Thiosemicarbazones and 2-Thio-4-(phthalimidoalkylidene)thiazolid-5-ones of N-Phthaloyl Amino Aldehydes. Preparation and Antibacterial Activity

J. Kolačny, N. Štimac, B. Sajko, B. Balenović and B. Urbas

Research Department, »Pliva« Pharmaceutical and Chemical Works, Zagreb, Croatia, Yugoslavia

Received March 29, 1954

Thiosemicarbazones of the formula I and 2-thio-4-(phthalimidoalkylidene)thiazolid-5-ones of the formula II derived from glycine, L-alanine, β-alanine, DL-leucine, DL-valine, L-tyrosine, DL-serine and α-amino-n-butyric acid were prepared and tested against *Staphylococcus aureus, B. pyocyaneus, E. coli* and *Enterococcus.*

A few years ago Fling, Minard and Fox[1] showed that N-phthaloyl derivatives of α-amino acids have similar antibacterial properties as D-amino acids[2, 3]. Thus D and L-N-phthaloyl valines and N-phthaloyl leucines were antibacterial in concentration of about 3 mg./ml. when tested on *Lactobacillus arabinosus*[2, 3]. or *Escherichia coli*[1].

We prepared further N-phthaloyl-α-amino acid derivatives for the testing of antibacterial activity. From the N-phthaloyl aldehydes of naturally occurring α-amino acids we prepared thiosemicarbazones of formula I, because, as Domagk et al. have shown[4], thiosemicarbazones of aromatic and heterocyclic aldehydes are very active chemotherapeutic agents.

$$C_6H_4(CO)_2NCH(R)CH : N \cdot NH(C : S)NH_2$$

I

a, R = H
b, R = CH_3CH_2
c, R = $(CH_3)_2CHCH_2$
d, R = $C_2H_5OCH_2$
e, R = p-$(CH_3O)C_6H_4CH_2$
f, R = $(CH_3)_2CH_2$

Furthermore, from N-phthaloyl-α-amino aldehydes and 2-mercaptothiazolid-5-one we prepared 2-thio-4-(phthalimidoalkylidene)-thiazolid-5-ones of the formula II, following methods described earlier[5, 6, 7]. Synthesis of compounds of this type was stimulated by the fact that the antibiotic Mycobacidin, recently isolated by three independent groups of research workers[8, 9, 10] from the *Streptomyces* genus, especially from a new species *Streptomyces virginiae,* shows a high specific activity *in vitro* against mycobacteria. The structure of the compound was found to be 4-thiazolidone-2-caproic acid[10, 11] and verified by synthesis[11].

$$C_6H_4(CO)_2NCH(R)(CH_2)_nCH : \begin{matrix} NH \cdot C : S \\ | \\ CCO \end{matrix}$$

II

a, R = H; n = 1
b, R = CH_3; n = 0
c, R = $(CH_3)_2CH$; n = 0
d, R = $C_2H_5OCH_2$; n = 0
e, R = CH_3CH_2; n = 0

The substances Ia-f and IIa-e were tested for antibacterial activity against *Staphylococcus aureus, B. pyocyaneus, E. coli* and *Enterococcus* using the Food and Drug Administration method[16], and the Oxford Cup Assay Method[17]. For comparison, antibacterial activity of 2-mercaptothiazol-5-one and 2-thio-4-(propylidene)thiazolid-5-one was also tested. The results are summarized in Tables 1 and 2.

