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# Synthesis and Evaluation of the Anticancer Activity of Some Water Soluble Maleimide Derivatives

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Six maleimide derivatives were synthesized and their anticancer activity was studied. They demonstrated toxic rather than cytotoxic effect.

Biological alkylating agents react nonspecifically with many chemical groupings, many of which are of vital importance to cell growth.[2] Their reaction with the 7-position of guanine is now accepted to be the reaction responsible for their cytotoxic activity.[3] This did not rule, however, the importance of their reaction with sulfhydryl groups. Since blocking the SH-groups of macromolecules change significantly their structure which in turn impair their biological functions. It was observed that the development of resistance of tumor cells to alkylating agents was accompanied with an increase in their nonprotein sulfhydryl groups.[4] This phenomenon was once thought to be major factor in the development of resistance.

N-Ethylmaleimide, a typical SH-blocking agent, inhibits the growth of chick fibroblasts in tissue culture at a concentration of $10^{-3}$M[5] and render tumor cell more sensitive to irradiation. Tumor cells attenuated with ethylmaleimide enhance the immunity of the recipient animal.[6]

Accordingly, it was thought that water soluble maleimide derivatives may find some application in the chemotherapy of resistant tumor. Also, this will throw some light on the effect of blocking SH-groups on the sensitivity of tumor cells to anticancer agents.

The work described in this paper deals with the synthesis of some water soluble mono and dimaleimide derivatives and studies on their anticancer activity. Also, their effect on the white blood cells of rats was investigated.

The maleimides were synthesized by cyclization of the appropriate maleamic acids with acetic anhydride in the presence of anhydrous sodium acetate. Kovaeic[7] modified the method reported by Searle[8] by using slight excess of acetic anhydride and held the reaction at 92°. He reported 5—15% yield. However, we find that 2:1 molar ratio of acetic anhydride per maleamic acid group at 60—65° for 30 minutes results in better yields. Maleamic acids were prepared from the appropriate amine and maleic acid in dry ether and dioxane for dimaleamic acids in almost quantitative yields and they are used as such for cyclization. The analytical data are shown in Table I.

Toxicity and screening data are shown in Table II. In rats, the toxicity of dimaleimide is almost double that of the maleimide. In mice, only compound No. **881** showed abnormally high toxicity and it seems that different substitutions have no effect on the toxicity. No cytomorphological effects was observed on Yoshida sarcoma or AH-13 cells. In the L-1210

1) Location: *a) Cairo, Egypt*; *b) Kami-Ikebukuro 1-chome, Toshima-ku, Tokyo.*
2) W.C.J. Ross, "Biological Alkylating Agents," Butterworth, London, 1962 p. 32—63.
3) P. Brookes and P.D. Lawely, *Biochem. J.*, **80**, 496 (1961).
4) I. Hirono, *Nature*, **186**, 1059 (1960); C. R. Ball T. A. Conners, J. A. Double, V. Ujhazy and M. E. Whisson, *Int. J. Cancer*, **1**, 319 (1966).
5) E. Friedmann, *et al.*, *Brit. J. Pharmacol.*, **4**, 105 (1949).
6) Morton D. Prazer, *Cancer Res.*, **31**, 1488 (1971).
7) P. Kovacic and Richard W. Heim, *J.A.C.S.*, **71**, 1187 (1959).
8) N.E. Searle, U.S. Patent 24444536 (1948) [*C.A.*, **42**, 7340 (1948)].

Table I. Mono and Dimaleimides

| Compound No. | Starting amine | Salt | mp (°C) | Formula | Analysis (%) Calculated C | H | N | Found C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|
| **880** | $CH_3N(CH_2CH_2CH_2NH_2)_2$ | picrate[a] | 213—214 | $C_{21}H_{22}O_{11}N_6$ | 47.19 | 4.15 | 15.72 | 47.13 | 4.21 | 15.32 |
| **881** | $NH_2(CH_2)_3$-(N-bridged)-$(CH_2)_3NH_2$ | [b] | 181—182 | $C_{18}H_{24}O_4N_4$ | 59.95 | 6.71 | 15.75 | 60.11 | 6.69 | 15.40 |
| **882** | $(C_2H_5)_2NCH_2CH_2NH_2$ | picrate[c] | 119—120 | $C_{16}H_{19}O_9N_5$ | 45.15 | 4.50 | 16.52 | 45.27 | 4.49 | 16.29 |
| **883** | $(C_2H_5)_2NCH_2CH_2CH_2NH_2$ | picrate[c] | 150—151 | $C_{17}H_{21}O_9N_5$ | 43.77 | 4.16 | 17.09 | 43.20 | 3.83 | 16.69 |
| **884** | $(CH_3)_2NCH_2CH_2CH_2NH_2$ | picrate[c] | 144—145 | $C_{15}H_{17}O_9N_5$ | 46.44 | 4.81 | 15.99 | 46.45 | 4.85 | 15.59 |
| **885** | O-(N-bridged)-$NCH_2CH_2CH_2NH_2$ | picrate[a] | 209—211 | $C_{17}H_{19}O_{10}N_5$ | 45.01 | 4.22 | 15.50 | 45.19 | 4.25 | 15.07 |

