Supporting Information

2-Oxotetrahydroquinoline-Based Antimalarials with High Potency and Metabolic Stability

Vivek J. Bulbule, Kasey Rivas, Christophe L.M.J. Verlinde, Wesley C. Van Voorhis, and Michael H Gelb*

Departments of Chemistry, Biochemistry and Medicine, University of Washington, Seattle, WA 98195, USA.

Email: gelb@chem.washington.edu

Contents

1. General Methods S2
2. Plasmodium strains S2
3. P. falciparum culture S2
4. P. falciparum ED\textsubscript{50} determination S2
5. PfPFT IC\textsubscript{50} determination S3
6. Microsome Metabolism S4
7. Synthesis: Experimental procedures. S4
8. C18 reverse phase HPLC traces of target compounds S17 onwards
**Chemistry**

1. **General Methods**

Unless otherwise indicated, all anhydrous solvents were commercially obtained and stored under nitrogen. Reactions were performed under an atmosphere of dry nitrogen in oven dried glassware and were monitored for completeness by thin layer chromatography (TLC) using silica gel 60 F-254 (0.25 mm) plates with detection with UV light. $^1$H-NMR spectra were recorded on dilute solutions in CDCl$_3$, CD$_3$OD, D$_2$O or DMSO-d$_6$ at 300 or 500 MHz. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS). Coupling constants (J) are reported in Hz. Electrospray ionization mass spectra were acquired on a Bruker Esquire LC00066. Flash chromatography was carried out with silica gel (40-63 μm). Preparative reverse phase HPLC was performed on an automated Varian Prep star system using a gradient of 20% MeOH to 100% MeOH (with 0.1% trifluoroacetic acid) over 30 min using a YMC S5 ODS column (20x100 mm, Waters Inc.). All final compounds (those tested on Pf-PFT and on parasites cultures) were shown to be pure based on HPLC as described above.

2. **Plasmodium strains.** The *P. falciparum* strains used in this study were 3D7 (Netherlands [airport-associated malaria], chloroquine sensitive) and K1 (Thailand, chloroquine resistant, pyrimethamine resistant). Strain 3D7 was provided by Dr. Pradipsinh Rathod from the University of Washington. *P. falciparum* strain K1 and *P. berghei* isolate NK65 (used for rodent malaria experiments) were obtained from the MR4 Unit of the American Type Culture Collection (ATCC, Manassas, VA).

3. **P. falciparum culture.** Strains of *P. falciparum* were cultured *in vitro* using experimental techniques described by Trager and Jensen.$^{22}$ Cultures were maintained in RPMI-1640 (Sigma, St. Louis, MI) with 2 mM L-glutamine, 25 mM HEPES, 33 mM NaHCO$_3$, 20 μg/ml gentamicin sulfate and 20% (v/v) heat-inactivated human plasma type A+ (RP-20P). Type A+ erythrocytes were obtained from lab donors, washed three times with RPMI 1640, re-suspended in 50% RPMI-1640, and stored at 4°C. Parasites were grown in 10-ml of a 2% hematocrit/RP-20P (v/v) in 50-ml flasks under a 5% CO$_2$, 5% O$_2$, and 90% N$_2$ atmosphere.

4. **P. falciparum ED$_{50}$ determination.** Twenty microliters of PFTI in solution, dissolved in RP-20P from a 10 mM stock in dimethylsulfoxide, was added to each well of a 96-well
plate followed by the addition of 190 µl of an asynchronous *P. falciparum* culture at parasitemia and hematocrit of 0.5%. Plates were flushed with 5% CO₂, 5% O₂, and 90% N₂ then incubated at 37°C for 48 hr. [8-³H]-Hypoxanthine (0.3 µCi, 20 Ci/mmol, American Radiolabeled Chemicals) in 30 µl RP-20P was added to cultures and incubated for an additional 24 hr. Cells were harvested onto glass fiber filters by a cell harvester (Inotech Biosystems International, Inc Rockville, MD), and the radioactivity incorporated into the parasites was counted on a Chameleon™ 425-104 multilabel plate counter (Hidex Oy Turku, Finland). The background level detected with uninfected erythrocytes was subtracted from the data. The ³H-incorporation into infected RBCs with 1 µl DMSO vehicle alone represents 100% malaria growth. ED₅₀ values, the effective dose that reduces growth by 50%, were determined by linear regression analysis of the plots of ³H-hypoxanthine incorporation versus concentration of compound. Each compound was tested in duplicate, and the mean value is shown; individual measurements differed by less than three-fold.

