Identification of dithiolethiones with better chemopreventive properties than oltipraz

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Oltipraz affords protection against acute and chronic hepatotoxicity, while treatment with 1,3-dithiole-2-thione (D2T), which previously has been shown to be an intermediate predictor of chemopreventive activity. Rats were pretreated with dithiolethiones (0.3 mmol/kg body wt, three times a week per os) and challenged with two acutely toxic doses of AFB1 (0.5 mg/kg body wt, once daily for two successive days per os). Inhibition of hepatotoxicity was measured by changes in body weight gain during AFB1 challenge, reduction in levels of hepatic enzymes in serum and diminution of bile duct cell proliferation. Nine dithiolethiones spanning a range of responses in this toxicity screen were further tested for their ability to prevent AFB1-induced tumorigenicity, as assessed by a reduction in hepatic burden of putative preneoplastic foci. Six dithiolethiones were found to be considerably more effective than oltipraz in preventing AFB1-induced tumorigenesis. In general, dithiolethiones that were very effective in inhibition of acute hepatotoxicity were also found to be effective in prevention of hepatic tumorigenesis.

Introduction

Dithiolethiones offer considerable promise as cancer chemoprevention drugs. For example, oltipraz [2] (see Table I for chemical names and corresponding identification numbers) is an effective chemopreventive agent in rodent models of experimental carcinogenesis in target organs such as the pancreas, lung, forestomach, colon, urinary bladder, trachea, liver, mammary gland and skin (1 and references therein). Oltipraz has advanced through Phase I clinical trials to determine its pharmacokinetics and dose-limiting side effects during chronic administration to humans (2,3). A short-term Phase IIa clinical intervention trial has been completed recently in Qidong, Jiangsu Province, People’s Republic of China, to define a dose and schedule of oltipraz [2] for reducing levels of validated biomarkers of exposure to the human hepatocarcinogen aflatoxin B1 (AFB1) (4) and to characterize dose-limiting toxicities (5).

In humans, two dithiolethiones are known to possess pharmacological properties other than cancer chemoprevention. For example, oltipraz [2] was originally used as an investigational drug for the treatment of schistosomiasis. Single oral doses of oltipraz [2] have achieved cure rates of >90% in field trials (6). 5-(4-Methoxyphenyl)-3H-1,2-dithiole-3-thione (ADT) is used currently as a choleretic and to stimulate salivary secretion and is marketed as an over-the-counter drug in many countries (7). Thus, general tolerance of dithiolethiones and their acceptance in humans has been examined.

Oltipraz [2] and other dithiolethiones are potent inducers of enzymes involved in the maintenance of the reduced glutathione pools as well as enzymes involved in electrophile detoxification, such as NAD(P)H:quinone reductase (QR), epoxide hydrolase, UDP-glucuronosyl transferase and glutathione S-transferase (GST) (8). The enhancement of electrophile detoxification through induction of phase II enzymes has been recognized as a characteristic action of many chemopreventive agents (9). In contrast to marked induction of phase II enzymes, cytochrome P450 levels and other phase I enzyme activities were only slightly elevated by oltipraz [2] (10).

Oltipraz affords protection against acute and chronic hepatotoxicity. Pretreatment of rats or mice with oltipraz [2] resulted in the inhibition of acute hepatotoxicity of carbon tetrachloride (11), acetaminophen (11), allyl alcohol (12) and AFB1 (13,14).

Mechanisms of chemoprevention by dithiolethiones are not fully understood. With oltipraz [2], the enhancement of carcinogen detoxification pathways appears to be a major component of prevention against AFB1 hepatocarcinogenesis (15,16). We have synthesized or obtained over 60 dithiolethiones and other chemically related compounds with the objective of identifying more effective chemopreventive compounds as well as some important chemical structural motifs that confer chemoprevention. These structure–activity studies might help to clarify the mechanism by which dithiolethiones inhibit carcinogenesis. For example, our initial anti-tumorigenesis studies of a few dithiolethiones showed that treatment with 0.1 or 0.3 mmol/kg body wt [1], [3], ADT or oltipraz [2] resulted in the inhibition of preneoplastic lesions induced by AFB1, while treatment with 1,3-dithiole-2-thione (D2T), which...
dithiolethiones were known compounds and were prepared by the literature
procedure or as described elsewhere (18,19). Compound [13] was prepared
from the known 3

