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Short communication

Synthesis of some 2-[(benzazole-2-yl)thioacetylamino]thiazole derivatives and their antimicrobial activity and toxicity

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Abstract

Some 2-[(benzazole-2-yl)thioacetylamino]thiazole derivatives (**III**) were synthesized by reacting 4-methyl-2-(chloroacetylamino)thiazole derivatives (**I**) with benzazol-2-thiole (**II**) in acetone in the presence of K_2CO_3. The chemical structures of the compounds were elucidated by ^{1}H NMR and FAB$^+$-MS spectral data. The prepared compounds were tested for antimicrobial activity and toxicity.

Keywords: Thiazole; Benzothiazole; Benzimidazole; Benzoxazole; Antimicrobial activity

1. Introduction

The benzothiazole ring is present in various marine or terrestrial natural compounds, which have useful biological activities [1–4]. Benzothiazole and its bioisosteres; benzoxazole and benzimidazole, were studied for their antitumor, antiviral and antimicrobial activities [5–9]. In the last few years, it was reported that the 2 and 5-substituted benzothiazole, benzoxazole and benzimidazole derivatives had antimicrobial activities against some Gram-positive, Gram-negative bacteria and the yeast *Candida albicans,* and these compounds provided a wide variety of in vitro antimicrobial effects especially against the enterobacter *Pseudomonas aeruginosa* and the yeast *C. albicans* [10–16].

In view of these observations, some novel 2-[(benzazole-2-yl)thioacetylamino]thiazole derivatives have been synthesized in order to examine their in vitro antimicrobial activities against different Gram-positive, Gram-negative bacteria and the yeast *C. albicans* in comparison with control drugs.

2. Chemistry

In the present work, 4-methyl-2-(chloroacetylamino)thiazole (**I**) was prepared by reacting 2-amino-4-methylthiazole with chloroacetyl chloride in accordance with the method described in the literature [17–19].

Benzazol-2-thiole (**II**) derivatives used in the synthesis were prepared according to the methods reported in the literature [20].

The reaction of 4-methyl-2-(chloroacetylamino)thiazole (**I**), benzazol-2-thiole (**II**) and anhydrous potassium carbonate in acetone gave the 2-[(benzazole-2-yl)thioacetylamino]thiazole derivatives (**III a–s**) as shown in (Scheme I).

Some characteristics of the synthesized compounds are shown in Table 1. Analytical and spectral data (IR, ^{1}H NMR, FAB$^+$-MS) confirmed the structures of the new compounds.

3. Biology

3.1. Antimicrobial activity

Antimicrobial activities of compounds were tested using microbroth dilution method [21–23]. Tested microorganism strains were; *Staphylococcus aureus* (*B-767*), *Escherichia coli* (*B-3704*), *Bacillus subtilis* (*NRS-744*), *Streptococcus faecium* (*B-3502*), *Staphylococcus epidermidis* (*B-4268*) and *C. albicans* (isolate obtained from Osmangazi Uni. Fac. of Medicine).

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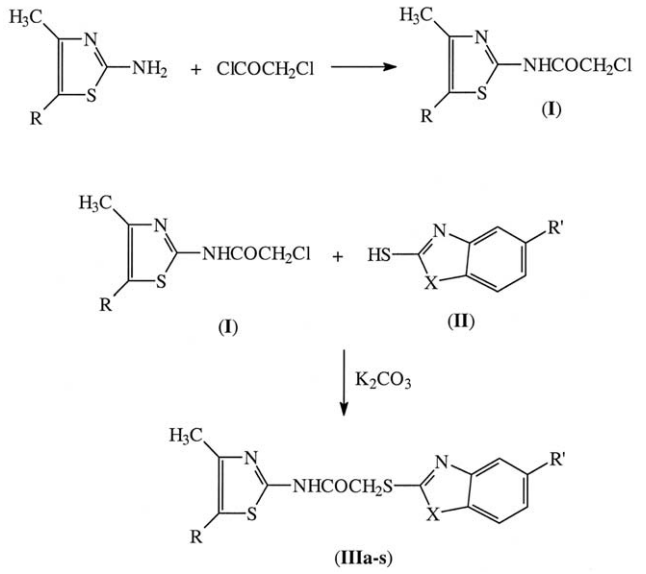

Scheme 1. The general synthesis reactions.

