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Supplemental Data for Drug Metabolism and Disposition

Pharmacokinetics and Metabolism of Delamanid, a Novel Anti-Tuberculosis Drug, in Animals and Humans: Importance of Albumin Metabolism *In Vivo*

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Supplemental Methods

Synthesis of M1 from delamanid with a single step reaction.

Method A. To a solution of **delamanid** (4.0 g, 7.5 mmol) in THF (40 mL) was added 25% *aq*. NH_3 solution (20 mL) at room temperature. The reaction mixture was refluxed for 20 min at 100 °C in sealed tube apparatus (ca. 0.8 MPa). The mixture was concentrated *in vacuo*. Purification of the residue by column chromatography (surface-modified silica gel with aminopropyl group; eluent, CH_2Cl_2/ MeOH=100/1) provided **M1** (1.6 g, 47%) as an amorphous solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.38 (3H, s), 1.74 (2H, dddd, *J* = 12.2, 9.2, 8.2, 3.7 Hz), 2.00–2.07 (2H, m), 2.93 (2H, ddd, *J* = 12.2, 9.2, 3.1 Hz), 3.30 (1H, d, *J* = 12.2 Hz), 3.36 (2H, ddd, *J* = 12.2, 5.8, 3.7 Hz), 3.53 (1H, d, *J* = 12.2 Hz), 3.85 (1H, d, *J* = 10.4 Hz), 3.88 (1H, d, *J* = 10.4 Hz), 4.54 (1H, tt, *J* = 8.2, 3.7 Hz), 5.80 (2H, brs), 6.84 (2H, d, *J* = 9.2 Hz), 6.91 (2H, d, *J* = 9.2 Hz), 7.08 (2H, d, *J* = 9.2 Hz), 7.28 (2H, dq, *J* = 9.2, 0.6 Hz).

Method B. To a solution of **delamanid** (1.0 g, 1.9 mmol) in THF (20 mL) was added 1,3-diaminopropane (1.6 mL, 19 mmol) at room temperature. The reaction mixture was stirred for 16 h. The solvent was removed *in vacuo*. After addition of water, the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over sodium sulfate. Removal of the solvent and purification of the crude product by column chromatography (surface-modified silica gel with aminopropyl group; eluent, CH_2Cl_2/MeOH=100/1) provided

M1 (0.67 g, 74%).

Synthesis of M1 with a stepwise procedure. (Kitano et al., 2009)

(*R*)-1-Azido-2-methyl-3-(4-(4-(4-trifluoromethoxy-phenoxy)-piperidin-1-yl)-phenoxy)-propan-2-ol (2). To a suspension of (*R*)-1-(4-((2-methyloxiran-2-yl)-methoxy)phenyl)-4-(4-(trifluoro-methoxy)phenoxy)piperidine (**1,** Tsubouchi et al., 2008) (1.7 g, 4.0 mmol) in MeOH (40 mL) and water (5 mL) were added NH_4Cl (0.48 g, 8.9 mmol) and sodium azide (1.5 g, 20 mmol) and the mixture was refluxed for 16 h. The reaction mixture was allowed to cool to room temperature and the solvent was removed *in vacuo*. After addition of water, the mixture was extracted with AcOEt. The extract was washed with water, and then with a saturated aqueous solution of NaCl. The combined organic layers were dried over sodium sulfate. Removal of the solvent and purification of the crude product by column chromatography (silica gel; eluent, hexane/ AcOEt =65/35) provided **2** (1.8 g, 98%) as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.33 (3H, s), 1.80–2.25 (4H, m), 2.85–3.10 (2H, m), 3.25–3.50 (4H, m), 3.80 (1H, d, J = 9.1 Hz), 3.86 (1H, d, J = 9.1 Hz), 4.25–4.50 (1H, m), 6.50–7.00 (6H, m), 7.14 (2H, d, J = 9.6 Hz).