acid hydrolysis to the corresponding acid, followed by conversion to the amide
with ammonium acetate and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide
metho-p-toluenesulfonate. Full experimental details of this preparation will be
published elsewhere (T.J.Curphey, manuscript in preparation). The identity of
different analogs was established by correspondence of physical properties
to published values and by 1H- and 13C-NMR spectroscopy. Purity was
assessed by TLC and HPLC and was >95% in all cases, as judged by
integrated UV absorbance in HPLC chromatograms. The dithiolethione analogs
were gavaged as a finely ground powder suspended in a saturated and viscous
solution of sucrose (14).

Serum alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH)
activities were measured spectrophotometrically using diagnostic kits (Sigma,
St Louis, MO). Bromodeoxyuridine (BrDU) was purchased from Sigma. Anti-
BrDU monoclonal antibody was supplied by Becton Dickinson (San Jose,
CA) and immunostaining of BrDU-labeled DNA was performed with the
peroxidase–anti-peroxidase detection system (Signet Laboratories, Dedham,
MA). The primary GST Yp antibody was purchased from Biotrin International
(Dublin, Ireland) and its localization was accomplished with a PAP immuno-
 enzymatic staining kit (Dako, Carpinteria, CA).

Male F344 rats (90–100 g) were purchased from Charles River Breeding
Laboratory (Wilmington, MA). Animals were fed pelleted purified AIN-76A
diet (Harlan, Madison, WI), but without the dietary antioxidant ethoxyquin.
Rats were allowed 5 days to acclimate to the facilities before treatment.

Strategy for selection of dithiolethione analogs

Over a score of dithiolethiones have been evaluated for phase II enzyme
induction in Hepa 1c1c7 cells in culture (20). A subset from this in vitro
screen plus a few additional analogs were selected for evaluation and are
listed in Table 1. Chemical structures of the dithiolethiones studied herein are
shown in Figure 2. Several considerations were used in the selection of
dithiolethione analogs for the study. Compounds [1] and [3] have already
been shown to be more active than oltipraz [2] in chemoprevention studies
against the formation of AFB1-induced foci (14) and were tested again.
Compounds [10] and [11] have been shown to reduce AFB1-adduction to
hepatic DNA, implying chemopreventive activity (15). Four pairs of positional
isomers (i.e. carbon 4 versus 5 of the dithiolethione ring system: [3] and [4];
[5] and [6]; [10] and [11]; [12] and [13]) were included for structure–activity
comparisons. For an additional comparison of aliphatic side chain size, we
added compound [7] and two analogs with a fused ring system of either five
[8] or six carbons [9]. Four additional analogs ([14], [15], [16] and [17]) were
closely chosen to represent other types of substituents.

Induction of phase II enzymes

Rats were gavaged with dithiolethione analogs (1 mmol/kg body wt)
and killed 48 h following treatment. Phase II enzymes were measured as described
previously (21). Briefly, livers were homogenized in 4 vol (w/v) 50 mM
Tris–HCl buffer, pH 7.0, containing 0.25 M sucrose. Homogenates were
centrifuged at 10 000 g for 15 min and the obtained supernatant was then
centrifuged at 105 000 g for 60 min. The resulting fluid was used for GST
assay, using 1-chloro-2,4-dinitrobenzene as substrate (22), and QR assay (23).

Acceptance of test compounds

To precisely know the dose and because of limited availability of many
compounds, treatment with the putative chemopreventive agents was by
gavage. In a preliminary experiment (14), growth of the rats during a 1 week
period was limited by some dithiolethione analogs at doses >0.3 mmol/kg
body wt. Because of generally accepted concern that inhibition of growth
would modify the carcinogenic response (24–26), we assessed growth when
the dosages was at the rate of every other day over a 6 day period. This
schedule approximates the timing and the total dose of oltipraz (2) in previous
studies where it was fed in the diet (15–17). This protocol has been described
in detail previously (14). Briefly, groups of four rats received three doses of
an analog at 0.3 mmol/kg body wt by gavage every other day. During this
time, general acceptance and tolerance of dithiolethiones by the animals
were assessed.

