CHEMMEDCHEM
Chemistry & Drug Discovery

Supporting Information

HTS, Chemical Hybridization, and Rational Drug Design Identify a Chemically Unique Anti-tuberculosis Agent-Coupling Serendipity and Rational Approaches to Drug Discovery

Jialin Mao, [†, ‡] Baojie Wan, [‡] Yuehong Wang, [‡] Scott G. Franzblau, [*, ‡] and Alan P. Kozikowski[*, †]

Department of Medicinal Chemistry and Pharmacognosy and Institute for Tuberculosis

Research, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street,

Chicago, Illinois 60612

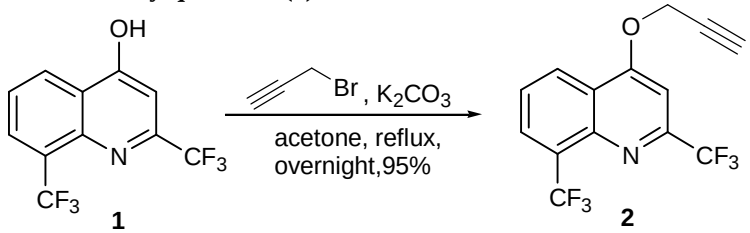
E-mail: kozikowa@uic.edu, sgf@uic.edu

Experimental section

A. Chemistry

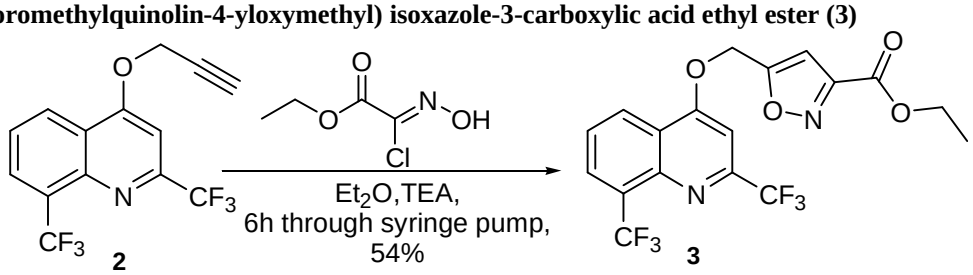
General Information. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometer at 400 MHz and 100 MHz or 300 MHz and 75 MHz respectively with TMS as an internal standard. The following abbreviations are used: DCM = dichloromethane; THF = tetrahydrofuran; Standard abbreviation indicating multiplicity was used as follows: s = singlet, d = doublet, t = triplet, q = quadruplet and m = multiplet. Mass spectra were measured in the EI or ESI mode at an ionization potential of 70 *e*V; HRMS experiment was performed on Q-TOF-2TM (Micromass). TLC was performed with Merck 250-mm $60F_{254}$ *silica gel* plates. Column chromatography was performed using Merck silica gel (40-60 mesh). Analytical HPLC was carried out on an Ace 5AQ column (25 cm × 4.6 mm), with detection at 254 and 366 nm on a Shimadzu SPD-10A VP detector; flow rate = 2.0 $mLmin^{-1}$; from 10% acetonitrile in water to 100% acetonitrile with 0.05% TFA.

4-Prop-2-ynyloxy-2, 8-bis-trifluoromethylquinoline (2)



To a solution of the 2, 8-bis-trifluoromethylquinolin-4-ol (**1**) (1.000 g, 3.6 mmol) in dry acetone (25 ml) was added excess anhydrous K_2CO_3 (5 g) and the mixture was refluxed for 0.5 h. To the mixture, propargyl bromide (0.53 g, 3.6 mmol) was added dropwisely. The resulting mixture was refluxed for an additional period of 14 h and then cooled, filtered and the filtrate was evaporated, and then dried *in vacuo* to obtain a white solid (1.1 g, 95%). It was then carried to the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): *d* 2.69 (t, *J* = 2 Hz, 1H), 5.05 (d, *J* = 2 Hz, 2H), 7.26(s, 1H), 7.66 (t, *J* = 7.6 Hz, 1H), 8.14 (d, *J* = 7.2 Hz, 1H), 8.44 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) *d* 56.8, 75.9, 77.8, 98.3, 119.5, 122.3, 124.9, 126.3, 128.1, 128.4, 129.4, 144.6, 149.4, 161.6.

