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Preparation and antiplasmodial activity of 3',4'-dihydro-1'*H*-spiro(indoline-3,2'-quinolin)-2-ones

Synthesis of antiplasmodial spiroindolones

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ABSTRACT

A series of 3',4'-dihydro-1'*H*-spiro(indoline-3,2'-quinolin)-2-ones were prepared by the inverse electron demand aza Diels-Alder reaction (Povarov reaction) of imines derived from isatin and substituted anilines, and the electron rich alkenes *trans*-isoeugenol and 3,4-dihydro-2*H*-pyran. These compounds were assessed for *in vitro* antiplasmodial activity against drug-sensitive and drug-resistant forms of the *P. falciparum* parasite. Three compounds derived from 3,4-dihydro-2*H*-pyran and four compounds derived from *trans*-isoeugenol showed antiplasmodial activity in the low micromolar range against the drug-resistant FCR-3 strain (1.52 – 4.20 μM). Only compounds derived from *trans*-isoeugenol showed antiplasmodial activity against the drug-sensitive 3D7 strain (1.31 - 1.80 μM).

KEY WORDS

Spiroindolone, Povarov reaction, antiplasmodial activity

1 INTRODUCTION

Malaria is a disease caused by parasitic protozoa of the genus *Plasmodium*. An estimated 435 000 malaria deaths were reported in 2017, with 92% of these occurring in sub-Saharan Africa and due to *P. falciparum* infection (WHO, 2018). Although there has been a 48% decline in the number of malaria fatalities globally over the last 15 years, the rate of decline has decreased significantly since 2014 owing to a number of factors, including insecticide resistance and increasing reports of drug resistant strains of the parasite. There is thus still a need for the identification of new antimalarial agents with novel modes of action.

Cipargamin (also known as NITD609 or KAE609 **1**, Figure 1) is a spiroindolone targeting a parasitic P-type cation-transporter ATPase4 and is the first antimalarial drug candidate with a novel mechanism of action identified in the last 25 years (Rottmann et al., 2010). Significantly, cipargamin displays potent activity against both sexual and asexual stages of the parasite, and thus shows promise as a transmission blocking agent, capable of preventing the spread of malaria to healthy individuals (Upton et al., 2015; van Pelt-Koops et al., 2012). We became interested in spiroindolones owing to their potent antimalarial properties and noted that they can be prepared by means of an inverse electron demand aza Diels-Alder reaction (Povarov reaction) from relatively simple precursors.

Of interest to us is the use of isatin **3** in the Povarov reaction, which affords spiroindolones such as **2** (Scheme 1). Aromatic imines derived from isatin have successfully been used in the Povarov reaction with vinylbenzenes (Kouznetsov, Bello Forero, & Amado Torres, 2008; Shi, Xing, Zhu, Tan, & Tu, 2013), vinylindoles (Zhang et al., 2014), 3,4-dihydro-2*H*-pyran (Ramesh, Elamparuthi, & Raghunathan, 2008), acetylene dicarboxylates (Karmakar, Kayal, Bhattacharya, & Maiti, 2014) β-enamino esters (Gao, Sun, & Yan, 2014) and α,β-unsaturated *N,N*-dimethylhydrazones (Bianchini, Ribelles, Becerra, Ramos, & Menendez, 2016) to afford spiroindolones. Herein we describe the synthesis and biological evaluation of a series of substituted spiroindolones **2** prepared by Povarov reaction of ketimines with electron rich alkenes. To the best of our knowledge, these compounds have not previously been assessed for antiplasmodial activity *in vitro*.

2 METHODS AND MATERIALS

2.1 Chemistry

All reagents were purchased from Sigma-Aldrich (South Africa) and used as received. Dichloromethane used for reactions was distilled from calcium hydride under an inert atmosphere prior to use. Absolute ethanol was obtained from commercial sources without any further purification. Solvents for column chromatography (ethyl acetate and hexane) were purified by distillation prior to use.

Thin Layer Chromatography (TLC) was carried out using Merck aluminium foil F254 backed plates coated with silica gel 60. Column chromatography was performed on Macherey-Nagel silica gel 60 particle size 0.063 – 0.200 mm, while Merck silica gel (particle size 0.035 – 0.070 mm) was employed for flash chromatography.

^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopic data were recorded on a Bruker 300 or 500 MHz spectrometer using specified deuterated solvents. For those compounds soluble in deuterated chloroform ($CDCl_3$), chemical shifts were referenced against the internal standard tetramethylsilane (TMS) which occurs at zero parts per million. Chemical shifts are recorded in ppm, while coupling constants are recorded in Hertz. High Resolution Mass Spectra (HRMS) were obtained on a SYNAPT G2 HDMS mass spectrometer (ESI).

2.1.1 General procedure for the synthesis of aryl-imines (4a-n)

Isatin (3.00 g, 20.4 mmol) and the appropriately substituted aniline (1.0 eq, 20.4 mmol) were dissolved in absolute ethanol (120 ml) and treated with glacial acetic acid (0.8 ml). The resulting heterogeneous mixture was stirred at reflux (100°C) under a nitrogen atmosphere for 5-15 hours. The reaction was then cooled and concentrated by removal of the solvent to allow the product to precipitate out of solution. The mixture was filtered and the filter cake was further purified by recrystallization from ethanol to afford the desired imine. Imines **4a**-**n** were prepared using this procedure. A representative example is given below.

3-(*p*-Tolylimino)indolin-2-one (Kouznetsov et al., 2008) (**4a**) was isolated as a mixture of *E*:*Z* isomers in a ratio of 3:1. **^{1}H NMR** (300 MHz, DMSO-d_6) ***E* Isomer** δ 10.97 (1H, s, NH), 7.38 – 7.22 (3H, m, ArH), 6.93 – 6.85 (3H, m, ArH), 6.72 (1H, td, *J* = 7.7, 1.0 Hz, ArH), 6.49 (1H, dd, *J* = 7.8, 1.2 Hz, ArH), 2.35 (3H, s, CH_3); ***Z* isomer** δ 10.85 (1H, s, NH), 7.57 (1H, dd, *J* = 7.5, 1.2 Hz, ArH), 7.43 (1H, td, *J* = 7.7, 1.3 Hz, ArH), 7.11 (2H, d, *J* = 8.0 Hz, ArH), 7.05 (1H, td, *J* = 7.6, 0.9 Hz, ArH), 6.99 – 6.92 (2H, m, ArH), 6.91 – 6.81 (1H, m, ArH, signal overlapping with ArH of E-isomer), 2.30 (3H, s, CH_3); **^{13}C NMR** (75 MHz, DMSO-d_6) ***E* Isomer** δ 163.5, 154.7, 147.8, 146.9, 134.2, 134.1, 129.9, 125.2, 121.6, 117.4, 115.7, 111.4, 20.5; ***Z* Isomer** δ 158.4, 152.5, 146.1, 145.4, 133.9, 133.9, 128.7, 122.5, 122.2, 119.6, 115.7, 110.6, 20.5; **HRMS** (m/z), calculated for $C_{15}H_{13}N_2O$: 237.1028, found $(M + H)^+$: 237.1022.

