The Potential of Secondary Metabolites from Plants as Drugs or Leads Against Protozoan Neglected Diseases - Part II

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Abstract: Infections with protozoan parasites are a major cause of disease and mortality in many tropical countries of the world. Diseases caused by species of the genera *Trypanosoma* (Human African Trypanosomiasis and Chagas Disease) and *Leishmania* (various forms of Leishmaniasis) are among the seventeen "Neglected Tropical Diseases" (NTDs) defined by the WHO. Furthermore, malaria (caused by various *Plasmodium* species) can be considered a neglected disease in certain countries and with regard to availability and affordability of the antimalarials. Living organisms, especially plants, provide an innumerable number of molecules with potential for the treatment of many serious diseases. The current review attempts to give an overview on the potential of such plant-derived natural products as antiprotozoal leads and/or drugs in the fight against NTDs.

In part I, a general description of the diseases, the current state of therapy and need for new therapeuticals, assay methods and strategies applied in the search for new plant derived natural products against these diseases and an overview on natural products of terpenoid origin with antiprotozoal potential were given.

The present part II compiles the current knowledge on natural products with antiprotozoal activity that are derived from the shikimate pathway (lignans, coumarins, caffeic acid derivatives), quinones of various structural classes, compounds formed via the polyketide pathways (flavonoids and related compounds, chromenes and related benzopyrans and benzofurans, xanthones, acetogenins from Annonaceae and polyacetylenes) as well as the diverse classes of alkaloids.

In total, both parts compile the literature on almost 900 different plant-derived natural products and their activity data, taken from over 800 references. These data, as the result of enormous efforts of numerous research groups world-wide, illustrate that plant secondary metabolites represent an immensely rich source of chemical diversity with an extremely high potential to yield a wealth of lead structures towards new therapies for NTDs. Only a small percentage, however, of the roughly 200,000 plant species on earth have been studied chemically and only a small percentage of these plants or their constituents has been investigated for antiprotozoal activity. The repository of plant-derived natural products hence deserves to be investigated even more intensely than it has been up to present.

Keywords: Neglected tropical diseases, *Trypanosoma*, *Leishmania*, *Plasmodium*, natural product, lignan, coumarin, caffeic acid, flavonoid, chalcone, aurone, chromene, xanthone, acetogenin, polyacetylene, alkaloid.

1. INTRODUCTION

Neglected tropical diseases (NTDs) are a group of mostly life-threatening or disabling infections affecting more than a billion people world-wide. Most affected are poor populations in developing countries. People suffering from NTDs hence constitute an unattractive market to private-sector research and development (R&D) investment.

The infections caused by protozoan parasites, namely, human African trypanosomiasis (HAT) caused by *Trypanosoma brucei gambiense* and *T. b. rhodesiense*, Chagas disease caused by *Trypanosoma cruzi*, various forms of leishmaniasis caused by *Leishmania* spp., as well as malaria caused by various *Plasmodium* spp., are among the most devastating NTDs. The search for new drugs against these diseases is an urgent need and natural sources such as plants with their extremely diverse secondary metabolites may play an important role.

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A comprehensive introduction to these diseases, their current therapies, the necessity to develop new drugs, the potential of natural products as druglike lead structures in general and an overview of the particular potential of terpenoid plant secondary metabolites has been given in part I of this review [1].

The present part II continues with natural product classes other than terpenoids, namely, phenylpropanoids, polyketides and the large group of alkaloids.

Much of the literature has previously been reviewed in a number of instances. Most existent reviews, however, are dedicated to a particular disease or other aspect, whereas others are confined to few particularly interesting compounds [2-8]. The last comprehensive review on natural products against neglected diseases was published by Ioset in 2008 [7].

The present review attempts to summarize the literature not covered by this previous work as well as the more recent reports published since then. It will certainly not be possible to avoid that particularly important reports already cited in previous reviews are mentioned again; on the other hand, it is an impossible task to cover all literature. The authors therefore apologize to all who feel that their important contributions are missing.

The names of protozoan organisms mentioned throughout this review are abbreviated as follows: *Trypanosoma brucei; Tb; T. brucei gambiense; Tbg; T. brucei rhodesiense; Tbr; T. brucei brucei; Tbb; T. congolense; Tcon; T. cruzi; Tcr; Leishmania amazonensis; Lam; L. braziliensis; Lbra; L. enriettii; Lenr; L. donovani; Ldon; L. infantum; Linf (syn. L. chagasi; Lcha); L. major; Lmaj; L. mexicana; Lmex; L. panamensis; Lpan; L. peruviana; Lper; L. tropica; Ltro; Plasmodium falciparum; Pfc; P. berghei; Pber; P. vinckei petteri; Pvin; P. yoelii; Pyoe*.

2. PHENYLPROPANOIDS AND OTHER METABOLITES OF THE SHIKIMATE PATHWAY

2.1. Lignans and Neolignans

Lignans are dimeric phenylpropanoids formed by C-C linkage between the β-carbon atoms of both propanoid side chains. Most lignans are synthesized via two main pathways, namely, by dimerization of monolignols such as coniferyl alcohol or by dimerization of allylphenols/propenylphenols. Dimeric phenylpropanoids with other modes of C-C linkage are termed neolignans [9].

Especially the monolignol-based compounds are extremely widespread in higher plants and currently over 2500 different lignan and neolignan structures are known [10]. Many lignans are well known to possess cytotoxic/cytostatic, antimicrobial and other activities. An excellent collection of reviews on these issues can be found in [11]. A great number of lignans have also been tested for antiprotozoal activity. The structures of monolignol-based lignans (**1**-**15**) are depicted in Fig. (**1A**), those of propenylphenolic origin (**16**-**41**) in Fig. (**1B**) and those of neolignans and some related compounds (**42**-**53**) in Fig. (**1C**).

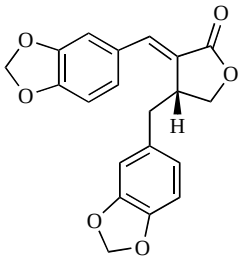
Dibenzylbutyrolactone lignans (Fig. **1A**) isolated from *Zanthoxylum naranjillo* (Rutaceae) were tested *in vitro* against *Tcr* strains, revealing partial activity for (-)-hibalactone (**1**), (-)-kaerophyllin (**2**) and (-)-cubebin (**3**) at concentrations of 25 and 50 μg/mL. The isolated lignan (-)-methylpluviatolide (**4**) was highly effective *in vitro* (88-99% inhibition at 25 μg/mL) and *in vivo*, inhibiting the development of the disease in healthy animals and also exhibiting activity against bloodstream forms of *Tcr* strains. However, it was not active against amastigote forms [12]. Studies with five partially synthesized (-)-cubebin derivatives displayed promising *in vitro* activities on free amastigote forms for (-)-hinokinin (**5**) (IC_{50} = 0.7 μM) [13]. Intracellular amastigotes of *Tcr* incubated with **5** presented an IC_{50} value of 18.36 μM and epimastigote forms an IC_{50} at 0.67 μM. *In vivo* assays with compound **5** on mice treated with 40 mg/kg/day also showed significant reduction of parasitemia [14]. More recently, the lignans (-)-cubebin (**3**) and (-)-hinokinin (**5**) were evaluated for their activity in oral treatment of mice chronically infected with *Tcr* in which **3** was more effective than **5**. Treatment with either compound led to a larger reduction in tissue parasitism of all evaluated organs than with the positive control benznidazole [15]. Trypanocidal structure-activity relationship studies for racemic *cis*- and *trans*-methylpluviatolide demonstrated better results for the *trans*-stereoisomer with an IC_{50} of 89.3 μM and further showed that only the respective (-)-enantiomer was active against trypomastigote forms of *Tcr* (IC_{50}≈18.7 μM) [16].

A recent study against *Tcr* trypomastigotes with the dibenzylbutyrolactone lignans hinokinin (**5**) and kusunokinin (**6**) as well as the furofuran lignans fargesin (**8**), epieudesmin (**9**) and sesamin (**10**) isolated from *Aristolochia cymbifera* (Aristolochiaceae), reported IC_{50} values of 94.5, 51.5, 89.9, 121.1 and 102.8 μM, respectively. When tested against intracellular *Tcr* amastigotes, the lignans exhibited IC_{50} values of >135, >129, >141, >141 and 17.0 μM, respectively [17]. Interestingly, IC_{50} values for hinokinin (**5**) reported in this study diverged significantly from those previously published [13, 14].

(+)-Veraguensin (**16**) and (-)-grandisin (**17**) are tetrahydrofuran type lignans (Fig. **1B**) which were isolated from *Virola surinamensis* (Myristicaceae) and exhibited trypanocidal activity against *Tcr*. Both compounds led to total trypomastigote lysis at a concentration of 5 μg/mL after 24h incubation [18]. Other tetrahydrofuran lignans were isolated from leaves of *Nectandra megapotamica* (Lauraceae) and tested against the Y strain of *Tcr*. An IC_{50} value of 2.2 μM and lysis of 94% of the parasites at 32 μM were reported for machilin G (**18**). Galgravin (**19**), nectandrin B (**20**), calopeptin (**21**), aristolignin (**22**) and ganschisandrine (**23**), in comparison, displayed lower activity with IC_{50} values of 4.4, 47.3, 12.6, 34.8 and 12.2 μM, respectively [19]. Five other tetrahydrofuran lignans isolated from *Peperomia blanda* (Piperaceae) also demonstrated *in vitro* trypanocidal activity when assayed against epimastigotes of *Tcr* strain Y. With an IC_{50} value of 9.6 μg/mL the lignan *rel*-(7*R*,8*S*,7'*S*,8'*S*)-4,5,4',5'-dimethylenedioxy-3,3'-dimethoxy-7,7'-epoxylignan (**24**) was shown to be the most active compound in this study. The other isolated compounds (**25**-**28**) exhibited IC_{50} values ranging between 12.6 and 25.4 μg/mL [20]. Three tetrahydrofuran lignans isolated from *Piper solmsianum* (Piperaceae) were furthermore found active against trypomastigote forms of *Tcr*. The most active lignan, *rel*-(7*R*,8*R*,7'*R*,8'*R*)-3,4,3',4'-dimethylenedioxy-5,5'-dimethoxy-7,7'-epoxylignan (**29**), presented an IC_{50} value of 3.47 μg/mL [21].

The furanolignans **33**, **34** and **36** were recently isolated from *Larrea tridentata* (Zygophyllaceae) [22] and tested for antiprotozoal activity together with two compounds, (+)-veraguensin (**16**) and tetrahydrofuroguaiacin B (**35**), previously isolated from *Illicium floridanum* (Illiciaceae) [23]. The compounds showed quite moderate activity against *Tbr* (bloodstream forms), *Tcr* (intracellular amastigotes) and *Ldon* (axenic amastigotes) with IC_{50} values in the range of 17.8 (**34**) to 188 (**16**) μM against *Tbr*, 162 (**34**) to 200 (**35**) μM against *Tcr* and 10.7 (**16**) to 102 (**36**) μM (*Ldon*). Against *Pfc*, (+)-veraguensin (**16**) was the most active compound of this group with an IC_{50} value of 8.3 μM followed by **34** with 12.0 μM [24].

Justicidin B (**13**), an arylnaphthalene lignan (Fig. **1A**) isolated from *Phyllanthus piscatorum* (Phyllanthaceae; formerly Euphorbiaceae), displayed strong activity against the trypomastigote forms of *Tbr* (IC_{50} = 0.2 μg/mL) and moderate activity against *Tcr* (IC_{50} = 2.6 μg/mL). Piscatorin (**14**), a C-9 hydroxylated derivative of **13** also isolated from *P. piscatorum*, in comparison exhibited milder activity against *Tbr* and *Tcr* with IC_{50}



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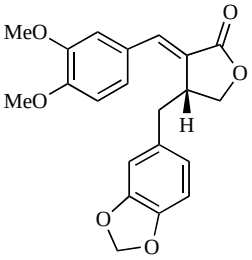

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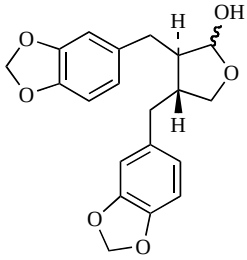

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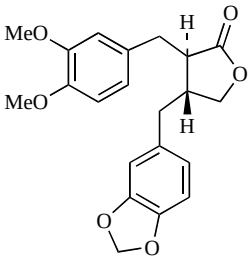

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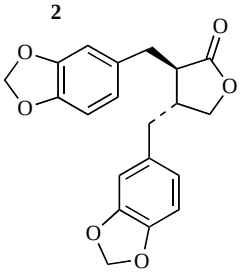

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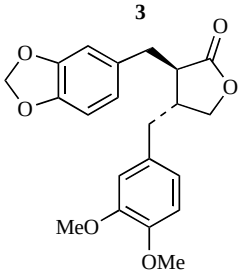

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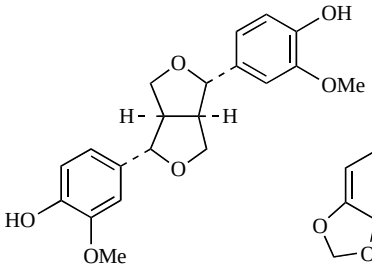

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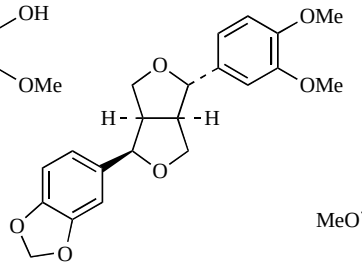

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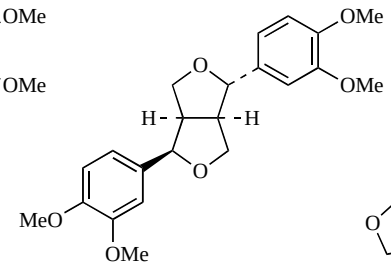

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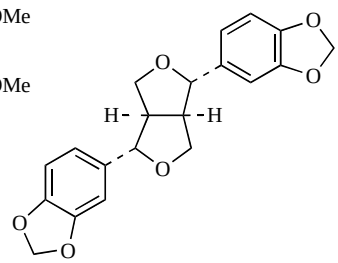

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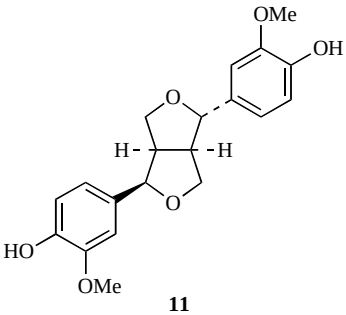

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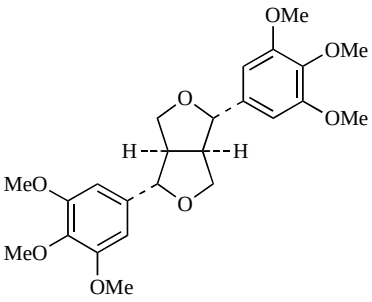

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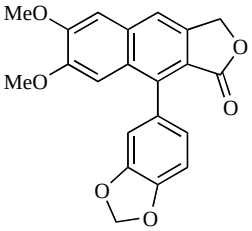

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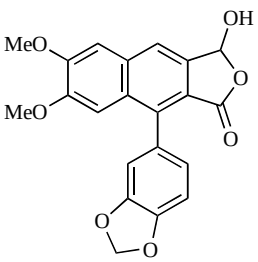

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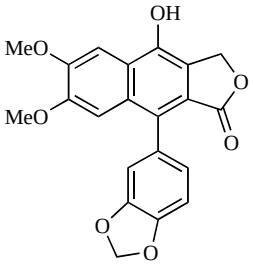

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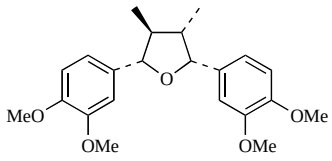

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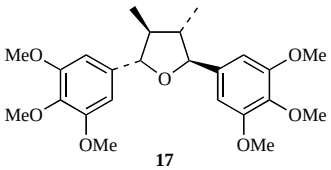

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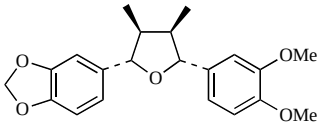

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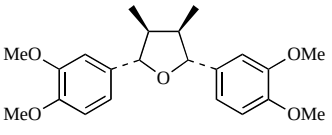

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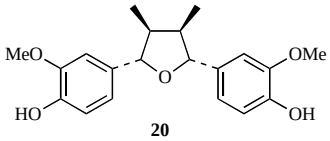

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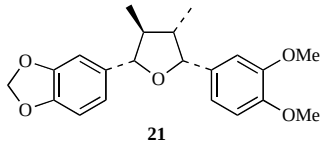

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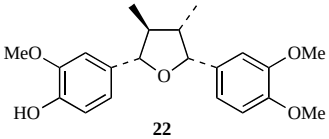

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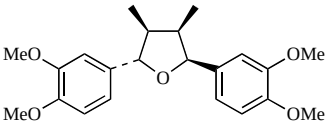

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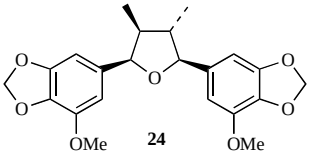

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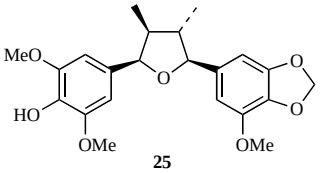

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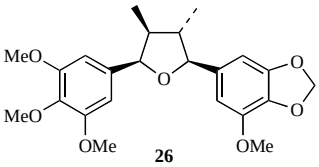

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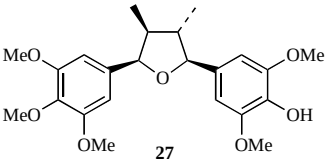

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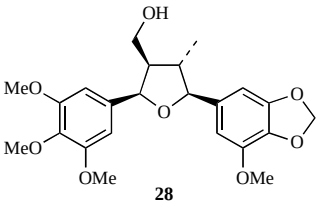

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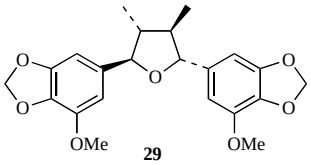

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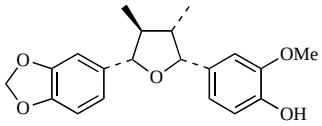

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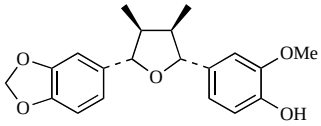

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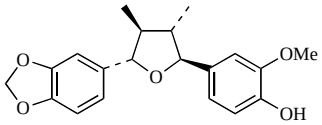

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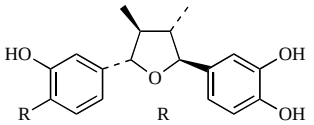
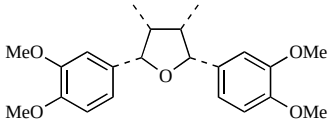


33 OMe
34 OH



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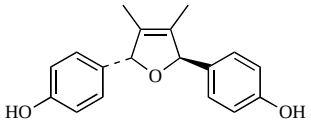

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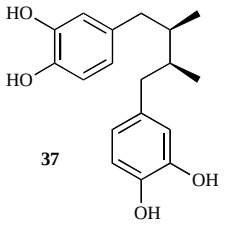

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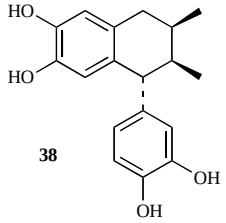

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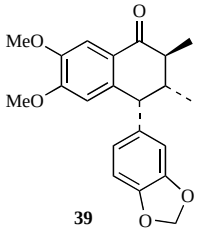

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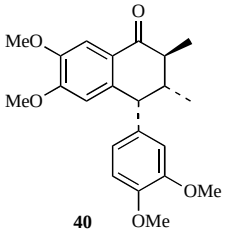

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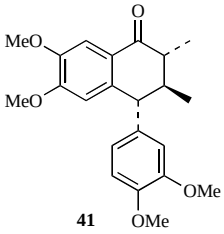

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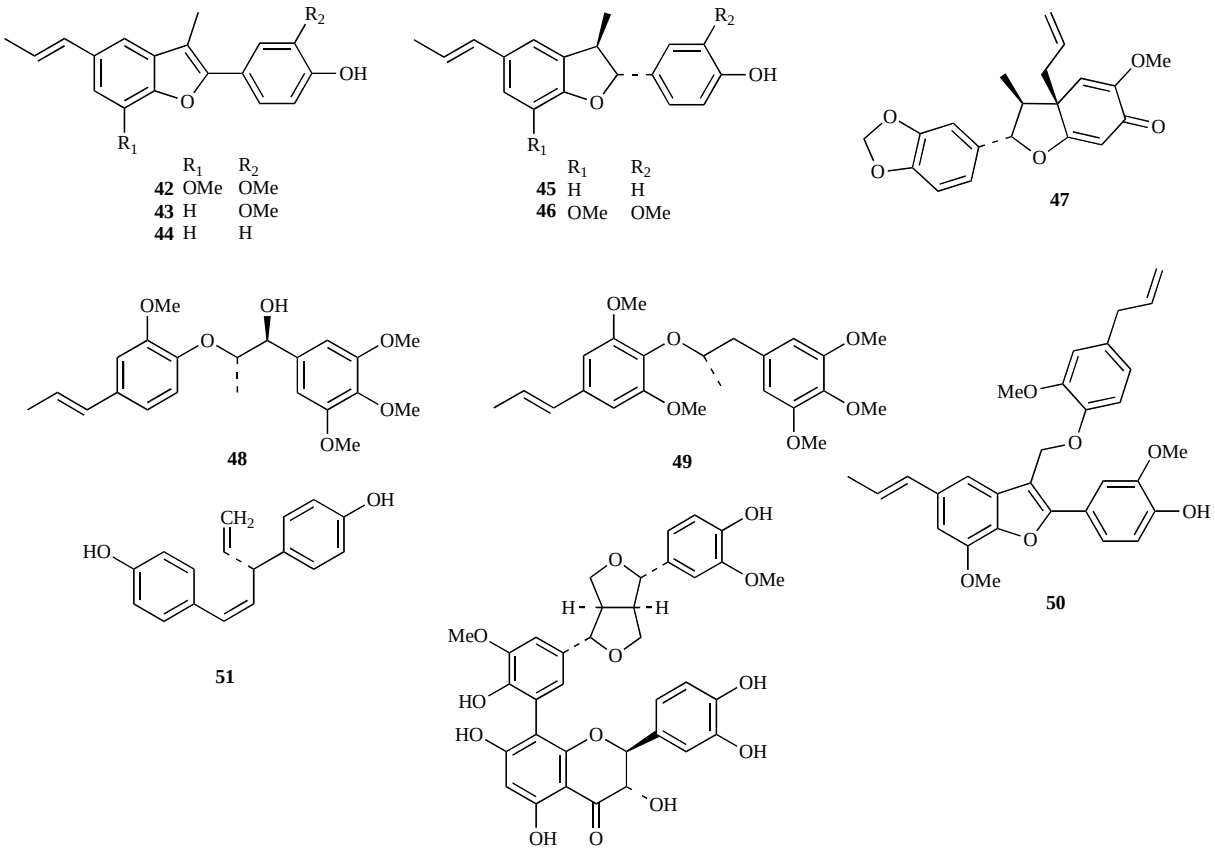
B

(Fig. 1). Contd…..



C

Fig (1). Structures of lignans and neolignans with antiprotozoal activity.

A. Monolignol-derived lignans.

B. Propenylphenol-derived lignans.

C. Neolignans, a norlignan (**51**) and a lignan-flavanonol-dimer (**52**).

values of 2.3 and >4 μg/mL, respectively. Both, **13** and **14** were not particularly selective, the IC_{50} values vs. four mammalian cell types ranging between 0.2 and 4.7 μg/mL (**13**) and 10->15 μg/mL (**14**) [25].

Potentially interesting lignans isolated from *Aristolochia taliscana* (Aristolochiaceae), austrobailignan-7 (**30**) and fragransin E_1 (**31**) (Fig. **1B**), were tested against *Tcr* epimastigotes, immobilizing these after 48h at MIC_{100} of 75 μg/mL and 50 μg/mL respectively. The neolignans (Fig. **1C**) eupomatenoid-7 (**42**) and licarin-A (**45**), isolated from the same source, showed MIC_{100} values of 25 μg/mL and 40 μg/mL, respectively [26].

A considerable *in vitro* trypanocidal activity was observed for neolignans (Fig. **1C**) isolated from *Piper regnelli* var. *pallescens*. Eupomatenoid-5 (**43**), eupomatenoid-6 (**44**) and conocarpan (**46**), assayed against epimastigote forms of *Tcr*, displayed IC_{50} values of 7.0, 7.5 and 8.0 μg/mL, respectively. Low cytotoxicity against Vero cells was observed for **43.** Methylation of hydroxy groups of active molecules led to a reduction in activity [27]. Furthermore, another study reported that **43** is able to induce ultrastructural alterations on epimastigote and amastigote forms of *Tcr* [28].

The important triatomine vector for the transmission of *Tcr*, *Rhodnius prolixus*, has also been the focus of several studies with lignans and neolignans. For instance, the furofuran-lignan pinoresinol (**7**) was tested against *R. prolixus* and demonstrated to be toxic to its larvae in high concentrations while lower ones (1-100 μg/mL) displayed anti-feedant as well as dose-dependent anti-moulting activity [29]. The neolignan burchellin (**47**) as well as the dibenzylbutane lignan nordihydroguaiaretic acid (NDGA) (**37**) were tested on *R. prolixus* prior to and after infection with *Tcr* epimastigotes. Treatment with both compounds before infection significantly reduced the number of parasites in the insects gut. When insects were fed with *Tcr* epimastigotes and treated simultaneously with a single dose of **37** or **47**, the number of parasites in the gut and feces was decreased in case of **37** while **47** was only partially active. However, neither compound was able to significantly reduce the number of gut parasites when applied 20 days after infection [30].

NDGA (**37**), the major lignan of *Larrea tridentata*, after re-isolation from this source together with the furanolignans **33**, **34** and **36**, the aryltetralin **38** and some analogues [22], was also tested for *in vitro* activity against *Tbr*, *Tcr*, *Ldon* and *Pfc*. NDGA was active against the respective parasites with IC_{50} values of 4.5, 33.1, 12.0 and 7.7 μM while the IC_{50} for cytotoxicity against L6 cells was 33.1 μM. Interestingly, cyclization to the aryltetralin system of **38** had a significant influence on activity. In comparison with the dibenzylbutane **37**, the tetralin **38** was 2.6, 5.6, 3.7, and 1.5 times less active against *Tbr*, *Tcr*, *Ldon* and *Pfc* but also 4.4 times less cytotoxic [24].

Burchellin (**47**) and licarin A (**45**) isolated from northeastern Brazilian Lauraceae, were also tested for activity against *Tcr*

epimastigotes and for potential toxicity on peritoneal macrophages. They were reported to be without effect on the macrophages but their antiprotozoal activity was also very low. After 96h, IC_{50} values for **47** of 463 μM and **45** of 756 μM were detected in the epimastigote assay. IC_{50} values for trypomastigote inhibition were 960 μM and 520 μM, respectively [31].

Diphyllin (**15**) (Fig. **1A**), a C-7 hydroxylated derivative of justicidin B (**13**)**,** isolated from *Haplophyllum bucharicum* (Rutaceae), was evaluated for activities against different *Linf* forms. When assayed against promastigote forms, the compound exhibited mild activity (IC_{50} = 14.4 μM), but against intracellular amastigote forms **15** displayed strong specific inhibitory activity (IC_{50} = 0.2 μM). This property was mainly considered as a result from modulation of macrophage phagocytosis [32]. Considering the observed effects and structural similarity with **13** and **14**, testing of derivatives of these lignans for potential antileishmanial activity could therefore be interesting and allow insights into structure-activity relationships.

An antiprotozoal norlignan from the root of *Asparagus africanus* (Asparagaceae), (+)-nyasol (**51**, Fig. **1C**), was tested against *Lmaj* promastigotes. It inhibited the growth of promastigotes with an IC_{50} value of 12 μM and showed moderate activity against *Pfc* schizonts with an IC_{50} of 49 μM [33].

The antileishmanial activity of the 8.O.4' neolignans surinamensin (**48**) and 3,4,5-trimethoxy-8-[2',6'-dimethoxy-4'-(*E*)-propenylphenoxy]-phenylpropane (**49**) (Fig. **1C**), isolated from *Virola* species, and of 25 synthetic related products has also been evaluated. The highest *in vitro* activity and selectivity against *Linf* promastigotes and amastigotes was found for some synthetic β-ketosulfides [34].

A study on constituents of *Ocotea duckei* (Lauraceae) led to the isolation of the furofuran lignan yangambin (**12**) (Fig. **1A**). In assays testing the activity against the promastigote forms of *Lcha* and *Lam*, yangambin showed IC_{50} values of 49.0 μg/mL and 64.9 μg/mL, respectively [35]. A more recent study observed that incubation of *Leishmania* promastigotes with **12** led to alterations in the parasite cell-morphology, changes in physiological characteristics and induction of cell death by apoptosis as well as autophagy [36].

Analysis of the constituents of *Ocimum sanctum* (Lamiaceae) led to the identification of seven novel neolignan derivatives (Fig. **1C**) which were evaluated for their antileishmanial activity. Assays performed with promastigotes of *Lmaj* revealed the highest activity for 5-allyl-3-(4-allyl-2-methoxyphenoxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydro-benzofuran (**50**), with an IC_{50} value of 9.1 μg/mL [37].

Tetrahydrofuran lignans (**18**-**23**, Fig. **1B**) isolated from *Nectandra megapotamica* and previously reported to possess trypanocidal activity [19], were also evaluated for their *in vitro* antileishmanial and antimalarial activity. Against *Ldon* promastigotes, machilin G (**18**) and veraguensin (**16**) displayed the highest activity, with IC_{50} values of 18 μg/mL and 36 μg/mL. In the assay against *Pfc*, calopeptin (**21**) exhibited moderate activity with an IC_{50} of 3600 ng/mL (D6 clone) and 3900 ng/mL (W2 clone). None of the isolated lignans displayed cytotoxic effects against Vero cells [38].

Antiplasmodial activity was observed for six aryltetralone lignans from *Holostylis reniformis* (Aristolochiaceae). Among the compounds tested against *Pfc*, the three most active ones, (7'*R*,8*S*,8'*R*)-4,5-dimethoxy-3',4'-methylenodioxy-2,7'-cyclolignan-7-one (**39**), (7'*R*,8*S*,8'*R*)-3',4,4',5-tetramethoxy-2,7'-cyclolignan-7-one (**40**) and (7'*R*,8*R*,8'*S*)-3',4,4',5-tetramethoxy-2,7'-cyclolig-nan-7-one (**41**) (Fig. **1B**), demonstrated IC_{50} values ≤ 0.32 μM. Cytotoxicity tests with Hep G2A16 cells exhibited low toxicity for these lignans [39]. The neolignan talaumidin (**32**) (Fig. **1C**) from *Pycnanthus angolensis* (Myristicaceae) revealed weaker antiplasmodial activity, displaying an IC_{50} value of 20.7 μg/mL against the chloroquine-resistant Dd2 strain of *Pfc* [40].

A compound with an unusual structure formed by condensation of pinoresinol (**7**) with the flavanonol taxifolin, pseudotsuganol (**52**), was investigated for antileishmanial activity together with a variety of oligomeric procyandidins (see section 4.1.2 below). While displaying low activity against extracellular forms of *Ldon* and low cytotoxicity against RAW macrophages, it was reported to be highly efficient against intracellular forms with an EC_{50} value of 0.9 nM [41].

2.2. Coumarins

Coumarins (structures see Fig. (**2**)) are monomeric phenylpropanoids formed by cyclization of *ortho*-hydroxycinnamic (= *o*-coumaric) acid or related acids leading to coumarin (**1**) and related simple compounds. Structural diversity is mainly introduced by prenylation followed by various cyclizations and further structural modifications. Another type of coumarins with a 4-phenyl substituent (4-phenylcoumarins) is also termed neoflavonoids. Such compounds are also treated in this section.

Simple unsubstituted coumarin (**1**) and scopoletin (**2**) were isolated from *Amburana cearensis* (Fabaceae) and *Guarea rhophalocarpa* (Meliaceae), respectively [42, 43]. Compound **1** was active against *Lam*, *Lbra* and *Ldon* at IC_{50} = 50 μg/mL and also displayed activity against *Pfc* at IC_{50}= 9 μg/mL. Compound **2**, on the other hand, did not show antiplasmodial activity and displayed inexpressive activity against *Ldon* (IC_{50} = 374 and > 90 μM to promastigote and amastigote forms, respectively).

Four simple coumarins (**3**-**6**) were isolated from *Pelargonium sidoides* (Geraniaceae) and evaluated against intracellular forms of *Ldon* [44]. In comparison with the positive control pentostam (EC_{50}= 2.6 μg/mL), these compounds displayed very weak activity (EC_{50} >25 μg/mL).

The *in vitro* bioactivity of chromatographic fractions and compounds obtained from *Polygala sabulosa* (Polygalaceae) against *Tcr* was tested [45]. The prenylated coumarin **7** was the most active compound against both epimastigote and trypomastigote forms, with IC_{50} values determined as 10.5 and 88.2 μg/mL, respectively.

Thamnosma rhodesica (Rutaceae), along with some acridone derivatives, yielded five coumarins (**8**-**12**) which were tested for antileishmanial activity [46]. The percentages of survival of free-living promastigote and of amastigote forms of *Lmaj* were determined after treatment with each compound at 10 μM. These compounds displayed inhibition in the range from 34.9 to 99.1 % against promastigote forms. Compound **11** was the only coumarin reported to be active against amastigote forms (79.0 % inhibition at 10 μM). Two of the acridones showed somewhat higher activity.

Toddalia asiatica (Rutaceae) is a plant used traditionally in Kenya by many communities for the treatment of malaria [47]. The bioguided fractionation of its extracts afforded the coumarin **13**, which displayed IC_{50} = 16.2 and 8.8 μg/mL, respectively, against K39 and V1/S strains of *Pfc*. However, it is interesting to note that the crude MeOH extract of this plant was more active than **13**, suggesting the presence of other active derivatives, which could act individually or by synergy with **13**.

Two prenylated pyrenocoumarins **14** and **15** from *Clausena harmandiana* (Rutaceae) were tested against the K1 strain of *Pfc* [48]. Structurally, compound **15** differs from **14** by an additional prenyl substituent and a hydroxy group in the aromatic ring instead of a methoxy group. However, **15** displayed IC_{50}= 0.1 - 0.7 μg/mL while **14** was distinctly less active, with IC_{50}= 8.5 - 12.3 μg/mL.

