Mechanism of Mouse Skin Tumor Promotion by Chrysurbin

J. DiGiovanni, P. C. Decina, W. P. Prichett, J. Cantor, K. K. Aalfs, and M. M. Coombs

The Wistar Institute, Philadelphia, Pennsylvania 19104 (P. C. D., W. P. P., J. C.); The University of Texas System Cancer Center, Science Park—Research Division, Smithville, Texas 78957 (J. D., K. K. A.); and The Imperial Cancer Research Fund, P. O. Box No. 123, Lincoln’s Inn Fields, London, WC2A 3PX England (M. M. C.)

ABSTRACT

The skin tumor-promoting ability of 1,8-dihydroxy-3-methyl-9-anthrone (chrysurbin) was compared with that of 12-O-tetradecanoylphorbol-13-acetate (TPA) and 1,8-dihydroxy-9-anthrone (anthralin) in SENCAR mice. Although dose-response comparisons indicated that chrysurbin was several orders of magnitude less potent than TPA for promoting papilloma formation, this anthralin was 1.5 to 2 times more potent than anthralin. Maximal papilloma responses were achieved by 15 weeks of promotion with TPA whereas at least 25 weeks of promotion were necessary to achieve maximal papilloma responses with chrysurbin or anthralin indicating marked differences in tumor latency between the two classes of compounds. Interestingly, at optimal promoting doses, chrysurbin gave a carcinoma response (22% with 0.3 carcinomas per mouse at 45 weeks) similar to that of TPA suggesting that this compound may be more efficient at promoting carcinomas than papillomas. In two-stage promotion experiments, chrysurbin was incapable of functioning independently as a Stage I or II promoter despite its complete promoting activity.

Chrysurbin and TPA were compared at optimal promoting doses for their ability to induce: (a) skin edema, (b) epidermal hyperplasia, and (c) epidermal ornithine decarboxylase. In each case, distinct differences were noted between the two compounds. When taken together, the data support the hypothesis that anthracene-derived skin tumor promoters work at least in part by a mechanism different from the phorbol esters.

INTRODUCTION

Phorbol esters are the most widely studied skin tumor promoters, and TPA is the most potent member and prototype of this class of chemical compounds (reviewed in Refs. 7, 10, and 16). Much is known about the mechanism of action of TPA and those events that appear to be critical for its skin tumor-promoting properties. Within a few h after topical application of TPA to the skins of mice, one can observe an inflammatory response (37), and by 24 h, a marked increase in the percentage of dark basal keratinocytes (19, 20, 27). In addition, by 24 to 48 h, a marked hyperplasia is evident ([27], and reviewed in Ref. 7). In addition to these cellular changes, by 4 to 6 h, one observes a marked increase in the activity of ODC (EC 4.1.1.17) with subsequent increases in polyamine levels at later times ([25, 26]; and reviewed in Ref. 7). These events appear to correlate most closely with the skin tumor-promoting action of TPA (reviewed in Ref. 31).

Recently, specific high-affinity phorbol ester membrane binding sites have been demonstrated in a variety of tissues using either particulate fractions or whole cells (reviewed in Ref. 4). Castagna et al. (8) demonstrated that phorbol esters capable of promoting skin tumors directly activated a partially purified protein kinase C in vitro. In addition, Niedel et al. (22) and Ashendel et al. (1) have now shown that the phorbol ester receptor copurifies with, and may be, a protein kinase C. Clearly, these findings may provide a mechanistic explanation for the multiplicity of effects that are observed after exposure of cells in vivo or in culture to TPA (reviewed in Refs. 2, 3, 10, and 34).

A wide variety of chemically diverse compounds are known to possess tumor promoting properties in mouse skin (reviewed in Ref. 39). A number of compounds which differ in chemical structure but induce many of the same cellular and biochemical responses as the phorbol esters include: teleocidin (14); dihydro-teleocidin B (13, 38); lynybatoxin or teleocidin A (39); and aplysiantoxin and debromoplysiantoxin (15). Despite the differences in chemical structure, teleocidin, aplysiantoxin, and debromoplysiantoxin appear to interact with the phorbol ester receptor and thus exhibit a similar mechanism of action (18, 40). Other skin tumor promoters such as anthralin (Chart 1, Compound Ia) and iodoacetic acid do not interact with the phorbol ester receptor (9, 35, 36). Little else is known about the mechanism of skin tumor promotion by these compounds or the host of other skin tumor promoters (which are reviewed in Refs. 2, 3, 7, 10, and 34).