Chloramphenicole and ketoconazole were used as control drugs. The observed data on the antimicrobial activity of the compounds and control drugs are given in Table 2.

3.2. Toxicity

Bioactive compounds are often toxic to shrimp larvae. Thus, in order to monitor these chemicals' in vivo lethality to shrimp larvae (*Artemia salina*), Brine-Shrimp Lethality Assay [24] was used. Results were analyzed with LC_{50} program to determine LC_{50} values and 95% confidence intervals [25]. Results are given in Table 3.

4. Result, discussion and conclusions

In the present work, 19 new compounds were synthesized. The structures of the obtained compounds were elucidated by spectral data. In the IR spectra, some significant stretching bands due N–H, C=O, C=N and C=C were at 3225–3220 cm^{-1}, 1670–1665 cm^{-1}, 1630 cm^{-1}, 1580 cm^{-1}, respectively. In the ^1H NMR spectra, the signal due to $COCH_2$ methylene protons, present in all compounds, appeared at 4.2–4.4 ppm, as singlets. NHCO proton was observed at 12.3–13.1 ppm as broad.

The shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity, and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticides, and cytotoxicity testing of dental materials [26], natural and synthetic organic compounds [24]. It has also been shown that, *A. salina* toxicity test results have a correlation with rodent and human acute oral toxicity data. Generally, a good correlation was obtained between *A. salina* toxicity test and the rodent data. Likewise, the predictive screening potential of the aquatic invertebrate tests for acute oral toxicity in man, including *A. salina* toxicity test, was slightly better than the rat test for test compounds [27].

In order to prevent the toxicity results from possible false effects originated from solubility of compounds and DMSO's possible toxicity effect, compounds were prepared by dissolving in DMSO in the suggested DMSO volume ranges [23].

MIC's were recorded as the minimum concentration of compound, which inhibits the growth of tested microorganisms. All of the compounds tested were showed significant antifungal activity against *C. albicans,* when compared with

Table 1
Some characteristics of the compounds

Compound	R	R′	X	m.p. (°C)	Yield (%)	Molecular formula
III-a	H	Cl	NH	140	78	$C_{13}H_{11}N_4OS_2Cl$
III-b	H	NO_2	NH	120	75	$C_{13}H_{11}N_5O_3S_2$
III-c	CH_3	H	NH	141	72	$C_{14}H_{14}N_4OS_2$
III-d	CH_3	CH_3	NH	200	76	$C_{15}H_{16}N_4O_3S_2$
III-e	$COOC_2H_5$	H	NH	71	69	$C_{16}H_{16}N_4O_3S_2$
III-f	$COOC_2H_5$	Cl	NH	172	79	$C_{16}H_{15}N_4O_3S_2Cl$
III-g	$COOC_2H_5$	CH_3	NH	167	71	$C_{17}H_{18}N_4O_3S_2$
III-h	$COOC_2H_5$	NO_2	NH	152	70	$C_{16}H_{15}N_5O_3S_2$
III-I	H	Cl	O	180	69	$C_{13}H_{10}N_3O_2S_2Cl$
III-j	H	NO_2	O	139	77	$C_{13}H_{10}N_4O_4S_2$
III-k	CH_3	H	O	102	75	$C_{14}H_{13}N_3O_2S_2$
III-l	CH_3	Cl	O	155	80	$C_{14}H_{12}N_3O_2S_2Cl$
III-m	CH_3	CH_3	O	144	81	$C_{15}H_{15}N_3O_2S_2$
III-n	CH_3	NO_2	O	169	78	$C_{14}H_{12}N_4O_4S_2$
III-o	$COOC_2H_5$	H	O	164	74	$C_{16}H_{15}N_3O_4S_2$
III-p	$COOC_2H_5$	Cl	O	185	77	$C_{16}H_{14}N_3O_3S_2Cl$
III-q	$COOC_2H_5$	NO_2	O	208	79	$C_{16}H_{14}N_4O_6S_2$
III-r	CH_3	H	S	85	72	$C_{14}H_{13}N_3OS_3$
III-s	$COOC_2H_5$	H	S	156	74	$C_{16}H_{15}N_3O_3S_3$