The pyranocoumarin 3-(1,1'-dimethylallyl)-lomatin (**16**) was the most active isolated constituent from *Raulinoa echinata*

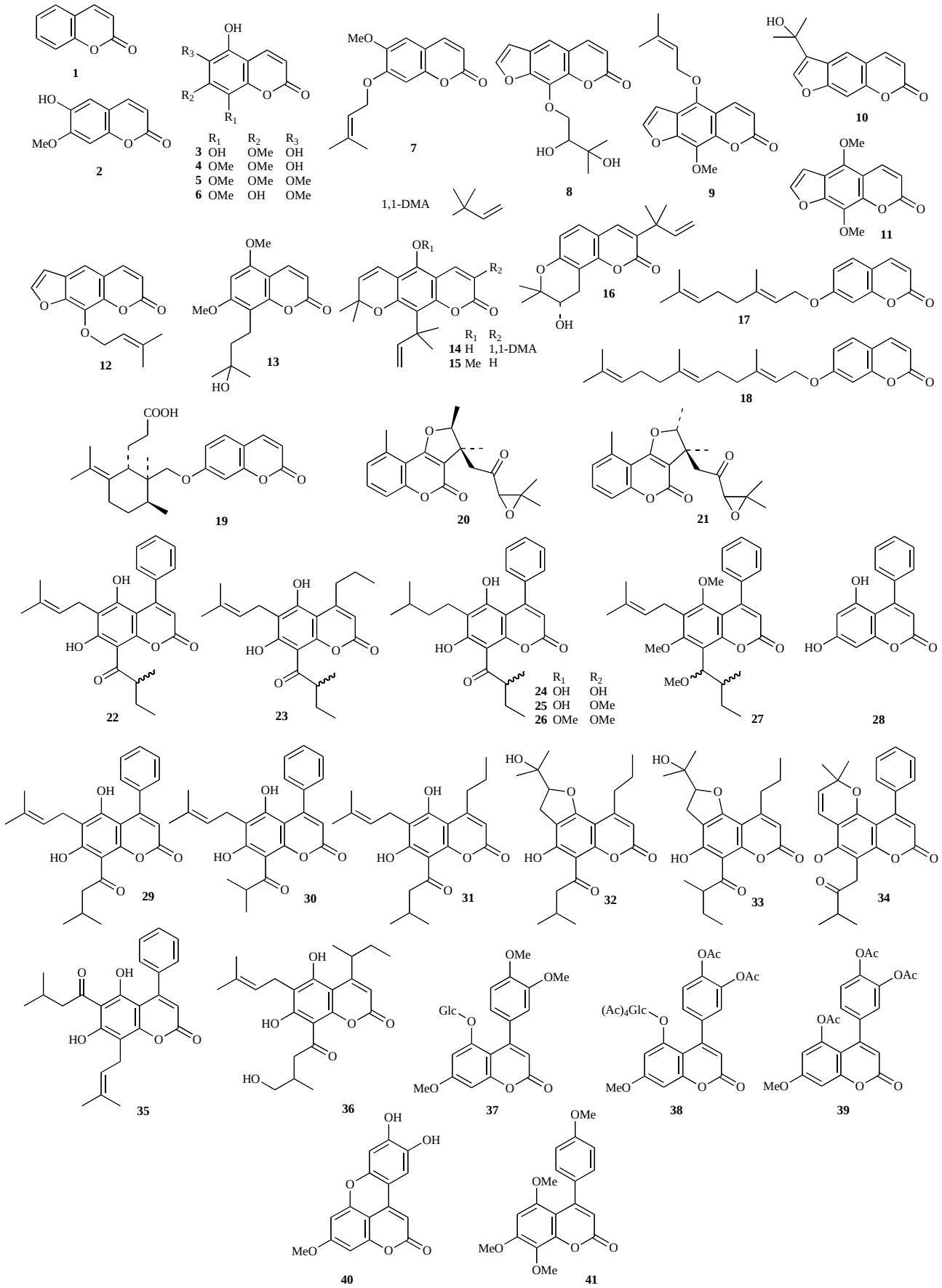


Fig. (2). Structures of antprotozoal coumarins and 4-phenylcoumarins (neoflavonoids).

(Rutaceae) from which it was isolated together with sesqui- and triterpenes (see part I [1]). **16** inhibited the *in vitro* growth of *Tcr* trypomastigotes by 72% when applied at 100 μg/mL [49].

Three coumarins with terpenoid ether moieties (**17**-**19**) were isolated from *Ferula szowitsiana* (Apiaceae), which displayed *in vitro* activity against promastigote forms of *Lmaj* [50]. Compounds **17** and **18**, which possess linear terpenoid side chains, showed moderate activity (IC_{50}= 17.1 and 13.3 μM, respectively), while compound **19** with a cyclic sesquiterpenoid side chain was only weakly active (IC_{50}= 164.8 μM).

Two stereoisomeric 5-methylcoumarins (**20** and **21**) isolated from *Vernonia brachycalyx* (Asteraceae), were tested *in vitro* for antiplasmodial activity against a chloroquine-susceptible (3D7) and a chloroquine-resistant strain (Dd2) of *Pfc* [51]. Compounds **20** and **21** inhibited the growth of the Dd2 strain (IC_{50} = 54 and 54 μM) more potently than that of the 3D7 strain (IC_{50} = 160 and 111 μM). Both compounds were also tested against *Lmaj* and showed a similar level of inhibition with IC_{50} values of 37.0 and 13.4 μM, respectively.

Brenzan *et al.* reported on the isolation of two 4-phenylcoumarins (**22** and **23**) from *Calophyllum brasiliense* (Calophyllaceae) as well as preparation of five semi-synthetic derivatives (**24**-**28**) which showed antileishmanial activity against *Lam* with an IC_{50} range from 0.9 μM (promastigote forms) to 0.6 μM (intracellular amastigote forms) [52]. This work revealed some interesting relationship between coumarin chemical structure and antileishmanial activity.

A variety of further 4-phenylcoumarins were isolated from *Calophyllum brasiliense* (**24**, **29**-**34**) and from *Mammea americana* (**35**) (Calophyllaceae) and tested *in vitro* against epi- and trypomastigotes of *Tcr* [53]. Compounds **29**-**31** and **35** were shown to be the most potent, with MC_{100} values in the range from 15 to 90 μg/mL. Coumarins with a cyclic γ,γ-dimethylallyl substituent at C-6, such as **32**-**34**, showed MC_{100} values > 200 μg/mL and were considered inactive.

Kielmeyera albopunctata (Calophyllaceae) yielded three 4-phenylcoumarins of which compound **36** turned out to be active *in vitro* against the trypomastigote form of *Tcr*, killing 80% of the parasites when added at 125 μg/mL and 100% at 500 μg/mL [54].

A series of 4-phenyl coumarins such as **37**-**39**, isolated from *Hintonia latiflora* (Rubiaceae) [55], were assayed for activity against *Ldon*. The most active derivative **36** showed an IC_{50} value of 10.2 μM against promastigote forms. None of the compounds was active against amastigotes. Tests against *Tbr* revealed **38** as the most active derivative (IC_{50}= 10.7 μM). Additionally, these compounds were also evaluated for antiplasmodial activity against *Pfc* where they were not very active, the lowest IC_{50} (60.3 μM) being found for **39**. The 5,2'-oxido derivative **40** with an additional pyran ring was of very low activity (IC_{50} 444, >30 and 474 μM vs. *Ldon*, *Tbr* and *Pfc*, respectively).

The stem bark of *Exostema mexicanum* (Rubiaceae) is used in traditional medicine for malaria treatment in Latin America. Bioguided fractionation of its extract yielded 4-phenylcoumarins, among which O-methylexostemin (**41**) revealed the strongest antiplasmodial activity (IC_{50} = 3.60 μg/mL) [56].

2.3. Caffeic Adic Derivatives

Caffeic acid and esters have in a few cases (structures see Fig. (**3**)) been investigated for antiprotozoal activity.

Caffeic acid (**1**) and 4,5-di-O-*E*-caffeyolquinic acid (**6**) exhibited very weak trypanocidal activity against trypomastigote forms of *Tcr* showing IC_{50} values of 2104 and 286 μM, respectively [57]. Methyl caffeate (**2**) was inactive against *Pfc* [58]. Caffeic acid (**1**) was reported to inhibit promastigote forms of *Lam* (IC_{50} = 190 ng/μL). Amastigotes of the same species were inhibited at 410 ng/μL and a weaker effect on *Lbra* amastigotes was found [59].

Three simple aliphatic esters of caffeic acid (**3**-**5**) were isolated from leaves of *Piper sanguineispicum* (Piperaceae) and assessed for their antileishmanial potential against axenic amastigote forms of *Lam* [60]. Compounds **3** and **5** exhibited the best antileishmanial activity (IC_{50} = 2.0 and 1.8 μM, respectively), while **4** displayed weaker activity (IC_{50} = 10.0 μM).

From *Phlomis brunneogaleata* (Lamiaceae), two caffeic acid ester derivatives (**7** and **8**) were obtained which exhibited some anti-parasitic potential against *Ldon* (IC_{50} = 4.7 and 9.1 μg/mL, respectively) and *Pfc* (IC_{50} = 38.9 and >90 μg/mL, respectively), but were inactive against *Tcr* (IC_{50} > 90 μg/mL) [61].

3. QUINONES: BENZO-, NAPHTHO-, ANTHRAQUINONES

Quinones occur in a variety of plant families (Bignoniaceae, Lythraceae, Verbenaceae, Polygonaceae, Fabaceae, as well as some gymnosperms), lichens (*Cladonia*, *Xanthoria*), fungi (*Streptomyces*, *Penicillium*, *Aspergillus*), and bacteria (*Escherichia coli*, *Micrococcus lutens*, *Aerobacter aerogenes*, *Bacillus cereus*) and are also present in some arthropods (insect family Coccidae) and echinoderms.

Natural quinones are classified by their basic aromatic carbon skeleton as benzoquinones, anthraquinones and naphthoquinones

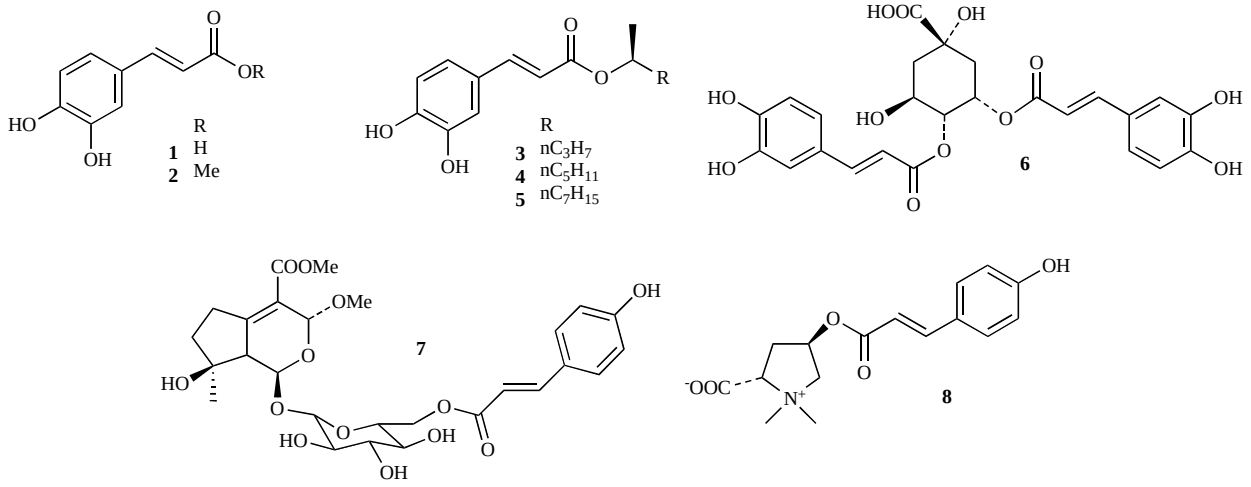


Fig. (3). Structures of antiprotozoal caffeic acid derivatives.

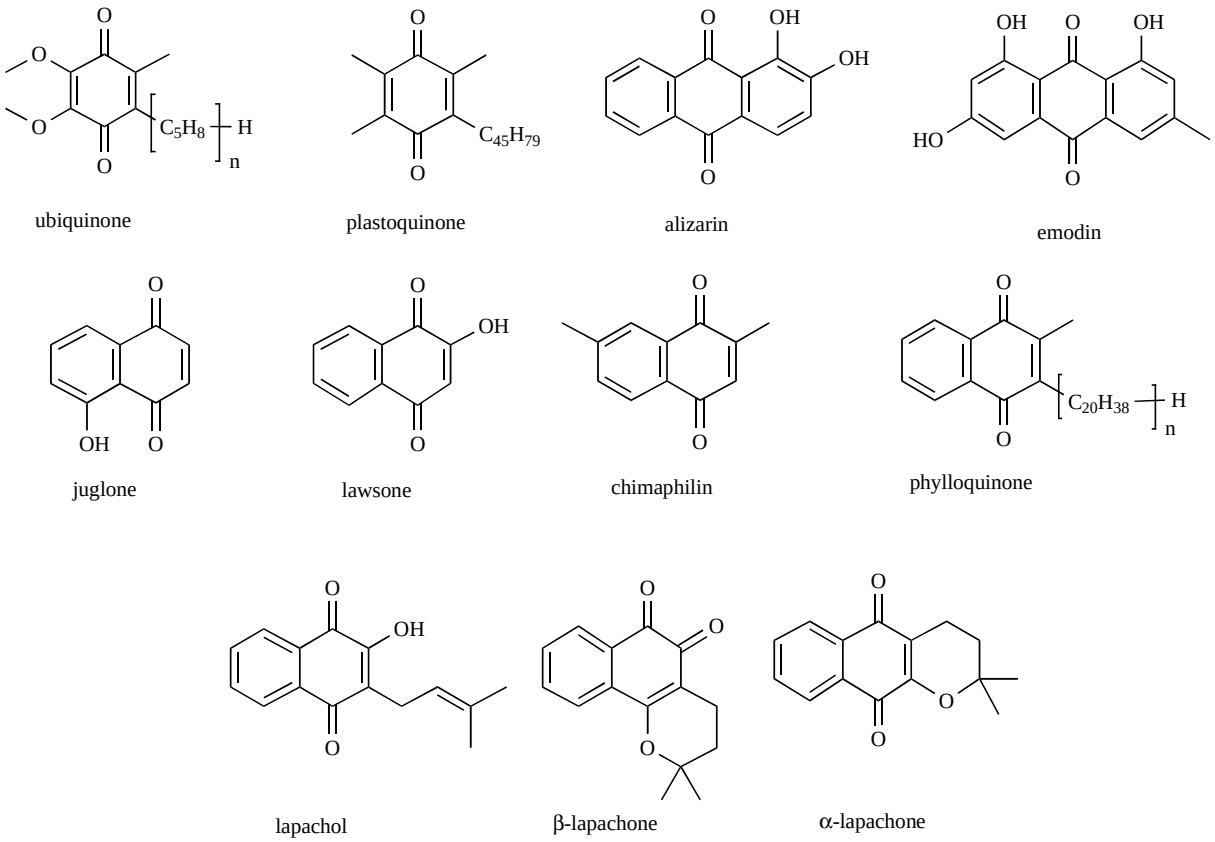


Fig. (**4).** Structures of some representative quinones.

(Fig. **4**). Benzoquinones are found in plants and insects and are sometimes involved in the defense against predators. Among relevant groups of benzoquinones there are ubiquinone and plastoquinone, which differ in the substitution pattern, lateral chain of different length and unsaturation levels. Ubiquinones, also known as coenzyme Q, are involved in respiratory chain processes. Anthraquinones are the major group of natural quinones with historical importance and use since ancient times. As example, alizarin obtained from the roots of *Rubia tinctorum* (Rubiaceae) is used as a dye. Extracts of several plants such as *Cassia angustifolia* (Fabaceae), *Rhamnus purshiana* and *R. frangula* (Rhamnaceae) and *Rheum palmatum* (Polygonaceae) contain laxative glycosides of anthraquinones like emodin. Naphthoquinones represent the majority of natural naphthalenes with a variety of structural motifs. They are considered privileged structures in medicinal chemistry due to their biological activities and structural properties [62], especially against tumor cells and pathogenic protozoa [63, 64]. Simple hydroxylated naphthoquinones are e.g. juglone, isolated from *Juglans* spp. and *Capparis ovata*, and lawsone from *Lawsonia inermis* and *Impatiens* spp. Lawsone is a compound present in henna (*L. inermis*), the crushed leaves of which are used world-wide as a cosmetic agent to stain the hair, skin, and nails. Chimaphylin, isolated from *Chimaphila umbellata*, is a well known representative of natural naphthoquinones with a methylated quinoid ring. Another naphthoquinone with an important biological role is phylloquinone (vitamin K1) with an isoprenic lateral chain, which acts as an enzymatic cofactor involved in the blood clotting cascade [65]. The well-studied naphthoquinones lapachol, α -lapachone and β-lapachone will be discussed in section 3.2.

3.1. Quinones and Unicellular Parasites

Quinone-containing plants have been used in diverse cultures as dyes, cosmetics, food and traditional medicines. Especially Native American Indians employed them for the treatment of various diseases [66, 67]. A variety of compounds was isolated from natural sources and characterized. The results of bioassays against parasites were compiled in several reviews, among others [68-75]. Natural quinones, especially monomeric and dimeric naphthoquinones, represent one of the major classes of natural products with significant biological activity against parasites of the genera *Leishmania*, *Trypanosoma* and *Plasmodium*. Their major disadvantage is the cytotoxicity to mammalian cells. In some cases, their mechanism of action was investigated and *in vivo* preclinical studies were performed. Table **1** lists publications about the effects of natural quinones (structures see Fig. (**5**)) on protozoan parasites; in the following, we will focus on studies related to lapachol, β-lapachone and derivatives.

3.2. Lapachol, β-Lapachone and Derivatives

Lapachol was originally isolated from the heartwood of *Tabebuia* spp. trees (Bignoniaceae) and is also found in other families such as Verbenaceae, Proteaceae, Fabaceae, Sapotaceae, Scrophulariaceae and Malvaceae [76]. The inner bark of *Tabebuia avellanedae*, commonly known as "pau d'arco", is used as an analgesic, anti-inflammatory, antineoplasic and diuretic drug by the local people in the northeastern regions of Brazil [77]. Together with lapachol, α-lapachone and β-lapachone are obtained as minor components. These two naphthoquinones can be easily obtained by the cyclization of the isoprenyl lateral chain of lapachol in acid medium.

Lapachol and β -lapachone, more than a century after their discovery, still attract the attention of many researchers. In this context, several groups are interested in the development of potentially useful new compounds containing the naphthoquinone system [78]. Today, the greatest interest in lapachol and β -lapachone involves their antitumoral and microbicidal effects.

Table 1. Natural quinones assayed against protozoan parasites: Structures see Fig. (5)

Quinone	Source	Parasite[a]	Ref.
Plumbagin (**1**) 3,3'-biplumbagin (**2**) 8,8'-biplumbagin (**3**)	*Pera benensis* (Euphorbiaceae) *Nepenthes thorelii* (Nepenthaceae)	*Tcr* (epi, ama) *Lam* (pro) *Lbra* (pro) *Ldon* (pro) *Lmex* (vv) *Lven* (vv)[b] *Pfc*	[127-131]
Diospyrin (**4**) 8'-Hydroxyisodiospyrin (**5**)	*Diospyros montana*, *Diospyros assimilis* (Ebenaceae)	*Tcr* (trypo, ama) *Ldon* (ama) *Tbb* (trypo) *Pfc*	[100, 124-126]
2,3,3-trimethyl-2-3-dihydronaphtho[2,3-*b*]furan-4,9-quinone (**6**)	*Calceolaria sessilis* (Calceolariaceae)	*Tcr* (epi)	[132]
2-(1-Hydroxyethyl)-naphtho[2,3-b]furan-4,9-quinone (**7**)	*Kigelia pinnata* (Bignoniaceae)	*Tbb* (trypo) *Tbr* (trypo) *Pfc*	[133, 134]
Isofuranonaphthoquinones (**8-13**)	*Bulbine capitata* (Xanthorrhoeaceae/ Asphodeloideae)	*Pfc*	[135]
Aloe-emodin (**14**)	*Stephania dinklagei* (Menispermaceae)	*Pfc* *Ldon* (pro) *Tbb* (trypo)	[136]
Purpurin (**15**)	*Rubia tinctorum* (Rubiaceae)	*Tcr* (trypo)	[137]
Embelin (**16**)	*Oxalis erythrorhiza* (Oxalidaceae)	*Tcr* (trypo) *Lam* (pro) *Ldon* (pro)	[138]
Hydropiperone (**17**)	*Peperomia galioides* (Piperaceae)	*Lam* (pro) *Lbra* (pro) *Ldon* (pro)	[139]
Diterpenoid 1,2-quinones (**18-21**)	*Perovskia abrotanoides* (Lamiaceae)	*Lmaj* (pro) *Pfc*	[140]
Cochlioquinone A (**22**) Isocochlioquinone A (**23**)	*Cochliobolus sp.* (Pleosporaceae, Ascomycota, Fungi)	*Lam* (ama)	[141]
Xestoquinone (**24**)	*Xestospongia sapra* (Petrosiidae, Porifera /sponges), Animalia)	*Pfc* *Pber* (vv)	[142]
Alisiaquinone C (**25**)	New Caledonian deep water sponge	*Pfc*	[143]

[a]In parentheses the evolutive form of the parasite assayed: trypo (trypomastigotes), ama (amastigotes), epi (epimastigotes), pro (promastigotes) and vv when the studies with experimental animals were performed.
[b]Lven: L. venezuelensis.

Using lapachol as a lead compound for potential antimalarial agents, atovaquone was identified [79, 80]. Atovaquone is a highly lipophilic drug that resembles the structure of ubiquinone. It acts selectively in sensitive parasites by affecting their mitochondrial electron transport and related processes such as ATP synthesis [81]. Currently, a combination of atovaquone and proguanil is being used to treat malaria [82] and is also useful for the treatment of patients with mild to moderate infection caused by *Pneumocystis carinii* [83]. Atovaquone displayed *in vitro* activity against *Ldon* and *Linf* but its efficacy in a murine model was low [84-87].

The great majority of the studies with lapachol and β-lapachone were conducted on pathogenic trypanosomatids, i.e. *Tcr* and *Leishmania* spp., parasites that are especially sensitive to oxidative stress conditions [88]. The mechanism of action of β -lapachone against *Tcr* was investigated. In epimastigotes, an increased generation of reactive oxygen species (ROS) through formation of the semiquinone radical was observed, leading to lipid peroxidation as well as inhibition of nucleic acid and protein synthesis [89-91]. At the ultrastructural level, β-lapachone-treated parasites presented alterations in nuclear, mitochondrial and plasma membranes as well as in the chromatin pattern, consistent with the measured inhibition of glucose and pyruvate oxidation due to decrease in ATP concentration [92]. Lapachol was also reported to be active *in vitro* on intracellular amastigotes of *Lbra* while *in vivo* it did not prevent the development of lesions in hamsters, suggesting that it could be transformed into an inactive metabolite [93]. Lapachol, isolapachol, dihydrolapachol and other derivatives were assayed *in vitr*o and *in vivo* against *Lam* and *Lbra*. Isolapachol acetate was the most active compound *in vitro* (IC_{50}= 1.6 and 3.4 μg/mL for *Lam* and *Lbra*, respectively). This compound was hence tested *in vivo* against *Lam* and found active [94]. Isolapachol acetate was also active against trypomastigotes of *Tcr* [95]. Investigating the influence of the redox potential of naphthoquinones on trypomastigotes, Goulart and

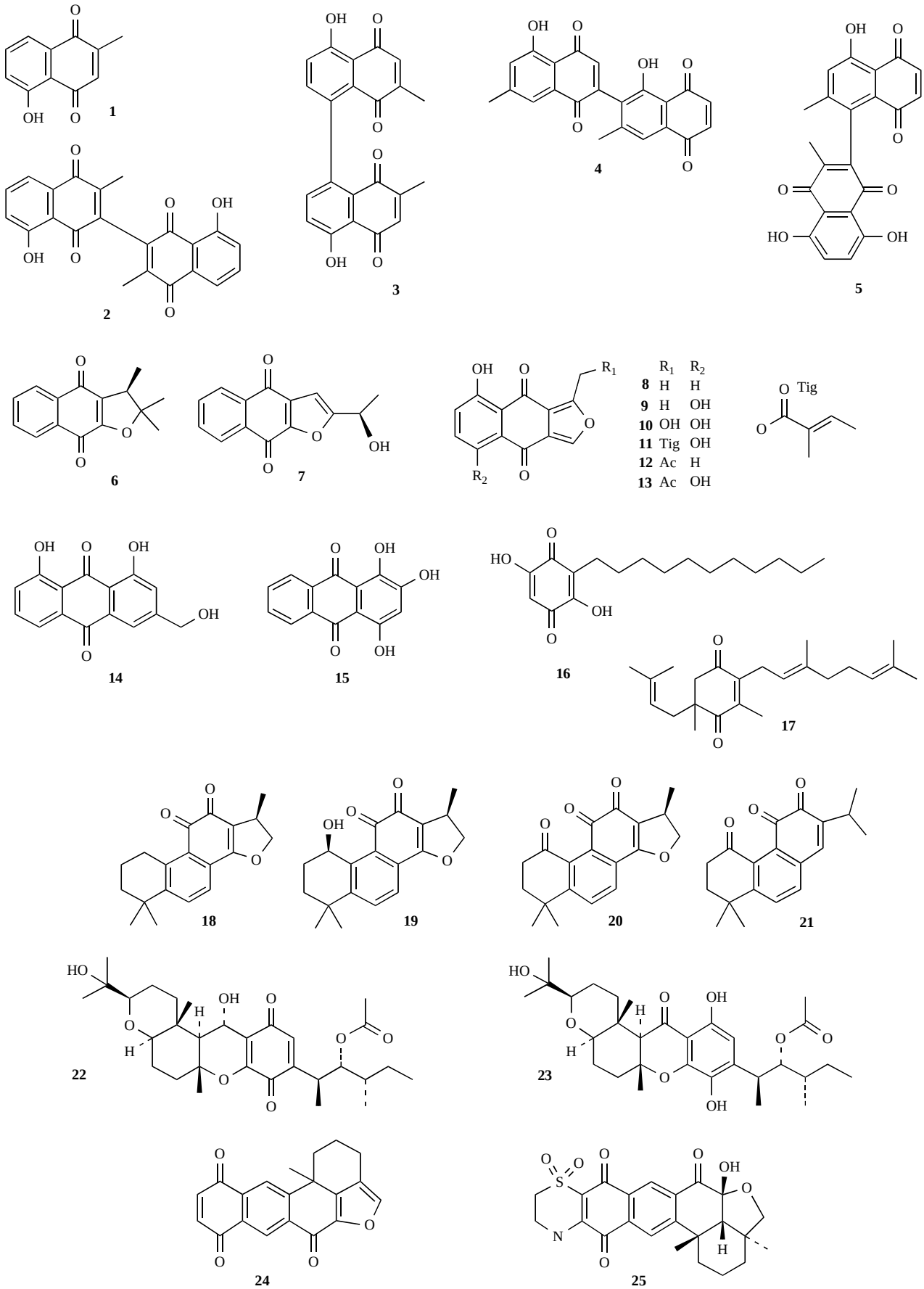


Fig. (5). Structures of natural quinones with antiprotozoal activity, compare Table **1**.

coworkers [96] observed a positive correlation between the trypanocidal activity and the easiness of reduction. Benzo[a]phenazine derivatives of these quinones were synthesized and tested *in vitro* against *Pfc* and selected for assays in *Pber*-infected mice, being the 3-sulfonic derivative from β-lapachone the most active, followed by β-lapachone, while lapachol was inactive [97].

Naphthoquinones have been identified as possible lead structures against pathogenic protozoans, but their potential usefulness is limited by their cytotoxicity and low bioavailability, pointing to the necessity of optimizing the natural product lead to potentiate its activity and reduce side effects [68, 71]. Several research groups have therefore synthesized a host of naphthoquinone derivatives and assayed them against *Tcr* [63], including series of *ortho*-naphthoquinones [91], isoxazolylnaphthoquinones [98, 99], plumbagin derivatives [100], 3,3'-[polyaminobis(carbonylalkyl)]-bis(1,4-naphthoquinones) [101, 102], thiophenquinones [103, 104] and furoquinolinediones [105, 106] and others. A vast number of synthetic naphthoquinone analogues of different structural classes, their activity against *Tcr*, as well as information on structure-activity relationships and cytological as well as mechanistic aspects of their antitrypanosomal action can be found in references [107-123].

The intensity of synthetic efforts on such analogues underlines their importance as leads for new drugs against NTDs.

4. POLYKETIDES AND METABOLITES DERIVED FROM PATHWAYS RELATED TO FATTY ACID BIOSYNTHESIS

4.1. Flavonoids and Related Compounds

Flavonoids are a very large group of mixed polyketides biosynthesized from a cinnamic acid derivative which is coupled with three acetate units. First cyclization products are chalcones which can then further be cyclised to flavan-4-ones (flavanones), biogenetic precursors of the flavanonols (3-hydroxyflavanones), flavones, flavonols (3-hydroxyflavones), flavan-3,4-diols (leukoanthocyanidins) and flavan-3-ols (proanthocyanidins). The biological activities of this chemically very diverse group are multiple and have been reviewed extensively. Flavonoids are most renowned for their anti-oxidative, anti-inflammatory and cytoprotective activities. Most importantly, flavonoids occur in all green plants and many of them are thus constituents of our common daily diet which makes them important protective factors for human health [144-147].

Flavonoids with a normal flavane carbon skeleton including some proanthocyanidins are shown in Fig. (**6A** and **6B**). Fig. (**6C**) reports structures of prenylated flavonoids, Fig. (**6D**) contains structures of biflavonoids.

4.1.1 Regular Flavonoids

A series of eleven common dietary flavonoids was tested for *in vitro* antiplasmodial activity against *Pfc* strains 3D6 (chloroquine sensitive) and 7G8 (chloroquine resistant). The flavone luteolin (**1**) was the most active compound against both strains with IC_{50} values of 11 and 12 μM, respectively, followed by its flavonol congener quercetin (**9**; 15 and 14 μM, resp.). Apigenin (**2**) and acacetin (**3**) were also active against the latter strain with IC_{50}= 14 μM. It is remarkable that **3** was selectively active against 7G8 and completely inactive (IC_{50}>100 μM) against the 3D6 strain while most other compounds affected the two cell lines at similar concentrations [148].

Luteolin (**1**), was recently isolated from *Melampyrum arvense* (Orobanchaceae) along with its 7-*O*-β-glucoside and the 3'-deoxy analog apigenin (**2**) as well as some compounds of other classes; **1** was active against *Tbr*, *Tcr*, *Ldon* and *Pfc* with IC_{50}s of 3.8, 17.0 3.0 and 4.2 μg/mL, respectively; **2** was distinctly less active against all parasites. Quite noteworthy, the glucoside of **1**, less active than **1** against the kinetoplastids, was even more active against *Pfc* (IC_{50}= 2.9 μg/mL= 6.5 μM) [149].

Luteolin (**1**) and apigenin (**2**) along with their 7-*O*-glucosides (**4** and **5**, resp.), apigenin-4'-*O*-glucoside (**6**) and rutin **14**, after isolation from *Achillea millefolium* (Asteraceae), were tested against chloroquine sensitive (D10) and resistant (W2) *Pfc*. **1** was the most active derivative against both strains (IC_{50}= 6.1 and 5.0 μg/mL, resp.), **2** being much less active (IC_{50}= 25.4 and 20.2 μg/mL). However, apigenin-7-*O*-gluoside (**5**) was more active than the corresponding luteolin derivative **4** (IC_{50}= 10.1 and 6.1 μg/mL vs. 26.2 and 26.8 μg/mL). **14** and the 4'-glucoside of apigenin (**6**) were distinctly less active. Several mono- and dicaffeoylquinic acid esters from the same plant did not show any significant activity [150].

Luteolin (**1**), along with some further flavonoids such as the C-glucosylflavone vicenin-2 (**28**), was also tested for activity against *Tcr* trypomastigotes after isolation from *Lychnophora pohlii* (Asteraceae). This stage of *Tcr* appeared to be quite insensitive against the tested compounds (also some sesquiterpene lactones, see part I [1]). **1** and **28** were the only flavonoids from this study for which IC_{50} values could be determined (1325 and 571 μM, respectively). All other flavonoids did not kill 50% of the parasites at the highest concentration tested (500 μM) [57].

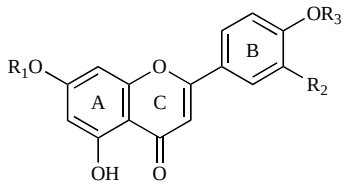
6-hydroxyluteolin-7-α-*O*-rhamnoside (**30**) from the Bromeliacea *Vriesea sanguinolenta* showed activity against chloroquine resistant (K1) and sensitive (NF54) strains of *Pfc* with IC_{50} values of 2.13 and 3.32 μM, respectively. The peracetylated derivative was somewhat more active with IC_{50}s of 0.52 and 0.62 μM, respectively [151].

Luteolin (**1**) and the corresponding flavanone, eriodictyol (**31**), after isolation from *Satureja parvifolia* (Lamiaceae) along with the two common triterpenes ursolic and oleanolic acid (see part I [1]), were reported to show antiplasmodial activity (*Pfc*, K1 and 3D7 strains; IC_{50} = 17 and 27 μg/mL, resp., for **31** and 6.4 and 6.3 μg/mL, resp., for **1**) as well as antritrypanosomal activity *in vitro* (14.4 and 2.3 μg/mL for **31** and **1**, resp., against *Tbr* trypomastigotes). They displayed much lower cytotoxicity against KB cells: IC_{50} = 174 and 13 μg/mL, respectively [152].

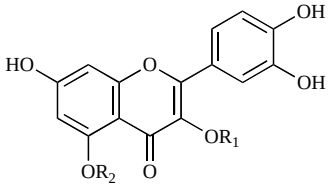
Three flavanones (eriodictyol and its 3'-mono- as well as 3',4'-dimethylether, **32** and **33**, resp.), two flavones (luteolin-3'-mono and -7,3'-dimethylether, **7** and **8**), a flavonol (quercetin-7,3'-dimethylether, **17**) and a flavanonol (taxifolin-3'-methylether **34**), isolated from *Lychnophora granmongolense* along with some sesquiterpene lactones (see part I [1]), were tested *in vitro* against *Tcr* trypomastigotes. Their activity was very low with IC_{50}s ranging from 833 to 2930 μM [153].

The common flavonols **9**, kaempferol (**20**) and trifolin (kaempferol-3-*O*-β-galactoside, **21**) as well as acetyl hyperoside (quercetin-3-*O*-β-galactoside acetate, **10**) isolated from *Consolida oliveriana* (Ranunculaceae) and their peracetates were reported to show *in vitro* antileishmanial activity against promastigotes of *Lper* and *Lbra* [154]. The most active compound was the octaacetate of hyperoside (**11**) with respective IC_{50} values of 7.4 and 6.2 μM and SI values of 17 and 20 (J774.2 macrophages) [154]. The activities reported for **9** (IC_{50}=60 and 30 μM, resp.) and **20** (IC_{50}=71 and 54 μM, resp.) were significantly lower than those reported by [155] for the respective compounds on *Ldon* axenic amastigotes (IC_{50}=10.1 and 3.3 μM). Based on ultrastructural changes in the parasite, the possibility that the compounds interact with tubulin was discussed [154].

