Scheme 2

11 → 12 (*m*CPBA; CH_2Cl_2, rt, 20 min)

Scheme 3[a]

30 + 31 →(a) 32: R_4 = tetrahydropyran-2-yl →(b) 28: R_4 = H

[a] Reagents: (a) $Pd(OAc)_2$, *rac*-BINAP, Cs_2CO_3, toluene, reflux, 30 min; (b) pyridinium *p*-toluenesulfonate, EtOH, 70 °C, 24 h.

Table 2. *In Vitro* and *in Vivo* Activity of Synthesized Compounds **3f** and **4−12**

Compd	R_5	MIC (μg/mL) against *M. tuberculosis* strains			log CFU reduction[b]
		$H_{37}Rv$[a]	$H_{37}Rv$ INH-resistant	$H_{37}Rv$ RFP-resistant	
3f	H	0.05	0.05	0.05	2.0
4	Cl	0.024	0.012	0.006	> 3.1
5	Me	0.012	0.024	0.012	2.9
6	MeO	0.05	0.1	0.05	0.72
7	CF_3	0.2	0.2	0.1	> 4.4
8	OCF_3	0.2	0.39	0.2	> 3.6
9	N	0.78	0.39	0.39	1.9
10	N O	0.78	0.78	0.39	1.3
11	N S	0.78	0.39	0.2	0.0
12	N S→O	6.25	6.25	6.25	ND[c]

[a] MIC of RFP against *M. tuberculosis* $H_{37}Rv$ = 0.1−0.39 μg/mL. [b] log CFU reduction in mouse lung relative to untreated controls by once-daily oral administration at 50 mg/kg for 10 days (n = 2) starting on the day after intravenous infection with 10^4 CFU of *M. tuberculosis* Kurono. [c] ND = not determined.

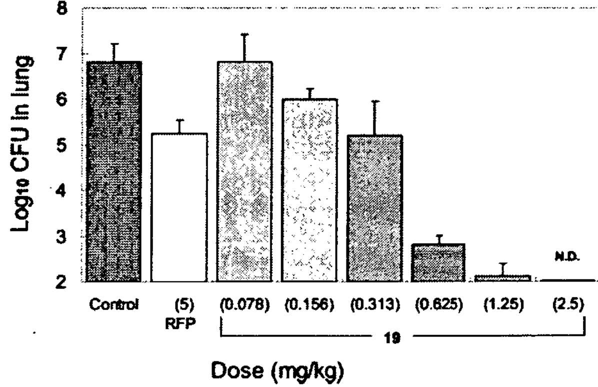

Figure 2. *In vivo* efficacy of compound **19** against *M. tuberculosis* Kurono. Mice were orally dosed once daily for 28 days (n = 6) starting on the day after intravenous infection with 10^4 CFU of mycobacteria.

vitro activity, but **19** was superior to **18** regarding *in vivo* potency (>3.8 log CFU reduction). The excellent *in vitro* activity of **19** was mirrored by its significant *in vivo* efficacy in the mouse acute model. Although **20** (*o*-OCF_3) and **21** (*m*-OCF_3), synthesized by converting the positions of a trifluoromethoxy group of **19** into *ortho* or *meta*, were found to have less potent *in vitro* activity than **19**; the *in vivo* efficacy of **21** was found to be similar to that of **19**.

Next, the compounds **8**, which showed potent *in vivo* efficacy, and **19**, which demonstrated the highest *in vitro* activity among all of the synthesized compounds, were then evaluated *in vivo* at oral doses of 0.5 and 10 mg/kg for 10 days (Table 4). In this *in vivo* test, RFP at 5 mg/kg was used as a reference compound. Compounds **8** and **19** both showed a significant decrease in bacterial load in this evaluation. The oral activity of **8** at a dose of 0.5 mg/kg was similar to that of RFP at 5 mg/kg, and even more notably, oral administration of **19** at a dose of 0.5 mg/kg produced a much better result than RFP at 5 mg/kg. Consequently, based on these evaluation results, compound **19** was selected for further scrutiny.

Finally, **19** was tested for *in vivo* efficacy at lower oral doses of 0.078−2.5 mg/kg once daily for 28 days in mice infected with *M. tuberculosis* Kurono (Figure 2) as a model system. The results for RFP at a dose of 5 mg/kg as a reference drug are

Table 3. *In Vitro* and *in Vivo* Activity of Synthesized Compounds **13–21**

compd	R_6	MIC (μg/mL) against *M. tuberculosis* strains: $H_{37}Rv$[a]	$H_{37}Rv$ INH-resistant	$H_{37}Rv$ RFP-resistant	log CFU reduction[b]
13	H	0.39	0.39	0.2	2.8
14	*p*-Cl	0.05	0.05	0.024	2.2
15	*p*-F	0.39	0.39	0.2	2.2
16	*p*-Me	0.78	0.39	0.39	0.6
17	*p*-MeO	0.39	0.39	0.2	0.1
18	*p*-CF_3	0.012	0.012	0.006	2.2
19	*p*-OCF_3	0.006	0.006	0.006	>3.8
20	*o*-OCF_3	0.39	0.39	0.2	3.0
21	*m*-OCF_3	0.024	0.024	0.024	>4.4

[a] MIC of RFP against *M. tuberculosis* $H_{37}Rv$ = 0.1–0.39 μg/mL. [b] log CFU reduction in mouse lung relative to untreated controls by once-daily oral administration at 50 mg/kg for 10 days ($n = 2$) starting on the day after intravenous infection with 10^4 CFU of *M. tuberculosis* Kurono.

Table 4. *In Vivo* Efficacy of Compounds **8** and **19** as Compared with RFP

compd	**19**		**8**		RFP
dose (mg/kg)	0.5	10	0.5	10	5
log CFU reduction[a]	2.5	>4.4	0.4	3.0	0.5

[a] 10-day treatment of mouse model infection with *M. tuberculosis* Kurono similar to Tables 1 and 2.

also presented. Compound **19** showed a dose-dependent and significant decrease in mouse pulmonary *M. tuberculosis* bacterial counts. In particular, the efficacy of **19** at 0.313 mg/kg was comparable to that of RFP at 5 mg/kg. This potent compound **19** had none of the mutagenicity previously associated with **1**.[20] Therefore, based on the screening results, we selected compound **19** as an orally active candidate for the treatment of tuberculosis.

Conclusions

Screening of this novel series of (*R*)-form optically active 6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole derivatives for *in vitro* antituberculosis activity and *in vivo* oral efficacy indicated that compounds with substituted phenoxymethyl groups and a methyl group at the 2-position are a new class of agents endowed with highly potent antituberculosis activity. Due to its excellent *in vitro* antituberculosis activity against both drug-susceptible and drug-resistant strains of *M. tuberculosis* $H_{37}Rv$ and its potent *in vivo* efficacy in mice infected with *M. tuberculosis* Kurono as a model system, compound **19** was concluded to be a promising orally active candidate for the treatment of tuberculosis. Most notably, compound **19** at an oral dose of 0.313 mg/kg for 28 days showed *in vivo* efficacy comparable to that of RFP at 5 mg/kg. Still more detailed biological data will be presented in a separate paper.[20] Compound **19** (OPC-67683)[20] is now under intensive development.

Experimental Section

General Methods. Reagents were used as supplied unless otherwise noted. All melting points were determined on a Yanaco MP-500D apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker DPX250 instrument operating at 250 MHz. Chemical shifts are shown in parts per million (ppm) on the δ scale downfield relative to tetramethylsilane as an internal standard, and coupling constants are shown in hertz (Hz). Optical rotations were measured on a JASCO DPI-370 digital polarimeter. Satisfactory spectral data were obtained for all of the new compounds. Satisfactory elemental analyses (±0.4%) were obtained for all crystalline derivatives. Chromatographic separations were performed on silica gel columns by gravity column (Kieselgel 60, 0.063–0.200 mm; Merck) chromatography.

Racemic compounds **3b–e** were essentially prepared according to the previously reported methods.[17,21] Compound **3a** has been previously reported.[17]

2-Benzyloxymethyl-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole (3b). Mp 125–126 °C. ^{1}H NMR ($CDCl_3$) δ 3.76 (1H, dd, J = 3.5 Hz, 11.2 Hz), 3.87 (1H, dd, J = 4.1 Hz, 11.2 Hz), 4.23–4.34 (2H, m), 4.59 (2H, s), 5.34–5.43 (1H, m), 7.23–7.41 (5H, m), 7.52 (1H, s). MS (DI) m/z 276 (M^+ + 1). Anal. ($C_{13}H_{13}N_3O_4$) C, H, N.

6-Nitro-2-phenethyloxymethyl-2,3-dihydroimidazo[2,1-*b*]oxazole (3c). Mp 115–116 °C. ^{1}H NMR ($CDCl_3$) δ 2.84 (2H, t, J = 6.6 Hz), 3.64–3.86 (4H, m), 4.09 (1H, dd, J = 6.2 Hz, 10.0 Hz), 4.21 (1H, dd, J = 8.6 Hz, 10.0 Hz), 5.25–5.41 (1H, m), 7.07–7.32 (5H, m), 7.46 (1H, s). MS (DI) m/z 289 (M^+). Anal. ($C_{14}H_{15}N_3O_4$) C, H, N.

2-Cinnamyloxymethyl-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole (3d). Mp 145–147 °C. ^{1}H NMR ($CDCl_3$) δ 3.80 (1H, dd, J = 3.6 Hz, 11.3 Hz), 3.90 (1H, dd, J = 4.0 Hz, 11.3 Hz), 4.20–4.34 (4H, m), 5.34–5.50 (1H, m), 6.22 (1H, ddd, J = 6.2 Hz, 12.4 Hz, 16.0 Hz), 6.57 (1H, d, J = 16.0 Hz), 7.20–7.39 (5H, m), 7.54 (1H, s). MS (DI) m/z 302 (M^+). Anal. ($C_{15}H_{15}N_3O_4$) C, H, N.

2-Methyl-6-nitro-2-phenoxymethyl-2,3-dihydroimidazo[2,1-*b*]oxazole (3e). Mp 117–119 °C. ^{1}H NMR ($CDCl_3$) δ 1.79 (3H, s), 4.03 (1H, d, J = 10.2 Hz), 4.09 (1H, d, J = 10.2 Hz), 4.24 (1H, d, J = 10.1 Hz), 4.50 (1H, d, J = 10.1 Hz), 6.84 (2H, dd, J = 2.0 Hz, 8.6 Hz), 7.01 (1H, t, J = 7.4 Hz), 7.20–7.31 (2H, m), 7.56 (1H, s). MS (DI) m/z 275 (M^+). Anal. ($C_{13}H_{13}N_3O_4$) C, H, N.

(*R*)-2-Chloro-1-[2-hydroxy-2-methyl-3-(4-nitrobenzoyloxy)]-propyl-4-nitroimidazole (24). A solution of 2-chloro-4-nitro-1*H*-imidazole (**22**)[23] (3 g, 20.34 mmol), (*R*)-form epoxide **23**[24] (5.31 g, 22.37 mmol), and triethylamine (0.57 mL, 4.07 mmol) in ethyl acetate (10 mL) was heated at 60–65 °C for 6 h. The reaction mixture was allowed to cool to room temperature and concentrated under reduced pressure. To the residue was added ethyl acetate (3 mL) and toluene (30 mL). The resulting precipitates were collected by filtration and recrystallized from ethyl acetate–isopropylether to give **24** (6.82 g, 87%) as colorless needles. Mp 122–123 °C. ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, s), 4.11–4.33 (4H, m), 5.61 (1H, s), 8.25 (2H, d, J = 8.9 Hz), 8.31–8.45 (3H, m). $[\alpha]_D^{26}$ 54.0° (c 1.04, CH_3CN). MS (DI) m/z 384 (M^+). Anal. ($C_{14}H_{13}ClN_4O_7$) C, H, N.

(*R*)-2-Chloro-1-(2,3-dihydroxy-2-methyl)propyl-4-nitroimidazole (25). To a solution of **24** (6.80 g, 17.67 mmol) in methanol (68 mL) was added potassium carbonate (122 mg, 0.88 mmol). After the solution was stirred at room temperature for 2 h, 6 M hydrochloric acid (0.3 mL) and magnesium sulfate (3 g) were added at 0 °C, and the resulting mixture was stirred for 1 h. The insoluble materials were filtered off through Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 20/1) and recrystallized from ethyl acetate–isopropylether to give **25** (4.09 g, 97%) as colorless needles. Mp 110–111 °C. ^{1}H NMR (DMSO-d_6) δ 1.01 (3H, s), 3.25 (2H, d, J = 5.3 Hz), 4.05 (2H, s), 5.01 (1H, s), 5.11 (1H, t, J = 5.4 Hz), 8.32(1H, s). $[\alpha]_D^{27}$ 17.4° (c 1.03, DMSO). MS (DI) m/z 235 (M^+). Anal. ($C_7H_{10}ClN_3O_4$) C, H, N.

