Supplementary Material for

Reversal Agent and Linker Variants of Reversed Chloroquines: Activities against *Plasmodium falciparum*

Simeon Andrews,[†] *Steven J. Burgess,*[†,§] *Deborah Skaalrud,*[†] *Jane Xu Kelly,*[#,†,§] *& David H. Peyton*[†,*]

[†]Department of Chemistry, Portland State University, P.O. Box 751, Portland, Oregon 97207-0751; USA, DesignMedix, Inc., 2828 SW Corbett Ave., Portland, Oregon, 97201; & [#]Portland Veterans Affairs Medical Center, Portland, Oregon, 97239, USA.

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* To whom correspondence should be addressed. Phone: 503 725-3875. Fax: 503 725-9525. E-mail: peytond@pdx.edu.

General Methods

***In vitro* drug susceptibility assays.** Both CQ^S (D6) and CQ^R (Dd2) *P. falciparum* maintained continuously in culture were used. Asynchronous cultures were diluted with uninfected erythrocytes and complete medium (RPMI-1640 with 0.5% Albumax II) to achieve 0.2% parasitemia and 2% hematocrit. In 96-well microplates, chloroquine (positive control) or **1** diluted in complete medium from 10 mM stock in DMSO were added to the cell mixture to yield triplicate wells with drug concentrations ranging from 0 to 10^{-4} M in a final well volume of 100 μL. After 72 hours of incubation under standard culture conditions (ref), plates were harvested and read by the SYBR Green I fluorescence-based method[24] using a 96-well fluorescence plate reader (Gemini-EM, Molecular Devices), with excitation and emission wavelengths at 497 and 520 nm, respectively. The fluorescence readings were plotted against log[drug], and the IC_{50} values were obtained from curve fitting performed by nonlinear regression using either Prism (GraphPad) or Excel (Microsoft) software.

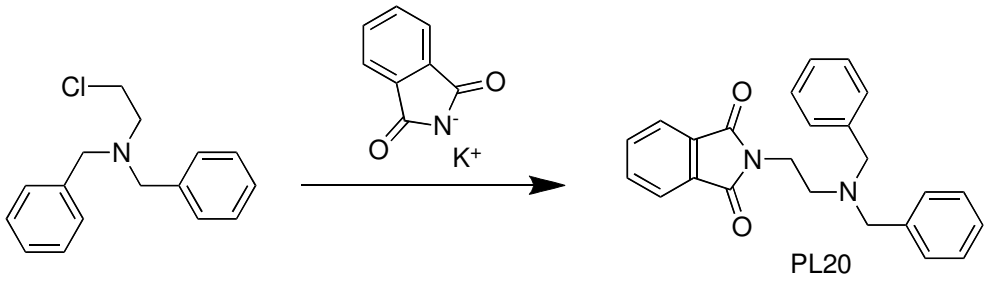
***In vitro* cytotoxicity assay.**

The general cytotoxic effect of RCQs on host cells was assessed by functional assay as described previously[1,2,3], using murine splenic lymphocytes induced to proliferate and differentiate by concanavalin A. Splenic lymphocytes isolated from C57B1/6J mice were washed twice in RPMI 1640 medium, and resuspended in complete RPMI containing 10% FBS, 50 μg/ml penicillin/streptomycin, 50 μM β-mercaptoethanol, and 1 μg/ml concanavalin A. Cells (100 μl/well) were then seeded into 96-well flat-bottom tissue culture plates containing drug solutions (100 μl) serially diluted with complete culture

medium to a final cell density $2x10^5$ per well. The plates were incubated for 72 h in a humidified atmosphere at 37 °C and 5% CO_2. An aliquot of a stock solution of resazurin (Alamar Blue, prepared in 1X PBS) was then added at 20 μl per well (final concentration 10 μM), and the plates were returned to the incubator for another 24 h. After this period, fluorescence in each well was measured in a Gemini EM plate reader with excitation wavelength at 560 nm and emission wavelength at 590 nm. IC_{50} values were determined by non-linear regression analysis of logistic concentration-fluorescence intensity curves (GraphPad Prism software).

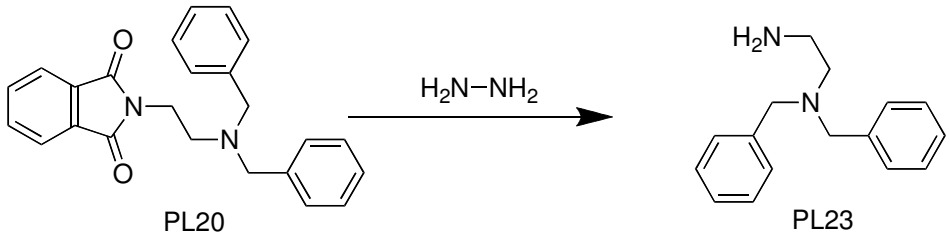
Syntheses

Reagents were from Aldrich and used as supplied, unless otherwise noted. The Chromatotron is a "Centrifugal Thin-Layer Chromatograph".[1] NMR characterizations were done using $CDCl_3$ solvent on a Tecmag Libra-modified NM-500 NMR spectrometer, operating at 499.8 MHz, or with a Bruker AMX-400 NMR spectrometer operating at 400.14 MHz for ^1H, or 100.62 MHz for ^{13}C observation. Spectra were processed with the program Swan-MR,[2, 3] or with DataChord (One Moon Scientific). The compounds were found to be pure to >95% by NMR and by HPLC, and the identifications were confirmed by mass spectrometry. Note that the syntheses were performed to give highly purified material in sufficient quantity for characterization and evaluation against *P. falciparum*; no attempts were made to optimize the yields.

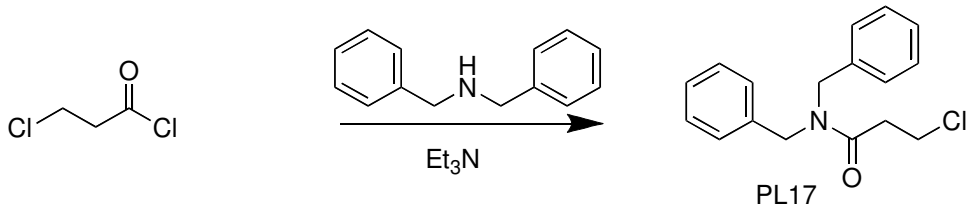


2-(2-(dibenzylamino)ethyl)isoindoline-1,3-dione (18). A suspension of *N*-(2-chloroethyl) dibenzylamine hydrochloride (TCI Chemicals, 10.00 g, 33.76 mmol) in 150 mL distilled H_2O and 100 mL EtOAc was made basic with 1 M NaOH until the aqueous portion was no longer cloudy. Layers were separated, and aqueous layer was extracted with 3 × 30 mL of EtOAc. Combined organic layers were concentrated to give the free base, **N,N-dibenzyl-2-chloroethanamine (17)** (8.73 g, 99.5% yield). ^1H-NMR ($CDCl_3$) δ

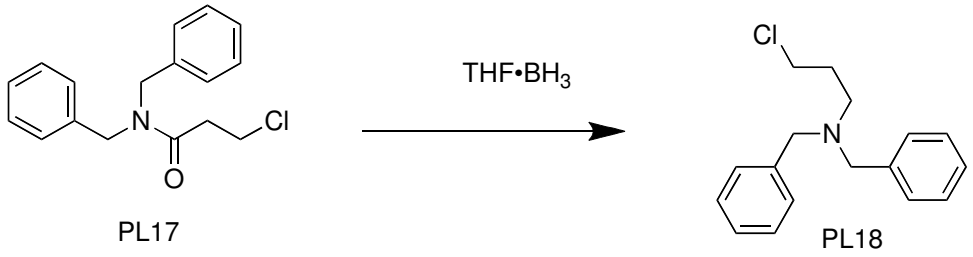
2.83 (t, J = 7.1 Hz, 2H), 3.49 (t, J = 7.1 Hz, 2H), 3.66 (s, 4H), 7.24 (m, 2H), 7.32 (t, J = 7.4 Hz, 4H), 7.37 (m, 4H). A mixture of **17** (2.00 g, 7.70 mmol) and potassium phthalimide (2.14 g, 11.55 mmol) in 40 mL DMF was refluxed for 20 hours. The mixture was brought to room temperature and added to 50 mL H_2O with 50 mL sat. $NaHCO_3$ before being extracted with 40 mL Et_2O, then 5 × 20 mL Et_2O. The combined organic layer was washed with 2 × 30 mL saturated aqueous $NaHCO_3$, then 30 mL brine before being dried over $MgSO_4$. The volume was reduced by rotoevaporation, then filtered, washing with cold Et_2O. This filtrate was then reduced in volume and filtered again before rotoevaporation to give **2-(2-(dibenzylamino)ethyl)isoindoline-1,3-dione** (**18**) (1.91 g, 60% yield). ^{1}H-NMR ($CDCl_3$) δ 2.72 (t, J = 5.9 Hz, 2H), 3.59 (s, 4H), 3.78 (t, J = 5.8 Hz, 2H), 7.11 (m, 6H), 7.22 (m, 4H), 7.72 (dd, J = 5.4, 3.1 Hz, 2H), 7.80 (dd, J = 5.5, 3.0 Hz, 2H).



