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Condensed Thiazoles, I: Synthesis of 5,7-Disubstituted Thiazolo[4,5-*d*]pyrimidines as Possible Anti-HIV, Anticancer, and Antimicrobial Agents

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Summary. A convenient and simple synthesis of 5-mercapto-3-phenyl-2-thioxo-2,3-dihydrothiazolo-[4,5-*d*]pyrimidin-7(6*H*)-one (**2**) and its 5,7-dichloro (**3**), 5,7-diazido (**4**), 5,7-diamino (**5**), 5,7-dimerapto (**6**), 5,7-dimethylthio (**7**), and 6-methyl-5-methylthio (**8**) derivatives is described. The prepared compounds were screened for their *in vitro* anti-HIV, anticancer, antibacterial, and antifungal activities.

Keywords. Thiazolo[4,5-*d*]pyrimidines.

Kondensierte Thiazole, 1. Mitt.: Synthese von 5,7-disubstituierten Thiazolo[4,5-*d*]pyrimidinen als potentielle anti-HIV, anticancerogene und antimikrobielle Verbindungen

Zusammenfassung. Die Synthese von 5-Mercapto-3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]-pyrimidin-7(6*H*)-on (**2**) und seiner 5,7-dichloro- (**3**), 5,7-diazido- (**4**), 5,7-diamino- (**5**), 5,7-dimercapto- (**6**), 5,7-dimethylthio- (**7**) und 6-methyl-5-methylthio-Derivaten (**8**) wird beschrieben. Die hergestellten Verbindungen wurden auf ihre *in vitro* anti-HIV, anticancerogenen, antibakteriellen und antimykotischen Aktivitäten geprüft.

Introduction

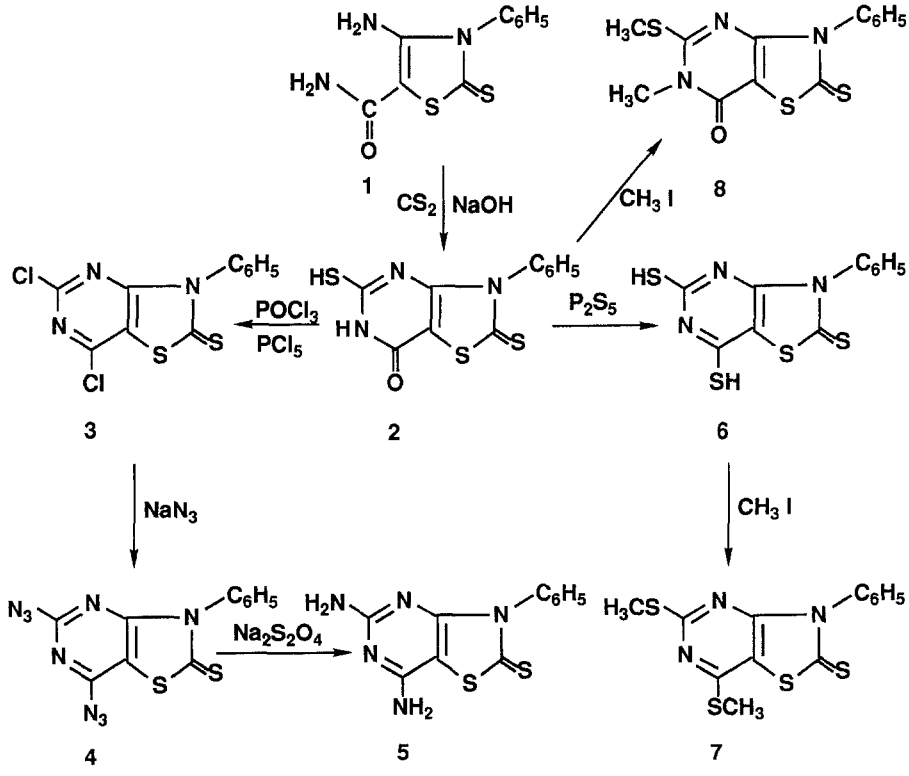
The synthesis of thiazolo[4,5-*d*]pyrimidines has been successfully accomplished by various methods. A 4-amino-5-ethoxycarbonylthiazole derivative has been cyclized to a thiazolo[4,5-*d*]pyrimidine by its reaction with phenyl isothiocyanate [1]. Many 4-amino-5-carbamoylthiazole derivatives have been cyclized to the corresponding thiazolo[4,5-*d*]pyrimidines using a triethyl orthoformate/acetic anhydride mixture [2–5]. Moreover, 4-amino-5-cyano thiazoles have been used to prepare the same fused ring system *via* their reaction with triethyl orthoformate, followed by treatment of the produced intermediate with hydrogen sulfide, guanidine, amines, and isothiocyanates [6, 7]. Other thiazolo[4,5-*d*]pyrimidines have been obtained from 4-amino-5-cyano, carbamoyl, or ethoxycarbonyl thiazoles *via* cyclization with acetic anhydride [8] or formic acid [9].

