Supporting Information

for

Synthesis and Evaluation of Artesunate-Indoloquinoline Hybrids as Antimalarial Drug Candidates

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Experimental Section

Chemistry

Materials and Methods. Column chromatographies were achieved on a silica gel column (230–400 mesh) using a gradient solvent system (hexane/ethyl acetate as the eluent unless otherwise specified). Melting points were determined on a J-Science RFS-10 hot stage microscope. The ^{1}H NMR and ^{13}C NMR spectra were measured on the Varian INOVA-600 spectrometer. Chemical shifts (δ ppm) were determined using

tetramethylsilane (TMS) as the internal reference. High resolution mass spectra were obtained on a Bruker micrOTOF II-SKA spectrometer.

General Procedure for the Synthesis of Artesunate-Indoloquinoline Hybrids 5–7.

Artesunate **4** (102 mg), EDTI (39 mg) and HOBt (27.6 mg) dissolve in CH_2Cl_2 were stirred for 1 h, then add N^1-(11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamine (**2**). The mixture was stirred at room temperature for 6 h. The mixture was monitored by TLC to ensure the progress of the reaction. The reaction mixture was diluted with CH_2Cl_2, washed with brine, dried over anhydrous $MgSO_4$. After concentrated in vacuo, the crude products were purified by flash chromatography using AcOEt-MeOH (1:10 V/V) as the eluent to yield pure **6** as solids.

Artesunate-[N^1-(5-methyl-5H-indolo[2,3-b]quinolin-11-yl)propane-1,3-diamine] hybride (**5a**):

Yield: 55%. ^{1}H NMR ($CDCl_3$) δ 8.37 (d, *J* = 8.3 Hz, 1H), 7.91 (d, *J* = 7.7 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.67 (d, *J* = 7.3 Hz, 1H), 7.61 (d, *J* = 8.6 Hz, 1H), 7.37 (t, *J* = 7.6 Hz, 2H), 7.15 (t, *J* = 7.5 Hz, 1H), 6.51 (dd, *J* = 14.4, 8.0 Hz, 2H), 5.68 (d, *J* = 9.8 Hz, 1H), 5.24 (s, 1H), 4.20 (s, 3H), 3.94–3.87 (m, 1H), 3.85–3.78 (m, 1H), 3.57–3.50 (m, 1H), 3.28 (dd, *J* = 14.1, 5.7 Hz, 1H), 2.82 (dd, *J* = 9.0, 5.5 Hz, 1H), 2.74–2.69 (m, 1H), 2.55 (dd, *J* = 9.2, 5.3 Hz, 1H), 2.51–2.40 (m, 2H), 2.27 (dd, *J* = 13.9, 3.8 Hz, 1H), 1.96–1.91 (m, 1H), 1.82–1.78 (m, 1H), 1.74–1.67 (m, 2H), 1.48 (ddd, *J* = 14.7, 9.9, 3.7 Hz, 3H), 1.37–1.27 (m, 4H), 1.15 (dd, *J* = 11.4, 6.5 Hz, 1H), 1.11–0.99 (m, 2H), 0.86 (dd, *J* = 15.8, 10.0 Hz, 4H), 0.69 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.8, 171.9, 156.9, 152.2, 148.4, 137.7, 130.4, 125.5, 124.0, 123.8, 121.9, 121.0, 118.7, 116.9, 116.5, 114.5, 106.6, 104.4, 92.3, 91.4, 80.1, 51.4, 45.0, 43.67, 37.1, 36.1, 36.0, 33.9, 32.8, 31.7, 31.3, 30.8, 29.9, 25.9, 24.4, 21.7, 20.1, 11.9. HRMS (ESI) calcd for $C_{38}H_{47}N_4O_7$ $[M+H]^+$ Exact Mass: 671.3445, found 671.3442.

Artesunate-[N^1-(2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-yl)-2,2-dimethylpropane-1,3-diamine] hybride (**5c**):

Yield: 47%. ^{1}H NMR ($CDCl_3$) δ 8.59 (d, *J* = 2.0 Hz, 1H), 7.93 (s, 1H), 7.88 (s, 1H), 7.81 (s, 1H), 7.73 (dd, *J* = 6.9, 2.3 Hz, 2H), 7.38–7.35 (m, 1H), 7.22 (ddd, *J* = 7.9, 6.8, 1.1 Hz, 1H), 7.06 (s, 1H), 5.63 (d, *J* = 9.9 Hz, 1H), 4.90 (s, 1H), 4.40 (s, 3H), 3.73–3.66 (m, 2H), 3.41–3.35 (m, 1H), 3.15–3.08 (m, 1H), 2.89 (ddd, *J* = 11.9, 7.6, 5.0 Hz, 2H), 2.72 (ddd, *J* = 13.5, 8.7, 4.8 Hz, 1H), 2.66–2.60 (m, 1H), 2.38 (ddd, *J* = 9.9, 7.1, 4.6 Hz, 1H), 2.23 (td, *J* = 14.3, 3.9 Hz, 1H), 1.92–1.85 (m, 1H), 1.74–1.67 (m, 1H), 1.39–1.33 (m, 1H), 1.31–1.27 (m, 1H), 1.23–1.13 (m, 5H), 1.05 (dd, *J* = 10.5, 4.1 Hz, 1H), 0.78 (s, 4H), 0.71 (s, 5H), 0.62 (s, 3H), 0.58 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 174.0, 172.2, 145.0, 143.6, 135.5, 131.7, 128.2, 126.1, 124.3, 123.5, 123.4, 122.6, 118.6, 118.1, 116.9, 114.8, 111.0, 104.2, 92.3, 91.3, 79.9, 54.3, 51.2, 46.5, 44.8, 38.8, 36.8, 36.0, 35.2, 33.8, 31.5, 30.7, 29.7, 25.7, 24.4, 24.0, 23.9, 21.5, 19.9, 11.7. HRMS (ESI) calcd for $C_{40}H_{50}ClN_4O_7$ $[M+H]^+$ Exact Mass: 733.3368, found 733.3364.

