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SYNTHESIS, ANTIMICROBIAL AND ANTICANCER ACTIVITIES OF 2-SUBSTITUTED ACETAMIDO-4-ETHOXYCARBONYLMETHYL-1,3- THIAZOLES

H. ALTINTAŞ* , Ö. ATEŞ*, A.GÜRSOY*, S. BİRTEKSÖZ, G.ÖTÜK****

SUMMARY

Nine novel 2-substituted acetamido-4-ethoxycarbonylmethyl-1,3-thiazoles were synthesized and screened for their in vitro antimicrobial activity. One of the compoundsshowed antibacterial activity against *Staphylococcus aureus* ATCC 6538 and antifungal activity against *Candida albicans* ATCC 10231. Compounds **4-7** chosen as prototypes were also evaluated in the National Cancer Institute's 3-cell line, one dose in vitro primary cytotoxicity assay.

The structures of synthesized compounds were confirmed by spectral methods (IR, ^{1}H-NMR and EI mass spectrometry) and elemental analyses.

ÖZET

2-Sübstitüe asetamido-4-etoksikarbonilmetil-1,3-tiyazol yapısında 9 yeni madde sentezlendi ve in vitro antimikrobiyal aktiviteleri incelendi. Bileşiklerin biri *Staphylococcus aureus* ATCC 6538'e karşı antibakteryel ve *Candida albicans* ATCC 10231'e karşı antifungal aktivite gösterdi. Prototip olarak seçilen **4-7** numaralı bileşiklerin tek doz in vitro primer sitotoksisite denemesi Ulusal Kanser Enstitüsünde yürütüldü. Sentezlenen bileşiklerin yapıları spektral metodlar (IR, ^{1}H-NMR ve EI kütle spektro metrisi) ve elementel analiz ile aydınlatıldı.

Key words: 2,4-Disubstituted 1,3-thiazoles, antimicrobial activity, synthesis

* Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Beyazıt 34116, Istanbul, Turkey

** Istanbul University ,Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Beyazıt 34116, Istanbul, Turkey

Corresponding author: e-mail: handanaltuntas@yahoo.com

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INTRODUCTION

The multifarious biological properties of 2-aminothiazoles which include antibacterial (1), analgesic (2) and antiinflammatory (3) activities have been well documented. While screening for various activities of 2-(∝-haloacyl)aminothiazol-4-acetic acid ethyl esters, cytotoxic (4), muscarinic (5) and cardiovascular activities (6) in addition to antifungal activity (7) were observed. The present paper reports the synthesis, characterization and antimicrobial screening of the 2-substituted acetamido-4-ethoxycarbonylmethyl-1,3-thiazoles. Four of the compounds **(4-7)** which were chosen by National Cancer Institute in Bethesda, Maryland, USA as candidates of anticancer agents have also been assayed for anticancer activity.

RESULTS AND DISCUSSION

2-Chloroacetylamino-4-ethoxycarbonylmethyl-1,3-thiazole **(2)** was prepared according to a reported procedure (7). 2-Substituted acetamido-4-ethoxycarbonylmethyl-1,3-thiazoles **(3-11)** were prepared by refluxing the 2-chloroacetylamino-4-ethoxycarbonylmethyl-1,3-thiazole with appropriate amine derivatives in benzene for 5-6 hours (Fig. 1).

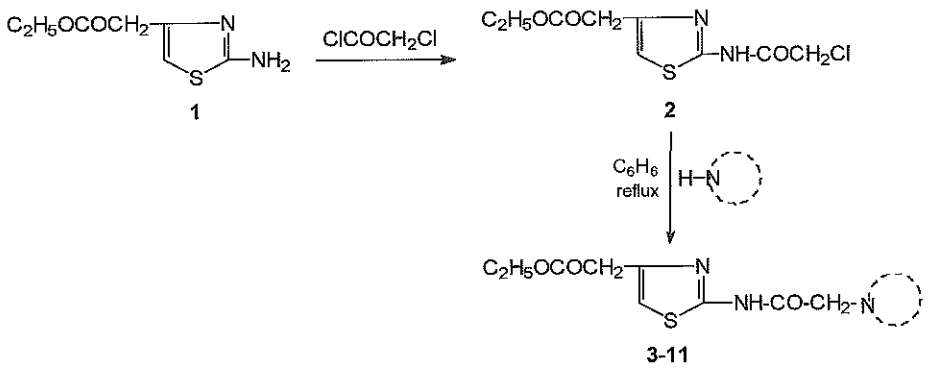
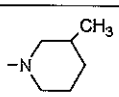
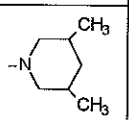
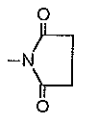


