Supporting Information

Structural Optimization of Quinolon-4(1*H*)-imines as Dual-Stage Antimalarials: Toward Increased Potency and Metabolic Stability

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1. **Material and methods.** All manipulations requiring anhydrous conditions were carried out in flame-dried glassware, with magnetic stirring and under a nitrogen atmosphere. Chemicals and anhydrous solvents were obtained from commercial sources (Sigma-Aldrich or Alfa Aesar) and were used as received. Analytical TLC plates, aluminium sheets with silica gel F250 (Merck), were visualised by UV light (254 nm or 366 nm) or stained with $KMnO_4$ or with a solution of ninhydrin in ethanol. Flash chromatography was performed on Kieselgel 60 GF_{254} silica (Merck) of 0,040-0,063 mm. Melting points were determined on a Kofler melting point apparatus. NMR spectra were recorded on a Bruker 400 Ultra-Shield (^{1}H, 400.13 MHz; ^{13}C, 100.61 MHz). Data are reported as follows: chemical shift, multiplicity (s: singlet, d: doublet, t: triplet, dd: doublet of doublets, dt: doublet of triplets, q: quartet, m: multiplet, br: broad), coupling constants (*J* in Hz), integration and assignment. Chemical shifts are given in parts per million (ppm) with the solvent signal as internal standard [$CDCl_3$: δ (^{1}H) 7.26 ppm and δ (^{13}C) 77.2 ppm, and CD_3OD: δ (^{1}H) 4.87 ppm and δ (^{13}C) 49.0 ppm].

4,6-Dichloroquinoline[1] **(6c)** and 4-chloro-6-(trifluoromethyl)quinoline[2] **(6d)** were prepared according to literature procedures and their analytical data were in agreement with those already published.

4,6-Dichloroquinoline (6c). Off-white solid (overall yield from **9a** = 26%). Mp 102–104 ºC (lit.[3] 101–104 ºC). ^{1}H NMR ($CDCl_3$) δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (d, *J* = 4.8 Hz, 1H); 8.39 (d, *J* = 2.2 Hz, 1H), 8.08 (d, *J* = 8.8 Hz, 1H), 7.78 (dd, *J* = 8.8, 2.2 Hz, 1H); 7.44 (d, *J* = 4.8 Hz, 1H).

4-Chloro-6-(trifluoromethyl)quinoline (6d). Beige solid (overall yield from **9b** = 30%). Mp 49–51 ºC. ^{1}H NMR ($CDCl_3$) δ 8.91 (d, *J* = 4.8 Hz, 1H), 8.57 (s, 1H), 8.28 (d, *J* = 8.8 Hz, 1H), 7.96 (d, *J* = 8.8 Hz, 1H), 7.62 (d, *J* = 4.8 Hz, 1H).

2. General procedure for 7-substituted quinolin-4-amines (7a-h). To a solution of 7-substituted 4-chloroquinoline in absolute ethanol (5 mL/mmol), was added 4-aminobiphenyl (1.2 equiv), and the mixture was refluxed 24 h. The mixture was then cooled to room temperature and the precipitate formed was filtered, and washed with acetone to afford the corresponding 7-substituted quinolin-4-amines.

3. General procedure for 6-substituted quinolin-4-amines (7i and 7j). To solution of 6-substituted 4-chloroquinoline in absolute ethanol (5 mL/mmol), was added 4-aminobiphenyl (2 equiv), and the mixture was refluxed 24 h. The reaction mixture was then cooled to room temperature, concentrated under vacuum, and the crude product was purified by flash chromatography (EtOAc, 100%) to afford the corresponding 6-substituted quinolin-4-amines.

4. Structural data of intermediates 7a-j.

***N*-([1,1'-Biphenyl]-4-yl)-7-chloroquinolin-4-amine (7a).** Yellow solid (348 mg, 98%). Mp 260–261 ºC. ^{1}H NMR (CD_3OD) δ 8.88 (d, *J* = 9.2 Hz, 1H), 8.68 (d, *J* = 6.8 Hz, 1H), 8.25 (s, 1H), 8.14 – 8.09 (m, 3H), 7.98 (d, *J* = 7.6 Hz, 2H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.77 (t, *J* = 7.2 Hz, 2H), 7.68 (t, *J* = 6.8 Hz, 1H), 7.27 (d, *J* = 7.2 Hz, 1H). ^{13}C NMR (CD_3OD) δ 142.9, 141.1, 140.0, 139.7, 139.4, 135.9, 128.7, 128.3, 127.8, 127.6, 126.6, 125.6, 124.8, 119.1, 116.0, 100.3.

