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Synthesis, Characterization and Evaluation of the Activity of Ten Mesoionic Compounds Against Microorganisms

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SUMMARY. Three mesoionic 1,3,4-thiadiazolium-2-thiolates and seven 1,3,4-triazolium-2-thiol chlorides have been synthesised and tested for activity against a range of microorganisms. The chemical structures were confirmed by Elemental Analysis, IR, Mass, ^{1}H and ^{13}C NMR spectrometry. The biological tests indicate that the compounds have substantial activity against Gram-positive and alcohol-acid-resistant bacteria; moderate activity against yeasts and little activity against other fungi and are inactive against Gram-negative bacteria.

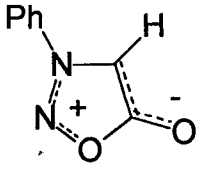
RESUMEN. "Síntesis, Caracterización y Evaluación de la Actividad de diez Compuestos Mesoiónicos contra Microorganismos". Tres mesoiónicos 1,3,4-tiadiazolio-2-tiolato y siete cloruros 1,3,4-triazolio-2-tiol se han sintetizado y se ha probado su actividad contra distintos microoganismos. Las estructuras químicas se determinaron por Análisis Elemental, IR, Masa y espectrometria de RMN ^{1}H e ^{13}C. Las pruebas biológicas indican que los compuestos tienen actividad sustancial contra bacterias Gram positivas y alcohol-ácido-resistentes, actividad moderada contra levaduras, escasa actividad contra otros hongos y son inactivos contra las bacterias Gram negativas ensayadas.

INTRODUCTION

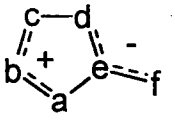
Compounds now classified as mesoionic were first prepared around 100 years ago by Fischer and Besthorn and by Busch *et. al.* [1-4]. Even so it was only in 1949 that the first definition was launched by Baker and Ollis [5,6] referring especially to the sydnones (**I**), although important fundamental concepts were put forward by Schönberg in 1938 [7].

The concept of mesoionic compounds evolved somewhat over the years although they were generally classified as aromatic betaines. However in 1996 Miller, Simas and their co-workers [8] put forward a new definition, *viz;* "Mesoionic compounds are heterocyclic betaines with a least one side-chain whose a-atom is also in the ring plane and with dipole moments of the order of 5D (1D = 3,33564x10^{-30} cm). Electrons are delocalized over two regions separated by what are essentially single bonds. One region which includes the α-atom of the side-chain is associated with the HOMO and negative π-charge whereas the other is associated with the LUMO and positive π-charge". This is equivalent to indicating that mesoionic compounds are not aromatic.

Structure **II** is a convenient generic representation of mesoionic compounds, according to this definition, in which *a,b,c,d,e* and *f* are usually C, N, O, S or Se.



I



II

As a result, mesoionic compounds interact strongly with many biomolecules, leading to a very wide range of biological activity: *inter alia* analgesic, anti-convulsant, anti-depressive/psycho-stimulant, anti-inflammatory, anti-malaria, an-

PALABRAS CLAVE: Compuestos mesoiónicos, Bacteria Gram negativa, Bacteria Gram positiva, Hongos, Levaduras.
KEY WORDS: Fungi, Gram-negative bacteria, Gram-positive bacteria, Mesoionic compounds, Yeasts.