These compounds have aquired a growing importance as anticancer [10] and antiviral [11–15] agents, being considered as thia analogues of the naturally occuring purine bases. Based on the above mentioned findings and in continuation of our previous work in the same area [16, 17], we describe here a simple method for the synthesis of some 5,7-disubstituted thiazolo[4,5-*d*]pyrimidines as well as their screening for antimicrobial, anti-HIV, and anticancer activities.

Results and Discussion

The synthesis of thiazolo[4,5-*d*]pyrimidines was accomplished *via* the cyclization of 4-amino-5-carbamoyl-3-phenylthiazole-2(3*H*)-thione (**1**) [2] with carbon disulfide in the presence of sodiumhydroxide to give 5-mercapto-3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7-(6*H*)-one (**2**). Chlorination of **2** with a mixture of phosphorous pentachloride and phosphorous oxychloride afforded the 5,7-dichloro compound **3**. Displacement of the chlorine atoms in this compound with sodium azide gave the 5,7-diazido derivative **4** which, upon reduction with sodium dithionite, gave the 5,7-diamino analogue **5**. Thiation of **2** with phosphorous pentasulfide yielded the 5,7-dimercapto derivative **6** which, upon methylation with methyl iodide, gave the 5,7-dimethylthio compound **7**. On the other hand, methylation of the parent compound **2** yielded the N-methyl-S-methyl derivative **8**.

Compounds **2**, **3** and **6** were screened for their anti-HIV activity following the NCI *in vitro* anti-AIDS Discovery Program [18], and for their anticancer activity against 60 human cell lines derived from 7 types of cancer (lung, colon, melanoma,



renal, ovarian, brain, and leukemia) following the NCI Preclinical Antitumor Drug Discovery Screen [19]. However, none of them was found to be active in these programs. In addition, all prepared compounds were tested for their *in vitro* antimicrobial activity against *Staphylococcus aureus* (ATCC 29523), *Escherichia coli* (HP 101), *Proteus vulgaris*, *Candida albicans* (NCTC 2708), *Aspergillus niger*, and *Penicillium species*. The agar diffusion technique [20] was adopted to determine the inhibition zones (*IZ*), and compounds with $IZ > 25$ mm were evaluated for their minimal inhibitory concentrations (*MIC*) against the most sensitive organisms. The compounds were found to possess good antifungal activity against *Aspergillus niger* ($IZ = 24$–32 mm, $MIC < 50 - < 25$ μg/ml) and *Penicillum species* ($IZ = 25$–34 mm, $MIC < 50$ μg/ml).

Experimental

Melting points were determined in open-glass capillaries on a Gallenkamp apparatus and are uncorrected. IR spectra were recorded as KBr discs on a Perkin Elmer 1430 spectrometer. ^{1}H NMR spectra were recorded on a Varian EM-390 NMR spectrometer at 90 MHz using tetramethylsilane as internal standard. LC-MS were recorded using a Vestec model 201. Microanalyses were carried out at the microanalytical unit, Faculty of Science, Cairo University; the experimental values were in good accordance with the calculated ones.

5-Mercapto-3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one ($C_{11}H_7N_3OS_3$; **2**)

To a stirred solution of **1** [2] (2.5 g, 10 mmol) in *DMF* (10 ml), carbon disulfide (0.6 ml, 10 mmol) and sodium hydroxide (0.4 g, 10 mmol) were added. The reaction mixture was stirred for 5 h at room temperature; the product obtained after dilution with water and neutralization with dilute hydrochloric acid was filtered, washed with water, and dried.

Yield: 1.76 g (60%); m.p.: 230–232 °C (aqueous *DMF*); IR 3390, 3240, 3170 (N–H), 2860 (S–H), 1650 (C=O), 1640 (C=N), 1610, 1510 (C=C), 1520 (δN–H), 1560, 1250, 1070, 980 (N–C=S), 1030 (C–S–C) cm^{-1}.

5,7-Dichloro-3-phenylthiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{11}H_5Cl_2N_3S_2$; **3**)

A solution of **2** (2.93 g, 10 mmol) and phosphorous pentachloride (4.16 g, 20 mmol) in phosphorous oxychloride (20 ml) was heated under reflux for 5 h. The excess phosphorous oxychloride was distilled off under vacuum, and the residue was triturated with cold water and neutralized with saturated sodium bicarbonate solution. The product obtained was filtered, washed with water, and dried.