Experiments *in vivo* with some of these substances are in progress, as well as experiments with *mycobacteria.*

TABLE I

Compound	micro-organism*	Food & Drug Administration Method[16,**] time in minutes 1	2	5	10	15	30	hrs. 24	control	Oxford Cup Assay Method[17,***] penetration in mm.
Ia	A	+++	—	—	—	—	—	—	+++	
	B	—	—	—	—	—	—	—	+++	
	C	—	—	—	—	—	—		+++	
	D	++	—	—	—	—	—		+++	
Ib	A	+++	++	—	—	—	—	—	+++	0
	B	—	—	—	—	—	—	—	+++	0
	C	—	—	—	—	—	—	—	+++	0
	D	+	+	—	—	—	—	—	+++	0
Ic	A	—	—	—	—	—	—	—	+++	20
	B	—	—	—	—	—	—	—	+++	26
	C	—	—	—	—	—	—	—	+++	20
	D	—	—	—	—	—	—	—	+++	20
Id	A	—	—	—	—	—	—	—	+++	16
	B	—	—	—	—	—	—	—	+++	0
	C	—	—	—	—	—	—	—	+++	15
	D	+++	+	+	—	—	—	—	+++	0
Ie	A	+++	+++	++	+	—	—	—	+++	
	B	—	—	—	—	—	—	—	+++	
	C	—	—	—	—	—	—	—	+++	
	D	+++	+++	+++	+++	+++	+++	+++	+++	
If	A	—	—	—	—	—	—	—	+++	15
	B	—	—	—	—	—	—	—	+++	0
	C	—	—	—	—	—	—	—	+++	18
	D	++	+	—	—	—	—	—	+++	0

(Normal growth: +++; 2/3 normal growth: ++; 1/3 normal growth: +; complete inhibition of growth: —)

TABLE II

Compound	micro-organism*	Food & Drug Administration Method[16], ** time in minutes 1	2	5	10	15	30	hrs. 24	control	Oxford Cup Assay Method[17], *** penetration in mm.
IIa	A	+++	+	—	—	—	—	—	+++	
	B	—	—	—	—	—	—	—	+++	
	C	—	—	—	—	—	—	—	+++	
	D	+++	+++	+	—	—	—	—	+++	
IIb	A	+++	++	+	—	—	—	—	+++	
	B	—	—	—	—	—	—	—	+++	
	C	—	—	—	—	—	—	—	+++	
	D	+++	+++	+++	++	+	+	+	+++	
IIc	A	+++	+++	+++	+++	+++	+++	+++	+++	13
	B	—	—	—	—	—	—	—	+++	0
	C	+++	+++	+++	+++	+++	+++	+++	+++	0
	D	+++	+++	+++	+++	+++	+++	+++	+++	0
IId	A	+++	+++	+++	+++	+++	+++	+++	+++	0
	B	+++	+++	+++	+++	+++	+++	+++	+++	0
	C	+++	+++	+++	+++	+++	+++	+++	+++	0
	D	+++	+++	+++	+++	+++	+++	+++	+++	0
IIe	A	+++	+++	+++	+++	+++	+++	+++	+++	0
	B	+++	+++	+++	+++	+	—	—	+++	0
	C	+++	+++	+++	+++	+++	+++	+++	+++	0
	D	+++	+++	+++	+++	+++	+++	+++	+++	0
****	A	—	—	—	—	—	—	—	+++	
	B	++	++	++	+	+	+	+	+++	
	C	+++	+++	+++	+++	+++	+++	+++	+++	
	D	+++	+++	+++	+++	+++	+++	+++	+++	
*****	A	+++	+++	+++	+++	+++	+++	+++	+++	
	B	++	++	++	+	+	+	+	+++	
	C	+++	+++	+++	+++	+++	+++	+++	+++	
	D	+++	+++	+++	+++	+++	+++	+++	+++	

* Micro-organisms are designated by capital letters as follows: A, *Staphylococcus aureus;* B, *B. pyocyaneus;* C, *E. coli;* D, *Enterococcus.*

** An aqueous 1% solution of the compound to be tested was used, with pH adjusted to 7.4—7.6.

*** An aqueous 2% solution of the compound to be tested was used, with pH adjusted to 7.4—7.6.

**** For comparison, antibacterial activity of 2-mercaptothiazol-5-one was tested.

***** For comparison, antibacterial activity of 2-thio-4-(propylidene)thiazolid-5-one was tested.

EXPERIMENTAL*

The microanalyses were carried out at the Chemical Institute, Faculty of Science, University of Zagreb, by Dr. L. Filipović and Z. Štefanac.

