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Antiparasitic Activities of Acridone Alkaloids from *Swinglea glutinosa* (Bl.) Merr.

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Onze alcalóides acridônicos isolados de *Swinglea glutinosa* (Bl.) Merr. foram avaliados para suas atividades *in vitro* contra linhagens de *Plasmodium falciparum* sensíveis a cloroquina 3D7, *Trypanosoma brucei rhodesiense* STIB9000 e *Leishmania donovani* L82. Ensaios com células KB foram também executados com o objetivo de se determinar o grau de toxicidade das substâncias ativas contra os parasitas. Nove dos compostos apresentaram IC_{50} entre 0,3 e 11,6 μM contra *P. falciparum*. Em contraste, um pequeno número de compostos mostrou atividade significativa contra *T. brucei rhodesiense* e nenhum apresentou atividade contra *L. donovani*. Entre os alcalóides três tiveram $IC_{50} < 1,0$ μM contra *P. falciparum*, enquanto que contra *T. b. rhodesiense* cinco mostraram $IC_{50} < 10$ μM. A caracterização dos alcalóides, 1,3,5-triidróxi-4-metóxi-10-metil-2,8-bis(3-metilbut-2-enil)acridin-9(10*H*)-ona (**1**), 2,3-diidro-4,9-diidróxi-2-(2-hidróxipropan-2-il)-11-metóxi-10-metilfuro[3,2-*b*]acridin-5(10*H*)-ona (**2**) e 3,4-diidro-3,5,8-triidróxi-6-metóxi-2,2,7-trimetil-2*H*-pirano[2,3-*a*]acridin-12(7*H*)-ona (**3**), é aqui discutida. Discute-se também a relação estrutura-atividade para todos os compostos ensaiados. O isolamento e dados espectrais para os alcalóides **1**-**3** estão sendo aqui descritos pela primeira vez, embora em trabalho anterior tenham sido relatadas as suas atividades citotóxicas.

Eleven acridone alkaloids isolated from *Swinglea glutinosa* (Bl.) Merr. were examined for *in vitro* activity against chloroquine-sensitive *Plasmodium falciparum* 3D7, *Trypanosoma brucei rhodesiense* STIB900 and *Leishmania donovani* L82. An assay with KB cells was developed in order to compare *in vitro* toxicity of alkaloids with the selective action on the parasites. Nine of the compounds had IC_{50} values ranging from 0.3 to 11.6 μM against *P. falciparum*. In contrast, a small number of compounds showed significant activity against *T. brucei rhodesiense* and none had activity against *L. donovani*. Among the alkaloids three had $IC_{50} < 1.0$ μM against *P. falciparum*, whereas against *T. b. rhodesiense* five had $IC_{50} < 10$ μM. The characterization of the acridone alkaloids, 1,3,5-trihydroxy-4-methoxy-10-methyl-2,8-bis(3-methylbut-2-enyl)acridin-9(10*H*)-one (**1**), 2,3-dihydroxy-4,9-dihydroxy-2-(2-hydroxypropan-2-yl)-11-methoxy-10-methylfuro[3,2-*b*]acridin-5(10*H*)-one (**2**) and 3,4-dihydro-3,5,8-trihydroxy-6-methoxy-2,2,7-trimethyl-2*H*-pyrano[2,3-*a*]acridin-12(7*H*)-one (**3**), is discussed, as well as the structure-activity relationship of all compounds assayed. Isolation and spectral data of alkaloids **1**-**3** are described for the first time although their citotoxicities to cancer cells have been described before.

Keywords: antiparasitic acridone alkaloids, malaria, *Swinglea glutinosa*, Rutaceae

Introduction

Parasitic protozoa are the causative agents of human and livestock diseases infecting hundreds of millions of people every year and are collectively one of most important causes of human misery.[1] Human African trypanosomiasis (HAT), or sleeping sickness, malaria, Chagas' disease and leishmaniasis are major health problems in many countries. HAT promoted by *Trypanosoma brucei rhodesiense* and *T. b. gambiense* is endemic in over 30 African countries threatening over 60 million people. HAT has reached epidemic proportions in some countries, such as Angola, southern Sudan, Uganda and the Democratic Republic of Congo.[2,3] Malaria has re-emerged as a major public health problem over the past three decades mainly because of the development of worldwide resistance of *Plasmodium*

falciparum to chloroquine, a drug which formed the basis for cheap and effective treatment and for prophylaxis of this disease.[4] Each year, approximately 300 to 500 million malaria infections lead to over one million deaths. In many endemic countries, malaria is responsible for economic stagnation, lowering the annual economic growth in some regions by up to 1.5%.[5,6] Leishmaniasis is a disease caused by protozoa of the genus *Leishmania*. According to WHO 88 countries are affected, with 350 million people at risk. 90% of cases of visceral leishmaniasis occur in India, Sudan, Bangladesh and Brazil.[7] Present chemotherapy for these diseases is inadequate or toxic, or becoming ineffective due to an increase in resistance.[8]

The family Rutaceae contains many secondary metabolites such as alkaloids, flavonoids, coumarins, limonoids and lignans with a large spectrum of biological activities.[9] Studies showed that acridone alkaloids are compounds with promising activity against *P. falciparum*,[10,11] and also have antiviral[12] and antiproliferative effects on cancer cell lines.[13,14] The Asian genera *Citrus* and *Swinglea* are members of the Rutaceae and are included in the subfamily Aurantioideae. *Citrus* species

have been investigated and characterized by possessing acridone alkaloids. These data stimulated an investigation of *Swinglea glutinosa* (Bl.) Merr. in a search for lead acridones.

From the MeOH extract of the stem bark of *S. glutinosa* three new acridone alkaloids were identified: 1,3,5-trihydroxy-4-methoxy-10-methyl-2,8-*bis*(3-methylbut-2-enyl)acridin-9(10*H*)-one (**1**), 2,3-dihydro-4,9-dihydroxy-2-(2-hydroxypropan-2-yl)-11-methoxy-10-methylfuro[3,2-*b*]acridin-5(10*H*)-one (**2**) and 3,4-dihydro-3,5,8-trihydroxy-6-methoxy-2,2,7-trimethyl-2*H*-pyrano[2,3-*a*]acridin-12(7*H*)-one (**3**). In addition, eight known alkaloids were characterized by the analysis of their NMR spectra and compared with reference data: 1,3,5-trihydroxy-2,8-*bis*(3-methylbut-2-enyl)-10-methyl-9-acridone (**4**),[10] glycocitrine-IV (**5**),[15] citrusinine-II (**6**),[16] citrusinine-I (**7**),[17] citibrasine (**8**),[16] 5-hydroxynoracronycine (**9**),[18] pyranofoline (**10**)[19] and *bis*-5-hydroxynoracronycine (**11**).[20] These alkaloids were tested *in vitro* against chloroquine-sensitive *P. falciparum* 3D7, *T. b. rhodesiense* STIB900, *L. donovani* L82 and their toxicity effects were also evaluated on KB cells.
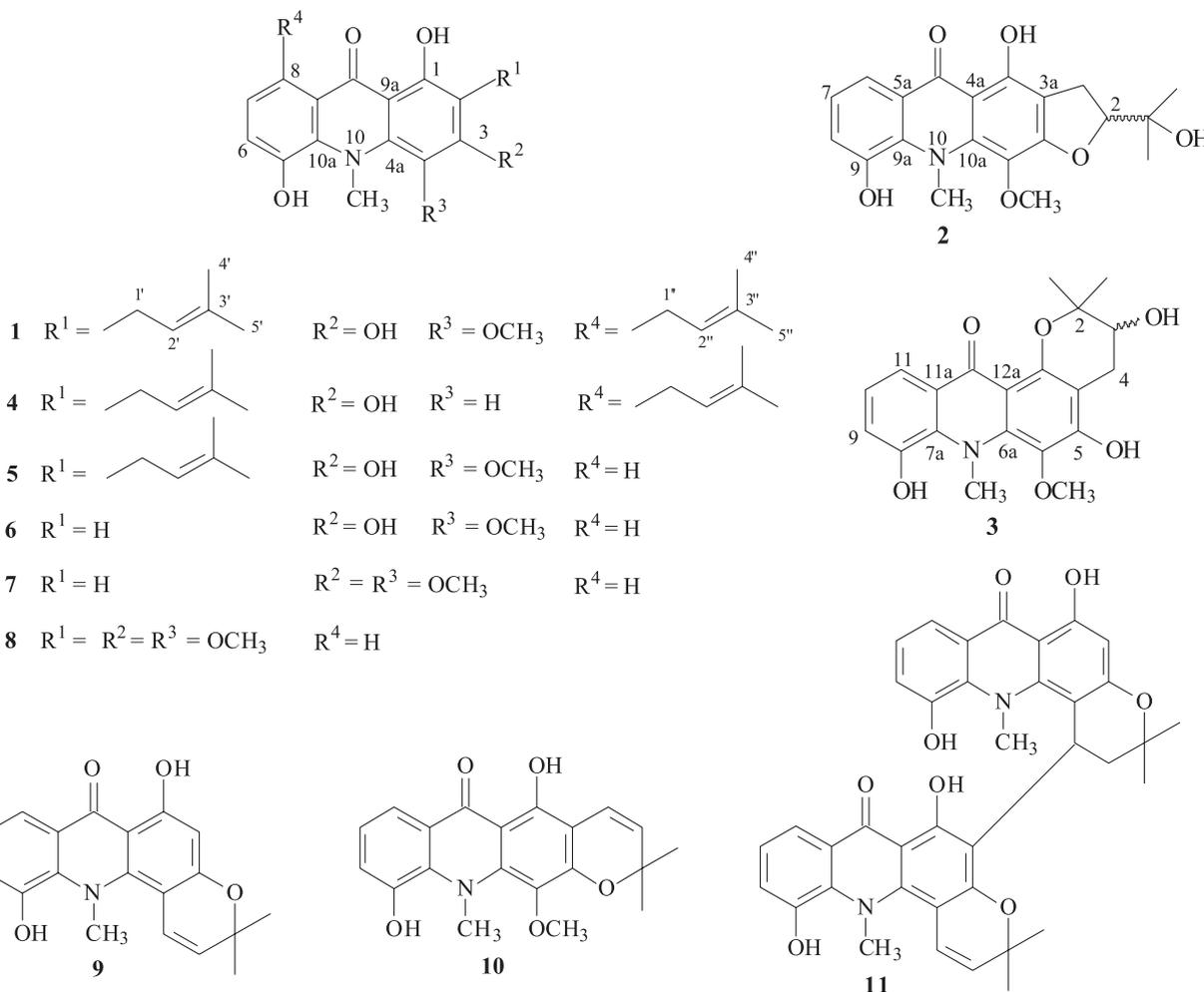
Results and Discussion