***N,N*–dibenzylethane-1,2-diamine (21).** Hydrazine monohydrate (1.45 mL, 30.0 mmol) was mixed with **18** (1.67 g, 4.51 mmol) in 25 mL EtOH and refluxed for 3 hrs. The mixture was brought to room temperature, filtered, then washed with hexanes before evaporating the filtrate. This crude product was subjected to purification with a Chromatotron (Silica, 1:9 MeOH/EtOAc) to give ***N,N*–dibenzylethane-1,2-diamine** (**21**) (0.68 g, 63% yield). ^{1}H-NMR ($CDCl_3$) δ 2.51 (t, J = 5.7 Hz, 2H), 2.74 (t, J = 5.8 Hz, 2H), 3.58 (s, 4H), 7.24 (m, 2H), 7.31 (m, 4H), 7.34 (m, 4H).

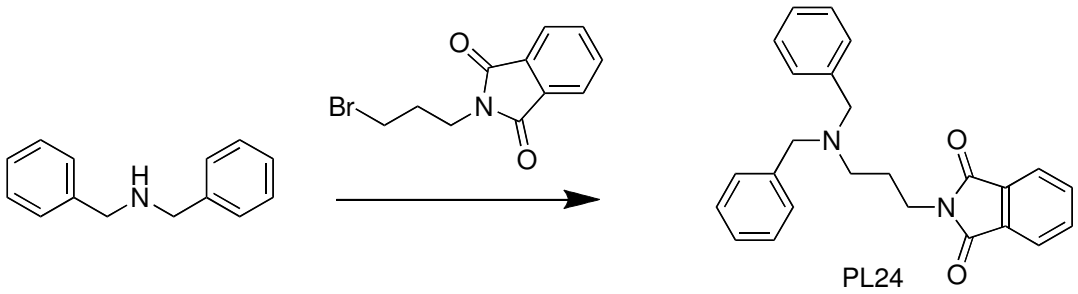


N,N-dibenzyl-3-chloropropanamide (15). Chilled 40 mL CH_2Cl_2 in ice before stirring in dibenzylamine (4.81 mL, 25 mmol) and triethylamine (4.18 mL, 30 mmol). 3-chloropropionyl chloride (2.52 mL, 26.2 mmol) was added dropwise to 10 mL CH_2Cl_2 over ice, keeping the mixture under 10ºC. After stirring for an hour, the mixture was filtered through Celite, washing with CH_2Cl_2, then the volume was reduced by rotoevaporation. The slurry was taken into EtOAc then filtered by gravity; the filtrate was concentrated by rotoevaporation. The resulting crude product was purified on a Chromatotron (silica, 9:90:1 EtOAc/hexanes/triethylamine), to give **N,N-dibenzyl-3-chloropropanamide (15**) (~ 3 g). ^{1}H-NMR ($CDCl_3$) δ 2.89 (t, J = 6.8 Hz, 2H), 3.91 (t, J = 6.8 Hz, 2H), 4.47 (s, 2H), 4.64 (s, 2H), 7.29 (m, 10H).

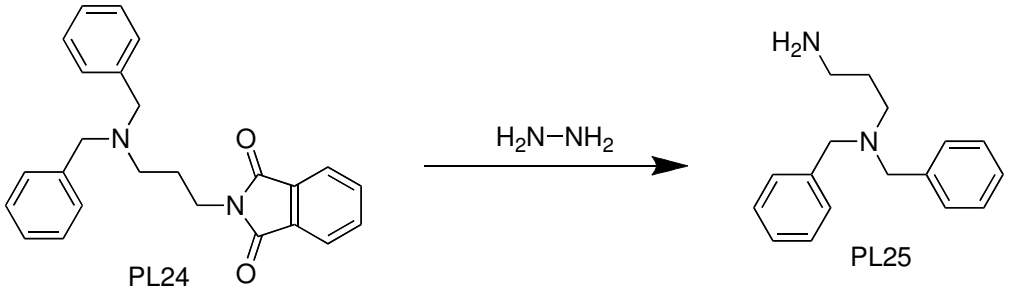


N,N-dibenzyl-3-chloropropan-1-amine (16). A solution of **15** (1.00 g, 3.47 mmol) in 50 mL THF was chilled over ice before adding borane-THF (Acros Organics, 1 mol/L, 10.42 mL, 10.42 mmol) with vigorous stirring. After stirring for an hour over ice, reaction was refluxed for 3 hours. The reaction was brought to room temperature, then 25 mL 2.5 M HCl was added. 25 mL of 5 M NaOH was slowly added to make the solution

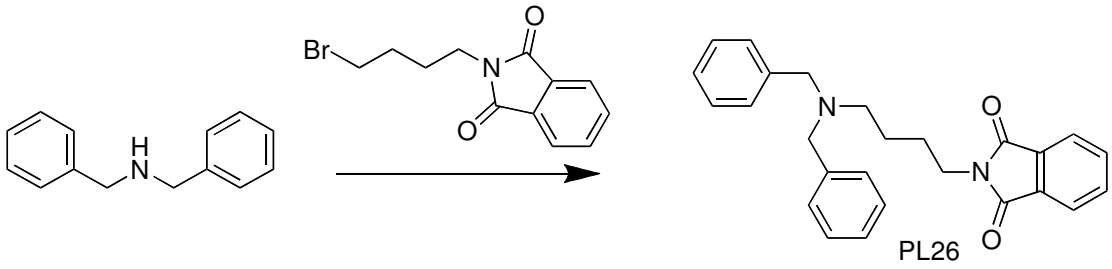
strongly basic. After removing the organic layer, the aqueous layer was extracted with 3 × 15 mL Et_2O, and this combined organic layer was washed with brine (15 mL), dried over $MgSO_4$, filtered, and concentrated by rotoevaporation to give 0.64 g of crude product. Purification was accomplished with column chromatography (silica, hexanes then 1:9 EtOAc/hexanes) followed by flash chromatography (silica, hexanes then 1:19 EtOAc/hexanes) to give **N,N-dibenzyl-3-chloropropan-1-amine** (**16)** (0.27 g, 28% yield). ^{1}H-NMR ($CDCl_3$) δ 1.93 (p, J = 6.8 Hz, 2H), 2.56 (t, J = 6.6 Hz, 2H), 3.53 (t, J = 6.9 Hz, 2H), 3.56 (s, 4H), 7.23 (tt, J = 1.8, 7.3 Hz, 2H), 7.31 (m, 4H), 7.34 (m, 4H).



2-(3-(dibenzylamino)propyl)isoindoline-1,3-dione (22). Dibenzylamine (1.13 g, 5.73 mmol) and *N*-(3-bromopropyl)-phthalimide (0.70 g, 2.61 mmol) were mixed in 25 mL xylenes and refluxed for 20 hrs. The mixture was cooled to room temperature, extracted with 5 × 30 mL 6 M HCl, then made basic with 50% NaOH. The aqueous layer was extracted with several EtOAc washes to yield 2 g of crude product. Purification was achieved on a Chromatotron (silica, 15:84:1 EtOAc/hexanes/triethylamine) to give **2-(3-(dibenzylamino)propyl)isoindoline-1,3-dione (22)** (0.76g, 38% yield). ^{1}H-NMR ($CDCl_3$) δ 1.88 (p, J = 7.3 Hz, 2H), 2.49 (t, J = 7.0 Hz, 2H), 3.56 (s, 4H), 3.67 (t, J = 7.6 Hz, 2H), 7.18 (tt, J = 7.3, 1.5 Hz, 2H), 7.27 (m, 4H), 7.35 (m, 4H), 7.70 (dd, J = 5.5, 3.0 Hz, 2H), 7.81 (dd, J = 5.3, 3.2 Hz, 2H).

