SYNTHESIS OF 6-MORPHOLINYL AND 6-PIPERAZINYL-4-ALKYL-4*H*-1,4-BENZO-THIAZIN-3-ONE DERIVATIVES WITH BACTERIOSTATIC ACTIVITY

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ABSTRACT

Synthesis, physical and analytical properties of 6-morpholinyl and 6-piperazinyl-4-alkyl-4*H*-benzo[1,4]thiazin-3-one derivatives are described. Some of these compounds show promising *in vitro* bacteriostatic activity against *Micrococcus flavus* or *Bacillus cereus.*

INTRODUCTION

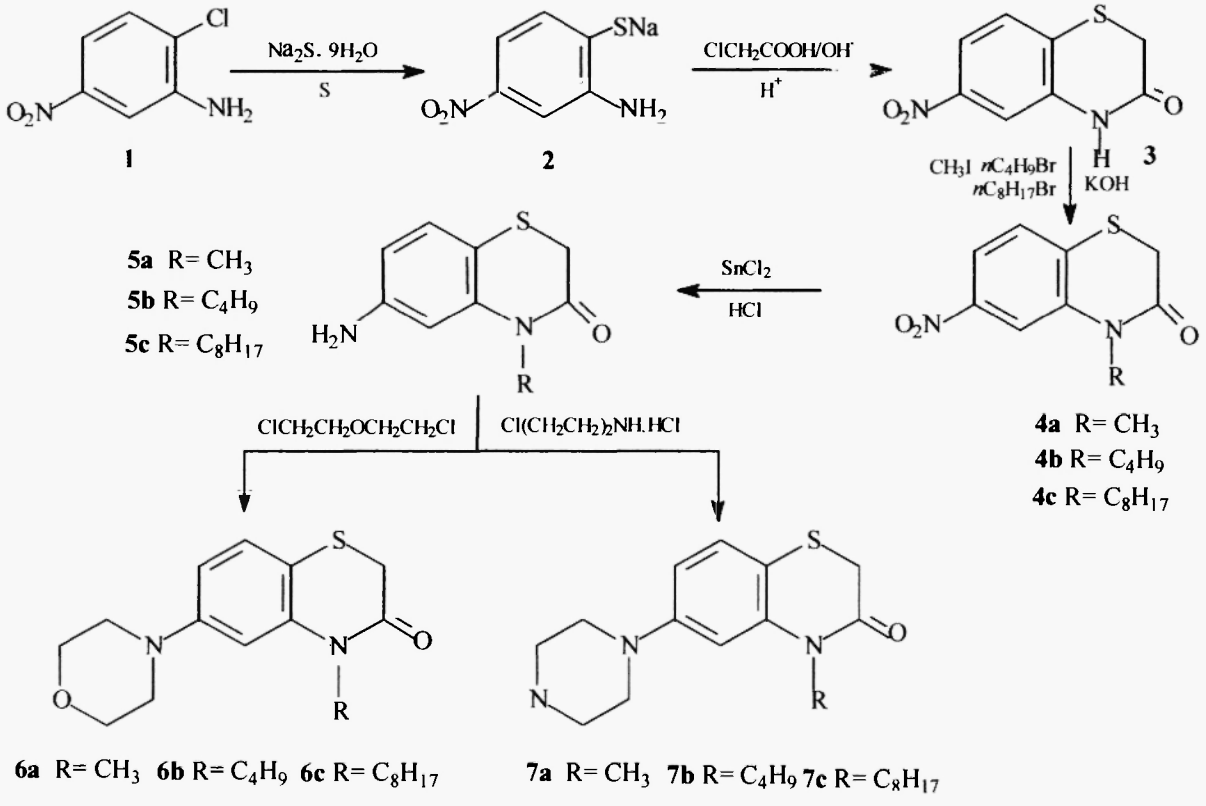
1,4-Benzothiazines have been extensively investigated [1]. Recently, moderate antibacterial activity was detected in 4-alkyl-2H-1,4-benzo-thiazin-3-ones [2]. Within the frame of a SAR study, the title compounds were prepared and tested against six microorganisms including gram positive and gram negative bacteria.

