Imidazo[1,2-*a*]pyridine-3-carboxamides are Active Antimicrobial Agents of *Mycobacterium avium* Infection *In Vivo*

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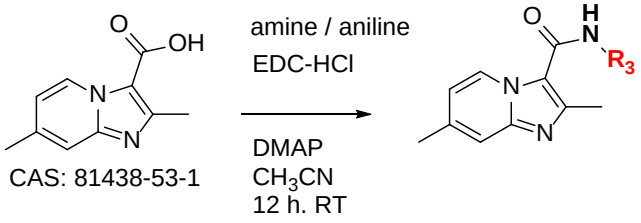
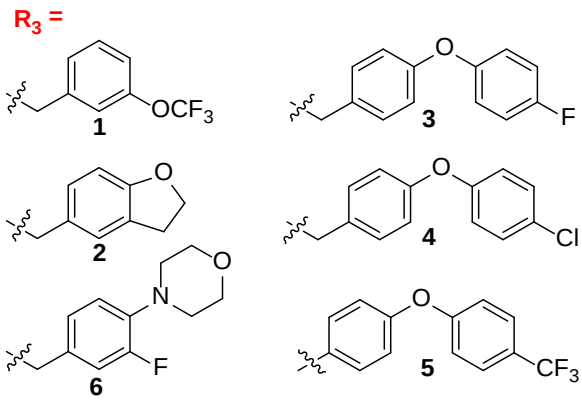
Synthesis of new compounds **1**, **2** and **6**.

We have previously described the general synthesis of these compounds, isomers of these compounds and the exact synthesis of compounds **3, 4, 5** (Ref 1). Preparation of all compounds can be consolidated to a single amide bond formation between the 2,7-dimethyl-imidazo[1,2-*a*]pyridine-3-carboxylic acid [CAS: 81438-53-1]and the desired amines or aniline. The coupling conditions used to prepare all the compounds studied are shown in Scheme S1.

Scheme S1. EDC-mediated coupling of 2,7-dimethyl-imidazo[1,2-*a*]pyridine-3-carboxylic acid with various amines and aniline to produce compounds **1 – 6**.



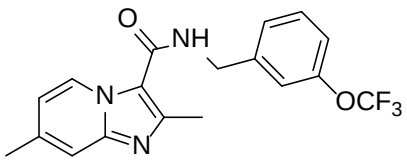
amine / aniline = 3-(trifuloromethoxy)benzyl amine [CAS: 93071-75-1], 4-(4-(trifluoromethyl)phenoxy)aniline [CAS: 57478-19-0], (2,3-dihydrobenzofuran-5-yl)methanamine [CAS: 55745-74-9], (4-(4-fluorophenoxy)phenyl)methanamine [CAS: 179057-32-0], (4-(4-chlorophenoxy)phenyl)methanamine [CAS: 774525-83-6], (3-fluoro-4-morpholinophenyl)methanamine [CAS: 870843-45-1]



