Antiplasmodial Triterpenoids from the Fruits of Neem, Azadirachta indica

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Antiplasmodial Triterpenoids from the Fruits of Neem, *Azadirachta indica*

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Eight known and two new triterpenoid derivatives, neemfruitins A (**9**) and B (**10**), have been isolated from the fruits of neem, *Azadirachta indica*, a traditional antimalarial plant used by Asian and African populations. *In vitro* antiplasmodial tests evidenced a significant activity of the known gedunin and azadirone and the new neemfruitin A and provided useful information about the structure−antimalarial activity relationships in the limonoid class.

Malaria is still a major health burden in many tropical countries, causing an intolerable number of child deaths, particularly in poor African countries. Almost half of the world's population is at risk of the vector born disease, and each year, an estimated 300−500 million malaria episodes occur, of which 1−3 million cases lead to death.[1] This situation is further aggravated by the wide distribution of multidrug-resistant *Plasmodium falciparum* strains. In fact, a dramatic increase in the number of fatal cases among patients having received modern drug treatment has been registered in recent years.[2]

Azadirachta indica A. Juss., a plant of the Meliaceae family, well known as neem, has been used for centuries by the Ayurvedic medicine system as a source for the treatment of a wide variety of ailments. Since the beginning of the 20th century it has become part of several African pharmacopeias, and, nowadays, the populations of many tropical and subtropical countries use homemade neem preparations against various illnesses including malaria.[3] The parts of the plant used, the dosage, and the method of preparation (more frequently aqueous decoctions, but also infusions and macerations) are not well established, since they vary from region to region. However, either fruits, seeds, barks, or, more frequently, leaves are utilized to this aim. Extracts of *A. indica* seeds and leaves are also widely applied as pesticides and insect repellents. The efficacy of such products has been unambiguously associated with their content of the highly functionalized and rearranged limonoid (C_{26} tetranortriterpenoid) azadirachtin-A and analogues.[4] Various biological effects have been evidenced also in mosquitoes, illustrating the potential of limonoid-rich products for the control of insect disease vectors.[5,6]

Extensive chemical investigations carried out in the last decades have led to the identification of a large number of secondary metabolites from *A. indica*, including at least 50 bioactive limonoids with either insecticidal, antibacterial, antitumor, or antiviral properties.[7] Studies aimed at isolating metabolites responsible for the *in vitro* antimalarial activity of neem extracts have indicated the limonoids nimbolide[8] and gedunin (**4**)[9,10] as the most potent components and likely responsible for the activity. Remarkably, gedunin has recently also been shown to be a potent Hsp90 inhibitor with potential anticancer activity.[11] On the other hand, although azadirachtin showed a very poor antimalarial activity on the erythrocytic stages, a transmission-blocking activity of azadirachtin-enriched neem seed extracts has recently been demonstrated *in vivo* using a murine malaria model.[12]

Chart 1

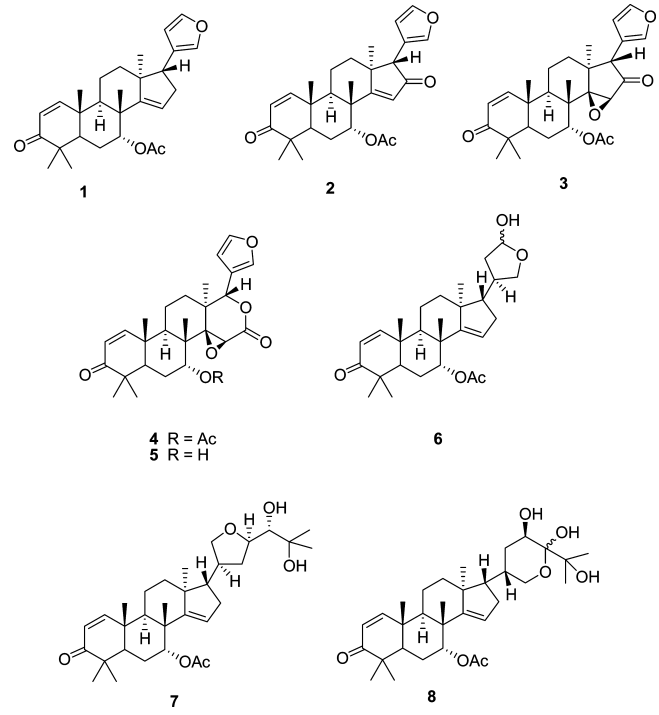
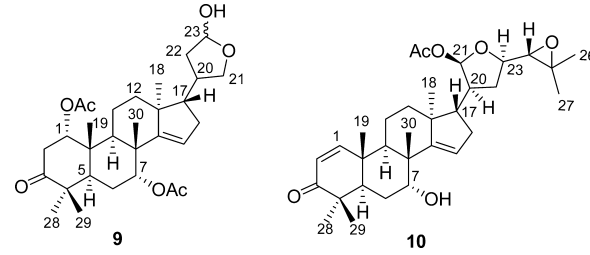