5. PfPFT IC₅₀ determination. The PFT assay used to determine the IC₅₀ values (inhibitor concentration that causes 50% enzyme inhibition) of the compounds is based on a PFT [³H] scintillation proximity assay (SPA) (TRKQ7010 Amersham Biosciences Corp Piscataway, NJ). Assays were carried out in assay buffer (pH 7.5, 50 mM HEPES, 30 mM MgCl₂, 20 mM KCl, 5 mM DTT, 0.01% Triton X-100), 1 µM human lamin-B carboxy-terminus sequence peptide (biotin-YRASNRSCAIM) and 1 µCi ³H-farnesylpyrophosphate (Amersham specific activity 15 to 20 Ci/mM) in a total volume of 50 µL, which included 1 µL of Pf-PFT inhibitor solution in DMSO and 5 µL of partially purified Pf-PFT. Assays in the absence of PfPFT inhibitor and PfPFT were included as positive and negative controls, respectively. Reaction mixtures were incubated at 37 °C for 60 minutes and terminated by addition of 70 µL of assay STOP solution and 5 µL SPA beads. The assay mixture was incubated at room temperature for 30 minutes. The assay was counted on a plate Chameleon™ 425-104 multilabel counter (Hidex Oy Turku, Finland). IC₅₀ values were calculated using linear regression analysis of the plots of the amount of radio-prenylation versus the concentration of compound.
6. Microsome Metabolism
Liver microsome metabolism assays were performed with female pooled microsomes (BD Biosciences, San Jose, CA). Reactions (400 μL) contained pH 7.4 0.1 M potassium phosphate buffer, 3 mM MgCl₂, 1 mM EDTA, 1 mM NADP+, 5 mM Glucose-6-phosphate, 1 U/ml Glucose-6-phosphate dehydrogenase (Sigma, St Louis, MO), and 0.5 mg/ml liver microsomes. Each reaction was incubated at 37 ºC for 10 minutes, then 2 μL of THQ at 200 μM stock in DMSO was added to give a final solution of 1 μM THQ in the reaction. At each time point, samples of the reactions were stopped with 3 x the volume of acetonitrile at containing an internal standard. THQ concentration and metabolites were quantified for each time point by liquid chromatography (LC)- electrospray ionization mass spectrometry (MS) analysis.

7. Synthesis : Experimental procedures.
Methyl 2-((1-methyl-1H-imidazol-5-yl)methylamino)-5-bromobenzoate :

![Chemical Structure]

A mixture of methyl 2-amino-5-bromobenzoate (5.0 g, 0.021mol), 1-Methyl-1-H-imidazole-5-carbaxaldehyde (2.87 g, 0.026 mol) and 100 mL of 50% trifluoroacetic acid in dichloroethane was warmed at 50 ºC under argon. After 2 hours triethylsilane (2.87 g, 4.0 mL, 0.024 mol) was added. After 48 hours, the solvent was removed under reduced pressure, and the crude product was partitioned between methylene chloride and aqueous saturated NaHCO₃. The organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (2x50 mL). The combine organic layer were dried over MgSO₄, filtered and concentrated. The crude residue was recrystallized with ethyl acetate:hexane or purified on a flash silica gel chromatography to afford 2 as a white solid. Yield: 3.6 g, 50%.

¹H-NMR (300 MHz, Methanol-d₄) δ 7.95 (s, 1H), 7.60 (s, 1H), 7.45 (d, J = 7.2, 1H), 6.95 (s, 1H), 6.85 (d, J = 7.2 Hz, 1H), 4.45 (s, 2H), 3.85 (s, 3H), 3.70 (s, 3H). MS m/z 325 (M+H⁺)
(2-((1-methyl-1H-imidazol-5-yl)methylamino)-5-bromophenyl)methanol :

To a suspension of LAH (420 mg, 0.011 mol) in THF (20 mL) was added 2 (3.59 g, 0.011 mol) in THF (25 mL) at 0 °C. Further stirring continued for 2 hours bringing down slowly to room temperature. Reaction mixture was quenched with aqueous saturated Na₂SO₄ solution at 0 °C and resulting slurry was stirred for another hour, and then filtered to remove sulfate salts, and the solvent was removed under reduced pressure to provide the alcohol 3 as a white solid. Yield: 2.35 g, 72%.

1H-NMR (300 MHz, Methanol-d₄) δ 7.65 (s, 1H), 7.30 (d, J = 7.2 Hz, 1H), 7.25 (s, 1H), 6.95 (s, 1H), 6.75 (d, J = 7.2 Hz, 1H), 4.60 (s, 2H), 4.40 (s, 2H), 3.75 (s, 3H). MS m/z 296 (M+H⁺)

Ethyl 3-acetamido-6-bromo-1,2,3,4-tetrahydro-1-((1-methyl-1H-imidazol-5-yl)methyl)-2-oxoquinoline-3-carboxylate (3):

To a stirred solution of alcohol above (1.13 g, 3.82 mmol) dissolved in thionyl chloride (5.0 mL) was added a drop of DMF and mixture was heated to reflux. After 1 hour excess thionyl chloride was removed under reduced pressure, and the resulting solid was dried under vacuum to give a white solid 4-bromo-2-(chloromethyl)-N-((1-methyl-1H-imidazol-5-yl)methyl) benzenamine dihydrochloride 2 (1.48 g, 90%) which was used without further purification.