Artesunate-[methyl 11-(3-aminopropylamino)-5-methyl-5H-indolo[2,3-b]quinoline-2-carboxylate] hybride (**5e**):

Yield: 43%. ^{1}H NMR ($CDCl_3$) δ 9.09 (d, *J* = 1.7 Hz, 1H), 8.30 (dd, *J* = 8.9, 1.8 Hz, 1H), 8.01 (d, *J* = 7.7 Hz, 1H), 7.73 (d, *J* = 7.9 Hz, 1H), 7.65 (d, *J* = 9.0 Hz, 1H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.21 (t, *J* = 7.2 Hz, 1H), 6.66 (s, 1H), 6.35 (s, 1H), 5.70 (d, *J* = 9.9 Hz, 1H), 5.27 (s, 1H), 4.26 (s, 3H), 4.00 (d, *J* = 3.2 Hz, 3H), 3.94 (d, *J* = 6.5 Hz, 1H), 3.88 (d, *J* = 7.0 Hz, 1H), 3.61 (d, *J* = 7.2 Hz, 1H), 3.41 (dd, *J* = 14.2, 5.8 Hz, 1H), 2.90–2.84 (m, 1H), 2.79–2.73 (m, 1H), 2.58 (dd, *J* = 8.9, 5.4 Hz, 1H), 2.54–2.50 (m, 1H), 2.50–2.45 (m, 1H), 2.35–2.28 (m, 1H), 1.99–1.94 (m, 1H), 1.90 (dd, *J* = 10.8, 5.7 Hz, 2H), 1.82 (s, 1H), 1.61–1.55 (m, 2H), 1.52 (dd, *J* = 13.6, 4.4 Hz, 1H), 1.35 (m, 4H), 1.22–1.11 (m, 3H), 0.91 (m 4H), 0.73 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.8, 171.97, 166.7, 166.3, 140.6, 130.8, 127.4, 126.0(2C), 123.7, 122.5, 121.7(2C), 119.9, 117.3, 116.7, 115.8, 114.7, 104.5, 92.4, 91.5, 80.2, 52.6, 51.5, 45.2, 44.6, 37.3, 36.3, 36.2, 34.1, 33.4, 31.8, 31.6, 31.1, 30.1, 26.0, 24.6, 21.9, 20.3, 12.0. HRMS (ESI) calcd for $C_{40}H_{49}N_4O_9$ $[M+H]^+$ Exact Mass: 729.3500, found 729.3502.

Artesunate-[methyl 11-(3-amino-2,2-dimethylpropylamino)-5-methyl-5H-indolo[2,3-b]quinoline-2-carboxylate] hybride (**5f**):

Yield: 51%. ^{1}H NMR ($CDCl_3$) δ 9.27 (d, *J* = 1.7 Hz, 1H), 8.32–8.28 (m, 1H), 8.02 (d, *J* = 7.7 Hz, 1H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.68–7.63 (m, 1H), 7.43–7.39 (m, 1H), 7.24–7.21 (m, 1H), 7.15 (s, 1H), 6.31–6.26 (m, 1H), 5.65 (d, *J* = 9.9 Hz, 1H), 5.09 (s, 1H), 4.26 (d, *J* = 5.1 Hz, 3H), 3.99 (s, 3H), 3.74 (ddd, *J* = 21.1, 13.5, 6.1 Hz, 2H), 3.60 (dd, *J* = 14.4, 8.0 Hz, 1H), 3.03 (dd, *J* = 14.4, 5.7 Hz, 1H), 2.97 (ddd, *J* = 17.7, 9.7, 4.8 Hz, 1H), 2.79 (ddd, *J* = 17.7, 6.2, 4.7 Hz, 1H), 2.67 (ddd, *J* = 14.5, 9.7, 4.6 Hz, 1H), 2.61–2.55 (m, 1H), 2.39 (ddd, *J* = 9.9, 7.1, 4.6 Hz, 1H), 2.28 (td, *J* = 14.3, 3.9 Hz, 1H), 1.97–1.91 (m, 1H), 1.81–1.76 (m, 1H), 1.39–1.32 (m, 3H), 1.30 (s, 3H), 1.22–1.18 (m, 1H), 1.10 (td, *J* = 11.3, 6.7 Hz, 1H), 0.93–0.88 (m, 1H), 0.86 (d, *J* = 6.2 Hz, 3H), 0.82 (s, 3H), 0.74 (t, *J* = 10.9 Hz, 2H), 0.66 (s, 3H), 0.58 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.2, 172.1, 166.5, 164.8, 148.5, 140.4, 131.8, 130.5, 126.6, 125.3, 124.1, 122.4(2C) 122.3, 119.3, 117.2, 116.1, 114.3, 104.2, 92.3, 91.3, 80.0, 60.3, 54.2, 52.3, 51.3, 46.6, 44.8, 38.6, 36.8, 36.1, 33.8, 31.5, 30.9, 29.7, 25.8, 24.4, 24.0, 23.7, 21.4, 20.0, 11.6. HRMS (ESI) calcd for $C_{42}H_{53}N_4O_9$ $[M+H]^+$ Exact Mass: 757.3813, found 757.3809.

