



***Comparative Evaluation of Twelve
Pyrimidinedione Inhibitors of HIV-1 For Further
Preclinical and Clinical Development***

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Overview of Pyrimidinediones

- The pyrimidinedione inhibitors were developed by Samjin Pharmaceuticals, Ltd. and are currently under advanced preclinical development as anti-HIV therapeutics and topical microbicides
- 78 congeners were synthesized and evaluated in our preclinical HIV development programs
- Each of the pyrimidinediones were highly potent inhibitors of both HIV-1 and HIV-2

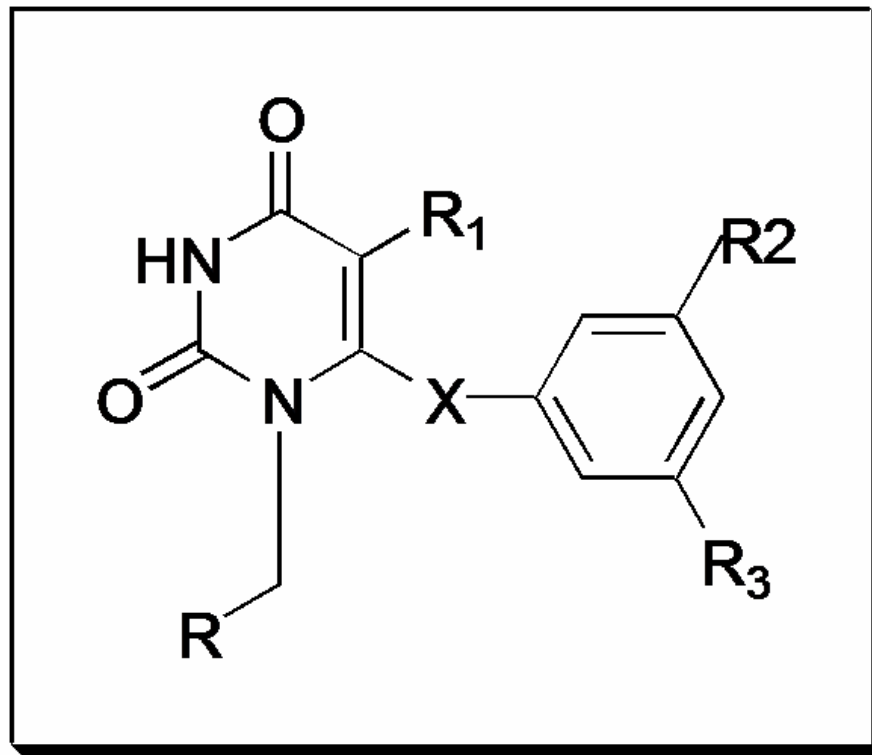
Overview of Pyrimidinediones

- The compounds have been determined to be “mixed” type inhibitors of HIV-1 RT, affecting both the K_m and the V_{max} . The K_i approximates the *in vitro* cell-based anti-HIV activity. The compounds are inactive against HIV-2 RT.
- The compounds inhibit entry of both HIV-1 and HIV-2 through interaction with a unique pre-fusion, conformational target formed upon co-culture of virus and target cells

Definition of Pyrimidinediones for Further Preclinical and Clinical Development

- Of the 78 congeners, 12 compounds were selected for further evaluation based on their antiviral and toxicity profile.
- The selected compounds were comparatively evaluated in a variety of cell-based and biochemical assays to select the most potent inhibitors for therapeutic and microbicidal use:
 - Efficacy versus HIV-1 and HIV-2 in PBMCs and monocytes-macrophages
 - Virus attachment and RT inhibition
 - Combination efficacy and toxicity with other anti-HIV compounds
 - Resistance and their relative ability to prevent the outgrowth of virus.
- The compounds were evaluated for bioavailability in mice to define the compounds which have the greatest level of oral bioavailability.