Diethyl acetamidomalonate (1.65 g, 7.62 mmol) was slowly added to a suspension of sodium hydride (550 mg, 95% powder, 22.9 mmol) in dry DMF (20 mL) at 0 °C. The mixture was allowed to warm to room temperature, and stirring under nitrogen was continued until a clear yellow solution was obtained and no more H₂ evolved (ca 2 hours). Then a solution of 2 (1.48 g, 3.82 mol) in dry DMF (20 mL) was added and the mixture was refluxed under nitrogen for 12 hours. The reaction was quenched with saturated
NH₄Cl (5 mL) combined aqueous DMF was evaporated under reduced pressure to give the crude product. Purification of the crude product by column chromatography (SiO₂; EtOAc-MeOH, 8:2) gave a dense yellow liquid 3. Yield: 1.07 g, 65%.

1H-NMR (300 MHz, Methanol-d₄) δ 8.90 (s, 1H), 7.35 (d, J = 8.0, 1H), 7.55 (s, 1H), 7.49- 7.41 (m, 2H), 7.08 (d, J = 8.0 Hz, 1H), 5.45 (d, J = 16.0 Hz, 1H), 5.15 (d, J = 16.0 Hz, 1H), 4.09 (q, J = 3.0 Hz, 2H), 2.05 (s, 3H), MS m/z 449 (M+H⁺) and 451 (M+H⁺).

3-amino-6-bromo-3,4-dihydro-1-((1-methyl-1H-imidazol-5-yl)methyl)quinolin-2(1H)-one (4):

A solution of 3 (1.07 g, 2.38 mmol) in 12 M HCl was heated to reflux for 12 hours. The mixture was concentrated and dried to give crude solid as the hydrochloride. The free amine was obtained by treating the methanolic solution of the above solid with saturated NaHCO₃ solution. The combined mixture was evaporated to dryness to get the solid which was purified by column chromatography (SiO₂; CHCl₃-MeOH, 9:1) gave a crystalline brown color solid 4. Yield: 600 mg, 75%.

1H-NMR (300 MHz, D₂O) δ 8.50 (s, 1H), 7.35 (d, J = 8.0, 1H), 7.40 (s, 1H), 7.20 (s, 1H), 6.95 (d, J = 8.0 Hz, 1H), 5.35 (d, J = 16.0 Hz, 1H), 5.20 (d, J = 16.0 Hz, 1H), 4.29 (d, J = 16.0 Hz, 1H), 4.25 (d, J = 16.0 Hz, 1H), 3.75 (s, 3H), 3.29-3.10 (m, 2H). MS m/z 335 (M+H⁺) and 337 (M+H⁺).

General procedure for Sulfonation.

A solution of 3-amino-6-bromo-3,4-dihydro-1-((1-methyl-1H-imidazol-5-yl)methyl)quinolin-2(1H)-one (6) (5 mmol), N, N-diisopropylethyl amine (15 mmol) in 25 mL of anhydrous CH₃CN and required sulfonyl chloride (10 mmol) was added in portion at room temperature and stirred overnight. A light colored precipitate was isolated by vacuum filtration. More often, product was obtained by flash chromatography on silica gel column eluting with 20% MeOH/ethyl acetate. Yields 85-95%.

General procedure for N-alkylation.
To the suspension of required sulfonamide (5 mmol) and Cs₂CO₃ (7.5 mmol) in dry DMF (5 mL) was added the appropriate alkyl halide (5.4 mmol), and the mixture was stirred at room temperature overnight under argon. After addition of water (20 mL), the solution was extracted with ethyl acetate (3 x 20 mL). The organic layer was extracted with brine (3 x 10 mL). The combine organic layers were dried over MgSO₄ and evaporated under reduce pressure. The residue was purified by HPLC. Appropriate fractions were collected, and the pure product was obtained as the trifluoroacetate salt. Yields 50-60%.

**General procedure for nitriles.**

Microwave heating was carried out with a SANYO (MODEL # EM-220M), 900 W, producing irradiations at 2450 MHz. The required analog containing bromine (2.0 mmol) was dissolved in (1.0 mL) of anhydrous DMF in round bottom flask with teflon septum. Argon was bubbled through the solution for 10 min by inserting needle through the cap. Zn(CN)₂ (2.5 mmol) was added, and argon was passed through solution for additional 10 min. Pd(PPh₃)₄ (0.02 mmol) was added followed by brief bubbling of argon. The reaction mixture was exposed to microwave irradiation for 3 min. The reaction was allowed to reach to room temperature before reaction was quenched with water. The solvent was removed under reduced pressure. The crude residue was purified with RP HPLC. Appropriate fractions were collected and the pure product was obtained as the trifluoroacetate salt. Yields 60-78%.

