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Activity of AT-2266 Compared with Those of Norfloxacin, Pipemidic Acid, Nalidixic Acid, and Gentamicin Against Various Experimental Infections in Mice

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AT-2266 (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid) showed marked activity in vivo when administered orally to mice bearing systemic, pulmonary, dermal, or urinary tract infections due to variety of organisms. The activity of AT-2266 was uniformly higher than those of norfloxacin, pipemidic acid, and nalidixic acid against all of the infections. The activity of AT-2266 administered orally was almost comparable to that of gentamicin administered subcutaneously against urinary tract infections due to gram-negative organisms but was generally lower against other infections. AT-2266 exhibited significant activity against infections due to gentamicin-resistant and nalidixic acid-resistant organisms.

AT-2266 (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid) is a pyridonecarboxylic acid derivative with broad and potent antibacterial activity (16). It is well absorbed by the oral route, distributed to tissues at concentrations higher than plasma levels, and excreted into urine in large quantities (12). Preliminary in vivo studies have shown that AT-2266 administered orally is generally more effective than are related compounds against systemic and urinary tract infections in mice (16). The present paper details results of comparative evaluations of the activities of AT-2266, norfloxacin, pipemidic acid, nalidixic acid, and gentamicin against various experimental infections in mice.

MATERIALS AND METHODS

Drugs. AT-2266 (10), norfloxacin (6), pipemidic acid (9), and nalidixic acid (15) were prepared in our laboratories as described previously. Although AT-2266 and pipemidic acid were prepared as sesquihydrate and trihydrate, respectively, doses or concentrations were expressed in terms of anhydrous compound. All compounds were dissolved in water with equimolar NaOH for determinations of minimal inhibitory concentrations (MICs) and suspended in 0.2% carboxymethyl cellulose for oral administration. Gentamicin sulfate (Shionogi Co., Ltd.) was dissolved in water for both in vitro and in vivo experiments.

Organisms. The organisms utilized for experimental infections were stock strains stored at −70°C in our laboratories and subcultured just before use.

Determinations of MICs. MICs were determined by the twofold agar dilution method as described previously (17). The respective media used for preculture and MIC determinations were brain heart infusion agar (Difco Laboratories) and blood agar (heart infusion agar [Eiken] plus 10% defibrinated rabbit blood) for *Streptococcus pyogenes*, and Trypto-Soy broth (Eiken) and heart infusion agar for the other organisms. One loopful of an overnight culture which had been adjusted to an optical density of 0.3 at 625 nm was inoculated onto 10-ml drug-containing agar layers in petri dishes. Bacterial inocula contained approximately 10^6 CFU, and bacterial growth was observed after 20 h of incubation at 37°C. The MIC was defined as the lowest drug concentration which prevented visible bacterial growth.

Assessment of therapeutic activity. In vivo activities of compounds were studied in four model infections in mice, i.e., systemic, pulmonary, dermal, and urinary tract infections, which were essentially the same as those described previously (18) except for some modifications and additions. Each dosing group consisted of 8 Std-ddY mice weighing 18 to 22 g.

Systemic infections were induced by inoculating male mice intravenously (*Staphylococcus aureus*) or intraperitoneally (other organisms) with 4 to 180 times the 50% lethal doses of the bacteria suspended in 0.4 ml of menstruums: 0.8% saline for *Staphylococcus aureus*, brain heart infusion (Difco) for *Streptococcus pyogenes*, nutrient broth (Eiken) for *Salmonella typhimurium*, and Trypto-Soy broth containing 4% mucin for the other organisms. Therapeutic achievements, in terms of percentage of survivors, were evaluated 1 week post-inoculation with all infections, except those caused by *Staphylococcus aureus* and *Salmonella typhimurium*, for which a 2-week evaluation interval was employed. Survival rates of untreated controls rarely exceeded 10%.

Pulmonary infections were induced in female mice by instilling intranasally 3 to 20 times the 50% lethal

doses of organisms suspended in 0.05 ml of 0.8% saline or Trypto-Soy broth in the case of *Klebsiella pneumoniae*. More than 97% of untreated mice died, usually with pulmonary hemorrhage or consolidation. Survival for 5 days after challenge was used as the end point for therapeutic efficacy, except for infections with *K. pneumoniae*, in which survival 14 days after challenge was employed.