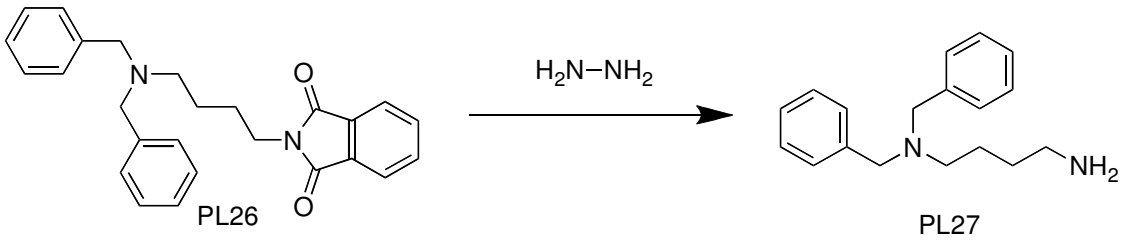


***N*[1],*N*[1]-dibenzylpropane-1,3-diamine (23).** A mixture of **22** (1.03 g, 2.68 mmol) and hydrazine (0.17 mL, 3.5 mmol) in 20 mL EtOH was refluxed for 3 hrs. The solvent was removed by rotoevaporation, and the residue was filtered over Celite, washing with EtOAc. Purification was achieved on a Chromatotron (silica, EtOAc, then 1:1 EtOAc:MeOH) to give ***N*[1],*N*[1]-dibenzylpropane-1,3-diamine (23)** (0.13 g, 20% yield). ^{1}H-NMR ($CDCl_3$) δ 1.66 (p, J = 6.6 Hz, 2H), 2.48 (t, J = 6.6 Hz, 2H), 2.73 (br t, 2H), 3.55 (s, 4H), 7.23 (t, J = 7.2 Hz, 2H), 7.31 (t, J = 7.3 Hz, 4H), 7.34 (m, 4H).

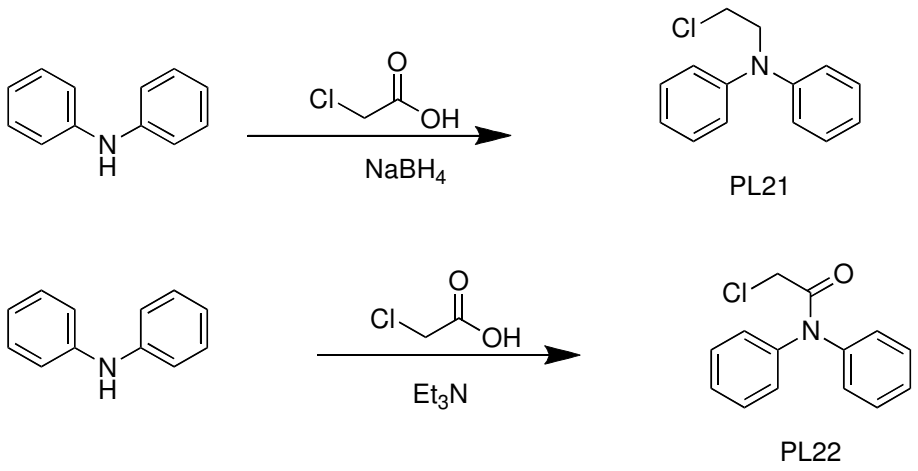


2-(4-Dibenzylaminobutyl)-isoindole-1,3-dione (24). A mixture of *N*-(bromobutyl)-phthalimide (3.54 g, 12.55 mmol) and dibenzylamine (5.45 g, 27.61 mmol) in xylenes (50 mL) was refluxed for 3 days. The mixture was cooled and poured into 50 mL 3 M HCl. This mixture was made basic with 50% aqueous NaOH, then extracted with EtOAc and the organic layer was concentrated by rotoevaporation. An initial purification was carried out by column chromatography (silica, 1:9 EtOAc/hexanes, then EtOAc, then 1:1 EtOAc:MeOH). The product concentrated by rotoevaporation, stirred

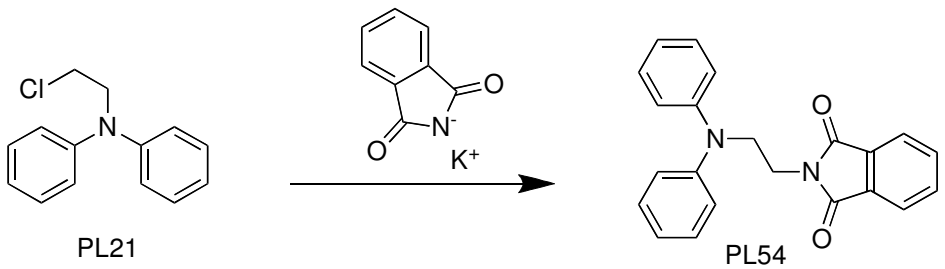
with 1:9 EtOAc/hexanes, chilled, and filtered to give **2-(4-Dibenzylaminobutyl)-isoindole-1,3-dione (24)** (2.71 g, 49% yield) as a solid with 10% dibenzylamine impurity. ^{1}H-NMR ($CDCl_3$) δ 1.53 (p, J = 7.4 Hz, 2H), 1.66 (p, J = 7.4 Hz, 2H), 2.45 (t, J = 6.9 Hz, 2H), 3.53 (s, 4H), 3.60 (t, J = 7.2 Hz, 2H), 7.20 (t, J = 7.3 Hz, 2H), 7.28 (m, 4H), 7.34 (m, 4H), 7.70 (dd, J = 3.1, 5.4 Hz, 2H), 7.83 (dd, J = 3.1, 5.4 Hz, 2H).



N^1,N^1-dibenzylbutane-1,4-diamine (25). A mixture of **24** (2.44g, 6.12 mmol) and hydrazine monohydrate (1.97 mL, 40.8 mmol) in EtOH (25 mL) was refluxed overnight. Once cooled, the mixture was filtered over Celite, and washed with EtOAc. The crude oil was purified by column chromatography (alumina, EtOAc) to give **N^1,N^1-dibenzylbutane-1,4-diamine (25)** (0.25 g, 15% yield). ^{1}H-NMR ($CDCl_3$) δ 1.45 (p, J = 7.1 Hz, 2H), 1.54 (p, J = 7.0 Hz, 2H), 2.43 (t, J = 6.8 Hz, 2H), 2.61 (t, J = 6.9 Hz, 2H), 3.55 (s, 4H), 7.23 (m, 2H), 7.31 (t, J = 7.5 Hz, 4H), 7.35 (m, 4H).



N-(2-chloroethyl)-N-phenylaniline (19) and 2-chloro-N,N-diphenylethanamide (20). Chloroacetic acid (8.10 g, 85.7 mmol) was dissolved in toluene (200 mL), and $NaBH_4$ (2.50 g, 66.0 mmol) was added portion wise. Once evolution of gas had ceased, diphenylamine (2.19 g, 12.9 mmol) was added and the reaction was refluxed overnight. The reaction was cooled to rom temperature and 50 mL 2 M NaOH was added. The aqueous layer was separated, then extracted with 50 mL EtOAc. The combined organic layers were washed with 50 mL of brine before being dried ($MgSO_4$), filtered, and concentrated. The resultant dark oil was dissolved in 15 mL CH_2Cl_2. Chloroacetyl chloride (1.4 mL, 17.6 mmol) and triethylamine (3 mL, 21.5 mmol) were added to the mixture in three portions over two hours until all the diphylamine had reacted, as evaluated by TLC. Products were purified by column chromatography (silica, 30-60 ºC petroleum ether, with increasing polarity to 1:1 EtOAc/petroleum ether) to give both **N-(2-chloroethyl)-N-phenylaniline (19)** [(0.3 g, 10% yield); ^{1}H-NMR ($CDCl_3$) δ 3.68 (t, J = 7.5 Hz, 2H), 4.05 (t, J = 7.5 Hz, 2H), 6.99 (m, 6H), 7.29 (t, J = 7.9 Hz, 4H)] and **2-chloro-N,N-diphenylethanamide (20)** [(1.5 g, 47% yield); ^{1}H-NMR ($CDCl_3$) δ 4.03 (s, 2H), 7.35 (br m, 10H).



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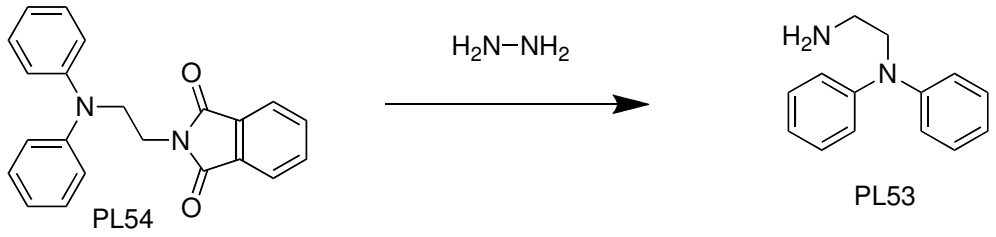
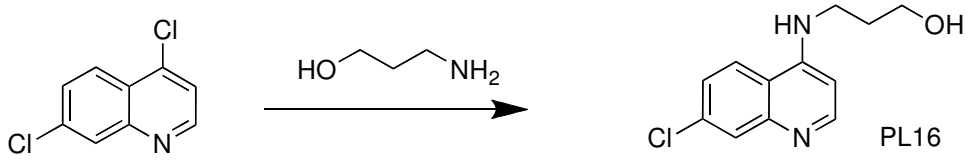
2-(2-(diphenylamino)ethyl)isoindoline-1,3-dione (30). Combined **19** (0.89 g, 3.84 mmol) with potassium phthalimide (1.07 g, 5.76 mmol) in 40 mL DMF and heated to 140 ºC for 20 hrs. The reaction was cooled to room temperature, then 25 mL sat.

