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SOME 3-ALKYL-6-(2-ARYL-2-OXO-1-ETHYL)-7-OXOTHIAZOLO[4,5-*d*]PYRIMIDIN-2(3*H*)-THIONE DERIVATIVES AND THEIR ANTIMICROBIAL ACTIVITIES

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SUMMARY

In this study, some 3-alkyl-6-(2-aryl-2-oxo-1-ethyl)-7-oxothiazolo[4,5-*d*]pyrimidin-2(3*H*)-thione derivatives have been synthesized by reacting 3-alkyl-7-oxothiazolo[4,5-*d*]pyrimidin-2(3*H*)-thiones with various ω-bromoacetophenones. The antibacterial and antifungal activities of the obtained compounds have been investigated and it is reported that some of the compounds showed remarkable antimicrobial activities.

ÖZET

Bu çalışmada, 3-alkil-7-oksotiyazolo[4,5-*d*]pirimidin-2(3*H*)-tiyonlarla değişik ω-bromoasetofenonlar reaksiyona sokularak, bazı 3-alkil-6-(2-aril-2-okso-1-etil)-7-oksotiyazolo[4,5-*d*]pirimidin-2(3*H*)-tiyon türevleri sentezlenmiştir. Elde edilen bileşiklerin antibakteriyel ve antifungal etkileri incelenmiş ve bazı bileşiklerin kayda değer antimikrobiyal etki gösterdikleri saptanmıştır.

Key words: 7-Oxothiazolo[4,5-*d*]pyrimidin-2(3*H*)-thione, thiazole, antimicrobial activity

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J. Fac. Pharm. Istanbul
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INTRODUCTION

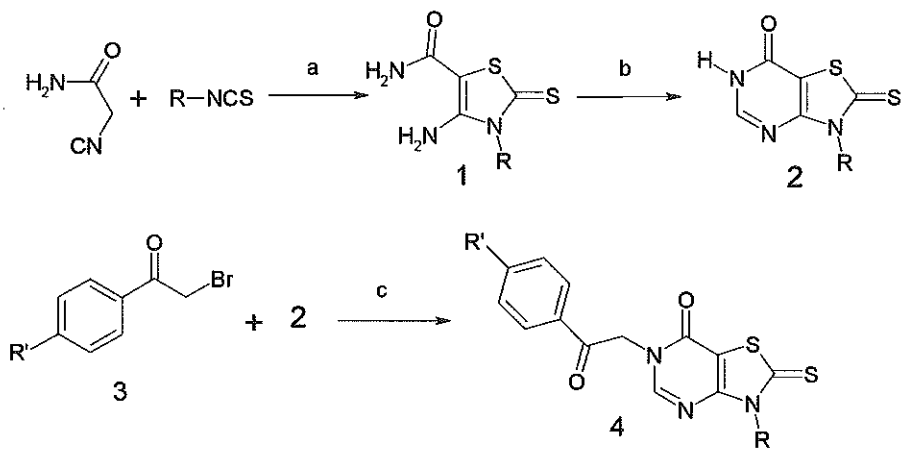
The substituted thiazol-2(3*H*)-thione derivatives can be regarded as analogues of rhodanines and/or dithiocarbamates, which possess well known antimicrobial activities. It was reported that the antimicrobial activities of rhodanine and dithiocarbamates might be due to the *in situ* formation of isothiocyanates (1,2). Although, it was thought that thiazol-2(3*H*)-thiones could not be converted into isothiocyanates contrary to rhodanines or dithiocarbamates, they are reported to be well known antimicrobial and anticancer compounds (3,4).

On the other hand, thiazolopyrimidines which have been reported to possess antimicrobial, anticancer and antiviral activities can be considered as analogues of purine bases of nucleotides (5-19).

In line of these findings 3-alkyl substituted 6-(2-aryl-2-oxo-1-ethyl)-7-oxothiazolo[4,5-*d*]pyrimidin-2(3*H*)-thione derivatives have been synthesized and their antimicrobial and antifungal activities have been tested.

