Supplemental Material

Isoquinoline-based analogs of the cancer drug clinical candidate tipifarnib as anti-*Trypanosoma cruzi* agents

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Synthesis of Compounds

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 $CDCl_3$ or CD_3OD at 300 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 an Bruker Esquire LC00066. Flash chromatography was carried out with silica gel (40-63 micron). 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) at 12 mL/min over 30 min using a YMC S5 ODS column (20x100 mm, Waters Inc.). All compounds tested on parasites were purified to a single peak by HPLC as described above. HPLC purified compounds were submitted to ^{1}H-NMR analysis, and compounds were submitted to testing on parasites if the ^{1}H-NMR spectrum was free of detectable non-compound peaks (estimated purity at least 95%).

2-Amino-1-(3-chlorophenyl)ethanol (5).

Trimethylsilyl cyanide (2.5 mL, 18.5 mmol) was added to a solution of *m*-chlorobenzaldehyde **4** (1.62 mL, 14.2 mmol) and catalytic ZnI_2 in dry CH_2Cl_2 (60 mL) under an atmosphere of nitrogen. The reaction mixture was stirred at room temperature for 3 hours, and washed with water, dried over Na_2SO_4. The solution was then concentrated to give a colorless liquid which was used without further purification in the next step. A solution of O-silylchlorophenyl cyanohydrin in THF (30 mL) was added dropwise to a suspension of $LiAlH_4$ (1.08g, 28.4 mmol) in THF (30 mL), which was cooled with an ice water bath. The reaction mixture was stirred at room

temperature for 2 hours, then cooled in an ice bath as water was slowly added. After stirring for 30 min at room temperature, the mixture was filtered, the precipitate was washed with additional THF and again filtered. The combined filtrates were dried over Na_2SO_4, filtered and concentrated to give the desired product (1.7 g, 9.95 mmol) in 70% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.32-7.19 (m, 3H), 4.65-4.56 (m, 1H), 3.00 (dd, 1H, *J* = 12.0 and 5.4 Hz), 2.78 (dd, 1H, *J* = 12.0 and 5.4 Hz), 2.40-2.00 (br S, 3H).

2-Amino-1-(2,6-difluorophenyl)ethanol.

The compound was prepared by following the procedure described for the synthesis of **5**. 2,6-Difluorobenzaldehyde (5 g, 35.18 mmol) was treated with TMSCN (6.1 mL, 45.74 mmol) and followed by reduction with $LiAlH_4$ (2.67 g, 70.36 mmol) to afford the product (3.83 g, 22.16 mmol) in 63% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.18 (m, 1H), 6.98-6.82 (m, 2H), 5.10-5.00 (m, 1H), 3.29-3.16 (m, 1H), 3.14-2.90 (m, 1H), 2.58-2.30 (br S, 3H).

2-(4-Bromobenzylamino)-1-(3-chlorophenyl)ethanol (6).

To a solution of compound **5** (1.2 g, 6.99 mmol) in methanol (30 mL) was added *p*-bromobenzaldehyde (1.42g, 7.69 mmol). To this reaction mixture triethylamine (1.06 mL, 7.68 mmol) was added and stirred for 6 hours under a nitrogen atmosphere. The reaction mixture was cooled and $NaBH_4$ (531 mg, 13.9 mmol) was added. The mixture was stirred overnight. After completion of the reaction methanol was evaporated, the residue was dissolved in CH_2Cl_2 and washed with water, brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography on silica gel to give the title compound **6** (1.78 g, 5.24 mmol) in 75% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (d, 2H, *J* = 8.1 Hz), 7.29-7.05 (m, 6H), 4.65-4.56 (m, 1H), 3.76-3.61 (m, 2H), 2.80 (dd, 1H, *J* = 12.0 and 3.3 Hz), 2.71-2.55 (m, 2H).

2-(4-Bromobenzylamino)-1-(2,6-difluorophenyl)ethanol.

The compound was prepared by following the procedure described for the synthesis of compound **6**. 2-Amino-1-(2,6-difluorophenyl)ethanol (2.1 g, 12.13 mmol) was treated with *p*-bromobenzaldehyde (2.47 g, 13.34 mmol) followed by reduction with $NaBH_4$ (921 mg, 24.26 mmol) to gave the product (2.98g, 8.73 mmol) in 72% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54-7.42 (m, 2H), 7.32-7.18 (m, 3H), 6.94-6.80 (m, 2H), 5.24-5.14 (m, 1H), 3.86 (d, 2H, *J* = 6.0 Hz), 3.42-3.29 (m, 1H), 3.25-3.11 (m, 1H), 2.96-2.84 (m, 1H).

