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Synthesis of deuterium labelled chloroquine, hydroxychloroquine and their metabolites

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ABSTRACT

This paper describes the synthesis of deuterium-labelled chloroqiune, hydroxychloroquine and their metabolites. Mass spectrometry analysis of these compounds revealed over 98% deuterium enrichment.

Keywords: deuterium-labelled; chloroquine; hydroxychloroquine; metabolite; synthesis

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INTRODUCTION

The popularity of chloroquine and hydroxychloroquine for malaria treatment in many Third World countries emanates from it being cheap, widely available, relatively well tolerated, and having a rapid onset of action [1-4]. Besides being active against malaria, they are used to treat rheumatoid arthritis [5-6] and cutaneous lupus erythematosus (LE) and rashes associated with systemic lupus erythematosus (SLE) [7-8]. They are also used in some photosensitivity disorders and occasionally in other inflammatory skin conditions [9-10]. In addition, chloroquine and hydroxychloroquine are two inexpensive agents that have been shown to achieve some level of anti-HIV activity [11]. Metabolism studies revealed that chloroquine and hydroxychloroquine are mainly metabolized to the still active metabolite desethylchloroquine and desethylhydroxychloroquine respectively firstly and then convert to the same metabolite bisdesethylchloroquine, as shown in Figure 1 [12-13]. Although ^{3}H&^{14}C chloroquine and hydroxychloroquine have been prepared for pharmacological studies, the synthesis of their stable labeled internal standard has not been described in details. In this paper, the synthetic route to [$^{2}H_4$] chloroquine, [$^{2}H_5$] hydroxychloroquine and their metabolites were described in detail.

EXPERIMENTAL SECTION

General

All reagents were obtained from Sigma-Aldrich and CDN Isotope. Mass spectra were recorded using a Quattro micro API mass spectrometer. ^{1}H NMR spectra were recorded on a Bruker 300 MHz instrument (Bruker Corporation, Germany). Chemical purities were determined by an Agilent 1200 HPLC with a XDB-C18 column, 5 μm, 4.6 mm×150 mm (Agilent, USA).

Synthesis of (Z)-5-hydroxypentan-2-one oxime (2)

A mixture of 5-hydroxypenta-2-one (1) (80 g, 0.78 mol) and hydroxylamine (65 g, 0.94 mol) in water (640 mL) was added KOH (37 g, 0.67 mol) slowly under ice/water bath. The reaction solution was stirred for 8 h at room temperature under N_2. The reaction solution was cooled to 0 °C and acidified to pH 8 by adding conc. HCl. The solution was extracted by EtOAc (200 mL×8). The organic layers were combined, dried by Na_2SO_4 and

concentrated under reduced pressure to give (2) as a light yellow oil (41.36 g, 90%).

