

Synthesis, Infrared Spectra, Conformation, and Antiyeast Activity of 2-(Alkoxy carbonylmethylthio)-6-aminobenzothiazoles

^aE. SIDÓOVÁ, ^bA. PERJÉSSY, ^aD. LOOS, and ^cT. KUČHTA

^aInstitute of Chemistry, Faculty of Natural Sciences, Comenius University, CS-842 15 Bratislava

^bDepartment of Organic Chemistry, Faculty of Natural Sciences, Comenius University, CS-842 15 Bratislava

^cDepartment of Microbiology and Virology, Faculty of Natural Sciences, Comenius University, CS-842 15 Bratislava

Received 29 November 1991

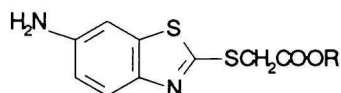
2-(Alkoxy carbonylmethylthio)-6-aminobenzothiazoles were synthesized. Their conformation and electronic structure were studied both by means of IR spectra and theoretical methods (MMP1 and CNDO/2). Some derivatives have shown good antiyeast activity against *Candida krusei* and *Saccharomyces cerevisiae*.

2-Alkylthio-6-aminobenzothiazoles have shown good antimycobacterial [1] and antiyeast [2, 3] activity. It has been a challenge for us to investigate the corresponding esters, which have been synthesized in a similar way as 2-alkylthio-6-aminobenzothiazoles [1]. Twelve 2-(6-aminobenzothiazolylthio)acetic acid esters have been synthesized (Table 1), ten of them having been identified

for the first time. Ethyl [4] and allyl [5] esters were synthesized earlier and some other derivatives were used as intermediates without isolating the pure compounds [6, 7].

The structure of the synthesized compounds was confirmed by infrared spectra. The wavenumbers of C=O stretching vibrations of 2-(alkoxy carbonylmethylthio)-6-aminobenzothiazoles I–XII measured

Table 1. Characterization of the Prepared 2-(Alkoxy carbonylmethylthio)-6-aminobenzothiazoles



Compound	R	Formula M_r	$w_i(\text{calc.})/\%$ $w_i(\text{found})/\%$				Yield %	M.p./°C $n_D(20^\circ\text{C})$
			C	H	N	S		
I	CH ₃	C ₁₀ H ₁₀ N ₂ O ₂ S ₂	47.22	3.96	11.01	25.21	93	85–86.5
		254.33	46.97	3.89	10.95	25.30		
III	(CH ₂) ₂ CH ₃	C ₁₂ H ₁₄ N ₂ O ₂ S ₂	51.04	5.00	9.92	22.71	84	61.0–62.5
		282.39	51.19	5.02	9.90	22.85		
IV	CH(CH ₃) ₂	C ₁₂ H ₁₄ N ₂ O ₂ S ₂	51.04	5.00	9.92	22.71	79	82.0–83.0
		282.39	50.76	4.99	9.82	22.62		
VI	CH ₂ —C≡CH	C ₁₂ H ₁₀ N ₂ O ₂ S ₂	51.78	3.62	10.06	23.04	93	96.5–97.5
		278.35	51.77	3.61	10.12	22.92		
VII	(CH ₂) ₃ CH ₃	C ₁₃ H ₁₆ N ₂ O ₂ S ₂	52.68	5.44	9.45	21.63	88	59.0–60.5
		296.41	52.66	5.41	9.51	21.34		
VIII	CH(CH ₃)C ₂ H ₅	C ₁₃ H ₁₆ N ₂ O ₂ S ₂	52.68	5.44	9.45	21.63	81	52.0–54.0
		296.41	52.80	5.42	9.52	21.94		
IX	(CH ₂) ₂ CH(CH ₃) ₂	C ₁₄ H ₁₈ N ₂ O ₂ S ₂	54.16	5.84	9.02	20.66	97	1.6030
		310.45	54.07	5.91	8.80	20.38		
X	(CH ₂) ₅ CH ₃	C ₁₅ H ₂₀ N ₂ O ₂ S ₂	55.53	6.21	8.63	19.76	77	29.0–30.5
		324.47	55.25	6.21	8.41	19.90		
XI	CH(CH ₃)(CH ₂) ₅ CH ₃	C ₁₇ H ₂₄ N ₂ O ₂ S ₂	57.92	6.86	7.95	18.19	79	63.0–64.5
		352.52	57.94	6.89	8.09	18.40		
XII	CH ₂ C ₆ H ₅	C ₁₈ H ₁₄ N ₂ O ₂ S ₂	58.16	4.27	8.48	19.41	87	115.0–116.0
		330.43	58.04	4.22	8.54	19.46		

