Aminoalkyl Derivatives of 2,4'-Bithiazole-4-carboxylic Acid, the Intercalating Part of Bleomycin

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The synthesis of three 2,4'-bithiazole derivatives related to the antibiotic antitumor bleomycin is reported. These compounds substituted by aminoalkyl chains on 2' and/or 4-positions were prepared by the Hantzsch synthesis between new thioamides and methyl 2-(2-bromoacetyl)thiazole-4-carboxylate and will be useful for the study of the intercalative moiety of bleomycin.

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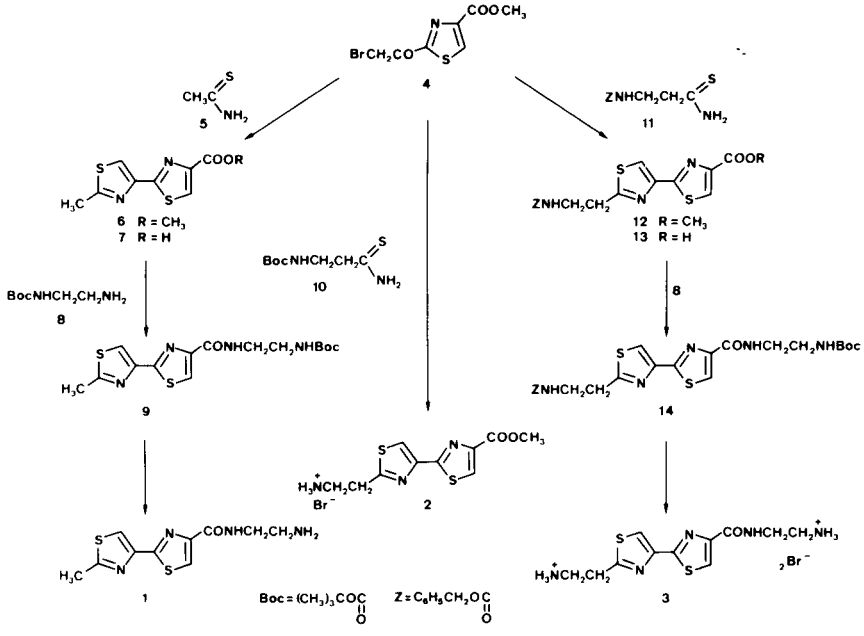
The role of the bithiazole moiety of the antitumor antibiotic bleomycin [1] in the DNA binding process has been studied by several techniques applied to the natural drug [2,3], degradation products [4] or simpler synthetic bithiazole models [5-7]. The suggested mode of interaction is an intercalation of the coplanar bithiazole rings between the base-pairs. To specify the manner of insertion we have proposed the use of spin-labeled probes [8] able to give interesting information, by esr spectroscopy, about the size of the intercalation site and subsequently, the geometry of the complex formed by the antibiotic and its target in the binding region.

It has been reported that another important feature involved in the DNA-bleomycin binding was the presence of substituents at the 2'- and 4- positions of the bithiazole ring. Moreover the role of the cationic terminus, in particular, in the formation of electrostatic bondings with the backbone phosphates has been emphasized [5].

We propose here the synthesis of some bithiazole models **1**, **2**, **3**, substituted by aminoalkyl chains on the 2'-position or on the 4-carboxamide group.

The thiazole rings were obtained by the general procedure of Hantzsch [9] which consists in the cyclization between a thioamide and a bromomethylketone group. The bithiazoles **1**, **2**, and **3** were prepared starting from 2-(2-bromoacetyl)thiazole-4-carboxylate **4** [10] by the same way.

The amide **1** was synthesized by coupling of 2'-methyl-2,4'-thiazole-4-carboxylic acid (**7**) with *N*-*t*-butyloxycarbonyl-1,2-diaminoethane [8] in the presence of dicyclohexylcarbodiimide (DCC) and *N*-hydroxybenzotriazole (HOBt) in conditions described previously [8]. The cleavage of the



t-butyloxycarbonyl group (Boc) was achieved in classical conditions to afford the expected compound **1**.

Methyl 2'-(2-aminoethyl)-2,4'-thiazole-4-carboxylate (**2**) was prepared by Hantzsch synthesis between the bromomethylketone **4** and the thioamide **10**. The bithiazole **2** was obtained directly as a hydrobromide, the Boc-protecting group being removed during the course of the reaction by the liberated hydrogen bromide.

