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

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Summary. Some 3-substituted 2-thioxothiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones (**2a, b**; **4**) have been synthesized and converted to their 7-chloro (**3**, **5**), 7-diethanolamino (**6a, b**), 7-*bis*(2-chloroethyl)amino (**7**), 7-azido (**8**), 7-amino (**9**), 7-hydrazino (**10**), 7-mercapto (**11a, b**), and 7-methylthio (**12**) derivatives. These compounds were evaluated for their *in vitro* antimicrobial, anti-HIV, and anticancer activities.

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

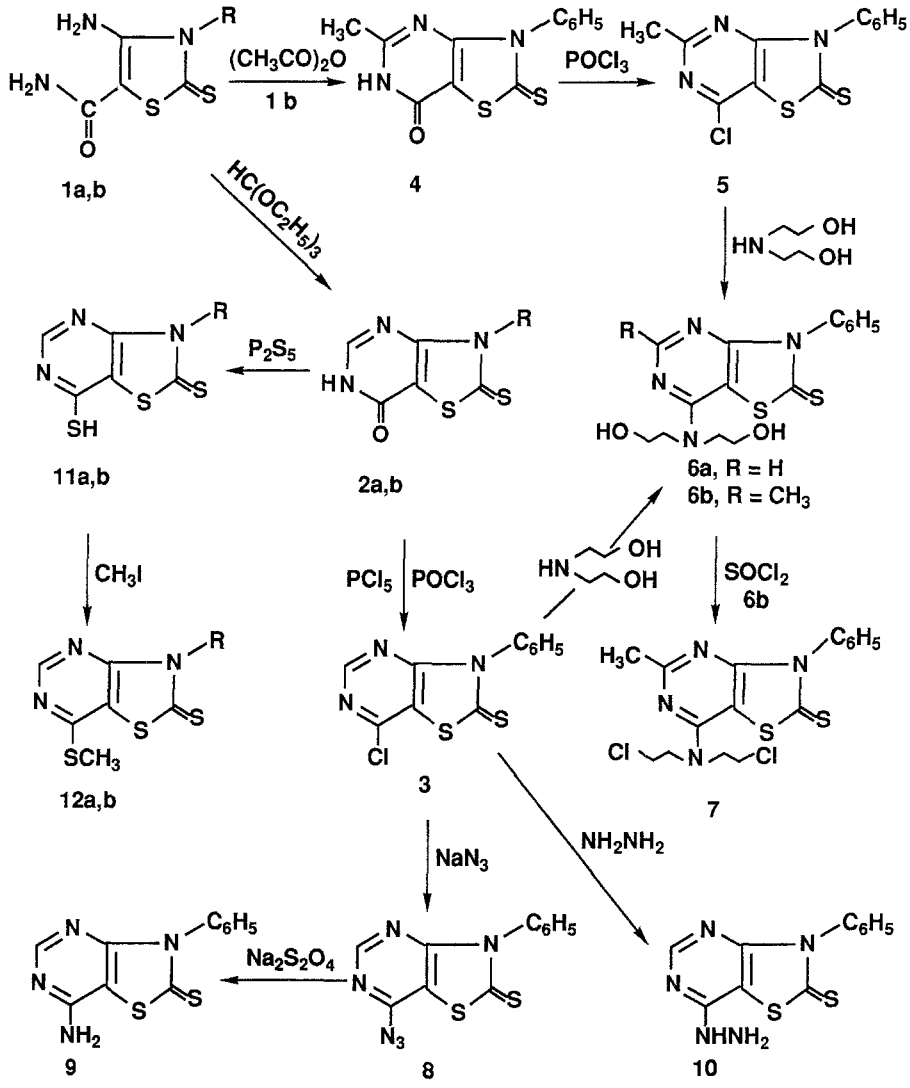
Kondensierte Thiazole, 2. Mitt.: Synthese von 7-substituierten Thiazolo[4,5-*d*]pyrimidinen als potentielle anti-HIV, anticancerogene und antimikrobielle Verbindungen

Zusammenfassung. Einige 3-substituierte 2-Thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (**2a, b**; **4**) wurden hergestellt und zu 7-Chloro- (**3**, **5**), 7-Diethanolamino- (**6a, b**), 7-*bis*(2-Chloroethyl) amino- (**7**), 7-Azido- (**8**), 7-Amino- (**9**), 7-Hydrazino- (**10**), 7-Mercapto- (**11a, b**) und 7-Dimethylthioderivaten (**12a, b**) umgesetzt. Diese Verbindungen wurden auf ihre *in vitro* antimikrobiellen, anti-HIV und anticancerogenen Aktivitäten geprüft.

