A test compound is considered to be positive for mutagenic effect if it produces a dose-related increase in the number of revertants (at least doubling of the number of revertant colonies compared to concurrent control values).

Results: The results are presented in Table 29. No significant dose-related increased incidence of revertant colonies was observed for any of the tester strains after treatment with the test compound with or without metabolic activation. Respective positive control compounds produced significant increases in mutant frequencies, demonstrating the sensitivity of the strains as well as the activity of the S-9 mix.

It is noted that precipitation occurred at the two highest concentrations (500 and 1000 µg/plate), but not at lower concentrations (100 µg/plate or lower), with all tester strains in this study. According to ICH Guidelines for testing of relatively insoluble compounds, the lowest precipitating concentration should be used as the top concentration, but not exceeding 5 mg/plate, for bacterial tests.

[Note: A repeat Ames test (Study Number 004447) with OPC-13013 was conducted (November - December, 1987) at concentrations of 156, 313, 625, 1250, 2500 and 5000 µg/plate using S.typhimurium TA1535, TA1537, TA100 and TA98, and E.coli WP2uvrA tester strains. No significant dose-related increased incidence of revertant colonies was observed in any of the tester strains, with or without metabolic activation, at concentrations tested in the study. It is noted that precipitation occurred at the lowest concentration (156 µg/plate) tested with most of the tester strains. Although concentrations lower than 156 µg/plate were not tested in this study, in the previous study (Study No.211108-0698) no precipitation occurred at concentrations of 100 µg/plate or lower.]

Table 29. Reversion Test of OPC-13013 with TA1535

compound	amount (μg/plate)	-S-9 revertants/plate	-S-9 mean	+S-9 revertants/plate	+S-9 mean
DMSO	-	8, 11, 7	9	6, 4, 6	5
OPC-13013	1	7, 7, 5	6	5, 8, 4	6
	5	2, 6, 8	5	4, 7, 5	5
	10	4, 4, 5	4	12, 10, 9	10
	50	5, 5, 11	7	11, 9, 8	9
	100	7, 5, 15	9	8, 4, 8	7
	500	9^P, 3^P, 4^P	5^P	6^P, 11^P, 9^P	9^P
	1000	5^P, 5^P, 3^P	4^P	6^P, 2^P, 7^P	5^P
ENNG	5	401, 457, 532	463	NT, NT, NT	NT
2AA	2	8, 3, 5	5	110, 78, 109	99

NT: not tested

P: precipitation

Table 29. continued Reversion Test of OPC-13013 with TA1537

compound	amount (μg/plate)	-S-9 revertants/plate	-S-9 mean	+S-9 revertants/plate	+S-9 mean
DMSO	-	4, 4, 9	6	6, 8, 11	8
OPC-13013	1	4, 7, 3	5	11, 9, 11	10
	5	6, 4, 4	5	10, 12, 6	9
	10	10, 5, 9	8	7, 11 11	10
	50	3, 3, 5	4	8, 5, 7	7
	100	3, 9, 9	7	7, 4, 11	7
	500	6^P, 7^P, 7^P	7^P	9^P, 8^P, 10^P	9^P
	1000	3^P, 9^P, 5^P	6^P	5^P, 5^P, 7^P	6^P
ACR	80	1518, 1714, 1325	1519	NT, NT, NT	NT
2AA	2	9, 7, 8	8	108, 73, 80	87

NT: not tested

P: precipitation

Table 29. continued

Reversion Test of OPC-13013 with TA1538

compound	amount (μg/plate)	-S-9 revertants/plate	-S-9 mean	+S-9 revertants/plate	+S-9 mean
DMSO	-	4, 7, 6	6	17, 10, 16	14
OPC-13013	1	8, 10, 5	8	9, 12, 9	10
	5	3, 5, 7	5	22, 10, 15	16
	10	4, 3, 6	4	10, 7, 9	9
	50	5, 4, 6	5	9, 19, 17	15
	100	5, 5, 5	5	11, 11, 12	11
	500	8^P, 6^P, 3^P	6^P	9^P, 13^P, 13^P	12^P
	1000	4^P, 3^P, 7^P	5^P	13^P, 9^P, 10^P	11^P
2NF	2	337, 384, 331	351	NT, NT, NT	NT
2AA	0.5	13, 6, 10	10	138, 151, 199	163