A similar cyclization was achieved to yield the bithiazole derivative **12** starting from the same aminoethylthioamide but protected by a benzyloxycarbonyl *(Z)* group. The sequential pathway leading to compound **9** from bithiazole **6** was applied to the *Z*-aminoethylbithiazole **12** to afford the bis-protected bithiazole **14** in an excellent yield. The main interest of the method lies in the selective protection of the two different amino groups of side chains on 2'- and 4-positions. The bi-substituted bithiazole **14** constitutes a starting material for further syntheses by selective cleavage of either *Z*-group (by catalytic reduction) or Boc-group (in trifluoroacetic medium).

EXPERIMENTAL

Melting points were determined on a Tottoli (Büchi 510) melting point apparatus and are uncorrected. The ir spectra were obtained on a Beckman Acculab I spectrophotometer. The ^{1}H nmr spectra were obtained on a Brücker WP 80 SY spectrophotometer. Chemical shifts are reported in ppm from TMS as an internal standard and are given in δ units. Elemental analyses were performed by the "Service Central d'Analyses", CNRS, Vernaison, France.

N-(2'-Methyl-2,4'-bithiazolyl-4-carboxy)-1,2-diaminoethane (**1**).

A solution of 0.640 g of DCC (3.1 mmoles) and a suspension of 0.480 g of HOBt (3.1 mmoles) in dichloromethane (30 ml) at 0° were added to a suspension of 0.680 g (3 mmoles) of 2'-methyl-2,4'-bithiazole-4-carboxylic acid (**7**) [7] in dichloromethane (30 ml). The mixture was stirred at 0° for 1 hour. A solution of 0.480 g (3 mmoles) of *N*-Boc-1,2-diaminoethane **8** [10] in dichloromethane (20 ml) was then added. The reaction mixture was stirred at 0° for 2 hours, then overnight at room temperature. After filtration of dicyclohexylurea (DCU), the solution was washed with 1 *N* hydrochloric acid (20 ml), 1 *M* sodium bicarbonate (20 ml) and water (20 ml) and dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure afforded the crude protected amide **9** as a colorless oil; ir: 1690 cm^{-1} (O-CO). The crude product **9** was dissolved in trifluoroacetic acid (10 ml) and allowed to stand at room temperature for 10 minutes. Excess of trifluoroacetic acid was removed by evaporation under reduced pressure and the residue was neutralized by 1 *M* sodium bicarbonate. After extraction by dichloromethane, drying of the organic layer by sodium sulfate and evaporation of the solvent, the expected product **1** was isolated as a colorless oil which solidified by trituration in petroleum ether. The white solid was recrystallized from ethanol to give **1**, 0.630 g (78%), mp 115-117°; ir (potassium bromide): 2940, 2860 (CH_2), 1630 cm^{-1} (C=O); nmr (DMSO-d_6): δ 2.7 (s, CH_3, 3H), 3.2-3.6 (m, CH_2, 4H), 8.3 (s, CH, 1H), 8.5 (s, CH, 1H).

Anal. Calcd. for $C_{10}H_{12}N_4OS_2$: C, 44.8; H, 4.5; N, 20.9. Found: C, 44.5; H, 4.3; N, 20.7.

N-Boc-3-Aminopropanethioamide (**10**).

N-Boc-3-Aminopropionitrile.

To a solution of 6.4 g (50 mmoles) of 3-aminopropionitrile fumarate (Aldrich) in dichloromethane (30 ml), triethylamine (30 ml) and di-*t*-butyldicarbonate [12] (10.9 g, 50 mmoles) in dichloromethane (20 ml) were added. The stirred mixture was refluxed for 12 hours. After filtration of triethylamine hydrochloride, washing with water, and drying over anhydrous sodium sulfate, the evaporation of the solvent under reduced pressure gave a colorless oil which was purified by distillation; 8.2 g (96%) $bp_{0.1}$ = 95° (which solidified by cooling, mp <35°); ir: 3380 (NH), 2220 (C≡N), 1700 cm^{-1} (O-CO); nmr (deuteriochloroform): δ 1.5 (s, CH_3, 9H), 2.9 (t, CH_2, 2H), 3.5 (dt, CH_2, 2H, $J_{CH_2\text{-}CH_2}$ = 6 Hz, $J_{CH_2\text{-}NH}$ = 6 Hz), 5.6 (m, NH, 1H).

N-Boc-3-Aminopropanethioamide.

A solution of *N*-Boc-3-aminopropionitrile (5.1 g, 30 mmoles) in dimethylformamide (30 ml) and diethylamine (2 ml) was poured in a stainless steel bomb and allowed to react with hydrogen sulfide under 5 atmospheres pressure for 12 hours at 20°. Solvent was evaporated and the residual solid recrystallized from ethanol giving 2.9 g (48%) of white crystals; ir: 1660 (O-CO), 1650 cm^{-1} (C=S); nmr (deuteriochloroform): δ 1.5 (s, CH_3, 9H), 2.9 (t, CH_2, 2H), 3.4 (dt, CH_2, 2H, $J_{CH_2\text{-}CH_2}$ = 6 Hz, $J_{CH_2\text{-}NH}$ = 6 Hz), 5.8 (m, NH, 1H), 8.8 (m, NH_2, 2H).

