Synthesis and antifungal activity of some 2-benzothiazolylthioacetyl amino acid and peptide derivatives

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Summary:

A series of benzothiazolylthioacetyl amino acid and peptide derivatives including glycoside, hydrazide and hydrazone moieties were synthesized with the aim of evaluating their antifungal activity. Their chemical structures were confirmed by ^{1}H NMR, IR, mass spectrometry and elemental analyses. Out of the thirty two tested compounds three derivatives have enhanced activity as fluconazole at 100 ppm and six at 1000 ppm toward *Candida albicans* whereas, they were actually inactive toward *Aspergillus flavus*.

Keywords: 2-mercaptobenzothiazole, amino acids, peptides, tyrosine, hydroxyproline, threonine, serine, methionine, glycosides, hydrazides, hydrazones, antifungal activity.

Introduction

2-Substituted benzothiazoles and their derivatives have attracted much attention of chemists and pharmacologists because of their broad spectrum of biological activities and applications as accelerators in rubber vulcanization, antioxidants, dyes, polymers and photographic materials. [1-4] 2-Mercaptobenzothiazole, the first known benzothiazole derivative, was found to be effective antifungal agent. [5-10] Some peptide antibiotics are known to exhibit remarkable antifungal activity, such as iturins, bacillomycins, mycosubtilin, pradimicins and many others. [11-14]

These observations prompted us to undertake systematic study of the synthesis and antifungal evaluation of some mercaptobenzothiazolyl amino acid and peptide derivatives. The 2-mercaptobenzothiazole derivatives were synthesized as shown in schemes 1 and 2. The selection of the amino acids L-serine, L-tyrosine and DL- threonine is based on their presence as major constituents of the antifungal antibiotics iturins A and D, bacillomycins L, D and F and mycosubtilin. [11] In addition L-hydroxyproline is found in the antifungal antibiotic echinocandin B [15] whereas L-methionine is one of the constituent amino acids of the antifungal compound AK-3, isolated from *Synechocystis sp*. [16]