6-Bromo-4-(3-chlorophenyl)-1,2,3,4-tetrahydroisoquinoline .

2-(4-Bromobenzylamino)-1-(3-chlorophenyl)ethanol **6** (1.5 g, 4.41 mmol) in CH_2Cl_2 (30 mL) was added slowly into a suspension of $AlCl_3$ (1.75 g, 13.2 mmol) in CH_2Cl_2 (30 mL). The mixture was refluxed for 3 hours under nitrogen atmosphere and then poured over ice and basified to pH 9.5 with 10% NaOH solution. The layers were separated and the aqueous layer was extracted with CH_2Cl_2, the combined organic layer was washed with brine and dried over Na_2SO_4. The organic layer was evaporated to yield the product (1.28g, 3.97 mmol) in 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26-7.13 (m, 3H), 7.03-6.86 (m, 4H), 4.10-3.90 (m, 3H), 3.30 (dd, 1H, *J* = 12.9 and 5.4 Hz), 2.98 (dd, 1H, *J* = 12.9 and 6.0 Hz).

6-Bromo-4-(2,6-difluorophenyl)-1,2,3,4-tetrahydroisoquinoline

The compound was prepared by following the procedure described for the synthesis of 6-bromo-4-(3-chlorophenyl)-1,2,3,4-tetrahydroisoquinoline. 2-(4-Bromobenzylamino)-1-(2,6-difluorophenyl)ethanol (4.15 g, 12.13 mmol) was reacted with $AlCl_3$ (4.84g, 36.39mmol) to afford the product (3.62g, 11.16 mmol) in 92% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.18 (m, 2H), 7.06-6.82 (m, 3H), 4.64-4.48 (m, 1H), 4.22-3.96 (m, 2H), 3.50-3.32 (m, 1H), 3.30-3.12 (m, 1H).

6-Bromo-4-(3-chlorophenyl)-2-trityl-1,2,3,4-tetrahydroisoquinoline (7).

To a stirred solution of 6bromo-4-(3-chlorophenyl)-1,2,3,4-tetrahydroisoquinoline (900 mg, 2.79 mmol) in CH_2Cl_2 was added Et_3N (0.466 mL, 3.35 mmol) and the solution was stirred in an ice bath. Catalytic DMAP and triphenylmethyl chloride (1.16 g, 4.19 mmol) were added in one portion, and the reaction mixture was stirred vigorously under nitrogen atmosphere at room temperature for 18 hours. The reaction mixture was quenched with water, and extracted with CH_2Cl_2, and dried over Na_2SO_4. The organic layer was evaporated to yield the crude product which was purified by flash chromatography on silica gel to get the pure product **7** (1.46 g, 2.59 mmol) in 93% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48-7.36 (m, 5H), 7.34-7.10 (m, 17H), 4.40-4.31 (m, 1H), 3.50 (s, 2H), 2.98-2.82 (m, 1H), 2.60-2.44 (m, 1H).

6-Bromo-4-(2,6-difluorophenyl)-2-trityl-1,2,3,4-tetrahydroisoquinoline.

The compound was prepared by following the procedure described for the synthesis of **7**. 6-Bromo-4-(2,6-difluorophenyl)-1,2,3,4-tetrahydroisoquinoline (1.95 g, 6 mmol) was reacted with trityl chloride (5g, 18 mmol) to produce the product (3.05 g, 5.4 mmol) in 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62-6.74 (m, 21H), 5.08-4.96 (m, 1H), 3.93 (d, 1H, *J* = 15.0 Hz), 3.36-3.24 (m, 1H), 3.14-3.04 (m, 1H), 2.28-2.12 (m, 1H).

4-Chloro-*N*-methoxy-*N*-methylbenzamide (9).