Experimental Section

Chemistry. All anhydrous solvents, reagent grade solvents for chromatography and starting materials were purchased from either Aldrich Chemical Co. (Milwaulkee, WI) or Fisher Scientific (Suwanee, GA) unless otherwise noted. Water was distilled and purified through a Milli-Q water system (Millipore Corp., Bedford, MA). General methods of purification of compounds involved the use of silica cartridges purchased either Practichem, LLC. (www.practichem.com) and/or recrystallization. The reactions were monitored by TLC on precoated Merck 60 F_{254} silica gel plates and visualized using UV light (254 nm). All compounds were analyzed for purity by HPLC and characterized by ^{1}H and ^{13}C NMR using Bruker 300MHz NMR and/or Bruker 500 MHz NMR spectrometers. Chemical shifts are reported in ppm (δ) relative to the residual solvent peak in the corresponding spectra; chloroform δ 7.26 and δ 77.23, methanol δ 3.31 and δ 49.00 and coupling constants (*J*) are reported in hertz (Hz) (where, s = singlet, bs = broad singlet, d = doublet, dd = double doublet, bd = broad doublet, ddd = double doublet of dublet, t = triplet, tt – triple triplet, q = quartet, m = multiplet; a = apparent) and analyzed using MestReC NMR data processing. ^{19}F NMR were run without a standard and are uncorrected. Mass spectra values are reported as *m/z*. Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected (Benzoic acid melting point was measured at 114 – 115°C, literature 122.4°C). The HPLC HRMS analyses were carried out on a Dionex RSLC (UPLC) System with a Bruker MicroOTOF-Q II, using a Dionex Acclaim RSLC 120 C_{18}, 2.2 um, 120 Å, 2.1 x 100 mm column run at 40 °C. Mobile phases: (A) Millipore purified water with 0.1% formic acid at a flow rate of 0.5 mL min^{-1} and UV dection at 254 nm. (B) HPLC grade acetonitrile with 0.1% formic acid at a flow rate of 0.5 mL min^{-1} and UV dection at 254 nm. Gradient A was 10 min run with a linear gradient of 70% A to 30% B to start (time = 0 mins) and then 100% B at 6 min, 100% B at 8 min, 70% A to 30% B at 8.1 min and finally 70% A to 30% B at 10 min. Gradient B was 20 min run from 0.0 to 2.0 min B=10%; at 18.0 min B=100%, at 18.1 min B=10% and the run finishes at 20 min with B=10%. Flow - 0.4 mL/min. High performance liquid chromatography analyses for checking purity (>95% area) of compounds were performed on Dionex HPLC as described above and by HRMS. All final compounds were found to have > 95% purity by HPLC unless otherwise noted. All reactions were conducted under argon unless otherwise noted. Solvents were removed *in vacuo* on a rotary evaporator. MIC values reported are the average of three individual measurements.

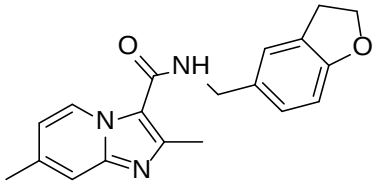
Preparation and characterization of compounds **1, 2 and 6**.



2,7-dimethyl-*N*-(3-(trifluoromethoxy)benzyl)imidazo[1,2-*a*]pyridine-3-carboxamide (**1**).

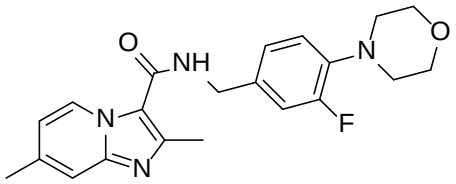
2,7-dimethylimidazo[1,2-a]pyridine-3-carboxylic acid [CAS: 81438-53-1] (200 mg, 1 mmol) and EDC-HCl (287 mg, 1.5 mmol) were dissolved in 15 ml of dry CH_3CN and the reaction stirred for 10 min at room temperature. Next, 3-trifluoromethoxybenzyl amine (0.15 mL, 1.06 mmol) and DMAP (305 mg, 2.5 mmol) were added and the reaction was stirred for 12 h at room temperature under argon. The reaction was concentrated *in vacuo*. The residue was taken up in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ (2x), 5% aqueous acetic acid (2x), brine and then dried over sodium sulfate. The drying agent was removed

by filtration and the organic layer was concentrated *in vacuo*. Resulting residue was purified by silica gel column (with 10% EtOAc: CH_2Cl_2 to remove upper running amine and polarity was increased to 50% EtOAc: CH_2Cl_2 to collect product) or by recrystallization with hot CH_3CN to give 265 mg of 2,7-dimethyl-*N*-(3-(trifluoromethoxy)benzyl)imidazo[1,2-*a*]pyridine-3-carboxamide (**1**) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 9.24 (d, *J* = 7.1 Hz, 1H), 7.36 (t, *J* = 7.9, 7.9 Hz, 1H), 7.31-7.20 (m, 3H), 7.13 (d, *J* = 8.0 Hz, 1H), 6.73 (d, *J* = 7.1 Hz, 1H), 6.26 (bs, 1H, NH), 4.69 (d, *J* = 5.9 Hz, 2H), 2.65 (s, 3H), 2.40 (s, 3H). ^{13}C (126 MHz, $CDCl_3$) δ ppm 161.2, 149.5, 146.5, 145.5, 140.9, 138.5, 130.1, 127.2, 125.7, 120.7 (q, *J* = 257.4 Hz), 120.0, 119.9, 115.8, 115.0, 114.7, 42.7, 21.3, 16.7. Melting point = 110-111^{o}C. HRMS (EI), M+1 calcd. for $C_{18}H_{17}F_3N_3O_2$, 364.1267; found 364.1271. HPLC t_R = 2.8 min (99% pure) gradient A.



