[Chem. Pharm. Bull. 21(7)1560—1563(1973)]

UDC 547.269.3'233.057.09 : 615.277.3.015.11

Synthesis and Evaluation of the Anticancer Activity of a New Series of Methanesulfonates

Mahmoud M. El-Merzabani[1a] and Yoshio Sakurai[1b]

Cancer Institute, Cairo University[1a] and Cancer Institute, Japanese Foundation for Cancer Research[1b]

(Received March 26, 1973)

Nine new methanesulfonic acid ester of aminoglycols were prepared. Their anticancer activities were evaluated against Yoshida sarcoma, mouse leukemia L-1210 and rat leukemia DBLA-6. From these compounds 3,3'-(morpholinopropylimino)di-1-propanol, dimethanesulfonate (ester), dihydrochloride (No. **888**) and 3,3'-(dibutylaminopropylimino)di-1-propanol, dimethanesulfonate (ester), dihydrochloride (No, **893**) were found to be very active. No. **893** was unique in its effect against rat leukemia DBLA-6(GV) by the intravenous inoculation system.

In our preceding studies we had reported the synthesis and anticancer activity of several new methanesulfonate of aminoglycols.[2]

$$R\cdot N(CH_2CH_2CH_2OSO_2CH_3)_2$$

R=H No. **864** R=CH_3 No. **838**

Of significance here is the fact that, despite their close similarity in chemical structure and reactivity, they showed different antitumor spectrum against experimental animal tumors.[3]

Clinical data so far obtained proved their outstanding activity against chronic myeloid leukemia, specially in cases resistant to busulfan,[4] malignant lymphomas and Hodgkin's disease.[5]

This potent anticancer activity of No. **838** and No. **864** is no doubt due to the presence of the two active 3-mesyloxypropyl groups. However, the different antitumor activities of these two compounds can be attributed either to the slight difference in their basic character, the ability of No. **864** to form hydrogen bonds with some active centers in the tumor cells or to its ability to penetrate through the membrane of the naturally resistant tumor cells such as AH-7974 and AH-66 more easier than No. **838**.

It was, therefore, thought that synthesis of some new derivatives with different carrier groups (R) or separating the two active 3-mesyloxypropyl groups on two different nitrogen atoms may lead to some potent derivatives.

The work described in this paper deals with the synthesis and the anticancer activity of nine new derivatives against experimental tumors.

The compounds were synthesized by reacting the appropriate aminoalcohols with methanesulfonic anhydride in acetonitrile for 24—48 hr at room temperature,[2] or reacting the aminoalcohols with methanesulfonyl chloride with cooling in dry acetone. The analytical data are shown in Table I. The aminoalcohols were prepared by reducing the corresponding

1) Location: *a) Cairo, Egypt; b) Kami-Ikebukuro 1-Chome, Toshima-ku, Tokyo.*

2) Y. Sakurai and M.M. El-Merzabani, *Chem. Pharm. Bull.* (Tokyo), **11**, 1218 (1963); *idem, ibid.*, **12**, 954 (1964); *idem, ibid.*, **13**, 1363 (1965).

3) M.M. El-Merzabani and Y. Sakurai, *Gann*, **56**, 575 (1965); *idem, ibid.*, **56**, 589 (1965); *idem, ibid.*, **58**, 199 (1967).

4) M. Hirano, M. Miura, H. Kakizawa, A. Morita, T. Uetani, R. Ohno, K. Kawashima, H. Nishiwaki, and K. Yamada, *Cancer Chemother. Rep.*, **56**, 47 (1972).

5) N. Gad-el-Mawla, F. Hammuda, M.M. El-Merzabani, M.I.R. Hamza, A. Osman, A.S. Ibrahim, Y. Sakurai, and A.L. Abul-Naser, *Cancer. Chomother. Rep.*, in press.