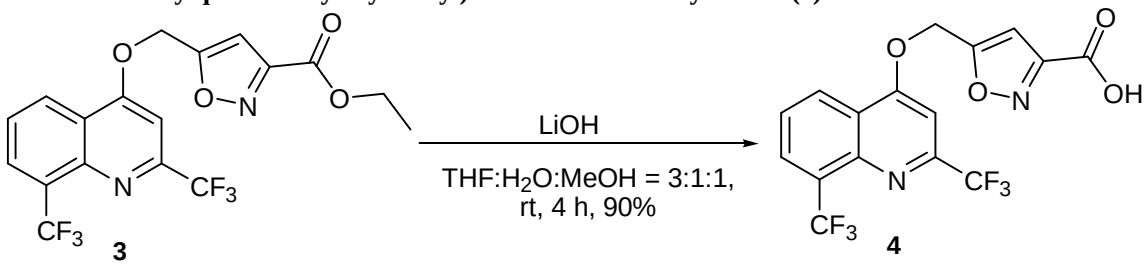
5-(2, 8-Bis-trifluoromethylquinolin-4-yloxymethyl) isoxazole-3-carboxylic acid ethyl ester (3)



2 **3**

To a vigorously stirred solution of ethyl chlorooximidoacetate (1.57 g, 10.34 mmol) and 4-prop-2-ynyloxy-2, 8-bis-trifluoromethylquinoline (**2**) (1.1 g, 3.4 mmol) in Et_2O (15 mL) was added triethylamine (1.45 mL, 10.34 mmol) in Et_2O (12 mL) via syringe pump over 6 h period. The mixture was diluted with Et_2O (20 mL), the organic layer separated, washed with H_2O (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford an oil which was purified by column chromatography (EtOAc : hexane = 1 : 4) to give the product as a white solid (0.8 g, 54%). ^{1}H NMR (400 MHz, $CDCl_3$): *d* 1.46 (t, *J* = 7.2 Hz, 3H), 4.50 (q, *J* = 7.2 Hz, 2H), 5.50 (s, 2H), 6.94 (s, 1H), 7.24 (s, 1H) ,7.73 (t, *J* = 7.2 Hz, 1H), 8.20(d, *J* = 7.2 Hz, 1H), 8.46 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR 61.4, 62.6, 97.7, 105.0, 121.5, 125.7, 126.0, 126.7, 128.4, 128.7, 129.7, 144.4, 149.0, 156.8, 159.0, 161.6, 167.0; MS (APCI-LC MS) *m/z* 435.0 (M^+ + H, 100); FAB-HRMS calcd for $[C_{18}H_{12}F_6N_2O_4 + Na]^+$:457.0593; found: 457.0592; $[C_{18}H_{12}F_6N_2O_4 + H]^+$:435.0774; found: 435.0773; HPLC purity: 99%.

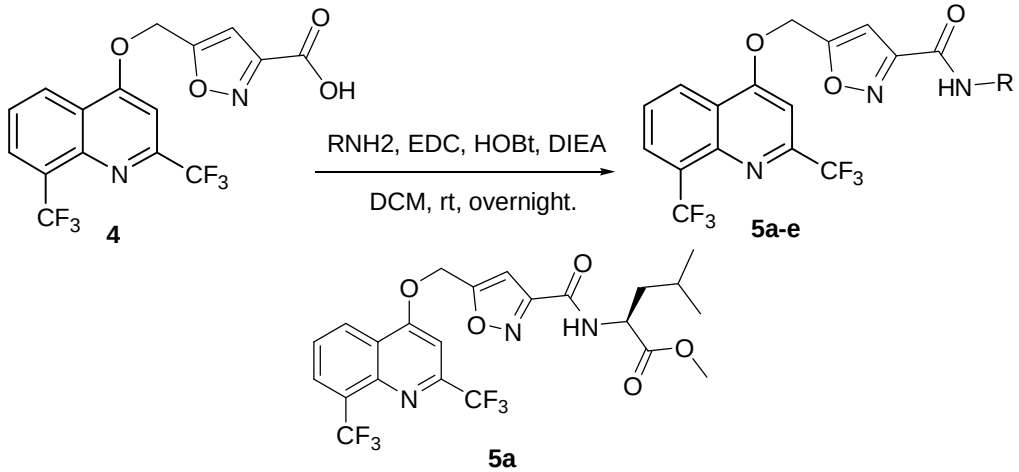
5-(2, 8-Bis-trifluoromethylquinolin-4-yloxymethyl)-isoxazole-3-carboxylic acid (4)