The 6-morpholinyl and 6-piperazinyl-4-alkyl-4*H*-1,4-benzo-thiazin-3-one derivatives were synthesized from 2-chloro-5-nitro-aniline. Action of sodium sulfide and sulfur on this starting compound led to 2-amino-4-nitrobenzenethiol sodium salt which was cyclized in 6-nitro-4*H*-1,4-benzo-thiazin-3-one by using chloroacetic acid. *N*-alkylation at position 4 followed by reduction of the nitro group, led to the 4-alkyl-6-amino-4*H*-1,4-benzo-thiazin-3-ones before these compounds be alkylated to provide the 6-morpholinyl and the 6-piperazinyl-4-alkyl-4*H*-1,4-benzo-thiazin-3-one.

CHEMISTRY

The treatment of 2-chloro-5-nitroaniline, **1,** with sodium sulfide and sulfur [3] gave the 2-amino-4-nitrobenzenethiol (sodium salt), **2,** which was cyclized with chloroacetic acid to give the 4*H*-1,4-benzo-thiazin-3-one, **3**. *N*-alkylation with alkyl halides like methyl iodide, *n*-butyl or *n*-octyl bromide, using KOH in methanolic solution as a base [4] afforded compounds, **4**. Reduction of the nitro group by $SnCl_2$ in acidic medium [5] gave the corresponding 4-alkyl-6-amino-4*H*-1,4-benzo-thiazin-3-ones, **5**.

Reaction of the 6-amino group with bis-2-chloroethyl ether or bis-(2-chloroethyl) amine hydrochloride in basic medium [6,7] gave 6-morpholinyl-4-alkyl-4*H*-1,4-benzo-thiazin-3-one, **6,** and 6-piperazinyl-4-alkyl-4*H*-1,4-benzo-thiazin-3-one,**7**, as shown in the scheme.



6a R= CH_3 **6b** R= C_4H_9 **6c** R= C_8H_{17}

7a R= CH_3 **7b** R= C_4H_9 **7c** R= C_8H_{17}

BIOLOGICAL ACTIVITY

The minimal inhibitory concentration (MIC) against six microorganisms was estimated *in vitro* for compounds **6b**, **6c**, **7a** and **7b**. With respect to this, *Micrococcus flavus* (DAUFPE 323), *Staphylococcus aureus* (IC 06), *Salmonella enteritidis* (DAUFPE 415), *Bacillus cereus* (DAUFPE 11), *Escherichia coli* (IC 02) and *Proteus vulgaris* (IC 03) were used. Procedure is detailed in ref. [2].

Compounds **6b** and **6c** are only active on the Gram-positive bacillus *M. flavus* with a MIC of 8 μg/mL and 64 μg/mL respectively while compound **7b** is active either on *M. flavus* with a 0.5 μg/mL MIC or *B. cereus* with a 1 μg/mL MIC. The other compounds do not show bacteriostatic activity, even at a 128 μg/mL concentration.

MIC values for ciprofloxacine, which was selected as reference, are 0.25 μg/mL with *M.flavus* , 4 μg/mL with *S.aureus* and *P.vulgaris*, 8 μg/mL with *S.enteritidis* and 2 μg/mL with *B.cereus* and *E.coli*.

EXPERIMENTAL

Melting points were determined on a Buchi apparatus. 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. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AC-200 spectrometer in DMSO-d_6. Chemical shifts are expressed in ppm. Electronic impact (70eV) mass spectra were recorded with a R-1010C Delsi-Nermag spectrometer.

Chemical data on **2**, **3**, **4a** and **5a** are given in ref. [8], and those on **4b** and **5b** are gathered in ref. [9].