Results and Discussion

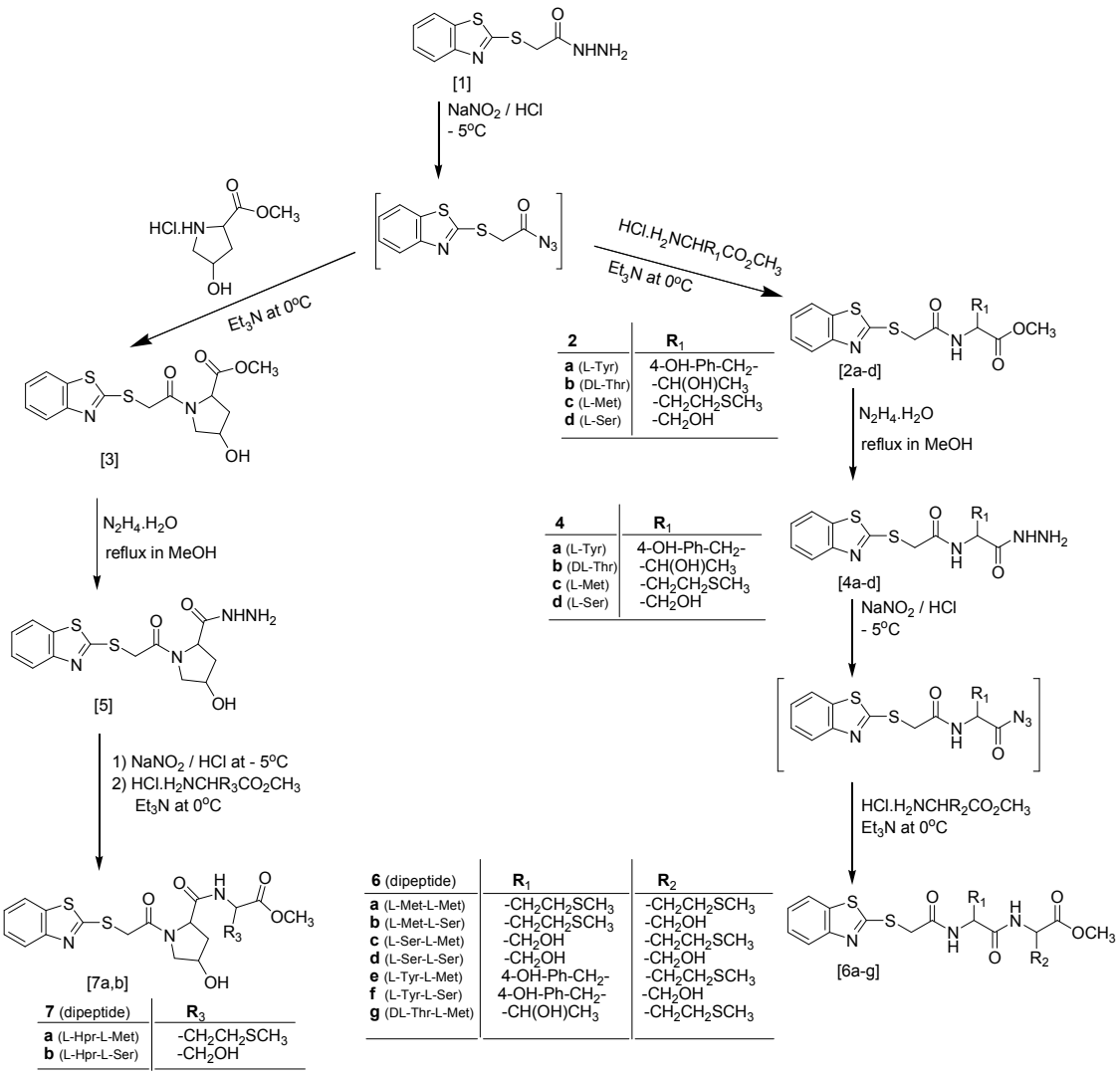
Chemistry

In the present study, 2-benzothiazolylthioacetyl amino acid ester derivatives **2a-d** and **3** were prepared from hydrazide **1** [17] via the racemization-free azide coupling method. [18, 19] Hydrazide **1** was treated with nitrous acid ($NaNO_2$ / HCl) in strong acid medium at low temperature. The resulting azide is unstable at higher temperature, so it was extracted by cold ethyl acetate, neutralized and washed also at low temperature.

The azide solution in ethyl acetate reacted with amino acid methyl esters hydrochloride, previously treated with triethylamine in ethyl acetate at low temperature, to produce 2-benzothiazolylthioacetyl amino acid ester derivatives **2a-d** and **3** in good yields and their chemical structures were confirmed by ^{1}H NMR and elemental analysis. The ^{1}H NMR spectra showed, doublet signal at 8.3 ppm for the NH proton of the peptide bond (except the hydroxyproline derivative **3**), multiplet signals between 6.8 and 8.0 ppm for the four aromatic protons, multiplet signal at 4.5-5.0 ppm for α-CH proton of the amino acids and singlet signal at 3.6 ppm for the three protons of OCH_3 of the ester groups. Also, the geminal coupling between the two protons of the thioacetyl group SCH_2CO appears between 3.8 – 4.3 ppm as two doublet signals. The other signals for the amino acid side chains are reported in the experimental part.

The dipeptide derivatives **6a-g** and **7a, b** were prepared from their corresponding amino acid ester derivatives **2a-d** and **3** after conversion to hydrazides **4a-d** and **5** by boiling with excess hydrazine hydrate in methanol**.** The ^{1}H NMR, IR spectra and elemental analyses of **4a-d** and **5** confirmed their structures as shown from the data reported in the experimental part. The dipeptide derivatives **6a-g** and **7a, b** were obtained by the azide-coupling method in 30-43 % yields (Scheme 1). The ^{1}H NMR spectra revealed, two doublets in the range 7.5 – 8.5 ppm for the two NH protons of the peptide bonds, other two multiplets in the range 4.0 – 5.0 ppm for the α-CH protons of the two

amino acids, in addition to other several signals corresponding to protons of the individual side chains.



