Chemical and Cytotoxic Activity of three main Sesquiterpenoids from *Warburgia ugandensis*

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**ABSTRACT**

Plant materials of *Warburgia ugandensis* have long been used in traditional African medicine due to their broad spectrum of biological activity. Here, we describe an efficient and mild isolation of three major compounds polygodial 1a, warburganal 2a, and muzigadial 2b using Soxhlet extraction and combined preparative separation methods. Furthermore, 9-deoxymuzigadial was reported for the first time from this species. Since in silico analysis predicted drug-likeness, the compounds were tested against leukemia and solid cancer cell lines as well as human primary cells in vitro. The most susceptible cells were THP-1 indicating a selective effect against monocytes pointing towards a potential treatment of monocytic leukemia. The most robust cells were primary gingival fibroblasts offering selectivity also to solid cancers. The results in this study shed new light on long-known chemical compounds from traditional medicine and pave the way for in vitro studies to advance the substances to the next level towards the use as anti-cancer drugs.

Introduction

According to the World Health Organization, cancer is currently one of the major health challenges facing the world \([1]\) and ranks as the most common cause of death in the 21\(^{st}\) century \([2]\). In 2020, 9.96 million deaths were reported, which is expected to increase to 16.3 million deaths by 2040 \([3]\). To combat this growing issue, screening of natural biomass sources is a promising approach in the development of innovative anti-cancer drugs. Previously, natural products have often played an important role in the search for new drugs and lead structures for active ingredients \([4]\). Therefore, the selection of plants as a source for novel therapeutics is an appropriate strategy since plants naturally produce a wide range of secondary metabolites \([5]\).

In traditional African medicine, *Warburgia ugandensis* is highly valued and frequently used to treat a wide range of diseases \([6]\). In fact, in several phytochemical studies, antibacterial \([7\,–\,9]\), antifungal \([8\,\text{and}\,9]\), antiparasitic \([10\,\text{and}\,11]\) as well as antioxidant \([12]\) and cytotoxic \([9]\) activities were reported for *W. ugandensis* extracts. Interestingly, compared to other members of evergreen native tree species, *W. ugandensis* extracts had an oral LD\(_{50}\) > 5000 mg/kg body weight in mice, and no mortality was recorded in dose levels ranging from 500 to 5000 mg/kg body weight indicating safety for human use \([13]\).

In the extracts of stem bark, drimane- and coloratane-type sesquiterpenoids were identified including the three major bioactively relevant compounds polygodial 1a, warburganal 2a, and muzigadial 2b \([6,\,14\,\text{–}\,16]\) (Fig. 1) together with other known oxo-functionalized drimane-type sesquiterpenoids mukaadial and ugandensialdial \([17]\). Other drimane-type sesquiterpenoids such as 9-epi-polygodial \([46]\), drimenin \([47]\), pereniporin B, cinnamolide, cinnamolide-3\(^{-}\)-hydroxymethyl, deacetyl-gandensolide \([6]\), and dendocarbin A \([18]\) were observed in the analyzed bark extract. The coloratane-type sesquiterpenoids, the rearranged bicyclic drimane-type ones, muzigadiolide, 11α-hydroxy...
muzigadiolide, and 7α-hydroxy-8-drimen-11,12-olide have also been reported as compounds in the stem bark of *W. ugandensis* [17] (Fig. S1 in Supporting Information).

The path of natural product extraction to the pure target compounds requires many time-consuming steps. In this work, we present an isolation strategy using mild extraction conditions for effective and reproducible isolation of 1a, 2a, and 2b from *W. ugandensis* stem bark. Furthermore, the drug-likeness was investigated using the in silico prediction tool SwissADME. In order to derive new applications in human health care, the extract, relevant fractions as well as the isolated compounds were tested against different human cancer cell lines in vitro.

This study brings already known compounds into new spotlight as potential anti-cancer therapeutics and highlights a new model for a possible mechanism of action.