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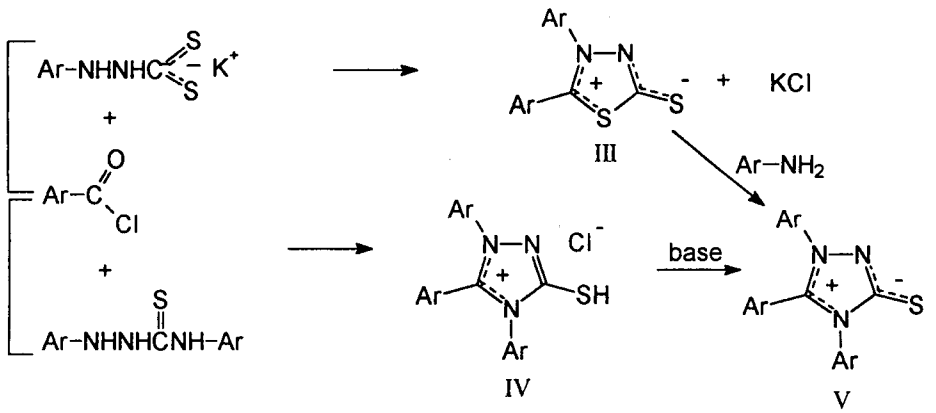
ti-neoplasic, anti-pyretic activity. They also possess ascaricidal, bactericidal, CNS-stimulant, coccidiostatic, diuretic, fungicidal, hypoglicemic, hypotensive, insecticidal, sympathomimetic and tuberculostatic activity as well as non-competitive inhibition of monoamineoxidase and spasmolytic activity in smooth muscle [9 - 36].

Mesoionic compounds of the 1,3,4-thiadiazolium-2-thiolate system (**III**) are readily prepared by the reaction of potassium β-aryldithiocarbazinate with carboxylic acid chlorides. Mesoionic compounds of the 1,3,4-triazolium-2-thiolate system (**V**) are conveniently prepared by two synthetic routes. The first involves the reaction of mesoionic 1,3,4-thiadiazolium-2-thiolates (**III**) with primary amines and the second involves the reaction of carboxylic acid chlorides with 1,4-diaryl-thiosemicarbazides (Scheme 1).

In the present work we describe the synthesis and characterisation of ten mesoionic compounds and tests of their activity against a range of microorganisms. Three of these are 1,3,4-thiadiazolium-2-thiolates (**III**) and seven are 1,3,4-triazolium-2-thiolate hydrochlorides (**IV**) (see Table 1).

MATERIALS AND METHODS

Mass spectra were obtained on a Finnigan GCQ Mat type quadrupole-ion trap spectrometer. IR spectra were obtained on a Bruker IFS66 spectrometer with the sample in a KBr disc. ^{1}H and ^{13}C NMR spectra were obtained on a VARIAN UNITY PLUS 300 MHz spectrometer - the samples being dissolved in DMSO-d_6 with TMS as reference. Elemental Analysis was carried out on a Perkin Elmer Elemental Microanalyser. The melt-



Scheme 1.

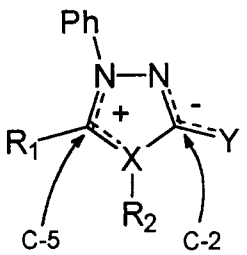
Structure	Mesoionic compound	X	Y	R^1	R^2
	MI.1	S	S	C_6H_5-	-
	MI.2	S	S	pCl-C_6H_4-	-
	MI.3	S	S	styryl	-
	MI.4	N	S/HCl	C_6H_5-	C_6H_5-
	MI.5	N	S/HCl	pCl-C_6H_4-	C_6H_5-
	MI.6	N	S/HCl	pCl-C_6H_4-	pCl-C_6H_4-
	MI.7	N	S/HCl	pCl-C_6H_4-	pF-C_6H_4-
	MI.8	N	S/HCl	styryl	C_6H_5-
	MI.9	N	S/HCl	Furanyl	C_6H_5-
	MI.10	N	S/HCl	2-NO_2-furanyl	C_6H_5-

Table 1. Chemical structure of the Mesoionic compounds studied.
MI.1 - 3 are 1,3,4-thiadiazolium-2-thiolates. MI.4 - 10 are 1,3,4-triazolium-2-thiolate hydrochlorides

ing points were determined on a Kofler hot-plate apparatus combined with a Carl-Zeiss microscope and are uncorrected.

