MESOIONIC 5-ALKYL-1,3-DITHIOLIUM-4-THIOLATES: SYNTHESIS AND BRINE SHRIMP TOXICITY

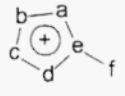
Paulo Afonso de Almeida, Tânia Maria Sarmento da Silva and Aurea Echevarria*
Departamento de Quimica, ICE, Universidade Federal Rural do Rio de Janeiro,
23851-970, Seropedica (RJ) Brazil

Abstract. A series of twelve 1,3-dithiolium-4-thiolate mesoionic compounds were synthesized and characterized. The synthetical approach starting from α-bromoalkancic acids to obtain the corresponding 2-N-morpholino-dithiocarbamoyl-carboxylic acids that by on-pot reaction with carbon disulfide and acetic anhydride in triethylamine formed not isolate intermediates, 1,3-dithiolium-4-olates. After, the 2-N-morpholino-5-alkyl-1,3-dithiolium-4-thiolates were obtained by retro 1,3-dipolar addition reactions. The alkyl moiety linked to C-5 of heterocyclic ring permitted the increase of the hydrophobic character and this effect was evaluated on *Artemia salina* lethality. The results indicated a bell-shaped relationship between the number of carbon of side chain in mesoionic derivatives and LD_{50} in brine shrimp toxicity assays.

Introduction

Mesoionic compounds have received much more attention and have been extensively studied because of their special structure, reaction behaviour, and wide-ranging biological activities (1-5). Recently, these compounds have been used in a broad range of new material applications including nonlinear optical effects (6), analytical reagents (7) (e. g. Nitron®), and photographic materials (8).

The mesoionic compounds can be considered as belonging to a class of mesomeric heterocyclic betaines (9). These betaines are planar five-member heterocycles with at least one side-chain whose α-atom is also in that plane and with dipole moments of 5 D's order. In addition, they also have eight π-electrons in association with both five atoms comprising the ring and the side chain α-atom, and cannot be represented satisfactorily by any one covalent or polar structure, these compounds are called mesoionic (6,10,11). The general formula **1** where a - f represent suitably substituted by carbons or heteroatoms.



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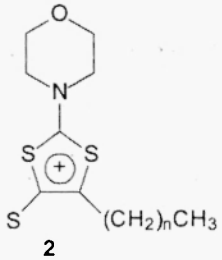


2

Previously we have studied several mesoionic systems such as 1,3,4-tiadiazolium-2-aminide (5,12), 1,3,4-triazolium-2-thiolate (13), and 1,2,3-oxadiazolium-2-olate (14,15), and these studies were very useful in structural elucidation and anti-tumour evaluation. In this work a special attention for 1,3-dithiolium-4-thiolate class (**2**), particularly our goal is to introduce a lipophylic moiety to afford a new mesoionic series with significant hydrophobic character with ceramide-like analogy. Ceramide are important metabolites, with polar and apolar moieties, related to cellular signalization mechanisms (16). Thus, in this paper we report the synthesis of twelve 2-N-morpholino-5-alkyl-1,3-dithiolium-4-thiolates (**2a – l**), where the alkyl group $(CH_2)_n$-CH_3 with n = 1 – 8, 10, 12, 14 and 16 to permit the increase of hydrophobic character. In order to verify the preliminary effects on lipophylic increment we used the brine shrimp (*Artemia salina* Leach) bioassay (17). This bioassay determines the lethality of compounds toward brine shrimp larvae and doing predicts about the ability to kill cancer cell culture, and also a wide range of other pharmacological effects (17,18).

Experimental

Melting points uncorrected were determined on Kofler hot stage. ^1H and ^{13}C NMR spectra were recorded on a Brucker AC-200 (200 MHz and 50.3 MHz, respectively). The ^1H and ^{13}C chemical shifts (δ) in $CDCl_3$ are given in ppm relative to tetramethylsilane as internal standard, and J values are given in Hz. Infrared spectra were recorded in KBr pellets, using a Perkin-Elmer 1420 spectrophotometer. U.V. spectra (nm) were taken on Hitachi U2000 spectrometer in methanol. MS spectra were obtained on a Shimadzu QP5050A. Yields are based on isolated products with sufficient purity.

General procedure for the preparation of α-bromo-alkanoic acids (**3a** – **l**): In a bottom of 150 mL attached at condenser with gas apparatus containing 65 mmol of alkanoic acid, 68 mmol of bromine and 22 mmol of phosphorus trichloride was heating at 80°C by seven hours and at 100°C by one hour more. When used alkanoic acids with 10 or more carbons atoms were putted additional 5.0 mL of water and at final reaction the products were extracted with chloroform. ^1H NMR identified all compounds.

