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Potential anti-microbials. II. Synthesis and *in vitro* anti-microbial evaluation of some thiazolo[4,5-*d*]pyrimidines

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Summary — Some thiazolo[4,5-*d*]pyrimidines were prepared in order to investigate their antimicrobial activity. A significant inhibitory effect was recorded for many compounds against *Staphylococcus aureus* ATCC 25923 (**3**, **6**, **9a**; MIC = 10–20 μg/ml), *Escherichia coli* ATCC 25922 (**3**, **5**, **9b**, MIC = 40 μg/ml) and *Candida albicans* DSM 70443 (**6**, MIC = 50 μg/ml).

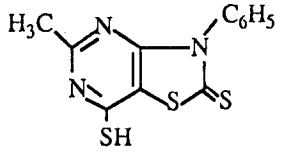
thiazolo[4,5-*d*]pyrimidines / anti-bacterial / anti-fungal

Introduction

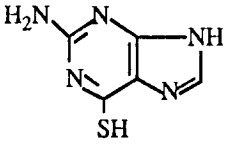
In a previous paper [1], we have described an easy method for the synthesis of some 3,6-diarylthiazolo[4,5-*d*]pyrimidines to be evaluated as anti-microbial agents. As a result of the interesting *in vitro* anti-microbial potencies recorded for some derivatives of this bicyclic system, which resembles the natural purines with respect to size and to the 5-membered ring fused to the pyrimidine nucleus, we now report the synthesis and *in vitro* anti-microbial activity of some 5-methylthiazolo[4,5-*d*]pyrimidin-2(3*H*)-thiones with various substituents at N-3, N-6 and C-7 (scheme 1). The structural similarities between compound **6** and 6-thioguanine (fig 1) may add a special criterion to the possible activity of this compound.

Chemistry

Many thiazolo[4,5-*d*]pyrimidine derivatives were succesfully synthesized either by reacting substituted 4-aminothiazole-5-carboxamides with ethyl *ortho*-formate-acetic anhydride mixture [2–5] or by cyclizing 4-ethoxymethyleneaminothiazole-5-carbonitriles with ammonia [5], hydrogen sulfide [6] or guanidine and allyl amine [7]. In the preceding paper [1], we described the syntheses of some 2,3-dihydro-3,6-diaryl-5-mercaptothiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones through condensation of some ethyl 4-amino-thiazole-5-carboxylates with isothiocyanates. In conjunction with this work, we report here two additional synthetic approaches to some 5-methylthiazolo-[4,5-*d*]pyrimidin-7(6*H*)-thiones having an amino substitution (**3**) or remaining unsubstituted (**4**) at N-6 (scheme 1). Thus the reaction of ethyl 4-amino-2,3-dihydro-3-phenyl-2-thioxothiazole-5-carboxylate **1d** with acetyl chloride gave the corresponding 4-acetamido derivative **2**, which when treated with hydrazine hydrate formed 6-amino-2,3-dihydro-5-methyl-3-phenyl-2-thioxothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one **3**, whereas the 6-unsubstituted analogues **4a–c** were obtained in excellent yields by refluxing 4-amino-2,3-



6



6 - Thioguanine

Fig 1. The structural similarities between compound **6** and 6-thioguanine.