Biological Testing Assay

Activity against *Plasmodium falciparum*

In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay,[1,2] using the chloroquine and pyrimethamine resistant *P. falciparum* K1 strain that originate from Thailand (Thaitong et al. 1983)[3] and strain susceptible to known antimalarial drugs (*P. falciparum* NF54) (Ponnudurai et al. 1981),[4] and all the test compounds were compared for activity with the standard drug chloroquine (Sigma C6628). Compounds were dissolved in DMSO at 10 mg/mL and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/l), $NaHCO_3$ (2.1 g/l), neomycin (100 U/mL),

Albumax^{R} (5 g/l) and washed human red cells A^{+} at 2.5% haematocrit (0.3% parasitaemia). Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. The 96-well plates were incubated in a humidified atmosphere at 37 °C; 4% CO_2, 3% O_2, 93% N_2. After 48 h 50 μl of ^{3}H-hypoxanthine (=0.5 μCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate™ cell harvester (Wallac, Zurich, Switzerland), and the red blood cells transferred onto a glass fibre filter then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid, and counted in a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves by linear regression (Huber 1993)[5] using Microsoft Excel.

In vitro cytotoxicity against L6 cells

Assays were performed in 96-well microtiter plates, each well containing 100 μl of RPMI 1640 medium supplemented with 1% L-glutamine (200mM) and 10% fetal bovine serum, and 4000 L6 cells (a primary cell line derived from rat skeletal myoblasts) (Page et al., 1993 and Ahmed et al., 1994).[6,7] Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. After 70hours of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10 μl of Alamar Blue was then added to each well and the plates incubated for another 2 hours. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. The IC50 values were calculated by linear regression (Huber 1993) from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA).

Detergent mediated assay for *β*-haematin inhibition

The β-haematin formation inhibition assay method described by Carter *et al.*[3,7] was modified for manual liquid delivery. Three stock solutions of the samples were prepared by dissolving the pre-weighed compound in DMSO and after sonication, diluting with DMSO to give 20 mM, 2 mM and 0.4 mM solutions of each sample. These were delivered to a 96-well plate in duplicate to give concentrations ranging from 0–1000 μM (final concentration) with a total DMSO volume of 10 μL in each well after which deionised H_2O (70 μL) and NP-40 (20 μL; 30.55 μM) were added. A 25 mM haematin stock solution was prepared by sonicating haemin in DMSO for one minute and then suspending 178 μL of this in a 1M acetate buffer (pH 4.8). The homogenous suspension (100 μL) was then added to the wells to give final buffer and haematin concentrations of 0.5 M and 100 μM respectively. The plate was covered and incubated at 37 °C for 5–6 h in a water bath. Analysis was carried out using the pyridine-ferrichrome method developed by Ncokazi and Egan.[8] A solution of 50% (v/v) pyridine, 30% (v/v) H_2O, 20% (v/v) acetone and 0.2 M HEPES buffer (pH 7.4) was prepared and 32 μL added to each well to give a final pyridine concentration of 5% (v/v). Acetone (60 μL) was then added to assist with haematin dispersion. The UV-vis absorbance of the plate wells was read on a SpectaMax plate reader. Sigmoidal dose-response curves were fitted to the absorbance data using GraphPad Prism v3.02 to obtain a 50% inhibitory concentration (IC_{50}) for each compound. Prediction of physical properties and multiple correlation analysis were carried out using the ChemSW Molecular Modeling Pro Plus v.6.36 software.

In Vivo Anti-Malarial Efficacy Studies

In vivo anti-malarial activity was determined as previously described by Peters.[9] In vivo efficacy studies in mice were conducted according to the rules and regulations for the protection of animal rights ("Tierschutzverordnung") of the Swiss "Bundesamt für Veterinärwesen". They were approved by the veterinary office of Canton Basel-Stadt, Switzerland.

Notes and references

1 R. E. Desjardins, C. J. Canfield, J. D. Haynes, J. D. Chulay, *Antimicrob. Agents Chemother.,* 1979, **16**, 710–718.

2 H. Matile, J. R. L. Pink, In *Immunological Methods, vol. IV*; I. Lefkovits, B. Pernis, Eds, Academic Press: San Diego, CA, USA, 1990, pp 221–234.