*General procedure for the preparation of 2-N-morpholino-dithiocarbamoyl-alkanoic acids (**5a** – **l**)*: 25.1 mmol of Et_3N was added at 0°C with stirring to a solution of 23 mmol of morpholine in 10 mL of anhydrous benzene. After 10 minutes, 23.2 mmol of CS_2 was added to afford the intermediate **4** (not isolated), and was put on 22.9 mmol of α-bromo-alkanoic acids (**3a** – **l**) in 20 mL of anhydrous benzene. The mixture was maintained at room temperature and stirred for 16 hours. The solvent was removed under reduced pressure, washed in chloroform with three successive portions of 5N HCl, and distilled water until pH 5. The organic phase was drying over anhydrous sodium sulphate and removed under reduced pressure. The products were obtained as a clear brown wax and characterized by ^1H NMR, ^{13}C NMR and IR.

2-N-morpholino-dithiocarbamoyl-propanoic acid (**5a**): (75% yield), oil. ^1H NMR δ 4.77 (1H, q, J = 7.49 Hz, H-4), 4.40-3.80 (4H, m, CH_2N), 3.73 (4H, t, J = 4.66 Hz, CH_2O), 1.59 (d, J = 7.38 Hz, CH_3). ^{13}C NMR δ 195.01 (C-2), 176.12 (CO_2H), 65.96 (CH_2O), 50.92 (CH_2N), 48.22 (C-4), 16.66 (CH_3).

2-N-morpholino-dithiocarbamoyl-butanoic acid (**5b**): (90% yield), oil. ^1H NMR δ 4.77 (1H, q, J = 7.49 Hz, H-4), 4.40-3.90 (4H, m, CH_2N), 3.75 (4H, t, J = 4.71 Hz, CH_2O), 2.10-1.87 (2H, m, H-5), 1.05 (t, J = 7.36 Hz, CH_3). ^{13}C NMR δ 194.98 (C-2), 175.81 (CO_2H), 65.82 (CH_2O), 55.04 (C-4), 50.74 (CH_2N), 24.39 (C-5), 1 7.01 (CH_3).

2-N-morpholino-dithiocarbamoyl-pentanoic acid (**5c**): (85% yield), oil. ^1H NMR δ 4.77 (1H, q, J = 7.49 Hz, H-4), 4.40-3.85 (4H, m, CH_2N), 3.75 (4H, t, J = 4.44 Hz, CH_2O), 2.03-1.84 (2H, m, H-5), 1.48 (2H, m, H-6), 0.93 (t, J = 7.27 Hz, CH_3). ^{13}C NMR δ 195.43 (C-2), 176.00 (CO_2H), 66.05 (CH_2O), 53.47 (C-4), 51.01 (CH_2N), 32.94 (C-5), 20.38 (C-6), 13.95 (CH_3).

2-N-morpholino-dithiocarbamoyl-hexanoic acid (**5d**): (93% yield), oil. ^1H NMR δ 7.51 (1H, sl, OH), 4.73 (1H, q, J = 7.09 Hz, H-4), 4.40-3.85 (4H, m, CH_2N), 3.74 (4H, t, J = 4.65 Hz, CH_2O), 2.03-1.85 (2H, m, H-5), 1.42-1.26 (4H, m, H-6 and H-7), 0.87 (t, J = 7.00 Hz, CH_3). ^{13}C NMR δ 195.28 (C-2), 175.85 (CO_2H), 65.96 (CH_2O), 53.74 (C-4), 50.89 (CH_2N), 30.69 (C-5), 29.09 (C-6), 22.12 (C-7), 13.62 (CH_3).

2-N-morpholino-dithiocarbamoyl-heptanoic acid (**5e**): (77% yield), oil. ^1H NMR δ 7.64 (1H, sl, OH), 4.74 (1H, q, J = 7.14 Hz, H-4), 4.40 - 3.90 (4H, m, CH_2N), 3.74 (4H, t, J = 4.82 Hz, CH_2O), 2.03-1.85 (2H, m, H-5), 1.30 (2H, s, H-6), 1 27 (4H, s, H-7 to H-8), 0.85 (t, J = 7.00 Hz, CH_3). ^{13}C NMR δ 195.01 (C-2), 175.57 (CO_2H), 65.75 (CH_2O), 53.71 (C-4), 50.62 (CH_2N), 30.94 (C-5 and C-7), 26.45 (C-6), 21.93 (C-8), 13.56 (CH_3).

2-N-morpholino-dithiocarbamoyl-octanoic acid (**5f**): (97% yield), oil. ^1H NMR δ 7.76 (1H, sl, OH), 4.74 (1H, t, J = 7.14 Hz, H-4), 4.40-3.85 (4H, m, CH_2N), 3.77 (4H, t, J = 3.95 Hz, CH_2O), 2.08-1.82 (2H, m, H-5), 1.45-1 25 (8H, m, H-6 to H-9), 0.84 (t, J = 5.72 Hz, CH_3). ^{13}C NMR δ 195.25 (C-2), 176.36 (CO_2H), 65.99 (CH_2O), 53.83 (C-4), 50.81 (CH_2N), 31.30 (C-8), 30.97 (C-5) 28.81 (C-7), 26.98 (C-6), 22.36 (C-9), 13.90 (CH_3).