Improved preparation of 2-mercaptothiazol-5-one

A description of the preparation of 2-mercaptothiazol-5-one was given earlier by Cook, Heilbron and Levy[5]. This method is suitable for the preparation of smaller quantities, whereas for preparations on a somewhat larger scale, the following procedure proved to be useful: Aminoacetonitrile sulfate, prepared according to Organic Syntheses[12], was dried *in vacuo* and ground in a warmed mortar and this procedure repeated several times. The dried and finely powdered aminoacetonitrile sulfate (30 g.) was transferred to an Erlenmeyer flask, and methanol (30 ml.) added. The flask was stoppered with a rubber stopper fitted with a calcium chloride tube and a dropping funnel through which a solution of sodium (7 g.) in absolute methanol (150 ml.) was gradually added with stirring. During this procedure, which took half an hour, the reaction mixture was kept at 0°. The addition of sodium methoxide was discontinued as soon as the red color of phenolphthalein, which was added to the reaction mixture as indicator, did not disappear at once. The sodium sulfate was then filtered off and washed with absolute methanol (25 ml.). The combined filtrate and washings were evaporated *in vacuo* under nitrogen at a temperature not higher than 40°. After the two thirds of the solvent had distilled off, a few milliliters of the above mentioned sodium methoxide solution were added, just enough for the red color to reappear, and then the rest of methanol was quickly evaporated. (The evaporation process should not take more than fifteen minutes.) To the residue, left in the same vessel, sodium ethylate was added (1 ml. of a solution of 0.1 g. of sodium in 3.5 ml. of ethanol) and dry, freshly distilled acetone (17 ml.) poured over the mixture. After standing for an hour with occasional shaking the reaction mixture solidified to *5-imino-2,2-dimethyloxazolidine*. The oxazolidine was then dissolved in water (20 ml.) and the solution evaporated *in vacuo*. The residue was dissolved in absolute ethanol (125 ml.) and carbon disulfide (7 ml.) added. A precipitate separated, which was oily at first, but crystallized after standing overnight and scratching. The crystals were collected and washed with a small quantity of methanol, and 20 g. of *carbamylmethylammonium carbamylmethyldithiocarbamate* was obtained. The compound was dried, finely powdered and dissolved with shaking in concentrated hydrochloric acid (50 ml.) which was previously cooled to 0°. When the solution was completed, water (100 ml.) was added, and the mixture left overnight at 0°. *The 2-mercaptothiazol-5-one* was filtered off and washed with cold water. Yield 10 g., m .p. 300° (decomp.).

The N-*phthaloyl amino aldehydes* required for this work were prepared following the procedures described by Balenović and coworkers[13, 14].

Preparation of thiosemicarbazones of N-*phthaloyl aldehydes* [I].

General procedure. To a saturated ethanolic solution of N-phthaloyl amino aldehyde, an equimolar quantity of a saturated aqueous solution of thiosemicarbazide was added. After standing for 48 hours, the crystals which had separated were collected and recrystallized to constant m. p. from ethanol-water (1 : 1).

N-*phthaloyl glycine aldehyde thiosemicarbazone* [Ia]

Prepared from N-phthaloyl glycine aldehyde and thiosemicarbazide. Colorless prisms, m. p. 213-213.5°.

Anal. 10.490 mg. subst.: 19.46 mg. CO_2, 3.71 mg. H_2O
$C_{11}H_{10}N_4O_2S$ (262.28) calc'd: C 50.37; H 3.84%
found: C 50.62; H 3.96%

* All melting points are uncorrected.

α-Phthalimidobutyraldehyde thiosemicarbazone [Ib]

Prepared from α-phthalimidobutyralhedyde and thiosemicarbazide. White leaflets, m. p. 205.5—207°.

