Synthesis and microbiological activity of some 2*H*-1,4-Benzothiazin-3-one derivatives

V. L. de M. Guarda[1,3]; M. Perrissin[1]; F. Thomasson[1]; E. A. Ximenes[2]; S. L. Galdino[2]; I. R. Pitta[2] and C. Luu-Duc[1]

[1] Laboratoire de Chimie Pharmacie, CNRS UPRES-A 5077
Universite Joseph-Fourier, Grenoble I, F 38706 La Tronche Cedex

[2] Departamento de Antibioticos
Universidade Federal de Pernambuco, 50.670-901 Recife/PE (Brasil)

[3] Faculdade de Farmacia
Universidade Federal de Ouro Preto, 35.400-000 Ouro Preto/MG (Brasil)

Abstract
Synthesis, physical and analytical properties of 6-alkylacylamino-4-methyl-2*H*-1,4-benzothiazin-3-one derivatives are described. These new compounds were prepared by acylation and/or alkylation of the amino group by phase transfer catalysis. Acid hydrolysis of alkylacylamino-2*H*-1,4-benzothiazin-3-ones afforded N-alkylamino-benzothiazin-3-ones. Some of them were evaluated *in vitro* for bacteriostatic activity.

Introduction

Chemical and pharmacological properties of phenothiazines and 1,4-benzothiazines have been widely studied. They are reported in the GUPTA and OJHA's review (1). Some benzothiazines exhibited antimicrobial and anticancer activity (2-4).
The 6-alkylacylamino-4-methyl-2*H*-1,4-benzothiazin-3-one derivatives described in this paper are synthesized from 2-chloro-5-nitro-aniline. The action of sodium sulfide and sulfur on this compound led to 2-amino-4-nitrobenzenethiol (sodium salt) which is cyclized to 6-nitro-2*H*-1,4-benzothiazin-3-one with chloracetic acid. The N-alkylation of the 4-position followed by the reduction of the nitro group led to 6-amino-4-methylbenzothiazin-3-ones. The 6-NH_2 is acylated then alkylated. Sulfuric acid hydrolysis provided 6-alkylamino-4-methyl-2*H*-1,4-benzothiazin-3-one derivatives.
Five compounds were tested for bacteriostatic activity against a lot of microorganisms including cocci, gram positive and gram negative bacteria.