Chart 2



As part of our ongoing research for new antimalarial leads from natural sources,[13−16] we have carried out a detailed phytochemical investigation of the fruit of an African sample of *A. indica*, collected in Burkina Faso. This analysis led to the isolation of 10 pure triterpenoid derivatives (**1**−**10**), two of which were new molecules, named neemfruitins A (**9**) and B (**10**). This paper describes the structural characterization of these new metabolites and reports on the *in vitro* antimalarial activity of all the isolated triterpenoid derivatives.

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Table 1. *In Vitro* Antimalarial Activity of the Extracts and of the Triterpenoids from the Fruits of *A. indica* (**1**−**10**) against D10 (CQ-S) and W2 (CQ-R) Strains of *P. falciparum*[a]

	D10 IC_{50} μM	W2 IC_{50} μM
fruit (EtOAc phase)[b]	1.31 ± 0.48	1.92 ± 0.84
fruit (BuOH phase)[b]	3.18 ± 0.38	3.35 ± 0.35
fruit (H_2O phase)[b]	>50	>50
azadirone (**1**)	1.63 ± 0.23	1.21 ± 0.30
azadiradione (**2**)	5.96 ± 0.76	3.40 ± 0.82
epoxyazadiradione (**3**)	3.30 ± 0.75	2.16 ± 0.73
gedunin (**4**)	1.66 ± 0.37	1.31 ± 0.42
deacetylgedunin (**5**)	5.14 ± 1.23	3.29 ± 0.59
desmethyllimocin B (**6**)	4.80 ± 0.53	2.59 ± 0.70
protoxylocarpin G (**7**)	5.00 ± 0.66	2.40 ± 0.90
spicatin (**8**)	5.40 ± 0.75	2.74 ± 0.79
neemfruitin A (**9**)	2.82 ± 0.70	1.74 ± 0.25
neemfruitin B (**10**)	9.49 ± 1.08	9.98 ± 2.16
chloroquine	0.03 ± 0.01	0.34 ± 0.09

[a] Data are means ± SD of three different experiments in duplicate. [b] These data are in μg/mL.

Preceding the detailed phytochemical investigation of the neem fruit, it was ascertained that this plant part actually contains antimalarial compounds by testing a crude fruit extract in a murine malaria model (*Plasmodium berghei*). In mice treated with the extract at a daily oral dosage of 200 mg/kg over 9 days and exposed to infectious mosquito bites on day 3 of treatment, parasitaemia levels reduced by 45% (CI95 40%−50%; $p < 0.001$) compared to control animals were observed, confirming the choice of this plant part as study object.

Dried fruits (25 g) of *A. indica* were exhaustively extracted with MeOH, and the obtained material was then partitioned between H_2O and EtOAc to yield a brown organic extract (2.75 g). The water layer was further partitioned against *n*-BuOH, yielding a polar organic extract (2.50 g) and a water extract (8.1 g). These three phases were subjected to a preliminary screening on *P. falciparum* blood stage cultures to assess the *in vitro* antimalarial activity. The results indicated that the EtOAc phase was the most promising for further investigation (Table 1). It was therefore subjected to MPLC chromatography over silica gel followed by repeated HPLC purifications to afford the new limonoid neemfruitin A (**9**) and the new apotirucallane neemefruitin B (**10**), in addition to the known metabolites azadirone (**1**),[17] azadiradione (**2**),[18] epoxyazadiradione (also called nimbinin) (**3**),[19] gedunin (**4**),[20] deacetylgedunin (**5**),[21] desmethyllimocin B (**6**),[22] protoxylocarpin G (**7**),[23] and spicatin (**8**).[24] Protoxylocarpin G (**7**) has recently been isolated from seed kernels of *Xylocarpus granatus*,[23] and this is the first report of its presence in *A. indica*. As already noticed in other studies, the neem fruit extract did not contain any azadirachtin.[25]