**Results and discussion**

**Efficient isolation of three sesquiterpenoids in one process**

By a process coupling a mild Soxhlet extraction and two combined flash column chromatographies (for detailed description see chapter 2 in Supporting Information), three sesquiterpenoids identified as polygadal 1a, warburganal 2a, and muzigadial 2b (chapter 3 in Supporting Information) were efficiently isolated from *W. ugandensis*. In this process, ground stem bark material (30 g) was extracted for 5 h using dichloromethane (350 mL). The solvent was evaporated leaving the extract (3.58 g, 11.93 wt%), which was fractionated by CombiFlash® chromatography. Fractions eluted between 7.1 and 8.3 min were combined and concentrated providing the relevant fraction 1 (RF1; 390 mg, 1.30 wt%). Fractions eluted between 8.3 and 10.4 min were combined and concentrated providing relevant fraction 2 (RF2; 591 mg, 1.97 wt%) (Fig. S3 in Supporting Information). RF1 and RF2 were fine purified by a second flash chromatography to obtain polygadal 1a (191 mg, 0.64 wt %) from RF1 as well as warburganal 2a (322 mg, 1.07 wt%) from RF2. Additionally, after a longer bark storage time, 9-deoxymuzigadial 1b (44 mg, 0.15 wt%) was found in RF1 in NMR analyses in less concentration (1a = 0.52 wt%; 1b = 0.15 wt% yield) while muzigadial 2b (226 mg, 0.75 wt%) was obtained from RF2 in higher concentration (2a = 0.02 wt%; 2b = 0.75 wt%). However, the similar polarity of 1a and 1b as well as 2a and 2b resulted in separation issues for initial purification steps.

The isolation process was monitored by thin layer chromatography and high performance liquid chromatography. Thereby, the compounds 1a, 2a, and 2b were continuously detected (Table 1, Fig. S4 in Supporting Information) allowing a straightforward purification and isolation. Based on the currently available literature, 1b is reported for the first time as a compound of *W. ugandensis*. Previously, 1b was isolated only from species of other genera such as *Pseudowintera* and *Canella* [20, 21].

The isolation process was completed within three days including two overnight drying steps. The rapidness of the extraction method reflects its high efficiency. Here, Soxhlet extraction with dichloromethane already yielded 7 wt% extract within one extraction cycle (about 1 h), which could be improved to about 12 wt% by increasing the number of cycles (extraction time) (Fig. S5 in Supporting Information). In comparison to studies which performed dichloromethane maceration, only 4 wt% and 5 wt% extract yields were obtained after 48 h and 72 h [22, 23]. Moreover, using maceration, solid plant parts must be removed from the extract which can complicate the procedure [24]. The process presented here including Soxhlet extraction is not affected by this particular difficulty, since the solid plant material is efficiently excluded from the extract by the extraction thimble. However, although Soxhlet extraction is not suitable for thermolabile compounds compared to maceration [25], the use of dichloromethane, which has a low boiling point of 40°C under normal pressure, allows a mild extraction at a relatively low temperature. Moreover, Soxhlet extraction is an automatic, continuous method and still consumes only small amounts of solvent [25]. Thus, the mild Soxhlet extraction described here is not only very efficient, but also sustainable regarding solvent consumption.

Since this study focused on an efficient isolation, stem bark instead of leaves was chosen as starting material for extraction as higher extract yields were obtained from stem bark material than from leaves (Fig. S5 in Supporting Information). Furthermore, for the stem bark extract, a higher number of intense spots not originating from chlorophyll and other photosynthetic molecules were detected on thin layer chromatography plates indicating higher concentrations of extracted secondary plant metabolites including the compounds 1a, 1b, 2a, and 2b (Fig. S6 in Supporting Information). In fact, it was reported that *W. ugandensis* stem bark extracts were more bio-active than extracts from leaves [7].