Antitrypanosomal *in vitro* activity against *Tcr* epimastigotes was very recently reported for a series of 11 flavonol glycosides from *Delphinium staphisagria* (Ranunculaceae). The peracetylated derivative of astragalin (astragalin heptaacetate, **23**) was the most

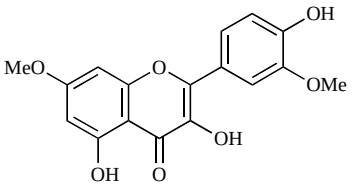


	R_1	R_2	R_3
1	H	OH	H
2	H	H	H
3	H	H	Me
4	Glc	OH	H
5	Glc	H	H
6	H	H	Glc
7	H	OMe	H
8	Me	OMe	H



	R_1	R_2
9	H	H
10	Gal-Ac	H
11*	Gal(Ac)4	H
12	Rha	Ac
13	Glc	H
14	Glc-6-Rha	H
15	Rha-2-Ara*p*	H
16	Ara*f*	Galloyl

*phenolic OH groups acetylated



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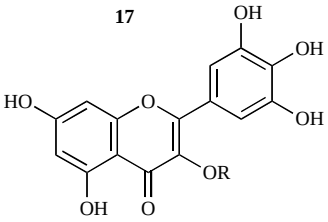
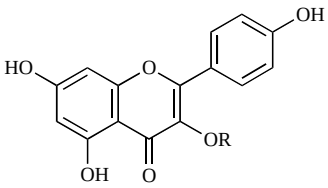
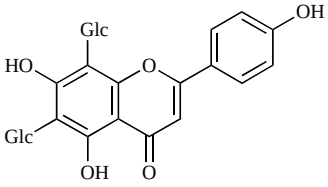


R	
H	**18**
Ara*f*	**19**



R	
20	H
21	Gal
22	Glc
23*	Glc(Ac)4
24	Gal(2-Xyl)-6-Rha
25	Glu-6-Rha
26	Gal-2-Xyl
27	Rha-2-Ara*p*

*phenolic OH groups acetylated



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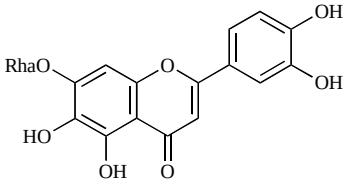
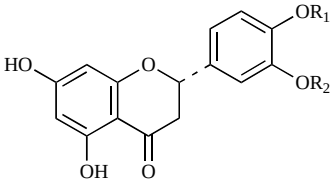
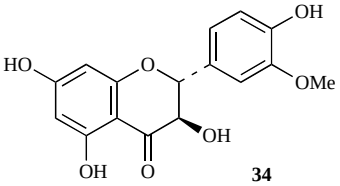


29
30 peracetate



R_1	R_2	
H	H	**31**
H	Me	**32**
Me	Me	**33**



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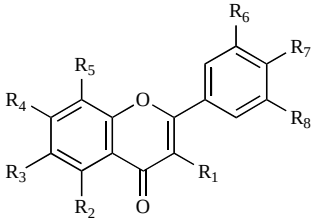
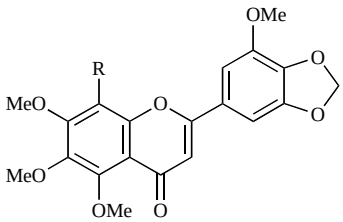


	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
35	H	OH	H	OMe	H	OMe	OH	H
36	OMe	OH	OMe	OMe	H	OMe	OH	H
37	OMe	OH	OMe	OH	H	OMe	OH	H
38	OMe	OH	OMe	OH	H	OH	OH	H
39	OMe	OH	H	OH	H	OH	OH	H
40	OMe	OH	H	OMe	H	OMe	OMe	H
41	H	OH	H	OMe	H	OMe	OMe	H
42	OH	OH	H	OMe	H	OMe	OMe	H
43	H	OH	OMe	OH	H	OH	OH	H
44	H	OH	OMe	OH	H	H	OH	H
45	OMe	OH	OMe	OH	H	H	OMe	H
46	OMe	OH	OMe	OMe	H	H	OH	H
47	H	OMe	OMe	OMe	OMe	OMe	OMe	OMe
48	H	OMe	OMe	OMe	H	OMe	OMe	OMe
49	H	OMe	OMe	OMe	H	OMe	OH	OMe
50	H	OMe	H	OMe	H	OMe	OMe	H
51	OMe	OH	H	OMe	H	OH	OMe	OMe
52	OMe	OH	H	OMe	H	OMe	OMe	OMe
53	H	OMe	H	OMe	H	OMe	OMe	OMe

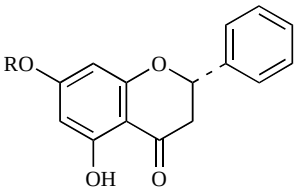
Gal: β-D-galactose
Glc: β-D-glucose
Rha: α-L-rhamnose
Ara*f*: α-L-arabinofuranose
Ara*p*: α-L-arabinopyranose
Xyl: β-D-Xylose



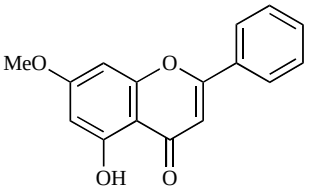
R	
54	OMe
55	H

A

(Fig. 6). Contd…..



R
56 Me
57 H



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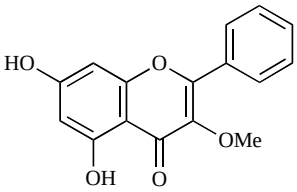

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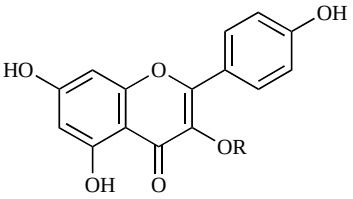
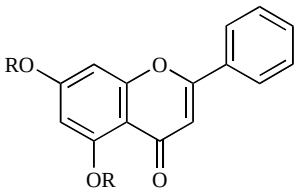
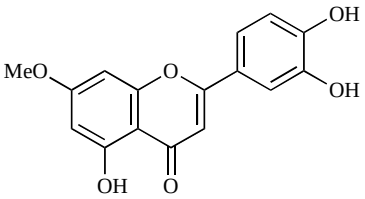


R
60 Glc-6-*O*-*p*-Cum



R
61 H
62 Me



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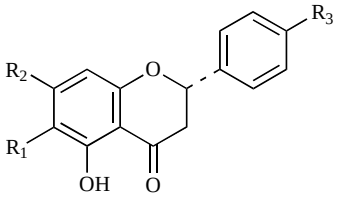
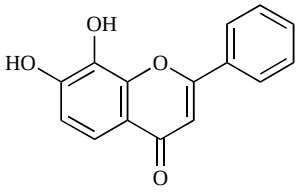


	R_1	R_2	R_3
64	H	OMe	OH
65	OH	OH	OMe



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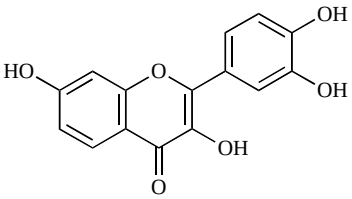

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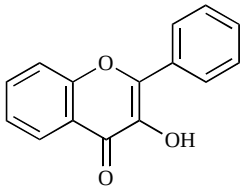

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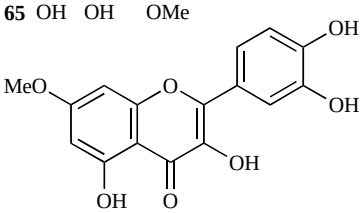

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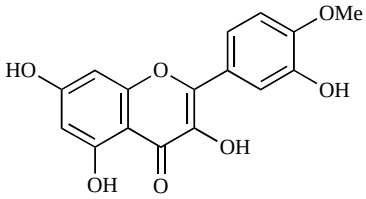

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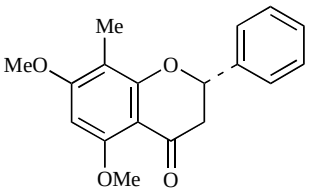

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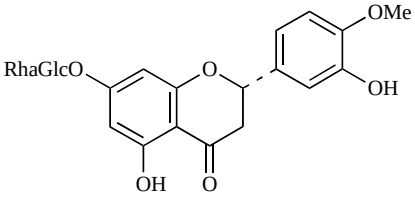

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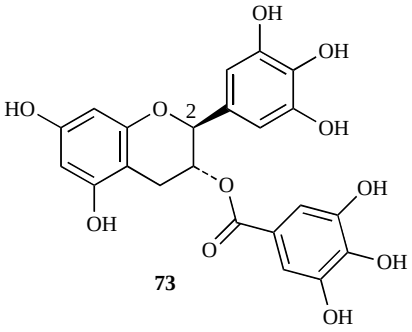

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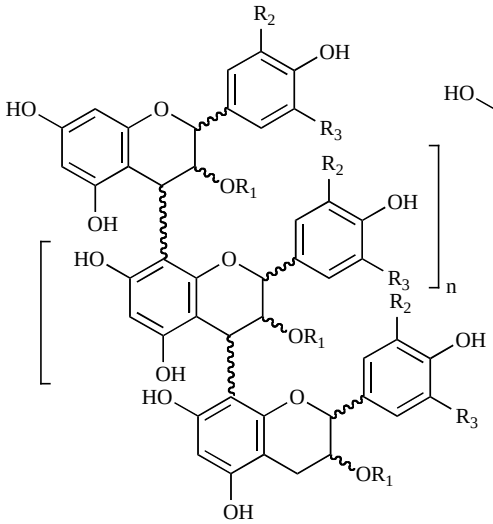
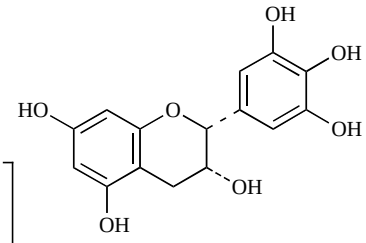


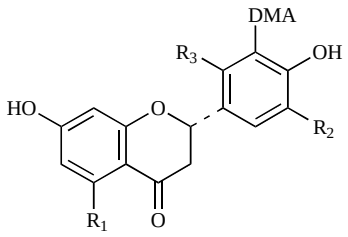
74a



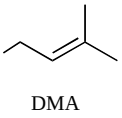
74

B

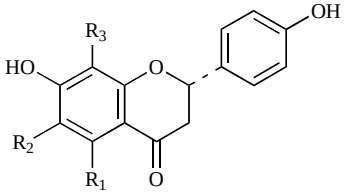
(Fig. 6). Contd…..



	R_1	R_2	R_3
75	H	DMA	H
76	OH	DMA	H
77	OH	OH	DMA



DMA



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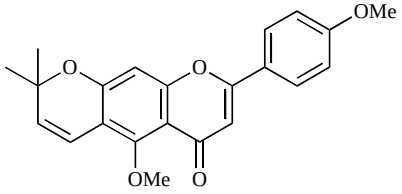
78	DMA	DMA	OH
79	OH	DMA	DMA



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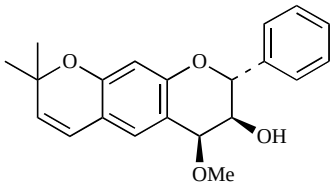

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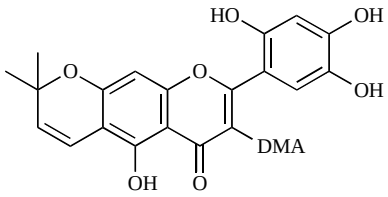

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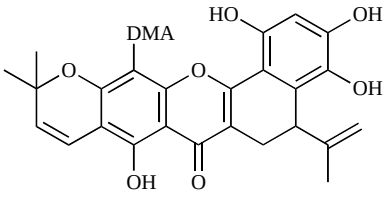

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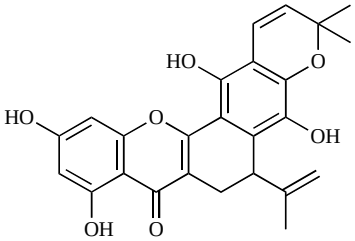

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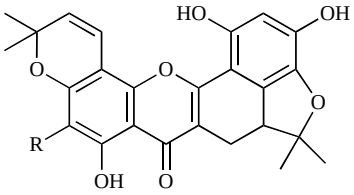
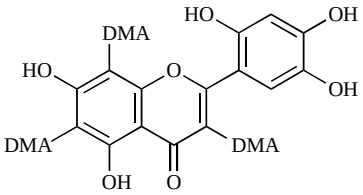


	R
85	DMA
86	H



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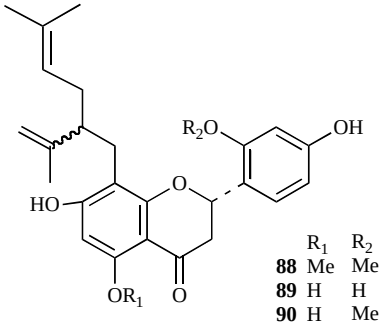

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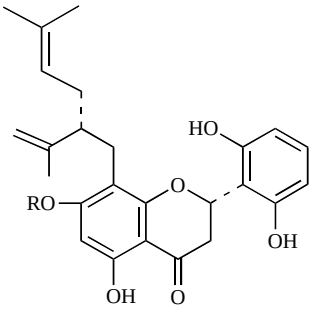
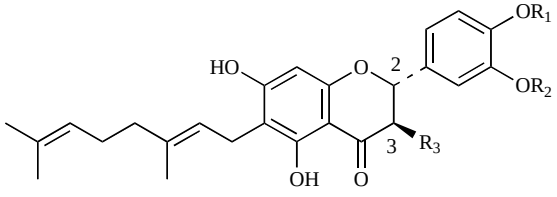
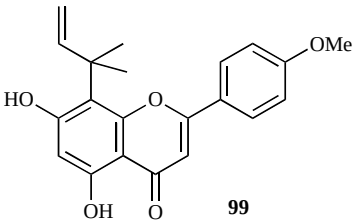
88	Me	Me
89	H	H
90	H	Me
91	Me	H



	R
92	Me
93	H



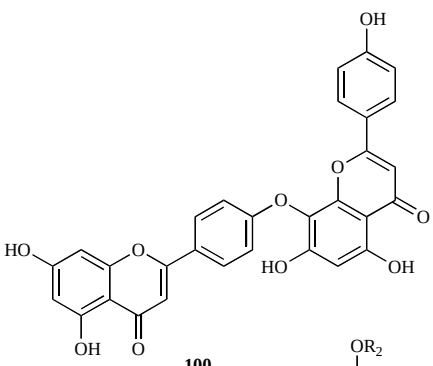
	R_1	R_2	R_3	
94	H	H	H	
95	H	Me	H	
96	Me	H	H	
97	H	Me	OH	
98	H	Me	H	$\Delta^{2,3}$



99

C

(Fig. 6). Contd…..



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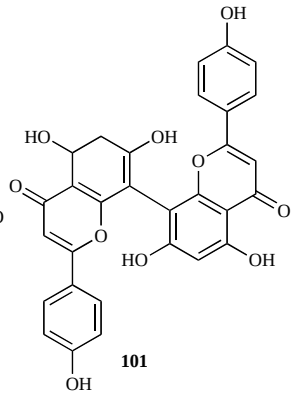

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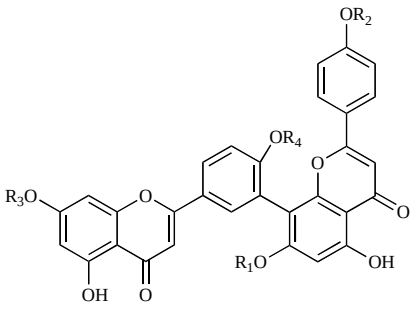
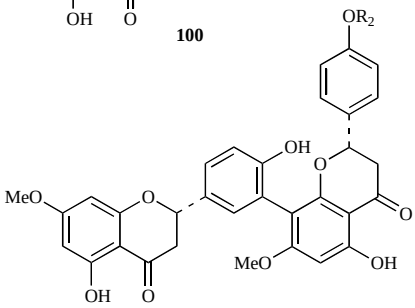


	R_1	R_2	R_3	R_4
102	H	H	H	H
103	H	Me	H	Me
104	Me	H	Me	Me
105	H	Me	H	Me
106	H	Me	Me	Me
107	Me	Me	Me	Me



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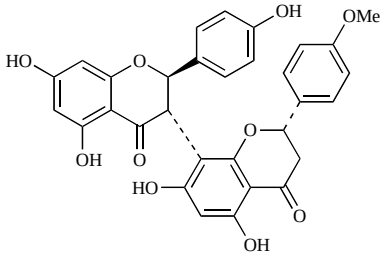

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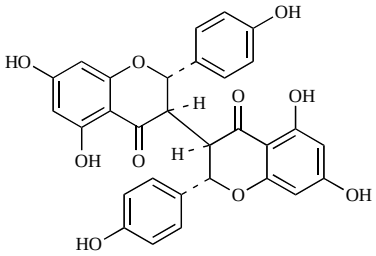

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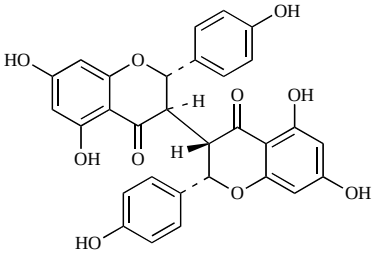

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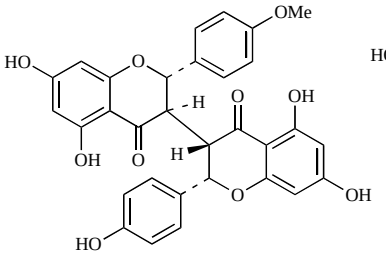

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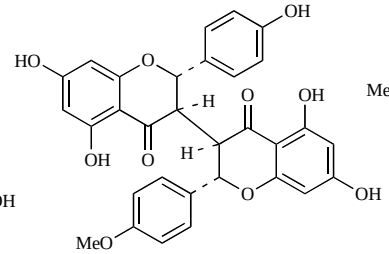

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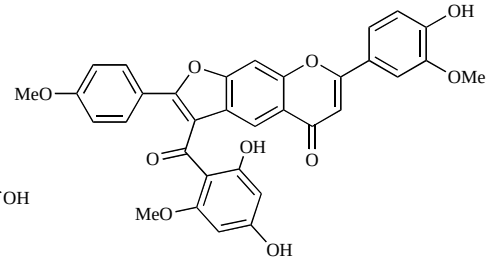

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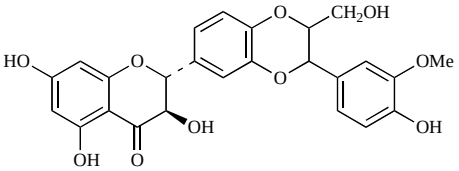

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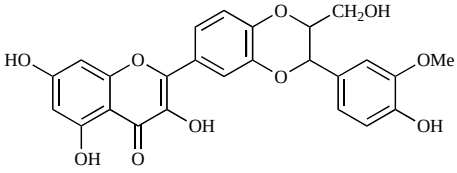


116

D

Fig. (6). Structures of flavonoids with antiprotozoal activity.

A. Simple flavonoids.

B. Simple flavonoids and some proanthocyanidins (**73**, **74**).

C. Prenylated flavonoids.

D. Biflavonoids and related compounds.

active derivative with an IC_{50} value of 0.8 μM and an SI of 205 (determined on Vero cells) [156]. Interestingly, peracetyl analogs with a second sugar moiety at C-7-*O* (paeonoside and petiolaroside decaacetates, structures not shown) were significantly less active. The infection rates of promastigotes on Vero cells and formation of intracellular amastigotes as well as trypomastigote forms were also tested *in vitro* and shown to be significantly reduced by the flavonoids. *In vivo* experiments in an acute mouse model (5 x 1 mg/kg/day of flavonoid) revealed a decrease in parasitaemia which correlated with the *in vitro* activity and was better than that of benznidazole applied in the same dose. Furthermore the survival rates were higher than those of the positive control group (100 vs. 80%) [156]. These flavonoids hence appear very interesting as potential anti-chagasic drugs or leads.

Three kaempferol glycosides (**24**-**26**) from *Hydrangea macrophylla* var. *thunbergii* (Hydrangeaceae) were reported to show *in vitro* activity against *Pfc*. They were tested together with some further flavonol glycosides such as quercitrin, isoquercitrin and rutin (**12**-**14**) at concentrations between 0.05 and 5 ng/mL. The compounds were reported to inhibit approximately 60% of parasite proliferation at a concentration as low as 0.05 ng/mL which appears extremely low in comparison with data reported for similar compounds in other investigations. No or very low cytotoxicity was observed. Quite curiously however, most of the compounds displayed their activity against *Pfc* without a clear concentration-dependency and it was also not possible to kill 100% of the parasites with higher concentrations [157].

The antileishmanial activity of *Kalanchoe pinnata* (Crassulaceae) has been demonstrated *in vitro* and *in vivo* [158]. Quercitrin (**12**), isolated from this plant, was demonstrated to kill intracellular amastigotes of *Lam in vitro* with IC_{50} values of 1 μg/mL [159] and 8 μg/mL (18 μM) [160]. Therefore, the aglycone quercetin (**4**) as well as quercitrin (**12**) and the quercetin-3-*O*-arabinorhamnoside (**15**) from *K. pinnata* were tested *in vivo* against *Lam* in infected BALB/c mice. Intragastric gavage treatment for 30 days with 16 mg flavonoid/kg/day yielded similar efficacy as pentostam (i.p., 8 mg/kg, twice per week) used as reference drug. Reduction of parasitemia on day 68 ranged from 57 % (**12**) up to 76 % (**4**) in comparison with 62% for pentostam [158]. A homologous arabinorhamnoside of luteolin (**27**) as well as 8-methoxyisoquercitrin (not shown), isolated from the same plant, were found much less active (IC_{50} > 100 μM) against *Lam in vitro* than **12** (18 μM) and **15** (78 μM) [160].

A quercetin arabinofuranoside esterified at C-5-*O* with gallic acid (**16**) from *Calycolpus warszewiczianus* (Myrtaceae) was reported to show weak antiplasmodial activity against the chloroquine-resistant W2 strain of *Pfc* with an IC_{50} of 14.5 μM. The cytotoxic IC_{50} against Vero Cells was 86.5 μM. A second compound from this plant with somewhat lower activity was myricetin-3-*O*-α-L-arabinofuranoside (**19**; IC_{50}= 27.8 μM) [161].

Six flavones and flavonols (including **9** and several methyl ethers, **35**-**39**) from *Chromolaena hirsuta* (Asteraceae) were reported to possess *in vitro* antitrypanosomal activity against *Tcr* trypomastigotes. However, the reported IC_{50} values ranging between 102 (**36**) and 352 μg/mL (**37**), these activities were not impressively high. Three of the compounds (**9**, **38**, **39**) were also reported active against *Lam* promastigotes, compound **39** being the most active with IC_{50}= 87.9 μg/mL [162].

Methylated flavones from *Lychnophora salicifolia*, namely, quercetin-3,7,3′,4′-tetramethylether (**40**), luteolin-7,3′,4′-trimethyl-ether (**41**) and quercetin-7,3′,4′-trimethyl ether (**42**), displayed very low *in vitro* activity against *Tcr* trypomastigotes (IC_{50} = 697, 847 and 217 μM, respectively) [163].

Eupafolin (**43**), a 6-methoxyflavone from *Eupatorium perfoliatum* (Asteraceae) was recently shown to possess some antileishmanial (*Ldon* axenic amastigotes) and antiplasmodial (*Pfc*) activity. This flavonoid, isolated along with several guaianolide sesquiterpene lactones (see part 1 [1]), yielded IC_{50} values of 10.2 and 6.6 μM against the mentioned parasites, respectively. It might hence contribute, together with the terpenoids, to the reported folk medicinal use of the plant against malaria [164].

Two 6-methoxylated flavonoids, hispidulin (**44**) from *Ambrosia tenuifolia* and santin (**45**) from *Eupatorium buniifolium* (both Asteraceae growing in Argentina), were found to show *in vitro* activity against *Tcr* epimastigotes (both IC_{50} =47 μM) and trypomastigotes (IC_{50} = 62 and 42 μM, resp.) as well as *Lmex* promastigotes (IC_{50} = 6 and 32 μM, resp.). The IC_{50}s for cytotoxicity against murine T cells were > 50 μM in both cases [165].

Two out of four flavonoids isolated from *Trixis vauthieri* (Asteraceae), namely, the flavone penduletin (**46**) and the flavanone sakuranetin (**64**), displayed some *in vitro* activity against *Tcr* trypomastigotes. Both compounds were tested in a concentration of 500 μg/mL and showed 100 and 86 % trypanocidal activity at this relatively high concentration [166].

Five highly methoxylated/methylenedioxygenated flavones (**47**-**49**, **54**, **55**) were isolated from Sudanese *Ageratum conyzoides* (Asteraceae). While the crude dichloromethane extract of this plant had shown promisingly high *in vitro* activity especially against *Tbr* (IC_{50}=0.78 μg/mL; SI= 47 with L6 cells), none of the constituents isolated in this study (flavonoids and a chromene) showed activity in this range. The most active compound against *Tbr* was 4'-hydroxy-5,6,7,3',5'-pentamethoxyflavone (**49**) with an IC_{50} of 3.0 μg/mL (7.8 μM). This flavone also showed activity in the same concentration range with IC_{50} values of 3.6 μg/mL against *Ldon* and *Pfc* but was inactive against *Tcr* (IC_{50}>30 μg/mL) and not cytotoxic against L6 cells (IC_{50}>90 μg/mL). 5,6,7,3',4',5'-hexamethoxyflavone (**48**) was slightly more active against *Pfc* (3.0 μg/mL) but also more cytotoxic (SI = 2). Compounds **47** and **55** showed weak activity against *Tcr* intracellular amastigotes (IC_{50}= 26.4 and 19.5 μg/mL). The high activity of the crude extract could not be reconstituted when the isolated flavonoids were tested as a mixture in approximately their natural ratio. Since a chromene isolated from the same extract also displayed very little activity against *Tbr* (IC_{50} = 78 μg/mL), the active principle of the extract had to remain unidentified [167] (compare section 3.1 of part I [1] and section 4.2 of the current work).

A flavone very similar to the ones just mentioned, 5,7,3',4'-tetramethoxyflavone (**50**), isolated from *Kaempferia parviflora* (Zingiberaceae), has also been reported to possess *in vitro* antiplasmodial activity. Compound **50** showed an IC_{50} against *Pfc* of 4.06 μg/mL [168], which was comparable to that of the compounds from [167].

From *Neoraputia magnifica* (Rutaceae), methylated and methylenedioxygenated flavonoids very similar to those of the last mentioned studies were isolated. 5,7,3',4',5'-pentamethoxyflavone (**53**) was isolated along with **55**, two further methylenedioxy-flavonoids and four chalcone derivatives. The flavonoid **53** and a mixture of two methylenedioxy analogues were found to inhibit *Tcr* glyceraldehyde-3-phosphate dehydrogenase (*Tc*GAPDH) showing approximately 50% enzyme inhibition at 20-30 μg/mL; a mixture of two of the chalcones (see secion 4.1.6 below) was less active [169].

In a series of five flavonoids (four with unsubstituted B-ring) isolated from *Lychnophora markgravii* (Asteraceae) and tested against *Lam* amastigotes *in vitro*, the flavanone pinostrobin **56** and its flavone analog tectochrysine **58** showed the most promising activity (IC_{50} = 0.31 and 0.55 μM, respectively). The related compounds pinocembrin (**57**) and galangin-3-methylether (**59**) were somewhat less active (IC_{50}= 3.45 and 2.89 μM). Quite noteworthy is the relatively high activity of tiliroside (**60** = kaempferol-3-*O*-(6-*O*-*p*-cumaroyl)-glucoside; IC_{50}= 1.1 μM) [170] also known from linden flowers.

Pinostrobin and pinocembrin (**56** and **57**) were also included in a study on the activity of various kinds of natural products against *Ldon*, *Tcr*, *Tbr* and *Pfc*, along with the acetate of **57** as well as chrysin **61** and 7-*O*-methylluteolin **63**. Activity against *Ldon* promastigotes varied between 35.7 (**63**) and 550 μM (**56**). None of the compounds showed activity against amastigotes of this parasite. **57**, **61** and **63** showed moderate activity against *Tbr* trypomastigotes (IC_{50}= 10.5, 13.5 and 13.3 μM, respectively) and *Pfc* (K1 strain; IC_{50}= 155, 195 and 46 μM) [55].

A flavanone (**65**) isolated from *Baccharis retusa* (Asteraceae) was assayed *in vitro* against promastigotes of various *Leishmania* species (*Lcha*, *Lam*, *Lmaj*, *Lbra*) and showed IC_{50} values between 40 and 54 μg/mL. Activity against *Lcha* amastigotes was in the same concentration range. Its activity against *Tcr* was also assayed where it displayed an IC_{50} of 20.39 μg/mL against promastigotes but was inactive against intracellular amastigotes [171].

The probably largest set of flavonoids with representatives of all major subtypes (flavones, flavonols, flavanones, flavan-3-ols, isoflavonoids) was investigated by Tasdemir *et al*. [155] for *in vitro* activity against *Ldon* (axenic amastigotes), *Tbr* (trypomastigotes) and *Tcr* (intracellular amastigotes). Cytotoxicity was assessed with L6 cells (SI values); some compounds of other related classes of phenolics were also included. The most active compounds within the respective subgroups were: flavones (32 compounds, including 5 glycosides and 1 biflavone): luteolin (**1**) (*Ldon*: IC_{50}=0.8 μg/mL; SI=12), 7,8-dihydroxyflavone (**66**) (*Tbr*: IC_{50}=0.068 μg/mL; SI=116) and chrysin dimethylether (**62**) (*Tcr*: IC_{50}=3.9 μg/mL; SI=6); flavonols (25 compounds including 5 glycosides): fisetin (**67**) (*Ldon*: IC_{50}=0.7 μg/mL; SI=64), 3-hydroxyflavone and rhamnetin (**68**, **69**) (*Tbr*: both IC_{50}=0.5 μg/mL; SI=21, >180, resp.), tamarixetin (**70**) (*Tcr*: IC_{50}=6.4 μg/mL; SI=7); flavanones (7 compounds, including 2 glycosides): 5,7-dimethoxy-8-methylflavanone (**71**) (*Ldon*: IC_{50}=2.4 μg/mL and *Tcr*: IC_{50}=13.6 μg/mL; SI=16 and 3, resp.), neohesperidin (**72**) (*Tbr*: IC_{50}=11.5 μg/mL; SI>7.8); Isoflavones (6 compounds): biochanin A (structure **2** in Fig. **7**) (*Ldon*: IC_{50}=2.5 μg/mL; SI=26), genistein (structure **1** in Fig. **7**)(*Tbr*: IC_{50}=1.3 μg/mL; SI=16), 3'-hydroxydaidzein (structure **3** in Fig. **7**) (*Tcr*: IC_{50}=4.7 μg/mL; SI=4.5); flavan-3-ols (catechins; 10 compounds): (-)-gallocatechingallate (**73**) (*Ldon*: IC_{50}=8.9 μg/mL; *Tbr*: IC_{50}=3.7 μg/mL; SI=1.7 and 4, resp.), (-)-epigallocatechin (**74**) (*Tcr*: IC_{50}=80.7 μg/mL; SI=0.2).

It is interesting to note that, in spite of the large and coherent data set, it was not possible to draw clear conclusions on structure-activity relationships for any of the studied activities. It was not possible to derive QSAR models by classical as well as 3D-QSAR methods [155]. It may thus be concluded that the activity of the various compounds is not caused by a common mechanism which is an essential requirement for the successful application of QSAR methods.

Several of the more active compounds were also tested *in vivo* against *Ldon* (BALB/c mice, HU3 strain; 30 mg/kg i.p. for 5 days) and *Tbb* (STIB795). In the former assay, only quercetin (**9**) displayed moderate activity (15% reduction of parasitaemia, while in the latter 7,8-dihydroxyflavone led to an increase of survival time but did not cure the mice (relapse after withdrawal of the drug) [155].

Mechanistic Aspects of Flavonoid Action

Taken the interesting potential of flavonoids as antiprotozoal agents, a number of studies have shed light on potential mechanisms that can be held responsible for the activity of these compounds.

In the above cited study on antimalarial activity of common dietary flavonoids, luteolin (**1**) was found to arrest the intraerythrocytotic cycle of *Pfc* at the early trophozoite stage [148]. Cell cycle arrest by **1** and quercetin (**9**) had also been reported previously for *Ldon* where an influence on DNA-topoisomerase II was found as likely mechanism of action [172]. Quercetin (**9**) was also reported to inhibit induction of bradyzoite development in *Toxoplasma gondii*, by inhibiting the synthesis of heat shock proteins [173].

Another study has shown that **9** deprives ribonucleotide reductase in *Ldon* of iron due to its chelating property, and thereby inhibits parasite DNA synthesis. In this study it was also demonstrated that the activity of **9** is enhanced by serum albumin and that myricetin (**18**) is less active [174]. **9** and some related flavonoids were also demonstrated to arrest the development of anaemia in *Ldon* infected animals, probably due to the antioxidant and radical scavenging activity that ameliorates oxidative stress in the post-infection period [175].

The effect of **9** on human african trypanosomiasis (HAT) was reported to be due not only to a direct trypanocidal effect (*Tbg*: $IC_{50}\approx$ 2 μM) but also to a decrease in pro-inflammatory response of the host cells (decrease of TNF-α and NO synthesis in activated human macrophages) [176], which is in line with the well known anti-inflammatory properties of such flavonoids [144-147].

Most recently, **9** has been demonstrated to inhibit *Tb* hexokinase I, an enzyme essentially required for this parasite's survival, with an IC_{50} of 4.1 μM [177]. *Tb* bloodstream forms rely entirely on glycolysis for ATP synthesis and their hexokinase as the first key enzyme of glycolysis is thus a very promising target for anti-HAT drugs.

Very noteworthy is the finding that **1**, **9**, and some further flavonoids with di- or trihydroxy substituted B-ring are potent inhibitors of fatty acid synthesis in *Pfc*. Inhibitory effects in the low micromolar and submicromolar concentration range were found against FabG, FabZ and FabI (β-ketoacyl reductase, β-hydroxyacyl dehydratase and enoylreductase subunits of *Pfc* fatty acid synthase), the latter being generally most sensitive. Most prominently, myricetin (**18**) showed an IC_{50} against FabI of only 0.4 μM [178]. This interesting mechanism, also found for some procyanidin derivatives (see section 4.1.2 below) should hence be -at least partly- responsible for the antiplasmodial activity of these flavonoids.

Flavonoids as MDR Reversal Agents

Besides direct toxicity against protozoan parasites exhibited by flavonoids, these compounds as well as some isoflavonoids and chalcones (see sections 4.1.5 and 4.1.6 below) are also known as agents that can revert the multidrug resistance (MDR) of the parasites.

This phenomenon has long been known in case of human cancer cells [179, 180], but also in case of protozoan parasites [181]. It has been thoroughly reviewed already in 2002 [182].

Quite recently, two flavonols, **51** and **52** with a high degree of methylation from *Aeonium lindleyi* (Crassulaceae), from which they were isolated together with some leishmanicidal diterpenes (see part I [1]), were demonstrated to reverse drug resistance to daunomycin in a multidrug resistant (MDR) *Ltro* strain to some degree [183]. However, the capability to reverse MDR is not a common property of all flavonoids as was recently demonstrated in a study on (8-1;1)-dimethylallylkaempferide (**99**), silibinin (**115**) and dehydrosilibinin (**116**), in which **99** and **116** showed some direct antiplasmodial activity (see below) but none of the compounds was able to reverse chloroquine resistance [184].

Whatsoever, flavonoids occur very frequently in plant extracts, especially in such from green parts, so that the capability to interact with MDR transporters may be interesting with respect to the activity of crude extracts which are sometimes more active than their isolated constituents. It can easily be conceived that the antiprotozoal activity of other secondary metabolites may be higher

in an extract, where such reversal agents are present, than in isolated form.

4.1.2. Proanthocyanidins

Monomeric proanthocyanidins (flavan-3-ols or "catechins") and leukoanthocyanidins (flavan-3,4-diols) are formed by reduction of flavanonols. They usually occur in the form of oligo- and polymers (for some representative structures see Fig. (**6B**)) formed by condensation of two or more subunits which are also termed "condensed tannins". The most common type of linkage (4-8-type) in such oligomers is represented in structure **74a**. Such polyphenols have many biological activities associated with their tendency to interact with literally all kinds of proteins.

As already mentioned in section 4.1.1, monomeric procyanidins such as (-)-epigallocatechin and (-)-gallocatechin gallate (**73** and **74**) have shown *in vitro* antiprotozoal activity [155]. A number of studies have investigated the antiprotozoal potential of such compounds.