NT: not tested

P: precipitation

Table 29. continued

Reversion Test of OPC-13013 with TA100

compound	amount (μg/plate)	-S-9 revertants/plate	-S-9 mean	+S-9 revertants/plate	+S-9 mean
DMSO	-	87, 74, 89	83	73, 86, 61	73
OPC-13013	1	84, 89, 70	81	77, 67, 68	71
	5	67, 95, 67	76	90, 74, 70	78
	10	94, 84, 76	85	67, 54, 55	59
	50	85, 71, 96	84	66, 73, 65	68
	100	72, 67, 65	68	79, 67, 79	75
	500	77^{P}, 75^{P}, 73^{P}	75^{P}	61^{P}, 68^{P}, 58^{P}	62^{P}
	1000	77^{P}, 90^{P}, 86^{P}	84^{P}	68^{P}, 53^{P}, 92^{P}	71^{P}
MMS	200	399, 469, 426	431	NT, NT, NT	NT
2AA	0.5	76, 83, 63	74	262, 216, 288	255

NT: not tested

P: precipitation

Table 29. continued

Reversion Test of OPC-13013 with TA98

compound	amount (μg/plate)	-S-9 revertants/plate	-S-9 mean	+S-9 revertants/plate	+S-9 mean
DMSO	-	16, 13, 9	13	17, 17, 15	16
OPC-13013	1	13, 8, 14	12	16, 16 15	16
	5	14, 13, 17	15	27, 17, 17	20
	10	10, 14, 16	13	16, 17, 16	16
	50	12, 11, 10	11	18, 23, 15	19
	100	7, 12, 15	11	17, 19, 19	18
	500	14^P, 9^P, 12^P	12^P	20^P, 12^P, 21^P	18^P
	1000	15^P, 8^P, 9^P	11^P	18^P, 16^P, 19^P	18^P
2NF	1	82, 82, 108	91	NT, NT, NT	NT
2AA	0.5	15, 19, 11	15	196, 177, 178	184

NT: not tested

P: precipitation

Table 29. continued Reversion Test of OPC-13013 with WP2uvrA

compound	amount (μg/plate)	-S-9 revertants/plate	-S-9 mean	+S-9 revertants/plate	+S-9 mean
DMSO	-	10, 23, 14	16	19, 19, 22	20
OPC-13013	1	13, 18, 16	16	10, 23, 25	19
	5	13, 22, 9	15	31, 20, 21	24
	10	16, 12, 28	19	25, 17, 12	18
	50	17, 22, 19	19	26, 19, 19	21
	100	22, 12, 18	17	19, 19, 16	18
	500	23^{P}, 18^{P}, 20^{P}	20^{P}	27^{P}, 19^{P}, 13^{P}	20^{P}
	1000	28^{P}, 18^{P}, 14^{P}	20^{P}	17^{P}, 14^{P}, 28^{P}	20^{P}
ENNG	2	787, 785, 902	825	NT, NT, NT	NT
2AA	80	20, 20, 27	22	209, 272, 285,	255

NT: not tested

P: precipitation

Ames Test of Cilostazol Metabolite OPC-13015

Testing Facility:

Study Number: 003680

Study Dates: November 25, 1986 to February 23, 1987

GLP Compliance: Not addressed; however, it is stated that the study was performed according to the "Guidelines for safety Testing of Drugs" issued by the Japanese Ministry of Health and Welfare.

Lot No. of the Test Compound: 4F83M

Concentrations Tested: 156, 313, 625, 1250, 2500 and 5000 µg OPC-13015/plate.

Solvent: DMSO

Tester Strains : S.typhimurium strains TA1335, TA1537, TA100 and TA98, and E.coli WP2uvrA

Metabolic Activation System: Rat liver S-9 fraction (phenobarbital and beta-naphthoflavone induced)

Positive Control Substances Not Requiring S-9 Mix: N-Ethyl-N'-nitro -N-nitrosoguanidine (ENNG; strains TA1535 and WP2uvrA), ICR-191 (TA1537), methyl methane sulfonate (MMS; TA100) and 2-nitrofluorene (2NF; TA98)

Positive Control Substance Requiring S-9 Mix: 2-Aminoanthracene (2AA, all strains - to test the activity of S-9 mix)

All positive control compounds were dissolved in DMSO except MMS, which was dissolved in sterile distilled water.