General Structure of Pyrimidinediones



Structure of Selected Pyrimidinediones

SAR	R1	R2	R3	X	R
16	Et	Me	Me	O	Cyclopropyl
17	iPr	Me	Me	O	Cyclopropyl
18	Et	Me	Me	C=O	Cyclopropyl
19	iPr	Me	Me	C=O	Cyclopropyl
20	Et	Me	Me	S	Cyclobutyl
22	Et	Me	Me	O	Cyclobutyl
40	Et	Me	Me	O	Phenyl
45	iPr	Me	Me	S	1-Cyclopenten-1-yl
49	iPr	Me	Me	C=O	1-Cyclopenten-1-yl
56	Et	Me	Me	O	3-Cyclopenten-1-yl
62	Et	Me	Me	C=O	3-Cyclopenten-1-yl
63	iPr	Me	Me	C=O	3-Cyclopenten-1-yl

Inhibition of HIV-1 and HIV-2 in CEM-SS Cells

Compound	HIV-1 _{III B} EC ₅₀ (nM)	HIV-2 _{ROD} EC ₅₀ (nM)	Therapeutic Index
SAR 16	1.0	280	444,000
SAR 17	0.2	50	119,500
SAR 18	0.2	40	>2,500,000
SAR 19	0.2	20	730,000
SAR 20	2.0	250	61,000
SAR 22	10	>1000	22,300
SAR 40	4.0	110	12,850
SAR 45	2.0	160	12,100
SAR 49	0.5	30	48,600
SAR 56	2.0	110	155,000
SAR 62	0.2	40	2,087,500
SAR 63	0.4	20	218,250

Inhibition of Clinical Strains of HIV-1 and HIV-2 in PBMCs

Compound	HIV-1 Clade B (US/92/727) EC ₅₀ (nM)	HIV-2 EC ₅₀ (nM)	Therapeutic Index
SAR 16	0.24	<0.1	>2,083,300
SAR 17	0.23	<0.1	787,826
SAR 18	0.33	<0.1	>1,515,151
SAR 19	0.42	<0.1	>1,190,476
SAR 20	1.73	<0.1	76,127
SAR 22	>10	0.45	>50,000
SAR 40	2.44	<0.1	26,352
SAR 45	1.62	<0.1	70,802
SAR 49	0.54	<0.1	62,777
SAR 56	2.40	0.18	>208,333
SAR 62	0.13	<0.1	>3,846,154
SAR 63	0.36	<0.1	>1,388,888

Virus Entry Inhibition

Compound	EC ₅₀ (μM)	Therapeutic Index
SAR 16	0.073	>6849
SAR 17	0.022	5486
SAR 18	0.021	>23,810
SAR 19	0.017	7371
SAR 20	0.21	1519
SAR 22	>1	>500
SAR 40	0.55	907
SAR 45	0.024	4646
SAR 49	0.027	3974
SAR 56	0.23	>2174
SAR 62	0.059	>8475
SAR 63	0.050	>10,000