2-N-morpholino-dithiocarbamoyl-nonanoic acid (**5g**): (86% yield), oil. ^1H NMR δ 7.92 (1H, sl, OH), 4.75 (1H, t, J = 6.68 Hz, H-4), 4.40-3.85 (4H, m, CH_2N), 3.76 (4H, s, CH_2O), 2.04-1.86 (2H, m, H-5), 1.43-1 24 (10H, m, H-6 to H-10), 0.85 (t, J = 6.56 Hz, CH_3). ^{13}C NMR δ 195.28 (C-2), 176.27 (CO_2H), 66.02 (CH_2O), 53.83 (C-4), 50.95 (CH_2N), 31.51 (C-9), 30.94 (C-5), 28.97 (C-8), 28.78 (C-7), 27.03 (C-6), 22.42 (C-10), 13.93 (CH_3).

2-N-morpholino-dithiocarbamoyl-decanoic acid (**5h**): (100% yield), oil. ^{1}H NMR δ 8.44 (1H, s, OH), 4.73 (1H, t, *J* = 7.05 Hz, H-4), 4.40-3.85 (4H, m, CH_2N), 3.73 (4H, t, *J* = 3.88 Hz, CH_2O), 2.01-1.77 (2H, m, H-5), 1.40-1.21 (12H, m, H-6 to H-11), 0.83 (t, *J* = 5.86 Hz, CH_3). ^{13}C NMR δ 195.13 (C-2), 175.80 (CO_2H), 65.99 (CH_2O), 53.83 (C-4), 50.92 (CH_2N), 31.60 (C-10), 30.97 (C-5), 29.06 (C-7, C-8, and C-9), 27.00 (C-6), 22.45 (C-11), 13.93 (CH_3).

2-N-morpholino-dithiocarbamoyl-dodecanoic acid (**5i**): (93% yield), oil. ^{1}H NMR δ 7.10 (1H, sl, OH), 4.74 (1H, t, *J* = 7.14 Hz, H-4), 4.40-3.85 (4H, m, CH_2N), 3.76 (4H, t, *J* = 4.74 Hz, CH_2O), 2.05-1.78 (2H, m, H-5), 1.43-1.20 (16H, m, H-6 to H-13), 0.87 (t, *J* = 7.00 Hz, CH_3). ^{13}C NMR δ 195.49 (C-2), 175.63 (CO_2H), 66.05 (CH_2O), 53.80 (C-4), 51.04 (CH_2N), 31.30 (C-12), 30.94 (C-5), 29.18 - 30.94 (C-7 to C-11), 27.09 (C-6), 22.54 (C-13), 13.99 (CH_3).

2-N-morpholino-dithiocarbamoyl-tetradecanoic acid (**5j**): (98% yield), oil. ^{1}H NMR δ 6.30 (1H, sl, OH), 4.74 (1H, t, *J* = 7.15 Hz, H-4), 4.40-3.85 (4H, m, CH_2N), 3.76 (4H, t, *J* = 4.70 Hz, CH_2O), 2.06-1.79 (2H, m, H-5), 1.43-1.30 (20H, m, H-6 to H-15), 0.85 (t, *J* = 6.45 Hz, CH_3). ^{13}C NMR δ 195.28 (C-2), 175.99 (CO_2H), 66.02 (CH_2O), 53.86 (C-4), 50.92 (CH_2N), 31.73 (C-14), 31.00 (C-5), 29.18 (C-7 to C-13), 27.06 (C-6), 22.51 (C-15), 13.96 (CH_3).

2-N-morpholino-dithiocarbamoyl-hexadecanoic acid (**5k**): (66% yield), oil. ^{1}H NMR δ 6.10 (1H, sl, OH). 4.73 (1H, t, *J* = 7.16 Hz, H-4), 4.40-3.85 (4H, m, CH_2N), 3.76 (4H, t, *J* = 4.10 Hz, CH_2O), 2.06 - 1.79 (2H, m, H-5), 1.45-1.20 (24H, m, H-6 to H-17), 0.85 (t, *J* = 6.37 Hz, CH_3). ^{13}C NMR δ 195.52 (C-2), 174.84 (CO_2H), 66.05 (CH_2O), 53.74 (C-4), 51.25 (CH_2N), 31.79 (C-16), 30.94 (C-5), 29.21 (C-7 to C-15), 27.09 (C-6), 22.54 (C-17), (13.99 CH_3).
2-N-morpholino-dithiocarbamoyl-octadecanoic acid (**5l**): (95% yield), oil. ^{1}H NMR δ 4.73 (1H, t, *J* = 7.13 Hz, H-4), 4.40-3.85 (4H, m, CH_2N), 3.76 (4H, t, *J* = 4.45 Hz, CH_2O), 1.76-2.10 (2H, m, H-5), 1.22-1.44 (28H, m, H-6 to H-19), 0.85 (t, *J* = 6.37 Hz, CH_3). ^{13}C NMR δ 195.52 (C-2), 174.96 (CO_2H), 66.05 (CH_2O), 53.77 (C-4), 51.25 (CH_2N), 31.79 (C-18), 31.00 (C-5), 29.24 (C-7 to C-17), 27.09 (C-6), 22.57 (C-19), 13.99 (CH_3).