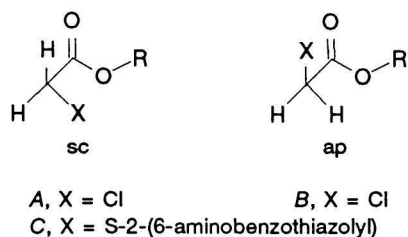
Compound II (R = C₂H₅), yield = 89 %, m.p. = 95.5–97.0 °C (according to Ref. [4] 92 °C). Compound V (R = CH₂—CH=CH₂), yield = 90 %, m.p. = 91.0–92.0 °C (according to Ref. [5] 89 °C).

Table 2. Infrared Spectral Data ($\tilde{\nu}/\text{cm}^{-1}$) and Taft Substituent Constants $\sigma^*(R)$ for Compounds I—XII

Compound	$\nu(\text{C}=\text{O})$		$\sigma^*(R)^a$
	CHCl_3	CCl_4	
I	1741.8	1749.2	0.000
II	1736.0	1745.0	-0.100
III	1736.2	1742.5	-0.115
IV	1731.0	1738.3	-0.190
V	1738.9	1745.3	0.180 ^b
VI	1748.7	1755.8	0.468 ^c
VII	1734.5	1741.7	-0.130
VIII	1730.8	1737.5	-0.210 ^b
IX	1734.9	1741.1	-0.126
X	1734.6	1742.5	-0.130
XI	1730.1	1737.6	-0.227 ^d
XII	1738.7	1743.8	0.215

a) Taken from [8]. b) Taken from [9]. c) Calculated using the equation $\sigma^*(\text{CH}_2\text{X}) = 0.36 \sigma^*(\text{X})$ and the value $\sigma^* = 1.3$ for CH_2C taken from [9]. d) Calculated by means of the equations $\sigma^*(\text{CXYZ}) = \sigma^*(\text{CH}_2\text{X}) + \sigma^*(\text{CH}_2\text{Y}) + \sigma^*(\text{CH}_2\text{Z})$.

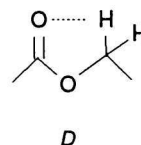
in tri- and tetrachloromethane are listed in Table 2. On comparing these data with the wavenumbers of $\nu(\text{C}=\text{O})$ vibrations measured for a series of monochloroacetates [10], which exist in two conformations A and B, 2-(alkoxycarbonylmethylthio)-6-aminobenzothiazoles in both solvents (CHCl_3 and CCl_4) exhibit only one $\nu(\text{C}=\text{O})$ absorption band and therefore these compounds exist exclusively in only one conformation in respect of the hindered rotation around the $\text{CH}_2\text{—CO}$ bond.



The mentioned absorption band in CCl_4 solution has been found in the range of $\tilde{\nu} = 1737\text{—}1756 \text{ cm}^{-1}$ well corresponding to the analogical values of $\nu(\text{C}=\text{O})$ for the conformation A of monochloroacetates ($1740\text{—}1756 \text{ cm}^{-1}$), which is an evidence for the fact that compounds I—XII exist in the conformation C analogous to the conformation A of monochloroacetates. It can be supposed that in the monochloroacetates the strong repulsion between the lone electron pairs on the chlorine atom and the oxygen atom of the RO group causes the twisting of these atoms to the most distant mutual position and therefore beside conformation A there exists a less stable antiperiplanar conformation B as well. In the case of the investigated compounds I—XII the repulsion between the lone electron pairs of the sulfur atom of the 2-thio group

and of the oxygen atom in the RO group is much weaker and therefore the compounds remain in only one, thermodynamically more advantageous conformation C which is close to the synclinal conformation A of the monochloroacetates.