**1-Methyl-1H-imidazole-4-sulfonic acid [6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-amide**

\[
\text{1H-NMR (300 MHz, D}_2\text{O)} \delta 8.55 (s, 1H), 8.12 (s, 1H), 7.75 (s, 1H), 7.32 (d, } J = 7.2 \text{ Hz, 1H), 7.28 (s, 1H), 7.11 (s, 1H), 6.88 (d, } J = 7.2 \text{ Hz, 1H), 5.12 (d, } J = 16.0 \text{ Hz, 1H), 5.00 (d, } J = 16.0 \text{ Hz, 1H), 4.28 (t, } J = 6.0 \text{ Hz, 1H), 3.75 (s, 3H), 3.70 (s, 3H), 2.95 (d, } J = 6.0 \text{ Hz, 2H). MS m/z 479 (M+H\textsuperscript{+}) and 481 (M+H\textsuperscript{+})}
\]

**1-Methyl-1H-imidazole-4-sulfonic acid [6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-amide**
\( ^1\text{H-NMR (300 MHz, Methanol-}d_4 \text{)} \delta 8.87 (s, 1H), 8.62 (d, J = 6.0 \text{ Hz}, 1H), 8.08-8.00 (m, 1H), 7.73-7.57 (m, 3H), 7.56-7.30 (m, 2H), 5.45 (d, J = 18.0 \text{ Hz}, 1H), 5.28 (d, J = 18.0 Hz, 1H), 4.61 (d, J = 3.0 \text{ Hz}, 0.5H), 4.58 (d, J = 3.0 \text{ Hz}, 0.5H), 3.93 (s, 3H), 3.41-3.07 (m, 2H). \text{MS m/z 423.2 (M+H\textsuperscript{+}).} \)

1,5-dimethyl-1H-imidazole-4-sulfonic acid [6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-amide

\( ^1\text{H-NMR (300 MHz, Methanol-}d_4 \text{)} \delta 8.80 (s, 1H), 8.00 (s, 1H), 7.40-7.52 (m, 3H), 7.12 (d, J = 7.2 \text{ Hz}, 1H), 5.38 (d, J = 16.0 \text{ Hz}, 1H), 5.20 (d, J = 16.0 \text{ Hz}, 1H), 4.35 (d, J = 2.0 \text{ Hz}, 0.5H), 4.30 (d, J = 2.0 \text{ Hz}, 0.5H), 3.90 (s, 3H), 3.70 (s, 3H), 3.00-3.30 (m, 2H), 2.50 (s, 3H). \text{MS m/z 493 (M+H\textsuperscript{+}) and 495 (M+H\textsuperscript{+}).} \)

3,4-Dihydro-2H-benzo[b][1,4]dioxepine-7-sulfonic acid [6-bromo-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-amide

\( ^1\text{H-NMR (300 MHz, Methanol-}d_4 \text{)} \delta 8.80 (s, 1H), 7.40-7.50 (m, 5H), 7.02 (d, J = 7.2 \text{ Hz}, 1H), 7.10 (d, J = 7.2 \text{ Hz}, 1H), 5.30 (d, J = 16.0 \text{ Hz}, 1H), 5.15 (d, J = 16.0 \text{ Hz}, 1H), 4.20-4.32 (m, 4H), 4.10-4.20 (m, 1H) 2.98-3.20 (m, 2H), 2.12-2.28 (m, 2H). \text{MS m/z 547 (M+H\textsuperscript{+}), 547 (M+H\textsuperscript{+}) and 549 (M+H\textsuperscript{+}).} \)

N-tert-Butyl-2-[[6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-(3-fluoro-benzenesulfonyl)-amino]-acetamide (28)
H-NMR (300 MHz, Methanol-\(d_4\)) \(\delta\) 8.91 (s, 1H), -7.71-7.81 (m, 1H), 7.69-7.63 (m, 3H), 7.62-7.50 (m, 1H), 7.49-7.42 (m, 1H), 7.41-7.29 (m, 2H), 5.30 (d, \(J = 16.0\) Hz, 1H), 5.18 (d, \(J = 16.0\) Hz, 1H), 4.38 (d, \(J = 2.5\) Hz, 0.5H), 4.13 (d, \(J = 2.5\) Hz, 0.5H), 4.10 (d, \(J = 18.0\) Hz, 1H), 3.93 (s, 3H), 3.63 (d, \(J = 18.0\) Hz, 1H), 3.29-3.05 (m, 2H). MS m/z 553 (M+H\(^+\)).

N-tert-Butyl-2-[[6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-[(3-ethoxy-4-hydroxy-benzenesulfonyl)-amo]-acetamide (27)

H-NMR (300 MHz, Methanol-\(d_4\)) \(\delta\) 8.90 (s, 1H), 7.70-7.62 (m, 2H), 7.55 (s, 1H), 7.45-7.52 (m, 2H), 7.35 (d, \(J = 8.0\) Hz, 1H), 7.35 (d, \(J = 8.0\) Hz, 1H), 7.10 (d, \(J = 8.0\) Hz, 1H), 5.42 (d, \(J = 16.0\) Hz, 1H), 5.30 (d, \(J = 16.0\) Hz, 1H), 4.30 (q, \(J = 3.0\) Hz, 4H), 4.00 (d, \(J = 18.0\) Hz, 1H), 3.98 (s, 3H), 3.70 (d, \(J = 18.0\) Hz, 1H), 3.55 (t, \(J = 2.5\) Hz, 1H), 3.18 (d, \(J = 2.5\) Hz, 0.5 H), 3.13 (d, \(J = 2.5\) Hz, 0.5 H), 2.25 (q, \(J = 3.0\) Hz, 4H), 1.30 (s, 9H). MS m/z 607 (M+H\(^+\)).