Table 2
MIC values of the compounds as μg/ml

Compound	*S. aureus*	*E. coli*	*B. subtilis*	*S. faecium*	*S. epidermidis*	*C. albicans*
III-a	31.25	62.5	62.5	31.25	3.90	4
III-b	15.62	62.5	62.5	31.25	7.81	8
III-c	7.81	62.5	62.5	3.90	15.62	8
III-d	62.5	62.5	7.81	7.81	15.62	16
III-e	31.25	62.5	62.5	62.5	15.62	4
III-f	31.25	62.5	62.5	62.5	15.62	8
III-g	15.62	62.5	62.5	15.62	15.62	8
III-h	62.5	125	62.5	62.5	31.25	16
III-j	31.25	62.5	15.62	1.95	7.81	4
III-l	62.5	62.5	31.25	62.5	15.62	4
III-m	15.62	62.5	15.62	3.90	15.62	8
III-n	31.25	62.5	62.5	31.25	15.62	16
III-o	62.5	62.5	62.5	62.5	15.62	8
III-p	31.25	62.5	62.5	62.5	62.5	8
III-q	31.25	62.5	62.5	62.5	31.25	4
III-r	3.90	62.5	62.5	31.25	7.81	4
III-s	31.25	62.5	62.5	31.25	62.5	16
A	7.81	62.5	31.25	31.25	1.95	–
B	–	–	–	–	–	8

Control compounds: **A**, chloramphenicole; **B**, ketoconazole.

ketoconazole. It was also observed that these compounds have antimicrobial activity against all tested bacteria when compared with chloramphenicole.

In consideration of these data, it has been seen that; especially 2- and 5-substituted benzazole derivatives have shown antimicrobial activities against some Gram-positive, *E. coli* and the yeast *C. albicans*.

The only non-toxic compound **III-j** is the one of the most effective compound against *C. albicans* and other bacteria at the same time.

In comparison of the results of toxicity and antimicrobial activity tests, it is seen that the antimicrobial activity of the compounds are not due to their general toxicity effect, however their antimicrobial activity can be possibly because of their selective antimicrobial effect.

5. Experimental

5.1. Chemistry

All melting points (m.p.) were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck). Spectroscopic data were recorded by the following instruments. IR, Shimadzu IR-435 spectrophotometer; ^{1}H NMR, Bruker 250 MHz spectrometer.

5.1.1. General procedure for synthesis of the compounds

*5.1.1.1. 5-Substituted-4-methyl-2-(chloroacetylamino)thiazole (**I**):* Chloroacetyl chloride (0.01 mol) and triethylamine (0.01 mol) were added to a solution of 5-substituted-2-amino-4-methylthiazole (0.01 mol) in anhydrous benzene and the mixture was treated as described in literature.

*5.1.1.2. 2-[(Benzazole-2-yl)thioacetylamino]thiazole derivatives (**III a–s**):* A mixture of 5-substituted-4-methyl-2-(chloroacetylamino)thiazole (**I**) (0.01 mol), benzazol-2-thiole **II** (0.01 mol) and K_2CO_3 (0.01 mol) in acetone (50 ml) was refluxed for 8 h. After cooling, the solution was evaporated until dryness. The residue was washed with water and recrystallized from ethanol.

III a–e, g, h: IR (KBr, cm^{-1}): 3225 (NH), 1665 (C=O), 1630 (C=N), 1580 (C=C).

III-a: ^{1}H NMR (DMSO-d_6): δ (ppm): 2.70 (3H, s, CH_3), 4.20 (2H, s, $COCH_2$), 7.10 (1H, s, thiazole C_5), 7.60 (1H, d [J = 8.31 Hz], benzimidazole C_6), 7.80 (1H, s, benzimidazole C_4), 7.85 (1H, d [J = 8.40 Hz], benzimidazole C_7), 10.50 (1H, s, benzimidazole N–H), 12.70 (1H, br., NHCO). MS (FAB) [M+1]: *m/z* 339.8.