4-Chlorobenzoic acid **8** (20.0 g, 0.128 mols) was placed in a 500 mL round-bottomed flask. Thionyl chloride (120 mL) was added, and the mixture was refluxed overnight. Thionyl chloride was removed under reduced pressure to produce a red-colored oil. Anhydrous toluene was added and then removed under reduced pressure two times. The crude product was dissolved in anhydrous dichloromethane (200 mL). Then *N*,*O*-dimethylhydroxylamine · HCl (13.73 g, 0.140 mols) was added, and anhydrous pyridine (52 mL, 0.640 mols) was added over a period of 10 min. The reaction was stirred under nitrogen at room temperature overnight. Volatiles were removed under reduced pressure. The solid was partitioned between $CHCl_3$ and water. The organic phase was washed with brine and then collected and dried over anhydrous magnesium sulfate. Solvent was removed to produce a red-colored oil, which was purified on silica with 5% MeOH/CH_2Cl_2 as eluent; 22.9 g produced, yield 90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (d, J= 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 3.54 (s, 3H), 3.36 (s, 3H). ESI-MS m/z 200.4 ($M + H^+$).

(4-Chlorophenyl)(1-methyl-1*H*-imidazol-5-yl)methanone (11).

A flame-dried 125 mL round-bottomed flask was charged with a stir bar, pressurized with dry nitrogen gas and charged with freshly distilled *N*-methylimidazole **10** (3.0 mL, 37.6 mmol). The flask was sealed with a new rubber septum, and anhydrous THF (30 mL) was added. The solution was stirred for about 10 min, and then the temperature was lowered to -78 °C and stirred for an additional 10 min. Then freshly titrated *n*-butyl lithium (16.2 mL, 41.3 mmol, 2.5 M in hexane) was added dropwise through the septum over a period of 10 min under an atmosphere of dry nitrogen. A slight color change to pale yellow was observed. This was allowed to stir at this temperature for 45 min, and then 99% chlorotriethylsilane (6.3 mL, 37.6 mmol) was added dropwise over 5 min. The reaction was allowed to stir for 1 h at -78 °C, at which point freshly titrated *n*-butyl lithium (15.0 mL, 37.6 mmol) was added dropwise through the septum over a period of 10 min and allowed to stir at -78 °C for an additional 45 min. A separate flask was flame-dried and charged with Weinreb amide **9** (5.0 g, 25.1 mmol) and sealed. Then anhydrous THF (15 mL) was added and the mixture was stirred at room temperature for 10 min, and then at the appropriate time, transferred via cannula to the flask containing the in situ generated C-2 triethylsilyl protected *N*-methylimidazole at a slow rate to maintain low temperature as indicated by the slow sublimation of CO_2. The mixture was left to stir overnight and became a deep-red color. The reaction was quenched by the addition of 1 M HCl until the pH of the aqueous phase was no longer basic, as indicated by litmus paper, and then allowed to stir for one hour. The pH

of the aqueous phase was adjusted to above 8 with 1.5 M NaOH, and the mixture was partitioned between $CHCl_3$ and water. The organic phase was washed with brine and dried with anhydrous $MgSO_4$. Solvent was removed under reduced pressure to produce a reddish solid. Product was purified by recrystallization from CH_2Cl_2 to produce 4.24 g of fluffy golden crystals, 75.6% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, *J*= 8.4 Hz, 2H), 7.68 (s, 1H), 7.59 (s, 1H), 7.50 (d, *J* = 8.4 Hz, 2H), 4.03 (s, 1H). ESI-MS *m*/*z* 221.4 ($M + H^+$).

(4-Chlorophenyl)(4-(3-chlorophenyl)-2-trityl-1,2,3,4-tetrahydroisoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol (12).

6-Bromo-4-(3-chlorophenyl)-2-trityl-1,2,3,4-tetrahydroisoquinoline **7** (1.3 g, 2.3 mmol) was dissolved in dry THF (10 mL) and treated with *n*-BuLi (1.38 mL, 2.5M in hexane) at –78^{o}C under a positive pressure of argon. The reaction mixture was stirred at –78^{o}C under argon for 45 min, then (4-Chlorophenyl)(1-methyl-1*H*-imidazol-5-yl)methanone **11** (506 mg, 2.3 mmol) in THF (5 mL) was added and mixture was stirred at –78^{o}C for 30 min, and then warmed to room temperature and stirred overnight. The reaction mixture was quenched with saturated NH_4Cl aqueous solution, the organic residues were extracted with EtOAc and dried over Na_2SO_4. Flash chromatography of the crude product on silica gel yielded the desired product **12** (908 mg, 1.29 mmol) in 56% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50-6.91 (m, 27H), 6.25 (s, 1H), 4.40-4.30 (m, 1H), 3.68-3.42 (m, 2H), 3.29 (s, 3H), 2.95-2.81 (m, 1H), 2.69-2.51 (m, 1H).

