ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2006, p. 1352–1364 Vol. 50, No. 4
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.4.1352–1364.2006


# Antitrypanosomal and Antileishmanial Activities of Flavonoids and Their Analogues: In Vitro, In Vivo, Structure-Activity Relationship, and Quantitative Structure-Activity Relationship Studies

Deniz Tasdemir,[1]* Marcel Kaiser,[2] Reto Brun,[2] Vanessa Yardley,[3] Thomas J. Schmidt,[4] Fatma Tosun,[5] and Peter Rüedi[1]

*Organisch-Chemisches Institut, Universität Zürich, Zürich, Switzerland[1]; Departement Medizinische Parasitologie und Infektionsbiologie, Schweizerisches Tropeninstitut, Basel, Switzerland[2]; Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom[3]; Institut für Pharmazeutische Biologie und Phytochemie, Westfälische Wilhelms-Universität Münster, Münster, Germany[4]; and Department Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey[5]*

Received 14 March 2005/Returned for modification 11 April 2005/Accepted 8 December 2005

**Trypanosomiasis and leishmaniasis are important parasitic diseases affecting millions of people in Africa, Asia, and South America. In a previous study, we identified several flavonoid glycosides as antiprotozoal principles from a Turkish plant. Here we surveyed a large set of flavonoid aglycones and glycosides, as well as a panel of other related compounds of phenolic and phenylpropanoid nature, for their in vitro activities against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, and *Leishmania donovani*. The cytotoxicities of more than 100 compounds for mammalian L6 cells were also assessed and compared to their antiparasitic activities. Several compounds were investigated in vivo for their antileishmanial and antitrypanosomal efficacies in mouse models. Overall, the best in vitro trypanocidal activity for *T. brucei rhodesiense* was exerted by 7,8-dihydroxyflavone (50% inhibitory concentration [$IC_{50}$], 68 ng/ml), followed by 3-hydroxyflavone, rhamnetin, and 7,8,3′,4′-tetrahydroxyflavone ($IC_{50}$s, 0.5 μg/ml) and catechol ($IC_{50}$, 0.8 μg/ml). The activity against *T. cruzi* was moderate, and only chrysin dimethylether and 3-hydroxydaidzein had $IC_{50}$s less than 5.0 μg/ml. The majority of the metabolites tested possessed remarkable leishmanicidal potential. Fisetin, 3-hydroxyflavone, luteolin, and quercetin were the most potent, giving $IC_{50}$s of 0.6, 0.7, 0.8, and 1.0 μg/ml, respectively. 7,8-Dihydroxyflavone and quercetin appeared to ameliorate parasitic infections in mouse models. Generally, the test compounds lacked cytotoxicity in vitro and in vivo. By screening a large number of flavonoids and analogues, we were able to establish some general trends with respect to the structure-activity relationship, but it was not possible to draw clear and detailed quantitative structure-activity relationships for any of the bioactivities by two different approaches. However, our results can help in directing the rational design of 7,8-dihydroxyflavone and quercetin derivatives as potent and effective antiprotozoal agents.**

Trypanosomiasis and leishmaniasis have reemerged over the last few decades as important threats to human health and economical development. Human African trypanosomiasis, also known as sleeping sickness, is caused by *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* and is a major cause of mortality and morbidity in sub-Saharan Africa. Currently, human African trypanosomiasis occurs in 36 African countries and about 60 million people are at risk (16). *Trypanosoma cruzi* is the causative agent of American trypanosomiasis (also known as Chagas' disease), which is a major endemic disease in Latin America. The number of people infected is estimated to be from 16 million to 18 million, with a further 100 million considered at risk (7). Leishmaniasis is a vector-borne disease, affecting 72 developing countries and 13 of the least developed countries. Visceral leishmaniasis due to *Leishmania donovani* is the most severe form of *Leishmania* infections. The annual incidence of visceral leishmaniasis is estimated to be 500,000 cases. The overall prevalence of visceral leishmaniasis is 12 million people, and the population at risk is 350 million (6). If they are left untreated, both *Trypanosoma* and *Leishmania* infections can be fatal. The current treatment regimens, based on chemotherapy, for these parasitic diseases are limited and are not ideal, as they are often associated with severe side effects. The emergence of drug-resistant parasites presents an additional and major problem. All these facts underline the urgent need for the development of new, cheap, safe, and easy-to-administer molecules for the treatment of these infectious diseases.

Flavonoids are a large group of polyphenolic compounds possessing a basic flavan nucleus with two aromatic rings (the A and the B rings) interconnected by a three-carbon-atom heterocyclic ring (the C ring). The most widespread flavonoids contain a double bond between C-2 and C-3 ($\Delta^{2,3}$) and a keto function at C-4 of ring C, which is attached to ring B at C-2 (flavone) or at C-3 (isoflavone). As a result of a number of further modifications on all three rings, particularly on ring C, flavonoids represent one of the largest and the most diverse class of plant secondary metabolites. These compounds are naturally present in vegetables, fruits, and beverages and thus are important components of the daily Western diet. Flavonoids have been known for a long time to exert diverse biological effects (biofla-

* Corresponding author. Mailing address: Institute of Organic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland. Phone: 41-1-635 42 13. Fax: 41-1-635 68 12. E-mail: deniz@oci.unizh.ch.

vonoids) and in particular to act as antioxidants and preventive agents against cancer (10). They are also common constituents of medicinal plants, and the therapeutic effects of many traditional medicines have been ascribed to these phytochemicals. We and others have recently identified several types of flavonoids as antiprotozoal principles of plant extracts (3, 9, 12, 17, 32, 33), but a comprehensive study of their structure-activity relationships (SARs) has not been conducted so far. Therefore, it was our interest to assess the in vitro trypanocidal and leishmanicidal activities of a large flavonoid library, as well as a panel of other related phenolic and phenylpropanoid compounds; draw SARs, if possible; and determine their antiparasitic selectivities. The present study underlines the in vitro ability of flavonoids and structurally related phenolic compounds to inhibit trypanosomal and leishmanial infections without significant toxicity to mammalian cells. However, their low bioavailability indicates the need for detailed mechanistic studies together with the development of parasite-specific formulations and delivery systems.

## MATERIALS AND METHODS

**Materials.** Aldrich Chemical Co. (Milwaukee, WI) supplied flavone, 6-hydroxyflavone, 3,6-dihydroxyflavone, 3,7-dihydroxyflavone, and *trans*-ferulic acid samples, while Sigma (St. Louis, MO) supplied 5- and 7-hydroxyflavones, all catechin-type compounds, scopoletin, 4-hydroxycoumarin, umbelliferone, 4-methylumbelliferone, gallic acid, 2,3- and 3,4-dihydroxybenzoic acids, and *o*- and *p*-coumaric acids. 3-Hydroxyflavone was purchased from ABCR GmbH & Co. (Karlsruhe, Germany). 5,7-Dihydroxyflavone (chrysin), apigenin, luteolin, galangin, kaempferol, fisetin, quercetin dihydrate, myricetin, morin dihydrate, catechol, pyrogallol, and all other cinnamic acid derivatives were purchased from Fluka (Buchs, Switzerland). Roth (Karlsruhe, Germany) supplied vitexin, rutin, quercetin-3,7,3′,4′-tetramethylether, 7,8-dimethoxyflavone, naringenin, 5,7-dimethoxy-8-methylflavanone, 5,4′-dihydroxy-6,7-dimethoxyflavanone, hesperidine, and neohesperidine. Ladanein, 5,4′-dihydroxy-6,7-dimethoxyflavanone, cirsimaritin, and genkwanin were previously isolated from plants of the family Lamiaceae (2, 13, 21, 34), whereas luteolin-7-*O*-rutinoside was previously obtained from *Gonocytisus angulatus* (35). Their purities (>95%) were determined by thin-layer chromatography and $^1$H nuclear magnetic resonance imaging. All remaining compounds were purchased from Extrasynthese (Genay, France). The commercial chemicals were of the highest grade available (purities, 95 to 99%).

**In vitro assay for *Leishmania donovani*.** Amastigotes of *Leishmania donovani* strain MHOM/ET/67/L82 were grown in axenic culture at 37°C in SM medium (5), at pH 5.4, supplemented with 10% heat-inactivated fetal bovine serum under an atmosphere of 5% $CO_2$ in air. One hundred microliters of culture medium with $1 \times 10^5$ amastigotes from axenic culture with or without a serial drug dilution was seeded in 96-well microtiter plates. Seven threefold dilutions covering a range from 30 to 0.041 μg/ml were used. After 72 h of incubation, 10 μl of Alamar Blue was added to each well. The plates were incubated for another 2 h and read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA) by using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The data were analyzed by using the software Softmax Pro (Molecular Devices Cooperation). The decrease of fluorescence (which indicated inhibition) was expressed as the percentage of the fluorescence of the control cultures and was plotted against the drug concentrations. The 50% inhibitory concentrations ($IC_{50}$s) were calculated from the sigmoidal inhibition curves.

**In vitro assays for *Trypanosoma brucei rhodesiense* and L6 cell cytotoxicity.** Minimum essential medium (50 μl) supplemented, as described previously (1), with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions covering a range from 90 to 0.123 μg/ml were prepared. Then, $10^4$ bloodstream forms of *Trypanosoma brucei rhodesiense* STIB 900 in 50 μl were added to each well, and the plate was incubated at 37°C under a 5% $CO_2$ atmosphere for 72 h. After addition of 10 μl of Alamar Blue to each well, the plates were incubated for another 2 to 4 h and read in a Spectramax Gemini XS microplate fluorometer by using an excitation wavelength of 536 nm and an emission wavelength of 588 nm (31). Fluorescence development was expressed as the percentage of that for the control, and $IC_{50}$s were determined. Cytotoxicity was assessed by using the same assay with rat skeletal myoblasts (L6 cells).

**In vitro assay for *Trypanosoma cruzi*.** Rat skeletal myoblasts (L6 cells) were seeded in 96-well microtiter plates at 2,000 cells/well in 100 μl RPMI 1640 medium with 10% fetal bovine serum and 2 mM L-glutamine. After 24 h, the medium was removed and replaced by 100 μl per well containing 5,000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (LacZ) gene. Forty-eight hours later, the medium was removed from the wells and replaced by 100 μl fresh medium with or without a serial drug dilution. After 96 h of incubation, the substrate chlorophenol red-β-D-galactopyranoside (CPRG)-Nonidet (50 μl) was added to all wells. A color reaction developed within 2 to 6 h and was read photometrically at 540 nm. Data were transferred into a graphic program (e.g., Microsoft Excel), sigmoidal inhibition curves were determined, and $IC_{50}$s were calculated.