3 S. Thaithong, G. H.Beale, M. T. Chutmongkonkul, *Roy. Soc. Trop. Med. H.,* 1983, **77**, 228–231.

4. T. Ponnudurai, A. D. Leeuwenberg, J. H. Meuwissen, *Tropical and Geographical Medicine*, 1981, **33**, 50–54.

5 W. Huber, J. C. Koella, *Acta Trop*., 1993, **55**, 257–261.

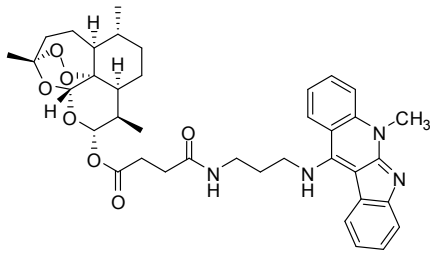
6 B. Page, M. Page, C. Noel, *Int. J. Oncol.,* 1993, **3**, 473–476.

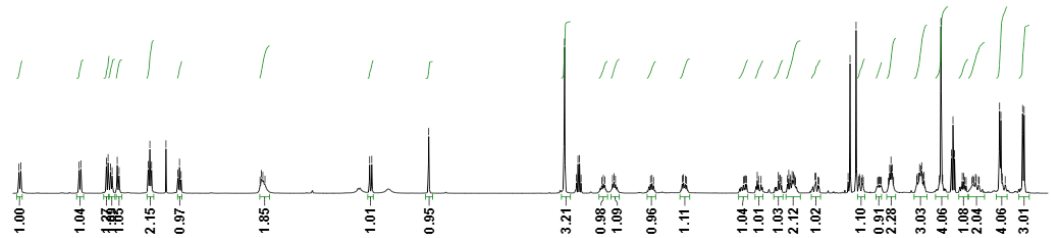
7 S. A. Ahmed, R. M. Gogal, J. E. Walsh, *J. Immunol. Methods,* 1994, **170**, 211–224.

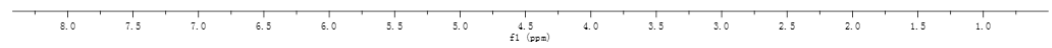
8 K. K. Ncokazi, T. J. Egan, *Anal. Biochem.*, 2005, **338**, 306–319.

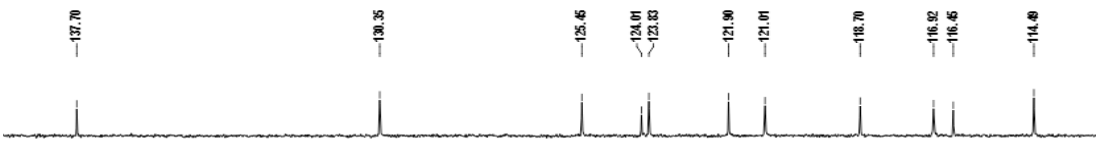
9 (a) L. Vivas, L, Rattray, L.B. Stewart, B.L. Robinson, B. Fugmann, R.K. Haynes, W. Peters, S.L. Croft, J. Antimicrob. Chemother. 59 (2007) 658–665. (b) W. Peters, 1987. Chemotherapy and Drug Resistance in Malaria, Volume 1 (1987), Academic Press, London.