Figure 1. Synthesis of the compounds

Experimental data for compounds **3-11** are presented in Table 1. The spectral details of these compounds have been given in the experimental. In the IR spectra of the compounds, the characteristic N-H and C=O absorption bands were observed at 3428-

Table 1. Physical data of Compounds **3-11**

	R	Formula (M.W.)	Yield (%)	M.p. (^{0}C)	Elemental Analysis (%) Calcd./found			
					C	H	N	S
3		$C_{11}H_{17}N_3O_3S.2H_2O$ (307.37)	90	189	42.98 43.18	6.89 6.15	13.67 12.92	10.43 10.84
4		$C_{13}H_{21}N_3O_3S.H_2O$ (317.40)	88	wax	49.19 48.51	7.30 7.22	13.24 13.22	10.10 10.03
5		$C_{13}H_{19}N_3O_3S.3/2H_2O$ (324.39)	93	wax	48.13 48.33	6.84 7.29	12.95 13.52	9.88 9.13
6		$C_{13}H_{19}N_3O_4S.H_2O$ (331.38)	95	wax	47.12 47.76	6.39 6.05	12.68 12.65	9.68 10.15
7		$C_{14}H_{21}N_3O_3S.H_2O$ (329.41)	98	wax	51.04 50.63	7.04 6.35	12.76 12.75	9.73 10.46
8		$C_{15}H_{23}N_3O_3S.H_2O$ (343.43)	87	wax	52.46 52.84	7.34 6.94	12.24 12.46	9.34 9.13
9		$C_{15}H_{23}N_3O_3S.1/2H_2O$ (334.42)	89	wax	53.87 53.55	7.23 7.60	12.57 12.32	9.59 8.63
10		$C_{16}H_{25}N_3O_3S.H_2O$ (357.46)	91	wax	53.76 53.64	7.61 6.91	11.76 12.02	8.97 9.39
11		$C_{13}H_{15}N_3O_5S.3H_2O$ (379.38)	90	145	41.15 41.30	5.58 5.95	11.08 10.84	8.45 8.90

3216 cm^{-1}, 1738-1732 cm^{-1} (ester), 1701-1659 cm^{-1} (amide), respectively. In the ^{1}H-NMR spectra, the protons were observed in the expected regions. EI-MS of Compound **8** which was chosen as prototype was taken and displayed molecular ion at m/z 325 which confirmed its molecular weight. Also, $(M+2)^+$ peak was observed besides (M^+) peak, due to sulfur. In the spectrum, peaks observed at m/z 100 and 112 showed the presence of 2-aminothiazole and 2-methyl-4-thia-1,6-diazabicyclo[3.1.0]-hexa-2,5-diene fragments. Compounds **3-11** were evaluated for *in vitro* antibacterial and antifungal activity against representative bacteria: *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella*

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pneumoniae ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi, Shigella flexneri* and *Proteus mirabilis* ATCC 14153 and fungi: *Candida albicans* 10231 using the microbroth dilution method. One of the compounds **(11)** was found to be effective against *S.aureus* ATCC 6538 and *C.albicans* ATCC 10231. The minimum inhibitory concentrations (MIC) are given in Table 2. Four of our compounds **(4-7)** were chosen to be screened for potential anticancer activity and it was found that their growth inhibitions were not satisfactory to pass to the second stage of the assay. Results of anticancer activity are shown in Table 3.

