Synthesis and Antipancreatitis Activities of Novel *N*-(2-Sulfonylamino-5-trifluoromethyl-3-pyridyl)carboxamide Derivatives as Phospholipase A_2 Inhibitors

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Novel *N*-(2-sulfonylamino-5-trifluoromethyl-3-pyridyl)carboxamide derivatives have been prepared and evaluated as phospholipase A_2 (PLA_2) inhibitors. Among these compounds, IS-741 (sodium salt of 1j), which showed the highest and the most stable therapeutic effect on acute hemorrhagic pancreatitis induced by the closed duodenal loop method in rats, was selected as a candidate for further development.

Key words antipancreatitis agent; phospholipase A_2 inhibitor; *N*-pyridylsulfonamide; IS-741

Phospholipase A_2 (PLA_2) is an enzyme which catalyze the hydrolysis of membrane phospholipids at the 2-position to produce arachidonic acid and lysophospholipids. PLA_2 has become a prime target for pharmacological and biochemical studies because of its role as an initial enzyme in the production of various chemical mediators such as prostaglandins, leukotrienes and platelet activating factor. It is also important in the pathogenesis of acute pancreatitis,[1-3] though trypsin has long been considered of key importance in pancreatitis. Activated PLA_2 splits lecithin into toxic lysolecithin and fatty acid after trypsin activation[4] and the amount of lysolecithin increases during hemorrhagic pancreatitis.[1,2] Furthermore, PLA_2, lipase and colipase are thought to be essential in the pathogenesis of fatty necrosis, with the initial cell membrane damage being ascribed to PLA_2.[5] Damage to the celiac plexus by the action of PLA_2 may contribute to the aggravation of acute pancreatitis.[6]

Accordingly, the action of PLA_2 to hydrolyze phospholipid, an essential constituent of the cell membrane,[7] is considered to cause the destruction of epithelial cells of the pancreatic duct, provoking acute pancreatitis and damage to the retroperitoneal nerve plexus and leading to shock as well as disturbance of the pancreatic blood flow, which aggravates pancreatitis.[7] In a study using the sodium taurocholate-induced acute pancreatitis model, the anti-protease drug aprotinin had no beneficial effect, but procaine hydrochloride, a PLA_2 inhibitor, was efficacious.[8] In addition, chlorpromazine, xylocaine and *p*-bromophenacyl bromide, which are PLA_2 inhibitors, were effective in experimental pancreatitis models.[9-11] Trypsinogen-enterokinase activation is not the only route of PLA_2 activation; another route is involved in the pancreaticobiliary junction.[12] In brief, the treatment of acute pancreatitis with protease inhibitors alone is not sufficiently effective.

As a result of our studies, novel compounds **1** were found to possess PLA_2-inhibitory activity and to exhibit a therapeutic effect on acute hemorrhagic pancreatitis induced by the closed duodenal loop method in rats.

In this paper, we describe the preparation of these compounds **1** and their pharmacological activities.

Synthesis *N*-(2-Sulfonylamino-5-trifluoromethyl-3-pyridyl)-carboxamides **1** were prepared as shown in Chart 1. 2-Sulfonylamino-5-trifluoromethylpyridines **3** were synthesized by sulfonylamidation of 2-chloro-5-trifluoromethylpyridine **2**. 3-Nitro-2-sulfonylamino-5-trifluoromethylpyridines **4** were synthesized by nitration of **3** Reduction of **4** with iron powder in acetic acid afforded 3-amino-2-sulfonylamino-5-trifluoromethylpyridines **5**. Condensation of **5** with the corresponding acid chlorides (method A) or acids (method B) gave the target compounds **1** in good yields. Physicochemical data for compounds **1** are listed in Table 1.

Pharmacological Results and Discussion The effect of compounds **1** on porcine pancreatic PLA_2 was evaluated by photometric assay using the acyl-CoA synthetase/acyl-CoA oxidase (ACS–ACO) method.[13] The *in vitro* results are summarized in Table 1. It was found that introduction of hydrophobic substituents R^2, such as cycloalkyl groups,