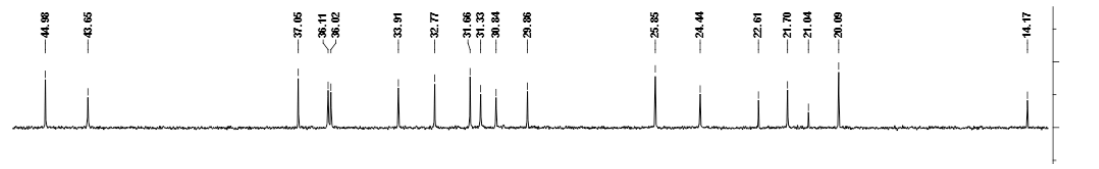
5a

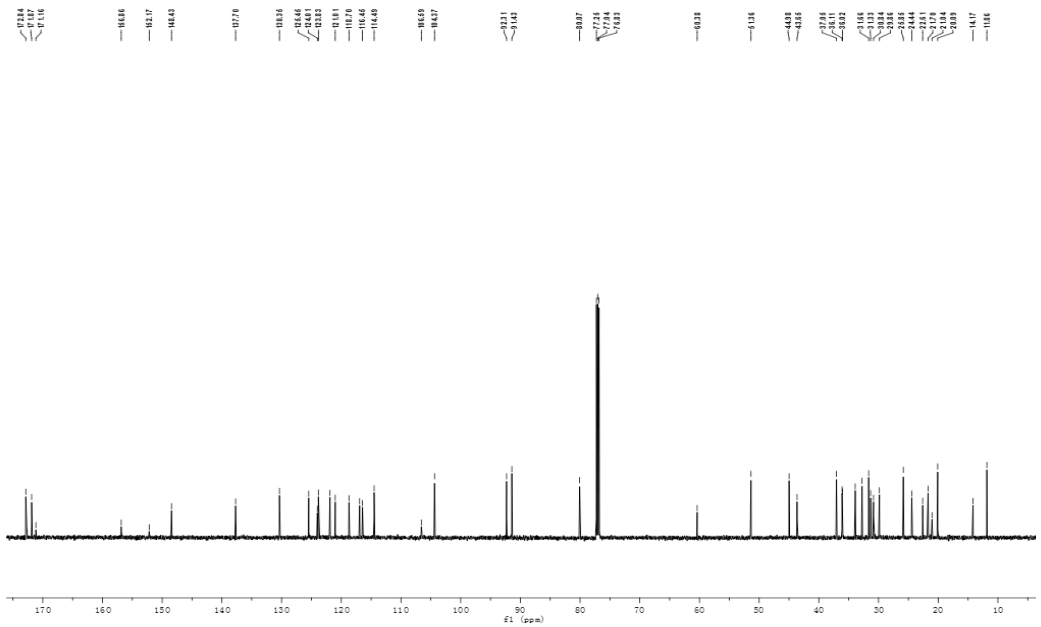












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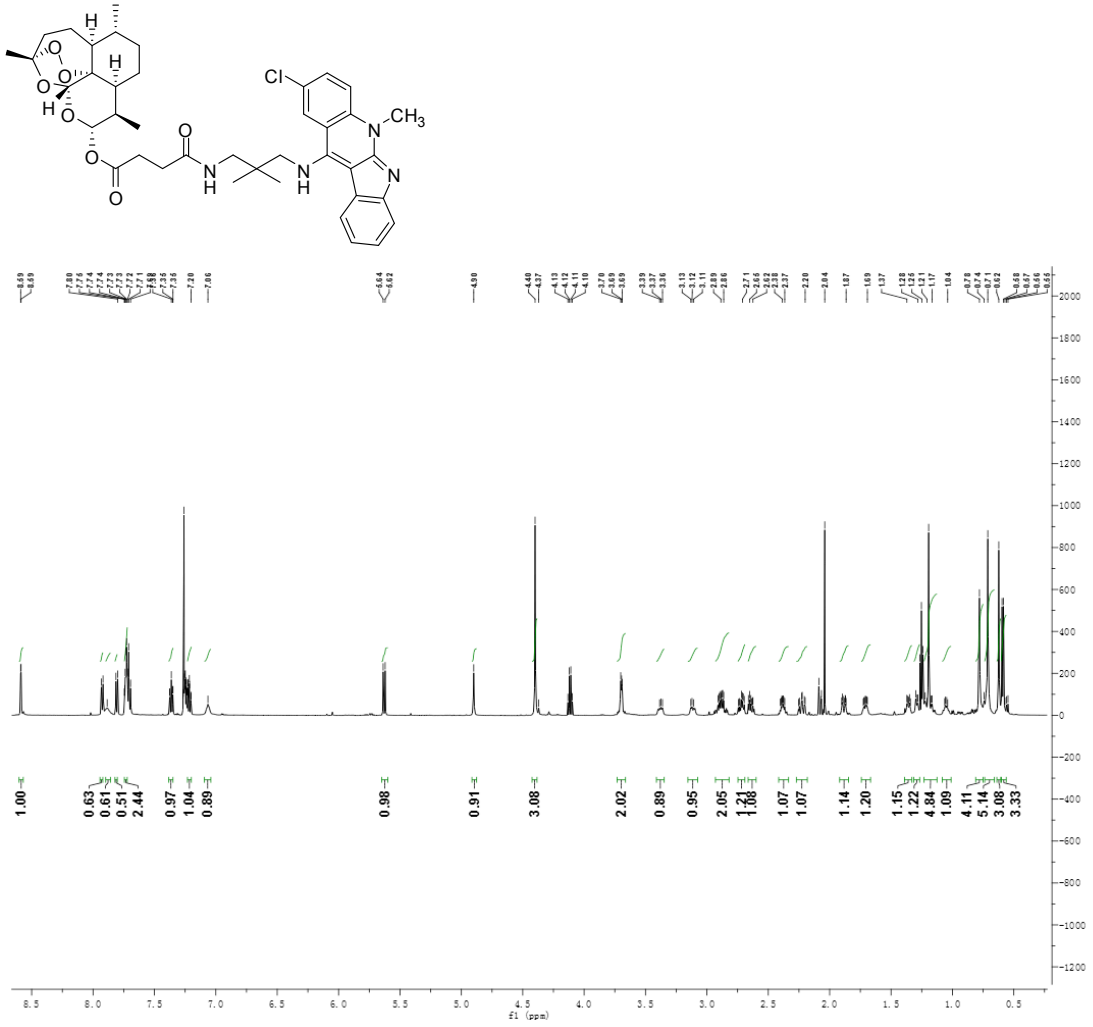