Interestingly, it was observed that the heights of the peaks representing compounds 1a, 2a, and 2b in the extract’s high performance liquid chromatogram changed over storage time of the stem bark. For compound 2b, the peak height in the chromatogram of freshly ground stem bark was increased compared to that of ground stem bark from the same plant but stored for 1 year. In contrast, the peak heights representing compounds 1a and 2a declined with longer storage time of the ground stem bark (data not shown). From these observations, it can be assumed that drimane sesquiterpenoids such as 1a and 2a are less stable in ground *W. ugandensis* stem bark than coloratanes like 2b. Based on these results, the choice of the explicit stem bark material depends on the target compound; its degradation in the raw material should therefore be investigated to guarantee an effective and sustainable isolation process regarding the starting material.

**Drug-likeness prediction**

Structure-related physicochemical parameters and absorption, distribution, metabolism, and excretion (ADME) properties were evaluated to predict the drug-likeness of 1a, 1b, 2a, and 2b using the free web tool SwissADME [26]. All four compounds were predicted to exhibit favorable properties regarding oral bioavailability, gastrointestinal absorption, skin permeability as well as metabolic elimination and thus rated as drug-like, which was already reported for 2b [23] (for detailed data and discussion see chapter 5 and Table S3 in Supporting Information).
After their use as antibiotics for infectious diseases, herbal agents became more and more relevant as therapeutics with anti-cancer properties. In this context, 1a and derivatives thereof were recently shown to exhibit cytotoxic activity against hematopoietic cell lines e.g. K-562 (chronic myeloid leukemia) and Nalm-6 (acute B lymphoblastic leukemia) [27]. With regard to these results, in the present study, the extract, RF1, RF2, and the compounds 1a, 2a, and 2b were tested against non-malignant peripheral blood mononuclear cells (PBMC) as well as against the blood cancer cell lines MV4-11 (biphenotypic B myelomonocytic leukemia) and THP-1 (acute monocytic leukemia) in vitro. The treatment with 1% Triton X-100 served as positive control representing the maximum cell lysis effect to be reached by our test compounds. All test materials led to significantly decreased cell viabilities at 100 µg/mL (Fig. 2A) indicating a strong cytotoxic effect at this concentration. Since 1b was only found in NMR analysis but could not be isolated as pure substance, it was not possible to test its cytotoxic activity so far.

To evaluate a selective anti-cancer activity of 1a, 2a, and 2b, the respective selectivity index (SI) defined as the quotient of the toxic

Table 1

<table>
<thead>
<tr>
<th>Analysis Specification[a]</th>
<th>Key figure</th>
</tr>
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<tbody>
<tr>
<td>RF1 RF2 1a 1b 2a 2b</td>
<td></td>
</tr>
<tr>
<td>CombiFlash® CC Silica phase, CH:EA gradient</td>
<td>Elution time [min] 7.1–8.3 7.1–8.3 8.3–10.4 8.3–10.4</td>
</tr>
<tr>
<td>TLC Silica phase, CH:EA – 4:1</td>
<td>Rf [-] 0.24 0.24 0.17 0.17</td>
</tr>
<tr>
<td>TLC Silica plate, CH:EA – 3:2</td>
<td>Rf [-] 0.62 0.62 0.53 0.53</td>
</tr>
<tr>
<td>HPLC C8 phase, water:ACN gradient</td>
<td>tR [min][b] 17.58 17.60 15.18 14.93</td>
</tr>
<tr>
<td>GC–MS Electron ionization</td>
<td>tR [min] 7.38 7.40 7.44 7.45</td>
</tr>
<tr>
<td>ES–MS ESI positive; time-of-flight</td>
<td>M&lt;sub&gt;u&lt;/sub&gt; [g/mol] 234 232 250 248</td>
</tr>
<tr>
<td>Mass balancing of RF</td>
<td>Yield [mg][c] 390 591</td>
</tr>
<tr>
<td>Change during bark storage of the compounds derived from fresh bark material</td>
<td>Ratio 95.5 4.5 77.5 22.5</td>
</tr>
<tr>
<td>Yield [mg][c] 191 322 94[a] 94[a]</td>
<td></td>
</tr>
<tr>
<td>Yield [wt%] 0.64 0.03 1.07 0.31</td>
<td></td>
</tr>
<tr>
<td>Change during bark storage of the compounds derived from stored bark material (1 year)</td>
<td>Ratio 78.0 22.0 2.0 98.0</td>
</tr>
<tr>
<td>Yield [mg][c] 156[a] 44[a] 5[a] 226</td>
<td></td>
</tr>
<tr>
<td>Yield [wt%] 0.52[a] 0.15[a] 0.02[d] 0.75</td>
<td></td>
</tr>
</tbody>
</table>