RESULTS AND DISCUSSION

The synthesis of the intermediate and target compounds were performed by the reactions illustrated in Scheme 1.



a : S_8, $(C_2H_5)_3N$, C_2H_5OH, b : $(C_2H_5O)_3CH$, $(CH_3CO)_2O$, c : K_2CO_3, CH_3COCH_3

Scheme 1

Compounds **1**, namely; 3-alkyl-4-amino-5-carboxamidothiazole-2(3*H*)-thiones; were prepared in excellent yields following the methods described by Gewald (3,5,20). It involved the reaction of cyanoacetamide with sulphur and an appropriate alkyl or arylisothiocyanates in the presence of triethylamine as a basic catalyst. The aminothiazoles, **1**, so obtained were used for the preparation of the starting compounds **2**, i.e. 3-alkyl-7-oxo-thiazolo[4,5-*d*] pyrimidin-2(3*H*)-thiones (20). To achieve this cyclization the aminothiazoles were heated in a mixture of triethyl orthoformate and acetic anhydride. The target compounds **4** were prepared by reacting the thiazolopyrimidines, **2**, with the appropriate 2-bromoacetophenones, **3**, in acetone in the presence of potassium carbonate.

Spectroscopic data confirm the structures of the compounds. IR spectra of **4** exhibited characteristic carbonyl bands due to 7-oxo-thiazolo[4,5-*d*]pyrimidin-2(3*H*)-thiones and benzoyl residues. In the NMR spectra methylene and C_5-H protons of thiazolopyrimidine that are common in all compounds resonated at the expected regions. Some of the characteristics of the synthesized compounds are given in Table 1.

Table 1. Some characteristics of the compounds **4**

Comp.	R	R'	M.p.(°C)	Yield (%)	Mol. Formula/Anal. (C,H,N,S)
4a	$-CH_3$	-H	202-203	72	$C_{14}H_{11}N_3O_2S_2$
4b	$-CH_3$	$-CH_3$	205-206	75	$C_{15}H_{13}N_3O_2S_2$
4c	$-CH_3$	$-0CH_3$	184-185	65	$C_{15}H_{13}N_3O_3S_2$
4d	$-CH_3$	-Cl	258-259	80	$C_{14}H_{10}ClN_3O_2S_2$
4e	$-CH_2CH_3$	-H	164-165	68	$C_{15}H_{13}N_3O_2S_2$
4f	CH_2CH_3	$-CH_3$	299-300	70	$C_{16}H_{15}N_3O_2S_2$
4g	CH_2CH_3	$-OCH_3$	226-227	72	$C_{16}H_{15}N_3O_3S_2$
4h	CH_2CH_3	-Cl	339-340	75	$C_{15}H_{12}ClN_3O_2S_2$

Compounds **4** were evaluated for antibacterial and antifungal activity against representative bacteria- Gram(-)bacteria *Escherichia coli* and Gram(+)bacteria *Staphylococcus aureus* - and fungi - *Candida albicans* as shown in Table 2. An antibacterial agent, chloramphenicol, and an antifungal agent, ketoconazole, were used as standards. In the light of the test results, we may conclude that the tested compounds have remarkable antibacterial and antifungal activities. However, no correlation between the structure and biological activities of the compounds was found.

J. Fac. Pharm. Istanbul
38(1) (2005-2006)

Table 2. Antimicrobial activities of the compounds (MIC, μg ml^{-1}).

Compounds	*E. coli*	*S. aureus*	*C. albicans*
4a	5	5	5
4b	5	5	5
4c	5	5	5
4d	10	5	5
4e	5	10	5
4f	10	10	7.5
4g	10	10	7.5
4h	10	10	7.5
Chloramphenicol	5	5	-
Ketoconazole	-	-	7.5

EXPERIMENTAL

Chemistry

Melting points were determined by using an Electrothermal 9100 digital melting point apparatus and are uncorrected. Spectroscopic data were recorded on the following instruments, FTIR: Shimadzu 8400 FTIR spectrophotometer, ^{1}H-NMR: Bruker DPX 400 NMR spectrometer in DMSO-d_6 and Mass: AGILENT 1100 MSD mass spectrometer. Analyses for C, H, N were within 0.4 % of the theoretical values. ω-Bromoacetophenone derivatives (21) and cyanoacetamide (22) were prepared according to the literature methods. 3-Alkyl-4-amino-5-carboxamidothiazole-2(3*H*)-thione derivatives **(1)** were obtained by reacting the appropriate alkyl/arylisothiocyanate derivatives with cyanoacetamide in the presence of triethylamine in ethanol (3,5,20).

