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Laboratory note

Synthesis, cytostatic and trichomonacide activities of 3,5-bis-(halomethyl)pyrazoles

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Summary — Reduction of 1-methyl- and 1-benzyl-diethylpyrazole-3,5-dicarboxylates **2a** and **2b** with diisobutylaluminium hydride in toluene gave 3,5-bis-(hydroxymethyl)pyrazoles **4a** and **4b** in very good yields. Alternatively, **4a** was obtained by reduction of *S,S*-diethyl 1-methylpyrazole-3,5-dicarbothioate **3''** with lithium aluminium hydride in lower yield. Treatment of above diols **4a** and **4b** with thionyl chloride or phosphorous tribromide in dimethoxyethane gave stable 3,5-bis-(chloromethyl)- and 3,5-bis-(bromomethyl)pyrazoles **5a**, **6a** and **6b**. All the compounds synthesized were evaluated as being cytostatic in *in vitro* cultures of HeLa cells and both 1-methyl- and 1-benzyl-3,5-bis-(bromomethyl)pyrazoles **6a** and **6b** were found to be powerful cytostatic agents. In biological tests against *Trichomonas vaginalis*, *Entamoeba invadens* and *Candida albicans*, **6a** and **6b** showed significant trichomonacide activities.

Résumé — **Synthèse et activité cytostatique et trichomonacide de bis(halométhyl)-3,5 pyrazoles.** *Les dérivés méthyl-1 et benzyl-1 bis-(hydroxyméthyl)-3,5-pyrazole* ***4a*** *et* ***4b*** *ont été synthétisés par réduction des diéthylesters correspondants* ***2a*** *et* ***2b*** *avec l'hydrure de diisobutylaluminium avec de très bons rendements. Le dialcool* ***4a*** *a été aussi obtenu à partir du thioester* ***3''*** *par réduction à l'hydrure de lithium aluminium avec un faible rendement. Sous l'action du chlorure de thionyle ou du tribromure de phosphore,* ***4a*** *et* ***4b*** *ont été transformés en dérivés halométhylés* ***5a***, ***6a*** *et* ***6b*** *respectivement. L'activité cytostatique, trichomonacide, amoebicide et fungicide* in vitro *de tous les composés synthétisés a été étudiée. Les dérivés bromométhylés* ***6a*** *et* ***6b*** *inhibent puissamment la croissance des cellules HeLa et ont montré aussi une activité trichomonacide significative.*

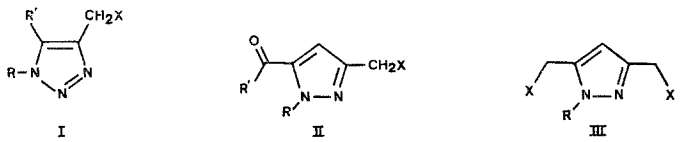
3,5-bis-(hydroxymethyl)pyrazoles / 3,5-bis-(halomethyl)pyrazoles / cytostatic agents / trichomonacide activity

Introduction

Some therapeutic drugs with alkylating properties have simultaneously shown anti-cancer and anti-parasitic activities. Thus, the azaserine, active against sarcoma 180 and other tumors and leukemias [1, 2] also shows inhibition of *Trypanosoma equiperdum* [3]. The above mentioned anti-cancer activity seems to be due to the azaserine ability to covalently bind to the enzyme responsible for the conversion of formylglycinamide ribonucleotide into formylglycinamidine ribonucleotide (purine nucleotide pathway). However, the anti-parasitic activity has been attributed to its interference with the pyrimidine biosynthesis (conversion of uridine triphosphate into cytidine triphosphate).

Another different example is mechloroethamine, which remains the therapeutic drug of choice in the treatment of advanced Hodgkin's disease due to its ability to bind to DNA by bifunctional alkylation of guanine [4] and it has also been used in the treatment of mycosis fungoide [5].

Recently, both the cytostatic activities and the chemical alkylating abilities of a series of halomethyl-1,2,3-triazoles **I** [6] and halomethylpyrazoles **II** [7, 8] (Scheme 1) have been reported. A study of their modes of action demonstrated that **I** and **II** act as alkylating agents [9]. In fact, most of the 1-glycosyl derivatives of 3-bromomethyl-and 3-iodomethylpyrazole **II** showed significant *in vitro* activities (X = I ⩾ Br > Cl) and *in vivo* they were reported to be



Scheme 1.

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effective against ECA and P388 tumor systems [10]. However, the corresponding 1-H substituted analogues of **II** were completely inactive.

Since the most active alkylating agents are usually bifunctional, the same authors attempted, unsuccessfully, the synthesis of 1H-3,5-bis-(bromomethyl)pyrazole. This fact was then attributed to the instability of such compounds due to their high reactivity [11].

Now, we have obtained stable 3,5-bis-(halomethyl)-pyrazoles **III**, which, in spite of not being 1-glycosyl- but 1-methyl- and 1-benzyl-substituted, have shown strongcytostatic activities in agreement with their expected alkylating properties.

The present paper describes for the first time the syntheses of above mentioned halomethylpyrazoles **III** (R = CH_3, Bz, X = Cl, Br) and the 3,5-bis-(hydroxymethyl)pyrazole precursors **III** (R = CH_3, Bz, X = OH). All these compounds, as well as the starting esters and thioesters, have been evaluated *in vitro* as cytostatic and as anti-parasitic agents.

