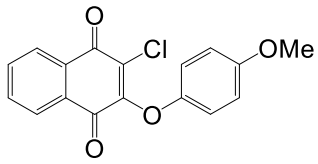
Supplementary Chemistry: Synthesis and Characterization

Chloro-naphthoquinone derivatives

Methyl 1,4-bis(2-(diethylamino)ethoxy)-2-naphthoate, MDN-6

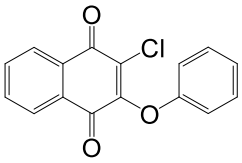
The synthesis procedure of MDN-6 started with the addition of a solution of methyl 1,4-dihydroxy-2-naphthoate (500 mg, 2.29 mmol) and 2-diethylaminoethyl chloride hydrochloride (1.18 g, 6.87 mmol) in DMF (10 mL) K_2CO_3 (6.33 g, 45.83 mmol) and then the mixture stirred for 16 h at room temperature. The reaction mixture was later poured into an ice-water (40 mL) and extracted into CH_2Cl_2 (1 x 50 mL). The mixed organic layers were rinsed with water (1 x 50 mL), desiccated over anhydrous Na_2SO_4 and evaporated to give brown residue that was further purified using column chromatography. ($MeOH/CH_2Cl_2$ = 0.5/9.5) as eluent to afford methyl 1,4-bis(2-(diethylamino)ethoxy)-2-naphthoate (150 mg, 16%) as colorless oil; ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 8.49 (d, J = 8.3 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H), 8.0 (s, 1H), 7.85-7.82 (m, 1H), 7.75-7.72 (m, 1H), 4.09 (t, J = 5.95 Hz, 4H), 3.90 (s, 3H), 2.88 (t, J = 6.0 Hz, 4H), 2.52-2.50 (m, 8H), 1.0-0.94 (m, 12H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 165.2, 156.7, 134.3, 130.8, 130.0, 128.2, 12 7.1, 124.9, 120.2, 116.3, 74.9, 52.9, 47.3, 12.3 ppm.



2-chloro-3-(4-methoxyphenoxy)-1,4-naphthoquinone, N15

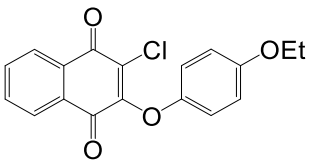
To a solution of 227 mg of 2,3-dichloronaphthoquinone (1 mmol) and 149 mg of 4-methoxyphenol (1.2 mmol) in THF (10 mL), 325 mg of Cs_2CO_3 (1 mmol) was added. At the room temperature, the reaction mixture was stirred for 76 hrs. H_2O and ethyl acetate were used

partition the mixture. The organic phase was rinsed with brine, dried ($MgSO_4$), and evaporated under reduced pressure to obtain a residue. This residue was purified by column chromatography on silica gel (hexane/EtOAc = 9/1) to afford 289 mg (92%) of 2-chloro-3-(4-methoxyphenyloxy)-1,4-naphthoquinone. mp 113℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.20 (dd, *J* = 9.0, 2.2 Hz, 1H), 8.03 (dd, *J* = 9.0, 2.3 Hz, 1H), 7.79-7.75 (m, 2H), 6.97 (d, *J* = 6.0 Hz, 2H), 6.85 (d, *J* = 6.0 Hz, 2H), 3.79 (s, 3H) ppm; ^{13}C NMR ($CDCl_3$): δ_C 178.9, 178.6, 156.5, 154.3, 150.8, 134.9, 134.7, 133.4, 131.6, 131.1, 127.7, 127.5, 118.3, 115.1, 56.0 ppm; EIMS (70eV): *m/z* (rel intensity/%) = 316 (M^+, 10), 314 (M^+, 28), 251 (13), 191 (13), 163 (24), 135 (26), 123 (12), 99 (31), 76 (58), 63 (100), 50 (100).



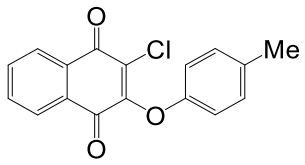
2-Chloro-3-phenoxy-1,4-naphthoquinone, A29

mp 138-139℃; ^{1}H NMR (300MHz, $CDCl_3$): δ_H 8.22 (dd, *J* = 9.0, 2.1 Hz, 1H), 8.06 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.80-7.76 (m, 2H), 7.34 (t, *J* = 7.8 Hz, 2H), 7.16 (d, *J* = 7.5 Hz, 1H), 7.02 (d, *J* = 8.1 Hz, 2H) ppm; EIMS: *m/z* (relative intensity/%) = 286 (M^+, 22), 284 (M^+, 65), 249 (52), 221 (40), 165 (44), 123 (29), 77 (97), 51 (100).



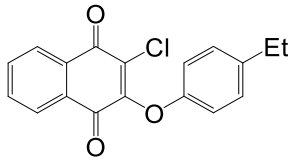
2-Chloro-3-(4-ethoxyphenoxy)-1,4-naphthoquinone, N16

mp 145-146℃; ^{1}H NMR (300MHz, $CDCl_3$): δ_H 8.20 (dd, *J* = 9.0, 2.1 Hz, 1H), 8.04 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.79-7.75 (m, 2H), 6.96 (d, *J* = 6.6 Hz, 2H), 6.84 (d, *J* = 6.9 Hz, 2H), 4.00 (q, *J* = 6.9 Hz, 2H), 1.40 (t, *J* = 6.9 Hz, 3H) ppm; EIMS: *m/z* (relative intensity/%) = 328 (M^+, 66), 299 (31), 264 (30), 236 (28), 190 (29), 162（46), 135 (51), 99 (100), 75 (92), 64 (88).