Protection from acute hepatotoxicity of AFB1

Protection against AFB1 toxicity was assessed as previously described (14).
Beginning 2 days following the last treatment with dithiolethiones, rats were
challenged with two acutely toxic doses of AFB1 (0.5 mg/kg body wt, 24 h
apart). Because of the large number of compounds tested, four replicate
effects were carried out, each with its own AFB1 and no-AFB1 control
groups. ALT and SDH activities and BrDU incorporation into bile duct cells
(BDC) were measured. Rats were given two doses of BrDU (100 mg/kg body
wt i.p., 5 and 2 h prior to autopsy) and killed 28 h after the second dose of
AFB1. A minimum of 350 BDC were counted in a total of 20–30 periportal
units per rat. The labeling index was calculated by dividing the number of labeled
nuclei by the total number of nuclei counted. No single parameter clearly

![Fig. 1. Chemical structures of two dithiolethiones studied previously.](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>Dithiolethione</th>
<th>Hepatic activity (treated/control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3H-1,2-dithiole-3-thione</td>
<td>10.2/3.0</td>
</tr>
<tr>
<td>2</td>
<td>4-methyl-1-pyrazinyl-3H-1,2-dithiole-3-thione (oltipraz)</td>
<td>2.7/1.9</td>
</tr>
<tr>
<td>3</td>
<td>5-methyl-3H-1,2-dithiole-3-thione</td>
<td>9.5/3.6</td>
</tr>
<tr>
<td>4</td>
<td>4-methyl-3H-1,2-dithiole-3-thione</td>
<td>10.8/2.9</td>
</tr>
<tr>
<td>5</td>
<td>5-ethyl-3H-1,2-dithiole-3-thione</td>
<td>7.8/2.8</td>
</tr>
<tr>
<td>6</td>
<td>4-ethyl-3H-1,2-dithiole-3-thione</td>
<td>8.4/2.7</td>
</tr>
<tr>
<td>7</td>
<td>3-tert-buty1-3H-1,2-dithiole-3-thione</td>
<td>6.1/2.7</td>
</tr>
<tr>
<td>8</td>
<td>5,6-dihydro-4H-cyclopenta-1,2-dithiole-3(4H)-thione</td>
<td>7.3/2.4</td>
</tr>
<tr>
<td>9</td>
<td>4,5,6,7-tetrahydro-3H-1,2-benzodithiole-3-thione</td>
<td>4.2/2.1</td>
</tr>
<tr>
<td>10</td>
<td>5-phenyl-3H-1,2-dithiole-3-thione</td>
<td>5.4/2.3</td>
</tr>
<tr>
<td>11</td>
<td>4-phenyl-3H-1,2-dithiole-3-thione</td>
<td>1.2/1.5</td>
</tr>
<tr>
<td>12</td>
<td>5H-1,2-dithiole-3-thioxo-5-carboxylic acid amide</td>
<td>3.7/1.8</td>
</tr>
<tr>
<td>13</td>
<td>3H-1,2-dithiole-3-thioxo-4-carboxylic acid amide</td>
<td>5.9/2.0</td>
</tr>
<tr>
<td>14</td>
<td>4-methyl-3-mercaptopro-3H-1,2-dithiole-3-thione</td>
<td>5.1/1.3</td>
</tr>
<tr>
<td>15</td>
<td>5-dimethylamino-1,2,4-dithiazole-3-thione</td>
<td>3.5/1.2</td>
</tr>
</tbody>
</table>
| 16  | 5-(N,N-dimethyl-2-aminoethenyl)-4-methyl-3H-1,2-
dithiole-3-thione | 20.8/4.4                      |
| 17  | 5-mercapto-4-methyl-3H-1,2-dithiole-3-thione, dimethylammonium salt | 9.8/1.7                        |

*Measured 48 h following a single per os treatment with 1 mmol
dithiolethione/kg body wt.