2.1.2 General procedure for the synthesis of 2',3',4',4a',6',10b'-hexahydrospiro[indoline-3,5'-pyrano[3,2-*c*)quinolin]-2-ones (2a-h)

Aryl imines **4a**-**h** (1.8 mmol) were each dissolved in anhydrous dichloromethane (30 ml) and treated with $BF_3{\cdot}OEt_2$ (0.9 mmol) under an inert atmosphere and allowed to stir for fifteen minutes. 3,4-Dihydro-2*H*-pyran (2.7 mmol) was then added dropwise, and the reaction progress monitored by TLC. Typically, after 72 hours, the reaction mixture was treated with saturated aqueous $NaHCO_3$ solution (20 ml) and extracted with ethyl acetate (3 × 20 ml). The organic layer was dried (Na_2SO_4), concentrated under vacuum, and the resulting residue purified by either recrystallization (hexane/EtOAc) or silica gel column chromatography (30-50% EtOAc/hexane). The following products were prepared by this method:

9'-Methyl-2',3',4',4a',6',10b'-hexahydrospiro-[indoline-3,5'-pyrano[3,2-*c*]quinolin]-2-one (2a) (Ramesh et al., 2008)
^{1}H NMR (300 MHz, DMSO-d_6) δ 10.32 (s, 1H), 7.18 (td, *J* = 7.5, 1.5 Hz, 1H), 7.07 (d, *J* = 2.2 Hz, 1H), 6.91 – 6.80 (m, 4H), 6.52 (d, *J* = 8.1 Hz, 1H), 6.19 (s, 1H), 5.07 (d, *J* = 5.2 Hz, 1H), 3.55 – 3.48 (m, 1H), 3.47 – 3.36 (m, 1H), 2.21 (s, 3H), 2.01 – 1.94 (dd, *J* = 13.4, 4.3 Hz, 1H), 1.82 – 1.73 (m, 1H), 1.54 – 1.32 (m, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 182.7, 147.2, 145.7, 140.2, 134.0, 133.8, 132.4, 130.3, 128.8, 126.7, 122.7, 119.5, 114.7, 74.7, 68.6, 66.0, 42.3, 30.0, 25.6, 25.4; **HRMS** (m/z), calculated for $C_{20}H_{21}N_2O_2^+$: 321.1598, found $(M + H)^+$: 321.1603.

7'-Fluoro-2',3',4',4a',6',10b'-hexahydrospiro-[indoline-3,5'-pyrano[3,2-*c*]quinolin]-2-one (2b)
^{1}H NMR (500 MHz, DMSO-d_6) δ 8.39 (s, 1H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.21 (t, *J* = 7.7 Hz, 1H), 7.05 (d, *J* = 7.4 Hz, 1H), 6.99 – 6.90 (m, 2H), 6.88 (d, *J* = 7.8 Hz, 1H), 6.78 (td, *J* = 7.9, 5.0 Hz, 1H), 5.29 (d, *J* = 5.0 Hz, 1H), 4.40 (s, 1H), 3.67 – 3.61 (m, 1H), 3.60 – 3.53 (m, 1H), 2.15 – 2.03 (m, 2H), 1.67 – 1.53 (m, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 178.9, 151.1 (d, $J_{C\text{-}F}$ = 240.0 Hz), 139.2, 134.0, 131.8 (d, $J_{C\text{-}F}$ = 12.3 Hz), 129.4, 124.2, 123.1 (d, $J_{C\text{-}F}$ = 3.2 Hz), 123.0, 121.5 (d, $J_{C\text{-}F}$ = 2.5 Hz), 117.9 (d, $J_{C\text{-}F}$ = 7.2 Hz), 114.0 (d, $J_{C\text{-}F}$ = 17.9 Hz), 110.4, 69.7, 64.2, 62.0, 37.8, 25.3, 20.7; **HRMS** (m/z), calculated for $C_{19}H_{18}N_2O_2F^+$: 325.1347, found $(M + H)^+$: 325.1364.

7'-Chloro-9'-methyl-2',3',4',4a',6',10b'-hexahydro-spiro[indoline-3,5'-pyrano[3,2-*c*]quinolin]-2-one (2c)
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.32 (s, 1H), 7.24 – 7.16 (m, 1H), 7.13 – 7.03 (m, 2H), 6.85 (d, *J* = 4.3 Hz, 2H), 6.83 (d, *J* = 7.6 Hz, 1H), 6.05 (s, 1H), 5.13 (d, *J* = 5.2 Hz, 1H), 3.62 – 3.48 (m, 1H), 3.37 (td, *J* = 11.1, 3.2 Hz, 1H), 2.24 (s, 3H), 2.04 – 1.97 (m, 1H), 1.83 – 1.76 (m, 1H), 1.56 – 1.41 (m, 2H), 1.35 – 1.25 (m, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 177.0, 140.7, 138.0, 134.9, 128.7, 128.5, 126.2, 126.1, 123.2, 121.3, 119.8, 117.3, 109.5, 69.1, 63.3, 60.7, 36.9, 24.7, 20.1, 19.9; **HRMS** (m/z), calculated for $C_{20}H_{20}N_2O_2Cl^+$: 355.1208, found $(M + H)^+$: 355.1207.

8'-Chloro-2',3',4',4a',6',10b'-hexahydrospiro-[indoline-3,5'-pyrano[3,2-*c*]quinolin]-2-one (2d) (Ramesh et al., 2008)
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.34 (s, 1H), 7.27 (d, *J* = 7.6 Hz, 1H), 7.25 – 7.22 (m, 1H), 7.21 – 7.17 (m, 1H), 6.91 – 6.85 (m, 2H), 6.84 (d, *J* = 7.7 Hz, 1H), 6.73 (t, *J* = 7.7 Hz, 1H), 6.26 (s, 1H), 5.16 (d, *J* = 5.2 Hz, 1H), 3.59 – 3.49 (m, 1H), 3.37 (td, *J* = 11.2, 3.3 Hz, 1H), 2.06 – 1.93 (m, 1H), 1.86 – 1.77 (m, 1H), 1.56 – 1.44 (m, 2H), 1.37 – 1.23 (m, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 176.9, 140.7, 140.4, 134.9, 128.6, 128.3, 125.9, 123.2, 121.3, 119.8, 117.3, 117.0, 109.5, 69.0, 63.2, 60.7, 36.8, 24.7, 20.1; **HRMS** (m/z), calculated for $C_{19}H_{18}N_2O_2Cl^+$: 341.1051, found $(M + H)^+$: 341.1066.

9'-Chloro-2',3',4',4a',6',10b'-hexahydrospiro-[indoline-3,5'-pyrano[3,2-*c*]quinolin]-2-one (2e) (Ramesh et al., 2008)
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.40 (s, 1H), 7.27 – 7.14 (m, 2H), 7.06 (dd, *J* = 8.6, 1.7 Hz, 1H), 6.95 (d, *J* = 7.3 Hz, 1H), 6.92 – 6.81 (m, 2H), 6.66 (s, 1H), 6.63 (d, *J* = 8.6 Hz, 1H), 5.08 (d, *J* = 5.0 Hz, 1H), 3.55 (d, *J* = 11.0 Hz, 1H), 3.44 – 3.41 (m, 1H), 2.03 – 1.91 (m, 1H), 1.89 – 1.80 (m, 1H), 1.58 – 1.40 (m, 2H), 1.37 – 1.28 (m, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 177.0, 143.1, 140.4, 134.4, 128.6, 127.8, 126.4, 123.4, 121.5, 119.9, 119.2, 115.5, 109.4, 68.8, 64.8, 62.9, 36.6, 24.4, 20.1; **HRMS** (m/z), calculated for $C_{19}H_{18}N_2O_2Cl^+$: 341.1051, found $(M + H)^+$: 341.1053.