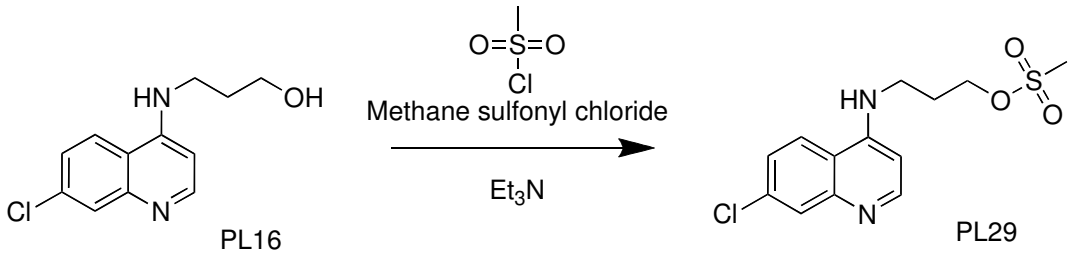
$NaHCO_3$ and 75 mL H_2O were added. The layers were separated and then the aqueous layer was extracted with 40 mL, then 20 mL of EtOAc, followed by 20 mL Et_2O and another 60 mL EtOAc. The organic fractions were combined and washed with 30 mL brine, dried over $MgSO_4$, and concentrated by rotoevaporation. The product was purified with a Chromatotron (silica, 5%-15% EtOAc in Hexanes with 1% triethylamine) to give **2-(2-(diphenylamino)ethyl)isoindoline-1,3-dione (30)** (0.88g, 90% yield). ^{1}H-NMR ($CDCl_3$) δ 4.02 (m, 4H), 6.91 (t, J = 7.3 Hz, 2H), 7.07 (m, 4H), 7.24 (m, 4H), 7.70 (dd, J = 3.0, 5.4 Hz, 2H), 7.80 (dd, J = 3.1, 5.4 Hz, 2H).



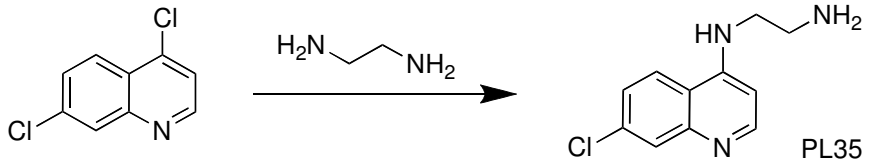
N^1,N^1-diphenylethane-1,2-diamine (29). A mixture of **30** (0.88 g, 2.6 mmol) and hydrazine monohydrate (0.62 mL, 12.8 mmol) in EtOH (25 mL) was refluxed for 3 hrs. The mixture was filtered, and the solid was washed with hexanes. The combined filtrate was concentrated by rotoevaporation and purified by column chromatography (silica, 1:9 MeOH/EtOAc) to give **N^1,N^1-diphenylethane-1,2-diamine (29)** (0.27 g, 50% yield). ^{1}H-NMR ($CDCl_3$) δ 2.98 (br t, 2H), 3.82 (t, J = 6.6 Hz, 2H), 6.96 (t, J = 7.3 Hz, 2H), 7.02 (d, J = 7.9 Hz, 4H), 7.26 (m, 4H).



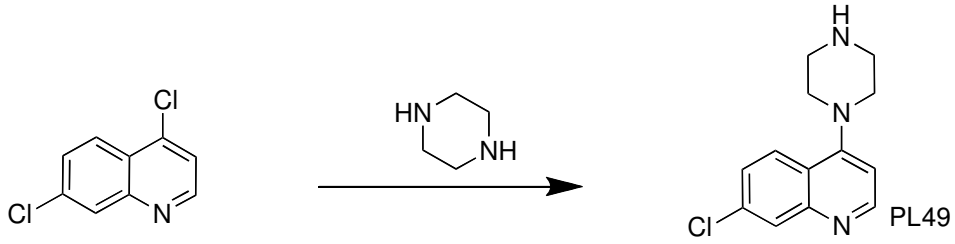
3-(7-chloroquinolin-4-ylamino)propan-1-ol (14). A solution of 4,7-dichloroquinoline (5.05 g, 25.5 mmol) and 3-amino-1-propanol (30 mL, 395 mmol) was heated to 135 ºC for 3 days. The mixture was then poured into 250 mL H_2O and stirred until cool. The solid was claimed by filtration, washed with H_2O, and dried. Recrystallization from EtOH gave **3-(7-chloroquinolin-4-ylamino)propan-1-ol (14)** (5.21 g, 86% yield). ^{1}H-NMR (CD_3OD) δ 1.15 (p, J = 6.4 Hz, 2H), 2.67 (t, J = 7.0 Hz, 2H), 2.92 (t, J = 5.9 Hz, 2H), 5.75 (d, J = 5.6 Hz, 1H), 6.60 (d, J = 8.8 Hz, 1H), 6.96 (s, 1H), 7.26 (d, J = 8.9 Hz, 1H), 7.55 (d, J = 5.5 Hz, 1H).



3-(7-chloroquinolin-4-ylamino)propyl methanesulfonate (26). A mixture of **14** (2.00 g, 8.98 mmol) and triethylamine (2.5 mL, 17.9 mmol) in 40 mL THF was chilled to -10°C over ice/MeOH. Methane sulfonyl chloride (0.84 mL, 10.8 mmol) was slowly added, keeping temperature below 0 ºC. The reaction was then stirred over ice for an additional 30 minutes before being poured into sat. $NaHCO_3$ (50 mL). The aqueous layer was extracted with 40 mL EtOAc, then 3 × 20 mL of EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated to give **3-(7-chloroquinolin-4-ylamino)propyl methanesulfonate (26)** (0.97 g, 34% yield). ^{1}H-NMR ($CDCl_3$) δ 2.18 (p, J = 6.0 Hz, 2H), 3.07 (s, 3H), 3.59 (q, J = 6.2 Hz, 2H), 4.43 (t, J = 5.7 Hz, 2H), 5.44 (br t, 1H), 6.43 (d, J = 5.3 Hz, 1H), 7.40 (dd, J = 8.9, 2.2 Hz, 1H), 7.70 (d, J = 8.9 Hz, 1H), 7.97 (d, J = 2.2 Hz, 1H), 8.55 (d, J = 5.4 Hz, 1H).

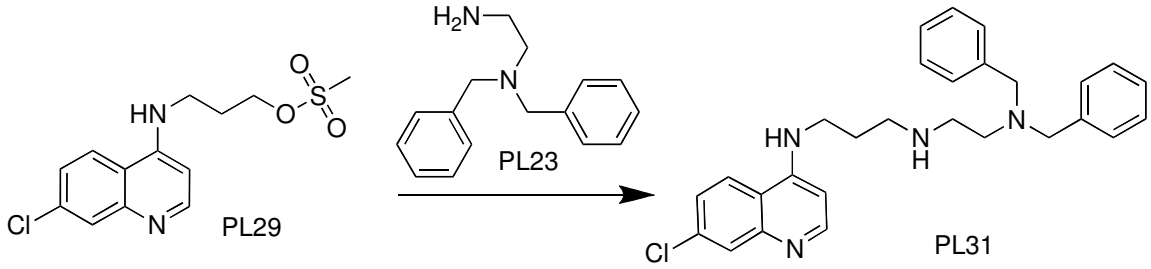


N^1-(7-chloroquinolin-4-yl)-ethane-1,2-diamine (27). A mixture of 4,7-dichloroquinoline (5.01 g, 25.30 mmol) and ethylenediamine (MCB, 20 mL, 300 mmol) was refluxed for 5 hours, then cooled and poured into 25 mL EtOAc / 50 mL H_2O. The organic layer was separated and filtered, then washed with H_2O and EtOAc. This filtrate was separated, and the aqueous layer was extracted with 25 mL hot EtOAc. The combined organic layer was concentrated and dried by rotoevaporation to give **N^1-(7-chloroquinolin-4-yl)-ethane-1,2-diamine (27)** (4.10g, 73% yield). ^{1}H-NMR ($CDCl_3$) δ 3.14 (t, J = 5.7 Hz, 2H), 3.34 (q, J = 5.4 Hz, 2H), 5.75 (b s, 1H), 6.42 (d, J = 5.1 Hz, 1H), 7.38 (dd, J = 1.4, 8.9 Hz, 1H), 7.74 (d, J = 8.8 Hz, 1H), 7.96 (d, J = 1.3 Hz, 1H), 8.54 (d, J = 5.1 Hz, 1H).