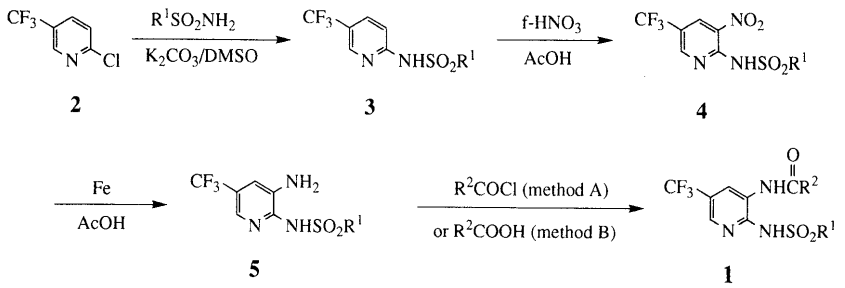

Chart 1

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Table 1. Physical Properties and PLA_2-Inhibitory Activities of *N*-(2-Sulfonylamino-5-trifluoromethyl-3-pyridyl)carboxamides **1**

No.	R^1	R^2	Method	Yield (%)	mp (°C)	IC_{50} (mM)	Solubility[a] (mg/ml)
1a	CH_3	CH_3	A	72	142—145	>5.0	ND
1b	CH_3	$(CH_2)_3CH_3$	A	93	113—114	>5.0	ND
1c	CH_3	$(CH_2)_5CH_3$	A	63	94—96	0.84	2
1d	CH_3		A	88	152—154	>5.0	ND
1e	CH_3		A	92	153—155	3.8	10
1f	CH_3		A	79	110—116	0.98	<1
1g	CH_3		B	77	143—145	1.2	6
1h	CH_3		B	82	126—130	0.71	<1
1i	CH_2CH_3		A	91	157—161	2.1	16
1j	CH_2CH_3		A	85	159—160	0.52	5
1k	CH_2CH_3		B	69	163—166	0.61	4
1l	CH_2CH_3		B	62	147—148	0.80	4
1m	CH_2CH_3		B	43	163—165	1.7	10
1n	CF_3		A	88	175—178	0.59	<1
1o	$CH(CH_3)_2$		A	82	174—177	2.3	ND
1p	$CH(CH_3)_2$		A	91	152—154	0.55	1
1q	$(CH_2)_2CH_3$		A	86	148—150	0.27	2
1r	$(CH_2)_3CH_3$		A	83	152—154	0.13	<1
	Quinacrine					3.3	ND

a) As the sodium salt in saline solution. ND: not determined.

significantly increased the inhibitory activity. Compounds bearing a cyclohexyl substituent (**1f**, **1j**, **1n**, **1p—r**) were about 10 times more potent than quinacrine. With regard to the influence of substitution at R^1, compounds bearing a C_3–C_4 alkyl substituent were slightly more potent than compounds bearing a C_1–C_2 alkyl substituent.

Experimental pancreatitis can be induced by various methods. The experimental pancreatitis in rats induced by the closed duodenal loop method has many similarities to human pancreatitis from the viewpoint of the early pathogenetic mechanism.[14] Effectiveness in this assay was evaluated in terms of macroscopic findings, which were transformed to scores for grade and extent of hemorrhage, edema or necrosis in the pancreas or fatty necrosis in the abdominal cavity. Compounds, which proved to be active *in vitro* and to be soluble in water as the sodium salt, were tested *in vivo*. The results of the *in vivo* tests are summarized in Table 2. Compounds **1c**, **1p**, **1q**, which had low water-solubility, and compound **1i**, which was highly water-soluble, showed no therapeutic effect on acute hemorrhagic pancreatitis. But, compounds (**1e**, **1g**, **1j**, **1m**), which were moderately water-soluble, were effective. Compound **1j** showed the highest and the most stable effect among the test compounds. It suppressed the pancreatic change with higher potency than nafamostat mesilate.

In conclusion, the novel compound IS-741 (sodium salt of **1j**), which exhibited potent antipancreatitis activity in this model, was selected as a candidate for a new anti-pancreatitis agent. Further pharmacological and medicinal investigations on IS-741 are in progress.

Experimental

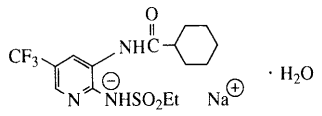
Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL JNM-GSX400 spectrometer with tetramethylsilane as an internal standard. FAB-Mass spectra (FAB-MS) were recorded on a JEOL SX-102A spectrometer.