III-b: ^{1}H NMR (DMSO-d_6): δ (ppm): 2.70 (3H, s, CH_3), 4.30 (2H, s, $COCH_2$), 7.10 (1H, s, thiazole C_5), 7.65 (1H, d [J = 8.61 Hz], benzimidazole C_6), 7.80 (1H, s, benzimidazole C_4), 7.90 (1H, d [J = 8.61 Hz], benzimidazole C_7), 10.50 (1H, s, benzimidazole N–H), 12.70 (1H, br., NHCO).

III-c: ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm): 2.15 and 2.25 (6H, two s, thiazole C_4 and C_5–CH_3), 4.40 (2H, s, $COCH_2$), 7.10–7.60 (4H, m, aromatic protons), 10.60 (1H, s, benzimidazole N–H), 12.30 (1H, br, NHCO).

III-d: ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm): 2.10 and 2.20 (6H, two s, thiazole C_4 and C_5–CH_3), 2.40 (3H, s, benzimidazole C_5–CH_3), 4.30 (2H, s, $COCH_2$), 6.90–7.60 (3H, m, aromatic protons), 10.80 (1H, s, benzimidazole

Table 3
Toxicity assay

Chemical	Cons. (μg/ml)	Mortality [a]	Toxicity	LC_{50}	Upper 95% lim.	Lower 95% lim
III-a	10	0	Harmful	199.53	357.26	111.43
	100	2				
	1000	10				
III-b	10	0	Harmful	316.23	628.26	159.17
	100	2				
	1000	8				
III-c	10	0	Harmful	681.29	–	–
	100	0				
	1000	6				
III-d	10	4	Harmful	100.00	8385.68	1.19
	100	5				
	1000	6				
III-e	10	3	Toxic	21.54	9.87	47.01
	100	9				
	1000	10				
III-f	10	5	Very toxic	10.00	–	–
	100	8				
	1000	10				
III-g	10	4	Toxic	14.68	–	–
	100	10				
	1000	10				
III-h	10	4	Toxic	17.78	71.13	4.45
	100	8				
	1000	10				
III-j	10	0	Non-toxic	1000.00		
	100	0				
	1000	5				
III-l	10	0	Harmful	398.11	1005.52	157.62
	100	2				
	1000	7				
III-m	10	0	Harmful	251.19	388.81	162.28
	100	1				
	1000	10				
III-n	10	5	Very toxic	10.00	–	–
	100	10				
	1000	10				
III-o	10	5	Very toxic	10.00	–	–
	100	9				
	1000	10				
III-p	10	4	Toxic	15.85	50.29	4.99
	100	9				
	1000	10				
III-q	10	4	Toxic	17.78	71.13	4.45
	100	8				
	1000	10				
III-r	10	5	Very toxic	10.00	–	–
	100	9				
	1000	10				
III-s	10	2	Harmful	100.00	403.21	24.80
	100	5				
	1000	8				

[a] Ten organisms (*A. salina*) tested for each concentration.

N–H), 12.30 (1H, br., NHCO). MS (FAB) [M+1]: *m/z* 365.4.

III-e: ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm): 1.30 (3H, t, $COOCH_2$–CH_3), 2.55 (3H, s, thiazole C_4–CH_3), 4.30 (2H, q, $COOCH_2$), 4.40 (2H, s, $COCH_2$), 7.15–7.55 (4H, m, aromatic protons), 12.50 (1H, s, benzimidazole N–H), 13.10 (1H, br., NHCO).

III-g: ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm): 1.20 (3H, t, $COOCH_2$–CH_3), 2.30 (3H, s, benzimidazole C_5–CH_3), 2.40 (3H, s, thiazole C_4–CH_3), 4.20 (2H, q, $COOCH_2$),

4.30 (2H, s, $COCH_2$), 6.90 (1H, d, J = 8.30 Hz, benzimidazole C_6), 7.20 (1H, s, benzimidazole C_4), 7.30 (1H, d [J = 8.13 Hz], benzimidazole C_7), 12.50 (1H, s, benzimidazole N–H), 12.70 (1H, br., NHCO). MS (FAB) [M+1]: *m/z* 391.4.