*a*) acetone/EtOH *b*) EtOH/ether *c*) EtOH

Table II. Toxicity and Anticancer Activity of Maleimides

| Compd. No. | Rat[a] $LD_{50}$ mg/kg | Rat[a] MTD mg/kg | $IC_{50}$[b] mM | $LD_{50}$[c] mg/kg | Dose mg/kg×d | Mice[d] Route | Mice[d] Response T/C | IST[b] % |
|---|---|---|---|---|---|---|---|---|
| **880**[e] | 17.5 | 10 | $1.1\times10^{-2}$ | 48 | 5×5 | *i.p.* | 7.9/7.6=104 | 4 |
| | | | | | 10×2 | *i.p.* | 8.0/7.6=106 | 6 |
| **881** | 7.5 | 5 | $1.5\times10^{-2}$ | 3.5 | 5×5 | *i.p.* | 7.7/7.6=101 | 1 |
| | | | | | 10×2 | *i.p.* | 7.7/7.6=101 | 1 |
| **882**[e] | 37.5 | 25 | $3.2\times10^{-1}$ | 48 | 5×5 | *i.p.* | 8.2/7.6=108 | 8 |
| | | | | | 10×5 | *i.p.* | 8.1/7.6=107 | 7 |
| **883**[e] | 37.5 | 25 | $1.5\times10^{-2}$ | 36 | 5×5 | *i.p.* | 8.7/8.1=107 | 7 |
| | | | | | 10×5 | *i.p.* | 9.1/8.1=112 | 12 |
| | | | | | 5×5 | *i.v.* | 8.6/8.1=106 | 6 |
| **884**[e] | 37.5 | 25 | $2.0\times10^{-2}$ | 48 | 5×5 | *i.p.* | 8.2/8.1=101 | 1 |
| | | | | | 10×5 | *i.p.* | 8.3/8.1=103 | 3 |
| | | | | | 5×5 | *i.v.* | 8.6/8.1=106 | 6 |
| **885**[e] | 37.5 | 25 | $3.6\times10^{-3}$ | 48 | 5×5 | *i.p.* | 8.3/8.1=103 | 3 |
| | | | | | 10×5 | *i.p.* | 8.4/8.1=104 | 4 |
| | | | | | 5×5 | *i.v.* | 8.0/8.1=9.9 | — |

*a*) Rats bearing ascites hepatoma AH-13, by the method reported by T. Yoshida, *et al.*, *Gann*, **45**, 489 (1954).
*b*) 50% inhibition concentration *in vitro* culture by the method reported by A. Moriwaki, *Chem. Pharm. Bull.* (Tokyo), **10**, 462 (1962).
*c*) Intact $CDF_1$ mice by single *i.v.* injection.
*d*) $CDF_1$ mice bearing $10^5$ cell L1210, IST=increase in survival time determined by the protocol of DR & D: Cancer Chemotherapy Reports, **25**, 1 (1962).
*e*) Compounds tested as diphenyldisulfonate.

screening system all the tested compounds were evaluated as negative according to the criteria of DR & D.[9] *In vitro* culture they have strong killing effect on Yoshida sarcoma cells. Compound No. **885** was the most active ($IC_{50}$ $3.6\times10^{-3}$ mM) and compound No. **882** was the least active ($IC_{50}$ $3.2\times10^{-1}$ mM). Although these maleimide effect the viability of AH-13 cells

9) DR & D protocol, *Cancer Chemotherapy Reports*, **25**, 1 (1962).

*in vitro* culture as tested by the trypan blue exclusive test they did not demonstrate any significant increase in the life-span of rats bearing the same tumor and strong accumulative toxic effect was observed Fig. 1.

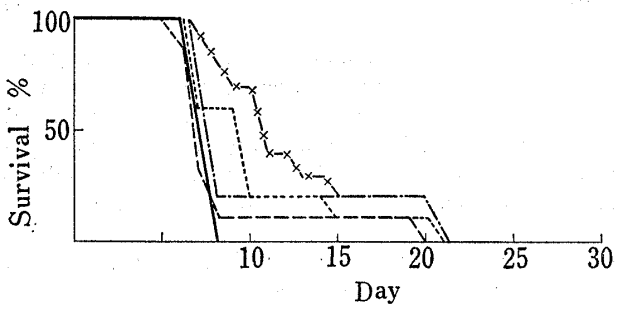


Fig. 1. Effect of No. **880** and **881** on the Life-span of Rats Bearing AH-13.

control
No. **880** 2.5 mg/kg/d.
No. **880** 5 mg/kg/d.
No. **881** 2.5 mg/kg/d.
No. **881** 5 mg/kg/d.