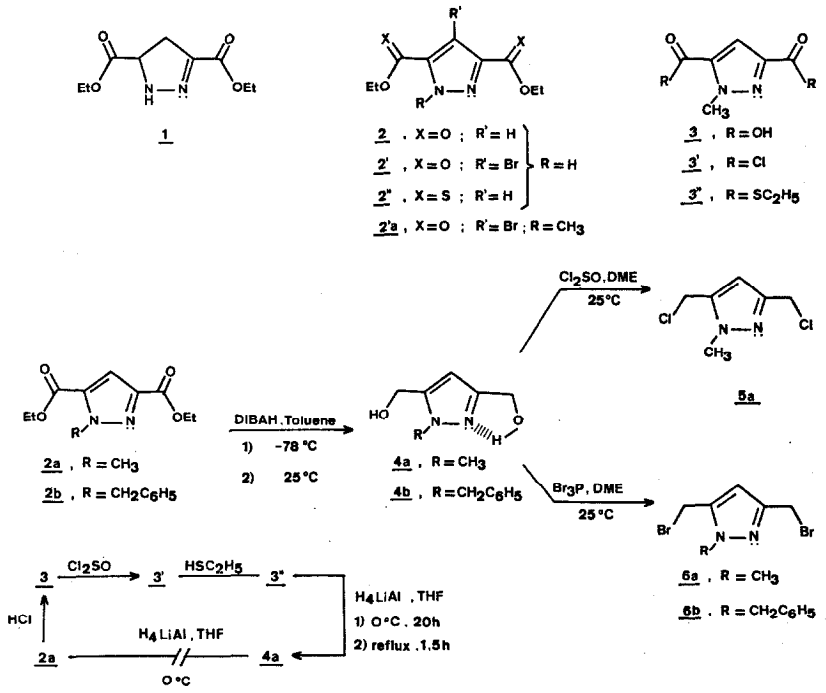
Chemistry

The 3,5-bis-(hydroxymethyl)-and 3,5-bis-(halomethyl)pyrazoles of structure **III** (Scheme 1) have been prepared as indicated in Scheme 2.

Synthesis of starting esters and thioesters

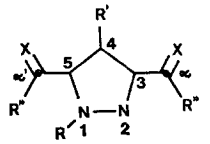
The diethyl 1H-pyrazole-3,5-dicarboxylate **2** (mp = 54—55°C, M^+ 212) used as the starting material was prepared as previously described for the dimethyl ester analogue [12] by oxidation of diethyl Δ^2-pyrazoline-3,5-dicarboxylate **1** with bromine (molar ratio 1:1) in 93% yield. Its 4-bromo-derivative **2′** (mp = 108—109°C, M^+ 290) was obtained in 81% yield when the above oxidation of **1** was carried out in the presence of an excess of bromine (molar ratio 1:2). Alkylation of **2** and **2′** in acetone with methyl iodide or benzyl chloride and potassium carbonate gave **2a** (94%), **2b** (96%) and **2′a** (90%).

There are references in the literature for the preparation of *S,S*-diethyl 1H-pyrazole-3,4-dicarbothioate [13] but, to the best of our knowledge, there has not been any report on *S,S*-diethyl-1-methyl-3,5-dicarbothioate **3″** (mp = 57—58°C) which has been prepared as indicated in Scheme 2 [**2a** → **3** (M^+ 170) → **3′** (M^+ 206) → **3″** (M^+ 258)] in 72% overall yield. In order to compare the cytostatic and anti-parasitic activities of **2** and its thionoester, **2″** (mp = 143—144°C, M^+ 244) was also prepared for the first time by reflux of **2** with Lawesson's reagent [14] in anhydrous xylene under nitrogen atmosphere [15] in 90% yield. The structural assignments of the above mentioned derivatives were made on the basis of their analytical and spectroscopical data from MS, IR, UV and ^1H NMR (see Experimental protocols), as well as on the most relevant chemical shifts of ^{13}C NMR spectra which are reported in Table I. The unsubstituted pyrazoles **2**, **2′** and **2″** show one signal for carbons C-3 and C-5 and the same is true for carbons C-α and C-α′. However in their *N*-methyl and *N*-benzyl derivatives, the C-3 and the C-α carbons are deshielded in relation to the C-5 and C-α′ ones (≃ 6—8 ppm and 2—3 ppm, respectively). Some differences can also observed in the signals from carbon C-4 in the 4-bromopyrazol **2′**, which appears at a higher field (11.3 ppm) than its 4-H substituted analogue **2**. In addition, it is interesting to note the



Scheme 2.

Table I. ^{13}C NMR spectroscopic data (δ, ppm) [a].



Comp. nº	X	R''	R'	R	C-3	C-4	C-5	C-α	C-α'	C-N
1	O	OEt	H	H	142.5	34.8	61.2	162.1	171.6	---
2	O	OEt	H	H	140.0	111.3	140.0	160.5	160.5	---
2'	O	OEt	Br	H	138.1	100.0	138.1	159.5	159.5	---
2''	S	OEt	H	H	147.5	108.2	147.6	200.3	200.3	---
3	O	OH	H	CH_3	141.9	113.1	134.7	162.5	160.5	40.0
3'	O	Cl	H	CH_3	144.5	120.3	137.6	160.6	157.8	41.7
3''	O	SEt	H	CH_3	148.1	109.5	139.6	185.4	182.5	40.7
2a	O	OEt	H	CH_3	142.1	113.8	134.0	161.5	159.2	40.4
2b	O	OEt	H	Bz	142.6	114.4	136.2	161.7	159.0	55.9
2'a	O	OEt	Br	CH_3	140.2	101.5	133.0	160.6	158.7	42.0
4a	H_2	OH	H	CH_3	150.8	103.6	142.9	57.2	53.9	35.8
4b	H_2	OH	H	Bz	151.7	103.9	143.3	57.4	54.0	52.1
5a	H_2	Cl	H	CH_3	147.6	106.5	138.9	38.7	34.7	36.5
6a	H_2	Br	H	CH_3	147.6	106.8	139.0	24.8	20.2	36.5
6b	H_2	Br	H	Bz	148.0	107.6	139.1	24.9	20.1	53.6

[a] In $CDCl_3$ solution except compounds **3**, **4a** and **4b** which were registered in DMSO d_6.

deshielding found for the carbon atoms C-α and C-α' in the dithionoester **2''** ($\simeq$ 40 ppm) in relation to its starting oxoester **2**.