HR-ESIMS data established the molecular formula of neemfruitin A (**9**) as $C_{30}H_{44}O_7$. The ^{1}H NMR spectrum of **9** (Table 2, $CDCl_3$) showed seven methyl singlets, two of which (δ_H 2.09 and 1.98) belong to acetyl groups, four broad singlets between δ_H 5.00 and 5.50, and a number of multiplets between δ_H 3.00 and 4.20. A combined analysis of ^{13}C NMR (Table 2) and HSQC data revealed the presence of three carbonyl resonances (two ester carbonyls at δ_C 170.5 and 170.1 and a ketone carbonyl at δ_C 213.8) as well as a trisubstituted double bond (δ_C 159.2; δ_H 5.30, δ_C 119.1), accounting for four of the nine unsaturation degrees implied by the molecular formula. Therefore, neemfruitin A must be pentacyclic. The ^{13}C NMR spectrum of **9** contained also the resonances of two oxygenated methine carbons (δ_H 5.05, δ_C 76.7; δ_H 5.22, δ_C 74.6), one oxymethylene (δ_H 4.20 and 3.44, δ_C 72.2), and a hemiacetal group (δ_H 5.48, δ_C 97.8).

The 2D NMR COSY spectrum of **9** allowed the arrangement of the proton multiplets into four spin systems (indicated in bold in Figure 1), one of which is a C_7 moiety spanning the sp^2 methine at C-15 to the hemiacetal proton and including an oxymethylene

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Neemfruitins A (**9**) and B (**10**)[a]

	9		**10**	
pos.	δ_H, mult. (*J* in Hz)	δ_C, mult.	δ_H, mult. (*J* in Hz)	δ_C, mult.
1	5.05, bs	76.7, d	7.12, d (10.2)	161.8, d
2a	3.04, dd (17.1, 3.6)	41.3, t	5.84, d (10.2)	127.2, d
2b	2.48, dd (17.1, 1.5)			
3		213.8, s		205.8, s
4		42.4, s		44.3, s
5	2.31[b]	47.8, d	2.07[b]	44.7, d
6a	1.98[b]	25.1, t	1.85[b]	24.0, t
6b	1.80, m		1.80[b]	
7	5.22, bs	74.6, d	3.98, bs	72.0, t
8		42.5, s		44.8, s
9	2.50[b]	35.3, d	2.08[b]	36.8, d
10		41.9, s		40.5, s
11a	1.80[b]	16.5, t	1.75[b]	16.8, t
11b	1.70[b]		1.73[b]	
12a	1.90[b]	34.0, t	1.84[b]	33.4, t
12b	1.50[b]		1.51[b]	
13		47.2, s		53.1, s
14		159.2, s		161.9, s
15	5.30, bs	119.1, d	5.52, bd (6.5)	119.9, d
16a	2.20[b]	35.3, t	2.25[b]	35.4, t
16b	2.10[b]		2.20[b]	
17	1.54, m	59.2, d	1.93[b]	46.7, d
18	1.00, s	21.3, q	1.03, s	19.5, q
19	1.18, s	19.1, q	1.10, s	18.9, q
20	2.63, m	38.4, d	2.37, m	44.7, d
21a	4.20, t (8.0)	72.2, t	6.26, d (6.6)	96.8, d
21b	3.44, t (8.0)			
22a	2.49[b]	35.5, t	2.09[b]	32.3, t
22b	1.58[b]		1.73[b]	
23	5.48, bs	97.8, d	3.95[b]	80.3, d
24			2.67, d (7.2)	67.0, d
25				57.8, s
26			1.33, s	23.2, q
27			1.29, s	25.9, q
28	1.08, s	22.0, q	1.08, s	21.3, q
29	1.18, s	25.2, q	1.16, s	27.1, q
30	1.19, s	27.3, q	1.12, s	27.4, q
21-OAc				170.7, s
			2.07, s	21.2, q
1-OAc		170.1, s		
	1.98, s	21.0, q		
7-OAc		170.5, s		
	2.09, s	21.2, q		