General procedure for the preparation of 2-N-morpholino-5-alkyl-1,3-dithiolium-4-thiolates (**2a – l**): 12.8 mmol of (**5a – l**) was dissolved in 60 mL of anhydrous benzene at 0°C and was added 215 mmol of CS_2 and 43 mmol of anhydrous Et_3N at same temperature. Then was added 132 mmol of acetic anhydride, when the reaction mixture became carmine-red, and allowed to attain ambient temperature with stirring for 20 hours. The products (**2a – l**), obtained as a yellow precipitate, were filtered off and purified by recrystallization from acetone.

2-N-morpholino-5-methyl-1,3-dithiolium-4-thiolate (**2a**): 85% yield; M.p. 194–196°C. IR 1538 (C=S$^+$ and C=C), 1478 (C=C), 2908, 2870, 1255, 1109, 1033, 883 cm^{-1}. UV λ_{max} (log ε) 407 (4.27), 273 (4.88), and 205 (4.61). MS m/z (%) 233 (M$^+$, 70), 86 (100), 130 (90). ^{1}H NMR δ 3.88 (4H, t, *J* = 5.3 Hz, CH_2O), 3.56 (4H, t, *J* = 5.2 Hz, CH_2N), 2.26 (3H, s, CH_3), 1.56 (H, s, SH).
2-N-morpholino-5-ethyl-1,3-dithiolium-4-thiolate (**2b**): 80% yield; M.p. 191–193°C . IR 1546 (C=S$^+$ and C=C), 1483 (C=C), 2926, 2865, 1431, 1255, 1109, 1066, 883 cm^{-1}. UV λ_{max} (log ε) 407 (4.05), 273 (4.66), and 205 (4.66). MS *m/z* (%) 247 (M$^+$, 65), 130 (100), 86 (95), 60 (20), 73 (12), 44 (28). ^{1}H NMR δ 3.88 (4H, t, *J* = 4.74 Hz, CH_2O), 3.61 (4H, t, *J* = 3.68 Hz, CH_2N), 2.75 (2H, q, *J* = 7.46 Hz, H-12), 1.69 (H, s, SH); 1.18 (3H, t, *J* = 7.40 Hz, CH_3). ^{13}C NMR δ 183.06 (C-2), 117.56 (C-4), 64.58 (CH_2O), 52.03 (CH_2N), 21.68 (C-13), 13.87 (CH_3).

2-N-morpholino-5-propyl-1,3-dithiolium-4-thiolate (**2c**): 85% yield; M.p. 167–169°C. IR 1535 (C=S$^+$ and C=C), 1473 (C=C), 2923, 2869, 1246, 1107, 1033, 887 cm^{-1}. UV λ_{max} (log ε) 409 (4.17), 275 (4.83) and 210 (4.61). MS m/z 261 (M$^+$, 50), 130 (100), 86 (90). ^{1}H NMR δ 3.88 (4H, t, *J* = 5.01 Hz, CH_2O), 3.60 (4 H, t, *J* = 5.12 Hz, CH_2N), 2.68 (2H, t, *J* = 7.68 Hz, H-13), 1.69 (H, s, SH); 1.58 (2H, m, *J* = 7.34 Hz, H-14), 0.96 (3H, t, J = 7.23 Hz, CH_3). ^{13}C NMR δ 184.51 (C-2), 150.88 (C-5), 119.51 (C-4), 64.92 (CH_2O), 51.71 (CH_2N), 30.64 (C-13), 22.71 (C-14), 13.62 (CH_3).

2-N-morpholino-5-butyl-1,3-dithiolium-4-thiolate (**2d**): 65% yield; M.p. 154–156°C. IR 1548 (C=S$^+$ and C=C), 1473 (C=C), 2928, 2862,1249,1105,1023, 882 cm^{-1}. UV λ_{max} (log ε) 407 (4.27), 273 (4.88) and 205 (4.61). MS m/z 275 (M$^+$, 40), 130 (100), 86 (90), 76 (30). ^{1}H NMR δ 3.88 (4H, t, *J* = 5.00 Hz, CH_2O), 3.61 (4H, t, *J* = 3.70 Hz, CH_2N), 2.72 (2H, t, *J* = 7.55 Hz, H-13), 1.65 (H, s, SH); 1.57 (2H, dd, *J* = 14.26, 7.27 Hz, H-14), 1.44 (2H,dd, *J* = 14.26, 7.13 Hz, H-15), 0.90 (3H, t, *J* = 7.13 Hz, CH_3). ^{13}C NMR δ 184.42 (C-2), 150.50 (C-5), 119.67 (C-4), 64.92 (CH_2O), 51.72 (CH_2N), 30.64 (C-13), 28.44, (C-14), 22.12 (C-15), 13.70 (CH_3).