4-Octyl-6-nitro-4H-1,4-benzo-thiazin-3-one, **4c**
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 octyl bromide (1.93 g, 10 mmol) was added. The solution was heated at 50°C under stirring for 15 h. After cooling, the crude liquid product (4-octyl-6-nitro-4*H*-1,4-benzo-thiazin-3-one) was extracted with cyclohexane (3x 50 mL) and purified by column chromatography on silica gel with a mixture of toluene:ethyl acetate (8:2) as eluent. Yield, 78%; liquid; Rf, 0.85 (toluene:ethyl acetate, 7:5); IR (υ cm^{-1}): 2920, 2860, 1680, 1525, 1345, 1140, 740; ^{1}H NMR (δ ppm, DMSO-d_6): 0.82 (t, J=6.6Hz, CH_3), 1.20 (m, CH_2 chain, 10H), 1.51 (m, CH_2 chain), 3.61 (s, CH_2 ring), 4.05 (t, J=7.1Hz, NCH_2), 7.69 (d, J=8.5Hz, 1H), 7.86 dd, J=8.5 and 2.2Hz, 1H), 7.99 (d, J=2.2Hz, 1H); ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 13.8 (CH_3), 22.0 (CH_2 chain), 28.4-25.7 ($4CH_2$), 29.7 or 31.0 (CH_2 chain, CH_2 ring), 43.2 (CH_2N), 112.4 (CH), 117.5 (CH), 128.8 (CH), 132.8 (C), 139.2 (C), 146.3 (CNO_2), 164.1 (CO); MS *m/z* (%): 322 (27.5), 275 (34), 210 (100), 195 (53), 181 (28.3), 149 (25.4), 135 (27.5), 41 (44.3).

6-Amino-4-octyl-4H-1,4-benzo-thiazin-3-one, **5c**
Compound **4c** was added portion-wise, over a 15 min period, (1.13 g, 3.5 mmol) to a cold and stirred solution of stannous chloride dihydrate (3.72 g) in concentrated HCl (3.8 mL). The crude was then left for 15 min at room temperature before to be heated under refluxing conditions for 2 h. After cooling, the precipitate of 6-amino-4-octyl-4*H*-1,4-benzo-thiazin-3-one hydrochloride (mp 163-165°C) was put in a suspension in water. A 20% aq. sodium hydroxide solution was added until pH 10, to give the

corresponding amine. The oily compound was extracted with chloroform (3 x 50 mL), washed with 10% aq. NaOH, dried over anhydrous magnesium sulfate. Solvent was evaporated. The crude was purified by column chromatography on silica gel with toluene:ethyl acetate (8:2) as eluent. Yield, 91%; mp, 163-165°C; Rf, 0.61 (toluene:ethyl acetate, 7:5); IR (υ cm^{-1}): 3450, 3350, 2920, 2830, 1650, 1600, 1490, 1380, 1140, 760; ^1H NMR (δ ppm, DMSO-d_6): 0.84 (t, J=6.6Hz, CH_3), 1.23 (m, CH_2 chain, 10H), 1.52 (m, CH_2 chain), 3.31 (s, CH_2 ring), 3.81 (t, J=7.1Hz, NCH_2), 5.21 (s, NH_2), 6.28 (dd, J=8.3 and 2.1Hz, 1H), 6.53 (d, J=2.1Hz, 1H), 6.98 (d, J=8.3Hz, 1H); ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 13.8 (CH_3), 22.0 (CH_2), 26.0-28.6 ($4CH_2$), 31.1 or 31.5 (CH_2 chain, CH_2 ring), 43.4 (CH_2N), 103.7 (CH), 107.5 (C), 109.3 (CH) 128.4 (CH), 139.7 (C) 148.4 (CNH_2), 165 (CO); MS *m/z* (%): 292 (100), 180 (52.4), 165 (38.1), 151 (98.5), 135 (32), 41 (52.7).