Materials and methods

**Chemicals and animals**

AFB1 was obtained from Aldrich (Milwaukee, WI) and was dissolved in
tricaprylin for administration by gastric intubation. Except for [13], all
dithiolethiones were known compounds and were prepared by the literature
procedure or as described elsewhere (18,19). Compound [13] was prepared
from the known 3H-1,2-dithiole-3-thioxo-4-carboxylic acid ethyl ester
(R.L.Hodgson and E.J.Smutny, US patent 3 394 146, 1968) by acetic/sulfuric
Dithiolethiones with greater efficacy than oltipraz

Fig. 2. Chemical structures of the dithiolethiones examined within this study.

characterizes hepatotoxicity elicited by AFB$_1$; therefore, we used several facile measures. For each of four assessments of toxicity, the rankings of the compounds were approximately similar, therefore, for simplicity of presentation, an overall sum of the ranks was calculated.

Inhibition of hepatic tumorigenesis

Nine dithiolethiones were evaluated in a tumorigenesis experiment. Compounds [1], oltipraz [2] and [3] have been previously evaluated (14) and were included for comparative purposes. Based upon the ability to protect against AFB$_1$ toxicity, five analogs better and one worse than oltipraz [2] were selected.

Dithiolethiones were gavaged at 0700–0800 h, Monday, Wednesday and Friday for three successive weeks. AFB$_1$ (25 µg/rat/day, five days a week for two successive weeks) was given by gavage at 1300 h, starting on Monday of the second week. As in the acute toxicity experiment above, rats were exposed to the dithiolethiones beginning 1 week prior to exposure to AFB$_1$. All rats were killed 5 weeks after the end of the AFB$_1$ treatment. Livers were analyzed by light microscopy for foci expressing the placental form of GST (GST-P) (27). Details of these protocols have been published (14).

Statistical analysis

Because the analogs were tested in four separate experiments, all toxicological data are presented as a percentage of inhibition of toxicity. Body weight and GST-P-positive focal data were analyzed by one way ANOVA followed by Bonferroni multiple comparison tests. The variances for two variables, the number of foci/cm$^3$ and the volume percentage of the liver occupied by the GST-P-positive foci, were found to be approximately proportional to the size of the variable. Therefore, in order to stabilize the variance, the data for these variables were logarithmically transformed prior to ANOVA. The Spearman rank correlation coefficient ($r_s$) was used to compare the in vivo enzyme activities or toxicological indices with the GST-P focal burden. Additionally, in Figure 3, the Pearson product-moment correlation ($r$) was used to compare focal volume percent and in vivo enzyme activities.

Results

Induction of phase II enzymes

Results of the hepatic enzyme assays are presented in Table I. Of 17 dithiolethiones tested, 15 induced hepatic QR activity greater than oltipraz [2], with only compound [11] showing less response. Eleven analogs were better inducers of hepatic GST activity than oltipraz [2].

Protection from acute hepatotoxicity of AFB$_1$

Rats in the groups receiving [1], [8] and [13] continued to gain weight even after the challenge with toxic doses of AFB$_1$ (Table II). Additionally, pretreatment with eight other compounds, [3], [4], [5], [6], [7], [9], [11] and [17], resulted in greater protection (i.e. less loss of body weight) from the toxic effects of AFB$_1$ than afforded by oltipraz [2].

Except for [15], pretreatment with all dithiolethiones protected against AFB$_1$ cytotoxicity, i.e. these dithiolethiones resulted in less release of SDH and ALT to the serum as compared with the AFB$_1$-treated group. As with weight gain, compounds [1], [8] and [13] afforded the greatest protection (Table II).

For rats not exposed to AFB$_1$, the BDC labeling was low, in the range 0.3–1.1%, and labeling increased to 31–36% with AFB$_1$ treatment. This high BDC proliferation rate was reduced by pretreatment with all dithiolethiones except for [3], [12], [16] and [17] (Table II). Again, compounds [1], [8] and [13] afforded the greatest protection from this classic effect of AFB$_1$.

Using all four toxicological indices (i.e. growth during AFB$_1$ treatment, level of each hepatic enzyme in blood and BDC proliferation), all 17 analogs were ranked (Table II). Ten dithiolethiones were found to be more effective in inhibiting AFB$_1$-induced hepatotoxicity than oltipraz [2] using the sum of ranks as an indicator.

Previously, we had shown that a strong correlation existed between preventing hepatotoxicity and chemoprevention of
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Fig. 3. Correlation of in vivo enzyme activity with liver GST-P-positive focal burden for several dithiolethiones.