Preparation of the mesoionic compounds

Mesoionic 1,3,4-thiadiazolium-2 - thiolates - (MI.1 - 3)

Potassium β-phenyldithiocarbazinate was dissolved in the minimum of water. An equimolar quantity of the appropriate aroyl chloride was then added in small amounts, with magnetic stirring. A viscous precipitate appeared at the bottom of the flask but stirring was continued overnight. The precipitate was then filtered off, washed with hot ethanol then recrystallized from acetonitrile.

Mesoionic 1,3,4-triazolium-2-thiolate hydrochlorides - (MI.4 - 10)

1,4-diphenylthiosemicarbazide was suspended in humid dioxane with magnetic stirring. An equimolar quantity of the appropriate aroyl chloride was then added in small amounts. The solution turned yellow and subsequently formed a yellow crystalline precipitate. However, the reaction mixture was stirred overnight, before the precipitate was filtered off under vacuum. It was washed sequentially with small portions of dioxane, ethanol and diethyl ether, then recrystallized from ethanol, adding a few drops of trifluoroacetic acid when necessary.

Characterisation of the products

4,5-diphenyl-1,3,4-thiadiazolium-2-thiolate - MI.1

Elemental analysis: calculated for $C_{14}H_{10}N_2S_2$; C,62.22; H,3.70; N,10.37 %; found: C,62.20; H,3.71; N,10.35 %; IR spectrum ν (cm^{-1}) 3042 (ν C_{Ar}-H), 1349 (ν NC-S^-, thiolate); Mass spectrum m/z (%) 270 - M^+ (28,13%); ^1H NMR spectrum δ (ppm) 7.60 (m -10 H, aromatic); ^{13}C NMR spectrum δ (ppm) 180.1 (C-2), 163.2 (C-5) and M.p. 228 °C.

4-phenyl-5-p-chlorophenyl-1,3,4-thiadiazolium-2-thiolate - MI.2

Elemental analysis: calculated for $C_{14}H_9ClN_2S_2$; C,55.17; H,2.95; N,9.19 %; found: C,55.10; H,2.95; N,9.17 %; IR spectrum ν cm^{-1}, 3047 (ν C_{Ar}-H), 1332 (ν NC-S^-); Mass spectrum m/z (%) M^+ 304 (16,24%); ^1H NMR spectrum δ (ppm) 7.22 - 7.50 (m - 9H, aromatic); ^{13}C NMR spectrum δ (ppm), 183.2 (C-2), 165.1 (C-5) and M.p. 234 °C.

4-phenyl-5-styryl-1,3,4-thiadiazolium-2-thiolate - MI.3

Elemental analysis: calculated for $C_{16}H_{12}N_2S_2$; C,64.77; H,4.05; N,9.45 %; found: C,64.74; H,4.05; N,9.42 %; IR spectrum ν (cm^{-1}) 3050 (νC_{Ar}-H), 1614 (ν HC_{Ar}=C_{Ar}H), 1345 (ν NC-S^-, thiolate), 1041 (ν C_{Ar}-H out-of-plane deformation); Mass spectrum m/z (%) 296 (M^+, 17.55), 238 (11.75), 206 (17.01), 147 (100), 115 (3.36) 103 (22.45), 77 (11.53); ^1H NMR spectrum δ (ppm) 6.47 (δ, ^1H, J 16.5 Hz), 6.58 (δ, 1H, J 16.5 Hz), 7.20 - 7.52 (m - 10 H, aromatic); ^{13}C NMR spectrum δ (ppm) and 183.1 (C-2), 163.6 (C-5) and M.p. 236 - 238 °C.