4-Alkyl-6-morpholinyl-4H-1,4-benzo-thiazin-3-ones, **6.** General procedure.
A mixture of compound **5** (10 mmol), 2-bis-chloroethyl-ether (10 mmol), sodium carbonate (15 mmol) and 10mL of methanol was heated at 150°C during 24h in a sealed tube. After cooling, hot ethanol was added and the mixture was filtered. The morpholino substituted derivatives were purified by crystallization from water or by column chromatography.

4-Methyl-6-morpholinyl-4H-1,4-benzo-thiazin-3-one, **6a**
Compound was crystallized from water. Yield, 50%; mp 78-80°C; Rf. 0.88 (chloroform:ethanol, 9:1); IR (υ cm^{-1}): 3400, 3300, 2960, 2850, 1650, 1595, 1050; ^1H NMR (δ ppm, DMSO-d_6): 3.11 (m, $2CH_2$, morpholine), 3.32 (s, CH_2), 3.4 (s, CH_3), 3.72 (m, $2CH_2$, morpholine), 6.66 (d, J=8.5Hz, CH), 6.74 (broad s, CH), 7.2 (d, J=8.5Hz, CH); ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 30.6 (CH_3), 31.6 (CH_2), 48.4 ($2CH_2$, morpholine), 66.0 ($2CH_2$, morpholine), 105.4 (CH), 110.3 (CH), 111.5 (C), 128.2 (CH), 140.6 (CN), 150.7 (CN, morpholine), 165.5 (CO); MS *m/z* (%): 264(100), 206(53.5), 177(10), 163(15.7), 135(7.4), 42(18.3).

4-Butyl-6-morpholinyl-4H-1,4-benzothiazin-3-one, **6b**
Compound was purified by column chromatography on silica gel with chloroform:ethanol (9:1) as eluent. Yield, 40%; mp, 85-85°C; Rf, 0.47 (toluene:ethyl acetate, 8:2); IR (υ cm^{-1}): 2960, 2850, 1650, 1595,1140; ^1H NMR (δ ppm, DMSO-d_6): 0.85 (t, J=7.1Hz, CH_3), 1.36 (m, CH_2), 1.46 (m, CH_2), 3.10 (m, $2CH_2$, morpholine), 3.37 (s, CH_2, ring), 3.73 (m, $2CH_2$, morpholine), 3.99 (t, J=6.8Hz, NCH_2), 6.65 (d, J=8.4Hz, CH), 6.78 (broad s, CH), 7.21 (d, J=8.5Hz, CH); ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 13.6 (CH_3), 19.1 (CH_2), 28.9(CH_2), 31.2 (CH_2, ring), 42.6(NCH_2), 48.4 ($2CH_2$, morpholine), 66 ($2CH_2$, morpholine), 105.5 (CH), 110.5 (CH), 112.7(C), 128.5 (CH), 139.6 (CN), 150.7 (CN, morpholine), 165.1 (CO); MS *m/z* (%): 306(100), 192(48.7), 177(29), 163(42), 149(16.4), 135(15.2), 61(19.6), 43(26.9).

4-Octyl-6-morpholinyl-4H-1,4-benzo-thiazin-3-one, **6c**
Compound was purified by column chromatography on silica gel with toluene:methanol (95:5) as eluent. Yield, 45%; liquid; Rf, 0.61 (toluene:ethyl acetate,