[a] Detailed description in Supporting Information. [b] corrected to internal standard. [c] from 30 g ground bark material. [d] theoretically determined. [e] theoretically determined assuming 200 mg of 1a + 1b.

Abbreviations: ACN, acetonitrile; CC, column chromatography; CH, cyclohexane; EA, ethyl acetate; ES–MS, electrospray ionization – mass spectrometry; GC–MS, gas chromatography – mass spectrometry; HPLC, high performance liquid chromatography; RF1/RF2, relevant fraction 1/2; Rf, retention factor; TLC, thin layer chromatography, tR, retention time.

Cytotoxicity against hematopoietic cells

After their use as antibiotics for infectious diseases, herbal agents became more and more relevant as therapeutics with anti-cancer properties. In this context, 1a and derivatives thereof were recently shown to exhibit cytotoxic activity against hematopoietic cell lines e.g. K-562 (chronic myeloid leukemia) and Nalm-6 (acute B lymphoblastic leukemia) [27]. With regard to these results, in the present study, the extract, RF1, RF2, and the compounds 1a, 2a, and 2b were tested against non-malignant peripheral blood mononuclear cells (PBMC) as well as against the blood cancer cell lines MV4-11 (biphenotypic B myelomonocytic leukemia) and THP-1 (acute monocytic leukemia) in vitro. The treatment with 1% Triton X-100 served as positive control representing the maximum cell lysis effect to be reached by our test compounds. All test materials led to significantly decreased cell viabilities at 100 µg/mL (Fig. 2A) indicating a strong cytotoxic effect at this concentration. Since 1b was only found in NMR analysis but could not be isolated as pure substance, it was not possible to test its cytotoxic activity so far.

To evaluate a selective anti-cancer activity of 1a, 2a, and 2b, the respective selectivity index (SI) defined as the quotient of the toxic