Table 2. MIC values (μg/mL) of compound 3

Compound	***S.aureus*** **ATCC 6538**	***C.albicans*** **ATCC 10231**
3	156	78
Cefuroxime sodium	1.2	-
Clotrimazole	-	4.9

Table 3. One dose primary anticancer assay results of compounds **4-7***

Compound	(Lung) NCI-H460	(Breast) MCF7	(CNS) SF-268
4	75	109	113
5	118	121	106
6	106	117	95
7	110	107	112

* Percent growth of treated cells compared to control

EXPERIMENTAL

Chemistry

Chemicals used in this study were supplied by Merck-Schuchard (Munich, Germany) and Fluka (Buchs, Switzerland). Melting points were determined on a Buchi 530 melting point apparatus (Flawil, Switzerland) in open capilleries and are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer (Milan, Italy) at the Scientific and Technical Research Council of Turkey, Ankara Testing and

Analyses Laboratory (Ankara-Turkey). The purities of the compounds were controlled by TLC on silicagel $HF_{254+366}$ (E.Merck, Darmstadt, Germany). IR spectra were recorded as potassium bromide (ν in cm^{-1}) (BDH, Poole, England) discs, using a Perkin-Elmer Model 1600 FT-IR spectrophotometer (Norwalk, Connecticut, USA). ^{1}H-NMR spectra were obtained on a Bruker AC-L 200 and 200 MHz spectrophotometer (Rheinstätten, Germany) using deutero dimethylsulfoxide (DMSO-d_6) (E.Merck, Darmstadt, Germany) as solvent and tetramethylsilane (TMS) as internal standard. All chemical shifts are reported as δ (ppm) values and spin-spin couplings J are expressed in Hz. EI/MS were determined on a VG Zab Spec (70 eV) mass spectrometer (Manchester, England).

2-Chloroacetylamino-4-ethoxycarbonymethyl-1,3-thiazole **(2)** (7) 0.01 mol of 4-ethoxycarbonylmethyl-2-amino-1,3-thiazole **(1)** in 4 mL of dry benzene and 1 mL of dry pyridine was stirred with 0.01 mol (0.8 mL) of chloroacetyl chloride in 3 mL of dry benzene for 1 h at room temperature. The crude product was washed first with water to remove the acid and then with hot ethanol.

2-Substituted acetamido-4-ethoxycarbonylmethyl-1,3-thiazoles **(3-11)** (8) To a stirred suspension of 0.05 mole of **2** in 100 mL of benzene, an appropriate amine (0.075 mole) was added dropwise and this mixture was then refluxed for 5-6 hours. After cooling, the benzene layer was washed several times with water. The organic phase was treated with anhydrous sodium sulfate. Removal of the organic phase under vacuum gave the pure product **(4-10)** or the product which was recrystallised from ethanol **(3,11)**.Yield, melting points and elemental analysis are given in Table 1.