Table II. Inhibition of AFB$_1$-induced hepatotoxicity by pretreatment with dithiolethiones

<table>
<thead>
<tr>
<th>Compound</th>
<th>Body weight gain (g/day)</th>
<th>SDH activity inhibition (%)</th>
<th>ALT activity inhibition (%)</th>
<th>BDC proliferation inhibition (%)</th>
<th>Sum of ranks$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dithiolethiones alone$^b$</td>
<td>AFB$_1$ challenge$^b$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1]</td>
<td>+4</td>
<td>+3</td>
<td>97</td>
<td>99</td>
<td>92</td>
</tr>
<tr>
<td>[3]</td>
<td>+3</td>
<td>−1</td>
<td>48</td>
<td>18</td>
<td>−2</td>
</tr>
<tr>
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<tr>
<td>[5]</td>
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<td>0</td>
<td>58</td>
<td>49</td>
<td>46</td>
</tr>
<tr>
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<td>−1</td>
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<td>37</td>
<td>30</td>
</tr>
<tr>
<td>[7]</td>
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<td>93</td>
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<td>81</td>
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<td>[9]</td>
<td>−2</td>
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<tr>
<td>[10]</td>
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<td>8</td>
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<td>−1</td>
<td>20</td>
<td>8</td>
<td>−6</td>
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<tr>
<td>[14]</td>
<td>+3</td>
<td>−2</td>
<td>56</td>
<td>38</td>
<td>3</td>
</tr>
<tr>
<td>[15]</td>
<td>−3$^e$</td>
<td>−4</td>
<td>0</td>
<td>−35</td>
<td>21</td>
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<tr>
<td>[16]</td>
<td>+3</td>
<td>−3</td>
<td>44</td>
<td>9</td>
<td>−20</td>
</tr>
<tr>
<td>[17]</td>
<td>+3</td>
<td>−1</td>
<td>45</td>
<td>7</td>
<td>−1</td>
</tr>
<tr>
<td>No AFB$_1$</td>
<td>+4</td>
<td>+3</td>
<td>97</td>
<td>99</td>
<td>99</td>
</tr>
</tbody>
</table>

$^a$Four rats per group. Inhibition as compared with the AFB$_1$-treated group.

$^b$Mean value for 6 days pretreatment with dithiolethiones.

$^c$Mean value for 2 days pretreatment with AFB$_1$. In the AFB$_1$-treated group body weight loss was 3 g/day.

$^d$For all 17 dithiolethiones, each measure of toxicity (i.e. growth during AFB$_1$ treatment, ALT and SDH activities and BDC proliferation) was ranked from least to most severe and these four ranks were summed. The lowest sum of ranks corresponds to the greatest protection.

$^e$All four rats experienced weight loss and two rats that received [15] had such a substantial weight loss (up to 25% of pre-dose weight) that they were not treated with AFB$_1$. Analysis of their serum samples indicated no signs of hepatic toxicity. The remaining two rats were challenged with AFB$_1$ as described.

$^f$By convention, tie values are indicated as half way between the two possible ranks.

Inhibition of hepatic tumorigenesis

In this experiment, all rats grew at comparable rates except for those in groups [7] and [12], where growth retardation during AFB$_1$ treatment was similar to the AFB$_1$-treated group (data not shown). However, at termination of the experiment, the mean body weight in all groups did not differ significantly from the mean body weight in the AFB$_1$-treated group nor did the liver weights differ (data not shown).

Treatment with all dithiolethiones resulted in significant inhibition of AFB$_1$-induced tumorigenesis (Table III). Except for the oltipraz [2] and [9] groups, in which all rats had foci, at least one of eight animals in each of the other dithiolethione groups had no detectable GST-P-positive foci. Foci were only very rarely encountered in the no-AFB$_1$ control rats, whereas in the livers of the AFB$_1$-treated rats, ~8 foci/cm$^2$ tissue were observed. As is already well documented (28), statistical analysis of the observed focal data is not appropriate, therefore, these data were first subjected to morphometric transformation.