Introduction

Several methods for the synthesis of thiazolo[4,5-*d*]pyrimidines have been described in the literature, starting either from 4-aminothiazoles [1–5] or from 6-aminopyrimidines [6–8]. The importance of these compounds as antiviral [9–11] and anticancer [12] agents is due to their structural similarity to purine bases. Recently we have described three methods for the synthesis of such fused ring system [13, 14]. As a continuation of this work, we report here the synthesis of new 7-substituted derivatives of thiazolo[4,5-*d*]pyrimidines as well as their evaluation for anti-HIV, anticancer, and antimicrobial activities.

[#] For part I of this series, see Monatsh Chem **127:** 1203



1a, b, 2a, b, 11a, b, 12a, b: a: $R = CH_2CH=CH_2$
b: $R = C_6H_5$

Results and Discussion

The synthesis of the starting thiazolo[4,5-*d*]pyrimidines **2a, b** and **4** was achieved by cyclization of 4-amino-5-carbamoylthiazole-2(3*H*)-thiones **1a, b** with either triethyl orthoformate [2] or acetic anhydride [14]. Chlorination of **2b** and **4** afforded the 7-chloro compounds **3** and **5**. Treatment of **3** with sodium azide gave the 7-azido derivative **8** which, upon reduction with sodium dithionite, afforded the 7-amino analogue **9**. Substitution of the chlorine atom of **3** with hydrazine hydrate resulted in the 7-hydrazino derivative **10**. The chloro compounds **3** and **5** were also utilized for the synthesis of the 7-diethanolamino derivatives **6a, b**. Reaction of **6b** with thionyl chloride gave the 7-*bis*(2-chloroethyl)amino derivative **7** which exists in the aziridinium form in *DMSO*-d_6 as proved by the ^1H and ^{13}CNMR spectra,

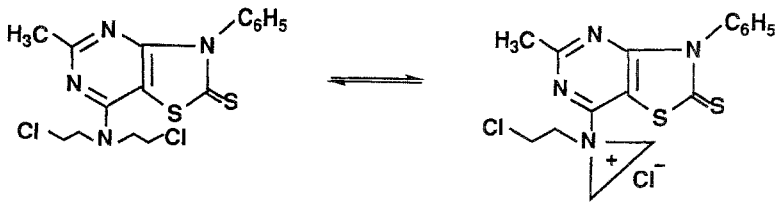


Fig. 1.

whereas the FD mass spectrum shows molecular ion peaks corresponding to both forms (Fig. 1). Thiation of **2a, b** with phosphorous pentasulfide gave the 7-mercapto compounds **11a, b** which upon methylation produced the 7-methylthio analogues **12a, b**.

Compounds **2a, b, 3, 4, 6a,b, 8, 9, 11a,b,** and **12b** were screened for their anti-HIV activity according to the NCI *in vitro* anti-AIDS Discovery Program [15] 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 [16]; they were found to be inactive. In addition, all prepared compounds were evaluated for their antibacterial activity against *Staphylococcus aureus* (ATCC 29523), *Escherichia coli* (HP 101), and *Proteus vulgaris*, and for their antifungal activity against *Candida albicans* (NCTC 2708), *Aspergillus niger*, and *Penicillium species* by measurement of their inhibition zones using the agar diffusion method [17] and measurement of their MIC values using the serial dilution method [18]. Pronounced antifungal activity was observed against the fungi *Aspergillus niger* ($IZ = 20$–40 mm, $MIC < 50 - < 25$ μg/ml) and *Penicillium species* ($IZ = 30$–38 mm, $MIC < 50$ μg/ml).