Spectral data of 3: IR [ν, cm^{-1}, KBr]: 3216 (NH), 1733 (C=O, ester), 1701 (C=O, amide).
Spectral data of 4: IR [ν, cm^{-1}, KBr]: 3284 (NH), 1738 (C=O, ester), 1694 (C=O, amide).
Spectral data of 5: IR [ν, cm^{-1}, KBr]: 3428 (NH), 1732 (C=O, ester), 1694 (C=O, amide).
Spectral data of 6: IR [ν, cm^{-1}, KBr]: 3272 (NH), 1732 (C=O, ester), 1694 (C=O, amide).
Spectral data of 7: IR [ν, cm^{-1}, KBr]: 3272 (NH), 1732 (C=O, ester), 1682 (C=O, amide). ^{1}H-NMR [δ, ppm, DMSO-d_6]: 1.18 (t, J=7.1 Hz, 3H, CH_3-CH_2O), 1.39-1.92 (m, 6H, piperidine $C_{3,4,5}$-H), 3.44 (br.s, 4H, piperidine $C_{2,6}$-H), 3.69 (s, 2H, CO-CH_2), 4.08 (q, J=7.1 Hz, 2H, CH_2-O), 4.78 (s, 2H, CH_2-N), 7.00 (s, 1H, thiazole C_5-H), 11.50 (br.s, 1H, N-H).
Spectral data of 8: IR [ν, cm^{-1}, KBr]: 3286 (NH), 1738 (C=O, ester), 1694 (C=O, amide). ^{1}H-NMR [δ, ppm, DMSO-d_6]: 1.03 (d, J=6.3 Hz, 3H, piperidine C_2-CH_3), 1.23 (t, J=7.2 Hz, 3H, CH_3-CH_2O), 1.32-1.99 (m, 6H, piperidine $C_{3,4,5}$-H), 3.37-3.50 (m, 3H, piperidine $C_{2,6}$-H), 3.67 (s, 2H, CO-CH_2), 4.15 (q, J=7.2 Hz, 2H, CH_2-O), 4.65 (s, 2H, CH_2-N), 6.77 (s, 1H, thiazole C_5-H), 9.27 (s, 1H, N-H). EI/MS [m/z (rel.int.%)]: 327 $(M+2)^+$ (23), 326 $(M+1)^+$ (100) (base peak), 325 (M^+) (3), 112 (10), 100 (6).
Spectral data of 9: IR [ν, cm^{-1}, KBr]: 3417 (NH), 1733 (C=O, ester), 1693 (C=O, amide). ^{1}H-NMR [δ, ppm, DMSO-d_6]: 0.82, 0.87 (dd, J=6.7, 6.5 Hz, 3H, C_3-CH_3), 1.18 (t, J=7.1 Hz, 3H, CH_3-CH_2O), 1.53-1.94 (m, 5H, piperidine $C_{3,4,5}$-H), 3.39 (s, 3H, piperidine C_2-H and C_6-$H_{ax.}$), 3.69 (s, 3H, CO-CH_2 and piperidine C_6-$H_{eq.}$), 4.08 (q, J=7.1 Hz, 2H, CH_2-O), 4.70 (s, 2H, CH_2-

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N), 7.01 (s, 1H, thiazole C5-H), 9.16 (br.s, 1H, N-H).
Spectral data of 10: IR [ν, cm^{-1}, KBr]: 3282 (NH), 1738 (C=O, ester), 1694 (C=O, amide).
Spectral data of 11: IR [ν, cm^{-1}, KBr]: 3216 (NH), 1734 (C=O, ester), 1659 (C=O, amide).

Antimicrobial Activity

All of the compounds were screened *in vitro* for their antimicrobial activity against various bacteria and fungus. Antimicrobial activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi, Shigella flexneri, Proteus mirabilis* ATCC 14153 and *Candida albicans* ATCC 10231 were determined by the microbroth dilutions technique using the National Committee for Clinical Laboratory Standarts (NCCLS) recommendations (9, 10). Mueller-Hinton broth for bacteria, RPMI-1640 medium for yeast strain were used as the test medium. Serial two-fold dilutions ranging from 5000 μg/mL to 4.9 μg/mL were prepared in medium. The inoculum was prepared using a 4-6h broth culture of each bacteria and 24h culture of yeast strains adjusted to a turbidity equivalent to a 0.5 Mc Farland standard, diluted in broth media to give a final concentration of $5x10^5$ cfu/mL for bacteria and $0.5x10^3$ to $2.5x10^3$ cfu/mL for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation.
The trays containing Mueller-Hinton broth were incubated at 35 °C for 18-20h and the trays containing RPMI-1640 medium were incubated at 35°C for 46-50h. The MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth. The MIC values of the Compound **11** are given in Table 2.

Anticancer Activity

Primary anticancer assay was performed in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda (11-13). Four compounds chosen as prototypes **4-7** were evaluated in the 3-cell line panel consisting of NCI-H460 (Lung), MCF7 (Breast) and SF-268 (CNS). The compounds were added at a single concentration (0.4 M) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, Sulforhodramine B (SRB). Results for each compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds which inhibit the growth of any of the 3 cell lines to 32% or less than control growth are automatically forwarded for testing in the 60 cell line assay.The growth inhibitions were not satisfactory to pass to the second stage of the assay. Anticancer assay results are shown in Table 3.

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