Compound **1** was isolated as an amorphous powder, the molecular formula was determined as $C_{25}H_{29}NO_5$ by HRESIMS, showing an $[M+H]^+$ peak at *m/z* 424.2124. The UV absorptions bands at λ_{max}/nm: 222, 265, 285, 332 and 410 nm, IR absorptions at ν_{max}/cm^{-1}: 3500 and 1625 cm and the characteristic signal of a hydrogen-bonded hydroxyl proton at δ 14.42 in the ^{1}H NMR spectra, exchangeable with D_2O, suggested the presence of a hydroxyl group. These data together with analyses of the ^{13}C NMR spectrum, suggested that compound **1** had a 1-hydroxy-9-acridone framework.[16-22]

The ^{1}H NMR spectrum of the compound **1** showed two AB type aromatic protons at δ 7.17 (1 H, d, *J* 8.0Hz, H-6) and 6.96 (1H, d, *J* 8.0Hz, H-7), one *N*-methyl group at δ 3.74 and one *O*-methyl group at δ 3.76. Also signals of two trisubstituted double bonds at δ 5.41(1H, m, H-2"), 5.31 (1H, m, H-2'), two methylene protons at δ 4.01 (2H, d, *J* 7.0Hz, H-1"), 3.40 (2H, d, *J* 7.0Hz, H-1'), the former protons being deshielded by the 9-carbonyl group, and four vinyl methyl groups [δ 1.80 (3H,d, *J* 0.9Hz, H-4' *E*), 1.65 (3H,d, *J* 1.0Hz, H-5' *Z*), 1.74 (3H,d, *J* 0.9Hz, H-4" *E*), 1.69 (3H,d, *J* 1.0Hz, H-5" *Z*)] were observed, which suggested the presence of two prenyl groups (3-methylbut-2-enyl). Their positions were confirmed at C-2 and C-8 by HMBC.

The position of a prenyl group at C-2 was confirmed by the correlation of the methylene protons at δ 3.40 (H-1') with C-1 (δ 158.1), C-2 (δ 109.4), C-3 (δ 156.1), C-2' (δ 123.6) and C-3' (δ 131.4) in the HMBC spectrum. In addition, the correlation of 1-OH at δ 14.42 with C-1 and C-9a (δ 107.6) confirmed that the first prenyl group was attached to C-2. The position of the second prenyl group was confirmed by the correlation of the deshielded methylene protons at δ 4.01 (H-1") with C-7 (δ 125.4), C-8 (δ 135.0), C-2" (δ 125.4) and C-3" (δ 131.2), confirming that the second prenyl group was attached to C-8. The *N*-methyl protons showed correlation with C-4a and C-5a at δ 140.2 and 139.4, respectively. The *O*-methyl protons showed correlation with C-4 at δ 128.8. This value is consistent with the acridone possessing an *O*-methyl group at C-4.[17,23] Based on these data, the structure of **1** was characterized as 1,3,5-trihydroxy-4-methoxy-10-methyl-2,8-*bis*(3-methylbut-2-enyl)acridin-9(10*H*)-one. Table 1 shows the data of ^{1}H, ^{13}C NMR and correlations observed in the HMBC experiment.

Compound **2** was isolated as an amorphous powder, the molecular formula $C_{20}H_{21}NO_6$ was determined on the basis of HRESIMS, exhibiting an $[M+H]^+$ peak at *m/z* 372.1447. The UV and IR spectra were identical to those of **1**, however some differences were observed in the ^{1}H and ^{13}C NMR. In the ^{1}H NMR spectrum, the characteristic signal of a

Table 1. ^{1}H and ^{13}C NMR and HMBC data for compound **1** (acetone-d_6)

Position	$^{13}C^a$ δ	$^1H^b$ δ (mult., *J* in Hz)	HMBC^b (H-C)
1	158.1		
1-OH		14.42 (s)	C-1, C-2
2	109.4		
3	156.1		
4	128.8		
4^a	140.2		
5	147.3		
5^a	139.4		
6	120.0	7.17 (d, 8.0)	C-5, C-8, C-5a
7	125.4	6.96 (d, 8.0)	C-8a, C-1"
8	135.0		
8a	123.6		
9	185.5		
9a	107.6		
N-CH$_3$	45.3	3.74 (s)	C-4a, C-5a
O-CH$_3$	59.8	3.76 (s)	C-4
1'	22.4	3.40 (d, 7.0)	C-1, C-2, C-3, C-3', C-2'
2'	123.6	5.31 (m)	C-4', C-5'
3'	131.4		
4'	25.9	1.65 (d, 1.0)	C-2', C-3', C-5'
5'	17.8	1.80 (d, 0.9)	C-2', C-4', C-3'
1"	34.3	4.01 (d, 7.0)	C-7, C-8, C-2", C-3"
2"	125.4	5.41 (m)	C-4', C-5"
3"	131.2		
4"	25.9	1.74 (d, 0.9)	C-2", C-3", C-5"
5"	18.0	1.69 (d, 1.0)	C-2", C-3", C-4"

^{a} Recorded at 100 MHz; ^{b} Recorded at 400 MHz.

hydrogen-bonded hydroxy proton at δ 14.42, exchangeable with D_2O suggested the presence of a hydroxyl group. These data suggested that compound **2** had a 4-hydroxy-5-acridone skeleton.[16-22]

The ^{1}H NMR spectrum of **2** showed an ABX type aromatic spin system at δ 7.15 (1 H, t, *J* 7.8Hz, H-7), 7.29 (1H, dd, *J* 7.8, 1.4Hz, H-8) and 7.80 (1H, dd, *J* 7.8, 1.4Hz, H-6), this last proton being deshielded by the 5-carbonyl group. The spectrum also showed one *N*-methyl group at δ 3.85 and one *O*-methyl group at δ 3.89. The presence of a hydroxyisopropyldihydrofuran moiety was suggested by an oxymethine proton at δ 4.88 (1H, dd, *J* 9.4, 7.8Hz, H-2), methylene protons as two dd in an AB system at δ 3.20 (1H, dd, *J* 15.5, 7.8Hz, H-3a) and 3.26 (1H, dd, *J* 15.5, 9.4Hz, H-3b), two methyl groups at δ 1.33 (3H, s, H-2') and 1.29 (3H, s, H-3'). The signal at δ 93.0 and 71.5 in the ^{13}C NMR spectrum supported the presence of this substituent in the acridone nucleus.[23]

The presence of a linear orientation of the hydroxyisopropyldihydrofuran moiety was confirmed by correlations of the methylene protons at δ 3.20 (H-3) with C-3a (δ 109.4), C-2 (δ 93.0) and C-1' δ 71.5 and the correlations of the 4-OH at δ 14.31 with C-4a (δ 108.4), C-4 and C-3a in the HMBC spectrum. The spectrum also showed the correlation of the signals of the *N*-methyl protons at δ 3.85 with δ 143.5 and 138.0 assigned to C-11a and C-9a, respectively. The *O*-methyl proton at δ 3.89 showed correlation with C-11 at δ 126.6. The correlation at H-6, H-7 and H-8 in the HMBC spectrum allowed the assignments of the carbons C-9, C-9a, C-8, C-7, C-6, C-5a and C-5. Based on the above evidence, compound **2** could be defined as 2,3-dihydro-4,9-dihydroxy-2-(2-hydroxypropan-2-yl)-11-methoxy-10-methylfuro[3,2-*b*]acridin-5(10*H*)-one. Table 2 shows ^{1}H, ^{13}C NMR data for **2** and the correlations observed in the HMBC.

Table 2. ^{1}H and ^{13}C NMR and HMBC data for compound **2** (acetone-d_6)

Position	^{13}C^a δ	^{1}H^b δ (mult., *J* in Hz)	HMBC^b (H-C)
2	93.0	4.88 dd (9.4, 7.8)	C-2'/C-3', C-1',C-3'
2^a	161.0		
3	28.6	3.20 dd (15.5, 7.8, Ha) 3.26 dd (15.5, 9.4, Hb)	C-4, C-3a, C-2a, C-2
3^a	109.4		
4	155.2		
4-OH		14.31 s	C-4a, C-4, C-3a
4a	108.4		
5	182.6		
5a	125.4		
6	117.2	7.80 dd (7.8, 1.4)	C-8, C-9a, C-5
7	120.4	7.15 t (7.8)	C-9, C-5a
8	123.4	7.29 dd (7.8, 1.4)	C-9a, C-6
9	148.8		
9^a	138.0		
11	126.6		
11^a	143.5		
1'	71.5		
2'	26.1	1.33 s	C-2, C-1', C-3'
3'	27.5	1.29 s	C-2, C-1', C-2''
N-CH$_3$	46.9	3.85 s	C-11a, C-9a
O-CH$_3$	60.9	3.89 s	C-11

^{a} Recorded at 100 MHz; ^{b} Recorded at 400 MHz.