(*R*)-2-Chloro-1-(2-methyl-2,3-epoxypropyl)-4-nitroimidazole (27). To a solution of **25** (10 g, 42.44 mmol) in pyridine (20 mL) was added methanesulfonyl chloride (7.29 g, 63.66 mmol) at below 15 °C dropwise over 30 min. After the solution was stirred for 2 h, 6 M hydrochloric acid (63 mL) was added to the reaction mixture at below 30 °C. The resulting mixture was extracted with ethyl acetate (75 mL × 2), and the combined organic layer was washed with brine, dried over magnesium sulfate, and filtered. The

filtrate was concentrated under reduced pressure, and to the residue was added toluene (75 mL). The resulting precipitates were collected by filtration to afford crude **26**. To a solution of this crude **26** in ethyl acetate (100 mL) was added 1,8-diazabicyclo[5.4.0]-7-undecene (7.10 g, 46.68 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was washed with brine, dried over magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethy acetate/hexane = 1/1) to give the (*R*)-form epoxide **27** (6.93 g, 75%) as colorless needles. Mp 72−73 °C. ^{1}H NMR ($CDCl_3$) δ 1.38 (3H, s), 2.62 (1H, d, *J* = 4.0 Hz), 2.78 (1H, d, *J* = 4.0 Hz), 4.00 (1H, d, *J* = 14.9 Hz), 4.38 (1H, d, *J* = 14.9 Hz), 7.87 (1H, s). $[\alpha]_D^{26}$ 31.1° (*c* 2.02, $CHCl_3$). MS (DI) *m/z* 217 (M^+). Anal. ($C_7H_8ClN_3O_3$) C, H, N.

(*S*)-2-Chloro-1-(2-methyl-2,3-epoxypropyl)-4-nitroimidazole (29). This compound was obtained by the same procedure as described for **27** from 2-chloro-4-nitro-1*H*-imidazole (**22**) and (*S*)-2-methyl-2,3-epoxypropyl 4-nitrobenzoate.[24] Mp 72−73 °C. ^{1}H NMR ($CDCl_3$) δ 1.39 (3H, s), 2.63 (1H, d, *J* = 4.0 Hz), 2.79 (1H, d, *J* = 4.0 Hz), 4.00 (1H, d, *J* = 14.9 Hz), 4.38 (1H, d, *J* = 14.9 Hz), 7.88 (1H, s). $[\alpha]_D^{27}$ −29.2° (*c* 1.18, $CHCl_3$). MS (DI) *m/z* 217 (M^+). Anal. ($C_7H_8ClN_3O_3$) C, H, N.

1-[4-(Tetrahydropyran-2-yloxy)phenyl]-4-(4-trifluoromethoxyphenoxy)piperidine (32g). A mixture of 2-(4-bromophenoxy)-tetrahydropyran (**30**)[26] (30 g, 116.67 mmol) and 4-(4-trifluoromethoxyphenoxy)piperidine (**31g**)[27] (30.30 g, 115.60 mmol) in the presence of palladium acetate (1 g, 4.64 mmol), *rac*-2,2′-bis-(diphenylphosphino)-1,1′-binaphthyl (4.30 g, 6.96 mmol), and cesium carbonate (49 g, 150.39 mmol) in toluene (300 mL) was refluxed under a nitrogen atmosphere for 30 min. The reaction mixture was allowed to cool to room temperature, and ethyl acetate (300 mL) and water (200 mL) were added. The thus-obtained mixture was filtered through Celite. The organic layer was separated, washed with brine, dried over magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethy acetate/hexane = 1/20) to give **32g** (32.60 g, 64%) as a yellow crystalline powder. ^{1}H NMR ($CDCl_3$) δ 1.55−1.75 (3H, m), 1.81−2.20 (7H, m), 2.95−3.04 (2H, m), 3.38−3.42 (2H, m), 3.55−3.66 (1H, m), 3.87−3.99 (1H, m), 3.56−4.45 (1H, m), 5.29−5.32 (1H, m), 6.89−7.01 (6H, m), 7.11−7.16 (2H, m).

4-[4-(4-Trifluoromethoxyphenoxy)piperidin-1-yl]phenol (28g). A mixture of **32g** (30.10 g, 68.81 mmol) and pyridinium *p*-toluenesulfonate (5.20 g, 20.69 mmol) in ethanol (450 mL) was heated at 70 °C for 24 h. The reaction mixture was allowed to cool to room temperature and concentrated under reduced pressure. Saturated sodium hydrogen carbonate aqueous solution (100 mL) was added to the residue, which was extracted with dichloromethane (200 mL). The organic layer was washed with brine, dried over magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (dichloromethane/ethyl acetate = 10/1) to give **28g** (22.90 g, 94%) as a pale yellow crystalline powder. ^{1}H NMR ($CDCl_3$) δ 1.88−2.02 (2H, m), 2.06−2.16 (2H, m), 2.92−3.02 (2H, m), 3.30−3.39 (2H, m), 4.36−4.44 (1H, m), 4.74 (1H, s), 6.71−6.78 (2H, m), 6.85−6.94 (4H, m), 7.10−7.16 (2H, m).

Other phenol derivatives **28a−f** and **28h,i** were synthesized by the same procedure as described for **28g**. Compounds **28a−i** were immediately used for the next reaction.

4-(4-Phenoxypiperidin-1-yl)phenol (28a). ^{1}H NMR ($CDCl_3$) δ 1.89−2.03 (2H, m), 2.04−2.18 (2H, m), 2.92−3.02 (2H, m), 3.31−3.41 (2H, m), 4.39−4.49 (1H, m), 4.92 (1H, s), 6.70−6.78 (2H, m), 6.84−6.98 (5H, m), 7.24−7.33 (2H, m).

4-[4-(4-Chlorophenoxy)piperidin-1-yl]phenol (28b). ^{1}H NMR ($CDCl_3$) δ 1.87−2.01 (2H, m), 2.04−2.16 (2H, m), 2.91−3.02 (2H, m), 3.29−3.39 (2H, m), 4.34−4.44 (1H, m), 4.85 (1H, s), 6.71−6.78 (2H, m), 6.82−6.92 (4H, m), 7.20−7.26 (2H, m).

4-[4-(4-Fluorophenoxy)piperidin-1-yl]phenol (28c). ^{1}H NMR ($CDCl_3$) δ 1.86−2.00 (2H, m), 2.04−2.16 (2H, m), 2.90−3.00 (2H, m), 3.30−3.40 (2H, m), 4.29−4.39 (1H, m), 4.72 (1H, s), 6.71−6.78 (2H, m), 6.83−7.01 (6H, m).

4-[4-(4-Methylphenoxy)piperidin-1-yl]phenol (28d). ^{1}H NMR ($CDCl_3$) δ 1.87−2.01 (2H, m), 2.04−2.16 (2H, m), 2.29 (3H, s), 2.90−3.00 (2H, m), 3.30−3.40 (2H, m), 4.33−4.43 (1H, m), 4.85 (1H, s), 6.71−6.78 (2H, m), 6.80−6.92 (4H, m), 7.06−7.10 (2H, m).

4-[4-(4-Methoxyphenoxy)piperidin-1-yl]phenol (28e). ^{1}H NMR ($CDCl_3$) δ 1.86−2.00 (2H, m), 2.05−2.13 (2H, m), 2.88−2.99 (2H, m), 3.31−3.41 (2H, m), 3.77 (3H, s), 4.25−4.35 (1H, m), 4.72 (1H, s), 6.72−6.77 (2H, m), 6.80−6.92 (6H, m).

4-[4-(4-Trifluoromethylphenoxy)piperidin-1-yl]phenol (28f). ^{1}H NMR ($CDCl_3$) δ 1.90−2.05 (2H, m), 2.08−2.20 (2H, m), 2.94−3.05 (2H, m), 3.30−3.40 (2H, m), 4.46−4.56 (1H, m), 4.64 (1H, s), 6.72−6.80 (2H, m), 6.86−6.93 (2H, m), 6.96−7.00 (2H, m), 7.52−7.56 (2H, m).

4-[4-(2-Trifluoromethoxyphenoxy)piperidin-1-yl]phenol (28h). ^{1}H NMR ($CDCl_3$) δ 1.91−2.16 (4H, m), 2.91−3.07 (2H, m), 3.25−3.40 (2H, m), 4.40−4.53 (1H, m), 4.70 (1H, s), 6.76 (2H, dd, *J* = 2.3 Hz, 6.7 Hz), 6.81−7.05 (4H, m), 7.12−7.28 (2H, m).

4-[4-(3-Trifluoromethoxyphenoxy)piperidin-1-yl]phenol (28i). ^{1}H NMR ($CDCl_3$) δ 1.89−2.18 (4H, m), 2.94−3.06 (2H, m), 3.27−3.41 (2H, m), 4.35−4.51 (1H, m), 4.71 (1H, s), 6.71−6.96 (7H, m), 7.25−7.35 (1H, m).

(*R*)-2-Methyl-6-nitro-2-{4-[4-(4-trifluoromethoxyphenoxy)piperidin-1-yl]phenoxymethyl}-2,3-dihydroimidazo[2,1-*b*]oxazole (19). To a mixture of **27** (127.56 g, 586.56 mmol) and 4-[4-(4-trifluoromethoxyphenoxy)piperidin-1-yl]phenol (**28g**) (165.70 g, 468.95 mmol) in *N*,*N*-dimethylformamide (1600 mL) was added 60% sodium hydride (22.51 g, 562.74 mmol) at 0 °C portionwise. After the mixture was stirred at 50 °C for 2 h under a nitrogen atmosphere, the reaction mixture was cooled in an ice bath and carefully quenched with ethyl acetate (230 mL) and ice water (50 mL). The thus-obtained mixture was poured into water (3000 mL) and stirred for 30 min. The resulting precipitates were collected by filtration, washed with water, and dried at 60 °C overnight. This crude product was purified by silica gel column chromatography using a dichloromethane and ethyl acetate mixture (5/1) as solvent. The appropriate fractions were combined and evaporated under reduced pressure. The residue was recrystallized from ethyl acetate (1300 mL)−isopropyl alcohol (150 mL) to afford **19** (119.11 g, 48%) as a pale yellow crystalline powder. Mp 195−196 °C. ^{1}H NMR ($CDCl_3$) δ 1.77 (3H, s), 1.87−2.16 (4H, m), 2.95−3.05 (2H, m), 3.32−3.41 (2H, m), 4.02 (1H, d, *J* = 10.2 Hz), 4.04 (1H, d, *J* = 10.2 Hz), 4.18 (1H, *J* = 10.2 Hz), 4.36−4.45 (1H, m), 4.49 (1H, d, *J* = 10.2 Hz), 6.76 (2H, d, *J* = 6.7 Hz), 6.87−6.94 (4H, m), 7.14 (2H, d, *J* = 8.6 Hz), 7.55 (1H, s). $[\alpha]_D^{28}$ −9.9° (*c* 1.01, $CHCl_3$). MS (DI) *m/z* 535 (M^+ + 1). Anal. ($C_{25}H_{25}F_3N_4O_6$) C, H, N.

Compounds **3f**, **4−11**, **13−18**, **20**, and **21** were prepared by the same procedure as described for **19**.

(*R*)-2-Methyl-6-nitro-2-phenoxymethyl-2,3-dihydroimidazo[2,1-*b*]oxazole (3f). Mp 151−153 °C. ^{1}H NMR ($CDCl_3$) δ 1.79 (3H, s), 4.03 (1H, d, *J* = 10.2 Hz), 4.09 (1H, d, *J* = 10.2 Hz), 4.24 (1H, d, *J* = 10.1 Hz), 4.50 (1H, d, *J* = 10.1 Hz), 6.84 (2H, dd, *J* = 1.8 Hz, 8.5 Hz), 7.01 (1H, t, *J* = 7.2 Hz), 7.21−7.31 (2H, m), 7.55 (1H, s). MS (DI) *m/z* 275 (M^+). Anal. ($C_{13}H_{13}N_3O_4$) C, H, N.

(*R*)-2-(4-Chlorophenoxymethyl)-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole (4). Mp 185−187 °C. ^{1}H NMR ($CDCl_3$) δ 1.78 (3H, s), 4.04 (1H, d, *J* = 3.2 Hz), 4.08 (1H, d, *J* = 3.2 Hz), 4.21 (1H, d, *J* = 10.1 Hz), 4.49 (1H, d, *J* = 10.1 Hz), 6.78 (2H, d, *J* = 9.0 Hz), 7.19−7.29 (2H, m), 7.56 (1H, s). MS (DI) *m/z* 309 (M^+). Anal. ($C_{13}H_{12}ClN_3O_4$) C, H, N.