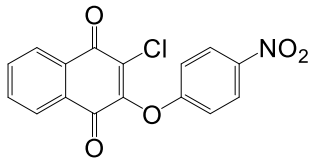
2-Chloro-3-(p-tolyloxy)-1,4-naphthoquinone, N13

mp 146-147℃; ^{1}H NMR (300MHz, $CDCl_3$): δ_H 8.21 (dd, *J* = 9.0, 2.1 Hz, 1H), 8.04 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.79-7.75 (m, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 6.91 (d, *J* = 8.4 Hz, 2H), 2.33 (s, 3H) ppm; EIMS: *m/z* (relative intensity/%) = 300 (M^+, 37), 298 (M^+, 100), 263 (85), 235 (22), 207 (64), 179 (54), 163 (29), 135 (58), 123 (58), 99 (67), 91 (73), 65 (92).



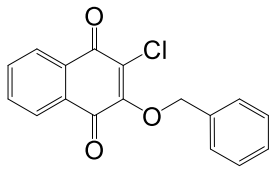
2-Chloro-3-(4-ethylphenoxy)-1,4-naphthoquinone, N14

mp 121-122℃; ^{1}H NMR (300MHz, $CDCl_3$): δ_H 8.22 (dd, *J* = 9.0, 2.1 Hz, 1H), 8.05 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.80-7.76 (m, 2H), 7.15 (d, *J* = 8.4 Hz, 2H), 6.93 (d, *J* = 6.9 Hz, 2H), 2.63 (q, *J* = 7.5 Hz, 2H), 1.23 (t, *J* = 7.5 Hz, 3H) ppm; EIMS: *m/z* (relative intensity/%) = 314 (M^+, 29), 312 (M^+, 77), 297 (100), 277 (29), 191 (34), 163 (61), 135 (38), 99 (74), 77 (99), 51 (35).



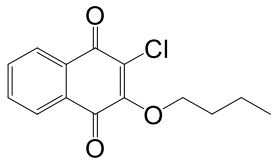
2-Chloro-3-(4-nitrophenoxy)-1,4-naphthoquinone, N17

mp 181-182℃; ^{1}H NMR (300MHz, $CDCl_3$): δ_H 8.28-8.25 (m, 3H), 8.02 (dd, *J* = 9.0, 2.7 Hz, 1H), 7.87-7.79 (m, 2H), 7.11 (d, *J* = 9.0 Hz, 2H) ppm; EIMS: *m/z* (relative intensity/%) = 331 (M^+, 25), 329 (M^+, 51), 301 (20), 220 (26), 191 (18), 163 (53), 135 (30), 99 (51), 76 (100), 63 (41), 50 (65).



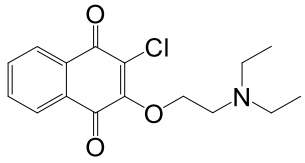
2-Benzyloxy-3-chloro-1,4-naphthoquinone, N7

mp 78-80°C; ^{1}H NMR (300MHz, $CDCl_3$): δ_H 8.14-8.07 (m, 2H), 7.74 (dd, *J* = 9.0, 3.0 Hz, 2H), 7.43 (d, *J* = 7.5 Hz, 2H), 7.38-7.35 (m, 3H), 5.65 (s, 2H) ppm; EIMS: *m/z* (relative intensity/%) = 300 (M^+, 15), 298 (M^+, 58), 263 (13), 181 (10), 123 (15), 91 (88).



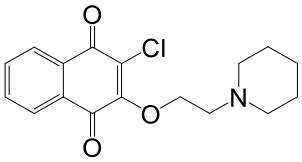
2-Butoxy-3-chloro-1,4-naphthoquinone, N8

oil; ^{1}H NMR (300MHz, $CDCl_3$): δ_H 8.14 (dd, *J* = 9.0, 2.7 Hz, 1H), 8.08 (dd, *J* = 9.0, 2.4 Hz, 1H), 4.57 (t, *J* = 6.3 Hz, 2H), 1.79 (quintet, *J* = 6.6 Hz, 2H), 1.52 (sextet, *J* = 7.5 Hz, 2H), 0.98 (t, *J* = 7.5 Hz, 3H) ppm; EIMS: *m/z* (relative intensity/%) = 266 (M^+, 16), 264 (M^+, 40), 221 (10), 208 (100), 180 (98), 173 (24), 123 (44).



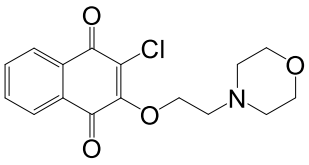
2-Chloro-3-(2-diethylaminoethoxy)-1,4-naphthoquinone, N11

oil; ^{1}H NMR (300MHz, $CDCl_3$): δ_H 8.14 (dd, *J* = 9.0, 2.7Hz, 1H), 8.09-8.03 (m, 1H), 7.73-7.70 (m, 2H), 4.80 (t, *J* = 4.8 Hz, 2H), 2.90 (t, *J* = 4.8 Hz, 2H), 2.58 (q, *J* = 6.9 Hz, 4H), 0.87 (t, *J* = 6.9 Hz, 6H) ppm; EIMS: *m/z* (relative intensity/%) = 310 (M^+, 1), 308 (M^+, 3), 250 (3), 149 (22), 111 (26), 97 (39), 83 (43), 71 (53), 57 (100).