Thus, e.g. Kolodziej *et al.* [41] studied the antileishmanial effects of various mono-, oligo- and polymeric proanthocyanidin derivatives on extracellular and intracellular forms of *Ldon in vitro*. Most prominently, some of the compounds exhibited activity on the intracellular amastigote form at nanomolar concentrations. Polymeric procyanidingallate (**74a**, R_1=Galloyl, R_2=H, R_3=OH; C-2*R*) was reportedly the most active compound with an IC_{50} of only 0.7 nM; its toxicity vs. RAW macrophages was >10 nM. As a simple structure-activity relationship, the activity was related to the presence of an acyl moiety at C-3. It was furthermore found that the test compounds increased the formation of pro-inflammatory factors by the macrophages (TNF-α, NO, IFN-γ) so that the procyanidins may contribute to the crucial transformation of macrophages from host cells to leishmanicidal effector cells [41]. Based on studies showing that proanthocyanidin-rich extracts as well as individual compounds such as epigallocatechin gallate (EGCG: C-2 epimer of **73**) are inhibitors of ornithine decarboxylase (ODC), an enzyme necessary for trypanothione synthesis, the potential of Green Tea (*Camellia sinensis*, Theaceae) extract as a new therapeutical for *Leishmaniasis* has recently been emphasized [185].

Oligomeric proanthocyanidins (mixtures of di- to nonameric epicatechins) isolated from the Cola Nut (*Kola acuminata*, Malvaceae), were reported to inhibit *in vitro* growth of bloodstream forms of *Tb* (IC_{50} = 14.7 μg/mL) [186]. *In vivo* experiments with infected mice showed that the effect was rather a trypanostatic than a trypanocidal effect since no decrease of parasitemia was observed, but the animals had an increased survival time after i.v. application of 14 mg/kg/day. An inverse correlation of the *in vitro* effect with the serum albumin content of the media was also found [186], which is quite expectable taken the "tannin" character of such compounds.

Gallocatechin gallate (GCG, **73**) and EGCG from green tea were furthermore reported to be very active *in vitro* against *Tcr* bloodstream forms. They were reported to kill the trypomastigotes at extremely low concentrations, with "MBC_{50}" values of 0.12 and 0.53 pM, respectively. At a concentration of 100 nM both compounds also reduced the number of infected macrophages by 50%. Therefore, besides the potential of lead compounds for antichagasic therapeutics, the authors proposed to use these compounds to sterilize blood from *Tcr* parasites [187].

Green tea and constituents have also been reported to possess antimalarial potential. Epigallocatchin- and Epicatechin gallates were found to inhibit *Pfc in vitro* growth with IC_{50}s of ca 30 and 10 μM (3d7 and FCR-1/FVO strains) [188]. Several mechanistic studies have shown that the antiplasmodial effects of such compounds are (a) not due to interference with folate metabolism [188], (b) not due to inhibition of hexose transporters [189] and (c) probably related to interference with the parasites' fatty acid biosynthesis [178, 190]. Thus, momomeric procyanidin gallates, but not the unesterified compounds, were shown to inhibit components of the fatty acid synthase complex of *Pfc* (FabG, FabZ and FabI) in low micromolar and submicromolar concentration [178]. EGCG was more recently shown to be a slow-tight binding nanomolar inhibitor of Enoyl-ACP reductase (=FabI) [190].

Taken together, these studies indicate an interesting potential of procyanidins. Development of an orally applicable therapy for any of the diseases based on such compounds will, however, be immensely difficult, due to their chemical and metabolic instability and poor oral bioavailability [191].

4.1.3. Prenylated Flavonoids

A variety of prenylated flavanones are known to occur characteristically in Fabaceae along with isoflavonoids (see next section). Such compounds also occur sporadically in other plant families. Structures mentioned in this section are shown in Fig. (**6C**).

Several species of the genus *Erythrina* (Fabaceae) have been shown to contain prenylflavanones with antiprotozoal activity. Abyssinone-IV (**75**) [192] as well as abyssinone-V (**76**) and abyssinin-III (**77**) [193] were the prenylflavanones with most potent *in vitro* antiplasmodial acivity isolated from *E. abyssinica*. They were tested along with a variety of further related compounds from this plant against the D6 and W2 strains of *Pfc* and yielded the following respective IC_{50} values (μM): **75**: 9.0, 7.7 [192]; **76**: 4.9, 6.1; **77**: 5.8, 5.2 [193]). **76**, also found in *E. sacleuxii* [194] was recently demonstrated to act synergistically together with artemisinin [195].

Similar compounds were also identified in *E. fusca*, of which lonchocarpol A (**78**) was the most potent against *Pfc*, strains K1 (chloroquine insensitive) and FCR-3 (sensitive) with IC_{50} values of 1.6 and 3.0 μg/mL and a cytotoxic IC_{50} against MRC-5 cells of 12.0 μg/mL. Three further prenylflavanones yielded antiplasmodial IC_{50} values between 3.9 and >12.5 μg/mL [196]. Another prenylflavanone (**79**), isolated along with several related compounds and some isoflavonoids from the same plant in another study [197], also displayed activity against *Pfc* K1 (IC_{50}= 9.2 μg/mL) but was also cytotoxic against three mammalian cell lines in the same concentration range. A pterocarpane from the same study was similar in activity (see section 4.1.5 below) [197].

Plants of the genus *Lonchocarpus* (Fabaceae) yielded a series of prenylated flavonoids which were tested *in vitro* against promastigotes of various *Leishmania* species (*Lbra*, *Ldon*, *Lam*), *Tcr* epimastigotes, as well as against *Pfc*. The most active compound against the kinetoplastids was the flavone **80** with reported IC_{50}s of 5.6 μg/mL agaist each of the mentioned kinetoplastids and a cytotoxic IC_{50} against P388 murine leukemia cells of 34 μg/mL. This compound was inactive against *Pfc*. On the other hand, a 4-methoxy-flavane-3-ol derivative **81**, inactive against the kinetoplastids, showed some antiplasmodial activity (IC_{50}= 3.6 μg/mL) and low cytotoxicity (IC_{50}= 76 μM) [198].

From the root bark of *Artocarpus styracifolius*, a plant from the Moraceae family growing in Vietnam, 8 complex prenylated flavones were isolated and tested against chloroquine resistant *Pfc* (FcB1 strain) and *Tbb*. Three of these showed antiplasmodial activity with IC_{50} values below 2 μM, namely, heterophyllin (**82**) (1.20 μM), artonin B (**83**) (1.56 μM) and styracifolin B (**87**) (1.12 μM). Their IC_{90} values were about twofold higher and their IC_{50} values for cytotoxicity against KB and MRC-5 tumor cells were in the range from 3.8 to 11.3 μM. They also displayed some activity against *Tbb*, however in the same concentration range as cytotoxicity, i.e. IC_{50}= 6.7, 8.8 and 6.9 μM, respectively [199].

From *Artocarpus rigidus*, a series of six similar compounds was isolated and tested *in vitro* against *Pfc*. Three compounds (**84**-**86**)

were active with IC_{50} values of 7.9, 2.4 and 3.7 μg/mL, respectively. Compound **85** showed some selectivity since it did not display cytotoxicity up to 20 μg/mL against KB, BC and NCI-H187 cancer cells [200].

Four flavanones bearing a lavandulyl monoterpenoid prenyl side chain were isolated from *Sophora flavescens* (Fabaceae). The *in vitro* antiplasmodial activity of three compounds (**88**-**90**) was at the same level with IC_{50} values of 2.4, 2.6 and 2.1 μM, respectively. The compounds displayed no selectivity with respect to their cytotoxicity data (FM3A cell line): IC_{50}= 3.3, 0.04 and 1.1 μM, respectively. The activity of the fourth derivative (**91**) was dramatically lower (IC_{50} for *Pfc* and cytotoxicity both > 20 μM) which is quite noteworthy considering the great structural similarity to **88**-**90** [201].

Two lavandulyl flavanones, exiguaflavanones A and B (**92**, **93**), structurally related to those from *Sophora flavescens*, were obtained from *Artemisia indica* (Asteraceae). Their *in vitro* activity against *Pfc* (K1 strain) was somewhat lower than the ones just mentioned, with IC_{50}s of 4.6 and 7.1 μg/mL, respectively. Two further compounds, a pterocarpane and a benzofurane derivative (see below), were less active than the flavanones [202].

Four flavanones substituted with a geranyl monoterpenoid side chain as well as one homologous flavone derivative were recently found in *Mimulus bigelovii* (Phrymaceae) and tested against *Ldon* (axenic amastigotes) and *Tbb* trypomastigotes. Interestingly, the flavanones (**94**, **95**+**96**, **97**) were more active against *Ldon* than the flavone **98** (IC_{50}= 4.8, 7.5 and 7.2 vs. 14.6 μg/mL, respectively) whereas against *Tbb* the latter was more active (IC_{50}= 1.9 μg/mL) than **95**+**96** (7.2 μg/mL) and **97** (3.3 μg/mL) and almost as active as diplacone **94** (1.4 μg/mL) [203].

A prenylated kaempferide derivative with a 3,3-dimethylallyl moiety attached by 3-8 linkage (**99**) was reported to inhibit *in vitro* growth of five *Pfc* strains with IC_{50} and IC_{90} values ranging from 2.1 to 10.1 μM and from 7.7 to 26.5 μM, respectively [184]. In the same study, the flavonolignan dehydrosilibinin (**116**, Fig. **6D**) was also tested and found similarly active (IC_{50} from 1.7 to 23.9 μM, IC_{90} from 5.4 to 41.7 μM). The drug resistant strain 7G8 was most sensitive against both compounds [184].

4.1.4. Biflavonoids

Dimeric flavonoids occur much less frequently than monomers. Various existing dimerization modes give rise to considerable structural diversity. They are often found in Anacardiaceae, Clusiaceae and in some gymnosperms. A number of such derivatives (structures shown in Fig. **(6D)**) have been reported to act against protozoan pathogens.

A series of nine biflavones (**100**-**107**) were tested by Weniger *et al.* against a panel of protozoan parasites [204]. The most active compound of this series against *Pfc* (K1) was lanaroflavone (**100**) which displayed an IC_{50} value of 0.48 μM. A similar value was reported for this compound after its original isolation from *Campanosperma panamense* (Anacardiaceae) [205]. Sciadopitysin (**106**) and Ginkgetin (**104**) were also quite active against *Pfc* with IC_{50}s of 1.4 and 2.0 μM [204]. Isoginkgetin (**105**) was the most active derivative against *Ldon* axenic amastigotes (IC_{50}= 1.9 μM). *Tcr* was the least sensitive against the biflavones, the highest activity being displayed by ginkgetin (**104**: IC_{50}= 11 μM). **105** was again the most active against *Tbr* (IC_{50}= 3.5 μM). Cytotoxicity against L6 rat skeletal myoblasts was somewhat lower for all active derivatives (ranging from 9 (**104**) to 68 (**106**) μM) so that there appears to be some selectivity. Cupressusflavone (**101**) and amentoflavone (**102**) as well as amentoflavone tetramethylether (**107**) did not show any significant activity against any of the protozoa.

From *Rhus retinorrhoea* (Anacardiaceae), a biflavanone derivative (**108**) was isolated which showed antiplasmodial activity against the W2 and D6 strains of *Pfc* (IC_{50}= 0.98 and 2.8 μg/mL), while not showing any cytotoxicity [206].

Several biflavanones with a 3-8'-linkage showing antiprotozoal activity were described from *Garcinia livingstonei* (Clusiaceae) [207], *Ormocarpum kirkii* (Fabaceae) [208] and *Wikstroemia indica* (Thymelaeaceae) [209].

From *G. livingstonei*, besides several xanthone derivatives (see section 4.3 below), three biflavanones were isolated, of which one (**109**) had significant antiplasmodial activity (IC_{50} against *Pfc* 6.0 μM) and also showed effects against *Tbb* and *Tcr* (IC_{50}≈ 28 and 35 μM) [207].

From *O. kirkii*, besides other constituents, two biflavanones (**110**, **111**) showed moderate activity against some of the tested protozoa. **111** was active against *Pfc* K1 (IC_{50}= 7.3 μM) while the isomer **110** was more active against *Tcr* (IC_{50}= 19.9 μM). However, selectivity was quite low, IC_{50}s for **110** and **111** against MRC-5 cells being 18.3 and 29.0 μM, respectively [208].

Quite interestingly, two biflavanones isolated from *W. indica* (**112**, **113**) with very similar structures to those just mentioned showed much stronger antiplasmodial activity when tested against *Pfc* (K1 and FCR3 strains). Against the drug-resistant K1 strain, IC_{50} values of 0.54 and 0.56 μg/mL (0.96 and 1.00 μM) were measured and against the sensitive FCR3 strain, the values were similarly low (0.54 and 0.34 μg/mL, respectively) [209]. **112** and **113** differ from the much less active **111** and **110** merely in the methylation of one OH group. Thus, methylation in this series appears to be of profound impact on antiplasmodial activity and systematic studies on this issue would be highly interesting.

A dimeric flavonoid compound in which a flavone unit is linked to a second subunit of the chalcone type, (chalcones actually represent the biogenetic precursors of flavonoids, see section 4.1.6), is cissampeloflavone (**114**), isolated from *Cissampelos pareira*, a plant of the Menispermaceae family growing in South America. This compound has been demonstrated to show interesting antiprotozoal activity, especially with respect to trypanosomes. It showed *in vitro* ED_{50} values against *Tcr* (intracellular amastigotes) and against *Tbr* (trypomastigotes) of 2.09 and 0.61 μg/mL and at the same time only very low cytotoxicity against KB cells (106 μg/mL) which make it a promising candidate for further studies [210]. No reports could be found, however, on a potential *in vivo* activity of this compound.

4.1.5. Isoflavonoids and Derivatives

Isoflavonoids are derived from normal flavonoids by rearrangement of ring B from C-2 to C-3. Apart from normal and prenylated isoflavones, a variety of derivatives formed by further cyclization such as pterocarpanes and rotenones as well as benzofuranoid degradation products have been reported to show antiprotozoal activity. Isoflavonoids and their derivatives typically occur in Fabaceae (Leguminosae) but sporadically are also isolated from other plant families. Therefore, family names are only reported in this section in cases where such compounds were isolated from other families than Fabaceae. The structures of isoflavonoids and their derivatives mentioned here are depicted in Fig. (**7**).

Normal isoflavone derivatives such as genistein (**1**), biochanin A (**2**), prunetin (**4**), formononetin (**5**) and calycosin (**6**) have been reported from *Andira inermis* (Fabaceae). These compounds were tested against *Pfc* strains poW and Dd2 (chloroquine-sensitive and -resistant, respectively). Genistein (**1**) was the most active compound (IC_{50}= 2.0 and 4.1 μg/mL, resp.), followed by calycosin (**6**: IC_{50}= 4.2 and 9.8 μg/mL, resp.). Prunetin (**4**), biochanin A (**2**) and formononetin (**5**) were distinctly less active, with IC_{50}s ranging from 28 (**3**) to >50 (**4**) μg/mL against the more sensitive poW strain [211]. Methylation at C-4'-OH appears to render the compounds less active as exemplified by direct comparison of **3** with **2** and **5**.

Biochanin A (**2**), thus weakly active against *Pfc*, was also tested against *Tcr* trypomastigotes and *Lcha* promastigotes after isolation from *Cassia fistula*. It was reported to show activity with IC_{50} values of 18.3 μg/mL (*Tcr*) and 19.0 μg/mL (*Lcha*) and to be less toxic to LLC-MK2 macrophages (IC_{50}=42.6 μg/mL) [212].

Genistein (**1**) was tested for activity against *Ldon* (axenic amastigotes) and *Tbb* (trypomastigotes) after isolation from *Psorothamnus arborescens*. Against *Tbb* it showed activity with IC_{50}= 4.2 μM whereas it was much less active against *Ldon* (IC_{50}= 73.0 μM) and also less toxic against mammalian cells (IC_{50}= 32.9 μM, Vero cells) [213].

Dalparvone (**7**) was the only active compound of 11 flavonoids/isoflavonoids and related derivatives from *Dalbergia parviflora* tested against *Pfc*. It displayed an IC_{50} value of 8.2 μg/mL (24.8 μM) but was only little less toxic against mammalian cells (KB and NCI-H187 cells; IC_{50}= 12.2 and 15.2 μg/mL, resp.) [214]. A related isoflavone (**8**) from *Dalbergia louvelii* showed somewhat higher activity against *Pfc* (IC_{50}= 6.8 μM) [215].

A related compound, afrormosin (**9**) from *Dipteryx odorata*, was reported to inhibit the glyceraldehyde-3-phosphate dehydrogenase of *Tcr* (*Tc*GAPDH). The IC_{50} value of 84 μM was rather high [216], so that it is not clear whether this will play a role in a live parasite within a host cell. No indication on a trypanocidal effect of **9** was found. Three structurally similar isoflavones from *Virola surinamensis* (Myristicaceae; **10**-**12**), at any rate, were not active against *Tcr* trypomastigotes (all IC_{50}> 50 μg/mL) [18], whereas the lignans of this plant showed activity (see section 2.1.).

Two isoflavones with methylenedioxygenated B ring (**13**, **14**) were isolated from *Millettia puguensis*. Only **13** showed low activity against *Linf* (stage not reported) with an IC_{50} of 32 μM while the isomer **14** was inactive. Both were inactive against *Tcr*, *Tbb* as well as *Pfc* [217].

Isoflavones with a prenylated B ring were reported from *Erythrina sacleuxii* (**15**, **16**) [194] and from *Psorothamnus arborescens* (**17**) [213]. The former two were found to exhibit *in vitro* activity against *Pfc*, strains D6 and W2, with IC_{50} values of 17.6 and 22.5 μM (**15**), and 6.3 and 8.7 μM (**16**), respectively [194]. Compound **17** was tested against *Ldon* (axenic amastigotes) and *Tbb* (trypomastigotes) yielding IC_{50} values of 13.0 and 12.1 μM, respectively, while its cytotoxicity against Vero cells was somewhat lower (IC_{50}= 37.5 μM) [213].

Prenylated isoflavanones of the sophoronol series were isolated from *Sophora mollis*. Of 12 isolated compounds, sophoronol E (**18**) was the most active *in vitro* against *Pfc* (D10 strain) with an IC_{50} of 12.8 μM. Its antiplasmodial effect was also fairly selective in relation to toxicity against CHO cells (SI=13) [218].

Barbigerone (**19**), an isoflavone with a prenyl sidechain cyclised to a chromene structure, was reported as a constituent of *Millettia usaramensis*, from which it was isolated together with a variety of rotenone derivatives such as 12a-epimillettosin (**20**). Both, **18** and **19** showed a similar level of activity against *Pfc*, W2 and D6 strains, respectively, with IC_{50} values of 27 μM (**18**, both strains) and 22 and 19 μM, respectively (**19**) [219].

An isoflavane with a B-ring oxidized to a *p*-benzoquinone, abruquinone B (**21**) from *Abrus precatorius*, showed a relatively strong *in vitro* effect against *Pfc*, K1 strain. Its IC_{50} value was 1.5 μg/mL (3.8 μM). At the same time, **21** showed little toxicity against Vero-, KB- and BC cells (IC_{50} >50, 9.9 and 5.7 μg/mL, respectively). Quite noteworthy, a corresponding hydroquinone derivative (not shown) was inactive against *Pfc* [220].

Another derivative from *Erythrina abyssinica* (see above), the isoflavene **22**, showed weaker activity against *Pfc* than the abyssinin and abyssinone derivatives mentioned in the previous section. Its IC_{50}s against *Pfc* W2 and D6 strains were 28 and 18 μM, respectively [192].

Three structurally related isoflavanes from *Smirnowia iranica* (**23**-**25**) were tested *in vitro* against *Pfc* (3D7 strain) and various *Leishmania* species. Their antiplasmodial IC_{50} values were 10, 33 and 58 μM, respectively. In the antileishmanial assays, **23** displayed the strongest activity when tested against promastigotes of *Ldon*, *Linf* and *Lmaj* (IC_{50}= 6.9, 9.2 and 7.9 μM, respectively). Tests against intracellular amastigotes of *Ldon* and *Linf* yielded values between 81 and 112 μM for the three derivatives [221].

Pterocarpane derivatives such as maackiain (**26**) and others have frequently shown antiprotozoal activity. Compond **26** was isolated from various plants already mentioned, i.e. *Millettia puguensis* [217], *Artemisia indica* [202], *Sophora mollis* [218] and *Psorothamnus arborescens* [213]. Its activity against *Tcr* was reported to be about 4 times higher (IC_{50}= 10 μM) than its toxicity against MRC-5 cells; no activity against *Linf*, *Tbb* or *Pfc* was found in this study [217]. Consistently, others reported very low activity against *Pfc* with IC_{50}s of 47 μg/mL (183 μM) (K1 strain) [202] and of 177 μM (D10 strain) [218]. Taken the inactivity of **26** against *Tbb* reported consistently by [218] and [213], it is quite surprising that a hydroxylated analog, **27**, isolated from *Psorothamnus arborescens*, was reported to be active against *Tbb* at an IC_{50} value as low as 3.7 μM [213]. It thus appears that a 7,8-dihydroxy (*o*-hydroquinoid) structure has a dramatic influence on these compounds' activity. However, **27** was also cytotoxic at the same concentration level (IC_{50}= 3.3 μM against Vero cells) [213] so that this effect unfortunately is not selective.

Two structural analogs of **26** additionally substituted with isoprenyl moieties, **28** and **29**, were isolated from *Harpalyce brasiliana*. They displayed antitrypanosomal activity against *Tcr* epimastigotes *in vitro* with IC_{50}s of 12.2 and 13.3 μg/mL and were non-toxic against isolated human peripheral blood mononuclear cells (PBMCs; IC_{50} both >50 μg/mL) [222].

A pterocarpane-like derivative isolated from *Andira inermis*, andirol A (**30**), as well as the benzofuranoid andinermals A and C and andinermol (**36**-**38**; presumably formed by degradation of andirol-like precursors) were tested for antiplasmodial activity (*Pfc*, strains poW and Dd2). Compound **30** showed relatively modest activity (IC_{50}=43.9 and 124 μM, resp.) [223]. The activity of andinermol (**36**) was distinctly higher with IC_{50}s of 24 and 38 μM [223]. The aldehyde derivatives andinermals A and C (**37** and **38**, respectively) were the most active antiplasmodials in this group, the former with IC_{50}= 6.1 and 11.3 μM [223, 224], the latter with IC_{50}= 17.9 and 19.1 μM [224] against the two respective strains.

Erythrina species, as already mentioned, yielded prenylated flavanones and isoflavanes with antiplasmodial activity. Besides these, pterocarpanes such as shinpterocarpine (**31**) from *E. sacleuxii*, erythrabyssin II (**32**) from *E. abyssinica* and **33** from *E. fusca* were described to be active against *Pfc*. Compound **31** yielded IC_{50} values of 6.6 and 8.3 μM against the D6 and W2 clones, and was thus active at the same level as the prenylated isoflavones from this plant mentioned above. The prenylated flavanone abyssinone-V (see above), also a constituent of this species, was only slightly more active [194]. Erythrabyssin-II (**32**) was similarly active (IC_{50}= 8.1 and 6.5 μM) against the respective clones; it was more active than the related pterocarpene derivative **34** (IC_{50}= 22 and 21 μM, resp.) [192]. The monoisoprenyl derivative **33** from *E. fusca* displayed an IC_{50} of 9.1 μg/mL (28 μM) against the K1 strain but was cytotoxic against three mammalian cell lines in the same concentration range [197].

Likely degradation products of pterocarpanes were isolated from *Artemisia indica* (Asteraceae), (**35**) [202] and from *Dalbergia louvelii* (**39** and **40**) [215]. The former was investigated for antiplasmodial activity (*Pfc*, K1) together with the lavandulyl-flavanones and maackiain already mentioned. Its activity was higher than that of the pterocarpane but lower than that of the exiguaflavanones (see previous section), with an IC_{50} of 27 μg/mL

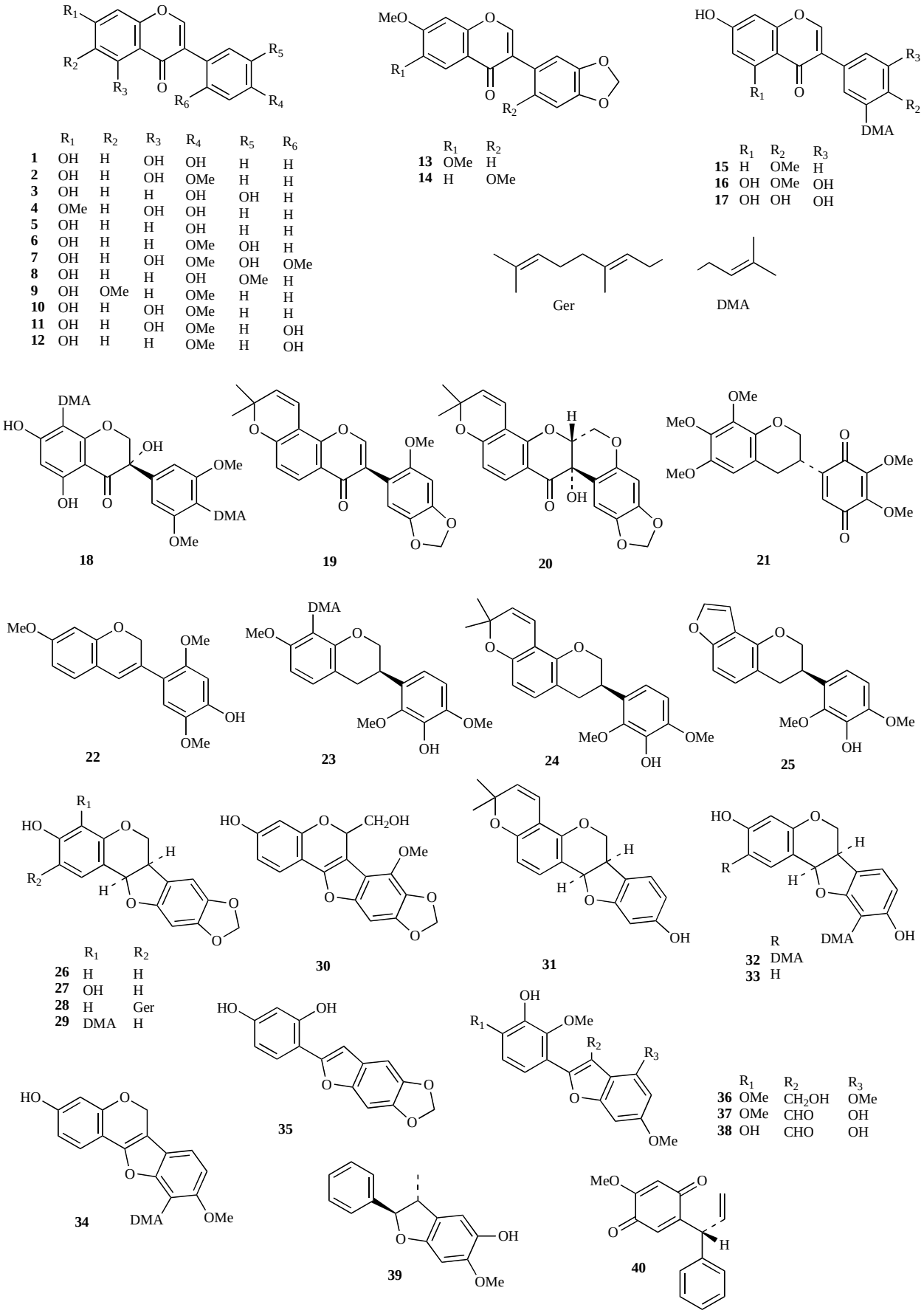


Fig. (7). Structures of isoflavonoids (including pterocarpanes, rotenoids and some further related compounds) with antiprotozoal activity.

[202]. Compounds **39** (obtusafuran) and **40** (4''-methoxy-dalbergione) were active against *Pfc* in the same concentration range as the isoflavone **8** (see above) and the chalcone isoliquiritigenin (see following section) isolated from the same plant, namely, with IC_{50}s of 8.7 and 5.8 mM, respectively [215].

4.1.6. Chalcones

Chalcones are the biogenetic precursors of flavonoids. In most plants, they are isomerized to flavanones by specific enzymes. However, in some plant species, chalcones are -at least in part- not transformed into flavanones but accumulated as such. A number of chalcone derivatives have been demonstrated to be particularly interesting as antiprotozoal agents. The structures of the chalcones mentioned here are shown in Fig. (**8**).

The flavone-chalcone dimer cissampeloflavone has already been mentioned in section 4.1.4. on dimeric flavonoids.

Simple chalcones such as **1**-**8** and also a variety of prenylated chalcones such as **9**-**17** have been intensely studied with respect to antileishmanial activity. Thus, e.g. compound **1**, isolated from *Piper rusbyi* (Piperaceae), was found to act *in vitro* on promastigote forms of *Lbra*, *Lam* and *Ldon* with an IC_{50} of 11.2 μM and to be active *in vivo* after s.c. injection of 5 mg/kg/day (28 days) on mice infected with *Lam*. The lesion size was reduced by 33% in comparison with untreated animals [225]. Similarly, chalcone **2**, isolated from *Piper aduncum*, was active against *Lam* promastigotes and intracellular amastigotes *in vitro*. The ED_{50} for the former was 0.5 μg/mL while the latter required 40 μg/mL to reduce macrophage infection by 50% and 24 mg/μL to diminish parasite load by 50%. The compound did not exhibit toxicity towards mouse macrophages [226].

A set of 19 chalcones was tested for antileishmanial activity by Kayser and Kiderlen against promastigotes of *Lmaj, Ldon, Linf* and *Lenr* and against intracellular amastigotes of *Ldon*. The IC_{50} values for many of the compounds against the extracellular forms were quite low, compounds **7** and **9** showing the highest activity against *Ldon* promastigotes (IC_{50}=0.07 and 0.09 μg/mL). Against the intracellular forms, IC_{50}s were somewhat higher; yet, several derivatives showed values below 1 μg/mL, namely, compounds **3**-**8** (0.55, 0.40, 0.39, 0,41, 0.44, 0.46 μg/mL, respectively). The selectivity indices, however, determined with murine bone marrow-derived macrophages, were not convincingly high, ranging from 0.46 (**6**) to 2.07 (**3**) for the compounds mentioned here. Based on this comparatively large set of chalcones, the authors discussed some structure-activity relationships on a qualitative basis [227]. In this set of chalcones, also three prenylated chalcones with dimethylallyl (DMA) moieties were included, of which **9** was the most active against *Ldon* amastigotes (IC_{50}=1.03, SI=0.34) [227].

Similar compounds with antiprotozoal activity have been described from several plant species of the genera *Crotalaria* (**10**, **11**), *Humulus* (**12**), *Lonchocarpus* (**13**-**15**) and *Glycyrrhiza* (**16**).

Isocordoin (**11**) was isolated from *Lonchocarpus xuul* (Fabaceae) and tested along with congeners **13**-**15** and some further derivatives against *Lmex* promastigotes and *Tcr* epimastigotes. Acetylation as in **13** led to an increase in activity against *Lmex* whereas O-methylation as in **14** and **15** led to a decrease (IC_{50}: **11**: 7.7; **13**: 3.1; **14**: 11.7, **15**: 23.0 μM). With respect to *Tcr*, acetylation and O-methylation led to a similar increase in activity (**11**: 7.0; **13**: 1.8; **14**: 1.5 μM). Surprisingly, **15**, missing one oxygen substituent in comparison with the other compounds, was much less active (IC_{50}= 40.1 μM). However, **15** also was by far the least cytotoxic derivative (MDCK cells) and thus showed selectivity indices of 358 (*Lmex*) and 205 (*Tcr*). Whatsoever, compound **14** was probably the most promising candidate against *Tcr* since it was also fairly selective (SI=109) but far more active than **15** [228].

Compound **16** was isolated from Chinese licorice root (*Glycyrrhiza* sp., Fabaceae) along with a derivative with a second prenyl moiety on ring B cyclized to a benzopyrane structure element (**18**). The former displayed antileishmanial activity against *Ldon* promastigotes inhibiting 50% growth at a concentration between 5 and 10 μg/mL, the latter was less active (>20 μg/mL) [229].

The probably most interesting chalcone from Chinese licorice is licochalcone A (**22**). It was found to be active against *Leishmania* spp. [230-232] and also to show interesting antimalarial activity [233]. In *Lmaj* and *Ldon* promastigote cultures, **22** killed 50% of the parasites at 2.5-4 μg/mL and was even more active against intracellular amastigotes (EC_{50}< 0.5 μg/mL) [230]. In mice infected with *Lmaj* and in hamsters infected with *Ldon*, it demonstrated *in vivo* efficacy [231] and was shown to be an effective inhibitor of leishmanial fumarate dehydrogenase with an IC_{50} of 1.2 μM [232].

Activity of **22** against *Pfc* was found in *in vitro* studies with strains 3D7 and Dd2 where it displayed IC_{50} values of 0.1-0.5 μg/mL. *In vivo*, it led to >90% reduction of parasitemia in *Pyoe* infected mice at doses of 5, 10 and 15 mg/kg (2 doses daily over 3 days). At 15 mg/kg it also increased the survival so that only one out of five mice died of the infection [233].

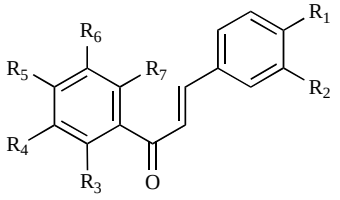
Antimalarial activity was also found with chalcones from *Crotalaria* (Fabaceae) species (**10**, **17**, **19**-**21**). Compound **10** from *C. orixensis* inhibited the schizont differentiation of *Pfc* (strain NF54) by 100% at concentrations of 10 and 50 μg/mL. The diprenylated chalcone **17** from *C. medicagenia* was even more effective leading to the same effect at 2 μg/mL. Three dihydrochalcone derivatives with a benzopyrane moiety on the A ring (**19**-**21**) from *C. ramosissima* were similar in activity to **10** (100% inhibition at 10, 50 and 50 μg/mL) [234].

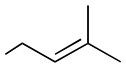
Antimalarial activity is also reported for the prenylchalcone xanthohumol (**12**) from hop flowers (*Humulus lupulus*, Cannabaceae), which was tested against *Pfc* along with 7 further chalcones and was found to be the most active compound in this set. IC_{50} values of 8.2 and 24.0 μM were reported against the poW and Dd2 strains respectively [235]. A later study reported IC_{50}s of 9.3 and 2.8 μM against the D6 and W2 strains of *Pfc* [236]. **12** was demonstrated to inhibit glutathione-dependent hemin degradation by the plasmodia, exposing them to increased concentrations of this toxic metabolite [235].

In vivo activity against *Pber* in mice was recently described for chalcone **23**, which was tested along with three related compounds and found the only active derivative in this study [237]. **23** displayed partial activity when administered in a dose of 160 mg/kg for five days. Of five mice, two were completely cured, two were initially cured but showed a relapse and one mouse died. The investigated compounds' biotransformation and pharmacokinetics were also studied [237] but will not be discussed here.

Dihydrochalcones **24** and **25** isolated from *Piper elongatum* (Piperaceae) were demonstrated to show antileishmanial activity. They were tested *in vitro* against promastigote forms of *Lbra*, *Ltrop* and *Linf* together with some synthetic devivatives (**26**-**28**). *Lbra* appeared particularly sensitive to the acetylated derivatives **26**-**28** so that respective IC_{50} values of 0.66, 0.44 and 0.79 μg/mL were measured, while the natural compounds **24** and **25** yielded IC_{50}s of 27 and 28 μg/mL, respectively. Unfortunately, **26**-**28** did not show selectivity, their cytotoxic IC_{50} values against J774 murine macrophages being 0.5-1.0 μg/mL. The other parasites were less sensitive and the acetylated compounds showed IC_{50}s in the range of 8-14 μg/mL. Interestingly, however, the natural product **25** was more active against *Ltrop* and *Linf* with IC_{50}s of 3.8 and 6.4 μg/mL and also was moderately selective (cytotoxic IC_{50} = 20.0 μg/mL) [238].