9'-Bromo-2',3',4',4a',6',10b'-hexahydrospiro-[indoline-3,5'-pyrano[3,2-*c*]quinolin]-2-one (2f) (Ramesh et al., 2008)
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.42 (s, 1H), 7.25 – 7.19 (m, 2H), 6.95 (d, *J* = 7.4 Hz, 1H), 6.93 – 6.88 (m, 1H), 6.85 (d, *J* = 7.7 Hz, 1H), 6.75 (s, 1H), 6.68 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.64 (d, *J* = 2.0 Hz, 1H), 5.05 (d, *J* = 5.1 Hz, 1H), 3.60 – 3.49 (m, 1H), 3.45 – 3.39 (m, 1H), 2.00 – 1.91 (m, 1H), 1.86 (dt, *J* = 10.0, 4.8 Hz, 1H), 1.63 – 1.38 (m, 2H), 1.36 – 1.26 (m, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 177.2, 145.7, 140.6, 134.3, 132.5, 129.1, 128.9, 123.5, 121.7, 116.5, 116.2, 112.9, 109.6, 68.9, 64.9, 63.0, 36.9, 24.5, 20.25.

7',9'-Dimethyl-2',3',4',4a',6',10b'-hexahydrospiro-[indoline-3,5'-pyrano[3,2-*c*]quinolin]-2-one (2g)
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.29 (s, 1H), 7.16 (td, *J* = 7.1, 2.4 Hz, 1H), 6.97 (d, *J* = 2.0 Hz, 1H), 6.85 – 6.78 (m, 3H), 6.75 (d, *J* = 2.0 Hz, 1H), 5.48 (s, 1H), 5.11 (d, *J* = 5.3 Hz, 1H), 3.56 – 3.44 (m, 1H), 3.41 – 3.33 (m, 1H), 2.19 (s, 3H), 2.06 – 1.94 (m, 4H), 1.73 (dt, *J* = 10.2, 4.8 Hz, 1H), 1.56 – 1.29 (m, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 177.6, 140.5, 140.0, 135.4, 130.1, 128.3, 124.8, 124.66, 123.4, 121.9, 121.2, 117.4, 109.4, 69.5, 63.6, 60.4, 36.9, 24.9, 20.3, 20.1, 17.5; **HRMS** (m/z), calculated for $C_{21}H_{23}N_2O_2^+$: 335.1754, found $(M + H)^+$: 335.1761.

2',3',4',4a',6',10b'-Hexahydrospiro[indoline-3,5'-pyrano[3,2-*c*]quinolin]-2-one (2h) (Ramesh et al., 2008)
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.36 (d, *J* = 3.2 Hz, 1H), 7.34 – 7.14 (m, 2H), 7.07 – 6.80 (m, 4H), 6.72 – 6.53 (m, 2H), 6.40 (d, *J* = 8.7 Hz, 1H), 5.11 (d, *J* = 5.1 Hz, 1H), 3.51 (d, *J* = 11.0 Hz, 1H), 3.41 (td, *J* = 10.7, 2.9 Hz, 1H), 2.01 – 1.77 (m, 2H), 1.60 – 1.21 (m, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 176.3, 143.1, 139.4, 133.8, 127.5, 126.9, 125.9, 122.4, 120.3, 116.3, 115.5, 112.9, 108.4, 68.2, 62.1, 59.7, 35.9, 23.6, 19.1; **HRMS** (m/z), calculated for $C_{19}H_{19}N_2O_2^+$: 307.1441, found $(M + H)^+$: 307.1448.

2.1.3 General procedure for the synthesis of 4'-(4-hydroxy-3-methoxyphenyl)-3'-methyl-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-ones (2i-2p)

Aryl imines **4a-n** (1.8 mmol) were each dissolved in anhydrous dichloromethane (30 ml) and treated with $BF_3{\cdot}OEt_2$ (1.8 mmol) under an inert atmosphere and allowed to stir for fifteen minutes. *Trans*-isoeugenol (1.5 eq, 2.7 mmol) was then added, and the reaction progress monitored by TLC. Typically, after 72 hours, the reaction mixture was treated with saturated aqueous $NaHCO_3$ solution (20 ml) and extracted with ethyl acetate (3 × 20 ml). The organic layer was dried (Na_2SO_4), concentrated under vacuum, and the resulting residue purified by either recrystallization (hexane/EtOAc) or silica gel column chromatography (30-50% EtOAc/hexane). The following products were prepared by this method:

4'-(4-Hydroxy-3-methoxyphenyl)-3',6'-dimethyl-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2i) (Kouznetsov et al., 2008)
^{1}H NMR (500 MHz, $CDCl_3$) δ 10.50 (s, 1H), 8.82 (s, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 6.95 (d, *J* = 7.3 Hz, 1H), 6.90 (t, *J* = 7.5 Hz, 1H), 6.86 (d, *J* = 7.7 Hz, 1H), 6.75 – 6.65 (m, 3H), 6.56 (d, *J* = 8.0 Hz, 1H), 6.45 (d, *J* = 8.0 Hz, 1H), 6.32 (s, 1H), 6.22 (s, 1H), 3.75 (d, *J* = 11.8 Hz, 1H), 3.70 (s, 3H), 2.32 (dq, *J* = 13.1, 6.7 Hz, 1H), 2.02 (s, 3H), 0.31 (d, *J* =

6.5 Hz, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 177.7, 147.4, 144.9, 141.5, 141.2, 134.6, 132.0, 129.4, 128.3, 127.4, 124.5, 123.8 (2C), 121.8, 121.4, 115.5, 114.2, 113.0, 109.3, 64.8, 55.5, 46.5, 39.7, 20.2, 13.1; **HRMS** (m/z), calculated for $C_{25}H_{25}N_2O_3^+$: 401.1860, found $(M + H)^+$: 401.1865.

8'-Fluoro-4'-(4-hydroxy-3-methoxyphenyl)-3'-methyl-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2j)

^{1}H NMR (500 MHz, DMSO-*d*6) δ 10.45 (s, 1H), 8.84 (s, 1H), 7.33 – 7.26 (m, 1H), 7.23 (td, *J* = 7.7, 1.2 Hz, 1H), 7.05 (dd, *J* = 7.5, 1.2 Hz, 1H), 6.95 (td, *J* = 7.5, 1.0 Hz, 1H), 6.86 (dd, *J* = 7.8, 1.0 Hz, 1H), 6.76 – 6.70 (m, 3H), 6.59 (t, *J* = 7.8 Hz, 2H), 6.26 (s, 1H), 3.85 (d, *J* = 12.1 Hz, 1H), 3.71 (s, 3H), 2.32 (dq, J = 13.1, 6.6 Hz, 1H), 0.31 (d, *J* = 6.6 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO) δ 177.0, 151.1, 149.2, 140.7, 134.8, 132.7, 132.6, 128.6, 123.3, 122.6, 121.4, 120.6, 120.5, 115.9, 115.9, 113.7, 113.5, 109.5, 69.0, 62.9, 61.0, 36.9, 24.7, 20.2.