The wavenumbers of the $\text{C}=\text{O}$ stretching vibrations of the investigated compounds I—IV and VII—XI, measured in both solvents (CHCl_3 and CCl_4), similarly as in the case of monochloroacetates [10] give statistically significant correlations with Taft induction constants $\sigma^*(R)$ of the substituents R (listed in Table 2). Analogically as in the case of monochloroacetates, here the compounds V, VI, and XII exhibit an exception, as their alkyl groups contain α -methylene, the acidity of which is sufficient [11] for the formation of intramolecular hydrogen bond of the type D with the CO group.



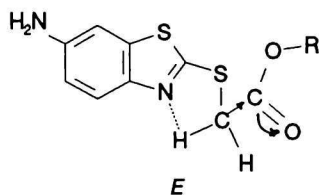
As a result of this hydrogen bond the wavenumbers of $\nu(\text{C}=\text{O})$ of the compounds V, VI, and XII are observed to be shifted by $12\text{—}18 \text{ cm}^{-1}$ to lower values against the values resulting from the $\tilde{\nu}(\nu(\text{C}=\text{O}))$ vs. $\sigma^*(R)$ correlation. The results of the correlation analysis in comparison with analogous results for monochloroacetates are given in Table 3. It follows from the table that in 2-(alkoxycarbonylmethylthio)-6-aminobenzothiazoles the inductive effects of the substituents R are transferred on the $\text{C}=\text{O}$ group much more effectively (slope $\rho = 54.53 \text{ cm}^{-1}$ (for CCl_4)) than in monochloroacetates ($\rho = 35.69$ and 37.92 cm^{-1} (for CCl_4)), which is evidently caused by strong electron-donating effect of 6-amino-2-benzothiazolylthio group in comparison with the electron-withdrawing effect of the chlorine. This effect is probably still more amplified by the existence of the intramolecular hydrogen bond of the type E, which we suppose

Table 3. The Statistical Results of Linear Correlations of Wavenumbers of $\text{C}=\text{O}$ Stretching Vibrations with the Substituent Constants $\sigma^*(R)$

Series	Solvent	<i>n</i>	<i>r</i>	ρ	<i>q</i>	<i>s</i>
C	CCl_4	9	0.980	54.53	1749.1	0.8
C	CHCl_3	9	0.994	52.59	1741.6	0.4
A [10]	CCl_4	10	0.992	37.92	1749.6	1.4
B [10]	CCl_4	10	0.986	35.69	1773.0	1.8
B [10]	CHCl_3	10	0.981	40.16	1760.2	2.3

n - number of experimental points in the correlation; *r* - correlation coefficient; ρ - slope; *q* - intercept; *s* - standard deviation.

between the α -methylene group and the lone electron pair of the nitrogen atom of the benzothiazole skeleton.



The conformation and the electronic structure of the synthesized compounds were studied by theoretical methods as well and the results were compared with the experimental data.

The structures of the optimal conformations were calculated by the method of molecular mechanics (MMPI) [12] and the electronic structure, especially the charge densities and Wiberg indices by the quantum-chemical method CNDO/2 [13]. Various conformations of methyl esters (I) and in some cases ethyl esters (II) as well were chosen for theoretical study. Monochloroacetates were studied, when the alkyl group was methyl.

For monochloroacetates the synclinal conformation appeared to be energetically more favourable than the antiperiplanar one. The difference of the total energy (CNDO/2) is $\Delta E_{\text{tot}} = 5 \text{ kJ mol}^{-1}$ and that of the steric energy (MMPI) is $\Delta E_{\text{ster}} = 2.5 \text{ kJ mol}^{-1}$. The calculated torsion angle $\text{Cl}-\text{C}-\text{C}-\text{O}$ is 111° .

Similar results were obtained also in the case when Cl was substituted by SH group but the difference of the total energy was more pronounced ($\Delta E_{\text{tot}} = 17 \text{ kJ mol}^{-1}$). The torsion angle remained unchanged in this case but its rather significant change to 120° occurred when Cl was substituted by H. The substituents SH and Cl cause increasing of the charge density on the oxygen of the CH_3O group and decreasing of the value of Wiberg index of the $\text{C}=\text{O}$ bond.