Compound **3** was isolated as an amorphous powder, the molecular formula was determined as $C_{20}H_{21}NO_6$ by HRESIMS, showing an [M + H]$^+$ peak at *m/z* 372.1447.

The UV absorption band at λ_{max} 265, 285, 332 and 410 nm, IR absorptions at ν_{max} 3509 and 1620 cm^{-1} and comparison with ^{1}H and ^{13}C NMR data characterized an acridone nucleus.[16-22] The lack of a characteristic signal of hydrogen-bonded hydroxy proton in the ^{1}H NMR spectrum, in acetone-d_6 and DMSO-d_6, and the shielded value of C-12 at δ 177.7 in the ^{13}C NMR spectrum suggested that there is no intramolecularly hydrogen-bonded hydroxyl group as observed for **1** and **2**. The ^{1}H NMR spectrum showed an ABX type aromatic spin system at δ 7.04 (1H, t, *J* 7.8Hz, H-10), 7.15 (1H, dd, *J* 7.8, 1.5Hz, H-9) and 7.64 (1H, dd, *J* 7.8, 1.5 Hz, H-11). The spectrum also showed one *N*-methyl group at δ 3.65 and one *O*-methyl group at δ 3.77. The presence of a 3-hydroxy-2,2-dimethyldihydropyran moiety was suggested by an oxymethine signal at δ 3.88 (1H, m, H-3), two methylene protons Ha at δ 3.00 (1H, dd, *J* 16.8, 5.7Hz, Ha-4), Hb 2.61 (1H, dd, *J* 16.8, 7.8Hz, Hb-4) and two methyl groups at δ 1.43 (3H, s, H-1') and 1.28 (3H, s, H-2'). The resonances at δ 69.1 and 78.1 in the ^{13}C NMR spectrum supported the presence of this substituent on the acridone nucleus.

In the HMBC spectrum of **3,** the methylene protons at δ 2.61 (H-4) showed correlations with C-4a (δ 103.9), C-5 (δ 153.3), C-3 (δ 69.1) and C-2 (δ 78.1), indicating that the substituent was located at an angular position. This spectrum also showed the correlation of the *N*-methyl protons at δ 3.65 with C-6a (δ 143.2) and C-7a (δ 137.1). The *O*-methyl protons at δ 3.77 showed correlation with C-6 at δ 130.4; this value is consistent with an acridone having an *O*-methyl group in this position.[17,23] Correlations of H-9, H-10 and H-11 allowed the assignments of the carbons C-8, C-8a, C-9, C-10, C-11, C-11a and C-12. Therefore, compound **3** was identified as 3,4-dihydro-3,5,8-trihydroxy-6-methoxy-2,2,7-trimethyl-2*H*-pyrano[2,3-*a*]acridin-12(7*H*)-one. Table 3 shows the data of ^{1}H, ^{13}C NMR and the correlation observed in the HMBC.

The eleven acridone alkaloids isolated from *S. glutinosa* were tested for *in vitro* activity against *P. falciparum*, *T. b. rhodesiense* and *L. donovani*. An assay with KB cells indicated *in vitro* cytotoxicity. The results are summarized in Table 4. To facilitate the discussion, IC$_{50}$ values were assigned as IC$_{50}^{T,P,K}$ against *T. b. rhodesiense*, *P. falciparum* and KB cells, respectively.

Nine out of the eleven acridone alkaloids showed IC$_{50}^P$ below 10 µM, four showed IC$_{50}^T$ below 10 µM and none displayed significant activity against *L. donovani*.

Related acridone alkaloids from *Thamnosma rodesica* (Bak. F.), showed activity against promastigote and amastigote forms of *Leishmania major*. These

Table 3. ^1H and ^{13}C NMR and HMBC data for compound **3** (acetone-d_6)

position	$^{13}C^a$ δ	$^1H^b$ δ (mult., J in Hz)	HMBC^b (H-C)
2^a	151,8*		
2	78.1		
3	69.1	3.88 m	
4	27.3	3.00 dd (16.8, 5.7, Ha) 2.61 dd (16.8, 7.8, Hb)	C-3, C-2, C-1
4^a	103.9		
5	153.3*		
6	130.4		
6^a	143.2		
8	149.0		
7^a	137.1		
9	118.8	7.15 dd (7.8, 1.5)	C-7a, C-11
10	123.1	7.04 t (7.8)	C-8, C-11a
11	117.8	7.64 dd (7.8, 1.5)	C-7a, C-9, C-12
11a	130.7		
12	177.7		
12a	110.9		
1'	20.5	1.43 s	C-3, C-2, C-2'
2'	26.0	1.28 s	C-3, C-2, C-1'
N-CH$_3$	44.9	3.65 s	C-4a, C-5a
O-CH$_3$	59.7	3.77 s	C-4

^{a} Recorded at 100 MHz; ^{b} Recorded at 400 MHz; * Assignments may be exchanged.

alkaloids have a methyl 2,3-dihydroxypropanoate chain at C-3 and C-4,[23] indicating that the substitution in the acridone skeleton is important for activity in this class of compounds.

According to the data, compound **5**, with one prenyl group at C-2, was the most active against *P. falciparum* with IC_{50}^P 0.3 µM. Comparison of **5**, **1** (IC_{50}^P 2.6 µM) and **4** (IC_{50}^P 2.6 µM) indicates that the second prenyl at C-8 was responsible for reducing the activity of this series. This fact was also observed by Weniger *et al.*,[10] who performed the assay with four alkaloids from *S. glutinosa* on a Nigerian chloroquine-sensitive strain of *P. falciparum*. Analysis of the results for compounds **2**, **3**, **9**, **10** and **11** suggests that the presence of a pyran ring is important for activity against *P. falciparum* and the position of this group, angular pyrano[2,3-*c*] (**9**) or linear pyrano[3,2-*b*] (**10**), did not alter the results of IC_{50}^P.

The activity against *P. falciparum* observed for **6**, **7** and **8**, IC_{50}^P 8.9, 29.9 and 6.1 µM, respectively, shows that presence of an *O*-methyl group at C-2 improves the activity.

From the 11 alkaloids tested against *T. b. rhodesiense*, compound **9** was the most active with an IC_{50}^T 1.0 µM.

Alkaloids **1**-**3** had their citotoxicity to cancer cells described in an earlier paper,[30] however here we disclose for the first time their isolation, spectral data and structure elucidation.

Table 4. *In vitro* activity against *P. falciparum* 3D7, *T. b. rhodesiense* STIB 900, *L. donovani* L82 and KB cells^a

Compounds	IC$_{50}$ in µM			
	P. falciparum	*T. b. rhodesiense*	*L. donovani*	KB cell
1	2.6 ± 0.4	9.6 ± 1.1	> 70	19.8 ± 3.7
2	3.0 ± 0.2	4.3 ± 0.9	> 80	> 100
3	11.6 ± 1.2	28.5 ± 2.9	> 80	80.7 ± 6.8
4	2.6 ± 0.6	14.4 ± 1.8	> 76	50.2 ± 10.7
5	0.3 ± 0.02	12.0 ± 0.8	> 70	14.2 ± 3.5
6	8.9 ± 0.6	7.8 ± 2.6	> 76	> 100
7	29.9 ± 2.1	13.6 ± 1.8	> 80	84.9 ± 12.8
8	6.1 ± 1.8	8.9 ± 0.9	> 99	> 89.9
9	0.6 ± 0.04	1.0 ± 0.1	> 56	11.6 ± 1.5
10	0.4 ± 0.03	17.0 ± 1.9	> 35	63.5 ± 7.3
11	5.7 ± 1.1	21.7 ± 5.3	> 46.4	49.4 ± 4.8
chloroquine diphosphate^b	0.003 ± 0.0002			
pentamidine^c		0.035 ± 0.0057		
pentostan^d			15 ± 3.4	
podophyllotoxin^e				0.0028 ± 0.00005

^{a} The mean IC$_{50}$ values of the test compounds and standard drug (n = 3, ±, σ, n = number of tests performed in three series). Drugs used as positive control: ^{b}*Plasmodium falciparum*; ^{c}*T. brucei rhodesiense*; ^{d}*Leishmania donovani* and ^{e}toxicity.

Experimental

General

Optical rotations were measured using a Perkin Elmer polarimeter. IR spectra were recorded on a Bomem M-B Series spectrophotometer. UV absorptions were recorded using a Varian 500 SCAN UV-Vis-NIR spectrophotometer. ^1H and ^{13}C NMR data were recorded on Bruker ARX-200 and Bruker DRX-400 spectrometers. Spectra were recorded in acetone-d_6 and DMSO-d_6 with TMS as internal standard. All 2D NMR data were recorded at 400MHz (Bruker DRX-400), HSQC *J* 145 Hz; HMBC *J* 8 Hz. HR-MS data were recorded on a Micromass Q-Tof (QqTOF) spectrometer; column chromatography was on silica gel 60 (Merck) and Sephadex LH-20 (Pharmacia). Preparative HPLC was performed on a Shodex Asahipak GS-310 2G column. TLC was carried out using Merck aluminum-backed silica gel 60 F$_{254}$.

Plant material

Leaves, stem and root bark were collected in Campinas (SP) at the Instituto Agronômico de Campinas, and dried in the shade. The plant was identified by Prof. Dr. Maria Inês Salgado. A voucher specimen is deposited at the Herbarium of the Departamento de Botânica of the Universidade Federal de São Carlos (HUFSCar) as number 7110.