2-Chloro-3-(2-piperidin-1-ylethoxy)-1,4-naphthoquinone, N9

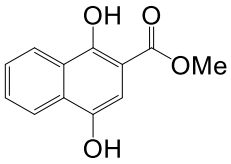
oil; ^{1}H NMR (300MHz, $CDCl_3$): δ_H 8.09 (dd, *J* = 9.0, 2.1 Hz, 1H), 8.02 (dd, *J* = 9.0, 2.1 Hz, 1H), 4.71 (t, *J* = 4.8 Hz, 2H), 2.61 (t, *J* = 4.8 Hz, 2H), 2.30 (br. s, 4H), 2.01 (s, 4H), 0.88-0.76 (m, 2H) ppm; EIMS: *m/z* (relative intensity/%) = 321 (M^+, 38), 319 (M^+, 100), 279 (43), 167 (9), 149 (11), 98 (32).



2-Chloro-3-(2-morpholin-4-ylethoxy)-1,4-naphthoquinone, N10

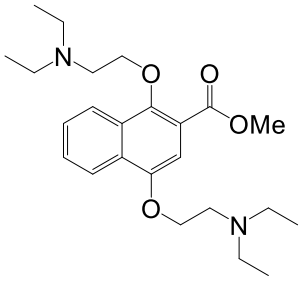
mp 104-105℃; ^{1}H NMR (300MHz, $CDCl_3$): δ_H 8.17 (dd, *J* = 8.9, 2.4 Hz, 1H), 8.09 (dd, *J* = 8.9, 2.3 Hz, 1H), 7.77-7.73 (m, 2H), 4.77 (t, *J* = 4.6 Hz, 2H), 3.26 (t, *J* = 4.1 Hz, 4H), 2.69 (t, *J* = 4.6 Hz, 2H), 2.39 (t, *J* = 4.5 Hz, 4H) ppm; EIMS: *m/z* (relative intensity/%) = 323 (M^+, 9), 321 (M^+, 37), 208 (3), 163 (3), 113 (6), 100 (100), 56 (12).

Naphthoate derivatives



Synthesis of methyl 1,4-dihydroxy-2-naphthoate

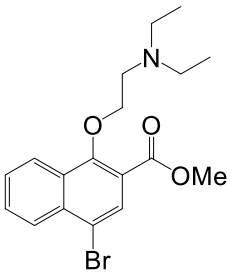
To a stirred solution of 1,4-dihydroxy-2-naphthoic acid (25.0 g, 122.44 mmol) and $NaHCO_3$ (12.86 g, 153.05 mmol) in DMF (200 mL), CH_3I (26.07 g, 183.66 mmol) was added dropwise for 10 minutes at 0 °C. Then the reaction mixture was stirred at rt for 16 hrs. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was poured into ice-cold water (800 mL) and stirred for 10 mins, filtered the off-white solid formed, washed with water (300 mL) and dried in vacuum to give methyl 1,4-dihydroxy-2-naphthoate (24.3 g, 91%). mp 194-195 °C; ^{1}H NMR (DMSO-d_6): δ_H11.44 (s, 1H), 9.19 (s, 1H), 8.32 (d, J = 8.1 Hz, 1H), 8.18 (d, J = 8.1 Hz, 1H), 7.63-7.50 (m, 2H), 7.14 (s, 1H), 3.98 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d6): δ_C170.87, 153.04, 145.48, 129.37, 129.06, 126.75, 125.19, 123.54, 122.52, 105.08, 104.13, 52.90 ppm.



Synthesis of methyl 1,4-bis(2-(diethylamino)ethoxy)-2-naphthoate, N6 (MDN-6)

To a solution of methyl 1,4-dihydroxy-2-naphthoate (500 mg, 2.29 mmol) and 2-diethylaminoethyl chloride hydrochloride (1.18 g, 6.87 mmol) in DMF (10 mL) K_2CO_3 (6.33

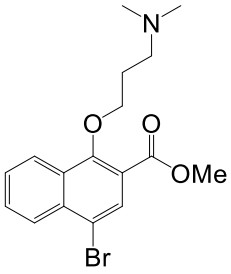
g, 45.83 mmol) was added and then stirred for 16 hrs at room temperature. The reaction mixture was poured into an ice-water (40 mL) and extracted into CH_2Cl_2 (1 x 50 mL). The combined organic layers were washed with water (1 x 50 mL), dried over anhydrous Na_2SO_4 and evaporated to give brown residue which was further purified by column chromatography ($MeOH/CH_2Cl_2$ = 0.5/9.5) as eluent to afford methyl 1,4-bis(2-(diethylamino)ethoxy)-2-naphthoate (150 mg, 16%) as colorless oil; ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 8.49 (d, J = 8.3 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H), 8.0 (s, 1H), 7.85-7.82 (m, 1H), 7.75-7.72 (m, 1H), 4.09 (t, J = 5.95 Hz, 4H), 3.90 (s, 3H), 2.88 (t, J = 6.0 Hz, 4H), 2.52-2.50 (m, 8H), 1.0-0.94 (m, 12H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 165.2, 156.7, 134.3, 130.8, 130.0, 128.2, 127.1, 124.9, 120.2, 116.3, 74.9, 52.9, 47.3, 12.3 ppm.



