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## Cytotoxic constituents from *Exostema mexicanum* and *Artemisia afra*, two traditionally used plant remedies

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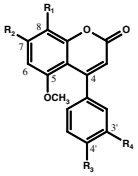
During our ongoing research on antimalarial plant remedies, we investigated *Exostema mexicanum* Gray, a Rubiaceae, which is used to treat malaria in Central America [1] and *Artemisia afra* Jacq. (Asteraceae) an appraised febrifuge from Southern Africa [2]. By means of bioassay-guided fractionation using *in vitro* cultures of *Plasmodium falciparum*, we were able to isolate a series of active constituents representing 4-phenylcoumarins (*E. mexicanum*) or sesquiterpene lactones (*A. afra*) [3, 4]. The present paper deals with the cytotoxicity of these compounds against the endothelial cell line ECV-304.

All 4-phenylcoumarins displayed marked toxicity against ECV-304 cells which is in agreement with findings that related compounds from *E. acuminatum* showed cytotoxic effects against a panel of human tumor cell lines [5]. Their activity against the chloroquine-resistant clone Dd2 of *P. falciparum* in comparison was four to hundred times lower. This is expressed by selectivity indices (SI), which are defined as the ratio of the respective biological responses [6] <1. Whereas well-known antimalarial agents such as artemisinin were found to give a selectivity index >1000, generally cytotoxic agents produce ratios of <10. Interestingly, compounds **2** and **3** which possessed the lowest ($IC_{50}$ > 50 μg/ml) and the best antiplasmodial activity ($IC_{50}$ 1.6 μg/ml), respectively, were both very potent against ECV-304 with $IC_{50}$ values about 0.5 μg/ml, thus suggesting a different mode of action for both activities.

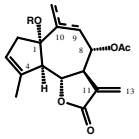
Of the sesquiterpene lactones tested, the rupicolin derivatives **8** and **9** proved to be most active, whereas 1α,4α,8α-trihydroxyguaia-2,9,11(13)-triene-12,6α-olide-8-O-acetate (**12**) did not show any toxic effect at the highest concentration tested (200 μg/ml). This is quite astonishing as **12** differs from **9** only in the position of the double bond (Δ2 *vs.* Δ3) and the presence of the 4-hydroxy group in the cyclopentene ring system with the exomethylene substructure at C-11 remaining unchanged. Obviously, an additional exomethylene group at C-10 is able to enhance both the cytotoxic and the antiplasmodial activity, because the Δ9-isomers of **8** and **11** (**9** and **12**) exhibited lower values in both test systems.

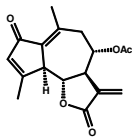
In conclusion, as the antiplasmodial activity of the isolated compounds is in most cases lower than the toxicity

| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| **1** | H | $OCH_3$ | OH | OH |
| **2** | H | OH | $OCH_3$ | OH |
| **3** | $OCH_3$ | $OCH_3$ | $OCH_3$ | H |
| **4** | OH | $OCH_3$ | OH | H |
| **5** | $OCH_3$ | $OCH_3$ | OH | OH |
| **6** | OH | $OCH_3$ | OH | OH |

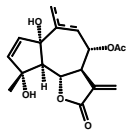


| | R | Δ |
|---|---|---|
| **7** | OH | 10 |
| **8** | H | 10 |
| **9** | H | 9 |



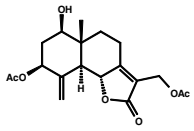


**10**



**11** (Δ10)

**12** (Δ9)



**13**

**Table:** ***In vitro*** **cytotoxic and antiplasmodial effects of 4-phenylcoumarines and sesquiterpene lactones isolated from** ***Exostema mexicanum*** **and** ***Artemisia afra***

| Compound | Mean $IC_{50}$ values (μg/ml)* | | |
|---|---|---|---|
| | ECV-304 | Dd2** | SI*** |
| *4-Phenylcoumarins* | | | |
| 3′,4′-Dihydroxy-5,7-dimethoxy-4-phenylcoumarin (**1**) | 3.2 | 12.8 | 0.25 |
| 3′,7-Dihydroxy-4′,5-dimethoxy-4-phenylcoumarin (**2**) | 0.55 | >50 | <0.01 |
| 4′,5,7,8-Tetramethoxy-4-phenylcoumarin (**3**) | 0.4 | 1.6 | 0.25 |
| 4′,8-Dihydroxy-5,7-dimethoxy-4-phenylcoumarin (**4**) | 1.6 | 16.5 | 0.10 |
| 3′,4′-Dihydroxy-5,7,8-trimethoxy-4-phenylcoumarin (**5**) | 0.54 | 14.0 | 0.04 |
| 3′,4′,8-Trihydroxy-5,7-dimethoxy-4-phenylcoumarin (**6**) | 3.3 | 18.0 | 0.18 |
| *Sesquiterpene lactones* | | | |
| 1-Desoxy-1α-peroxy-rupicolin A-8-*O*-acetate (**7**) | 10.6 | 17.5 | 0.61 |
| Rupicolin A-8-*O*-acetate (**8**) | 0.8 | 10.8 | 0.07 |
| Rupicolin B-8-*O*-acetate (**9**) | 2.5 | 31.8 | 0.08 |
| 11,13-Dehydromatricarin (**10**) | 25.7 | 12.5 | 2.10 |
| 1α,4α-Dihydroxybishopsolicepolide (**11**) | 5.3 | 11.7 | 0.45 |
| 1α,4α,8α-Trihydroxyguaia-2,9,11(13)-triene-12,6α-olide-8-*O*-acetate (**12**) | >200 | 20.4 | >9.80 |
| Eudesmaafraglaucolide (**13**) | 60.4 | >50 | <1.21 |
| *Control* | | | |
| Chloroquine × 2 $H_3PO_4$ | 30.0 | 0.073 | 411.0 |

* Number of experiments: 8 (ECV-304); 3 (Dd2).
** *P. falciparum* clone Dd2, for details see [3, 4].
*** Selectivity index (SI) is defined as the ratio of cytotoxicity over antiplasmodial activity

against ECV-304 cells, the two plant remedies might not be very suitable for antimalarial treatment. This is especially true for *E. mexicanum* the 4-phenylcoumarins of which possess relatively high cytotoxicity *in vitro*.

## Experimental

*3.1. Materials*

All substances used in the cytotoxicity assay were isolated in our lab [3, 4] and analysed for structure and impurities. The purity of the substances (> 90%) was checked by HPLC and TLC.

*3.2. In vitro cytotoxicity assay*

The cytotoxicity of the substances was estimated by a proliferation assay using the MTT-assay [7]. Test substances were dissolved in acetone and diluted with medium to the desired concentrations. Human endothelial cells (ECV-304) were cultivated in Eagle Medium 199 supplemented with 10% fetal calf serum in 96-well plates in an atmosphere of 5% $CO_2$ at 37 °C in a humidified environment. Endothelial cells were seeded at a density of approximately 1000 cells per well. After 24 h they were supplemented with 100 µl test substance in medium and cultivated for further 4 days. The cell viability was measured by the MTT-assay using DMSO to dissolve the formed purple formazan. The absorbance was quantified at 580 nm with an ELISA plate reader.
Data are presented as the mean of 8 parallel samples for each concentration. The $IC_{50}$ values were calculated by linear regression.

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Received November 11, 2001
Accepted December 20, 2001

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