Synthesis of 3,5-bis-(hydroxymethyl)- and 3,5-bis-(halomethyl)pyrazoles

Although it had previously been reported that reduction of dimethyl-1-triphenylmethyl-pyrazole-3,5-dicarboxylate with lithium aluminium hydride (LAH) in tetrahydrofuran (THF) gave the corresponding diol in quantitative yield [16], we could not obtain **4a** by reduction of **2a** using the above reagent under the same conditions. This unexpected result seems to be in agreement with those published by Greco *et al.* [13] who unsuccessfully tried to obtain 1H-3,4-bis-(hydroxymethyl)pyrazole by treating diethyl-1H-pyrazole-3,4-dicarboxylate with LAH in THF. However, since thioesters are reduced more readily than ordinary esters with LAH [17], in a way similar to that described by Greco *et al.* [13], we have obtained 1-methyl-3,5-bis-(hydroxymethyl)pyrazole **4a** in 68% yield by treatment of **3''** with LAH in THF. Furthermore, we have also verified that diisobutylaluminum hydride (DIBAH) is a much more convenient reducing agent of diethyl-1H-pyrazole-3,5-dicarboxylates than LAH. Thus, treatment of **2a** and **2b** with a toluene solution of DIBAH in molar ratio 1:4 (at — 78°C until the starting ester has disappeared and warming thereafter at 25°C until total reduction of the intermediate aldehydes) gave **4a** and **4b** in 96% and 90% yields, respectively.

In their IR spectra, the OH group nearer to the 1-methyl- or 1-benzyl-substituted nitrogen is responsible for the sharp band at 3380 cm^{-1} in both diols, while the broad absorption at 3500—2500 cm^{-1} may be mainly due to the hydrogen interactions between the other hydroxyl group and the N-2 nitrogen atom. In the ^1H NMR spectra of **4a** and **4b** (both recorded in DMSO d_6) the two OH protons, which appear as triplets by coupling with the neighboring methylene group (J = 6 Hz), are not equivalent. The OH proton nearest to the lone pair of the N-2 nitrogen atom, following the same reasoning as above, is deshielded (0.70 ppm in **4a** and 0.35 ppm in **4b**) in relation to the *N*-methyl or *N*-benzyl vicinal OH proton [18]. The halomethyl derivatives 3,5-bis-(chloromethyl)pyrazole **5a** and 3,5-bis-(bromomethyl) pyrazole **6a** and **6b** were obtained by reacting **4a** or **4b** with thionyl chloride or phosphorous tribromide (with dimethoxyethane as the solvent at room temperature) with 89, 80 and 92% yields, respectively.

The main structural difference between the above mentioned haloderivatives arises from the chemical shift displacements at high field found for the C-α and C-α' carbon atoms in the bromomethylpyrazoles **6a** and **6b**, as compared to the same carbons in the ^{13}C NMR spectrum of its chloromethyl analogue **5a** (see Table I).

Cytostatic activity

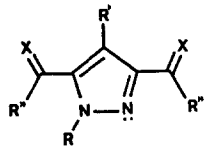
All the pyrazoles reported in this paper were evaluated as cytostatic agents against HeLa cell cultures (Table II). The diethyl esters **2**, **2'**, **2a**, **2b** and **2'a** were inactive. However, the *S,S*-diethyl-1-methyl-3,5-dicarbothioate **3''** and the thiono derivative **2''** showed some activity. In contrast to the three halomethylpyrazoles, which were the more cytostatic compounds, the starting hydroxymethyl derivatives **4a** and **4b** were totally inactive. As expected, the cytostatic activity increased with the alkylating ability of the halomethyl groups. Thus, in the 1-methyl-substituted 3,5-bis-(chloromethyl)pyrazole **5a**, the cytostatic activity was lower than in both 1-methyl- and 1-benzyl-derivatives of 3,5-bis-bromomethylpyrazole **6a** and **6b**. The latter showed a strong *in vitro* activity (ED_{50} = 1 μg/ml) higher than that previously reported for the 1-glycosyl-3-bromomethylpyrazole-5-carboxylates and 1-glycosyl-3-bromomethylpyrazole-5-carboxamides of general structure **II** [Gl = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl, X = Br: R = COOEt (ED_{50} = 5 μg/ml) and R = $CONH_2$ (ED_{50} = 4 μg/ml); Gl = 2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl, X = Br: R = COOEt (ED_{50} = 2 μg/ml) and R = $CONH_2$ (ED_{50} = 3 μg/ml), Gl = 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl), X = Br: R = COOEt (ED_{50} = 2.5 μg/ml) and R = $CONH_2$ (ED_{50} = 2 μg/ml)] [7].

The *in vivo* cytostatic evaluation and the mode of action of these 3,5-bis-(bromomethyl)pyrazoles **6a** and **6b** are presently under study.

Anti-parasitic activities

The activities of compounds **2**, **2'**, **2''**, **2a**, **2b**, **2'a**, **3''**, **4a**, **4b**, **5a**, **6a** and **6b** were evaluated *in vitro* against the following parasites: *Trichomonas vaginalis*, *Entamoeba invadens* and *Candida albicans* using metronidazole as the

Table II. *In vitro* anti-fungal, amebicidal, trichomonacidal and cytostatic activities of compounds **2′**, **2′a**, **2″**, **3″**, **5**, **6a** and **6b**[a].



Compound nº	X	R″	R′	R	M.P. (°C) (solvent)	Antifungal Activity (μg/mL) MIC	MFC	Amebicidal Activity (μg/mL) MIC	MCC	Trichomonacidal Activity (μg/mL) MIC	MCC	Cytostatic Activity (μg/mL) ED_{50}
2′	O	OEt	Br	H	108-109 (n-hexane)	400	800	Inactive		400	---	100
2′a	O	OEt	Br	CH_3	53-54 (n-hexane)	---	400	Inactive		Inactive		100
2″	S	OEt	H	H	143-144 (n-hexane)	200	---	400	---	200	---	10
3″	O	SEt	H	CH_3	57-58 (EtOH,H_2O)	200	---	Inactive		200	---	30
5a	H_2	Cl	H	CH_3	35-36 (n-hexane)	200	---	100	200	100	200	5
6a	H_2	Br	H	CH_3	70-71 (n-hexane)	200	---	200	400	25-50	100	1
6b	H_2	Br	H	Bz	52-53 (n-hexane)	---	400	100	200	25	50	1
Amphothericin B						0.79						
Ketoconazole						0.19						
Metronidazole								25	50-100	4-8	8	
6-Mercaptopurine												~0.1