N-((2,3-dihydrobenzofuran-5-yl)methyl)-2,7-dimethylimidazo[1,2-a]pyridine-3-carboxamide (**2**).

2,7-Dimethylimidazo[1,2-a]pyridine-3-carboxylic acid [CAS: 81438-53-1] (4 g, 21 mmol) and the EDC-HCl (4.8 g, 25.2 mmol) were dissolved in 250 mL dry CH_3CN and the reaction stirred for 10 min at room temperature. Next, the 5-(aminomethyl)-2,3-dihydrobenzo[b]furan (CAS: 55745-74-9, 3.5 g, 23.1 mmol) and DMAP (3.1 g, 25.2 mmol) were added and reaction stirred for 16 hours under argon. Reaction was concentrated *in vacuo*. Residue was taken up in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution (2x), 5% aqueous acetic acid solution (2x), brine and then dried over sodium sulfate. Drying agent was removed by filtration and organics were concentrated *in vacuo*. Resulting residue was purified by silica gel column or by recrystallization with hot CH_3CN to give 4.2 grams of *N*-((2,3-dihydrobenzofuran-5-yl)methyl)-2,7-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide (**2**) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 9.28 (d, *J* = 7.1 Hz, 1H), 7.37 (s, 1H), 7.21 (s, 1H), 7.10 (d, *J* = 7.8 Hz, 1H), 6.75 (d, *J* = 7.8 Hz, 1H),6.04 (bs, 1H, NH), 4.58 (d, *J* = 7.4 Hz, 1H), 4.55 (app. t, 2H, hidden), 3.19 (t, *J* = 8.6, 8.6 Hz, 1H), 2.63 (s, 3H), 2.41 (s, 3H). ^{13}C (126 MHz, $CDCl_3$) δ ppm 161.4, 159.7, 146.3, 138.4, 130.3, 127.7, 127.3, 127.2, 124.7, 115.7, 115.0, 114.9, 109.4, 71.4, 43.2, 29.7, 21.3, 16.7. Melting point = 140-141.5^{o}C. HRMS (EI), M+1 calcd. for $C_{19}H_{20}N_3O_2$, 322.1550; found 322.1568. HPLC t_R = 1.2 – 1.4 min (99% pure), gradient A.



N-(3-fluoro-4-morpholinobenzyl)-2,7-dimethylimidazo[1,2-a]pyridine-3-carboxamide (**6**).

2,7-Dimethylimidazo[1,2-a]pyridine-3-carboxylic acid [CAS: 81438-53-1] (175 mg, 0.92 mmol) and the EDC-HCl (211 g, 1.1 mmol) were dissolved in 15 mL dry CH_3CN and the reaction stirred for 10 min at room temperature. Next, the (3-fluoro-4-morpholinophenyl)methanamine [CAS: 870843-45-1] (219 mg, 1.0

mmol) and DIPEA (0.18 mL, 1.0 mmol) were added and reaction stirred for 16 hours under argon. Reaction was concentrated *in vacuo*. Residue was taken up in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution (2x), 5% aqueous acetic acid solution (2x), brine and then dried over sodium sulfate. Drying agent was removed by filtration and organics were concentrated *in vacuo*. Resulting residue was purified by silica gel column or by recrystallization with hot CH_3CN to give 207 mg of *N*-(3-fluoro-4-morpholinobenzyl)-2,7-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide (**6**) as an off white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 9.27 (d, J = 7.1 Hz, 1H), 7.31 (s, 1H), 7.10-7.03 (m, 2H), 6.90 (t, J = 8.6 Hz, 1H), 6.75 (dd, J = 7.1 1.2 Hz, 1H), 6.13 (bs, 1H, NH), 4.60 (d, J = 5.7 Hz, 2H), 3.88-3.82 (m, 4H), 3.09-3.02 (m, 4H), 2.65 (s, 3H), 2.41 (s, 3H). ^{13}C (126 MHz, $CDCl_3$) δ ppm 161.46, 155.63 (d, J = 247.2 Hz), 146.45, 145.26, 139.32 (d, J = 8.6 Hz), 138.53, 133.12 (d, J = 7.0 Hz), 127.32, 123.58 (d, J = 2.8 Hz), 118.85 (d, J = 2.9 Hz), 115.84, 115.60 (d, J = 21.4 Hz), 115.00, 114.78, 66.96, 50.8 (d, J = 2.8 Hz, 2C), 42.54 (s, 2C), 21.35, 16.8. Melting point = 173 - 174^{o}C. HRMS (EI), M+1 calcd. for $C_{21}H_{24}FN_4O_2$, 383.1898 found 383.1878. HPLC t_R = 7.2 – 7.3 min (98% pure), gradient B.