7-Chloro-*N*-(4-chlorophenyl)quinolin-4-amine (7b). Yellow solid (421 mg, 93%). Mp 260–262 ºC. ^{1}H NMR (CD_3OD) δ 8.58 (d, *J* = 9.1 Hz, 1H), 8.43 (d, *J* = 7.1 Hz, 1H), 7.99 (d, *J* = 1.9 Hz, 1H), 7.83 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.66 – 7.58 (m, 2H), 7.54 – 7.47 (m, 2H), 6.92 (d, *J* = 7.1 Hz, 1H). ^{13}C NMR (CD_3OD) δ 156.1, 143.1, 140.1, 139.3, 135.6, 133.3, 130.3, 127.9, 126.9, 124.8, 119.2, 116.2, 100.3.

***N*-(4-Bromophenyl)-7-chloroquinolin-4-amine (7c).** Yellow solid (387 mg, 100%). Mp 283–285 ºC. ^{1}H NMR (CD_3OD) δ 8.57 (d, *J* = 9.1 Hz, 1H), 8.43 (d, *J* = 7.1 Hz, 1H), 7.99 (d, *J* = 2.0 Hz, 1H), 7.83 (dd, *J* = 9.1, 2.0 Hz, 1H), 7.80 – 7.74 (m, 2H), 7.48 – 7.41 (m, 2H), 6.94 (d, *J* = 7.1 Hz, 1H). ^{13}C NMR (CD_3OD) δ 155.7, 143.2, 140.1, 139.4, 136.2, 133.1, 127.9, 127.1, 124.8, 121.0, 119.3, 116.1, 100.3.

7-Chloro-*N*-phenylquinolin-4-amine (7d). Yellow solid (550 mg, 100%). Mp 241–243 ºC. ^{1}H NMR (CD_3OD) δ 8.62 (d, *J* = 9.1 Hz, 1H), 8.40 (d, *J* = 7.1 Hz, 1H), 7.99 (d, *J* = 2.0 Hz, 1H), 7.81 (dd, *J* = 9.1, 2.0 Hz, 1H), 7.67 – 7.56 (m, 2H), 7.56 – 7.45 (m, 3H), 6.90 (d, *J* = 7.1 Hz, 1H). ^{13}C NMR (CD_3OD) δ 156.0, 142.8, 140.1 (s), 139.2, 136.7, 129.9, 128.0, 127.8, 125.3, 124.9, 119.1, 115.9, 100.1.

7-Chloro-*N*-(p-tolyl)quinolin-4-amine (7e). Yellow solid (471 mg, 94%). Mp 280–283 ºC. ^{1}H NMR (CD_3OD) δ 8.59 (d, *J* = 9.1 Hz, 1H), 8.37 (d, *J* = 7.1 Hz, 1H), 7.97 (d, *J* = 2.0 Hz, 1H), 7.80 (dd, *J* = 9.1, 2.0 Hz, 1H), 7.47 – 7.31 (m, 4H), 6.85 (d, *J* = 7.1 Hz, 1H), 2.45 (s, 3H). ^{13}C NMR (CD_3OD) δ 156.1, 142.7, 140.0, 139.2, 138.3, 134.0, 130.4, 127.7, 125.2, 124.8, 119.1, 115.9, 100.1, 19.8.

7-Chloro-*N*-(4-fluorophenyl)quinolin-4-amine (7f). Yellow solid (453 mg, 92%). Mp 255–257 ºC. ^{1}H NMR (CD_3OD) δ 8.58 (d, *J* = 9.1 Hz, 1H), 8.40 (d, *J* = 7.1 Hz, 1H), 7.99 (d, *J* = 2.0 Hz, 1H), 7.82 (dd, *J* = 9.1, 2.0 Hz, 1H), 7.58 – 7.48 (m, 2H), 7.41 – 7.30 (m, 2H), 6.83 (d, *J* = 7.1 Hz, 1H). ^{13}C NMR (CD_3OD) δ 163.2, 160.8, 156.7, 143.0, 140.1, 139.2, 128.04, 127.9, 127.8, 127.7, 124.8, 119.2, 116.9, 116.6, 115.9, 100.1.

7-Chloro-*N*-(4-phenoxyphenyl)quinolin-4-amine (7g). Yellow solid (150 mg, 63%). Mp 208–209 ºC. ^{1}H NMR (CD_3OD) δ 8.60 (d, *J* = 9.1 Hz, 1H), 8.40 (d, *J* = 7.1 Hz, 1H), 7.98 (d, *J* = 1.9 Hz, 1H), 7.81 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.52 – 7.38 (m, 4H), 7.25 – 7.14 (m, 3H), 7.14 – 7.05 (m, 2H), 6.88 (d, *J* = 7.1 Hz, 1H). ^{13}C NMR (CD_3OD) δ 157.5, 156.5, 156.3, 142.7, 140.0, 139.1, 131.4, 129.8, 127.8, 127.2, 124.8, 123.9, 119.3, 119.1, 115.8, 100.1.