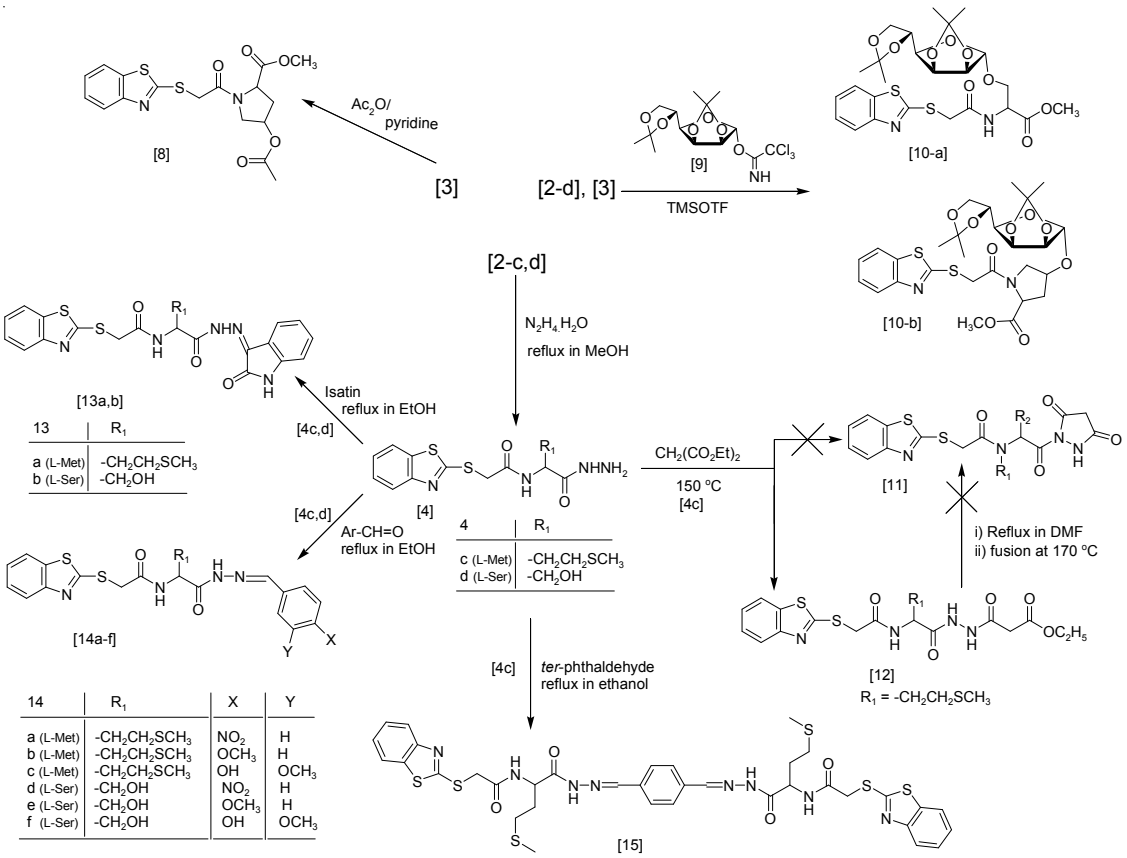
Scheme 1: Synthesis of benzothiazolylthioacetyl amino acid and peptide derivatives.

Some other derivatives, bearing physiologically important functional groups such as, acetylated derivative **8**, glycosides **10a, b**, hydrazide derivative **12**, and hydrazones **13a, b, 14a-f, 15** have been prepared for comparison (Scheme 2).

Acetylation of the hydroxyl group of the amino acid derivatives **2a, b,** and **d** with acetic anhydride in the presence of pyridine failed to give pure products. However, exceptionally compound **3** gave the acetyl derivative **8** in good yield, which was easily

purified by crystallization from ethyl acetate / petroleum ether. The ^{1}H NMR spectrum of **8** showed a singlet signal at 2.02 ppm for the three protons of the acetyl group $COCH_3$.

Next, our target was the synthesis of the glycopeptides **10a,b** by the procedure reported by Schmidt et al.[20] Glycosylation of **2d** and **3** as an alcohol-acceptor precursor, with O-(2:3,5:6-di-O-isopropylidene-α-D-mannofuranose)trichloroacetimidate **(9)** as donor precursor in the presence of catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTF) as Lewis acid afforded **10a,b** in 35.8 % and 28 % yield respectively.



13	R_1
a (L-Met)	$-CH_2CH_2SCH_3$
b (L-Ser)	$-CH_2OH$

4	R_1
c (L-Met)	$-CH_2CH_2SCH_3$
d (L-Ser)	$-CH_2OH$

14	R_1	X	Y
a (L-Met)	$-CH_2CH_2SCH_3$	NO_2	H
b (L-Met)	$-CH_2CH_2SCH_3$	OCH_3	H
c (L-Met)	$-CH_2CH_2SCH_3$	OH	OCH_3
d (L-Ser)	$-CH_2OH$	NO_2	H
e (L-Ser)	$-CH_2OH$	OCH_3	H
f (L-Ser)	$-CH_2OH$	OH	OCH_3

Scheme 2: Reactions of some 2-benzothiazolylthioacetyl amino acid derivatives.