8'-Chloro-4'-(4-hydroxy-3-methoxyphenyl)-3',6'-dimethyl-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2k)

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.94 (s, 1H), 7.27 (t, *J* = 7.0 Hz, 1H), 7.07 – 6.94 (m, 3H), 6.91 (d, *J* = 7.4 Hz, 1H), 6.79 (d, *J* = 7.4 Hz, 1H), 6.75 (s, 1H), 6.62 (s, 1H), 6.36 (s, 1H), 6.21 (s, 1H), 3.85 (d, *J* = 11.8 Hz, 1H), 3.76 (s, 3H), 2.44 – 2.26 (m, 1H), 2.09 (s, 3H), 0.37 (d, *J* = 5.7 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 177.6, 147.6, 145.2, 142.0, 137.5, 133.9, 131.9, 128.7, 128.5, 127.6, 126.0, 125.3, 123.7, 121.8 (2C), 117.2, 115.7, 115.2, 110.6, 109.4, 64.1, 55.7, 46.4, 19.8, 13.0; **HRMS** (m/z), calculated for $C_{25}H_{24}ClN_2O_3^+$: 435.1470, found $(M + H)^+$: 435.1485.

7'-Chloro-4'-(4-hydroxy-3-methoxyphenyl)-3'-methyl-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2l)

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.60 (s, 1H), 8.74 (s, 1H), 7.22 (t, *J* = 7.3 Hz, 1H), 6.98 (t, *J* = 7.7 Hz, 1H), 6.93 – 6.82 (m, 2H), 6.77 (d, *J* = 7.1 Hz, 1H), 6.71 – 6.65 (m, 3H), 6.62 (t, *J* = 7.4 Hz, 2H), 6.48 (d, *J* = 7.7 Hz, 1H), 3.75 (d, *J* = 10.2 Hz, 1H), 3.67 (s, 3H), 2.37 – 2.26 (m, 1H) 0.52 (d, *J* = 6.2 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 176.6, 147.3, 147.1, 144.6, 141.7, 135.9, 134.3, 130.8, 128.7, 128.1, 123.0, 122.9, 121.9, 120.9, 118.8, 115.3, 114.1, 113.5, 109.8, 63.8, 55.7, 46.5, 44.7, 13.4; **HRMS** (m/z), calculated for $C_{24}H_{22}ClN_2O_3^+$: 421.1313, found $(M + H)^+$: 421.1323.

6'-Chloro-4'-(4-hydroxy-3-methoxyphenyl)-3'-methyl-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2m)

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.57 (s, 1H), 8.89 (d, *J* = 4.1 Hz, 1H), 7.23 (t, *J* = 7.1 Hz, 1H), 7.06 – 7.02 (m, 2H), 6.96 (d, *J* = 6.9 Hz, 1H), 6.87 (d, *J* = 7.8 Hz, 1H), 6.76 (d, *J* = 7.7 Hz, 1H), 6.74 – 6.69 (m, 2H), 6.59 (d, *J* = 8.1 Hz, 1H), 6.56 – 6.43 (m, 2H), 3.82 (d, *J* = 11.9 Hz, 1H), 3.72 (s, 3H), 2.32 (dq, *J* = 13.6, 6.4 Hz, 1H), 0.31 (d, *J* = 6.6 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 177.5, 147.7, 145.2, 145.0, 141.5, 133.6, 131.7, 131.2, 130.9, 128.7, 124.0, 122.8, 122.2, 121.5, 115.7, 115.6, 113.0, 112.6, 109.6, 63.9, 55.6, 45.8, 40.1, 13.0; **HRMS** (m/z), calculated for $C_{24}H_{22}ClN_2O_3^+$: 421.1313, found $(M + H)^+$: 421.1331.

6'-Bromo-4'-(4-hydroxy-3-methoxyphenyl)-3'-methyl-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2n) (Kouznetsov et al., 2008)

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.50 (s, 1H), 8.81 (s, 1H), 7.21 (td, *J* = 7.6, 1.7 Hz, 1H), 6.96 (d, *J* = 7.4 Hz, 1H), 6.92 (t, *J* = 7.5 Hz, 1H), 6.87 (d, *J* = 7.8 Hz, 1H), 6.78 – 6.67 (m, 3H), 6.57 (d, *J* = 8.0 Hz, 1H), 6.46 (d, *J* = 8.1 Hz, 1H), 6.33 (s, 1H), 6.23 (s, 1H), 3.76 (d, *J* = 11.6 Hz, 1H), 3.72 (s, 3H), 2.33 (dd, *J* = 11.9, 6.5 Hz, 1H), 0.32 (d, *J* = 6.4 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 178.3, 148.0, 145.5, 142.1, 141.8, 135.1, 132.6, 130.0, 128.9, 128.0, 125.1, 124.4 (2C), 122.4, 122.0, 116.1, 114.8, 113.7, 109.9, 64.6, 56.1, 47.1, 40.6, 13.7.

4'-(4-Hydroxy-3-methoxyphenyl)-3',6',8'-trimethyl-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2o)

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.45 (s, 1H), 8.81 (s, 1H), 7.19 (t, *J* = 7.3 Hz, 1H), 6.95 – 6.86 (m, 2H), 6.84 (d, *J* = 7.6 Hz, 1H), 6.71 (d, *J* = 7.9 Hz, 1H), 6.66 (s, 1H), 6.63 (s, 1H), 6.53 (d, *J* = 7.5 Hz, 1H), 6.19 (s, 1H), 5.56 (s, 1H), 3.75 (d, *J* = 11.9 Hz, 1H), 3.69 (s, 3H), 2.38 – 2.20 (m, 1H), 2.00 (s, 3H), 1.98 (s, 3H), 0.30 (d, *J* = 6.2 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 178.1, 147.5, 144.9, 141.8, 139.3, 134.9, 132.5, 129.0, 128.2, 127.4, 124.1, 123.8, 123.7, 121.8, 121.5, 121.4, 115.5, 113.2, 109.2, 64.9, 55.6, 46.6, 40.1, 20.2, 17.6, 13.2; **HRMS** (m/z), calculated for $C_{26}H_{27}N_2O_3^+$: 415.2016, found $(M + H)^+$: 415.2036.

4'-(4-Hydroxy-3-methoxyphenyl)-3'-methyl-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2p)
(Kouznetsov et al., 2008)
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.83 (s, 1H), 7.21 (t, *J* = 7.2 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 6.95 – 6.84 (m, 3H), 6.73 (d, *J* = 7.4 Hz, 1H), 6.70 (s, 1H), 6.58 (d, *J* = 7.3 Hz, 1H), 6.52 (d, *J* = 7.5 Hz, 1H), 6.50 – 6.41 (m, 3H), 3.79 (d, *J* = 11.8 Hz, 1H), 3.70 (s, 3H), 2.41 – 2.28 (m, 1H), 0.32 (d, *J* = 5.6 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 177.8, 147.6, 145.1, 143.6, 141.6, 134.4, 132.2, 129.2, 128.5, 126.8, 123.9, 123.8, 122.0, 121.6, 116.3, 115.5, 114.0, 113.1, 109.5, 64.0, 55.6, 46.4, 40.1, 13.2; **HRMS** (m/z), calculated for $C_{24}H_{23}N_2O_3^+$: 387.1703, found $(M + H)^+$: 387.1716.