A test substance is considered to be positive if:

a) the average number of revertant colonies per plate is greater than twice that of the negative control and

b) a dose-response relationship is seen between concentration and the number of revertant colonies.

Results: The results are presented in Tables 30 to 34. No significant dose-related increased incidence of revertant colonies was observed for any of the tester strains after treatment with the test compound with or without metabolic activation. Respective positive control compounds produced significant increases in revertant colonies, demonstrating the sensitivity of the strains as well as the activity of the S-9 mix.

[It is noted that precipitation occurred at all concentrations with all tester strains in this study. However, in the dose-finding study, no precipitation occurred at the lowest concentration (50 µg/plate).]

Table 30.

Mutagenicity study of OPC-13015 in TA100.

Compound	Dose (μg/plate)	S-9	Revertant colonies per plate [a]							
			Expt.-1				Expt.-2			
			Plate-1	Plate-2	Plate-3	Mean	Plate-1	Plate-2	Plate-3	Mean
DMSO	–	–	96	100	124	107	103	111	116	110
OPC-13015	156	–	P 104	P 113	P 121	113	P 82	P 92	P 104	93
	313	–	P 86	P 88	P 107	94	P 90	P 94	P 104	96
	625	–	P 94	P 98	P 118	103	P 90	P 96	P 109	98
	1250	–	P 110	P 113	P 113	112	P 85	P 100	P 106	97
	2500	–	P 86	P 90	P 107	94	P 86	P 103	P 106	98
	5000	–	P 87	P 96	P 97	93	P 87	P 91	P 103	94
H_2O	–	–	105	127	130	121	109	110	117	112
MMS	200	–	718	719	753	730	673	699	807	726
DMSO	–	+	115	119	130	121	91	98	104	98
OPC-13015	156	+	P 106	P 107	P 119	111	P 103	P 105	P 116	108
	313	+	P 95	P 112	P 112	106	P 101	P 112	P 114	109
	625	+	P 94	P 102	P 102	99	P 110	P 115	P 125	117
	1250	+	P 88	P 89	P 106	94	P 96	P 105	P 107	103
	2500	+	P 80	P 103	P 105	96	P 89	P 100	P 111	100
	5000	+	P 92	P 99	P 101	97	P 93	P 98	P 102	98
2AA	1	+	948	1118	1574	1213	1276	1318	1324	1306

a, Mean value of three replicate plates; P, Precipitation

Table 31.

Mutagenicity study of OPC-13015 in TA98.

Compound	Dose (μg/plate)	S-9	Revertant colonies per plate [a]							
			Expt.-1				Expt.-2			
			Plate-1	Plate-2	Plate-3	Mean	Plate-1	Plate-2	Plate-3	Mean
DMSO	–	–	15	15	16	15	21	21	24	22
OPC-13015	156	–	P 15	P 16	P 16	16	P 18	P 19	P 29	22
	313	–	P 18	P 20	P 21	20	P 15	P 18	P 23	19
	625	–	P 14	P 22	P 25	20	P 19	P 20	P 22	20
	1250	–	P 12	P 17	P 18	16	P 17	P 20	P 23	20
	2500	–	P 8	P 14	P 14	12	P 7	P 10	P 12	10
	5000	–	P 14	P 14	P 15	14	P 8	P 10	P 12	10
2NF	1	–	108	120	121	116	128	128	136	131
DMSO	–	+	27	28	33	29	20	22	27	23
OPC-13015	156	+	P 23	P 26	P 34	28	P 24	P 32	P 33	30
	313	+	P 23	P 23	P 24	23	P 23	P 27	P 32	27
	625	+	P 22	P 27	P 29	26	P 21	P 21	P 24	22
	1250	+	P 19	P 21	P 23	21	P 23	P 24	P 27	25
	2500	+	P 16	P 18	P 22	19	P 20	P 21	P 26	22
	5000	+	P 18	P 19	P 20	19	P 14	P 20	P 22	19
2AA	0.5	+	496	530	573	533	512	543	620	558

a, Mean value of three replicate plates; P, Precipitation

Table 32.

Mutagenicity study of OPC-13015 in TA1535.