Yield: 1.89 g (60%); m.p.: 285–287 °C (acetone); IR 1720, (ClC=N), 1710 (ClC=N), 1590, 1490 (C=C), 1540, 1270, 1070, 860 (N–C=S), 1245, 1030 (C–S–C) cm^{-1}.

5,7-Diazido-3-phenylthiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{11}H_5N_9S_2$; **4**)

Sodium azide (1.30 g, 20 mmol) was added to a stirred solution of **3** (3.14 g, 10 mmol) in dry acetone (20 ml). The reaction mixture was heated under reflux for 2 h and cooled. The product obtained after addition of water was filtered, washed with water, and dried.

Yield: 2.03 g (62%); m.p.: 153–155 °C (ethanol); IR: 2120 (N_3), 1710 (C=N), 1590, 1485 (C=C), 1570, 1260, 1050, 980 (N–C=S), 1250, 1030 (C–S–C) cm^{-1}; LC-MS (*m/z*, %): 328 ($M^{+\cdot}$ + 1, 75.5), 312 (100).

5,7-Diamino-3-phenylthiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{11}H_9N_5S_2$; **5**)

To a solution of **4** (3.27 g, 10 mmol) in ethanol (20 ml), a solution of sodium dithionite (2.0 g) in water (10 ml) was gradually added. The reaction mixture was heated under reflux for 1 h. Ethanol was evaporated on a water bath, and the product obtained after cooling was filtered, washed with water, and dried.

Yield: 1.68 g (61%); m.p.: 180–182 °C (ethanol); IR: 3460, 3350, 3300, 3200 (N–H), 1680, 1660 (C=N), 1620, 1490 (C=C), 1560 (δN–H), 1530, 1290, 1065, 840 (N–C=S), 1255, 1030 (C–S–C) cm^{-1}; LC-MS (*m/z*, %): 276 ($M^{+\bullet}$ + 1, 60), 260 (M^+–NH, 100).

5,7-Dimercapto-3-phenylthiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{11}H_7N_3S_4$; **6**)

A mixture of **2** (2.93 g, 10 mmol) and phosphorous pentasulfide (4.44 g, 20 mmol) in xylene (20 ml) was heated under reflux for 5 h and then cooled. Petroleum ether was added and the product obtained was filtered, washed with ethanol, and dried.

Yield: 1.55 g (50%); m.p.: 205–207 °C (aqueous *DMF*); IR: 3500, 3300 (N–H), 2870 (S–H), 1640 (C=N), 1590, 1510 (C=C), 1560, 1250, 1050, 970 (N–C=S), 1035 (C–S–C) cm^{-1}.

5,7-Dimethylthio-3-phenylthiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{13}H_{11}N_3S_4$; **7**)

To a mixture of **6** (3.09 g, 10 mmol) and anhydrous potassium carbonate (2.76 g, 20 mmol) in dry acetone (20 ml), methyl iodide (2.84 g, 1.24 ml, 20 mmol) was added. The reaction mixture was heated under reflux for 3 h and cooled. The product obtained after addition of water was filtered, washed with water, and dried.

Yield: 2.09 g (62%); m.p.: 210–212 °C (ethanol); IR: 1670, 1640 (C=N), 1590, 1510 (C=C), 1535, 1250, 1070, 860 (N–C=S), 1230, 1030 (C–S–C) cm^{-1}; ^1H NMR (CF_3COOH): 2.5 (s, 3H, C_5–SCH_3), 2.9 (s, 3H, C_7–SCH_3), 7.2–7.7 (m, 5H, Ar–H) ppm; LC-MS (*m/z*, %): 338 ($M^{+\bullet}$ + 1, 86), 322 (28.8), 260 (100).

6-Methyl-5-methylthio-3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one ($C_{13}H_{11}N_3OS_3$; **8**)

To a mixture of **2** (2.93 g, 10 mmol) and anhydrous potassium carbonate (2.76 g, 20 mmol) in dry acetone (20 ml), methyl iodide (2.84 g, 1.24 ml, 20 mmol) was added. The reaction mixture was heated under reflux for 3 h and cooled. The product obtained after addition of water was filtered, washed with water, and dried.

Yield: 2.28 g (71%); m.p.: 297–299 °C (aqueous *DMF*); IR: 1680 (C=O), 1630 (C=N), 1590, 1510 (C=C),1560, 1255, 1030, 870 (N–C=S), 1230, 1075 (C–S–C) cm^{-1}; ^1H NMR (CF_3COOH): 2.3 (s, 3H, SCH_3), 3.7 (s, 3H, NCH_3), 7.2–7.7 (m, 5H, Ar–H) ppm.

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