[a] Data taken in $CDCl_3$. For neemfruitin A (**9**) data of the major epimer have been listed. ^{1}H NMR data of the minor epimer are reported in the Experimental Section. [b] Overlapped with other signals.

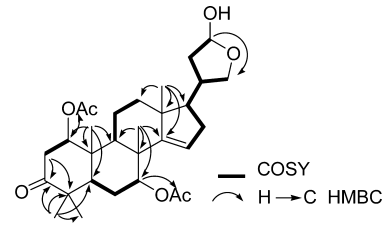

Figure 1. COSY and key H→C HMBC correlations detected for neemfruitin A (**9**).

branching at C-20. The HMBC data (Figure 1) allowed the connection of the moieties and the assembly of the protolimonoid-type neemfruitin A planar structure. In particular, cross-peaks of Me-19, Me-28, and Me-29 indicated the structure of ring A, including the placement of an oxymethine at C-1 and the ketone carbonyl at C-3. Analogously, cross-peaks of Me-18, Me-19, and Me-30 disclosed the structure of rings B, C, and D, placing the second oxymethine at C-7. Both H-1 and H-7 showed ^3J HMBC cross-peaks with an ester carbonyl, thus inferring the attachment of the two acetyl groups. Finally, the key cross-peak of H-23 with

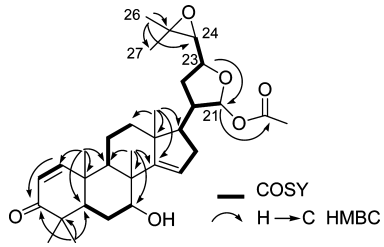

Figure 2. COSY and key H→C HMBC correlations detected for neemfruitin B (**10**).

C-21 indicated the presence of a γ-lactol ring with an oxygen atom connecting C-21 and the hemiacetal C-23.

Collectively, the above data led to the assignment of the planar structure of **9**. It should be noted, however, that both ^{1}H and ^{13}C NMR spectra of **9** included a series of signals attributable to a minor compound. However, all our attempts to further purify compound **9** failed. Once the structure of compound **9** was disclosed, these signals were readily rationalized by the presence of an equilibrating mixture (ca. 3:1 ratio) of the two epimers at C-23. ^{1}H and ^{13}C NMR resonances of the minor epimer have been reported in the Experimental Section.

The relative configuration of neemfruitin A (**9**) was assigned on the basis of ROESY cross-peaks aided by analysis of proton−proton coupling constants. The correlations of Me-19 with both Me-29 and Me-30 were indicative of the *cis* relationships of these groups, for which we have indicated in **9** the β-orientation, by assuming that neemfruitin A shares the absolute configuration invariably found for *Azadirachta* limonoids. Analogously, following the α-orientation of Me-28, the ROESY correlations Me-28/H-5, H-5/H-9, and H-9/Me-18 indicated the α-orientation of all these groups. Both H-1 and H-7 must be in equatorial (β) orientation on the basis of their very small coupling constants with protons on the adjacent methylenes. The ROESY cross-peaks H-1/Me-19 and H-7/Me-30 further supported this assignment. The cross-peak of Me-18 with H-20 indicated the β-orientation of H-17, while, given the free-rotating nature of the C-17/C-20 bond and the lack of unambiguous information, the configuration at C-20 has been left undetermined.