General Procedure for Preparation of *N*-(5-Trifluoromethyl-2-pyridyl)-sulfonamides 3 A solution of **2** (30.00 g, 165 mmol), an appropriate sulfonamide[15] (210 mmol) and K_2CO_3 (52.00 g, 376 mmol) in dimethylsulfoxide (DMSO) (200 ml) was stirred at 110—120 °C for 7 h. The reaction mixture was cooled, then water (1000 ml) was added and the aqueous layer was washed with ethyl ether. The cooled water layer

Table 2. Therapeutic Effect in Terms of Macroscopic Findings

No.	Dose (mg/kg)	$N^{a)}$	Therapeutic effect[b] (%)
1j	0.625	3	41
	1.25	3	45
	2.5	3	45
	10	3	43
	20	2	67
1e	2.5	3	32
	5	3	37
	10	3	32
	25	3	62
1g	2.5	5	10
	10	3	59
IS-741	2.5	8	46
	10	5	55
1m	10	3	42
1k	10	3	24
1l	10	3	19
1i	10	3	1
1c	10	3	15
1p	10	3	16
1q	10	3	16
Nafamostat mesilate	10	18	25

a) Number of animals. *b*) Defined in the text.



IS-741

Fig. 1

was adjusted to pH 4 with concentrated hydrochloric acid, and precipitated crystals were collected by filtration and dried to give **3**. The yields were 65—84%. The spectral data are listed in Table 3.

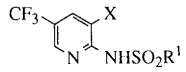
General Procedure for Preparation of *N*-(3-Nitro-5-trifluoromethyl-2-pyridyl)sulfonamides 4 An appropriate **3** (177 mmol) was added to acetic acid (112.5 ml). Under heating at 105 °C to 110 °C, fuming nitric acid (94%) (17.3 ml, 388 mmol) was added dropwise, and the mixture was allowed to react for a further 6 h. It was left to cool to 80 °C, and then poured into ice water (1 l). Precipitated crystals were collected by filtration, washed with water and dried to give **4**. The yields were 89—93%. The spectral data are listed in Table 3.

General Procedure for Preparation of *N*-(3-Amino-5-trifluoromethyl-2-pyridyl)sulfonamides 5 An appropriate **4** (33.4 mmol) was suspended in acetic acid (100 ml), and reduced iron (7.45 g, 134 mmol) was added portionwise at 65 °C. After 1 h, water (50 ml) was added and excess iron was removed by filtration. The filtrate was poured into water (80 ml), and adjusted to pH 5 with 30% aqueous NaOH. The precipitated crystals were collected by filtration, washed with water and dried to give **5**. The yields were 75—94%. The spectral data are listed in Table 3.

General Procedure for Preparation of *N*-(2-Sulfonylamino-5-trifluoromethyl-3-pyridyl)carboxamides 1 Method A: An appropriate **5** (1.96 mmol) was dissolved in tetrahydrofuran (10 ml), and an appropriate acid chloride (2.34 mmol) in tetrahydrofuran (1 ml) was dropwise added thereto under cooling with ice. The mixture was stirred for 1 h and further allowed to react at room temperature overnight. After completion of the reaction, the solvent was evaporated, and the obtained crystals were washed with ethyl ether to give **1**. The yields are listed in Table 1. The spectral data are listed in Table 4.

Method B: An appropriate **5** (1.96 mmol) was added to a solution of 4-dimethylaminopyridine (260 mg, 2.14 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (410 mg, 2.14 mmol) and an appropriate acid (2.14 mmol) in dichloromethane (10 ml) with stirring at room temperature. The mixture was stirred for 24 h at room temperature. Then, 10% HCl was added and the whole was extracted with dichloromethane. The extract was washed with brine, then dried. The solvent was evaporated, and the obtained crystals were washed with ethyl ether to give **1**. The yields are listed in Table 1. The spectral data are listed in Table 4.

Table 3. *N*-(5-Trifluoromethyl-2-pyridyl)sulfonamide Derivatives (**3**—**5**)