Anal. Calcd. for $C_8H_{16}N_2O_2S$: C, 47.1; H, 7.8; N, 13.7. Found: C, 46.9; H, 7.8; N, 13.5.

2'-(2-Aminoethyl)-4-methoxycarbonyl-2,4'-bithiazole Hydrobromide (**2**).

A mixture of the thioamide **10** (2.04 g, 10 mmoles) and methyl 2-(2-bromoacetyl)thiazole-4-carboxylate (**4**) [10] (2.64 g, 10 mmoles) in ethanol (50 ml) was stirred for 12 hours at 20°. The Boc-protecting group was cleaved during the course of the reaction and the product **2** was isolated after filtration as a hydrobromide hydrate and recrystallized from methanol giving **2**, 3 g (82%), as a white solid, mp 244°; ir: 1715 cm^{-1} (C=O); nmr (DMSO-d_6): δ 3.3-3.9 (m, CH_2, 4H), 3.4 (s, H_2O, 2H), 3.9 (s, CH_3, 3H), 8.3 (s, CH, 1H), 8.6 (s, CH, 1H).

Anal. Calcd. for $C_{10}H_{12}BrN_3O_2S_2 \cdot H_2O$: C, 32.6; H, 3.8; N, 11.4. Found: C, 32.9; H, 3.8; N, 11.2.

N-Z-3-Aminopropanethioamide (**11**).

N-Z-3-Aminopropionitrile.

To a solution of 6.4 g (50 mmoles) of 3-aminopropionitrile fumarate in 1 *N* sodium hydroxide (40 ml) was added dropwise a solution of benzyl chloroformate (8.5 g, 50 mmoles) in 1 *N* sodium hydroxide (50 ml) at 0° over a period of 30 minutes. The mixture was stirred at room temperature, for 30 minutes following addition. The solution was then extracted by ether (3 × 250 ml) and the organic layer was washed with 1 *N* hydrochloric acid and 1 *M* sodium bicarbonate and dried over anhydrous sodium sulfate. After removal of the ether, the resulting solid was recrystallized from ethanol giving 8.5 g (82%) of white crystals, mp <35°; ir: 3340 (NH), 2960-2930 (CH_2), 2240 (C≡N), 1700 cm^{-1} (O-CO); nmr (deuteriochloroform): δ 2.5 (t, CH_2, 2H), 3.4 (dt, CH_2, 2H, $J_{CH_2\text{-}CH_2}$ = 6 Hz, $J_{CH_2\text{-}NH}$ = 6 Hz), 5.1 (s, CH_2, 2H), 5.6 (t, NH, 1H), 7.3 (s, phenyl, 5H).

Anal. Calcd. for $C_{11}H_{12}N_2O_2$: C, 64.7; H, 5.9; N, 13.7. Found: C, 64.9; H, 5.8; N, 13.5.

N-Z-3-Aminopropanethioamide.

A solution of *N-Z*-3-Aminopropanethioamide (3.06 g, 15 mmoles) in dimethylformamide (20 ml) and diethylamine (1 ml) were submitted to the action of hydrogen sulfide in the above conditions. Solvent was evaporated to dryness under reduced pressure and the residual grey solid recrystallized from ethanol to give 3.1 g (89%) of white crystals; ir: 1675 (O-CO), 1625 cm^{-1} (C=S); nmr (deuteriochloroform): δ 2.7 (t, CH_2, 2H), 3.4 (dt, CH_2, 2H, $J_{CH_2\text{-}CH_2}$ = 6 Hz, $J_{CH_2\text{-}NH}$ = 6 Hz), 5.2 (s, CH_2, 2H), 5.7 (t, NH, 1H), 7.3 (s, phenyl, 5H), 9.1 (m, NH_2, 2H).

Anal. Calcd. for $C_{11}H_{14}N_2O_2S$: C, 55.5; H, 5.9; N, 11.8. Found: C, 55.2; H, 6.1; N, 11.7.

2'-(*N-Z*-2-Aminoethyl)-4-methoxycarbonyl-2,4'-bithiazole (**12**).