Table I. Methanesulfononic Esters Dihydrochloride of Aminoglycols

Compound No.	Structural formula	mp °C	Formula[a]	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
886	$(C_2H_5)_2N\cdot CH_2CH_2NR_2$	103—104[b]	$C_{14}H_{34}O_6N_2S_2Cl_2$	36.43	7.42	6.09	36.01	7.40	6.30
887	$(CH_3)_2N\cdot CH_2CH_2CH_2NR_2$	93—95[b]	$C_{13}H_{32}O_6N_2S_2Cl_2$	34.89	7.21	6.29	34.31	7.45	6.45
892	$(C_2H_5)_2N\cdot CH_2CH_2CH_2NR_2$	98—100[c]	$C_{15}H_{36}O_6N_2S_2Cl_2$	37.88	7.63	5.92	37.74	7.87	5.85
888	$O(CH_2CH_2)_2N\cdot CH_2CH_2CH_2NR_2$	94—95[b]	$C_{15}H_{34}O_7N_2S_2Cl_2$	36.80	7.00	5.75	36.84	7.17	5.99
893	$(C_4H_9)_2N\cdot CH_2CH_2CH_2NR_2$	99—100[c]	$C_{19}H_{44}O_6N_2S_2Cl_2$	42.92	8.34	5.29	42.99	8.65	5.30
890	$CH_3N(R)CH_2CH_2N(CH_3)R$	133—135[c]	$C_{12}H_{30}O_6N_2S_2Cl_2$	33.24	7.00	6.49	33.19	7.12	6.69
891	$CH_3N(R)(CH_2)_3N(CH_3)R$	111—112[c]	$C_{13}H_{32}O_6N_2S_2Cl_2$	34.89	7.21	6.29	34.94	7.68	6.89
894	$CH_3N(R)(CH_2)_4N(CH_3)R$	105—106[c]	$C_{14}H_{34}O_6N_2S_2Cl_2$	36.43	7.43	6.10	36.13	7.35	6.17
889	$R\text{-}N(CH_2CH_2)_2N\text{-}R$	139—140[c]	$C_{12}H_{28}O_6N_2S_2Cl_2$	33.37	6.54	6.52	33.48	6.80	6.92

a) dihydrochloride, R=-$CH_2CH_2CH_2OSO_2CH_3$ *b*) from MeOH/ether, *c*) from hot EtOH

Table II. Preparation of Ethoxycarbonylethylamines and Aminoglycols

Starting diamines	Boiling points R=$CH_2CH_2COOC_2H_5$		R=$CH_2CH_2CH_2OH$	
$(C_2H_5)_2N\ CH_2CH_2NR_2$[a]	b_2	172—174	b_3	157—159
$(CH_3)_2N\ CH_2CH_2CH_2NR_2$[a]	b_1	141—142	b_1	151—153
$(C_2H_5)_2N\ CH_2CH_2CH_2NR_2$[a]	b_1	140—142	b_2	159—161
$(C_4H_9)_2N\ CH_2CH_2CH_2NR_2$[a]	b_1	185—186	$b_{0.5}$	165—167
$O(CH_2)_2N\ CH_2CH_2CH_2NR_2$[a]	b_1	166—167	b_1	180—181
$CH_3NR(CH_2)_2NRCH_3$	b_2	128—129	b_1	120—121
$CH_3NR(CH_2)_3NRCH_3$[b]	b_1	137—139	b_1	144—146
$CH_3NR(CH_2)_4NRCH_3$[b]	b_1	146—147	b_1	152—153
$R\text{-}N(CH_2CH_2)_2N\text{-}R$	b_1	125—127	b_1	139—141

a) The diamines were kindly supplied by Koei Kagaku Kogyo k.k..
b) M. Ishidate, Y. Sakurai and K. Maruyama, *Chem. Pharm. Bull.* (Tokyo), **5**, 435 (1957).

bis[2-(ethoxycarbonyl)ethyl]amines with $LiAlH_4$ in dry ether. The (ethoxycarbonyl)ethylamines were prepared by condensing the appropriate amines with ethyl acrylate in absolute EtOH[6] (see Table II).

The primary screening data using rats-bearing Yoshida sarcoma by single *i.p.* injection 72 hr after tumor transplantation are shown in Table III. All the tested compounds showed

6) P. Mozingo and J. H. McCracken, *Org. Synthesis, coll.* 3, 258 (1955).

Table III. Primary Screening Data with Rats-bearing Yoshida Sarcoma

Compound No. [a]	*In vivo* [b]			*In vitro*	
	MED mg/kg	MTD mg/kg	LD_{50} mg/kg	IC_{50} [c] mM	MEC [d] mM
886	0.1	10	17.5	1.8×10^{-3}	1×10^{-2}
887	0.1	5	7.5	4.6×10^{-4}	1×10^{-3}
888	0.5	100	175	4.9×10^{-3}	3×10^{-2}
889	0.1	5	7.5	9.1×10^{-4}	3×10^{-3}
890	0.25	25	37.5	1.7×10^{-3}	1×10^{-2}
891	1.0	100	175	2.4×10^{-2}	1×10^{-1}
892	0.25	10	17.5		
893	2.5	250	375		
894	0.5	100	175		

a) Compounds tested as dihydrochlorides.
b) By the method reported by T. Yoshida, *et al.*, *Gann*, **45**, 489 (1954).
c) 50% inhibition concentration *in vitro* culture by the method reported by A. Moriwaki, *Chem. Pharm. Bull.* (Tokyo), **10**, 462 (1962).
d) minimum effective concentration

strong carcinostatic effect with varying degree of toxicities, compounds No. **887** and No. **889** were the most toxic and the most potent against *in vitro* cultured Yoshida sarcoma (IC_{50} 4.6×10^{-4} and 9.1×10^{-4} mM respectively). Compounds No. **888** and No. **893** were the most interesting as far as their chemotherapeutic indices are concerned.