No.	R^1	X	mp (°C)	^{1}H-NMR ($CDCl_3$) δ (ppm)
3a	CH_3	H	189—191	3.25 (3H, s), 7.41 (1H, m), 7.94 (1H, dd, J=2.4, 8.8 Hz), 8.67 (1H, s)
3b	CH_2CH_3	H	161—162	1.41 (3H, t, J=7.6 Hz), 3.34 (2H, q, J=7.6 Hz), 7.46 (1H, d, J=8.8 Hz), 7.93 (1H, dd, J=2.4, 8.8 Hz), 8.68 (1H, s)
3c	$CH(CH_3)_2$	H	181—184	1.41 (6H, d, J=6.8 Hz), 3.45 (1H, qq, J=6.8 Hz), 7.25 (1H, d, J=8.8 Hz), 7.88 (1H, dd, J=2.4, 8.8 Hz), 8.64 (1H, s)
3d	$(CH_2)_2CH_3$	H	157—159	0.98 (3H, t, J=7.6 Hz), 1.85 (2H, m), 3.21 (2H, m), 7.39 (1H, d, J=8.8 Hz), 7.86 (1H, dd, J=2.4, 8.8 Hz), 8.61 (1H, s)
3e	$(CH_2)_3CH_3$	H	148—150	0.83 (3H, t, J=8.0 Hz), 1.39 (2H, m), 1.75 (2H, m), 3.23 (2H, m), 7.39 (1H, d, J=8.8 Hz), 7.86 (1H, dd, J=2.0, 8.8 Hz), 8.61 (1H, s)
3f	CF_3	H	215—218	7.84 (1H, d, J=9.2 Hz), 8.34 (1H, dd, J=2.4, 9.2 Hz), 8.62 (1H, s)[a]
4a	CH_3	NO_2	138—139	3.58 (3H, s), 8.83 (1H, s), 8.91 (1H, s)
4b	CH_2CH_3	NO_2	158—159	1.42 (3H, t, J=8.0 Hz), 3.70 (2H, d, J=8.0 Hz), 8.74 (1H, d, J=1.6 Hz), 8.77 (1H, d, J=1.6 Hz), 9.88 (1H, br s)
4c	$CH(CH_3)_2$	NO_2	138—140	1.46 (6H, d, J=6.8 Hz), 4.17 (1H, qq, J=6.8 Hz), 8.74 (1H, d, J=2.0 Hz), 8.80 (1H, d, J=2.0 Hz), 9.80 (1H, br s)
4d	$(CH_2)_2CH_3$	NO_2	109—112	1.12 (3H, t, J=7.6 Hz), 2.00 (2H, m), 3.72 (2H, m), 8.82 (1H, s), 8.89 (1H, s)
4e	$(CH_2)_2CH_3$	NO_2	76—78	0.91 (3H, m), 1.41 (2H, m), 1.86 (2H, m), 3.68 (2H, m), 8.78 (1H, d, J=1.6 Hz), 8.82 (1H, d, J=1.6 Hz), 9.90 (1H, br s)
4f	CF_3	NO_2	126—127	8.78 (1H, d, J=2.0 Hz), 8.88 (1H, d, J=2.0 Hz)
5a	CH_3	NH_2	128—130	3.13 (3H, s), 6.84 (1H, s), 7.30 (1H, s)
5b	CH_2CH_3	NH_2	118—119	1.33 (3H, t, J=7.2 Hz), 3.12 (2H, q, J=7.2 Hz), 6.76 (1H, d, J=2.0 Hz), 7.22 (1H, s)
5c	$CH(CH_3)_2$	NH_2	155—157	1.34 (6H, d, J=6.8 Hz), 3.22 (1H, qq, J=6.8 Hz), 6.76 (1H, d, J=2.0 Hz), 7.20 (1H, s)
5d	$(CH_2)_2CH_3$	NH_2	82—84	1.01 (3H, t, J=7.2 Hz), 1.82 (2H, m), 3.08 (2H, m), 6.76 (d, J=1.6 Hz), 7.19 (1H, s)
5e	$(CH_2)_3CH_3$	NH_2	102—103	0.87 (3H, m), 1.38 (2H, m), 1.76 (2H, m), 3.09 (2H, m), 6.75 (1H, s), 7.19 (1H, s)
5f	CF_3	NH_2	122—124	4.83 (2H, br s), 7.03 (1H, d, J=2.0 Hz), 7.35 (1H, s)

a) $(CD_3)_2CO$.