A mixture of **11** (2.38 g, 10 mmoles) and bromomethylketone **4** (2.64 g, 10 mmoles) in ethanol (50 ml) was stirred for 12 hours at room temperature. The expected product crystallized from the reaction solvent and was filtered. Recrystallization from ethanol gave 2.7 g (67%) of **12**, mp 141-142°; ir: 1715 (C=O), 1680 cm^{-1} (O-CO); nmr (deuteriochloroform): δ

3.2 (t, CH_2, 2H), 3.6 (t, CH_2, 2H), 4.0 (s, CH_3, 3H), 5.1 (s, CH_2, 2H), 5.4 (m, NH, 1H), 7.3 (s, phenyl, 5H), 8.0 (s, CH, 1H), 8.1 (s, CH, 1H).

Anal. Calcd. for $C_{18}H_{17}N_3O_4S_2$: C, 53.6; H, 4.2; N, 10.4. Found: C, 53.5; H, 4.2; N, 10.2.

2'-(*N-Z*-2-Aminoethyl)-2,4'-bithiazole-4-carboxylic Acid (**13**).

The ester **12** (2.02 g, 5 mmoles) was dissolved in 30 ml of methanol and sodium hydroxide (0.8 g in 5 ml of water) was added. The mixture was stirred at room temperature for 3 hours. The solution was neutralized by 0.2 *N* hydrochloric acid. Removal of the solvents was followed by the addition of boiling ethanol. Sodium chloride was separated by filtration. The filtrate was evaporated to dryness and the residual solid was recrystallized from a mixture methanol-water (2:1) to give 1.54 g (79%) of white crystals, mp 208-209°; ir: 1690 (shoulder, O-CO), 1675 cm^{-1} (CO); nmr (DMSO-d_6): δ 3.1-3.7 (m, CH_2, 4H), 5.1 (s, CH_2, 2H), 6.0 (m, NH, 1H), 7.4 (s, phenyl, 5H), 7.5 (m, OH, 1H), 8.2 (s, CH, 1H), 8.5 (s, CH, 1H).

Anal. Calcd. for $C_{17}H_{15}N_3O_4S_2$: C, 52.4; H, 3.9; N, 10.8. Found: C, 52.0; H, 4.1; N, 10.7.

N-[2'-(*N''-Z*-2-Aminoethyl)-2,4'-bithiazole-4-carboxy]-*N'*-Boc-1,2-diaminoethane (**14**).

A solution of 0.700 g of DCC (3.4 mmoles) and a suspension of 0.530 g of HOBt (3.4 mmoles) in dichloromethane (50 ml) at 0° were added to a solution of 1.25 g (3.2 mmoles) of compound **13** in dichloromethane (50 ml). The mixture was stirred at 0° for 1 hour. A solution of *N*-Boc-1,2-diaminoethane **8** (0.51 g, 3.2 mmoles) in dichloromethane (30 ml) was added and the mixture was stirred at 0° for 2 hours, then overnight at room temperature. DCU was removed by filtration and the filtrate washed with 1 *N* hydrochloric acid (20 ml), 1 *M* sodium bicarbonate (20 ml) and water (20 ml) then dried over anhydrous sodium sulfate. The solvent was evaporated *in vacuo* giving **14** as a white solid. Recrystallization from a mixture ethanol-ether gave 1.26 g (74%) of white crystals, mp 76-78°; ir: 1700 (O-CO); nmr (DMSO-d_6): δ 1.5 (s, CH_3, 9H), 3.3-3.9 (m, CH_2, 8H), 4.3 (s, NH, 1H), 5.2 (s, CH_2, 2H), 5.6 (s, NH, 1H), 7.4 (s, phenyl, 5H), 7.9 (s, NH, 1H), 8.0 (s, CH, 1H), 8.2 (s, CH, 1H).

N-[2'-(2-Aminoethyl)-2,4'-bithiazolyl-4-carboxy]-1,2-diaminoethane Dihydrobromide (**3**).

The Boc-*Z*-diprotected compound **14** (1.06 g, 2 mmoles) was dissolved in a solution of anhydrous hydrogen bromide (3 g) in acetic acid (20 ml). The cleavage was found effective after a contact of 15 minutes. The solvent and the excess of hydrogen bromide were removed by evaporation. The crude oily residue was triturated in anhydrous ether to afford a white solid which was recrystallized from ethanol giving 0.7 g (78%) of white crystals, mp 262-265°; ir: 1640 cm^{-1} (C=O); nmr (DMSO-d_6): δ 3.2-3.9 (m, CH_2, 8H), 8.2 (s, CH, 1H), 8.3 (s, CH, 1H).

Anal. Calcd. for $C_{11}H_{17}Br_2N_5OS_2$: C, 28.8; H, 3.7; N, 15.3. Found: C, 28.5; H, 3.7; N, 15.0.

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