N-tert-Butyl-2-[[6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-[(1,5-dimethyl-1H-imidazole-4-sulfonyl)-amo]-acetamide (26)
$^1$H-NMR (300 MHz, Methanol-$d_4$) δ 8.93 (s, 1H), 8.00 (s, 1H), 7.71-7.64 (m, 2H), 7.49 (s, 1H), 7.32 (d, $J = 9.0$ Hz, 1H), 5.29 (s, 2H), 4.83 (s, 2H), 4.40 (d, $J = 2.0$ Hz, 0.5H), 4.36 (d, $J = 2.0$ Hz, 0.5H), 3.95 (s, 3H), 3.67 (s, 3H), 3.00-3.30 (m, 2H), 2.50 (s, 3H), 1.35 (s, 9H). MS m/z 553 (M+H$^+$).

Pyridine-2-sulfonic acid [6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-[4-methanesulfonyl-benzyl]-amide (25)

$^1$H-NMR (300 MHz, Methanol-$d_4$) δ 8.82 (s, 1H), 8.65 (d, $J = 4.0$ Hz, 1H), 8.01-7.97 (m, 3H), 7.87 (d, $J = 8.0$ Hz, 1H), 7.73-7.62 (m, 6H), 7.46 (s, 1H), 7.29 (d, $J = 8.0$ Hz, 1H), 5.23 (s, 2H), 5.15-4.90 (m, 3H), 3.89 (s, 3H), 3.28-3.24 (m, 1H), 3.14 (s, 3H), 3.12-3.07 (m, 1H). MS m/z 591.1 (M+H$^+$).

1-Methyl-1H-imidazole-4-sulfonic acid [6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-pyridin-2-ylmethyl-amide (24)

$^1$H-NMR (300 MHz, Methanol-$d_4$) δ 9.07 (s, 1H), 8.52 (d, $J = 8.0$ Hz, 1H), 7.90-7.83 (m, 2H), 7.70 (s, 1H), 7.68-7.55 (m, 2H), 7.48-7.38 (m, 3H), 7.29 (d, $J = 8.0$ Hz, 1H), 5.38 (d, $J = 16.0$ Hz, 1H), 5.23 (d, $J = 16.0$ Hz, 1H), 4.39 (d, $J = 2.0$ Hz, 0.5H), 4.33 (d, $J = 2.0$ Hz, 0.5H), 3.93 (s, 3H), 3.78 (s, 3H), 3.02-3.28 (m, 2H). MS m/z 517 (M+H$^+$).

1-Methyl-1H-imidazole-4-sulfonic acid [6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-(2-fluoro-benzyl)-amide (23)
1H-NMR (300 MHz, Methanol-d$_4$) $\delta$ 8.90 (s, 1H), 7.72 (s, 1H), 7.78-7.49 (m, 4H), 7.35-7.60 (m, 2H), 7.19-7.12 (m, 1H), 7.05-6.95 (m, 1H), 5.35 (d, $J = 16.0$ Hz, 1H), 5.25 (d, $J = 16.0$ Hz, 1H), 4.78 (d, $J = 16.0$ Hz, 1H), 4.52 (d, $J = 16.0$ Hz, 1H), 3.93 (s, 3H), 3.72 (s, 3H), 3.15 (d, $J = 2.5$ Hz, 0.5 H), 3.22 (d, $J = 2.5$ Hz, 0.5 H). MS m/z 534 (M+H$^+$).

1-Methyl-1H-imidazole-4-sulfonic acid [6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-(2-methyl-benzyl)-amide (22)

1H-NMR (300 MHz, Methanol-d$_4$) $\delta$ 8.85 (s, 1H), 7.71-7.61 (m, 3H), 7.56 (s, 1H), 7.40-7.15 (m, 5H), 5.45-5.28 (m, 4H), 4.50-4.32 (m, 1H), 3.90 (s, 3H), 3.78 (s, 3H), 3.19-2.85 (m, 2H), 2.27 (s, 3H). MS m/z 530 (M+H$^+$).

1-Methyl-1H-imidazole-4-sulfonic acid [6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-(2,4-difluoro-benzyl)-amide (21)

1H-NMR (300 MHz, Methanol-d$_4$) $\delta$ 8.85 (s, 1H), 7.73 (s, 1H), 7.71-7.62 (m, 3H), 7.61 (s, 1H), 7.52 (s, 1H), 7.32 (d, $J = 8.0$ Hz, 1H), 7.0-6.83 (m, 2H), 5.34 (d, $J = 16.0$ Hz, 1H), 5.26 (d, $J = 16.0$ Hz, 1H), 5.09-4.95 (m, 1H), 4.71 (d, $J = 18.0$ Hz, 1H), 4.67 (d, $J = 16.0$ Hz, 1H), 3.94 (s, 3H), 3.76 (s, 3H), 3.24-3.15 (m, 2H). MS m/z 552 (M+H$^+$).
1-Methyl-1H-imidazole-4-sulfonic acid [6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-[4-pyrazol-1-yl-benzyl]-amide (19)

\[
\text{NC} \begin{array}{c}
\text{N} \\
\text{N} \\
\text{O} \\
\text{O} \\
\text{N}
\end{array}
\text{S} \begin{array}{c}
\text{O} \\
\text{O} \\
\text{N} \\
\text{N}
\end{array}
\]