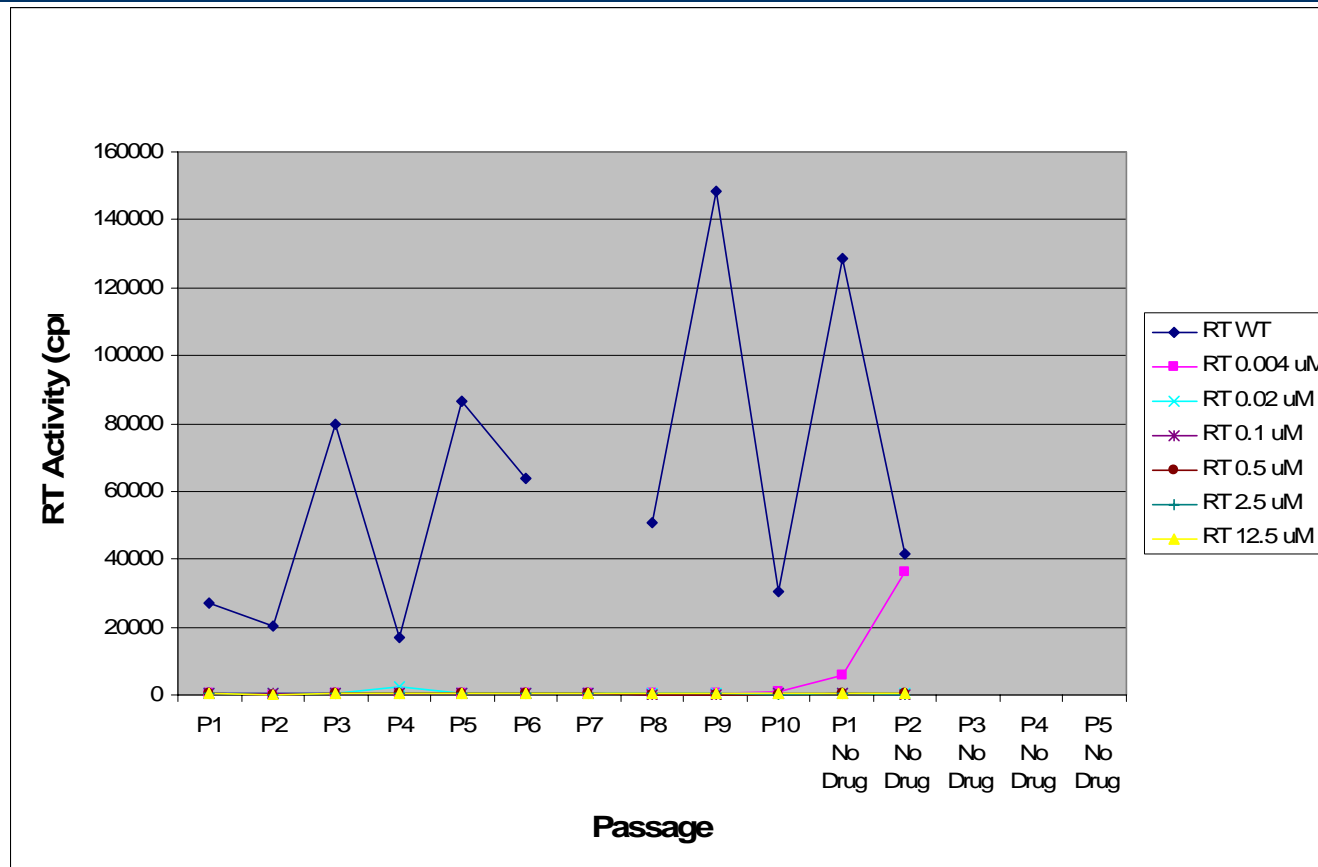
Inhibition of Virus and RT with K103N Mutation

Compound	Site Directed NL4-3 _{K103N} CEM-SS Cells EC ₅₀ (μM) (Fold Resistance)	K103N Enzymatic Assay IC ₅₀ (μM) (Fold Resistance)
SAR 16	0.04 (80)	0.093 (S)
SAR 17	0.02 (67)	0.088 (29)
SAR 18	0.004 (7)	0.45 (10)
SAR 19	0.003 (7.5)	0.48 (11)
SAR 20	0.05 (25)	0.93 (72)
SAR 22	0.45 (113)	2 (S)
SAR 40	0.14 (28)	0.08 (S)
SAR 45	0.07 (70)	>100 (1150)
SAR 49	0.02 (20)	0.09 (S)
SAR 56	0.11 (37)	0.09 (S)
SAR 62	0.01 (50)	0.82 (13)
SAR 63	0.01 (20)	85.6 (243)