***N*-([1,1'-Biphenyl]-4-yl)-7-(trifluoromethyl)quinolin-4-amine (7h).** Yellow solid (301 mg, 95%). Mp 292–293 ºC. ^{1}H NMR (CD_3OD) δ 9.02 (d, *J* = 9.2 Hz, 1H), 8.68 (d, *J* = 6.8 Hz, 1H), 8.42 (s, 1H), 8.17 (d, *J* = 7.6 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 7.6 Hz, 2H), 7.61 (d, *J* = 8.8 Hz, 2H), 7.52 (t, *J* = 7.6 Hz, 2H), 7.42 (t, *J* = 7.2 Hz, 1H), 7.04 (d, *J* = 7.2 Hz, 1H). ^{13}C NMR (CD_3OD) δ 143.9, 141.2, 141.0, 139.7, 135.9, 134.4, 128.7, 128.4, 127.6, 126.6, 125.5, 124.9, 122.6, 117.9, 116.0, 101.2.

***N*-([1,1'-Biphenyl]-4-yl)-6-chloroquinolin-4-amine (7i).** Yellow solid (148 mg, 100%). Mp 225–228 ºC. ^{1}H NMR (CD_3OD) δ 8.75 (d, *J* = 1.8 Hz, 1H), 8.48 – 8.38 (m, 1H), 8.04 (dd, *J* = 9.0, 1.9 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 7.4 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 1H), 7.04 (d, *J* = 7.0 Hz, 1H). ^{13}C NMR (CD_3OD) δ 146.4, 144.9, 143.6, 141.2, 139.9, 138.3, 137.1, 132.6, 131.5, 130.5, 129.4, 126.2, 125.9, 122.4, 104.4.

***N*-([1,1'-Biphenyl]-4-yl)-6-(trifluoromethyl)quinolin-4-amine (7j).** Yellow solid (204 mg, 100%). Mp 233–236 ºC. ^{1}H NMR (CD_3OD) δ 9.08 (s, 1H), 8.49 (d, *J* = 6.8 Hz, 1H), 8.23 (d, *J* = 8.8 Hz, 1H), 8.12 (d, *J* = 8.8 Hz, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 7.8 Hz, 3H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 1H), 7.08 (d, *J* = 6.8 Hz, 1H). ^{13}C NMR (CD_3OD) δ 155.6, 144.7, 140.7, 139.7, 136.1, 128.9, 128.7, 128.3, 127.5, 126.6, 125.5, 125.3, 122.6, 121.3, 117.3, 101.2.

5. X-ray crystallographic data for compound 5c

Crystal data

$C_{29}H_{30}ClN_3$	γ = 93.145 (2)°
Mr = 456.01	V = 2352.59 (15) Å3

Triclinic, *P*1	Z = 4
a = 10.6941 (4) Å	Mo *K*α radiation, λ = 0.7107 Å
b = 13.1890 (5) Å	μ = 0.19 mm−1
c = 17.9026 (6) Å	T = 100 K
α = 108.731 (2)°	0.44 × 0.22 × 0.05 mm
β = 98.280 (2)°	

Data collection

Bruker APEXII CCD diffractometer	8596 measured reflections
Absorption correction: multi-scan (*SADABS*; Bruker, 2005)	5495 reflections with $I > 2\ \sigma(I)$
T_{min} = 0.676, T_{max} = 0.745	R_{int} = 0.067
33771 measured reflections	

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.054	H-atom parameters constrained
$wR(F^2)$ = 0.121	$\Delta\rho_{max}$ = 0.26 e Å^{-3}
S = 1.04	$\Delta\rho_{min}$ = -0.32 e Å^{-3}
8596 reflections	
595 parameters	