3 **4**

To a solution of compound **3** (0.8 g, 3.4 mmol) in THF/H_2O/MeOH (3 : 1 : 1, 15 mL) was added lithium hydroxide monohydrate (0.6 g, 13.6 mmol). The solution was allowed to stir for 4 h at ambient temperature and the solvent was removed *in vacuo*. The residue was dissolved in water and extracted with EtOAc (3 × 15 mL). The aqueous layer was acidified to PH 2 with 6 M HCl and extrated with EtOAc (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give a colorless product (0.67 g, 90%). ^{1}H NMR (400 MHz, CD_3OD): *d* 5.75 (t, 1H), 7.07 (s, 1H), 7.63 (s, 1H), 7.81 (t, *J* = 7.6 Hz, 1H), 8.26 (d, *J* = 7.2 Hz, 1H), 8.46 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): *d* 62.0, 97.4, 105.0, 121.4, 125.5, 126.3, 126.7, 128.4, 128.7, 129.6, 144.4, 149.3, 157.2, 159.0, 162.4, 172.2; MS (APCI-LC MS) *m/z* 407.2 (M^+ + H, 100); FAB-HRMS calcd for $[C_{16}H_8F_6N_2O_4 + H]^+$:407.0467; found:407.0469; HPLC purity: 99%.

General procedure for the preparation of isoxazolecarboxamides (5a-e)



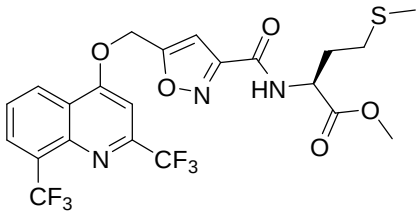
4 **5a-e**

5a

To a stirring solution of compound **4** (50 mg, 0.12 mmol) in anhydrous DCM (5 mL) at 0°C was added 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) (46 mg, 0.24 mmol) and 1-hydroxybenzotriazole hydrate (HOBt) (32.4 mg, 0.24 mmol). After 15 mins, *L*-leucine methyl ester (40 mg, 0.24 mmol) dissolved in dichloromethane (5 mL) was added following diisopropylethylamine (0.1 ml, 0.57 mmol) and stirred the reaction mixture from 0 °C to room temperature overnight. The reaction mixture was diluted with ethyl acetate (10 mL) and washed with water (10 mL). The organic phase

was further washed with saturated $NaHCO_3$ solution (3 × 5 mL) and brine (3 × 5 mL). The organic phase was dried ($MgSO_4$) and evaporated in *vacuo*. The pure product **5a** (36.4 mg, 54%) was obtained by column chromatography (EtOAc : hexane = 1 : 4). ^{1}H NMR (400 MHz, $CDCl_3$): *d* 1.14 (m, 6H), 1.8 (m, 3H), 3.80 (s, 3H), 4.80 (m, 1H), 5.55 (t, 1H), 6.96 (s, 1H), 7.25 (s, 1H), 7.86 (t, *J* = 7.6 Hz, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 8.47 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*): *d* 22.7, 28.9, 43.2, 50.5, 51.1, 60.9, 97.9, 103.3, 121.4, 125.8, 126.3, 126.7, 128.4, 128.7, 129.6, 144.4, 149.3, 156.7, 159.2, 167.6, 173.4, 175.7; MS (APCI-LC MS) *m/z* 534.1 (M^+ + H, 100); FAB-HRMS calcd. for $[C_{23}H_{21}F_6N_3O_5$ + Na$]^+$: 556.1277; found: 556.1274; HPLC purity: 99%.

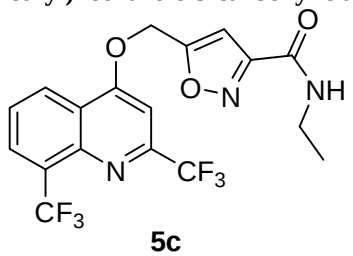
2-{[5-(2,8-Bis-trifluoromethylquinolin-4-yloxymethyl)-isoxazole-3-carbonyl]-amino}-4-methylsulfanyl-butyric acid methyl ester (5b)



5b

The general procedure was followed using *L*-methionine methyl ester instead of *L*-leucine methyl ester in the reaction. ^{1}H NMR (400 MHz, CD_3OD): *d* 2.12 (s, 3H), 2.17(m, 2H), 2.64 (m, 2H), 3.79 (s, 3H), 4.82 (m, 1H), 5.76 (s, 2H), 7.07 (s, 1H), 7.64 (s, 1H), 7.82 (t, *J* = 8 Hz, 1H), 8.26 (d, *J* = 8 Hz, 1H), 8.56 (d, *J* = 8.0 Hz, 1H) ; ^{13}C NMR (100 MHz, DMSO-*d6*): *d*13.4, 28.9, 29.4, 51.0, 51.2, 61.2, 97.9, 103.4, 121.6, 125.8, 126.3, 127.1, 128.0, 128.7, 129.1, 144.4, 148.8, 159.2, 161.9, 167.7, 170.2, 171.5; MS (APCI-LC MS) *m/z* 552.1 (M^+ + H, 100) FAB-HRMS calcd for $[C_{22}H_{19}F_6N_3O_5S$ + Na$]^+$:574.0847; found:574.0841; HPLC purity: 99%.