(*R*)-1-Amino-2-methyl-3-(4-(4-(4-trifluoromethoxy-phenoxy)-piperidin-1-yl)-phenoxy)-propan-2-ol (3). A mixture of azide **2** (2.7 g, 5.7 mmol) and palladium on activated carbon

(10 wt % Pd, 0.27 g) in EtOH (50 mL) was stirred at room temperature under a hydrogen atmosphere at normal pressure. The reaction mixture was filtered over Celite. Removal of the solvent from the combined filtrates afforded **3** (2.1 g, 83%) as a gray amorphous solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.26 (3H, s), 1.80–2.25 (4H, m), 2.64 (1H, d, J = 12.9 Hz), 2.80–3.15 (2H, m), 2.98 (1H, d, J = 12.9 Hz), 3.25–3.40 (2H, m), 3.79 (2H, s), 4.30–4.50 (1H, m), 6.75–7.00 (6H, m), 7.13 (2H, d, J = 9.1 Hz).

(*R*)-5-Methyl-5-(4-(4-(4-trifluoromethoxy-phenoxy)-piperidin-1-yl)-phenoxymethyl)-4,5-di-hydro-oxazol-2-ylamine (M1). To a solution of 2-aminoalcohol (**3**) (4.9 g, 11 mmol) in anhydrous MeOH (50 mL) were added sodium acetate (2.0 g, 24 mmol) and cyanogen bromide (1.5 g, 13 mmol) at room temperature. The reaction mixture was stirred for 2.5 h at room temperature and then refluxed for 3.5 h. The reaction mixture was allowed to cool to room temperature and the solvent was removed *in vacuo*. After addition of a saturated aqueous solution of $NaHCO_3$, the mixture was extracted with AcOEt. The combined organic layers were dried over sodium sulfate. Removal of the solvent and purification of the crude product by column chromatography (surface-modified silica gel with aminopropyl group; eluent, CH_2Cl_2/ MeOH =100/1) and recrystallization from hexane–AcOEt provided **M1** (3.5 g, 67%) as a white powder, mp 109–111 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.38 (3H, s), 1.74 (2H, dddd, J = 12.2, 9.2, 8.2, 3.7 Hz), 2.00–2.07 (2H,

m), 2.93 (2H, ddd, *J* = 12.2, 9.2, 3.1 Hz), 3.30 (1H, d, *J* = 12.2 Hz), 3.36 (2H, ddd, *J* = 12.2, 5.8, 3.7 Hz), 3.53 (1H, d, *J* = 12.2 Hz), 3.85 (1H, d, *J* = 10.4 Hz), 3.88 (1H, d, *J* = 10.4 Hz), 4.54 (1H, tt, *J* = 8.2, 3.7 Hz), 5.80 (2H, brs), 6.84 (2H, d, *J* = 9.2 Hz), 6.91 (2H, d, *J* = 9.2 Hz), 7.08 (2H, d, *J* = 9.2 Hz), 7.28 (2H, dq, *J* = 9.2, 0.6 Hz).

Synthesis of M3 and M2. (Kitano et al., 2009)

(*R*)-3-Bromo-2-hydroxy-2-methyl-propionic acid ethyl ester (5). To a solution of (*R*)-3-bromo-2-hydroxy-2-methyl-propionic acid (**4** is commercially available) (0.50 g, 2.8 mmol) in anhydrous EtOH (10 mL) was added $TsOH \cdot H_2O$ (52 mg, 0.28 mmol) and the mixture was refluxed for 7 h. The reaction mixture was allowed to cool to room temperature and the solvent was removed *in vacuo*. After addition of a saturated aqueous solution of $NaHCO_3$, the mixture was extracted with AcOEt. The extract was washed with an aqueous solution of $NaHCO_3$, and then with a saturated aqueous solution of NaCl. The combined organic layers were dried over sodium sulfate. Removal of the solvent provided ethyl ester **5** (0.48 g, 83%) as a light yellow oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.33 (3H, t, *J* = 7.1 Hz), 1.54 (3H, s), 3.48 (1H, d, *J* = 10.3 Hz), 3.49 (1H, s), 3.70 (1H, d, *J* = 10.3 Hz), 4.22–4.37 (2H, m).