4'-(4-Hydroxy-3-methoxyphenyl)-3'-methyl-8'-(trifluoromethyl)-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2q)
^{1}H NMR (500 MHz, DMSO-*d*6) δ 10.45 (s, 1H), 8.84 (s, 1H), 7.33 – 7.26 (m, 1H), 7.23 (td, *J* = 7.7, 1.2 Hz, 1H), 7.05 (dd, *J* = 7.5, 1.2 Hz, 1H), 6.95 (td, *J* = 7.5, 1.0 Hz, 1H), 6.86 (dd, *J* = 7.8, 1.0 Hz, 1H), 6.76 – 6.70 (m, 3H), 6.59 (t, *J* = 7.8 Hz, 2H), 6.26 (s, 1H), 3.85 (d, *J* = 12.1 Hz, 1H), 3.71 (s, 3H), 2.32 (dq, *J* = 13.1, 6.6 Hz, 1H), 0.31 (d, *J* = 6.6 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-*d*6) δ 177.7, 147.8, 145.3, 142.1, 141.1, 133.6, 133.3, 131.6, 128.6, 128.1, 126.4, 124.9 (q, $J_{C\text{-}F}$ = 271.0 Hz), 124.7, (q, $J_{C\text{-}F}$ = 6.1 Hz), 123.9 (2C), 121.9, 115.7, 115.2, 111.2, (q, $J_{C\text{-}F}$ = 28.4 Hz), 109.4, 64.9, 55.7, 45.8, 38.7, 12.9; **HRMS** (m/z), calculated for $C_{25}H_{22}F_3N_2O_3^+$: 455.1577, found $(M + H)^+$: 455.1583.

6'-Chloro-4'-(4-hydroxy-3-methoxyphenyl)-3',8'-dimethyl-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2r)
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.52 (s, 1H), 8.91 (s, 1H), 7.21 (t, *J* = 7.4 Hz, 1H), 6.98 (d, *J* = 7.1 Hz, 1H), 6.92 (t, *J* = 7.3 Hz, 1H), 6.90 – 6.82 (m, 2H), 6.74 (d, *J* = 7.9 Hz, 1H), 6.70 (s, 1H), 6.56 (s, 1H), 6.29 (s, 1H), 5.99 (s, 1H), 3.80 (d, *J* = 11.9 Hz, 1H), 3.71 (s, 3H), 2.36 – 2.18 (m, 1H), 2.02 (s, 3H), 0.30 (d, *J* = 5.9 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 177.9, 147.7, 145.2, 141.8, 140.7, 133.8, 132.2, 128.5, 127.5, 126.2, 125.4, 123.9, 123.6, 121.9 (2C), 119.0, 115.7, 113.0, 109.4, 64.9, 55.7, 46.2, 39.1, 17.5, 13.0; **HRMS** (m/z), calculated for $C_{25}H_{24}ClN_2O_3^+$: 435.1470, found $(M + H)^+$: 435.1476.

4'-(4-Hydroxy-3-methoxyphenyl)-3'-methyl-7'-(trifluoromethyl)-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2s)
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (s, 1H), 8.73 (s, 1H), 7.21 (td, *J* = 7.7, 0.9 Hz, 1H), 7.16 (t, *J* = 7.9 Hz, 1H), 6.95 – 6.84 (m, 3H), 6.80 (d, *J* = 7.5 Hz, 2H), 6.64 (d, *J* = 8.1 Hz, 1H), 6.57 (d, *J* = 1.6 Hz, 1H), 6.40 (dd, *J* = 8.1, 1.8 Hz, 1H), 5.75 (s, 1H), 3.95 (d, *J* = 10.1 Hz, 1H), 3.64 (s, 3H), 2.26 (dq, *J* = 13.3, 6.6 Hz, 1H), 0.50 (d, *J* = 6.7 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 176.6, 146.9, 146.7, 144.9, 141.8, 136.2, 130.7, 128.8, 128.0 (q, $J_{C\text{-}F}$ = 29.2 Hz), 127.3, 124.6 (q, $J_{C\text{-}F}$ = 274.6 Hz), 122.92, 122.89, 122.0, 121.3, 119.7, 116.5 (q, $J_{C\text{-}F}$ = 6.3 Hz), 115.2, 113.7, 109.9, 63.3, 55.8, 45.1, 40.1, 13.6; **HRMS** (m/z), calculated for $C_{25}H_{22}F_3N_2O_3^+$: 455.1577, found $(M + H)^+$: 455.1572.

4'-(4-Hydroxy-3-methoxyphenyl)-3'-methyl-6'-(trifluoromethyl)-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2t)
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.63 (s, 1H), 8.90 (s, 1H), 7.27 (s, 1H), 7.25 – 7.20 (m, 2H), 7.10 (d, *J* = 7.5 Hz, 1H), 6.97 (t, *J* = 7.6 Hz, 1H), 6.89 (d, *J* = 7.7 Hz, 1H), 6.79 – 6.72 (m, 2H), 6.70 (s, 1H), 6.66 (d, *J* = 8.5 Hz, 1H), 6.61 (d, *J* = 8.0 Hz, 1H), 3.88 (d, *J* = 12.0 Hz, 1H), 3.71 (s, 3H), 2.33 (dq, *J* = 13.5, 6.7 Hz, 1H), 0.32 (d, *J* = 6.5 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 177.5, 147.7, 146.9, 145.3, 141.6, 132.9, 131.6, 128.8, 126.0 (q, $J_{C\text{-}F}$ = 5.2 Hz), 125.2 (q, $J_{C\text{-}F}$ = 270.0 Hz), 124.2, 124.00 (q, $J_{C\text{-}F}$ = 3.4 Hz), 123.4, 122.3, 122.0, 115.8 (q, $J_{C\text{-}F}$ = 5.0 Hz), 115.5, 113.4 (2C), 109.6, 63.9, 55.7, 45.6, 38.7, 13.0; **HRMS** (m/z), calculated for $C_{25}H_{22}F_3N_2O_3^+$: 455.1577, found $(M + H)^+$: 455.1587.

7'-Bromo-4'-(4-hydroxy-3-methoxyphenyl)-3'-methyl-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2u)
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.60 (s, 1H), 8.76 (s, 1H), 7.21 (t, *J* = 7.5 Hz, 1H), 6.92 – 6.84 (m, 3H), 6.82 (d, *J* = 7.9 Hz, 1H), 6.74 (d, *J* = 7.5 Hz, 1H), 6.68 – 6.62 (m, 4H), 6.45 (d, *J* = 8.0 Hz, 1H), 3.69 (d, *J* = 10.0 Hz, 1H), 3.66 (s, 3H), 2.34 – 2.26 (m, 1H), 0.53 (d, *J* = 6.3 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 176.6, 147.5, 147.0, 144.6, 141.7, 135.8, 130.7, 128.7, 128.5, 125.1, 124.4, 123.0, 122.3, 121.9, 121.3, 115.3, 114.8, 114.1, 109.8, 63.8, 55.7, 48.4, 45.2, 13.5; **HRMS** (m/z), calculated for $C_{24}H_{22}{}^{79}BrN_2O_3^+$: 465.0808, found $(M + H)^+$: 465.0814