2-N-morpholino-5-pentyl-1,3-dithiolium-4-thiolate (**2e**): 60% yield; M.p. 147–149°C. IR 1558 (C=S$^+$ and C=C), 1473 (C=C), 2926, 2859, 1253, 1111, 1028, 885 cm^{-1}. UV λ_{max} (log ε) 407 (4.27), 273 (4.88) and 205 (4.61). MS *m/z* (%) 289 (M$^+$, 31), 246 (10), 130 (100), 86(80), 76 (60), 57 (20), 44(21). ^{1}H NMR δ 3.87 (4H, t, *J* = 5.11 Hz, CH_2O), 3.58 (4 H, t, *J* = 5.11 Hz, CH_2N), 2.69 (2H, t, *J* = 7.75 Hz, H-13), 1.69 (H, s, SH), 1.56 (2H, t, *J* = 7.27 Hz, H-14), 1.30 (4H, t, *J* = 3.63 Hz, H-15 and H-16), 0.88 (3H, t, *J* = 6.94 Hz, CH_3). ^{13}C NMR δ 183.83 (C-2), 150.08 (C-5), 119.03 (C-4), 64.26 (CH_2O), 51.07 (CH_2N), 30.51 (C-13), 28.47 (C-14), 28.08 (C-15), 21.62 (C-16), 13.16 (CH_3).

2-N-morpholino-5-hexyl-1,3-dithiolium-4-thiolate (**2f**): 51% yield; M.p. 151–153°C. IR 1550 (C=S$^+$ and C=C), 1483 (C=C), 2929, 2861, 1257, 1114, 1030, 884 (CH_2) cm^{-1}. UV λ_{max} (log ε) 408 (4.00), 273 (4.60) and 209 (4.38). MS m/z 289 (M$^+$,

60), 130 (100), 86 (90), 233 (20). ^{1}H NMR 3.88 (4H, t, *J* = 4.96 Hz, CH_2O), 3.59 (4 H, t, *J* = 5.08 Hz, CH_2N), 2.72 (2H, t, *J* = 7.68 Hz, H-13), 1.65 (H, s, SH), 1.57(2H, s, H-14), 1.40 (6H, s, H-15 to H-17), 0.85 (3H, s, CH_3). ^{13}C NMR δ 184.43 (C-2), 150.44 (C-5), 119.74 (C-4), 64.58 (CH_2O), 52.03 (CH_2N), 31.31 (C-13), 29.36 (C-14), 28.72 (C-15,16), 22.26 (C-17), 13.81 (CH_3).

2-N-morpholino-5-heptyl-1,3-dithiolium-4-thiolate (**2g**): 45% yield; M.p. 151–153°C. IR 1547 (C=S$^+$ and C=C), 1481 (C=C), 2926, 2859, 1261, 1114, 1030, 886 cm^{-1}. UV λ_{max} (log ε) 407 (3.42), 272 (4.11) and 214 (3.82). MS m/z 319 (M$^+$, 20), 130 (100), 86 (90). ^{1}H NMR δ 3.88 (4H, t, *J* = 5.00 Hz, CH_2O), 3.60 (4H, t, *J* = 4.93 Hz, CH_2N), 2.71 (2H, t, *J* = 7.60 Hz, H-13), 1.59 (H, s, SH), 1.56(2H, t, *J* = 6.95 Hz, H-14), 1.24 (8H, sl, H-15 to H-18), 0.85 (3H, t, *J* = 5.88 Hz, CH_3). ^{13}C NMR δ 184.39 (C-2), 150.22 (C-5), 119.75 (C-4), 64.81 (CH_2O), 51.65 (CH_2N), 31.42 (C-13), 29.38 (C-14), 28.93 (C-15), 28.78 (C-16), 28.70 (C-17), 22.30 (C-18), 13.81 (CH_3).

2-N-morpholino-5-octyl-1,3-dithiolium-4-thiolate (**2h**): 62% yield; M.p. 151–153°C. IR 1546 (C=S$^+$ and C=C), 1476 (C=C), 2925, 2853, 1255, 1115, 1030, 884 cm^{-1}. UV λ_{max} (log ε) 409 (4.05), 274 (4.63) and 210 (4.43). MS *m/z* (%) 331 (M$^+$, 17), 233 (10), 130 (55), 97 (10), 86 (40), 76 (30), 57 (28), 44 (100). ^{1}H NMR δ 3.90 (4H, t, *J* = 5.00 Hz, CH_2O), 3.59 (4H, t, *J* = 5.00 Hz, CH_2N), 2.71 (2H, t, *J* = 7.55 Hz, H-13), 1.66 (H, s, SH), 1.57 (2H, t, *J* = 7.22 Hz, H-14), 1.24 (10H, sl, H-15 to H-19), 0.84 (3H, t, *J* = 6.28 Hz, CH_3). ^{13}C NMR δ 184.28 (C-2), 150.13 (C-5), 119.60 (C-4), 64.77 (CH_2O), 51.63 (CH_2N), 31.46 (C-13), 29.33 (C-14), 29.03 (C-15), 28.94 (C-16), 28.85 (C-17), 28.66 (C-18), 22.27 (C-19), 13.77 (CH_3).

