

Antiplasmodial hirsutinolides from *Vernonia staehelinoides* and their utilization towards a simplified pharmacophore

Pamisha Pillay ^{a,*}, Robert Vlegaar ^b, Vinesh J. Maharaj ^a, Peter J. Smith ^c,
Carmen A. Lategan ^c, Frank Chouteau ^d, Kelly Chibale ^{d,e}

^a Biosciences, CSIR, RSA, P.O. Box 395, Pretoria 0001, South Africa

^b Department of Chemistry, University of Pretoria, Pretoria 0002, South Africa

^c Division of Pharmacology, University of Cape Town, Observatory, 7925, South Africa

^d Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa

^e Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa

Received 21 December 2006; received in revised form 14 February 2007

Abstract

The dichloromethane extract of the leaves of *Vernonia staehelinoides* Harv. (Asteraceae) showed *in vitro* activity ($IC_{50} \sim 3 \mu\text{g/ml}$) against the chloroquine-sensitive (D10) and the chloroquine-resistant (K1) strains of *Plasmodium falciparum*. Through conventional chromatographic techniques and bioassay-guided fractionation two structurally-related hirsutinolides displaying *in vitro* antiplasmodial activity ($IC_{50} \sim 0.2 \mu\text{g/ml}$ against D10) were isolated and identified by spectroscopic data. Compounds **1**, 8 α -(2-methylacryloyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-acetate, and **2**, 8 α -(5'-acetoxyseneciyoxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-acetate were found to be cytotoxic to mammalian Chinese Hamster Ovarian (CHO) cells at similar concentrations but proved to be attractive scaffolds for structure–activity relationship studies. Two main privileged substructures, a 2(5*H*)-furanone unit and a dihydrofuran-4-one unit, were identified as potential pharmacophores which may be responsible for the observed biological activity. Mucochloric and mucobromic acids were selected as appropriate 2(5*H*)-furanone substructures and these were shown to have comparable activity against the D10 and superior activity against the K1 strains relative to the hirsutinolide natural product. Mucochloric and mucobromic acids also show selective cytotoxicity to the malaria parasites compared to mammalian (CHO) cells *in vitro*. The antiplasmodial data obtained in respect of these two acids suggests that the 2(5*H*)-furanone substructure is a key pharmacophore in the observed antiplasmodial activity.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: *Vernonia staehelinoides*; *Plasmodium falciparum*; Hirsutinolides; Antiplasmodial activity; Cytotoxicity; Pharmacophores; 2(5*H*)-furanone; Mucochloric and mucobromic acids

1. Introduction

Malaria is recognised as one of the leading public health problems in sub-Saharan Africa. The evolution and spread of drug-resistant forms of the causative agent *Plasmodium falciparum* and the limited number of chemotherapeutic agents available to treat and control the disease has highlighted the need for new chemically diverse, effective drugs.

Historically, plants have provided a major source of anti-malarial agents and novel template compounds (Schwikkard and van Heerden, 2002).

Vernonia staehelinoides Harv. (Asteraceae), was identified as a potential source of new antimalarial drugs through a national multidisciplinary-consortium project aimed at scientifically validating South African medicinal plants for the treatment of malaria (Clarkson et al., 2004). *Vernonia staehelinoides* is indigenous to South Africa and is commonly known as “blouteebossie”. It is a multi-stemmed perennial shrublet with stems and leaves covered with

* Corresponding author. Tel.: +27 12841 2215; fax: +27 12841 4790.
E-mail address: ppillay@csir.co.za (P. Pillay).