(*R*)-2-Methyl-2-(4-methylphenoxymethyl)-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole (5). Mp 177−179 °C. ^{1}H NMR ($CDCl_3$) δ 1.78 (3H, s), 2.28 (3H, s), 4.02 (1H, d, *J* = 7.2 Hz), 4.06 (1H, d, *J* = 7.2 Hz), 4.20 (1H, d, *J* = 10.1 Hz), 4.49 (1H, d, *J* = 10.1 Hz), 6.74 (2H, d, *J* = 8.3 Hz), 7.08 (2H, d, *J* = 8.3 Hz), 7.55 (1H, s). MS (DI) *m/z* 289 (M^+). Anal. ($C_{14}H_{15}N_3O_4$) C, H, N.

(*R*)-2-(4-Methoxyphenoxymethyl)-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole (6). Mp 179−180 °C. ^{1}H NMR ($CDCl_3$)

δ 1.77 (3H, s), 3.76 (3H, s), 4.02 (1H, d, J = 2.6 Hz), 4.06 (1H, d, J = 2.6 Hz), 4.17 (1H, d, J = 10.2 Hz), 4.50 (1H, d, J = 10.2 Hz), 6.71–6.86 (4H, m), 7.55 (1H, s). MS (DI) *m/z* 305 (M^+). Anal. ($C_{14}H_{15}N_3O_5$) C, H, N.

(*R*)-2-Methyl-6-nitro-2-(4-trifluoromethylphenoxymethyl)-2,3-dihydroimidazo[2,1-*b*]oxazole (7). Mp 188–190 °C. ^{1}H NMR ($CDCl_3$) δ 1.81 (3H, s), 4.08 (1H, d, J = 10.3 Hz), 4.18 (1H, d, J = 10.3 Hz), 4.29 (1H, d, J = 10.3 Hz), 4.50 (1H, d, J = 10.3 Hz), 6.93 (2H, d, J = 8.7 Hz), 7.50–7.59 (3H, m). MS (DI) *m/z* 343 (M^+). Anal. ($C_{14}H_{12}F_3N_3O_4$) C, H, N.

(*R*)-2-Methyl-6-nitro-2-(4-trifluoromethoxyphenoxymethyl)-2,3-dihydroimidazo[2,1-*b*]oxazole (8). Mp 176–178 °C. ^{1}H NMR ($CDCl_3$) δ 1.79 (3H, s), 4.06 (1H, d, J = 6.8 Hz), 4.10 (1H, d, J = 6.8 Hz), 4.23 (1H, d, J = 10.1 Hz), 4.49 (1H, d, J = 10.1 Hz), 6.84 (2H, d, J = 9.0 Hz), 7.13 (2H, d, J = 9.0 Hz), 7.56 (1H, s). MS (DI) *m/z* 359 (M^+). Anal. ($C_{14}H_{12}F_3N_3O_5$) C, H, N.

(*R*)-2-Methyl-6-nitro-2-[4-(piperidin-1-yl)phenoxymethyl]-2,3-dihydroimidazo[2,1-*b*]oxazole (9). Mp 217–219 °C. ^{1}H NMR ($CDCl_3$) δ 1.45–1.57 (5H, m), 1.61–1.78 (4H, m), 2.94–3.08 (4H, m), 4.00 (1H, d, J = 7.4 Hz), 4.04 (1H, d, J = 7.4 Hz), 4.17 (1H, d, J = 10.1 Hz), 4.49 (1H, d, J = 10.1 Hz), 6.75 (2H, d, J = 6.8 Hz), 6.89 (2H, d, J = 6.8 Hz), 7.54 (1H, s). MS (DI) *m/z* 358 (M^+). Anal. ($C_{18}H_{22}N_4O_4$) C, H, N.

(*R*)-2-Methyl-2-[4-(morpholin-4-yl)phenoxymethyl]-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole (10). Mp 233–235 °C. ^{1}H NMR (DMSO-d_6) δ 1.67 (3H, s), 2.92–3.00 (4H, m), 3.61–3.71 (4H, m), 4.08–4.22 (3H, m), 4.36 (1H, d, J = 10.9 Hz), 6.80 (2H, d, J = 6.8 Hz), 6.88 (2H, d, J = 6.8 Hz), 8.15 (1H, s). MS (DI) *m/z* 360 (M^+). Anal. ($C_{17}H_{20}N_4O_5$) C, H, N.

(*R*)-2-Methyl-6-nitro-2-[4-(thiomorpholin-4-yl)phenoxymethyl]-2,3-dihydroimidazo[2,1-*b*]oxazole (11). Mp 227–229 °C. ^{1}H NMR ($CDCl_3$) δ 1.77 (3H, s), 2.69–2.80 (4H, m), 3.31–3.71 (4H, m), 4.01 (1H, d, J = 5.5 Hz), 4.05 (1H, d, J = 5.5 Hz), 4.18 (1H, d, J = 10.1 Hz), 4.49 (1H, d, J = 10.1 Hz), 6.77 (2H, d, J = 6.7 Hz), 6.86 (2H, d, J = 6.7 Hz), 7.55 (1H, s). MS (DI) *m/z* 376 (M^+). Anal. ($C_{17}H_{20}N_4O_4S$) C, H, N.

(*R*)-2-Methyl-6-nitro-2-[4-(4-phenoxypiperidin-1-yl)phenoxymethyl]-2,3-dihydroimidazo[2,1-*b*]oxazole (13). Mp 195–197 °C. ^{1}H NMR ($CDCl_3$) δ 1.77 (3H, s), 1.86–2.18 (4H, m), 2.92–3.08 (2H, m), 3.31–3.47 (2H, m), 4.01 (1H, d, J = 5.8 Hz), 4.05 (1H, d, J = 5.8 Hz), 4.18 (1H, d, J = 10.2 Hz), 4.37–4.55 (2H, m), 6.78 (2H, dd, J = 2.2 Hz, 6.8 Hz), 6.84–7.00 (5H, m), 7.20–7.33 (2H, m), 7.55 (1H, s). MS (DI) *m/z* 450 (M^+). Anal. ($C_{24}H_{26}N_4O_5$) C, H, N.

(*R*)-2-{4-[4-(4-Chlorophenoxy)piperidin-1-yl]phenoxymethyl}-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole (14). Mp 183–184 °C. ^{1}H NMR ($CDCl_3$) δ 1.77 (3H, s), 1.84–2.14 (4H, m), 2.92–3.04 (2H, m), 3.29–3.43 (2H, m), 4.01 (1H, d, J = 6.5 Hz), 4.05 (1H, d, J = 6.5 Hz), 4.18 (1H, d, J = 10.2 Hz), 4.33–4.45 (1H, m), 4.49 (1H, d, J = 10.2 Hz), 6.71–6.92 (6H, m), 7.16–7.27 (2H, m), 7.55 (1H, s). MS (DI) *m/z* 484 (M^+). Anal. ($C_{24}H_{25}ClN_4O_5$) C, H, N.

(*R*)-2-{4-[4-(4-Fluorophenoxy)piperidin-1-yl]phenoxymethyl}-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole (15). Mp 191–193 °C. ^{1}H NMR (DMSO-d_6) δ 1.59–1.76 (5H, m), 1.92–2.10 (2H, m), 2.80–2.98 (2H, m), 3.24–3.41 (2H, m), 4.10–4.20 (3H, m), 4.37–4.51 (2H, m), 6.78 (2H, d, J = 8.6 Hz), 6.90 (2H, d, J = 8.6 Hz), 6.92–7.12 (4H, m), 8.16 (1H, s). MS (DI) *m/z* 468 (M^+). Anal. ($C_{24}H_{25}FN_4O_5$) C, H, N.

(*R*)-2-Methyl-2-{4-[4-(4-methylphenoxy)piperidin-1-yl]phenoxymethyl}-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole (16). Mp 199–201 °C (decomp.). ^{1}H NMR ($CDCl_3$) δ 1.77 (3H, s), 1.86–2.14 (4H, m), 2.29 (3H, s), 2.88–3.04 (2H, m), 3.29–3.45 (2H, m), 4.00 (1H, d, J = 6.3 Hz), 4.04 (1H, d, J = 6.3 Hz), 4.17 (1H, d, J = 10.1 Hz), 4.33–4.43 (1H, m), 4.49 (1H, d, J = 10.1 Hz), 6.71–6.92 (6H, m), 7.08 (2H, d, J = 8.4 Hz), 7.55 (1H, s). MS (DI) *m/z* 464 (M^+). Anal. ($C_{25}H_{28}N_4O_5$) C, H, N.

(*R*)-2-{4-[4-(4-Methoxyphenoxy)piperidin-1-yl]phenoxymethyl}-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazole (17). Mp 193–195 °C. ^{1}H NMR ($CDCl_3$) δ 1.77 (3H, s), 1.82–2.14 (4H, m), 2.86–3.02 (2H, m), 3.31–3.45 (2H, m), 3.77 (3H, s), 4.00 (1H, d, J = 6.2 Hz), 4.04 (1H, d, J = 6.2 Hz), 4.18 (1H, d, J = 10.1 Hz), 4.22–4.35 (1H, m), 4.49 (1H, d, J = 10.1 Hz), 6.71–6.92 (8H, m), 7.55 (1H, s). MS (DI) *m/z* 480 (M^+). Anal. ($C_{25}H_{28}N_4O_6$) C, H, N.

(*R*)-2-Methyl-6-nitro-2-{4-[4-(4-trifluoromethylphenoxy)piperidin-1-yl]phenoxymethyl}-2,3-dihydroimidazo[2,1-*b*]oxazole (18). Mp 179–181 °C. ^{1}H NMR ($CDCl_3$) δ 1.77 (3H, s), 1.88–2.20 (4H, m), 2.92–3.10 (2H, m), 3.27–3.43 (2H, m), 4.01 (1H, d, J = 5.8 Hz), 4.05 (1H, d, J = 5.8 Hz), 4.18 (1H, d, J = 10.2 Hz), 4.43–4.57 (2H, m), 6.78 (2H, d, J = 6.8 Hz), 6.90 (2H, d, J = 6.8 Hz), 6.98 (2H, d, J = 8.6 Hz), 7.47–7.60 (3H, m). MS (DI) *m/z* 518 (M^+). Anal. ($C_{25}H_{25}F_3N_4O_5$) C, H, N.

(*R*)-2-Methyl-6-nitro-2-{4-[4-(2-trifluoromethoxyphenoxy)piperidin-1-yl]phenoxymethyl}-2,3-dihydroimidazo[2,1-*b*]oxazole (20). Mp 152–153 °C. ^{1}H NMR ($CDCl_3$) δ 1.77 (3H, s), 1.86–2.19 (4H, m), 2.95–3.12 (2H, m), 3.28–3.44 (2H, m), 4.10 (1H, d, J = 10.2 Hz), 4.04 (1H, d, J = 10.2 Hz), 4.18 (1H, d, J = 10.2 Hz), 4.42–4.56 (2H, m), 6.78 (2H, dd, J = 2.3 Hz, 6.9 Hz), 6.83–7.07 (4H, m), 7.14–7.28 (2H, m), 7.55 (1H, s). MS (DI) *m/z* 534 (M^+). Anal. ($C_{25}H_{25}F_3N_4O_6$) C, H, N.

(*R*)-2-Methyl-6-nitro-2-{4-[4-(3-trifluoromethoxyphenoxy)piperidin-1-yl]phenoxymethyl}-2,3-dihydroimidazo[2,1-*b*]oxazole (21). Mp 184–186 °C. ^{1}H NMR ($CDCl_3$) δ 1.77 (3H, s), 1.88–2.17 (4H, m), 2.96–3.06 (2H, m), 3.31–3.41 (2H, m), 4.02 (1H, d, J = 10.2 Hz), 4.04 (1H, d, J = 10.2 Hz), 4.18 (1H, d, J = 10.2 Hz), 4.40–4.48 (1H, m), 4.50 (1H, d, J = 10.2 Hz), 6.74–6.94 (7H, m), 7.24–7.31 (1H, m), 7.55 (1H, s). MS (DI) *m/z* 534 (M^+). Anal. ($C_{25}H_{25}F_3N_4O_6$) C, H, N.

(*S*)-2-Methyl-6-nitro-2-phenoxymethyl-2,3-dihydroimidazo-[2,1-*b*]oxazole (3g). This compound was obtained by the same procedure as described for **19** using (*S*)-form epoxide **29** instead of **27**. Mp 153–155 °C. ^{1}H NMR ($CDCl_3$) δ 1.79 (3H, s), 4.04 (1H, d, J = 10.2 Hz), 4.09 (1H, d, J = 10.2 Hz), 4.24 (1H, d, J = 10.1 Hz), 4.50 (1H, d, J = 10.1 Hz), 6.83 (2H, dd, J = 2.0 Hz, 8.6 Hz), 7.01 (1H, t, J = 7.4 Hz), 7.20–7.31 (2H, m), 7.56 (1H, s). MS (DI) *m/z* 275 (M^+). Anal. ($C_{13}H_{13}N_3O_4$) C, H, N.