[a]Compounds **2**, **2a**, **2b**, **4a** and **4b** were inactive.
Amebicidal and trichomonacidal activities were measured after 48 h of incubation.

reference for the amoebicide and trichomonacide activities and amphothericin B and ketoconazole as the references for anti-fungal activity (see Table II). The diethyl esters **2**, **2a** and **2b** and their hydroxymethyl derivatives **4a** and **4b** were inactive, while the thioester **3″** and the thionoester **2″** showed some activity as anti-fungal and anti-trichomonias agents. The most relevant data are provided by the halomethyl derivatives **5a**, **6a** and **6b**. Compounds **5a** and **6b** had a *MIC* of 100 μg/ml in the amebicidal test, and the two 3,5-bis-(bromomethyl)pyrazoles 1-methyl- and 1-benzyl-substituted, presented significant trichomonacidal activities: **6a**: *MIC* between 25—50 μg/ml; **6b**: *MIC* = 25 μg/ml.

In order to evaluate the possible correlation between the trichomonacidal and cytostatic activities, 3,5-bis-(iodomethyl)derivatives of **III** are now being prepared.

Experimental protocols

Chemistry

Melting points were measured on a Köfler hot-stage apparatus, ^1H and ^{13}C NMR were recorded with a Varian EM-390 and a Bruker WP 80 SY spectrometers using Me_4Si as the internal standard. Chemical shifts are given in ppm (δ scale). The mass spectra on an Hitachi Perkin—Elmer RMU-6MG at 70 eV. (Mass numbers are given in *m/z* with relative intensities in parentheses). The IR spectra, the UV spectra were obtained on Perkin—Elmer 257 and Perkin—Elmer 550 SE spectrometers, respectively.

Analytical TLC was performed on aluminum sheets coated with a 0.2 mm layer of silica gel 60 F_{254} (Merck). Chromatographic separations were performed either on columns, using the flash chromatography technique [19] on silica gel 60 (Merck), 200—400 mesh, or by preparative layer chromatography on 20 × 20 cm glass plates coated with a 2 mm layer of silica gel PF_{254} (Merck). Compounds were detected with UV light (254 nm) and/or iodine chamber. Elemental analyses were carried out by the Analytical Department of the Instituto de Química Orgánica General, Madrid, Spain. For all the compounds mentioned analyses of C, H, N, S, Cl and Br were within ± 0.4% of the theoretical values.

Lithium aluminium hydride (LAH) was purchased from Carlo Erba and diisobutylaluminum hydride supplied in a toluene solution (20%) was from Fluka.

Diethyl Δ²-pyrazoline-3,5-dicarboxylate **1**
Following the method previously described for the dimethyl-1H-pyrazole-3,5-dicarboxylate [12], the reaction of 25 g (220 mmol) of ethyl diazoacetate with 22 g (220 mmol) of ethyl acrylate afforded 44.8 g (95% yield) of **1**. mp = 58—59°C (*n*-hexane). Anal. $C_9H_{14}N_2O_4$ (C, H, N). MS *(m/e)*, 95 (100), 214 (M^+, 7). UV (ethanol), λ_{max} (log ε), 288 (3.89) nm. IR (KBr), ν (cm^{-1}), 3370, 1730, 1710, 1585. ^1H NMR ($CDCl_3$) δ: 6.74 (m broad, 1H, NH, disappears with D_2O); 4.40 (m, 1H, *H*C-5); 3.24 (m, 2H, H_2C-4); 4.30 (q, 4H, CH_3—CH_2—OCO); 1.30 (t, 6H, CH_3—CH_2—OCO).

Diethyl 1H-pyrazole-3,5-dicarboxylate **2**
The oxidation of the above pyrazoline **1** (22 g, 103 mmol) with bromine in molar ratio 1:1 afforded 20.3 g of **2** in 93% yield. mp = 54—55°C (*n*-pentane). Anal. $C_9H_{12}N_2O_4$ (C, H, N). MS *(m/e)*, 167 (100), 212 (M^+, 29). UV (ethanol), λ_{max} (log ε), 224 (3.9) nm. IR (KBr), ν (cm^{-1}), 3200, 1725, 1565, 1560, 1540. ^1H NMR ($CDCl_3$) δ: 11.4 (m br, 1H, N*H*, disappears with D_2O); 7.37 (s, 1H, HC-4); 4.50 (q, 4H, CH_2—OCO); 1.40 (t, 6H, CH_3—CH_2—OCO).

Diethyl 1H-4-bromopyrazole-3,5-dicarboxylate **2′**
When the above mentioned oxidation of **1** (4.4 mmol) was performed with two equivalents of bromine, a mixture of **2a** and **2′** was obtained. They were separated by preparative TLC on silica gel using a mixture of chloroform—ethyl acetate—ethanol—25% aqueous solution of ammonium hydroxide (v/v, 5:5:5:1) to give 0.093 g (10%) of **2** (R_f = 0.74) and 1.03 g (81% yield) of **2′** (R_f = 0.53) as a cristalline solid. mp = 108—109°C (*n*-hexane). Anal. $C_9H_{11}Br\ N_2O_4$ (C, H, N, Br). MS *(m/e)*, 43 (100), 290 (M^+, 1), 292 (M^+ + 2, 1). UV (ethanol)

λ_{max} (log ε), 209 (4.28), 232 sh (3.75), 253 sh (3.48). IR (KBr), ν (cm^{-1}), 3240, 1725, 1540, ^1H NMR (CCl_4) δ: 12.5 (m broad, 1H, NH, disappears with D_2O); 4.32 (q, 4H, H_2C—OCO); 1.35 (t, 6H, H_3C—OCO).