1,4,5-triphenyl-1,3,4-triazolium-2-thiol chloride - MI.4

Elemental analysis: calculated for $C_{20}H_{16}ClN_3S$; C,65.66; H,4.37; N,11.49%; found: C,65.64; H,4.37; N,11.46 %; IR spectrum ν (cm^{-1}) 3015 (ν C_{Ar}-H), 2760 (ν NC-SH, thiol); Mass spectrum m/z (%) 329 (32.4 $(M\text{-}HCl)^+$), 180 (32.56), 135 (100); ^1H NMR spectrum δ (ppm) 7.4 -7.6 (m - 15H, aromatic); ^{13}C NMR spectrum δ (ppm) 169.8 (C-2), 148.2 (C-5) and M.p 262 °C.

1,4-diphenyl-5-p-chlorophenyl-1,3,4-triazolium-2-thiol chloride - MI.5

Elemental analysis: calculated for $C_{20}H_{15}Cl_2N_3S$; C,60.00; H,3.22; N,9.66 %; found: C,60.01; H,3.21; N,9.60 %; IR spectrum ν (cm^{-1}) 3052 (ν C_{Ar}-H), 2761 (ν NC-SH, thiol); 980 (ν C-Cl); Mass spectrum m/z (%) 363 (100 $(M\text{-}HCl)^+$); ^1H NMR spectrum δ (ppm) 7.13 (δ - 2H, aromatic, J 6.5 Hz), 7.22 (d - 2H, aromatic, J 6.5 Hz), 7.32 - 7.53 (m - 10H, aromatic); ^{13}C NMR spectrum δ (ppm) 171.77 (C-2), 144.47 (C-5) and M.p. 269 - 270 °C.

1,5 -bis-p-chlorophenyl-4-phenyl-1,3,4-triazolium-2-thiol chloride - MI.6

Elemental analysis: calculated for $C_{20}H_{14}Cl_3N_3S$; C,55.23; H,3.22; N,9.60; found: C,55.20; H,3.22; N,9.59 %; IR spectrum ν (cm^{-1}) 3053 (ν C_{Ar}-H), 2712 (ν NC-SH, thiol), 991 (ν C-Cl); Mass spectrum m/z (%) 396 (100 $(M\text{-}HCl)^+$) 170 (20.5); ^1H NMR spectrum δ (ppm) 7.20 - 7.50 (m - 13H, aromatic); ^{13}C NMR spectrum δ (ppm) 170.0 (C-2), 144.2 (C-5) and M.p. 245 - 247 °C.

1-p-fluorphenyl-4-phenyl-5-p-chlorophenyl-1,3,4-triazolium-2-thiol chloride - MI.7

Elemental analysis: calculated for $C_{20}H_{14}Cl_2FN_3S$; C,62.74; H,3.60; N,10.98 %; found: C,62.72;

H,3.60; N,10.96 %; IR spectrum ν (cm^{-1}) 3051 (ν C_{Ar}-H), 2710 (ν NC-SH, thiol), 1016 (ν C-F), 930 (ν C-Cl); Mass spectrum m/z (%) 381(100 $(M-HCl)^{+\cdot}$), 198 (44.4), 170 (32.4); ^1H NMR spectrum δ (ppm) 7.3 - 7.8 (m - 13H, aromatic); 13C NMR spectrum d (ppm) 170.3 (C-2), 144.0 (C-5) and M.p. 265 - 267 °C.

1,4-diphenyl-5-styryl-1,3,4-triazolium-2-thiol chloride - MI.8

Elemental analysis: calculated for $C_{22}H_{18}ClN_3S$; C,67.43; H,4.59; N,10.72 %; found: C, 67.42; H,4.59; N,10.71 %; IR spectrum ν (cm^{-1}), 3019 (ν C_{Ar}-H), 2932 (ν C-H), 2877 (ν C-H), 2750 (ν NC-SH thiol), 1045 (ν CAr-H out-of-plane deformation); Mass spectrum m/z (%) 355 (77,24 $(M-HCl)^{+\cdot}$), 206 (21.4), 135 (34.6), 77 (100); ^1H NMR spectrum δ (ppm) 6.47 (δ, 1H, J 16,5 Hz), 6.58 (δ, ^1H, J 16,5 Hz) 7.10 - 7.60 (m, 15H, aromatic); ^{13}C NMR spectrum δ (ppm) 171.7 (C-2), 144.4 (C-5) and M.p. 284 - 286 °C.