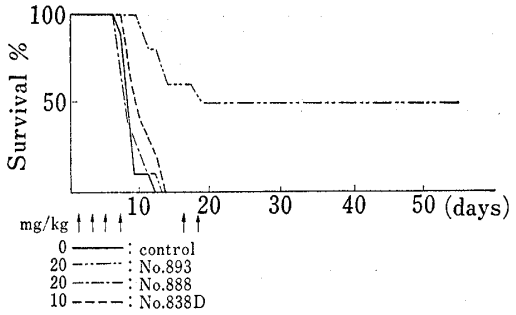

Fig. 1. Effect of No. **893**, No. **888** and No. **838D** on the life-span of Rats Bearing DBLA-6(GV)
($i\cdot v\cdot$-$i\cdot p\cdot$ system)

The percentage cure rates of rats-bearing Yoshida sarcoma treated by repeated injections at appropriate doses were determined. Table III shows the effect achieved with these compounds at their optimal doses. In general, compounds in which the active 3-mesyloxypropyl groups are placed on two different nitrogen atoms were less active than No. **838** type compounds. Of interest to notice, compound No. **887** and No. **892** are the dimethyl and diethyl derivatives of the same parent compound but the latter was completely inactive and produce no cures. Compound No. **887** has almost the same effect as No. **888** and No. **893**. However,

Table IV. Percentage Cure Rates of Rats-bearing Yoshida Sarcoma by Some Methanesulfonates at Their Optimal Doses

Compound No.	Dose mg/kg/d ×12	Survival %	
		1 month	2 months
886	1	70	40
887	1	80	70
888	10	80	70
889	0.5	70	50
890	2.5	70	50
891	10	50	30
892	1	0	0
893	10	90	80
894	10	0	0

it showed relatively high toxicity and 1 mg/kg seems to be the maximum tolerated dose. Similar results were obtained with rat leukemia DBLA-6 (GP)[7] in the *i.p.-i.p.* treatment system. However, when the tumor was transplanted *i.v.* and the drug administered *i.p.*, compound No. **893** was more active than No. **838** and No. **888**. It was also observed that compound No. **893** retain some of its activity against rats bearing DBLA-6 (GV) in the intravenous inoculation system but not No. **838** or **888** as shown in Fig. 1.

In L-1210 screening system, 2 mg/kg was found to be the optimal and maximum tolerated dose for No. **887**. No. **888** was found active over a wide dose range of 5—200 mg/kg and by different schedules. Its activity was also confirmed in mouse leukemia P-388.

Aoshima and Sakurai[8] reported the detection of DBLA-6 (GV) leukemic cells in the bone marrow of rats 24 hr after *i.v.* transplantation of 10^6 leukemic cells. The unique effect of compound No. **893** on this system seems to be due to its ability to diffuse into bone marrow more easier than No. **838** and No. **888**.

Work now is in progress to evaluate further the anticancer activity of No. **888** and No. **893** in wide spectrum of ascites hepatoma and quantitatively evaluate their activities in comparison with No. **838**.

The details of the animal experiments will be published elsewhere.

Experimental

General Method for Preparation of the Aminoglycols——Ether solution of 0.1 M of bis[2-(ethoxycarbonyl)-ethyl]amine was added dropwise with stirring and cooling to ether solution of 0.1 M $LiAlH_4$. After 6—8 hr stirring at room temperature, water was added and the precipitated $Al(OH)_3$ was filtered off and washed with ether. After evaporation of the solvent, the residue was fractionated at reduced pressure (see, Table II).

General Method for Preparation of the Methanesulfonates——A) A solution of 0.05 mole of aminoglycol in 100 ml CH_3CN was added with stirring to a solution of 0.11 mole methanesulfonic anhydride in 50 ml CH_3CN. Stirring was continued for 24—48 hr at room temperature. The solvent was then evaporated at reduced pressure and the residue was dissolved in 25 ml abs. EtOH containing HCl gas in 5 N concentration, added with ether and kept overnight at −5°. The separated white crystals, usually in more than 75% yield, was recrystallized from hot EtOH or MeOH/ether.

B) A solution of 0.05 mole aminoalcohol in 100 ml dry acetone was added to a well stirred and cooled solution of 0.11 mole of methanesulfonyl chloride in 200 ml acetone. Stirring was continued for 3—6 hr at room temperature. The separated crystals were recrystallized from hot EtOH or MeOH/ether.

Acknowledgement The authors express their thanks to Mr. Y. Sudo for his technical assistance. This study was partially supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Japan.

7) M. Aoshima and Y. Sakurai, *Gann*, **63**, 281 (1972).
8) M. Aoshima and Y. Sakurai, *Gann.*, **64**, in press.