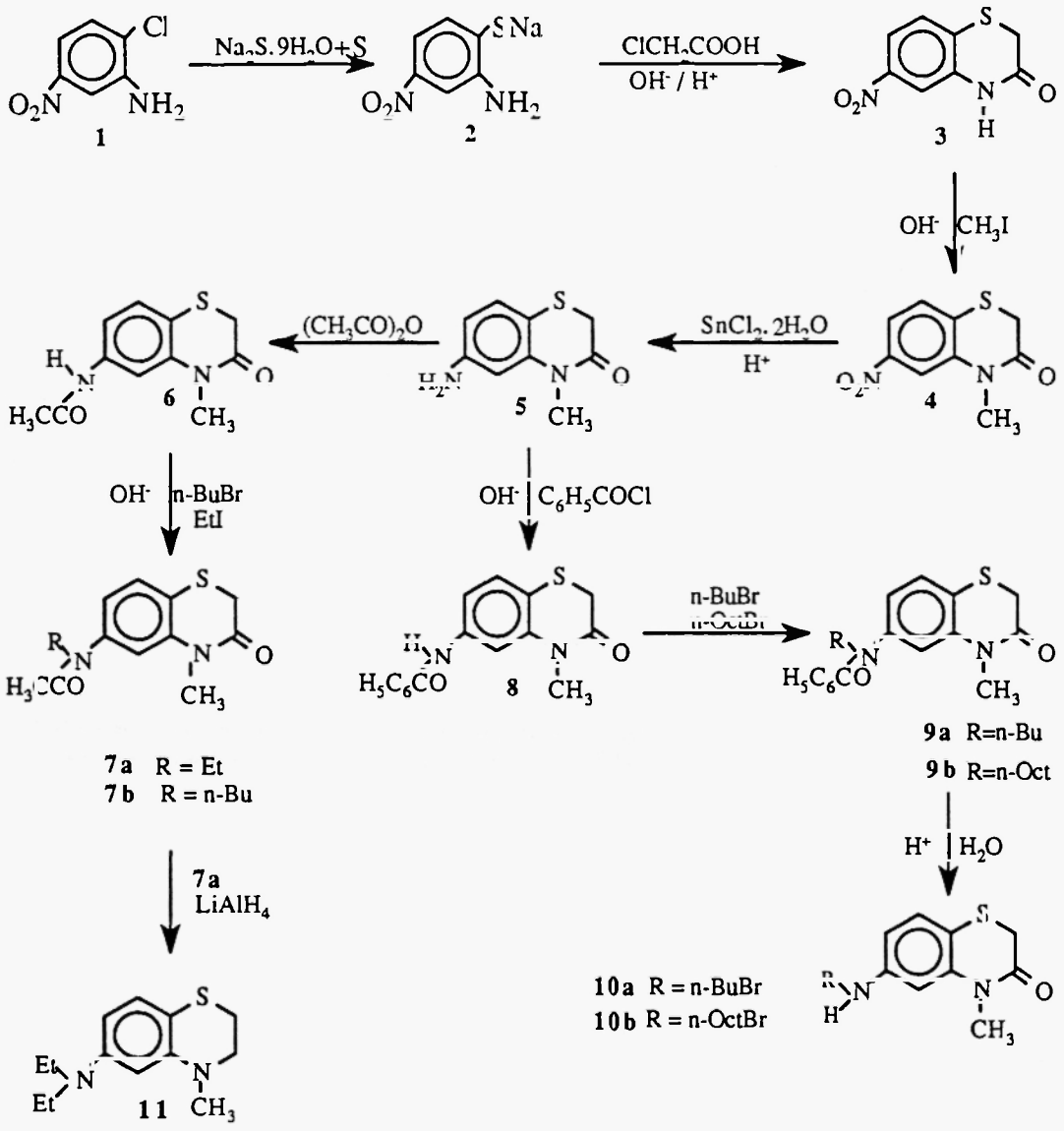
Chemistry

2*H*-1,4-benzothiazine derivatives can be prepared in several ways reported in the literature (1). Treatment of 2-chloro-5-nitroaniline **1** with sodium sulfide and sulfur (5) gave 2-amino-4-nitrobenzenethiol (sodium salt) **2** which is cyclized to 2*H*-1,4-benzothiazin-3-one **3** with chloracetic acid. N-methylation with methyl iodide using KOH as a base in methanolic solution (6) afforded **4**. The reduction of the nitro group by $SnCl_2$ in acidic medium (7) gave the corresponding amine : 6-amino-4-methyl-2*H*-1,4-benzothiazin-3-one **5**. N-acylation of the amino group followed by phase transfer catalysed alkylation in basic medium (8) led to N-alkylacyl derivs **7a, 7b** and **9a, 9b**. The N-alkylation with alkyl halides (ethyl iodide, n-butyl bromide and n-octyl bromide) in the presence of excess powder sodium hydroxide/potassium carbonate and 10 mol-% of tetrabutylammonium bromide as catalyst proceeds in boiling toluene. Acid hydrolysis of **9a** and **9b** afforded 6-alkylamino-4-methyl-2*H*-1,4-benzothiazin-3-one derivatives **10a** and **10b**. The reduction of **7a** with aluminium lithium hydride (9) led to 6-diethylamino-4-methyl-2*H*-1,4-benzothiazine **11** (Scheme I).

Material and methods

All melting points were determined with a capillary Buchi apparatus and are uncorrected. Thin layer chromatography was performed on silica pre-coated plates (Merck $60F_{254}$). IR spectra were recorded in KBr tablets (1%) with a Perkin-Elmer 1310 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-200 spectrometer in DMSO-d_6 or $CDCl_3$. Chemical shifts are expressed in ppm. Electronic impact (70eV) mass spectra were recorded with a R-1010C Delsi-Nermag spectrometer.