Neemfruitin B (**10**) was isolated as a colorless, amorphous solid with the molecular formula $C_{32}H_{46}O_6$ (established by HR-ESIMS), implying 10 degrees of unsaturation. The stereostructure of **10** was established on the basis of full 1D and 2D (COSY, HSQC, HMBC) NMR analysis, following the aforementioned approach for compound **9** (NMR assignment, Table 2). In particular, combined analysis of COSY and HSQC spectra allowed the elucidation of the four spin systems depicted in bold in Figure 2, with a large moiety connecting the sp^2 methine at δ_H 5.52 (C-15) with the oxymethine at δ_H 2.67 (δ_C 67.0; C-24) and including a deshielded methine (δ_H 6.26 C-21) branching. Analysis of the HMBC cross-peaks (see Figure 2) allowed the assembly of the tetracyclic system of rings A−D, whose structure is similar to that of compounds **1** and **6**−**8**, with the single exception of functionalization at C-7, which should be a nonacetylated hydroxy group (H-7 resonates at δ_H 3.98 in **10** and at 5.20 in **7**). Since this tetracyclic system included seven unsaturation degrees and two oxygen atoms, the side chain moiety should account for the three remaining unsaturation degrees and should include four oxygen atoms. The HMBC cross-peak of H-23 (δ_H 3.95) with C-21 (δ_C 96.8) suggested the presence of an oxygen bridge between C-23 and C-21, giving rise to a tetrahydrofuran ring. In addition, H-21 showed an HMBC cross-peak with an acetyl carbonyl (δ_C 170.7), thus identifying C-21 as an acetylated hemiacetal carbon, in agreement with its downfield resonance. Finally, since both Me-26 and Me-27 showed HMBC cross-peaks with two oxygenated carbons (the unprotonated C-25 and the oxymethine C-24), the last unsaturation degree and the last oxygen atom can both be accounted for by the presence of an epoxide ring connecting C-24 and C-25. This is also in agreement with the resonance of H-24 (δ_H 2.67), a typical value of oxirane methines. Thus, the gross structure of neemfruitin B (**10**) was defined as a new monoacetylated apotirucallane triterpenoid.

The relative configuration of the tetracyclic core of **10** was assigned by inspection of its ROESY spectrum and was the same as that detected for compound **9**. The side chain configuration of **10** was deduced on the basis of the high similarity of its ^{1}H/^{13}C NMR data with those of acetyltoosendantriol,[26] a triterpenoid derivative isolated from *Melia toosendan* possessing the same structure of **10** as for ring D and the entire side chain (from C-20 to C-27) and whose stereochemical details had been secured by X-ray analysis.[26] The observed ROESY correlation of H-23 with H-20 and H-21 fully agrees with this assignment.

The triterpenoid derivatives isolated (**1**−**10**) were assayed *in vitro* against D10 (chloroquine sensitive, CQ-S) and W2 (chloroquine resistant, CQ-R) strains of *Plasmodium falciparum* using the pLDH assay. Results are compiled in Table 1.

Interestingly, the tested compounds were found to be more active on the chloroquine-resistant clone (W2), a behavior previously observed with antimalarials of the endoperoxide class.[13−15] The most active compounds were the three limonoids (C_{26}) azadirone (**1**), gedunin (**4**), and neemfruitin A (**9**), while, on the contrary, the apotirucallane (C_{30}) derivative **10** emerged to be the less active compound. Interestingly, the EtOAc and BuOH fractions showed good antiplasmodial activity, with IC_{50} values ranging from 1.31 to 3.35 μg/mL, comparable to those of the most active isolated molecules. Given the relatively low abundance of highly active limonoids in these extracts, their prominent activity cannot be explained only on the basis of additive effects, but a synergistic action between the constituent molecules should be taken into account.

The comparison of the structures of compounds **7** and **8** with that of **1** indicates that their different activities should be attributed to the differences in the side chain. Also interesting is the comparison between the activities of **1** and **2**, clearly indicating that the presence of a second conjugated carbonyl group at C-16 is deleterious for the antimalarial activity.

It is important to notice that gedunin (**4**) has been identified as the most potent antimalarial limonoid of both the bark[9] and leaves[10] of *A. indica*. We have now found that the fruits of the same plant contain two limonoids that are as active as gedunin, namely, azadirone (**1**) and the new neemfruitin A (**9**). A previous study[10] identified the conjugated enone system, the furan ring, and the acetoxy group at C-7 as critical for the antimalarial activity of gedunin (**4**). The comparable activity of neemfruitin A (**9**), lacking the double bond at C-1/C-2 and showing a lactol ring in place of the furan ring, indicates that the above relationships, and in particular the need of a Michael acceptor in ring A, found for the D-*seco*-limonoid gedunin, do not apply to tetracarbocyclic limonoids. The pharmacophoric portions of limonoid antimalarials are evidently still far from being identified. Only a more detailed investigation on the mechanisms of their antimalarial action, which remain elusive, could allow a better understanding of the structural requirements needed by an active molecule.