General Procedure for the synthesis of methyl 4-bromo-1-(2-(diethylamino)ethoxy)-2-naphthoate, N2

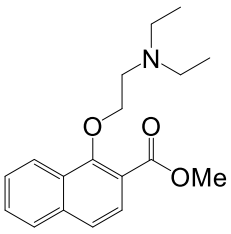
To a solution of methyl 4-bromo-1-hydroxy-2-naphthoate (1.0 g, 3.56 mmol) and 2-dimethylaminoethyl chloride hydrochloride (1.8 g, 10.67 mmol) in DMF (10 mL) K_2CO_3 (9.83 g, 71.15 mmol) was added and then stirred for 16 h at room temperature. The reaction mixture was poured into an ice-water (70 mL) and extracted into CH_2Cl_2 (1 x 100 mL). The combined organic layers were washed with water (1 x 100 mL), dried over anhydrous Na_2SO_4 and

evaporated to give brown residue which was further purified by column chromatography (MeOH/CH_2Cl_2 = 0.25/9.75) as eluent to afford methyl 4-bromo-1-(2-(diethylamino)ethoxy)-2-naphthoate (812 mg, 60%) as a colorless oil; ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 8.49 (d, J = 8.3 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H), 8.0 (s, 1H), 7.85-7.82 (m, 1H), 7.75-7.72 (m, 1H), 4.09 (t, J = 5.95 Hz, 2H), 3.90 (s, 3H), 2.88 (t, J = 6.0 Hz, 2H), 2.56-2.50 (m, 4H), 1.0-0.95 (m, 6H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 165.2, 156.7, 134.3, 130.8, 130.0, 128.2, 127.1, 124.9, 120.2, 116.3, 74.9, 52.9, 47.3, 12.3 ppm;



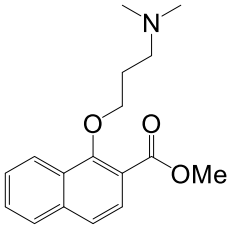
Methyl 4-bromo-1-(3-(dimethylamino)propoxy)-2-naphthoate, N4

^{1}H NMR (500 MHz, DMSO-d_6): δ_H 8.49 (d, J = 8.3 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H), 8.0 (s, 1H), 7.85-7.82 (m, 1H), 7.75-7.72 (m, 1H), 4.09 (t, J = 5.95 Hz, 2H), 3.90 (s, 3H), 2.88 (t, J = 6.0 Hz, 2H), 2.56-2.50 (m, 4H), 1.0-0.95 (m, 6H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 165.2, 156.7, 134.3, 130.8, 130.0, 128.2, 127.1, 124.9, 120.2, 116.3, 74.9, 52.9, 47.3, 12.3 ppm.



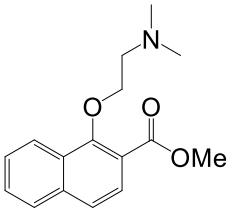
Methyl 1-(2-(diethylamino)ethoxy)-2-naphthoate, N1

^{1}H NMR (500 MHz, DMSO-d_6): δ_H 8.39 (d, J = 7.9 Hz, 1H), 7.99-7.98 (m, 1H), 7.77-7.73 (m, 2H), 7.68-7.62 (m, 2H), 4.10 (t, J = 6.0 Hz, 2H), 3.90 (s, 3H), 2.89 (t, J = 6.0 Hz, 2H), 2.56 (q, J = 14.2, 7.91 Hz, 4H), 0.99 (t, J = 2.0 Hz, 6H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 166.7, 156.6, 136.5, 128.9, 128.6, 128.3, 127.2, 126.6, 123.9, 119.6, 74.6, 52.9, 52.7, 47.5, 12.4 ppm.



Methyl 1-(3-(dimethylamino)propoxy)-2-naphthoate, N3

^{1}H NMR (500 MHz, DMSO-d_6): δ_H 8.26-8.24 (m, 1H), 8.00-7.98 (m, 1H), 7.77-7.73 (m, 2H), 7.69-7.62 (m, 2H), 4.09 (t, J = 6.5 Hz, 2H), 3.90 (s, 3H), 2.48 (t, J = 7.0 Hz, 2H), 2.18 (s, 6H), 2.02-1.98 (m, 2H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 166.7, 156.5, 128.9, 128.5, 128.4, 127.3, 126.6, 123.9, 123.6, 119.7, 74.6, 56.1, 52.6, 45.5, 28.2 ppm.



General Procedure for the Synthesis of methyl 1-(2-(dimethylamino)ethoxy)-2-naphthoate, N5

To a solution of methyl 1-hydroxy-2-naphthoate (1.0 g, 4.95 mmol) and 2-dimethylaminoethyl chloride hydrochloride (2.14 g, 14.84mmol) in DMF (10 mL), K_2CO_3 (13.67 g, 98.91 mmol) was added and the mixture was then stirred for 96 h at room temperature. The reaction mixture was poured into an ice-water (80 mL) and extracted with CH_2Cl_2 (1 x 100 mL). Combined organic layers were washed with water (1 x 100 mL), dried over anhydrous Na_2SO_4, and evaporated to obtain a brown residue which was further purified by column chromatography with MeOH/CH_2Cl_2 = 0.25/9.75 as eluent to afford methyl 1-(2-(dimethylamino)ethoxy)-2-naphthoate (450 mg, 34%) as a yellow oil;. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 8.34-8.32 (m, 1H), 8.01-7.98 (m, 1H), 7.78-7.74 (m, 2H), 7.69-7.63 (m, 2H), 4.14 (t, J = 5.65 Hz, 2H), 3.90 (s, 3H), 2.75 (t, J = 5.7 Hz, 2H), 2.27 (s, 6H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 166.6, 156.6, 136.5, 129.0, 128.6, 128.3, 127.3, 126.6, 124.0, 123.7, 119.7, 74.1, 59.1, 52.7, 46.0 ppm.