Extraction and isolation

The parts of the plant were extracted separately with n-hexane at room temperature for 3 days, filtered and evaporated under reduced pressure at 40 ºC. This procedure was repeated 3 times to yield the crude hexane extract. The residue was extracted, as above, using MeOH to yield the crude MeOH extract. The crude MeOH extract (60 g) was fractioned by VCC over 1 kg of silica gel 60 (70-239 mesh, Merck) eluted with 1.5 L of the solvents: 100% hexane (fraction 1), 100% CH_2Cl_2 (fraction 2), 3:1 CH_2Cl_2:MeOH (fraction 3); 2:1 CH_2Cl_2:MeOH (fraction 4); 1:1 CH_2Cl_2:MeOH (fraction 5) and 100% MeOH (fraction 5). Fractions 2 and 3 were combined and chromatographed on silica gel using CH_2Cl_2 as mobile phase, the polarity was increased by addition of 5%, 10%, 13%, 18%, 20% and 25% of MeOH in a gradient system to yield 15 fractions. Lupeol was crystallized from fractions 1 to 3. Fraction 5 was rechromatographed on Si gel, being eluted with 1:3 n-hexane-acetone followed by Sephadex LH-20 with MeOH to give alkaloids **1** (16.3 mg), **4** (30.4 mg),

5 (3.5 mg), **8** (11.2 mg), **9** (13.4 mg), **10** (9.8 mg) and **11** (5.4 mg). Further purification of fraction 9 on Sephadex LH-20 with MeOH and HPLC using MeOH as mobile phase, UV detection at 254 and 365nm, and a flow rate of 3.0 mL min^{-1} afforded compounds **6** (13.5 mg), **7** (11.2 mg) and **8** (7.3 mg). Fraction 10 was dissolved in MeOH and submitted to Sephadex LH-20, being eluted with MeOH to give compounds **2** (5.2 mg) and **3** (4.3 mg).

1,3,5-Trihydroxy-4-methoxy-10-methyl-2,8-bis(3-methylbut-2-enyl)acridin-9(10H)-one, (1)

Amorphous powder, IR (liquid film) ν_{max}/cm^{-1}: 3500, 2964, 1625, 1566. UV (MeOH) λ_{max}/nm (log ε) 265 (3.47), 285 (3.08), 332 (3.02) and 410 (2.57). ^1H, ^{13}C NMR and HMBC correlations see Table 1. HRESIMS m/z 424.2124 [M + H]$^+$ (calc. for $C_{25}H_{30}NO_5$, 424.2124).

2,3-Dihydro-4,9-dihydroxy-2-(2-hydroxypropan-2-yl)-11-methoxy-10-methylfuro[3,2-b]acridin-5(10H)-one, (2)

Optically inactive, amorphous powder, IR (liquid film) ν_{max}/cm^{-1}: 3475, 2964, 1630. UV (MeOH) λ_{max}/nm (log ε): 259 (4.21), 274 (4.62), 284 (4.65). ^1H, ^{13}C NMR and HMBC correlations see Table 2. *m/z* 372.1447 [M + H]$^+$ (calc. for $C_{20}H_{22}NO_6$, 372.1447).

3,4-Dihydro-3,5,8-trihydroxy-6-methoxy-2,2,7-trimethyl-2H-pyrano[2,3-a]acridin-12 (7H)-one, (3)

$[\alpha]_D^{25}$ −21.4 (*c*, 0.0022 MeOH), amorphous powder, IR (liquid film) ν_{max}/cm^{-1}: 3509, 2978, 1620, 1572. UV (MeOH) λ_{max}/nm (log ε): 264 (3.39), 285 (3.13), 332 (2.99). ^1H, ^{13}C NMR and HMBC correlations see Table 3. HRESIMS *m/z* 372.1447 [M + H]$^+$ (calc. for $C_{20}H_{22}NO_6$, 372.1447).

Biological assays

Stock solutions of the compounds, plus control drugs, were prepared at a concentration of 20 mg mL^{-1} in DMSO (Sigma, UK), and diluted to appropriate concentrations prior to assays. IC$_{50}$ values were calculated with MSXLFIT (IDBS, UK).

P. falciparum

Chloroquine-sensitive *P. falciparum* strain 3D7 was maintained in human A$^+$ erythocytes in RPMI 1640 medium (Sigma, UK) supplemented with Albumax II at 37 ºC in a 5% CO_2-air mixture. Asynchronous (65-75% ring stage) of *P. falciparum* intraerythrocytic cultures were set up as above, with 1% parasitemia, 2.5% hematocrit, in triplicate in 100 μL of medium in 96 well, flat-bottomed Microtest III tissue plates. Drugs were added in a threefold dilution

series and cultures incubated for a total of 48 h at 37 °C in a 5% CO_2-air mixture. After 24 h, {^{3}H} hypoxanthine (0.2 mCi) was added to each well.[25,26] At the end of the assay, plates were rapidly freeze-thawed, harvested using a Tomtec Mach III cell harvester (Tomtec, CT) onto a 96-well format filtermat and Meltilex™ solid scintillant (both Wallac, Finland) added prior to reading in a Microbeta 1450 scintillation counter (Wallac, Finland) at 1 min *per* well.

T. brucei rhodesiense

T. b. rhodesiense STIB900 bloodstream form trypomastigotes were maintained in HMI-18 medium,[27] with 15% heat-inactivated fetal calf serum (Harlan Sera Lab, UK) at 37 °C in a 5% CO_2-air mixture. Prior to drugging, trypomastigotes were washed and resuspended in fresh medium at a concentration 2×10^5 trypanosoma/mL and 100 µL of this suspension was added to the drug dilutions. The top concentration for the test compounds was 30 mg mL^{-1}. Pentamidine was included as the standard drug. Plates were incubated for 72 h at 37 °C in a 5% CO_2-air mixture.[28] At 72 h AlamarBlue was added to the plates. Plates were read after 4-5 h on a Gemini fluorescent plate reader (Sofimax Pro. 3.1.1, Molecular Devices, UK) at EX/EM 530/585 nm with a filter cut-off at 550 nm.

L. donovani

L. donovani L82 amastigotes were harvested from an infected hamster (*Mesocricetus auratus*) spleen and used to infect murine peritoneal exudate macrophages (PEM) at a ratio of 7:1. In brief, infected cells were exposed to drug for a total of 5 days.[29] The percentage of infected cells was evaluated microscopically and the percentage inhibition in comparison with untreated controls was calculated.

Cytotoxicity assays

96-well plates were seeded with KB cells at 4×10^4 mL^{-1} (100 µL *per* well). Drugs at 300, 30, 3 and 0.3 µg mL^{-1} were added in fresh overlay after 24 h, in triplicate at each concentration. Plates were incubated for 72 h at 37 °C in a 5% CO_2-air mixture. At 72 h AlamarBlue was added to the plates. Plates were read after 4-5 h on a Gemini fluorescent plate reader (Sofimax Pro. 3.1.1, Molecular Devices, UK) at EX/EM 530/585 nm with a filter cut-off at 550 nm. IC_{50} values were calculated against the blanks and control samples.

Acknowledgments

Supplementary Information

Available free of charge at http://jbcs.org.br, as PDF file.

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Supplementary Information

Antiparasitic Activities of Acridone Alkaloids from *Swinglea glutinosa* (Bl.) Merr.

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Figure S1. ^{1}H NMR spectrum of compound **1** (CDCl$_3$, 400 MHz).