Anal. 7.543 mg. subst.: 14.98 mg. CO_2, 3.27 mg. H_2O
$C_{13}H_{14}N_4O_2S$ (290.32) calc'd: C 53.78; H 4.86%
found: C 54.19; H 4.86%

N-*phthaloyl*-DL-*leucine aldehyde thiosemicarbazone* [Ic]

Prepared from N-phthaloyl-DL-leucine aldehyde and thiosemicarbazide. Colorless prisms, m. p. 195—196°.

Anal. 7.260 mg. subst.: 15.09 mg. CO_2, 3.72 mg. H_2O
$C_{15}H_{18}N_4O_2S$ (318.39) calc'd: C 56.58; H 5.70%
found: C 56.72; H 5.73%

O-*Ethyl*-N-*phthaloyl*-DL-*serine adlehyde thiosemicarbazone* [Id]

Prepared from O-ethyl-N-phthaloyl-DL-serine aldehyde and thiosemicarbazide. Colorless leaflets, m. p. 163—165°.

Anal. 10.205 mg. subst.: 19.74 mg. CO_2, 4.63 mg. H_2O
$C_{14}H_{16}N_4O_3$ (320.37) calc'd: C 52.49; H 5.03%
found: C 52.77; H 5.08%

O-*Methyl-N-phthaloyl*-L-*tyrosine aldehyde thiosemicarbazone* [Ie]

Prepared from O-methyl-N-phthaloyl-L-tyrosine aldehyde and thiosemicarbazide. Colorless prisms, m. p. 142°.

Anal. 8.530 mg. subst.: 18.71 mg. CO_2, 3.61 mg. H_2O
$C_{19}H_{18}N_4O_3S$ (382.43) calc'd: C 59.67; H 4.74%
found: C 59.86; H 4.74%

N-*Phthaloyl*-DL-*valine aldehyde thiosemicarbazone* [If]

Prepared from N-phthaloyl-DL-valine aldehyde and thiosemicarbazide. Colorless prisms, m. p. 205.5—206.5°.

Anal. 9.762 mg. subst.: 19.78 mg. CO_2, 4.81 mg. H_2O
$C_{14}H_{16}N_4O_2S$ (304.36) calc'd: C 55.24; H 5.29%
found: C 55.30; H 5.51%

Preparation of 2-thio-4-(phthalimidoalkylidene) thiazolid-5-ones of N-phthaloyl amino aldehydes [II]

General procedure. Condensation of N-phthaloyl amino aldehydes with 2-mercaptothiazol-5-one was carried out following the procedure described by Billimoria and Cook[5] for the condensation of aldehydes with 2-mercaptothiazol-5-one.

2-Thio-4-(2'-phthalimidopropylidene)thiazolid 5-one [IIa] was prepared following Balenović and Cerar[15].

2-Thio-4-(1'-phthalimidopropylidene)thiazolid-5-one [IIb] was prepared followingBalenović and Cerar[15].

2-Thio-4-(2'-methyl-1'-phthalimidobutylidene)thiazolid-5-one [IIc]

Prepared from N-phthaloyl-DL-valine aldehyde and 2-mercaptothiazol-5-one. Colorless leaflets, m. p. 195—196°.

Anal. 11.182 mg. subst.: 22.75 mg. CO_2, 3.95 mg. H_2O
$C_{16}H_{14}N_2O_3S_2$ (346.40) calc'd: C 55.47; H 4.07%
found: C 55.51; H 3.95%

2-Thio-4-(2'-ethoxy-1'-phthalimidobutylidene)thiazolid-5-one [IId]

Prepared from O-ethyl-N-phthaloyl-DL-serine aldehyde and 2-mercaptothiazol-5-one. Colorless leaflets, m. p. 155.5—157°.

Anal. 10.022 mg. subst.: 19.37 mg. CO_2, 3.30 mg. H_2O
$C_{16}H_{14}N_2O_4S_2$ (362.40) calc'd: C 53.02; H 3.89%
found: C 52.73; H 3.68%

2-Thio-4-(1'-phthalimidobutylidene)thiazolid-5-one [IIe]

Prepared from α-phthalimidobutyraldehyde and 2-mercaptothiazol-5-one. Colorless prisms, m. p. 182—183.5°.