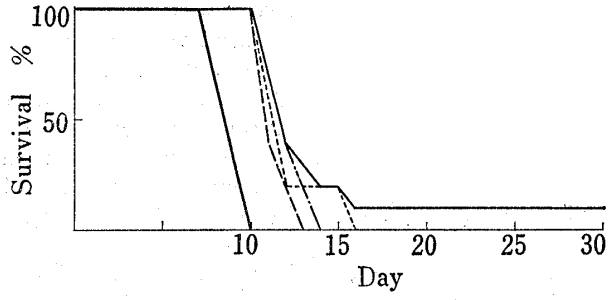


Fig. 2. Combination Effect of **838 D** and **880** or **881** on the Life-span of Rats Bearing Resistant Yoshida Sarcoma

control
10mg/kg/d. **838D**
10mg/kg/d. +2.5 mg/kg **880**
10mg/kg/d. +5 mg/kg **880**
10mg/kg/d. +2.5 mg/kg **881**

To test if the blocking of SH-groups has any effect on the sensitivity of resistant Yoshida sarcoma cells, combination of **838D** and maleimides were tried. As shown in Fig. 2, combination of maleimide and **838D** was less effective than **838D** alone.

To investigate the cause of this strong toxicity, the effect of maleimides on the white blood cell count of intact rats was studied after single *i.p.* injection. As shown in Table III, no significant depressing effect was observed with compound No. **880** and **885**; however, with No. **885** slight depression was observed at 48 hr after the administration and the animals recovered on the 7th day. Autopsy of rats died by delayed toxic effect showed marked atrophy of the liver and spleen with the development of considerable amount of bloody ascites fluid.

Table III. Effect of Maleimides on the Leucocytic Count of Donryu Rats

| Compound No. | No. of rats | Dose mg/kg | Days 0 | 1 | 2 | 3 | 4 | 5 | 7 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 15 | 0 | 10047 ±1670 | 9646 ±2111 | 10338 ±2039 | 10667 ±2600 | — | 10857 ±1947 | — | 11494 ±2057 |
| **880** | 15 | 10 | 9986 ±1256 | 12283 ±3107 | 9533 ±1631 | 10129 ±1700 | — | 9717 ±2240 | — | 10127 ±1985 |
| **880** | 5 | 5 | 10440 ±928 | 11295 ±2866 | 11104 ±2748 | 10566 ±2676 | — | 10797 ±1065 | — | 10066 ±1479 |
| Control | 5 | — | 10961 ±1875 | — | 10281 ±2355 | 11167 ±1941 | 11809 ±1099 | — | 11866 ±1113 | 11585 ±2084 |
| **882** | 3 | 20 | 9990 ±1144 | — | 7521 ±591 | 7577 ±409 | 9285 ±1279 | — | 10368 ±522 | 10034 ±1447 |
| **885** | 4 | 20 | 11209 ±1064 | — | 12241 ±5816 | 10975 ±2928 | 12088 ±3519 | — | 12180 ±1769 | 10454 ±614 |

Count was performed by after single *i.p.* injection on 0-day (counts/mm$^3$) countIter couer coulter counter.

The results represented in this paper indicate that this class of compounds is very toxic with no appreciable anticancer activity on the tumor tested. Also it showed that blocking of the SH-groups of resistant tumor cells did not effect their sensitivity of alkylating agents.

## Experimental

**General Method for Synthesis of Maleamic Acids**——Ether solution of 0.1M appropriate amine was added dropwise with stirring to ether solution of 0.1 M maleic anhydride. After 3 hr stirring at room temperature, the white powder was filtered off and washed with ether to remove unreacted starting material, dried and were cyclized without further purification.

**General Method for Synthesis of Maleimide Derivatives**——A mixture of 0.05M maleamic acid, 0.5 g anhydrous sodium acetate and 0.12M acetic anhydride (per maleamic acid group) was heated for 30 min at 60—65°. The reaction mixture was then carefully neutralized with sodium bicarbonate and extracted with ether. The combined ether extract was dried with anhydrous sodium sulfate and ether was evaporated at reduced pressure at room temperature. The residual oil was converted to its picrate for analysis and to the water soluble diphenyldisulfonate for animal experiments. Analytical data see Table I.

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