1-p-chlorophenyl-4-phenyl-5-(2-furanyl)-1,3,4-triazolium-2-thiol chloride - MI.9

Elemental analysis: calculated for $C_{18}H_{13}Cl_2N_3$ OS; C,55.50; H,3.30; N,12.58 %; found: C,55.48; H,3.28; N,12.49 %; IR spectrum ν (cm^{-1}), 3150 and 3080 (ν C_{Ar}-H), 2748 (ν NC-SH thiol), 878 (ν C-Cl); Mass spectrum m/z (%), 353 (85.84 $(M-HCl)^{+\cdot}$); ^1H NMR spectrum d (ppm) 6.2 (d, 1H - furanyl), 6.5 (δ, ^1H - furanyl), 7,5 (m, 10H - aromatic); ^{13}C NMR spectrum δ (ppm) 168.3 (C-2), 146.8 (C-5) and M.p. 235 °C.

1,4-diphenyl-5-(4-nitro-2-furanyl)-1,3,4-triazolium-2-thiol chloride - MI.10

Elemental analysis: calculated for $C_{18}H_{13}ClN_4$ O_2S; C,54.00; H,3.25; N,14.00 %; found: C,54.03; H,3.22; N,14.02 %; IR spectrum ν (cm^{-1}), 3150 and 3045 (ν C_{Ar}-H), 2714 (ν NC-SH thiol) 1572 and 1361 (ν NO_2, asymmetric and symmetrical stretching); Mass spectrum m/z (%) 364 (6,14 $(M-HCl)^{+\cdot}$); ^1H NMR spectrum δ (ppm) 7.2 (d, ^1H - furanyl) 7.7 (m, 11H - aromatic); ^{13}C NMR spectrum δ (ppm) 167.8 (C-2), 146.5 (C-5) and M.p. 263 °C

Biological studies

Microorganisms representative of Gram-positive and Gram-negative bacteria, alcohol-acid-resistant bacteria, yeasts and filamentous fungi were selected from the collection of cultures of the Department of Antibiotics of the Federal University of Pernambuco. Specifically we used (a) *Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis,* Gram-positive bacteria; (b) *Escherischia coli* and *Pesudomonas aeruginosa,* Gram-negative bacteria; (c) *Mycobacterium smegmatis,* alcohol and-acid-resistant bacteria; (d) *Candida albicans,* yeast and (e) *Monilia sitophila,* filamentous fungus.

Preparation of the inoculum

The stock cultures were transplanted to appropriate culture media, incubated at temperatures of 35 °C for bacteria and 30 °C for yeasts and filamentous fungi and allowed to develop during 24-48 h, depending on the requirements of the microorganisms. From these cultures, suspensions were prepared in saline solution, were standardised by the 0,5 perturbation of the scale of McFarland.

Antimicrobial Activity tests

The levels of activity against microorganisms were measured by the diffusion in paper-discs [37], using Mueller-Hinton-agar (Difco), Sabouraud-agar, modified and glucose-extract of yeast-agar [38].

The samples were prepared by dissolution of the mesoionic compounds in DMSO in a concentration of 15 mg/ml. The paper discs with 6 mm diameter were moistened with 20 µl of solutions of the substances studied: each disc with a total of 300 µg of material. For each test of activity there was a parallel control test in which the disc was moistened with 20 µl of the solvent. The moistened discs were then placed on the surface in the middle of a Petri dish - the medium being sown with the microorganism being tested. Halos of inhibition around the paper discs were evaluated. A test was considered positive when the inhibition halo had a diameter ≥ 10 mm.