**In vivo activity assessments.** The assay procedures, the administration route, and the doses of the test compounds have been selected on the basis of the standard operating procedure that has been devised within the TDR-WHO mandate. All compounds were injected by the intraperitoneal (i.p.) route, based on our experience that a new test compound has the best chance to show activity when it is administered abdominally. The selected standard dosage should be high enough but should still ensure the absence of toxicity. The agreed-upon standard operating procedure also allows the screeners to compare data (with those for positive control drugs) and to perform duplicate experiments.

**In vivo assay for *Leishmania donovani*.** Female BALB/c mice (weight, 20 g; Charles Rivers, United Kingdom) were infected with $2 \times 10^7$ amastigotes in a 0.2-ml bolus via a lateral tail vein. The mice were then randomly sorted into groups of five mice each. *L. donovani* HU3 amastigotes were harvested from a passage animal immediately prior to infection (4). At 7 days postinfection, the mice were dosed intraperitoneally at 30 mg/kg of body weight/day for 5 consecutive days. At 14 days postinfection, all mice were killed; the livers were weighed; and impression smears were made on glass slides, which were then fixed and stained. Antileishmanial activity was evaluated by counting the number of amastigotes per 500 host cell nuclei and multiplying that value by the weight of the liver (mg). That value was then compared to the values for the untreated controls. Treatment with the standard drugs pentostam (sodium stibogluconate, 15 mg/kg subcutaneously [s.c.] for 5 days) and oral miltefosine (30 mg/kg for 5 days) was also included as a positive control.

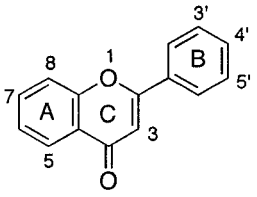
**In vivo assay for *Trypanosoma brucei brucei*.** Groups of four female NMRI mice weighing 20 to 25 g were infected i.p. on day 0 (d0) with $10^5$ bloodstream forms of *Trypanosoma brucei brucei* STIB 795, which is a derivative of strain 427. Mice were treated on 4 consecutive days (d3 to d6 postinfection) with 50 mg/kg by the i.p. route. One group served as untreated controls, and two other groups were treated with the standard drugs pentamidine (5 mg/kg for 4 days) and melarsoprol (2 mg/kg for 4 days), respectively. The levels of parasitemia of the mice were checked by examination of tail blood on day 7 and thereafter twice a week. The day of death of the mice was recorded.

**Molecular modeling and QSAR analyses.** Molecular models were generated with the program package MOE (27). For documentation of the quantitative structure-activity relationship (QSAR) descriptors calculated by MOE, see http://www.chemcomp.com/Journal_of_CCG/Features/descr.htm. The energies of the initial geometries were minimized by using the MMFF94x force field. For each compound, a stochastic conformational search was carried out, and the energies of the lowest-energy conformers found were minimized by using the semiempirical AM1 Hamiltonian. Descriptors for the partial least-squares (PLS) analysis were generated on the basis of the lowest-energy conformer of each compound by using the MOE module MOE Descriptor, and PLS analysis was carried out by using MOE QuaSAR. For modeling by the use of the Raptor program Biographics Laboratory 3R, Basel, Switzerland [http://www.biograf.ch/]), the molecules were aligned as described in the Results and imported into Raptor in mol2 format. For each compound, the two lowest-energy B-ring rotamers were included. Test set compounds were selected randomly. Binding site modeling was carried out by using Raptor version 2.0 (20) and applying default settings.

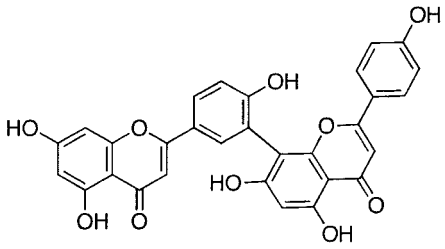
## RESULTS

**In vitro antileishmanial activity.** All 105 compounds were investigated for their activities against axenic *L. donovani* amastigotes, the clinically relevant form of the parasite; and the results are illustrated in Tables 1 to 7. With few exceptions, all flavone and flavon-3-ol type aglycones showed significant antileishmanial activity, with fisetin ($IC_{50}$, 0.6 μg/ml), 3-hydroxyflavone ($IC_{50}$, 0.7 μg/ml), and luteolin ($IC_{50}$, 0.8 μg/ml) being the most potent.

TABLE 1. In vitro antiprotozoal and cytotoxic activities of flavones (aglycones and glycosides) and biflavonoids[a]




Flavone




Biflavone (Amentoflavone)

| Class | No. for QSAR | Compound | Position(s) of OH | Position(s) of OMe (or Me) | $IC_{50}$ (μg/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *L. donovani* | *T. brucei rhodesiense* | *T. cruzi* | L6 cells |
| | | Standards | | | 0.34[b] | 0.0026[c] | 0.328[d] | 0.008[e] |
| Flavone | 1 | Flavone | | | 5.0 (8.5)[f] | 6.4 (6.6) | 25.8 (1.6) | 42.3 |
| Aglycone | 2 | 5-Hydroxyflavone (primuletin) | 5 | | 5.5 | 10.6 | >30 | >90 |
| | 3 | 5-Methoxyflavone | | 5 | 13.4 (2.8) | 15.0 (2.5) | 6.1 (6.2) | 37.7 |
| | 4 | 6-Hydroxyflavone | 6 | | 5.4 (7.5) | 3.3 (12.2) | 23.1 (1.7) | 40.4 |
| | 5 | 6-Methoxyflavone | | 6 | >30 | 29.4 | 19.6 | >90 |
| | 6 | 7-Hydroxyflavone | 7 | | 4.1 (10.8) | 6.6 (6.7) | >30 | 44.1 |
| | 7 | 7-Methoxyflavone | | 7 | 29.2 (1.3) | 14.3 (2.7) | 7.0 (5.5) | 38.4 |
| | 8 | Chrysin | 5,7 | | 2.2 (14.8) | 5.3 (6.1) | 20.6 (1.6) | 32.5 |
| | 9 | Tectochrysin | 5 | 7 | >30 | 86.2 | 12.8 | >90 |
| | 10 | Chrysin dimethylether | | 5,7 | >30 | 7.9 (2.7) | 3.9 (5.5) | 21.5 |
| | 11 | 6,7-Dihydroxyflavone | 6,7 | | 6.2 (2.6) | 1.9 (8.5) | 18.0 (0.9) | 16.1 |
| | 12 | 7,8-Dihydroxyflavone | 7,8 | | 1.7 (4.6) | 0.068 (116.2) | 6.6 (1.2) | 7.9 |
| | 13 | 7,8-Dimethoxyflavone | | 7,8 | 9.6 (5.0) | 6.1 (7.9) | >30 | 48.4 |
| | 14 | 7,4′-Dihydroxyflavone | 7,4′ | | 11.1 (7.6) | 6.9 (12.3) | >30 | 84.7 |
| | 15 | Apigenin | 5,7,4′ | | 1.9 (9.5) | 5.1 (3.5) | 21.8 (0.8) | 18.1 |
| | 16 | Genkwanin | 5,4′ | 7 | 9.4 | 8.0 | >30 | >90 |
| | 17 | Ladanein | 5,6 | 7,4′ | 2.1 (14.5) | 2.5 (12.2) | 25.2 (1.2) | 30.6 |
| | 18 | Cirsimaritin | 5,4′ | 6,7 | 3.9 (4.3) | 3.3 (5.1) | 19.7 (0.9) | 16.7 |
| | 19 | Scutellarein | 5,6,7,4′ | | 2.6 (18.5) | 4.6 (10.5) | >30 | 48.2 |
| | 20 | 3′,4′-Dihydroxyflavone | 3′,4′ | | 2.0 (8.2) | 1.1 (14.9) | 10.1 (1.6) | 16.4 |
| | 21 | 7,3′,4′-Trihydroxyflavone | 7,3′,4′ | | 5.5 (3.8) | 4.5 (4.7) | 22.6 (0.9) | 21.0 |
| | 22 | Luteolin | 5,7,3′,4′ | | 0.8 (11.8) | 3.7 (2.5) | 21.4 (0.4) | 9.4 |
| | 23 | Diosmetin | 5,7,3′ | 4′ | 7.1 (10.9) | 6.1 (12.7) | >30 | 77.6 |
| | 24 | Luteolin tetramethylether | | 5,7,3′,4′ | >30 | 4.4 | 17.3 | >90 |
| | 25 | 7,8,3′,4′-Tetrahydroxyflavone | 7,8,3′,4′ | | 8.8 (2.5) | 0.5 (43.2) | 24.5 (0.9) | 21.6 |
| | 26 | 7,8,3′,4′-Tetramethoxyflavone | | 7,8,3′,4′ | >30 | 67.2 | >30 | >90 |
| Glycoside | | Apigenin-7-*O*-glucoside | 5,4′; 7-*O*-Glu | | 2.1 (34.6) | 22.7 (3.2) | >30 | 72.6 |
| | | Luteolin-5-*O*-glucoside | 7,3′,4′; 5-*O*-Glu | | 3.0 | 39.0 | >30 | >90 |
| | | Luteolin-7-*O*-glucoside[g] | 5,3′,4′; 7-*O*-Glu | | 1.1 | 60.6 | >90 | >90 |
| | | Luteolin-7-*O*-rutinoside | 5,3′,4′; 7-*O*-Rut | | 2.6 (24.1) | 28.7 (2.2) | >30 | 62.7 |
| | | Vitexin | 5,7,4′ | 8-C-Glu | >30 | 55.7 | >30 | >90 |
| Biflavone | | Amentoflavone | The structure is shown above | | 6.0 (12.3) | 22.7 (3.2) | >30 | 73.6 |

[a] The $IC_{50}$s are mean values from at least two replicates (the variation is a maximum of 20%). Abbreviations: Glu, glucose; Rut, rutinose (6-*O*-rhamnopyranosylglucose); OMe, methoxyl; Me, methyl.
[b] Reference compound, miltefosine.
[c] Reference compound, melarsoprol.
[d] Reference compound, benznidazole.
[e] Reference compound, phodophyllotoxin.
[f] Selectivity indices are given in parentheses.
[g] These data are from our previous work (17).

Their $IC_{50}$s were almost comparable to that of miltefosine ($IC_{50}$, 0.34 μg/ml), the antileishmanial drug used in the clinic. Also, quercetin was a potent leishmanicidal agent, with an $IC_{50}$ of 1.0 μg/ml. Among the remaining compounds, 18 had $IC_{50}$s that ranged from 1.1 to 3.0 μg/ml. These 18 compounds included 6 flavones and 4 flavone glycosides, 5 flavon-3-ols, 1 flavanone, 1 isoflavone, and 1 coumarin derivative. Thirty compounds (11 flavones, 6 flavonols, 1 biflavone, 1 flavanone, 1 isoflavone, 1 flavan-3-ol, 2 simple phenolic compounds, and 7 cinnamates) displayed $IC_{50}$s that ranged from 3.1 to 10 μg/ml.