Scheme I - Synthesis of 2*H*-1,4-Benzothiazin-3-one derivatives



2-amino-4-nitrobenzenethiol (sodium salt) **2**

A stirred solution of 2-chloro-5-nitroaniline (1.72 g, 10 mmol) in 20 mL of hot absolute ethanol was treated gradually by a hot solution of sodium sulfide nonahydrate (2.4 g, 10 mmol) and sulfur (0.48 g, 15 mmol). The mixture was refluxed for 30 min. After cooling, 2-amino-4-nitrobenzenethiol sodium salt was filtered and dried. Yield 93%. This sodium salt is identified by free thiol. After filtration, the aqueous solution of sodium salt is acidified in the cold with diluted HCl (10%) when 2-amino-4-nitrobenzenethiol crystallized. mp 99°C - lit 96-98°C (10); Rf 0.71 (toluene/ethyl acetate 6/4); IR (ν cm^{-1}): 3400, 3310, 2540, 1500, 1340, 740 ; ^{1}H NMR (δ ppm, DMSO-d_6): 6.20(s, 2H); 7.26(dd, 1H), 7.34(d, 1H), 7.56(d, 1H) ; ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 108.4(CH), 109.9(CH), 123.3(C), 134.1(CH), 148.9(C), 149.5(C)

6-nitro-2H-1,4-benzothiazin-3-one **3**
Equimolecular amounts (10 mmol) of compound **2**, chloracetic acid and sodium hydroxide in 50 mL of water are refluxed for 30 min. After cooling, the reaction mixture is acidified with 10% HCl and filtered. The solid product contains a mixture of 2-amino-4-nitro-benzenethioglycolic acid and 6-nitro-2*H*-1,4-benzothiazin-3-one. The precipitate is suspended in absolute ethanol, acidified with 1 mL of concentrated HCl and heated at 60° for 15 min. After hot filtration, the precipitate is crystallized from ethanol. Yield 81% ; mp 243-245°C - lit 245°C (11); Rf 0.84 (benzene/acetone 1/1) ; IR (ν cm^{-1}): 3320, 1680, 1510, 1340, 740 ; ^{1}H NMR (δ ppm, DMSO-d_6): 3.61(s, 2H), 7.58(d, 1H, J=9.2Hz), 7.60(d, 1H, J=2.3Hz), 7.70(dd, 1H, J=9.2 and 2.3Hz), 10.91(s, 1H) ; ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 28.1(CH_2), 111.0(CH), 117.2(CH), 127.9(C), 128.5(CH), 137.8(C), 145.9(C), 164.6(CO)

4-methyl-6-nitro-2H-1,4-benzothiazin-3-one **4**
A mixture of compound **3** (1.05 g, 5 mmol), potassium hydroxide (0.56 g, 10 mmol) and DMSO (10 mL), methanol (12.5 mL) was stirred for 10 min before methyl iodide (1.42 g, 10 mmol) was added. The solution was heated at 50°C under stirring for 15 h. After cooling, the crude product precipitated when water was added. The precipitate was washed with water, dried, then recrystallized from ethanol. Yield 80% ; mp 183-185°C ; Rf 0.76 (chloroform/ethanol 9/10) ; IR (ν cm^{-1}): 2940, 1690, 1510, 1340, 745 ; ^{1}H NMR (δ ppm, DMSO-d_6): 3.36(s, 3H), 3.64(s, 2H), 7.67(d, 1H, J=8.5Hz), 7.80(dd, 1H, J=2.3 and 8.5Hz), 7.96(d, 1H, J=2.3Hz) ; ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 29.3(CH_2), 31.5(CH_3), 112.2(CH), 117.5(CH), 128.5(CH), 131.9(C), 140.4(C), 146.3(C), 164.6(CO); MS, m/z(%): 224(100), 181(65.1), 149(33.8), 95(45.0)

6-amino-4-methyl-2H-1,4-benzothiazin-3-one **5**
To a cold and stirred solution of 3.72 g of stannous chloride dihydrate in 3.8 mL of concentrated HCl was added portionwise, over a 15 min period, 0.8 g (3.5 mmol) of compound **4**. The mixture was then left for 15 min at laboratory temperature and refluxed for 2 h. After cooling, the precipitate was suspended in water and a 20% sodium hydroxide solution was added (pH 10) to give the corresponding amine, which was collected and recrystallized from water. Yield 96% ; mp 228-230°C (hydrochloride) ; mp 131-133°C (amine) ; Rf 0.61 (chloroform/ethanol 9/1) ; IR (ν cm^{-1}): 3430, 3340, 2900, 1655 ; ^{1}H NMR (δ ppm, DMSO-d_6): 3.35(s, 2H), 3.24(s, 3H), 5.24(s, 2H), 6.28(dd, 1H, J=2.3 and 8.2Hz), 6.46(d, 1H, J=2.3Hz), 6.98 (d, 1H, J= 8.2Hz) ; ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 31.2(CH_2), 31.3(CH_3), 103.6(CH), 106.7(C), 109.2(CH), 128.3(CH), 140.8(C), 148.5(C), 165.6(CO) ; MS, m/z(%): 194(100), 165(17.7), 151(29.5), 149(28.3)