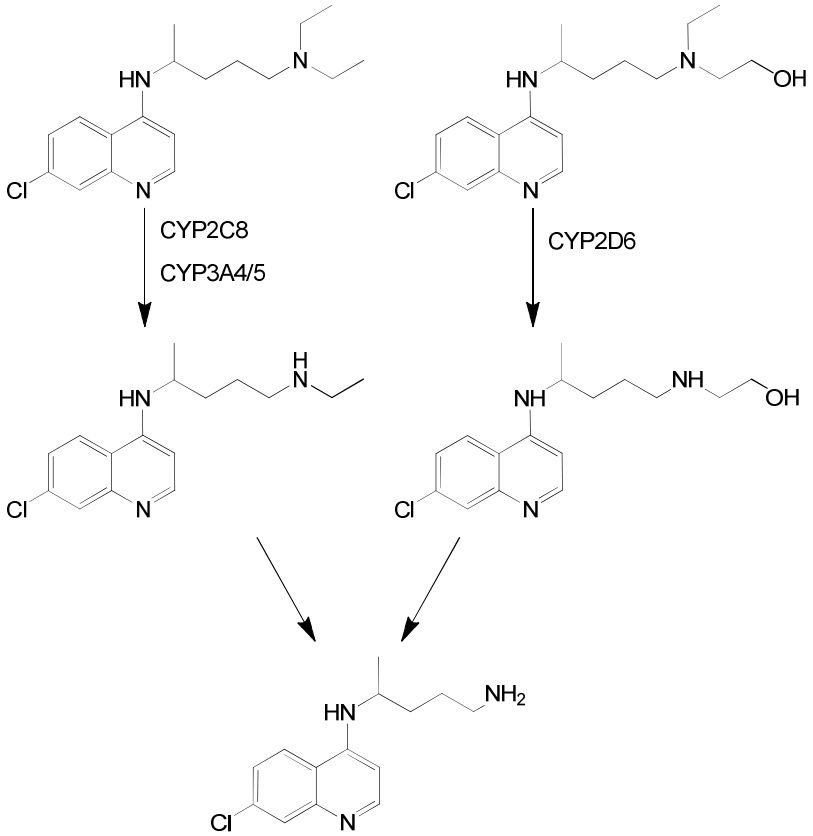


Figure 1. The metabolite routes of chloroquine and hydroxychloroquine

Synthesis of 4-aminopentan-1-ol (3)

A slurry of (2) (15 g, 0.13 mol) and Raney Ni (5 g) in MeOH (60 mL) was placed in a stainless steel pressure vessel, which was pressurized to 0.8 Mpa with H_2 gas. The reaction was mechanically stirred for 4 h at 65 °C. The mixture was cooled to room temperature. The Raney Ni was removed by filtration through celite, and the solid was rinsed with MeOH (25 mL×3). The combined filtrates were concentrated to dryness to give (3) as a little blue oil (13.1 g, 98%).

^{1}H NMR (300 MHz, $CDCl_3$): δ3.66 (t, 2H, J= 7.1 Hz), 2.89 (m, 1H), 2.81 (brs, 3H), 1.69 (m, 3H), 1.35 (m, 1H), 1.12 (d, 3H, J=6.8 Hz).

Synthesis of 4-(7-chloroquinolin-4-ylamino)pentan-1-ol (4)

To a solution of compound (3) (27 g, 0.262 mol) and 4, 7-dichloroquinoline (25.9 g, 0.131 mol) was stirred at 140 °C for 5 h. The reaction mixture was cooled to 100°C, poured into water (100 mL) and stirred to induce crystallization. The resulting solid was collected by filtering and rinsed thoroughly with water (50 mL×3). The crude product was recrystallized from methanol/ethanol to give (4) as colorless solid (16.8 g, 48.41%).

^{1}H NMR (DMSO, 300 Hz): δ 8.36 (d, 1H, J=7.5 Hz), 8.34 (d, 1H, J= 1.5 Hz), 7.75 (d, 1H, J= 7.5 Hz), 7.40(d, 1H, J=7.5 Hz), 6.93 (d, 1H, J=7.5 Hz), 6.42 (d, 1H, J=2.5 Hz), 4.56 (brs, 1H), 1.45-1.85 (m, 4H), 1.19 (t, 3H, J=6.8 Hz).

Synthesis of N-(5-bromopentan-2-yl)-7-chloroquinolin-4-amine hydrobromide (5)

To a solution of 48% hydrobromic acid (45.5 mL) was added conc. H_2SO_4 (9.7 mL). Compound (4) (13 g, 49.1 mmol) was dissolved in the acid solution, and the resulting solution heated to 105°C as rapidly as possible. The mixture was stirred continuously for 15 min. The solution was cooled to room temperature and diluted with water (50 mL). The solution was extracted with CH_2Cl_2 (50 mL×6). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give (5) as a grey solid (16.5 g, 82.3%).