In conclusion, the present analysis of the fruits of *A. indica* revealed the presence of a new apotirucallane and a new limonoid (neemfruitin A, **9**). This latter compound represents, together with gedunin (**4**) and azadirone (**1**) (a major component of the organic extract), the most active antimalarial limonoid of this part of the tree. Owing to the importance placed on the traditional use of neem remedies for the treatment of malaria patients, this investigation could be potentially useful in the view of developing improved

standardized herbal formulations that can be produced locally in African and Asian countries.

Experimental Section

General Experimental Procedures. Optical rotations ($CHCl_3$) were measured at 589 nm on a P2000 Jasco polarimeter. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a Varian INOVA spectrometer. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H 7.26, δ_C 77.0). Homonuclear ^1H connectivities were determined by the COSY experiment; one-bond heteronuclear $^1H-^{13}C$ connectivities by the HSQC experiment; two- and three-bond $^1H-^{13}C$ connectivities by gradient-HMBC experiments optimized for a $^{2,3}J$ of 8 Hz. Through-space ^1H connectivities were evidenced by using a ROESY experiment with a mixing time of 500 ms. Low- and high-resolution ESIMS spectra were performed on a LTQ OrbitrapXL (Thermo Scientific) mass spectrometer. MPLC was performed on a Büchi apparatus using a silica gel (230−400 mesh) column; HPLC were achieved on a Knauer apparatus equipped with a refractive index detector. LUNA (normal phase, SI60, 250 × 4 mm) (Phenomenex) columns were used, with elution with EtOAc/*n*-hexane mixtures and 0.8 mL/min as flow rate.

Plant Material. Fresh, uncrushed, ripe neem fruits were collected from the Oubritenga (Ziniaré) region, Burkina Faso, in June 2008. The tree was identified by Prof. Jeanne Millogo, professor of Botanics at the Life Science Unit (University of Ouagadougou), and a voucher specimen (N°2 NFE) has been deposited in the Laboratory of Ecology at the University of Ouagadougou.

Extraction and Isolation. After elimination of seeds, fruits of *A. indica* were dried (25 g, dry weight) and repeatedly extracted with MeOH (4 × 500 mL), and the obtained material was partitioned between H_2O and EtOAc to yield a brown organic extract (2.75 g). The water extract was then partitioned against *n*-BuOH, yielding a polar organic (2.50 g) and a water (8.1 g) phase. The EtOAc extract was subjected to chromatography over a silica column (230−400 mesh), eluting with a solvent gradient of increasing polarity from *n*-hexane to EtOAc. Fractions eluted with *n*-hexane/EtOAc (9:1) were subjected to repeated HPLC chromatographies (*n*-hexane/EtOAc, 95:5, isocratic, flow rate 0.8 mL/min), affording azadirone (**1**, 112.1 mg, t_R 12 min) and epoxyazadiradione (**3**, 135.4 mg, t_R 16 min). Fractions eluted with *n*-hexane/EtOAc (8:2) were rechromatographed by HPLC (*n*-hexane/EtOAc, 85:15, isocratic, flow rate 0.8 mL/min) to give gedunin (**4**, 11.3 mg, t_R 7 min) and deactylgedunin (**5**, 7.3 mg, t_R 13 min). Fractions eluted with *n*-hexane/EtOAc (7:3) contained pure azadiradione (**2**, 118.5 mg). Fractions eluted with *n*-hexane/EtOAc (6:4) were subjected to repeated HPLC purifications (*n*-hexane/EtOAc, 65:35, isocratic, flow rate 0.8 mL/min) to yield neemfruitin B (**10**, 4.5 mg, t_R 11 min), protoxylocarpin G (**7**, 2.2 mg, t_R 18 min), and desmethyllimocin B (**6**, 14.6 mg, t_R 19 min). Fractions eluted with *n*-hexane/EtOAc (1:1) were rechromatographed by HPLC (*n*-hexane/EtOAc, 55:45, isocratic, flow rate 0.8 mL/min), affording pure neemfruitin A (**9**, 3.5 mg, t_R 6 min) and spicatin (**8**, 2.8 mg, t_R 12 min).