Anti-mycobacterial activity assessment

The Alamar Blue assay:

the cultures of *M. tuberculosis* strains of H37Rv (ATCC 27294) and XDR (KMRC 00203-00197) were grown in Middlebrook 7H9 broth (BD, USA) supplemented with 10% albumin dextrose catalase (ADC) (BD, USA) and 0.2% glycerol and inoculated into 96-well plates at a final concentration of 1 $\times 10^5$ cfu/ml in 200 µl of volume with preselected concentrations of MDN-6 or control drugs (200-0.02 µg/ml). As a positive control, we used an equal number of bacterial cells with solvent (DMSO), and as a negative control, we used the fresh medium without any bacteria. After 7 days of incubation at 37°C, 20 µl of freshly prepared 0.2% resazurin solution was provided to each well, and the plates were then incubated at 37°C for a further 48 hours, or until the blue color became pink. The lowest concentration at which the color change was inhibited was selected as the test compound's minimum inhibitory

concentration (MIC). Fluorescence readings were measured at 570 and 600 nm using a multi-label reader (Victor X3, Perkin Elmer, USA), and then the 50% minimum inhibitory concentrations (MIC_{50}) were determined.

The luminescent cell viability assay:

A luminescent cell viability assay kit (G8231, Promega) was used to enumerate the viability of H37Rv (ATCC 27294) and XDR cells (KMRC 00203-00197) in the presence of MDN-6 and the reference drugs. Briefly, in 96-well plates, 1×10^5 cfu/ml concentration of mycobacterial cells were treated with a preselected concentration of test drugs (200-0.02 µg/ml) in 200 µl Middlebrook 7H9 broth (BD, USA) supplemented with 10% ADC and 0.2% glycerol and were incubated at 37 °C for 7 days. Without any drugs, the equal concentration of bacterial cells with solvent (DMSO) was used as a positive control, while the fresh medium without any bacteria or drugs was used as a negative control. After completion of incubation, 50 µl drug-treated mycobacterial cells were collected from each well, thoroughly resuspended in 50 µl of freshly prepared BacTiter-Glo reagent, and further incubated at room temperature for 10 min on an orbital shaker. Then the luminescence was measured with a multi-label plate reader (Perkin Elmer Victor X3) and finally enumerated the viability of H37Rv and XDR cells from the luminescence reading.

CFU counting assay:

A CFU counting assay was used to determine the quantitative bactericidal efficiency of MDN-6 applying the prior published protocol. In brief, *M. tuberculosis* cultures in a final concentration of 1×10^5 cfu/ml were treated with preselected doses of test compounds in 96-well plates. After 7 days of treatment at 37 °C, mycobacterial cells were diluted in fresh Middlebrook 7H9 broth (BD, USA), and spread onto Middlebrook 7H10 (BD, USA) agar plates. Mycobacterial colonies were then counted after completion of the incubation of 21 days.

Synergistic property evaluation

In 96-well plates, H37Rv and XDR cells at a concentration of 1 $\times 10^5$ cfu/ml were exposed to 2-fold serial dilutions of each drug either alone or in combination. Plates were then incubated at 37 °C. Without any drug, the same concentration of bacterial cells with solvent (DMSO) was used as a positive control, while the fresh medium alone was used as a negative control. Following 7 days of incubation at 37°C, 20 µl of freshly prepared 0.2% resazurin solution was added to each well to make a final concentration of 0.02% for resazurin. Plates were then incubated at 37°C until the blue color changed to pink. Color changes were assessed, and fractional inhibitory concentration indices (FICI) were calculated using the following formula

$$FICI = \frac{MIC\ (antibiotic\ combined\ with\ compound)}{MIC\ (antibiotic\ alone)} + \frac{MIC\ (compound\ combined\ with\ antibiotic)}{MIC\ (compound\ alone)}$$

FIC index values were interpreted as follows: ≤ 0.5, synergy; > 0.5 to 0.75, partial synergy; > 0.75 to 1.0, additive effect; > 1.0 to 4.0, indifference; and > 4.0, antagonism.

Post-antibiotic effect (PAE)

H37Rv (ATCC 27294) cells at their early log phase (OD_{600} 0.2) was exposed to MDN-6, RIF, INH, STR, or EMB at the same concentration of 10 µg/ml for 2 hours in Middlebrook 7H9 broth (BD, USA). The exact concentration of bacterial cells with only solvent (DMSO) was used as a positive control and only the fresh medium was used as a negative control. Antibiotics were removed after 2 hours of drug treatment at 37°C by washing three times with pre-warmed fresh Middlebrook 7H9 broth through centrifugation at 3600 g for 10 minutes. Finally, we resuspended the washed pellets in a fresh Middlebrook 7H9 broth and incubated at 37°C for

two weeks and determined their growth saturation point (OD_{max}). OD_{600} was recorded for each culture before and after the exposure of drugs, as well as at 24-hour intervals afterwards. The PAE duration was calculated by subtracting the time needed for the antibiotic-treated culture to reach 50% of the drug-free culture's OD_{max} from the time required for the drug-free control culture to reach the similar point.