Starting with the flavones (Table 1), the insertion of a single OH group at the benzo-γ-chromone portion of the flavone structure did not have a notable influence, but insertion of two OH functions significantly enhanced the leishmanicidal potential. Particularly important positions were C-5, C-7, and C-8. Hydroxylation on ring B had some impact on the activity, but a clear SAR could not be observed. For instance, luteolin, which contains a catechol (3′,4′-dihydroxyphenyl) moiety and a 5,7-dihydroxybenzochromone structure, was twice as efficacious ($IC_{50}$, 0.8 μg/ml) as apigenin, which contains a *p*-hydroxyphenyl side chain ($IC_{50}$, 1.9 μg/ml). In turn, apigenin was only slightly more active than chrysin ($IC_{50}$, 2.2 μg/ml), which has an unsubstituted B ring. On the other hand, 7-hydroxyflavone was more active ($IC_{50}$, 4.1 μg/ml) than 7,4′-dihydroxyflavone ($IC_{50}$, 11.1 μg/ml) and was equally as active as 7,3′,4′-trihydroxyflavone ($IC_{50}$, 5.5 μg/ml). A good antileishmanial potential was exerted by 3′,4′-dihydroxyflavone itself ($IC_{50}$, 2.0 μg/ml). The best combination was represented by luteolin, which has four OH groups at C-5, C-7, C-3′, and C-4′. 7,8-Dihydroxyflavone had the second best activity among the flavone aglycones ($IC_{50}$, 1.7 μg/ml), but the addition of a catechol structure into the B ring (7,8,3′,4′-tetrahydroxyflavone) diminished the activity almost fivefold ($IC_{50}$, 8.8 μg/ml). Replacement of the OH groups, either on the benzochromone skeleton or on the side chain, by methoxyl groups decreased the activity at least twofold. For example, apigenin had an $IC_{50}$ of 1.9 μg/ml, whereas the $IC_{50}$ of genkwanin (7-methoxyapigenin) was 9.4 μg/ml. Moreover, it seemed that the higher the numbers of methoxyl functions that there were, the lower the leishmanicidal potential was, for example, as in the case of luteolin ($IC_{50}$, 0.8 μg/ml), diosmetin ($IC_{50}$, 7.1 μg/ml), and luteolin-tetramethylether ($IC_{50}$, >30 μg/ml). Attachment of one or more sugar units at the C-5 or the C-7 position of the flavone skeleton, at least for the compounds apigenin and luteolin studied here, caused a slight to appreciable reduction in antileishmanial potency. The apigenin dimer amentoflavone, the only biflavonoid investigated here, also had some antileishmanial effect ($IC_{50}$, 6.0 μg/ml) (Table 1).

The compounds with the highest levels of antileishmanial activity were found in the flavon-3-ol (flavonol) series (Table 2). The simplest flavonol, 3-hydroxyflavone ($IC_{50}$, 0.7 μg/ml), was seven times more potent than flavone ($IC_{50}$, 5.0 μg/ml). The compounds with further hydroxylations at position 5, 6, or 7 were also active; but they were not as potent as 3-hydroxyflavone. The number and the pattern of hydroxylation on ring B had an influence on the activity, but again, clear SARs could not be determined. The best combination was presented by fisetin ($IC_{50}$, 0.6 μg/ml), which possessed four OH groups at C-3, C-7, C-3′, and C-4′. In some cases, an association with the presence of a catechol moiety in ring B and higher leishmanicidal activity was apparent; e.g., quercetin with a catechol function ($IC_{50}$, 1.0 μg/ml) was almost three times more active than kaempferol with a *p*-hydroxyphenyl ring ($IC_{50}$, 2.9 μg/ml) and morin with two *meta*-positioned OH functions at C-2′ and C-4′ ($IC_{50}$, 2.8 μg/ml). However, kaempferol was in turn less active than galangin ($IC_{50}$, 1.5 μg/ml), which bears an unsubstituted B ring. Remarkably, the presence of a pyrogallol function (three *ortho*-hydroxyl functions at C-3′, C-4′, and C-5′) on ring B was unfavorable, because myricetin had activity comparable to those of only galangin and quercetin. Moreover, robinetin was nine times less active ($IC_{50}$, 5.9 μg/ml) than its catecholic counterpart, fisetin ($IC_{50}$, 0.6 μg/ml). As in the case of the flavone subset, the replacement of hydroxyl groups by methoxyl substituents anywhere on the entire structure significantly reduced or completely abolished the leishmanicidal activity. Such a relationship was observed for 3-hydroxyflavone and 3-methoxyflavone ($IC_{50}$s, 0.7 and >30 μg/ml, respectively). Glycosidation of C-3 or C-5 hydroxyl groups led to a dramatic reduction in the activity (Table 2).

All flavanone aglycones except for (+)-taxifolin had some leishmanicidal potential, with 5,7-dimethoxy-8-methylflavanone being the most potent ($IC_{50}$, 2.4 μg/ml), whereas the two flavanone glycosides were inactive (Table 3). Comparison of the leishmanicidal activities of naringenin, eriodictyol, and (+)-taxifolin with those of their unsaturated ($\Delta^{2,3}$) derivatives, namely, apigenin, luteolin, and quercetin, respectively, implied the importance of the double bond function between C-2 and C-3.

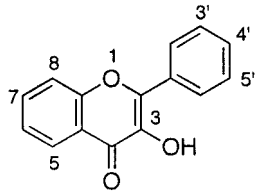
(−)-Gallocatechingallate ($IC_{50}$, 8.9 μg/ml) and (−)-epigallocatechingallate ($IC_{50}$, 19.1 μg/ml) were the only flavan-3-ol type compounds with weak leishmanicidal activities (Table 4). A comparison of the flavon-3-ols and their flavan-3-ol counterparts was indicative of the essentiality of both the keto function at C-4 and the $\Delta^{2,3}$ double bond for antileishmanial potency. For example, quercetin demonstrated remarkable potency, whereas quercetin analogues [(+)-catechin or (−)-catechin and (+)-epicatechin or (−)-epicatechin] were inactive at the highest concentration tested ($IC_{50}$s, >30 μg/ml). The same trend was observed between myricetin ($IC_{50}$, 1.3 μg/ml) and (−)-epigallocatechin or (−)-gallocatechin ($IC_{50}$s, >30 μg/ml). In order to gain more structural characteristics for antiprotozoal activity, five simple phenolics, namely, catechol, pyrogallol, gallic acid, and 2,3-dihydroxybenzoic and 3,4-dihydroxybenzoic acids, were also tested (Table 6). Note that only catechol and pyrogallol and not gallic or 3,4-dihydroxybenzoic acid inhibited the growth of *L. donovani* amastigotes.

Among the isoflavone subclass, daidzein and prunetin were devoid of activity, while the remaining isoflavones, biochanin A ($IC_{50}$, 2.5 μg/ml), genistein ($IC_{50}$, 8.0 μg/ml), and 3′-hydroxydaidzein ($IC_{50}$, 24.0 μg/ml), possessed some activity (Table 5). The coumarins, which contain a benzo-α-chromone skeleton instead of the benzo-γ-chromone skeleton found in flavonoids, had either slight or no activity ($IC_{50}$s, >20 μg/ml); the exception was the linear furanocoumarin bergaptol, which exhibited an $IC_{50}$ of 2.5 μg/ml (Table 5).

Finally, a number of hydroxy derivatives of cinnamic acid, the biogenetic precursor of flavonoids and coumarins, were evaluated (Table 7). Except for cinnamic acid, methyl cinnamate, and α-acetamido cinnamate, all substituted cinnamates displayed moderate activity. Noteworthy were the findings that caffeic and ferulic acids exhibited equivalent potencies ($IC_{50}$, 5.6 μg/ml) and that hydrocaffeic acid was twofold less active than caffeic acid, pointing out the importance of the double bond for antileishmanial potential.

**In vitro anti-*Trypanosoma brucei rhodesiense* activities.** All compounds were also assayed for their in vitro activities against the trypomastigote forms (mammalian stage) of *T. brucei rhodesiense*. As shown in Tables 1 to 7, 7,8-dihydroxyflavone demonstrated the highest growth-inhibitory activity ($IC_{50}$, 68 ng/ml); this was followed by the activities of three molecules with the same $IC_{50}$ (0.5 μg/ml), 3-hydroxyflavone, rhamnetin, and 7,8,3′,4′-tetrahydroxyflavone, as well as catechol ($IC_{50}$, 0.8 μg/ml). Beside these compounds, 13 compounds exhibited remarkable trypanocidal poten-

TABLE 2. In vitro antiprotozoal and cytotoxic activities of flavon-3-ols (flavonols)[a]