Table 4. ^{1}H-NMR Spectral Data for Compounds **1**

No.	^{1}H-NMR ($CDCl_3$) δ (ppm)
1a	2.20 (3H, s), 3.08 (3H, s), 7.50 (1H, s), 8.33 (1H, s), 8.80 (1H, s), 12.29 (1H, br s)
1b	0.89 (3H, t, J=8.0 Hz), 1.35 (2H, m), 1.65 (2H, m), 2.39 (2H, t, J=7.6 Hz), 3.07 (3H, s), 7.46 (1H, s), 8.31 (1H, s), 8.84 (1H, s), 12.29 (1H, br s)
1c	0.95 (3H, m), 1.34—1.51 (6H, m), 1.75 (2H, m), 2.51 (3H, t, J=8.0 Hz), 3.20 (3H, s), 3.99 (1H, m), 7.64 (1H, s), 8.44 (1H, s), 8.95 (1H, d, J=2.0 Hz)
1d	1.84—2.03 (2H, m), 2.16—2.36 (4H, m), 3.07 (3H, s), 3.18 (1H, m), 7.49 (1H, s), 8.23 (1H, s), 8.86 (1H, s)
1e	1.56—2.02 (8H, m), 2.81 (1H, m), 3.20 (3H, s), 7.62 (1H, s), 8.44 (1H, s), 8.91 (1H, d, J=2.0 Hz), 11.86 (1H, br s)
1f	1.14—1.90 (10H, m), 2.27 (1H, m), 3.08 (3H, s), 7.47 (1H, s), 8.37 (1H, s), 8.85 (1H, s)
1g	1.75—2.41 (6H, m), 2.60—2.67 (1H, m), 3.19 (3H, s), 5.75—5.79 (2H, m), 7.58 (1H, s), 8.54 (1H, s), 8.93 (1H, s), 12.33 (1H, br s)
1h	1.49—2.02 (12H, m), 2.48—2.55 (1H, m), 3.16 (3H, s), 7.58 (1H, s), 8.39 (1H, s), 8.91 (1H, s), 12.32 (1H, br s)
1i	1.39 (3H, t, J=7.6 Hz), 1.53—2.02 (8H, m), 2.77 (1H, m), 3.18 (2H, q, J=7.6 Hz), 7.54 (1H, s), 8.43 (1H, s), 8.87 (1H, s), 12.40 (1H, br s)
1j	1.13—1.91 (10H, m), 1.35 (3H, t, J=7.2 Hz), 2.26 (1H, m), 3.14 (2H, q, J=7.2 Hz), 7.48 (1H, s), 8.41 (1H, s), 8.83 (1H, s), 12.35 (1H, br s)
1k	1.47 (3H, t, J=7.6 Hz), 1.54—2.07 (12H, m), 2.54 (1H, m), 3.26 (2H, q, J=7.6 Hz), 7.61 (1H, s), 8.46 (1H, s), 8.93 (1H, s), 12.44 (1H, br s)
1l	1.47 (3H, t, J=7.6 Hz), 1.80—2.45 (6H, m), 2.64—2.72 (1H, m), 3.26 (2H, q, J=7.6 Hz), 5.77—5.83 (2H, m), 7.62 (1H, s), 8.61 (1H, s), 8.96 (1H, s), 12.46 (1H, br s)
1m	1.48 (3H, t, J=7.6 Hz), 1.66—1.83 (4H, m), 2.31—2.41 (4H, m), 3.26 (2H, q, J=7.6 Hz), 6.90 (1H, m), 7.59 (1H, s), 8.86 (1H, s), 8.98 (1H, s), 12.47 (1H, br s)
1n	1.14—1.91 (10H, m), 2.32 (1H, m), 7.66 (1H, s), 8.44 (1H, s), 9.18 (1H, d, J=2.0 Hz)
1o	1.34 (6H, d, J=6.8 Hz), 1.55—1.95 (8H, m), 2.72 (1H, m), 3.22 (1H, qq, J=6.8 Hz), 7.44 (1H, s), 8.40 (1H, s), 8.81 (1H, d, J=2.0 Hz), 12.40 (1H, br s)
1p	1.20—2.05 (10H, m), 1.42 (6H, d, J=6.8 Hz), 2.34 (1H, m), 3.30 (1H, qq, J=6.8 Hz), 7.53 (1H, s), 8.51 (1H, s), 8.90 (1H, d, J=1.6 Hz), 12.49 (1H, br s)
1q	1.02 (3H, t, J=7.6 Hz), 1.13—1.90 (12H, m), 2.28 (1H, m), 3.10 (2H, m), 7.49 (1H, s), 8.40 (1H, s), 8.83 (1H, s)
1r	0.89 (3H, t, J=7.6 Hz), 1.13—1.91 (14H, m), 2.27 (1H, m), 3.11 (2H, m), 7.48 (1H, s), 8.40 (1H, s), 8.82 (1H, d, J=1.6 Hz), 12.34 (1H, br s)