\[\text{H-NMR (300 MHz, Methanol-} \text{d}_4 \text{)} \delta 8.86 (\text{s, 1H}), 8.21 (\text{d, } J = 2.0 \text{ Hz, 1H}), 7.77-7.72 (\text{m, 3H}), 7.69-7.62 (\text{m, 4H}), 7.57 (\text{s, 1H}), 7.54-7.49 (\text{m, 2H}), 7.48 (\text{s, 1H}), 7.29 (\text{d, } J = 6.0 \text{ Hz, 1H}), 6.56-6.53 (\text{m, 1H}), 5.33 (\text{d, } J = 18.0 \text{ Hz, 1H}), 5.26 (\text{d, } J = 18.0 \text{ Hz, 1H}), 5.01 (\text{d, } J = 4.0 \text{ Hz, 0.5H}), 4.95 (\text{d, } J = 4.0 \text{ Hz, 0.5H}), 4.75 (\text{d, } J = 18.0 \text{ Hz, 1H}), 4.47 (\text{d, } J = 16.0 \text{ Hz, 1H}), 3.93 (\text{s, 3H}), 3.71 (\text{s, 3H}), 3.48 (\text{t, } J = 10.0 \text{ Hz, 1H}), 3.21 (\text{t, } J = 6.0 \text{ Hz, 0.5H}), 3.16 (\text{t, } J = 6.0 \text{ Hz, 0.5H}). \text{ MS } m/z 582 (\text{M+H}^+)\]

2-[[6-Cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydroquinolin-3-yl]-(1-methyl-1H-imidazole-4-sulfonyl)-amino]-succinic acid di-tert-butyl ester (18)

\[
\text{NC} \begin{array}{c}
\text{N} \\
\text{N} \\
\text{O} \\
\text{O} \\
\text{N}
\end{array}
\text{O} \begin{array}{c}
\text{O} \\
\text{O} \\
\text{N} \\
\text{N}
\end{array}
\]

\[\text{H-NMR (300 MHz, Methanol-} \text{d}_4 \text{)} \delta 8.96 (\text{s, 1H}), 7.80-7.65 (\text{m, 4H}), 7.59 (\text{s, 1H}), 7.29 (\text{d, } J = 7.6 \text{ Hz, 1H}), 5.35 (\text{d, } J = 16.0 \text{ Hz, 1H}), 5.16 (\text{d, } J = 16.0 \text{ Hz, 1H}), 5.11-5.01 (\text{m, 1H}), 4.99 (\text{s, 2H}), 4.16 (\text{d, } J = 16.0 \text{ Hz, 1H}), 3.98 (\text{s, 3H}), 3.85 (\text{d, } J = 16.0 \text{ Hz, 1H}), 3.78 (\text{s, 3H}), 1.52 (\text{s, 9H}), 1.49 (\text{s, 9H}). \text{ MS } m/z 654 (\text{M+H}^+)\]

[[6-Cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-(1-methyl-1H-imidazole-4-sulfonyl)-amino]-acetic acid tert-butyl ester (17)
$^1$H-NMR (300 MHz, Methanol-$d_4$) $\delta$ 8.89 (s, 1H), 7.75-7.69 (m, 3H), 7.67 (d, $J$ = 7.6 Hz, 1H), 7.52 (s, 1H), 7.29 (d, $J$ = 7.6 Hz, 1H), 5.32 (d, $J$ = 16.0 Hz, 1H), 5.20 (d, $J$ = 16.0 Hz, 1H), 5.08 (d, $J$ = 3.0 Hz, 0.5H), 5.03 (d, $J$ = 3.0 Hz, 1H), 4.17 (d, $J$ = 16.0 Hz, 1H), 3.95 (s, 3H), 3.88 (d, $J$ = 16.0 Hz, 1H), 3.77 (s, 3H), 3.49-3.35 (m, 2H). MS m/z 540.5 (M+H$^+$).

1-Methyl-1H-imidazole-4-sulfonic acid [6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-(5-trifluoromethyl-furan-2-ylmethyl)-amide (16)

$^1$H-NMR (300 MHz, Methanol-$d_4$) $\delta$ 8.90 (s, 1H), 7.73 (s, 1H), 7.71-7.65 (m, 2H), 7.52 (s, 1H), 7.32 (d, $J$ = 7.6 Hz, 1H), 6.90 (m, 1H), 6.50 (d, $J$ = 7.6 Hz, 1H), 5.35 (d, $J$ = 16.0 Hz, 1H), 5.28 (d, $J$ = 16.0 Hz, 1H), 5.16-5.06 (m, 1H), 4.79 (d, $J$ = 16.0 Hz, 1H), 4.48 (d, $J$ = 16.0 Hz, 1H), 3.97 (s, 3H), 3.75 (s, 3H), 3.62-3.49 (m, 1H), 3.25-3.15 (m, 2H). MS m/z 574.5 (M+H$^+$).