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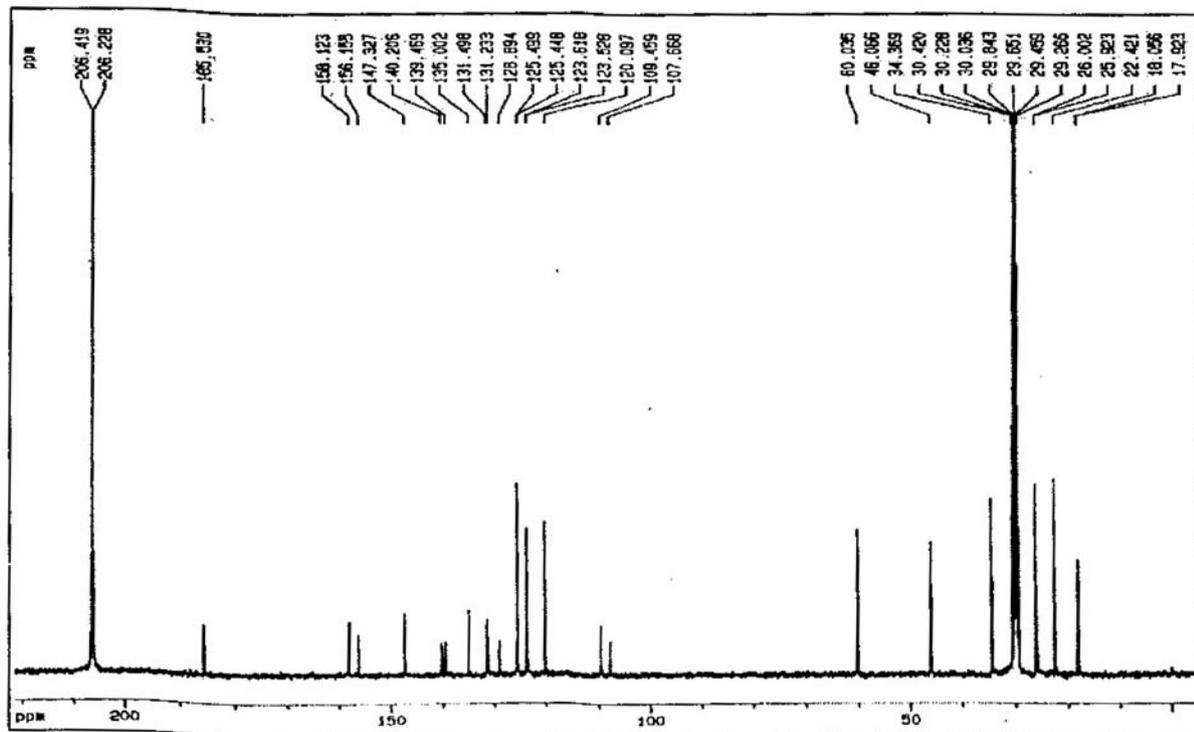
Figure S2. ^{13}C NMR spectrum of compound **1** (CDCl$_3$, 100 MHz).
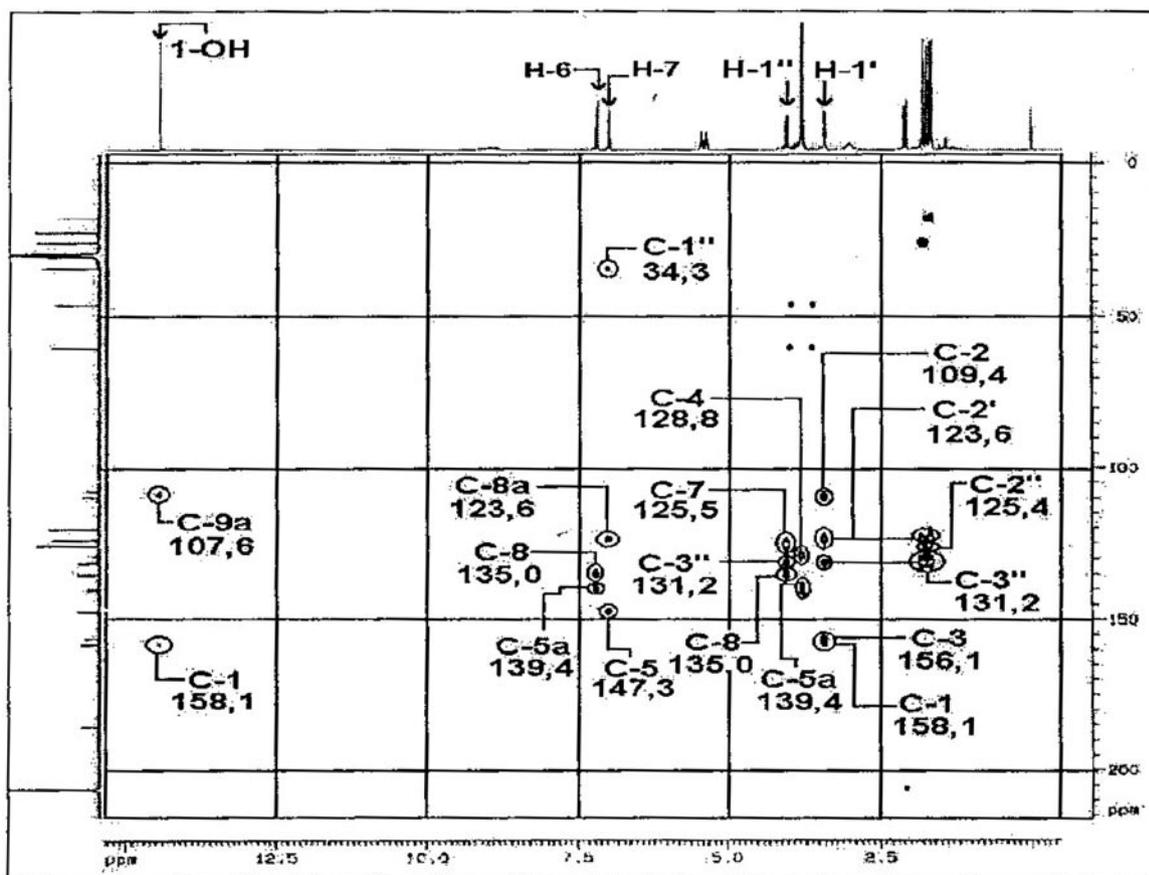
Figure S3. HMBC spectrum of compound d **1** (CDCl$_3$).
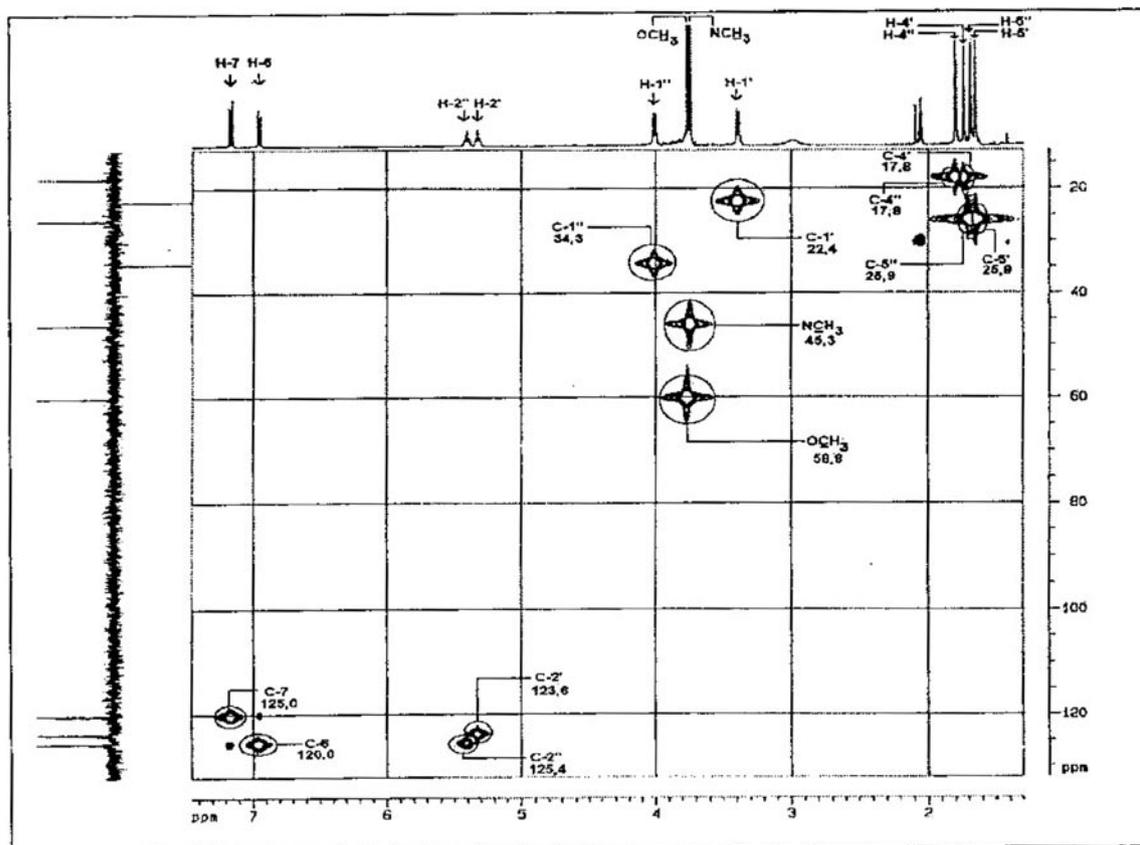
Figure S4. HMQC spectrum of compound **1** (CDCl$_3$).