Fig. 2. Effects of W. ugandensis plant materials and its single compounds on the viability of human hematopoietic cells. A) 100 µg/mL of the extract (E), relevant fractions 1 (RF1) and 2 (RF2), 1a, 2a, and 2b, respectively, were tested against non-malignant human peripheral blood mononuclear cells (PBMC) as well as the cancer cell lines MV4-11 and THP-1. 1% Triton X-100 was used as positive control (PoC) indicating complete cell lysis. Cell viabilities were compared to those of untreated cells (negative control, NeC) (*** p < 0.001; **** p < 0.0001 (unpaired t-test with/without Welch’s correction)). B) Dose-response curves of 1a, 2a, and 2b against PBMC, MV4-11, and THP-1. Cell viabilities were normalized to viability of untreated cells. Non-linear regression was used to display sigmoidal curves of the cell viability allowing the determination of the respective half-maximum inhibitory concentration (IC<sub>50</sub>). The experiments were performed in triplicates including three biological replicates with three technical replicates each, and the data are expressed as arithmetic mean ± standard error of the mean indicated by error bars.
concentration against non-malignant cells and the effective concentration against malignant cells was calculated. Therefore, dose–response curves were recorded to determine the half-maximum inhibitory concentration (IC₅₀) of the compounds (Fig. 2B). The IC₅₀ values of the test materials against MV4-11 ranged from 0.65 µg/mL (2.62 µM of compound 2b) to 1.62 µg/mL (6.91 µM of compound 1a), which were comparable to the IC₅₀ results against PBMC leading to SI around 1 (Fig. 2B, Table 2). Generally, a SI > 10 shows high selectivity of a compound, which could hence serve as a therapeutically useful substance [28]. Thus, the test materials were assumed not to be selective to biphenotypic B myelomonocytic leukemia cell line. In contrast, THP-1 cells were the most susceptible cells with IC₅₀ values between 0.11 µg/mL (0.44 µM of compound 2b) and 0.52 µg/mL (2.22 µM of compound 1a) and SI between 3.79 (compound 2a) and 6.45 (compound 2b). Although the test materials were not fully selective to acute monocytic leukemia cells, the results indicate a selective effect on monocytic cells. Indeed, it was observed that, at the respective IC₅₀, PBMC completely lack CD14⁺ cells representing monocytes. In contrast, CD3⁺, CD19⁺ and CD56⁺ cells representing T, B, and natural killer cells were still detected with comparable percentages as PBS treated cells (Figure S8 in Supporting Information). This indicates that the strong decrease of PBMC viability was most likely due to the inhibition of the monocye population. As a speculation, the IC₅₀ value of the test materials is expected to be higher against lymphocytes alone than against PBMC (lymphocytes + monocytes) leading to a higher selectivity for malignant THP-1 cells related to healthy lymphocytes. This theoretically higher SI, especially of compound 2b, could exceed the targeted value of 10. 2b but also 1a and 2a are therefore considered to be valuable candidates for the potential treatment of acute monocytic leukemia and should be further investigated in vitro using primary patient material.

Moreover, in vivo experiments should be performed for safety and efficacy testing to clarify the impact of a treatment on healthy monocytic cells as simplified models of cancer cells and PBMC in culture cannot adequately model the complexity of the hematopoietic system as well as the human body. Based on the currently available literature, the compounds were not yet tested before against MV4-11 and THP-1 cell lines as well as PBMC as primary cell type since no previous study was found.

**Cytotoxicity against adherent cells**

Previously, polygodial and its derivatives were tested against the adherent cell lines MCF-7 (breast cancer), PC-3, and DU-145 (both prostate cancer) showing promising selectivity when comparing with non-malignant colon epithelial cells [29]. In the present study, non-malignant human gingival fibroblasts (GF) as well as the malignant cell lines Sk-Mel29 (malignant melanoma) and LN-229 (glioblastoma multiforme) were chosen for cytotoxicity analyses of the compounds. As stated before, the treatment with 1% Triton X-100 served as positive control for the maximum cell lysis effect to be reached by our test compounds. Except 1a against GF, all test materials led to significantly reduced cell viabilities, whereby the effect was more pronounced against Sk-Mel29 and LN-229 than against GF (Fig. 3A) indicating a cell-type dependent cytotoxicity of the compounds.

Cell-type dependence could be confirmed by the SI values. Therefore, analogous to the previous section, the respective IC₅₀ of the compound against Sk-Mel29 and LN-229 cell lines determined on the basis of their dose–response curves (Fig. 3B) were related to those against non-malignant GF. A SI value of >10 is the target for sufficient selectivity in vitro [28]. Sk-Mel29 and LN-229 cell lines showed similar dose–responses with IC₅₀ values between 0.61 µg/mL (2.46 µM of compound 2b) and 1.83 µg/mL (7.81 µM of compound 1a) as well as between 0.68 µg/mL (2.74 µM of compound 2b) and 5.25 µg/mL (22.40 µM of compound 1a), respectively (Fig. 3, Table 3). In contrast, GF were the most robust cells in this study with IC₅₀ values of at least 36.53 µg/mL (147.11 µM of compound 2b). The respective SI values for Sk-Mel29 and LN-229 cells were > 10 up to about 60 indicating tumor-cell specificity. Thus, a local treatment of malignant melanoma and glioblastoma might be considered and further evaluated in subsequent in vitro and in vivo efficacy and safety studies.