2-N-morpholino-5-decyl-1,3-dithiolium-4-thiolate (**2i**): 39% yield; M.p. 147–149°C. IR 1558 (C=S$^+$ and C=C), 1483 (C=C), 2922, 2854, 1252, 1113, 1030, 885 cm^{-1}. UV λ_{max} (log ε) 408 (3.49), 273 (4.11) and 230 (3.74). MS *m/z* (%) 359 (M$^+$, 20), 233 (15), 130 (55), 86 (40), 76 (30), 44 (100). ^{1}H NMR δ 3.88 (4H, t, *J* = 5.03 Hz, CH_2O), 3.59 (4 H, t, *J* = 5.02 Hz, CH_2N), 2.72 (2H, t, *J* = 7.54 Hz, H-13), 1.62 (H, s, SH), 1.59 (2H, t, *J* = 7.22 Hz, H-14), 1.23 (12H, sl, H-15 to H-20), 0.84 (3H, t, *J* = 6.28 Hz, CH_3). ^{13}C NMR δ 184.43 (C-2), 150.11 (C-5), 119.85 (C-4), 64.87 (CH_2O), 51.73 (CH_2N), 31.59 (C-13), 29.42-28.75 (C-14 to C-19), 22.38 (C-20), 13.87 (CH_3).

2-N-morpholino-5-dodecyl-1,3-dithiolium-4-thiolate (**2j**): 35% yield; M.p. 139–141°C. IR, 1539 (C=S$^+$ and C=C), 1470, 2919, 28501252, 1116, 1035, 872 cm. UV λ_{max} (log ε) 407 (3.98), 275 (4.52) and 213 (4.34). MS m/z 387 (M$^+$, 10), 312 (50), 130 (30), 86 (40), 76 (100). ^{1}H NMR δ 3.88 (4H, t, *J* = 5.00 Hz, CH_2O), 3.60 (4 H, t, *J* = 5.02 Hz, CH_2N), 2.69 (2H, t, *J* = 7.68 Hz, H-13), 1.55 (2H, sl, H-14), 1.22 (18H, sl, H-15 to H-22), 0.84 (3H, t, *J* = 6.28 Hz, CH_3). ^{13}C NMR δ 184.44 (C-2), 150.67 (C-5), 119.65 (C-4), 64.17 (CH_2O), 51.72 (CH_2N), 31.67 (C-13), 29.42-28.82 (C-14 to C-21), 22.44 (C-22), 13.90 (CH_3).

2-N-morpholino-5-tetradecyl-1,3-dithiolium-4-thiolate (**2k**): 62% yield; M.p.147-149°C. IR, 1554 (C=S$^+$ and C=C), 1480 (C=C), 2921, 28521254, 1115, 1033, 885 cm^1. UV λ_{max} (log ε) 407 (4.16), 271 (4.71) and 212 (4.41). MS m/z 415 (M+, 10), 382 (10), 233 (10), 130 (100), 86 (80). ^{1}H NMR δ 3.88 (4H, t, *J* = 5.06 Hz, CH_2O), 3.57 (4H, t, *J* = 5.06 Hz, CH_2N), 2.73 (2H, t, *J* = 7.73 Hz, H-13), 1.57 (2H, s, H-14), 1.22 (22H, s, H-15 to H-24), 0.84 (3H, t, *J* = 6.28 Hz, CH_3). ^{13}C NMR δ 184.58 (C-2), 150.65 (C-5), 119.85 (C-4), 65.01 (CH_2O), 51.83 (CH_2N), 31.78 (C-13), 29.54-28.92 (C-14 to C-23), 22.55 (C-22), 14.00 (CH_3).

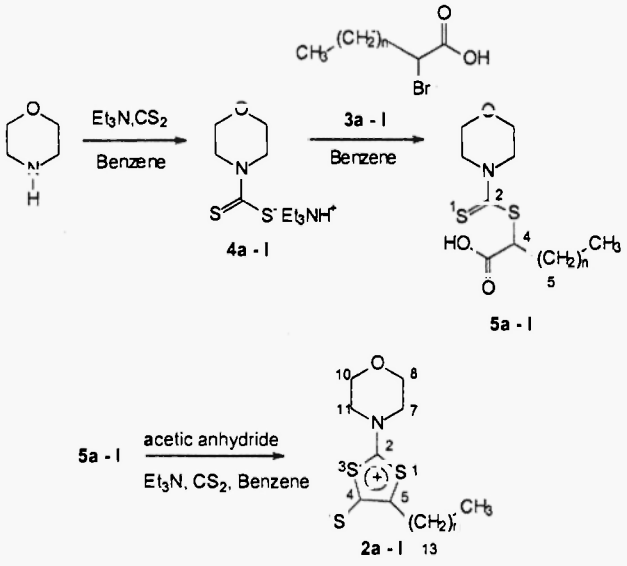
2-N-morpholino-5-hexadecyl-1,3-dithiolium-4-thiolate (**2l**): 28% yield; M.p. 143-145°C. IR 1553 (C=S$^+$ and C=C), 1479 (C=C), 2922, 2853, 1255, 1114, 1032, 884 cm^{-1}. UV λ_{max} (log ε) 407 (4.75), 275 (4.70), 207 (4.49). MS *m/z* (%) 443 (M$^+$, 5), 247 (10), 130 (30), 86 (42), 76 (55), 44(100). ^{1}H NMR δ 3.88 (4H, t, *J* = 5.00 Hz, CH_2O), 3.58 (4H, t, *J* = 4.76 Hz, CH_2N), 2.72 (2H, t, *J* = 7.63 Hz, H-13), 1.63 (H, s, SH), 1.59 (2H, s, H-14), 1.22 (26H, s, H-15 to H-26), 0.85 (3H, t, *J* = 6.50 Hz, CH_3). ^{13}C NMR δ 184.58 (C-2), 150.65 (C-5), 119.87 (C-4), 64.97 (CH_2O), 51.76 (CH_2N), 31.74 (C-13), 29.52-28.87 (C-14 to C-25), 22.51 (C-26), 13.96 (CH_3).