Dermal infections were induced by subcutaneous inoculation of female mice in a depilated area of the back with 16 times the 50% infective dose of *Pseudomonas aeruginosa* suspended in 0.2 ml of 0.8% saline. In untreated, control mice, skin abscesses were usually formed 2 days after inoculation and within 4 days developed into focal necrotic areas of about 20 mm in diameter. Absence of skin abscesses 4 days after inoculation served as the indicator of therapeutic accomplishment.

Urinary tract infections were induced in female mice lightly anesthetized with sodium pentobarbital (Dainippon Pharmaceutical Co., Ltd.) by instilling 10 to 100 times the 50% infective doses of organisms in 0.1 ml of Trypto-Soy broth directly into the exposed bladders. Drinking water was restricted for 24 h before and 24 h after inoculation. Mice were sacrificed 5 days after inoculation. Kidneys were removed aseptically and bisected, and two halves were pressed on an agar layer of appropriate medium. The media employed were *Staphylococcus* medium no. 110 (Nissan) for *Staphylococcus aureus*, King medium A (Nissan) for *P. aeruginosa*, and MacConkey agar (Nissan) for other organisms. In untreated control mice, bacterial growth was always detected on the agar surface after 20 h of incubation at 37°C. The absence of organisms in both kidneys was the sole end point of therapeutic achievement.

TABLE 1. MICs of AT-2266, norfloxacin, pipemidic acid, nalidixic acid, and gentamicin for the organisms used to induce experimental infections

Organism	MIC (μg/ml) of:				
	AT-2266	Norfloxacin	Pipemidic acid	Nalidixic acid	Gentamicin
Escherichia coli					
P-5101	0.1	0.05	3.13	3.13	3.13
174	1.56	0.78	12.5	200	0.39
Klebsiella pneumoniae					
13	0.2	0.2	6.25	12.5	1.56
1775b	0.39	0.2	3.13	6.25	0.78
7530	3.13	3.13	50	100	0.78
Proteus mirabilis					
P-3003	0.78	0.2	3.13	12.5	0.39
287	0.78	0.39	6.25	>200	0.39
Proteus morganii					
Kono	0.2	0.1	3.13	6.25	0.78
Salmonella typhimurium					
S-9	0.1	0.05	3.13	3.13	3.13
Serratia marcescens					
S-7	0.39	0.2	1.56	3.13	1.56
S-9	0.39	0.2	1.56	1.56	1.56
6700	0.39	0.2	3.13	3.13	0.39
1650u	1.56	1.56	12.5	12.5	0.78
1735IIu	0.78	0.78	6.25	6.25	0.78
Pseudomonas aeruginosa					
12	0.78	0.78	25	100	1.56
Ky-32	0.78	0.39	12.5	50	1.56
Ky-22	1.56	1.56	25	200	50
Ky-24	3.13	3.13	50	100	100
Ky-43	3.13	1.56	25	200	100
Ky-44	3.13	1.56	25	200	100
Staphylococcus aureus					
50774	0.78	0.78	25	50	0.39
Streptococcus pyogenes					
A65	12.5	3.13	100	>200	3.13

TABLE 2. Comparative activities of AT-2266, norfloxacin, pipemidic acid, nalidixic acid, and gentamicin against systemic infections in mice