8:4); IR (υ cm^{-1}): 3400, 3300, 2930, 2850, 1670, 1600, 1140; ^1H NMR (δ ppm, DMSO-d_6): 0.84 (t, J=6.3Hz, CH_3), 1.2 (m, $5CH_2$), 1.48 (m, CH_2), 3.09 (m, $2CH_2$, morpholine), 3.36 (s, CH_2, ring), 3.72 (m, $2CH_2$, morpholine), 3.98 (t, J=7.1Hz, NCH_2), 6.65 (dd, J=8.6 and 2.3Hz, CH), 6.77 (d, J=2,2Hz, CH), 7.2 (d, J=8.5Hz, CH); ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 13.8 (CH_3), 22 (CH_2), 25.7-28.4 ($4CH_2$), 31.1 (CH_2), 31.2 (CH_2), 42.8 (NCH_2), 48.4 ($2CH_2$· morpholine), 65.9 ($2CH_2$· morpholine), 105.5 (CH), 110.5 (CH), 112.7 (C), 128.4 (CH), 139.5 (CN), 150.6 (CN, morpholine), 165 (CO); MS *m/z* (%): 362(100), 221(5.7), 192(18), 177(7.2), 163(5.9), 41(9.5).

4-Alkyl-6-piperazinyl-4H-1,4-benzo-thiazin-3-ones, **7.** General procedure.
Equimolecular amounts (10mmol) of compound **5** and bis-chloroethylamine hydrochloride in butanol (15mL) were refluxed for 6h. After light cooling, sodium carbonate (26mmol) was added and the mixture was refluxed again for 4h. After cooling, the hydrochloride precipitated and was separated by filtration. Treatment with 10% aq. NaOH until pH 10, led to the free base. Compound **7a** was separated by filtration and recrystallized. The liquid compounds **7b** and **7c** were extracted with chloroform. Organic phase was washed two times with water (20mL), dried with magnesium sulfate and the solvent was evaporated. Compounds were purified by column chromatography.

4-Methyl-6-piperazinyl-4H-1,4-benzo-thiazin-3-one, **7a**
Compound was crystallized from methanol. Yield, 40%; mp, 86-89°C; Rf, 0.64 (chloroform:ethanol, 9:1 added with 2 drops of 28% aq. ammonia); IR (υ cm^{-1}): 3300, 2960, 2800, 1650, 1580, 1140, 800; ^1H NMR (δ ppm, DMSO-d_6): 2.82 (m, $2CH_2$, piperazine), 3.05 (m, $2CH_2$, piperazine), 3.33(s, CH_3), 3.40 (s, CH_2), 6.62 (dd, J=7,3 and 2,4Hz, CH), 6.71 (d, J=2.4Hz, CH), 7,17 (d, J=8.5Hz, CH), 9.79 (s, NH); ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 30.9 (CH_2), 31.5 (CH_3), 45.5 ($2CH_2$, piperazine), 49.3 ($2CH_2$, piperazine), 105.4 (CH), 110.4 (CH), 110.8 (C), 128 (CH), 140.7 (CN), 151.2 (CN, piperazine), 165.5 (CO). MS *m/z* (%): 263(91.3), 221(100), 206(17), 163(12), 83(18.9), 56(36.7), 42(26).

4-Butyl-6-piperazinyl-4H-1,4-benzo-thiazin-3-one, **7b**
Compound was purified by column chromatography on silica gel with chloroform:ethanol:28% aq. ammonia (9:1:0.5) as eluent. Yield, 26%; liquid; Rf, 0.55 (chloroform:ethanol, 9:1 added of 2 drops of 28% aq. ammonia); IR (υ cm^{-1}): 3400, 3300, 2960, 1650, 1590, 1130, 810; ^1H NMR (δ ppm, DMSO-d_6): 0.85 (t, J=7.2Hz, CH_3), 1.24 (m, CH_2), 1.44 (m, CH_2), 2.87 (m, $2CH_2$, piperazine), 3.08 (m, $2CH_2$, piperazine), 3.5 (s, CH_2), 3.99 (t, J=7.1Hz, NCH_2), 6.63 (dd, J=8.6 and 2.3Hz, CH), 6.76 (d, J=2.3Hz, CH), 7.19 (d, J=8.5Hz, CH), 8.3 (s, NH); ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 13.6 (CH_3), 19.1 (CH_2), 28.9(CH_2), 31.2 (CH_2, ring), 40.7 (NCH_2), 48.5 ($2CH_2$, piperazine), 48.8 ($2CH_2$, piperazine), 105.7 (CH), 110.8 (CH), 112.3 (C), 128.4 (CH), 139.5 (CN), 151 (CN, piperazine), 165 (CO). MS *m/z* (%): 305(48), 263(100), 192(19.4), 177(36.2), 163(42.4), 135(14.4), 56(19.1), 42(27.3).