Experimental

Melting point were determined in open-glass capillaries on a Gallenkamp apparatus and are uncorrected. The 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 *TMS* as internal standard. ^{13}C NMR spectra were recorded on a Varian VXR-300 NMR spectrometer at 75 MHz. LC-MS were recorded using a Vestec Model 201. Mass spectra were obtained using a Mat-711 mass spectrometer. Microanalyses were carried out at the microanalytical unit, Faculty of Science, Cairo University; the experimental values were in good accordance with the calculated ones. Compounds **4** and **5** were prepared as reported previously [14].

3-Allyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one ($C_8H_7N_3OS_2$; **2a**)

2a was prepared from **1a** (10 mmol) and triethyl orthoformate using the method reported by *Gewald* [2].

Yield: 2.04 g (90%); m.p.: 207–209 °C (aqueons *DMF*); IR: 3220 (N–H), 1670 (C=O), 1650 (C=N), 1590, 1500 (C=C), 1550 (δN–H), 1535, 1270, 1090, 920 (N–C=S), 1220, 1040 (C–S–C) cm^{-1}; ^{1}H NMR (CF_3COOH): 5.1 (d, $J = 12$ Hz, 2H, $NCH_2CH{=}CH_2$), 5.0–5.3 (m, 2H, $NCH_2CH{=}CH_2$), 5.4–6.0 (m, 1H, $NCH_2CH{=}CH_2$), 8.4 (s, 1H, C_5–H) ppm.

3-Phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidine (**2b**)

2b was prepared from **1b** and triethyl orthoformate using the reported method [2]; m.p.: 234 °C.

7-Chloro-3-phenylthiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{11}H_6ClN_3S_2$; **3**)

A solution of **2b** (2.61 g, 10 mmol) and phosphorous pentachloride (2.08 g, 10 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 saturated sodium bicarbonate solution. The product obtained was filtered, washed with water, and dried.

Yield: 1.95 g (70%); m.p.: 162–164 °C (acetone); IR: 1710, 1670 (C=N), 1590, 1490 (C=C), 1550, 1260, 1030, 970 (N–C=S), 1220 (C–S–C) cm^{-1}; ^1H NMR (CF_3COOH): 7.2–7.7 (m, 5H, Ar–H), 8.9 (s, 1H, C_5–H) ppm.

7-(bis(2-Hydroxyethyl)amino)-3-phenylthiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{15}H_{16}N_4O_2S_2$; **6a**)

To a solution of **3** (2.79 g, 10 mmol) in dry acetone (20 ml), diethanolamine (2.1 g, 1.9 ml, 20 mmol) was added. The reaction mixture was heated under reflux for 5 h, concentrated, and cooled. The product obtained after addition of water was filtered, washed with water, and dried.

Yield: 2.16 g (62%); m.p.: 165–167 °C (ethanol); IR: 3500–3200 (O–H), 1660 (C=N), 1580, 1490 (C=C), 1525, 1260, 1050, 980 (N–C=S), 1240, 1040 (C–S–C) cm^{-1}; LC-MS (*m/z*, %): 349 ($M^{+\cdot}+1$, 100); ^1H NMR (*DMSO*-d_6): 4.2 (br s, 8H, $N(CH_2CH_2OH)_2$, 7.2–7.8 (m, 5H, Ar–H), 8.4 (s, 1H, C_5-H) ppm.

7-(bis(2-Hydroxyethyl)amino)-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{16}H_{18}N_4O_2S_2$; **6b**)

6b was prepared similarly to **6a** from **5** (2.94, 10 mmol) and diethanolamine (2.1 g, 1.9 ml, 20 mmol).