We reported recently that a derivative of anthralin with a methyl group in lateral ring Position 3 (1,8-dihydroxy-3-methyl-9-anthrone or chrysurbin) possessed skin tumor-promoting activity (Chart 1, Compound Ib) (11). In the present investigation, we have compared the tumor-promoting activity of chrysurbin with that of anthralin and TPA. In addition, we have provided a comparison between chrysurbin and TPA for several cellular and biochemical responses believed to be important for tumor promotion by TPA. Our results support the hypothesis that chrysurbin works at least in part by a mechanism different from the phorbol esters.

MATERIALS AND METHODS

Chemicals. DMBA was purchased from the Eastman Kodak Co. (Rochester, NY) and further purified as described previously (11). TPA and mezarin were obtained from Chemicals for Cancer Research (Eden Prairie, MN). Anthralin (Compound Ia) and chrysurbin (Compound Ib) were supplied by ICN Pharmaceuticals, Inc. (K and K Laboratories Division, Plainview, NY). Both of these compounds were purified using the procedures reported in a previous study (11). Emodin anthrone (Compound Ic) was prepared by reduction of emodin (Compound IIa) as follows: emodin (technical, 0.5 g), purchased from Aldrich Chemical Co., Ltd., Gillingham, Dorset, U.K., was dissolved in dichloromethane, and the yellow solution was freed from a small amount of brown insoluble...
material by filtration. Chromatography on a column of silica gel (30 x 3 cm; diameter; Fisons, Loughborough, U.K.) gave a first fraction, eluted with 10 to 20% ethyl acetate in dichloromethane, consisting of physcion (1,8-dihydroxy-6-methoxy-3-methyl-9,10-anthraquinone, Compound IIb) (78 mg) as a pale orange crystalline solid, m.p. 201-202°C after crystallization from hot n-butyl alcohol

\[ \text{C}_{18}\text{H}_{12}\text{O}_{5} \]

Required: C 67.60, H 4.26
Found: C 67.75, H 4.24

λ<sub>max</sub> 264, 289, and 435 nm (anion, 512 nm); diacette, m.p. 185°C (literature, 188-189°C).

Later fractions from the column eluted with 20 to 50% ethyl acetate in dichloromethane yielded emodin (1,6,8-trihydroxy-3-methyl-9,10-anthraquinone, Compound IIa) (340 mg) as orange crystals, m.p. 258-260°C. λ<sub>max</sub> 255, 294, and 457 nm (anion, 527 nm).

Pure emodin (340 mg) was dissolved in hot glacial acetic acid (25 ml), and to this was added stannous chloride (5 g) dissolved in concentrated hydrochloric acid (12 ml) in portions during 3 h under reflux. The orange color faded with the separation of a yellowish solid. When cold, the solid was collected, washed well with water, and dried giving the crude anthrone (267 mg). This material and that (288 mg) from a second, identical preparation were then recrystallized from hot n-butyl alcohol (30 ml) to give pale fawn crystals of emodin anthrone (1,6,8-trihydroxy-3-methyl-9-anthrone, Compound l)c (490 mg) m.p. approximately 275°C with decomposition

\[ \text{C}_{18}\text{H}_{12}\text{O}_{5} \]

Required: C 70.30, H 4.72
Found: C 70.29, H 4.71

λ<sub>max</sub> 260, 271, and 357 nm; nuclear magnetic resonance (δ-dimethylsulfoxide-d<sub>4</sub>) 2.92 (s, 3H; methyl), 4.78 (s, 2H; méthylène), 6.68, 6.82, 7.10, 7.19 (each d, J ~ 1.5 Hz, 1H; aromatic protons), and 11.40, 12.80, and 12.98 (each 1H; hydroxyl protons).