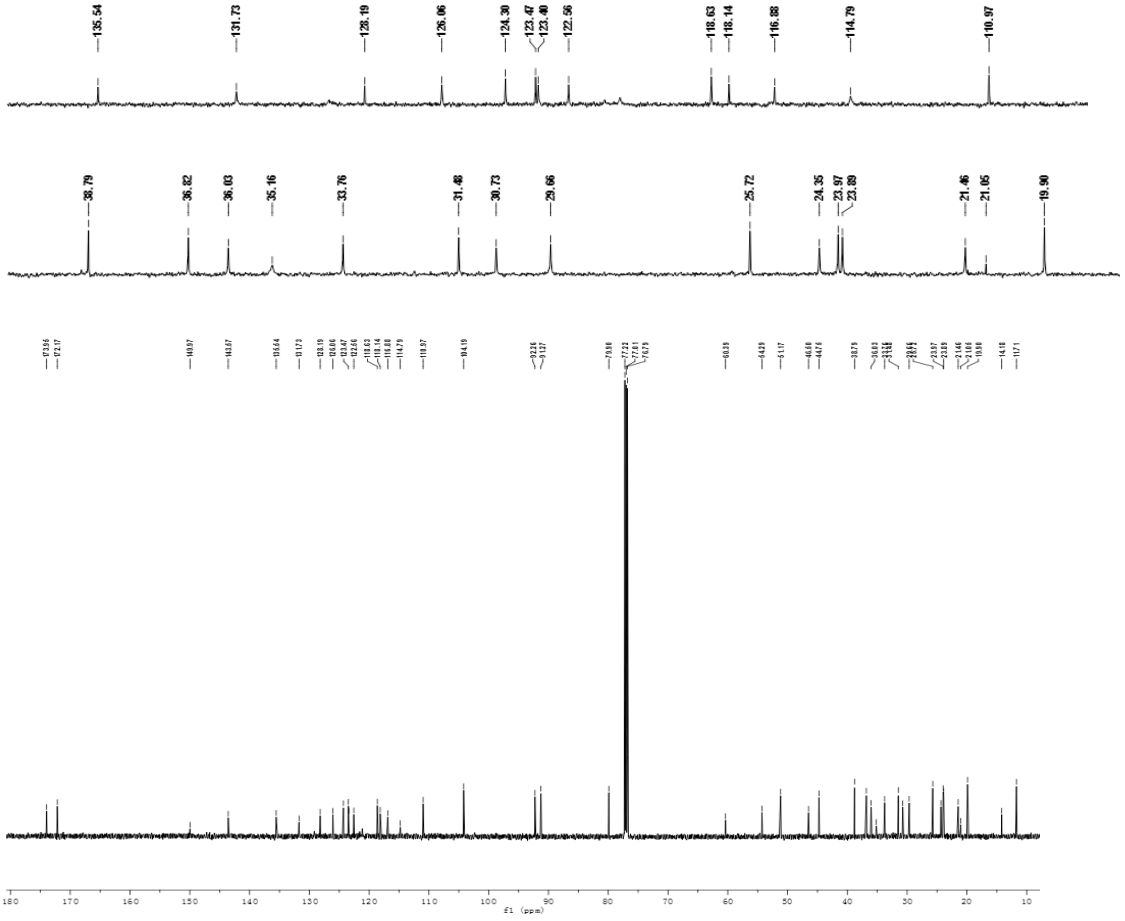
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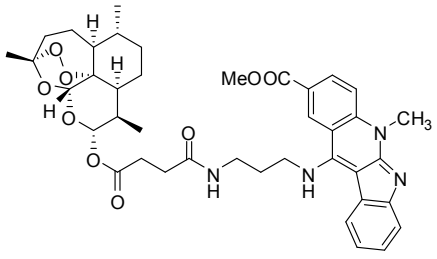
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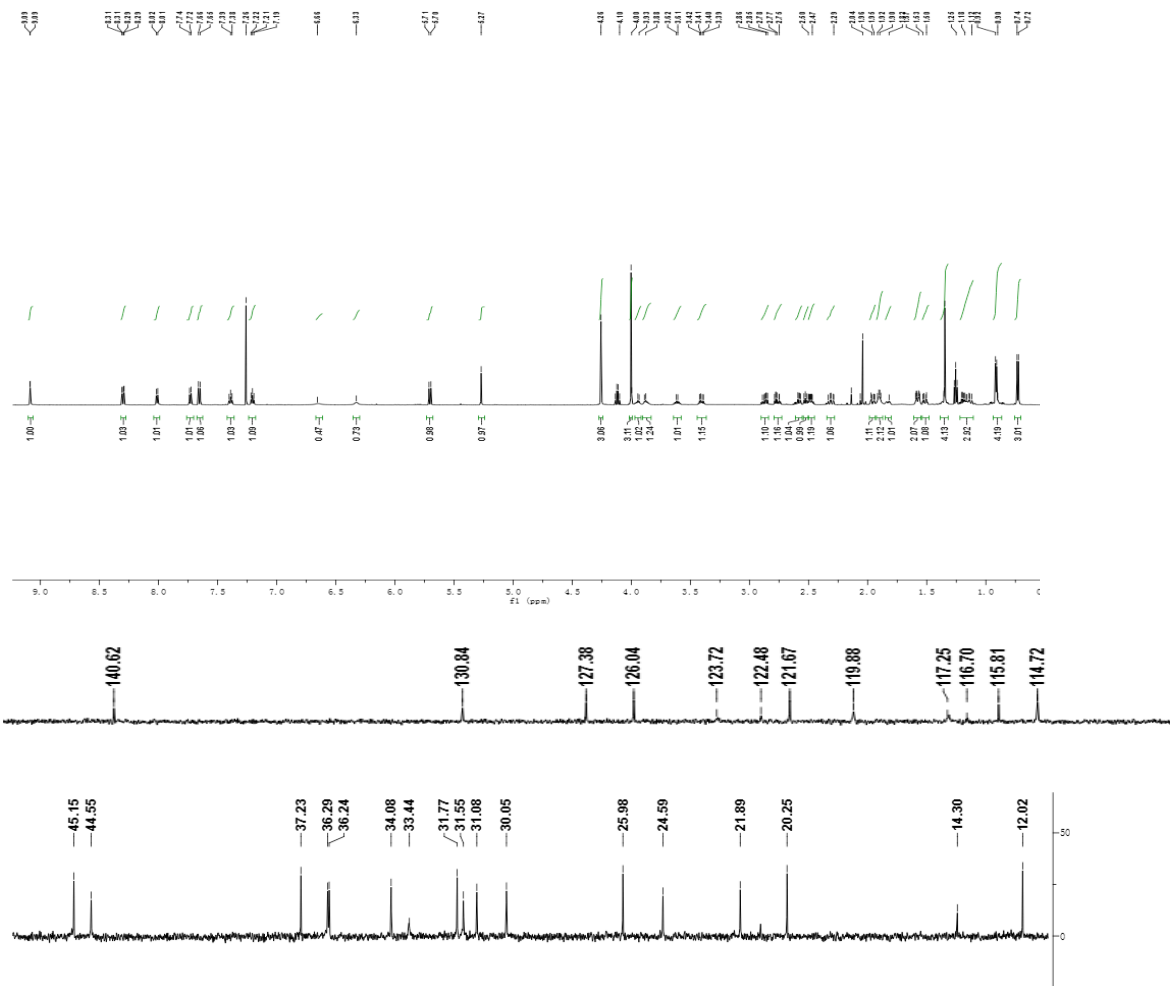