6-acetylamino-4-methyl-2H-1,4-benzothiazin-3-one **6**
A mixture of 0.97 g (5 mmol) of compound **5** and 10 mL of acetic anhydride is refluxed for 10 min. The excess of anhydride is hydrolyzed by 10 mL of water and the mixture is refluxed for 5 min more. The precipitate is washed with water and recrystallized from ethanol/water. Yield 88% ; mp 215-218°C Rf 0.58 (chloroform/ethanol 9/1) ; IR (ν cm^{-1}): 3320, 2860, 1680, 1645 ; ^{1}H NMR (δ ppm, DMSO-d_6): 2.03(s, 3H), 3.28(s, 3H), 3.46(s, 2H), 7.23-7.29(m, 2H), 7.53(d, 1H), 10.02(s, 1H) ; ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 24.0(CH_3), 30.4(CH_2), 31.4(CH_3), 108.5(CH), 113.7(CH), 115.8(C), 127.9(CH), 138.6(C), 140.1(C), 165.3(CO), 168.3(CO) ; MS, m/z(%): 236(94.9), 194(100), 149(38.7)

6-acetylalkylamino-4-methyl-2H-1,4-benzothiazin-3-ones **7**
A mixture of 1.18 g (5 mmol) of compound **6** dissolved in toluene (100 mL), 1.4 g of potassium carbonate, 7 g of sodium hydroxide et 0.16 g of tetrabutylammonium bromide is refluxed. The solution of ethyl iodide or butyl bromide (7.5 mmol) in toluene (10 mL) is added. Stirring is continued for 4 h at refluxed temperature. After cooling, the mixture is filtered and the filtrate added with water;

the organic phase is separated, washed with water (2x50 mL), dried over anhydrous magnesium sulfate and evaporated. The crude product is recrystallized from ethanol 95%.

6-acetylethylamino-4-methyl-2H-1,4-benzothiazin-3-one **7a**

Yield 67% ; mp 119-120°C ; Rf 0.68 (chloroform/ethanol 9/1) ; IR (ν cm^{-1}): 2970, 2860, 1640-1670(broad) ; ^1H NMR (δ ppm, DMSO-d_6): 1.00(t, 3H, J=7.1Hz), 1.77(s, 3H), 3.34(s, 3H), 3.54(s, 2H), 3.61(q, 2H, J=7.1Hz), 6.95(dd, 1H, J=8.1 and 1.4Hz), 7.20(d, 1H, J=1.4Hz), 7.44(d, 1H, J=8.1Hz) ; ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 12.9(CH_3), 22.5(CH_3), 29.9(CH_2), 31.5(CH_3), 43.0(CH_2), 117.9(CH), 121.8(C), 122.7(CH), 128.4(CH), 140.8(C), 141.5(C), 165.0(CO), 168.5(CO) ; MS, m/z(%): 264(89.2), 222(36.8), 207(100), 70(76.2)

6-acetylbutylamino-4-methyl-2H-1,4-benzothiazin-3-one **7b**

Yield 36% ; mp 105-106°C ; Rf 0.70 (chloroform/ethanol 9/1) ; IR (ν cm^{-1}): 2920, 2860, 1660 (broad) ^1H NMR (δ ppm, DMSO-d_6): 0.83(t, 3H, J=7.2Hz), 1.22-1.38(m, 4H), 1.77(s, 3H), 3.34(s, 3H), 3.54(s, 2H), 3.61(t, 2H, J=7.2Hz), 6.96(dd, 1H, J= 8.1 and 1.4Hz), 7.19(d, 1H, J=1.4Hz), 7.44(d, 1H, J= 8.1Hz) ; ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 13.6(CH_3), 19.4(CH_2), 22.5(CH_3), 29.4(CH_2), 29.9(CH_2), 31.6(N-CH_3), 47.7(N-CH_2), 117.8(CH), 121.6(C), 122.6(CH), 128.4(CH), 140.8(C), 141.8(C), 164.9(CO), 168.7(CO) ; MS, m/z(%): 292(59.1), 236(9.4), 207(100), 194(12.3), 43(72.5)