Animals. Female SENCAR mice were obtained from the Oak Ridge National Laboratory, Oak Ridge, TN, or Harlan Sprague-Dawley, Indianapolis, IN. At 7 to 9 weeks of age, the backs of the mice were carefully shaved with surgical clippers. Mice were allowed to rest for 2 days, and only those mice in the resting phase of the hair growth cycle were used. All chemicals were applied topically to the shaved area in 0.2 ml acetone, and control animals were treated with an equal volume of acetone.

Tumor Induction Experiments. Each experimental group contained 30 preshaved mice. Mice were initiated with 10 nmol DMBA and beginning 1 to 2 weeks after initiation received topical applications of TPA, anthralin, chrysarobin, mezerein, or combinations thereof. The incidence of papillomas and/or carcinomas was observed and recorded weekly. The data presented in this paper represent maximal tumor responses and are presented as the average number of tumors per mouse and the percentage of mice surviving with papillomas. For some groups, carcinoma data is expressed as a percentage of the mice at risk at the time of appearance of the first carcinoma.

Histology. All mice for histological experiments were used at 7 to 9 weeks of age as described above for the tumor experiments. Mice were treated with either 3.4 nmol TPA, 220 nmol anthralin, or 220 nmol chrysarobin as a single application. For the determination of epidermal formation, procedures similar to that described by Slaga et al. (33) were used where mice were treated with a single application of promoter and sacrificed 24 h later. For the determination of hyperplasia, skins were removed and small sections were fixed in 3% glutaraldehyde and then embedded in epon. One-μm thick sections were stained with toluidine blue and then examined for the number of nucleated cells in the interfollicular epidermal (IFE) cell layers (19).

ODC Assay. At various times after single or multiple (5) treatments, SENCAR mice were sacrificed by cervical dislocation. The epidermal

CANCER RESEARCH VOL. 45 JUNE 1985

2585

Downloaded from cancerres.aacrjournals.org on April 20, 2017. © 1985 American Association for Cancer Research.
CHRYSAROBIN SKIN TUMOR PROMOTION

Table 1

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Dose (nmol)</th>
<th>% of mice with papillomas</th>
<th>Papillomas/mouse</th>
<th>% of mice with carcinomas</th>
<th>Carcinomas/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPA</td>
<td>0.425</td>
<td>14</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.85</td>
<td>86</td>
<td>5.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.70</td>
<td>100</td>
<td>14.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.40</td>
<td>100</td>
<td>14.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.80</td>
<td>100</td>
<td>14.00</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Chrysarobin</td>
<td>50.00</td>
<td>0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>66</td>
<td>2.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>220.00</td>
<td>68</td>
<td>2.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>880.00</td>
<td>93</td>
<td>3.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>220.00</td>
<td>88</td>
<td>4.39</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>220.00</td>
<td>88</td>
<td>4.39</td>
<td></td>
<td>22</td>
</tr>
</tbody>
</table>

Values are an average of 2 separate experiments.

Table 2

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Treatment protocol and dose</th>
<th>Papillomas/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TPA, 3.4 nmol</td>
<td>Mezerein, 3.4</td>
</tr>
<tr>
<td>2</td>
<td>Acetone, 0.2 ml</td>
<td>Mezerein, 3.4</td>
</tr>
<tr>
<td>3</td>
<td>Chrysarobin, 22 nmol</td>
<td>Mezerein, 3.4</td>
</tr>
<tr>
<td>4</td>
<td>Chrysarobin, 220 nmol</td>
<td>Mezerein, 3.4</td>
</tr>
<tr>
<td>5</td>
<td>TPA, 3.4 nmol</td>
<td>Chrysarobin, 22</td>
</tr>
<tr>
<td>6</td>
<td>TPA, 3.4 nmol</td>
<td>Chrysarobin, 22</td>
</tr>
<tr>
<td>7</td>
<td>TPA, 3.4 nmol</td>
<td>Chrysarobin, 22</td>
</tr>
</tbody>
</table>

The animals receiving 6.8 nmol of TPA and 220 nmol of chrysarobin were treated for a total of 45 weeks, and the carcinoma response at this time is also shown in Table 1. Twenty % of the mice receiving TPA bore a single carcinoma, yielding 0.20 carcinomas per mouse. Twenty-two % of the animals receiving 6.8 nmol of chrysarobin bore carcinomas yielding an average of 0.30 carcinomas per mouse. All carcinomas were verified histologically as papillary squamous cell carcinomas with varying degrees or grades of differentiation and invasiveness.