^{1}H NMR (DMSO, 300 Hz): δ 1.19 (3H, t, J= 6.8 Hz), 1.43-1.68 (4H, m), 1.45-1.85 (4H, m), 3.42 (2H, t, J=7.1 Hz), 4.56 (1H, brs), 6.42 (1H, br d, J= 5.4 Hz), 6.93 (1H, d,), 7.40(1H, d), 7.76 (1H, d), 8.52 (2H, dd),

Synthesis of N^4-(7-chloroquinolin-4-yl)-N^1-ethylpentane-1, 4-diamine (6)
To a solution of (5) (11g, 26.92 mmol) in methanol (100 mL) was added ethylamine solution (70%, 110 mL) under ice/water bath. The solution was stirred for 8 h at room temperature. TLC showed little starting material remained. The solution was co-evaporated with ethanol (150 mL×3) to afford a yellow solid. The solid was purified by column chromatography on silica gel column, eluted with CH_2Cl_2/saturated methanol ammonia (10: 0.3) to afford (6) as a silver gray solid (3.62 g, 46.07%).

Synthesis of [$^2H_5$] chloroquine diphosphate (7)
To a suspension of (6) (1.8 g, 6.17 mmol) and K_2CO_3 (0.85 g, 6.17 mmol) in dry DMF (18 mL) was added [$^2H_5$] ethyl iodide slowly. The reaction solution was stirred for 8 h at room temperature. The mixture was diluted with water (150 mL) under water/ice bath and extracted with EtOAc (100 mL×9). The combined organic layers were evaporated to dryness to afford a colorless liquid. The crude product was purified by column chromatography on silica gel column, eluted with CH_2Cl_2/ saturated methanol ammonia (10: 0.7) to afford a silver gray solid (3.62 g, 46.07%). The solid (0.5 g, 1.54 mmol) was dissolved in dry ethanol (4 mL) and heated under reflux. The solution was adjusted to pH 5 by adding H_3PO_4 (0.36 g, 85%). The mixture was stirred continuously for 3 h. The resulting solid was collected by filtering, and compound (10) was obtained as a white solid (0.651g, 81.2%).

^{1}H NMR (DMSO) δ 8.38 (dd, 2H, J= 7.5, 7.5 Hz), 7.74 (d, 1H, J=2.1 Hz), 7.42(d, 1H, J=9.0 Hz), 7.02 (d, 1H, J= 7.5 Hz), 6.50 (brd, 1H, J= 5.4 Hz), 2.35-2.75 (m, 5H), 1.45-1.85 (m, 4H), 1.22 (d, 3H, J= 6.3 Hz), 1.02 (t, 3H, J= 6.9 Hz). MS-EI, (m/z): 325.2 (100), 326.2 (22), 327.2 (36), 328.2 (8). HPLC (XDB-C18, wavelength= 240 nm, CH_3OH/10mmol/L K_2HPO_4=83/17, 1.0 mL/min): t_R 6.79 min (98.7%). Isotopic enrichment determined by MS was over 98%.

Synthesis of [$^2H_4$]2-(ethylamino)ethanol (9)
To a stirred solution of [$^2H_4$]2-bromoethanol (8) (2 g, 15.51 mmol), $CH_3CH_2NH_2$ (5.59 g, 124.04 mmol) in EtOH/H_2O (25 mL) was heated at 85°C for 8 h. The solution was cooled by ice/water bath and acidified by conc. HCl to pH 2. The mixture was co-evaporated by EtOH to remove water out. White solid and oily solution was appeared. Na_2CO_3 solid (15 g) and ether (100 mL) were added to solution to adjust pH=10. The organic layer was separated, and the aqueous layer was extracted with ether (100 mL×5). The combined organic phases were concentrated under reduced pressure to give (9) as a colorless liquid (1.44 g, 28.1%).