The *O*-glycofuranosyl trichloroacetimidate **9** is characterized by its stability at room temperature for long periods and gives the *α*-anomer derivatives (Scheme 2). The structures of **10a,b** were identified from the ^{1}H NMR and elemental analysis. In the ^{1}H NMR spectra the sugar protons appeared as singlet at 5.34 ppm for H-1 of **10a** and at 5.09 ppm for H-1 of **10b**, confirming the α-anomer of the glycosides **10a,b**. The doublet at 4.59 ppm (J = 5.8 Hz) was attributed to H-2, whereas multiplet at 4.78-4.71 ppm

assigned for H-3**.** The singlet at 3.68 ppm and the four singlet signals at 1.41, 1.33, 1.29 and 1.22 ppm were assigned to OCH_3 and 4 CH_3 of isopropylidene groups respectively.

Reaction of the hydrazide **4c** with diethyl malonate led to the formation of the linear hydrazino ethyl acetate derivatives **12**. ^{1}H NMR spectra of the product showed singlet signal at **3.25 ppm** for (–CO-CH_2-CO-), multiplet signal at **4.12-4.06 ppm** and triplet signal at **1.16 ppm** for OCH_2CH_3 group indicative of the formation of linear product **12** rather than the cyclic one **11**. Trials to obtain the corresponding pyrazolidinedione derivative **11** either by fusion of **12** or by heating in DMF were unsuccesful.

Hydrazone derivatives have gained importance due to their application in pharmaceutical chemistry. The biological activity associated with these compounds was attributed to the presence of the (–CONHN=CH–) moiety. [21] In the present study, some hydrazones have been synthesized by refluxing the hydrazides **4c,d** with different carbonyl compounds such as isatin, p-nitrobenzaldehyde, anisaldehyde, vanillin and benzene-1,4-dicarbaldehyde. The structures of the hydrazones **13a,b**, **14a-f** and **15** were investigated by IR, ^{1}H NMR, mass spectrometry and elemental analyses. (See the experimental part). The ^{1}H NMR spectra of hydrazones **14a**, **14b** and **14c** (Table 1) revealed the existence of a mixture from E- and Z-stereoisomers (Fig. 1) in which the E-form predominates. This conclusion is supported by a previously published data on similar compounds. [22, 23]

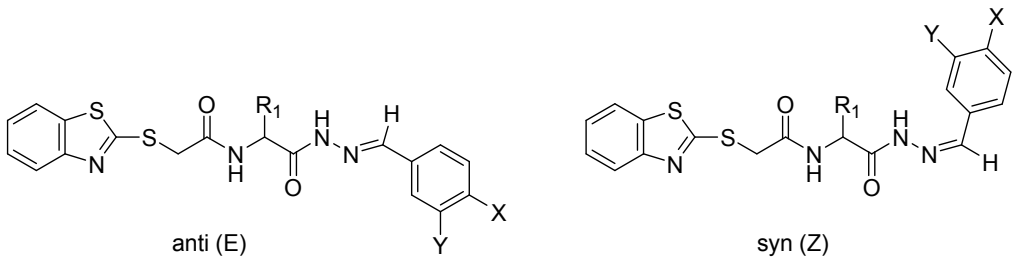


Fig.1: E- and Z-Forms of Compounds 14a-c

Table1: Data of ^{1}H NMR Spectra of Compounds 14a-c

compound		Chemical shifts, δ, ppm						
		NH, s	NH, d	=CH,s	NCH, m	OCH_3, s	SCH_3, s	Other signals
14a	E (54.5%)	9.91	8.42	Involved in aromatic range	5.60-5.54	--	1.89	8.23-8.04 (2H, m, ArH), 7.94-7.72 (5H, m, 4ArH, CH), 7.45-7.31 (2H, m, ArH), 4.14-3.92 (2H, m, CH_2S), 2.53-2.42 (2H, m, CH_2S), 2.26-2.01 (2H, m, CH_2)
	Z (45.5%)	10.59	8.56		4.80-4.68	--	1.94	
14b	E (60%)	9.39	8.36	7.71	5.60-5.55	3.83	1.85	7.97-7.90 (2H, m, ArH), 7.60-7.54 (2H, m, ArH), 7.40-7.29 (2H, m, ArH), 6.91-6.80 (2H, m, ArH), 4.16-3.86 (2H, m, CH_2S), 2.47-2.38 (2H, m, CH_2S), 2.29-2.05 (2H, m, CH_2)
	Z (40%)	10.19	8.49	7.75	4.78-4.66	3.80	1.94	
14c	E (53.3%)	10.66	8.36	8.17	5.39-5.85	3.70	1.67	7.78-7.55 (2H, m, ArH), 7.26-7.10 (3H, m, ArH), 6.82-6.62 (2H, m, ArH), 3.97-3.73 (2H, m, CH_2S), 2.67 (1H, s, OH), 2.31-2.21 (2H, m, CH_2S), 2.00-1.82 (2H, m, CH_2),
	Z (46.7%)	10.88	8.49	7.59	4.58-4.47	3.68	1.47	