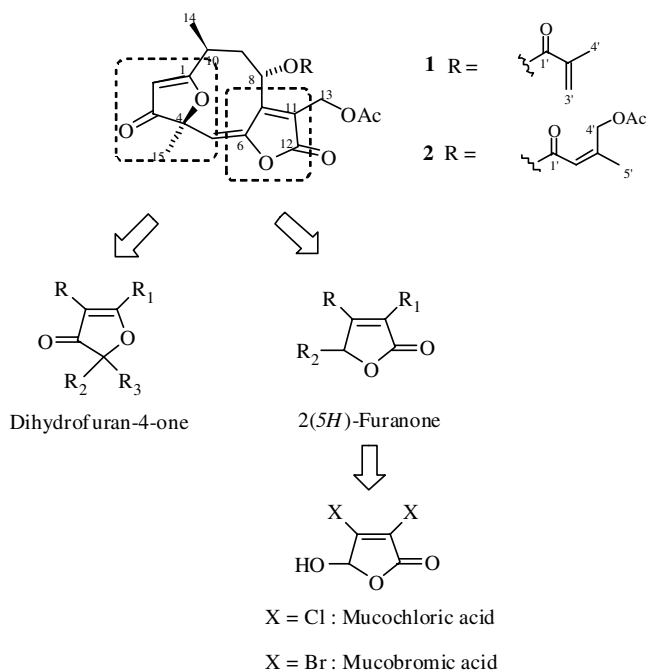


Fig. 1. Structures of hirsutinolides **1** and **2**, and the identified pharmacophores.

numerous short hairs, giving it a greyish appearance. Leaves are very narrow and long flexuous stems give rise to numerous flower heads.

V. staehelinoides is reported to be used medicinally but no details have been specified (Watt and Breyer-Brandwyk, 1962). Previous phytochemical investigations of this plant revealed that the roots contain squalene, stigmasterol and sitosterol while the aerial parts afforded caryophyllene, α -humulene, germacrene D and the novel glaucolide, 3 β -hydroxy-stilpnomentolide-8-*O*-(5-acetoxysenecioate) (Bohlmann et al., 1982).

The *in vitro* antiplasmodial activity of the dichloromethane extract of this plant warranted further investigation to identify the biologically active components. Bioassay guided fractionation identified hirsutinolides **1**, 8 α -(2-methylacryloyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-acetate (Bohlmann et al., 1983), and **2**, 8 α -(5'-acetoxyseneciolyoxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-acetate (Bohlmann et al., 1983; Tully et al., 1987), (Fig. 1) as the active components. This paper reports on the isolation and biological activity of these compounds and their utilization towards identifying a novel simplified pharmacophore.

2. Results and discussion

2.1. Identification of the active ingredients

Dried, ground leaves of *Vernonia staehelinoides* were extracted sequentially with dichloromethane, dichloromethane/methanol (1:1) and water. The extracts were bio-

assayed *in vitro* against the chloroquine-sensitive D10 and the chloroquine-resistant K1 strains of *Plasmodium falciparum*. The dichloromethane and dichloromethane/methanol extracts were found to be active with IC₅₀ values of 3.0 and 6.0 μ g/ml against D10 and 2.8 and 4.5 against K1; respectively. The aqueous extract was relatively inactive (IC₅₀ > 10 μ g/ml).

The dichloromethane extract was subjected to bioassay-guided fractionation using silica gel column chromatography and antiplasmodial activity against the D10 strain as the biological indicator. This resulted in the isolation and identification of hirsutinolides **1** and **2** as the active ingredients. Bioassay-guided fractionation of the 1:1 dichloromethane/methanol extract led to the same active ingredients.

The structures of compounds **1** and **2** were elucidated using spectroscopic techniques. The two ketohirsutinolides differed only in the ester groups at the C-8 position, with compound **1** containing a 2-methylpropenoyl moiety and compound **2** having a 4-acetoxy-3-methyl-2*Z*-butenoyl group. The *Z* configuration of the 2' double bond in hirsutinolide **2** followed from the strong NOE observed between H-2' and H-5'. This was confirmed by comparison of the H-4' chemical shift (δ_H 5.17) (Table 1) with published data for methyl *Z*- and *E*-4-acetoxy-3-methyl-2-butenates: the *Z*-CH₂ signals were shown to occur at δ_H 5.15, whereas the *E*-CH₂ signals were observed at δ_H 4.56 (Tully et al., 1987). The structures of the compounds were confirmed by comparison with ¹H NMR data reported in the literature (Bohlmann et al., 1983). This comparison did reveal that the original reporters had transposed the H-13 and H-4' resonances of compound **2**. In this account an unequivocal assignment of these resonances based on 2D NMR data, which the original reporters did not have access to, is reported (Table 1). The ¹³C data of compounds **1** and **2** are also reported in Table 1.