Yield: 2.72 g (75%); m.p.: 180–182 °C (ethanol); IR: 3600–3200 (O–H), 1680, 1650 (C=N), 1590, 1490 (C=C), 1550, 1260, 1050, 900 (N–C=S), 1240, 1040 (C–S–C) cm^{-1}; ^1H NMR (*DMSO*-d_6): 2.26 (s, 3H, CH_3), 3.6–3.7 (two t, $J = 7$ Hz, each 4H, $N(CH_2CH_2OH)_2$, 4.9 (t, 2H, two OH), 7.3–7.5 (m, 5H, ArH) ppm; ^{13}C NMR (*DMSO*-d_6): 25.55 (CH_3), 51.64 (NCH_2), 59.21 (CH_2OH), 89.92 (C_{7a}), 134.07, 128.44, 128.83, 129.13 (C_1, $C_{2,6}$, $C_{3,5}$, C_4 of C_6H_5), 155.0 (C_{3a}), 156.0 (C_5), 163.30 (C_7), 191.0 (C_2) ppm.

7-(bis(2-Chloroethyl)amino)-5-methyl-3-phenylthiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{16}H_{16}Cl_2N_4S_2$; **7**)

To a solution of **6b** (3.62 g, 10 mmol) in dry chloroform (20 ml), a solution of thionyl chloride (1.5 ml, 20 mmol) in dry chloroform (5 ml) was added dropwise under stirring. Stirring was continued for 1 h at room temperature. The reaction mixture was then heated under reflux for 2 h on a water bath during which the product separated, and cooled. Chloroform was removed by distillation under vacuum, and the residue obtained was triturated with ether, filtered, and dried.

Yield: 3.19 g (80%); m.p.: 220–222 °C (ethanol/ether); IR 2940, 2860 (N^+), 1690, 1640 (C=N), 1570, 1490 (C=C), 1540, 1260, 1050, 870 (N–C=S), 1245, 1030 (C–S–C) cm^{-1}; ^1H NMR (*DMSO*-d_6): 2.53 (s, 3H, CH_3), 4.04–4.06 (m, 4H, $2CH_2$), 4.25 (t, $J = 9.5$ Hz, 2H, CH_2N), 4.78 (t, $J = 9.5$ Hz, 2H, CH_2Cl), 7.4–7.6 (m, 5H, Ar–H) ppm; FD-MS (*m/z*, %): 402(2), 400(8), 398(25) and 365(33), 363(100) corresponding to $C_{16}H_{16}Cl_2N_4S_2$ and $[C_{16}H_{16}ClN_4S_2]^+Cl^-$, respectively; EI-MS (*m/z*, %): 299 (1.2), 272 (1.2), 259 (1.2), 228 (1.2), 223 (1.2), 191 (1.2), 182 (1.2), 165 (2.4), 147 (3.6), 143 (7.0), 135 (100), 108 (6), 91 (6), 77 (47), 51 (15); ^{13}C NMR (*DMSO*-d_6): 21.3 (CH_3), 41.1 (CH_2Cl), 47.9 (aziridine-CH_2), 48.4 (aziridine-CH_2), 48.7 (CH_2N), 95.3 (C_{7a}), 128.5, 129.8, 130.1, 134.8 ($C_{2,6}$, $C_{3,5}$, C_4, C_1 of C_6H_5), 149.7 (C_{3a}), 159.4 (C_7), 159.6 (C_5), 189.4 (C_2) ppm.

7-Azido-3-phenylthiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{11}H_6N_6S_2$; **8**)

Sodium azide (0.65 g, 10 mmol) was added to a stirred solution of **3** (2.79 g, 10 mmol) in dry acetone. 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 (71%); m.p.: 275–277 °C (ethanol); IR: 2120 (N_3), 1680, 1640 (C=N), 1580, 1490 (C=C), 1555, 1260, 1070, 865 (N–C=S), 1225, 1040 (C–S–C) cm^{-1}.

7-Amino-3-phenylthiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{11}H_8N_4S_2$; **9**)

To a solution of **8** (2.86 g, 10 mmol) in ethanol (20 ml), a solution of sodium dithionite (1.0 g) in water (10 ml) was gradually added. The reaction mixture was heated under reflux for 1 h. Ethanol was then evaporated on a water bath, and the product obtained after cooling was filtered, washed with water, and dried.

Yield: 1.82 g (70%); m.p.: 175–177 °C (ethanol); IR: 3300, 3100 (N–H), 1660 (C=N), 1580, 1490 (C=C), 1520 (δN–H), 1550, 1230, 1070, 880 (N–C=S), 1020 (C–S–C) cm^{-1}; LC–MS (*m/z*, %): 261 (100, $M^{+\bullet} + 1$).