(*R*)-2-Methyl-6-nitro-2-{4-[(1-oxo-thiomorpholin)-4-yl]phenoxymethyl}-2,3-dihydroimidazo[2,1-*b*]oxazole (12). To a solution of **11** (85 mg, 0.23 mmol) in dichloromethane (5 mL) was added 70% *m*-chloroperbenzoic acid (59 mg, 0.24 mmol), and the resulting mixture was stirred at room temperature for 20 min. Sodium thiosulfate aqueous solution (10%, 15 mL) was added to the reaction mixture, which was extracted with dichloromethane (20 mL). The organic layer was washed with saturated sodium hydrogen carbonate aqueous solution (15 mL) and brine, dried over magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure. The residue was recrystallized from methanol–isopropylether to afford **12** (59 mg, 67%) as a colorless crystalline powder. Mp 198–200 °C. ^{1}H NMR ($CDCl_3$) δ 1.77 (3H, s), 2.82–2.96 (4H, m), 3.33–3.45 (2H, m), 3.78–3.90 (2H, m), 4.02 (1H, d, J = 5.5 Hz), 4.06 (1H, d, J = 5.5 Hz), 4.20 (1H, d, J = 10.2 Hz), 4.49 (1H, d, J = 10.2 Hz), 6.80 (2H, d, J = 6.8 Hz), 6.91 (2H, d, J = 6.8 Hz), 7.56 (1H, s). MS (DI) *m/z* 392 (M^+). Anal. ($C_{17}H_{20}N_4O_5S$) C, H, N.

***In Vitro* Antituberculosis Activity.** MICs of test agents against both drug-susceptible and drug-resistant strains of *M. tuberculosis* $H_{37}Rv$ were determined essentially according to the previously reported method.[22] Test drugs were each dissolved in dimethyl sulfoxide (DMSO), and the solutions were diluted serially with DMSO in 2-fold dilutions to the desired concentrations. All strains were grown in Middlebrook 7H9 broth. Stock cultures stored frozen at −80 °C were diluted and adjusted to approximately 10^6 CFU/mL. The bacterial suspension containing about 10^6 CFU/mL was spotted onto 7H11 agar plates containing test drugs using a multipoint inoculator (Sakuma Seisakusho). After cultivation at 37 °C for 14 days, MICs were determined as the minimum concentrations of drugs completely inhibiting visible growth of organism.

***In Vivo* Efficacy for 10 Days.** The basic therapeutic efficacy of test agents was determined in mouse models of acute bacterial infection with *M. tuberculosis* Kurono.[10] In brief, the designated compound was suspended in 5% gum arabic. ICR male mice (Japan

SLC) weighing 20–25 g were infected intravenously with 10^4 CFU of mycobacteria through a caudal tail vein and treated once daily at oral doses of 0.5–50 mg/kg for 10 days ($n = 2$) starting on the day after infection. Animals were sacrificed on day 11, approximately 24 h after administration of the final drug dose. Lungs were aseptically removed and ground in a contained tissue homogenizer. The number of viable organisms was determined by dilution plating on 7H11 agar plate and incubating at 37 °C for 14 days prior to counting. Mean log colony forming units (CFU) reduction values were calculated from mycobacterial counts of test groups relative to untreated controls.

***In Vivo* Efficacy for 28 Days.** The designated compound was suspended in 5% gum arabic. ICR male mice (Japan SLC) weighing 20–25 g were infected intravenously with 10^4 CFU of mycobacteria through a caudal tail vein and treated once daily at various oral doses for 28 days ($n = 6$) starting on the day after infection. Animals were sacrificed on day 29, approximately 24 h after administration of the final drug dose. Lungs were aseptically removed and ground in a contained tissue homogenizer. The number of viable organisms was determined by dilution plating on 7H11 agar plate and incubating at 37 °C for 14 days prior to counting. Bacterial counts of test groups were measured and compared with the counts from untreated controls.

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Supporting Information Available: Result of elemental analysis for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Screening for Novel Antituberculosis Agents that are Effective Against Multidrug Resistant Tuberculosis

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Abstract: The challenges in preventing and controlling tuberculosis are further complicated by the deadly rise of multi-drug-resistant tuberculosis (MDR-TB). Recognizing the seriousness of the situation, we initiated a program to screen new agents that would satisfy these unmet needs and have a favorable safety profile. Mycobacteria are well known for their lipid-rich properties. In *Mycobacterium tuberculosis*, mycolic acid in particular has been established the wall component related to the pathogenesis in the host. There are approximately 250 identified genes related to biosynthesis of the lipid turnover that contain InhA, the main target of isoniazid. Thus, the logical approach for developing a chemotherapy agent against tubercle bacilli included screening compounds that could inhibit the biosyntheses of mycolic acid and that had a novel chemical structure to ensure improved efficacy against MDR-TB. Some of the screening systems established for those purposes and some of the candidates are outlined.

Keywords: Tuberculosis, mycolic acid inhibitor, nitrodehydroimidazooxazole, mycolic acid, BRM test.

INTRODUCTION

Among the various infectious diseases seen worldwide, tuberculosis (TB) remains the disease that inflicts the highest death toll [1]. In 1993, the World Health Organization declared TB to be a global emergency. Reportedly, 32% of the world's population are currently infected with *Mycobacterium tuberculosis*, and each year 8 million people develop active TB and 2 million die as a result [2]. The annual rate of increase in TB incidence is 3% globally, but is as high as 7% in Eastern Europe and higher than 10% in some African countries [3].

The situation is further fuelled by the deadly rise of multidrug-resistant TB (MDR-TB). Epidemics of MDR-TB can spread quickly from city to city, from country to country, and even from continent to continent. A survey of 72 countries suggested that the problem of MDR-TB is more widespread than previously thought and is most likely worsening [4], and some experts estimate that between 185,000 and 415,000 new cases of MDR-TB develop each year [5].

The current TB drug regimen has several disadvantages, and there a number of desirable qualities that a new anti-TB drug should have. The first is shortened treatment duration. Currently, patients require between 6 and 9 months of treatment. This long treatment period leads to lack of compliance, which in turn can be responsible for relapse and the emergence of MDR-TB strains. With MDR-TB posing a major threat, a new TB drug would have to also address this issue. It would also be desirable for the new drugs to have fewer side effects than existing chemotherapeutics, thus providing better overall safety. We have carried out our TB research program with these expectations in mind. When patients present at the clinic, they typically start out with a regimen of 4 drugs – isoniazid, rifampicin, pyrazinamide, and either streptomycin or ethambutol – for the first 2 months in the initial phase, followed by isoniazid and rifampicin for 4 months in the continuation phase. During treatment, the tubercle bacilli are still multiplying, although at a slower rate. During this process, mutant bacilli resistant to one or more of the drugs can emerge and cause the patient to relapse with MDR-TB.

The action mechanisms of the conventional TB drugs illustrated in Fig. (**1**) provide valuable information as to their resistance mechanisms [6,7,8,9,10]. Isoniazid is activated by KatG to form a complex with NAD, which then inhibits InhA [6]. Thus, mutation of either the Kat G gene or the InhA gene produces isoniazid resistance. Since the target of rifampicin is the RNA polymerase beta subunit, mutation in the *rpoB* gene gives rise to rifampicin resistance [7]. Bacteria resistant to both isoniazid and rifampicin, formally designated as MDR-TB, have been increasing and spreading worldwide.

STRATEGY

Recognizing the seriousness of the situation, we initiated a program to screen for new antituberculosis agents that would satisfy these unmet needs. To establish a strategy for our approach, we looked at the process from infection to disease for tuberculosis as illustrated in Fig. (**2**). Infection typically occurs by inhaling airborne *Mycobacterium tuberculosis*. Within the first two years, 5% of newly infected individuals develop the disease. The tubercle bacilli survive as "persisters" in the remaining individuals, and of those about 5% contract active TB at some point later in life, but 90% never develop the disease. These phenomena suggest 2 broad areas of approach: the more common approach is to target the pathogen, which is done through administering antimicrobial agents, and the other is to target the host by stimulating the self-defense system through

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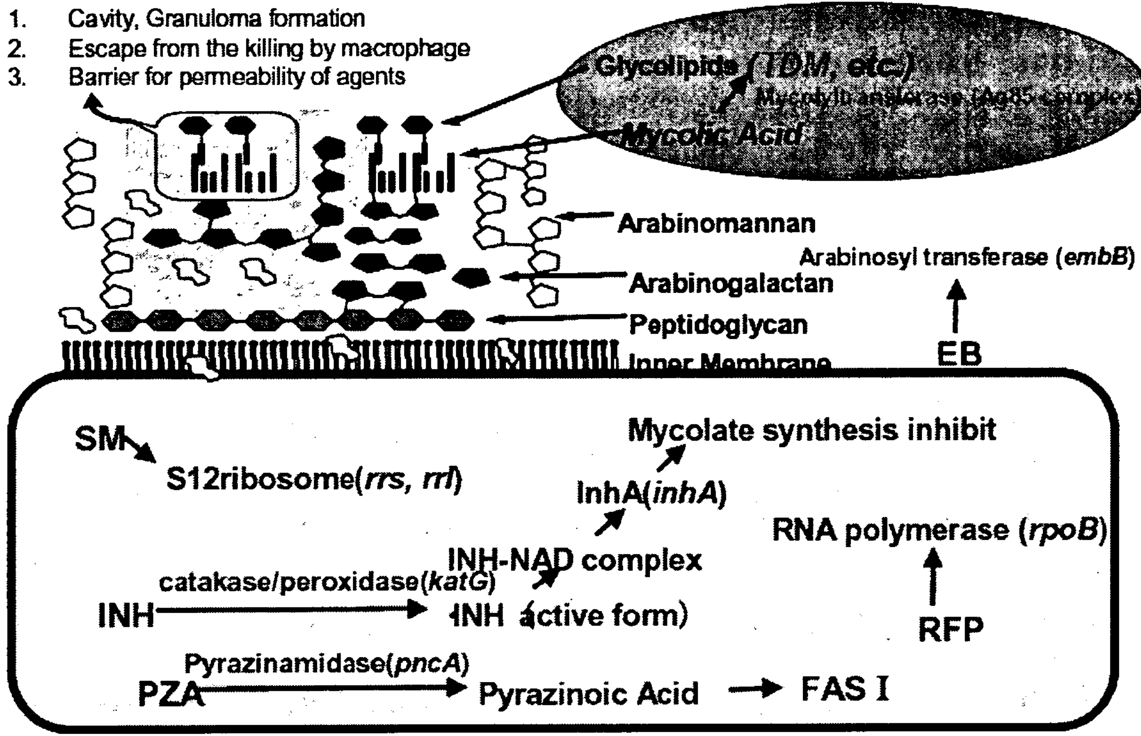


Fig. (1). Mechanism of Action of Conventional TB Drugs.

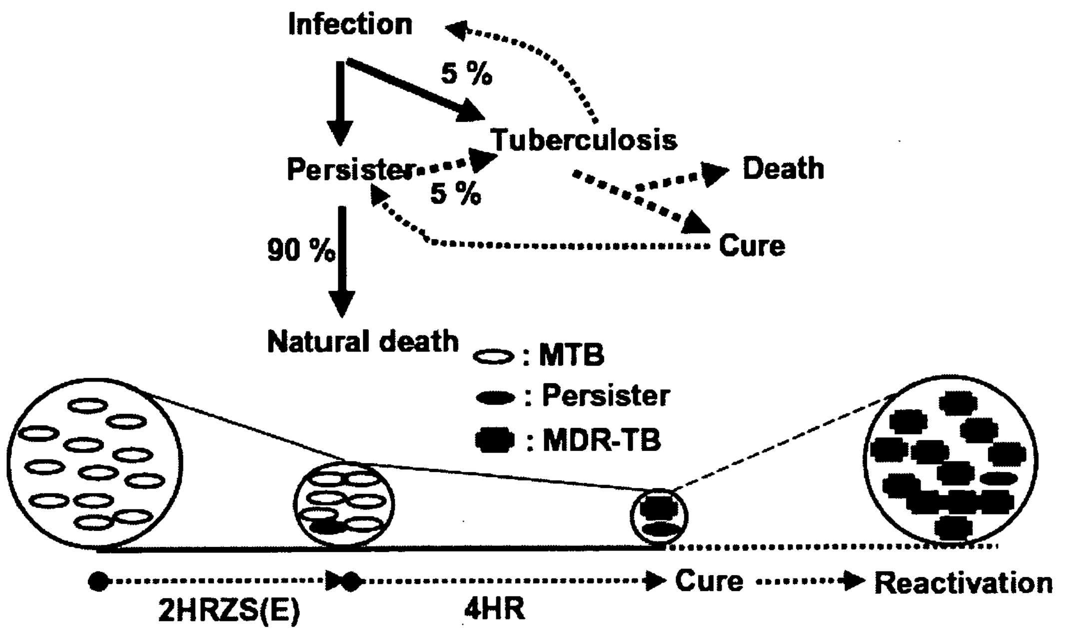


Fig. (2). The Tuberculosis Disease Process and the Emergence of MDR-TB.

vaccines, gene therapy, immunotherapy, and cytokine therapy [11, 12, 13, 14]. An overall approach for TB therapy should include a delicate balance between these two approaches.