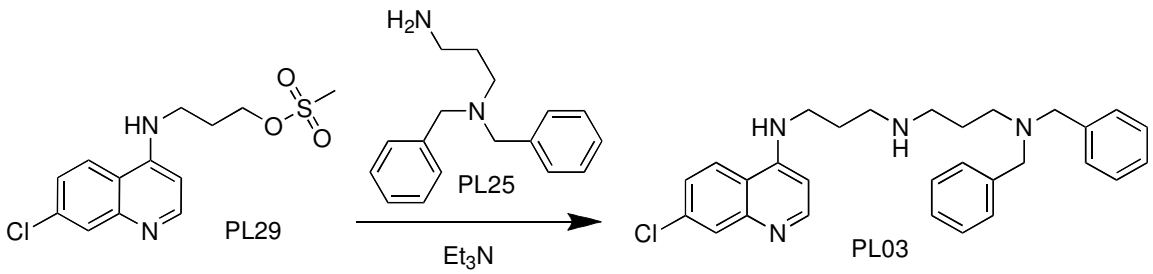


7-chloro-4-(piperazin-1-yl)quinoline (28). A mixture of 4,7-dichloroquinoline (2.05 g, 10.1 mmol) and piperazine (8.73 g, 101 mmol) in EtOH (30 mL) was refluxed for 4 hrs. The solvent was removed by rotoevaporation, and the resulting solid taken up in 40 mL sat. $NaHCO_3$ and 20 mL EtOAc. The separated organic layer was washed with brine, dried over $MgSO_4$, and rotoevaporation gave **7-chloro-4-(piperazin-1-yl)quinoline (28)** (2.04 g, 82% yield) as a yellow powder. ^{1}H-NMR ($CDCl_3$) δ 3.18 (m,

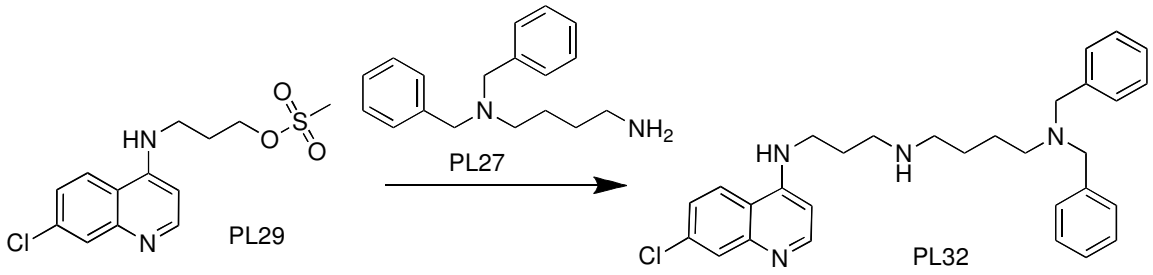
8H), 6.84 (d, *J* = 5.0 Hz, 1H), 7.43 (dd, *J* = 2.0, 9.0 Hz, 1H), 7.96 (d, *J* = 9.0 Hz, 1H), 8.04 (d, *J* = 2.0 Hz, 1H), 8.73 (d, *J* = 5.0 Hz, 1H).



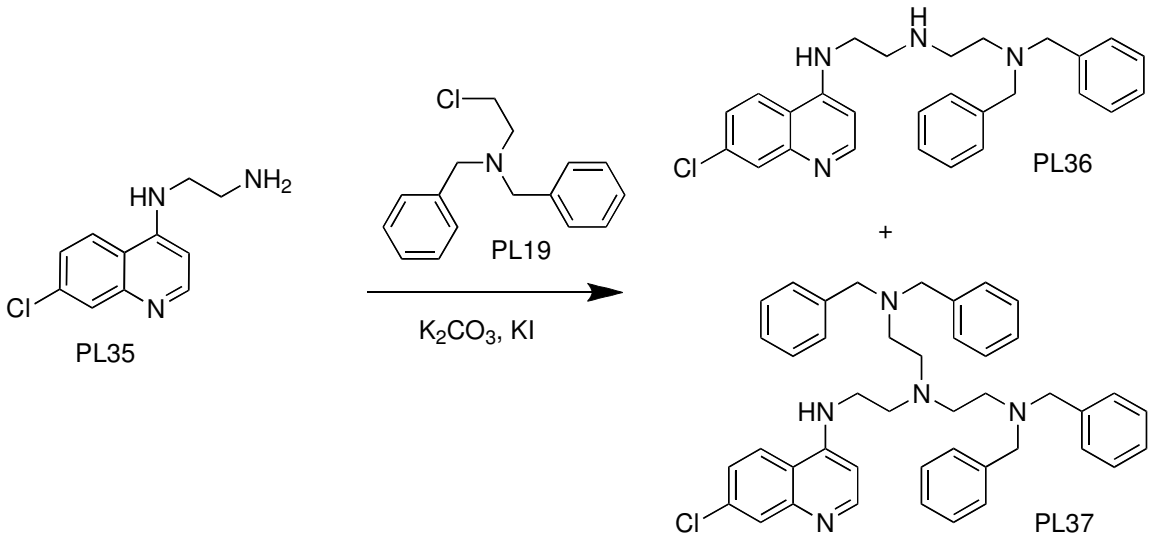
N-(7-Chloroquinolin-4-yl)-N'-(2-dibenzylaminoethyl)-propane-1,3-diamine (4). A mixture of **21** (0.22g, 0.92 mmol), triethylamine (180 µL, 1.29 mmol), and **26** (0.27 g, 0.86 mmol) in 10 mL THF was refluxed for three days. The solvent was removed by rotoevaporation, and mixture was taken up in 10 mL EtOAc and 10 mL sat. $NaHCO_3$. The layers were separated, and the aqueous layer was extracted with 2 × 10 mL EtOAc. The combined organic layers were washed with 5 mL brine, dried over $MgSO_4$, and concentrated to give 0.26 g of crude product, which was then purified using a Chromatotron (silica, hexanes/EtOAc mixes) to give **N-(7-Chloroquinolin-4-yl)-N'-(2-dibenzylaminoethyl)-propane-1,3-diamine (4)** (0.0154g, 4% yield). ^{1}H-NMR ($CDCl_3$) δ 1.82 (m, 2H), 2.64 (t, *J* = 5.0 Hz, 2H), 2.70 (b s, 4H), 3.32 (b t, 2H), 3.64 (s, 4H), 6.26 (d, *J* = 5.3 Hz, 1H), 6.97 (dd, *J* = 1.4, 8.9 Hz, 1H), 7.22 (m, 2H), 7.31 (m, 4H), 7.35 (m, 4H), 7.40 (m, 1H), 7.89 (m, 2H), 8.47 (d, *J* = 5.3 Hz, 1H). ^{13}C NMR δ (ppm)($CHCl_3$-d): 151.9, 150.7, 148.9, 139.4, 134.6, 128.8, 128.5, 128.3, 127.3, 125.0, 122.1, 117.6, 98.1, 59.3, 53.2, 49.3, 47.3, 44.2, 27.2.



N-(7-Chloro-quinolin-4-yl)-N'-(3-dibenzylaminopropyl)-propane-1,3-diamine (2). A mixture of **23** (0.05 g, 0.2 mmol), **26** (0.15 g, 0.45 mmol), and triethylamine (50 mL, 0.36 mmol) in 10 mL THF was refluxed for 1 week. The solvent was removed by rotoevaporation and the mixture was added to 10 mL EtOAc and 10 mL sat. $NaHCO_3$. The aqueous layer was extracted with 10 mL EtOAc, then the combined organic fractions were washed with 10 mL sat. $NaHCO_3$, 10 mL brine, then dried over $MgSO_4$, filtered, and concentrated by rotoevaporation to give 0.1 g of crude extract, which was purified on a Chromatotron (silica, 30:69:1 EtOAc/hexanes/triethylamine, with increasing polarity to 5:94:1 MeOH/EtOAc/triethylamine) to give **N-(7-Chloro-quinolin-4-yl)-N'-(3-dibenzylaminopropyl)-propane-1,3-diamine (2)** (0.02 g, 22% yield). ^{1}H-NMR ($CDCl_3$) δ 1.80 (m, 4H), 2.53 (t, J = 6.5 Hz, 2H), 2.67 (t, J = 6.5 Hz, 2H), 2.76 (t, J = 5.4 Hz, 2H), 3.33 (t, J = 5.8 Hz, 2H), 3.55 (s, 4H), 6.27 (d, J = 5.5 Hz, 1H), 7.23 (m, 2H), 7.29 (t, J = 7.6 Hz, 4H), 7.32 (m, 4H), 7.69 (d, J = 8.8 Hz, 1H), 7.84 (br s, 1H), 7.91 (d, J = 2.0 Hz, 1H), 8.48 (d, J = 5.3 Hz, 1H). ^{13}C NMR δ (ppm)($CHCl_3$-d): 151.5, 150.7, 148.7, 139.1, 135.0, 129.0, 128.4, 128.0, 127.3, 127.0, 125.1, 122.5, 117.6, 98.2, 58.6, 51.4, 50.9, 48.4, 48.4, 42.8, 26.5, 25.9. HRMS: calc.: 473.2472; found: 473.2460 $[M+H]^+$, for $C_{20}H_{33}ClN_4+H^+$.