6-benzoylamino-4-methyl-2H-1,4-benzothiazin-3-one **8**

To a suspension of 1 g (5.15 mmol) of benzothiazine **5** in 20 mL of NaOH 20%, 2 mL of benzoyl chloride were added dropwise. The mixture is stirred for 10 min. The precipitate is separated, washed with water and recrystallized from ethanol 95%. Yield 97% ; mp 185-186°C ; Rf 0.42 (toluene/ethyl acetate 6/4) ; IR (ν cm^{-1}): 3280, 2840, 1660, 1640 ; ^1H NMR (δ ppm, DMSO-d_6): 3.32(s, 3H), 3.50(s, 2H), 7.36(d, 1H), 7.55(m, 1H), 7.76(d, 1H), 7.96(dd, 2H), 7.53-7.77(m, 3H), 10.33(s, 1H) ; ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 30.4(CH_2), 31.4(CH_3), 109.8(CH), 114.9(CH), 116.6(C), 127.5(2CH), 127.8(CH), 128.3(2CH), 131.6(CH), 134.6(C), 138.5(C), 140.1(C), 165.3(CO), 165.5(CO) ; MS, m/z(%): 298(37.8), 105(100), 77(53.9)

6-benzoylalkylamino-4-methyl-2H-1,4-benzothiazin-3-ones **9**

The method of alkylation by phase transfer catalysis described for the synthesis of the compound **7** has been used.

6-benzoylbutylamino-4-methyl-2H-1,4-benzothiazin-3-one **9a**

The compound is recrystallized from methanol 50%. Yield 64% ; mp 103-104°C ; Rf 0.48 (toluene/ethyl acetate 6/4) ; IR (ν cm^{-1}): 2960, 2870, 1670, 1640 ; ^1H NMR (δ ppm, $CDCl_3$): 0.93(t, 3H), 1.38(m, 2H), 1.60(m, 2H), 3.07(s, 3H), 3.34(s, 2H), 3.91(t, 2H), 6.55(d, 1H), 6.81(dd, 1H), 7.25(m, 6H) ; ^{13}C NMR (δ ppm, $CDCl_3$, BB decoupling and DEPT): 13.8(CH_3), 20.2(CH_2), 29.9(CH_2), 31.1(CH_2), 31.8(N-CH_3), 50.1(N-CH_2), 117.9(CH), 121.3(C), 121.5(CH), 127.9(2CH), 128.4(2CH), 128.6(CH), 129.7(CH), 136.2(C), 140.5(C), 142.6(C), 165.1(CO), 170.2(CO); MS, m/z(%): 354(23.8), 160(11.3), 105(100), 77(41.1)

6-benzoyloctylamino-4-methyl -2H-1,4-benzothiazin-3-one **9b**

The oily compound is purified by column chromatography with silicagel and chloroform/ethanol (9/1) as eluent. Yield 52% ; Rf 060 (toluene/ethyl acetate 6/4) ; IR (ν cm^{-1}): 2920, 2850, 1650 (broad) ; ^1H NMR (δ ppm, DMSO-d_6): 0.83(t, 3H), 1.22(m, 10H), 1.50(m, 2H), 3.06(s, 3H), 3.44(s, 2H), 3.82(t, 2H), 6.82(dd, 1H, J=8.2 and 2.0Hz), 6.98(d, 1H, J=2.0Hz), 7.21-7.28(m, 6H) ; ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 13.9(CH_3), 22.0(CH_2), 26.2-28.6(CH_2), 30.0(CH_2), 31.4(N-CH_3), 49.2(N-CH_2), 118.0(CH), 120.5(C), 122.1(CH), 127.8(2 CH),

127.9(2CH), 128.8(CH), 129.2(CH), 136.6(C), 140.1(C), 141.9(C), 164.8(CO), 169.3(CO) ; MS, m/z(%): 410(19.0), 298(15.9), 207(20.3), 105(100), 77(69.4)

6-alkylamino-4-methyl -2H-1,4-benzothiazin-3-ones **10**

A suspension of compound **9** (2 mmol) in 5 mL of 70% sulfuric acid is refluxed at 147-150°C for 30 min. After cooling, water and 10 mL of a solution of concentrated ammonium hydroxide are added; the organic phase is extracted with chloroform.