RESULTS AND DISCUSSION

Chemical Studies

The mesoionic compounds of the 1,3,4-thiadiazolium-2-thiolate series (MI.01 - 03) were synthesised by a standard method, *viz.* reaction of aroyl chlorides with potassium β-phenyldithiocarbazinate, The latter was prepared immediately before use owing to its instability. The mesoionic compounds of the 1,3,4-triazolium-2-thiolate series were prepared as their hydrochlorides (MI.04 - 10) by reactions of aroyl chlorides with 1,4-diphenyl-thiosemicarbazide in humid dioxane allowing reaction to proceed overnight. In these conditions the desired compounds, which are the

thermodynamic products [39] are obtained in the form of the hydrochlorides. The spectrometric data and Elemental Analyses are in complete accord with the proposed structures.

Activity Studies

The ten mesoionic compounds synthesised were tested for activity against microorganisms selected (see Table 2) and their effectiveness as potential therapeutic agents was estimated as follows. Paper-discs with 6 mm diameter with 300 µg of substance were placed on the surface of the culture medium. Inhibition halos (IH) were measured after an appropriate incubation time for each microorganisms. The 300 mg quantity was selected as being the maximum used in earlier studies [40, 41] *e.g.* with nitrofurantoin and sulfafurazole. Results were considered positive for inhibition halos ≥ 10 mm. Values of 10 - 15 mm correspond to low activity; 15 - 20 mm to moderate activity and ≥ 20 mm to high activity.

Gram-positive bacteria: *Staphylococcus aureus, Bacillus subtilis* and *Streptococcus faecalis.* Compound MI.8 was the most active against *Staphylococcus aureus* (IH, 25,5 mm). Compound MI.10 was the most active against *Bacillus subtilis* (IH, 20.0 mm) and *Streptococcus faecalis* (IH, 26.0 mm).

Alcohol-acid-resistant bacteria: *Mycobacterium smegmatis.* The most active compounds were MI.4 (IH, 23.5 mm); MI.5 (IH, 21.5 mm) and MI.10 (IH, 20.0 mm).

Yeasts: *Candida Albicans.* The most active compound was MI.10 (IH, 21.0 mm).

Gram-negative bacteria: *Escherichia coli* and *Filamentous fungi: Monilia sitophila.* The compounds were virtually inactive; only MI.10 had low activity, viz. IH, 11.0 mm for *Escherichia coli* and IH, 13.0 mm against *Monilia sitophila.*

It is noteworthy that in the conditions used the 1,3,4-thiadiazolium-2-thiolates are inactive. There are indications of activity when in more concentrated solution in DMSO (results not shown in the present work) .

The widest activity spectrum was presented by MI.10, *viz.* 1,4-diphenyl-5-(2-nitrofuranyl)-1,3,4-triazolium-2-thiol chloride.

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Samples	**Microorganisms**							
300 µg	***S.aureus***	***B.subt***	***S.faecalis***	***E. coli***	***P. aerug.***	***M. smeg***	***C.albicans***	***M. sitophila***
MI.1*	-	-	-	-	-	-	-	-
MI.2*	-	-	-	-	-	-	-	-
MI.3*	-	-	-	-	-	-	-	-
MI.4	16.5	15.0	12.0	-	-	23.5	13.5	-
MI.5	14.0	15.0	12.0	-	-	21.5	11.5	-
MI.6	-	11.0	-	-	-	14.0	13.5	-
MI.7	16.0	12.5	-	-	-	17.5	17.5	10.0
MI.8	25.5	-	-	-	-	21.5	16.5	-
MI.9	10.0	13.0	-	-	-	17.5	11.0	
MI.10	20.0	20.0	26.0	11.0	-	20.0	21.0	13.0
Control	-	-	-	-	-	-	-	-

* Inactive in the conditions used for the tests

Table 2. Activity of the mesoionic compounds against the microorganisms specified: data are diameters of the inhibition halos around the paper discs (in mm) Each value is the average of five tests.

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