8'-Chloro-4'-(4-hydroxy-3-methoxyphenyl)-3'-methyl-3',4'-dihydro-1'*H*-spiro[indoline-3,2'-quinolin]-2-one (2v)

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.49 (s, 1H), 8.85 (s, 1H), 7.23 (t, *J* = 7.7 Hz, 1H), 7.09 (d, *J* = 6.9 Hz, 1H), 6.99 (d, *J* = 7.6 Hz, 1H), 6.93 (t, *J* = 7.5 Hz, 1H), 6.86 (d, *J* = 7.7 Hz, 1H), 6.74 (d, *J* = 8.0 Hz, 1H), 6.70 (s, 1H), 6.57 (d, *J* = 8.0 Hz, 1H), 6.51 – 6.44 (m, 2H), 6.35 (s, 1H), 3.84 (d, *J* = 11.8 Hz, 1H), 3.70 (s, 3H), 2.33 (dq, *J* = 13.6, 7.1, 6.5 Hz, 1H), 0.32 (d, *J* = 6.6 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 177.6, 147.7, 145.2, 142.0, 139.8, 133.6, 131.9, 128.6, 128.2, 127.2, 126.0, 123.8, 121.9, 121.6, 117.1, 116.4, 115.6, 113.1, 109.4, 64.1, 55.6, 46.2, 39.9, 13.0; **HRMS** (m/z), calculated for $C_{24}H_{22}ClN_2O_3^+$: 421.1313, found $(M + H)^+$: 421.1325.

2.1.4 General procedure for the one-pot synthesis of spiroindolones

A mixture of isatin (1 mmol), the appropriately substituted aniline (1.0 eq, 1 mmol) and either 3,4-dihydro-2*H*-pyran or isoeugenol (1.0 eq, 1 mmol) were dissolved in acetonitrile (10 ml) and treated with $InCl_3$ (0.2 eq, 0.2 mmol). The resulting heterogeneous mixture was stirred at room temperature under a nitrogen atmosphere for 72 hours. The reaction was quenched with water (10 ml) and extracted with ethyl acetate (3 × 20 ml). The organic layer was dried (Na_2SO_4), concentrated under vacuum, and the resulting residue purified by silica gel column chromatography (30-50% EtOAc/hexane).

2.2 *In vitro* antiplasmodial assays

2.2.1 ^{3}H-Hypoxanthine incorporation assay against FCR-3 strain. The chloroquine-resistant Gambian FCR-3 strain was cultured *in vitro* according to the method described by Jensen and Trager (Trager & Jensen, 1976) and van Zyl *et al* (Van Zyl, Seatlholo, & Viljoen, 2010) under an optimal atmosphere of 5% CO_2, 5% O_2, 90% N_2 in human erythrocytes taken from volunteers of various blood groups and Rh factors. For experimental purposes the cultures were synchronized with 5% D-sorbitol when the parasites were in the ring stage (Lambros & Vanderberg, 1979). The antimalarial activity of the various compounds was determined using the tritiated hypoxanthine incorporation assay (Desjardins, Canfield, Haynes, & Chulay, 1979). The parasite suspension, consisting of predominately the ring stage, was adjusted to a 0.5% parasitaemia and 1% haematocrit in hypoxanthine-free RPMI-1640 culture medium with 10% human plasma and exposed to 6 to 7 concentrations of each compound for a single cycle of parasite growth. All assays were carried out using untreated parasites and uninfected red blood cells as controls. Labelled ^{3}H-hypoxanthine was added after 24 h and the parasitic ^{3}H-DNA harvested after a further 24 h incubation period at 37°C in a humidified environment. The concentration that inhibited 50% of parasite growth (IC_{50} value) was determined from the sigmoidal log dose response curve generated by the Enzfitter® and GraphPad Prism® software.

2.2.2 pLDH assay against 3D7 strain. Three-fold serial dilutions of the test compounds were incubated with 3D7 strain *P. falciparum* parasites (2% parasitaemia, 1% haematocrit) in 96-well plates containing RPMI 1640 medium supplemented with 25 mM HEPES, 0.5% (w/v) Albumax II, 22 mM glucose, 0.65 mM hypoxanthine, 0.05 mg/mL gentamicin and 1% (v/v) human erythrocytes. Incubations were initiated when parasites were predominantly in the trophozoite stage and continued for 48 h at 37°C in sealed containers filled with an atmosphere of 5% CO_2, 5% O_2, 90% N_2. Subsequently, a colourimetric assay for parasite lactate dehydrogenase (pLDH) activity in individual wells was carried out (Makler et al., 1993). Twenty µL of culture was removed from the individual wells and transferred to a second 96-well plate containing 125 µL per well pLDH assay reagent (44 mM Tris buffer, pH 9, containing 0.18 M L-lactic acid, 0.13 mM acetylpyridine adenine dinucleotide, 0.39 mM nitrotetrazolium blue chloride, 0.048 mM phenazine ethosulfate and 0.16% (v/v) Triton X-100) and incubated at ambient temperature for 10 – 30 minutes, after which Abs_{620} was measured in a Spectramax M3 plate reader. Absorbance values were used to calculate percentage parasite viability relative to control wells containing untreated parasite cultures. IC_{50} values for individual compounds were calculated from plots of % viability vs. log[compound] by non-linear regression using GraphPad Prism®.

3 RESULTS AND DISCUSSION

The Povarov reaction of ketimines derived from isatin typically requires the use an acid catalyst owing to the low reactivity of the ketimine. Lewis acids, such as indium trichloride (Bianchini et al., 2016; Ramesh et al., 2008), antimony pentachloride (Karmakar et al., 2014) and boron trifluoride (Kouznetsov et al., 2008), *p*-toluenesulfonic acid (Gao et al., 2014), as well as chiral phosphoric acids (Shi et al., 2013; Zhang et al., 2014) have been reported to catalyse this reaction. We wanted to assess the ability of a range of catalysts to

facilitate the Povarov reaction of a ketimine derived from isatin. To this end, ketimine **4a** was prepared for a model study by reaction of isatin **3** with *p*-toluidine in absolute ethanol (Scheme 2). The imine was then treated with 3,4-dihydro-2*H*-pyran in the presence of an acid catalyst as described in Table 1, to afford spiroindolone **2a** as a single regioisomer.

In each case, the *cis* product was isolated from the reaction mixture as the major product, with only traces of the *trans* product identified. Raghunathan and co-workers reported the use of $InCl_3$ in this reaction affording the desired product as a mixture of isomers in a yield of 66% (77:23 *cis*:*trans*) (Ramesh et al., 2008). In our hands, only the *cis* product was isolated in a yield of 42% under these conditions (entry 1 of Table 1). Increasing the temperature of the reaction or catalyst loading did not significantly improve the yield of the reaction (entry 2 and 3 of Table 1), with starting material recovered in each case (approximately 35%), even when the reaction was run for extended periods of time. By comparison, CuOTf, Montmorillonite K10 and Montmorillonite KSF afforded the product in comparatively lower yields (entry 4, 5 and 6 of Table 1). For $ZnCl_2$, a change in solvent resulted in improved yields of product (entry 10 and 11 of Table 1), while in other cases this had little effect (eg. for Montmorillonite K10, entry 7 and 8 of Table 1). Increasing the temperature of the reactions catalysed by $ZnCl_2$ and Montmorillonite K10, or the use of microwave (MW) irradiation, resulted in a slight decrease in the yield of product isolated (compare entries 7, 8 and 9, and 11, 12 and 13 of Table 1), while only starting material was recovered for the reactions with *p*-TsOH and $Ti(O^iPr)_4$ as catalyst (entry 14, 15 and 16 of Table 1). The use of catalytic $BF_3{\cdot}OEt_2$ afforded a reasonable yield of the product (entry 17 of Table 1), with a higher yield of product obtained when 0.5 mole equivalents of $BF_3{\cdot}OEt_2$ was used (entry 18 of Table 1). Increasing this further resulted in a decrease in product yield (entry 19 of Table 1). We therefore chose to employ $BF_3{\cdot}OEt_2$ as catalyst in the synthesis of a range of spiroindolones derived from 3,4-dihydro-2*H*-pyran.

