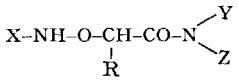
α-Aminooxy-Acid Derivatives with Potent Antituberculotic Effect

It is known[1] that certain α-aminooxy-acids (AOA) and their derivatives have a growth-inhibition effect on *Mycobacterium tuberculosis*. In spite of this, attempts to find a potent derivative in this group have been unsuccessful. First a rational process has been worked out for preparing optically active α-AOA. Starting from L- and D-amino acids, respectively, optically active α-bromo-carboxylic acids have been prepared, which, reacted with N-Z- and N-BOC-hydroxylamines, give the corresponding optically active N-Z- and N-BOC-α-AOA respectively. However, starting from optically inactive α-halogen-carboxylic acids in the above way, racemic N-Z- and N-BOC-α-AOA are obtained which can be resolved by different bases. After removing the protecting groups, by the usual way, the free α-AOA can be obtained. Thus, several hitherto unknown optically active α-AOA have been prepared. The advantage of this procedure is that the intermediates, such as protected α-AOA, can be directly used for preparing different derivatives. The activation of the carboxyl group of the protected AOA was carried out by methods well known from peptide chemistry e.g. activation by DCC, m.a. activated esters. The about 100 derivatives prepared so far can be divided into 3 groups, namely α-AOA-amides, α-AOA-hydrazides and α-AOA-hydroxamic acid derivatives. These compounds can be

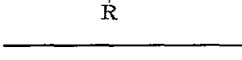
[1] C. B. FAVOUR, J. Bact. *55*, 1 (1948). – D. MCHALE, J. GREEN and P. MAMALIS, J. chem. Soc. *1960*, 225. – E. TESTA, B. J. R. NICOLAUS, L. MARIANI and G. PAGANI, Helv. chim. Acta *46*, 766 (1963).

Table I. α-AOA-amides



No.	X	R	Y	Z	in vitro inhibition mcg/ml								
					$H_{37}R_v$	INH res.	Strepto-mycin res.	PAS res.	*M. kan-sasii*	*Staph. aur.* haem. Wood	*Bact. subti-lis*	*E. coli*	*Ps. pyo-cyanea*
7	H	H	H	H	10–25	10–25	10–25	10–25	–	–	–	–	–
11	H	H	H	C_6H_5	0.1–1	0.1–1	0.1–1	0.1–1	–	–	–	–	–
12	H	H	H	C_6H_4(pCl)	1–5	1–5	1–5	1–5	–	–	–	–	–
23	Ac	H	H	C_6H_5	0.1–1	0.1–1	0.1–1	0.1–1	–	>50	>50	>50	>50
30	H	H	Me	C_6H_5	>50	>50	>50	>50	–	–	–	–	–
33	H	H	H	C_6H_4(oOMe)	0.1–1	0.1–1	0.1–1	0.1–1	–	>50	>50	>50	>50
49	H	H	H	C_6H_4(pOH)	5–10	5–10	10–25	–	5–10	>50	>50	>50	>50
56	H	H	H	C_6H_4(pNO_2)	1–5	1–5	1–5	–	1–5	>50	>50	>50	>50
60	H	H	H	CH_3NH–CO–	25–50	25–50	>50	–	>50	>50	>50	>50	>50
71	H	H	H	H_2N–CS–	25–50	25–50	25–50	–	25–50	>50	>50	>50	>50
69	H_2N–CO	H	H	C_6H_5	>50	>50	>50	–	>50	>50	>50	>50	>50
41	H	CH_3 (L)	H	C_6H_5	1–5	1–5	1–5	–	–	–	–	–	–
47	H	CH_3 (D)	H	C_6H_5	5–10	5–10	10–25	–	10–25	–	–	–	–
62	H	Bzl (DL)	H	C_6H_4(oOMe)	1–5	1–5	1–5	–	1–5	>50	>50	>50	>50
64	H	C_6H_5 (DL)	H	C_6H_4(oOMe)	25–50	25–30	>50	–	>50	>50	>50	>50	>50

Table II. α-AOA-hydrazides



No.	X	R	Ac	in vitro inhibition mcg/ml								
				$H_{37}R_v$	INH res.	Strepto-mycin res.	PAS res.	*M. kan-sasii*	*Staph. aur.* haem. Wood	*Bact. subtilis*	*E. coli*	*Ps. pyo-cyanea*
2	H	H	H	1–5	1–5	1–5	1–5	–	–	–	–	–
3	H	H	AO-acetyl	5–10	5–10	5–10	1–5	–	–	–	–	–
4	H	H	His	>50	>50	>50	>50	–	–	–	–	–
5	H	H	Gly	>50	>50	>50	>50	–	–	–	–	–
6	AO-acetyl	H	AO-acetyl	25–50	25–50	25–50	25–50	–	–	–	–	–
10	H	CH_3 (L)	Gly	>50	>50	>50	–	>50	>50	>50	>50	>50
5A	H	H	i-nikotinoyl	0.07	5–10	0.1–1	0.1–1	1–10	>50	>50	>50	>50
8	H	CH_3 (L)	i-nikotinoyl	0.1–1	>50	0.1–1	–	5–10	>50	>50	>50	>50
9	H	Bzl (L)	i-nikotinoyl	1–5	>50	1–5	–	5–10	>50	>50	>50	>50
11	H	Bzl (D)	i-nikotinoyl	1–5	>50	1–5	–	5–10	>50	>50	>50	>50