4-Octyl-6-piperazinyl-4H-1,4-benzo-thiazin-3-one, **7c**

Compound was purified by column chromatography on silica gel with chloroform:ethanol:28% aq. ammonia (9:1:0.5) as eluent. Yield, 22%; liquid; Rf, 0.47 (toluene:ethyl acetate, 6:4); IR (υ cm^{-1}): 3340, 2940, 2850, 1670, 1595, 1140, 810, 760; ^1H NMR (δ ppm, DMSO-d_6): 0.83 (t, J=6.7Hz, CH_3), 1.20 (m, $5CH_2$), 1.47 (m, CH_2), 2.82 (m, $2CH_2$, piperazine), 3.04 (m, $2CH_2$, piperazine), 3.36 (s, CH_2, ring), 3.98 (t, J=7.1Hz, NCH_2), 6.63 (dd, J=8.6 and 2.3Hz, CH), 6.74 (d, J=2.3Hz, CH), 7.18 (d, J=8.6Hz, CH), (s, NH, has not been observed);. ^{13}C NMR (δ ppm, DMSO-d_6, BB decoupling and DEPT): 13.8 (CH_3), 21.9 (CH_2), 25.7-28.4 ($4CH_2$), 31(CH_2), 31.2 (CH_2), 42.8 (NCH_2), 45.3 ($2CH_2$, piperazine), 49.2 ($2CH_2$, piperazine), 105.6 (CH), 110.7 (CH), 112 (C), 128.4 (CH), 139.5 (CN), 151.1 (CN, piperazine), 165 (CO). MS *m/z* (%): 361(72.1), 319(100), 207(12.7), 192(12.4), 177(19), 163(16.7), 56(21.4), 43(14.2).

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REFERENCES

[1] R.R. Gupta and K.G. Ojha, in R.R. Gupta (ed.) *Phenothiazines and 1,4-Benzothiazines-Chemical and Biochemical Aspects*, Elsevier, Amsterdam, 1988, pp.163.

[2] V.L.M. Guarda, M. Perissin, F. Thomasson, E.A. Ximenes, S.L. Galdino, I.R. Pitta, C. Luu-Duc and J. Barbe, *Eur. J. Med. Chem.*, **38,** 769 (2003).

[3] K.Fries, M. Vorbrodt and G. Siebert, *Ann.*, **454**, 121(1927).

[4] L. Ngadi A.M. Galy, J.P. Galy, J. Barbe, A. Cremieux, J. Chevalier and D. Sharples, *Eur. J. Med. Chem.*, **25**, 67 (1990).

[5] V. Cecchetti, S. Dominici, A. Fravolini and F. Schiaffella, *Eur. J. Med. Chem.*, **19**, 29 (1984).

[6] E. Cerkovnikov, N. Skarica, P. Stern, *Arkhiv Kem.*, **18**, 37 (1946); *Chem. Abstr.*, **48**, 1942i (1948).

[7] L. Thunus, C.L. Lapiere, N. Verbeke, *Ann. Pharm. Fr.*, **4**, 353 (1980).

[8] V.L.M. Guarda, M. Perrissin, F. Thomasson, E.A. Ximenes, S.L. Galdino, I.R. Pitta. and C. Luu-Duc, *Heterocycl. Commun.*, **6**, 49 (2000).

[9] V.L.M. Guarda, M. Perrissin, F. Thomasson, E.A. Ximenes, S.L. Galdino, I.R. Pitta. and C. Luu-Duc, *Il Farmaco*, **56**, 689 (2001).

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