Synthesis of diethyl esters 1-methyl- and 1-benzyl-substituted

General procedure

A solution of 100 mmol of methyl iodide (or benzyl chloride) in 200 ml of anhydrous acetone was added to a solution of 100 mmol of **2** or **2'** in an acetone solution (500 ml) and 225 mmol of potassium carbonate. The mixture was refluxed (8—10 h) until TLC (silica gel: *n*-hexane—chloroform—acetone, v/v, 4:4:1) indicated the formation of the new product (**2** and **2'**, R_f = 0.47, **2a** and **2'a** R_f = 0.76, **2b** R_f = 0.85). After cooling to room temperature, the residual solid was filtered off and the resulting solution evaporated to dryness. The residue was dissolved in chloroform (3 × 100 ml) and washed repeatedly with water (3 × 100 ml). The organic layer was dried with magnesium sulfate, and evaporated to dryness under reduced pressure. The solid residue was purified, as indicated in each case.

Diethyl 1-methylpyrazole-3,5-dicarboxylate **2a**

According to the general procedure reaction, **2** and methyl iodide afforded a solid which after crystallization from *n*-hexane gave **2a** as a white solid in 94% yield. mp = 71—72°C. Anal. $C_{10}H_{14}N_2O_4$ (C, H, N). MS *(m/e)*, 181 (100), 226 (M^+, 19), 227 (M^+ + 1, 5). UV (ethanol), λ_{max} (log ε), 224 (4.0) nm. IR (KBr), ν (cm^{-1}), 1740, 1725, 1530. ^1H NMR ($CDCl_3$) δ: 7.35 (s, 1H, *H*C-4), 4.41 (q, 2H, H_2C—OCO—C—3); 4.36 (q, 2H, H_2C—OCOC—5); 4.25 (s, 3H, CH_3—N); 1.40 (t, 3H, CH_3—CH_2OCOC—3); 1.38 (t, 3H, CH_3—CH_2OCOC-5).

Diethyl 1-benzylpyrazole-3,5-dicarboxylate **2b**

Reaction of **2** (15 g, 71 mmol) and benzyl chloride (8.25 ml), according to the general procedure, gave a residue which crystallized from *n*-hexane to give 20.5 g, of **2b** in 96% yield. mp = 74—75°C. Anal. $C_{16}H_{18}N_2O_4$ (C, H, N). MS *(m/e)* 225 (100), 302 (M^+, 70), 303 (M^+ + 1, 12). UV (ethanol), λ_{max} (log ε), 220 (4.1), 242 sh (3.8) nm. IR (KBr), ν (cm^{-1}) 1730, 1720. ^1H NMR ($CDCl_3$) δ: 7.35 (s, 1H, *H*C-4); 7.25 (s, 5H, C_6H_5); 5.83 (s, 2H, H_2C—C_6H_5); 4.40 (q, 2H, H_2C—OCOC-3); 4.28 (q, 2H, H_2C—OCOC-5); 1.38 (t, 3H, H_3C—CH_2OCOC-3); 1.30 (t, 3H, H_3C—CH_2OCOC-5).

Diethyl 1-methyl-4-bromopyrazole-3,5-dicarboxylate **2'a**

According to the general procedure, the reaction of **2'** (3.5 g, 12 mmol) with methyl iodide (2.05 g, 14.4 mmol) for 5 h, gave 3.30 g (90% yield) of a crystalline solid. mp = 53—54°C (*n*-hexane). Anal. $C_{10}H_{13}BrN_2O_4$ (C, H, N, Br). MS *(m/e)*, 81 (100), 304 (M^+, 47), 306 (M^+ + 2, 45). UV (ethanol) λ_{max} (log ε), 210 (4.45), 253 sh (3.71) nm. IR (KBr), ν (cm^{-1}), 1735, 1715, 1270, 1230, ^1H NMR ($CDCl_3$) δ: 4.41 (q, 4H, CH_2—OCO); 4.20 (s, 3H, CH_3—N); 1.35 (t, 3H, CH_3—CH_2—OCO).

Synthesis of dicarbothioate 3''

1-Methylpyrazole-3,5-dicarboxylic acid **3**

The ester **2a** (50 mmol) in 6 N HCl (310 ml) was heated under reflux for 4 h, cooled to room temperature and the precipitate formed was filtered off. After crystallization from water and drying under vacuum, the above solid gave 8.3 g (88% yield) of **3**. mp = 250—252°C. Anal. $C_6H_6N_2O_4 \cdot 1\ H_2O$ (C, H, N). MS *(m/e)* 153 (100), 170 (M^+, 91), 171 (M^+ + 1, 9). UV (ethanol), λ_{max} (log ε) 224 (3.8), 243 sh (3.5) nm. IR (KBr), ν (cm^{-1}), 3600—2500, 1700. ^1H NMR (DMSO d_6) δ: 7.5 (m broad, 2H, *H*OCO, disappears with D_2O); 7.28 (s, 1H, *H*C-4); 4.22 (s, 3H, CH_3—N).

1-Methylpyrazole-3,5-dicarbonyl chloride **3'**

A mixture of **3** (42 mmol) and thionyl chloride (32 ml) was heated under reflux for 1 h. Removal of the excess thionyl chloride gave 8.71 g of **3'** as a syrup in quantitative yield. Anal. $C_6H_4Cl_2N_2O_2$ (C, H, N, Cl). MS *(m/e)*, 153 (100), 206 (M^+, 0.6), 208 (M^+ + 2, 0.5), 210 (M^+ + 4, 0.2). IR (neat), ν (cm^{-1}), 1775, 1765, 1450, 1200, 1145, 865, 835. ^1H NMR ($CDCl_3$), δ: 7.68 (s, 1H, *H*C-4); 4.28 (s, 3H, H_3C—N).