7-Hydrazino-3-phenylthiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{11}H_9N_5S_2$; **10**)

A solution of **3** (2.79 g, 10 mmol) in absolute ethanol (10 ml) was gradually added to a stirred solution of 99% hydrazine hydrate (5 ml) in ethanol (10 ml). The reaction mixture was heated under reflux for 2 h and cooled. The product obtained after addition of water was filtered, washed with ethanol, and dried.

Yield: 1.65 g (60%); m.p.: 240–242 °C (*DMF*); IR: 3350, 3250 (N–H), 1640 (C=N), 1595, 1490 (C=C), 1520 (δN–H), 1540, 1280, 1070, 950 (N–C=S), 1240, 1040 (C–S–C) cm^{-1}.

3-Allyl-7-mercaptothiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_8H_7N_3S_3$; **11a**)

A mixture of **2a** (2.25 g, 10 mmol) and phosphorous pentasulfide (2.22 g, 10 mmol) in xylene (20 ml) was heated under reflux for 5 h and then cooled. The product obtained after addition of petroleum ether was filtered, washed with ethanol, and dried.

Yield: 1.76 g (73%); m.p.: 250–252 °C (aqueous *DMF*); IR: 3100 (N–H), 2640 (S–H), 1640 (C=N), 1585, 1485 (C=C), 1535 (δN–H), 1550, 1280, 1080, 930 (N–C=S), 1255, 1020 (C–S–C) cm^{-1}.

3-Phenyl-7-mercaptothiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{11}H_7N_3S_3$; **11b**)

11b was prepared similarly to **11a** from **2b** (2.61 g, 10 mmol) and phosphorous pentasulfide (2.22 g, 10 mmol).

Yield: 2.08 g (75%); m.p.: 275–277 °C (aqueous *DMF*); IR: 3100 (N–H), 2630 (S–H), 1640 (C=N), 1575, 1470 (C=C), 1530 (δN–H), 1540, 1225, 1065, 970 (N–C=S), 1210, 1020 (C–S–C) cm^{-1}.

3-Allyl-7-methylthiothiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_9H_9N_3S_3$; **12a**)

To a mixture of **11a** (2.41 g, 10 mmol) and anhydrous potassium carbonate (1.38 g, 10 mmol) in dry acetone (20 ml), methyl iodide (1.42 g, 0.62 ml, 10 mmol) was added. The reaction mixture was heated under reflux for 3 h and then cooled. The product obtained after addition of water was filtered, washed with water, and dried.

Yield: 1.78 g (70%); m.p.: 100–102 °C (*DMF*); IR: 1640 (C=N), 1580, 1500, (C=C), 1560, 1260, 1070, 960 (N–C=S), 1230, 1040 (C–S–C) cm^{-1}; ^1H NMR (CF_3COOH): 2.7 (s, 3H, SCH_3), 4.95 (d, J = 12 Hz, 2H,

$NCH_2CH{=}CH_2$), 5.0–5.25 (m, 2H, $NCH_2CH{=}CH_2$), 5.8–6.0 (m, 1H, $NCH_2CH{=}CH_2$), 8.9 (s, 1H, C_5-H) ppm.

3-Phenyl-7-methylthiothiazolo[4,5-d]pyrimidine-2(3H)-thione ($C_{12}H_9N_3S_3$; **12b**)

12b was prepared similarly to **12a** from **11b** (2.77 g, 10 mmol) and methyl iodide (1.42 g, 10 mmol).

Yield: 2.12 g (73%); m.p.: 198–200 °C (*DMF*); IR: 1640 (C=N), 1580, 1500 (C=C), 1560, 1260, 1070, 960 (N–C=S), 1230, 1040 (C–S–C) cm^{-1}; ^1H NMR (CF_3COOH): 2.9 (s, 3H, SCH_3), 7.1–7.9 (m, 5H, ArH), 8.7 (s, 1H, C_5-H) ppm.

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