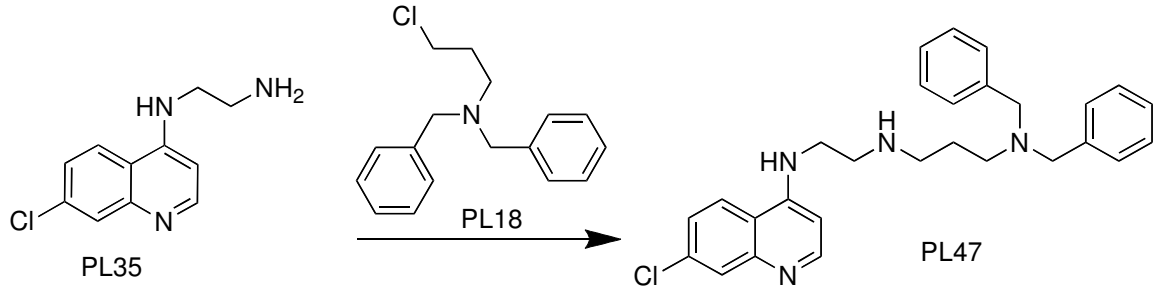


N^1,N^1-dibenzyl-N4-(3-(7-chloroquinolin-4-ylamino)propyl)butane-1,4-diamine (5). A mixture of **26** (0.28 g, 0.89 mmol), **25** (0.25 g, 0.93 mmol), and triethylamine (0.43 mL, 1.36 mmol) in 15 mL dry THF was refluxed for three days. The solvent was removed by rotoevaporation and the mixture was added to 15 mL EtOAc and 15 mL sat. $NaHCO_3$. The aqueous layer was extracted with 2 × 5 mL EtOAc, and then the combined organic layers were washed with 5 mL brine, dried over $MgSO_4$, and concentrated to yield the crude product, which was purified first by column chromatography (silica, 99:1 EtOAc/NH_3OH^+), then using a Chromatotron (silica, 49:49:2 EtOAc/hexanes/triethylamine with increasing polarity to 5:93:2 MeOH/EtOAc/triethylamine), to give **N^1,N^1-dibenzyl-N4-(3-(7-chloroquinolin-4-ylamino)propyl)butane-1,4-diamine (5)** (15 mg, 13% yield). ^{1}H-NMR (CD_3OD) δ 1.53 (m, 4H), 1.93 (p, J = 6.9 Hz, 2H), 2.40 (t, J = 6.3 Hz, 2H), 2.51 (t, J = 6.6 Hz, 2H), 2.72 (t, J = 7.0 Hz, 2H), 3.42 (t, J = 6.9 Hz, 2H), 3.51 (s, 4H), 6.54 (d, J = 5.6 Hz, 1H), 7.19 (t, J = 7.2 Hz, 2H), 7.27 (t, J = 7.4 Hz, 4H), 7.33 (m, 4H), 7.40 (dd, J = 1.9, 9.0 Hz, 1H), 7.78 (d, J = 1.9 Hz, 1H), 8.08 (d, J = 8.7 Hz, 1H), 8.36 (d, J = 5.5 Hz, 1H). ^{13}C NMR δ (ppm)(CHCl•-d): 151.8, 150.7, 148.9, 139.8, 134.8, 128.8, 128.3, 128.2, 126.9, 124.9, 122.2, 117.6, 98.3, 58.5, 53.0, 49.6, 49.1, 43.7, 27.3, 27.0, 24.9. HRMS: calc.: 487.2628; found: 487.2625 $[M+H]^+$, for $C_{30}H_{35}ClN_4+H^+$.



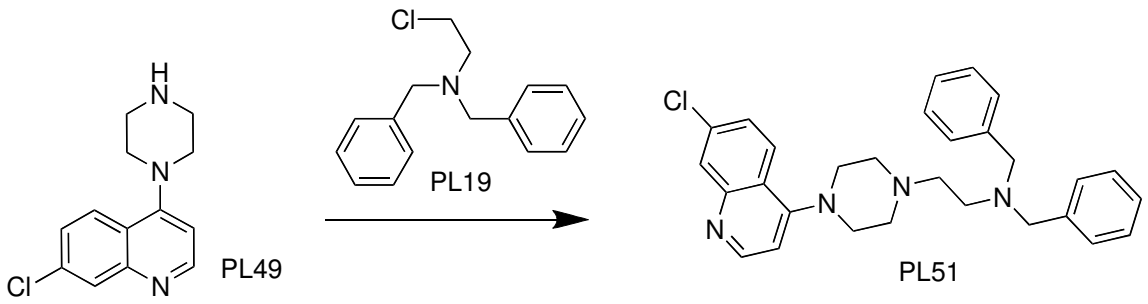
N^1,N^1-dibenzyl-N^2-(2-(7-chloroquinolin-4-ylamino)ethyl)ethane-1,2-diamine (6) and N^1,N^1-dibenzyl-N^2-(2-(7-chloroquinolin-4-ylamino)ethyl)-N^2-(2-(dibenzylamino)ethyl)ethane-1,2-diamine (7). A mixture of **27** (0.30 g, 1.4 mmol), **17** (0.35 g, 1.4 mmol), iodine crystals (0.45 g, 2.7 mmol), and K_2CO_3 (0.19 g, 1.4 mmol) in acetonitrile (10 mL) was refluxed under N_2 for 1 day, followed by concentration by rotoevaporation. The residue was taken up into 15 mL EtOAc and 15 mL sat. $NaHCO_3$; the aqueous layer was extracted with 2 × 5 mL EtOAc. The combined organic fraction was washed with 5 mL brine before being dried over $MgSO_4$, concentrated, and washed through Celite with EtOAc to give 0.51 g of a crude yellow oil which was purified by column chromatography (alumina, EtOAc), to give **N^1,N^1-dibenzyl-N^2-(2-(7-chloroquinolin-4-ylamino)ethyl)ethane-1,2-diamine (6)** (0.03 g, 5% yield). ^{1}H-NMR ($CDCl_3$) δ 2.65 (m, 4H), 2.73 (t, J = 5.6 Hz, 2H), 3.20 (q, J = 4.9 Hz, 2H), 3.59 (s, 4H), 5.85 (b t, 1H), 6.36 (d, J = 5.3 Hz, 1H), 7.23 (m, 2H), 7.29 (m, 4H), 7.32 (m, 5H), 7.60 (d, J = 8.9 Hz, 1H), 7.95 (d, J = 1.9 Hz, 1H), 8.53 (d, J = 5.2 Hz, 1H). ^{13}C NMR δ (ppm)(CHCl•-d): 152.0, 150.0, 149.2, 139.5, 134.9, 128.9, 128.7, 128.4, 127.1, 125.3,

121.5, 121.5, 117.4, 99.1, 59.2, 53.1, 47.0, 46.6, 41.9. HRMS: calc.: 445.2159; found: 445.2165 $[M+H]^+$, for $C_{27}H_{29}ClN_4+H^+$. Also **N^1,N^1-dibenzyl-N^2-(2-(7-chloroquinolin-4-ylamino)ethyl)-N^2-(2-(dibenzylamino)ethyl)ethane-1,2-diamine (7)** (0.19g, 42% yield). ^{1}H-NMR ($CDCl_3$) δ 2.47 (t, J = 6.5 Hz, 4H), 2.55 (t, J = 6.5 Hz, 4H), 2.64 (t, J = 5.5 Hz, 2H), 3.04 (q, J = 5.0 Hz, 2H), 3.50 (s, 8H), 5.79 (b t, 1H), 6.29 (d, J = 5.3 Hz, 1H), 7.16 (m, 4H), 7.21 (t, J = 7.2 Hz, 8H), 7.27 (m, 9H), 7.37 (d, J = 8.7 Hz, 1H), 7.95 (d, J = 1.3 Hz, 1H), 8.51 (d, J = 5.1 Hz, 1H). ^{13}C NMR δ (ppm)(CHCl•-d): 152.0, 149.7, 149.1, 139.3, 134.8, 128.8, 128.7, 128.2, 127.0, 125.2, 121.3, 117.4, 99.3, 59.1, 52.3, 52.2, 51.5, 39.8. HRMS: calc. 668.3520:; found: 668.3522 $[M+H]^+$, for $C_{43}H_{46}ClN_5+H^+$.