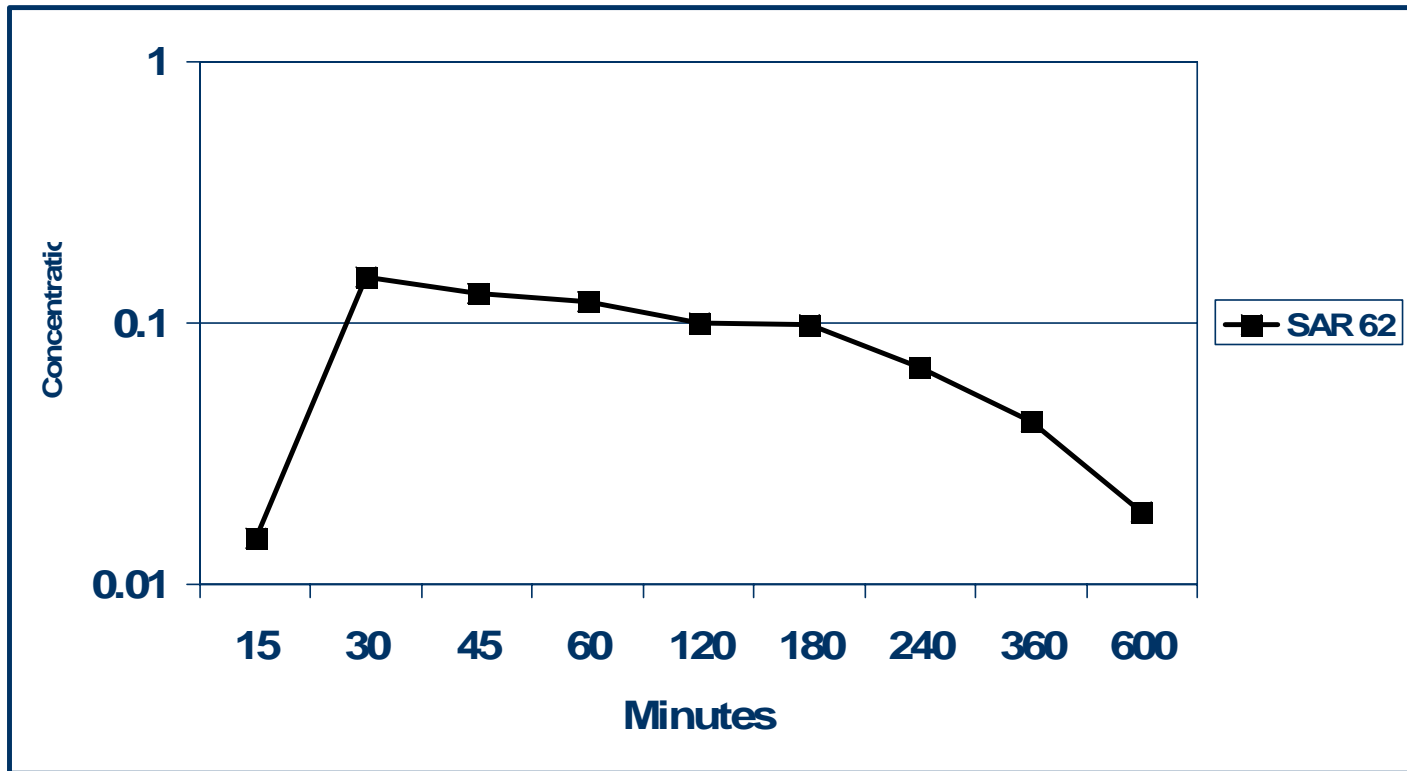
Suppression of Virus Outgrowth (Sterilizing Concentrations)

Compound	EC ₅₀ (μ M) (Fold Increase) Continuous Drug Pressure	EC ₅₀ (μ M) (Fold Increase) Drug Pressure Removed
SAR 16	0.25 (250)	0.25 (250)
SAR 17	0.05 (250)	0.25 (1,250)
SAR 18	0.25 (1,250)	0.25 (1,250)
SAR 19	0.05 (250)	0.05 (250)
SAR 20	12.5 (6250)	62.5 (31,250)
SAR 22	12.5 (1,250)	62.5 (6,250)
SAR 40	1.0 (250)	1.0 (250)
SAR 45	12.5 (6250)	62.5 (31,250)
SAR 49	0.125 (250)	0.125 (250)
SAR 56	12.5 (6250)	12.5 (6,250)
SAR 62	0.5 (2500)	12.5 (6,250)
SAR 63	0.02 (50)	0.02 (50)
UC781	0.025 (50)	0.025 (50)