Synthesis of [$^2H_4$]hydroxychloroquine sulfate (10)
A mixture of compound (5) (0.85 g, 2.59 mmol) and compound (9) (0.97 g, 10.38 mol) was stirred for 2 days at room temperature under sealing. TLC showed no starting material remained. The mixture was diluted with water (6 mL) and basified with Na_2CO_3 solid to pH 9. The aqueous solution was extracted with CH_2Cl_2 (100 mL×5). The combined organic layers were concentrated under reduced pressure to give yellow oil. The crude product was purified by column chromatography on silica gel column, eluted with CH_2Cl_2/MeOH (10: 0.2) to afford a colorless solid (0.6 g, 68.1%). The solid (0.45 g, 1.13 mmol) was dissolved in dry ethanol (8 mL) under an ice-water bath, and the solution was acidified by alcoholic solution of sulfuric acid (3 mL, 0.7 mL conc. H_2SO_4 in 28 mL ethanol) to pH 5. The mixture was stirred for 3 hours. The resulting precipitate was filtered, and the compound (10) was obtained as a white solid (0.47g, 83.2%).

^{1}H NMR (D_2O, 300 Hz): δ 8.17 (d, 1H, J=7.5 Hz), 8.12 (d, 1H), 7.76 (d, 1H), 7.53(d, 1H,), 6.73 (br d, 1H, J= 5.4 Hz), 4.02 (brs, 1H), 3.52 (m, 1H), 3.13 (m, 1H), 1.45-1.85 (m, 4H), 1.43-1.68 (m, 3H), 1.14 (t, 3H, J= 6.8 Hz), 1.04 (t, 1H, J= 5.8 Hz). MS-EI (m/z): 170.7 (100), 171.4 (37), 340 (68), 341.2 (16), 342.2 (24). HPLC (XDB-C18, wavelength= 240 nm, CH_3OH/10mmol/L CH_3COONH_4+0.03% TEA=62/38, 1.0 mL/min): t_R=8.95 min (98.5%). Isotopic enrichment determined by MS was over 98%.

Synthesis of N^4-(7-chloroquinolin-4-yl)pentane-1, 4-diamine (11)
A solution of (5) (21.00 g, 51.4 mmol) in methanolic ammonia (210 mL) was stirred at room temperature for 8 h. The solution was evaporated to dryness to give solid. The solid was purified by column chromatography on silica gel column, eluted with CH_2Cl_2/saturated methanol ammonia (10:1) to afford (11) as a colorless solid (6.21 g, 45.8%).

Synthesis of [$^2H_3$] N-(4-(7-chloroquinolin-4-ylamino)-pentyl)acetamide (13)
To a solution of (11) (1.2 g, 4.55 mmol) and Et_3N (0.92, 9.1 mmol) in CH_2Cl_2 was added [$^2H_3$] acetyl chloride (13) (0.445 g, 5.46 mmol) slowly for 30 min. The reaction solution was stirred for 30 min at room temperature. The solution was cooled by an ice/water bath, basified by saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (50 mL×5). The organic layers were combined and concentrated under reduced pressure to give a white solid. The solid was purified by column chromatography on silica gel column, eluted with CH_2Cl_2/saturated methanol ammonia (10:

0.5) to afford (13) as a white solid (1.34 g, 95.3%).

Synthesis of [$^2H_5$] desethylchloroquine (14)
To a suspension of (13) (0.96g, 3.11 mmol) and $LiAlD_4$ (0.261 g, 6.22 mmol) in THF (9.6 mL) was refluxed for 1 h. The reaction solution was cooled to room temperature and diluted with water (50 mL). The solution was extracted with EtOAc (60 mL×4), and the combined organic layers were concentrated under reduced to give a liquid. The crude product was purified by chromatography on a silica gel column and then eluted with CH_2Cl_2/saturated methanol ammonia (10:1) to afford (14) as a white solid (0.62 g, 67.1%).