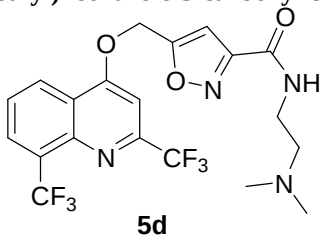
5-(2,8-Bis-trifluoromethyl-quinolin-4-yloxymethyl)-isoxazole-3-carboxylic acid ethylamide (5c)



5c

The general procedure was followed using ethylamine instead of *L*-leucine methyl ester in the reaction. ^{1}H NMR (300 MHz, $CDCl_3$): *d* 1.29 (d, *J* = 9.6 Hz, 3H), 3.51(q, *J* = 9.2 Hz, 2H), 5.52 (s, 1H), 6.81(brs, 1H), 6.97(s, 1H) ,7.24 (s, 1H) ,7.71 (t, *J* = 8.0 Hz, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 8.47 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): *d* 15.2, 35.4, 62.0, 97.4, 105.0, 121.4, 125.5, 126.3, 126.7, 128.4, 128.7, 129.6, 144.4, 149.5, 156.8, 159.3, 161.6, 173.4; MS (APCI-LC MS) *m/z* 434.2 (M^+ + H, 100); FAB-HRMS calcd for $[C_{18}H_{13}F_6N_3O_3$ + H$]^+$:434.0939; found:434.0936; HPLC purity: 99%.

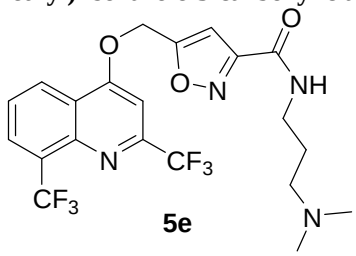
5-(2,8-Bis-trifluoromethyl-quinolin-4-yloxymethyl)-isoxazole-3-carboxylic acid (2-dimethylamino-ethyl)-amide (5d)



5d

The general procedure was followed using *N, N*-dimethylethylenediamine instead of *L*-leucine methyl ester in the reaction. The pure product was obtained by column chromatography (MeOH : DCM = 1 : 19). ^{1}H NMR (400 MHz, CD_3OD): *d* 2.45(s, 6H) , 2.69 (m, 2H), 3.60 (m, 2H), 5.72(s, 2H), 7.08 (s, 1H), 7.61 (s, 1H), 7.81 (t, *J* = 7.6 Hz, 1H), 8.26 (d, *J* = 7.6 Hz, 1H), 8.57 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR 39.7, 43.4, 57.1, 62.3, 97.6, 103.2, 121.4, 125.8, 126.3, 126.9, 128.4, 128.7, 129.2, 144.8, 149.0, 156.8, 159.0, 161.4, 173.8; MS (APCI-LC MS) *m/z* 477.1 (M^+ + H, 100); FAB-HRMS calcd for $[C_{20}H_{18}F_6N_4O_3$ + H$]^+$:477.13559; found: 477.1347; HPLC purity: 99%.

5-(2,8-Bis-trifluoromethyl-quinolin-4-yloxymethyl)-isoxazole-3-carboxylic acid (3-dimethylamino-propyl)-amide (5e)



5e

The general procedure was followed using 3-(dimethylamino)-1-propylamine instead of *L*-leucine methyl ester in the reaction. The pure product was obtained by column chromatography (MeOH : DCM = 1 : 19). ^{1}H NMR (400 MHz, CD_3OD): *d* 1.96 (m, 2H), 2.57 (s, 6H), 2.86 (m, 2H), 3.48 (m, 2H), 5.77 (s, 2H), 6.99 (s, 1H), 7.64 (s, 1H), 7.73 (t, *J* = 7.6 Hz, 1H), 8.26 (d, *J* = 7.6 Hz, 1H), 8.55 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, CD3OD):*d* 29.1, 38.4, 44.8, 57.3, 62.2, 98.0, 105.0, 121.4, 125.5, 126.3, 126.7, 128.4, 129.2, 129.6, 145.4, 149.2, 156.8, 159.8, 161.6, 174.1; MS (APCI-LC MS) *m/z* 491.2 (M^+ + H, 100); FAB-HRMS calcd for $[C_{21}H_{20}F_6N_4O_3 + H]^+$:491.1518; found:491.1514; HPLC purity: 99%.

B. Bioassay

CYP3A4 inhibition

Inhibition of human recombinant CYP3A4 was determinated by using the Vivid$^{@}$ Green Screening Kit (P2857, Invitrogen Co.) and following the manufacture instruction.