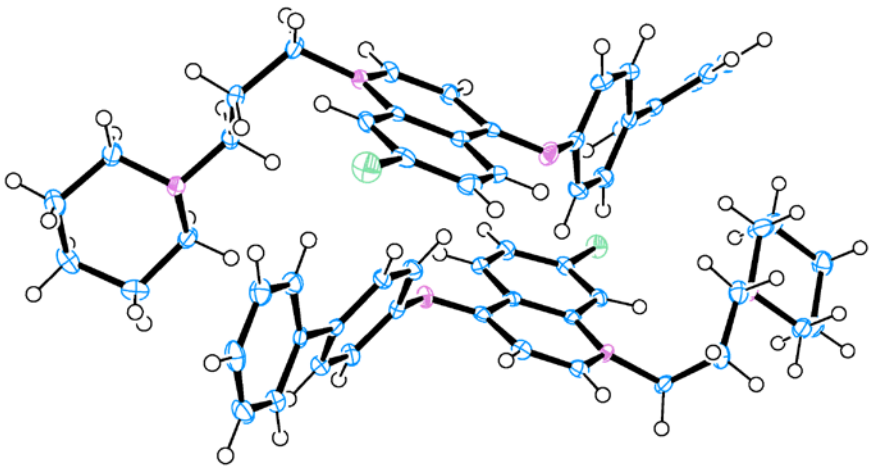


Figure S1. ORTEP view of the molecular structure of **5c**.