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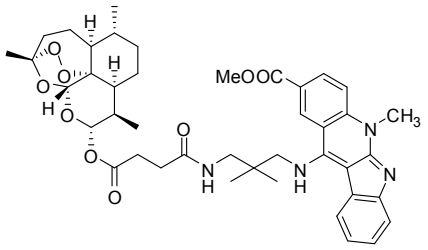
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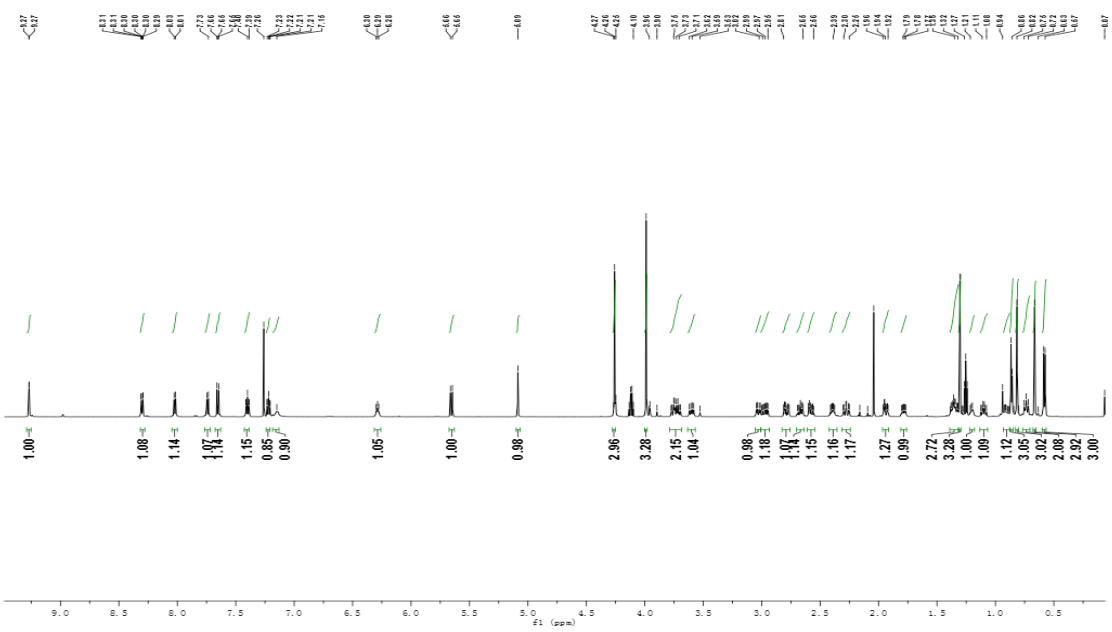