Treatment with [1], [3] and [5], [6], [7] and [8] significantly decreased the number of GST-P-positive foci/cm$^2$ compared with the AFB$_1$-treated group, whereas treatment with oltipraz [2], [9] and [12] did not. While it seemed that, except for [1] and [7], treatment with dithiolethiones reduced the size of the GST-P-positive foci, the difference in focal diameter between tumorigenesis (14). As seen in Table II, 10 dithiolethiones were more effective and six were less effective than oltipraz [2] in mitigating against AFB$_1$ toxicity. Based on their ability to block toxicity, [3] and [12] were predicted to have chemopreventive activity less than oltipraz [2], while [1] and [5], [6], [7], [8] and [9] were expected to have activity greater than oltipraz [2]. These nine dithiolethiones were subsequently tested for their ability to protect against AFB$_1$-induced hepatic tumorigenesis.
the groups was not statistically significant. The volume percentage of liver occupied by GST-P-positive foci (or simply focal volume percent) is analogous to tumor burden. This parameter age of liver occupied by GST-P-positive foci (or simply focal burden as did the other dithiolethiones.

The focal volume percent for the analogs was ranked from the most effective inhibitor, analog [1], to the least effective, analog [12] (Table III). Except for [3], the rank order based on acute toxicity screening was identical to the rank order based on inhibition of tumorigenesis. Moreover, volume percent of the GST-P-positive foci was highly significantly inversely correlated with all four indices of acute hepatic toxicity (for weight phase II enzyme induction activities by dithiolethiones and implies that protection against, the highly predictive nature of induction of phase II enzyme preparation). Based on these studies, we selected 16 dithiolethiones with chemopreventive properties, the GST and QR values from in vivo induction of QR and GST (20) and from their ability to induce oltipraz [2] were initially identified from in vitro data on the induction of QR and GST (T.J.Curphey, manuscript in preparation). Based on these studies, we selected 16 dithiolethiones (see Table I), of which most showed greater induction of hepatic QR and/or GST than that engendered by oltipraz [2]. To further prioritize the selected compounds, we employed a strategy used previously (14). Specifically, the candidate dithiolethiones were initially tested for their ability to induce hepatic QR and GST (20) and from their ability to induce hepatic QR and GST (T.J.Curphey, manuscript in preparation). Based on these studies, we selected 16 dithiolethiones (see Table I), of which most showed greater induction of hepatic QR and/or GST than that engendered by oltipraz [2]. To further prioritize the selected compounds, we employed a strategy used previously (14). Specifically, the candidate dithiolethiones were initially tested for their ability to mitigate against the toxic effects of AFB1. The rationale behind this approach is our observation that dithiolethiones that reduce the acute toxic effects of AFB1 are the same ones that are chemopreventive against AFB1-induced hepatic tumorigenesis (14). From those chemicals that protected against the acute toxic effects of AFB1, we selected several active dithiolethiones and other interesting structural analogs (see discussion below) to test their ability to prevent AFB1-induced hepatic tumorigenesis. This tiered approach proved very effective.

Of the compounds evaluated for their chemopreventive properties, the GST and QR values from in vivo enzyme induction (Table I) were inversely correlated with the focal volume percent values (Table III). Figure 3 graphically depicts the highly predictive nature of induction of phase II enzyme activity by dithiolethiones and implies that protection against, at least, these early events of AFB1 hepatic carcinogenesis