Organism	Challenge dose (CFU/mouse)	Drug[a]	No. of doses[b]	ED_{50} (mg/kg)	95% Confidence limit (mg/kg)
Escherichia coli P-5101	9×10^6	AT-2266	2	1.8	1.2–2.6
		Norfloxacin		4.7	3.3–6.6
		Pipemidic acid		12.8	8.9–18.6
		Nalidixie acid		29.2	21.7–39.2
		Gentamicin		0.50	0.37–0.67
Klebsiella pneumoniae 13	3×10^6	AT-2266	2	3.5	2.9–4.2
		Norfloxacin		6.5	5.2–8.1
		Pipemidic acid		23.0	12.7–41.7
		Nalidixic acid		84.0	56.0–126
		Gentamicin		0.40	0.3–0.6
Proteus morganii Kono	7×10^6	AT-2266	2	2.5	2.1–3.0
		Norfloxacin		3.1	2.1–4.7
		Pipemidic acid		67.3	33.2–137
		Nalidixic acid		87.3	62.0–123
		Gentamicin		0.22	0.17–0.27
Salmonella typhimurium S-9	5×10^5	AT-2266	8	1.8	1.3–2.5
		Norfloxacin		2.6	1.7–4.2
		Pipemidic acid		11.4	7.1–18.3
		Nalidixic acid		19.6	15.6–24.7
		Gentamicin		1.2	0.85–1.75
Serratia marcescens S-7	5×10^4	AT-2266	2	1.2	0.9–1.5
		Norfloxacin		2.8	1.7–4.5
		Pipemidic acid		8.1	5.6–11.6
		Nalidixic acid		13.6	8.5–21.6
		Gentamicin		0.60	0.4–0.9
Serratia marcescens S-9	5×10^4	AT-2266	2	2.0	1.4–2.8
		Norfloxacin		3.7	2.5–5.6
		Pipemidic acid		8.0	5.9–10.8
		Nalidixic acid		11.5	6.4–20.8
		Gentamicin		1.1	0.5–2.1
Pseudomonas aeruginosa 12	4×10^3	AT-2266	2	9.0	8.0–9.9
		Norfloxacin		15.5	13.3–17.9
		Pipemidic acid		70.8	31.1–161
		Nalidixic acid		267	197–362
		Gentamicin		4.6	3.8–5.7

Experiments were repeated two to six times to confirm the reproducibility of results, and accumulated data were used for calculation of the 50% effective doses (ED_{50}s) and 95% confidence limits, which were calculated by probit analysis (11) and the method of Litchfield and Wilcoxon (8), respectively.

RESULTS

Susceptibility of infecting organisms to AT-2266 and reference compounds. Table 1 shows the MICs of AT-2266, norfloxacin, pipemidic acid, nalidixic acid, and gentamicin for the infecting organisms used. The MICs of AT-2266, ranging from 0.1 to 12.5 μg/ml, were equal to or 2 to 4 times higher than those of norfloxacin, 1/4 to 1/32 those of pipemidic acid, 1/4 to less than 1/256 those of nalidixic acid, and 1/32 to 4 times higher than those of gentamicin.

Comparative activities against systemic infections. The therapeutic activities of AT-2266 were compared with those of norfloxacin, pipemidic acid, nalidixic acid, and gentamicin in systemic infection models in mice (Table 2). The ED_{50}s of AT-2266 ranged from 1.2 to 24.0 mg/kg, except for infections with *Streptococcus pyogenes*, for which its ED_{50} was 92.1 mg/kg. The ED_{50}s of norfloxacin (2.6 to 185 mg/kg) were uniformly higher than those of AT-2266 even against infections with organisms that were more susceptible

TABLE 2—*Continued*

Organism	Challenge dose (CFU/mouse)	Drug[a]	No. of doses[b]	ED_{50} (mg/kg)	95% Confidence limit (mg/kg)
Pseudomonas aeruginosa Ky-32	3×10^4	AT-2266	2	7.1	5.6–9.1
		Norfloxacin		11.5	8.1–16.2
		Pipemidic acid		119	79.6–179
		Nalidixic acid		283	236–340
		Gentamicin		5.1	3.9–6.7
Pseudomonas aeruginosa Ky-22	5×10^4	AT-2266	2	17.7	7.9–40.2
		Norfloxacin		33.9	27.0–42.6
		Pipemidic acid		218	120–394
		Nalidixic acid		345	253–472
		Gentamicin		>100	NC[c]
Pseudomonas aeruginosa Ky-24	5×10^4	AT-2266	2	24.0	15.6–37.0
		Norfloxacin		120	72.2–198
		Pipemidic acid		400	NC
		Nalidixic acid		>400	NC
		Gentamicin		70.8	31.1–161
Pseudomonas aeruginosa Ky-43	5×10^4	AT-2266	2	21.9	17.3–27.8
		Norfloxacin		105	75.4–145
		Pipemidic acid		254	128–504
		Nalidixic acid		305	203–458
		Gentamicin		>100	NC
Pseudomonas aeruginosa Ky-44	8×10^3	AT-2266	2	22.9	17.8–29.4
		Norfloxacin		67.5	51.5–88.4
		Pipemidic acid		261	179–383
		Nalidixic acid		>400	NC
		Gentamicin		>100	NC
Staphylococcus aureus 50774	5×10^8	AT-2266	2	10.0	7.1–14.1
		Norfloxacin		21.9	16.8–28.4
		Pipemidic acid		215	162–286
		Nalidixic acid		>400	NC
		Gentamicin		0.78	0.58–1.05
Streptococcus pyogenes A65	3×10^7	AT-2266	2	92.1	82.4–103
		Norfloxacin		185	148–231
		Pipemidic acid		>400	NC
		Nalidixic acid		>400	NC
		Gentamicin		7.2	5.0–10.5