For improved efficacy against MDR-TB, we screened for compounds with a new structure and mechanism of action. Mycobacteria are well known to be wax-rich bacteria, and a main component of the wax is mycolic acid, which is present only in mycobacteria and not in gram-positive or gram-negative bacteria or in mammalian cells. Genomic analysis has verified the lipid richness of tubercle bacilli, showing that there are almost 250 distinct enzymes involved in the lipid metabolism of tubercle bacilli [15]. Glycolipids such as trehalose dimycolate are well known as a virulence factors related to cavity and granuloma formation, survival in macrophages, and a barrier to the permeability of drugs [16, 17, 18]. We thus regarded the mycolic acid cell-wall component as an ideal target for a new agent, not only for

exerting antituberculosis activity but also for decreasing the virulence of the bacteria. In view of the important role of mycolic acids in mycobacteria, we screened for inhibitors of mycolic acid synthesis that had potent antibacterial activity.

INHIBITORS OF MYCOLIC ACID BIOSYNTHESIS

Using random screening, we found several compounds that inhibited mycolic acid biosynthesis. Some of the structures of the hit compounds are shown in Fig. (3). Compound A in particular possessed a unique profile. Many derivatives of this compound were then synthesized, and one candidate, OPC-37306, was brought forward as a candidate for further evaluation.

A profile summary of OPC-37306 is shown in Fig. (4). The compound is a newly synthesized dihydrophenazine derivative that showed dose-dependent inhibitory activity against mycolic acid biosynthesis (Fig. (**4b**)) and exhibited potent *in vitro* antituberculosis activity, with minimal inhibitor concentrations (MICs) in the range of 0.1 to 0.2 μg/mL (Fig. (**4c**)). Interestingly, this compound did not kill gram-positive or gram-negative bacteria (data not shown), likely because of its inhibition of mycolic acid biosynthesis, which is specific to mycobacteria. We then examined whether this compound could show potency in an experimental tuberculosis model in mice. The results indicated OPC-37306 to have dose-dependent therapeutic efficacy that was more potent than that of rifampicin.

Other structures of interest that we focused on were urea-type derivatives and dihydroimidazooxazole derivatives. These compounds also showed TB-specific activity that included drug resistant bacteria through an inhibition of mycolic acid biosynthesis. The *in vitro* antituberculosis activity of the urea-type derivatives was potent, with MIC values of around 0.1μg/ml, and these derivatives also did not show any activity against gram-positive and gram-negative bacteria. The urea-type derivatives, however, did not show any therapeutic efficacy in an experimental tuberculosis mouse model. The reason for the lack of efficacy has not been clarified, but it could be due to reasons such as poor absorption and high protein binding.

OPC-J is one of the dihydroimidazooxazole derivatives that inhibited mycolic acid biosynthesis. This compound also demonstrated mycobacteria-specific antituberculosis activity and did not show any activity against gram-positive or gram-negative bacteria. Nitroimidazole-type compounds, which are based on the structure of nitro-heterocyclic compounds including various 5- and 2-nitroimidazoles and 5-nitrofurans, are known to be effective against a number protozoan and bacterial infections in humans and animals [19]. These compounds, however, are also known to commonly possess mutagenicity, which poses a major hurdle to their development. CGI is a nitroimidazole that showed potent antituberculosis activity but was not developed because of its mutagenicity [20, 21].

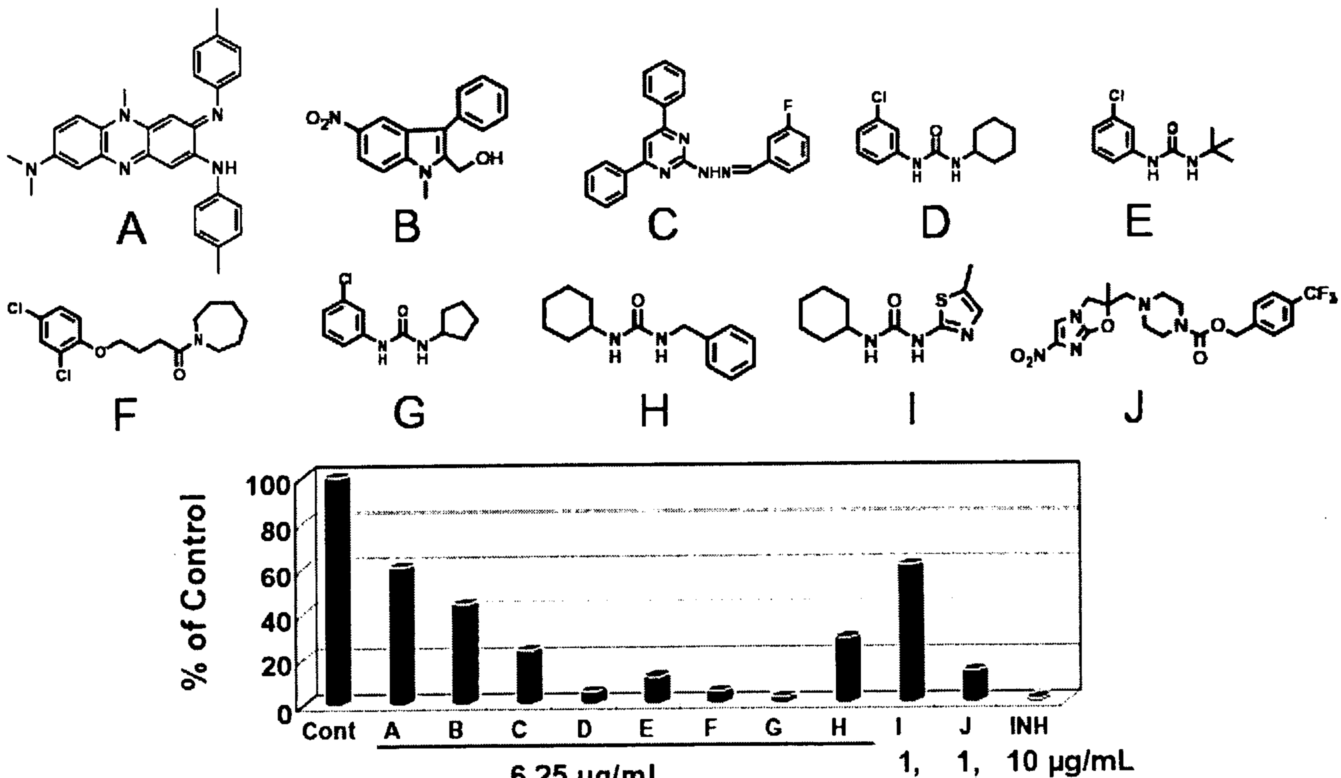
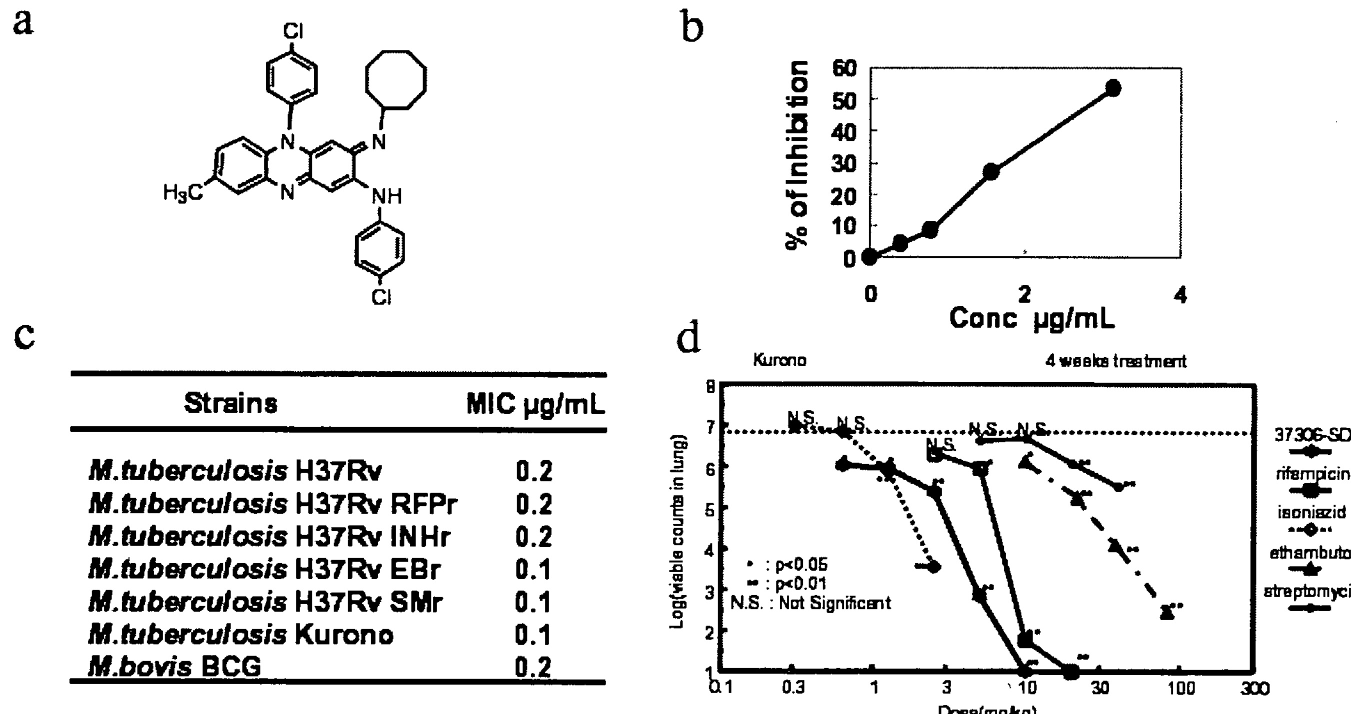


Fig. (3). Inhibitors of Mycolic Acid Biosynthesis: The compound A, B, C, D, E, F, G, H, I, and J showing the inhibitory activity against mycolic acid biosynthesis in Mycobacteria was randomly selected and compared with that of INH, a well-known inhibitor of mycolic acid synthesis. ^{14}C-labeled acetic acid was incorporated to mycolic acid by incubation with *M. bovis* BCG cell cultures in the presence of 6.25 μg/ml of A, B, C, D, E, F, G, or H, or 1μg/ml of I, J, or INH. ^{14}C-labeled fatty acid and mycolic acid s were detected using thin-layer chromatography, and analyzed by BAS-2500 (Fujifilm). The radioactivity of each fatty acid and mycolic acid was calculated using PhotoStimulated Luminescence, expressed as the percentage of incorporation in untreated controls.



Strains	MIC µg/mL
M.tuberculosis H37Rv	0.2
M.tuberculosis H37Rv RFPr	0.2
M.tuberculosis H37Rv INHr	0.2
M.tuberculosis H37Rv EBr	0.1
M.tuberculosis H37Rv SMr	0.1
M.tuberculosis Kurono	0.1
M.bovis BCG	0.2

Fig. (4). Antituberculosis Profile of OPC-37306

(a) Structure of OPC-37306. (b) Inhibitory activity against mycolic acid biosynthesis; ^{14}C-labeled acetic acid was incorporated to mycolic acid by incubation with Mycobacteria cell cultures in the presence of 0.39, 0.78, 1.56, 3.13 µg/ml of OPC-37306. ^{14}C-labeled fatty acid and mycolic acids were detected using thin-layer chromatography, and analyzed by BAS-2500 (Fujifilm). The radioactivity of mycolic acids were calculated using PhotoStimulated Luminescence, expressed as the percentage of incorporation in untreated controls. Percent of inhibitory activity was plotted in the figure. (c) Susceptibility test; The *in vitro* antimycobacterial activity of the OPC-37306 against *M. tuberculosis* H37Rv, H37Rv RFPr (resistance to RFP), H37Rv INHr (resistance to INH), H37Rv EBr (resistance to EB), H37Rv SMr (resistance to SM), Kurono, and *M.bovis* BCG were examined by an agar dilution method using 7H11 agar plates. The MIC was determined as the lowest concentration that inhibited visible growth of the organism on the agar medium after incubation. (d) Therapeutic efficacy; ICR mice (n=5) were inoculated intravenously with 0.2 ml of suspension containing *M. tuberculosis* Kurono at 10^4 CFU/ml. The designated compound (OPC-37306: 0.625-10 mg/kg, RFP: 2.5-20 mg/kg, INH: 0.313-1.56 mg/kg, EB: 10-80 mg/kg, SM: 5-40 mg/kg mg/kg) suspended in 5% gum arabic or saline for SM was then administered orally once daily for 4 weeks. At the end of the treatment period, the mice were euthanized (exsanguination through the abdominal inferior vena cava) under ether anesthesia, and the lung was aseptically excised. A lung homogenate for each mouse was prepared by pestling the lung evenly with a glass homogenizer after adding sterile distilled water to the excised lungs, and the homogenate was then diluted further with distilled water. A smear plate for each lung homogenate was then prepared by spreading 0.1 ml of each diluted solution on a 7H11 agar plate using a spreader. All plates after spreading the homogenate solution were incubated at 37YC and counted for formed colonies after 14 days. Statistical analysis was conducted using SAS software (SAS Institute Japan, R. 8.1) on the number of viable bacteria in the lung of mice surviving until necropsy on the 29th day after inoculation. The significance level of the test was set at 5%. A test for dose dependency was performed using linear regression analysis based on log-transformed values of the viable bacterial counts in the lung. When dose dependency was confirmed, the Williams test (lower-tailed) was subsequently performed, and when dose dependency was not confirmed, the Dunnett's test (two-tailed) was subsequently performed against each of the control groups. The care and handling of the animals was in accordance with "Guidelines for Animal Care and Use in Otsuka Pharmaceutical Co., Ltd."