6. NMR spectra of compound 5o

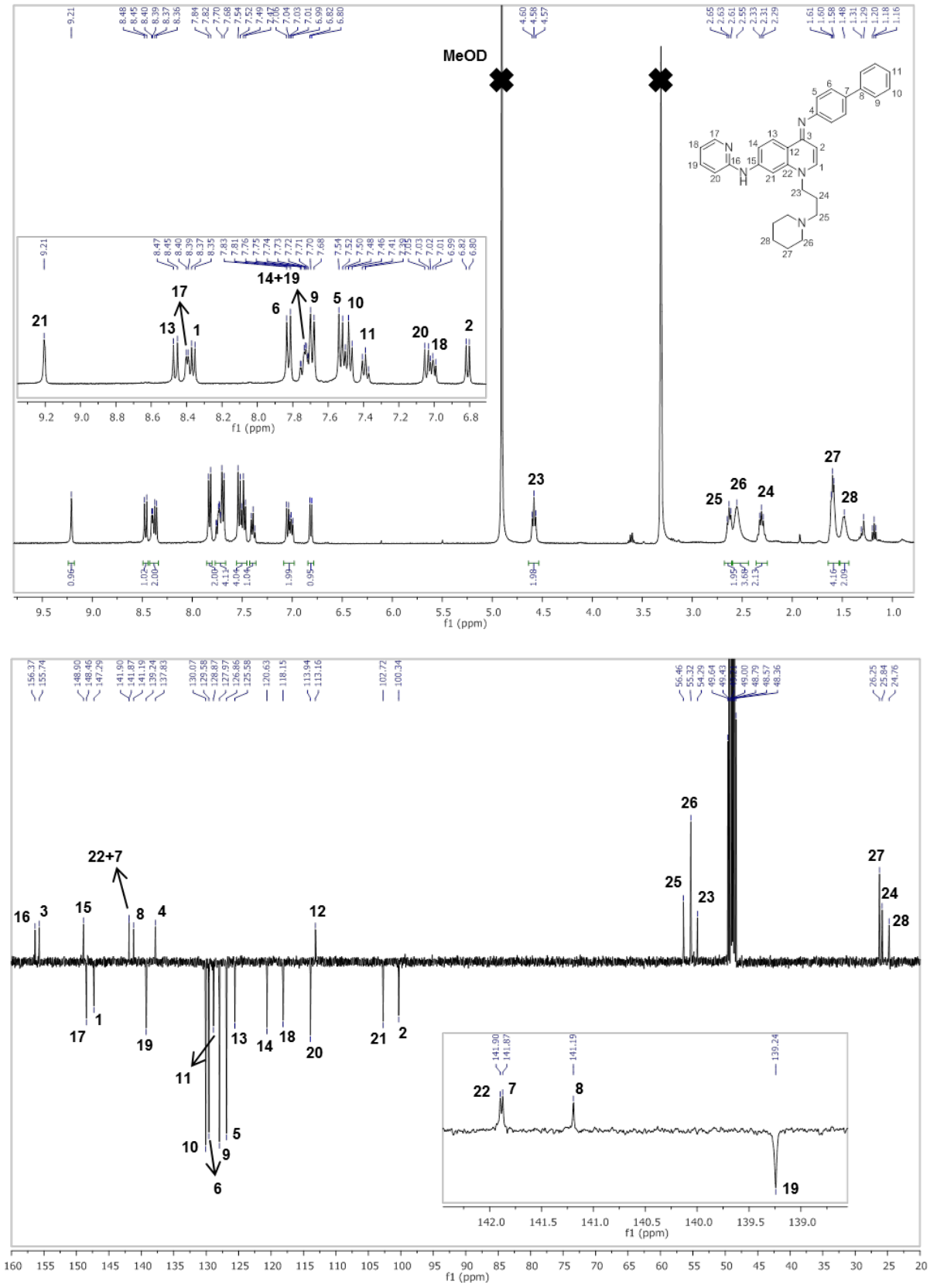
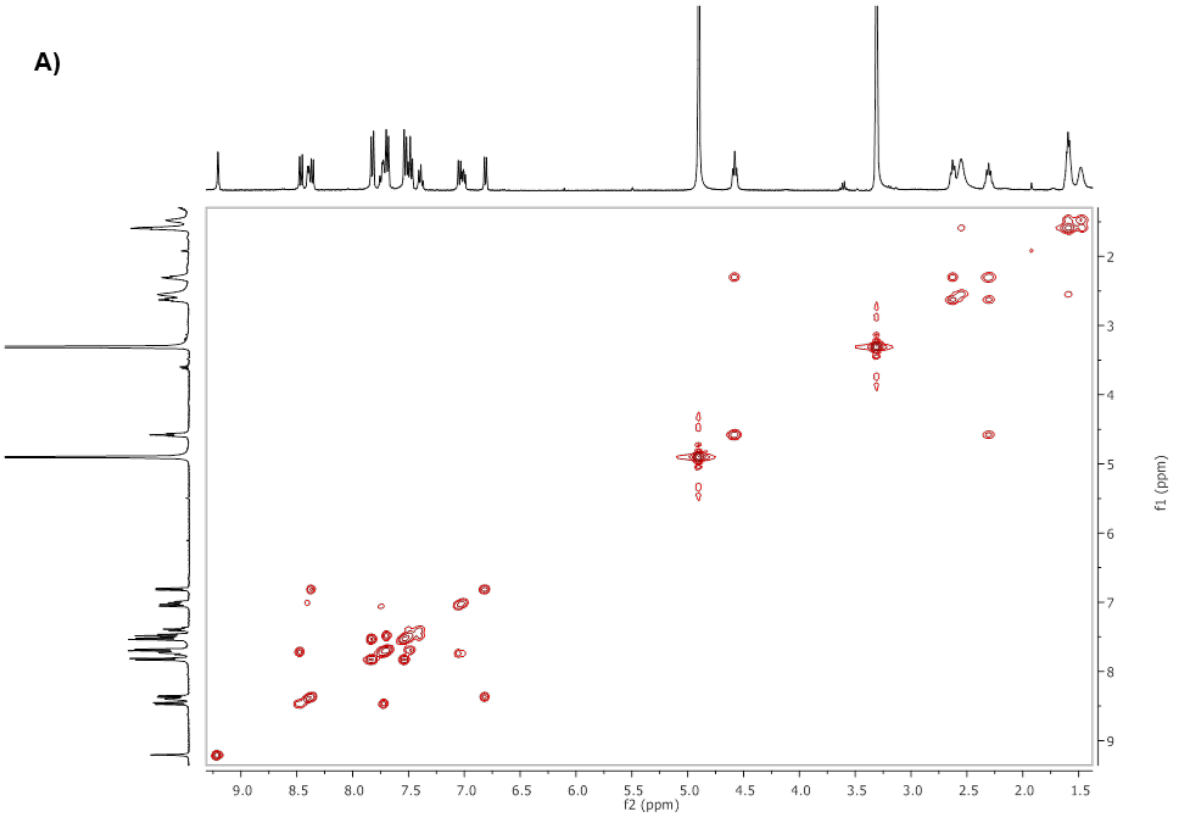