^{1}H NMR (DMSO, 300 Hz) δ 9.11 (2H, d, J=8.4 Hz), 8.83 (1H, d, J=9.3 Hz), 7.42(1H, d, J=6.9 Hz), 8.05 (1H, d, J=1.8 Hz), 7.75 (1H, d, J= 9.0 Hz), 6.94 (1H, d, J= 7.2 Hz),4.11(1H, brd), 2.84 (2H, m), 1.88(1H, m), 1.68 (3H, m), 1.29 (3H, d, J= 6.3 Hz). MS-EI, (m/z): 296.2 (28), 297.2 (100), 298.2 (38), 299.2 (26), 300.2 (9). HPLC (XDB-C18, wavelength= 240 nm, CH_3OH/10mmol/L K_2HPO_4=75/25, 1.0 mL/min): t_R=6.79 min (99.2%). Isotopic enrichment determined by MS was over 98%.

Synthesis of [$^2H_4$] desethylhydroxychloroquine (15)
To a stirred solution of (11) (5 g, 18.95 mmol) and [$^2H_4$] 2-bromoethanol (1.22 g, 9.47 mmol) in methanol (100 mL) was under ice/water bath. The solution was stirred for 8 h at room temperature. TLC showed little starting material remained. The solution was co-evaporated with ethanol (150 mL×3) to give yellow solid. The solid was purified by column chromatography on silica gel column, eluted with CH_2Cl_2/ saturated methanol ammonia (10: 0.3) to afford (15) as a silver gray solid (1.36 g, 46.1%).

^{1}H NMR ($CDCl_3$, 300 Hz) δ 8.48 (1H, d, J=5.4 Hz), 7.92 (1H, d, J=2.1 Hz), 7.70 (1H, d, J=9.0 Hz), 7.32 (1H, d, J=2.1 Hz), 7.29 (1H, d, J= 2.4 Hz), 6.38 (1H, d, J= 5.4 Hz), 5.41 (1H, brd), 3.70(1H, m), 2.74 (2H, m), 1.88(2H, m), 1.68 (2H, m), 1.29 (3H, d, J= 6.3 Hz). MS-EI, (m/z): 311.8 (100), 312.8 (43), 313.8 (16), 314.8 (6). HPLC (XDB-C18, wavelength= 240 nm, CH_3OH/10mmol/L K_2HPO_4=65/35, 1.0 mL/min): t_R=8.67 min (99.9%). Isotopic enrichment determined by MS was over 98%.