(*S*)-2-Hydroxy-2-methyl-3-(4-(4-(4-trifluoromethoxyphenoxy)piperidin-1-yl)phenoxy)-

propionic acid ethyl ester (7). To a solution of (*R*)-bromoester (**5**) (17 g, 80 mmol) in anhydrous EtOH (250 mL) were added 4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)-phenol (**6**, Tsubouchi et al., 2008) (26 g, 73 mmol) and 20% NaOEt–EtOH solution (33 mL). The mixture was stirred for 16 h at 90 °C under an argon atmosphere. After the reaction mixture was allowed to cool to room temperature, it was poured into an aqueous solution of ice–NH_4Cl and extracted with CH_2Cl_2. The combined organic layers were dried over sodium sulfate. Removal of the solvent and purification of the crude product by column chromatography (surface-modified silica gel with aminopropyl group; eluent, hexane/ AcOEt =65/35) provided **7** (33 g, 94%) as a light brown oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.26 (3H, t, J = 7.1 Hz), 1.47 (3H, s), 1.80–2.20 (4H, m), 2.85–3.15 (2H, m), 3.25–3.45 (2H, m), 3.53 (1H, s), 3.93 (1H, d, J = 9.0 Hz), 4.15 (1H, d, J = 9.0 Hz), 4.25 (2H, q, J = 7.1 Hz), 4.30–4.50 (1H, m), 6.70–7.00 (6H, m), 7.14 (2H, d, J = 9.9 Hz).

(*S*)-2-Imino-5-methyl-5-((4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)phenoxy)-methyl)oxazolidin-4-one (**M3**). To a suspension of guanidine hydrochloride (7.4 g, 78 mmol) in anhydrous EtOH (50 mL) was added 20% NaOEt–EtOH solution (27 mL) at 0 °C under an argon atmosphere. Subsequently, hydroxyester (**7**) (15 g, 31 mmol) in anhydrous EtOH (100 mL) was added dropwise at 0 °C. After 48 h of stirring at room temperature under an argon atmosphere, ice was added and the mixture was adjusted to pH 5–6 with 6*N*

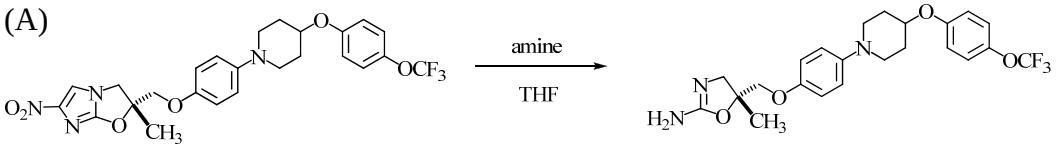
HCl (11 mL). After addition of an aqueous solution of NH_4Cl, the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over sodium sulfate. Removal of the solvent and purification of the crude product by column chromatography (surface-modified silica gel with aminopropyl group; eluent, CH_2Cl_2/ MeOH=20/1) and recrystallization from 2-propanol provided imino-ketone **M3** (8.4 g, 57%) as a white powder, mp 178–180 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.39 (3H, s), 1.73 (2H, dddd, J = 12.5, 9.2, 8.2, 3.7 Hz), 2.00–2.07 (2H, m), 2.93 (2H, ddd, J = 12.2, 9.2, 3.6 Hz), 3.36 (2H, ddd, J = 12.2, 5.8, 3.7 Hz), 4.07 (2H, s), 4.54 (1H, tt, J = 8.2, 3.7 Hz), 6.78 (2H, d, J = 9.2 Hz), 6.89 (2H, d, J = 9.2 Hz), 7.08 (2H, d, J = 9.2 Hz), 7.28 (2H, dq, J = 9.2, 0.9 Hz), 8.33 (1H, brs), 8.57 (1H, brs).

(4*RS*,5*S*)-2-amino-5-methyl-5-((4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)-phenoxy)methyl)-4,5-dihydrooxazol-4-ol (**M2**). To a solution of imino-ketone **M3** (4.2 g, 88 mmol) in CH_2Cl_2 (200 mL) was added dropwise diisobutylaluminium hydride (33 mL, 31 mmol; 0.93 *M* in hexane) at −40 °C under an argon atmosphere. After 4.5 h of stirring at −40 °C, MeOH (10 mL) was added and the mixture was stirred under warming to room temperature. After addition of CH_2Cl_2 and an aqueous solution of NH_4Cl, the precipitate was separated from the filtrate. After the precipitated material was dissolved with aqueous acetic acid, the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over sodium sulfate. Removal of the solvent and purification of the crude product by