A series of ketimines **4b**-**h** were then prepared by reaction of isatin **3** with substituted anilines, followed by Povarov reaction with 3,4-dihydro-2*H*-pyran in the presence of $BF_3{\cdot}OEt_2$ to afford spiroindolones **2b**-**h** in moderate yields (Table 2), with starting material recovered in most cases. Once again, the *cis* product was isolated as the major product from each reaction.

Other electron rich alkenes were tested in the Povarov reaction, including *trans*-isoeugenol **5** and ethyl vinyl ether. While spiroindolone products derived from *trans*-isoeugenol were formed using $BF_3{\cdot}OEt_2$ as catalyst, we were unable to isolate any of the desired products when ethyl vinyl ether was used as the electron rich alkene. As shown in Scheme 3, when imine **4a** derived from isatin **3** and *p*-toluidine was reacted with *trans*-isoeugenol **5** using the optimised conditions described above for the Povarov reaction with 3,4-dihydro-2*H*-pyran, the desired *trans* product **2i** was isolated in a yield of 32%. Increasing the amount of $BF_3{\cdot}OEt_2$ used in the reaction to one mole equivalent resulted in the product being isolated in a yield comparable to that reported previously (Kouznetsov et al., 2008).

Ketimines **4b**-**n** were then reacted with *trans*-isoeugenol in the presence of 1 mole equivalent of $BF_3{\cdot}OEt_2$ to afford spiroindolones **2j**-**v**, in moderate yields (Table 3). With the use of *trans*-isoeugenol as the substrate, the *trans* product was isolated from the reaction mixture as the major product in each case.

We then tested the one-pot reaction of isatin **3**, *p*-toluidine and 3,4-dihydro-2*H*-pyran to form the product **2a** in a single step (entry 1 of Table 4). In this case, we found that the use of $InCl_3$ was best to facilitate the one-pot preparation of spiroindolones **2**, as reported by Raghunathan (Ramesh et al., 2008). The one-pot reaction resulted in a higher overall yield of product **2a**, and therefore we repeated the one-pot reaction on selected substrates that were particularly low yielding in the two step process (Table 4). With the exception of **2v** (entry 5 of Table 4), the yields of isolated products improved significantly, suggesting that the one-pot method is superior to the two step approach for these compounds.

Compounds prepared were then assessed for antiplasmodial activity in a whole cell *P. falciparum* screen against a drug-sensitive strain (3D7) and a drug-resistant strain (FCR-3) of the parasite. Those compounds that were found to inhibit parasite growth by 50% or more at a concentration of 5 μM (for the FCR-3 strain) or by 75% or more at a concentration of 20 μM (for the 3D7 strain) were then assessed further and IC_{50} values obtained (Table 5).

As can be seen from the results in Table 5, a wider range of spiroindolones showed moderate antiplasmodial activity against the drug resistant FCR-3 strain, than against the drug sensitive 3D7 strain. Compounds derived from an aniline bearing a substituent in the 3-position did not show any antiplasmodial activity. Only compounds derived from isoeugenol and an aniline bearing an electron withdrawing group in either the 2- or the 4-position of the aromatic ring showed activity against the 3D7 strain (**2j**, **2n**, **2r**, entry 5, 7 and 8 of Table 5). Two of these compounds also showed activity against the FCR-3 strain (**2n** and **2r**, entry 7 and 8 of Table 5), along with an additional two compounds derived from isoeugenol (**2i** and **2k**, entry 4 and 6 of Table 5). Furthermore, three compounds derived from 3,4-dihydro-2*H*-pyran showed moderate activity against the FCR-3 strain (**2a**, **2f** and **2g**, entry 1, 2 and 3 of Table 5). Significantly, all the compounds displaying activity against the FCR-3 strain were derived from an aniline bearing either a methyl substituent or a halogen at the 4-position of the aromatic ring, irrespective of whether isoeugenol or 3,4-dihydro-2*H*-pyran was used as the diene in the Povarov reaction.

4 CONCLUSIONS

We have prepared a series of 3',4'-dihydro-1'*H*-spiro(indoline-3,2'-quinolin)-2-ones by the Povarov reaction of electron rich alkenes and ketimines derived from isatin and assessed them for antiplasmodial activity *in vitro*. Eight of the compounds prepared displayed antiplasmodial activity in the low micromolar range against either the 3D7 or the FCR-3 strain. Interestingly, compounds derived from 3,4-dihydro-2*H*-pyran did not show activity against the 3D7 strain but did show activity in the low micromolar range against the FCR-3 strain. Compounds derived from isoeugenol displayed moderate antiplasmodial activity against both drug-resistant and drug-sensitive strains of the parasite. Although the compounds were less potent than Chloroquine, the structure-activity relationships observed from this study have been utilised in the design of a second generation of analogues currently under investigation in our laboratories.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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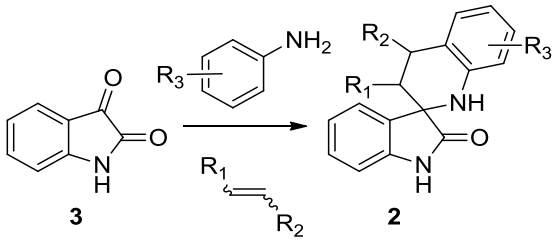
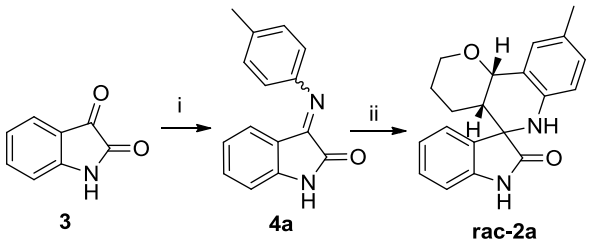
FIGURES AND SCHEMES:

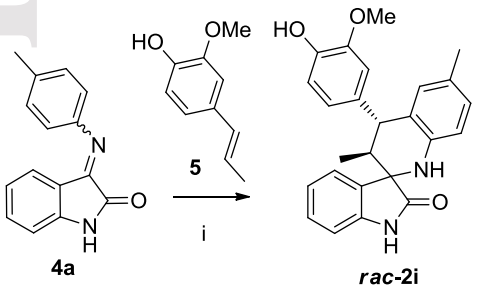
FIGURE 1 Cipargamin, **1** and spiroindolones **2** prepared by Povarov reaction.