1-Methyl-1H-imidazole-4-sulfonic acid (4-cyano-benzyl)-[6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-amide (15)

$^1$H-NMR (300 MHz, Methanol-$d_4$) $\delta$ 8.88 (s, 1H), 7.75 (s, 1H), 7.71-7.57 (m, 7H), 7.52 (s, 1H), 7.28 (d, $J$ = 8.0 Hz, 1H), 5.28 (s, 2H), 4.95-5.06 (m, 1H), 4.82 (d, $J$ = 16.0 Hz, 1H), 4.46 (d, $J$ = 16.0 Hz, 1H), 3.94 (s, 3H), 3.75 (s, 3H), 3.48-3.36 (m, 1H), 3.20 (d, $J$ = 3.0 Hz, 0.5H), 3.15 (d, $J$ = 3.0 Hz, 0.5H). MS m/z 541.4 (M+H$^+$).

1-Methyl-1H-imidazole-4-sulfonic acid benzyl-[6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-amide (14)

S13
1H-NMR (300 MHz, Methanol-\(d_4\)) \(\delta\) 8.85 (s, 1H), 7.74 (s, 1H), 7.67-7.61 (m, 1H), 7.60 (s, 1H), 7.55 (s, 1H), 7.48 (s, 1H), 7.37-7.31 (m, 2H), 7.30-7.23 (m, 4H), 5.06-5.15 (m, 1H), 4.70 (d, \(J = 16.0\) Hz, 1H), 4.42 (d, \(J = 16.0\) Hz, 1H), 3.92 (s, 3H), 3.74 (s, 3H), 3.19-3.07 (m, 2H). MS m/z 526.6 (M+H\(^+\)).

1-Methyl-1H-imidazole-4-sulfonic acid [6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl)-(2-methyl-allyl)-amide (13)

1H-NMR (300 MHz, Methanol-\(d_4\)) \(\delta\) 8.85 (s, 1H), 7.76 (s, 1H), 7.73 (s, 1H), 7.69-7.65 (m, 2H), 7.33 (d, \(J = 7.6\) Hz, 1H), 5.41 (d, \(J = 16.0\) Hz, 1H), 5.23 (d, \(J = 16.0\) Hz, 1H), 5.10 (m, 2H), 4.70 (s, 2H), 4.33 (d, \(J = 2.0\) Hz, 0.5H), 4.29 (d, \(J = 2.0\) Hz, 0.5H), 3.93 (s, 3H), 3.80 (s, 3H), 3.00-3.02 (m, 2H), 1.73 (s, 3H). MS m/z 480.3 (M+H\(^+\)).

3-yl)-(1-methyl-1H-imidazole-4-sulfonyl)-amino]-N-tert-butyl-acetamide (12)

1H-NMR (300 MHz, Methanol-\(d_4\)) \(\delta\) 8.90 (s, 1H), 7.85 (s, 1H), 7.85 (s, 1H), 7.73 (s, 1H), 7.53 (s, 1H), 7.69-7.65 (m, 2H), 7.33 (d, \(J = 7.6\) Hz, 1H), 5.41 (d, \(J = 16.0\) Hz, 1H), 5.23 (d, \(J = 16.0\) Hz, 1H), 5.10 (m, 2H), 4.70 (s, 2H), 4.33 (d, \(J = 2.0\) Hz, 0.5H), 4.29 (d, \(J = 2.0\) Hz, 0.5H), 3.93 (s, 3H), 3.80 (s, 3H), 3.26-3.02 (m, 2H), 1.73 (s, 3H). MS m/z 593 (M+H\(^+\)).
N-tert-Butyl-2-[[6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-([1-methyl-1H-imidazole-4-sulfonyl]-amino)-acetamide (11)

\[
\begin{align*}
\text{NC} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\text{O} & \quad \text{S} \\
\text{O} & \quad \text{O} \\
\text{N} & \quad \text{N} \\
\text{O} & \quad \text{N} \\
\text{H} & \quad \text{H}
\end{align*}
\]

\(^1\)H-NMR (300 MHz, Methanol-\(d_4\)) \(\delta\) 8.93 (s, 1H), 7.91 (s, 1H), 7.80 (s, 1H), 7.66 (d, \(J = 8.0\) Hz, 1H), 7.65 (s, 1H), 7.50 (s, 1H) 7.33 (d, \(J = 8.0\) Hz, 1H), 5.34 (d, \(J = 16.0\) Hz, 1H), 5.26 (d, \(J = 16.0\) Hz, 1H), 4.83 (s, 2H), 4.43 (d, \(J = 2.0\) Hz, 0.5H), 4.39 (d, \(J = 2.0\) Hz, 0.5H), 3.95 (s, 3H), 3.80 (s, 3H), 3.00-3.30 (m, 2H), 1.32 (s, 9H). MS m/z 539 (M+H\(^+\)).