Flavon-3-ol

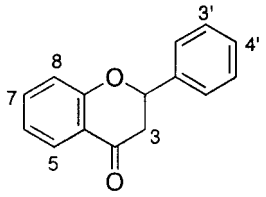
| Class | No. for QSAR | Compound | Position(s) of OH | Position(s) of OMe (or Me) | $IC_{50}$ (μg/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *L. donovani* | *T. brucei rhodesiense* | *T. cruzi* | L6 cells |
| | | Standards | | | 0.34[b] | 0.0026[c] | 0.328[d] | 0.008[e] |
| Flavon-3-ol | 27 | 3-Hydroxyflavone | 3 | | 0.7 (20.6)[f] | 0.5 (28.8) | 7.7 (1.9) | 14.4 |
| Aglycone | 28 | 3-Methoxyflavone | | 3 | >30 | 24.5 | 14.3 | >90 |
| | 29 | 3,6-Dihydroxyflavone | 3,6 | | 2.5 (4.1) | 3.5 (2.9) | 7.0 (1.5) | 10.2 |
| | 30 | 3,7-Dihydroxyflavone | 3,7 | | 3.3 (10) | 1.7 (19.4) | 9.0 (3.7) | 32.9 |
| | 31 | 7-Methoxyflavonol | 3 | 7 | 11.9 | 2.4 | 19.8 | >90 |
| | 32 | Galangin | 3,5,7 | | 1.5 (16.5) | 16.5 (1.5) | 19.8 (1.3) | 24.8 |
| | 33 | Kaempferol | 3,5,7,4′ | | 2.9 (12.9) | 9.2 (4.1) | 23.9 (1.6) | 37.5 |
| | 34 | Kaempferide | 3,5,7 | 4′ | 11.3 | 25.4 | 20.6 | >90 |
| | 35 | Morin | 3,5,7,2′,4′ | | 2.8 | 38.3 | >30 | >90 |
| | 36 | Fisetin | 3,7,3′,4′ | | 0.6 (64.2) | 3.3 (11.7) | >30 | 38.5 |
| | 37 | Quercetin | 3,5,7,3′,4′ | | 1.0 (37.1) | 8.3 (4.5) | >30 | 37.1 |
| | 38 | Tamarixetin | 3,5,7,3′ | 4′ | 16.8 (2.5) | 14.8 (2.8) | 6.4 (6.6) | 42.0 |
| | 39 | Isorhamnetin | 3,5,7,4′ | 3′ | 3.8 (10.7) | 8.9 (4.6) | >30 | 40.6 |
| | 40 | Rhamnetin | 3,5,3′,4′ | 7 | 4.6 | 0.5 | 12.1 | >90 |
| | 41 | 3,4′-Dimethylquercetin | 5,7,3′ | 3,4′ | 7.9 (0.4) | 4.5 (0.7) | 17.7 (0.2) | 3.0 |
| | 42 | Quercetin-3,7,3′,4′-tetramethylether | 5 | 3,7,3′,4′ | 7.8 (1.7) | 47.4 (0.3) | >30 | 13.1 |
| | 43 | Quercetagetin | 3,5,6,7,3′,4′ | | 10.9 (2.7) | 3.1 (9.5) | 17.1 (1.7) | 29.4 |
| | 44 | Robinetin | 3,7,3′,4′,5′ | | 5.9 (9.1) | 5.3 (10.1) | >30 | 53.5 |
| | 45 | Myricetin | 3,5,7,3′,4′,5′ | | 1.3 (28.2) | 15.9 (2.3) | >30 | 36.7 |
| | 46 | Syringetin | 3,5,7,4′ | 3′,5′ | >30 | 6.1 (3.5) | >30 | 21.5 |
| | 47 | Myricetin trimethylether | 3,5,7 | 3′,4′,5′ | >30 | 6.0 (13.6) | >30 | 81.7 |
| Glycoside | | Kaempferol-3-*O*-glucoside | 5,7,4′; 3-*O*-Glu | | 20.4 | 70.1 | >30 | >90 |
| | | Kaempferol-3-*O*-rutinoside | 5,7,4′; 3-*O*-Rut | | >30 | 84.5 | >30 | >90 |
| | | Hyperoside | 5,7,3′,4′; 3-*O*-Gal | | >30 | 23.2 | >30 | >90 |
| | | Quercitrin | 5,7,3′,4′; 5-*O*-Rha | | 17.7 | 27.9 | >30 | >90 |
| | | Rutin | 5,7,3′,4′; 3-*O*-Rut | | >30 | 42.1 | >30 | >90 |

[a] The $IC_{50}$s are mean values from at least two replicates (the variation is a maximum of 20%). Abbreviations: Glu, glucose; Rut, rutinose (6-*O*-rhamnopyranosyl-glucose); Gal, galactose; Rha, rhamnose; OMe, methoxyl; Me, methyl.
[b] Reference compound, miltefosine.
[c] Reference compound, melarsoprol.
[d] Reference compound, benznidazole.
[e] Reference compound, phodophyllotoxin.
[f] Selectivity indices are given in parentheses.

tial, with $IC_{50}$s ranging from 1.0 to 3.0 μg/ml. Of these were three flavones, two flavon-3-ols, three isoflavones, three simple phenolics, and two caffeic acid derivatives. Twenty-eight compounds (15 flavones, 10 flavon-3-ols, 3 flavan-3-ols, and 2 isoflavones) displayed anti-*T. brucei rhodesiense* potential, with $IC_{50}$s between 3.1 and 10 μg/ml.

Although all flavone and flavonols exhibited lethal effects on *T. brucei rhodesiense* trypomastigotes, it was generally more difficult to interpret the data for trypanocidal activity, particularly for the flavones (Table 1). Flavone itself, as well as its mono- or dihydroxybenzochromone derivatives, had some activity, with 7,8-dihydroxyflavone and 6,7-dihydroxyflavone ($IC_{50}$, 1.9 μg/ml) being the most potent compounds. A clear trend for the effect of the hydroxylation pattern on ring B was not observed, but generally, the compounds with a catechol function at the side chain appeared to have higher trypanocidal activities. 7,8,3′,4′-Tetrahydroxyflavone ($IC_{50}$, 0.5 μg/ml) and 3′,4′-dihydroxyflavone ($IC_{50}$, 1.1 μg/ml) represented the compounds with the best potential. Different trends were observed by methylation of the OH groups on the flavone structure. In many cases, methylation was strongly disfavored and resulted in an enormous reduction in trypanocidal activity. The most noteworthy examples of this situation were represented by two pairs: 7,8-dihydroxyflavone and 7,8-dimethoxyflavone ($IC_{50}$s, 0.068 and 6.1 μg/ml, respectively) and 7,8,3′,4′-tetrahydroxyflavone and 7,8,3′,4′-tetramethoxyflavone ($IC_{50}$s, 0.5 and 67.2 μg/ml, respectively). In some cases, methylation had a less significant effect on the activities of the flavones. Apigenin-genkwanin

TABLE 3. In vitro antiprotozoal and cytotoxic activities of flavanone aglycones and glycosides[a]



Flavanone

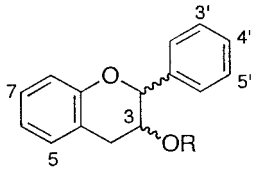
| Class | No. for QSAR | Compound | Position(s) of OH | Position(s) of OMe (or Me) | $IC_{50}$ (μg/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *L. donovani* | *T. brucei rhodesiense* | *T. cruzi* | L6 cells |
| Flavanone | 49 | Naringenin (2*S*) | 5,7,4′ | | 5.0 | 46.1 | >30 | >90 |
| | 50 | 5,7-Dimethoxy-8-methylflavanone | | 5,7;8-Me | 2.4 (15.5)[b] | 11.7 (3.2) | 13.6 (2.7) | 37.3 |
| | 51 | 5,4′-Dihydroxy-6,7-dimethoxyflavanone | 5,4′ | 6,7 | 13.2 (3.0) | 25.4 (1.6) | >30 | 39.4 |
| | 52 | Eriodictyol (2*S*) | 5,7,3′,4′ | | 10.4 (5.3) | 24.3 (2.3) | 14.5 (3.8) | 55.6 |
| | 53 | (+)-Taxifolin (2*R*,3*R*) | 3,5,7,3′,4′ | | >30 | 14.6 | >30 | >90 |
| Glycoside | | Hesperidine (2*S*) | 5,3′; 7-*O*-Rut | 4′ | >30 | 44.5 | >30 | >90 |
| | | Neohesperidine | 5,3′; 7-*O*-Glu-Rha | 4′ | >30 | 11.5 | >30 | >90 |

[a] The $IC_{50}$s are mean values from at least two replicates (the variation is a maximum 20%). The reference compounds and their $IC_{50}$s are shown in Tables 1 and 2. Abbreviations: Glu, glucose; Rut, rutinose; Rha, rhamnose; OMe, methoxyl; Me, methyl.
[b] Selectivity indices are given in parentheses.

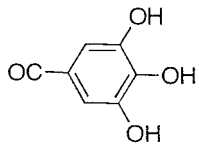
($IC_{50}$s, 5.1 and 8.0 μg/ml, respectively) and scutellarein-ladanein-cirsimaritin ($IC_{50}$s, 4.6, 2.5, and 3.3 μg/ml, respectively) or luteolin-diosmetin-luteolin tetramethylether ($IC_{50}$s, 3.7, 6.1, and 4.4 μg/ml) had such a relationship. A different behavior was observed between chrysin ($IC_{50}$ 5.3 μg/ml) and its methoxylated derivatives. Tectochrysin, the monomethoxy derivative, was almost inactive ($IC_{50}$, 86.2 μg/ml), whereas chrysin dimethylether had trypanocidal activity ($IC_{50}$, 7.9 μg/ml) almost comparable to that of chrysin.

The most potent members that emerged from the flavon-3-ol subset were 3-hydroxyflavone and rhamnetin ($IC_{50}$s 0.5 μg/ml) (Table 2). Among the compounds with a dihydroxychromone substructure, the most active one was 3,7-dihydroxyflavone ($IC_{50}$, 1.7 μg/ml). As in the case of galangin ($IC_{50}$, 16.5

TABLE 4. In vitro antiprotozoal and cytotoxic activities of flavan-3-ol subclass[a]



Flavan-3-ol

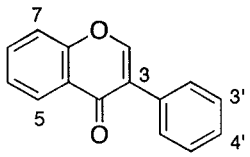


Galloyl moiety

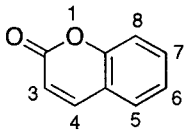
| Compound | No. for QSAR | R | Positions of OH | $IC_{50}$ (μg/ml) | | | |
|---|---|---|---|---|---|---|---|
| | | | | *L. donovani* | *T. brucei rhodesiense* | *T. cruzi* | L6 cells |
| (+)-Catechin (2*R*,3*S*) | 54 | H | 3,5,7,3′,4′ | >30 | 14.5 | >90 | >90 |
| (−)-Catechin (2*S*,3*R*) | 55 | H | 3,5,7,3′,4′ | >30 | 22.1 | >90 | >90 |
| (+)-Epicatechin (2*S*,3*S*) | 56 | H | 3,5,7,3′,4′ | >30 | 16.8 | >90 | >90 |
| (−)-Epicatechin (2*R*,3*R*) | 57 | H | 3,5,7,3′,4′ | >30 | 21.2 | >90 | >90 |
| (−)-Gallocatechin (2*S*,3*R*) | 58 | H | 3,5,7,3′,4′,5′ | >30 | 16.3 (1.1)[b] | >90 | 18.5 |
| (−)-Epigallocatechin (2*R*,3*R*) | 59 | H | 3,5,7,3′,4′,5′ | >30 | 4.0 (3.7) | 80.7 (0.2) | 14.6 |
| (−)-Catechingallate (2*S*,3*R*) | 60 | Galloyl | 3,5,7,3′,4′ | >30 | 15.2 | >90 | >90 |
| (−)-Epicatechingallate (2*R*,3*R*) | 61 | Galloyl | 3,5,7,3′,4′ | >30 | 22.7 | >90 | >90 |
| (−)-Gallocatechingallate (2*S*,3*R*) | 62 | Galloyl | 3,5,7,3′,4′,5′ | 8.9 (1.7) | 3.7 (4.0) | >90 | 14.8 |
| (−)-Epigallocatechingallate (2*R*,3*R*) | 63 | Galloyl | 3,5,7,3′,4′,5′ | 19.1 (0.8) | 9.3 (1.6) | >90 | 14.7 |

[a] The $IC_{50}$s are mean values from at least two replicates (the variation is a maximum of 20%). The reference compounds and their $IC_{50}$s are shown in Tables 1 and 2.
[b] Selectivity indices are given in parentheses.