Dithiolethiones with greater efficacy than oltipraz

| No. | GSTP foci/cm² liver⁴ | Mean focal area (mm²/100) | Foci/cm³ liver | Focal diameter (mm) | Vol.% GST-P⁺ foci (×100) | Inhibition of vol.% GSTP foci (%) | Rank⁶ (vol.%)
|-----|----------------------|---------------------------|----------------|---------------------|--------------------------|---------------------------------|---------
| AFB₁ | 8.3 ± 1.2 | 8.0 ± 1.7 | 322 ± 54⁴ | 282 ± 29 | 70.3 ± 25.1⁵ | 32.5 | 4
| [12] | 1.3 ± 0.3 | 3.0 ± 0.6 | 68 ± 16⁴ | 176 ± 21 | 4.5 ± 1.2⁵ | 49.6 | 6
| [2] | 1.2 ± 0.2 | 2.8 ± 0.6 | 130 ± 50⁶ | 153 ± 23 | 3.7 ± 1.0⁸ | 94.7 | 8
| [9] | 1.1 ± 0.2 | 3.2 ± 0.4 | 87 ± 21¹⁴ | 168 ± 27 | 3.5 ± 0.7¹⁸ | 95.0 | 7
| [7] | 0.1 ± 0.04 | 9.6 ± 4.7 | 7 ± 5² | 324 ± 150 | 1.0 ± 0.4⁴ | 98.6 | 6
| [5] | 0.3 ± 0.1 | 1.9 ± 0.3 | 19 ± 7² | 159 ± 14 | 0.6 ± 0.2² | 99.1 | 4.5
| [6] | 0.4 ± 0.1 | 1.4 ± 0.3 | 40 ± 11¹ | 128 ± 22 | 0.6 ± 0.2² | 99.1 | 4.5
| [3] | 0.4 ± 0.2 | 1.1 ± 0.2 | 35 ± 15¹ | 117 ± 24 | 0.4 ± 0.2² | 99.4 | 4.5
| [8] | 0.2 ± 0.04 | 1.6 ± 0.5 | 14 ± 4² | 118 ± 10 | 0.3 ± 0.1¹⁸ | 99.6 | 2
| [1] | 0.1 ± 0.05 | 4.3 ± 1.6 | 7 ± 6² | 297 ± 110 | 0.2 ± 0.1⁶ | 99.7 | 1
| No-AFB₁ | 0.05 ± 0.03 | 1.7 ± 0.5 | 3 ± 2² | 191 ± 45 | 0.1 ± 0.1⁶ | 99.9 | 1

⁴Mean ± SE; n = 8, except for the AFB₁-treated group, where n = 5.
⁵On average 7–9 cm² liver/animal were analyzed, except for the AFB₁-treated group, where because of the large number of foci observed only 2–3 cm² were analyzed.
⁶The extent of protection was ranked based on the volume percentage of the liver occupied by the GST-P-positive foci. The lowest rank corresponds to the greatest chemoprevention.
⁷Statistically different (P < 0.05) from the no-AFB₁ control group.
⁸Statistically different (P < 0.05) from oltipraz [2].
⁹Statistically different (P < 0.05) from the AFB₁-treated group.
¹⁰Statistically different (P < 0.05) from each other.

Table III. Chemoprevention of AFB₁-induced hepatic carcinogenesis by pretreatment with dithiolethiones

Discussion

The first objective of these studies was successful. At least six dithiolethiones with chemopreventive properties that exceeded oltipraz [2] have been identified (Table III). Herein, we have confirmed our previous observation that compounds [1] and [3] are more efficacious than oltipraz [2] (14,21). Additionally, four new, promising dithiolethiones were identified, [5], [6], [7] and [8]. Based solely upon the toxicological screening data of Table II, [13] would likely have been more efficacious than oltipraz [2], but we did not have enough of it synthesized for it to be fully tested. The conditions of testing to identify these six dithiolethiones were stringent, in that we purposefully tested these dithiolethiones at equal molar doses against an extremely effective dose (0.3 mmol/kg body wt) of oltipraz [2]. In other words, a successful compound had to be better than oltipraz, which itself reduced the AFB₁-induced focal burden by >90%. As seen previously, this reduction in focal volume percent was attended by a decrease in the number of foci and less often by a decrease in the mean focal size (17,21). This outcome further supports previous studies indicating that oltipraz effects the initiation events of carcinogenesis and not the events that primarily drive the growth of foci (15).

Our strategy for identifying compounds with greater chemoprotective properties than oltipraz [2] relied upon a tiered approach. Candidate dithiolethiones with activity greater than oltipraz [2] were initially identified from in vitro data on the induction of QR and GST (20) and from their ability to induce in vivo hepatic QR and GST (T.J.Curphey, manuscript in preparation). Based on these studies, we selected 16 dithiolethiones (see Table I), of which most showed greater induction of hepatic QR and/or GST than that engendered by oltipraz [2]. To further prioritize the selected compounds, we employed a strategy used previously (14). Specifically, the candidate dithiolethiones were initially tested for their ability to mitigate against the toxic effects of AFB1. The rationale behind this approach is our observation that dithiolethiones that reduce the acute toxic effects of AFB1 are the same ones that are chemopreventive against AFB1-induced hepatic tumorigenesis (14). From those chemicals that protected against the acute toxic effects of AFB1, we selected several active dithiolethiones and other interesting structural analogs (see discussion below) to test their ability to prevent AFB1-induced hepatic tumorigenesis. This tiered approach proved very effective.