S,S-*Diethyl 1-methylpyrazole-3,5-dicarbothioate 3''*

From ethanethiol (10.42 g, 168 mmol) and pyridine (5.0 g) added to a solution of **3'** (8.7 g, 42 mmol) in dry benzene (75 ml) cooled between 5—10°C, a large volume of gas evolved. When the evolution ceased, the mixture was allowed to attain room temperature and was stirred for 72 h. The white solid formed was dissolved by adding water (300 ml) and the resulting solution extracted with ether (4 × 75 ml). The combined extracts were washed successively with 5% sodium carbonate (3 × 70 ml), dilute HCl (3 × 70 ml) and water (3 × 70 ml). Evaporation of the dried ($MgSO_4$) organic layer gave the dicarbothioate **3'** as a yellow oil which was purified by flash chromatography on silica gel using a mixture of *n*-hexane—ethyl acetate (v/v, 2:1) as the eluent. The appropriate fractions combined gave 7.95 g (73% yield) of chromatographically pure **3''** (R_f = 0.80) which crystallized from *n*-hexane. mp = 57—58°C. Anal. $C_{10}H_{14}N_2O_2S_2$ (C, H, N, S). MS *(m/e)*, 197 (100), 258 (M^+, 9), 260 (M^+ + 2, 1). UV (ethanol), λ_{max} (log ε), 195 (4.1), 221 (4.3), 271 (4.2) nm. IR (KBr), ν (cm^{-1}), 1715, 1705, 1515, 1455, 1205, 1150, 900, 885, 740. ^1H NMR ($CDCl_3$) δ: 7.33 (s, 1H, HC-4); 3.05 (q, 4H, —H_2C—SCO); 1.35 (t, 6H, H_3C—CH_2SCO); 4.20 (s, 3H, H_3C—N).

O,O-*Diethyl 1H-pyrazole-3,5-dicarbothioate 2''*

A mixture of 3 g (14.15 mmol) of **2** and 13.74 g (33.97 mmol) of 2,4-*bis*-(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent) and anhydrous xylene (20 ml) was refluxed under nitrogen until TLC analysis showed that the ester had reacted (24 h). After cooling to room temperature, the excess of Lawesson's reagent was filtered off and the organic solvent was evaporated to dryness. The residual syrup was purified by flash chromatography on silica gel using a mixture of *n*-hexane—dichloromethane—acetone (v/v, 5:5:1) as the eluent. Removal of the solvents from the eluate gave 4.5 g of a yellow oil which after crystallization from ethanol—water gave 3.11 g (90%) of thionoester **2''**. mp = 143—144°C. Anal. $C_9H_{12}N_2O_2S_2$ (C, H, N, S). MS *(m/e)* 200 (100), 244 (M^+, 7), 246 (M^+ + 2, 2). UV (ethanol), λ_{max} (log ε) 238 (4.1), 295 (4.2) nm. IR (KBr), ν (cm^{-1}), 3190, 1545, 1395, 1300, 1280, 1235, 1205, 1020, 845. ^1H NMR ($CDCl_3$) δ: 10.25 (m broad, 1H, N*H*, disappears with D_2O); 7.28 (q, 1H, *H*C-4); 4.66 (q, 4H, H_2C—OCS); 1.45 (t, 6H, H_3C—CH_2—OCS).

Synthesis of 3,5-bis-(hydroxymethyl)derivatives: Method A with DIBAH as the reducing agent

General procedure

A solution of diethyl ester (10.0 mmol) in anhydrous toluene (15 ml) under nitrogen atmosphere was cooled at —78°C and treated with a 1.2 M toluene solution of diisobutylaluminum hydride (≃ 34 ml, 40 mmol). The mixture was stirred at — 78°C until TLC analysis (silica gel, AcOEt) showed that the starting ester had reacted. Then, the reaction was allowed to reach room temperature and stirred until a new TLC control showed that the intermediate aldehydes had disappeared. After the reaction was completed, it was cooled at 0°C and hydrolyzed with methanol. The aluminum hydroxide was filtered off and the organic layer was evaporated to dryness under reduced pressure to give the corresponding diol as a solid which was purified by crystallization.

1-Methyl-3,5-bis-(hydroxymethyl)pyrazole **4a**

According to the general Method A, **2a** (84 mmol), was reacted with DIBAH (366 mmol) for 6 h at — 78°C (**2a**, R_f = 0.91) and after 36 h at 25°C (intermediate aldehydes R_f = 0.68 and R_f = 0.60) afforded **4a** (R_f = 0.17) as a white solid which crystallized into prismatic needles. mp = 91—92°C (AcOEt), 11.45 g (96% yield). Anal. $C_6H_{10}N_2O_2$ (C, H, N). MS *(m/e)*, 142 (M^+, 100), 143 (M^+ + 1, 18). UV (ethanol) λ_{max} (log ε) 226 (3.6), 214 sh (3.4) nm. IR (KBr), ν (cm^{-1}), 3360, 3580—2500, 1220, 1150, 1015, 815. ^1H NMR (DMSO d_6) δ: 6.08 (s, 1H, *H*C-4); 5.18 (t, 1H, *H*O—CH_2C-3, J = 6 Hz, disappears with D_2O); 4.88 (t, 1H, *H*O—CH_2C-5, J = 6 Hz, disappears with D_2O); 4.45 (d, 2H, HO—C*H*$_2$C-5, J = 6 Hz, collapses to a singlet with D_2O); 4.32 (d, 2H, HO—C*H*$_2$—C-3, J = 6 Hz, collapses to a singlet with D_2O); 3.70 (s, 3H, H_3C—N).

1-Benzyl-3,5-bis-(hydroxymethyl)pyrazole **4b**

Following the general Method A, the benzyl ester **2b** (66.2 mmol) was treated with DIBAH (276 mmol) for 2.5 h at — 78°C (**2b**, R_f = 0.95) and 150 h at 25°C (intermediate aldehydes: R_f = 0.83 and R_f = 0.70) to afford **4b** (R_f = 0.40) as a white solid which crystallized into prismatic needles. mp = 92—93°C (AcOEt), 13.0 g (90%). Anal.