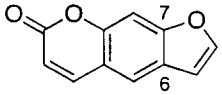
TABLE 5. In vitro antiprotozoal and cytotoxic activities of isoflavones and coumarines[a]



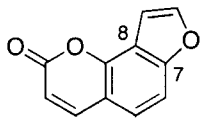
Isoflavone



Coumarine



6,7-Furanocoumarine



7,8-Furanocoumarine (Angelicin)

| Class | No. for QSAR | Compound | Position(s) of OH | Position of OMe (or Me)[b] | $IC_{50}$ (μg/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *L. donovani* | *T. brucei rhodesiense* | *T. cruzi* | L6 cells |
| Isoflavone | 64 | Daidzein | 7,4′ | | >30 | 5.5 | >90 | >90 |
| | 65 | Genistein | 5,7,4′ | | 8.0 (2.6)[c] | 1.3 (16.1) | 23.4 (0.9) | 20.9 |
| | 66 | Demethyltexasin | 6,7,4′ | | 15.9 (1.3) | 2.4 (8.8) | >30 | 21.0 |
| | 67 | 3′-Hydroxydaidzein | 7,3′,4′ | | 24.0 (0.9) | 1.62 (13.1) | 4.7 (4.5) | 21.3 |
| | 68 | Prunetin | 5,4′ | 7 | >30 | 12.9 (0.4) | 8.0 (0.7) | 5.8 |
| | 69 | Biochanin A | 5,7 | 4′ | 2.5 (26.3) | 3.2 (20.6) | 20.0 (3.3) | 65.8 |
| Coumarine | | Scopoletin | 7 | 6 | >30 | 31.0 | >90 | >90 |
| | | 4-hydroxycoumarine | 4 | | 23.5 | >90 | >90 | >90 |
| | | Umbelliferone | 7 | | 27.9 | 51.8 | >90 | >90 |
| | | 4-Methylumbeliferone | 7 | 4-Me | 27.1 (3.2) | 40.4 (2.2) | >90 | 87.0 |
| 6,7-Furanocoumarine | | Bergapten | | 5 | >30 | 44.6 | >90 | >90 |
| | | Bergaptol | 5 | | 2.5 (35.4) | 22.1 (4.0) | 68.0 (1.3) | 88.5 |
| 7,8-Furanocoumarine | | Angelicin | The structure is shown above | | 24.5 (2.3) | 18.7 (3.0) | 84.6 (0.7) | 55.2 |

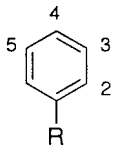
[a] The $IC_{50}$s are mean values from at least two replicates (the variation is a maximum of 20%). The reference compounds and their $IC_{50}$s are shown in Tables 1 and 2.
[b] OMe, methoxyl; Me, methyl.
[c] Selectivity indices are given in parentheses.

μg/ml), additional OH groups on the benzochromone ring (particularly at C-5) unexpectedly reduced the trypanocidal potential. Similar to the finding observed for flavones, an obvious trend for the impact of the number and the pattern of hydroxylation on ring B was absent. However, by using galangin (with a B ring completely free of hydroxyl groups) as a model, it was notable that the presence of a *p*-hydroxyphenyl side chain (e.g., kaempferol [$IC_{50}$, 9.2 μg/ml]) improved the activity, which, in turn, was almost comparable to that observed for quercetin ($IC_{50}$, 8.3 μg/ml), which contains a 3′,4′-*ortho*-dihydroxyphenyl moiety. Again, the presence of a third *ortho*-hydroxyl function (pyrogallol) in ring B was unfavorable with respect to the trypanocidal activity (myricetin [$IC_{50}$, 15.9 μg/ml]). Nevertheless, the activities of these compounds were still much higher (fourfold) than that observed for morin ($IC_{50}$, 38.3 μg/ml), which bears two *meta*-positioned hydroxyl groups in ring B. The influence of the methylation of the OH groups on both benzochromone and the side chain was quite unclear. The most interesting examples were quercetin and its methoxy derivatives. The 3′- or 4′-monomethylethers of quercetin, tamarixetin, and isorhamnetin had trypanocidal activities ($IC_{50}$s, 14.8 and 8.9 μg/ml) either equal to or less than that of quercetin ($IC_{50}$, 8.3 μg/ml). 3,4′-Dimethylquercetin had a twofold greater potency ($IC_{50}$, 4.5 μg/ml), whereas quercetin-3,7,3′,4′-tetramethylether was five times less active ($IC_{50}$, 47.4 μg/ml) than the title compound. Most strikingly, rhamnetin, which contains a methoxyl group at C-7, had prominent trypanocidal activity ($IC_{50}$, 0.5 μg/ml). Again, the dimethyl- and trimethylether derivatives of myricetin had higher in vitro potentials than myricetin itself. All flavone or flavonol glycosides were far less potent than their nonglycosidic counterparts (aglycones), and their activities ranged from 23.2 to 84.5 μg/ml (Table 2).

All members of the flavanone subclass were found to have some potential to inhibit the growth of African trypanosomes (Table 3). When the trypanocidal activities of the flavones, e.g., apigenin ($IC_{50}$, 5.1 μg/ml) and cirsimaritin ($IC_{50}$, 3.3 μg/ml), were compared to those of their flavanone counterparts, namely, naringenin and 5,4′-dihydroxy-6,7-dimethoxyflavanone, respectively ($IC_{50}$s, 46.1 and 25.4 μg/ml, respectively), the key role of the $\Delta^{2,3}$ double bond for bioactivity became apparent. Except for (−)-epigallocatechin and (−)-gallocatechingallate ($IC_{50}$s, 4.0 and 3.7 μg/ml), the compounds from the flavan-3-ol subclass were also only moderately active (Table 4). This highlights the fact that not only the $\Delta^{2,3}$ double bond but also the ketone function at C-4 are important for the trypanocidal activity. The exceptions were (−)-gallocatechin ($IC_{50}$, 16.3 μg/ml) and (−)-epigallocatechin, which were as active or more active than their analog compound, myricetin ($IC_{50}$, 15.9 μg/ml).

All isoflavone aglycones tested were highly active, with genistein (5,7,4′-OH) being the most potent and prunetin (5,4′-dihydroxy,7-methoxy) being the least potent (Table 5). Noteworthy is that methylation of the hydroxyl groups on the benzochromone ring has a greater (negative) effect (prunetin [$IC_{50}$, 12.9 μg/ml]) on activity than methylation on the side chain (biochanin A [$IC_{50}$, 3.2 μg/ml]). The coumarins were

TABLE 6. In vitro antiprotozoal and cytotoxic activities of simple phenolics[a]



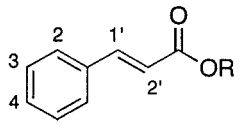

| Compound | R | Position(s) of OH | $IC_{50}$ (μg/ml) | | | |
|---|---|---|---|---|---|---|
| | | | *L. donovani* | *T. brucei rhodesiense* | *T. cruzi* | L6 cells |
| Catechol | H | 3,4 | 3.5 (3.3)[b] | 0.8 (14.5) | 7.5 (1.5) | 11.6 |
| Pyrogallol | H | 3,4,5 | 4.8 (3.0) | 2.0 (7.4) | >30 | 14.7 |
| Gallic acid | COOH | 3,4,5 | >30 | 1.6 (9) | 67.0 | 14.4 |
| 2,3-Dihydroxybenzoic acid | COOH | 2,3 | >30 | 62.5 | >90 | >90 |
| 3,4-Dihydroxybenzoic acid | COOH | 3,4 | >30 | 2.9 | >90 | >90 |

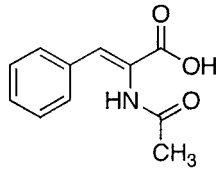
[a] The $IC_{50}$s are mean values from at least two replicates (the variation is a maximum of 20%). The reference compounds and their $IC_{50}$s are shown in Tables 1 and 2.
[b] Selectivity indices are given in parentheses.

only slightly active or inactive against *T. brucei rhodesiense* (Table 5). Among the phenylpropanoids tested, caffeic and hydrocaffeic acids exhibited the highest growth inhibition against *T. brucei rhodesiense* (Table 7). Both compounds contain an *ortho*-dihydroxyphenyl structure, which appears to be crucial for the trypanocidal activity. Ferulic acid, the 3-methoxy derivative of caffeic acid, was far less potent (30-fold) than caffeic acid. Four of five simple phenolic compounds, catechol, pyrogallol, gallic acid, and 3,4-dihydroxybenzoic acid, which contain two or three OH groups positioned *ortho* to each other revealed significant trypanocidal activities, with $IC_{50}$s ranging from 0.8 to 2.9 μg/ml (Table 6). The activity of 2,3-dihydroxybenzoic acid was only marginal ($IC_{50}$, 62.5 μg/ml).

**In vitro anti-*Trypanosoma cruzi* activity.** Contrary to the activity observed for African trypanosomes, the test compounds displayed much weaker growth inhibition against the trypomastigote forms of American *T. cruzi* (Tables 1 to 7). The highest potentials were exhibited by chrysin dimethylether and the isoflavone 3′-hydroxydaidzein ($IC_{50}$s, 3.9 and 4.7 μg/ml, respectively). Nine additional compounds represented by three flavones, four flavonols, one isoflavone, and a simple phenolic compound revealed anti-*Trypanosoma cruzi* activities, with

TABLE 7. In vitro antiprotozoal and cytotoxic activities of cinnamic acid derivatives[a]




α-Acetamido cinnamic acid

| Compound | Position(s) of OH | Position of OMe[b] | R | Other substituent | $IC_{50}$ (μg/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *L. donovani* | *T. brucei rhodesiense* | *T. cruzi* | L6 cells |
| *trans*-Cinnamic acid | | | H | | >30 | 83.0 | >30 | >90 |
| Methyl-cinnamate | | | Me[c] | | >30 | >90 | >30 | 20.4 |
| α-Acetamido cinnamic acid[d] | | | H | | >30 | 49.8 | >30 | >90 |
| *o*-Coumaric acid | 2 | | H | | 6.0 | 43.5 | >30 | >90 |
| *m*-Coumaric acid | 3 | | H | | 8.5 | 42.2 | >30 | >90 |
| *p*-Coumaric acid | 4 | | H | | 6.8 | 37.2 | >30 | >90 |
| 3-Methoxycinnamic acid | | 3 | H | | 9.2 | 39.7 | >30 | >90 |
| 4-Methoxycinnamic acid | | 4 | H | | 9.6 | 37.4 | >30 | >90 |
| *Trans*-4-nitro-cinnamic acid | | | H | 4 $NO_2$ | 16.6 (0.2)[e] | 75.4 (0.04) | >30 | 2.7 |
| Caffeic acid | 3,4 | | H | | 5.6 (9.5) | 1.1 (48.5) | >30 | 53.3 |
| Hydrocaffeic acid($\Delta^{1',2'}$saturated) | 3,4 | | H | | 12.9 (1.3) | 1.2 (14.2) | >30 | 17.0 |
| Ferulic acid | 4 | 3 | H | | 5.6 | 33.0 | >30 | >90 |

[a] The $IC_{50}$s are mean values from at least two replicates (the variation is a maximum of 20%). The reference compounds and their $IC_{50}$s are shown in Tables 1 and 2.
[b] OMe, methoxyl.
[c] Me, methyl.
[d] The structure is shown above.
[e] Selectivity indices are given in parentheses.