A derivative of chrysarobin, emodin anthrone Chart 1, (Compound Ic), was also tested for skin tumor-promoting activity. Emodin anthrone differs from chrysarobin by the addition of a hydroxyl group in lateral ring Position 6. This derivative was tested at doses of 220, 440, and 880 nmol/mouse in DMBA-initiated mice. After 28 weeks of promotion, there were no detectable tumors in the mice.

Two-Stage Promotion. The ability of chrysarobin to function as a Stage I or II promoter was examined using the 2-stage promotion scheme described by Slaga et al. (30). This protocol provides a framework to examine the mechanism of action of nonphorbol ester as well as phorbol ester skin tumor promoters. If anthralin or chrysarobin possess only some of the actions of phorbol esters, this might explain their lower efficacy as skin tumor promoters. Table 2 summarizes the results of an experiment to determine Stage I- or II-promoting ability of chrysarobin. For these experiments, all mice were initiated with 10 nmol DMBA and 1 week later received twice-weekly applications of 3.4 nmol TPA for 15 weeks or 220 nmol chrysarobin for 30 weeks had 0.10 or 0.0 papillomas/mouse, respectively. Values are an average of 2 separate experiments.

It is important to note that chrysarobin did promote papilloma development in the mice of Group 7; however, the tumor response was not different from that obtained in Group 3 that

Downloaded from cancerres.aacrjournals.org on April 20, 2017. © 1985 American Association for Cancer Research.
received acetone during Stage I. Thus, although chrysarobin is a complete promoter, it does not possess properties similar to TPA or mezerein that would allow it to function as a Stage II promoter. If this were the case, one would expect a much greater tumor response in the animals of Group 7. It should also be noted that the duration of treatment with chrysarobin was shorter than the experiments shown in Table 1. This explains the lower papilloma response, since the tumor response had not reached a plateau. The experiments in Table 2 have been repeated giving similar results.

Hyperplasia and Edema Formation. The effect of a single treatment with optimal promoting doses of TPA, chrysarobin, and anthralin on the number of nucleated IFE cell layers and on edema formation is shown in Table 3. The data indicate that both chrysarobin and anthralin were capable of inducing hyperplasia, but the magnitude of the response was lower than that observed with TPA. In addition, the kinetics of induction of hyperplasia were different. With chrysarobin, a slight hyperplasia was observed at 24 and 48 h after treatment and a greater level at 72 and 96 h after treatment. A similar result was obtained with anthralin. With TPA, hyperplasia was maximal by 48 to 72 h after application and by 96 h had begun to decline. Interestingly, both chrysarobin and anthralin produced edema to a greater extent than did TPA.

Effect of Chrysarobin on Epidermal ODC. Time course experiments were performed to determine the effect of single and multiple applications of 220 nmol chrysarobin on epidermal ODC. A single application of 220 nmol chrysarobin gave rise to a slight induction of ODC activity with a maximum value at 64 h (0.75 ± 0.25 nmol CO2/mg protein/60 min) (Table 4). The acetone (0.2 ml)- and TPA (3.4 nmol)-treated control groups for this single-application experiment had ODC activities of 0.06 ± 0.02 and 0.06 ± 0.005, respectively, 64 h after application. Doses above 220 nmol chrysarobin did not give further increases in epidermal ODC activity. Little or no increase in epidermal ODC activity was detected prior to 24 h after treatment, and enzyme activity returned to control levels by 96 h after treatment. When chrysarobin (220 nmol) was applied 5 times at intervals similar to a promotion experiment, a slight induction of ODC activity also was observed, but the time course of induction was markedly different from that observed with the single-application experiment. In this experiment, peak ODC activity (0.22 ± 0.002) was observed at 4 h after the last application and fell to control values (0.021 ± 0.007) by 12 h after the last application (Table 4). Epidermal ODC activity was dramatically increased 4 h after the last treatment when mice received 5 applications of 3.4 nmol TPA (10.87 ± 1.08). In these experiments, doses above 220 nmol chrysarobin did not give further increases in epidermal ODC activity.