The compound 2b showed the highest toxicity towards all cell lines tested in this study followed by compound 2a, while compound 1a was less effective. This is consistent with results reported previously where 2a exhibited an at least three-fold stronger inhibitory effect on the human tumor cell line KB (mouth epidermoid carcinoma) than 1a [18]. These findings might be explained by the additional hydroxyl group at position C-9 in the molecule structure of 2a and 2b. Furthermore, the coloratane-type skeleton of 2b seems to have a stronger impact on human cells than the drimane-type 2a and 1a. For further improvements, the coloratane skeleton might be the ideal target for structural modifications, such as oxo-functionalization of the exocyclic methylene group.

**Effect on cell morphology**

In the present study, swelling of hematopoietic cells, detachment, and rounding of adherent cells as well as partial or complete leakage of the cell content were observed when treated with the respective IC₅₀ of the compounds (Fig. 4) indicating an increased cell permeability. This was also observed on 1a and 2a treated human cancer cells in previous studies [29,30]. It is already known, that 1a acts as detergent on yeast cell membrane causing an increased permeability and leakage of cell components [31]. These observations provide another crucial hint that the cell membrane is the target site for the mode of action not only of 1a but also of 2a and 2b.

**Conclusion**

Due to the high similarity of healthy/non-cancerous and malignant cells, the main challenge of cancer treatment is the distinction between these cells to minimize side effects. To highlight the great potential of the compounds 1a, 2a, and 2b as selective anti-cancer drugs, we developed a model, which takes already known modes of action of the compounds, especially of 1a, into account and explains why specific and relevant cancer cells and monocytes are more susceptible than healthy cells and lymphocytes, respectively (Fig. 5). As already mentioned, 1a is known to target the cell membrane by acting as detergent [31], but the cell membrane is a non-specific rather than a specific target since it is present in every cell. However, different membrane fatty acid profiles were reported between healthy and malignant cells as well as between lymphocytes and monocytes [32]. For instance, primary monocytes were shown to have the highest content of mono- and polyunsaturated fatty acids compared to T and B lymphocytes, and also cancer cell lines were reported to exhibit a higher amount of monounsaturated fatty acids than their healthy primary counterparts [32]. It is conceivable that a specific membrane fatty acid profile present in monocytes and some cancer cells could lead to a stronger interaction with the compounds followed by membrane rupture and induction of necrosis. Polygodial is furthermore known to generate reactive oxygen species (ROS) [33] as

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**Table 2**

Half-maximum inhibition concentration (IC₅₀) of the compounds 1a, 2a, and 2b against human peripheral blood mononuclear cells (PBMC) and human blood cancer cell lines MV4-11 and THP-1 as well as selectivity index (SI) related to PBMC.

<table>
<thead>
<tr>
<th>Compound</th>
<th>PBMC IC₅₀ (µg/mL)</th>
<th>MV4-11 IC₅₀ (µg/mL)</th>
<th>SI</th>
<th>THP-1 IC₅₀ (µg/mL)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>2.55</td>
<td>10.88</td>
<td></td>
<td>1.62</td>
<td>6.91</td>
</tr>
<tr>
<td>2a</td>
<td>1.06</td>
<td>4.23</td>
<td></td>
<td>0.80</td>
<td>3.20</td>
</tr>
<tr>
<td>2b</td>
<td>0.71</td>
<td>2.86</td>
<td></td>
<td>0.65</td>
<td>2.62</td>
</tr>
</tbody>
</table>
sigmoidal curves of the cell viability allowing the determination of the respective half-maximum inhibitory concentration (IC$_{50}$) (ns: not statistically significant with p > 0.05; * p < 0.01; ** p < 0.001; *** p < 0.0001 (unpaired t-test with/without Welch’s correction)). B) Dose-response curves of 1a, 2a, and 2b against GF, Sk-Mel29, and LN-229. Cell viabilities were normalized to viability of untreated cells. Non-linear regression was used to display sigmoidal curves of the cell viability allowing the determination of the respective half-maximum inhibitory concentration (IC$_{50}$). The experiments were performed in triplicates including three biological replicates with three technical replicates each, and the data are expressed as arithmetic mean ± standard error of the mean indicated by error bars.

Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ GF (µM)</th>
<th>IC$_{50}$ Sk-Mel29 (µM)</th>
<th>SI Sk-Mel29/GF</th>
<th>IC$_{50}$ LN-229 (µM)</th>
<th>SI LN-229/GF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>&gt; 100$^a$</td>
<td>&gt; 426.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>41.41</td>
<td>165.42</td>
<td></td>
<td>1.25 4.99</td>
<td>33.13</td>
</tr>
<tr>
<td>2b</td>
<td>36.53</td>
<td>147.11</td>
<td></td>
<td>0.61 2.46</td>
<td>59.89</td>
</tr>
</tbody>
</table>

[a] no inhibitory effect up to 100 µg/mL (426.75 µM).
further chemical modification in order to generate therapeutics with improved properties.

**Experimental section**

**General experimental procedures**

The isolation process is described in detail in Supporting Information (Fig. S2). GC–MS spectra of the compounds dissolved in dichloromethane (VWR International GmbH, Darmstadt, Germany) were recorded on Agilent 6890 N spectrometer combined with Agilent 5975B VL MSD detector. ESI-MS spectra were recorded in an ESI positive mode on the Bruker micrOTOF™ mass spectrometer in deuterated chloroform (CDCl₃; VWR International GmbH, Darmstadt, Germany). Proton (¹H) NMR and carbon (¹³C) NMR spectra were recorded on Varian Mercury Plus spectrometers (100 MHz, 400 MHz) in CDCl₃. Chemical shifts were reported in ppm (δ). For an exact allocation of the signals, 2D spectra (¹H, ¹H correlation spectroscopy, heteronuclear...
multiple bond correlation) were involved in the structure analysis.

**Plant material**

The *W. ugandensis* stem bark material was collected from private plantations in Mutufu, Sironko District (Eastern Uganda) in August 2017 and exported in accordance with the International Plant Protection Convention 1951 and the Phytosanitary Convention for Africa 1971 (Licence/authority number: UQIS 0002931/93/PC (E); responsible person: Dr. Andreas Schubert, Fraunhofer IZI, Leipzig). The plants originated from the tree nursery of the National Forestry Resources Research Institute.

**In silico investigations**

Physicochemical and ADME properties were predicted and interpreted using the web tool SwissADME (available at [http://www.swissadme.ch/](http://www.swissadme.ch/)) as it is described in detail in [26].

**Cell lines**

PBMC were obtained by density gradient centrifugation of blood from healthy donors (collected by the Institute for Transfusion Medicine of University Clinic of Leipzig, Germany and approved by the Ethics Committee of the Faculty of Medicine of the University Clinic of Leipzig, Germany, as no ethical or scientific concerns were raised (file reference: Committee of the Faculty of Medicine of the University Clinic of Leipzig, Germany and approved by the Ethics Review Committee of the University Clinic of Leipzig, Germany) were cultivated in RPMI 1640 medium (Gibco®) supplemented with 10% FBS. THP-1 (DSMZ no.: ACC 102), PBMC, MV4-11 (DSMZ no.: ACC 102), THP-1 (DSMZ no.: ACC 16) and Sk-Mel29 (accession number: CVCL_6031; kindly provided by Prof. Dr. Barbara Seliger, University of Halle, Germany) were cultivated in RPMI 1640 medium + 10% FBS (both Gibco®, Thermo Fisher Scientific, Waltham, MA, USA). Human gingival fibroblasts (CLS 300703) were cultivated in DMEM/F12 (Gibco®) + 10% FBS. LN-229 (ATCC® CRL-2611; kindly provided by Prof. Dr. Frank Gauntz, University Clinic of Leipzig, Germany) were cultivated in DMEM (Gibco®) + 10% FBS.