TABLE 8. In vivo activities of selected flavonoids against *L. donovani* in HU3 in BALB/c mice

| Compound tested | Dosing regimen (mg/kg)[a] | Injection | Total dose (mg/kg) | % Inhibition ± 95% CL[b] | Standard error of mean |
|---|---|---|---|---|---|
| Sbv[c,d] | 15 | s.c. | 75 | 63.65 ± 21.45 | 10.9 |
| Miltefosine[d] | 30 | p.o.[e] | 150 | 96.62 ± 0.88 | 0.4 |
| Luteolin | 30 | i.p. | 150 | 1.90 ± 25.99 | 13.3 |
| Luteolin-7-*O*-glucoside | 30 | i.p. | 150 | 0 | 13.9 |
| 3-Hydroxyflavone | 30 | i.p. | 150 | 0 | 12.5 |
| Fisetin | 30 | i.p. | 150 | 0 | 4.0 |
| Quercetin | 30 | i.p. | 150 | 15.39 ± 14.24 | 7.3 |
| Myricetin | 30 | i.p. | 150 | 0 | 10.4 |

[a] All doses were given for 5 days.
[b] CL, confidence limits.
[c] pentavalent antimony (active ingredient of sodium stibogluconate).
[d] Standard drugs.
[e] p.o., perorally.

$IC_{50}$s less than 10 μg/ml. Among the remaining compounds, the flavones ($n = 13$), flavonols ($n = 6$), and isoflavones ($n = 2$) had some weak activity ($IC_{50}$s, 10 to 30 μg/ml); and all others were practically inactive. 5,7-Dimethoxy-8-methylflavanone and eriodictyol were the only compounds that showed some inhibitory potential ($IC_{50}$s, 13.6 and 14.5 μg/ml, respectively), although they lack the $\Delta^{2,3}$ double bond.

**Cytotoxicity for mammalian cells.** A primary cell line (L6) derived from rat skeletal myoblasts was used for the determination of the relative (selective) toxicities of the test compounds. The selectivity indices (SIs; $IC_{50}$ value for cytotoxicity divided by $IC_{50}$ value for antiprotozoal activity) of the compounds with $IC_{50}$s less than 90 μg/ml against L6 cells were calculated and given for each parasite (Tables 1 to 7). Overall, the highest cytotoxicity for mammalian cells was exerted by *trans*-4-nitro-cinnamic acid ($IC_{50}$, 2.7 μg/ml), which, interestingly, had either no or marginal toxicity for the parasites tested (Table 7). This was followed by 3,4′-dimethylquercetin and the isoflavone prunetin ($IC_{50}$s, 3.0 and 5.8 μg/ml, respectively).

Three of the four compounds with marked activity against *L. donovani* (3-hydroxyflavone, fisetin, and quercetin) had slight or no toxicity for mammalian cells (SIs, 20 to 64); only luteolin had a lower therapeutic index (SI, 11.8). Except for 7,8-dihydroxyflavone (SI, 4.6), 3,6-dihydroxyflavone (SI, 4.1), and 3′,4′-dihydroxyflavone (SI, 8.2), the majority of the remaining compounds with significant leishmanicidal activities, e.g., myricetin, galangin, scutellarein, ladanein, and apigenin, proved to be weakly cytotoxic (SIs, ≥10). It was also noteworthy that the flavone glycosides had broader selectivity indices (SIs, >24). The other smaller SI values shown in Tables1 to 7 generally stem from low antileishmanial activities.

The cytotoxicity of the most potent anti-*Trypanosoma brucei rhodesiense* agent, 7,8-dihydroxyflavone, had great selectivity for this parasite (SI, 116.2). Rhamnetin also appeared to be very safe toward mammalian L6 cells ($IC_{50}$, >90 μg/ml). The other trypanocidal compounds, namely, 3-hydroxyflavone, catechol, 7,8,3′,4′-tetrahydroxyflavone, and 3′,4′-dihydroxyflavone, possessed some cytotoxicity for L6 cells ($IC_{50}$s, ca. 12 to 22 μg/ml); however, the calculated SIs were quite reasonable (28.8, 14.5, 43.2, and 14.9, respectively). Interestingly, caffeic acid (SI, 48.5) was far less toxic than hydrocaffeic acid (SI, 14.2), suggesting that the reduction of the double bond increases the cytotoxicity. Several compounds with appreciable activities, e.g., gallic acid, pyrogallol, 6,7-dihydroxyflavone, luteolin, cirsimaritin, and (−)-gallocatechingallate, displayed some toxicity, resulting in low SIs that ranged from 2.5 to 9, but most of the remaining compounds with notable trypanocidal activities appeared to be selectively toxic to *T. brucei rhodesiense*.

Due to a moderate anti-*Trypanosoma cruzi* potential, the SIs of the most active compounds, i.e., chrysin dimethylether and 3′-hydroxydaidzein, appeared to be low (SIs, about 5). This held true for the remaining nine compounds with moderate trypanocidal activities ($IC_{50}$s, <10 μg/ml), as their SI values ranged from 1.2 to 6.6.

**In vivo antileishmanial activity.** Six flavonoids, luteolin, luteolin-7-*O*-glucoside, 3-hydroxyflavone, fisetin, quercetin, and myricetin, were tested in vivo with BALB/c mice infected with the *L. donovani* HU3 strain. All samples were injected i.p. at a dose of 30 mg/kg for 5 days. The positive control drugs were oral miltefosine and sodium stibogluconate, which were administered s.c. Only quercetin showed some in vivo activity by inhibiting the infection 15.3%, whereas all other flavonoids were completely inactive (Table 8).

**In vivo trypanocidal (anti-*T. brucei brucei*) activity.** Six selected compounds (3-hydroxyflavone, 7,8-dihydroxyflavone, genistein, catechol, caffeic acid, and hydrocaffeic acid) were also tested for their in vivo efficacies in mice infected with *T. brucei brucei* (STIB795). All compounds were applied i.p. at 50 mg/kg. None of the compounds was able to completely eradicate the parasites. The most potent in vitro trypanocidal agent, 7,8-dihydroxyflavone ($IC_{50}$, 68 ng/ml), significantly reduced the level of parasitemia; but the mice relapsed and showed a mean survival of 13 days, which represents an extension of the length of survival of 6 days for the untreated control group (Table 9). As two flavonoids, 3-hydroxyflavone and genistein, proved to be inactive, the other active compounds, rhamnetin and 7,8,3′,4′-tetrahydroxyflavone, were not tested in vivo.

**QSARs.** Attempts were made to establish common QSARs for the compounds of the flavone, flavonol, flavane-3-ol, and isoflavone groups. The $IC_{50}$ data for each of the biological effects under study were transformed to a molar scale. The four sets of biological activities under study were investigated for their degree of cross-correlation in order to assess the possibility that a common structure-activity relationship exists for the observed effects against the different parasites and

TABLE 9. In vivo activities of selected compounds against *T. brucei brucei* STIB795 mouse model

| Compound tested | Dosing regimen (mg/kg)[a] | Injection | Total dose (mg/kg) | No. of mice cured/ no. of mice infected | Mean survival time (days) |
|---|---|---|---|---|---|
| Control[b] | | | | 0/4 | 6.0 |
| Pentamidine[c] | 5.0 | i.p. | 20 | 4/4 | >30 |
| Melarsoprol[c] | 2.0 | i.p. | 8 | 4/4 | >30 |
| 3-Hydroxyflavone | 50 | i.p. | 200 | 0/4 | 5.5 |
| 7,8-Dihydroxyflavone | 50 | i.p. | 200 | 0/4 | 13 |
| Genistein | 50 | i.p. | 200 | 0/4 | 6.5 |
| Catechol | 50 | i.p. | 200 | 0/4 | 5.75 |
| Caffeic acid | 50 | i.p. | 200 | 0/4 | 5.75 |
| Hydrocaffeic acid | 50 | i.p. | 200 | 0/4 | 5.75 |

[a] All doses were given for 4 days.
[b] Untreated control used to determine the mean survival.
[c] Standard drugs.

mammalian cells, in which case largely identical mechanisms should underlie the observed effects. However, the degree of cross-correlation was very low between the four sets of biological data. A maximum correlation coefficient ($r$) of 0.44 was observed between the *T. brucei rhodesiense* and L6 cytotoxicity data, indicating that the data do not follow a common structure-activity relationship; i.e., that the biological effects are based on largely different mechanisms.

In order to elucidate possible QSARs for each compound with biological activity, molecular models were generated for each of the nonglycosidic compounds ($n = 68$; for QSAR numbering, see Tables 1 to 5; amentoflavone was the sole biflavone in the series but was excluded since such singular structures can generally be expected to lead to problems with statistical modeling). A large variety of 241 molecular descriptors (numerical expressions encoding information on many aspects of the compounds' electronic, steric, and other molecular properties; a full list of these descriptors may be obtained from one of the authors [T.J.S.]) was calculated on the basis of the lowest-energy conformer of each compound. The resulting matrix of independent variables was then investigated for correlations with the biological data [as $-\log IC_{50}$ (M) = $pIC_{50}$ (negative decadic logarithm of molar $IC_{50}$)] by using PLS analysis. This method is widely used in QSAR and quantitative structure-property relationship studies in order to combine the information content of large descriptor matrices in a new set of few information-rich latent variables (PLS analysis components) which best mirror the influences of certain structural properties on bioactivity (22).

None of the series of biological data led to a statistically relevant correlation with the descriptors taken into account. The correlations obtained in this way by PLS analysis in no case reached a value greater than 0.5 for the cross-validated correlation coefficient ($q^2$), which is generally considered to indicate a biologically relevant QSAR model. Since this difficulty might be due to the fact that compounds from different structural subgroups follow separate QSARs, PLS analysis calculations were also performed for the subset of flavones and flavonols and for both of these groups separately. However, none of the correlations obtained by PLS analysis reached the critical value. As a possible explanation, the molecular features important for the activities under study may not have been adequately represented in the descriptor matrix. This might, e.g., be the case because the activity is due to interactions with a common (putative) receptor site in a particular common orientation.