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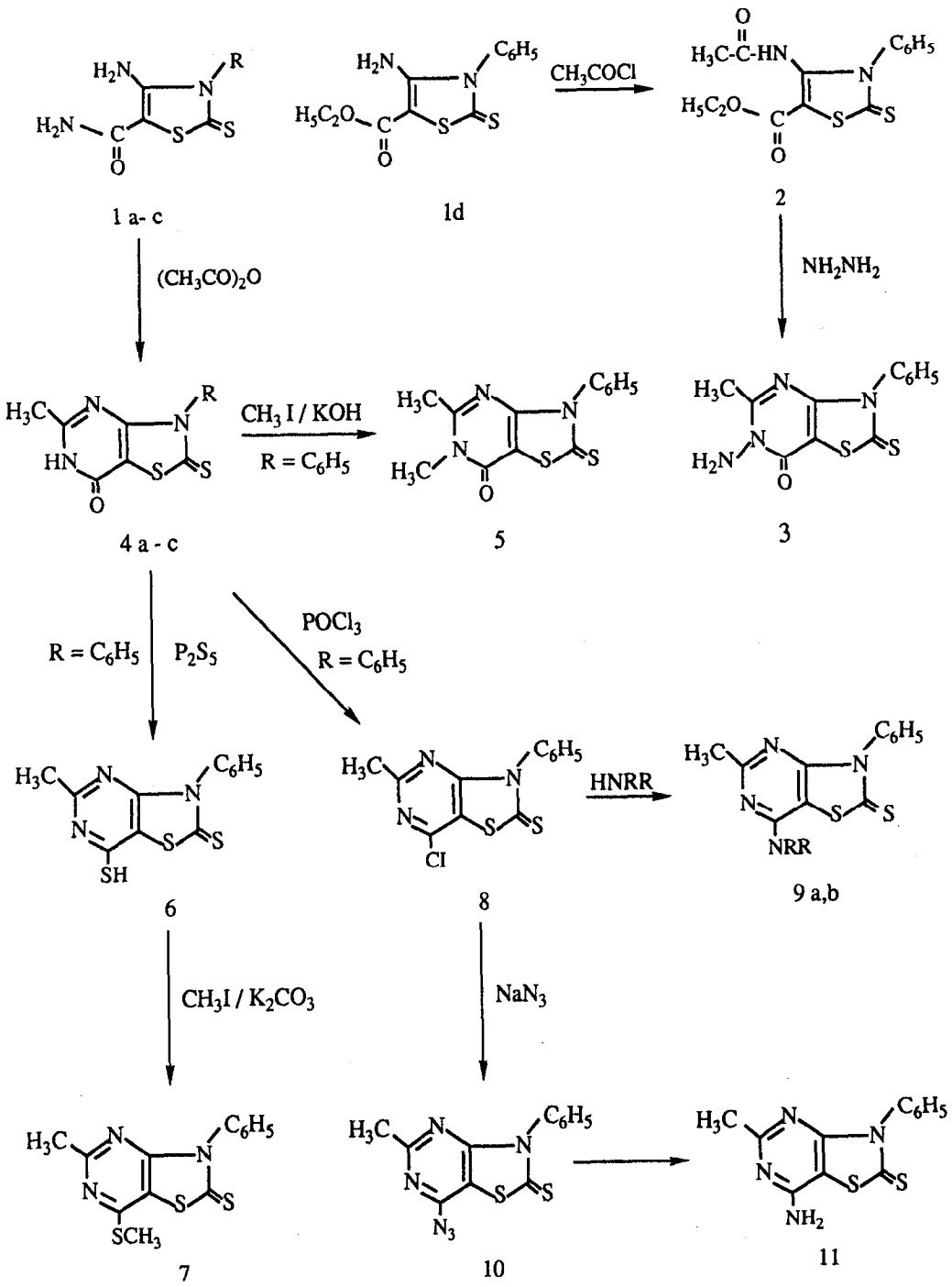

Scheme 1. (**1,4**) **a**: R = CH_2:$CHCH_2$; **b**: R = C_6H_5; **c**: R = 4-ClC_6H_4; **9a**: NRR = $N(C_2H_5)$; **9b**: NRR = morpholino.

dihydro-3-substituted-2-thioxothiazole-5-carboxamides **1a–c** with acetic anhydride. Methylation of **4b** with methyl iodide afforded 2,3-dihydro-5,6-dimethyl-3-phenyl-2-thioxothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one **5**. The appearance of a methyl singlet at δ 3.5 in its ^{1}H-NMR spectrum and a strong amide C=O absorption band at 1680 cm^{-1} in the IR spectrum confirmed *N*- and not *O*-methylation. On the other

hand, 5-methyl-7-methylmercapto-3-phenylthiazolo-[4,5-*d*]pyrimidin-2-(3*H*)-thione **7** was obtained by direct thiation of **4b** with phosphorus pentasulfide followed by treatment of the formed 7-mercapto derivative **6** with methyl iodide. The ^{13}C-NMR of compound **6** revealed two signals corresponding to C-5 and C–SH at 172.89 and 153.54, respectively, and another signal for the 2-thioxo at 191.20 δ ppm. Chlorination of **4b** with phosphoryl chloride yielded 7-chloro-5-methyl-3-phenylthiazolo[4,5-*d*]pyrimidin-2-(3*H*)-thione **8** in good yield. Its ^{13}C-NMR spectrum showed C-5 and C–Cl at 166.51 and 149.35, in addition to the 2-thioxo at 190.38 δ ppm. Nucleophilic displacement of the 7-chloro atom with diethylamine and morpholine gave the 7-diethylamino **9a** and 7-morpholino derivative **9b**, respectively. The 7-amino compound **11** was prepared by sodium dithionite reduction of the 7-azido analogue **10**, which is readily accessible from **8** and sodium azide.