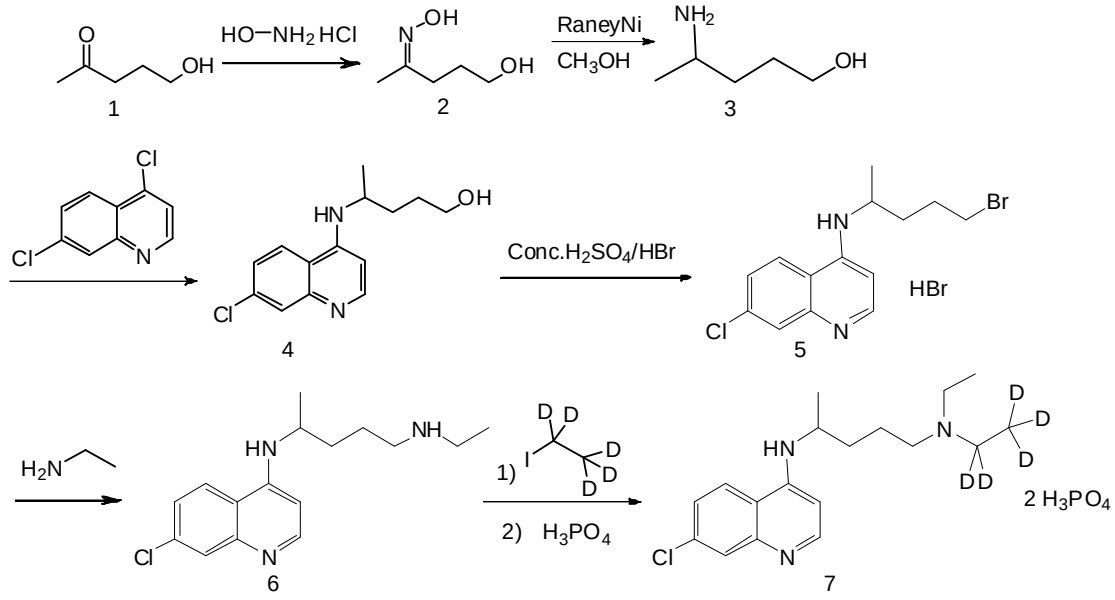
RESULTS AND DISCUSSION

Although chloroquine, hydroxychloroquine and desethylchloroquine have been readily prepared via several synthetic routes [15-22], the synthesis of [$^2H_5$] chloroquine, [$^2H_4$] hydroxychloroquine, [$^2H_5$] desethylchloroquine and their metabolites have not been described previously. Scheme 1 presents the general synthetic scheme for preparing [$^2H_5$] chloroquine. 5-hydroxypenta-2-one (1) was condensed with hydroxylamine hydroxychloride in the presence of KOH to yield (Z)-5-hydroxypentan-2-one oxime (2). Hydrogenolysis of compound (2) with Raney nickel and hydrogen (0.8 Mpa) afforded 4-aminopentan-1-ol (3). Nucleophilic substitution of the chlorine atom in 4, 7-dichloroquinoline with 4-aminopentan-1-ol (3) at 140 °C produced 4-(7-chloroquinolin-4-ylamino)pentan-1-ol (4) [23]. Compound (4) was brominated by 48% HBr in the presence of conc. H_2SO_4 was afford the bromoquine derivative (5), which was alkylated with 70% ethylamine solution to give diamine (6). The diamine (6) was further alkylated with [$^2H_5$] ethyl iodide in presence of K_2CO_3 to give [$^2H_5$] chloroquine. After purification of the free base by column chromatography, [$^2H_5$] chloroquine diphosphate (7) was prepared.

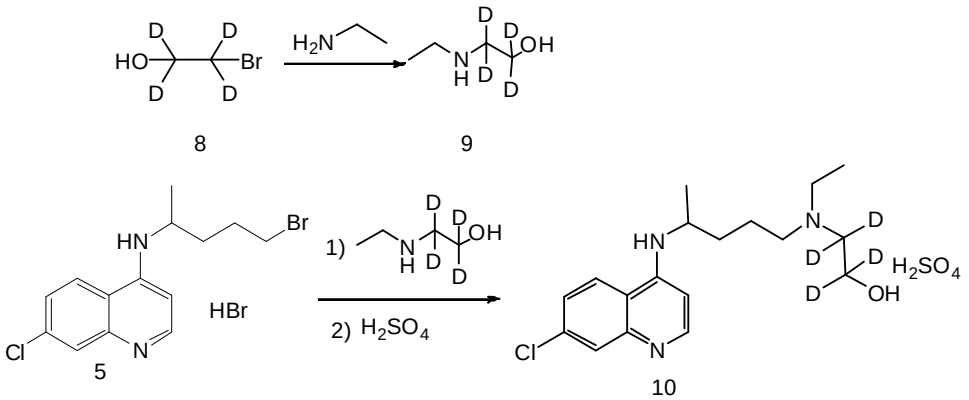
Scheme 2 presents the general synthetic scheme for preparing [$^2H_4$] hydroxychloroquine. [$^2H_4$] 2-bromoethanol (8) was alkylated with 70% ethylamine solution to give [$^2H_4$] 2-(ethylamino)ethanol (9),[22] which was treated with bromoquine derivative (5) to give [$^2H_4$] hydroxychloroquine. After purification of the free base by column chromatography, [$^2H_4$] hydroxychloroquine sulfate (10) was prepared.

Scheme 3 presents the general synthetic scheme for preparing [$^2H_5$] desethylchloroquine. The ammoniation of compound (5) in saturated methanol ammonia solution in a sealed tube afforded the diamine (11). Acylation of compound (11) was carried out with [$^2H_3$] acetyl chloride (12) in dry CH_2Cl_2 in presence of Et_3N to give [$^2H_3$] amide (13), which was reduced by $LiAlD_4$ to give [$^2H_5$] desethylchloroquine (14).