General method for the preparation of 3-substituted 6-(2-aryl-2-oxo-1-ethyl)-7-oxothiazolo[4,5-d]pyrimidin-2(3H)-thione, 4a-h.

A mixture of **2** (5 mmol), an appropriate **3** (5 mmol) and potassium carbonate (6 mmol) in acetone (100 ml) was refluxed for two hours. The solvent was evaporated at low temperature. The residue was washed with water and then ethanol. The raw product was recrystallized from ethanol.

4a IR(KBr)υ_{maks}(cm^{-1}) : 1687 (C=O), 1615-1510 (C=N ve C=C), 1234 (C=S). ^{1}H-NMR(400 MHz)(DMSO-d6) δ (ppm): 3.70 (3H, s, 3-CH_3), 5.65 (2H, s, -$COCH_2$), 7.61-7.65 (2H, m, benzoyl $C_{3,5}$-H), 7.74-7.78 (1H, m, benzoyl C_4-H), 8.00-8.01 (2H, m, benzoyl $C_{2,6}$-H), 8.44 (1H, s, thiazolopyrimidine C_5-H). MS(ES) m/z: 320 (M+3,

% 4), 319 (M+2, %8), 318 (M+1, %45), 317 (M^+, %32), 316 (M-1, %100).

4b IR(KBr) $\upsilon_{maks}(cm^{-1})$: 1685 (C=O), 1604-1427 (C=N ve C=C), 1242 (C=S). ^{1}H-NMR(400 MHz)(DMSO-d_6) δ (ppm) : 2.54 (3H, s, Ar-CH_3), 3.70 (3H, s, 3-CH_3), 5.68 (2H, s, -$COCH_2$-), 7.71 (2H, d, j:8.63 Hz, benzoyl $C_{3,5}$-H), 8.11 (2H, d, j:8.62 Hz, benzoyl $C_{2,6}$-H), 8.62 (1H, s, thiazolopyrimidine C_5-H).

4c IR(KBr) $\upsilon_{maks}(cm^{-1})$: 1676 (C=O), 1598-1457 (C=N ve C=C), 1178-1038 (C-O), 1230 (C=S). ^{1}H-NMR(400 MHz)(DMSO-d_6) δ (ppm) : 3.70 (3H, s, 3-CH_3), 3.88 (3H, s, Ar-OCH_3), 5.62 (2H, s, -$COCH_2$-), 7.13 (2H, d, j:8.94 Hz, benzoyl $C_{3,5}$-H), 8.07 (2H, d, j:8.91 Hz, benzoyl $C_{2,6}$-H), 8.62 (1H, s, thiazolopyrimidine C_5-H).

4d IR(KBr) $\upsilon_{maks}(cm^{-1})$: 1692 (C=O), 1618-1485 (C=N ve C=C), 1242 (C=S). ^{1}H-NMR(400 MHz)(DMSO-d_6) δ (ppm) : 3.71 (3H, s, 3-CH_3), 5.67 (2H, s, -CH_2-CO-), 7.69 (2H, d, j:8.67 Hz, benzoyl $C_{3,5}$-H), 8.08 (2H, d, j:8.63 Hz, benzoyl $C_{2,6}$-H), 8.45 (1H, s, thiazolopyrimidine C_5-H).