Figure S2. ^{1}H- and ^{13}C-NMR spectra of compound **5o** in $CDCl_3$ and peak assignment.

A)



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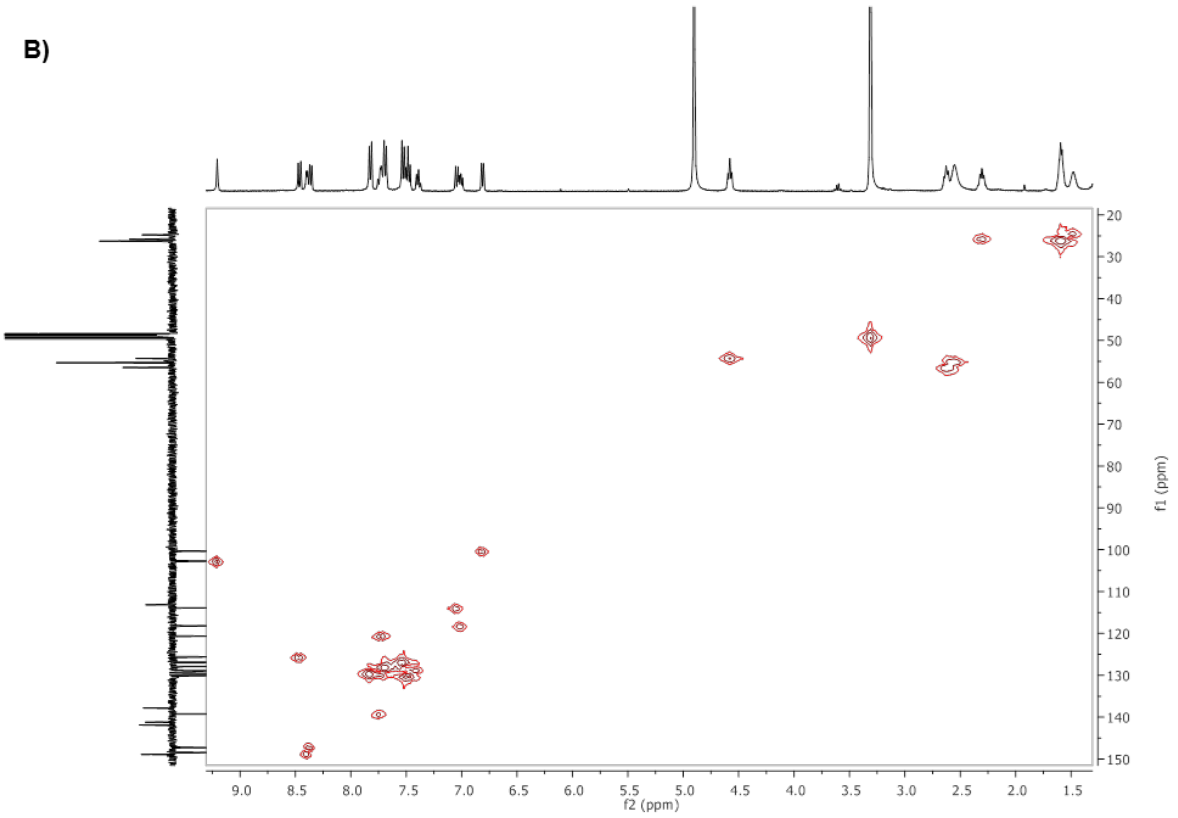