It has been suggested that hirsutinolides are not natural products but are rather artefacts formed from glaucolides on exposure to slightly acidic silica gel during chromatography (Borkosky et al., 1997; Tully et al., 1987). However, compounds **1** and **2** were easily detected on TLC through their characteristic colours (orange and brown, respectively, with vanillin) and *R_f* values (0.29 and 0.23, respectively with acetone–hexane–dichloromethane 1:4.5:4.5) prior to any silica gel chromatography suggesting that these may not be artefacts; unless the acid-catalyzed rearrangement could have been effected during the TLC analysis, itself.

Although compounds **1** and **2** have been isolated previously from other South African *Vernonia* species (Bohlmann et al., 1983; Tully et al., 1987) this is the first report on the isolation of these compounds from *V. staehelinoides*.

2.2. Biological activity

There are no previous reports of compounds **1** and **2** being investigated for any biological activity. In this account the compounds are demonstrated to have significant

Table 1
¹H and ¹³C NMR data (399.96 MHz, in CDCl₃) for hirsutinolides **1** and **2**

Position	1		2	
	δ _C	δ _H (mult., <i>J</i> in Hz)	δ _C	δ _H (mult., <i>J</i> in Hz)
1	194.9	—	194.9	—
2	98.3	5.40 (<i>s</i>)	99.9	5.43 (<i>s</i>)
3	202.3	—	202.2	—
4	86.9	—	86.9	—
5	117.9	5.89 (<i>s</i>)	117.4	5.88 (<i>s</i>)
6	146.2	—	146.2	—
7	151.1	—	151.2	—
8	65.7	6.45 (<i>br d</i> , 9.0)	65.6	6.23 (<i>br d</i> , 8.0)
9a	40.2	2.77 (<i>ddd</i> , 5.1, 9.0, 14.9)	40.1	2.75 (<i>ddd</i> , 5.5, 8.0, 14.7)
9b	—	1.70 (<i>dd</i> , 11.0, 14.9)	—	1.70 (<i>dd</i> , 9.0, 7.0)
10	31.3	3.01 (<i>ddq</i> , 5.1, 11.0, 7.0)	31.9	3.01 (<i>ddq</i> , 5.5, 9.0, 7.0)
11	132.7	—	131.8	—
12	166.1	—	166.2	—
13a	55.7	4.91 (<i>d</i> , 13.2)	55.8	4.93 (<i>d</i> , 13.1)
13b	—	5.09 (<i>d</i> , 13.2)	—	5.02 (<i>d</i> , 13.1)
14	15.5	1.26 (<i>d</i> , 7.0)	15.4	1.25 (<i>d</i> , 7.0)
15	20.7	1.58 (<i>s</i>)	20.6	1.59 (<i>s</i>)
1'	166.9	—	164.0	—
2'	135.7	—	116.5	5.75 (<i>br m</i> ^a)
3'a	127.0	5.63 (<i>dq</i> , 1.6, 1.6)	156.8	—
3'b	—	6.22 (<i>br dq</i> , 1.6, 1.0)	—	—
4'a	18.1	1.93 (<i>dd</i> , 1.6, 1.0)	63.6	5.12 (<i>br dm</i> ^a , 15.5)
4'b	—	—	—	5.22 (<i>br dm</i> ^a , 15.5)
5'	—	—	21.5	1.91 (<i>br m</i> ^a)
13-O-COCH ₃	170.2	—	170.2	—
13-O-COCH ₃	20.7	2.03 (<i>s</i>)	20.7	2.03 (<i>s</i>)
4'-O-COCH ₃	—	—	170.6	—
4'-O-COCH ₃	—	—	20.7	2.05 (<i>s</i>)

^a Allylic coupling of ~1 Hz.

in vitro antiplasmodial activity against the D10 strain of *P. falciparum*. The difference in their C-8 ester groups does not appear to significantly influence the observed antiplasmodial activities of compounds **1** and **2** as they were equipotent against the chloroquine-sensitive (D10) strain. There was a marginal difference in their observed activity against the chloroquine-resistant strain (Table 2).