Neemfruitin A (9): colorless, amorphous solid; $[\alpha]_D$ −17.2 (*c* 0.2 in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) Table 2; ^1H NMR data for the minor epimer (only resonances differing from those of the major epimer are reported) δ_H 5.54 (1H, H-23, bs), 3.98 (1H, H-21a, t, J = 8.0 Hz) 3.60 (1H; H-21b, t, J = 8.0 Hz), 2.81 (1H, H-20, m), 2.25 (1H, H-22a, overlapped), 1.63 (1H, H-22a, overlapped), 0.96 (3H, Me-18, s); ^{13}C NMR ($CDCl_3$, 125 MHz) Table 2; (+) ESIMS *m/z* 539 $[M + Na]^+$; HR-ESIMS *m/z* 539.2979 (calcd for $C_{30}H_{44}O_7Na$ 539.2985).

Neemfruitin B (10): colorless, amorphous solid; $[\alpha]_D$ +5.3 (*c* 0.4 in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) Table 2; (+) ESIMS *m/z* 549 $[M + Na]^+$; HR-ESIMS *m/z* 549.3200 (calcd for $C_{32}H_{46}O_6Na$ 549.3192).

***In Vitro* Drug Susceptibility Assay on *P. falciparum*.** The CQ-sensitive (D10) and the CQ-resistant (W2) strains of *P. falciparum* were cultured *in vitro* as described by Trager and Jensen.[27] Parasites were maintained in human type A-positive red blood cells at 5% hematocrit in RPMI 1640 (Gibco BRL, $NaHCO_3$ 24 mM) medium with the addition of 1% AlbuMaxII (Invitrogen, Milano, Italy), 0.01% hypoxantine, 20 mM Hepes (Euroclone), and 2 mM glutammine (Euroclone). The cultures were maintained at 37 °C in a standard gas mixture consisting of 1% O_2, 5% CO_2, and 94% N_2. Test compounds were dissolved in either H_2O or DMSO and then diluted with medium to achieve the required concentrations (final DMSO concentration <1%, which is nontoxic to the parasite). Compounds were placed in serial dilutions into 96-well flat-bottom microplates (COSTAR). Asexual parasite stages derived from asynchronous cultures with parasitaemia of 1−1.5% were aliquoted into the plates (final hematocrit 1%) and incubated for 72 h at 37 °C. Parasite growth was determined spectrophotometrically (OD_{650}) by measuring the activity of the parasite lactate dehydrogenase (LDH), according to a modified version of Makler's method in control and treated cultures.[28] Chloroquine was used as reference control. The antiplasmodial activity is expressed as the 50% inhibitory concentrations (IC_{50}). Each IC_{50} value presented in Table 1 is the mean and standard deviation of three separate experiments performed in duplicate.

***In Vivo* Antimalarial Activity.** The *in vivo* antimalarial activity was assessed with the rodent malaria model *Plasmodium berghei*−*Anopheles stephensi*−BALB/c mice. Groups of six mice (5-week-old females) were treated for 9 days with an EtOH extract of neem fruits, administered orally twice a day at a dose of 100 mg/kg (200 mg/kg/d), or with control solution. The treatment was well tolerated by all animals over the entire period of extract administration. On treatment day 3, mice were exposed to ∼20 bites of infectious mosquitoes, i.e., *Anopheles* females harboring *P. berghei* sporozoites in their salivary glands. The day following the last treatment (day 10), the percent parasitaemia was assessed by the examination of Giemsa-stained thin blood smears at the light microscope (1000× magnification). Reduction in parasite proliferation as a result of treatment was estimated by comparing the mean parasitaemia values of treated and control group mice from two experimentations using the Student's *t* test.

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Supporting Information Available: Copies of NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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