[a] Drugs were administered orally except for gentamicin, which was administered subcutaneously.
[b] 2, Two doses, immediately after challenge and 6 h later; 8, eight doses, immediately after challenge, and 6, 24, 30, 48, 54, 72, and 78 h later.
[c] NC, Not calculable.

to norfloxacin in vitro. Without exception, the ED_{50}s of pipemidic acid (8.0 to >400 mg/kg) and nalidixic acid (11.5 to >400 mg/kg) were higher than those of AT-2266 and norfloxacin. The ED_{50}s of gentamicin (0.22 to 7.2 mg/kg) were generally lower than those of AT-2266, except for infections due to gentamicin-resistant *P. aeruginosa* strains Ky-22, Ky-24, Ky-43, and Ky-44, for which the ED_{50}s of AT-2266 (17.7 to 24.0 mg/kg) were lower than those of gentamicin (70.8 to >100 mg/kg).

Comparative activities against localized infections. Comparisons of the activities of AT-2266, norfloxacin, pipemidic acid, nalidixic acid, and gentamicin against pulmonary, dermal, and urinary tract infections in mice were also carried out (Table 3).

In pulmonary infections, the ED_{50}s of AT-2266 (7.7 to 47.9 mg/kg) were about 1/2 to 1/9 those of norfloxacin (16.7 to 426 mg/kg), about 1/7 to 1/19 those of pipemidic acid (81.7 to >400 mg/kg), and about 1/8 to 1/52 those of nalidixic

TABLE 3. Comparative activities at AT-2266, norfloxacin, pipemidic acid, nalidixic acid, and gentamicin against localized infections in mice

Type of infection and organism	Challenge dose (CFU/mouse)	Drug[a]	No. of doses[b]	ED_{50} (mg/kg)	95% Confidence limit (mg/kg)
Pulmonary					
K. pneumoniae 1775b	2×10^5	AT-2266	2	13.7	9.6–19.4
		Norfloxacin		127	58.9–275
		Pipemidic acid		101	36.2–280
		Nalidixic acid		109	62.3–192
		Gentamicin		0.30	0.2–0.5
S. marcescens 6700	8×10^7	AT-2266	3	13.1	8.8–19.6
		Norfloxacin		37.3	25.4–54.8
		Pipemidic acid		253	145–442
		Nalidixic acid		392	171–613
		Gentamicin		1.2	0.9–1.6
S. marcescens 1650u	8×10^7	AT-2266	2*	43.3	27.1–69.2
		Norfloxacin		271	141–522
		Pipemidic acid		>400	NC[c]
		Nalidixic acid		>400	NC
		Gentamicin		2.6	1.5–4.6
S. marcescens 1735IIu	8×10^7	AT-2266	2*	16.2	13.0–20.1
		Norfloxacin		65.9	42.3–103
		Pipemidic acid		144	84.9–244
		Nalidixic acid		126	93.1–171
		Gentamicin		0.60	0.4–0.9
P. aeruginosa 12	2×10^7	AT-2266	3	7.7	5.8–10.3
		Norfloxacin		16.7	11.9–23.5
		Pipemidic acid		81.7	52.9–113
		Nalidixic acid		>400	NC
		Gentamicin		1.4	0.8–2.3
P. aeruginosa Ky-24	8×10^7	AT-2266	2*	47.9	29.2–78.5
		Norfloxacin		426	255–712
		Pipemidic acid		>400	NC
		Nalidixic acid		>400	NC
		Gentamicin		37.1	27.6–50.0
P. aeruginosa Ky-44	8×10^7	AT-2266	2*	31.0	20.7–46.4
		Norfloxacin		186	106–325
		Pipemidic acid		438	256–749
		Nalidixic acid		>400	NC
		Gentamicin		66.0	44.8–97.2

acid (109 to >400 mg/kg). The ED_{50}s of gentamicin (0.30 to 66.0 mg/kg) were about 1/6 to 1/46 those of AT-2266, except for infections with *P. aeruginosa* Ky-24, against which the two agents were essentially equally active, and *P. aeruginosa* Ky-44, against which AT-2266 was approximately twice as active as was gentamicin.