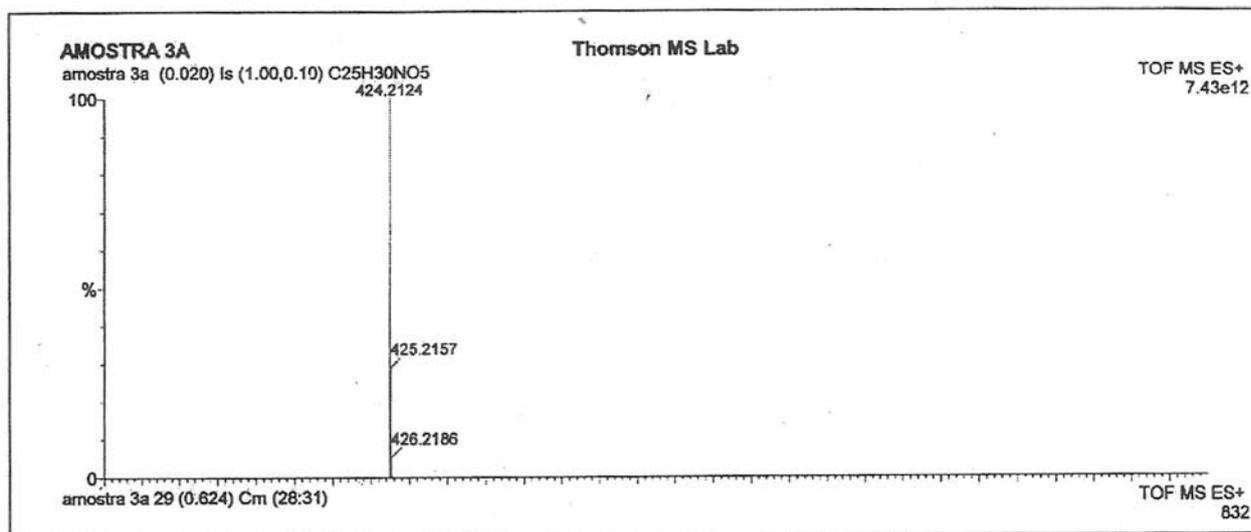
Figure S5. High resolution mass spectrum of compound **1**.
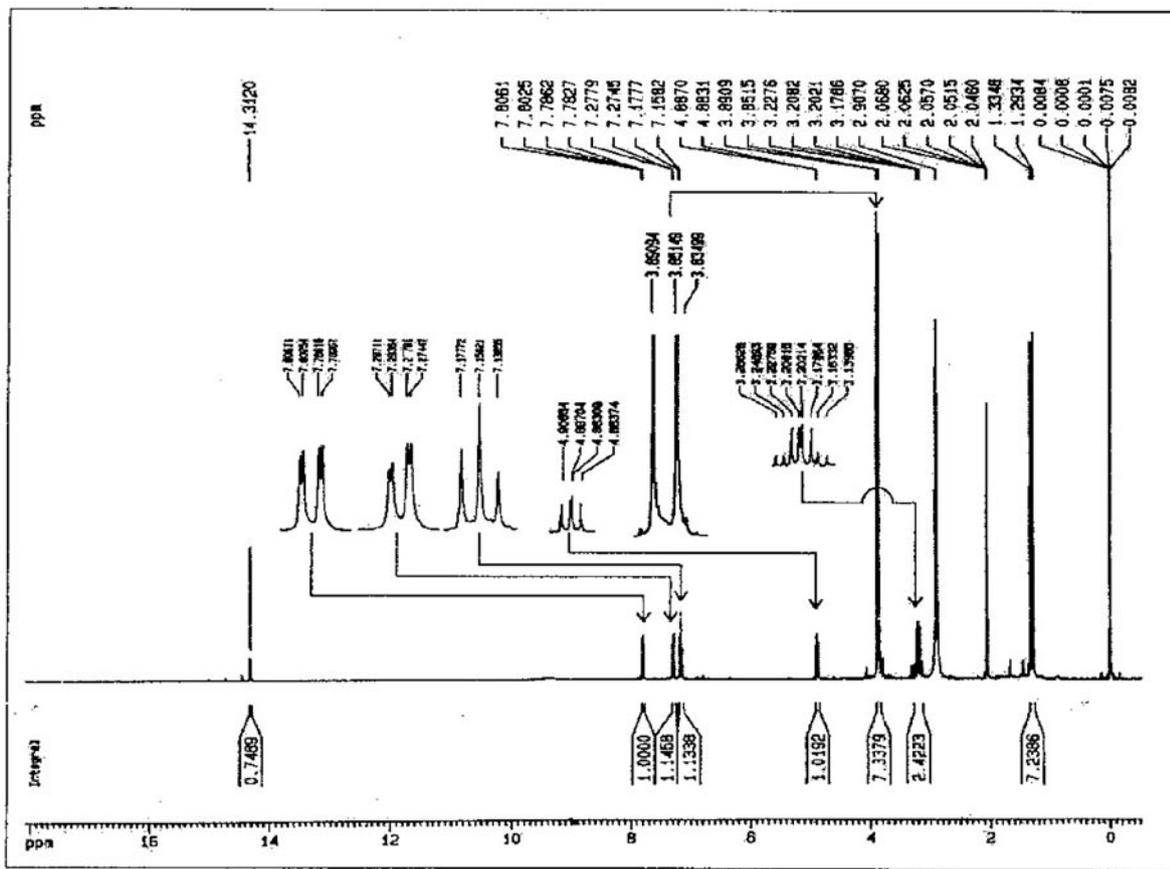
Figure S6. ¹H NMR of compound **2** (CDCl₃, 400 MHz).
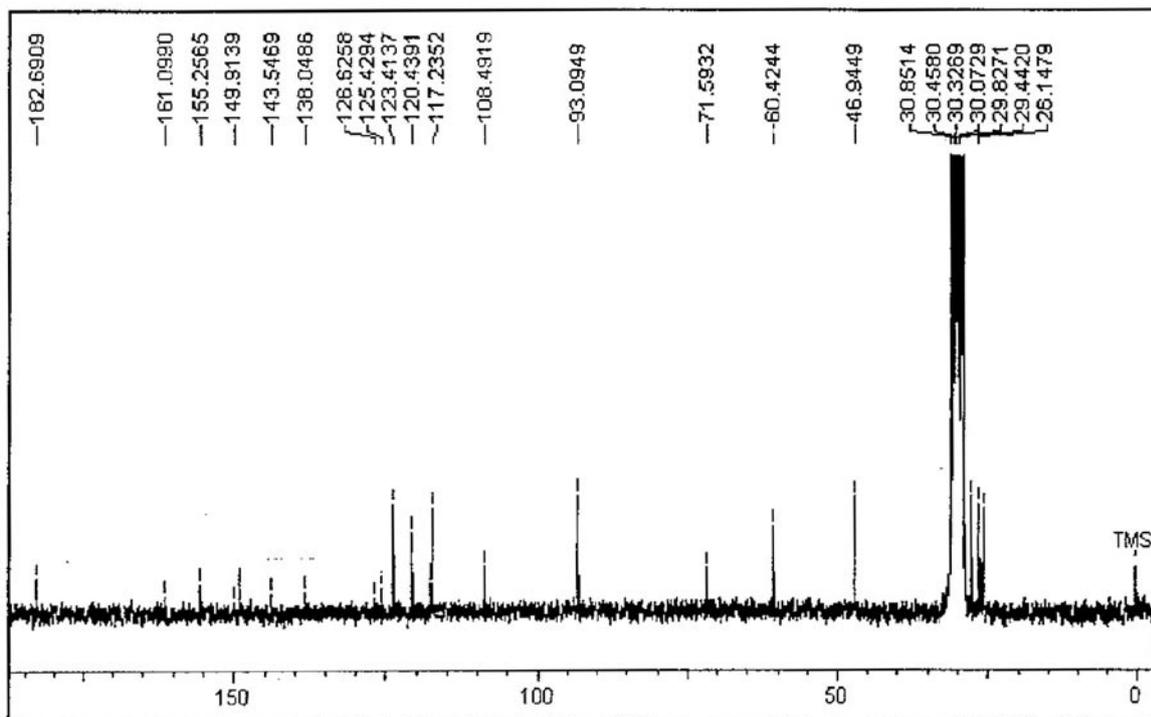
Figure S7. ¹³C NMR of compound **2** (CDCl₃, 100 MHz).
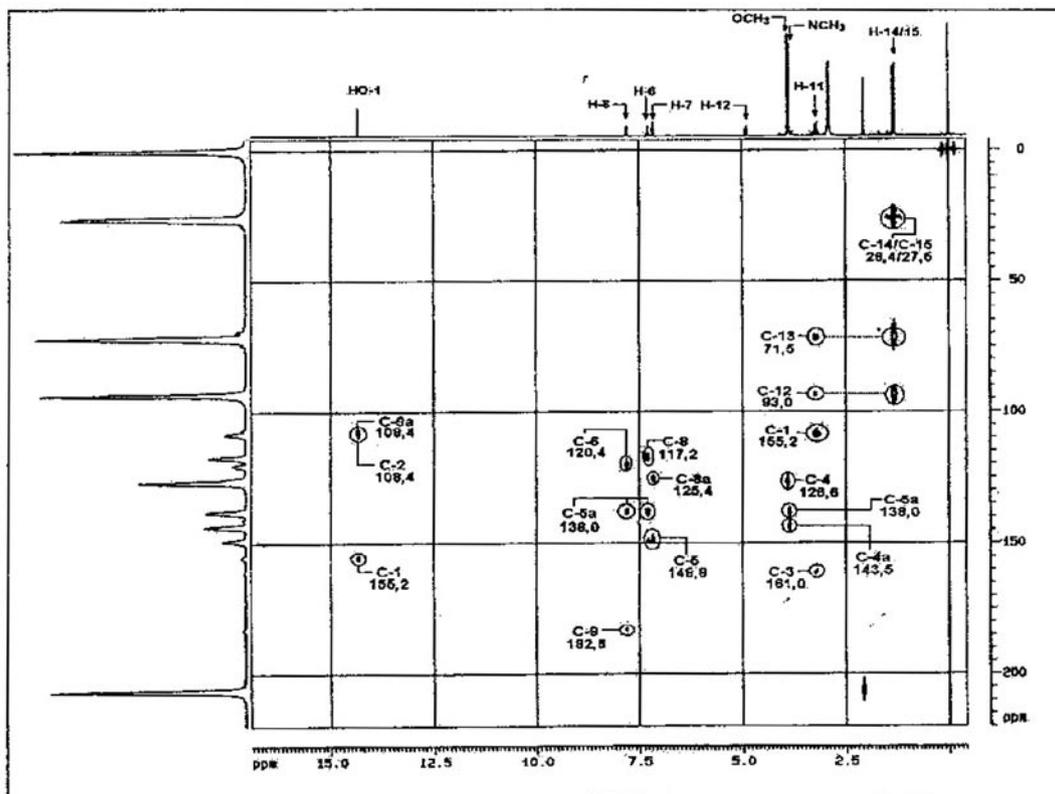
Figure S8. HMBC spectrum of compound **2** (CDCl$_3$).