Chalcones with larger linear terpenoid substituents were isolated from *Boronia bipinnata* (Rutaceae), namely, **31** and **32** bearing a farnesyl- or geranyl moiety, respectively, attached to ring





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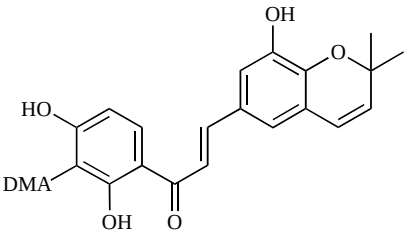
1	H	H	OH	H	OMe	H	Me
2	H	H	OH	H	OMe	H	H
3	OH	H	OH	H	OH	H	H
4	OMe	H	OH	H	OH	H	H
5	OMe	OMe	OH	H	H	H	H
6	OMe	H	OH	H	OMe	H	H
7	H	H	OH	OMe	OMe	H	OMe
8	OMe	OMe	OMe	H	OMe	H	OMe
9	OH	H	OH	DMA	OH	H	OH
10	OH	OMe	OH	DMA	OH	H	H
11	OH	H	OH	DMA	OH	H	H
12	OH	H	OH	DMA	OH	H	OMe
13	H	H	OAc	DMA	OAc	H	H
14	H	H	OMe	DMA	OMe	H	H
15	H	H	H	DMA	OMe	H	H
16	OH	DMA	OH	DMA	OH	H	H
17	OH	H	OH	DMA	OH	DMA	H



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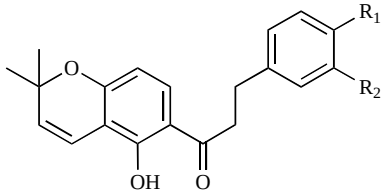
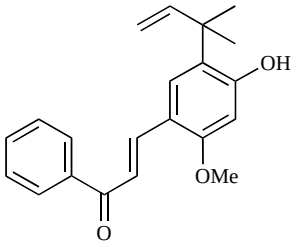


	R_1	R_2
19	OH	H
20	OMe	H
21	OH	OH



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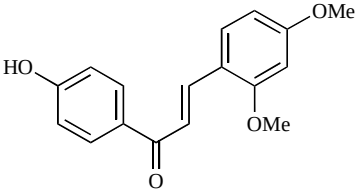

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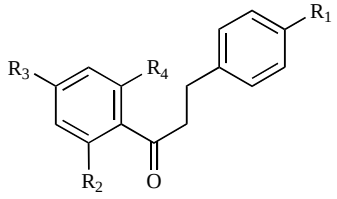
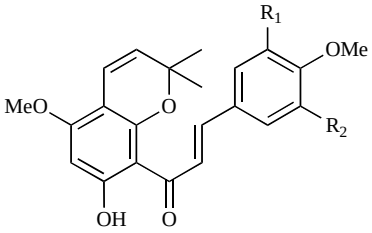
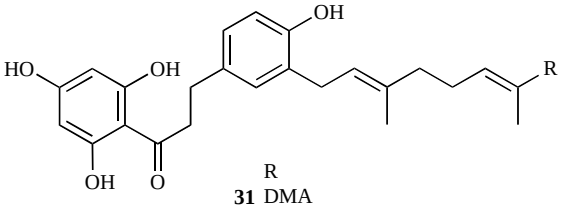


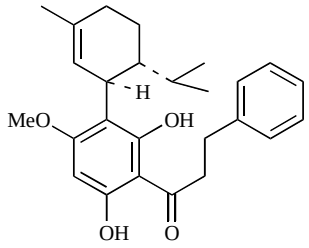
	R_1	R_2	R_3	R_4
24	H	OH	OMe	OH
25	OH	OH	OMe	OH
26	OAc	OH	OAc	OAc
27	OAc	OAc	OAc	OAc
28	OAc	$Glc(Ac)_4$	OAc	OAc



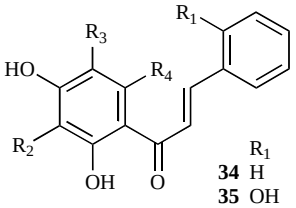
	R_1	R_2
29	OMe	OMe
30	H	OMe



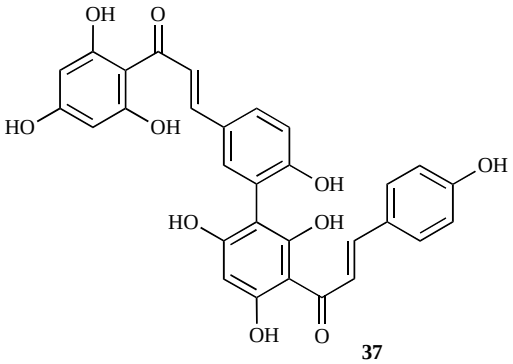
	R
31	DMA
32	Me



33



	R_1	R_2	R_3	R_4
34	H	Me	Me	OMe
35	OH	Me	Me	OMe
36	H	CHO	CHO	OH



37

Fig. (8). Structures of chalcones with antiprotozoal activity.

B. These two derivatives were found to inhibit hemoglobinase II, an enzyme essentially required by *Plasmodium* species to degrade hemoglobin for nutrition. The reported IC_{50} values of 64 and 51 μM are quite high, however, so that it is not clear whether this can play a role in malaria infection [239].

Similarly, a mixture of chalcones **29** and **30**, isolated from *Neoraputia magnifica* (Rutaceae) along with flavonoids (see above) and two further chalcones, were reported to inhibit TcGAPDH and would thus interfere with the vital energy metabolism of *Tcr*. However, the activity was quite low (45% inhibition at 105 μg/mL) [169] so that involvement in antirypanosomal effects *in vivo* would not appear very likely.

A chalcone with a cyclic monoterpenoid substituent, (-)-methyllinderatin (**33**) was isolated by activity-guided fractionation together with seven further related chalcones from *Piper hostmannianum* (Piperaceae). **33** was the most active compound in this series against *Pfc* strains F32 and FcB1 with *in vitro* IC_{50} values of 5.64 and 5.27 μM, respectively. In relation to cytotoxicity against MCF7 cancer cells, the selectivity indices were 12 and 13. Compound **33** was therefore also tested *in vivo* against *Pber* infection in mice. When administered at 20 mg/kg/day over 3 days, it led to an 80% reduction of parasitemia [240].

Unusual chalcones with C_1 substituents at positions 3 and 5 of ring A were isolated from *Psorothamnus polydenius* (Fabaceae) [241] and from *Friesodielsia obovata* (Annonaceae) [242]. Compounds **34** and **35** from the former species, bearing methyl groups in the mentioned positions, were active against axenic amastigotes of *Ldon* and against *Tbb* trypomastigotes *in vitro*. Their IC_{50} values were 5.0 and 7.5 μg/mL, respectively, against the former. Against *Tbb*, IC_{50}s of 6.3 and 6.8 μg/mL were determined. Comparison with cytotoxicity data against Vero cells (IC_{50}= 12.9 and 13.3 μg/mL, resp.) and against J744 macrophages (IC_{50}=7.7 and 9.3 μg/mL, resp.) indicates that selectivity is low [241]. Friesodielsial (**36**), bearing aldehyde groups in positions 3 and 5, was isolated together with a dimethyl analogue and some other constituents from the second mentioned species. **36** proved to possess modest antiplasmodial activity with an IC_{50} of 23 μg/mL against the K1- and of 29 μg/mL against the NF54 strain of *Pfc* [242].

Dimeric chalcones are known to occur in *Rhus* species (Anacardiaceae). Rhuschalcone VI from *R. pyroides* was shown to be toxic to a free living kinetoplastid, *Bodo caudatus*, a distinct relative of *Leishmania* and *Trypanosoma* spp. [243]. Activity tests of **36** against the relevant kinetoplastid pathogens would hence appear interesting.

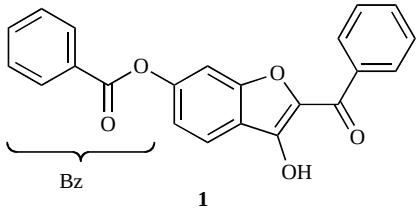
4.1.7. Aurones

Aurones can be formed from chalcone precursors by a mode of cyclization alternative to that leading to flavanones. Instead of the six-membered ring, a five-membered analog is closed leading to the aurone skeleton. Aurones are far less widespread in plants than flavonoids and comparatively few such compounds have been reported as potential antiprotozoal agents. The structures of aurones mentioned here are depicted in Fig. (**9**).

In their initial report on antileishmanial activity of naturally occurring aurones, Kayser *et al.* tested the *in vitro* activity of seven aurones (**1**-**7**) against extracellular forms of *Lmaj*, *Ldon*, *Lin*f and *Lenr* as well as against intracellular forms of *Ldon*. The EC_{50} values for most compounds against the extracellular forms were in the low micromolar and submicromolar range. Most interestingly, compounds **1**-**4** and **7** showed IC_{50}s <2 μg/mL against intracellular amastigotes of *Ldon*. The most active derivative was the benzoate **1** with and IC_{50} of only 0.04 μg/mL. This compound also displayed selectivity since its cytotoxic IC_{50} against bone marrow-derived macrophages was 2.32 μg/mL, corresponding to an SI of 58 [244]. The glucoside **6** was the least active compound in this set. It was subsequently shown with an even larger set of aurones, that some of these compounds may seriously affect mitochondrial function of *Lmaj* by effectively inhibiting fumarate reductase. Three compounds, **2**, **8** and **9**, inhibited the activity of this enzyme by over 95% at 25 nM. Compound **2** was the most effective with 63% inhibition at 6.25 nM [245].

An aurone glycoside (**11**) isolated from *Gomphrena agrestis* (Amaranthaceae) was found inactive against *Lam* amastigotes [246].

Antiplasmodial activity was also reported for aurones by Kayser *et al.* in a study on 12 compounds tested against the K1 and NF54 strains of *Pfc*. Again, quite strong *in vitro* effects were obtained, all compounds showing IC_{50} values <1 μM. Compounds **9** and **10** were the most active with IC_{50}s of 0.03 and 0.007 μM, respectively, and selectivity indices of >100 and >500 were obtained with KB cells [247].



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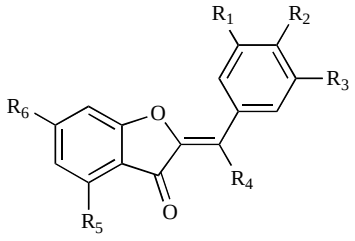

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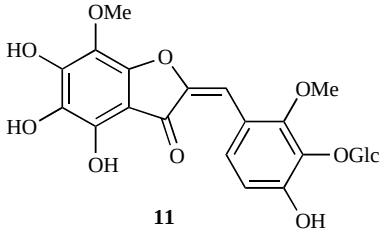
3	H	H	H	H	H	OH
4	H	H	H	OH	H	OMe
5	H	H	H	OH	OBz	OBz
6	OH	OH	OH	H	Glc	OH
7	OH	OH	H	H	H	OH
8	H	OH	H	H	H	OH
9	H	OH	OMe	H	OH	OH
10	OMe	OAc	OMe	H	OAc	OAc



11

Fig. (9). Structures of aurones with antiprotozoal activity.

In spite of these very promising *in vitro* data, no *in vivo* studies have been performed by the Kayser group (O. Kayser, personal communication) on antiprotzoal potential of aurones and apparently no such investigations have been published by others.

4.2. Chromenes and Related Benzopyrane and Benzofuran Derivatives

Chromene and related benzopyranoid systems occur frequently as substructures in secondary metabolites such as flavonoids (see e.g. structures **80**-**86** in Fig. **6C, 19**, **20**, **24** in Fig. **7** or **18**-**21**, **29**, **30** in Fig. **8**). They are formed by cyclization of *ortho*-prenylated phenol structures and may hence also occur in the form of "pure" chromenes formed from simple triketide phenolics after prenylation and further cyclization. A number of such compounds have been studied for antiprotozoal activity. Their structures are presented in Fig. (**10**).

A series of twelve compounds, 7 chromenes (**1**-**7**) and 5 chromanes, i.e. 3,4-dihydro derivatives (**8**-**12**), was investigated for antichagasic activity by Batista *et al.*. Compounds **2**-**5** were isolated from *Piper aduncum*, **6** from *P. gaudichaudianum* (Piperaceae), the others were semisynthetic analogues. The compounds were tested *in vitro* against epimastigotes of *Tcr*. IC_{50} values ranged from 2.8 (**7**) to >500 μM (**1**) and three derivatives (**8**, **11**,**12**) were inactive. The best compound in this array (**7**) was about 4 times more active than benznidazole. Interestingly, hydrogenation of the chromene- and dimethylallyl (DMA) double bonds led to a dramatic decrease of activity (compare e.g. **7** with its hydrogenated analog **12**) [248].

One of the chromenes from the study just cited, gaudichaudianic acid (**6**), although reported initially to be of moderate activity (IC_{50}= 33.8 μM [248]), was studied further by the same group and it was found that anti-*Tcr* activity is influenced by the absolute configuration at the compound's sole asymmetric carbon. After chromatographic separation of the enantiomers, the activity was found to be much lower for each pure enantiomer than for the racemate (IC_{50}: racemate: 55.8 μM; (+)-*S*-**6** (depicted in Fig. **10**): 177 μM; (-)-*R*-**6**: 224 μM). As a very interesting example for the influence of stereochemistry on biological activity, the *S*-form was more active than its enantiomer. Tests with various mixtures in different ratios showed that the 1:1 mixture was most efficient. Interestingly, the activity was more strongly decreased by an increasing amount of *R*-**6** than by increasing the concentration of the *S*-form [249].

Three chromenes isolated from *Peperomia obtusifolia* (Piperaceae) along with two furofuranolignans and two C-glycosyl flavones, were submitted to antitrypanosomal testing against *Tcr* epimastigotes and proved to be the more active principles in this plant (IC_{50}: 27.0, 3.1 and 47.5 μM for **13**, **14** and **15**). The selectivity indices for **13** and **14**, determined with mouse peritoneal macrophages, with values of 405 and 402 were quite high [250], so that especially **14** would deserve further attention.

From *Ageratum conyzoides* (Asteraceae), besides methylated flavonoids (see section 4.1.1), the chromene encecalol dimehylether **16** was isolated. It was much less active against the parasites under study than the flavonoids. Against *Tbr* and intracellular *Tcr* its IC_{50} values were 78 and 29 μg/mL, respectively. None of the isolated compounds could explain the excellent *in vitro* activity of the crude extract (0.78 μg/mL against *Tbr*) [167]. It was then found that **16** had been formed during workup by methanolysis from the actual native chromene encecalol angelate **17** which is very unstable in the presence of protic solvents. **17** was subsequently tested against *Tbr*, *Tcr*, *Ldon* and *Pfc*. Although not being very active against the first three parasites (IC_{50}=50, 19 and 15 μg/mL) and thus probably not being responsible for the antitrypanosomal activity of the crude extract, **17** displayed *in vitro* activity against *Pfc* with an IC_{50} of 6.0 μg/mL [251]. Synthetic preparation of a series of stable analogs of **17** has yielded several products with very promising *in vitro* activity against *Pfc* (Harel, D.; Brun, R.; Schmidt, T.J. and Wünsch, B., unpublished).

Two chromanone analogues (**18a** and **18b**) similar to **17**, were found in *Calea uniflora* (Asteraceae), along with the corresponding deacyl derivative **19**. In tests for activity against *Lmaj* promastigotes it was found that **19** was inactive but that a mixture of the E and Z isomers **18a** and **18b** showed *in vitro* activity with an IC_{50} <25 μg/mL (55% inhibition at 25 μg/mL, 81.5 and 88.9 % at 50 and 100 μg/mL, respectively) [252].

A series of 10 chromenone derivatives was isolated from *Harrisonia perforata* (Simaroubaceae) and tested against *Pfc*, strain K1. Compound **20** was the only one with antiplasmodial activity in this series with an IC_{50} of 10.5 μg/mL while some further derivatives showed antibacterial activity [253].

A chromone derivative (**21**) isolated from an African *Ancistrocladus* species (Ancistrocladaceae) along with a variety of naphthylisoquinoline alkaloids (see section 5.2) displayed some *in vitro* activity against *Tbr* and *Tcr* with IC_{50} values of 21.5 and 19.7 μg/mL, respectively. Unlike most of the alkaloids it was inactive against *Pfc* and *Ldon*, but also not cytotoxic to L6-cells [254].

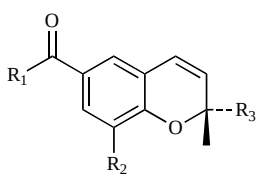
From *Artocarpus integer* (Moraceae), the stilbenochromene **22** and two closely related prenylated stilbene derivatives, **23** and **24** were isolated. All three compounds were active against the K1 strain of *Pfc* with IC_{50} values of 9.4, 1.7 and 8.2 μg/mL, respectively [255].

Interesting stilbenochromenes (**25**, **26**, **28**), apparently formed by addition of a monoterpene instead of a prenyl (hemiterpene) moiety, were isolated along with some biogenetically related compounds, e.g. **27**, **29**, from *Machaerium multiflorum* (Fabaceae) [256, 257]. The compounds were tested against *Pfc* (W2 and D6 clones). *In vitro* IC_{50} values of 0.6 and 1.5 μg/mL were reported for **25** against the respective clones. Compound **26** was found even more active with IC_{50}s of 0.12 and 0.72 μg/mL; SI values determined with Vero cells showed reasonable selectivity, e.g. >40 for **28** with respect to W2 [256]. In a second study, **26**, **27** and **29** were also found active against the same *Pfc* clones, **27** representing the most active and selective derivative (IC_{50} 0.64 and 0.22 μg/mL against *Pfc* D6 and W2, resp.; SI >7.4 and >22). **27**, in the same study, was also most active against *Ldon* promastigotes (IC_{50}=0.9 μg/mL) [257]. These compounds with structural resemblance to cannabinoids thus possess an interesting antiprotozoal potential.

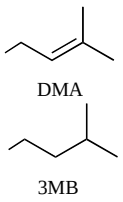
Indeed, cannabinoid related compounds from *Cannabis sativa* (Cannabaceae) have recently also been demonstrated to possess antiprotozoal activity. Thus, 4-terpinylcannabinolate (**30**) was shown to act on *Pfc*, D6 and W2 clones, with IC_{50} values of 2.7 and 2.4 μg/mL, respectively [258]. Cannabigerolic acid (**33**) exhibited antileishmanial activity with IC_{50}= 12 μg/mL against *Ldon* promastigotes [259]. The cannabichromene derivatives **31** and **32** and the cannabigerol derivatives **34** and **35** were tested for *in vitro* activity against *Ldon* and *Pfc*. Against the former (life stage not reported), they were slightly active with IC_{50}s between 11 (**34**) and 58 μM. Against *Pfc*, only **32** and **34** were active with IC_{50}s of 7.2 μM (both vs. D6) and 4.0 (**32**) and 6.7 (**34**) μM [260]. Considering the immense diversity of *Cannabis sativa* metabolites, more systematic studies on such compounds will be of high interest.

4.3. Xanthones

Xanthones (structures see Fig. **11**) are formed by cyclization of benzophenone precursors and are found mainly in tropical Clusiaceae but also occur in Gentianaceae and some other families as well as in certain fungi. Xanthones have been reported to show *in vitro* and *in vivo* antiprotozoal activities and are often relatively simple to synthesize which makes this class of compound interesting for the development of drugs. Hydroxyxanthones are in general active against *Pfc in vitro*. Their mode of action is believed

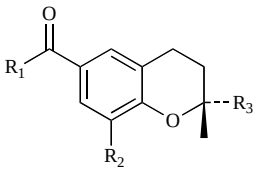


	R_1	R_2	R_3
1	OH	H	Me
2	OMe	H	Me
3	OMe	OH	Me
4	OMe	DMA	Me
5	OMe	OAc	Me
6	OH	DMA	DMA
7	OMe	DMA	DMA

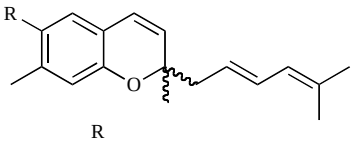


DMA

3MB



	R_1	R_2	R_3
8	OMe	H	Me
9	OMe	OH	Me
10	OMe	DMA	Me
11	OH	3MB	3MB
12	OMe	3MB	3MB

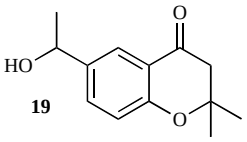


R

13 COOH

14 H

15 DMA



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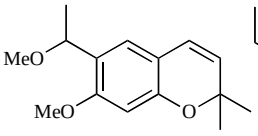

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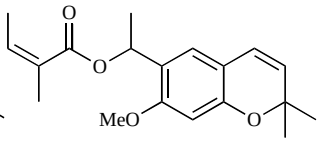

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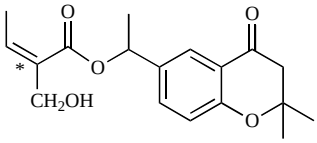
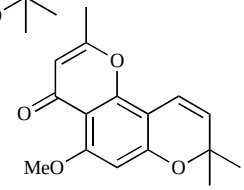


18a: *Z

18b: *E



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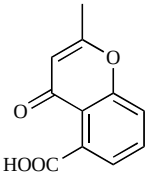

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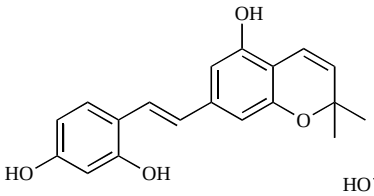

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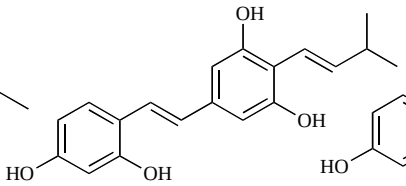

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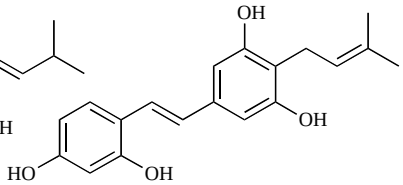

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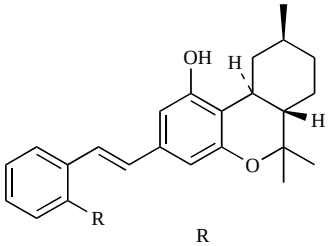
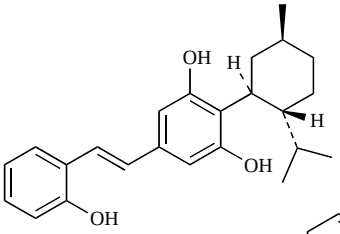


R

H **25**

OH **26**



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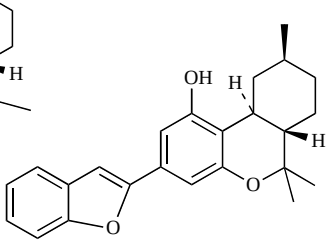

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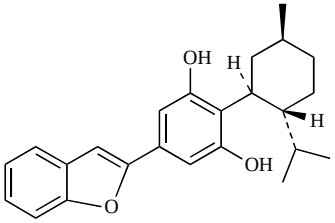

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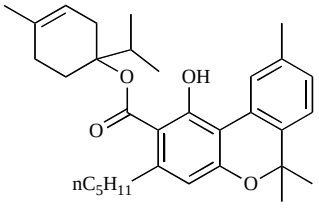

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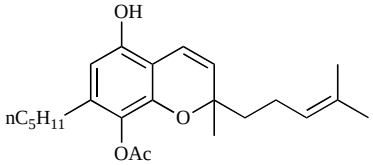

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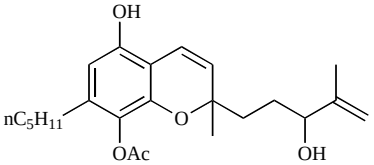

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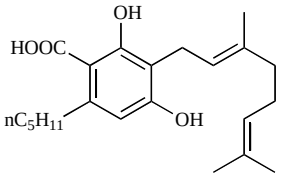

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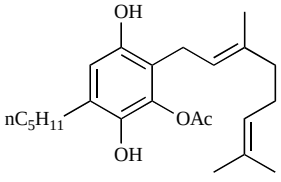

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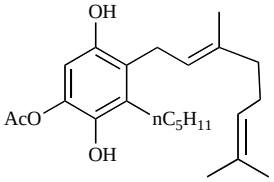

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Fig. (10). Structures of chromenes and related compounds with antiprotozoal activity.

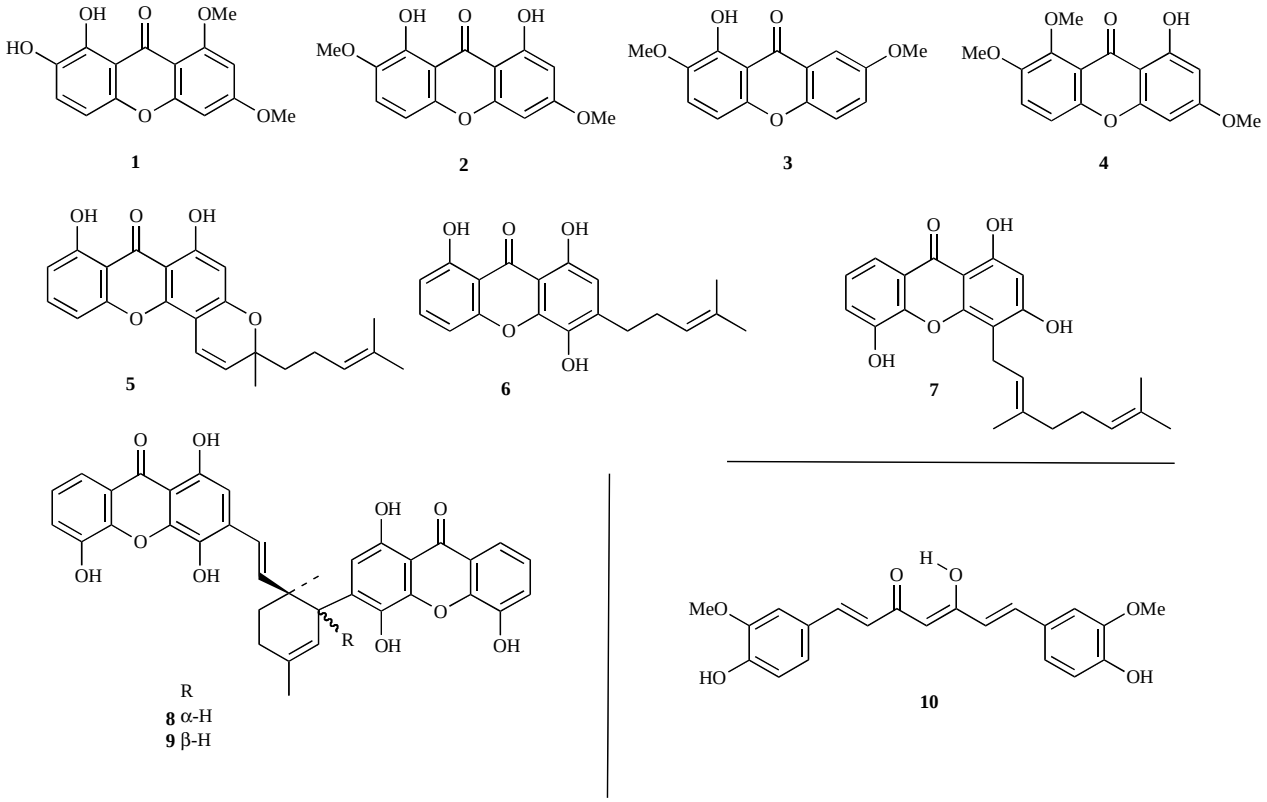


Fig. (11). Structures of xanthones (**1**-**9**) with antiprotozoal activity and of curcumin (**10**).

to be due mainly to a general mechanism involving the malaria parasite digestive vacuole. Many potent antimalarials, such as 4-aminoquinolines (e.g. chloroquine, mefloquine and primaquine), artemisinin and its derivatives, and also xanthones are believed to accumulate in the digestive vacuole where they inhibit the polymerization of heme into polymeric hemozoin. The latter is released during proteolytic degradation of hemoglobin and toxic to the parasites [261]. Studies on synthetic hydroxyxanthones as inhibitors of spontaneous heme aggregation have revealed specific structure-activity relationships within this class of antimalarial compounds. Thus, hydroxylation or oxygenation on carbons 3, 4, 5 and/or 6 of the xanthone nucleus is believed to be critical for complexation to heme and for antimalarial activity. Xanthones are believed to block hemozoin formation and accumulation of soluble heme-xanthone complexes is thought to be responsible for increases in the osmotic pressure in the digestive vacuole ultimately leading to parasite death through lysis of this organelle. *In vitro* heme-complex formation and solubility under acidic conditions -as prevalent in the digestive vacuole- were optimized in a series of synthetic xanthones [261], although no *in vivo* data are available for these compounds.

Several tetraoxygenated xanthones are worthy of note for their *in vivo* antimalarial and other antiprotozoan properties. Recent reviews have demonstrated that xanthones obtained from species of the Clusiaceae family exhibit moderate antiprotozoal activity against several *Pfc* strains. Thus, xanthones from *Garcinia mangostana* (IC_{50}=5.1-7.0 μM) [261], *Pentadesma butyraceae*, *Cratoxylum generum* and *Garcinia griffithii* (IC_{50}=3.0-8.3 μM) exhibit antimalarial activity [262].

1,2-dihydroxy-6,8-dimethoxy-xanthone (**1**) is active in a murine model of malaria [262] and has recently been isolated from the roots of *Andrographis paniculata* (Acanthaceae). This xanthone has also been shown to be active against *Tbb* and *Linf* (IC_{50}= 16 and 28 μM, respectively). Another compound isolated from the roots of the same plant, 1,8-dihydroxy-3,7-dimethoxy-xanthone (**2**), was active *in vitro* against intracellular amastigotes of *Tcr* (IC_{50}= 14 μM) [263] but not active against *Tbb*. Quite interestingly, two further very similar xanthones (**3** and **4**) from the same source were inactive against *Tcr*, *Tbb* and *Linf* [263].

Several xanthones isolated from *Swertia alata* (Gentianaceae) were all found to offer some protective effect against malaria parasite invasion. However, only xanthone **2**, also a constituent of this plant, exhibited significant *in vitro* activity and was therefore further tested *in vivo* in the *Plasmodium berghei* rodent model, in which it produced the most significant *in vivo* reduction of parasitemia at a dose of 10 mg/kg [264].

The prenylated xanthones **5**-**9** were obtained from *Garcinia livingstonei* (Clusiaceae) along with some biflavonoids (see section 4.1.4) [207]. They were tested *in vitro* against *Tbb*, *Tcr* (intracellular amastigotes), *Linf* (intracellular amastigotes) and *Pfc*. Against *Tbb*, compounds **6** and **8** were the most active with IC_{50} values of 0.87 and 0.4 μM, respectively. Against *Tcr*, IC_{50}s were between 4.0 and 8.0 μM for the five xanthones. Antileishmanial activity was less impressive, IC_{50} values could only be determined for **6** and **8** (27 and 32 μM, where **8** was toxic to the macrophages used for testing). These two compounds were also active against *Pfc* with IC_{50}s of 10.0 and 6.7 μM. Cytotoxicity against MRC-5 cells was low (IC_{50} >32 μM) for **5**-**7**, whereas compound **8** showed an IC_{50} of only 2 μM and is hence not very selective. Significant activity differences between **8** and its epimer **9**, which was generally much less active, appear quite noteworthy. The favourable data for activity and selectivity of **6** would make this simple prenyl xanthone derivative an interesting object for further studies [207].

4.4. Curcumin and Related Arylheptanoids

Arylheptanoids such as curcumin (structure **10** in Fig. **11**) are formed by reaction of a cinnamic acid derivative (feruloyl-CoA)

with a single unit of malonyl-CoA and a second unit of the activated cinnamic acid derivative. They occur mainly in Zingiberaceae such as *Curcuma xanthorrhiza* and *C. longa*. Curcumin has long been known to possess antiprotozoal potential against *Trypanosoma*, *Leishmania* and *Plasmodium* species. Apparently, no new natural compounds of this type were discovered in the last years. It appears worth mentioning here, that synthetic analogues with improved activity against *Tbb* and *Lmex* [265] as well as *Pfc* [266] have been designed and that some attempts were made to develop drug delivery systems based on nanoparticles [267, 268]. With respect to the earlier literature, the reader is referred to Ioset's review of 2008 [7].

4.5. Annonaceous Acetogenins

The Annonaceae represent the largest family in the order Magnoliales with about 135 genera and 2500 species which are widely distributed in tropical and sub-tropical regions. Apart from isoquinoline alkaloids, which have been reported from various genera of this family (see section 5.2 below), the annonaceous acetogenins (ACGs; structures see Fig. **12**) constitute a characteristic class of natural products isolated exclusively from members of this family [269]. Due to the latter fact, the family name is not mentioned further in this section.

ACGs are generally characterized by being white waxy substances composed of C_{32} to C_{37} long-chain fatty acids combined with a 2-propanol unit at C-2 to form a methyl-substituted terminal α,β-unsaturated γ-lactone. They usually cyclize to form one, two, or three tetrahydrofuran (THF) or tetrahydropyran (THP) rings near the middle of the aliphatic chain. In case of more than one THF ring, the cyclic structures may be adjacent or nonadjacent near the centre of the hydrocarbon chain and accompanied by one or two flanking hydroxyl groups. Various hydroxyls, double bounds, carbonyls, and acetyls can be located throughout the molecule. Accordingly, ACGs have been classified into mono-THF, adjacent bis-THF, nonadjacent bis-THF, tri-THF, adjacent tetrahydrofuran-tetrahydropyran (THF-THP), nonadjacent THF-THP, mono-THP, and ACGs containing only γ -lactones. Biogenetically, ACGs are derived from the polyketide pathway. It has been suggested that the THF or THP cores are generated by polyepoxidation of an unconjugated polyene followed by domino cyclizations. ACGs have been associated with a number of biological activities including antiprotozoal, insecticidal, antimitotic, cytotoxic, fungicidal and immunosuppressive effects. It is generally accepted that the mode of action of acetogenins is the inhibition of NADH–ubiquinone oxidoreductase (complex I) in mitochondria [270].

To date, more than 500 ACGs have been reported from various members of the family Annonaceae during the last three decades and these compounds have been recently regarded as likely source of potential candidates for future drugs [271].