Determination of anti-nontuberculous mycobacterial (NTM) effect

All 27 NTM strains (1×10^5 cfu/ml) were inoculated into 96-well plates in 200 µl volume of Middlebrook 7H9 broth (BD, USA) containing a predetermined concentration of MDN-6 or control drugs (200-0.02 µg/ml) and incubated at 37°C for 1 to 7 days according to their doubling time. Fresh media with an equal amount of bacteria and solvent (DMSO) and only fresh media were used as positive control and negative control, respectively. After incubation, the lowest concentration of a test drug that inhibited the visual bacterial growth was determined as the MIC of the test drug.

Assessment of activity against gram-positive and gram-negative bacteria

Briefly, bacteria in a final concentration of 1×10^5 cfu/ml were prepared in cation-adjusted Muller Hilton broth (CAMHB, Sigma) containing predetermined concentrations (0.02-200 µg/ml) of test drugs were seeded into 96-well plates and incubated at 37°C for 18-24 hours. For *Corynebacterium* spp. and *Streptococcus* spp., we added an extra 3% lysed horse blood (LHB) to the CAMHB. Fresh media containing bacteria and solvent (DMSO) and fresh media without bacteria or DMSO were used as positive control and negative control, respectively. After incubation, the lowest concentration of a test drug that inhibited the visual bacterial growth was defined as the MIC of the test drug.

Appendix 1. Anti-tubercular activities of MDN-6 and control drugs against drug-resistant strains *M. tuberculosis* strains collected from the Korean Mycobacterium Resource Center (KMRC) (Cheongju, Chungbuk, Korea).

Strains	**MIC (μg/ml)**					
	MDN-6	**INH**	**RIF**	**STR**	**EMB**	**PZA**
XDR *M. tuberculosis* 1	0.02	12.5	>200	0.78	6.25	>200
XDR *M. tuberculosis* 2	0.02	3.13	>200	50	6.25	>200
XDR *M. tuberculosis* 3	0.19	3.13	>200	>200	6.25	>200
XDR *M. tuberculosis* 4	0.19	12.5	6.25	0.78	6.25	>200
XDR *M. tuberculosis* 5	0.19	12.5	>200	50	6.25	>200
XDR *M. tuberculosis* 6	0.19	12.5	100	50	6.25	>200
XDR *M. tuberculosis* 7	0.19	6.25	>200	0.78	12.5	>200
XDR *M. tuberculosis* 8	0.19	12.5	>200	>200	6.25	>200
XDR *M. tuberculosis* 9	0.19	50	>200	200	6.25	>200
XDR *M. tuberculosis* 10	0.02	>200	6.25	<0.09	6.25	>200
XDR *M. tuberculosis* 11	0.19	6.25	>200	200	6.25	>200
XDR *M. tuberculosis* 12	3.12	12.5	250	250	6.25	>200
MDR *M. tuberculosis* 1	12.5	12.5	>200	100	6.25	>200
MDR *M. tuberculosis* 2	12.5	12.5	>200	1.56	6.25	>200
MDR *M. tuberculosis* 3	6.25	3.13	>200	0.39	3.13	>200
MDR *M. tuberculosis* 4	12.5	25	>200	0.78	6.25	>200
MDR *M. tuberculosis* 5	12.5	12.5	3.13	3.13	6.25	>200
MDR *M. tuberculosis* 6	3.12	25	>200	25	6.25	>200
MDR *M. tuberculosis* 7	6.25	50	>200	200	6.25	>200
MDR *M. tuberculosis* 8	12.5	12.5	>200	3.13	6.25	>200
MDR *M. tuberculosis* 9	25	25	>200	25	3.13	>200
MDR *M. tuberculosis* 10	25	3.13	3.13	>200	0.78	>200
MDR *M. tuberculosis* 11	0.02	12.5	3.13	3.13	6.25	>200
MDR *M. tuberculosis* 12	0.02	12.5	250	100	6.25	>200

Abbreviations: INH, isoniazid; RIF, rifampicin; STR, streptomycin; PZA, pyrazinamide; EMB, ethambutol; MIC, minimum inhibitory concentration; MDR, multidrug-resistant; XDR, extensively drug-resistant.

Appendix 2. *In vitro* MIC_{50} results of MDN-6 and control drugs against drug-sensitive and single drug-resistant *M. tuberculosis* strains tested using the resazurin broth microdilution method.