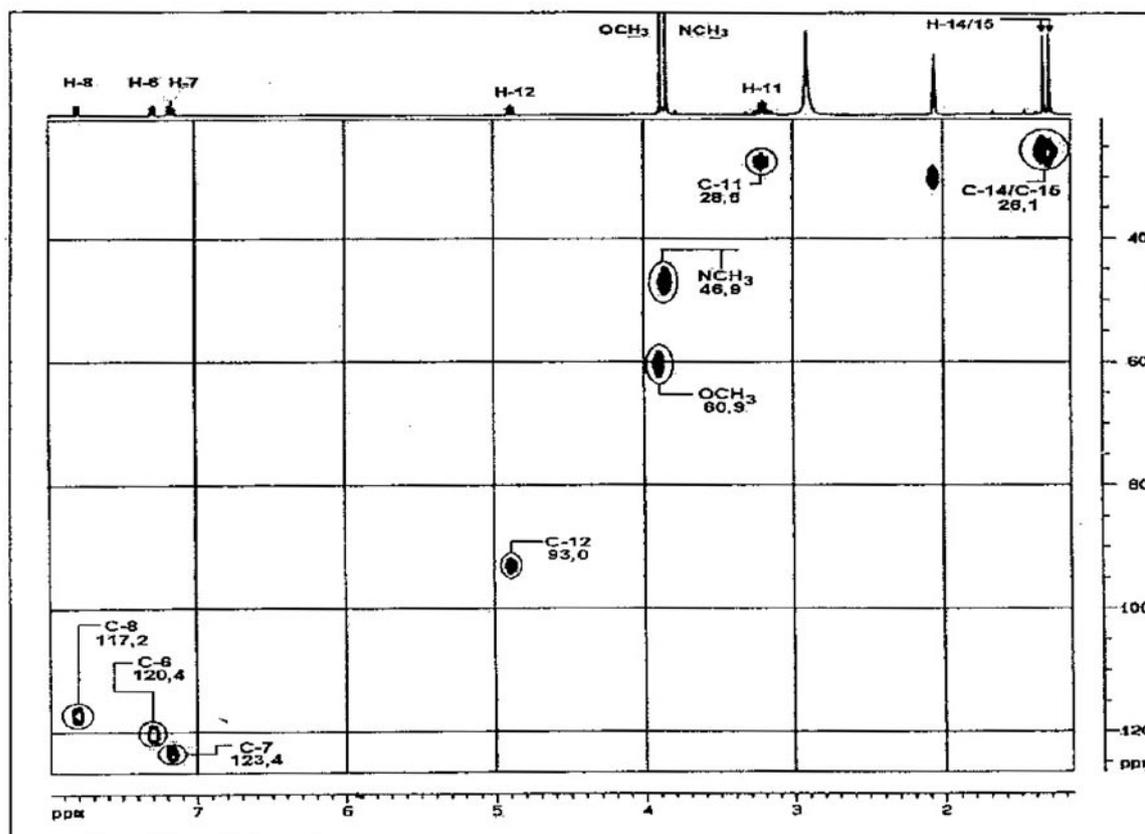
Figure S9. HMQC spectrum of compound **2** (CDCl$_3$).
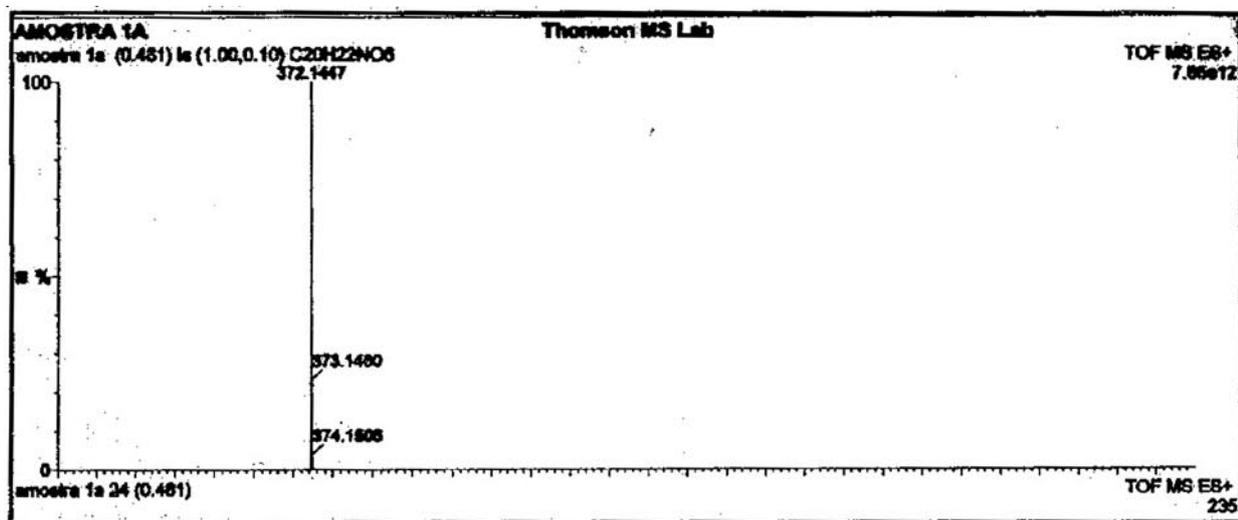
Figure S10. High resolution mass spectrum of compound **2**.
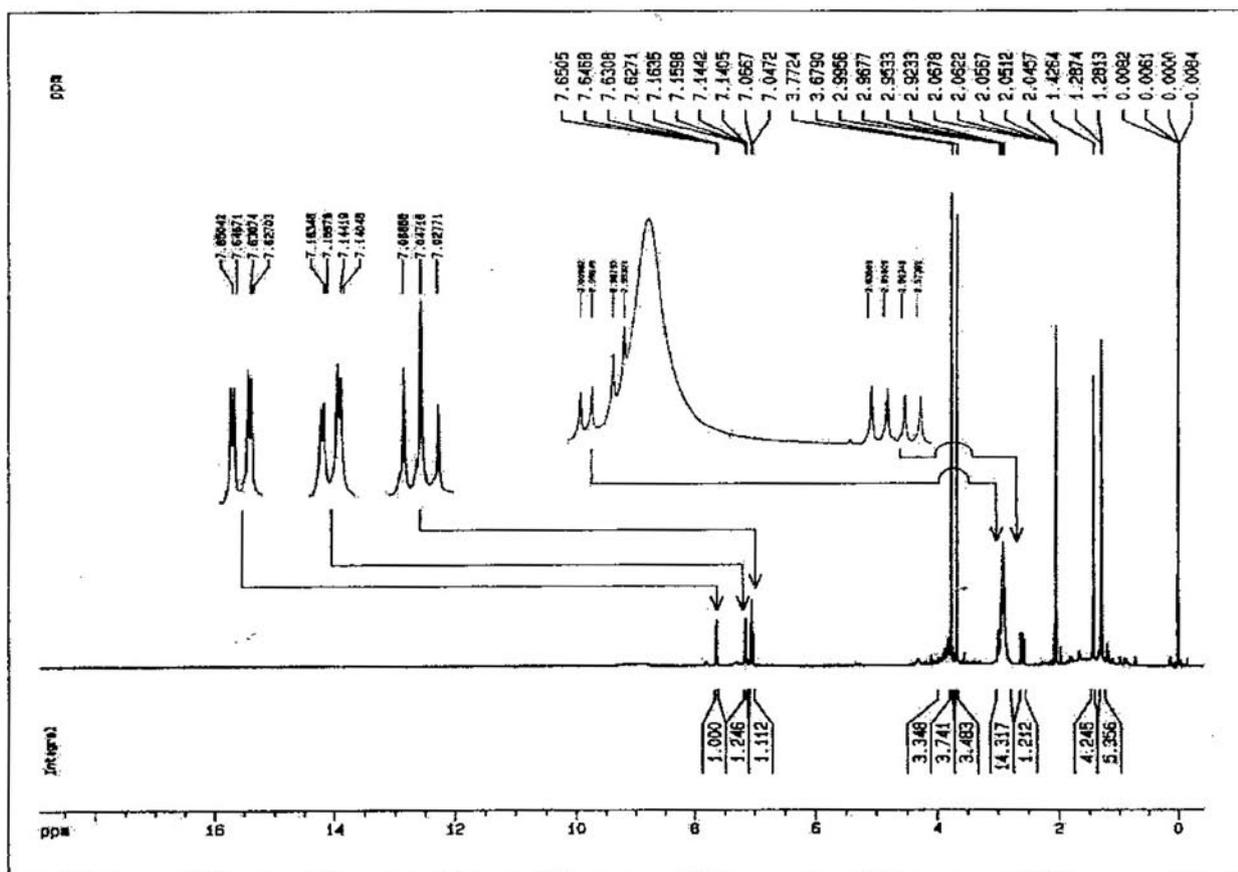
Figure S11. ¹H NMR of compound **3** (CDCl₃, 400 MHz).
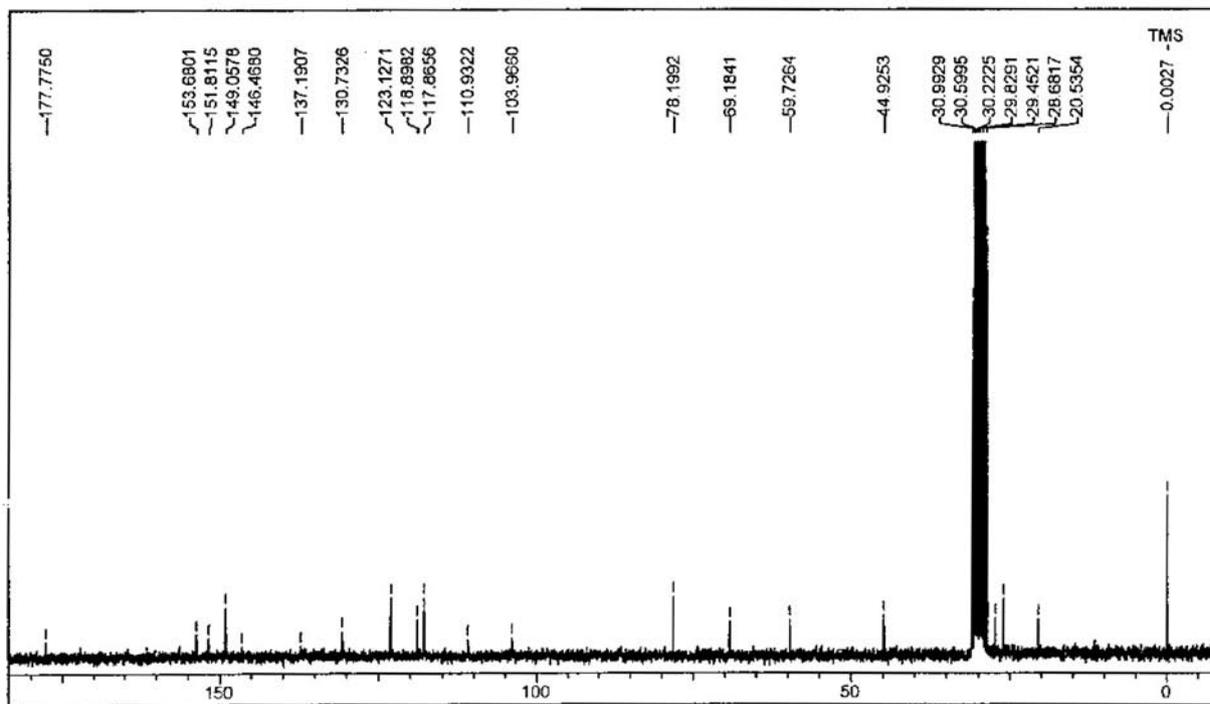