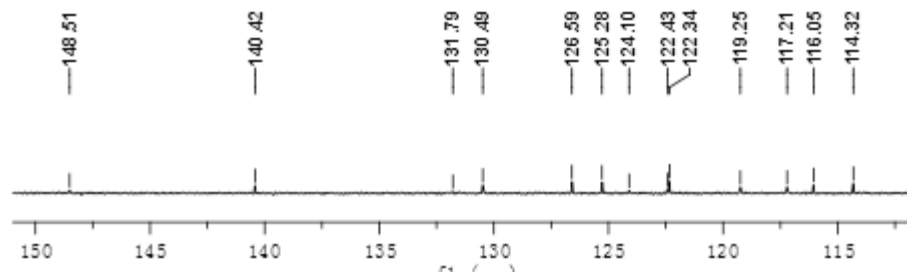

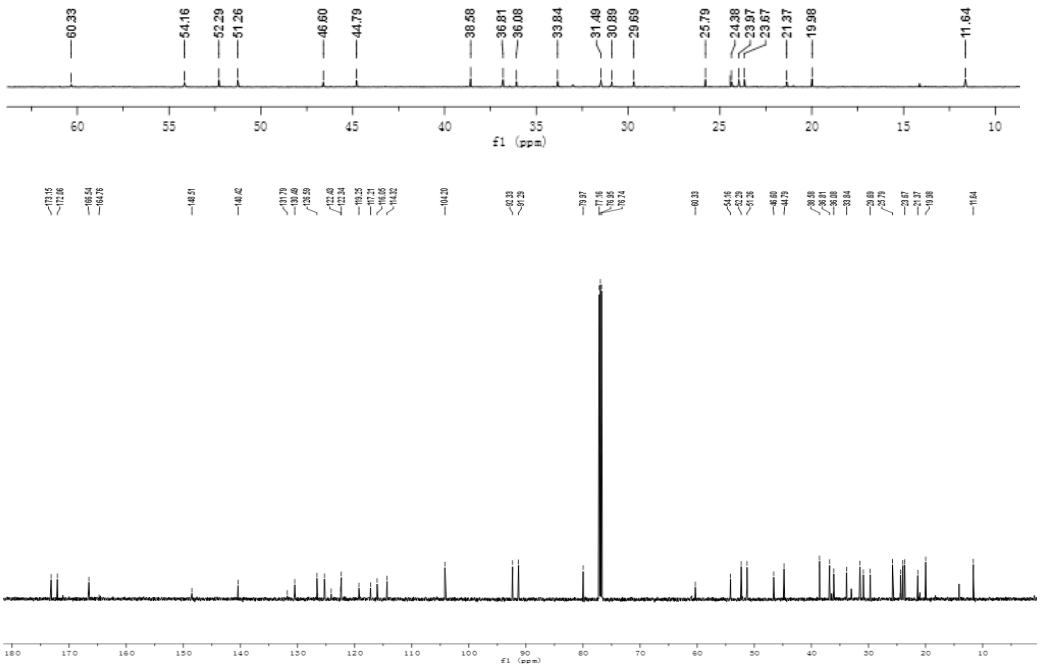

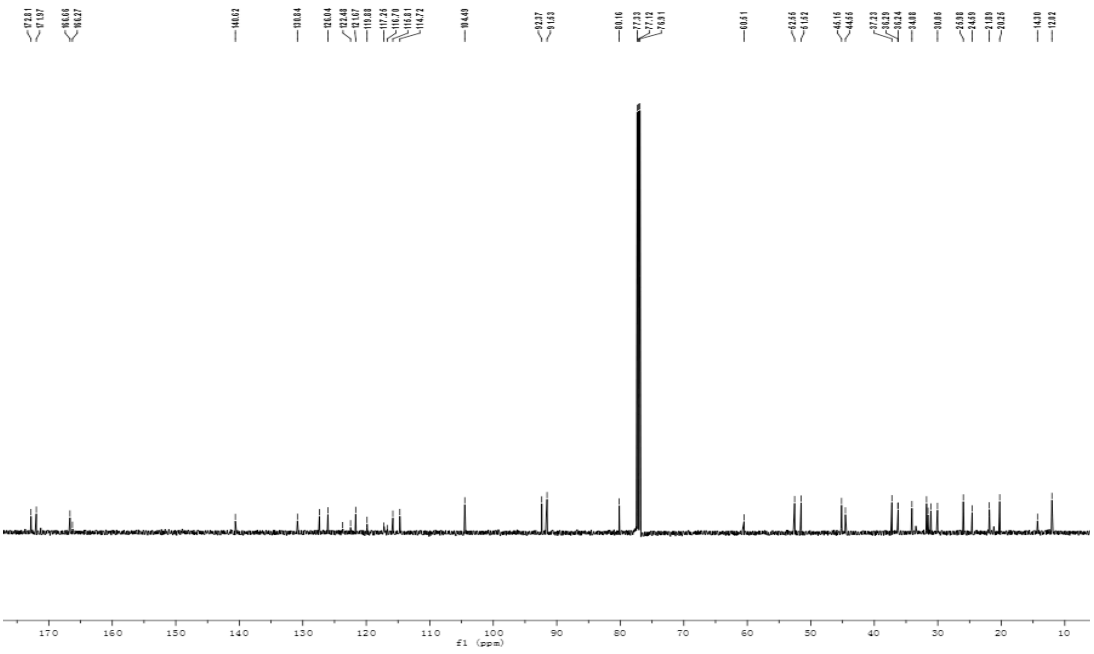
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