(4-Chlorophenyl)(4-(2,6-difluorophenyl)-2-trityl-1,2,3,4-tetrahydroisoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol.

The compound was prepared by following the procedure described for the synthesis of **12**. 6-Bromo-4-(2,6-difluorophenyl)-2-trityl-1,2,3,4-tetrahydroisoquinoline (1.73 g, 3.05 mmol) was treated with BuLi (1.83 mL, 4.58 mmol) followed by addition of compound **11** (640 mg, 2.9 mmol) to afford the product (1.12 g, 1.58 mmol) in 52% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62-6.66 (m, 26H), 6.26 (s, 1H), 5.06-4.94 (m, 1H), 3.96 (d, 1H, *J* = 15.0 Hz), 3.50-3.16 (m, 5H), 2.28-2.14 (m, 1H).

(4-Chlorophenyl)(isoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol.

Compound was prepared from 6-bromoisoquinoline as described for the synthesis of compound **12**. 6-Bromoisoquinoline (208 mg, 1 mmol) was treated with BuLi (0.5 mL, 1.2 mmol) followed by addition of compound **11** (220 mg, 1 mmol) to afford the product (209.8 mg, 0.6 mmol) in 60% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 9.74 (s, 1H), 9.02 (s, 1H), 8.48 (d, 1H, *J* = 9.0 Hz),

8.42 (s, 1H), 8.22 (s, 1H), 8.05 (dd, 1H, *J* = 9.0 and 3.0 Hz), 7.50-7.41 (m, 4H), 7.02 (s, 1H), 3.70 (s, 3H).

(4-Chlorophenyl)(4-(3-chlorophenyl)-1,2,3,4-tetrahydroisoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol.

(4-Chlorophenyl)(4-(3-chlorophenyl)-2-trityl-1,2,3,4-tetrahydroisoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol **12** (300 mg, 0.42 mmol) was dissolved in 20% TFA in CH_2Cl_2 (20 mL) and stirred under a nitrogen atmosphere at room temperature for overnight. The reaction mixture was concentrated on rotary evaporator and basified with $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined organic layer was washed with water, brine and dried over Na_2SO_4, evaporation of organic solvent yielded product (167 mg, 0.36 mmol) in 85% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-6.61 (m, 12H), 6.23 (s, 1H), 4.13-3.93 (m, 3H), 3.39-3.28 (m, 1H), 3.23 (s, 3H), 3.04-2.92 (m, 1H).

(4-Chlorophenyl)(4-(2,6-difluorophenyl)-1,2,3,4-tetrahydroisoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol.

The compound was prepared by following procedure described for the synthesis of compound (4-chlorophenyl)(4-(3-chlorophenyl)-1,2,3,4-tetrahydroisoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol. (4-Chlorophenyl)(4-(2,6-difluorophenyl)-2-trityl-1,2,3,4-tetrahydroisoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol (260 mg, 37 mmol) was treated with 20% TFA in CH_2Cl_2 (20 mL) to produce the product (136.8 mg, 0.29 mmol) in 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46-6.60 (m, 11H), 6.28 (s, 1H), 4.60-4.48 (m, 1H), 4.26-4.02 (m, 2H), 3.51-3.38 (m, 1H), 3.32 (s, 3H), 3.28-3.12 (m, 1H).

(4-Chlorophenyl)(4-(3-chlorophenyl)isoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol (13).

To a solution of (4-Chlorophenyl)(4-(3-chlorophenyl)-1,2,3,4-tetrahydroisoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol (167 mg, 0.36 mmol) in dioxane (30 mL) was added MnO_2 (628 mg, 7.22 mmol) and the resulting mixture was stirred under nitrogen atmosphere at reflux temperature for 24 hours. The reaction mixture was filtered through a pad of celite and the filtrate was concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel to afford the pure product **13** (106 mg, 0.23 mmol) in 64% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 9.26 (s, 1H), 8.39 (s, 1H), 8.20 (d, 1H, *J* = 9.0 Hz), 7.82 (dd, 1H, *J* = 9.0 and 3.0 Hz), 7.71-7.64 (m, 2H), 7.49-7.20 (m, 8H), 6.22 (s, 1H), 3.48 (s, 3H).