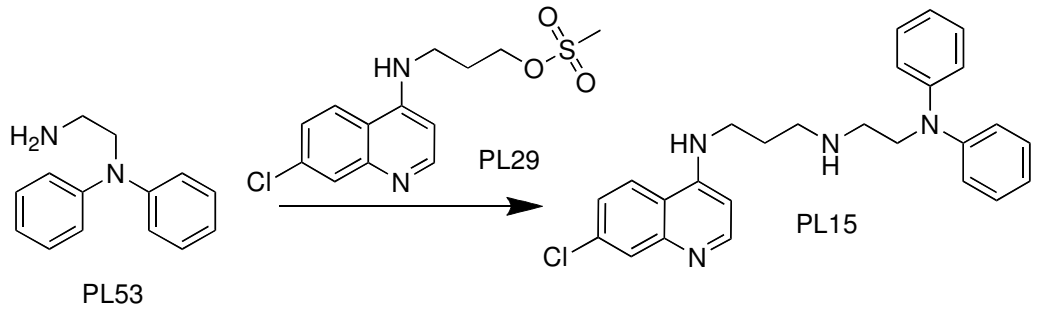
N^1,N^1-dibenzyl-N^3-(2-(7-chloroquinolin-4-ylamino)ethyl)propane-1,3-diamine (9). A mixture of **16** (0.22 g, 0.80 mmol), **27** (0.50g, 2.26 mmol), iodine crystals (0.19 g, 1.50 mmol), and K_2CO_3 (0.42, 3.0 mmol) in EtOH (15 mL) was refluxed for 5 hrs, cooled, and the solvent was evaporated. The mixture was added to 10 mL EtOAc / 10 mL sat. $NaHCO_3$, then the aqueous layer was extracted with 3 × 10 mL EtOAc. The combined organic layers were washed with 10 mL brine, then dried over $MgSO_4$. Concentration by rotoevaporation gave 0.37 g of crude product, which was purified by column chromatography (alumina, 9:1 EtOAc/hexanes) to give **N^1,N^1-dibenzyl-N^3-(2-(7-chloroquinolin-4-ylamino)ethyl)propane-1,3-diamine (9)** (5.2 mg, 1% yield). ^{1}H-NMR ($CDCl_3$) δ 1.83 (m, 2H), 2.51 (t, J = 6.0 Hz, 2H), 2.72 (t, J = 6.3 Hz, 2H), 2.97 (dist t,

2H), 3.44 (dist t, 2H), 3.51 (s, 4H), 6.30 (d, *J* = 5.5, 1H), 7.23 (m, 10H), 7.33 (dd, *J* = 1.8, 8.9 Hz, 1H), 7.88 (d, *J* = 9.0 Hz, 1H), 7.90 (d, *J* = 1.7 Hz, 1H), 8.47 (d, *J* = 5.4 Hz, 1H). ^{13}C NMR δ (ppm)(CHCl•-d): 152.1, 150.0, 149.2, 139.7, 134.8, 128.9, 128.8, 128.3, 127.0, 125.2, 121.3, 117.5, 99.2, 58.7, 51.4, 47.5, 47.5, 42.1, 27.2. HRMS: calc. 459.2315:; found: 459.2301 $[M+H]^+$, for $C_{28}H_{31}ClN_4+H^+$.

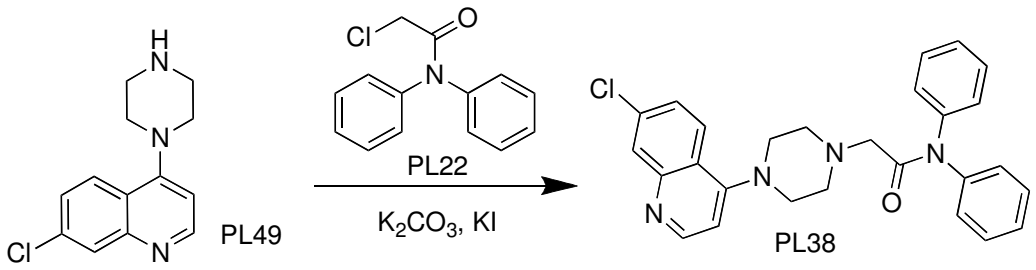


N,N-dibenzyl-2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanamine (11). A mixture of **28** (0.49 g, 1.99 mmol), **17** (0.49 g, 1.54 mmol), K_2CO_3 (0.51 g, 3.69 mmol), and a few grains of KI in acetonitrile (21 mL) was refluxed for 17 hrs, then concentrated by rotoevaporation. The resulting slurry was taken up into 40 mL H_2O / 20 mL EtOAc, then the aqueous layer was extracted with 2 × 10 mL EtOAc. The organic layer was washed with 15 mL sat. $NaHCO_3$, then 20 mL brine before drying over $MgSO_4$ and concentrating by rotoevaporation. The resulting crude oil was purified by column chromatography (silica, EtOAc), to give **N,N-dibenzyl-2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanamine (11)** (0.24 g, 27% yield). ^{1}H-NMR ($CDCl_3$) *δ* 2.65 (b s, 8H), 3.18 (b s, 4H), 3.64 (s, 4H), 6.81 (d, *J* = 5.0 Hz, 1H), 7.24 (m, 2H), 7.32 (t, *J* = 7.4 Hz, 4H), 7.40 (m, 5H), 7.91 (d, *J* = 9.0 Hz, 1H), 8.03 (d, *J* = 1.9 Hz, 1H), 8.70 (d, *J* = 5.0 Hz, 1H). ^{13}C NMR δ (ppm)(CHCl•-d): 157.0, 151.9, 150.2, 139.7, 134.9, 128.9, 128.8,

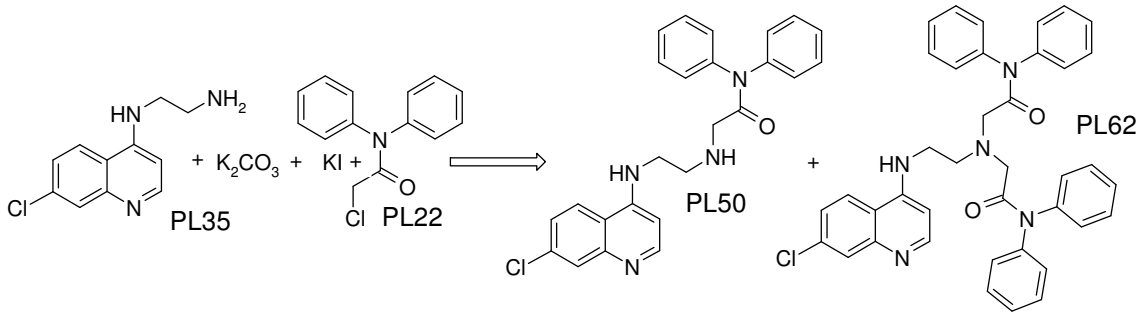
128.2, 126.9, 126.0, 125.2, 122.0, 108.9, 58.9, 56.5, 53.3, 52.2, 50.8. HRMS: calc. 471.2315:; found: 471.2316 $[M+H]^+$, for $C_{29}H_{31}ClN_4+H^+$.



N^1-(7-chloroquinolin-4-yl)-N^3-(2-(diphenylamino)ethyl)propane-1,3-diamine (3). A mixture of **29** (0.12 g, 0.57 mmol), **26** (0.17 g, 0.54 mmol), and triethylamine (0.109 mL, 0.78 mmol) in THF (10 mL) was refluxed for 4 days. The mixture was brought to room temperature, concentrated by rotoevaporation, and the residue was taken up in 10 mL EtOAc / 10 mL sat. $NaHCO_3$. The aqueous layer was extracted with 2 × 10 mL EtOAc, then the combined organic layer was washed with 5 mL brine before drying over $MgSO_4$ and concentrating by rotoevaporation to give 0.18 g of crude product, which was purified by two sequential purifications on a Chromatotron (silica, 30:69:1 EtOAc/hexanes/triethylamine, increasing polarity to 5:94:1 MeOH/EtOAc/triethylamine) to give **N^1-(7-chloroquinolin-4-yl)-N^3-(2-(diphenylamino)ethyl)propane-1,3-diamine (3)** (3.83 mg, 1% yield). ^{1}H-NMR ($CDCl_3$) δ 1.90 (dist p, 2H), 2.90 (dist t, 2H), 2.96 (dist t, 2H), 3.38 (b s, 2H), 3.96 (dist t, 2H), 6.31 (d, J = 5.2 Hz, 1H), 7.00 (m, 6H), 7.18 (dd, J = 1.8, 8.9 Hz, 1H), 7.27 (m, 4H), 7.44 (b s, 1H), 7.65 (d, J = 8.8 Hz, 1H), 7.93 (d, J = 1.5 Hz, 1H), 8.50 (d, J = 5.2 Hz, 1H). ^{13}C NMR δ (ppm)(CHCl•-d): 151.8, 150.6, 148.8, 148.2, 134.9, 129.5, 128.4, 125.2, 122.0, 121.8, 121.2, 117.5, 98.4, 52.2, 49.5, 47.7, 43.9, 27.5. HRMS: calc.: 431.2002; found: 431.1988 $[M+H]^+$, for $C_{26}H_{27}ClN_4+H^+$.