In order to determine the specificity of the antiplasmodial activity of the hirsutinolides, it was considered necessary to obtain information about their general cytotoxicity. For this, the compounds were tested for cytotoxicity against a Chinese Hamster Ovarian (CHO) cell line, using the microculture tetrazolium (MTT) assay and Emetine as a positive control (Table 2).

The selectivity index (SI) value for compound **1** (Table 2) indicates that it is approximately 11 times more potent to the parasites than to mammalian cells. For compound **2** this ratio is of the order of 4, indicating less selectivity. In considering a recent publication (Pink et al., 2005) outlining criteria for antiparasitic drug discovery, a compound can be considered a hit if it is:

Table 2

In vitro antiplasmodial activity, cytotoxicity and selectivity index (SI) values for chloroquine, emetine, hirsutinolides **1** and **2**, and mucochloric and mucobromic acids

Compound	D10, IC ₅₀ (ng/ml)	K1, IC ₅₀ (ng/ml)	CHO, IC ₅₀ (μg/ml)	SI ^a
Chloroquine	12	182	18.5	1542
1	260	1800	2.9	11
2	240	2600	0.9	4
Mucochloric acid	152	137	4.8	32
Mucobromic acid	422	359	6.3	15
Emetine	—	—	0.06	—

^a SI, cytotoxicity CHO IC₅₀/antiplasmodial D₁₀ IC₅₀.

- Active *in vitro* against whole protozoa with an IC₅₀ of ≤1 μg/ml.
- Selective (at least 10-fold more active against the parasite than against a mammalian cell line).

Based on these criteria, only compound **1** can be considered a hit while compound **2** was not selective enough to kill the parasites without damaging mammalian cells.

2.3. Pharmacophore identification

Overall, the active components of *V. staehelinoides* may not be considered as viable antimalarial drug leads as their activity does not compare to that of standard clinically used drugs such as chloroquine. Moreover, compound **2** is non-selective while compound **1** shows only limited selectivity. The hirsutinolides do, however, provide attractive new scaffolds for chemical modification with the view of exploring structure-activity relationships. This could be achieved *via* one or more of the following three approaches namely;

- modification of core structures, depending on the availability of appropriate quantities of the hirsutinolides from natural sources;
- total synthesis from readily available and/or accessible starting materials. A synthesis and/or semi-synthesis plan amenable to chemical library generation could then be planned and subsequently implemented;
- synthesis of derivatives around the basic structure of privileged sub-structures identified from the hirsutinolides.

Of the three aforementioned approaches, the last approach was considered to be the most attractive within the context of the hirsutinolides. These natural products have two main privileged substructures which may be the pharmacophores responsible for the observed antiplasmodial activity of the hirsutinolides: a 2(5*H*)-furanone unit and a dihydrofuran-4-one unit as shown in Fig. 1. These substructures are known to possess varying biological activities. The 2(5*H*)-furanone moiety occurs in many

natural products that exhibit biological activities ranging from antibiotic (Miao and Andersen, 1991; Davidson and Ireland, 1990), cytotoxic (Ortega et al., 2000; Bohlman et al., 1982) and antitumor properties (Hung et al., 1990) to inhibition of cholesterol biosynthesis (Kuhnt et al., 1990). On the other hand the dihydrofuran-4-one moiety is known for its antitumoral activity (Smith et al., 1981; Kupchan et al., 1976; LeQuesne et al., 1978).

In order to verify the aforementioned pharmacophore hypothesis, appropriate privileged furanone substructures were identified and evaluated for antiplasmodial activity. Acifran, which contains a dihydrofuran-4-one unit, was found to have no antiplasmodial activity at a concentration of 1.0 µg/ml disputing the possibility that the dihydrofuranone moiety is a key pharmacophore in the observed antiplasmodial activity of the hirsutinolides.