The results of the investigation of 2-(alkoxycarbonylmethylthio)-6-aminobenzothiazoles were not so unambiguous. The changes of the Wiberg indices of the $\text{C}=\text{O}$ bond in seven various conformations are very small ($\Delta W_i < 0.01$) and therefore the changes of the wavenumber values of $\text{C}=\text{O}$ stretching vibrations are small as well. The influence of 6-amino-2-benzothiazolyl group manifested itself by the increase of negative charge on the sulfur atom and on the oxygen atom of the $\text{C}=\text{O}$ group, by the increase of the $\text{C}=\text{O}$ bond order and by the decrease of the $\text{C}-\text{OR}$ bond order, i.e. also by the shift of the negative charge towards the oxygen of the $\text{C}=\text{O}$ group. In the antiperiplanar conformation, which gave the best values of steric and total energy, the torsion angle is

122° , which is very close to the same type of conformation with monochloroacetates. The synclinal conformation is energetically less advantageous. The difference between the steric energy of synclinal and antiperiplanar conformation is 4 kJ mol^{-1} and the difference of the total energy is 5 kJ mol^{-1} . The value of Wiberg indices of the $\text{C}=\text{O}$ bond is in both cases nearly the same (1.820). The torsion angle of the synclinal conformation for $\text{S}-\text{C}-\text{C}-\text{OR}$ is 16° . The difference between the relative stability of both conformations is very small, therefore it can be very easily influenced by interaction with the solvents or by forming of hydrogen bond. When changing the values of torsion angles in order to get optimal planar structures for both conformers, no Wiberg indices were obtained which would point to forming of a hydrogen bond.

In order to determine the optimal geometric structure of synclinal and antiperiplanar conformers not only methyl esters but also ethyl esters were studied. The antiperiplanar conformer of the ethyl ester is more stable than the synclinal one similarly as with the methyl esters. The difference in the total energy is 3 kJ mol^{-1} and that of the steric energy is 4 kJ mol^{-1} . The torsion angle for antiperiplanar conformation is 123° and for the synclinal one 18° . The change of torsion angle is not influenced by the change of the charge on the oxygen of the OR group, therefore it is possible to suppose a steric influence of the 6-amino-2-benzothiazolyl group.

The results obtained for the structure of the methyl and ethyl ester conformers do not differ and their relative stability is equal as well. The obtained theoretical results are in a good agreement with the experimental ones and point to the preference of synclinal conformation C for the existence of 2-(alkoxycarbonylmethylthio)-6-aminobenzothiazoles, as well as to the fact that the electronic effects of the substituents R are transferred much more readily in them than in the analogical monochloroacetates.

The synthesized compounds were tested for antimicrobial activity. Their antifungal activity against *Aspergillus niger* did not reach the activity of 2-mercaptobenzothiazole (MBT) used as standard. The antiyeast activity with the majority of the compounds against *Saccharomyces cerevisiae* CCY 28-3 is better than that of MBT and the best ones are superior to MBT by more than one order. IC_{50} , i.e. the concentration causing 50 % inhibition of the growth of the culture in comparison with the control was found (in mol dm^{-3}): 11 for 2-octyl (XI), 12 for benzyl (XII), 13 for isopentyl (IX) and n-hexyl (X) derivatives. The values obtained for the standards were: 14 for 6-amino-2-n-pentylthiobenzothiazole, the most efficient compound among

2-alkylthio-6-aminobenzothiazoles and 197 for MBT, the effective component of Dermacid, a preparation with antimycotic activity.

Some derivatives manifested good antiyeast activity against the clinical pathogen *Candida krusei*. The best obtained IC_{50} values ($\mu\text{mol dm}^{-3}$) are: 44 for benzyl (XII) and 47 for n-hexyl (X) derivatives, while MBT is represented by the value 245. The results of the antimicrobial tests have verified the supposed biological activity of the synthesized compounds.

EXPERIMENTAL

Yields, melting points determined with a Kofler micro hot-stage and elemental analyses of the compounds are listed in Table 1. The infrared spectra were recorded in the range of $\tilde{\nu} = 1600\text{--}1800\text{ cm}^{-1}$ in tri- and tetrachloromethane with a Specord 75 spectrophotometer (Zeiss, Jena) in 0.1 and 1.0 cm cells. The concentration of the solutions was chosen so that the maximal transmittance of bands in the investigated range should be 25–30 %. The frequency scale of the apparatus was calibrated according to a polystyrene reference spectrum and the absorption band maxima were read with the accuracy of $\pm 0.1\text{ cm}^{-1}$.