Investigation into the mutagenicity of these compounds has revealed that the mutations occur as a result of damage to the DNA by O_2 and NO radicals that are formed during metabolism of the nitro residue. One seemingly logical means to avoid the mutations would be to prevent the nitrogen from being metabolized, but there is no theoretical way to modify the structure in such a manner.

To overcome the hurdles associated with mutagenicity, we looked at the possibility of avoiding the problem by testing all of the nitroimidazole derivatives in our library using the Ames test. The strains used in the Ames test were *Salmonella typhimurium* TA98 (frame shift), *Salmonella typhimurium* TA100 (GC base pair), *Salmonella typhimurium* TA1535(GC base pair), *Salmonella typhimurium* TA1537(GC base pair), and *Escherichia coli* wp2 (frame shift). Using this method we observed that the number of revertant colonies formed on the plates varied with the compound structures. Some of these data are summarized in Fig. (5). These findings appear to indicate that the mutagenic potency of the nitrodihydroimidazooxazole-based compounds is dependent on the functional groups attached to the core structure. Most of the nitroimidazole derivatives we tested showed mutagenicity,

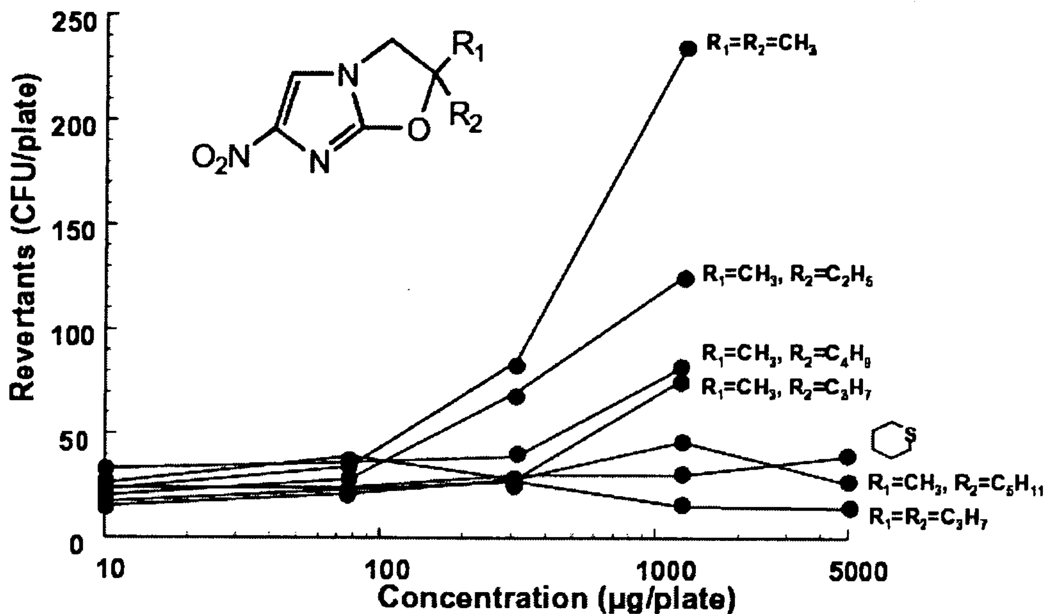


Fig. (5). Ames Test for the Dihydroimidazooxazole Derivatives: The BRM test was performed in accordance with OECD Guideline 471 using *Salmonella tyiphimurium* TA98. Each bacterial strain was precultured at 37°C for 18 h using a nutrient broth (Nissui). After adjustment to 2.4 at OD660 nm, each bacterial suspension was added to a test tube containing the designated compound. After a 20-minute incubation at 37°C, top agar was added to each test tube and the contents were poured into minimum essential medium (Oriental Yeast). The number of revertants was counted 48 h after incubation at 37°C.

Table 1. Relationship between Ames Test and MIC Values For Selected Dihyrdoimidazooxazoles

	Ames-	MIC mg/mL
	67%	0.2 – 25
	100%	0.39 – 1.56
	94%	0.39 -> 100
	83%	0.012 – 3.13

(Table 1) Contd....

	Ames-	MIC mg/mL
	100%	0.39
	98%	<0.006 – 1.56
	58%	0.012 – 1.56

Ames test: BRM test was performed in accordance with OECD Guideline 471 using *Salmonella tyiphimurium* TA98, TA100, TA1535, TA1537, and TA102 or *Escherichia coli* WP2*uvrA*. The data are expressed as Ames-negative percentage among the tested compounds.
Susceptibility test: The in vitro antimycobacterial activity of the compounds against *Mycobacterium tuberculosis* H37Rv were examined and evaluated by an agar dilution method using 7H11 agar plates. The MIC was determined as the lowest concentration that inhibited visible growth of the organism on the agar medium after incubation. The data are shown as the range of the MIC among each structural type.

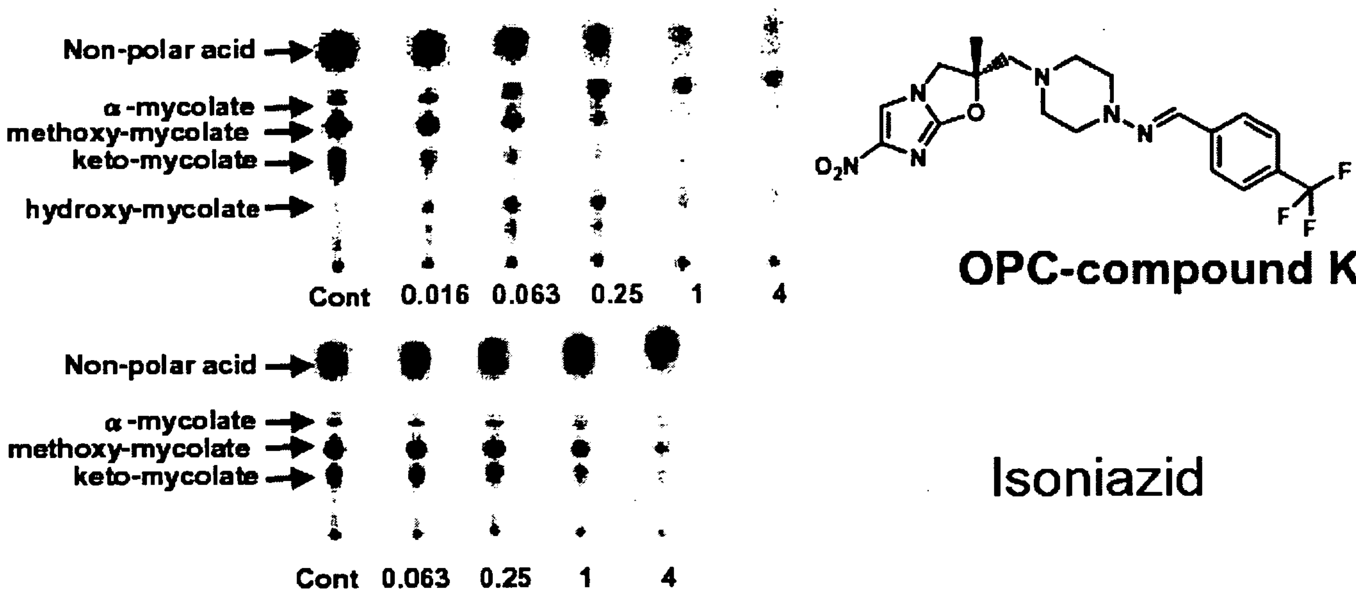


Fig. (6). Inhibitory Activity of Compound K and Isoniazid on the Biosynthesis of Mycolic Acids in the BCG Strain: The inhibitory activity of the compound K against mycolic acid synthesis in *M. bovis* BCG was determined and compared with that of INH, a well-known inhibitor of mycolic acid synthesis. ^{14}C-labeled acetic acid was incorporated to mycolic acid by incubation with *M. bovis* BCG cell cultures in the presence of compound K or INH as a reference. ^{14}C-labeled fatty acid and mycolic acid subclasses were detected using thin-layer chromatography, and analyzed by BAS-2500 (Fujifilm).

particularly those with dimethyl residues. However, 22% of the tested compounds did not show mutagenicity, providing a hint to help us overcome the hurdles associated with mutagenicity.

We then introduced various types of residues to the dihydroimidazooxazole structures and evaluated their mutagenicity and *in vitro* antituberculosis activities. Carbamate-type residues overall were 67% Ames negative, but those

residues that exchanged nitrogen for oxygen were 100% Ames negative. Introduction of spiral-, piperazine-, heterocylic-, phenoxy-, and piperidine-type residues produced respective Ames negativity results of 94%, 83%, 100%, 98%, and 58%. In terms of antituberculosis activity, piperazine-, piperidine-, and phenoxy-type residues all showed high potency, with phenoxy-type residues producing the best overall results. A summary of the results are shown in Table (**1**).

Our optimized imidazooxazole compound OPC-K inhibited the biosynthesis of mycolic acid dose dependently, as also shown for isoniazid. However, while isoniazid inhibited the synthesis of alpha, methoxy, and keto mycolic acids, OPC-K only affected the synthesis of methoxy and keto mycolic acids (Fig. **6**). OPC-K showed potent *in vitro* antituberculosis activity against standard TB strains,, with MICs ranging from 0.025 to 0.39μg/ml (Table **2**). The *in vivo* therapeutic efficacy of OPC-K was also evaluated, and the results showed OPC-K to be more potent than rifampicin (Fig. (**7**)).

Table 2. Minimum Inhibitory Concentrations of Compound K Against *M.tuberculosis*

Strains	MIC mg/mL
M. tuberculosis H37Rv	0.39
M. tuberculosis H37Rv RFPr	0.025
M. tuberculosis H37Rv INHr	0.2
M. tuberculosis H37Rv EBr	0.2
M. tuberculosis H37Rv SMr	0.39
M. tuberculosis Kurono	0.2
M. bovis BCG	0.05

Susceptibility test: The *in vitro* antimycobacterial activity of the compound K against *M. tuberculosis* H37Rv, H37Rv RFPr (resistance to RFP), H37Rv INHr (resistance to INH), H37Rv EBr (resistance to EB), H37Rv SMr (resistance to SM), Kurono, and *M.bovis* BCG were examined by an agar dilution method using 7H11 agar plates. The MIC was determined as the lowest concentration that inhibited visible growth of the organism on the agar medium after incubation.

The relationship between the inhibition of mycolic acid subclasses and the MIC values was evaluated to confirm whether the target of these compound series is the metabolism of biosynthesis of mycolic acid. The results indicated that antituberculosis activities were well correlated to the inhibitory activity of methoxy and keto mycolic acid biosynthesis but not for alpha-mycolic acid or fatty acid (Fig. (**8**)). We thus concluded that a mechanism involved in the antituberculosis activity of the compound deals with the inhibition of mycolic acid.