Of the compounds evaluated for their chemopreventive properties, the GST and QR values from in vivo enzyme induction (Table I) were inversely correlated with the focal volume percent values (Table III). Figure 3 graphically depicts the highly predictive nature of induction of phase II enzyme activity by dithiolethiones and implies that protection against, at least, these early events of AFB1 hepatic carcinogenesis.
labeled as 85%. A wealth of evidence testifies that agents that increase detoxification pathways for AFB1 would be chemopreventive (21). Similarly, strong correlations were seen between indices of hepatotoxicity and focal volume percent. While the nature of the relationship between the hepatotoxic effects and tumorigenic effects of AFB1 is largely unknown, there is little doubt that chemicals that ameliorate acute toxicity also protect against tumor development. For example, upon challenge with acutely toxic doses of AFB1, the parent dithiolethione [1] afforded complete protection against the growth inhibitory effects of AFB1, whereas the rats receiving only AFB1 lost weight (Table II, footnote c).

Previously, we observed that both oltipraz [2] and the parent dithiolethione [1] afforded complete protection against the growth inhibitory effects of AFB1, whereas the rats receiving only AFB1 lost weight (Table II, footnote c). The parent dithiolethione [1] largely prevented all AFB1-induced BDC proliferation and corroborated previous observations in this identical animal model (14). In contrast, oltipraz [2] provided much less protection as assessed by all four markers of hepatic toxicity. The sum of ranks of these four toxicological markers placed oltipraz [2] in the midst of the selected compounds (Table II).

Mechanistic insights of exactly how the dithiolethiones blunt tumorigenesis are certainly not clear (32,33). Identification of chemical structural motifs that confer chemoprevention would be highly desirable and certainly would aid in elucidating mechanisms of chemoprevention. We can make only tentative observations regarding structural activity. As already demonstrated and largely confirmed herein, both the parent dithiolethione [1] and the 5-methyl member [3] were more effective than oltipraz [2] in alleviating hepatic toxicity of AFB1 and inhibiting development of foci (14). For an unexplained reason, [3] was less effective in protecting against acute toxicity in this study than in our previous study (14), although the comparison between this and the former study for protection against foci development yielded identical results and showed [3] to be nearly as good as [1]. Several other simple alkyl-substituted dithiolethiones (e.g. [5]–[8]) were more active than oltipraz [2]. These aforementioned dithiolethiones are certainly more lipophilic than oltipraz. This is especially interesting in the light of the report by French workers (34) who, using our previously published in vitro data (20), concluded that less lipophilic dithiolethiones were the most active chemopreventive agents. Our findings in the present instance lend little support to this generalization, which was in fact based not on the full set of our in vitro data, but on a carefully selected subset (five out of seven disubstituted dithiolethiones, the remaining two excluded because of their failure to fit the correlation). Indeed, a more extensive structure–activity study of enzyme induction in vivo (T.J.Curphey, manuscript in preparation) has failed to find much correlation between lipophilicity and biological activity of dithiolethiones. It appears as if the position of this addition (i.e. carbon 4 or 5 of the ring) may not be critical, since compounds [5] and [6] protected against AFB1-induced foci to similar extents. The difference in biological activity between compounds [8] and [9] was quite large (Tables I–III). This observation is particularly interesting considering that the aliphatic addition to the parent structure differed only by a single carbon (Figure 2). It is not possible to know at what level these structural changes render their action. The effectiveness of these dithiolethiones could be explained in a trivial sense by differences in biological stability, absorption and metabolism or these effects could be manifestations of differing molecular and/or chemical interactions at some critical target.

Based upon the inhibition of putative preneoplastic foci, we have identified several dithiolethiones possessing cancer chemoprevention activity equal to or greater than oltipraz [2]. Furthermore, we have defined a series of short-term toxicological tests to identify candidate chemopreventive agents to mitigate against AFB1 carcinogenicity.

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References

Dithiolethiones with greater efficacy than oltipraz


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