$C_{12}H_{14}N_2O_2$ (C, H, N). MS *(m/e)* 91 (100), 218 (M^+, 40), 219 ($M^+ + 1$, 6). UV (ethanol), λ_{max} (log ε), 206 (3.7), 223 sh (3.5) nm. IR (KBr), ν (cm^{-1}), 3360, 3520—2520, 1160, 1055, 1030, 1005, 835, 735. ^1H NMR (DMSO d_6) δ: 7.20 (m, 5H, C_6H_5); 6.20 (s, 1H, *H*C-4); 5.30 (s, 2H, H_2C—C_6H_5); 5.30 (t, 1H, *H*OCH$_2$C-3, J = 6 Hz, disappears with D_2O); 4.95 (t, 1H, *H*OCH$_2$C-5, J = 6 Hz, disappears with D_2O); 4.48 (d, 2H, HOC*H*$_2$C-3, J = 6 Hz, collapses to a singlet with D_2O); 4.38 (d, 2H, HOC*H*$_2$-C-5, J = 6 Hz, collapses to a singlet with D_2O).

Method B with LAH as the reducing agent

1-Methyl-3,5-bis(hydroxymethyl)pyrazole ***4a***

To a suspension of 1.23 g (32 mmol) of LAH in THF (100 ml) was added a solution of *S,S*-diethyl-1-methylpyrazole-3,5-dicarbothioate **3″** (2.5 g, 10 mmol) in THF (50 ml) in small portions at 5°C. After stirring for 20 h at 5°C, the mixture was warmed to reflux for 1.5 h and then cooled to 0°C and hydrolyzed with a water—methanol solution (v/v, 85:15) (100 ml). The metallic hydroxides were filtered off and washed with ethanol. The organic layer was separated and the aqueous one was extracted with chloroform (3 × 100 ml). These extracts were then combined with the above organic layer, dried ($MgSO_4$) and evaporated to dryness. A residual syrup was obtained and purified by flash chromatography on silica gel using toluene—acetone—ethanol (v/v, 1:1:1) as the eluent. Removal of the solvent from the appropriate fractions gave **4a** (R_f = 0.55) as a syrup which crystallized into prismatic needles from ethyl acetate. mp = 91—92°C (0.92 g, 68% yield).

Synthesis of 3,5-bis-(halomethyl)pyrazoles

1-Methyl-3,5-bis-(chloromethyl)pyrazole ***5a***

A solution of thionyl chloride (15 ml) in 1,2-dimethoxyethane (DME) (30 ml) was slowly added to a stirred solution of 1-methyl-3,5-*bis*-(hydroxymethyl)pyrazole **4a** (2 g, 14 mmol) in DME (100 ml). The mixture was kept at room temperature while stirring, until TLC analysis (silica gel, *n*-hexane—ether, v/v, 1:1) showed that the reaction was completed (**5a**, R_f = 0.56). The mixture was poured into 200 g of crushed ice, vigorously stirred and neutralized with sodium bicarbonate. The organic layer was separated and the aqueous one was extracted with chloroform (4 × 100 ml). Then all the organic extracts were washed with water and dried (Na_2SO_4). Evaporation of the solvents to dryness left **5a** as an oil which crystallized from *n*-hexane after several days. mp = 35—36°C (2.2 g, 89 %). Anal. $C_6H_8Cl_2N_2$ (C, H, N, Cl). MS *(m/e)*, 143 (100), 178 (M^+, 21), 180 ($M^+ + 2$, 14), 182 ($M^+ + 4$, 2). UV (ethanol), λ_{max} (log ε) 205 (3.6), 224 sh (3.5) nm. IR (KBr), ν (cm^{-1}), 2940, 1450, 1270, 1020, 810, 745, 730, 675. ^1H NMR, (CCl_4) δ: 6.22 (s, 1H, *H*C-4); 4.47 (s, 2H, ClC*H*$_2$C-3); 4.40 (s, 2H, ClC*H*$_2$C-5); 3.82 (s, 3H, H_3C—N).

1-Methyl-3,5-bis-(bromomethyl)pyrazole ***6a***

Phosphorous tribromide (2.0 ml) was slowly added to a stirred solution of 1-methyl-3,5-*bis*-(hydroxymethyl)pyrazole **4a** (1.32 g, 9.3 mmol) in anhydrous DME (80 ml). The mixture was stirred at room temperature until a TLC control (silica gel, *n*-hexane—diethyl ether, v/v, 2:1) indicated that the reaction was completed (20 h). The mixture was worked up as above. Evaporation of the solvent gave **6a** (R_f = 0.35) as a white solid which crystallized from *n*-hexane into prismatic needles. mp = 70—71°C (2 g, 80% yield). Anal. $C_6H_8Br_2N_2$ (C, H, N, Br). MS *(m/e)* 187 (100), 266 (M^+, 3), 268 ($M^+ + 2$, 5), 270 ($M^+ + 4$, 3), UV (ethanol) λ_{max} (log ε) 230 (3.9), 212 (3.8) nm. IR (KBr), ν (cm^{-1}) 3025, 2970, 1370, 1215, 1160, 1020, 820, 810, 710, 660. ^1H NMR ($CDCl_3$) δ: 6.33 (s, 1H, HC-4); 4.40 (s, 4H, Br—C*H*$_2$C); 3.83 (s, 3H, C*H*$_3$—N).

1-Benzyl-3,5-bis-(bromomethyl)pyrazole ***6b***

Following the above procedure, phosphorous tribromide (6 ml) was reacted with 1-benzyl-3,5-*bis*-(hydroxymethyl)-pyrazole **4b** (6.0 g, 27.5 mmol) in 230 ml of DME at room temperature for 20 h and gave 8.72 g (92%) of **6b**. mp = 52—53°C (*n*-hexane). Anal. $C_{12}H_{12}Br_2N_2$ (C, H, N, Br). MS *(m/e)* 263 (100), 342 (M^+, 5), 344 ($M^+ + 2$, 10), 346 ($M^+ + 4$, 5). UV (ethanol) λ_{max} (log ε) 209 (3.4), 235 sh (3.1) nm. IR (KBr), ν (cm^{-1}), 3020, 2960, 1460, 1330, 1210, 825, 815, 770, 730, 715, 695. ^1H NMR ($CDCl_3$) δ: 7.23 (m, 5H, C_6H_5); 6.37 (s, 1H, *H*C-4); 5.38 (s, 2H, H_2C—C_6H_5); 4.43 (s, 2H, Br—C*H*$_2$C-3); 4.23 (s, 2H, BrC*H*$_2$C-5).