(4-Chlorophenyl)(4-(2,6-difluorophenyl)isoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol.

The compound was prepared by following the procedure described for the synthesis of **13**. (4-Chlorophenyl)(4-(2,6-difluorophenyl)-1,2,3,4-tetrahydroisoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol (136.8 mg, 0.29 mmol) was reacted with MnO_2 (511 mg, 5.88 mmol) at reflux temperature to afford the product (81.3 mg, 0.18 mmol) in 60% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.36 (s, 1H), 7.91 (d, 1H, *J* = 8.4 Hz), 7.64 (dd, 1H, *J* = 8.7 and 1.5 Hz), 7.44 (s, 1H), 7.38-6.83 (m, 8H), 6.37 (s, 1H), 3.30 (s, 3H).

4-(3-Chlorophenyl)-6-((4-chlorophenyl)(methoxy)(1-methyl-1H-imidazol-5-yl)methyl)isoquinoline (14).

(4-Chlorophenyl)(4-(3-chlorophenyl)isoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol **13** (30 mg, 0.065 mmol) was dissolved in thionyl chloride (2 mL) and stirred at room temperature under an atmosphere of dry N_2 for 3 hours. $SOCl_2$ was removed under reduced pressure. The residue was taken up in CH_2Cl_2 and concentrated under vacuum. The resulting solid was dissolved in MeOH (5 mL) and stirred under nitrogen atmosphere at reflux temperature for overnight. The mixture was cooled to room temperature and evaporated in vacuo. The crude residue was purified by flash column chromatography on silica gel to afford pure product **14** (24.6 mg, 0.052 mmol) in 80% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 9.87 (s, 1H), 9.17 (s, 1H), 8.60-8.49 (m, 2H), 8.20-8.08 (m, 2H), 7.76 (s, 1H), 7.65-7.40 (m, 8H), 3.54 (s, 3H), 3.24 (s, 3H). MS *m/z* 474.1 ($M + H^+$).

6-((4-Chlorophenyl)(methoxy)(1-methyl-1H-imidazol-5-yl)methyl)-4-(2,6-difluorophenyl)isoquinoline (15).

The compound was prepared by following the procedure described for the synthesis of **14**. (4-Chlorophenyl)(4-(2,6-difluorophenyl)isoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol (37 mg, 0.08 mmol) was treated with neat $SOCl_2$ (2 mL) and followed by MeOH (5 mL) at reflux temperature to afford the product (32mg, 0.07 mmol) in 84% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 9.95 (s, 1H), 9.09 (s, 1H), 8.85 (s, 1H), 8.47 (d, 1H, *J* = 9.0 Hz), 8.06 (d, 1H, *J* = 9.0 Hz), 7.85 (s, 1H), 7.74-7.62 (m, 2H), 7.52-7.38 (m, 6H), 3.51 (s, 3H), 3.25 (s, 3H). MS *m/z* 476.1 ($M + H^+$).

6-((4-Chlorophenyl)(methoxy)(1-methyl-1H-imidazol-5-yl)methyl)isoquinoline (16).

The compound was prepared by following the procedure described for the synthesis of **14**. (4-Chlorophenyl)(isoquinolin-6-yl)(1-methyl-1H-imidazol-5-yl)methanol (50 mg, 0.143 mmol) was treated with neat $SOCl_2$ (3 mL) followed by MeOH (5 mL) at reflux temperature to afford the product (43 mg, 0.12 mmol) in 83% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 9.65 (s, 1H), 9.14 (s, 1H), 8.64 (s, 1H), 8.60-8.46 (m, 3Hz), 8.20 (dd, 1H, *J* = 8.7 and 1.8 Hz), 7.89 (s, 1H), 7.68 (d, 2H, *J* = 9.0 Hz), 7.55 (d, 2H, *J* = 9.0 Hz), 3.54 (s, 3H), 3.39 (s, 3H). MS *m/z* 364.4 ($M + H^+$).