In dermal infections, the ED_{50} of AT-2266 (15.3 mg/kg) was about 1/2 that of norfloxacin (27.3 mg/kg), about 1/11 that of pipemidic acid (173 mg/kg), <1/13 that of nalidixic acid (>200 mg/kg) and about 11 times greater than that of gentamicin (1.4 mg/kg).

In urinary tract infections, the ED_{50}s of AT-2266 (1.5 to 17.8 mg/kg) were about 1/2 to 1/7 those of norfloxacin (3.4 to 55.8 mg/kg), 1/2 to 1/12 those of pipemidic acid (3.4 to 161 mg/kg), and 1/4 to 1/160 those of nalidixic acid (6.6 to >400 mg/kg). The ED_{50}s of gentamicin (0.50 to 7.3 mg/kg) were similar to those of AT-2266 (1.5 to 5.0 mg/kg), except for infections with *Staphylococcus aureus*, against which the ED_{50} of gentamicin (1.6 mg/kg) was about 1/11 that of AT-2266 (17.8 mg/kg).

Comparative activities against systemic infections due to nalidixic acid-resistant organisms. Since AT-2266 was active against nalidixic acid-resistant organisms in vitro (16), its in vivo

TABLE 3—*Continued*

Type of infection and organism	Challenge dose (CFU/mouse)	Drug[a]	No. of doses[b]	ED_{50} (mg/kg)	95% Confidence limit (mg/kg)
Dermal					
P. aeruginosa 12	9×10^6	AT-2266	6	15.3	11.8–20.0
		Norfloxacin		27.3	NC
		Pipemidic acid		173	146–205
		Nalidixic acid		>200	NC
		Gentamicin		1.4	0.8–2.3
Urinary tract					
E. coli P-5101	2×10^7	AT-2266	6*	1.5	0.9–2.4
		Norfloxacin		3.4	2.4–5.0
		Pipemidic acid		3.4	2.1–5.4
		Nalidixic acid		6.6	4.4–9.9
		Gentamicin		0.50	0.2–1.2
K. pneumoniae 13	2×10^5	AT-2266	6*	3.1	2.0–4.8
		Norfloxacin		17.7	7.1–43.8
		Pipemidic acid		11.9	6.7–21.0
		Nalidixic acid		40.8	18.4–90.7
		Gentamicin		2.0	1.2–3.2
P. mirabilis P-3003	2×10^5	AT-2266	6*	2.5	1.6–3.9
		Norfloxacin		11.5	6.0–22.1
		Pipemidic acid		23.6	14.9–37.3
		Nalidixic acid		68.7	34.6–136
		Gentamicin		2.5	1.5–4.1
S. marcescens S-9	3×10^7	AT-2266	6*	5.0	3.0–8.1
		Norfloxacin		25.7	14.9–44.5
		Pipemidic acid		18.6	11.4–30.1
		Nalidixic acid		40.7	19.4–84.0
		Gentamicin		7.3	4.6–11.7
P. aeruginosa 12	2×10^4	AT-2266	6*	2.4	1.4–4.1
		Norfloxacin		16.1	8.4–30.9
		Pipemidic acid		29.1	16.7–50.8
		Nalidixic acid		384	138–1070
		Gentamicin		1.6	0.9–2.8
S. aureus 50774	2×10^5	AT-2266	6*	17.8	11.4–28.0
		Norfloxacin		55.8	28.3–110
		Pipemidic acid		161	102–253
		Nalidixic acid		>400	NC
		Gentamicin		1.6	0.9–2.9

[a] Drugs were administered orally, except for gentamicin, which was administered subcutaneously.