Biological methods

In vitro *cytostatic activity*

The previously described method [20] was followed. Minimal Eagle's medium [21] (Difco, code 5675) supplemented with 10% fetal calf serum (Difco) was used. HeLa cells (10^5/ml) were incubated at 37°C in Leighton tubes. After 2—3 h, the cells were attached to the glass and the compound to be tested, suspended in sterile saline containing 0.05% Tween 80 (v/v), was then added. The volume of this suspension was 10% of the final incubation mixture. Incubation was carried out at 37°C for 72 h. As a positive control, 6-mercaptopurine was always included ($ED_{50} \simeq 0.1$ μg/ml). Cell growth was estimated by measuring the cell proteins following the colorimetric method of Oyama and Eagle [22].

In vitro *trichomonacide activity*

Cultures of standard strains preserved in liquid nitrogen (strain G) were performed in Diamond medium (TYM) without agar. The products to be tested were added to the cultures at the different preestablished doses 6 h after reseeding (h 0) were taken. After 24 and 48 h of contact between the compounds and protozoa, counts were determined using the method of lactic acid release into the culture. Parallel growth and activity control tubes were run in each experiment. Metronidazole was used as the reference drug. Minimal inhibitory concentration *(MIC)* and minimal cytocidal concentration *(MCC)* were established in accordance with the definitions proposed by Escario *et al.* [23].

In vitro *amebicidal activity*

Entamoeba invadens was used as the experimental model. The cultures were grown in *Entamoeba* medium (Difco). The compounds to be tested at the initial dose of 400 μg/ml were added to the culture 6 h after reseeding (log phase). Doses were reduced in active cases, in order to determine the *MIC* and *MCC*. Counts were made after 48 h of contact of the compounds with the protozoa. In this study, in contrast to the trichomonal activity test, it was necessary to use the classical counting method in a hematocymeter. Metronidazole was used as the reference compound.

Anti-fungal activity

Candida albicans (Strain 1001) preserved in YED medium was used as the experimental model for anti-fungal screening. The anti-fungal activity was determined in SAH medium (Sabourand's agar—honey with chloramphenicol) and in 24-well, that-bottomed Nuclon plates. 1 ml of medium was added to each well. The plates thus prepared, and once dry, were kept at 4°C until use. For the test, yeasts cultured in SAH medium for 20 h at 37°C were used, *i.e.*, at log growth stage, diluted in a sterile saline solution at a final concentration of 10^5/ml [23, 24]. Each well was seeded with 10 μl of this suspension. Half an hour after seeding the products to be tested were added at the preestablished dosage, dissolved in a maximum of 25 μl of DMSO. Amphotericin B and ketoconazole were the reference drugs, also with DMSO as the solvent.

In the case of anti-fungal assays, *MIC* expresses the concentration of the drug, that inhibits the growth of the yeast in the test tubes but does not inhibit their growth after reseeding.

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References

1 Moore E. C. & Le Page G. A. (1957) *Cancer Res.* 17, 804
2 Duvall L. R. (1960) *Cancer Chemother. Rep.* 7, 65
3 Momparler R. L. & Jaffe J. J. (1965) *Biochem. Pharmacol.* 14, 255
4 Livingston R. B. & Carter S. K. (1970) *in: Single Agents in Cancer Chemotherapy* IFI/Plenum, New York

5 Greenspan E. M. & Bruckner H. W. (1975) *in: Clinical Cancer Chemotherapy* (Greenspan E. M., ed.), Raven Press, New York, Ch. 3
6 De las Heras F. G., Alonso R. & Alonso G. (1979) *J. Med. Chem.* 22, 496
7 García-López M. T., Herranz R. & Alonso G. (1979) *J. Med. Chem.* 22, 807
8 García-López M. T., Domínguez M. J., Herranz R., Sánchez-Pérez M., Contreras A. & Alonso G. (1980) *J. Med. Chem.* 23, 567
9 Contreras A., Sánchez-Pérez R. M. & Alonso G. (1978) *Cancer Chemother. Pharmacol.* 1, 243
10 Sánchez-Pérez R. M. (1982) Doctoral Thesis, Universidad Complutense de Madrid
11 García-López M. T., Herranz R. & Alonso G. (1980) *J. Heterocyclic Chem.* 17, 13
12 Buchner E. & Papendidiek A. (1893) *Ann. Chem.* 273, 232
13 Greco C. V. & Pellegrini F. (1972) *J. Chem. Soc. Perkin I*, 720
14 Pedersen B. S., Schetbye S., Clausen K. & Lawesson S. O. (1978) *Bull. Soc. Chim. Belg.* 87, 293
15 Baxter S. L. & Bradshaw J. S. (1981) *J. Org. Chem.* 46, 831
16 Gilman N. W. & Fryer R. I. (1977) *J. Heterocyclic Chem.* 14, 1171
17 Wolfram M. L. & Karabinos J. V. (1944) *J. Am. Chem. Soc.* 66, 909
18 Elguero J. (1984) *in: Comprehensive Heterocyclic Chemistry* (Katritzky A. R. & Rees C. W., eds.), Pergamon Press Ltd., Oxford, pp. 167
19 Still W. C., Kahn M. & Mitra A. (1978) *J. Org. Chem.* 43, 2923
20 Geran T. I., Greenberg N. H., McDonald M. M., Schumacher A. M. & Abbot B. J. (1959) *Cancer Chemother. Rep.*, pt 3, 3, 432
21 Eagle H. (1954) *Science* 130, 432
22 Oyama V. I. & Eagle H. (1956) *Proc. Soc. Exp. Biol. Med.* 91, 305
23 Escario J. A., Sánchez-Souza A., Gomez-Criado C., Jiménez M. L., Fernández-Jorg E. A. & Baquero F. (1985) *Ann. Inst. Pasteur* 136A, 371
24 Defever K. S., Whelan W. L., Rogers A. L., Beneke E. S., Veselenak J. M., Drouhet E. & Dupont B. (1978) *Bull. Soc. Fr. Mycol. Med.* 2, 165