Mucochloric and mucobromic acids were initially identified as appropriate privileged 2(5*H*)-furanone substructures (Fig. 1). Relative to the “lead” hirsutinolide natural products, compounds **1** and **2**, these significantly simplified structures (mucochloric and mucobromic acids) show comparable activity against D10 and superior activity against K1 strains of *P. falciparum* (Table 2). Furthermore, mucochloric and mucobromic acids are equally active in D10 and K1 strains of *P. falciparum*. This equipotency suggests that chloroquine cross-resistance is not observed with mucochloric and mucobromic acids. For such significantly simplified structures compared to the rather complex hirsutinolide natural product, the *in vitro* data are noteworthy. Besides, they show selective cytotoxicity to the malaria parasites compared to mammalian cells (CHO) *in vitro* with selectivity indices of 32 and 15, respectively (Table 2).

3. Concluding remarks

The identification of antiplasmodial hirsutinolides from *V. staehelinoides* suggests that they may play a role in the medicinal properties of the plant, but their potential for the development of antimalarial drugs is limited due to inherent cytotoxicity and lack of selectivity. The results did however lead to the identification of potential pharmacophores, a 2(5*H*)-furanone unit and a dihydrofuran-4-one unit. The antiplasmodial data obtained in respect of mucochloric and mucobromic suggests that the 2(5*H*)-furanone is at least one of the key pharmacophores responsible for the observed antiplasmodial activity.

4. Experimental

4.1. General experimental procedures

Mass spectra were recorded on a VG 70SEQ HRMS instrument, in the positive EI mode with a resolution of 7500 at 8 kV. Nuclear magnetic resonance spectra were

recorded on a Varian 400 MHz Unity spectrophotometer in [²H]-chloroform with TMS as internal standard. Optical rotations were measured in chloroform at room temperature on a Perkin-Elmer 241 polarimeter at 589 nm (Na D-line) using a 1 dm cell.

The fractionation process involved column and thin layer chromatographic techniques. Different sized columns, ranging from 1.5 to 6 cm in diameter, were used depending on the amount of sample and the purification stage. Silica gel column chromatography was conducted using silica gel 60 (0.063–0.2 mm) and flash silica gel chromatography was carried out using 35–75 µm flash silica gel (Merck Art). Solvents used in chromatography were of technical grade and were distilled before use. TLC analysis was conducted on 0.20 mm pre-coated (SIL-25 UV254) glass-backed plates using acetone–hexane–dichloromethane (1:4.5:4.5). The plates were developed using a vanillin-concentrated H₂SO₄ (1 g/100 ml) spray reagent and then heated.

Mucobromic acid (racemic) and mucochloric acid (racemic) were purchased from Sigma–Aldrich and used as received.

4.2. Plant material

Vernonia staehelinoides Harv. plant material was collected in April 1999 in the Magaliesburg region of the Gauteng province in South Africa. Voucher specimen (EN00323) containing flowers and aerial parts of the plants were identified and retained at the National Herbarium (Pretoria). Plant material was dried in an oven at 45 °C for 48 h and stored at room temperature until processing.

4.3. Extraction and isolation

Dried plant material (120 g) was finely ground and extracted with dichloromethane (1 l) at room temperature for 4 h with occasional stirring. The solvent was evaporated under reduced pressure to yield over 4 g (3.3%) of crude extract of which 1.5 g was subjected to open column chromatography over silica gel using a gradient eluent of increasing polarity (ethyl acetate–hexane 1:9 → ethyl acetate–hexane 3:2). Fractions were pooled according to their behaviour (*R_f* values) on TLC. Six fractions in total were subsequently bio-assayed against *P. falciparum* D10. Three of these showed improved antiplasmodial activity (D10 IC₅₀ values 1.6, 1.0 and 0.9 µg/ml) relative to the crude extract (D10 IC₅₀ 3.0 µg/ml).

Using *in vitro* antiplasmodial activity against the D10 *P. falciparum* strain as the biological indicator, the active fractions were subjected to repeated flash chromatography and column chromatography to identify the active compounds. The bioassay-guided fractionation process yielded compounds **1** (Bohlmann et al., 1983) and **2** (Bohlmann et al., 1983; Tully et al., 1987). The compounds were identified using spectral data such as ¹H, ¹³C, DEPT, HSQC, HMBC, COSY and NOESY NMR spectra, as well as

EI-MS spectra, and by direct comparison with published spectral data and structures (Bohlmann et al., 1983).