The methanolic and hexane extracts of the dried powdered seeds of *Annona senegalensis* showed activity against *Lmaj* promastigotes at a concentration of 200 μg/mL after 24 h incubation but exhibited no activity against *Ldon*. The bloodstream forms of *Tbb* were totally destroyed at a concentration of 50 μg/mL after an incubation period of 24 hours. The dichloromethane extract, however, displayed activity in the range of 50 to 100 μg/mL against all kinetoplastids under investigation after 24 and 96 h incubation [272]. Bioactivity-guided fractionation of the dichloromethane extract led to the isolation of a mono-THF-γ-lactone, senegalene (**1**) and 4 bis-THF-γ-lactones, namely, molvizarine (**2**), asimicine (**3**), rolliniastatine-2 (**4**) and squamocine (**5**). Compounds **1**, **3** and **5** displayed antileishmanial activity against *Lmaj* and *Ldon* with minimum effective concentrations (MECs) ranging between 25 and 50 μg/mL [272]. Rolliniastatine-2 (**4**), which differs from molvizarine (**2**) by two additional methylene groups in the chain between the unsaturated γ -lactone and the tetrahydrofuran ring, was five times less active against *Lmaj* and *Ldon*. It seems that change of stereochemistry at C-23, C-24 into *threo* (asimicine, **3**) slightly increased activity in comparison with rolliniastatine-2 (**4**). The absence of a hydroxy group at C-28 and its presence at C-4 of rolliniastatine-2 (**4**) significantly reduced the activity against *Lmaj* and *Ldon*. The monofuranic acetogenin senegalene (**1**) was the most active against *Tbb* with an MEC of 50 μg/mL after 1 h and of 10 μg/mL after 24 h incubation periods. Senegalene (**1**) also exhibited activity against *Lmaj* and *Ldon* at concentrations of 50 and 25 μg/mL, respectively. Pentamidine was approximately twice as efficient as senegalene (**1**), squamocine (**5**) and molvizarine (**2**). On the other hand, the activity observed against *Tbb* was not encouraging enough to compare the antitrypanosomal activity with a reference drug [272]. The cytotoxicity of all the isolated acetogenins and more particularly that of difuranic molvizarine (**2**) and squamocine (**5**) was higher against KB than against VERO cell lines, whereas the monofuranic senegalene (**1**) showed slightly less cytotoxicity [272].

Bioactivity-directed fractionation of the dichloromethane fraction of the methanolic stem bark extract of *Rollinia emarginata* based on antileishmanial *in vitro* activity resulted in the isolation of 5 acetogenins, namely, squamocin (**5**), rolliniastatin-1 (**6**), sylvaticin (**7**), rollidecin B (**8**) and liriodenine (structure not shown and no acetogenin of this name listed in CAS) [273]. Compounds **5**, **6** and liriodenine were able to lyse the promastigotes of *Lbra*, *Lam* and *Ldon* with IC_{100} values of 5μg/mL while 10 μg/mL of **7** were required for the same effect. Rollidecin B (**8**) was 10 times less active [273]. The aforementioned three most active acetogenins against *Leishmania* strains also exhibited significant trypanocidal activity in mice infected with *Tcr* at a concentration of 250 μg/mL. Compounds **6** and **5** reduced the parasite load in infected murine blood by 89 and 67 %, respectively. Although no detailed structure-activity relationship can be identified, it appears that leishmanicidal activity is inversely correlated with the number of hydroxyl groups. In fact, the maximum antiprotozoal activity was observed in acetogenins with three hydroxy groups as in **5** and **6** while **7** and **8** containing four hydroxy groups exhibited less activity [273].

The dichloromethane extract of seeds of *Annona glauca* was active against *Lbra*, *Lam* and *Ldon*. Accordingly, 9 known acetogenins were isolated and their leishmanicidal and trypanocidal activity evaluated *in vitro* against the promastigote forms of the three *Leishmania* species and the bloodstream forms of *Tcr* [274]. The mono-THF acetogenins annonacin A (**9**) and goniothalamicin (**10**) showed promising activity against the *Leishmania* promastigotes with EC_{100} values of 10 and 5 μg/mL, respectively. Glaucanisin (**11**), squamocin (**5**), annonacin A (**9**) and annonacin (**12**) were also reported to exhibit activity against *Tcr* reducing the parasites by 78%, 67%, 71% and 85%, respectively, but the concentrations required for these effects were not unambiguously specified [274]. Although it is not easy to establish clear structure-activity relationships, it appears that the mono-THF acetogenins tend to be more leishmanicidal than the bis-THF acetogenins. Among the latter, the width of the alkyl chain seems to be associated with the leishmanicidal activity of glaucanisin (**11**), rolliniastatin-2 (**4**), squamocin (**5**), molvizarine (**2**) and parviflorin (**13**). In effect, the C_{35} bis-THF acetogenins molvizarine (**2**) and parviflorin (**13**) were inactive against *Leishmania* species whereas the C_{37} bis-THF acetogenins glaucanisin (**11**), rolliniastatin-2 (**4**) and squamocin (**5**) presented an IC_{100} value of 25 μg/mL. Glaucafilin (**14**), annonacin A (**9**), annonacin (**12**) and goniothalamicin (**10**), which are C_{35} mono-THF acetogenins with four hydroxy groups, presented IC_{100} values of 25, 10, 25 and 5 μg/mL, respectively, against *Leishmania* spp. The difference in activity between annonacin (**12**) and goniothalamicin (**10**) as well as between annonacin A (**9**) and annonacin (**12**) is probably due to the position of THF-ring and its relative configuration [274].

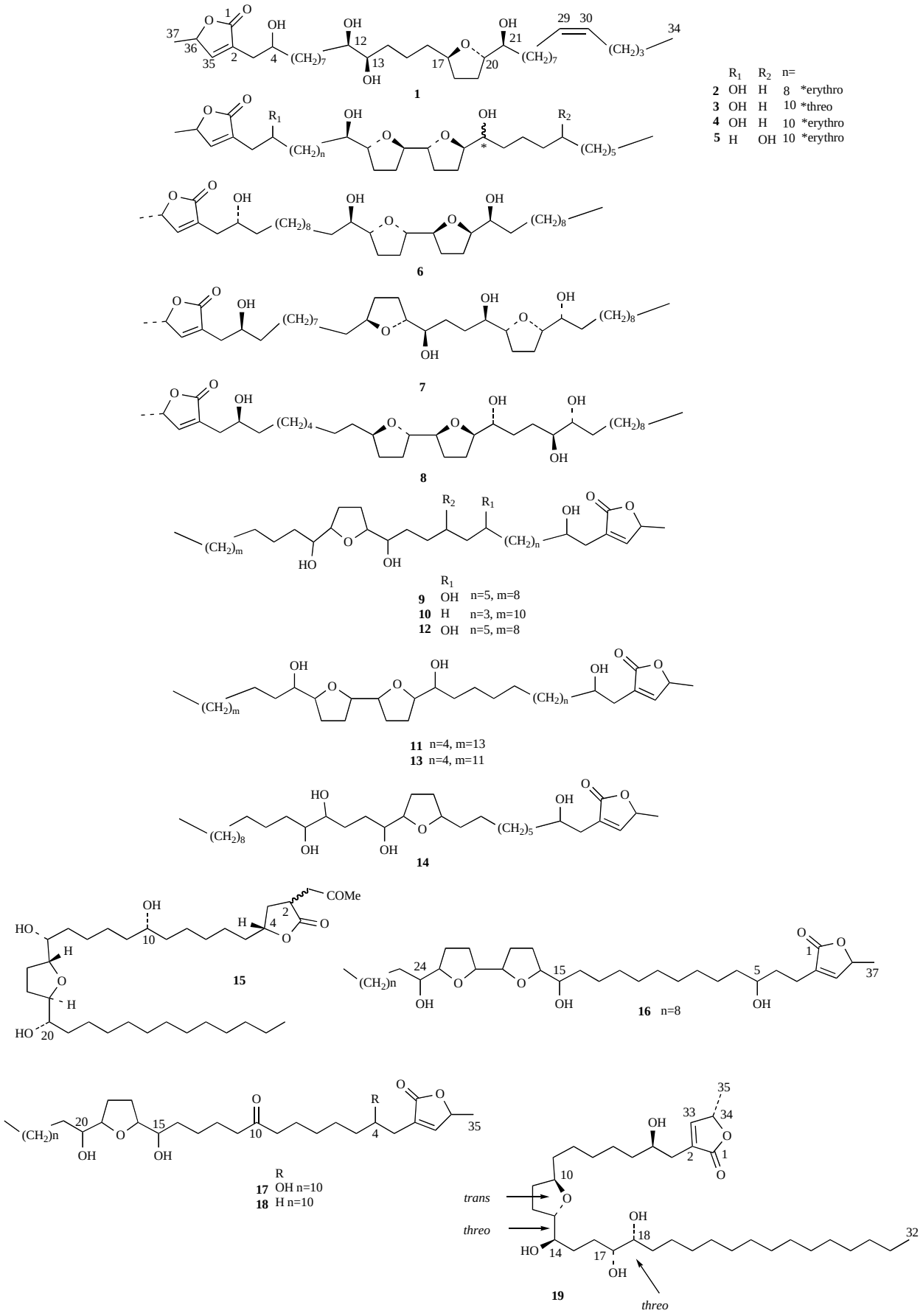


Fig. (12). Structures of acetogenins from Annonaceae with antiprotozoal activity.

In another study aimed at the identification of new antileishmanial lead compounds, 12 ACGs were evaluated for their inhibitory effect on the promastigote forms of wild-type and four drug-resistant lines of *Ldon*. The compounds were also tested on amastigote-infected and non-infected macrophages and "therapeutic indices" (certainly better to be termed selectivity incides, SI) were determined. The IC_{50}s against promastigotes were in the range 4.7–47.3 μM. Rolliniastatin-1 (**6**) of the adjacent bis-THF series exhibited the strongest activity with IC_{50}= 4.7 μM. In the intramacrophage amastigote *in vitro* model, **7** compounds exhibited measurable antileishmanial activity with IC_{50} values in a range of 2.5–29.7 μM. Compound **6** once more was the most interesting compound with an IC_{50} of 2.5 μM and the best "therapeutic index" of 18.08. The mono-THF isoannonacin (**15**) was found to be active against intra-macrophagic amastigotes (IC_{50}= 6.2 μM) with a "therapeutic index" of 2.05 and also displayed significant activity against drug-resistant strains (IC_{50} from 5.1 to 9.8 μM) [275]. Preliminary *in vivo* experiments on the *Ldon* LV9/Balb/C mice model showed a decrease of 48% of the liver parasitic burden after an intraperitoneal treatment with **15** at 10 mg/kg, administered as a single injection. Although the exact mechanism of action is not known, some influences of structural features on activity were discussed by the authors. The presence of free hydroxy groups adjacent to the bis-THF rings as in rolliniastatin-1 (**6**) was recognized to be favourable, but not essential, for activity. This was assumed to be due to the high polarity rather than hydrogen bond-donating ability. The length of the alkyl spacer was also demonstrated to be very important for activity, the optimal length being approximately 13 carbon atoms. This discussion, sometimes contradictory to other published results, proves the complexity of the structure-activity relationships of ACGs. Further studies are certainly required in order to elucidate the exact leishmanicidal mechanism of action of ACGs.

Recent phytochemical work on the leaves of *A. squamosa* and the seeds of *A. muricata* led to the isolation of a new C_{37} trihydroxylated bis-THF acetogenin (**16**) from *A. squamosa* and two known ACGs namely, annonacinone (**17**) and corossolone (**18**). Compound **16** exhibited IC_{50} values of 26.4 and 25.3 μg/mL against *Lcha* promastigotes and intracellular amastigotes, respectively, while annonacinone (**17**) and corossolone (**18**) displayed IC_{50} values of 37.6 and 25.9 μg/mL, respectively, against *Lcha* promastigotes. In the amastigote assay, annonacinone (**17**) and corossolone (**18**) exhibited IC_{50} values of 13.5 and 28.7 μg/mL, respectively. The cytotoxicity assay for the three acetogenins showed results ranging from 43.5 to 59.5 μg/mL [276].

A recent antiprotozoal screening of 10 acetogenins isolated by one of the present authors (SAK) from three annonaceous species resulted in the isolation of several acetogenins, including the mono-THF ACG gigantetrocin (**19**) from the African plant *Uvariopsis congolana*. This compound -originally reported from the Asian Annonacea *Goniothalamus giganteus* [277]- exhibited very significant antitrypanosomal activity specifically against *Tcr* (IC_{50} = 0.019 μg/mL) and less activity (IC_{50} = 0.88 μg/mL) against *Tbr* trypomastigotes with relatively low cytotoxity (IC_{50} = 5.5 μg/mL). The activity of **19** against *Ldon* was weaker than against the trypanosomes (IC_{50}= 1 μg/mL). Against chloroquine resistant *Pfc* only moderate activity was detected (IC_{50} = 6.72 μg/mL). Isoannonacin (**15**), also included in this study, showed weak activity with IC_{50}= 19.4 and 25.7 μg/mL against *Tcr* and *Tbr*, respectively (Khalid, S.A. *et al*., unpublished results). The discrepancy in selectivity between these two ACGs may be related to structure-dependent membrane factors and the possibility that metabolic inactivation plays a role in the biological activity of these compounds. It has been demonstrated that ACGs are inhibitors of mitochondrial NADH-ubiquinone oxidoreductase [278] and that *Tcr* is more prone to inhibition by ACGs on the basis of NADH oxidase than other related kinetoplastids [279]. The ACGs' cytotoxicity seems to be dependent on species/cell and membrane factors. Metabolic inactivation plays an important role in the biological activity of these compounds [279]. Recent application of chemometric methods generated significant exploratory and predictive correlations. Such models can be taken into consideration in designing new antichagasic agents acting as NADH-oxidase inhibitors with special emphasis on the unbalance of the hydrophilic and hydrophobic profiles in relation to the total molecular surface [280].

Although ACGs seem to be selectively more toxic towards the kineteoplastid parasites than against *Plasmodium*, some preliminary screening involving crude extracts showed certain antimalarial activity. Among the six plants screened for their antiplasmodial activity, the ethanol extract of the leaves of *Annona muricata* exhibited IC_{50} of 39.9 μg/mL against various isolates of *Pfc* [281]. *In vitro* tests of three ACGs on a chloroquine-resistant *Pfc* strain resulted in IC_{50} values between 5 to 10 μM. It was demonstrated that these compounds partially inhibit the parasites' adenylate translocase. As a structure-activity relationship, it was found that at least one THF moiety is required for maximal antiplasmodial activity. The presence of synergistic action between ACGs and chloroquine was also observed [282].

Due to the intriguing activity of ACGs, especially against the kinetoplastid protozoan pathogens, this class of secondary metabolites can be expected to play a significant role in drug discovery against these parasites.

4.6. Polyacetylenes

Acetylenic natural compounds (often called polyacetylenes, although they are not polymers and many metabolites may contain only a single acetylenic bond; structures see Fig. **13**) constitute a bioactive group of relatively unstable and reactive phytochemicals, succumbing either to oxidative, photolytic, or pH-dependent decomposition [283]. Polyacetylenes are typically characterized as strong photosensitizers with the primary mechanism of action described as a photodynamic or nonphotodynamic disruption of membranes (involving singlet oxygen or oxygen-independent processes, respectively). Many polyacetylenes also possess considerable light-independent toxicity [284]. In higher plants they have been isolated from approximately 12 families, with the majority being isolated from the botanically closely related families Apiaceae [285, 286] Araliaceae [287, 288] and Asteraceae [289-291]. Polyacetylenes from Apiaceae and Araliaceae are classically characterized by aliphatic C_{17} chains, whereas the structural diversity among Asteraceae (responsible for more than the half of known structures) includes thiophenes, alkamides, lactones, furans, spiroketals, aromatics and others [283, 284], including glycosides [292]. Regarding bioactivity, polyacetylenes have shown to possess considerable cytotoxic [293, 294] and antimicrobial [290, 295] activity as well as immunological activity [296], also observed for some marine compounds [297, 298]. Therefore, polyacetylenes could be of interest for the investigation of their anti-protozoan activity profile in order to assess their potential as agents against protozoan NTDs. Quite few efforts have been spent in this field so far, but some recent results have shown that further studies could be encouraging.

Bidens pilosa (Asteraceae) is used in Brazil to treat malaria. Its extract containing both polyacetylenes and flavonoids, caused growth inhibition of plasmodia *in vitro* and *in vivo*. The *in vivo* tests (*Pber*, mouse) showed that the ethanol extract (250 mg/kg) reduced parasitemia on the fifth day (36% reduction, $\rho \leq 0.05$) in comparison with non-treated control mice; by the seventh day, reduction was 29%. The major polyacetylene component was identified as 1-phenyl-1,3-diyn-5-en-7-ol-acetate (**1**), but was not tested in isolated form due to its instability [299, 300]. The authors suggested that other *Bidens* species should be further investigated.

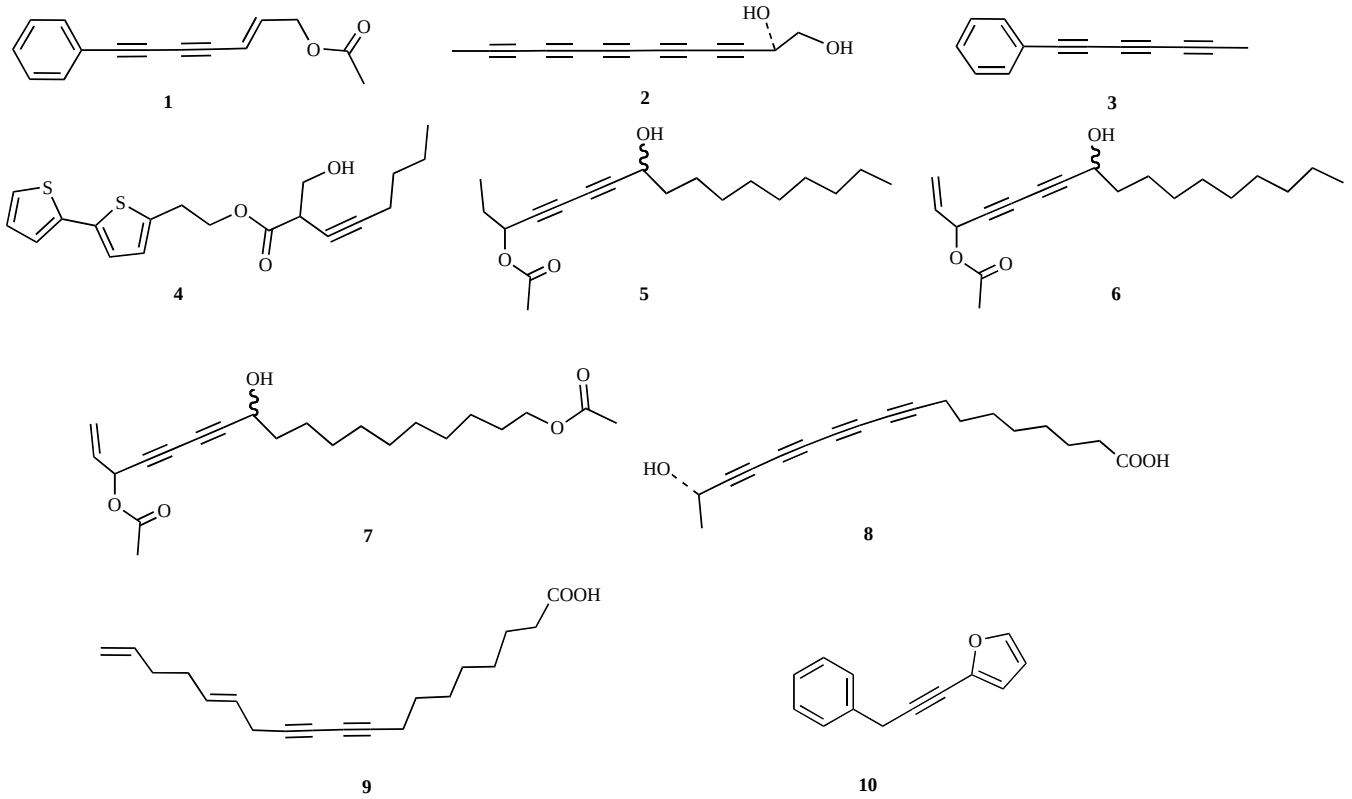


Fig. (13). Structures of polyacetylenes with antiprotozoal activity.

Recently, a new compound from *B. pilosa*, the linear polyacetylenic diol (**2**) exhibited potent antimalarial activity *in vitro* (IC_{50}= 0.35 µg/mL), as well as *in vivo* by way of intravenous injection, carried out in mice infected with the *Plasmodium berghei* NK-65 strain. In this experiment, the average parasitemia of 32.8% observed in control mice was decreased to 12.1% ($p < 0.05$) by the administration of 0.8 mg/kg of **2** for 4 days. In the same experiment, chloroquine (12 mg/kg) decreased the parasitemia to 1.1% after 4 days. The authors assert that polyacetylenes represent a promising new class of drugs effective in the treatment of malarial and bacterial diseases [301]. From the same plant species, the acetylenic compound 1-phenyl-1,3,5-heptatriyne (**3**) was isolated and found to be active *in vitro* at an IC_{50} of 6.0 µg/mL in reducing the parasitemia of *Pfc* (NF54 strain) growing in human blood [302].

From another Asteraceae, *Tagetes erecta*, an acetylenic compound (**4**) showed significant schizonticidal activity *in vitro* using the schizont maturation inhibition assay against chloroquine sensitive and resistant strains of *Pfc*, with IC_{50} of 26 and 53 nM, respectively [303].

A biomonitored screening of 13 Tanzanian medicinal plants searching for antiprotozoal activity revealed the petroleum ether extract of the root bark of *Cussonia zimmermannii* (Araliaceae) as the most promising sample. Bioguided chromatographic fractionation led to the isolation of four polyacetylenes, and testing of the isolated pure compounds **5** - **7** showed compound **5** to be the least active in all antiparasitic *in vitro* assays (*Tbr*, *Pfc*, *Tcr* and *Ldon* amastigotes, axenic as well as in infected macrophages). Compound **6** showed promising activities in the *Tcr* and *Ldon* (axenic and infected macrophages) assays with IC_{50} values of 0.65, 0.13, and 0.32 µM, respectively (0.20, 0.039, and 0.098 µg/mL). The cytotoxicity was relatively high, so that the selectivity was in a moderate range (SI= 18 and 37, respectively, for *Tcr* and intracellular *Ldon*). Polyacetylene **7** also showed interesting activities in the *Tcr* and *Ldon* (axenic) assays with IC_{50} values of 0.40 and 0.14 µM, respectively (0.15 and 0.054 µg/mL). The SI value of 150 (*Tcr*) was considered high [304].

In another ethnopharmacological survey, carried out with plants from the Amazonian region, some plants were selected that are used to treat suggestive symptoms of parasitic infections. One of the most potent extracts, used traditionally as an anthelmintic, was an infusion of the stem bark of *Minquartia guianensis* (Olacaceae). In this bark, the polyacetylene minquartynoic acid (**8**) is present in large amounts (2-3% of the dry weight), which displayed moderate *in vitro* activity against *Pfc* and *Lmaj* (IC_{50} of 3.0 and 1.4 µg/mL, respectively) [305].

The phototoxicity of thiophenes and of some straight chain polyacetylenes to insects is well established. Protozoan neglected diseases are vectorized by insects so that it could be useful to investigate compounds potentially able to reduce the vectorization of these parasites. Thus, for example, a straight chain polyacetylene, tridecapentaynene (**9**) from *Rudbeckia hirta* (Asteraceae) was highly toxic to *Aedes atropalpus* (Diptera: Culicidae) [306].

Very recently, carlina oxide (**10**) from the roots of *Carlina acaulis* (Asteraceae), a plant used in traditional medicine due to its antimicrobial properties, has been shown to exhibit strong and selective activity against *Tbb*. Carlina oxide was active against trypomastigotes of *Tbb* with an IC_{50} value of 1.0 µg/µL and was 446 times less toxic against HeLa cells [307], so that it represents a very promising natural lead.

Given these examples, further investigations on this class of natural compounds in order to explore its indicative potential against protozoan diseases certainly appear worthwhile. The major challenge with regard to bioactive polyacetylenes is, however, to overcome their susceptibility to oxidation by air and light and their spontaneous polymerization. Therefore, development of an applicable therapy based on these compounds would require an efficient means of drug delivery which might be difficult to devise.

5. ALKALOIDS

The antiprotozoal activities of alkaloids of higher plants have been frequently reviewed with more than a dozen references [2, 5, 7, 68, 71, 308-316]. The present review intends to cover the literature as from 2007 with special bias towards the NTDs to avoid duplication and yet to highlight recent development since then. Nevertheless, a few milestones predate 2007 which are considered of significance and indispensable to complement the overview so that they are reiterated within the context of the present review. Meanwhile, due to the diversity of the biosynthetic origin of the various structural types of alkaloids, we believe that the most practical classification for the purpose of this review is in accordance to their chemical structures. Accordingly, we classified the antiparasitic alkaloids into five main groups, namely, quinoline, isoquinoline, indole, steroidal, diterpenoidal and miscellaneous alkaloids.

5.1. Quinoline Alkaloids

Due to the significance of quinoline alkaloids and their contribution to drug discovery, exemplified by the isolation of quinine (**1**) as the first antimalarial drug ever discovered from the bark of the *Cinchona* tree (Rubiaceae) more than 190 years ago [317], coupled with its immense direct impact on the industrial production of a range of synthetic antimalarials based on 4-substituted (e.g. chloroquine and mefloquine) or 8-substituted quinoline (e.g. primaquine), it is quite justifiable to honour this class of alkaloids at the outset of this section. Their structures (**1**-**28**) are represented in Fig. (**14A**).

Apparently, quinine (**1**) and its congeners quinidine (**2**), cinchonidine (**3**) and cinchonine (**4**) have a rather potent effect on *Tcr* by completely inhibiting epimastigote replication *in vitro* at 5 μg/mL (14 μM) [68]. Furthermore, the synthetic 8-aminoquinoline analogue, sitamaquine (**5**), is currently undergoing phase IIb clinical trials for the treatment of visceral leishmaniasis [318]. Although the mechanism of action of quinoline derivatives against kinetoplastid parasites remains unknown, evidence has emerged very recently about the detailed mechanism of action of sitamaquine (**5**) against *Leishmania*. **5** was shown to cause a dose-dependent inhibition of complex II (succinate dehydrogenase) of the respiratory chain in digitonin-permeabilized promastigotes, accompanied by a drop in intracellular ATP levels and a decrease of the mitochondrial electrochemical potential. These effects are associated with increases of reactive oxygen species and intracellular Ca^{2+} levels, a higher percentage of the population with sub-G(1) DNA content, and with exposure of phosphatidylserine. Taken together, these effects of **5** finally lead to an apoptosis-like death of *Leishmania* parasites [318]. This mechanism seems to be largely different from the suggested antiplasmodial mechanism of action of quinoline-type drugs, which is presumably based on binding to hemozoin, resulting in the accumulation of toxic heme which ultimately kills the parasite [319].

Six quinolinone alkaloids have been identified in a crude extract of *Haplophyllum acutifolium* (syn. *Haplophyllum perforatum*) (Rutaceae) by direct on-line hyphenation of high-performance liquid chromatography, photo-diode array detection, mass spectrometry, solid-phase extraction and nuclear magnetic resonance spectroscopy (HPLC-PDA-MS-SPE-NMR). The known quinolone acutine (**6**) and a new related alkaloid, hapacutine E (**7**), displayed moderate *in vitro* antiplasmodial activity against chloroquine-sensitive *Pfc* (3D7 strain) with IC_{50} values of 2.17 and 3.79 μM, respectively. However, the cytotoxicity profiles of these compounds have not been provided [320].

Eleven acridone alkaloids isolated from the botanically related taxon *Swinglea glutinosa* (Rutaceae) were tested *in vitro* against chloroquine-sensitive *Pfc* (3D7), *Tbr* (STIB900) and *Ldon* (L82). Nine of the alkaloids exhibited IC_{50} values ranging from 0.3 to 11.6 μM against *Pfc*. In contrast, a small number of compounds showed significant activity against *Tbr* and none had activity against *Ldon*. Three alkaloids had IC_{50}< 1.0 μM against *Pfc*, whereas five compounds had IC_{50}< 10 μM against *Tbr*. Compound **8** (glycocitrine-IV), with one prenyl group at C-2, was the most active against *Pfc* with IC_{50}= 0.3 μM. Comparison of **8** (IC_{50}= 0.3 μM) with **9** (IC_{50}= 2.6 μM) indicates that the second prenyl at C-8 might be responsible for reducing antiplasmodial activity in this series. Evaluation of the antiplasmodial potency of compounds **10**, **11**, **12**, **13** and **14** suggests that the presence of a pyran ring is important for activity, whereas the position of this group, angular pyrano[2,3-*c*] (**12**) or linear pyrano[3,2-*b*] (**13**), did not alter the results. Compounds **15** (citrusinine-II), **16** (citrusinine-I) and **17** (citibrasine), exhibited antiplasmodial activities with IC_{50} values of 8.9, 29.9 and 6.1 μM, respectively. The authors considered the improvement in the activity of compound **17** over **15** and **16** to be correlated with the presence of an *O*-methyl group at position C-2 [321].

From the stem bark of the African tree *Teclea gerrardii* (Rutaceae), commonly used in the traditional medicine as a febrifuge, five acridones and two furanoquinolines were isolated and their antimalarial activity tested against the CQS D10 strain of *Pfc*. Only the furanoquinoline evoxine (**18**) and the acridone arborinine (**19**) showed moderate antiplasmodial activity with IC_{50} values of 24.5 μM and 12.3 μM, respectively [322].

In vitro assessment of the chloroquine sensitive strain NF54 of *Pfc* with 3 μM concentration of the four furanoquinolines isolated from *Teclea afzelii* (Rutaceae), kokusaginine (**20**), maculine (**21**), tecleaverdoornine (**22**) and montrifoline (**23**), resulted in a partial suppression of the parasites. The best antiplasmodial activity was seen after treatment with kokusaginine (**20**). Testing of **20** at concentrations ranging from 2 to 8 μM resulted in a partial inhibitory effect at 4 μM and an even stronger effect at 8 μM. However, the effect can be considered as moderate when compared with chloroquine, which showed a complete growth inhibition of the parasites at a concentration of 0.032 μM [323].

The infusion of the bark of *Helietta apiculata* (Rutaceae) is used in Paraguay for wound healing in relation with leishmanial ulcers and for its anti-inflammatory properties. Bioassay-directed fractionation using *Lam* (MHOM/BR/PH8), *Linf* (MHOM/FR/91/LEM 2259), and *Lbra* (MHOM/BR/75/M-2903) *in vitro* culture led to the isolation of five furoquinolines, maculine (**21**), dictamine (**24**), γ -fagarine (**25**), skimmianine (**26**) and flindersiamine (**27**), along with four known coumarins. Among the isolated alkaloids, only γ-fagarine exhibited activity against the promastigote forms of the three *Leishmania* spp. (IC_{50} between 17 and 30 μM). *In vivo* treatment of *Lam*-infected Balb/C mice with γ -fagarine (**25**) at 10 mg/kg/d (p.o.) over 14 days produced a significant reduction of the lesion weight by 66.9% and a drastic reduction of the lesional parasites by 97.4%. The good *in vivo* result of γ-fagarine in spite of its relatively weak *in vitro* activity was related by the authors to the formation of potential metabolites by cytochrome P450 enzymes. Two of the coumarins, (-)-heliettin and (+)-3-(1'-dimethylallyl)-decursinol (not shown) also gave promising *in vivo* results after s.c. injection [324].

Among seven quinolone alkaloids isolated from the leaves of *Raputia heptaphylla* (Rutaceae) along with some *seco*-limonoids, the pyranoquinoline alkaloid N-methyl-8-methoxyflindersine (**28**) showed antileishmanial activity against extracellular promastigotes of *Lpan* whose viability was reduced with an EC_{50} value of 14.3 μg/mL [325].

5.2. Isoquinoline Alkaloids

The structures of isoquinoline alkaloids (**29**-**87**) are presented in Fig. (**14A**-**14C**).

Among the 9 alkaloids isolated from the tuber of *Stephania rotunda*, 4 isoquinolines were subjected to *in vitro* evaluation against *Pfc*, along with the aqueous and dichloromethane extracts. The aporphine alkaloid dehydroroemerine (**29**) and the bisbenzylisoquinoline alkaloid cepharanthine (**30**) showed the best *in vitro* antimalarial activity against W2 with IC_{50} values of 0.36 and 0.61 μM, respectively. In cytotoxicity assays on human monocytic THP1 cells, **29** and **30** showed IC_{50} values of 10.8 and 10.3 μM and their SI were 30 and 16.9, respectively [326].

Evaluation of the antitrypanosomal activity of the dichloromethane/methanol (1:1) extract of the stem bark of the Cameroonian medicinal plant *Garcinia lucida* (Clusiaceae) showed inhibition of the bloodstream form *Tbb* (IC_{50}= 4.9 μg/mL). A bioguided fractionation of this extract resulted in the isolation of three benzo[*c*]phenanthridine alkaloids, namely, dihydrochelerythrine (**31**), 6-acetonyldihydrochelerythrine (**32**), and its new derivative, lucidamine A (**33**). A semisynthetic derivative of **33** was prepared and designated as lucidamine B (**34**). The four compounds exhibited antitrypanosomal activity with IC_{50}s of 0.8, 0.39, 14.1 and 4.1 μg/mL respectively, with no toxicity on Vero cells. Dihydrochelerythrine (**31**) was the most potent compound (IC_{50}= 0.8 μM), with more than 44-fold selectivity for *Tbb* over Vero cells. When those compounds were tested on the axenic amastigote form of *Ldon*, **31** was the most potent (IC_{50}= 2.0 μM), followed by **32**, **34** and **33** (IC_{50}= 6.6, 6.8 and 10.8 μM, respectively). **31** and its derivatives were thus also proven to be potential antileishmanial candidates [327].

The isoquinoline alkaloid protopine (**35**) and its tetrahydro derivative cheilanthifoline (**36**) were found to be associated with the antimalarial activity of *Corydalis calliantha* (Fumariaceae), which is commonly used in Bhutanese folk medicine to treat malaria. Both, **35** and **36**, showed promising *in vitro* antiplasmodial activities against *Pfc* wild type (TM4) as well as multidrug resistant (K1) strains with IC_{50} values in the range of 2.78-4.29 μM. The results validate the clinical use of this plant in traditional medicine and revealed cheilanthifoline (**36**) to be a promising bioactive candidate for further investigation [328].

The antiplasmodial and antitrypanosomal activities of *Triclisia sacleuxii* (Menispermaceae), used to treat either malaria and/or fevers in Tanzanian traditional medicine, were investigated on chloroquine-sensitive (3D7) and chloroquine-resistant (W2) *Pfc* strains as well as on *Tb*. Ethanolic extracts from roots, stems and leaves as well as crude fractions with tertiary and quaternary alkaloids were considered. While the ethanolic extracts and quaternary crude alkaloid fractions exhibited no significant activity, the tertiary alkaloid fraction from roots and stems revealed interesting growth inhibition against *Pfc* and *Tb* strains. The IC_{50} were 1.04 and 0.89 μg/mL for roots, 2.50 and 0.91 μg/mL for stems, respectively. The tertiary alkaloid fraction from leaves also showed a promising antitrypanosomal activity (IC_{50}= 1.85 μg/mL). Phytochemical analysis of the root fraction yielded four major bisbenzylisoquinoline alkaloids, phaeanthine (**37**), N-methylapateline (**38**), 1,2-dehydroapateline (**39**) and 1,2-dehydrotelobine (**40**), which displayed *in vitro* antitrypanosomal activity with IC_{50} values of 2.68, 1.19, 1.06 and 1.11 μM, respectively. They also demonstrated antiplasmodial activity on *Pfc* 3D7 with IC_{50}s of 1.72, 0.93, 1.39 and 12.4 μM and on the chloroquine-resistant W2 with IC_{50}s of 0.35, 1.10, 1.63 and 1.52 μM, respectively. However, these alkaloids also displayed cytotoxicity towards the human MCR-5 cell line with little difference between the antiprotozoal and cytotoxic concentrations so that it was concluded by the authors that these compounds cannot be considered likely direct antimalarial drug candidates [329].

It is worth mentioning at this juncture that phaeanthine (**37**) had previously been isolated from the related species *Triclisia patens* and exhibited activity against *Tb* trypomastigotes (IC_{50}= 1.73 μM) but demonstrated cytotoxicity to murine macrophages at the active concentration [330]. Likewise, investigation of 1,2-dehydrotelobine (**40**), previously isolated from *Stephania erecta* (Menispermaceae), displayed antiplasmodial activity on a chloroquine-sensitive strain (D6) with IC_{50} of 0.54 μM and on a chloroquine-resistant strain (W2) with IC_{50} of 0.45 μM, accompanied by high cytotoxicity [331].