column chromatography (silica gel; eluent, CH_2Cl_2/MeOH/25% *aq*. NH_3 soln.=50/10/1) provided amino-alcohol **M2** (3.5 g, 83%) as a white amorphous solid (a mixture of two diastereomers = *ca*. 60:40). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.31 (3H, s), 1.69–1.78 (2H, m), 2.00–2.08 (2H, m), 2.92 (2H, ddd, *J* = 12.5, 8.9, 3.1 Hz), 3.35 (2H, ddd, *J* = 12.5, 5.8, 3.1 Hz), 3.80 (1.2H, s), 3.97 (0.4H, d, *J* = 10.7 Hz), 4.01 (0.4H, d, *J* = 10.7 Hz), 4.54 (1H, tt, *J* = 8.2, 3.7 Hz), 4.94 (0.4H, d, *J* = 3.1 Hz), 5.01 (0.6H, d, *J* = 3.1 Hz), 5.31 (1H, brs), 6.07 (2H, brs), 6.81 (0.8H, d, *J* = 9.2 Hz), 6.83 (1.2H, d, *J* = 9.2 Hz), 6.90 (1.2H, d, *J* = 9.2 Hz), 6.92 (0.8H, d, *J* = 9.2 Hz), 7.08 (2H, d, *J* = 9.2 Hz), 7.28 (2H, d, *J* = 9.2 Hz).

Supplemental Figures

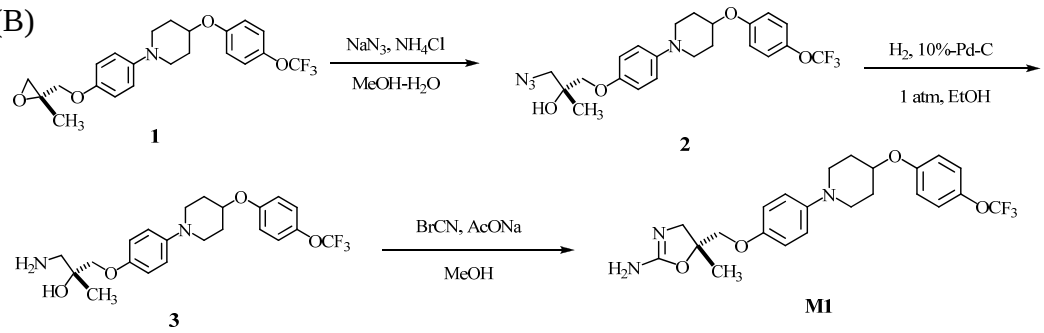
(A)



delamanid **M1**

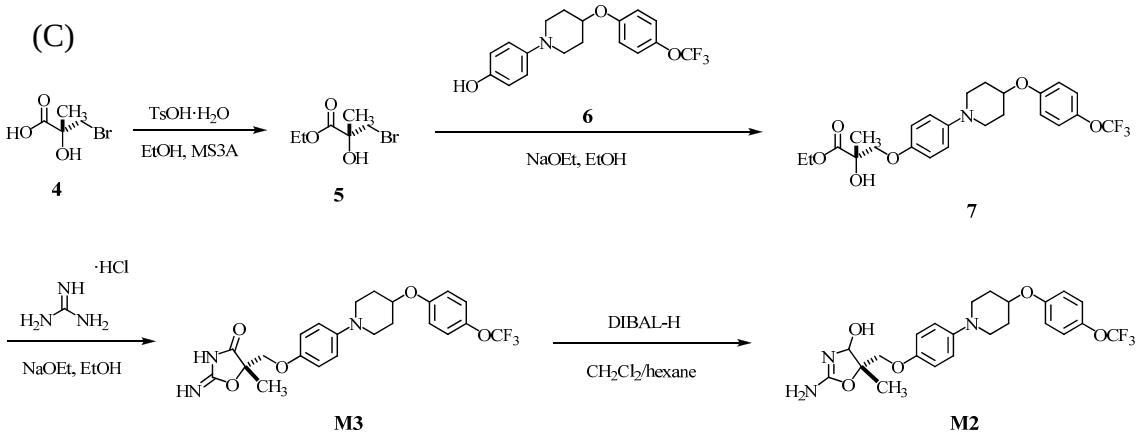
method	amine	apparatus	temp. (°C)	time (hr)	press. (MPa)	**M1** (% yield)
A	25% aq. NH_3	sealed tube	100	0.33	0.8	47
B	H_2N⌒NH_2	open system	25	16	0.1	74

(B)