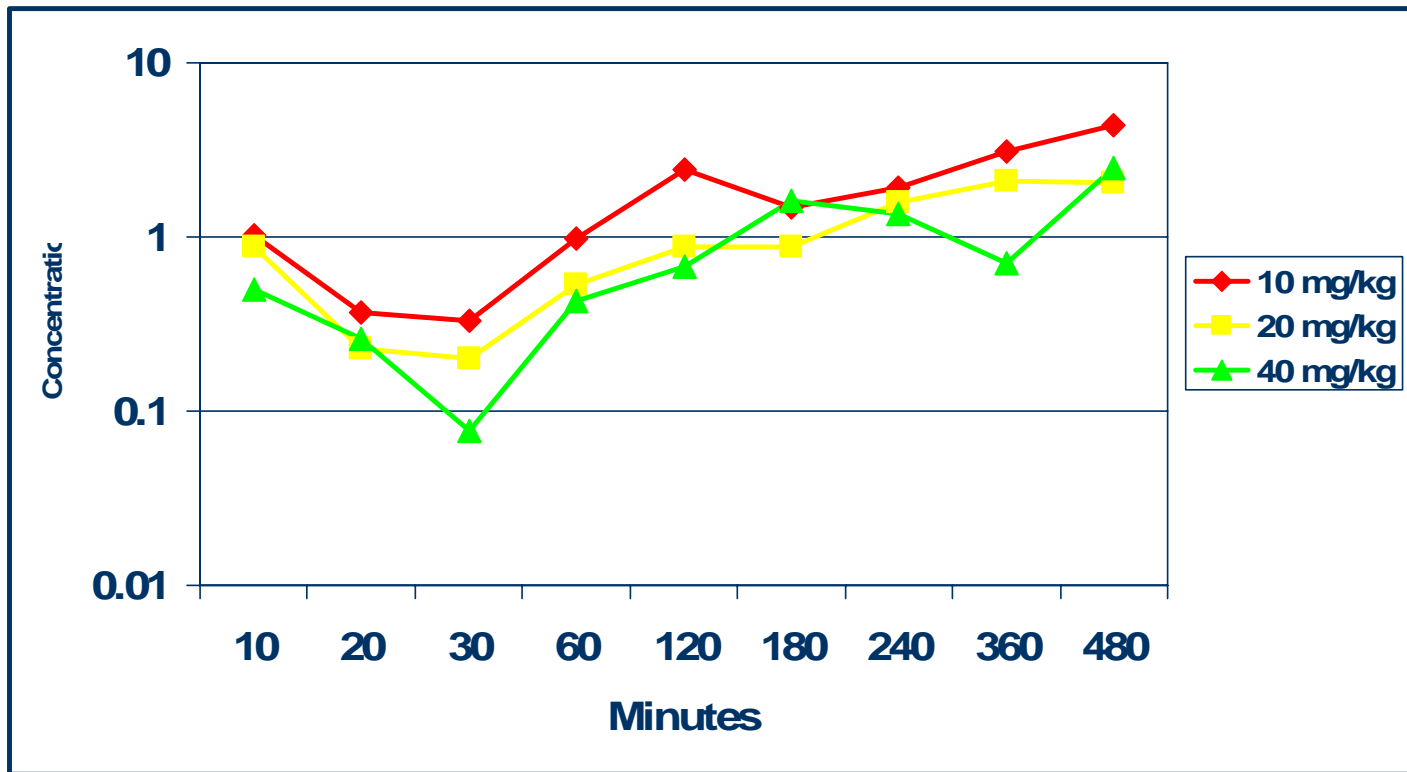
Compound 63: Inhibition of Virus Outgrowth (Sterilization Assay)