DISCUSSION

Our present results suggest that chrysarobin is a more potent skin tumor promoter than anthralin and that both compounds are clearly less active than TPA. When compared with TPA, chrysarobin was at least two orders of magnitude less potent for promoting papilloma development. In a previous report, we stated that on a weight basis chrysarobin was ~43 times less active than TPA for promoting papilloma formation (11). However, in these earlier experiments only one dose was used, and thus, our quantitative comparison was less accurate.

In addition, papilloma formation with chrysarobin and anthralin promotion appeared less dependent on the dose compared with TPA promotion. It is important to point out that at the suboptimal promoting doses of TPA used (i.e., 0.425 and 0.85 nmol), the time of appearance of the first papilloma as well as the duration of treatment required to reach a plateau in the papilloma response was much longer than at optimal promoting doses (i.e., doses of 1.7 nmol or larger). For the 0.425 and 0.85 nmol doses, papillomas were not observed before 10 weeks of promotion, and a total of 25 weeks of promotion were required to reach a plateau in the papilloma response. The apparent lack of a dose-response relationship with chrysarobin and anthralin may be related to a similar phenomenon. Thus, doses of 100 nmol/mouse and higher of chrysarobin (or anthralin) are essentially optimal promoting doses, and the 50-nmol dose is a suboptimal dose requiring a longer duration of treatment. Our experiment with chrysarobin and anthralin was terminated at 27 weeks (except for the 220-nmol group receiving chrysarobin) and it may have been necessary to continue promotion in the 50-nmol chrysarobin and anthralin groups for a longer period of time to achieve a significant papilloma response. Our previous studies using crude chrysarobin did show a dose-response relationship for promoting papilloma formation in SENCAR mice (11).

A comparison of the carcinoma response between TPA- and chrysarobin-promoted mice (at a single dose level) yielded an interesting observation. The ratio of carcinomas:papillomas was higher in the chrysarobin-treated mice suggesting that this compound may be more efficient for promoting the development of carcinomas than papillomas. Bock and Burns (5) noted that anthralin was effective at promoting skin papillomas in mice, but they also reported that of the 36 Swiss mice bearing tumors, 8 carcinomas were observed. This contrasted with 6 carcinomas appearing in 66 tumor-bearing mice that had been promoted with croton oil. Thus, in their study, anthralin also appeared to

<table>
<thead>
<tr>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 3 Effect of a single topical application of TPA, anthralin, or chrysarobin on mouse epidermis</td>
</tr>
<tr>
<td>Groups of 3 to 4 mice each were treated as shown and then sacrificed 1, 2, 3, or 4 days after treatment for the determination of hyperplasia induction or at 24 h for the determination of edema formation.</td>
</tr>
<tr>
<td>Number of nucleated IFE cell layers, days after treatment</td>
</tr>
<tr>
<td>Treatment (dose)</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Acetone (0.2 ml)</td>
</tr>
<tr>
<td>TPA (3.4 nmol)</td>
</tr>
<tr>
<td>Chrysarobin (220 nmol)</td>
</tr>
<tr>
<td>Anthralin (220 nmol)</td>
</tr>
</tbody>
</table>

a The degree of edema was classified from 0 to 5 (with 0 = no effect and 5 = maximum response).
be more efficient for promoting the development of malignant tumors than benign tumors. These results and our present data suggest that further work might uncover other tumor promoters that have been considered very weak in terms of their papilloma-promoting ability while giving a higher carcinoma response than expected.