**Resazurin-based cytotoxicity assay**

To measure cell viability, alamarBlue™ cell viability reagent (Thermo Fisher Scientific, Waltham, MA, USA) was used. In preliminary tests, the length of reagent incubation time and cell number were determined for each cell line following the recommended procedure of the manufacturer (data not shown). 90 µL of cell suspension containing the respective cell number were filled into wells of a black flat bottom 96 well microplate (FLUOTRAC™, Greiner Bio-One International GmbH, Kremsmünster, Austria). Tissue growing cell lines were preincubated allowing the cells to adhere. 10 µL of the test material’s solutions were added resulting in final concentrations between 0.01 µg/mL and 100 µg/mL, each with 0.5% DMSO. As positive control, 1% Triton X-100 (Merck KGaA, Darmstadt, Germany) with 0.5% DMSO was used. PBS (Gibco®) with 0.5% DMSO was used as growth control. The respective cell medium without cells acted as blank. The plates were incubated at 37 °C and 5% CO₂. Depending on the results of the preliminary test, after 4–20 h, 10 µL of the cell viability reagent were added. The plates were further incubated at 37 °C and 5% CO₂ until 24 h total incubation time was reached. The emitted fluorescence was measured at 590 nm with an excitation wavelength of 530 nm using the Tecan Infinite® X-100 microplate reader (Tecan Trading AG, Männedorf, Switzerland). The fluorescence intensity correlated directly to the number of viable cells in the culture. After subtracting the blank value from each value, the cell viability was normalized to untreated cells in the growth control.

**Flow cytometry**

PBMC distribution was analysed at BD FACSCanto™ II (Becton, Dickinson and Company, Franklin Lakes, NY, USA) after a 24 h incubation with the determined IC₅₀ of the test materials. The following antibodies were used: FITC mouse anti-human CD3 (clone UCHT1; BD Pharmingen™), APC mouse anti-human CD19 (clone HIB19; BD Pharmingen™), PerCP mouse anti-human CD14 (clone MoP9; BD Biosciences) and PE-Cy7 mouse anti-human CD56 (clone NCAM16.2; BD Biosciences).

**Statistics**

Graph Pad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA) was used for all statistical analyses and the generation of the dose–response curves. The F test was performed to compare the variances of the data sets. For non-significantly different variances (p > 0.2), an unpaired t-test was performed, while an unpaired t-test with Welch’s correction was used, if significantly different variances (p < 0.2) were determined. Probabilities of p < 0.05 were considered as statistically significant and are indicated by asterisks (* p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001). To determine IC₅₀ values, nonlinear regression of the dose–response curves was performed using the equation log (inhibitor) vs. response (normalized data) with variable slope (four parameters).

**Author contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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**Declaration of Competing Interest**

Andreas Schubert has patent #WO201113834A1 issued to Fraunhofer Gesellschaft zur Förderung der Angewandten Forschung eV. Andreas Schubert declares that he is involved in a company that cultivates *Warburgia ugandensis* for human and animal healthcare applications.

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**Appendix A. Supplementary data**

Overview of *W. ugandensis* compounds; detailed description of the isolation process; NMR and chromatographic data; *in silico* analysis; PBMC distribution after compound treatment (PDF)

Supplementary data to this article can be found online at [https://doi.org/10.1016/j.rechim.2021.100242](https://doi.org/10.1016/j.rechim.2021.100242).

**References**