Biological investigation and discussion

Table I lists the results of *in vitro* microbiological screening of 10 compounds against *Staphylococcus aureus* and *Bacillus subtilis* as Gram-positive bacteria, *Escherichia coli* as Gram-negative bacteria, in addition to *Candida albicans* and *Saccharomyces cerevisiae* fungi. The data indicate that the 3-allyl derivative **4a** was moderately active against *B subtilis*, *E coli* and *C albicans* (MIC = 80–90 μg/ml) but was inactive against *S aureus* and *S cerevisiae*. Similar activity was recorded for the 3-phenyl derivative **4b** but with loss of the anti-fungal properties against the strain used in this study. A good spectrum of activity was recorded when the hydrogen at N-6 was replaced by an amino group (**3**) or methyl group (**5**), and the highest activity was noted for (**3**) against *S aureus* (MIC = 10 μg/ml). In addition, structure variations at C-7 could enhance the activity. Thus good activity was found against *S aureus* for 7-mercapto **6** and 7-diethylamino **9a** (MIC = 20 μg/ml) and against *E coli* for 7-morpholino compounds **9b** (MIC = 40 μg/ml). In contrast, the 7-chloro compound **8** exhibited only slight anti-fungal potency (MIC = 80–100 mg/ml), while the 7-azido compound **10** was moderately active against *E coli* (MIC = 70 μg/ml).

The data indicate that amino (**3**) or methyl (**5**) substituents at N-6 seem to be essential for good activity, especially against *S aureus* and *E coli*. The free SH at C-7 also seems to be necessary (**6**) because its methylation led to nearly inactive compound (**7**). Moreover, structure variations at C-7 may promote more specific action. None of the tested compounds were superior to the reference antibiotics.

Experimental protocols

Chemistry

The melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus and are uncorrected. The infrared spectra were recorded on a Perkin–Elmer 1430 ratio recording IR spectrophotometer, using samples in potassium bromide disks. ^{1}H-NMR spectra were measured on a Varian EM-390 at 90 MHz using trifluoroacetic acid (unless otherwise stated) with tetramethylsilane (TMS) as the internal standard. ^{13}C-NMR spectra were measured on a Varian XL-200 using DMSO as the solvent and internal standard. Analyses indicated by elemental symbols were within ± 0.4% of the theoretical values and were performed by the Microanalytical Unit, University of Cairo, Egypt.

Ethyl-4-acetamido-2,3-dihydro-3-phenyl-2-thioxothiazole-5-carboxylate 2

Under anhydrous conditions, acetyl chloride (0.7 ml, 10 mmol) was added to a stirred solution of **1d** [2] (2.8 g, 10 mmol) in acetic anhydride (20 ml). After refluxing for 5 h, the reaction

Table I. Minimal inhibitory concentration (μg/ml).

Cmpd	*S aureus*	*B subtilis*	*E coli*	*C albicans*	*S cerevisiae*
3	10	20	40	60	20
4a	>200	80	90	90	>200
4b	>200	70	90	>200	>200
5	40	70	40	70	160
6	20	40	160	50	80
7	>200	50	>200	>200	>200
8	>200	>200	>200	80	180
9a	20	50	180	160	80
9b	120	80	40	80	50
10	>200	100	70	100	>200
A[a]	1	1	–	–	–
S	4	–	3	–	–
N	–	–	–	2	–

[a]A: ampicillin; S: streptomycin; N: nystatin.

mixture was poured into crushed ice and the separated product was filtered, washed with water, dried and recrystallized from ethanol; yield: 80%, mp: 140°C. IR ν cm^{-1}: 3300, 2980, 2920, 1735, 1670, 1570, 1490, 1320, 1200, 1070. ^{1}H-NMR: δ 1.4 (t, 3H, CH_3–CH_2), 2.2 (s, 3H, CH_3CO), 4.4 (q, 2H, CH_3–CH_2), 7.2–7.7 (m, 5 Ar*H*). Anal ($C_{14}H_{14}N_2O_3S_2$) C, H, N, S.

6-Amino-2,3-dihydro-5-methyl-3-phenyl-2-thioxothiazolo[4, 5-d]-pyrimdin-7(6H)-one ***3***
To a solution of **2** (3.2 g, 10 mmol) in absolute ethanol (20 ml), hydrazine hydrate 99% (5 ml) was added. The reaction mixture was heated under reflux for 1 h. After cooling, the isolated product was recrystallized from dimethylformamide; yield: 30%, mp: 220–223°C. IR ν cm^{-1}: 3300, 3200, 1650, 1590, 1550, 1300, 1240, 1030. ^{1}H-NMR: δ 2.9 (s, 3H, CH_3), 7.2–7.6 (m, 5 Ar*H*), 8.8 (s, 2H, NH_2). Anal ($C_{12}H_{10}N_4OS_2$), C, H, N, S.