Twelve isoquinolines were isolated from the roots of *Thalictrum flavum* (Ranunculaceae) and assessed for their antimalarial and antileishmanial activity against *Pfc* and *Lmaj*, respectively. The ubiquitous quaternary protoberberine alkaloid berberine (**41**) was identified as the major component with appreciable antimalarial activity (IC_{50}= 0.4 μg/mL). Its analogue, pseudoberberine (**42**), isolated for the first time from this plant, was found similarly active (IC_{50}= 0.5 μg/mL). Six bisbenzylisoquinolines were identified, including thalfoetidine (**43**) (IC_{50} = 2.1 μg/mL) and its demethyl analog northalfoetidine (**44**) (IC_{50}= 2.8 μg/mL). Moreover, bisbenzylisoquinoline-, aporphine-, phenanthrene- and benzylisoquinoline alkaloids, previously isolated from other *Thalictrum* species, were newly described in the roots of *T. flavum*. The tertiary isoquinolines, especially bisbenzylisoquinolines, were found to be leishmanicidal against *Lmaj*. Thalfoetidine (**43**) appeared as the most potent (IC_{50}= 17 μg/mL) but northalfoetidine (**44**) (IC_{50} = 39 μg/mL) is of particular interest since its leishmanicidal activity was not associated with high cytotoxicity [332].

In a screening of Paraguayan plants for antileishmanial activity, the alkaloidal crude extract of the popular plant *Ocotea lancifolia* (Lauraceae) was investigated. Fractionation yielded thirteen known isoquinoline alkaloids, namely, coclaurine, N-methylcoclaurine, crostparine, glaziovine, caaverine, laurotetanine, nordomesticine, norisoboldine, norantenine, corytuberine, domesticine, isoboldine, and (*S*)-pallidine. The *in vitro* screening of these alkaloids against the promastigote forms of *Lbra*, *Lam* and *Ldon* revealed that only the noraporphines caaverine (**45**) and nordomesticine (**46**) were active against the three *Leishmania* species (IC_{100}= 10 and 25 μg/mL, respectively). **45** and **46** also exhibited weak antitrypanosomal activity *in vitro* against *Tcr* with IC_{50} of 155 and 105 μg/mL, respectively. Alkaloid **45,** the most active compound of this study against *Leishmania* species and *Tcr*, unfortunately was found to be hepatotoxic (LC_{50}=16 μg/mL) [333].

The monoterpenoid isoquinoline alkaloid emetine (**47**) occurs along with its analogues in three plant families, namely, Alangiaceae, Icacinaceae, and Rubiaceae. One major source of emetine and its congeners is the root of *Carapichea ipecacuanha* (syn. *Cephaelis* or *Psychotria ipecacuanha*, Rubiaceae). Emetine (**47**) has long been known to possess antiparasitic activities against *Entamoeba histolytica*, *Protostrongylus rufescens*, *Ldon*, *Tcr and Tb* [334]. It has also been independently demonstrated that **47** has *in vitro* activity against *Tbb* bloodstream forms by inducing apoptosis as a result of its interaction with DNA and inhibition of parasite protein biosynthesis [335]. However, high acute and subacute toxicity renders **47** rather unsuitable for clinical applications. Testing the antiparasitic activity of its dehydro derivative (*i.e.* dehydroemetine) which has lower toxicity and accumulative action might be interesting.

The antitrypanosomal effect and cytotoxicity of 34 alkaloids of the piperidine, pyridine, tropane, isoquinoline, indole, quinolizidine, quinoline, purine, and steroidal types on the growth of *Tb* and *Tcon* have been investigated *in vitro* using Alamar Blue® and human myeloid leukaemia HL-60 cells. Berbamine, berberine, cinchonidine, cinchonine, emetine, ergotamine, quinidine, quinine, and sanguinarine showed trypanocidal activities with ED_{50} (50% effective dose) values below 10 μM. Among them, berberine (**41**), emetine (**47**), and quinidine (**2**) were the most active compounds

with ED_{50} values and minimum inhibitory concentrations comparable to those of the antitrypanosomal drugs suramin and diminazene aceturate [336].

The decoction of the leaves of *Argemone mexicana* (Papaveraceae) is widely used in Sudanese traditional medicine for the treatment of malaria and early stage trypanosomiasis. *In vitro* evaluation of the aqueous extract displayed prominent antitrypanosomal activity against *Tbr* (IC_{50}= 0.09 μg/mL). Bioactivity-directed fractionation of this extract resulted in the isolation of the quaternary benzo[*c*]phenanthridine alkaloid sanguinarine (**48**) (IC_{50} = 0.05 μg/mL). The *in vitro* cytotoxicity of **48** on three cell lines (HT-29, MRC-5 and NBZa) was less than antitrypanosomal activity by factor of 60, 17 and 70, respectively (IC_{50} values: 3.0, 0.85 and 3.5 μg/mL). Thus, sanguinarine (**48**) appears to show selective toxicity towards *Tbr* [337]. Based on its quaternary nature and polarity it is anticipated, however, that sanguinarine would not be able to treat the late-stage of the disease due to its inability to cross the blood-brain barrier. Testing of *A. mexicana* against chloroquine resistant strains of *Pfc* revealed that activity is almost confined to the aqueous extract (IC_{50} = 4.5 μg/mL). Sanguinarine (**48**), however, exhibited relatively weak antiplasmodial activity (IC_{50}= 2.65 μg/mL) [337].

Five isoquinoline alkaloids were isolated from subterranean stem bark of *Duguetia furfuracea* (Annonaceae). The isolated compounds displayed antiprotozoal activity against trypomastigotes of *Tcr* and promastigotes of *Lbra*. Among the isolated compounds, the aporphine alkaloid duguetine (**49**) exhibited the most prominent activity against *Tcr* (IC_{50}= 9.32 μM) while its β -*N*-oxide (**50**) exhibited the least trypanocidal activity (IC_{50}= 30.79 μM). The oxoaporphine alkaloid, dicentrinone (**51**) exhibited intermediate activity (IC_{50}= 18.83 μM). On the other hand, **51** displayed the highest activity against *Lbra* promastigotes (IC_{50}= 0.01 μM), followed by **50** (IC_{50}= 0.11 μM), **49** (IC_{50}= 4.32) and *N*-methylglaucine (**52**) (IC_{50}= 4.88 μM). It is relevant to mention that duguetine (**49**) and its β-*N*-oxide **50** showed considerable cytotoxic activity against three cancer cell lines [338].

The benzylisoquinoline **53** and an aporphine alkaloid (**54**), isolated from *Doryphora sassafras* (Monimiaceae), were evaluated for their antiplasmodial *in vitro* activity against *Pfc* strains 3D7 and Dd2. They exhibited IC_{50} values of 3.0 and 4.4 μM, respectively. Compound **53** was tested for cytotoxicity toward a human embryonic kidney cell line (HEK293) and showed no activity at 120 μM [339].

The structurally related aporphine alkaloid xylopine (**55**), isolated from *Guatteria amplifolia* (Annonaceae), proved to be active against *Lmex* and *Lpan* (IC_{50}= 3 and 6 μM, rep.), and showed a 37-fold higher toxicity towards *Lmex* than to macrophages, the regular host cells of *Leishmania* spp. [340].

Investigation of the antimalarial activity of the decoction of the stem bark of *Strychnopsis thouarsii* (Menispermaceae), a plant used in the traditional medicine of Madagascar, proved its validity as antiplasmodial agent in the rodent model by targeting *Pyoe*. The number of parasites in the hepatocytes at the early liver stage was reduced with IC_{50} values of 79.6 and 12.5 μg/mL by an aqueous decoction and an ethanolic extract, respectively. Bioassay-guided fractionation of *S. thouarsii* stem bark extract using the *Pyoe* liver stage parasite inhibition assay led to to the isolation of the morphinan alkaloids tazopsine (**56**), sinococuline (**57**), 10-*epi*-tazopsine (**58**) and 10-*epi*-tazoside (**59**) [341]. Except for 10-*epi*-tazoside (**59**), which did not show any antimalarial activity (IC_{50}> 390 μM), compounds **56**-**58** exhibited a significant and selective inhibitory activity against *Pyoe* liver stage *in vitro*. Tazopsine (**56**) showed the most potent effect with an IC_{50} value of 3.1 μM and an SI of 14.0 (determined with primary mouse hepatocytes). Sinococuline (**57**) was slightly less active (IC_{50}=4.5 μM) and equally less toxic on the primary mouse hepatocytes (SI=13.8), while 10-*epi*-tazopsine (**58**) was 5-fold less active but also less toxic on host cells than **56**, yielding a higher selectivity index (SI= 24.1). The presence of a free hydroxy group with (*R*)-stereochemistry enhanced the inhibitory effect against *Pyoe* liver stage, while presence of (*S*)-stereochemistry decreased significantly the toxicity on mouse primary hepatocytes. The linkage of this hydroxy group with a glucose unit via a β-glucosidic bond led to a loss in activity. This could be attributed to steric bulk created by the glucose unit. A total inhibition was observed for tazopsine (**56**) at a concentration as low as 7.1 μM, whereas *Pyoe* parasites could still be observed in cultures treated with high concentrations of the reference drug primaquine (38.6 μM of primaquine only inhibited 80% of parasites [341]).

The naphthylisoquinoline (NIQ) alkaloids are a rapidly growing class of natural products remarkable in many respects. Structurally, they are characterized by an unusual and biogenetically unique framework of acetate origin including a methyl substituent at C-3 and a *meta*-oxygenation pattern at C-6 and C-8. NIQs, as natural biaryls with an axis that connects the isoquinoline part to the naphthalene moiety, show axial chirality. To date, NIQ alkaloids are restricted in their chemotaxonomical distribution to the Ancistrocladaceae and Dioncophyllaceae, both small families of tropical origin within the order Caryophyllales. Dioncophyllaceae comprise only three species: *Triphyophyllum peltatum* (a carnivorous liana), *Habropetalum dawei* and *Dioncophyllum thollonii*. Closely related are the Ancistrocladaceae with only one genus, *Ancistrocladus*, consising of about 25 species found in the palaeotropic rain forests of Africa and Asia.

The activities of various naturally occurring naphthylisoquinoline (NIQ) alkaloids and their synthetic analogues were tested *in vitro* using *Lmaj* promastigotes and the macrophage cell line J774.1. Dioncophylline C (**60**), ancistroheynine B (**61**), *ent*-dioncophylleine A (**62**), and *N*-phenyl-6,8-dimethoxy-1,3-dimethylisoquinolinium chloride (**63**) inhibited the growth of J774.1 macrophages (IC_{50}: 32.83, 29.90, 34.83, and 43.70 μM, respectively) at concentrations similar to those needed to inhibit the proliferation of parasites. In contrast, compound **64** inhibited the growth of macrophages at concentrations (IC_{50}= 39.20 μM) higher than those needed to inhibit the proliferation of *Lmaj* (IC_{50}= 26.58 μM) but this level of antileishmanial activity is quite low. Ancistrocladiniums A (**65**) and B (**66**) and the synthetic analogue (**67**) displayed higher antileishmanial activity (IC_{50}= 4.90, 1.24 and 2.91 μM, respectively) and were toxic against the macrophage cell line at concentrations significantly higher than those needed to inhibit parasite cell growth (IC_{50}= 31.76, 11.21 and 10.15 μM, respectively). The IC_{50} for amphotericin B, used as a reference compound, was 2.51 μM for *Lmaj* promastigotes, without affecting the growth of J774.1 macrophages. Together, these results suggest that the efficacy of **65**-**67** against *Lmaj* is similar to that of amphotericin B, although they are more toxic against the macrophage cell line J774.1. Macrophages are important target cells in the therapy of leishmaniasis because they are critical for the clearance of intracellular parasites via the production of cytokines and reactive nitrogen metabolites [342]. Surprisingly, the effect of the studied alkaloids seems not to be associated with the stimulation of host macrophages to produce nitric oxide or secrete cytokines relevant for the leishmanicidal function. A question that remains, however, is why low inhibitor concentrations decreased the infection rate of macrophages but did not kill promastigotes. This effect might be the consequence of an increased sensitivity of amastigotes or a selective activity of the NIQs against amastigotes. Alternatively, it may be caused by a selective uptake mechanism through the macrophage plasma membrane with enhanced concentrations of the compounds reaching the parasitophorous vacuole within the host cell. Whatsoever, the data suggest that **65**-**67** can be considered as lead candidates for new leishmanicidal drugs and the authors concluded that their results shed some light

on structural features involving the importance of the quaternary nitrogen atom and its counter-ion for antileishmanial activity. They anticipated that these findings might help in the selection and synthesis of novel synthetic NIQs based on QSAR-guided design [342].

From the roots of a recently discovered Congolese *Ancistrocladus* taxon with close affinities to *A. congolensis*, six NIQ alkaloids, 5'-*O*-demethylhamatine (**68**), 5'-*O*-demethyl-hamatinine (**69**), 6-*O*-demethylancistroealaine A (**70**), 6,5'-*O*,*O*-didemethylancistroealaine A (**71**), 5-*epi*-6-*O*-methylancistrobertsonine A (**72**), and 5-epi-4'-*O*-demethylancistrobertsonine C (**73**) were isolated along with a benzopyranone carboxylic acid (see section 4.2) [254]. Alkaloids **68**-**73** as well as **74**, a compound isolated from *A. abbreviatus*, were found to exhibit weak antiplasmodial activities against the K1 strain of *Pfc* (resistant to chloroquine and pyrimethamine) [254], though not reaching the good results of other NIQs previously reported [343-345]. The antitrypanosomal activities of **68**-**74** against *Tbr* and *Tcr* were moderate to weak. Compound **72** exhibited activity against *Ldon* comparable to those of the most active NIQ alkaloids previously tested. The two *O*-demethyl derivatives **70** and **71** of the highly antileishmanially active ancistroealaine A (**75**) [346], by contrast, exhibited only weak activity against *Ldon*, thus indicating a high degree of O-methylation as an important precondition for good antileishmanial activity within this chemical class. The likewise isolated N,C-coupled ancistrocladinium A (**65**) previously described for leaf extracts of this *Ancistrocladus* species, had been shown to exhibit promising bioactivities, especially against *Leishmania* [347]. Therefore, further bioactivity tests and synthetic studies in combination with detailed structure-activity relationship investigations have been conducted to further optimize the activity of such N,C coupled NIQ alkaloids [342].

The continuous search for novel NIQ alkaloids with antiparasitic activity resulted very recently in the isolation of the first N,8'-coupled NIQ alkaloids with free phenolic OH groups, 4'-*O*-demethylancistrocladinium A **76** and 6,4'-*O*-didemethylancistrocladinium A (**77**) from the leaves and bark of the Vietnamese liana *Ancistrocladus cochinchinensis*, along with the known parent compound **65** and four C,C-coupled representatives [348]. **76** exhibited good antiplasmodial activity against the chloroquine- and pyrimethamine-resistant K1 strain of *Pfc*, similar to the parent compound ancistrocladinium A (**65**). The antiprotozoal activities for the bisphenolic derivative **77** were moderate to weak against *Tbr, Tbb*, as well as *Tcr*, *Ldon* amastigotes and *Lmaj* promastigotes, while the mono-phenolic analog **76** showed an excellent activity against *Tcr*, much better than the fully O-methylated parent compound [347]. Apparently, the one free OH group of **76** represents an optimum for activity against *Tcr* and *Ldon*. Meanwhile, the additional OH group present in **77** seems to hamper substantially the antiparasitic activity against the tested parasites [348].

Recent investigation of the alkaloidal constituents in the aerial part and bulbs of *Lycoris traubii* (Amaryllidaceae) resulted in the isolation of one new lycorine-type alkaloid named LT1 (**78**) and some of its derivatives [349]. Compound LT1 (**78**) and lycorine (**79**) as well as its derivatives (**80**-**87**) have been assessed *in vitro* for their antimalarial activity by using the drug-resistant K1 strain and the drug-sensitive FCR3 strain of *Pfc*. Among the tested compounds, **85**, **86**, LT1 (**78**) and **83** showed high antimalarial activities with IC_{50} values of 0.67, 0.37, 0.60, and 0.62 μg/mL for the K1 strain and of 0.53, 0.30, 0.45, and 0.49 μg/mL for the FCR3 strain, respectively. The cytotoxicities of these compounds have been evaluated on the human diploid embryonic cell line MRC-5. Among the tested compounds, **85**, **86**, LT1 (**78**) and **83** showed higher SIs with ratios of 21.7, 13.5, 13.5, and 10.2 for the K1 strain, respectively. The other compounds showed SIs lower than **84** [349]. *In vitro* bioactivity tests using *Tbb* were performed on isolated alkaloid **78** and lycorine derivatives (**80**, **81**, **82**, **83**, **86**, and **87**). Pentamidine, suramin, and eflornithine were used as standards. 2-*O*-Acetyllycorine (**82**) showed significant inhibitory activity against *Tbb* (strain GUTat 3.1) with an IC_{50} of 0.15 μg/mL (approved drugs pentamidine, suramin, and eflornithine had IC_{50} values of 0.00158, 1.58, and 2.27 μg/mL, respectively), and showed low cytotoxicity of 6.11 μg/mL as well as a high selectivity index (SI) of 40.7. 1-*O*-acetyllycorine (**80**), 1,2-di-*O*-acetyllycorine (**81**), and 1-*O*-(30*R*)-hydroxybutanoyllycorine (**83**) exhibited low antitrypanosomal activities while 1-*O*-propanoyllycorine (**86**), 1-*O*-butanoyllycorine (**87**) and the natural compound 1-O-(30*S*)-hydroxybutanoyllycorine (**78**) showed moderate activities. Although 1, 2-di-*O*-acetyllycorine (**81**) was reported to exhibit potent activity against *Tbr*. These results showed that it had low inhibitory activity against *Tbb*, probably because of the difference in strain [349].

5.3. Indole Alkaloids

The structures of the mentioned indole alkaloids (**88**-**123**) are shown in (Fig. **14C** and **14D**).

The two simple indole alkaloids **88** and **89**, isolated from *Clausena harmandiana* (Rutaceae), exhibited antiplasmodial activity with IC_{50} values of 15.5 and 12.2 μM, respectively, against the *Pfc* strain K1 [350].

The azafluorenone 5-hydroxy-6-methoxyonychine (**90**) obtained from *Mitrephora diversifolia* (Annonaceae) has been shown to be active against *Pfc* strains 3D7 and Dd2, with IC_{50} values of 9.9 and 11.4 μM, respectively [351].

The genus *Kopsia*, which is widely distributed in Southeast Asia, represents a rich source of novel indole alkaloids characterized by an intriguing variety of carbon skeletons and interesting biological activity. A number of indole alkaloids have been isolated from *K. griffithii* and three of them, harmane (=demethoxy-**95**), pleiocarpine and buchtienine (not shown), displayed leishmanicidal activity against the promastigote forms of *Ldon* [352]. No reference so far has been made to antiparasitic activity of 6-oxoleuconoxine (**91**), also from *K. griffithii*, and three alkaloids, kopsinitarine E (**92**), kopsijasminine (**93**), and kopsonoline (**94**) from *K. teoi*, isolated in a more recent study [353].

Bioassay-guided isolation from the seeds of *Peganum harmala* (Nitrariaceae, formerly Zygophyllaceae) yielded two β -carboline alkaloids, harmine (**95**) and harmaline (**96**) as well as two quinazoline alkaloids, vasicinone (**97**) and deoxyvasicinone (**98**). **95** and **96** exhibited antiplasmodial activity against *Pfc* with IC_{50} values of 8.0 and 25.1 μg/mL respectively. The quinazolines **97** and deoxyvasicinone (**98**) did not show any antiplasmodial activity (IC_{50}>10 μg/mL) [354].

The indole alkaloids ellipticine (**99**) and aspidocarpine (**100**) were isolated from the bark of the Amazonian plants *Aspidosperma vargasii and A. desmanthum* (Apocynaceae), respectively. They were subjected to *in vitro* evaluation against the multidrug-resistant K1 strain of *Pfc*. Both, **99** and **100**, presented significant inhibition of parasite growth (IC_{50} = 0.073 and 0.019 μM, respectively) [355]. Very recently, **99** was also tested *in vitro* against *Tbr* (bloodstream forms), *Tcr* (intracellular amastigotes) and *Ldon* (axenic amastigotes) where it yielded IC_{50} values of 0.32, 2.43 and 11.50 μM, respectively. The cytotoxicity against L6 cells, however, was also very high (IC_{50}= 0.93 μM) (Schmidt, T.J. and Brun, R., unpublished).

The genus *Strychnos* comprises about 200 species and can be subdivided into three geographically distinct groups occurring in Central and South America, Africa, and Asia. The liana *Strychnos icaja*, besides being used as an ordeal poison, is also occasionally used in African traditional medicine to treat malaria. The roots of this species are particularly rich in antiplasmodial indole alkaloids

including some bisindole derivatives such as 18-hydroxyisosungucine (**101**) and strychnogucine B (**102**) as well as the trisindolomonoterpene alkaloid strychnohexamine (**103**). These alkaloids, posessing a strychnine substructure, have demonstrated potent and selective antiplasmodial properties [356]. *In vitro* antiplasmodial activity was reported for the quasi-symmetric bisindolomonoterpenoid alkaloid isosungucine (**104**), which yielded IC_{50} values of 1.32 and 0.27 μM against chloroquine-sensitive (FCA) and -resistant (W2) strains of *Pfc*, respectively. This result has encouraged the *in vivo* evaluation of this alkaloid against the *Pvin* murine strain in the Peters 4-day suppressive test. Interestingly, **104** suppressed the parasitemia by almost 50% on day 4 when administered intraperitoneally at a dose of 30 mg/kg/d [357].

Four novel bis-indole alkaloids designated as flinderoles A−C (**105**-**107**) exhibited selective antimalarial activities with IC_{50} values between 0.15-1.42 μM. Flinderole A (**105**) and isoborreverine (**108**) have been isolated from *Flindersia acuminata* (Rutaceae) while dimethylisoborreverine (**109**) and flinderoles B (**106**), and C (**107**) were obtained from *Flindersia ambionensis*. These unprecedented rearranged bis-indole alkaloids showed selective antiplasmodial activities, with IC_{50} values ranging from 0.08 to 1.42 μM against the *Pfc* strain Dd2. Their selectivity (SI=14-29) was assessed using the mammalian cell line HEK-293 [358].

In a recent study, alkaloids isolated from extracts of *Flindersia amboinensis* (Rutaceae), *Stephania zippeliana* (Menispermaceae) and *Voacanga papuana* (Apocynaceae) from Papua New Guinea as well as *Flindersia acuminata* from Australia were examined for their antiparasitic activity against *Pfc* and *Tbb* as well as for cytotoxicity against the mammalian cell lines HEK 293 and HeLa. The most active compound, dimethylisoborreverine (**109**), showed submicromolar activity, with IC_{50} values between 20 nM and 810 nM against drug-sensitive and drug-resistant *Pfc* strains, along with moderate selectivity. Stage specificity studies revealed that *Pfc* trophozoite-stage parasites were more susceptible to **109** than ring- or schizont-stage parasites. Trophozoites treated with **109** showed changes in food vacuole morphology, with an apparent reduction in hemozoin formation that does not appear to be inhibited via the direct binding of heme. These findings suggesting an interesting potential for indole alkaloids from *Flindersia* spp., the antiplasmodial activities of the aforementioned five alkaloids **105**-**109**, along with voacamine (**110**), were evaluated using *Pfc* strains with different drug-resistance profiles (3D7, FCR3, HB3 and K1). Some differences in the IC_{50} values among strains were observed. These compounds were also tested for antitrypanosomal activity against *Tbb* and the bis-indole alkaloid isoborreverine (**108**) exhibited the highest activity (IC_{50} = 2.87 μM) [359].

Voacamine (**110**), isolated from the leaves of *Peschiera fuchsiaefolia* (Apocynaceae), was previously demonstrated to be the most active compound among the alkaloids found in this taxon [360].

Search for new bioactive alkaloids in tropical plants of Malaysia and Indonesia yielded a series of bisindole alkaloids from the bark of *Hunteria zeylanica* (Apocynaceae), bisnicalaterines A-D (**111**-**113, 115**) as well as the monomer nicalaterine A (**114**), with potent antiplasmodial activity. The antimalarial activity for **111**-**115** against *Pfc* 3D7 strain was evaluated. Nicalaterine A (**114**) and bisnicalaterine C (**113**) showed potent antiplasmodial activity (IC_{50}= 0.11 and 0.05 μM, respectively) with considerable selectivity (SI> 450 and > 1000, respectively). Bisnicalaterine C (**113**) with an extended conformation was 20 times more effective than its stereoisomer bisnicalaterine B (**112**) possessing a twisted conformation. Bisnicalaterine D (**115**), also adopting a twisted conformation, showed practically no antimalarial activity. The conformation around the C-16 - C-9' bond thus appears to be of crucial impact on antimalarial activity [361].

In the course of a phytochemical investigation of Sudanese medicinal plants used for treatment of malaria, the chloroform extract from the root bark of *Nauclea latifolia* (Rubiaceae) was subjected to bioactivity directed fractionation. It yielded the glucosidic monoterpene indole alkaloid strictosamide (**116**), which exhibited relatively weak antiparasitic activities with IC_{50} values of 54.1, 55.7, 29.7 and 18.5 μg/mL against *Tbr* (trypomastigotes), *Tcr* (intracellular amastigotes), *Ldon* (axenic amastigotes) and *Pfc* (chloroquine-resistant K1 strain), respectively. The cytotoxicity of **116**, however, was 51.9 μg/mL which indicates some selectivity only towards *Ldon* and *Pfc* (Khalid, S.A. *et al.*, unpublished).

It is of interest to note that a number of other species of this genus (e.g. *Nauclea orientalis, N. diderrichii* and *N. pobeguinii*) are frequently used in the African traditional medicine as antimalarials and some commercial formulations based on *N. latifolia* are currently marketed in the DR Congo for uncomplicated malaria [362]. The antiplasmodial activity of these extracts has been attributed mainly to strictosamide (strictosidine lactam) and other related indole alkaloids. There is a real controversy, however, regarding the reported *in vitro* IC_{50} values of strictosamide against the chloroquine-resistant K1-strain with extreme values of 0.37 μg/mL [363] and 547 μg/mL [364]. Furthermore, there is some degree of skepticism about the ability of the strictosamide to enter the parasite and reach the intracellular target due to its glycosidic nature. Nevertheless, the *in vivo* results give credence for the oral use of the decoction in the African traditional medicine considering the envisaged metabolic activation of strictosamide as a result of its de-glucosylation to yield mainly its aglycone which is capable to penetrate the parasite and reach its intracellular target. However, rapid recrudescence is considered as one of the main drawbacks of this alkaloid [362]. The metabolic fate of strictosamide has recently been demonstrated in rat bile using ion trap-TOF mass spectrometry and mass defect filter technique [365]. The presence of other metabolites bearing a 4,9-dihydro-3*H*-β-carboline moiety resulting from the metabolic transformation of strictosamide or its derivatives may partly contribute to our understanding of the antiparasitic activity associated with the traditional use of strictosamide-containing plants. β -Carbolines have attracted attention due to the variety of their biological activities resulting from intercalation into DNA as well as inhibition of topoisomerase and monoamine oxidase, beside their antiparasitic activities [313].

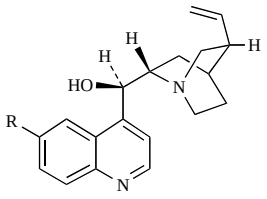
Continuing search for antitumor and antimalarial agents in the stem and bark of *Nauclea officinalis* resulted in the isolation of five new indole alkaloids designated as naucleofficines A-E (**117**-**121**), as well as two known indoles, naucleidinal (**122**) and angustoline (**123**) [366]. Compounds **118** and **119** are rare examples of monoterpene indole alkaloids with a glucopyranosyloxy group attached to the position C-12. *In vitro* screening of the antimalarial activity of the mentioned seven compounds showed weak to moderate inhibitory activity against *Pfc* [366].

5.4. Steroidal Alkaloids

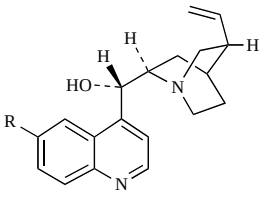
Structures of steroidal alkaloids **124**-**138** are depicted in Fig. (**14D** and **14E**).

Sarcococca hookeriana (Buxaceae) is an evergreen shrub whose root extracts are commonly used by the rural communities in Nepal against gout. Two new steroidal alkaloids, hookerianamides J (**124**) and K (**125**), as well as eight known compounds (**126**-**133**) have been isolated from the whole air-dried plant [367]. Compounds **124**-**133** were tested *in vitro* for leishmanicidal activity against *Lmaj*. All compounds displayed moderate to potent leishmanicidal activity, with compound **133** showing the best potency (IC_{50}= 1.5 μM), comparable to the positive controls amphotericin B (IC_{50} 0.5 μM) and pentamidine (IC_{50}= 7.5 μM).

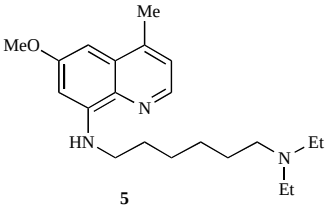
Glycoalkaloids have been frequently been reported from Solanaceae species. Five of them, α-chaconine (**134**), α-solanine



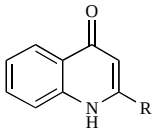
R
1 OMe
3 H

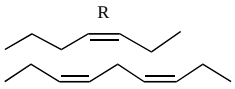


R
2 OMe
4 H



5





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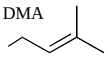
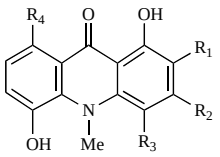
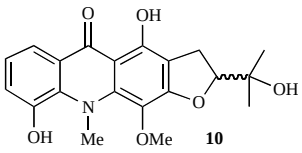


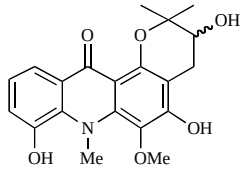
DMA



	R_1	R_2	R_3	R_4
8	DMA	OH	OMe	H
9	DMA	OH	H	DMA
15	H	OH	OMe	H
16	H	OMe	OMe	H
17	OMe	OMe	OMe	H



10



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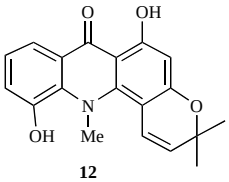
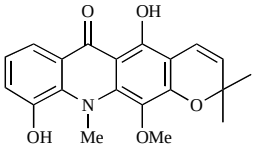


12



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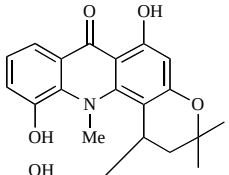

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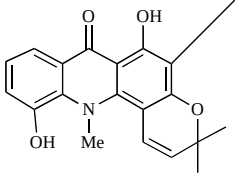

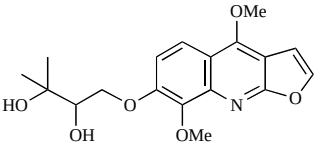

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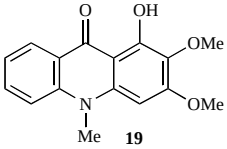

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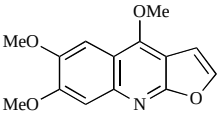

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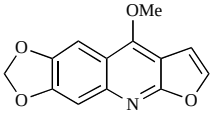

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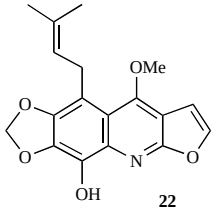

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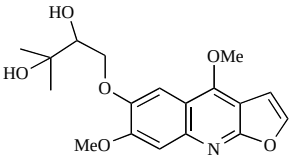

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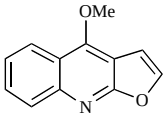

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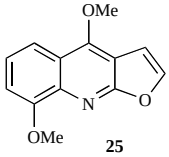

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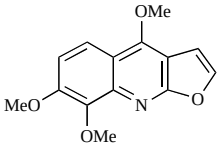

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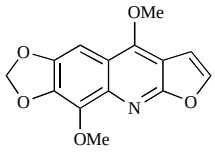

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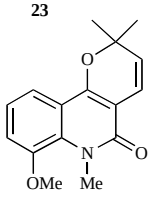

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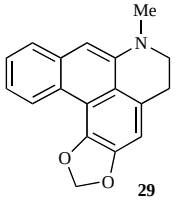

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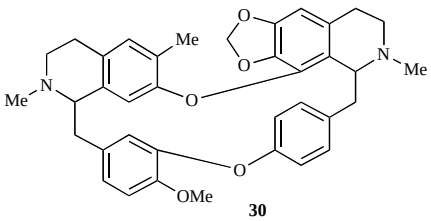

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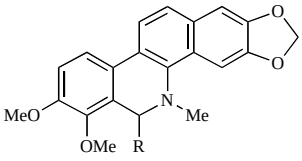
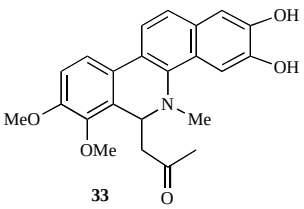


R
31 H
32 CH_2COMe



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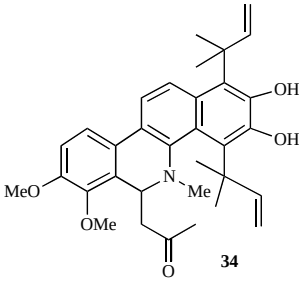

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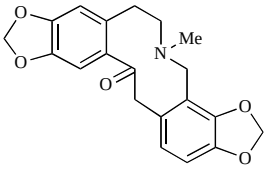

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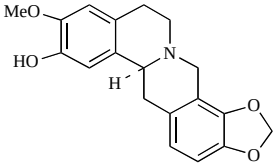
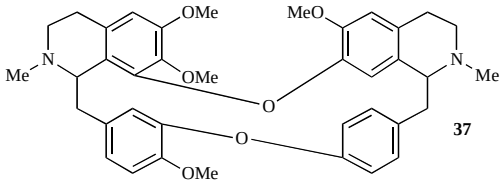


36

A

(Fig. 14). Contd…..