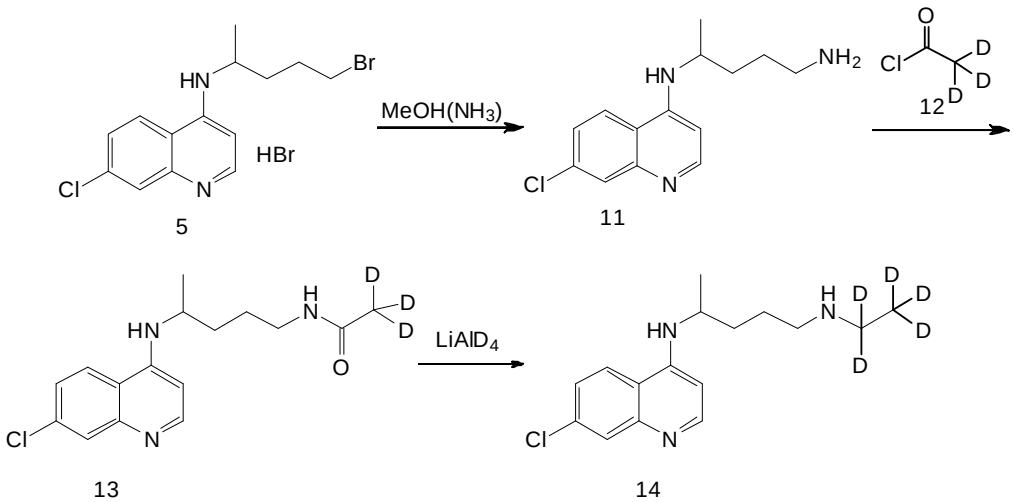
Scheme 4 presents the general synthetic scheme for preparing [$^2H_4$] desethylhydroxychloroquine (15). The diamine (11) was alkylated with [$^2H_4$] 2-bromoethanol (8) to give [$^2H_4$] desethylhydroxychloroquine (15).



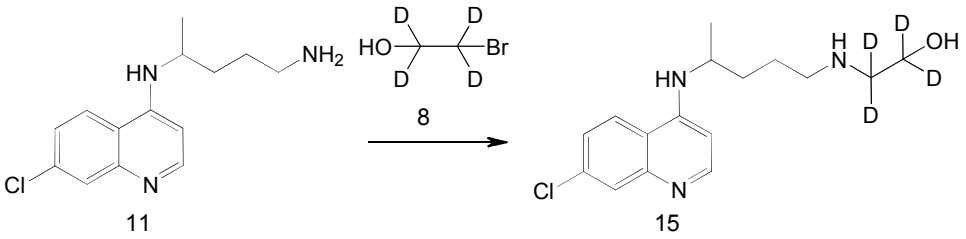
Scheme 1 Synthesis of [$^2H_5$] chloroquine



Scheme 2 Synthesis of [$^2H_4$] hydroxychloroquine



Scheme 3 Synthesis of [$^2H_5$] desethylchloroquine



Scheme 4 Synthesis of [$^2H_4$] desethylhydroxychloroquine

HPLC results showed that [$^2H_5$] chloroquine (7), [$^2H_4$] hydroxychloroquine (10), [$^2H_5$] desethylchloroquine (14) and [$^2H_4$] desethylhydroxychloroquine (15) were obtained with over 98% chemical purity. Mass spectrometry analysis of compound (7), compound (10), compound (14) and compound (15) revealed over 98% deuterium enrichment.

CONCLUSION

The present study describes the synthesis of deuterium-labelled chloroqiune, hydroxychloroquine and their metabolites. Mass spectrometry analysis of these compounds revealed over 98% deuterium enrichment. The compounds can be used as internal standards in metabolism studie.

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