[b] 2, Two doses, immediately after challenge and 6 h later; 3, three doses, immediately after challenge and 1 and 3 h later; 2*, two doses, immediately after challenge and 3 h later; 6, six doses, immediately after challenge and 3, 6, 24, 27, and 30 h later; 6*, six doses, 3, 8, 24, 30, 48, and 54 h after challenge.

[c] NC, Not calculable.

activities against the organisms in systemic infections were assessed (Table 4). Nalidixic acid was ineffective against all infections, even at a dose of 400 mg/kg. AT-2266 was active against all infections, with ED_{50}s of between 7.5 to 72.3 mg/kg. These doses were lower than the doses of norfloxacin (11.0 to >200 mg/kg) and pipemidic acid (50.0 to >400 mg/kg) that were required for the same result.

DISCUSSION

AT-2266 is a pyridonecarboxylic acid derivative with a broad antibacterial spectrum covering gram-positive, gram-negative, and glucose-

TABLE 4. Comparative activities of AT-2266, norfloxacin, pipemidic acid, and nalidixic acid against systemic infections with nalidixic acid-resistant organisms in mice

Organism	Challenge dose (CFU/mouse)	Drug[a]	No. of doses[b]	ED_{50} (mg/kg)	95% Confidence limit (mg/kg)
Escherichia coli 174	4×10^5	AT-2266	2	16.8	10.2–27.5
		Norfloxacin		29.3	14.3–59.8
		Pipemidic acid		138	56.1–337
		Nalidixic acid		>400	NC[c]
Proteus mirabilis 287	4×10^5	AT-2266	2	7.5	5.7–9.9
		Norfloxacin		11.0	7.8–15.5
		Pipemidic acid		50.0	NC
		Nalidixic acid		>400	NC
Klebsiella pneumoniae 7530	4×10^5	AT-2266	2	72.3	51.0–102
		Norfloxacin		>200	NC
		Pipemidic acid		>400	NC
		Nalidixic acid		>400	NC

[a] Drugs were administered orally.
[b] 2, Two doses, immediately after challenge and 6 h later.
[c] NC, Not calculable.

nonfermentative organisms (16). Its in vitro antibacterial activity is generally much higher than those of pipemidic acid and nalidixic acid (16) and similar to that of norfloxacin (2), which has been reported to be more active than frequently used β-lactams and aminoglycosides against most gram-negative organisms (4, 5, 13). AT-2266 is well absorbed orally, and its levels in urine, bile, and tissues are generally higher than those in plasma (12). These antibacterial and pharmacological properties of AT-2266 suggest that the compound might be effective against infections with various organisms at a variety of sites. This possibility was examined in the present study, in which systemic, pulmonary, dermal, and urinary tract infections were induced in mice. AT-2266 was effective orally against all infections and was uniformly more active than was norfloxacin, which had been reported to be highly active, in vivo (1), even when the infecting organisms were more susceptible to norfloxacin in vitro. AT-2266 was generally more effective than pipemidic and nalidixic acids against all infections. Pipemidic acid is reportedly more effective than oral antibiotics such as ampicillin and cephalexin against experimental infections due to gram-negative organisms (18) and in clinical trials (3, 7, 14). Therefore, it is reasonable to think that AT-2266 is probably more effective than such oral antibiotics. AT-2266 administered orally was generally less effective against systemic, pulmonary, and dermal infection models than was gentamicin administered subcutaneously but had activity comparable to that of gentamicin in the urinary tract infection model. High concentrations of AT-2266 in urine (12) may account for this result. AT-2266 administered orally was generally more effective than gentamicin administered subcutaneously against infections with gentamicin-resistant strains of *P. aeruginosa*. The activity of AT-2266 against *P. aeruginosa* is noteworthy because there are few agents that are active against gentamicin-resistant *P. aeruginosa* strains. AT-2266 was also active against infections with nalidixic acid-resistant organisms. Incomplete cross-resistance between pyridonecarboxylic acid derivatives observed in vitro (16, 18) was thus confirmed in vivo also.

These results indicate that AT-2266 may be a potent oral antibacterial agent applicable to variety of infections, including those not controlled by existing pyridonecarboxylic acids. Clinical trials of AT-2266 are in progress.

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