An attempt was therefore made to establish a QSAR model for the activity against *T. brucei rhodesiense* (which shows the largest variance of the four sets of biological data) by using the receptor-binding site modeling program Raptor (20). Raptor is based on an alignment of the molecules in the assumed bioactive conformation and orientation. Based on the aligned molecules, the shape of a putative receptor pocket is modeled along with its surface properties in terms of hydrogen bonding and hydrophobic interactions and the induced fit phenomena which best reproduce the binding affinity of each compound, as estimated from the $IC_{50}$ data. The data set was split into a training set of 53 compounds, which is used by the program to build a receptor model, and a test set of 14 compounds, which are not taken into account during receptor modeling and whose activities (binding affinities) are predicted with the model obtained. As in the PLS analysis modeling study, it turned out that the statistical significance of the correlation between the binding energies predicted by the Raptor model and those calculated from the $IC_{50}$s was low. Figure 1A shows a plot of the predicted versus the experimental $pIC_{50}$s. The average root mean square errors of both the training and the test sets are quite high (factors of 2.3 and 2.8 in the $IC_{50}$s, respectively), and the correlation between the predicted and the experimental $pIC_{50}$s was very poor ($q^2 = 0.36$ and 0.09 for the training and the test sets, respectively). In order to test whether this slight positive correlation was of any biological relevance, a scramble test was carried out, in which the bioactivity data were randomly assigned to the compounds of the training set and the model-building process was repeated in essentially the same way. In cases where a model reflects a "true" SAR, the model resulting from a scramble test must generally lead to a much poorer correlation between the predicted and the experimental data than that obtained with the real biological data (20). In the case of the present data set, the result of the scramble test was of approximately the same statistical quality as that described above, clearly showing that the latter is a result of a chance correlation (Fig. 1B). It must be concluded that the compounds in the present data set do not follow a common structure-activity relationship that can be deciphered by two independent QSAR methods. This may be due to the structure of the data set in association with a relatively small variance in the activities in the four sets of biolog-

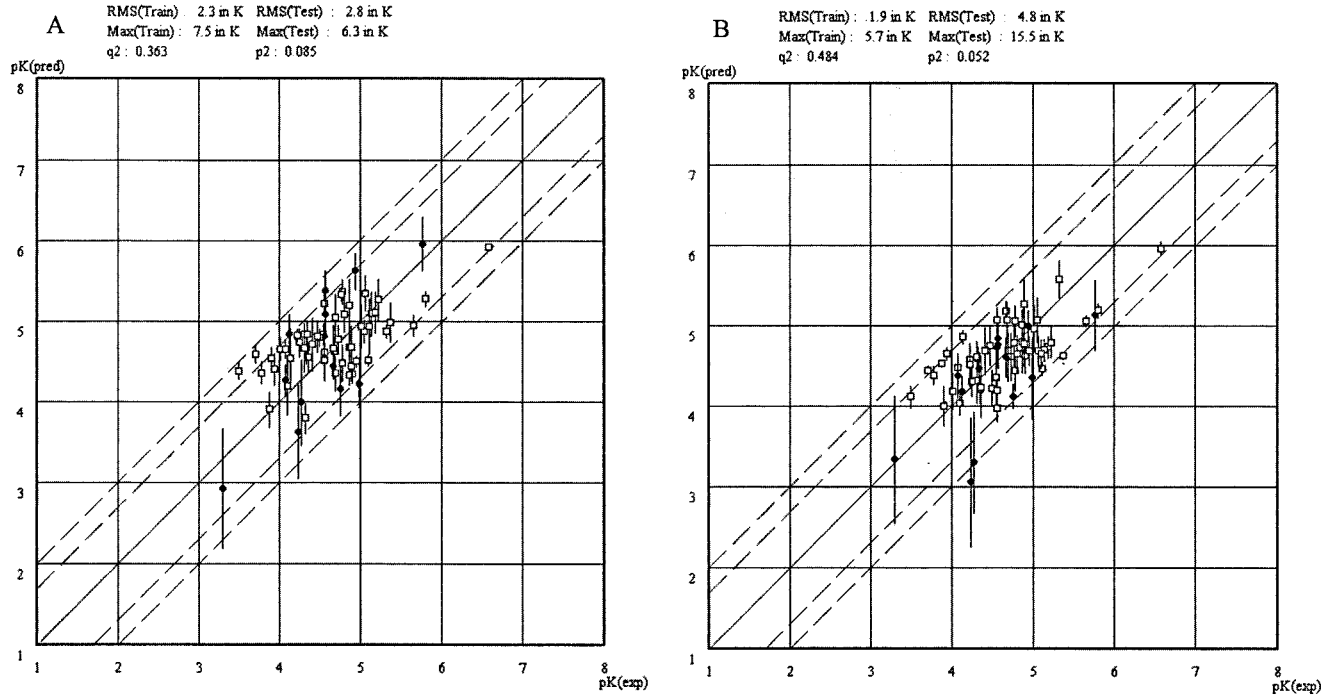



FIG. 1. (A) Plot of the $pIC_{50}$s predicted by the Raptor model versus the experimental data. (B) Result of a scramble test obtained under the same conditions with randomly assigned biological activity. Filled circles, test set; open squares, training set; RMS, root mean square; Max, maximum; pK(pred), predicted negative decadic logarithm of binding constant; pK(exp), experimental negative decadic logarithm of binding constant (as derived from $IC_{50}$ value); p2, squared correlation coefficient of predicted versus experimental data for the test set; K, thousands.

ical data and/or by the nonexistence of a true common mechanism of action.

## DISCUSSION

We previously reported on the antiprotozoal activities of two flavonoid glycosides (17). This prompted us to screen a large set of polyphenolic compounds mainly of a flavonoid nature and thereby to attempt to gain deeper insights into the structural characteristics necessary for their in vitro trypanocidal and leishmanicidal activities. As selectivity is the other relevant characteristic for defining lead candidates, the cytotoxic potentials of the compounds for primary mammalian cells were also determined and compared to the corresponding antiparasitic activities. We believe that this is the first (and the most) comprehensive study performed with polyphenols so far. The activities against *L. donovani* and *T. brucei rhodesiense* of many of the compounds investigated here (including the most potent ones, such as 3-hydroxyflavone, fisetin, myricetin, 3,7-dihydroxyflavone, 7,8-dihydroxyflavone, and catechol) are being reported for the first time. It is noteworthy that some flavones, such as 3-hydroxyflavone, 3,6-dihydroxyflavone, 3,7-dihydroxyflavone, 3′,4′-dihydroxyflavone, cirsimaritin, ladanein, and biochanin A, potently inhibit both *T. brucei rhodesiense* and *L. donovani* parasites with almost equivalent $IC_{50}$s. The first four compounds are of special interest, as they are also able to kill *Trypanosoma cruzi* to some extent. On the other hand, some of the phenolic compounds tested (e.g., luteolin, quercetin, gallic acid, and 3,4-dihydroxybenzoic acid) are well known for their inhibitory activities against *Leishmania* and *Trypanomosoma* parasites (18, 23, 26); however, the $IC_{50}$s obtained in our study are generally much lower (2- to 40-fold). We believe that these discrepancies derive from the use of different methods and different life cycle stages of the parasites. The $IC_{50}$s for *Leishmania donovani* of caffeic acid and a few of the catechins were consistent with the values described in the literature (19, 30). On the other hand, some of our results conflict with data reported previously: Paveto and coworkers have recently reported that green tea catechins, particularly gallocatechingallate and epigallocatechingallate, inhibit both bloodstream trypomastigote and amastigote forms *T. cruzi* at pM concentrations (29). In our studies, all catechin derivatives were practically inactive against *T. cruzi*. Instead, (−)-gallocatechingallate and (−)-epigallocatechingallate were reasonably toxic against bloodstream trypomastigote forms of *T. brucei rhodesiense*. As catechin-type compounds are well known for their instability, we tested them immediately after dissolving them in dimethyl sulfoxide in order to exclude the use of decomposed material. These differences most likely stem from the different formats of the assays.

Based on the information obtained, it is difficult to decipher empirical SARs and/or QSARs among the plant polyphenols investigated in the current study. Noteworthy is the finding that the majority of the most active trypanocidal and leishmanicidal compounds have a typical flavone structure ($\Delta^{2,3}$ and C-4 keto function) and/or an unhindered catechol substructure (either on ring B or on the benzochromone skeleton, e.g., 7,8-dihydroxyflavone). The other clear trend observed for flavones and flavonols was that the methylation of the OH groups was dis-

favored and reduced the leishmanicidal activity significantly. However, this trend was not so clear among the isoflavone subclass, and in fact, the situation between genistein (4′-OH) and biochanin A (4′-methoxy) was the opposite. The influence of methylation of the hydroxyl groups on trypanocidal activity was irregular. It generally potentiated the activities of the flavonols and the caffeic acid derivatives, but the situation was mostly the opposite for flavones. Hence, in line with the difficulties with the interpretation of the data for SARs on an empirical basis, it was also not possible to establish any clear QSARs based on those data by the different modeling approaches applied here. This may be explained by a variety of reasons. The first possibility is that a common SAR simply does not exist for the compounds under study. Second, the range of biological data (i.e., the difference between the most active and the least active compounds) was relatively low for three of the four data sets (*L. donovani*, *T. cruzi*, and L6 cells); and in the case of the *T. brucei rhodesiense* data, although the spread was wider, only a few highly active compounds were present. In the absence of a sufficiently large spread in the dependent variable (here, biological activity), it is generally difficult to obtain statistically significant linear correlations. Finally, despite the large variety of properties taken into account in two different QSAR approaches (descriptors with proven relevance in QSAR correlations, as well as a validated three-dimensional binding site modeling technique), it is still possible that the structural factors underlying the variance of the biological effects are not represented in the chosen molecular descriptions in an adequate way.

Polyphenols, such as flavonoids, aromatic acids, and cinnamates, are abundant constituents of our diet; and evidence for their role in the prevention of many degenerative diseases is emerging (25). Previously, it was believed that these molecules could not be absorbed at all after oral ingestion; however, this view has been overturned by the pioneering work of Hollman and coworkers in 1995 (11). It is now clear that flavonoids are absorbed into plasma; however, the level of absorption is generally very low and the extent of absorption, bioavailability, and biological activity is highly dependent on the nature of the flavonoid. Bioavailability studies have shown that the circulating form of most flavonoids is as a conjugate (formed by deglycosylation, glucuronidation, sulfation, and methylation reactions mediated by a range of enzymes in the small intestine, liver, and colon), which is then excreted into bile and urine (36). For example, after the oral consumption of quercetin, one of the most prevalent flavonoids found in edible plants, no free aglycone is detectable in the plasma; but its conjugates are detectable (24). The bioactivities of the conjugated polyphenols remain virtually uninvestigated. Furthermore, since metabolism might transform one class of flavonoid into another (28), new pharmacological activity or the loss of previous activity can be observed. All these facts may underlie the absence of notable in vivo activity for the majority of the polyphenols investigated here. However, 7,8-dihydroxyflavone and quercetin appeared to have some in vivo activity. Therefore, they could serve as pharmacophore models for the rational design of synthetic analogs with higher in vitro and in vivo activities and more favorable chemical properties.