CONCLUSIONS

We successfully synthesized nitro-imidazooxazole derivatives that were free from mutagenicity by introducing unique residues and found no correlation between

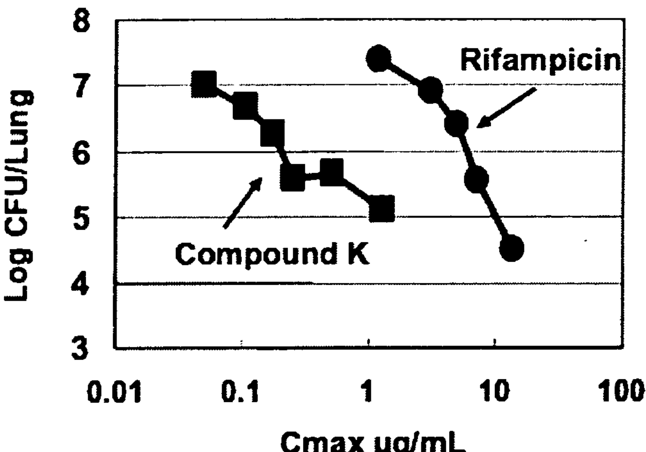


Fig. (7). Therapeutic Efficacies of Compound K and Rifampicin in an Experimental Tuberculosis Mouse Model: Therapeutic efficacy; ICR mice (n=5) were inoculated intravenously with 0.2 ml of suspension containing *M. tuberculosis* Kurono at 10^4 CFU/ml. The designated compound (Compound K: 0.039, 0.078, 0.156, 0.313, 0.625, and 1.25 mg/kg, RFP: 1.25, 2.5, 5, 10, and 20 mg/kg) suspended in 5% gum arabic was then administered orally once daily for 4 weeks. At the end of the treatment period, the mice were euthanized (exsanguination through the abdominal inferior vena cava) under ether anesthesia, and the lung was aseptically excised. A lung homogenate for each mouse was prepared by pestling the lung evenly with a glass homogenizer after adding sterile distilled water to the excised lungs, and the homogenate was then diluted further with distilled water. A smear plate for each lung homogenate was then prepared by spreading 0.1 ml of each diluted solution on a 7H11 agar plate using a spreader. All plates after spreading the homogenate solution were incubated at 37YC and counted for formed colonies after 14 days. The care and handling of the animals was in accordance with "Guidelines for Animal Care and Use in Otsuka Pharmaceutical Co., Ltd."

mutageneticity and antituberculosis potency (Fig. (**9**)). Based on these results, we were convinced that dihydroimidazooxazole derivatives could be a candidate for the development of a new-class of TB agents effective against MDR-TB.

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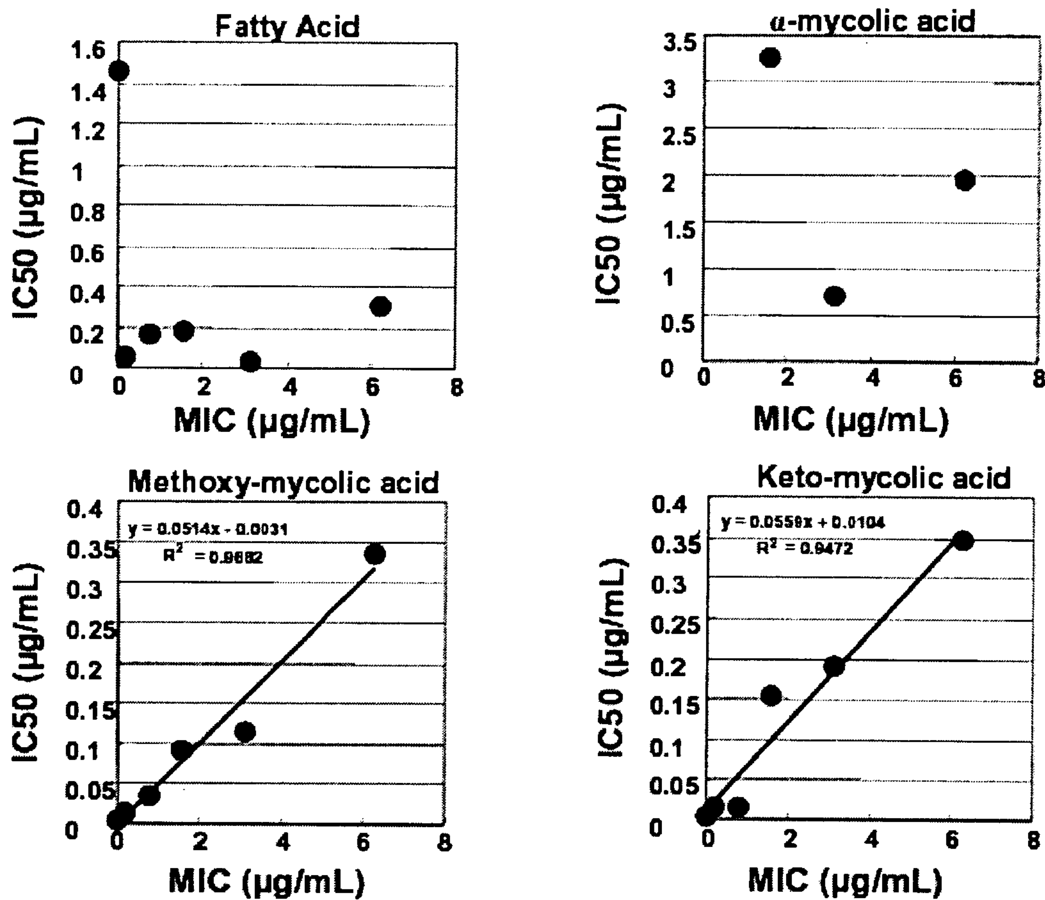

Fig. (8). Relationship Between Mycolic Acid Biosynthesis and MIC Values
Compounds, which show a different MIC value, were randomly selected, and IC_{50} value (concentration required to inhibit by 50%) of each compound was calculated by linear regression analysis based on an inhibitory activity of each mycolic acid subclass and fatty acid biosynthesis. And these values were plotted on the figures to analysis a relationship between the inhibitory activity against mycolic acid biosynthesis and the MIC value.

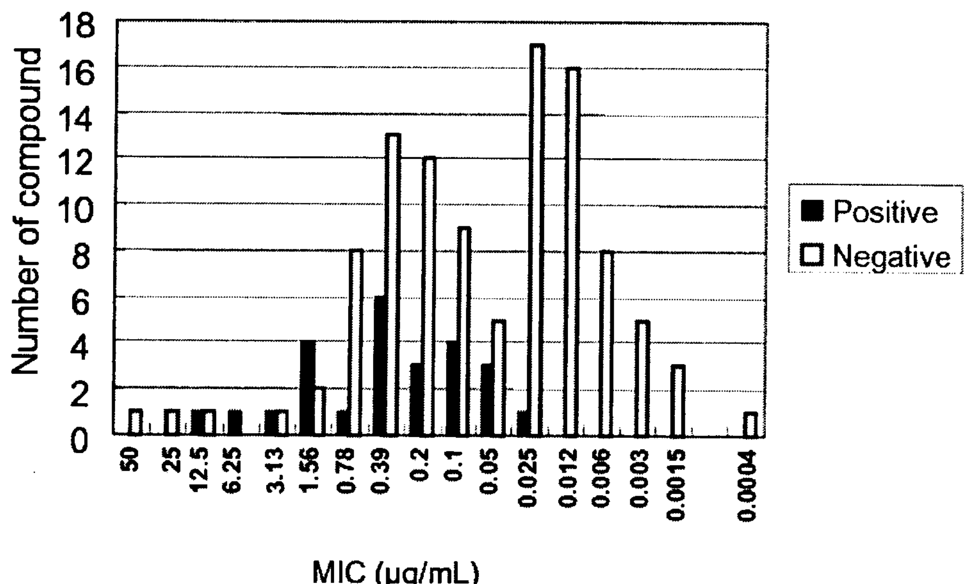

Fig. (9). Relationship Between Mutagenicity and Antimycobial ActivityInsert here footnotes for Figure, showing experimental conditions BRM test was performed in accordance with OECD Guideline 471 using *Salmonella tyiphimurium* TA98, TA100, TA1535, TA1537, and TA102 or Escherichia coli WP2uvrA in the absence and presence of S9-mix.The number of mutagenic or non-mutagenic compounds which showed respective MIC values was plotted.

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特集 多剤耐性結核の現状と今後

7．新たな抗結核薬開発の必要性と世界の現状

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特 集 多剤耐性結核の現状と今後

7．新たな抗結核薬開発の必要性と世界の現状

松本 真*

世界の総人口の約３分の１に相当する約 20 億人の人が結核菌に感染している。また近年，ヒト免疫不全ウイルス（以下 HIV）/ 後天性免疫不全症候群（以下 AIDS）の世界的な蔓延に伴い，結核患者数も増加に転じ，過去のどの時代よりも多い人が結核に感染している状態となっている。また，多剤耐性結核菌の出現が治療を難治化し，交通網の発達により，世界中の人々が多剤耐性結核菌を含む結核菌の感染の危機に直面している。このような現状を解決するために，新しい結核治療薬の開発が切望されている。現在 40 年ぶりの新規抗結核薬となる OPC-67683 および TMC-207 が，臨床試験段階に入っている。

Key Words：結核症／新規抗結核薬／OPC-67683／TMC-207／MFLX

Ⅰ 世界の結核の現状

世界で知られる種々な感染症の中で，結核は死亡者数第１位の単独の感染症として今も尚猛威を振るっている[1]。1993 年に WHO は，結核緊急事態宣言を発表した。世界の人口の 32%（約 20 億人）が結核菌に感染しており，そのうち毎年 800 万人が伝染性の活動性結核を発病し，200 万人が死亡していると報告されている[2]。結核発病の年間増加率は，世界で３%であるが，東欧では７%程度と高く，アフリカ諸国では 10%以上に達するとされている[3]。

日本においては，かつて「国民病」といわれた結核は，ここ 30 年間に激減したが，1985 年以降感染の減少率は鈍化し，1997 年には新規登録患者数，罹患率等が増加に転じ，1999 年に厚生大臣による「結核緊急事態宣言」が出されるに至っている。現在は再び減少に転じているが，現在も年間３万人以上の新規発病患者が報告されている。

現在の結核の問題は，大きく多剤耐性菌の問題，ヒト免疫不全ウイルス（HIV）/ 後天性免疫不全症候群（AIDS）の世界的な蔓延に伴う結核患者の増加の問題が挙げられる。

多剤耐性結核（MDR-TB）は，主には不完全な治療により発生し，その発生は結核治療において致命的な状況をもたらしている。一旦発生した多剤耐性菌は，一昔前とは異なり，交通手段の発達，それによる人々の移動の増加に伴い，町から町へ，国から国へ，更には大陸から大陸へ，すばやく広がる。72 カ国で調査した結果[4]，多剤耐性結核は以前考えられていた以上に蔓延し，悪化の一途を辿っている。専門家らは，世界で年に 18.5 ～ 41.5 万の新規の多剤耐性結核が発現するであろうと推定している[5]。日本では，治療中の活動性感染者の中で約 2,500 人が多剤耐性結核に感染しており，適切な治療法がないのが現状である。

今日，結核は過去 15 年以上前から蔓延している HIV/AIDS との致死的な相乗作用によって複雑化されている。結核と HIV との二重感染は，潜在性結核感染を活動性結核に転換する最も有力な危険因子となる。一方で，結核菌感染は HIV の病態経過を加速させ AIDS を発病させる。現在，世界

Current global situation of TB and need for new drugs

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で1,200万人の患者が二重感染しており，結核はHIV陽性患者の間で主な死亡原因となっている。潜在性結核感染の患者がHIVに二重感染すると，活動性結核に進展する危険性が30～50倍増加する[6]。

結核は，かつて撲滅されるだろうと考えられていた。しかし，近年巻き返しが起こり，歴史上のどの時点よりも多くの人々が感染している。もし，この傾向が続くとすると20年後には，10億人もが新規に感染し，3,600万人は死亡するであろうと考えられている[6]。

II　今日の化学療法の現状

今日の結核の化学療法は，リファンピシンとピラジナミドの登場した1970年代まで遡る。British Medical Research Councilによって確立された現在の標準治療は，初め2カ月間のリファンピシン，イソニアジド，エタンブトール，ピラジナミドの併用による初期治療と，それに続く4カ月間のリファンピシンとイソニアジドによる維持治療からなっている。WHOはDOTS（directly observed treatment，short course：直接監視下短期化学療法）療法の中で，この治療法を推奨している。しかし，6カ月の治療期間は相当長い期間であり，服薬し続けることは容易なことではない。特に発展途上国においては，コンプライアンスが悪く，不完全な治療により薬剤耐性の問題が深刻化している。多剤耐性結核に対する治療には十分な効力がなく，且つ高価で重篤な副作用のある二次選択薬を使った組み合わせで治療しなければならないのが現状である。

過去40年間，政府機関や企業が新しい抗結核薬の開発にあまり興味を示さなかった結果として，新しい作用機序を持った新しい抗結核薬は1966年のリファンピシン以来上市されていない。したがって，今日WHOを初め世界結核薬開発同盟（Global Alliance for TB Drug Development）は，次の4つを可能にする新しい化学療法剤の登場を求めている。（1）総治療期間の短縮，（2）多剤耐性結核に対する有効性の改善，（3）潜在性結核感染を標的とする，（4）投与方法等を改良しコンプライアンスを向上させる。

III　現在開発途上にある抗結核薬

前臨床段階の化合物や初期の化合物を含めると数多くの化合物が報告されているが，今回は具体的に臨床試験に移行している化合物について紹介してみたい。

1．モキシフロキサシン（MFLX）

モキシフロキサシン（MFLX）（図1）は，グラム陽性菌およびグラム陰性菌を主体とする感染症の治療薬として開発され，幅広い抗菌スペクトラムを有する薬剤である。従来からニューキノロンには抗結核活性があることはわかっていたが，*in vivo*での効力は低く，臨床的有効性についても賛否両論であった。