III-h: ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm): 1.30 (3H, t, $COOCH_2$–CH_3), 2.50 (3H, s, thiazole C_4–CH_3), 4.20 (2H, q, $COOCH_2$), 4.30 (2H, s, $COCH_2$), 7.50 (1H, d [J = 8.85 Hz], benzimidazole C_7), 8.00 (1H, dd [J = 6.54 Hz], benzimidazole C_6), 8.20 (1H, d [J = 2.31 Hz], benzimidazole C_4), 12.10 (1H, s, benzimidazole N–H), 12.30 (1H, br, NHCO).

III k, l, n–p: IR (KBr, cm^{-1}): 3220 (NH), 1667 (C=O), 1630 (C=N), 1580 (C=C).

III-k: ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm): 2.10 and 2.20 (6H, two s, thiazole C_4 and C_5–CH_3), 4.40 (2H, s, $COCH_2$), 7.20–7.70 (4H, m, aromatic protons), 12.30 (1H, br, NHCO). MS (FAB) [M+1]: *m/z* 320.4.

III-l: ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm): 2.15 and 2.25 (6H, two s, thiazole C_4 and C_5–CH_3), 4.45 (2H, s, $COCH_2$), 7.40–7.80 (3H, m, aromatic protons), 12.40 (1H, br, NHCO).

III-n: ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm): 2.10 and 2.20 (6H, two s, thiazole C_4 and C_5–CH_3), 4.45 (2H, s, $COCH_2$), 7.85 (1H, d [J = 8.82 Hz], benzoxazole C_6), 8.20 (1H, d [J = 8.91 Hz], benzoxazole C_7), 8.45 (1H, s, benzoxazole C_4), 12.30 (1H, br, NHCO).

III-o: ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm): 1.20 (3H, t, $COOCH_2$–CH_3), 2.50 (3H, s, thiazole C_4–CH_3), 4.20 (2H, q, $COOCH_2$), 4.40 (2H, s, $COCH_2$), 7.30–7.65 (4H, m, aromatic protons), 12.60 (1H, br, NHCO). MS (FAB) [M+1]: *m/z* 378.4.

III-p: ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm): 1.20 (3H, t, $COOCH_2$–CH_3), 2.45 (3H, s, thiazole C_4–CH_3), 4.20 (2H, q, $COOCH_2$), 4.40 (2H, s, $COCH_2$), 7.30–7.75 (3H, m, aromatic protons), 12.30 (1H, br, NHCO).

III-r: IR (KBr, cm^{-1}): 3223 (NH), 1670 (C=O), 1630 (C=N), 1580 (C=C).

III-r: ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm): 2.10 and 2.20 (6H, two s, thiazole C_4 and C_5–CH_3) 4.40 (2H, s, $COCH_2$), 7.20–7.95 (4H, m, aromatic protons), 12.30 (1H, br, NHCO). MS (FAB) [M+1]: *m/z* 336.4.

5.2. Biology

5.2.1. Antimicrobial activity

Microdilution broth susceptibility assay was used for the antimicrobial evaluation of the compounds, whereas antifungal susceptibility of the *C. albicans* was examined according to NCCLS reference method for broth dilution antifungal susceptibility testing of yeasts [23]. Chloramphenicole was used as standard antibacterial agent and ketoconazole was used as antifungal agent. And both are prepared as described in the related references.

5.2.2. Toxicity

Brine-shrimp toxicity assay was used to determine cytotoxicity levels of the compounds. The compounds prepared by dissolving in DMSO in the suggested DMSO volume ranges [24]. Artificial seawater is prepared by dissolving 3.8 g sea salt per liter of sterile water. The eggs of the brine-shrimp *A. salina*, readily available as a fish food in pet shops, placed in artificial seawater and allowed 48 h at room temperature to hatch and mature. After brine-shrimp larvae have matured (after 2 days), 10 of them placed into each vial contains; 10, 100 and 1000 μg/ml concentrations of the compounds which were prepared by dissolving in DMSO and then added to 5 ml of artificial seawater. After 24 h have elapsed, number of surviving shrimps counted and recorded with the aid of 3× magnifying glasses. Larvae were considered dead, if they did not exhibit any internal or external movement during several seconds of observation. Results were analyzed with the LC_{50} program to determine LC_{50} values and 95% confidence intervals.

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