Compound	Dose (μg/plate)	S-9	Revertant colonies per plate [a]							
			Expt.-1				Expt.-2			
			Plate-1	Plate-2	Plate-3	Mean	Plate-1	Plate-2	Plate-3	Mean
DMSO	–	–	7	10	11	9	7	8	9	8
OPC-13015	156	–	P 8	P 11	P 12	10	P 5	P 7	P 10	7
	313	–	P 6	P 6	P 8	7	P 6	P 8	P 8	7
	625	–	P 7	P 8	P 11	9	P 5	P 8	P 11	8
	1250	–	P 6	P 7	P 11	8	P 6	P 12	P 13	10
	2500	–	P 6	P 7	P 8	7	P 1	P 3	P 8	4
	5000	–	P 8	P 8	P 11	9	P 7	P 8	P 10	8
ENNG	5	–	71	93	102	89	132	155	177	155
DMSO	–	+	9	9	11	10	5	8	11	8
OPC-13015	156	+	P 7	P 8	P 14	10	P 6	P 8	P 9	8
	313	+	P 6	P 6	P 8	7	P 6	P 7	P 13	9
	625	+	P 4	P 7	P 10	7	P 7	P 8	P 12	9
	1250	+	P 6	P 6	P 10	7	P 4	P 8	P 8	7
	2500	+	P 5	P 6	P 6	6	P 5	P 7	P 11	8
	5000	+	P 3	P 4	P 4	4	P 4	P 7	P 12	8
2AA	2	+	213	218	275	235	182	200	233	205

a, Mean value of three replicate plates; P, Precipitation

Table 33.

Mutagenicity study of OPC-13015 in TA1537.

Compound	Dose (μg/plate)	S-9	Revertant colonies per plate [a]							
			Expt.-1				Expt.-2			
			Plate-1	Plate-2	Plate-3	Mean	Plate-1	Plate-2	Plate-3	Mean
DMSO	–	–	6	8	9	8	6	8	13	9
OPC-13015	156	–	P 5	P 6	P 6	6	P 5	P 9	P 10	8
	313	–	P 5	P 6	P 6	6	P 6	P 6	P 7	6
	625	–	P 2	P 6	P 7	5	P 5	P 6	P 7	6
	1250	–	P 4	P 6	P 6	5	P 3	P 3	P 4	3
	2500	–	P 3	P 6	P 6	5	P 3	P 3	P 4	3
	5000	–	P 2	P 4	P 8	5	P 1	P 2	P 2	2
ICR-191	1	–	605	625	686	639	179	214	237	210
DMSO	–	+	6	7	12	8	9	9	9	9
OPC-13015	156	+	P 4	P 5	P 8	6	P 6	P 6	P 7	6
	313	+	P 8	P 10	P 10	9	P 5	P 5	P 11	7
	625	+	P 7	P 8	P 9	8	P 4	P 5	P 6	5
	1250	+	P 4	P 8	P 9	7	P 4	P 8	P 9	7
	2500	+	P 6	P 6	P 9	7	P 2	P 3	P 7	4
	5000	+	P 1	P 2	P 7	3	P 2	P 3	P 4	3
2AA	2	+	261	281	306	283	494	498	508	500

a, Mean value of three replicate plates; P, Precipitation

Table 34.

Mutagenicity study of OPC-13015 in WP2 *uvr*A.

Compound	Dose (μg/plate)	S-9	Revertant colonies per plate [a]							
			Expt.-1				Expt.-2			
			Plate-1	Plate-2	Plate-3	Mean	Plate-1	Plate-2	Plate-3	Mean
DMSO	–	–	7	10	11	9	11	11	14	12
OPC-13015	156	–	P 7	P 10	P 12	10	P 8	P 16	P 16	13
	313	–	P 10	P 10	P 12	11	P 11	P 13	P 13	12
	625	–	P 9	P 11	P 11	10	P 10	P 18	P 20	16
	1250	–	P 7	P 10	P 12	10	P 10	P 10	P 10	10
	2500	–	P 5	P 5	P 8	6	P 7	P 9	P 11	9
	5000	–	P 3	P 10	P 12	8	P 9	P 9	P 9	9
ENNG	2	–	156	181	195	177	178	195	220	198
DMSO	–	+	9	10	10	10	10	14	14	13
OPC-13015	156	+	P 8	P 14	P 14	12	P 12	P 12	P 13	12
	313	+	P 11	P 11	P 14	12	P 13	P 14	P 14	14
	625	+	P 9	P 11	P 13	11	P 13	P 14	P 14	14
	1250	+	P 7	P 7	P 13	9	P 11	P 17	P 18	15
	2500	+	P 7	P 11	P 11	10	P 8	P 9	P 13	10
	5000	+	P 8	P 11	P 12	10	P 9	P 10	P 13	11
2AA	20	+	489	492	570	517	627	685	704	672

a, Mean value of three replicate plates; P, Precipitation

Ames Test of Cilostazol Metabolite OPC-13213

Testing Facility:

Study Number: 003682

Study Dates: November 25, 1986 to February 23, 1987

GLP Compliance: Not addressed; however, it is stated that the study was performed according to the "Guidelines for Safety Testing of Drugs", issued by the Japanese Ministry of Health and Welfare.

Lot No. of the Test Compound: 4A73

Doses Tested: 156, 313, 625, 1250, 2500 and 5000 µg OPC-13213/ plate.

Solvent: DMSO

Tester Strains: S. Typhimurium strains TA1535, TA1537, TA100 and TA 98, and E.coli WP2uvra

Metabolic Activation System: Rat liver S-9 fraction (phenobarbital and β-naphthoflavone induced)

Positive Control Substances Not Requiring S-9 Mix: ENNG (TA1535 and WP2uvrA), ICR-191 (TA1537), MMS (TA100) and 2NF (TA98)

Positive Control Substance Requiring S-9 Mix: 2AA (all strains for testing the activity of S-9 mix)

All positive control compounds were dissolved in DMSO except MMS, which was dissolved in sterile distilled water.

A test substance is considered to be positive if:

a) the average number of revertant colonies per plate is greater than twice that of the negative control and

b) a dose-response relationship is observed between concentration and the number of revertant colonies.

Results: Results are presented in Tables 35 to 39. No significant dose-related increased incidence of revertant colonies was observed for any of the tester strains after treatment with the test compound with or without metabolic activation. Respective positive control compounds produced significant increases in revertant colonies, demonstrating the sensitivity of the test strains as well as the activity of the S-9 mix.

[It is noted that precipitation occurred at all concentrations except at the lowest concentration (156 µg/plate).]

Table 35.

Mutagenicity study of OPC-13213 in TA100.

Compound	Dose (μg/plate)	S-9	Revertant colonies per plate [a]							
			Expt.-1				Expt.-2			
			Plate-1	Plate-2	Plate-3	Mean	Plate-1	Plate-2	Plate-3	Mean
DMSO	–	–	106	107	113	109	117	118	118	118
OPC-13213	156	–	82	104	115	100	99	102	111	104
	313	–	P 102	P 105	P 112	106	P 100	P 101	P 102	101
	625	–	P 90	P 102	P 105	99	P 92	P 97	P 99	96
	1250	–	P 103	P 105	P 106	105	P 96	P 97	P 109	101
	2500	–	P 105	P 108	P 114	109	P 99	P 107	P 109	105
	5000	–	P 102	P 103	P 115	107	P 100	P 103	P 105	103
H_2O	–	–	103	110	116	110	118	121	122	120
MMS	200	–	533	594	602	576	711	735	760	735
DMSO	–	+	95	107	113	105	96	115	115	109
OPC-13213	156	+	108	109	109	109	97	102	107	102
	313	+	105	105	114	108	100	101	113	105
	625	+	P 99	P 104	P 120	108	P 104	P 104	P 105	104
	1250	+	P 104	P 104	P 113	107	P 97	P 103	P 109	103
	2500	+	P 95	P 95	P 116	102	P 102	P 103	P 110	105
	5000	+	P 101	P 102	P 105	103	P 90	P 95	P 102	96
2AA	1	+	1223	1374	1397	1331	1606	1636	1693	1645

a, Mean value of three replicate plates; P, Precipitation

Table 36.

Mutagenicity study of OPC-13213 in TA98.