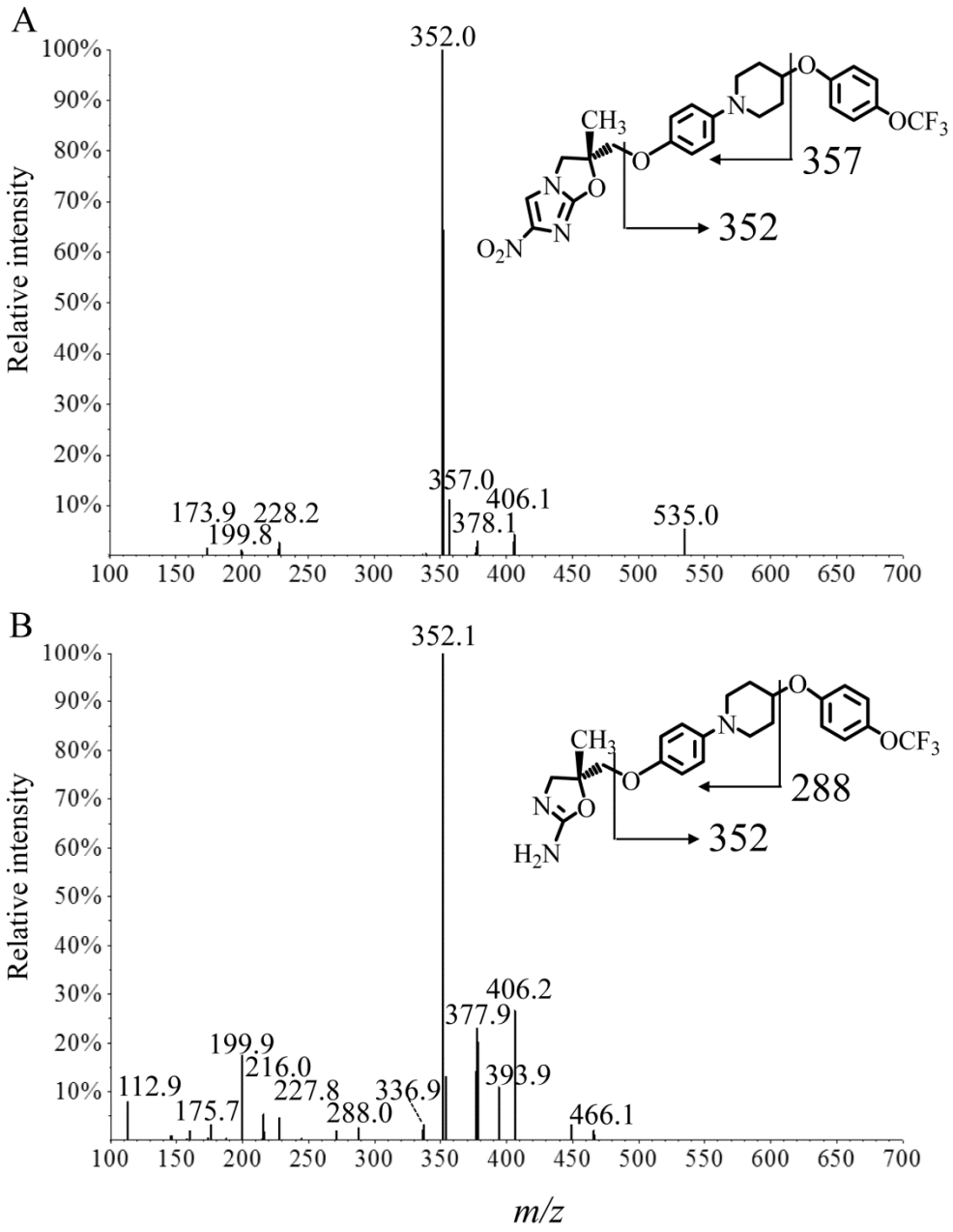
1 **2** **3** **M1**

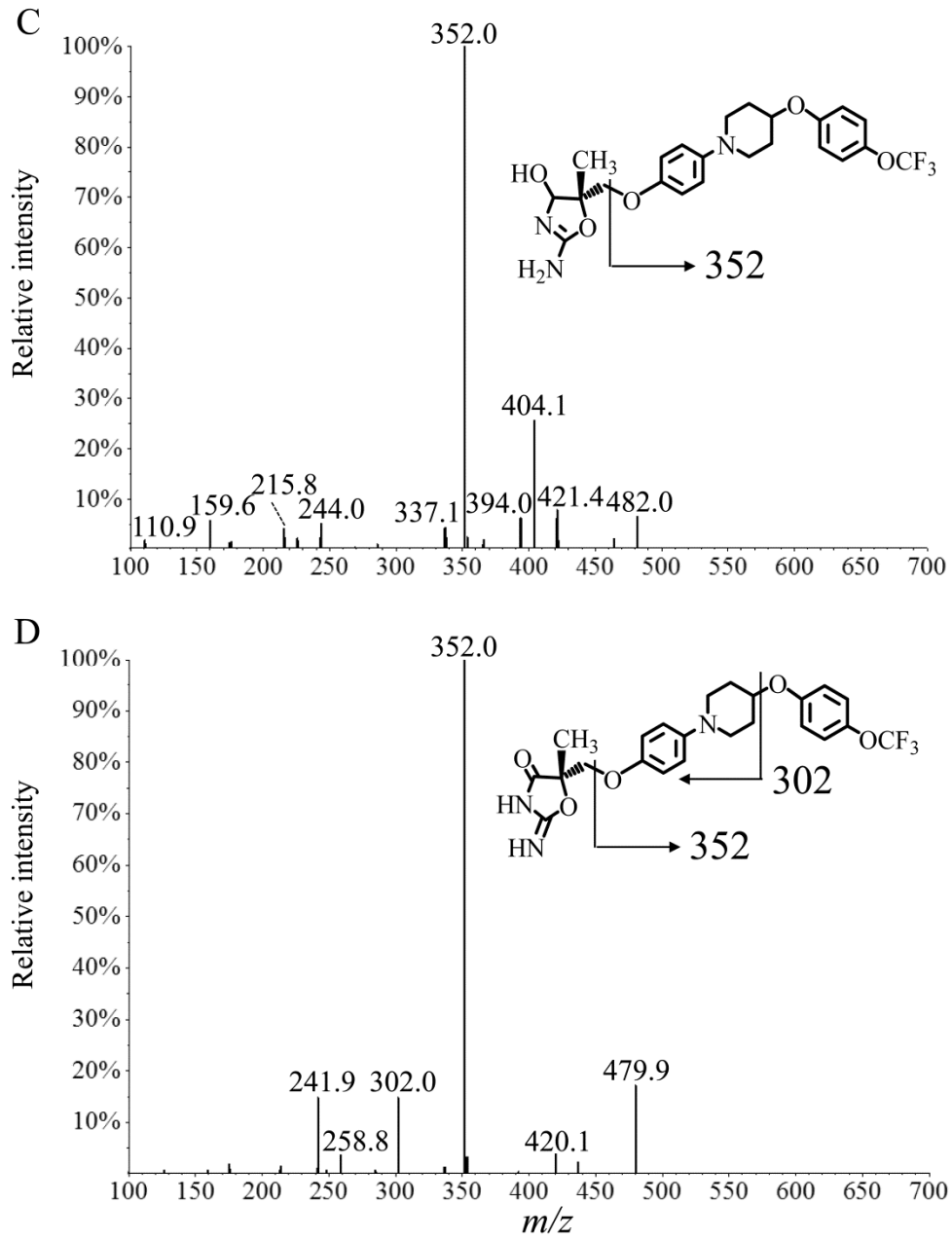
(C)