Sodium Salt of *N*-[(2-Ethylsulfonylamino)-5-trifluoromethyl-3-pyridyl]cyclohexanecarboxamide Monohydrate IS-741 A suspension of **1j** (3.0 g, 7.91 mmol) in ethanol (20 ml) was heated at 60 to 70 °C, then 8 N sodium hydroxide aqueous solution (1.26 g, 7.91 mmol) was added dropwise, and mixture was stirred for 30 min. It was cooled to 40 °C and ethyl ether (40 ml) was added. The mixture was cooled to room temperature, and the precipitated crystals were collected by filtration, washed with ethyl ether and dried at 60 °C under reduced pressure to give IS-741 (2.46 g, 74%). mp 268 °C (dec.); FAB-MS *m/z*: 424 (M+Na), 402 (M+H). *Anal.* Calcd for $C_{15}H_{19}F_3N_3O_3SNa \cdot H_2O$: C, 42.95; H, 5.05; N, 10.02. Found: C, 42.80; H, 4.92; N, 9.88.

Biology 1. Assay of PLA_2 Activity This assay system used a substrate of mixed micelles of phosphatidylcholine and cholate, taken from Volwerk *et al.*[16] Dipalmitoyl phosphatidylcholine (10 μmol) and sodium cholate (60 μmol) were dried under N_2. These materials were suspended in 2 ml of 250 mM NaCl/Tris–HCl, pH 8 and used as the substrate. The reaction mixture (total volume of 50 μl) contained (final concentrations) 1 μg/ml porcine pancreatic phospholipase A_2, 2 mM dipalmitoyl-PC, 12 mM sodium cholate, 10 mM $CaCl_2$, 100 mM NaCl, 1 mg/ml BSA, 100 mM Tris–HCl, pH 8 and test sample at various concentrations. The reaction was started by addition of the substrate to a mixture of PLA_2 and a test sample, and incubation was continued at 37 °C for 30 min. The production of free fatty acids was measured by the ACS–ACO method.[13] After incubation, 80 μl of colorization reagent A (NEFA C-Test Wako; 1.46 mM coenzyme A, 9 mM ATP, 3 mM 4-aminoantipyrine, 0.54 u/ml ACS, 5.4 u/ml ascorbate oxidase, 50 mM phosphanate, pH 7) was added to the reaction mixture and incubation was continued at 37 °C for another 10 min. Then 160 μl of colorization reagent B (NEFA C-Test Wako; 5.5 u/ml ACO, 6.8 u/ml peroxidase, 1.2 mM 3-methyl-*N*-ethyl-*N*-(β-hydroxyethyl)aniline) was added and incubation was continued at 37 °C for another 10 min. The production of dye was evaluated by measuring the absorbance at 540 nm. Three replicates were used for each determination of PLA_2 activity.

2. Effects on Experimental Acute Pancreatitis in Rats Animals: Male rats of the Sprague-Dawley strain, weighing about 200 g were obtained from Charles River Japan Co. Rats were divided at random into drug treatment groups (Table 2). A similarly constituted vehicle group was used with each compound treatment group. Animals were allowed food during the experiment. Induction of experimental acute pancreatitis: Each rat was laparotomized by midline incision under halothane–NO_2 anesthesia. Experimental pancreatitis was induced by the method of Rao *et al.*[17] with some modifications. A closed loop was created by ligating the duodenum with silk at two points on both sides of the common bile duct. The length of the closed loop was approximately 1.5 cm. After the procedure the abdomen was closed.

Treatment: Rats of each group were injected with 0.625—25 mg/kg of compounds, 10 mg/kg of nafamostat mesilate or 5% glucose (vehicle) into the caudalis vein immediately after closure of the abdomen. Macroscopic examination: All rats were observed 6 h after induction. Each finding was transformed to a score reflecting the grade and extent of hemorrhage, edema or necrosis in the pancreas or fatty necrosis in the abdominal cavity. Scoring of each finding was as follows: 0=nil, 0.5=minimal, 1=slight, 2=moderate or 3=marked. Each score was summed up to obtain the total score.

Calculation of therapeutic effect was as follows:

$$\frac{\text{total score in the vehicle group} - \text{total score in the compound group}}{\text{total score in the vehicle group}} \times 100$$

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