Biological assay

The brine shrimp lethality bioassay was performed following the reported procedure (17) with some modifications. The growth medium was prepared with a water solution 38g/L of sea salt and in small divided tank adds shrimp eggs to one cover side. Lamp above other side was putted to attract hatched shrimp through perforations in the dam. After 48h the shrimp are mature as nauplii and prepared to assay. Test compounds were dissolved in three drops of Cremophor®, 2mL DMSO and saline solution to complete 5 ml of total volume. After, appropriate volumes were added to tubes with 5 mL of saline solution containing 10 nauplii to afford final drug concentration of 1000, 100, 10 and 1 μg/mL, in quadruplicate for each concentration. The control samples containing Cremophor® and DMSO, in same conditions, do not cause significant brine shrimp mortality. After 24 h of incubation under light, the number of dead and survivors brine shrimp in each tube was counted. The LD_{50} were calculated by graphics from drug concentration vs. lethality percentage using a Probit scale adjust. Data analysis was performed with Origin 6.0 software (Microcal Software).

Results and Discussion

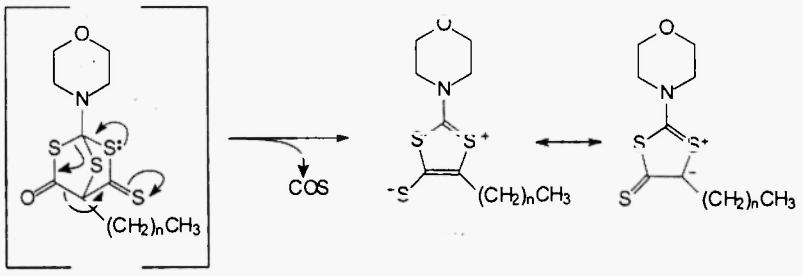
The 2-N-morpholino-5-alkyl-1,3-dithiolium-4-thiolates (**2a – l**) were prepared by general procedure shown in Scheme 1. To start, the α-bromo-alkanoic acids (**3a –l**) were prepared by traditional procedure (19) with bromine and phosphorous trichloride. When the fatty acids containing 10 or more carbon atoms were used water addition at the final of reaction to avoid the intermediate α-bromoalkylacetyl bromide, reinforcing the Hell-Volhard-Zeilinski reaction, afforded the α-bromo-alkanoic acids in quantitative yields. The 2-N-morpholino-dithiocarbamoyl-carboxilic acids (**5a – l**) were obtained in one-pot procedure. The triethylammonium-2-N-morpholino-dithiocarbamate (**4**) was initially formed by nucleophilic addition of morpholine to carbon disulfide in the presence of triethylamine in benzene. After 10 minutes the appropriate α-bromo-alkanoic acids in benzene were added at 25°C temperature for 16 hours and by substitution nucleophilic reaction afforded the corresponding **5a – l**, obtained in 66 to 100% yield as a clear brown wax.



n = 0 (**2a**), 1 (**2b**), 2 (**2c**), 3 (**2d**), 4 (**2e**), 5 (**2f**), 6 (**2g**), 7 (**2h**), 9 (**2i**), 11 (**2j**), 13 (**2k**) and 15 (**2l**)

Scheme 1

The mesoionic compounds (**2a – l**) were synthesized according to the modified procedure previously described (20) to the analogues with different and single side chain. The intermediates 2-N-morpholino-dithiocarbamoyl-carboxilic acids in anhydrous benzene, triethylamine and carbon disulfide, were added in acetic anhydride at 0°C. When the reactions started, a change of yellow to carmine-red colour was observed, and after 20h stirring the target products were obtained as yellow solids. The **2a – l** were purified from acetone afforded orange crystals with yields into 45 to 85%. In the synthesis of **2i – l** (with exception of **2k** 62%), the increase of length of side-chain linked at C-5 to heterocyclic ring, caused a high hindered in cyclic intermediate (not isolated) affording the target mesoionic compounds by retro-1,3-dipolar addition reaction, eliminating carbon oxysulfide, with lower yield, 39 to 28% (Scheme 2).