Biological evaluation

The antifungal activities of the synthesized compounds were measured using the well technique of the agar diffusion method with comparison to fluconazole and 2-mercaptobenzothiazole **MBT.** All the tested compounds were screened for antifungal activities *in vitro* against *Aspergillus flavus* as a representative of mold fungi and *Candida albicans* as a representative of yeasts. The recorded data, [Tables 2 & 3], lead to the following conclusions:

- None of the synthesized and tested compounds had comparable activity to fluconazole towards both *Aspergillus flavus* and *Candida albicans*.
- All the synthesized and tested benzothiazolyl derivatives were devoid of antifungal activity (except **10a**) against the mold *Aspergillus flavus.*
- It is evident that *Candida albicans* is more sensitive to benzothiazolyl amino acid and dipeptide derivatives than *Aspergillus flavus.*
- It is clear that out of the thirty two tested compounds three derivatives have enhanced activity as fluconazole at 100 ppm and six at 1000 ppm toward *Candida albicans.*

Table2: The antifungal effects of the synthesized compounds against mold (*Aspergillus flavus*) and yeast (*Candida albicans*) fungi indicated by the diameter of the inhibition zones (well method).

Compd.	*Aspergillus flavus*				*Candida albicans*			
	100ppm	**300ppm**	**500ppm**	**1000ppm**	**100ppm**	**300ppm**	**500ppm**	**1000ppm**
MBT	--	--	+	++	+	+	++	+++
2a	--	--	--	--	+	+	++	+++
2b	--	--	--	--	--	--	--	+
3	--	--	--	--	--	+	+	+++
4c	--	--	--	--	--	+	+	++
5	--	--	--	--	--	--	--	++
6f	--	--	--	--	--	--	--	+
7a	--	--	--	+	--	--	--	++
7b	--	--	--	+	--	--	--	++
8	--	--	--	--	+	+	++	+++
10a	--	+	+	++	--	--	+	++
10b	--	--	--	--	--	--	--	+
12	--	--	--	--	--	--	--	+
13a	--	--	--	--	--	+	++	+++
13b	--	--	--	--	--	+	++	+++
14a	--	--	--	--	+	+	+	++
14c	--	--	--	--	--	+	++	+++
14d	--	--	--	--	--	--	--	+
15	--	--	--	--	--	--	+	++
fluconazole	+	++	+++	+++	+	++	+++	+++

Zone diameter of growth inhibition: (--) inactive, (+) < 10 mm, (++) 10-15 mm, (+++) >16 mm.

- The tyrosine and *O*-acetyl hydroxyproline methyl ester derivatives **2a** and **8** respectively were the most potent compounds against *Candida albicans* among the tested benzothiazolyl derivatives.
- Conversion of the benzothiazolyl amino acid ester derivatives to the corresponding hydrazides does not improve appreciably their antifungal activity with exception of the methionine derivative **4c**.
- Hydrazone derivatives showed higher activity than the corresponding hydrazides especially these of isatin **13a, b** and methionine **14a, c**.
- In general all the tested dipeptide derivatives were actually inactive with exception of **7a, b**, which showed slight activity against both *Candida albicans* and *Aspergillus flavus.*

Table3: The MIC and the MFC of the synthesized compounds compared with MBT and fluconazole

	A. flavus		*C. albicans*	
Compd.	MIC values (µg/ml)	MFC values (µg/ml)	MIC values (µg/ml)	MFC values (µg/ml)
MBT	500	--	100	500
2a	--	--	100	500
2b	--	--	1000	--
3	--	--	300	--
4c	--	--	300	--
5	--	--	1000	--
6f	--	--	1000	--
7a	1000	--	1000	--
7b	1000	--	1000	--
8	--	--	100	500
10a	300	500	500	500
10b	--	--	1000	--
12	--	--	1000	--
13a	--	--	300	300
13b	--	--	300	500
14a	--	--	100	500
14c	--	--	300	--
14d	--	--	1000	--
15	--	--	500	--
fluconazole	100	300	100	300

MIC: the minimum inhibitory concentration MFC: the minimum fungicidal concentration
(--) inactive

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