Compound	Dose (µg/plate)	S-9	Revertant colonies per plate [a]							
			Expt.-1				Expt.-2			
			Plate-1	Plate-2	Plate-3	Mean	Plate-1	Plate-2	Plate-3	Mean
DMSO	–	–	15	18	20	18	18	20	20	19
OPC-13213	156	–	17	21	22	20	19	21	22	21
	313	–	P 15	P 19	P 20	18	17	19	21	19
	625	–	P 13	P 19	P 21	18	P 17	P 21	P 22	20
	1250	–	P 17	P 19	P 21	19	P 18	P 19	P 21	19
	2500	–	P 13	P 17	P 20	17	P 15	P 16	P 16	16
	5000	–	P 13	P 15	P 16	15	P 14	P 14	P 16	15
2NF	1	–	96	113	133	114	143	144	154	147
DMSO	–	+	26	27	29	27	27	28	32	29
OPC-13213	156	+	21	24	27	24	23	29	34	29
	313	+	25	26	29	27	24	24	33	27
	625	+	P 20	P 24	P 34	26	P 31	P 32	P 33	32
	1250	+	P 29	P 33	P 39	34	P 22	P 22	P 26	23
	2500	+	P 18	P 21	P 24	21	P 23	P 23	P 29	25
	5000	+	P 18	P 20	P 22	20	P 23	P 23	P 25	24
2AA	0.5	+	472	488	511	490	561	565	573	566

a, Mean value of three replicate plates; P, Precipitation

Table 37.

Mutagenicity study of OPC-13213 in TA1535.

Compound	Dose (μg/plate)	S-9	Revertant colonies per plate [a]							
			Expt.-1				Expt.-2			
			Plate-1	Plate-2	Plate-3	Mean	Plate-1	Plate-2	Plate-3	Mean
DMSO	–	–	7	9	11	9	9	9	13	10
OPC-13213	156	–	9	10	11	10	7	9	13	10
	313	–	P 7	P 8	P 8	8	8	14	14	12
	625	–	P 7	P 11	P 12	10	P 7	P 9	15	10
	1250	–	P 6	P 6	P 11	8	P 8	P 10	12	10
	2500	–	P 9	P 12	P 14	12	P 8	P 9	12	10
	5000	–	P 8	P 8	P 12	9	P 7	P 9	13	10
ENNG	5	–	85	96	104	95	121	134	140	132
DMSO	–	+	7	10	11	9	7	9	10	9
OPC-13213	156	+	6	8	10	8	8	9	10	9
	313	+	7	8	14	10	7	8	12	9
	625	+	P 8	P 8	P 14	10	P 7	P 8	P 13	9
	1250	+	P 7	P 7	P 8	7	P 8	P 9	P 13	10
	2500	+	P 6	P 7	P 8	7	P 10	P 13	P 15	13
	5000	+	P 7	P 8	P 10	8	P 9	P 11	P 11	10
2AA	2	+	134	174	183	164	235	237	251	241

a, Mean value of three replicate plates; P, Precipitation

Table 38.

Mutagenicity study of OPC-13213 in TA1537.

Compound	Dose (μg/plate)	S-9	Revertant colonies per plate a							
			Expt.-1				Expt.-2			
			Plate-1	Plate-2	Plate-3	Mean	Plate-1	Plate-2	Plate-3	Mean
DMSO	–	–	7	7	9	8	8	9	11	9
OPC-13213	156	–	7	7	8	7	10	10	11	10
	313	–	P 3	P 6	P 8	6	P 12	P 13	P 13	13
	625	–	P 3	P 8	P 10	7	P 11	P 12	P 12	12
	1250	–	P 5	P 6	P 8	6	P 8	P 9	P 10	9
	2500	–	P 4	P 7	P 8	6	P 9	P 10	P 10	10
	5000	–	P 5	P 5	P 7	6	P 7	P 8	P 9	8
ICR-191	1	–	572	602	622	599	557	577	584	573
DMSO	–	+	7	9	10	9	11	12	13	12
OPC-13213	156	+	5	7	9	7	9	10	12	10
	313	+	6	8	10	8	9	12	13	11
	625	+	P 6	P 7	P 8	7	P 9	P 10	P 12	10
	1250	+	P 5	P 11	P 13	10	P 7	P 7	P 9	8
	2500	+	P 4	P 9	P 9	7	P 7	P 8	P 8	8
	5000	+	P 7	P 8	P 8	8	P 5	P 6	P 6	6
2AA	2	+	356	412	468	412	530	566	580	559

a, Mean value of three replicate plates; P, Precipitation