4.3.1. 8α -(2-Methylacryloyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-O-acetate (**1**)

Colourless gum; $[\alpha]_D -77.4$ (c 0.31, CHCl_3); ^1H and ^{13}C NMR (CDCl_3) data in Table 1; EI-MS (positive-ion mode) m/z : 316.0868 $[\text{M}-\text{C}_3\text{H}_5\text{COOH}]^+$ (calcd for $\text{C}_{17}\text{H}_{16}\text{O}_6$: 316.0947).

4.3.2. 8α -(5'-Acetoxyseneciolyoxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-O-acetate (**2**)

Colourless gum; $[\alpha]_D -209.8$ (c 0.51, CHCl_3); ^1H and ^{13}C NMR (CDCl_3) data in Table 1; EI-MS (positive-ion mode) m/z : 316.0885 $[\text{M}-\text{C}_7\text{H}_{10}\text{O}_4]^+$ (calcd for $\text{C}_{17}\text{H}_{16}\text{O}_6$, 316.0947).

4.4. Biological activity

4.4.1. Antiplasmodial assaying

Parasite viability was measured using parasite lactate dehydrogenase (pLDH) activity according to the methods described by Makler et al. (1995) and Clarkson et al. (2003). The chloroquine-sensitive (D10) and chloroquine-resistant (K1) strains of *Plasmodium falciparum* were continuously cultured according to the methods described by Trager and Jensen (1976). Fractions and pure compounds were dissolved in 10% methanol and were further diluted in complete medium on the day of the experiment. The starting concentration for a full dose–response was 100 $\mu\text{g}/\text{ml}$, which was diluted 2-fold in complete medium to give 10 concentrations, with the lowest concentration being 0.195 $\mu\text{g}/\text{ml}$. The IC_{50} values were obtained from the dose response curves, using non-linear dose–response curve fitting analyses with GraphPad Prism v. 4.00 software.

4.4.2. Cytotoxicity assaying

Compounds were tested for *in vitro* cytotoxicity against a Chinese Hamster Ovarian (CHO) cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). Samples were dissolved in methanol:water (1:9). Stock solutions (2 mg/ml) were prepared and were stored at -20°C until use. The highest concentration of methanol to which the cells were exposed to had no measurable effect on the cell viability. Emetine was used as the positive control in all cases. The initial concentration of emetine was 100 $\mu\text{g}/\text{ml}$, which was serially diluted in complete medium with 10-fold dilutions to give six concentrations, the lowest being 0.001 $\mu\text{g}/\text{ml}$. The same dilution technique was applied to all test samples with an initial concentration of 100 $\mu\text{g}/\text{ml}$ to give five concentrations, with the lowest concentration being 0.01 $\mu\text{g}/\text{ml}$. The concentration of test samples that inhibits 50% of the cells (IC_{50} values) were obtained from dose–response curves, using a non-linear dose–response curve fitting analyses via GraphPad Prism v. 2.01 software.

Acknowledgements

This work was financially supported by the Department of Science and Technology which awarded an innovation fund to five South African institutions (The Medical Research Council, South African National Biodiversity Institute, Council for Scientific and Industrial Research, University of Cape Town and University of Pretoria) to scientifically validate South African medicinal plants for the treatment of malaria.