SCHEME 1 Reaction of isatin **3** in Povarov reaction to afford spiroindolones **2.**



SCHEME 2 Reagents and conditions: (i) *p*-toluidine, abs. EtOH, gl. AcOH, reflux, 15h, 96 %; (ii) 3,4-dihydro-2*H*-pyran, conditions as described in Table 1.



SCHEME 3 Reagents and conditions: (i) $BF_3{\cdot}OEt_2$; CH_2Cl_2, rt, 32% (0.5 eq $BF_3{\cdot}OEt_2$); 45% (1.0 eq. $BF_3{\cdot}OEt_2$).

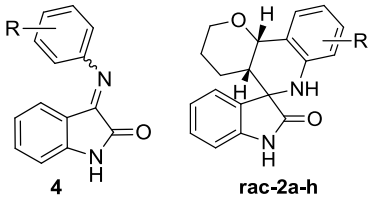
TABLES:

TABLE 1 Screening of catalysts for Povarov reaction of ketimine **4a** with 3,4-dihydro-2*H*-pyran

	Catalyst	Solvent	Temp	Time (h)	Yield of 2a
1	$InCl_3$ (20 mol %)	CH_3CN	rt[†]	72	42 %
2	$InCl_3$ (20 mol %)	CH_3CN	40°C	24	47 %
3	$InCl_3$ (50 mol %)	CH_3CN	rt	72	48 %
4	CuOTf (20 mol %)	CH_2Cl_2	rt	72	38 %
5	Montmorillonite K10 (0.2 mass eq.)	CH_2Cl_2	rt	72	34 %
6	Montmorillonite KSF (0.2 mass eq.)	CH_2Cl_2	rt	72	28 %
7	Montmorillonite K10 (1 mass eq.)	CH_2Cl_2	rt	72	42 %
8	Montmorillonite K10 (1 mass eq.)	CH_3CN	reflux	72	38 %
9	Montmorillonite K10 (1 mass eq.)	CH_3CN	65°C[‡]	3	28 %
10	$ZnCl_2$ (20 mol %)	CH_2Cl_2	rt	72	No reaction
11	$ZnCl_2$ (20 mol %)	CH_3CN	rt	72	36 %
12	$ZnCl_2$ (20 mol %)	CH_3CN	reflux	72	22 %
13	$ZnCl_2$ (20 mol %)	CH_3CN	65°C[‡]	3	30 %
14	*p*-TsOH (5 mol %)	CH_2Cl_2	rt	72	No reaction
15	$Ti(O^iPr)_4$ (10 mol %)	CH_2Cl_2	rt	72	No reaction
16	$Ti(O^iPr)_4$ (10 mol %)	CH_3CN	reflux	24	No reaction
17	$BF_3{\cdot}OEt_2$ (20 mol %)	CH_2Cl_2	rt	72	45%
18	$BF_3{\cdot}OEt_2$ (50 mol %)	CH_2Cl_2	rt	72	63%
19	$BF_3{\cdot}OEt_2$ (1 mol eq.)	CH_2Cl_2	rt	72	15%

[†]rt = room temperature; [‡]reactions conducted under MW irradiation (150W)

TABLE 2 Spiroindolones **2** prepared by Povarov reaction of imines **4** with 3,4-dihydro-2*H*-pyran

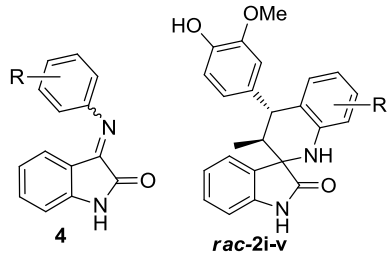


4 **rac-2a-h**

Entry	R	Yield %		Yield %		RSM %[†]
1	4-Me	**4a**	96	**2a**	63	15
2	2-F	**4b**	73	**2b**	68	11
3	2-Cl, 4-Me	**4c**	35	**2c**	22	48
4	3-Cl	**4d**	84	**2d**	49	26
5	4-Cl	**4e**	65	**2e**	54	18
6	4-Br	**4f**	82	**2f**	60	22
7	2,4-diMe	**4g**	75	**2g**	62	14
8	H	**4h**	58	**2h**	45	33

[†]RSM % = % starting material recovered

TABLE 3 Spiroindolones **2** prepared by Povarov reaction of imines **4** with *trans*-isoeugenol



4 ***rac*-2i-v**

Entry	R	Yield %		Yield %		RSM %[†]
1	4-Me	**4a**	96	**2i**	45	34
2	2-F	**4b**	73	**2j**	25	62
3	2-Cl, 4-Me	**4c**	35	**2k**	28	49
4	3-Cl	**4d**	84	**2l**	36	41
5	4-Cl	**4e**	65	**2m**	52	24
6	4-Br	**4f**	82	**2n**	48	29
7	2,4-diMe	**4g**	75	**2o**	65	9
8	H	**4h**	58	**2p**	54	25
9	2-CF_3	**4i**	46	**2q**	24	68
10	2-Me, 4-Cl	**4j**	82	**2r**	64	18
11	3-CF_3	**4k**	67	**2s**	42	32
12	4-CF_3	**4l**	58	**2t**	56	22
13	3-Br	**4m**	72	**2u**	45	30
14	2-Cl	**4n**	80	**2v**	30	66

[†]RSM % = % starting material recovered

TABLE 4 Spiroindolones **2** prepared by one-pot reaction[†]

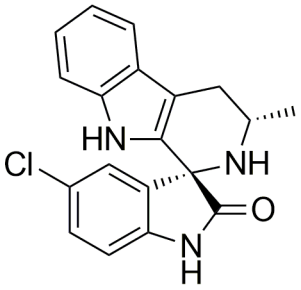
Entry	Compound	Yield %	RSM %[‡]
1	**2a**	72	12
2	**2c**	45	21
3	**2i**	53	26
4	**2k**	52	38
5	**2v**	31	42

[†]$InCl_3$ (20 mol %), CH_3CN, rt, 72 hr; [‡]RSM % = % starting material recovered

TABLE 5 *In vitro* antiplasmodial activity of spiroindolones **2**

Entry	Compound	IC_{50} 3D7 strain (μM)[†]	IC_{50} FCR-3 strain (μM)[‡]
1	**2a**	Not active	3.75 ± 0.39
2	**2f**	Not active	3.73 ± 1.57
3	**2g**	Not active	3.21 ± 1.27
4	**2i**	Not active	2.28 ± 1.73
5	**2j**	1.48 ± 1.50	Not active
6	**2k**	Not active	4.20 ± 1.29
7	**2n**	1.80 ± 1.86	1.67 ± 0.71
8	**2r**	1.31 ± 1.18	1.52 ± 0.84
9	CQ[§]	0.0127 ± 0.0030[¶]	0.110 ± 0.002

Data are presented as the mean ± SD of three experiments; [†]IC_{50} values determined by pLDH assay; [‡]IC_{50} values determined by ^{3}H-hypoxanthine incorporation assay; [§]CQ = chloroquine; [¶]similar IC_{50} value obtained for CQ using ^{3}H-hypoxanthine incorporation assay



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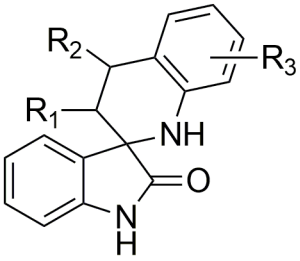

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