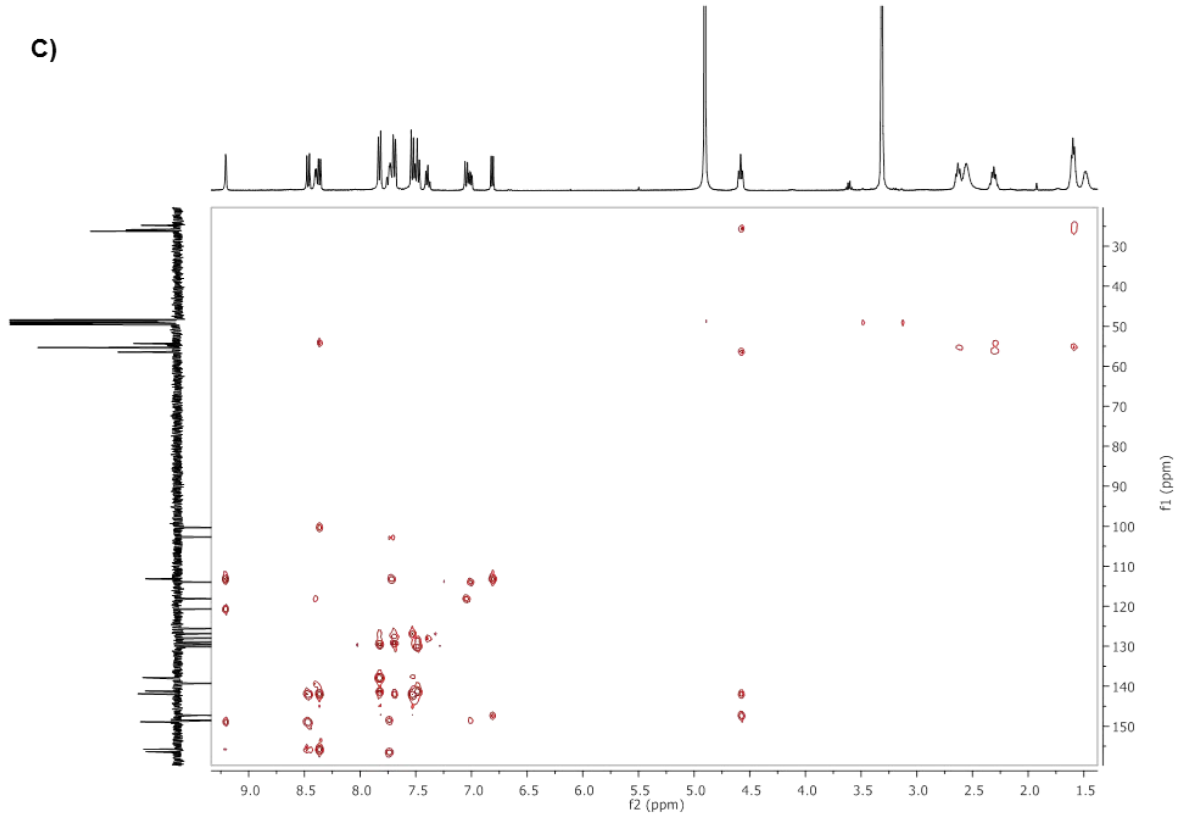

Figure S3. Bidimensional spectra of compound **5o** in $CDCl_3$: A) COSY, B) HMQC and C) HBMC.

7. HPLC analysis of final compounds 5a-v

To determine the purity of final compounds (**5a-v**), HPLC experiments were conducted using an ELITE LaChrom VWR HITACHI equipment (Pump + controller L-2130, UV detector L-2400) and a column LiChroCART, RP-18e, 5 µm. Table S1 summarizes HPLC retention times and peak area obtained for compounds **5a-v**. All quinolon-4(1*H*)-imines tested in biological assays were ≥ 95% pure.

Table S1. HPLC experimental conditions and purity of compounds **5a-v**.

Compd	Mobile Phase (mL) ACN : H_2O : TFA	Retention Time (min)	Area (%)
5a	40 : 60 : 0	4.91	99.1
5b	35 : 65 : 0	5.40	99.8
5c	35 : 65 : 0	6.23	99.7
5d	30 : 70 : 0	4.57	95.4
5e	42 : 58 : 0.5	3.88	97.4
5f	21 : 79 : 0	4.98	99.9
5g	21 : 79 : 0	9.37	95.1
5h	21 : 79 : 0	5.58	97.1
5i	35 : 65 : 0	5.48	100
5j	35 : 65 : 0	11.7	95.8
5k	40 : 60 : 0	4.37	99.4
5l	35 : 65 : 0	7.35	99.9
5m	35 : 65 : 0	3.73	95.7
5n	37 : 63 : 0	10.47	96.2
5o	45 : 55 : 0.5	4.51	97.2
5p	37 : 63 : 0	7.35	96.0
5q	35 : 65 : 0	6.01	99.5
5r	45 : 55 : 0.5	5.21	100
5s	35 : 65 : 0	6.11	100
5t	45 : 55 : 0.5	6.24	100
5u	45 : 55 : 0.5	5.55	100
5v	45 : 55 : 0.5	7.08	100

8. LC-MS analysis of compound 5u

The LC analyses were performed on a Waters Alliance 2695 (Waters®, Ireland) equipped with a quaternary pump, solvent degasser, auto sampler and column oven, coupled to a Photodiode Array Detector Waters 2996 PDA (Waters®, Ireland).

The separation was performed on a reversed-phase column (dC-18, 150x2.1mm; 5 µm; Atlantis®) at 35 ^{o}C using an injection volume of 20 µL. The mobile phase consisted of water (acidified with 0.5% formic acid) (A): Acetonitrile (B) at a flow rate of 0.30 mL/min and the eluting conditions applied consisted of 1 min at 10% of B followed by a linear gradient up to 90% B for 9 min. The system was stabilized during 1 min at 90% eluent B, and then re-equilibrated with 90% eluent A for 8 min. Photodiode Array Detector was used to scan

wavelength absorption from 210 to 600 nm. A triple quadrupole mass spectrometer was operated using an electrospray ionization source (ESI) in the positive ion mode. Acquisition and data processing were performed using the MassLynx software (Waters). Source temperature was 120 ºC and desolvation temperature was 350 ºC. Cone gas flow and desolvation gas flow were 50 and 750 L/Hr respectively. The capillary voltage was set at 3.0 kV and cone voltage 30 V.

In figure S4 is presented the chromatogram obtained 6 h after incubation in microssomes. At retention time (t_r) 8.72 min can be observe a peak detected at *m/z* 456 corresponding to the protonated molecule $[M+H]^+$ of **5u**. In the same figure can be observed how concentration of protonated molecule $[M+H]^+$ decreases, from t = 0 h to t = 6 h and the metabolite *m/z* 331 at t_r = 9.68 min is formed as a result of *N*-dealkylation of **5u**. The corresponding mass spectra is presented in figure S5 and is in accordance with the molecular formula of **5u** and isotopic pattern matches the theoretical isotopic pattern of a molecule with a chloride atom, calculated from the natural abundance of the chloride isotopes.