Figure S12. ^{13}C NMR of compound **3** (CDCl$_3$, 100 MHz).
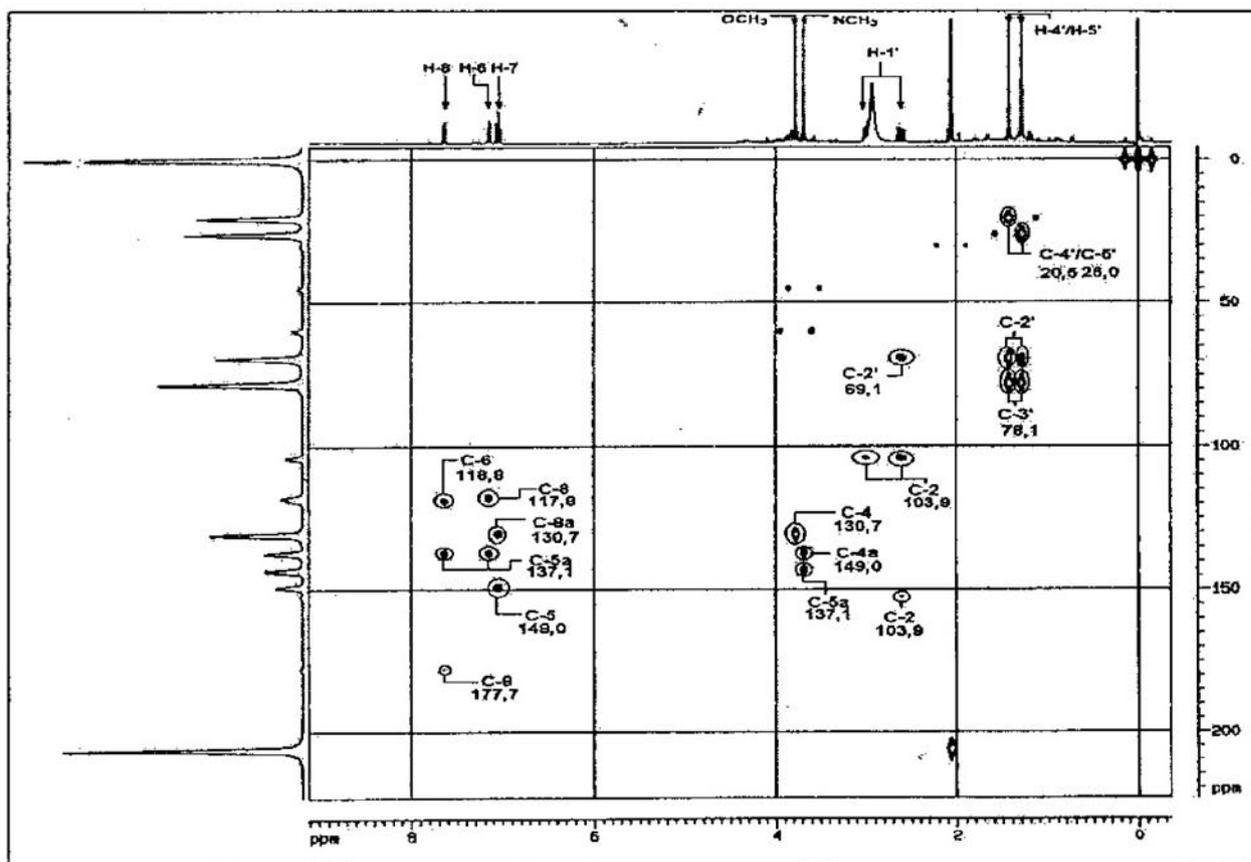
Figure S13. HMBC spectrum of compound **3** (CDCl$_3$).
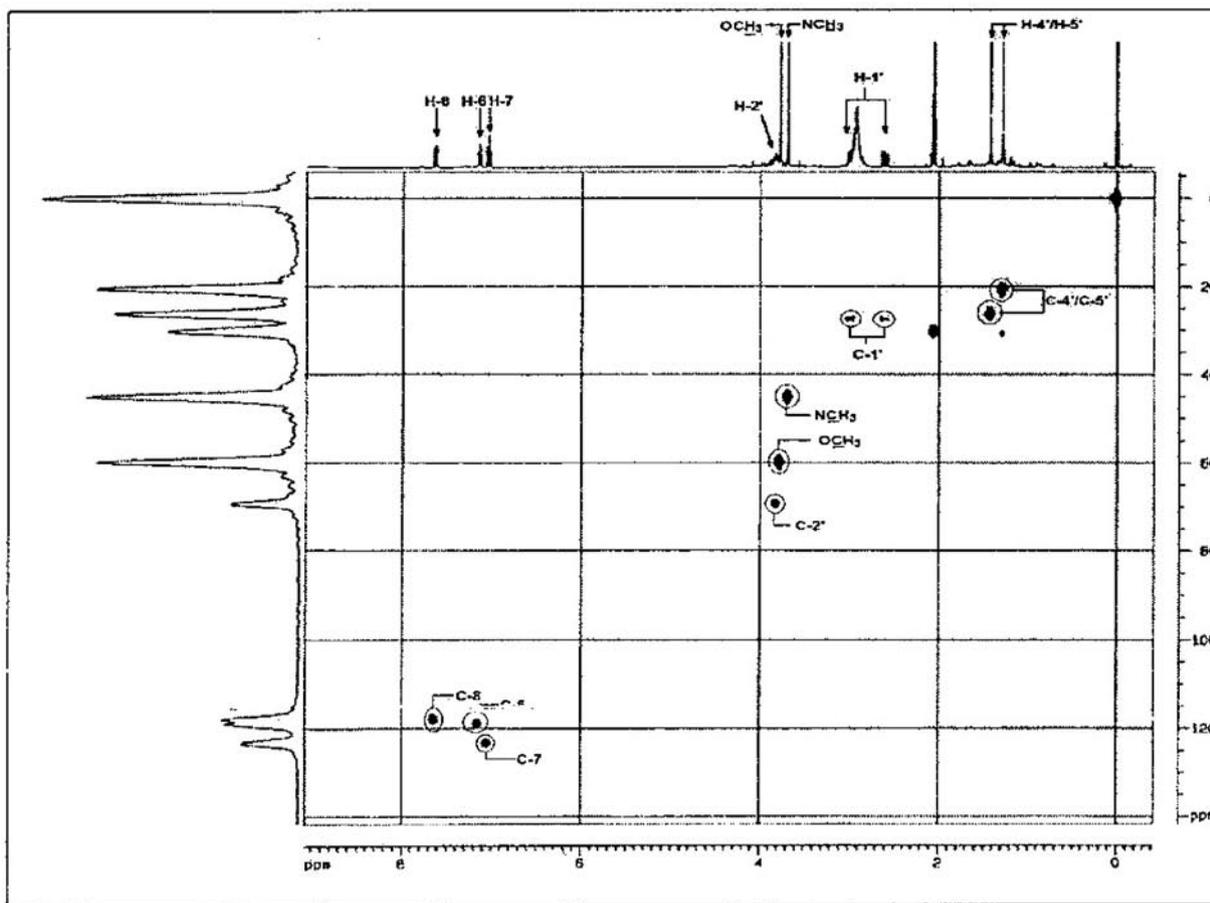
Figure S14. HMQC spectrum of compound **3** (CDCl$_3$).
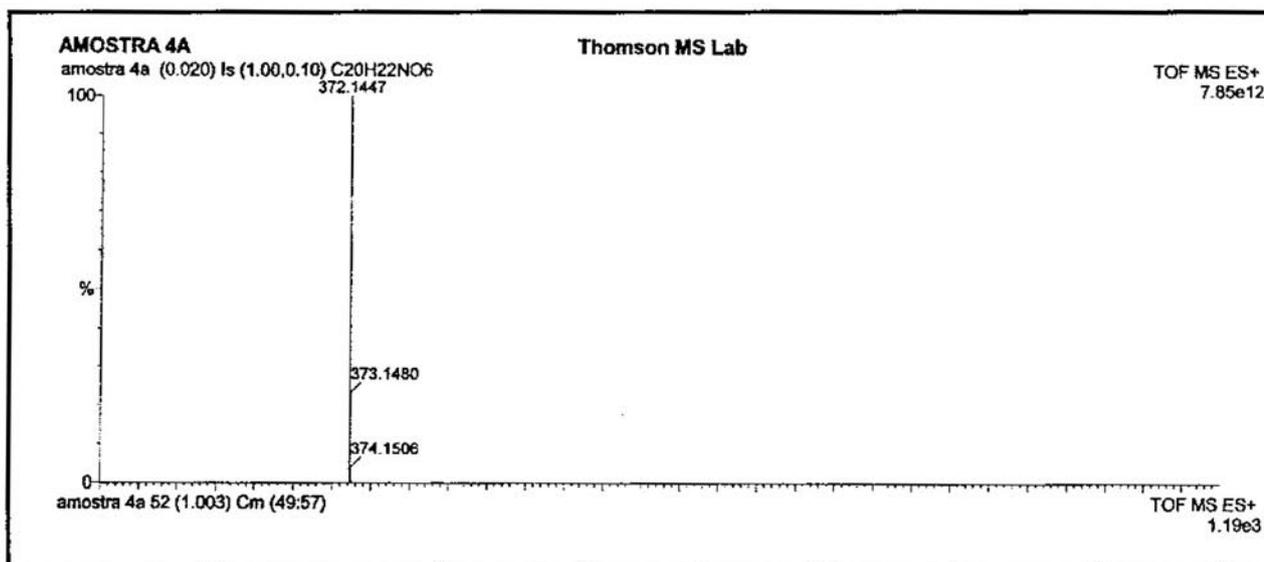
Figure S15. High resolution mass spectrum of compound **3**.