1990年代後半にJ Grossetら[7]およびWR Bishaiら[8]のグループにより，MFLXは従来のキノロンに比較し，*in vitro*および*in vivo*共に優れた抗結核活性があることが示された。その後，同研究グループより実験的マウス結核症モデルを用い

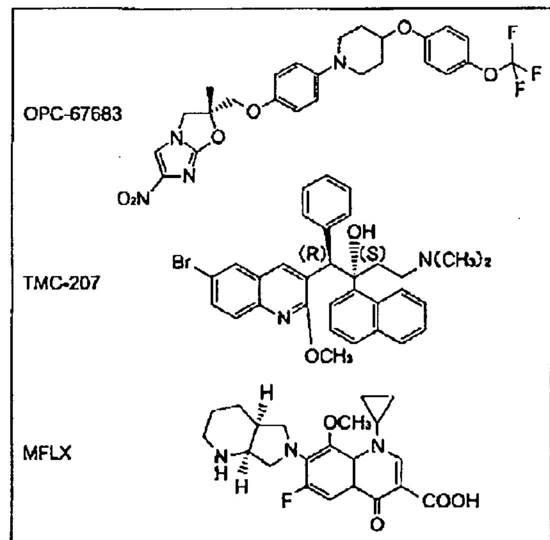

図1　現在開発中の主な抗結核薬

MFLXはグラム陽性菌およびグラム陰性菌を主体とする感染症の治療薬として開発され，幅広い抗菌スペクトラムを有する薬剤である。OPC-67683は大塚製薬株式会社微生物研究所にて新規に見出された，ニトロイミダゾオキサゾール誘導体である。TMC-207はヤンセンファーマ株式会社により見出された，ジアリルキノリン誘導体である。

たMFLXと既存薬剤との組み合わせに関する研究が行われ，イソニアジドにMFLXを置き換えることで，1カ月から2カ月の短期化を達成できることを示した（表1）[9]。また，結核治療において再発という問題を考えることが重要であるが，同グループにより，再発率についてもMFLXをイソニアジドに置き換えることで，既存薬剤より短期治療であっても既存薬と治療機関と同等以上の再発率であることが示された（表2）[10]。

これらの結果を基に，米国CDC（Centers for Disease Control and Prevention）を中心として立ち上げられたコンソーシアム，Tuberculosis Trials Consortium（TBTC）がMFLXを含むレジメについて臨床試験を行った。マウスを用いた実験結果からはMFLXをイソニアジドに置き換えたレジメが最も優れた成績を示していたが，イソ

表1　肺内生菌数の平均対数値

治療レジメ	治療期間（月） 0	2	3	4	5	6
A. Infected, untreated	7.80±0.21	7.63±0.41	7.24±0.41	8.06±0.81	7.68±0.51	7.34±0.50
B.2RHZ/4RH		3.36±0.32	1.89±0.40	0.39±0.32	0	0
C.2RHMZ/4RHM		2.74±0.48	1.26±0.33	−0.29±0.58	0	0
D.2RHM/4RH		3.70±0.25	2.11±0.26	1.32±0.37	−0.10±0.57	−0.65±0.32
E.2RMZ/4RM		0.90±0.58	−0.47±0.43	0	0	0
F.2MHZ/4MH		4.21±0.25	3.67±0.12	3.39±0.18	2.63±0.35	1.98±0.22

（文献9より抜粋）

結核菌H37Rv株をマウスにエアロゾル感染させ，19日目から治療を開始し，経時的な肺内生菌数の計測を行っている。治療は，非治療群（A群），標準療法（B群）に対し，モキシフロキサシンを標準療法に追加した群（C群），標準療法使用薬剤の何れか1剤と置き換えた群（D，E，F群）を設定し，6カ月までの治療効果を比較検討している。

H＝イソニアジド：25mg/kg，M＝モキシフロサシン：100mg/kg，R＝リファンピシン：10mg/kg，
Z＝ピラジナミド：150mg/kg

表2　治療終了後の再発率

	治療期間（月） 3		4		5		6
治療レジメ	数	CFU分布*	数	CFU分布*	数	CFU分布*	数
2RHZ/4RH	11/12	2.7～3.7	5/12	2.1～3.4	1/16	0.6	0/12
1RMZ/4RM	4/12	1.7～3.0	0/12	0	0/12	0	Not done
2RMZ/3RM	2/12	0.3～2.9	0/12	0	0/13	0	Not done
5RMZ	4/12	3.4～3.7	0/12	0	0/12	0	Not done

（文献10より抜粋）

結核菌H37Rv株をマウスにエアロゾル感染させ，19日目から治療を開始し，3カ月，4カ月，5カ月および6カ月の期間治療を行い，治療終了後3カ月後のマウス肺内生菌の有無と菌数を測定することで，再発率を算出している。

H＝イソニアジド：25mg/kg，M＝モキシフロサシン：100mg/kg，R＝リファンピシン：10mg/kg，
Z＝ピラジナミド：150mg/kg
＊再発したマウスの肺内菌数の対数値

ニアジドは第1選択薬としてFirst-lineで使用される薬剤であり，本薬剤を治療レジメから外すことに倫理的抵抗があったためか，MFLXを含む新しいレジメ試験はエタンブトールとの置き換えにより，2カ月間の投与でエタンブトール群との比較試験が行われた。その結果，4週目および6週目の喀痰中の結核菌陰性率は，MFLXを含むレジメにおいて有意に高かったが，期待に反して2カ月目の陰性化率においては，両レジメとも同等の結果であった[11]。現在，実験的マウス結核症モデルにおいて最も強い効力が確認されたMFLX，リファンピシンおよびピラジナミドの3剤により臨床試験が行われており，その結果が待たれるところである。

また，MFLXの後に開発されたガチフロキサシンについても，現在WHOの主導のもと臨床試験が行われているが，詳細なデータは発表されていない。

2．OPC-67683

OPC-67683（図1）は大塚製薬株式会社微生物研究所にて新規に見出された，ニトロイミダゾオキサゾール誘導体である。*in vitro*の活性は現在開発中の化合物を含め全ての抗結核薬の中で最も強い活性を示している。本薬剤の作用機作の1つとして，サブクラスミコール酸であるメトキシおよびケトミコール酸の生合成を阻害することが判明しており，既存の抗結核薬とは異なった新しい作用メカニズムにより効力を発揮する。これら本薬剤の特徴を簡単に示す[12]。

1）結核菌に対する*in vitro*の効力

臨床分離株（67株）に対するMIC_{90}値は0.024 μg/mL（範囲：0.003～0.024 μg/mL）を示し，既存薬とは交叉耐性を示さないことから，多剤耐性結核菌にも有効と考えられる（表3）。また，既存の抗結核薬と拮抗作用がないことが確認されている。これは，単剤での治療が難しい結核治療を考えると，既存薬との組み合わせで，新しいレジメが構築できる可能性を示すものである。また，本化合物はグラム陽性菌，グラム陰性菌，主な腸内細菌などに対しては全く活性を示さず，結核菌を主体とする抗酸菌特異的な活性を示す。

2）細胞内結核菌に対する効力

結核菌は細胞内に寄生し増殖することが知られており，また既存薬剤の細胞内結核菌に対する効力が十分ではないことがいわれている。したがって細胞内結核菌に効力を示すことは，結核治療薬の1つの大きなプロファイルとして要求されるところである。OPC-67683の細胞内結核菌に対する活性をヒト単球由来のTHP-1細胞をマクロファージに分化誘導し，結核菌標準株である

表3 OPC-67683の既存薬剤感受性株および耐性株に対する最小発育阻止濃度

臨床分離株（試験菌株数）		最小発育阻止濃度（μg/mL）	
		MIC_{90}*	95%信頼区間
RFP感受性株	(31)	0.01248	0.01097～0.01535
RFP耐性株	(36)	0.01221	0.01050～0.01583
INH感受性株	(31)	0.01194	0.01054～0.01452
INH耐性株	(36)	0.01279	0.01094～0.01679
EB感受性株	(56)	0.01213	0.01081～0.01440
EB耐性株	(11)	0.01341	0.01073～0.02450
SM感受性株	(49)	0.01203	0.01077～0.01416
SM耐性株	(18)	0.01340	0.01068～0.02298

（文献12より抜粋）

臨床分離の67菌株に対する最小発育阻止濃度の範囲は，0.006～0.024 μg/mLであった。
* MIC_{90}値はプロビット法により算出した。
RFP：リファピシン，INH：イソニアジド，EB：エタンブトール，SM：ストレプトマイシン

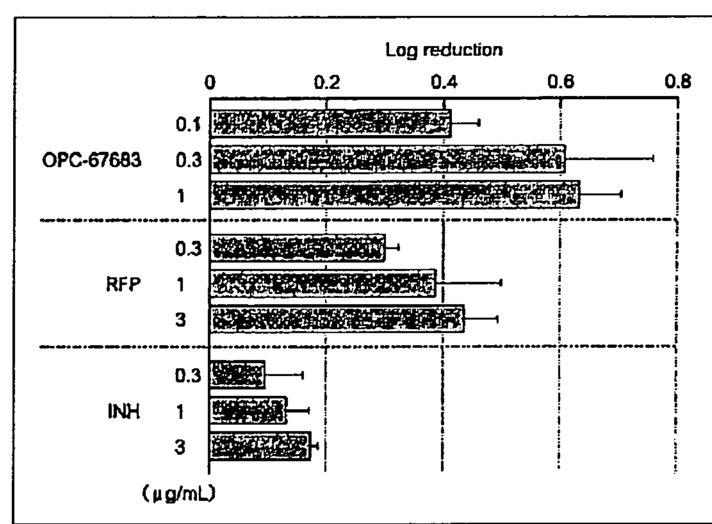

図2 短時間接触における細胞内結核菌に対する殺菌力

ヒト単球由来THP-1細胞をマクロファージに分化誘導し，結核菌H37Rv株を感染させた後，OPC-67683，RFPまたはINHを4時間接触させ，68時間後の細胞内生菌数を求め，コントロールからの対数減少度で効力を表した。

（文献12より抜粋）

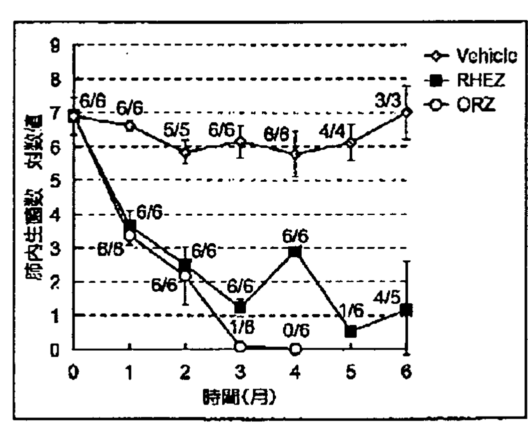

図3 OPC-67683を含む新しいレジメと既存薬標準療法との治療効果の比較試験成績

結核菌Kurono株を気管内接種し，慢性結核症モデルを作製し，2RHEZ/4RHと2ORZ/2ORのスケジュールで連日強制経口投与を行い，肺内生菌数をモニタリングすることで，OPC-67683を含む新しいレジメの効力を，標準療法と比較した。

O：OPC-67683；2.5mg/kg，R：リファンピシン；5mg/kg，H：イソニアジド；10mg/kg，E：エタンブトール；100mg/kg，Z：ピラジナミド；100mg/kg

（文献12より抜粋）

H37Rv株を感染させ，効力を測定した結果，短時間の作用（4時間，0.1 μg/mL）においても強い効力を示すことがわかっている（図2）。

3）実験モデルでの薬物動態

マウスに0.156～40 mg/kgまで経口投与し，経時的に血中および肺内濃度を測定した結果，用量相関的に吸収が確認され，0.625 mg/kg投与時に約100 ng/mLのC_{max}（最高血中濃度）が得られた。また，肺内へは血中の約3倍移行することが確認され，ほとんどの結核症の発症の場が肺であることを考えると望ましいプロファイルであると考えられる。

4）実験的マウス結核症モデル

結核菌Kurono株を感染させ，結核症が慢性化した4週目より治療を開始し，単剤での治療効果を確認したところ，0.313 mg/kg以上の投与量において有意に治療開始時の菌量を減少させている。同実験において，既存薬の同様の効果を発揮する用量は，OPC-67683より8倍～500倍高い用量を必要とする。

また，同モデルにおいて現在の4剤併用療法（リファンピシン，イソニアジド，エタンブトール，およびピラジナミドの4剤併用の2カ月強化療法と，リファンピシンとイソニアジドの2剤併用の4カ月の維持療法）を対照に，より強い効力を発揮する併用レジメを検討した結果，OPC-67683，リファンピシン，ピラジナミドの3剤2カ月強化療法にOPC-67683とリファンピシンの2カ月維持療法において，既存の標準療法を少なくとも2カ月以上短期化できることが確認され