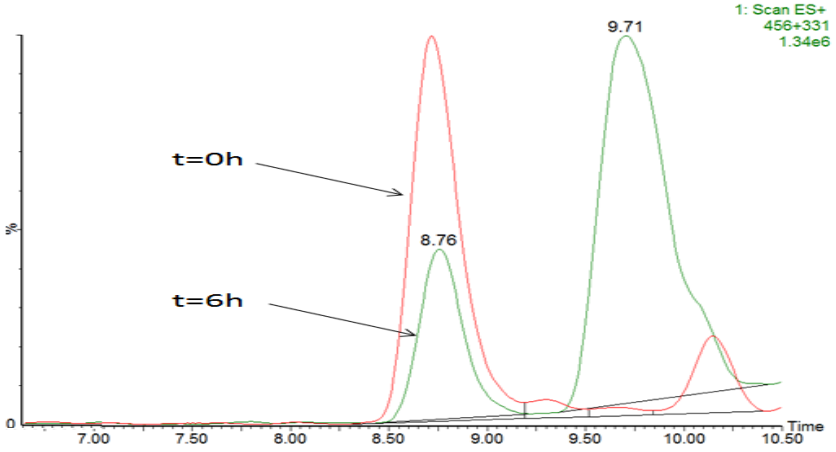

Figure S4. Chromatogram of compound **5u** in the microsomal reaction mixture after 6 h incubation in rat liver microsomes**:** *m/z* 456 at t_r = 8.72 min correspond to the $[M+H]^+$ of **5u** and *m/z* 331 at t_r = 9.68 min correspond to the formed metabolite as a result of *N*-dealkylation of $[M+H]^+$**.**

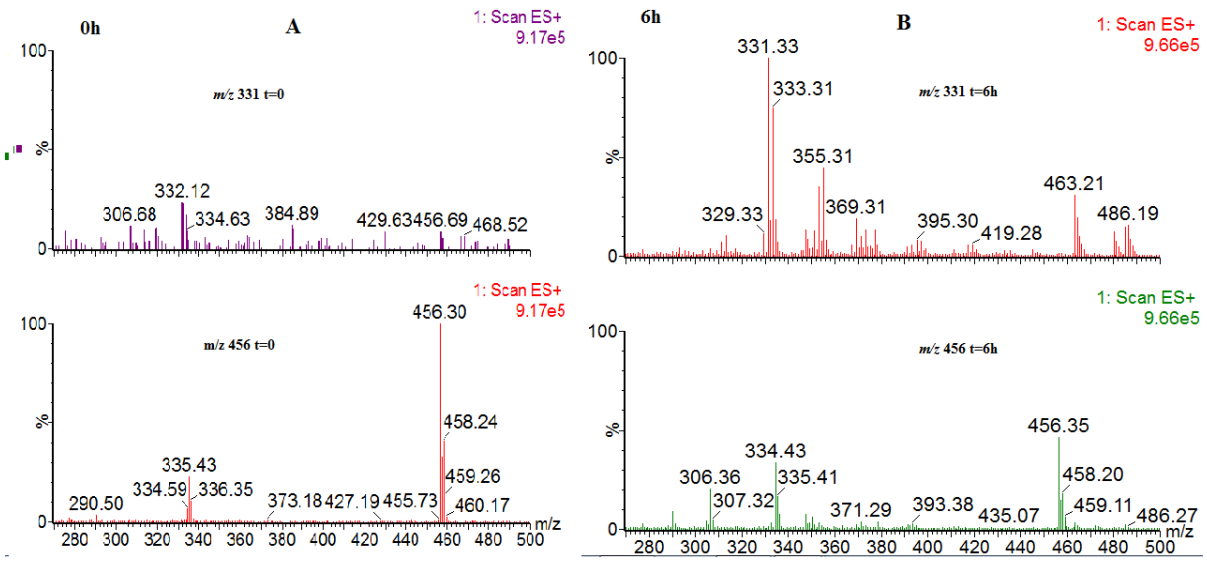

Figure S5. MS spectra of the peaks detected at t_r = 8.72 min (predominant $[M+H]^+$ *m/z* 456) and t_r = 9.68 min (predominant *m/z* 331) in the microsomal reaction mixture after: A) 0 h and B) 6 h incubation.

9. References:

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