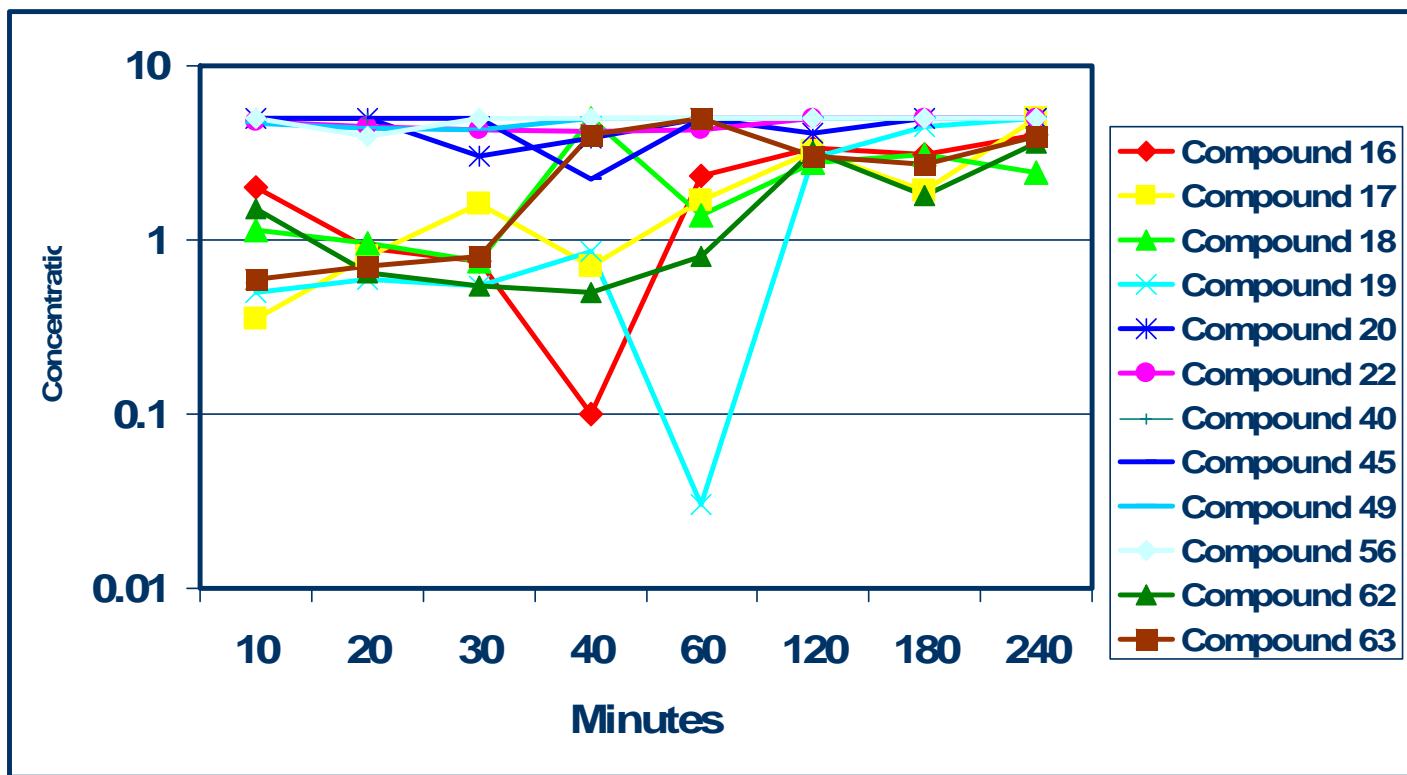
Oral Bioavailability in Mice of SAR 62 (Quantified using HPLC)



Oral Bioavailability in Mice of SAR 62 (Quantified using anti-HIV-1 Bioassay)



Oral Bioavailability of Compounds in Mice (Quantified by anti-HIV-1 Bioassay)



Conclusions

- 78 congeners were evaluated, yielding 12 potential clinical candidates.
- Five highly potent inhibitors were defined based on efficacy and toxicity profiles [16, 18, 19, 62, 63]
- Four inhibitors exhibited higher levels of oral bioavailability in mice [16, 18, 19, 62]
- Compound 63 performed exceptionally well in the virus outgrowth assay and had reasonably high oral bioavailability.

Acknowledgements

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- The microbicidal potential of the pyrimidinediones is the subject of Poster 164: *Development of highly potent pyrimidinedione inhibitors as topical microbicides.*