3-Allyl-2,3-dihydro-5-methyl-2-thioxothiazolo[4,5-d]pyrimidin-7(6H)-one ***4a***
A solution of **1a** [2] (2.15 g, 10 mmol) in acetic anhydride (20 ml) was refluxed for 3 h during which the product was partially crystallized out. After cooling, the separated white crystals were filtered, washed with ethanol, dried and recrystallized from dimethylformamide; yield: 78%, mp: 235–238°C. IR ν cm^{-1}: 3600–3300, 2800, 1660, 1580, 1550, 1480, 1400, 1310, 1250, 1040. ^{1}H-NMR: δ 1.9 (d, 2H, CH_2–CH=CH_2), 2.8 (s, 3H, CH_3), 4.8 (dd, 2H, CH_2–CH=CH_2), 5.2 (m, 1H, CH_2–CH=CH_2). Anal ($C_9H_9N_3OS_2$) C, H, N, S.

2,3-Dihydro-5-methyl-3-phenyl-2-thioxothiazolo[4,5-d]pyrimidin-7(6H)-one ***4b***
As described for **4a** from the reaction of **1b** [2] with acetic anhydride; yield: 80%, mp: 335–338°C (dimethylformamide). IR ν cm^{-1}: 3600–3200, 2800, 1660, 1590, 1550, 1470, 1400, 1300, 1250, 1220, 1040. ^{1}H-NMR: δ 2.2 (s, 3H, CH_3), 7.2–7.6 (m, 5 Ar*H*). Anal ($C_{12}H_9N_3OS_2$) C, H, N, S.

3-(4-Chlorophenyl)-2,3-dihydro-5-methyl-2-thioxothiazolo-[4,5-d]pyrimidin-7(6H)-one ***4c***
As described for **4a** from the reaction of **1c** with acetic anhydride; yield: 81%, mp: > 350°C (dimethylformamide). IR ν cm^{-1}: 3100–2800, 1670, 1580, 1550, 1290, 1220, 1060. ^{1}H–NMR (DMSO–d$_6$): δ 2.5 (s, 3H, CH_3), 7.1 (d, 2 Ar*H*), 7.6 (d, 2 Ar*H*). Anal ($C_{12}H_8ClN_3OS_2$) C, H, Cl, N, S.

2,3-Dihydro-5,6-dimethyl-3-phenyl-2-thioxothiazolo[4,5-d]pyrimidin-7(6H)-one ***5***
Methyl iodide was added to a solution of **4b** (2.75 g, 10 mmol) and an equimolar amount of potassium hydroxide (0.6 g) in absolute ethanol (20 ml). The reaction mixture was refluxed for 5 h during which the product partially crystallized out. After cooling, the isolated product was recrystallized from dimethylformamide; yield: 75%, mp: 270–272°C. IR ν cm^{-1}: 3000–2800, 1680, 1590, 1320, 1260, 1040. ^{1}H-NMR (DMSO–d$_6$): δ 2.4 (s, 3H, CH_3 at C-5), 3.5 (s, 3H, NCH_3), 7.2–7.6 (m, 5 Ar*H*) Anal ($C_{13}H_{11}N_3OS_2$) C, H, N, S.

5-Methyl-7-mercapto-3-phenylthiazolo[4,5-d]pyrimidine-2-(3H)-thione ***6***
A mixture of **4b** (2.75 g, 10 mmol) and phosphorus pentasulfide (2.2 g, 10 mmol) in xylene (20 ml) was refluxed for 5 h. After cooling, the product was filtered, boiled with ethanol, then refiltered, dried and recrystallized from dimethylformamide; yield: 77%, mp: 322–325°C. IR ν cm^{-1}: 3200–2800, 1570, 1310, 1250, 1220, 1040. ^{13}C-NMR: δ 21.48 (CH_3 at C-5); 121.01 (C-7a); 129.08, 129.27, 129.50, 129.76, 130.02, 135.75 (6 Ar*C* at N-3); 153.34 (C-7); 160.80 (C-3a); 172.89 (C-5); 191.20 (C=S). Anal ($C_{12}H_9N_3S_3$) C, H, N, S.

5-Methyl-7-methylmercapto-3-phenylthiazolo[4,5-d]pyrimidin-2(3H)-thione ***7***
A mixture of **6** (2.91 g, 10 mmol) and an equimolar amount of potassium carbonate (1.38 g) in dry acetone (30 ml) was refluxed with methyl iodide (0.62 ml, 10 mmol) for 4 h, then filtered while hot, concentrated and cooled. The separated crystals were recrystallized from acetone; yield: 70%, mp: 220–222°C. IR ν cm^{-1}: 2900, 1590, 1550, 1290, 1250, 1050. ^{1}H-NMR (DMSO–d$_6$): δ 2.4 (s, 3H, CH_3 at C-5), 2.6 (s, 3H, SCH_3), 7.2–7.6 (m, 5 Ar*H*). Anal ($C_{13}H_{11}N_3S_3$) C, H, N, S.