Strains	**MIC (μg/ml)**				
	MDN-6	**INH**	**RIF**	**STR**	**EMB**
***M. tuberculosis* H37Ra**	12.5	0.19	0.01	0.19	0.39
***M. tuberculosis* H37Rv**	12.5	0.19	0.04	0.19	0.78
INH-resistant *M. tuberculosis*	3.12	25	0.04	0.19	0.78
RIF-resistant *M. tuberculosis*	12.5	0.19	>200	0.19	0.78
STR-resistant *M. tuberculosis*	25	0.19	0.04	12.5	0.78
PZA-resistant *M. tuberculosis*	25	0.19	0.04	0.19	0.78

Abbreviations: INH, isoniazid; RIF, rifampicin; STR, streptomycin; PZA, pyrazinamide; EMB, ethambutol

Appendix 3. MICs of MDN-6 and three control drugs against 27 nontuberculous mycobacteria (NTM)

NTMs	**Strain No.**	**MIC (μg/ml)**			
		MDN-6	**INH**	**RIF**	**STR**
M. abscessus	KMRC 00136-61038	>50	>50	>50	25
M. avium	KMRC 00136-41012	>50	25	1.56	3.13
M. fortuitum	KMRC 00136-60002	>50	25	0.19	6.25
M. intracellulare	KMRC 00136-43007	>50	25	<0.09	0.78
M. marinum	KMRC 00136-21108	12.5	25	<0.09	<0.09
M. phlei	KMRC 00136-19002	>50	>50	25	0.19
M. szulgai	KMRC 00136-61005	>50	1.56	<0.09	0.19
M. xenopi	KMRC 00136-42003	>50	1.56	0.19	0.19

M. gilvum	KCTC-19423	>50	12.5	0.19	0.19
M. smegmatis	KCTC-9108	>50	25	25	0.19
M. bovis	NCCP 14790	>50	0.19	<0.09	0.19
M. kansasii	KMRC 00136-20004	12.5	0.19	0.04	0.19
M. arupense	KMRC 00136-15004	>50	50	0.1	0.1
M. aubagnense	KMRC 00136-72001	>50	>50	1.56	>50
M. bolletti	KMRC 00136-52003	>50	>50	>50	25
M. colombiense	KMRC 00136-86001	>50	12.5	0.19	0.78
M. conceptionense	KMRC 00136-79001	>50	25	25	3.12
M. chitae	KMRC 00136-80001	>50	12.5	6.25	>50
M. gordonae	KMRC 00136-32003	>50	>50	3.12	>50
M. goodie	KMRC 00136-28003	>50	25	>50	0.19
M. heraklionease	KMRC 00136-81001	>50	>50	0.19	50
M. kyorinense	KMRC 00136-82002	>50	3.12	50	0.78
M. masisiliense	KMRC 00136-13017	>50	12.5	0.19	0.78
M. marseiliense	KMRC 00136-83001	>50	>50	>50	25
M. neoaurum	KMRC 00136-18001	>50	3.12	0.19	0.78
M. paregrinum	KMRC 00136-75003	>50	6.25	25	3.12
M. phocaicum	KMRC 00136-22005	>50	>50	25	6.25

Abbreviations: KMRC, Korean Microorganism Resource Center; KCTC, Korean Collection for Type Cultures. NCCP, National Culture Collection for Pathogens

Appendix 4. Effect of test compounds against gram-positive and gram-negative bacterial strains

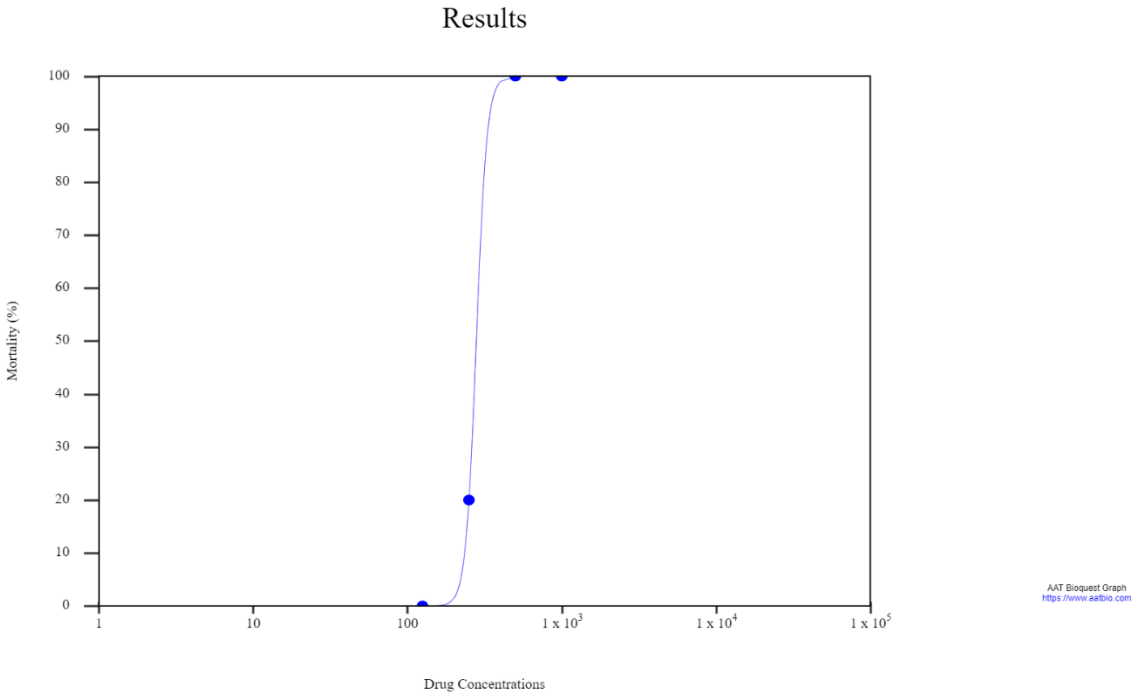
Bacterial Strains	**NCCP No.**	**MIC values (μg/ml)**					
Gram-negative		**MDN-6**	**INH**	**RIF**	**STR**	**VAN**	**MET**
Acinetobacter baumannii	14782	>50	>50	3-6	6-12	>50	3.12-6.25
Citrobacter freundii	14766	>50	>50	>50	1.5-3	>50	>50
Enterobacter aerogenes	14761	>50	>50	6-12	3-6	>50	>50
Escherichia coli	14762	>50	>50	6-12	>25	>50	>50
***Escherichia coli* O157**	14541	>50	>50	6-12	6-12	>50	>50
Klebsiella pneumoniae	14764	>50	>50	6-12	3-6	>50	>50
Proteus mirabilis	14763	>50	>50	3-6	6-12	>50	>50
Proteus vulgaris	14765	>50	>50	6-12	25	>50	>50
Pseudomonas aeruginosa	14781	>50	>50	24	>25	>50	>50

