkette sowie weitere disubstituierte Verbindungen darzustellen und auf H_2-antagonistische Wirksamkeit zu prüfen. Zudem sollte der Einfluß einer Methylverzweigung der Oxypropylkette sowie der Methylierung des Oxadiazolringes am N-4 auf die pharmakologische Wirksamkeit untersucht werden.

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