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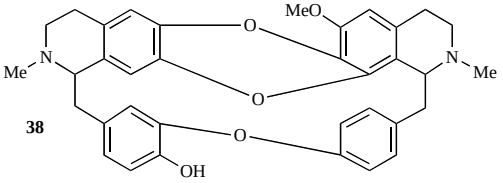

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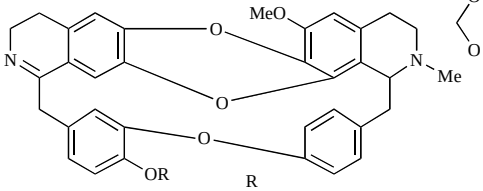
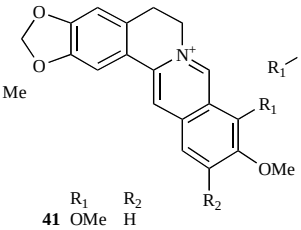
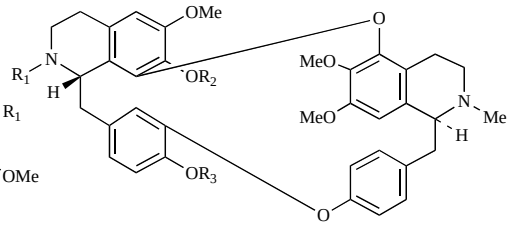


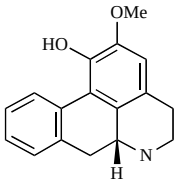
	R
39	H
40	Me



	R_1	R_2
41	OMe	H
42	H	OMe



	R_1	R_2	R_3
43	Me	Me	H
44	H	Me	H



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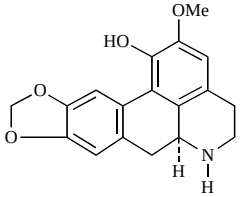

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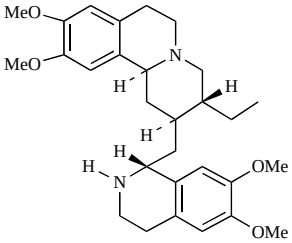

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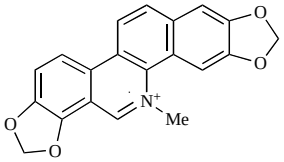

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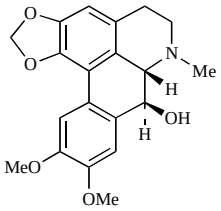

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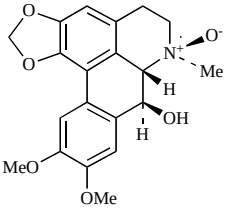

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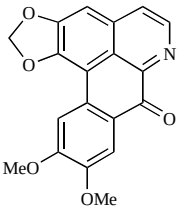

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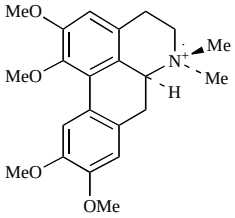

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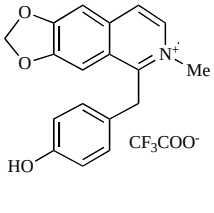

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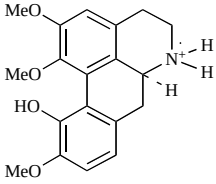

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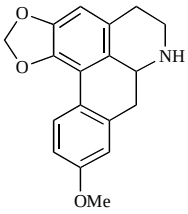

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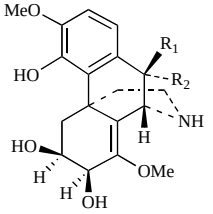
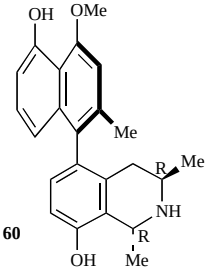


	R_1	R_2
56	OH	H
57	H	H
58	H	OH
59	H	OGlc



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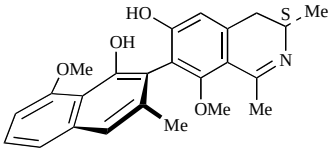

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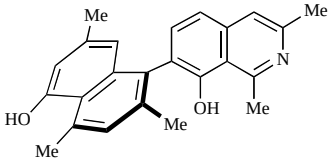
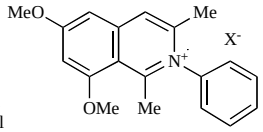
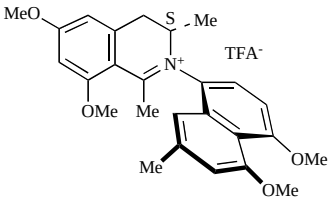


62



	X
63	Cl
64	ClO_4



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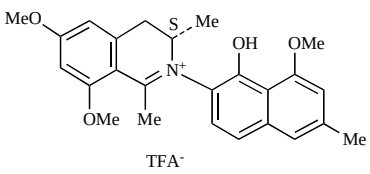

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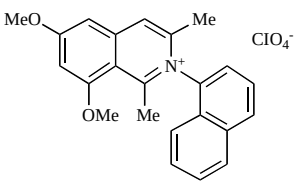
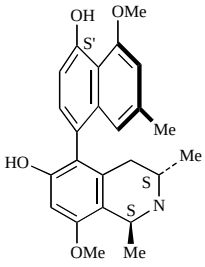


67

B

(Fig. 14). Contd…..



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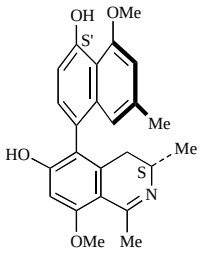

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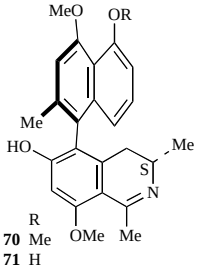
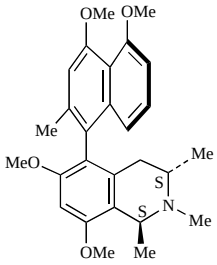


R
70 Me
71 H



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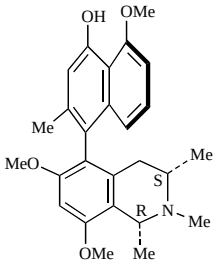

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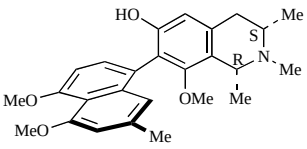

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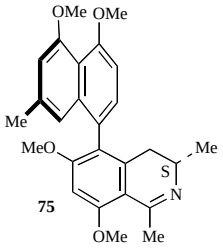

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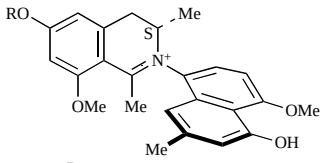
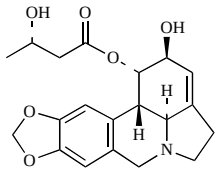


R
76 Me
77 H



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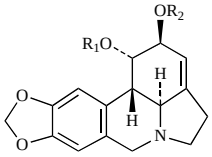
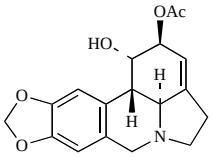


R1 R2
79 H H
80 Ac H
81 Ac Ac



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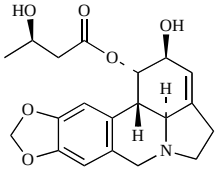

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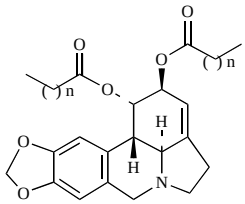
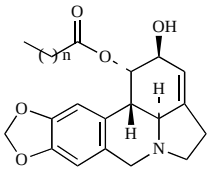


84 n=1
85 n=2



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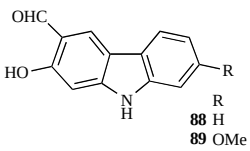
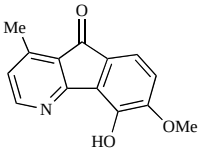
87 n=2



R
88 H
89 OMe



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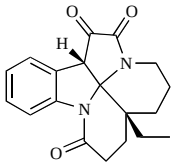

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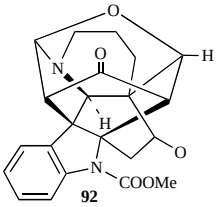

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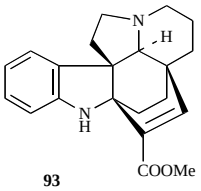

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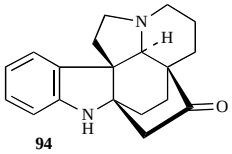

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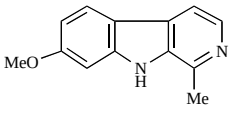

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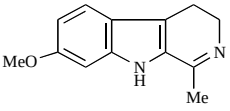

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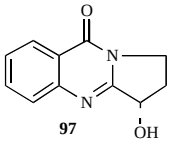

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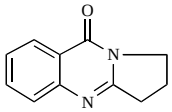

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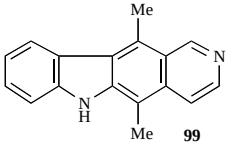

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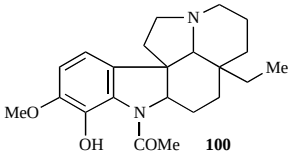

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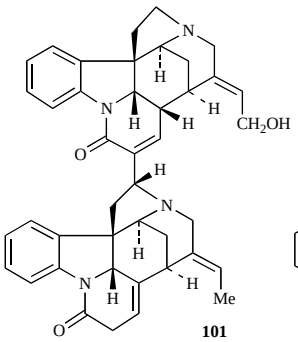

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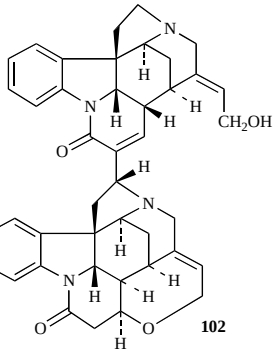

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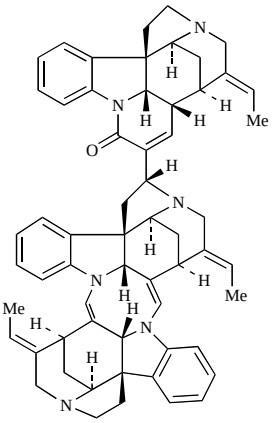
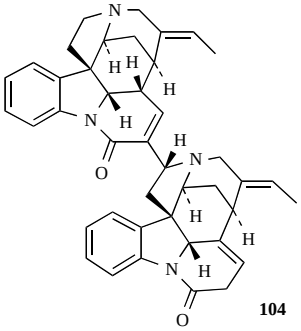


103

C

(Fig. 14). Contd…..



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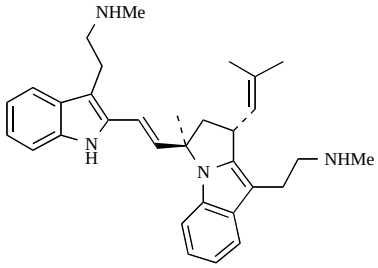

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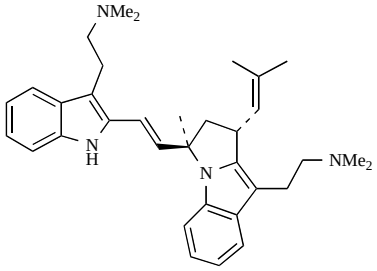

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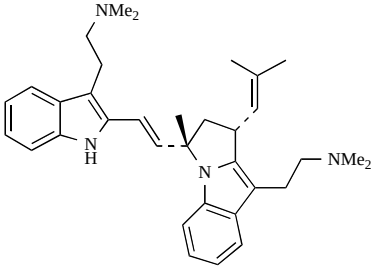

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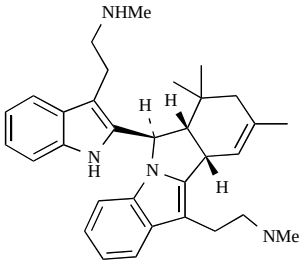

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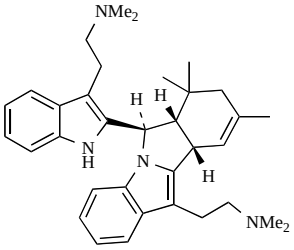

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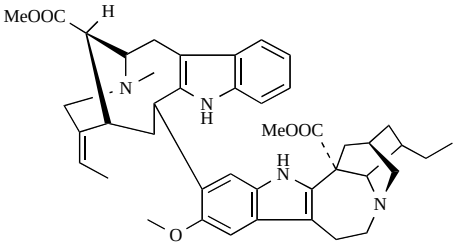

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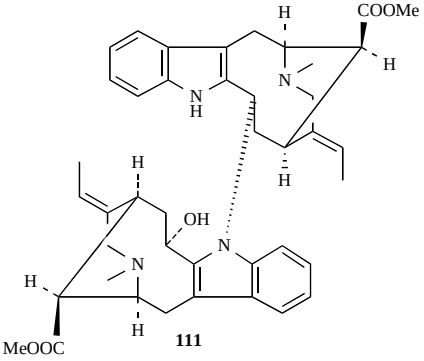

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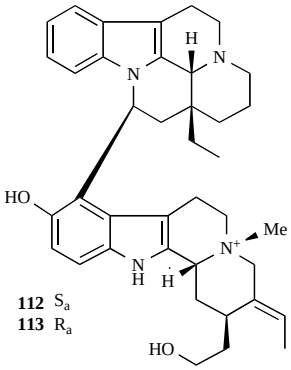
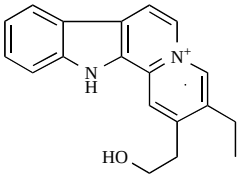


112 S_a
113 R_a



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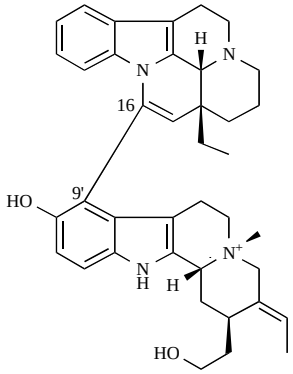

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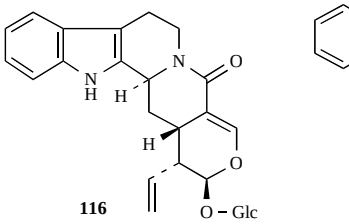

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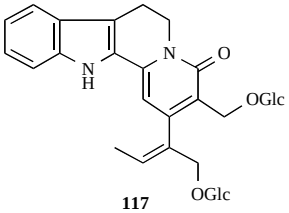

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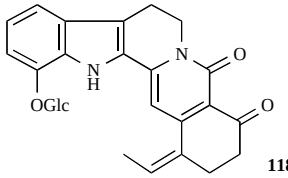

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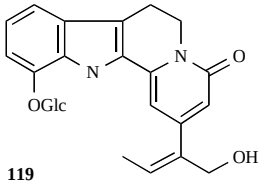

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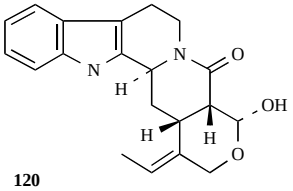

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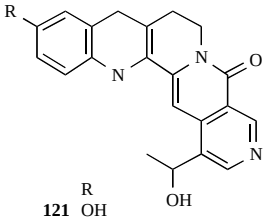
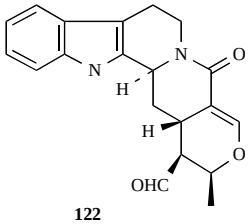


121 OH
123 H



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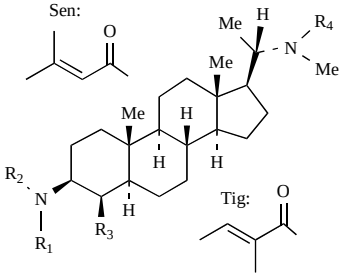
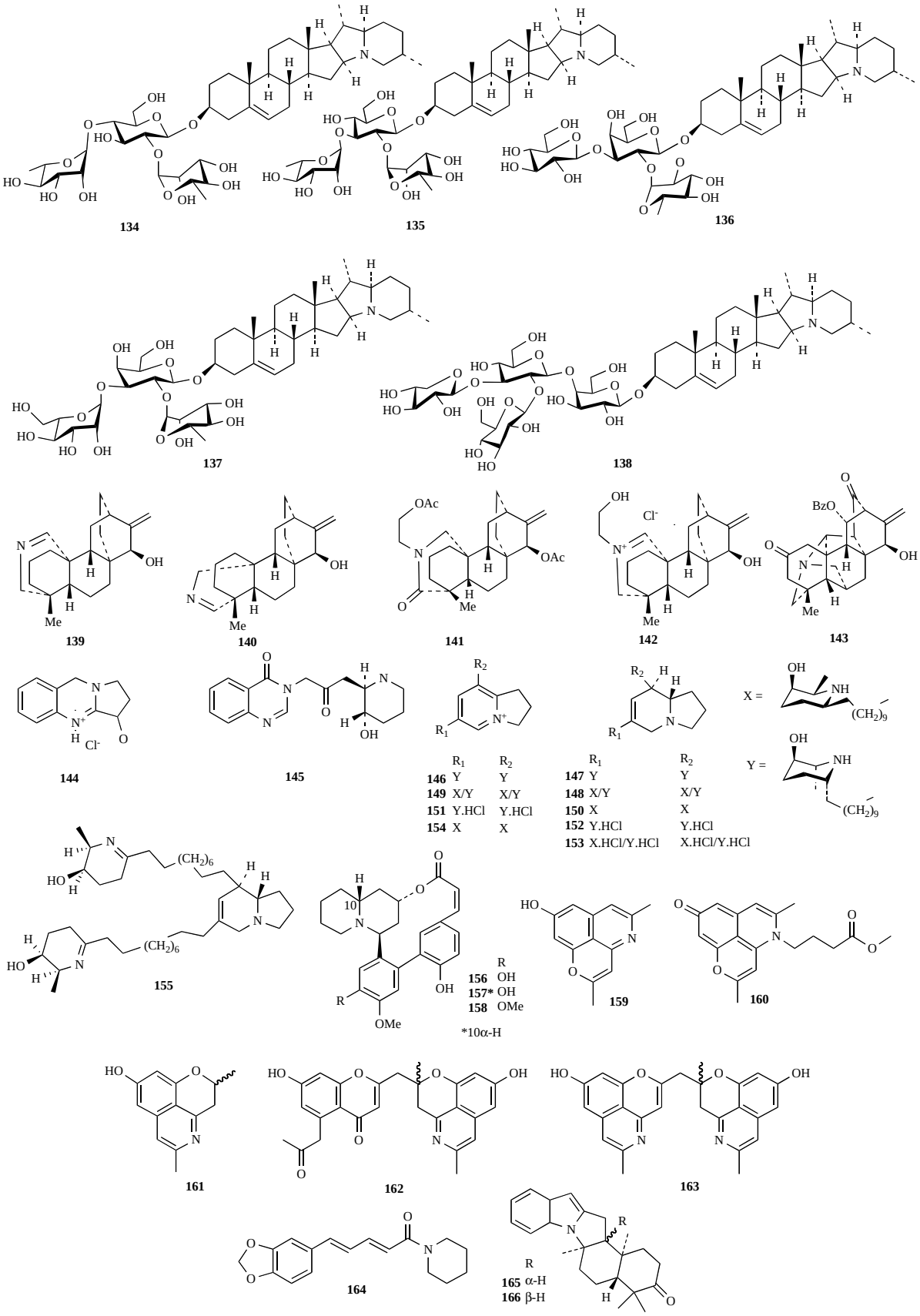


	R_1	R_2	R_3	R_4	
124	H	Sen	OH	Me	unsat.
125	Me	Me	H	Me	$\Delta^{16,17}$
126	H	CHO	=O	Me	$\Delta^{4,5\ \&\ 14,15}$
127	Me	COPh	H	H	$\Delta^{2,3}$
128	H	H	H	Me	
129	Me	Me	H	Me	
130	H	Sen	=O	H	
131	H	Sen	OAc	H	$\Delta^{5,6}$
132	H	Tig	=O	H	
133	H	Tig	OAc	H	$\Delta^{2,3\ \&\ 5,6}$

D

(Fig. 14). Contd…..



E

Fig. (14). Structures of antiprotozoal alkaloids.

(**135**), α-solamargine (**136**), α-solasonine (**137**), and tomatine (**138**) have recently been evaluated *in vivo* with a 4-day parasitemia suppression test with *Pyoe* 17XL infected mice [368]. **134** showed a dose-dependent suppression of malaria infection with an ED_{50} of 4.49 mg/kg/d and a therapeutic index (TI) of ~9. At a dose of 7.50 mg/kg/d, the parasitemia suppression values of **134**, **138**, **136**, **137** and **135** were 71.4, 65.3, 64.9, 57.5, and 41.3 %, respectively. At 3.75 mg/kg/d, the parasitemia suppression of **134** was 42.66 % [368].

Several steroidal glycoalkaloids of the solanidane or spirosolane type from plants of the genus *Solanum* (Solanaceae) have been tested *in vitro* against epimastigote and trypomastigote forms of *Tcr*. Among them, the α-spirosolanes **136** and **137** as well as the solanidanes **134** and **135**, almost completely inhibited the growth of the epimastigote form of *Tcr* at 5.7 μM. These glycoalkaloids were more effective than ketoconazole at the same concentration [369]. Later studies confirmed the role of the carbohydrate moiety in the formation of sterol-complexes with the parasite membrane [370].

5.5. Diterpenoid Alkaloids

The structures of diterpenoid alkaloids (**139**-**143**) are reported in Fig. (**14E**). Such diterpenes are characteristic constituents of certain Ranunculaceae such as *Aconitum*, *Consolida* and *Delphinium* species.

Screening of C_{19}- or C_{20}-diterpenoid alkaloids for antitrypanosomal activity against *Tcr* resulted in the identification of only five compounds among the 64 diterpenoid alkaloids as active against *Tcr* epimastigotes. Azitine (**139**), isoazitine (**140**) and 15,22-*O*-diacetyl-19-oxodihydroatisine (**141**) displayed moderate effects on the parasite, while atisinium chloride (**142**) and 13-oxocardiopetamine (**143**) showed more prominent inhibitory activity against *Tcr* epimastigote, with activity levels similar to that of the reference drug benznidazole [371].

The *in vitro* anti-proliferative effects of diterpenoid alkaloids against *Linf* were described for several atisine-type compounds [372]. From a total of 43 compounds tested, including several classes of C_{19}-and C_{20}-diterpenoid alkaloids, **140** exhibited the highest toxicity against the extracellular *Linf* parasites (IC_{50} of 22.58 μM). This IC_{50} was lower than that obtained with the antileishmanial reference drug pentamidine isethionate. **139** and **141** also showed pronounced effects against *Linf* promastigotes (IC_{50}= 30.92 and 27.16 μM, respectively). In general, this leishmanicidal activity was associated with a lack of toxicity to murine macrophages. It seems that these alkaloids act fundamentally at the level of the cytoplasmic membrane of the parasites, as well as in some organelle membranes. Additional studies are required to confirm the mechanism of action [372].

The crude total alkaloid extract of *Aconitum orochryseum* (Ranunculaceae) exerted a similar level of antiplasmodial activity against TM4 (wild type) and K1CB1 (chloroquine- and antifolate-resistant) *Pfc* strains with IC_{50} values of 20.4 and 19.2 μg/mL, respectively [373]. However, the antiplasmodial activity increased markedly for purified atisinium chloride (**142**), the major alkaloid of this plant. **142** demonstrated IC_{50} values of 1.51μg/mL (4.02 μM) and 1.35μg/mL (3.6 μM) against both the TM4 and K1 strains of *Pfc*, respectively. It is of particular interest to mention that *A. orochryseum* is recommended by traditional healers in Bhutan for the treatment of malaria-associated fever [373].

5.6. Miscellaneous Alkaloids

Structures of various alkaloids of miscellaneous origin (**144**-**166**) are shown in Fig. (**14E**).

Bioassay guided fractionation of *Peganum harmala* (Nitrariaceae, formerly Zygophyllaceae) seeds resulted in the identification of the quinazoline alkaloid peganine hydrochloride dihydrate (**144**) as an orally active antileishmanial lead molecule [374]. Compound **144** exhibited *in vitro* activity against *Ldon*, killing both extracellular promastigotes as well as intracellular amastigotes residing within murine macrophages. Dose-dependent cell death of intracellular amastigotes occurred at concentrations between 30 and 100 μg/mL, reaching approximately 90% at 85 μg/mL (IC_{90}). The IC_{50} was determined to be 41 μg/mL. Miltefosine, used as reference, had an IC_{50} of 5 μg/mL. It was suggested on the basis of molecular docking studies that **144** induces apoptosis-like cell death in *Ldon* by inhibiting the parasites' DNA topoisomerase I. **144** also exhibited *in vivo* activity against visceral leishmaniasis in hamsters when administered p.o. at doses of 100 and 200 mg/kg/d for 5 days, which led to 79.6% and 87.5% inhibition of parasite growth, respectively [374].

The interest in antiparasitic activity of quinazoline alkaloids dates back to the late 1940s, when the quinazolinone alkaloid febrifugine (**145**) was identified as the bioactive compound from the Chinese herb Chang Shan (*Dichroa febrifuga* Hydrangeaceae) [375]. This plant drug has been traditionally used as antimalarial. Adverse side effects have precluded the use of febrifugine as a clinical drug. However, there is a recent upsurge in interest to synthesize novel febrifugine analogues with lower toxicity and some of the new synthetic quinazolinone derivatives possess a therapeutic index over ten times superior to that of febrifugine and the commonly used antimalarial drug chloroquine [376].

A new potent antiinfective and antiparasitic compound, prosopilosidine (**146**), was isolated from the extract of the leaves of *Prosopis glandulosa* var. *glandulosa* (Fabaceae/Mimosoideae) along with further indolizidine alkaloids (**147**-**150**, **154**). The dihydrochloride salts of **146, 147** and **148** (compounds **151**-**153**) were prepared [377]. Among these compounds, the dihydroindolizinium derivatives (**146** and **149**) exhibited the most potent activity and high SI against *Pfc*. They showed IC_{50} values of 39 and 95 ng/mL and 42 and 120 ng/mL, respectively, against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains, which were similar to the standard antimalarial drug chloroquine (IC_{50}= 17 and 140 ng/mL, resp.). The dehydroindolizidine bases prosopilosine (**147**) and isoprosopilosine (**148**) (IC_{50}= 120 and 230 ng/mL against D6 strain and 83 and 150 ng/mL against W2 strain, resp.), were less potent than **146** and **149** but more toxic against mammalian kidney fibroblast (Vero) cells (IC_{50}= 5600 and 1800 ng/mL for **147** and **148** vs >23 800 ng/mL for **146** and **149**, respectively). Therefore, **146** and **149** exhibited higher SI values against *Pfc* D6 and W2 strains (SI > 610, 567 and SI > 250, 198, respectively) than the corresponding analogues **147**, **148** and **150** (SI= 47, 24, 23 and SI 22, 12, 13, respectively), suggesting that dihydroindolizinium quaternary alkaloids **146** and **149** are better candidates for further *in vivo* antimalarial studies than the tertiary bases **147**, **148** and **150**. The dihydrochloride salts **151**, **152** and **153**, prepared from compounds **146**, **147**, and **148**, respectively, retained weak activities compared to their corresponding bases. Compound **146** was selected for preliminary *in vivo* antimalarial screening at two doses, 1 and 2 mg/kg, and displayed an ED_{50} value of ≈2 mg/kg against *Pber* in infected mice, thereby exhibiting ≈48% suppression of parasitemia after 3 days of treatment. **146** also caused 40.5% suppression in parasitemia at 1 mg/kg/day for 3 days. No significant toxicity was observed under treatment with **146** at these doses. Higher doses were not tested since the estimated maximum tolerated dose of compound **146** was 2.5 mg/kg [377].

Compounds **146**-1**50** also demonstrated potent *in vitro* activity against *Ldon* promastigotes (IC_{50} = 0.26-0.80 μg/mL) and were as potent as the standard control pentamidine. All of the indolizidine compounds were tested for cytotoxic activity against selected human cancer cell lines, namely, SK-MEL, KB, BT-549, and SK-OV-3. **146** and **149** were weakly active toward all of these cancer

cell lines but untoxic to Vero (monkey kidney fibroblast) cells and LLCPK1 (pig kidney epithelial) cells (IC_{50}= 21.3-25 μg/mL) [377].

Another indolizidine alkaloid, named $\Delta^{1,6}$-juliprosopine (**155**) has been reported recently from the leaves of *Prosopis glandulosa* var. *glandulosa* collected from a different location [378]. This compound exhibited much less antiplasmodial activity than the previously mentioned analogues against *Pfc* D6 and W2 strains (IC_{50} values of 560 and 600 ng/mL, respectively). This work suggested that the qualitative and quantitative nature of the bioactive alkaloidal profile in *P. glandulosa* varies significantly due to its geographical location.

Alkaloids of the biphenylquinolizidine lactone type were isolated from *Heimia salicifolia* (Lythraceae) [379]. *Epi*-Lyfoline (**156**) exhibited moderate antiplasmodial activity against *Pfc* (D6 and W2 clones, both IC_{50}= 2.8 μg/ mL), while the C-10 epimer (H-10α) lyfoline (**157**) was found to be inactive. Vertine (**158**) was less active (IC_{50} vs. both clones= 4.76 μg/mL), and differs from **156** by an additional methoxy group at C-4''. Thus, the antiplasmodial activity of these alkaloids is strongly influenced by the stereochemistry of the quinolizidine moiety as well as by the methoxylation pattern.

Two novel aromatic alkaloids, cassiarins A (**159**) and B (**160**), with an unprecedented tricyclic skeleton were isolated from the leaves of *Cassia siamea* (Fabaceae), which have been widely used in traditional medicine, particularly for the treatment of periodic fever and malaria [380]. Cassiarin A (**159**) showed prominent *in vitro* antiplasmodial activity against *Pfc* (IC_{50}= 0.005 μg/mL; 0.02 μM), whereas cassiarin B (**160**) showed only moderate activity (IC_{50}= 6.9 μg/mL). **159** was highly selective with regard to the cytotoxicity on P388 cells (IC_{50}=35 μg/mL). Cassiarin B (**160**) was even less cytotoxic (IC_{50} >100 μg/mL). These results were further consolidated by *in vivo* work using a rodent 4-day suppressive test protocol which yielded ED_{50} values of 0.17 mg/kg (i.p.) for **159** and 0.21 mg/kg for chloroquine [381].

Further structurally related alkaloids, cassiarins C-E (**161-163**), displayed moderate *in vitro* antiplasmodial activity with IC_{50} values ranging between 2.3 and 24.2 μM and no apparent cytotoxicity (IC_{50}> 100 μM) [382].

It is well known that the piperidine amide alkaloid piperine (**164**) is the main constituent of the fruit of *Piper nigrum* (Piperaceae). Various biological activities have been attributed to piperine, including insecticidal and nematocidal activity, inhibition of liver metabolism as well as antiprotozoal properties. The leishmanicidal activity of **164** has only been demonstrated for the Old World species *Ldon* but new results have emerged recently [383] providing evidence of the susceptibility of the New World cutaneous species *Lam* to **164**. At a concentration of 50 μM, **164** inhibited 53%, 92% and 96% of promastigote growth at 24, 48 and 72 h post treatment, respectively. Among the tested piperine analogues, tetrahydropiperine and phenylamide were the most active compounds inhibiting promastigote growth [384].

The indolosesquiterpene alkaloids polysin (**165**) and greenwayodenrin-3-one (**166**) and some related compounds from *Polyalthia suaveolens* (Annonaceae) were recently discovered to possess anti-trypanosomal activity. A mixture of **165** and **166** displayed an *in vitro* ED_{50} of 18 μM against *Tb*. The other compouds were somewhat less active. These alkaloids were found to inhibit the glycolytic enzymes phosphofructokinase, GAPDH and aldolase and may hence be interesting lead structures in the search for new drugs against human african trypanosomiasis [385].

CONCLUSIONS AND OUTLOOK

This review (including part I on terpenoids [1]) has presented the current literature knowledge on the antiprotozoal activity of almost 900 different secondary metabolites isolated from plants.

It must be mentioned that a large number of articles dealing only with crude extracts or fractions, although representing important information, could not be considered. Moreover, this review is confined to plant products and it should be kept in mind that a plethora of active natural products has also been isolated from fungi and marine organisms, so that the actual number of known natural products with antiprotozoal potential is even much higher.

Many of the compounds mentioned in this review were demonstrated to show very interesting *in vitro* activity, but the reader will also find reports on compounds with comparatively low activity. It is important to emphasize that certainly all of this information is highly valuable, be it as a guide to select (or avoid) further plants to study, be it as information to be used to estimate the overall usefulness of a particular group of compounds or in the context of structure-activity relationship (SAR) studies that may eventually lead to new lead compounds. On the other hand, a high number of compounds with potent *in vitro* activity can be found which would have justified *in vivo* animal studies, but in many of these cases no reports on such studies exist. A likely reason is the restricted quantity of natural products that can be isolated from plants and the fact that their structures are often too complex for easy synthesis. It is also likely that in many cases *in vivo* experiments *were* performed but led to negative results that were never published, which is a very dissatisfactory situation. *In vitro* active compounds lacking *in vivo* activity probably suffer from poor pharmacokinetics, strong protein binding or metabolic instability. Such liabilities might be overcome by simple chemical modifications. These compounds should therefore not simply be abandoned but further investigated to find the reasons for their lack of *in vivo* efficacy.

It is also possible that *in vivo* experiments were not carried out because a compound displayed high cytotoxicity against mammalian cells, as usually assessed in parallel to the antiprotozoal activity assays. It has to be emphasized however, that selectivity indices are important for a general orientation but must not be seen as an absolute criterion to decide whether a compound should be moved forward to an animal model. There are examples of compounds that showed a very favourable selectivity index (SI) *in vitro* but lateron turned out to be inactive or even toxic to animals; on the other hand, there are also examples for compounds with rather moderate *in vitro* activity and SIs that have turned out to be active and untoxic *in vivo*. Such an example is the sesquiterpene lactone helenalin (section 4.2.1 of part I [1]), which displayed high *in vitro* activity against *Tbr* (IC_{50}= 0.05 μM) and a moderate SI of about 20 [386]. *In vivo* tests with this compound in a mouse model showed that it was untoxic to the animals but that there was also no antitrypanosomal effect (Schmidt, T.J. & Brun, R., unpublished). To stay in the same class of compounds, the sesquiterpene lactone cynaropicrin displayed a less impressive *in vitro* IC_{50} (0.3 μM) in comparison with helenalin and an SI that was even lower than that of helenalin [387]. Nevertheless, *in vivo* experiments have very recently shown that cynaropicrin efficiently reduces parasitemia in infected mice [387]. Thus, high *in vitro* activity and selectivity is certainly not all that counts, especially if only one mammalian cell line was used for the cytotoxicity determination, as is often the case.

It becomes evident from this review, that further continued efforts to find new potential lead or drug candidates in plants are certainly highly worthwhile. At the same time, it can also be concluded that further work on already existing compounds, some already abandoned, would often be useful, e.g. in order to produce them in sufficient quantities for *in vivo* studies or –in other cases- to find out the reasons for their apparent lack if *in vivo* efficacy.

Natural products remain an extremely rich source of new molecules which can be developed to new leads for NTDs. Interesting new mechanisms have often emerged from studies on

natural products and many promising new structures have been found among them, but chances are very small that a native natural product without chemical modification will become the active constituent of an applicable medication for an NTD.

A large number of synthetic studies have been published in which natural products have been modified to produce whole series of analogues, leading to interesting insights into SAR and, sometimes, to candidate molecules for further development. Methods of rational drug design, i.e. computer aided studies aiming at an optimization of natural lead structures are currently being applied in many cases. Only few such studies could be cited in this review. However, a third part reviewing the existing literature on these issues is in preparation, which will be published in due course.

Taken the huge number of existing natural compounds with known antiprotozoal activity, it may appear surprising that not many natural products have so far been developed into applicable medication for the diseases under study. Quinine and artemisinin still remain the only successful examples. Why is that so? It may at least in part be related to the way in which research in this field is carried out, i.e. often in small groups and with little effort to form larger consortia. It is interesting to note, e.g., that there are few groups screening compounds against a wider panel of parasite species. This approach could identify activities of natural products against parasites other than one particular target pathogen and make better use of isolated pure compounds. It is very easy to imagine that many compounds exist in isolated form which have been tested only against one particular parasite (or even an irrelevant life stage) that could turn out more useful against other protozoa if only they were tested.

A group of over 20 scientists working on different aspects of drug discovery, including most authors of this review, have therefore recently established a consortium with the aim of carrying out research in a more coordinated and efficient manner. This network, acting under the name "Research Network Natural Products against Neglected Diseases (ResNetNPND)" was formally established in April 2011 and the collaborators have committed themselves to performing joint and coordinated research with the ultimate goal to develop new drugs for the NTDs mentioned in this review. Scientists with serious interest in joining such a larger initiative are cordially invited to visit the ResNetNPND website at http://www.uni-muenster.de/ResNetNPND/index.html.

CONFLICT OF INTEREST

None declared.

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