Scheme 2

The structures proposed are consistent with the chemical shifts assignments in 1D ^{1}H and ^{13}C NMR (HBBD and PEDANT) and 2D $^{1}H - ^{13}C$ HMBC (^{1}H x ^{13}C – COSY, $^{2,3}J$ (CH)). The chemical shifts of carbon atoms of heterocyclic ring in **2a – l**, presented C-2 and C-5 at high frequencies when compared with values of other carbon atoms (see Table I).

Table I. Effects of 1,3-dithiolium-4-thiolate derivatives on survival of brine shrimp nauplii.

Mesoionic Compound	n[a]	LD_{50} [b] (μM)
2a	1	870
2b	2	372
2c	3	479
2d	4	182
2e	5	309
2f	6	195
2g	7	105
2h	8	12
2i	10	112
2j	12	123
2k	14	195
2l	16	513

[a] Number of carbon atoms in side chain attached at C-5 of heterocyclic ring.

[b] Compound concentration required to kill brine shrimp by 50%. All assays were performed in quadruplicate.

We found δ 183.06 – 184.58 for C-2 and δ 150.08 – 150.88 for C-5. Thus, for the **2h** derivative, for example, the spin-spin interactions of H-7 (δ 3.59) with C-2 (δ 184.28, ^{3}J (CH)) and C-8 (δ 64.77, ^{2}J (CH)); and of H-13 (δ 2.71) with C-5 (δ 150.13, ^{2}J (CH)) and C-4 (δ 119.60, ^{3}J (CH)), were observed in the HMBC spectrum. The chemical shifts of C-4 atoms showing lower frequencies δ 119.03 – 119.87 due to the greater electron-donating effect caused by the sulphur atom negatively charged directly attached. The other carbon atoms showed the expected chemical shifts.

The brine shrimp nauplii have been used previously in a number of bioassay systems (18) and the compounds are tested at initial concentrations of 10, 100 and 1000 ppm (or μg/ml) in vials containing 5 ml of brine and ten shrimps in each of five replicates. Survivors are counted after 24h. These data are processed to estimate LD_{50} values with 95%

confidence intervals for statistically significant comparisons of potencies. The literature report a positive correlation between brine shrimp toxicity and 9KB cells cytotoxicity and has been observed that ED_{50} values for general cytotoxicities are about one-tenth LD_{50} values in the brine shrimp test (21).

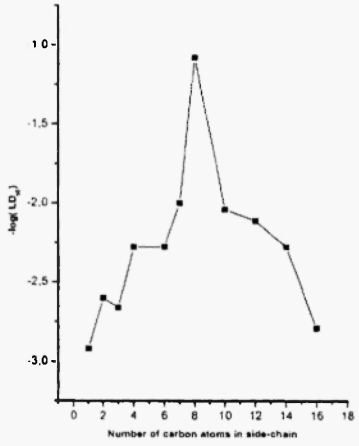
The Table II show the results obtained in brine shrimp assay. In order to verify a possible relationship between the chemical structure of 1,3-dithiolium derivatives and the biological response, was plotted the –log LD_{50} values against the number carbon atoms of side chain attached on mesoionic ring. The continuous growth of an alkyl chain or of methylene units increases the hydrophobic part of the molecule.

Table II. NMR-chemical shifts for main carbon atoms of mesoionic derivatives.[a]

	C-2	C-4	C-5		C-2	C-4	C-5
2a	n.o.[b]	n.o.[b]	n.o.[b]	**2g**	184.39	119.75	150.22
2b	183.06	117.56	n.o.[B]	**2h**	184.28	119.60	150.13
2c	184.51	119.51	150.88	**2i**	184.43	119.85	150.11
2d	184.42	119.67	150.50	**2j**	184.44	119.65	150.67
2e	183.83	119.03	150.08	**2k**	184.58	119.85	150.65
2f	184.43	119.74	150.44	**2l**	184.58	119.87	150.67

[a] δ (ppm) in $CDCl_3$ solutions at 50.3 MHz. [b] n.o. = no observed peak

The Graphic 1 indicates a bell shaped curve with serrated variations (zigzag) on left side, where the **2h** (n = 8) has been the most active derivative. Zigzag variations are well known in homologous series for physical properties such as melting points and solubilities. Thus, sometimes is observed alternating variations of biological activities according to whether the number of carbon atoms is even or odd (22-24). Furthermore, the curves with an activity maximum are the most common, and it is presumed that they reflect the existence of an optimal partition coefficient associated with the easiest crossing of biological membranes. Bell-shaped curves are also seen when using isolated cells, for which it can be demonstrated that the receptor is in outside the membrane (25). Another possibility is a lipophylic pocket chains making up part of the bilayer, examples being leukotriene D_4-agonist/antagonists (26). The study of the activities of some homologous compounds can identify which term is associated with the highest potency, using an easy bioassay.



Graphic 1. Effect of carbon chain length on brine shrimp lethality.

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