Salmonella enteritidis	14771	>50	>50	12-24	3-6	>50	>50
***Salmonella paratyphi* A**	14759	>50	>50	12-24	6-12	>50	>50
Salmonella typhimurium	16207	>50	>50	12-24	>25	>50	>50
Serratia marcescens	14770	>50	>50	12-24	>25	>50	>50
Shigella boydii	14745	>50	>50	0.4-0.8	>25	12.5-25	>50
Shigella flexneri	14744	>50	>50	1.5-3	>25	>50	>50
Shigella sonnei	14773	>50	>50	3-6	3-6	>50	>50
Shigella dysenteriae	14746	>50	>50	1.5-3	>25	>50	>50
		MIC values (μg/ml)					
Gram-positive	NCCP No.	MDN-6	INH	RIF	STR	VAN	MET
Staphylococcus aureus	14780	>50	>50	< 0.1	6-12	1.5-3	1.56
***Staphylococcus aureus* MRSA**	14769	>50	>50	< 0.1	3-6	0.8-1.5	>50
Staphylococcus epidermidis	14768	>50	>50	< 0.1	1.5-3	0.8-1.5	>50
Corynebacterium diphtheriae	10353	>50	>50	< 0.1	3-6	0.8-1.5	>50
Streptococcus pneumoniae	14774	>50	>50	< 0.1	12-24	0.8-1.5	1.5-3
Streptococcus pyogenes	14783	>50	>50	< 0.1	12-24	0.1	0.4-0.8
Streptococcus sanguinis	14775	>50	>50	< 0.1	12-24	0.4-0.8	0.8-1.6

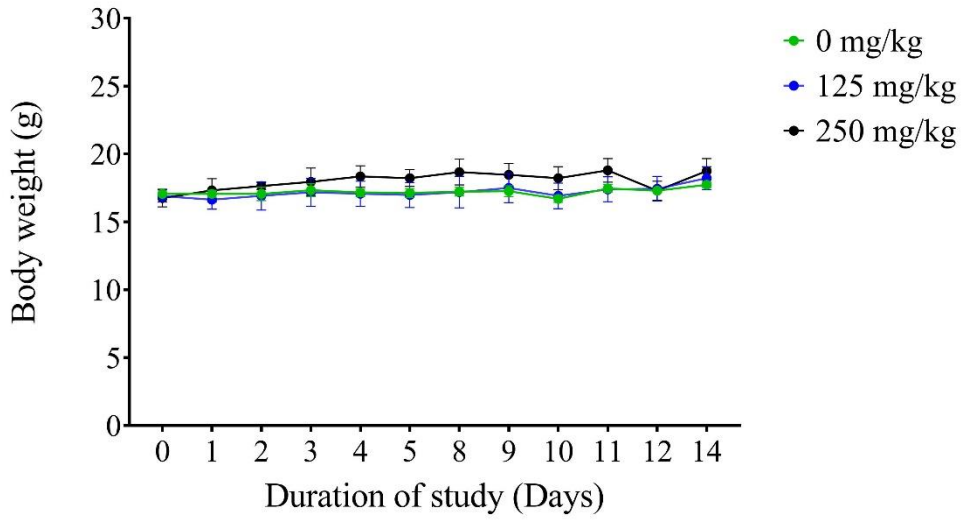
Abbreviations: NCCP, National Culture Collection for Pathogens

Appendix 5. Summary of deaths during the acute oral toxicity test.

Group	**Dose (mg/kg)**	**No. of animals**	**Days after dosing**														
			0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
G1	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G2	125	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G3	250	5	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
G4	500	5	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
G5	1000	5	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0



Appendix 6. Dose-response mortality curve of oral MDN-6 in BALB/c mice. Percentage lethality values are plotted against the concentration of the drug.



Appendix 7. Body weights of mice during the acute oral toxicity test for MDN-6.

Appendix 8. Summary of clinical signs during the acute oral toxicity test.

Sex	Group	Dose (mg/kg)	No. of animals	Clinical sign	Hours (Day 0) after dosing					Days after dosing													
					0.5	1	2	4	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Female	G1	0	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	G2	125	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	G3	250	5	NOA	5	0	0	0	0	5	4	0	0	0	0	0	0	0	0	0	0	0	0
				Abnormal gait	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				Irregular respiration	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				Prone position	0	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				Death	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	G4	500	5	Abnormal gait	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				Irregular respiration	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				Prone position	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				Death	0	5	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	G5	1000	5	Abnormal gait	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				Irregular respiration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				Prone position	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				Death	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Abbreviation: NOA, No observable abnormality