Table III. α-AOA-hydroxamic acid derivatives
X–NH–O–CH(R)–CO–NH–O–Y

No.	X	R	Y	in vitro inhibition mcg/ml								
				$H_{37}R_v$	INH res.	Streptomycin res.	PAS res.	*M. kansasii*	*Staph. aur.* haem. Wood	*Bact. subtilis*	*E. coli*	*Ps. pyocyanea*
1	H	H	Bzl(pCl)	0.1–1	1–5	1–5	0.1–1	–	>50	>50	>50	>50
19	H	H	Bzl	5–10	5–10	10–25	5–10	–	–	–	–	–
17	H	H	Bzl(pNO_2)	5–10	5–10	5–10	5–10	–	–	–	–	–
16	AO-acetyl	H	Bzl(pCl)	1–5	1–5	5–10	5–10	–	–	–	–	–
28	Gly	H	Bzl(pNO_2)	>50	>50	>50	>50	–	–	–	–	–
34	H	H	dodecyl	0.1–1	0.1–1	1–5	1–5	–	–	–	–	–
53	H	Bzl (L)	Et	5–10	1–5	10–25	–	1–5	>50	>50	>50	>50
54	H	Bzl (L)	dodecyl	10–25	5–10	5–10	–	1–5	>50	>50	>50	>50
59	H	CH_3 (DL)	Bzl(pCl)	5–10	10–25	10–25	–	10–25	>50	>50	>50	>50
68	H	C_6H_5 (DL)	Bzl(pCl)	>50	>50	>50	–	>50	>50	>50	>50	>50

The in vitro experiments were carried out in liquid culture-medium[2] with *M. tub.* $H_{37}R_v$ strain and its resistant variations resp. after inoculation with 0.1 mg and incubation for 3 weeks at 37 °C. The upper limit of the given figures resulted in complete inhibition. The investigation of other bacterium strains were carried out in bouillon culture-medium.

prepared with excellent yield for example by reacting protected α-AOA-OPCP esters with corresponding amines and hydrazides respectively. Some representatives of the 3 groups and the in vitro activities of the compounds are listed in the Tables.

The most potent compounds were investigated under in vivo conditions, too. It was found that 7.5 mg of the compound No. 5/A in Table II inhibited the generalization of *M. tuberculosis* infection in a 3-month-experiment on guinea-pigs. Similarly good results were obtained from experiments carried out on white mice. The compounds show fairly low toxicity in acute experiments.

Zusammenfassung. Es wurde festgestellt, dass einzelne α-Aminooxy-carbonsäure-Derivate, d.h. α-AOA-Amide, α-AOA-Hydrazide und α-AOA-Hydroxamsäure, in vitro und auch in vivo eine ausgeprägte Hemmung auf verschiedene *M. tuberculosis*-Stämme aufweisen.

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[2] I. TÁRNOK and I. SZABÓ, Z. Tuberk. *120*, 74 (1963).

Censored Distribution Techniques in Analysis of Toxicological Data

Truncated and censored samples are both common in bioassays. Truncation occurs if the population assayed cannot have values beyond a given cut-off point or if the number of observations exceeding the cut-off point is unknown[1]. Censoring refers to situations where observations beyond the cut-off point are not measured, but the number of such observations is known.

Recently, two different approaches to analyzing censored data have been compared. We report here details of the statistical techniques involved. The large body of data available permitted assessment of the internal consistency of the results obtained by these techniques; such assessment is not possible when only a few data sets are to be analyzed. Thus, the validity of the assumptions made could be verified.

The data examined are part of a large series designed to evaluate the effects of 100 MDOP compounds and related open-ring analogs on the hepatic microsomal enzyme function in the mouse as measured by the prolongation of hexobarbital narcosis[2]. For each replicated experiment 16 control mice were given 75 mg/kg of hexobarbital which would induce sleep for about 30 min and 5 groups of 8 mice each were given in addition doses of 2.5, 10, 40, 160 and 640 mg/kg, respectively, of the MDOP compound. The mice were observed for 300 min and the duration of narcosis recorded. For the active compounds some mice in the higher dose groups slept for longer than 300 min but the duration was not known.

When deaths occurred after the cut-off point and no record was available of whether the mice regained consciousness before death, it could be argued that truncation occurred. However, the approach was taken that all sleeping mice that were alive at the cut-off point had extended narcosis and their later death did not affect the sample size, thus censoring prevailed. The sample size of 8 was reduced if the mice died before 300 min as these deaths could be attributed to causes other than drug effect.

Most of the 100 drugs were tested at least twice, and if the reproducibility of the results was not satisfactory when inspected visually, they were replicated again, resulting in a total of 240 replications. Of these, 114