2-[[6-Bromo-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydroquinolin-1-Methyl-1H-imidazole-4-sulfonic acid [6-cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl]-[methanesulfonyl-benzyl]-amide (9)

\[
\begin{align*}
\text{NC} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\text{O} & \quad \text{S} \\
\text{O} & \quad \text{O} \\
\text{S} & \quad \text{O} \\
\text{O} & \quad \text{N} \\
\text{N}
\end{align*}
\]

\(^1\)H-NMR (300 MHz, Methanol-\(d_4\)) \(\delta\) 8.89 (s, 1H), 7.88 (d, \(J = 8.0\) Hz, 1H), 7.76 (s, 1H), 7.72-7.58 (m, 5H), 7.52 (s, 1H), 7.29 (d, \(J = 8.0\) Hz, 1H), 5.28 (s, 2H), 5.00-5.0 (m, 1H), 4.83 (d, \(J = 16.0\) Hz, 1H), 4.51 (d, \(J = 16.0\) Hz, 1H), 3.92 (s, 3H), 3.75 (s, 3H), 3.23-3.16 (m, 1H), 3.11-3.06 (m, 1H). MS m/z 594.3 (M+H\(^+\)).

4-[[6-Cyano-1-(3-methyl-3H-imidazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydroquinolin-3-yl]-[1-methyl-1H-imidazole-4-sulfonyl]-amino]-methyl]-piperidine-1-carboxylic acid methyl ester (8)
$^1$H NMR (300 MHz Methanol-$d_4$, ppm), $\delta$ 8.89 (s, 1H), 7.82-7.62 (m, 4H), 7.52 (s, 1H), 7.30 (d, $J$ = 8 Hz, 1H), 5.41 (d, $J$ = 16.0 Hz, 1H), 5.26 (d, $J$ = 16.0 Hz, 1H), 4.16-4.05 (m, 2H), 3.98 (s, 3H), 3.81 (s, 3H), 3.70 (s, 3H), 3.21-3.10 (m, 2H), 2.97-3.07 (m, 2H), 2.85-2.65 (m, 3H), 1.91-1.78, (m, 3H), 0.09-1.12 (m, 2H). MS (EI) m/z 567.6 (M+H$^+$).

**HPLC of target compounds:**

On the following pages are the HPLC traces (see above for experimental details) of compounds 8, 9, 16, 11, 14, 15, 17, 19, 25 and 27 in that order.
Data File:  c:\star\data\1213_03_data\vjb-305-2
Injection Date:  12/27/2006 03:06:01
Injection Method:  c:\star\methods\bms-thq-5-vjb.mth
Run Time (min):  29.970
Instrument (Inj):  Gelb Lab HPLC

Injection Notes:

Method Notes
No Method Notes
**Injection Date:** 10/28/2007 04:06:01

**Injection Method:** c:\star\methods\bms-thq-5-original.mth

**Run Time (min):** 29.975

**Instrument (Inj):** Gelb Lab HPLC

---

**Peak** | **Ret. Time** | **Width** | **Area** | **% Area**
---|---|---|---|---
1 | 2.122 | 6.8 | 1177206 | 2.44
2 | 18.146 | 31.4 | 47165972 | 97.56

**Total Area:** 48343176

**% Area:** 100.00

**Injection Notes:**

---

**Method Notes**

No Method Notes
**Injection Date:** 10/28/2007 05:23:03  
**Injection Method:** c:\star\methods\bms-thq-5-original.mth  
**Run Time (min):** 29.975  
**Instrument (Inj):** Gelb Lab HPLC

---

### Chromatogram Details

**Peaks:**

<table>
<thead>
<tr>
<th>Peak No</th>
<th>Ret. Time</th>
<th>Width</th>
<th>Area</th>
<th>% Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Injection Notes:**

No Method Notes
Data File: c:\star\data\1213_03_data\vjb-276r01
Injection Date: 10/30/2006 12:40:30
Injection Method: c:\star\methods\bms-thq-5-vjb.mth
Run Time (min): 29.970
Instrument (Inj): Gelb Lab HPLC

<table>
<thead>
<tr>
<th>Peak No</th>
<th>Ret. Time</th>
<th>Width</th>
<th>Area</th>
<th>% Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.300</td>
<td>38.9</td>
<td>52542836</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Injection Notes:

Method Notes
No Method Notes
UW Chemistry Department
Gelb Lab, HPLC Report

Data File: c:\star\data\1213_03_data\vjb-24r-jn
Injection Date: 10/29/2007 01:02:07
Injection Method: c:\star\methods\bms-thq-5-original.mth
Run Time (min): 29.975
Instrument (Inj): Gelb Lab HPLC

Injection Notes:

Method Notes
No Method Notes
UW Chemistry Department
Gelb Lab, HPLC Report

Data File: c:\star\data\1213_03_data\vjb-28-jm
Injection Date: 10/29/2007 01:36:37
Injection Method: c:\star\methods\bms-thq-5-original.mth
Run Time (min): 29.975
Instrument (Inj): Gelb Lab HPLC

Injection Notes:
Method Notes
No Method Notes
**UW Chemistry Department**  
**Gelb Lab, HPLC Report**

**Data File:**  
c:\star\data\1213_03_data\vjb-325-21

**Injection Date:**  03/21/2007 05:01:23

**Injection Method:**  c:\star\methods\bms-thq-5-vjb-1.mth

**Run Time (min):**  34.970

**Instrument (Inj):**  Gelb Lab HPLC

---

**Injection Notes:**

**Method Notes**  
No Method Notes