7-Chloro-5-methyl-3-phenylthiazolo[4,5-d]pyrimidin-2(3H)-thione ***8***
A solution of **4b** (2.75 g, 10 mmol) in phosphoryl chloride (15 ml) was heated under reflux for 4 h and worked up as described in [8]; yield: 90%, mp: 170–172°C. IR ν cm^{-1}: 3040, 1590, 1550, 1290, 1230, 1030. ^{13}C-NMR: δ 25.27 (CH_3 at C-5); 117.15 (C-7a); 128.78, 129.38, 129.54, 129.79, 130.05, 135.45 (6 Ar*C* at N-3); 149.35 (C-7); 161.09 (C-3a); 166.51 (C-5); 190.38 (C=S). Anal ($C_{12}H_8ClN_3S_2$) C, H, Cl, N, S.

7-Diethylamino-5-methyl-3-phenylthiazolo[4,5-d]pyrimidin-2(3H)-thione ***9a***
It was prepared by refluxing **8** (2.94 g, 10 mmol) with diethylamine (1.0 ml, 20 mmol) in dry acetone for 3 h. After cooling the product was filtered, washed with ethanol and recrystallized from dimethylformamide; yield: 70%, mp: 155–157°C. IR ν cm^{-1}: 2950, 1550, 1480, 1400, 1350, 1320, 1300, 1250, 1200, 1090, 1050. ^{1}H-NMR: δ 1.4 (t, 6H, 2 CH_3–CH_2), 2.5 (s, 3H, CH_3 at C-5), 3.8 (q, 4H, 2 CH_3–CH_2), 7.2–7.7 (m, 5 Ar*H*). Anal ($C_{16}H_{18}N_4S_2$) C, H, N, S.

5-Methyl-7-morpholino-3-phenylthiazolo[4,5-d]pyrimidin-2-(3H)-thione ***9b***
Like **9a**, it was similarly prepared from **8** (2.94 g, 10 mmol) and morpholine (0.87 ml, 20 mmol); yield: 85%, mp: 255–257°C. IR ν cm^{-1}: 3000–2800, 1560, 1490, 1240, 1040. ^{1}H-NMR: δ 2.6 (s, 3H, CH_3), 3.9–4.3 (br, s, 8H, morpholino), 7.2–7.8 (m, 5 Ar*H*). Anal ($C_{16}H_{16}N_4OS_2$) C, H, N, S.

7-Azido-5-methyl-3-phenylthiazolo[4,5-d]pyrimidin-2(3H)-thione ***10***
It was prepared from **8** (2.94 g, 10 mmol) and sodium azide (0.65 g, 10 mmol) as reported in [1]; yield: 80%, mp: 165–168°C (ethanol). IR ν cm^{-1}: 3060, 2160, 2120, 1560, 1240, 1040. ^{1}H-NMR: δ 2.5 (s, 3H, CH_3), 7.0–7.6 (m, 5 Ar*H*).Anal ($C_{12}H_8N_6S_2$) C, H, N, S.

7-Amino-5-methyl-3-phenylthiazolo[4,5-d]pyrimidin-2(3H)-thione ***11***
It was prepared from **10** (3.0 g, 10 mmol) and sodium dithionite (1.0 g), following our procedure reported in [1]; yield: 60%, mp: 330–333°C. IR ν cm^{-1}: 3300, 3100, 1660, 1580, 1300, 1240, 1030. ^{1}H-NMR: δ 2.6 (s, 3H, CH_3), 7.3–7.8 (m, 7H, 5 Ar*H* + NH_2). Anal ($C_{12}H_{10}N_4S_2$) C, H, N, S.

Microbiological methods

Test organisms and culture media
Staphylococcus aureus ATCC 25923, *Bacillus subtilis* DSM 347b, and *Escherichia coli* ATCC 25922 were cultivated in

nutrient broth, while *Candida albicans* DSM 70443 and *Saccharomyces cerevisiae* IMG 70014 were grown in liquid Sabouraud.

Minimal inhibitory concentration (MIC) measurements
Minimum inhibitory concentrations (MICs) were determined by the broth dilution technique [9] as previously described [1]. Ampicillin, streptomycin and nystatin were used during the test procedure as reference antibiotics.

Acknowledgments

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