For the determination of antifungal effect on yeasts *in vitro* liquid synthetic medium was used (5 cm³ in test tubes), the cultivation was static at 28 °C. The compounds were tested immediately after solving in dimethyl sulfoxide, they were dosed to the medium so that the concentration of solvent in the medium should not exceed 1 vol. %. Afterwards the inoculum was added to the medium: with *Saccharomyces cerevisiae* 10^5 cells cm⁻³ and with *Candida krusei* 10^4 cells cm⁻³. The growth of the yeast was followed photometrically and from the dependence $A/A_{\text{control}} = f(\log c)$ (where A is absorbance and c concentration of the compound in the medium) the values IC_{50} were read by nonlinear regression.

2-(Alkoxy-carbonylmethylthio)-6-aminobenzothiazoles I–XII

6-Amino-2-benzothiazolinethione (9.1 g; 0.050 mol) was suspended by mechanical stirring in the solution of potassium hydroxide (3.6 g; 0.056 mol)

in water (10 cm³). Dimethylformamide (30 cm³) was added and immediately after complete dissolution of the solid alkyl chloroacetate (0.050 mol) was added in one portion. After 30 min of further stirring the reaction mixture was poured onto ice (800 g) and after solidifying of the product water was added to get total volume of 2000 cm³ and the solid was isolated by suction.

The compounds were crystallized from ethanol (I–VII and XII), ethanol–petroleum ether (VIII and XI) or ethanol–diethyl ether–petroleum ether (X) using charcoal. Some compounds with lower m.p. (VIII, X, and XI) were dried at 5 °C wrapped in filter paper. The isopentyl derivative IX, a viscous liquid, was purified by extraction of the impurities with petroleum ether and dried by filtering through a funnel filled with calcium chloride.

Acknowledgements. Our thanks are due for the intermediate alkyl chloroacetates to Professor RNDr. V. Sutoris, CSc. (Department of Organic Chemistry) and for analyses to Ing. E. Grejplová (Institute of Chemistry, Laboratory of Elemental Analysis, Faculty of Natural Sciences, Comenius University, Bratislava).

REFERENCES

- Sidóová, E., Odlerová, Ž., Volná, F., and Blöckinger, G., *Chem. Zvesti* 33, 830 (1979).
- Kuchta, T., Bujdaková, H., Sidóová, E., and Neslušanová, E., *Acta Fac. Rerum Nat. Univ. Comenianae, Microbiologia* 17, 18, 49 (1990).
- Kuchta, T., Bujdaková, H., and Sidóová, E., *Folia Microbiol. (Prague)* 34, 504 (1989).
- Torizo Takahashi and Jutaro Okada, *J. Pharm. Soc. Jpn.* 73, 802 (1953); *Chem. Abstr.* 48, 9365d (1954).
- Sutoris, V. and Blöckinger, G., *Czech.* 157793 (1975).
- Lipthay, T. and Foltínová, P., *Acta Fac. Rerum Nat. Univ. Comenianae, Chimia* 29, 9 (1981).
- Lipthay, T., Foltínová, P., and Blöckinger, G., *Univ. Comenianae Fac. Paedag. Tyrnaviensis, Chémia* 8, 185 (1977).
- Exner, O., *Struktura a reaktivita.* (Structure and Reactivity.) The House of Technics of the Czechoslovak Scientific-Technical Society, Pardubice, 1970.
- Zhdanov, Yu. A. and Minkin, V. I., *Korrelyatsionnyi analiz v organicheskoi khimii*, p. 307–319. Izdatel'stvo Rostovskogo Universiteta, Rostov na Donu, 1966.
- Perjéssy, A. and Sutoris, V., *Acta Fac. Rerum Nat. Univ. Comenianae, Chimia* 20, 71 (1974).
- Radel, J. and Harrah, L. A., *J. Chem. Phys.* 36, 1571 (1962).
- Burkert, U. and Allinger, N. L., *Molecular Mechanics.* American Chemical Society, Washington, 1982.
- Pople, J. A. and Beveridge, D. L., *Approximate Molecular Orbital Theory.* McGraw-Hill, New York, 1970.

Translated by E. Sidóová