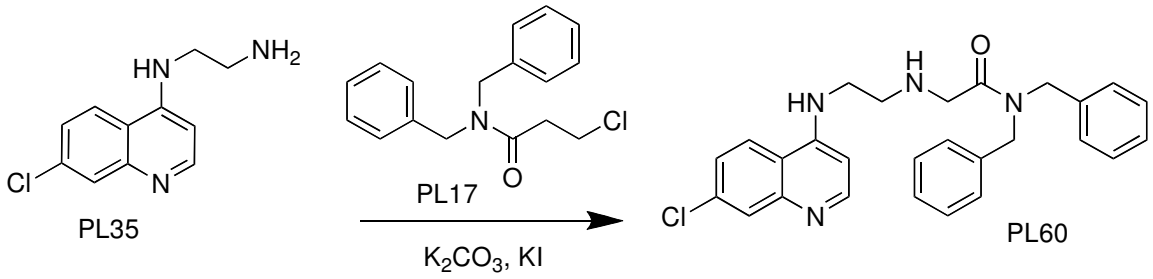


2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-N,N-diphenylethanamide (8). A mixture of **20** (0.33 g, 1.3 mmol), **28** (0.33 g, 1.3 mmol), K_2CO_3 (0.37 g, 2.6 mmol), and KI (0.22g, 1.3 mmol) in acetonitrile (15 mL) was refluxed for 5 hours. The mixture was brought to room temperature, and the solvent was removed by rotoevaporation before the residue was taken up in 10 mL EtOAc / 10 mL sat. $NaHCO_3$. The layers were separated and the aqueous layer was extracted with 2 × 5mL EtOAc. The combined organic layer was washed with 5 mL brine before drying over $MgSO_4$; rotoevaporation gave 0.65 g of crude product, which was purified by column chromatography (alumina, 9:1 EtOAc/hexanes) to give **2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-N,N-diphenylethanamide (8)** (0.55 g, 91% yield). ^{1}H-NMR ($CDCl_3$) δ 2.85 (b t, 4H), 3.24 (b t, 4H), 3.26 (s, 2H), 6.82 (d, J = 5.0 Hz, 1H), 7.83 (b m, 10H), 7.39 (dd, J = 1.8, 8.9 Hz, 1H), 7.91 (d, J = 8.9 Hz, 1H), 8.02 (d, J = 1.9 Hz, 1H), 8.71 (d, J = 4.9 Hz, 1H). ^{13}C NMR δ (ppm)(CHCl•-d): 169.4, 157.0, 152.0, 150.2, 142.4, 134.9, 129.4(broad; amide cis/trans not resolved), 129.4, 128.9, 127.0(broad; amide cis/trans not resolved), 126.1, 125.2, 122.0, 109.0, 60.4, 53.0, 52.2. HRMS: calc.: 457.1795; found: 457.1790 $[M+H]^+$, for $C_{27}H_{25}ClN_4O+H^+$.



2-(2-(7-chloroquinolin-4-ylamino)ethylamino)-N,N-diphenylethanamide (10) and 2-{[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-(diphenylcarbamoyl)-methyl]-amino}-N,N-diphenyl-acetamide (13). A mixture of **20** (0.18 g, 0.73 mmol), **27** (0.50 g, 2.2 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and KI (0.12g, 0.72 mmol) in acetonitrile (10 mL) was refluxed for 1 day. After bringing the mixture to room temperature, the solvent was removed by rotoevaporation, and the residue was taken up in 15 mL EtOAc / 15 mL sat. $NaHCO_3$. The aqueous layer was extracted with 2 × 5mL EtOAc, then the combined organic layer was washed with 5 mL brine before drying over $MgSO_4$ and concentrating by rotoevaporation to give 0.24 g of crude product, which was purified by column chromatography (alumina, EtOAc, then 5:95 MeOH/EtOAc) to give **13** (0.07 g, 30% yield); ^{1}H-NMR ($CDCl_3$) δ 3.07 (m, 2H), 3.18 (m, 2H), 3.55 (s, 4H), 6.20 (d, J = 5.5 Hz, 1H), 7.22 (m, 10H), 7.34 (m, 11H), 7.70 (b s, 1H), 7.89 (s, 1H), 8.27 (d, J = 8.9 Hz, 1H), 8.47 (d, J = 5.2 Hz, 1H); ^{13}C NMR δ (ppm)(CHCl•-d): 171.1, 151.7, 150.8, 141.9, 134.7, 129.5(broad; amide cis/trans not resolved), 129.4, 127.9(broad; amide cis/trans not resolved), 127.8, 126.6(broad; amide cis/trans not resolved), 125.0, 123.5, 117.8, 98.3, 56.5, 52.6, 41.2, in addition to a yellow slurry. This yellow slurry from above was further purified by flash chromatography (silica, 1:9 MeOH: EtOAc) to give **2-(2-(7-chloroquinolin-4-ylamino)ethylamino)-N,N-diphenylethanamide** (**10)** (0.08 g, 25%

yield). ^{1}H-NMR ($CDCl_3$) δ 3.03 (dist t, 2H), 3.31 (dist q, 2H), 3.36(s, 2H), 6.29 (b s, 1H), 6.35 (d, J = 5.4 Hz, 1H), 7.47-7.27 (m, 11H), 7.87 (d, J = 8.9 Hz, 1H), 7.94 (d, J = 2.0 Hz, 1H), 8.50 (d, J = 5.4 Hz, 1H). ^{13}C NMR δ (ppm)(CHCl•-d): 171.7, 151.7, 150.3, 148.9, 135.0, 129.8(broad; amide cis/trans not resolved), 129.6(broad; amide cis/trans not resolved), 128.5(broad; amide cis/trans not resolved), 128.3, 125.3, 121.9, 117.5, 98.9, 51.7, 47.7, 42.7. HRMS: calc.: 431.1638; found: 431.1638 $[M+H]^+$, for $C_{25}H_{23}ClN_4+H^+$.



N,N-dibenzyl-2-(2-(7-chloroquinolin-4-ylamino)ethylamino)ethanamide (12). A mixture of **27** (0.42g, 0.90 mmol), **15** (0.26 g, 0.95 mmol), K_2CO_3 (0.28 g, 1.9 mmol), and KI (0.16 g, 0.95 mmol) in acetonitrile (15 mL) was refluxed overnight. After bringing the mixture to room temperature and rotoevaporation, the residue was taken up in 15 mL EtOAc / 15 mL sat. $NaHCO_3$. The aqueous layer was extracted with 2 × 5 mL EtOAc. An emulsion layer was formed upon the addition of 10 mL brine and 5 mL EtOAc to the combined organic layer, which was discarded with aqueous layer. The organic layer was dried over $MgSO_4$, filtered, then concentrated by rotoevaporation to give crude product, which was purified by column chromatography (alumina, 1:9 MeOH:EtOAc) to give fractions containing **12**, which were concentrated and re-purified by column chromatography (alumina, 1:3 EtOAc:hexanes, followed by 3:1 EtOAc:hexanes, and finally with EtOAc) to give **N,N-dibenzyl-2-(2-(7-chloroquinolin-4-ylamino)ethylamino)ethanamide (12)** (0.22 g, 51% yield). ^{1}H-NMR ($CDCl_3$) δ:

(amide cis/trans resolved) 3.06 (dist t, 2H), 3.35 (m, 2H), 3.60 (s, 2H), 4.41 (s, 2H), 4.69 (s, 2H), 6.36 (d, *J* = 5.5 Hz, 1H), 6.73 (br s, 1H), 7.14 (d, *J* = 7.3 Hz, 2H), 7.34 (m, 8H), 7.98 (d *J* = 2.1 Hz, 1H), 8.01 (d, *J* = 9.0 Hz, 1H), 8.54 (d, *J* = 5.5 Hz, 1H). ^{13}C NMR δ (ppm)(CHCl•-d): (amide cis/trans resolved) 171.9, 151.2, 150.1, 147.1, 136.7, 135.7, 135.6, 129.2, 128.8, 128.3, 128.0, 127.8, 126.7, 126.2, 125.6, 122.5, 117.1, 98.5, 49.7, 49.1, 47.6, 42.7.

References

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