Table S1. MICs of compounds **1 – 6** against *M. tuberculosis* strain H37Rv**.** MICs and VERO toxicity IC50 are shown in μM.

Compound	Mol. Wt.	Mtb GAS media	Mtb 7H12 media	VERO toxicity IC50
ND-9873 (1)	363.34	1.3	0.22	>50
ND-10885 (2)	321.38	0.043	0.145	>50
ND-9758 (3)	389.43	<0.2[a]	<0.2[a]	9.5
ND-9759 (4)	405.88	<0.2[b]	<0.2[b]	13.3
ND-9903 (5)	425.41	<0.2[c]	0.39[c]	>50
ND-10890 (6)	382.44	0.24	0.42	>50
Rifampin	822.95	0.023	0.025	>50
Ethambutol	204.31	0.97	>2	>50
Clarithromycin	747.95	ND	ND	ND
Azithromycin	748.88	ND	ND	ND

[a]MIC previously published as 0.004 $\pm$ 0.002 μM using two readouts of growth (optical density and fluorescence) (Ref 2).

[b]MIC previously published as 0.006 $\pm$ 0.004 μM using two readouts of growth (optical density and fluorescence) (Ref 2).

[c]MIC previously published as 0.7 $\pm$ 0.1 μM using two readouts of growth (optical density and fluorescence) (Ref 2).

Table S2. MIC of **ND-10885 (2)** against various strains of *M. avium*. MICs in μM.

	Strain 101 (serotype 1)	Strain 104 (serotype 1)	Strain B-92 (serotype 1)	Strain 25546-759 (serotype 5)	Strain 34540-Wales (serotype 6)	Strain SJB#2 (serotype 8)	Strain 17584-285 (serotype 9)	Strain 1602-1965 (serotype 10
ND-10885	2.49	2.49	38.89	4.98	38.89	77.79	19.45	9.72
DMSO	>311.2	>311.2	>311.2	>311.2	>311.2	>311.2	>311.2	>311.2

Table S3. Mean plasma pharmacokinetic parameters for compounds **1**, **2**, and **6** following a single 1 mg/kg IV dose administered to mice.

Parameter	Units	**ND-9873 (1)** (Mean)	**ND-10885 (2)** (Mean)	**ND-10890 (6)** (Mean)
Route		IV Bolus	IV Bolus	IV Bolus
Original Dose	mg/kg	1	1	1
AUC	ng*Hours/mL	1341	4110	2960
AUC Interval		(0-24 Hours)	(0-24 Hours)	(0-24 Hours)
AUC Extrapolated	ng*Hours/mL	1341	4115	2960
Co (concentration at time 0)	ng/mL	2960.7	9192.0	1936.0
T1/2	Hours	1.22	0.69	2.19
Vdarea	mL/kg	1868	232	1070
Vdss	mL/kg	475	126	648
CL	mL/min/kg	20.0	4.13	5.63

IV formulation: DMA 25%v/v, EtOH 15%v/v, propylene glycol 10%v/v, 2 pyrrolidone 25%v/v, purified water 25%v/v.

REFERENCE

1. **Moraski GC**, **Markley LD**, **Hipskind PA**, **Boshoff H**, **Cho S**, **Franzblau SG**, **Miller MJ**. 2011. Advent of Imidazo[1,2-a]pyridine-3-carboxamides with Potent Multi- and Extended Drug Resistant Antituberculosis Activity. ACS Med Chem Lett **2**:466–470.

2. **Moraski GC**, **Markley LD**, **Cramer J**, **Hipskind PA**, **Boshoff H**, **Bailey M**, **Alling T**, **Ollinger J**, **Parish T**, **Miller MJ**. 2013. Advancement of Imidazo[1,2-*a*]pyridines with Improved Pharmacokinetics and Nanomolar Activity Against Mycobacterium tuberculosis. ACS Med Chem Lett **4**:675–679.