6-butylamino-4-methyl -2H-1,4-benzothiazin-3-one **10a**

This compound is recrystallized from ethanol 95%. Yield 91%; mp 89-92°C; Rf 0.90 (chloroform/ethanol 9/1) ; IR (ν cm^{-1}): 3390, 2930, 2940, 2860, 1650 ; ^1H NMR (δ ppm, DMSO-d_6): 0.89(t, 3H), 1.34-1.51(m, 4H), 2.99(dt, 2H), 3.27(s, 3H), 3.35(s, 2H), 5.71(t, 1H), 6.27(dd, 1H, J=8.4 and 2.1Hz), 6.41(d, 1H, J=2.1Hz), 7.02(d, 1H, J=8.4Hz) ; ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 13.7(CH_3), 19.7(CH_2), 29.9(CH_2), 31.2(CH_2), 31.4(N-CH_3), 42.4(N-CH_2), 128.4(CH), 140.8(C), 148.8(C), 165.6(CO) ; MS, m/z(%): 250(43.3), 207(100), 136(5.6), 77(5.6)

4-methyl-6-octylamino-2H-1,4-benzothiazin-3-one **10b**

The compound is recrystallized from methanol. Yield 68% ; mp 62-64°C ; Rf 0.49 (toluene/ethyl acetate 8/2) ; IR (ν cm^{-1}): 3350, 2910, 2850, 1670 ; ^1H NMR (δ ppm, DMSO-d_6): 0.85(broad t, 3H), 1.25(m,10H), 1.50(m, 2H), 2.98(m, 2H), 3.27(s, 3H), 3.35(s, 2H), 5.70(broad t, 1H), 6.25(dd, 1H, J=8.3Hz), 6.41(d, 1H) , 7.03(d, 1H, J=8.3Hz) ; ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 13.9(CH_3), 22.1(CH_2), 26.6-28.8(CH_2), 31.2(2 CH_2), 31.4(N-CH_3), 42.8(N-CH_2), 101.9(CH), 106.4(C), 107.1(CH), 128.3(CH), 140.9(C), 148.8(C), 165.7(CO) ; MS, m/z(%): 306(65.6), 207(100), 136(4.8), 105(2.5)

6-diethylamino-4-methyl-2H-1,4-benzothiazine **11**

A mixture of 1.32 g (5 mmol) of acylated compound **7a** in 10 mL of anhydrous THF, 0.95 g of $LiAlH_4$ is refluxed for 24 h. After cooling, ethyl alcool is added to destroy excess of $LiAlH_4$. After filtration and evaporation of the filtrate, the oily compound is purified by column chromatography with toluene/ethyl acetate added with 1% of ammonium hydroxide. Yield 42% ; Rf 0.78 (chloroform/ethanol 9/1) ; IR (ν cm^{-1}): 2960, 2860 ; ^1H NMR (δ ppm, DMSO-d_6): 1.06(t, 6H, J=7.0Hz), 2.89(s, 3H), 2.92-2.97(m, 2H), 3.25(q, 4H, J=7.0Hz), 3.41-3.47(m, 2H), 5.97(d, 1H), 5.99(dd, 1H, J=7.7Hz), 6.71(d, 1H, J= 7.7Hz) ; ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 12.5(2 CH_3), 25.7(CH_2S), 39.5(N-CH_3), 43.8(N- CH_2), 51.6(CH_2N), 97.2(CH), 102.4(CH), 103.7(C), 127.6(CH), 145.5(C), 146.5(C) ; MS, m/z(%): 236(100), 221(55.8), 192(48.5), 177(30.5), 163(10.8)

Biological activity

The minimal inhibitory concentration (MIC) was performed *in vitro* for five compounds: **4**, **5**, **6**, **7a** and **10a** against six microorganisms: *Micrococcus flavus*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis*.