A complete carcinogenesis protocol is characterized by a low incidence of papillomas which develop more slowly than in a DMBA initiation-TPA promotion protocol and a high carcinoma yield. For example, a 50-nmol weekly dose of DMBA to the backs of SENCAR mice yields ~2.0 papillomas/mouse with 60% of the mice bearing papillomas after 22 weeks. In this same protocol, carcinomas can be seen as early as 20 weeks, and by 40 weeks 100% of the mice will have carcinomas with an average of ~1.5 to 2.0 carcinomas/mouse (12, 29). Thus, this protocol is extremely efficient at producing squamous cell carcinomas. The longer latency of papilloma development observed with chrysarobin and anthralin and the greater ratio of carcinoma:papillomas obtained with chrysarobin suggest that the mechanism of skin tumor promotion by chrysarobin may be more like the promotion stage which occurs during complete carcinogenesis. An examination of the chemical structures of anthralin and chrysarobin supports this notion since these compounds are really hydrocarbon derivatives (Chart 1) derived from anthracene.

Boutwell (6) showed that promotion could be divided into 2 steps, "conversion and propagation," using a limited number of croton oil treatments followed by repetitive applications of turpentine. This 3-stage protocol gave rise to a significant tumor response but less than that observed with croton oil used as a complete promoter. Slaga et al. (30) using this scheme demonstrated that mezerein is a very good second-stage promoter. The possibility exists that chrysarobin possesses only some of the actions of the phorbol esters and lacks certain characteristics specific to either Stage I or II of promotion which could explain its lower promoting efficacy in terms of papilloma formation. The results in Table 2, however, demonstrate that chrysarobin did not function independently as a Stage I or II promoter although it is a complete promoter. These data indicate that in the context of the operational definitions of 2-stage promotion (using TPA and mezerein), chrysarobin could not substitute for either compound.

Chrysarobin and anthralin were both capable of producing epidermal hyperplasia, but the kinetics of this response was different from that observed with TPA. Raick (28) has shown that the weak tumor promoters EPP and cantharidin (17) are capable of producing epidermal hyperplasia and an increased mitotic index but that the time sequence of these events is different from that observed with TPA. With TPA, the increase in number of nucleated cell layers precedes the increase in mitotic index, whereas with EPP and cantharidin the order of these events is reversed. With EPP, the maximal increase in the number of nucleated cell layers of the IFE occurs between 72 and 120 h. Although we have not measured the mitotic index following treatment with chrysarobin (or anthralin), the time course of hyperplasia was similar to that reported for EPP. These results again suggest inherent differences in the way epidermal cells respond to TPA and chrysarobin.

A single application of 220 nmol chrysarobin gave rise to an ~10-fold increase in epidermal ODC activity. Interestingly, the time course of this induction was markedly different from that established for a single application of TPA, which gives a maximal increase between 4 and 6 h (6, 22). Multiple applications of 220 nmol chrysarobin also gave rise to an ~10-fold increase in epidermal ODC but with a time course more similar to that of TPA. Previous investigations by O'Brien (23) demonstrated that a large dose (2.2 µmol) of anthralin induced an 18- to 20-fold increase in epidermal ODC activity 48 h after a single application. Furthermore, when multiple applications of anthralin (6 applications of 2.2 µmol each) were tested, a similar induction of ODC was observed but with a time course of induction more like that observed with multiple applications of TPA. In our hands, doses above ~800 nmol of chrysarobin produced considerable epidermal toxicity and are well above optimal promoting doses. Thus, the mechanism of induction of ODC by chrysarobin appears to be different from that of TPA. The time course of induction of ODC by chrysarobin (and anthralin) is more like that observed with DMBA (23, 41) again suggesting a possible similarity in mechanism. Further work is currently in progress examining this as well as the effects of various inhibitors of TPA-induced ODC on the ability of chrysarobin to induce epidermal ODC activity and promote skin tumors. Hopefully, these studies will allow us to determine if the low level of ODC induction is important for the mechanism of skin tumor promotion by chrysarobin.

ACKNOWLEDGMENTS

The authors wish to thank J. M. Singer and E. P. Fisher for their excellent technical assistance and B. Zapalac for typing the manuscript.

REFERENCES

CHRYSAROBIN SKIN TUMOR PROMOTION


Mechanism of Mouse Skin Tumor Promotion by Chrysalobin