References

- Bohlmann, F., Ates (Gören), N., Jakupovic, J., 1983. Hirsutinolides from South African *Vernonia* species. *Phytochemistry* 22, 1159–1162.
- Bohlmann, F., Wallmeyer, M., Jakupovic, J., 1982a. Glaucolide from *Vernonia staehelinoides*. *Phytochemistry* 21, 1445–1447.
- Bohlmann, F., Zdero, C., King, R.M., Robinson, H., 1982b. Hirsutinolides and other sesquiterpene lactones from *Vernonia* species. *Phytochemistry* 21, 695–699.
- Borkosky, S., Bardón, A., Catalán, C.A.N., Díaz, J.G., Herz, W., 1997. Glaucolides, hirsutinolides and other sesquiterpene lactones from *Vernonia pinguis*. *Phytochemistry* 44, 465–470.
- Clarkson, C., Campbell, W.E., Smith, P., 2003. In vitro antiplasmodial activity of abietane and totarane diterpenes isolated from *Harpagophytum procumbens* (Devil's Claw). *Planta Med.* 8, 720–724.
- Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O.M., Pillay, P., Matsabisa, M.G., Bhagwandhin, N., Smith, P.J., Folb, P.I., 2004. In vitro antiplasmodial activity of medicinal plants native to or naturalized in South Africa. *J. Ethnopharmacol.* 92, 177–191.
- Davidson, B.S., Ireland, C.M., 1990. Lissoclinolide A polyketide lactone from *Lissoclinum patella*. *J. Nat. Prod.* 53, 1036–1038.
- Hung, J.H., Pummangura, S., Chaichantipyuth, S., Patarapanich, C., Fanwick, P.E., Chang, C.J., McLaughlin, J.L., 1990. New bioactive heptenes from *Melodorum fruticosum* (Annonaceae). *Tetrahedron* 46, 5043–5054.
- Kuhnt, D., Anke, T., Besl, H., Bross, M., Herrmann, R., Mocek, U., Steffan, B., Steglich, W., 1990. Antibiotics from basidiomycetes. XXXVII. New inhibitors of cholesterol biosynthesis from cultures of *Xerula melanotricha* Dorfeld. *J. Antibiot.* 43, 1413–1420.
- Kupchan, S.M., Sigel, C.M., Matz, M.J., Gilmore, C.J., Bryan, R.F., 1976. Structure and stereochemistry of jatrophone, a novel macrocyclic diterpenoid tumor inhibitor 132. *J. Am. Chem. Soc.* 98, 2295–2300.
- LeQuesne, P.W., Lavery, S.B., Menachery, M.D., Brennan, T.F., Raffauf, R.F., 1978. Antitumor plants. Part 6. Novel modified germacranolides and other constituents of *Eremanthus elaeagnus* Schultz-Bip (*Compositae*). *J. Chem. Soc. Perkin Trans. 1*, 1572.
- Makler, M.T., Ries, J.M., Williams, J.A., Bancroft, J.E., Piper, R.C., Gibbons, B.L., Hinrichs, R.C., 1995. Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. *Am. J. Trop. Med. Hyg.* 48, 739–741.
- Miao, S., Andersen, R.J., 1991. Rubrolides A–H, metabolites of the colonial tunicate *Riterella rubra*. *J. Org. Chem.* 56, 6275–6280.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65, 55–63.
- Ortega, J.J., Zubia, E., Ocana, J.M., Naranjo, S., Salva, J., 2000. New rubrolides from the ascidian *Synoicum biochmanni*. *Tetrahedron* 56, 3963–3967.

- Pink, R., Hudson, A., Mouriès, M., Bendig, M., 2005. Opportunities and challenges in antiparasitic drug discovery. *Nat. Rev. Drug Discov.* 4, 727–740.
- Smith III, A.B., Levenberg, P.A., Jerri, P.L., Scarborough Jr., R.M., Wovkulich, P.M., 1981. Synthesis and reactions of simple 3(2*H*)-furanones. *J. Am. Chem. Soc.* 103, 1501–1513.
- Schwikkard, S., van Heerden, F.R., 2002. Antimalarial activity of plant metabolites. *Nat. Prod. Rep.* 19, 675–692.
- Trager, W., Jensen, J.B., 1976. Human malaria parasites in continuous culture. *Science* 193, 673–675.
- Tully, L.E., Carson, M.S., McMurray, T.B.H., 1987. A novel sesquiterpene lactone from *Vernonia erinacea*. *Tetrahedron Lett.* 28, 5925–5928.
- Watt, J.M., Breyer-Brandwyk, M.G., 1962. *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. Livingston, London, p. 254.