The microorganisms were cultivated on Mueller-Hinton agar. Inocula were prepared from 18 h-old subcultures at 37°C in this broth. The turbidity of culture suspension was adjusted to obtain 0.5 on McFarland scale, i.e. 10^8 UFC/mL. The solutions of each compound to be tested were prepared at the concentration of 1280 μg/mL in a mixture of DMSO/Tween 80/distilled water (1/1/8) then in distilled water from mother solutions. Serial dilutions were made with sterile distilled water according to a geometric progression of ratio 2, such as the concentrations of Petri plates were in the range of 128-0.5 μg/mL. In the same way, we have verified that DMSO, at the 1/10 dilution, exhibited no intrinsic activity against tested microorganisms. The ciprofloxacin was used as the reference antibiotic.

The bacterial suspensions were streaked with a loop of 0.05 mL. After 18h-incubation at 37°C, the reading was compared to control plate which did not contain tested compound. The MIC was defined as the lowest drug concentration for which there is no bacterial growth (13). The results of the MIC

show that tested compounds inhibited the most microorganisms at concentration of 1284 µg/mL. Compounds **4** and **5** (MIC = 1 µg/mL), **6** and **10a** (MIC = 0.5 µg/mL) and **7a** (MIC = 32 µg/mL) inhibited *M. flavus* . Compound **5** is active against *K. pneumoniae* (MIC = 2 µg/mL) and *S. aureus* (MIC = 8 µg/mL). For ciprofloxacin, the results of MIC are : 0.25 µg/mL against *M. flavus*, 2 µg/mL against *K. pneumoniae* and 4 µg/mL against *S. aureus*.
M. flavus DAUFPE-323 comes from the collection of Department of Antibiotics; *K. pneumoniae* and *S. aureus* are wild strains isolated from contaminated food.

Conclusion

Some new *2H*-1,4-benzothiazin-3-one derivatives have been synthesized and characterized by their physical and analytical properties. The fragments observed by electronic impact mass spectrometry are in agreement with the proposal structures.
Five compounds were evaluated as potential antimicrobial agents against six microorganisms. Their activity is inferior to ciprofloxacin, the reference antibiotic, except for the compound **5** against *K. pneumoniae*.

Aknowledgments

The authors thank the CNPq (Conselho Nacional de Desenvolvimento Cientifico e Tecno-logico, Brasil) and the International Cooperation Agreement (CAPES/COFECUB) for their support.

References

(1) R.R. Gupta and K.G. Ojha, Phenothiazines and 1,4-Benzothiazines : Chemical and Biochemical Aspects, Elsevier, Amsterdam, 1988, 4, pp 163

(2) G. Grandolini, V. Ambrogi, C. Rossi, M.C. Tiralti and L. Tuttobello, Eur. J. Med. Chem. - Chim. Ther. 21, 455 (1986)

(3 R.R. Gupta, P.K. Dev, M.L. Sharma, C.M. Rajoria, A. Gupta and M. Nyati, Anti-cancer Drugs 4, 589 (1993)

(4 D.K. Todorov, M.V. Ilarionova, R.R. Gupta, J. Molnar and N. Motohashi, Heterocycl. Commun. 1, 153 (1995)

(5) K. Fries, M. Vorbrodt and G. Siebert, Ann. 454, 121 (1927)

(6) A.M. Ngadi, J.P. Galy, J. Barbe, A. Cremieux, J. Chevalier and D. Scharples, Eur. J. Med. Chem. 25, 67 (1990)

(7) V. Cecchetti, S. Dominici, A. Fravolini and F. Schiaffella, Eur. J. Med. Chem.- Chim. Ther. 19, 29 (1984)

(8) A. Koziara and S. Zawadzki, Synthesis 7, 527 (1979)

(9) J. Bourdais, French Patent 1,438,225 *in* Chem. Abstr. 65, 20141h (1966)

(10) Shell International Research, Neth Appl 6.515.923 *in* Chem. Abstr. 65, 15391c (1966)

(11) A. Martani, A. Fravolini and G. Grandolini, Annali di chimica 58,1126 (1968)

(12) R. Cleeland and E. Grunberg, Laboratory evaluation of new antibiotics in vitro and in experimental animal infection. In U. LORIAN *Antibiotics in Laboratory Medecine*, Williams and Wilkins, Baltimore, 1986, pp 825

(13) P. Courvalin, F. Goldstein, A. Philippon and J. Sirot, L'Antibiogramme, MPC/Vigot, Bruxelles, 1985, pp 191

Received on October 15, 1999