4e IR(KBr) $\upsilon_{maks}(cm^{-1})$: 1680 (C=O), 1596-1420 (C=N ve C=C), 1251 (C=S). ^{1}H-NMR(400 MHz)(DMSO-d6) δ (ppm) : 1.30 (3H, t, CH_2-CH_3), 4.38 (2H, q, CH_2-CH_3), 5.62 (2H, s, -$COCH_2$-), 7.64-7.77 (3H, m, benzoyl $C_{3,5}$-H), 7.98-8.07 (2H, m, benzoyl $C_{2,6}$-H), 8.46 (1H, s, thiazolopyrimidine C_5-H).

4f IR(KBr) $\upsilon_{maks}(cm^{-1})$: 1688 (C=O), 1611-1432 (C=N ve C=C), 1232 (C=S). ^{1}H-NMR(400 MHz)(DMSO-d_6) δ (ppm) : 1.30 (3H, t, CH_2-CH_3), 2.43 (3H, s, Ar-CH_3), 4.38 (2H, q, CH_2-CH_3), 5.65 (2H, s, -$COCH_2$-), 7.43 (2H, d, j:8.02 Hz, benzoyl $C_{3,5}$-H), 8.00 (2H, d, j:8.02 Hz, benzoyl $C_{2,6}$-H), 8.63 (1H, s, thiazolopyrimidine C_5-H).

4g IR(KBr) $\upsilon_{maks}(cm^{-1})$: 1689 (C=O), 1596-1426 (C=N ve C=C), 1168-1030 (C-O), 1226 (C=S). ^{1}H-NMR(400 MHz)(DMSO-d_6) δ (ppm) : 1.30 (3H, t, CH_2-CH_3), 3.88 (3H, s, Ar-OCH_3), 4.37 (2H, q, CH_2-CH_3), 5.64 (2H, s, -$COCH_2$-), 7.13 (2H, d, j:8.94 Hz, benzoyl $C_{3,5}$-H), 8.07 (2H, d, j:8.91 Hz, benzoyl $C_{2,6}$-H), 8.64 (1H, s, thiazolopyrimidine C_5-H).

4h IR(KBr) $\upsilon_{maks}(cm^{-1})$: 1682 (C=O), 1601-1422 (C=N ve C=C), 1230 (C=S). ^{1}H-NMR(400 MHz)(DMSO-d_6) δ (ppm) : 1.31 (3H, t, CH_2-CH_3), 4.38 (2H, q, CH_2-CH_3), 5.71 (2H, s, -CH_2-CO-), 7.69 (2H, d, j:8.75 Hz, benzoyl $C_{3,5}$-H), 8.08 (2H, d, j:8.73 Hz, benzoyl $C_{2,6}$-H), 8.47 (1H, s, thiazolopyrimidine C_5-H). MS(ES) m/z : 369 (M+4, %3), 368 (M+3, % 7), 367 (M+2, %10), 366 (M+1, %42), 365 (M^+, %18), 364 (M-1, %100).

Antimicrobial Activity

The study was designed to compare MIC values obtained by the NCCLS reference M27-A2 broth macrodilution method (23,24). MIC readings were performed twice

J. Fac. Pharm. Istanbul
38(1) (2005-2006)

with each chemical agent. For both the antibacterial and antimycotic assays the compounds were dissolved in DMSO. Further dilutions of the compounds and standard drug in test medium were prepared at the required quantities of 100, 75, 50, 25, 15, 10, 7.5, 5, 2.5, 1, 0.5 μg ml^{-1} concentrations with Mueller-Hinton broth and Sabouroud dextrose broth.

In order to ensure that the solvent *per se* had no effect on bacteria and yeast growth, a control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments and found inactive in culture medium.

All the compounds were tested for their *in vitro* growth inhibitory activity against human pathogenic *Staphylococcus aureus* (ATCC 6538) as Gram-positive bacteria and *Escherichia coli* (ATCC 25922) as Gram-negative bacteria and yeast *Candida albicans* (isolated at the Faculty of Medicine Osmangazi University, Eskişehir, Turkey). Chloramphenicol and ketoconazole were used as standard drugs. Data concerning antibacterial and antifungal activity of the compounds and the standard drugs are given in Table 2.

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