Anal. 9.825 mg. subst.: 19.62 mg. CO_2; H 3.15 mg. H_2O

$C_{15}H_{12}N_2O_3S_2$(332.38) calc'd: C 54.20; H 3.64%

found: C 54.48; H 3.59%

REFERENCES

1. M. Fling, F. Minard and S. W. Fox, *J. Am. Chem. Soc.* **69** (1947) 2466.
2. S. W. Fox, M. Fling and G. N. Bollenbock, *J. Biol. Chem.* **155** (1944) 465.
3. M. Fling and S. W. Fox, *J. Biol. Chem.* **160** (1945) 329.
4. G. Domagk, R. Behnisch, F. Mietsch and H. Schmidt, *Naturwissenschaften* **33** (1946) 315.
5. A. H. Cook, I. M. Heilbron and A. L. Levy, *J. Chem. Soc.* **1948**, 201.
6. J. D. Billimoria and A. H. Cook, *J. Chem. Soc.* **1949**, 2323.
7. K. Balenović, I. Jambrešić and I. Furić, *J. Org. Chem.* **17** (1952) 1459.
8. W. E. Grundy, A Whitman, E. G. Rdzok, E. J. Rdzok, M. E. Hanes and J. C. Sylvester, *Antibiotics and Chemotherapy* **2** (1952) 399.
9. B. A. Sobin. *J. Am. Chem. Soc.* **74** (1952) 2947.
10. J. R. Schlenk and A. F. De Rose, *Arch. Biochem. and Biopyhs.* **40** (1952) 263.
11. W. M. McLamore, W. D. Celmer, V. V. Bogert, F. C. Pennings and I. A. Solomons, *J. Am. Chem. Soc.* **74** (1952) 2946.
12. W. K. Anslow and H. King, *Org. Syntheses, Coll. Vol.* **1** (1941) 298.
13. K. Balenović, N. Bregant, D. Cerar, D. Fleš and I. Jambrešić, *J. Org. Chem.* **18** (1953) 297.
14. K. Balenović, N. Bregant, D. Dvornik and N. Štimac, *J. Org. Chem.* (in press).
15. K. Balenović and D. Cerar, (unpublished work).
16. J. A. Kolmer and F. Boerner, *Approved Laboratory Techniques*, 4th Ed. 1945, D. Appleton Century Com., New York, page 513.
17. E. P. Abraham, C. M. Fletcher, H. W. Florey, A. D. Gardner, N. G. Heatley, M. A. Jennings, *Lancet*, **2** (1941) 177.

IZVOD

Tiosemikarbazoni i 2-tio-4-(ftalimidoalkiliden)tiazolidoni-5 N-ftaloil-aminokiselina. I. Priprava i antibakterijska aktivnost

J. Kolačny, N. Štimac, B. Sajko, B. Balenović i B. Urbas

Prije nekoliko godina pokazano je[1, 2, 3], da N-ftaloilni derivati aminokiselina imaju slična antibakterijska svojstva kao i pripadne D-aminokiseline.

U svrhu ispitivanja antibakterijskog djelovanja priredili smo derivate nekih N-ftaloil-α-aminokiselina, i to tiosemikarbazone [I] i 2-tio-4-(ftalimidoalkiliden)tiazolidone-5 [II] glicina, L-alanina, β-alanina, DL-leucina, DL-valina, L-tirozina, DL-serina i α-amino-n-maslačne kiseline.

Ovi su derivati ispitani na migkroorganizmima *Staphylococcus aureus, B. pyocyaneus, E. coli* i *Enterococcus*. Rezultati tih ispitivanja dani su u tablicama 1 i 2.

Ispitivanje spomenutih spojeva *in vivo*, i na mikro-organizmima, nastavlja se.

ISTRAŽIVAČKI INSTITUT »PLIVA«
TVORNICA FARMACEUTSKIH I KEMIJSKIH PROIZVODA
ZAGREB

Primljeno 29. ožujka 1954.