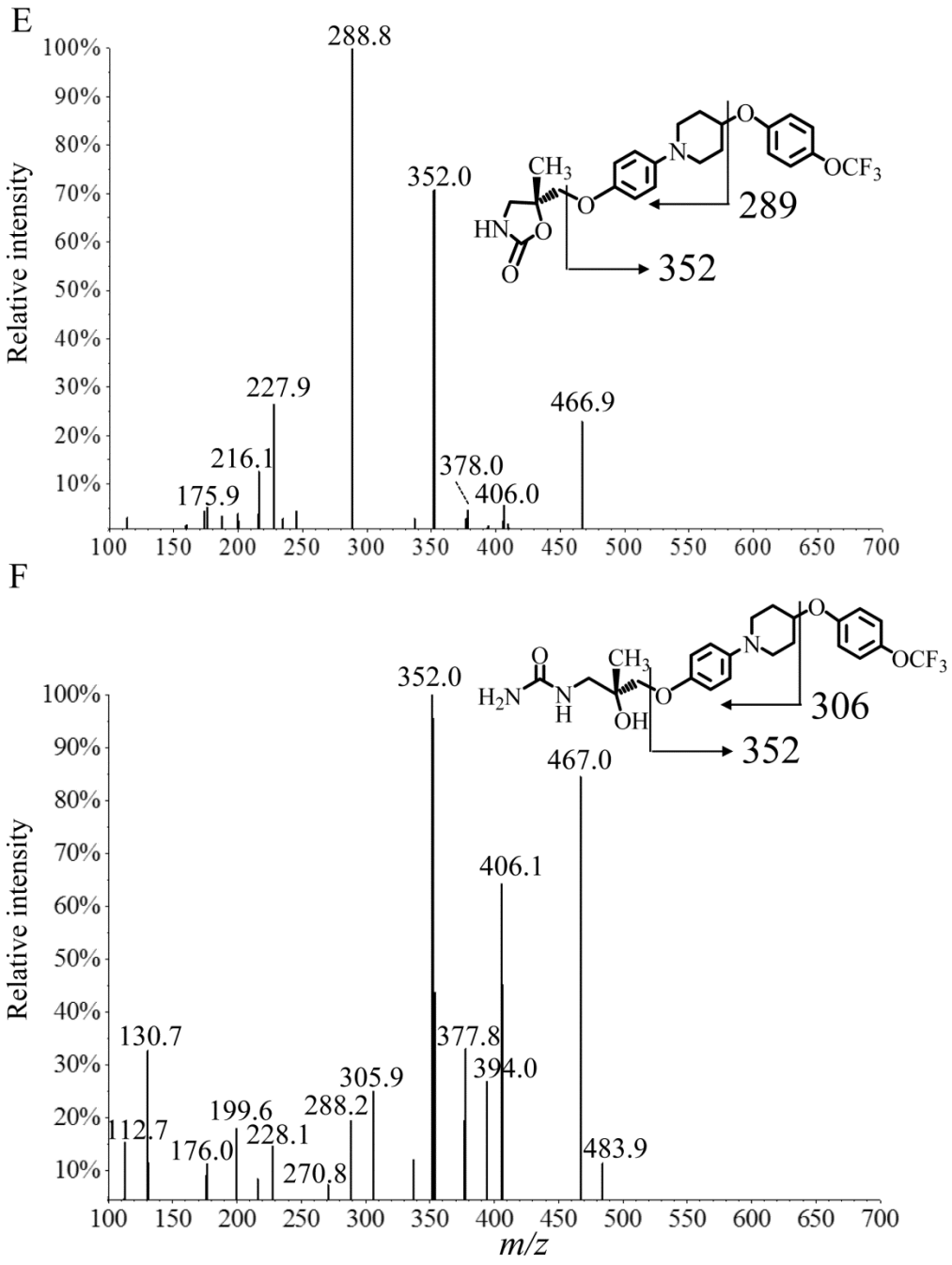


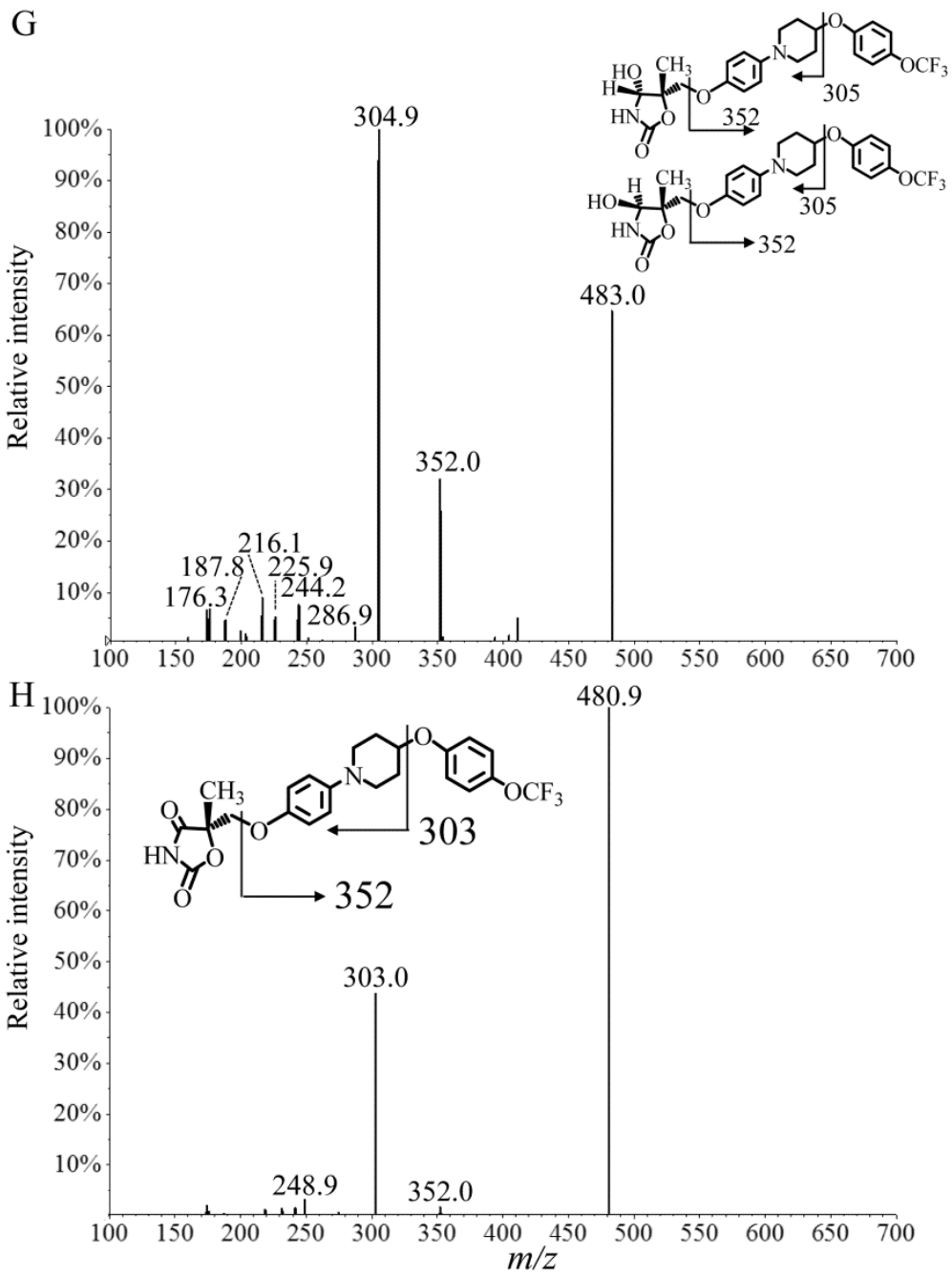
4 **5** **6** **7** **M3** **M2**

Supplemental Fig. 1. Synthesis scheme of standard M1 (A and B), M2 (C), and M3(C)


A
352.0
357
352
357.0
406.1
378.1
173.9
228.2
199.8
535.0
Relative intensity
B
352.1
288
352
406.2
377.9
199.9
216.0
112.9
227.8
336.9
393.9
175.7
288.0
466.1
Relative intensity
m/z


C
352.0
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
Relative intensity
HO
CH3
N
O
O
N
O
OCF3
H2N
352
404.1
215.8
159.6
244.0
337.1
394.0
421.4
482.0
110.9
100
150
200
250
300
350
400
450
500
550
600
650
700
D
352.0
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
Relative intensity
CH3
O
N
O
OCF3
HN
O
HN
302
352
241.9
302.0
258.8
420.1
479.9
100
150
200
250
300
350
400
450
500
550
600
650
700
m/z


E
Relative intensity
288.8
352.0
227.9
466.9
216.1
378.0
406.0
175.9
289
352
F
352.0
306
352
467.0
406.1
130.7
377.8
394.0
305.9
288.2
199.6
228.1
112.7
176.0
270.8
483.9
m/z



Supplemental Fig. 2. MS/MS spectra of delamanid (A, *m/z* 535),M1 (B, *m/z* 466), M2 (C, *m/z* 482), M3 (D, *m/z* 480), M4 (E, *m/z* 467), M5 (F, *m/z* 484), M6 and M7 (G, *m/z* 483), and M8 (H, *m/z* 481).

Supplemental References

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