Chemotherapy of parasitic diseases still represents a major challenge. Drug efficacy is mostly limited by the inability of the pharmaceutical to reach its target in a sufficient concentration and for a sufficient duration. Compartmentalization of the pathogen in a parasitophorous vacuole, as in the case of *Leishmania*, requires the drug to cross an additional membrane. Hence, only a few recommended antiparasitic agents are able to reach the pathogens at their specific locations, leaving the rest to be distributed throughout the body without beneficial effects. On the other hand, the high dosage and the length of application often result in serious side effects (15). At this point, it seems that site-directed drug formulation could be a very useful means to solve these problems. Optimization of drug carrier and drug delivery systems (e.g., liposomes, polymeric nano- and microparticles, and nanosuspensions) have also been proven to be very effective for the treatment of visceral leishmaniasis (8, 14). We believe that adaptation of new drug formulations and delivery strategies will certainly improve the in vivo antiparasitic activities and bioavailabilities of flavonoids (particularly those of 7,8-dihydroxyflavone and quercetin) and reduce the risk of adverse effects.

In conclusion, the flavonoids and their analogues tested here proved to have very promising in vitro antiprotozoal potentials with minimum or no cytotoxicity. By screening a library composed of more than 100 flavonoids and similar polyphenolics, however, we were able to draw only a few SARs, indicating that the detection of SARs within the polyphenol class is a very complex task. As polyphenols are one of the largest groups of natural products, we conclude that a much larger library needs to be tested in order to reach meaningful SARs. It was particularly noteworthy that, despite their excellent in vitro activities, most of the polyphenols selected were devoid of in vivo efficacy. Nevertheless, the current study suggests the in vivo potentials of 7,8-dihydroxyflavone and quercetin and encourages us to use a medicinal chemistry approach to develop more potent derivatives as lead compounds. Furthermore, we are currently trying to identify the potential molecular target(s) of these two compounds. The determination of their exact mechanism(s) of action in association with an efficient drug delivery strategy will eventually reveal a deeper understanding about the real antiparasitic potential of flavonoids.

## ACKNOWLEDGMENTS

D. T. acknowledges the fellowship provided by the Dr. Helmut Legerlotz Foundation of the University of Zurich. This investigation received financial support from UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

## REFERENCES

1. **Baltz, T., D. Baltz, C. Giroud, and J. Crockett.** 1985. Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei*, *T. equiperdum*, *T. evansi*, *T. rhodesiense* and *T. gambiense*. EMBO J. **4:**1273–1277.
2. **Bürgi, C. B.** 1991. Isolierung, Strukturaufklaerung und Synthese von antioxidativ wirksamen langkettigen Alkylphenolen aus *Plectranthus albidus* (Labiatae). Ph.D. thesis. University of Zurich, Zurich, Switzerland.
3. **Camacho, M. D. R., J. D. Phillipson, S. L. Croft, D. Marley, G. C. Kirby, and D. C. Warhurst.** 2002. Assessment of the antiprotozoal activity of *Galphimia glauca* and the isolation of new nor-secofriedelanes and nor-friedelanes. J. Nat. Prod. **65:**1457–1461.
4. **Croft, S. L., and V. Yardley.** 1999. Animal models of visceral leishmaniasis, p. 783–787. *In* O. Zak (ed.), Handbook of animal models of infection. Academic Press, Inc., New York, N.Y.
5. **Cunningham, I.** 1977. New culture medium for maintenance of tsetse tissues and growth of trypanosomatids. J. Protozool. **24:**325–329.
6. **Desjeux, P.** 2004. Leishmaniasis: current situation and new perspectives. Comp. Immunol. Microbiol. Infect. Dis. **27:**305–318.
7. **Dias, J. C. P., A. C. Silveira, and C. J. Schofield.** 2002. The impact of Chagas

disease control in Latin America. A review. Mem. Inst. Oswaldo Cruz **97:** 603–612.
8. **Fusai, T., Y. Boulard, R. Durand, M. Paul, C. Bories, D. Rivollet, A. Astier, R. Houin, and M. Deniau.** 1997. Ultrastructural changes in parasites induced by nanoparticle-bound pentamidine in a *Leishmania major*/mouse model. Parasite **4:**133–139.
9. **Grael, C. F., S. Albuquerque, and J. L. Lopes.** 2005. Chemical constituents of *Lychnophora pohlii* and trypanocidal activity of crude plant extracts and of isolated compounds. Fitoterapia **76:**73–82.
10. **Harborne, J. B., and C. A. Williams.** 2000. Advances in flavonoid research since 1992. Phytochemistry **55:**481–504.
11. **Hollman, P. C. H., J. H. M. de Vries, S. D. van Leeuwen, M. J. B. Mengelers, and M. B. Katan.** 1995. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. Am. J. Clin. Nutr. **62:**1276–1282.
12. **Jordão, C. O., W. Vichnewski, G. E. P. de Souza, S. Albuquerque, and J. L. C. Lopes.** 2004. Trypanocidal activity of chemical constituents of *Lychnophora salicifolia* Mart. Phytother. Res. **18:**332–334.
13. **Juch, M.** 1997. Isolierung, Strukturaufklaerung und Synthese von langkettigen Alkyloxy-catecholen aus Labiaten als Antioxidanten und Inhibitoren von Lipoxygenase. Ph.D. thesis. University of Zurich, Zurich, Switzerland.
14. **Kayser, O.** 2000. Nanosuspensions for the formulation of aphidicolin to improve drug targeting effects against Leishmania infected macrophages. Int. J. Pharm. **196:**253–256.
15. **Kayser, O., and A. F. Kiderlen.** 2003. Delivery strategies for antiparasitics. Expert Opin. Investig. Drugs **12:**197–207.
16. **Kennedy, P. G. E.** 2004. Human African trypanosomiasis of the CNS: current issues and challenges. J. Clin. Investig. **113:**496–504.
17. **Kırmızıbekmez, H., I. Çalıs, R. Perozzo, R. Brun, A. A. Dönmez, A. Linden, P. Rüedi, and D. Tasdemir.** 2004. Inhibiting activities of the secondary metabolites of *Phlomis brunneogaleata* against parasitic protozoa and plasmodial enoyl-ACP reductase, a crucial enzyme in fatty acid biosynthesis. Planta Med. **70:**711–717.
18. **Koide, T., M. Nose, M. Inoue, Y. Ogihara, Y. Yabu, and N. Ohta.** 1998. Trypanocidal effects of gallic acid and related compounds. Planta Med. **64:**27–30.
19. **Kolodziej, H., and A. F. Kiderlen.** 2005. Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania* parasitised RAW 264.7 cells. Phytochemistry **66:**2056–2071.
20. **Lill, M. A., A. Vedani, and M. Dobler.** 2004. Raptor: combining dual-shell representation, induced-fit simulation, and hydrophobicity scoring in receptor modeling: application toward the simulation of structurally diverse ligand sets. J. Med. Chem. **47:**6174–6186.
21. **Liu, G., and P. Rüedi.** 1996. Phyllocladanes (13-beta-kaurenes) from *Plectranthus ambiguus.* Phytochemistry **41:**1563–1568.
22. **Livingstone, D.** 1995. Data analysis for chemists—applications to QSAR and chemical product design. Oxford University Press, Oxford, United Kingdom.
23. **Mamani-Matsuda, M., J. Rambert, D. Malvy, H. Lejoly-Boisseau, S. Daulouède, D. Thiolat, S. Coves, P. Courtois, P. Vincendeau, and M. D. Mossalayi.** 2004. Quercetin induces apoptosis of *Trypanosoma brucei gambiense* and decreases the proinflammatory response of human macrophages. Antimicrob. Agents Chemother. **48:**924–929.
24. **Manach, C., and J. L. Donovan.** 2004. Pharmacokinetics and metabolism of dietary flavonoids in humans. Free Radic. Res. **38:**771–785.
25. **Manach, C., A. Scalbert, C. Morand, C. Remesy, and L. Jimenez,.** 2004. Polyphenols: food sources and bioavailability. Am. J. Clin. Nutr. **79:**727–747.
26. **Mittra, B., A. Saha, A. R. Chowdury, C. Pal, S. Mandal, S. Mukhopadhyay, S. Bandyopadhyay, and H. K. Majumder.** 2000. Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. Mol. Med. **6:**527–541.
27. **MOE.** 2005. MOE Molecular Operating Environment [Online.] http://www.chemcomp.com/. Chemical Computing Group, Montreal, Quebec, Canada.
28. **Nikolic, D., and R. B. van Breemen.** 2004. New metabolic pathways for flavanones catalyzed by rat liver microsomes. Drug Metab. Dispos. **32:**387–397.
29. **Paveto, C., M. C. Güida, M. I. Esteva, V. Martino, J. Coussio, M. M. Flawiá, and H. N. Torres.** 2004. Anti-*Trypanosoma cruzi* activity of green tea (*Camellia sinensis*) catechins. Antimicrob. Agents Chemother. **48:**69–74.
30. **Radtke, O. A., L. Y. Foo, Y. Lu, A. F. Kiderlen, and H. Kolodziej.** 2003. Evaluation of sage phenolics for their antileishmanial activity and modulatory effects on interleukin-6, interferon and tumor necrosis factor-α-release in RAW 264.7 cells. Z. Naturforsch. C **58c:**395–400.
31. **Räz, B., M. Iten, Y. Grether-Bühler, R. Kaminsky, and R. Brun.** 1997. The Alamar Blue assay to determine drug sensitivity of African trypanosomes (*T. b. rhodesiense* and *T. b. gambiense*) in vitro. Acta Trop. **68:**139–147.
32. **Takeara, R., S. Albuquerque, N. P. Lopes, and J. L. C. Lopes.** 2003. Trypanocidal activity of *Lychnophora staavioides* Mart. (Vernonieae, Asteraceae). Phytomedicine **10:**490–493.
33. **Taleb-Contini, S. H., M. J. Salvador, J. M. Balanco, S. Albuquerque, and D. C. R. de Oliveira.** 2004. Antiprotozoal effect of crude extracts and flavonoids isolated from *Chromolaena hirsuta* (Asteraceae). Phytother. Res. **18:**250–254.
34. **Tasdemir, D., I. Çalıs, and O. Sticher.** 1998. Labdane diterpenes from *Leonurus persicus*. Phytochemistry **49:**137–143.
35. **Tosun, F., and Ç. Akyüz.** 1997. Flavonoids of *Gonocytisus angulatus*. Pharm. Sci. **3:**377–378.
36. **Williamson, G., D. Barron, K. Shimoi, and J. Terao.** 2005. In vitro biological properties of flavonoid conjugates found in vivo. Free Radic. Res. **39:**457–469.